HTC-17

17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology

May 18-20, 2022 – Ghent, Belgium

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WELCOME FROM THE CONFERENCE CHAIR

17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology

SYMPOSIUM VENUE

De Aula
Voldersstraat 9
9000 Ghent, Belgium

SHORT COURSE VENUE

Novotel
Hoogpoort 52
9000 Ghent, Belgium

ORGANIZING SECRETARIAT

Conference Office
htc17@kuleuven.be
KU Leuven, Leuven, Belgium
www.htc-17.com

WELCOME FROM THE CONFERENCE CHAIR

Dear Colleagues and Friends,

It is my immense pleasure to welcome you – on behalf of the entire organizing committee– to the 17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-17), in "De Aula" in Ghent, Belgium. HTC-17 is organized under the auspices of the Royal Flemish Chemical Society (KVCV) and the Royal Society of Chemistry (RSC).

The HTC-17 conference is the premier platform for state-of-the-art developments in separation technologies and hyphenated techniques. The conference will encompass two parallel sessions consisting of plenary lectures, keynote lectures, tutorials and oral presentations. Posters will be on display throughout the entire conference. The symposium will also host an attractive technical exhibition where vendors will present their newest instruments and developments, topped with technical seminars. I would like to thank all our sponsors and exhibitors for their support of the conference. The scientific program will cover both fundamental and practical aspects of liquid and gas chromatography, including UHPLC-MS, multidimensional LC, GC(×GC)-MS, SFC, etc. The program will furthermore include topics such as sample preparation, miniaturization and automation, in-silico chromatographic method development and data mining, system design and optimization, chip technology… We even have a Covid-19 session! New trends and technologies for a wide range of applications will be presented, including (bio-)pharmaceuticals, proteins and other macromolecules, environmental applications, food, beverages and natural products…

We moreover have a nice number of awards lined up, including the HTC Innovation Award, sponsored by LCGC Europe, that will be presented to a scientist who has made innovative contributions to the field of separation science, on Thursday morning. The HTC award will go to the most innovative oral presentation given during the conference, and will be presented during the Closing Ceremony. The three most innovative poster contributions will receive HTC poster awards. The best poster on sample preparation will moreover receive an award from Advances in Sample Preparation (Elsevier). Finally, we will also present awards for the three best videos received for the HTC YouTube contest, sponsored by Journal of Chromatography A and Journal of Chromatography Open (Elsevier) on Thursday evening!

As always, we will ensure abundant networking opportunities during the conference, with an informal Belgian Beer tasting event on Wednesday evening and the Conference Dinner, which will be held in the beautiful Poortackere Monasterium, on Thursday evening. Please also join us for the Farewell Drink on Friday.

On behalf of the entire organizing team of HTC-17, welcome to Ghent!

Deirdre Cabooter

University of Leuven

Chair of HTC-17

COMMITTEES

SCIENTIFIC COMMITTEE

Frédéric Lynen, Ghent University, Belgium (Chair)

John Langley, University of Southampton, UK (Co-Chair)

Jean-François Focant, Université de Liège, Belgium

Marja-Liisa Riekkola, University of Helsinki, Finland

Valérie Pichon, École Supérieure de Physique et de Chimie Industrielles, France

Gert Desmet, Vrije Universiteit Brussel, Belgium

Caroline West, Université d'Orléans, France

Lucie Nováková, Charles University, Czech Republic

Sebastiaan Eeltink, Vrije Universiteit Brussel, Belgium

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Melissa Hannah-Brown, Pfizer, UK

Rob Edam, Shell, The Netherlands

LOCAL ORGANIZING COMMITTEE

Deirdre Cabooter, KU Leuven, Belgium (Chair)

Frédéric Lynen, Ghent University, Belgium (Co-chair)

Sebastiaan Eeltink, Vrije Universiteit Brussel, Belgium (Co-chair)

John Langley, University of Southampton, UK

Ken Broeckhoven, Vrije Universiteit Brussel, Belgium

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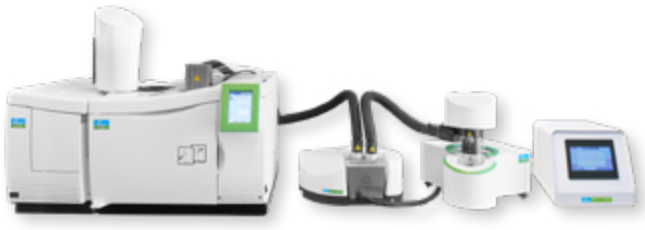

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JOURNAL OF CHROMATOGRAPHY A

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SOCIAL EVENTS

BELGIAN BEER TASTING

The traditional Belgian Beer tasting event will take place on Wednesday 18 May 2022, starting at 18.30 in the Peristilium and Foyer of the conference center De Aula. Your voucher will be provided at the registration desk. Enjoy a choice of high-quality Belgian beers and selected snacks.

CONFERENCE DINNER

The HTC-17 Conference Dinner will be held on Thursday 19 May 2022 in Monasterium Poortackere, Oude Houtlei 56, 9000 Ghent. Located in the "land (akker) in front of the gate (poort)", the Sint-Aubertus Beguinage was built in 1278. In the 19th century, a monastery and a new chapel were built. It is in this chapel that the conference dinner will take place.

The venue is about 500 m from the HTC-conference center "De Aula" (10 min walking distance).

The dinner will start with an aperitif at 19:00, followed by a seated dinner at 20:00. Please note that pre-registration for the dinner was required.

FAREWELL DRINK

On Friday afternoon, 20 May 2022, HTC-17 will reach its end. Join your symposium friends for a last exchange of ideas and experiences while enjoying a Belgian beer or another drink. All participants and accompanying persons are kindly invited, no registration or voucher needed.

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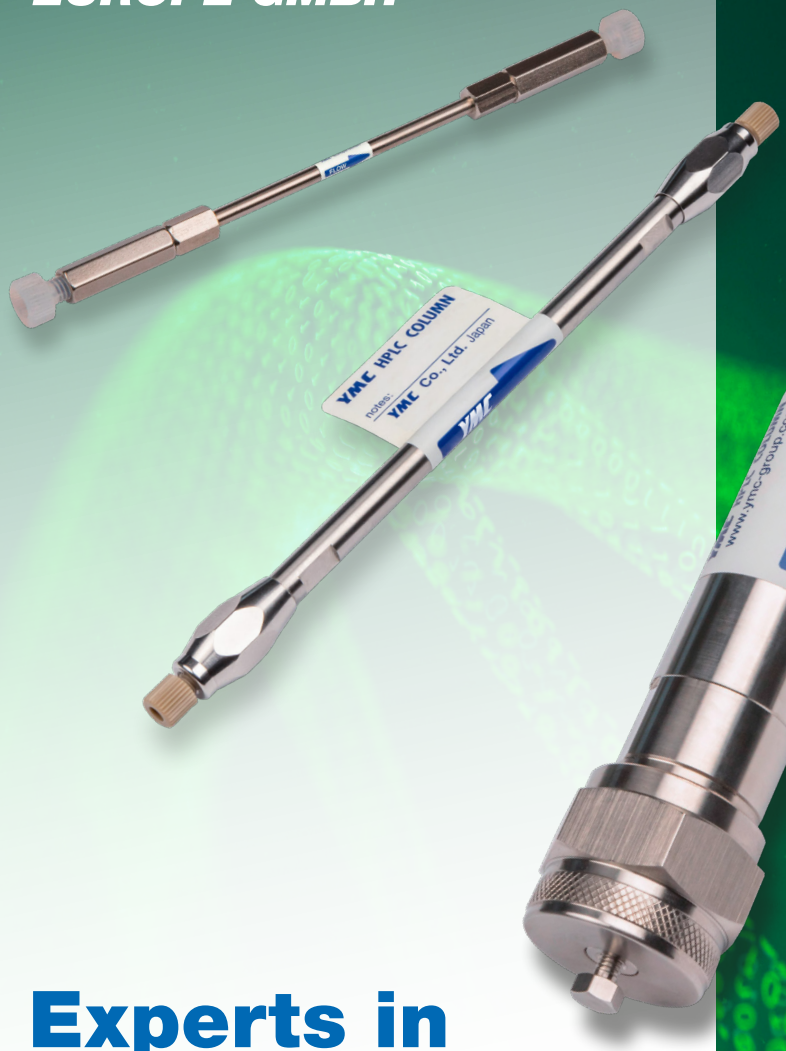

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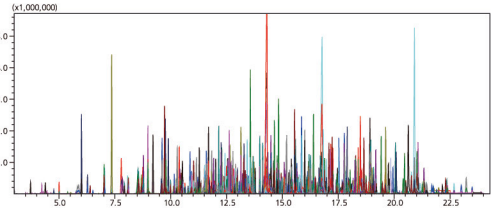

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AWARDS

2022 HTC INNOVATION AWARD

The HTC Innovation Award was launched in 2018 to celebrate the work of scientists who are innovatively evolving the field of hyphenated techniques. LCGC Europe will present the award at the event. Applications from separation scientists worldwide were welcomed and LCGC Europe readers were invited to nominate themselves or others in the course of 2021.

The winner has been selected by the HTC-17 Scientific Committee and the HTC-17 Industry Board based on the following criteria:

- The winner has made an innovative contribution to the field of separation science by introducing new methodologies, new instrumentation, or new techniques in the field with a strong focus on applicability.
- The winner is a scientist under 45 years of age.

The winner of the 2022 HTC Innovation Award will be announced and present his/her research at HTC-17.

Previous HTC Innovation Award winners:

- Caroline Huhn (2018)
- Ryan T. Kelly (2020)

HTC AWARD

The most innovative oral presentation given during the conference will receive the HTC Award. The winner will be selected by the HTC-17 Scientific Committee and the HTC-17 Industry Board. The HTC award will be presented during the closing session of HTC-17 on Friday afternoon.

Previous HTC-Award winners are:

- Janusz Pawliszyn (1996)
- N. Semenov (1998)
- Heidi Goenaga-Infante (2000)
- Aviv Amirav (2002)
- Gert Desmet (2004)
- Luigi Mondello (2006)
- Robert Shellie (2008)
- Oliver Trapp (2010)
- Tuulia Hyötyläinen (2012)
- Frank David (2014)
- Paola Dugo (2016)
- André De Villiers (2020)

POSTER AWARDS

The three most innovative poster contributions will receive HTC-poster awards worth 500 Euro (first place), 300 Euro (second place) and 200 Euro (third place). To qualify for a poster award, you must have declared yourself a candidate upon submitting your abstract. Prof. Ken Broeckhoven will lead a jury consisting of a large number of recognized international experts on hyphenated separation techniques.

HTC TUBE

The three best HTC TUBE videos will receive awards of 750 Euro (first place), 500 Euro (second place) and 250 Euro (third place), sponsored by Journal of Chromatography A and Journal of Chromatography Open, both Elsevier journals.

SAMPLE PREP AWARD

The best poster on sample preparation will receive an award of 500 Euro, sponsored by "Advances in Sample Preparation", an Elsevier journal.

GENERAL INFORMATION

CONGRESS VENUE

The conference will be held in '**De Aula**'

De Aula
Voldersstraat 9
9000 Ghent Belgium
https://www.ugent.be/re/en/contact/directions-aula.htm

The historical city of Ghent is located 60 km from Brussels international airport that has connections to over 120 destinations in Europe and is directly accessible by train from the airport. The Brussels South airport (Charleroi) that accommodates many low-cost airlines is located at 80 km from Ghent.

HOW TO REACH THE VENUE

The conference venue is within walking distance of most hotels in the city of Ghent.

By Tram:
From the Ghent Sint-Pieters train station, take Tram 1 (every 6 min.) or Tram 24 (every 20 min.) and exit at stop 'Korenmarkt' or 'Korte Meer'. Tickets are available at every stop at the ticket machine.

By Road:
Ghent is located near the intersection of the E40 (Brussels-Calais) and E17 (Antwerp-Lille) highways. The Ghent city centre may be the largest pedestrianised shopping area in Flanders, but you can easily park your car nearby. The P-route leads you to the various car parks that give access to the city centre. As soon as you enter the city, the digital signs of the P-route parking guidance system will quickly and conveniently direct you to the parking facility of your choice.

By Rail:
In addition to the connection to the airport, Ghent has direct rail links to many cities in Belgium such as Brussels, Antwerp and Liège that host international train stations with high speed train links to France, The Netherlands, Germany and the UK. Ghent station (Gent Sint-Pieters) is located near the edge of the city and is a 2.5 km walk from the conference venue, the historical city center and hotels.

By Plane:
Brussels Airport is located in Zaventem, 15 km from the center of Brussels and 60 km from the conference venue in Ghent. The airport has over 120 direct connections to European cities, 12 to the USA and Canada, and more than 20 in Asia. The train station is located conveniently at the lower floor of the airport. The Brussels South airport (Charleroi) is located at 80 km from Ghent and has a shuttle service every 30 min to the Brussels-Midi train station

OFFICIAL LANGUAGE

English is the official Symposium language. No translations will be provided.

SHORT COURSES AND SYMPOSIUM DATES

Short courses: Tuesday, May 17, 2022 from 09.00 to 17.00
Symposium: from Wednesday, May 18, 2022 at 09.00 to Friday May 20, 2022 at 17.00

REGISTRATION DESK OPENING HOURS

Tuesday May 17	08:00 – 09:00	(for short course only, please note that this registration moment is planned in the Short course venue Novotel)
Tuesday May 17	16:00 – 18:00	(pre-registration for conference, please note that this registration moment is planned in De Aula, the conference venue)
Wednesday May 18	08:00 – 18:00	
Thursday May 19	08:00 – 18:00	
Friday May 20	08:00 – 15:00	

Badge and conference bag will be available at the HTC-17 registration desk. All participants and exhibitors have to wear the name badge in the conference area, visible at all times.

SCIENTIFIC SESSIONS

Tuesday May 17	09:00 – 17:00	Short courses
Wednesday May 18	09:00 – 18:30	Scientific sessions
	From 18:30	Belgian beer tasting (tickets required)
Thursday May 19	08:30 – 18:00	Scientific sessions
	From 19:00	Conference dinner (tickets required)
Friday May 20	09:00 – 15:45	Scientific sessions
	From 15:45	Farewell Drink

EXHIBITION

The exhibition is an important component of the conference, so please take the time to thank the exhibitors for their generous support of the program by visiting the booths located in the Peristilium and the Foyer.

Exhibition area opening hours:

Wednesday May 18	09:00 – 17:30
Thursday May 19	09:30 – 17:00
Friday May 20	09:30 – 15:00

Exhibitor pass and media pass only allow entry in the Exhibition Area and do not allow entry in the scientific session.

LUNCHES

Wednesday May 18	12:45 – 14:00
Thursday May 19	12:50 – 14:00
Friday May 20	12:50 – 14:00

COFFEE BREAKS

Wednesday May 18	10:45 – 11:15	15:55 – 17:00
Thursday May 19	10:45 – 11:15	15:40 – 16:10
Friday May 20	10:40 – 11:10	

INSTRUCTIONS FOR SPEAKERS

All presentations must be in PowerPoint 4:3 format and must be copied to the laptop in your session at the latest 20 min before the start of your session. Speakers are recommended to arrive at least 15 minutes before the start of the session to introduce themselves to the session chairs. Speakers are to respect time limits for their talks as provided by their chairmen.

ORAL COMMUNICATION SESSIONS

- Please arrive at your session at least 15 minutes before the start of the session to introduce yourself to the session chair and to submit your presentation.
- Kindly note that session chairs are under very strict instructions to keep their sessions on schedule. There are two sessions running in parallel with strict time constraints.
- Time allowed for presentation: 30 min for a keynote lecture or tutorial lecture, 20 min for an oral presentation and 10 min for an industry pitch. This timing includes discussion, so please limit your presentation to allow time for questions.

POSTER SESSIONS

Posters should be printed in portrait A0 format. Suitable materials to attach the posters to the board will be provided at the registration office, do not use any other material to attach your poster to avoid damage to the poster boards.

All posters will be on display during the entire conference. Posters have to be set up on Wednesday May 18 between 08:00 and 09:00 and removed on Friday before 14:30. If you don't remove your poster as mentioned above, the Organizing Secretariat will remove it (Organizing Secretariat assumes no responsibility of problems or damage).

Posters will be organized according to topic:

- FOOD: Food analysis / Food safety and trace analysis
- NAT: Natural product analysis
- MACRO: Macromolecule analysis
- ENV: Environmental and pollutant analysis
- SAMP: Sample preparation
- COL: Column Technology and Stationary phase development
- SYS: System design & optimization
- MET: Method development and artificial intelligence, data mining and curation
- OMIC: Omics (lipidomics, metabolomics, proteomics)
- PHA: (Bio-)pharmaceutical analysis (proteins, oligonucleotides, MAbs, viruses)

The best poster award winners will be announced on Friday, May 20, 2022 during the Closing Plenary Session at 15:30.

SPECIAL ISSUE PUBLICATION – ELSEVIER – JCA

All authors of both oral and poster presentations are kindly invited to submit manuscripts based on their presentation(s) at the HTC-17 meeting for publication in Journal of Chromatography A with the intention of publishing in a Special Issue that is dedicated to this symposium.

CONFERENCE WIFI

Free WIFI access is available throughout the conference venue. Please connect to guestHtc17 (password: **we9WrwKK**).

GENERAL INFORMATION

The use of still or video cameras and cell phones is prohibited during oral sessions and in the poster and exhibition areas without the explicit consent of the poster presenter or exhibitor

ONSITE REGISTRATION

Onsite registration can be made only by credit card.

Participant type	On-site
Academic /Industry	750 €
Student*	450 €
1-day registration	250 €
Accompanying person	250 €
Conference dinner**	75 €
Beer tasting**	30 €

* Students must provide an official proof of their full-time student status.

** This event has a maximum capacity.

Registration Fees (Academic, Industry and Student) include:

- Access to oral and poster sessions
- Access to the exhibition halls
- Coffee breaks and lunches on Wednesday, Thursday, and Friday
- Farewell drink on Friday
- Badge and conference bag

Accompanying Person Fee includes:

- Coffee breaks and lunches on Wednesday, Thursday, and Friday
- Farewell drink on Friday

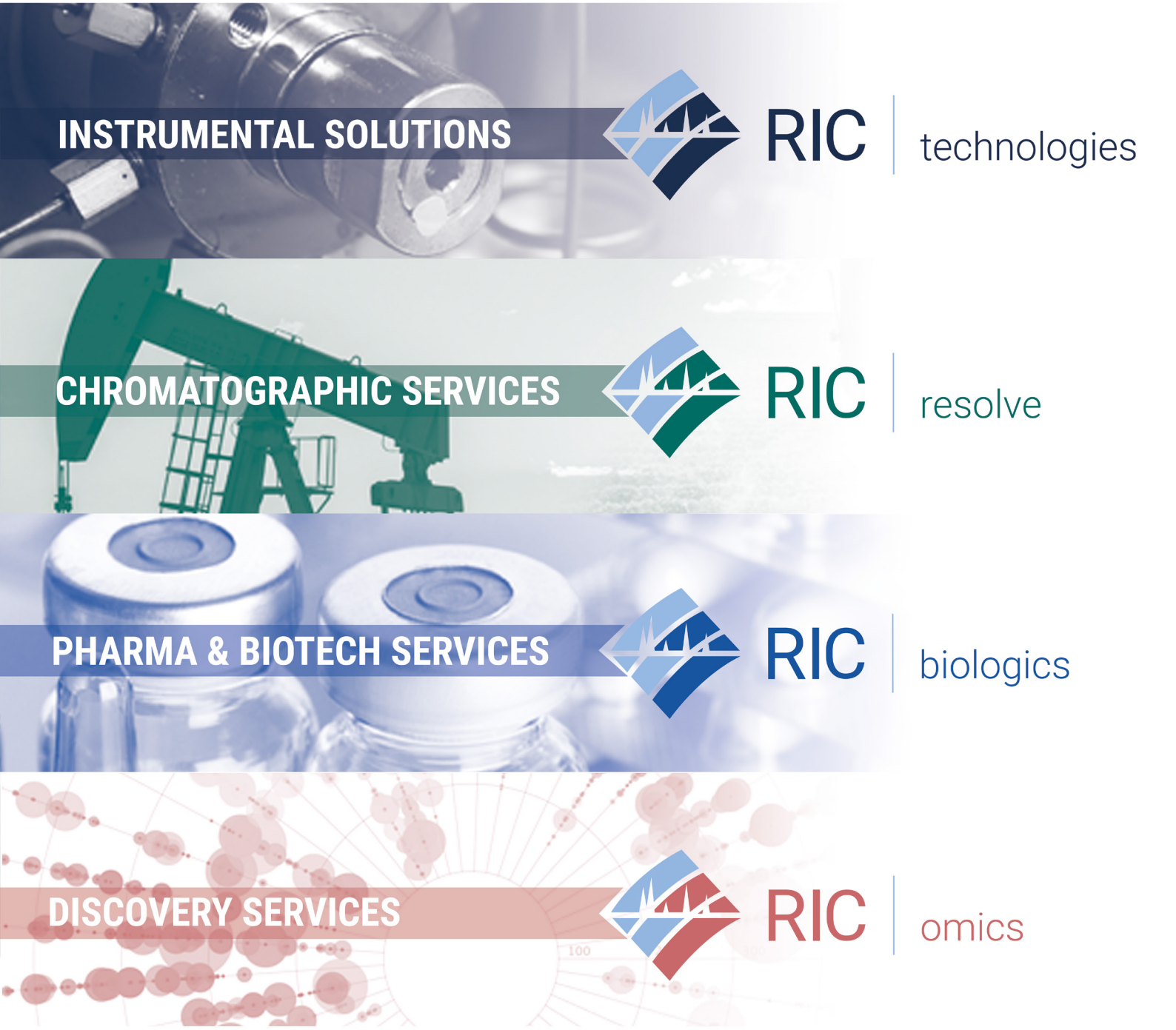
MEETING LOCATIONS

Oral presentations take place in the De Aula lecture hall and the Academie Raadzaal, at the ground floor.

The exhibition of the sponsors is in the Foyer and in the Peristilium.

Coffee/tea breaks and lunches take place next to the exhibitors.

ANALYTICAL QUESTIONS HOLDING YOU BACK?



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SPECIAL ISSUE PUBLICATION – JOURNAL OF CHROMATOGRAPHY A

All authors of both oral and poster presentations are kindly invited to submit manuscripts based on their presentation(s) at the HTC-17 meeting for publication in Journal of Chromatography A with the intention of publishing in a Special Issue that is dedicated to this symposium.

The Special Issue essentially rules out possible delays in publication for contributors to the special issue. Please see below the publication process:

- All papers will go through the normal peer review process per journal standard;
- Papers will be published as soon as they are accepted in earliest available regular journal volumes at ScienceDirect, which ensures very fast publication for individual authors;
- There will be a Footnote included in each accepted paper, indicating at which conference it was presented;
- The collection of finally accepted papers will be prepared and hosted on a dedicated Special Issue site – with links to the papers on Science Direct, retaining all original citation details.
- Submission instructions:
- Submission link: JCA: https://ees.elsevier.com/chroma
- First-time users will need to register;
- Please select Special Issue short title "VSI: HTC-17 conference " during the submission process;
- Please follow the step-by-step guide in completing the submission procedure;
- Submission deadline: 31st August 2022

When preparing your manuscript(s), carefully follow the Guide to Authors of the journal, which you can find at the journal's homepage site. In the cover letter please mention that your manuscript is intended for the HTC-17 Special Issue.

Please note that all manuscripts will be subjected to the mandatory selection process for the journal selected, including the strict peer review procedure; therefore, acceptance for presentation at the conference is not a guarantee for publication in the journal.

SHORT COURSE 1

A fundamental and practical look at separation mechanisms beyond RPLC: HIC, HILIC, SFC, SEC, IEX, MM, PGC

Lecturers: Davy Guillarme, Caroline West

In the ever-changing landscape of Liquid Chromatography (LC), reversed phase (RP) liquid chromatography is still the workhorse for most applications and first choice in method development. There are, however, a large number of applications where RPLC cannot produce an acceptable separation quality and in many instances other separation techniques can provide superior performance than RPLC. The goal of this short course is to provide insight in a selection of these techniques, their underlying mechanisms and applications. These insights will additionally allow for a better selection of orthogonal separation mechanisms for application in multidimensional LC separations.

In the first part of this short course, Davy Guillarme will highlight the basic principles of Ion-exchange (IEX), Size-exclusion (SEC) and Hydrophobic Interaction Chromatography (HIC). The type of mobile phases and stationary phases will also be described to better understand how retention and selectivity can be tuned. The main applications of IEX, SEC and HIC in the field of small molecules analysis as well as protein biopharmaceuticals will be highlighted, to better understand when those specific chromatographic modes have to be used and what could be the added value over RPLC mode. Finally, the detection modes that can be used with these modes of chromatography will also be critically discussed.

In the second part of the course, David McCalley will discuss Hydrophilic interaction chromatography (HILIC), a complementary LC technique that is particularly suited to the analysis of polar and ionised compounds that tend to be poorly retained in RPLC. While it is simple to apply HILIC in practice, the underlying mechanisms of retention can be more complex and deserve consideration by practitioners interested in method development or evaluation. This course should enable participants to understand the basis of hydrophilic retention together with contributions from ionic and other processes; how to judge whether a compound is suitable for HILIC separation; how to select appropriate columns and mobile phases and to enable an appreciation of the advantages and limitations of the technique.

In the final part of the course, Caroline West will discuss the possibilities of Mixed-mode chromatography, porous graphitic carbon stationary phases and Supercritical Fluid Chromatography (SFC). Traditional RPLC is mostly done on conventional stationary phases with little chemical diversity (mostly alkyl or aromatic ligands), offering limited possibilities to optimize selectivity. Other, more exotic stationary phases like porous graphitic carbon (PGC) and mixed-mode (MM) stationary phases involve the combined use of two (or more) retention mechanisms in a single chromatographic system (e.g. RP and HILIC, RP and IEX, etc.). An overview of the current knowledge on these stationary phases, their complementarity to RPLC and sample applications will be proposed. SFC on the other hand uses mobile phases composed of pressurized carbon dioxide and a liquid co-solvent. It uses all stationary phases normally employed in HPLC (from polar silica gel to non-polar C18-bonded silica), for achiral and chiral separations. The basic theory of SFC will be explained, then guidelines to select the operating conditions based on the analyte nature will be given. Specific applications will be presented, especially the transfer of HPLC methods to SFC.

SHORT COURSE 2

Capillary Electrophoresis-Mass Spectrometry for Metabolomics: Principles and Applications

Lecturers: Rawi Ramautar, Marlien van Mever

The main objective of this course is to provide you with an overview of the main capillary electrophoresis-mass spectrometry (CE-MS) approaches used in bioanalysis and metabolomics, including their working/separation mechanisms.

The course is composed of both lectures and cases showing in particular the applicability of CE-MS for volume-restricted biological samples and for compound classes that are (still) difficult to analyze with chromatographic-based separation techniques. Lectures are focused on explaining the basics of the various capillary electrophoresis separation modes and on the use of coated capillaries for specific bio-analytical applications. Ample attention is devoted to the coupling of CE to MS using both the classical and the recently developed interfacing designs. The possibilities enabled by CE-MS using improved interfacing designs is shown for challenging, material-limited biological questions. The reproducibility of CE-MS for metabolomics studies is also considered by highlighting the Metabo-Ring trial.

The utility of the state-of-the-art CE-MS approaches in metabolomics is demonstrated by discussing a few recent studies in more detail. For example, it is shown that neurotransmitters can be directly analyzed in rat microdialysis samples without using derivatization and sample pretreatment. Highly polar metabolites, such as ATP, ADP and AMP, can be analyzed in extracts from just a limited number of mammalian cells with an exquisite detection sensitivity, opening up the possibility to assess the adenylate energy charge in studies dealing with microscale cell cultures. Finally, the potential of CE-MS for large-scale and quantitative clinical metabolomics studies is also addressed. This course is given in an interactive way by using tools such as for example the Mentimeter.

SCIENTIFIC PROGRAM

Tuesday May 17th, 2022

	Room 1	Room 2
	Short course 1: A fundamental and practical look at separation mechanisms beyond RPLC: HIC, HILIC, SFC, SEC, IEX, MM, PGC Speakers: Davy Guillarme, David McCalley, Caroline West	**Short course 2: possibilities of CE and CE-MS for bioanalysis (and in particular metabolomics)** Speaker: Rawi Ramautar
8:45	Registration and welcome	Registration and welcome
9.00	Introduction	Introduction to course (teachers and learning goals)
9.15	IEX, SEC and HIC: Fundamentals and retention mechanisms Davy Guillarme	Principles of capillary electrophoresis
10.15	Coffee break	Coffee break
10:45	IEX, SEC and HIC: applications + Q&A Davy Guillarme HILIC: Fundamentals David McCalley	CE-MS: principles and interfacing designs
12.15	Lunch	Coffee break
13:15	HILIC: Retention mechanisms and applications + Q&A David McCalley	CE-MS for volume-restricted metabolomics – part 1: reproducibility
14.00	Mixed Mode Stationary phases and Porous Graphitic Carbon Caroline West	CE-MS for volume-restricted metabolomics – part 2: volume-restricted body fluids
14.45	Coffee break	Coffee break
15:15	SFC: Fundamentals and applications + Q&A Caroline West	CE-MS for volume-restricted metabolomics – part 3: microscale cell cultures
16.00	Overall Q&A, summary and discussion	Overall Q&A, summary and discussion

Wednesday May 18th, 2022

	AULA	Academieraadzaal
	HTC-17 Opening Ceremony	
9:00	Symposium chair HTC opening ceremony	
9:15	Plenary Lecture: Perdita Barran (University of Manchester, UK Translation of biomarkers to clinical assays: lessons from COVID and Joy	
10:00	Plenary Lecture: Jef Focant (Université de Liège, BE) Recent advances in medical applications of GCxGC	
10:45	Coffee Break & Exhibition	
	session: Hyphenated Gas Chromatography	**session: hyphenation and modulation in (bio-) macromolecular analysis**
11:15	KN: Philip Marriott (Monash University, AU) You REALLY want to do GC-FTIR? Some recent applications of the technique.	KN: ML Riekkola (University of Helsinki, FI) On-line coupled immunoaffinity chromatography - asymmetrical flow field-flow fractionation for the isolation and fractionation of subpopulations of the biomacromolecules
11:45	OR: Frank David (RIC, Kortrijk, BE) The role of GCxGC-TOFMS in fragrance characterization	OR: Bastiaan Duivelshof (University of Geneva, CH) Replacing historical glycan profiling strategies for biopharmaceutical products with quantitative Middle-Up HILIC-HRMS analysis
12:05	OR: Thomas Groeger The analysis of tracer compounds in particulate matter – from offline GC×GC and TOFMS towards at- and online analysis.	OR: Christoph Gstöttner (Leiden University Medical Center, NL) Multidimensional liquid chromatographic approaches for automated bottom-up analysis of antibody-derived therapeutics
12:25	OR: Eppe Gauthier (University of Liege, BE) Novel strategy to screen multi-classes of halogenated pollutants by Gas Chromatography-Atmospheric Pressure Chemical Ionization-Trapped Ion Mobility-Mass Spectrometry (GCAPCI-TIMS-MS)	OR: Li Peng (University of Tubingen, DE) Targeted lipidomics analysis of phosphoinositide signaling network in biological samples by liquid chromatography with tandem mass spectrometry
12:45	Lunch Break, Vendor Seminars, Exhibition & Posters	
13:15	vendor seminar: PERKIN ELMER	Vendor Seminar: LECO
	session: Fundamental Chromatography	**session Modern industrial applications (Chair: XX)**
14:00	KN: Gert Desmet (VUB, BE) Classic and New Views on the Eddy-Dispersion in Liquid Chromatography	KN: Thomas De Vijlder (J&J, Beerse, BE) Analytical Challenges in Therapeutic Oligonucleotide Development, and how to Solve them with Chromatography and Mass Spectrometry.
14:30	KN: Martina Catani (University of Ferrara, IT) Exploring retention of natural cannabinoids on polysaccharide chiral stationary phases under reversed-phase liquid chromatographic conditions	KN: Jan Blomberg and Richard Oldfield (Shell Technology, NL) Thermal gradient GC: Opportunities in combining Discrimination-Free Sample-Introduction with Ultra-fast Chromatography.
15:00	OR: Jörgen Samuelsson (Karlstad University, Sweden) Fundamental studies aiming at improved selectivity in oligonucleotide ion-pair chromatographic separations	OR: Ton Brooimans (Covestro/University of Amsterdam, NL) Coupling of Non-Aqueous Ion-Exchange to Size Exclusion Chromatography in Two-dimensional microstructural analysis of acid-functional polymers

	AULA	Academieraadzaal
15:20	OR: Ali Moussa (VUB, BE) Modelling of analyte profiles and band broadening generated by interface loops used in multi-dimensional liquid chromatography	IP1: Laura McGregor (Sepsolve, UK) Battle of the brands: Using GC×GC–TOF MS to compare the VOC profiles of food and beverages
15:30		IP2: Wim Decrop (Thermo) Novel low-flow LC technologies hyphenated with HRAM MS are pushing the boundaries of LC-MS bottom-up proteomics
15:40	Coffee Break, Exhibition & Posters	
	Sample Preparation, Miniaturization & Automation	**session: In-silico chromatographic method development and data mining**
16:10	KN: Valerie Pichon (École Supérieure de Physique et de Chimie Industrielles, FR)" Selective extraction devices based on imprinted polymers for trace analysis of molecules and ions in complex samples	KN: Stefanuto Pierre-Hugues (Université de Liege, BE) "Development of data processing workflow for untargeted multidimensional chromatography"
16:40	OR: Christophe Stove (Ghent University, BE) microsampling: hyphenated chromatographic applications (working title)	OR: Thomas van Laethem (University of Liège, BE) In-silico screening for the development of robust reversed-phase liquid chromatography methods of known small molecules based on QSRR and desirability index
17:00	OR: Thorsten Teutenberg Hyphenation, automation, digitalization: do we still need analytical scientists for running the lab of the future?	OR: Bos Tijmen (Vrije Universiteit Amsterdam, NL) Detailed molecular characterization of complex cellulose ethers by LC-MS and probability-based deconvolution
17:20	OR: Sigrid Deprez (Ghent University, Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, BE) Fully Automated Dried Blood Spot Extraction coupled to Liquid Chromatography-tandem Mass Spectrometry for Therapeutic Drug Monitoring of Immunosuppressants	OR: Alexander Kensert (KULeuven, BE) Graph neural networks for improved retention time predictions and molecular identification
17:40		
17:50	**Interactive session** moderators: J. Vercammen & I. Francois chromatography goes to court	
18:50	Poster presentations and Belgian Beer tasting	

Thursday May 19th, 2022

	AULA	Academieraadzaal
	session: Multidimensional comprehensive liquid chromatography	**session: Hyphenated protein chromatography**
9:00	KN: Sabine Heinisch (Institut des Sciences Analytique, Lyon, FR) Some unexpected advantages of on-line RPLC x RPLC over RPLC	KN: Govert Somsen (Vrije Universiteit Amsterdam, NL) Hyphenative analysis of proteins
9:30	KN: André de Villiers (Stellenbosch University, SA) Comprehensive two-dimensional LC hyphenated to ion mobility spectrometry and high resolution MS for tannin analysis	KN: Christian Neusuess (Aalen University, DE) NanoLC-CZE-MS: Development of a new Platform and Future Perspectives
10:00	OR: Mimi den UiL (University of Amsterdam, NL) Online photodegradation hyphenated with twodimensional liquid chromatography	OR: Amarande Murisier (University of Geneva, CH) Direct SEC-MS for the detailed characterization of monoclonal antibody size variants: what are the critical parameters for a straightforward hyphenation?
10:20	OR: Soraya Chapel (Université de Lyon, FR; KULeuven, BE) The Total Breakthrough phenomenon: Description and Application in On-Line Comprehensive 2D-LC	OR: Guusje van schaick (Leiden University Medical Center, NL) Robust native LC–MS platform for in-depth proteoform characterization
10:40	Coffee Break, Exhibition & Posters	
	session: Hyphenated Supercritical Fluid Chromatography	**session: unconventional hyphenated Separation modes**
11:10	KN: Caroline West (Université d'Orléans, FR) The new frontiers of SFC-MS	TUT: Rob Haselberg (Vrije Universiteit Amsterdam, NL) Hyphenation & Capillary Electrophoresis – current status and future directions
11:40	KN: Lucie novakova (Charles University, CZ) Tips and tricks for optimization of conditions in SFC-MS coupling	KN: Elena Dominguez Vega (Leiden University Medical Center, NL) Proteoform-selective antibody effector function monitoring using affinity CE-MS
12:10	OR: Isabelle Francois From Flower to Extract to Isolated Cannabinoids using Supercritical CO2	OR: Chiara De Luca (University of Ferrara, IT) Potentiality of Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) process to boost the purification performance of a peptide
12:30	OR: Jörgen Samuelsson (Karlstad University, Sweden) Prediction of retention shifts in SFC for improved method transfer and robustness	OR: Adriaan Ampe (UGent, BE) Investigation of the potential of mixed solvent mobile phases in temperature-responsive liquid chromatography (TRLC)
12:50	Coffee Break, Vendor Seminars , Exhibition & Posters	
13:00	vendor seminar: SHIMADZU	vendor seminar: SCIEX
	session: (Bio-) and pharmaceutical analysis	**session: High Throughput Gas chromatography**
14:00	KN: Koen Sandra (RIC, Kortrijk, BE) One, two, three, four and more chromatographic dimensions in biopharmaceutical analysis	TU: Jacob de Zeeuw (RESTEK, NL) Basics of Low Pressure Gas Chromatography / Vacuum GC, A robust solution for 3x faster GC/MS analysis
14:30	KN: Davy Guillarme (University of Geneva, CH) Simplifying the characterization of complex biopharmaceutical products through the use of unexpected chromatographic conditions	KN: Peter Boeker (University of Bonn, DE) Latest developments and improvements in high throughput flow field thermal gradient GC
15:00	OR: Simona Felletti (University of Ferrara) Investigation of mass transfer kinetics of nonpsychoactive cannabinoids under achiral reversed phase conditions on C18 2.7 um superficially porous particles	OR: An Adams (Dow, Terneuzen, NL) Use of TD-GCxGC/FID/MS for Analysis of Trace VOCs in LDPE Materials and its correlation with Olfactory Sensory Panel Testing

	AULA	Academieraadzaal
15:20	OR: Pauline Bosman (PSL University, FR) Standardisation of saliva collection modes using a targeted metabolomic approach	OR: Gaida Meriem (University of Liège, Belgium) Modeling the GC×GC separation as individual subsystems under vacuum outlet conditions
15:40	Coffee Break, Exhibition & Posters	
	session: System design and optimization	**session: Hyphenated Environmental Analysis**
16:10	OR: Mariusz Belka (Medical University of Gdańsk, PO) 3D printed devices and sorbents – application for extraction procedures in bioanalysis	OR: Selina Tisler (University of Copenhagen, Denmark) Matrix effects in LC-MS: Evaluation and correction approaches using wastewater analysis as an example
16:30	OR: Thomas Themelis (VUB, BE) Performance Limits and Design Aspects for Spatial Comprehensive Three-Dimensional Isoelectric Focusing × Size-Exclusion Chromatography × Reversed-Phase Liquid Chromatography for the Analysis of Intact Proteins	OR: Penchao Cao (PSL University, Paris, FR) Development of ion-imprinted polymers for the selective extraction of Cu (II) ions in environmental waters
16:50	OR: Sadriaj Donatela (KULeuven, BE) Database of molecular diffusion coefficients of representative biopharmaceuticals under reversed-phase liquid chromatography conditions	OR: Kevin Humbert (Normandie University, UNIROUEN, FR) Development of methodologies for quantifying simultaneously PAHs and PCBs in sediment cores: advantages and drawbacks of chromatography coupled to thermal or solvent extraction and hyperspectral imaging.
17:10	OR: Leon E. Niezen (University of Amsterdam, NL) Thermal modulation to enhance two-dimensional liquid chromatography separations of polymers	OR: Caroline Gauchotte-Lindsay (James Watt School of Egineering) University of Glasgow, United Kingdom Non-targeted analysis of organic contaminants in complex environmental matrices- Elucidation of high-level (bio) chemical mechanisms
17:30	**HTC tube contest**	

Friday May 20th, 2022

	AULA	Academieraadzaal
9:00	**session xx** HTC innovation award Plenary Lecture by the awardee	
	session: Covid19 & analytics	**session: Miniaturization and chip technology**
9:50	KN: Martijn Schenning (J&J, Analytical Assays in Analytical Development, Leiden, NL) Employing the analytical platform toolbox for rapid COVID-19 vaccine development	KN: Sebastiaan Eeltink (VUB, BE) Spatial 3D-LC
10:20	OR: John Barr (Centre for Disease Control, US) Comprehensive Analysis of the Glycan Complement of SARS-CoV-2 Spike Proteins Using Signature Ions Triggered Electron-Transfer/Higher- Energy Collisional Dissociation (EThcD) Mass Spectrometry	OR: Tiny Deschrijver (Janssen Pharmaceutica, BE) Use of micropillar array columns for high resolution separation of phosphorothioated oligonucleotides and their diastereomers
10:40	Coffee Break, Exhibition & Posters	
	session: hyphenated mass spectrometry	**Analysis of food, beverages and natural products**
11:10	KN: (Michael Lämmerhofer) (University of Tübingen, DE) Strategies for enhancing selectivity in pharmaceutical analysis by orthogonal columns and column coupling	KN: Hans-Gerd Janssen (Unilever Research, NL) Fast and ultra-fast GC-MS with automated data interpretation in the development of sustainable foods
11:40	KN: Edwin de Pauw (University of Liege, BE) "Coupling plate chromatography to Imaging Mass Spectrometrty"	KN: Giorgia Purcaro (Gembloux Agro-Bio Tech, University of Liege, BE) A fully automated LC-GCxGC-TOF MS/FID platform for MOSH and MOAH determination in food
12:10	OR: Jeremy Molineau Université d'Orléans, FR) UC-MS and RPLC-MS complementary analysis for small pharmaceutical peptides	OR: Pascal Cardinael (University of Rouen Normandy, FR) GC-HRMS and GC×GC-HRMS, versatile tools for characterization of complex matrices and quantification of targeted compounds. Applications on biofuels, compounds emitted by plants, multiresidue of pesticides and contaminant analysis in food and cosmetics.
12:30	OR: Mauro De Pra Multidetector UHPLC analysis of polysorbate 80	OR: Andrea Hochegger (Graz University of Technology, AT) An Approach for the Deeper Analytical Characterization of Mineral Oil Aromatic Hydrocarbons
12:50	Coffee Break, Vendor Seminars , Exhibition & Posters	
13:00	vendor seminar: Aqilent	vendor seminar: THERMO FISHER
	session Hyphenated Liquid Chromatography	
14:00	Plenary Lecture: Dave Mc Calley (University of West England, UK) Recent advances in hydrophilic interaction liquid chromatography	
14:45	Plenary Lecture: Peter Schoenmaekers (Universiteit van Amsterdam, NL) Hyphenation for large-molecule samples	

	AULA	Academieraadzaal
	HTC-17 CLOSING SESSION	
15:30	Symposium Chair poster awards and HTC award	
15:45	Symposium Chair Symposium closure	
15:50	*Farewell Drink*	

PLENARY LECTURE

Translation of biomarkers to clinical assays: lessons from COVID and Joy?

Perdita Barran

Manchester Institute of Biotechnology, The University of Manchester, United Kingdom

In this talk I will discuss why and how we have performed metabolomics of sebum in order to diagnose Parkinson's Disease (PD). The methods that we have used include, odor analysis, thermal desorption gas chromatography mass spectrometry (TD-GC-MS), HPLC- MS, direct infusion MS and chemometrics. I will describe how these methods can be used to stratify PD and the prospect for early diagnosis. I will also describe the use of mass spectrometry to diagnose COVID-19 and our efforts to determine prognostic markers of COVID.

PLENARY LECTURE

Recent advances in medical applications of GC×GC

Pierre-Hugues Stefanuto[1], Delphine zanella[1], Thibaut Dejong[1], Thibault Massenet[1], Nicolas Di Giovanni[1], Laurie Giltay[2], Monique Henket[2], Françoise Guissard[2], Florence Schleich[2], Julien Guiot[2], Béatrice André[3], Michel Malaise[3], Judith Potjewijd[4], Marie-Alice Meuwis[5], Edouard Louis[5], Renaud Louis[2], Jef Focant[1]

[1]Organic and Biological Analytical Chemistry Group, University of Liège, Belgium; [2]Respiratory Medicine, GIGA I3, CHU Sart-Tilman, Belgium; [3]Rheumatology Department, CHU Liege, Belgium; [4]Department of Internal Medicine, Maastricht UMC, Maastricht, The Netherlands; [5]Translational Gastroenterology Department, GIGA I3, CHU Sart-Tilman, Belgium

Over the last decade, comprehensive gas chromatography couped to time-of-flight mass spectrometry (GC×GC-TOFMS) has demonstrated to be one of the most effective method for the characterization of (semi-)volatile mixture in complex matrices (petroleum, flavor and fragrance, food, environment, forensics, metabolomics, and many more). What makes GC×GC-TOFMS popular is obviously its enhanced peak capacity, but also its propensity to offer a large set of additional identification criteria such as two retention times, retention indices, spatially organized elution orders (structured chromatograms), library searchable mass fragmentograms, exact masses,... allowing to univocally identify separated molecules.

A particular medical application of GC×GC-TOFMS is volatilomics, or volatile organic compound (VOC) metabolomics. In that context, blood is a matrix of choice for which the level of invasiveness for specimen collection is well accepted by patients. Blood allows for example non-target metabolomic profiling of inflammatory bowel diseases (IBDs) such as Crohn's disease (CD) or also of colorectal cancer for which inflammation/remission biomarkers can thus be screened for [1,2].

With a view to further reduce the invasiveness, exhaled air (breath) has for long been a matrix of interest. Its analysis does not require any derivatization nor particular preparation, and the constitutive VOCs are representative of the metabolic pathways that take place inside the patient through lung/blood exchanges. Such a breathomics approach has been demonstrated for lung cancer and other chronic respiratory diseases (CRDs) like asthma and chronic obstructive pulmonary disease (COPD), but also interstitial lung disease (ILD) [3-5].

These recent advances are however tarnished by a lack of robustness, inter-comparability, standardization, and quality control. This prevents from an international cohesion of the approaches and limits the transferability to hospitals. It will only be at the price of stronger and more implemented robust analytical strategies that breathomics will have a real meaning in medical applications. The potential is significant, especially when coupled with existing clinical methods (e.g. Fractional exhaled Nitric Oxide FeNO, immunotesting, CT scan, ...) for early diagnosis, for monitoring response to treatment, and for precision medicine, but there is still a bit of a way to go, and a passable road is to be put through...

[1] DiGiovanni, N. et al. Untargeted Blood Metabolic Profiling by GC×GC-HRTOF-MS. J. Prot. Res. 19, 1013–1028 (2020).

[2] DiGiovanni, N. et al. Specificity of metabolic colorectal cancer biomarkers in serum through effect size. Metabolomics. 16, 88, 1-16 (2020).

[3] Pesesse, R et al. Multimodal chemometric approach for the analysis of human exhaled breath in lung cancer patients by TD-GC × GC-TOFMS. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1114–1115, 146–153 (2019).

[4] Schleich, F. N. et al. Exhaled Volatile Organic Compounds are Able to Discriminate between Neutrophilic and Eosinophilic Asthma. Am. J. Respir. Crit. 200, 444-453 (2019).

[5] Zanella, D. et al. Breathomics to diagnose systemic sclerosis using thermal desorption and comprehensive two-dimensional gas chromatography high-resolution time-of-flight mass spectrometry. Anal. Bioanal. Chem. 413, 3813-3822 (2021).

[6] Stefanuto, P.-H. et al. Breath Volatolomics for Medical Applications. Are We There Yet? AM. J. Biomed. Sci. Res. 11, 001621 (2020).

Keywords: GCxGC, medical, biomarkers, disease

PLENARY LECTURE

The favourable features of hydrophilic interaction chromatography (HILIC) as a complimentary technique to reversed phase (RPLC)

David McCalley

University of the West of England, United Kingdom

This lecture will consider the favourable features of hydrophilic interaction chromatography (HILIC) which have led to its emergence as a complimentary technique to reversed phase (RPLC), especially for the analysis of polar and ionised solutes that have little retention in RPLC. For those solutes amenable to both mechanisms, HILIC often provides orthogonal separation to RPLC, which together with its sensitivity advantage, makes it a very suitable method for 2-dimensional LC-electrospray mass spectrometry. Sensitivity improvements using HILIC have been noted in other detection techniques that involve evaporation of the mobile phase such as the charged aerosol detector. Other advantages of HILIC include the widely different selectivity of different HILIC stationary phases, whereas different RP materials have much more similar properties. Perceived difficulties of HILIC include irreproducible retention, difficulties with gradient elution, and poor peak shapes. Some of these difficulties arise from the longer equilibration time of the column in HILIC. This problem has been considered and some solutions are proposed, including the use of repeatable partial equilibration in gradient elution. Other causes of poor peak shape include mismatch of the injection solvent and the mobile phase; this problem will be considered and possible remedies suggested.

Recently, some doubt has been expressed over the measurement of the column void volume in HILIC, which enables calculation of the retention factor- a more fundamental measure of retention than the retention time. A new procedure involving a linear free energy relationship (LFER) approach is evaluated and consideration is made as to whether this method has significant advantages over traditional methods such as the use of toluene as a void volume marker.

Keywords: HPLC, HILIC, separation mechanisms

PLENARY LECTURE

Hyphenation for large-molecule samples

Peter Schoenmakers

Van 't Hoff Institute for Molecular Science (HIMS), University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

Chromatography is indispensable for a vast fraction (if not a majority) of analytical measurements. In many important domains of science, industry and society, chromatography is used an awful lot and there are many of us chromatographers. Can we be proud of our field? And how it has developed over the more than 30 years spanned by HTC? HTC is the meeting on Hyphenated Techniques IN chromatography. Using the word IN suggests some INward looking, but at times INtrospection may be healthy. What is it that we do well? A lot! We can separate almost any two analytes and we can separate hundreds of compounds in very complex mixtures. What can we do better? And what is it that we cannot do? The uncomfortable truth is: also a lot! We may be pretty happy with the detectors we have for GC, but we have seen remarkably little progress in detectors for LC. We cannot separate extremely complex mixtures (although there is an increased demand to do so). It is rewarding to explore what we can hyphenate withIN chromatography, e.g. heart-cut 2D-GC or 2D-LC, or even-more rewarding, comprehensive two-dimensional chromatography (GC×GC or LC×LC).

But to be honest, although we, IN chromatography, have gotten better at our jobs, we should be humble. We need help. And more than just a bit. The fabulous success of Hyphenated Techniques IN Chromatography in the laboratory (not just at symposium venues) has come from embracing other fields, especially mass spectrometry. The true "hyphenator" approaches the homo universalis of the 21st century. To design, develop and apply hyphenated techniques properly, we need to master engineering science. Transport phenomena (fluid dynamics) determine chromatographic efficiency. Some knowledge of mechanical and electronic engineering also come in handy. Chromatographic retention and selectivity is covered by thermodynamics and physical chemistry, including surface chemistry. Derivatizing samples and interpreting mass spectra involve organic chemistry. Other detectors require knowledge of spectroscopy and electrochemistry. To understand the analytical questions we are confronted with, we need to understand many aspects of life science and biology, food science, environmental science, etc. And we increasingly need computer science and mathematics to control our systems and interpret our data.

By focussing on large molecules, such as polymers and proteins we are being challenged on all these aspects – and some more. For the characterization of large-molecule samples hyphenated techniques are often essential. Polymer samples are especially compatible with LC×LC, because of their high complexity, but low sample dimensionality. The latter aspect also make them prime candidates for computer-aided interpretation of mass spectra. Protein MS has seen fantastic progress, as long as it is preceded by adequate separations.

Examples from large-molecule characterization will be used to illustrate the immense progress made in hyphenated techniques with chromatography.

Keywords: Chromatography, hyphenated techniques, large-molecule samples

KEYNOTE LECTURE

Thermal gradient GC: Opportunities in combining Discrimination-Free Sample-Introduction with Ultra-fast Chromatography

Jan Blomberg, Richard Oldfield

Shell Global Solutions International B.V., The Netherlands

In traditional gas chromatography, going fast is only feasible if sample introduction systems are comparably fast.

Flash vaporization is by far the most simple and robust and typically combines relatively fast sample introduction with acceptable narrow introduction bandwidths. It does, however, suffer from non-representative sample introduction (discrimination). This becomes ever more pronounced when trying to cover ever wider boiling ranges.

Simulated Distillation by GC (SimDist) puts stringent limitations on the amount of discrimination a sample-introduction device is allowed to exhibit. To minimize sample discrimination SimDist typically relies on dedicated inlets that combine temperature programmed vaporization with direct (non-splitting) transfer to large internal diameter columns. This, however, results in low-resolution and comparatively slow separations.

Programmable Temperature Vaporizers are reputedly known to eliminate sources of discrimination, but compatibility with narrow bore columns can only be obtained by peak focusing techniques. Cold-trap thermo-desorption has been used in combination with PTV injection to achieve input band widths which are compatible with narrow bore columns 1.

In this presentation we will elaborate on the impact of thermal gradient GC in realizing ultra-fast SimDist.

[1] A. van Es, High speed narrow bore capillary gas chromatography, Huethig, Heidelberg (1992) p. 58-70

Keywords: Thermal Gradient GC, sample discrimination, sample introduction

KEYNOTE LECTURE

Latest developments and improvements in high throughput flow field thermal gradient GC

Peter Boeker[1,2], Peter J. Müller[1,2], Jan Leppert[1]

[1]*University of Bonn, Germany;* [2]*HyperChrom SA, Luxembourg*

Since the first paper on Flow-Field Thermal Gradient Gas Chromatography in 2014, and the first presentation on the HTC-14 in 2016, the new method of gas chromatography has been developed much further. The chromatographic resolution of the new method was already at a very high level early on. However, not only resolution, but retention time stability, robustness and user-friendliness had to be optimized.

Retention time stability through active cooling

If gas chromatography is not carried out in a hermetically sealed oven, interfering influences from the ambient conditions are much more difficult to compensate for. In extended measurements, we found drift effects and fluctuations in retention times. One of the main causes of systematic drift of retention times was identified as heating of the separation capillary support in the course of a measurement sequence. To solve this problem at its root, the support structure was equipped with a cooling circuit. Since this was not possible with conventional manufacturing techniques, an additive manufacturing process, metal 3D-printing, is now being used. GC start temperatures of 20°C are now possible. Cooling takes about 10s.

Purged column connectors

High-quality gas chromatography often requires the use of guard columns or transfer lines. However, connector technology is becoming increasingly difficult with very fast chromatographic signals. Even very small dead volumes lead to significant tailing effects on narrow peaks. Therefore, it was decided to use purged connectors to solve the problem. The use of purged connectors makes it possible, after injection of a sample and transfer to the separation capillary, to perform chromatography with clean carrier gas that is not contaminated with residual solvents or matrix from the injector.

Injection technology

The pure process of gas chromatography is trivial compared to the sample introduction. A very crucial question was whether the fast GC is compatible with the previous methods of sample introduction. After various research projects, this question can be answered positively.

In the area of split/splitless injection, tests were made with own injector designs. In the end, an optimized design for fast GC was obtained. Some new effects occur when fast measurements are made in close temporal succession. This concerns in particular adsorptions and permeations in connection with the liner O-ring. Here, the effects were investigated with the various materials available, such as Viton, Kalrez and also the rather rarely used graphite.

User-friendliness

A high degree of user-friendliness has now also been achieved with the fast GC. This was developed and tested in cooperation with a large laboratory group. The separation capillaries can be exchanged just as easily as in the classic air bath oven GC. All gas chromatographic knowledge has also retained its validity in the very fast thermal gradient GC. Therefore, the 'method translation' according to Klee and Blumberg can be used as a starting point to transfer previous methods to faster ones.

Thermal gradient GC simulation is also addressed as a tool for understanding chromatographic processes and optimization.

Keywords: Ultra-fast GC, Thermal Gradient GC, GC-MS, High-Throughput

KEYNOTE LECTURE

Exploring retention of natural cannabinoids in chiral liquid chromatography

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Cannabis Sativa L. is one of the of the most controversial plants in the world. The most studied class of compounds produced by the plant is that of cannabinoids, which are responsible for both the therapeutic and psychotropic effects.

Although some cannabinoids are achiral (e.g., cannabigerol, CBG and cannabinol, CBN), many others (such as Δ9-tetrahydrocannabinol, Δ9-THC and cannabidiol, CBD) contain one or more asymmetric carbons. Production of cannabinoids by the plant has thought to be stereoselective for a long time, with (-)-trans forms preferentially synthesized. As a result, analytical methods employed to characterize cannabis products have been usually based only on achiral determination, irrespective of stereochemistry.

However, since the number of discovered cannabinoids exceeds 100, the biological activity of each single chiral cannabinoid is still largely unknown. Only recently, due to the increasing popularity of cannabis products and recent legalization, there is a growing interest towards the characterization of cannabis samples also in terms of enantiomeric purity and stereostability.

The lack of studies on chirality of cannabinoids is also due to the fact that reference standards are not available for every single chiral cannabinoid. A quick search in literature databases shows that there are only few peer-reviewed papers available where chiral LC has been applied to the separation of chiral cannabinoids [1-3]. These works date back to the beginning of nineties, and they report the separation of enantiomers of CBD and Δ8-THC, together with other synthetic cannabinoids, on an amylose-based chiral stationary phase (CSP) under normal-phase (NP) conditions. More recently, different companies have published technical notes showing the separation of Δ8-THC and Δ9-THC again on polysaccharide CSPs under NP conditions. However, there is a lack of fundamental studies on retention properties of cannabinoids on CSPs and they have never been tested under reversed-phase (RP) conditions.

This work reports about recent studies made by our research group aimed at characterizing retention behavior of natural cannabinoids on different chiral stationary phases (CSPs), including polysaccharide and Pirkle-type ones. These CSPs have been operated under different chromatographic conditions (including NP and RP) and the influence of stationary phase chemistry on enantio- and chemo-selectivity has been explored. Future challenges and pitfalls in this field will be also discussed.

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Keywords: cannabinoids, retention, selectivity, polysaccharide chiral stationary phases

KEYNOTE LECTURE

Coupling plate chromatography to Imaging Mass Spectrometrty

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Matrix Assisted Laser Desorption Ionization is now a well-established technique in analytical and bioanalytical Sciences. However, compared to electrospray and related methods, its coupling with separation methods received less attention despite some very attractive features. Interfacing MALDI with a separation method is most often performed by offline deposition of the elution profile on a MALDI plate followed by a classical MALDI workflow (LC-MALDI). The possibility to keep a physical memory of the separation owing to the very low sample consumption of MALDI is an interesting advantage. Thanks to the progress in Imaging Mass Spectrometry (IMS) and in particular the high repetition rate Lasers and dedicated software for image analysis, it is now possible to use IMS to visualize the whole elution profile in a workable time. An option for separation presenting a growing interest is thin layer chromatography and is variants. Mono, bi- dimensional or radial separations require quite basic hardware and consumables. Separations on thinner TLC plates (HPTLC, UTLC) consume less solvent, are usually faster and can be performed in parallel. The single use of the plates cancels cross-contamination. In MALDI, the wide choice of matrices allows bringing specificity in the ionization step. In situ derivatizations pre-charging the analytes are available, already developed for classical optical detection. During MALDI ionization, ions receive an amount of internal energy that can lead to fragmentation when they are still in the source. The process has been named “in Source Decay”, by opposition of the fragmentation of the same ions bearing less internal energy and fragmenting later during the flight, a process named “Post source Decay”. The fragmentation rate constant depends on the relative “hardness” of the matrix. In source decay fragmentation can be chemically assisted by the MALDI matrix. Its mechanism has been largely studied in particular when hydrogen radicals are involved. This can be transposed to TLC. Reactive matrices can induce in source fragmentation that can be used for fast sequencing long tags of biopolymers, a growing application. Recent additional by ion mobility is a clear prospect for future developments. In this short presentation, the recent developments in TLC MALDI coupling will be presented together with specific applications in the biomolecules field with a specific focus to MALDI imaging as powerful detector.

Keywords: MALDI Imaging, HPTLC, UTLC, lipids analysis, In source Decay fragmentation

KEYNOTE LECTURE

Classic and New Views on the Eddy-Dispersion in Liquid Chromatography

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The band broadening processes occurring in liquid chromatography are very complex. Due to the combined effort of many great scientists building upon each other's work in the past decades, the most important parts of the puzzle have now been solved. To date, this has produced a relatively clear picture of the different individual band broadening contributions, how they are linked together, and how they can be used to qualitatively explain most of the experimental facts and findings.

However, despite the many achievements made in the past, there are still a number of important and intriguing questions that remain: is there a direct link between particle size distribution and column efficiency?, is there a possibility to quantitatively relate the values of the A, B, and C-term to the bed structure?, how does radial dispersion exactly contribute to the observed eddy-dispersion?,… . Many of these questions still await a definitive answer and can only be answered using sophisticated modelling of the fine details of the column. During the presentation, an account of the most recent findings of this modelling work will be given.

Keywords: band broadening, column technology, theory

KEYNOTE LECTURE

Analytical Challenges in Therapeutic Oligonucleotide Development, and how Chromatography and Mass Spectrometry can help solving them

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Janssen R&D, Belgium

Oligonucleotides (ASO, siRNA, miRNA, etc.) are an emerging class of therapeutic modalities. With 14 oligonucleotide medicines licensed world-wide (including 10 in the last 5 years), and many more in clinical trials, there is an increased demand from the pharmaceutical industry for the development and application of adequate analytical technology. Due to their size, and specific physicochemical properties (e.g., polyanionic backbone, potential for higher-order structure formation), the comprehensive analysis of oligonucleotide therapeutics is inherently more challenging compared to traditional small molecule (SM) medicines. Nonetheless, the lack of oligonucleotide-specific regulatory guidance forces the pharmaceutical industry to follow guidelines originally developed for SM. These analytical challenges will be tackled next to the recent developments and applications in analytical technology ranging from in-depth characterization to routine monitoring. The emphasis on liquid chromatography and (hyphenation to) mass spectrometry as the prime techniques will be elaborated.

Keywords: Therapeutic oligonucleotides, Liquid chromatography, Mass spectrometry

KEYNOTE LECTURE

Comprehensive two-dimensional LC of phenolic compounds using HILIC and RP-LC: How far can we go?

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The combination of HILIC and RP-LC separations in comprehensive two-dimensional LC (LC×LC) is attractive due to the high degree of orthogonality of these separation modes, despite the challenges associated with their hyphenation. For the improved separation of complex mixtures of plant phenolics, on-line HILIC×RP-LC in particular has found extensive application. However, the extreme complexity of such samples means that their complete characterization, even when hyphenating LC×LC with advanced mass spectrometric detection, remains elusive.

In this contribution, we evaluate the current performance limits of on-line LC×LC-MS where HILIC and RP-LC are combined for phenolic analysis. A comparison of the kinetic performance limits of on-line HILIC×RP-LC and RP-LC×HILIC will be presented and discussed in terms of the requirements for phenolic analysis. Experimental data for a range of phenolic compounds present in wine, grapes, herbal tea, cannabis, and tannin extracts will be used to support the theoretical predictions, and demonstrate the performance of these methods for particular analyte classes.

Particular emphasis will be placed on the separation of condensed tannins, since the very large number of isomeric species present in these samples present an especially severe challenge. We will show how even with state-of-the-art LC×LC-MS methods, the performance is insufficient for the characterization of oligomeric condensed tannins. Finally, means of improving LC×LC separation performance using multiple heart-cutting instrumentation and stop-flow operation, as well as the incorporation of ion mobility spectrometry to further improve compound identification will be explored. Preliminary data for real-life samples will be used to critically assess the potential of these approaches, and highlight further work that should be performed to enable more detailed characterization of complex phenolic samples.

Keywords: LCxLC, HILIC, RP-LC, MS, phenolics

KEYNOTE LECTURE

Simplifying the characterization of complex biopharmaceutical products through the use of unexpected chromatographic conditions

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The therapeutic success of monoclonal antibodies (mAbs) in the treatment of cancer has contributed to their rise, ranking six mAbs and derived products among the 10 best-selling drugs in 2020. From production to patient administration, mAbs are subject to numerous chemical and enzymatic modifications that can alter their biological activity and pharmacological profile. A complete characterization of therapeutic proteins and their variants must then be performed by analytical methods, to ensure product safety and inter-batch reproducibility.

The aim of this presentation will be to detail some innovative chromatographic approaches recently developed in our laboratory, to improve the possibilities offered by chromatographic techniques for the analysis of therapeutic proteins.

A first innovative approach is based on the use of ultra-short columns (only a few millimeters) in reverse phase liquid chromatography (RPLC) and ion exchange chromatography (IEX), to obtain separations as efficient as with standard size columns, but with significantly reduced analysis times [1,2]. This behavior is due to the fact that large proteins have very high S values (slope of the log k relation as a function of %ACN), and an on-off (also known as bind-elute) retention mechanism. Analyses of mAbs and immunoconjugates (ADCs) could thus be successfully performed in only a few tens of seconds.

The second approach is based on the use of special gradient conditions, allowing to significantly improve the selectivity between different protein isoforms. Based on the fact that the protein retention mechanism is on-off, it is possible to add one or more isocratic steps during the RPLC analysis to increase the distance between the chromatographic peaks [3]. Thanks to this principle, we were able to demonstrate infinite selectivity between protein chromatographic peaks simply by adding a suitable isocratic step. We also tested the possibility of using negative gradients instead of an isocratic step to obtain even higher selectivity between closely related protein isoforms [4].

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Keywords: protein analysis, biopharmaceuticals, monoclonal antibody, chromatography

KEYNOTE LECTURE

Proteoform-selective antibody effector function monitoring using affinity CE-MS

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Monoclonal antibodies (mAbs) consist of an antigen binding domain (Fab) and a crystalizable fragment (Fc). This Fc domain has several key functionalities, such as recruitment of immune components via different Fcγ receptors (FcRs), activation of the complement system and recycling of the antibody via binding to the neonatal Fc Receptor (FcRn) which determine the half-life of antibodies. These interactions are strongly influenced by structural features of the Fc domain and, therefore, small variations in the Fc region (e.g. glycosylation, oxidation) can severely impact their binding. Unfortunately, common approaches, such as SPR provide an overall affinity response for all different mAb proteoforms and assessment of their individual binding require tedious production or enrichment of specific proteoforms. In our lab, we have exploited for the first time the capabilities of Capillary Electrophoresis hyphenated with Mass Spectrometry (CE-MS) to study the binding affinity of antibodies and FcRs in a proteoform-resolved fashion.

We have developed different methods based on mobility-shift affinity CE-MS to study the binding of mAbs to various FcRs, namely FcRn, FcγRIIa and FcγRIIb. To this end, the FcR receptors were added to the background electrolyte whereas the mixture of antibody proteoforms was injected in the CE. As a first case, we studied the interaction towards FcRn which determine antibody half-life. We will show that, by adding different amounts of FcRn to the background electrolyte, we are able to determine the relative affinity of different proteoforms based on the shifts in their mobility. We observed differences in the mobility for singly and doubly oxidized mAbs with respect to the unmodified antibodies indicating lower binding affinity. For FcγRIIa (activating) and FcγRIIb (inhibitory) receptors, glycosylation of the antibody was key for the binding. Hemiglycosylated antibodies showed a strong decrease in the binding towards both FcγRIIs. Within glycoforms, differences were also observed with high mannose forms showing lower binding compared to complex type glycoforms.

The developed approach offers unique possibilities to study in solution binding of individual proteoforms and simultaneously to address their heterogeneity. Furthermore, as the receptor is free in the solution, higher-order structures can be formed reflecting the in vivo situation in contrast to immobilized receptors (e.g. affinity LC or SPR). We believe that our approach will have a tremendous influence on the study of the interactions of mAb proteoforms with different FcRs in biopharma. Understanding these interactions is essential for developing new drugs as well as defining (and redefining) critical quality attributes of biopharmaceuticals.

Keywords: antibodies, proteoforms, affinity capillary electrophoresis, mass spectrometry, effector funtions

KEYNOTE LECTURE

Some unexpected advantages of on-line RPLC x RPLC over RPLC

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A very large degree of orthogonality is theoretically expected in LC x LC by combining very different separation mechanisms (e.g. HILIC with RPLC). However, in this case, solvent strength mismatch may dramatically reduce the peak capacity in the second dimension and hence the effective peak capacity. Combining two different RPLC systems is less efficient in term of orthogonality but the solvents are fully compatible. In addition to the effective peak capacity, other quality descriptors are of major importance. These include method sensitivity, analysis time, column lifetime or MS coupling performance. It is often assumed that a higher peak capacity must be obtained at the cost of a higher dilution (lower sensitivity) and therefore that RPLC x RPLC should be less sensitive than RPLC.

Here, it will be shown for ionisable compounds that a critical analysis time exists above which RPLC x RPLC outperforms RPLC in term of peak capacity. Furthermore, it will be demonstrated theoretically and experimentally that much higher peak intensities can be obtained in online RPLC x RPLC due to the focusing effect in the second dimension, which allows, for a given analysis time, to gain both separation and sensitivity. Finally, both the column lifetime in the second dimension and the advantage of MS coupling with RPLC x RPLC rather than RPLC will be discussed from experimental studies on protein digests and pharmaceuticals.

Keywords: on-line RPLC x RPLC; peak capacity; sensitivity, ionizable compounds, MS coupling

KEYNOTE LECTURE

Fast and ultra-fast GC-MS with automated data interpretation in the development of sustainable foods

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There are many application fields of GC where analysis time is relevant. This is for example the case if the results of the analysis are needed to take decisions on accepting or rejecting ingredient batches, or if very large numbers of samples have to be analysed. A high analysis speed is also needed if rapidly changing processes have to be monitored. Over the years several methods have been developed to obtain fast separations. These include the use of narrow-bore columns, short columns operated at high velocity, vacuum outlet conditions etc, but also selective mass spectrometry can be seen as a way to speed-up separations. In addition to the actual separation and detection step, also the sample preparation time and the time needed for interpretation of the data are key factors in determining the throughput and 'time-to-result' in GC.

In this presentation options for fast and ultra-fast GC-MS will be discussed and applications of the methods in the field of food analysis will be presented. The techniques discussed will cover the entire workflow of a typical GC analysis including fast and automated methods for on-line sample preparation such as static and dynamic head-space, (arrow) SPME and cryo-trapping. Fast separation methods will be shown as will advanced software tools that allow data reduction and 'relevant-compound' detection in the huge data sets generated by fast GC and comprehensive GC×GC-MS methods. For the latter methods data processing nowadays is the most time-consuming step. GC×GC allows to generate massive amounts of data in a short time, but it also enables ultra fast (<5 seconds) single dimension separations. In that application the modulator is used as a cryo-trap and the second-dimension column as the ultra fast separation step.

Applications of fast and ultra fast GC with automated data interpretation will be shown. These include high throughput methods for identification of natural food preservatives, tools to study the flavour burst of ice-cream when melting in the mouth, and high-resolution tools for identification of off-flavours in aged plant-based ice creams and meat alternatives.

Keywords: Fast GC-MS, Food analysis, High throughput analysis, Data processing

KEYNOTE LECTURE

Strategies for enhancing selectivity in pharmaceutical analysis by orthogonal columns and column coupling

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In the last decades, there has been a strong focus in liquid chromatography on advancing stationary phase particle morphologies and chromatographic efficiencies. It led to the introduction of monoliths, sub-2µm and core-shell particle technology which greatly advanced peak capacities of modern columns. More and more stationary phase chemistries become available based on such modern particle technology. Challenging separations in (Bio) Pharmaceutical analysis are, however, not only limited by peak capacity but primarily insufficient selectivity and other practical aspects such as incompatibility of various chromatographic modes with ESI-MS detection. Moreover, new therapeutic modalities may require the combination of more than one analytical methodology to get a full picture of the quality of a therapeutic. Thus, the demand of more diverse chromatographic modalities is steadily increasing and analytical chemists are advised to develop their chromatographic understanding further beyond mere reversed-phase retention and selectivity principles.

In this contribution some ideas and concepts on the implementation of orthogonal selectivity in the course of an analytical workflow will be presented by selected examples.

The stationary phase chemistry drives thermodynamics which in turn retention and selectivity.

Hence, to forecast selectivities of stationary phase relies on detailed understanding of the phase chemistry. Some examples will be given how the surface chemistry can be tuned to provide new selectivities, improved stability and enhanced kinetic properties. Furthermore, concepts to understand the stationary phase orthogonality will be discussed. Column inline coupling is a concept that can be applied to combine orthogonal selectivities that are not available by a single column alone. It is not a new concept but maybe underestimated in the context of tailoring selectivities to specific needs. Combination of orthogonal columns is also of great utility for the requirement of comprehensive impurity profiling and selection of columns for multidimensional separations. Applications where such column coupling and multidimensional separations offer great flexibility and enhance separation power are discussed in the context of pharmaceutical applications.

In this presentation examples of less know stationary phases, orthogonal column chemistries and column coupling in the context of multidimensional separations will be discussed as a chromatographer's rich toolbox for burning applications in pharmaceutical analysis including impurity profiling enantioselective amino acid analysis, oligonucleotide separations and analysis of therapeutic peptides.

Keywords: multidimensional LC, impurity profiling, therapeutic peptides, oligonucleotides, enantioselective amino acid analysis

KEYNOTE LECTURE

You really want to do GC-FTIR? Some recent applications of the technique

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For decades, the promise of GC-FTIR has excited the interest of researchers and analysts as a tool to provide a unique selectivity in GC analysis with true orthogonal identification to mass spectrometry. But it largely has not lived up to its promise due to a number of factors. We can summarise these as (I) poor sensitivity, especially for light-pipe interfaces; and (ii) poor stability and maintenance difficulty.

Having a long-held interest in technologies for improved strategies for confirmation of structures in GC analysis, from MS/MS, GC×GC-MS and structured retentions, prep-GC with NMR and X-Ray analysis, it was apparent that an information rich spectroscopic method such as FTIR should – again – be investigated for its GC capabilities.

The conclusion we made is that GC-FTIR has certainly lived up to its potential in respect of its unique structural identification, and provides molecular structural details beyond the oft-times-limited ability of MS to distinguish spectra of structurally-related compounds. In addition, the opportunity to support identification with molecular modelling and simulation of structure is something which cannot be readily reproduced with other techniques. Here, we will mention a number of applications, from E/Z isomers, interconversions of E/Z oxime isomers, synthetic brominated products, and degradation of a phosphate material, as specific examples that bring out the best in the novel opportunities available with GC-FTIR analysis.

Keywords: spectroscopic detection, gas chromatography, oxime interconversion in GC, IR simulation

KEYNOTE LECTURE

Characterisation of a New Online NanoLC-CZE-MS Platform and Application for the Glycosylation Profiling of Alpha-1-Acid Glycoprotein

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The ever-increasing complexity of biological samples to be analysed by mass spectrometry has led to the necessity of sophisticated separation techniques, including multidimensional separation. Despite a high degree of orthogonality, the coupling of liquid chromatography (LC) and capillary zone electrophoresis (CZE) has not gained notable attention in research.

We present a heart-cut nanoLC-CZE-MS platform to analyse intact proteins. NanoLC and CZE-MS are coupled using a four-port valve with an internal nanoliter loop. NanoLC and CZE-MS conditions were optimised independently to find ideal conditions for the combined setup. Harnessing the increased loadability of the nanoLC, the nanoLC-CZE-MS setup exhibits a 280-fold increased concentration sensitivity compared to CZE-MS alone. The platform was used to characterise intact human alpha-1-acid glycoprotein (AGP), a heterogeneous glycoprotein. With the nanoLC-CZE-MS approach, 368 glycoforms can be assigned at a concentration of 50 µg/mL as opposed to the detection of only 186 glycoforms from 1 mg/mL by CZE-MS alone. Additionally, we demonstrate that glycosylation profiling is accessible for dried blood spot analysis (25 µg/mL AGP spiked), indicating the general applicability of our setup to biological matrices. The combination of high sensitivity and orthogonal selectivity in both dimensions makes the here presented nanoLC-CZE-MS approach capable for detailed characterisation of intact proteoforms from complex biological samples and in physiologically relevant concen-trations. Further developments and future applications of nanoLC-CE-MS will be discussed.

Keywords: Multidimensionl Separation, Intact Protein, Glycoprotein, Heart-Cut

KEYNOTE LECTURE

Optimization of conditions in SFC-MS coupling

Lucie Nováková, Kateřina Plachká, Taťána Gazárková, František Švec

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The coupling of supercritical fluid chromatography and mass spectrometry (SFC-MS) is more challenging compared to coupling liquid chromatography and mass spectrometry. Although the volatile nature of CO_2 used in the SFC mobile phase may appear to favor desolvation and transfer in the gas phase thus facilitating the hyphenation, several issues must be handled. An important attribute of the supercritical mobile phase is its high compressibility that can result in a substantial cooling effect and precipitation of analytes once the pressure is released after the back pressure regulator (BPR). This way, chromatographic integrity, including selectivity and separation efficiency, can be compromised. Moreover, the non-polar nature of CO_2 is not particularly suited for electrospray ionization. Therefore, dedicated interfaces are needed to confront these problems and to maintain stable SFC-MS conditions. Several interfaces are currently commercially available, among them (i) pre-BPR splitter with sheath pump and (ii) BPR with sheath pump with no splitter belong among the most widely used approaches. The sheath pump is used in both interfaces to deliver make-up solvent and to prevent the above-described issues. The compositions of both mobile phase and make-up solvent have an important effect on MS response and require detailed optimization in particular applications. Volatile solvents, e.g. methanol and ethanol, as well as additives such as ammonium salts of organic acids, organic acids, and bases in organic modifiers are used as the make-up solvents. A small amount of water in the make-up solvent based on an organic modifier can in some cases also enhance the MS sensitivity. Despite numerous published studies on SFC-MS, this coupling usually relies on experimental optimization.

We evaluated the effect of the make-up solvent composition on SFC-MS response, using different stationary phases to reflect variations in retention of analytes, with a large set of make-up solvents including alcohols (methanol, ethanol, isopropanol, and combinations of methanol with water) and volatile additives in methanol (ammonia, formic acid, acetic acid, ammonium formate, and ammonium acetate) at several different concentrations. The evaluation was carried out using two organic modifiers in the mobile phase and 95 compounds differing in physicochemical properties. We were searching for behavioral trends of different make-up solvents and for relationships between physicochemical properties and MS response. Using our experimental data and regression analysis, we derived equations for the prediction of MS response as a function of the make-solvent.

The study was supported by the GAČR project 21-27270S, and by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

Keywords: SFC, SFC-MS, make-up solvent, MS response

KEYNOTE LECTURE

Selective extraction devices based on imprinted polymers for trace analysis of molecules and ions in complex samples

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The determination of compounds (organic molecules or inorganic compounds such as metal ions) at very low levels of concentration is still a real analytical challenge in various application fields. The evolution of the instrumentation allows a significant improvement of sensitivity and analysis time. However, the analysis of ultra-traces present in complex matrices often requires a step of purification and preconcentration prior to the analysis. Therefore, extraction sorbents based on a molecular recognition mechanism can be developed and used for the selective extraction of target compounds and some structural analogs, thus rendering their quantitative analysis more reliable and sensitive. For this, molecularly imprinted polymers (MIPs) and ion imprinted polymers (IIPs) have already shown a high potential for the selective extraction of target analytes from complex matrices.

Different imprinted sorbents were synthesized and evaluated for their potential to selectively extract a target analyte but also structurally related compounds from various complex matrices (real water, soils or biological fluids…). The conditions of synthesis were adapted in order to generate specific cavities for the selective trapping of the analytes. Their potential for the selective trapping of organic compounds or metal ions from environmental matrices and biological fluids will be presented, thus highlighting the advantages and limitations of MIPs and IIPs in terms of use, stability, selectivity, capacity and on-line coupling with HPLC.

Most of the developments were achieved by introducing the MIP particles in disposable SPE cartridges. However, these selective tools are also particularly necessary when developing miniaturized devices due to the decrease in resolution resulting from the use of short length separation devices. In this context, totally miniaturized analytical systems were developed for the quantitative analysis of target molecules in complex samples. Miniaturized MIPs were prepared by in-situ synthesis of imprinted monoliths in capillaries (I.D. 100 µm). The repeatability of the synthesis was assessed. At last, these miniaturized selective sorbents were coupled on-line to nanoLC and even directly to UV for the determination of organic compounds in biological fluids.

Keywords: imprinted polymers, sample preparation, selectivity, miniaturization

KEYNOTE LECTURE

A fully automated LC-GC×GC-TOF MS/FID platform for MOSH and MOAH determination in food

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The coupling of an LC system with a GC one was first published by Majors in 1980 and the first automated system was presented in 1987 by Ramsteiner, shortly after followed by the first commercial instrument (Dualchrom 3000), introduced in 1989 by Carlo Erba (Italy). The LC–GC systems were intensively studied in 1980s and 1990s when different types of possible interfaces were explored. A particularly intensive work was carried out by Grob and his group. LC–GC can be used to reach efficient sample clean-up and/or a group-type separation of the analytes, thus proving to be very suitable for both clean samples, when high selectivity and sensitivity is required, and for complex ones. Despite the many advantages, the LC–GC technique never widely spread until the scientist were forced to face the challenging problem of mineral oil hydrocarbons (MOH) evaluation in food. Since then, not only the LC-GC-FID has became the reference method for this analysis, but even more dimensions has been exploited to unravel the complexity of the unresolved complex mixture (UCM) detected in food). LC-GC allows to separate the two main fraction of interest, namely saturated (MOSH) and aromatic (MOAH) hydrocarbons, but the chromatographic profile obtained is a hump of unresolved substances. Therefore, GC×GC-ToFMS/FID is the most promising solution to characterize the MOSH and MOAH fraction in detail.

Within this context, a fully integrated and automated platform, namely LC-GC×GC-ToFMS/FID is proposed to merge the routine and the confirmatory method in a single analysis. A novel software algorithm was used in order to improve the reliability of GC×GC quantification in MOSH and MOAH where a different quantification logic is needed. The advantages and the performance of the proposed platform will be discussed.

Keywords: LC-GCxGC, MOSH, MOAH, Contaminant, Food

KEYNOTE LECTURE

On-line coupled immunoaffinity chromatography – asymmetrical flow field-flow fractionation for the isolation and fractionation of subpopulations of biomacromolecules

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Many human biomacromolecules, such as extracellular vesicles (EVs) and lipoproteins, have a crucial role in physiological and pathological processes. Both can be further divided to subpopulations with differences in size, function, composition and/or origin. Better understanding of their biological function and role in pathological processes, as well as their exploitation in new diagnostic or therapeutic applications need detailed knowledge of their molecular composition. To meet this requirement a new, reliable, efficient and fast methods are needed to allow their reproducible isolation.

In this study, immunoaffinity chromatography with polymeric monolithic disks, immobilized with antibodies, was successfully on-line coupled to asymmetrical field-flow fractionation (IAC-AsFlFFF) for the isolation and fractionation of EVs, exosomes, exomeres, and apolipoprotein B-100 (apoB-100) containing lipoproteins from human blood plasma. The system was equipped with multiple detectors, such as ultraviolet, multiangle light- scattering, dynamic light-scattering, and diode array, to provide further chemical and physical characteristics of their subpopulations under 120 nm. On-line coupled IAC-AsFlFFF was an excellent tool for fast, reliable, and reproducible isolation and fractionation of challenging biomacromolecules from human plasma with a high purity and high yields of subpopulations.

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[2] Evgen Multia, Thanaporn Liangsupree, Matti Jussila, José Ruiz-Jimenez, Marianna Kemell, and Marja-Liisa Riekkola, Automated on-line isolation and fractionation system for nanosized biomacromolecules from human plasma, Anal. Chem. 92 (2020) 13058–13065.

Keywords: on-line coupled affinity chromatography – asymmetric flow field-flow fractionation, extracellular vesicles, lipoproteins, isolates

KEYNOTE LECTURE

One, two, three, four and more chromatographic dimensions in biopharmaceutical analysis

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Biotech drugs, once considered a risky and nearly impossible route to explore, are today dominating the pharma landscape. In the footsteps of the biotech pioneers walking this bumpy road, our chromatographic community rose to the challenge and sharpened the analytical toolbox.

Throughout the years we have seen products become increasingly complex and witnessed analytics becoming more and more performant.

This talk will reflect on the recent advances in the analysis of biologics; from the coupling of historical incompatible chromatographic methods with mass spectrometry, to the combination of ever more separation modes in multidimensional set-ups and the implementation of chemical and enzymatic reactors in the flow path for primary and higher order structural assessment.

Keywords: biopharmaceuticals, LC-MS, multidimensional LC

KEYNOTE LECTURE

Hyphenative analysis of proteins

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Separation techniques hyphenated with mass spectrometry (MS) undeniably have become primary tools for the structural characterization of proteins in complex mixtures. Whereas MS-based workflows provide detailed compositional information, they often also induce a loss of the native conformation and functional state of the analyzed proteins. This leaves questions open relating to their original three-dimensional (3D) structure and presence of conformers, aggregates and complexes. The Higher Order Structure Analysis (HOSAna) project aims to establish hyphenated analytical systems that can provide valuable data on size, chemical composition, conformation and supramolecular structure of intact macromolecules. Here we present several appealing project outcomes in the field of native protein analysis, involving size-exclusion chromatography (SEC), asymmetrical flow field-flow fractionation (AF4) or trapped ion mobility spectrometry (TIMS), all in hyphenation with time-of-flight MS.

We studied the impact of volatile mobile phases on the retention, ionization and denaturation of proteins analyzed by SEC-MS. Measured SEC distribution coefficients and protein-ion charge state distributions obtained by native MS were used to probe protein unfolding. Notably, several supposedly mild eluent compositions induced nonideal SEC behavior and/or protein unfolding. From the obtained results, mobile phase compositions that do not compromise native states were proposed.

A new platform coupling AF4 to native MS was developed to obtain a better understanding on protein aggregation and denaturation processes. The system appeared highly useful for the stability study of a biotherapeutic enzyme, whose main active form is a tetrameric oligomer. Presence of octameric and dodecameric structures in the native product were revealed, while dissociation into various oligomers as result of stress (pH, temperature, agitation) could be monitored, disclosing denaturing pathways. At the same time, obtained accurate protein masses indicated formation of deamidated tetramers.

TIMS provides unprecedented ion-mobility resolution and shows strong potential for analysis of protein 3D structures. However, to accurately determine (changes in) protein conformation by measuring ion mobilograms, it is imperative that experimental conditions do not alter molecular shapes. TIMS employs DC voltages to guide ions through a buffer gas across the different instrument stages. We systematically investigated the potential influence of these DC voltages on the conformation of various proteins to define native TIMS conditions. A set of DC values could be established which assured native analysis of most proteins at satisfactory sensitivity.

Keywords: native protein separation, native mass spectrometry, higher order protein structure, protein conformation

KEYNOTE LECTURE

The new frontiers of SFC-MS

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Supercritical fluid chromatography (SFC) has faced several waves of interest and disinterest through times. In the past decade, instrument developments have favored its reemergence in analytical laboratories. Significant improvements were made, particularly to enhance its sensitivity and robustness, but also to favor hyphenation to mass spectrometry (MS). However, further technological upgrades are desirable, which would further promote SFC-MS progress.

Regarding application domains, SFC is now more used than before, for achiral as much as chiral separations, and for ever more polar compounds. The wide choice of stationary phases and the flexibility offered by mobile phase compositions and operating parameters allow the exploration of new domains that were previously considered inaccessible.

In this presentation, I will illustrate the recent evolutions of SFC-MS with different sample applications. I will also discuss the current barriers limiting its expansion to unexplored territories.

Keywords: Supercritical fluid chromatography, Mass spectrometry, Applications, Unified chromatography

TUTORIAL

Basics of Low Pressure Gas Chromatography / Vacuum GC, A robust solution for 3x faster GC/MS analysis

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Fast gas chromatography (GC) using mass spectrometry (MS) has always been challenging as we have to use a column with sufficient restriction under vacuum-outlet conditions. Short 0.10mm columns will work, but are practically challenging to work with in regards to injection and capacity.

In 2001 a new concept was presented for speeding up GC-MS analysis using short 0.53mm capillary columns (directly connected at the MS inlet) connected to a restriction column at the inlet, enabling a high vacuum inside the 0.53mm analytical column. Technique is known as LPGC (Low Pressure Gas Chromatography) or sometimes called "Vacuum GC". Under the conditions created, very fast separations were performed as the optimal carrier gas velocity is a function of the actual pressure inside the capillary. Typically 3x shorter run times are obtained. We trade here some efficiency in for speed and robustness. Since a MS detector is used, no need full chromatographic separation is required. This technique was immediately adopted by Lehotay around 2003 for fast pesticide screening in Quechers extracts of food samples. Together with Lehotay, an optimized pre-assembled robust column for LPGC for fastest possible pesticide screening was developed and will be discussed in detail. In this tutorial the basics of LPGC are discussed and the impact it has on the chromatography of for example, pesticides in food. We will also zoom into the impact of matrix on pesticide response and ways how to work with this.

Keywords: LPGC, Vacuum GC, Fast GC/MS, Pesticides

TUTORIAL

Hyphenation & Capillary Electrophoresis – current status and future directions

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Capillary electrophoresis (CE) is a highly efficient separation technique, that has found its applicability in many fields or research and industry. Historically, separations of minute amounts of hydrodynamically- or electrokinetically-introduced sample are visualized using optical detection schemes. However, this often results in a low detection sensitivity and fairly information-poor electropherograms. With ever-increasing complexity of samples and subsequently analytical queries, this classical combination might not suffice in giving the desired answers anymore.

To resolve that, hyphenation has also found its way in the field of electrophoretic separations. Sample concentration and clean-up can be included in-capillary using either elegant injection schemes or immobilized chromatographic materials. For more information-rich data sets, CE is commonly hyphenated with mass spectrometry (MS). The key here, however, is the interface that allows the transfer of the analytes to the gas phase while mainting a closed electrical circuit. On the detection side, the ongoing MS developments related to novel fragmentation schemes and gas phase separations has lead to even more information-rich datasets. Lastly, online multidimensional separation have also been introducted in the CE field as a way to reduce sample complexity and resolve incompatibility issues.

In this tutorial, I will discuss where the field of hyphenated CE currently stands. Abovementioned aspects will be covered and the state of the art will be highlighted. There will be ample time for questions and discussions.

Keywords: capillary electrophoresis, 2DCE, mass spectrometry, interfacing, SPE

ORAL

Use of TD-GCxGC-FID/MS for Analysis of Trace VOCs in LDPE Materials and its Correlation with Olfactory Sensory Panel Testing

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Over the last decade, consumer markets (e.g. automotive, paints & coatings and food packaging) have driven a reduction in the amount of volatile organic components (VOCs) in finished products. Recycling plastics creates the potential for increased odor-active volatiles. Because the human nose is extremely sensitive (able to detect VOCs in the parts-per-billion to parts-per-trillion range) it is challenging to develop an analytical method to detect and/or identify VOCs at human sensory levels. Thus, the industry has relied upon the use of human sensory panels for the characterization of odor of finished product materials. But human sensory panels are limited in the number of samples that can be analysed within a given time period resulting in a backlog of samples. Additionally, panelists are not always able to identify the odor-active compound(s), plus there are potential safety risks to participants. This presentation will cover the development of direct thermal desorption coupled with comprehensive GCxGC analysis and Mass Spectrometric detection (TD-GCxGC-FID/TOFMS) for separation and identification of VOCs from low-density polyethylene (LDPE) materials, and the correlation of instrumental data with human sensory panel odor results.

Keywords: VOC analysis, odor-active volatiles, thermal desorption, TD-GCxGC-FID/MS

ORAL

Investigation of the potential of mixed solvent mobile phases in temperature-responsive liquid chromatography (TRLC)

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Temperature-responsive liquid chromatography (TRLC) allows for extensive retention and selectivity tuning through temperature in HPLC. This is achieved through the use of stationary phases comprising a temperature-responsive polymer which is able to undergo a reversible change of its surface from hydrophilic to hydrophobic upon an increase of the temperature. This interesting behaviour allows for retention behaviour to be controlled purely by a change in temperature,1,2 allowing for conventionally used mobile phase gradients to be replaced by temperature gradients. As a result, this technique allows for analyses to be performed under purely aqueous conditions.3,4 However, despite the promising nature of such form of retention control under isocratic mobile phase conditions, TRLC can suffer from excessive retention upon analysis of highly apolar solutes even at lower column temperatures where the surface of the polymer behaves hydrophilic. This is related both to a residual apolarity of the polymer chain, as well as due to the high inherent logP values and reduced water solubility of the more highly apolar compounds. Despite this apparent drawback, analyses in TRLC have already been performed under non-purely aqueous conditions and the use of co-solvents to the water have been proven to be possible.5–7 However, the exact impact for introduction of organic solvents was not yet investigated in a systematic way. Therefore, in this study the advantages and drawbacks for the use of organic co-solvents (methanol and acetonitrile) in TRLC was assessed on two types of temperature-responsive phases: poly-N-N-isopropylacrylamide (PNIPAAm) and poly-N-isopropylacrylamide (PNNPAAm). For this, the influence of organic co-solvents on the efficiency of the stationary phase as well as the cloud point temperature of the polymers is investigated with two representative test mixtures comprising of parabens and apolar steroids.

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Keywords: temperature-responsive chromatography, mixed mobile phase, PNIPAAm

ORAL

3D printed devices and sorbents – application for extraction procedures in bioanalysis

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There is still a need for novel tools for simple, efficient, and selective extraction of analytes proceeding LC-MS bioanalysis. Additive manufacturing, also called 3D printing, can possibly contribute in terms of rapid prototyping of novel extraction devices but also by direct fabrication of advanced sorbents.

Fused deposition modeling, currently the most popular mode of 3D printing, was used to fabricate different kinds of sorbents, subsequently used for analytical assays such as: determination of glimepiride in water, a set of steroids in human plasma, as well as drug candidates in in vitro enzymatic incubations aimed to study their metabolic stability. It was presented also how the size and shape can be customized to fulfil particular requirements of an assay. The analytes were quantified using LC-MS as a final analytical technique. The primary results showed that composite material, commercially known as LAYFOMM 60, can be successfully used for analyte extraction in the SPME procedure. Factors influencing extraction efficiency, as solvents for sorption and desorption along with the time of those steps, were assessed [1]. Next, the analytical system for the simultaneous extraction of steroids in a 96-well format was developed thanks to a feature of 3D printing to manufacture the complex geometry of a sorbent. The designed procedure required a small volume of a sample and solvents, was semi-automated and was successfully validated in terms of bioanalytical validation. The developed system was also compared with available alternatives (C-18 sorbent) and possible advantages were discussed [2]. In the final application, it was shown that metabolic stability assay, which is usually performed in a small volume due to the cost of enzymes and co-factors, can also be beneficial by being 3D-printed. The size of a sorbent was customized to allow extraction from a small volume of post-incubation mixture [3].

We also demonstrate the usefulness of custom in-lab fabrication on an example of a new device for dispersive solid phase extraction. The set of four fully printed parts is aimed to perform the extraction without leakage, unwanted evaporation, and most importantly avoiding contamination of the final extract with sorbent particles.

To show further perspective in this field the novel composite material containing silica particles embedded in the polymer matrix was developed and assessed in view of its extraction efficiency.

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Keywords: extraction, bioanalysis, 3D printing

ORAL

Detailed molecular characterization of complex cellulose ethers by LC-MS and probability-based deconvolution

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Cellulose ethers (CEs) are derivatized celluloses, which primary resource, i.e. wood, is renewable. CEs may comprise different substituents, such as ethyl, methyl, hydroxy ethyl, hydroxy propyl, carboxymethyl, and more. These substituents, and combinations thereof, give CEs useful properties which are widely applied in the paint, pharmaceutical and food industry to enhance the quality and efficacy of products.

The degree of substitution (DS) of cellulose ethers is of crucial interest since it determines the properties of the final product. Distributions at the molecular level influence CE properties, such as solubility, viscosity, and biodegradability. Current GC-based methods for the structural analysis of CEs are time-consuming and bothersome. Protocols developed for ethyl hydroxyethyl cellulose (EHEC) and methyl ethyl hydroxyethyl cellulose (MEHEC) involve reductive cleavage and permethylation. However, with this approach information on methyl substitution is lost unless deuterated reactants are used.

In this work, we present a new strategy for the structural elucidation of CEs based on LC-MS and automated data deconvolution. Industrial EHEC and MEHEC products were taken as representative model samples. Following acid hydrolysis, the samples were analysed by UHPLC-MS employing an acid-resistant reversed-phase stationary phase, positive electrospray ionization, and a quadrupole time-of-flight mass spectrometer.

The DS of methyl and ethyl groups and MS of ethyloxide (EO) groups could partly be resolved by LC-MS. However, some constitutional isomers (i.e. having the same mass) were not chromatographically separated resulting in convoluted distributions of methyl/ethyl combinations. To solve this issue, a probability-based deconvolution approach for monomers of EHEC and MEHEC was developed, allowing their deconvolution based on the measured data. Using the new method, the average DS of the tested CE samples could be determined, revealing differences. In some EHEC samples, significant amounts of methylated species were found which could be traced back to a recycling step in the production.

The developed workflow for CE analysis includes automated information extraction and visualization, facilitating use in an industrial environment. The workflow covers aspects such as peak detection and signal shift and peak area correction, and provides illustrative LC-MS heat maps highlighting the DS differences among CE samples.

Keywords: cellulose ethers, monomer composition, probability-based deconvolution

ORAL

Standardisation of saliva collection modes using a targeted metabolomic approach

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Recent advances in metabolomic, proteomic and genomic have allowed the identification and characterization of salivary components and some of them can be used as biomarkers for the diagnostic or monitoring of many diseases [1-3]. Differences in saliva collection, processing and storage methods could explain the discrepancies observed between studies [4,5]. Therefore, standardization of saliva collection, storage and processing conditions as well as the development of tools to detect multiple biomarkers are needed. Indeed, it is important to verify if the collection method affects the saliva composition to ensure the reliability of the results obtained.

In order to perform the most comprehensive analysis of the salivary metabolome, which contains compounds belonging to a wide range of polarity and mass, two modes of chromatography, namely reverse-phase liquid chromatography (RP-LC) and hydrophilic interaction liquid chromatography (HILIC), were used and coupled to mass spectrometry (MS) used in positive and negative modes. The optimization of the analytical conditions was done using a mixture of 90 compounds (fatty acids, amino acids, steroid hormones, vitamins, sugars ...) representative of the salivary metabolome in terms of polarity (log P from -4.7 to 10.5) and molecular weight (from 60 to 430 g.mol-1), naturally present in saliva and described in the literature as having differential salivary expressions in various pathologies. Calibration curves with concentrations ranging from 0.25 to 18 µg.mL-1 were then established in the four analytical combinations (RPLC and HILIC/ MS in positive and negative modes) for these 90 compounds.

In order to standardize the sample collection, storage and processing conditions, targeted LC/MS analysis of salivary samples collected from healthy controls were performed. The three most commonly used collection modes, that is spitting, aspiration and Salivette®, were compared with and without prior mouth rinsing. It is well known that the mode of collection can affect the salivary composition. Indeed, the presence of mucus, cells and bacteria in the samples, especially for samples collected by spitting or aspiration, can induce a bias in the analysis. Conversely, the Salivette® reduce the number of bacteria or cells present in the sample and led to the loose of some compounds that may be retained on the polymer. Analysing LC/MS profile of saliva collected from these three sampling methods therefore make it possible to compare them and then to standardize the conditions of collection, storage and treatment of saliva, to emancipate from individual variability and, finally, to obtain absolute quantifications for the reference compounds.

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Keywords: Metabolomic, Saliva, RPLC/MS, HILIC/MS

ORAL

Coupling of Non-Aqueous Ion-Exchange to Size Exclusion Chromatography in Two-dimensional microstructural analysis of acid-functional polymers

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There is an ever-increasing demand for environmentally friendly coatings/resins, which are commonly found in water-borne polymer applications. These polymers are often designed using a wide variety of monomers and polymerization techniques, resulting in tailor-made resins for targetted applications. The microstructure of such polymers is immensely complex, and there is a continuous drive to understand the exact molecular build-up of these resins as it would enable a more efficient and targetted approach to further improve copolymer designs.

Two-dimensional LC has been applied to study copolymer architecture for some years, but the current approaches lack information on the microstructural architecture of the stabilizing monomers which are used in low concentrations in such copolymers (most commonly acrylic- or methacrylic acid). As these monomers are critical for colloidal stabilization and polymer end-product properties, such as 2K-reactivity, adhesion and physical/chemical resistances, the microstructure of these monomer types is of significant importance.

In this contribution, we will share our advances in the analysis of these monomer types using two-dimensional LC. We have coupled Non-Aqueous Ion-Exchange Chromatography (which specifically separates copolymers according to the number of acid groups present in the polymer1) with Size Exclusion Chromatography (separating the polymers on size in solution). We will describe separation behaviour and orthogonality, and show examples of the stabilizing monomer microstructure and its effect on the appearance of copolymer particles.

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Keywords: polymer analysis, multi-dimensional LC, acid distribution, polymer microstructure

ORAL

Development of ion-imprinted polymers for the selective extraction of Cu (II) ions in environmental waters

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Nowadays, there is a growing need in environmental areas for the analysis of analytes at trace level in very complex samples and Solid Phase Extraction (SPE) is the most widely used technique for sample handling in this context. Concerning the analysis of inorganic ions, a variety of resins are commercially available as SPE sorbents but still lack selectivity at present. This problem can be solved by developing ion-imprinted polymers (IIPs), having specific recognition cavities. The synthesis of IIPs started conventionally with the complexation between a template ion and one or several appropriate monomeric ligands. The complexes are then immobilized in a highly cross-linked polymer matrix after the copolymerization with a crosslinking agent. Finally, template ions are removed from the polymer leaving tailored cavities that are complementary to template ions in size and coordination geometries.

The aim of this study was to introduce and compare two different approaches for the preparation of a new IIP targeting Cu(II) ions. One approach was based on the use of the target ion (i.e. Cu (II)) as the template ion while the second one used an analog ion as the template ion. With these two approaches, several IIPs were synthesized with a monomeric ligand and a crosslinking agent selected according to a bibliographical study, but by varying the duration of complexation and the volume and nature of porogen. In parallel, a non-imprinted polymer (NIP) was synthesized as a reference identically for each synthesis condition but in absence of template ion. These IIPs and NIPs were characterized with SPE protocols in order to investigate the selectivity of each IIP with respect to its NIP and its specificity towards Cu(II) ions in presence of other ions as potential interferents. The SPE protocols were optimized for each IIP to eliminate the interfering ions retained by non-specific interactions, and next eluate only Cu(II). This was done by analyzing each fraction (percolation, washings, and elution) by inductively coupled plasma mass spectrometry (ICP-MS). For the most promising IIPs, the capacities, the breakthrough volumes, and the enrichment factors were subsequently determined with their optimized protocols. The structure of these IIPs was also characterized by thermogravimetry analysis (TGA), nitrogen adsorption/desorption isotherms, and scanning electron microscopy (SEM). Finally, applications to real samples (mineral water, river water, and seawater) as well as the study of their possible reuse were carried out, demonstrating the high potential of these synthesized sorbents for different and complex samples.

Keywords: Ion-imprinted polymer (IIP), Copper, Solid-phase extraction (SPE), Selectivity, Preconcentration.

ORAL

GC-HRMS and GC×GC-HRMS, versatile tools for characterization of complex matrices and quantification of targeted compounds. Applications on biofuels, compounds emitted by plants, multiresidue of pesticides and contaminant analysis in food and cosmetics

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The development of the coupling of gas chromatography (GC) to high-resolution mass spectrometry (HRMS) has made it possible to combine the high separation power of GC with the selectivity and sensitivity of HRMS. A GC-Q-Orbitrap system provides a high mass resolving power (up to 120,000 full width at half maximum (FWHM) (m/z 200)) combined with a high mass accuracy (<3 ppm), which is needed to avoid isobaric interferences. Nevertheless, for GC-Q-Orbitrap spectra, some differences are observed in ion abundance in comparison with those recorded with a quadrupole mass spectrometer. So, for untargeted compound analysis different strategies were presented to enhance compound identification. Applications on the characterization of biofuels [1],]plant exudates, odorous compounds emitted from flax by the different part of the plants will be presented using GC-Q-Orbitrap and GC×GC-Q-Orbitrap. Results were compared with those obtained GC/MS using a simple quadrupole showing the advantages and drawbacks of these approaches. Concerning targeted compound analysis, the high resolving power and the high mass accuracy allowed for a drastic reduction of the noise and thus decreasing the limit of detection (LOD). So, GC-Q-Orbitrap method and GC-triple-quadrupole method were evaluated and compared for the screening and quantification of 100 pesticides and contaminants in different complex food matrices, such as wheat, rapeseed, cumin and black tea [2]. Application of GC×GC- Q-Orbitrap for the identification of aromatic compounds in lipsticks was also described allowing to avoid false positive contamination.

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Keywords: Gas Chromatography, High-Resolution Mass Spectrometry, Pesticides

ORAL

The Total Breakthrough phenomenon: Description and Application in On-Line Comprehensive 2D-LC

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In liquid chromatography (LC), differences in eluent strength between the sample solvent and the mobile phase usually give rise to undesirable effects on the separation, which may range from slight broadening to severe peak deformation and even peak splitting. In the most extreme case, the retention factor of the analyte at the head of the column is so small at the time of injection that part of the analyte goes through the column with so little interaction with the stationary phase that it elutes very close to the column dead time. This phenomenon is known as breakthrough.

Under breakthrough conditions, the retained peak is usually strongly distorted, resulting in a continuous trail of molecules between the breakthrough peak and the retained peak and/or in the appearance of multiple retained peaks. However, we have recently reported conditions in which large injected volumes could give rise to a single and fully symmetric retained peak in addition to the breakthrough peak [1]. We called this phenomenon "Total Breakthrough" in reference to the complete disappearance of the molecules between the breakthrough peak and the retained peak above a critical injection volume.

In the present work, we describe the results of an in-depth study aimed at better understanding and defining the conditions for the emergence of breakthrough and Total Breakthrough phenomena in LC. The effects of a broad range of parameters, including the nature of the solute, the nature of the stationary phase, the injection and elution conditions, the column temperature, and the sample concentration were investigated. While breakthrough can occur for any compound provided that the injection volume is large enough, only the presence of positive charges on the molecule allowed to observe the Total Breakthrough phenomenon. It was found that only the injection conditions and the analyte retention had an impact on the occurrence of both phenomena. This finding allowed us to establish two injection volumes above which each respective phenomenon are observed. It is shown that these two critical volumes depend only on the column dead volume and the analyte retention factor in the injection solvent.

Finally, an application of this so far unexplored phenomenon is presented for the analysis of peptides in on-line comprehensive two-dimensional liquid chromatography (LC x LC) combining hydrophilic interaction liquid chromatography (HILIC) and reversed-phase liquid chromatography (RPLC). One of the most limiting problems in on-line HILIC x RPLC is related to the injection of strong solvent in the second dimension, which deteriorates the separation. Here, we demonstrate that excellent peak shapes can be obtained under Total Breakthrough conditions, which is an attractive alternative approach to those frequently used in HILIC x RPLC. In addition to an 80% increase in peak capacity obtained compared to RPLC x RPLC, the competitiveness of this HILIC x RPLC approach compared to flow splitting or on-line solvent dilution is clearly demonstrated.

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Keywords: Injection solvent strength, Breakthrough phenomena, Two-dimensional liquid chromagraphy, Ionizable compounds, Peptides

ORAL

The role of GCxGC-TOFMS in fragrance characterization

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In the past 20 years, comprehensive gas chromatography (GC×GC) has been applied for the characterization of fragrances and perfumes. The advantages of GC×GC compared to one-dimensional GC are mainly demonstrated by the increased peak capacity, resulting in a larger number of separated solutes.

In fragrance industry, the implementation of GC×GC technologies in R&D and in quality control still suffers from uncertainties in method set-up (GC×GC parameter selection), method robustness, complex data handling and difficult quantification.

At RIC, we have performed a number of studies applying both one-dimensional GC-MS and GC×GC -MS to fragrance and perfume samples for characterization, detection and quantification of suspected fragrance allergens and for perfume authenticity verification.

In this presentation, an overview of the results of these studies will be presented. Attention will be paid to GC×GC method parameter selection, aiming at the use of a generic approach that is helpful for method installation, verification, and implementation. A comparison of the quantitative results obtained for suspected fragrance allergen analysis with GC-MS and GC×GC -MS will also be presented.

Finally, some approaches for fragrance and perfume authentication based on achiral and chiral analysis will be given.

Keywords: fragrances, comprehensive GC

ORAL

Potentiality of Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) process to boost the purification performance of a peptide

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Pharmaceutical products, such as biomolecules, must satisfy very strict purity specifications, because of quality and safety reasons. Therefore, the necessity to operate one or more purification steps to obtain high quality drugs is indisputable. Critical impurities chemically very similar to the target product are generated during the synthesis and are generally removed by means of preparative single-column chromatographic techniques (=batch methods) [1,2]. Batch methods struggle to separate completely the peptide of interest from other groups of impurities, because of their similarity and of high loading of sample processed in preparative conditions, which cause peaks overlapping [3]. The typical situation encountered in these cases is the so-called center-cut separation, where the target elutes as intermediate between two other groups of impurities less and more retained respectively. The direct consequence of this apparently insurmountable overlapping is a yield-purity trade-off, a limit intrinsic to batch chromatography according to which it is possible to obtain either high purity or high recovery of the peptide of interest, depending on whether the overlapping windows are collected or not [4]. This trade-off leads to drawbacks in the overall performance and economy of the process. Multicolumn chromatographic processes, operating in continuous and countercurrent mode, can alleviate this limitation by performing internal recycling of the overlapping portions of the chromatogram [5]. Also, theoretical studies can help deciding the experimental conditions to be used, by knowing the adsorption behaviour of the major components.

The technique used in the frame of this research is twin-column Multicolumn Countercurrent Solvent Gradient Purification (MCSGP), which has been applied to the purification of an industrial crude of a bioactive decapeptide. It has been demonstrated that MCSGP leads to promising results, including a remarkable improvement in process performance (up to 6 times higher) from the point of view of recovery, productivity and solvent consumption, with respect to the corresponding batch run. The automation of the process on industrial scale would lead to great reproducibility which would reflect in improved consistency in product quality.

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Keywords: multicolumn chromatography, peptides purification, continuous chromatography, downstream processes

ORAL

Online photodegradation hyphenated with two-dimensional liquid chromatography

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Organic compounds can undergo photochemical conversion through exposure to (UV) light. Sometimes this is exploited, for example in water purification, but it is often undesirable. For example, the fading of cultural-heritage objects, reducing their esthetical value, or the change of flavour or nutritional value as a result of degradation of food ingredients.

Many techniques have been developed to study photodegradation. Several commonly applied methods were included in the present study for benchmarking purposes, viz. Xenotest, Microfading-Tester, and light-box (Spectrolinker) experiments. However, these methods tend to be laborious, time consuming and error prone, if only because manual extractions are typically required. The resulting information tends to be inconsistent and unsatisfactory. Often it is difficult to establish links between the degradation products and the starting materials, which impairs the construction of quantitative degradation models.

An on-line hyphenated system, in which samples can be prepared (first separation stage), selectively illuminated (degradation stage), and characterized in detail (second separation and identification stages) will have enormous potential for fast, efficient and reliable studies in many different fields. In analytical-chemistry terms, such a system concurs with a comprehensive two-dimensional liquid chromatography – diode-array detection / mass spectrometry (LC×LC-DAD/MS) with reaction modulation. In this presentation a number of steps towards the realization of such a system will be described.

Recently, a low-volume light-cell was developed based on a liquid-core waveguide (LCW) technique, which allowed online degradation studies [1]. In this work, such a novel LCW cell was coupled to LC-DAD/MS and this online setup was compared with the benchmark techniques described above.

Already, the online hyphenation of the LCW cell and LC is creating new options for efficient photodegradation studies, bringing the ultimate goal of a fully online, automated LC×LC-DAD/MS system with reaction modulation within reach.

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Keywords: light-induced degradation, reaction modulation, LC×LC

ORAL

Fully Automated Dried Blood Spot Extraction coupled to Liquid Chromatography-tandem Mass Spectrometry for Therapeutic Drug Monitoring of Immunosuppressants

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Background: In therapeutic drug monitoring (TDM), there is an increased interest in dried blood microsampling. Sampling of dried blood spots (DBS) on filter paper is the best known strategy, as a less invasive alternative to venous blood sampling. Key advantages are the possibility to perform home sampling and the amenability for automation. One of the key requirements for this strategy is the availability of sensitive Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) configurations.

In this study, we validated and compared LC-MS/MS methods for the quantification of four immunosuppressants (tacrolimus, everolimus, sirolimus and cyclosporin A) in blood and in DBS, for the latter using a fully automated DBS-MS 500 autosampler. Additionally, the applicability of the DBS method was evaluated.

Methods: Method development included optimization of the fully automated extraction procedure. After method validation, this optimized procedure was applied on venous left-over samples from patients taking immunosuppressants at Ghent University Hospital and was compared with an optimized procedure in whole blood. Paired venous DBS and whole blood samples were analyzed in duplicate, using fully automated extraction for DBS. Agreement between the automated DBS and whole blood method was assessed using Bland-Altman comparison. Both an analytical and clinical acceptance limit were pre-defined at more than 67% of all paired samples within 20% of the mean of both samples and more than 80% of all paired samples within 20% of the whole blood concentration, respectively.

Results: The LC-MS/MS methods were successfully validated based on international guidelines, for the DBS method also taking into account DBS-specific parameters. Reproducible (CV<15%) IS-compensated relative recovery values were obtained. However, a hematocrit-dependent relative recovery was observed for the DBS, with lower hematocrit values yielding higher relative recoveries (and vice versa). Relative to the reference hematocrit of 0.37, this difference exceeded 15% at hematocrit extremes (0.18 and 0.60). Also for the quantification of patient samples, an impact of the hct on DBS quantitation was observed for all analytes. Since a significant hct trend was present, a correction algorithm was set up for the DBS results, based on the hct value of the liquid whole blood. Using the hct conversion formula [DBScorrected]=[DBSmeasured]/(1.6305 – 0.0156*hct), which was based on the tacrolimus data set, the hct-effect could be alleviated for all analytes. After correction, both analytical and clinical acceptance criteria were met for all analytes.

Conclusion: Automated DBS analysis, coupled to LC-MS/MS, shows great potential for routine TDM of immunosuppressants, avoiding any manual sample handling (from card to result, with no hands-on).

Keywords: Therapeutic Drug Monitoring, Immunosuppressants, Dried Blood Spots, Automated extraction, Liquid Chromatography-Tandem Mass Spectrometry

ORAL

Use of micropillar array columns for high resolution separation of phosphorothioated oligonucleotides and their diastereomers

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Oligonucleotide based drugs, such as Antisense (ASO) and small interference ((si)RNA), have gained a lot of interest as therapeutic molecules and are currently a part of the portfolio of many pharmaceutical companies. To support their development programs or study exposure after administration, analytical tools are essential to investigate impurity profiles or degradation pathways, a. As these products contain several closely related oligonucleotide structures/ impurities development of analytical methods with very high resolution and selectivity is required for the analysis and characterization. In some cases the complexity is even increased because of the introduction of a phosphorothioate linkage in the backbone. The presence of such PS linkages creates chiral centres, producing diastereomers with Rp and Sp configuration that may have different pharmacological and physicochemical properties. Depending on the number of PS linkages in an oligonucleotide structure, the complexity increases as 2n (n = # internal PS linkages) isomers are formed requiring diastereoselective methods for oligo analysis. Current ion pair reversed phase chromatography combined with UV detection and electrospray ionization (ESI) mass spectrometry is considered the most powerful method for oligonucleotide analysis making identification and quantification of co-eluting structurally closely related impurities feasible.. MS analysis of these structures is however complex due to co-elution and the relatively large molecular weight resulting in several multiply charged ions for each analyte. An analytical method in which all impurities are resolved, making UV based quantification possible, would thus be preferred. This presentation will present the potential of micropillar array columns for high resolution oligonucleotide analysis focussed on resolving closely related oligo impurities, degradants, or metabolites and diastereomers.

Keywords: oligonucleotides, micropillar array columns, impurity profiling, diastereomers

ORAL

Replacing historical glycan profiling strategies for biopharmaceutical products with quantitative Middle-Up HILIC-HRMS analysis

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The identification and accurate quantitation of the various glycoforms found on therapeutic monoclonal antibodies (mAb) is of crucial importance due to their role in the immunogenicity and clinical efficacy of these products. Current reference techniques for glycan analysis are based on the release of the N-glycans of the mAb, followed by labelling with a fluorescence dye (2-AB, RFMS or Procaine) and analysis using hydrophilic interaction chromatography (HILIC) with fluorescence detection (FD). This workflow is not only considered time-consuming and laborious, but also causes the loss of important site-specific information and is unable to detect other important post-translational modifications (PTMs) present on mAbs.

Recently, the use of HILIC-MS for the analysis of protein subunits (25-50 kDa) has emerged as a powerful technique for the qualitative glycan analysis of mAbs, fusion proteins and antibody-drug conjugates1,2. Since these highly informative mAb subunits can be generated rapidly (~ 1 hour) after enzymatic digestion (e.g., IdeS, Papain) and chemical reduction of the disulfide bonds, the sample preparation time can be drastically reduced, and important site-specific glycan information can be maintained. By using wide-pore HILIC stationary phases (300 Å), the subunits can be chromatographically resolved prior to introduction in the mass spectrometer and provide accurate mass information on the glycosylation profile as well as other important PTMs.

In this work, we evaluated the quantitative performance of glycan analysis on subunit level and compared the relative quantitation accuracy with the reference 2-AB and RFMS released glycan approaches. A wide range of commercially available mAbs was selected with diverse glycan characteristics, including cell line difference, glycoengineered products and biosimilars, and used to evaluate the potential of the subunit approach as cost- and time-effective alternative to the released glycan methods. It was observed that accurate quantitation results could be obtained on the glycosylation profile and that the middle-up analysis holds a great potential in use as multi-attribute monitoring method.3

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Keywords: Glycans, HILIC-MS, Relative quantitation, Biopharmaceuticals, Subunits

ORAL

Novel strategy to screen multi-classes of halogenated pollutants by Gas Chromatography-Atmospheric Pressure Chemical Ionization-Trapped Ion Mobility-Mass Spectrometry (GC-APCI-TIMS-MS)

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Over the past two decades, it has become increasingly evident that the presence of organohalogen contaminants in the environment, called persistent organic pollutants (POPs), is a growing concern. The recent incident of the release of a large amount of perfluoroalkyl substances (PFAS) over decades into the soil in the vicinity of a PFOS production plant near Antwerp (Belgium) reminds us of how these environmental issues are still relevant and of public health concern. These incidents lead competent authorities to proactively set up monitoring plans that require the screening of as many compounds as possible that are recognized and established to be toxic or potentially toxic. In addition, there is a need to set up policies that focus on compounds that are little or poorly studied at all, in order to address the hidden side of the problem.

Thus, novel analytical methods and strategies that allow rapid screening and quantitation of multiple POPs simultaneously, while providing improved separation and identification capabilities, are in high demand. We propose to focus our efforts mainly on ion mobility rather than on the ion mass. In this context, the research developed here aims at combining a method integrating the use of trapped ion mobility (TIMS) with a GC-APCI-TOFMS instrumentation. Gas chromatography (GC, Bruker 456-GC; equipped with Rxi-5SilMS column, 30mx0.25mmx0.25?m, Restek) has been hyphenated to atmospheric pressure chemical ionization (APCI, GC-APCI II, Bruker) and trapped ion mobility spectrometry (TIMS)-Time of Flight Mass Spectrometry (GC-APCI-TIMS-TOFpro, Bruker) to characterize a complex mixture of emerging halogenated POPs. A solution comprising a total of 152 compounds was prepared in n-Nonane (99%, Alfa Aesar) and run by GC-APCI-TIMS-MS. This mixture contained 7 PCDDs and 10 PCDFs (NK-ST-B4 mix, Wellington), 82 PCBs (PAR-H mix, Wellington), 4 PXDDs and 2 PXDFs (where X are mixed Cl, Br substitution; individual standards, Wellington), 23 PAHs (EPA & EU-PAH-STK mixes, Wellington), 20 BDEs (BDE-MXC mix, Wellington), 2 PBBs (individual standards, AccuStandard) and 2 other brominated compounds (BTBPE and DBDPE, Wellington).

In TIMS mode, Collision Cross Sections (CCS) versus m/z trendlines were examined in details. The power fit functions (of the type y=axb) highlighted that each class of contaminant was characterized by its own trendline. It was found that for given mass, the experimental CCS in nitrogen buffer gas of the chlorinated compounds were higher than that of the brominated compounds but lower than that of planar PAHs. In addition, the primary factor contributing the difference in CCS among a family of halogenated compounds was discernibly identified as the halogenation degree, while the fine ion mobility separation of isomeric congeners could also be related to key structural parameters such as the number of chlorine/bromine atoms in ortho position for PXBs or next to the oxygen atom for PXDFs. In addition, the ion mobility resolution provided by TIMS proved valuable in deconvoluting the signal from isobars compounds that were not separated by either the GC conditions used here or the high resolution ToF MS.

Keywords: gas chromatography, Ion mobility, mass spectrometry, persistent organic pollutants, food safety

ORAL

Investigation of mass transfer kinetics of non-psychoactive cannabinoids under achiral reversed phase conditions on C18 2.7 um superficially porous particles

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In the last decades, following an increment in the production of hemp and recreational cannabis, more and more attention has been paid in the development of analytical methods for the accurate measurement of the potency of cannabis products prior commercialization [1].

Reversed-phase liquid chromatography (RPLC) is one of the most popular methods currently used for the separation, identification, and quantification of cannabinoids by employing C18 stationary phases in combination with aqueous/acetonitrile (ACN) or aqueous/methanol (MeOH) mobile phases.

Up to now, potency testing has been devoted to the investigation of only a limited number of cannabinoids, despite their number exceeds one hundred and is continuously growing.

This points the attention to the urgent need of developing new separation methods able to detect and quantify the largest number of compounds in a single run. To this end, the understanding of all the factors affecting the separation may help in the development of highly efficient and ultrafast methods for potency testing of cannabinoids from real samples.

In this study, a systematical evaluation of the kinetic performance of a 150x2.1 mm column packed with 2.7 µm C18 superficially porous particles is presented for 5 neutral non-psychoactive cannabinoids. More precisely, mass transfer kinetics were studied through the independent evaluation of each term of the van Deemter equation. The coupling of proper models of diffusion in porous media and stop flow techniques (eg. peak parking) [2] were used in order to gain knowledge about different contributions to band broadening and finally to be able to compare the kinetic performance of the column with different analytes and mobile phase compositions.

The investigation of the effect of the chemical structure of the analytes and the mobile phase composition on the kinetic performance of columns is essential to understand the basis of band broadening for terpenophenolic compounds and to push forward the limits of high efficient and ultrafast liquid chromatography [3].

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Keywords: cannabinoids, mass transfer, van deemter, liquid chromatography

ORAL

From Flower to Extract to Isolated Cannabinoids using Supercritical CO2

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The cannabis and hemp industry is rapidly gaining traction in Europe. Although flowers still hold largest market share, the concentrates segment is expected to witness highest growth in future years boosted by increased demand of governments, doctors as well as patients.

The medicinal cannabis business in Europe is approaching this trend with high focus on quality control and sustainability. The quality of the biomass is of utmost importance and next to an appropriate cannabinoid profile, the absence of pesticides, fungus and heavy metals is key to ensure patient safety. Next to stringent quality requirements to the cannabis cultivation, the extraction and post processing are of significant importance, as well as the quality monitoring and the control of batch-to-batch consistency of this natural product.

The current contribution describes a workflow for the extraction and consequent purification to individual cannabinoids using supercritical carbon dioxide (scCO2). The use of of scCO2 represents significant advantages when used as an extraction solvent or as a mobile phase to perform purification, as CO2 is non-toxic and non-flammable and is furthermore considered as a green and sustainable solvent.

Keywords: SFE, SFC, cannabis, CO2

ORAL

Modeling the GC×GC separation as individual subsystems under vacuum outlet conditions

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Method development in comprehensive two-dimensional gas chromatography (GC×GC) can be considered as a rather tedious task in light of the complex interplay between the involved experimental parameters [1]. Moreover, due to the wide applicability of this technique in the analysis of complex samples, method optimization has become more challenging and time consuming. Therefore, a renewed interest in modeling GC×GC separations has sprung. In fact, establishing accurate modeling procedures helps bypass demanding trial and error optimizations, thus significantly decreasing the number of runs preceding the actual chromatographic separation.

Typically, the GC×GC separation is modeled as a whole complex set. However, in this research, the separation is modeled as individual subsystems in which the primary (1D) and secondary (2D) columns are treated separately and the cryogenic modulator is considered as a consecutively second injection device. In this scheme, the tR are modeled by performing separate one-dimensional GC (1D-GC) runs on the 1D and 2D columns. For the 1D-GC runs performed on the 2D column, a special experimental setup was conceived in order to maintain working with the same flow rate as in a typical GC×GC separation. This setup, helps circumvent the negative inlet pressure issue entailed by the use of a short GC column for 1D-GC purposes and enhances the peak shape quality by minimizing the peak broadening.

In the current framework, the separations are modeled using a thermodynamic-based model that involves a thermodynamic treatment of the GC equilibrium constant [2]. This model uses retention data retrieved from isothermal runs and simulates the temperature-programmed 1D-GC runs as series of infinitesimal isothermal time intervals during which both the retention factor and the carrier gas velocity are considered constant. Accurate retention time predictions were successfully achieved on the primary column [3]. The performance of the modeling approach was evaluated using different test samples, namely the Grob test mix, the fragrance materials test mix and a series of saturated linear alkanes, and experimental conditions (constant inlet pressure, constant flow rate and different temperature programs). Average modeling errors of 0.43%, 0.33% and 0.15% were registered across all the studied temperature programs when the thermodynamic-based model was applied under constant flow rate mode. The same modeling approach will be applied to predict retention times on the secondary column. However, it is still a work in progress.

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Keywords: multidimensional gas chromatography, retention time prediction, thermodynamic modeling, method development and optimization

ORAL

Non-targeted analysis of organic contaminants in complex environmental matrices- Elucidation of high-level (bio)chemical mechanisms

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We present here our approaches for non-targeted (NT) two-dimensional gas chromatography coupled with mass spectrometry (GCxGC-MS) workflows to describe (bio)chemical transformations of complex environmental samples. We aim to simplify complex data to elucidate high-level mechanisms of (bio)engineering processes. By using NT analysis, chemical signatures and chemical markers of the reactions can be identified and related to the reaction conditions and parameters.

We focus here on the formation of semi-volatile organic compounds (SVOCs) during underground coal gasification (UCG)1 from two different experimental set-ups. Coal tar samples were collected from a shallow in situ UCG experiment and processed (or waste) waters from two ex situ experiments. Exhaustive extraction of the samples was carried out by novel and robust ultra-sound assisted methods. Samples were analysed by GC-MS, through a workflow we presented previously, and by GCxGC-MS. The NT GCxGC-MS data workflow include tile alignment of the chromatograms and multivariate statistical analysis of feature tables such as principal component analysis, hierarchical clustering analysis and partial least square-discriminant analysis. It enabled us to better describe the coal gasification processes compared to the quantitative analysis of targeted compounds2 and to identify markers of the reactions that could be used in future experiments to monitor progress and yield.

While our workflows are adapted from metabolomics, our applications present specific challenges. Namely, our samples contain several tens of thousands of closely related hydrocarbons, we often have low numbers of sample per "class" and we are interested mostly in times series. Therefore, we will also highlight the QA/QC process we are developing to ensure findings reflect the state of the system and are not biased by the workflow.

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Keywords: GCxGC-MS, data mining, multivariate analysis, non targeted analysis

ORAL

Multidimensional liquid chromatographic approaches for automated bottom-up analysis of antibody-derived therapeutics

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One of the fastest growing class of biotherapeutics are monoclonal antibodies (mAbs). The interest in this type of therapeutic proteins is attributed to their high specificity for their targeting antigen. MAbs and new antibody formats are complex glycoproteins, exhibiting a wide range of heterogeneities. As these heterogeneities can affect the pharmacological properties of the protein, it is important to perform extensive characterization. Characterization of mAbs is commonly performed by bottom-up approaches, involving sample preparation and peptide analysis by liquid chromatography-mass spectrometry (LC-MS). Sample preparation for conventional bottom-up approaches is very time consuming and can increase the risk of inducing artificial modifications as many off-line steps (denaturation, reduction, alkylation and digestion) have to be performed. Other drawbacks, includes long incubation times (several hours) and low efficiency of tryptic digestion. To overcome these issues, we developed a multidimensional LC (mD-LC) set-up for fast routine analysis of formulated conventional mAbs and newer antibody formats. In the current presentation I will focus on the optimization of different parameters (1D -and 2D flow and temperature) to increase the retention of small polar peptides in the RP-C18 column. The applicability of the method was demonstrated by the analysis of different (stressed and non-stressed) mAbs. This new automated approach enables mAb characterization in less than 2h with sequences coverages between 96-98%. The proposed mD-LC approach permitted the direct injection of formulated mAb samples without the need of any sample preparation or pre-separation of antibody variants.

Keywords: multidimensional LC mass spectrometry, automation, on-line bottom-up, therapeutic antibodies, post-translational modifications

ORAL

An Approach for the Deeper Analytical Characterization of Mineral Oil Aromatic Hydrocarbons

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The contamination of food with mineral oil residues is still a widely discussed problem. Although there had been significant advancements in the last years, there a remaining knowledge gaps in analysis, exposure assessment, hazard characterisation and risk assessment1.

Mineral oil hydrocarbons are divided into a saturated and an aromatic fraction. While the mineral oil saturated hydrocarbons (MOSH) are not considered to have a genotoxic relevance, the mineral oil aromatic hydrocarbons (MOAH) may include potential mutagenic and carcinogenic substances2. State-of-the-art analysis is done using the online-coupling of LC-GC-FID, but analysis reveals only unresolved humps with unknown origin. Confirmatory techniques using multi-dimensional chromatography, e.g. 2D-comprehensvie GC×GC with various detector types are needed to allow for an adequate substance class identification, recognition of false-positive values and therefore correct quantification of the generated humps. One of the probably biggest knowledge gap is related to positvie MOAH results found in food samples. There are still adequate hazard characterisation and risk assesments missing, because of the unknown and very complex compostion the MOAH has1.

Therefore, this work deals with the deep characterization of MOAH sub-fractions using the hyphenation of different analytical techniques: In a first step, mineral oil products, food contact materials and food samples are analyzed for their MOSH and MOAH contamination using the current state-of-art. Afterwards, the MOAH is separated and second, is subjected to a HPLC fractionation according to ring numbers3. Using those separated fractions, on the one hand, relevant substance classes are identified using 2D-comprehensive GC×GC-ToF. On the other hand, hazard characterization of this sub-fractions is made using the AMES-test. The information of both approaches is combined to allow for the needed health risk assessment of the samples tested positive for MOAH.

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Keywords: Hazard characterisation & risk assessment

ORAL

Development of methodologies for quantifying simultaneously PAHs and PCBs in sediment cores: advantages and drawbacks of chromatography coupled to thermal or solvent extraction and hyperspectral imaging

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Polycyclic aromatic compounds (PAHs, PCBs) are widespread contaminants usually investigated in sediments, which act as sinks for these toxic persistent lipophilic compounds. Conventional approaches often use gas chromatography coupled to mass spectrometry (GC-MS) as a powerful tool to separate, identify and quantify them, but a preliminary step of extraction is required. The latter generally necessitate the use of toxic organic solvents and extend the duration of the overall analytical process, particularly for sediment cores analyses.

In this study, two extraction methodologies were optimized using experimental designs for multi-residual analysis and then compared: microwave assisted extraction (MAE) and thermal desorption (TD). Indeed solvent-free TD is frequently used for analysing PAHs from atmospheric particles but multi-residual analyses (PAHs/PCBs simultaneously) after TD from sediments are unusual. Several factors that may influence extraction recoveries were studied, including matrix parameters (sediment mass, organic matter content) and processing parameters (such as solvent volume for MAE). A full factorial design 23 was used to optimise MAE extraction. Optimization of TD parameters was more complex as dozens of factors could be more or less influent, so a definitive screening design (DSD) was performed to screen the most influential factors (among 6 studied) and model the extraction recoveries at the same time.

After the optimization of extraction conditions, matrix effects were evaluated using the standard addition procedure and quality assurance and control (QA/QC) were implemented for comparing MAE and TD for sediment analysis. Although MAE process was easier to optimize and less sensitive to matrix effects, sediment thermal desorption could significantly improve the analytical process, due to direct coupling with TD-GC-MS/MS and complete automation. Moreover, it offered higher spatial resolution, particularly for sediment core analysis, due to the 1000-times lower sample size (mg instead g).

If TD-GC-MS/MS could significantly improve laboratory throughput for sediment analysis compared to MAE-GC-MS, a comparison was also carried out with hyperspectral imaging to determine the value of this methodology for sediment core analysis. Indeed, this recent, promising and non-destructive technology has the advantages of reducing drastically the analysis time (min instead of days) and has a considerably higher spatial resolution for sediment core analysis (µm instead of cm). But substantial work should continue to improve PAHs/PCBs signal identification and quantification, with a deeper understanding of matrix effects.

Keywords: Environnmental Chemistry, Pollutants, PAHs, PCBs, sediment cores

ORAL

Multi-detector UHPLC analysis of polysorbate 80

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Polysorbate (PS) is a non-ionic surfactant widely used in pharmaceutical, biopharmaceutical, cosmetic, and beverage formulations. Several types of PS are available, with PS 20, PS 60, and PS 80 being most frequently used in pharmaceutical products. All commercially available PS are complex mixtures of several hundreds of molecules. This complexity is a consequence of the inherently heterogeneous raw materials used for the synthesis, and the synthetic pathway that leads to the final product. In the case of PS 80, the product is obtained by esterification of oleic acid with sorbitan polyoxyehthylene (POE); the oleic acid originates from natural sources and contains other fatty acid impurities such as palmitic, linoleic, and stearic acids. These impurities will participate in the esterification reactions. The additional presence of the precursor and side product of sorbitan, along with different degrees of ethoxylation, contributes to the overall sample complexity.

The control of PS as chemical raw material for (bio)pharmaceutical formulations is difficult because of the complexity described above. Regardless, such control is needed since variations from lot-to-lot are expected to occur. Variation of the relative population of esters, and polyoxyethyleted polyols, can affect the behavior of PS 80 as an excipient in biotherapeutic formulations. Understanding the quality of PS 80 by analyzing the raw material is a potential time and cost-saving approach, as it would decrease the need of costly root-cause analyses when formulations obtained with a particular batch of PS do not meet the required standards. A full quantitative characterization of a PS sample is, with the current technology, an extremely complex task that consumes considerable analytical resources. It is not required that every component of a PS is quantified. However, simple analytical techniques capable of profiling the main features of PS sample and enabling lot-to-lot comparison, or sample degradation, are highly desirable.

In this work we propose a reversed-phase UHPLC multi-detector approach for the analysis of PS 80 raw material. Charged Aerosol Detector (CAD) and a Single Quadrupole MS are used for the quantitative and qualitative analysis respectively. The method enables to identification of expected PS single components by combining information on retention time and m/z values. With this approach we could characterize PS 80 raw material from different productions. Moreover the method was applied to PS 80 samples enzymatically degraded. The analysis could provide accurate quantitation of the degradation rate at different time points using the CAD signal. The analysis of MS and retention data enabled the assignment of putative identity of the main degradation products, thereby clarifying the main degradation pathway.

Keywords: surfactants, formulation, single quadrupole, mass detection, CAD

ORAL

Graph neural networks for improved retention time predictions and molecular identification

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Liquid chromatography (LC) is an important analytical tool used in all stages of drug discovery and development, and is for example used to identify and quantify degradation products and impurities, and to determine the drug candidate in bioanalytical samples during clinical trials. To improve the analysis of these compounds, machine learning (ML) models can be developed to predict their retention times (RT).1 In this study, a new generation of ML models, namely graph neural networks (GNNs),2 are developed to improve the accuracy of RT predictions to better filter out false positives in the identification of molecules. Classical ML models to predict RTs are based on so-called descriptors of molecules (e.g., Log P and total polar surface area (TPSA)). These descriptors are fixed numerical representations of molecules which are directly operated on by the ML algorithm. Although these descriptors have proven highly predictive, they are not optimized to predict RTs. In contrast, a GNN optimizes the numerical representation of molecules, based on their atoms and the bonds between these atoms, to predict RTs. Each molecule is treated as a graphs G = (V, E), where V is a set of atoms (vertices) and E a set of bonds (edges).

In this work, a number of interesting variants of GNNs, including graph convolutional networks (GCNs), message-passing neural networks (MPNNs) and graph attention networks (GATs), are used for the purpose of improving RT predictions and molecular identification for different LC modes. These GNNs all abstract out information about the molecule based on complex aggregations of information from local structures within the molecular graph.

Preliminary results indicate that the GNNs perform overall significantly better than classical ML models (such as random forests and support vector machines) for RT predictions in LC (about 4 to 25% lower absolute error for all LC modes investigated). Utilizing both the graph structure of a molecule and the "low-level" molecular structures such as atoms and bonds, is demonstrated to better abstract out information from the molecule for the downstream task (namely, RT predictions). Importantly, improved models for predicting RTs could lead to significant improvements in identifying molecules in practice, which could significantly reduce time, effort and costs in e.g., drug discovery and development.

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Keywords: deep learning, graph neural networks, retention time predictions, molecular identification

ORAL

Targeted lipidomics analysis of phosphoinositide signaling network in biological samples by liquid chromatography with tandem mass spectrometry

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Phosphoinositide (PIPx) signaling network plays central roles in membrane dynamics and signal transduction of key functions like cellular growth, proliferation, differentiation, migration and adhesion [1]. Arising from phosphorylation at 3', 4' and 5' positions of the inositol ring of phosphoinositol (PI), the highly regulated PIPx family consists of seven groups and three regioisomers in two groups (PIP and PIP2) can be formed [2]. In phospholipase C-mediated hydrolysis of phosphoinositol-4,5-bisphosphate (PI(4,5)P2), second messengers sn-1,2-diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (I(1,4,5)P3) are generated, which are responsible for calcium release from intracellular stores. Similar to PIPx, a diverse inositol phosphate (IPx) family was formed with kinase and phosphatase reactions. Numerous studies have revealed the importance of fatty acyl chains (for PIPx), degree of phosphorylation, and phosphorylation positions under physiological and pathological states. Although methods for PI and DG are available, analysis of PIPx and IPx remains challenging due to their low abundance, instability, high charge density, and presence of regioisomers. A comprehensive workflow addressing abovementioned issues is still missing.

Here, we established an integrated workflow which enables lipidomics analysis of phosphoinositide signaling network in three levels: A) general lipid profile including PI and DG with different fatty acyl side chains [3], B) PIPx profile [4], and C) IPx profile, from a single aliquot of biological samples. For PI and DG, the sample was extracted with neutral solvent mixture and separated with reverse phase liquid chromatography (RP-LC). Sequential window acquisition of all theoretical fragment ion spectra (SWATH) was utilized for MS data acquisition for better coverage. Stable isotope labeled (SIL) lipids from different classes were spiked as internal standards (IS). For PIPx analysis, the residue from the neutral extract was extracted with acidified solvent mixture. The organic layer containing PIPx was methylated to improve stability, chromatographic and ionization performance. Separation of regioisomers was achieved with chiral polysaccharide stationary phase. SWATH was taken for full coverage of different fatty acyl chains. The aqueous layer was purified with TiO_2 and methylated for IPx analysis. A cholesterylether-bonded RP-type column was chosen for separation of regioisomers including I(1,4,5)P3. A differential isotope labeling strategy was used to generate IS. Therefore, comprehensive analysis of phosphoinositide signaling network was achieved with the same aliquot. This workflow was applied to different biological samples, including NIST SRM1950 plasma, cultured HeLa cells, human platelets, etc. The results show that this workflow holds the potential to advance the measurement in studies of phosphoinositide signaling network.

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Keywords: lipidomics, phosphoinositide, inositol phosphate, derivatization, SWATH

ORAL

Fused deposition modeling based 3D-printing of solid phase cartridges: development and implementation

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In this work fused deposition modeling (FDM) based 3D-printing is used for the manufacturing of cartridges for solid phase extraction. The novelty of the approach is that the 3D-printing is used for the simultaneous printing of the porous retentive bed, the cartridge housing and wall as a single printed monolith. This process allows for enhanced freedom in the used cartridge format, shape and dimensions as compared to the conventional packed bed formats. Cartridges were printed by polylactic acid (PLA) and polypropylene (PP), whereby the latter currently appear the most promising in terms of applications with emphasis on environmental analyses. Cartridges were optimized in terms of strut orientations, pore size, wall thickness and bed length. The obtained performance was tested with e.g. 16 priority polluting poly-aromatic hydrocarbon, illustrating that large aqueous volumes of PAH solutions can be loaded and eluted in fairly narrow plugs also on these alternatively manufactured SPE cartridges.

ORAL

UC-MS and RPLC-MS complementary analysis for small pharmaceutical peptides

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Identifying impurities in drug products is a duty to ensure patient safety. This task is reserved to analytical chemists, who must separate the impurities from active components, quantify and identify them. Chromatographic techniques associated to mass spectrometry are the gold standard methods. Our work focusses on biomolecular active substances and more specifically peptides. Peptides are oligomers or polymers of amino acids. They may be natural or synthetic, linear or cyclic, and they may have various biological activities such as antidiabetic, anticancer, ... 1

RPLC-MS method has been applied for peptide purity analysis at Servier Research Laboratories since 2016. However, this method provides satisfying results to only 80% of the pharmaceutical peptides. To overcome some deficiencies of this method and to offer a complementary analysis, we explored a new method based on unified chromatography (UC) hyphenated to mass spectrometry. UC is operating with pressurized CO2 associated to a liquid solvent, in proportions ranging from 0 to 100%. The "unified" term is related to the 3 different chromatographic modes joint in a single experiment, namely supercritical fluid chromatography (SFC), enhanced-fluidity liquid chromatography (EFLC) and liquid chromatography (LC) (see figure below). This combination perfectly fits the wide diversity encountered in peptide samples.

This talk focuses on short-chain peptides (< 1 000 Da) analysis using UC-MS and exposes the difficulties encountered in the analysis of biomolecules 2. Comparisons between UC-MS and RPLC-MS results will be discussed on the same set of peptides to highlight the complementarity of the methods.

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Keywords: Peptides, Supercritical Fluid Chromatography, Unified Chromatography

ORAL

Modelling of analyte profiles and band broadening generated by interface loops used in multi-dimensional liquid chromatography

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With the increased interest in multi-dimensional LC separations in recent years, several sophisticated commercial 2D-LC systems have been introduced to the market. However, there is still a lack of a complete theoretical foundation upon which sound developments can be made. One parameter that is not fully understood is the shape and variance of the analyte band entering the second-dimension column when injected from an open loop interface in two-dimensional liquid chromatography. This is however important as it is connected to several other variables encountered when developing 2D-LC methods, including the first-dimension flow rate, the sampling (modulation) time and the loop volume.

In this presentation both numerical simulation methods and experimental measurements were used to understand and quantify the dispersion occurring in open tubular interface loops. Variables included are the analyte diffusion coefficient (Dmol), loop filling and emptying rates (Ffill & Fempty), loop inner diameter or radius (Rloop) and loop volume (Vloop). For a straight loop capillary, we find that the concentration profile (as measured at the loop outlet) depends only on a single dimensionless parameter and the ratio of the filling and emptying flow rates Fempty/Ffill. A model depending only on these two parameters was developed to predict of the peak variance resulting from the filling and emptying of a straight capillary operated in the first-in-last-out (FILO) modulation mode. In the first-in-first-out (FIFO) modulation mode a model with the same function was also developed however in this case the filling fraction of the loop (f) needed to be included as it plays a role in determining the peak shape and variance in this modulation mode. It was found that the FILO mode yields lower variances than the FIFO mode because the emptying of the loop in the opposite direction of its filling partially counteracts the dispersion created by the parabolic flow profile during the filling phase.

Comparison of the concentration profiles and the corresponding variances obtained by either numerical simulation or experiments with straight capillaries shows the results generally agree very well. These results are important to improve the overall quality of future 2D-LC separations, due to the sensitivity of small second dimension columns to peak dispersion due to the injection plug.

Keywords: loop dispersion, numerical simulations, peak variance model, modulation, two-dimensional liquid chromatography

ORAL

Direct SEC-MS for the detailed characterization of monoclonal antibody size variants: what are the critical parameters for a straightforward hyphenation?

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Antibody-based drugs can undergo several enzymatic and chemical posttranslational modifications and degradations, leading to the creation of variants such as size variants. Their identification can be critical, especially for variants resulting from the aggregation which are known as high molecular weight species (HMWS). These may generate an adverse immune reaction after administration to the patient and thus alter the clinical efficacy of the drug.

Among the several analytical strategies to characterize the size variants of therapeutic proteins, size-exclusion chromatography (SEC) is one of the gold standards for separation and quantitation of HMWS and fragments. However, this technique suffers from the difficult identification of the resolved chromatographic peaks. The hyphenation of SEC with mass spectrometry (MS) can overcome this drawback. For this purpose, volatile salts have to be used in the LC mobile phase to allow a direct coupling, while allowing an analysis of the protein in its native form.

Previous attempts to couple SEC with MS were performed, but suffer from low sensitivity, poor peak shapes and limited aggregates recovery. The lack of column inertness has been highlighted as preventing the success of the SEC-MS hyphenation, due to unwanted interactions between proteins and the column hardware.

In our recent work, we have evaluated the possibilities offered by a new generation of metal-free SEC columns to perform direct SEC-MS of antibody-based products with ammonium acetate as mobile phase additive. The prototype metal-free SEC column hardware used in this work was a polyether ether ketone (PEEK) infused stainless steel tube including PEEK frits. This prototype metal-free SEC column was systematically compared with a conventional stainless-steel SEC column hardware packed with the same stationary phase material.

The benefits of metal-free column hardware were demonstrated. It appears that peak symmetry, separation of low molecular weight species (LMWS), and recovery of HMWS were significantly improved for the different biopharmaceutical products on the metal-free SEC column. It has also been demonstrated that the largest differences between standard and metal-free SEC columns were observed for the most basic antibody-based drugs (high isoelectric point). This confirms that electrostatic interactions between the protein and the metallic parts of the column (frits and inlet tube) could be responsible for the issues observed when performing SEC analysis with volatile mobile phases.

Finally, our results highlight that an inappropriate column could bias the quantification of size variants when using MS-compatible mobile phases. Therefore, metal-free column, such as the PEEK-lined column, should be preferentially selected for SEC-MS analysis.

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Keywords: SEC-MS, Monoclonal antibody, Metal-free stationary phase

ORAL

Thermal modulation to enhance two-dimensional liquid chromatography separations of polymers

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Many materials used in a wide range of fields consist of polymers that feature great structural complexity. One particularly suitable technique for characterizing these complex polymers, that often feature correlated distributions in e.g. microstructure, chemical composition, or molecular weight, is comprehensive two-dimensional liquid chromatography (LC×LC). For example, using a combination of reversed-phase LC and size-exclusion chromatography (RPLC×SEC). Efficient and sensitive LC×LC often requires focussing of the analytes between the two stages. For the analysis of large-molecule analytes, such as synthetic polymers thermal modulation (or cold trapping) may be feasible because of the exponential increase of retention with molar mass and their sensitivity towards small changes in temperature. Due to the temperature differences required this approach can be readily implemented using conventional LC systems. The approach was studied for the analysis of a styrene/butadiene "star" block copolymer. Trapping efficiency is evaluated qualitatively by monitoring the effluent of the trap with an evaporative light-scattering detector and quantitatively by determining the recovery of polystyrene standards from RPLC×SEC experiments. The recovery was dependent on the molecular weight and the temperatures of the first-dimension column and of the trap, and ranged from 46% for a molecular weight of 2.78 kDa to 86% (or up to 94.5% using an optimized set-up) for a molecular weight of 29.15 kDa, all at a first-dimension-column temperature of 80 C and a trap temperature of 5 C. Additionally a strategy to reduce the pressure pulse from the modulation has been developed, bringing it down from several tens of bars to only a few bar, potentially increasing the life-time of the trap columns.

Keywords: Thermal Modulation, Polymer Analysis, Thermal Focussing

ORAL

Database of molecular diffusion coefficients of representative biopharmaceuticals under reversed-phase liquid chromatography conditions

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High Performance Liquid Chromatography (HPLC) is key to evaluate biopharmaceuticals in terms of identity, purity and stability. Reversed-phase liquid chromatography (RPLC), the most widely used HPLC mode, is ideally suited for their analysis. To improve the separation of biopharmaceuticals, it is crucial to improve the fundamental understanding of the parameters governing the band broadening of these molecules. This can be obtained by a detailed assessment of the individual contributions to their mass transfer. For this purpose, a precise knowledge of the molecular diffusion coefficient (Dm) of biopharmaceuticals is required.

Only little experimental data is available for the Dm-values of biopharmaceuticals under RPLC relevant conditions. Furthermore, none of the available equations that can be used to calculate Dm-values, allows to account for any conformational changes that might occur. Therefore, the goal of this work was to create a database of Dm-coefficients of frequently used biopharmaceuticals under relevant RPLC conditions that can serve future fundamental investigations.

The Taylor-Aris method is often employed to determine Dm-coefficients, and is a very simple and absolute method. The Taylor-Aris method measures the peak-broadening of an analyte in an open tube under laminar conditions, wherein longitudinal diffusion can be ignored, the sample is fully radially equilibrated and the contribution of the extra-column variance to the total variance is negligible. Moreover, since the open tubes are typically coiled for practical reasons, the influence of secondary flows should be insignificant [1-2].

In this presentation, the impact of each aforementioned condition on the accuracy of the Dm measurements is first discussed. Secondly, it is investigated how the analysis time of a Taylor-Aris experiment can be reduced, without compromising the accuracy of the obtained Dm-values. This results in a set-up wherein all conditions are met, while providing accurate Dm-values within an analysis time of 20-30 minutes. Finally, this set-up is used to measure Dm-coefficients of representative biopharmaceuticals under relevant chromatographic conditions.

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Keywords: Biopharmaceuticals, Molecular Diffusion Coefficient, Taylor-Aris, RPLC

ORAL

Fundamental studies aiming at improved selectivity in oligonucleotide ion-pair chromatographic separations

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Oligonucleotides constitute a growing field of various important therapeutic modalities targeting the genomic expression. Their complicated synthesis and degradation pathways offer many challenges for separation science, including analysis and purification.

Here we will present a closer investigation of ion pair chromatographic (IPC) separation systems relevant for oligonucleotides. This work has been performed in an academic-industrial collaboration funded by the Swedish Knowledge Foundation together with AstraZeneca.

First, the effect on selectivity of stationary phase pore size will be discussed from an experimental and theoretical perspective [1]. A theoretical model was created, correlating accessible surface area to the oligonucleotide size. Here we will show how the accessible surface area are correlated to the selectivity. Experiments were carried out on four different columns with pore sizes between 60-300Å. Results show that selectivity gains are possible by using 300Å versus 100Å pore sizes.

Second, the presentation will cover the recent fundamental study of other factors affecting oligonucleotide selectivity in IPC [2]. Here, two different cases were studied: (1) solutes with different charges, for example short and longmer impurities and (2) solutes with same charge but different hydrophobicity, for example the diastereomer. In the first case, selectivity was found to increase with increasing electrostatic potential due to the adsorption of the ion-pairing reagent, decreasing cosolvent fraction and decreasing temperature. In the second case the selectivity increases with decreasing cosolvent fraction and temperature. Gradient elution was also investigated for full length heteromeric oligonucleotides. An ion-pair reagent concentration gradient was introduced and evaluated as an alternative gradient mode, relying on the fact that oligonucleotide retention is predominantly driven by electrostatic interactions. This mode was found to increase the selectivity of the phosphodiester impurity of phosphorothioated oligonucleotides.

[1] J. Bagge, M. Enmark, M. Leśko, F. Limé, et al., Impact of stationary-phase pore size on chromatographic performance using oligonucleotide separation as a model, J. Chromatogr. A. 1634 (2020) 461653. https://doi.org/10.1016/j.chroma.2020.461653.

[2] M. Enmark, S. Harun, J. Samuelsson, E. Örnskov, et al., Selectivity limits of and opportunities for ion pair chromatographic separation of oligonucleotides, J. Chromatogr. A. 1651 (2021) 462269. https://doi.org/10.1016/j.chroma.2021.462269.

Keywords: ion pair chromatography, retention modeling, oligonucleotides

ORAL

Prediction of retention shifts in SFC for improved method transfer and robustness

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Method transfer in supercritical fluid chromatography (SFC) is more complex than in LC due to the compressibility of the mobile phase. If SFC continues to gain more popularity, it will be necessary to demonstrate scientific knowledge about the underlying reasons for retention shifts for example during method transfer, due to the strict regulations of pharmaceutical product control and the quality by design ICH regulations. Here we focused on retention shifts due to the pressure drop and how the robustness of the separation system depends on the MeOH adsorption.

In SFC the retention of a solute depends on the temperature, density, pressure, and co-solvent fraction. Because the most important factor controlling the retention is the co-solvent, it was recently proposed to calculate the co-solvent molarity from density and mole fraction co-solvent [1]. This simple idea allows the construction of the isomolar plot which shows the variation in molarity with co-solvent and pressure. More importantly, it can be used to predict retention of solutes. This could be used as a strategy to compensate retention shifts due to changing column pressure drop during method transfer and will be presented and exemplified for the separation of pharmaceutical enantiomers. This straightforward approach relies on the isomolar plot and adjusting the co-solvent fraction delivered by the pump and does not require back-pressure adjustments.

The robustness of a separation method is an equally important consideration during method development and was also recently investigated [2]. We will focus on how to adsorption of MeOH changes with changes with pressure and temperature and how this affects the retention of several solutes. It was found that robustness decreased with lower pressure and higher temperature, which could clearly be correlated to changes in the amounts of MeOH adsorbed to the stationary phase. The choice of sufficiently high pressure and low temperature will be especially important when transferring a method from classical SFC to ultrahigh-performance SFC (UHPSFC).

[1] M. Enmark, J. Samuelsson, T. Fornstedt, A Retention-Matching Strategy for Method Transfer in Supercritical Fluid Chromatography: Introducing the Isomolar Plot Approach, Anal. Chem. 93 (2021) 6385–6393. https://doi.org/10.1021/acs.analchem.0c05142.

[2] E. Glenne, M. Leśko, J. Samuelsson, T. Fornstedt, Impact of Methanol Adsorption on the Robustness of Analytical Supercritical Fluid Chromatography in Transfer from SFC to UHPSFC, Anal. Chem. 92 (2020) 15429–15436. https://doi.org/10.1021/acs.analchem.0c03106.

Keywords: SFC, method transfer, retention, robustness

ORAL

Development of data processing workflow for untargeted multidimensional chromatography

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During the last decade, comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS) has become a mature technique. The constant improvement of the hardware (e.g., high speed MS, modulators) and controlling software have generated robust commercial systems. GC×GC-MS has now entered a new phase of extended applications in different fields. Indeed, GC×GC-MS is now commonly used to conduct large scale studies, giving full access to its high-resolution power for targeted and mostly untargeted screening. This high-resolution in the separation is particularly helpful for complex samples untargeted screening, as commonly encountered in metabolomics. The current challenges of the technique are now localized on the data management side, where powerful chemometric tools are required to unlock GC×GC-MS full potential [1].

In order to tackle those challenges, we have been investigating specific chemometric tools for GC×GC-MS in the aim of developing user friendly data processing solutions. An optimized processing workflow is a key to ensure data quality. To understand the impact of each data processing step, each of the critical steps of data preprocessing, feature selection, model building, and validation have been investigated in detail using different data sets.

From this investigation, we have designed a guide for users who desire to establish robust and reproducible GC×GC-MS data processing workflows. This guide also evaluates the user impact in the data processing and how it can bias the data. We have also investigated the development of the next generation of chemometric tools, looking to artificial intelligence and machine learning.

[1] PH Stefanuto, A Smolinska, JF Focant, Advanced chemometric and data handling tools for GC×GC-TOF-MS: Application of chemometrics and related advanced data handling in chemical separations, TrAC Trends in Analytical Chemistry, Volume 139, 2021.

Keywords: data mining, metabolomics, GCxGC, multidimensional technique

ORAL

Hyphenation, automation, digitalization: do we still need analytical scientists for running the lab of the future?

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In recent years, there is a clear trend towards digitalization and more automation in the analytical lab. Traditionally, specialists with a different education are needed (e.g. analytical chemists, data-scientist, IT-specialists as well as mechanical engineers).

In this presentation it will be explained how instrument miniaturization can transform the laboratory. Also it is exemplarily shown that essential programming and IT-skills for lab-automation and digitalization are not needed if innovative software is used that supports the analytical chemist to automate and digitize complete workflows.

Keywords: automation, digitalization, miniaturization

ORAL

Performance Limits and Design Aspects for Spatial Comprehensive Three-Dimensional Isoelectric Focusing × Size-Exclusion Chromatography × Reversed-Phase Liquid Chromatography for the Analysis of Intact Proteins

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Spatial comprehensive three-dimensional chromatography (3D-LC) is an emerging separation technology that can potentially offer unprecedented resolving power. The maximum peak capacity that can be generated corresponds to the product of the three peak capacities of the individual development stages, provided that orthogonal retention mechanisms are applied. When considering protein analysis and separation in the space domain, there are limitations on which modes can be combined. Furthermore, based on the choice of separation mechanisms and experimental conditions applied in the three individual developments, the design requirements, i.e., the number of channels and the channel length should vary. Here, we study the advantages and disadvantages that these aspects can bring when designing a spatial 3D-LC chip targeting protein analysis.

Based on Pareto optimization we discuss the optimal chip design and assess the performance limits of a spatial 3D-LC device operated in xLC× xLC× tLC mode, combining isoelectric focusing (IEF), aqueous size exclusion chromatography (SEC) and gradient reversed-phase (RP) in the first (1D), second (2D) and third dimension (3D) respectively. Peak capacities and peak production rates are calculated for each individual dimension and the influence of the channel diameter and length on these objectives is studied, taking into account the spatial nature of the development in the first two dimensions. Moreover, the effect that the number of channels in each dimension has on the obtained separation of the previous one is investigated using a newly proposed equation to calculate the loss in peak capacity due to suboptimal sample transfer. Finally, some aspects of chip prototyping as well as integration of orthogonal separation mechanisms, based on the above-mentioned considerations, are demonstrated.

Keywords: Pareto-optimality, multi-dimensional LC, spatial 3D-LC, microfluidic chips

ORAL

Matrix effects in LC-MS: Evaluation and correction approaches using wastewater analysis as an example

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Matrix effects are well known in liquid chromatography (LC) electrospray ionization mass spectrometry (ESI-MS), and result in signal suppression or enhancement that impairs the accuracy and comparability of the analytical measurements. This study describes a method to evaluate and compensate for matrix effects in enriched wastewater extracts using LC ESI-high resolution MS (HRMS) [1]. As a first step, the dilution of wastewater extracts was evaluated, showing the loss of low-response compounds by dilution, but also the loss of compounds by signal suppression in samples with high enrichment factors. To ensure that low-response compounds will not fall below the detection limit, a strategy to compensate for the matrix effects at higher enrichment factors is necessary. As a second step, the observed matrix effect at higher REFs was corrected by the retention time dependent matrix effect. We showed strong evidence that the primary mechanism responsible for the matrix effects observed in analysis of wastewater is the competition for available charge between the matrix and analytes of interest during the electrospray ionization. A new scaling of the matrix effect was introduced, which demonstrates that the total ion chromatogram (TIC) can be used to predict the matrix effect as effectively as post column infusion approaches. Thus, the TIC can be used for an easy correction of the retention time dependent matrix effect, which accounts for ca. 85% of the observed matrix effect. As a final step, the residual 15 % structure specific matrix effect was predicted and corrected by quantitative structure-property relationships (QSPR), which led to a further correction of the matrix effect to 0±7%. The method showed better correction of the matrix effect for non-target compounds when compared to matrix correction by a set of generic, non-matched internal standards. This study provides new approaches for generating reliable, consistent data, which allows comparison of unknown compounds in samples with unknown matrix effect.

[1] Tisler, S., Pattison, D.I & Christensen, J.H. (2021): Correction of Matrix Effects for Reliable Non-target Screening LC–ESI–MS Analysis of Wastewater, Analytical Chemistry, doi.org/10.1021/acs.analchem.1c00357

Keywords: non-target screening, matrix effect, LC-HRMS, wastewater

ORAL

In-silico screening for the development of robust reversed-phase liquid chromatography methods of known small molecules based on QSRR and desirability index

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Reversed phase liquid chromatography is one of the most widely used analytical methods for the analysis of mixtures of chemical compounds. The development stage can be extensive due to the multitude of possible stationary and mobile phases and analytical parameters. Thorough screening of the different possible combinations is time-consuming and costly, even when using a systematic approach such as experimental planning. The development of quantitative structure-retention relationship (QSRR) models can accelerate this screening phase for mixtures of known composition by allowing "in silico" screening of experimental conditions and selecting pre-optimal conditions.

Ninety-eight molecules were selected to cover a broad range of LogP values, molecular weights and include both ionizable and non-ionizable molecules. An experimental dataset (retention times) was generated in the laboratory on three different HPLC systems (Waters Alliance) with gradients ranging from 100% buffer to 5% buffer in 20 and 60 minutes and at five different pH (2.7, 3.5, 5, 6.5 and 8). The organic modifier selected is methanol.

First, the selected molecular descriptors describing the physicochemical properties of the analytes of each compound are calculated. Then, different QSRR machine learning (ML) models derived from relationships between chromatographic parameters and molecular descriptors are trained for each condition. Next, a response surface model (RSM) is trained for each compound on the predictions of the ML models. The RSM equation is selected depending on the values of pKa of the compound regarding the pH range covered experimentally.

The last step is the multiple criteria decision analysis (MCDA) using the desirability index for the selection of the optimal conditions. The most important criterion is the separation of the different analytes as it is the main purpose of chromatography. Another criterion that is considered is the robustness of the method to the analysis parameters. The third criterion is the maximum retention time per condition. This criterion is used to limit the analysis time.

Applying this strategy showed that the combined performances of the QSRR model and the RSM are encouraging. An R2 of 0.87 and a root mean squared error (RMSE) of 3.56 minutes resulted from the comparison of the actual retention times and the retention times predicted by the RSM model for an external test set of six compounds.

The MCDA allows the definition of multiple regions of the experimentally covered space where the predicted peaks are completely separated that represent approximatively 7% of the original area.

The results of the application of this strategy demonstrates that the combination of QSRR, RSM and MCDA offers the possibility to assist usefully the experimental screening phase by computational methods when developing chromatographic techniques for known sets of molecules. This also reduces the dependence of the method development to experienced users.

Keywords: HPLC, Small pharmaceutical compounds, Reverse phase liquid chromatography, Quantitative Structure Retention Relationship, Multi-criteria optimization

ORAL

Robust native LC–MS platform for in-depth proteoform characterization

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Proteins are macromolecules that perform key roles in most processes in life. The biological function of these proteins is closely related to their structure. Therefore, structural changes, caused by e.g. post-translational modifications (PTMs), may have profound effects on protein activity. For instance, the presence and nature of glycosylation can influence protein stabilization and regulate interactions with receptors in vivo. As a result, it is of great importance to be able to characterize and monitor the impact of PTMs on protein function (i.e. establishment of structure-function relationships). For this purpose, analytical techniques that permit characterization of proteins while maintaining their functional state are crucial. In particular, native protein separations hyphenated with mass spectrometry (MS) are emerging as a powerful tool to study these aspects in a fast and straightforward manner.

Here, we developed a native liquid chromatography (LC)-MS platform to resolve proteoforms without interrupting their functional integrity. The platform allows structural characterization by online MS coupling of resolved proteoforms and their functional assessment using activity assays. Different native LC modes were employed to separate proteoforms based on size (size exclusion chromatography), charge (ion exchange chromatography), and presence of glycation (boronate affinity chromatography). Proper ionization of the separated proteoforms was ensured by optimization of the hyphenation strategy using a post-column flow splitter and dopant enriched desolvation gas. The post-column flow splitter allowed coupling of an analytical-scale separation and a nano-electrospray source. In this way, the performed separation was robust and permitted proteoform isolation, while permitting high ionization efficiency. For the dopant gas enrichment, we evaluated the effect of different organic modifiers, including acetonitrile (ACN), isopropanol (IPA) and methanol (MeOH), on protein ionization. While the use of IPA and MeOH improved the ionization and desolvation, ACN resulted in lower quality mass spectra. We demonstrated the usefulness of the native LC–MS platform to establish structure-function relationships for different applications, including biotherapeutics, industrials enzymes, and endogenous proteins.

Keywords: native liquid chromatography, hyphenation, native mass spectrometry

ORAL

The analysis of tracer compounds in particulate matter – from offline GC×GC and TOFMS towards at- and online analysis

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The chemical loading of particulate matter in aerosols comprises a multitude of (semi-)volatile and non-volatile organic compounds. Such compounds are either directly emitted by biogenic and anthropogenic processes or secondarily produced and altered by atmospheric processes. In each case, the chemical fingerprint could consist of thousands of compounds, which can affect human health, environment and climate. Within this study, we present an analytical scenario for a comprehensive analysis of the organic matter in PM. We focus on the application of gas chromatographic and time-of-flight mass spectrometric techniques with the aim to identify and quantify the chemical fingerprint.

The formation and atmospheric aging of secondary organic aerosols (SOA) in the atmosphere involves different complex reactions including interaction with seed material, photochemical reactions and different oxidation processes [1]. This can rapidly alter the physicochemical composition of aerosols and may thus affect their impact on human health and environment. For this study, the photochemical transformation of aerosols was simulated in controlled laboratory experiments by means of a potential aerosol mass (PAM) oxidation flow reactor [2]. Naphthalene and beta-pinene were applied as an anthropogenic and biogenic precursor model compound, respectively. The produced SOA was comprehensively characterized in terms of its physical and biological parameters. Particulate matter was sampled on quartz fiber filters. For the comprehensive analysis of the particle-bound vaporizable organic species, a thermal desorption (TD) two-dimensional gas chromatography (GC×GC) time-of-flight mass spectrometry (TOFMS) method was developed and used to investigate differences between experimental aging conditions in the PAM reactor, such as variations in relative humidity, temperature or lamp power. The chemical characterization included the comparison of individual compounds, evaluation of their degree of oxidation and the classification of chemical groups. This comprehensive approach targets a broad volatility and polarity range of SOA. More heavy compounds, which are not accessible by gas chromatography, were analyzed by direct inlet probe (DIP), where the sample is directly introduced into the ion source of a high-resolution TOFMS system [3]. Here we show an application of TD-GC×GC-TOFMS to gain fundamental insight into the chemical composition of SOA and the complementarity of a DIP-HRTOFMS approach to extend the accessible total mass of SOA for time-of-flight mass spectrometry.

The study clearly shows the advantages of a close interaction of on- and offline techniques, but also the potential merit and resulting benefits of a comprehensive online technique that would diminish the advantages of chromatographic and mass spectrometric systems.

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[2] Kang E. et al., Atmos. Chem. Phys. Discuss. 7 (2007) 9925–9972

[3] Käfer U. et al., Talanta 202 (2019) 308–316

Keywords: comprehensive gas chromatography, time-of-flight mass spectrometry, secondary organic aerosols

INDUSTRY PITCH

Novel low-flow LC technologies hyphenated with HRAM MS are pushing the boundaries of LC-MS bottom-up proteomics

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The modern LC-MS technologies became a standard in proteomics research, translational studies, and penetrated clinical applications. While the advances in mass-spectrometry during recent years resulted in sensitivity, speed, resolution, and mass-accuracy improvements the increased variety of LC-MS applications possess a challenge for existing low-flow LC systems to provide required application versatility, flow and pressure range, long-term robustness, system intelligence, and throughput capabilities. Thus, the development of new LC instrumentation and separation workflows is required to enable applications from single-cell proteomics to high-throughput targeted quantitative assays for large sample cohorts profiling. Here we describe the next-generation all-in-one UHPLC system specifically designed for nano-, capillary- and micro-flow high-sensitivity LC-MS applications and analysis of complex samples.

The novel autosampler and separation pump design with the built-in system controller, touch user interface and remote control enables standardized low-flow LC-MS workflows and guided method creation. The binary pump delivers pressure up to 1500 bar and yields high-resolution separations with ultra-long columns. We achieved ca. 20 s PWHM for 4 hours gradients using 75 µm x 75 cm long columns and identified above 9000 proteins and > 100000 peptide groups with single-shot data-dependent acquisition. The coupling of two 75 cm long columns led to ca.15% PWHM reduction and further peak capacity increase. The wide-flow pressure footprint with active flow control permitted the development of high-throughput LC-MS methods using high-pressure 15 cm columns with 75 and 150 µm ID. The reference methods with the throughput of 180, 100, 60, 30, or 24 samples per day can be easily adjusted to accommodate different sample types and column chemistries. The developed methods showed excellent reproducibility with 70-90% of peptides and proteins identified and quantified across three sites worldwide independent of the method duration. The robustness testing covered > 1000 injections and up to 6 months of continuous operation for multiple systems. The thorough study of carryover sources in low-flow LC-MS analysis and advanced multi-liquid wash routines allowed to practically eliminate system carryover and reduce overall application carryover to < 0.1% for complex samples. The versatility provided by the extended flow capabilities, low gradient delay, and wide pressure range enables robust micro-flow LC-MS separations on 1.0 mm I.D. columns interface using the analytical flow ESI source with MS. With the throughput of up to 400 samples per day, the new low-flow UHPLC system enables targeted LC-MS screening in translational studies of large sample cohorts.

Keywords: low-flow UHPLC, nano-LC, peptides, proteins, single-cell proteomics

INDUSTRY PITCH

Battle of the brands: Using GC×GC–TOF MS to compare the VOC profiles of food and beverages

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Unlike counterfeit goods, replica and imitation products are legal, as they do not use the branded product's trademark. In the food and beverage industry, imitation products attempt to mimic the taste experience of popular branded products.

However, flavour profiles are extremely complex and consist of a broad range of chemical classes – the combination of which ultimately determines the consumer's preference for a particular brand. It is important to be able to confidently identify these volatiles during product development, as well as in quality and authenticity studies.

Traditional sample preparation methods, such as headspace and SPME, are widely used, but often limited in terms of sensitivity. We demonstrate the use of high-capacity sorptive extraction with novel trap-based focussing to provide enhanced sensitivity and improved chromatographic performance. This improved performance, coupled with improved separation by GCxGC and highly-sensitive detection by time-of-flight mass spectrometry (TOF MS), gains greater insight into sample composition.

However, sampling, separation and detection is just the beginning – the resulting datasets must then be reduced to discover significant differences and ultimately allow meaningful conclusions to be reached.

Here, we will demonstrate the use of a new chemometrics platform to transform complex data sets into useable results. Firstly, alignment of the raw data is applied, to account for potential retention time drifts. Next, advanced feature discovery identifies key differentiators across sample classes using all of the raw data. This innovative approach ensures that trace peaks are not ignored and enables automated workflows to be adopted, minimising laborious pre-processing steps and accelerating analytical workflows.

We show that this efficient end-to-end workflow is easy to use for the comparison of brand and imitation food products, including crisp flavourings, soft drinks and beer.

Keywords: GCxGC, volatiles, chemometrics, untargeted, mass spectrometry

POSTER

TLC analysis of anthocyanins in fruits of some cultivar varieties and hybrids of Rubus occidentalis

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The fruits of black raspberry (Rubus occidentalis L.) are a rich source of metabolites with antioxidant, anti-inflammatory and anticancer activities [1,2]. These compounds belong to different classes of polyphenols, namely anthocyanins, ellagitannins and flavonoids. According to literature data it fruits of R. occidentalis contains 4-6 times more anthocyanin compounds than fruits of more popular species, namely Rubus idaeus L. [3,4].

The aim of the study was to analyse anthocyanin compounds in the fruits of different cultivar varieties of black raspberry (5) and hybrids of R. occidentalis and R. idaeus (6) using chromatographic methods –TLC and also HPLC.

TLC analysis conditions were optimized based on mobile and stationary phase selection. The best separation was obtained on HPTLC plates coated with silica gel Si60 using ethyl acetate: water: formic acid (100: 30: 30, v/v/v) as mobile phase. A positive effect of lowering the separation temperature on its efficiency was observed. Finally, the HPTLC separations were carried out in a vertical glass chamber, at a temperature of 4°C, over a distance of 70 mm (30 min). HPTLC separation enabled identification of all anthocyanins – mainly derivatives of cyanidin and also pelargonidin, present in fruits of analysed cultivars (6 compounds) and hybrids (7 compounds) against 8 anthocyanin standards. The presence of 3-O-xylosylrutinoside cyanidin and 3-O-glucosylrutinoside cyanidin – compounds characteristic respectively for R. occidentalis and R. idaeus, was confirmed by HPLC-DAD-ESI-MS method. The developed HPTLC method was validated for quantitative analysis. The anthocyanin content was determined using densitometry and videoscanning. Significant differences in concentration of anthocyanins between fruits of cultivar varieties and hybrids of R. occidentalis were showed. The developed HPTLC method is fast, inexpensive and enables the analysis of many samples in a short time. Additionally, it enables the distinction between black and red raspberries on the basis of the obtained TLC chromatographic profiles.

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Keywords: Rubus occidentalis, anthocyanins, TLC

POSTER

Direct determination of water in solid pharmaceutical bulk products by full evaporation headspace gas chromatography with thermal conductivity detection

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Several pharmaceutical substances contain adsorbed water or are hydrates. This water can eventually lead to decreased shelf life, microbial contamination or instability of the product. Therefore, the determination of water present in pharmaceutical products is mandatory. Depending on the substance and the precision required, different methods may be prescribed. The most common one is loss on drying. Although quite simple, this method is not specific and only allows the determination of the total amount of volatile matter. A more specific test for water is the Karl Fischer titration (KFT) involving a redox reaction. In this case, the substance is dissolved in a suitable solvent and then titrated with the KFT reagent. Although methanol is the most commonly used solvent, in some cases other solvents or mixtures may be needed, requiring careful development and validation. Moreover, the presence of oxidizing agents can interfere and some samples are prone to react with components of the titrant.

Considering these limitations and the fact that water can be volatilized quite easily, gas chromatography (GC) has been proposed as an alternative method. Although there exist many different ways to deal with sample introduction, headspace (HS) has become very popular and it has been reported for water analysis already. However, the use of solvents to dissolve the sample is also needed in this case. The dissolution creates a dilution and limits the application of the method when samples do not properly dissolve in the solvent. In addition, the use of large volumes can saturate the HS. So, a solvent-free method would be beneficial. An option is the full evaporation technique (FET), where a small amount of sample is directly weighed in a HS vial.

Hence, in this study, the use of FET-HS-GC for the determination of water in solid samples was explored. This simplifies considerably the method since no diluent has to be searched and HS saturation is avoided. Blank corrections were performed to compensate for atmospheric moisture variation. Moreover, the performance of mass spectrometry (MS) and thermal conductivity detection (TCD) was compared. The method showed excellent figures of merit when working with TCD, such as $R2 > 0.99$ and $RSD < 5\ \%$ for each level of the calibration curve. Eight samples were studied at different equilibration temperatures to find the optimal working conditions for each case and the results were compared to the ones obtained by KFT. Both methods showed restrictions and proper case by case optimization/validation turned out to be necessary. Simple visual observations indicated if the results observed were reliable. Hyphenation with a flame ionization detector in series with the TCD proved to be useful for testing residual solvents in a simultaneous run.

Keywords: gas chromatography, water determination, full evaporation headspace, thermal conductivity detection

POSTER

Gas chromatographic method with minimal sample consumption for quality control of 13C-mixed triglycerides used in clinical diagnosis

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Exocrine pancreatic insufficiency can be comfortably diagnosed by a breath test using the mixed triglyceride 2-octanoyl(1-13C)-1,3 distearoyl glycerol (13C-MTG). However, it is not fully accepted as a routine test yet as no vendor provides a certified product for clinical applications. Current recommended methods for quality control of triglycerides are not compatible with the produced expensive small batches of 13C-MTG since they require a relatively large amount of sample. In addition, existing methods consist of multiple steps and are rather time consuming. In order to enable the use of 13C-MTG in routine clinical work, two aspects must be considered: the compound structure must be confirmed and its composition must be controlled. Moreover, the proposed method should consume as little sample and time as possible. In this study, the use of two simple pretreatments with a single analysis method are proposed for the quality control of 13C-MTG samples. The pretreatments were optimized by applying response surface models.

In the first pretreatment, lipase from Rhizomucor miehei was used as sn-1,3 regiospecific enzyme that catalyzes the esterification of the released fatty acids into butyl esters when the reaction takes place in 1-butanol. This single step enzymatic reaction was combined with a single step extraction, followed by GC-MS analysis. This approach proved to be useful for confirmation of the 13C-MTG structure, consuming only 10 mg of sample.

In a second stage, the use of a boron trifluoride – methanol reaction was optimized, minimizing the use of sample, reagents and time, to determine the sample composition via the quantitation of fatty acids by GC-MS. Hereto, fatty acid methyl esters were produced in a micro scale under mild conditions followed by a single step extraction. The external calibration procedure combined with MS in single ion monitoring mode led to good precision (RSD < 1.4 %), linearity ($R^2 > 0.995$) and accuracy (recovery close to 100 %). Analysis of a sample by this method for the quantitation of caprylic and stearic acid accounted for nearly 100 % of the theoretical value.

In this way, two similar pretreatments with minimal sample consumption and only one GC-MS instrument are needed for the analysis of 13C-MTG samples, largely simplifying and miniaturizing current methods.

Keywords: gas chromatography, mass spectromGas chromatography, mass spectrometry, mixed triglycerides, regiospecific enzymatic reaction

POSTER

Hyphenation of Preparative Liquid Chromatography to Laser-Based Mid-Infrared Spectroscopy for Monitoring of Proteins in Chromatographic Effluents

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Laser-based mid-infrared (IR) spectroscopy is a novel technique for the analysis of aqueous protein solutions. Compared to conventional Fourier-transform IR (FTIR) instrumentation, external cavity-quantum cascade laser (EC-QCLs) based spectrometers offer significant advantages such as larger applicable optical path lengths and higher sensitivity. These advantages open a wide range of possible applications, including monitoring of proteins from complex separation processes.

In this study, a preparative liquid chromatography (LC) system was hyphenated to an EC-QCL based mid-infrared spectrometer. The laser was operated between 1750 and 1350 cm-1 to cover the most important IR regions for protein quantification and secondary structure analysis. Robust spectra acquisition during flow-through operation was assured by the large optical path length (25 µm) of the equipped transmission cell. To demonstrate the high potential of LC-QCL-IR coupling, two different model systems based on ion-exchange chromatography (IEX) and size exclusion chromatography (SEC) were applied. In IEX, a pronounced challenge was caused by the applied sodium chloride gradient which caused undesired mid-IR absorbance bands that overlap with protein bands. Here, a novel background compensation approach, capable of eliminating these bands, was introduced. In this way, high-quality protein spectra were obtained, comparable to reference off-line measurements. In case of SEC, proteins with similar molecular weights, showing co-eluting behaviour under the applied chromatographic conditions, were analyzed. As proteins from overlapping peaks cannot be distinguished with conventional UV detectors, the collected fractions are typically analyzed by laborious and time-consuming off-line methods. In contrast, laser-based mid-IR spectroscopy combined with chemometrics enabled quantification of the individual proteins, based on their difference in secondary structure. The demonstrated advantages of QCL-IR detectors for liquid chromatography reveal high potential for complementing off-line methods and established detectors for monitoring of proteins.

Keywords: mid-infrared spectroscopy, quantum cascade laser, liquid chromatography, proteins, secondary structure

POSTER

Chromium Determination in Leather: Challenges of Extraction and Speciation

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Leather industry plays an essential role in the world's economy; however, it also has a negative environmental impact due to the generation of significant quantities of wastes, some of which are classified as hazardous chemicals. Moreover, hexavalent chromium, a known carcinogenic and mutagenic, can be found in leather products and cause allergic dermatitis or trigger other diseases[1]. For this reason, it is important to quantify the total chromium in these products, as well as the oxidation state in which this element is found due to the fact that trivalent chromium (Cr(III)) is considered an essential nutrient involved in glucose regulation, and hexavalent chromium (Cr(VI)) represents a risk to human health. In aqueous systems, the oxidation state of chromium is determined by redox potential and pH, which poses a difficulty in the speciation of chromium, as the sample preparation protocols can lead to an interconversion between Cr(III) and Cr(VI), thus distorting the results. In addition, there is no current standard describing a method to extract and quantify both species simultaneously[2]. During this work, six pre-treatment procedures were tested, the total chromium was determined by ICP-AES and speciation was studied by ion chromatography coupled to ICP-MS. The best extraction results were obtained with mineralization method (around 1.6% w/w of total Cr in dry leather), but in such conditions the speciation is not preserved. However, EDTA extraction led to very good extraction results (around 1.5% w/w of total Cr in dry leather) and allowed the extraction of Cr(VI) and Cr(III) as a Cr-EDTA complex. Chromium isotopic standards (53Cr(III) (97.01%) and 50Cr(VI) (97.36%)) were used in order to study the possible Cr interconversions resulting from the EDTA extraction conditions and allowed to prove that Cr(III) forms Cr-EDTA complex and Cr(VI) remains free. Moreover, it was proved that this extraction medium almost preserved the speciation, 53Cr(III) was not transformed into 53Cr(VI) and 1.5% of initial 50Cr(VI) was transformed into 50Cr(III) and detected as 50Cr(III)-EDTA complex. With this study, we present a validated extraction procedure for Cr(VI) and Cr(III) in leather samples which allowed a quantitative extraction while maintaining the speciation[3].

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Keywords: chromium, speciation, extraction, ion chromatography, ICP-MS

POSTER

Analysis of Phenolic Compounds using HPLC and GC Methods in Different Types of Balsamic Vinegars

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Phenolic compounds affect the properties of food products and are important antioxidants. Balsamic vinegar is one of the best known and most popular vinegars in the world and is a good source of phenolic compounds. The quality of balsamic vinegars as well as the content of phenolic substances varies depending on the production method. In the present work, we have developed liquid chromatographic method coupled with MS and MS/MS detection for quantitative determination of the content of phenolic compounds in balsamic vinegars. Optimization of MS/MS parameters and separation conditions, i.e. column selection, composition of mobile phase and gradient profile, was performed for 32 standards of phenolic compounds. A total of 14 samples of different types of balsamic vinegar were analyzed with simple sample preparation. The profile of phenolic compounds was completed by semiquantitative analysis of volatile organic compounds using GC-MS and GC-FID methods. VOCs extraction was performed by the HS-SPME method, for which the parameters of extraction temperature, extraction time and volume of saturated NaCl solution were first optimized. The achieved results were compared with already existing works. Gallic acid, protocatechuic acid, caffeic acid and p-coumaric acid have been identified as the major phenolic compounds in balsamic vinegars.

Keywords: balsamic vinegars, phenolic compounds, GC, HPLC

POSTER

Detailed Characterization of Advanced Oxidation Products in Wastewater via Comprehensive Temperature Responsive LCxLC-HRMS

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Many pollutants present in wastewater are chemically persistent and difficult to degrade through conventional processes. The implementation of Advanced Oxidation Processes (AOPs) offers promise therein as it allows degrading organic pollutants in aqueous media through the formation of reactive oxygen species such as the hydroxyl radical swiftly reacting with organic pollutants [1]. It is thereby crucial to identify and quantify the chemicals occurring in wastewater prior to and after such treatments, for optimization of the processes and to allow ascertaining a reduction in toxicity.

In this context, comprehensive chromatographic separation techniques coupled with Mass Spectrometry can offer powerful analytical tools for the identification of the many additionally formed, and a priori unknown, oxidized species. The variety of oxidized chemical compounds can depict a broad range in chemical properties (e.g., molecular weight, atomic and structural composition, polarity, volatility, etc.), requiring the implementation of existing, and the development of novel, comprehensive chromatographic separation platforms. In this work, LCxLC-MS platform is developed to detect, identify, and quantify, the products formed during AOPs. The potential of Thermo-responsive liquid chromatography (TRLC) is explored as the 1st dimension in LCxLC. As TRLC is operated with pure water as a mobile phase, this provides benefits by allowing for enhanced, and almost universal, peak refocussing in the 2nd dimension separation. This can lead to better peak capacities and overall improved detection sensitivities [2]. The method is tested with carbamazepine as a representative solute. Finally, the novel platform is tested with genuine wastewater samples before and after AOP based treatment.

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Keywords: comprehensive LC, AOPs, degradation products, HRMS

POSTER

Thermo-responsive Liquid Chromatography as a tool for gradient analysis with Refractive Index Detector

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Despite the huge potential of Refractive Index Detectors (RID), their use is limited in HPLC (High-Performance Liquid Chromatography) because of the sensitivity to solvent and temperature changes. RID responds to any change in refractive index comparing it with a reference cell filled with the mobile phase. The main advantage is that it is reasonably universal because the refractive index is a bulk property of a substance, therefore, any analyte can be detected, which is not the case with other detectors such as UV that requires molecules containing chromophores, or Mass Spectrometers that requires ionizable molecules [1]. The problem arises by the fact that the refractive index is dependent on the density of a substance, therefore, a change in temperature or solvent composition that led to density changes causes instability in the signal. This limits the current use of the detector to only isocratic analysis.

Thermo-responsive stationary phases show promise to mitigate this issue. Thermo-responsive polymers are water-soluble smart polymers, that change their conformation when exposed to an external stimulus, in this case, temperature. The responsive polymers used in this work depict a Lower Critical Solution Temperature behaviour. In this case, at high temperature the polymer dehydrates from water (because monomer-monomer forces are dominant while at low temperature the polymer chains interact with water molecule and a unique hydrophilic phase is formed. This offers a change in chromatographic retention with temperature when the polymer is coupled to silica packed into an HPLC column. This allows for tuning of the selectivity and retention of the analytes passing through the column. Thanks to the use of pure water as the mobile phase and the possibility to operate a temperature gradient this allows obtaining comparable results to solvent gradients in terms of peak selection and reduced elution time. This approach offers a promising alternative for the combination of gradients analyses with RID [2].

In this work, thermo-responsive HPLC is used to demonstrate the possibilities to perform gradient analysis using RID with non-UV detectable molecules. A study on baseline stability is conducted to illustrate that temperature gradients can effectively be used because it is possible to stabilize the baseline drift against temperature changes via post-column thermostatization. The approach is demonstrated with a representative mixture of free fatty acids and primary alcohols on PNIPAAm (Poly(N-isopropylacrylamide)) and PDEAAm (Poly(N,N-diethyl acrylamide)) type TRLC columns.

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Keywords: refractive index, temperature gradient, thermo-responsive

POSTER

The analysis of cannabinoids in e-cigarettes liquids using LC-HR-MS and LC-UV

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The use of cannabidiol or CBD products has skyrocketed the last five years due to the alleged therapeutic benefits, along with a low potential for abuse and lack of the typical psychoactive effects associated with the use of cannabis products containing high levels of tetrahydrocannabinol (THC). The use of CBD in e-liquids has come under scrutiny mid-2019, due the outbreak in the USA of e-cigarette, or vaping, product use-associated lung injury (EVALI) that was linked to the use of CBD and THC containing e-liquids. The current hypothesis states that vitamin E acetate, present in the CBD liquids of the victims, is one of the main causes of EVALI as this chemical is present in the lungs of almost all EVALI patients [1]. In Belgium only CBD-containing e-liquids with a total THC content lower than 0.2% are legal, provided that they do not contain vitamin E acetate. In order to verify if the different CBD-containing e-cigarette liquids, that are available to the Belgian population, are indeed legal, a method was developed to screen for the presence of vitamin E acetate and to screen and quantify accurately the major cannabinoids CBD, CBD-A, Δ9-THC and Δ9-THC-A. Therefore it is pivotal that such methodology enables also the separation and distinction of the abovementioned major cannabinoids from the more minor cannabinoids that could also be present in these e-liquids. Moreover, as a recent study demonstrated that a large fraction of CBD can be transformed into other chemical components upon heating, including THC [2]. In order, to avoid any method induced artefact, liquid chromatographic separation conditions were preferred to the classical gas chromatographic conditions which require a heating of the e-liquid. Our developed methodological screening strategy, employing liquid chromatography coupled to high resolution accurate mass spectrometry, consisted of isocratic screening methodology for cannabinoids and a separate screening method for vitamin E acetate. Next, a downstream quantification methodology for CBD, CBD-A, Δ9-THC and Δ9-THC-A by UHPLC-DAD method was developed and successfully validated using the 'total error' approach, utilising accuracy profiles. The obtained accuracy profiles show that the β-expectation tolerance intervals did not exceed the acceptance limits of ± 10%, meaning that 95% of future measurements will be included in the [-10%, 10%] bias limits. Next, 20 real-life samples acquired on the Belgium market were investigated, illustrating that the proposed methodologies and strategy can be used in routine analysis for the quality control of e-liquids.

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Keywords: cannabinoïds, e-cigarettes, high resolution accurate mass spectrometry, liquid chromatography

POSTER

Comprehensive two-dimensional liquid chromatography with high-resolution mass spectrometry as a generic method to study the degradation of environmentally relevant micropollutants

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The contamination of natural water resources with emerging pollutants is of growing interest since potentially harmful chemicals, such as pharmaceuticals, pesticides and surfactants, among others, can enter the environment through wastewater streams [1]. Current advanced oxidation processes (AOPs) are inefficient to fully remove those contaminants and often lead to a complex sample containing multiple degradation products showing a wide range of physicochemical properties [2]. To evaluate the presence, degradation and removal of several contaminants, liquid chromatography coupled to high-resolution mass spectrometry is usually chosen. However, due to the high complexity of the matrices, adequate chromatographic resolution is often challenging to achieve under a single set of separation conditions, leading to matrix effects in the MS interface and more ambiguous structure confirmation [3-4]. To drastically enhance separation performance, a comprehensive two-dimensional liquid chromatography system (LC × LC) coupled to high-resolution tandem quadrupole time-of-flight (QTOF) mass spectrometry was developed and applied to screen environmental contaminants in complex matrices. The system was optimized using standard compound mixtures of pharmaceuticals and pesticides, to characterize chromatographic separation, selectivity and orthogonality. Several stationary phases were evaluated in terms of peak capacity and ease of hyphenation with mass spectrometry. A combination of reversed-phase in both dimensions (RP × RP) was found to improve chromatographic resolution. System selectivity was eventually increased by using different mobile phase conditions. The hyphenation of LC × LC with QTOF MS was optimized for untargeted analysis. Finally, the developed method was applied to tentatively identify degradation products formed during advanced oxidation processes of contaminated water samples.

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Keywords: Comprehensive two-dimensional liquid chromatography, high resolution mass spectrometry, emerging pollutants analysis

POSTER

Advanced analytical tools based on GC×GC-MS for the characterization of biomass and biofuel samples

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Biomass feedstocks, mainly composed of lipids (oleaginous) and carbohydrates (lignocellulosic), are among promising candidates to possibly fulfill requirements as substitutes of crude oils as primary sources of chemical energy feedstock. In the light of this consideration, a detailed characterization of major and minor biomass and biofuel components is of primary importance. As an example, amongst others, heteroatom-containing compounds can be a cause of the corrosion, thus limiting and/or deactivating catalytic processes needed to transform the biomass into fuel.

The use of advanced gas chromatography techniques, in particular, multidimensional gas chromatography (MDGC) coupled to mass spectrometry (MS), has been widely exploited in the field of petroleomics over the past 30 years and has also been successfully applied to the characterization of volatile and semi-volatile compounds during the processing of biomass feedstock. In this context, different analytical strategies and analytical platforms for biomass and biofuels characterization, using mainly comprehensive 2D-GC (GC×GC) coupled with TOF-MS are employed, together with the application of liquid chromatography (LC) as a pre-separation step before the GC analysis for minor component characterization.

Keywords: biomass feedstock, comprehensive 2D-GC, lipid minor components, bio-oil, oxygen-containing compounds

POSTER

Ion Mobility Spectrometry as an Additional Separation Dimension for the Screening of Contaminants of Emerging Concern (CECs) – Database Compilation and Application to Plastics Samples

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Ion mobility mass spectrometry (IM-MS) derived collision cross section (CCS) values can serve as a valuable additional identification parameter within screening analyses of compounds of emerging concern (CECs), such as alternative plasticizers, organophosphate flame retardants, perfluoroalkyl chemicals and others. However, their application for compound identification requires the availability of reference databases of CCS values which can be matched with experimental data. The unavailability of database values for many CECs and limited information about their use on different instrumental set-ups hampers compound identification in environmental samples.

To fill this knowledge gap, reference DTCCSN2 values of more than 150 CECs and their metabolites were acquired[1]. Comprehensive quality assurance guidelines were implemented in the workflow of acquiring the DTCCSN2 values to ensure reproducible experimental conditions. For >110 compounds, more than one ion (i.e. adducts) is reported yielding a database containing DTCCSN2 values for >320 ions. The obtained dataset allows the characterization of trends observed for the CCS-m/z relationship of the investigated classes of CECs and can serve as a valuable tool in suspect target studies on CECs. Additionally, IM-MS allowed the separation of several commonly occurring isomers of some organophosphate flame retardants, plasticizers and their metabolites. To assess the use of the complied database on different IM-MS instrumental set-ups, a sub-selection of 56 CECs was acquired using travelling wave (TW) IM-MS and observed deviations between DTCCSN2 andTWCCSN2 were investigated. The main aim was to assess the comparability of CCS values obtained by different instrumental set-ups and – based on the observed relative errors – to evaluate whether databases containing CCS values of CECs can be used independent of the instrument they were acquired on.

Ultimately, plastics samples covering several commonly used materials were analyzed applying a suspect screening approach using liquid chromatography coupled to IM-MS. The obtained IM-MS data was matched against the developed DTCCSN2 database as well as previously published CCS databases aiming to characterize additives present in plastics which might be missed by commonly used target methods. Additionally, this approach aimed to access the added value of an additional separation dimension for compound identification within suspect screening studies.

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Keywords: ion-mobility separation, emerging contaminants

POSTER

Identification of novel halogenated naturally occurring compounds in sea sponge by high-resolution mass spectrometry and combined screening approaches

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Marine sponges are a rich source of bioactive naturally occurring halogenated compounds (NHCs) such as bromophenols (BPs), bromoanisoles (BAs) and hydroxylated or methoxylated analogues of polybrominated diphenyl ethers (HO-PBDEs, MeO-PBDEs) and bromobiphenyls (HO-BBs, MeO-BBs). The presented study used liquid chromatography high-resolution mass spectrometry to identify new hydroxylated NHCs which might be missed by commonly applied target gas chromatographic methods. Sea sponge samples of Lamellodysidea sp. and Callyspongia sp. were analyzed using a combined approach which included suspect and non-target screening methodologies.[1] For suspect screening, a suspect list was developed containing BPs, HO-PBDEs, MeO-PBDEs, HO-BBs and MeO-BBs with different levels of halogenation. The suspect screening results confirmed the findings of previous studies conducted on sea sponge samples of Lamellodysidea sp. and Callyspongia sp. Additionally, in Lamellodysidea sp. and Callyspongia sp., 13 and 4 newly identified NHCs are reported, respectively, including heptabrominated diOH-BDE, monochlorinated pentabrominated diOH-BDE, hexabrominated OH-MeO-BDE and others. The fragmentation spectra of the newly identified compounds provided additional information about the positions of the halogens and the hydroxy groups allowing to report most of the compounds with confidence level 3 according to Schymanski et al.[2]

Through non-target screening, 31 and 20 polyhalogenated compounds were identified in Lamellodysidea sp. and Callyspongia sp. samples, respectively. Based on the obtained fragmentation spectra, polybrominated dihydroxylated diphenoxybenzenes (diOH-PBDPB) could be identified. These included hepta-, octa- and nonabrominated diOH-BDPBs which were detected in both sponge species. To our knowledge, this study is the first report on the environmental presence of OH-PBDPBs.

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Keywords: naturally occurring halogenated compounds, non-target screening, high-resolution mass spectrometry

POSTER

Optimization of a sample preparation method for analysis of lipids in plasma using GC×GC-TOF-MS

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Lipidomics, or the comprehensive analysis of lipids, is rapidly expanding and providing critical information to the field of biosciences. Lipids are structurally complex molecules that serve a wide range of biological functions[1]. Therefore, the study of lipid compositions of biological specimens can possibly help to analyze and to understand their related influences in various pathophysiological conditions. When considering lipidomics, liquid chromatography (LC) mass spectrometry (MS)-based techniques are logically most often used[2]. Enhanced separation capacities and lower limits of detection are however still needed but challenging to be achieved in LC-MS(/MS). Chemical derivatization has the potential to make at least some families of lipids more 'gas chromatography (GC)-amenable', allowing GC-MS to also be considered[3].

The present paper reports on the development of a GC-specific sample preparation method dedicated to measurements by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOF-MS) to determine free fatty acids and fatty acids bound in sources such as triglycerides in plasma.

For GC separation, the fatty acid components of lipids have to be converted into more volatile derivatives, usually methyl esters, by chemical derivatization. Thus, a two-step sample extraction and derivatization, base-catalyzed transesterification and acid-catalyzed esterification approach was developed to improve measurement efficiency and obtain distinguishable structured chromatographic separation for the different saturated and unsaturated fatty acids in plasma. Moreover, predictable shifts in retention times were also observed because of the positioning of the double bond and its number in the chemical structure. Furthermore, specific care was taken in optimizing factors like time and temperature at each stage of the extraction. A standard mixture with unsaturated fatty acids having double bonds up to six, a pooled human plasma, and a NIST standard (SRM 1950) were analyzed with the optimized method, demonstrating the potential value of the proposed approach to complement classical LC-MS approaches.

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Keywords: GCxGC-TOF-MS, Lipidomics

POSTER

Mapping O-GlcNAcylation sites of tau with IP-LC-MSMS methodology to develop a new biomarker assay for O-GlcNAc hydrolase inhibition

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In Alzheimer's disease (AD) brain, tau is found hyperphosphorylated and aggregated in paired helical filaments, a histopathological hallmark of the disease1. O-β-linked N-acetylglucosaminylation (O-GlcNAcylation), regulated by two antagonist enzymes O-GlcNAc transferase (OGT) and O-GlcNAc hydrolase (OGA), modulates Tau phosphorylation and slows down its aggregation in vitro2. Furthermore, pharmacological increase of O-GlcNAcylation upon treatment with inhibitors of OGA such as Thiamet-G leads to neuroprotective effects in AD mouse models3, thereby constituting a potential strategy to tackle neurodegenerative diseases4.

Tau O-GlcNAcylation in cerebrospinal fluid (CSF) could thus serve as a biomarker proxy of central brain OGA inhibition in future preclinical and clinical studies. However, due to the low abundance of tau in CSF (three orders of magnitude lower than in the brain parenchyma)5 and because 99.9% of CSF tau corresponds to N-terminal fragments cleaved between amino acid residues 222 and 225 (based on isoform 2N4R)5, the use of the main described tau C-terminal S4003 O-GlcNAcylation site will be extremely challenging. The goal of the current study was thus to identify new tau O-GlcNAcylation sites in the development of a CNS OGA inhibition biomarker in human.

Using a total tau antibody (Ab), both full-length tau and fragments were immunoprecipitated from tau P301S transgenic mouse brain homogenates (BH), digested with trypsin and analyzed by UHPLC-MSMS (6500+, Sciex). Assay development to determine tau S400 O-GlcNAcylation levels as positive control, demonstrated an average 12-fold increase in tau S400 O-GlcNAcylation in the brain of Thiamet-G treated mice. More importantly, nanoLC-HRMS (Q Exactive HF, Thermo Scientific) analysis of O-GlcNAcylated peptides originating from recombinant O-GlcNAcylated human tau digested with multiple enzymes (trypsin, asp-N and glu-C) allowed us to gather high-quality LC-MSMS data, to be used as reference during identification of low concentration O-GlcNAc-peptides in mouse BH extracts. In an effort to mitigate matrix effects from highly abundant unmodified peptides in tryptic digests, and easy loss of O-GlcNAc-moieties by in-source fragmentation, a selective clean-up by chemo-enzymatic derivatization of O-GlcNAc peptides was optimized: nanoLC-HRMS analysis rendered confirmation of the previously identified O-GlcNAc-sites in recombinant O-GlcNAcylated human tau. However, due to considerable losses during sample preparation, it was decided to analyze mouse BH digests without additional purification. NanoLC-QQQMS analysis (6500+, Sciex) revealed the presence of the three N-terminal and mid-domain O-GlcNAc-sites previously identified in recombinant O-GlcNAcylated human tau, reported here for the first time in mouse BH. Three low abundant tau O-GlcNAc-sites located in the N-terminal and mid domain of tau were identified, namely S208, S191 and a third O-GlcNAc-site at either S184 or S185, in line with previously described in vitro studies2.

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Keywords: tau O-GlcNAcylation, IP-LC-MSMS, Alzheimer's disease, biomarker discovery

POSTER

Segmentation-based natural cubic splines and k-nearest neighbours for automated chromatogram smoothening

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Noise, arising from physical sources such as thermal fluctuations or environmental causes such as small temperature changes or building vibrations, is inevitable in chromatograms. Excessive noise hampers subsequent data analysis, whereby derivative-based peak detection algorithms will suffer the most, thereby hindering the accurate detection and quantification of analytes. Currently applied smoothing methods such as the Savitzky-Golay filtering rely on input of the operator for the choice of appropriate parameter values, which makes it inconvenient for automation. It can furthermore suffer from significant information loss and inaccuracies in some cases.

In this work, two machine-learning related methodologies, namely natural cubic splines, and k-nearest neighbours, are applied as smoothing algorithms [1, 2]. Both methods smoothen the noisy chromatogram by modelling the experimental chromatogram, thereby retaining as much of the desired information as possible and removing the noise. The natural cubic splines method divides the chromatogram into equal sized segments known as knots and approximates these as a set of third-degree polynomials where the second derivative at the endpoint of each knot is set to zero to ensure smoothness throughout the modelled curve. K-nearest neighbours models the underlying chromatogram by averaging the noisy curve out using the k nearest neighbours of each datapoint. In this work, the algorithms are applied on segmented chromatograms, whereby a chromatogram is divided into peak and inter-peak areas. Optimal model parameters (number of knots/ number of neighbours) are determined on each segment using the Durbin-Watson criterion [3, 4]. Finally, smoothness between consecutive segments is enforced using polynomial regression linking the last datapoints of one segment with the first datapoints of the following segment.

It is shown that both methods perform significantly better than the Savitzky-Golay filtering on both simulated and experimental chromatography data. Both approaches are simple, fast, and fully automated smoothing methods as they do not require any input of the operator.

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Keywords: Machine learning, HPLC, smoothening, denoising

POSTER

Parallel artificial liquid membrane extraction of organophosphorus nerve agent degradation products from environmental and biological samples

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Alkyl methylphosphonic acids (AMPAs) are the hydrolysis products of the main nerve agents (soman, sarin, cyclohexyl-sarin, VX and Russian-VX) and they are important environmental and biological markers of used of theses nerve agents. Their analysis is still challenging because of their very polar (log P ranging from -0.8 to 0.8) and acidic character. An emerging miniaturized high-throughput microextraction technique named "Parallel artificial liquid membrane extraction (PALME)" [1] coupled with liquid chromatography–tandem mass spectrometry was, for the first time, investigated for the extraction and enrichment of AMPAs from environmental waters and urine samples. The effect of various extraction parameters on enrichment factors (nature of the membrane, the extraction solvent, the pH values of both donor and acceptor phases, agitation speed and extraction time) was studied in pure media (spiked deionized water samples). The impact of temperature and ionic strength on extraction efficiency were also investigated for the first time.

The developed method led to an almost exhaustive extraction for 4 of the 5 targeted AMPAs from pure water and to high enrichment factor (42.4 for the less polar compound). The performance of this method in terms of efficiency and repeatability was evaluated. The LOQs (S/N≥10) were in the range of 0.009–1.141 ng mL-1, linearity above 0.9973 for all the AMPAs and with RSD values below 11%. This method was also applied to river and waste waters. No matrix effect was observed and the LOQs were in the range of 0.011 to 1.210 ng mL-1. The major limitation of PALME regarding the low enrichment factors was overcome in this study thanks to the selection of new type of donor plates (deep well) than the ones commonly used in PALME. A detailed comparison between the results previously obtained with hollow fiber liquid-phase microextraction (HF-LPME) [2][3] and PALME for the extraction of AMPAs demonstrated the ease of transfer of method from HF-LPME to PALME which could lead to an easier development of PALME for many compounds from the large amount of HF-LMPE data available in the literature. Moreover, the developed method was improved to be more environmentally friendly and "green" by using the deep eutectic solvents (DES) for the first time in PALME as an alternative to the toxic and volatile organic solvents. The study of the potential of this method for urine is still in progress, but the results already obtained are promising and will also be presented.

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Keywords: Sample prepration, chemical warfare agent degradation products, LC-MS/MS analysis, environmental and biological samples, Deep Eutectic Solvents (DES)

POSTER

Innovative approach for the analytical characterization of monoclonal antibodies based on affinity chromatography

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Over the past decades, biopharmaceuticals, and among them, monoclonal antibodies (mAbs), have emerged as an important class of therapeutic proteins and have become key treatments. Their main areas of application are within the field of oncology, as well as infectious and immune diseases. MAbs are complex macromolecules that are produced in biological systems using recombinant DNA technology. As result of their complex manufacturing process and throughout the lifecycle up to the formulated product, antibody-based drugs are prone to several enzymatic and chemical post-translational modifications (e.g. oxidation, deamidation, glycosylation, glycation, fragmentation). Therefore, proper analytical methods are required to determine their structures and understand their behavior.

Numerous chromatographic approaches are available for the analytical characterization of mAbs. In addition to physiochemical characterization of mAbs, there are methods such as affinity chromatography that enable a more functional characterization, providing information on the antibody-dependent cellular cytotoxicity (ADCC) and residence time in the body. It involves interaction of the biomolecule with a ligand covalently immobilized to a solid stationary phase. Different types of affinity chromatography ligands are available, allowing to potentially target different post-translational modifications.

In this work, various innovative analytical methods were developed for FcRn and FcγRIIIa affinity columns. First, a generic method was developed on each column for different types of mAbs. In order to be fully MS-compatible, the nature of the selected mobile phases was carefully examined. Subsequently, a strategy involving the use of multiple isocratic steps to improve the resolution of very closely eluting peaks was implemented to exploit the "on-off" type elution behavior of mAbs in affinity chromatography. Finally, this whole strategy provides a complete panel of analytical methods that can be implemented according to the requirements and the analytical constraints.

Keywords: Affinity chromatography, FcRn, FcγRIIIa, Monoclonal antibodies

POSTER

Profile of Thermal Gradient of FFTGGC

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The hyper-fast Flow Field Thermal Gradient GC (FF-TG-GC) [1] adds a spatial thermal gradient as additional parameter for the separation of volatile components. This thermal gradient is a controlled variation of the temperature over the length of a chromatographic column. The separation column is resistance-heated whereas a variable air flow generated by a fan cools the column creating the thermal gradient. In combination with a temperature program the variation of the column temperature in time (temporal gradient) is possible.

Knowledge about the actual profile of the thermal gradient is useful for simulations of chromatographic phenomena, such as retention time or separation of analytes [2]. Measurements with external infrared temperature sensors placed along the lengths of the GC column where used to measure the real profile of the thermal gradient. The intensity of the fan and initial temperature where varied. The data is used to create 3D-maps to show the influence of the varied values. A model was found that that can used to predict the gradient profile by different configurations. Simulations using the thermal gradient model are compared to measurements for verification.

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Keywords: Thermal Gradient GC, Numerical Simulation, Method Development

POSTER

Trial-and-Error vs. QbD – A Story about Mitigation in Method Development from Pharmaceutical Industries

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A LC-UV test method for assay and related substances has been developed by one of the CMO's for a project in late stage. During the feasibility test in an internal stability lab, it was found that retention time shifting and peak width broadening started after dozens of injections. This brought us concerns of impact on the robustness and correctness of results of future stability and commercial analysis.

By urgent investigation, it was further found that the poor robustness was due to the incompatibility between the column and other chromatographic parameters. A test with columns of several lots has been set-up. After 500 consecutive injections, the peak width broadening stopped whereas retention time shifting remained in a seemingly random way to both directions.

Per retrospective review into the developmental report, the method has been developed in a trial-and-error way. The initial chromatographic parameters were derived from the compendial method followed by tuning of gradient and column temperature. With the tuned gradient and temperature, method development came back to mobile phase optimization and column selection. The column was selected because of relative better separation and lower column pressure.

As mitigation action, our team in the Higi site started urgent method development on a QbD platform with Fusion software. Two waves of experiments were designed: general screening on column and pH in aqueous mobile phase as Wave 1, and further optimization on column temperature and gradient as Wave 2. The automatic executing of designed experiments has been completed within 3 days. A draft test method on a more robust column was obtained as outcome. After some efforts in fine-tuning, a final test method was confirmed and validated for the coming stability studies.

This story demonstrates many challenges in the daily method development in pharmaceutical industries: difference strategies, practice and capability in CMO/ECL's, time pressure on robust method development, etc. Using a QbD approach by deploying a method screening platform such as Fusion can deliver reliable methods with designed quality in a short lead time, which is proven a solution for these challenges.

Keywords: fusion, automation, data science, method development, industry

POSTER

Selecting optimal scouting runs for retention modelling in reversed phase liquid chromatography

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Many applications in modern liquid chromatography, such as the optimization of resolution in method development, the validation of the robustness of a method, and the unambiguous identification of an analyte, rely on accurate retention time predictions. For this purpose, empirical models relating the retention factor of an analyte to an independent experimental variable, such as the fraction of strong eluting solvent in the mobile phase, are often used. Frequently used models in liquid chromatography are the linear solvent strength (LSS) model [1] and the Neue-Kuss (NK) model [2]. To obtain an adequate retention model for a compound of interest, a number of experimental input or scouting runs, that can be executed in isocratic mode and/or gradient elution mode, are required. The minimum number of scouting runs is typically equal to the number of fitting parameters in the model (i.e., two for the LSS model, three for the NK model). The selection of scouting runs can have a large impact on the resulting prediction accuracy, whereby the selection of scouting runs leading to the highest prediction accuracy moreover seems to be analyte dependent. When complex mixtures containing multiple analytes are evaluated, the selection of scouting runs for an adequate retention time prediction of all compounds becomes even more challenging.

In this study, the effect of different sets of scouting runs on the obtained retention time prediction is evaluated. More specifically, the retention time prediction accuracy in isocratic and gradient elution mode is examined when experimental isocratic and/or gradient retention data are modelled to the NK model. It is investigated how well the obtained retention models obtained from isocratic and gradient data transfer to retention predictions in isocratic and gradient elution modes, in all possible combinations. It is demonstrated that the selection of the initial scouting runs is very important to obtain a satisfactory prediction accuracy, and that this selection depends on the retention properties of the compound under consideration. This implies that there is no 'universal' set of initial scouting runs that can be applied to any compound under any set of separation conditions.

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Keywords: retention modelling

POSTER

Analysis of polybutadiene polydispersity by ion mobility coupled with a time of flight mass spectrometry

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Polydispersity and chain end identification are key parameters used to characterize polymeric compounds. A well-known technique to retrieve this information is mass spectrometry classically coupled to matrix assisted laser desorption Ionization (MALDI). Other ionization sources such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are also being considered for the transfer of polymers from solution to the MS instrument. Nevertheless, the analysis of polybutadiene by mass spectrometry is challenging because this polymer has a low ionization capacity related to its electroneutrality, and it is poorly soluble in aqueous solvent, such as methanol or acetonitrile, commonly used for ESI and APCI. Here, we propose a method for characterizing the chain length and the chain ends of polybutadiene by direct infusion onto an ion mobility mass spectrometer.

The study is conducted on a timsTOF Pro from Bruker (Bremen, Germany). This is a trapped ion mobility spectrometer (tims) coupled to high resolution time-of-flight (ToF) mass analyser. The tims cell is capable of separating and accumulating ions according to their mobility in gas-phase (here, in N2). This mobility (k0) is related to the projection of the conformation (mass, size and shape) of the polymer in gas phase and therefore to the three-dimensional structural information of the polymers. The analysis of the high resolution mass spectra for every separated conformation gives access to additional structural information such as the polymer length and the nature of its chain ends.

Two ion sources, ESI and APCI, are being tested and optimized to improve the polymer signal. However, as polybutadiene is poorly ionized, a doping agent has been added to the solution. To promote the ionization, lithium has good properties to bind to polybutadiene. Two lithium salts have been tested to cationize the polymer: lithium chloride (LiCl) and lithium lactate (CH3CH(OH)COOLi). The ratio between the amount of polybutadiene and lithium salts has been optimized to increase the polymer signal while avoiding multiple doping agent binding which would complicate the data interpretation. A stronger signal has been observed with lithium lactate which has been selected for analysis at a ratio of 1:5 (Polybutadiene:Lithium lactate, concentration ratio). The ionization is optimized for low polar molecules on an ESI source set to positive mode and the mass spectrometer operating between 50 to 2000m/z in full scan mode. Trapped ion mobility scanning parameters have been optimized to improve separation in high purity dry nitrogen in the interest scan range between 0.8 and 2.0 V.s/cm² expressed in 1/k0.

Kendrick mass defect filtering has been used to process the data and extract the polybutadiene signal. Families can thus be distinguished by the unit of repetition of the monomer (used as Kendrick mass reference), by the state of oxidation and by the polymer length to polybutadiene. The interplay of ion mobility mass spectrometry and Kendrick analysis allow rapid characterization of the polybutadiene present in the solution using a direct infusion method.

Keywords: Polybutadiene, polydispersity, timsTOF, lithium, Kendrick

POSTER

Monitoring the effect of BACE inhibition on beta-galactoside alpha-2,6-sialyltransferase activity in hepatocytes by sialic acid analysis

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β-site amyloid precursor protein cleaving enzyme (BACE) is a membrane bound aspartic protease, which is expressed in brain and in lower levels in peripheral tissue e.g. liver tissue. β-Galactoside α2,6-sialyltransferase (ST6Gal I), a membrane-bound protein which is highly expressed in the liver, is mainly cleaved by BACE1 and catalyses α2,6-sialylation of Gal β 1,4-GlcNAc structures on N-glycans. Inhibition of BACE1 may disrupt detoxification by ST6Gal1, resulting in reduced levels of α2,6-sialylation of soluble glycoproteins, and potentially inducing liver injury.

Here, protein α2,6-sialylation was measured in hepatocytes using enzymatic glycan removal with PNGase F, followed by procainamide labelling and chromatographic separation of labelled N-glycans by hydrophilic interaction liquid chromatography (HILIC) in combination with high resolution Q-TOF mass spectrometry. Cell pellets contained mainly α2,6-sialylated glycan A2G2S2 and in lesser amount α2,6-sialylated A2G2S1, while in the supernatants the reverse was noticed. Glycan mapping of hepatocytes incubated with stable isotopically labelled 13C6-N-acetyl neuraminic acid (13C6-NANA) revealed de novo α2,6-sialylation. 13C6-NANA was mainly incorporated in the cell pellets of hepatocytes, mainly on A2G2S2, and its level increased with increased incubation time.

Incubation of 13C6-NANA with and without BACE inhibitors showed that both Verebecestat ($p< 0.01$) and Atabecestat ($p<0.001$) inhibit the α2,6-sialylation on A2G2S2 present in the cell pellets of hepatocytes. Also, chelerythrine, known as an inhibitor of protein kinase C which blocks ST6Gal1 induced sialylation, inhibits α2,6-sialylation of A2G2S2.

In this study, we have demonstrated that hepatocyte incubation with 13C6-NANA allows to selectively and sensitively detect de novo α2,6-sialylation of hepatic glycoproteins. We have also demonstrated that BACE inhibition reduces the levels of α2,6-sialylation of glycoproteins present in the hepatocyte cell pellets.

Keywords: sialic acid, BACE inhibition, beta-galactoside alpha-2, 6-sialyltransferase 1, liquid chromatography-mass spectrometry

POSTER

Simultaneous multiple SPME fibers sampling to extract sample full potential

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In omics research setting, access to sample is usually a key factor of the experimental design. In some cases, samples can be abundant and readily available. However, in the case of biological matrices, samples can be difficult to obtain and the chemical integrity difficult to maintain. In the context of microbiome research, stool samples are difficult to obtain, difficult to homogenize, difficult to store [1,2]. Unstable samples make difficult potential combinations of different analytical techniques without introducing bias. For example, when solid-phase micro extraction fiber (SPME) is employed for VOCs analysis, only one extraction is possible. If something goes wrong, the entire sample is lost.

In this study, we evaluate simultaneous multiple SPME fibers sampling. Using three fibers simultaneously, we generated three technical replicates from one biological sample. Each fiber was then analyzed by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) in separated runs. The robustness of the procedure was tested on a 24-standards mixture, human whole stool matrix, and human whole stool samples from the National Institute of Standards and Technology (NIST). In addition, we studied two fiber storage conditions: storage at room temperature and at -20°C. We also used this procedure to enhance the detection and the identification of compounds by injecting fibers from a unique sample on different instruments. We combined the sensitivity of the PegasusTM BT 4D Cryo modulator (LECO Corporation) and the power of the high-resolution detector with the PegasusTM 4D HRT (LECO Corporation) both set up with the same column set (non-polar phase in the first dimension and the mid polar phase in the second dimension).

For the storage condition, the RSD mean value based on the area of 18 of the 24 standards is 38.4 % for the storage of fibers at room temperature and 19.8 % for the storage at -20°C. The cold temperature storage allows decreasing the RSD mean value by a factor of two. On human whole stool matrix, we target two fecal biomarkers, the indole and the benzaldehyde in order to compare the two instruments. In the limit of detection side, the mean area values for those two compounds on the PegasusTM BT 4D are 100 times higher than the PegasusTM 4D HRT. The PegasusTM BT 4D detects more than 11 times more compounds than the PegasusTM 4D HRT. However, the high resolution of the PegasusTM 4D HRT system offers stronger mass accuracy and enable a more robust compound identification. When dealing with complex-biological matrices, the ability to combine low limit of detection on one instrument and high MS resolution on a second one represents a large added value.

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Keywords: GCxGC-TOFMS, Microbiome, SPME

POSTER

A new GPC-HPLC-UV/VIS method for fast and detailed mapping of complete lignin depolymerisation product pools

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In recent years, lignocellulosic biomass, consisting of cellulose, hemicellulose and lignin, has been recognized as the cheapest and most abundantly available inedible biomass, which could serve as the most scalable and economically viable bio-source for production of biofuels and high-value chemicals. [1-4] However, currently, lignin, the 'cement' between cellulose and hemicellulose, is largely used as a low-value fuel due to its heterogeneity, structural complexity and low reactivity. [5] Research towards lignin depolymerisation into functionalised bio-aromatics, i.e., sustainable building blocks for polymers, is highly promising [3,4] but screening of catalysts and reaction conditions is constrained by the lack of dedicated analytical techniques, capable of fast and detailed mapping of the entire product pool. Set technique needs to separate the complex product pool according to a wide range of molecular weights and functionalities, related to depolymerisation degree and reaction selectivities respectively, as these are the most determining characteristics for valorisation of the products. Moreover, reaction screening requires fast, global and preferably graphical comparison of a large number of samples. Within this work, a multiple heart-cutting (mLCxLC) coupling of gel permeation chromatography (GPC) and reversed-phase LC (GPC-HPLC-UV/VIS) setup has been developed to fill the analytical gap. Analysis of monomeric and dimeric lignin model compounds with the GPC-HPLC-UV/VIS method demonstrated a unique effect of hydroxyl groups, the most important functionality for valorisation, on the retention times in both dimensions, i.e., a reduction of both 1tR and 2tR. Analysis of 4 different depolymerisation pools confirms their inherent complexity wherein peak density increases exponentially with increasing hydrodynamic volume, i.e., decreasing 1tR, which results in a zone with low peak density and one with high peak density. The occurrence and intensity of peaks in the former and the overall size and position of the latter can be used to identify differences in depolymerisation degree and reaction selectivities between the samples. These results validate the capability of the setup as a tool for concise compositional comparison of monomers, dimers and higher molecular weight products, with regards to molecular weight and functionalities, especially OH-groups, which is vital for the screening stages in catalyst development and optimization of reaction conditions. The technique was developed as an offline setup but the interface between the dimensions will be automated. Additionally, the developed setup, and its balance between analysis speed and analysis detail, can be adapted to fit other applications, serving as a platform setup for analysis of a plethora of complex mixtures, by, e.g., using mass spectrometry instead of UV/VIS at later screening stages, when the number of samples is lower, to increase analysis detail at the cost of a reduced analysis speed.

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Keywords: analytical methods, two dimensional liquid chromatography, lignin depolymerization, bio-aromatics, catalysts development

POSTER

On-line LC x LC-HRMS for obtaining predictive data in the suspected analysis of organic micropollutants and transformation products in environmental samples

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As the consumption of chemicals continues to increase, questions about the impact of these products on health and the environment are frequently raised. It is therefore important to develop reliable analytical methods to detect these compounds in environmental samples (water and soil) to ensure that environmental standards are met and that forbidden products are no longer used.

Water samples are usually analyzed by suspect screening by coupling ultra-high performance liquid chromatography and high-resolution mass spectrometry (UHPLC-HRMS). This analytical strategy consists in searching the sample for organic micropollutants and their transformation products (TPs) whose presence is suspected. However, data provided by mass spectrometry (molecular weight and isotopic profile) are often insufficient to discriminate suspected molecules. Standard retention can be a valuable help but this approach cannot be applied to TPs, the related standards being most of the time unavailable. Another approach is the use of Quantitative Structure Retention Relationship (QSRR) models, which relate the chromatographic retention to molecular descriptors such as molecular weight or octanol-water partition coefficient. Several models have been reported and until now, each model developed has used a single chromatographic dimension [1]. The separation power is significantly increased in comprehensive two-dimensional liquid chromatography (LC x LC) making the QSRR approach very attractive for increasing the confidence level associated with the identification of a suspected compound.

The aim of this work was to develop a QSRR model in each dimension of a LC x LC analysis. 68 standards were considered. All of them can be found in water samples (pesticides, pharmaceutical products and agrochemicals). Molecular descriptors, easily calculated or found in databases, were selected. A 60-minute LC x LC-HRMS method was developed using two sufficiently orthogonal stationary phases for our compounds: a zirconia-based phase coated with porous graphitic carbon (PGC) and a C18 silica-based phase. The two resulting QSRR models were tested on river samples and on a solution of TPs. The results were compared to those obtained with a unidimensional classical method. The comparison criteria were the coefficient of determination, the confidence interval, the number of possible molecules among the suspected ones and the reliability of the model for complex environmental matrices.

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Keywords: Suspect screening, QSRR model, 2D-LC, LC x LC

POSTER

Optimisation of oligonucleotide separations on ion-exchange chromatography

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Nucleic acid therapeutics such as antisense, siRNA and aptamers are expected to play an important role as next-generation pharmaceuticals together with antibody drugs. These drugs demand chromatographic purification and analysis that can recognize slight structural differences following synthesis.

Non-porous anion exchange columns are widely used for analysis of oligonucleotides. In this poster, effective method development of 20 mer single-stranded DNA and RNA is pursued using BioPro IEX QF, which is a nonporous high-performance anion exchange column. Also, the influence of following parameters on the separation are clarified; 1. concentration of counter ion added to mobile phase, 2. types of salt contained in buffer solution, and 3. column temperature. An optimised method that can separate oligonucleotides with single-base difference in length is proposed.

Keywords: oligonucleotides, AEX, method parameters, optimisation

POSTER

Hydrophobic interaction chromatography; insights on retention mechanism and separation potential for monoclonal antibodies

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Over the past two decades, there has been a rapid increase in the use of biopharmaceuticals such as a recombinant monoclonal antibody (mAb), for the treatment of cancer or immune-mediated diseases. To ensure that biopharmaceuticals meet the safety and efficacy level, these products need to be well characterized. However, during the production of these therapeutic molecules, some modifications may occur e.g. post and co-translational modifications which may result in aggregate formation, loss of biological activity, low yield, and increase in sample complexity. Liquid chromatography has emerged as the gold standard for the analysis and characterization of these molecules. In this regard, hydrophobic interaction chromatography (HIC), which is based on the interaction between hydrophobic patches of proteins and weakly hydrophobic ligands of the stationary phase, offers several possibilities for sample characterization and method development. On the one hand, HIC is mainly used for the characterization of biopharmaceuticals, on the other hand, retention mechanisms and optimization parameters are yet to be fully explored. Here, we demonstrate the significance of column chemistry and mobile phase-type in the analysis of trastuzumab reference material. Furthermore, the separation potential of HIC in the analyses of oxidative variants was explored, highlighting optimization parameters and characterizing the oxidative variants in an attempt to better understand the retention mechanism in HIC for improved method development.

Keywords: native separation, monoclonal antibodies, hydrophobic interaction

POSTER

A case study of (TAGGGT)2 oligonucleotide G-quadruplex by non-equilibrium kinetic capillary electrophoresis coupled to native ion mobility mass spectrometry

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Oligonucleotides are are capable to form G-quadruplex structures in the presence of various cations, including ammonium, sodium or potassium ions. Melting point experiments using Circular Dichroism detection, nuclear magnetic resonance, native mass spectrometry and native ion mobility mass spectrometry where extensively used to investigate the structures of these G-quadruplexes in solution and the gas phase. Nonetheless some discrepancies appear concerning the experimental conditions allowing the preservation of the G-quadruplex structures in the liquid phase and after the transfer to the gas phase.

We propose to introduce non-equilibrium capillary electrophoresis hyphenated to native ion mobility mass spectrometry to investigate the contribution of the main experimental parameters such as the concentration of ammonium or potassium cations in the background electrolyte, composition of the electrolytes and temperature, sheath liquid composition of the CE-MS interface, and sample preparation of the G-quadruplexes including the annealing conditions.

The chemical modification of the backbone from the classical phosphodiester (P=O) to the phosphorothioated equivalent (P=S) was investigated on (TAGGGT)2 G-quadruplexes based on ammonium or potassium cations. Kinetic and equilibrium parameters in solution were extracted from capillary electrophoresis in native or native-like conditions and favorably confronted to breakdown curve experiments as well as collision induced unfolding experiments. Our preliminary results also highlighted the contribution of the organic modifiers added to the G-quadruplexes solution to preserve the G-quadruplex structures during the transfer in the gas phase by electrospray ionization to prevent the heating of the ion during the desolvation process.

Keywords: oligonucleotides, G-quadruplex, capillary electrophoresis, ion mobility, mass spectrometry

POSTER

Sensitive and Selective Analysis of Wood Sugars and Uronic Acids for Biofuel Research with Electrochemical Detection

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Carbohydrates are week acids with pKa values between 12 and 14. Consequently, they can be completely or partially ionized under basic conditions with pH >12. The sources for the different kinds of monosaccharides can vary between food samples like honey or fruits, to scientific applications like glycopeptides or they can be products of fermentation processes like the here analysed wood monosaccharides. The mixture of the seven hemicellulosic sugars fucose, rhamnose, arabinose, galactose, glucose, xylose and mannose mixed with the two uronic acids galacturonic acid and glucuronic acid, extracted from wood by heat or chemical pretreatment, are of special interest in the research for new biofuels. They are considered to be more sustainable and are expected to become a competitive commercial alternative to fuel made from corn and other food sources. A mixture of the seven monosaccharides and two uronic acids could be baseline-separated with high S/N ratios in nanomolar concentrations with AZURA High Performance Anion Exchange Chromatography (HPAEC) and a pulsed 4-step PAD potential waveform for the detection. An easy to perform method using different concentrations of NaOH allows a fast and reproducible analysis even in lowest concentrations. Besides the research for biofuels, the investigated sugars are components in numerous processes in nature and food applications. Thus, the current application is suitable for several issues where carbohydrates need to be specifically separated and analyzed.

Keywords: Carbohydrates, HPAEC, natural products, Biofuel

POSTER

Evaluation of common lipid extraction methods for untargeted lipidomic study in platelets

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Efficient extraction of lipids in biological matrix is a key or first step for lipidomic study. Although the LC-MS technology enables detecting most of the known lipid classes, the results are highly limited by the extraction protocol with the number of lipids classes that can be simultaneously extracted. In this study, we explored the different extraction solvent systems including biphasic systems MTBE/MeOH/H2O (MTBE) [1] and Hexane/IPA/1M acetic acid (Hexane) [2] and monophasic systems MTBE/MeOH/CHCl3 (MMC) [3] and IPA/H2O (IPA) [4] for lipid extraction of platelets, followed by the investigation of the impact of extraction cycles for each method. MMC method displayed the best extraction recovery and efficiency for most of the lipid classes while IPA showed the priority for extraction of polar lipids like phospholipids. Further, a comparison of 4 different cell disruption methods based on the IPA method (vortexing, water bath ultrasonication, pestle and cell disruptor) and 2 different cell lysis methods which can be applied for large-scale study based on the IPA and MMC method (water bath ultrasonication and homogenizer) were evaluated. In general, 4 different cell disruption methods performed similar extraction efficiency for platelet lipids while extraction protocol with homogenizer showed significant improvement compared to sonication.

In this presentation, the sample preparation procedure for untargeted lipidomic study will be discussed. The priority on different lipid classes of different extraction protocols will be concluded.

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Keywords: Extraction methods, Untargeted lipidomics, Platelets lipidomics

POSTER

Determination of lambda-cyhalothrin metabolites in urine samples from operators and bystanders, using optimized and conventional cropping systems

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Lambda cyhalothrin (α-cyano-3- phenoxybenzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2- dimethylcyclo-propanecarboxylate) is a widely used synthetic pyrethroid whit a potential neurotoxicity and hepatoxicity [1]. Human exposure to this pesticide can be assessed by measuring specific and unspecific metabolites in human biological samples, like urine. In this study 3-phenoxybenzoic acid (3-PBA) and [3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane carboxylic acid (CFMP) were the selected metabolites to be investigated in biological matrices.

This work is stablished within the context of Perfect Life project [2] in which conventional and optimized application techniques were compared to reduce pesticides human exposure. A total of 54 volunteers (40 bystanders and 14 operators) were involved in controlled field trials in which lambda-cyhalothrin was applied using conventional (20 bystanders and 7 operators) and optimized systems (20 bystanders and 7 operators). Three urine samples were collected from each volunteer (total of 162 samples), a previous sample to the application and two samples after application (one of them after 8 hours and another after 24 hours). Target metabolites were analysed using Liquid Chromatography coupled to Mass Spectrometry (LC-MS/MS) and obtained results do not show a clear relationship between CFMP concentrations and exposure field trials, however, specific 3-PBA metabolite was found in urine at highest concentrations (15 ng/mL urine) when volunteers had been exposed to conventional treatments. Nevertheless, calculated Hazard Quotients showed that low health risk due to pesticide exposure was expected for the population under study.

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Keywords: lambda-cyhalothrin, human exposure, metabolites, LC-MS/MS, biological sample

POSTER

Identification of unknown substances in ambient air (PM10) by LC-Orbitrap-Tribrid-HRMS: Differences between urban, rural and industrial areas of the Valencian Region (Spain)

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A fast and automated strategy has been developed for identifying unknown substances in the atmosphere (concretely, in the particulate matter, PM10) using LC-Orbitrap Tribrid-HRMS (MS3). 49 compounds were tentatively identified using very restrictive criteria regarding exact mass, retention time, isotopic profile and both MS2 and MS3 spectra. Pesticides, pharmaceutical active compounds, drugs, plasticizers as well as metabolites were the most identified compounds. To verify whether the developed methodology was suitable, 11 substances were checked with their analytical standards and all of them were confirmed.

Different profiles for industrial, rural and urban areas were examined using LC-Orbitrap Tribrid-HRMS (MS3) and Compound Discoverer TM (CD) data processing software. Principal Component Analysis (PCA) model allowed us to separate the obtained data of the three assessed area.

When the profiles obtained in the three evaluated areas were compared using a Volcano plot (rural area was taken as reference), 11 compounds were confirmed as being discriminant: 3 of them (3-hydroxy-2-methylpyridine, 3-methyladenine and nicotine) were more likely to be found in industrial sites; 10 compounds (3-hydroxy-2-methylpyridine, 3-methyladenine, azoxystrobin, cocaine, cotinine, ethoprophos, imidacloprid, metalaxyl-M, nicotine and pyrimethanil) were more probable in the case of urban sites; finally, triisopropanolamine was more likely to be detected in rural locations.

Keywords: PM10, Orbitrap Tribrid, Unknown analysis, pollutants profiles

POSTER

Lambda-cyhalothrin inhalation exposure of applicators and bystanders using optimized and conventional application techniques

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France, Spain, Italy and Germany are considered the main agricultural producers in the European Union (EU) and in consequence they account for the two thirds of total pesticide sales in the EU [1]. During 2018 period, pesticides sales to Spain and Italy were over 50-80 million of kilograms that were applied in crops to keep them healthy and prevent from disease and infestations. However, pesticide application leads to pesticides human exposure that result in a health risk depending on the amount of pesticides, how long exposure lasts, the route of exposure and, also, the type of pesticide [2].

In this sense, this work provides scientific results from a European LIFE project, called Pesticide Reduction using Friendly and Environmentally Controlled Technologies (PERFECT LIFE) which main objective is to demonstrate the reduction of environmental pesticides contamination using optimized application techniques based on Optimal Volume Rate Adjustment tools (OVRA) and drift reducing tools (SDRT) [3]. Optimized application systems were used in two types of crops (Spanish citrus crops and Italian vineyard crops) and compared with conventional application systems. For this propose, lambda-cyhalothrin was applied in several seasonal campaigns and air samples were taken for the different application techniques. Air samples were analysed by Gas Chromatography coupled to tandem Mass Spectrometry (GC-MS/MS) and a 70% and 10% reduction of airborne pesticides was reported in citrus and vineyard crops respectively. Additionally, risk assessment of inhalation exposure was performed with the obtained concentrations and resulted Hazard Quotients showed that low health risk due to pesticide exposure was expected for the population under study.

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Keywords: Lambda-cyhalothrin, Risk assessment, GC-MS/MS, Pesticides, Application techniques

POSTER

Untargeted screening, identification and risk assessment of unknown substances (IAS/NIAS) in plastic food contact materials by GC-Q-Orbitrap HRMS

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In Europe, plastic materials and articles intended to come into contact with food shall comply with the Commission Regulation (EU) No 10/2011 [1], which contains the 'Union list of authorized monomers, other starting substances, macromolecules obtained from microbial fermentation, additives and polymer production aids' (intentionally added substances, IAS) that can be used for the manufacture of plastic food contact materials (FCM). Moreover, overall and specific migration limits (SML) are also established. However, during the manufacture processes and uses of plastic FCM, reaction and degradation products can occur (non-intentionally added substances, NIAS). For this reason, the risk associated with the presence and potential release of NIAS should be assessed before authorization of FCM [2].

In this work, a fast and automated analytical procedure has been developed and validated for the tentative identification and risk assessment of unknown migrant substances (IAS/NIAS) in plastic FCM by GC-Q-Orbitrap HRMS. The proposed approach combines untargeted GC-HRMS full scan data acquisition coupled to Compound Discoverer™ 3.2 software for automated data processing and compound identification. To perform the tentative identification of the detected features, a restrictive set of identification criteria was used, including match with NIST Mass Spectral Library, exact mass of annotated fragments, and retention index calculation. After the tentative identification, a risk assessment of the identified substances was performed by using the threshold of toxicological concern (TTC) approach [3].

This strategy was applied to recycled low-density polyethylene (LDPE), which could be used as FCM. In the analyzed sample, 374 features were detected, of which 83 were tentatively identified after examination of the identification criteria. Most of them were additives, such as plasticizers, used in a wide variety of plastic applications; oligomers of LDPE; and substances with chemical, industrial, or cosmetic applications. The risk assessment was performed, and the obtained results shown that there was not risk associated with the release of the identified substances.

The proposed analytical methodology, which includes solvent extraction, GC-HRMS analysis, tentative identification, and risk assessment can be applied for the untargeted screening analysis of unknown substances (IAS/NIAS) in different plastic FCM, showing its great utility and versatility.

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Keywords: Food Contact Materials, Gas Chromatography, High-resolution mass espectrometry, Non-intentionally added substances, Untergeted analysis

POSTER

Combined solid phase extraction purification method for heparan sulfate analysis

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Glycosaminoglycans (GAGs) are long-chained macromolecules that are built up of sugar disaccharide repeating units. These molecules are often attached to proteins as post- translational modifications, thus having an important role in many biological processes. Heparan sulfate is one of the subclasses of GAGs that has the most variable sulfation features of all. The repeating disaccharide units can be sulfated at several positions and can contain up to three sulfate groups. This structural variability gives a wide range of polarity to them and makes HPLC separation quite complicated. After enzymatic digestion, a purification step is necessary to remove contaminants and salts from the sample. However, due to the diverse physicochemical properties of these disaccharides, developing an all-fit method is still a challenging task.

We aimed to develop a solid phase extraction (SPE) method, that could be used for purification of HS disaccharides extracted from small size biological samples.

In the first phase of the project, we developed a method operating with self-packed cotton SPE spin tips (HILIC-type resin). We tested the effect of several parameters on the recovery of HS disaccharides: the brand and amount of cotton used, quality and concentration of acids used for loading, pH and salt content of the elution. The operation temperature and solvent volumes were also optimized. According to our results, the developed system binds well the more polar, more sulfated components, while a significant loss was observed for the non-sulfated and singly-sulfated components during sample loading.

In the second phase, we tested graphite-based commercial SPE systems for GAG purifications. During our experiments, we combined and applied the manufacturers recommended protocols to all three type of solid phases, in order to test the effect of these protocols on different resins. Our experiments showed that graphite-based resins are ideal for purification of non-sulfated and singly sulfated components, but the more sulfated ones are bound too strongly to the solid phase.

As a result, we found that the two optimized methods complement each other, therefore to achieve high recovery and undistorted sulfation pattern, a combination of the two methods is necessary. The flow-through of the cotton SPE is further purified on the graphite phase and the two elution fractions are combined.

Finally, to test the applicability of the combined method on small-size biological samples, we purified the digested samples of heparan sulfate chains originating from bovine kidney (HSBK) and porcine intestinal mucosa (HSPIM). Results showed significant improvements as compared to previously used methods.

Keywords: solid phase extraction, sample purification, glycomics, glycosaminoglycan, HILIC

POSTER

A robust methodology based on the use of a volatile surfactant in micellar electrokinetic chromatography – tandem mass spectrometry for the determination of pesticide residues in pollen and honeybee samples

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In this work, we propose for the first time an electrophoretic method based on micellar electrokinetic chromatography tandem mass spectrometry (MEKC-MS/MS) for the simultaneous determination of nine neonicotinoids (NNIs) together with the fungicide boscalid in pollen and honeybee samples. The use of these pesticides has been related with the colony collapse disorder (CCD) produced in honeybees due to their toxic effects to beneficial and non-target insects such as pollinators1. Based on a risk assessment of the European Food Safety Authority (EFSA), EU restricted their use to protect honeybees. In 2018 the application of imidacloprid, clothianidin, and thiamethoxam was restricted to greenhouse uses2 and in 2020, the approval of thiacloprid was not renewed3. Maximum residues levels (MRLs) for different commodities (or lower limit of analytical determination in such matrixes for which their use is forbidden, including apiculture products), have been established by the EU.

In the proposed method the electrophoretic separation was performed using ammonium perfluorooctanoate (APFO, 50 mM, pH 9) as both volatile surfactant and electrophoretic buffer compatible with MS detection, allowing the direct coupling of MEKC-MS. A stacking strategy to achieve an on-line pre-concentration of the target compounds, known as sweeping, was carried out to improve peak efficiency and sensitivity. Furthermore, a scaled-down QuEChERS was developed as sample treatment, involving a lower organic solvent consumption, using Z-Sep+ as dispersive sorbent in the clean-up step. A triple quadrupole mass spectrometer was operating in positive ion electrospray mode (ESI+) under multiple reaction monitoring (MRM). The main parameters affecting MS/MS detection as well as the composition of the sheath-liquid (ethanol/ultrapure water/formic acid, 50:45.5:0.5 v/v/v) and other electrospray variables were optimized to achieve satisfactory sensitivity and reproducibility. Procedural calibration curves were established in pollen and honeybee samples with LOQs below 11.6 µg kg-1 and 12.5 µg kg-1, respectively. Satisfactory precision, (% RSD lower than 15.2 %) and recoveries higher than 70 % were obtained for both samples. The method was applied to real samples of pollen an honeybees. Two samples of pollen were found positives in imidacloprid and thiamethoxam; and imidacloprid was also found in a dead honeybee sample. The method is an environmentally friendly, efficient, sensitive and useful alternative to the commonly used LC methods for the determination of NNIs and boscalid in pollen and honeybee samples.

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Keywords: capillary electrophoresis, mass spectrometry, neonicotinoids, scaled-down QuEChERS, honeybees

POSTER

How the storage conditions impact the analysis of human milk lipidome – the untargeted approach

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A comprehensive insight into the human milk (HM) lipid composition and its dynamics is crucial to understand the health benefits offered by breastfeeding and to impart optimal infant nutrition (via amelioration of infant formula composition). The lipidomic approach enables gaining in-depth knowledge about the composition and dynamics of HM lipidome. Changes in HM metabolite composition can be associated with the growth of the child (adaptation to the needs of the growing baby) or with the influence of external factors that can affect the dynamics of HM composition. Therefore, evaluation of the changes in HM lipids content occurring during the lactation can contribute to a better understanding of the mechanisms of adaptation of the HM composition in response to the individual nutritional needs of the growing child.

In lipidomic research, the stability of the human milk (HM) lipidome throughout storage is critical to avoid misleading findings or misinterpretations. Due to a lack of comprehensive research on the HM lipidome stability, the current investigation was conducted to elucidate a potential variation in the HM lipid profiles of samples under the different storage conditions. The impact of storage conditions, as well as the combination of storage temperature and time, on the profile of HM lipids, was investigated using an untargeted LC-Q-TOF-MS-based method. The samples were kept at temperatures ranging from 4 to -80°C for 4 to 84 days and exposed to up to three freeze-thaw cycles. The findings revealed that four days of storage at 4°C, as well as three freeze-thaw cycles, can lead to a change in the HM lipid contents. The observed variations in concentration levels of several lipid species in samples stored at -20°C versus levels of those lipids in samples stored at -80°C were not statistically significant, and interindividual variance was maintained regardless of sample storage condition. It was founded that storing HM samples at -20°C for up to three weeks and -80°C for up to 12 weeks ensures that the HM lipidome of the samples remains stable.

To the best of our knowledge, this is the first study on the influence of storage conditions on HM lipidome stability using the LC-Q-TOF-MS-based untargeted lipidomic strategy. Moreover, recommendations for the HM sample storage for a lipidomic study of HM samples were proposed based on the study findings.

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Keywords: human milk, storage, lipidome stability, LC-MS lipidomics

POSTER

Application of 3D-printed PET-G/carbon nanotubes composite in extraction procedures

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Microextraction is widely used for clean-up of sample matrix and preconcentration of analyte. The most commonly used type of microextraction is SPME, however, its bioanalytical application is limited, mainly due to economic reasons.

The main goal of this study was to investigate the possibility to apply PETG-carbon nanotubes (PETG-CNT) composite filament for 3D printing as a sorbent for extraction. PETG-CNT can be easily processed by fused deposition modeling to achieve the desired shape of a sorbent. CNT possess sorption properties, however, it is crucial to expose them on the surface of the sorption device. According to our experiments, the 3D printed object can be activated using the mixture of solvents partially dissolving the PETG polymer matrix.

The material available on the market was used in the research (3DXSTAT ESD-PETG, 3DXTECH, Grand Rapids, USA). To better understand the sorption mechanism and structure of the material scanning electron microscopy and porosimetry measurements were carried out. Both the surface morphology and pore size we significantly affected by solvent treatment.

The extraction efficiency was tested using an LC-MS technique using C18 column and a single quadrupole mass spectrometer. The preliminary results for imipramine extraction showed 37% efficiency in water samples. Such a method is very time efficient as printing 12 extraction devices and preparing them for the extraction procedure take less than 30 minutes. Water-based samples were extracted using reversible sorption to carbon nanotubes executed by changed lipophilicity of solvent and further processed by LC-MS analysis. In results, we discuss parameters that influence extraction recovery and performance of our newly developed extraction protocol. The main advantage of the proposed method is low costs comparing with classic SPME approaches as well as the ability to design sorbent that meets current needs, eg. scaling up (or down) the procedure, mixing by a magnetic stir bar or applying biomimetic shapes.

Keywords: 3D printing, carbon nanotubes composite, extraction, sample preparation

POSTER

Development of aptamer-based supports for cadmium ion extraction of complex samples

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Aptamers are single strands of synthetic DNA or RNA with a few dozen to a hundred nucleotide bases. They have the ability to form specific interactions with molecules or ions, which gives them great potential for the extraction and selective concentration of compounds of interest present in complex samples. While the use of aptamers for the extraction of molecules of various sizes (from small organic compounds, pesticides, mycotoxins… to proteins) has been widely described[1], the use of aptamers for ions has mainly been described for the development of sensors but not that much in solid phase extraction (SPE).

This project aims to develop solid sorbents functionalized with aptamers for the selective extraction of ions present in environmental samples. The chosen target ion here is the cadmium ion, Cd2+. Its toxicity for humans implies the development of methods to monitor it at trace levels. To date, only sensors using aptamers specific to Cd2+ have been described. In order to develop an oligoextraction method, it was necessary to identify in the literature an aptamer with a high affinity for the targeted ion, to synthesize it and then to immobilize it by covalent grafting onto a sorbent. Once the oligosorbent prepared, it was necessary to optimize a selective extraction protocol by selecting the conditions of percolation of the sample, of washing to eliminate potential interferents retained by non-specific interactions and then of elution of the ion of interest which is then quantified by inductively coupled plasma mass spectrometry (ICP-MS).

Firstly, a literature review was carried out to identify sequences described as specific for Cd2+[2,3]. Selected sequences as well as so-called control sequences (scramble sequences with the same nucleotide bases, but placed in a different order or another sequence with a very different conformation identified by molecular modelling) were grafted onto Sepharose and the grafting yields were measured by ion-pair liquid chromatography coupled to a UV detector. The grafting yields obtained are between 45% and 63%.

SPE procedures were then applied to these different supports using different percolation media as they can strongly affect the conformation of the aptamer and thus modify the target ion-aptamer interactions. These experiments were also carried out by studying the retention of other di-charged ions in order to investigate the specificity of the retention mechanism. As a result of this work, different media favoring the cadmium retention were identified.

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Keywords: Aptamers, Cadmium ion extraction, Sample preparation, ICP-MS

POSTER

Evaluation of different lectin-based sorbents for the extraction of the isoforms of human chorionic gonadotropin, the pregnancy hormone

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The characterization of the glycosylation state of an essential hormone that is secreted during pregnancy, the human chorionic gonadotropin (hCG), is of great interest, as differences in hCG glycosylation are associated with some pathologies during gestation. This hormone is comprised of two subunits, the alpha subunit (hCGα) with 2 N-glycosylation sites and the beta subunit (hCGβ) with 2 N- and 4 O-glycosylation sites. Therefore, numerous isoforms of hCG can exist. To achieve such a challenging goal as the elucidation of the glycosylation of hCG, a novel analytical method has been recently developed [1], which allowed the detection of the hCG isoforms at the intact level by using nano-reversed phase liquid chromatography coupled to high resolution mass spectrometry (nanoLC-HRMS, Orbitrap MS). The detection of 42 hCGα and 33 hCGβ isoforms was achieved when analyzing the recombinant hCG-based drug Ovitrelle® (rhCG) and of 84 hCGα and 17hCGβ isoforms in a purified hCG of urinary origin (uhCG).

However, to characterize hCG glycosylation in complex biological samples, efficient sample pre-treatment steps prior to the nanoLC-HRMS analysis should be developed to selectively extract hCG out of the numerous other proteins of the sample. For this reason, the potential of lectin-based sorbents for solid phase extraction was evaluated. Lectins are proteins that specifically recognize glycan motifs linked to glycoproteins. Two lectins were selected, the Concanavalin A lectin (Con A) that recognizes N-linked oligo-mannose glycans and Jacalin lectin that is mainly specific for O-glycosylation. Con A was successfully grafted on Cyanogen Bromide activated Sepharose with an average grafting yield of 99.2% (RSD=1.0%, n=7) whatever the lectin to gel density, which was ranging between 3.0 and 12.5 mg/ml. The experimental capacity of the sorbent having the highest lectin density was found to be 104 µg of uhCG/ml of gel. The nanoLC-HRMS analysis of the percolation, washing and elution fractions revealed that all 84 hCGα subunits had affinity with the sorbent with an average normalized extraction recovery of 98.1% ± 5.4% and 100% for the 17 hCGβ. The corresponding values for the rhCG were 99.8% ± 0.2% and 100% for hCGα and hCGβ isoforms respectively. On the other hand, a commercial Separopore® solid support functionalized with Jacalin lectin with a lectin density of 4.5 mg/ml exhibited an experimental capacity of 12.5 µg of rhCG/ml of gel. In addition, average normalized extraction recoveries of 99.9% ± 0.1% and 100% of were found for all the hCGα and hCGβ isoforms, respectively. These results obtained for purified samples of hCG are promising for the selective extraction of hCG isoforms from biological samples. Comparative studies with the use of a home-made Jacalin lectin affinity sorbent and with a commercialized Con A one are currently in progress and will also be presented in the symposium.

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Keywords: intact protein analysis, glycoproteins, lectin affinity sorbents, solid phase extraction, nanoLC-HRMS

POSTER

Novel LC-NMR method allows simultaneous quantitative and qualitative analysis of polymer mixtures

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There are several methods to determine properties of polymer mixtures. Liquid Chromatography (LC) is one of the most common tools. The separation of polymer mixtures according to their characteristics is very helpful but still cannot provide all the information we need. On the other hand, Nuclear Magnetic Resonance (NMR) delivers more insight to structural information by performing different 1D- and 2D-experiments which helps to investigate the polymers and their properties.

We combined both systems mentioned above and were able to establish a novel method to analyze polymer mixtures regarding chemical composition and molar mass of each compound. The hyphenation of LC and NMR allowed for the separation according to chain lengths followed by the investigation of quantitative and qualitative information of the structures of the polymer mixture. Therefore, onflow- and stopflow experiments using non-deuterated solvents were performed to analyze small amounts of compounds in the flow cell. With a previously performed calibration, both the molar masses and the chemical composition could be determined. In addition, it is possible to perform a complete structural analysis of the previously separated components to also obtain information about the structure and microstructure along to the already mentioned properties.

Keywords: LC-NMR, NMR, Polymer Mixtures

POSTER

Multi-Angle Light Scattering detection to characterize carbohydrate at Cargill's Research and Innovation Center

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In the last few years, large carbohydrates could be used as a valuable replacement for reducing sugars (glucose, saccharose) in foods. However, as the physico-chemical properties of large carbohydrates differ from sugars and small oligosaccharides, R&D labs had to adapt their analytical strategies towards more advanced technologies. Size exclusion chromatography coupled with a multi-angle light scattering detector (SEC-MALS) has proofed its value for comprehensive characterization of oligo- and polysaccharides.

During SEC-MALS, carbohydrates are separated following their hydrodynamic volume and detected by measuring the scattered light from a laser with up to 18 photodiodes positioned at various relative angles. Compiling data from the different photodiodes allows to retrieve the absolute molecular weight and molecular weight distribution. MALS results are only available when a quantitative detector is used, for carbohydrate the addition of a refractive index detector (RID) allows also estimates on recovery and purity. Finally, combined with a viscosimeter detector, intrinsic viscosity, substitution degree and conformation of the molecule can be evaluated.

Alternatives such as SEC-RID return relative molecular weights calibrated against external reference standards. These results can be considerably biased depending on the type of standards used. Or high-performance anion exchange chromatography coupled with amperometry detection (HPAEC-PAD) is often used to complement SEC-MALS data with information on the oligosaccharide profile up to Dp 140 (25,000 Da).

This contribution demonstrates the pros and cons of SEC-MALS and alternative methods for the characterization of fructans. Combining data from different characterization techniques enabled to identify some key differences among samples.

Keywords: Carbohydrates, light scattering, characterization, molecular weight, macromolecules

POSTER

Lipidomic analysis of the Cyanobacterium with the use of high-performance chromatography coupled to high-resolution mass spectrometry

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Cyanobacteria have become attractive models for metabolic engineering, intending to explore them as microbial cell factories. However, the study of cyanobacterial lipids' composition and variation, and the assessment of the lipids' functional and structural roles have been largely overlooked. The goal of the study was to expand the cyanobacterial lipidomic analytical pipeline by using an untargeted lipidomics approach.

Cyanobacterial lipid extracts were analyzed in an untargeted approach with the use of reversed-phase liquid chromatography–quadrupole-time of flight mass spectrometry (RPLCQ-TOF-MS) in positive ionization mode. The database search based on measured m/z value and mass spectra interpretation revealed information about fatty acyl chain length and level of saturation of Synechocystis sp. PCC 6803 lipid species. The employed method enabled the detection of Synechocystis sp. PCC 6803 lipids such as monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols (DGDG), sulfoquinovosyldiacylglycerols (SQDG) and phosphatidylglycerols (PG).

The employed method based on high-performance liquid chromatography and high-resolution mass spectrometry allowed for the sensitive detection and detailed analysis of lipid composition variation, with cells grown in alternative cultivation setups, and with a genetically modified strain. The comparative lipidomic analysis resulted in detecting differences in total lipid content, alterations in fatty-acid unsaturation level, and adjustments of specific lipid species among the identified lipid classes.

Keywords: lipidomics, cyanobacterial lipids, LC-MS, ultra-performance liquid chromatography, mass spectrometry

POSTER

Using Kendrick plot as a rapid visualization tool for lipidomics in complex sample using direct infusion mass spectrometry and reverse phase liquid chromatography-mass spectrometry

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Introduction. Lipidomics is an emerging field of growing interest that has developed rapidly over the past decade. Lipidomics is the study of lipids from a qualitative and quantitative perspective. The rapid profiling of lipids extracted from cell samples by mass spectrometry (MS) is the most promising tool for the diagnosis of various pathologies such as diabetes or obesity. However, non-targeted lipidomics from biological samples remains a challenge due to the high structural diversity of lipids (i.e. hydrophilic head nature, hydrophobic tail length, unsaturation level/position(s) and cis-trans isomerism) and to the complexity of such matrices. It is known that cells contain tens to hundreds of thousands of lipids at concentrations ranging from amol to nmol/mg protein.

The present work, developed in the context of the Eurlipids project (Euregio Meuse-Rhine Interreg, http://eurlipids.com/) introduces the use of Kendrick plots as a rapid visualization tool for selectively extracting the lipid signals from data generated by direct infusion mass spectrometry and liquid chromatography-mass spectrometry (LC-MS). Each lipid family is easily identified according to its theoretical Kendrick plot pattern (using -CH_2- scale). Lipids sharing the same unsaturation level but variable CH_2 units are aligned horizontally while they are aligned toward a specific angle if the number of unsaturation changed for the same number of -CH_2- units (or if another more appropriate Kendrick base is applicable). The m/z and the intensity of all the lipids from these patterns can be extracted and assigned on the basis of one of the most intense member of the series using exact mass measurements and the LIPID MAPS database (Nature Lipidomics Gateway, www.lipidmaps.org).

Material and methods. The dry yeast used for baking was ground and the lipids were extracted according to the methanol/MTBE extraction method. On the one hand, lipid extracts were directly infused on a 9.4T FT-ICR instrument (SolariX XR 9.4T, Bruker Daltonics, Bremen, Germany) equipped with a robotic nanoflow ion source NanoMate HD (Advion BioSciences, Ltd., Ithaca, NY). On the other hand, lipid extracts were separated by reverse-phase (C18) liquid chromatography performed on a Waters Acquity UPLC I-Class system (Milford, MA, USA) coupled with a 7T LTQ-FT Ultra mass spectrometer (LTQ-FT Ultra 7T, Thermo Fisher, San Jose, CA).

Main results. A comparison between the Kendrick plot of the yeast lipid extracts collected by direct infusion and LC-MS using a C18 column can be performed. This comparison was carried out using the superposition of Kendrick plots using an in-house Python software called MSKendrickFilter [1]. The analysis of lipids/adducts specific to direct infusion or LC-MS was also performed. Finally, the comparison of the LC-MS method and the direct infusion of lipids with respect to the Kendrick plot will be discussed.

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Keywords: Kendrick plot, Lipidomics, Direct infusion, LC-MS

POSTER

Band broadening in multicapillary columns with diffusional bridging – alleviating the polydispersity problem

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In gas chromatography, it is well-established that a porous-layer open-tubular column provides the best trade-off between peak capacity and pressure drop [1]. In liquid chromatography, however, the capillary diameter of such a column would have to be comparable to the particle diameter of a packed bed column in order to compete with it. Such a micrometre-scale capillary would be unsuitable because of its limited flow rate and sample capacity.

Therefore, the idea to bundle a vast number of micrometre-scale capillaries to function as a millimetre-scale column is, at least in principle, promising [2]. However, the inevitable differences in diameter among the capillaries translate to differences in velocity, which in turn translate to differences in retention time between the capillaries. This so-called polydispersity problem causes the appearance of an additional plate height term, proportional to the column length, thus limiting the achievable number of plates to a fixed level which is very low.

Fortunately, the polydispersity problem can be alleviated if the walls between the capillaries were to consist of mesoporous material, allowing the analytes to occasionally move from one capillary to another [3]. This so-called diffusional bridging reduces the polydispersity problem to a mass transfer problem, causing the additional plate height term to become akin to the C-term. Thus, the upper limit on the achievable number of plates is lifted.

Recently, we have derived analytical expressions for the case of a two-capillary system, providing insight into the aforementioned alleviation of the polydispersity problem. The plate height's dependence on all relevant parameters has been studied, both in the case of plug flow and parabolic flow. These analytical expressions could be seen as a mathematical demonstration of the benefits of diffusional bridging.

Continuing our research on this subject, we aim to bridge the gap between the idealised mathematical models and a real multicapillary column. Of particular interest is the generalisation of the plate-height expressions from a two-capillary system to an n-capillary system. This involves the study of both short-range (neighbouring capillaries) and long-range (trans-column) mass transfer within a multicapillary column.

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Keywords: diffusional bridging, multicapillary column, plate height expression, polydispersity problem

POSTER

Evaluation of different reversed-phase columns for the separation of proteins using the gradient kinetic plot model

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The chromatographic analysis of macromolecules like proteins is a challenging task even after the progress made in column technologies in the past decades. Biopharmaceuticals like monoclonal antibodies are especially difficult to analyse due to their complex structural heterogeneity. Still, there is a high interest for both the industry and the scientific community to get high quality chromatographic separations for these analytes to allow for a full characterization also of minor proteoforms in such biopharmaceuticals.

The focus in this work is to provide some guidance about the proper selection of suitable columns for protein analysis based on the gradient kinetic plot model presented by Desmet et al. [1]. The gradient kinetic plots provide information about what peak capacity can be achieved within a certain analysis time. The higher the peak capacity and the shorter the analysis time in which it can be achieved, the better is the column performance. Each column is compared at its respective performance optimum considering the maximum allowed backpressure of the column hardware. The study included 13 different columns and compares different stationary phase chemistries, particle sizes, pore sizes, stationary phase morphologies and stationary phase supports. First, the validity of the gradient kinetic plot model for protein columns was demonstrated using two columns from the same type and only changing the column length. The plots for the two column lengths showed a good overlapping indicating the model is valid for protein samples, too. Second, a sample set covering the range from small to medium size proteins (12 to 66 kDa: cytochrome c, lysozyme, myoglobin, β-lactoglobulin and BSA) up to the high molecular mass of a monoclonal antibody (148 kDa, NISTmAb) was used for the experiments. The gradient range was optimized using DryLab software and the same gradient steepness was kept constant for all columns. The performance for the different proteins was used to check in which molecular mass range each column is performing best. The BioShell A400 Protein C4 column had the best performance for proteins from 12 to 66 kDa but using the intact NISTmAb as test analyte, the Advance Bio RP-mAb C4 and the BioResolve RP mAb columns were superior. Furthermore, the evaluation of different stationary phase morphologies including totally porous, core shell particles and monolithic columns enabled a comparison which technology is currently the best one. The results indicate that core shell columns are currently the best, but some totally porous and monolithic columns provide still good results. Monolithic columns benefit from low back pressure even at higher flow rates thus they do not need the use of ultra-high pressure systems.

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Keywords: gradient kinetic plot, protein analysis, reversed phase chromatography, column comparison

POSTER

Prediction and simulation tools for more efficient method development

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Developing quality methods requires an understanding of the entire chromatographic problem. How do analytes' physicochemical characteristics affect their retention? How do controllable factors, such as gradient, temperature, and pH, affect desired measures, such as resolution? While repeated experiments supply that knowledge, they also consume significant time, manpower, and resources.

Software can help chromatographers understand the separation problem in less time and with fewer experiments. Here, we present ACD/Method Selection Suite, a method development assistant that includes tools for predicting physicochemical properties and simulating separations.

The prediction tool was used to predict the pKa of dazatinib and two impurities, and their logD at different pHs. Ranges were identified where the dominant ionic form was robust to changes in pH. These areas are particularly suitable for initial pH screening experiments.

The simulator tool was used to optimize the separation of three compounds in 3D mode. A 2x2x3 matrix of experimental conditions was designed to vary gradient, temperature, and pH, and a 3D rotating-cube model was produced to visualize predicted resolution at each point. The 3D map also marked areas of poor robustness. While a few of the initial conditions failed to resolve all three compounds, the simulator suggested several conditions that produced good resolution.

The user retains control over the simulator and can adjust settings based upon their chromatographic knowledge. For example, modelling equations can be customized to improve model accuracy, and the success criteria can be modified.

This work demonstrates two separate tools to help chromatographers understand factors governing analyte separation. The use of such software can reduce the time and number of experiments needed to develop a separation method. Such tools could be further extended by combining them with other useful chromatographic algorithms like retention time prediction, to optimize separations containing new but related analytes with a minimal number of experiments.

Keywords: method development, 3D optimization, pH prediction

POSTER

QC-friendly approach for the determination of formaldehyde in pharmaceutical chemicals

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As a reactive C1 building block, formaldehyde is widely acknowledged to be of key importance in the chemical and pharmaceutical industry, particularly for the synthesis of complex chemical structures.[1] Because of this intrinsically high reactivity, aldehyde-type impurities, in the form of remaining starting material or excipient related degradant, have been shown to adversely impact numerous drug product characteristics, including safety, efficacy as well as chemical stability, and this primarily through the formation of adducts with available nucleophilic functional groups. Prompt and regulated evaluation of the formaldehyde content is therefore vital to prevent unexpected problems during late-stage product development.[2,3] Nevertheless, several inherent physiochemical properties of formaldehyde, such as a low molecular weight, high solubility and negligible UV activity, have greatly complicated the search for a suitable approach that ensures accurate assessment of this compound with sufficient sensitivity. As a result, current methods for trace-level analysis heavily rely on HPLC and GC based techniques that are preceded by complex multistep and non-selective derivatization procedures to improve their detectability towards formaldehyde.[2] From the perspective of a quality control (QC) laboratory, such additional steps in routine batch analysis are however preferably avoided to limit the analysis time and risk of errors. The development of a derivatization-free method for the reliable determination of formaldehyde is therefore considered highly desirable in terms of efficiency.

In general, gas chromatography (GC) with headspace (HS) sampling and flame ionization detection (FID) is regarded as one of the most straightforward approaches for the quantification of such low molecular weight, volatile impurities in a wide variety of pharmaceutical chemicals.[2,4] Despite the very low sensitivity towards formaldehyde itself, we succeeded in applying this technique for the development of a more QC-friendly analytical tool that permits trace-level analysis of formaldehyde without prior derivatization or additional steps. In this study, essential sample preparation features, such as the reference standard and dilution solvent, were identified, together with key instrument parameters, including the injection speed and injection volume. Ultimately, the optimized method was effectively validated according to ICH requirements Q2(R1),[5] demonstrating the accuracy, precision, specificity, detection limit, quantification limit, linearity and range of the analytical method for the determination of formaldehyde at concentration levels as low as 100 ppm (w/w).

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Keywords: Gas Chromatography, GC-HS-FID, Formaldehyde, Pharmaceuticals, QC-Friendly Approach

POSTER

Development of reliable and reproducible methods to measure molecular and longitudinal diffusion in supercritical fluid chromatography

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The improvement of supercritical fluid chromatography (SFC) instrumentation enhanced its reliability and utility over the past decade. The further development of high speed and high resolution separations is however obstructed by the lack of accurate models for axial dispersion in SFC. This work is a first step to tackle this by developing more reliable methods to measure molecular and longitudinal diffusion in SFC.

The molecular diffusion coefficient (Dmol) affects all aspects of separation efficiency. Dmol drives longitudinal diffusion, enhances mass-transfer in packed bed SFC and counteracts peak dispersion due to packing inhomogeneities. Dmol can be determined using the Taylor-Aris peak broadening method, where the dispersion in an open tubular capillary is measured. The capillary has to be coiled in the lab because the required tube length is impractical. Hence, the flowrate in the capillary must be small, not to cause secondary flow effects that affect radial dispersion and thus the apparent diffusion coefficient. This set-up was further adapted to increase the reliability and reproducibility in SFC conditions. The first modification was the insertion of a column before the capillary to ensure Dmol was measured in the desired mobile phase and not in the initial sample plug. Furthermore, a fraction collection loop was incorporated after the column. This second modification avoids the trial-and-error approach to find the optimal column, instrument and mobile phase conditions to achieve symmetric peak shapes, as only a small fraction of an overloaded peak is reinjected into the capillary. A final modification was the addition of a by-pass of the mobile phase flow after the column, which made it possible to use higher pump flow rates, improving stability and accuracy of the pump operation and reducing residence time in the column.

Next the effective diffusion coefficient (Deff) was measured using stop-flow experiments where the mobile phase flow rate is stopped when the solute is halfway through the column for a time tpark and then resumed. During this period tpark, longitudinal diffusion is the only source of axial dispersion. This set-up was adapted for SFC as the pressure and hence the supercritical conditions are no longer maintained when the flow is stopped. In addition, due to the strong compressibility of the mobile phase, restarting the flow rate is accompanied by transient start-up effects that vary flowrate and retention in a non-reproducible way. Therefore, a two-column variant of the set-up was developed where two identical columns are coupled in parallel. When the analyte is halfway through the first column, the flow is switched to the second column, parking the peak in the first column (still pressurized) and maintaining the flow and pressure in the SFC system.

This experimental data, together with theoretical models, will help us better understand the diffusion process in the packed bed, by modeling longitudinal diffusion and mass transfer contribution to band broadening in SFC. In the future, this will allow us to better investigate, predict and optimize separation performance in SFC, hereby guiding further instrument development (e.g. higher operating pressure, smaller particles, optimal columns dimensions,...).

Keywords: supercritical fluid chromatography, axial dispersion, molecular diffusion, longitudinal diffusion

POSTER

De Novo Structural Elucidation Principle via In Silico Chromatographic Retention Index Prediction for Micropollutants in Wastewater

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Micropollutants, such as pharmaceuticals, industrial chemicals, steroid hormones, etc. are defined as anthropogenic chemicals and can be found in water. It is seen as a serious threat, not just to aquatic life but also to humans, which requires the availability of tools allowing structural elucidation and ideally, fast identification of unknowns. Over the past decade, high-performance liquid chromatography, coupled with high resolution mass spectrometry (HPLC-HRMS), has been increasingly used in the analysis of environmental and treated wastewater samples. However, HRMS prediction software cannot always reliably predict the elemental composition of (larger) molecules while structural information obtained by MS remains limited. This hinders the identification and structural characterization of unknowns in wastewater.

In this research, a Quantitative Structure- Retention Relationship (QSRR) approach is used to build predictive retention index (RI) models to assist in the identification of unknowns. Development of algorithms based on LC retentive data allows confirmation or invalidation of the ensuing hypothesized structural formulas. The novelty of this work is that for the first time a complete workflow is provided allowing narrowing down the possibilities in de novo structural elucidation of in principle any carbon, hydrogen or oxygen containing organic solute (< 500 Da) purely based on chromatographic (RPLC) retention.

Keywords: In silico prediction, QSRR, Micropollutants

POSTER

LC-MS based methodology for monitoring of chiral and achiral emerging contaminants as a tool for wastewater surveillance

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Liquid chromatography coupled with mass spectrometry (LC-MS) is a technique with many applications. Among them, wastewater surveillance is currently the most needed as it can provide information on environmental water pollution, the spread of epidemics or excessive drug consumption. In practice, this is done by monitoring particular contamination indicators, also known as markers. Markers useful for these purposes belong to emerging contaminants (ECs), which are represented by pharmaceutical residues, artificial sweeteners and various compounds released as a result of functioning in everyday life. ECs are not commonly included in government regulation or environmental monitoring programs due to limited knowledge about their ecotoxicity, behavior in the environment and potential negative impact on human health and aquatic ecosystems. Moreover, it is well documented that ECs are not very amenable to removal in conventional wastewater treatment plants (WWTPs). Therefore, the main source of them in environmental waters is municipal and industrial wastewater due to continuous discharges from WWTPs [1, 2]. On the other hand, treated wastewater is considered to be a remedy for limited drinking water resources, which emphasizes the importance of ECs monitoring. Besides, wastewater analysis can provide information on the condition and preferences of the population living in the study area. For instance, wastewater surveillance can supply data on the occurrence and transmission of disease-causing viruses or bacteria. However, the occurrence of asymptomatic patients or those with non-specific or mild symptoms is generally not included in the recording of the actual number of cases. This offers the possibility of also using other markers to monitor the epidemiological situation. In fact, ECs, particularly non-steroidal anti-inflammatory drugs (NSAIDs), whose consumption increases during the illness period, may have great potential in this case. Thus it was decided to exploit the potential of ECs as Covid-19 disease co-markers and a tool to reflect the condition and preferences of the population living in North Poland.

During wastewater surveillance, two different chromatographic approaches (chiral and reversed-phase liquid chromatography) were applied for analyses. A silica gel column modified with vancomycin as a chiral selector was used for enantioselective separation, whereas wastewater profiling and ECs identification were performed using an untargeted LC-Q-TOF-MS-based method. The levels of ECs were determined with LC-MS/MS-based method. Solid-phase extraction (SPE) was performed to isolate and enrich the analytes. Furthermore, the dSPE procedure was developed to reduce the amount of solvents used to achieve a more environmentally friendly sample preparation step. The green character of both sample preparation protocols combined with LC-MS determination was evaluated using the Green Analytical Procedure Index (GAPI) and the Analytical Greenness calculator (AGREE). The results obtained were quite similar, so it was decided to continue using the SPE-LC-MS approach.

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Keywords: emerging contaminants, wastewater surveillance, LC-MS

POSTER

HPLC-MS/MS Analysis of Pyrrolizidine Alkaloids in Comfrey and cosmetic products containing its extracts

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Comfrey (Symphytum officinale L.) is a medicinal plant traditionally used in the treatment of musculoskeletal system disorders, such as blunt injuries, fractures, muscle and joint pains. However, due to its' content of hepatotoxic pyrrolizidine alkaloids (PAs), EMA restricts comfrey root use only to topical application in short therapies (up to 10 days), with daily dose of PAs not exceeding 0,007 µg/kg [1,2]. In some European countries, special technological processes are used to obtain comfrey products with reduced content or free from pyrrolizidine alkaloids. However, in Poland, comfrey root is present only in cosmetic products, that are not subject to these regulation and quality control regarding quantification of their ingredients [2]. Little is also known about the chemical composition of comfrey leaves, which are reported to contain lower amounts of PAs [3]. Therefore, the aim of our research was to evaluate the content of pyrrolizidine alkaloids in comfrey roots, leaves, and some cosmetic products available on the market.

HPLC-MS/MS qualitative and quantitative analysis of pyrrolizidine alkaloids in plant raw materials (comfrey roots and leaves obtained from botanical gardens and herbal stores) and cosmetic products was performed in MRM mode on Kinetex-C18 (100 x 2.1 mm, 2.6 µm) column under gradient elution with increasing concentration of a mixture ACN/H2O/HCOOH 50:50:0.1 (v/v/v) in H2O/HCOOH 100:0.1 (v/v) with a flow of 0.2 ml/min at, reduced in comparison to previous studies, column temperature of 25°C.

As a result, the presence of pyrrolizidine alkaloids previously reported by Trifan et al. [4] was confirmed in 8 different samples of comfrey roots, that showed great variation in their qualitative and quantitative composition. On the other hand, analyzed comfrey leaves obtained from 4 different sources, had more stable chemical composition, with the dominance of lycopsamine, intermedine and their N-oxides in all analyzed samples, while the other PAs were present only in trace amounts. Moreover, the pyrrolizidine alkaloids content was evaluated in three cosmetic products containing comfrey root extract.

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Keywords: comfrey, symphytum officinale, pyrrolizidine alkaloids, HPLC-MS/MS, cosmetics

POSTER

A Fast Ultra-Low Noise Current Amplifier for Flame Ionization Detectors

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Due to its combination of robustness, sensitivity and vast linear range, the flame ionization detector has been one of the workhorses of gas chromatography for decades. One of its key components is the amplifier measuring the small current generated by ionization in the hydrogen flame. The amplifier has to be sufficiently fast to follow the peaks eluting from the column, linear over the linear range of the FID and at the same time should provide as low as possible noise to reduce the limits of detection. Obviously, the continuous development of gas chromatography has led to an equal increase in required performance. The continuously increasing speed of separations, both in fast unidimensional gas chromatography and in the last dimension of comprehensive multidimensional gas chromatography, has led to an equal increase in required current amplifier speed. At the same time, the lower sample capacity of the typically used thinner columns reduces signal intensities. Thus, today's amplifiers need to provide less noise despite being faster, all that while maintaining a large linear range.

Here, we present a capacitive current amplifier combining a novel reset circuit for its integrator with a special mixed signal data processing architecture, allowing it to achieve a unique combination of extremely low noise, high speed and wide linear range. While usable for a wide range of applications, this amplifier is especially suitable for detecting the output current of a flame ionization detector coupled to a fast gas chromatograph. With a filter bandwidth suitable for peaks with a full width at half maximum of 50 ms, the measured noise current standard deviation is 3.4 fA, meaning that peaks with an amplitude of only 10 fA can be detected. This is equivalent to only about 3000 charges in said peak. When using a filter bandwidth suitable for peaks with a full width at half maximum of 2 s, peaks with an amplitude below 1 fA become measurable. At the same time, a linear range of more than six orders of magnitude is achieved with a single amplifier and no range switching, meaning that the amplifier is able to follow peaks of arbitrary height right next to each other. Furthermore, due to the utilized reset circuit, the amplifier has an extremely stable zero point, drifting by less than ±0.5 fA over the course of several days.

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Keywords: Ultra-Low Noise FID Amplifier, Hyper-Fast GC, Capacitive Current Amplifier

POSTER

Sequence determination of copolymers by pyrolysis-Gas Chromatography

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Synthetic polymers find a wide application in daily life, with applications ranging from coatings to packaging, to automotive parts and cosmetics. A polymer is not a single defined molecule but a collection of molecules featuring distributions in molecular weight, chemical composition, end-groups etc. One of these distributions, the sequence distribution, describes the order of monomers in a copolymer. The sequence distribution affects material properties and to develop new sustainable and high-performance polymers, it is important to determine the sequence length in relation to the final properties [1].

The measurement of the sequence is notoriously challenging, as it requires the measurement of intact subunits i.e. dimers/trimers. NMR serves as the gold standard [2]. Ideally, NMR quantify the copolymer sequence length directly, however the resolution between various subunits such as trimers is often lacking. This limits the NMR approach to sequence determination of copolymers consisting of maximal 3 monomers.

Pyrolysis-GC (py-GC) is an alternative method which can be applied to copolymer sequence determination [3]. The polymer is fragmented in smaller subunits, which could be related to the sequence length. To investigate the possibilities and thus the application range, a py-GC approach was developed, optimised and investigated for the sequence analysis of copolymers. This py-GC approach was compared to sequence analysis by NMR, outlining the application range of py-GC. Furthermore, py-GC was applied after SEC to study the sequence length over the MWD of copolymers, which is the first step into the determination of the correlations between various distribution i.e. sequence length across the MWD.

In this lecture, we outline the possibilities of py-GC for sequence analysis of copolymers and compare this with NMR analysis. This will clearly show the advantages of py-GC in copolymer sequence analysis and the application range on complex copolymers. And finally, we will show how we applied py-GC after SEC to determine the sequence length over the MWD range. We will close this lecture with an outlook for further research.

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Keywords: Polymer, Sequence, Pyrolysis-GC, Size Exclusion Chromatography

POSTER

Sweet, sweeter Stevia – The whole story from Analytical Method Development to a robust and effective online SPE purification of Steviolglycosides with preparative HLPC

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Steviolglycosides are the main sweetening compounds in stevia rebaudiana and can be used as natural sugar substitutes with up to 400 times higher sweetening effect compared to sucrose or glucose. To enable a commercial usage in quality assurance and for the production of ultrapure reference compounds, the plant extract needs to be purified. Sample cleanup via solid phase extraction (SPE) and similar methods are time-consuming and cost-intensive procedures and the purification of complex plant extracts requires special care. We herewith introduce a robust and sensitive online SPE sample preparation method for the purification of six steviolglycosides using reversed phase HPLC. A short analytical gradient method or an isocratic method, both with optional preceding online SPE were optimized. Afterwards the isocratic method was up-scaled for a preparative 22 mL/min approach achieving a short and robust purification method for steviaglycosides from plant extracts with maximum throughput efficiency. In a review of the method development process the automated online SPE purification will be compared to the common batch SPE workup and advantages and drawbacks of both methods will be evaluated in depth.

Keywords: steviolglycosides, online SPE, HPLC, method development

POSTER

Resolution of co-eluting peaks to obtain pure mass spectra from UHPLC-ESI-HRMSe data by use of a flexible-PARAFAC2 algorithm: Improved identification and quantification of unknown compounds

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A major challenge in processing of complex data obtained from chromatography hyphenated to mass spectrometry is to resolve chromatographically co-eluting compounds and thereby obtain pure mass spectra containing adduct-, fragment- and precursor ions. Data dependant acquisition using tandem mass spectrometry is the most common method to obtain pure mass spectra from co-eluting compounds. However, this has several drawbacks e.g. risk of under sampling chromatographic peaks, and introduction of a bias in the mass spectral dimension towards selected precursor ions. In this study, we present a workflow based on multiway curve-resolution of ultrahigh pressure liquid chromatography high resolution mass spectrometry data obtained by broadband data-independent acquisition obtained by MSE operation (UHPLC-HRMSE). The multiway curve resolution allows grouping of mass-spectral peaks, which correlate across samples and elution profiles even for peaks with minor changes in peak shape and retention time shifts. To the authors' knowledge, this is the first-time multiway curve-resolution has been applied to UHPLC-HRMSE data.

The aim of the study was to provide proof-of-concept that the high energy and low energy trace obtained in MSE could be addressed, simultaneously, using multiway curve-resolution to resolve co-eluting compounds and obtain pure mass spectra. The dataset consisted of UHPLC-HRMSE data of plant tissue (n=93), soil (n=59), and drainage water as well as nearby stream water (n=75) from a Lupinus angustifolius L. crop field study. A flexible-PARAFAC2 algorithm with non-negativity constraints on all three modes was fitted to 16 chromatographic intervals on the three-way arrays (m/z × retention time × samples) using the summed trace of the high energy and low energy MSE trace. Decomposing the summed trace, will obtain a combined mass spectrum of precursor, fragment, and adduct ions, which has the potential to improve compound identification as more diagnostic m/z's will be present in the mass spectrum. The three environmental matrices were decomposed, independently. Mass spectral loadings and pure mass spectra were considered similar if they had a cosine value > 0.85.

The performance of the models was best in the soil and plant matrix followed by the water matrix. Similarity between the mass spectral loadings and the pure spectra of 14 spiked compounds was obtained for most compounds in soil (13 out of 14 compounds) and plant samples (12 out of 14 compounds), but only for half of the compounds in the water matrix (8 out of 14). The worse performance in the water matrix was believed to be caused by larger variations in the retention time, thus, this data violated the underlying assumption of the data being trilinear to a higher degree. The flexible-PARAFAC2 algorithm was found to resolve chromatographically co-eluting compounds in 37 out of 48 of the decomposed retention time intervals, detect non-gaussian peaks, and perform grouping of mass spectral peaks.

This workflow presents a possibility for more exhaustive compound detection and, possibly, better compound identification for UHPLC-HRMSE data, due to its ability to resolve co-eluting peaks and group mass spectral peaks which correlate across samples, and elution profiles.

Keywords: Non-target screening, Multiway Curve-Resolution, Data-processing, Lupinus angustifolius L., Chemometrics

POSTER

Pyrolysis-GC×GC-QTOF for Improved Characterization of Crude Oils

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Crude oil consists of an extremely large amount of compounds covering a wide range of volatility and chemistry. This makes detailed composition characterization highly challenging.

Pyrolysis coupled to comprehensive two-dimensional gas chromatography-high resolution mass spectrometry (py-GC×GC-HRMS) is a very powerful technique for the characterization of complex, heavy matrices such as crude oils. The two-dimensional resolution provides enhanced separation of the pyrolysis products, leading to improved classification for groups and individual analytes. Additionally, the 2D pyrograms make sample comparison easier and more informative. In addition, high resolution and accurate mass can deliverextra selectivity and identification power, especially for compounds with heteroatoms (e.g. N, S, O).

Here we show the use of py-GC×GC for improved characterization and comparison of crude oils and the advantages arising from HRMS detection for speciation of heteroatoms such as sulfur-containing compounds.

Keywords: GCxGC, High Resolution Mass Spectrometry, QTOF, Pyrolysis, Crude oil

POSTER

Development of a novel methodology for determination of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs) in meconium of newly born by HRGC-HRMS

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The vulnerability of the human fetus and infants has been repeatedly demonstrated to the effects of organic pollutants (OP) due to their rapid growth and organ development, cell differentiation, and immaturity of metabolism. Meconium, the first intestinal evacuation of the newborn, has been suggested as a non-invasive way to assess accumulated prenatal exposure in the long-term due to their easy collection and the large amount of sample that can be collected [1]. In this work, a new analytical strategy to analyze polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs) in meconium samples has been developed. A total of 9 meconium samples were collected at the University and Polytechnic La Fe Hospital (Valencia, Spain) from 2020. Pressurized fluid extraction (PLE) was used as extraction methodology with n-hexane and dichlorometane (1:1) followed by a clean-up step using the multicolumn system Power-PrepTM, consisted on a sequential array of three different columns: silica ABN, alumina, and carbon columns. The analysis of PCDD/F and dl-PCBs were performed using gas chromatography coupled to high-resolution mass spectrometry. Nineteen out of the 29 analyzed congeners were detected in any of the analyzed mecounium samples, with eight of them being detected in all samples (OCDD, PCB-77, PCB-123, PCB-118, PCB-105, PCB-167, PCB-157 and PCB-156). Average concentrations of individual congeners ranged from 0.003 (PCB-123) to 0.05 (PCB-118) pg TEQ/g, ,The total concentrations of the sum of PCDD, PCDF, and dl-PCBs in the analyzed samples (considering upper-bound) ranged from 4.38 to 22.92 pg TEQ/g lipid, with an estimated geometric mean (GM) of 10.01 pg TEQ/g lipid.

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Keywords: meconium, dioxins, sample preparation, GC-HRMS, human biomonitoring

POSTER

Tentative identification of unknown compounds in tears using liquid chromatography-Orbitrap Tribrid high-resolution mass spectrometry

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The tear film is an heterogeneous fluid layer wich protects the ocular surface. It conteins proteins, enzymes, lipids, metabolites, electrolytes and in some cases drugs or other compounds [1]. For the identification of unknown compounds in tears a new approach has been implemented, which integrates intelligent data acquisition with AcquireX linked to liquid chromatography-Orbitrap Tribrid high-resolution mass spectrometry using data dependent-tandem mass spectrometry and processing with the Compound Discoverer™ software for tentative identification. Data were acquired both in positive and negative modes. The identification criteria used were exact mass, isotope pattern and tandem mass spectrometry spectra match.

Following this aprroach, 39 substances were tentatively identified. Most of the identified compounds were aminoacids, hormones, other internal metabolites and drugs. In order to confirm these compounds, standards will be used to check the retention time identification criteria.

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Keywords: tears, liquid chromatography, high resolution mass spectometry, unknown analysis, human biomonitoring

POSTER

Exploring biocompatible alternatives to traditional LC systems and columns to avoid non-specific adsorption of oligonucleotides

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Non-specific adsorption is a recurring issue that takes place during the characterization of biopharmaceutical products in liquid chromatography. Analytes may adsorb on metallic surfaces of the fluidic path, interacting with stainless steel parts of the column and instrumentation. In practice, this could increase peak tailing and asymmetry, lower sample recovery, or even worse, lead to sample loss. Consequently, reliability and reproducibility of results are directly impacted.

Oligonucleotides have a propensity toward non-specific adsorption. Their backbone composed of numerous electron-rich functional groups, namely phosphates makes them prone to interactions with materials traditionally used in chromatographic analyses.

Hence, there is a need of permanent solutions to minimize the interactions of analytes with metallic surfaces. To this end, novel materials applied to columns and systems are emerging in the last few years. Referred to as being biocompatible, they are composed of hybrid organic-inorganic material, polyether ether ketone (PEEK), MP35N or titanium, and they show great promise in mitigation of adsorption.

What is the real impact of these novel surface technologies on the adsorption of oligonucleotides? Are they all equivalent?

To answer these questions, different biocompatible columns were evaluated and compared to their stainless-steel analogue toward the analysis of oligonucleotides, in both ion-pairing reversed phase (IP-RP) and hydrophilic interaction chromatography (HILIC) modes. Chromatographic systems that differ from their flow path material were also explored to be able to conclude on a potential optimal column-system combination to avoid non-specific adsorption during the characterization of oligonucleotides.

Keywords: oligonucleotides, adsorption, biocompatible

POSTER

Properties of spatial thermal gradient gas chromatography

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In gas chromatography, one of the driving forces of peak broadening is the accelerating velocity of the analyte band. Naturally, every gas chromatographic separation has a positive gradient of the linear velocity due to gas decompression and acceleration of the mobile phase towards the outlet of the column, most significantly in GC-MS. This increase of the velocity stretches the width of the band of the analyte molecules and results in wider peaks in the chromatogram. The controlled variation of the temperature over the length of the chromatographic column, resulting in a spatial thermal gradient, can partly counteract this positive velocity gradient and can reduce the width of the analyte band during the migration through the column. Under certain circumstances the peak width can be reduced. Another property of the thermal gradient is the reduction of the temperature to which an analyte is exposed during separation. In addition, a thermal gradient also changes other properties of the chromatographic system, for example flow and mobile phase velocity.

A numerical simulation model of gas chromatographic separation with a spatial thermal gradient is used to investigate the changes of the properties of the separation system by the added thermal gradient. The influence of different forms of a thermal gradient (linear or non-linear) can be investigated. With the simulation the optimal settings for a thermal gradient can be estimated for which the performance of a separation can be improved.

Keywords: Thermal Gradient GC, Numerical Simulation, Method Development

POSTER

Speciation analysis of arsenic in sea urchins (Paracentrotus lividus) from North-West Mediterranean Sea

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Arsenic (As) speciation in marine ecosystems has been the subject of much attention for decades and seafood was identified as a source of major exposure to As through human consumption. Various species have been identified in seafood; among these, the most toxic species are the inorganic forms (arsenite (As(III)) and arsenate (As(V))), followed by methylated forms (monomethylarsonic acid (MA) and dimethylarsinic acid (DMA)) which are considered as carcinogenic. It is also worth to note that in the fishery products, predominant arsenic species is the (AsB), which is considered as non toxic.

So far, there are extremely few studies dealing with the speciation analysis of arsenic in urchins. Therefore, obtaining occurrence data on arsenic speciation at national and European level in in such foodstuff is necessary in order to assess more accurately the toxicological impact of this element via the consumption of the urchins. This is a challenging task given the extremely low levels of arsenic species in such samples (especially the inorganic fraction) and the complexity of the matrix.

The determination of inorganic arsenic (As the sum of As(III) and As(V)), monomethylarsonic acid (MA), dimethylarsinic acid (DMA) and arsenobetaine (AsB) was carried out by anion exchange HPLC coupled to Inductively Coupled Plasma Mass Spectrometry after extraction with H2O by Microwave Assisted Extraction(MAE).

Arsenic speciation analysis was carried out in gonad tissue of sea urchins (Paracentrotus lividus) from coastal areas of North-West Mediterranean Sea. For this purpose, 39 samples were collected in 2017 from 13 different sites: 7 stations were located around an industrial effluent (la Ciotat (Cio), Calanques (Cal), Riou (Rio), Couronne (Cou), Carry (Car), Frioul (Fri), les Embiez (Emb). Urchins were also sampled from 6 locations far away from the industrial effluent and any industrialized area including 2 stations located in a protected area (Porquerolles (Por), Port Cros (PC)), 2 stations in Corsica (Canari (Car) and Bastia Nord (Bas)) and 2 stations considered as reference station (Cap Garonne (CG) and Théoule (The)).

Keywords: arsenic, speciation, urchins, mediterranean sea, ICP-MS

POSTER

Novel stationary phases for non-ion-pairing reversed-phase liquid chromatography of synthetic oligonucleotides and its potential for hyphenated techniques

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Nowadays, ion-pairing reversed-phase liquid chromatography (IP-RP-LC) is still the dominating state-of-art method for the analysis of nucleic acid related compounds, such as antisense-oligonucleotides (ASO), small-interfering ribonucleic acid (siRNA) or other DNA or RNA molecules. Despite of its reliable performance and robustness, the usage of a high number of ion-pairing reagents by IP-RP-LC complicates the hyphenation with mass spectrometry (MS) for an advanced characterization of the analytes. In this work, we tested two alternative stationary phases, viz. polybutylene terephthalate- and pentafluorostyrene bonded silica, for the separation of generically synthesized Patisiran as siRNA strands (antisense, sense and annealed) giving some unexpected selectivity without any presence of ion-pairing reagents. With that, a further MS and tandem MS (MS/MS) characterization was possible to be carried out. Additionally, other chromatographic methods like hydrophilic interaction liquid chromatography (HILIC) and mixed-mode chromatography coupling with MS detection were used for the impurity separation of Patisiran strands. The results of normalized retentions were subsequently investigated by principal component analysis (PCA) towards similarities in retention pattern of the corresponding impurity species on the distinct stationary phases. The PCA also revealed the orthogonality of the used one-dimensional (1D) LC methods which can be recombined in a further second-dimensional (2D) LC setup to enhance the selectivity for more separation power.

Keywords: Oligonucleotide separation, Tandem MS, LC-MS Hyphenation, 2D-LC

POSTER

Development of a "greener" HPLC-UV method for the analysis of reducing sugars in fruit juice and indigenous fruits using acetone as an alternative solvent

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Chromatographic techniques can be made more environmentally friendly, i.e. "greener", by a number of different strategies. One approach is to replace the toxic mobile phase solvents such as acetonitrile with greener alternatives. Acetone, in particular, has proven to be a suitable alternative to acetonitrile, since the two solvents have similar physicochemical properties, including solubility, miscibility and viscosity properties. However, due to acetone's high ultraviolet (UV) cut-off wavelength (330 nm), it normally cannot be used as a mobile phase solvent when performing high performance liquid chromatography (HPLC) analysis with UV detection. In this study, a reversed phase HPLC-UV method, using acetone-containing mobile phase, was developed for the determination of reducing sugars in fruit juice and indigenous fruits. Pre-column derivatisation of analytes via reductive amination with p-aminobenzoic acid ethyl ester (ABEE) was performed to enable photometric detection at 307 nm. The method was directly compared to a method that utilized acetonitrile-containing mobile phase. Although the detection wavelength of the ABEE derivatives is below the UV cutoff wavelength of acetone, it is high enough above acetone's absorbance maximum (~280 nm) to enable satisfactory detection of the derivatives. Hence, the method compared well with the acetonitrile method, providing sufficient sensitivity to facilitate the quantitation of glucose and fructose in all the fruits and juices investigated in this study. The method developed in this study provides a greener alternative to sugar analysis for laboratories that are limited to using HPLC-UV equipment.

POSTER

Chemical fingerprinting of polar and neutral lipids from shea kernels using an LC-ESI-QTOF-MS method for their quality assessment

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The shea tree is a wild-growing sustainable crop. It is indigenous to the savanna belt in sub-Saharan Africa. It is considered a sacred tree by many communities and ethnic groups and plays an essential role in religious and cultural ceremonies. Furthermore, kernels are collected, stored, pre-treated by West African (WA) women, and sold for export and refining. Outside African countries, shea is used primarily in chocolate manufacturing, but it is also gaining popularity in the food and cosmetic industries, and especially as a natural, plant-based, and bio-sustainable alternative to the palm-oil-based bakery and confectionery solutions. The quality of shea oil fluctuates, and both the presence and abundance of polar and neutral lipids may play a crucial role in its quality assessment. Degradation products from the fats, such as free fatty acids (FFAs) and diacylglycerol (DAG), are abundant, often 10-20% of the extractable lipids. Fresh kernels have less than 2% FFA, highlighting the need to understand and control their degradation process.

The aim of the presented research was to establish a workflow for the analysis of the polar and neutral lipid fractions from shea kernels of different quality as a new way of carrying out a more comprehensive quality assessment of shea products because, currently, quality assessment is done based solely on the FFA content. Firstly, the liquid-liquid (LLE) extraction, followed by the solid-phase extraction (SPE) fractionation of the polar and neutral lipids, was developed and optimized. Secondly, the non-target analysis of the shea kernels classified as a good, medium, and bad quality with liquid chromatography-high resolution mass spectrometry (LC-HRMS) using both positive and negative electrospray ionization (ESI), followed by multivariate analysis was established. For the full characterization of the kernel lipids, three chromatographic platforms were developed. Principal component analysis (PCA) of the LC-ESI-QTOF-MS revealed the differences between kernels of different quality and allowed to identify of compounds that are key in the differentiation of the kernels.

Keywords: lipidomics, food quality, shea kernels

POSTER

Headspace GC-MS workflows for untargeted analysis of volatile metabolites in biological matrices: a compromise between high recovery and broad coverage

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Introduction: Microbial volatile metabolites act as modulators of intra- and inter-kingdom relationships and may influence host health. Mechanistic investigations are required to further understand these interactions and identify novel biomarkers of disease. We aimed to develop an untargeted metabolomics workflow using headspace SPME-GC-MS and raw data processing tools to analyse volatiles in biological matrices for application in oral and gut health models.

Methods and Results: Pooled samples from ex vivo faecal fermentations and supernatants from in vitro bacterial single- and multi-species culture were used in method development. A mixture of matrix-matched heterogenous chemical standards (heptane, acetone, ethanol, C2-C8 carboxylic acids, indole, p-cresol) was used to monitor performance and confirm matrix effects. Manual extractions were performed in 4mL vials and sample volumes were optimised for a balance between sensitivity and linear range for most metabolites. Extracted analytes were desorbed in the GC inlet at 300°C with 1:1 split, separated on a ZB-WAX capillary column (35-250°C over 60 min), and detected on a mass spectrometer operating in scan mode (30-300 m/z).

A combination of uni- and multi-variate approaches were used to design a method development workflow for rapid optimisation of matrix-dependent extraction conditions. Univariate evaluation of SPME fibre phase, ionic strength and pH was performed to maximise overall recovery. A Carboxen/PDMS fibre displayed a superior ability to extract biologically relevant small aldehydes, ketones, and alcohols. Addition of NaCl to saturation positively affected recovery, and pH adjustment to 2 with H_2SO_4 improved quantitative recovery of protonated forms of low pKa compounds (e.g., carboxylic acids). Multivariate optimisation of temperature, equilibration time, and extraction time was performed using a face-centred central composite design. Polynomial response surface analysis revealed substantial differences between chemical classes. Recovery was typically highest at 80°C, excluding alcohols and ketones (highest recovery at 40°C). Optimal values were set to maximise metabolome coverage, according to the composition of each biological matrix. Desirability values at optimal conditions varied substantially between classes (0.24-0.92).

Methods were validated for repeatability on pooled biological samples and for limits of detection and quantitation on matrix-matched standards. The latter were in the low µM range, in line with expected biological concentrations. Three raw data processing packages (eRah, xcms and PARADISe) were evaluated on a model SPME-GC-MS dataset, with a focus on peak table quality (spectral deconvolution, alignment, and library matching). The PARAFAC2-based PARADISe had superior flexibility regarding variable peak widths/shapes and changing baselines. Processing time for a typical untargeted dataset was in the order of hours for eRah and xcms and days for PARADISe (64-bit PC, i7 processor, 16 GB RAM).

Conclusion: Optimal volatile extraction conditions were set at pH 2, 60° C, equilibration time of 30 minutes, extraction time of 60 minutes, and NaCl to saturation, on a CAR/PDMS SPME fibre. Substantial differences in desirability values for chemical classes highlight the compromise between recovery of specific chemical classes and wide metabolome coverage. Despite longer processing times, PARAFAC2 modelling was the best compromise. Exploring microbial volatilomes using these methods holds promise for novel biomarker discovery and further mechanistic insights.

Keywords: microbiome, oral health, gut health, volatilomics, solid-phase microextraction (SPME)

POSTER

Breathomics approach to investigate systemic sclerosis using thermal desorption and comprehensive two-dimensional gas chromatography high-resolution time-of-flight mass spectrometry

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Systemic sclerosis (SSc) is a chronic and heterogenous auto-immune disease of unknown origin characterized by fibrosis, inflammation, vascular damages, and involvement of internal organs. Organ involvement appears at the early stage of the disease[1,2]. Interstitial lung disease (ILD) is one of the most common types of pulmonary involvement, responsible for the disease severity, and leading to high morbidity and mortality. One of the challenges in SSc remains the early diagnosis of patients with a high risk of disease progression driving mortality[3]. There is an unmet need for biological markers enabling SSc early diagnosis, prognosis, disease progression monitoring, and improving patients' classification for more targeted therapies. Ideally, new diagnostic methods for SSc should be simple, fast, accurate, and cost-effective. Comprehensive two-dimensional gas chromatography (GC×GC) has a great potential for exhaled breath analysis. The increased peak capacity and sensitivity of GC×GC, provided by the combination of two capillary columns of different stationary phases by means of a modulator, enable the chromatographic separation and detection of thousands of compounds from a complex matrix[4]. For this reason, we carried out an exploratory study on SSc[5]. Basically, breath samples were collected in 5L Tedlar® bags. Volatiles contained in the sampling bag were then transferred onto Tenax®GR/Carbopack™B thermal desorption tubes (Markes International Ltd., Llantrisant, UK) and finally released and separated into a Pegasus GC-HRT 4D (LECO Corporation, St Joseph, MI, USA) through a mid-polar Rxi-624SilMS (30 m × 0.25 mm × 1.4 µm) as first column (dimension) and a polar Stabilwax (2 m × 0.25 mm ×0.5µm) as second dimension. The exhaled breath of 32 patients and 30 healthy subjects was therefore analyzed. The high resolving power of this approach and the use of statistical models enabled the identification of 16 compounds discriminating SSC patients from healthy ones[5]. However, further investigations had to be held to reach a better disease classification. In fact, the biomarkers highlighted here could be related to the scarring (fibrosis) of the lungs making these non-specific to SSCs. The second phase of the study aims to go deeper in patient stratification. Three groups were investigated: 50 SSC patients, 50 SSC-fibrosis patients and 50 ILD ones. The samples were collected at Maastricht medical center and CHU of Liège. All samples were then analyzed in the OBiACHem lab. Currently, a classification model is under construction to stratify patients based on their fibrosis status.

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Keywords: Volatile organic compounds, Exhaled breath, Biomarkers, Comprehensive two-dimensional gas chromatography, High-resolution mass spectrometry

POSTER

Improved characterization of malodours in recycled plastics using TD–GC×GC–TOF MS

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The analysis of plastic has gained increased attention in recent years, due to the global push to move to a circular economy – meaning the increased use of recycled materials to reduce waste. Manufacturers are being urged to produce or use more post-consumer recycled (PCR) plastics, especially for food and beverage packaging.

PCR plastics require more rigorous quality control (QC) measures to ensure that they will not produce volatile emissions that could be considered harmful or have a negative impact on the packaged product (e.g., malodours).

However, there are severe limitations with the existing methods used for the detection of odours from plastics, such as human sensory panels and gas chromatography coupled with mass spectrometry (GC–MS).

Human sensory panels are restricted to sensory information and do not provide chemical identities, while GC-MS is often unable to resolve the complex volatile profiles from plastics, meaning that key odorants may be overlooked. Therefore, with these approaches, it is often not possible to identify the characteristic compounds responsible for high odour in recycled plastics, meaning that the recycling process cannot be improved, and QC failures continue to occur.

Here, we show how thermal desorption (TD) coupled with comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry (TD–GC×GC–TOF MS) can address these challenges, by providing high sensitivity and improved separation of the odour profiles, prior to confident identification of the individual analytes.

We will also demonstrate how TD–GC×GC–TOF MS can provide confident characterisation of complex odour profiles from recycled plastics, for fast and simple identification of the compounds causing QC failures. Once the key odorants are known, methods can be easily translated to TD–GC×GC–FID for routine screening in QC labs at production sites.

Keywords: Polymer, odours, GCxGC, mass spectrometry, thermal desorption

POSTER

Development and characterization of immobilized enzymatic reactors for on-line comprehensive bottom-up proteomics

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Conventional comprehensive proteomics workflows suffer from significant variation in sample preparation due to excessive pipetting and vessel changes during biochemical modifications. A number of techniques have been developed to combat this variation, including, one-pot sample preparation[1], novel integrated fluidic setups (iPAD)[2] and oil-air droplet systems (OAD)[3]. A major disadvantage of these techniques is the multiple handling steps necessary to add different reagents which ultimately will impact sample loss, additionally, the slow kinetic performance of proteolysis leads to multi-hour protein digestion times.

Immobilized enzymatic reactors (IMERs) provide a facile methodology to address both the variation as well as the slow digestion times in global proteomics studies. IMER technology involves the covalent attachment of bioactive proteins to a framework which allows for ultrafast protein digestion (minute to second scale) by catalyzing the proteolysis of proteins.

Monolithic frameworks represent a particularly apt format to produce IMERs due to the tunable porosity which can allow for optimal surface area to porosity ratio to efficiently immobilize bioactive enzymes in comparison to open tubular columns, as well as low backpressure allowing faster flowrates and subsequent digestion speeds in comparison to packed particle columns.

Herein, we present the development of novel on-line IMER LC-MS workflow optimized for minimal peptide carryover and digestion reproducibility. A tertiary monomeric polymer was produced in a single porogenic solvent to produce monoliths inside 100 µm I.D capillaries capable of being integrated into nanoLC-MS setups. We optimized the hydrophobicity to reduce non-specific hydrophobic interactions to decrease sample carryover as well as fine-tuned the maximal concentration immobilized trypsin enzyme.

In this way it was possible to comprehensively characterize complex proteome samples in less than 1 hour with good repeatability. The beforementioned IMERs represent a suitable technology to be seamlessly integrated into current nanoLC-MS setups, removing the need for conventional sample preparation methods.

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Keywords: IMER, Comprehensive global proteomics, Sample preparation, LC-MS

POSTER

SPME-GC/MS for pesticides analysis: Palm wine case of study

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Palm wine is a widely consumed product on the African continent. It is produced by fermenting the spontaneous sap of palm trees and is a nutritious drink (1) which is consumed without any treatment. A previous study (2) focused on the aromatic profiles of palm wines and five main families of VOCs were highlighted by HS-SPME-GC/MS, esters being predominant. The aim of the present study is to check the presence of pesticides in palm wines from the Ivory Coast. Indeed, pesticides are used around the world to provide maximum agricultural production to feed ever-growing populations. However, it presents a real danger to humans above a certain concentration. To successfully analyze pesticides potentially present in palm wines, a HS-SPME-GC/MS method was developed. SPME can be automatized and does not require solvent, is easy to do, relatively low cost and fast.

Different fibers (PDMS, PA, DVB/Carbon WR/PDMS) were tested and the best conditions were determined by experimental design with 48 trials (equilibration time, extraction time, temperature, salt, desorption time and temperature). Various pesticides (organochlorines, organophosphates, methylparathion, terbutylazine, diazinon) were separated by a ZB5MS + column following a gradient program. The method was validated in terms of linearity, limit of detection and quantification, accuracy and repeatability, and allowed the quantification of 36 pesticides in 43 min. Among the 32 samples analyzed in triplicate, 7 pesticides have been detected in 10 samples (dichlorvos, methyl parathion, pirimiphos methyl, p,p'-DDE, p,p'-DDD, 4,4'-DDT, endrine ketone). Dichlorvos was the only one detected at levels above the European maximal limits.

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Keywords: SPME-GC/MS, palm wine, pesticides, experimental design

POSTER

Fingerprinting by mass spectrometry and infrared spectroscopy for food fraud detection

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Spectroscopic techniques (near infrared (NIR) and mid-infrared (MIR) and hyperspectral imaging), gas chromatography coupled to mass spectrometry (GC-MS) and proton-transfer reaction time-of-flight mass spectrometry (PTR-MS), combined with chemometrics, were examined to evaluate their potential for food fraud detection in dried oregano, as an example for spices and herbs, and rice, as an example of cereals. Data fusion, the combination of data from multiple techniques, was also tested. Because variety and cultivation area are the major factors affecting the market price of rice, the main authenticity issues are the partial substitution of one variety or cultivar with another, or the mislabeling of the geographical origin of the rice[1]. A previous study was shown that about 25% of the analyzed commercial oregano samples were adulterated; up to 70% of bulk agents can be detected in sample[2]. For oregano, geographical origin and variety assessment, processing control and adulteration with look-alike agents, e.g. sumac, myrtle, olive leaves and cistus leaves were examined. In total 257 rice samples and 358 oregano samples were analyzed by all five analytical techniques. The Gaussian Process Latent Variable Model (GP-LVM) was selected as technique and applied to obtain a reduced two-dimensional space[3].

For oregano, differentiation between the different origins (Italy, Turkey, Israel and South-America) was successfully done, with a prediction rate over 90%, except for South American samples. Batch-to-batch control could be performed with infrared spectroscopy. Adulteration was detected successfully as well, but quantification could not be performed under 10% adulteration. Data fusion models showed little or no improvement on single technique models.

For rice, differentiation between the different origins was successfully done and GP-LVM plots show different clusters for the different countries. Clusters for varieties and subvarieties were also seen in the GP-LVM plots. Validation for all experiments was performed. Prediction rates for variety assessment (indica-japonica-glutinous rice) were above 95% with hyperspectral imaging. For origin assessment, GC-MS provided the best results with prediction rates above 85%. Data-fusion experiments were done to improve prediction rate. The combination of NIR and GC-MS was shown to be the best combination with prediction rates above 90% for origin assessment.

Overall, food fraud detection by untargeted analysis, both by GC-MS and infrared spectroscopy was shown to be possible. Data fusion can improve some models, but difficult questions, such as the quantification of adulteration remain challenging for non-targeted approaches.

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Keywords: food fraud, artificial intelligence, GC-MS, PTR-MS, infrared spectroscopy

POSTER

Detailed characteristics of human milk gangliosides throughout the lactation with the use of ultra-performance liquid chromatography coupled to high-resolution mass spectrometry

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Gangliosides are one of the chemical compounds present in human milk. They are complex lipids composed of a ceramide and oligosaccharide chain that can contain N-acetylneuraminic acid (SA). Gangliosides molecules are structurally diversified due to the variation in the ceramide part, that corresponds to the various length and saturation levels of the sphingosine base and fatty acid substituents, therefore their comprehensive analysis constitutes a challenge for analytical chemists. In this study, we employed ultra-performance liquid chromatography coupled to high-resolution mass spectrometry (UPLC-HRMS) to monitor the composition of gangliosides in human milk samples of several healthy volunteers through the whole lactation, from the first month of lactation until the end of breastfeeding. The use of this analytical platform enabled the evaluation of the compositional dynamics with the focus on many gangliosides species instead of the total ganglioside content approach. The detected gangliosides belonged to the GM3 and GD3 classes. The use of ultra-performance liquid chromatography in a reversed-phase mode enabled the separation of GM species accordingly to the length of the ceramide part. The 1.7 particle-sized column with C8 stationary phase was used to provide the best resolution of the chromatographic method. A high-resolution mass spectrometer equipped with quadrupole and time of flight analyser was employed to identify gangliosides based on the accurate measurement of the m/z ratio and to characterise their structure. MS/MS experiments in positive ionisation mode were performed to determine the structure of the ceramide part of ganglioside species. Financing: This research received funding from National Science Centre, Poland (2018/29/B/NZ7/02865).

Keywords: gangliosides, ultra-performence chromatography, LC-MS

POSTER

Multivariate Optimization and Refinement Program for the Efficient Analysis of Key Separations (MOREPEAKS)

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Method development in one- and, especially, two-dimensional liquid chromatography (LC×LC) can be very time consuming. Our group has aimed to aid in this cumbersome task by creating the Program for the Interpretive Optimization of Two-dimensional Resolution (PIOTR)[1]. By utilizing retention modelling, i.e. modelling the elution time of compounds as a function of modifier content, thousands of theoretical chromatograms can be simulated within minutes. Thereafter, an optimal separation can be chosen from a Pareto optimality plot. In recent years, PIOTR has been reworked into the Multivariate Optimization and Refinement Program for the Efficient Analysis of Key Separations (MOREPEAKS). This new software is freely available from https://cast-amsterdam.org/. Utility tools have been incorporated to aid with optimization, such as peak detection tools[2] and peak-tracking tools for LC-MS and LC×LC-MS data[3,4]. Retention models for difficult-to-understand retention mechanisms, such as hydrophilic-interaction liquid chromatography, have been investigated within this software, to establish the best choice within the optimalization software[5,6]. In this poster presentation, an overview of the possibilities of MOREPEAKS is presented.

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Keywords: Method development, Retention modelling, Software, Peak-tracking

POSTER

Separation of isomer and isobar compounds from complex halogenated POP mixtures by GC-TIMS-MS

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Nowadays, the ubiquitous presence and continuous emission of a wide range of pollutants in the environment is of particular concern due to the adverse effects they represent for human kind and ecosystems. In particular, the food, feed and environmental monitoring of regulated Persistent Organic Pollutants (POPs) such as dioxins, furans, PCBs and other halogenated POPs is performed by well-established reference methods such as GC-EI-magnetic sectors high resolution mass spectrometry in selected ion monitoring (SIM) or GC triple quadrupole in tandem mass spectrometry mode. These classical approaches are compliant with current regulations, but emerging technologies that could also meet the legal performance criteria while offering a more versatile approach need to be assessed. Indeed, the number of compounds of concern to be monitored is steadily increasing, including poorly studied, overlooked and emerging compounds. Thus, it is essential to build solutions that are based on a different vision of analytical chemistry and develop non-targeted methods for the analysis of POPs.

Here, we propose to develop a novel non-targeted analytical strategy that relies on the direct coupling of gas chromatography (GC) with trapped ion mobility (TIMS)-mass spectrometry (MS) to probe and monitor emerging halogenated persistent organic pollutants in complex food/environmental samples. Compared to classical methods, our approach shall increase the number of contaminants monitored per analysis while at the same time providing additional separation, sensitivity and identification capabilities, thanks to the determination of ion-specific collision cross sections (CCS). To evaluate the capabilities of our approach, we prepared a standard mixture containing more than 120 contaminants bellowing to 10 different families of POPs (including mixed chlorinated-brominated compounds). The mixture was injected splitless on 30 m gas chromatographic column (Rxi-5SilMS column, 30mx0.25mmx0.25?m, Restek) hyphenated to a timsTOF Pro mass spectrometer equipped with an GC-APCI source (Bruker).

It was found that, for a given class of contaminant, the halogenated congeners were readily separated in the ion mobility dimension by halogenation degree. Moreover, it was noted that isomeric congeners were also separated according to specific substitution pattern, such as the degree of ortho substitution for PCBs and BDEs, and the degree of substitution at the positions adjacent to the oxygen atom(s) in dioxins (positions 1,4,6,9) and furans (positions 4,6). While the TIMS was unable to resolve GC coeluting isomers, it showed very promising prospects for the separation of coeluting isobars requiring high mass resolving power. A noticeable case was the coelution of four congeners, two of which (PCB 157 and 2Br,378Cl-Dioxin) were isobars requiring more than 35 000 mass resolving power to be distinguished in the m/z dimension, but base-line separated in the ion mobility dimension. These preliminary results thus confirm the potential of the use of TIMS as an additional separation dimension in GC-MS for the non-targeted analysis of complex halogenated contaminant mixtures.

Keywords: Gas chromatography, Ion mobility, Mass spectrometry, Persistent organic pollutants, Food safety

POSTER

Full-comprehensive chiral x RP 2D-LC-MS analysis of conjugated fatty acid isomers

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In pharmaceutical lipid-formulations conjugated fatty acids can be considered as an impurity, generated by oxidation of the unsaturated fatty acid over a hydroperoxide [1]. Although many positive health benefits are ascribed to conjugated fatty acids [2, 3], they have to be identified once the identification threshold is exceeded. For the characterisation of unknown compounds, it appears that mass spectrometry is often considered as the gold standard. But not in all cases mass spectrometry alone can deliver satisfying results regarding the complete identification. This is especially the case for isomeric/isobaric compounds when they do not show characteristic MS2-spectra. In our research we showed the benefit of additional UV-spectra-evaluation from LC-DAD-measurements for the identification of conjugated fatty acids. By this approach, a differentiation of the groups (unconjugated fatty acids; conjugated dienes, trienes and tetraenes) by their absorbance maximum and spectra-shape was possible. To some extend the determination of the configuration of the double bonds was possible, since each double bond with Z-configuration would shift the absorbance maximum by 2.5 to 3.0 nm [4-6].

Coeluting fatty acids and their isomers have to be separated, since they can produce mixed UV-spectra, making a clear statement about the identity during the impurity-profiling difficult. Reversed phase (RP) chromatography with C18-columns is a very simple separation method for hydrophobic compounds like fatty acids but shows a lack of selectivity for the different isomeric conjugated fatty acids. In total, 10 out of 18 analytes in the five investigated mixes were coeluting with the screening gradient. Increasing the complexity of the three-dimensional structure by utilizing different RP-selectors like C30-carbon chains or cholesterol only slightly improved the separation. Using amylose-/cellulose-based chiral stationary phases (CSPs) led to a drastic increase of complexity of the three-dimensional stationary phase structure. Although they are rarely used for the separation of non-chiral compounds, they showed in our research significantly improved separation of the investigated isomers. The amylose-based CSPs showed better selectivity than the cellulose-based CSPs. Especially the Chiralpak IA-U and IG-U-column had the best performance, being able to separate 16 out of the 18 investigated analytes with the final LC-method. However, for more complex mixtures the higher peak capacity from two-dimensional LC is necessary. The combination of the reversed phase column and amylose-based CSP is favourable since they show orthogonality and good compatibility, since they are both operated under RP-conditions. This gave a good basis for the development of a full-comprehensive 2D-LC method.

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Keywords: 2D-LC-MS, Impurity-profiling, Fatty acids, Chiral

POSTER

Analysis of Mineral Oil Residues in Food and Foodpackaging using LC-GCxGCMS Technique

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Mineral oil components such as Mineral oil saturated hydrocarbons (MOSH) and Mineral oil aromatic hydrocarbons (MOAH) can be identified almost everywhere in the Environment in fatty components in cosmetics, food and transport packaging materials. Both compounds, the MOSH and also the MOAH are easily absorbed by the human body from food, and can accumulate in body fat and in the organs. Yet, it cannot be excluded that MOAH fractions may contain carcinogenic compounds. That´s why European Community (EC) is under pressure for fixing analytical determination limits of MOSH and MOAH in food and food packaging [1]. Because of their complexity it is not possible to resolve MOH mixtures into individual components for quantification. However, it is possible to quantify the concentration of total MOSH and MOAH fractions, as well as certain sub-classes, using methods based on gas chromatography (GC). The system combines HPLC (LC-40BXR) and GC (GC-2030) technology with flame ionization detection (FID) for a highly efficient analysis covering preparation, pre-separation and automated processes. The Shimadzu MOSH/MOAH analyzer has been designed specifically for sensitive and fast detection of mineral oil contaminations based on the European Norm DIN EN 16995:2017: Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID Analysis [2]. In case of a detected MOAH concentration in food it is important to understand the origin of the contamination and the toxicity. Also, cross-contamination generated from MOSH, POSH, PAO and others should be excluded. That is why according to the European norm 16995:2017 GCMS is required. More effective is GC x GCMS technique or comprehensive GCMS. Comprehensive GCMS is a powerful technique that provides the two-dimensional chromatography data acquisition, resulting in a significantly improved resolution and sensitivity. Data processing is done via Chromsquare software (Chromaleont srl, Messina).

[1] EU: COMMISSION RECOMMENDATION (EU) 2017/84 of 16 January 2017

[2] EN16995:2017 Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis

Keywords: Mineraloil, food analysis, HPLC, GC, sample preparation

POSTER

Simultaneous separation of polar and non-polar organic micropollutants by multidimensional liquid chromatography combining HILIC and RPLC

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An increasing problem for the adequate supply of freshwater is the global occurrence of organic micro-pollutants (OMPs) in surface and groundwater. OMPs, such as pharmaceuticals, pesticide residues, personal care products, and (synthetic) hormones, are organic chemicals with highly variable structures that occur at trace concentrations (ng/L – µg/L) and can have ecotoxicological and/or human health effects. To guide the development of novel advanced water treatment technologies for the removal of OMPs from surface and groundwaters, innovative analytical methods to characterize and quantify relevant OMPs are required. Since these OMPs occur in complex mixtures, and have various physicochemical properties including a wide polarity range, their analysis remains challenging, especially when the characterization of both polar and non-polar components is required. Most studies in environmental analysis are therefore performed using multiple liquid chromatography-mass spectrometry (LC–MS) methodologies. However, a single efficient method that allows the complete analysis of the entire sample is often preferred, especially when only small amounts of sample are available. A possible approach to achieve this, is by using multidimensional LC, capable of delivering a higher separation performance for complex samples, by transferring fractions from a first-dimension column to a second-dimension column, with a different stationary phase, and therefore a different selectivity for the analytes present in the sample.

In this study, we aim at combining analytical methods with orthogonal separation capabilities into a multidimensional liquid chromatography method, to analyze complex mixtures of OMPs in one single analysis. A complex sample, containing relevant polar and non-polar OMPs for wastewater treatment analysis, is separated by coupling a HILIC column to a RPLC column, using different multidimensional LC methodologies including heart-cutting and selective comprehensive LC. Due to the incompatibility of the mobile phases, the consecutive on-line analysis of HILIC and RPLC can cause peak shape deformation in the second dimension. A possible solution for this problem is active solvent modulation, a method in which a specifically designed valve allows the on-line dilution of the first-dimension fractions before transfer into the second-dimension column by means of restriction capillaries. The application of active solvent modulation is therefore investigated in this study as well.

Keywords: organic micropollutants, multidimensional liquid chromatography, HILIC, RPLC

POSTER

Development of new analytical technologies to profile protein-protein interactions network with hydrophobic interaction chromatography

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Over 80% of proteins exert their function as part of larger assemblies. Interactions between biomolecules have proven to be critical as aberrant protein-protein interactions (PPIs) are known to cause a number of neurologic disorders, such as Alzheimer and Creutzfeldt-Jacob disease. To identify the partners within biomolecular interaction networks, it is essential to profile PPIs directly in complex life-science matrices. In-vitro and in-vivo biophysical and biochemical methods are generally used to profile PPIs. Yet, these techniques prove to be both inaccurate and imprecise and cannot be applied to analyze PPIs directly in complex clinical matrices.

Ergo, the aim of this study is to develop new techniques based on native liquid chromatography (LC) modes coupled with a native mass spectrometry (MS) detection. Typically, the kinetic performance in 1D-LC is much lower compared to reversed phase liquid chromatography (RP-LC) separations. This is because native LC columns are packed with relatively large (3-5 µm) particles, instead of sub-2-µm particles as commonly used in RP-LC experiments.

To address this issue, polymer-monolithic capillary columns were synthesised in situ in 200 µm i.d. capillaries with a chemistry tuned for hydrophobic interaction chromatography mode. Different synthesis conditions were screened and physical characterisation of these monoliths was done with SEM imaging and measurements of their permeability (Darcy's law). The monoliths were then implemented in a homemade analytical set-up that allows the delivery of low flows (ca. 1 µL min-1) using a conventional pump. The flow was controlled using a tuneable split capillary. All samples were injected on the set-up using a miniaturised homemade sample loop of 60 nL. Finally, the platform was coupled with a UV detection. Based on the injection of a mixture of myoglobin and lysozyme, the conditions of injection and separation were optimised. Then, the separation performance of each of these columns was assessed by evaluation of the peak capacity for different flow rates. The obtained results were compared to packed columns filled with 4 µm non-porous polymethacrylate particles inside 200 µm i.d. capillaries. The most promising columns were then used to profile a PPI network around human beta actin based on the interaction of 5 proteins.

Keywords: native LC, miniaturisation, monolith, packed bed, protein-protein interactions

POSTER

Semi-automated method for in-line removal of matrix components from food for the analysis of residual pesticides by LC-MS/MS

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In-line sample prep (ILSP) provides a semi-automated cleanup procedure for the analysis of pesticide residues in food by LC-MS/MS. ILSP selectively retains matrix components from the sample extract and can be utilized as a standalone workflow or integrated into an existing QuEChERS workflow. In these experiments, ILSP was applied to multiple challenging commodities representing a wide range of compositions including spinach, soybean meal, avocado, whole orange, black tea, and hibiscus tea for the analysis of 61 pesticides. This solution provides a novel, semi-automated approach to reduce the abundance of matrix components entering the analytical column and MS source resulting in a decrease in instrument contamination and an improvement in data quality.

Keywords: Sample Preparation, Automation, Pesticide Analysis

POSTER

A novel and reliable solid-phase extraction method for the simultaneous extraction of polar and non-polar organic micropollutants from wastewater

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Water pollution is one of the largest environmental threats as toxic waste produced by human activities, especially organic micropollutants (OMPs) originating from pharmaceuticals, pesticides, personal care products, and industrial compounds, easily enter water supplies through wastewater systems [1]. Current wastewater treatment methods are not efficient enough to fully degrade those pollutants, generating different degradation products along with their intact parent pollutants. These compounds often have diverse physicochemical properties and are typically contained in a complex matrix [2]. To assist in the identification and toxicological evaluation of such compounds, a dedicated analytical methodology was developed using a complex mixture of 57 representative OMPs determined in wastewater, covering different chemical species and a wide polarity range. For the separation and detection of these analytes, methods based on reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) coupled to a quadrupole time-of-flight mass spectrometer (Q-TOF MS), were developed and optimized. To reach trace concentrations of OMPs (ng/L – mg/L) in wastewater, in combination with these techniques, a solid-phase extraction (SPE) based sample preparation method was developed. SPE sorbents suitable for polar compounds including (HLB) (hydrophilic-lipophilic balanced), Isolute ENV+ (hydroxylated polystyrene-divinylbenzene copolymer), and GCB (graphitized carbon black) were individually evaluated for the retention of these analytes [3]. The results indicated that while most of the analytes were fully retained on each of the SPE sorbents, some of the very polar ones were only retained on one SPE sorbent. Therefore, to simultaneously extract non-polar, polar and very polar compounds, a multi-layer or mixed-bed SPE method, combining these sorbents, was developed and optimized in terms of type, order and bed size. Additionally, a suitable elution strategy was developed to obtain high extraction recoveries for these analytes. Finally, the developed and optimized method was validated and successfully applied for the analysis of real wastewater samples.

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Keywords: solid-phase extraction, LC-MS, non-polar and polar micropollutants, wastewater

POSTER

Comparison of high concentration capacity headspace technique for brewed coffee characterization follow by GC×GC-MS

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Volatile and semi-volatile components are highly relevant to characterize different samples, among which food. These compounds contribute to the peculiar aroma profile of foods and are widely used to assess quality and authenticity. The most common technique used to characterize the headspace of food samples is the used of high-concentration capacity (HCC) technique and in particular solid-phase microextraction (SPME). Recently HiSorb technique has been introduced, which can be considered an intermediate tool between SPME and stir-bar sorptive extraction (SBSE), allowing an easy automation as SPME but with higher sample capacity as SBSE.

The performance of the novel Hisorb probe have been compared with the traditional SPME approach. Time and temperature conditions were carefully optimized, and the possibility of performing multiple cumulative trapping was investigated as well. The analyses were performed on a multidimensional comprehensive gas chromatographic system coupled with a quadrupole mass spectrometer (GC×GC-qMS) and equipped with a Flow modulator. The fingerprinting

Keywords: SPME, HiSorb, GCxGC-MS, Coffee

POSTER

Analysis of chiral pharmaceuticals through hyphenation of temperature-responsive chromatography and ultrafast chiral chromatography

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Major differences in pharmacokinetic behaviour and pharmacological activity have been reported between enantiomers of chiral pharmaceuticals. As a result, each of the enantiomers of a chiral pharmaceutical should be considered as a single active compound according to guidelines published by the FDA and EMA. Therefore, proper separation and chiral recognition of molecules used as active pharmaceutical ingredients and their metabolites play an important role in comprehension of the way they work. (1,2)

Chiral pharmaceutical separations require high resolving power to achieve satisfactory separation of analytes in complex (biological or synthetic) samples. This can be, in theory, achieved by comprehensive two-dimensional liquid chromatography (LC x LC) as this would allow to significantly increase peak capacity per unit of time. In practice, However, finding compatible solvents for the second dimension, can be challenging. On the other hand, the relatively slow speed of chiral separations has limited the use of chiral stationary phases as the second dimension in 2D-LC, especially in the comprehensive model. (3,4,5)

In this study, the combination of temperature-responsive and reversed-phase chiral liquid chromatography is assessed in terms of enantioselective separation of a broad range of pharmaceutical compounds (log p: 0.9-4.1). Applying temperature-responsive liquid chromatography (TRLC) in the first dimension allows for analysis to be performed under purely aqueous conditions, and therefore allows for complete and more generic refocusing of organic solutes prior to the second-dimension separation. (3,4) Combination thereof with small particle (sub-2 micron) based chiral stationary phases as second dimension in TRLCxChiral-RPLC allows for the construction of a chiral screening platform which in principle offers much promise for solving chiral screening issues. (5)

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Keywords: two dimensional liquid chromatography, temperature-responsive liquid chromatography (TRLC), Chiral Separation

POSTER

A Fast method for lipids screening using TLC and Mass Spectrometry imaging

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Introduction: Lipids share diverse biological functions and locations in the body while being structurally vast class of compounds. Disturbances in lipid biosynthesis are involved in numerous human diseases such as cancer and neurodegenerative and cardiovascular diseases. Nowadays MALDI mass spectrometry imaging (MSI) has gained enough robustness to efficiently map the spatial distribution of lipids in histological thin sections. In the case of liquid extracts, (U)HPLC-MS is used for quantification. Alternatively, direct infusion allows rapid qualitative lipids pattern evaluation. In this work, we developed a new method for non-targeted and semi-quantitative lipids profiling using radial separation on thin layer chromatography plate (TLC) coupled with MALDI MSI detection.

Method: Fast lipid screening would ideally avoid pretreatment of biological samples such as liquid-liquid or solid-liquid extraction. Here we propose a method of direct transfer of the lipids to the TLC. MALDI suffers from ion suppression effects that lower the dynamic range of observable lipids from complex mixtures. A fast pre-separation of lipid families on the MALDI-TLC support should limit this drawback while adding an additional identification parameter: the retention factor. The lipid family is determined according to its polar head that are used to perform their separation on TLC plates, testing different thicknesses of the phase, both in linear and radial modes. The performances of the two modes are discussed. The MALDI images of the plates were recorded using either a TOF and FT-ICR mass analyzer. Kendrick Mass Defects filtering was used for fast and non-targeted data analysis.

Preliminary data: TLC operates with a normal phase allowing the separation of the different families of lipids according to the hydrophobicity of their polar heads. The nature of the eluent mixture was optimized and several separation steps can be performed sequentially. The lipids were directly transferred by sample-support contact. During sample preparation, two major factors were identified to affect the MS images: matrix application and silica thickness. The matrix application is a critical step as the matrix must reach the analytes that can be embedded inside the TLC silica pores. The optimal amount of sprayed matrix depends on the silica thickness and the co-crystallization efficiency was different according to the deposition method of the matrix solution. Fluctuations in topology and local accumulation of matrix had to be avoided to retain the optimal lateral resolution, mass resolving power, and mass accuracy. The choice of the mode of separation, linear or radial, was guided by practical considerations allowing improved sample handling and constrained duration MSI detection. Additionally, the radial mode allows a larger number of samples to be run on the same TLC plate with minimal sample handling. Rapid image acquisition of few lines of pixels passing through the center of each spots allowed the automated acquisition and provided twice the separation image on both sides of the diameter. Other separation directions are still available for replicates or further deeper analysis, including ion mobility measurements.

Keywords: Lipids, thin layer chromatography, MALDI imaging

POSTER

Volume and composition of semi-adsorbed stationary phases in hydrophilic interaction liquid chromatography

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Hydrophilic Interaction Liquid Chromatography (HILIC) is an alternative chromatographic mode for the separation of polar compounds that show weak retention in reversed-phase liquid chromatography (RPLC). Like normal-phase liquid chromatography (NPLC), HILIC employs traditional polar stationary phases, but mobile phases are similar to those employed in RPLC: a polar mixture of organic solvents and water.

Polar bonded phases present a high affinity for the water from hydroorganic mobile phases. Therefore, some water is adsorbed (and immobilized) on the surface of the stationary phase, followed by several semi-adsorbed (and partially immobilized) water-rich transition layers created between bonded and mobile phases. The main retention mechanism in HILIC is based on the liquid-liquid partition of solutes between the mobile phase and the immobilized or semi-immobilized water-rich layers. The thickness of the water-rich layers is related to the water content of the mobile phase, but also on the functionalization and the support (silica or polymer) of the bonded phase [1].

The mobile phase volume flowing through the column (hold-up volume) can be accurately measured by a homologous series approach derived from the Abraham's solvation model [2]. The behaviour of the homologous series (e.g., n-alkyl benzenes, n-alkyl phenones, or n-alkyl ketones) also provides information about the predominant retention mode of the column (HILIC or RPLC). In HILIC, retention decreases with the size of the homologue, whereas in RPLC increases. When using mobile phases with high and medium proportions of acetonitrile or methanol the column works as a HILIC column, but for mobile phases with less than 20% acetonitrile or 40% methanol the retention behaviour is RPLC. Even a dual HILIC-RPLC behaviour is observed with mobile phases containing intermediate mobile phase compositions. The total solvent volume inside the column (flowing and adsorbed) can be pycnometrically measured using solvents of sufficiently different densities (i.e. water and acetonitrile or methanol), since the solvent weight can be related to its volume and composition. From the column weight and the difference between total solvent volume (pycnometry) and the mobile phase volume (homologous series), the volume and mean composition of the water-rich layers can be estimated [3].

In the present work six columns with stationary phases made with several functionalizations on silica support have been studied: zwitterionic sulfobetaine and phosphorylcholine, aminopropyl, pentafluorophenyl, polyvinyl alcohol, and 1,2-dihydroxypropyl. Water and acetonitrile or methanol mixtures have been studied as mobile phases [4]. Zwitterionic and aminopropyl columns show the highest water adsorption followed by polyvinyl alcohol and 1,2-dihydroxypropyl columns. On the contrary, pentafluorophenyl column does not show any water uptake capacity nor a HILIC behaviour. Water adsorption is significantly larger for acetonitrile than methanol mobile phases.

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Keywords: HILIC, Hydrophilic interaction liquid chromatography, Homologous series, Pycnometry, Hold-up volume

POSTER

Development of a heart cutting 2D-LC method for the analysis of emerging micropollutants in wastewater

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Agriculture, industry and domestic activities around the world are releasing emerging micropollutants (EMs) in wastewater, leading to an increasing occurrence of these compounds in the environment. Their effects on the environment and human health are currently largely unknown. Therefore, wastewater treatment is required to eliminate these potentially toxic compounds. Conventional wastewater treatment plants (WWTPs) are, however, ineffective in removing EMs. Electrochemical Advanced Oxidation Processes (eAOP) are novel, chemical-free water treatment processes, which operate at ambient temperature and pressure and can be powered by green energy sources. eAOPs produce a wide spectrum of degradation products and therefore, a complete chemical and toxicological evaluation is mandatory to assure the safe application of eAOPs in water treatment. The analysis of EMs and their degradation products is challenging, due to their vast numbers, often unknown, their low concentrations and the complexity of the matrices they present themselves in, requiring highly selective and sensitive techniques.

For this, a heart-cutting 2D-LC set-up that combines orthogonal stationary phase columns is developed. This set-up uses a hydrophilic interaction liquid chromatography (HILIC) column in the first dimension and a reversed-phase (RP) column in the second dimension (HILIC x RP). The set-up furthermore consists of three high-pressure switching valves, two restriction capillaries, two T-pieces and one RP trap column. The non-polar compounds that are not retained on the HILIC column are redirected to a sample loop by a precise switching of the valves through a high resistance restriction capillary. Meanwhile, the polar analytes are retained and separated on the HILIC column and will go directly to the detector. Subsequently, the configuration of the valves is changed to direct the non-polar compounds from the sample loop to a RP trap column, while diluting the sample with an aqueous mobile phase via a low resistance restriction capillary. After another switch of the valves, the non-polar compounds are guided to the RP column for separation and subsequent detection.

To identify and quantify the EMs and their degradation products, a hybrid quadrupole time-of-flight (QTOF) mass spectrometer is used.

To evaluate the efficiency and robustness of this analytical technique, an artificial sample containing 50 widely used pharmaceuticals is used. The pharmaceuticals are selected in such a way that they cover a wide spectrum of polarity (LogP= -5 to 5) and can hence represent both EMs and their more polar degradation products.

The enlarged peak capacity of the final 2D-LC-QTOF-MS method will be used to separate and identify EMs present in wastewater and/or surface waters and their potential degradation products generated by eAOP.

Keywords: Multi-dimensional techniques, Liquid Chromatography, Mass spectrometry, Environmental analysis, Pollutants electrochemical degradation

POSTER

Predicting retention on SPAM columns for their implementation in LC x LC

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Liquid chromatography (LC) is a key technique in the arsenal of an analytical chemist. However, unlike other separation techniques, such as capillary electrophoresis (CE) and gas chromatography (GC), LC does not provide a very high efficiency. As a result, relatively low peak capacities are obtained, which impairs adequate separation of complex samples. For the latter, comprehensive two-dimensional liquid chromatography (LC×LC) is more appropriate. The peak capacity can be increased significantly when going from one-dimensional LC (1D-LC) to LC×LC. Moreover, two very different (orthogonal) LC modes can be used to target different chemical properties of the sample. In LC×LC many fractions of the first dimension (1D) effluent are transferred to the second dimension (2D) column. Typically, a loop-equipped modulation valve is used to facilitate this transfer. However, disadvantages of this approach are potential incompatibility issues between the 1D and 2D solvent systems and successive dilution of the sample, which reduces sensitivity. Fortunately, these problems can be overcome by using an active-modulation strategy, such as stationary phase-assisted modulation (SPAM). In SPAM, low-volume trapping columns (e.g. guard columns) are used instead of loops with relatively large volumes. In this approach the 1D eluent is allowed to flow through the trapping column to waste, while the analytes are effectively retained. The analytes are then eluted from the trapping column in narrow bands through application of a 2D gradient.

The major requirement for SPAM is that the analytes must be retained on the trapping column while the1D eluent passes through. Conventionally, a dilution flow is used to decrease the elution strength of the 1D effluent and, thus, increase the retention of the analytes on the trapping column. Usually, this dilution flow is randomly chosen, and it does not preclude the possibility of losing early eluting compounds.

In this project, the application of retention modelling was investigated to predict the retention of compounds on a guard column, using scanning-gradient data from an analytical column. First, the retention factors on the analytical and guard columns were compared to evaluate the similarity between the two columns. Next, scanning gradients were performed on an analytical column and several semi-empirical models were established. The obtained retention parameters were used to predict the retention of the same compounds in isocratic elution from the guard column, so as to assess the performance of the models. Moreover, the set of scanning-gradient experiments was optimized to decrease the prediction error. Using the optimal set of experiments, predictions were made and evaluated for (simulated) dilution flows and an optimization strategy for applying SPAM in LC×LC was developed.

Keywords: stationary phase assisted modulation, retention modeling, two-dimensional liquid chromatography, method optimization, active modulation

POSTER

Comprehensive suspect screening for the identification of contaminants of emerging concern in urine of Flemish adolescents through LC-QTOF-MS

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Biomonitoring studies, such as the Flemish Environment and Health Study (FLEHS IV, 2016-2020), aim to assess human exposure to environmental chemicals. Whilst these studies are of high importance for the collection of quantitative data on the exposure to known contaminants, unknown or recently discovered contaminants of emerging concern (CECs) remain undetected. Since the toxicity of many CECs or their influence on the environment and humans are not yet well understood, they are not extensively included in (bio)monitoring programs. Therefore, complementary analytical approaches are needed to document the occurrence of CECs in humans. Suspect screening approaches based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) are valuable tools for the identification of CECs and their metabolites. LC-HRMS allows the simultaneous acquisition of HRMS data and MS/MS fragmentation spectra for a high number of analytes providing spectral information used for compound identification. The acquired data can subsequently be matched against a predefined list containing CECs suspected to be present in the samples (suspect list). The suspect list can also include metabolites of CECs predicted based on modifications of known contaminants or known metabolization pathways to allow the simultaneous detection of a high number of compounds.

The present study aimed to identify CECs and their metabolites not included in previous target FLEHS biomonitoring studies. Urine samples of 83 Flemish adolescents participating in the FLEHS IV (2016-2020) were analysed applying sample preparation and suspect screening methods based on a previously published approach with modifications [1]. After analysis in both negative and positive ionization modes, chromatograms were aligned and deconvoluted. A fold change (FC > 5) analysis was applied for the mass features by comparing urine samples with procedural blanks. Upregulated features were matched against the suspect list developed in the scope of this study. The suspect list included > 3,500 CECs from several compound classes, such as (alternative) plasticizers, organophosphate flame retardants, synthetic antioxidants, UV-light stabilizers, pesticides, and others. Additionally, metabolites of all included parent compounds were predicted and added to the suspect list. Thereby, metabolic reactions corresponding to oxidation (Phase I), glucuronidation, acetylation and methylation (all Phase II) were considered. This resulted in a suspect list containing > 15,000 compounds.

Data analysis was carried out applying two complementary approaches. Thereby, obtained MS/MS spectra were matched against open-source and in-house libraries allowing compound identification with the highest confidence levels of 1 according to Schymanski et al. [2]. The identified CECs and metabolites were compared with the list of target analytes available for the investigated samples from the FLEHS IV study. These results aimed to investigate the additional value of suspect screening for the analysis of human exposure to CECs and to potentially discover new metabolites to be added to the list of target analytes of upcoming FLEHS studies.

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Keywords: High-resolution mass spectrometry, Suspect screening analysis, Chemicals of emerging concern, Biomonitoring, FLEHS IV

POSTER

Development of a multi-attribute platform for monoclonal antibody characterization

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Monoclonal antibodies (mAbs) have revolutionized the treatment of life-threatening diseases and conquered the pharmaceutical market. mAbs are large, complex and heterogeneous molecules. One mAb product may comprise tens to hundreds of variants with respect to e.g. N-glycosylation, N- and C-terminal motifs, amino acid composition, disulfide bonds, fragmentation, and aggregation. This plethora of modifications defines the product and determines its quality. Consequently, many critical quality attributes (CQAs) have to be monitored during mAb development and subsequent production. Currently, each CQA requires a specific analytical approach. For example, size variants of mAbs are resolved at the intact protein level using SEC-UV. Amino-acid sequence variants are determined at the peptide level using RPLC-MS/MS after off-line enzymatic digestion of the mAb. These approaches often involve manual sample preparation that is error-prone, but crucial for in-process samples containing e.g. cellular debris, host proteins, and lipids. Recent developments in two-dimensional (2D) LC offer the possibility to perform high-resolution analysis on mAbs at the intact, sub-unit, and peptide level. This is the core of the current study, which aims to establish a fully integrated multi-attribute platform for in-depth CQA assessment of mAb samples from upstream/downstream processes and complex formulations using 2DLC technology.

The developed platform combines affinity chromatography purification with various LC modes and immobilized enzyme reactors (IMERs) to perform intact, sub-unit, and peptide level analysis on mAbs. The intact workflow involved Protein A chromatography (ProtA) coupled with SEC, RPLC, and IEX separations. ProtA was used to isolate the mAbs from cell culture media and a valve allowed multiple heart-cuts of the mAb band into storage loops. These fractions were successively analyzed by the second dimension chromatographic systems. Using UV absorbance and MS detection, CQAs related to exact mass, aggregation, glycosylation, and charge variants could be determined reliably in less than 60 min. For the middle-up workflow, an immobilized enzyme reactor (IMER) holding the protease IDeS was placed between the first and second LC dimensions. As the ProtA eluent is not compatible with the IMER digestion conditions, a strong cation exchange trap was introduced before the IMER to facilitate a solvent switch. The trapped antibody was subsequently flushed through the IMER at low flow to the RPLC column. The resulting mAb fragments were preconcentrated on the head of the column and subsequently separated. This approach allowed a detailed characterization of F(ab)2 and Fc/2 fragments including PTMs like glycoforms in less than 35 min. For the bottom-up workflow, a trypsin IMER was added to the platform to digest the isolated mAb. Again, a trap-and-elute approach was used to tackle incompatibility issues of ProtA and trypsin IMER conditions. RPLC-MS was used to analyze the resulting tryptic peptides. A protein sequence coverage of up to 85% was obtained within a total analysis time of 90 min. To conclude, Three fully online mAb-characterization workflows can be run on the developed 2D platform in less than 5 hours, enabling the determination of a large number of CQAs from single injections of in-process mAb samples requiring no sample preparation.

Keywords: monoclonal antibody, proteins and peptides, biopolymers

POSTER

Revealing charge heterogeneity of stressed trastuzumab at the subunit level

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Trastuzumab is known to be heterogeneous in terms of charge. Stressing trastuzumab under physiological conditions (pH 7.4 and 37oC) increases charge heterogeneity further. In our previous study, we have shown that the separation of charge variants of stressed trastuzumab at the intact protein level is challenging due to increasing complexity [1]. Here we report an approach for revealing charge heterogeneity of stressed trastuzumab at the subunit level by chromatographic separation after GingisKHAN® digestion. The basic pI of Fab fragments generated after enzymatic digestion allowed us to use the same pH gradient buffers for both intact protein and Fab level analysis. Fab fragments were fractionated by cation-exchange chromatography and the corresponding modifications assigned by LC-MS/MS peptide mapping. The reported approach here allows in-depth characterization of charge variants of antibodies that have two or more modification sites in the Fab region.

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Keywords: trastuzumab, charge variants, cation-exchange chromatography, pH gradient separation

POSTER

3D-printed system for dispersive solid phase extraction

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Dispersive solid phase extraction (DSPE) is considered a beneficial sample preparation in comparison with traditional solid phase extraction, mostly due to its simplicity and high recoveries. However, in a typical procedure extract has to be manually aspirated from a vessel containing centrifuged particles of sorbent. This step can lead to contamination of chromatograph and cause loss of analyte. Due to the abovementioned disadvantages, DSPE technique is much more popular in the removal of unwanted substances rather than in the extraction of compounds of interest.

We propose a new design of a set of simple vessels manufactured by 3D printing that help to overcome the main drawbacks of a typical DSPE sample preparation protocol. The set of four fully printed parts is made of polypropylene with fused deposition modeling technique. The set is aimed to perform the extraction without leakage, unwanted evaporation, and most importantly avoiding contamination of the final extract with sorbent particles. We show that a complete analytical system can be designed, improved, manufactured and applied within one laboratory at a low cost. The usefulness of the developed sample preparation vessel is demonstrated on an example of imipramine extraction and compared with the performance of the protocol lacking 3D printed parts.

Keywords: sample preparation, dispersive solid phase extraction, 3D printing

POSTER

Doubly Hyphenated Method (LC-MS/MS-Fluorometric) for Determination of Nucleoside Triphosphates and Analogs in Peripheral Blood Mononuclear Cells (PBMCs)

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We report a validated doubly hyphenated technique (LC-MS/MS-fluorometry) for determination of nucleoside triphosphates and analogs (TPs) in peripheral blood mononuclear cells (PBMCs). The analytical process involves sequential lysis of a PBMC sample, extraction and selective determination of TPs in the total extract volume by LC-MS/MS, and subsequent determination of sample cell counts by use of a DNA-specific fluorogenic intercalant to flourometrically quantify DNA in the residual pellet of the extracted sample. The amount of DNA is calibrated by standards prepared from commercially available purified reference human genomic DNA. Since a known, constant amount of DNA is present in every human PBMC (and within ±1.5% between males and females), the amount of DNA in a PBMC sample can be used to determine the number of PBMCs in the sample. The overall amount of TPs in a PBMC sample can then be presented as mass of TPs per million cells, which can be further converted to an intracellular molar concentration of TPs by use of the known mean volume of a PBMC and the TP molecular weight. Although the initial PBMC sample lysis for TP extraction and the corresponding TP quantification method need to be developed and validated for the specific TP(s) of interest, the subsequent determination of PBMC number in the residual pellet sample applies to any PBMC sample, regardless of drug analyte; therefore validation and application of the cell count method is independent of drug analysis and can be performed once.

As an example, we present a hyphenated method validated for simultaneous determination of tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) in a PBMC sample by LC-MS/MS and determination of the PBMC count by fluorometric detection. Both TFV-DP and FTC-TP are TP metabolites of dosed prodrugs (tenofovir alafenamide fumarate and emtricitabine, respectively) that are administered in combination as treatment for HIV. Validation results for both the analyte and PBMC components of the LC-MS/MS-fluorometric hyphenated method are summarized and meet relevant expectations of regulatory guidances for bioanalytical methods.

Keywords: LC-MS/MS-fluorometry, PBMCs, cell counting, nucleoside triphosphates

POSTER

Performance Limits and Design Aspects for Spatial Comprehensive Three-Dimensional Isoelectric Focusing × Size-Exclusion Chromatography × Reversed-Phase Liquid Chromatography for the Analysis of Intact Proteins

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Spatial comprehensive three-dimensional chromatography (3D-LC) is an emerging separation technology that can potentially offer unprecedented resolving power. The maximum peak capacity that can be generated corresponds to the product of the three peak capacities of the individual development stages, provided that orthogonal retention mechanisms are applied. When considering protein analysis and separation in the space domain, there are limitations on which modes can be combined. Furthermore, based on the choice of separation mechanisms and experimental conditions applied in the three individual developments, the design requirements, i.e., the number of channels and the channel length should vary. Here, we study the advantages and disadvantages that these aspects can bring when designing a spatial 3D-LC chip targeting protein analysis.

Based on Pareto optimization we discuss the optimal chip design and assess the performance limits of a spatial 3D-LC device operated in xLC× xLC× tLC mode, combining isoelectric focusing (IEF), aqueous size exclusion chromatography (SEC) and gradient reversed-phase (RP) in the first (1D), second (2D) and third dimension (3D) respectively. Peak capacities and peak production rates are calculated for each individual dimension and the influence of the channel diameter and length on these objectives is studied, taking into account the spatial nature of the development in the first two dimensions. Moreover, the effect that the number of channels in each dimension has on the obtained separation of the previous one is investigated using a newly proposed equation to calculate the loss in peak capacity due to suboptimal sample transfer.

Keywords: Pareto-optimality, spatial 3D-LC, microfluidics

POSTER

Glycosaminoglycan alterations in benign prostate hyperplasia and prostate cancer

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Glycosaminoglycans (GAGs) are large linear polysaccharides bearing different sulfation motifs along the chain according to their functions. They are mainly attached to proteins forming proteoglycans. The quantity and sulfation of GAGs can substantially change under different physiological conditions, the most interesting of which are diseases like cancer. However, more research has to be performed in the upcoming years to determine the exact changes and the biological information they carry. Thus, we decided to investigate the GAG content of benign and malignant prostate diseases.

We have investigated the chondroitin-sulfate (CS) and heparan-sulfate (HS) content of samples originating from patients with benign prostate hyperplasia (BPH) (n=16) and prostate cancer (low (n=20), intermediate (n=16) and high (n=16) risk groups based on Gleason grading). Risk groups were balanced for age. The GAG chains of the tissues was degraded into the disaccharide building blocks using bacterial lyase enzymes and then purified in a graphite-based pipet tip SPE system. For the measurements, we used a nanoHPLC-MS/(MS) method which was previously developed [1] by our group using HILIC-WAX capillary column with ammonium formate salt gradient for separating and quantifying GAG disaccharides.

We found that both the overall quantity and the sulfation motifs of HS chains were the same among the investigated groups. CS chains, however, showed significant changes. The total quantity of CS monotonously increased from BPH through cancer progression. This implicates the upregulation of CS-bearing proteoglycans and importance of further investigation. The non-sulfated CS disaccharide showed a significant decrease with cancer progression. The singly sulfated components exerted a shift: while the relative abundance of 4-O-sulfated disaccharide decreased monotonously from BPH to high risk prostate cancer, the opposite could be observed for 6-O-sulfated one. Furthermore, 6-O-sulfated disaccharide changed significantly between BPH and all the different severity groups of prostate cancer.

Finally, we investigated whether the overall survival of the patients can be estimated based on glycosaminoglycan levels. We found that the non-sulfated and doubly sulfated CS disaccharides and the doubly and triply sulfated HS disaccharides along with the total HS quantity carry potential to survival estimation.

Our findings provide a good basis for future potential glycosaminoglycan-based classification of prostate malfunctions.

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Keywords: cancer, glycosaminoglycan, prostate, HPLC-MS

POSTER

Heart-cut two-dimensional liquid chromatography with online photodegradation

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Organic compounds can change under the influence of light. It is undesirable in food, when healthy food ingredients (e.g. vitamins) degrade or when toxic components are formed. Food samples are complex mixtures of thousands of compounds, making analysis difficult. When any of these compounds undergo photodegradation, the complexity of the sample increases further, which makes them even more challenging to analyze. It can be difficult to establish a strong link between the degradation products and the starting compounds, which results in poor degradation-prediction models. Moreover, large sample volumes and long analysis times are often needed.

Within the TooCOLD project, Toolbox for studying the Chemistry Of Light-Induced Degradation, a light cell has been developed to perform online photodegradation. This cell is based on the liquid-core-waveguide (LCW) principle. The sample is illuminated along the channel [1]. The LCW cell consists of an inner tubing made of Teflon AF, placed inside an outer tubing. Teflon AF is an amorphous and translucent polymer, made from tetrafluoroethylene (TFF) and 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole (PDD). It has a refractive index (RI) of 1.29-1.31, which is lower than that of water (RI = 1.33), methanol (RI = 1.333), and acetonitrile (RI = 1.34). This implies that light introduced at an appropriate angle to a liquid-filled LCW cell will travel through the cell due to total internal reflection.

This novel LCW cell was coupled online to a liquid-chromatography separation and the photodegradation efficiency was compared with that of other, more established, photodegradation techniques. The degradation of components in the liquid core is faster than with other techniques, due to higher light intensities. Also, no sample preparation is needed, and low sample volumes are possible (60 µl). In this project, the LCW cell was inserted at the transfer stage in a multiple-heartcut two-dimensional liquid chromatography (mHC-2DLC) method. First, a multiple-heartcut 2DLC method was optimized with an elongated second dimension. Next, the LCW cell was coupled between the multiple-heartcut valve (mHCV) and a second six-port valve, coupled to the second dimension. To demonstrate the system, a mixture of several organic colorants was chosen. This mixture was separated in the first dimension and the peaks of interest were transferred to the loops in the mHCV. The separated compounds were degraded in the LCW cell and then transferred to the second-dimension separation. The degradation products were identified in the final effluent.

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[2] M.J. den Uijl, A. Lokker, B. van Dooren, P.J. Schoenmakers, B.W.J. Pirok, M.R. van Bommel, Comparing different light-degradation approaches for the degradation of crystal violet and eosin Y, Dye. Pigment. (2021) 109882. https://doi.org/10.1016/j.dyepig.2021.109882.

Keywords: light degradation, online reaction, food dyes, cultural heritage, two-dimensional liquid chromatography

POSTER

Probing the effects of cortisol and specific receptor involvement in zebrafish larvae using a novel capillary electrophoresis-mass spectrometry metabolomics workflow

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The zebrafish (Danio rerio) is increasingly used as a model system in biomedical research. Zebrafish embryos and larvae can fill the gap between cultured cells and mammalian animal models, because they can be obtained in large numbers, are small in size and can easily be manipulated genetically. Given that zebrafish larvae are intrinsically material-limited samples, we have developed a new capillary electrophoresis-mass spectrometry (CE-MS) workflow for profiling (endogenous) metabolites in extracts from individual zebrafish larvae and pools of small numbers of larvae. During method development, special attention was paid to the selection of an appropriate homogenization and efficient metabolite extraction strategy.

The developed CE-MS workflow was used to analyse the metabolite levels in extracts from pools of 1, 2, 4, 8, 12, 16, 20 and 40 zebrafish larvae, and showed a linear response (R2>0.97) for at least 11 selected endogenous metabolites. The repeatability was satisfactory, with inter-day RSD values for peak area of 9.4-18.1% for biological replicates (n=3 over three days). Furthermore, the method allowed analysis of over 70 endogenous metabolites in a pool of 12 zebrafish larvae, and still about 30 endogenous metabolites in a single zebrafish larvae, thereby showing the value of CE-MS for probing metabolic changes at an individual level and studying individual variation within a population (rather than average readouts). This also enables us to correlate the metabolic profile of an individual larva with other phenotypical traits, such as behavioral readouts.

Just like humans, zebrafish secrete the hormone cortisol upon the perception of a stressor. Two receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) mediate the action of cortisol. These receptors have different affinities for cortisol, with GR being activated at high cortisol levels, and MR being already activated at basal cortisol levels. The involvement of GR and MR in mediating the effects of cortisol on the metabolite levels in zebrafish larvae was investigated by using GR and MR knockout zebrafish. Our results revealed distinct metabolic profiles for the analysis of extracts from a pool of 12 zebrafish larvae among the various groups, which enabled us to determine distinct roles for GR and MR in mediating the metabolic effects of cortisol.

Keywords: Capillary Electrophoresis, Glucocorticoid receptor, Metabolomics, Mineralocorticoid receptor, Zebrafish larvae

POSTER

Development and validation of a targeted LC-MS/MS quantitation method to monitor cell culture expression of tetanus neurotoxin during vaccine production

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The tetanus neurotoxin (TeNT) is one of the most toxic proteins known to man, which prior to the use of the vaccine against the TeNT producing bacteria Clostridium tetani, resulted in a 20% mortality rate upon infection. The clinical detrimental effects of tetanus have decreased immensely since the introduction of global vaccination programs, which depend on sustainable vaccine production. One of the major critical points in the manufacturing of these vaccines is the stable and reproducible production of high levels of toxin by the bacterial seed strains. In order to minimize time loss, the amount of TeNT is often monitored during and at the end of the bacterial culturing. The different methods that are currently available to assess the amount of TeNT in the bacterial medium suffer from variability, lack of sensitivity, and/or require specific antibodies. In accordance with the consistency approach and the three Rs (3Rs), both aiming to reduce the use of animals for testing, in-process monitoring of TeNT production could benefit from animal and antibody-free analytical tools. Here, we describe the development and validation of a new and reliable antibody free targeted LC-MS/MS method that is able to identify and quantify the amount of TeNT present in the bacterial medium during the different production time points up to the harvesting of the TeNT just prior to further upstream purification and detoxification. The quantitation method, validated according to ICH guidelines and by the application of the total error approach, was utilized to assess the amount of TeNT present in the cell culture medium of two TeNT production batches during different steps in the vaccine production process prior to the generation of the toxoid. The amount of TeNT generated under different physical stress conditions applied during bacterial culture was also monitored.

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Keywords: 3Rs principles, Antibody free protein quantification, Consistency approach, Targeted LC-MS/MS, Tetanus vaccine

POSTER

Closing the Loop in Automated Liquid Chromatographic Method Development

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Method development for liquid chromatographic (LC) separations often involves intensive labor over the span of multiple days – if not weeks. To minimize the method-development time, smart solutions continue to be developed. One example is the use of retention modelling, where retention times of compounds are modelled as a function of mobile-phase compositions (e.g. based on multiple isocratic or gradient runs) and allow for the prediction of analyte retention at different compositions. Various models have been developed to increase the accuracy of prediction. Using such retention models for each sample component, entire separations can be predicted for a given combination of method parameters. By comparing different simulated separations, optimal method parameters may be obtained. However, the dimensionality of this problem increases with each additional method parameter that is included in the optimization. Several research groups have devoted attention to developing tools that solve this problem. One example is the implementation of evolutionary algorithms to examine the parameter space for multi-step gradients1. While method optimization approaches have been extensively investigated with various success, their implementation in fully automated workflows remains difficult.

In the present research, a method-development algorithm was developed for automated optimization of reversed-phase LC separations of a dye mixture, employing diode-array detection (DAD). This was performed using an in-house built interface that allows the algorithm to communicate with and control the LC system. The entire workflow will be presented and its various components will be explained, namely: peak detection and peak tracking to extract retention times from the LC-DAD data, calculation of the optimal gradient profile using an optimization algorithm, and automated application of the algorithm to the separation of the dye mixture.

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Keywords: Retention Modelling, Method Development, Automation, Liquid Chromatography, Gradient Elution

POSTER

Chromatography and mass spectrometry to study cleaning effects on Asian lacquered cultural heritage objects

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Durable Anacardiaceae thermosetting polymers transform upon light ageing into extremely sensitive carboxylated surfaces. The polymeric chemistry, consisting of enzyme catalysed catecholic macromolecular matrices, concurrently dispersed with glycoproteins and polysaccharides, is complex and largely understudied. Cleaning of such degraded Asian lacquer surfaces of cultural heritage objects utilizing solvents and aqueous solutions becomes hence a high-risk procedure.

To evaluate the effect of solvents on the degraded lacquer surface, solid samples from aged lacquer mock-up surfaces and a historical object are analysed by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), before and after cleaning using different solvents and water. Solvent and aqueous extracts are analysed by state of the art gas chromatography-mass spectrometry (GC-MS) and liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), providing not only detailed information on extracted compounds, but also on degraded compounds which allows to hypothesize on degradation pathways. Pooling data of surface- and leachables analyses, in combination with artificial ageing cycles after cleaning allows to predict long term effects of the cleaning procedures.

Keywords: Ancardiaceae polymers, Pyrolysis-GC-MS, Cultural heritage analysis

POSTER

Chemical speciation of Chromium in Water Samples: Method Development and Validation

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Chromium (Cr) is a transition element that exists in oxidation states ranging from −2 to +6. The common stable ones in the environment are trivalent Cr(III) and hexavalent Cr(VI) chromium. Cr(III) is an important micronutrient for the human body, while Cr(VI) is highly toxic and carcinogenic. The environmental concentrations of both oxidation states is low. Due to the differences in toxicity between Cr(VI) and Cr(III) compounds, speciation of Cr is very important. Therefore, an improved sensitive and robust method for the simultaneous determination of Cr(III) and Cr(VI) in water samples has been developed. The method uses a hyphenated micro liquid chromatography (µLC) system coupled to inductively coupled plasma mass spectrometry (ICP-MS). The optimised method incorporates a pH adjusted EDTA complexation step to stabilise Cr(VI) and Cr(III). The µLC system uses an anion exchange micro-sized column to separate the Cr species. Cr(III) and Cr(VI) were separated with different retention times at 170 and 230 sec, respectively. The method was optimized and validated by spiking Cr(III) and Cr(VI) in various water samples. Furthermore, the method was validated using a drinking water proficiency testing material sample. The developed method can be used for rapid routine determination of chromium species with high precision and reliability.

Keywords: speciation, chromium, water, method development

POSTER

Sample pretreatment and determination of sodium, potassium, calcium, magnesium and chloride in parenteral nutrition solution by ion chromatography

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Parenteral nutrition (PN) is administered intravenously and it contains essential nutrients such as electrolytes, glucose, amino acids and trace elements. These nutritional solutions are typically prepared in hospital pharmacies. To avoid the risk for patients caused by errors in the electrolyte concentrations, quality control is necessary for every batch of PN solutions. Currently, sodium, potassium, magnesium and calcium are usually analyzed by potentiometric methods. However, the repeatability and accuracy of these methods are rather poor and the large amount of amino acids in PN solutions may interfere with the analysis of the ions. Therefore, other analytical techniques are required.

In this work, ion chromatography (IC) was applied for analysis of the ions (sodium, potassium, calcium, magnesium and chloride). Since IC is known to be prone to interference from ions from other sources like glassware, precautions had to be taken.

For the determination of the cations, a total ash method was applied as sample pretreatment to remove the interfering amino acids from the PN solution. During the development of the total ash method, the influence of different acidic solutions, heating temperature and crucibles was studied. An acidic solution was added before heating to provide an acidic environment that facilitated charring. Methanesulfonic acid (MSA) was chosen because MSA introduced less interference compared to nitric acid and sulfuric acid. After charring, an acidic solution was necessary to dissolve properly the residue. Hereto, 30 mM MSA was added since this is also used as mobile phase. Three heating temperatures (500 °C, 550 °C and 600 °C) were compared. Heating at 550 °C for 1 hour was the optimal condition considering charring efficiency. Concerning the containers, quartz crucibles were preferred for total ash since they released no interfering substances and showed higher temperature tolerance compared to polytetrafluoroethylene (PTFE), porcelain and aluminum oxide (Al-24) crucibles.

In a next step, the IC method was validated. The method showed good linearity with correlation coefficients > 0.999. Relative standard deviations for six replicates were 0.2 % – 0.6 % for repeatability and 0.3 % – 0.9 % for intermediate precision. The recovery values amounted to 99.1 % – 101.2 %. Finally, the method was successfully applied to analyze inorganic ions in PN solutions.

Keywords: sample pretreatment, PN solution, inorganic ions, ion chromatography

POSTER

Online supercritical fluid extraction – supercritical fluid chromatography hyphenated to mass spectrometry (SFE-SFC-MS) for nonpolar and polar compounds from milk thistle seeds

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Plants, as milk thistle (Silybum marianum), are a valuable source of polar and nonpolar compounds with interesting biological effects. The main nonpolar compounds are triglycerides, which are also the main components of all vegetable oils. They are an essential part of human nutrition and their imbalance can cause several diseases. In addition, specific polar compounds – flavonolignans, have been found in large amounts in a standardized extract from milk thistle. Flavonolignans are a group of organic chemical compounds that are derivatives of flavonoids and have different biological activity. For example, silymarin is well known for its anti-hepatotoxic and anti-inflammatory properties and has been used as a natural cure for centuries.

In order to extract and analyse both nonpolar (triglycerides) and polar compounds (flavonolignans) from milk-thistle seeds through a unique methodology, an online supercritical fluid extraction – supercritical fluid chromatography (SFE-SFC) method was developed. Due to direct on-column transfer of the extracted compounds to SFC, different transfer approaches were compared to enhance transfer quality. In this respect, nonpolar and polar compounds caused different issues, especially as polar compounds required a significant portion of co-solvent in the extraction. Indeed, this step could be problematic for the top-column SFC trapping.

First, online extraction kinetic measurements were conducted to measure (i) the time required to achieve complete defatting of the seeds before starting to extract the flavonolignans, then (ii) the time required to achieve the extraction of flavonolignans.

Once the appropriate extraction times were determined, the analysis of triglycerides was performed thanks to a highly selective SFC method using 5 tandem C18 columns and atmospheric pressure chemical ionization mass spectrometry (APCI-MS). Then the polar fraction of flavonolignans containing several isomers (including positional isomers and diastereomers) was successfully analysed in SFC on a chiral stationary phase (Chiralpak IB).

The aim of this presentation is to discuss the versatility of online SFE-SFC and how challenging the coupling can be, especially when both non-polar and polar molecules must be analysed in a single sample.

Keywords: supercritical fluid chromatography (SFC), supercritical fluid extraction (SFE), on-line SFE-SFC, hyphenated systems

POSTER

Exploitation of the modulation freedom by on-column peak refocusing in TRLC×RPLC – speeding up 2D-LC via combined temperature and flow rate gradients

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In recent years, TRLC was introduced as a separation mode that offers interesting features when implemented in the first dimension (1D) of 2D-LC. The separation mode is based on the coupling of stimuli-responsive polymer to the silica supporting material. Commonly, poly(N-isopropyl acrylamide) is used, which depicts increasing retention of hydrophobic compounds for rising temperatures. One of the major benefits of this type of stationary phase is that these effects occur in water, thus allowing for the usage of purely aqueous mobile phases. In combination with RPLC, this establishes a great advantage in terms of robust modulation, as full solute refocusing at the 2D column head is obtained without methodical complexity for solutes which can be retained in RPLC. With more commonly used LC×LC combinations, this transfer of 1D effluent is often hampered due to the peak distorting effects of transferring high organic content. It was shown in TRLC×RPLC, that the volume transferred can be chosen freely, tested for transfer volumes of 10-2000 µL without peak broadening. The combination of the analyte refocusing and the resulting almost unlimited transferable injection volumes in 2D also paves the way for the implementation of flow rate gradients in the 1D. In this way, in this work, the possibilities offered by TRLC in 2D-LC are explored to reduce analysis times while maintaining or improving the overall method performance. This can be done via the usage of single-component type gradients (comprising either a decreasing temperature or increasing flow rate gradient) or combined dual-component gradients (combining temperature and flow rate gradients). It is shown that a change in flow rate (e.g. from 100 – 400 µL/min) and the consequent increase in modulation volume has little to no effect on the 2D separation. The possibilities of the approach are demonstrated through the separation of representative mixtures of small pharmaceuticals and food additives.

Keywords: 2D-LC, TRLCxRPLC, modulation freedom, flow rate gradient

POSTER

Solving the dilution problem in LC×LC via exploitation of the solute refocusing effect in TRLC×RPLC

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Achievable sensitivity of online 2D-LC systems is still hampered compared to conventional (U)HPLC, as sensitivity is often traded off to increase peak capacity. This limitation is mostly imposed by the modulation problem. To maximize orthogonality, less compatible separation modes are combined. Thus, solvents of high elution-strength are transferred between the two dimensions. A possible solution is the use of narrow columns in the 1D, maintained at low flow rates to naturally decrease the transferred volumes, but consequently, sample-loading capacities are also lowered. To minimize these effects and allow for fast gradients, the second dimension (2D) column then often must be rather broad, operated at high flow rates. The combination of a 1 mm column in the 1D paired with a 4.6 mm column in the 2D, however, leads to a 20-fold loss in sensitivity for concentration-sensitive detectors due to the dilution of the sample in the 2D flow when assuming comparable flow velocities. The latter is in reality even further exacerbated in LC×LC due to the shorter residence times in the fast flow operated second dimension.

RPLC×RPLC allows for some more freedom in column choices, nowadays more often a 2 mm ID column is combined with a 3 mm ID column, also lowering detection sensitivity. Rarely, 2 mm columns are implemented in the 2D, as this is mostly only applicable if aqueous mobile phases are used in the 1D, e.g in IEX×RPLC. Nevertheless, it was shown that 2D-LC is promising, also for the determination of low abundant impurities at a 0.05% level in pharmaceutical quality control if methodical parameters are optimized for high sensitivity. Here, temperature-responsive liquid chromatography (TRLC) is paired with RPLC. Such stationary phases allow for separation in a purely aqueous mobile phase, which solves some of the methodical complexities discussed above. Because full solute refocusing at the 2D column head is obtained in TRLC×RPLC, the transferred volume of 1D effluent is not a determining factor. Thus, the dilution problem experienced with all concentration-sensitive detectors in comprehensive 2D-LC offers the prospect to be overcome, as columns of the same diameter in both dimensions can be used (2.1×2.1 mm I.D.). Optimally, the detrimental dilution problem can even be inversed towards a refocusing based sensitivity enhancement, by combining a broader 1D column with a narrower 2D column (2.1×1 mm I.D.). In this work these aspects are demonstrated through comparative LOD determinations for various column I.D. combinations in comprehensive TRLC×RPLC-UV. Addiontally, split-less transfer of the 2D effluent to the MS from the 1 mm column is introduced. This strategy allows more facile and more sensitive 2D-LC-MS/UV implementations.

Keywords: LCxLC, TRLCxRPLC, analyte refocussing

POSTER

Design of next-generation polymer-monolithic stationary phases targeting ultra-high resolving power of peptides, proteins, and oligonucleotides

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Polymer monolithic stationary phases have emerged as an good alternative for packed column formats. To increase the resolving power of packed bed columns, the particle size can be optimized. However, downscaling the particle size leads to increase in pressure proportional to the fourth power. The porous structure of polymer monoliths can be to some extent optimized such that macropore size and globule size are tuned independently. This study involves the optimization of the macropore structure of poly(styrene-co-divinylbenzene) monoliths and their performance assessment for biomolecule gradient separations. Effects of flow rate and gradient duration on peak capacity and chromatographic retention have been studied and a performance comparison has been made using a commercially-available monolithic capillary column and a significant increase in column performance was observed. The potential of a novel monolithic stationary phase developed in 100 µm i.d. column formats have been demonstrated for high-throughput and high-resolution LC-MS analysis of proteomic sample mixtures on the peptide level, and emerging therapeutic biomolecules including monoclonal antibodies, and single- and double-stranded oligonucleotides.

Keywords: proteomic, nanoLC, monoliths, oligonucleotides

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