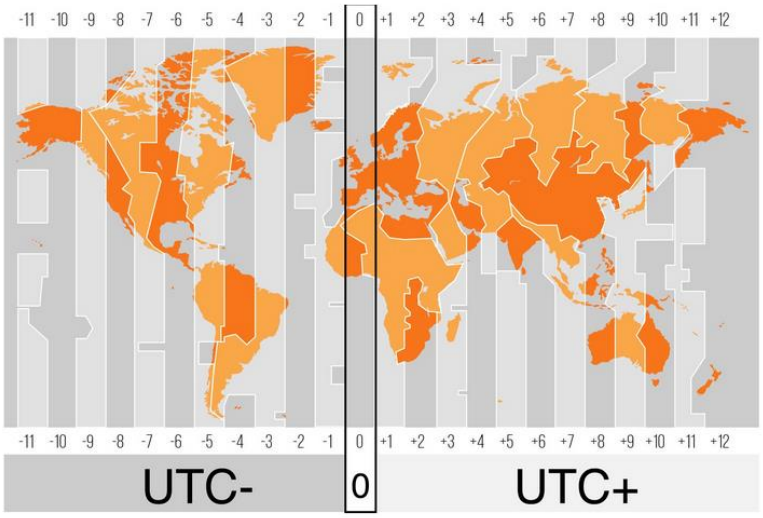
18th GC×GC SYMPOSIUM GUIDEBOOK

(All times in UTC, so please convert to your location accordingly)

https://www.timeanddate.com/worldclock/converter.html



Conference access information will be sent to the email address you provided when you registered. If you have not yet registered, please do so (for free) at www.gcxgc-symposium.com.

Thank you to our sponsors for making this event possible. It is your generous support that enriches the conference program and allows us to operate the conference with free registration for all attendees.

Conference Details

The meeting will take place on **GoToWebinar**, a different event will be created for each symposium day. If you registered on the symposium webpage, you should have received an email providing links to register for 5 separate days of the conference. You can also find the links below:

Day 1: https://attendee.gotowebinar.com/register/6941505393188326667
Day 2: https://attendee.gotowebinar.com/register/5985911041395449099
Day 3: https://attendee.gotowebinar.com/register/4476467393924186379
Day 4: https://attendee.gotowebinar.com/register/7447572112547093515
Day 5: https://attendee.gotowebinar.com/register/6918478321168472587

You will receive an email at the start of each conference day reminding you of your registered sessions and providing the connection link. There is a 15-minute pre-conference connection period built into the schedule where we can assist with virtual platform troubleshooting and provide the opening remarks for the day. Please log in at the start of this 15-minute window so that we are able to assist you.

Award Presentations will be taking place on Monday the 7th and Tuesday the 8th. On the first day, Dr. Hans-Gerd Janssen will be receiving the 2020 Scientific Achievement Award; Dr. Frank Dorman and Jack Cochran will receive the 2021 Scientific Achievement Award. On the second day, Dr. Pierre-Hugues Stefanuto will receive the 2020 John B Phillips Award; Dr. Katelynn Perrault will receive the 2021 John B Phillips Award.

Burst Presentations will take place on Wednesday the 9th in a single session in 6 different rooms. Burst presentations are 10-minute pre-recorded presentations available now at the gallery link below. Burst presenters will summarize their work in a 3- to 5-minute presentation the day of the event. The remainder of the session will be facilitated by a moderator to assist in discussion and Q&A. A burst discussion room will be available during the Gather event.

Gallery of pre-recorded talks: https://tinyurl.com/2xxu3ru

Live Burst Session Links:

- **Chemometrics:** https://global.gotomeeting.com/join/905917613
- **Foodomics and Metabolomics:** https://global.gotomeeting.com/join/995152397
- **Forensic and Environmental:** https://global.gotomeeting.com/join/951443949
- **Instrumentation:** https://global.gotomeeting.com/join/334666125
- **Bioanalytical:** https://global.gotomeeting.com/join/199179549
- **Petroleomics:** https://global.gotomeeting.com/join/787652069

Gather Networking Event will take place on Thursday after the conference. The Gather room can be accessed at https://gather.town/i/Xt6Oxec2 ; Password: GCXGC2021

Monday, June 7, 2021

01:00 – 01:05 PM Opening Address
P. Marriott (Monash University, Australia), Symposium Chair
PH Stefanuto (University of Liège, Belgium), Organizing Committee Chair
J. Harynuk (University of Alberta, Canada), Organizing Committee Co-Chair

01:05 – 02:35 PM Introductory Short Course on GC×GC
L. Fell (LECO Corporation, USA), Moderator
T. Gröger (Helmholtz Zentrum, Germany)
C. Kelly (LECO Corporation, USA)
PH Stefanuto (University of Liège, Belgium)
B. Weggler (Cynora GmbH, Germany)

02:35 – 02:45 PM Virtual Coffee Break #1

02:45 – 04:15 PM Scientific Achievement Awards Session
JF Focant (University of Liège, Belgium), Awards Chair

02:45 – 02:50 PM	2020 Scientific Achievement Award Presentation: P. Schoenmakers (Vrije Universiteit, The Netherlands)	
02:50 – 03:15 PM	Award Lecture A1	**Hans-Gerd Janssen** (Unilever Foods Innovation Center, and Wageningen University, The Netherlands) High-Speed Capillary GC as Enabler and Resultant of Comprehensive Two-Dimensional GC (GC×GC)
03:15 – 03:25 PM	2021Scientific Achievement Award Presentations: D. Patterson (EnviroSolutions Consulting, USA)	
03:25 – 03:50 PM	Award Lecture A2	**Jack Cochran** (Retired, USA) GC×GC×JC: A Multi-Dementia-nal Journey of Friends and Science
03:50 – 04:15 PM	Award Lecture A3	**Frank Dorman** (Penn State University, Waters Corporation, and Dartmouth College, USA) A Look Backwards and Forwards with My Experiences in GC×GC: -or- How to Have Some Success by Surrounding Yourself with Talented People

04:15 – 04:25 PM Virtual Coffee Break #2

04:25 – 04:45 PM Sponsor's Corner #1
L. McGregor (SepSolve Analytical, UK)
"Increasing dimensionality in GC×GC–TOF MS"

04:45 – 05:30 PM GC×GC Discussion Session
T. Goreckí (University of Waterloo, Canada), Session Co-Chair
P. Marriott (Monash University, Australia), Session Co-Chair

05:30 PM Day 1 Adjournement
PH Stefanuto (University of Liège, Belgium), Organizing Committee Chair
J. Harynuk (University of Alberta, Canada), Organizing Committee Co-Chair

Tuesday, June 8, 2021

01:00 – 01:05 PM Day 2 Opening Remarks
JF Focant (University of Liège, Belgium), Awards Chair

01:05 – 02:05 PM John B. Phillips Awards Session
HG Janssen (Wageningen University, The Netherlands), Session Chair

01:05 – 01:10 PM	2020 John B. Phillips Award Presentation: E. Reiner (Wellington Laboratories, and University of Toronto, Canada)	
01:10 – 01:35 PM	Award Lecture A4	**Pierre-Hugues Stefanuto** (University of Liège, Belgium) Advanced Data Processing as the Unexpected Hyphenation to Reveal the Full Potential of GC×GC-TOF MS
01:35 – 01:40 PM	2021John B. Phillips Award Presentation: S. Forbes (Université du Québec à Trois-Rivières, Canada)	
01:40 – 02:05 PM	Award Lecture A5	**Katelynn Perrault** (Chaminade University of Honolulu, USA) A Secret Guide to Learning, Using, and Teaching GC×GC

02:05 – 02:15 PM Virtual Coffee Break #1

02:15 – 02:35 PM Sponsor's Corner #2
F. Stilo (University of Turin)
"Time to Have a Paradigm Shift in Food Quality Measurements: New Opportunities Opened by Comprehensive 2D GC"

02:35 – 03:45 PM Foodomics Session I
L. Mondello (University of Messina, Italy), Session Chair

02:35 – 02:40 PM	Session Introduction	**L. Mondello** (University of Messina, Italy) **C. Cordero** (University of Turin, Italy)
02:40 – 03:05 PM	Keynote Lecture L1	**Carlo Bicchi** (University of Turin, Italy) From Multidimensional Chromatography and Enantioselective GC to Comprehensive 2D GC – A Tribute to Geo Schmarr
03:05 – 03:20 PM	Keynote Lecture L2	**Claudia Zini** (Universidade Federal do Rio Grande do Sul, Brazil) A Simple and Low Cost Lab Made GC-O (Step by Step) and an (Expensive?) GC×GC /MS: A Couple That is Needed for Wine Odor Analysis and Improvement
03:20 – 03:35 PM	Keynote Lecture L3	**Chiara Cordero** (University of Turin) Artificial Intelligence Smelling Machines Bases on GC×GC Technology
03:35 – 03:45 PM	Foodomics I Question and Answer Session	

03:45 – 03:55 PM Virtual Coffee Break #2

03:55 – 04:55 PM Foodomics Session II
C. Cordero (University of Turin, Italy), Session Chair

03:55 – 04:10 PM	Keynote Lecture L4	**Luigi Mondello** (University of Messina, Italy) GC×GC-sd-FTIR/MS for Reliable Identification in the Foodomics Field
04:10 – 04:25 PM	Keynote Lecture L5	**Taylor Hayward** (Apeel Sciences, USA) Volatile Profiling of Mandarins by Reversed Flow Modulated Comprehensive Two-Dimensional Gas Chromatography and Mass Selective Detection (GC×GC-MSD)
04:25 – 04:40 PM	Keynote Lecture L6	**Mariosimone Zoccali** (University of Messina) Untargeted Profiling and Differentiation of Geographical Wine Samples Using Headspace SPME-GC×GC-TOF MS Coupled with Tile-Based Fisher Ratio Analysis
04:40 – 04:55 PM	Keynote Lecture L7	**Giorgia Purcaro** (University of Liège/Gembloux) LC-GC×GC-TOFMS/FID: Multidimensionality as a Powerful Alley for the Improvement of Sample Preparation and Data Accuracy for MOSH and MOAH Analysis
04:55 – 05:10 PM	Foodomics II Question and Answer Session	

05:10 PM Day 2 Adjournement
PH Stefanuto (University of Liège, Belgium), Organizing Committee Chair

Wednesday, June 9, 2021

01:00 – 01:05 PM Day 3 Opening Remarks
PH Stefanuto (University of Liège, Belgium), Organizing Committee Chair

01:05 – 02:05 PM Forensics/Environmental Session I
F. Dorman (Penn State University, USA), Session Chair

01:05 – 01:10 PM	Session Introduction	**F. Dorman** (Penn State University, USA) **T. Goreckí** (University of Waterloo, Canada)
01:10 – 01:25 PM	Keynote Lecture L8	**Tommy Saunders** (Activated Research Company, USA) Simplified Stop-Flow GC×GC Modulation
01:25 – 01:40 PM	Keynote Lecture L9	**Alina Muscalu** (Ministry of the Environment, Canada) Comprehensive Two-Dimensional Gas Chromatography – Routine Testing and Discovery for Environmental Analysis
01:40 – 01:55 PM	Keynote Lecture L10	**Caroline Gauchotte-Lindsay** (University of Glasgow, UK) Non-Targeted "Metabolomics" and Biomarker Discovery for Environmental Applications
01:55 – 02:05 PM	Forensics/Environmental I Question and Answer Session	

02:05 – 02:15 PM Virtual Coffee Break #1

02:35 – 03:15 PM Forensics/Environmental Session II
T. Goreckí (University of Waterloo, Canada), Session Chair

02:15 – 02:30 PM	Keynote Lecture L11	**José Gomes** (Universidade Federal de Minas Gerais, Brazil) Analysis of $PM_{2.5}$ Volatile Organic Compounds Sampling in the Ambient Air of a Brazilian Urban Area Using High Resolution GC×GC/Q-TOFMS
02:30 – 02:45 PM	Keynote Lecture L12	**Chadin Kulsing** (Chulalongkorn University, Thailand) Splitter-Based Non-Cryogenic Artificial Trapping Comprehensive Heartcut (SNATCH): A Disruptive Modulation Mechanism in GC×GC
02:45 – 03:00 PM	Keynote Lecture L13	**Flavio Franchina** (University of Ferrara, Italy) High-Dimensional Analytical Strategies for Accurate Metabolite Profiling: Application to Cannabis
03:00 – 03:15 PM	Forensics/Environmental II Question and Answer Session	

03:15 – 03:35 PM Sponsor's Corner #3
D. Peroni and G. Stani (SRA Instruments)
"New SRA OPTIMODE PLUS: Exploiting the Full Potential of Thermal GC×GC"

03:35 – 03:45 PM Virtual Coffee Break #2

03:45 – 04:55 PM Parallel Burst Sessions
PH Stefanuto (University of Liège, Belgium), Organizing Committee Chair
J. Harynuk (University of Alberta, Canada), Organizing Committee Co-Chair

03:45 – 03:50 PM	Introduction to Burst Parallel Session Outline PH Stefanuto (University of Liège, Belgium)
Details of each parallel session are available on the next page	

04:55 PM Day 3 Adjournement
PH Stefanuto (University of Liège, Belgium), Organizing Committee Chair

Parallel Burst Session Schedule

Click here for the full gallery, or click on individual presenter's name to access their talk directly

	Chemometrics	Foodomics/Metabolomics	Forensics/Environmental	Instrument Development	Bioanalytical	Petroleomics
Session Moderators	L. McGregor (SepSolve Analytical, UK) B. Weggler (Cynora, Germany)	G. Purcaro (University of Liège/Gembloux, Belgium) A. Casilli (Firmenich, USA)	K. Perrault (Chaminade University of Honolulu, USA) PH Stefanuto (University of Liège, Belgium)	J. Binkley (LECO, USA) F. Franchina (University of Ferrara, Italy)	J. Hill (University of British Columbia, Canada) H. Bean (Arizona State University, USA)	H. Boswell (Chevron, USA) A. Giri (SABIC, The Netherlands)
03:50 – 3:55 PM	Session Introduction (Chemometrics)	Session Introduction (Foodomics/Metabolomics)	Session Introduction (Forensics/Environmental)	Session Introduction (Instrument Development)	Session Introduction (Bioanalytical)	Session Introduction (Petroleomics)
03:55 – 4:00 PM	**Sarah Prebihalo** (University of Washington, USA) Control-Normalized Fisher Ratio Analysis of Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry Data for Enhanced Biomarker Discovery in a Metabolomic Study of Orthopedic Knee-Ligament Injury (B1)	**Juliane Welke** (Universidade Federal do Rio Grande do Sul, Brazil) GC×GC /MS as a Tool to Assess the Impact of a Biofungicide on the Volatile Profile of Chardonnay Wines (B7)	**Kyra Murrell** (Penn State University, USA) Determination of Contaminants of Emerging Concern and Their Transformation Products in Treated-Wastewater Irrigated Soil and Corn (B13)	**Barbara Giocastro** (University of Messina, Italy) Evaluation of Different Internal Diameter Modulation Columns Within the context of Solid-State Modulation (B19)	**Delphine Zanella** (University of Liège, Belgium) In vitro Modelling of Lung Inflammatory Processes (B25)	**Haleigh Boswell** (Chevron, USA) A Fit for Purpose Data Analysis Approach for Distribution of Aromatics in Diesels (B31)
04:00 – 04:05 PM	**Trenton Davis** (Arizona State University, USA) Missing Data Imputation in Untargeted Metabolomics (B2)	**Olga Vyviurska** (Slovak University of Technology in Bratislava, Slovakia) Geographic Classification of Botrytized Wines using Flow-Modulated Comprehensive Two-Dimensional Gas Chromatography (B8)	**Darshil Patel** (Université du Québec à Trois-Rivières, Canada) Identifying the Transition of Odour from the Ante-Mortem to Post-Mortem Period (B14)	**Palathip Kakanopas** (Chulalongkorn University, Thailand) Development of Retention Index Based Approach for Simulation of GC×GC Results (B20)	**Caitlin Cain** (University of Washington, USA) Development of an Enhanced Total Ion Current Chromatogram Algorithm to Improve Untargeted Peak Detection (B26)	**Ivan Aloisi** (University of Messina, Italy) Use of Comprehensive Two-Dimensional Gas Chromatography – High Resolution Time-of-Flight Mass Spectrometry for the Investigation of Organic Sulphur Compounds in Coal Tar (B32)
04:05 – 04:10 PM	**Grant Ochoa** (University of Washington, USA) Class Comparison Enabled Methodologies for Improving Analyte Quantitation and Identification with GC×GC-TOFMS (B3)	**Keisean Stevenson** (University of British Columbia, Canada) Breath Biomarkers for Tuberculosis in Children using GC×GC-TOFMS (B9)	**Grace Idowu** (University of Manitoba, Canada) GC×GC – Method Development for Analysis of Polycyclic Aromatic Compounds (PACs) in Environmental Samples (B15)	**John Chow** (University of Waterloo, Canada) Flexible Second Dimension Temperature Programming System for GC×GC (B21)	**Emily Higgins Keppler** (Arizona State University, USA) Characterizing the Valley Fever Volatile Metabolome for Breath Test Development (B27)	**Yun Zou** (University of Liège, Belgium) Insights into Olefin Oligomerization Products Based on GC×GC-PI-TOFMS (B33)
04:10 – 04:15 PM	**Nadine Gawlitta** (Helmholtz Zentrum, Germany) Comparative Analysis of Particle-Bound Semi-Volatile Organic Compounds by IDTD-GC×GC-TOFMS: Identification of Compounds Specific for Allergy-Protective Environments (B4)	**Ryan Dias** (University of Alberta, Canada) Forensic Identification and Metabolomic Profiling of Two Oak Species by GC×GC-TOFMS (B10)	**Nadin Boegelsack** (Mount Royal University, Canada) Addressing Challenges in Analyzing Arson Debris by Implementing GC×GC-MS (B16)	**Timothy Trinklein** (University of Washington, USA) Exploring Total-Transfer Comprehensive Three-Dimensional Gas Chromatography (B22)	**Allix Coon** (State University of New York/Albany, USA) Play it by Ear: The Utilization of GC×GC -MS Analysis of Earwax as a Means of Diagnosing Disease (B28)	**Nuttanee Tungkijanansin** (Chulalongkorn University, Thailand) System Design, Method Development and Application of Splitter-Based Non-Cryogenic Artificial Trapping Comprehensive Heartcut (SNATCH) Modulation Mechanism (B34)
04:15 – 04:20 PM	**Simone Squarra** (University of Turin, Italy) Setting Key-Processing Parameters for Effective Template Patterns Alignment and Transform for Saliva Metabolites Fingerprinting by Comprehensive Two-Dimensional Gas Chromatography (B5)	**Adrien Garcia** (Université Côte d'Azur, France) GC×GC-TOFMS Applied to the Enantioselective Analysis of Extraterrestrial Samples (B11)	**Rushali Dargan** (Université du Québec à Trois-Rivières, Canada) Volatile Profiling of Cadaver Dog Training Aids using Comprehensive Two-Dimensional Gas Chromatography (B17)	**Pannipa Janta** (Chulalongkorn University, Thailand) Strategies Towards Simpler Configuration and Higher Peak Capacity with Comprehensive Multidimensional Gas Chromatography (B23)	**Sonia Schoenich** (University of Washington, USA) Minimum Variance Optimized Fisher Ratio Analysis of GC×GC-TOFMS Data for the Discovery of Metabolites in Farmed Pacu Fish (B29)	**Breno Pollo** (University of Campinas, Brazil) Optimization of Vac-HSSPME+GC-MS for Determination of Biomarker Profiles on Oil Source Rocks using Multivariate Approaches (B35)
04:20 – 04:25 PM	**Ewenet Mesfin** (University of Alberta, Canada) Initiation of Non-Negative PARAFAC2 Using Independent Component Analysis (B6)	**Lena Dubois** (University of Liège, Belgium) Volatile Fingerprinting of Boar Taint by GC×GC-TOFMS (B12)	**Cathrin Veenaas** (Örebro University, Sweden) Characterization of Contaminants in the Canadian Arctic Marine Environment (B18)	**Yada Nolvachai** (Monash University, Australia) Diving Deep Into Super-Resolved GC×GC (B24)	**Paulina Piotrowski** (NIST, USA) Characterization of Microbiomes Using GC×GC (B30)	**Paige Sudol** (University of Washington, USA) Investigation of the Limit of Discovery using Tile-Based Fisher Ratio Analysis with Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (B36)
04:25 – 04:55 PM	Q/A & Discussion Session (Chemometrics)	Q/A & Discussion Session (Foodomics/Metabolomics)	Q/A & Discussion Session (Forensics/Environmental)	Q/A & Discussion Session (Instrument Development)	Q/A & Discussion Session (Bioanalytical)	Q/A & Discussion Session (Petroleomics)

Thursday, June 10, 2021

01:00 – 01:05 PM Day 4 Opening Remarks
PH Stefanuto (University of Liège, Belgium), Organizing Committee Chair

01:05 – 02:05 PM Instrument Development Session I
P. Tranchida (University of Messina, Italy), Session Chair

01:05 – 01:10 PM	Session Introduction	**P. Tranchida** (University of Messina, Italy) **J. Harynuk** (University of Alberta, Canada)
01:10 – 01:25 PM	Keynote Lecture L14	**Philip Marriott** (Monash University, Australia) The Trajectory to GC×GC and Comprehensive MDGC. It's all about Modulation!
01:25 – 01:40 PM	Keynote Lecture L15	**Tadeusz Goreckì** (University of Waterloo, Canada) Multidimensional Inspirations
01:40 – 01:55 PM	Keynote Lecture L16	**Joshua Whiting** (Sandia National Laboratories, USA) TBD
01:55 – 02:05 PM	Instrument Development I Question and Answer Session	

02:05 – 02:15 PM Virtual Coffee Break #1

02:35 – 03:15 PM Instrument Development Session II
J. Harynuk (University of Alberta, Canada), Session Chair

02:15 – 02:30 PM	Keynote Lecture L17	**Robert Shellie** (Deakin University, Australia) Rapid Detection of Bacteria in Food using HS-GC×GC
02:30 – 02:45 PM	Keynote Lecture L18	**Karl Jobst** (Memorial University of Newfoundland, Canada) Novel Multidimensional Separation Techniques to Sequence the Exposome
02:45 – 03:00 PM	Keynote Lecture L19	**Peter Tranchida** (University of Messina, Italy) Options of 1D GC, Flow-Modulation Signal-Enhanced 1D GC and Flow-Modulation GC×GC in a Single Instrument: a Proof-of-Concept
03:00 – 03:15 PM	Instrument Development II Question and Answer Session	

03:15 – 03:35 PM Sponsor's Corner #4
C. Cody and B. van der Meer (JEOL)
"A New High-Resolution GC×GC-TOFMS with Multiple Ionization Methods: the AccuTOF-GC Alpha"

03:35 – 03:45 PM Virtual Coffee Break #2

03:45 – 04:45 PM Metabolomics/Bioanalytical Session I
JF Focant (University of Liège, Belgium), Session Chair

03:45 – 03:50 PM	Session Introduction	**JF Focant** (University of Liège, Belgium) **H. Bean** (Arizona State University, USA)
03:50 – 04:05 PM	Keynote Lecture L20	**Jane Hill** (University of British Columbia, Canada) New Tools for Breath Analysis Studies using GC×GC
04:05 – 04:20 PM	Keynote Lecture L21	**Heather Bean** (Arizona State University, USA) Three C's for Engaging your Brain's Visual Center to Extract Biological Information from Untargeted GC×GC Metabolomics Data
04:20 – 04:35 PM	Keynote Lecture L22	**Michael Wilde** (University of Leicester, UK) Exhaled breath analysis reveals distinct signatures for acute cardiorespiratory breathlessness
04:35 – 04:45 PM	Instrument Development I Question and Answer Session	

04:45 - 04:55 PM Virtual Coffee Break #3

04:55 – 06:00 PM Metabolomics/Bioanalytical Session II
H. Bean (Arizona State University), Session Chair

04:55 – 05:10 PM	Keynote Lecture L23	**Jean-François Focant** (University of Liège, Belgium) TD-GC×GC-HRTOFMS for Breath Profiling of Systemic Sclerosis
05:10 – 05:25 PM	Keynote Lecture L24	**Leandro Hantao** (University of Campinas, Brazil) Opportunities for GC×GC-Based -Omics Methods in Latin America
05:25 – 05:45 PM	Keynote Lecture L25	**Azzura Stefanucci** (University "G. d'Annunzio" of Chieti-Pescara, Italy) Antioxidant and Biological Activity of Peperone Dolce and Piccante di Altino
05:45 – 06:00 PM	Instrument Development II Question and Answer Session	

06:00 – 06:30 PM Gather Networking Session
(For details, click here)

Friday, June 11, 2021

01:00 – 01:05 PM Day 5 Opening Remarks
PH Stefanuto (University of Liège, Belgium), Organizing Committee Chair

01:05 – 02:05 PM Chemometrics Session I
R. Synovec (University of Washington, USA), Session Chair

01:05 – 01:10 PM	Session Introduction	**R. Synovec** (University of Washington, USA) **S. Prebihalo** (Food and Drug Administration, USA)
01:10 – 01:25 PM	Keynote Lecture L26	**James Harynuk** (University of Alberta, Canada) Robust Feature Extraction from Raw GC×GC-TOFMS Data with PARAFAC2×2
01:25 – 01:40 PM	Keynote Lecture L27	**Steve Reichenbach** (University of Nebraska, USA) Discriminant Visualization and Analysis Applied to Comprehensive Two-Dimensional Gas Chromatography for Geographic Classification of Olive Oils
01:40 – 01:55 PM	Keynote Lecture L28	**Liz Humston-Fulmer** (LECO Corporation, USA) Differentiation of Regular and Barrel-Aged Maple Syrup with Tile-Based Fisher Ratio using ChromaTOF Tile
01:55 – 02:05 PM	Instrument Development I Question and Answer Session	

02:05 – 02:15 PM Virtual Coffee Break #1

02:35 – 03:15 PM Instrument Development Session II
S. Prebihalo (Food and Drug Administration, USA), Session Chair

02:15 – 02:30 PM	Keynote Lecture L29	**Robert Synovec** (University of Washington, USA) Class Comparison Enabled Data Analysis for GC×GC-TOFMS
02:30 – 02:45 PM	Keynote Lecture L30	**Benedikt Weggler** (Cynora GmbH, Germany) A Unique Data Analysis Framework and Open Source Benchmark Data Set for the Analysis of Comprehensive Two-Dimensional Gas Chromatography Software
02:45 – 03:00 PM	Keynote Lecture L31	**Kristin Favela** (Southwest Research Institute, USA) Non-Targeted Analysis GC×GC-TOFMS using Floodlight and Searchlight: Machine Learning Assisted Data Analysis
03:00 – 03:15 PM	Instrument Development II Question and Answer Session	

03:15 – 03:35 PM Sponsor's Corner #5
L. Fell (LECO Corporation)
"What's going on at LECO now? Tiling, Lean and GC×GC "

03:35 – 03:45 PM Virtual Coffee Break #2

03:45 – 04:45 PM Petroleomics Session I
HG Janssen (Wageningen University, The Netherlands), Session Chair

03:45 – 03:50 PM	Session Introduction	**HG Janssen** (Wageningen University, The Netherlands) **C. Siegler** (Dow Corporation, USA)
03:50 – 04:05 PM	Keynote Lecture L32	**Ralf Zimmermann** (Helmholtz Zentrum, Germany) The Investigation of Bitumen Aging on a Molecular Level: A Complementary Approach Based on Comprehensive Two-Dimensional Gas Chromatography High-Resolution Time-of-Flight Mass Spectrometry and High-Resolution Fourier-Transform Ion-Cyclotron Mass Spectrometry
04:05 – 04:20 PM	Keynote Lecture L33	**Carin von Mühlen** (State University of Rio de Janeiro, Brazil) Average Theoretical Peak Time as a Metric to Analytical Speed in Monodimensional and Multidimensional Gas Chromatographic Separations
04:20 – 04:35 PM	Keynote Lecture L34	**Bruce King** (US Army Futures Command CCDC Chemical Biological Center, USA) Comprehensive Chromatography and Comparison of Operationally Relevant Atmospheres
04:35 – 04:45 PM	Instrument Development I Question and Answer Session	

04:45 - 04:55 PM Virtual Coffee Break #3

04:55 – 06:00 PM Petroleomics Session II
C. Siegler (Dow Corporation, USA), Session Chair

04:55 – 05:10 PM	Keynote Lecture L35	**Melissa Dunkle** (Dow Benelux, The Netherlands) Evaluation of Pyrolysis Oils from Mixed Waste Plastics by GC×GC
05:10 – 05:25 PM	Keynote Lecture L36	**Anupam Giri** (SABIC, The Netherlands) Pyro-GC×GC-HRMS: Unlocking Heavies for In-Depth Characterization
05:25 – 05:45 PM	Keynote Lecture L37	**Chris Reddy** (Woods Hole Oceanographic Institute, USA) A Pressing Need for GC×GC-Based Untargeted Analysis of Dumped Waste in the Ocean
05:45 – 06:00 PM	Instrument Development II Question and Answer Session	

06:00 – 06:05 PM Richard D. Sacks Awards Presentation
JF Focant (University of Liège), Awards Chair
J. Harynuk (University of Alberta), Organizing Committee Co-Chair
J. Whiting (Sandia National Laboratories), Honorary Awards Committee Member

06:05 – 06:15 PM Closing Remarks and Symposium Closure
P. Marriott (Monash University, Australia), Symposium Chair
PH Stefanuto (University of Liège), Organizing Committee Chair

18th GC×GC SYMPOSIUM
BOOK OF ABSTRACTS

Award Abstracts

High-speed capillary GC as Enabler and Resultant of Comprehensive Two-Dimensional Gas Chromatography (GC×GC)

Hans-Gerd Janssen[1,2], Herrald Steenbergen[1], Ed Rosing[1], Tim Spiering[1]

[1]Unilever Foods Innovation Centre, Wageningen, the Netherlands
[2]Laboratory of Organic Chemistry, Wageningen University, Wageningen, the Netherlands

Comprehensive two-dimensional gas chromatography (GC×GC) uses a very fast GC separation with typical run times of 2 to 10 seconds in the second dimension. As such, it only became possible after the introduction of fast GC (and of course the introduction of modulators by the late John Phillips). Fast GC from that perspective is an enabler of comprehensive GC×GC. Over the years, GC×GC has become much more widespread than fast GC and there are not many laboratories that perform very fast single dimension GC analyses.
One area where fast GC is a relevant method is in the monitoring of rapidly changing concentrations. This is for example the case in the study of very fast chemical reactions in the (petro-)chemical industry, or in food flavour research. A ‘flavour burst’ is an exciting sensation that occurs e.g. when crunching a candy in your mouth. The typical duration of such an event is 10 to 30 seconds, meaning that accurate assessment of the burst profile requires very fast analyses with run times of a few seconds or less. Techniques such as proton-transfer reaction monitoring (PTR) MS or secondary-ion flow tube (SIFT) MS can deliver these high sampling rates, but are expensive, difficult to operate, not very sensitive and provide limited resolution.
In the present contribution we will describe the history of GC×GC starting from the enabling technique of fast GC. The presentation will end with an innovative application of GC×GC where the modulator is used as an enrichment device and the second dimension provides the high separation speed needed to monitor flavour bursts. Basically, this brings GC×GC back to where it originated from: fast GC with a cryotrap inlet.

GC×GC×JC: A Multi-Dementia-nal Journey of Friends and Science

Jack Cochran[1], Frank Dorman[2], Mark Merrick[3]

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[2]Waters Corporation, Milford, Massachusetts, USA
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GC×GC has proven itself to be a very powerful technique for dissecting complex samples, especially when combined with mass spectrometry. I (Jack Cochran) was fortunate to be at LECO Corporation in the early 2000s when the combination of those techniques was commercialized, a good marriage, since LECO had a fast-recording time-of-flight mass spectrometer (TOFMS). Just as importantly, they had developed excellent GC×GC-TOFMS software that not only controlled all hardware, but also made data visualization and processing relatively quick and easy. GC×GC was on its way to being a practical problem-solving method of analysis. It was an exciting time to be a gas chromatographer!
This presentation will be a brief travelogue of the lead author's experience with GC×GC, as well as acknowledgment of the help of many collaborators and friends along the way. Although it may be challenging to do virtually, I hope I can convey the enthusiasm I still have for the technique. And, if I have time, I'll share my next adventure with (citizen) science.

A Look Backwards and Forwards with My Experiences in GC×GC: -or- How to Have Some Success by Surrounding Yourself with Talented People

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Back in the 1990's the still young GC×GC community allowed me to hang out with them and get involved with this really exciting technique. I remember the first few times I met with some of the people that are now considered leaders of this community and I tried to wrap my mind around (pun intended) this method of re-modulating the separations that I have been focused on working so hard to try to optimize. It was clear, even in my early experiences, that this technology could achieve separations that us 1-D chromatographers could only dream about; I was hooked! I also had an academic outlet in addition to my industrial collaborations and this allowed me to begin more directed research. I found what may of the readers have also found – students and young scientists are attracted to this technique as well and it can solve real issues that we are otherwise too limited to address as fully as we can with GC×GC. Couple this to a mass spectrometer that is fast enough to keep up, and the world of separations science was truly energized.

I hope to, in this presentation, cover such personal excursions as column selectivity and thermodynamic modeling which is where my initial entry into this field began and continues to some extent. I will also highlight several applied techniques where I would offer that something was learned that a non-multidimensional technique would not have revealed. I will also try to offer a few thoughts on where we might be headed as a community. Lastly, and most importantly, I hope to pay homage to some of those that I have encountered along the journey. They are the true reason for this humbling award. I was just lucky enough to have been able to interact with this amazing cast of colleagues, and the science that sprang from these collaborations was exciting and fun. I hope to "see" you all there and I look forward to where we all go from here!

Advanced Data Processing as the Unexpected Hyphenation to Reveal the Full Potential of GC×GC-TOFMS

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After 30 years of existence, GC·GC has crossed multiple milestones to become a mature analytical technique. The constant development of modulators and mass spectrometers technology have conducted to a generation of versatile and powerful commercial instruments. Based on these development, GC×GC-MS applications have evolved to become a key player in all kind of untargeted Omics research.

Indeed, GC×GC-MS can now be used to conduct large scale studies, giving full access to its high-resolution power for targeted and mostly untargeted screening. The current challenges are now localized on the data management side, where powerful chemometric tools are required to unlock GC×GC-MS full potential.

In this award presentation, I will discuss our journey towards the development of large scale GC×GC-MS studies and the concomitant development of the data processing solutions. This presentation will summarize the most commonly apply techniques to extract significant information from complex data set. In addition, I will also discuss the key steps of GC×GC-MS data processing workflow and the current tools available. Finally, some future perspectives on the development of the next generation of chemometric tools, especially machine learning, will be discussed.

A Secret Guide to Learning, Using, and Teaching GC×GC

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It is no secret that comprehensive two-dimensional gas chromatography (GC×GC) users are strong advocates for a technique that has broad and exciting potential in many areas of the life sciences. The increased peak capacity and peak detectability have improved many applications from petroleomics to food science to environmental analysis. Not only is GC×GC a powerful technique, but it is has recently grown exponentially in terms of modulator, detector, and software options. As a newcomer to the field of GC×GC, one could argue that the landscape is becoming increasingly challenging to navigate at first glance. As a result, while the GC×GC community begins to increasingly advocate for GC×GC and provide new technology options for users, the perceived difficulty of adopting GC×GC remains high to those outside of the field. In this presentation, information from first-hand experience in becoming a GC×GC user will be discussed including:

- the path of an early career researcher in learning, acquiring, and using various GC×GC instruments.
- developments within a research field of forensic science with the incorporation of GC×GC over 1D GC techniques.
- common barriers to making convincing arguments on using multidimensional GC approaches over 1D GC techniques.
- how education and incorporation of simple teaching approaches could be a gateway to increased adoption of GC×GC.

Keynote Lecture Abstracts

From Multidimensional Chromatography and Enantioselective GC to Comprehensive 2D-GC. A Tribute to Geo Schmarr

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This presentation is a critical review of the evolution of the gas chromatographic techniques mainly when applied to food analysis over the last 30 years (1990-2019) “read” in the context of the scientific career and production of Hans-Georg (Geo) Schmarr. This context will also reflect the parallel scientific activities of Geo's and the authors' laboratories.

The survey will cover Geo's research work from the beginning, when he started with his PhD under the Armin Mosandl's supervision, and contributed both to the theoretical development of cyclodextrins as GC stationary phases for enantiomeric recognition, and to their application in authentication, especially in routine, in the food industry.

His research work was the basis for important developments of one-step methods based on heart-cut GC-enantioselective GC (EsGC) and its combination with Isotopic Ratio Mass Spectrometry (IRMS). His studies were applied to control the authenticity of foodstuffs through their volatile chiral markers and to detect their adulterations and origins. Since the early 2000s, he approached comprehensive 2D-GC and focussed his efforts to promote its application in food analysis, especially in wines and fruits, and to make it attractive to the food industry.

A Simple and Low Cost Lab Made GC-O (Step by Step) and an (Expensive?) GC×GC /MS: a Couple that is Needed for Wine Odor Analysis and Improvement

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A step by step approach to easily adapt a gas chromatograph with a flame ionization detector (GC-FID) as a GC-olfactometer (O), as well as a detailed description of the operation, acquisition and interpretation of olfactometric data by the OSME method is presented. A Merlot wine is used to exemplify the whole strategy and its volatiles are characterized resulting in 43 volatiles in 1D-GC/MS and 142 in GC×GC /MS. GC-O indicated the presence of 24 odor-active compounds and GC×GC /MS showed additional 14 co-eluting odor-active components, some of them responsible for distinct and confusing odor perceptions by the odor panel. Twelve odor-active compounds were found to be a positive contribution to Merlot odor, although three of its major volatiles contributed negatively. The following Figure shows the system for obtaining odor data of wine volatile compounds using GC-O analysis.

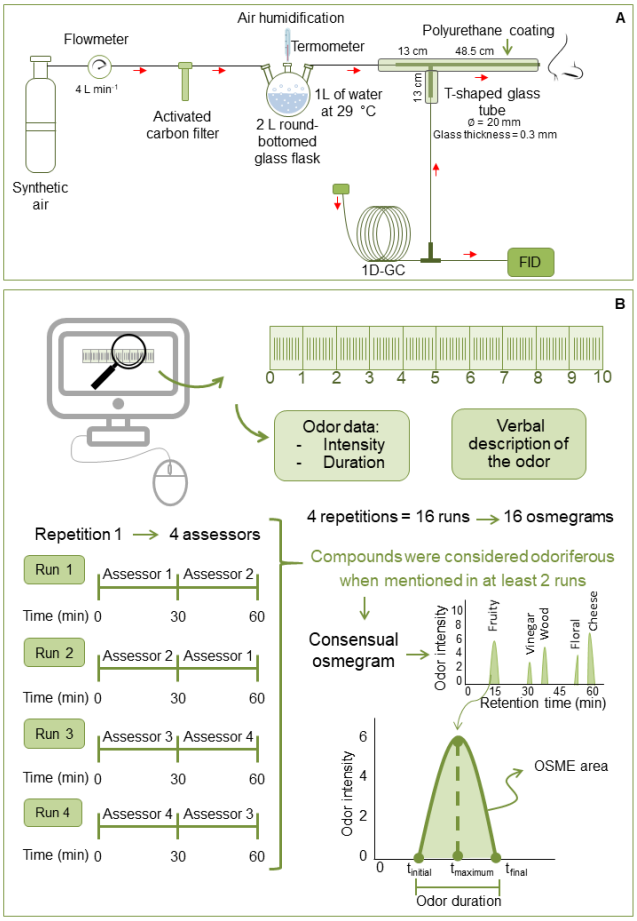

Figure. (A) Details of the set up adaptation of a GC-FID to perform the olfactometric analysis. (B) Collection and evaluation of data obtained using the OSME method of the GC-O.

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Artificial Intelligence Smelling Machines Based on GC×GC Technology

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Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOF MS) has been recently applied as core technology of a Sensomics-based expert system (SEBES) [1] capable to predict key-aroma signatures of food without using human olfaction. The strategy, also referred to as *Artificial Intelligence Smelling,* conceptually opens many different opportunities for odorants pattern detection, accurate quantification avoiding time-consuming sample preparation/extraction steps, and samples sensory qualification/discrimination based on computer vision strategies.

The contribution illustrates the potentials of GC×GC platforms in the context of *Artificial Intelligence Smelling* through a key-food product of relevance for confectionery industry: high-quality hazelnuts (*Corylus avellana* L.).

Sensory quality of raw hazelnuts depends to the presence of key-aroma compounds in well-balanced proportions, and of unpleasant odorants mainly deriving by lipid oxidation processes and/or enzyme-catalyzed reactions carried out by bacteria and molds [2,3].

Odorant patterns strongly correlated to sensorial defects (e.g., *rancid*, *rancid-stale*, *mould*, *mould-rancid-solvent*, *rancid-solvent* etc.) can be effectively detected by composite-class images generated by combining 2D chromatographic signals from samples showing specific off-odors. Computer vision helps in highlighting marker odorants while minimizing interferents contribution. Moreover, by exploring the 3D-data matrix (i.e., GC×GC-TOF MS), markers are reliably identified to support a better understanding on the spoilage phenomenon.

When the GC×GC is combined to parallel detection by MS/FID and automated headspace (HS) solid-phase microextraction (SPME), the platform can perform accurate quantification on an extended list of volatiles/odorants. The identification of key-aromas, and related signatures, is done in a single-step analysis. As by an *Artificial Intelligence Smelling* machine, reliable sensory qualification of hazelnuts becomes possible.

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GC×GC-*sd*-FTIR/MS for Reliable Identification in the Foodomics Field

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The hyphenation of a mass spectrometry (MS) and a recently developed solid deposition Fourier Transform Infrared Spectroscopy (sd-FTIR) with a flow-modulation comprehensive two-dimensional gas chromatography (FM GC×GC) enabling untargeted analysis of complex food samples is herein proposed for the first time. After GC×GC separation, the outlet flow of the second dimension column is diverted to the two detectors. Considering the FTIR side the eluent is deposited as a continuous track of sample, and acquisition of solid phase transmission spectra occurs through cryogenically-cooled ZnSe sample disc. The interferometer simultaneously collects a set of time-ordered IR spectra from the deposit track, and in addition allows for post-run data collection, through re-analysis of deposited samples. The FTIR approach is very useful for the characterization of functional groups, due to his strong "fingerprinting" capabilities and may be advantageous when complementary information is required, for instance to discriminate between molecular structures with identical mass and/or to confirm MS-based identifications. The usefulness and the advantages in the foodomics of a GC×GC system with flow modulator coupled in parallel with a dual detection, a solid phase IR and a mass spectrometer will be discussed.

Volatile Profiling of Mandarins by Reversed Flow Modulated Comprehensive Two-Dimensional Gas Chromatography and Mass Selective Detection (GC×GC-MSD)

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Volatile profiling of fruits can provide great insight into quality and flavor. Mandarins are known to have aromas of citrus with a hint of vanilla and spicy components that are attributed to a combination of terpenes, but primarily limonene. Amongst these major flavor contributors, investigations have observed and identified more than 40 compounds including aldehydes, alcohols, esters, and terpenes[1]. The analysis of such a complex mixture can be a challenge in separation and quantification.

Flow modulated comprehensive two dimensional gas chromatography (GC×GC) was used to increase separation of the volatile compounds in mandarins and was coupled to a mass selective detector (MSD). Typically, a time of flight mass spectrometer is used with flow modulated GC×GC as it is capable of handling high flow rates coming from the second dimension column. However recent advancements in this field have shown the use of an MSD with flow modulated GC×GC by two strategies: lower flow separations[2] and flow splitting[3].

Presented here is the application of both of these strategies to enable identification and quantification of volatiles in cucumbers. In the low flow setup, narrow bore capillary columns of inner diameters of 100 um are used to enable flows entering the MSD to be < 2.0 mL/min. While this setup uses sub-optimal flow rates in the first dimension, excellent separation is observed in the two dimensional chromatographic space. In the flow splitting mode, column effluent was split between an MSD and a flame ionization detector (FID). Advantages in quantification were observed in this mode as the FID provides a more universal response towards these compounds of interest.

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Untargeted Profiling and Differentiation of Geographical Variants of Wine Samples Using Headspace SPME-GC×GC-ToFMS Coupled with Tile-based Fisher Ratio Analysis.

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The volatile fraction of food, also called the food volatilome, is increasingly used to develop new fingerprinting approaches. Characterization of the food volatilome is important for achieving desired flavor profiles in food production processes, with winemaking being one popular area of interest. In the present research, headspace solid-phase microextraction (HS-SPME) coupled to flow-modulated comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (FM GC×GC-ToFMS) was developed to characterize geographical-based differences in the volatilome of five Sicilian Grillo wines. All wines were produced with the same vinification method in 2019. To minimize the influence of minor bottle-to-bottle differences, three bottles of the same wine were randomly selected, and three samples were collected per bottle, resulting in nine sample replicates per wine. Particular emphasis was devoted to the operational conditions of a novel low duty cycle flow modulator. A fast FM GC×GC-ToFMS method with a modulation time of 700 ms and a re-injection period of 80 ms (such FM conditions enabled a higher duty cycle) was developed, with the resulting wrap-around not impacting analyte identification. Following GC×GC-ToFMS analysis, the instrumental software (ChromaTOF Tile software) was exploited to identify class-distinguishing analytes in the dataset *via* a Fisher ratio calculation. A tile size of 10 modulations on the first dimension and 45 spectra on the second dimension was used to encompass average peak widths and accounting for minor retention time shifting. The resulting hitlist contained hundreds of class-distinguishing hits with p-values less than 0.05. A principal component analysis (PCA) scores plot of the quantified peak areas of these hits showed five distinct clusters for each type of wine studied, with the “Feudo Arancio” replicates appearing the most distinct, likely due to this being the only sample produced in the southern region of Sicily, fully demonstrating the potential of the proposed approach.

LC-GC×GC-ToFMS/FID: Multidimensionality as a Powerful Alley for the Improvement of Sample Preparation and Data Accuracy for MOSH and MOAH Analysis

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Few samples really benefit for additional dimensions, and mineral oil, more specifically mineral oil saturated and aromatic hydrocarbons (MOSH and MOAH) are one of this. The reference method for this analysis is the LC-GC-FID method developed by Grob and Biedermann in 2009 [1]. In this method, the LC step is used as a preliminary sample preparation step to separate MOSH and MOAH and the rest of the sample (mainly lipid components). Beside LC-GC, comprehensive gas chromatography (GC×GC) has been proposed to better investigate the MOSH and MOAH fraction identity.
In this work, we proposed a fully integrated platform, namely LC-GC×GC-ToFMS/FID, coupled with a novel integration algorithm, to improve results reliability. Every dimension involved was an important alley for sample preparation allowing an additional "chromatographic sample purification" of the MOSH and MOAH hump from the interferences still remaining after the previous LC step [2].

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Simplified Stop-Flow Comprehensive GC×GC Modulation

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Comprehensive two-dimensional gas chromatography (GC×GC) is a powerful technique for enhanced separation capabilities with widely ranging applications, but it is not yet widely adopted. This is primarily due to perceptions around complexity and cost. Many of the modulation systems currently available rely on thermal modulation with consumable liquid nitrogen or expensive refrigeration systems. Recent improvements in valving and improved flow technologies have allowed flow modulators to become increasingly relevant and advantageous. Here, we discuss a technique that implements stop-flow modulation which simplifies systems to allow for easy installation and operation. This technique can effectively reduce the barriers to entry for GC×GC, resulting in a democratization of this powerful technique.
In this presentation, we will:

- Discuss the novel modulation technology, which employs a three-way solenoid valve connected to an accumulation capillary and tee. The tee connects the two columns, and sampling for the second dimension occurs inside the accumulation capillary, allowing for fast, quantitative transfer [1].
- Provide example data showing the performance of the modulator for a variety of applications.
- Introduce a free, open-source multidimensional chromatography visualization tool, which was developed to aid analysts in understanding their data with no up-front cost.

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Comprehensive Two-Dimensional Gas Chromatography - Routine Testing and Discovery for Environmental Analysis

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Environmental samples contain hundreds or thousands of non-targeted compounds with various chemical and physical properties, some of which may pose a risk to the environment or human health. Their identification can be very challenging as most routine analytical techniques are transparent to non-targeted compounds. Comprehensive two-dimensional gas chromatography (GC×GC) is known to be one of the most powerful techniques for the separation of complex environmental samples, especially for the analysis of compound groups that contain congeners or homologues. Since 2010, we have been using GC×GC-µECD for routine analysis of polychlorinated biphenyls, organochlorine pesticides and herbicides, trifluralin and more recently toxaphene congeners in different matrices: solid samples (sediments, soil, sludge), biota (fish, clams, and mussels), water (effluent, ground water, surface water, and drinking water), and passive samplers. Quality control and quality assurance procedures, implemented as outlined the international standard (ISO 17025) requirements, showed that the method performs very well. As of today, our laboratory has implemented three fully validated GC×GC-µECD methods, two of them being accredited to ISO17025 standard, which are successfully applied to "real-life" samples.

By using a technique such as GC×GC-µECD for routine analysis, the identification of other nontargeted halogenated contaminants present in the environmental samples became possible. The GC×GC-µECD method combined with GC×GC-TOF-MS for further characterization proved to have many benefits, including the identification of nontargeted compounds and the ability to re-direct the samples to validated methods for the identified compounds when available, identifying and tracking down the source of contamination based on the targeted and non-targeted contaminants patterns observed in the 2D chromatograms, identifying short-chain chlorinated paraffins (SCCPs) as ubiquitous contaminant class in sediment samples. The GC×GC chromatograms of samples analyzed routinely for targeted compounds such as PCBs and pesticides can now be further sorted, and data further processed when non-target pesticides, SCCPs, OH-PCBs, PCDEs or other halogenated non-targeted compounds are found. Further, previously saved data can be qualitatively and quantitatively interpreted, and historical trends can be determined offering several advantages to conventional approaches.

Examples of a number of non-target identified compounds are presented to emphasize that using a technique such as GC×GC and complementary detectors, µECD and TOFMS, for the routine testing of environmental samples is also an excellent screening approach for detecting non-targeted contaminants. These findings also emphasize the importance of expanding the list of target contaminants in routine analysis of environmental samples and the need for more studies to assess the continuous exposure of the organisms/environment to these compounds or mixtures of compounds.

Non-targeted "Metabolomics" and Biomarker Discovery for Environmental Applications

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We previously developed and demonstrated GC×GC -ToFMS methods for the near-exhaustive analysis of environmental coal tars and the forensic analysis of their provenance [1-3]. We will present here examples across environmental engineering where non-targeted analysis by GC×GC -ToFMS provided information about the transformations and processes undergone by the samples and enabled chemical markers to be identified. These markers can then act as indicators of the state/ health of the studied system. We identified markers of the progress of the underground gasification of coal using non-targeted analysis and multivariate statistics on liquid by-products and compared pipelines using GC-MS and GC×GC -ToFMS. We have also for the first time integrated, in the same multivariate analysis, GC×GC data and 16S RNA sequencing of coal tar contaminated soils, discovering chemical and microbial indicators of soil biodegradation regimes [4]. We are aiming to establish sample and data workflows that can be applied across environmental engineering technologies, in which markers are discovered using robust analytical and statistical (machine learning) methods and then chemically identified using high resolution mass spectrometry. Good quality control and quality assurance (QA/QC) are crucial to ensure that findings are representative of the studied processes; we explore how we can learn from metabolomics approached and adapt them to environmental samples to establish QA/QC for sample extraction and analysis and for data analysis.

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Analysis of $PM_{2.5}$ Volatile Organic Compounds sampling in the ambient air of a Brazilian urban area using high resolution GC×GC /Q-TOFMS

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Air quality in large cities has worsened in recent years affecting directly people's health. In this study, the research focus was to analyze the non target volatile organic compounds (VOCs) in particulate matter ($PM_{2.5}$) using comprehensive two-dimensional gas chromatography with high resolution mass spectrometry (GC×GC /Q-TOFMS/MS). The system used was an Agilent 7250 GC/ Q-TOFMS unit coupled with an Agilent 7890B GC using a two-stage ZX2 cooled loop modulation from Zoex (Houston, USA). The columns set chosen was a D^1 5% phenyl methylpolysiloxane (DB – 5MS; 30 m×0.25 mm× 0.25 mm) column coupled with a D^2 polyethylene glycol phase (Stabilwax; 2 m×0.15 mm×0.15 mm). Data were acquired by MassHunter software Agilent (Santa Clara, USA) and processed using GC Image GC×GC Software, version 2.9 GC Image (Lincoln NE, USA).

Sampling was performed using a high-volume (Hi-Vol) sampler (Energética, RJ, Brazil) for suspended particles with aerodynamic diameter smaller than 2.5 μm ($PM_{2.5}$). Samples were collected in quartz filter GE Whatman (Clifton, USA) during 24 h at Universidade Federal de Minas Gerais (UFMG) campus (19°51'47.3"S 43°57'30.2"W), Belo Horizonte, Brazil. The sample preparation was carried out in a vial containing 20.0 mL of ultrapure water, 150.0 μL of acetonitrile, and 6 discs (diameter 0.6 cm) sample of the quartz. The vial was sealed and agitated in an ultrasound bath for 2 min. Extraction was performed for 45 min at a temperature of 70 °C by direct immersion of the fiber (DI-CF-SPME). The fiber 65 μm PDMS/DVB Supelco (Bellefonte, USA) was cooled during extraction with liquid nitrogen[1]. After extraction, the fiber was taken for desorption in the GC×GC /QTOF-MS injector at 270 °C for 2 min. The Fig. 1 shows chromatogram 2D of a $PM_{2.5}$ sample.

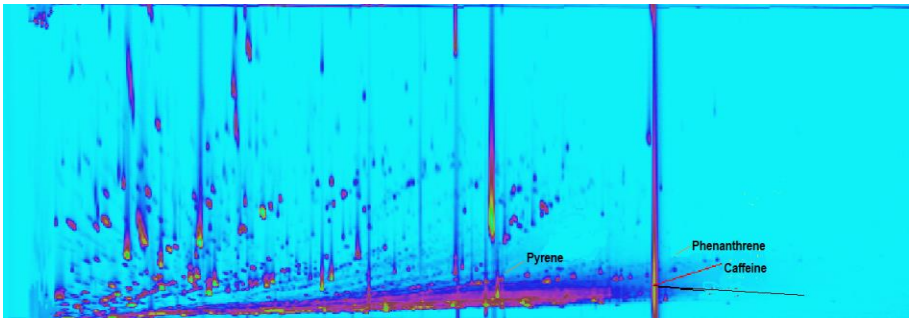

Fig. 1 – GC×GC Chromatogram of $PM_{2.5}$ sample after extraction by CF-SPME.

Many classes of volatiles and semi volatiles compounds were identified such as polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs (nitro-PAHs) and oxygenated PAHs (oxy-PAHs). These classes of compounds are commonly found in the air of urban regions. However, caffeine was also identified as shown Fig 1. One of the main sources of caffeine in urban areas is domestic sewage. Near the sampling site there is sewage overflow from the collection networks. After drying in ambient conditions, the solids present in this effluent are deposited on public roads, and then transferred to atmospheric air due to vehicle traffic. This can justify the presence of caffeine in the analyzed sample

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Splitter-Based Non-Cryogenic Artificial Trapping Comprehensive Heartcut (SNATCH): A Disruptive Modulation Mechanism in GC×GC

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Limitations of the existing modulation technologies applied in comprehensive two dimensional gas chromatography (GC·GC) have been recognized. These may involve ineffective modulation of highly volatile or low volatile compounds as well as cryogen consumption with the thermal modulation, low efficiency in second dimensional (^{2}D) separation and short modulation period (P_M) with flow/pulse modulation, lack of peak focusing effect or column bleeding accumulation with valve based/stopped flow modulation, or extremely long analysis time with comprehensive multiple injection/heartcut analysis. A desirable goal is to develop a disruptive modulation mechanism breaking these limitations.

In this presentation, we will:

- introduce a novel modulation mechanism called Splitter-based Non-cryogenic Artificial Trapping Comprehensive Heartcut (SNATCH) enabling modulation of highly volatile compounds with "artificially cryogenic" focusing effect without use of any cryogenic device, additional pressurized pulses or stopped flow.
- present possible system configuration and experimental approach to perform such mechanism with the focus on the concept illustration and the method development.
- provide example application of the developed system for "artificially cryogenic" modulation of a mixture of highly volatile compounds using a long ^{2}D (60 m) column and long P_M (ε4 min).

High-Dimensional Analytical Strategies for Accurate Metabolite Profiling: Application to Cannabis

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Metabolite analysis, particularly metabolite profiling of complex biological matrices, is essential in many fields of life and environmental science. Usually, metabolomic studies are carried out using advanced analytical techniques, with mass spectrometry (MS) playing a central role. Although MS allows detecting more components than can actually be separated by any pre-fractionation step, the precision and robustness of the identification and the quantification of the analytes remain a problem if coelutions occur. Chromatographic separation contributes to the isolation of hundreds of metabolites and thus improves the reliability of measurements.

Especially in the case of natural extracts that may have complex compositions, both high-resolution separation and high-selective detection are beneficial. Often overlooked, sample preparation truly opens the circle of the analytical strategy for detailed metabolite profiling, and it is critical for the reliability of the final results. Closing the loop, multivariate analysis and non-targeted analysis make the handling of such high-dimensional information possible.

The present contribution demonstrates the development of analytical strategies for multiclass and small metabolite profiling in cannabis products. The method involved sorptive extraction (SBSE), optimized for capturing an extended range of metabolites. The separation and detection steps considered the hyphenation of high-resolution techniques, namely mono- and multi-dimensional capillary gas chromatography, with low- and high-resolution MS. In this context, the power of accurate MS information supported analyte confirmation and facilitated structural elucidation. Finally, a multivariate approach using non-targeted data analysis enabled a global metabolite composition study and discrimination of the different cannabis flowers. The overall methodology also allowed for quantitative targeted analysis of regulated cannabinoids.

The Trajectory to GC×GC and Comprehensive MDGC. It's all about Modulation!

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The terms comprehensive two-dimensional gas chromatography, and comprehensive GC-MS are both seen in the literature, along with non-recommended terms such as comprehensive GC×GC. 'Comprehensive' is intended to mean analysis of all components, but does this also mean identification of 'all'? This is surely rather ambitious, because the closer we look at a chromatogram, normally the more components we see, especially at lower abundance, so we'd really need to specify an abundance threshold. Comprehensive GC–MS – meaning using GC–MS to analyse all compounds – is fine provided there is no confounding of identification, i.e. that all compounds can be identified; clearly confounding may arise for unresolved components. GC×GC should solve much of the resolution problem arising with single column analysis, although some especially complex samples challenge this, e.g. branched and cyclic alkanes in petrochemicals, and naphthenic acids in process water.

Comprehensive MDGC is differentiated from comprehensive two-dimensional GC, and has not been widely implemented in the past. The method applies classical heart cut MDGC in a repetitive sense – i.e. with repeat injections – with contiguous heart cuts sampled in each successive sequential analysis, to eventually cover the whole sample. This has an analogy with LC×LC, where storage loops and repetitive analyses are not uncommon.

This presentation will examine different implementation procedures we have developed for this task. Applications might be use of a long ^{2}D chiral column where a short column is not practical for enantioselective separation; improved resolution of petrochemical samples; and oxidation products in thermally stressed biofuels. Of interest is the scope for increased resolution of compounds compared with GC×GC, although it is a more time-consuming analytical separation method.

Multidimensional Inspirations

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Comprehensive two-dimensional gas and liquid chromatography (GC×GC and LC×LC) have the potential to provide peak capacities and selectivities that are impossible to accomplish using conventional one-dimensional separations. Even though the techniques share the same principle, they have evolved practically independently of each other. Transfer of fractions between the two dimensions in LC×LC is typically accomplished using a simple interface consisting of two sample loops and a multiport valve, whereas GC×GC requires a special interface called a modulator. Columns used in the two dimensions of GC×GC are never fully orthogonal because analyte volatility always affects the separation, whereas in LC×LC fully orthogonal separation mechanisms are typically used. Consequently, a lot of research effort is focused on modulator development in GC×GC, while little attention is paid to optimization of second dimension separations. On the other hand, numerous research groups work on coupling dissimilar (and often incompatible) separation mechanisms in the two dimensions of LC×LC. It is our opinion that each technique can benefit from the developments of the other one if this typical approach is modified. In GC×GC, a significant improvement in peak capacity can be achieved by using temperature programming in the second dimension. In LC×LC, good orthogonality and high peak capacity can be achieved using the same separation mechanisms in both dimensions provided parallel gradients are used. The talk will explore lessons we can learn from both techniques and show how the best features of one method can be transferred to the other.

BioVOC Biomarker Identification and Instrument Development for Field Detection

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There is a growing body of work demonstrating the correlation between biogenic volatile organic compounds (BioVOCs) and biological states, from canines being trained to detect COVID-19, to the use of breath tests to detect tuberculosis and other disease states. Utilizing these BioVOC signatures in the field for a variety of applications requires two separate but equally important development efforts targeting the “front side” and “backside” of the BioVOC field detection process. The front side is biomarker collection, analysis, and identification which establishes the link between biomarkers and biological states of interest. The backside is the development of portable instrumentation able to look for the identified biomarkers in a rapid, portable manner in order to provide actionable information to a decision-maker in a timely fashion. In this presentation we will present on both sides of this challenge.

Sandia National Laboratories is a multi-mission laboratory managed and operated by National Technology & Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International Inc., for the U.S. Department of Energy’s National Nuclear Security Administration under contract DE-NA0003525.

Rapid Detection of Bacteria in Food Using HS-GC×GC

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There is growing demand for rapid detection of bacteria in food, driven in part by the food industry's increasing interest in embracing Manufacturing 4.0 technologies to streamline production of safe food. This project's genesis came about during one of the author's international travel (when such a thing was still permitted). Explosive Trace Portals, or puff portals, were introduced into airports to detect explosives and illegal drugs. Our approach is draws on the puffer portal concept in two ways: we aim to detect presence of bacteria by detecting microbial volatile organic compounds emanating from contaminated samples, we aim to do this without recourse to sample preconcentration techniques. Notwithstanding the relatively widespread use of SPME-GC×GC-MS for microbial detection, our hypothesis is that direct headspace analysis is more amenable to near real-time at sample analysis. Thus, this presentation will discuss recent results using HS-GC×GC for rapid detection of *E. coli* in Ultrahigh Temperature Treated milk. We have used a model microbe and sterile food matrix to explore possibilities of rapid bacteria detection. To date, our time-to-signal is 24 h shorter than the conventional workflow using microbiological assays, using a 15 min HS equilibration followed by 5 min HS-GC×GC analysis of enriched sample containing as few as single cell bacteria load. While further work is required to assess specificity and further reduce enrichment time, these preliminary results support our hypothesis.

Novel Multidimensional Separation Techniques to Sequence the Exposome

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The *exposome* represents all environmental exposures to a human or organism during a lifetime [1]. The concept was developed to underline the important relationship between environment and health. Currently, large swaths of the exposome remain unknown. *Nontargeted screening* (NTS) is an innovative approach involving high resolution mass spectrometry and comprehensive multidimensional separations aimed at identifying environmentally significant chemicals without prior knowledge of their structure [2].
This contribution will describe:

- Novel experimental and computational approaches [3] that address some of the key analytical challenges associated with NTS, *viz*. prioritizing the interpretation of up to tens of thousands of mass spectra produced by multidimensional instrumentation; characterizing technical mixtures with myriad constituent compounds; and detecting trace level contaminants, especially in quantity-limited matrices like serum and dried blood spots.
- A top-down exposomics study of a cohort (n=125) of pregnant mothers at low (n=57) and high risk (n=68) of placental insufficiency. Using GC×GC hyphenated with atmospheric pressure chemical ionization, unknown and suspected persistent organic pollutants were screened using less than 200 µL of blood.
- A bottom-up exposomics study of household dust wherein ion mobility was used as an additional dimension to assign structures to unknowns.
- The potential for computational chemistry to help guide the tentative identification of unknown pollutants in the absence of authentic standards.

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Options of 1D GC, Flow-Modulation Signal-Enhanced 1D GC and Flow-Modulation GC×GC in a Single Instrument: a Proof-of-Concept

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In this presentation, we will introduce the concept of creating three analytical options in a single GC instrument with two flame ionization detectors (FIDs): 1D GC, flow-modulation (FM) signal-enhanced (SE) 1D GC and FM GC×GC. Analyses were performed by using an FM GC×GC instrument (in this case with one FID) equipped with a flow modulator characterized by seven ports and an external accumulation loop. Ports 6 and 7 received volumes of first-dimension effluent from the loop, and were connected to a 0.76 m × 0.20 mm ID uncoated column segment and to a mid-polarity 5 m × 0.32 mm ID × 0.25 μm d_f column. Both columns were characterized by the same flow resistance. When the uncoated column was connected to the FID, then either 1D GC-FID or FM SE GC-FID could be performed. If, on the other hand, the mid-polarity column was linked to the FID, then an FM GC×GC separation was performed. Applications were performed on a fuel sample and on a mixture of pesticides, with signal-to-noise ratios, limits of quantification and peak widths measured for a variety of analytes under the different analytical circumstances.

New Tools for Breath Analysis Studies using GC×GC

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Multidimensional chemical analyses have expanded our knowledge of the volatile and semi-volatile molecules in biomarker discovery circles, including breath analysis. With this expansion of known molecules in the breath volatilome, comes a need for new tools – from breath samplers to "housekeeping" indexes to informatic. In this presentation, I focus on some new work our team has developed, specifically, (a) the development of a "housekeeping" breath index concept and (b) the role and decisions associated with the use of informatic tools, such as data value or frequency cutoffs and how to incorporate machine learning approaches. I will highlight two *in vivo* studies, one evaluating the healthy breath of macaques and the other seeking biomarkers of active infection from the breath of patients with lung infections caused by non-tuberculosis mycobacteria.

Three C's for Engaging your Brain's Visual Center to Extract Biological Information from Untargeted GC×GC Metabolomics Data

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The ability to detect hundreds to thousands of volatile metabolites in a single sample makes GC×GC a powerful tool for untargeted metabolomics analysis and biomarker discovery through the comparative analysis of sample groups (e.g., disease and control) [1]. However, the goal of turning the chromatographic data into biological information is rarely straight forward, as issues such as missing data, sparse matrices, high sample group variance, and too few samples relative to the number of chemical features can confound statistical approaches for biomarker identification. Applying correlation, clustering, and color to your data (Three C's) and exploiting the brain's ability to identify patterns from vast amounts of visual information can provide an entry point for interpreting the large and often messy GC×GC data matrices generated from biological studies.

In this presentation, we will:

- Provide examples of how calculating correlations and performing clustering analysis can reveal systemic biological and chemical patterns in untargeted metabolomics analyses of large sample sets.
- Go beyond principal components analysis (PCA) for visualizing complex data.

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Exhaled Breath Analysis Reveals Distinct Signatures for Acute Cardiorespiratory Breathlessness

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Breathlessness due to cardio-respiratory diseases accounts for more than 1 in 8 of all emergency admissions to hospital [1]. Diagnostic evaluation of acute breathlessness is reliant on blood-based biomarkers and radiological tests. These biomarkers have clinical utility in patients with single pathologies, but have poor discriminatory power in patients with multifactorial presentations of acute breathlessness and are particularly challenging to interpret in the context of pre-admission treatment exposure [2].

The transformative potential of *breathomics* is becoming increasingly recognised in research and therapeutic development in respiratory diseases, particularly with the emergence of powerful separation technologies such as comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC -MS). Herein, we will explore breathomics in the acute care setting, within a real-world, prospective study of acutely unwell hospitalised patients presenting with breathlessness due to severe exacerbations of cardio-respiratory aetiology (asthma, chronic obstructive pulmonary disease, heart failure or pneumonia) and healthy controls (n=277) [3]. Breath analysis was underpinned by robust biomarker development protocols using TD-GC×GC -FID/MS, which are integral to the standardisation and integration of breath analysis in large translational studies [4, 5].

Volatile organic compounds (VOCs) derived from breath, measured by GC×GC-MS, drive topological identification of acute disease and causal subgroups. Linear combinations of relevant exhaled breath metabolites were used to produce breath biomarker risks scores, which demonstrated high combined diagnostic sensitivity and specificity for acute breathlessness, agnostic to treatment exposure in discovery and replication (79% sensitivity, 85% specificity). Receiver operating characteristic (ROC) analyses confirmed overall excellent to good specificity in both discovery and replication cohorts for acute asthma (0.96, 0.78), acute COPD (0.96, 0.77), community acquired pneumonia (0.95, 0.86) and heart failure (0.99, 0.89) highlighting the potential of breath biomarkers as a diagnostic triage tool in acute care.

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TD-GC×GC-HRTOFMS for Breath Profiling of Systemic Sclerosis

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Systemic sclerosis is a rare autoimmune disease associated with rapid evolving interstitial lung disease, responsible for the disease severity and mortality. Specific biomarkers enabling the early diagnosis, prognosis and associated with the disease progression are highly needed. Volatile organic compounds in exhaled breath are widely available, non-invasive, and have the potential to reflect metabolic processes occurring within the body. Comprehensive two-dimensional gas-chromatography coupled to high resolution mass spectrometry was used to investigate the potential of exhaled breath to diagnose systemic sclerosis.

The exhaled breath of 32 patients and 30 healthy subjects was analyzed. The high resolving power of this approach enabled the detection of 356 compounds in the breath of systemic sclerosis patients, which was characterized by an increase of mainly terpenoids and hydrocarbons. In addition, the use of 4 complementary statistical approaches (two-tailed equal variance t-test, fold-change, partial least square discriminant analysis, and random forest) resulted in the identification of 16 compounds that can be used to discriminate systemic sclerosis patients from healthy subjects. Receiver operating curves were generated and provided an accuracy of 90%, a sensitivity of 92%, and a specificity of 89%. The chemical identification of eight compounds predictive of systemic sclerosis was validated using commercially available standards. The analytical variations together with the volatile composition of room air were carefully monitored during the timeframe of the study to ensure the robustness of the technique. This study represents the first reported evaluation of exhaled breath analysis for systemic sclerosis diagnosis and provide surrogate markers for such disease.

Opportunities for GC×GC Based-Omics Methods in Latin America

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Latin America consists of low- and middle-income economies with very heterogenous levels of development. High inflation rates and devalued local currencies dampens the adoption of modern analytical solutions in research and development facilities. In this context, although comprehensive two-dimensional gas chromatography (GC·GC) has proven its potential for the analysis of volatile and semi-volatile compounds in many proof-of-concept studies [1-3], there are still barriers that must be overcome by the community to effectively implement GC·GC for routine analysis [4]. In this presentation, we will evaluate the analytical scope of flow-modulated GC·GC taking into consideration the volatility range of the analytical method. Interesting "-omic" applications in food and beverage industry, as well as in oil and gas industry will be presented to showcase opportunities that can benefit from GC·GC and/or chemometric methods.

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Antioxidant and biological activity of Peperone Dolce and Piccante di Altino

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Capsicum annum appeared on European tables since the 16th century. The morphology of sweet pepper type "Paesanello di Altino" or "A cocce capammonte" has fruit turned upwards and was recognized by the Abruzzo region as traditional typical product. The sweet pepper from Altino is peculiar to the area between the Sangro and Aventino rivers, in the province of Chieti.

The dried sweet peppers were pulverized inside large wooden mortars called "pillars". The pepper powder was widely used as a condiment for pasta or for the preparation of sausages. The denomination sweet pepper of Altino is reserved to a single morphological type characterized by an intense red color. The pepper contains a fair amount of sugar, it is rich in vitamins C, vitamin A, calcium, phosphorus and potassium. Its characteristic flavor is due to the presence of an alkaloid called capsaicin [1].

Studies are underway to investigate the potential application of sweet and hot pepper of Altino, as a rich source of natural antioxidants. Thanks to the antiseptic function, the pepper has characterized by a "long shelf life" which justifies its presence in diverse foods [2].

In the present work we have investigated the antioxidant and neuroprotective effects of different extracts of two varieties of *Capsicum annuum: Peperone dolce and peperone piccante di Altino* [3].

Each extract has been quantitatively analyzed to determine polyphenols and flavonoids contents by means of HPLC method, revealing a strong quantity of catechin and naringenin in microwave (MW) extracts of sweet and hot pepper respectively. Carvacrol is also present in decoction extract obtained with *n*-hexane for hot pepper. This extract resulted to have the highest phenolic content, while soxhlet extract of sweet pepper contains the best total flavonoid content.

Hot pepper and sweet pepper decoctions show good antioxidant properties in DPPH, ABTS, CUPRAC, FRAP, phosphomolybdenum assays and metal chelating activity. Enzyme inhibition tests were performed revealing a good inhibitory activity *in vitro* of MW extracts of hot and sweet pepper on α-tyrosinase. Finally we performed the cytotoxic activity test using MTT-based cell viability analysis against PC3 prostate cancer cells. MW extract of hot pepper and soxhlet extract of sweet pepper in methanol are able to reduce the % of cell survival to 20% and 3% at 1 mg/mL respectively suggesting a potential application as nutraceuticals in the management of prostatic cancer.

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Robust Feature Extraction from Raw GC×GC-TOFMS Data with PARAFAC2×2

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GC×GC-TOFMS excels when it comes to delivering sensitivity and selectivity, and it is widely used for probing complicated chemical problems. Uptake of GC×GC for studies with large numbers of samples has been slow, owing mostly to issues with performing data analysis and curating peak tables across hundreds or thousands of samples. A largely automated solution for robust and reliable automated identification and quantification of analyte signals in these large studies remains elusive. Most researchers rely primarily on commercial offerings, and for some commercial software, considering peak alignment across more than 150 or so samples is at best a challenge, and at worst an impossibility with these largely black box tools. Additionally, the number of parameters required to analyse GC×GC-TOFMS data leads to inconsistent results between researchers, and calls into question the objectivity of the analysis and its subsequent findings.

Parallel Factor Analysis 2 (PARAFAC2) is a parsimonious algorithm that has been used to quantify and identify analytes of interest in user-defined regions of one-dimensional GC-MS chromatograms, which present data as an $I \times J \times K$ third-order tensor of I spectral acquisitions, J mass-to-charge ratios, and K samples. It has been particularly influential through its distribution as the freely available analysis package: PARADISe [1]. Since GC×GC-TOFMS data presents as an $I \times J \times K \times L$ tensor of I spectral acquisitions (^2t_R), J mass-to-charge ratios, K modulations (^1t_R), and L samples, application of PARAFAC2 is not straightforward for these data. A possible work-around is to unfold the modulations to create an $IK \times J \times L$ tensor. In doing so, the degrees of freedom increases and the number of replicates decreases.

Herein, we present a novel approach to processing GC×GC-TOFMS data sets, using PARAFAC2×2. PARAFAC2×2 calculates two coupled PARAFAC2 models to account for drift in both the first- and second-dimension retention modes. At convergence, the two models are averaged to estimate for the unique score tensors for each sample and their resultant mass spectra and relative quantities. In this contribution the PARAFAC 2×2 algorithm is introduced, along with tools to estimate the number of chemical factors in the region of interest based on Projection Pursuit Analysis and clustering by OPTICS.

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Discriminant Visualization and Analysis Applied to Comprehensive Two-Dimensional Gas Chromatography for Geographic Classification of Olive Oils

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A new method for discriminant visualization and analysis can effectively overcome the difficult challenges of high-dimensional variable spaces with relatively small training sets such as are presented by classification with chemical features from comprehensive multidimensional chromatography. The visualization method, dubbed the Graphical Classification Interface (GCI), presents an interactive two-dimensional graph that is premised on the traditional assumptions of linear discriminant analysis, particularly multivariate normality and homoscedasticity.

GCI plots all (or selected) features for an individual sample with the abscissa for the item's feature value relative to the feature's Bayesian decision boundary and the ordinate for the feature's signed Fisher ratio. The result is a simple visual montage in which a positive slope indicates features consistent with Class 0 of the training set and a negative slope indicates features consistent with Class 1. The GCI also is suited for visualizing the results of linear discriminant analysis (LDA). For LDA visualization, the feature weights are used for scaling (rather than the features' standard deviations in the signed Fisher ratio).

Here, GCI is demonstrated on a data set from comprehensive two-dimensional gas chromatography (GC×GC) analysis of extra virgin olive oil (EVOO) samples from the VIOLIN project [1]. The data set is drawn from 73 samples from 2016-2018 harvests within seven different regions of Italy with nearly 600 matched chemical features per sample, including both targeted and untargeted features. LDA is highly effective for discriminating these EVOO samples by region of origin, with better than 90% accuracy in leave-one-out cross validation testing. GCI provides simple-to-interpret visualizations in which the chemical features that are most salient for classification are clearly distinguished and easily interrogated via the GUI.

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Differentiation of Regular and Barrel-Aged Maple Syrup with Tile-Based Fisher Ratio using ChromaTOF Tile

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Bourbon barrel-aging is a trend that has spread through a wide range of food and beverage markets where a charred oak barrel that had previously been used to age bourbon is repurposed for aging a different food or beverage product. This process typically lasts for several months and can cause significant changes in the product. The food or beverage can absorb flavors from the oak barrels and residual bourbon in the barrels, and flavors originally present in the food or beverage can infuse into the barrel and/or undergo reactions with time. This process tends to add both complexity of flavor and value to the products and has been used for beers, liquors, wines, sauces, syrups, coffees, and teas. In this work, we explore the differences between a barrel-aged maple syrup and a syrup that was not barrel-aged using comprehensive two-dimensional gas chromatography (GC×GC), time-of-flight mass spectrometry (TOFMS), and class-differentiating data mining software. GC×GC -TOFMS creates rich data and data mining tools help reveal the most important information and address the analytical questions. This combination of analytical tools helped isolate individual analytes in the complex data, determine those most likely to differ between the two types of syrups, and tentatively identify those differences. Many of the trends (higher or lower in barrel-aged syrup), were consistent by chemical functional groups. Alcohols, esters, and alkanes tended to be higher in barrel-aged syrups while nitrogen-containing rings (pyrazines and pyridines), sulfur-containing compounds, aldehydes, and ketones tended to be higher in the regular syrups. This sample comparison and characterization of the differences was facilitated by these analytical tools and workflow.
In this presentation, we will:

- Determine, identify, and characterize the chemical differences between bourbon barrel-aged syrup and syrup that was not barrel-aged.
- Apply Tile-based Fisher Ratio analysis with LECO's ChromaTOF Tile software to rapidly compare the raw data across the sample set to find class-differentiating features, the specific chemicals that distinguish the bourbon barrel-aged maple syrup from the regular maple syrup.

Class Comparison Enabled Data Analysis for GC×GC-TOFMS

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Comprehensive two-dimensional (2D) gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) has emerged as a powerful instrumental platform for the analysis of a wide variety of complex samples. However, since a massive amount of data is often obtained, to glean useful information more fully from the data, advanced data analysis tools are needed. To address this need, we have been examining and further developing different approaches to maximize the performance of tile-based Fisher-ratio (F-ratio) analysis, which can provide an in-depth understanding of the chemical composition differences for hypothesis-driven, class-comparison experimental designs. Although GC×GC-TOFMS provides exceptional peak capacity and detection selectivity, it is still common that analytes of interest may be marred by peak overlap, hindering reliable analyte quantitation and identification. Fortunately, tile-based F-ratio analysis can be extended to address such issues. Recently, statistical methods that determine mass channel purity have been developed, enabling the accurate quantitation and identification of analytes in complex peak overlap situations, with superior performance relative to deconvolution and decomposition chemometric methods. In this presentation, we will describe how the data output from tile-based F-ratio analysis is utilized to compute metrics for signal uniqueness and peak shape consistency between classes so that the purity of mass channel may be inferred. It will be shown that the pure mass channel information can be used for analyte hit quantitation and isolation of the analyte hit spectrum at very low 2D chromatographic resolution levels. Additionally, we have been investigating how to more beneficially apply the F-ratio calculation to improve “discoverability” of class-distinguishing analytes. These efforts broaden the scope of F-tile-based F-ratio analysis to a wider range of application scenarios.

A Unique Data Analysis Framework and Open Source Benchmark Data Set for the Analysis of Comprehensive Two-Dimensional Gas Chromatography Software

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Two-dimensional gas chromatography is amongst the most powerful separation technology currently available. Since its advent in 1990, it has become an established method which is readily available. However, one of its most challenging aspects, especially in hyphenation with mass spectrometry is the high amount of chemical information it provides for each measurement. The GC×GC community agrees that there the highest demand for action is found[1–3]. In response, the number of software packages allowing for in-depth data analysis of GC×GC data has risen over the last couple of years. These packages provide sophisticated tools and algorithms allowing for more streamlined data evaluation. However, these tools/algorithms and the functionality differs drastically within the available software packages.
This study focuses on two main objectives establishing first, an open-source dataset for benchmarking, and second, streamlined evaluation guidelines and thus allowing for an unanimously and comprehensive comparison for GC×GC software. Thereby, the Benchmark data includes, a set of standard compound measurements and a set of chocolate aroma profiles. On this foundation, eight readily available GC×GC software packages were investigated for fundamental and advanced functionality.

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Non-Targeted Analysis GC×GC -TOFMS using Floodlight and Searchlight: Machine Learning Assisted Data Analysis

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GC×GC -TOFMS has advanced the field of gas chromatography by enabling a high resolution separation that increases the detectability of low level species. Increasingly, NTA via GC×GC -TOFMS is being used to interrogate samples for exposomic purposes; sample matrices include consumer care products, household dust and serum [1]. Samples assayed by GC×GC -TOFMS using a non-targeted analysis (NTA) workflow may have thousands of compounds. With a lack of automated high-throughput screening methods available, tradeoffs must be made in regard to which samples can be investigated using an NTA workflow. This work aims to address this gap in available high-throughput screening technologies (Floodlight) and subsequent pattern analysis (Searchlight) for GC×GC NTA methods. Floodlight is a supervised learning-based method for high-throughput screening of MS data. Development utilized 30,000 high-quality spectra. The program couples spectral data with metadata to assess spectra quality and provide decision support to analysts. Searchlight employs a neural network approach to enable complex pattern analysis allowing the user to query a sample set for anomaly detection, concentration trends, sample comparison and nearest neighbor. Floodlight and Searchlight have been validated using chemical signatures in facial masks by comparison to results of the more tedious, manual curation process and have been found to produce comparable results in a fraction of the time [2].
In this presentation, we will:

- Describe the development and functionality of the Floodlight and Searchlight programs.
- Present a use case for consumer face masks.
- Discuss future applications and needs for high throughput GC×GC -TOFMS NTA.

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The Investigation of Bitumen Aging on a Molecular Level: A Complementary Approach Based on Comprehensive Two-Dimensional Gas-Chromatography High-Resolution Time-of-Flight Mass Spectrometry and High-Resolution Fourier-Transform Ion-Cyclotron Mass Spectrometry

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Bitumen is obtained in high margins in the distillation process of fossil energy sources such as heavy sour crude oils. In some cases, the properties are also modified by blending other heavy fossil vacuum gas oils and polymers or forced oxidation. The vast majority of the production is used as binding material in asphalt concrete for roads. During the life of the bitumen, aging processes occur that can negatively change its chemical and physical properties. As a result, increased corrosion damage to structures can occur, which can lead to substantial economic damage. While the macroscopic damage to e.g. roads in the form of fractures is obvious, its causes and the associated chemical changes in the bitumen are unclear. Commonly short-term and long-term aging are considered. Short-term aging occurs during the mixing and paving process at temperatures of 150-160 °C. In this study, short-term aged Bitumen is characterized deploying high-resolution mass spectrometry and state-of-the-art gas chromatography as analytical techniques to describe chemical changes

In this presentation, we will:

- Compare analytical data obtained from GC × GC – high resolution time-of-flight mass spectrometry with high resolution Fourier-transform ion cyclotron mass spectrometry. The interplay and complementarity of both high-resolution techniques will be shown to address different chemical compartments involved in the aging process.
- describe the general aging process on the basis of a model bitumen and will give an time course for the chemical changes in bitumen
- visualize and statistically evaluate the changes in certain chemical classes based on the distinctive strengths of the analytical techniques to differentiate and group isomeric and isobaric compounds.

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Average Theoretical Peak Time as a Metric to Analytical Speed in Monodimensional and Multidimensional Gas Chromatographic Separations

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The definition of a chromatographic analysis speed based simply on analysis time is an outdated concept to define conventional chromatography, fast chromatography, and emerging high-resolution techniques such as comprehensive two-dimensional and comprehensive three-dimensional chromatography [1-4]. Here, the metric average theoretical peak time (ATPT) is proposed for separation speed, considering conventional and multidimensional separations. ATPT can be defined as the time (in milliseconds per peak) needed to elute a theoretical peak in a chromatographic system. This metric was applied in several contexts to demonstrate its robustness to evaluate chromatographic separations for different techniques and analytical conditions. Applications also demonstrate the advantages of the use of ATPT as a method development metric tool.
In this presentation, we will:

- present the concept of a unified classification of gas chromatographic analysis speed based on time and efficiency.
- define the metric for average theoretical peak time (ATPT).
- apply the ATPT concept to several cases of study.

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Comprehensive Chromatography and Comparison of Operationally Relevant Atmospheres

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Chemical warfare agent sampling is performed using a variety of methods, such as direct liquid or solid collection, aerosol and vapor sampling, wipe sampling, or even via clothes and biological fluids of victims. CWAs range from high volatility compounds such as the G-agents, to low volatility compounds such as VX. Teams trained in the sampling of environments contaminated with chemical and biological (CB) materials are burdened with highly cumbersome PPE and handheld detectors as well as a limited working time due to breathing air constraints. During their time on target, they are tasked with characterizing the site, the materials present, and collecting samples for further analysis, all while providing real-time feedback to a command post.

There is an interest in low burden vapor characterization of CWA contaminated sites with passive sorbent samplers. We demonstrate an easy to use solution that provide reasonable detection limits as well as targeted and untargeted characterization of CWA contaminated sites that facilitates the comparison of multiple sites.

We also present preliminary data from aerosol and vapor sampling of several opioids that have undergone oxidative pyrolysis in a bench scale toxic chamber.[1-2] These highly complex samples contain a combination of opioid payload, pyrotechnic burn mix and breakdown products of both in aerosol and vapor form [3] which were analyzed with GC×GC.

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Evaluation of Pyrolysis Oils from Mixed Waste Plastics by GC×GC

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The issues around waste plastic have gained global attention, and many Value Chain Partners are working towards a plastic's circular economy. Dow has evaluated multiple pyrolysis oil samples produced around the world from waste plastics as a potential circular feedstock. Typically, these have been found not suitable to be used as a drop-in replacement of fossil-naphtha to produce circular plastics. To facilitate application of such oils as a circular feedstock, a much better understanding of the pyrolysis oil composition is required. This calls for the development of more advanced characterization techniques to identify a variety of hydrocarbons as well as organic molecules with hetero-atoms such as Cl, O, and/or N.

Pyrolysis oils originating from waste plastic are complex and contain unsaturated hydrocarbons which are not present in fossil feedstocks. Therefore, detailed hydrocarbon analysis (DHA) or GC×GC with FID detection is inadequate to fully characterize the hydrocarbon composition of such samples, especially when peaks are closely eluting, or even co-eluting. In this study, the GC-VUV method previously described for the analysis of liquid hydrocarbon streams[1] has been applied to the analysis of pyrolysis oils [2]. While GC-VUV ca provide superior PIONA quantification based on the UV spectra, little information was obtained for the heteroatoms.

Pyrolysis products are also rich in heteroatom containing hydrocarbons, especially oxygen, where commercially available methods are restricted within C_1 and C_5 oxygenates. To obtain a better understanding of the heteroatom composition, GC×GC coupled to Time of Flight Mass Spectrometry (TOF-MS) was used and preliminary results will be shown.

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Pyro-GC×GC-HRMS: Unlocking Heavies for In-Depth Characterization

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Analysis of heavy constituents of petrochemical products or by-products remains a challenging task due to both low volatility, making them inaccessible for gas chromatography, as well as low solubility in common solvents, making them inaccessible for liquid chromatography. Moreover, analysis of semi-solid or solid residues from petroleum refineries, such as vacuum residues, fouling deposits from steam cracker facilities or polymeric residues, requires a completely different approach to sample introduction. There is always a thrust to elevate the elution of heavier compounds for chromatographic separation. In addition, finding an effective approach to characterize non-elutable heavies also remained important.
In this study, first, an attempt has been made to elute native heavy species in such samples, by optimizing several aspects of the GC process, including column length, flow, injector and oven temperatures etc. Second, pyrolyzer injector coupled to GC×GC-HRMS was deployed to introduce intact molecules at lower temperature, as well as thermally degraded pyrolysis products at pyrolysis temperature, from semi-solid or solid residues. Thirdly, the thermal decomposition pathways of several polymers were investigated for better understanding of their degradation mechanisms.

Applying pyrolysis GC×GC coupled to EI-MS allowed separation of hydrocarbon classes resulting from polymer decomposition, along with heteroatom-containing species, in a grouped, structured manner. However, due to the high complexity of evolving constituents and extensive fragmentation occurring with EI, the identification of most of the pyrolysis products remained inconclusive. To minimize fragmentation, pyrolysis GC×GC was coupled to photo-ionization (PI) high-resolution mass spectrometry. Low energy PI yielded enhanced sensitivity and selectivity as a result of the dramatic reduction in fragmentation, as well providing an increase in the number of compounds identified. Applying of high-resolution MS combined with soft photo-ionization was found to be extremely useful and will be discussed.

A Pressing Need for GC×GC-Based Untargeted Analysis of Dumped Waste in the Ocean

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A recent story in the *Los Angeles Times* featured our GC×GC analyses of industrial waste within discarded barrels off the coastline of California. Underwater mapping with a wide range of remotely operated vehicles has identified 1000s of barrels dumped in the middle of the last century. It is assumed that the waste was generated by Montrose Chemical Corporation (MCC), the largest global supplier of DDT (~800,000 tons total production) at their Los Angeles County plant from 1947 to 1982.

Using advanced sampling techniques, we collected the material from several barrels and identified a wide range of chemicals consistent with DDT-based production including DDT, DDE, DDD, DDMu, Dicofol, and TCPMe (collectively referred to as ΣDDX). Yet, a much wider continuum of chemicals were identified in four distinct regions (mass islands) of the GC×GC chromatograms ranging from light saturated hydrocarbons, linear alkyl-benzene detergents, mid-range aromatic hydrocarbons, and petroleum-derived sterane, diasterane, and hopanoid biomarker compounds. Although the ΣDDX compounds were present in high abundance, they only represented 15-20% of the total mass of GC×GC amenable material in these complex waste-stream mixtures. While this work is preliminary, we believe that the dumped barrels also contain waste from multiple industrial waste-stream sources.

The heightened awareness of this previously undocumented environmental hazard has motivated numerous stakeholders to demand immediate removal of the barrels. There is no doubt that this is one approach to resolving this current problem, but we argue that the next steps demand an extensive collection of materials from other barrels and advanced analytical analysis. Based on our initial work, there are countless chemicals in these samples that are not regulated and listed on typical target-analyte lists. It would be unfortunate that risk-based decisions on future remediation options would consider only a handful of the chemicals in these barrels, especially when the barrels may contain different waste from other companies.

Industrial waste dumped into the Gulf of Mexico has also been recently recognized as a potentially significant problem. There are calls for expanded studies of these wastes around oil-production platforms near known dump sites. GC×GC has been an invaluable tool for studying the source, transport, and fate of pollutants in the environment and provided previously unknown and unattainable insights on problems that directly affect society and earth. We believe that the increased resolution afforded by GC×GC is essential for adding critical and powerful scientific content on how to mitigate dumped waste in the ocean.

In this presentation we present GC×GC-HRT data on environmental legacy waste-stream products dumped of the California coast.

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Burst Session Presentation Abstracts

Control-Normalized Fisher Ratio Analysis of Comprehensive Two-Dimensional Time-of-Flight Mass Spectrometry Data for Enhanced Biomarker Discovery in a Metabolomic Study of Orthopedic Knee-Ligament Injury

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Comprehensive two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass spectrometry (TOFMS) is more frequently being used for metabolomic studies due to the increased peak capacity provided by a second separation dimension [1,2]. However, there remain challenges in data handling of these complex data, and chemometric tools are often utilized to unravel relevant information from GC×GC-TOFMS data [3,4]. Recently, an innovative form of Fisher Ratio (F-ratio) has been developed and applied to the investigation of changes in human plasma for patients with injury to their anterior cruciate ligament (ACL) [5].
In this presentation, we will:

- compare metabolite hit list obtained from the standard F-ratio (^{S}F-ratio) of patients vs. controls, and that from "control-normalized F-ratio" (^{CN}F-ratio), which utilizes the within-class variance of the control class only.
- discuss the complementary nature of ^{S}F-ratio and ^{CN}F-ratio analyses using the top 30 "hits" from each hit list.
- demonstrate the class distinguishing ability of hits which pass the *t*-test, using principal component analysis (PCA).

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Missing Data Imputation in Untargeted Metabolomics

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Missing data is a significant issue in metabolomics that is often neglected when conducting data pre-processing, particularly when it comes to imputation. This can have serious implications for downstream statistical analyses and lead to misleading or uninterpretable inferences. Handling missing values depends heavily on the missingness type (missing completely-at-random, missing at-random, missing not-at-random), and while methods do exist to assess mechanisms of missingness, many rely on inherent assumptions about the missingness mechanisms themselves, and others cannot be utilized in metabolomics data that commonly contain significantly more features than samples. Additionally, methods that exclude features with high missingness such as the "80% rule" introduce the risk of excluding potentially important features (e.g. biomarkers), and approaches for imputing missing values must be carefully evaluated as misuse can lead to spurious results.

In this presentation, we will:

- provide a brief overview of missing data in the context of GC·GC (missingness type, how to identify missingness).
- outline approaches for imputing missing values in GC·GC data, particularly when there is replication in the experimental design.

Class Comparison Enabled Methodologies for Improving Analyte Quantitation and Identification with GC×GC-TOFMS

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Although GC×GC-TOFMS is a powerful instrumental platform for the analysis of complex samples, it is still common that analytes of interest may be marred by peak overlap, hindering reliable analyte quantitation and identification. Class comparison methods, specifically tile-based Fisher ratio analysis which facilitates the supervised discovery of analytes of interest [1,2,3], can also be extended to address such issues. Recently, statistical methods that determine mass channel (m/z) purity have been developed, enabling the accurate quantitation and identification of analytes in complex environments, with superior performance relative to deconvolution and decomposition chemometric methods [4,5]. In this presentation, we will describe how the data output from tile-based F-ratio analysis is utilized to compute metrics for signal uniqueness and peak shape consistency between classes so that the purity of m/z may be inferred. It will be shown that the pure m/z information can be used for analyte hit quantitation and isolation of the analyte hit spectrum at very low 2D chromatographic resolution levels.

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Comparative Analysis of Particle-Bound Semi-Volatile Organic Compounds by IDTD-GC·GC-ToFMS: Identification of Compounds Specific for Allergy-Protective Environments

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In the last decades the prevalence of allergies increased worldwide [1]. Industrialization and lifestyle changes in context with biodiversity loss and increasing air pollution seem to be major reasons for this trend [2]. Simultaneously, certain farming environments seem to protect from allergy development [3, 4]. Main factors leading to this protective effect are besides consumption of raw cow milk, the presence of cow and straw [5]. A comparative study investigating the difference between a protective (cow) and non-protective (sheep) farming environment has been conducted to identify significant differences in the chemical pattern of particle-bound semi-volatile organic compounds (SVOCs).

In this presentation, we will:

- demonstrate the direct analysis of particle-bound SVOCs of farming environments via in-situ derivatization thermal desorption two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (IDTD-GC·GC-ToFMS)
- compare the SVOC pattern of a protective and a non-protective environment and identify compounds specific for the protective environment by performing analysis of variances-principal component analysis (ANOVA-PCA)
- exemplary determine concentrations for these specific compounds found in one of the cow sheds by means of an external calibration or a standard addition approach after GC·GC analysis

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Setting Key-Processing Parameters for Effective Template Patterns Alignment and Transform for Saliva Metabolites Fingerprinting by Comprehensive Two-Dimensional Gas Chromatography

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Comprehensive two dimensional gas chromatography with time of flight mass spectrometry (GC×GC-TOF MS) represents one of the most powerful analytical platform for chemical investigation of complex samples. However, it produces large and complex sets of data, rich of information, but whose consistency might be affected by random fluctuations of system performances or changes in experimental parameters.

This study focuses on human saliva metabolites signatures explored by GC×GC-TOF MS followed by untargeted and targeted (*UT*) pattern recognition, i.e., *UT fingerprinting* [1]. *K*ey-process parameters are examined for their impact on false negative matches and for consistent cross-alignment of data. Signal-to-noise ratio detection and MS spectrum similarity thresholds were systematically varied to generate reference patterns (i.e., templates) to be used for effective cross-alignment. To compensate for retention time misalignment, supervised procedures accompanied by global polynomial 2nd order transforms were tested.

Case Study-I refers to a diet intervention by meals rich in advanced glycation end products (AGEs). The *UT fingerprintin*g was applied to identify markers arising from a AGEs rich diet vs. a control diet. *Case Study-II* deals with metabolically healthy (MHO) and unhealthy (MUHO) obesity and saliva signatures were captured by TOF MS acquiring in Tandem Ionization™ (TI) conditions.

The two datasets showed marked, random pattern shifts. By combining S/N and MS similarity thresholds to global polynomial 2nd order transforms and supervised re-alignment of patterns, the matching rate of reference 2D peaks increased from 51% to 84%. Once re-aligned, peak and peak-region features were explored by supervised pattern recognition to reveal potential markers.

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Initialization of Non-Negative PARAFAC2 using Independent Component Analysis

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A flexible coupling PARAFAC2 algorithm enables researchers to model experiments with chromatographic drift without constraining the elution profiles to be orthogonal. While offering a more accurate determination of latent chemical phenomena, the flexible coupling has not been proven to guarantee the uniqueness of the solution; this increases the risk of the algorithm converging to poorly descriptive local minimum. Independent Component Analysis (ICA) is a rapid matrix factorization technique that can be used to initialize a Non-Negative PARAFAC2 model, and can be deployed quickly and without non-negativity constraints using an appropriate sign-determination step.

Using the Joint Approximation Diagonalization of Eigen-matrices (JADE) implementation of ICA, this initialization strategy is compared against initialization using PARAFAC, Singular Value Decomposition (SVD), and random latent co-vectors on several experimental datasets. This technique has potential for applications to larger datasets - in particular those encountered as intermediate steps for decompositions of GC×GC-TOFMS data.

GC×GC /MS as a Tool to Assess the Impact of a Biofungicide on the Volatile Profile of Chardonnay Wine

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Bacillus-based biocontrol agents play a fundamental role in the field of biofungicides used in vineyards. Species of this bacterium have antifungal potential through space/nutrient competition with pathogens and synthesis of lipopeptides, polyketides, siderophores, volatile compounds, and enzymes [1]. *Bacillus velezensis* P1 isolated from the aquatic environment of the Amazon region (Brazil), proved to be a promising strain to control toxigenic fungi in culture media [2]. Wine quality is influenced by fungi control strategies since fungicide residues remain on grapes after harvest and are transferred to the must during winemaking [3]. The objective of this study was to evaluate the impact of the use *Bacillus velezensis* P1 as biofungicide during Chardonnay grapes production on the volatile profile of the wines analyzed by comprehensive two-dimensional gas chromatography coupled to mass spectrometry detection (GC×GC/MS). Two types of wines were analyzed: (i) wines made with grapes treated with a biocontrol strategy and (ii) wines made with grapes that did not receive antifungal treatment (control). Headspace-solid phase microextration (HS-SPME) was used for sampling volatile compounds as follows: 1 mL of sample, 30% of NaCl (m/v), 55 °C for 45 min [4]. The compounds were desorbed in the GC inlet at 250 °C for 5 min. GC×GC/MS was performed using SLB-5ms (30 m · 0.25 mm · 0.25 μm) and SLB-35ms (1.5 m · 0.25 mm · 0.25 μm) columns in first and second dimensions, respectively. The GC ovens were heated as follows: initial temperature of 50°C for 1 min, programmed at 3 °C min^{-1} to 260 °C (5 min). Fisher ratio, hierarchical clustering analysis (HCA) and heat map were used to verify the differences between samples. Wines made with grapes treated with biofungicide were differentiated from control wines mainly by higher levels of varietal compounds such as monoterpenes (linalool, citronellol, myrcenol, and α-fenchene) and sesquiterpenes (bisabolene, farnesol, and copaborneol). These compounds were found in low concentrations in wines; therefore, their detection by one-dimensional analysis systems would be a challenge. In addition to being a more environmentally friendly option, the use of biofungicide in grapes contributed to the expression of varietal aromas in wines.

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Geographic Classification of Botrytized Wines using Flow-Modulated Comprehensive Two-Dimensional Gas Chromatography

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A challenging area in the field of comprehensive two-dimensional gas chromatography (GC×GC) is incorporation of flow modulation setup into routine analysis. Whereas GC×GC with thermal modulation dominates in omics studies for food aroma characterization and analysis. However, advances and the continuous development of flow modulation technology support improvements in resolution, peak capacity, and separation efficiency of flow modulated comprehensive two-dimensional gas chromatography (FM-GC×GC) [1]. One of limitations to exploiting the technique is more demanding assignment of experimental conditions. Different mathematical models for processing data could simplify this process and help to predict chromatographic parameters [2-4] In this presentation, we will:

- apply central composite design for optimization of experimental parameters of FM-GC×GC method (modulation period, carrier gas flow in the primary and secondary column). It will involve Pareto charts to determine effects statistically significant for response area and peak tailing. The optimal experimental conditions will be obtained with polynomial functions, artificial neural networks (ANN) and afterward response surface methodology (RSM) coupled to Desirability approach.
- apply the FM-GC×GC method to determine geographical origin of botrytized wines based on enantiomeric distribution of chiral compounds.

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Breath Biomarkers for Tuberculosis in Children using GC×GC-ToFMS

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Tuberculosis (TB), was the 8th and 7th leading cause of death in low-income and lower-middle income countries respectively, in 2019.[1] In that year, 10 million persons acquired TB, of which 1.2 million where children under 15.[2] Additionally, the disease claimed the lives of 1.5 million people.[2] In 2017, 233,000 children under the age of 15 died from TB.[3] Underdiagnosed and under reported cases contribute to these deaths with 69% of cases in patients under 5, and 40% of cases in patients 5-14 estimated to be missed.[3] There are still challenges with reliably diagnosing TB in children, as the gold standard tests, microbial culture and nucleic acid amplification, require biofluid (often sputum), and are relatively insensitive. This requirement renders these tests non-child-friendly as children frequently cannot produce the sputum needed.[4] Although sputum-induction is possible, it proves challenging in certain healthcare settings.[5-7] Overall, these methods are inadequate for many children, and as such, there exists a need for child-friendly, sputum-independent, and accurate diagnostic methods for pediatric TB. To this end, breath biomarkers demonstrate great promise.

In this presentation, we will share the results of a pilot study in which we:

- putatively identified 4 breath biomarkers for pediatric TB via TD-GC×GC-ToFMS analysis
- built a machine learning model that classified children with confirmed TB from those with other lower respiratory tract infections (sensitivity 80% and specificity 100% across cross validation folds).[8]

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Forensic Identification and Metabolomic Profiling of Two Oak Species by GC×GC-TOFMS

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Many methods have been developed to track and identify illicit wood products or, on the other hand, to evaluate their legality. However, previous techniques have various limitations in terms of their reliability for robust species identification, costs, or sample type requirements. One significant challenge is that heartwood is typically the tissue of choice for sampling. However, its collection requires coring trees of a minimum diameter (i.e., "age"). This process is invasive, requires specialized tools, trained personnel, is time-consuming, and can potentially expose interior tissues of trees to pathogens. Additionally, as heartwood decays, consistency of results suffers.

In this presentation, we will:

- compare metabolite profiles obtained from GC · GC –TOFMS analysis of two oak species (Quercus rubra and Q. macrocarpa) and multiple tissue types (e.g., sapwood, branches, leaves, microcores). The goal is to simplify current identification methods and expand applicability of methods to other easier-to-collect sample tissues like branches, leaves, twigs, or bark for identification.
- apply chemometric models to GC · GC data to differentiate oak species and tissues

GC×GC-TOFMS Applied to the Enantioselective Analysis of Extraterrestrial Samples

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Life's essential molecules are asymmetric. Proteins use exclusively amino acids with L-configuration whereas DNA and RNA are composed of ribose and 2-deoxyribose with D-configuration. The origin of this symmetry breaking is key to understanding the origin of life. Based on *in-situ* observations and laboratory studies, this handedness might occur when chiral biomolecules are synthesized asymmetrically through interaction with circularly polarized light (CPL) in interstellar space [1]. Due to a true chiral influence of CPL [2], the interaction with racemic or achiral molecules generates slightly enantiomerically enriched mixtures.

In this regard, the enantioselective analysis of chiral biomolecules and their precursors in authentic and simulated extraterrestrial matter is crucial to deciphering the events that led to biological homochirality. In recent years, significant progress has been made to develop high-performance analytical protocols for the enantioseparation of amino acids [3], monocarboxylic acids [4], as well as aldehydes and ketones [5]. The detection of sugars in interstellar analogue ices [6],[7] and in meteorites [8] suggests that life's molecular building blocks are formed in interstellar environments. However, the enantioseparation of sugars represents a rather difficult task due to the low quantities available in such samples and the need to suppress the intramolecular hemiacetals formation of C5 and C6 sugars to decrease the sample's complexity and the limit of detection of individual sugars.

This presentation will show that GC×GC-TOFMS in combination with adequate derivatization strategies is a suitable tool for the detection, quantification, and enantioseparation of biomolecules such as sugars up to C6 carbohydrates as well as amino acids and will summarize the related latest results of our group.

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Volatile Fingerprinting of Boar Taint by GC×GC-TOFMS

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As a response to growing ethical constraints, the meat production industry has agreed to abandon surgical castration of male piglets towards 2018. However, raising uncastrated male pigs increases the risk commercializing meat with an undesirable taste know as boar taint. The main compounds contributing to the fecal, urine, and sweat-like taste or smell are androstenone, skatole, and indole. Different analytical methods have been proposed and validated for their quantification in plasma or fat tissue [1]. However, the application of these methods is oftentimes not applicable directly in a slaughterhouse routine due to time constraints related to the high throughput of industrial meat production processes. Only a few on-site options exist but are still at testing stage [2]. Therefore, it is common practice to conduct olfactive screening based on so called 'soldering iron sensory methods' carried out by trained assessor. Tainted carcasses are then pushed aside from commercialization. This is currently the fastest and least onerous procedure to determine boar taint presence but it is believed to suffer from inter-individual variations and limited correlation to instrumental measurements. The incidence of boar taint at commercial slaughters in Belgium is estimated to between 3 and 7% [3]. In this study, back fat and in-vivo samples (saliva, hair, semen) were analyzed with comprehensive two-dimensional gas chromatography (GC×GC) coupled to time-of-flight mass spectrometry (TOFMS) for volatile fingerprinting.
In this presentation, we will:

- discuss the problematic of current techniques for the detection of boar taint.
- explore how GC·GC-TOFMS can be applied for targeted and non-targeted analysis of back fat samples.
- compare analytical data obtained by GC·GC-TOFMS, UHPLC-MS and sensor panels.
- demonstrate the potential of GC·GC-TOFMS for boar taint fingerprinting.

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Determination of Contaminants of Emerging Concern and Their Transformation Products in Treated-Wastewater Irrigated Soil and Corn

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Irrigation of agricultural land with treated wastewater is commonly used in response to water shortages but there is concern about the environmental fate and transport of contaminants present in the irrigation wastewater. This study aimed to examine the presence of wastewater sourced contaminants in soil and field grown corn (*Zea mays*) crops spray irrigated with treated wastewater. Due to the complexity of these environmental matrices, traditional chromatographic techniques are not effective for the separation and characterization of contaminants of emerging concern (CECs) in the samples. GC × GC-TOFMS has been shown to be an effective technique for the characterization of organic contaminants in a variety of complex environmental samples, such as surface waters and soils [1,2].
In this presentation, we will:

- examine treated-wastewater irrigated soil and corn crops from the Penn State Living Filter for common organic pollutants and contaminants of emerging concern.
- use both targeted and non-targeted GC · GC- TOFMS data to compare the treated-wastewater irrigated samples to control site samples.
- detail the newly identified chloro-dimethyl-benzotriazole compounds detected in the treated-wastewater irrigated soil and corn root samples. These previously unidentified compounds are most likely formed in the chlorine disinfection step at the wastewater treatment plant and remain in the water until sprayed for crop irrigation at the Living Filter spray fields.

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Identifying the Transition from Antemortem to Postmortem Decomposition Odour

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Decomposition odour research investigates the volatile organic compounds (VOCs) released from decomposing human remains. Currently, there are very few studies focusing on the VOCs released during the early postmortem period and the transition from antemortem odour to postmortem odour (1)(2). Understanding the transition from the antemortem to the postmortem odour is critical to locate and recover victims of mass disasters (1). A sample collection and analytical method was developed in this study to identify the VOCs released during the early postmortem period. The VOC samples were collected from human remains donated to the facility for Research in Experimental and Social Thanatology (REST) and placed in an outdoor environment in Bécancour (Québec). Samples were collected for an extended period according to the accumulated degree days (ADD) as seasonal fluctuations impacted the rate of transition to decomposition VOCs. Donors were received at the REST facility within 6-72 hours of death (early postmortem period). The VOCs were collected from the headspace of the donor onto stainless steel dual sorbent tubes and analysed using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS). The analytical and sample collection method identified compounds from different chemical classes such as alcohols, ketones, sulfur-containing, aliphatics and aldehydes commonly associated with decomposition odour in the early postmortem period. The odour profiles demonstrated a variable range of chemical classes. This knowledge broadens our understanding of the VOCs released from cadavers in the early postmortem period, which could assist in more rapidly locating victims in mass disaster scenarios and search and rescue operations. Knowledge of the early postmortem period VOC profile can also enhance the training of cadaver-detection dogs to recognise the distinct odour during this timeframe.

In this presentation, we will:

- Present an optimised method for collecting and analysing VOCs from human remains during the early post-mortem period and the analytical output using a newer and more sensitive Pegasus®BT 4D GC×GC-TOFMS instrument (LECO Corporation, St Joseph, MI, USA).

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GC×GC - Method Development for Analysis of Polycyclic Aromatic Compounds (PACs) in Environmental Samples

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The application of gas chromatography coupled with mass spectrometry has been a dominant technique employed for the separation, identification, and quantification of volatile environmental contaminants present in complex matrices. Polycyclic aromatic compounds (PACs) are ubiquitous environmental contaminants and are among the most complex class of environmental compounds known with their complexity largely source-dependent.[1] They consist of multiple compounds and the number of theoretically possible isomers can reach into the thousands.[2] The high-resolution power of comprehensive gas chromatography was applied to aid in the separation, identification, and quantitation of PACs in different environmental matrices. Currently, attention is given more to group type separation of PACs. However, individual PACs can reveal important information on how the PACs were formed and this information may be used to determine sources of PACs in environmental samples and/or in ecotoxicology studies.

In this presentation, we will discuss: the development and validation of an analytical method for evaluating PACs and its application to three environmental samples; mussel tissue (*Mytilus edulis*), lubricating oil and coal; application of the method to detecting and identifying novel compounds – halogenated PAHs- in biota sample in the Canadian environment; and an ongoing expansion of our compound list in the method to comprehensively identify and measure PACs in environmental samples and assess what proportion of PACs are from coal tar-based products compared to other sources including diesel particulate, asphalt sealants, roofing material, tire and road rubbers diesel particulate and motor oils.

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Addressing Challenges in Analyzing Arson Debris by Implementing GC × GC-MS

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Arson cases have one of the lowest conviction rates for major crimes, with a large percentage of unsolved cases. The presence of trace levels of ignitable liquid residues (ILR) in arson debris samples are frequently obscured by abundant matrix compounds which may have similar physico-chemical attributes, making identification challenging. Comprehensive gas chromatography (GC · GC) has proven to be a powerful tool for improving sensitivity and selectivity in ILR analysis [1,2] and was shown to decrease the potential for false negative results from interferences [3].

In this presentation, we will discuss the:

- Application of design of experiment principles to method development of flow-modulated GC · GC-MS ILR analysis. We will highlight the criteria for column selection, modulator settings and run parameters, as well as their cumulative effects
- Development of retention time indices for GC · GC analysis of ILR and the advantages for sample comparison across arson cases and laboratories.
- Importance of sample integrity, and explore potential marker compounds (or ratio of compounds) which may be used to identify cross-contamination and associated false positives.

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Volatile Profiling of Cadaver Dog Training Aids using Comprehensive Two-dimensional Gas Chromatography

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Cadaver detection dogs (CDDs) are used to search for and locate whole or parts of human remains. CDDs detect the volatile organic compounds (VOCs) released during the process of human decomposition [1]. To reinforce their capability of detecting the targeted odor, CDDs are often trained on chemical formulations, animal remains and human remains although there is evidence that formulations and animal remains do not accurately represent the odor of human remains [2]. Currently, the Ontario Provincial Police (OPP) uses remains obtained from live amputation surgeries of limbs from diabetic patients as CDD training aids. There is limited knowledge about the VOC composition of such limbs and their appropriateness as an alternative to human remains for CDD training purposes, which formed the aim of the current study. In this study, VOCs were collected using sorbent tubes and analyzed with comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC–TOFMS). Compounds belonging to chemical classes including acids, alcohols, aldehydes, ketones, sulfur and nitrogen containing compounds, aliphatic and cyclic hydrocarbons, were identified. Among all the training aid types used by the OPP – teeth, blood, foot, foot tissue, foot bones; teeth were found to be the least suitable in their representation of the human decomposition VOC profile. These results allow us to understand the type of VOCs that CDDs are exposed to during training and to compare the profile with VOCs from decomposed cadavers, which represent the VOC profile they are exposed to during operational casework.

In this presentation, we will:

- Understand the use of GC×GC to identify volatile organic compounds in the complex air matrix associated with decomposing human remains.

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Characterization of Contaminants in the Canadian Arctic Marine Environment

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Every day, large amounts of chemicals are, in most cases unknowingly, released to the environment. These chemicals can travel on global or local scales and amongst the different environmental compartments based on their physiochemical properties. Although Arctic environments are often remote and not close to industry, the Arctic is considered a sink for global pollutants [1]. The goal of this study is to conduct a comprehensive screening and characterization of new and emerging anthropogenic and naturally derived organic pollutants in water, sediment and biota samples collect from the Canadian Arctic marine environment.

We collected nine large volume water samples, 16 surface sediment samples and 77 biological samples (fish and invertebrates) in the Hudson Bay area in the Canadian Arctic. Sediment and biota samples were extracted with microwave assisted extraction and underwent sulfur removal with activated copper and lipid removal with gel permeation chromatography, respectively. Water samples were collected on XAD sorbent and extracted with DCM. All samples were analyzed using comprehensive two-dimensional gas chromatography (GC×GC) coupled to high resolution time-of-flight mass spectrometry. In addition, a few selected sediment samples were analyzed by GC-Orbitrap for comparison.

The data files resulting from the GC×GC analysis were aligned and a pre-selection was performed to firstly, increase the quality of the data and, secondly, to reduce the amount of data. Multivariate statistics such as principle component analysis and hierarchical clustering are used to determine patterns and similarities between the sample types. Results indicate a difference in chemical composition between the three different types of matrix (water, sediment and biota). Moreover, distinct clusters form for different types of biota samples, as for example fish, shrimp and shrimp eggs.

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Evaluation of Different Internal Diameter Modulation Columns within the Context of Solid-State Modulation

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The solid-state modulator is a recently developed consumable-free thermal modulator used within the context of comprehensive two-dimensional gas chromatography. Its consumable-free nature is guaranteed by the presence of a thermal-electric cooler device located between two heated chambers, everything located outside the gas chromatograph oven. The motion of a trapping capillary through these three thermal zones enables a dual-stage modulation.
The present contribution is focused on the evaluation of the solid-state-modulator modulation performance in relationship to different modulation capillary geometries. Specifically, two coated modulation capillaries with the same length, but with different internal diameters (0.25 and 0.18 mm) were used. The effects of gas linear velocity, modulator temperature, and modulation period were evaluated in several applications involving a mixture of standard alkanes and a sample of diesel fuel. Fundamental gas chromatography parameters (peaks widths, resolution) were measured under the different experimental conditions. Detailed information is provided on gas flow optimization, with particular emphasis on the efficiency of chromatography band reinjection onto the second-dimension column. The results obtained from the present investigation highlight how the modulation capillary characteristics have a great impact on the overall comprehensive two-dimensional gas chromatography separation. Specifically, considering the results herein attained, the use of a 0.18 mm ID × 0.18 μm d_f modulation column is advisable compared to a 0.25 mm ID × 0.25 μm d_f one.

Development of Retention Index Based Approach for Simulation of GC × GC Results.

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In this work, retention index (*I*) based calculation approach is established to simulate first and second retention time (^1t_R and ^2t_R) and peak profile for a given volatile compound as well as contour plots of samples in comprehensive two-dimensional gas chromatography (GC×GC). This involves (1) curve fitting based on Kovats retention index equation and van den Dool and Kratz relationship in order to simulate the correct ^1t_R of alkane ($^1t_{R,corr}$) based on a training set of experimental data, (2) calculation of correct ^2t_R ($^2t_{R,corr}$) by using a nonlinear equation with six constant parameters that were obtained according to adjusted ^2t_R equation followed by generation isovolatility curves from $^1t_{R,corr}$ and $^2t_{R,corr}$ data of each alkane, (3) prediction of ^1t_R and ^2t_R for each analyte based on their given ^1I and ^2I data from literatures, and (4) summation of peak profiles for all the expected compounds in a sample in order to simulate the contour plot [1,2]. All the calculations and curve fittings were carried out by using *Solver* in Microsoft Excel, and generation of the contour plots was performed using MATLAB. The approach was applied to simulate the results for a given set of first and second dimensional retention indices (^1I and ^2I) of the compounds in several samples including saffron (*Crocus sativas* L.), *cachaça*, *Boswellia papyrifera*, acacia flowers, honey, incense powder and smoke [1,3,4,5]. The simulated ^{2}D contour plots were then compared with the experimental results and discussed. This approach can be applied to support experimental design in GC × GC in the future with a large dataset of volatile compounds from the retention index library based on three types of columns (polar, semi-polar and non-polar column).

In this presentation, we will

- present the concept and software for simulation of GC×GC results (^{2}D contour plots).
- show the simulated results and compare with the experiments e.g., saffron (*Crocus sativas* L.), *cachaça*, *Boswellia papyrifera*, acacia flowers, honey, incense powder and smoke.

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Flexible Second Dimension Temperature Programming System for GC×GC

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Comprehensive two-dimensional gas chromatography (GC×GC) has gone through three decades of research and development. Over this time, much of the instrumental development has been focused on the modulator, the most critical component of the GC×GC system. In contrast, little effort has been focused on optimizing the separation in the second dimension (^{2}D) column. One exception is the secondary oven, which provides a constant temperature offset between the secondary column and the GC oven. This, in turn, provides additional flexibility in method development to combat wraparound peaks. However, even with the secondary oven, the ^{2}D separations are practically isothermal and are subject to the general elution problem. Applying a positive temperature offset to the ^{2}D column to reduce wraparound peaks results in loss of resolution between less retained compounds. To remedy this issue, a ^{2}D temperature programming system capable of producing rapid and reproducible heating rates of > 10 °C/s have been developed. A sample of diesel was separated to compare the differences in peak capacity between a GC×GC separation with and without a constant temperature offset, and with ^{2}D temperature programming. The flexibility of the system will be highlighted with a perfume sample, using different ^{2}D temperature programs during a GC×GC separation. With this system, we were able to improve peak capacity and resolution with a design that was focused on creating compatibility with any GC×GC system.

Exploring Total-Transfer Comprehensive Three-Dimensional Gas Chromatography

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Improved instrumentation for comprehensive three-dimensional gas chromatography with time-of flight mass spectrometry (GC^3-TOFMS) is described. The new platform addresses shortcomings in previous GC^3-TOFMS designs, namely that one or both modulators had a duty cycle < 100%, making the potential gain in detection sensitivity over GC × GC modest at best. Thus, the GC^3-TOFMS instrument described provides total-transfer (100% duty cycle) with both modulators. The instrument is based on the facile modification of a commercial thermally modulated comprehensive GC × GC-TOFMS platform for modulation from the ^{1}D column to the ^{2}D column, with recently described dynamic pressure gradient modulation (DPGM) as the second modulator from the ^{2}D column to the ^{3}D column, which is a total-transfer flow modulation technique. The tandem total-transfer modulation approach enables signal enhancements from ^{1}D to ^{3}D as high as 177 ($\overline{x}$ = 130, s = 47) to be obtained. Column selection is explored, including the use of a highly polar ionic liquid phase on ^{3}D. Application to real-world samples will be presented, including derivatized porcine serum and a jet fuel with a high content of organosulfur compounds.

Strategies Towards Simpler Configuration and Higher Peak Capacity with Comprehensive Multidimensional Gas Chromatography

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Experimental and data analysis approaches in multidimensional gas chromatography (MDGC) comprising comprehensive multiple heart-cut (H/C) and comprehensive two dimensional GC (GC×GC) were developed with an example application illustrated for analysis of technical glycol precursor sample. GC×GC system employed a long ^{1}D (60 m) and a short ^{2}D (5 m) columns with flow modulator and a Deans switch (DS) as a splitter; whilst, the H/C system applied solely a DS located between long ^{1}D (60 m) and ^{2}D (60 m) columns without use of cryogenic trapping devices. The effects of injection time and ^{2}D column flow in GC×GC and the impacts of H/C window and number of injections (total analysis time) in H/C analysis were investigated. The analysis performance for each condition was evaluated according to peak capacity and number of separated compounds. The continuum between the two techniques was then established via the relationship between analysis time and analysis performance. The separation performances were improved with longer analysis time so that the suitable condition was selected within this compromise. Under the selected conditions, volatile compounds in technical glycol precursor sample were identified according to match between the experimental MS spectra and first dimensional retention indices (^1I) with that from NIST2014 database and literatures. An hour analysis with GC×GC resulted in total peak capacity of 798, number of separated peaks of 61 and average MS match score of 887±35; whilst, the corresponding numbers were improved to be 9198, 107 and 898±24, respectively, with the 25 h comprehensive H/C analysis [1] .

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Diving Deep Into Super-Resolved GC×GC

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Data interpretation and processing plays a major role in comprehensive two-dimensional gas chromatography (GC×GC). Based on the previous work, [1] where improved compound localisation and data presentation in GC×GC was reported, here aspects of 'super-resolved' derivation of the 2D data are further described. Centroiding (peak apex) representation, where the first- and second-dimension retention times (^1t_R and ^2t_R, respectively) are presented as x-y coordinates, allows a pin-point peak positioning allocation in the 2D plot. ^1t_R was derived from an exponentially modified Gaussian (EMG) fitting model for near-Gaussian distributed subpeaks with polynomial fitting for highly asymmetrical peaks, and parabolic fitting for under-sampled peaks. This assigns a precise value to the elution time of a compound on the ^{1}D column, which is now represented as its apex. ^2t_R was approximated by weighted average of ^2t_R values for all modulated peaks. Peak area, represented as the coordinate height of the peak area magnitude, is the summation of areas of the modulated peaks belonging to the same compound. Using this approach, the work further explores the technique for aspects of additional information mining, for instance using mass spectrum specificity as a general guide to support the relevance to peak position markers.

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In Vitro Modelling of Lung Inflammatory Processes

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Exhaled breath analysis has a high potential for early non-invasive diagnosis of lung conditions. The inflammation processes associated with oxidative stress yield to the conversion of membranes components into volatile organic compounds (VOC) secreted by the lungs. The characterization and understanding of the inflammatory metabolic pathways involved into VOC production is necessary to define proper medication. In this study, lung inflammation was simulated *in-vitro* on lung epithelial cells. We compared the VOC production following a conventional oxidative stress *in-vitro* using hydrogen peroxide (H_2O_2) with a biological model using inflammatory sputum from asthmatic patients. The VOC were extracted and analyzed by solid-phase microextraction comprehensive two-dimensional gas chromatography hyphenated to time-of-flight mass spectrometry. In the oxidative stress experiments, we exposed the epithelial cells to 0.1 mM H_2O_2 for 1 h. In the biological stress experiment, the epithelial cells were exposed to 50 % (*v/v*) inflammatory and non-inflammatory pool of sputum supernatants for 24 h. These optimal conditions were used to induce metabolic response, releasing specific metabolites, without causing significant cellular apoptosis. According to the type of inflammation induced, different VOCs were produced by the cells. For both chemical and biological challenges, an increase of carbonyl compounds and hydrocarbons was observed. However, 36% of the specific VOCs were produced only after a biological stress. Taken together, these results highlight that *in-vitro* VOC analysis is a very promising approach to characterize complex lung inflammatory mechanisms. The future implementation of multi-omics screening could reveal new information on the molecular mechanisms involved in lung inflammation.

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Development of an Enhanced Total Ion Current Chromatogram Algorithm to Improve Untargeted Peak Detection

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Data analysis for comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry relies upon accurate peak detection [1]. Often, this is performed on the total ion current chromatogram (TIC), which is the summed signal from all mass spectral channels. Despite detecting many of the most abundant peaks, a larger fraction of peaks remains undetected in the standard TIC due to their signal being below the limit of detection [2]. In this presentation, we will present the development of an untargeted peak detection method termed the "enhanced TIC algorithm" to find peaks obscured by the background noise. The algorithm utilizes the entire mass spectral dimension to find regions of analytical signal above a threshold while zeroing the noise. The resulting chromatographic data is summed together to create the enhanced TIC. The utility of the enhanced TIC algorithm is first demonstrated using serial dilutions from a 10 ppth test mixture. Application of reported algorithm on chromatograms collected at 1 and 10 ppm recovered 62% and 93%, respectively, of the original peaks observed, while the standard TIC recovered only 0% and 45%, respectively. The improvement in signal enhancement is also shown on a separation of a yeast cell metabolite extract, where the enhanced TIC found 33-64% more peaks than the standard TIC. Simulated chromatograms with lower signal-to-noise are more accurately modeled by the statistical overlap theory after enhanced TIC processing compared to those processed by the standard TIC. The enhanced TIC algorithm demonstrates an immense benefit in peak discovery to improve data analysis efforts for multidimensional chromatography [3].

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Characterizing the Valley Fever Volatile Metabolome for Breath Test Development

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Valley fever (coccidioidomycosis) is an endemic fungal pneumonia of the North and South American deserts. In endemic and highly populated areas of the US, e.g., Phoenix and Tucson in Arizona and the San Joaquin Valley in California, up to 30% of community-acquired pneumonias may be caused by Valley fever. The current diagnostics for Valley fever are severely lacking due to poor sensitivity (via serology) and invasiveness (via biopsy), leading to delayed diagnosis, inappropriate treatment with antibiotics, lost productivity, and increased medical costs (1-3). There is a critical need for sensitive and non-invasive diagnostics for detecting and identifying Valley fever lung infections. Our long-term goal is to substantially shorten the time-to-diagnosis for Valley fever through the development of sensitive and specific breath-based diagnostics for coccidioidomycosis lung infections. In the near-term, we are using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) to identify volatile organic compound biomarkers of *Coccidioides immitis* and *C. posadasii* infections via metabolomics analyses of *in vitro* cultures, murine model lung infections, and lung specimens from humans with Valley fever. Herein, we present recent data on the volatile profiles of *C. immitis* and *C. posadasii* grown *in vitro* as spherules and as mycelia (4), two life cycles the fungi adopt in the host and the environment, respectively.

In this presentation, we will:

- compare the *Coccidioides* volatilome by species and life cycle
- explore multivariate analyses of the *Coccidioides* volatilome, demonstrating the volatile metabolome of *Coccidioides* is more dependent on life cycle (spherule vs. mycelia) than species

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Play it by Ear: The Utilization of GC×GC-MS Analysis of Earwax as a Means of Diagnosing Disease

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Although blood and tissue are primary materials used in disease diagnosis, their cumbersome and invasive collection has spurred increasing interest in developing alternative approaches that utilize non-traditional matrices. Earwax is an underutilized matrix that is receiving more attention due to its ease of accessibility. As a lipid-rich material, it has the potential to reflect lipid profile changes correlated to disease. However, its chemical complexity can impede its analysis using traditional techniques. We utilized two-dimensional gas chromatography-mass spectrometry (GC×GC-MS) to establish the chemical profile of earwax. The profile was then compared to the earwax profile of patients with Ménière's disease to assess whether there were differences that could be used as the basis of disease determination.
In this presentation, we will:

- report the various compound classes detected including alkanes, alkenes, fatty acids, esters, triglycerides and cholesterol esters. In earwax that was directly analyzed without saponification, it will be shown that 44 compounds were identified which is the greatest number ever detected or observed in un-saponified earwax samples.
- compare the profile of earwax derived from healthy donors to the profile of those with Ménière's disease, a disorder of the inner ear. It will be demonstrated that major distinctions between the two included differences in triglyceride profiles, and levels of fatty acids. As a result, the observations pave the way for the use of this biological matrix in medical diagnostics.

Minimum Variance Optimized Fisher Ratio Analysis of GC×GC-TOFMS Data for the Discovery of Metabolites in Farmed Pacu Fish

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Pacu fish raised in an integrated rice farming system in Argentina are a major source of revenue and sustenance in Argentina, however there are concerns that herbicides or other contaminants may infiltrate the pacu fish environment and significantly change their metabolome. To address this issue, GC×GC-TOFMS with tile-based Fisher ratio (F-ratio) analysis is excellent for discovery of class-distinguishing analytes [1,2,3], ideally suited to study the complex metabolome of these fish. A preliminary study using principal component analysis (PCA) indicated that several metabolite concentrations differ between the control and farmed pacu fish, revealing that most metabolites were downregulated in the farmed fish [4]. In this presentation, we dig deeper into this dataset to investigate an advantageous way of applying F-ratio analysis to obtain a comprehensive list of class-distinguishing analytes between the control and farmed pacu fish. To do so, instead of using the standard F-ratio calculation that normalizes the class-to-class variance by the sum of the within-class variance from all classes, we examine and apply a minimum variance optimized (MVO) F-ratio calculation. The MVO F-ratio is defined as the class-to-class variance normalized by the minimum variance of either the control fish class or the farmed fish class, instead of the sum of the within-class variance from both classes. Additionally, to facilitate analyte discovery in derivatized metabolomic samples by F-ratio analysis, an algorithmic method to readily remove the artifact hits due to the derivatization process from the hitlist will be demonstrated that greatly reduces the number of hits to examine.

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Characterization of Microbiomes using GC·GC

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The microbiome constitutes of the microbial communities that live on and within our bodies. These microbes have been implicated in numerous health problems, such as cancer, depression, and autoimmune diseases. As science strives to develop diagnostics and therapeutics based on the microbiome, we at the National Institute of Standards and Technology (NIST) aim to introduce harmonization within the microbiome measurement community. As such we have engaged in efforts to develop methodologies to characterize bacterial cultures of microbes commonly found in probiotics and fecal samples for small molecule metabolites through solid phase microextraction (SPME) coupled to comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC·GC-TOFMS). These efforts highlight preliminary steps in the development of a microbiome reference material.

In this presentation, we will:

- Describe a SPME-GC·GC-ToFMS method for the characterization of diverse microbiome samples.
- Compare volatile metabolite profiles obtained from GC·GC separations of bacterial cultures, human, and canine fecal materials.

A Fit for Purpose Data Analysis Approach for Distribution of Aromatics in Diesels

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Aromatics can cause detrimental effects to refining operations by deactivating catalysts. This drives the need for a greater understanding of the aromatic species present within various feedstocks and products. Group-type analysis of aromatic species in diesels by comprehensive two-dimensional gas chromatography (GC×GC) is a well-established technique widely employed in the petroleum industry. However, the boiling ranges of the aromatic groups are needed to understand where aromatics are going to interact in refining operations. Furthermore, data processing must be meaningful and provide context specific information in a timely and straightforward manner.

In this presentation, we will:

- Demonstrate the use of a commercial software package to obtain simulated distillation curves of diesels by GC×GC-FID. This fit for purpose processing technique allowed for a targeted analysis to be completed in a timely manner and directly support continuous refining operations. The entire workflow is self-contained and includes an easy-to-use software interface for prompt turnaround that was repeatable between samples.
- Evaluate the simulated distillation of the total aromatics in diesels by GC×GC, which were validated with and found to be comparable to that of ASTM D7169.
- Investigate the yield curves of the individual aromatic groups by ring number and discuss how the boiling point distribution of the aromatic rings allowed for deeper interpretation of the crude processing and distillation.

Use of Comprehensive Two-Dimensional Gas Chromatography-High Resolution Time-of-Flight Mass Spectrometry for the Investigation of Organic Sulphur Compounds in Coal Tar

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Coal tar is a complex mixture of organic compounds obtained as a by-product from the coal carbonization. It contains several different chemical classes of compounds, among which sulphur derivates. The content of sulphur-based compounds depends from the conditions in which the coal is formed. The attention towards the organic sulphur compounds (OSCs) is mainly related to their dangerous effects on the environment and human health. The present investigation is based on the characterization of different classes of OSCs in coal tar by the use of cryogenically modulated (CM) comprehensive two-dimensional gas chromatography-high resolution time-of-flight mass spectrometry (GC · GC-HR ToFMS). In such a context, a targeted analysis was carried out leading to the identification of 60 OSCs belonging to 14 different classes. Furthermore, absolute quantification was performed by using eight pure standard sulphur compounds, and 1-fluoronaphtalene as internal standard. Finally, the overall analytical performance of CM GC · GC-HR ToFMS was also evaluated, it confirming to be a very powerful analytical technique.

Insights into Olefin Oligomerization Products Based on GC×GC-PI-TOFMS

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Catalyzed olefin oligomerization has been widely used to produce fuels and chemicals in the petrochemical industry [1]. The feedstock light olefins, namely ethylene, propene, butene, and pentene, are catalyzed over acidic catalysts or metal-based catalysts under typical oligomerization conditions [1]. In this study, we investigated the composition of dodecene products catalyzed by acidic catalysts, solid phosphoric acid (SPA) and zeolite, from various feedstocks, propene, propene + butene, butene, propene + nonene during oligomerization reaction.

The instrument applied in this study is two-dimensional gas chromatography (GC · GC) coupled photoionization (PI) - time of flight mass spectrometry (TOFMS). The distributions of olefin congeners, dodecene structural subgroups, and dodecene isomers were obtained by the developed method [2]. Various data sets enabled the multimodal characterization of the dodecene products from different production pathways.
By using the data set of dodecene structural subgroup distribution, the SPA and zeolite catalyzed products can be distinguished by principal component analysis (PCA) and hierarchical clustering analysis (HCA). In general, zeolite produced more linear dodecene isomers, and SPA produced more branched isomers. The T-test and partial least squares-discriminant analysis (PLS-DA) identified a few important features / dodecene isomers which could be used as catalyst indicator. Based on the data set of dodecene isomer distribution, the dodecene products from different feedstocks can also be distinguished except for propene and propene + nonene groups, which indicated the product composition from these two feedstocks did not have significant differences. Two samples with unknown feedstock composition were cumulated close to the groups of propene and propene + nonene in PCA scores. This suggested that butene was not contained in their feedstocks.

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System Design, Method Development and Application of Splitter-Based Non-Cryogenic Artificial Trapping Comprehensive Heartcut (SNATCH) Modulation Mechanism

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A novel modulation approach in multidimensional gas chromatography (MDGC) called as Splitter-based Non-cryogenic Artificial Trapping Comprehensive Heartcut (SNATCH) was developed offering the capabilities that could not be obtained from the conventional modulation technologies, such as artificial trap of highly volatile compounds or single injection analysis with long modulation period (P_M) and enhanced ^{2}D peak capacity using flow/valve based modulation. The approach employed a first dimensional (^{1}D) HP5-MS column (30 m), a second dimensional (^{2}D) DB-WAX column (60 m), a restrictor and a Deans switch (DS) applying a periodic multiple heartcut strategy throughout the comprehensive analysis. The essential equipments are not provided in this abstract. Thus, you could please come to our presentation for the details. The equipments enable the artificial trapping of pulses from ^{1}D separation prior periodically selective heartcut (H/C) to transfer the trapped pulses onto the ^{2}D column. This was performed without any extra pneumatic or cryogenic devices.

In this presentation, we will:

- provide the essential equipment details allowing the SNATCH modulation mechanism.
- propose methods to adjust sampling time (t_{samp}) and P_M for the application in comprehensive MDGC and provide the proof of concept for artificial cryogenic modulation of highly volatile compounds applyin t_{samp}/P_M of 2, 4, 8 and 16.
- demonstrate application of the SNATCH method for analysis of a gas mixture and petrochemical sample.

Optimization of Vac-HSSPME+GC–MS for Determination of Biomarker Profiles on Oil Source Rocks using Multivariate Approaches.

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The evaluation of oil source rocks depends on the characterization of the organic matter present on samples; some compounds can be assigned as biomarkers and associated to properties of the oil relevant to their potential commercial value. These compounds (mainly C20+ hydrocarbons found in trace amounts) are structurally similar to natural products present on the organisms that originated the oil and are formed from their diagenetic alteration [1,2]. Usual procedures involve lengthy and cumbersome demanding extraction steps as Soxhlet extraction, TLC and open-column liquid chromatography before GC-MS(/MS) analysis [1]. We propose here the combination of Vacuum-assisted Headspace SPME (Vac-HSSPME) and GC-MS to simplify and speed up the process. This is not a mere straightforward replacement: the target compounds have very low vapor pressures (~10^{-7} bar or less) and are not analytes typically isolated using headspace manipulation procedures.

In this poster, we will:

- Evaluate data from Doehlert design matrix for Vac-HSSPME+GC-MS source-rock samples collected from stratigraphic exploratory oil wells. The evaluated response was total peak area for the steranes monitored through GC–MS on SIM (m/z = 217). The algorithm for the factorial planning (model fitting and generation of response surface) plots was implemented on MATLAB®. The optimum operation conditions were found to be 15 min extraction und temperature of 250 °C, using a 7 μm PDMS SPME fiber.
- Compare results for selected samples to those obtained using conventional Soxhlet extraction. Apart from the steranes, other relevant biomarkers compounds were identified, such as C27 (13β, 17α, (20S) diasterane) among others. Fingerprinting was found to be comparable. The procedure was also validated for quantitation of these compounds and showed high sensitivity and sensibility despite the use of in-house developed apparatus.

References

[1] Peters, K. E., Walters, C. C., Moldowan., M., The Biomarker Guide. Biomarkers and Isotopes in Petroleum Exploration and Earth History. Cambridge, New York 2005.
[2] Killops, S., Killops, V., Introduction to Organic Geochemistry. *Introd. to Org. Geochemistry* 2005, 30–70.

Investigation of the Limit of Discovery using Tile-Based Fisher Ratio Analysis with Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry

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Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) is followed by tile-based Fisher ratio (F-ratio) analysis to investigate the "limit of discovery" for low concentration levels of sulfur-containing compounds in JP8 jet fuel. A mixture of 14 sulfur-containing compounds was spiked at 30 ppm, 15 ppm, 3 ppm and 1.5 ppm into the neat fuel prior to GC×GC-TOFMS analysis with a "reversed" column format (aka polar first dimension (1D) and non-polar second dimension (2D) column). Prior standard implementation of tile-based F-ratio analysis utilized an average F-ratio requiring a minimum of 3 mass channels (m/z) with the highest F-ratios. Herein, we explore the notion that use of the top F-ratio m/z for hitlist ranking is superior to the standard implementation for analytes near their limit-of-quantitation (LOQ), defined as an analyte concentration that produces a signal equal to ten times the standard deviation of the baseline noise (10σn). Hitlist ranking comparisons revealed that using only the top F-ratio m/z resulted in impressive improvements in discoverability for the low concentration comparisons. Specifically, for the 3 ppm versus neat hitlist, 1,4-oxathiane (LOQ = 2.5 ppm) improved from hit 114 via standard F-ratio analysis, to hit 25. For the 1.5 ppm versus neat hitlist, 2-propylthiophene (LOQ = 0.64 ppm) improved from hit 59 to 17, benzo[b]thiophene (LOQ = 1.1 ppm) from hit 98 to 28, and 2,5-dimethylthiophene (LOQ = 1.3 ppm) from hit 262 to 39. Additional hitlist ranking comparisons revealed the importance of proper tile size selection, as analyte discoverability deteriorated upon using either an inappropriately too small or too large of a tile [1].

References

[1] P.E. Sudol, G.S. Ochoa, R.E. Synovec, J. Chromatogr. A 1644 (2021) 462092.

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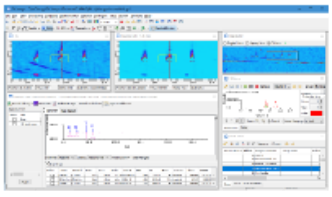
GC Image software imports many chromatographic, MS, and UV file formats, including proprietary formats from Agilent, Shimadzu, JEOL, Thermo, Sciex, Waters, and other vendors, and standard formats such as CSV, CDF, mzData, and mzXML.

Raw Data Alignment & Processing

GC Image software provides multidimensional, multispectral data processing including 2D baseline correction, peak detection, multivariate peak unmixing, and library search. Also, raw data alignment can be done using automated matching, alignment points, or a custom transform.

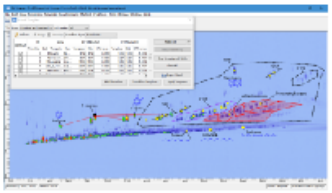
Multifaceted Visualization & Data Review

GC Image software offers a variety of interactive visualizations including 1D, 2D, and 3D chromatographic views, and tabular views. The Review Mode allows rapid inspection of analytical results. Customizable modes allow for user defined window layouts and review-focused interaction between the views.



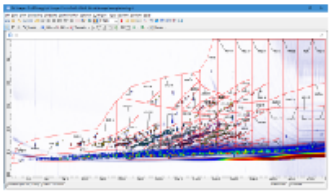
Advanced Peak Alignment, Identification & Analysis

GC Image software allows highly effective chemical identification and analysis with advanced Smart Template™ and CLIC™ technologies to recognize chromatographic patterns and spectral signatures. Automated template matching algorithm performs 2D retention time alignment assisted by constraints written in CLIC. Simple or complex CLIC expressions can be built using a collection of retention time, multi spectral, and peak characteristic functions to perform both qualitive and quantitative analysis.



Comprehensive Group Analysis

GC Image software provides sophisticated graphical objects to define 2D retention-time windows for manual integration or group analysis. Mesh objects can also be created to easily define comprehensive groups of peaks or chromatographic regions.

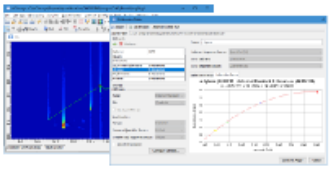


Generic Retention Index Calibration

GC Image software provides generic functions to perform calibration from retention times to retention indices or other chemical properties. Custom functions can be used in addition to linear, piece-wise linear, and polynomial functions. 2D region-based boiling point calibration is supported for SimDist analyses with GCxGC, and molecular weight calibration is supported for 2DLC (HPLC/GPC).

Calibration & Quantification

GC Image software supports external and internal standard calibration, and provides tools for creating and examining calibration tables from batches of processed chromatograms easily. Combined with Smart Template™, the software provides automated quantification and reporting.

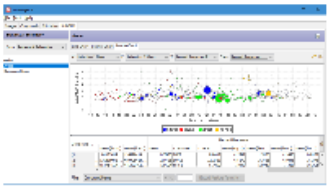


Mass Spectrometry, HRMS & MS/MS

GC Image software supports data from a wide range of multispectral detectors, including MS and DAD/PDA. An array of tools support spectral matching, library search, and selected ions/channels analysis, with options for handling adducts. Also, the software supports high resolution MS and MS/MS with additional tools for mass calibration and exact-mass, isotope, and mass defect analyses.

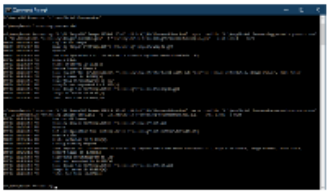
Multi-sample Analyses

The Investigator tool supports comparing chromatograms, peaks, and peak sets across multiple samples, and examining statistical characteristics and trends with pairwise differences, PCA, Fisher ratios, and F-tests. Customizable charts help to identify interesting features, outliers, and class differences.



Command Line Interface

The Command Line interface allows processing a single raw data file or reprocessing a chromatographic image with a Method or scripting. The interface can be used to create workflows that integrate multiple tools or software for automating routine analysis or complicated processing with a large number of data files.



What's New in Version 2020

Recent releases of the GC Image software contain many improvements. Here are some highlights:

- Support for NIST 2020 MS and MS/MS Libraries
- Command Line Interface for Batch Processing and Advanced Workflows
- New Improvements for Retention Index Calibration Including Support for GPC
- Improved Support of Background Subtraction for Batch Processing

Visit our website for more details on the latest version **2020** releases.

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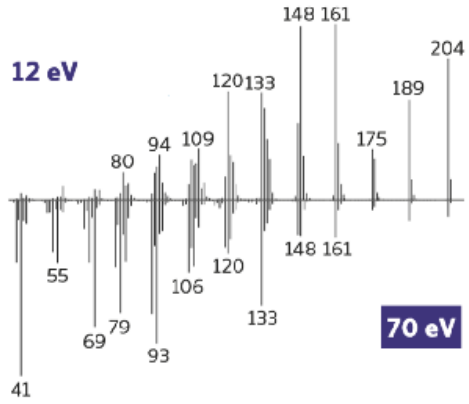

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