GC×GC-(HR)TOFMS : Why we Love it !

JF Focant et al.

Acknowledgements

- ✓ JEOL, LECO, Agilent, Thermo…
- ✓ Restek, Sigma-Aldrich/Supelco, SGE
- ✓ JSB, Gerstel, Markes…

GC×GC is Very Young

- ✓ GC×GC was invented just a few years ago
- ✓ This is why it is only used by experts
- ✓ It is much more expensive than regular GC
- ✓ It is much more complex than regular GC
- ✓ Runs in GC×GC last for ages
- ✓ GC×GC is only for petroleum sample analyses
- ✓ …

The Early Days

First report on GC×GC

Journal of Chromatographic Science, Vol. 29, June 1991

Expedited Paper

Comprehensive Two-Dimensional Gas Chromatography using an On-Column Thermal Modulator Interface

Zaiyou Liu and **John B. Phillips***
Department of Chemistry & Biochemistry, Southern Illinois University, Carbondale, Illinois 62901

A thermal desorption modulator prepared on an open-tubular gas chromatographic column continuously generates fast chromatograms sampled from a flowing stream.

A sample first separated by one column is separated a second time by an independent column. All substances in the sample mixture pass through both columns.

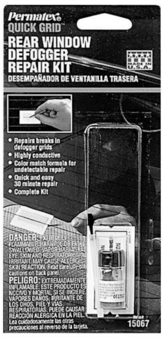
‘Comprehensive’

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission. 227

Modulation

- ✓What is a modulator?
  - Interface between the two columns that samples narrow bands from the eluate of the ^{1}D column,
  - For fast re-injection into the ^{2}D column, producing fragments that are analyzed sequentially.

First report on GC×GC



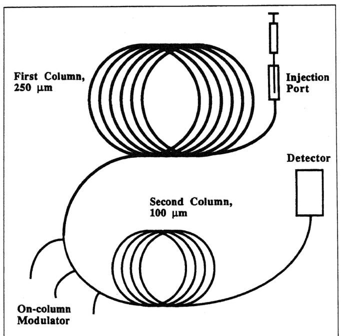


Figure 1. A two-dimensional gas chromatograph.

JM Dimandja @ work

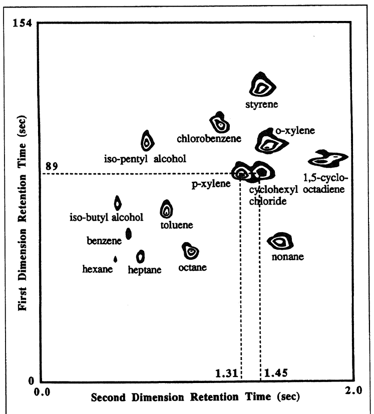
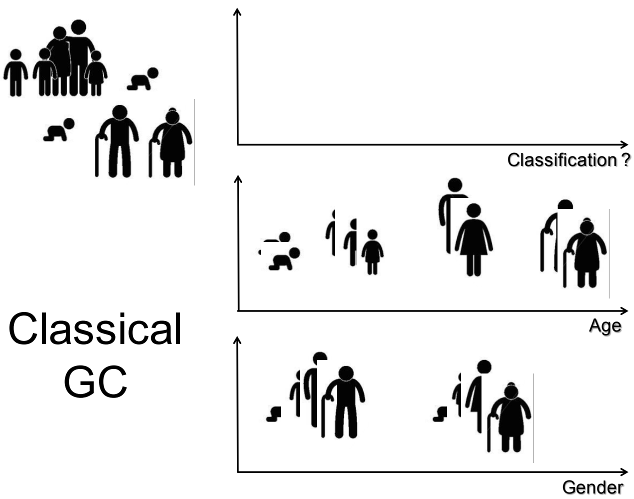
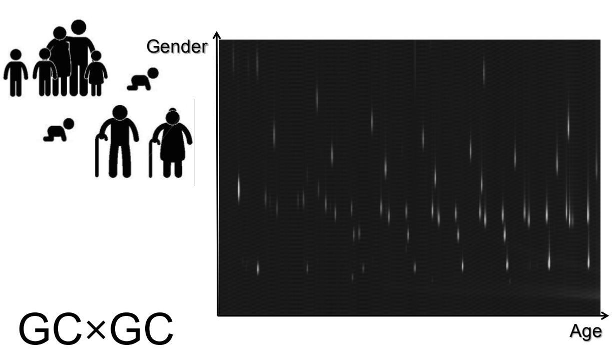


Figure 2. A two-dimensional gas chromatogram. The oven temperature was isothermal at 93°C. The carrier gas was hydrogen at 85 cm/s through the first column and 450 cm/s through the second column.

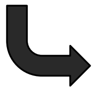
The Idea



Classical GC

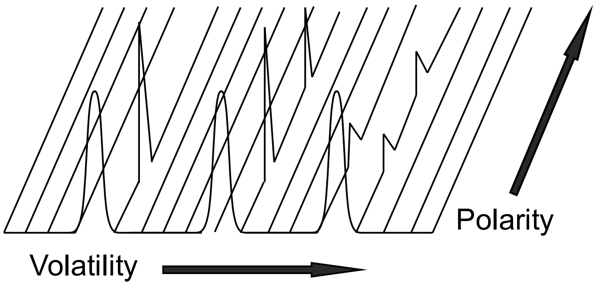


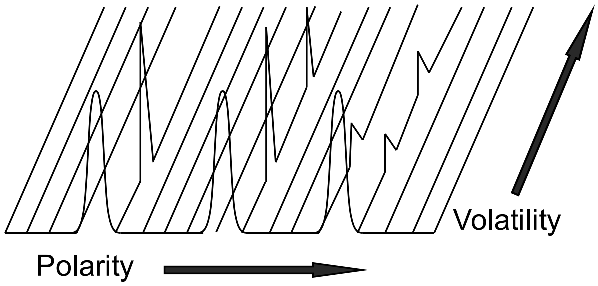
GC×GC

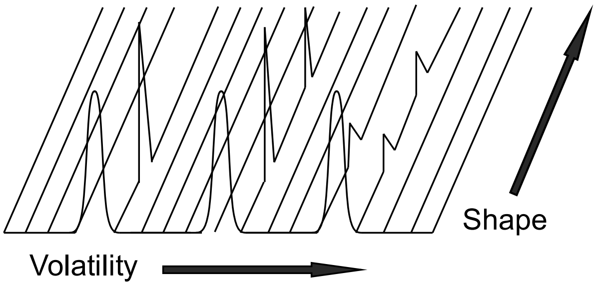


$$\text{Peak capacity } (n_c) = {}^{1}D_{nc} \times {}^{2}D_{nc}$$

$$t_{run}\ 1DGC = t_{run}\ GC\times GC$$



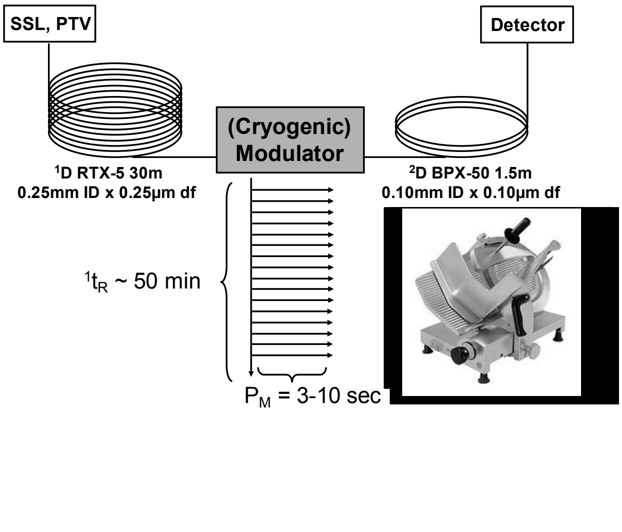


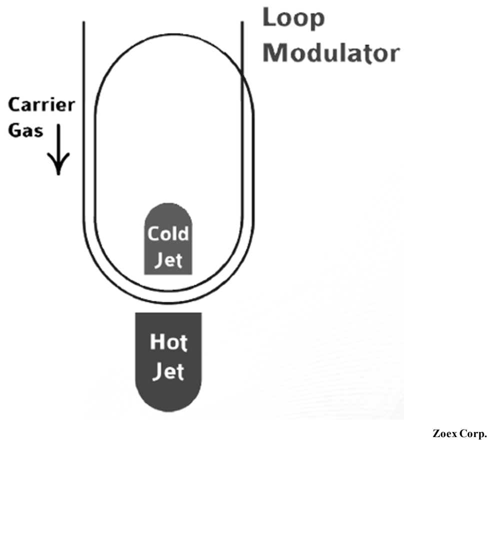


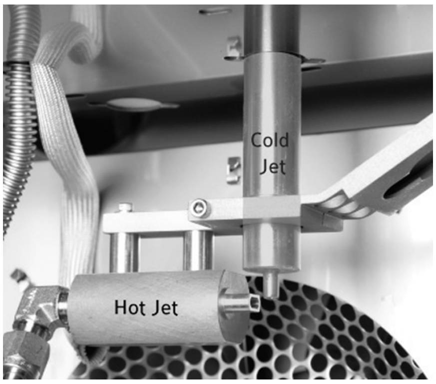
…

The Principle

Instrumental Setup

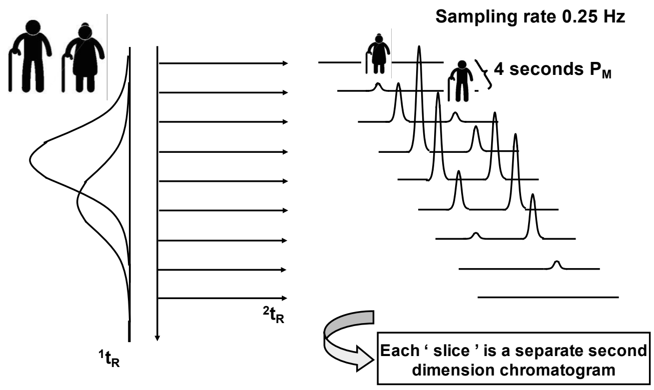




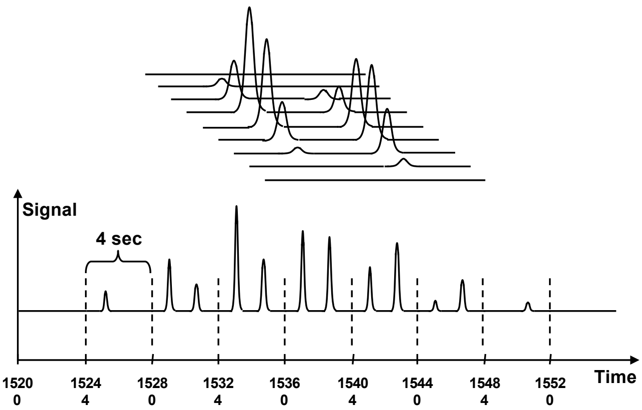


Zoex Corp.

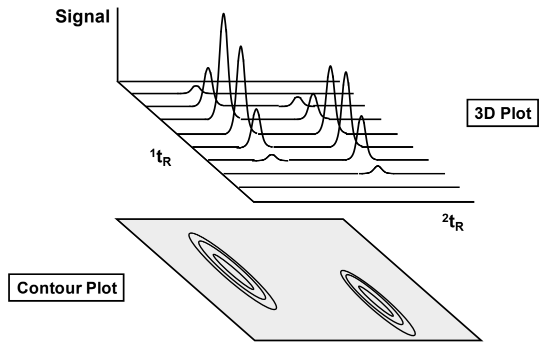
Modulation Process

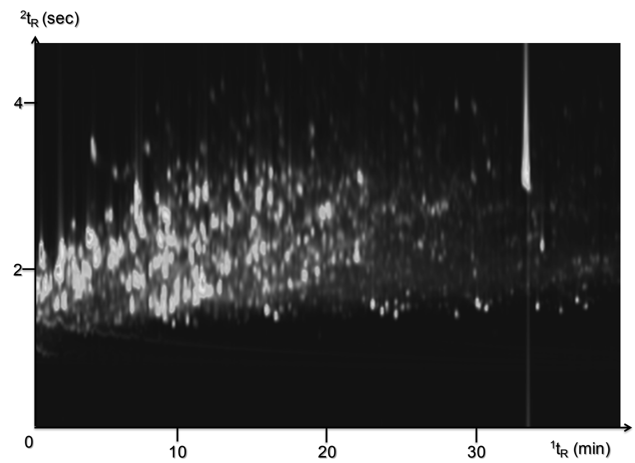


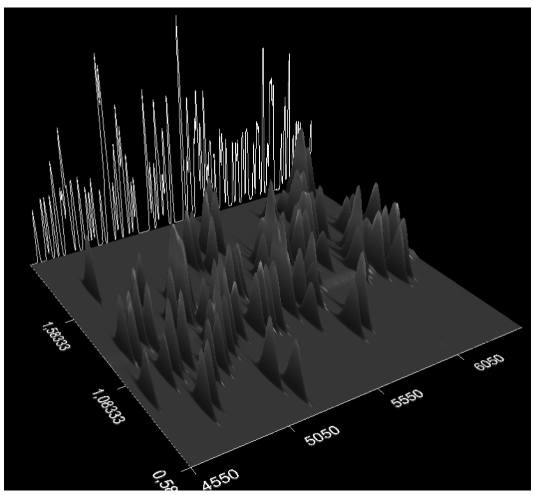
Signal at the Detector



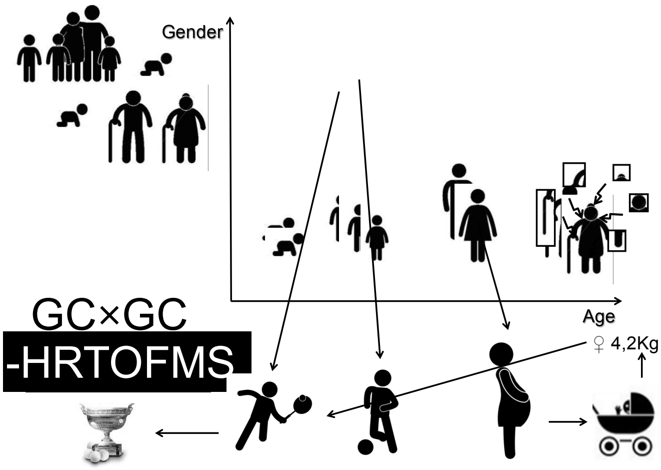
Displaying the Data

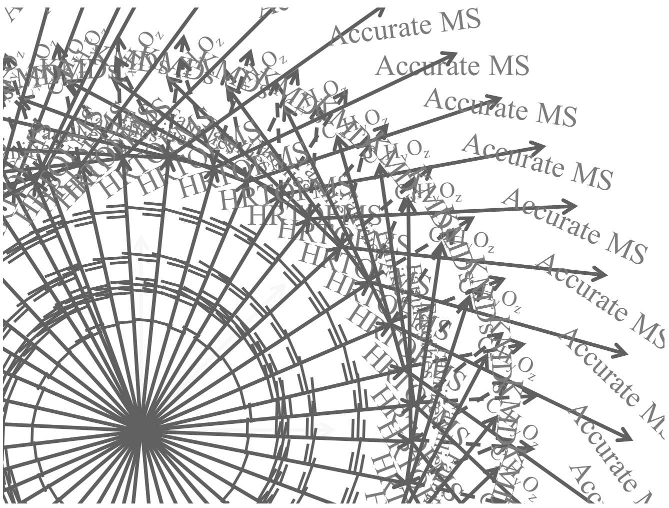


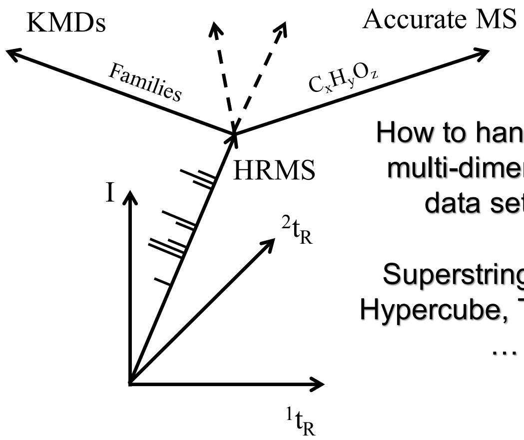




The Added Value of Mass Spectrometry

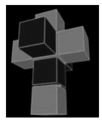




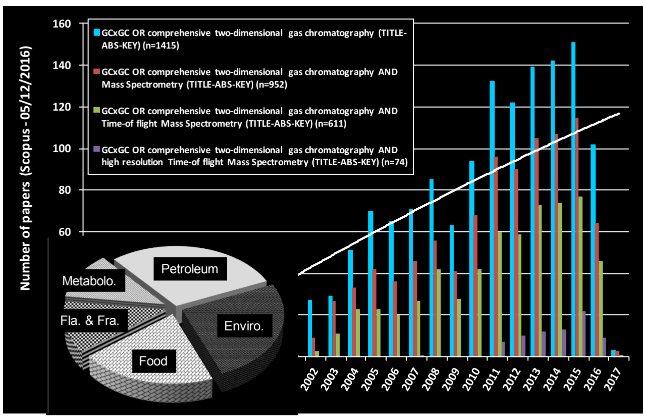


How to handle such multi-dimensional data sets ??

Superstring theory, Hypercube, Tesseract, …



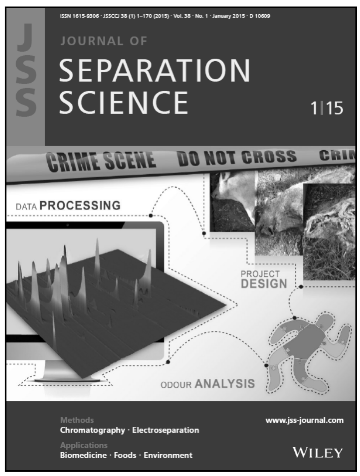
State of the Art GC×GC



Case Study #1:

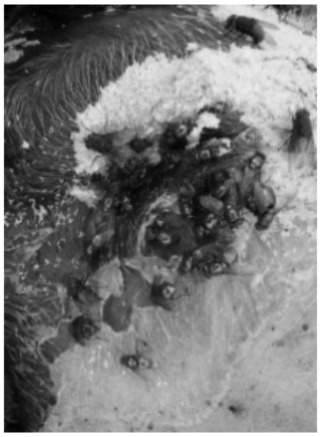
Cadaveric

Decomposition



Evolution of VOC release over time…

J. Dekeirsschieter *et al.* PLoS ONE 2012, 7, e39005

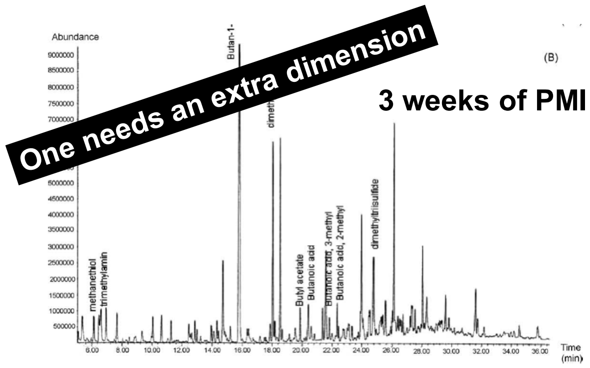


TD-GC×GC-TOFMS

➡

Data Processing

1DGC Cadaveric VOCs

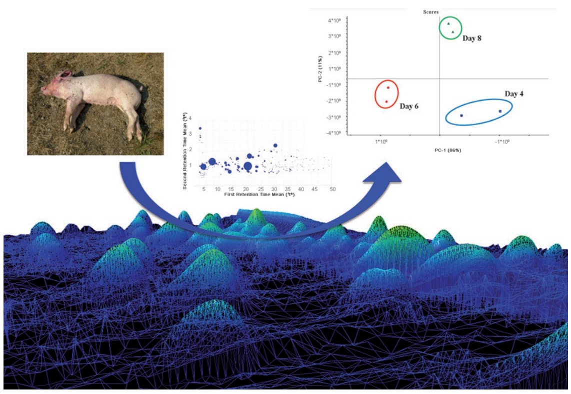


J. Dekeirsschieter *et al.* Forensic Sci Int 2009, 189, 43-56

GC×GC-TOFMS gives us…

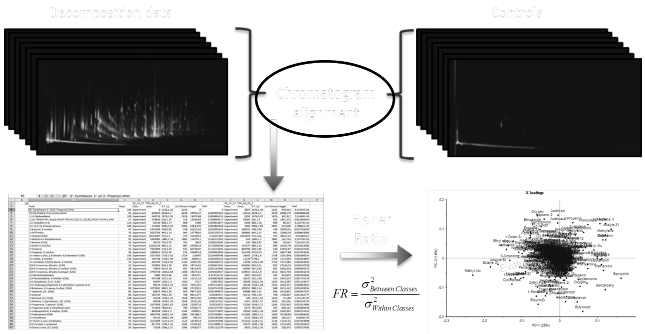
- ✓ Multi-dimensional date sets (sensitivity ++)
- ✓ Access to 'hiden' peaks
- ✓ $^{1}t_R$, $^{2}t_R$, deconvoluted MS signals, …
- ✓ > thousands of peaks (4-6 slices per peak)
- ✓ Several Gb file sizes

➡ **Making sense of such large data sets starts to be THE challenge…**

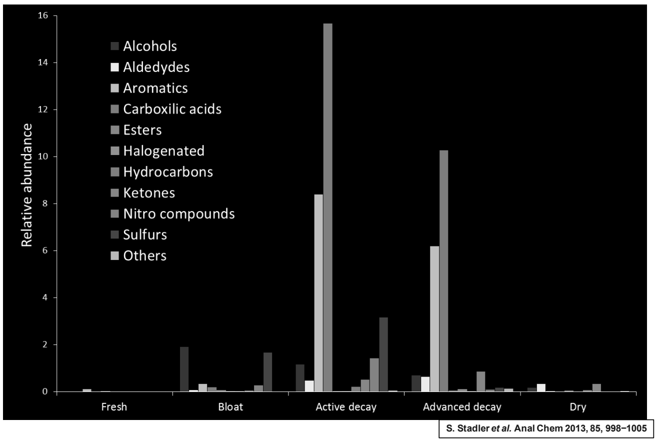


P.H. Stefanuto *et al.* ChemPlusChem 2014, DOI: 10.1002/cplu.201402003

TD-GC×GC-TOFMS

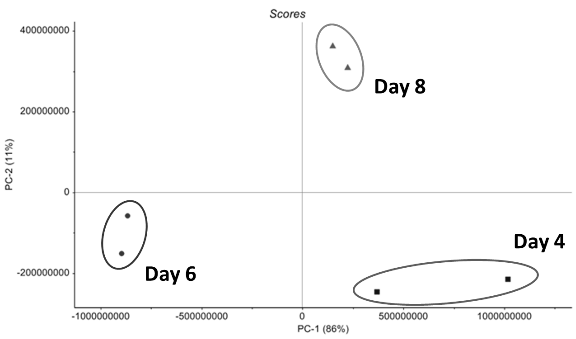


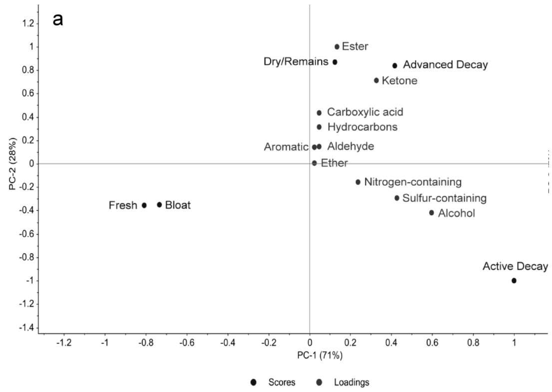
Trial carried out with pigs *vs* controls



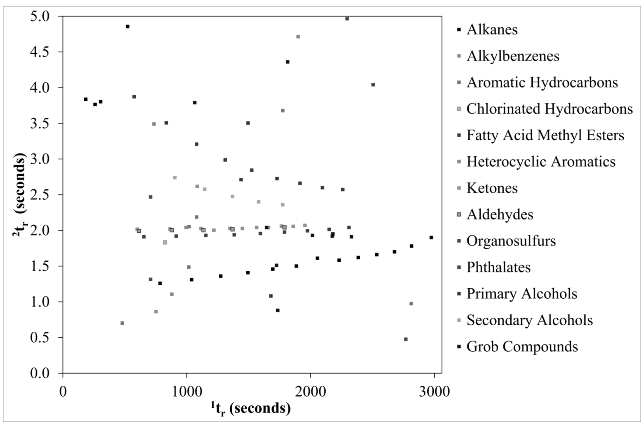
S. Stadler *et al.* Anal Chem 2013, 85, 998–1005

e.g. Active Decay Trend



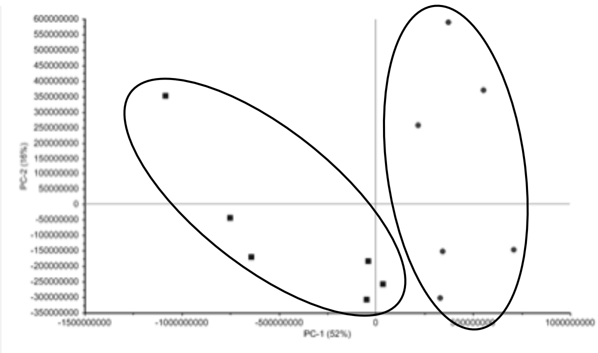


S. Forbes *et al.* PLoS ONE 2014, 9, e113681

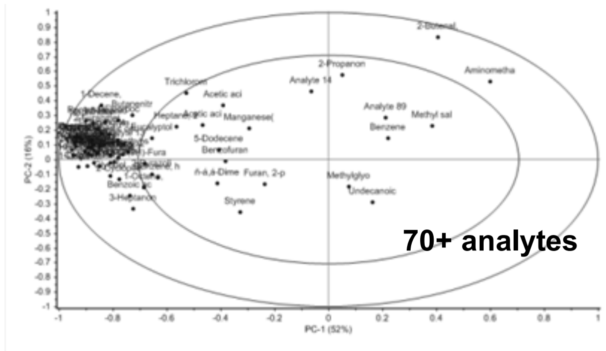


K. Perrault *et al.* J Sep Sci 2015, 38, 73-80

PCA (Ctrl *vs* HumInsIncl)

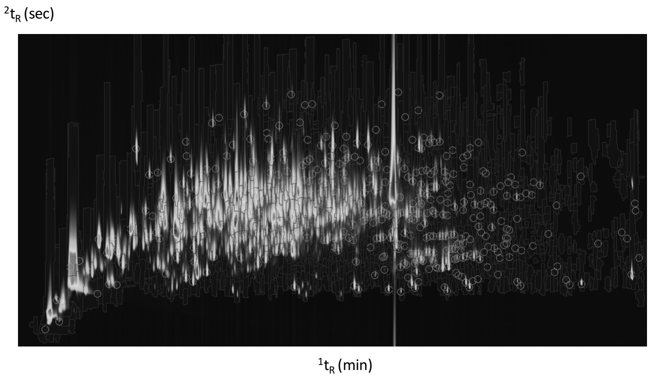


Correlation Loadings

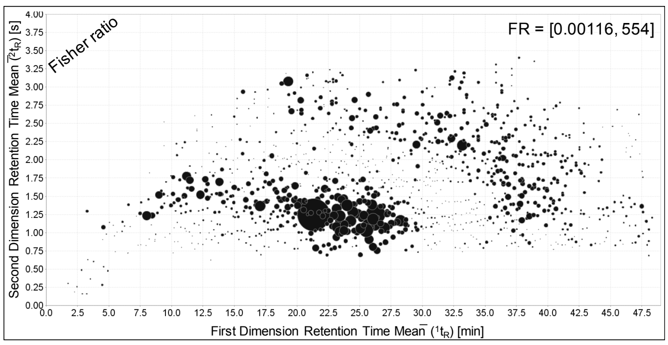


P.H. Stefanuto *et al.* Anal Bioanal Chem 2015, in DOI 10.1007/s00216-015-8683-5

Pixel-Based Approach

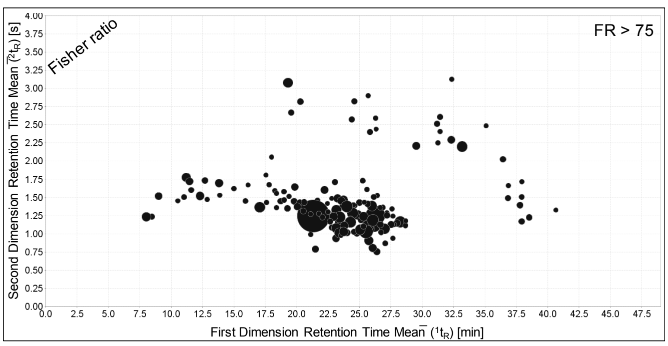


Fisher Ratio Plots



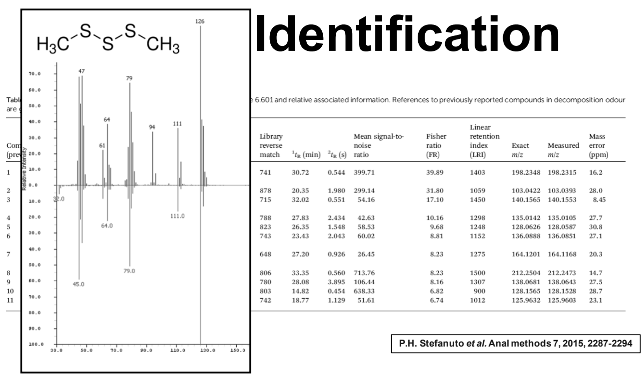
2D Fisher ratio bubble plot for percent responses of compounds detected in 24 chromatograms

Fisher Ratio Plot Cutoff



2D Fisher ratio bubble plot for percent responses of compounds detected in 24 chromatograms

Identification

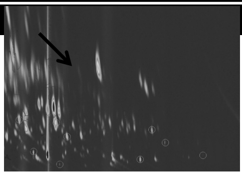


P.H. Stefanuto *et al.* Anal methods 7, 2015, 2287-2294

^1t_R, ^2t_R, LRI, Lib Search, Molecular Formula, …

Mass Accuracy

Compounds	Formula	Exact mass	Real mass	Diff (ppm)
DMDS	$C_2H_6S_2$	93,9915	93,9911	-4
DMTS	$C_2H_6S_3$	125,9631	125,9632	1
DMTeS	$C_2H_6S_4$	157,9367	157,9352	-9
DMPeS	$C_2H_6S_5$	189,8839	189,9073	123



Barely any signal

Case Study #2: Cancer Research

Estimated New Cases

Males			Females		
Prostate	220,800	26%	Breast	231,840	29%
Lung & bronchus	115,610	14%	Lung & bronchus	105,590	13%
Colon & rectum	69,090	8%	Colon & rectum	63,610	8%
Urinary bladder	56,320	7%	Uterine corpus	54,870	7%
Melanoma of the skin	42,670	5%	Thyroid	47,230	6%
Non-Hodgkin lymphoma	39,850	5%	Non-Hodgkin lymphoma	32,000	4%
Kidney & renal pelvis	38,270	5%	Melanoma of the skin	31,200	4%
Oral cavity & pharynx	32,670	4%	Pancreas	24,120	3%
Leukemia	30,900	4%	Leukemia	23,370	3%
Liver & intrahepatic bile duct	25,510	3%	Kidney & renal pelvis	23,290	3%
All Sites	**848,200**	**100%**	**All Sites**	**810,170**	**100%**

Estimated Deaths

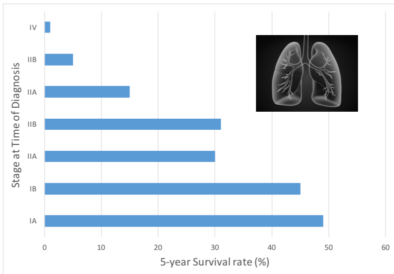
Males			Females		
Lung & bronchus	86,380	28%	Lung & bronchus	71,660	26%
Prostate	27,540	9%	Breast	40,290	15%
Colon & rectum	26,100	8%	Colon & rectum	23,600	9%
Pancreas	20,710	7%	Pancreas	19,850	7%
Liver & intrahepatic bile duct	17,030	5%	Ovary	14,180	5%
Leukemia	14,210	5%	Leukemia	10,240	4%
Esophagus	12,600	4%	Uterine corpus	10,170	4%
Urinary bladder	11,510	4%	Non-Hodgkin lymphoma	8,310	3%
Non-Hodgkin lymphoma	11,480	4%	Liver & intrahepatic bile duct	7,520	3%
Kidney & renal pelvis	9,070	3%	Brain & other nervous system	6,380	2%
All Sites	**312,150**	**100%**	**All Sites**	**277,280**	**100%**

FIGURE 1. Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2015.
Estimates are rounded to the nearest 10 and cases exclude basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Siegel, R.L. et al., Cancer Statistics, 2015. CA Cancer J. Clin. 2015; 65: 5-29.

'Further reduction in cancer death rates can be accelerated by applying existing cancer control knowledge across all segments of the population, with an emphasis on those in the lowest socioeconomic bracket…'

Siegel, R.L. et al., Cancer Statistics, 2015. CA Cancer J. Clin. 2015; 65: 5-29.



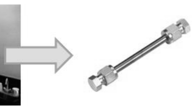
American Cancer Society, 2016.

Fast Cheap Early Detection

Biomarkers of Disease

VOCs as Biomarkers…

- ✓ Exhaled breath contains lots of VOCs
- ✓ Health status fingerprint
- ✓ Ease of accumulation
- ✓ Ease of sampling
- ✓ Fast and non-invasive



Exhaled Breath Analysis (EBA)

- ✓ Disease-related endogenous volatile biomarkers
- ✓ Not that new…

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 10, pp. 2374–2376, October 1971

Quantitative Analysis of Urine Vapor and Breath by Gas–Liquid Partition Chromatography

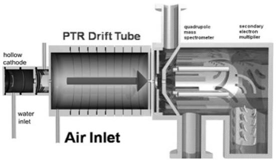
(orthomolecular medicine/vitamins/controlled diet)

LINUS PAULING*, ARTHUR B. ROBINSON*, ROY TERANISHI†, AND PAUL CARY*

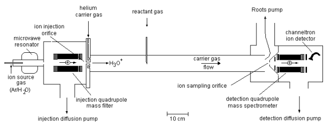
* Department of Chemistry, Stanford University, Stanford, California 94305; and † Western Regional Laboratory, U.S. Department of Agriculture

Contributed by Linus Pauling, July 29, 1971

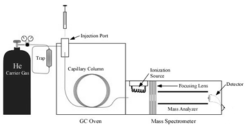
VOC Measurements



PTR-MS



SIFT-MS



GC-MS

E-nose

GC×GC-(HR)TOFMS

Sorbent Tubes: Tenax® and carbopack®

TD: Markes, desorption at 300°C for 3min

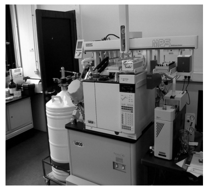
GC×GC-TOFMS: LECO Peg 4D, JEOL 4G

Columns: Rtx-5 (30m x 0.18mm x 0.2µm) as ^{1}D and Rxi-17 (1m x 0,1mm x 0.1µm) as ^{2}D

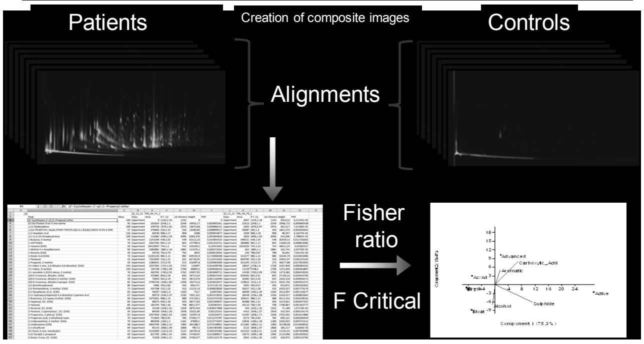
Oven T program: 45°C (0,2min); 5°C/min until 245°C (1min); 30°C/min until 280°C (5min).

Modulation period: 4 s

MS: EI TOF at 70 eV , 25-100 Hz

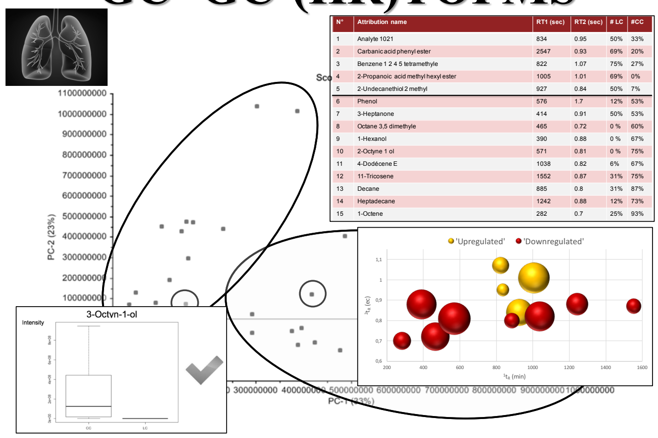


GC×GC-(HR)TOFMS

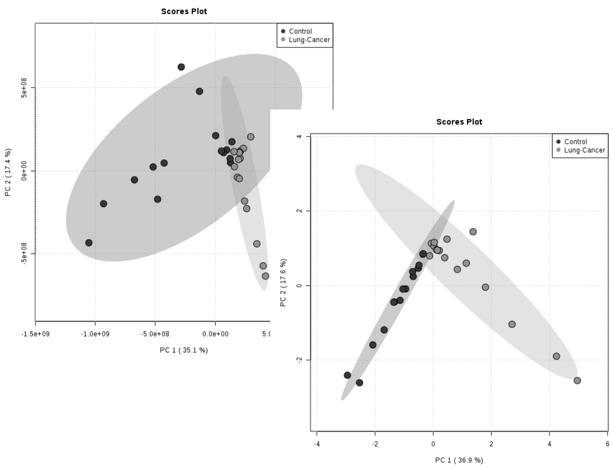


Various (un)supervised statistics (PCA, HCA, PLS, …)

GC×GC-(HR)TOFMS

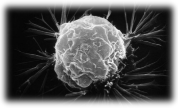


N°	Attribution name	RT1 (sec)	RT2 (sec)	# LC	#CC
1	Analyte 1021	834	0.95	50%	33%
2	Carbanic acid phenyl ester	2547	0.93	69%	20%
3	Benzene 1 2 4 5 tetramethyle	822	1.07	75%	27%
4	2-Propanoic acid methyl hexyl ester	1005	1.01	69%	0%
5	2-Undecanethiol 2 methyl	927	0.84	50%	7%
6	Phenol	576	1.7	12%	53%
7	3-Heptanone	414	0.91	50%	53%
8	Octane 3,5 dimethyle	465	0.72	0 %	60%
9	1-Hexanol	390	0.88	0 %	67%
10	2-Octyne 1 ol	571	0.81	0 %	75%
11	4-Dodécene E	1038	0.82	6%	67%
12	11-Tricosene	1552	0.87	31%	75%
13	Decane	885	0.8	31%	87%
14	Heptadecane	1242	0.88	12%	73%
15	1-Octene	282	0.7	25%	93%



GC×GC-(HR)TOFMS

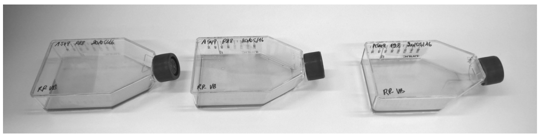
- ✓ Accessing patients and getting controls is somewhat complicated
- ✓ We need to gain orthogonal information's
- ✓ Another 'source' of VOCs can be considered
- ✓ What about VOCs produced by cancer cells?



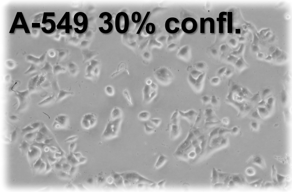
- ✓ Would we see a specific signature???

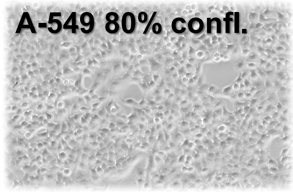
Let's Grow some Cells…

- ✓ Cell lines : MCF-7 breast cancer
  A-549 lung cancer
- ✓ Culture in DMEM @ 37°C under contr. CO_2
- ✓ T-75 boxes (20mL DMEM), triplicated



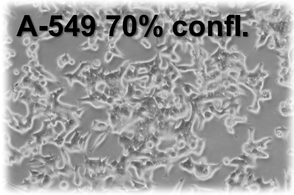
Sampling Based on Confluency



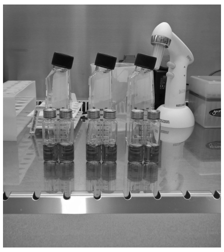


Sample	2-days (n)	3-days (n)	5-days (n)
MCF-7	6	-	6
A-549	-	6	6
DMEM[(a)]	6	-	-

[(a)] DMEM samples were fresh growth media

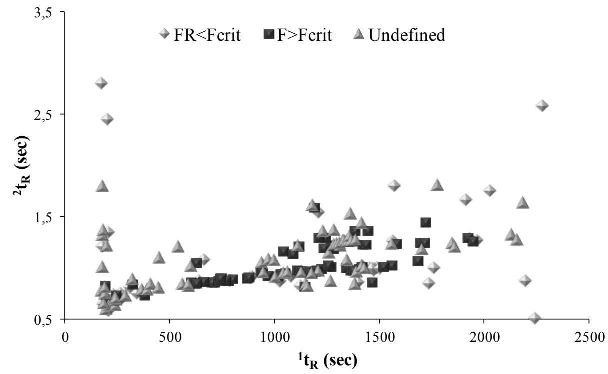


Measures on 'Used' DMEM

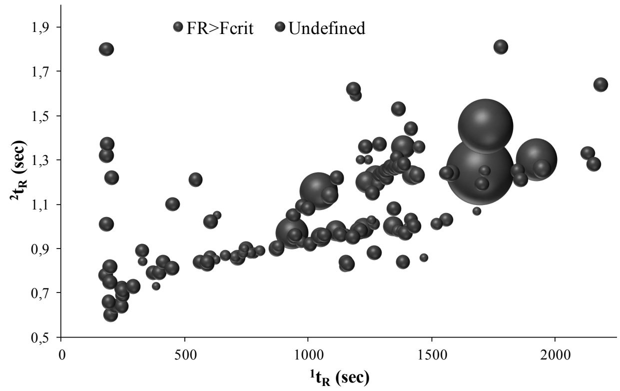


- PDMS 100µm SPME, 2h at 37°C, 250°C, split 10
- Rtx®-5MS (30m, .25, .25) × Rxi®-17Sil (1m, .15, .15)
- P_M 4s, T_{offset} 10°C
- 100 Hz, 35-450 amu, 70eV
- 2h cycle time

Apex of Composite Image



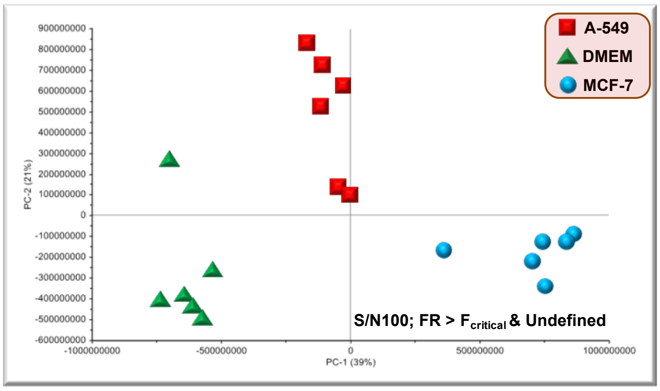
Apex of 'VIP' Analytes



PCA MCF-7 *vs* A-549 *vs* DMEM

Sampling at
Day-2 for MCF-7
Day-3 for A-549

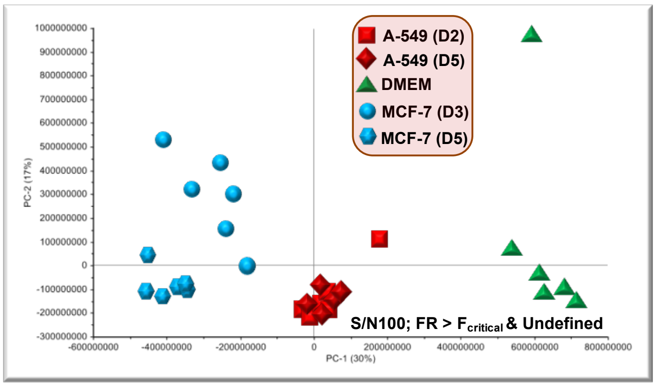
PCA MCF-7 *vs* A-549 *vs* DMEM



PCA MCF-7 *vs* A-549 *vs* DMEM

Sampling at
Day-2 & Day-5 for MCF-7
Day-3 and Day-5 for A-549

PCA MCF-7 *vs* A-549 *vs* DMEM

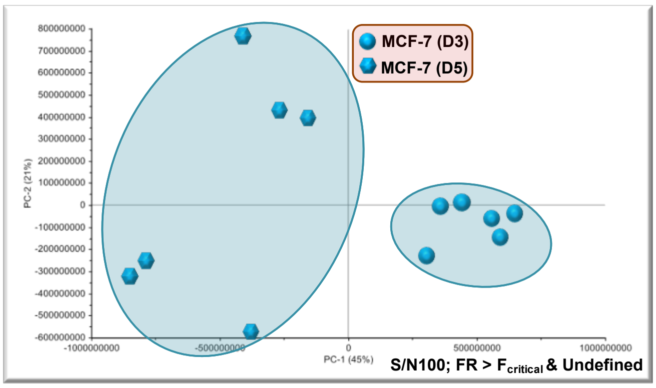


PCA MCF-7 Day-2 *vs* Day-5

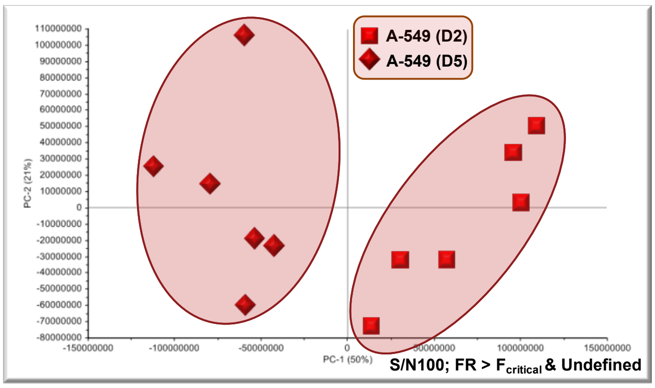
PCA A-539 Day-3 *vs* Day-5

Time trend?

PCA MCF-7 Day-3 *vs* Day-5

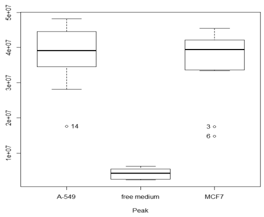


PCA A-539 Day-3 *vs* Day-5

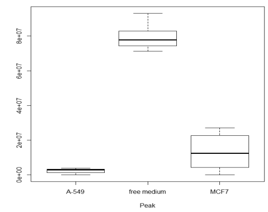


What Analytes ?

✓ DMEM specific analytes…



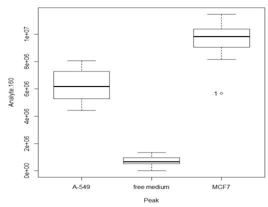
Tertamethyl-heptadecane



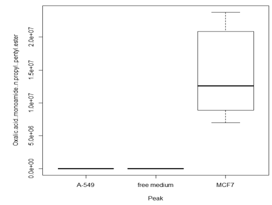
Benzoic acid 2-ethylhexyl ester

What Analytes ?

✓ Cancer cell specific analytes…



Analyte 160…



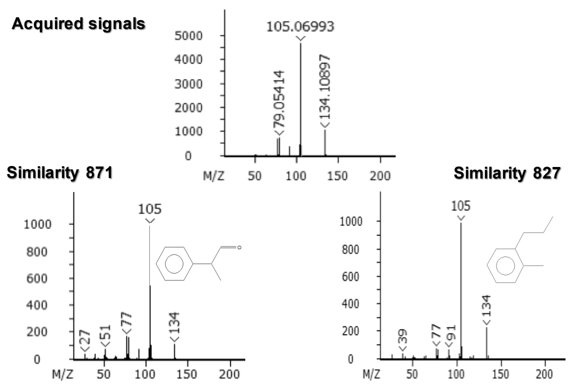
Oxalic acid amide ester…

What Analytes ID ?

Name	Peak S/N	Area	$^{1}t_R$ (min)	$^{2}t_R$ (sec)	Similarity	Formula	Mass accuracy (ppm)
N-Nitrosodimethylamine	1285	453980	04:25	1,89	967	C2H6N2O	**-0,20**
Pyridine	1421	823806	04:31	1,67	983	C5H5N	**-0,79**
Aniline	1232	668108	11:25	2,45	993	C6H7N	**0,18**
Phenol	1629	676747	11:34	2,11	896	C6H6O	**0,75**
Benzyl alcohol	1163	346729	13:31	2,38	981	C6H7	**-0,89**
p-Cresol	1425	408924	14:19	2,18	988	C7H8O	**0,67**
Benzene, nitro-	630	404963	15:28	2,58	979	C6H5	**-0,09**
Phenol, 4-nitro-	297	107768	17:10	2,47	753	C6H5NO3	**0,17**
Naphthalene	2476	1397062	19:04	2,49	986	C10H8	**0,56**
p-Chloroaniline	1501	549794	19:40	2,75	786	CH9N2OP2	**-0,72**
Naphthalene, 1-methyl-	1921	760645	22:55	2,46	934	C11H10	**0,09**
Phenol, 2,4,5-trichloro-	554	212178	25:01	2,41	870	C2N2O5S2	**1,02**
o-Nitroaniline	313	107524	26:37	0,18	948	C5H5	**0,42**
Benzene, 1,3-dinitro-	285	77777	27:55	0,23	937	C6H3	**-0,51**
m-Nitroaniline	551	184202	29:01	0,35	973	C5H5	**-0,22**
Phenol, 2-nitro-	146	64895	30:28	2,89	881	C6H5NO3	**1,24**
Diethyl Phthalate	997	611829	32:13	2,61	998	C8H5O3	**0,48**
p-Nitroaniline	388	116437	32:31	0,69	994	C5H5	**0,42**
Benzene, hexachloro-	1121	371228	35:31	2,55	912	C6Cl6	**0,15**
Phenanthrene	3274	1037472	37:19	0,18	900	H20NO5S2	**0,60**
Anthracene	3190	966141	37:34	0,17	912	C14H10	**0,82**
Carbazole	3229	938545	38:46	0,52	991	C12H9N	**0,98**
Dibutyl phthalate	1182	546177	41:46	2,43	828	CH13N2S3	**-0,54**
Fluoranthene	2155	983907	44:10	0,49	993	C16H10	**0,39**
Pyrene	3549	995653	45:22	0,71	985	C16H10	**0,38**
Chrysene	2764	699204	50:31	2,82	988	C18H12	**0,70**
Benzo[a]pyrene	817	566400	54:22	1,09	976	C20H12	**0,84**
Benzo[ghi]perylene	114	598974	58:40	1,98	896	C22H12	**0,29**

What Analytes ID ?

- ✓ Exact mass identification of putative biomarkers…
- ✓ Duplication of selected samples on HRTOF



Mass	Mass Acc. (ppm)	Calc. Mass	Formula
134,10897	271,15	134,07262	$C_9H_{10}O$

Mass	Mass Acc. (ppm)	Calc. Mass	Formula
134,10897	-0,23	134,10900	$C_{10}H_{14}$

Interest of low eV, combi EI/PI, …

Take Home Message

- ✓ GC×GC-HRTOFMS is powerful tool (complex data)
- ✓ Supervised statistics needed (biological diversity)
- ✓ The cell culture approach reduces ‘flat tables’
- ✓ Next steps are:
  - Extract robust analyte identities
  - Compare analytes from cells to breath VOCs
  - Get primary cultures (biopsies) started on CRC