Nouvelles techniques couplées pour l'analyse d'arômes, parfums – Automatisation de la préparation d'échantillons

Bart Tienpont, Christophe Devos, Frank David and Pat Sandra

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What do we expect?

- Volatility range BROAD
- Polarity range WIDE
- Complex samples HIGHLY
- Matrix interferences OMG

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Solutions in reach

- Sample preparation
  - Enrichment?
  - selective or generic?
- Separation: GC or GC-GC or GCxGC ?
- Detection: MS/ODP and… sensors?

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1. Sample Preparation

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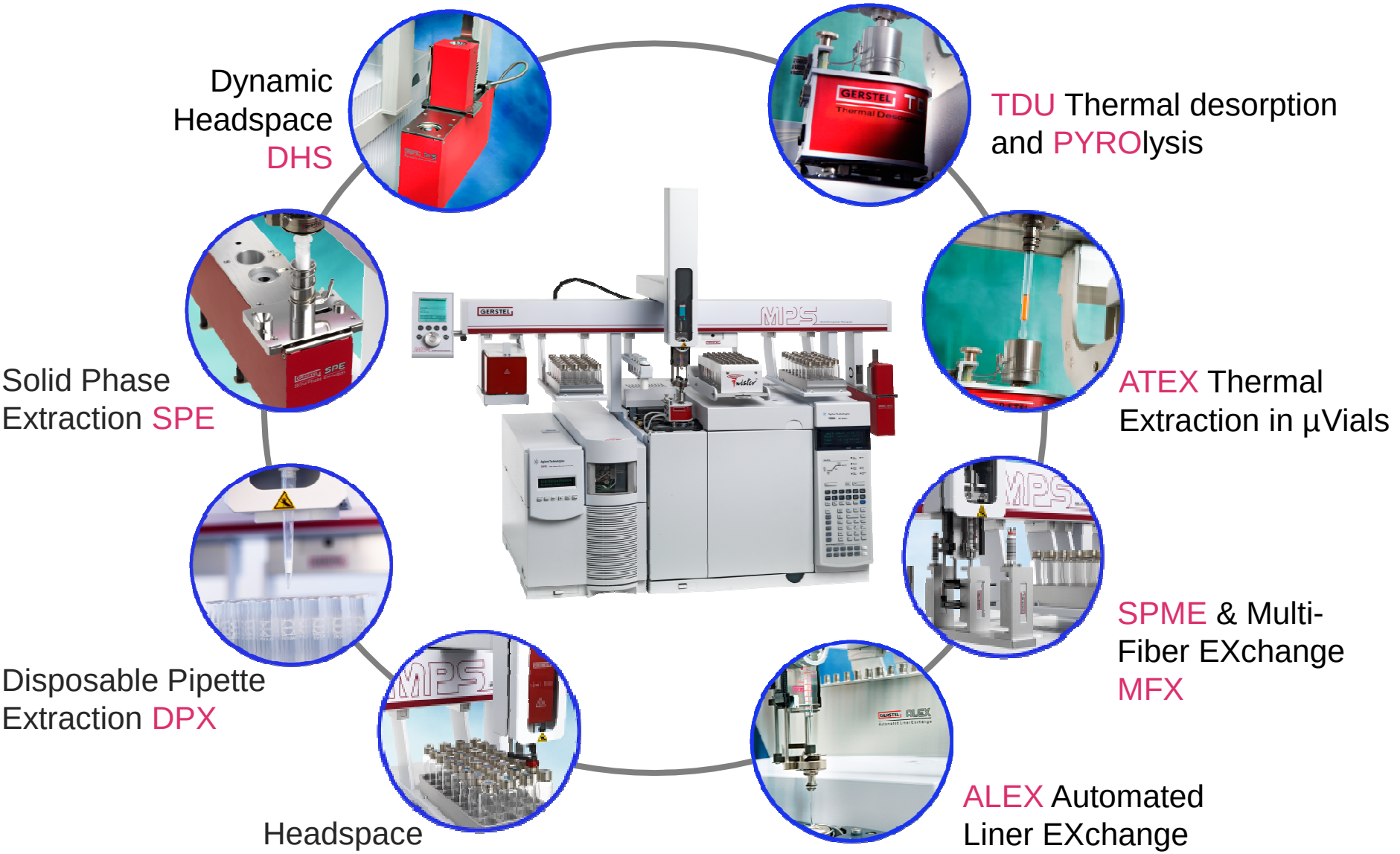
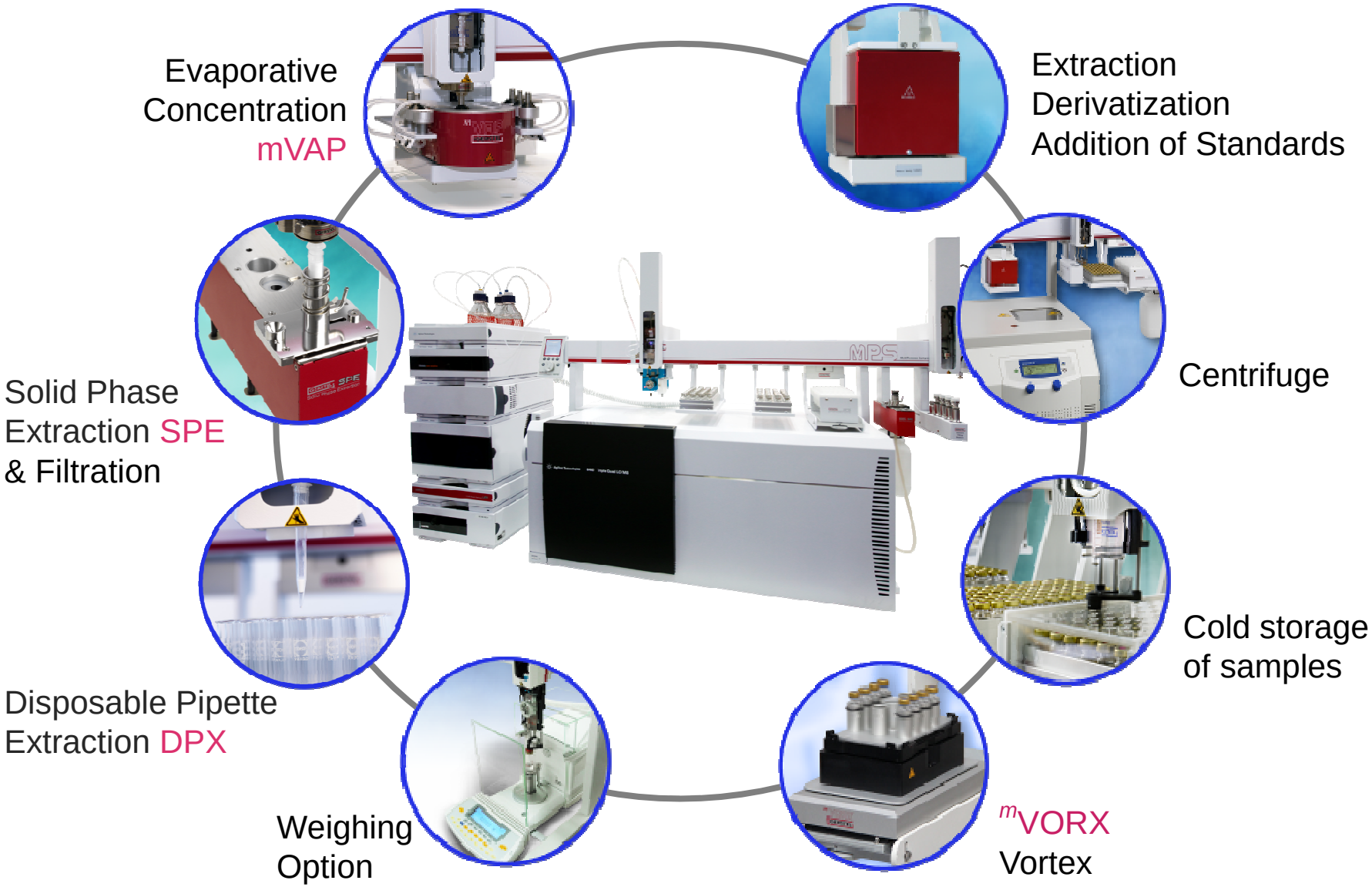
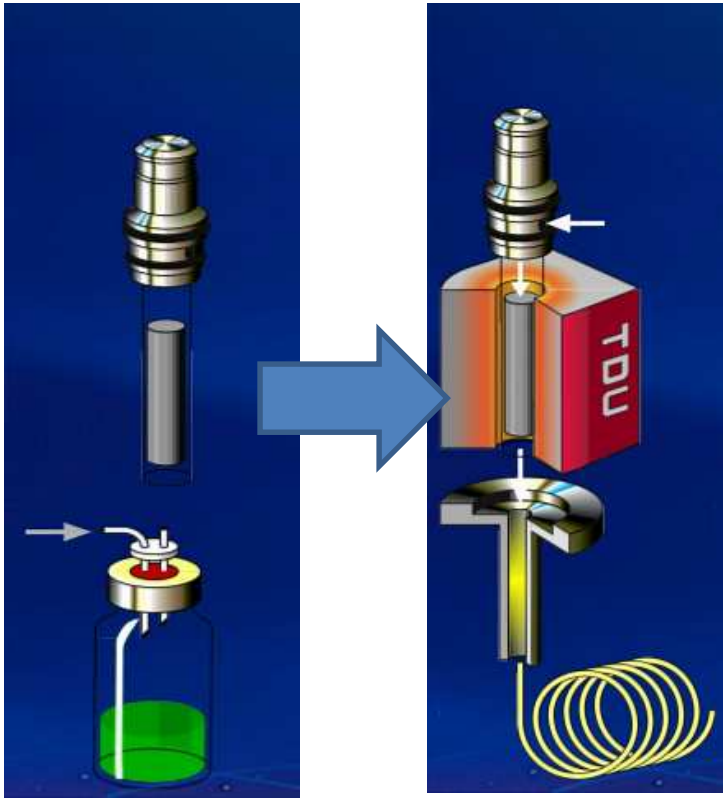

Dynamic Headspace DHS
TDU Thermal desorption and PYROlysis
Solid Phase Extraction SPE
ATEX Thermal Extraction in µVials
SPME & Multi-Fiber EXchange MFX
Disposable Pipette Extraction DPX
ALEX Automated Liner EXchange
Headspace


Evaporative
Concentration
mVAP
Extraction
Derivatization
Addition of Standards
Solid Phase
Extraction SPE
& Filtration
Centrifuge
Cold storage
of samples
Disposable Pipette
Extraction DPX
Weighing
Option
mVORX
Vortex

Dynamic Headspace (DHS)



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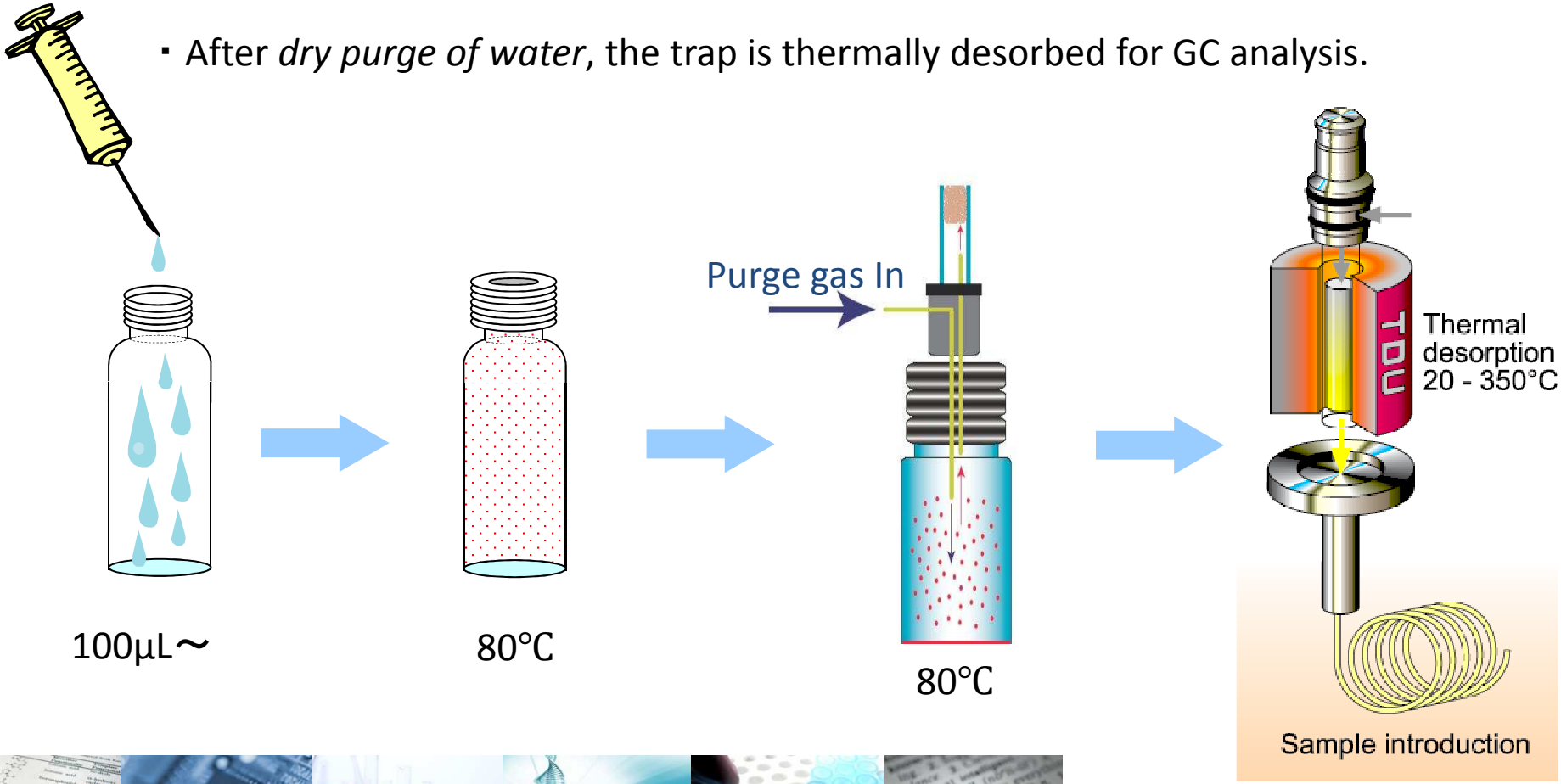
Large Volume FEDHS (LV-FEDHS)

- 100 µL of aqueous sample !
  purged with inert gas at an elevated temperature (80ºC) using DHS.
- Volatile and semi-volatile analytes are transferred into the trap (Tenax)
- After *dry purge of water*, the trap is thermally desorbed for GC analysis.



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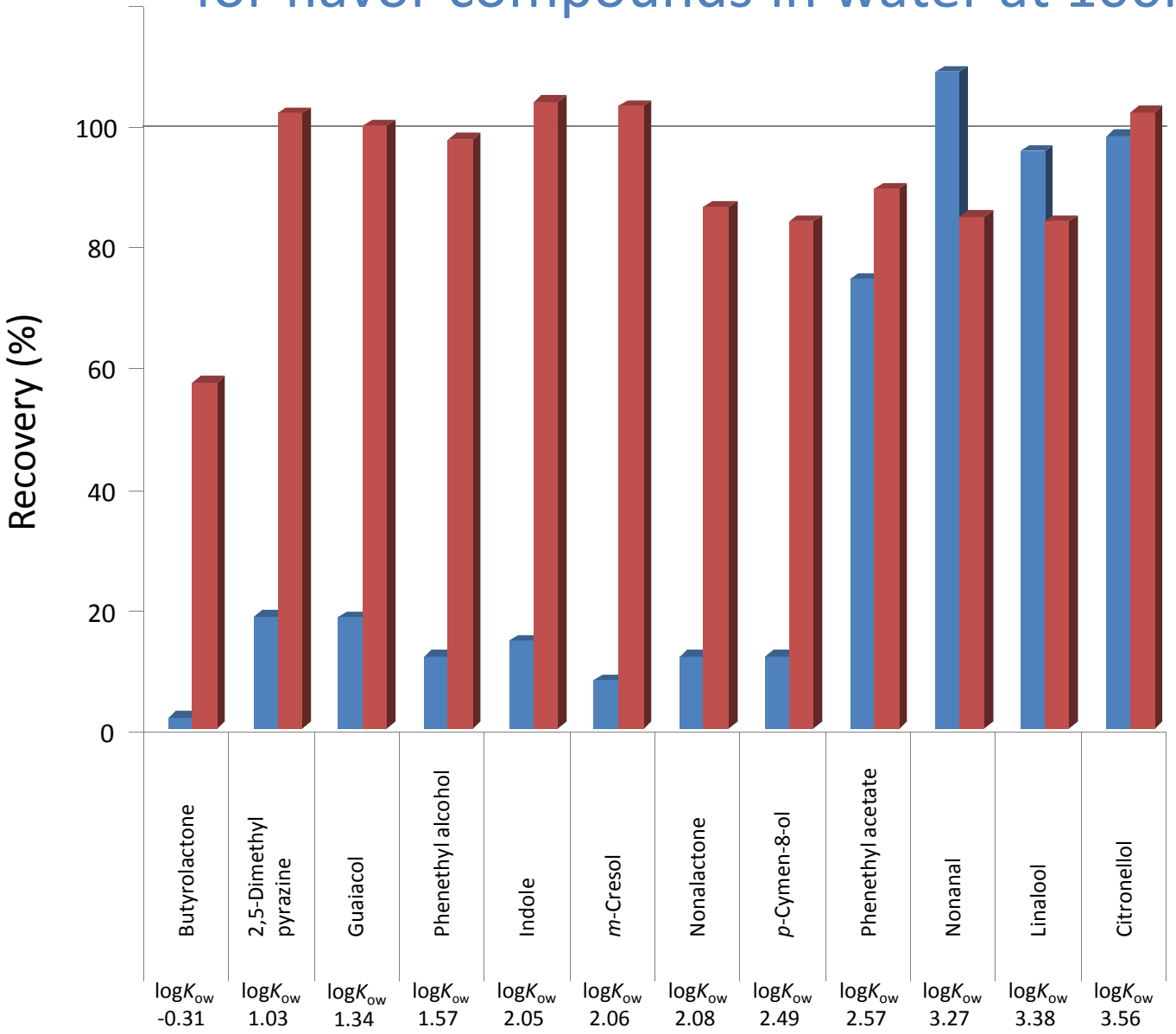
Comparison of recovery between DHS and FEDHS for flavor compounds in water at 100ng/mL



DHS

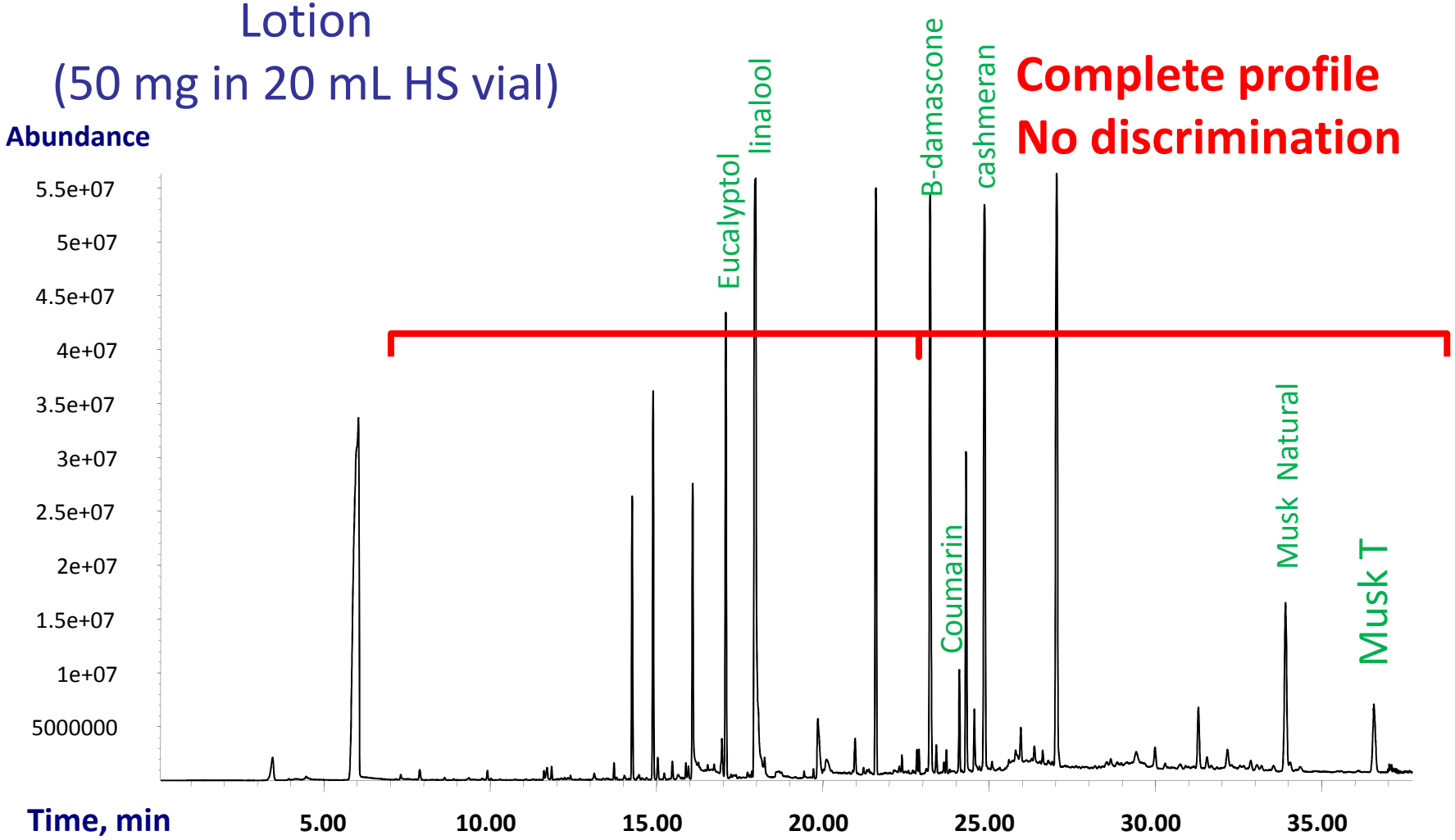
Sample: 1 mL
Sample temp: 25°C
Purge volume: 3 L
Trap temp: 40°C

LVFET

Sample: 100 µL
Sample temp: 80°C
Purge volume: 3 L
Trap temp: 40°C

FEDHS of Flavors and Fragrances in Cosmetics

Lotion
(50 mg in 20 mL HS vial)



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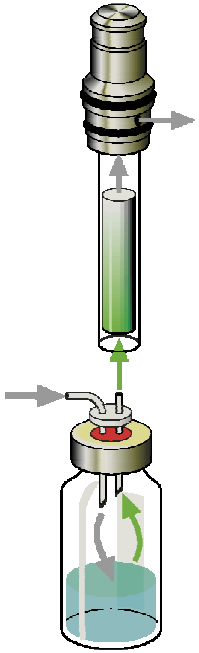
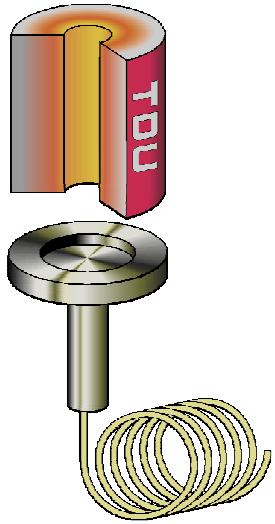
Sequential Dynamic Headspace Sampling - Multi-Volatile Method (MVM) for Aroma Analysis of Beverages

Dynamic Headspace

Method 1: Very Volatile Analytes





25◦C - carbon-based adsorbent trap
150 mL @ 50 mL/min

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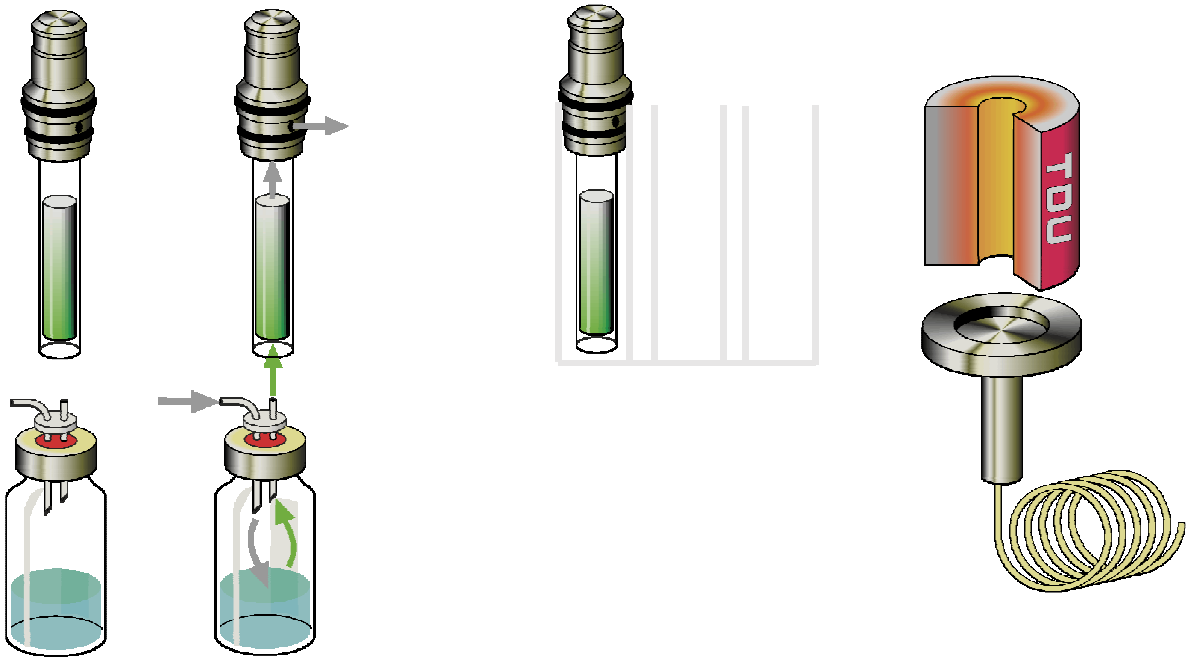
Sequential Dynamic Headspace Sampling - Multi-Volatile Method (MVM) for Aroma Analysis of Beverages

Dynamic Headspace

Method 1 Method 2 Volatile Analytes Semi Volatile Analytes



25◦C - carbon-based adsorbent trap
650 mL @ 100 mL/min

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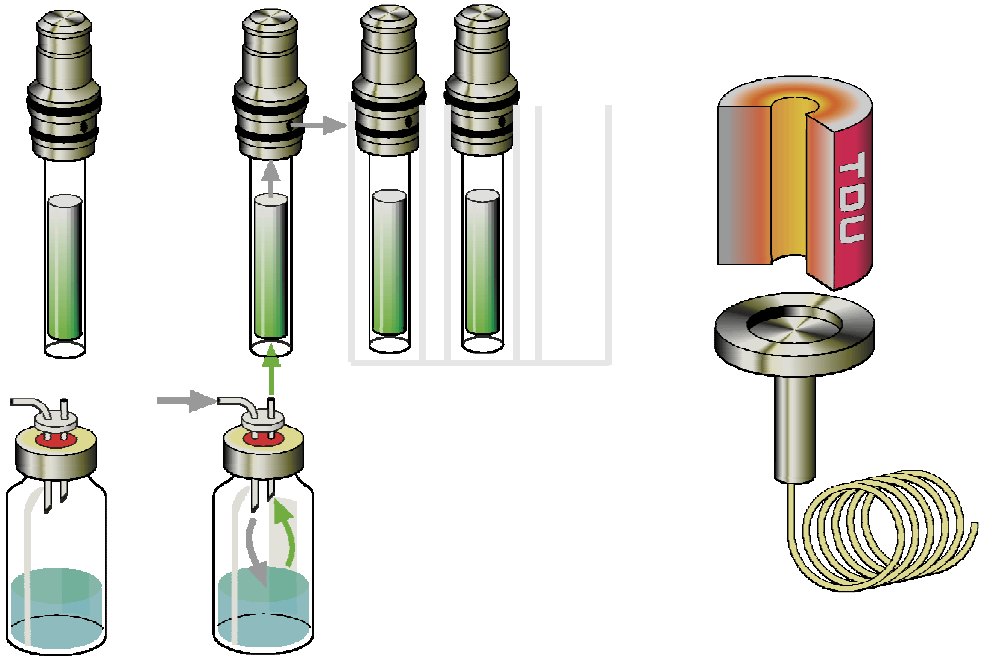
Sequential Dynamic Headspace Sampling - Multi-Volatile Method (MVM) for Aroma Analysis of Beverages

Dynamic Headspace

Method 2: Volatile and Semi-Volatile Analytes and hydrophillic analytes



80◦C - Tenax TA trap
3L @ 100 mL/min

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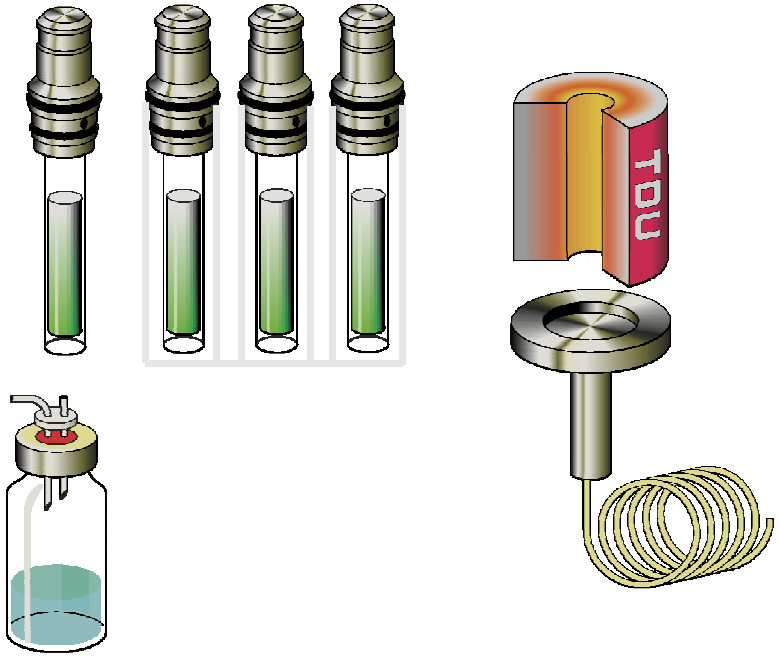
Sequential Dynamic Headspace Sampling - Multi-Volatile Method (MVM) for Aroma Analysis of Beverages

Dynamic Headspace

Method 3: Volatile, non volatile and hydrophillic analytes



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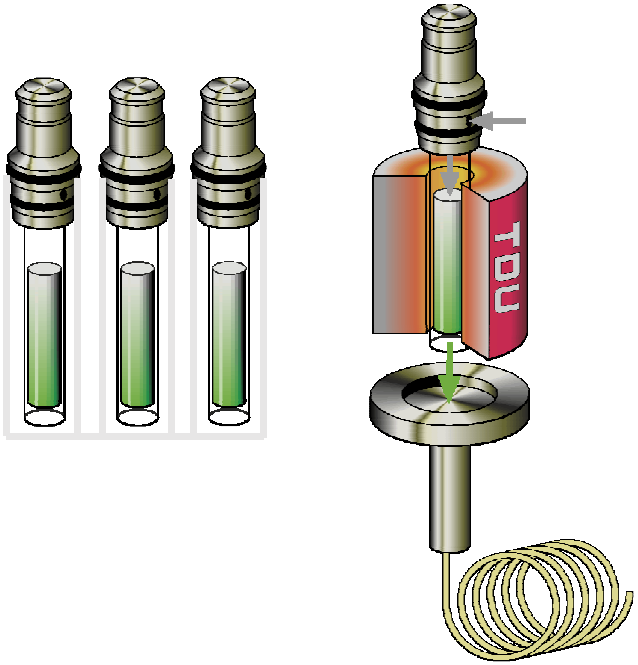
Sequential Dynamic Headspace Sampling - Multi-Volatile Method (MVM) for Aroma Analysis of Beverages

Dynamic Headspace

Method 4: TDU Multi Desorption



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Sequential Dynamic Headspace Sampling - Multi-Volatile Method (MVM) for Aroma Analysis of Beverages

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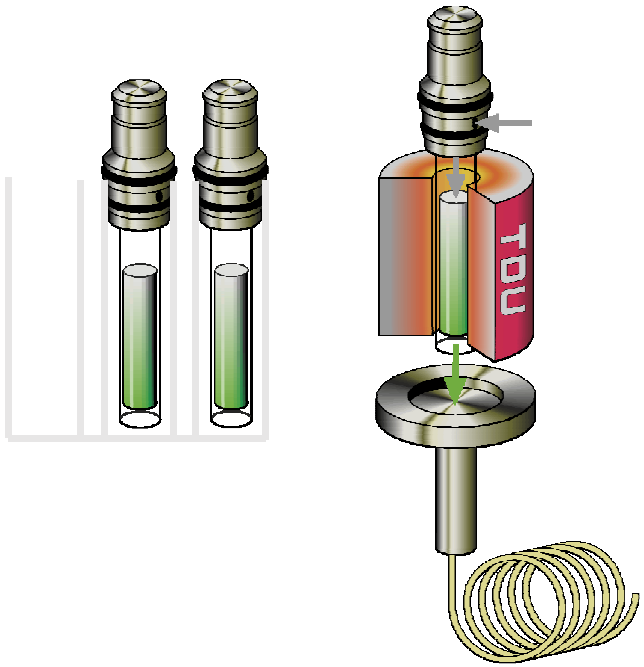
Method 4: TDU Multi Desorption



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Sequential Dynamic Headspace Sampling - Multi-Volatile Method (MVM) for Aroma Analysis of Beverages

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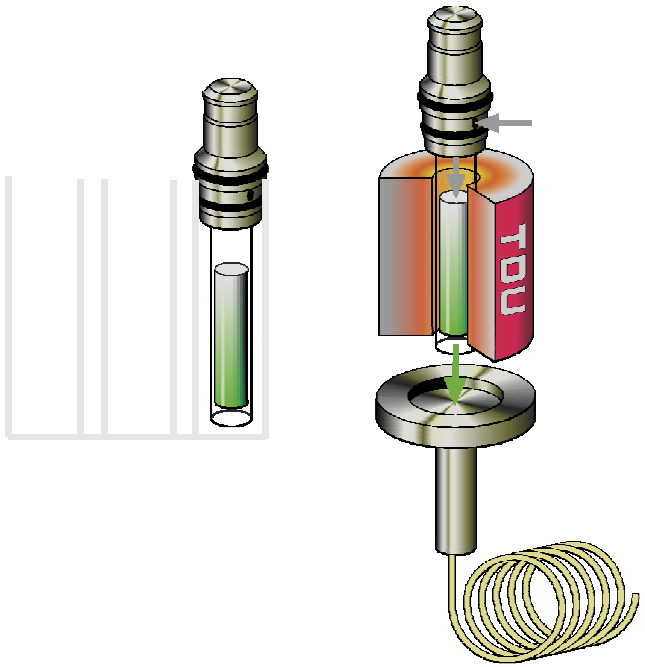

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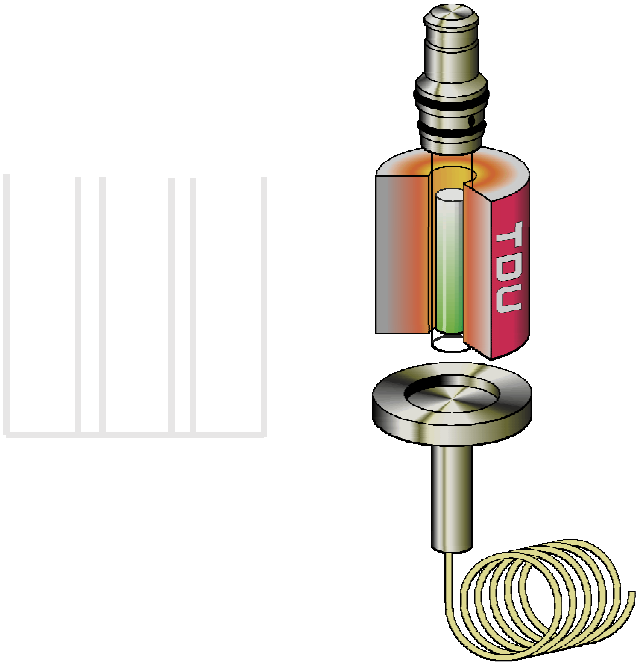
Method 4: TDU Multi Desorption



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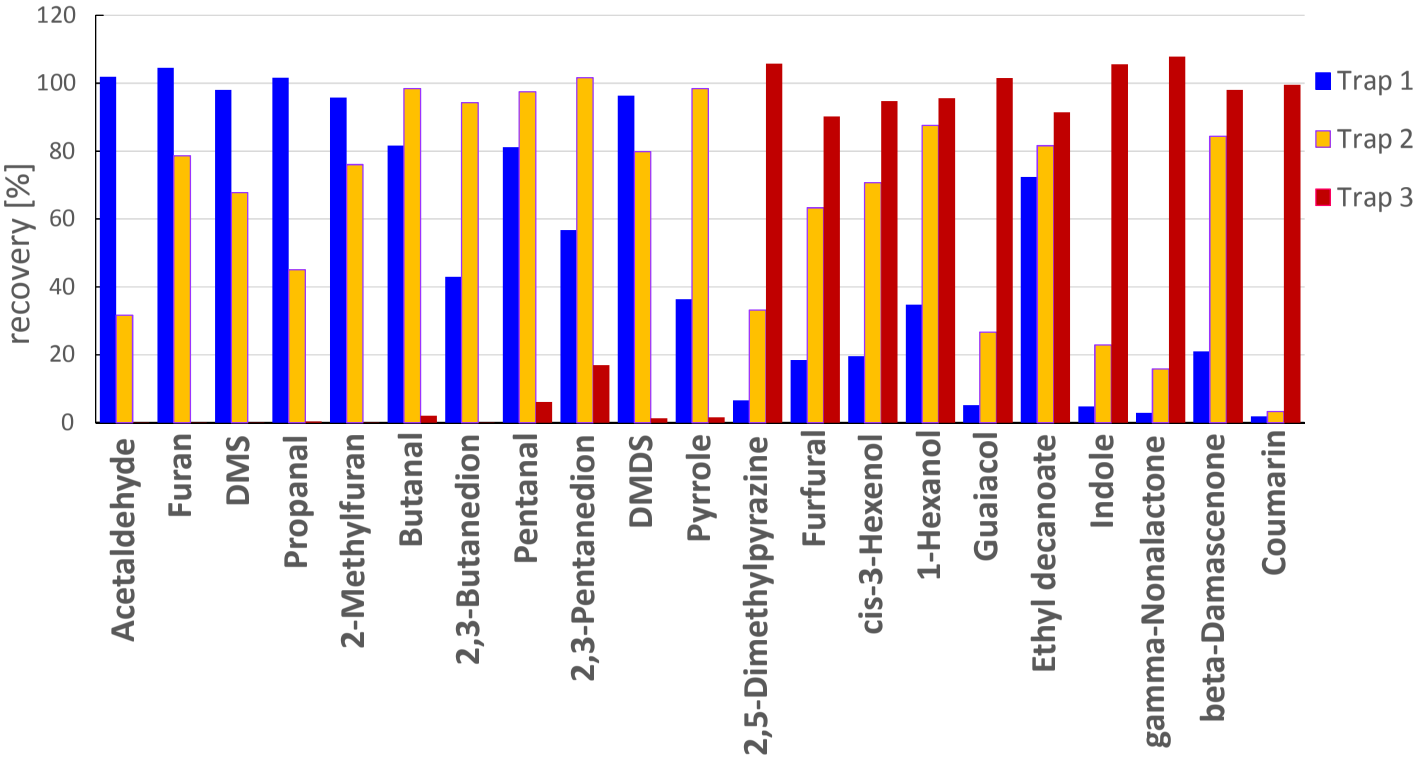
Brewed Coffee MVM Extraction



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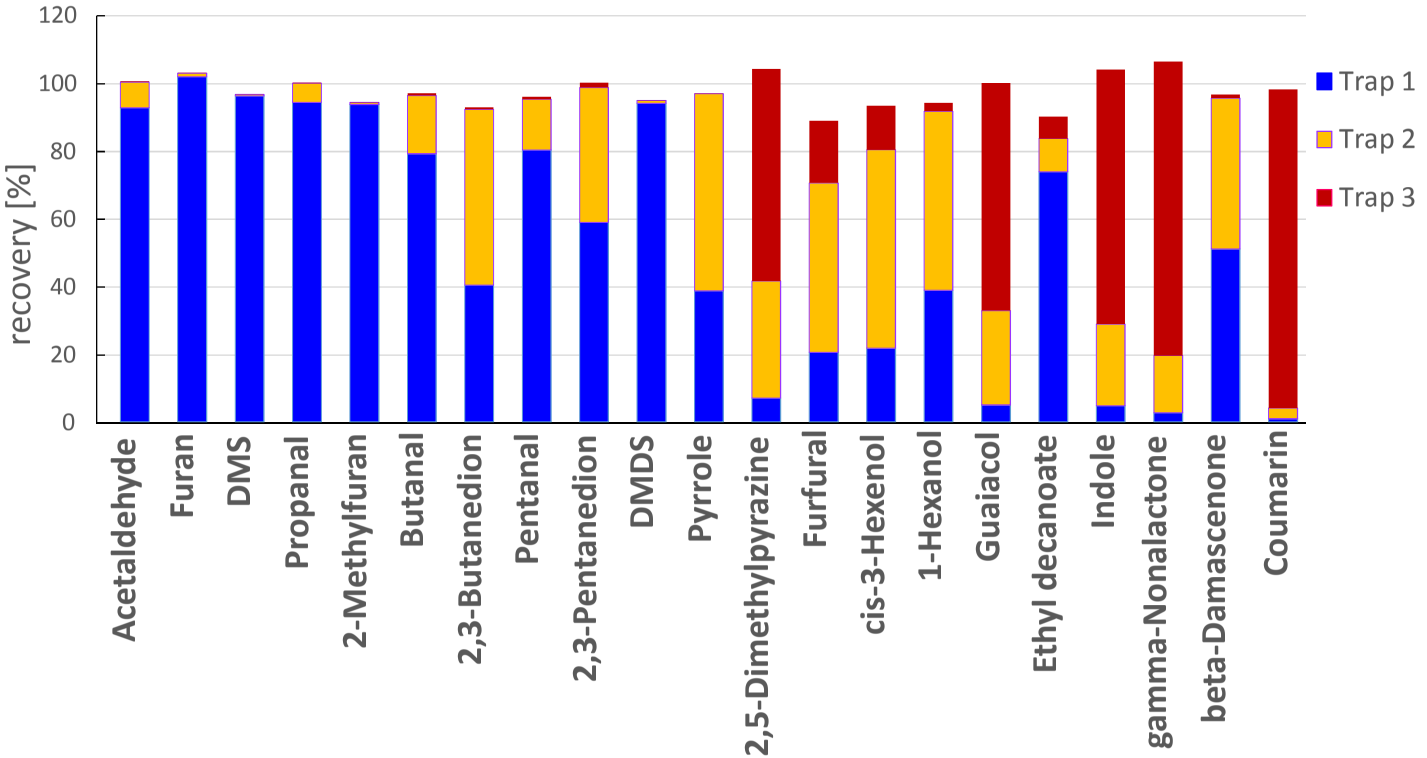
Brewed Coffee MVM Extraction



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Wine Analysis using SBSE-GC-MS

Céline Franc, Frank David, Gilles de Revel , JCA

Multi-residue methods

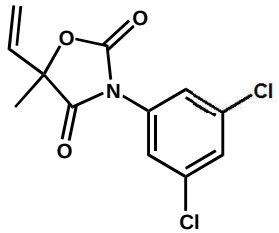
- 2007 : Off-flavours

  IBMP, EP, EG, TCA, TeCA, PCA, TBA, Géosmine

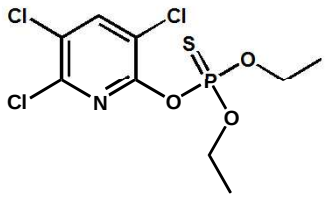
- 2010 : Markers of wine aroma (fruity)

  C13-norisoprenoides and lactones

- 2010 : Pesticide residues



Vinclozoline



Chlorpyriphos

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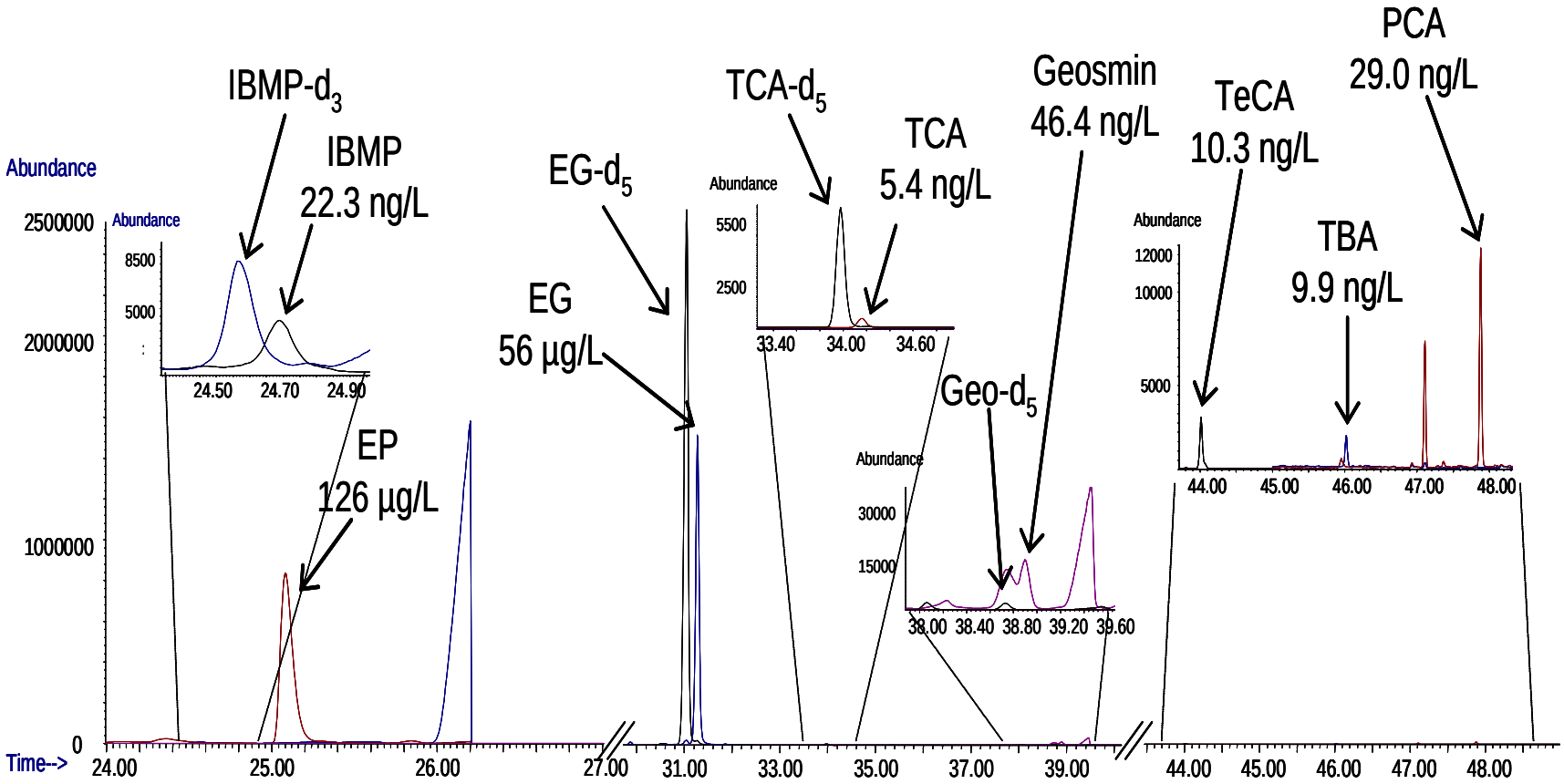
Multiresidue Analysis of Wine Defects

Céline Franc, Frank David, Gilles de Revel , JCA



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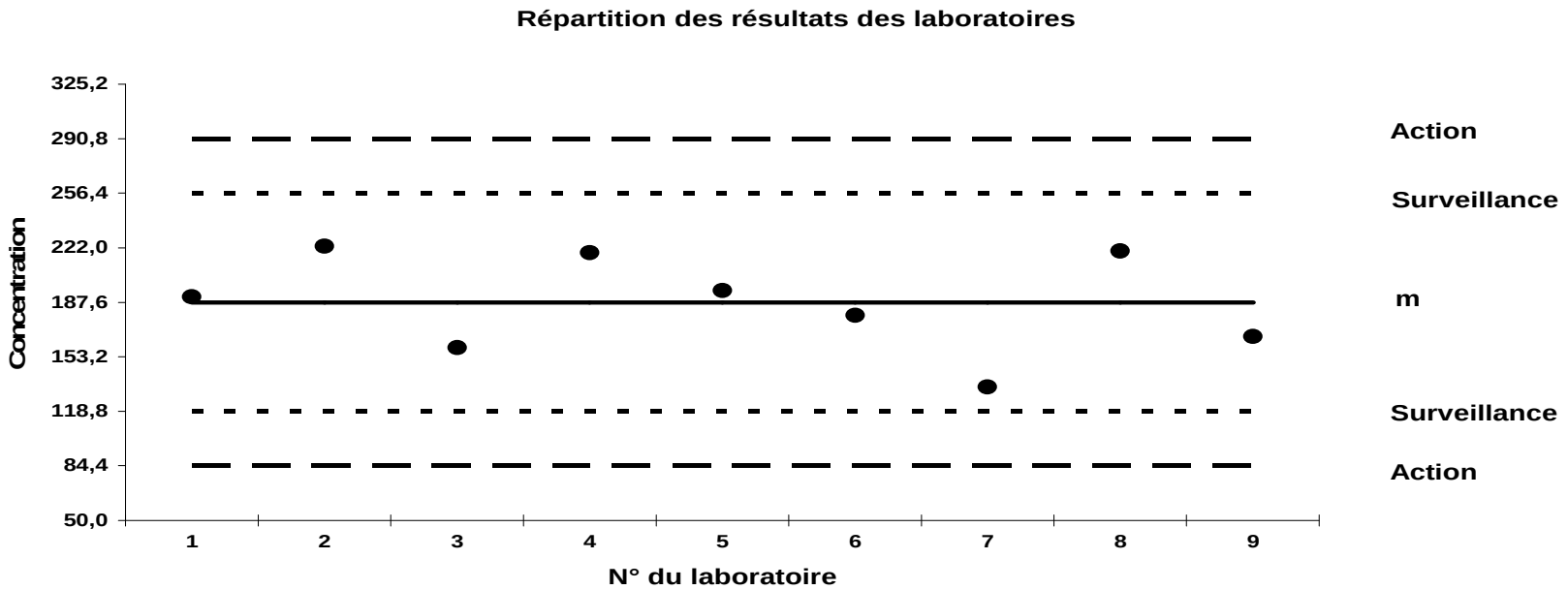
Application : Round-Robin test
SBSE-GC-MS « off-flavors"

2009 : inter-laboratory test, 8 compounds – 9 laboratoires

Volatile phenols

nombre de laboratoires retenus :	9		
valeur assignée m :	187,6		
écart-type s* :	34,4		
limites de surveillance :	118,8	;	256,4
limites d'action :	84,4	,	290,8



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SBSE procedure for vegetables and fruit

15 g sample + 15 mL ACN

Ultra-Turrax + ultrason (15 min)

(=QuEChERS)

1 mL extract

+ 10 mL water

P. Sandra, B. Tienpont and F. David J. Chromatogr., 1000 (2003) 299-309

SBSE: 60 min

TD-RTL-CGC-MS

Modified ratio water/organic Multi-Twister extraction

N. Ochiai, K. Sasamoto, H. Kanda, T. Yamagami, F. David, B. Tienpont and P. Sandra
J. Sep. Sci 28 (2005) 1083-1092

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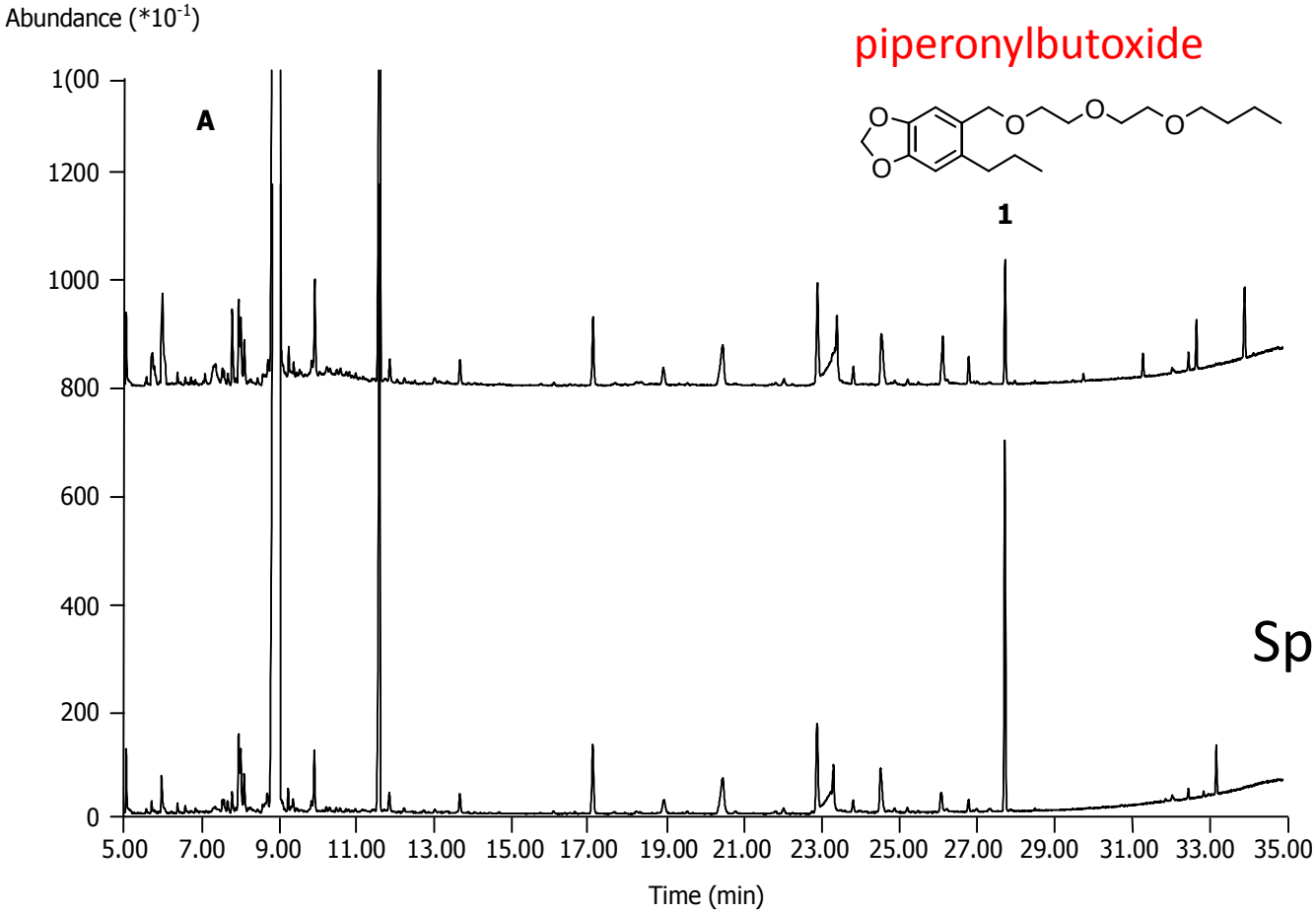
Analysis of Baby Food by SBSE-TD-GC-MSD (mixed vegetables, rice, chicken)



piperonylbutoxide

1

Non-spiked

Spiked - 2 ppb

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QC on solid material

‘Passive’ Extraction

10 corks – leaching during 24h

in 500ml wine simulant (10 % Ethanol)

<u>SBSE extraction for leachable haloanisoles:</u>

- 100ml « extract (10 % Ethanol) »
- IS: TCA-d5 (10ng/l),
- 2 h extraction with 20 mm x 0.5 mm stir bar

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Polarity?

3 Strategies

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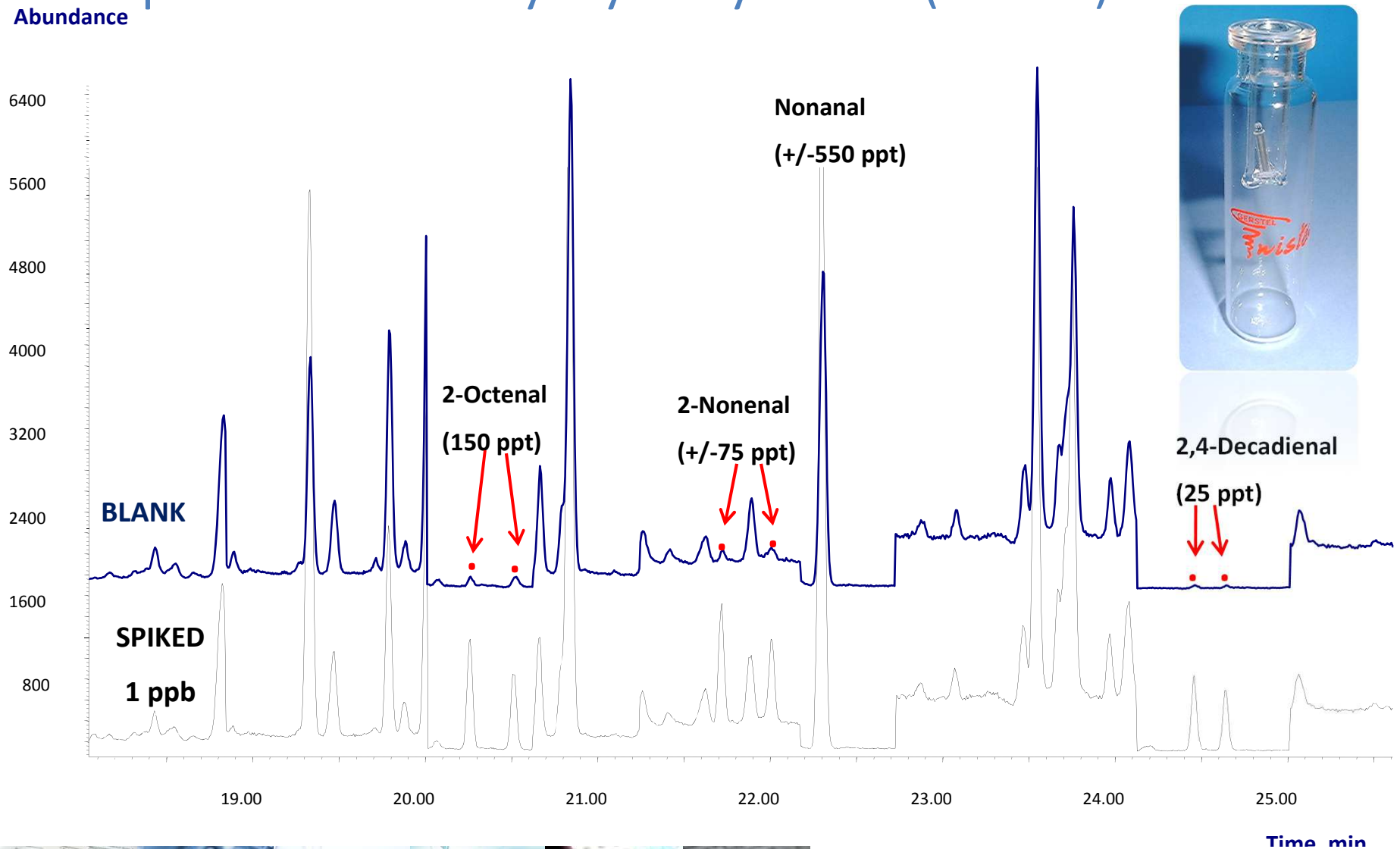
Derivatization HSSE

pentafluorobenzyl hydroxylamine (PFBHA) - BEER



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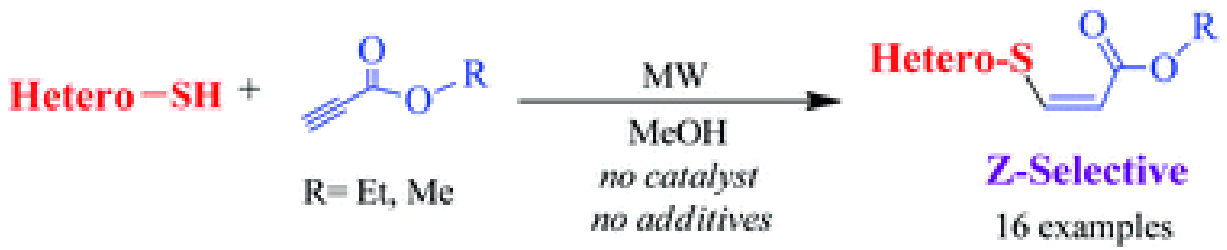
Analysis of Thiols (wine, beer,...)

- Polyfunctional thiols [3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP)] are important aroma compounds. Current methods lack specificity and sensitivity.

- Derivatization with alkyl propiolate (ethyl propriolate) can be performed in-situ and, combined with SBSE, high sensitivity and good selectivity are obtained for the detection of the thioacrylates.



- *See: N. Ochiai et al.*

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EG Silicon Twister

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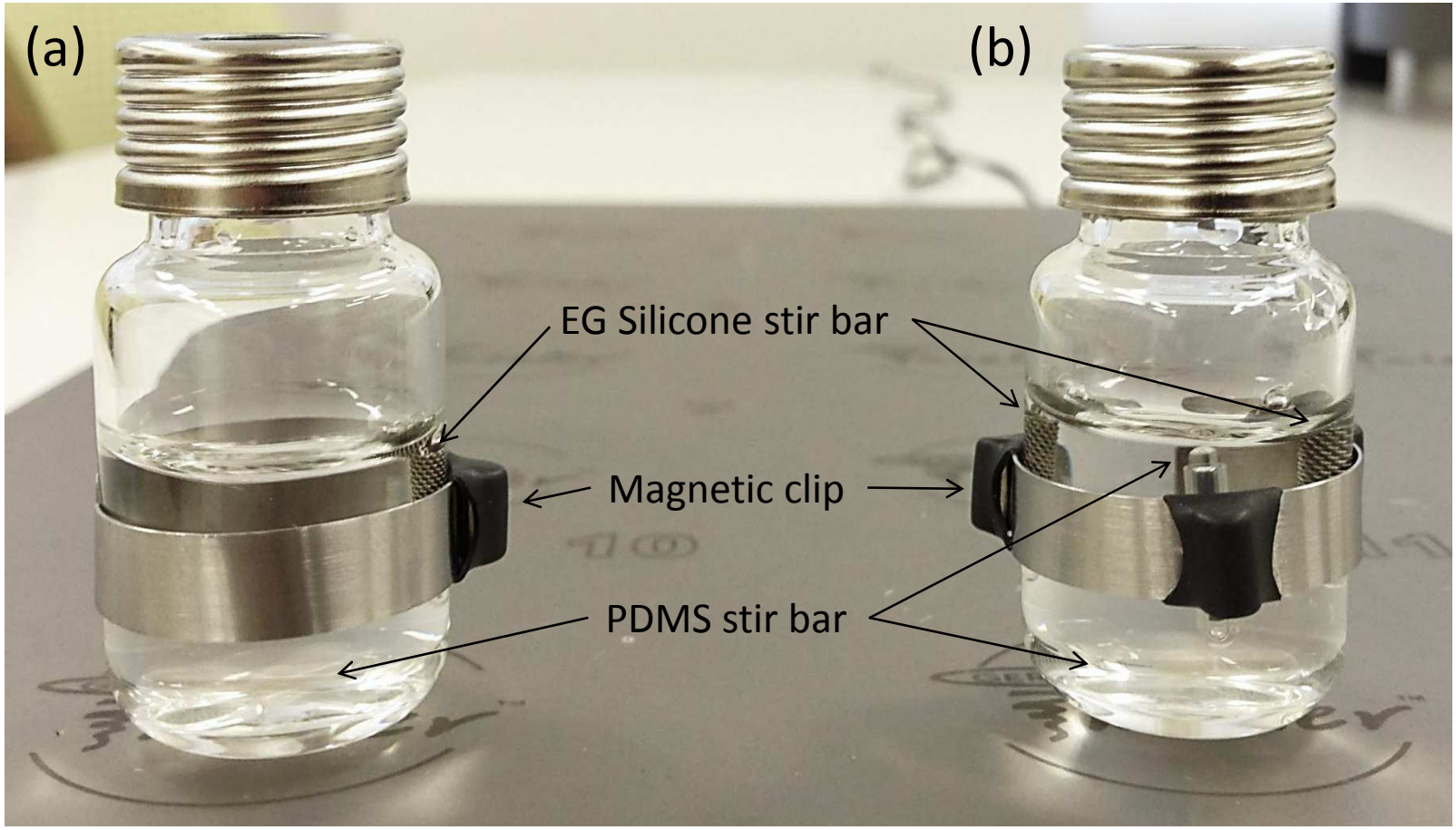
mSBSE using different coatings



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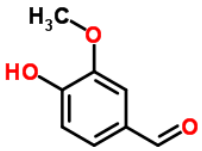
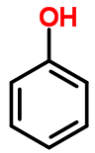
Comparison of recovery of phenolic compounds between EG Silicon, PDMS and Seq-SBSE



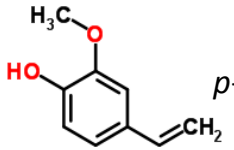
Vanillin (logKow: 1.05) (x 10)

Guaiacol (logKow: 1.34)



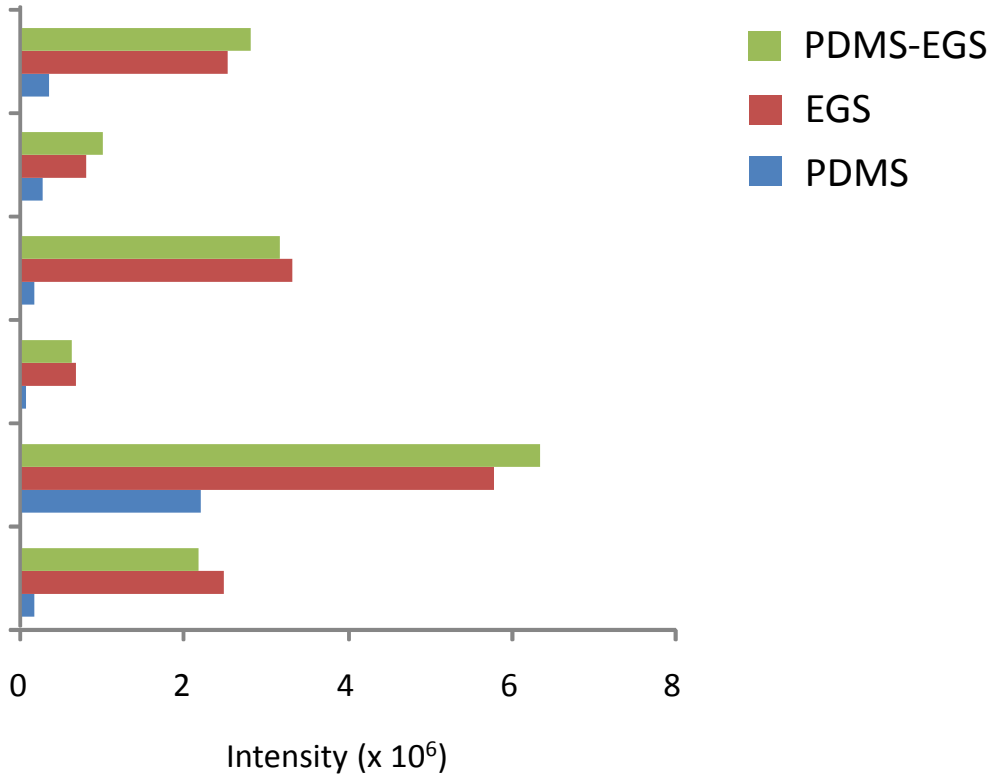
Phenol (logKow: 1.51)

p-cresol (logKow: 2.06)



p-Vinyl guaiacol (logKow: 2.24)

p-Vinyl phenol (logKow: 2.41)



J.I. Cach et al, J. Pharm Biomed Anal 78-79 (2013) 255: Analysis of Bisphenols in Cosmetics

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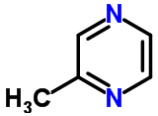
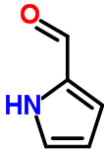
Comparison of recovery of nitrogen heterocyclic compounds between EG Silicon, PDMS and Seq-SBSE



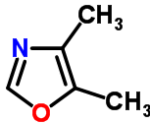
Methyl pyrazine (logKow: 0.49)



2-Acetyl pyrrole (logKow: 0.56)

2-Formyl pyrrole (logKow: 0.60)

Pyridine (logKow: 0.80)



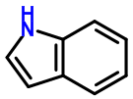
2,5-Dimethyl pyrazine (logKow: 1.03)

4,5-Dimethyl oxazole (logKow: 1.31) x10

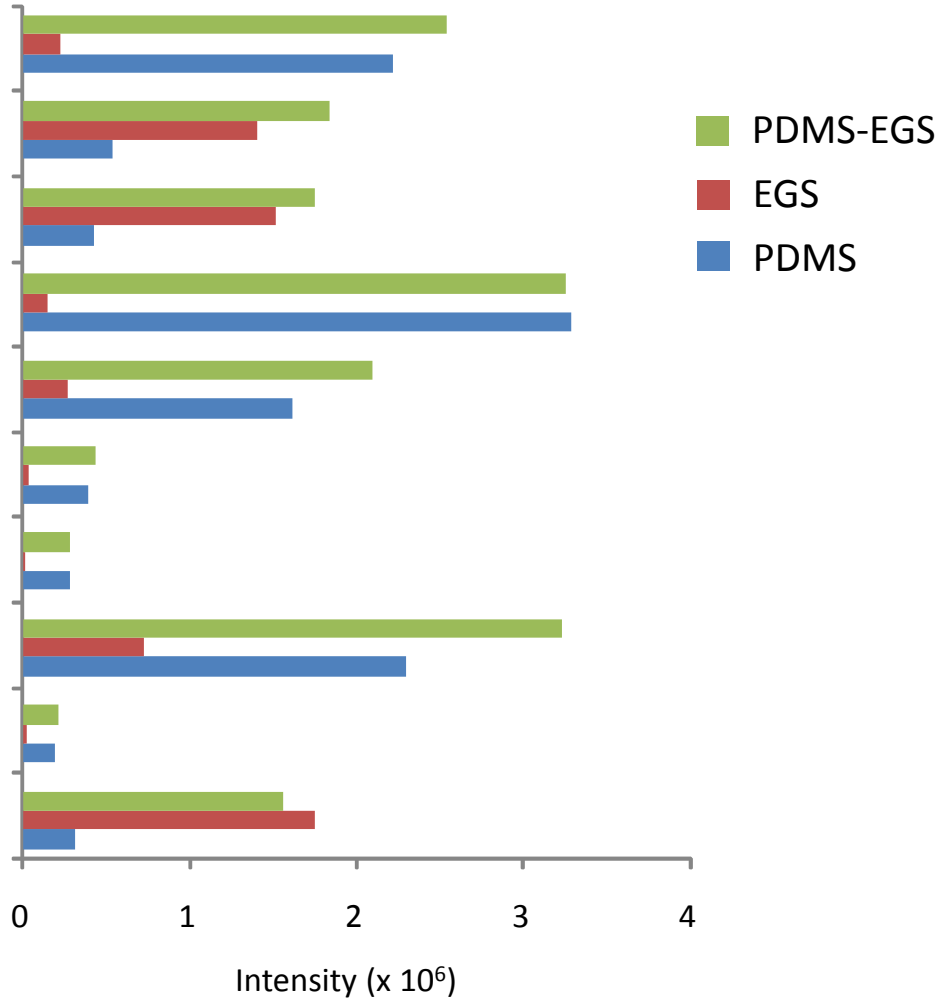
3,4-Dimethyl isoxazole (logKow: 1.31) x10

5-Methyl pyrazine (logKow: 1.53)

2,4,5-trimethyl oxazole (logKow: 1.86)



Indole (logKow: 2.05)



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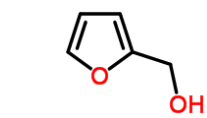
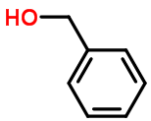
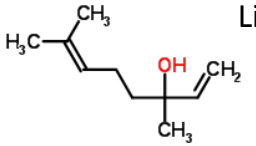
Comparison of recovery of alcohols between EG Silicon, PDMS and Seq-SBSE



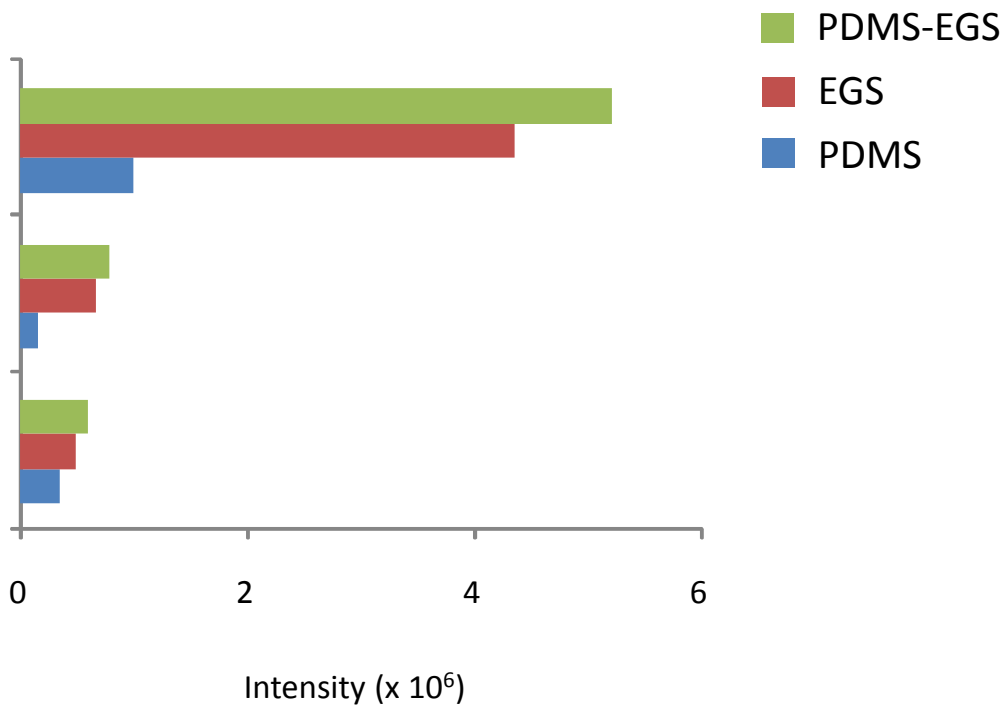
Furfuryl alcohol (logKow: 0.45)



Benzyl alcohol (logKow: 1.08)



Linalool (logKow: 3.38) x100



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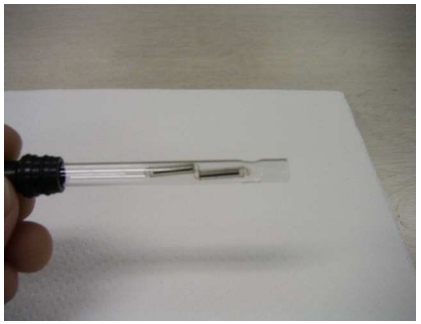
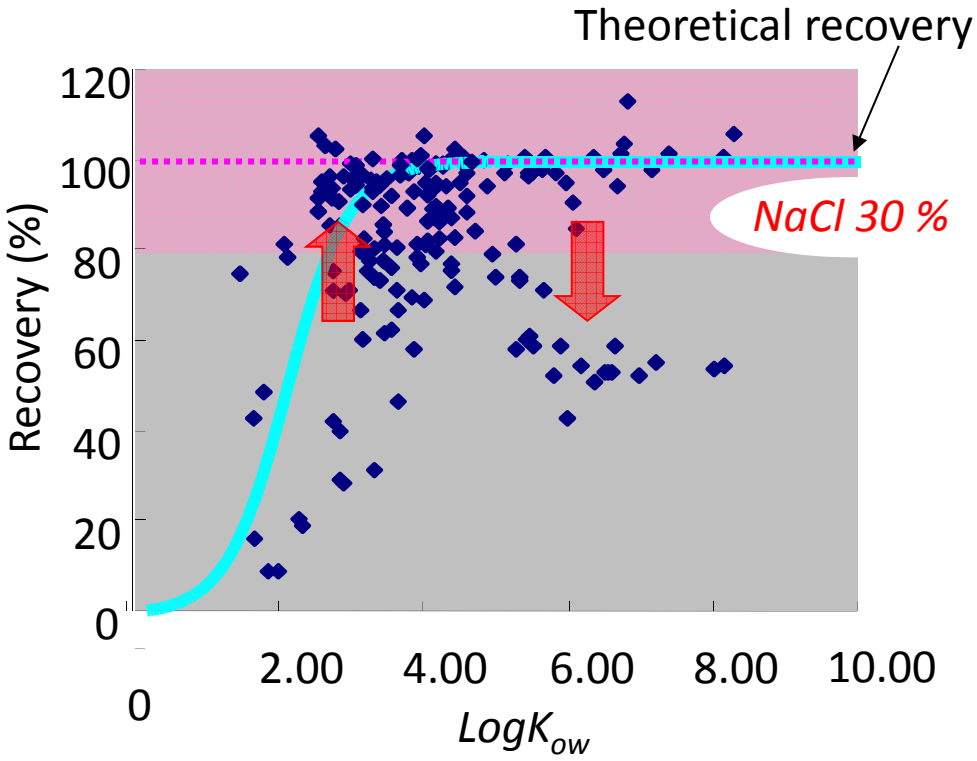
Sequential Stir Bar Sorptive Extraction

1. SBSE in pure water
   - Hydrophobic compounds
     LogKow > 4

2. Add Salt to same sample - SBSE
   - Hydrophylic compounds
     LogKow < 4





Recovery of 88 pesticides by SBSE

N. Ochiai et al, J. Chromatography 1200 (2008) 72.

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2. Improve Analytical Performance

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Capillary Flow Technology (CFT)

(purged) ultimate union

(purged) 2 or 3-way splitter

Deans Switch

GCxGC flow modulator

Quickswap

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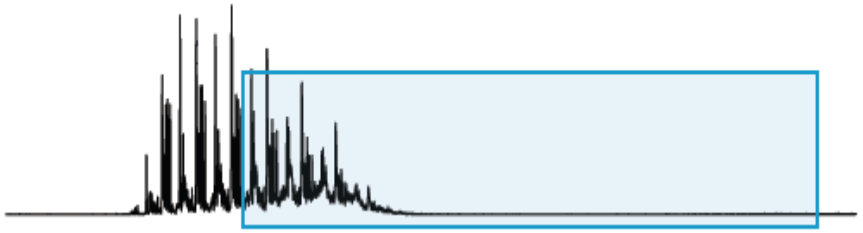
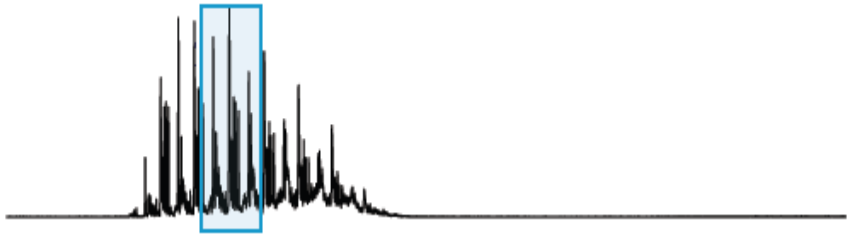
Agilent CFT capabilities

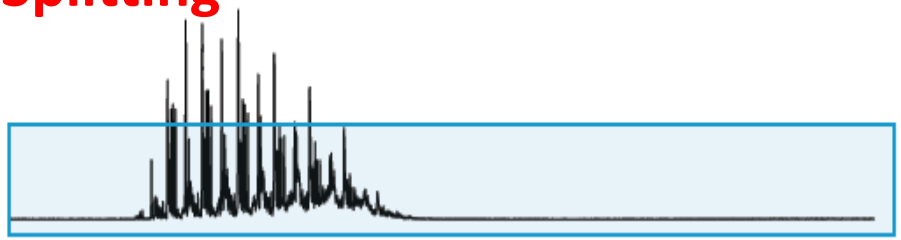
Back Flush



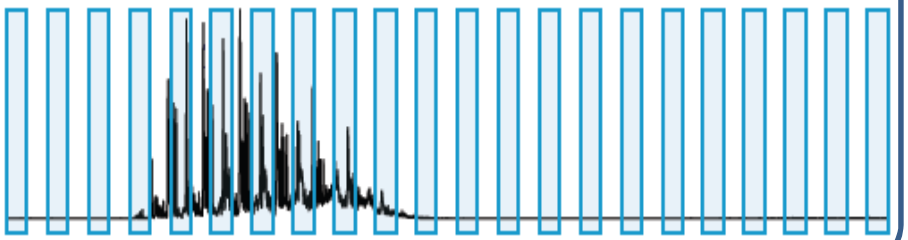
Heart Cutting (Deans Switch)



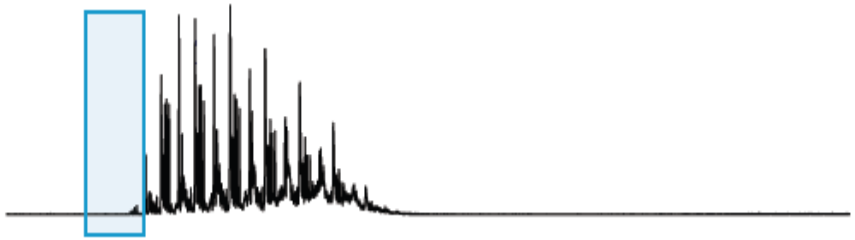
Splitting



GC x GC



Solvent Bypass



Specialties

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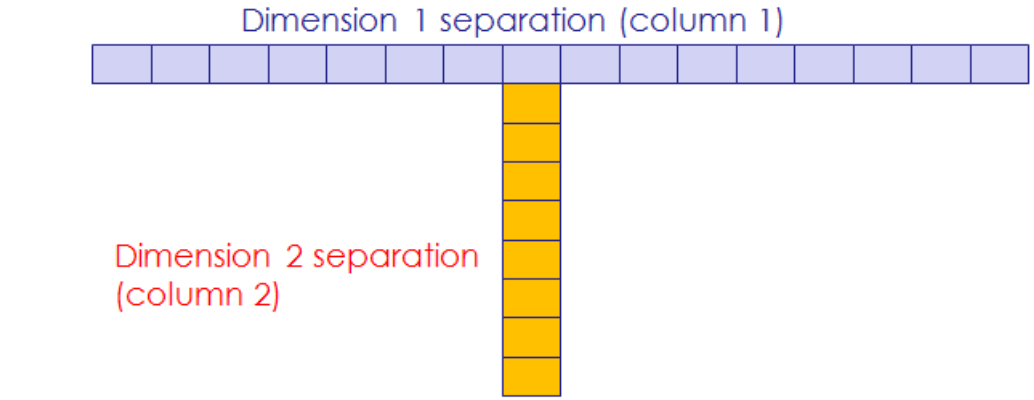
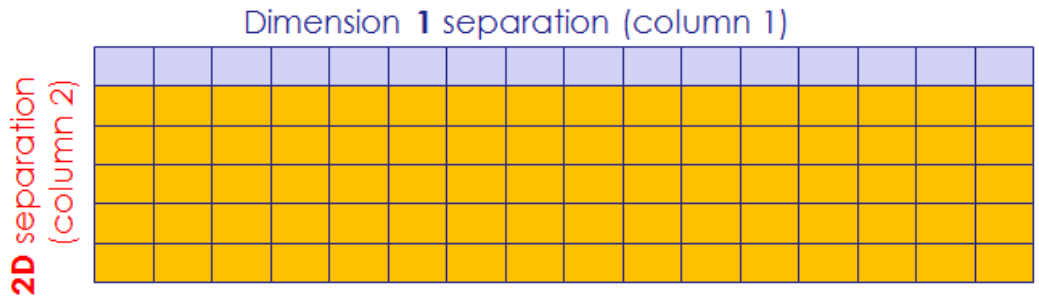
2D strategies – 4+ techniques

Heart-cut 	GC-GC
	LC-LC*
Comprehensive 	GCxGC
	LCxLC*

* SFC is an alternative

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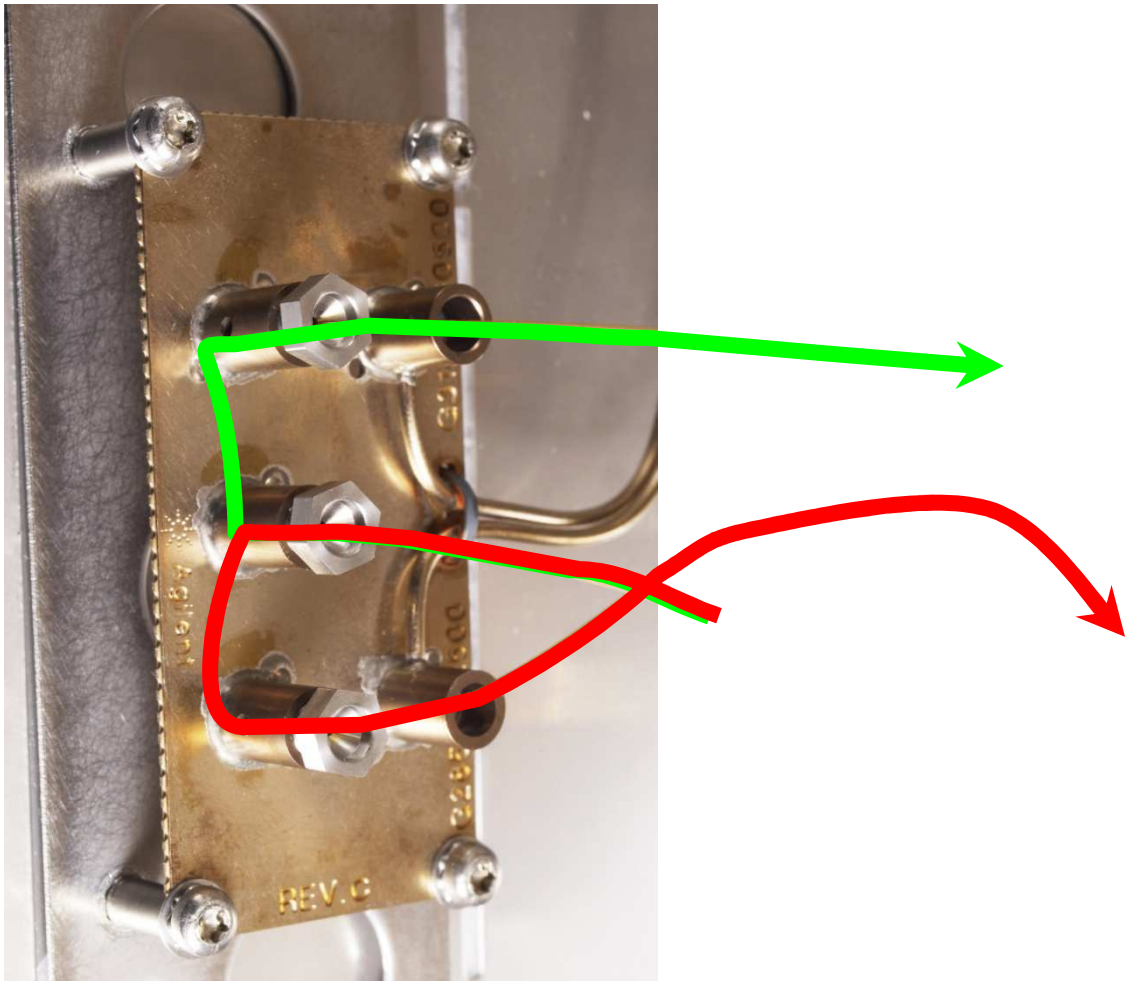
Agilent Deans Switch = *flow selector*



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Selectable 1D or 2D GCMS (RIC/ Gerstel KK)

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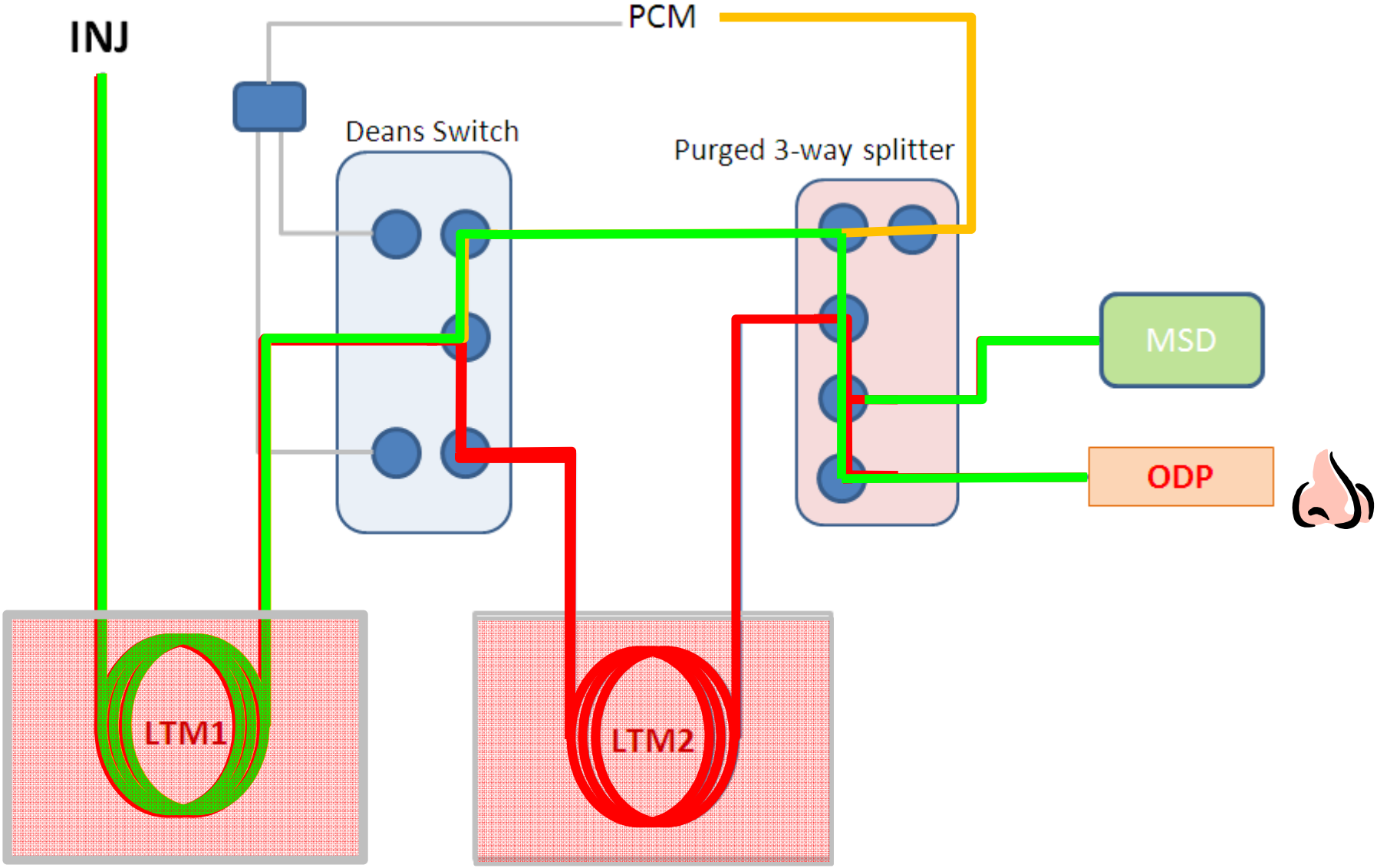
Selectable 1D or 2D GCMS (RIC/ Gerstel KK)



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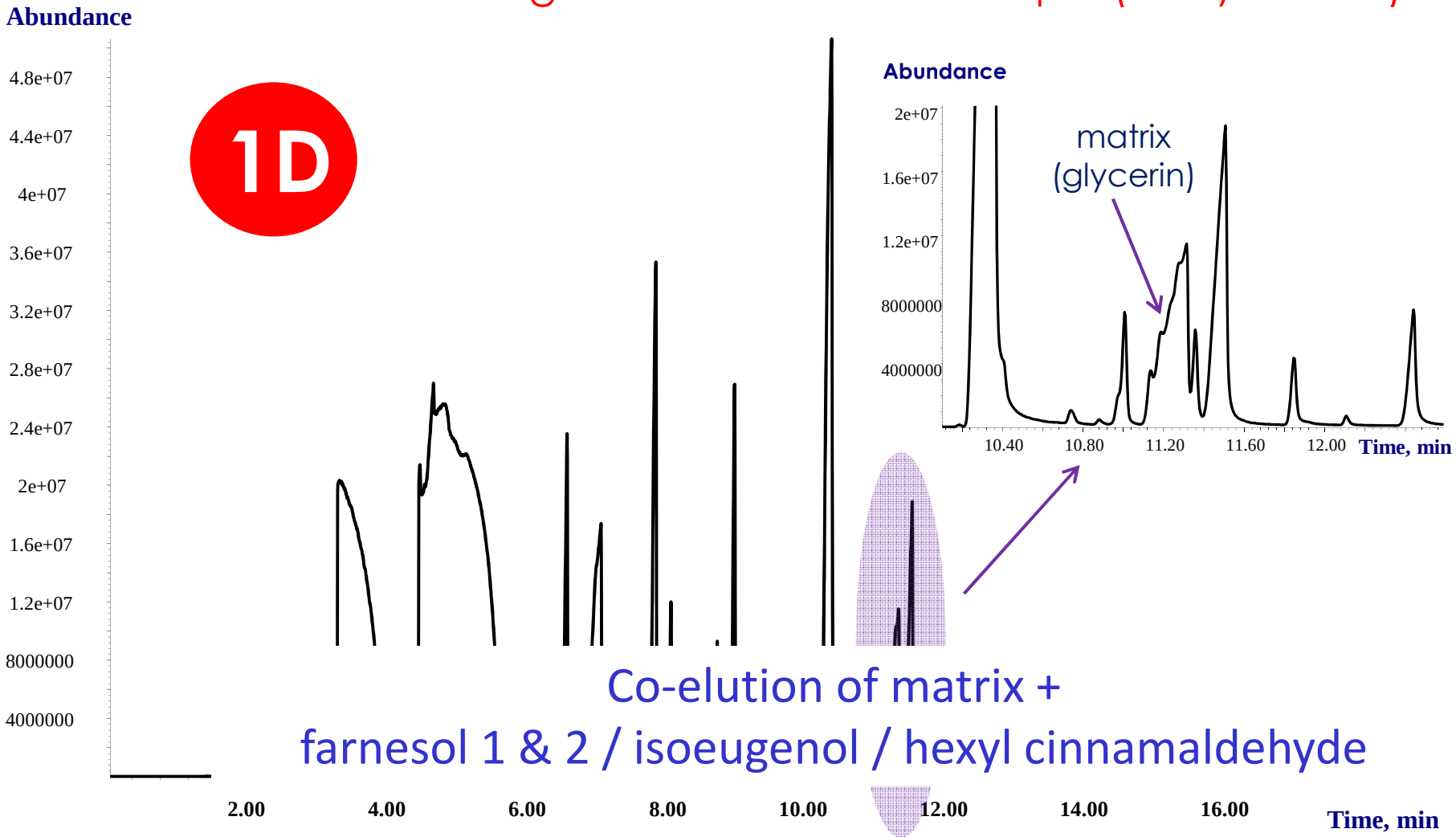
Selectable 1D/2D analysis of allergens in cosmetics

[1]D chromatogram of a cosmetic sample (body cream)



Co-elution of matrix +
farnesol 1 & 2 / isoeugenol / hexyl cinnamaldehyde

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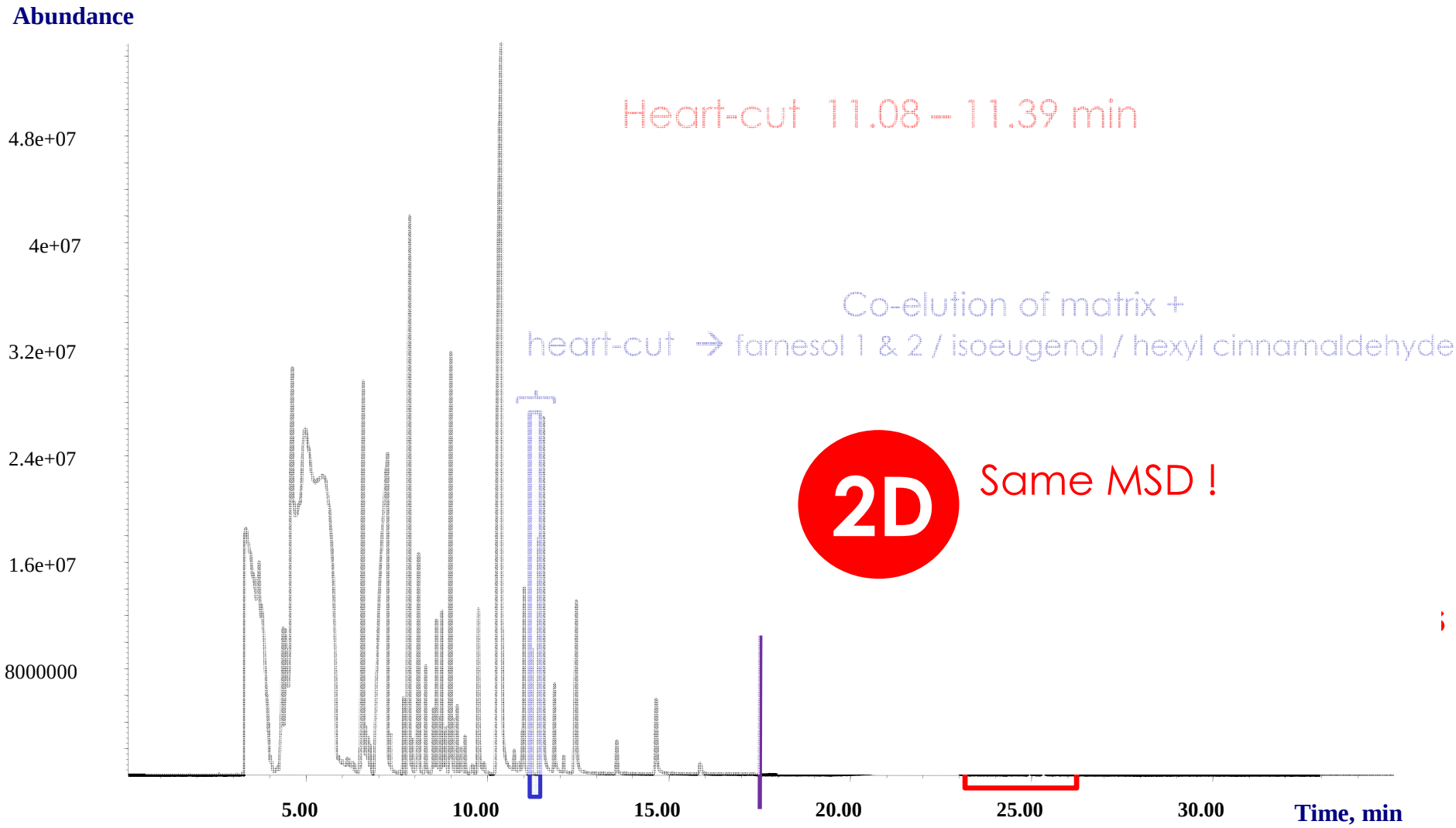
Selectable 1D/2D analysis of allergens in cosmetics



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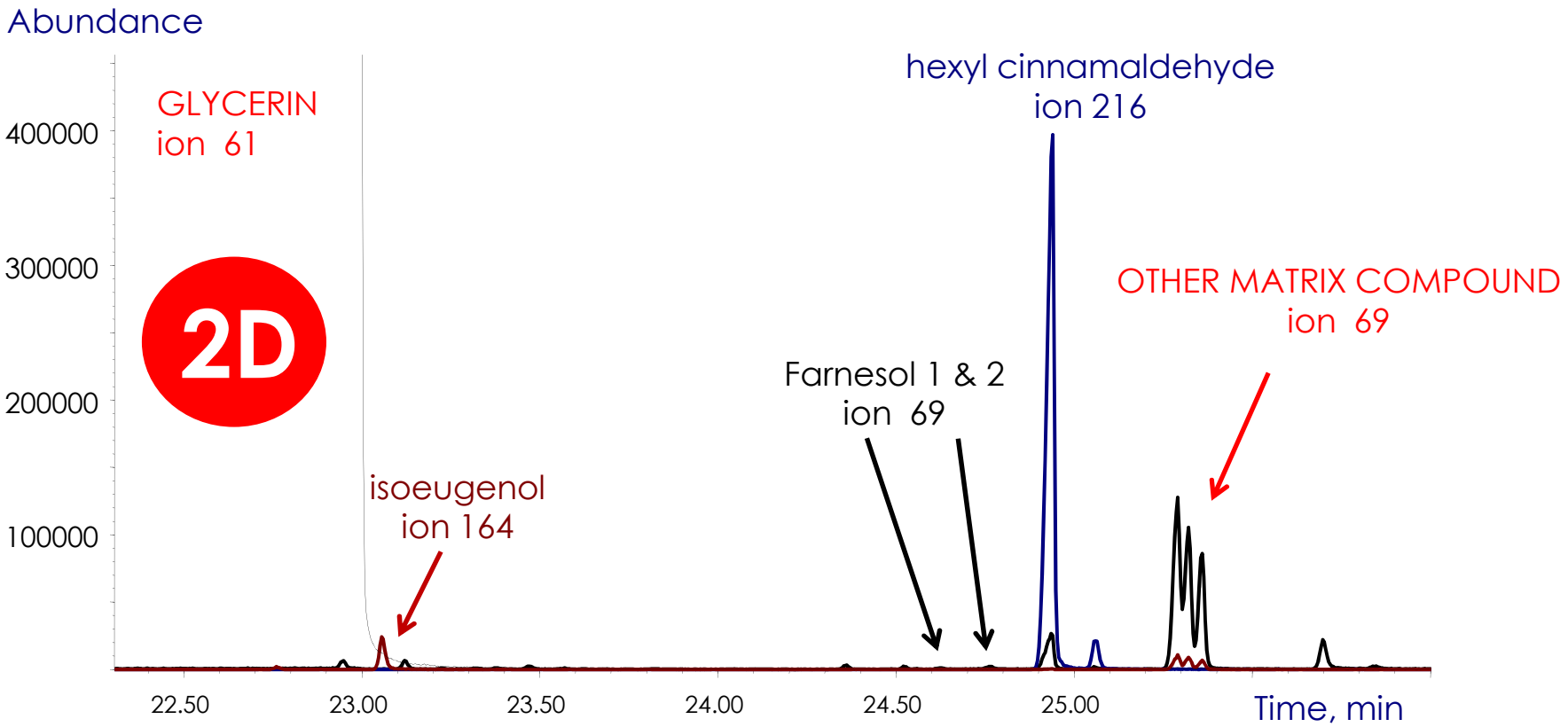
Sel. 1D/2D analysis of allergens in cosmetics

EIC (m/z 164, 69, 216)



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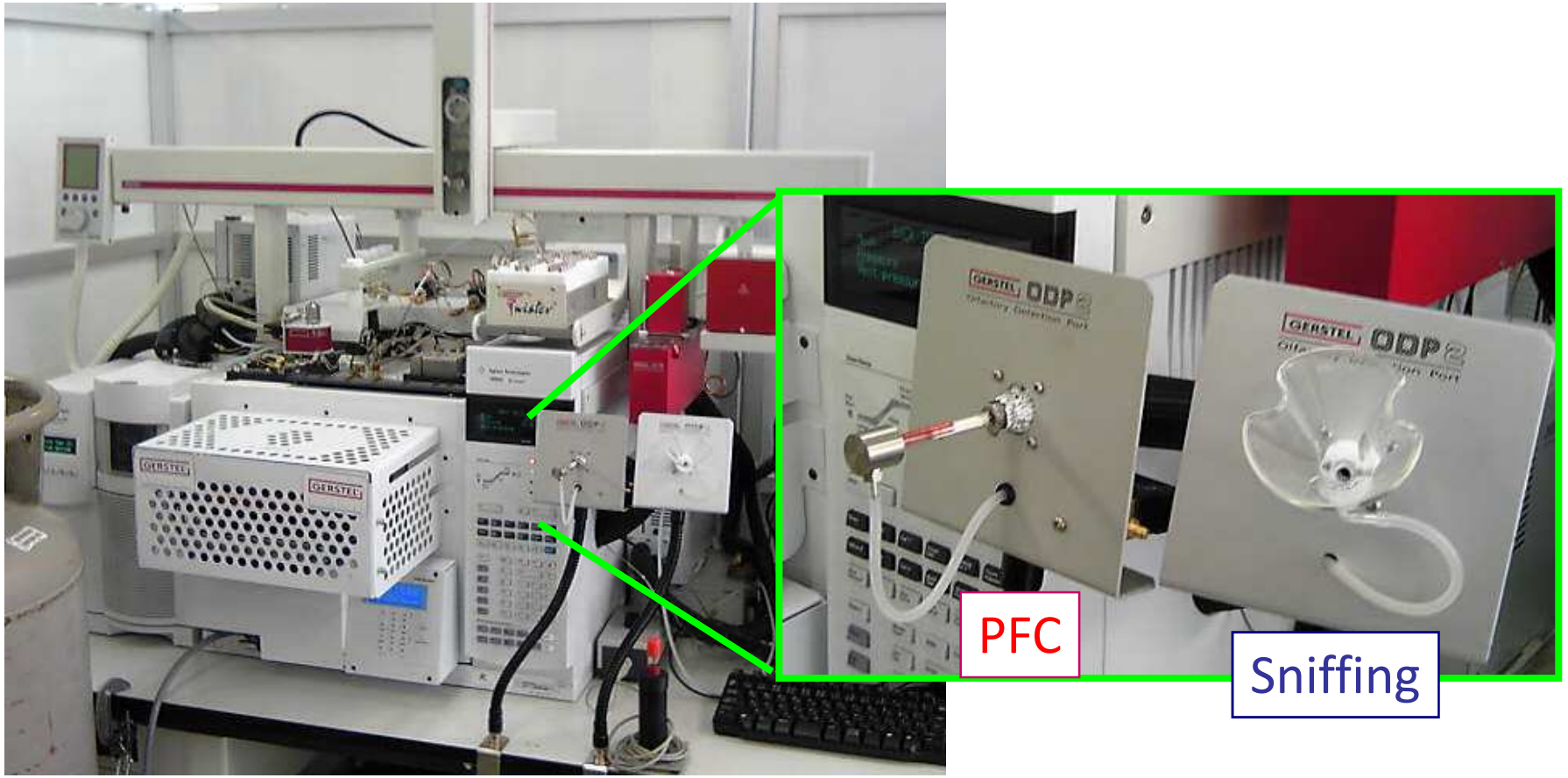
Selectable 1D/2D GC-O/MS with single PFC device



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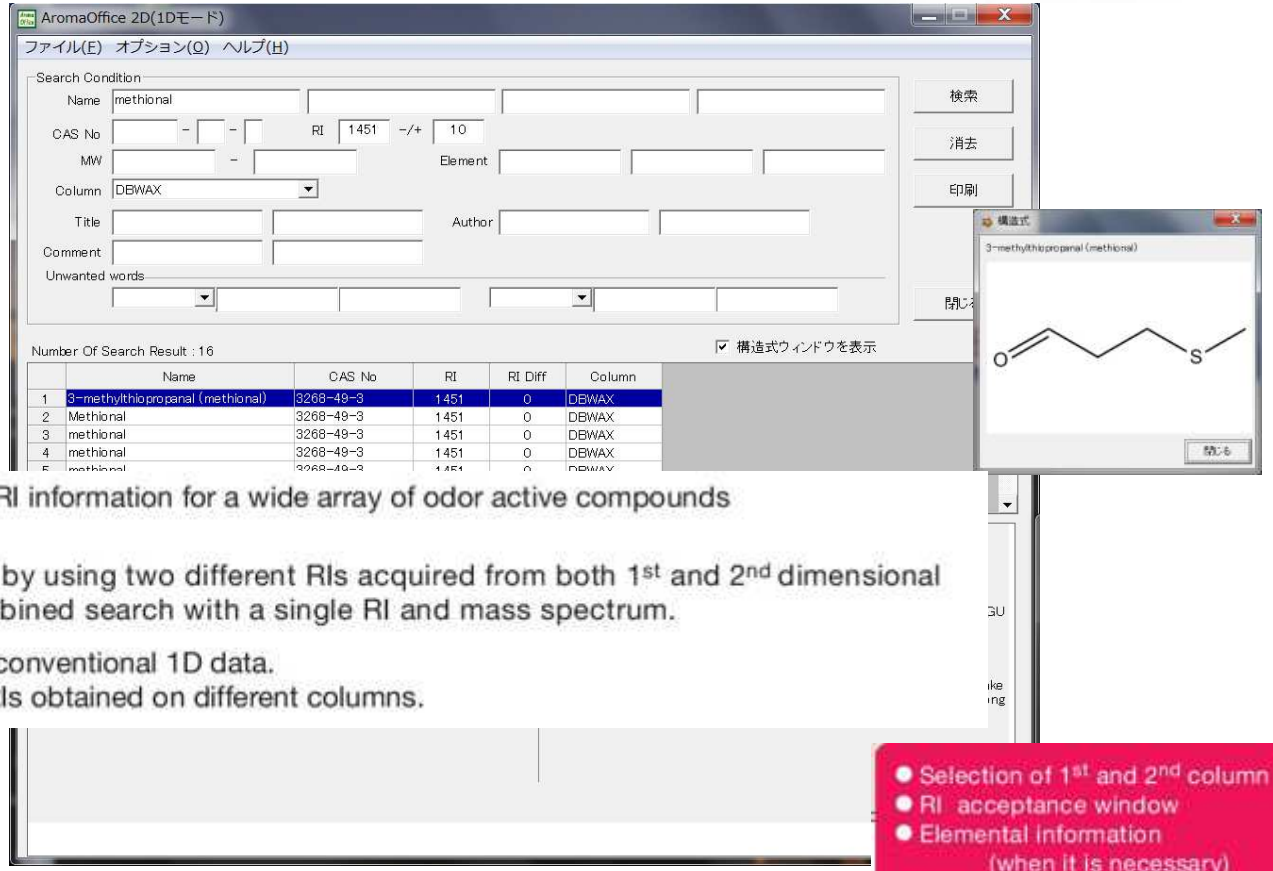
Advanced Flavor and Fragrance Analysis using Two-dimensional (2D) GC-MS

Aroma Office 2D Ver. 1.0

Flavor and Fragrance Compound Database for 2D GC-MS



- The searchable database contains RI information for a wide array of odor active compounds from many literature references.
- A cross search can be performed by using two different RIs acquired from both 1st and 2nd dimensional chromatograms, as well as a combined search with a single RI and mass spectrum.
- An RI search can be performed on conventional 1D data.
  A cross search can be used for 2 RIs obtained on different columns.

- Selection of 1st and 2nd column
- RI acceptance window
- Elemental information (when it is necessary)

Library Search Report

DataFile	C:\msdchem\1\data\DEMO_Aroma Office\DBWAX_Floralys5%.D								
PeakNo	RT	RI	Area	Area%	Hit	Name	CASNo	Entry	Library
1)	5.614	1179	84198258	0.355	99	D-Limonene	005989-27-5	15682	NIST11.L
					91	Limonene	000138-86-3	15667	NIST11.L
					76	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	005989-54-8	15879	NIST11.L
2)	11.239	1442	1.05E+08	0.442	83	7-Octen-2-ol, 2,6-dimethyl-	018479-58-8	28299	NIST11.L
					74	2-Octanol, 2-methyl-6-methylene-	018479-59-9	28320	NIST11.L
3)	12.814	1514	2.59E+08	1.093	97	1,6-Octadien-3-ol, 3,7-dimethyl-	000078-70-6	26774	NIST11.L
4)	16.448	1688	76859118	0.324	99	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.al	003691-11-0	64530	NIST11.L
					99	.alpha.-Bulnesene	1000374-19-9	64321	NIST11.L
					83	.alpha.-Guaiene	003691-12-1	64292	NIST11.L
5)	17.225	1727	5.52E+08	2.327	96	Citronellol	000106-22-9	28222	NIST11.L
					96	6-Octen-1-ol, 3,7-dimethyl-, (R)-	001117-61-9	28325	NIST11.L
					70	2-Octen-1-ol, 3,7-dimethyl-	040607-48-5	28296	NIST11.L
8)	17.6	1747	1.04E+08	0.44	80	Nopyl acetate	000128-51-8	67677	NIST11.L
					64	Bicyclo[4.1.0]hept-3-ene, 7,7-dimethyl-3-vinyl-	113003-13-7	22846	NIST11.L
7)	18.704	1803	3.77E+08	1.587	94	Geraniol	000106-24-1	26608	NIST11.L
					72	Geranyl vinyl ether	1000132-11-4	45595	NIST11.L
					72	2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (E)-	000105-86-2	47027	NIST11.L
8)	19.52	1846	6.32E+08	2.663	94	Phenylethyl Alcohol	000060-12-8	9920	NIST11.L
					70	Spiro[2,4]hepta-4,6-diene	000765-46-8	2472	NIST11.L

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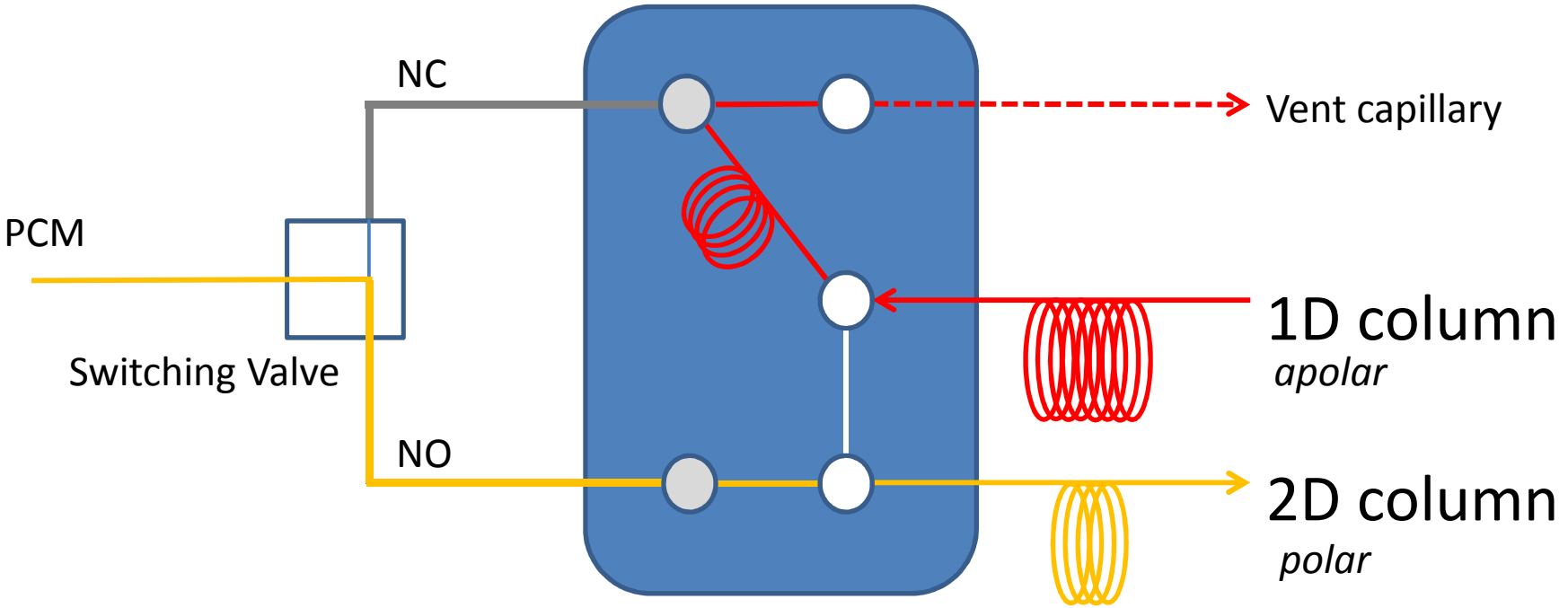
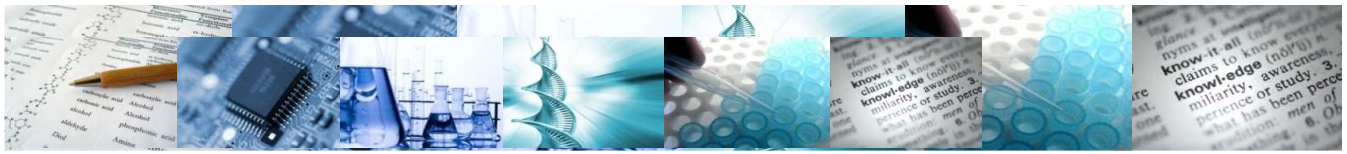
Comprehensive GCxGC
Flow Modulator





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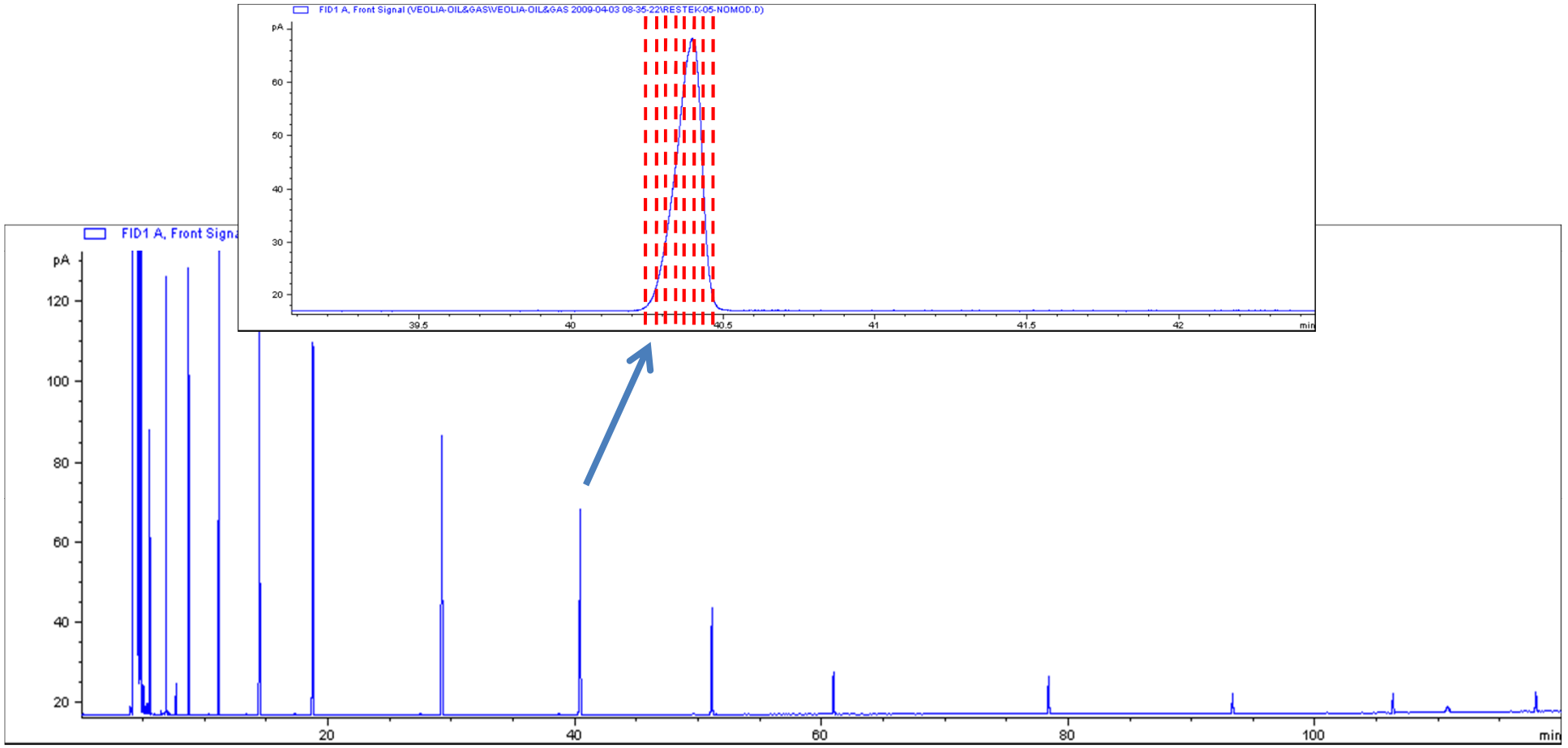
1D analysis (ASTM HC mix)



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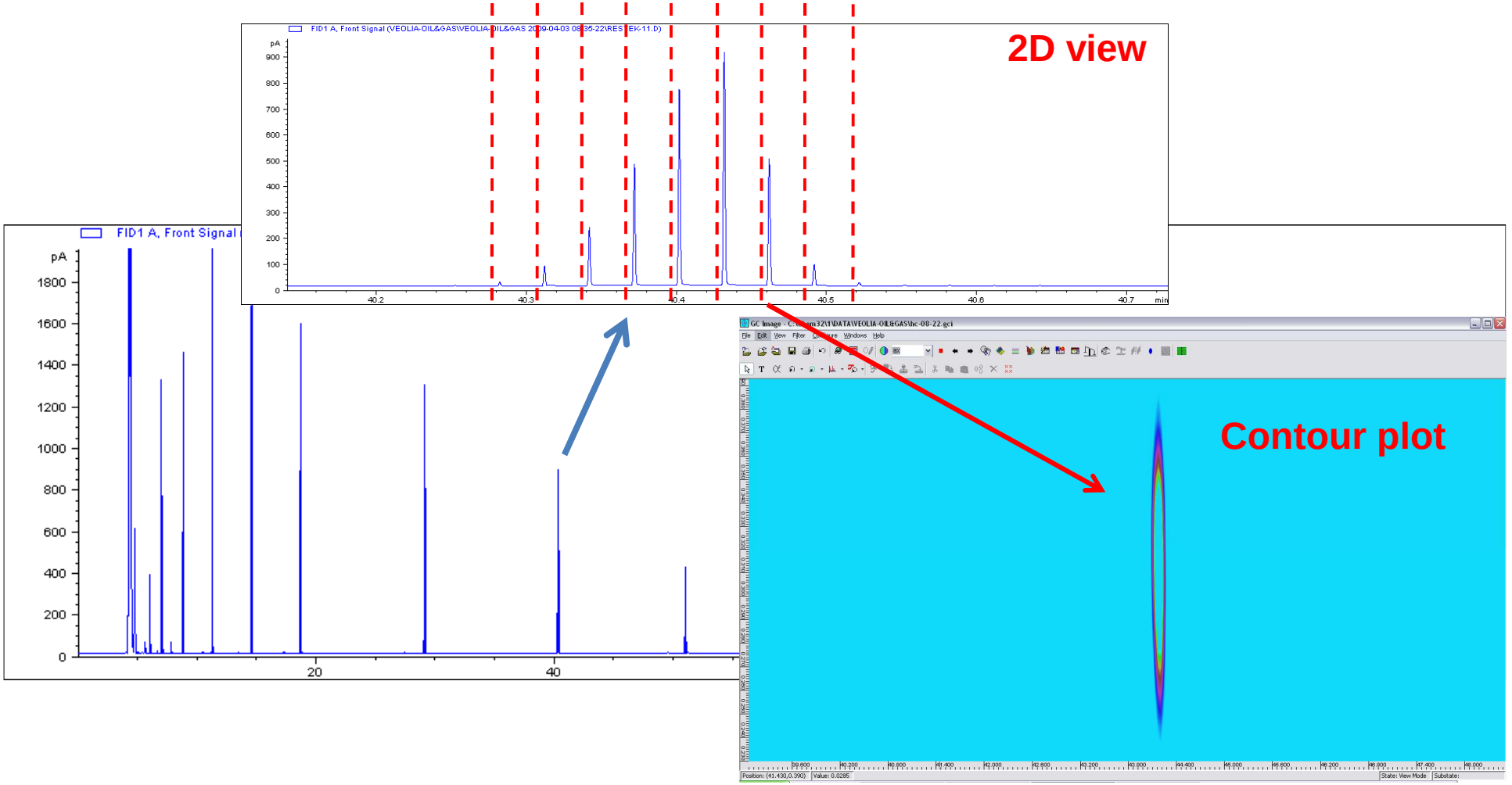
GCxGC modulated signal



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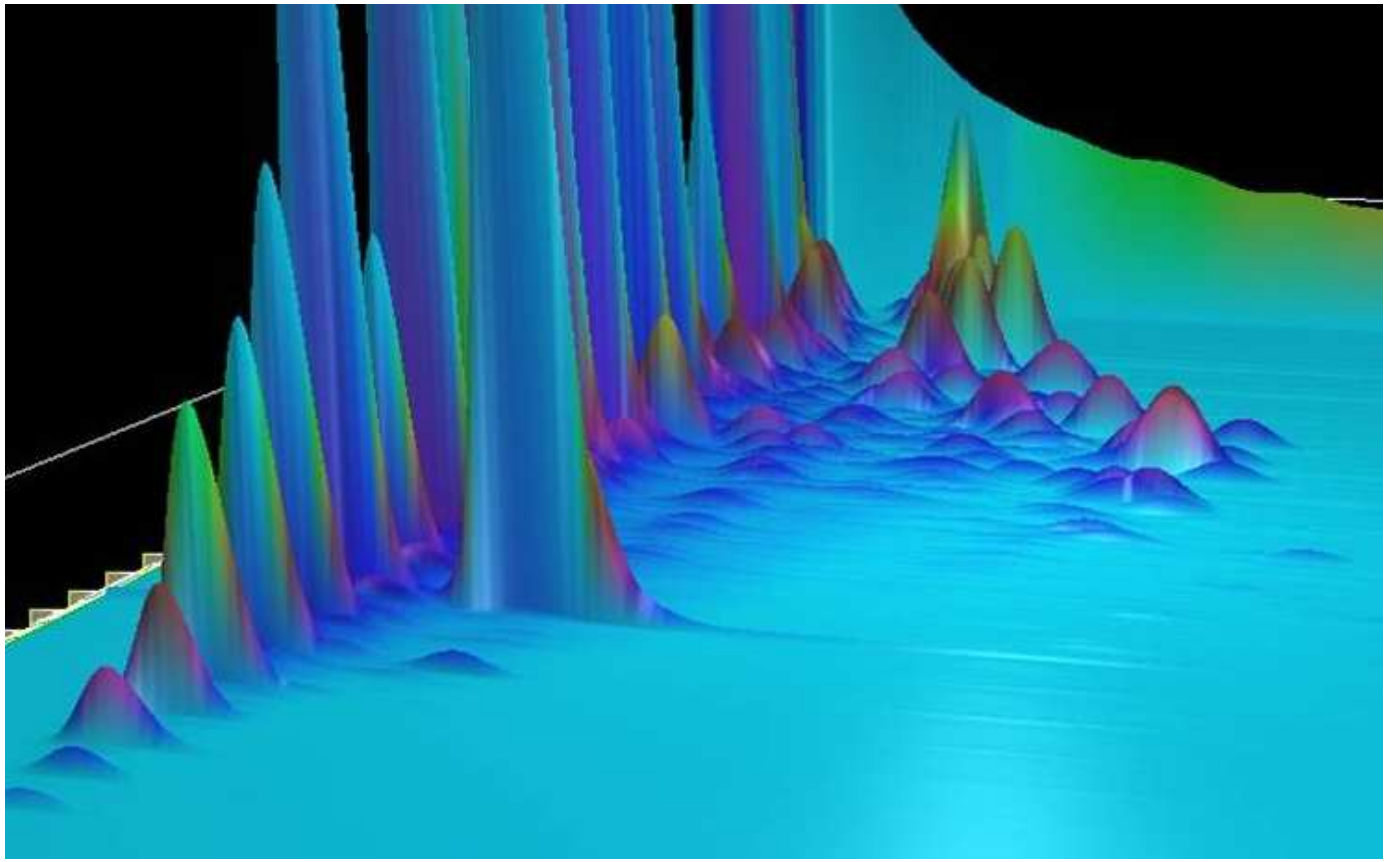
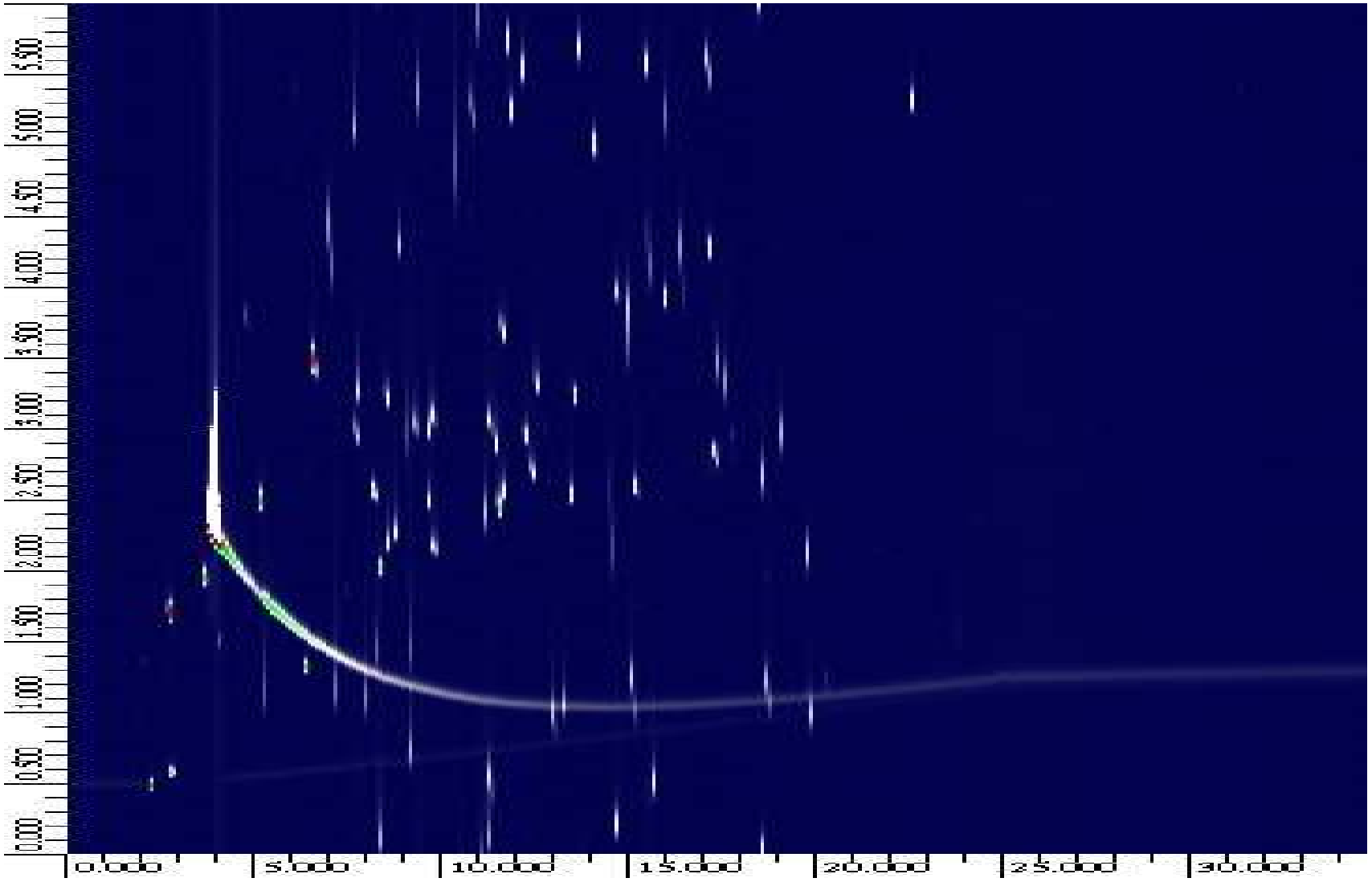
3D view



GCxGC: Perfume - good quality



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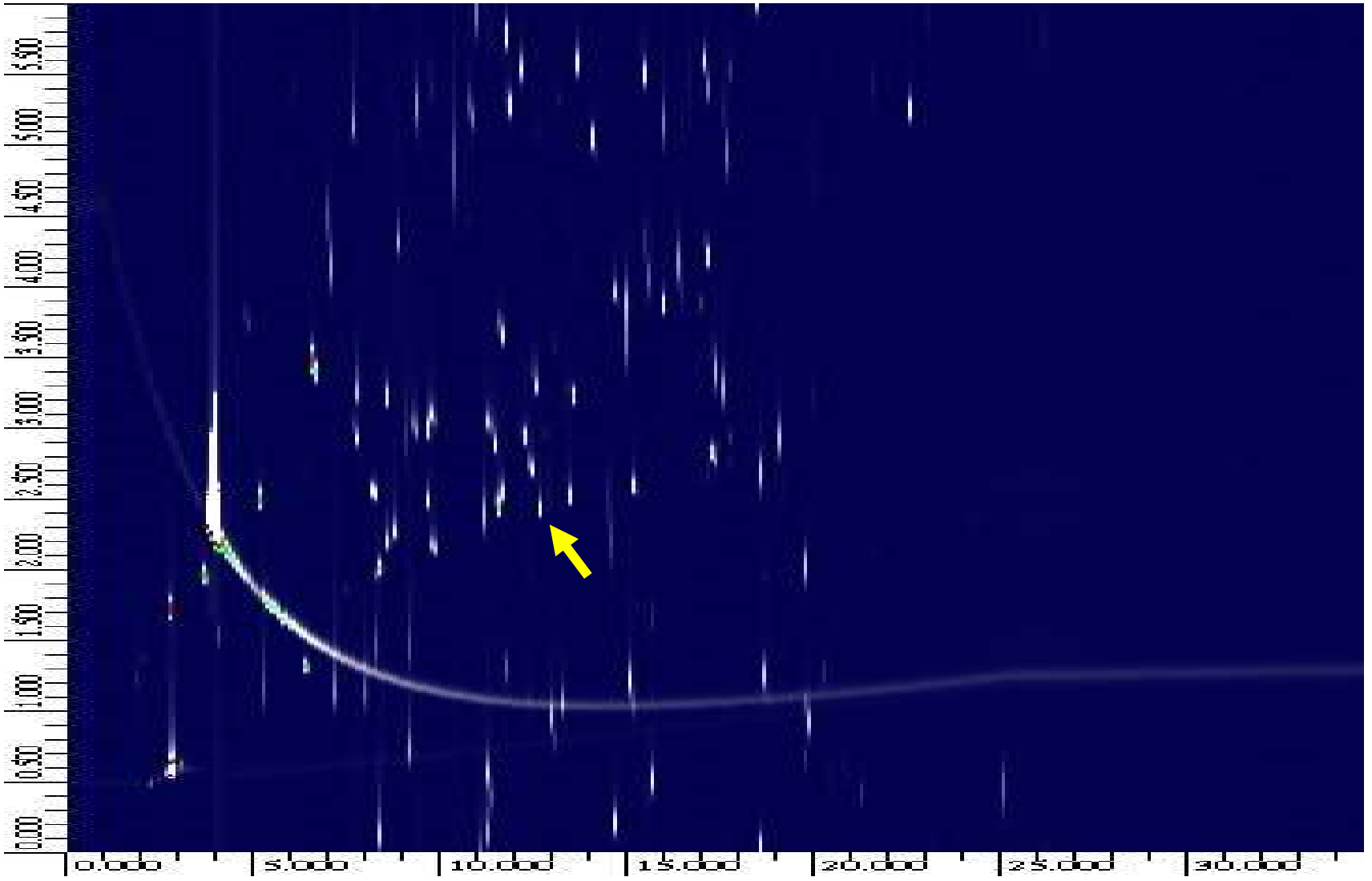
GCxGC: Perfume with defect



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3. Detection

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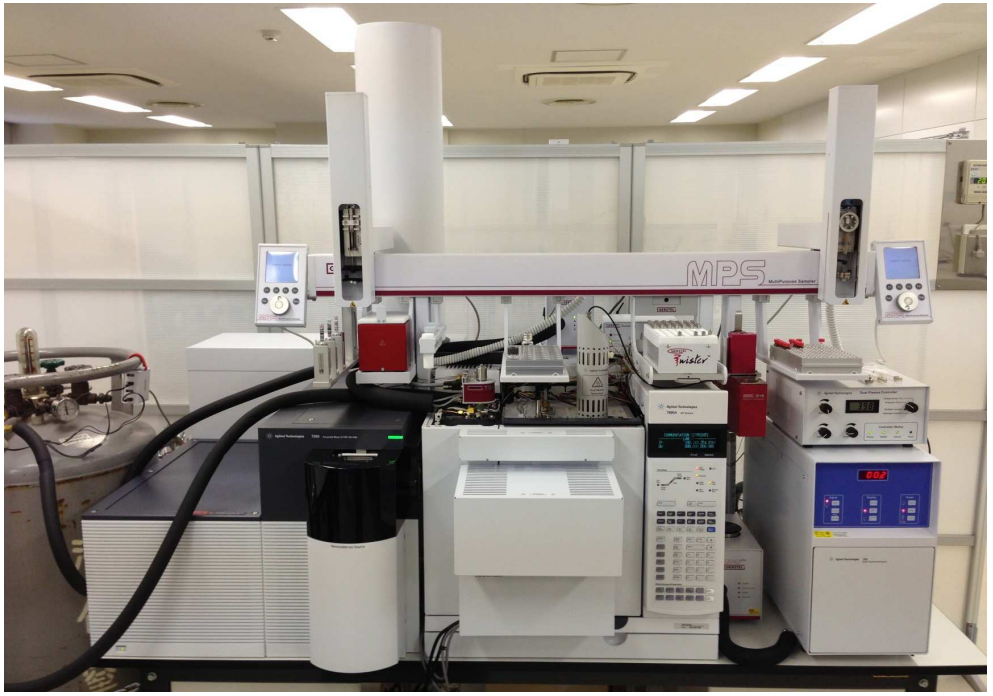
Detection: Unleash the power of MS

– High sensitivity
– Accurate mass (TOF)
– EI, CI, APCI, SMB
– MS/MS



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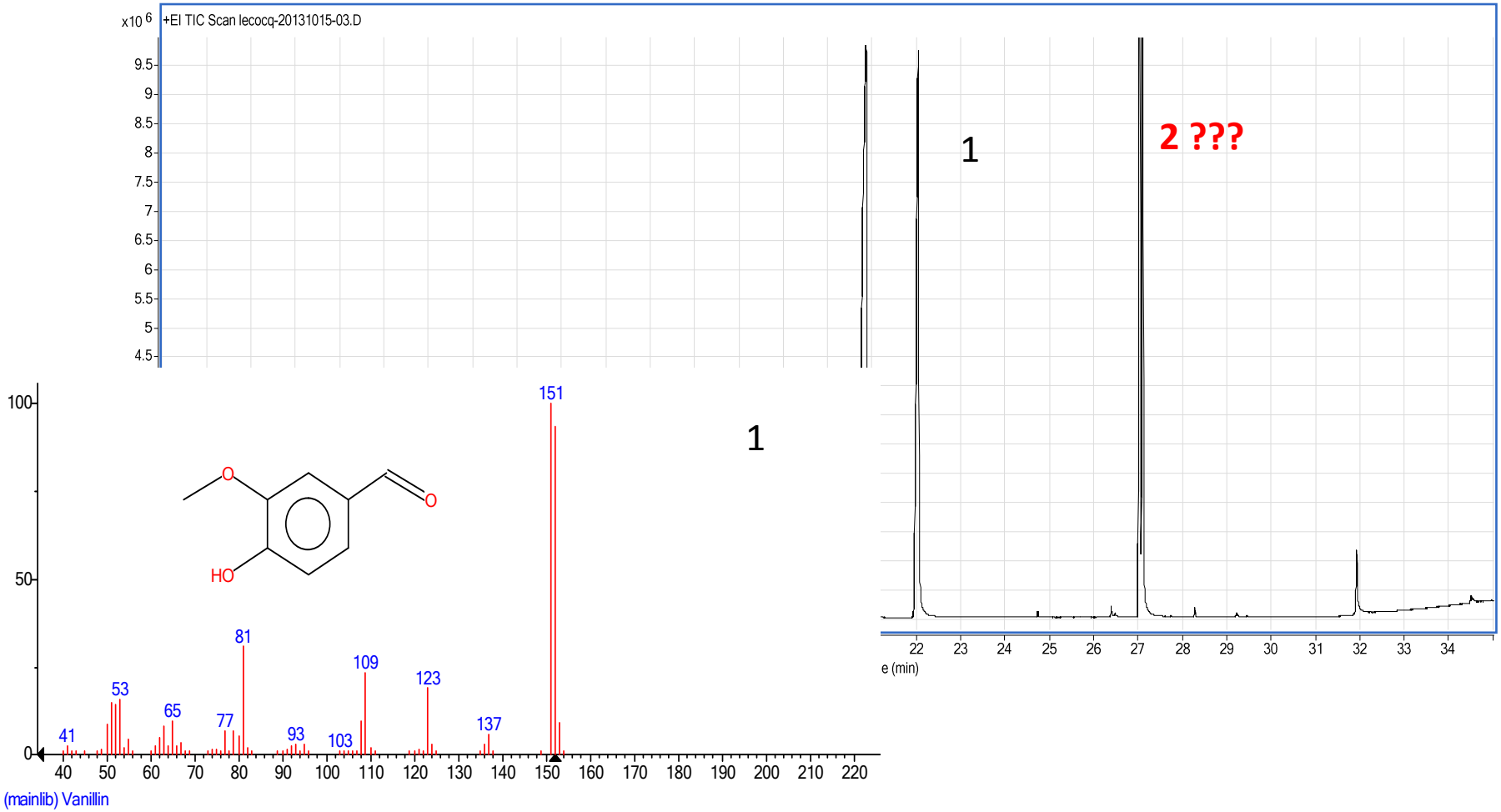
GC-Q-TOF: high resolution, high sensitivity



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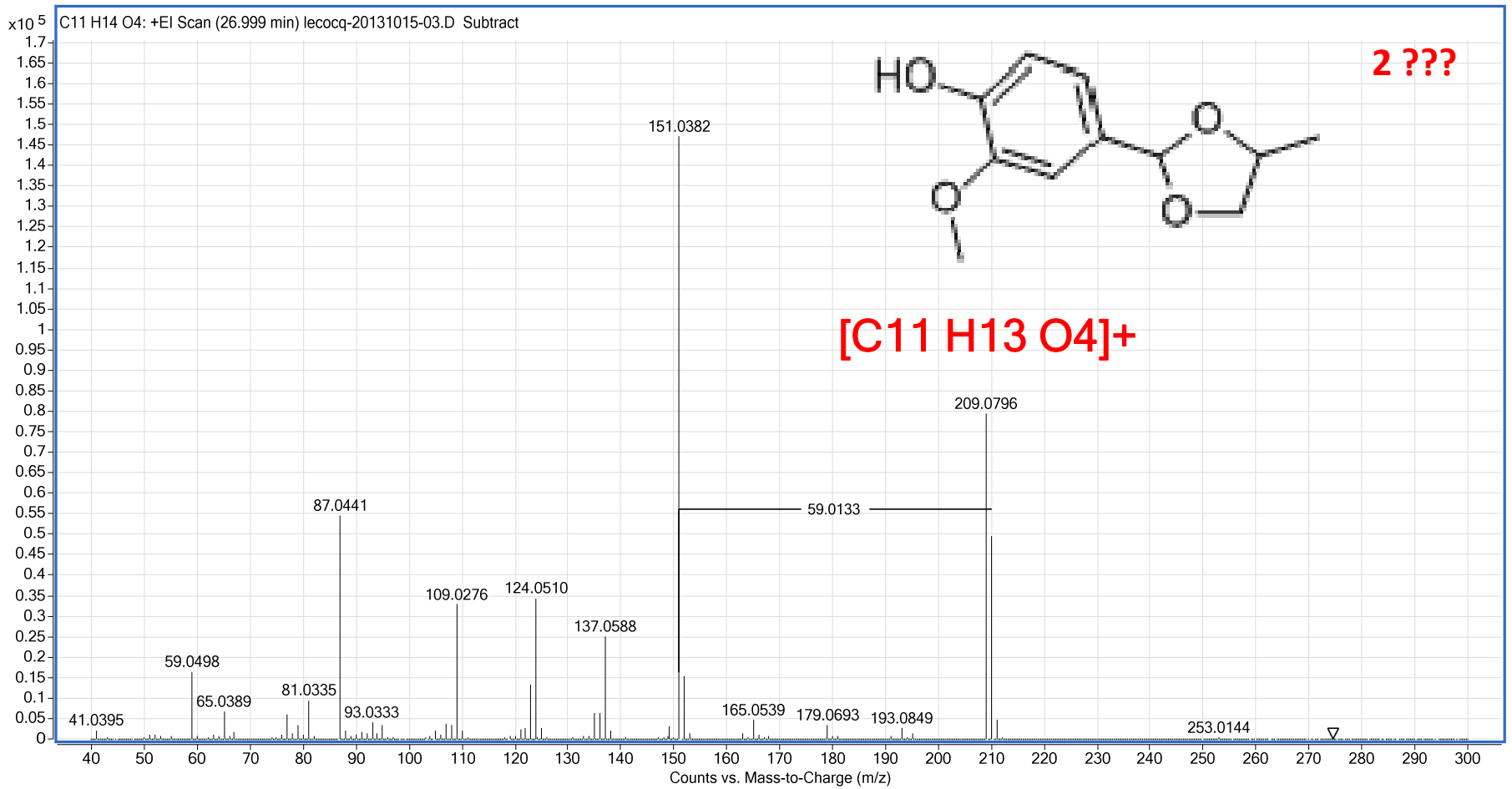
GC-Q-TOF: high resolution, high sensitivity



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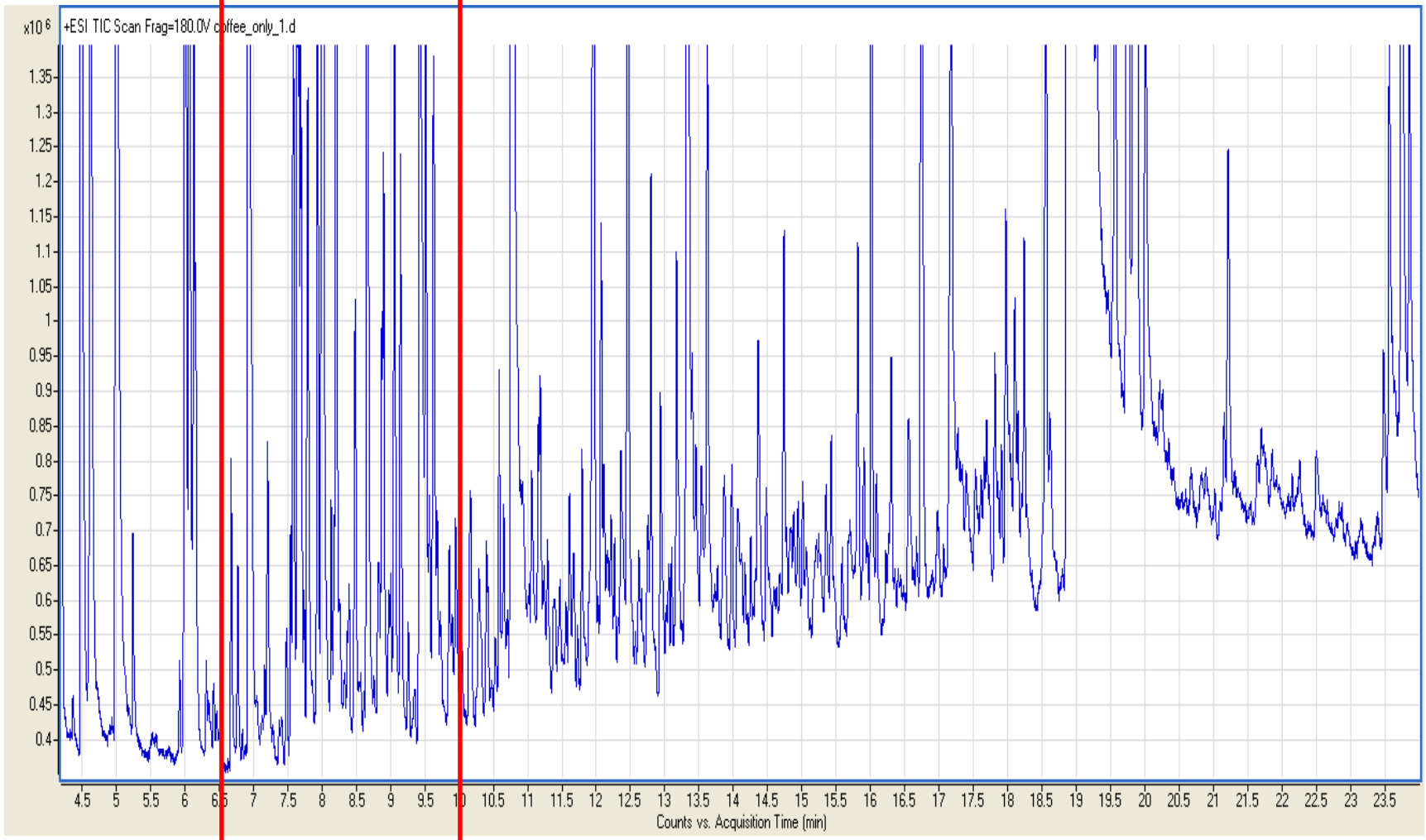
GC-Q-TOF: high resolution, high sensitivity

TIC of coffee extract (non-spike) (data from Gerstel KK)



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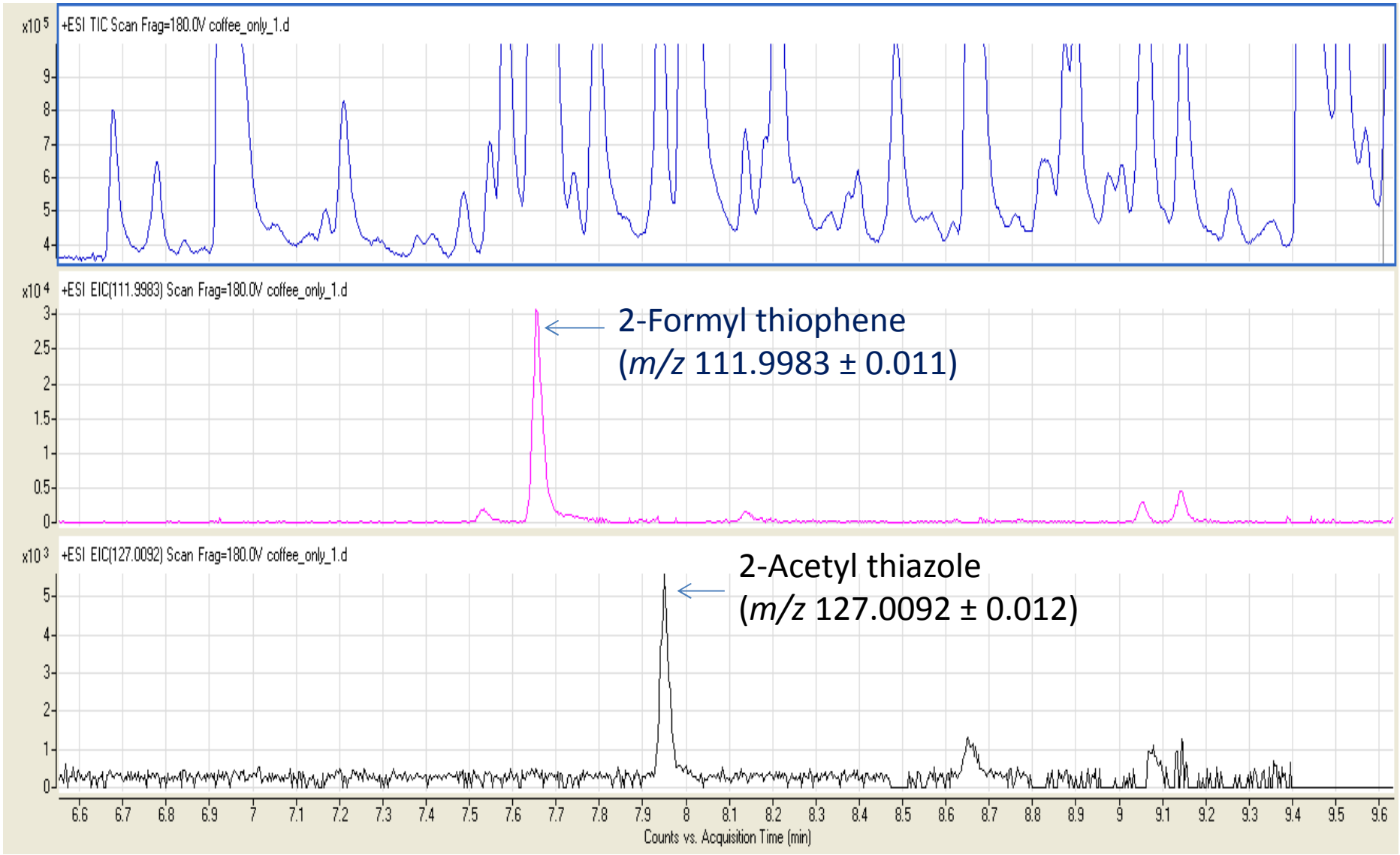
TIC and mass chromatograms of coffee extract (non-spike)



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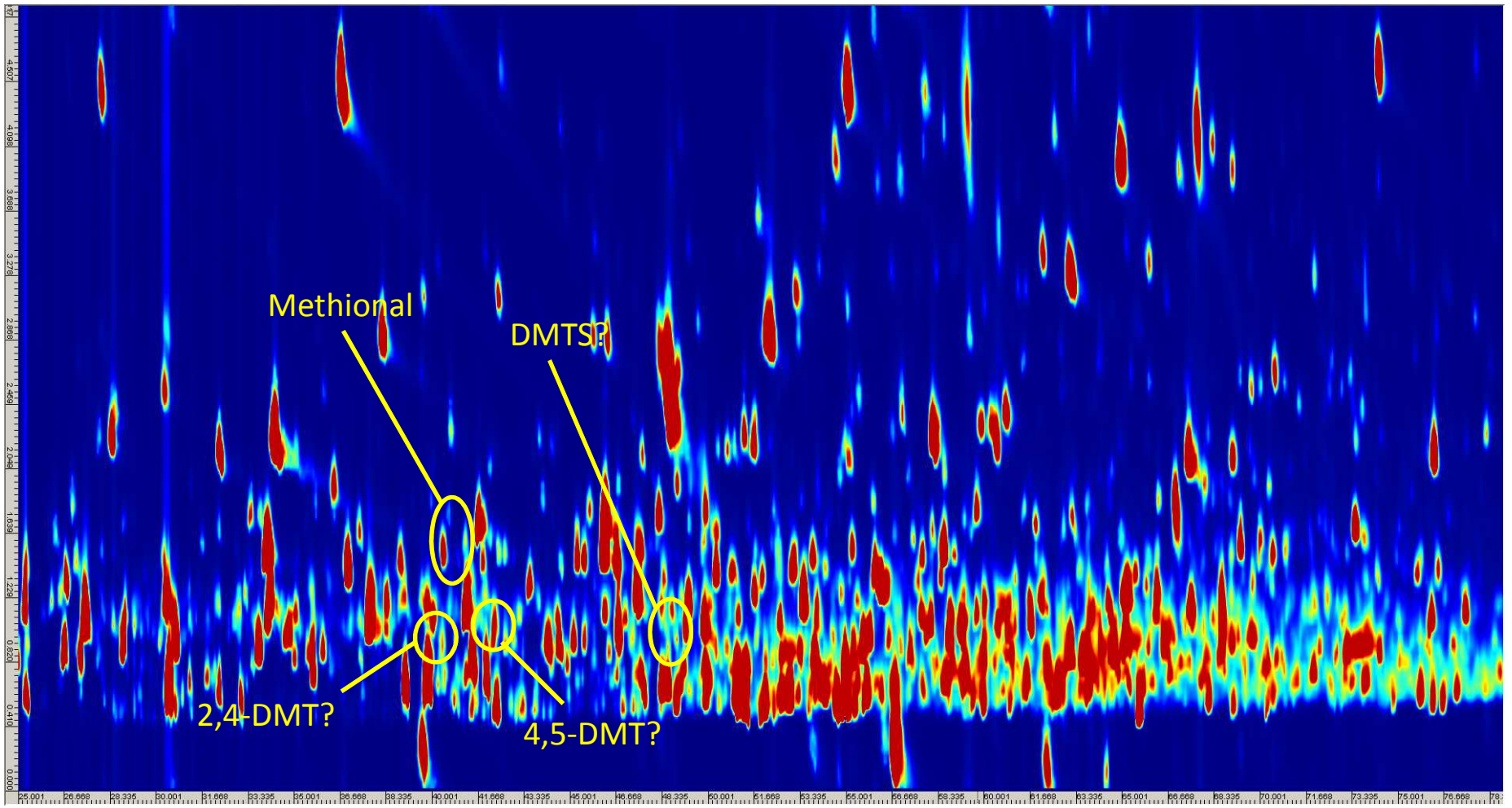
GCxGC-QTOF-MS



Tobacco smoke

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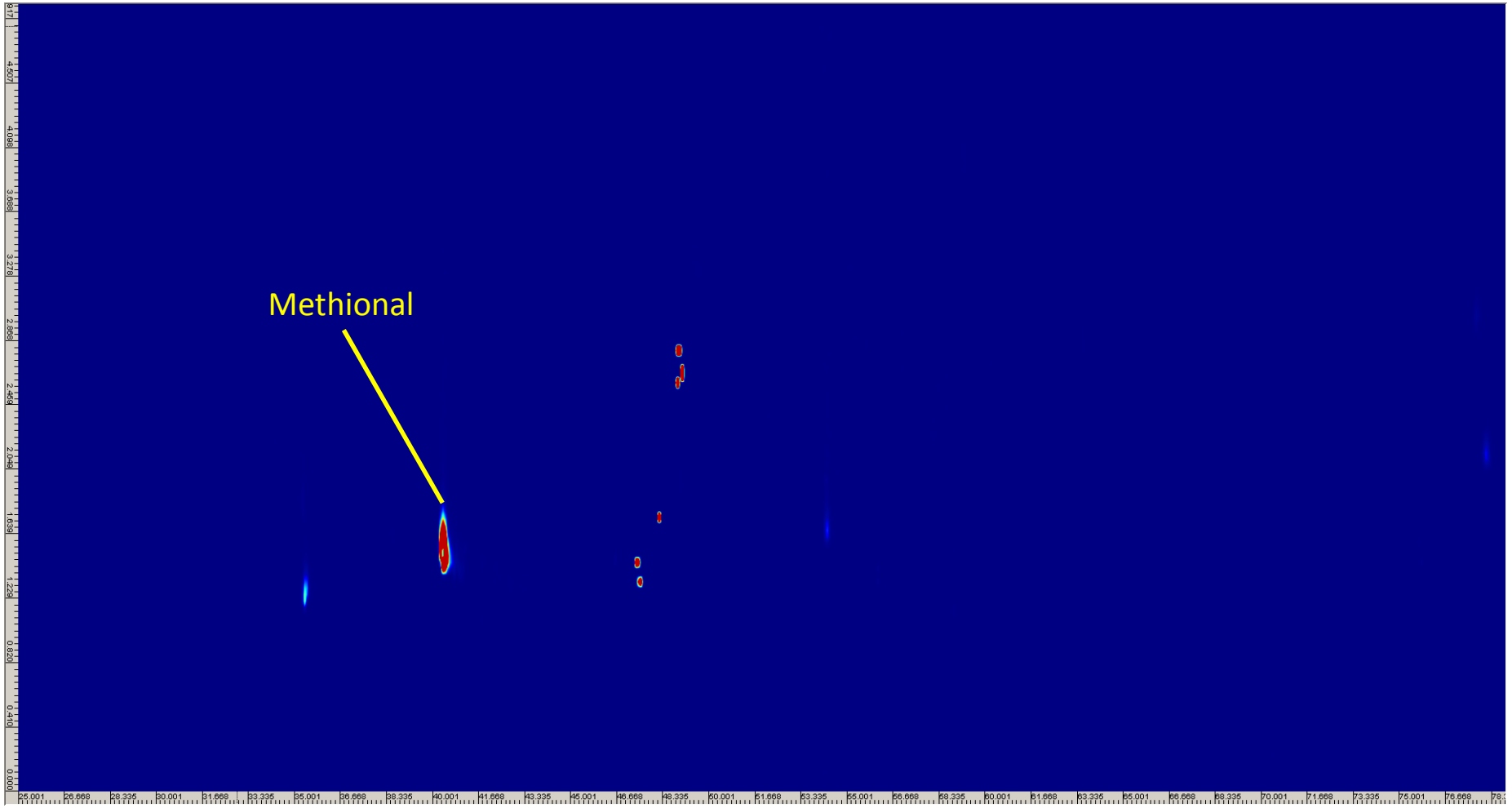
^{2}D mass chromatogram (*m/z* 104.0290±50 mDa)



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Now we have: FEDHS-GC-GC-QTOF-MS and FEDHS-GCxGC-QTOFMS

What about Odor?

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SHS-E-nose

Objective approach

1 g, SHS @ 80°C (20 min)
Injection 2 mL
Alpha MOS Enose Fox 4000
Synthetic dry air: 150 mL/min
Acquisition time: 500 s
Acquisition period: 0.5 s

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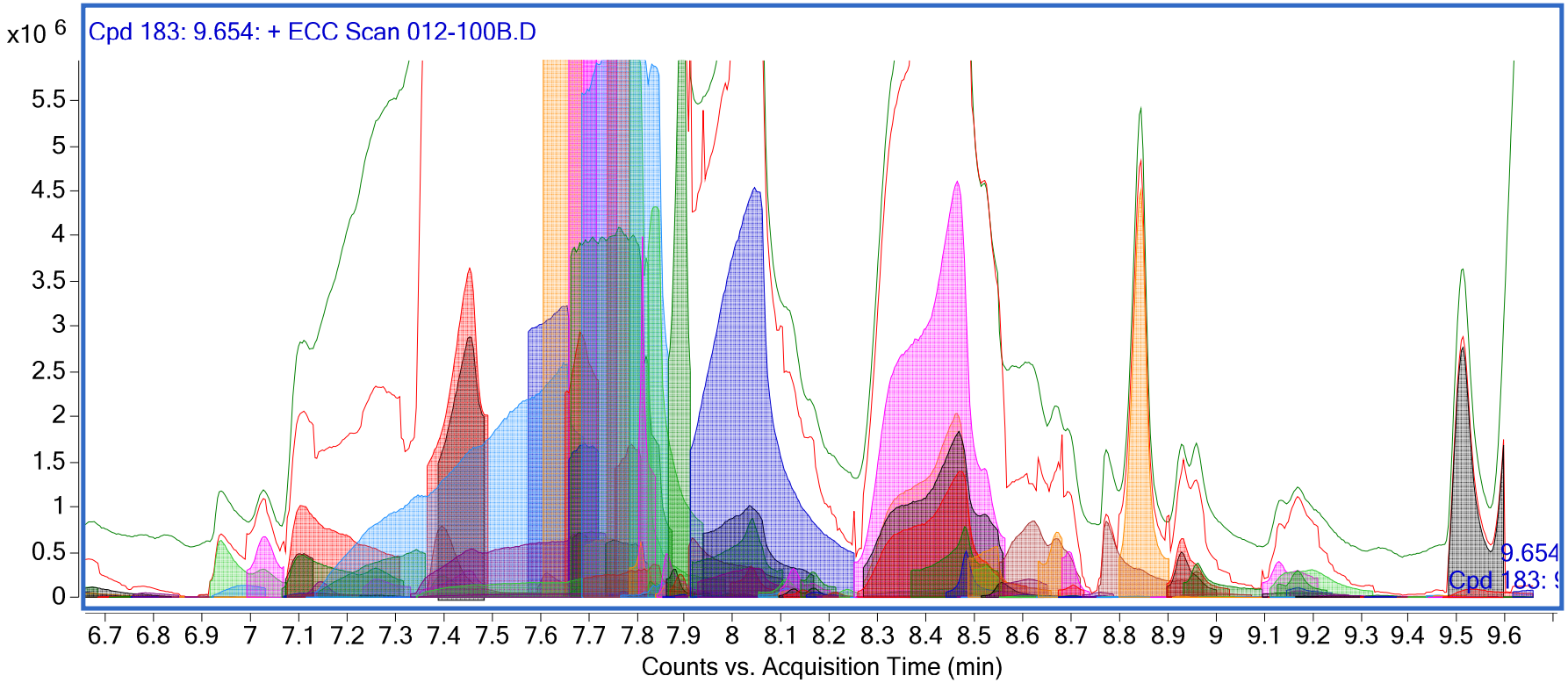
GC-MS Deconvolution



- 183 compounds in the ‘odour’ zone
- Concentration range 5 orders of magnitude

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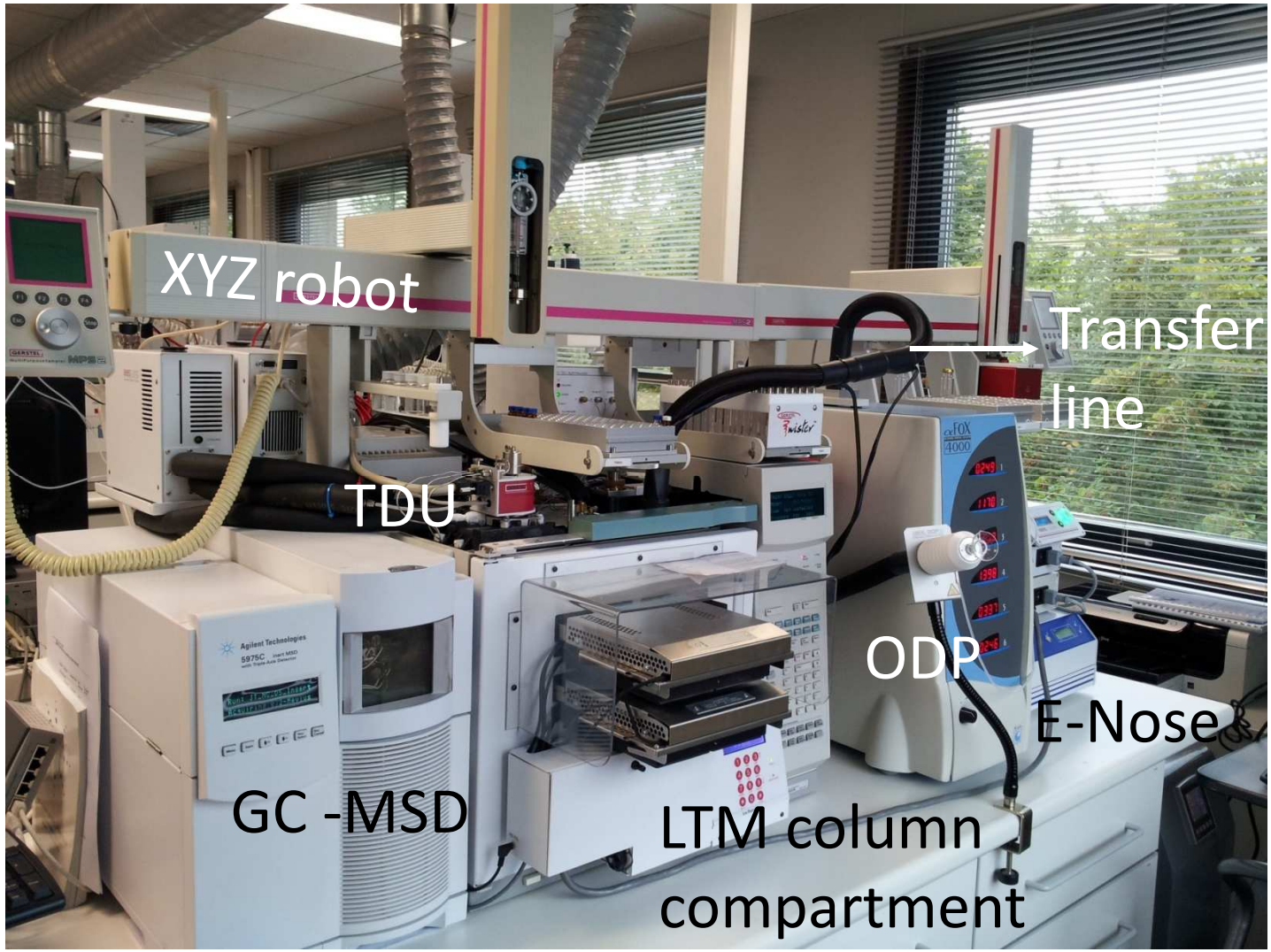
New GC-E-nose Configuration



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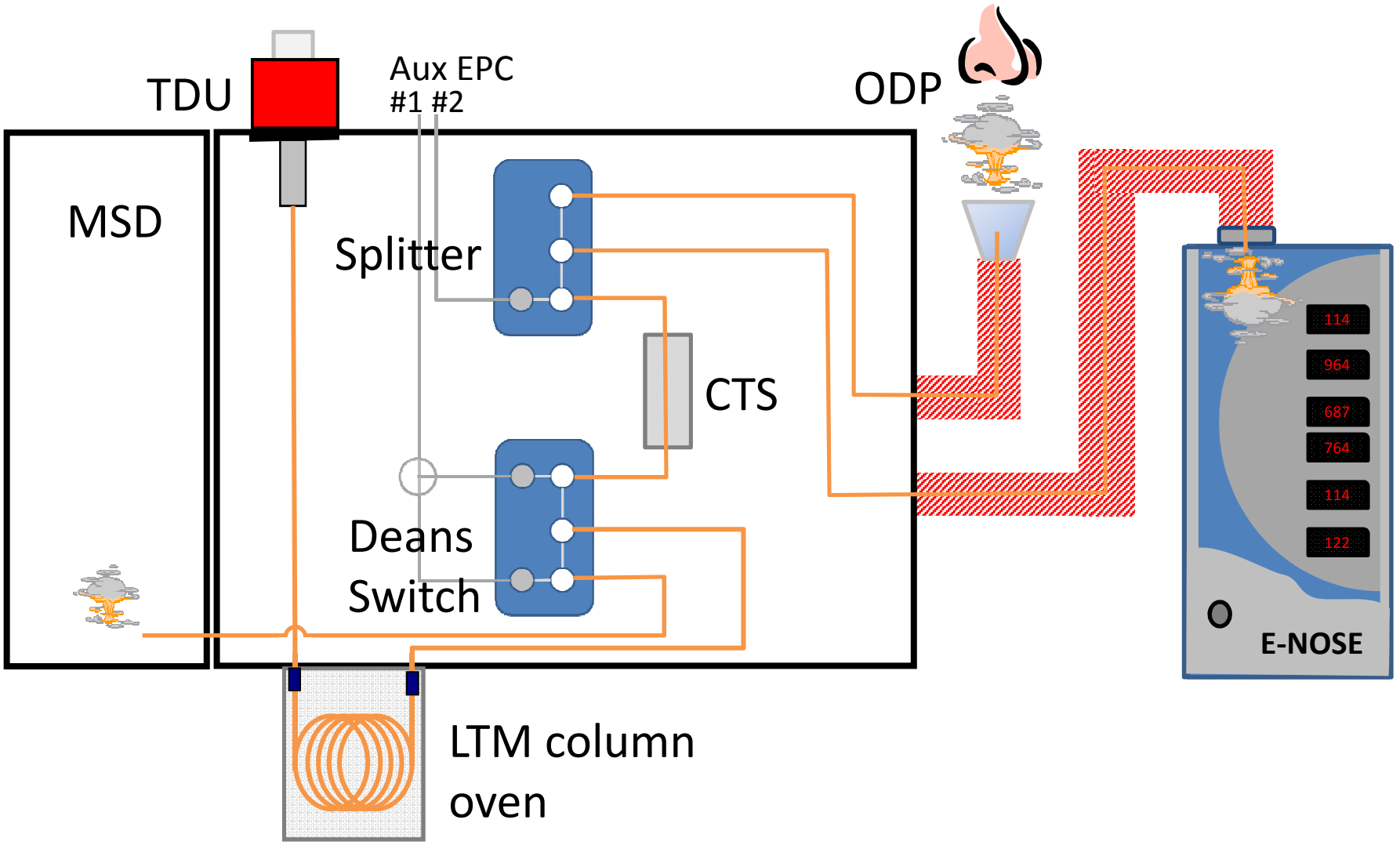
Configuration



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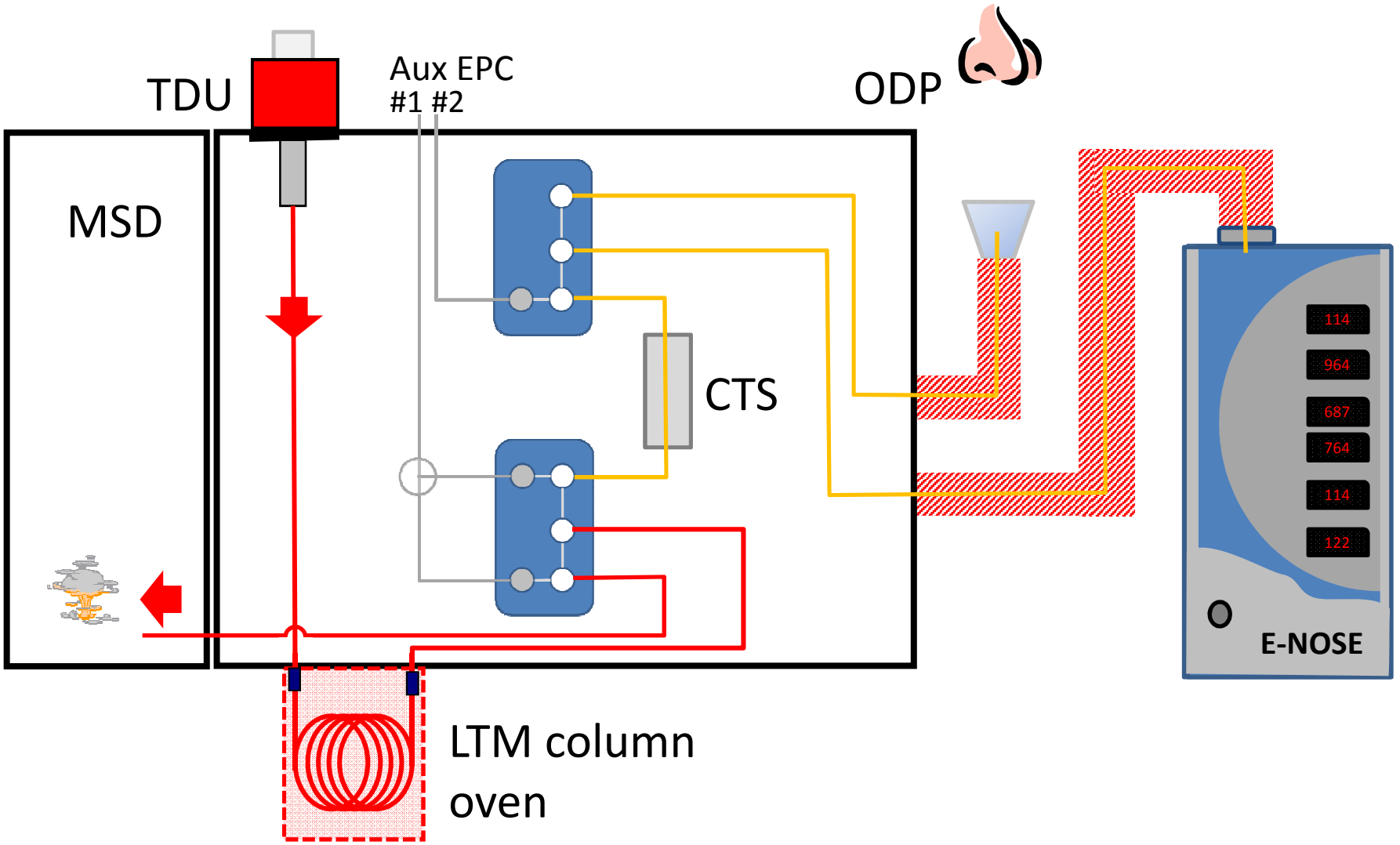
Step 1. GC-MS Analysis



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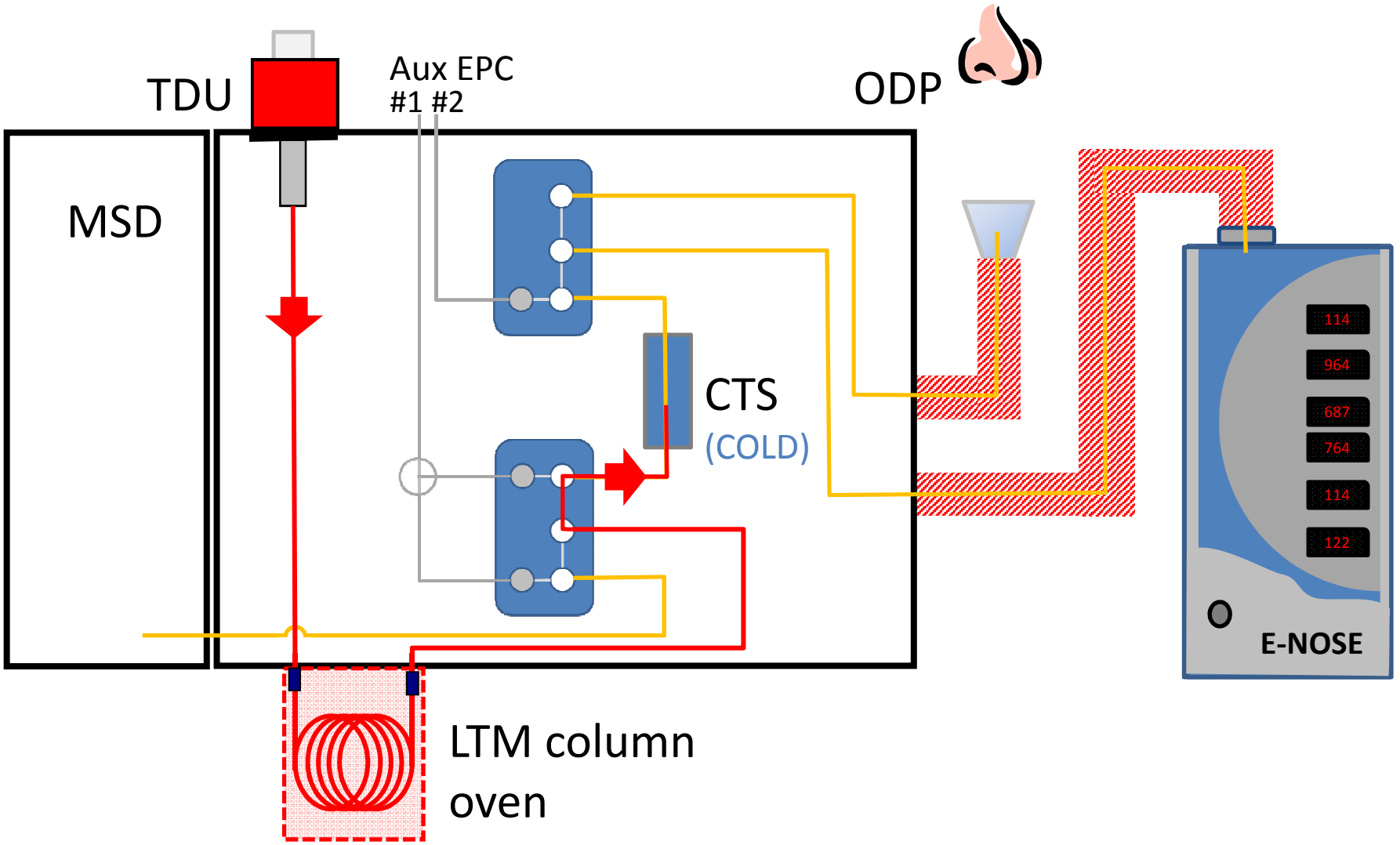
Step 2. Collection of Fractions (heart-cut)



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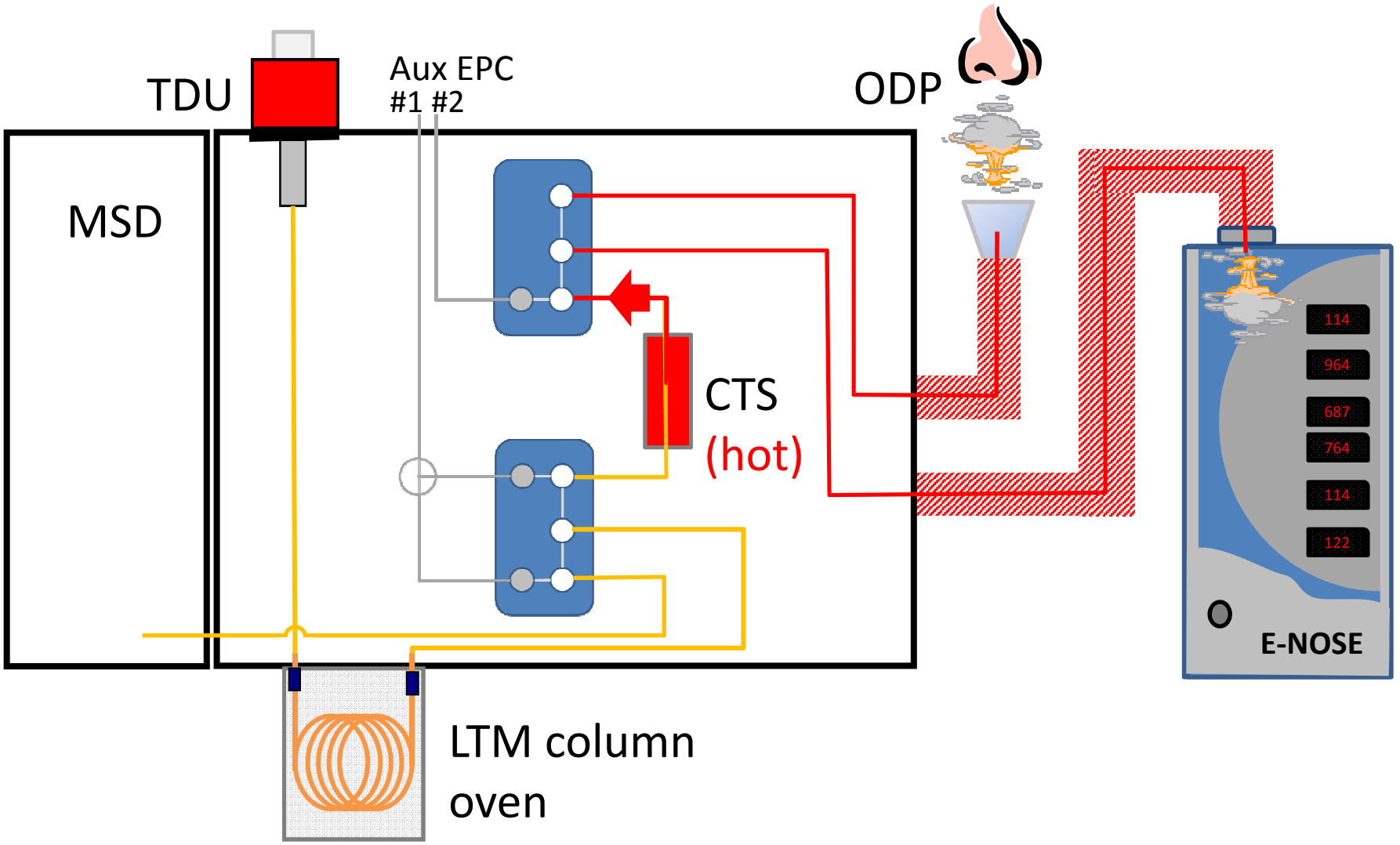
Step 3. Transfer of Fractions to Noses



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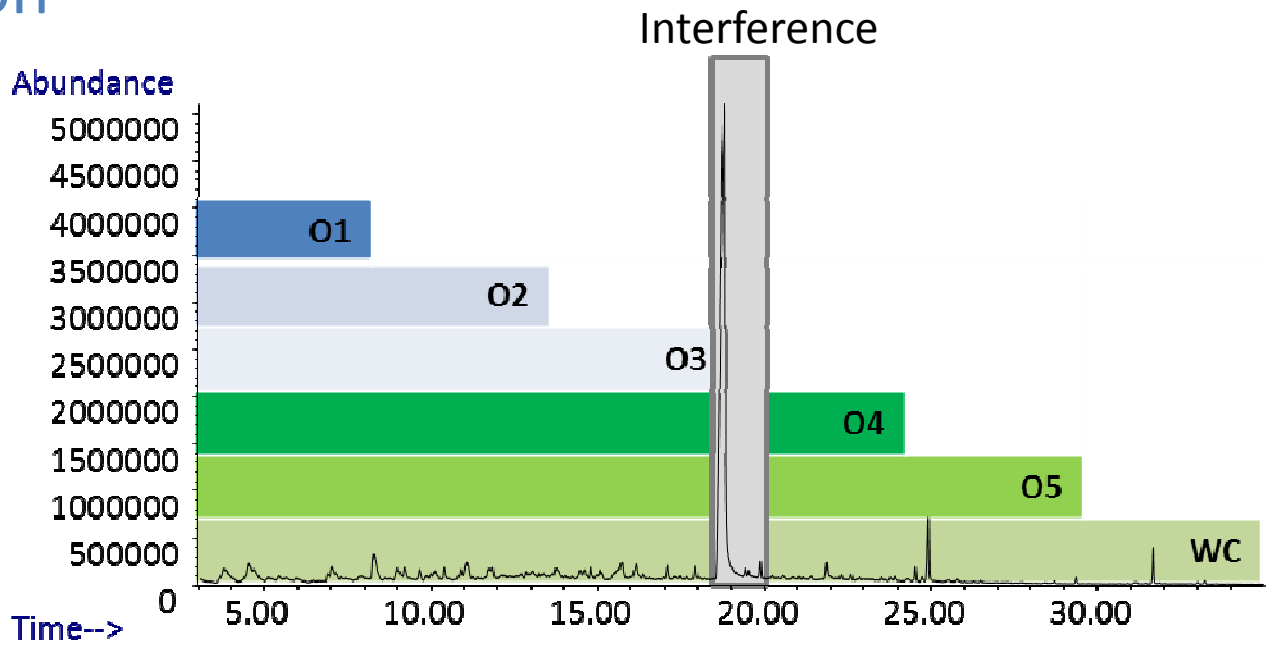
2 Operation modes

1. Omission



2. Addition

- Perceptual interaction (heart-cut)
- Recombination

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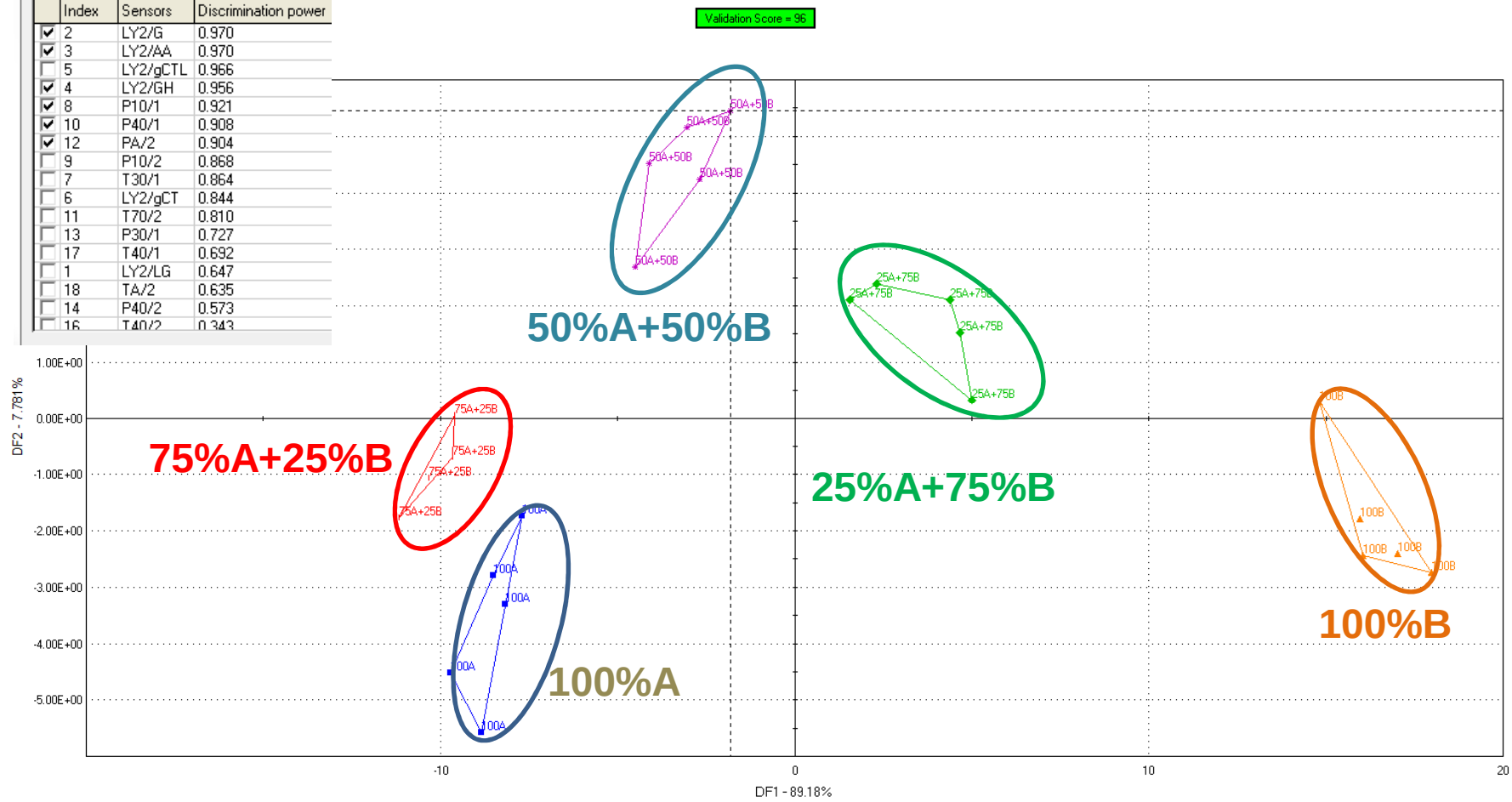
Discriminant Function Analysis (DFA) for ∑(HC)

6 Sensors

Sensor selection

	Index	Sensors	Discrimination power
☑	2	LY2/G	0.970
☑	3	LY2/AA	0.970
☐	5	LY2/gCTL	0.966
☑	4	LY2/GH	0.956
☑	8	P10/1	0.921
☑	10	P40/1	0.908
☑	12	PA/2	0.904
☐	9	P10/2	0.868
☐	7	T30/1	0.864
☐	6	LY2/gCT	0.844
☐	11	T70/2	0.810
☐	13	P30/1	0.727
☐	17	T40/1	0.692
☐	1	LY2/LG	0.647
☐	18	TA/2	0.635
☐	14	P40/2	0.573
☐	16	T40/2	0.343



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… omics?

Discovering the difference in your data

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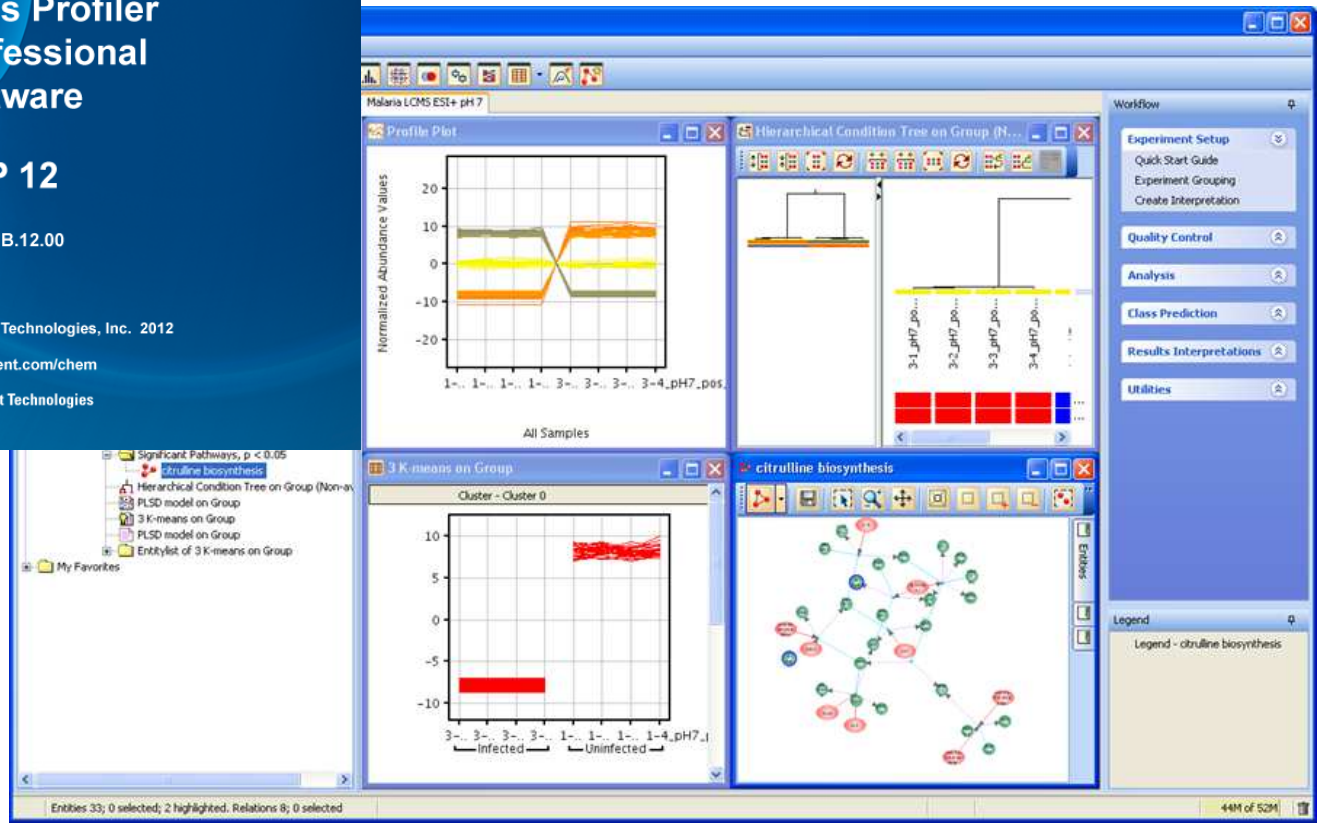
Mass Profiler Professional software



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Mass Profiler Professional software

- Analyze complex MS data (GC-MS, LC-MS, CE-MS, ICP-MS)
- Compare and classify sample groups
- Identify differences between samples
- *Untargeted*
- Application areas: proteomics, metabolomics, food safety, environmental, forensics, toxicology, …

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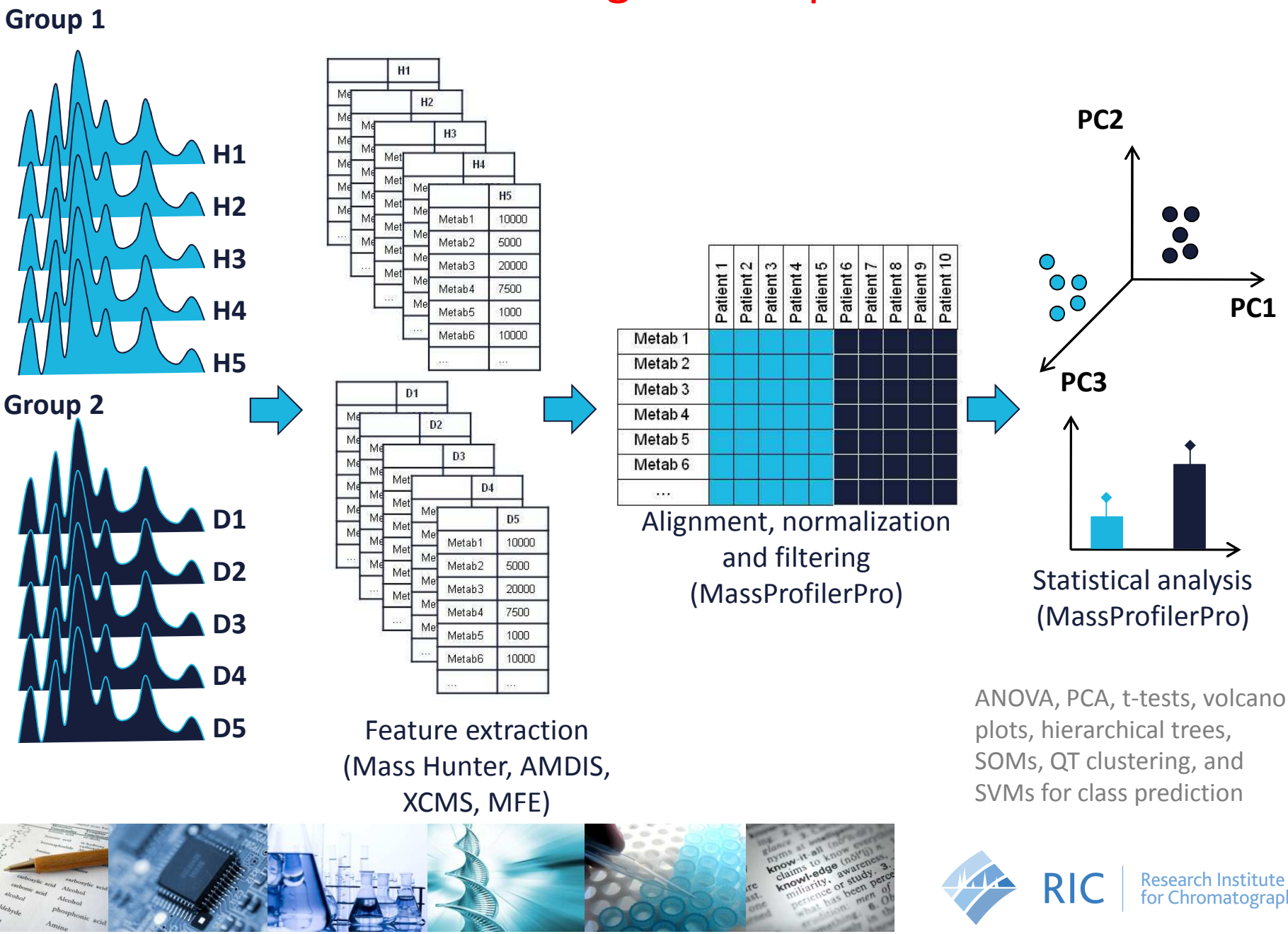
Workflow: from chromatograms to potential markers



ANOVA, PCA, t-tests, volcano plots, hierarchical trees, SOMs, QT clustering, and SVMs for class prediction

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Conclusions

- volatility range — DHS (LVDHS)
- Polarity range — SBSE + variation
- Complex samples — 2D-GC
- matrix interferences — 2D + MS

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