GC Q-TOF Analysis of Fluorinated Alkyl Compounds in Bio-solid Matrix

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Outline

Brief Review: The human Genome and exposome

Measuring the exposome

Analysis of Fluorotelomer alcohols (FTOH)

Summary

Q&A

GC Q-TOF analysis of fluorinated alkyl compounds in bio-solid matrix

THE HUMAN GENOME AND EXPOSOME

Sequencing and mapping

Elucidation of gene expression and protein function

Identification biochemical pathways implicated in chronic diseases

- Cancer, diabetes, vascular and neurodegenerative

Opportunities for improved treatment and patient management

- Tailored drug treatments and gene therapy

Wild, CP. Cancer Epidemiol Biomarkers Prev 2005;14:1847-1850

Application of genetic testing

Identify individuals at greatest risk for chronic diseases

New possibilities for disease prevention.

- Moves away from individual observation (clinical) towards population observation (epidemiology)
- Reduced morbidity and mortality
  - Utilizing public health measures

Our inheritance, our future: realising the potential of genetics in the NHS. Retrieved April 15, 2013 from, http://webarchive.nationalarchives.gov.uk/+/www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4006538

Exposure-Disease associations

Environmental exposures play an important role in many chronic diseases

Most exposure-disease associations remain ill defined

Genetic variants or in the human genome are of high prevalence but low penetrance

- Environmental exposures may contribute to population disease burden
- Questions if genetic knowledge has or will improve understanding of disease etiology at the population level

Vineis P, Schulte P, McMichael AJ. Lancet 2001;357:709 – 12

Peto J. Nature 2001;411:390 – 5

Cappuccio FP. Int J Epidemiol 2004;33:387 – 8

The Exposome and Exposomics

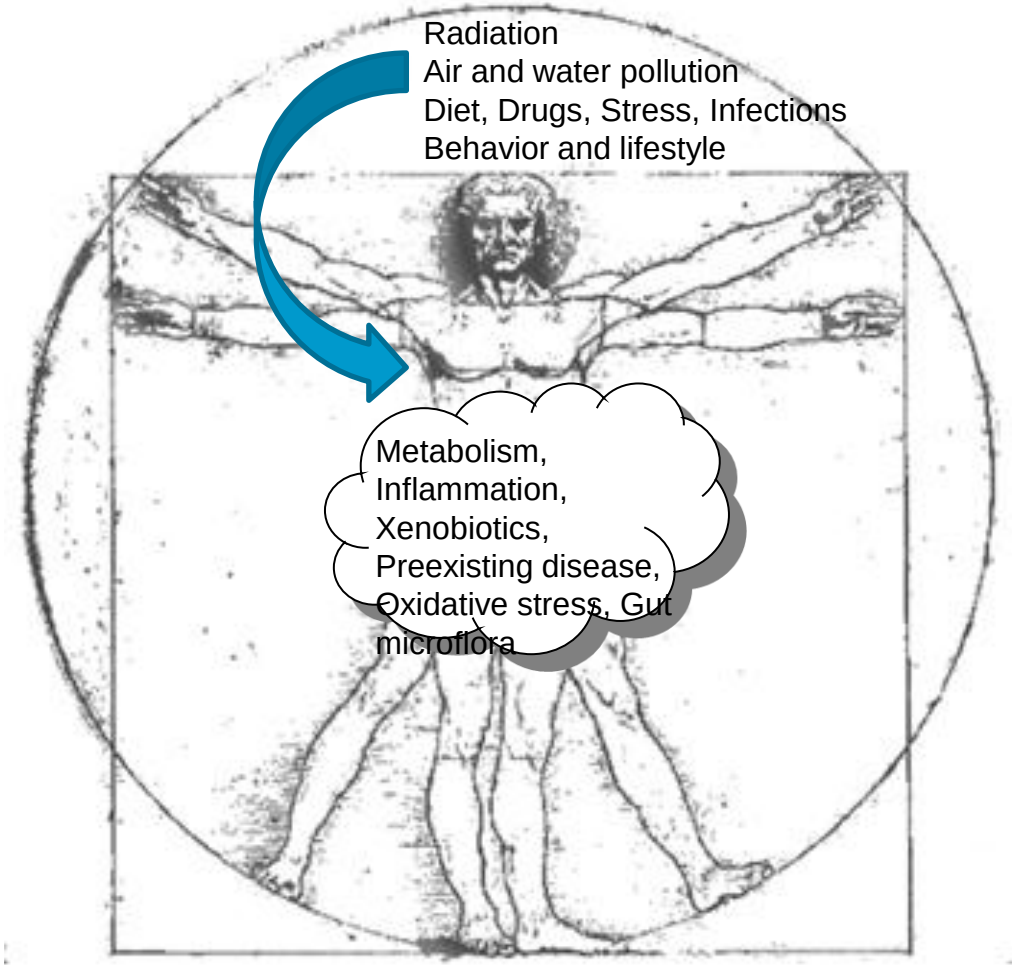
Exposome:

- CP Wild (2005) defined the exposome as the sum of all exogenous and endogenous exposures over a complete lifetime.
  - *Exogenous:* food, air, water and lifestyle choices
  - *Endogenous*: biosynthesized chemicals and metabolites

Exposomics:

- Measuring the exposome using “omics” tools: LC and GC mass spectrometry, NMR, bioinformatics

New paradigm: exposome and exposomics



S.M. Rappaport, J Expo Sci Environ Epidemiol, (2011) 21, pg. 6

Exposomics Funding and Studies

US:

- CDC, NIOSH, NIEHS http://www.cdc.gov/niosh/topics/exposome/
- EPA http://epa.gov/ncct/edr/non-monotonic.html

UK Biobank:

- Long-term follow-up of health (500,000 people ages 40 – 69) http://www.ukbiobank.ac.uk/2012/06/following-health/

EC:

- €17.3 million, Four year study. Nature 491:647 (2012)

China:

- National Basic Research Program of China J. Trans. Med. 10:A41 (2012)

Agilent and Exposomics in 2013

ASMS

- Monitoring Steroidal analogues in clinical and environmental chemistry: one model for exposomics.
- The role of GC/QTOF in exposomics

Agilent Environmental Seminars

- Harmonized GC Triple Quadrupole analysis of steroidal analogues for clinical and environmental monitoring of the exposome

Genetic Engineering & Biotechnology News

The future of GC/Q-TOF in environmental analysis

- Advanced Techniques in Gas Chromatography-Mass Spectrometry for Environmental Chemistry

Agilent Academic Newsletter

- Top-Down Exposomics: Profiling Urinary Organic Acids

U Penn Multi-omics symposium

- Exposomics and Exposure: Biological and Environmental Monitoring of 17β-Estradiol

GC Q-TOF analysis of fluorinated alkyl compounds in bio-solid matrix

MEASURING THE EXPOSOME

Measuring the exposome

Purpose: identify epidemiological risk factors

Multi-omics tools

EWAS: Exposome-wide Association Studies

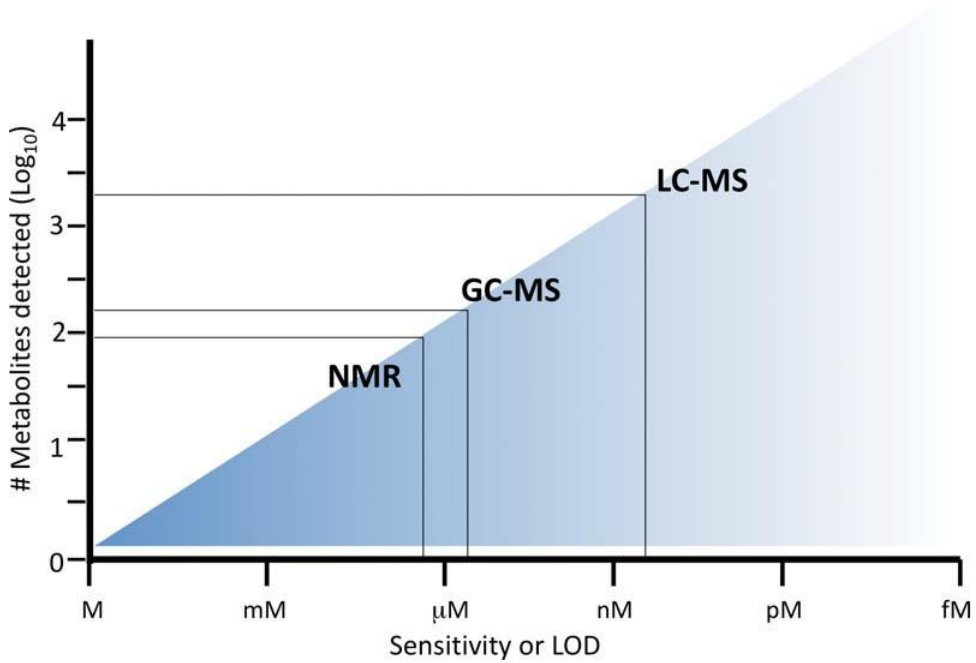
Hybrid analytical methods: simultaneous targeted and non-targeted analysis

Mass spectrometry, sensor technology, imaging technology

Wild, CP. Int J Epidemiol 2012;41:24-32

Instrumentation

Estimated limits of detection (x-axis) and the typical number of compounds identified (y-axis) by NMR, GC/MS and LC/MS for typical metabolomics studies



Wishart D.S. Exploring the Human Metabolome by Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry. Lutz N, Sweedler J, Wevers R, (eds.) In: Methodologies for Metabolomics: Experimental Strategies and Techniques, New York, NY, 2013. Reprinted with the permission of Cambridge University Press and David S. Wishart

Agilent GC/MS portfolio in exposomics

Metabolomics, food safety, water analysis, toxicology, clin chem, epidemiology, disease correlation

7890B/

- 7200A, 5977A, 5975T, 240IT, 7000B

What to measure? What would harm us?

Sediments, debris, chemical spills, fuel leaks, fires, open air burning...

Salted farm lands

Huge amount of debris to be treated

- Particulate matters from temporary storage sites

Radioactive debris

Chemical pollution from all those sources

- Impact on ecosystem
- Impact on human health

Targeted and Untargeted Analysis

Targeted Analysis

- List of chemicals to be monitored
- Standards for quantification

Untargeted Analysis

- When targets are unknown
- No standard available

Need to combine targeted and untargeted analysis especially in times of trouble

- Understand fate and transport
- Predict / determine toxicity
- Correlate exposure to disease

Bottom-up vs. top-down exposomics

Bottom-up:

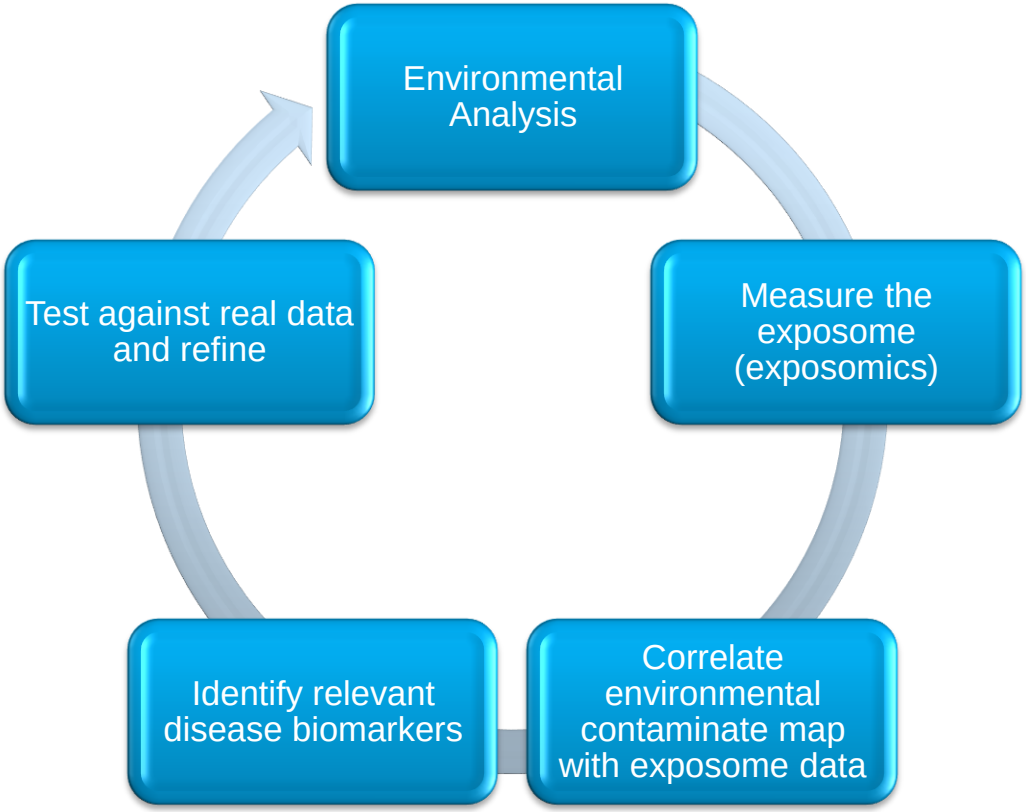
- Samples air, water, food, etc.
- Ties exposures directly to their immediate sources
- Misses essential features of the internal chemical environment

Top-down:

- Chemicals measured in blood, urine, tissue, etc.
- Identifies all potentially important exposures
- Provides no information about exogenous sources

Rappaport, SM. J Expos Sci Env Epidemiol 2011;21:5–9

Really need to look at the whole:
Cyclical Exposomics



Biomarkers

Exposure vs. Disease

Not always one and the same

Organic acidurias

- Genetic: Maple syrup Urine disease
- Exogenous: exposure xylene isomers results in increased levels of methyl benzoate and methylhippurate
- Iatrogenic sources: administration of valproic acid results elevated levels of in 3-hydroxyisovalerate, 5-hydroxyhexanoate, 7-hydroxyoctanoate, p-hydroxyphenylpyruvate and dicarboxylic
- Gastrointestinal dysbiosis: increased urinary levels of citramalic acid, 5-hydroxy-methyl-furoic acid and tartaric acid arising respectfully from *Saccharomyces*, *Aspergillus* and *Candida* species overgrowth in the bowel
  - Tartaric aciduria from yeast overgrowth in the bowel, there has been at least one report of the manifestation of Autism like symptoms in twin brothers

Lord RS, Alexander **Bralley** J Alt Med Rev 2008;13:205-215
Kumps A, Duez P, Mardens Y. Clin Chem 2002;48:708–717
Shaw W, Kassen E, Chaves E. Clin Chem 1995;41:1094-1104

Meet in the middle exposomics

Exposure and disease

Identifying the overlap between exposure markers and predictive biomarkers of disease

Biomarkers of exposure elevated in certain disease states:

- Causal link of exposure and disease

Potentially new avenues of prevention by identifying environmental factors of disease

Chadeau-Hyam M, Athersuch TJ, Keun HC, et al. Biomarkers 2011;16(1):83-88

GC Q-TOF analysis of fluorinated alkyl compounds in bio-solid matrix

ANALYSIS OF FTOH

Fluorotelomer Alcohols (FTOH)

Source unknown, but probably intermediate degradation products of fluorinated polymers

Oxidize to form fluorinated carboxylic acid, some of which are toxic

Methods needed for studying their transport and fate in the environment

Have the form $F_3C(CF_2)_{N-1}(CH_2)_MOH$

N:M FTOH is the shorthand notation

Purpose of Study

Develop robust, sensitive and selective GC-MS/MS method in bio-solid matrix from waste water treatment plants

Study the fate and transport of fluorotelomers in the environment

Investigate degradation pathways of fluoropolymers

Gain understanding of human exposure and toxicity

- Evaluate gas chromatography-quadrupole time of flight mass spectrometry (GC Q-TOF MS)

FTOH Disease associations

Endocrine disruption

Estrogen-Like Properties of Fluorotelomer Alcohols as Revealed by MCF-7 Breast Cancer Cell Proliferation[1]

- FTOH act as xenoestrogens *in vitro*
- Structural similarities of these compounds and 4-noylphenol (reference xenoestrogen)
- Ligands for the estrogen receptor

Fluorotelomer alcohols induce hepatic vitellogenin through activation of the estrogen receptor in male medaka (Oryzias latipes)[2]

- Vitellogenin: biomarker for estrogen EDC exposure
- Dose-dependent interaction between medaka estrogen receptor alpha (ERalpha) and coactivator TIF2
- Estrogenic effects for medaka ERalpha descended in the order of estradiol>> 6:2 FTOH > 8:2 FTOH

[1]Maras M, Vanparys C, MuylleEnviron F, et al. Health Perspect 2006;114(1):100–105.

[2]Ishibashi H, Yamauchi R, Matsuoka M, et al. Chemosphere 2008;71(10):1853-9.

GC/Q-TOF Method

15 m HP-INNOWax column (19091N-131)

Oven Program

- 60 °C for 1 min
- 3 °C/min to 75 °C for 0 min
- 20 °C/min to 210 °C for 0 min

MMI: 2 µl cold, splitless injections

- 65 °C (0.01 min), 300 °C/min to 250 °C

Two minute post column Backflush

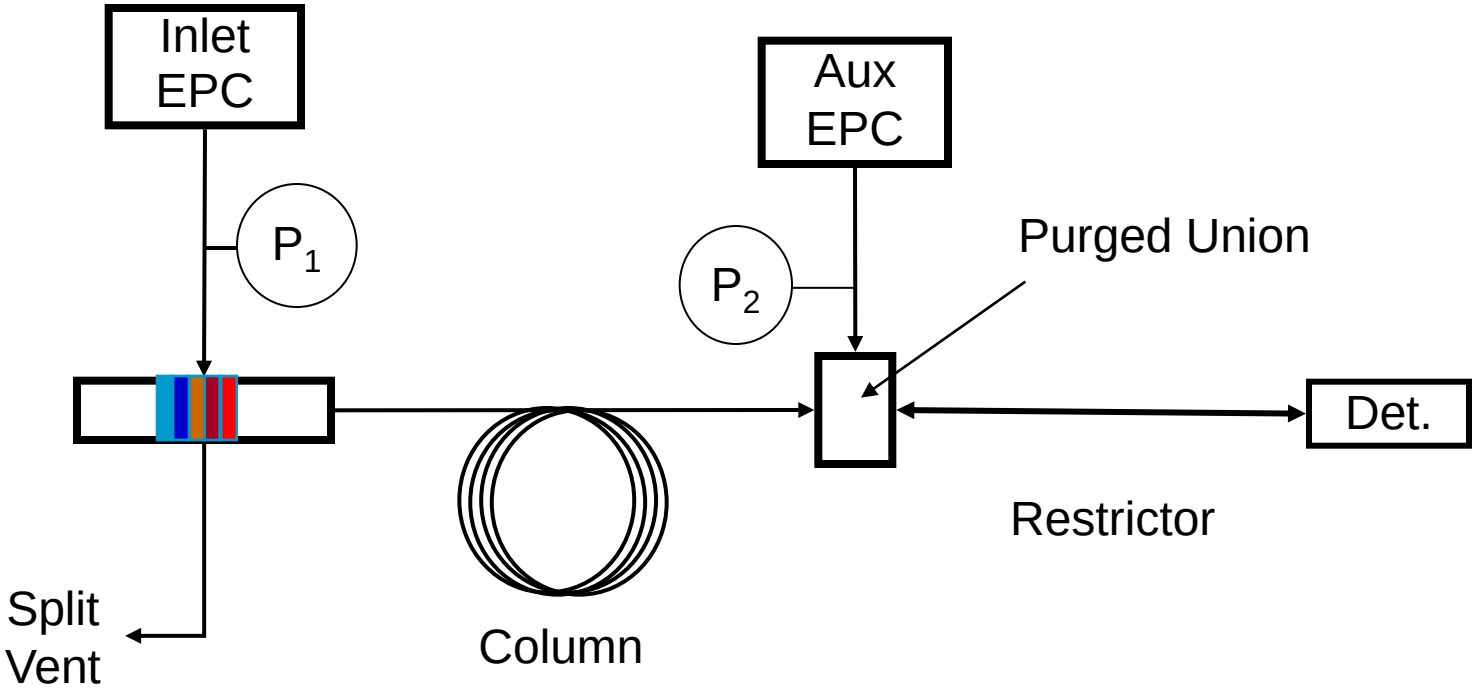
Positive CI, 20% CH_4

Capillary Flow Technology: Backflush

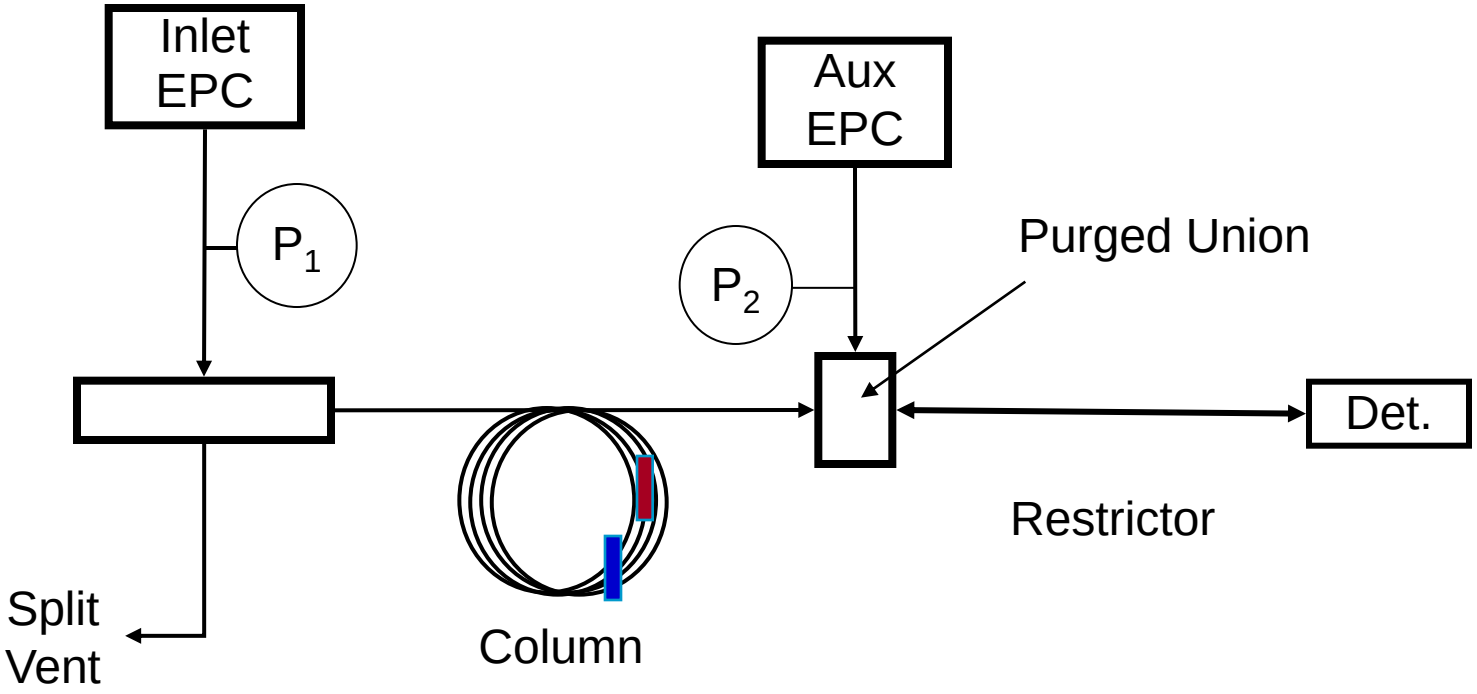
Advantages

- Reduces analysis time (more samples per day)
- Reduces contamination (and cleaning frequency) for the source
- Increases lifetime of analytical column
- Consistent GC retention times
- Consistent MS spectra through a sample sequence
  - Reduces chemical noise
  - Higher quality quantitation

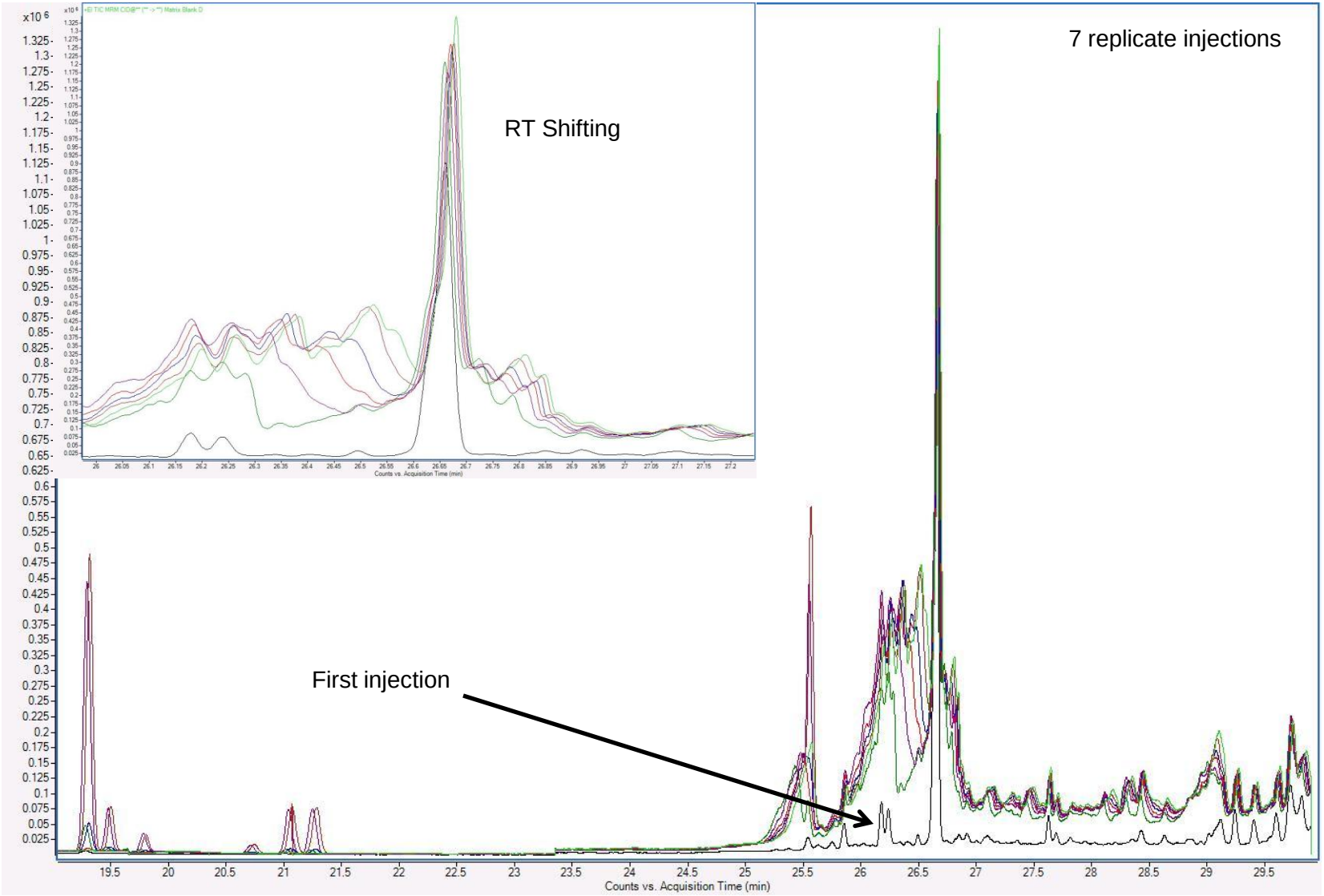
Post-Column Example – Analysis



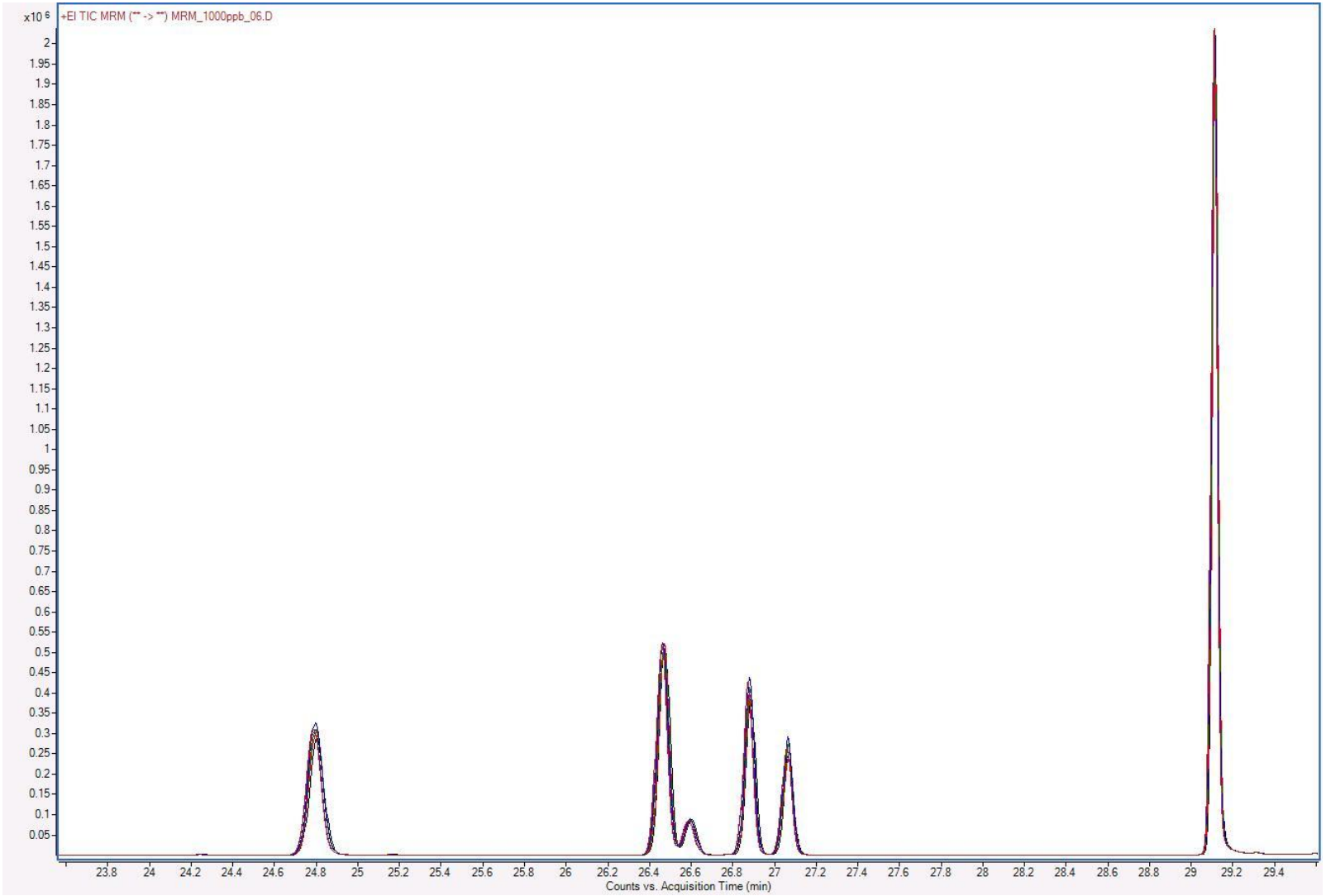
Post-Column Example - Backflush



No BF


RT Shifting
7 replicate injections
First injection
Counts vs. Acquisition Time (min)

With BF


x10 6
+EI TIC MRM (** -> **) MRM_1000ppb_06.D
2
1.95
1.9
1.85
1.8
1.75
1.7
1.65
1.6
1.55
1.5
1.45
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
23.8
24
24.2
24.4
24.6
24.8
25
25.2
25.4
25.6
25.8
26
26.2
26.4
26.6
26.8
27
27.2
27.4
27.6
27.8
28
28.2
28.4
28.6
28.8
29
29.2
29.4
Counts vs. Acquisition Time (min)

Standards and Samples

Solvent standards, 100 ng/µl in MTBE

Two bio-solid extracts prepared in EtOAc

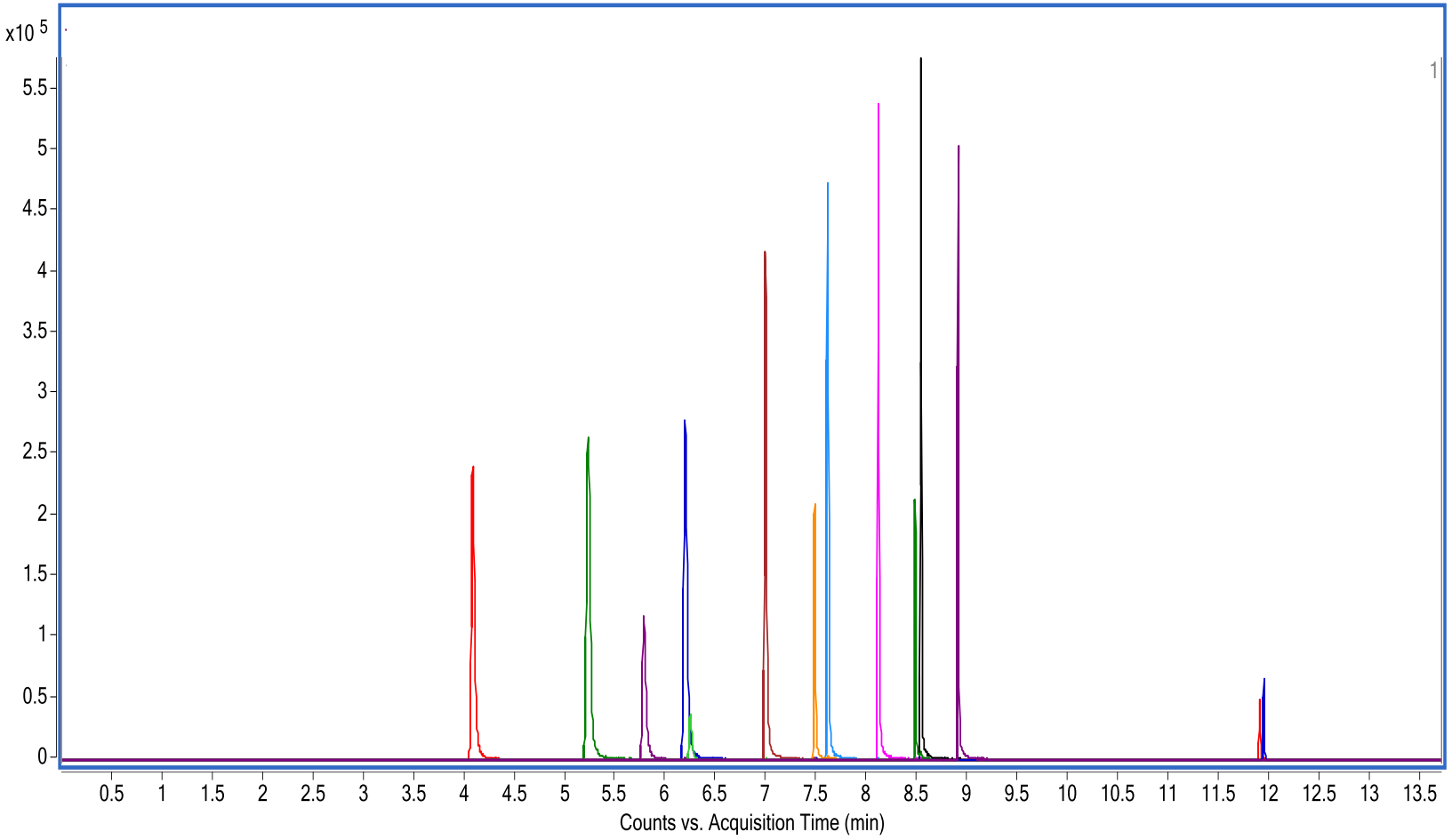
- One 1% and the other 5% lime treated
- Each extract split 50/50
  - One spiked with analytes and IS @ 50 ng/ml, the other not spiked

ID	Solvent	Vol (mL)	Description
1	Ethyl acetate	1	Extracted solvent, 1% lime treated biosolid
2	Ethyl acetate	1	Standards in extracted solvent
3	Ethyl acetate	1	Extracted solvent, 5% lime treated biosolid
4	Ethyl acetate	1	Standards in extracted solvent

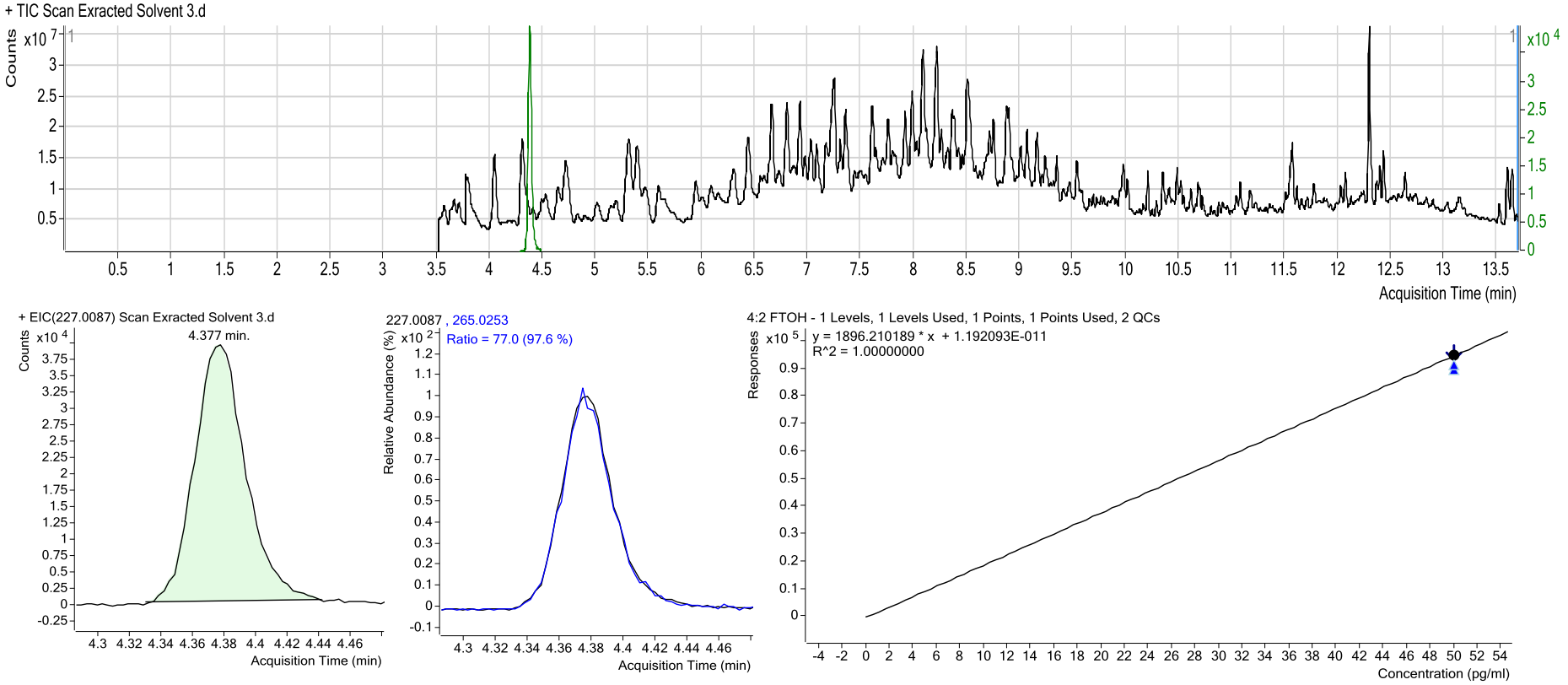
Analytes and ISTDs

Acronym	Type	r.t.	Exact Mass + H
4:2 FTOH	Target	4.078	265.0269
5:1 FTOH	Target	5.221	301.0081
6:2 FTOH	Target	5.773	365.0206
6:1 FTOH	Target	6.188	351.0049
7:2 sFTOH	Target	6.237	415.0174
7:1 FTOH	Target	6.981	401.0017
8:2 MFTOH	ISTD	7.449	469.0334
8:2 FTOH	Target	7.471	465.0142
8:1 FTOH	Target	7.598	450.9985
9:1 FTOH	Target	8.098	500.9953
10:2 FTOH	Target	8.470	565.0078
10:1 FTOH	Target	8.523	550.9921
11:1 FTOH	Target	8.886	600.9889
d7-MeFOSE	ISTD	11.868	565.0466
MeFOSE	Target	11.889	558.0026
d9-EtFOSE	ISTD	11.897	581.0748
EtFOSE	Target	11.928	572.0183

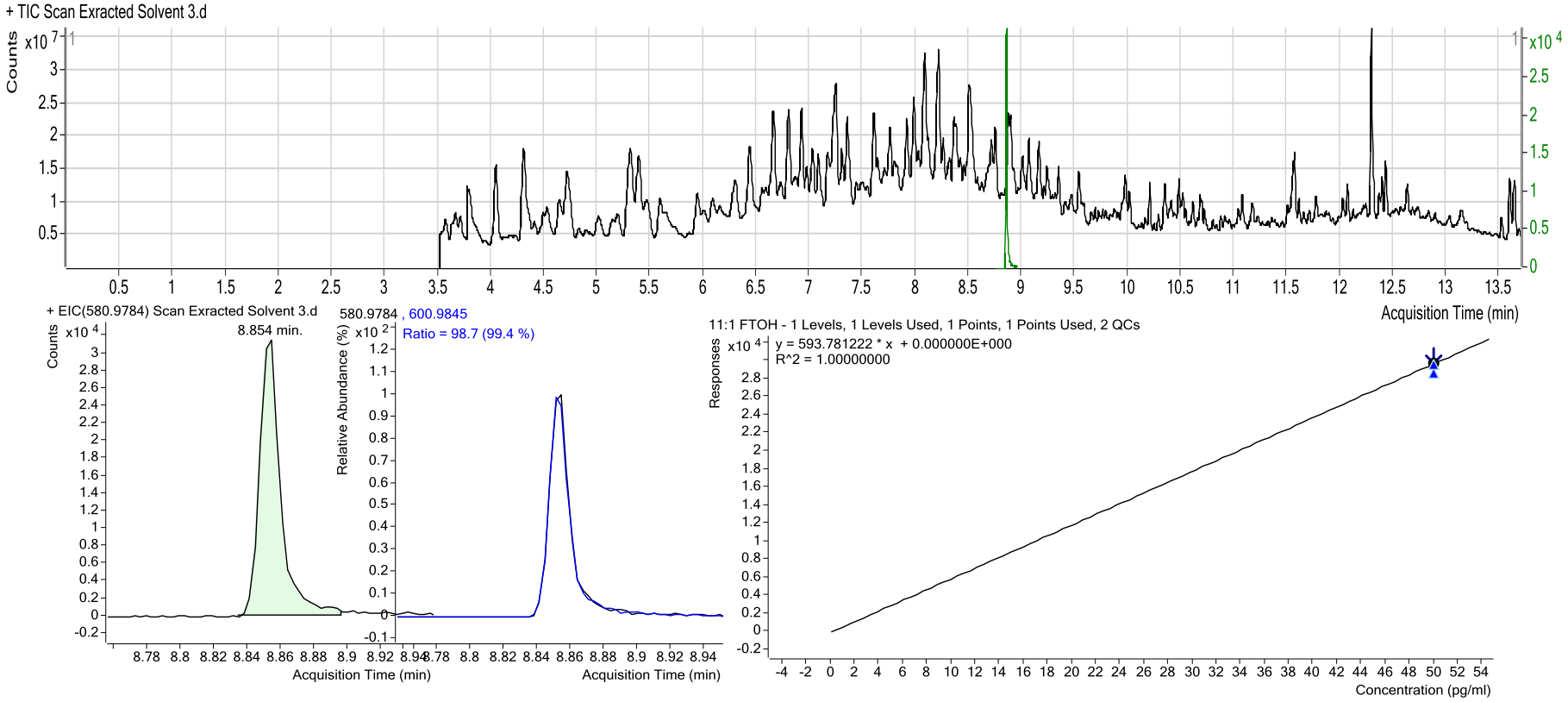
Chromatogram



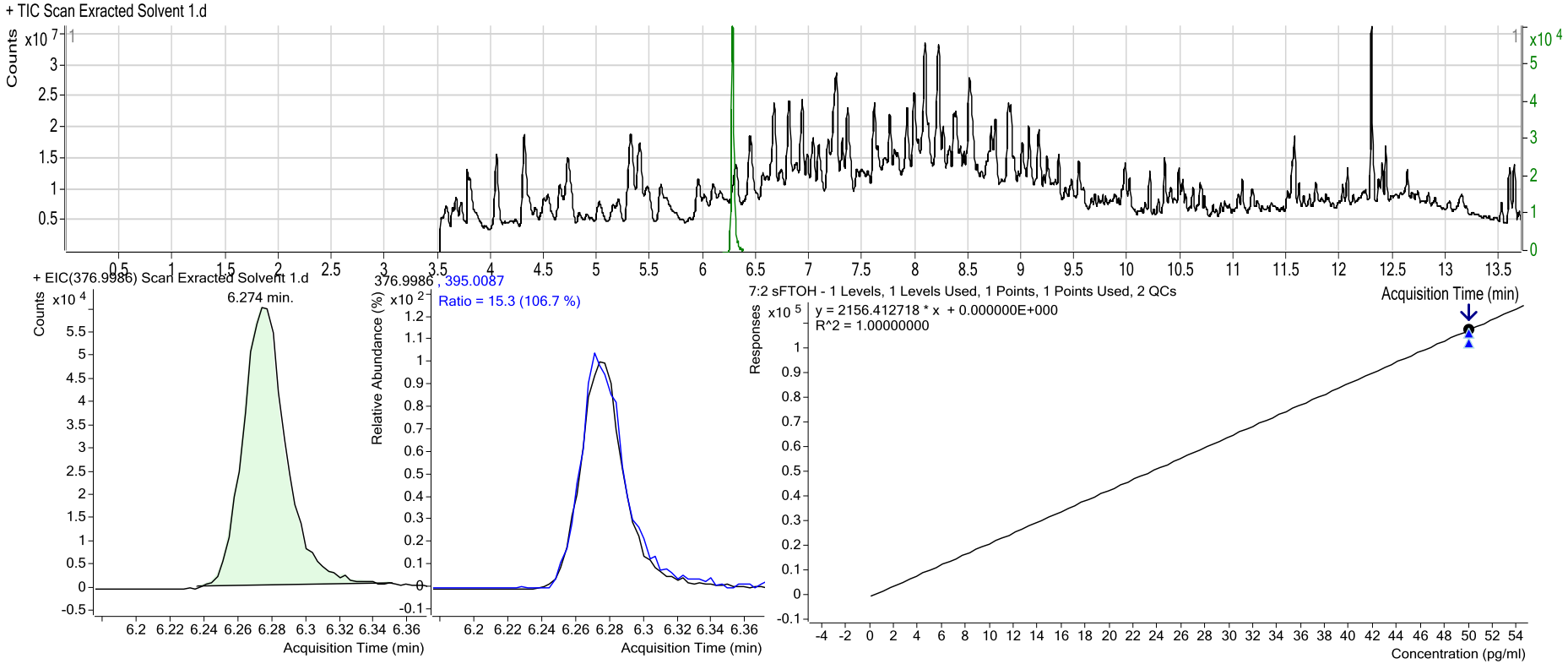
4:2 FTOH


+ TIC Scan Exracted Solvent 3.d
Counts
x10 7
Acquisition Time (min)
+ EIC(227.0087) Scan Exracted Solvent 3.d
4.377 min.
Counts
x10 4
Acquisition Time (min)
227.0087 , 265.0253
Ratio = 77.0 (97.6 %)
Relative Abundance (%)
x10 2
Acquisition Time (min)
4:2 FTOH - 1 Levels, 1 Levels Used, 1 Points, 1 Points Used, 2 QCs
y = 1896.210189 * x + 1.192093E-011
R^2 = 1.00000000
Responses
x10 5
Concentration (pg/ml)

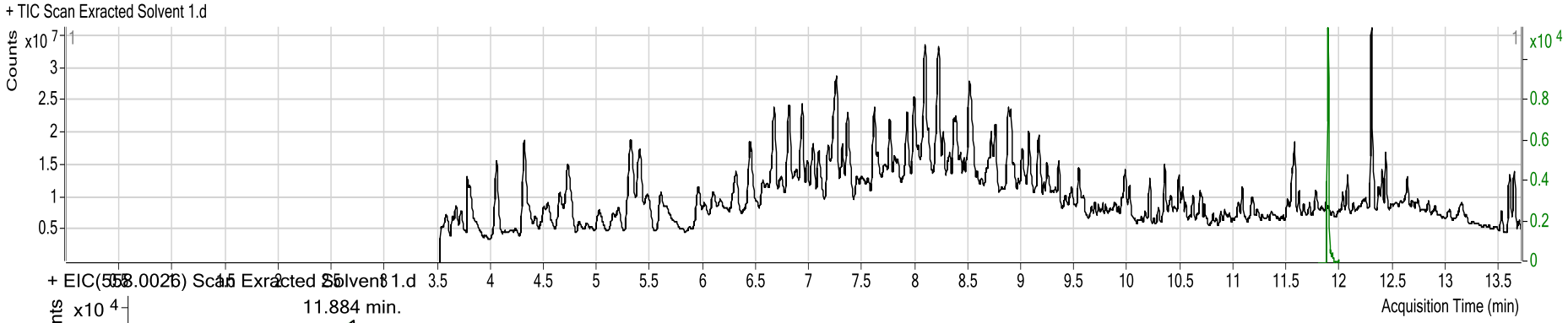
11:1 FTOH

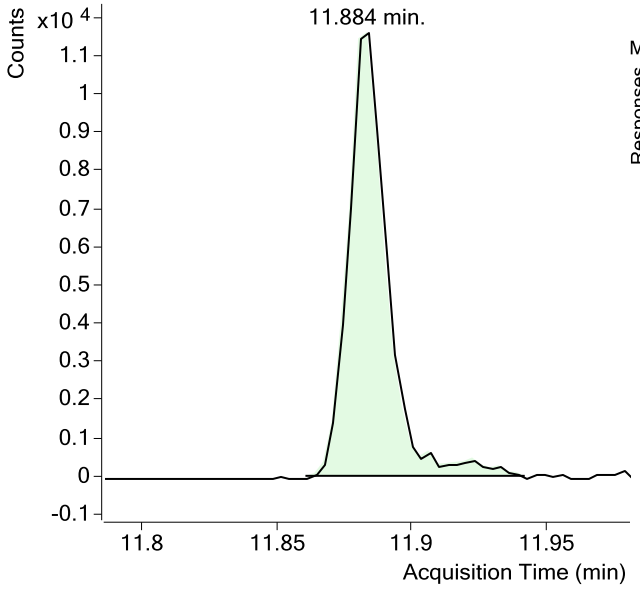

+ TIC Scan Exracted Solvent 3.d
Counts
Acquisition Time (min)
+ EIC(580.9784) Scan Exracted Solvent 3.d
8.854 min.
580.9784 , 600.9845
Ratio = 98.7 (99.4 %)
Relative Abundance (%)
Acquisition Time (min)
11:1 FTOH - 1 Levels, 1 Levels Used, 1 Points, 1 Points Used, 2 QCs
y = 593.781222 * x + 0.000000E+000
R^2 = 1.00000000
Responses
Concentration (pg/ml)

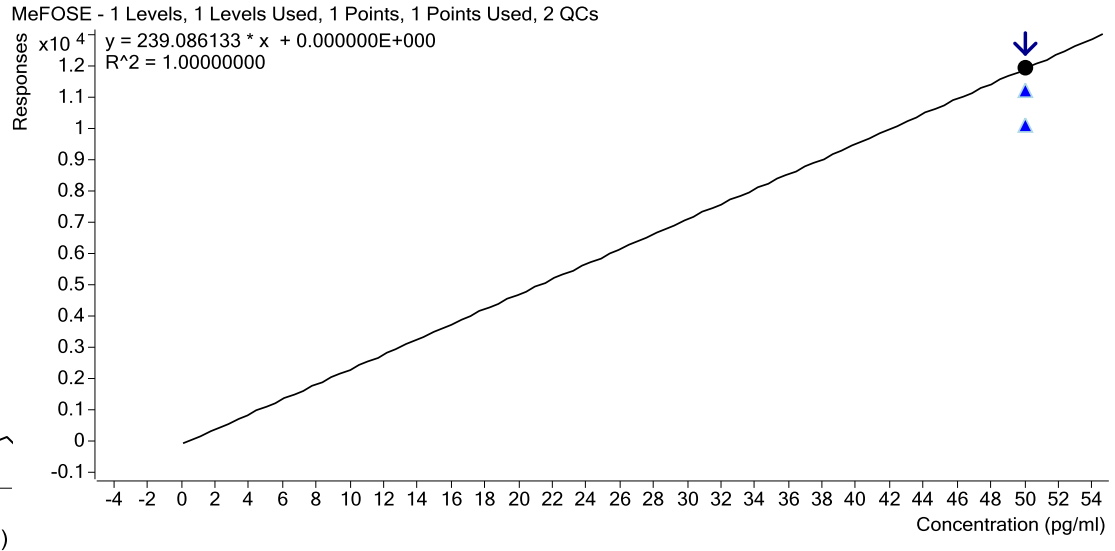
7:2 sFTOH


+ TIC Scan Exracted Solvent 1.d
Counts
x10 7
+ EIC(376.9986) Scan Exracted Solvent 1.d
6.274 min.
Counts
x10 4
376.9986 , 395.0087
Ratio = 15.3 (106.7 %)
Relative Abundance (%)
x10 2
Acquisition Time (min)
Acquisition Time (min)
7:2 sFTOH - 1 Levels, 1 Levels Used, 1 Points, 1 Points Used, 2 QCs
y = 2156.412718 * x + 0.000000E+000
R^2 = 1.00000000
Responses
x10 5
Concentration (pg/ml)

MeFOSE


+ TIC Scan Exracted Solvent 1.d
Counts
x10 7
Acquisition Time (min)
x10 4

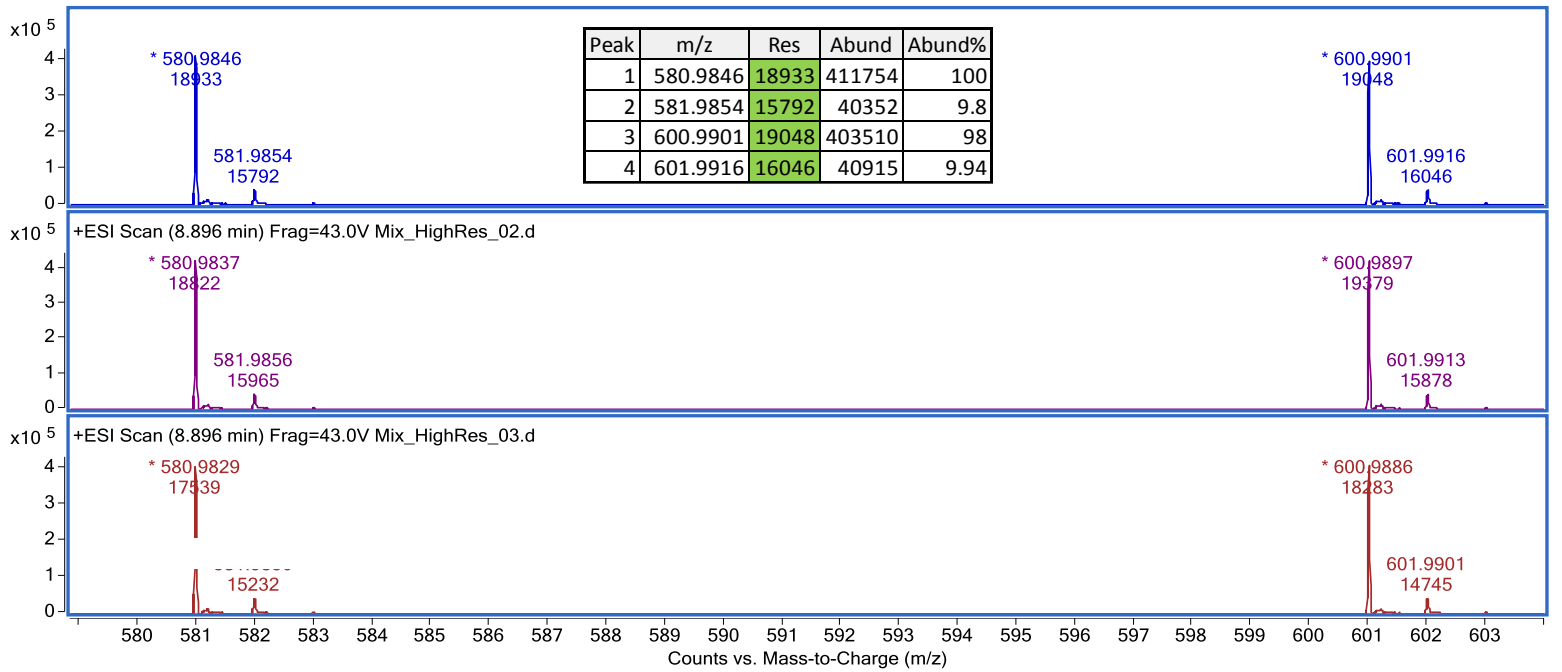

+ EIC(558.0026) Scan Exracted Solvent 1.d
11.884 min.
Counts
x10 4
Acquisition Time (min)


MeFOSE - 1 Levels, 1 Levels Used, 1 Points, 1 Points Used, 2 QCs
y = 239.086133 * x + 0.000000E+000
R^2 = 1.00000000
Responses
x10 4
Concentration (pg/ml)

Uncorrected Mass Accuracy (IRM off)

Acronym	Formula	Exact Mass + H	Observed Mass	Δppm
4:2 FTOH	$C_6H_5F_9O$	265.0269	265.0270	-0.3773
6:2 FTOH	$C_8H_5F_{13}O$	365.0206	365.0206	0.0000
8:2 FTOH	$C_{10}H_5F_{17}O$	465.0142	465.0140	0.4301
10:2 FTOH	$C_{12}H_5F_{21}O$	565.0078	565.0078	0.0000
7:2 sFTOH	$C_9H_5F_{15}O$	415.0174	415.0190	-3.8553
5:1 FTOH	$C_6H_3F_{11}O$	301.0081	301.0079	0.6644
6:1 FTOH	C7 H3 F13 O	351.0049	351.0050	-0.2849
7:1 FTOH	C8 H3 F15 O	401.0017	401.0016	0.2494
8:1 FTOH	C9 H3 F17 O	450.9985	450.9985	0.0000
9:1 FTOH	C10 H3 F19 O	500.9953	500.9956	-0.5988
10:1 FTOH	C11 H3 F21 O	550.9921	550.9922	-0.1815
11:1 FTOH	C12 H3 F23 O	600.9889	600.9896	-1.1647
MeFOSE	C11 H8 F17 N O3 S	558.0026	558.0042	-2.8674
EtFOSE	$C_{12}H_{10}F_{17}NO_3S$	572.0183	572.0167	2.7971

Resolution (50 pg on column)



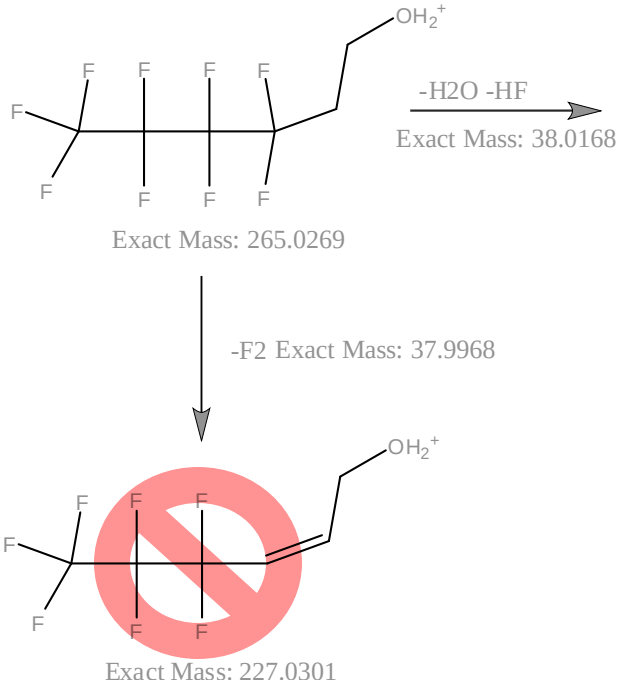
Peak	m/z	Res	Abund	Abund%
1	580.9846	18933	411754	100
2	581.9854	15792	40352	9.8
3	600.9901	19048	403510	98
4	601.9916	16046	40915	9.94

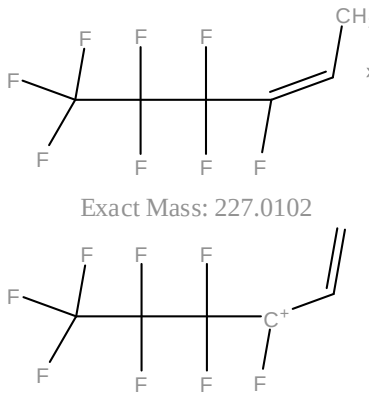
Loss Elucidation

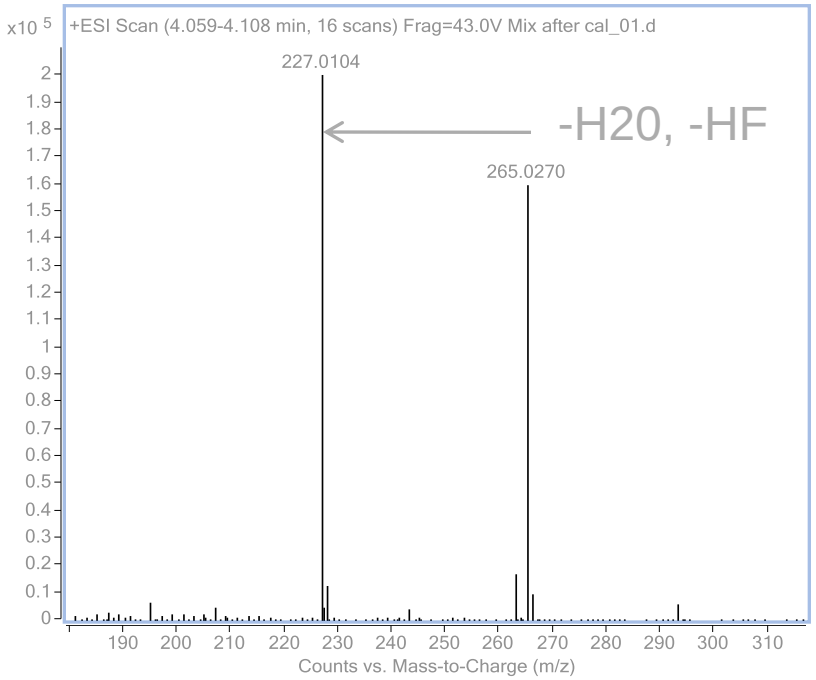
Mass accuracy in facilitated structure elucidation of fragment ions

- Nominal loss of 38 m/z for n:2 FTOH (n=4,6,7,8,10)
  - Loss of F_2 ruled out by mass
  - Loss of H_2O and HF confirmed
  - MS/MS confirmed –H_2O, -HF losses
- Loss of HF determined for n:1 FTOH (n=5,6,7,8,9,10,11)
- Loss of H_2O determined for perfluorosulfonamidoethanols

Structure Elucidation
Nominal loss = 38 m/z





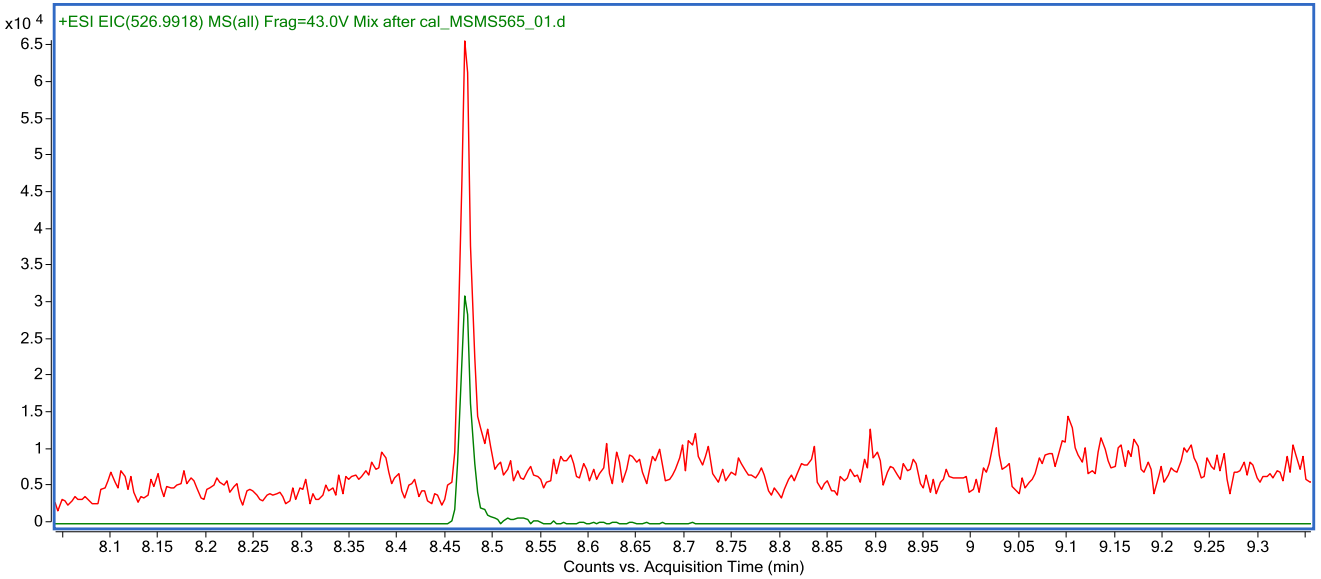


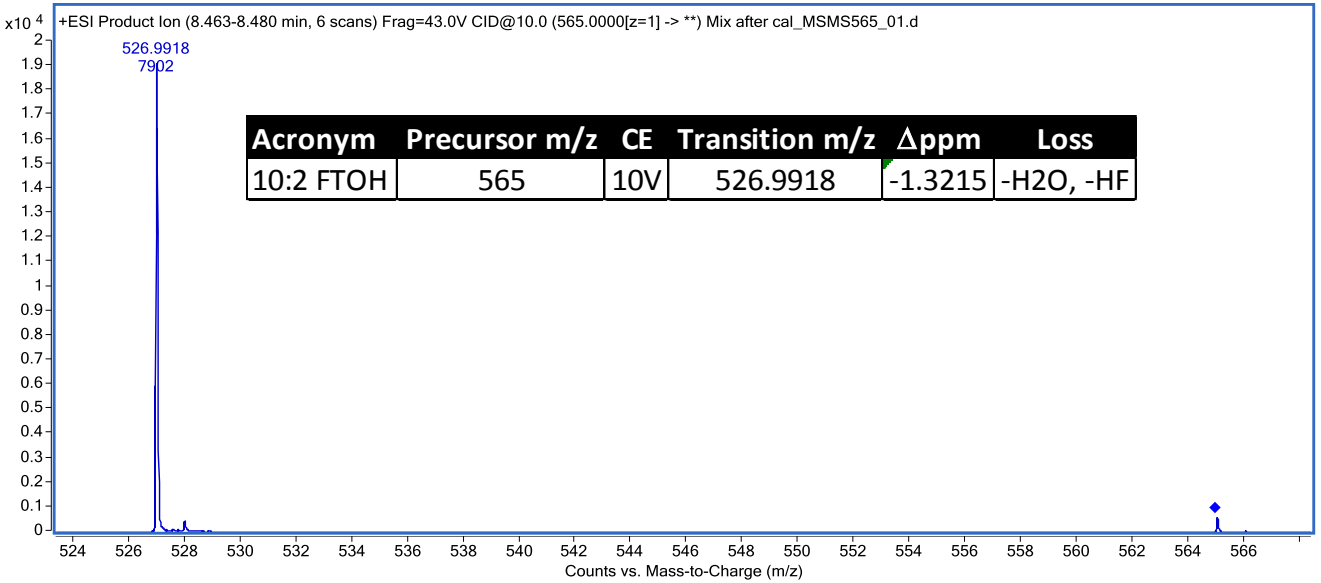
Acronym	Observed Base Peak m/z	Molecular ion -F_2 m/z	Δppm	Molecular ion -H_20, -HF m/z	Δppm
4:2 FTOH	227.0104	227.0301	86.7726	227.0102	-0.8810

Loss Elucidation & Mass Accuracy

Acronym	Formula	Loss	Exact Mass-Loss	Observed Mass 2	Δppm 2
4:2 FTOH	$C_6H_5F_9O$	-H2O, -HF	227.0102	227.0105	-1.3215
6:2 FTOH	$C_8H_5F_{13}O$	-H2O, -HF	327.0038	327.0040	-0.6116
8:2 FTOH	$C_{10}H_5F_{17}O$	-H2O, -HF	426.9972	426.9975	-0.7026
10:2 FTOH	$C_{12}H_5F_{21}O$	-H2O, -HF	526.9910	526.9915	-0.9488
7:2 sFTOH	$C_9H_5F_{15}O$	-H2O, -HF	377.0006	377.0011	-1.3263
5:1 FTOH	$C_6H_3F_{11}O$	-HF	281.0017	281.0019	-0.7117
6:1 FTOH	C7 H3 F13 O	-HF	330.9988	330.9990	-0.6042
7:1 FTOH	C8 H3 F15 O	-HF	380.9954	380.9956	-0.5249
8:1 FTOH	C9 H3 F17 O	-HF	430.9923	430.9927	-0.9281
9:1 FTOH	C10 H3 F19 O	-HF	480.9894	480.9896	-0.4158
10:1 FTOH	C11 H3 F21 O	-HF	530.9860	530.9864	-0.7533
11:1 FTOH	C12 H3 F23 O	-HF	580.9834	580.9835	-0.1721
MeFOSE	C11 H8 F17 N O3 S	$-H_2O$	539.9920	539.9943	-4.2593
EtFOSE	$C_{12}H_{10}F_{17}NO_3S$	$-H_2O$	554.0077	554.0100	-4.1516

MS/MS





Acronym	Precursor m/z	CE	Transition m/z	Δppm	Loss
10:2 FTOH	565	10V	526.9918	-1.3215	-H2O, -HF

Summary of Study

Backflush required for retention time reproducibility

- Ranged from 2.3% - 6.9% RSD in the matrix

Detection limits (LOD) as determined by a S/N ratio of ≥ 10:1

- Ranged from 2 fg – 100 fg on column

Excellent Mass Accuracy

- Typically < 2 ppm
- Range 0.1 ppm – 5 ppm

Mass Resolution > 12,000 for most samples (up to 20,000)

- Allowed differentiation from heavy chemical background

Summary of exposomics

Exposomics paradigm focuses on the environmental impact of exposure on disease

- New opportunities
  - Government funding and interest (academia and industry)
- Leverages GC/Q-TOF (GC/MS in general) power in environmental, food safety *and* biological monitoring
- Cyclical exposomics
  - Hybrid targeted / non-targeted panels focused on biologically relevant chemotypes
  - Multi disciplinary, multi-technique analyses

Software and Bioinformatics will be an integral component

- Multi-variant analysis
- Identify chemical fingerprints and biomarkers

GC/TOF and GC/Q-TOF in exposomics

Exposomics is a nascent field of study but GC/Q-TOF will be a primary tool

- Strong history in environmental analysis, current methods are easily ported
- Global monitoring of environmental contaminates
- Discovery new biomarkers for disease

Acknowledgements

The authors would like to gratefully acknowledge the United States Environmental Protection Agency and especially Shoji Nakayama, M.D., Ph.D. and Marc Mills, Ph.D. for providing standards and prepared samples for the study presented herein.

Disclaimer

The United States Environmental Protection Agency through its Office of Research and Development contributed to the research described here. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by USEPA.

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Thank you!

Questions?