



THE IMPLEMENTATION OF A SCREENING WORKFLOW FOR ION MOBILITY QUADRUOPOLE TIME-OF-FLIGHT MASS SPECTROMETRIC ANALYSIS OF PFOS ISOMERS

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Overview



- PFOS analysis collaboration between Waters Corporation and Örebro University
 - Challenges of PFOS analysis
- Instrumentation: Synapt G2-S and HDMS^E
 - Ion mobility
- Analysis of environmental samples for PFOS using HDMS^E
 - Experimental
 - Results
 - Screening approach vs Software enabled identification (Development of prototype software)
- Conclusions



Introduction: Poly and perfluoroalkyl substances (PFASs)

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PFCs research

	Figure 3: PFAAs composition profiles in different groups of sample matrices.									
Perfl in bl	Perfluorochemicals in women ages 16 to 49 years in blood serum, 1999-2008 Scientific Reports 5, Article number: 9313 doi:10.1038/srep09313									
n (ng/mL)	25		Houde M, Martin JW, Letcher RJ, Solomon KR, Muir DC (June 2006). "Biological monitoring of polyfluoroalkyl substances: A review". <u>Environ. Sci. Technol.</u> 40 (11): 3463–73. <u>doi</u> : A <u>10.1021/es052580b</u> . <u>PMID</u> <u>16786681</u> . <u>Supporting Information</u> (PDF).							
ı serun	20		Species	Geography	Year	Sample	PFOS (ppb)	A		
icals in	15		Bald eagle	Midwestern USA	1990–93	plasma	2,200	S		
ochem			Brandt's cormorant	California, USA	1997	liver	970			
irfluor	10		Guillemot	Baltic Sea	1997	egg	614			
of pei			Carrion crow	Tokyo Bay, Japan	2000	liver	464			
tratior	5		Red-throated loon	North Carolina, USA	1998	liver	861			
oncent			Polar bear	Sanikiluaq, Nunavut	2002	liver	3,100			
Ö	0		Harbor seal	Dutch Wadden Sea, Denmark	2002	muscle	2,725			
Data: Centers for I and National Cent		rs for I al Cent	Bottlenose dolphin	Charleston, South Carolina, USA	2003	plasma	1,315			
Note: are a	Note: To reflect ex are adjusted for th		Common dolphin	Mediterranean Sea, Italy	1998	liver	940			
America's Childrer		hildrer	Mink	Michigan, USA	2000-01	liver	59,500			

PFOS Analysis Challenges



- Matrix effects, retention time shifts.
- Correct PFOS isomer identification:
 - The physical, chemical and biological properties may be affected by perfluoromethyl branching.
 - Source elucidation.
 - Response factors of individual isomers.
- Increased scientific interest in toxicity, environmental transport, degradation and bioaccumulation of isomers.
- PFOS and TDCA as well as other cholic acids have similar isomeric profiles, retention times and MRM transitions (499 m/z →80m/z).
- Interferences can be mistaken for PFOS and lead to a positive bias.

Chemical Structures PFOS and Cholic Acid Interference's

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Taurochenodeoxycholic acid

 $C_{26}H_{45}NO_6S = [M-H]^- = 498.2889$

 $C_8HF_{17}O_3S = [M-H]^- = 498.9297$

Fragmentation Series for PFOS

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HDMS^E



- Uses high resolution MS and high efficiency ion mobility based measurements and separations.
- Both precursor ion and fragment ion information can be acquired in a single HDMS^E experiment.
- This technique offers some unique advantages to profiling complex matrices.
- HDMS^E can provide a route to specific and unambiguous identification, enabling the distinction of PFOS isomers.





Ion Mobility Spectrometry



- Ion mobility spectrometry (IMS) is a rapid, orthogonal, gas phase separation technique which allows another dimension of separation.
- Separation is driven by electric fields not under vacuum.
- Compounds can be differentiated based on size, shape and charge.

Ion Mobility Spectrometry (IMS)

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Small Compact

Large Extended

Slide courtesy of Severine Goscinny, ISP-WIV, Belgium

SYNAPT G2-S High Definition MS - Ion Mobility Explained



1. Increased sensitivity



Ion Mobility: an Orthogonal Dimension of Separation





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MS^E

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Environmental samples



- SLU project
 - Anna Rotander, Sara Persson

Mink



Extraction of environmental samples





ALLE WAY OUT

Experimental



- UPLC: Waters ACQUITY UPLC I-Class (equipped with PFC kit)
- Column: Waters ACQUITY UPLC BEH C18 (100 mm x 2.1 mm, 1.7 μm)
- Column temperature: 50°C Flow: 0.30 mL/min
- Mobile phase A: H₂O:MeOH/ACN 70:30 (80/20, 2 mM Ammonium Acetate)
 B: MeOH:ACN 80:20 (2 mM Ammonium Acetate)

• • • • • • • • • • • • • • • • • • •	Gradient			
	Time(min)	Flow Rate	%A	%B
	Initial	0.300	100.0	0.0
	0.50	0.300	100.0	0.0
	16.00	0.300	65.0	35.0
	22.00	0.300	65.0	35.0
	27.00	0.300	10.0	90.0
	27.10	0.300	0.0	100.0
	28.00	0.300	0.0	100.0
	28.10	0.450	100.0	0.0
	34.00	0.450	100.0	0.0



Experimental

MS: Waters SYNAPT G2-S

- Ionisation Mode: ES-
- Desolvation Temperature: 550 °C
- Acquisition Modes: IMS MS^E
- M/Z Range: 50-600
- Acquisition rate: 10 spectra/second
- Capillary Voltage: 2.3 kV
- Cone Voltage: 15 V
- Drift Gas: CO₂ and N₂
- Collision Energy Ramp: 35-75 eV
- IMS Wave Velocity Range: 400 m/s to 550 m/s
- IMS Wave Height: 40 V

Mobility Drift Gas	Mass	Polarisability (10e ⁻²⁴ cm ³)
Nitrogen N ₂	28.0123	1.7403
Carbon Dioxide	44.0098	2.9110

BPI Chromatogram for HDMS Analysis of Extract of Mink Liver for PFOS.

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50559 Components Detected





Extracted ion chromatograms for matrix interferences and the PFOS isomers





Mobility plots for the isobaric interferences and PFOS isomers using N₂ drift gas.



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Mobility plots for the isobaric interferences and PFOS isomers using CO₂ drift gas.





Ion Mobility Separation





Component drift plot showing drift times vs retention time for nominally isobaric interferences (A) and PFOS isomers (B)

Time and Mobility Aligned Fragmentation Waters

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Ion Mobility Resolved TDCA (Interference A) with MS^E Precursor and Fragmentation Spectra

🔿 🛛 Waters UNIFI - PFOS NEG ION MSE FILE 343 May 13, 2013: Analysis Center Search folders.. Q ቭ My Work C Explorer PFOS NEG ION MSE FILE... × -Review Investigate Report A (= Review Results 🕐 Limits 🔻 🗟 Process 🛭 Edit 🔻 🔯 Tools 💌 🚰 🛛 🛃 File 💌 Injections and Compo 🝷 🖪 UPLC SynaptG2-S... [1] TDCA A -9 🍸 Filters 🔻 Injecti 🔚 👒 🚽 🚖 View: *Accurate Mass Screening IMS C... 🝷 👔 🐮 🌟 🔞 Component Summary -Name 1 Component name Identification status Observed drift time (ms) m/z Mass error (ppm) Expected RT (min) Observed RT (min) Detector counts Adducts 1 UPLC_SynaptG2-S_120313 10 TCDCA B Identified 6.64 498.2903 1.64 22.52 22.52 183685 -H+ 11 TDCA A Identified 6.65 498.2913 3.59 20.88 20.89 1055397 -H+ ER 🎭 🏛 🗛 🗸 💠 E] 🛄 🔣 🏢 🚬 🗸 💠 Spectra 🗸 Item name: UPLC_SynaptG2-S_12031343_IEJ 0 X Item name: UPLC_SynaptG2-S_12031343_IEJ_Channel name: Low energy : Time 20.8890 +/- 0.0647 minutes : Drift Ti... 🔅 🗙 Channel name: TDCA A [-H+(6 ions)] : (25.0 PPM) 498.2913 4 ... Description: DL-09-007:205A 10 000ng/ul 498.2913e7 고 ^{2e7} TDCA A [Count < III. Intensity [2e6 Component: 🚳 444.2417 424.2158 [Counts] Status Name 150 200 100 250 300 350 400 450 500 Ø TCDCA B 1 Item name: UPLC_SynaptG2-S_12031343_IEJ Channel name: High energy : Time 20.8890 +/- 0.0647 minutes : Drift Ti... 🖈 🗙 2 TDCA A Description: DL-09-007:205A 10 000ng/ul le6 498.29084e7 3 PFOS G [stun 1e7 4 TCDCA B Ø PFOS M 5 Ø PFOS L Inten. 79.9569 124.0076 206.0500 480.2784 6 PFOS K 178.0183 276.1978 316.2278 372.2902 414.3007 0 7 350 PFOS J 100 150 200 250 300 400 450 500 10 20 30 Observed mass [m/z] < III. Retention time [min] 🔒 Administrator, UNIFI [Administrator] 0 \otimes

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PFOS (C) isomer, ion mobility resolved from isobaric interference TCDCA (A) at retention time 20.55 mins

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Injections and Components -	Tray: 1:8	UPLC_SynaptG2-S [1]	PFOS C (5mPFOS)	√ Filters				
Injections Con	mponent Summary 👻		View: *Accurate Mass Screeni	ng IMS C 🔹 👔 🐮 # 🔞 💷				
	Component name Identification status	Observed drift time (ms) m/z Mass er	rror (ppm) Expected RT (min) Observed RT (m	in) Detector counts Adducts				
1 UPLC_SynaptG2-S_12031343_1E0 1: 1	PFOS G (nPFOS) Identified	4.75 498.9228	-14.91 22.80	22.83 318131 -H+ 🗉				
2	PFOS F (1mPFOS) Identified	4.43 498.9319	3.40 22.40	22.41 233952 -H+				
3	PFOS E Identified	4.47 498.9310	1.66 21.48	21.48 106150 -H+				
4	PFOS D (isoPFOS) Identified	4.68 498.9318	3.12 21.20	21.14 469107 -H+				
5	PFOS C (5mPFOS) Identified	4.59 498.9321	3.72 20.52	20.52 392048 -H+				
Ch	romatograms =		Spectra -					
	nonacografiis		Item name UBLC SupertG2 S 1202124 Cha					
Cha	annel name: PFOS C (5mPFOS) [-H+(5 ions)] : (25.0 PPM) 498.9321 499.9332 50	Description: DL-09-007:205A 10 000ng	mer name. Low energy . Time 20.52 * × ×				
Components (UPLC_Syn: 🛞 🚊			∑ 1=7-]	498.93 2 68 ^{e7}				
Status Name 3								
3 🕜 PFOS E පු	FF			476.93087				
4 Ø PFOS D (isoPFOS)	2e6-	1	100 200	300 400 500				
5 O PFOS C (5mPFOS)			Item name: UPLC_SynaptG2-S_1203134 Cha	nnel name: High energy : Time 20.5 🖈 🗙				
6 ⊘ PFOS J (3mPFOS)	- PFOS C (5m 1e6 - 20.55	PFOS)	Description: DL-09-007:205A 10 000ng 1 F c 79,95674	498,93 16 \$e6				
7 Ø PFOS K				270.04407				
8 Ø PFOS L	0		129,95462 229,94858	/ 379.93841				
9 Ø PFOS M	5 10 15	20 25 30 35	100 200	300 400 500				
10 🕢 TCDCA B 🔹	Retention t	time [min]	Observe	d mass [m/z]				
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PFOS (D) isomer, ion mobility resolved Waters from isobaric interference TCDCA (A) at science OF WHAT'S POSSIBLE." retention time 21.14 mins

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				l riters *			
Injections 📑 😤 🖛 🚊	Component Summary 🗸		View: *Accurate Mass Scree	ning IMS C 🔹 🚺 🐮 # 🔞 📼			
Name Type	Component name Identification status	Observed drift time (ms) m/z Mass en	rror (ppm) Expected RT (min) Observed RT (min) Detector counts Adducts			
1 UPLC_SynaptG2-S_12031343_IEJ	1 PFOS G (nPFOS) Identified	4.75 498.9228	-14.91 22.80	22.83 318131 -H+			
	2 PFOS F (1mPFOS) Identified	4.43 498.9319	3.40 22.40	22.41 233952 -H+			
	3 PFOS E Identified	4.47 498.9310	1.66 21.48	21.48 106150 -H+			
	4 PFOS D (isoPFOS) Identified	4.68 498.9318	3.12 21.20	21.14 469107 -H+			
	5 PFOS C (5mPFOS) Identified	4.59 498.9321	3.72 20.52	20.52 392048 -H+			
	Chromatograms -	i 🖏 🎭 🏢 🛼 🗕 🖨 🛄	Spectra 🗸	II 🖬 🔜 III 🛬 🔸 🗖 🗖			
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	Channel name: PFOS D (isoPFOS) [-H+(5 ions)]	: (25.0 PPM) 498.9318 499.9335 50	Description: DL-09-007:205A 10 000ng	408 031782e7			
			딸 1e7-				
3 PEOS E	F F F						
4 PFOS D (isoPFOS)				300 400 500			
5 Ø PFOS C (5mPFOS)	PFOS D	(isoPFOS)	Item name: UPLC_SynaptG2-S_1203134 Cł	nannel name: High energy : Time 21.1 🖈 ×			
6 Ø PFOS J (3mPFOS)	21 22 21 21 21	1.14	Description: DL-09-007:205A 10 000ng	400 013#5/166			
7 🕢 РЕОБК			원 2e6 · 79.95676	498,9523			
8 Ø PFOS L	1		168.98987 229.94843	329.94180 429.93852			
9 Ø PFOS M	5 10 15	20 25 30 35	100 200	300 400 500			
10 🕢 TCDCA B 🔹	Retentio	n time [min]	Observe	ed mass [m/z]			
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Unassigned PFOS (E), ion mobility resolved from isobaric interference TDCA (A) at retention time 20.88 mins



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PFOS ISOMER C





PFOS ISOMER D





PFOS ISOMER E

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PFOS ISOMER F

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PFOS ISOMER G

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Summary for major PFOS isomers and matrix interferences



	PFOS ISOMER IDENTIFICATION				
PFOS Isomers	C 5mPFOS J 3mPFOS	D IsoPFOS	E 2,2- perfluoro- methyl PFOS (tentative)	F 1mPFOS	G nPFOS
Drift Time (ms)	4.59 4.27	4.68	4.47	4.43	4.75 -14.91
Mass Measurement Error	3.4 ppm -0.23 ppm	3.66 ppm	3.12 ppm	3.72 ppm	(2.68ppm HE)
Retention Time (mins)	20.55	21.14	21.48	22.40	22.80
TDCA Interferences	A TDCA	B TCDCA			
Drift Time (ms)	6.65	6.64			
Mass Measurement Error	3.59 ppm	1.64 ppm			
Retention Time (mins)	20.88	22.52			

A summary of drift times, retention times and isomer assigments for major PFOS isomers and co-eluting matrix.

Screening database



- A screening database was created for branched PFOS isomers based on observed retention times and unique product ions
 - 1m-PFOS, 3m-PFOS, 4m-PFOS, 5m-PFOS, 6m-PFOS, 4,4dim-PFOS, 3,5-dim-PFOS, 4,5-dim-PFOS, 5,5-dim-PFOS
- Environmental samples and a technical blend was analysed and compared against the database.
- The software filtered the results to include only isomers having a positively identified product ion.

Component summary for PFOS screening of mink liver extracts

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Software enabled identification

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Extracted ion chromatogram and spectrum for both low energy (top) and high energy (bottom) collision states. Using the structure, proposed product ions were deduced from the observed spectral peaks, as indicated by the blue molecule icons.

Drift time corrected spectrum





Spectrum for the same chromatographic peak, 1-PFOS, with interference from TDCA evident in the top spectrum. The bottom spectrum is from the same chromatographic peak and injection, but with the removal of any ions that do not share the same drift time as 1-PFOS.

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Conclusions



- Co-eluting isobaric biological interference's TDCA and TCDCA have been resolved from PFOS isomers using ion mobility.
- Utilising CO₂ as a mobility drift gas further enhanced the mobility resolution between PFOS isomers and isobaric TDCA / TCDCA interference isomers.
- The enhanced mobility resolution induced using CO₂ has a drift gas also enabled distinctive drift times to be obtained for the PFOS isomers.
- Single component precursor ion and fragmentation spectra have been generated for PFOS isomers and TDCA / TCDCA interference
- PFOS isomers can be characterised without contribution from isobaric interference isomers of TDCA / TCDCA.
- UPLC IMS MS^E facilitates accurate and informative data generation by isomerspecific analysis. This will be increasingly important as regulatory, scientific, and public awareness of the environmental impact of perflouralkylsubstances increases.

Acknowledgements

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Waters Centre of Innovation

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MAN TECHNOLOGY ENVIRONMENT RESEARCH CENTRE



Thank You!!!

Questions???

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