ÖREBRO UNIVERSITY

MAN TECHNOLOGY ENVIRONMENT RESEARCH CENTRE

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

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

**Ken Rosnack**
**ken_rosnack@waters.com**

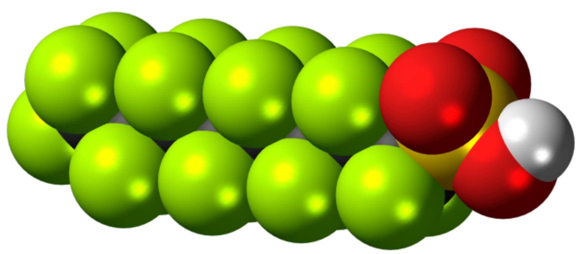
**Ingrid Ericson Jogsten and Bert van Bavel**
**(MTM Research Centre)**

**Mike McCullagh, Leonard Dillon, Mike Hodgkinson, Lauren Mullin, & Jennifer Burgess**
**(Waters Corporation)**

NEMC

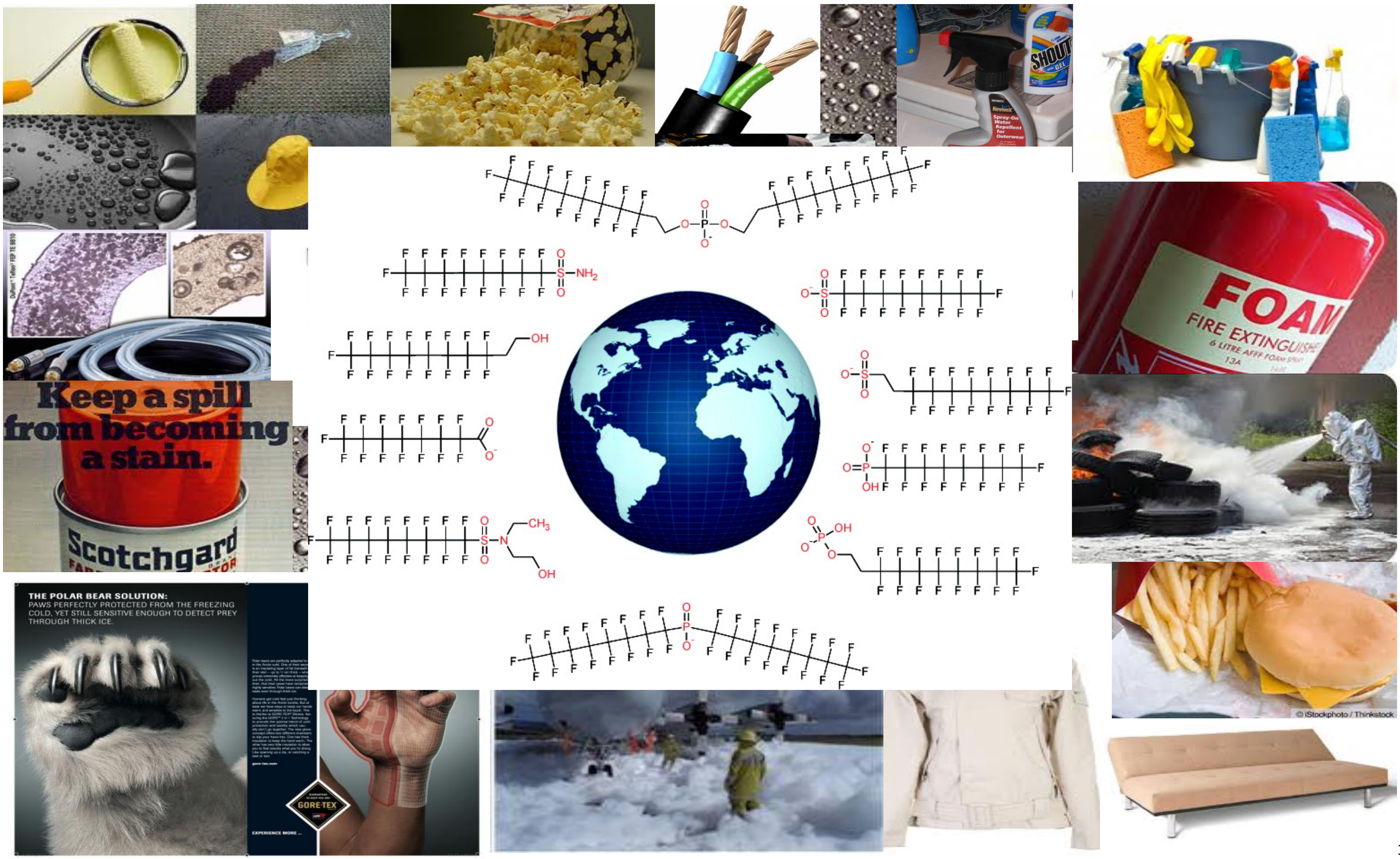
# Overview

- 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)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

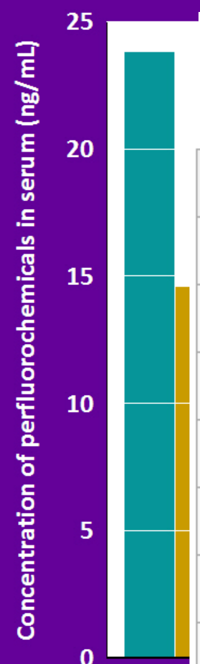


# PFCs research

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

**Perfluorochemicals in women ages 16 to 49 years: in blood serum, 1999-2008**




Data: Centers for I
and National Cent

Note: To reflect ex
are adjusted for th

*America's Children*

Figure 3: PFAAs composition profiles in different groups of sample matrices.

From

**Elevated levels of perfluoroalkyl acids in family members of occupationally exposed workers: the importance of dust transfer**

Jianjie Fu, Yan Gao, Thanh Wang, Yong Liang, Aiqian Zhang, Yawei Wang & Guibin Jiang

*Scientific Reports* **5**, Article number: 9313 | doi:10.1038/srep09313

Houde M, Martin JW, Letcher RJ, Solomon KR, Muir DC (June 2006). "Biological monitoring of polyfluoroalkyl substances: A review". *Environ. Sci. Technol.* **40** (11): 3463–73. doi: 10.1021/es052580b. PMID 16786681. Supporting Information(PDF).

| Species | Geography | Year | Sample | PFOS (ppb) |
|---|---|---|---|---|
| Bald eagle | Midwestern USA | 1990–93 | plasma | 2,200 |
| Brandt's cormorant | California, USA | 1997 | liver | 970 |
| Guillemot | Baltic Sea | 1997 | egg | 614 |
| Carrion crow | Tokyo Bay, Japan | 2000 | liver | 464 |
| Red-throated loon | North Carolina, USA | 1998 | liver | 861 |
| Polar bear | Sanikiluaq, Nunavut | 2002 | liver | 3,100 |
| Harbor seal | Dutch Wadden Sea, Denmark | 2002 | muscle | 2,725 |
| Bottlenose dolphin | Charleston, South Carolina, USA | 2003 | plasma | 1,315 |
| Common dolphin | Mediterranean Sea, Italy | 1998 | liver | 940 |
| Mink | Michigan, USA | 2000–01 | liver | 59,500 |

# PFOS Analysis Challenges

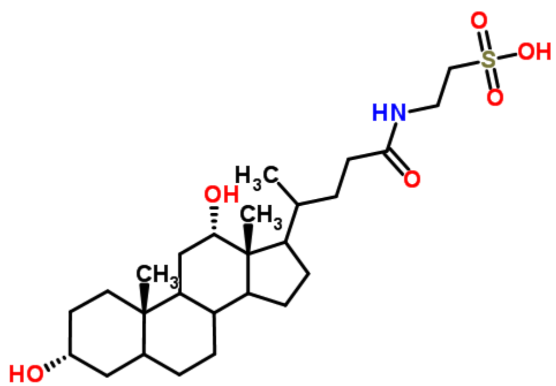
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

- 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

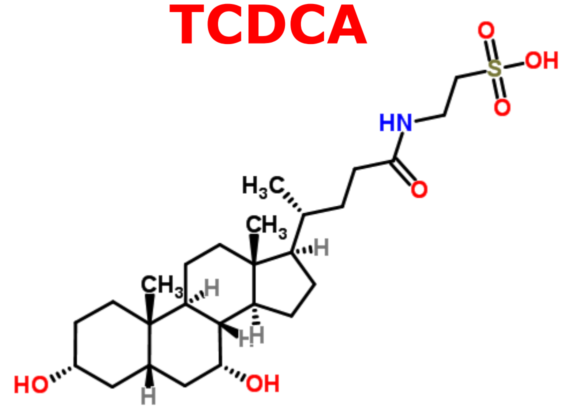
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

**TDCA**



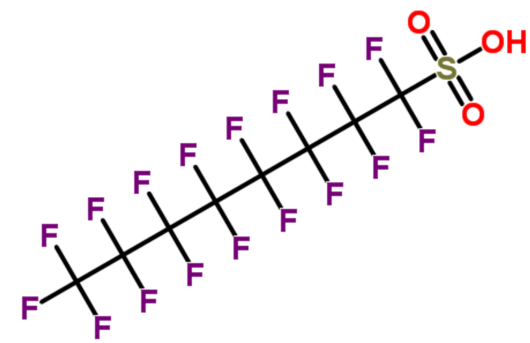

**Taurodeoxycholate**

**TCDCA**




**Taurochenodeoxycholic acid**

**PFOS**

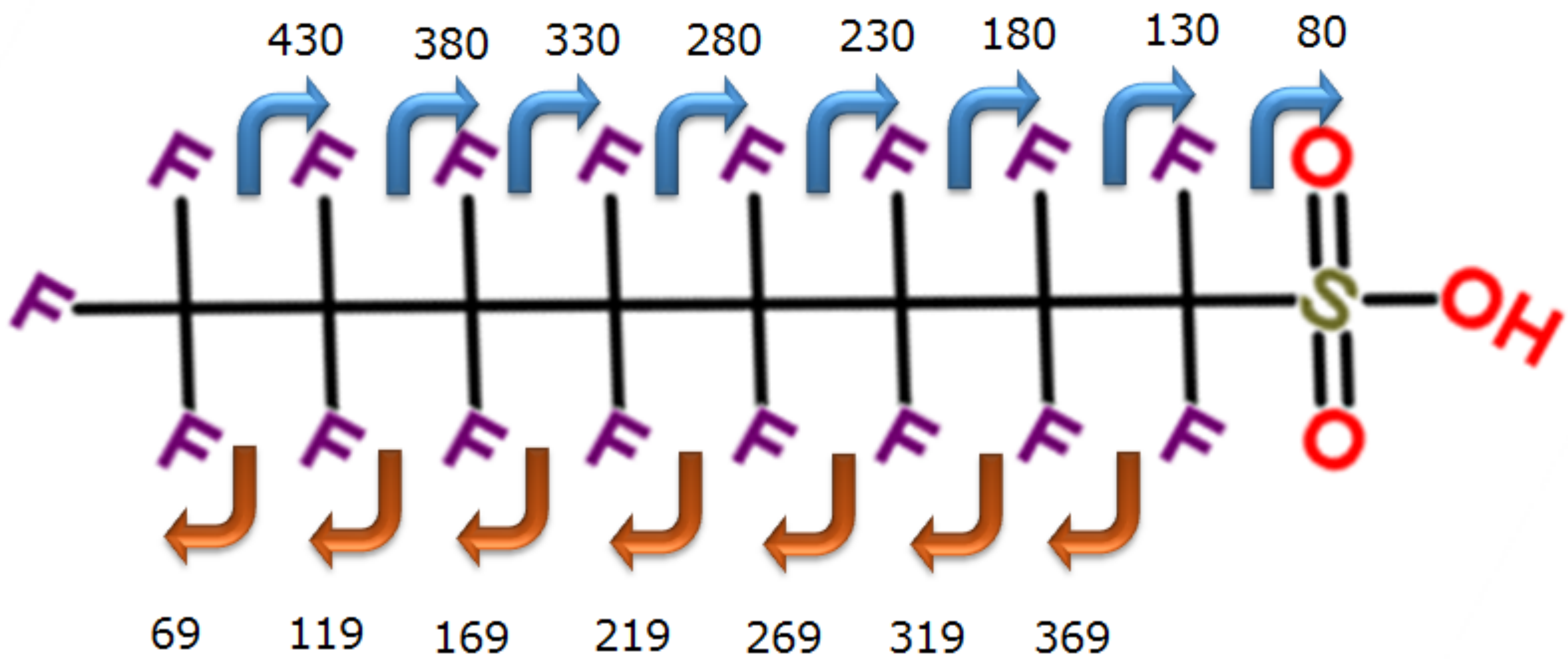



**Perfluorooctane sulfonate**

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

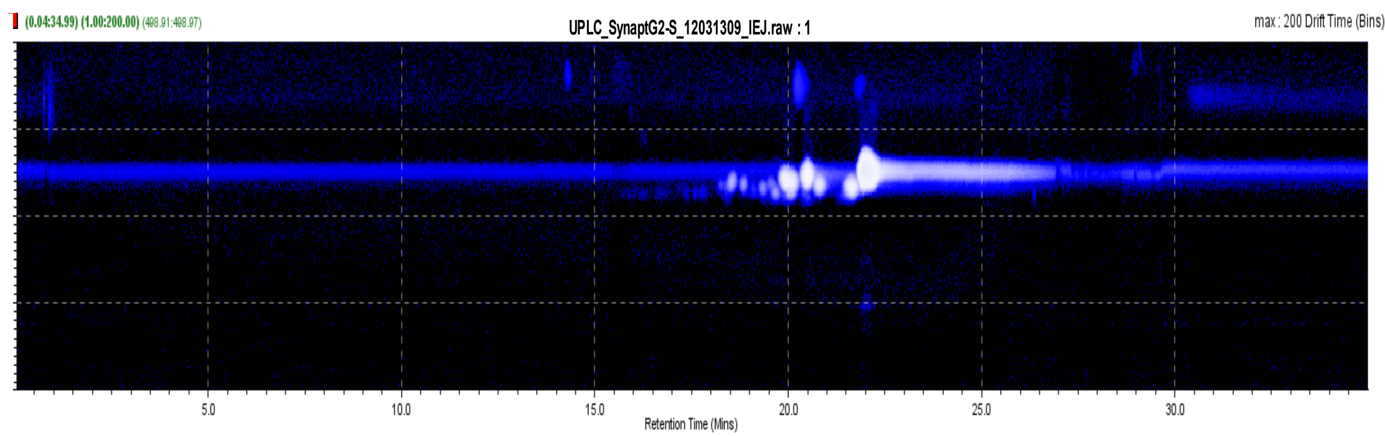
**$C_8HF_{17}O_3S$= [M-H]$^-$= 498.9297**

# Fragmentation Series for PFOS



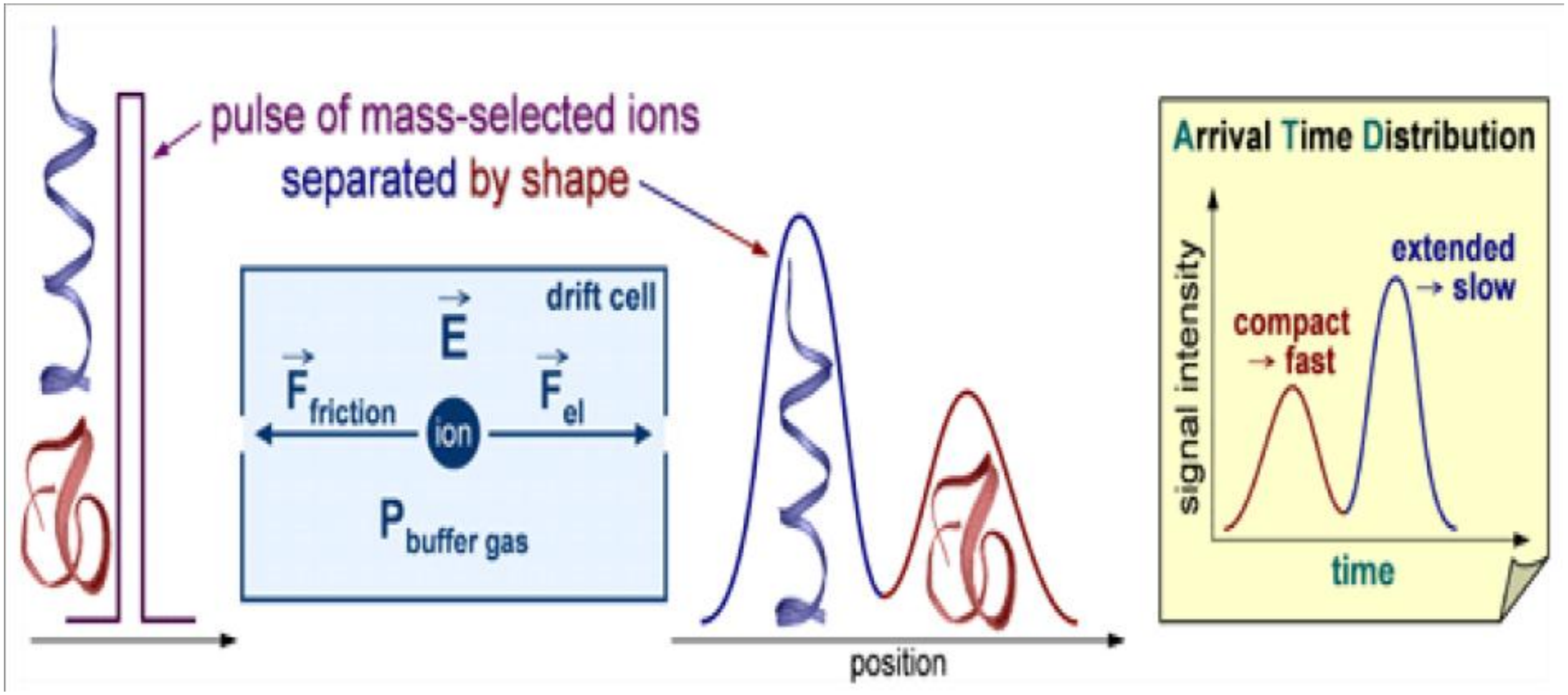
# HDMS$^E$

- 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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®




- 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)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

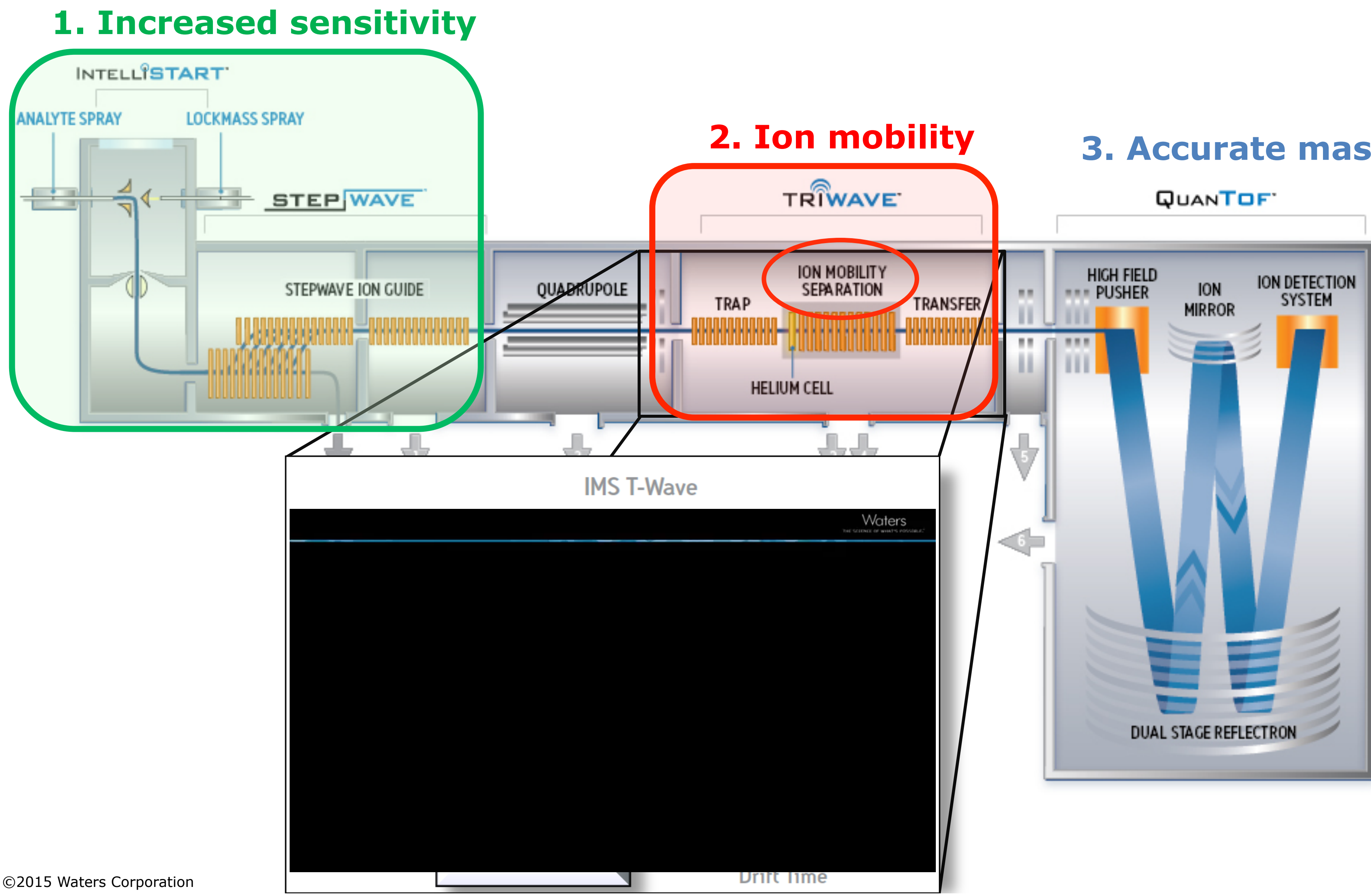
**Small**
**Compact**

Large
Extended

Slide courtesy of Severine Goscinny, ISP-WIV, Belgium

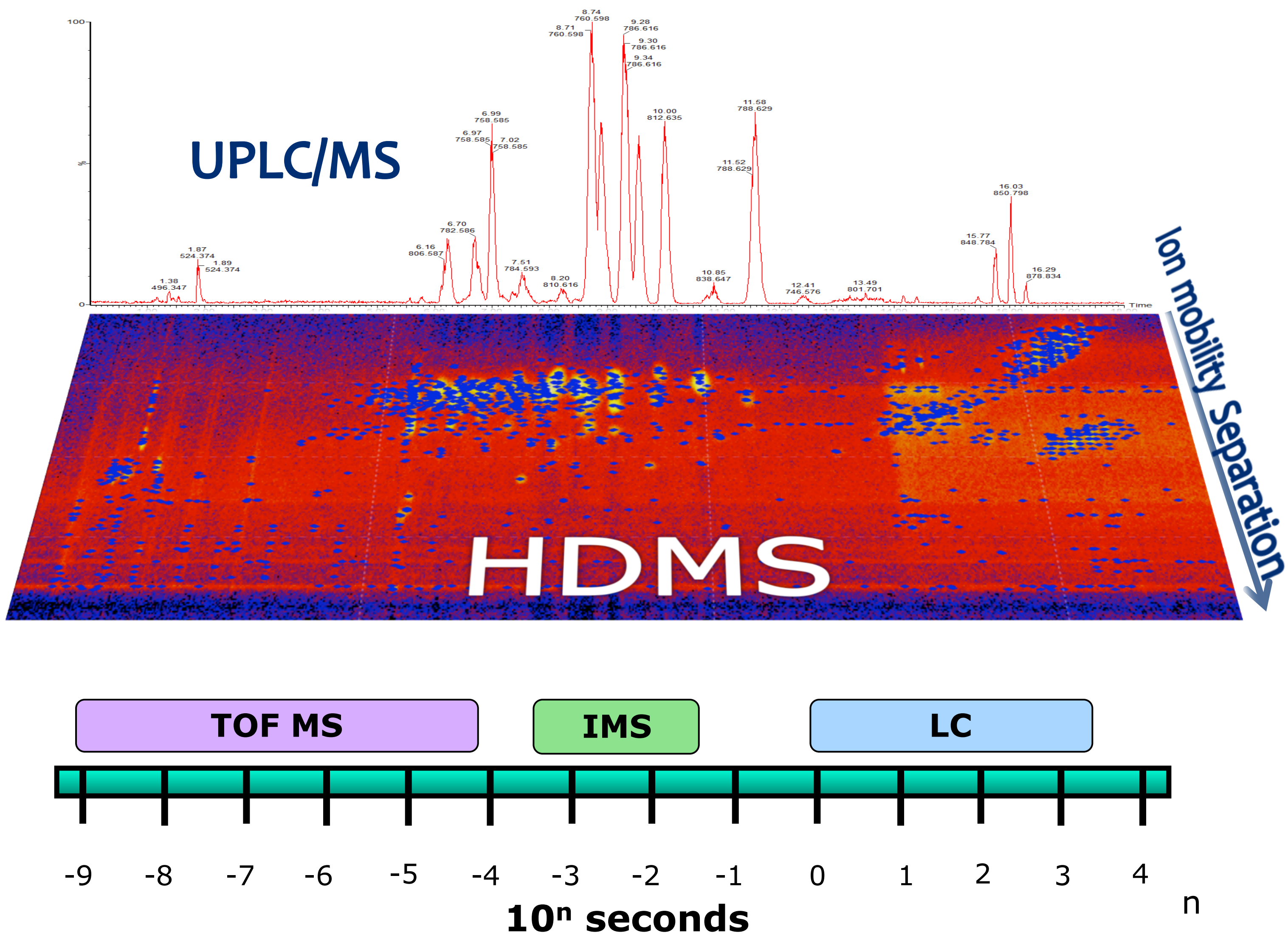
# SYNAPT G2-S High Definition MS
## *- Ion Mobility Explained*

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®



# Ion Mobility: an Orthogonal Dimension of Separation

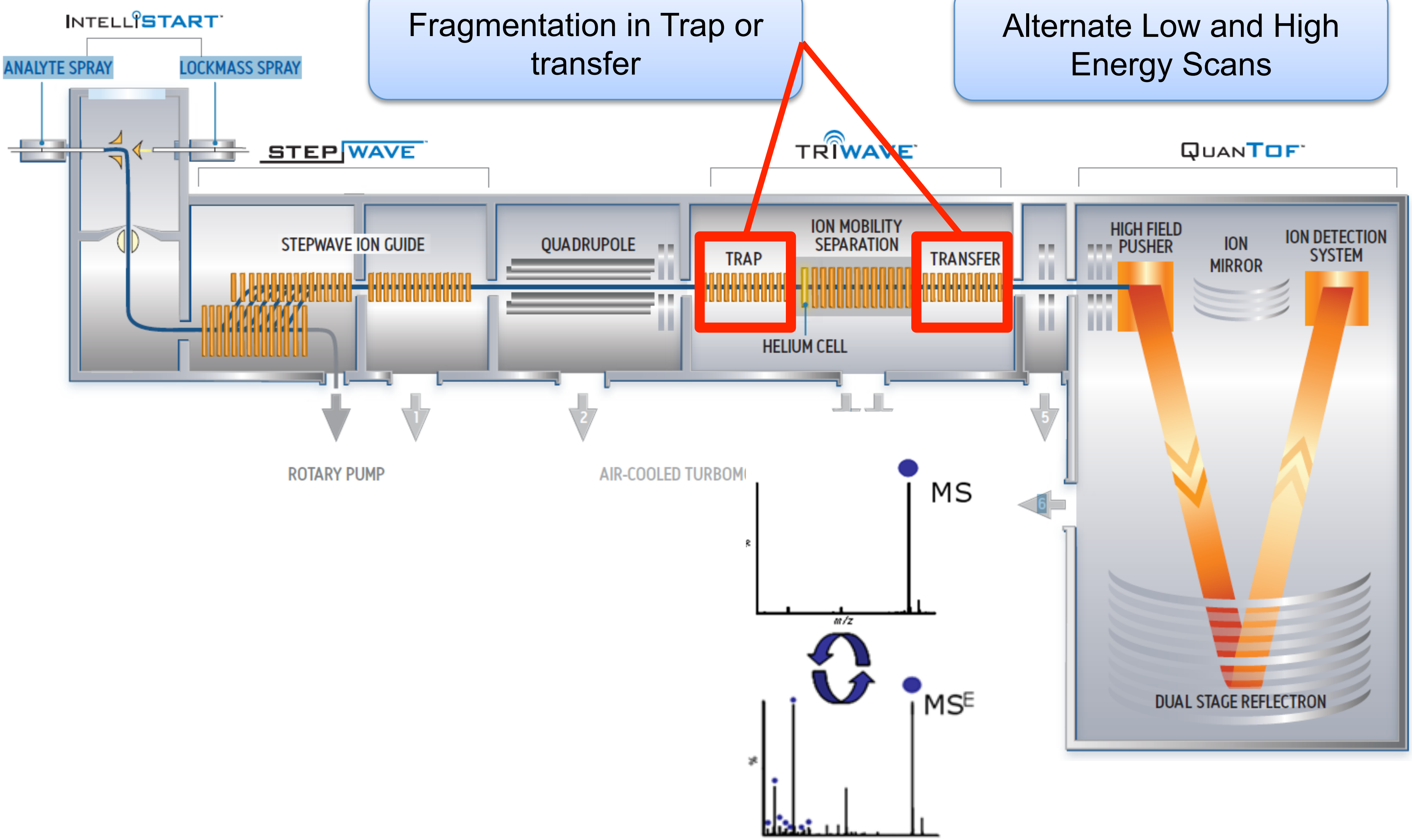
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®



# MS$^E$

Fragmentation in Trap or transfer

Alternate Low and High Energy Scans



# Environmental samples

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

- SLU project
  - Anna Rotander, Sara Persson
- Mink

# Extraction of environmental samples

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

1 g homogenized liver sample, Add labeled IS

↓

Repeated ACN extraction

↓

Vortex & ultrasonication

↓

SPE, Oasis WAX

↓

ENVI-carb

↓

Filtration, performance standards added

↓

Instrumental analysis

Condition: MeOH and water

Load samples

Wash: 4 ml NaAc (pH 4)
4 ml 20% MeOH

Dry cartridges

Elute: 4 ml MeOH (discarded)
4 ml $NH_4OH$

# Experimental

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

- ***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: $H_2O$: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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

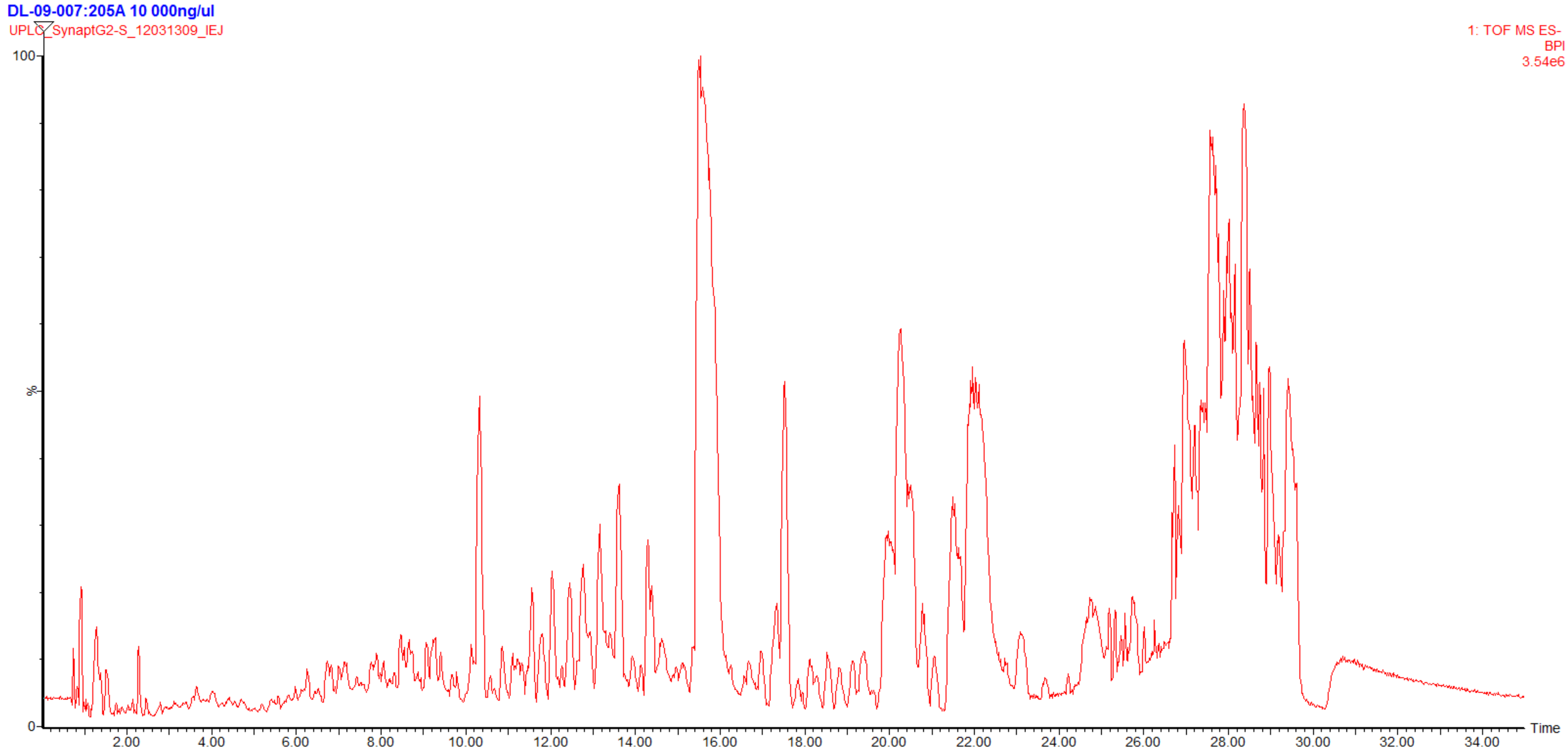
- ***MS: Waters SYNAPT G2-S***
- Ionisation Mode: ES-
- Desolvation Temperature: 550 °C
- Acquisition Modes: IMS $MS^E$
- M/Z Range: 50-600
- Acquisition rate: 10 spectra/second
- Capillary Voltage: 2.3 kV
- Cone Voltage: 15 V
- Drift Gas: $CO_2$ and $N_2$
- Collision Energy Ramp: 35-75 eV
- IMS Wave 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 |

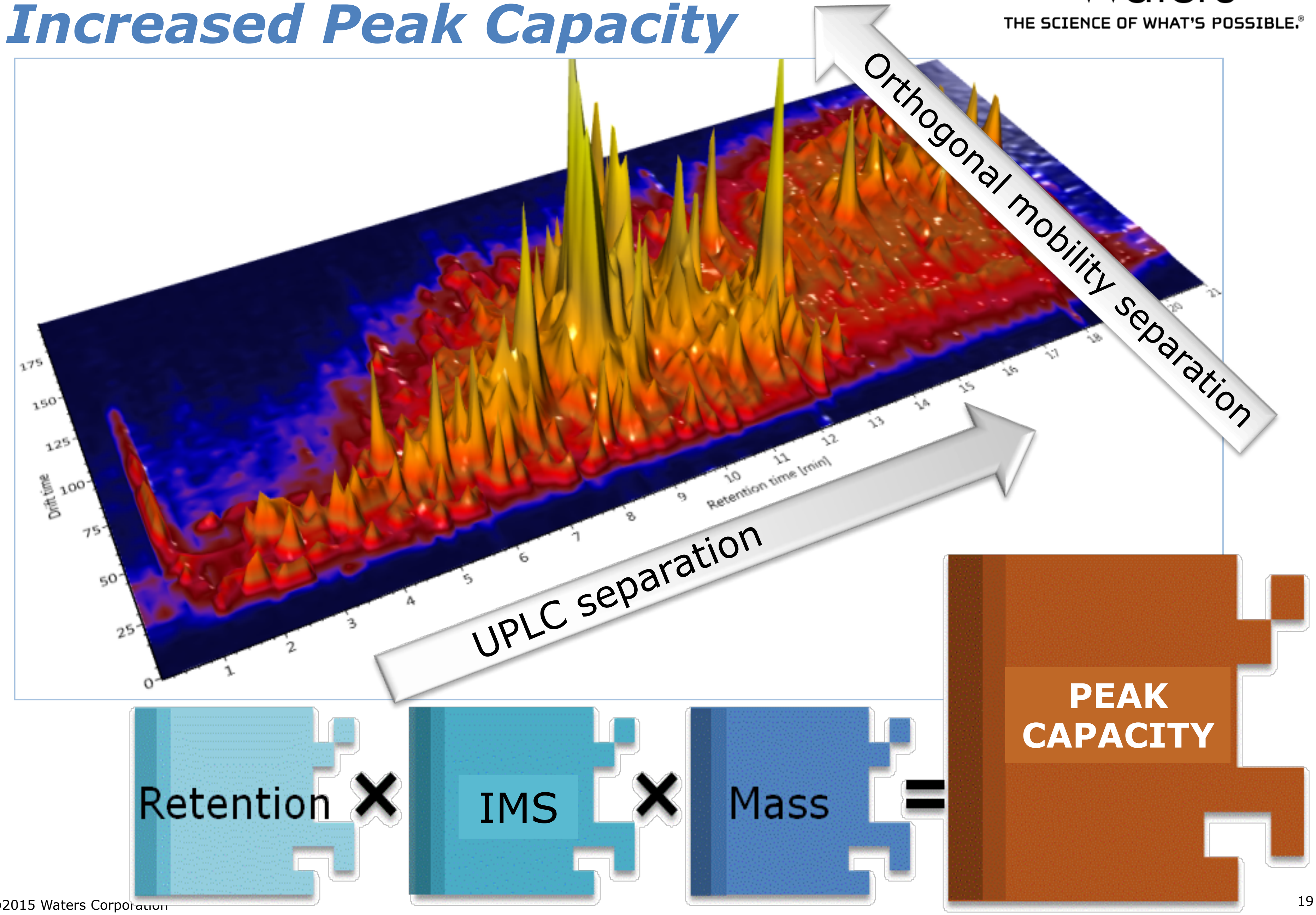
# BPI Chromatogram for HDMS Analysis of Extract of Mink Liver for PFOS.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

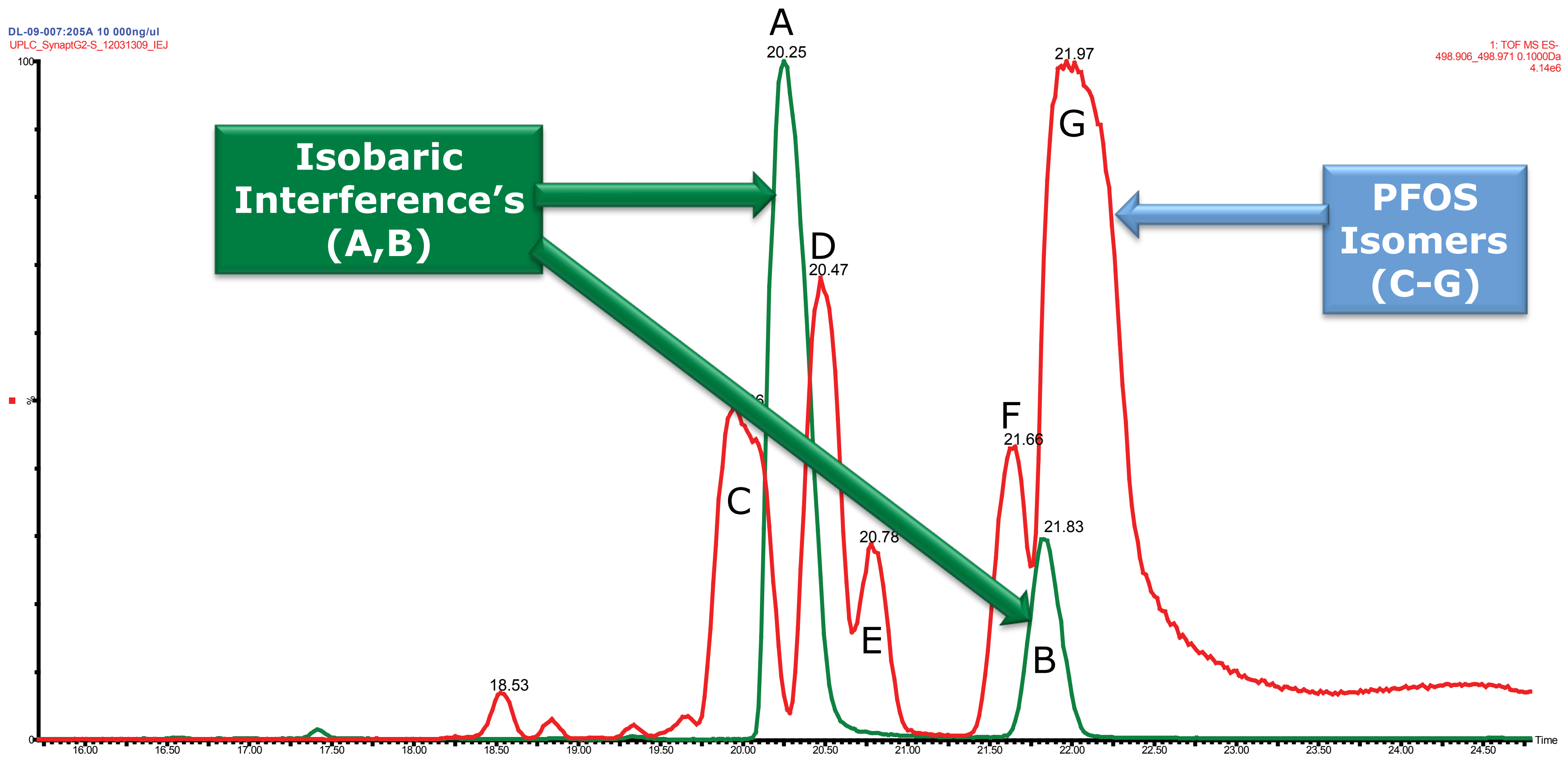
50559 Components Detected



# LC-HDMS$^E$ means…..
## *Increased Peak Capacity*



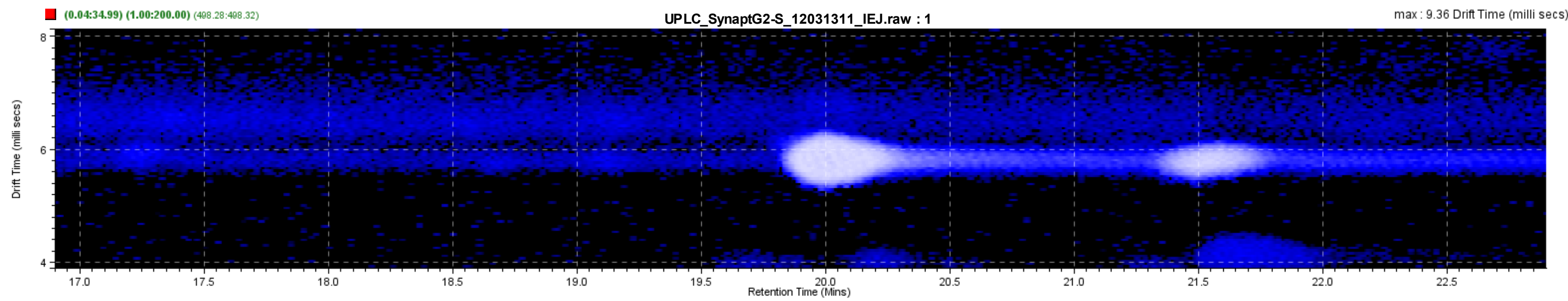
# Extracted ion chromatograms for matrix interferences and the PFOS isomers



# Mobility plots for the isobaric interferences and PFOS isomers using $N_2$ drift gas.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

## Ion Mobility Separation
## TDCA Interference's ($N_2$ Drift Gas)

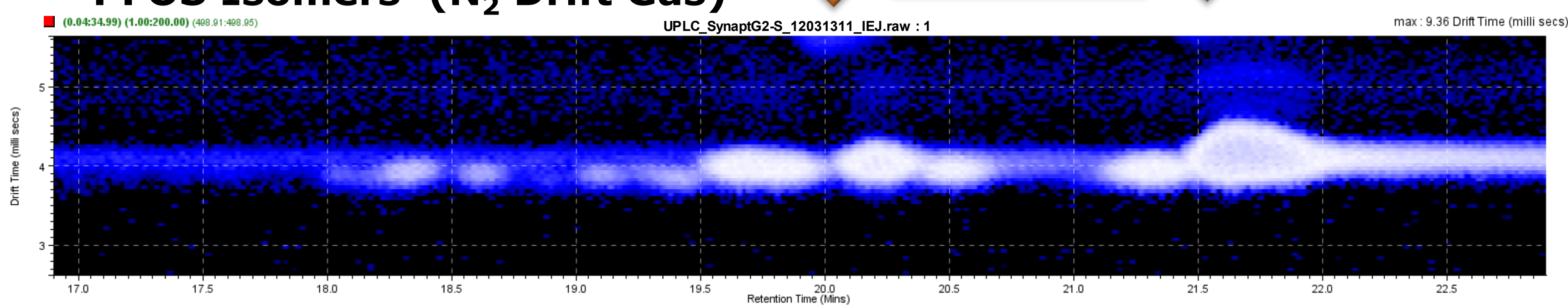



**Dt = 6 ms**

**Δ = 2 ms**

**Dt = 4 ms**

## Ion Mobility Separation
## PFOS Isomers ($N_2$ Drift Gas)

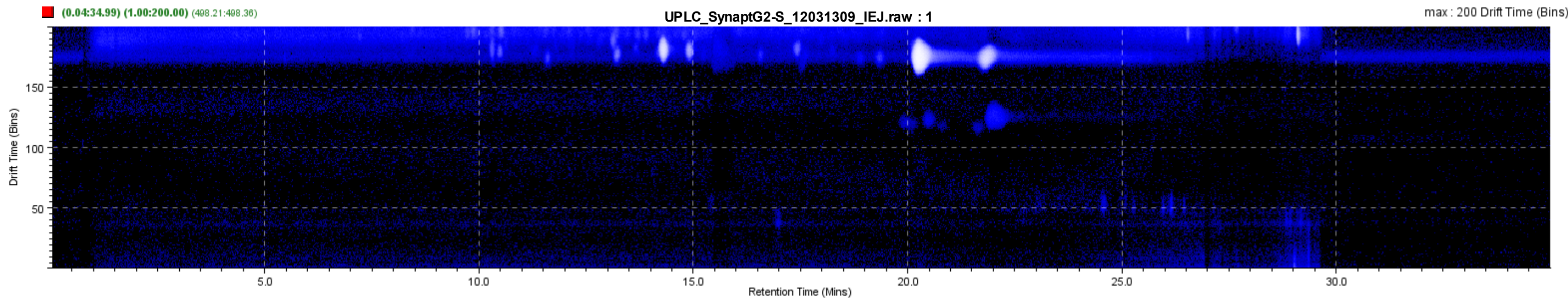


# Mobility plots for the isobaric interferences and PFOS isomers using $CO_2$ drift gas.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

## TDCA/TCDCA Interference's ($CO_2$ Drift Gas)

A

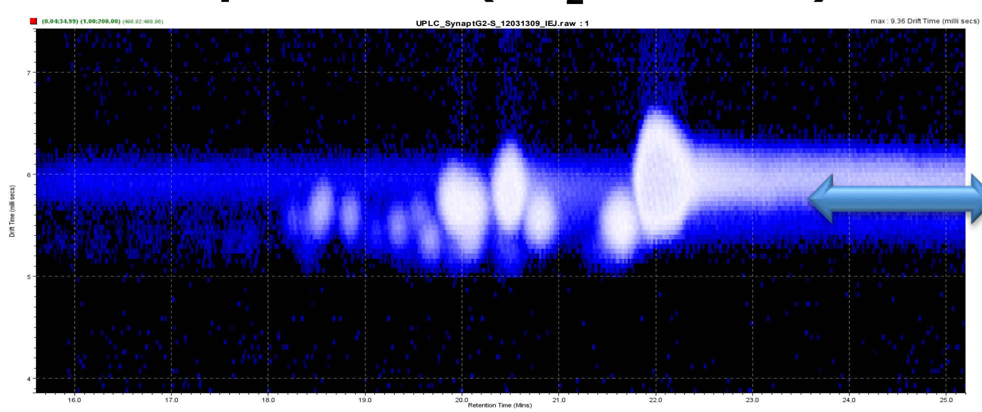



Dt = 9.2 ms

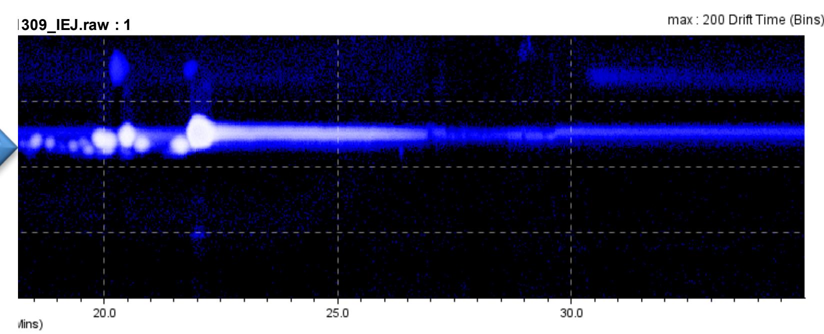
Δ = 2.7 ms

Dt = 6.5 ms

## PFOS Isomer Ion Mobility Separations ($CO_2$ Drift Gas)

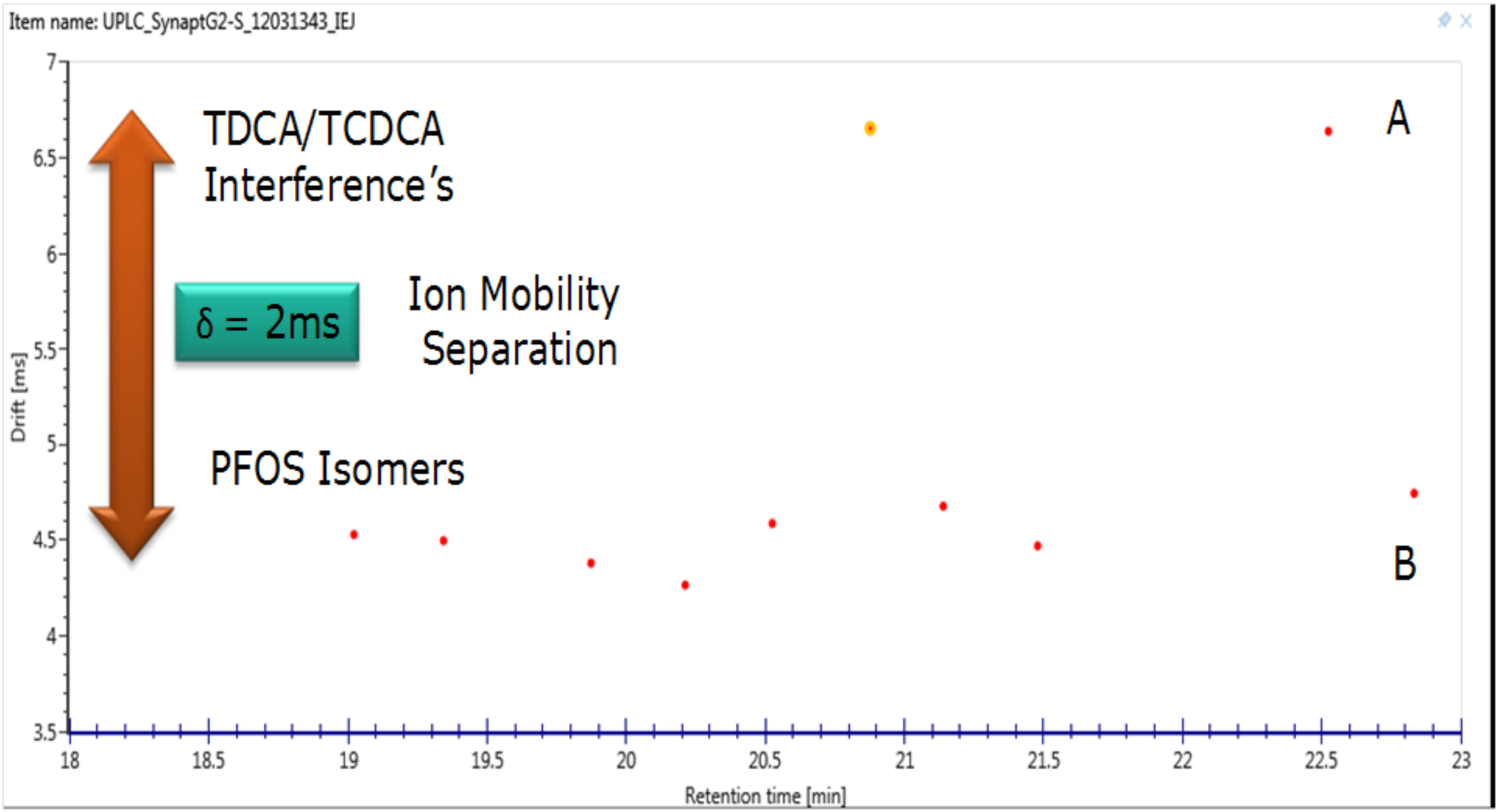



B



# Ion Mobility Separation

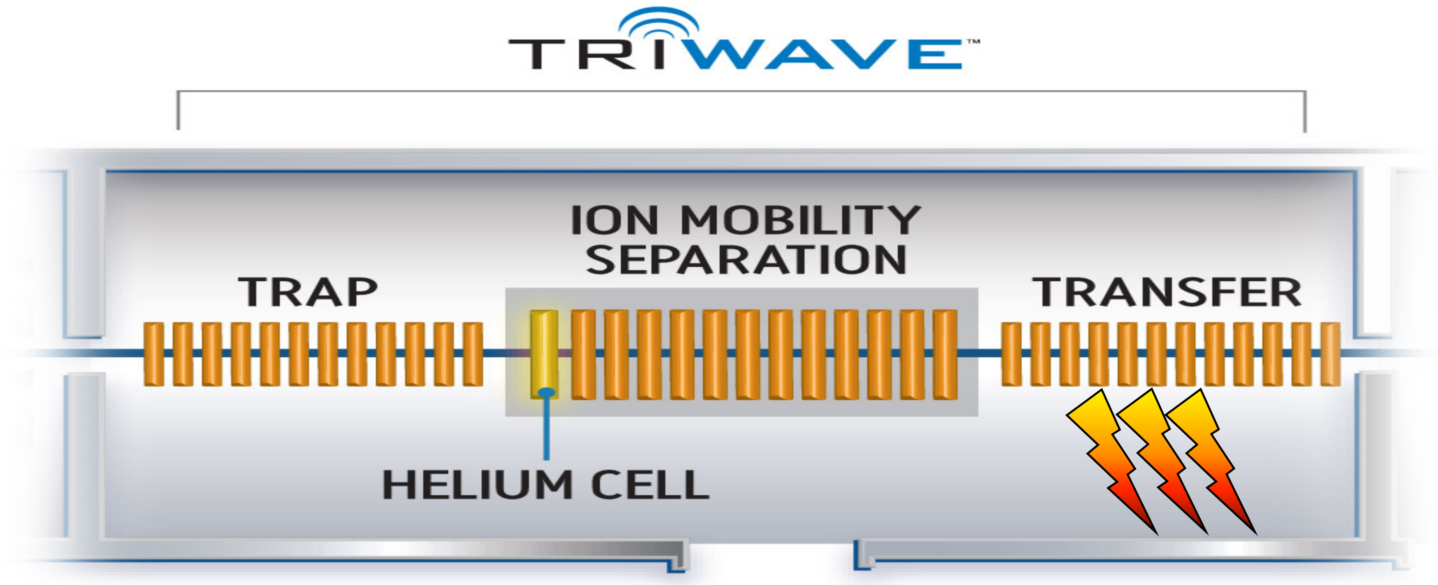
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

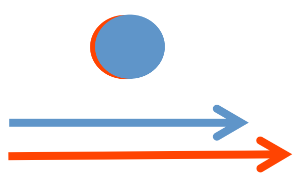



Component drift plot showing drift times vs retention time for nominally isobaric interferences (A) and PFOS isomers (B)

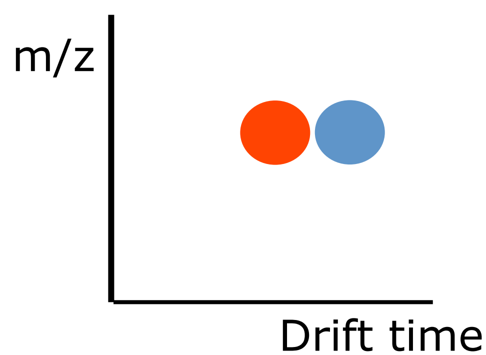
# Time and Mobility Aligned Fragmentation

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

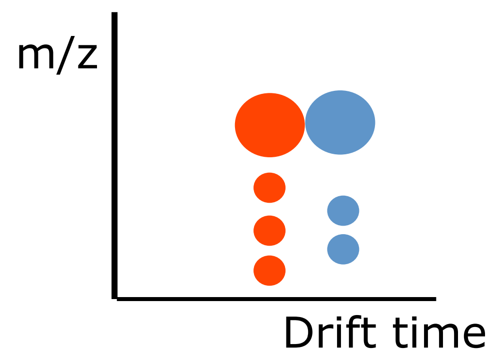


**Precursor ions**




**Precursor ions**



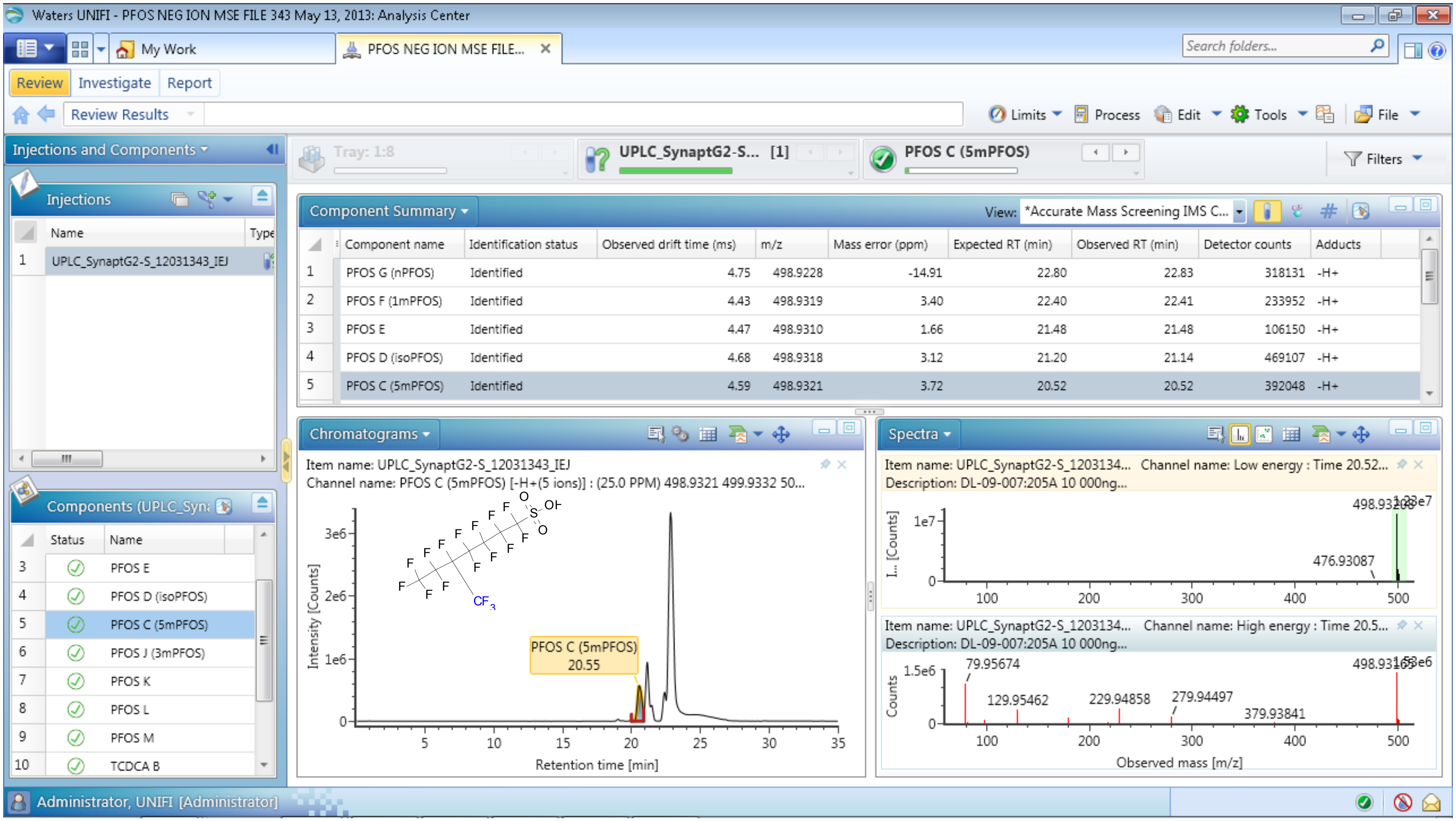

**Precursor and products are time aligned**

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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

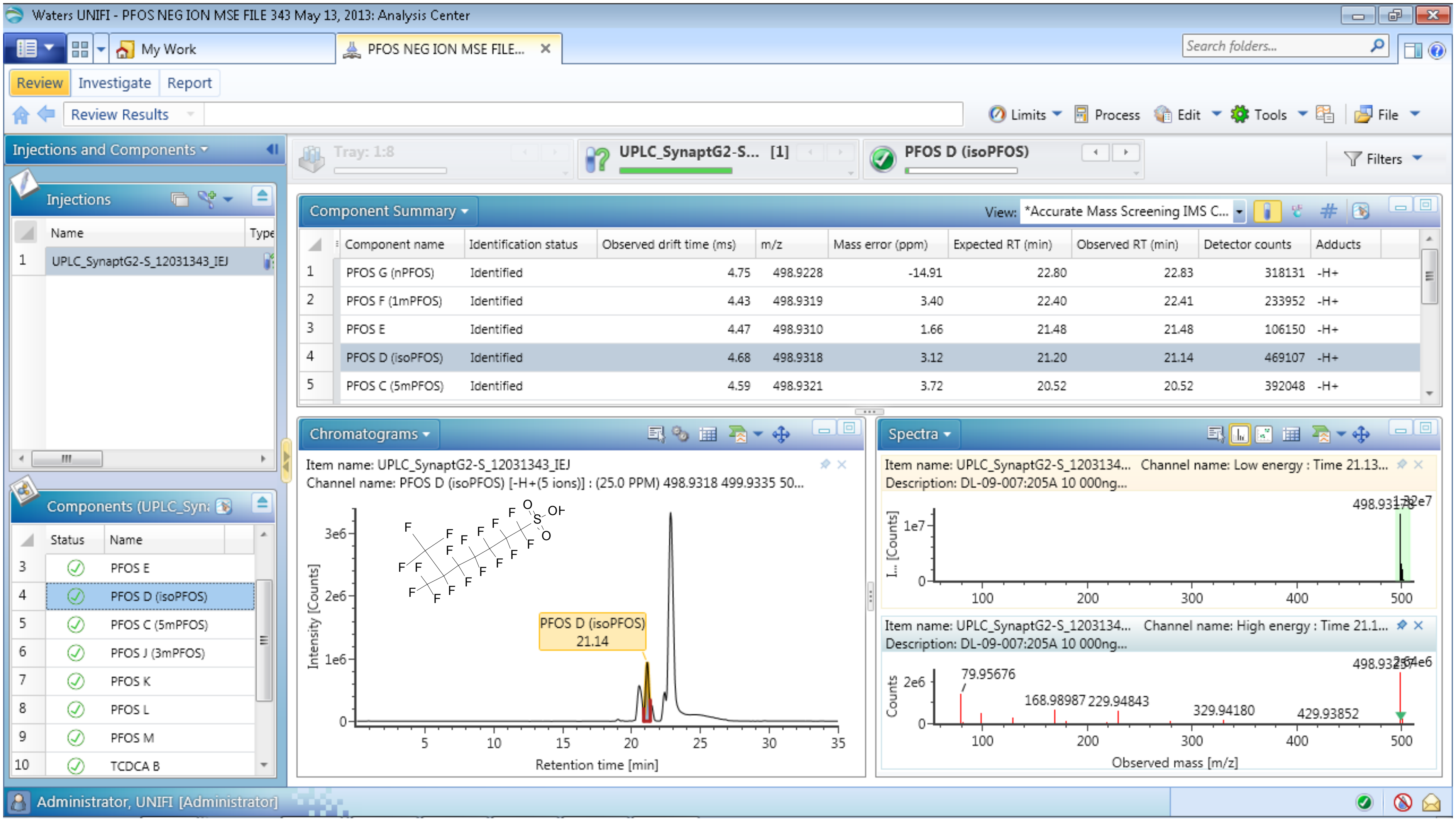
# PFOS (C) isomer, ion mobility resolved from isobaric interference TCDCA (A) at retention time 20.55 mins

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®



# PFOS (D) isomer, ion mobility resolved from isobaric interference TCDCA (A) at retention time 21.14 mins

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

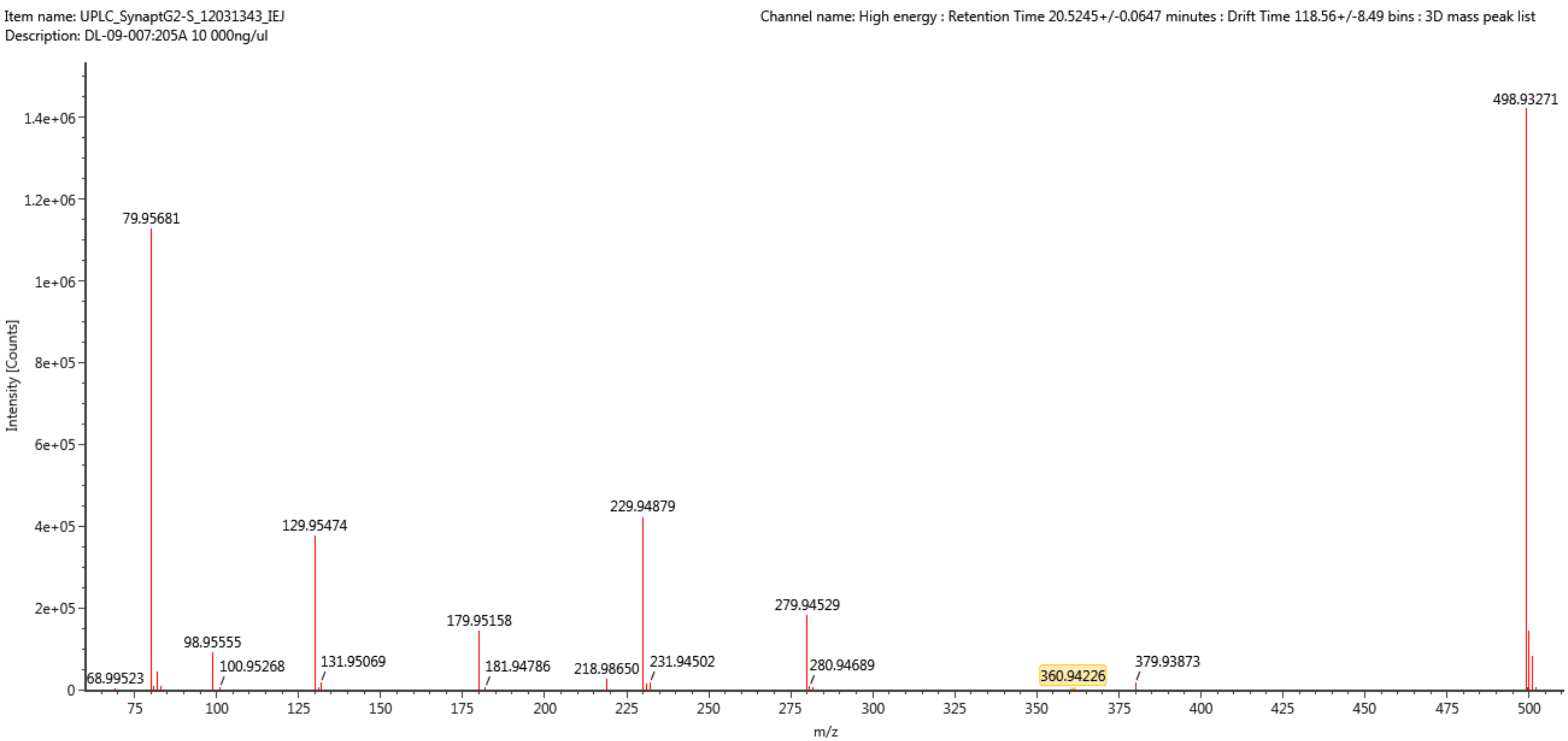


# Unassigned PFOS (E), ion mobility resolved from isobaric interference TDCA (A) at retention time 20.88 mins

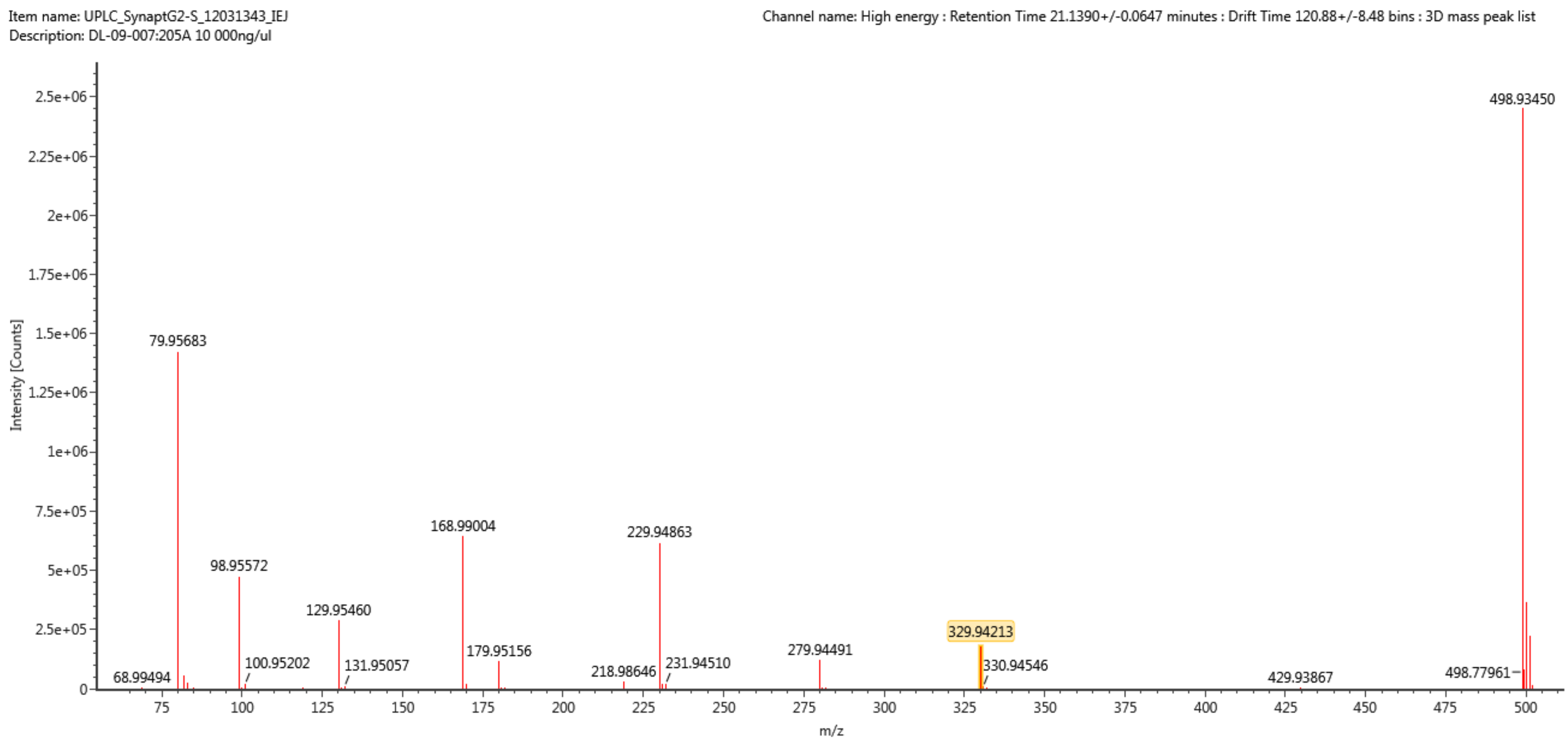
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

# PFOS ISOMER C

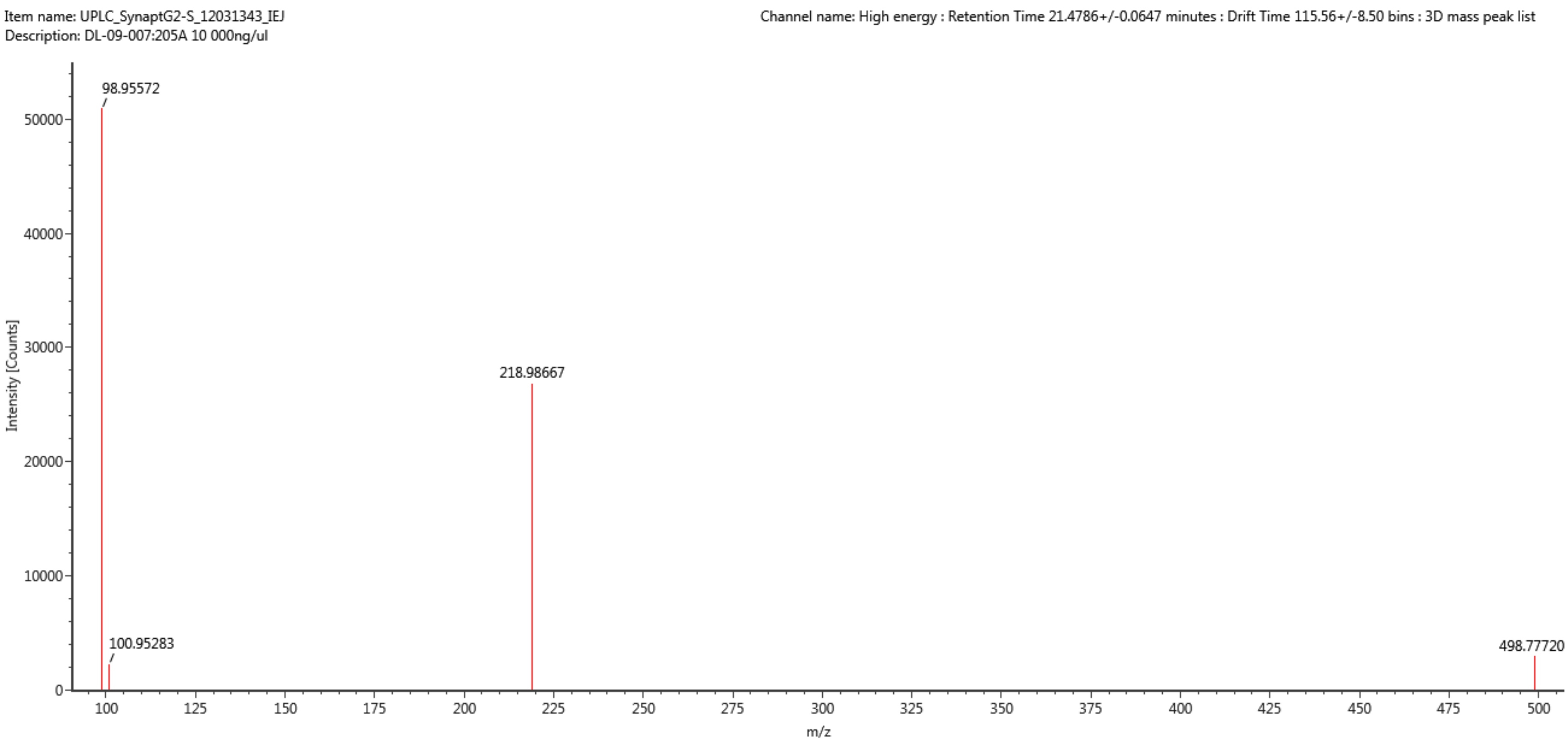
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®



# PFOS ISOMER D

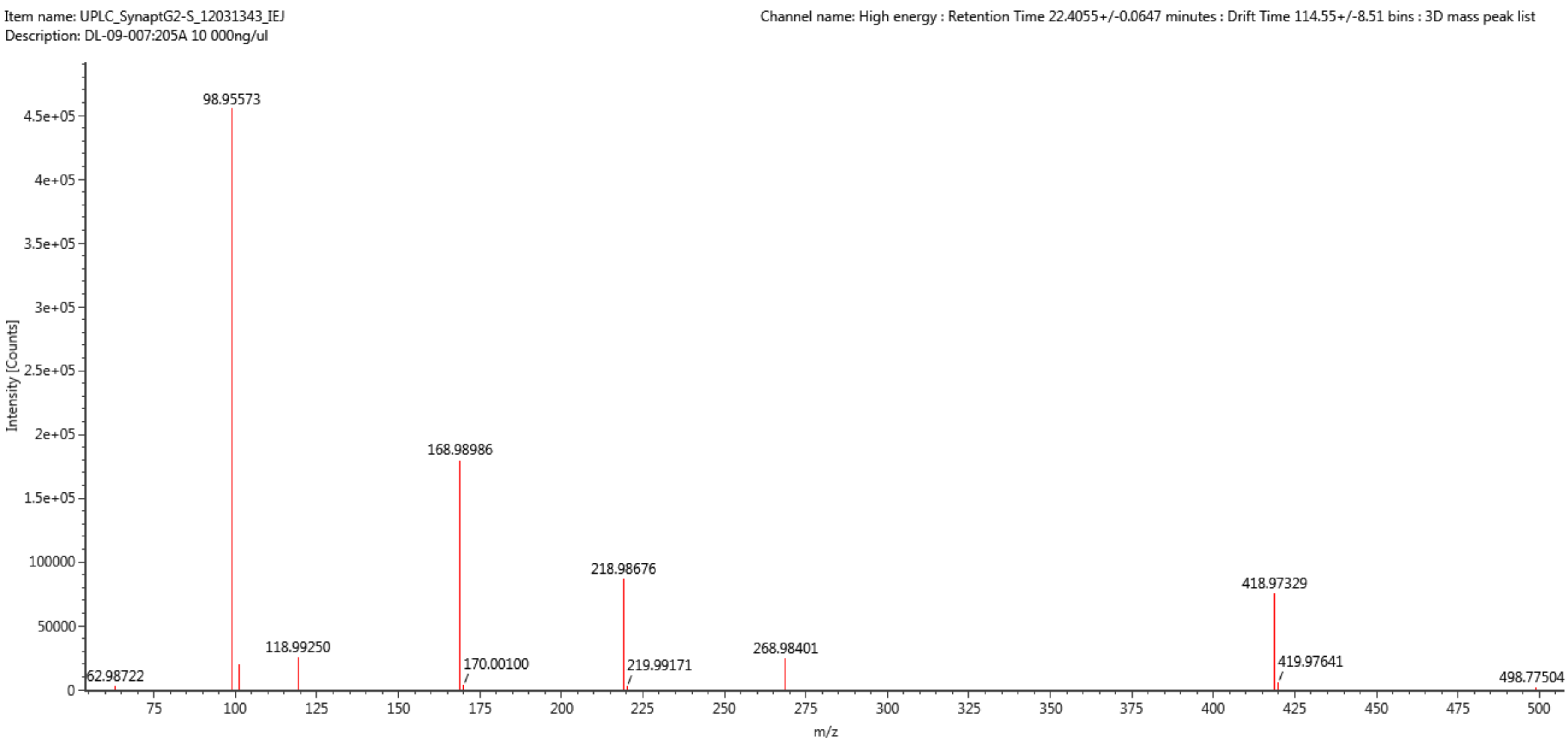


# PFOS ISOMER E

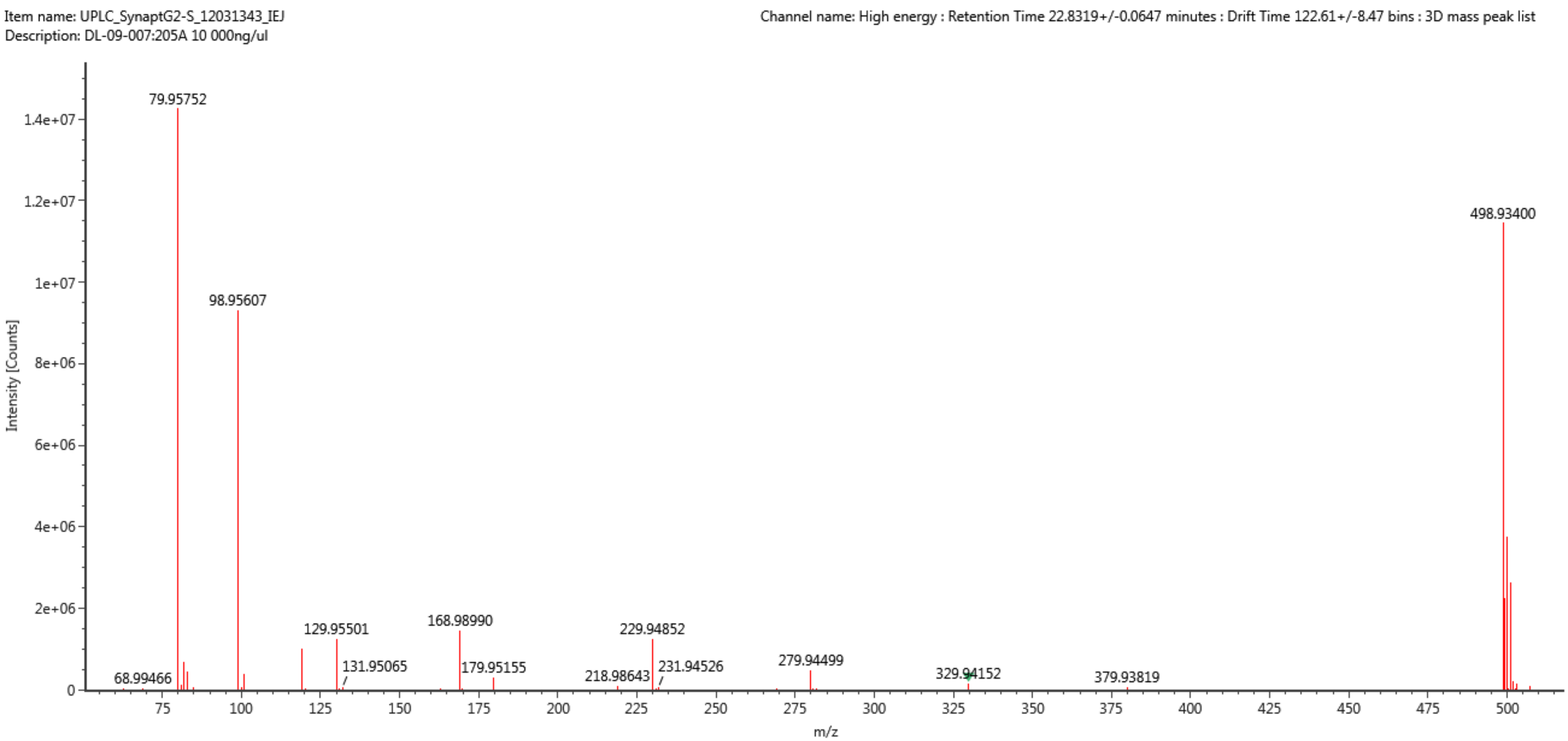


# PFOS ISOMER F

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®



# PFOS ISOMER G



# Summary for major PFOS isomers and matrix interferences

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

| | 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)<br><br>Mass Measurement Error | 4.59<br>4.27<br><br>3.4 ppm<br>-0.23 ppm | 4.68<br><br>3.66 ppm | 4.47<br><br>3.12 ppm | 4.43<br><br>3.72 ppm | 4.75<br><br>-14.91<br>(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)<br><br>Mass Measurement Error | 6.65<br><br>3.59 ppm | 6.64<br><br>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.***

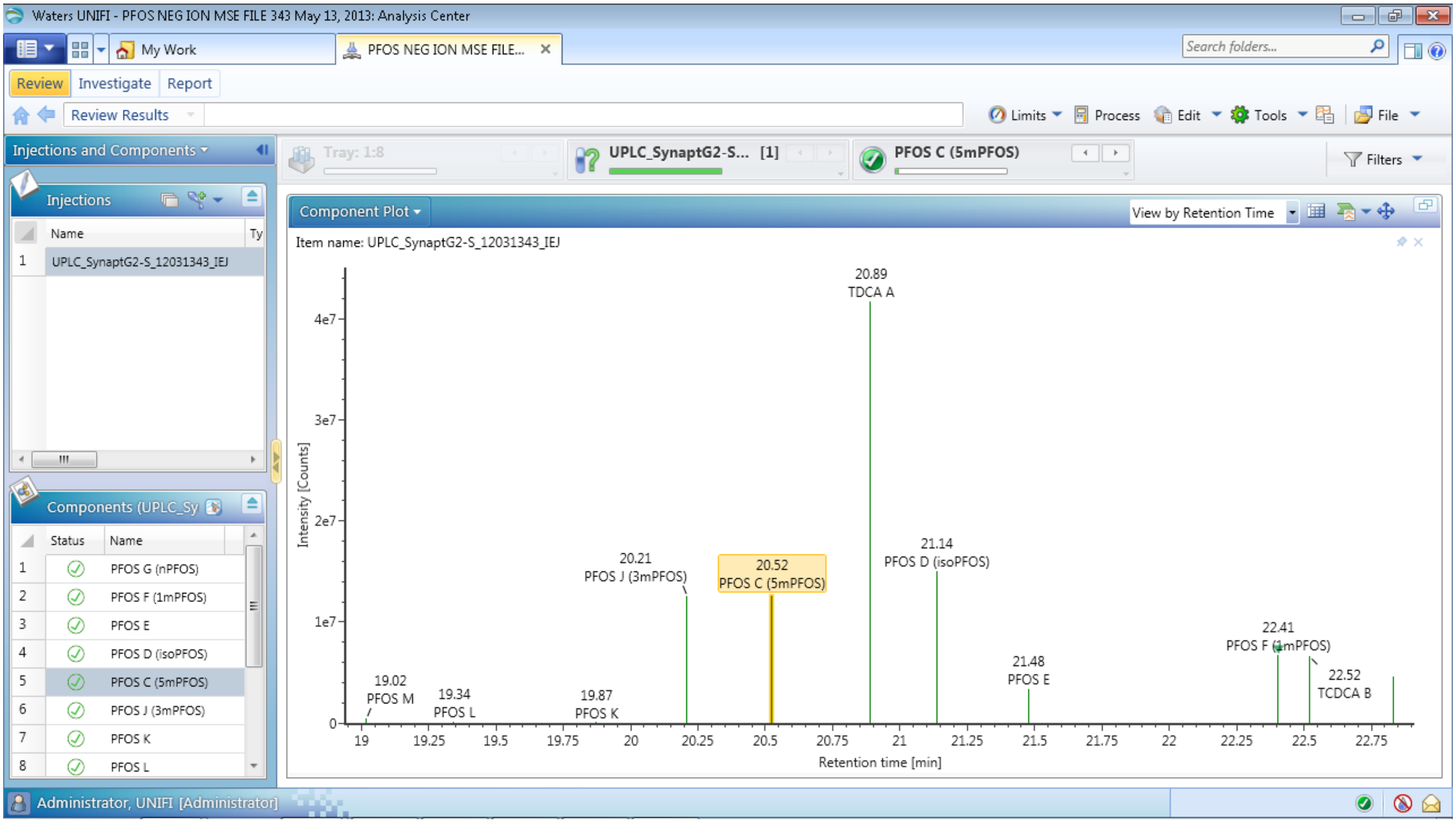
# Screening database

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

- 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.

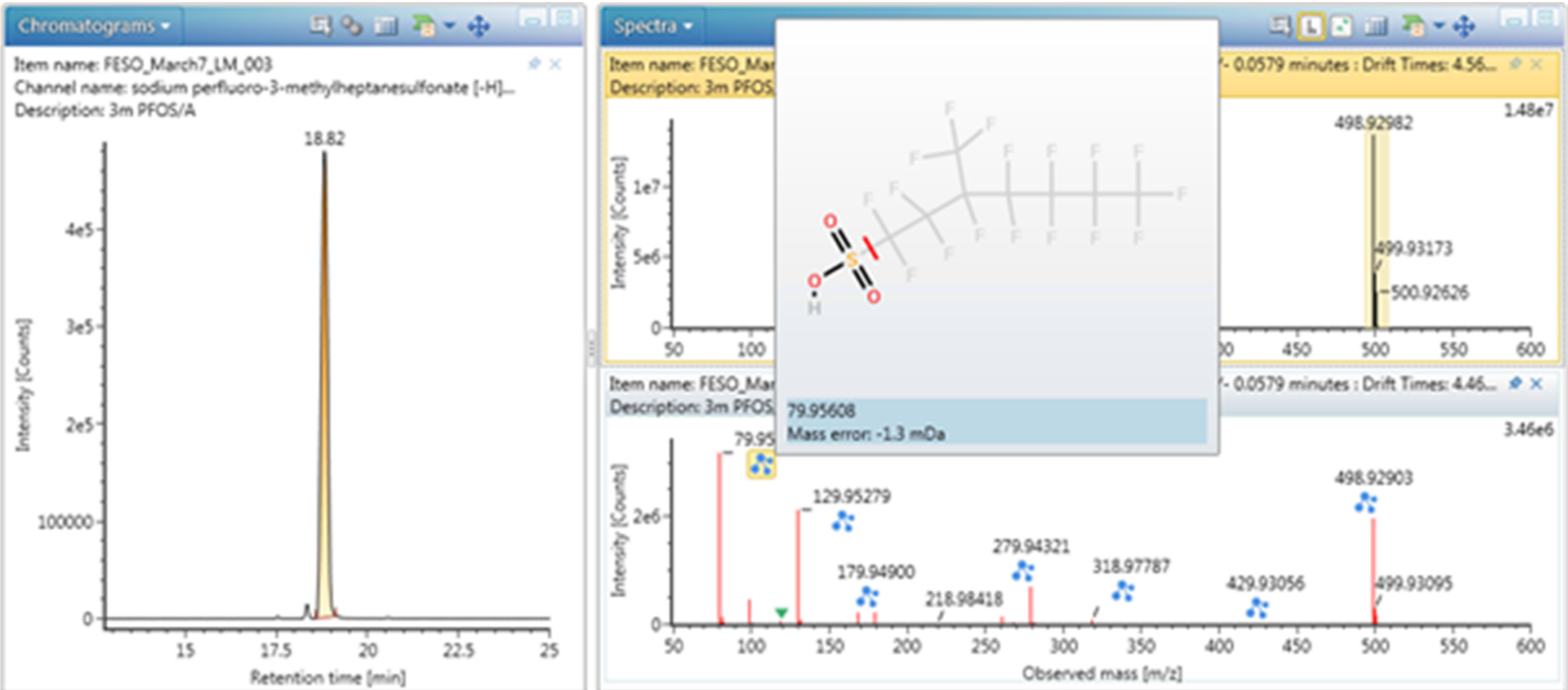
# Component summary for PFOS screening of mink liver extracts

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®



# Software enabled identification

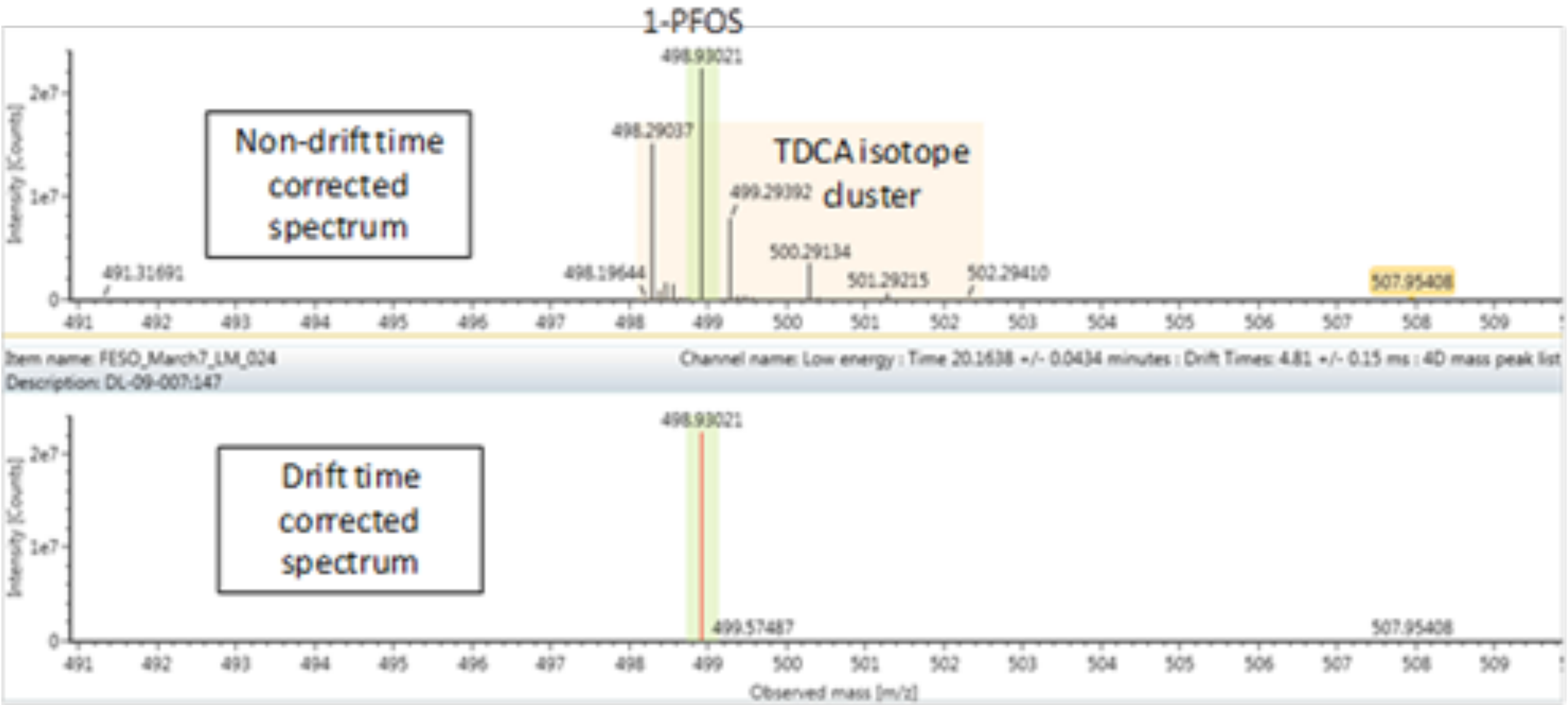
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®




**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

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®




**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.**

# Conclusions

- Co-eluting isobaric biological interference's TDCA and TCDCA have been resolved from PFOS isomers using ion mobility.
- Utilising $CO_2$ 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 $CO_2$ 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 isomer-specific analysis. This will be increasingly important as regulatory, scientific, and public awareness of the environmental impact of perflouralkylsubstances increases.

Acknowledgements

BENGT LUNDQVIST MINNE

Waters Centre of Innovation



Contact:
ingrid.ericson@oru.se

# Thank You!!!

# Questions???

Ken Rosnack
ken_rosnack@waters.com

Ingrid Ericson Jogsten and Bert van Bavel
(MTM Research Centre)

Mike McCullagh, Leonard Dillon, Mike Hodgkinson,
Lauren Mullin, & Jennifer Burgess
(Waters Corporation)