FluoroMatch Flow: An Open Source HRMS PFAS Annotation Tool

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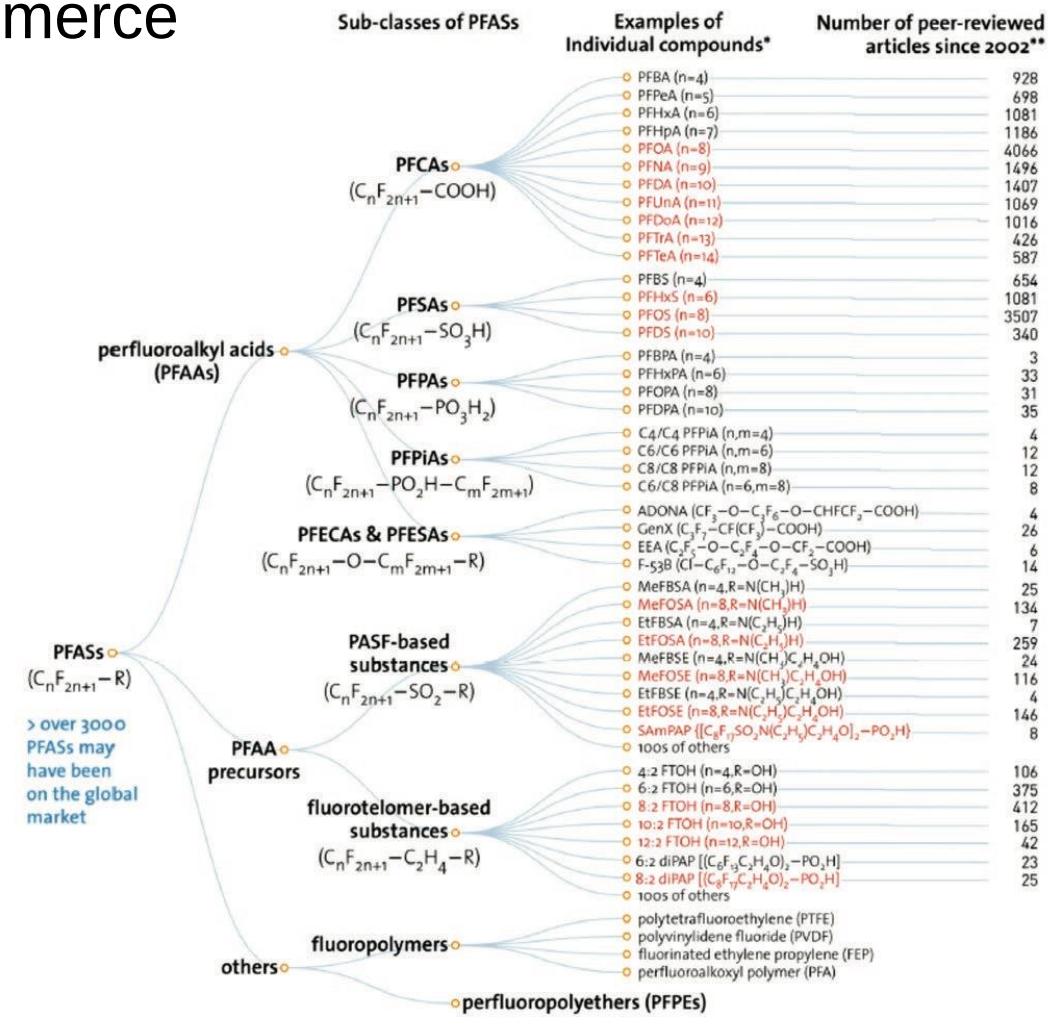
Agilent
Trusted Answers

PFAS Classifications and Terminology

EEA reports >4700 PFAS compounds in commerce

Common Acronyms

PFCA	**Perf**luoroalkyl**c**arboxylic **a**cid
PFOA	**Perf**luoro**o**ctanecarboxylic **a**cid
PFAS	**Perf**luoro**a**lkyl**s**ulfonate
PFOS	**Perf**luoro**o**ctane**s**ulfonate
PFASi	**Perf**luoro**a**lkyl**s**ulf**i**nate
FOSA	**Perf**luoro**o**ctane**s**ulfon**a**mide
FOSAA	**Perf**luoro**o**ctane**s**ulfon**a**mido**a**cetic acid
FOSE	**Perf**luoro**o**ctane**s**ulfonamido**e**thanol
FTOH	**F**luorinated **t**elomer alcohol (-**OH** functional group)
FTA	**F**luorinated **t**elomer **a**cid
FTUA	**F**luorinated **t**elomer **u**nsaturated **a**cid
FTS	**F**luorinated **t**elomer **s**ulfonate
PFAPA	**Perf**luoro**a**lkyl**p**hosphonic **a**cid
PFPi	**Perf**luoro**a**lkyl**p**hosph**i**nate
PAP	Mono-substituted **p**olyfluoro**a**lkyl**p**hosphate ester
diPAP	**Di**-substituted **p**olyfluoro**a**lkyl**p**hosphate ester
PFAI	**Perf**luoro**a**lkyl **i**odide
SFA	**S**emi**f**luorinated **a**lkane
FTI	**F**luorinated **t**elomer **i**odide
FTO	**F**luorinated **t**elomer **o**lefin
FTAC	**F**luorinated **t**elomer **ac**rylate



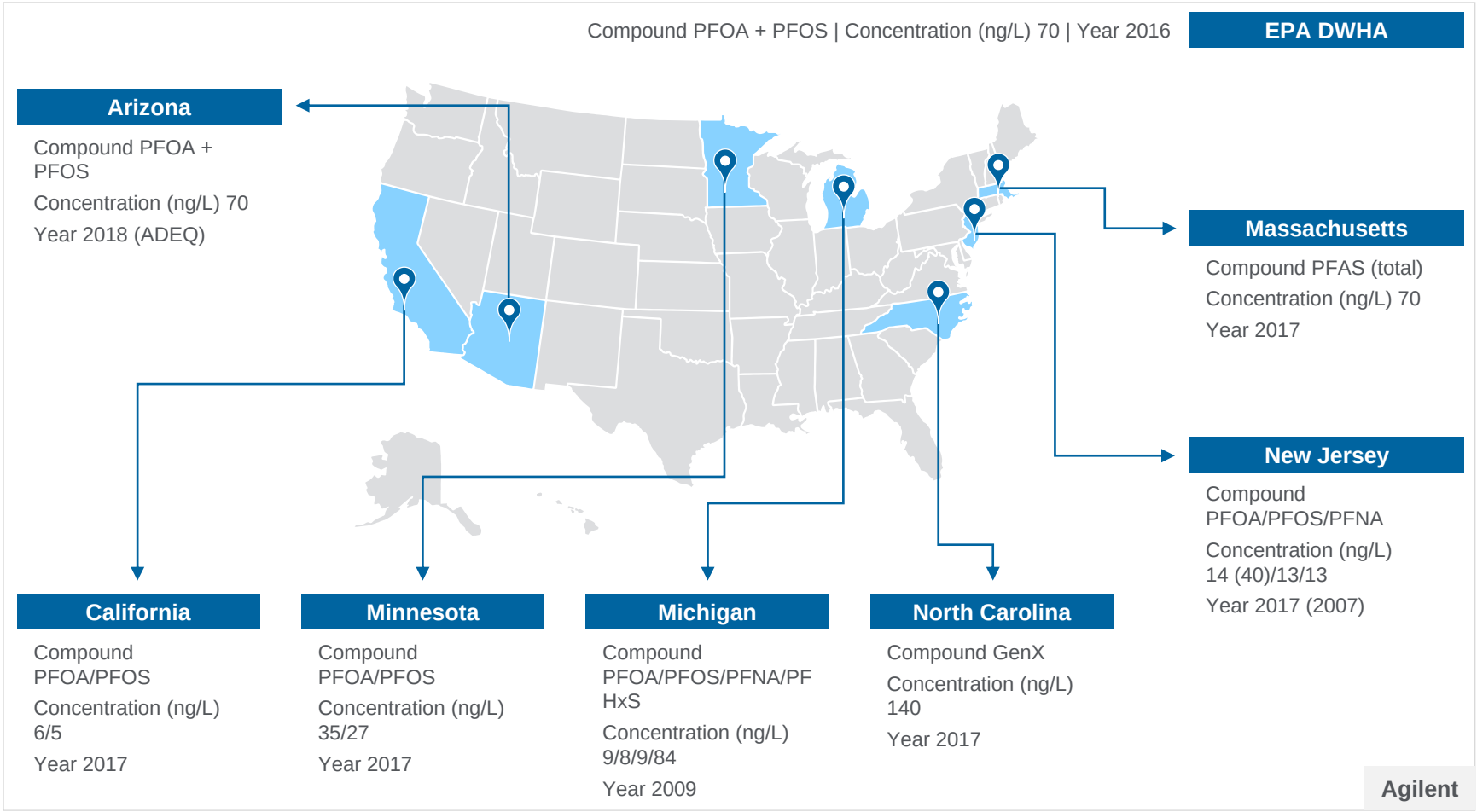
Wang, Z *et al.* (2017). *Environ. Sci. Technol.* 51, 2508-2518.
EEA: European Environmental Agency

Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water

PFAS regulation state-by-state – Numerical Limits

According to the Environmental Working Group, an advocacy group that tracks pollution and supports tougher standards:

- PFAS contamination has been found in at least 172 sites in 40 states
- More than 1,500 drinking water systems serving 110 Million people may be contaminated with perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS)



Environmental Protection Agency (EPA)

Recently Proposed Regulation for PFAS in Drinking Water and the Environment.

March 2021, the EPA issued a final regulatory determination to regulate perfluorooctanoic acid (**PFOA**) and perfluorooctane sulfonic acid (**PFOS**) as contaminants under **Safe Drinking Water Act (SDWA).**

March 29, 2023: The EPA proposed individual maximum contaminant levels (MCLs) of **4.0 ng/L or ppt** for **PFOA** and **PFOS**. EPA also proposed to use a Hazard Index (HI) approach to protecting public health from mixtures of **PFHxS, HFPO–DA and its ammonium salt, PFNA, and PFBS** because of their known and additive toxic effects and occurrence and likely co-occurrence in drinking water.

On April 13, the EPA proposed regulating seven PFAS, besides PFOA and PFOS, and their salts and structural isomers must be tested for all wastewater discharges, in soil and sediment for any construction, land permitting or infrastructure projects:

1. Perfluorobutanesulfonic acid (**PFBS**)
2. Perfluorohexanesulfonic acid (**PFHxS**)
3. Perfluorononanoic acid (**PFNA**),
4. Hexafluoropropylene oxide dimer acid (**HFPO–DA,** sometimes called GenX)
5. Perfluorobutanoic acid (PFBA)
6. Perfluorohexanoic acid (PFHxA)
7. Perfluorodecanoic acid (PFDA)

https://www.federalregister.gov/documents/2023/03/29/2023-05471/pfas-national-primary-drinking-water-regulation-rulemaking
https://www.federalregister.gov/documents/2023/04/13/2023-07535/addressing-pfas-in-the-environment

International Trend Is to Implement PFAS Regulation

Influence of exposure to perfluoroalkyl substances ...

https://www.sciencedirect.com/science/article/pii/S0160412017314952

Apr 01, 2018 · **Regulation** of **PFASs** in the US and Europe began earlier than in Asia; **PFASs** have been used with no **regulation** until recently in **Korea** (Webster, 2010). According to the international trend of **PFAS regulation**, we anticipate that **PFAS** exposure among people in **Korea** increased from the 1970s to the early 2000s, reaching a plateau around the mid 2000s, and then declined during the early 2010s.

Cited by: 46 **Author:** Sung-Hee Seo, Min-Hui Son, Sung-Deuk Ch...

Publish Year: 2018

PFAS in biosolids: A review of international regulations

Insights into global PFAS management and the Australian context

by Water Source — 03/03/2021 in Technical Papers, Public Health

28 min read

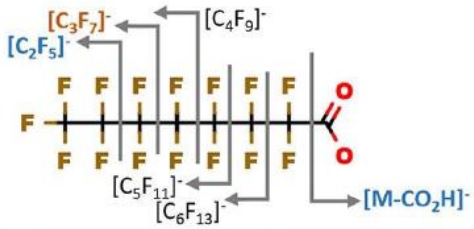
H Hall, D Moodie, C Vero.

First published in *Water e-Journal* Vol 5 No 4 2020.

- Sung-Hee Seo et al., Influence of exposure to perfluoroalkyl substances (PFASs) on the Korean general population: 10-year trend and health effects, Environment International, V. 113, 2018, Pages 149-161, https://doi.org/10.1016/j.envint.2018.01.025.
- https://watersource.awa.asn.au/publications/technical-papers/pfas-in-biosolids-a-review-of-international-regulations/

FluoroMatch Flow for Non-Targeted PFAS Annotation

>4700 Per/Polyfluoroalkyl substances (PFAS) compounds in commerce!*



Generate MS/MS spectra based on fragmentation rules or simply use the default 7000+ MS/MS spectra for annotation and processing of your nontargeted PFAS LC/Q-TOF data.

FluoroMatch Flow** simplifies the process with **drag-and-drop simplicity**. Import the correctly named files into FluoroMatch Flow, select an output directory, and click Run. The software performs **file conversion, peak picking, blank filtering, identification, and combining positive and negative mode data.

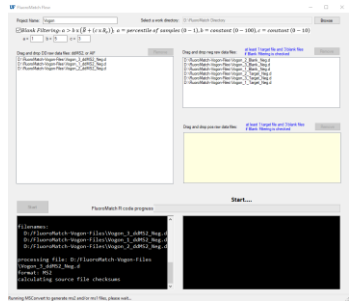
***FluoroMatch Generator** can be used to generate **MS/MS spectra based on class/type specific fragmentation rules** and SMILES strings indicating repeating units.*

Workflow

Separate & Detect

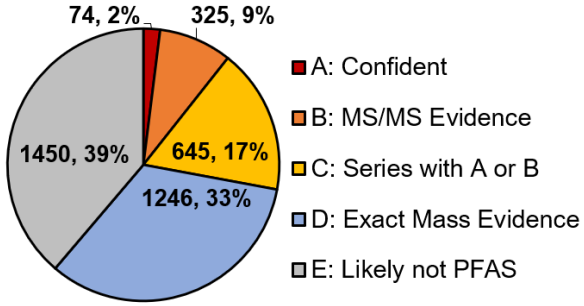
MassHunter ESI negative LC/Q-TOF data files can be dragged-and-dropped into FluoroMatch Flow.

FluoroMatch Flow



Simply follow the software's progress in the command-line interface boxes.

Automated Annotation



Thousands of PFAS automatically detected in aqueous film forming foam (AFFF) using an Agilent 6540 LC/Q-TOF.

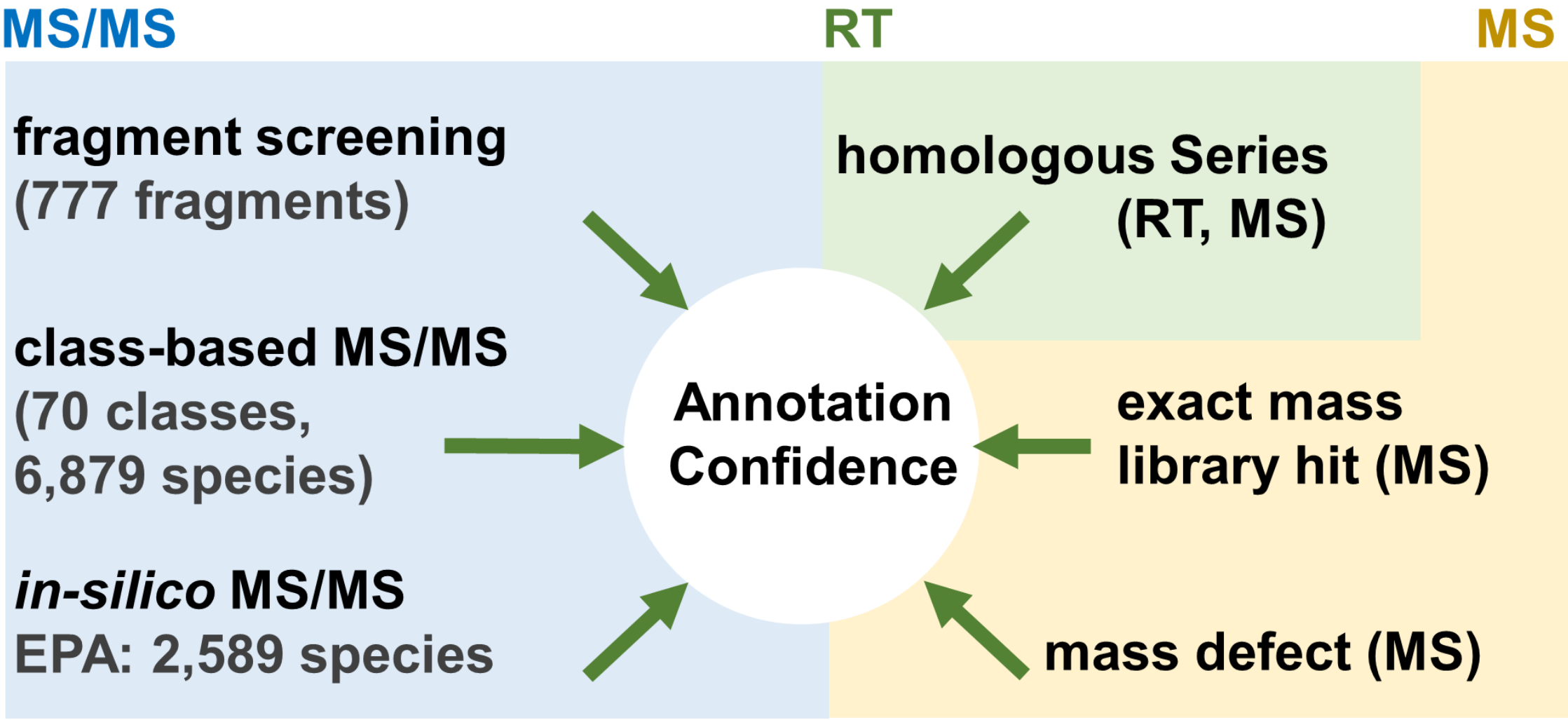
Advantages

- Screen 7000+ PFAS across 70+ subclasses.
- Automated – requires only a few clicks.
- FluoroMatch Flow automatically integrates retention time, mass, and fragmentation for confident PFAS annotation/identification.
- Generate predictive libraries based on fragmentation rules optimized on Agilent hardware.

- Future FluoroMatch projects to integrated ion mobility and GC/MS data.
- Future FluoroMatch Generator project to generate *in silico* libraries with predicted retention times.

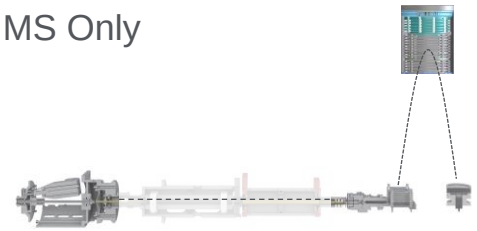
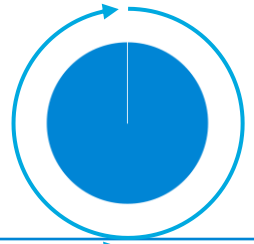
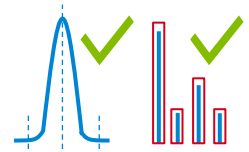
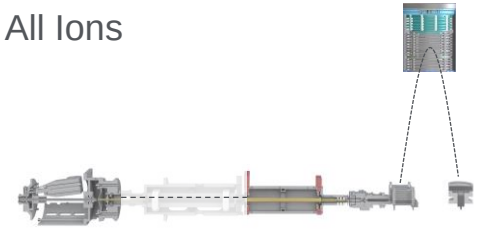
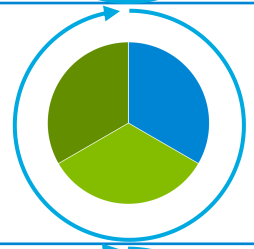
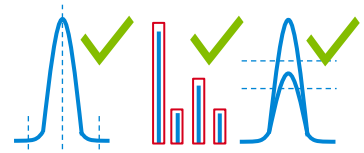
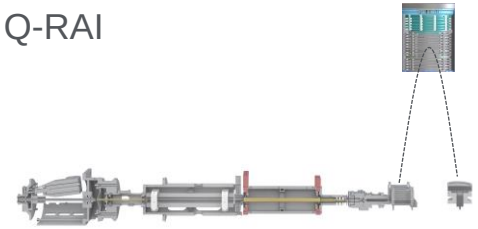
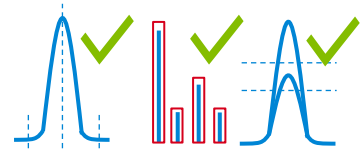
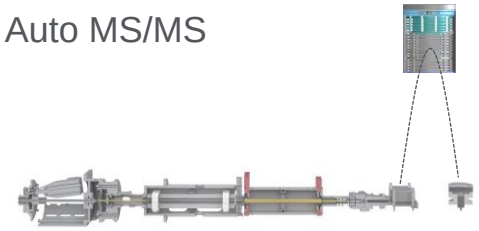
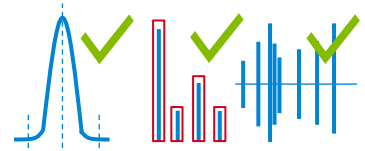
*https://www.eea.europa.eu/publications/emerging-chemical-risks-in-europe
https://comptox.epa.gov/dashboard/chemical-lists/pfasmaster
http://innovativeomics.com/software/fluoromatch-flow-covers-entire-pfas-workflow/

Confident PFAS Annotation Requires RT, MS, and MS/MS



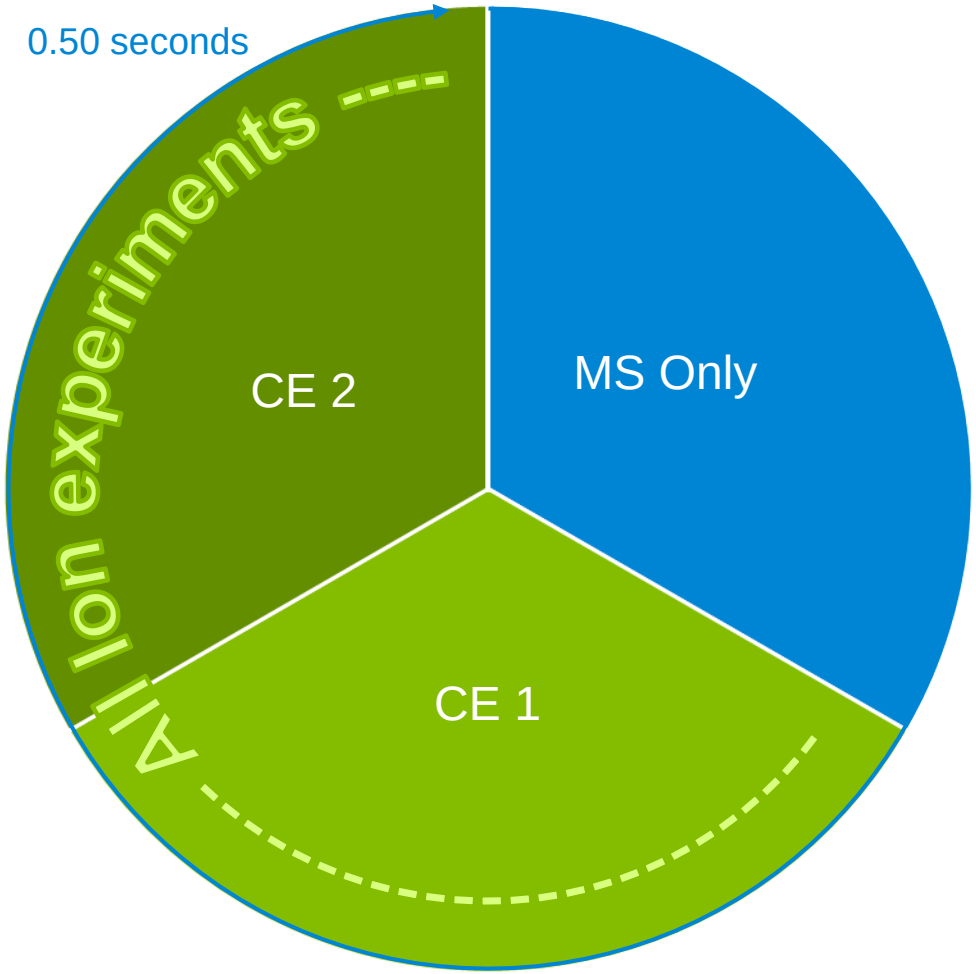
Agilent's Q-TOF Data Acquisition Strategies

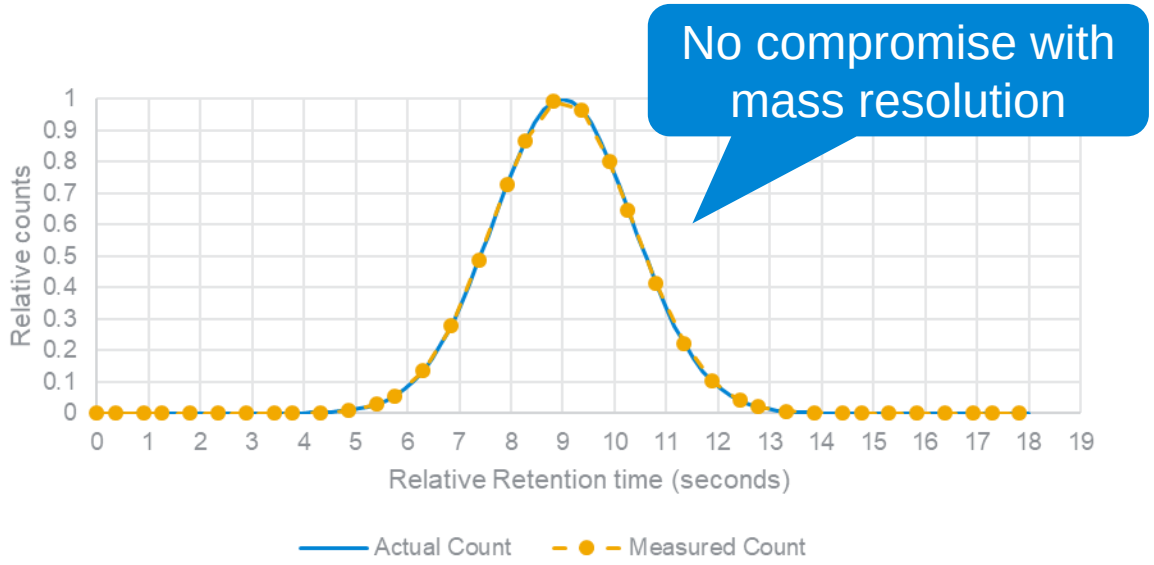
Balance between sensitivity and fragment confirmation quality

Acquisition Mode	Cycle distribution	Acquisition rate	Cycle time (seconds)	Data points per 6 s peak	Mass Resolution	DA Workflow
MS Only 		2 Hertz	0.50	12	>**30,000** FWHM at m/z **118** and >**60,000** FWHM at m/z **2,722** of spectral peak, with all instrument parameters set by autotune	
All Ions 		6 Hz for MS 6 Hz for All Ions	0.50	12		
Q-RAI 		8 Hz for MS 24 Hz for Q-RAI segments	0.49	12		
Auto MS/MS 		8 Hz for MS 16 Hz for MS/MS	0.54	11		

Data Acquisition Strategies

All Ions instrument cycle time

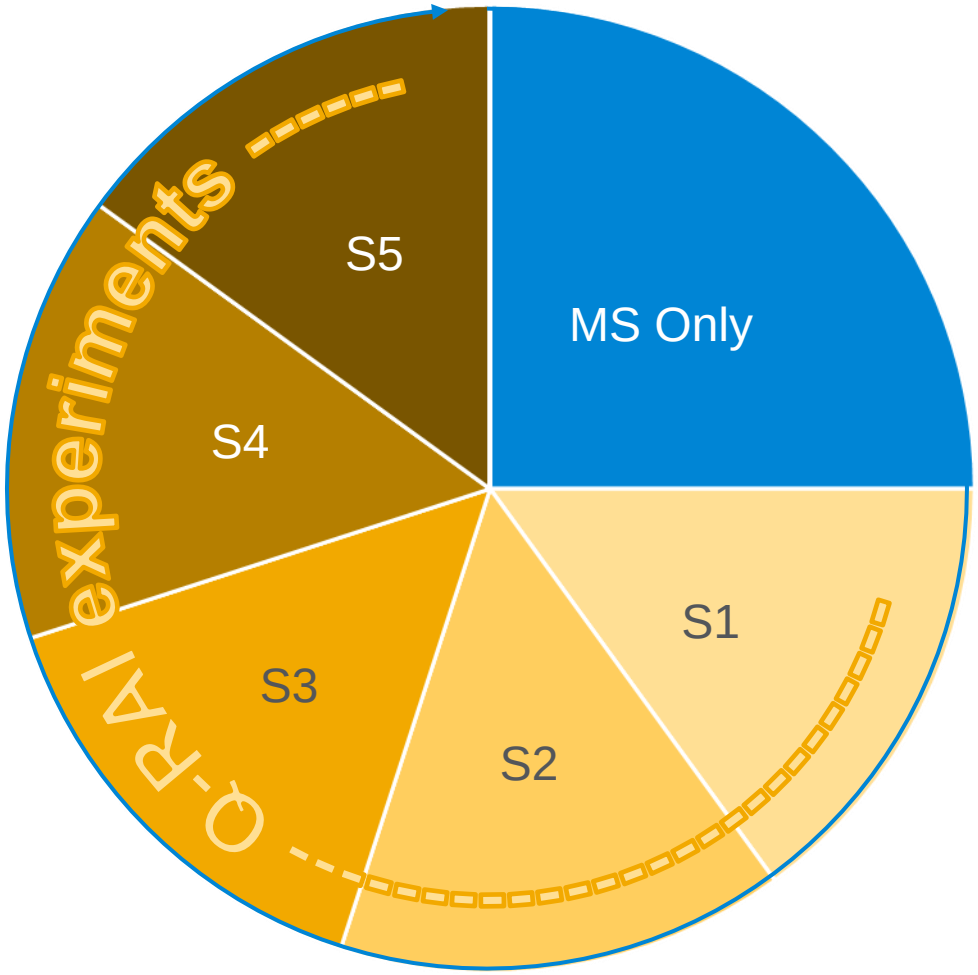




Parameter	Unit	Value
Typical chromatographic peak width	(seconds)	6
Number of CEs	(CEs)	3
MS acquisition rate	(spectra/sec)	6
Calculated cycle time	(seconds)	0.50
Data points per peak		**12.0**

Data Acquisition Strategies

Q-RAI instrument cycle time

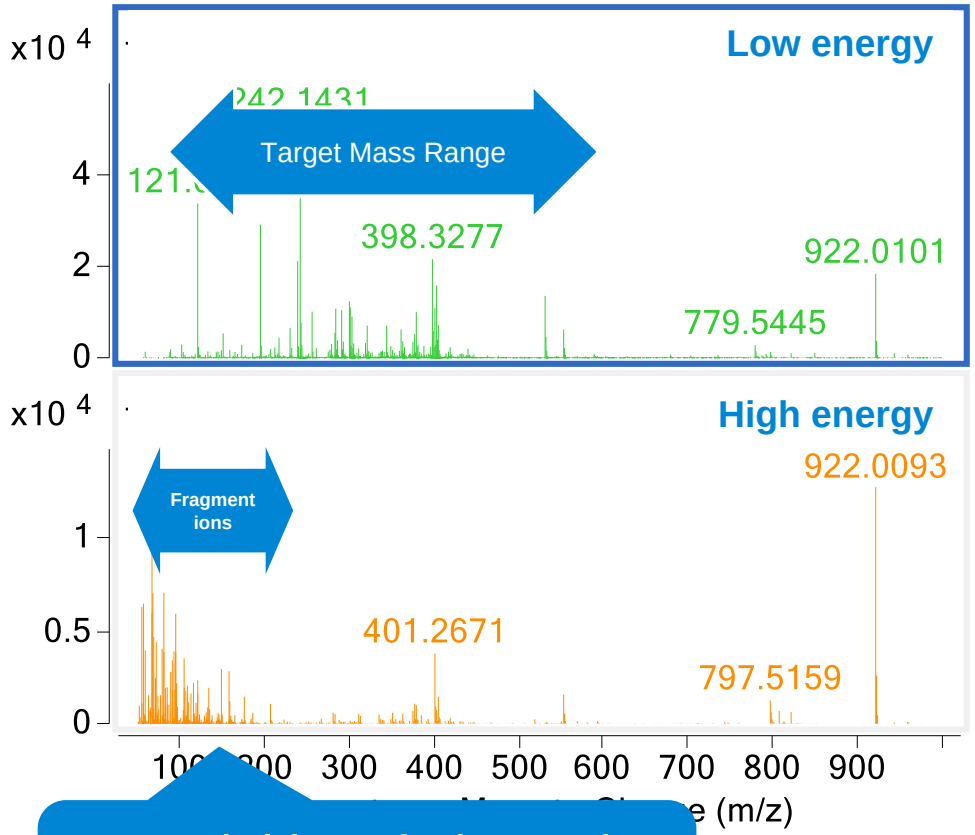


Parameter	Unit	Value
Typical chromatographic peak width	(seconds)	6
Number of Q-RAI segments	(segments)	5
MS acquisition rate	(spectra/sec)	8
MS/MS acquisition rate	(spectra/sec)	24
Calculated cycle time	(seconds)	0.49
Data points per peak width		**12**

Q-RAI = Quadrupole Resolved All Ions

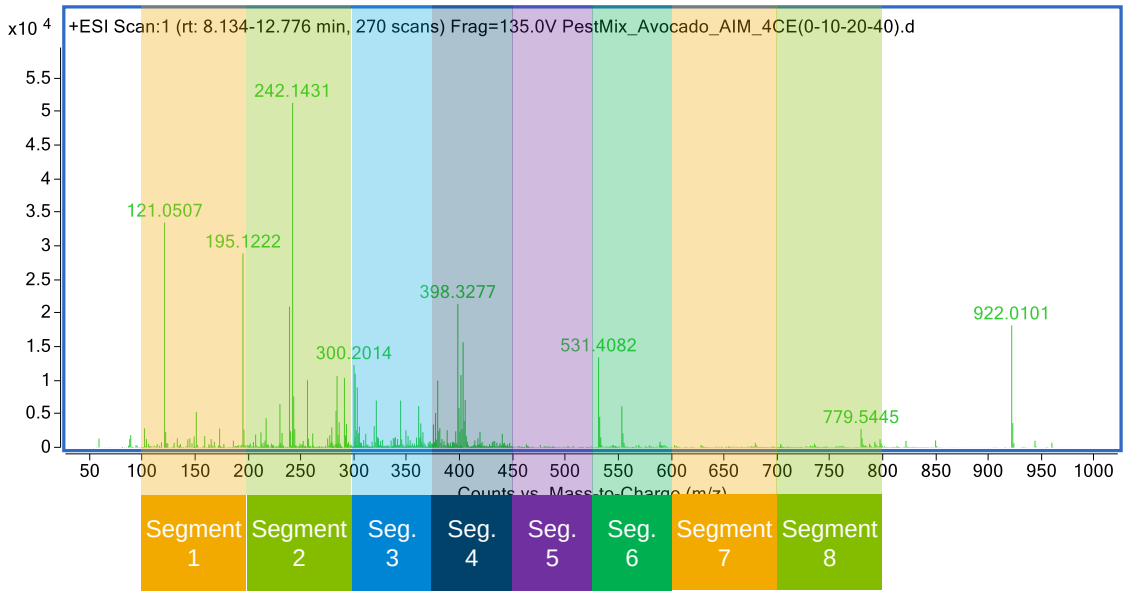
Data Acquisition Strategies

All Ions



Potential interfering noise in low m/z region

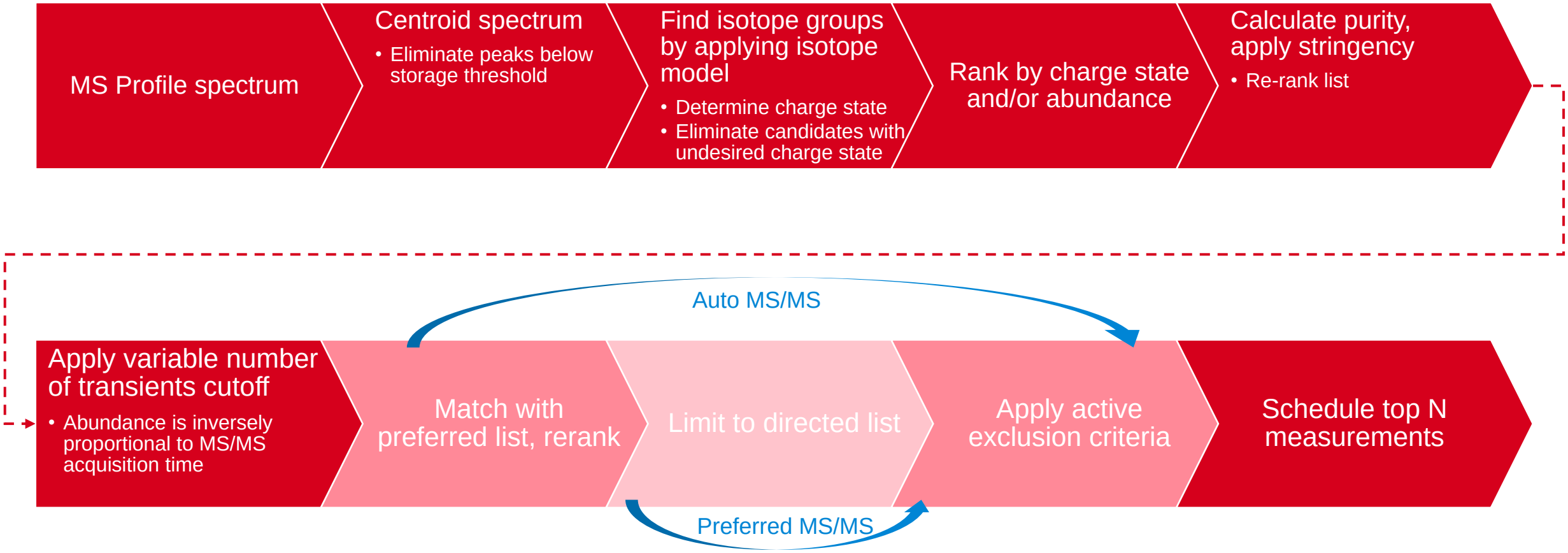
Quadrupole Resolved All Ions (Q-RAI)



Quadrupole filtering of wide bands to reduce complexity of the fragment spectra.

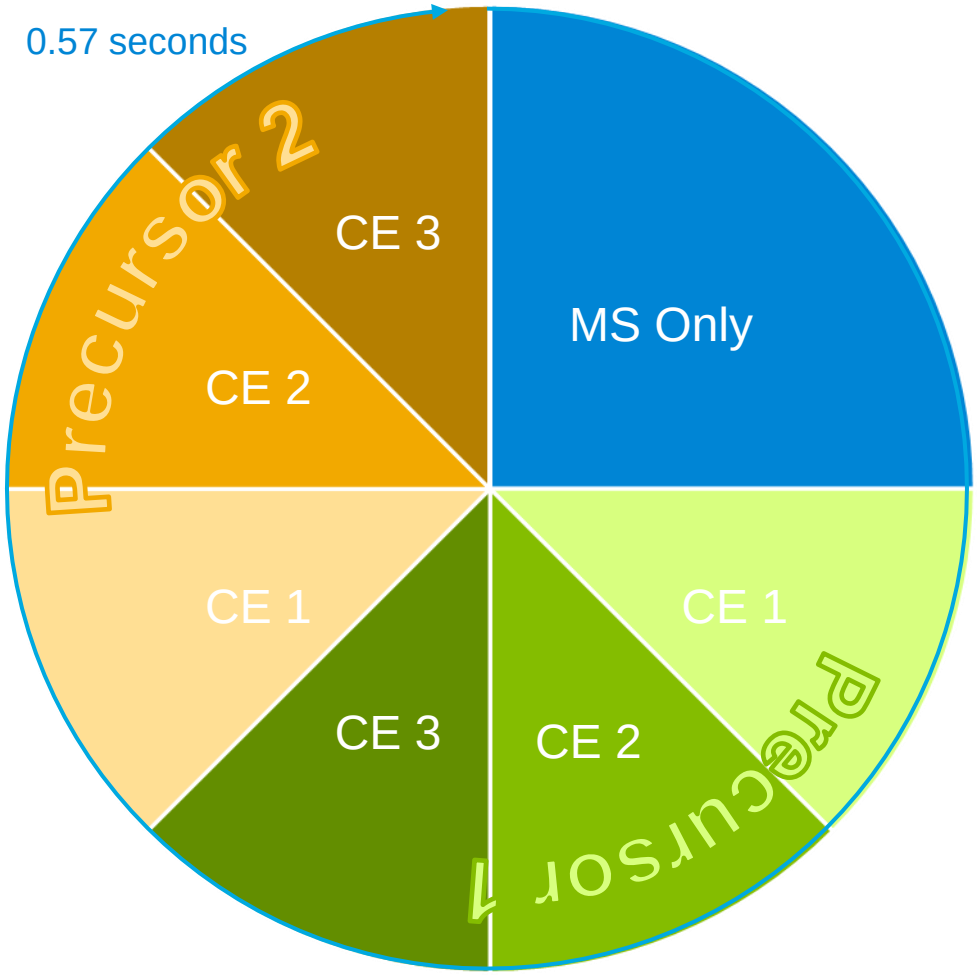
Data Acquisition Strategies

Auto MS/MS: What is it?

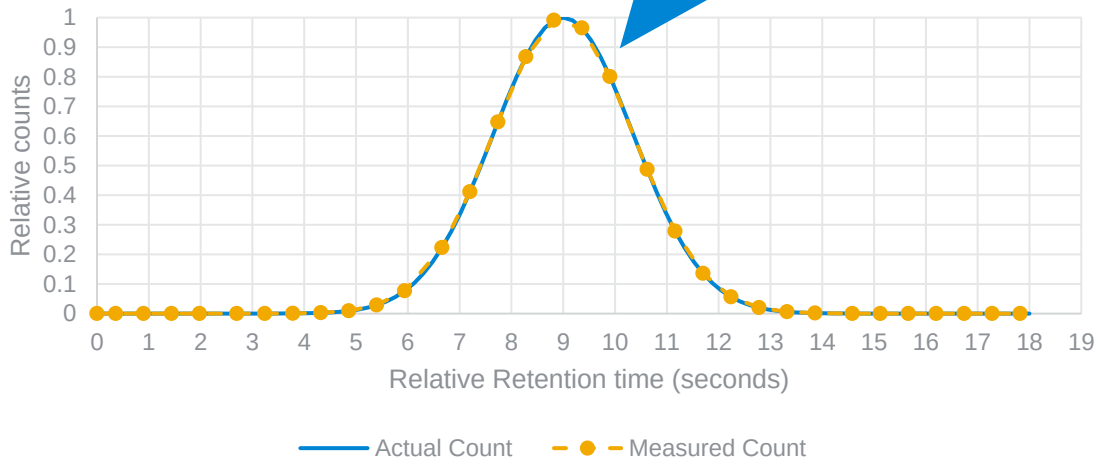


Data Acquisition Strategies

Auto MS/MS instrument cycle time



No compromise with mass resolution

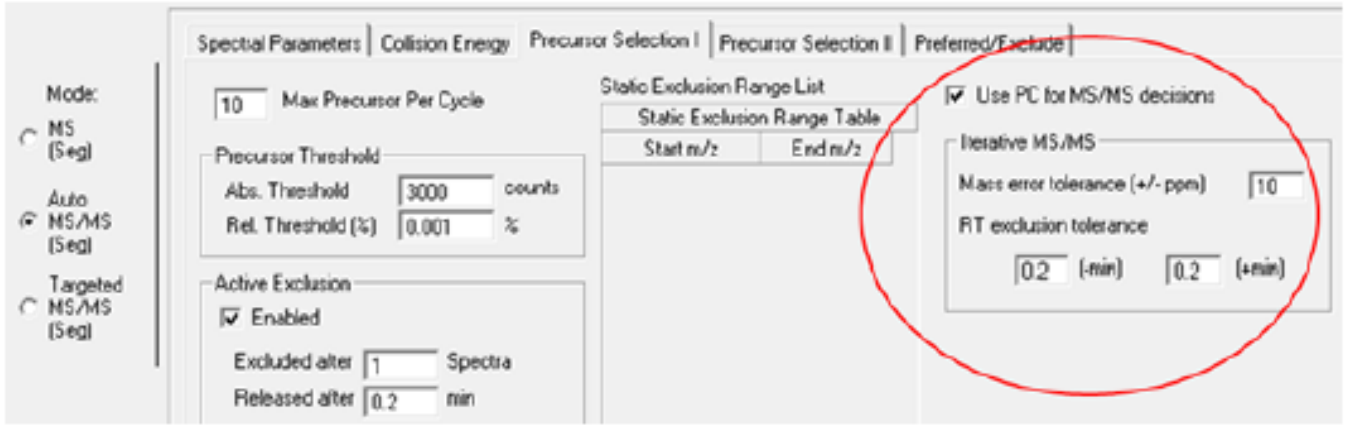


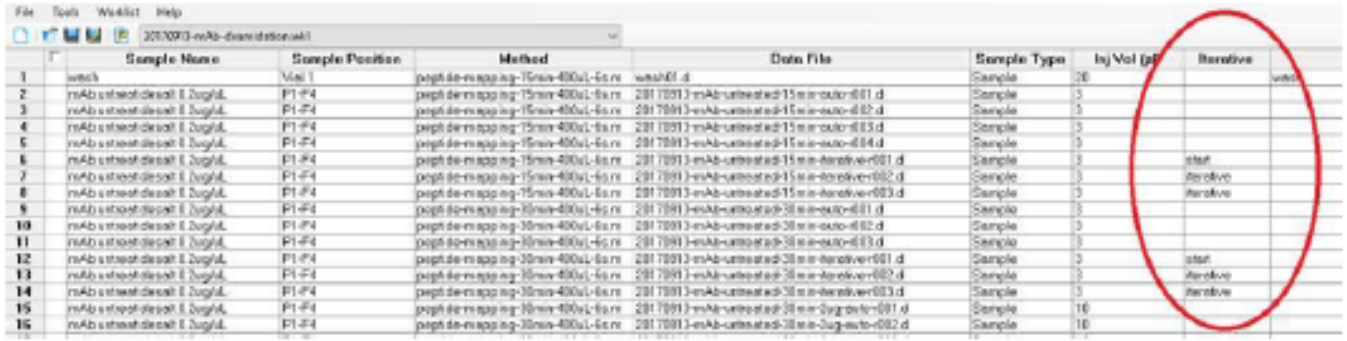
Parameter	Unit	Value
Typical chromatographic peak width	(seconds)	6
Number of MS/MS precursors	(Precursors)	2
Number of CE's/precursor	(CEs)	3
MS acquisition rate	(spectra/sec)	8
MS/MS acquisition rate	(spectra/sec)	16
Calculated cycle time	(seconds)	0.54
Data points per peak		**11**

Acquisition Modes Supported by FluoroMatch

Iterative MS/MS

To trigger Iterative MS/MS, you need to do two things. First setup parameters in LC-MS method, then trigger iterative method in worklist. You need to add the "Iterative" column to the worklist. By default, it is blank in the column. To trigger iterative, add the word "start" or "reset". Any word in the following rows will continue the precursor exclusion on a rolling basis. A blank row or standby script will put aside the exclusion list (i.e. not be used but also not clean up) until it meets another "start" or "reset".



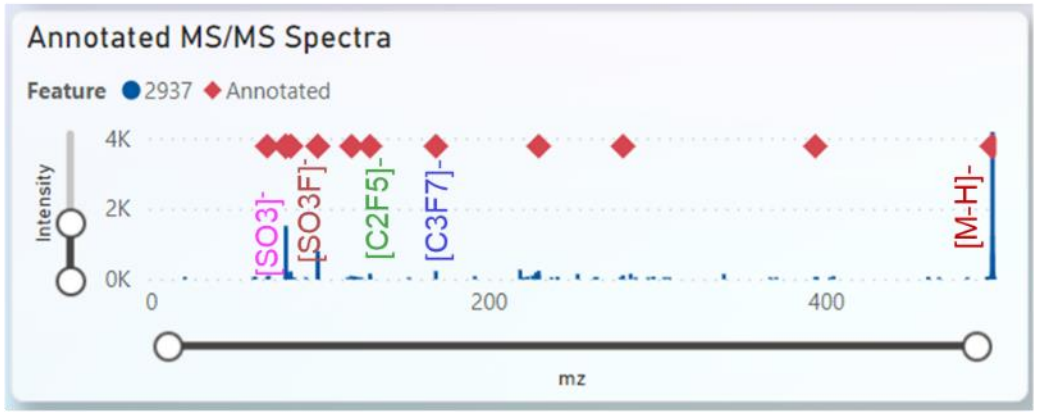


Acquisition Modes Supported by FluoroMatch

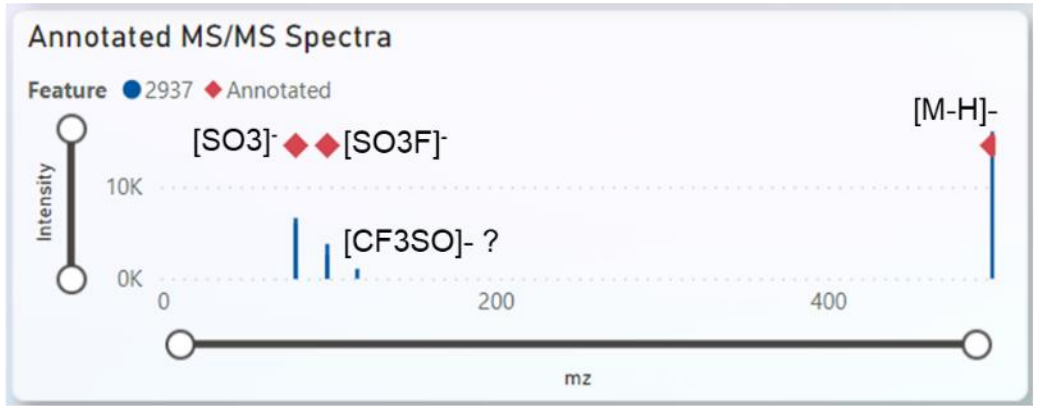
Auto MS/MS (Iterative MS/MS) and All Ions

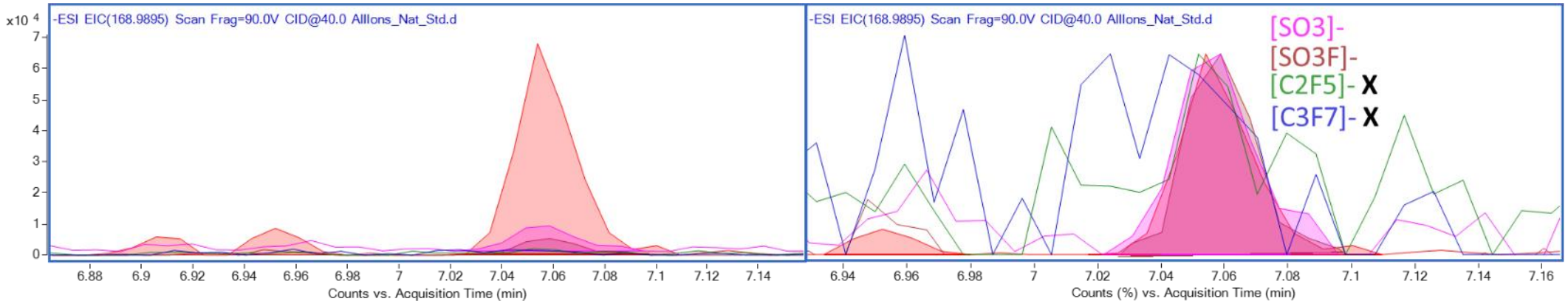
PFSA C8

DDA

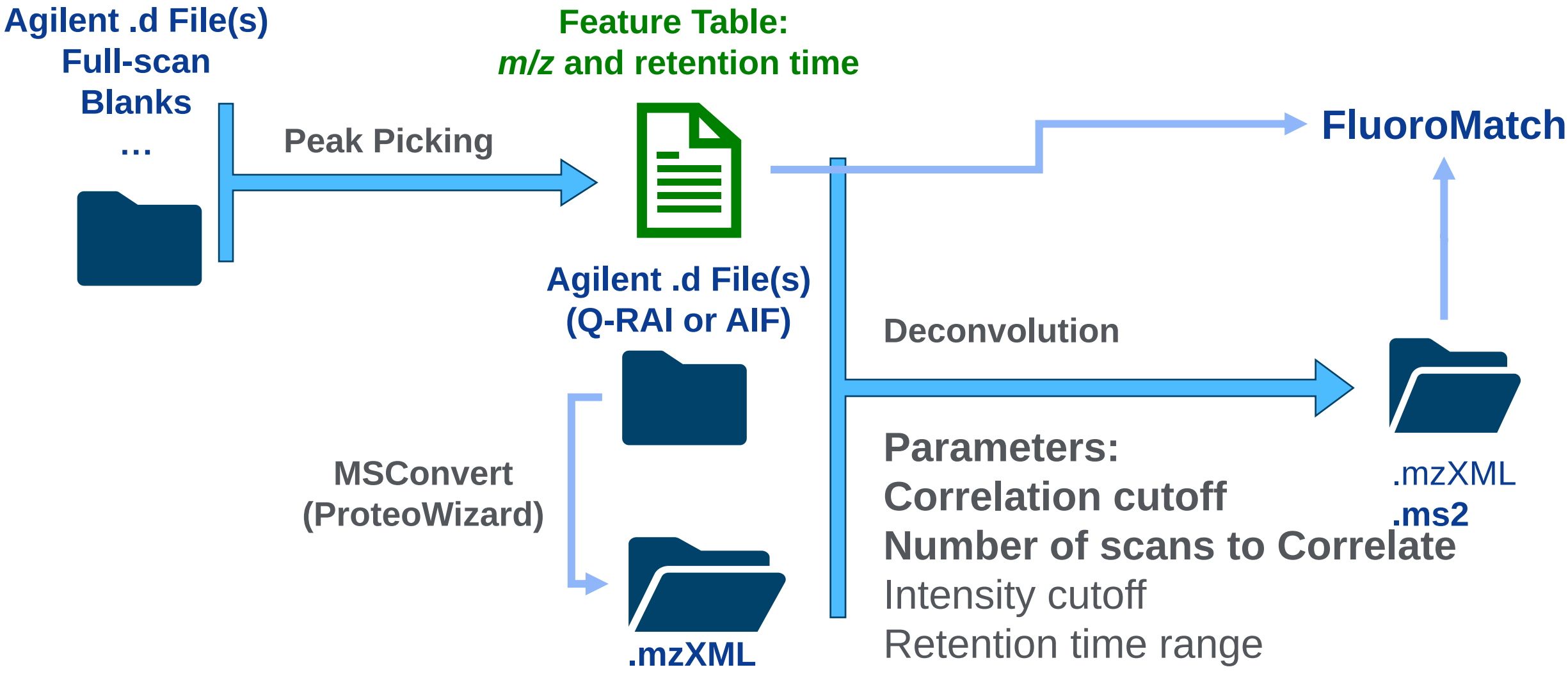


All-Ions



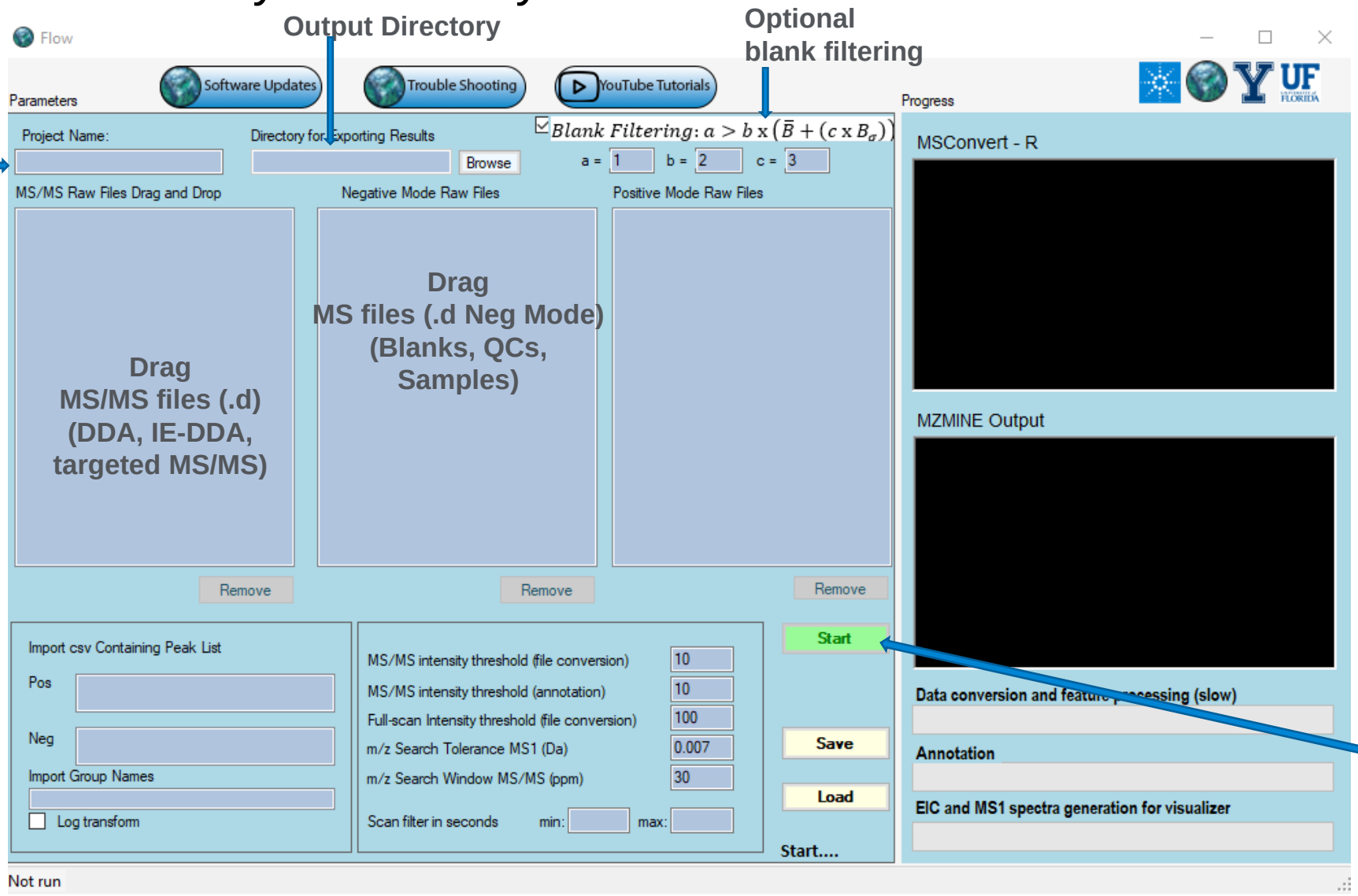


Generic FluoroMatch User Workflow



FluoroMatch Flow (Dependent on MZmine for Peak Picking)

FluoroMatch Flow to Systematically Annotate PFAS Molecules

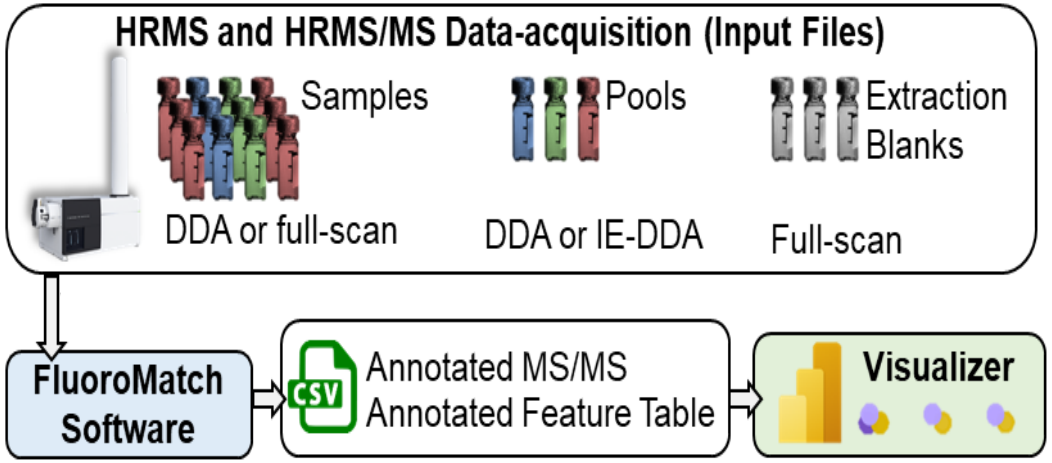


FluoroMatch Visualizer for *In Silico* PFAS Interpretation

FluoroMatch Visualizer is built upon Microsoft Power BI Desktop

Power BI Desktop necessary for interactive **FluoroMatch Visualizer**:

- **FluoroMatch 3.3** currently outputs a **Microsoft Power BI** readable **CSV** file with over 35 columns of information.
- Visualizer interface consists of three graphs, two filters, and four tables.
- Visualizer is interactive and **Crossfiltering** allows **simplified evaluation** of a feature, PFAS series, or other groups of features.

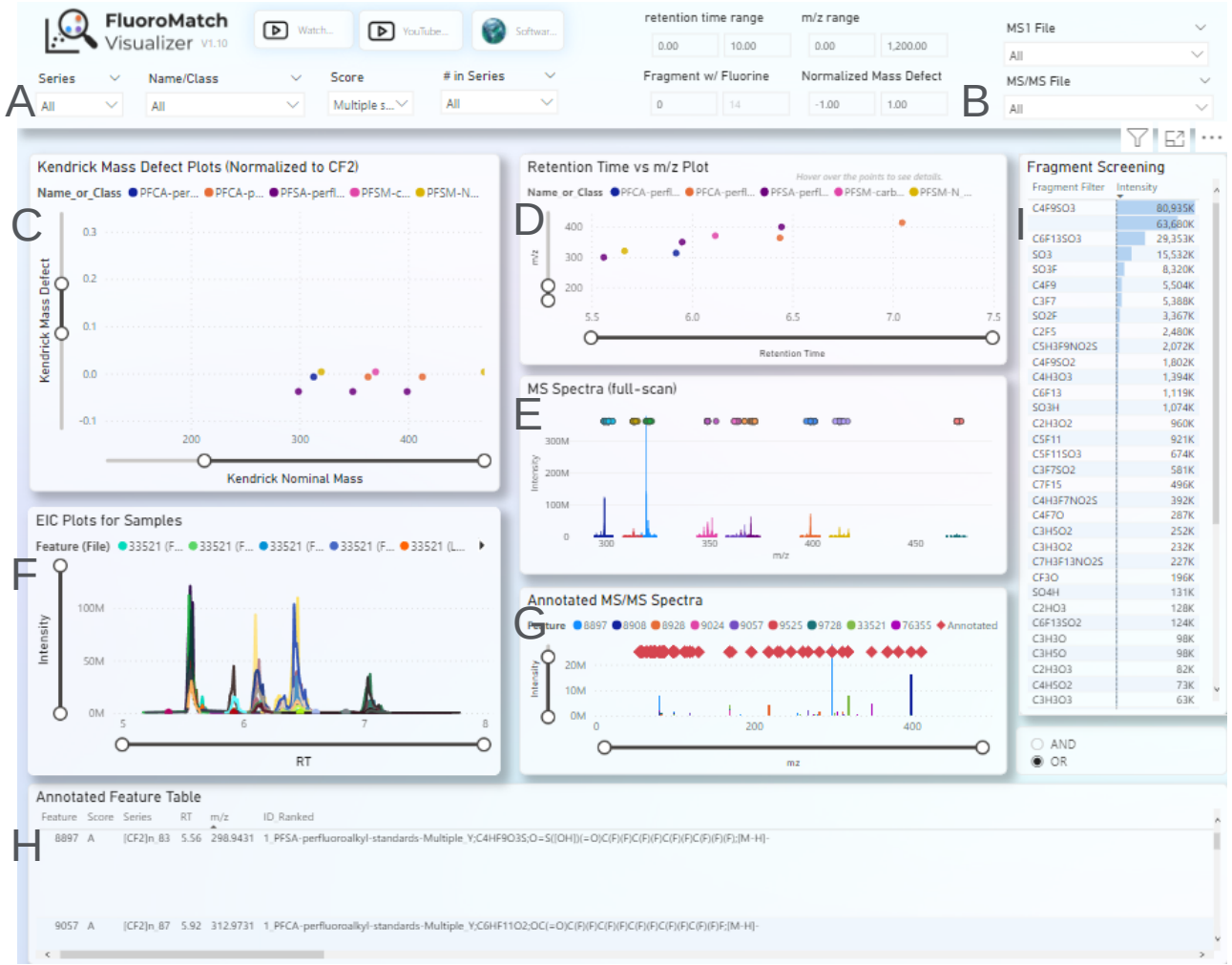


FluoroMatch Visualizer for *In Silico* PFAS Interpretation

Crossfiltering allows simplified evaluation of PFAS series

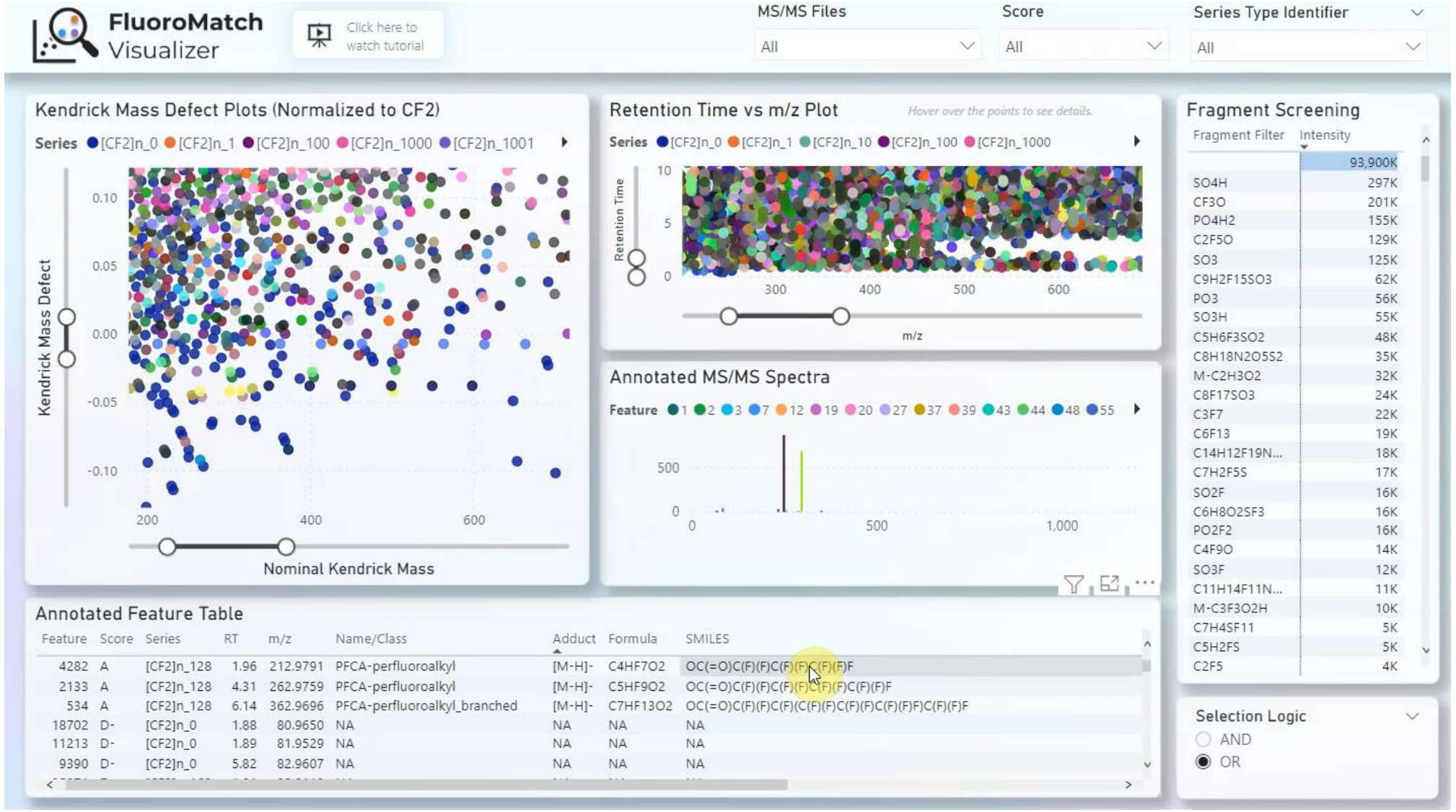
FluoroMatch Visualizer:

- Visualizer has **filters**:
  - A. Score, chemical, and number in series.
  - B. Files and MS/MS files.
- Visualizer interface consists of **five graphs**:
  - C. Normalized mass defect plot.
  - D. Interactive *m/z* vs. retention time.
  - E. Full-Scan MS Spectra.
  - F. Extracted Ion Chromatograms.
  - G. Annotated MS/MS Spectra.
- Visualizer includes **tables**:
  - H. Annotated and scored features.
  - I. Annotated fragments**.**



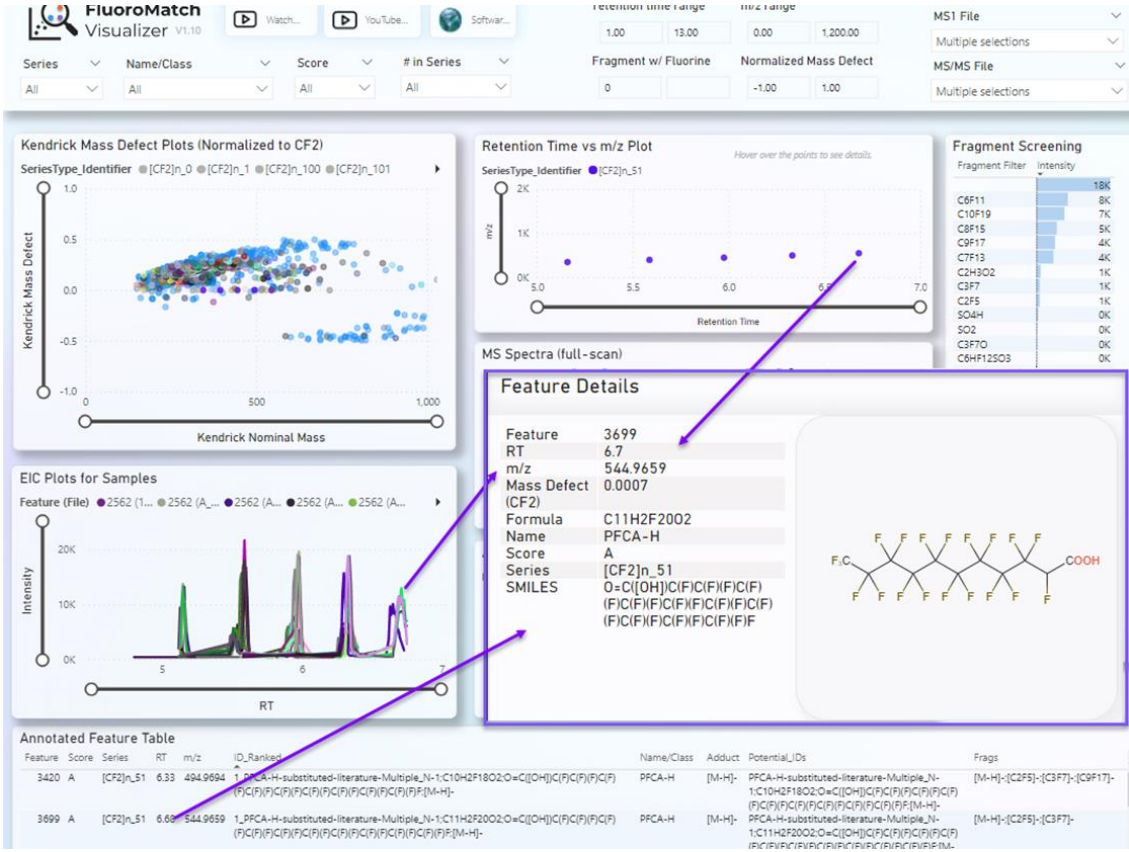
FluoroMatch Visualizer Demonstration

Series Shown in KMD and RT vs. m/z Plots



FluoroMatch 3.0: “MSmatch Visualizer” Power BI

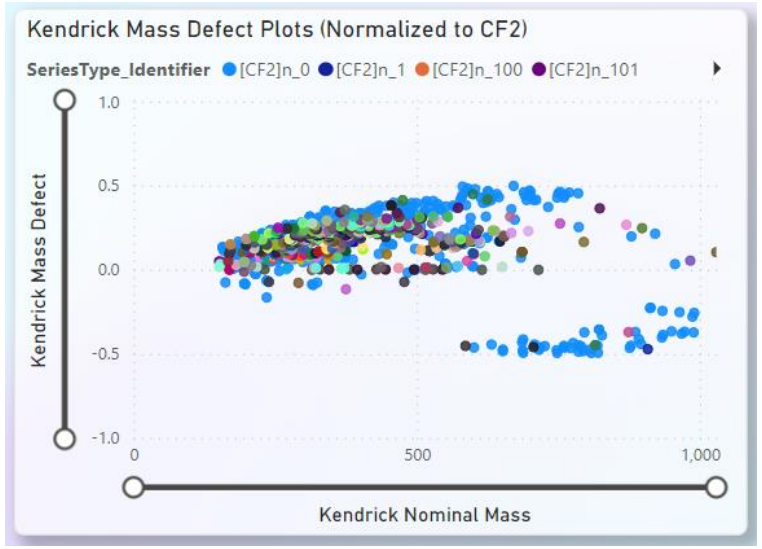
GUIPFCA-H Identified in Snow



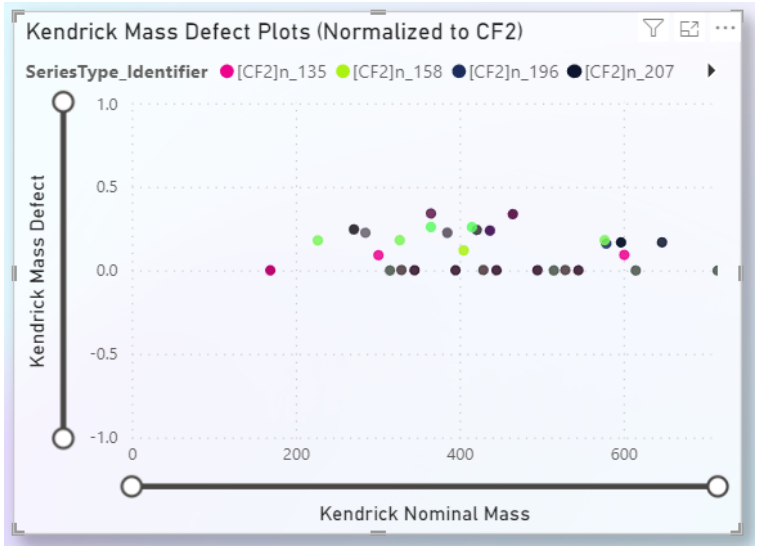
A series of five PFCA-Hs were detected in snow:

- Two had scores of “A”
- One had a score of “B+”
- Two had scores of “B”

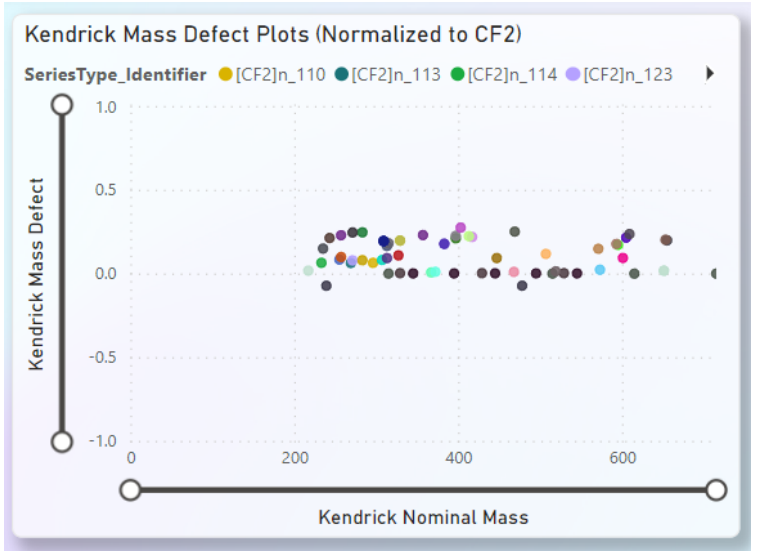
Evaluating Snow Features



Complex mixture of features



Filtered by features with >3 in a series

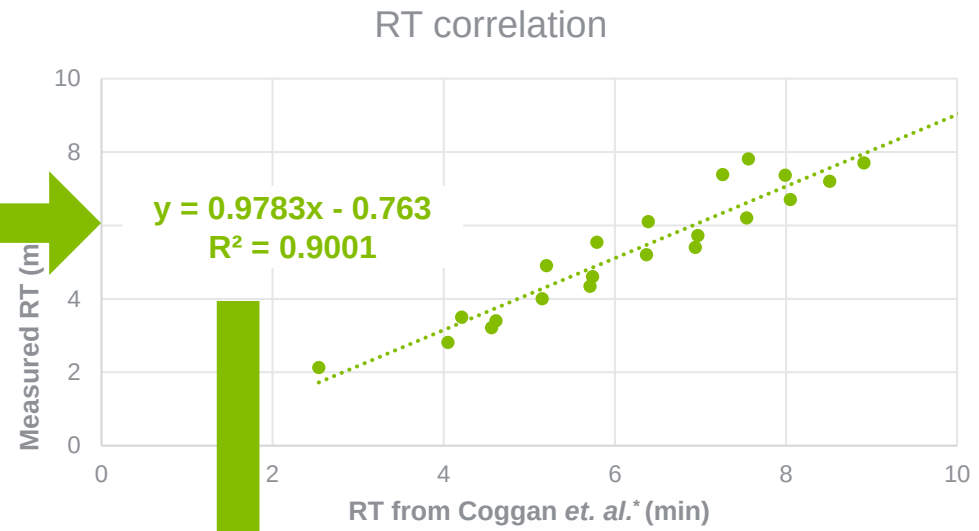


Filtered by Scores of > “B”

Building an *In Silico* PFAS Screening Method

Retention Time Projection

Compound Name	RT (min)
Perfluorobutanoic acid	2.13
Perfluoropentanoic acid	2.81
4:2 Fluorotelomer sulfonic acid	3.21
Perfluorohexanoic acid	3.40
Perfluorobutanesulfonic acid	3.50
Perfluoroheptanoic acid	4.00
6:2 Fluorotelomer sulfonic acid	4.34
Perfluorooctanoic acid	4.60
Perfluorohexanesulfonic acid	4.90
Perfluorononanoic acid	5.20
Perfluoroheptanesulfonic acid	5.54
8:2 Fluorotelomer sulfonic acid	5.40
Perfluorodecanoic acid	5.72
Perfluorooctanesulfonic acid	6.10
Perfluoroundecanoic acid	6.20
Perfluorododecanoic acid	6.70
Perfluorotridecanoic acid	7.20
Perfluorotetradecanoic acid	7.70
Perfluorooctanesulfonamide	7.37
2-(N-Methylperfluorooctanesulfonamido)acetic acid	7.38
2-(N-Ethylperfluorooctanesulfonamido)acetic acid	7.81
4,8-Dioxa-3H-perfluorononanoic acid	4.56
(2E)-3,4,4,5,5,6,6,7,7,8,8,8-Dodecafluoro-2-octenoic acid	4.44
2-Perfluorooctyl ethanoic acid	5.66
2-Perfluorodecyl ethanoic acid	6.83
2H-Perfluoro-2-octenoic acid	4.40
2H-Perfluoro-2-decenoic acid	5.64
2H-Perfluoro-2-dodecenoic acid	6.79
Ethyl heptafluorobutyrate	3.20
2H,2H,3H,3H-Perfluorooctanoic acid	4.41
3-Perfluoroheptylpropanoic acid	5.68
Perfluoropentanesulfonic acid	3.83
Perfluorononanesulfonic acid	6.07
Perfluorodecanesulfonic acid	6.60
Perfluorododecanesulfonic acid	7.52
Perfluoro(2-((6-chlorohexyl)oxy)ethanesulfonic acid)	5.84
2-[(8-Chloro-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-hexadecafluorooctyl)oxy]-1,1,2,2-tetrafluoroethanesulfonic acid	6.92
10:2 Fluorotelomer sulfonic acid	7.11
N-Methylperfluorooctanesulfonamide	8.15
8:2 Fluorotelomer sulfonamide	8.52
N-[(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluorooctyl)sulfonyl]glycine	6.14
N-Methyl-N-(2-hydroxyethyl)perfluorooctanesulfonamide	8.17
N-Ethyl-N-(2-hydroxyethyl)perfluorooctanesulfonamide	8.51
Phosphonic acid, (tridecafluorohexyl)-	3.22
PFOPA	4.35
Perfluorodecylphosphonic acid	5.57
6:2 Fluorotelomer phosphate diester	7.92
6:2/8:2 Fluorotelomer phosphate diester	8.48
8:2 Fluorotelomer phosphate diester	8.90
Bis(tridecafluorohexyl)phosphinic acid	7.39
(Heptadecafluorooctyl)(tridecafluorohexyl)phosphinic acid	8.07
Bis(heptadecafluorooctyl)phosphinic acid	8.60
Ammonium bis(N-ethyl-2-perfluorooctylsulfonaminoethyl)phosphate	9.35



21 measured RTs

32 projected RTs

Analytical and Bioanalytical Chemistry (2019) 411:3507–3520
https://doi.org/10.1007/s00216-019-01829-8

RESEARCH PAPER

Check for updates

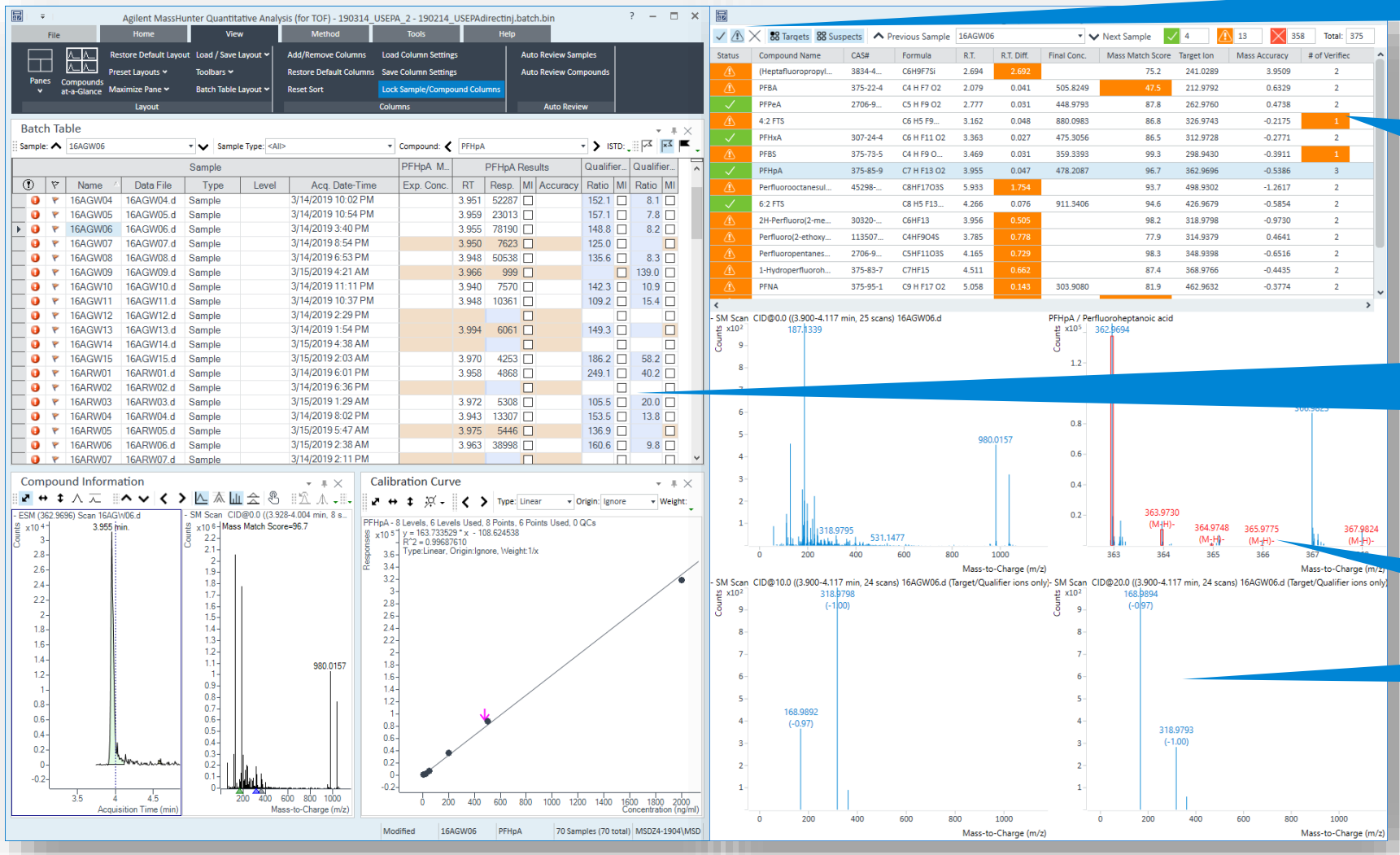
A single analytical method for the determination of 53 legacy and emerging per- and polyfluoroalkyl substances (PFAS) in aqueous matrices

Timothy L. Coggan[1] · Tarun Anumol[2] · James Pyke[2] · Jeff Shimeta[1] · Bradley O. Clarke[1]

Received: 30 November 2018 / Revised: 27 February 2019 / Accepted: 3 April 2019 / Published online: 9 May 2019

Use the *In Silico* Predictions to Tentatively Identify PFAS

Simultaneous Quantitation and Screening



Filter compounds that are Verified, Needs Review, Not Detected

Set outlier flag according to SANTE guidelines

MassHunter Quantitative 'Batch-at-a-glance' view

Review isotope pattern match

Review Fragments

See Agilent application note # 5994-1744EN for details on LC Screener setup.

Monitoring Suspect PFAS Using LC Screener

Screening summary PDF report

Screening Summary Report

Agilent | Trusted Answers

Sample name: 16AGW06 **Good** 4 **Warning** 17 **Error** 354

Status	Screening Summary Report	Formula	R.T.	R.T. Diff.	Match Score	Target Ion	Mass Accuracy	# of Qualified Ions	Final Conc.
!	(Heptafluoropropyl)trimethylsilane	C6H9F7Si	2.694	2.692		241.0289	3.95 PPM	2	
+	PFBA	C4 H F7 O2	2.079	0.041		212.9792	0.63 PPM	2	472.7851
+	PFPeA	C5 H F9 O2	2.777	0.031		262.9760	0.47 PPM	2	448.9793
!	4:2 FTS	C6 H5 F9 O3 S	3.162	0.048		326.9743	-0.22 PPM	1	880.0983
+	PFHxA	C6 H F11 O2	3.363	0.036		312.9728	-0.28 PPM	2	475.3056
!	PFBS	C4 H F9 O3 S	3.469	0.031		298.9430	-0.39 PPM	1	359.3393
!	3H-Perfluorobutanoic acid	C4H2F6O2	3.530	0.499		194.9886	-1.07 PPM	1	
!	Perfluorooctanesulfonate	C8HF17O3S	5.933	1.754		498.9302	-1.26 PPM	2	
+	6:2 FTS	C8 H5 F13 O3 S	4.266	0.076		426.9679	-0.59 PPM	2	911.3406
!	2H-Perfluoro(2-methylpentane)	C6HF13	3.956	0.505		318.9798	-0.97 PPM	2	
!	Perfluoro(2-ethoxyethane)sulfonic acid	C4HF9O4S	3.785	0.778		314.9379	0.46 PPM	2	
!	Perfluoropentanesulfonic acid	C5HF11O3S	4.165	0.729		348.9398	-0.65 PPM	2	
!	1-Hydroperfluoroheptane	C7HF15	4.511	0.662		368.9766	-0.44 PPM	2	
!	PFNA	C9 H F17 O2	5.058	0.143		462.9632	-0.38 PPM	2	303.9080
!	2,3,3,3-Tetrafluoro-2-(perfluoropentoxy)propan-1-ol	C8H3F15O2	4.526	0.718		414.9821	0.81 PPM	2	
!	1H-Perfluorohexane	C6HF13	3.956	1.326		318.9798	-0.97 PPM	2	
!	((Perfluorooctyl)ethyl)phosphonic acid	C10H6F17O3P	5.300	0.485		526.9710	4.37 PPM	1	
!	4-[3-(Perfluorobutyl)-1-propyloxy]benzyl alcohol	C14H13F9O2	6.167	0.221		383.0699	2.89 PPM	1	
!	(Perfluorooctyl)propanoyl chloride	C11H4ClF17O	5.927	0.137		508.9606	-2.22 PPM	1	
!	PFOS	C8 H F17 O3 S	5.933	0.167		498.9302	-1.23 PPM	2	63.0760
!	FOSA	[illegible]	[illegible]	0.015		[illegible]	[illegible] PPM	1	0.4484

Flagging RT outlier

Flagging number of verified ions

What Challenges Do PFAS Researchers Have?

The biggest problem is >12000 PFAS compounds identified in the EPA's PFASMASTER

- They are considered forever chemicals. **They don't readily degrade.**
- PFAS are found in UHPLC degassers, inlet lines, solvent bottle caps, glass filters, etc. (See **Consumables guide 5994-2357EN** and **application note 5994-0919EN for guidance**.)
- There are many PFAS compounds in commerce. However, there are **few standards**, and those are prohibitively expensive.
- Both targeted and nontargeted methods are required, depending on the study goals.
- Using a **PFC-Free Kit to Delay Background Contamination** helps reduce interferences.
- The **eMethod, application notes, and the Order Guide** are designed to **provide solutions** to address targeted needs.
- **Nontarget tools** like **FluoroMatch** software, methodology for modeling ***In Silico*** **PFAS libraries**, and the **LC Screener** aid putative identification of novel PFAS.

Note: PFASMASTER is a consolidated list of PFAS substances spanning lists of current PAFS of interest to researchers and regulators worldwide.

Appendix

Please contact me at stephan_baumann@Agilent.com with any questions or comments.

Quick Links:
PFAS Resources on Agilent.com:
https://www.agilent.com/en/solutions/environmental/water-analysis/pfas-in-water

EPA circular on PFAS Regulation in nonpotable waters and soils:
https://www.federalregister.gov/public-inspection/2023-07535/addressing-per--and-polyfluoroalkyl-substances-in-the-environment

Where to Download FluoroMatch Software:
http://innovativeomics.com/software/fluoromatch-flow-covers-entire-pfas-workflow/

Where to Download Microsoft Power BI Desktop:
https://www.microsoft.com/en-us/download/details.aspx?id=58494

Note: There are links to informative YouTube videos on the Innovative Omics website.