Application Note

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Quadrupole-Resolved All Ions (Q-RAI) Analysis of Select PFAS Chemicals on an Agilent 6546 LC/Q-TOF

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Abstract

The Agilent 6546 LC/Q-TOF introduces a new data-independent acquisition mode, Quadrupole-Resolved All Ions (Q-RAI). This application note evaluates the use of Q-RAI in the quantitative analysis of US EPA method 533 for per- and polyfluoroalkyl substances (PFAS). This successful method demonstrates ample sensitivity, reproducibility, and linearity to meet US EPA requirements.

Introduction

In Q-RAI acquisition mode, the quadrupole is used to filter precursor ions by groups of *m/z* before they enter the collision cell for transmission or fragmentation. The quadrupole isolation windows are user-set and may be as narrow as 10 amu or as wide as 100 amu. Each quadrupole isolation window has one or more associated collision energies. In this manner, the low energy precursor ion is associated with qualifying fragment ions from higher energy spectra while eliminating interfering ions coming from precursors outside of the quadrupole isolation window. The advantages of Q-RAI acquisition include a relatively simple, nontargeted method setup allowing retrospective analysis, fragment confirmation, and reduced noise and interference from coeluting fragments.

This work demonstrates the acquisition parameters and areas of optimization for the use of Q-RAI with US EPA method 533 for PFAS compounds. Method performance is also evaluated including linearity, reproducibility, and lowest concentration minimum reporting levels (LCMRLs).

Experimental

LC/MS instrument conditions

LC/MS analysis was performed using an Agilent 1290 Infinity II LC coupled to a 6546 LC/Q-TOF. LC configuration and method parameters are shown in Table 1. A delay column (see Table 1) was in place between the binary pump and multisampler to separate background contaminants from compounds originating in the sample vial. The 4.6 mm id column maintained low system backpressure. The analytical column (also listed in Table 1) resulted in baseline separation for most PFAS. The system was controlled by Agilent MassHunter acquisition software version 10.1. MS method parameters are shown in Tables 2 and 3. Data processing was performed with MassHunter quantitative analysis software version 11.0 and MassHunter qualitative analysis software version 10.0.

Table 1. LC instrument conditions.

Parameter	Value
LC	**Agilent 1290 Infinity II LC:** Agilent 1290 Infinity II multisampler (G7167B), Agilent 1290 Infinity II high-speed pump (G7120A), and Agilent 1290 Infinity II multicolumn thermostat (G7116B)
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18, 3.0 × 50 mm, 1.8 µm (p/n 959757-302)
Delay Column	Agilent ZORBAX RR StableBond C18, 4.6 × 50 mm, 3.5 µm (p/n 835975-902)
Column Temperature	50 °C
Injection Volume	10 µL
Mobile Phase	A) 20 mM ammonium acetate in water (LC grade) B) methanol (LC grade)
Gradient Flow Rate	0.4 mL/min
Gradient	Time (min) % B 0.0 5 0.5 5 3.0 40 16.0 80 18.0 80 20.0 95
Stop Time	20.0 min
Post Time	6.0 min

Table 2. MS instrument conditions.

Parameter	Value
MS	Agilent 6546 LC/Q-TOF with dual Agilent Jet Stream ESI source
Source Parameters	
Gas Temperature	230 °C
Gas Flow	4 L/min
Nebulizer	20 psi
Sheath Gas Temperature	375 °C
Sheath Gas Flow	12 L/min
Capillary Voltage (Neg)	2,000 V
Nozzle Voltage (Neg)	0 V
MS TOF	
Fragmentor	95 V
Skimmer	65 V
Oct 1 RF Vpp	750 V

Table 3. MS spectral parameters.

MS	
Mass Range	100 to 1,100 *m/z*
Acquisition Rate	2 spectra/s
MS/MS	
Mass Range	25 to 800 *m/z*
Acquisition Rate	11 spectra/s

Q-RAI isolation windows

This method uses data-independent acquisition run in electrospray negative mode using a 6546 LC/Q-TOF. Quantitation occurs on low energy accurate mass precursor ions with qualification by coelution of accurate mass product ions from higher collision energy spectra. Detailed method parameters for the Q-RAI isolations windows are shown in Table 4.

Method optimization

The quadrupole isolation widows are user-set to a maximum width of 100 amu. The method was developed as a generalized method with a midrange collision energy of 20 V. More isolation windows were added to ensure product ion formation for specific PFAS compounds requiring either higher or lower collision energies. When developing a Q-RAI method, it is important to consider the range of collision energies necessary to form fragment ions.

To reliably quantitate with acceptable relative standard deviations (RSDs), chromatography must be reproducible. To obtain reproducible peaks, there must be enough data points across the peak. The key factors that contribute to the number of data points across a peak are peak width (determined by LC conditions) and MS cycle time (determined by the spectral acquisition rates and the number of quadrupole windows). In setting up this method, an estimated peak width of 17 seconds was used, and the desired number of data points across the peak was 10 to 15. The number of quadrupole windows was minimized such that for each PFAS to be quantitated, at least one coeluting qualifying ion was generated.

Table 4. Q-RAI isolation windows.

Start *m/z*	End *m/z*	Window Width (amu)	Collision Energy (V)
98	198	100	20
196	296	100	20
294	394	10	20
392	492	100	20
490	590	100	20
588	688	100	20
686	786	100	20
210	270	60	5
510	570	60	10
330	410	80	40
440	502	62	40

Calibration standards and extracted waters

Calibration standards and extracted fortified water samples previously prepared for targeted MS/MS work were reanalyzed for this work. For more information, see app note 5994-3648EN.[1]

Results and discussion

Chromatography

Figures 1 and 2 show chromatograms from a 1.6 ng/mL (in vial) calibrator separated by compound groups.

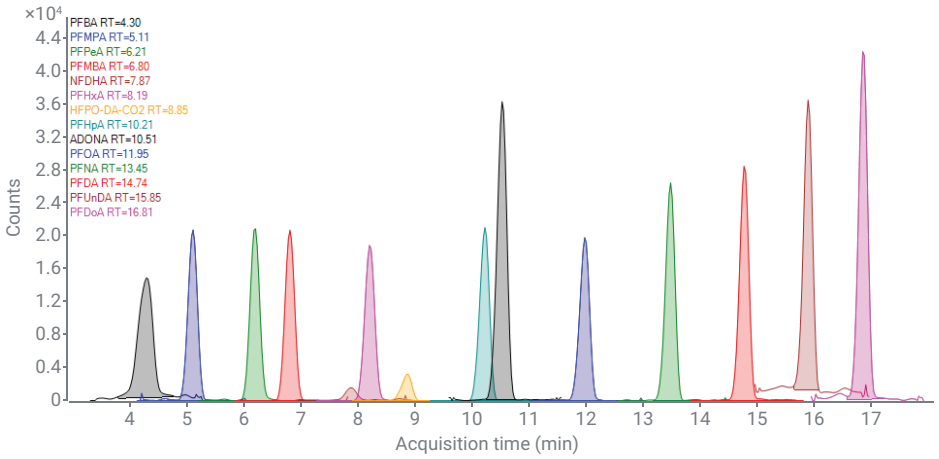

Figure 1. PFAS acids at 1.6 ng/mL in vial.

Method performance

All target compounds were quantitated on accurate mass precursor ions using quadratic fit, 1/x weighting, and forcing the origin. All targets have calibration curve fits with R^2 >0.997. Over 99% of precursor ions had sub-3 ppm mass accuracies. Targets were qualified by the presence of accurate mass coeluting diagnostic fragment ions.

System background was analyzed with a delay column. The LC/Q-TOF system was shown to be free from PFAS eluting at the retention times of the target compounds. Despite extracted lab reagent blanks showing trace levels of some PFAS, they were well below the LCMRL values.

Table 5 shows results of instrument performance as % RSD, which was calculated from repeated injections (n = 6) of a low-level calibrator (1.6 ng/mL in vial equivalent to 6.3 ng/L in water). All PFAS had RSD values less than the requirements of 20% noted in method 533. The table also demonstrates the LCMRL values on a 6546 LC/Q-TOF using Q-RAI acquisition, which are significantly lower than documented US EPA levels.

Untargeted and retrospective analysis

Data-independent quadrupole-resolved analysis has the reproducibility and sensitivity for quantitative analysis of targeted compounds while allowing retrospective analysis of untargeted or emerging PFAS compounds. Low-energy full MS spectra provide precursor identification of all compounds ionized in the source while the higher energy spectra allow coeluting fragment ion confirmation. The quadrupole isolation removes interferences from coeluting compounds outside the isolation window, providing confidence that the fragments belong to the precursor.

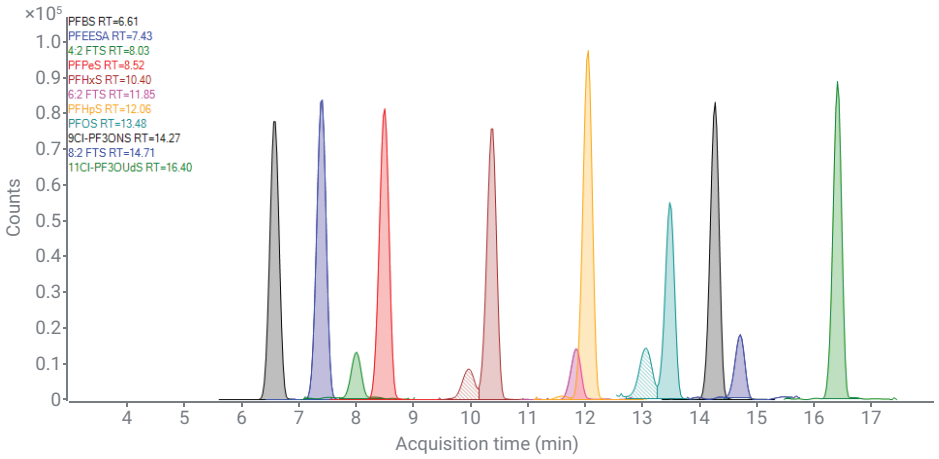

Figure 2. PFAS sulfonates and FTSs at 1.6 ng/mL in vial. Note the clear presence of branched isomers for PFHxS and PFOS.

Table 5. Instrument performance for US EPA method 533.

Analyte	RSD (n = 6) at 6.3 ng/L	Agilent 6546 LC/Q-TOF Q-RAI LCMRL (ng/L)	EPA Method 533 LCMRL (ng/L)
PFBA	4%	3.95	13.00
PFMPA	4%	3.09	3.80
PFPeA	3%	2.62	3.90
PFMBA	3%	2.03	3.70
PFEESA	2%	2.30	2.60
NFDHA	11%	8.60	16.00
PFHxA	2%	2.31	5.30
HFPO-DA -CO2	11%	2.73	3.70
PFHpA	4%	0.75	2.60
ADONA	1%	0.56	3.40
PFOA	6%	2.01	3.40
PFNA	5%	1.47	4.80
PFDA	6%	1.31	2.30
PFUnDA	7%	0.89	2.70
PFDoA	5%	1.58	2.20
4:2 FTS	7%	4.20	4.70
6:2 FTS	9%	4.70	14.00
8:2 FTS	11%	3.05	9.10
PFBS	1%	3.19	3.50
PFPeS	3%	2.20	6.30
PFHxS	3%	2.07	3.70
PFHpS	3%	2.39	5.10
PFOS	3%	1.67	4.40
9Cl-PF3ONS	1%	2.17	1.40
11Cl-PF3OUdS	2%	2.58	1.60

Conclusion

This application note demonstrates that the Agilent 6546 LC/Q-TOF operated in data-independent acquisition has ample sensitivity, reproducibility, and linearity to quantitatively analyze water samples for the PFAS compounds listed in US EPA method 533. The Q-TOF instrument in Q-RAI mode can achieve RSD values well below EPA requirements of 20% with all 25 compounds achieving values below 11% at a low calibration level. LCMRL values are near or predominately well below US EPA reported values. For the selected PFAS compounds, 24 out of 25 achieve LCMRL levels less than 5 ng/L. Using Q-RAI acquisition enables the collection of accurate mass precursor and fragment ions while simultaneously reducing or removing noise and interference, which originate from precursors outside of the quadrupole isolation window. Untargeted method setup and full scan spectra allow retrospective analysis of emerging PFAS.

Reference

1. Hunt, K. *et al.* Quantification of Per- and Polyfluoroalkyl Substances in Drinking Water, *Agilent Technologies application note*, publication number 5994-3648EN, **2021**.

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