Application Note

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Quantification of Per- and Polyfluoroalkyl Substances in Drinking Water

According to EPA Method 533 using an Agilent 6546 LC/Q-TOF

Authors

Kathy Hunt and Ralph Hindle
Vogon Laboratory Services
Cochrane, AB, Canada

Tarun Anumol
Agilent Technologies, Inc.
Wilmington, DE, USA

James Pyke and Chris Klein
Agilent Technologies, Inc.
Santa Clara, CA, USA

Abstract

United States Environmental Protection Agency (US EPA) Method 533 for the analysis of selected per- and polyfluorinated alkyl substances (PFAS) in drinking water is traditionally analyzed using liquid chromatography/triple quadrupole mass spectrometry (LC/TQ) for maximum sensitivity. This application note shows an instrument demonstration of the Agilent 6546 quadrupole time-of-flight LC/MS for the analysis of EPA Method 533 compounds to meet sensitivity levels and all performance criteria mentioned in Method 533, while also allowing users to perform screening of other PFAS or nontargeted identification of unknown and emerging PFAS with LC-Q/TOF.

The analysis of PFAS in Method 533 using LC/TQ including method parameters such as extraction recovery, method reporting levels, and precision are described in previously published application note 5994-1628EN. Method 533 requires solid phase extraction of 100 to 250 mL of drinking water, followed by solvent exchange to 1 mL of 80% methanol, which is then analyzed by LC/TQ. This application note replaces only the detector and uses an LC/Q-TOF instead of TQ to show performance criteria for EPA Method 533 can be met with the Agilent 6546 LC/Q-TOF.

Introduction

A quadrupole time-of-flight (Q-TOF) is a high-resolution accurate mass instrument that allows unambiguous identification of chemical formulas using mass accuracy. A Q-TOF can also be used for quantitation of a targeted list of compounds due to their ability to acquire data at high acquisition rates without loss in mass resolution, unlike trap-based high-resolution instruments. High acquisition rates are critical when up to 20 data points are required across the peak for accurate, robust, and sensitive quantitation as usually done with triple quadrupole (TQ) instruments. High resolution can also be used for retrospective analysis of acquired data for detection of suspected or unknown compounds.

There are four standard acquisition modes using typical HRMS instruments:

- MS (TOF only) mode provides full MS spectra without fragmentation and results in maximum sensitivity. With the All Ions technique, data are acquired using different conditions: (a) with a low value collision energy (typically 0 V, yielding in a full MS spectrum), and (b) one or multiple high energy values. The low energy spectra predominantly show just the molecular (or precursor) ions for the compounds and the high energy spectra provide the precursors plus their fragment ions, which allows for simultaneous collection of both precursor and fragmentation data.
- Auto MS/MS is a data-dependent acquisition mode, which triggers quadrupole mass filtering on precursors with abundances greater than a user-specified threshold.
- Targeted MS/MS mode is most similar to LC/TQ analysis. The quadrupole isolates precursor ions from a targeted list within specified retention time windows, providing acquisition of product ion spectra.
- Data-independent acquisition mode, referred to as Quadrupole Resolved All-Ions (Q-RAI), filters precursor ions using wide mass quadrupole isolation windows up to 100 amu before they enter the collision cell for fragmentation.

To meet the acquisition requirements for Method 533, Targeted MS/MS mode was selected, and quantitation performed on the product ion as would be the case with a traditional LC/TQ. Method acquisition rates and retention time windows were optimized for peak shape reproducibility to maximize sensitivity and to minimize the number of concurrent precursors.

Experimental

LC/MS analysis was performed using an Agilent 1290 Infinity II LC coupled to an Agilent 6546 LC/Q-TOF. LC conditions are provided in Table 1. A delay column 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 delay column maintains low system backpressure. The analytical column resulted in baseline separation for most PFAS. The system was controlled by Agilent MassHunter Acquisition software version 10.1. Data processing was performed with Agilent MassHunter Quantitative analysis software version 10.1 and MassHunter Qualitative analysis software version 10.0. Q-TOF method parameters are listed in Tables 2 and 3.

LC instrument conditions

Table 1. LC method parameters for EPA Method 533.

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 × 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 (HPLC grade) B) methanol (HPLC grade)
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 minutes
Post Time	6.0 minutes

Targeted MS/MS precursors

This method uses the Targeted MS/MS acquisition run in electrospray negative mode using a 6546 LC/Q-TOF.

Compound parameters including precursor ion selection and collision energy were previously optimized, see application note 5991-7951EN for more details. Delta retention times were kept as narrow as possible to minimize concurrent precursors. Parameters are shown in Table 4.

Quantitation was done on accurate mass product ion transitions.

Preparation of calibration standards

The high calibrator was prepared by dilution of a 500 ng/mL analyte dilution standard containing all analytes of interest. Serial dilutions of the high calibrator were prepared in 80% methanol for subsequent calibrators. The isotope dilution standard and internal standard were added before analysis.

Extraction

Water samples (250 mL) including blanks and lowest concentration minimum reporting level (LCMRL) replicate spikes, were fortified with isotope dilution analogues and were extracted using weak anion exchange cartridges according to Method 533. Samples were eluted with an ammonium hydroxide/methanol solution and concentrated to dryness before reconstituting with 80% methanol. The internal standard was added before analysis.

LC/MS instrument conditions

Table 2. LC/MS method parameters for EPA Method 533 on an Agilent 6546 LC/Q-TOF.

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. Spectral method parameters for EPA Method 533 on an Agilent 6546 LC/Q-TOF.

Spectral Parameters	
MS	
Mass Range	100 to 1,000 *m/z*
Acquisition Rate	50 spectra/s
MS/MS	
Mass Range	40 to 650 *m/z*
Acquisition Rate	5 spectra/s

Table 4. Compound method parameters for EPA Method 533 on an Agilent 6546 LC/Q-TOF (continued on next page).

Compound	Precursor Ion (*m/z*)	Retention Time (min)	Delta Retention Time (min)	Collision Energy (V)
PFBA	212.9813	4.25	0.6	8
$^{13}C_3$-PFBA	215.9893	4.25	0.6	8
$^{13}C_4$-PFBA	216.9926	4.25	0.6	8
PFMPA	228.9765	5.16	0.4	12
PFPeA	262.9788	6.18	0.4	8
$^{13}C_5$-PFPeA	267.9956	6.18	0.4	8
PFMBA	278.9739	6.85	0.4	12
HFPO-DA-CO_2	284.9778	8.95	0.4	4
$^{13}C_3$-HFPO-DA-$^{13}CO_2$	286.9845	8.95	0.4	4
NFDHA	294.9823	7.70	0.8	5
PFBS	298.9460	6.64	0.4	45
$^{13}C_3$-PFBS	301.9563	6.64	0.4	45
PFHxA	312.9762	8.37	0.4	8
PFEESA	314.9412	7.53	0.4	24
$^{13}C_5$-PFHxA	317.9930	8.36	0.4	8
4:2 FTS	326.9779	8.17	0.4	20
$^{13}C_2$-4:2 FTS	328.9844	8.17	0.4	20

Table 4. Compound method parameters for EPA Method 533 on an Agilent 6546 LC/Q-TOF.

Compound	Precursor Ion (*m/z*)	Retention Time (min)	Delta Retention Time (min)	Collision Energy (V)
PFPeS	348.9435	8.69	0.4	40
PFHpA	362.9737	10.42	0.4	0
$^{13}C_4$-PFHpA	366.9869	10.42	0.4	0
ADONA	376.9731	10.71	0.4	12
PFHxS	398.9409	10.59	0.4	49
$^{13}C_3$-PFHxS	401.9509	10.59	0.4	45
PFOA	412.9711	12.16	0.4	4
$^{13}C_2$-PFOA	414.9777	12.16	0.4	4
$^{13}C_8$-PFOA	420.9978	12.16	0.4	4
6:2 FTS	426.9725	12.06	0.4	24
$^{13}C_2$-6:2 FTS	428.9791	12.06	0.4	24
PFHpS	448.9384	12.25	0.4	52
PFNA	462.9683	13.64	0.4	4
$^{13}C_9$-PFNA	471.9985	13.64	0.4	4
PFOS	498.9357	13.67	0.4	50
$^{13}C_4$-PFOS	502.9492	13.67	0.4	50
$^{13}C_8$-PFOS	506.9625	13.67	0.4	50
PFDA	512.9656	14.92	0.4	4
$^{13}C_6$-PFDA	518.9859	14.92	0.4	4
8:2 FTS	526.9676	14.86	0.4	28
$^{13}C_2$-8:2 FTS	528.9738	14.86	0.4	28
^{9}Cl-PF_3ONS	530.9015	14.43	0.4	28
PFUnDA	562.9631	15.99	0.4	5
$^{13}C_7$-PFUnDA	569.9865	15.99	0.4	5
PFDoA	612.9604	16.94	0.4	5
$^{13}C_2$-PFDoA	614.9604	16.94	0.4	5
^{11}Cl-PF_3OUdS	630.8960	16.53	0.4	32

Results and discussion

Chromatography

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

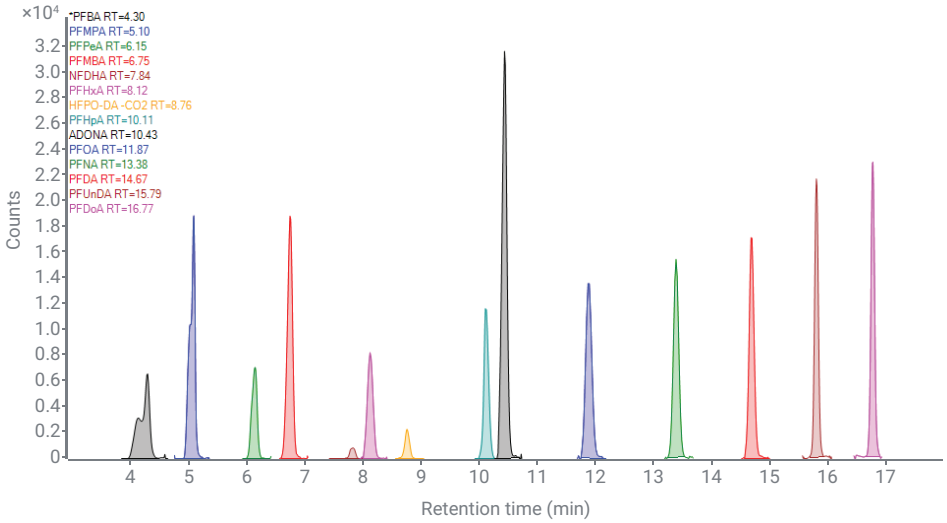

Figure 1. Separation of acids in 1.6 ng/mL PFAS calibrator.

Method performance

All target compounds were quantitated on transitions from precursor ions to accurate mass product ions using quadratic fit, 1/x weighting, and forcing the origin as required in Method 533. All targets have calibration curve fits with R^2 greater than 0.98 with the majority greater than 0.995. Over 98% of product ions had sub-2 ppm mass accuracies.

System background was evaluated. With use of a delay column, the LC/Q-TOF system was shown to be free from PFAS eluting at the retention time of the target compounds. While extracted lab reagent blanks showed trace levels of some PFAS, they were well below the LCMRL values.

Table 5 shows the results of instrument performance as relative standard deviation (RSD), which was calculated from repeated injections (n = 9) of a midlevel calibrator (6.3 ng/mL in vial, equivalent to 25 ng/L in water). All PFAS had RSD values well below the EPA requirement of 20% as noted in Method 533. The table also demonstrates the estimated LCMRL values on a 6546 LC/Q-TOF using Targeted MS/MS acquisition, which are significantly lower than the documented EPA levels. The LCMRL was calculated following guidelines defined in EPA Method 533.

Untargeted and retrospective analysis

A primary benefit of Q-TOF analysis is the ability to look for untargeted compounds both in the present and in the future. Full mass spectra are collected throughout the run. Even when acquisition is focused on targeted analysis and minimal time is spent acquiring full MS spectra, enough information is collected to correctly identify compounds that are present in high enough concentrations by a database search. Figure 3 shows the full MS 50 Hz spectra of the calibrator at 25 ng/mL. All 25 compounds are identified in MassHunter Qualitative

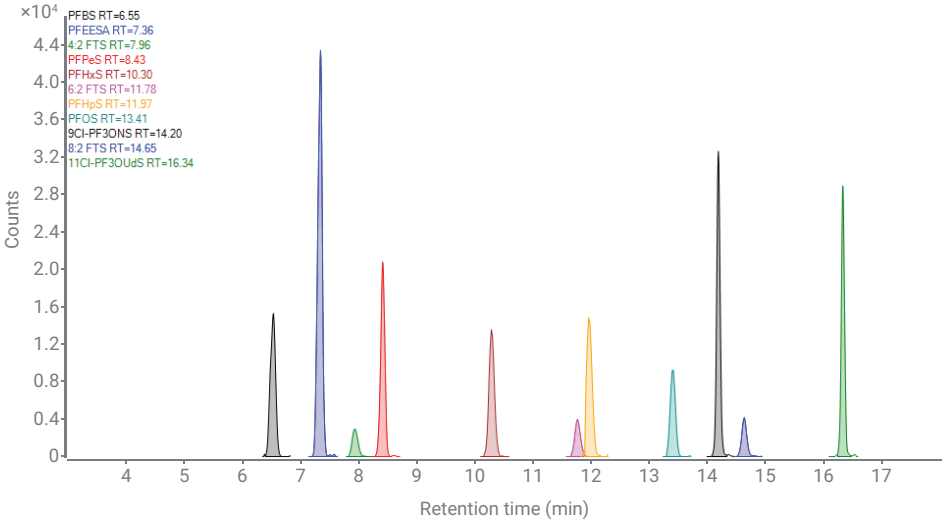

Figure 2. Separation of sulfonates and FTSs in 1.6 ng/mL PFAS calibrator.

Table 5. Agilent 6546 LC/Q-TOF performance for EPA Method 533.

Compound	RSD (n = 9) at 25 ng/L	Agilent 6546 LC/Q-TOF Targeted MS/MS LCMRL (ng/L)	EPA Method 533 Reported LCMRL (ng/L)
PFBA	2%	2.60	13.00
PFMPA	2%	0.45	3.80
PFPeA	3%	2.03	3.90
PFMBA	2%	0.62	3.70
PFEESA	4%	0.01	2.60
NFDHA	12%	5.93	16.00
PFHxA	4%	0.91	5.30
HFPO-DA-CO_2	9%	4.41	3.70
PFHpA	4%	1.62	2.60
ADONA	4%	0.61	3.40
PFOA	5%	1.31	3.40
PFNA	6%	1.40	4.80
PFDA	3%	1.21	2.30
PFUnDA	3%	0.35	2.70
PFDoA	4%	1.05	2.20
4:2 FTS	6%	2.91	4.70
6:2 FTS	8%	4.48	14.00
8:2 FTS	6%	3.57	9.10
PFBS	3%	0.61	3.50
PFPeS	2%	0.35	6.30
PFHxS	4%	0.99	3.70
PFHpS	4%	1.67	5.10
PFOS	4%	0.77	4.40
^{9}Cl-PF_3ONS	3%	0.65	1.40
^{11}Cl-PF_3OUdS	4%	0.66	1.60

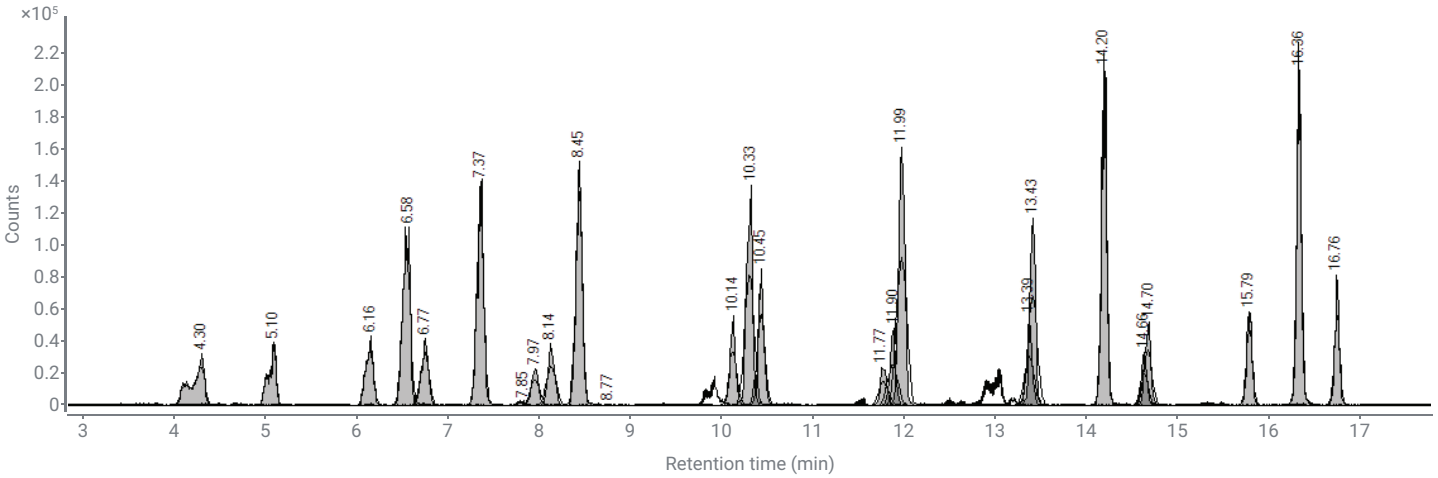

Figure 3. Full MS 50 Hz spectra of the PFAS calibrator at 25 ng/mL.

analysis software using the Find By Formula algorithm, 92% of which have match scores greater than 80%. When the lowest calibrator is evaluated (0.4 ng/mL in the vial, equivalent to 1.6 ng/L in water), 17 out of the 25 compounds are still identified with scores greater than 80%. Also, five more compounds were identified in the lowest calibrator, but with lower scores. Q-TOF analysis is a powerful tool for evaluating samples both quantitatively for targeted compounds and qualitatively for unknown or emerging contaminants.

Conclusion

This application note demonstrates that the Agilent 6546 LC/Q-TOF in Targeted MS/MS acquisition mode is suitable for meeting performance requirements in US EPA Method 533 for the quantitative analysis of PFAS. The 6546 LC/Q-TOF in Targeted MS/MS mode is able to achieve RSD values well below the EPA requirement of 20%, with 24 out of 25 compounds achieving values less than 10%. LCMRL values are near or predominately well below EPA reported values using the LC/TQ. Out of 25 compounds, 21 compounds achieve LCMRL levels less than 3 ng/L. LC/Q-TOF acquisition enables the simultaneous collection of targeted MS/MS data, which is required by the EPA for quantitation, and full MS spectra, which allows for screening and untargeted analysis of emerging PFAS.

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