

Improving Efficiency in the Determination of PFAS in Foods of Animal Origin

Margot Lee, Kari Organtini, Eimear Trivedi

Waters Corporation, United States

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Abstract

Benefits

- An accessible LC-MS/MS method developed for 44 PFAS to support compliance and monitoring testing of meats in accordance with EU Regulation 2022/2388 and Recommendation 2022/1431
- The sample preparation approach incorporates automated solid-phase extraction (SPE) cleanup and eliminates the need for an evaporation and reconstitution step, reducing time and risk of background interference from the pre-concentration step
- Reliable, yet efficient analytical method achieving separation of 44 PFAS in a short 11-minute runtime, while maintaining separation of analytes from isobaric and system background interferences
- Analytical parameters from the European Union Reference Laboratory (EURL) were set within the MS Quan Application in the waters_connect™ Software platform for data processing, improving data review time and consistency

Introduction

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants that can accumulate in a variety of foods commonly consumed by humans, including meats. As PFAS contamination continues to be identified across diverse food matrices, regulatory agencies have increased efforts to better understand dietary exposure and potential human health impacts. In previous studies, analytical workflows have been developed for the analysis of fish,¹ milk,² eggs,³ meat,⁴ fruit, vegetables, and baby foods⁵ to levels and performance required under EU Regulation 2022/2388,⁶ EU Recommendation 2022/1431,⁷ and EURL for POPs guidance on PFAS analysis.⁸ While these previous analytical workflows support the monitoring and compliance testing of meat from terrestrial animals, in this study, a streamlined sample preparation approach was explored to provide a more efficient and effective method for the reliable determination of PFAS in meat.

Method performance was evaluated in accordance with regulations and guidance in place in the European Union, focusing on meats of bovine, pig, and poultry – the lowest levels stipulated in Regulation 2022/2388. In total, 44 PFAS were evaluated in this study, addressing the 27 listed in Recommendation 2022/1431/EU, along with additional analytes of interest in emerging methodologies, including fluorotelomers.

µg/kg	PFOS	PFOA	PFNA	PFHxS	Max Sum
Regulated limits [6]	0.3	0.8	0.2	0.2	1.3
Method LOQ [7]	0.1	0.1	0.1	0.1	–

Table 1. Maximum levels set in EU Commission Regulation 2022/2388 meats of bovine animals, pork, and poultry and the limits of quantification (LOQ) required for the method as stipulated in Commission Recommendation 2022/1431.

Experimental

Sample Description

Detailed experimental information can be found in the [Lee and Organtini 2026 <](#)

<https://www.waters.com/nextgen/global/library/application-notes/2026/flame-grilled-and-fluorinated-pfas-analysis-in-barbeque-foods-using-a-miniaturized-quechers-workflow.html>> application note.⁹ In summary, store bought samples of beef, chicken, pork, and bratwurst were prepared as shown in Figure 1. Automation of the SPE procedure reduced hands-on sample preparation time by enabling simultaneous processing of eight samples in approximately one hour, allowing the analyst to focus on other tasks during the automated SPE procedure. In addition, direct analysis of the 5 mL SPE eluate eliminated the dry-down and reconstitution steps, further reducing analyst intervention and overall sample preparation time. For laboratories seeking lower-level detection, this flexible sample preparation procedure can support the concentration step, where preferred. Running a 10 µL injection, all analytes were acquired by LC-MS/MS in 11 minutes.

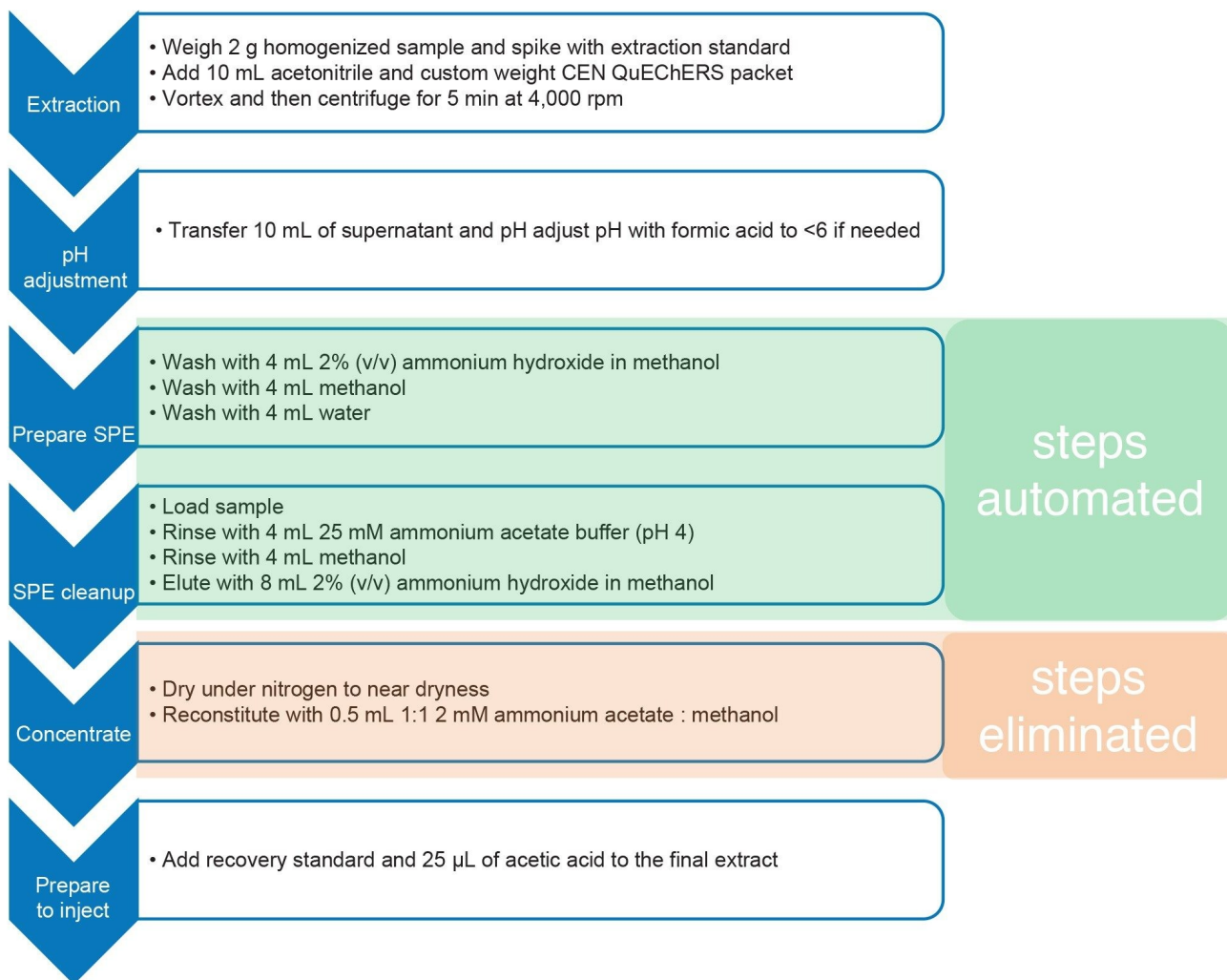


Figure 1. Summary of the sample preparation procedure.

Results and Discussion

Linearity and Chromatography

A ten-point solvent calibration curve was prepared and used for quantification of all food sample extracts, over a calibration range of 5–5,000 ng/L (corresponding to 0.025 μ g/kg–25 μ g/kg in the original sample). Calibration standards were analyzed throughout each sequence alongside spiked samples.

Linear regression with 1/x weighting was used for quantification. Good linearity was achieved for all analytes, including the four EU-regulated PFAS analytes, where back-calculated concentrations of the analytes were $\leq$ 20% of the true concentration.

The calibration range was selected to support quantification of PFAS at relevant concentrations, showing excellent system sensitivity. Representative chromatograms of the four EU-regulated PFAS at the lowest calibration level (5 ng/L) are shown in Figure 2. All analytes produced good peak shape with consistent retention times, well separated from isobaric and background interferences.

Method Validation of Meat Samples

Meat samples of beef burger, pork, chicken, and bratwurst (pork and veal sausage) were spiked to 0.1 $\mu\text{g/kg}$ in replicate ($n=3$) and analyzed in triplicate. Percent recoveries were calculated as apparent recoveries,¹⁰ as follows:

$$\% R = \frac{C_{ex} - C_{inc}}{C_{sp}}$$

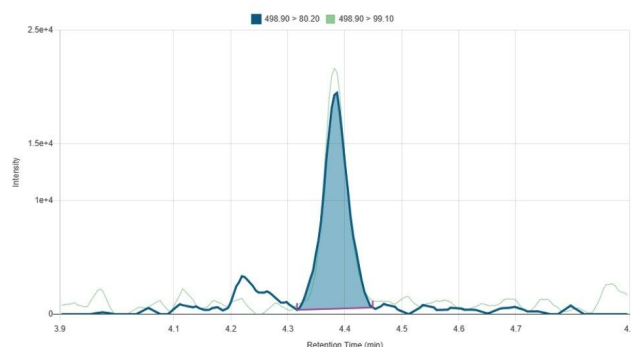
Where C_{ex} : quantity recovered from sample after extraction and clean-up.

C_{inc} : quantity detected in the original sample when analyzed as a blank or non-spiked sample.

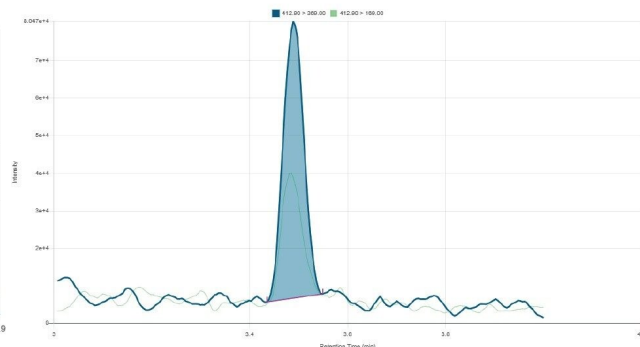
C_{sp} : known quantity spiked into the sample.

The average apparent recovery and within laboratory reproducibility of relative standard deviation (%RSD) was calculated from replicate samples, where results for the majority of analytes ($> 92\%$) were in accordance with the EURL for POPs guidance on PFAS analytical performance. For the four regulated analytes, apparent recoveries were close to 80 and 120%, with $\%RSD \leq 20\%$, while the remaining analytes met recommended tolerances for monitoring purposes with apparent recovery within 65 to 135% and $\%RSD \leq 25\%$. All results are summarized in Table 3.

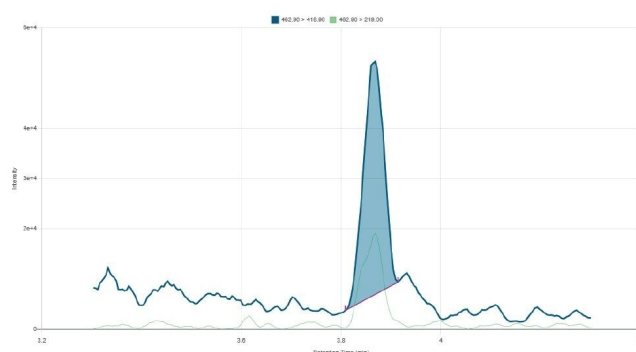
PFOS



PFOA



PFNA



PFHxS

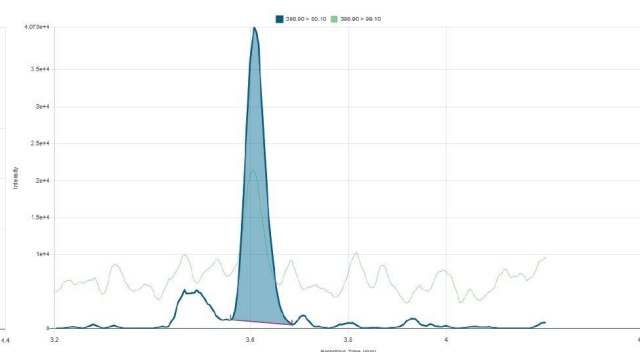
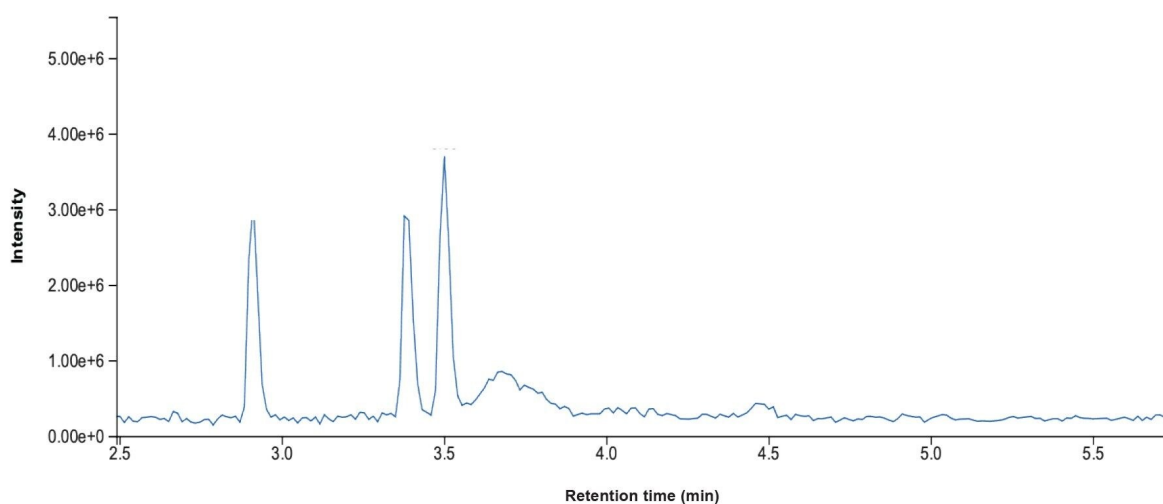


Figure 2. Representative chromatograms of the EU-regulated PFAS (PFOS, PFOA, PFNA, PFHxS) at the lowest calibration level (5ng/L).

A further example of this chromatographic performance is shown in Figure 3, where isobaric interferences cholic acids were baseline separated from PFOS. Cholic acids and their salts, including taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), and tauroursodeoxycholic acid (TUDCA), are bile acids, naturally present in biological samples, including edible meats. Due to their chemical characteristics, they may cause false detections of PFOS by triple quadrupole mass spectrometry, if not removed during clean-up or chromatographically resolved from the analyte peak of PFOS transition 499 > 80.

Cholic acids



PFOS

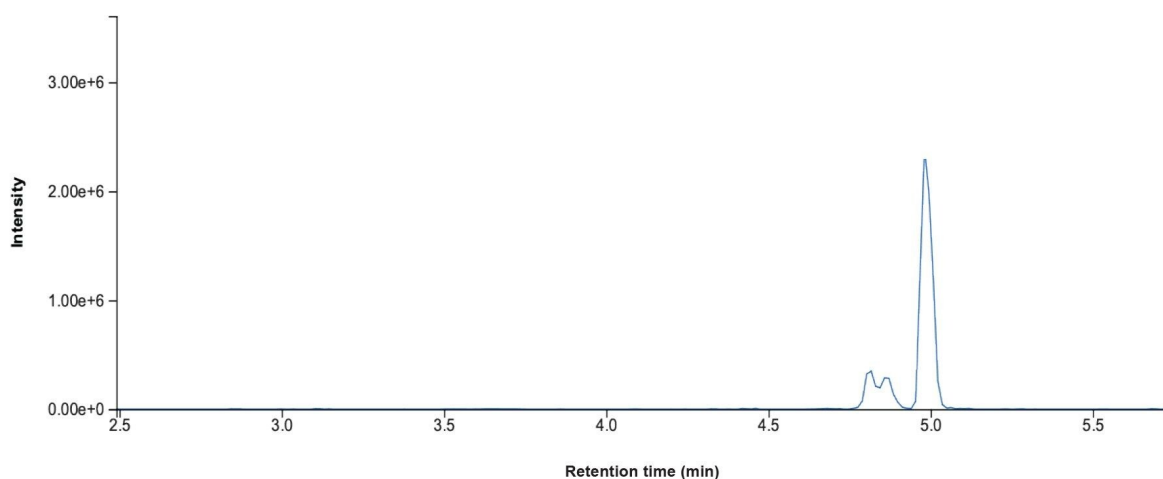


Figure 3. Example of PFOS chromatographically resolved from cholic acids, separated by more than one minute, reducing the risk of false detections when analyzing foods of animal origin.

Method Validation of Meat Samples

Meat samples of beef burger, pork, chicken, and bratwurst (pork and veal sausage) were spiked to 0.1 µg/kg in replicate (n=3) and analyzed in triplicate. The average apparent recovery and within laboratory reproducibility (%RSD) was calculated from these samples, where results for the majority of analytes (> 92%) were in accordance with the EURL for POPs guidance on PFAS analytical performance. For the four regulated analytes, apparent recoveries were close to 80 and 120%, with %RSD ≤ 20%, while the remaining analytes met

recommended tolerances for monitoring purposes with apparent recovery within 65 to 135% and %RSD $\leq$ 25%. All results are summarized in Table 3.

Compound	Bratwurst % Recovery	% RSD	Beef % Recovery	% RSD	Chicken % Recovery	% RSD	Pork % Recovery	% RSD
PFOS	77	9%	110	10%	88	6%	63	9%
PFOA	119	9%	111	3%	107	5%	87	5%
PFNA	123	14%	112	3%	107	6%	92	7%
PFHxS	102	5%	109	6%	73	4%	88	10%
PFBA	156	4%	130	1%	156	5%	157	23%
PFPeA	115	2%	107	4%	119	3%	108	6%
PFHxA	63	9%	63	12%	56	9%	53	13%
PFHpA	109	3%	110	4%	108	3%	93	5%
PFDA	113	10%	116	3%	115	3%	94	5%
PFDODA	90	13%	103	6%	104	4%	87	17%
PFBS	115	9%	101	1%	105	6%	94	16%
PFPeS	101	3%	104	5%	69	3%	78	17%
PFHpS	146	13%	96	8%	95	6%	85	4%
PFNS	129	18%	98	10%	92	5%	87	2%
PFDS	129	12%	97	4%	107	4%	97	2%
PFDODS	90	8%	103	3%	98	4%	95	2%
PFEESA	95	2%	102	3%	99	4%	95	8%
PFMPA	90	7%	99	2%	97	5%	93	9%
PFMBA	88	4%	97	3%	94	6%	83	12%
GenX	106	5%	113	1%	107	5%	83	12%
ADONA	99	3%	107	5%	97	4%	76	14%
FOSA	97	3%	101	2%	102	3%	92	8%
NMeFOSA	110	2%	100	6%	119	5%	105	10%
NEtFOSA	93	4%	103	5%	103	4%	101	9%
N-MeFOSAA	101	19%	109	11%	106	7%	104	15%
N-EtFOSAA	92	11%	95	16%	95	7%	74	16%
NMeFOSE	93	3%	92	3%	100	2%	100	11%
NEtFOSE	93	1%	100	3%	98	1%	98	8%
NFDHA	94	1%	99	7%	92	4%	86	18%
9CI-PF3ONS	109	8%	90	8%	89	3%	82	5%
11CI-PF3OUdS	133	11%	104	7%	97	5%	95	5%
4:2 FTS	71	11%	100	9%	80	23%	78	10%
6:2 FTS	130	3%	128	25%	131	9%	171	18%
8:2 FTS	94	10%	110	9%	105	6%	85	20%
3:3 FTCA	85	13%	87	4%	87	4%	44	28%
5:3 FTCA	90	2%	91	4%	76	3%	103	6%
7:3 FTCA	103	4%	113	8%	90	7%	160	3%
PFTrDS	106	9%	109	4%	109	5%	105	15%
PFTreDA	89	8%	104	9%	105	2%	97	6%
PFTriDA	121	10%	124	4%	89	10%	51	18%
PFUnDA	99	10%	104	7%	103	7%	89	2%
PFUnDS	58	3%	90	8%	80	7%	82	25%
Capstone A	13	63%	30	31%	19	23%	12	21%
Capstone B	100	20%	105	13%	71	27%	60	12%

Table 3. Mean recovery and relative standard deviation (% RSD) for PFAS fortified at 0.1 µg/kg in representative

food matrices.

The recovery standard (NIS), where used and spiked into all samples prior to injection, showed good recovery across all injections. An example of this is shown in Figure 4 for M8 PFOS across the analytical run as plotted within MS Quan Application, allowing for a quick check on batch performance during routine operation.

Recovery deviation

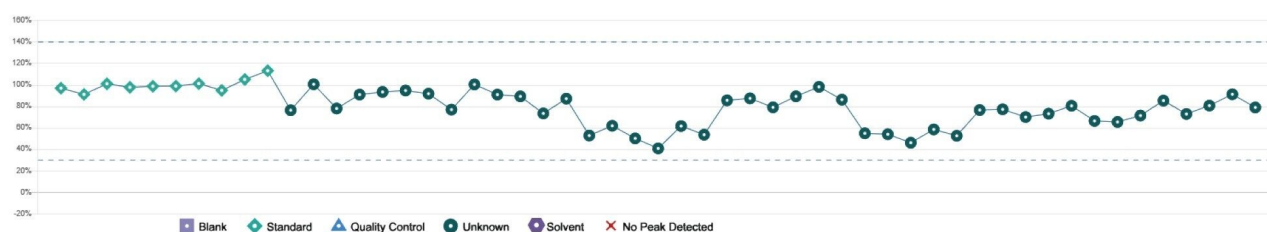


Figure 4. Plot extracted from MS Quan Application showing the trend of M8 PFOS used as a recovery standard, where all injections fell within the 30 to 140% tolerance (dotted blue lines) stated in the EURL for POPs guidance document.

Previous work demonstrated that capstone A may be lost during the SPE procedure.⁵ Recovery of capstone A was consistently lower than that of capstone B, likely because no compound-specific isotopically-labeled internal standard was available at the time of experimentation. Instead, the isotopically-labeled capstone B analogue was used as a surrogate internal standard for both analytes, which may not fully compensate for differences in analyte behavior experienced by capstone A throughout the workflow. Despite this limitation, these results demonstrate that the workflow provides reliable extraction, cleanup, and quantitation for a broad range of PFAS in complex food matrices, including meats, while maintaining performance at concentrations relevant to current regulatory requirements.

Identification

In accordance with EURL for POPs guidance, all identification criteria were applied, using customizable and automated tools within waters_connect Software and MS Quan Application. At least 2 MRM transitions were acquired per analyte, with ion ratios, relative retention times, and signal to noise (S/N) criteria set in the method.

All validation data was reviewed against these criteria to ensure LOQ in accordance with EURL for POPs guidance. An example is summarized for PFBA in the beef-spiked sample, where all criteria detailed in the table inset were met.

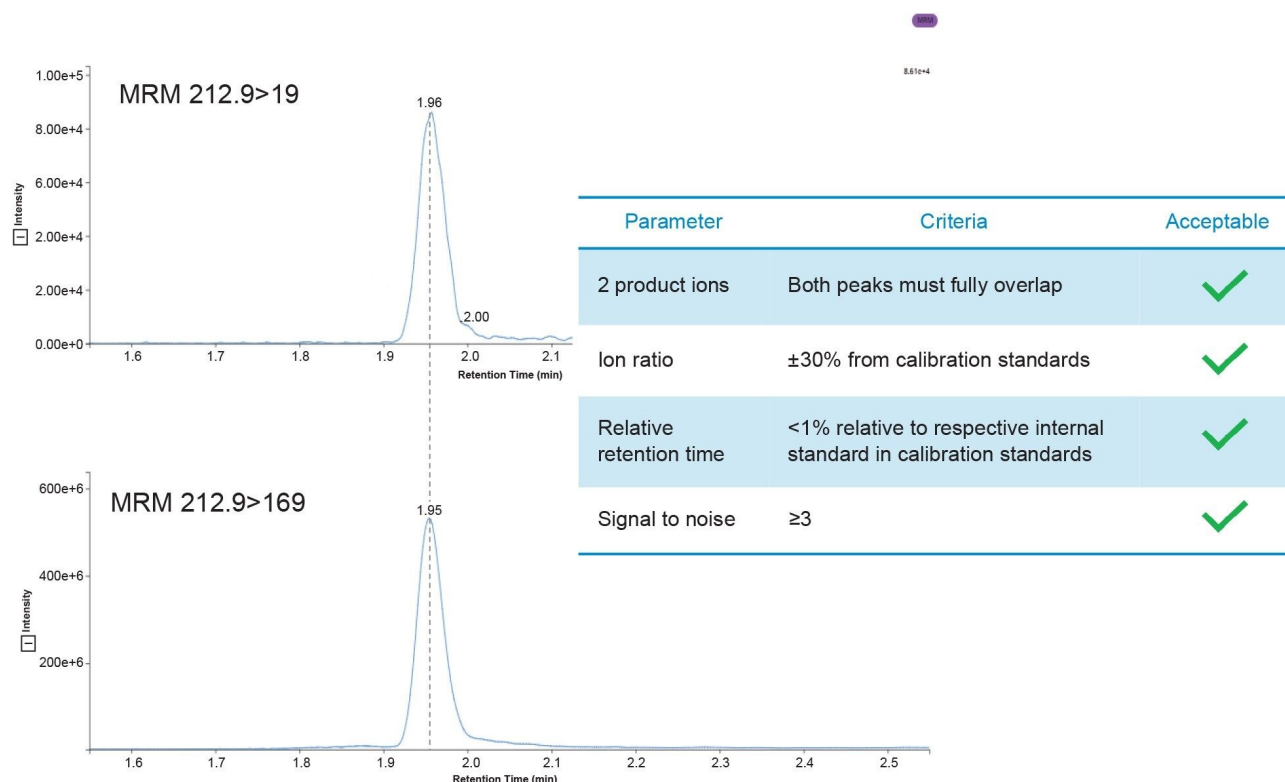


Figure 5. Representative chromatography of PFBA in beef fortified at 0.1 µg/kg, showing two transitions with full overlap of the quantifier and qualifier product ion transitions. The inlaid table summarizes the identification criteria, all of which were acceptable.

Conclusion

Streamlining the sample preparation protocol by reducing the sample weight, automating SPE clean up, and eliminating evaporation and reconstitution stages, created an efficient, yet reliable LC-MS/MS method, enabling food testing laboratories to meet high-throughput demand for meat samples analyzed in accordance with

current regulatory requirements. Removing the evaporation step from the workflow allowed for time savings, while reducing the risk of concentrating contamination of environmental PFAS from sources such as solvent, blow down gas, and equipment.

Overall, acceptable recoveries, precision and identification was achieved for all four meat samples at 0.1 µg/kg, in accordance with EURL for POPs guidance on PFAS analytical parameters, demonstrating method performance at the LOQ to support compliance and monitoring testing of meats. Combining technological advantages of automated SPE clean-up, advanced chromatography, MS performance, and waters_connect Software, an accessible, time efficient yet reliable workflow has been demonstrated for laboratories working to meet the demands of high-throughput in accordance with current EU regulatory requirements for PFAS in meats.

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