

Extraction of Polyfluorinated Compounds in Water Using EVOLUTE® EXPRESS WAX SPE Prior to LC-MS/MS Analysis

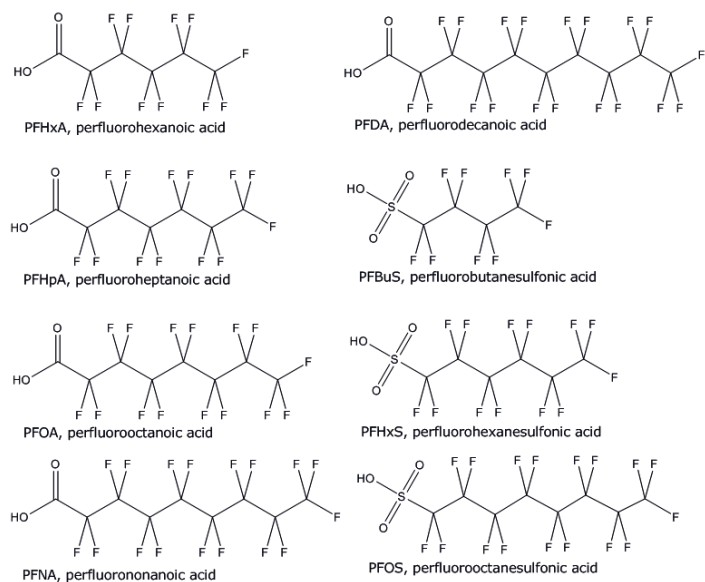


Figure 1. Structures of target polyfluorinated compounds.

Introduction

Polyfluorinated compounds (PFCs) such as PFOS (perfluorooctanesulfonic acid) and PFOA (perfluorooctanoic acid) are man-made fluorosurfactants. Due to their widespread industrial use and chemical stability, they have become ubiquitous in the natural environment. Exposure to PFCs has been linked to cancer, endocrine disruption and developmental delays.

The method described in this application achieves high reproducible recoveries for a number of common PFCs in water (chemical structures are shown in Figure 1), using an optimized weak anion exchange SPE procedure with EVOLUTE® EXPRESS WAX SPE columns.

EVOLUTE EXPRESS SPE products dramatically improve flow characteristics, and enhance sample preparation productivity providing clean, robust, sample preparation.

Analytes

Perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorobutanesulfonic acid (PFBuS), perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS)

Sample Preparation Procedure

Analytes were prepared at a concentration of 50 pg μL^{-1} in 18.2 M Ω cm water and stored at approximately 4 °C prior to use. A 20 μL aliquot of this solution was diluted to 1 L in 18.2 M Ω cm water to give a spiked concentration of 1 ng L^{-1} . No internal standards were used in this method.

Format

EVOLUTE® EXPRESS WAX 60 mg/3 mL SPE columns, part number 614-0006-BXG; with 150 mL reservoirs (p/n 120-1010-J), connected using PE Column Adapters (p/n 120-1101)

Condition

Condition the column with methanol (2 mL)

Equilibration

Equilibrate the column with 18.2 M Ω -cm water (2 mL)

Sample Loading

Load untreated water (100 mL) at approximately 6 mL min^{-1}

Wash 1

Elute interferences with 18.2 M Ω -cm water (2 mL)

Wash 2

Elute interferences with 1% formic acid (2 mL)

Wash 3

Elute interferences with methanol (2 mL)

Elution

Elute analytes with 1% concentrated ammonia solution (28–30% v/v) in methanol (3 x 0.8 mL)

Post Elution

Evaporate the eluate to dryness in a stream of air or nitrogen using a TurboVap® (1.5 bar at 40 °C for 1 hr). Reconstitute in 120 μL 40% methanol (aq).



HPLC Conditions

Instrument

Nexera UHPLC system (Shimadzu Europe GmbH, Germany)

Column

ACE Excel 1.7 C18 100 x 2.1 mm (Hichrom Ltd., UK)

Mobile Phase

A: water: acetonitrile (9:1 v/v) containing 2 mM ammonium acetate and 0.1% (v/v) formic acid

B: water: acetonitrile (1:9 v/v) containing 2 mM ammonium acetate and 0.1% (v/v) formic acid

Flow Rate

0.5 mL min⁻¹

Injection Volume

10 µL

Gradient

Initial to 0.5 min hold at 25% B

0.5 to 3.5 min linear ramp to 70% B

3.5 to 4 min linear ramp to 100% B

4 to 5.5 min hold at 100% B

5.5 to 6 min linear ramp to 25% B

6 to 9 min hold at 25% B

Total run time 9 min

(MS acquisition during initial 4.5 mins only)

Column Temperature

40 °C

Sample Temperature

20 °C

Table 1. Typical retention times for PFC analytes.

Analyte	Retention time (min)
perfluorohexanoic acid (PFHxA)	2.2
perfluorobutanesulfonic acid (PFBS)	2.2
perfluoroheptanoic acid (PFHpA)	2.4
perfluorooctanoic acid (PFOA)	3.0
perfluorohexanesulfonic acid (PFHxS)	3.1
perfluorononanoic acid (PFNA)	3.4
perfluorodecanoic acid (PFDA)	3.7
perfluorooctanesulfonic acid (PFOS)	3.9

Mass Spectrometry Conditions

Ions were selected in order to achieve maximum sensitivity in negative ion mode using multiple reaction monitoring.

Instrument

Triple Quad 5500 (AB Sciex, Framingham USA)

Carrier Gas

40

IonSpray Voltage

-2400

Temperature

450

Ion Source Gas 1 (GS1)

40

Ion Source Gas 2 (GS2)

50

Collision Gas

7

Entrance Potential, EP

-5.0

Setting Time

50 ms

Pause Between Mass Ranges

50 ms

Table 2. Mass Spectrometer properties for selected PFC analytes.

Analyte	Transition (m/z)	DP (V)	CE (V)	CXP (V)
perfluorohexanoic acid (PFHxA)	313.0>268.9	-40.0	-11.0	-14.0
perfluorobutanesulfonic acid (PFBS)	299.0>79.9	-75.0	-67.0	-8.0
perfluoroheptanoic acid (PFHpA)	363.0>319.0	-40.0	-13.0	-14.0
perfluorooctanoic acid (PFOA)	413.0>368.9	-55.0	-5.0	-20.0
perfluorohexanesulfonic acid (PFHxS)	399.0>80.0	-100	-85.0	-8.0
perfluorononanoic acid (PFNA)	463.0>419.0	-50.0	-13.0	-26.0
perfluorodecanoic acid (PFDA)	513.0>469.0	-58.0	-15.0	-27.0
perfluorooctanesulfonic acid (PFOS)	499.0>80.0	-100	-120	-10.0

Method Evaluation

Method linearity was determined by spiking known amounts of PFCs into 18.2 MΩ cm water. Calibration lines were constructed by spiking 100 µL of PFC stocks at variable concentrations into 250 mL 18.2 MΩ cm water giving final concentrations of 0.1, 0.5, 2, 4 and 5 ng L⁻¹. The samples were extracted in duplicate using the method detailed above to give duplicate calibration curves. A 1/x weighting was applied and the regression coefficient (r) was determined using Analyst software.

Method LOQ was determined from the lowest matrix-spiked calibration standard giving a peak to peak signal:noise ≥ 10:1.

Performance of the method using Load-Wash-Elute and standard SPE protocols was compared using conditions summarized in Table 3. Extraction recoveries of analytes spiked in 18.2 MΩ cm water at 1 ng L⁻¹ were compared.



Table 3. Side by side comparison of the Biotage Load-Wash-Elute and standard SPE methods.

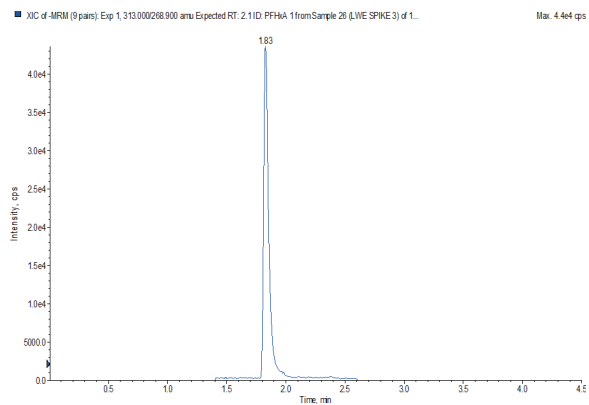
Extraction Step	Load-Wash-Elute Method	Standard SPE Method
Condition	N/A	2 mL methanol
Equilibrate	N/A	2 mL water
Sample Load	100 mL water (spiked at 1 ng L ⁻¹)	
Wash 1	2 mL water	
Wash 2	2 mL 1 % formic acid	
Wash 3	2 mL methanol	
Elution	3 x 0.8 mL 1 % ammonia solution in methanol	

Results

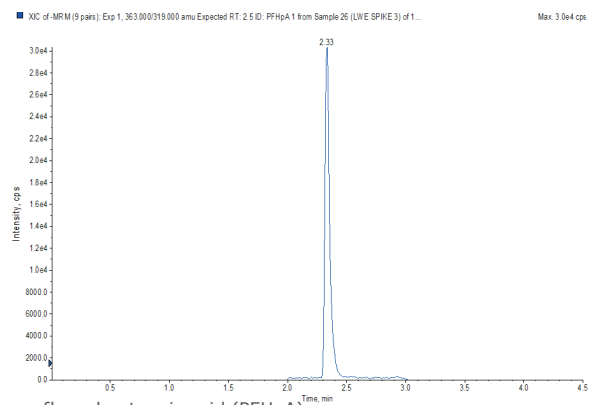
The extracted ion chromatograms in Figure 2 demonstrate good chromatography at 1 ng L⁻¹ from a spiked extraction of 100 mL 18.2 MΩ cm water. The analytes demonstrate acceptable linearity over the calibrated range of 0.1 ng L⁻¹ to 5 ng L⁻¹ as demonstrated by the calibration curves in Figure 3. The correlation coefficients and LOQ are tabulated in Table 4. A Load-Wash-Elute and standard SPE method demonstrate similar linearity and LOQ. Signal:noise at 0.1 ng mL⁻¹ is comparable between both methods, slightly higher using a standard SPE method compared to Load-Wash-Elute.

Table 4. PFC linearity coefficients and LOQ for Load-Wash-Elute and standard SPE methods.

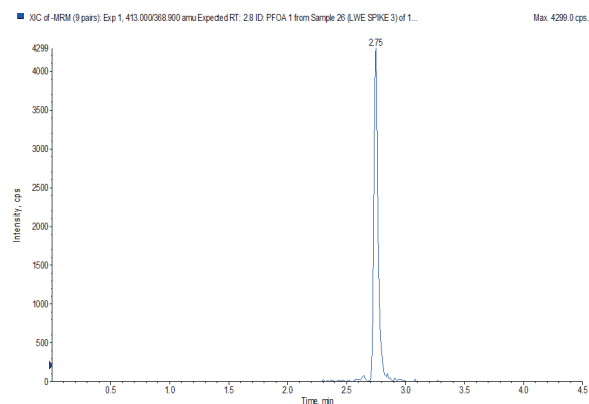
	Load-Wash-Elute method			Standard SPE method		
	Coefficient (r)	LOQ (ng L ⁻¹)	S/N (peak-to-peak)	Coefficient (r)	LOQ (ng L ⁻¹)	S/N (peak-to-peak)
perfluorohexanoic acid (PFHxA)	0.9984	0.1	145	0.9974	0.1	158
perfluorobutanesulfonic acid (PFBuS)	0.9978	0.1	192	0.9942	0.1	337
perfluoroheptanoic acid (PFHpA)	0.9984	0.1	177	0.9979	0.1	189
perfluorooctanoic acid (PFOA)	0.9980	0.1	102	0.9969	0.1	121
perfluorohexanesulfonic acid (PFHxS)	0.9994	0.1	140	0.9979	0.1	263
perfluorononanoic acid (PFNA)	0.9991	0.1	81	0.9974	0.1	72
perfluorodecanoic acid (PFDA)	0.9972	0.1	165	0.9942	0.1	153
perfluorooctanesulfonic acid (PFOS)	0.9989	0.1	10	0.9980	0.1	14



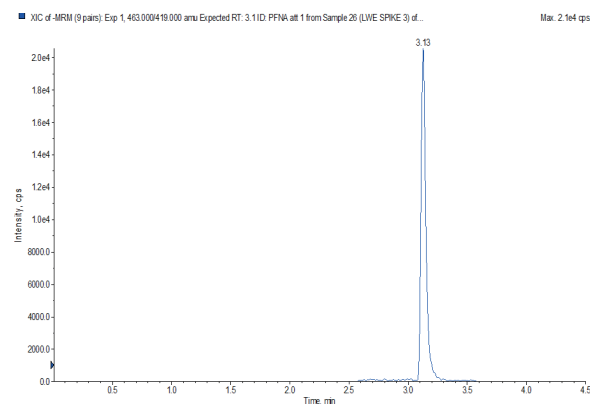
perfluorohexanoic acid (PFHxA)



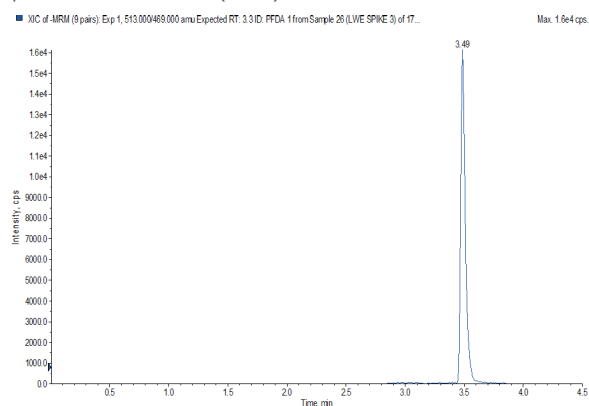
perfluoroheptanoic acid (PFHpA)



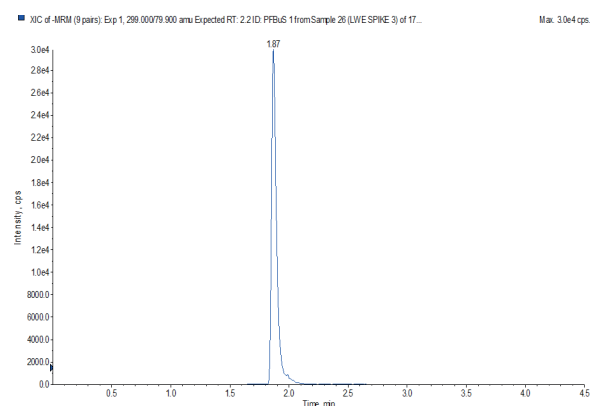
perfluorooctanoic acid (PFOA)



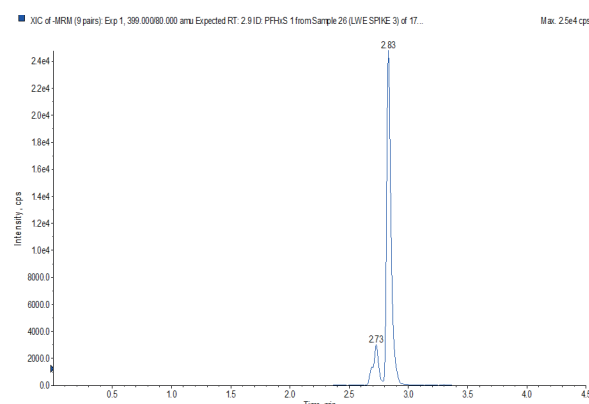
perfluorononanoic acid (PFNA)



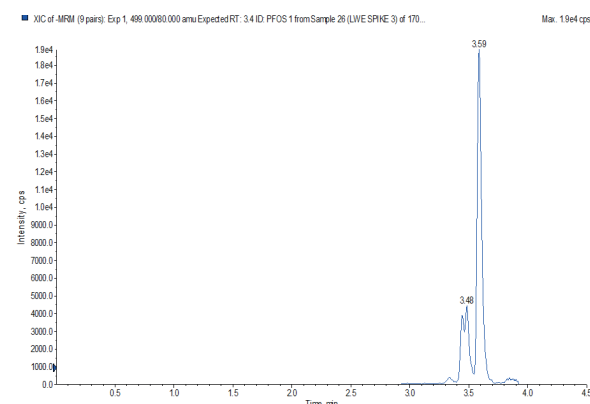
perfluorodecanoic acid (PFDA)



perfluorobutanesulfonic acid (PFBuS)

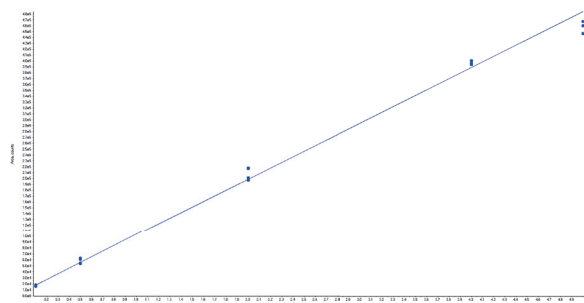


perfluorohexanesulfonic acid (PFHxS)

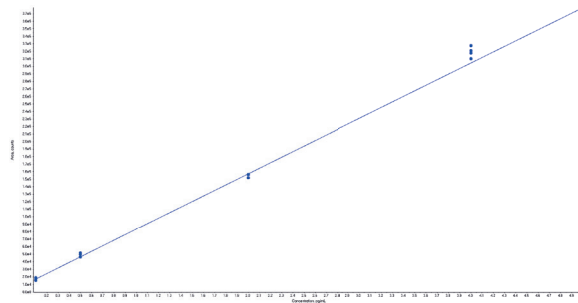


perfluorooctanesulfonic acid (PFOS)

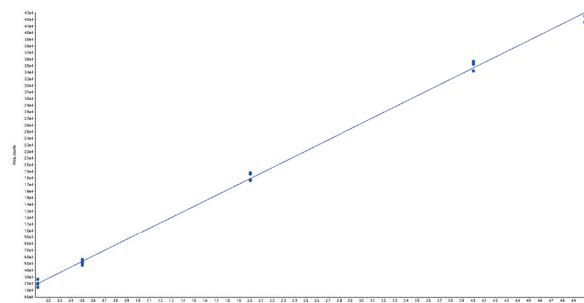
Figure 2. Representative extracted ion chromatograms of PFC compounds at 1 ng L^{-1} (extracted from 100 mL 18.2 M Ω cm water) using a Load-Wash-Elute method.



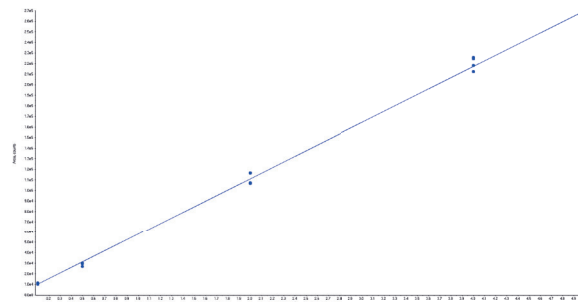
perfluorohexanoic acid (PFHxA)



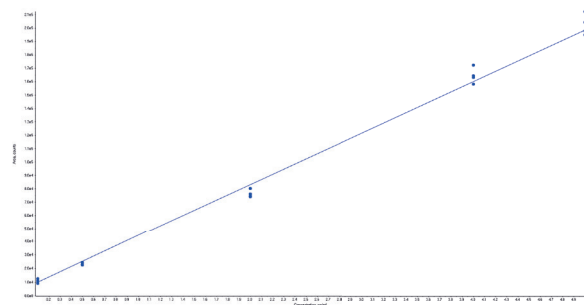
perfluoroheptanoic acid (PFHpA)



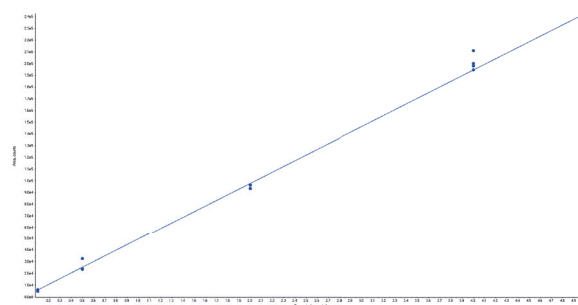
perfluorooctanoic acid (PFOA)



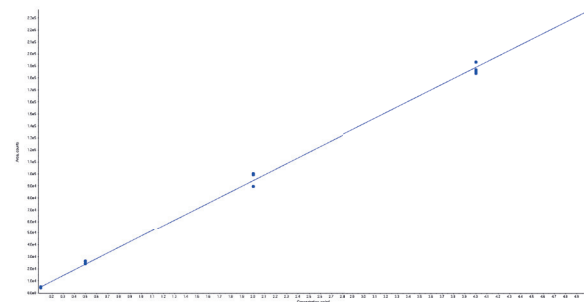
perfluorononanoic acid (PFNA)



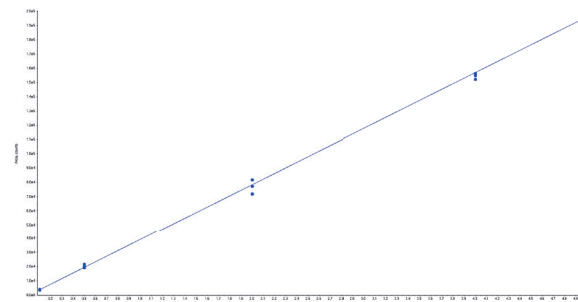
perfluorodecanoic acid (PFDA)



perfluorobutanesulfonic acid (PFBS)



perfluorohexanesulfonic acid (PFHxS)



perfluorooctanesulfonic acid (PFOS)

Figure 3. Calibration curves of PFC compounds spiked in 100 mL 18.2 MΩ cm water using a Load-Wash-Elute method.

The Load-Wash-Elute method detailed in Table 3 gave comparable extraction recoveries to a standard SPE method incorporating conditioning and equilibration steps (Table 5). Perfluorocarboxylic acid recoveries were slightly lower using a Load-Wash-Elute method compared to a standard SPE method. Perfluorosulfonic acid recoveries were slightly higher using a Load-Wash-Elute method compared to a standard SPE method. RSD for both methods were less than 10%.

Table 5. Extraction recovery and precision of Load-Wash Elute and standard SPE methods.

	Load-Wash-Elute method		Standard SPE method	
	Extraction recovery %	% RSD (n=5)	Extraction recovery %	% RSD (n=5)
perfluorohexanoic acid (PFHxA)	89	3.5	91	2.6
perfluorobutanesulfonic acid (PFBS)	76	7.9	92	4.8
perfluoroheptanoic acid (PFHpA)	88	1.8	93	6.0
perfluorooctanoic acid (PFOA)	89	4.3	92	6.1
perfluorohexanesulfonic acid (PFHxS)	84	4.4	93	6.9
perfluorononanoic acid (PFNA)	88	3.1	77	9.4
perfluorodecanoic acid (PFDA)	91	8.5	81	5.3
perfluorooctanesulfonic acid (PFOS)	84	9.6	82	5.4



Additional Notes

1. SPE method robustness may be further improved by the incorporation of a high pH conditioning step (e.g. 1% ammonia solution in methanol) prior to conditioning with methanol as part of a standard SPE methodology.
2. The method detailed in this application note lists a selection of PFCs, demonstrating method applicability. System changes were made to reduce the background level of PFCs, replacing the solvent delivery tubing of the LC pumps with non PTFE alternatives. Some PFCs e.g. heptafluorobutyric acid were not measured because the LC-MS system was not sufficiently adapted to remove background levels. The results demonstrate other PFCs would be expected to have similar method performance metrics when analyzed using a system with low PFC background levels.
3. Additional options to reduce PFC background levels include: thorough purging/flushing of the LC-MS system with methanol prior to analysis; thorough cleaning/rinsing of glassware whilst ensuring it has not been in contact with phosphoate-containing detergents; use of plastic vessels in contact with pre/post extracted samples; retrofitting non-PTFE polymeric tubing in all parts of the LC-MS system; installation of a PFC-scavenger column between the pumps and the injector.

Ordering Information

Part Number	Description	Quantity
614-0006-BXG	EVOLUTE® EXPRESS WAX 60 mg / 3 mL SPE cartridges	50
120-1010-J	ISOLUTE® Reservoir, 150 mL	25
120-1101	PE Column Adapters 1,3,6 mL Columns	10
121-2010	Biotage® VacMaster™-20 Sample Processing Station (with 10 mm Rack)	1
C103198	TurboVap® LV Without Racks 100/120V	1
C103199	TurboVap® LV Without Racks 220/240V	1

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