SHIMADZU

TP 314

Determination of PFAS in Pharmaceutical Water Used for Development of Injectable Drugs Using a High-Speed Triple Quadrupole

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are associated with risks to human health, including the development of tumors and other serious diseases that affect quality and life expectancy. [1] In this context, due to the dangerous nature of injectable drugs, a detailed extraction and leaching studies are necessary for the safe regulation of this type of product. [2] The objective of this study was to develop an analytical methodology for the detection of PFAS in the pharmaceutical water matrix, used for the preparation of injectable medicines, and to evaluate the possible effect of leaching and extraction in “serum bag” type packaging (**Figure 1**).

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Figure 1 – Cell culture sampling packaging (Millipore®) used in the leaching study. Image sourced from: https://www.merckmillipore.com/

2. Methods

Analyses were performed using an integrated LC-2060C liquid chromatograph coupled to an LCMS-8045 mass spectrometer, equipped with an electrospray ionization (ESI) source.

The LabSolutions software was used for sample injections, MRM event optimization, and data processing. The Connect and Insight software platforms were employed for interface condition optimization and data analysis, respectively.

Calibration curves were prepared by spiking pharmaceutical-grade water with the target standards and internal at 100 ng/L. After spiking, samples were injected directly into the system.

Sample preparation for the leaching study involved adding 50 mL of pharmaceutical-grade water to a cell culture sampling container (**Figure 1**), Millipore®, simulating an intravenous fluid bag (“serum bag"). Two units of this packaging were used: one was maintained at room temperature (RT) for 3 hours, and the other was kept in an oven at 60° C.

The same procedure was applied to the container's inlet septum (see **Figure 1**), which was removed and stored in Falcon-type tubes with 50 mL of pharmaceutical-grade water. This sample preparation was also performed using ethanol (EtOH) as the solvent to assess potential extraction effects.

Subsequently, the samples were aliquoted into vials and injected into the LCMS for analysis. Analytical conditions are summarized in **Table 1**.

Table 1 – Analytical conditions.

Analytical column	Ascentis® Express PFAS, 10mm x 3.0mm x 2.7 µm (PN 53564-U)
Delay column	Ascentis® Express PFAS DELAY, 5.0mm x 3.0mm x 2.7 µm (PN 53572-U)
Mobile phase	A – 5 mM de ammonium acetate in water B – MeOH
Gradient	0,00 a 0,50 min = 40% B; 0,50 a 6,00 min = 98% B; 6,00 a 9,00 min = 98% B; 9,00 a 9,10 min = 40% B; 9,10 a 13,00 min = 40% B
Flow	0.3 mL/min
Oven	50 ºC
Injection volume	50 µL
Heating block temperature	250° C
DL temperature	150 ° C
Interface temperature	300 ° C
Nebulizer gas	3 L/min
Drying gas	5 L/min
Heating gas	15 L/min
Interface voltage	- 1 kV

3. Results

Linearity tests were conducted over the range of 20 to 200 ng/L (vial concentration), and satisfactory results were obtained for all analyzed PFAS compounds ($R^2 > 0.99$).

Table 2 lists all monitored analytes (target compounds) and internal standards, along with the corresponding MRM transitions used. **Figure 2** shows the calibration curves obtained for the analytes PFOA, 4:2 FTS, and PFBA. **Figure 3** shows the results of leaching experiments.

Table 2 – MRM transitions employed for the target analytes and internal standards.

Analyte	MRM1	MRM2	ISTD
Targets			
PFBA	212.9>169.0	-	M2PFHxA
PFPeA	262.9>219.2	-	M2PFHxA
4:2FTS	326.8>307.1	326.8>80.9	MPFOS
PFBS	298.9>79.9	298.9>98.9	M2PFHxA
PFHxA	312.9>269.2	312.9>119.0	M2PFHxA
HFPO-DA	328.9>169.2	328.9>285.1	13C-HFPO-DA
PFHpA	362.8>319.0	362.8>169.1	M2PFHxA
PFPeS	348.8>80.0	348.8>99.0	M2PFHxA
6:2FTS	426.9>406.9	426.9>80.0	MPFOS
PFOA	412.8>369.0	412.8>169.1	M2PFOA
PFHxS	398.7>80.0	398.7>98.9	MPFOS
PFNA	462.8>419.1	462.8>219.0	M2PFOA
PFHpS	448.9>80.0	448.9>99.1	MPFOS
8:2FTS	526.8>507.0	526.8>81.0	MPFOS
N-Me-FOSAA	569.9>419.1	569.9>483.0	M2PFHxA
PFOS	498.9>79.7	499.0>98.9	MPFOS
N-EtFOSAA	583.9>419.0	583.9>168.9	M2PFHxA
PFUnA	563.0>518.9	563.0>269.1	M2PFDA
PFNS	548.9>98.8	548.9>79.9	MPFOS
PFHxDA	812.7>768.9	812.7>318.7	MPFOS
PFODA	912.7>868.9	912.7>169.1	M2PFHxA
ISTDs			
M2PFHxA	314.8>270.1	314.8>118.8	-
13C-HFPO-DA	286.9>169.2	286.9>185.0	-
M2PFOA	414.8>369.9	414.8>170.2	-
M2PFDA	514.8>219.9	514.8>270.0	-
MPFOS	502.9>79.8	502.9>99.1	-
D5-N-EtFOSAA	589.2>530.9	589.2>419.1	-

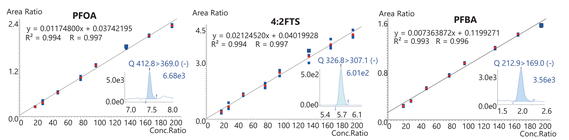

Figure 2 – Calibration curves and chromatogram of the lower limit of quantification (LOQ) for selected PFAS in pharmaceutical-grade water matrix.

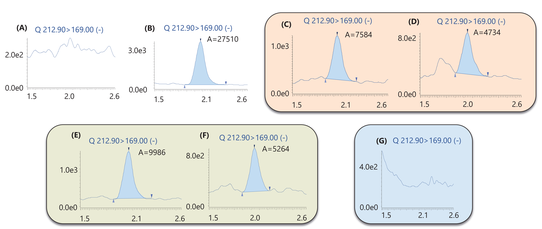

Figure 3 – PFBA monitoring: (A) Blank; (B) 20 ppt point of calibration curve; (C) Packaging with pharmaceutical water at room temperature for 3 h; (D) Packaging with pharmaceutical water at 60° C for 3 h; (E) Septum in pharmaceutical water at room temperature for 3 h; (F) Septum in pharmaceutical water at 60° C for 3 h; (G) Packaging with EtOH at room temperature for 3 h.

4. Conclusion

Using the LC-2060C system coupled to the LCMS-8045 mass spectrometer, a highly sensitive method was developed for the quantification of PFAS in pharmaceutical-grade water, with detection limits in the ppt range. This method was applied to leaching tests involving IV bag-type packaging, where PFBA was detected at levels below the calibration curve's lower limit. When EtOH was used as an alternative solvent, no PFAS were detected, suggesting that solvent polarity may influence the leaching behavior of PFBA from the tested packaging.

Reference

1) GRANDJEAN, P., & CLAPP, R. (2020). : A Journal of Environmental and Occupational Health Policy, 30(4), 223-246.
2) BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). RESOLUÇÃO RDC nº 17, de 16 de abril de 2010.