Method for
Analysis of PFAS in Pesticide Products
Containing Non-ionic Surfactants and Non-volatile Oils

Scope of Method and Application

This method is for the analysis of poly- and per-fluorinated alkyl substances (PFAS) in pesticide formulations containing non-ionic surfactants and oil. It is based on a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction approach, followed by Solid Phase Extraction (SPE) cleanup to remove excess oily substances, and analysis using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). This method is not applicable if formulations contain ionic surfactants (such as sodium lauryl sulfate, quaternary ammonium compounds, etc.) or only organic solvents/liquids (petroleum distillates, mineral oil, etc.). A different method Analysis of PFAS in Oily Matrix (epa.gov) can be used for pesticide products formulated in organic solvents/oils.

Note: Due to the wide occurrence of PFAS in the environment, it is highly recommended to verify that all supplies and equipment are free of PFAS above the limit of detection. Certain PFAS compounds have been found in SPE cartridges, SPE manifold, and filters during the method development.

This method is intended for use by analysts skilled in the performance of solid phase extractions, the operation of LC-MS/MS instrumentation, and the interpretation of the associated data. EPA has validated this method through the Analytical Chemistry Branch (ACB) of the Biological and Economic Analysis Division, Office of Pesticide Programs.

Sample Preparation

Solvents:

- Milli-Q water
- Ethyl acetate
- Hexane
- Methanol

Materials:

- QuEChERS salt mix (6 g $MgSO_4$/1.5 g NaCl)
- Ammonium acetate
- Solid Phase Extraction cartridge –Florisil 1 g/6 mL column
- Polypropylene test tubes 15 and 50 mL

Solutions:

- Mobile phase A: Aqueous 20 mM ammonium acetate
- Methanol/water (99/1, v/v)
- Hexanes/ethyl acetate (9/1, v/v)

Standards:

- Extraction Standard: Mixture of isotopically labeled PFAS standards, different from injection standards
- Injection Standard: Mixture of isotopically labeled PFAS standards, different from Extraction standards
- Native PFAS standard: Mixture of all the target PFAS compounds.

Equipment:

- Geno/Grinder or equivalent
- Centrifuge
- N-Evap or equivalent
- Sonicator
- Liquid chromatography/tandem mass spectrometry (LC-MS/MS)

Extraction Procedure:

1. Weigh approximately 4 grams of pesticide products into 50 mL polypropylene centrifuge tubes.
2. For the procedural blank, transfer approximately 4 grams of Milli-Q water into a 50 mL tube.
3. For blank spikes and matrix spikes, weigh approximately 4 grams of Milli-Q water and pesticide product, respectively, into 50 mL tubes.
4. Add appropriate amount of "Extraction Standard" into each sample.
5. Add appropriate amount of spiking solution containing PFAS to spike samples.
6. Mix by vortexing or shaking and then let samples equilibrate after addition of PFAS standards for 15 minutes.
7. Add 5 mL of Milli-Q water and 25 mL of ethyl acetate to each sample.
8. Shake each sample on Geno/Grinder for 20 minutes at 1000 rpm.
9. Add QuEChERS salt mix (6 g MgSO4/1.5 g NaCl) to each sample, shaking by hand to break all salt clumps.
10. Shake all samples on Geno/Grinder for 20 minutes at 1000 rpm, followed by centrifugation for 10 minutes at 4000 rpm.
11. Transfer 20 mL of organic supernatant to a new 50 mL centrifuge tube and concentrate to dryness under N_2 flow at 50°C-60°C. *Note*: Some oil may remain after concentration depending on product formulation.
12. Add 20 mL of hexane/ethyl acetate (9/1, v/v) to the dried extracts and sonicate for 30 minutes, followed by a round of brief hand-shaking and then centrifugation at 4000 rpm for 10 minutes.
13. For solid precipitates: Decant entire supernatant into a new 50 mL tube.
14. For biphasic layers: Carefully transfer 20 mL of organic supernatant to a new 50 mL tube.
15. Concentrate samples as much as possible as in Step 11. Then combine with 5 mL of hexane/ethyl acetate (9/1, v/v) and proceed to SPE cleanup.
16. Attach Florisil SPEs to manifold and condition with 10 mL of methanol, followed by 10 mL of hexane/ethyl acetate (9/1, v/v).

17. Load sample onto SPE, and wash with 10 mL of hexane/ethyl acetate (9/1, v/v). Do not let the column run dry.
18. Place collection tubes under the manifold and elute samples with 10 mL of methanol.
19. For all samples: Add appropriate amounts of "Injection Standard" mixture to all solutions.
20. Concentrate all samples to dryness. Reconstitute with 1 mL of methanol/water (99/1, v/v). *Note:* If precipitate is visible in tube, centrifuge the tubes.
21. Transfer the solutions to LC vials for instrument analysis with LC-MS/MS.

Sample Analysis and Procedure

Calibration:

- Prepare a calibration curve of at least 5 levels in the range of 0.02 – 20 ng/mL of "Native" compounds.
- Each calibration point should also have "Extraction Standards" and "Injection Standards" at, for example, 0.50 ng/mL.

Data Analysis Note:

- Quantitation calculations are based on the response ratio of "Native PFAS" signal to "Extraction Standard" signal.
- Matrix effects can be assessed by comparing responses of "Injection Standards" in samples and calibration sets.

LC-MS/MS Specifications/Parameters

Equipment:	Agilent 6470 LC-MS/MS or Equivalent
Mobile Phase A:	Aqueous 20 mM Ammonium Acetate
Mobile Phase B:	Methanol
Flow Rate:	0.400 mL/min
Solvent Gradient:	70% Mobile Phase A to 5% Mobile Phase A in 13 min.
Total Run Time:	26 minutes + 5 minutes Post Time Equilibration
MS Operation Mode:	Electrospray Negative Ionization (ESI) mode

List of Analyzed PFAS Compounds

Acronym	Chemical Name	Limits of Quantitation (ppb)	Comments
PFBA	Perfluoro-n-butanoic acid	0.40	High background
PFPeA	Perfluoro-n-pentanoic acid	0.40	High background
PFHxA	Perfluoro-n-hexanoic acid	0.40	
PFHpA	Perfluoro-n-heptanoic acid	0.40	
PFOA	Perfluoro-n-octanoic acid	0.40	
PFNA	Perfluoro-n-nonanoic acid	0.40	
PFDA	Perfluoro-n-decanoic acid	0.40	
PFUdA	Perfluoro-n-undecanoic acid	0.40	
PFDoA	Perfluoro-n-dodecanoic acid	0.40	
PFTrDA	Perfluoro-n-tridecanoic acid	0.40	
PFTeDA	Perfluoro-n-tetradecanoic acid	0.40	

Acronym	Chemical Name	Limits of Quantitation (ppb)	Comments
PFHxDA	Perfluoro-n-hexadecanoic acid	0.40	
PFODA	Perfluoro-n-octadecanoic acid	2.00	Low recovery
PFPeS	Perfluoro-1-pentanesulfonate, Potassium Salt	0.40	
PFHxS	Perfluoro-1-hexanesulfonate, Sodium Salt	0.40	
PFHpS	Perfluoro-1-heptanesulfonate, Sodium Salt	0.40	
PFOS	Perfluoro-1-octanesulfonate, Sodium Salt	0.40	
PFNS	Perfluoro-1-nonanesulfonate, Sodium Salt	0.40	
PFDS	Perfluoro-1-decanesulfonate, Sodium Salt	0.40	
PFDoS	Perfluoro-1-dodecanesulfonate, Sodium Salt	0.40	
FOSAA	Perfluoro-1-octanesulfonamidioacetic acid	2.00	
N-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	0.40	
N-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid	2.00	
11Cl-PF3OUDS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonate, Potassium Salt	0.40	
9-CL-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate, Potassium Salt	0.40	
4:2 FTS	1H, 1H, 2H, 2H-perfluorohexanesulfonate, Sodium Salt	2.0	
6:2 FTS	1H, 1H, 2H, 2H-perfluorooctanesulfonate, Sodium Salt	2.0	High background
8:2 FTS	1H, 1H, 2H, 2H-perfluorodecanesulfonate, Sodium Salt	0.40	
ADONA	Dodecafluoro-3H-4,8-dioxanonanoate, Sodium Salt	0.40	

Note: PFBA, PFPeA, and 6:2 FTS have high background levels in this procedure. PFODA have low recovery by this extraction procedure.