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STANDARD TEST METHOD

Determination of PFOA in articles of commerce: Disassembly, disjointment and mechanical preparation of solid samples, sample preparation of aqueous polymer systems, sample extraction, and LC-MS quantitative analysis.

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INTRODUCTION

The use of Per- and polyfluoroalkyl substances (PFAS) in manufacturing processes and for treatment of articles of commerce has drawn increased attention to their impact on human health and the environment. In many countries this has resulted in the adaptation of regulations affecting the acceptable levels of certain PFAS in articles of commerce. The current EU REACH POP restriction to limit PFOA in products to less than 25 parts per billion, poses particular technical challenges to determine conformance. Careful sample preparation, extraction, and analysis are critical to generating reliable data.

The purpose of this document is to provide a test method which will allow analytical laboratories to determine low levels (ppb) of PFOA in articles of commerce on a consistent and reliable basis. This method is a performance-based method which emphasizes the use of laboratory matrix spike samples (LMS) fortified with a known quantity of PFOA as the primary quality control (QC) samples used to verify adequate method performance. Other QC components include the use of $^{13}C_4$-PFOA surrogate recovery standard (SRS), or other isotope labeled analytes that are not used as internal standards (ISs), and fortified into samples prior to preparations, extraction and analysis. Recommended acceptance criteria for reportability of results are also provided and are based on the successful implementation of this method in the laboratory to the analysis of select fluoropolymer substances.

The applicability of this method to the analysis of other PFAS substances in articles of commerce should meet the same acceptance criteria as demonstrated by the adequate recovery of the PFAS target analytes from LMS samples and/or recovery of SRSs from samples.

The method is outlined in 3 Parts: Part 1 provides the strategies for sampling of articles of commerce along with the mechanical preparation of the samples, Part 2 describes the sample extraction and Part 3 describes the instrumental analysis.

WARNING – Persons using this method should be familiar with normal laboratory practices. This standard does <u>not</u> purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the laboratory staff and their management to establish appropriate safety and health practices and to ensure compliance with any applicable regulatory requirements.

Part 1: Disassembly, disjointment and mechanical sample preparation

1.1 Scope

Part 1 of this method provides strategies for sampling of articles of commerce along with the mechanical preparation of the samples based on IEC standard methods[1] and precedes Part 2 (Sample Extraction) and Part 3 (Instrumental Analysis) that are based on additional published methods[2,3] and are described later in this method.

This standard does not provide:

– Guidance on each and every product that could be classified as an article of commerce. Since there is a huge variety of commercial products available and they have various structures and processes, along with the continuous innovations in the industry, it is unrealistic to attempt to provide procedures for the disjointment of every type of commercial product available to the general public.

– Sampling procedures for packaging and packaging materials

– Guidelines for assessment of compliance.

1.2 Terms, definitions and abbreviations

1.2.1 Terms and definitions

For the purposes of this document, the following definitions apply.

1.2.1.1 Article
The reference to article, or article of commerce, refers to any commercially available product for which the determination of the amount of a substance present is desired to be determined.

1.2.1.2 Composite testing
The testing of two or more materials from a single commercial product that is treated as a single sample, but could be mechanically disjointed if necessary

1.2.1.3 Homogenous Sample
A homogenous sample can be described as a liquid or sufficiently sized resin or powder or a suspension of fine particles (aqueous, or other). Complex solid articles not of this type will typically require mechanical processing.

1.2.1.4 Certain PFAS substance
For this standard method the discussion of certain substances is reserved for PFOA and other PFAS substances that are be quantified in the article of interest.

1.2.2 Abbreviations

PFOA:	Perfluorooctanoic acid and its salts
PFAS:	Per- and polyfluoroalkyl substances
LLOQ:	Lower Limit of quantitation

1.3 Introduction to sampling strategy

1.3.1 Introductory remark

Obtaining a commercial sample (i.e. sampling) is the first step in analyzing a commercial product for the presence of certain substances. The strategy and process of sampling can be as important as the analytical measurement itself. Hence an effective sampling strategy requires a clear understanding of the product of interest, including its sold state (film, formed article, resin, powder, liquid, etc.), where and how it is sold, reasons for the analysis and the requirements that are to be met. More detailed guidance on effective sampling strategies can be found in IEC 62321-2:2013.

1.4 Sampling

Commercial articles (products) for sampling can be acquired via an indirect source (store, online, etc.) or may be acquired directly from the manufacturer. This standard does not provide detailed guidance for how to obtain articles of commerce.

1.4.1 Complete product

Sampling a complete product is the first, non-destructive step in the iterative sampling strategy where representative parts of the product can be analyzed in their present form without disassembly or disjointment.

However, "complete product" is a relative term. For example, a serge thread on a spindle is a complete product for its manufacturer, but the thread may be just a component of a sewn jacket. Complete products may be evaluated without disassembly if they have a very simple construction, or if the locations of expected certain substances are known and it is possible to test for them without disassembly.

1.4.2 Partial product disassembly

The product is disassembled down to its major components and subassemblies and each tested separately, where possible. See Annex E of IEC 62321-2:2013 for more information.

1.4.3 Complete product disassembly

Complete disassembly separates all components as far as possible, while still permitting reassembly to give an operational product. See Annex E of IEC 62321-2:2013 for more information.

1.4.4 Partial disjointment

Frequently, the detailed analysis of a complete product for certain substances requires disassembled subassemblies and components to be further separated by disjointment. However, it may be impractical for such a product to be disjointed completely into their constituent homogeneous materials. Hence sampling and partial disjointment of subassemblies and components shall typically not be done. Note: After disjointment, the separated subassemblies and/or components would not be able to be reassembled into an operational state.

1.4.5 Complete disjointment

The objective of complete disjointment is the complete separation of all components into their homogeneous materials and is typically not recommended for most commercial products. Generally, this is not practical because “complete disjointment” extends only as far as the separation of components into their homogeneous materials as far as the available tools and techniques permit.

1.4.6 Considerations of sampling and disjointment

1.4.6.1 Special remark

Disassembly and disjointment were discussed from the high level down to the detailed level. At the detailed level, certain difficulties become apparent. In theory, sampling to the homogeneous level could continue down to the nano-scale. In practice, however, such sampling is at best very difficult or unacceptably time consuming. Test method detection limits are dependent on sample masses, sizes or volumes. Testing below these masses, sizes, or volumes can render the test method unsuitable to confirm the presence or absence of a given substance below the allowable level.

Sampling and analysis of certain substances at the homogeneous material level are often difficult and a proper understanding of the basis of interpretation is crucial, particularly when dealing with composite samples.

1.4.6.2 Sample Size Required

Based on practical experience a recommended minimum sample amount is 10 grams of sample for analysis.

Samples with high concentrations of certain substances will require dilution which can increase LOQs.

In general, every combination of matrix, sample preparation technique and analytical method will have a minimum sample size required to achieve a particular reporting limit. As the size of the sample is reduced, the reporting limit increases. It is important to understand the relationship of sample size and reporting limit for the matrix, sample preparation technique and analytical method used to determine a certain substance. For this method, the minimum sample size needed for a single replicate preparation is 1 gram (1 mL liquids).

1.4.6.3 Determination of Sampling Position for Homogeneous Materials

Although commercial homogenous articles such as liquids, powders, resins and milled articles share the same physical or chemical properties, their compositions are not always completely uniform. Homogenization procedures should be adapted to the different product types:
It is advisable to shake all samples on a tumbling shaker for $\geq$ 1 hour to homogenize as much as possible prior to sampling, but too rigorous shaking of “metastable” dispersion may lead to precipitation having the opposite effect of the desired one

1.5 Conclusions and Recommendations for Sampling

The strategy and process of sampling are critical preliminary steps in the valid analysis of certain substances in commercial products. The quality of analytical results and specifically their representativeness of the analyzed sample are directly affected by the sampling technique. Selection of the sampling strategy depends on the substance(s) to be determined, their allowable limits, and the basis on which the restrictions are applied and possible exemptions.

1.6 Mechanical sample preparation

1.6.1 Overview

1.6.1.1 Field of Application

This standard provides strategies on processing samples obtained from an article of commerce for the purpose of determining certain substances by analytical means. Common techniques are covered for mechanical size reduction of articles, their sub- units or portions thereof, prior to the determination of certain substances in the article or article components via extraction and quantitative analysis. Polymer powders, nano-materials, fibers, pastes, fluorinated ionomers etc. may not require size reduction if they are known to be sufficiently homogenous and more than 95% of the material passes a 250 µm sieve. Examples of common articles requiring milling prior to extraction and analysis are compacted cakes, fabrics, and agglomerates, melt extruded pellets, elastomers and molded articles.

The user shall elect to apply one or more of the approaches, or compendium of approaches, to create homogenous samples suitable for extraction and analysis. Selection of the appropriate technique(s) depends on the article. Alternative methods of mechanical sample preparation can be used, provided that the required particle size (avg. particle size approx. 100 µm) of the sample is achieved without contaminating or compromising the sample.

1.6.1.2 Quality Assurance

Due to the risk of analytical bias resulting from contamination or from loss of material through dust emissions, it is important to select the appropriate equipment and cleaning procedures.

The laboratory shall demonstrate by experiment that a mechanical process does not result in contamination by, or loss of, detectable amounts of certain substances. Similarly, that the procedure employed for cleaning the mechanical sample preparation equipment prevents contamination of the sample with certain substances from the previous sample (carry-over). For example, processing and analyzing certified reference materials and blanks before or after processing a material known to contain significant levels of certain substances may be necessary. Use of certified reference materials is not mandatory. However, the materials used shall have a known regulated substance content to determine that the mechanical sample preparation and cleaning processes do not cause contamination or loss of regulated substances. The effectiveness of the mechanical sample preparation technique shall be continuously monitored by using quality control practices, including laboratory matrix spikes (LMS) and/or other quality control (QC) parameters (e.g. use of surrogate recovery standards, like $^{13}C_4$-PFOA or other isotope labeled analytes not used as internal standards).

1.6.2 Sample Processing Equipment and Materials

The following sample processing equipment and materials are required, subject to the type of material being prepared. Conditions for cryogenic fine milling are provided for the RetschTM CryoMill, however, alternative cryomilling equipment like the SPEX ® Freezer/Mill may be used for most products.

a) Scissors, heavy plate shears or RetschTM ZM 200 Grinding Mill (Centrifugal Mill) with 6 or 12 tooth stainless steel (SS) rotor and 1 mm stainless steel bottom sieve
b) Retsch CryoMill (Ball Mill), with SS grinding cartridge and 25 mm grinding ball (50 mL grinding cartridge, 5 g fill), or equivalent cryogenic mill capable of grinding to approximately 100 µm particles.
c) Shaker, end-over-end tumbling shaker or reciprocating shaker are sufficient.
d) Analytical balance capable of weighing accurately to 0.0001 g
e) Cleaning brushes (different sizes) and polishing cloths, or cotton balls/swabs
g) Assorted glassware (beakers, etc.)
h) Liquid nitrogen (LN2)
i) High Density Polyethylene (HDPE) bottles (assorted sizes)
j) Various organic solvents for cleaning mill parts (high purity methanol or ethanol, acetone, ASTM Type I water)
k) Ventilated hood or respirator, to minimize exposure to fine particles/dusts/aerosols and chemical vapors

l) Proper laboratory attire: safety glasses, laboratory coat, gloves (nitrile or other gloves as appropriate), etc.
m) Ventilated drying oven capable of $\geq$ 200°C
n) Precision sieves and accessories, nickel mesh, 50 µm and 150 µm sizes

1.6.3 Sample Processing Procedures

1.6.3.1 Manual cutting

If possible, manual cutting with shears or scissors is suitable for rough cutting and preparation of samples for further reduction by grinding, etc. Recommended maximum sample sizes is 10 x 10 mm, but will depend on the specification of the equipment used in the subsequent preparation processes:

1.6.3.2 Coarse grinding/milling

Metal components of the articles (i.e. buttons, zippers, bare wire) which would be considered free of certain substances should be removed prior to sample preparation to avoid damage to the centrifugal grinding mill teeth. Processing of metallic samples is not covered by this method.

Coarse grinding is suitable for reducing medium to hard samples to ~ 1 mm in diameter for later fine grinding to be more effective. Cooling soft samples with the LN_2 or dry ice prior to grinding is recommended.

An example of cryogenic preparation is to put the samples in a polyethylene bottle to cool with LN_2. Wait until the LN_2 has dissipated and then grind the samples in the grinding mill using a 4 mm stainless steel sieve. During grinding, maintain a sample temperature of $\leq$ –20 °C. Carefully sweep out and collect all particles. Refit the mill with a pre-weighed 1 mm stainless steel bottom sieve and reprocess the 4 mm material in similar fashion. Carefully sweep out the mill and collect all particles. Use a 5 min cooling period between grinding cycles. Reweigh the sieve to determine the quantity of $\leq$ 1 mm particles captured.

Thorough cleaning of the mill components between samples is crucial to avoid cross-contamination of certain substances as described in 1.6.3.4.

1.6.3.3 Fine grinding cryogenic milling

Some cryogenic mills are capable of handling materials $\leq$ 10 mm and can perform both coarse grinding and fine grinding via use of interchangeable grinding wheels/balls and sieves. Fine grinding via cryogenic milling is suitable for further reducing samples to ~100 µm in diameter. Be careful not to allow the LN_2 to come into direct contact with the sample in order to prevent spattering and sample loss.

Cryogenic milling will be described using a Retsch CryoMill (ball mill) with automatic LN_2 feed, as described below: Equipment: Retsch CryoMill with 50 mL SS grinding cartridge, fill quantity: 5g, and 1 SS grinding ball 25mm Ø and LN_2 automatic feed with LN_2 reservoir.

Sample Type	Cycles (No.)	Frequency (hz)	PCT (minutes)	MT (minutes)	ICT (minutes)
Elastomers (FKM, FFKM and FEPM) and agglomerates	1	30	Auto	2	2
Compacted Cake	5	30	Auto	2	2
Melt extruded pellets and molded articles	7	30	Auto	4	2
PCT: Precooling time (automatic set) MT: Milling time ICT: In-between cooling time					

In the case of FFKM compounds and articles, care has to be taken to avoid excessive energy input as it may lead to cryo-mechanical degradation of the polymer and the creation of mechanoradicals[4,5,6],

which can inflate the amount of target substance detected. Filling levels will impact the energy applied in the grinding process and can inflate test results as well.
In the case of cured FFKM elastomeric articles, pulverizing the product in a 2-roll mill as commonly used for compounding of elastomers can be an alternate sample preparation method to avoid or reduce the risk of mechanical degradation. Alternative methods for FFKM compounds and articles that do not require cryo-grinding are being investigated.

Cryogenically mill the sample powder and sweep out the milled material to collect all the powder.
The collected plastic-based sample material should be precision sieved to obtain a sufficiently homogeneous portion of known particle size range of 50 µm to 150 µm, by first sieving material through the 150 µm sieve, then the 50 µm sieve (keep contents captured on 50 µm sieve). Size range is 100 $\pm$ 50 µm (i.e. ~ 100 µm).

The collected FKM-, FFKM- and FEPM-based samples are visually inspected for homogenous size, verification via light-scattering is beneficial. Sieving of the samples is not recommended due to materials' elastomeric nature and the potential for particles to re-agglomerate into larger segments over time.

Thorough cleaning of the mill components between samples is crucial to avoid cross-contamination of certain substances as described in 1.6.3.4.

1.6.3.4 Cleaning Procedure for Mill Components

Thorough cleaning of the mill components between samples is crucial to avoid cross-contamination of certain substances.

The following cleaning procedure is recommended:

1) Remove residual polymer thoroughly with DI water and brush
2) If necessary, wipe away last polymer residue with clean polish cloth or cotton ball/swab
3) Rinse all parts with ethanol, use an ultrasonic bath as needed
4) Rinse all parts with DI Water, use an ultrasonic bath as needed
5) Rinse all parts with acetone and allow to dry
6) Repeat process 1-5 as needed.

Additionally, SS mill parts can be more thoroughly cleaned of certain substances by heating at $\geq$ 200°C in a ventilated drying oven for ≥ 2 hours.

1.7 Sample preparation of aqueous polymer systems/dispersions

1.7.1 Overview

Aim of this procedure is the preparation of extractable solid polymer samples from aqueous polymer systems like polymer lattices or polymer dispersions used in coating applications under mild drying conditions to prevent loss of (volatile) analytes. Two different approaches are used: Freeze drying or Coagulation followed by extraction of the coagulated dispersion

1.7.1 Freeze Drying
1.7.1.1 Quality Assurance

Due to the risk of analytical bias resulting from contamination or from loss of material through emissions, it is important to select the appropriate equipment and cleaning procedures.

The laboratory shall demonstrate by experiment that the freeze drying process does not result in contamination by, or loss of, detectable amounts of certain substances. Similarly, that the procedure employed for cleaning the freeze drying equipment prevents contamination of the sample with certain substances from the previous sample (carry-over). For example, processing and analyzing certified

reference materials and/or blanks before or after processing a sample known to contain significant levels of certain substances may be necessary. Use of certified reference materials is not mandatory. However, the materials used shall have a known regulated substance content to determine that the freeze drying processes do not cause contamination or loss of regulated substances. The effectiveness of sample preparation by freeze drying shall be continuously monitored by using quality control practices, including LMS, and/or other QC evaluations (e.g. use of surrogate recovery standards, like $^{13}C_4$-PFOA or other isotope labeled analytes not used as internal standards).

1.7.1.2 Sample processing equipment

Freeze drying equipment usable for drying aqueous polymer samples may strongly vary from lab to lab, so only a general description is given. The freeze drying process normally requires the following equipment (list may not be complete and has to be adjusted to the needs / prerequisites of the laboratory):

a) Uncontaminated sample containers providing a large sample surface, e.g. wide mouth glass or plastic vials / bottles, aluminum bowls etc. (preferable single use equipment to avoid carry-over of analytes)
b) Freeze dryer (commercial instrument e.g. Labconco Freezone Benchtop Systems or equivalent) or improvisational equipment (e.g. desiccator with vacuum hose, cooling trap and rotary vacuum pump)
c) Deep freezer / cooling device to prepare deep frozen aqueous polymer samples
d) Optional: cooling medium for cooling trap if used, e.g. acetone/dry ice or liquid nitrogen

The equipment also strongly depends on the sample size / amount of polymer that has to be dried. As large samples volumes with small surface areas need very long drying times, splitting of big samples into a higher number of small volume samples may be favorable.

1.7.1.3. Freeze drying process

As the freeze drying procedure and conditions also strongly depend on the equipment in use, a generic description of the process is provided.

Add surrogate recovery standards (SRS) e.g. $^{13}C_4$-PFOA or other standards in the desired concentration to the aqueous polymer system and homogenize the sample, for example by gentle shaking. Vigorous shaking and stirring has to be avoided, along with addition of larger amounts of standard solutions in organic solvents (e.g. Methanol), as coagulation of the polymer may occur resulting in inhomogeneous samples and strongly increased polymer particle sizes. Additionally, the freezing temperature may significantly decrease by adding organic solvents, making it hard or impossible to freeze the samples.
Deep freeze the sample(s) until completely frozen, ideally producing large area to volume ratios, e.g. by rolling of samples to produce a film on the vessel walls (see illustration) or by using flat bowls with large surface areas. (please review also: “A Guide to Freeze Drying for the Laboratory” Labconco Corporation, 8811 Prospect Avenue, Kansas City, Missouri 64132-2696, 816-333-8811, 800-821-5525, FAX: 816-363-0130, www.labconco.com © 2010 by Labconco Corporation Printed in the U.S.A. 3-53-8/10-James-3M-R8)

Once the samples are frozen, they can be attached / inserted into to the freeze drying equipment and vacuum is applied until the water is completely removed from the samples. According to equipment and sample amount this may take several hours or even days. If the freeze drying setup includes a cooling trap, ensure the trap is not blocked by ice and sufficient cooling medium is always present.

Note: *The content of the cooling trap can be used to evaluate possible loss of compounds of interest by analyzing the water and balancing the total mass and mass of sample and evaporated water after drying.*

In case the drying process was ended before dryness of the samples, keep the samples frozen, or freeze again if thawed, and continue the drying procedure until the samples are completely dry.

1.7.1.4 Cleaning procedure

To avoid contamination of samples and carry-over, use new clean single use equipment wherever possible, otherwise refer to section 1.6.3.4 Cleaning Procedure for Mill Components ("Cleaning Procedure for Mill Components") for possible cleaning methods. Before using organic solvents to clean freeze drying equipment, check the material compatibility to avoid any damage.

1.7.2 Coagulation Process

Dispersion are coagulated using a methanol/water mixture followed by centrifugation and possibly flitration. The methanol/water liquid is used for instrumental analysis.

Part 2: Sample Extraction

2.1 Scope

Part 2 of this method provides direction for the extraction of certain substances from the prepared samples generated in Part 1. Part 3 (Instrumental Analysis Parameters) are described later. The extraction procedures are based on the extraction described by Liu *et al.* 2009[3]. The method does not specify quality control aspects other than that obtained through the use of surrogate recovery standards (SRSs) and calibration standards. QC samples of special types may be prepared by some laboratories as extended quality control elements to further support their analysis conclusions.

2.1.1 Extraction Equipment and Materials

The following sample extraction equipment and materials are required, subject to the type of material being prepared.

a) Shaker, end-over-end tumbling shaker or reciprocating shaker are sufficient.
b) Analytical balance capable of weighing accurately to 0.0001 g
c) Assorted glassware (beakers, volumetric flasks, etc.)
d) HDPE bottles (assorted sizes)
e) Methanol (HPLC-MS grade or better) and Water (HPLC-MS grade or better)
f) 15 mL conical polypropylene centrifuge tubes (Falcon™, or equivalent)
g) Centrifuge capable of 5000 RPM and rotor for 15 mL conical centrifuge tubes
h) 22 x 75 mm glass test tubes, or equivalent
i) Ammonium acetate (HPLC-MS grade or better)
j) Ultrasonic water bath
k) Micropipettes (variable volumes, from 0.1 mL to 5 mL), and tips.
l) RapidVap® Vertex™ Nitrogen Evaporator, or equivalent
m) Autovials and caps
n) Milli-Q™ water purification system, or equivalent
o) Assorted PFAS reference substances and stable-isotope labeled PFASs (e.g. Wellington Laboratories) prepared as stocks at 1.00 mg/mL in methanol.

2.1.2 Extraction of Liquefied Samples

1) Prepare an aqueous solution of 2 mM ammonium acetate using Milli-Q™ pure water.
2) Dilute the aqueous 2 mm ammonium acetate with methanol 3:2 to get a solution of 60% methanol and 40% of aqueous ammonium acetate (60/40 MeOH-AA)
3) Accurately measure out 1 mL aliquots of homogenized liquid sample into each of three pre-weighed 15 mL centrifuge tubes.
4) Weight the tubes to determine the mass of sample in each, record the weights.
5) Fortify one of the three with a known quantity of certain substance(s) as a LMS sample to evaluate recovery.
6) Add 9 mL of 60/40 MeOH-AA (if sample is not miscible, use 9 mL methanol)
7) Sonicate 10 minutes
9) Centrifuge 5000 RPM for 10 minutes
10) Transfer 8 mL to a clean tube
11) Add known quantity of stable isotope labeled internal standard(s) and/or surrogate recovery standards, as appropriate, and mix.
12) Aliquot supernatant to autosampler vial and cap for analysis.

2.1.3 Extraction of Solid Samples (i.e. samples prepared in Part 1)

1) Accurately weigh 1 gram aliquots of solid sample, prepared in part 1 of this method (i.e. 50 to 150 µm particle size powder), into each of three pre-weighed 15 mL centrifuge tubes.
2) Accurately record the weights of sample in each.
3) Fortify one of the three with a known quantity of certain substance(s) as a LMS sample to evaluate recovery. (e.g. linear isomer PFOA for PFOA analysis). The remaining two will be sample and duplicate sample and do not get fortified.
    a In cases where prep time and or limited sample material are an issue, running samples in duplicate without a LMS is acceptable as long as both an IS and SRS are added in the next step to each duplicate extract.
4) Add 9 mL of methanol containing known quantity of stable-isotope labeled internal standard (IS) and/or surrogate recovery standard (SRS) as appropriate, and mix. For example, ^{13}C4-PFOA can serve as a SRS for PFOA and ^{13}C8-PFOA can serve as IS for PFOA. SRS and IS should be added within the mid-range of the anticipated calibration.
5) Shake for the appropriate time depending on material to be extracted (12-24 hours) as highlighted in example section
6) In cases where samples are fully or partially soluble in methanol, samples can be diluted 1:1 in HPLC grade water to crash the soluble polymer, avoiding precipitation and clogging the HPLC injector. After adding water, vortex mix the samples for 1 minute prior to proceeding to the next step.
7) Centrifuge 5000 RPM for 10 minutes
8) Transfer 8 mL of supernatant to clean tube
9) Aliquot clarified supernatant to autosampler vial and cap for analysis

2.1.4 Concentrating Extracts

If concentrating of extracts is desired, to achieve lower LLOQs, the following procedure can be performed. This will result in a 5-fold increase in measurement sensitivity for certain substances in the extract.

1) Accurately aliquot 5 mL of supernatant from step 3 of sections 2.1.2, or step 7 of 2.1.3, to a methanol-rinsed 22 x 75 mm glass test tube
2) Add 5 mL of aqueous 1 N NaOH
3) Evaporate the contents in Vertex evaporator at 50°C water bath temperature
4) Reconstitute with 1 mL of methanol
5) Add known quantity of stable-isotope labeled internal standard(s) and/or surrogate recovery standards, as appropriate, and mix.
6) Transfer to autosampler vial and cap for analysis.

2.1.5 Diluting Extracts

If dilution of extracts is desired to achieve results within the range of the calibration, in the event they exceed the calibration range, the following procedure can be performed. This will result in a 10-fold dilution. Successive repeated dilutions can result in 100-fold, 1000-fold, and so on.

1) Prepare a 100 mL of methanol solution containing IS and SRS at same concentrations as in sample extracts prepared in sections 2.1.2, 2.1.3 or 2.1.4
2) Accurately measure 1.00 mL of final extract from 2.1.2, 2.1.3 or 2.1.4 to a conical centrifuge tube.
3) Add 9 mL of the prepared methanol solution from step 1 of section 2.1.5 to each
4) Transfer an aliquot to an autovial and cap for analysis.

2.1.6 Preparation of Calibration Standards

1) Combine 1.00 mL of each stock solution of reference substance and SRS in a clean 100 mL volumetric flask. Bring to the mark with methanol solution (2.1.1.5 step 1) to make a combined stock solution with final concentration of each at 10 µg/mL of each.
2) Prepare a second multi-component stock by diluting 1.00 mL of the combined stock (step with methanol solution (2.1.1.5 step 1) in a 100-mL volumetric flask. Final concentration of each substance will be 0.100 µg/mL.
3) Add known quantity of stable-isotope labeled internal standard (IS) to each of ten 10-mL volumetric flasks. Then, prepare a set of 10 calibration standards in the 10 mL volumetric flasks from the combined stock solutions in step 2 and diluting with methanol solution (2.1.1.5 step 1), as appropriate. Final calibration standard range of 0.100 ng/mL to 100 ng/mL.
4) Analyze the set of calibration standards near the beginning of an analytical run
5) Analyze mid-range calibration standards during and at the end of an analytical run as continuing calibration verification (CCVs) to verify the maintained calibration during the analysis.
6) Calibration standard ranges may be expanded lower, or higher as needed using appropriate dilutions and similar calibration standard preparation procedures described above.

Part 3: Instrumental Analysis

3.1 Scope

Part 3 of this method provides direction for the Instrumental Analysis of prepared extracts generated following the procedures of Part 1 and Part 2. Analysis is performed by high performance liquid chromatography coupled with triple quadrupole mass spectrometric detection or other suitable (HR)MS systems like QTOF- or Orbitrap MS. Sample extracts are analyzed against calibration standards in an analytical run, as described below.

3.1.1 Analytical Equipment and Materials

The following analytical equipment and materials are required, subject to the type of material being prepared.

a) Suitable LC-MS system, eg. QQQ MS or equivalent: for instance: Applied Biosystems ™ API 5000 ™ triple quadrupole mass spectrometer(it is advisable to reduce the PTFE components of the HPLC when possible with non-PTFE replacements like stainless steel or PEEK, when possible)
b) Agilent 1200 HPLC system, or equivalent (degasser, autosampler, binary pump, column comp.)
b) Analytical balance capable of weighing accurately to 0.0001 g
c) Assorted glassware (beakers, mobile phase bottles, etc.
d) HDPE bottles (assorted sizes)
e) Methanol (HPLC-MS grade, or better)
f) Acetonitrile (HPLC-MS grade, or better)
f) Ammonium acetate (HPLC-MS grade, or better)
g) Milli-Q™ water purification system, or equivalent
h) Micropipettes (variable volumes, from 0.1 mL to 5 mL), and tips.
j) Analytical column: Betasil C8, reverse phase column, 150 x 4.6 mm, 5 µm (Thermo part no. 70205-154630). Alternative reverse phase columns and manufacturers may be used if demonstrated to give adequate peak shape and analyte peak resolution.

3.1.2 HPLC Conditions (exemplary)

1) Install the analytical column (e.g. Thermo Scientific™ BetaSil™ C8 or C18, reverse phase column, 150 x 4.6 mm, 5 µm). Column temperature 25.0 °C.
2) Prepare an aqueous solution of 2 mM ammonium acetate using Milli-Q™ pure water. Fill the mobile phase 1 bottle with aqueous 2 mM ammonium acetate, purge all lines in the HPLC, as needed.
3) Fill mobile phase 2 bottle with acetonitrile. Purge all lines in the HPLC, as needed.
4) Program the HPLC Gradient conditions as shown below:

Time (minutes)	Flow Rate (mL/minute)	Mobile Phase 1: Aqueous 2 mM ammonium acetate (%)	Mobile Phase 2: Acetonitrile (%)
0.00	0.750	95.0	5.0
2.00	0.750	95.0	5.0
10.0	0.750	5.0	95.0
12.0	0.750	5.0	95.0
12.5	0.750	95.0	5.0
15.0	0.750	95.0	5.0
Injection volumes: 1 to 20 µL are recommended, depending on instrument sensitivity Autosampler Tray at ambient temperature			

Special Note: *If background interference from the leaching of certain substances from components of the HPLC occurs, a pre-column (Betasil C18 2x50 mm, 5 µm) can be placed between the binary pump and the autosampler. This will result in a delayed interference peak that separates chromatographically from the sample analyte peak. However, this will increase back pressure on the system. Additionally, select PTFE components in the instrument may be replaced with non-PTFE components when available, or alternatively HPLC flows can be diverted around unnecessary components.*

3.1.3 Mass Spectrometer (MS/MS) Conditions (exemplary)

Mass spectrometer conditions described below are for a properly maintained and operated applied Biosystems API 5000 triple quadrupole mass spectrometer fitted with a TurboV™ electrospray ionization source. Other equivalent instruments from the same and other manufacturers may require optimization of the MS/MS parameters to achieve similar or better performance.

The TurboV™ source inlet is coupled to the outlet of the HPLC analytical column. The specific parameters provided are for analysis of PFOA, $^{13}C_4$-PFOA (SRS) and $^{13}C_8$-PFOA (IS). Other PFAS substances may require additional optimization of specific MS/MS parameters for adequate sensitivity to detect those analytes in the suggested calibration range. The MS/MS ions measured for each substance (when more than one) are typically summed during quantitation. Specific API 5000 parameter settings are as follows:

Special Note: *The MS/MS ions measured for each substance (when more than one are acquired) are typically summed during the data analysis phase.*

2.1.3.1 MS/MS Settings (API 5000)

Substance ID	**Q1 (*m/z*)**	**Q3 (*m/z*)**	**Dwell (msec.)**	**DP [a]**	**CE [a]**	**CXP [a]**
PFOA 1	413	369	20.0	-35.0	-14.0	-7.0
PFOA 2	413	219	20.0	-30.0	-14.0	-7.0
PFOA 3	413	169	20.0	-40.0	-14.0	-7.0
13C4-PFOA (SRS)	417	372	20.0	-40.0	-14.0	-7.0
13C8-PFOA (IS)	421	376	20.0	-40.0	-14.0	-7.0
Start and Stop voltages are identical for each parameter						

MS/MS Parameter	**API 5000 Setting**
Scan Type	MRM
Scheduled MRM	NO
Polarity	Negative
Scan Mode	N/A

Ion Source	Turbo Spray
Resolution (Q1)	Unit
Resolution (Q3)	Unit
Intesity Threshold	0.00 cps
Settling Time	0.00 msec
MR Pause	5.0070 msec
MCA	NO
Step Size	0.00 Da
CUR	35.0
GS1	45.0
GS2	45.0
TEM	450.0
ihe	ON
CAD	10.0
IS	-4500.0
EP	-10.0
CEM (Detector)	2200
DF (Detector)	300.0

Part 4: Examples and Acceptance Criteria

4.1Examples

Example 1: Sample preparation, extraction and LC-MS/MS analysis of a PTFE dispersion

151.83 g PTFE dispersion (solid content ~ 13% m/m) in a clean 250 ml PE bottle was spiked with 0.1 ml $^{13}C_4$-PFOA (SRS) in methanol with a concentration of 5 µg/ml = 500 ng $^{13}C_4$-PFOA, agitated gently and allowed to rest overnight at room temperature. 77.4 g of the spiked dispersion was deep frozen and then freeze dried in a desiccator equipped with a cooling trap (acetone / dry ice) and a rotary oil pump (p ~0,3 mbar). After 45h 10.3 g of dry PTFE powder was obtained, resulting in a solid content of 13.35% m/m and a theoretical $^{13}C_4$-PFOA concentration of 24.7 ng/g. 1.03 g of dry polymer was extracted with 3 ml Methanol at 50°C using a shaker at 250 RPM for 16 h and an aliquot of the extract (theoretical $^{13}C_4$-PFOA concentration 8.56 ng/ml) was analyzed for $^{13}C_4$-PFOA and PFOA. The analysis revealed a PFOA concentration of 8.5 ng/ml in extract, which calculates a PFOA content of 24.8 ng/g (µg/kg). The $^{13}C_4$-PFOA concentration was 7.35 ng/ml, resulting in an overall recovery rate of 86.0 % (21.4 ng/g polymer).

Example 2: Sample preparation, extraction and LC-MS/MS analysis of a PFA dispersion

52.95 g PFA dispersion (solid content ~ 27.5% m/m) was spiked in a clean 100 ml PE bottle with 0.075 ml $^{13}C_4$-PFOA in methanol with a concentration of 5 µg/ml = 375 ng $^{13}C_4$-PFOA, agitated gently and allowed to rest overnight at room temperature. The spiked dispersion was deep frozen and then freeze dried in a desiccator equipped with a cooling trap (acetone / dry ice) and a rotary oil pump (p ~0,3 mbar). After 45h 14.63 g of dry fine PFA powder was obtained, resulting in a solid content of 27.6 % m/m and a theoretical $^{13}C_4$-PFOA concentration of 25.6 ng/g. 0.98 g of dry polymer was extracted with 3 ml Methanol at 50°C using a shaker at 250 RPM for 16 h and an aliquot of the extract (theoretical $^{13}C_4$-PFOA concentration 8.4 ng/ml) was analyzed for $^{13}C_4$-PFOA and PFOA. The PFOA concentration was 6.4 ng/ml in extract, which results a PFOA content of 19.6 ng/g (µg/kg). The $^{13}C_4$-PFOA quantification revealed a concentration of 7.0 ng/ml, resulting in an overall recovery rate of 83.3 % (21.4 ng/g polymer).

Example 3: Sample preparation, extraction and LC-MS/MS analysis of a PFA sintered plate

About 5 g of a sintered PFA plate (cut in small pieces) was cryo-milled according to section 1.6.3.3 (7 x 4min) and 0.93 g of ground polymer was spiked with 25.5 ng $^{13}C_4$-PFOA in methanolic solution, resulting in a theoretical $^{13}C_4$-PFOA polymer content of 27.4 ng/g. After evaporation of the solvent, the polymer sample was extracted with 3 ml methanol at 50°C using a shaker at 250 RPM for 16 h and an aliquot of the extract (theoretical $^{13}C_4$-PFOA concentration 8.5 ng/ml) was analyzed for $^{13}C_4$-PFOA and PFOA. PFOA was quantified with 1.8 ng/ml in extract, resulting a PFOA content of 5.8 ng/g (µg/kg) in polymer. 8.2 ng/ml $^{13}C_4$-PFOA was found in the extract, giving a recovery rate of 96.5 % (26.5 ng/g in polymer).

Example 4: Sample preparation, extraction and LC-MS/MS analysis of a FFKM compound

5 g of a compounded FFKM (cut in small pieces) was cryo-milled according to section 1.6.3.3 (1 x 2 min) and 9 mL of extraction solution (methanol containing 2 ng/mL $^{13}C_8$-PFOA) was added to about 1 g of ground filled elastomer, resulting in a 18 ng/g concentration of $^{13}C_8$-PFOA. The sample was extracted with 9 ml methanol at room temperature using a wrist action shaker for 24 h and an aliquot of the extract (theoretical $^{13}C_8$-PFOA concentration 2 ng/ml) was analyzed for $^{13}C_8$-PFOA and PFOA. PFOA was quantified with 1.8 ng/ml in extract, resulting a PFOA content of 16.2 ng/g (µg/kg) in polymer.
The above extraction procedure is carried out on two or three 1g aliquots of ground polymer, with two being exact duplicates, where 9 mL extraction solution is added to the material as above, and the optional third being a spiked recovery sample, where 8 mL of extraction solution, and 1 mL of a 10 ng/mL solution of PFOA (also containing the same 2 ng/mL of $^{13}C_8$-PFOA) are added to the polymer. Thus, the native analyte is spiked at 10 ng/g of polymer.
Alternatively, ^{13}C4PFOA can be used for recovery calculation - also spiked into the extraction methanol while ^{13}C8PFOA is used as internal standard for PFOA calibration and added later to the LC-MS sample.

Example 5: Sample preparation, extraction and LC-MS/MS analysis of a FFKM article

About 5 g of a cured FFKM seals was crushed in a 2-roll mill – set to minimal gap (<1 mm). The material was passed twice through the mill which is kept at room temperature. The resulting course powder is homogenized. The remainder of the process follows example 4.

4.2 Acceptance Criteria

This method is a performance-based method and as such relies on the recovery of LMS and/or SRS recoveries for reportability of results. While results may be reported with poor recoveries, this is not recommended and re-preparation and analysis in effort to achieve acceptable results are highly encouraged.

The recommended analytical acceptance criteria are as such:

Precision: precision of results is demonstrated adequate when relative percent difference (RPD) of duplicate sample results is less than 30%, or for 3 or more replicate results the relative standard deviation (RSD) is less than 30%.

Fitted linear regression of calibration standards results (or quadratic regression when needed) should be performed using five (5) or more calibration standards and should have a correlation coefficient (R) of 0.995 or better, resulting in a coefficient of determination (R^2) of better than 0.990. Back calculation calibration standards (those included in the fit) should be within 100 $\pm$ 25% of their actual value. Standards which cannot meet that criterion should be excluded from the calibration curve

LMS and SRS recoveries should be within 100 $\pm$ 30% of their known fortification concentration and flagged in reported data for samples with associated LMS or SRS are outside of that range.

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