CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

ANALYSIS OF PERFLUORINATED ALKYLATED SUBSTANCES IN BIOTA SAMPLES BASED ON FAST AND SIMPLE ACTIVATED CHARCOAL CLEAN–UP PROCEDURE FOLLOWED BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY: INTERLABORATORY STUDY

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Introduction

- To assess health risks associated with a dietary intake of perfluoroalkylated substances (PFAS). European Food Safety Authority together with European Commission (2010/161/EU) recommended to member states to monitor PFAS in the food. The European project CON*ff*IDENCE aims to improve food safety in Europe by the development of fast and more cost-efficient methods for the detection of three target (PFAS): perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonamide (FOSA) in fish fillets, milk and feed commodities.

Aims of study

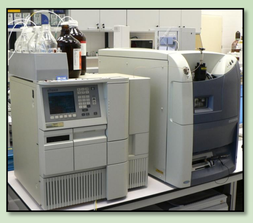
- To validate a simple, fast and cheap analytical approach for the determination of PFOS, PFOA and FOSA in fish fillets, in accordance with the Commission Decision 2002/657/EC.
- To organize the interlaboratory study after the method validation to assess the transferability of the developed procedure.

Analytical method

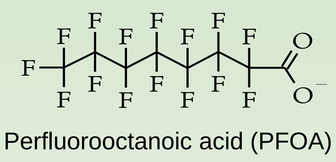
Extraction Methanol → **Clean-up Activated charcoal** → **LC-MS/MS**

Ultra Turrax

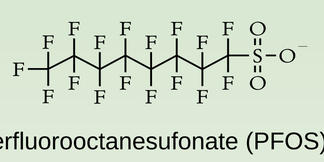
Shaking; Centrifugation; Filtration



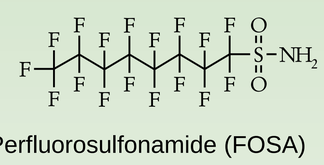
Alliance 2695 (Waters, USA) Premier XE (Waters, USA)



Perfluorooctanoic acid (PFOA)



Perfluorooctanesufonate (PFOS)



Perfluorosulfonamide (FOSA)

Studies proposals

- **Validation study**
  - Requirements: recovery 70–120%, LOQ 1 μg.kg^{-1}
  - The muscle of chub (*Leuciscus cephalus*) and milk (1.5% fat content) were used
  - Samples were fortified at levels 0.25, 0.5, 1, 1.5 and 2 μg.kg^{-1}
  - Six replicates at each level were tested
  - The specificity, the calibration curves, the recovery (trueness), the accuracy (repeatability and reproducibility), the robustness, decision limits (CC_α) and detection limits (CC_β) were established
- **Interlaboratory study**
  - Real-life contaminated fish muscle of chub containing mainly PFOS and FOSA (above LOQ) were employed
  - Homogeneity of material was assessed
  - Altogether 5 laboratories participated
  - Each participant received appropriate material and calibration standards which were also provided to laboratories

Results & Discussion

Table I Performance characteristics for fhe fish tissue

Analyte	Performance characteristics – fish muscle						
	Recovery (n=6; %)	RSD (n=6; %)	LOD (μg.kg^{-1})	LOQ (μg.kg^{-1})	Linearity range (μg.kg^{-1})	CC_α (μg.kg^{-1})	CC_β (μg.kg^{-1})
PFOS	107	9	0.075	0.15	0.15–15	0.015	0.21
PFOA	90	3	0.15	0.3	0.3–15	0.14	0.47
FOSA	90	4	0.15	0.3	0.3–15	0.18	0.49

Table II Performance characteristics for milk

Analyte	Performance characteristics – milk						
	Recovery (n=6; %)	RSD (n=6; %)	LOD (μg.kg^{-1})	LOQ (μg.kg^{-1})	Linearity range (μg.kg^{-1})	CC_α (μg.kg^{-1})	CC_β (μg.kg^{-1})
PFOS	92	6	0.3	0.75	0.75 – 75	0.21	0.87
PFOA	93	7	0.15	0.3	0.3 – 75	0.41	0.65
FOSA	81	12	0.15	0.3	0.3 – 75	0.55	0.77

- The presented method allows processing of **10 samples in one hour**.
- **The recoveries** at five levels ranged from **85–110%**, which is in agreement with the recommendation 2002/657/EC (70–120%).
- **The repeatability** was expressed as relative standard deviation (RSD) and ranged in 2–15%.
- **LOQ** for each analyte (0.15–0.75 μg.kg^{-1} for milk and 0.075–0.15 μg.kg^{-1} for fish muscle) complied with the level 1 μg.kg^{-1}, which is mentioned in the EC recommendation 2010/161/EU.
- **Decision limits (CC_α) and detection limits (CC_β)** were established - the values are shown in Table I and II.

RECOVERY, level 5 μg.kg^{-1}

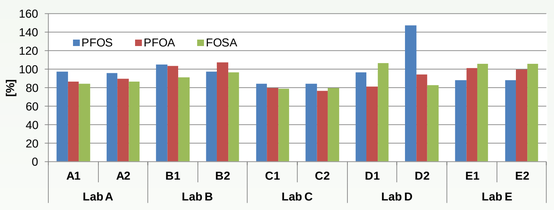
	Laboratories codes				
Analyte	Lab A	Lab B	Lab C	Lab D	Lab E
PFOS	103	81	87	102	93
PFOA	101	89	86	115	93
FOSA	102	85	85	84	96

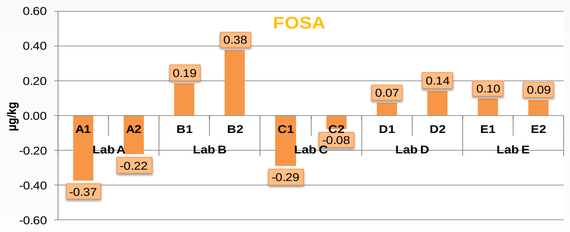
REPEATIBILITY, level 5 μg.kg^{-1}, n =6

PFOS	8	3	8	21	2
PFOA	5	2	2	7	1
FOSA	10	3	7	6	3

LOQ (μg.kg^{-1})

PFOS	0.56	0.4	0.3	3	0.15
PFOA	0.65	1	0.3	3	0.15
FOSA	0.11	0.4	0.3	3	0.15





- The recoveries of the analytical method reported by laboratories were calculated at spiking level 5 μg.kg^{-1} (designed value) and ranged from 93 to 115% for all analytes. Also repeatability and LOQs were in the range of 1–21% and 0.15–3 μg.kg^{-1}, for more detail see Tables.
- Target analytes in the unknown solution had a concentration of 2.5, 10 and 5 μg.L^{-1} of PFOS, PFOA and FOSA, respectively. These levels were determined with differences up to 24% from the designed concentrations. Mean concentrations and RSD (%) were calculated as follow: 2.3 (8%), 9.2 (12%) and 4.6 (12%) for PFOS, PFOA and FOSA, respectively. The extremely high value (reported by Lab D), which exceeded the difference of 45%, was excluded.
- The last figure illustrates the differences from the mean value for FOSA in the fish muscle sample (4 μg.kg^{-1}) reported by the participants, which ranged from 0.09 to 0.38 μg.kg^{-1}.

Conclusions

- The analytical procedure for determination of PFOS, PFOA and FOSA in fish muscle and milk was developed and validated in accordance with the Commission Decision 2002/657/EC.
- The method can be used for the routine monitoring of the PFAS in fish as shown by the interlaboratory study.

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