

# Minutes of the QUASIMEME Workshop

on

## Analysis of Perfluorinated Compounds

IVM, VU University Amsterdam,

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### ***Introduction to QUASIMEME (Michiel Kotterman)***

Kotterman presents the QUASIMEME proficiency testing organization and some recent developments. QUASIMEME provides proficiency tests for a wide range of contaminants and nutrients in (marine) environmental media such as seawater, sediment, biota etc. In important addition to this is that also technical learning workshops are organized (such as the present one) where people can exchange information on analytical chemical approaches. Furthermore, QUASIMEME provides a sharepoint in their web-site for exchanging information.

Some recent developments:

- Through collaboration with WEPAL (another Wageningen based proficiency test provider), QUASIMEME will soon be accredited as a provider for proficiency tests.
- The QUEST programme for data submission will be phased out in and replaced by an internet based application
- The web-site and the brochures will be updated and improved

The minutes and the presentations of the current meeting will be made available through the website. More information at [www.quasimeme.org](http://www.quasimeme.org)

### ***Bert van Bavel (MTM, Örebro University) - Introduction to the analysis of PFCs in Human samples.***

Van Bavel presented a chronological summary of the development of the analysis of organic fluor compounds in human samples. The first analysis by NMR were already presented in the international literature in the 1970s using NMR. The first more specific method, for PFOA, was presented in the middle of the 1980s using ion pair extraction with TBA and with GC/MS detection achieving detection limits of around 5 ug/ml. The most common method used in the beginning of 2000 is the so called Hansen method, using again ion pair extraction but now LC/MS detection. After this a large number of methods have been introduced including SPE and LL based methodology. This caused some discussion on the quality and compatibility of 'PFC' data. This issue was extensively discussed at a workshop in Hamburg in 2003. This workshop resulted in a future paper in ES&T where the several shortcomings of PFC analysis were identified which could hamper further development including purity of standards, contamination in the laboratory environment and instrumentation, problems in the quantification of different isomers and the lack of QA/QC studies and reference material. The organization of two rounds of QA/QC studies has improved this situation or at least given the possibility to quantify the uncertainty in the data.

### ***Christiaan Kwadijk (IMARES, WUR) - Extraction and clean-up of biological tissues***

Kwadijk presented a close look at the efficiency of a number of clean-up techniques for removal of co-extracted compounds such as lipids and other interferences. This was needed as approaches with minimal or no clean-up result in dirty extracts with interfering compounds. In addition, such extracts reduce the LC-MS performance and frequent cleaning is needed.

Kwadijk tested the ion pair extraction versus an extraction with acetonitrile. These extractions were combined with silica column clean-up, Powley clean-up (graphitized carbon), freezing out matrix interferences and hexane partitioning. Some observations:

- Ion pair extraction without clean-up results in a dirty MS instrument that requires cleaning frequently.
- Silica column clean-up, lipids can effectively be separated from the PFCAs and PFSA's, although PFOSA is lost with the lipid fraction.
- The Powley clean-up is effective, but residual water in the extract may lead to losses of some PFCs.
- Hexane partitioning leads to effective removal of most lipid contents
- Freezing of the extract (-20C) results in solidified lipids that can simply be separated from the extract.

The biota sample of the 1<sup>st</sup> ww interlaboratory study (ILS) was re-analysed with different methods to evaluate accuracy and showed that results vary per extraction/clean-up combination. The recommended procedure for removal of co-extracted lipids was hexane partitioning, followed by envicarb clean-up.

### ***Hanane Kadar (Laberca) - Low resolution triple quadrupole MS compared to high resolution***

#### ***Orbitrap MS for the analysis of PFCs***

Kadar evaluated the analysis of PFCs using the Thermo orbitrap MS system versus the Agilent 6410 triple quadrupole (QQQ) MS instrument. The orbitrap resulted in a better sensitivity compared to the QQQ instrument. Kadar used both systems for analysis of breast milk samples. These samples were extracted (after protein precipitation) by Oasis HLB SPE and clean-up was performed with Envicarb SPE. Approx. 80 % of the samples could be quantified with QQQMS, but the additional Orbitrap sensitivity resulted in an additional 20% of positive detected samples. The correlation between both instruments was good.

She also evaluated both systems with extracts of fish samples. Fish samples were freeze dried, extracted methanol, cleaned with Envicarb and after centrifugation analysed on both MS systems. The orbitrap resulted in at least 25 times better sensitivity compared to the QQQ instrument. In biological samples, a bile acid (TDCA) is present that co-elutes with PFOS on a C18 column and has a very similar mass (MW TDCA is 498.29 and MW of PFOS is 498.93) and the same fragment (SO<sub>3</sub><sup>-</sup>, m/z 80). The orbitrap high resolution enables mass separation of PFOS from the TDCA interferences, whereas in the QQQMS, the interference leads to an overestimation of the PFOS result if ignored. A possible solution could be the quantification based on the m/z 99 (FSO<sub>3</sub><sup>-</sup>) or 119 ion.

Finally, it should be noted that the benefits of the orbitrap comes only at a high initial investment and may therefore not be accessible to most laboratories.

***Robert Letcher (Environment Canada) –Analytical challenges and environmental relevance of branched and linear isomers of perfluorooctane sulfonate and related fluorinated compounds in biota***

Letcher presented the analysis of isomers for PFOS in biological samples, and the environmental relevance of the isomer profiles.

There are 10 branched and 1 linear isomer of PFOS. UPLC is capable of separating several isomers. The GC method developed by Letcher and co-workers enables separation of all 11 isomers. For GC analysis, derivatisation is needed. TBAH proved to be the best reagent for derivatisation in the GC-injector. The optimal temperature is 300C and up to 5 ul can be injected. A DB-5MS column is used for separation of the isomers and electron capture negative ionization-MS is used for detection of the PFOS-derivatives. Some carry over can occur. The LOQ of the method ranges from 0.09 to 0.60 ng/g wet weight (ww).

PFOSA and other substituted sulfonamides should not be neglected as a source for PFOS. These compounds can degrade biologically to the stable end-product PFOS. Selective enrichment of the linear PFOS isomer occurs higher in the food chain.

***Bert van Bavel (MTM, Orebro University) - Results of the 3<sup>rd</sup> international PFC interlaboratory studies (human)***

Van Bavel presents the preliminary results of the 3rd round of the Fluoros 2009 QA/QC study on human plasma and a standard solution. Two identical human plasma samples and a standard solution were analysed for 13 carboxylated and 4 sulphonates. The results from 21 laboratories were submitted before the set deadline. Most labs could report data for PFHpA, PFOA, PFNA, PFDA and PFHxS and PFOS. The variation between the laboratories was surprisingly good showing RSDs between all labs ranging from 19% and 21% for PFOA and 29% and 33% for PFOS. Although these results are preliminary they still gave a good indication of the 'state of the art' of PFC analysis. Problem areas including the quantification of branched and linear isomers and the quantification of the anion or the salt (sulphonates) were identified.

***Stefan van Leeuwen (IVM, VU University) - Results of the 3<sup>rd</sup> international PFC interlaboratory studies (environmental)***

Van Leeuwen presents the results of the last (3<sup>rd</sup>) interlaboratory study on environmental matrices. Thirty-six datasets were submitted and basic statistics were performed. The samples distributed were surface water, fish muscle tissue, dried sludge and a standard solution with undisclosed concentrations. An additional water sample was provided to the French participants in the framework of a study on the ISO standard 25101. Participants were asked to report concentrations in the samples as the salt for the sulfonates (anion + cation) and summarising branched and linear isomers assuming same response factors. Data submission not consistent (especially for the sulfonates).

For the standard solution, median and average were close, but relative standard deviations (RSDs) were sometimes high (up to 150%), due to some outliers (e.g. through calculation errors). Apart from these, the performance was similar to the 2<sup>nd</sup> ILS.

The performance for the water sample was reasonable (RSDs <50%) except for PFBS, PFOSA, PFDA, PFUnA and PFDoA. In case of PFOSA, PFUnA and PFDoA only 2-3 labs submitted data and these were variable.

The performance for the fish sample was substantially worse as in the 2<sup>nd</sup> ILS study. Only for PFOS a reasonable RSD was obtained (40%). In other cases, RSDs were much higher (up to 270% for PFOA). This sample was not spiked and therefore, the levels were lower (all below 3 ng/g wet weight), which may be part of the explanation. In addition, several labs used only mass

labeled PFOS to compensate losses and matrix effects in the whole procedure. The 2<sup>nd</sup> ILS study showed that this leads to inaccurate results.

A sludge sample was included for the first time. The results were reasonable (RSD <50%) for PFOS, PFOSA and PFDoA, but worse for PFBA, PFPeA, PFOA, PFDA, PFUdA, PFHxS and PFDS (all between 50-100%) and PFHxA, PFHpA, PFNA, PFBS (>100%). It seems that the complexity of the matrix, combined with the sometimes low levels and limited number of mass labeled standards used are the most likely causes for the bad performance for several compounds. More thorough study is needed to investigate the exact causes.

Preliminary conclusions of the study:

- Performance for water at same level as 2<sup>nd</sup> EILS, fish performance worse (outliers)
- First results sludge are reasonable
- Performance: unknown solution ~ water > fish > sludge
- Inconsistent confusion:
  - Salt vs acid
  - Quantification linear and branched
- Reporting errors (calculation errors)

### ***Pim de Voogt (IBED, University of Amsterdam) – Analytical challenges in PFC analysis***

De Voogt provides a number of practical insights in how to deal problems that can be encountered in the analysis of PFCs.

Blank problems can be encountered at several stages in the analysis and are related to fluorinated plastics such as PTFE and e.g. PVDF. The LC system contains PTFE tubing. Wherever possible, this should be replaced by e.g. PEEK or stainless steel. The degasser, made of PTFE should be excluded from the solvent circuit and replaced by ultrasonic or helium-purge degassing. Even then, traces of PFOA and PFNA can be observed due to several smaller LC parts that are more difficult to replace. By placing an additional LC precolumn in the eluent flow just before the injector will help to retard the LC system borne PFOA and PFNA so that it does not interfere the PFOA and PFNA signals from the sample.

Just before LC-MS analysis, small residual particles are often removed by filtration. However, filters can contain PVDF and PTFE membranes and these filters may look very similar to the filters that do not contain fluorinated plastics. A good alternative to filtration is centrifugation e.g. by 10000 rpm.

Dirty extracts can substantially reduce the MS performance. It's therefore recommended to switch the eluent to waste after the last PFC has eluted from the column. This could be done with an additional time-programmable valve between the column and the MS.

Solvent compatibility of the injected solution and the eluent is important in terms of peak shape. Injecting standards and extracts dissolved in pure ACN leads to peak shape distortion of PFCs. It is safe to MeOH/water (50:50 v/v) solutions in e.g. MeOH/water (30:70 v/v) eluent conditions. Some optimization may be required.

Extracts and standard solutions stored in containers and tubes should contain at least 20-30% methanol, because when stored in water only, some losses can occur over time due to adsorption to the container walls. De Voogt observed losses of especially the long chain PFCAs in a water solution stored in polypropylene tubes.

De Voogt recommends strongly having a good look at the mass spectrometry of the PFCs in your own instrument. Selection of the transitions should be based on your own spectra rather than copying the values from tables from other sources.

Commercial standards with high purity are commercially available. It's recommended to use these native standards and a mass labeled analogue for every target compound analysed to improve precision and accuracy. It is important to check all standards prior to use because they can contain small traces of the target compounds, resulting in inaccuracies. In combined standards, many small impurities can become substantial.

### **Anna Kärrman (MTM, Orebro University) - Challenges in quantification of PFCs in human samples**

Kärrman discusses the analysis of human samples.

Branched isomers can be separated using a UPLC set-up with a 100mm x 2.1 mm i.d. and 1.7 µm particle size, with a 200 µl/min slow gradient. Not all are separated, but a considerable improvement is achieved compared to the traditional set-up.

MS response factors vary substantial for the branched isomers compared to the linear one. Some isomers show less response (down to zero response) and some show more response. This also depends on the MS/MS transition. This may lead to overestimation or underestimation, depending on the isomeric composition in the sample. Therefore quantifying branched isomers against a standard with only the linear isomer may lead to inaccurate results.

In human blood, linear PFOA accounts for approx 95% of total PFOA and linear PFOS accounts for approx 60% of total PFOS and the 3-,4-,5- and 6-PFOS isomers contribute 25-35%.

Quantification can be done using standards consisting of (i) linear only, (ii) individual linear and individual branched isomers in known concentrations and (iii) a mixture of isomers that is not characterized, such as the Fluka standards. Kärrman demonstrates that the use of these different standards can result in substantially different total-PFOS concentrations, and therefore, care should be taken to compare old data and current data when different standard solutions were used for quantification.

An interference co-elutes with PFHxS, which is the natural hormone pregnadiol sulfonate. On a C18 column, this interference can generally not be separated from PFHxS. In addition the transition 399→80 and 399→99 of the hormone interferes with the transition of PFHxS. The transition 399→119 does not suffer from an interference.

Finally, Kärrman demonstrates a feature on the Waters triple quadrupole instrument called 'dual scan MRM' that can be helpful for concurrent identification of the target compounds and characterization of interferences.

### **Lutz Ahrens (GKSS Research Centre) - Analysis of PFCs in water samples**

Ahrens discusses the analysis of marine and freshwater samples for multiple compounds. He included multiple PFASs in his method. He removed perfluorsulfinate (PFOSi) as this compound showed to degrade to PFOS.

The ISO method analyses unfiltered water, but Ahrens chooses to work with filtered water (to prevent blocking of SPE due to particles). Filtration can be done by glass filters of polypropylene (PP) filters. PP filters may be contaminated with PFOA, so this should be checked beforehand. Also, some adsorption of PFCs from the sample on the filter may occur, and therefore, the filter should be rinsed after sample filtration.

Particles can be extracted by ultrasonation in MeOH. Water can be extracted by using SPE (Oasis Wax column) (method by Taniyasu et al. *J. Chr. A* 2005, 1093, 89-97). Extracts are analysed on a C18 column using a MeOH/water gradient (both with 10 mM NH<sub>4</sub>OAc). Detection takes place using a negative electrospray ionisation MS/MS.

Ahrens emphasizes that for analysis of low levels in water samples, it is of utmost importance to avoid blank contribution from the HPLC system (incl sample vial septa). He discusses the precautions as mentioned earlier by de Voogt as well. A intercomparison of two independent methods (GKSS and BSH) showed that very good comparability can be achieved.

Some future developments: the ISO method will increasingly be used; Ahrens promotes the filtration of the water sample prior to preconcentration; blanks should be further reduced because the low levels in water; the C3 and C2 carboxylic acids are generally not analysed, whereas the high levels present in water samples and analytical methods are needed for the phosphonic acids and

perfluoroalkylsulfonamidoacetic acids. Finally, Ahrens raises the question if passive samplers can be employed for monitoring levels in water.

***John Barber (CEFAS) - Analysis of PFASs in air.***

Barber presents a very comprehensive and complete overview of the analysis of PFASs in air and air particulates. Many details are available in his presentation. The volatile PFASs include Fluorotelomer alcohols (4:2, 6:2, 8:2, 10:2 and 12:2 FTOH), Fluorotelomer acrylates (6:2 and 8:2 FT acrylate), Fluorotelomer olefins (6:2, 8:2, 10:2 and 12:2 Ftolefin), Fluoroalkyl sulfonamides (PFOSA, NMeFOSA, NEtFOSA, NMeFBSA), Fluoroalkyl sulfonamidoethanols (NMeFOSE, NEtFOSE, NMeFBSE).

These compounds are generally extracted from air using PUF/XAD extraction, combined with a glass fiber filter (GFF) for retaining the airborne particulates. Extraction of the PFASs from the PUF/XAD is generally done with a medium polar solvent such as acetone or (m)ethylacetate. GC-MS with positive or negative chemical ionization are used for analysis of the extracts. Chromatography (peakshape) of PFASs with OH groups can get worse rapidly after injection of dirty sample extracts. Matrix effects (i.e. signal enhancement) occur in GC-MS analysis and many variables effect the strength of the enhancement. The use of many mass labeled internal standards improves the accuracy and is recommended. There are several problems that should be overcome in the analysis of Ftolefins, FTOHs and FOSA/Es in FBSA/Es and FTacrylates. Several problems being related to the (extreme) volatility of these compounds (see Barber's presentation for details). Branched isomers can affect the quantification results. Some early labs may use standards with an isomer mixture (often donated).

The ionic PFASs can also be trapped on PUF/XAD and GFF (particulates). They are effectively extracted using acetone. The use of acetone allows the extraction of both the neutrals and the ionics and extracts can be splitted for further analysis with GC-MS (neutrals) and LC-MS (ionics). Avoid using the graphitized carbon clean-up in an acetone extract. Solvent change to methanol is needed to avoid low recoveries. Storing extracts in the freezer results in precipitation and potentially losses of associated PFASs.

Active monitoring of air concentrations is generally done with PUF/XAD or PUF/PUF materials. Passive monitoring systems employ discs with PUF and XAD material. SPMDs can also be used. Interlaboratory studies showed that good comparability can be achieved among participants, as long as many mass labeled internal standards are used.