Perfluorooctane sulphonic acid/ perfluorobutane sulphonic acid (PFOS/PFBS)

Application	Determination in plasma and urine
Analytical principle	High performance liquid chromatography/ tandem mass spectrometric detection (LC/MS/MS)
Completed in	April 2003

Summary

The method described here serves to quantify perfluorooctane sulphonic acid (PFOS) and perfluorobutane sulphonic acid (PFBS) as well as their ammonium and alkali salts in the plasma and urine of persons exposed to these substances in the course of their work or in the environment.
For this purpose perfluorodecane-1-sulphonate and tetrabutylammonium hydrogen sulphate at a pH value of 10 are added (the former acts as an internal standard and the latter as an ion pair reagent). Then the two perfluoroalkane sulphonic acids are extracted from plasma or urine in *tert*-butylmethyl ether.
After evaporation of the solvent, the extraction residue is dissolved in methanol, and the analytes are subsequently separated by means of high performance liquid chromatography and quantified by tandem mass spectrometric detection. Calibration is performed using standards prepared in human plasma or in pooled urine from non-exposed persons and the calibration standards are treated in the same manner as the samples to be analysed.

Plasma

Perfluorooctane sulphonic acid (PFOS)

Within-series imprecision:	Standard deviation (rel.)	s_w = 4.5%, 3.7% or 6.7%
	Prognostic range	u = 10%, 8.3% or 14.9%
	at concentrations of 10, 50 or 100 µg PFOS per litre plasma and where n = 10 determinations	

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Between-day imprecision:	Standard deviation (rel.)	s_w = 6.7%, 5.0% or 7.1%
	Prognostic range	u = 14.9%, 11.1% or 15.8%
	at concentrations of 10, 50 or 100 µg PFOS per litre plasma and where n = 10 determinations	
Accuracy:	Recovery rate	r = 116% at 10 µg/L and 98% at 100 µg/L
Detection limit:	10 µg perfluorooctane sulphonic acid per litre plasma	

Perfluorobutane sulphonic acid (PFBS)

Within-series imprecision:	Standard deviation (rel.)	s_w = 4.9%, 4.9% or 4.0%
	Prognostic range	u = 10.9%, 10.9% or 8.9%
	at concentrations of 10, 50 or 100 µg PFBS per litre plasma and where n = 10 determinations	
Between-day imprecision:	Standard deviation (rel.)	s_w = 5.3%, 4.9% or 5.0%
	Prognostic range	u = 11.8%, 10.9% or 11.2%
	at concentrations of 10, 50 or 100 µg PFBS per litre plasma and where n = 10 determinations	
Accuracy:	Recovery rate	r = 93% at 10 µg/L and 94% at 100 µg/L
Detection limit:	10 µg perfluorobutane sulphonic acid per litre plasma	

Urine

Perfluorooctane sulphonic acid (PFOS)

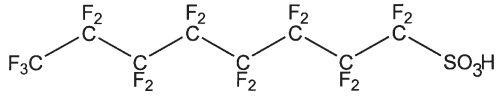
Within-series imprecision:	Standard deviation (rel.)	s_w = 9.5%, 5.1% or 4.4%
	Prognostic range	u = 21.2%, 11.4% or 9.8%
	at concentrations of 10, 50 or 100 µg PFOS per litre urine and where n = 10 determinations	
Between-day imprecision:	Standard deviation (rel.)	s_w = 10.2%, 5.7% or 5.5%
	Prognostic range	u = 22.7%, 12.7% or 12.3%
	at concentrations of 10, 50 or 100 µg PFOS per litre urine and where n = 10 determinations	
Accuracy:	Recovery rate	r = 108% at 10 µg/L and 110% at 100 µg/L
Detection limit:	10 µg perfluorooctane sulphonic acid per litre urine	

Perfluorobutane sulphonic acid (PFBS)

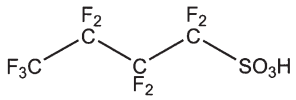
Within-series imprecision:	Standard deviation (rel.)	$s_w = 5.9\%$, 6.3% or 6.6%
	Prognostic range	$u = 13.2\%$, 14.0% or 14.7%
	at concentrations of 10, 50 or 100 µg PFBS per litre urine and where $n = 10$ determinations	
Between-day imprecision:	Standard deviation (rel.)	$s_w = 8.2\%$, 6.5% or 7.2%
	Prognostic range	$u = 18.3\%$, 14.5% or 16.7%
	at concentrations of 10, 50 or 100 µg PFBS per litre urine and where $n = 10$ determinations	
Accuracy:	Recovery rate	$r = 99\%$ at 10 µg/L and 106% at 100 µg/L
Detection limit:	10 µg perfluorobutane sulphonic acid per litre urine	

Perfluorooctane sulphonic acid (PFOS) and perfluorobutane sulphonic acid (PFBS)

Perfluorooctane sulphonic acid (PFOS) and perfluorobutane sulphonic acid (PFBS), their ammonium and alkali salts and their amides all have strong surface-active properties. They are used in considerable amounts for the production of wetting agents in metal cleaners, for metal processing, and especially for the manufacture of films and photographic paper as well as impregnating agents to render textiles, paper and leather resistant to water, oil and dirt [1].



Perfluorooctane sulphonic acid



Perfluorobutane sulphonic acid

Fig. 1. Structural formulae of perfluorooctane sulphonic acid (PFOS) and perfluorobutane sulphonic acid (PFBS)

Only a few investigations on the intake and elimination as well as the metabolism of the perfluoroalkane sulphonic acids in humans have been carried out, even in the case of PFOS that is used in large quantities worldwide.

Inhalation and cutaneous absorption are the dominant routes of intake when PFOS or PFBS and its derivatives are handled industrially. PFOS has a mean biological half-

life of 9 years [1]. Our own experience has shown that the half-life of PFBS is in the range of several weeks. The unchanged perfluorosulphonic acids are excreted both in the urine (a total of approx. 30% of the dosage administered to rats) and in the faeces [1].

The data on the toxicology of PFOS were summarised and evaluated by the OECD in a detailed report [2]. At present no information on the toxicology of PFBS is available.

Studies show that the persistence of PFOS has led to its widespread distribution in the environment [1], thus resulting in a background level of PFOS in the general population. Most evidence of this comes from the USA.

Table 1 shows an overview of reports in the literature of PFOS and PFBS concentrations in the blood of the general population. The mean detected concentrations of PFOS are approx. 40 µg/L plasma (cf. Table 1). No data has been published on PFBS to date. However, our own investigations show that no background level of PFBS above the detection limit of this method (10 µg/L plasma) is found in the general population.

Tab. 1. PFOS and PFBS concentrations in plasma in the general population

Environmental medicine investigations

Substance	n	µg/L plasma	References
PFOS	599 children Age: 2 to 12	Mean value: 35 (girls) 40 (boys) 95th percentile: 515	[3]
	645 adults (blood donors) Age: 20–69	Mean value: 35 95th percentile: 1656	[4]
	238 persons Age: 65–96	Mean value: 31 95th percentile: 175	[5]
	15 blood samples from the umbilical cord	Range: 1.6 to 5.3	[6]
PFBS	n = 356	Range: < 10	[7]

Tab. 2. Plasma concentrations of PFOS and PFBS detected in investigations in occupational medicine

Investigations in occupational medicine			
Substance	n	µg/L plasma	References
PFOS	263	Mean value: 1,320 Range: 60 to 10,060	[8]
	178	Mean value: 2,190 Maximum: 12,830	[9]
PFBS	170	Mean value: 333 Range: <10 to 2,057	[7]

In contrast, mean concentrations of 333 µg/L blood were determined for PFBS in occupational medicine (cf. Table 2). Table 2 presents a brief overview of the PFOS and PFBS concentrations detected in investigations carried out in the field of occupational medicine.

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1 General principles

After adding perfluorodecane-1-sulphonate and tetrabutylammonium hydrogen sulphate at a pH value of 10 (the former serves as an internal standard and the latter as an ion pair reagent), perfluorooctane sulphonic acid and perfluorobutane sulphonic acid are extracted from plasma or urine in *tert*-butylmethyl ether.
After evaporation of the solvent, the extraction residue is dissolved in methanol, and the analytes are subsequently separated by means of high performance liquid chromatography and quantified by tandem mass spectrometric detection. Calibration is performed using standards prepared in human plasma or in pooled urine from non-exposed persons and the calibration standards are treated in the same manner as the samples to be analysed.

2 Equipment, chemicals and solutions

2.1 Equipment

HPLC system comprising a ternary gradient pump (e.g. Waters Alliance 2695), a device for degassing the eluents, a column thermostat, an injection valve, an autosampler, a tandem mass spectrometric detector (e.g. Micromass Quattro Ultima) and a PC system for data evaluation

HPLC column:
Zorbax Eclipse XDB-C_8, length: 50 mm, inner diameter: 4.6 mm; particle diameter: 5 µm (e.g. from Agilent, Order No.: 946975-906)

Disposable syringe with anticoagulant (e.g. K-EDTA Monovettes from Sarstedt)

Laboratory centrifuge (e.g. Hettich Rotanta 460R)

Laboratory shaker (e.g. IKA Vibrax VXR basic)

Analytical balance (e.g. Mettler AE 200)

Magnetic stirrer (e.g. IKA IKAMAG RET-GS)

Vacuum centrifuge (e.g. Saur BA-VC-300H Vacuum Concentrator)

Variably adjustable pipettes, 10 to 100 µL and 100 to 1000 µL (e.g. Transferpette, from Brand)

13 mL Polypropylene tubes with screw caps (e.g. Sarstedt, Order No.: 60.541)

4 mL Polypropylene tubes (e.g. Sarstedt, Order No.: 73.705)

Transfer pipettes (e.g. Sarstedt, Order No.: 86.1171)

10, 100, 250 and 500 mL Volumetric flasks

250 mL Glass beaker

1 mL Crimp-cap sample vials with Teflon-coated caps for the HPLC autosampler (e.g. from Macherey-Nagel)

2.2 Chemicals

Potassium heptadecafluorooctane sulphonate (>98%) (e.g. Fluka, Order No.: 77282)

Potassium nonafluoro-1-butane sulphonate (>97%) (e.g. Fluka, Order No.: 60418)

Ammonium perfluoro-1-decane sulphonic acid (25% in water/2-butoxyethanol) (e.g. Aldrich, Order No.: 468150)

Methanol, LiChrosolv (>99.8%) (e.g. Merck, Order No.: 106018)

tert-Butylmethyl ether SupraSolv (e.g. Merck, Order No.: 101995)

Tetrabutylammonium hydrogen sulphate (min. 98%)
(e.g. Riedel-de Haën, Order No.: 65305)

Ammonium acetate (>97%) (e.g. Riedel-de Haën, Order No.: 32301)

Sodium carbonate, anhydrous, p.a. (>99%) (e.g. Merck, Order No.: 106392)

Sodium hydrogen carbonate (99.7 to 100.3%) (e.g. Merck, Order No.: 106329)

Sodium hydroxide (>99%) (e.g. Merck, Order No.: 106498)

Deionised water (e.g. produced by means of Millipore® technology)

Nitrogen, 99.996% (e.g. from Linde)

Argon, 99.9993% (e.g. from Linde)

2.3 Solutions

10 M NaOH solution:
Approximately 50 mL bidistilled water are placed in a 250 mL glass beaker. A total of 40 g sodium hydroxide are cautiously added (beware: heat is generated!). The solution is mixed until it is clear using a magnetic stirrer. After being allowed to cool, the solution is transferred to a 100 mL volumetric flask, the glass beaker is rinsed with bidistilled water and the rinsing water is added to the flask. Then the flask is filled to its nominal volume with bidistilled water.

1 M NaOH solution:
Approx. 50 mL water are placed in a 100 mL volumetric flask and 10 mL of the 10 M NaOH solution are added using a pipette. The flask is subsequently filled to its nominal volume with water.

0.5 M Tetrabutylammonium hydrogen sulphate solution (0.5 M TBAS):
16.9 g tetrabutylammonium hydrogen sulphate are weighed in a 250 mL glass beaker and dissolved in approx. 85 mL water while being stirred with a magnetic stirrer. Approx. 4 mL of the 10 M NaOH solution are added, during which the pH value is checked (pH meter) and the solution is stirred until a pH value of 10 is reached. It must be ensured that the last drops are added slowly, as the pH value changes spon-

taneously. The solution is subsequently filled to a total volume of 100 mL with bidistilled water.

0.25 M Sodium carbonate/sodium hydrogen carbonate buffer:
First 6.6 g sodium carbonate (anhydrous) and 5.35 g sodium hydrogen carbonate are weighed in a 250 mL glass beaker and then dissolved in approx. 200 mL bidistilled water. The solution is transferred to a 250 mL volumetric flask, the beaker is rinsed with bidistilled water and the rinsing water is added to the flask. Then the flask is filled to its nominal volume with bidistilled water.

Eluent C (100 mM ammonium acetate):
3.85 g Ammonium acetate are weighed into a 500 mL volumetric flask. The volumetric flask is filled to its nominal volume with water while the contents are being swirled at regular intervals.

All the solutions are stable at room temperature for at least 4 weeks. However, the pH value of the 0.5 M TBAS solution must be regularly checked before use, and it must be adjusted to a pH value of 10 by adding 1 M sodium hydroxide if necessary.

2.4 Calibration standards

Stock solution:
Approx. 112.4 mg potassium nonafluoro-1-butane sulphonate and approx. 107.4 mg potassium heptadecafluorooctane sulphonate are weighed exactly in a 100 mL volumetric flask. The flask is subsequently filled to its nominal volume with methanol. The concentration of the perfluorobutane sulphonic acid or perfluorooctane sulphonic acid is c = 1 g/L in each case.

Working solution A:
500 µL of the stock solution are pipetted into a 10 mL volumetric flask. The flask is subsequently filled to its nominal volume with methanol (50 mg/L).

Working solution B:
1.0 mL of working solution A is pipetted into a 10 mL volumetric flask. The flask is subsequently filled to its nominal volume with methanol (5 mg/L).

Working solution C:
1.0 mL of working solution B is pipetted into a 10 mL volumetric flask. The flask is subsequently filled to its nominal volume with methanol (500 µg/L).

Solution of the internal standard (ISTD)

Starting solution of the internal standard:
The commercially available solution of ammonium perfluoro-1-decane sulphonic acid (25% in water/2-butoxyethanol) serves as the starting solution of the internal standard.

Stock solution of the internal standard:
400 µL of the starting solution of the internal standard are pipetted into a 100 mL volumetric flask. The flask is then filled to its nominal volume with methanol (c = 1 g/L).

Spiking solution of the internal standard:
1 mL of the stock solution of the internal standard is pipetted into a 100 mL volumetric flask. The flask is then filled to its nominal volume with methanol (c = 10 mg/L).

The stock solutions of the calibration standards and of the internal standard are stable at 4 °C for approx. 6 months. However, the dilutions should be freshly prepared for each analytical series, as adsorptive effects on the glass cannot be ruled out.

Calibration standards:
The calibration standard solutions for the determination in urine or plasma are prepared in the pooled urine or pooled plasma of test persons who were not exposed to the analytes. In order to prepare pooled urine spontaneous urine samples are collected from the test persons in a suitable vessel; the urine is thoroughly mixed and stored at –20 °C until the standard and the control material are prepared.
Pooled human plasma is prepared after withdrawing approx. 5 mL of blood from non-exposed test persons using EDTA Monovettes and the plasma is separated as described in Section 3. The plasma samples from the test subjects are mixed in a suitable vessel and stored at –20 °C until the standards and the control material are prepared.
1 mL pooled urine or pooled plasma from non-exposed persons in a 13 mL polypropylene tube with a screw cap is spiked with the calibration standards and internal standard as shown in pipetting scheme in Table 3. The calibration range depends on the expected concentration in the real samples. A range of 5 to 250 µg/L has proved adequate in practice.
Only 0.1 mL of plasma or urine samples with a content greater than 250 µg/L is used for sample preparation as described in Section 3.1. Calibration is carried out in the same manner using 0.1 mL of the pooled urine or pooled plasma of non-exposed persons in accordance with the pipetting scheme in Table 3 (cf. Section 9.4).
A sample of water is included in each analytical series as a reagent blank and a urine or plasma blank sample is processed and analysed in order to detect any blank values.
The samples are subsequently shaken intensively on a laboratory shaker (Vortex) for 15 seconds and further treated as described in Section 3.1.

Table 3. Pipetting scheme for the preparation of calibration standards in pooled urine or pooled human plasma

Volume of the working solution			Volume of the spiking solution of the ISTD [µL]	Volume of the pooled urine or pooled plasma [mL]	Water [mL]	Concentration of the calibration standard [µg/L]
A [µL]	B [µL]	C [µL]				
–	–	–	10	1	–	Blank value
–	–	10	10	1	–	5
–	–	20	10	1	–	10
–	5	–	10	1	–	25
–	10	–	10	1	–	50
–	20	–	10	1	–	100
5	–	–	10	1	–	250
–	*10*	–	*10*	*0.1 **	*0.9*	*500*
–	*20*	–	*10*	*0.1 **	*0.9*	*1000*
–	*50*	–	*10*	*0.1 **	*0.9*	*2500*
–	*10*	–	*10*	*0.01 **	*0.99*	*5000*

** It is advisable to dilute the analytical samples with appropriately less spiking solution for analysis in the range of relevance to occupational medicine (cf. Section 5 and 9.4).*

3 Specimen collection and sample preparation

Blood:
Approx. 5 mL blood are withdrawn slowly using a disposable withdrawal system containing an anticoagulant (e.g. EDTA-K Monovettes, Sarstedt). The blood sample should be centrifuged as soon as possible at 1200 g for 10 minutes. The upper plasma phase is transferred to a sealable polypropylene tube using a transfer pipette.

Urine:
The urine sample is also transferred without further additives to a sealable polypropylene tube.
Both the plasma samples and the urine samples are stable for one month at +4 °C and for at least 6 months at –20 °C.

3.1 Sample preparation

Before analysis, the samples are thawed if necessary and thoroughly mixed. 1 mL plasma or 1 mL urine is pipetted into a 13 mL polypropylene tube with a screw cap, 10 µL of the spiking solution of the internal standard is added as stipulated in the pipetting scheme in Table 3 (Section 2.4) and the contents are mixed on the laboratory shaker (Vibrax) for 15 s.
Then 200 µL of the 0.5 M tetrabutylammonium hydrogen sulphate solution and 1 mL of the 0.25 M sodium carbonate/sodium hydrogen carbonate buffer are added. After adding 3 mL *tert*-butylmethyl ether to the sample, it is shaken intensively for 20 minutes on the laboratory shaker (Vortex).

The sample is centrifuged (2000 g, 20 min), then 2.5 mL of the upper organic phase are drawn off and transferred to a new 4 mL polypropylene centrifuge tube. The solution is evaporated to dryness in the vacuum centrifuge.
The residue is dissolved in 500 µL methanol and mixed for 30 s on the laboratory shaker (Vibrax). The sample is subsequently placed in an ultrasonic bath for a further 15 min in order to achieve complete dissolution of the residue. The centrifuge tube is then centrifuged for 5 min at 2000 g and the clear supernatant is transferred to an autosampler vial, sealed and stored at +4 °C until analysis.

4 Operational parameters

The analytical measurements were carried out on a coupled instrument comprising a Waters 2695 HPLC gradient pump and a Quattro Ultima LC/MS/MS system from Micromass.
Operational parameters for high performance liquid chromatography

Separation column:	Material:	Steel
	Length:	50 mm
	Inner diameter:	4.6 mm
	Column packing:	Zorbax Eclipse XDB-C_8, 5 µm
Separation principle:	Reversed phase	
Temperature:	30 °C	
Detection:	Tandem mass spectrometric detector	
Mobile phase:	Eluent A: Methanol Eluent B: Water Eluent C: 100 mM ammonium acetate (*The eluents must be degassed before use!*)	
Gradient:	See Table 4	

Table 4. Program of the gradient pump

Time [min]	Eluent A [vol.%]	Eluent B [vol.%]	Eluent C [vol.%]
0	50	40	10
0.5	90	0	10
9.5	90	0	10
10.5	50	40	10
12	50	40	10

Stop time:	14 minutes
Flow rate:	0.25 mL/min
Injection volume:	10 μL

All other parameters must be optimised in accordance with the manufacturer's instructions.

4.1 Operational parameters for mass spectrometry

Settings of the ion source:

Ionisation mode:	ESI negative
Source temperature:	120 °C
Desolvation temperature:	200 °C
Cone gas flow (L/h):	42
Desolvation gas flow (L/h):	510
Capillary (kV):	2.99
Cone (V):	100
Hex 1 (V):	0
Aperture (V):	0
Hex 2 (V):	0

Settings of the analyser:

LM 1 Resolution:	14.0
HM 1 Resolution:	14.0
Ion energy 1:	1.0
Entrance:	–1
Collision:	42
Exit:	5
LM 2 Resolution:	14.0
HM 2 Resolution:	14.0
Ion energy 2:	1.0
Multiplier (V):	650

The measurement conditions listed here were established for the configuration of instruments used in this case, and they must be optimised in accordance with the manufacturer's instructions if other instruments are used.

5 Analytical determination

In each case 10 µL of the plasma or urine samples processed as described in Section 3.1 are injected into the HPLC instrument.
Each analytical series includes a quality control sample, two reagent blank samples and a plasma or urine blank sample. If the resulting measurement values are outside the linear range of the calibration curve, the plasma or urine samples are diluted 1 : 10 with ultrapure water and processed anew with a similarly diluted calibration series (cf. Section 2.4).
The temporal traces of the ion transitions listed in Table 5 are recorded in the MRM mode of the tandem mass spectrometer (ESI negative mode):

Table 5. Retention times and detected ion transitions

Analyte	Retention time [min]	Ion transitions (MS/MS, ESI neg. mode)	
		Q 1	Q 3
Perfluorobutane sulphonic acid (PFBS)	5.38	298.8	80.3 *
Perfluorooctane sulphonic acid (PFOS)	6.63	498.8	80.3 99.3 * 130.3
Perfluorodecane sulphonic acid (ISTD)	7.31	599.0	80.0 *

*The masses marked * are used for quantitative evaluation.*

The retention times shown in Table 5 serve only as a guide. Users of the method must satisfy themselves of the separation power of the HPLC column they use and of the resulting retention behaviour of the substances.
Figure 2 gives an example of the chromatogram of a processed calibration standard prepared in pooled human plasma and spiked with 50 µg/L each of PFOS and PFBS. Figure 3 shows the chromatogram of a urine sample that was spiked with 50 µg/L each of PFOS and PFBS.

6 Calibration

The calibration standards prepared in plasma or urine as described in Section 2.4 are processed in the same manner as the plasma or urine samples prepared according to Section 3.1 and analysed in accordance with the instrumental parameters listed in Sections 4.1 and 4.2. In each case 10 µL of the processed calibration standards are injected into the HPLC.
The required reagent blank samples are processed as described in Section 3.1, whereby bidistilled water is used as the blank sample.
Then calibration curves are obtained by plotting the concentrations of the calibration standards as a function of the quotients of the peak areas of PFOS or PFBS with that of the internal standard perfluorodecane sulphonic acid. The regression coefficients of the calibration curve are calculated by the software of the instrument (e.g. MassLynx, Waters). Any reagent blank values and blank values that occur for the plasma or urine used for calibration must be taken into account and subtracted from the results if appropriate. A linear measurement range of 5 µg/L to 100 µg/L was determined for the instrument used in this case when a sample volume of 1 mL was used. A new calibration curve is plotted for each analytical series, whereby any dilution of the samples must be given special consideration (see Section 9.4, Sources of Error).
Figure 4 shows an example of the calibration curves for PFOS or PFBS in human plasma.

7 Calculation of the analytical result

The concentration in plasma or urine samples is calculated from the relevant calibration curves (cf. Section 6). After calculating the quotients of the peak areas of PFOS and PFBS with that of the internal standard, the corresponding concentration of the analytes in µg/L plasma or urine is read off the relevant calibration curve. This calculation may also be carried out by the evaluation software of the LC/MS/MS system (e.g. MassLynx, Waters) if appropriate.
Any reagent blank values must be subtracted from the analytical results for the real samples.

8 Standardisation and quality control

Quality control of the analytical results is carried out according to the guidelines of the Bundesärztekammer (German Medical Association) [10] and the special preliminary remarks in this series. Precision control is performed using spiked plasma or urine control samples containing a constant concentration of the analytes. As material for quality control is not commercially available, it must be prepared in the laboratory. For this purpose defined quantities of the analytes PFOS and PFBS are added to plasma or pooled urine. A six-month supply of this control material is prepared, divided into aliquots in 13 mL sealable polyethylene tubes with screw caps and

stored in the deep-freezer. The theoretical value and the tolerance range for this quality control material are determined in the course of a pre-analytical period (one analysis of the control material on each of 20 different days) [11, 12].

9 Evaluation of the method

9.1 Precision

In order to determine the precision in the series 1 mL of plasma or urine was spiked with PFBS and PFOS concentrations of between 10 µg/L and 100 µg/L, processed and analysed as described in the previous sections. Ten replicate determinations of the plasma or urine samples yielded the precision in the series documented in Table 6.

Table 6. Precision in the series for the determination of PFBS and PFOS in plasma or urine (n = 10)

Concentration [µg/L]	Plasma				Urine			
	PFBS		PFOS		PFBS		PFOS	
	S.D. (rel.) [%]	Prognostic range [%]	S.D. (rel.) [%]	Prognostic range [%]	S.D. (rel.) [%]	Prognostic range [%]	S.D. (rel.) [%]	Prognostic range [%]
10	4.9	10.9	4.5	10.0	5.9	13.2	9.5	21.2
50	4.9	10.9	3.7	8.3	6.3	14.0	5.1	11.4
100	4.0	8.9	6.7	14.9	6.6	14.7	4.4	9.8

In addition, the precision from day to day was determined. The same material was used as for the determination of the precision in the series. These plasma or urine samples were processed and analysed on each of 10 different days. The precision results are shown in Table 7.

Table 7. Precision from day to day for the determination of PFBS and PFOS in plasma or urine (n = 10)

Concentration [µg/L]	Plasma				Urine			
	PFBS		PFOS		PFBS		PFOS	
	S.D. (rel.) [%]	Prognostic range [%]	S.D. (rel.) [%]	Prognostic range [%]	S.D. (rel.) [%]	Prognostic range [%]	S.D. (rel.) [%]	Prognostic range [%]
10	5.3	11.8	6.7	14.9	8.2	18.3	10.2	22.7
50	4.9	10.9	5.0	11.1	6.5	14.5	5.7	12.7
100	5.0	11.2	7.1	15.8	7.2	16.7	5.5	12.3

9.2 Accuracy

Recovery experiments were carried out to test the accuracy of the method. Pooled human plasma or pooled urine was spiked with PFBS and PFOS in concentrations between 10 and 100 µg per litre for this purpose. Each of these plasma or urine samples was subsequently processed and analysed ten times in accordance with Section 3.1. The range of the resulting relative recovery rates is summarised in Table 8.

Table 8. Relative recovery rates for PFBS and PFOS in spiked pooled urine or pooled plasma samples

Concentration [µg/L]	Plasma Relative recovery rate [%]		Urine Relative recovery rate [%]	
	PFBS	PFOS	PFBS	PFOS
10	91–105	108–123	91–109	83–114
50	80–95	91–101	90–108	111–130
100	87–100	94–115	94–116	100–116

Furthermore, the accuracy of the method was investigated as part of the method evaluation in round-robin experiments on native human plasma samples involving 3 laboratories. The individual values for each of the samples obtained in the various laboratories are shown in Table 9.

Table 9. Individual results for real samples obtained in round-robin experiments to assay PFBS and PFOS in plasma

Sample	PFBS [µg/L] Laboratory 1 *	Laboratory 2	Laboratory 3	PFOS [µg/L] Laboratory 3	Laboratory 2
1	2045	1770	1767	47	47
2	42	103	46	242	336
3	<5	2	<10	848	700
4	<5	1	<10	55	51
5	1001	1070	1058	1008	875
6	9	12	<10	451	685
7	<5	1	<10	49	78
8	<5	1	<10	41	80
9	<5	2	<10	<10	34
10	1693	1280	1241	22	42

* Laboratory 1: only assay of PFBS

9.3 Detection limits

Under the conditions given here the detection limit, calculated as three times the signal/noise ratio of the analytical background noise in the temporal vicinity of the analyte signal, is approximately 5 µg/L for PFBS and PFOS for both the plasma matrix and the urine matrix. On account of the ubiquitous distribution of these substances and the consequent blank values the detection limit was not achievable in practice. As a result of this observation a quantitation limit of 10 µg/L plasma can be given here.

9.4 Sources of error

Relatively high reagent blank values were observed (up to approx. 10 µg/L). Therefore two reagent blank values and a plasma or urine blank value should be measured for each calibration, and these values must be subtracted from the results for the calibration standards. The double end-capped Zorbax Eclipse XDB-C_8 HPLC column described here was the only one of several RP18 phases tested that exhibited an inert and reproducible separation behaviour. The other phases showed memory effects which indicate an excessive residual activity of the separation material. In contrast to the test method described by the 3M company [13], the quantity of added tetrabutylammonium hydrogen sulphate was reduced, as excess phase transfer reagent can also carry over the analytes to the separation phase.
In addition, results were matrix-dependent if samples had to be diluted due to high values being obtained. In this case the diluted matrix caused distinctly higher deviation in the results compared to calibration using the concentrated matrix. Therefore dilution of the analytical sample must also be taken into account in the case of the calibration standards.
Although no systematic investigation was carried out on the extent to which false positive results may be caused by the intake of medication or drugs, the possibility seems unlikely.
For quality assurance it is important to ensure that the concentrated calibration standards are not stored at the same location where the samples are processed or stored, as elevated blank values may result.

10 Discussion of the method

Perfluorobutane sulphonic acid and perfluorooctane sulphonic acid of relevance to occupational and environmental medicine can be determined rapidly and reliably with the method presented here. Round-robin experiments carried out on native plasma samples by 3 laboratories as part of the method evaluation showed very good congruence of the measured values.
The characteristic data of the method are regarded as very good. The examiner was able to reproduce the reliability criteria of the method without problems.

The experience of the examiner has shown that the extraction of PFBS and PFOS can be carried out using 5 mL diethyl ether instead of 3 mL *tert*-butylmethyl ether. However, only 0.5 mL plasma were used in this case and the processed sample was taken up in a final volume of 200 µL methanol. The examiner reports that tetra-n-butylammonium hydroxide (20%) (Merck, No. 8.18759) can also be used as an ion pair reagent instead of the tetrabutylammonium hydrogen sulphate used by the authors.
Although the method is described as suitable for analysis of urine and also plasma as a matrix, determination in plasma is recommended due to the toxicological properties of the substances (accumulation).
The PFOS analyses carried out by the 3M company were also performed by means of an LC/MS/MS method. It proved impossible to establish the PFOS method developed by Hansen et al. at 3M [13] using the data described there. Therefore the method has been modified in certain points. It became evident that the quantity of the ion pair reagent had a considerable influence on the reproducibility of the results. The amount described in the method instructions from 3M led to strong adsorption effects on the HPLC column, and reproducible and valid results were achieved only by reducing the amount of reagent.

Instruments used:
HPLC 2695 from Waters with integrated autosampler, Quattro Ultima tandem mass spectrometric detector from Micromass, evaluation software: MassLynx, Waters.

11 References

[1] *3M Company:* SIDS initial report: perfluorooctane sulphonic acid and its salts. US EPA public docket AR-226. Saint Paul: 3M Company, 2000. Available under: *http://www.epa.gov/opptintr/pfoa/.*

[2] *OECD:* Hazard Assessment of Perfluorooctane Sulfonate (PFOS) and its salts. Organisation for economic co-operation and development. Paris 2004. *http://www.oecd.org/dataoecd/23/18/2382880.pdf.*

[3] *G. W. Olsen, T. R. Church* und *T. R. Hansen et al.:* Quantitative evaluation of perfluoroctanesulfonate (PFOS) and other fluorochemicals in the serum of children. J. Children's Health 2, 53–76 (2004).

[4] *G. W. Olsen, T. R. Church, J. P. Miller, J. M. Burris, K. J. Hansen, J. K. Lundberg, J. B. Armitage, R. M. Herron, Z. Medhdizadehkashi, J. B. Nobiletti, E. M. O'Neill, J. H. Mandel* und *L. R. Zobel:* Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. Environ. Health Perspect. 111, 1892–1901 (2003).

[5] *G. W. Olsen, T. R. Church, E. B. Larson, G. van Belle, J. K. Lundberg, K. J. Hansen, J. M. Burris, J. H. Mandel* und *L. R. Zobel: Serum concentrations of PFOS and other fluorochemicals in an elderly population from Seattle, Washington. Chemosphere 54, 1599–1611 (2004).*

[6] *K. Inoue, F. Okada, R. Ito, S. Kato, S. Sasaki, S. Nakajima, A. Uno, Y. Saijo, F. Sata, Y. Yoshimura, R. Kishi* und *H. Nakazawa:* Perfluoroctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ. Health Perspect. 112, 1204–1207 (2004).

[7] *G. Leng:* Unveröffentlichte Ergebnisse. Arbeitsmedizinische Vorsorgeuntersuchungen. Bayer Leverkusen 2001–2004

[8] *G. W. Olsen, J. M. Burris, M. M. Burlew* und *J. H. Mandel:* Epidemiologic assessment of workers serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. J. Occup. Environ. Med. 45, 260–270 (2003).

[9] *G. W. Olsen, J. M. Burris, J. H. Mandel* und *L. R. Zobel:* Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. J. Occup. Environ. Med. 41, 799–806 (1999).
[10] *Bundesärztekammer:* Richtlinie der Bundesärztekammer zur Qualitätssicherung quantitativer laboratoriumsmedizinischer Untersuchungen. Dt. Ärztebl. 100, A3335–A3338 (2003).
[11] *J. Angerer* und *G. Lehnert:* Anforderungen an arbeitsmedizinisch-toxikologische Analysen – Stand der Technik. Dt. Ärztebl. 37, C1753–C1760 (1997).
[12] *J. Angerer, Th. Göen* und *G. Lehnert:* Mindestanforderungen an die Qualität von umweltmedizinisch-toxikologischen Analysen. Umweltmed. Forsch. Prax. 3, 307–312 (1998).
[13] *K. J. Hansen, L. A. Clemen, M. E. Ellefson* und *J. O. Johnson:* Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environ. Sci. Technol. 35, 766–770 (2001).

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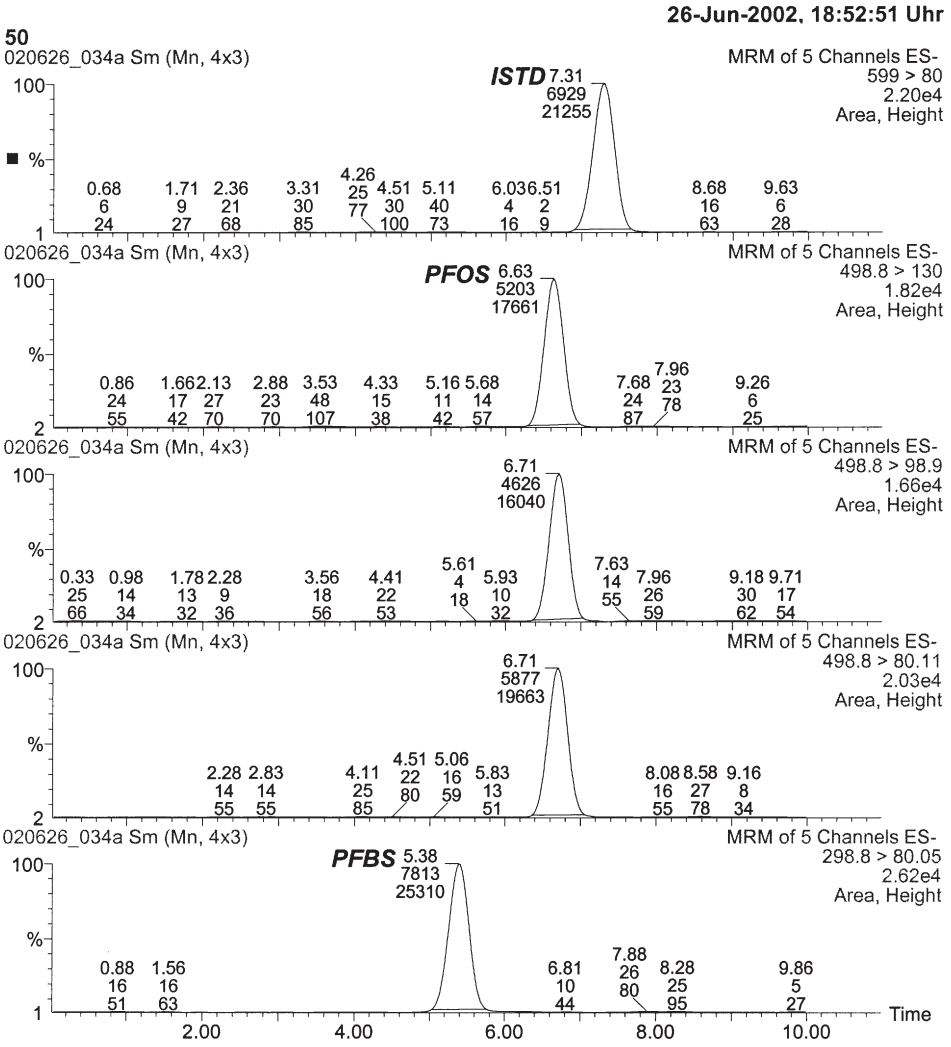


Fig. 2. Example of a chromatogram of a plasma sample spiked with 50 µg/L each of PFBS and PFOS

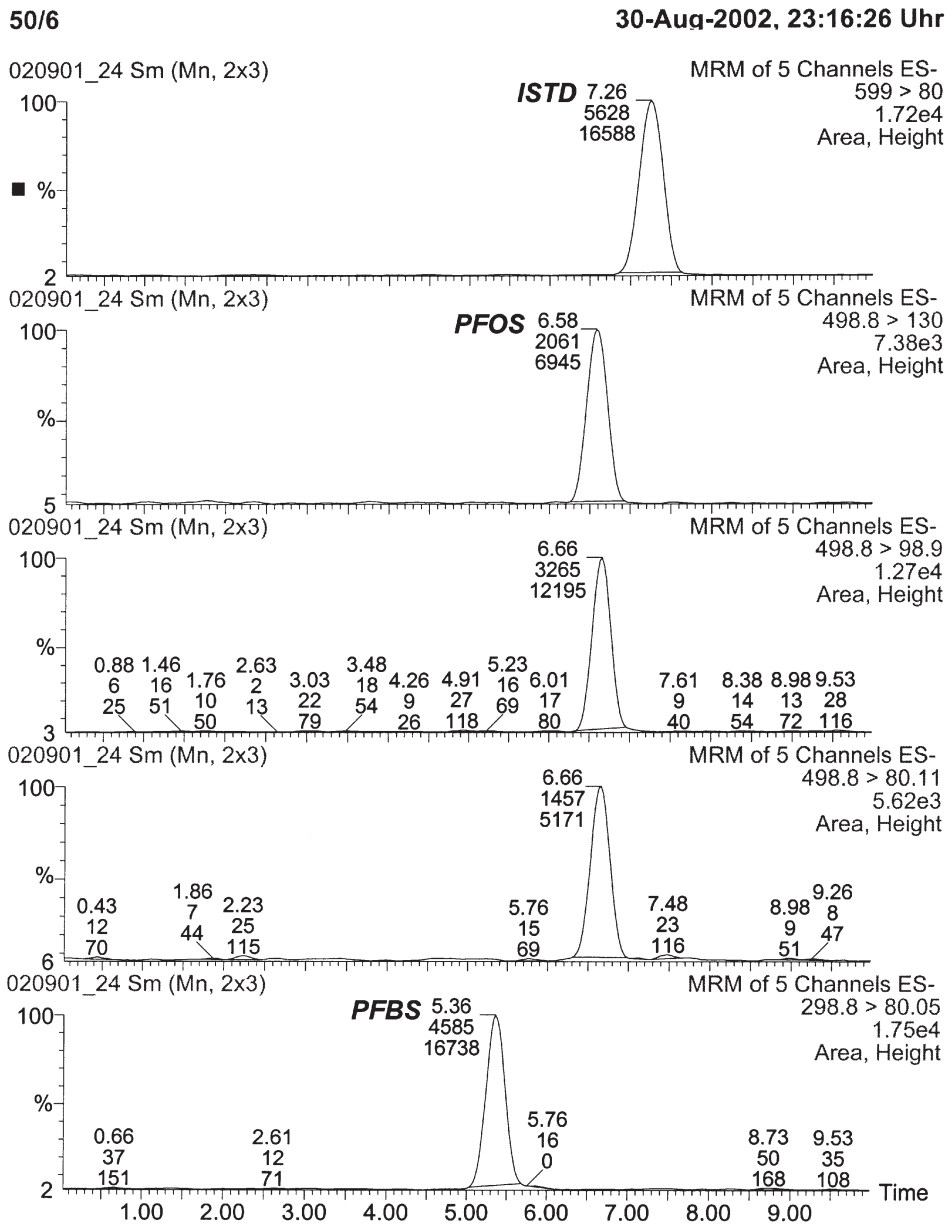
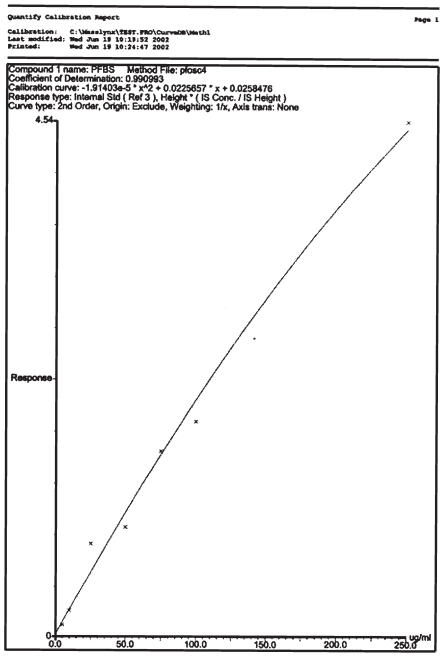


Fig. 3. Example of a chromatogram of a urine sample spiked with 50 µg/L each of PFBS and PFOS

Calibration curve PFBS



Calibration curve PFOS

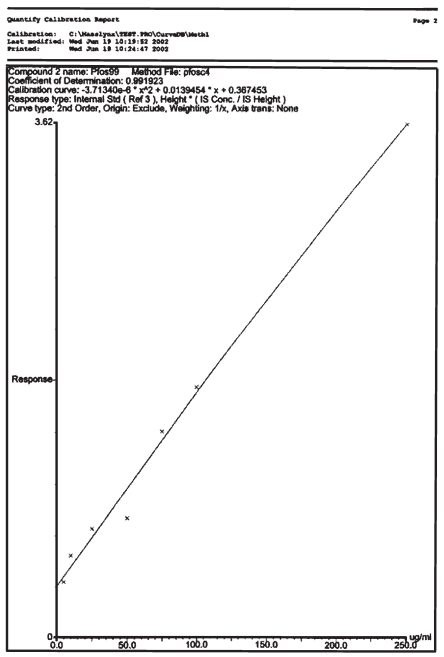


Fig. 4. Calibration curves for PFBS and PFOS, prepared in human plasma