Perfluoroctanoic acid in plasma

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Draft 22/11/01

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1 Basis of the procedure

Perfluoroctanoic acid is extracted with pH 10 as ion pair in tert-butylmethylether and derivatised with benzyl bromide. The determination takes place with GC/MS in the EGG - or NCI mode. Concentrations of interest for industrial medicine and environmental medicine in the plasma can be determined accurately.

2 Devices, chemicals, solutions

2,1 Devices

HP5890 with Split Splitless injection system and autoinjector
HP7673 mass-selective detector e.g. Kodiak 1200 v. Fa. Bear

Heating block with drillings for glass tube
Heatable evaporation block with blower
shaker

centrifuge (3000 - 4000 RPM) vacuum centrifuge (e.g. SpeedVac of SAVANT)
variable adjustable 100 µl and 1 ml pipettes (e.g. of EPPENDORF)
10 µl 100 µl glass syringes (e.g. of Hamilton)
Autosampler
2 ml vials (e.g. of HEWLETT-PACKARD)
10 ml glass vials with cap
20 ml glass vials with ground glass stoppers

2,2 chemicals

tert.Butylmethylether (TBME)
acetone
benzyl bromide Aldrich B17905
pig pool serum
linear Perfluoroctanoic acid
(PFOS) of Aldrich (Cat.Nr. 171468)
Perfluornonanoic acid (PFNS) of Aldrich (Cat.Nr. 394459)

2,3 solutions

-0,25 M carbonate buffers pH 10 beginning: 4.2 g NaHCO3 (Merck 106329) and 14.3 g Na2CO3 Decahydrate (Merck 106391) with water ad 200 ml solve
- 20 % (0.77 M) Tetrabutylammoniumhydroxide (TBA) in water of Merck (Kat.Nr. 818759)
- 0,5 M Tetrabutylammoniumhydroxide (TBA) in water beginning: 65 ml 20 % TBA + 35 ml water mix

2,3 comparison standards

2,3,1 initial solutions

Perfluoroctanoic acid (PFOA): Weigh 25 mg PFOA and with acetone add 25 ml exactly. End concentration: 1 mg/ml.

Perfluornonananoic acid (PFNA): Weigh and with acetone add 25 ml exactly. Final concentration 1 mg/ml.

2,3,2 Perfluoroctanoic acid dilution 1

In 10 ml measuring flasks an acetone , add 500 µl PFOA initial solution (1 mg/ml). With acetone fill to the mark. Final concentration: 50 µg/ml.

2,3,3 Perfluoroctanoic acid, dilution 2

In 10 ml measuring flasks add, to 100 µl PFOA dilution 2 (50 µg/ml) Fill to the mark with acetone. Final concentration 0.5 µg/ml.

2,3,4 Perfluornonanoic acid dilution 1 (internal standard)

In 10 ml measuring flasks take 10 µl PFNA initial solution (1 mg/ml). With acetone fill to the mark. Final concentration: 1 µg/mL

2,3,5 calibration standards

PFOA solutions are made by dilution with pig plasma calibration standards, which contain PFOA concentrations between 5000 and 5 µg/l.

Pipetting pattern

Volume of the PFOA dilution 1 (50 µg/ml) volume of the PFOA dilution 2 (0.5 µg/ml) pig plasma concentration in the comparison standard µl µl ml µg/l

µg/l = ppb

50 µl -- 0.5 5000 25 µl -- 0.5 2500 5 µl -- 0.5,500 -- 100 µl 0.5,100 -- 50 µL 0.5 50 -- 10 µL 0.5 10 -- 5 µL 0.5 5 -- -- 0.5 0

For questions according to industrial medicine it is sufficient to set calibration standards within the range 100 to 5000 µg/l. For measurements within the environmental medical purposes, standards are necessary within the range of 5 to at least 100 µg/l.

3 Sampling and sample preparation

3,1 Sampling and sample preparation

Sampling:

Blood withdrawal e.g. by means of EDTA - Sarsted Monovette. The plasma production can be accelerated by centrifugation. The blood withdrawal system should have been examined before use for zero blanks.

Execution:

1. The beginnings for samples and calibration standards take place in 10-ml-Gewinde-Roehrchen 2nd standard internal on 0,5 ml plasma 50 µl (PFNA Verd. 2) and 2 ml 0,25-M-Carbonate-Buffer(pH 10) gives and examines (with indicator paper) 3rd 1 ml 0,5-M-TBA-Loesung and 3 to pH value each ml TBME in addition-gives; then approx. 60 min intensively mixes (over head shakers, e.g. HEIDOLPH) 4th following centrifugation 5th 1.7 ml clear TBME excerpt into a Autosampler ampule transfers and

with approx. 35 C under air flow to dry ones restricts (is uncritical)
6. the arrears in 100 µl acetone takes up to put 20 µl benzyl bromide the solution to point-in-corrodes transfers and zudeckeln. Then approx. 45 min on approx. 75 C heat up.

(when investigations according to industrial medicine the derivatization can be accomplished alternatively in 500 µl acetone.)
7. Measurement in the EI mode or NCI mode

4 Conditions of work by gas chromatography

Detector: KODIAK 1200 (EI mode) gas chromatograph: HP 5890 II capillary column: RTX 5; -30 m, 0,25 mm I.D. x 0,5 µm film Taegergas: Helium 4,6 form: 11 psi temperature program: 1 min with 70 oC, then with 10 oC/min on 140 oC, with 30 C/min on 290 C and 2 min bake. Injector: Split splitless with 250 oC evaporator tubes: 2 mm I.D., task of sample deactivates: 2 µl Splitless time: 1 min transfer LINE: 280 oC electron collision ionization: 70 eV

EI MS conditions masses for PFOA: m/z 504 and 505 (Qualifier) masses for PFNA: m/z 554

NCI MSMS conditions: PFOA: Decomposition of m/z 413 after 369 PFNA: Decomposition from m/z 463 to 419 CI gas: Methane; Pouring jerk: 6.5 torr collision gas: Argon; 1.2 mTorr collision energy: 10 V

4 Analytic regulation

2µl is injected in each case in the GC.
Usually single injections are sufficient. If the measured values lie outside of the linearity of the calibration curve, then the samples are diluted and again regenerated. In each series a quality inspection sample is analyzed.

6 Calibration

For the production of a calibration curve comparison standards are set in pig plasma in accordance with the pipetting pattern in section 2,3,5 and regenerated as for plasma samples in section 3 described and analyzed according to section 4 by gas chromatography.

7 Computation of the results of analysis
On the basis the calibration curve, the associated concentration in the blood is determined.

8 Standardisation and quality assurance

For quality assurance will proceed, as in the TRgA 410 of the dangerous material regulation planned. With the individual analyses, precision and accuracy controls are analyzed.

9 Evaluation of the procedure

9,1 precision

For the determination of the precision doped pig plasma was examined. Measurements in the EI mode.

Spiking n Precision Precision mg/l into the series From day tons day VK (%) VK (%) 10 15 -- 8,2.5 10 4.77 -- 0.5 10 3.29 -- 0.1 10 5.00 --

9,2 accuracy

The correctness of the procedure was examined by regaining experiments with doped individual Human plasma proben. Measurements in the EI mode.

Spiking n Recovery guesses mg/l Mean (rank)

10 10 97 (89,1-107) 2.5 10 98.1 (78-107) 0.5 10 99 (80-110) 0.05 10 93 (78-116)

Selectivity comparison. Measurement of PFOA in the plasma vocationally not more exposed with EI-MS and NCI MSMS.

Person EI-GCMS NCI MSMS µg/l µg/l 1,9.6 10.8 2 5.4,6.6 3 3 3.5 4 4.3,4.8 5 3.5 5 6 2 3.1 7 3'1,5.1 8 5.2 7 9 3 3.7 10 4.8,5.4 blank 0.5,0,6

9,3 Detection limits

Under the indicated conditions of work and under taking a signal-to-noise ratio as a basis of 3:1 the detection limits for PFOA amount to 3 µg/l. in the EI mode. In the NCI MSMS mode is given the NWG at present by a blank value at a value of approx. 0.5 - to 1 µg/l. Those extrapolates detection limit amounts to approx. 0.2 µg/l.

9,4 Influences of noise

PFOA of test empties values at a value of approx. 0.5-1 were observed

µg/l. The causes are not finally clarified yet. The pig plasma used for calibration purposes is unloaded (PFOA < 1 µg/l) and from the blood withdrawal system no PFOA is set free.

Adsorption at Glass - or or plastic materials one did not observe.

10 Discussion of the method

The available GC-MS method been based on the procedure of M. Hanhijaervi et aluminium (1) for the quantification of PFOA by means of extraktive alkylation. From this the acid anion is extracted after integration with Tetrabutylammoniumhydroxide (TBA) as ion pair from the plasma and derivatised afterwards to the benzyl ester.

Already of M. Hanhijaervi et the aluminium (1) optimized buffer and TBA concentrations was invariably taken over. By the change of ethyl acetate against tert. Butylmethylether (TBME) as extracting agents the extraction temperature of 50 C could on ambient temperature be lowered and the evaporation of the excerpt to dry ones before the Derivatisierungsschritt be substantially accelerated.

After derivatisation with benzyl bromide in acetone the reaction mixture without further cleaning is chromatographed by means of GC-MS on a RTX5 30 m x 0.25 mm x 0.5 µm film thickness. GC columns with thinner film, e.g. HP5-MS 30 m x 0.25 mm x 0.25 µm, could be alternatively used. However the measuring solution had to be diluted of 0,1 to 0,5 ml, since in the more concentrated solution the benzyl bromide remainders unfavorably affected the peak form of PFOA with splitless injection (overloading the column).

Detection can take place with a MSD in the EGG or NCI mode (see spectra). If highest sensitivity is required, NCI GCMS or NCI GCMSMS is recommendable. The detection limit for NCI GCMS is given at present however by a test empties value in Hoehhe of approx. 0.5 µg/l. A parallel measurement with EI-GCMS and NCI GCMSMS at 10 did not load vocationally show that also with EGG-OFFICE-MS environmental medically relevant concentrations can be obtained at present sensitively and selectively. The determined PFOA of plasma results (2 - 11 ppb, n=10) is appropriate for a current HPLC MSMS study (Lit 2) in the lower range of values (< 5 to 35.2 ppb, n = 65).

During specific working place loading PFOA plasma concentrations within the lower ppm range were determined.

The procedure is uncomplicated, very sensitive and durable. The procedure characteristic data is good as very to designate. The procedure works is extended further to other fluoridated carbonic acids e.g. Perfluorheptane -, Perfuornonane and Perfluordecane acids. Likewise meaning Perfluoroctansulfononic acid can be determined however only by means of HPLC-MS (2).

Literature

1) M.Ylinen, H.Hanhijaervi et aluminium: Quantitative office determination OF Perfluorooctanic Acid as the benzyle ester in plasma and Urine. Arch.Environ.Contam.Toxicol. 14, 713-717 (1985)

2) K. J. Hansen et aluminium: Compound Specific, quantitative Characterization OF Organic Fluorochemicals in Biological Matrices. Environmental Science & Technology 35, 766-770 (2001)