Application of LGMS/MS Methodology to Determination of Telomer B Alcohols, Perfluorinated Acids, and Fluorosurfactants in Environmental Matrices.

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Introduction:

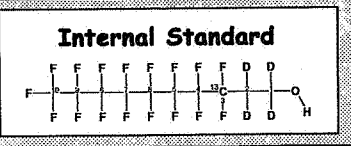
- analytical methodology for measurement of perfluoroalkyl substances in various biological and environmental matrices continues to be a challenging task despite tremendous progress in this field in recent years;
- the purpose of this work was the development of analytical methods for determination of Telomer B Alcohols (TBAs) and selected perfluorinated acids utilizing LC/MS and LC/MS/MS;
- Telomer B Alcohols are intermediates for manufacturing of a broad range of anionic, nonionic fluorosurfactants, and polymers;
- Telomer B Alcohols based products are broadly used in variety of consumer products as fluorosurfactants or water/soil repelling coatings for paper, textiles, and carpets;
- extraction and quantitation by LC/MS/MS of four selected perfluorinated acids, potential biodegradation products of 8-2 TBA, were investigated;

Telomer B Alcohols by LC/MS and LC/MS/MS

Introduction:

- TBAs chemical structure: $CF_3(CF_2)_x-CH_2-CH_2-OH$, x=3,5,7,9,11, ...
- Telomer B Alcohols are amenable to analysis by LC/MS or LC/MS/MS
- Telomer B Alcohols can be ionized by negative ion electrospray:
  - form deprotonated molecular ions (m/z: 363, 463, 563)
- in presence of acetate TBAs form predominantly acetate adduct;
- deprotonated molecular ions are prone to in-source fragmentation; need to be sampled at low cone voltage (10 V);

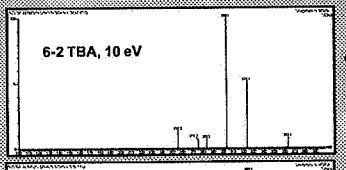
Internal Standard

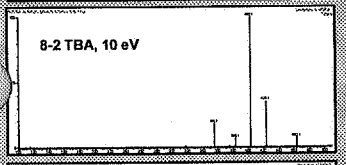


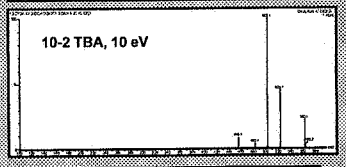
8-2 TBA by LC/MS

CID fragmentation

by LC/MS/MS







Fragmentation pattern for Telomer B Alcohols:
- loss of 4 HFs (4 x 20) and CO (28)

Chromatographic conditions:

Instrument: Agilent, HP 1100
Column: Xterra MS C18, 2.1 x 30 mm, 3.5 µm
Mobile phase: A: water B: methanol
Gradient:

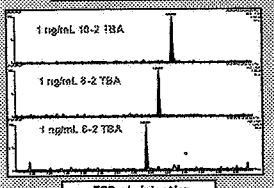
Time [min]	% B	Flow [mL/min]
0	50	0.25
0.1	50	0.25
0.14	600	0.35
7.4 (10.0)	100	0.25
7.1 (10.1)	50	0.25

* 500 µL injection

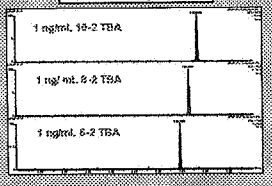
MS conditions:

Instrument: Quattro Micro; Waters
Ionization: electrospray, negative ions
Electrospray Capillary: 3.0 kV
Cone voltage: 10 V
Collision Energy: 12 eV
Source Temp.: 120 °C
Desolvation Temp.: 300 °C
Transitions: 6-2 TBA: 363 > 303
4-2 TBA: 463 >443
10-2 TBA: 563 > 503
IS, 6-2 TBA: 467 >405

50 µL injection



500 µL injection



Summary:

- detection and separation of Telomer B Alcohols by LC/MS/MS was demonstrated
- linear calibration curves were obtained for three examined TBAs: 0.5-100 ng/mL (50 µL injection) and 0.05-1.0 ng/mL (500 µL injection) for samples prepared in 50/50 % water/methanol;
- 50 ppt LOQs are achievable for examined TBAs in aqueous matrix by LC/MS/MS with 500 µL injection ;
- further validation of LC/MS/MS method for TBAs in authentic matrices is required;

Chromatographic conditions:

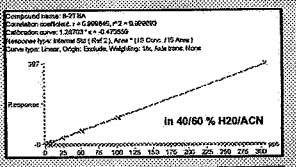
Instrument: Waters, Model 2795
Column: Luna 3u C18 (2), 20 x 2 mm
Injection volume: 20 µL
Mobile phase: A: water B: methanol
Gradient:

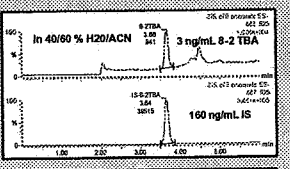
Time [min]	% B	Flow [mL/min]
0	70	0.25
1.0	70	0.25
2.0	100	0.25
6.0	100	0.25
6.1	70	0.25

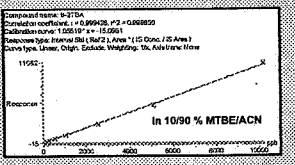
MS conditions:

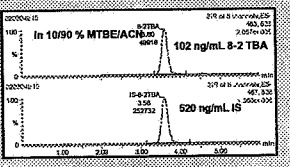
Instrument: ZQ; Waters
Ionization: electrospray, negative ions
Electrospray Capillary: 2.5 kV
Cone voltage: 16 V
Source Temp.: 120 °C
Desolvation Temp.: 500 °C
Ions monitored, 8-2 TBA: 563, 523
IS, 6-2 TBA: 467, 522

Example calibration curves and chromatograms









Summary:

- LC/MS methods for analysis of 8-2 TBA were designed to monitor signal of deprotonated molecular ion and acetate adduct;
- aqueous samples of 8-2 TBA can be quantitatively extracted with acetonitrile (1:2 water:ACN);
- samples can also be extracted with MTBE and MTBE extract injected after 1:10 dilution with acetonitrile;
- presented method allows injection of samples in 100% acetonitrile or 1:10 tetrahydrofuran (THF)/acetonitrile;
- ability to analyze 8-2 TBA by LC/MS allows simplification of sample preparation procedures and makes them compatible with those used for fluorinated acids analysis;
- ability to inject extracts originating from different solvents broadens the applicability of the method to different sample types (e.g. reduction of matrix effects for samples extracted with MTBE or analysis of polymers samples soluble in THF);
- linear calibration curves were obtained for 8-2 TBA ranging from 3 ng/mL to 10,000 ng/mL;
- sub-ng/mL LOQ for 8-2 TBA quantitation is achievable using LC/MS system (20 µL injection);

Perfluorinated acids by LC/MS/MS

Introduction:

- the following perfluorinated acids were investigated: perfluorooctanoic acid (PFOA), perfluorohexanoic acid (PFHA), 2-perfluorooctyl-2-ethanoic acid (2-PFOEA), 2H-hexadecafluoro-2-decenoic acid (2H-HDF-2-DA);
- mass spectrometric conditions were optimized for detection of perfluorinated acids;
- two different chromatographic methods were investigated;
- three different extraction procedure from bacterial culture were explored;

Methods

Method I:

Instrument: Waters 2795 and Quattro Micro

Column: Luna C18(2), 2.0 mm x 20 mm, 3 µm

Mobile phase:
A: 2 mM ammonium acetate in water
B: methanol

Gradient: Time [min]	% B	Flow [mL/min]
0	10	0.3
0.5	10	0.3
1.5	100	0.3
4.5	100	0.3
4.6	10	0.4
7	10	0.3

Injection volume: 10 µl

Method II:

Instrument: Waters 2795 and Quattro Micro

Column: Zorbax Rx-C8 2.1 mm x 150 mm, 5 µm

Mobile phase:
A: 0.15% (v/v) acetic acid in water
B: acetonitrile

Gradient: Time [min]	% B	Flow [mL/min]
0	5	0.4
0.8	5	0.4
1.0	80	0.4
5.0	80	0.4
5.1	5	0.4
9.5	5	0.4

Injection volume: 10 µl

MS conditions:

2-PFOEA, 477 > 393, CV 10 V, CE 20 eV
2H-HDF-2-DA, 457 > 393, CV 15 V, CE 15 eV
2H-HDF-2-DA, 457 > 393, CV 15 V, CE 15 eV
PFHA, 313 > 269, CV 15 V, CE 15 eV

Extraction

Sensitivity

Extraction methods:

Matrix: bacterial culture in E2BSMYE (0.1% YE) medium

1. Liquid-Liquid Extraction
   - acidify with H_2SO_4, extraction with MTBE (1:1, v/v, shaking 4 hr)
   - solvent-exchange to methanol

2. Ion-Pair Liquid Liquid Extraction
   - add 0.14M Na_2CO_3 pH 10 buffer
   - add 0.14M tetrabutyl ammonium hydrogensulfate (TBA)
   - extract 3x with MTBE (1.61:1, v/v, shaking 2 and 2x 1 hr)
   - solvent exchange to methanol (concentrate 6x)

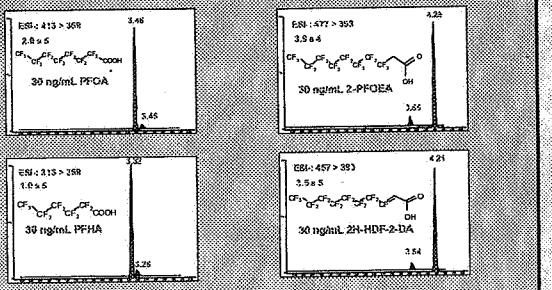
3. Protein Precipitation
   - denature with acetonitrile (1:1, v/v)
   - centrifuge, analyze

Comparison of extraction methods

Analysis using Method I

	% Recovery			
1. Liquid-Liquid Extraction	PFHA	PFOA	2H-HDF-2-DA	2-PFOEA
Samples, 200 ppb				
Day 0 (initial cell density), n = 6	61.8 ± 2.5	65.1 ± 4.3	63.7 ± 5.7	69.5 ± 8.3
Day 3 (full cell density), n = 3	65.9 ± 7.4	75.0 ± 0.2	33.9 ± 6.4	35.8 ± 5.3
Matrix QC, n = 4	108.3 ± 1.7	104.9 ± 2.7	100.5 ± 1.2	102.3 ± 5.1
Solvent QC, n = 2	107.7 ± 1.6	101.5 ± 2.8	97.4 ± 1.8	102.2 ± 7.3
Instrument QC (10-50 ppb), n = 12)	98.7 ± 1.7	97.9 ± 2.7	100.0 ± 2.2	97.0 ± 2.9
2. Ion-Pair Liquid-Liquid Extraction				
Samples, 200ppb				
Day 0 (initial cell density), n = 6	95.0 ± 4.7	93.0 ± 4.9	72.5 ± 4.4	76.2 ± 3.5
Matrix QC, n = 2	91.7 ± 1.0	104.3 ± 3.4	104.9 ± 2.5	98.4 ± 2.9
Solvent QC, n = 1	91.4	103.1	90.2	91.9
Instrument QC (10-50 ppb), n = 4	98.8 ± 2.8	100.4 ± 1.3	101.2 ± 7.0	99.8 ± 3.0
3. Protein Precipitation				
Samples				
Day 0, 200 ppb, n = 12	100.0 ± 3.1	102.6 ± 2.0	99.3 ± 2.9	95.6 ± 4.1
Day 0, 60 ppb, n = 6	93.1 ± 2.0	106.5 ± 2.3	102.7 ± 2.7	99.8 ± 5.8
Day 3, 200 ppb, n = 12	99.4 ± 9.4	102.1 ± 2.3	106.2 ± 2.5	103.4 ± 2.7
Day 3, 60 ppb, n = 6	93.8 ± 5.8	103.5 ± 5.0	99.3 ± 3.6	96.3 ± 5.8
Solvent QC				
200 ppb, n = 12	98.4 ± 3.6	100.1 ± 3.9	101.6 ± 3.3	99.1 ± 4.5
60 ppb, n = 12	99.5 ± 2.8	101.2 ± 2.4	103.3 ± 2.4	101.8 ± 3.7
Instrument QC (10-50 ppb), n = 20	98.3 ± 4.5	100.3 ± 3.9	101.0 ± 3.9	101.4 ± 5.5

Sensitivity difference: Method I vs. Method II



Summary:

- sensitivity of LC/MS/MS analysis of fluorinated acids can be significantly improved by appropriate selection of mobile phase. Approximately, 10 time increase of signal was observed when acetic acid/acetonitrile mobile phase was used vs. traditional ammonium acetate/methanol mobile phase;
- sensitivity observed for 2-PFOEA is approximately 10 times smaller than that observed for other acids investigated;
- better linearity of calibration curves was observed for Method II;
- Method II exhibited positive matrix effects for extracts obtained from protein precipitation extraction;
- extraction procedures: simple protein precipitation was most appropriate for all investigated fluorinated acids; MTBE extraction with either sample acidification or ion paring reagent did not work well for 2-PFOEA and 2H-HDF-2-DA;