Postprint of: Kubica P., Garraud H., Szpunar J., Lobinski R., Sensitive simultaneous determination of 19 fluorobenzoic acids in saline waters by solid-phase extraction and liquid chromatography–tandem mass spectrometry, Journal of Chromatography A, Vol. 1417, (2015), pp. 30-40, DOI: 10.1016/j.chroma.2015.09.024

Sensitive simultaneous determination of 19 fluorobenzoic acids in saline waters by solid-phase extraction and LC-MS/MS

Pawel Kubica,[a] Joanna Szpunar[b] and Ryszard Lobinski[b]

a *Department of Analytical Chemistry, Faculty of Chemistry, Gdansk University of Technology, 11/12 Narutowicza Str, Gdańsk, Poland*

b *CNRS/UPPA, Laboratoire de Chimie Analytique Bio-inorganique et Environnement (LCABIE-IPREM), Hélioparc, 2, av. Pr. Angot, 64053 Pau, France*

Abstract

A solid-phase extraction (SPE) procedure using a C_{18} stationary phase was optimized for preconcentration of 19 fluorinated derivatives of benzoic acid (FBA): mono- (3), di-(6), tri-(5) and tetra(1) fluorosubstituted in the ring, trifluoromethylbenzoic acid (5) and 3,5-bistrifluoromethyl benzoic acid from undiluted salt-rich (>20%) reservoir waters. Quantitative (>95%) retention/elution of 16 out of 19 analyte compounds was achieved allowing a 4-fold preconcentration factor accompanied by the elimination of >99% of salt. For the most polar three compounds: 2,6-dFBA, 2,3,6-tFBA and 2,4,6-tFBA the non-quantitative recoveries (>70%) were corrected by dedicated custom-synthesized deuterated internal standards. The FBAs were determined by HPLC - MS/MS revisited in terms of choice of column, elution conditions and MS/MS signal acquisition parameters allowing the baseline separation and a gain in sensitivity. For a sample intake of 4 mL, detection limits for all the compounds in a reservoir water sample containing more than 20% salt were between 0.01 and 0.05 ng/ml which represents a gain of a factor of 10-20 in comparison with the state-of the art HPLC-MS/MS procedures for samples of similar complexity.

Introduction:

Derivatives of benzoic acid with one or more fluorine atoms or one or more trifluoromethyl groups attached to the aromatic ring are the most common currently used non-radioactive passive water tracers for oil field applications {Serres-Piole, 2011 #1}. As a tracing campaign involves a set of several different compounds (out of more than 20 commercially available), there is a need for methods for their simultaneous determination in an oil reservoir water matrix. The low detection limits are critical as they determine the quantity of the tracers necessary to be used and thus the cost and environmental impact of the campaign. The matrix differs depending on the sample origin but it is usually rich in salts (reaching in some cases up to 30%) and organic constituents

The lowest detection limits (down to 0.01 ng/ml) were obtained by gas chromatography (GC) - MS but lengthy (24 h) and tedious sample preparation procedures including matrix removal and

derivatization were necessary{Müller, 2012 #6}. The incomplete and strongly compound-dependent yields required compound specific isotope dilution calibration that was proposed for several species to achieve accurate analysis. {Müller, 2014 #3}. {Müller, 2014 #4};

The alternative is the use of HPLC - MS/MS analysis to eliminate he derivatization step and thus to simplify the sample processing. The original work {Juhler, 2002 #7 } did not show any chromatogram, reported fairly high detection limits: 0.5-1 ng/ml for electrospray ionisation (ESI) and 10-20 ng/ml for atmospheric pressure chemical ionization (APCI), respectively, and was applied to simple matrices. The detection limits were considerably (ca. an order of magnitude) decreased by Serres-Pioles *at al.* {Serres-Piole, 2011 #1} with notable exception for tFBA for which hardly any improvement was observed). The maximum tolerated salt content did not exceed 1% which required considerable dilution of sample (10-20-times) drastically limiting the scope of method application.

Although the reported selectivity of HPLC separation of a set of 20 tracers usually studied was generally high, the baseline separation of all of them was not achieved in any of the published works {Isemura, 2009 #8;Juhler, 2002 #7;Müller, 2012 #5;Müller, 2012 #6;Müller, 2014 #3;Müller, 2014 #4;Serres-Piole, 2011 #2}. This caveat was compensated by the determination of the co-eluting compounds using different fragmentation reactions. On the other hand, the number of theoretical plates achieved in HPLC is important. Indeed, the poor specificity of fragmentation reactions (the loss of CO_2) used for quantification in combination with the unit resolution of quadrupole filter and matrix rich in organic acids may lead to the increase in baseline and false positives.

The above reasons spur the need for the development of methods allowing a considerable enrichment of FBAs with regard to salt and organic matrix. Solid phase extraction (SPE) is an attractive option for both matrix removal and analytes preconcentration {refs} prior to LC-MS/MS analysis of samples rich in salts. However, SPE of FBAs from reservoir water is a difficult task taking into account their high polarity and similarity to the organic matrix. The problems result, on one hand, from the difficulty to trap quantitatively simultaneously all the analytes while avoiding the retention of the matrix and, on the other hand, to release the trapped analytes quantitatively without substantial dilution. Another critical factor is the sample volume to be used for analysis as it determines the SPE time.

As a result of an extensive optimization study, Müller *et al.* reported fairly satisfactory recoveries (between 71% (2,5-dFBA) and 94 % (3-FBA)] for tap water {Müller, 2012 #5} but for reservoir waters the extraction efficiencies were very compound-dependent with the values between 18 (2,3,5,6-tetraFBA and 2,6-dFBA) and 90 % (2,4-dFBA) {Müller, 2012 #6}. Moreover, relatively large sample volumes (100 ml) processed {Müller, 2012 #5;Müller, 2012 #6} resulted in long analysis times. The recovery problems were partly (for 2 compounds: 26dFBA and 246tFBA) addressed by the use of custom synthetized deuterated internal standards {Müller, 2014 #4} {Müller, 2014 #3}.

The main goal of this work was the development of a rapid (small sample volume) SPE method allowing a direct multi-tracer (19 compounds) analysis in salt-rich (>20% salt) reservoir water

samples with an objective to reach at least an order of magnitude in terms of detection limits over the direct injection procedure {Serres-Piole, 2011 #1}. Additionally; an increase in the selectivity was investigated by reoptimisation of chromatographic conditions and probing the MS/MS mode using quadrupole-time-of-flight (QTOF) technology instead of triple quadrupole (QqQ).

Experimental conditions

Samples. Reservoir water samples of different origin with salt content ranging from 100 to more than 200 g/l were analyzed. The samples were stored at 4°C.

Reagents and standards. Acetonitrile, acetic acid, tetrahydrofuran, ammonia were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Ultrapure water (18 MΩ.cm) was obtained from a Milli-Q system (Millipore, Bedford, MA). The characteristics of the FBA standards used in this study are listed in **Table 1.** Deuterated 2,6-dFBA and 2,4,6-tFBA were a gift from Dr. K. Müller and Prof. Dr. A. Seubert. 4-fluorobenzoic acid-α-^{13}C-2,3,5,6-d4 was purchased from Sigma Aldrich (St. Louis, USA).

Materials. The SPE disposable cartridges (C_{18}, 500 mg, 3 mL) were supplied by Supelco (St. Louis, USA). Separations were carried out using an Acquity UPLC BEH C_{18} column, 150 mm x 2.1 mm x1.7 µm, Waters, Milford, MA) with a matching Vanguard precolumn.

Instrumentation. SPE was carried out using a Visiprep DL24 system from Supelco. Eluates were evaporated to dryness using an Eppendorf Concentrator Plus (Hauppauge, USA). An Acquity UPLC system (Waters) including a binary solvent pump, a cooled autosampler and a column oven was used. The detector was a XevoTQ (quadrupole-T-wave-quadrupole) MS with an orthogonal Z-spray-electrospray interface (Waters).

Procedures

Initial sample preparation procedure. Samples were filtered through 0.2 µm (13mm) syringe filter, GHP Acrodisc, Interchim, France). 4-fluorobenzoic acid-α-^{13}C-2,3,5,6-d_4) was added at 20 ng/mL. Deuterated 2,6-dFBA and 2,4,6-tFBA were added at 20 ng/ml if the corresponding compounds were to be determined.

Solid-phase extraction. The SPE cartridges were conditioned with two successive 2 ml volumes of acetonitrile followed by rinsing with two successive 2-ml volumes of water. Then, sample was loaded as two successive 2-mL aliquots. After loading of the sample, the sorbent was rinsed with a 2-mL volume of water to remove remaining salt and polar compounds. The cartridge was dried for 3 min under the stream of air. Then, the elution was performed with two successive 2-ml volumes of acetonitrile : 10% NH_4OH (8:2 v/v) (clarify the components of the mixture). The first portion of the eluting solvent was kept for 3 min to facilitate the desorption of analytes. The eluate was

collected and evaporated to dryness under vacuum (how long?). The residue was dissolved in 10% (*v/v*) acetonitrile.

Measurement conditions. A 50 µl aliquot was analyzed by HPLC - MS/MS. Mobile phase was composed by mixing 0.05% CH_3COOH (A) and 0.05% CH_3COOH in acetonitrile (B). The elution gradient was: 0 min (13% B), 1.3 min (13% B), 9 min (28 % B) and 13 min (80 % B). The column was equilibrated for 5 min. The flow rate was 0.45 ml/min, the column temperature was 45°C and the autosampler temperature was 5°C. MS/MS data acquisition was performed with the electrospray source operating in negative mode (ESI_{neg}) under the MRM conditions listed in **Table 2.**

Calibration. A calibration curve was constructed by plotting peak area for 7 concentrations (0.05, 0.1, 0.2, 0.5, 1, 10, 20 ng/ml).

Data processing. The Masslynx software (Waters, Milford, MA) was used to process data.

Results and discussion

LC - MS/MS determination of FBAs

The separation methods reported in the literature were based on isocratic elution in ion-chromatography {Müller, 2012 #5} or C_{18} reversed phase chromatography {Juhler, 2002 #7}. An improved selectivity in reversed-phase HPLC was obtained by gradient elution with slightly acidic methanol or acetonitrile {Serres-Piole, 2011 #2}. The latter procedure was the starting point for the optimization of the HPLC separation conditions in this work. In order to obtain the baseline separation and to reduce the co-elution with matrix components, it was decided to increase the length of the column. It allowed to triple the number of theoretical plates in comparison with the former work {Serres-Piole, 2011 #2} and to achieve the baseline separation of all the 19 FBAs within 13 min as shown in **Fig. 1.**

The calibration curves showed good linearity (r2 >0.999) and precision (n=3) below 3% (?) (*cf*. **Table 1** Supplementary Information). The detection limits calculated as 3x standard deviation of blank integrated at the corresponding retention times and the corresponding SRM divided by the slope of the calibration curve are summarized in **Table 3**. In the absence of the sample matrix, the LODs result from the ionization efficiency (strongly dependent on the changes in the organic modifier content during the elution gradient) and the chromatographic peak shape. The latter is not, however, a limiting factor in this work because UPLC signals are very sharp with the average peak width of **12**s. The low content of the organic modifier was likely to affect the ionization efficiency of the early eluting species (2,6-dFBA, 2,3,6-tFBA, 2,4,6tFBA and 2,3,4,5-tetraFBA) for which relatively high LODs are observed. Overall, the LODs compare favorably with those published elsewhere for LC-based methods. The most spectacular gain (10-fold) was obtained for the triFBA that are very sensitive to ionization conditions.

Table 3 also shows that the detection limits obtained for the triple quadrupole instrument compare favorably with QTOF of the similar generation operating in the MRM mode. However, it has to be admitted that the increased resolution of the TOF instruments is likely to increase confidence in the data close to the detection limits, eliminating false positives.

Optimisation of SPE conditions

Müller *at al*. {Müller, 2012 #6} published a comprehensive comparison study of five different SPE materials tested in a broad pH range (1-11); the best results were obtained for Oasis HLB-Plus (hydrophilic-lipophilic-balanced reversed-phase poly(divinylbenzene-co-N-vinylpyrrolidone sorbent) and Isolute ENV+ (hydroxylated polystyrene-divinylbenzene copolymer) at pH 3.4 and 1.5, respectively {Müller, 2012 #6}. Preliminary tests in these conditions for salt-rich reservoir waters produced very low (often 10-20%) and irreproducible recoveries. Also, the preliminary tests using Oasis HLB phase failed. Although they allowed high, quasi-quantitative recoveries of the analytes, the quantitative desorption of the latter turned out to be impossible. The most promising results were obtained with a C_{18} stationary phase that was similar to that of the column which was investigated in detail.

The optimization procedure included: (i) choice of the solvent for the initial conditioning step (acetonitrile or tetrahydrofuran); (ii) pH of the final condition step and sample (acidic, neutral, or alkaline); (iii) choice of the elution solvent (acetonitrile and tetrahydrofuran) and its pH. The conditions tested are summarized in **Table 4**. The results of the recoveries obtained during the optimization are summarized in **Fig. 2**.

A first hypothesis tested consisted in lowering pH to revert the dissociation of FBAs in order to increase their retention and then alkalize the solution for their elution. The acidification was initially carried out only during the conditioning step (1% acetic acid, pH 2.82) but the recoveries were lower than when the conditioning was carried out with water (*cf. e.g.* procedures IV and IX or X (procedure II). Hence, it was decided not to add acid neither during conditioning nor to the sample. Note that the recoveries in alkaline conditions (conditioning step and sample) (procedure III) were dramatically low (possibly also to the signal suppression because of the non-retained salt).

In terms of elution conditions, the use of ammonia resulted in recovery ratios of FBAs higher than 90% for most of the analytes. Two polar organic eluting solvents (ACN and THF) were tested together with ammonia. Recoveries from SPE procedures IX to XII were similar. Procedure X was chosen because the solution (8:2 organic/aqueous) was easier to evaporate than 5:5 organic/aqueous and acetonitrile was easier to evaporate than THF. Also, for 2,6-dFBA and 2,3,6-tFBA recoveries were significantly higher in comparison with other procedures.

Fig. 2. indicates that quantitative (>95%) recoveries (retention/elution) of 16 out of 19 analyte compounds were achieved from a salt-rich water matrix. The simultaneous elimination of >99% of

salt content and matrix simplification allowed an 4-fold preconcentration factor. For three compounds: 2,6-dFBA, 2,3,6-tFBA and 2,4,6-tFBA non-quantitative recoveries were observed.

The data in Fig. 2 was completed by verifying the recoveries from the pure water matrix by the method developed at three different concentration levels. The data are shown in **Table 5**. This systematic study shows that, *in fine*, only two compounds are problematic in terms of recoveries (2,6-dFBA, recovery ca. 50%) and 2,4,6-tFBA, recovery ca. 80%). It can be also concluded that the matrix does not practically affect the recoveries.

SPE - HPLC- MS/MS for the simultaneous multiple tracer analysis

Fig. 3. shows a chromatogram obtained for a concentration of 50 pg/ml spiked on a sample matrix containing 200 g/l of salt by the SPE method developed and the corresponding blanks. The concentration was chosen to correspond roughly to the detection limits of the procedure for water without preconcentration (instrumental detection limits). The figure clearly shows the peaks for all the compounds which exceed clearly the background demonstrating not only the absence of the need for sample dilution but also an effective preconcentration factor of up to 4 times resulting from the SPE preconcentration. The LODs are affected by the ionization efficiency (the degree of matrix removal and the content of acetonitrile at a given point of the chromatographic gradient), the peak shape and the baseline noise (again depending on the matrix).

The calibration curve data obtained for the procedure and the detection and quantification limits are summarized in **Table 6.** They confirm a 3-4-fold gain in detection limits resulting from the preconcentration factor in addition to the absence of the need of sample dilution prior to analysis.

Isotope dilution correction for the non quantitatively eluted compounds:

The recoveries most polar compounds 2,6-dFBA, 2,3,6-tFBA and 2,4,6-tFBA are not only non-quantitative but were also observed to vary by up to 30 % depending on the day and sample matrix. Therefore they have to be corrected for.

A convenient method proposed by Müller *et al.* [ref] is the use of deuterated standards The chromatograms **(Fig. 4**) show the perfect coelution of the doubly deuterated and non-deuterated standards which allows them to be measured in identical conditions. **Table 6** explains the benefits from the isotopically-labelled internal standard showing an efficient correction of the non-quantitative recoveries. Note that a single internal standard is enough to correct both of 2,3,6-tFBa and 2,4,6-tFBA recoveries as these compounds elute closely and share the reaction used for their quantification.

Validation of the method developed

In order to validate a method three synthetic samples containing all the tracers at the different concentration levles: 0.2, 1 and 10 ng/ml were prepared and analysed according to the procedure. The results shown in table 7 show consistent accuracies between 90-100% and precision between 2-5%.

Analysis of real samples: comparison with the direct analysis

The developed method was compared with the method based on the direct injection of diluted samples {Serres-Piole, 2011 #1}. The examples of chromatograms are shown in **Fig. 5.** The comparison shows an increase in sensitivity over at least an order of magnitude, allowing to detect peaks in the background not seen with the direct injection method, stabilize the baseline, and especially eliminate the false positives commonly encountered when integrating the peaks close to baseline. Note that the direct injection method developed elsewhere{ Serres-Piole, 2011 #1}w as slightly modified by diverting the chromatographic eluate off the detector for the first 15 s correct? to reduce the load of the salt on the column, as recently suggested by Bayen {Bayen, 2014 #10}.

Conclusions

The optimization of solid phase extraction allowed an efficient and straightforward simultaneous preconcentration of 19 fluorinated derivatives of benzoic acid commonly used as oil reservoir tracers. The simultaneous elimination of the salt eliminated the need for sample dilution allowing a gain of 10-20 in terms of detection limits in comparison with the figures of merit reported elsewhere in the literature for similar samples. The method uses a few ml of sample, is relatively rapid and can be readily automated.

References

Table 1. Standard compounds used in this study

Name	Abbreviation	Formula	Purity [%]	Supplier	Mass
2-fluorobenzoic acid	2-FBA	$C_7H_5O_2F$	99	Across Organics	140.11
3-fluorobenzoic acid	3-FBA	$C_7H_5O_2F$	99	Across Organics	140.11
4-fluorobenzoic acid	4-FBA	$C_7H_5O_2F$	98	Sigma-Aldrich	140.11
2,6-difluorobenzoic acid	2,6-dFBA	$C_7H_4O_2F_2$	98	Across Organics	158.10
2,5-difluorobenzoic acid	2,5-dFBA	$C_7H_4O_2F_2$	98	Across Organics	158.10
2,3-difluorobenzoic acid	2,3-dFBA	$C_7H_4O_2F_2$	98	Sigma-Aldrich	158.10
2,4-difluorobenzoic acid	2,4-dFBA	$C_7H_4O_2F_2$	99	Across Organics	158.10
3,5-difluorobenzoic acid	3,5-dFBA	$C_7H_4O_2F_2$	97	Sigma-Aldrich	158.10
3,4- difluorobenzoic acid	3,4-dFBA	$C_7H_4O_2F_2$	99	Across Organics	158.10
2,3,6-trifluorobenzoic acid	2,3,6-tFBA	$C_7H_3O_2F_3$	99	Sigma-Aldrich	176.10
2,4,6-trifluorobenzoic acid	2,4,6-tFBA	$C_7H_3O_2F_3$	98	Sigma-Aldrich	176.10
2,4,5-trifluorobenzoic acid	2,4,5-tFBA	$C_7H_3O_2F_3$	99.5	Across Organics	176.10
2,3,4-trifluorobenzoic acid	2,3,4-tFBA	$C_7H_3O_2F_3$	98	Sigma-Aldrich	176.10
3,4,5-trifluorobenzoic acid	3,4,5-tFBA	$C_7H_3O_2F_3$	98	Sigma-Aldrich	176.10
2-trifluoromethylbenzoic acid	2-tFmBA	$C_9H_5O_2F_3$	98	Across Organics	190.12
3-trifluoromethylbenzoic acid	3-tFmBA	$C_9H_5O_2F_3$	99	Sigma-Aldrich	190.12
4-trifluoromethylbenzoic acid	4-tFmBA	$C_9H_5O_2F_3$	98	Sigma-Aldrich	190.12
2,3,4,5-tetrafluorobenzoic acid	2,3,4,5-tetraFBA	$C_7H_2O_2F_4$	99	Sigma-Aldrich	194.08
3, 5-bis-trifluoromethylbenzoic acid	3,5-bisFmBA	$C_9H_4O_2F_6$	98	Sigma-Aldrich	258.12

Table 2. Reaction Monitoring parameters and operating parameters of ESI ion source

Name	Ion transition	Cone [V]	Collision [V]
2FBA	139.1 -> 95.0	22	12
3FBA		18	10
4FBA		22	12
IS (4-Fluorobenzoic acid-α-13C-2,3,5,6-d4)	144.0 -> 99.1	20	14
23dFBA	157.1 -> 113.0	14	12
24dFBA		16	10
26dFBA		10	8
25dFBA		14	10
34dFBA		20	14
35dFBA		14	10
26dFBA-d2	159.1 -> 115.1	14	11
234tFBA	175.1 -> 131.1	14	12
236tFBA		12	8
245tFBA		14	12
246tFBA		10	8
345tFBA		20	12
246tFBA-d2	177.1 -> 133.1	12	9
2tFmBA	189.2 -> 145.1	20	12
3tFmBA		26	14
4tFmBA		22	14
2345tetraFBA	193.2 -> 149.1	15	5
35bistFmBA	257.2 -> 213.1	22	16

Ion source parameters

Capillary [kV]	Desolvation temp. [˚C]	Cone gas [L/h]	Desolvation gas [L/h]
1.4	550	50	900

Table 3. HPLC-ESI MS/MS detection limits (ng/mL) for FBA tracers in water using deferent detection systems

Compound	**This method**	AB SCIEX TripleTOF® 6600*	Xevo TQ**	Xevo TQ***
2FBA	0.07	0.2	-	0,090
3FBA	0.09	0.2	0.086	0,150
4FBA	0.08	0.2	0.180	0,500
26dFBA*	0.20	0.2	-	0,003
2,5dFBA	0.05	0.2	0.068	0.500
2,3dFBA	0.03	2	0.02	0.050
2,4dFBA	0.03	0.2	0,023	0.090
3,5dFBA	0.04	0.2	0.022	0.035
3,4dFBA	0.06	0.2	0.020	0.040
2,3,6tFBA*	0.17	2	0.96	3
246tFBA*	0.13	0.2	-	0.300
2,4,5tFBA	0.02	0.2	0.650	1
2,3,4tFBA	0.03	2	0.31	0.500
3,4,5tFBA	0.03	0.2	0.29	0.900
2tFmBA	0.1	0.2	0.072	0.100
3tFmBA	0.1	0.2	0.030	0.039
4tFmBA	0.09	0.2	0.031	0.100
2,3,4,5tetraFBA	0.05	nd	0.24	0.700
3,5bisFmBA	0.04	nd	0.0004	0,003

** 10 µl injection, ACQUITY UPLC BEH C18 1.7 µm / 2.1 x 50 mm column*
***50 µl injection, ACQUITY UPLC BEH C18 1.7 µm / 2.1 x 50 mm column {*Serres-Piole, 2011 #2}
***15 µl *injection, ACQUITY UPLC BEH C18 1.7 µm / 2.1 x 50 mm column {*Serres-Piole, 2011 #2}
****Acquity UPLC® BEH Phenyl 2.1x50 mm 1.7µm

Table 4. Experimental conditions of the SPE procedures tested

	SPE I	SPE II	SPE III*	SPE IV	SPE V	SPE VI	SPE VII	SPE VIII	SPE IX	SPE X	SPE XI	SPE XII
Conditioning	2x2 mL ACN	2x2 mL ACN	2x2 mL ACN	2x2 mL ACN	2x2 mL ACN	2x2 mL ACN	2x2 mL THF	2x2 mL THF	2x2 mL ACN	2x2 mL ACN	2x2 mL THF	2x2 mL THF
	2x2 mL 1%AA	2x2 mL 1%AA	2x2 mL 1%NH_4OH	2x2 mL 1%AA	2x2 mL 1%AA	2x2 mL H_2O	2x2 mL H_2O	2x2 mL H_2O	2x2 mL H_2O	2x2 mL H_2O	2x2 mL H_2O	2x2 mL H_2O
Sample	4 mL	4 mL 1%AA	4 mL 1% NH_4OH	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL
Rinsing**	2 mL H_2O											
Drying in air stream**	4 min											
Elution**	2x2 mL ACN	2x2 mL ACN	-	2x2 mL ACN:1%NH_4OH (8:2)	2x2 mL ACN 1%AA	2x2 mL ACN:1%NH_4OH (8:2)	2x2ml THF:1%NH_4OH (8:2)	2x2 mL THF	2x2ml ACN:1%NH_4OH (5:5)	2x2ml ACN:10%NH_4OH (8:2)	2x2ml THF:1%NH_4OH (5:5)	2x2ml THF:10%NH_4OH (8:2)
Evaporation to dryness												
Dissolving of residue in 10% ACN												

* idea of the procedure was based on cleaning the sample without adsorption of analytes

** this step was omitted in case of SPE III

ACN – acetonitrile, AA – acetic acid, NH_4OH – ammonia, THF - tetrahydrofuran

Table 5. Recoveries of FBA standards from water samples by SPE in the optimal conditions (*cf.* Procedure) at the different concentration levels.

Compound	Recovery of 0.2 ng/mL, % (SD, n=3)	Recovery of 1ng/mL % (SD, n=3)	Recovery of 10 ng/mL % (SD, n=3)
2FBA	90 (2.7)	94 (3.4)	99 (3.4)
3FBA	95 (4.2)	96 (2.3)	102 (2.1)
4FBA	105 (4.9)	96 (4.5)	94 (3.7)
26dFBA*	52 (3.2)	51 (2.5)	49 (4.0)
25dFBA	99 (1.4)	98 (3.2)	96 (1.2)
23dFBA	94 (3.9)	103 (1.8)	98 (3.9)
24dFBA	96 (3.9)	98 (1.2)	104 (3.3)
35dFBA	93 (2.2)	90 (1.8)	90 (3.5)
34dFBA	95 (4.1)	93 (4.6)	92 (3.4)
236tFBA*	112 (2.9)	108 (3.5)	106 (4.1)
246tFBA*	76 (4.5)	82 (3.6)	84 (2.8)
245tFBA	97 (2.8)	101 (2.3)	103 (1.9)
234tFBA	96 (2.7)	102 (2.0)	97 (3.6)
345tFBA	93 (4.2)	95 (5.1)	93 (2.7)
2tFmBA	92 (3.4)	88 (3.7)	90 (2.0)
3tFmBA	87 (2.3)	92 (2.6)	89 (4.1)
4tFmBA	94 (3.0_	95 (2.5)	94 (4.0)
2345tetraFBA	98 (3.4)	103 (2.4)	108 (5.5)
35bisFmBA	94 (3.7)	100 (2.3)	101 (2.8)

, n - number of measurements

** early eluting compounds*

Table 6. Linearity, detection and quantification limits for the method developed applied to a reservoir water (>20% salt)

Name	Calibration curve equation for 1/x (8 points, n=3)	S_a	S_b	R^2	LOD [ng/mL]	LOQ [ng/mL]
2FBA	y=8804x - 70	40	75	0.9987	0.03	0.09
3FBA	y=16595x + 3262	104	162	0.9991	0.03	0.09
4FBA	y=12234x + 936	59	89	0.9988	0.02	0.06
26dFBA*	y=15951x + 540	168	187	0.9986	0.04	0.12
25dFBA	y=57762x + 2495	853	336	0.9998	0.02	0.06
23dFBA	y=34310x + 820	140	224	0.9986	0.02	0.06
24dFBA	y=53965x + 1117	251	311	0.9997	0.02	0.06
35dFBA	y=79825x + 3508	416	324	0.9999	0.01	0.03
34dFBA	y=69755x + 3231	877	287	0.9993	0.01	0.03
236tFBA*	y=6518x + 230	64	84	0.9984	0.04	0.12
246tFBA*	y=4986x – 65	11	55	0.9987	0.04	0.12
245tFBA	y=98181x + 3296	899	614	0.9995	0.02	0.06
234tFBA	y=91303x + 2057	1507	284	0.9991	0.01	0.03
345tFBA	y=115567x + 2969	1662	452	0.9989	0.01	0.03
2tFmBA	y=45379x + 6555	81	481	0.9997	0.03	0.09
3tFmBA	y=129965x + 5599	152	1021	0.9999	0.03	0.09
4tFmBA	y=95547x + 3384	265	841	0.9998	0.03	0.09
2345tetraFBA	y=8512x + 691	28	77	0.9998	0.03	0.09
35bisFmBA	y=129169x + 8247	955	755	0.9997	0.02	0.06

* early eluting compounds were quantified with their corresponding internal standards

S_a - standard deviation of the slope, S_b - standard deviation of the intercept, R^2 - coefficient of determination, LOD - limit of detection, LOQ - limit of quantitation, n - number of measurements

Table 7. Validation of the SPE-HPLC-MS/MS method developed for synthetic samples [blank reservoir water (ca. 20% salt) with FBA tracers spiked at 3 different concentrations].

Compound	Added [ng/ml]	Found [ng/mL] ± SD	Recovery [%]
2FBA	0.200	0.180 ± 0.005	90
	1	0.94 ± 0.03	94
	10	9.9 ± 0.3	99
3FBA	0.200	0.190 ± 0.008	95
	1	0.96 ± 0.02	96
	10	10.2 ± 0.2	102
4FBA	0.200	0.210 ± 0.009	105
	1	0.96 ± 0.05	96
	10	9.4 ± 0.4	94
26dFBA*	0.200	0.182 ± 0.007	91
	1	0.88 ± 0.04	88
	10	9.3 ± 0.4	93
25dFBA	0.200	0.198 ± 0.002	99
	1	0.98 ± 0.03	98
	10	9.6 ± 0.1	96
23dFBA	0.200	0.188 ± 0.007	94
	1	1.03 ± 0.02	103
	10	9.8 ± 0.4	98
24dFBA	0.200	0.192 ± 0.007	96
	1	0.98 ± 0.01	98
	10	10.4 ± 0.3	104
35dFBA	0.200	0.186 ± 0.004	93
	1	0.90 ± 0.02	90
	10	9.0 ± 0.4	90
34dFBA	0.200	0.190 ± 0.008	95
	1	0.93 ± 0.05	93
	10	9.2 ± 0.3	92
236tFBA	0.200	0.224 ± 0.005	112
	1	1.08 ± 0.04	108
	10	10.6 ± 0.4	106
246tFBA*	0.200	0.206 ± 0.005	103
	1	0.92 ± 0.06	92
	10	9.6 ± 0.4	96
245tFBA	0.200	0.194 ± 0.006	97
	1	1.01 ± 0.02	101
	10	10.3 ± 0.2	103
234tFBA	0.200	0.192 ± 0.005	96
	1	1.02 ± 0.02	102
	10	9.7 ± 0.4	97
345tFBA	0.200	0.186 ± 0.008	93
	1	0.95 ± 0.05	95
	10	9.3 ± 0.3	93
2tFmBA	0.200	0.184 ± 0.006	92
	1	0.88 ± 0.04	88
	10	9 ± 0.2	90
3tFmBA	0.200	0.174 ± 0.005	87
	1	0.92 ± 0.03	92
	10	8.9 ± 0.4	89
4tFmBA	0.200	0.188 ± 0.006	94
	1	0.95 ± 0.03	95
	10	10.4 ± 0.4	104
2345tetraFBA	0.200	0.196 ± 0.007	98
	1	1.03 ± 0.02	103
	10	10.8 ± 0.6	108
35bisFmBA	0.200	0.188 ± 0.007	94
	1	1.00 ± 0.02	100
	10	10.1 ± 0.3	101

* early eluting compounds were quantified with their corresponding internal standards

Captions to Figures

Figure 1. HPLC-MS/MS chromatograms obtained for 50 ng/mL standards.

a) 139-->95: 1) 2-fluorobenzoic acid, 2) 3-fluorobenzoic acid, 3) 4-fluorobenzoic acid;

b) 157-->113: 4) 2,6-difluorobenzoic acid, 5) 2,5-difluorobenzoic acid, 6) 2,3-difluorobenzoic acid, 7) 2,4-difluorobenzoic acid, 8) 3,5-difluorobenzoic acid, 9) 3,4-difluorobenzoic acid;

c) 175-->113: 10) 2,3,6-trifluorobenzoic acid, 11) 2,4,6-trifluorobenzoic acid, 12) 2,4,5-trifluorobenzoic acid, 13) 2,3,4-trifluorobenzoic acid, 14) 3,4,5-trifluorobenzoic acid;

d) 189-->145: 15) 2-(trifluoromethyl)benzoic acid, 16) 3-(trifluoromethyl)benzoic acid, 17) 4-(trifluoromethyl)benzoic acid;

e) 193-->149: 18) 2,3,4,5-tetrafluorobenzoic acid;

f) 257-->213: 19) 3,5-bis(trifluoromethyl)benzoic acid;

g) 144->99: 20) 4-Fluorobenzoic acid-α-13C-2,3,5,6-d4 (internal standard);

Figure 2. Recoveries obtained for the SPE procedures described in Table 4.

Figure 3. HPLC-MS/MS chromatograms of a reservoir water spiked with 50 pg/mL of each FBA (top chromatogram in each subfigure) and the corresponding blank (unspiked reservoir water) analysed by the developed procedure.

a) 139-->95: 1) 2-fluorobenzoic acid, 2) 3-fluorobenzoic acid, 3) 4-fluorobenzoic acid;

b) 157-->113: 4) 2,6-difluorobenzoic acid, 5) 2,5-difluorobenzoic acid, 6) 2,3-difluorobenzoic acid, 7) 2,4-difluorobenzoic acid, 8) 3,5-difluorobenzoic acid, 9) 3,4-difluorobenzoic acid;

c) 175-->113: 10) 2,3,6-trifluorobenzoic acid, 11) 2,4,6-trifluorobenzoic acid, 12) 2,4,5-trifluorobenzoic acid, 13) 2,3,4-trifluorobenzoic acid, 14) 3,4,5-trifluorobenzoic acid;

d) 189-->145: 15) 2-(trifluoromethyl)benzoic acid, 16) 3-(trifluoromethyl)benzoic acid, 17) 4-(trifluoromethyl)benzoic acid;

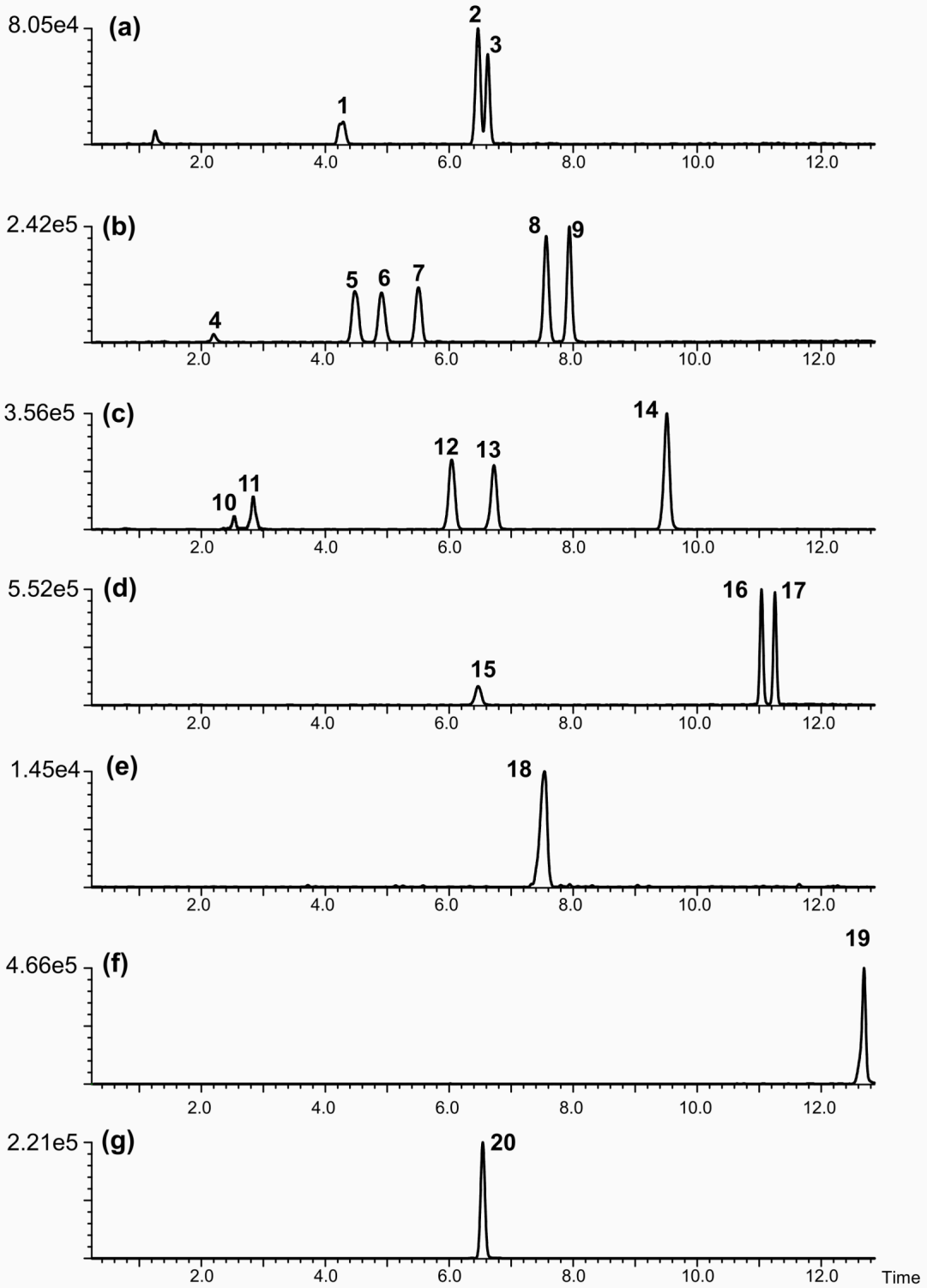
e) 193-->149: 18) 2,3,4,5-tetrafluorobenzoic acid;

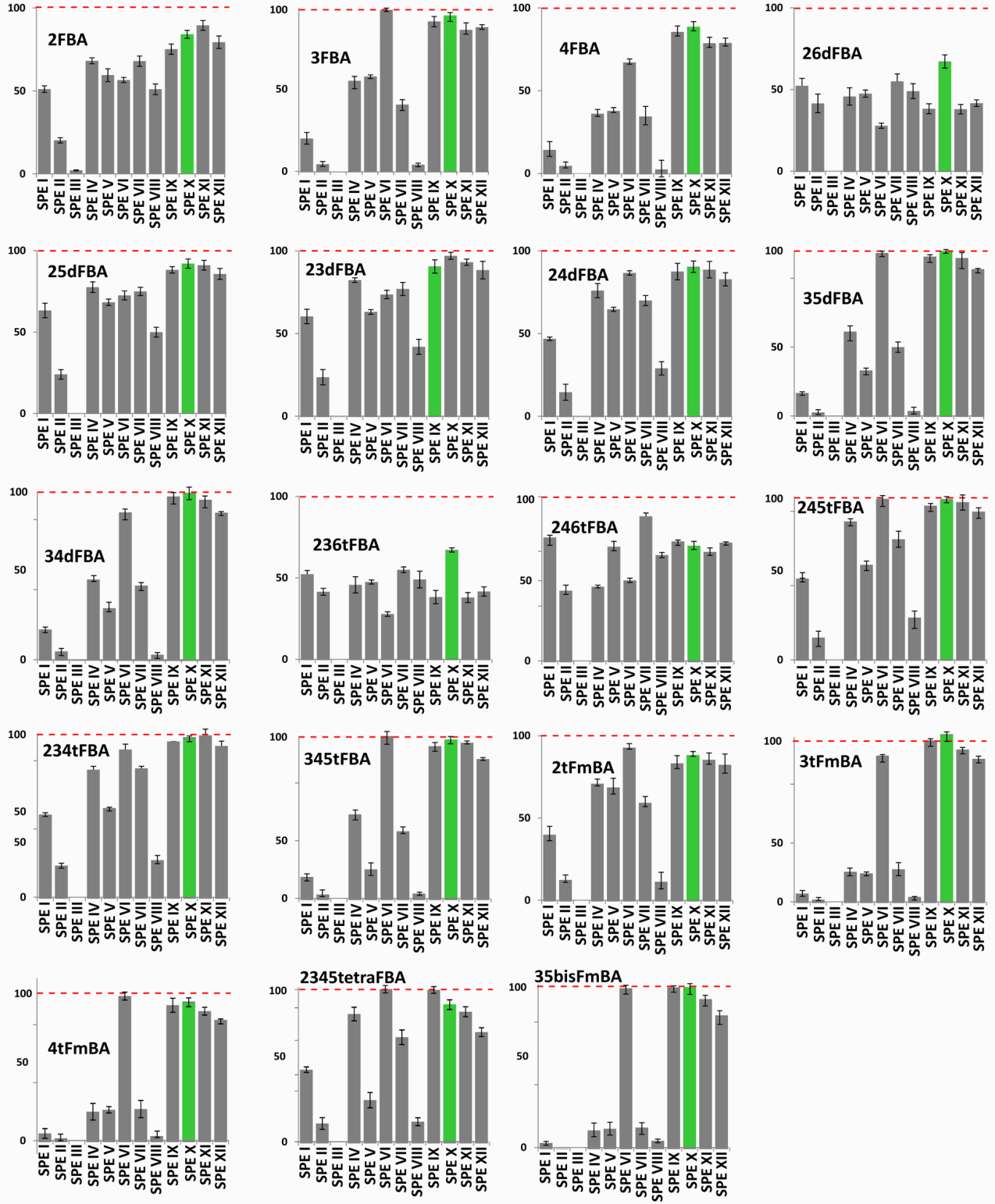
f) 257-->213: 19) 3,5-bis(trifluoromethyl)benzoic acid;

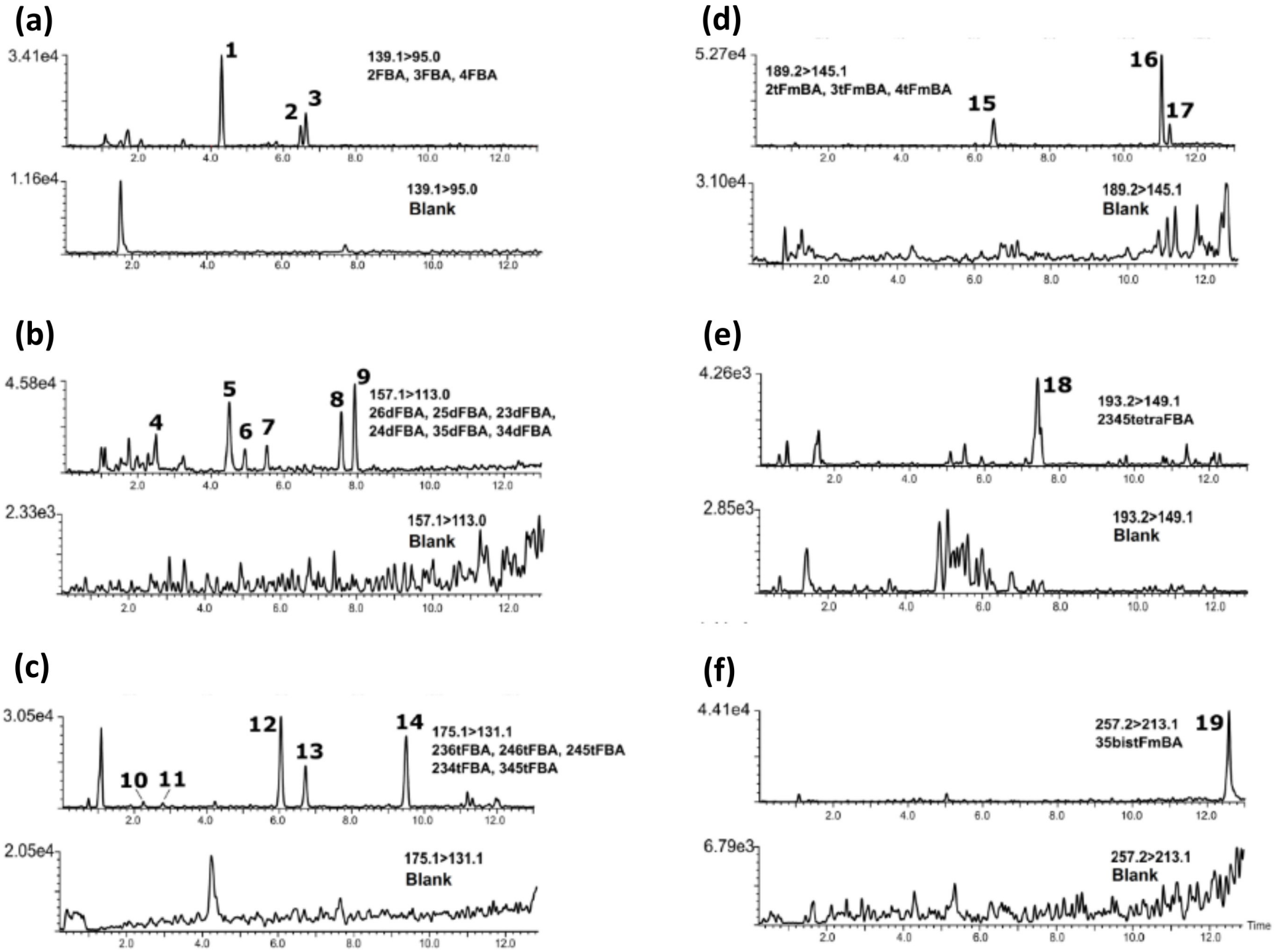
Figure 4. HPLC-MS/MS chromatograms early eluting compounds with specific internal standards:

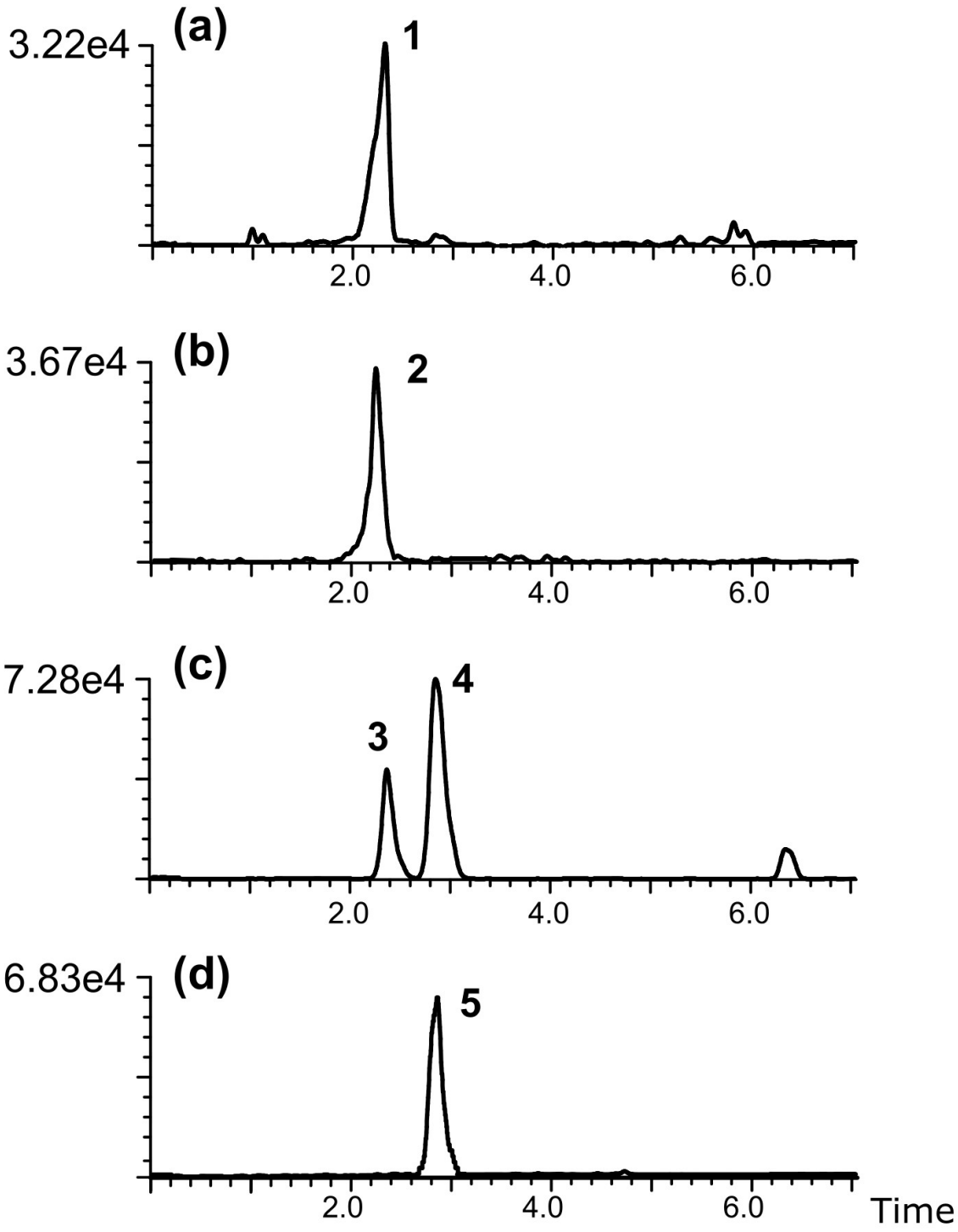
a) 157 --> 113: 1) 2,6- difluorobenzoic acid; b) 159-->115: 2) 2,6- difluorobenzoic acid - d_2; c) 177 --> 131: 3) 2,3,6-tFBA, 4) 2,4,6-TFBA; d) 177-->133: 5) 2,4,6-tFBA-d_2.

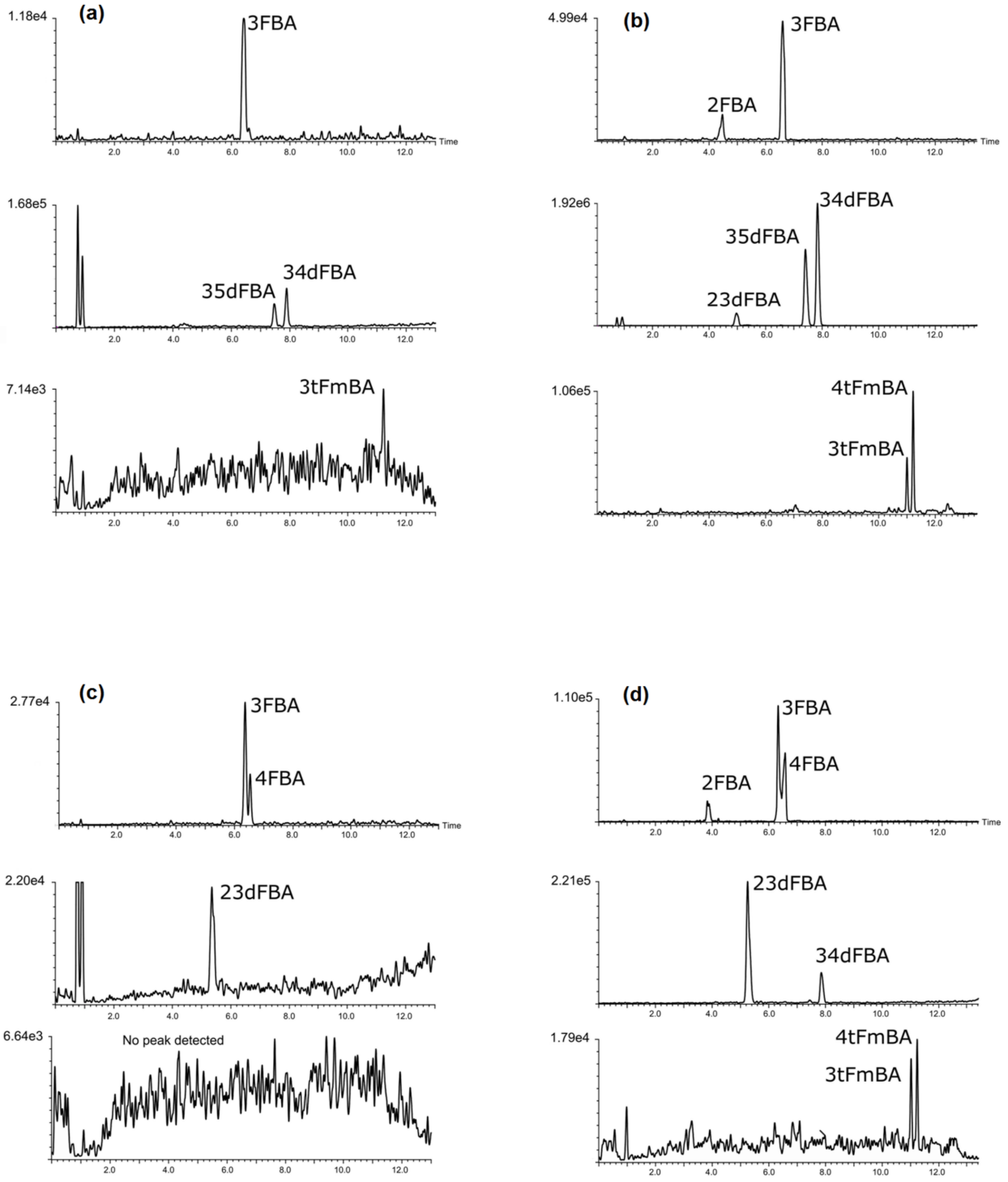
Figure 5. HPLC-MS/MS chromatograms of two (A and B) reservoir water samples. a,b - Sample A. c,d - Sample B. a,c- direct injection upon dilution (Ref. Coralie). b,d - analysed by the SPE-HPLC-MS/MS procedure developed.


8.05e4
(a)
1
2
3
2.42e5
(b)
4
5
6
7
8
9
3.56e5
(c)
10
11
12
13
14
5.52e5
(d)
15
16
17
1.45e4
(e)
18
4.66e5
(f)
19
2.21e5
(g)
20
2.0
4.0
6.0
8.0
10.0
12.0
Time


2FBA
3FBA
4FBA
26dFBA
25dFBA
23dFBA
24dFBA
35dFBA
34dFBA
236tFBA
246tFBA
245tFBA
234tFBA
345tFBA
2tFmBA
3tFmBA
4tFmBA
2345tetraFBA
35bisFmBA
100
50
0
SPE I
SPE II
SPE III
SPE IV
SPE V
SPE VI
SPE VII
SPE VIII
SPE IX
SPE X
SPE XI
SPE XII


(a)
3.41e4
1
2
3
139.1>95.0
2FBA, 3FBA, 4FBA
1.16e4
139.1>95.0
Blank
(b)
4.58e4
4
5
6
7
8
9
157.1>113.0
26dFBA, 25dFBA, 23dFBA,
24dFBA, 35dFBA, 34dFBA
2.33e3
157.1>113.0
Blank
(c)
3.05e4
10
11
12
13
14
175.1>131.1
236tFBA, 246tFBA, 245tFBA
234tFBA, 345tFBA
2.05e4
175.1>131.1
Blank
(d)
5.27e4
189.2>145.1
2tFmBA, 3tFmBA, 4tFmBA
15
16
17
3.10e4
189.2>145.1
Blank
(e)
4.26e3
18
193.2>149.1
2345tetraFBA
2.85e3
193.2>149.1
Blank
(f)
4.41e4
257.2>213.1
35bistFmBA
19
6.79e3
257.2>213.1
Blank
2.0
4.0
6.0
8.0
10.0
12.0
Time


3.22e4
(a)
1
2.0
4.0
6.0
3.67e4
(b)
2
2.0
4.0
6.0
7.28e4
(c)
3
4
2.0
4.0
6.0
6.83e4
(d)
5
2.0
4.0
6.0
Time


(a)
1.18e4
3FBA
Time
2.0
4.0
6.0
8.0
10.0
12.0
1.68e5
35dFBA
34dFBA
7.14e3
3tFmBA
(b)
4.99e4
3FBA
2FBA
1.92e6
34dFBA
35dFBA
23dFBA
1.06e5
4tFmBA
3tFmBA
(c)
2.77e4
3FBA
4FBA
2.20e4
23dFBA
6.64e3
No peak detected
(d)
1.10e5
3FBA
4FBA
2FBA
2.21e5
23dFBA
34dFBA
1.79e4
4tFmBA
3tFmBA