

Liquid Chromatography/ Mass Spectrometry

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Analysis of Fluorobenzoic Acids via UHPLC-MS/MS for Water Tracer Studies

Introduction

Fluorinated benzoic acids (FBAs), including a number of subgroups, are a distinct category of artificial, stable,

non-radioactive tracers that are commonly being used to investigate flow dynamics in geothermal, hydrothermal and oil well applications¹. In an oil field, FBAs are known to partition within the aqueous phase and can be monitored quantitatively between the injector and producer wells to enable quick operational decision making, with the goal of optimizing oil recovery².

With an increased need for oil water tracer measurements, new analytical methods are being developed to enhance the separation and detection of FBAs. Recent LC-MS/MS methods have been able to detect 0.05-50 ppb of FBA tracers in oil well water, the detectable limit depending on the subgroup³.

Herein, we present a reliable, rapid UHPLC-MS/MS method for the analysis of seventeen FBAs, using a reverse phase, C18 column. For this study, a suite of 14 FBAs tracers was analyzed. All tracers eluted within four minutes, with excellent chromatographic separation, reproducibility and resolution. Good linear fits were achieved for all FBA subgroups and the established LOQs (limits of detection) were in the ppt/ppb range, depending on the analyte.

Experimental

Hardware/Software

For the chromatographic separations, a PerkinElmer UHPLC System was used with a PerkinElmer QSight® 220 MS/MS detector. All instrument control, analysis and data processing was performed using the Simplicity 3Q™ software platform.

Method Parameters

The LC and MS/MS method/source parameters are shown in Tables 1, 2, and 3, respectively.

Table 1. LC method parameters.

Column:	Restek® Force 3.0 x 50 mm C18, 1.8 µm					
Mobile Phase	Solvent A: Water with 0.1% formic acid Solvent B: Acetonitrile with 0.1% formic acid	Step*	Time (min)	Flow rate (mL/min)	%A	%B
		1	Initial	0.85	95	5
		2	0.20	0.85	80	20
		3	1.80	0.85	60	40
		4	2.50	0.85	10	90
		5	3.20	0.85	10	90
		6	4.00	0.85	95	5
		7	7.50	0.85	95	5
		*All step solvent changes were linear				
Analysis Time						
Pressure						
Oven Temp.						
Injection Volume						

Table 2. MS/MS method parameters.

Experiment Group	FBA	Precursor Ion (m/z)	Product Ion(m/z)	CE (V)	CCL2 (V)	Dwell Time (ms)
0.00 – 1.62 min	2,6-dFBA	157.2	93.2	27	30	50
	2,6-dFBA**	157.2	113.2	35	37	50
	2,4,6-tFBA	175.2	111.2	39	45	50
	2,4,6-tFBA**	175.2	131.2	18	34	50
	2-FBA	139.3	95.2	35	23	50
	2-FBA**	139.3	75.2	37	30	50
1.60 – 2.18 min	2,5-dFBA	157.2	93.2	27	30	50
	2,5-dFBA**	157.2	113.2	35	37	50
	2,3-dFBA	157.2	93.2	27	30	50
	2,3-dFBA**	157.2	113.2	35	37	50
	3-FBA	139.3	95.2	35	23	50
	3-FBA**	139.3	75.2	37	30	50
	2,4,5-tFBA	175.2	111.2	39	45	50
	2,4,5-tFBA**	175.2	131.2	18	34	50
	2,3,4-tFBA	175.2	111.2	39	45	50
	2,3,4-tFBA**	175.2	131.2	18	34	50
2.00 – 2.44 min	2-tFmBA	189.3	145.2	22	33	50
2.22 – 4.00 min	2,3,4,5-tetraFBA	193.3	149.2	19	31	50
	2,3,4,5-tetraFBA**	193.3	173.0	20	38	50
	3,4,5-tFBA	175.2	111.2	39	45	50
	3,4,5-tFBA**	175.2	131.2	18	34	50
	3-tFmBA	189.3	145.2	22	33	50
	4-tFmBA	189.3	145.2	22	33	50
	3,5-bis-tFmBA	257.4	213.0	28	46	50

** Qualifier ion; CE (V) = Collision Energy; CCL2 (V) = Collision Cell Lens 2; EV (Entrance Voltage) was -25 for all.

Table 3. MS/MS source parameters.

Parameter	Setting
Ionization Mode	ESI, Negative
Drying Gas	120
HSID Temperature (°C)	300
Nebulizer Gas	200
Electrospray Voltage (V)	-4500
Source Temperature (°C)	410

Solvents and Standards

All solvents, reagents, and diluents used were HPLC-grade or better. For all dilutions, water:acetonitrile (7:3 v/v) was used.

The 17 FBA powder standards were all $\geq 98\%$ purity and were purchased from Apollo Scientific Ltd (Denton, Manchester, UK). The individual FBAs are shown in Table 4, along with their corresponding group, formula and nominal mass. All 1000-ppm FBA stock solutions were prepared using water:acetonitrile (7:3 v/v) and refrigerated prior to use.

Table 4. FBAs with corresponding group, formula and nominal mass.

FBA	FBA Subgroup	Formula	*Nominal Mass
2-FBA	monofluoro	C7H5O2F	140.1
3-FBA			
4-FBA			
2,3-dFBA	difluoro	C7H4O2F2	158.1
2,4-dFBA			
2,5-dFBA			
2,6-dFBA			
2,3,4-tFBA	trifluoro	C7H3O2F3	176.1
2,3,6-tFBA			
2,4,5-tFBA			
2,4,6-tFBA			
3,4,5-tFBA			
2-tFmBA	trifluoromethyl	C8H5O2F3	190.1
3-tFmBA			
4-tFmBA			
2,3,4,5-tetraFBA	tetrafluoro	C7H2O2F4	194.1
3,5-bis-tFmBA	bis-trifluoromethyl	C9H4O2F6	258.1

* Values obtained from the PubChem database.

0.01 g of each FBA was weighed and transferred into a separate 15 mL centrifuge tube. 10 mL of water:acetonitrile (7:3 v/v) was added and the contents were shaken vigorously, providing an individual stock concentration of 1,000 ppm for each analyte. For working standards, FBAs No. 1-11 (per Table 4) and 16 were diluted 100-fold (to 10 ppm), while FBAs No. 12-14 and 17 were diluted 1,000-fold (to 1 ppm), in order to compensate for the differences in the MS response. It should be noted that 3,4,5-tFBA was the exception in the trifluoro group, in that this analyte exhibited a greater response than the rest of the group.

To determine the retention time (RT) and MS response for each analyte, a 500- μ L aliquot of each working standard was transferred into a separate 2-mL glass vial, from which 4 μ L was injected.

For the chromatographic optimization and repeatability testing, a standard mix of 14 FBAs (excluding 2,3,6-tFBA; 2,4-dFBA and; 4-FBA) was prepared, using the 1000-ppm stock solutions and diluted with water:acetonitrile (7:3 v/v). This provided a 1-10 ppm standard mix, with analyte concentrations equal to those of the individual working standards, described above.

For calibrations, a 20-ppm 14 FBA standard mix was prepared from the individual 1000-ppm stock solutions. This stock standard was then serially diluted in 2-fold steps, to yield FBA calibrants in a range of 0.0488-10,000 ppb.

Results and Discussion

Chromatographic analysis of the seventeen individual FBAs showed that, as the retention time increased, so did the trend in MS response (Table 5). For the chromatographic separation of FBAs on a C18 column, the observed elution order under acidic mobile phase conditions was generally better than the predicted elution order, as shown by the logD values, corrected to pH 2.65. The less nonpolar FBAs eluted in 1.22-1.78 min, with a relatively lower response, while the intermediate to more nonpolar FBAs eluted in 1.84-2.86 min, with a relatively higher MS response. Of the seventeen FBAs, similar RTs were reported for the following pairs: 2,3,6-tFBA and 2,6-dFBA; 2,3-dFBA and 2,4-dFBA; 3-FBA and 4-FBA (as shown by the shaded pairs in Table 5).

For showing the chromatographic separation/repeatability and for calibration purposes, the 14 FBA standard mix excluded the 2,3,6-tFBA, 2,4-dFBA and 4-FBA, as these co-eluted with other FBAs (per shaded pairs in Table 5).

Table 5. Observed RT, predicted RT, concentration and MS response of individual FBAs.

FBA	Observed RT (min)	*LogD _{pH= 2.65}	Concentration (ppm)	MS Response (cps)
2,3,6-tFBA	1.22	1.47	10	8.2 E+03
2,6-dFBA	1.23	1.55	10	9.6 E+03
2,4,6-tFBA	1.42	1.78	10	1.5 E+04
2-FBA	1.51	1.69	10	9.2 E+03
2,5-dFBA	1.67	1.74	10	1.4 E+04
2,3-dFBA	1.76	1.75	10	1.6 E+04
2,4-dFBA	1.78	1.85	10	1.1 E+04
3-FBA	1.84	1.74	10	2.2 E+03
4-FBA	1.84	1.76	10	2.1 E+06
2,4,5-tFBA	1.95	1.94	10	3.8 E+05
2,3,4-tFBA	2.05	1.94	10	1.8 E+05
2-tFmBA	2.15	2.38	1	4.2 E+05
2,3,4,5-tetraFBA	2.29	1.99	10	5.8 E+05
3,4,5-tFBA	2.39	2.02	1	1.2 E+06
3-tFmBA	2.56	2.49	1	4.0 E+06
4-tFmBA	2.60	2.49	1	5.0 E+06
3,5-bis-tFmBA	2.86	3.36	1	1.5 E+07

* LogD values, based on pH 2.65, were calculated using MarvinSketch (ChemAxon). FBA pairs that showed similar observed RTs are shaded.

As shown in Figure 1 (A,B), good chromatographic separation of the 14 FBA standard mix (1-10 ppm, depending on analyte) was achieved in under 4 minutes. The relative MS response of 3-5-bis-tFmBA was clearly the strongest. The 14 individual FBA components were identified based on their observed RT when run separately.

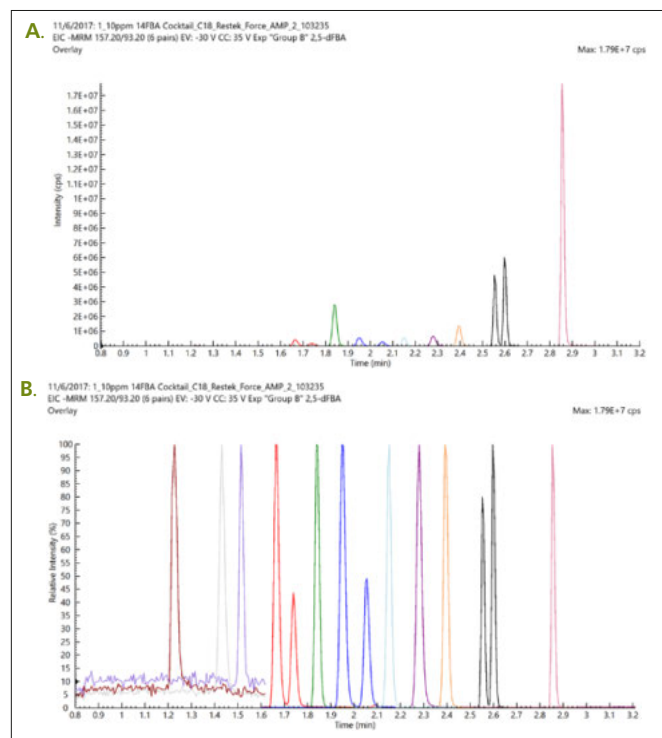


Figure 1. Overlaid MRM chromatograms of the 14 FBA standard suite (1-10 ppm). Panel A: The absolute intensity of quantifiers for the individual FBA components. Panel B: Normalized view, highlighting the chromatographic separation of the 14 analytes.

Per Figure 2, the chromatographic overlays of ten replicate injections of 14 FBA standard suite, demonstrated excellent reproducibility.

The linear-fits for each FBA class is shown in Figure 3 (A-F), represented by 3-FBA; 2,5-dFBA; 3,4,5-tFBA; 3-tFmBA; 2, 3, 4, 5-tetraFBA and; 3,5-bis-tFmBA. The R² values for all the FBAs were in the range of 0.991-0.999, demonstrating very good linearity.

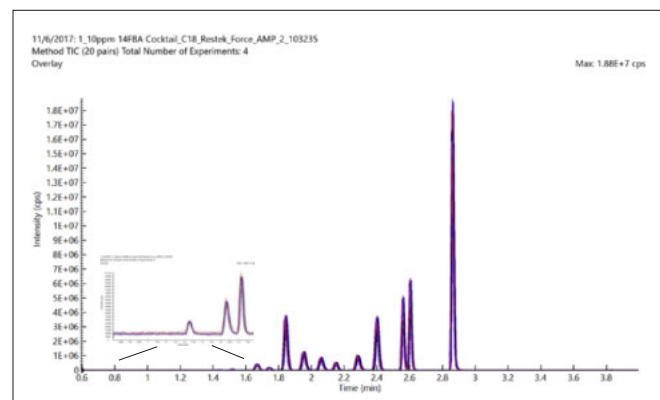


Figure 2. Overlay of ten replicate injections of the 14 FBA standard suite. Inset: Magnified view of the early eluting FBAs.

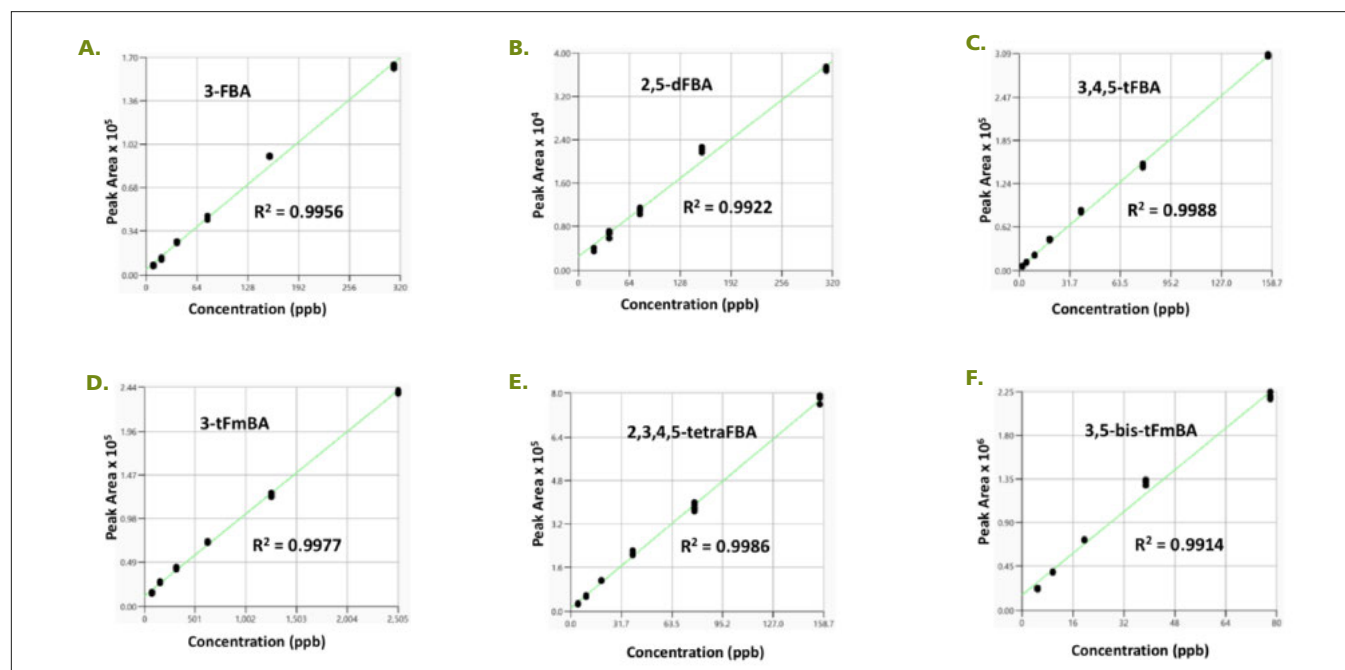


Figure 3. Linear-fit calibration curves for 3-FBA (A), 2,5-dFBA (B), 3,4,5-tFBA (C), 3-tFmBA (D) 2,3,4,5-tetraFBA (E) and 3,5-bis-tFmBA (F).

As shown in Table 6, the calculated LOQs (S/N = 10) for six FBAs, representing each of the six groups, ranged from 0.09-14.1 ppb. These were based on the low-level calibration standard.

Table 6. Calculated LOQs for representatives of the six FBA groups (based on the low-level calibration standard).

FBA Component	LOQ (ppb)
3-FBA	2.59
2,5-dFBA	14.07
3,4,5-tFBA	0.62
3-tFmBA	0.23
2,3,4,5-tetraFBA	10.89
3,5-bis-tFmBA	0.09

Conclusions

- A reverse phase LC-MS/MS method was developed for the analysis of a suite of fourteen FBAs, using a PerkinElmer UHPLC/QSight 220 MS/MS system.
- The described method, along with the selection of Restek® Force C18 column, provided exceptional chromatographic repeatability and peak resolution in under 4 minutes, with LOQs of 0.09-14.1 ppb, depending on analyte.
- Very good linear calibration fits were shown for the various FBA classes.

References

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2. Application of Radiotracer Techniques for Interwell Studies. IAEA Journal (2012). [online] <http://www-pub.iaea.org/MTCD/Publications/PDF/Pub1539_web.pdf>. (accessed November 14, 2017).
3. Serres-Piole C., Moradi-Tehrani N., Lobinski R. and Preud'homme H., Direct Sensitive Simultaneous Determination of Fluorinated Benzoic Acids in Oil Reservoir Waters by Ultra High-performance Liquid Chromatography-tandem Mass Spectrometry. J. Chromatogr. A 1218 (2011) 5872-5877.