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Analysing fluorobenzoate tracers in groundwater samples using liquid chromatography–tandem mass spectrometry A tool for leaching studies and hydrology

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Abstract

A sensitive LC–MS–MS method for the direct determination and quantification of 15 fluorobenzoic acids (FBAs) was developed. FBAs are used as conservative tracers for hydrological modelling of water flow and in studies of pesticides and other xenobiotic compounds. The use of FBAs is discussed in relation to other tracers (bromide, chloride, uranine). The method covers mono-substituted fluorobenzoic acid, difluorobenzoic acid, trifluorobenzoic acid, and tetrafluorobenzoic acid. The general detection limit in ground water was 1 μg/l using electrospray ionisation and 20 μg/l using atmospheric pressure chemical ionisation. Analysis time was less than 10 min, small sample volumes were needed and no clean-up was required.

Keywords: Water analysis; Environmental analysis; Fluorobenzoic acids; Benzoic acids; Organic acids

1. Introduction

Studying the fate of pesticides and other xenobiotics in soil and groundwater it is of foremost importance to have a reliable method for observing water movement in the soil. Several compounds have been used as conservative tracers [1], bromide being a classic example and fluorobenzoic acids (FBAs) have been introduced as an alternative [2]. The main advantage of using FBA tracers is the possibility of conducting several simultaneous leaching studies at the same location without tracer interference when employing specific detection of structural distinct FBAs. Additionally, the FBA tracers are well suited for multiple tracing experiments in fractured media were several conservative tracers are required.

From a study comparing the transport of 14 FBA isomers to a non-reactive tracer (Br^-) it has been found that FBAs can be very useful as non-reactive groundwater tracers. Thus, good performance was observed when only the matrix pH was approximately two pH units above the pK_a of the FBA used [3].

A reliable analytical method is required when using FBAs in hydrological studies. Previously the FBAs have been analysed using ion chromatography [4], HPLC–UV [5,6], and gas chromatography–mass spectrometry (GC–MS) analysis of derivatives [7,8]. However, using liquid chromatography–tandem mass spectrometry (LC–MS–MS) there is a potential for improving the sensitivity and specificity of the FBA analysis and the need for derivatisation procedures and labour-intensive clean-up steps can

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be eliminated. This is desirable, as frequent sampling is required to improve the description of the water movement.

The aim was to develop a method for fast, specific and sensitive quantification of fluorobenzoic acids that are used in hydrological studies. A LC–MS–MS method for the direct determination of FBAs was developed and the performance of the technique was demonstrated in a leaching study [9].

2. Material and methods

Details on the structure and purity of the fluorobenzoic acids standards are given in Table 1. All fluorobenzoic acids were purchased from ABCR (Karlsruhe, Germany) and all solvents were HPLC-grade (Romil, Cambridge, UK). The HPLC system consisted of a Waters Alliance 2690 (Waters, Milford, MA, USA) equipped with a 150×2 mm I.D. column packed with Luna 3 μm phenyl hexyl (Phenomenex, Torrance, CA, USA). All solvents were HPLC grade (Romil). Separation of FBA compounds was achieved under isocratic conditions using a solvent of methanol and diluted acetic acid (0.2%, v/v, in water). Total analysis time was less than 6 min for all compounds analysed. The injection volume was 10 μl, the flow rate was 0.2 ml/min and the column temperature was 25 °C. Detection and quantification were carried out using MS–MS as indicated in Table 2. Electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) mass spectrometry used a Micromass Quattro Ultima triple quadrupole mass spectrometer (Micromass, Manchester, UK). For negative mode ESI (ESI_{neg}), the ion source and desolvation temperatures were 120 and 250 °C, respectively. The flow rate for nitrogen desolvation gas was 440 l/h. For negative mode APCI ($APCI_{neg}$), the ion source and desolvation temperatures were 150 and 500 °C, respectively. The flow rates for nitrogen cone and desolvation gas were 100 and 500 l/h, respectively. Linear calibration curves were calculated by the linear least square method at five compound concentrations for ESI_{neg} (5, 25, 50, 100, and 200 μg/l) and $APCI_{neg}$ (32, 63, 125, 250, and 500 μg/l). For determination of detection limits (LODs) the standard deviations were estimated at 5.0 μg/l (ESI_{neg}) and 30 μg/l ($APCI_{neg}$) and calculated as $LOD = t_{(0.995,\ n=6)} \times s = 4.03 \times SD$. Further conditions for mass reaction monitoring using $APCI_{neg}$ and ESI_{neg} are shown in Table 2.

3. Results and discussion

A method was developed for the analysis of FBAs used as hydrological tracers. Typically, the FBAs chosen for hydrological studies are difluorobenzoic acids. However, it is desirable to use other groups

Table 1
Compounds included in the method with abbreviations used for each compound

Compound; purity (%, w/w)	CAS-No.	Abbreviation
2-Fluorobenzoic acid; 98%	[445-29-4]	2-FBA
3-Fluorobenzoic acid; 98%	[455-38-9]	3-FBA
4-Fluorobenzoic acid; 99%	[456-22-4]	4-FBA
2,3-Difluorobenzoic acid; 98%	[4519-39-5]	2,3-DFBA
2,4-Difluorobenzoic acid; 98%	[1583-58-0]	2,4-DFBA
2,5-Difluorobenzoic acid; 98%	[2991-28-8]	2,5-DFBA
2,6-Difluorobenzoic acid; 98%	[385-00-2]	2,6-DFBA
3,4-Difluorobenzoic acid; 98%	[455-86-7]	3,4-DFBA
3,5-Difluorobenzoic acid; 98%	[455-40-3]	3,5-DFBA
2,3,4-Trifluorobenzoic acid; 98%	[61079-72-9]	2,3,4-TFBA
2,3,6-Trifluorobenzoic acid; 98%	[2358-29-4]	2,3,6-TFBA
2,4,5-Trifluorobenzoic acid; 99%	[446-17-3]	2,4,5-TFBA
2,4,6-Trifluorobenzoic acid; 98%	[28314-80-9]	2,4,6-TFBA
3,4,5-Trifluorobenzoic acid; 98%	[121602-93-5]	3,4,5-TFBA
2,3,4,5-Tetrafluorobenzoic acid; 99%	[1201-31-6]	TeFBA

when the environmental conditions are agreeable (organic content, pH, etc.). For this reason, the method covers several classes of FBAs. The chromatography was carried out using a phenyl hexyl LC column and eluting with acidified methanol. Tandem mass spectrometry was used for identification and quantification of the tracers.

Analytical methods for quantification of FBAs have been published previously and ion chromatography for separation of fluorobenzoates [2] has been used in several studies. However, reversed-phase chromatography was chosen for the present study as use of ion chromatography and ionic buffers is less suitable for coupling with mass spectrometers due to the risk of salt formation in the inlet of the mass spectrometer.

Using RP-HPLC, a low pH is required for retention as the FBAs are small molecules having pK_a values less than 4. To favour non-ionised molecules, the optimal chromatography would be expected when using a solvent with pH one to two units below the pK_a of the FBA. However, due to possible redox reactions in the ion source, the pH may be lowered significantly at the interface [10]. Consequently it is not always possible to predict the optimal solvent pH for ionisation. Initially, the acid component of the eluent was optimised to give good retention and chromatography of the FBAs on the LC column. The ideal acid used for pH adjustment of the eluent in APCI- or ESI-MS–MS methods is volatile and its suppression of ionisation minimal. Three organic acids, trifluoroacetic acid, acetic acid, and formic acid were evaluated. Of these modifiers, the acetic acid is the most agreeable acid to work with, and a satisfactory retention was achieved. Optimising the solvent composition further it was observed that methanolic eluent gave a better sensitivity than when using ethyl acetate (results not shown). Using this combination of acetic acid and methanol in water the chromatographic retention of the compounds was characterised at three levels of organic modifier (Fig. 1). The FBAs could be separated within 6 min at

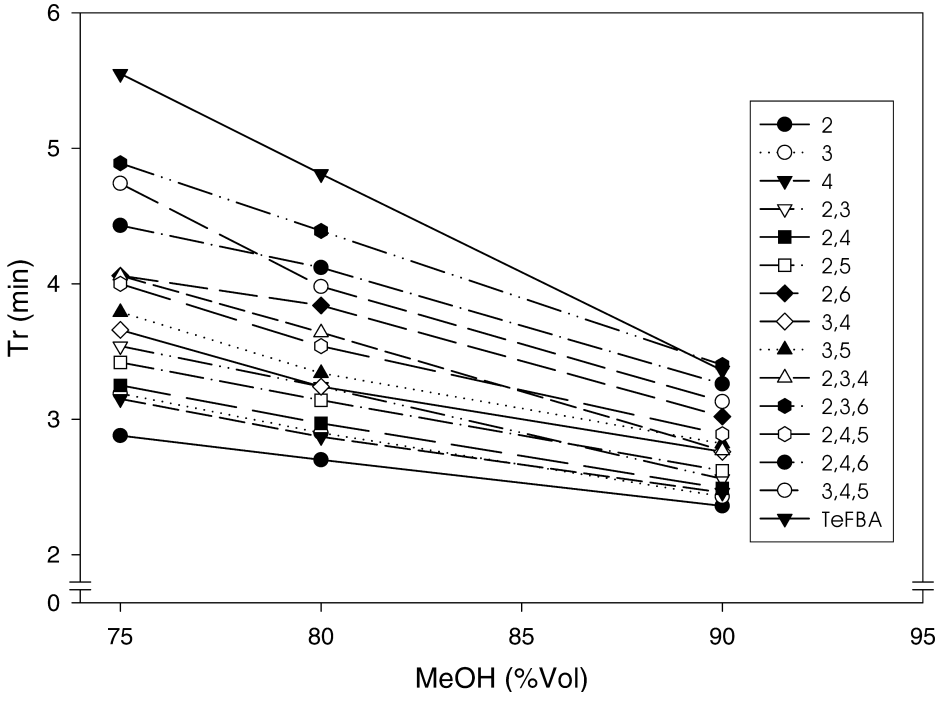

Fig. 1. Retention characteristics of FBAs using three different fractions of organic modifier in the eluent (LC conditions: methanol in diluted acetic acid, flow 0.2 ml/min, see text for details; Tr, retention time; numbers in the legend box refers to the substitution positions on the FBA).

Table 2
Conditions for $APCI_{neg}$ and ESI_{neg} and calibration data

Compound	P→F (m/z)	ESI_{neg}			$APCI_{neg}$		
		Settings (Ca/Co/Cl)	LOD (μg/l)	R^2	Settings (Ca, Io, Cl)	LOD (μg/l)	R^2
2-FBA	139→95	2.5/30/15	0.7	0.997	30/30/15	23	0.975
3-FBA	139→95	3.0/50/15	1.2	0.998	25/40/15	7	0.995
4-FBA	139→95	3.0/50/15	1.8	0.998	30/30/15	17	0.996
2,3-DFBA	157→113	2.8/20/10	1.1	0.999	25/30/10	10	0.996
2,4-DFBA	157→113	2.8/20/10	0.5	0.999	25/30/10	27	0.998
2,5-DFBA	157→113	3.5/25/10	1.5	0.986	25/30/15	16	0.998
2,6-DFBA	157→113	2.9/20/8	0.8	0.997	25/30/10	9	0.987
3,4-DFBA	157→113	3.0/35/15	0.2	0.999	25/30/15	7	0.997
3,5-DFBA	157→113	3.5/25/15	1.0	0.996	25/30/15	9	0.990
2,3,4-TFBA	175→131	2.5/10/10	0.7	1.000	30/30/10	13	0.999
2,3,6-TFBA	175→131	2.5/10/10	0.6	0.996	30/30/10	37	0.977
2,4,5-TFBA	175→131	2.5/20/10	0.2	0.998	30/30/10	18	0.998
2,4,6-TFBA	175→131	2.5/20/10	0.5	0.998	30/30/10	22	0.998
3,4,5-TFBA	175→131	2.5/20/15	0.3	0.993	30/30/15	15	0.998
TeFBA	193→149	2.5/25/10	0.1	0.986	25/20/10	NA	NA

The mass filter settings (m/z) for parent and fragment ions used for quantification (P→F), instrument settings (Ca, capillary/corona; Co, cone; Cl, collision, ionisation setting was 1.0), detection limits (LOD, 15 μl injected) and regression (R^2) of the calibration curve are shown.

75% methanol whereas at 90% methanol in the eluent the structural isomers started to coelute, but separation could still be obtained within groups of the FBAs.

The FBAs are measured by the (M-44) fragment. As this fragment is common to compounds within each group it is not possible to distinguish between isomers. Thus, if two or more isomers are used simultaneously they must be chromatographically resolved for quantification. Relating to the chromatographic behaviour of FBAs as shown in Fig. 1 it is possible to select a combination of FBAs where fast analysis is possible using a high organic content in the eluent. In the present study it has not been possible to find isocratic conditions where all 15 FBAs are separated in a short time (i.e. within 10 min). For example, even at 50% methanolic eluent the 3- and 4-FBAs were coeluting at 8.2 min and at these conditions the TeFBA would not elute within 10 min (results not shown). Also, from Fig. 1 it can be seen that at 75% methanol the FBAs 2,6-DFBA, 2,4,5-TFBA, and 2,3,4-TFBA will coelute. Whereas 2,6-DFBA can be resolved by the MS–MS, it is not possible to distinguish the isomers 2,4,5-TFBA, and 2,3,4-TFBA. Thus, separating all 15 FBAs would require a gradient method. Previously published LC methods have been able to separate seven FBAs using a gradient [6]. In general, a gradient method is less stable and several minutes are required for re-establishing the initial solvent conditions prior to a successive chromatographic run. Thus, isocratic conditions are preferred when aiming at a fast method. Also, in hydrological studies it is seldom necessary to employ more than three or four FBA tracers. Thus, using a high fraction of methanol allows for a fast, isocratic analysis when using selected combinations of mono-, di- and tri-substituted FBAs.

Using the optimised solvent system, the conditions for ionisation were optimised for both ESI and APCI. It was observed that positive mode was unsuitable for both ESI and APCI, and the subsequent optimisation was made using negative mode ESI and APCI only. This optimisation was made using direct injection of standards (syringe pump, 10 μl/min, 50 μg/l). Initially the conditions for the ionisation of the parent ion were optimised by varying the capillary and cone voltage. Then the fragmentation of the parent ion was optimised by increasing the accelerating voltage inside the collision cell within the range 5–40 eV. The optimal conditions are shown in Table 2. The optimised ESI_{neg} mass chromatograms for the compounds are shown in Fig. 2.

Statistical examination of the method demonstra-

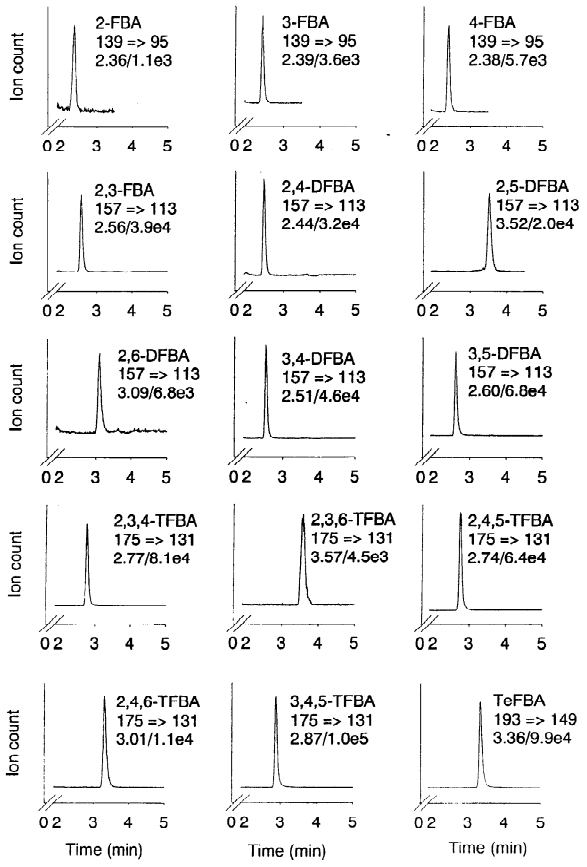

Fig. 2. Representative raw data from the ESI_{neg}-MS–MS chromatograms of FBAs (LC conditions: 90% (v/v) methanol in 10% (v/v) diluted acetic acid, flow 0.2 ml/min, 50 pg in 10 μl was injected, see text for details). The mass reaction monitoring chromatograms show the traces of the fragment ions (see Table 2 for details). The compound abbreviation, mass reaction, and retention time/maximum ion count are given for each peak.

ted linear correlation between area and tracer concentration (Table 2) and low detection limits were found using ESI_{neg}, whereas $APCI_{neg}$ could be used for most compounds at a higher level. The TeFBA compound had the poorest performance when using APCI as it was not possible to obtain a stable fragmentation from a direct injection of the standard.

In general, the method presented provides detection limits at the μg/l level (Table 2). It was found that ESI was more sensitive than APCI. Thus, in groundwater the general detection limit for $APCI_{neg}$ was 20 μg/l (equals 300 pg injected, Table 2). Comparing the method to previously published methods, the low detection limit at 1 μg/l or 15 pg injected when using ESI_{neg} is evident. Hence, in a previously published method using ion chromatography [2] the detection limit was 2 ng injected. Using another ion chromatography method, the pentafluorobenzoic acid (PFBA), 2,6-difluorobenzoic acid (2,6-DFBA), and *ortho*-trifluoromethylbenzoic acid (*o*-TFMBA) could be measured at levels 0.10–25 mg/l [4]. However, quantification may be problematic using ion chromatography due to possible interference from other ions such as NO_3^- and Cl^- even at concentrations as low as 1 mg/l [4,6].

A method for simultaneous analysis of seven fluorobenzoate isomers using RP-HPLC with UV detection has been published [6]. In this work, the overall detection limit was 0.5 mg/l. Another RP-HPLC method with UV detection allowed the direct determination of 16 fluorinated aromatic carboxylic acids to concentrations at 10 μg/l [5]. Also, gas chromatographic methods for detection of the FBAs have been developed. Using clean-up steps and derivatization, a detection limit of 0.1 μg/l could be achieved [7,8]. Thus, the present method is a decade or two more sensitive even without clean-up steps. Also the need for derivatization prior to analyses renders the GC approach labour-intensive and harmful derivatization reagents such as diazomethane are used in the procedure.

Several practical aspects must be considered when developing methods for field studies. Using the method presented less than 1 ml of water needs to be sampled and analysis can be made using filtration through a 0.45-μm nylon filter as the only clean-up step, rendering the method less labour-intensive. Due to the structure specific detection, the identification of the tracers is rather indisputable and the background noise on the signal is minimised. However, as in any analytical method there is a risk that matrix components may interfere with the detection and quantification of the tracers. Using LC–MS–MS, the matrix components may interfere with the ionisation of the tracers. For comparison, even simple spectrophotometric measurements of fluorescent tracers such as uranine may also be sensitive to matrix effects such as pH-induced change in fluorescence intensity [11]. Another type of fast and simple quantification of classical tracers is the use of ion selective electrodes for bromide measurements.

Agreement with ion chromatographic analysis has been shown [12]. However, matrix effects may be anticipated and difficult to predict. Combining the use of optimised chromatography, matrix identical calibration standards, and structure specific detection, the present method provides a tool where the risk of matrix interference is reduced.

The performance of the method was demonstrated in multiple tracing experiments in unsaturated fractured clayey till [9,13]. By combining selected tracers from the compounds covered by the method (2,3-DFBA and 2,6-DFBA) with classical tracers (chloride, bromide, uranine, and sulforhodamine B) a series of fracture flow studies were conducted on a single location. Samples were filtered and analysed without further clean-up using the ESI_{neg} method described and no matrix interference could be observed on the analysis of the FBAs. Based on the differences in diffusion coefficients between the FBAs and chloride/bromide, the influence of diffusive exchange on the solute transport was evaluated. Additionally, by including a FBA in every experiment, a reference tracer with similar diffusion coefficient was obtained, allowing direct comparison of different experiments. Thus, the FBA tracers can be used to obtain additional information on fracture transport processes and thereby serve as an important tool when studying and modelling fracture flow and transport. The LC–MS–MS method presented provides the analytical tool for such studies.

4. Conclusion

A sensitive LC–MS–MS method for the direct determination and quantification of 15 fluorobenzoic acids was developed. Negative mode ESI was found to be more sensitive than APCI. The method is fast and can be used for quantification of FBAs in leaching studies as an alternative or as a complement to commonly used tracers. In comparison to previously published work, the method is superior with respect to detection limits, number of compounds that can be directly analysed, small sample volume, fast analysis time, minimal sample clean-up, and specificity. Also, solvent consumption is low and there is a potential for automation. In conclusion, the method presented is a valuable tool for hydrological studies.

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