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Determination of benzylsuccinic acid in gasoline-contaminated groundwater by solid-phase extraction coupled with gas chromatography–mass spectrometry

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Abstract

Benzylsuccinic acid (BSA) and methylbenzylsuccinic acid (methyl-BSA) are unambiguous biotransformation products resulting from anaerobic toluene and xylene biodegradation, respectively. A solid-phase extraction method based on polystyrene–divinylbenzene sorbent was developed for the quantitative BSA determination in groundwater samples as an alternative to liquid–liquid extraction. Gas chromatography coupled with mass spectrometry was used for separation and detection. The recovery from spiked 1 l groundwater samples was 88 to 100%. The precision of the method, indicated by the relative standard deviation, was ±4% and the method detection limit was 0.2 μg/l. The concentration of BSA and methyl-BSA in groundwater samples from anaerobic BTEX (benzene, toluene, ethylbenzene and xylenes)-contaminated sites ranged from below the detection limit (3 μg/l) to 155 μg/l. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Gasoline contamination of the subsurface, mainly from underground storage tanks, is a widespread problem. Benzene, toluene, ethylbenzene and the xylene isomers, collectively known as BTEX are the components of major concern due to their high solubility [1,2] and their toxicity [3–5]. The US Environmental Protection Agency (EPA) drinking water criteria are 0.005 mg/l for benzene, 1 mg/l for toluene, 0.7 mg/l for ethylbenzene, and 10 mg/l for xylene isomers [6,7].

Limitations and costs of conventional ground water cleanup technologies and soil treatment methods have made monitored natural attenuation of BTEX an attractive approach for site remediation [8]. Monitored natural attenuation includes physical, chemical and biological processes that lead to reduction in contaminant concentrations. In particular, biodegradation is important for transforming BTEX to less toxic products. Since most sites with gasoline spills are anaerobic, BTEX degradation in the absence of oxygen is the process of interest for bioremediation. BTEX-degrading cultures were reported for denitrifying [9–19], sulfate-reducing [20–

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30], iron-reducing [23,31–34], manganese-reducing [35], phototrophic [36], and methanogenic [23,37–39] conditions.

Documenting in-situ biodegradation ideally should include multiple lines of evidence [8,40]. Identification of degradation products in field samples is particularly compelling evidence, especially when the detected products are unambiguous indicators of a specific biodegradation pathway. The anaerobic degradation pathway for toluene was investigated in detail during the last few years [41–58]. Benzylsuccinic acid (BSA) is the first intermediate during anaerobic microbial degradation of toluene. In a corresponding manner, methylbenzylsuccinic acid (methyl-BSA) results from anaerobic xylene degradation. Beller et al. [59] proposed to use BSA and methyl-BSA as unique indicators of the anaerobic degradation of toluene and the three xylene isomers because the relationship of BSA and methyl-BSA to their respective parent compounds is well understood, they do not have commercial or industrial uses, and they occur at detectable levels in the environment [59,60]. To the best of our knowledge, few reports document the occurrence or in-situ formation of these degradation products despite their potential as direct evidence for in situ biodegradation [59–63].

In previous studies, samples containing BSA and related compounds were extracted using liquid–liquid extraction, followed by methylation with diazomethane [59] or silanization with *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide [63]. Unfortunately, liquid–liquid extraction is cumbersome and typically uses high volumes of solvents. Solid-phase extraction is an attractive alternative to liquid–liquid extraction due to decreased solvent usage. Currently many formats and sorbent materials are available for solid-phase extraction. Classical solid-phase extraction utilizes packed columns of the sorbent of choice (typically 40 μm particles). Alternatively, the disk or membrane format has smaller particles (8 μm) embedded in a PTFE membrane, which allows for higher flow-rates due to smaller particle size and higher cross-sectional area compared to packed columns [64]. However, capacity, which is dependent on the mass of sorbent, is limited due to smaller amount of sorbent embedded in membranes.

Solid-phase extraction can be carried out in normal-phase, reversed-phase, size-exclusion or ion-exchange modes. The classical approach for acid analytes is to acidify samples and extract the free acids onto reversed-phase sorbents. Alternatively, extraction onto a strong anion-exchange (SAX) resin followed by in-vial derivatization and elution also was applied to other carboxylic acids [65,66]. The goal of this study was to develop and validate a solid-phase extraction method for analyses of BSA and methyl-BSA compounds in BTEX-contaminated groundwater samples.

2. Experimental

2.1. Reagents and standards

Benzylsuccinic acid (BSA; 99% purity) was purchased from Sigma (St. Louis, MO, USA). 2-Chlorolepidine (2CL, 99% purity) and 4-(trifluoromethyl)hydrocinnamic acid (4TFM, 95% purity) were purchased from Aldrich (Milwaukee, WI, USA). BSA, 2CL, and 4TFM were prepared in acetonitrile at 1 mg/ml. 4TFM was used as a surrogate standard and was spiked prior to the extraction while 2CL was used as internal standard and was spiked just prior to the derivatization reaction. Acetone, methanol, and acetonitrile (HPLC-grade) were purchased from Fisher Scientific (Fairlawn, NJ, USA). Ethyl acetate (HPLC-grade) was obtained from Mallinckrodt (Paris, KY, USA). Hydrochloric acid was obtained from J.T. Baker (Phillipsburg, NJ, USA). A diethyl ether solution of diazomethane was prepared from Diazald (Aldrich) according to standard procedures [67].

The solid-phase extraction sorbents in disk format evaluated for this study included the Empore polystyrene–divinylbenzene (PS–DVB) (Varian, Harbor City, CA, USA), and octadecyl-bonded silica (C_{18}). The sorbents in cartridge format included PS–DVB ENVI-Chrom P (Supelco, Bellefonte, PA, USA) and Bond Elut C_{18} (Varian).

2.2. Field sites and sampling

The first field site is a bulk-fuel terminal located in Willbridge near Portland, OR, USA and hereafter will be referred to as the Northwest Terminal. The unconfined aquifer at this site consists of a layer of fill (medium dense to fine grained sand and silty

Table 1
Geochemical characteristics of groundwater in wells at the two field sites

Parameter	Northwest Terminal			Kansas City site		
	Well CR12	Well CR13	Well CR15	Well 105s	Well 106s	Well 207
Benzene (mg/l)[a]	0.01	0.3	14	6.0	0.01	N/A[b]
Toluene (mg/l)[a]	0.4	5.9	0.38	0.27	ND	N/A[b]
Ethylbenzene (mg/l)[a]	0.18	1.5	0.62	0.50	0.01	N/A[b]
Xylene (mg/l)[a]	1.2	10.0	2.3	ND	ND	N/A[b]
NAPL observed	No	No	Yes	No	No	Yes
DO (mg/l)[c]	0.5	1.7	ND	0.7	1.8	ND
Nitrate (mg/l)[d]	ND	ND	ND	ND	ND	ND
Sulfate (mg/l)[e]	ND	ND	ND	7.6	6.9	1.8
Methane (mg/l)[f]	10	5.5	11	18	14	N/A

ND: Not detected; N/A: not analyzed or not applicable.
[a] EPA Method SW 8020 or EPA Method 8021 [75]; reporting limit=0.0005 mg/l for benzene, ethylbenzene, and toluene and 0.001 mg/l for xylenes.
[b] Sample not analyzed due to excess free product.
[c] Standard Method 4500-O.G. [76] or EPA 360.1 [77]; reporting limit=1 mg/l.
[d] EPA Method SW 846-9056 [75] or EPA 300.0 [77]; reporting limit=0.1 mg/l.
[e] EPA Method SW 846-9056 [75] or EPA 300.0 [77]; reporting limit 1 mg/l.
[f] RSK-175 standard operating procedure [78]; detection limit=0.002 mg/l.

sand) that rests on top of alluvium, which consists of clayey silt with sand interbedded with silty clays and clays. A 71 900 l (19 000 gallons) release of ethanol occurred in 1999. The site had a pre-existing dissolved hydrocarbon plume. Total BTEX concentrations prior to the study in June 2000 and were between 1 and 18 mg/l prior to the study (Table 1). Groundwater was anaerobic due to the existing hydrocarbon contamination and nitrate and sulfate were depleted and methane was detected (Table 1). The water table is approximately 2 to 3 m below land surface. Groundwater flows towards the east and the velocity is estimated to be about 100 m per year [68]. A total of four wells were sampled at this site.

The second field site is a former petroleum refinery near Kansas City, KS, USA and hereafter will be referred to as the Kansas City site. It was operated from 1930 until 1982. After installation of monitoring wells, presence of low-density non-aqueous phase liquid (LNAPL) was discovered over a 90-hectare area. Recovery operations initiated in 1984 recovered over $6 \cdot 10^6$ l; however, recent recovery efficiency has declined. Prior to this study in June and October 2000, BTEX concentrations ranged from 0.02 to 6.8 mg/l (Table 1) [69]. The unconfined aquifer consists of fine sand with clayey silt or silt (to ~3 m below land surface) covering sand (below ~3 m below land surface) [70]. Although groundwater flows generally southeast towards the Missouri River, in the spring, high stage elevations of the Missouri River cause the hydraulic gradient to shift reverse inward toward the site [71]. At the time of the experiments, groundwater velocities were 0.05 m/day [72] and the water table was about 7 to 9 m below land surface, which were low water table conditions for this site. Tests were conducted in wells that had 3-m screened intervals with the top of the screen located at 4.6 to 7 m below land surface.

Samples were obtained from each site from 2-in. I.D. poly(vinyl chloride) (PVC) wells (1 in.=2.54 cm). Before taking the sample, three times the well volume was purged where possible. The low water level in well 207 at the Kansas City site made it impossible to purge three times the well volume; therefore, 0.3 l was purged prior to sampling this well. Samples were collected in glass bottles (250 ml to 1 l), preserved with 5% (v/v) formalin, shipped on ice, and stored at 4 °C until analysis.

2.3. Solid-phase extraction

Prior to extraction, ground water samples were warmed to room temperature. The samples (0.5–1.0 l) were titrated to pH 2.0 with concentrated HCl. After acidification, the samples were spiked with 20

μl of 1 μg/μl of the 4TFM surrogate standard. The samples were then filtered under vacuum through 1 μm Whatman glass-fiber filters (Fisher Scientific).

Glass columns fitted with PTFE frits were packed with 0.5 g PS–DVB ENVI-Chrom P and attached to a vacuum manifold (Supelco). The columns were then preconditioned in three steps. First, 2.5 ml acetone was applied and the columns were allowed to dry. Second, 2.5 ml methanol was applied, after which the columns were kept wet until the end of the extraction. Third, the columns were washed with 4×5 ml of 0.01 *M* HCl. PTFE transfer lines were attached to the top of each column and used to transfer samples from the filtration flasks to the columns. Extractions were performed using a 5 ml/min flow-rate.

After extraction, the sorbent columns were dried overnight under vacuum by drawing air over the sorbent phase. To elute the columns, 2×2.5 ml of ethyl acetate was passed through each column, collected, and concentrated to ~0.5 ml under a stream of dry nitrogen. The eluate was transferred to a 2-ml autosampler vial by rinsing with 2×0.2 ml ethyl acetate. The autosampler vial contents were concentrated to 0.5 ml under a stream of dry nitrogen and then spiked with 20 μl of 1 μg/μl of the 2CL internal standard. The vial was then capped after adding 1.0 ml of diazomethane solution and the reaction was allowed to proceed for 5±1 min. The vial was then uncapped and the excess diazomethane was removed under a stream of dry nitrogen. The vial was recapped and placed on the autosampler for analysis.

2.4. Spike and recovery

Three sets of spike and recovery experiments were performed. First, five replicate 1 l tap water samples were spiked to give a final concentration of 20 μg/l BSA. Tap water samples were the only samples not prefiltered before the extraction. Quantitative recovery from tap water was only obtained if glass columns for the extraction cartridge and beakers containing the acidified BSA-spiked tap water were silanized. We suspect that interactions of the BSA with the glass surface caused losses from acidic tap water whereas the organic acids and phenols present in the groundwater samples may compete with BSA for sorption sites on the glass. Clean glassware was rinsed for about 15 s with DMDCS (dimethyldichorosilane 5% in toluene, Supelco) then washed two times with toluene, three times with methanol, and then air dried. Note that this procedure was necessary only for tap water samples and not for ground water samples. Glassware for the elution and the derivatization reaction were not silanized.

The second set of spike and recovery experiments was performed with a groundwater composite obtained by combining aliquots of several samples obtained from several wells from the Kansas City site. The composite sample first was determined to be blank and then seven 1-l aliquots of the composite sample were spiked with BSA to give a final concentration of 2 μg/l. In addition, over the course of the study, a total of 16 additional blank 1 l samples collected from a total of six wells from the Kansas City site and the Northwest Terminal were spiked to give a final concentration of 20 μg/l of BSA. Extractions were conducted at separate times and were not treated as a single batch of extractions.

2.5. Gas chromatography–mass spectrometry

All extracts were analyzed using a Hewlett-Packard Model 5890 gas chromatograph equipped with a Model 5972 mass-selective detector. The GC system was equipped with a 30 m×0.32 mm, 4 μm SPB-1 capillary column (Supelco). The injector was operated under splitless conditions at 250 °C with a 1 μl injection volume and helium as carrier gas. The initial oven temperature of 130 °C was increased at 2.5 °C/min to 225 °C and then raised at a rate of 10 °C/min up to 265 °C and held for 3 min to give a total run time of 45 min. The MS system was operated in the electron impact mode with a source temperature of 265 °C.

Three ions were acquired in single ion monitoring mode and used to identify and quantify each analyte in its methylated form (Table 2). Even though the molecular ions for BSA and methyl-BSA were only 20% of the base peak, the molecular ions were selected for purposes of maximum selectivity. The ion ratio 236/176 ranged from 0.09 to 0.11 and the ion ratio 236/91 ranged from 0.04 to 0.10.

Calibration curves for BSA were constructed from standards prepared in 0.5 ml ethyl acetate with 0.2 to

Table 2
Ions used to detect and quantify analytes of interest

Analyte	Quantitation ion (m/z)	Qualifier ion(s) (m/z)
BSA	236*	176/91
Methyl-BSA	250*	190/105
4TFM	172	232*/159
2CL	177*	179*/142

The molecular ion [M^+] is marked by an asterisk.

25 μg of BSA, 20 μg of the surrogate standard (4TFM), and 20 μg of the internal standard (2CL). Standards were methylated in a procedure similar to that for samples; however, that only 0.5 ml diazomethane was used. Note that less than quantitative diazomethylation of the standards would lead to an overestimation of the environmental concentrations determined by this approach. Surrogate-standard quantitation was used and gave linear calibration curves, typically with r^2 values of 0.999.

Although no authentic standard was available for methyl-BSA, fragmentation full-scan mode for peaks eluting 3.5 to 4.6 later than BSA were similar to those reported for methyl-BSA by others [29,51,59]. In contrast to BSA, the mass spectrum of methyl-BSA contains ions that are 14 mass units greater, which corresponds to the additional methyl group (Fig. 1). Because no authentic standard of methyl-BSA was available, the calibration curve of BSA was used for methyl-BSA quantification, assuming a response factor of 1. Multiple peaks with similar fragmentation patterns were observed for methyl-BSA, which likely to correspond to the different isomers of methyl-BSA (Fig. 2). Since we were not

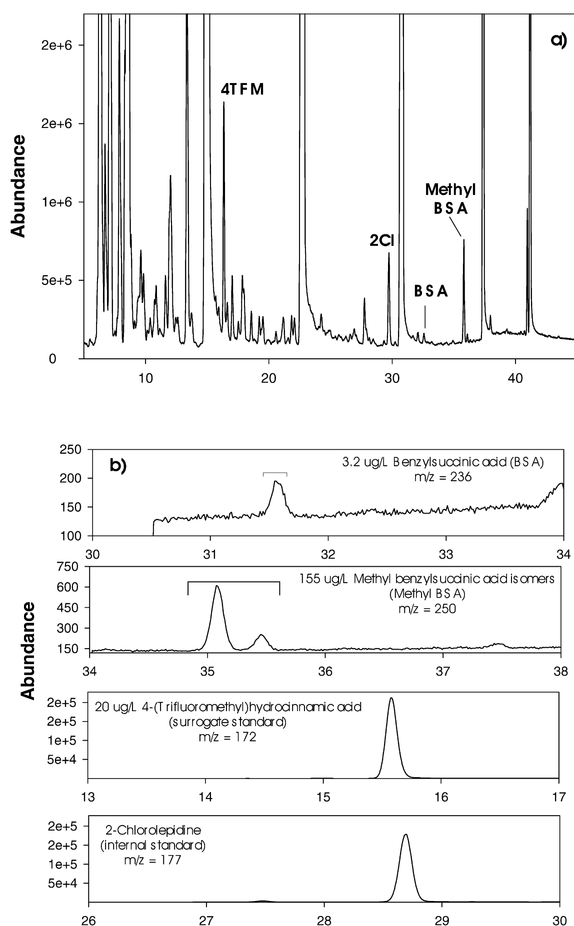

Fig. 1. Typical GC–MS chromatograms for samples obtained under (a) full scan and (b) selected ion monitoring (SIM) modes.

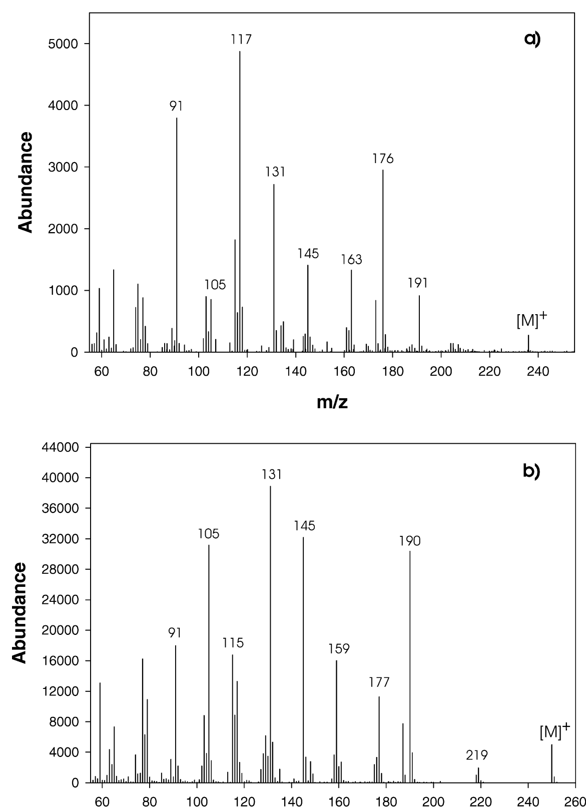

Fig. 2. Mass spectra of (a) BSA and (b) methyl-BSA in a groundwater sample from well CR13 at the Northwest Terminal site.

able to distinguish between isomers, peak areas for methyl-BSA were summed.

3. Results and discussion

3.1. Gas chromatography–mass spectrometry

The temperature gradient of the gas chromatograph was optimized towards fast separation while minimizing the variability of the BSA/2CL ratio. Chromatograms obtained for the same sample using temperature gradients ranging from 1 to 10 °C/min indicated that decreasing the gradient from 10 to 2.5 °C/min significantly reduced the baseline signal. In addition the BSA/2CL ratio became more reproducible and reached values similar to those obtained for standards. Decreasing the temperature gradient further to 1 °C/min did not further improve the reproducibility. Therefore, a temperature gradient of 2.5 °C/min was used for all subsequent experiments. Under these conditions, BSA eluted at 31.5 min and methyl-BSA isomers eluted later with peaks occurring from 34.9 to 35.6 min (Fig. 2).

3.2. Methylation reaction

To evaluate the influence of the reaction solvent, 20 μg each of BSA and 2CL were spiked into separate vials containing 0.5 ml of the following solvents: acetonitrile, methanol, diethyl ether, and ethyl acetate. The solution was allowed to react for 30 min with 0.5 ml diazomethane. After removing the diazomethane under a flow of dry nitrogen, the samples were analyzed by GC–MS. Methyl ester yields, determined as peak area of BSA relative to that of the area of the 2CL internal standard, were greatest for reactions conducted in ethyl acetate and methanol and lower for those conducted in diethyl ether and acetonitrile. Except for reactions conducted in acetonitrile and diethyl ether, standard deviations were not significantly different (*F*-test, 95%). Since ethyl acetate is less polar and has a smaller expansion factor, it was used as reaction solvent for all subsequent reactions.

Once the reaction solvent was selected, the reaction time was optimized using BSA and a range of chemicals selected for their potential as surrogate standards, including 4-fluorobenzoic acid, which was used by Beller et al. [59] and Elshahed et al. [63]. Reaction time was investigated by spiking a mixture of 20 μg of each BSA, 4TFM, 2CL, 4-fluorobenzoic acid (Sigma), 3-chlorocinnamic acid (Sigma), and 4-fluorocinnamic acid (Sigma) into 0.5 ml ethyl acetate and adding 0.5 ml diazomethane. Areas for the internal standard 2CL were stable over time as were the areas for the methyl esters of BSA, 4TFM and 4-fluorobenzoic acid as indicated by stable ion ratios (Fig. 3). However, the yield of the methyl esters of 4-fluorocinnamic acid and 3-chlorocinnamic acid, each of which have a double bond, declined with increasing reaction time. Although not evaluated in this study, phenylitaconic acid, which is the second intermediate of toluene degradation, has a double bond in its structure like of 4-fluorocinnamic acid and 3-chlorocinnamic acid. Careful control of reaction time is required if degradation products such as phenylitaconic acid are of interest. For this remainder of this study, a 5 min interval was used. Due to their reactivity, 3-chlorocinnamic acid and 4-fluorocinnamic acid were excluded as potential surrogate standards, which left 4TFM and 4-fluorobenzoic acid for further evaluation during the development of the solid-phase extraction approach.

3.3. Solid-phase extraction

Initial experiments focused on evaluation of strong anion-exchange (SAX) disks for BSA isolation using procedures similar to those developed for other carboxylic acids [65,66]. However, the capacity of 25 and 47 mm SAX disks was exceeded during the extraction 1 l field sample volumes as evidenced by breakthrough. As a result, this approach to solid-phase extraction was abandoned in favor of reversed-phase solid-phase extraction.

Breakthrough curves were developed for reversed-phase sorbents in disk and cartridge format. Groundwater samples from the Kansas City site, previously determined to be blank, were acidified with concentrated HCl to pH 2. The groundwater samples were then spiked to give a final concentration of 10 mg/l of BSA; high concentrations were used to achieve good detection. The spiked groundwater samples were then continuously applied to separate columns or disks. Samples for measurement of the outflow

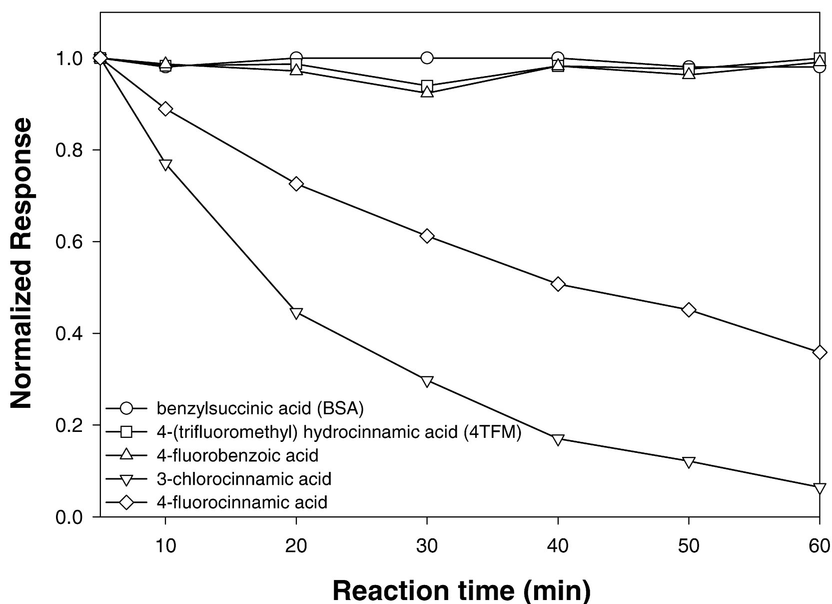

Fig. 3. Derivatization yield normalized to 2CL internal standard with time for BSA and chemicals evaluated as potential surrogate standards.

concentration were taken every 50 to 100 ml during the extraction and were analyzed by either high-performance liquid chromatography with diode-array detection (HPLC–DAD) or by GC–MS. To develop breakthrough curves, outflow concentrations relative to the applied concentrations were plotted versus the cumulative volume extracted.

HPLC–DAD was used in some experiments to simplify sample analysis because no derivatization step was required and the separation method was faster. Samples were analyzed on a Waters 2690 instrument with a Phenomenex Luna C_{18} column (150×4.6 mm, 5 μm). The mobile phase was 25 m*M* aqueous potassium phosphate buffer at pH 2.5. A solvent gradient was used changing from 30% to 60% methanol in 5 min. A Waters 996 diode array detector was used to monitor at 203 nm. For experiments involving 4TFM, which was not detected by the HPLC conditions, GC–MS was used for analyses of 1 ml. For GC the aqueous 1-ml sample was dried under a stream of dry nitrogen, redissolved in 0.5 ml ethyl acetate.

The 0.5-g PS–DVB cartridge exhibited the highest breakthrough capacity with no evidence of breakthrough up to 1.1 l and only 10% breakthrough at 1.4 l (Fig. 4). On the other hand, the 0.5-g C_{18} cartridge had 50% breakthrough at ~500 ml. The higher capacity of the 0.5-g PS–DVB cartridge was expected since PS–DVB is a 100% polymer sorbent compared to the 0.5 g octadecyl, which is about 20% (w/w) polymer and 80% (w/w) silica. Different sorbents in the 47-mm disk format gave 0 to 60% breakthrough at ~200 ml, which was insufficient for

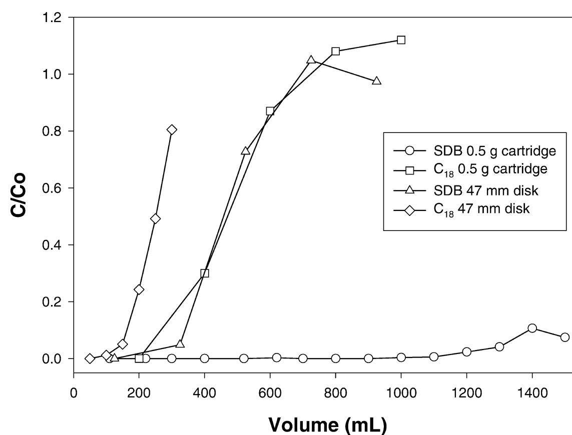

Fig. 4. Breakthrough curves for benzylsuccinic acid (BSA) extracted from spiked groundwater onto different types onto polystyrene–divinylbenzene (PS–DVB) and octadecylsilica (C_{18}) in cartridge and disk formats.

the expected sample volume required to detect anticipated environmental concentrations in the low-μg/l range [59,62,63]. The C_{18} in the disk format had a lower capacity than the C_{18} in the cartridge format even though both formats had about the same actual sorbent loading of ~0.1 g. The breakthrough capacity of PS–DVB in disk format was also lower than that expected based on comparison of the mass of sorbent in cartridge (~0.5 g) and disk (~0.3 g) format. Subsequent breakthrough experiments with 4TFM and 4-fluorobenzoic acid indicated that 4-fluorobenzoic acid broke through more quickly than BSA. On the other hand, 4TFM was retained more strongly than BSA. Based on these experiments, sample volumes up to 1 L, 0.5 g PS–DVB cartridges, and the surrogate standard of 4TFM were used in all subsequent experiments.

Because sorbents from breakthrough experiments were not eluted and sorbed masses not determined, it was important to test the compatibility of eluates from PS–DVB cartridges with diazomethane derivatization and GC–MS analyses. Plastic cartridges containing 0.5 g PS–DVB used to extract acidified water led to the formation of a precipitate during the methylation reaction step with diazomethane. Formation of the precipitate caused generally low and variable recoveries (2 to 140%) of BSA with an average recovery of 62%. As an alternative, the PS–DVB sorbent was packed into glass tube fitted with PTFE frits. Four spiked 500-ml samples from the Kansas City site were extracted and the sorbent was eluted with 2×2 ml ethyl acetate. To demonstrate completeness of elution, an additional third eluate (0.5 ml) was collected. In all four cases, the third fraction contained <1% of the amount of 4FTM and BSA collected in the first two fractions. Furthermore, no precipitation was observed during the methylation reaction step. Average recoveries from the Kansas City site groundwater were higher (86%) and less variable (standard error 2%) than those obtained during experiments with plastic columns. Therefore, glass tubes with PTFE frits filled with 0.5 g PS–DVB were eluted with 2×2.5 ml ethyl acetate for all subsequent experiments.

Ground water samples collected for the study contained varying amounts of brown-colored precipitate. The precipitate, presumably iron oxide, was not present at the time of sampling and did not form in acid-preserved samples. In order to check if BSA co-precipitated, non-acidified samples were spiked with BSA in the field and stored for 3 days during which varying amounts of precipitate formed. Filtered samples were then analyzed for BSA by HPLC (faster analyses). Average recovery for four replicates from each of four different wells were 100±4% (4% relative standard deviation; $n=16$), which indicated that BSA is not sorbed to the precipitate. Although BSA concentrations are not affected by iron precipitation, all samples were filtered to achieve faster flow-rates during the extraction process.

3.4. Accuracy and precision

The average recovery of 4TFM from tap water, measured relative to the 2CL internal standard, was 84±2% (average recovery ± standard deviation, $n=$ 5, Table 3). The precision, indicated by the relative standard deviation (RSD), was ±2.4%. The absolute recovery of BSA, measured relative to the 2CL

Table 3
Recovery of BSA and 4FTM spiked into 1 l samples of tap water, a groundwater composite, and selected individual groundwaters

Sample	Absolute 4FTM, recovery (%)[a]	Absolute BSA, recovery (%)[a]	Relative BSA recovery (%)[b]
Tap water ($n=5$)[c]	84±2 (2.4%)	86±2 (2.3%)	100±2 (2%)
Groundwater composite ($n=7$)[d]	98±3 (3.1%)	86±5 (5.8%)	88±2 (2.3%)
Individual groundwater samples ($n=16$)[c]	86±3 (3.5%)	88±4 (4.6%)	100±4 (4%)

Recovery is reported as the average±standard deviation (relative standard deviation).
[a] Absolute recovery is determined against the 2 CL internal standard.
[b] Relative BSA recovery is determined against the 4FTM surrogate standard.
[c] Spiked to give a final BSA concentration of 20 μg/l.
[d] Spiked to give a final BSA concentration of 2 μg/l.

internal standard, was 86±2% (2.3% RSD). The relative recovery of BSA, measured relative to the surrogate standard 4TFM, was 100±2% (2% RSD; Table 3).

Spike and recovery experiments also were performed using a groundwater composite because no single sample of sufficient volume was available to perform the necessary seven replicate analyses [73]. The absolute recoveries of 4TFM and BSA were 98±3% (3.1% RSD) and 86±5 (5.8% RSD), respectively. The relative recovery of BSA from the groundwater composite samples was 88±2% (2.3% RSD; Table 3). Over the course of the study, the absolute recoveries of 4TFM and BSA from 16 blank, individual groundwater samples were 86±3% (3.5% RSD) and 88±4 (4.6% RSD), respectively, while the relative recovery of BSA was 100±4% (4% RSD). Note that although the samples contained various amounts of iron precipitate, similar accuracy and precision were obtained for samples used for the three types of spike and recovery experiments.

The conventional method obtains detection limits from signal-to-noise ratios estimated from baseline variation and peak height [73]). To obtain the method detection limit of 0.2 μg/l the standard deviation of the seven groundwater composite replicate samples was multiplied by a factor of 3.14 (the Student's t-value for a one-tailed test at the 99% confidence interval with 6 degrees of freedom [73]). The method quantitation limit of 0.7 was calculated by multiplying the method detection limit by a factor of three [74].

Alternatively, the detection limit can be calculated as the standard deviation of repeated measurements of peak area [74]. Repeated injection ($n=7$) of two individual sample extracts that corresponded to a concentration of 2 μg/l gave a standard deviation that was not significantly lower (F-test on a 99% confidence level) than the standard deviation for the seven replicate extractions. Therefore, variations in the solid-phase extraction and derivatization method are no greater than the instrumental variation.

3.5. Application to groundwater samples

Samples from a total of six different wells from the Northwest Terminal and the Kansas City site were analyzed for BSA and methyl-BSA. Due to the limited amount of sample, replicate extraction analyses were not performed. Concentrations of BSA were 3.2 μg/l in well CR13, below the quantitation limit (0.7 μg/l) in well CR12, and below detection limit (0.2 μg/l) in wells CR15, 105s, 106s and 207 (Table 4). For the CR13 sample with 3.2 μg/l BSA, the 236/176 ion ratio was 0.088, which was within the range observed for the authentic BSA standard. These concentrations were equivalent to 0.02 mol% of background toluene concentrations detected in each well (Table 1).

Concentrations generally were higher for methyl-BSA than for BSA, which was previously observed [59]. Methyl-BSA concentrations were 5.7, 6.6, 10.4, and 155 μg/l for wells CR12, 105s, CR15 and CR13, respectively (Table 4). Although no authentic standard was available for comparison, the average ion ratio 250/190 for methyl-BSA was 0.16 while the 250/105 ion ratio was 0.12. Mass spectra published for methyl-BSA indicate an ion ratio 250/190 of approximately 0.2 [29,51,59]. No methyl-BSA was detected in well 207 and was below the quantitation limit (0.7 μg/l) in well 107s (Table 4). The concentrations of methyl-BSA corresponded to 0.2 to 0.7 mol% of the background xylene concentrations reported for each well (Table 1). The mol% of BSA and methyl-BSA are similar to those reported where BSA and methyl-BSA concentrations were three- to four-orders of magnitude lower than their respective parent BTEX concentrations [62,63]. Overall, BSA and methyl-BSA were detected in wells that had background concentrations of toluene

Table 4
Concentrations of BSA in single 0.25 to 1 l groundwater samples

Site	Well	BSA (μg/l)	Methyl-BSA (μg/l)
Northwest Terminal	CR12	<0.7[a]	5.7
	CR13	3.2	155
	CR15	ND	10.4
Kansas City site	105s	ND	6.6
	106s	ND	<0.7
	207	ND	ND

ND: Compound was not detected in this sample (detection limit=0.2 μg/l).

[a] Concentration was below quantitation limit (0.7 μg/l) but above detection limit of 0.2 μg/l.

and xylene but were not detected in groundwater that had no background toluene or xylene.

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