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**AN EXPANDED SUITE OF TRACERS  
FOR HYDROLOGICAL INVESTIGATIONS**

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**WRRRI Technical Completion Report No. 294**

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## ABSTRACT

Several fluorinated derivatives of benzoic acid (fluorobenzoate, FBA) have proven their usefulness as nonreactive tracers in soil and groundwater. In the first part of this research, four previously untested trifluorobenzoates (TFBA) and two tetrafluorobenzoates (TeFBA) were examined for their suitability as water tracers in saturated and unsaturated environments. Negative log acid dissociation constants ( $pK_a$ ) determined by base titration ranged from 2.71 to 3.54 at 298 K. Some sorption of FBAs was observed in batch equilibration experiments with lower pH, high organic C fraction soils. An algorithm was developed for calculating a linear partition coefficient ( $K_D$ ) for each FBA isomer based on  $pK_a$ , soil organic C, pH and an average organic C-based partition coefficient for protonated FBA ( $K_{oc}^*$ ).  $K_{oc}^*$  was estimated to be 18,200  $\pm$  6,300 L/kg when total FBA solution concentration is less than 5 mg/L. Column mobility experiments in sandy media under saturated and unsaturated conditions yielded breakthrough curves for TFBA and TeFBA isomers that were indistinguishable from Br<sup>-</sup>. The results presented herein suggest that all 16 ring-substituted FBAs can serve as nonreactive water tracers.

In the second part of this research, a method was developed to concentrate FBAs in natural water samples. The technique utilized solid-phase extraction of FBAs followed by elution and analysis by high performance liquid chromatography. Under the best conditions, a five-fold concentration of a suite of FBAs dissolved in a natural water matrix could be achieved with greater than 80% recovery. Attempts to further concentrate FBAs were hindered by limitations of the capacity of the solid-phase extraction material for large volumes of solution and/or by difficulties in eluting retained FBAs.

Keywords: fluorobenzoates, tracers, chromatography, solid-phase extraction

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## INTRODUCTION

Groundwater pollution due to chemical leaching from point and nonpoint sources is of critical concern due to the nation's heavy dependence upon groundwater. To protect groundwater quality, we need to understand the physical, chemical and biological processes which control chemical migration in the subsurface. Despite significant advances in characterizing and modeling hydrologic systems, we are still unable to predict accurately predict chemical migration through soils and aquifers.

Tracers have long been used to follow the movement of water through soils and aquifers. In the context of this report, "tracer" is defined as a water-soluble chemical that is stable and nonreactive during an investigation. Environmental water tracing has recently assumed increased national and international importance due to concerns over groundwater contamination. Determining the direction and velocity of water flow is the first step in analyzing subsurface chemical transport. Often the only way to determine a given parcel of water's origin is to add tracers to different potential water sources. Complex hydrological investigations may require several distinct tracers. For example, it is estimated that as many as 30 unique tracers will be required for hydrological characterization of the proposed high-level radioactive waste depository at Yucca Mountain, Nevada. Proposed hydrological characterization of the Culebra formation above the Waste Isolation Pilot Plant will require more than 20 unique tracers. Hazardous waste sites may require emplacement of different tracers at different points in the containment system to allow early detection and correction of failures. The greater the number of unique tracers, the more precisely a breach can be

located. Properly conducted tracer tests in many instances are prerequisites to preventing future groundwater contamination.

Presently the number of useful water tracers is limited. An effective tracer is nonreactive, that is, it is not significantly sorbed nor transformed chemically or biologically during the time frame of interest, and it is present naturally at low levels or not at all. Other considerations in choosing a tracer include ease of quantitation, detection limit, environmental hazards, and cost.

Neglecting radioisotopes, which often can't be used due to safety considerations, bromide ion is the only generally suitable inorganic tracer. Recently, several fluorinated benzoic acid derivatives have been used effectively as soil and groundwater tracers. This class of compounds has the potential to provide many new, chemically distinct, nonreactive water tracers. They are amenable to simultaneous analysis in small water samples, a distinct advantage in complex hydrological investigations requiring multiple tracers and hundreds or thousands of sample analyses. Expanding the suite of available tracers will benefit researchers, regulators, and persons charged with siting chemical and nuclear waste disposal facilities. Developing improved analytical techniques for these tracers will reduce tracer costs and allow larger-scale and more sensitive investigations, where tracer dilution is currently a limiting factor.

The goal of this research was thus to provide an expanded pool of effective water tracers which can be readily and economically utilized for hydrological investigations. There were two hypotheses upon which the research was based. The first hypothesis was that chemical analogues to the proven fluorobenzoate tracers (FBAs) would likewise be effective



water tracers. The second hypothesis was that improved analytical techniques for the proven and new fluorobenzoates could be developed. The research focused on the following objectives:

1. Test a new series of fluorobenzoic acid derivatives for suitability as soil and groundwater tracers.
2. Develop analytical methodologies to measure new and existing FBA tracers simultaneously in natural water samples.
3. Develop methods to lower FBA tracer detection limits in natural water samples.

All objectives were at least partially accomplished. We successfully evaluated six new FBAs for suitability as soil and groundwater tracers (Objective 1). As part of this work we developed an algorithm to predict potential sorption of FBAs as a function of soil organic matter content and pH. We developed analytical methods to quantify these six FBAs simultaneously in natural water samples (Objective 2). We did not, however, develop methods to analyze the new and previously tested FBAs together in the same samples. We also developed methods to lower FBA detection limits in natural water samples (Objective 3).

## **ORGANIZATION OF THIS REPORT**

The work under objectives 1 and 2 was summarized in a 1994 publication in the Soil Science Society of America Journal (Benson and Bowman 1994). The reader is encouraged to refer to this publication for discussion of the first two research objectives.

The remainder of this completion report describes progress made in achieving Objective 3. Our goal was to lower FBA detection limits by concentrating FBAs from dilute solutions in a manner which would allow direct analysis of the concentrate. Since FBAs can

cost as much as \$28 per gram, any increase in detection limit could result in large savings in material costs.

### **Previous Work Relevant to Objective 3.**

Bowman and Rice (1986a,1986b) studied 2,6-difluorobenzoic acid (2,6-DFBA) and pentafluorobenzoic acid (PFBA) in flood irrigated agricultural fields in Arizona. Boggs and Adams (1992) found these tracers to behave essentially identical to Br<sup>-</sup> in a large-scale, multiyear aquifer tracer test in Mississippi. Four FBAs were investigated by Bowman and Gibbens (1992) as possible groundwater tracers. These four DFBA isomers (2,3-, 2,5-, 3,4-, and 3,5-DFBA) had mobility characteristics similar to Br<sup>-</sup> and were stable for at least several months under saturated and unsaturated conditions. Benson and Bowman (1994) expanded on the possible FBA tracers to include four trifluorobenzoic acid (TFBA) and two tetrafluorobenzoic acid (TeFBA) isomers. They found all the tracers to be chemically stable over a seventy-day period, although there was concern about sorption to organic matter at specific pH and organic matter content conditions. They also found the transport properties of these FBAs to be indistinguishable from Br<sup>-</sup>. Two additional FBA tracers have recently become commercially available (2,3,5-TFBA and 2,3,5,6-TeFBA) and are included in this study.

Dombrowski and Stetzenbach (1994) reviewed tracer toxicity tests and concluded that fluorinated benzoic acids, when used as groundwater tracers, would be relatively safe for human exposure at the ultra-trace levels used for the tests.

The above studies show FBAs to be a desirable class of compounds for groundwater tracer studies. However, cost is of major importance when choosing groundwater tracers for

use. From this standpoint Br<sup>-</sup> is the most desirable at a few cents per gram. The FBAs can cost as much as \$28 per gram (for 3,4,5-TFBA). The high cost of the FBAs dictates that a method of lowering the detection limits be developed.

This portion of the research was designed to find methods to concentrate FBAs for direct analysis by high performance liquid chromatography (HPLC). After some preliminary studies, our goal was to identify a group of FBAs for which we could both obtain at least a five-fold increase in concentration and analyze simultaneously in a mixture via HPLC.

## MATERIALS AND METHODS

### FLUOROBENZOATE ANALYSIS

#### Materials

The fourteen FBA compounds used in this study are listed in Figure 1. All were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI, with the exception of carboxyl-labelled  $^{14}\text{C}$ -2,6-DFBA, which was custom synthesized by Sigma Chemical Co., St. Louis, MO. All Aldrich FBAs were 97% pure, according to the manufacturer. The purity of the  $^{14}\text{C}$ -labelled 2,6-DFBA was tested in this study. Except as otherwise noted, Type I water from a Millipore, Millford, MA, Milli-Q water purification system was used throughout this study.

#### Chromatographic Methods

High performance liquid chromatography (HPLC) was used to analyze all the FBAs, except  $^{14}\text{C}$  labeled 2,6-DFBA, which was analyzed using scintillation counting.

Initial HPLC analyses were conducted using a Model 510 pump, U6K injector and a 486 tunable absorbance detector from Waters Chromatography Division, Millipore Corp., Millford, MA and a 3396A integrator manufactured by Hewlett Packard, Wilmington, DE. The majority of HPLC runs were conducted using a 501 pump, U6K injector, 994 programmable photodiode array detector and 5200 printer/plotter, all from Waters. In all cases the detection wavelength was 205 nm. Peak heights were used to quantify analytes using external standards.

Two different reverse-phase separation columns were used. Both were 4.6-mm x 250-mm stainless steel columns packed with a silica-based anion exchanger. The Regis Chemical

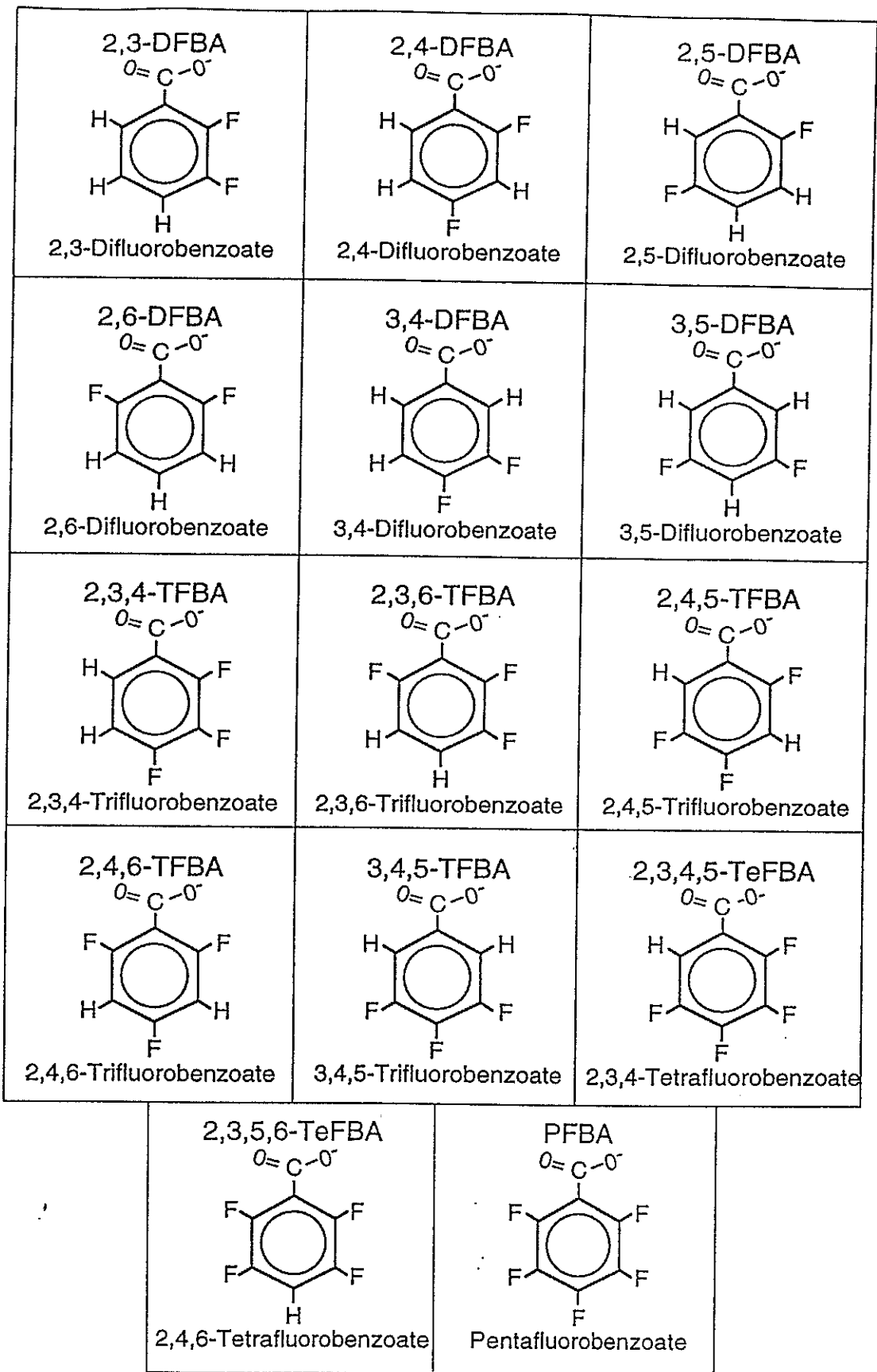


Figure 1. Fluorobenzoates tested in this study.

Co., Morton Grove, IL., column was packed with 5- $\mu$ m Spherisorb SAX. The Phenomenex, Torrance, CA, column was packed with 5- $\mu$ m Spherex 5SAX.

The mobile phase was a phosphate buffer ( $\text{KH}_2\text{PO}_4/\text{H}_3\text{PO}_4$ ) with 18% (v/v) acetonitrile added as an organic modifier. The phosphate concentration and pH varied, depending on the column age and chromatographic resolution. Buffer concentration ranged from 10 mM to 20 mM with the pH adjusted from 2.54 to 2.73 using  $\text{H}_3\text{PO}_4$  of the same molarity. The inorganic portion of the mobile phase was filtered through a 0.45- $\mu$ m nylon membrane filter. The acetonitrile was added after filtration. The eluent flow rate varied from 1.5 mL/min. to 1.7 mL/min., depending on system backpressure. Hamilton Co., Reno, NV, Series 800 glass 25- $\mu$ L syringes delivered the 25- $\mu$ L injection volume used for all the chromatographic analyses.

#### **$^{14}\text{C}$ Analyses**

Samples for  $^{14}\text{C}$  analysis were prepared by adding 10 mL of scintillation cocktail to 1 mL of sample in a glass scintillation vial with a foil-lined cap. Samples were kept in the dark until analysis. Scintillation counting was done using a Tri-Carb 460C Automatic Liquid Scintillation System manufactured by Packard Corp., Downers Grove, IL. All samples went through a 10-min count cycle.

#### **$^{14}\text{C}$ -2,6-DFBA Purity Test**

To check the purity of the  $^{14}\text{C}$ -2,6-DFBA, a 188-mg/L, 2- $\mu$ Ci/mL standard of this compound was injected into the HPLC. As the analysis was progressing, the effluent was collected in fractions over 1-min intervals. These fractions were then analyzed by scintillation counting. The distribution of  $^{14}\text{C}$  in the fractions was compared to the 2,6-DFBA chromatographic peak observed by UV detection at 205 nm.

## FLUOROBENZOATE CONCENTRATION BY SOLID PHASE EXTRACTION

### Solid Phase Extraction Procedure

Solid phase extraction (SPE) was investigated as a means to concentrate the tracers based on previous work by Stetzenbach et al. (1982) that showed Carbon-18 sorbents work well for trapping FBAs out of aqueous solutions. Four SPE media were investigated. Sep-Pak® "Classic"(C18, 360 mg), Sep-Pak® "Light"(C18, 130 mg), Sep-Pak® "Plus"(C8, 400 mg) and Empore® C18 disks were used. The Sep-Pak® cartridges were manufactured by Millipore Corporation, while the Empore® disk was manufactured jointly by Varian Corporation, Harbor City, CA and 3M Corporation, St. Paul, MN.

All Sep-Pak® cartridges were prepared by passing 10 mL methanol, then 10 mL water through them. Empore® C18 disks were prepared by soaking in acetone, then rinsing with water. The disk was housed in a reusable Teflon filter cartridge. All FBA test solutions were adjusted to  $1 < \text{pH} < 2$  with  $\text{H}_3\text{PO}_4$  before loading onto the cartridges. Unless otherwise noted, 10 mL of test solution was loaded at a rate of 1 mL/min  $\pm$  0.1 mL/min. Effluent off the cartridge during this step was labeled "waste." Different elution solutions were pushed through the SPE cartridge at 1 mL/min. and collected in increments of 1.0 or 2.0 mL. For all SPE cartridges, 30 mL of air was pushed through at the end of each solution addition to promote removal of excess fluid from the cartridge. All loadings were done by hand, or with a Waters Sep-Pak® vacuum manifold manufactured by Millipore Corp., Millford, MA.

## Elution Solution and Solid Phase Extraction Tests

Solid-phase-extraction tests were designed to indicate which Sep-Pak® best sorbed the FBAs and which phosphate buffer would elute the FBAs off in the smallest volume, thus maximizing the concentration.

A test solution containing 3,4-DFBA, 3,4,5-TFBA, 2,6-DFBA and PFBA at 10 mg/L each was loaded onto the three different Sep-Pak® cartridges. These FBAs were chosen because they represent the range of  $pK_a$  values of the 14 FBAs used in this study (Table 1). Analysis of the waste indicated whether all the FBAs had been retained on the SPE cartridge.

Table 1.  $pK_a$ s for fluorinated benzoic acids at 25°C (Benson and Bowman 1994).

| <u>Compound</u> | <u><math>pK_a</math></u> |
|-----------------|--------------------------|
| 3,4-DFBA        | 3.83(+/-0.01)            |
| 3,5-DFBA        | 3.59(+/-0.01)            |
| 2,4-DFBA        | 3.58(+/-0.01)            |
| 3,4,5-TFBA      | 3.54(+/-0.01)            |
| 2,3-DFBA        | 3.29(+/-0.01)            |
| 2,3,4-TFBA      | 3.30(+/-0.02)            |
| 2,5-DFBA        | 3.30(+/-0.01)            |
| 2,4,5-TFBA      | 3.28(+/-0.01)            |
| 2,3,4,5-TeFBA   | 3.08(+/-0.01)            |
| 2,6-DFBA        | 2.85(+/-0.01)            |
| 2,4,6-TFBA      | 2.83(+/-0.01)            |
| 2,3,6-TFBA      | 2.82(+/-0.01)            |
| PFBA            | 2.72(+/-0.01)            |
| 2,3,5,6-TeFBA   | 2.71(+/-0.01)            |



The retained FBAs were eluted off the cartridge with a phosphate buffer. Phosphate was used to minimize interference with the HPLC mobile phase. The concentration of phosphate and the pH of the elution solution were varied.  $\text{KH}_2\text{PO}_4$  was used for pHs below 4.5 while  $\text{K}_2\text{HPO}_4$  was used for higher pHs. Elution solution effluent was collected in 2-mL increments. The 2-mL increments were analyzed by HPLC to determine the volume of phosphate buffer needed to elute the FBAs off the SPE cartridge.

Ammonium acetate was investigated briefly as a possible elution solution, but was abandoned due to potential incompatibility problems with the HPLC mobile phase.

The best performing Sep-Pak® was compared to the Empore® C18 disk. A 100- $\mu\text{g/L}$  solution of  $^{14}\text{C}$  labeled 2,6-DFBA was prepared and loaded onto each cartridge. The most efficient phosphate buffer determined from the previous test was pushed through the cartridges and collected in 1-mL increments. The waste and elution increments were analyzed by scintillation counting.

After preliminary tests, we decided that the cartridge and elution solution that yielded at least a five-fold increase in concentration (elute fully in 2-mL or less) for at least one of the FBAs would be used for the remainder of the study.

### **Loading Capacity Tests**

One possible way to increase the concentration of the FBAs is to inject a larger volume of FBA solution through the Sep-Pak® thereby retaining more mass for subsequent elution. This was tested by using  $^{14}\text{C}$ -labeled 2,6-DFBA to determine the maximum amount of loading the Sep-Pak® "Light"(C18, 130 mg) could handle, and whether the maximum loading was dependent upon volume injected or concentration of FBA injected.

Two tests were done. One used a 100- $\mu\text{g/L}$  2,6-DFBA test solution and the other used a 50- $\mu\text{g/L}$  2,6-DFBA test solution. Both tests compared 10-mL, 20-mL, and 30-mL loadings. The elution solution was 20 mM  $\text{K}_2\text{HPO}_4/\text{H}_3\text{PO}_4$ , pH 8.0. Elution was in 1-mL increments to a total of 10 mL.

### **Fluorobenzoate Concentration Tests**

The purpose of these tests was to define conditions under which a suite of FBAs could be concentrated at least five-fold with near 100% recovery. This portion of the study was divided into three tests. The first test (Test 1) was designed to concentrate 10 mL of individual 100- $\mu\text{g/L}$  test solutions of each of the 14 FBAs listed in Table 1. Elution was with 20 mM  $\text{K}_2\text{HPO}_4/\text{H}_3\text{PO}_4$  at pH 7.81. Sequential 1-mL elution fractions were analyzed by HPLC, as was the waste.

For the second test (Test 2), the seven FBAs from Test 1 that were fully retained by the Sep-Pak® and that eluted in 2 mL or less (2,4-DFBA; 2,5-DFBA; 2,3-DFBA; 2,6-DFBA; 2,4,6-TFBA; 2,3,6-TFBA; and 2,3,5,6-TeFBA), were combined into a single solution containing 100- $\mu\text{g/L}$  of each FBA. This test was designed to concentrate and analyze the FBA mixture following the methodology described for Test 1.

The third test (Test 3) combined all five FBAs from Test 2 which were resolvable by HPLC (2,4-DFBA; 2,3-DFBA; 2,6-DFBA; 2,3,6-TFBA; and 2,3,5,6-TeFBA) into a single test solution which contained 100  $\mu\text{g/L}$  of each. Test solution concentration and analysis were performed according to Test 1 protocols.

Mobile phase used for the analysis of Test 3 FBAs was adjusted to 1.5 mM phosphate at a pH of 2.54 to optimize the resolution. The significant change in mobile phase

composition was needed to counteract decreased resolution due to an aging column. The acetonitrile fraction in the eluent remained unchanged at 18% (v/v).

### **Concentration Tests in a Natural Water Matrix**

Drainage water collected below an alfalfa field located in Las Nutrias, NM was used as the matrix for the FBA suite chosen in the previous section (2,4-DFBA; 2,3-DFBA; 2,6-DFBA; 2,3,6-DFBA; and 2,3,5,6-TeFBA). This was done to test the concentration/analysis procedure for FBAs dissolved in a typical shallow groundwater. The drainage water had an electrical conductivity of 1.1 mS/m, a Cl<sup>-</sup> concentration of 46 mg/L, and a NO<sub>3</sub><sup>-</sup> concentration of 0.33 mg/L. The five-component FBA test solution (100 µg/L of each tracer) was loaded, eluted and analyzed as in the "Fluorobenzoate Concentration Tests" described above. Elution increments of 2 mL were collected.

External standards for the HPLC analysis were prepared in Type I water. Las Nutrias water could not be used as the matrix for the standards because the nitrate present in the water interfered with 2,6-DFBA during chromatography. The nitrate was not present in the elution fractions, since it passed through the Sep-Pak® and into the waste during the concentration step. The 2,6-DFBA could not be quantified in the waste for this reason.

The mobile phase had to be adjusted again, due to a deteriorating HPLC column. Final mobile phase was 10 mM phosphate adjusted to pH 2.54 with 18% acetonitrile (v/v). Even with this change, the resolution between 2,3,6-TFBA and 2,3,5,6-TeFBA was poor. Due to increasing backpressure, flow rate had to be decreased from 1.7 mL/min. to 1.5 mL/min.

## RESULTS AND DISCUSSION

### $^{14}\text{C}$ LABELED 2,6-DFBA PURITY TEST

Figure 2 is a chromatogram comparing the UV detector response with the effluent fraction count rate for analysis of  $^{14}\text{C}$ -2,6-DFBA. Both detection modes show a concentration peak between 15 and 16 min. The apparent "lag" in the breakthrough of the  $^{14}\text{C}$  peak probably results from the discrete nature of the effluent collection and analysis. We concluded from the results depicted in Fig. 2 that the  $^{14}\text{C}$  activity in the labelled material was essentially all in the form of  $^{14}\text{C}$ -2,6-DFBA.

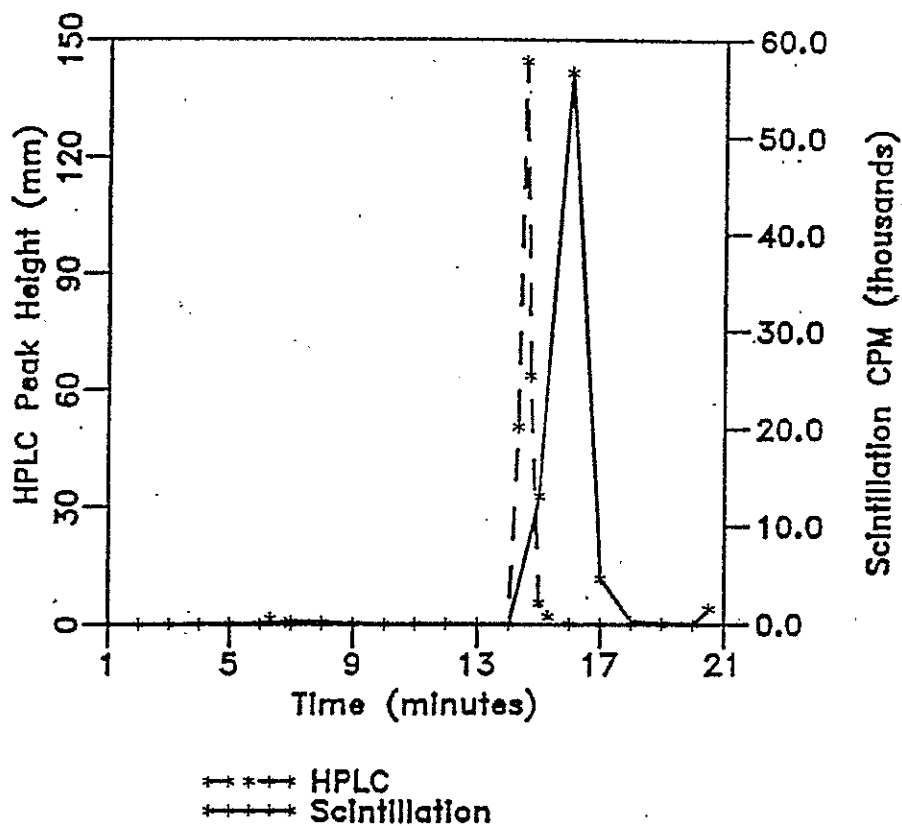


Figure 2: Distribution of  $^{14}\text{C}$  activity superimposed on chromatogram of  $^{14}\text{C}$ -labelled 2,6-DFBA.

## FLUOROBENZOATE CONCENTRATION BY SOLID PHASE EXTRACTION

### Elution Solution and Solid Phase Extraction Tests

Table 2 presents the results from these tests. 20 mM phosphate buffer at pH 3.00 was initially used to elute FBAs off the Sep-Pak® "Classic"(C18, 360 mg). This did not yield a five-fold increase in concentration for any of the FBA tests, and supported a hypothesis that the pH of the elution solution should be at least 2 units higher than the pK<sub>a</sub> of the species to be eluted. Increasing the elution pH to 6.05 gave much better results, and a pH of 7.81 increased recovery a bit more.

Table 2. Effect of phosphate concentration and pH on volume required for total elution of individual fluorobenzoates from three different Sep-Pak® SPE cartridges. Ten milliliters of a 10 mg/L solution initially loaded on each cartridge.

| <u>mM PO<sub>4</sub></u> | <u>pH</u> | <u>SPE</u> | <u>% <sup>14</sup>C- 2,6-DFBA<br/>in waste</u> | <u>Total Elution Volume (mL)</u> |                   |                 |                 |
|--------------------------|-----------|------------|--|----------------------------------|-------------------|-----------------|-----------------|
|                          |           |            |  | <u>3,4 DFBA</u>                  | <u>3,4,5-TFBA</u> | <u>2,6-DFBA</u> | <u>PFBA</u>     |
| 20                       | 3.00      | C18, 360mg | 0  | >36                              | >36               | 4               | 4               |
| 20                       | 6.05      | C18, 360mg | 0  | 14                               | 22                | 4               | 10              |
| 200                      | 6.05      | C18, 360mg | 0  | 18                               | >20               | 16 <sup>†</sup> | 16 <sup>†</sup> |
| 20                       | 6.05      | C8, 400mg  | 2.5  | 8                                | 10                | 4               | 6               |
| 200                      | 6.05      | C8, 400mg  | 6.5  | 10                               | 14                | 4               | 6               |
| 20                       | 6.05      | C18, 130mg | 0.2  | 4                                | 8                 | 2               | 4               |
| 20                       | 7.81      | C18, 130mg | 1.0  | 4                                | 6                 | 2               | 4               |

<sup>†</sup>2,6-DFBA and PFBA coeluted during HPLC analysis.

Increasing the phosphate concentration increased the elution volume in most cases. This may have been due to a "salting out" effect on the FBAs, hindering their release from the C-18 packing material.

Only the Sep-Pak® "Classic"(C18, 360mg) held all the FBAs during loading, but the required elution volumes were too high with this cartridge. Sep-Pak® "Plus"(C8, 400 mg) allowed as much as 6.5% of 2,6-DFBA to pass into the waste. The elution volumes with this cartridge were better than with the "Classic", but still not satisfactory. Sep-Pak® "Light"(C18, 130mg) allowed only 1% of 2,6-DFBA to pass into the waste, and yielded a five-fold concentration increase for 2,6-DFBA in the elution step. The 1% loss was considered acceptable, given the superior elution volumes for this cartridge. 2,6-DFBA was the only FBA detected in the waste with this cartridge. The Sep-Pak® "Light"(C18, 130 mg) was thus considered the best cartridge for our purposes and was used for all subsequent tests.

The Sep-Pak® "Light"(C18, 130 mg) performed better than the Empore® C18 disk in concentrating and eluting <sup>14</sup>C-2,6-DFBA. Both the Sep-Pak® the Empore® C18 disk efficiently trapped <sup>14</sup>C-2,6-DFBA, allowing less than 1.0% to pass into the waste. The Sep-Pak®, however, allowed 89% of the <sup>14</sup>C-2,6-DFBA to elute in the first mL, whereas the Empore® disk released only 3.0% of the FBA in the first mL. After 7 mL of elution, 90% of the <sup>14</sup>C-2,6-DFBA was eluted from the Sep-Pak® , whereas only 70% was eluted from the Empore® disk.

Based on the foregoing results, the Sep-Pak® "Light" (C18, 130mg) and a K<sub>2</sub>HPO<sub>4</sub>/H<sub>3</sub>PO<sub>4</sub> elution buffer of at least pH 7.8 were used in the remaining studies.

## Loading Capacity Tests

Results of the loading capacity tests for  $^{14}\text{C}$ -2,6-DFBA are given in Table 3. The larger the volume of 2,6-DFBA loaded onto the Sep-Pak®, the more 2,6-DFBA went into waste. Also, the larger the volume of 2,6-DFBA loaded onto the Sep-Pak®, the smaller the ultimate percent recovery. For each loading rate and volume, the majority of  $^{14}\text{C}$ -2,6-DFBA was recovered in the first mL of elution.

Table 3. Percent  $^{14}\text{C}$ - 2,6-DFBA recovered after 100- $\mu\text{g/L}$  and 50- $\mu\text{g/L}$  loadings onto Sep-Pak® "Light" cartridges (C18,130 mg) using various volumes, and elution with pH 7.81 phosphate buffer.

| Fraction (mL) | Concentration in Loading Solution |             |             |                    |             |             |
|---------------|-----------------------------------|-------------|-------------|--------------------|-------------|-------------|
|               | 100 $\mu\text{g/L}$               |             |             | 50 $\mu\text{g/L}$ |             |             |
|               | Volume Loaded                     |             |             | Volume Loaded      |             |             |
|               | <u>10mL</u>                       | <u>20mL</u> | <u>30mL</u> | <u>10mL</u>        | <u>20mL</u> | <u>30mL</u> |
|               | % Recovery                        |             |             |                    |             |             |
| Waste         | 2.6                               | 14.9        | 20.8        | 1.2                | 16.1        | 24.3        |
| 1             | 99.5                              | 73.2        | 41.0        | 90.6               | 61.8        | 29.9        |
| 2             | 0.4                               | 0.5         | 0.3         | 2.8                | 5.0         | 1.3         |
| 3             | 0.1                               | 0.0         | 0.1         | 1.0                | 0.8         | 0.2         |
| 4             | 0.1                               | 0.0         | 0.1         | 0.2                | 0.2         | 0.0         |
| 5             | 0.0                               | 0.0         | 0.1         | 0.2                | 0.2         | 0.0         |
| 6             | 0.0                               | 0.1         | 0.1         | 0.0                | 0.1         | 0.0         |
| 7             | <u>0.0</u>                        | <u>0.1</u>  | <u>0.0</u>  | <u>0.0</u>         | <u>0.1</u>  | <u>0.0</u>  |
| Total         | 102.7                             | 89.1        | 62.5        | 96.0               | 84.3        | 55.6        |

Only the 10-mL loading had all the  $^{14}\text{C}$ -2,6-DFBA recovered in the first mL of elution. The 20 and 30 mL loadings fared much worse, with as little as 56% recovered after a 7 mL elution.

Some of the 2,6-DFBA in the 20 and 30 mL loadings was not accounted for in the waste or elution fractions, apparently remaining on the Sep-Pak®.

Both 50- $\mu\text{g/L}$  and 100- $\mu\text{g/L}$  standards yielded similar results. The limiting factor appeared to be the volume passed through the Sep-Pak® to concentrate the  $^{14}\text{C}$ -2,6-DFBA. The results shown in Table 3 indicated that increasing the concentration of the FBAs could not be accomplished by increasing the volume of solution loaded onto the SPE cartridge beyond 10 mL.

### **Tracer Concentration Tests**

Concentration of single-FBA test solutions was evaluated first (Test 1). The results are listed in Table 4. Recoveries were much greater than 100% for several of the FBAs eluting in more than 2 mL. The reasons for these extreme recoveries are not known. Generally, the greater the elution volume, the higher the percent recovery calculated. This may have resulted from cumulative errors in analyzing the multiple fractions in which these tracers were detected.



Table 4. Results from concentration and elution of single-tracer solutions on Sep-Pak® "Light" (C18,130 mg) cartridges.

| <u>FBA</u>    | <u>HPLC Retention Time (minutes)</u> | <u>mL to Fully Elute</u> | <u>% in Waste</u> | <u>% Recovery</u> |
|---------------|--------------------------------------|--------------------------|-------------------|-------------------|
| 2,4-DFBA      | 3.0                                  | 2                        | 0.0               | 142               |
| 2,5-DFBA      | 3.6                                  | 2                        | 0.0               | 91                |
| 2,3-DFBA      | 3.7                                  | 2                        | 0.6               | 119               |
| 2,6-DFBA      | 6.3                                  | 1                        | 1.6               | 113               |
| 2,4,6-TFBA    | 6.3                                  | 2                        | 0.0               | 102               |
| 2,3,6-TFBA    | 7.1                                  | 2                        | 0.0               | 122               |
| 2,3,5,6-TeBA  | 7.3                                  | 2                        | 0.0               | 102               |
| 3,5-DFBA      | 3.2                                  | >5                       | 15.3              | 196               |
| 3,4-DFBA      | 3.5                                  | >5                       | 12.5              | 439               |
| 2,4,5-TFBA    | 3.8                                  | >3                       | 0.0               | 69                |
| 2,3,4-TFBA    | 3.9                                  | >4                       | 0.0               | 105               |
| 3,4,5-TFBA    | 4.2                                  | >5                       | 0.0               | 103               |
| 2,3,4,5-TeFBA | 4.9                                  | >5                       | 0.0               | 85                |
| PFBA          | 7.2                                  | 4                        | 0.0               | 182               |

The seven FBAs that eluted in 2 mL or less (top half of Table 4) were combined in a single suite (Test 2). Under the HPLC conditions used, 2,6-DFBA and 2,4,6-TFBA coeluted (Figure 3). Because 2,6-DFBA was eluted off the Sep-Pak® faster (1 mL) than 2,4,6-TFBA (2 mL), and because it is a more commonly used tracer, it was chosen to be part of the final suite.

The isomers 2,5- and 2,3-DFBA were not quantitatively resolvable (Figure 3). 2,3-DFBA was chosen over 2,5-DFBA for further testing because its peak height was greater and therefore yielded higher sensitivity.

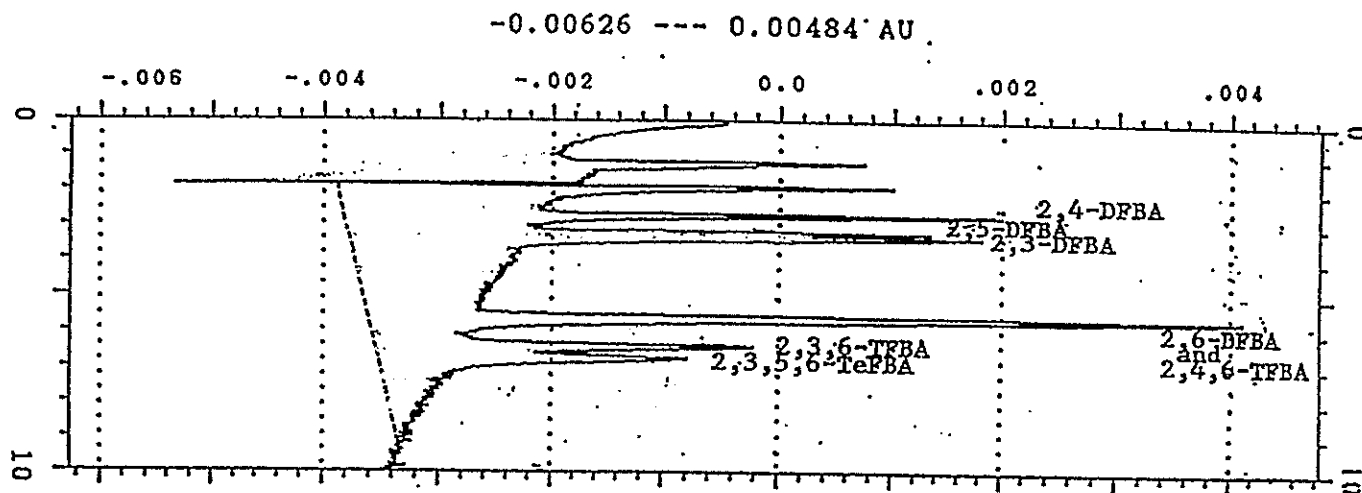


Figure 3. The seven FBA compounds that eluted in 2 mL or less. 1000  $\mu\text{g/L}$  standard in Type I water. Mobile phase: 0.015 M  $\text{KH}_2\text{PO}_4/\text{H}_3\text{PO}_4$ , pH 2.54, 18%  $\text{CH}_3\text{CH}$ , 1.7 mL/min.

The five remaining FBAs: 2,4-DFBA; 2,3-DFBA; 2,6-DFBA; 2,3,6-TFBA; and 2,3,5,6-TeFBA, were combined into one test solution for Test 3 (Figure 4). There were no problems through the concentration and analysis procedure. A correlation between recovery and elution order off the Sep-Pak® was noted in all the tests described above (Table 5).

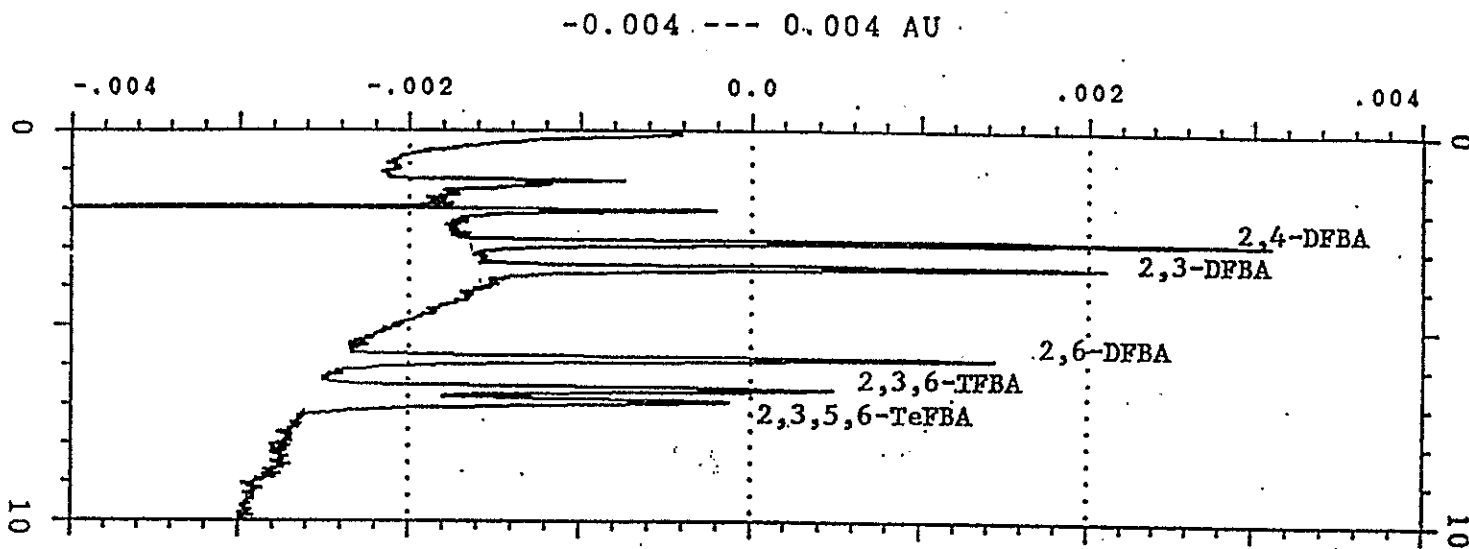


Figure 4. The five FBA compounds that had good resolution by HPLC. 1000  $\mu\text{g/L}$  standard in Type I Water. Mobile phase:  $\text{KH}_2\text{PO}_4/\text{H}_3\text{PO}_4$ , pH 2.54, 18%  $\text{CH}_3\text{CN}$ , 1.7 mL/min.

Table 5. Correlation between retention time and percent recovery for fluorobenzoates eluting off Sep-Pak® "Light" (C18,130 mg) cartridges in 2 mL or less.

| FBA (in order of elution) | % Recovery |        |        |
|---------------------------|------------|--------|--------|
|                           | Test 1     | Test 2 | Test 3 |
| 2,4-DBFA                  | 154        | 124    | 113    |
| 2,5-DFBA                  | 124        | 120    | †      |
| 2,3-DFBA                  | 121        | 106    | 107    |
| 2,6-DFBA                  | 115        | 102    | 86     |
| 2,4,6-TFBA                | 102        | 104    | †      |
| 2,3,6-TFBA                | 121        | 94     | 91     |
| 2,3,5,6-TeFBA             | 102        | 87     | 87     |

†This fluorobenzoate not included in this test.

## Concentration Tests in a Natural Water Matrix

Figure 5 shows a chromatogram for the five-FBA suite prepared in Las Nutrias drainage water. Results for concentration of the FBAs from the Las Nutrias drain water were similar to the results for the FBAs prepared in Type I water (compare Tables 5 and 6). The data in Table 6 show the results of two replications of the concentration/elution/analysis cycle for the FBAs in the Las Nutrias water. The lower recoveries for 2,3,6-TFBA and 2,3,4,6 TeFBA in the Las Nutrias matrix may be due to the relatively poor chromatographic separation of these two compounds in these tests (Fig. 5). Again, recoveries were somewhat lower for FBAs that eluted off the Sep-Pak® later. Although the initial experiments had shown that all the FBAs of Tables 5 and 6 were eluted off the Sep-Pak® in 2 mL or less, it's possible that some of the higher  $pK_a$ , more strongly retained FBAs were not completely eluted under these conditions. This would explain the less than quantitative recoveries of these compounds.

Table 6. Percent recoveries using Sep-Pak® "Light" (C18,130 mg) for fluorobenzoate standards in Las Nutrias water matrix.

| <u>FBA (in order of elution)</u> | <u>Run A</u> | <u>Run B</u> | <u>Average</u> |
|----------------------------------|--------------|--------------|----------------|
| 2,4-DFBA                         | 107          | 111          | 109            |
| 2,3-DFBA                         | 90           | 115          | 103            |
| 2,6-DFBA                         | 78           | 95           | 87             |
| 2,3,6-TFBA                       | 71           | 91           | 81             |
| 2,3,5,6-TeFBA                    | 70           | 93           | 82             |

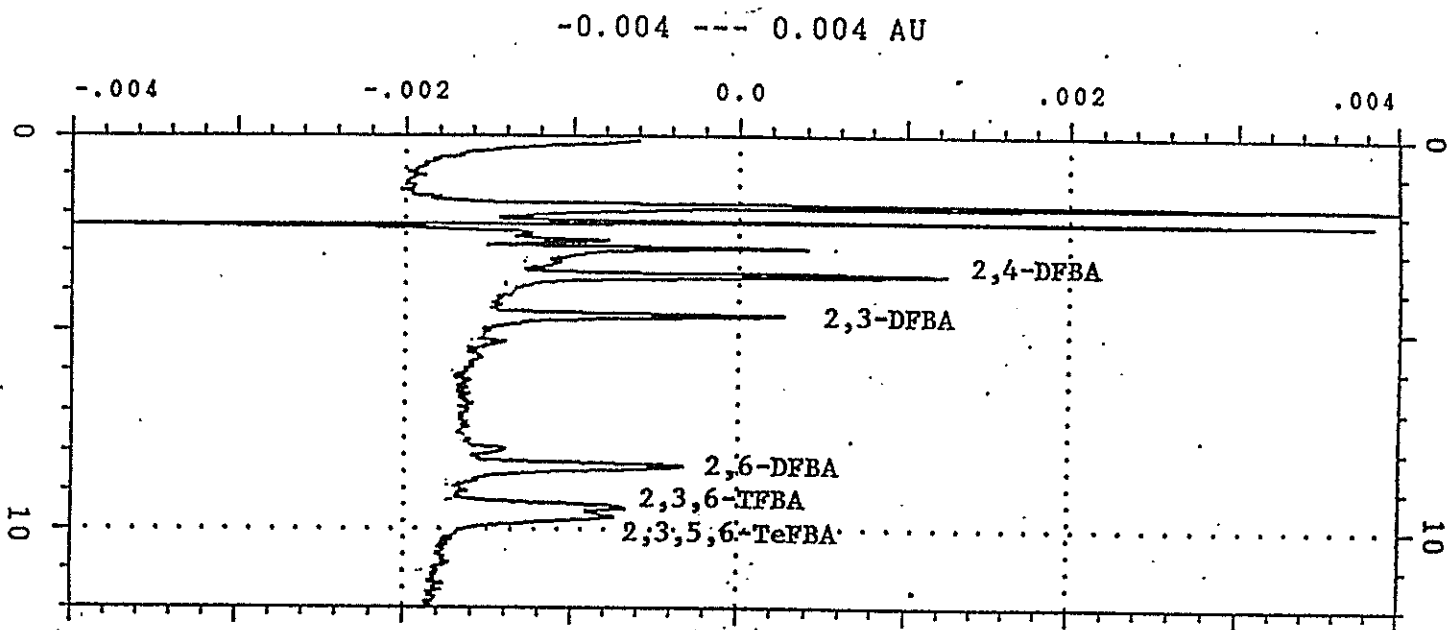


Figure 5. Test in Las Nutrias water matrix. Mobile phase: 0.015 M  $\text{KH}_2\text{PO}_4/\text{H}_3\text{PO}_4$ , pH=2.54, 18%  $\text{CH}_3\text{CN}$ , 1.7 mL/min.

Nevertheless, the results of Tables 5 and 6 show this select group of five FBAs can be concentrated five-fold with reasonable recoveries regardless of whether they're in a matrix of pure water or a water more representative of typical shallow groundwaters.

## SUMMARY AND CONCLUSIONS

The experiments described in this study determined a method to concentrate seven FBA compounds by a factor of five. Concentrating these compounds gives more flexibility for their use. A lower detection limit means a smaller amount of these expensive tracers needs to be used in any given experiment, or the tracer can be left in the groundwater to travel farther, and be diluted more, before retrieval.

Seven of the FBAs reviewed in this study (2,3-DFBA; 2,4-DFBA; 2,5-DFBA; 2,6-DFBA; 2,3,6-TFBA; 2,4,6-TFBA; and 2,3,5,6-TeFBA) can be concentrated by a factor of five using a Waters Sep-Pak® "Light" (C18, 130 mg) cartridge for the sorbent and a pH 7.81 phosphate buffer for the elution solution.

Five of these FBA tracers (2,4-DFBA, 2,3-DFBA, 2,6-DFBA, 2,3,6-TFBA and 2,3,5,6-TeFBA) can be concentrated in one run and analyzed simultaneously by HPLC. It would be possible to have different suites by trading 2,5-DFBA for 2,3-DFBA and 2,4,6-TFBA for 2,6-DFBA. These FBAs coeluted under our HPLC conditions, and could not be included in the same suite.

Increasing the concentration of the FBAs cannot be accomplished by increasing the volume of solution pushed through the Sep-Pak® beyond 10 mL. Increasing the volume causes a higher percentage of the FBA to be passed into the waste, and a lower percentage of the retained FBA to be recovered in the elution step.

Concentration and analysis of the FBAs was not affected by the presence of dissolved components of irrigation drainage water.

In summary, the recommended protocol for concentrating mixtures of 2,3-DFBA; 2,4-DFBA; 2,5-DFBA; 2,6-DFBA; 2,3,6-DFBA; 2,4,6-TFBA; and/or 2,3,5,6-TeFBA is:

1. Condition a Sep-Pak® "Light" (C18, 130 mg) cartridge by passing 10 mL of methanol through it, followed by 30 mL of air.
2. Rinse Sep-Pak® with 10 mL of Type I water, followed by 30 mL of air.
3. Using H<sub>3</sub>PO<sub>4</sub>, acidify 10 mL of the FBA solution to be concentrated to a pH of 1 to 2.
4. Pass 10 mL of the FBA solution through the Sep-Pak® cartridge, followed by 30 mL of air.
5. Elute the retained FBAs by passing 2 mL of 20 mM, pH 7.81 K<sub>2</sub>HPO<sub>4</sub> buffer through the Sep-Pak®, followed by 30 mL of air.
6. Analyze the eluted solution of step 4 by HPLC using the chromatographic conditions described in the Materials and Methods section.

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