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ANALYSIS OF SOIL EXTRACTS FOR INORGANIC AND ORGANIC TRACER ANIONS VIA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic technique allowing simultaneous quantification of Br^-, SCN^-, I^- and four fluorinated benzoic acid derivatives in soil extracts is described. The procedure requires minimal sample pretreatment and allows accurate measurements of tracer concentrations in the presence of high background levels of Cl^-, NO_3^- and naturally-occurring organic solutes, although high NO_2^- levels can cause interference. The technique has proven reliable for analyzing extracts from a variety of sources having wide variations in tracer concentrations.

INTRODUCTION

High-performance liquid chromatography (HPLC) combined with variable-wavelength ultraviolet (UV) photometric detection has recently been shown to be an effective technique for analysis of inorganic and organic anions. Cortes[1] used an amino column combined with UV detection at 205 nm to analyze mixtures of low-molecular-weight organic acids and inorganic anions. He pointed out the usefulness of such a system in the analysis of samples high in background levels of UV-transparent ions such as chloride and sulfate. Stetzenbach and Thompson[2] determined chloride, bromide, nitrate, thiocyanate and iodide in groundwater, making use of an anion-exchange column and UV detection at 195–205 nm. They found this technique superior to colorimetric and ion-selective methods in terms of speed and accuracy in quantifying these anions. Reeve[3] analyzed for inorganic anions using a cyano column in combination with an ion pairing reagent at 215 nm. He observed detection limits in the nanogram range. Thayer and Huffaker[4] used HPLC with UV detection at 210 nm to measure nitrite and nitrate levels in biological samples.

The ability to simultaneously measure sub-ppm levels of compounds of diverse chemical nature is particularly valuable in analyses of environmental samples. Such samples typically vary widely in the types and amounts of species they contain.

The present study arose from a need to evaluate the suitability of several types of anions for use as tracers of water movement in soils. Soil solutions typically con-

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tain a wide variety of inorganic and organic compounds[5]. A rapid, accurate technique was required to quantify the tracers at nanogram levels in the presence of much higher concentrations of naturally-occurring solutes, which included Cl^- (100–1000 ppm), NO_3^- (0–10 ppm), NO_2^- (0–10 ppm) and unidentified soluble organic substances. The tracers of interest were Br^-, I^-, SCN^- and four fluorinated derivatives of benzoic acid: 2,6-difluorobenzoic acid (2,6-DFBA), pentafluorobenzoic acid (PFBA), *o*-(trifluoromethyl)benzoic acid (*o*-TFMBA) and *m*-(trifluoromethyl)benzoic acid (*m*-TFMBA). Br^- and SCN^- have been used to monitor water movement in soils and aquifers. I^- has found less use as a soil–water tracer, probably due to the relative ease of oxidation of I^- to iodine. PFBA and *m*-TFMBA have been successfully used as tracers of groundwater flow[6]. They appear to be resistant to chemical and biological degradation and, as anions at near-neutral pH values, show little or no adsorption on most geologic materials. 2,6-DFBA and *o*-TFMBA have not previously been used as tracers, but show promise due to their chemical similarities to the other two fluorobenzoates. The fluorobenzoates should make particularly useful soil-water tracers since they are not native to the environment and because they are detectable at very low concentrations.

Little work appears to have been done regarding HPLC analysis of soil solutions. This paper describes an HPLC technique which allowed reliable measurement of the species of interest in soil extracts with a minimum of sample preparation. With minor modifications, the technique should be suitable for analysis of other anions of interest in natural waters.

EXPERIMENTAL

Chromatography

The HPLC instrumentation consisted of a Model 6000A pump, Model U6K syringe injector and Model 480 variable-wavelength UV absorption detector, all by Waters Assoc. (Milford, MA, U.S.A.)*. Signal output was monitored with a Beckman Model 1005 strip-chart recorder and a Hewlett-Packard Model 3390A recording integrator.

Analytical columns used for this work were packed with the strong anion exchanger Partisil 10 SAX (Whatman, Clifton, NJ, U.S.A.). The columns, each of which was 250 × 4.6 mm I.D., were prepared by Whatman or by Regis (Morton Grove, IL, U.S.A.). During analyses, the analytical column was preceded by a 50 × 4.6 mm I.D. guard column, packed with pellicular anion exchanger (Whatman). The mobile phase was pre-saturated with silica by passage through a 250 × 4.6 mm I.D. column packed with 37–53 μm silica and placed in the flow path before the injector. The mobile phase used in this work was 0.005 *M* Potassium dihydrogen phosphate (KH_2PO_4) buffer, pH adjusted to 4.0 with orthophosphoric acid, with 10% acetonitrile as an organic modifier. The flow-rate of the mobile phase was 2 ml/min.

* Mention of company and trade names is for the convenience of the reader and does not imply any preferential treatment or endorsement by the U.S. Department of Agriculture.

Chemicals

2,6-DFBA, PFBA and *o*-TFMBA were obtained from Aldrich (Milwaukee, WI, U.S.A.). *m*-TFMBA was obtained from PCR Research Chemicals (Gainesville, FL, U.S.A.). Each of these compounds had a purity in the range of 97–99% according to the manufacturers.

All inorganic chemicals used were reagent grade. Type I water for eluent preparation was generated using a Water-I water purifier (Gelman Sciences, Ann Arbor, MI, U.S.A.) with distilled water input. Acetonitrile for eluent preparation was OmniSolve HPLC grade (MCB Manufacturing Chemists, Gibbstown, NJ, U.S.A.). The aqueous portion of the eluent was filtered through a 0.45-μm nylon filter prior to the addition of acetonitrile.

Sample preparation

Samples of the soil solution were obtained by two methods. One method involved direct extraction of soil water in the field by applying vacuum to porous ceramic samplers buried in the soil. The other technique involved preparation of a paste by mixing water with bulk soil samples collected using an auger. Solution was extracted from the paste via suction filtration through Whatman No. 42 filter paper. Soil solution extracts collected by either method were then filtered using a centrifugal filter with a 0.2-μm nylon membrane (Rainin Instrument, Woburn, MA, U.S.A.). The filtered samples were introduced into the chromatograph by syringe injection without further pretreatment.

RESULTS AND DISCUSSION

Of major concern in the development of this analytical technique was the ability to accurately quantify the compounds of interest in the presence of high background levels of species naturally present in the soil extracts. The operating wavelength was chosen to maximize the response of the tracer ions (Br^-, SCN^-, I^-, *o*-TFMBA, *m*-TFMBA, 2,6-DFBA and PFBA) while minimizing the responses of the major inorganic contaminants (Cl^-, NO_3^- and NO_2^-). Fig. 1 presents the normalized detector responses *vs.* wavelength from 190 to 225 nm for the seven anionic tracers. With the exception of I^-, which shows an absorbance minimum at 210 nm, the compounds of interest display a regular increase in extinction coefficient with decreasing wavelength.

The wavelength responses for the major contaminants in a soil extract which contains no tracer are shown in Fig. 2. NO_2^- shows an absorbance maximum at 210 nm, with a gradual reduction in absorbance as the wavelength is lowered to 190 nm. Cl^- and NO_3^- elute together under the chromatographic conditions used (see below), and thus the response shown in Fig. 2 represents a combination of the individual ion wavelength responses. Although the relative amounts of NO_3^- and Cl^-, and thus the composite wavelength response, varied among soil samples, the trend of decreasing absorbance with increasing wavelength shown in Fig. 2 was typical. The absorbance of a Cl^- standard increases rapidly as the wavelength is reduced below 200 nm (Fig. 2). Since Cl^- was the contaminant with the highest concentration in the samples of interest, a working wavelength of 205 nm was found to be optimal for routine analyses. At 205 nm, NO_3^- absorbance is near maximum[2]; thus, the intensity of the NO_3^-/Cl^- peak was most sensitive to fluctuations in NO_3^- levels.

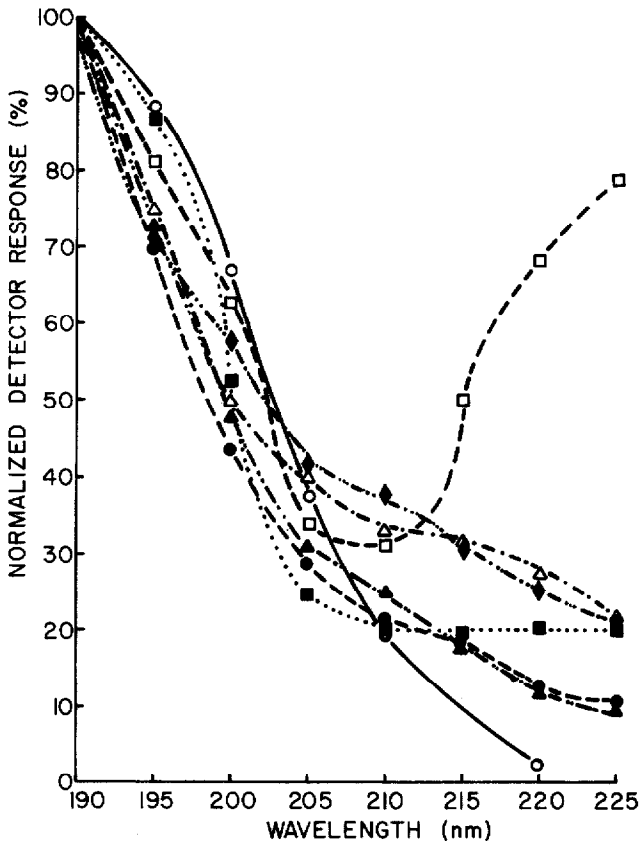

Fig. 1. Normalized detector responses of the tracer anions. ○, Br^-; □, I^-; △, SCN ; ●, 2,6-DFBA; ■, *m*-TFMBA; ▲, *o*-TFMBA; ◆, PFBA.

The chromatogram of a standard seven tracer mixture prepared in distilled water, with a detection wavelength of 205 nm, is illustrated in Fig. 3. The time required for a complete chromatogram was 22 min. The delay in elution of the first tracer peak (*m*-TFMBA), and the gap between elution of 2,6-DFBA and Br^-, were necessary in order to avoid interference from solutes naturally present in the soil solution. Fig. 4 shows the chromatogram of a typical soil extract which contains no tracer. The multiple peaks which elute with and immediately after the solvent are of unknown composition, but are most likely due to an array of charged and uncharged small organic molecules typically found in soil extracts. The peak at 11.8 min (k' = 5.7) is due to NO_2^-. This peak was not present in all extracts but, when present in large amounts, interfered with quantification of 2,6-DFBA (12.5 min, k' = 6.1). The other major contaminant peak in Fig. 4 (14.3 min, k' = 7.2) is composed of both Cl^- and NO_3^-.

Table I presents retention times and detection limits for the tracer standard solution of Fig. 3. Some comment is required on the elution order of the last three tracers (Br^-, SCN^- and I^-). Based on ionic radii considerations, simple ion-ex-

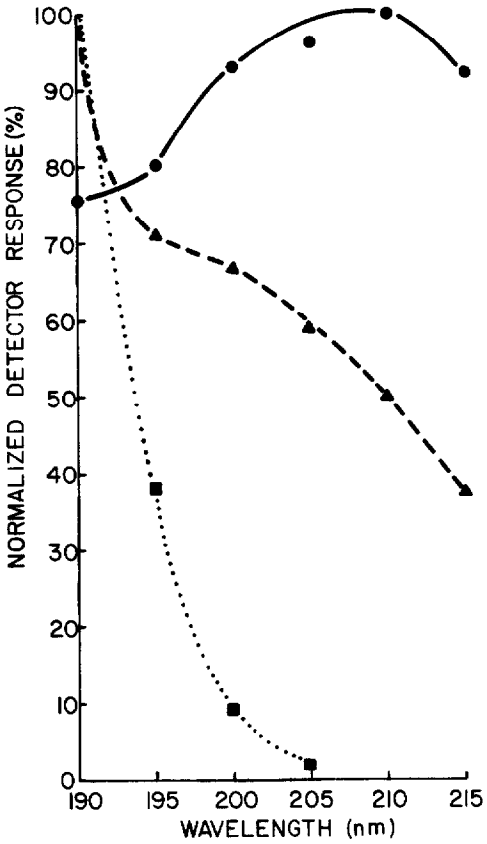

Fig. 2. Normalized detector responses for soil extract contaminants and a chloride standard. ●, NO_2^- (k' = 5.7); ▲, NO_3^- and Cl^- (k' = 7.2); ■, Cl^-.

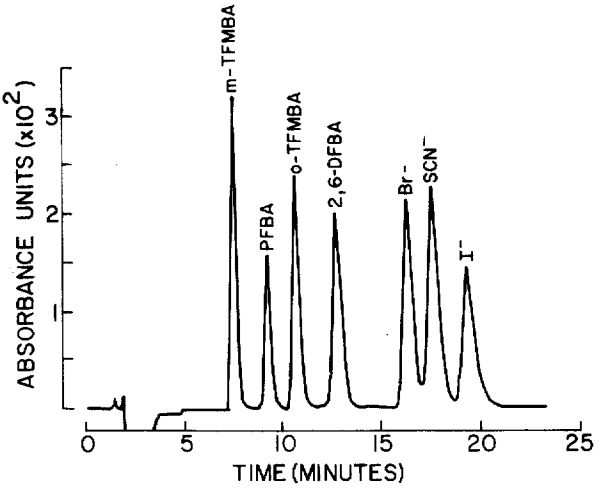

Fig. 3. Chromatogram, using the Regis column, of a standard tracer solution prepared in distilled water. Concentration of each anion equals 5 ppm; 100-μl injection; a.u.f.s. = 0.10; detection wavelength 205 nm; other chromatographic conditions as described in the Experimental section.

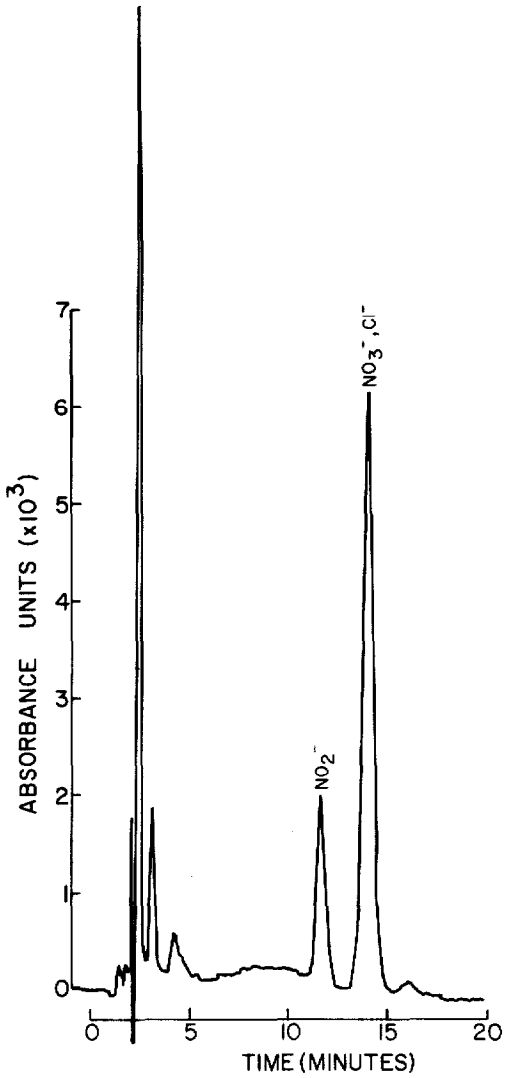

Fig. 4. Chromatogram, using the Regis column, of a soil extract which contains no tracer, showing major background components. 25-μl injection with a.u.f.s. = 0.01; detection wavelength 205 nm; other chromatographic conditions as described in the Experimental section.

change theory predicts an elution order Br^-, I^-, SCN^-. Indeed, this is the elution sequence noted with most ion-exchange resins. The elution reversal of I^- and SCN^- indicates the contribution of secondary retention mechanisms, in addition to simple anion exchange, with the Partisil 10 SAX packing. Stetzenbach and Thompson[2] likewise obtained the elution sequence Br^-, I^-, SCN^- with Partisil 10 SAX and a phosphate buffer eluent. The nature of the secondary mechanisms is not currently clear.

Detection limits for tracer standards were in the range of 1.2 to 3.5 ng (Table I). For analysis of the anions in soil extracts, practical limits for reliable quantifi-

TABLE I

RETENTION TIMES AND DETECTION LIMITS (AT 205 nm) FOR THE TRACER ANIONS

Column, Whatman Partisil 10 SAX; eluent, 0.005 *M* KH_2PO_4, pH 4.0, water–acetonitrile (90:10, v/v); a.u.f.s. 0.01.

Anion	*Retention time (min)*	*k'*	*Detection limit (ng)**	*r.s.d.** (%)*
m-TFMBA	7.3	3.2	1.2	2.5
PFBA	9.0	4.1	2.5	3.9
o-TFMBA	10.4	4.9	1.7	3.6
2,6-DFBA	12.5	6.1	2.1	2.9
Br^-	16.1	8.2	2.1	2.3
SCN^-	17.4	8.9	1.9	3.6
I^-	19.1	9.9	3.1	4.2

* Calculated as twice random baseline noise. Based on four 5-μl injections of a standard solution having a 5-ppm concentration of each anion.

** Relative standard deviation of detection limit.

cation were about an order of magnitude higher than the values shown in Table I. Recovery and precision data for a soil extract spiked with 25 ng of each anion are presented in Table II. Recoveries ranged from 91 to 138%, with generally greater accuracy obtained for the early-eluting fluoroorganic species.

A chromatogram for a typical tracer-containing soil extract is presented in Fig. 5. The presence of NO_2^-, Cl^-/NO_3^- and early-eluting contaminant peaks is evident. As with most of the soil extract solutions analyzed, the NO_2^- level is low in Fig. 5, and 2,6-DFBA could be accurately quantified. Occasionally, samples high in NO_2^- and/or low in 2,6-DFBA were encountered in which interference by the NO_2^- peak was severe. Fig. 6 is an example of such a chromatogram. In these cases, swamping of the 2,6-DFBA peak by NO_2^- precluded accurate determination of 2,6-DFBA.

TABLE II

RECOVERIES OF TRACER ANIONS ADDED TO SOIL EXTRACT

Chromatographic conditions as in Table I.

Anion	*Amount injected (ng)*	*Amount detected (ng)**	*Average recovery (%)*
m-TFMBA	25.0	26.3 (±1.1)	105
PFBA	25.0	26.1 (±1.1)	104
o-TFMBA	25.0	24.8 (±0.0)	99.1
2,6-DFBA	25.0	22.7 (±0.0)	90.6
Br^-	25.0	26.9 (±0.3)	108
SCN^-	25.0	34.6 (±0.0)	138
I^-	25.0	30.5 (±0.9)	122

* Based on three 100-μl injections of a soil extract spiked with 0.25 ppm of each anion. Figures in parentheses are standard deviations.

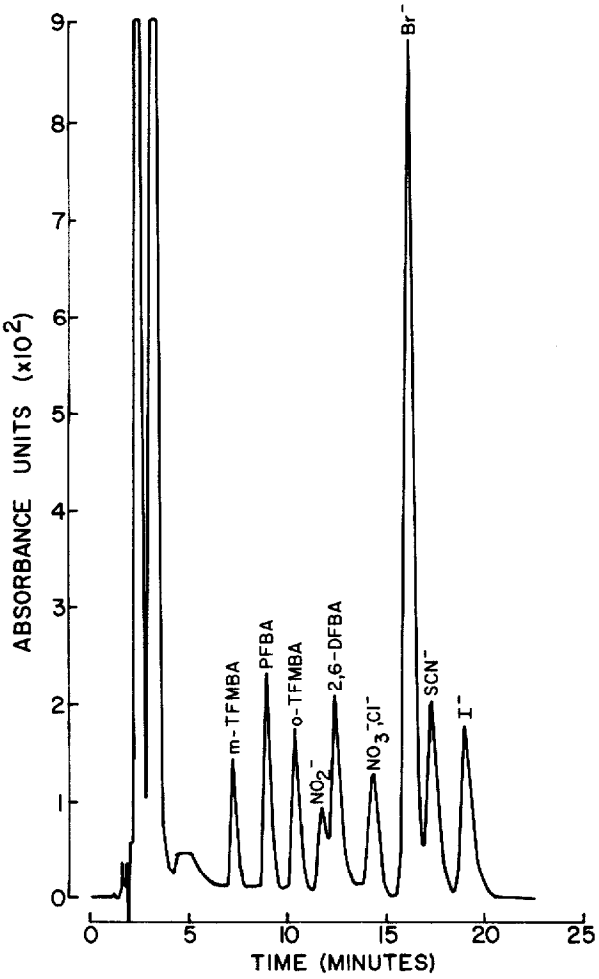

Fig. 5. Chromatogram, using the Regis column, of a tracer-containing soil extract, showing influence of background components. 100-μl injection; a.u.f.s. = 0.10; detection wavelength 205 nm; other chromatographic conditions as described in the Experimental section.

Attempts to convert NO_2^- to NO_3^- by hot acid treatment[7] failed to yield quantitative conversion in the soil extracts. Treatment with 3% hydrogen peroxide and exposure to sunlight effected quantitative conversion of NO_2^- to NO_3^-, but resulted in the oxidation of 2,6-DFBA and the other fluoroorganic compounds as well. Conversion of NO_2^- to nitrogen gas via treatment with sulfamic acid (NH_2SO_3H)[8] was quantitative in standards spiked with NO_2^- and did not affect tracer-ion peak heights; with soil extracts, however, NH_2SO_3H was consumed by the early eluting organic components. The addition of NH_2SO_3H in sufficient concentrations to remove NO_2^- in soil extracts resulted in new interferences from the reaction products. For routine analyses in which NO_2^- interference is a persistent problem, chromatographic conditions could be altered to give better separation from 2,6-DFBA. Alternatively, dual

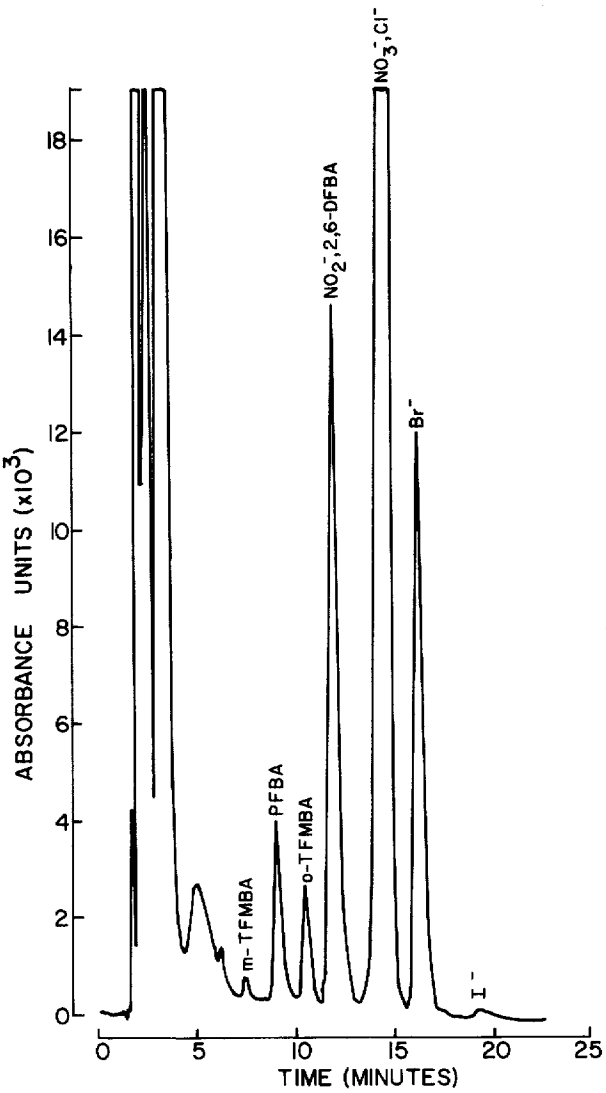

Fig. 6. Chromatogram, using the Regis column, of a tracer-containing soil extract, showing interference of 2,6-DFBA quantification due to a high NO_2^- level. 25-μl injection; a.u.f.s. = 0.02; detection wavelength 205 nm; other chromatographic conditions as described in the Experimental section.

wavelength UV detection with two detectors in series, or running samples twice at different wavelengths, would allow determination of 2,6-DFBA in the presence of NO_2^- overlap.

Although both the Regis and Whatman columns used were packed with the same material (Partisil 10 SAX), the Regis column proved superior in separating NO_2 from 2,6-DFBA. The Whatman columns, in fact, failed to provide this resolution even at low NO_2 levels. Columns from both manufacturers exhibited a de-

crease in resolving power over time, with or without regular methanol column rinsings. After about 350 h of operation at 2 ml/min with the phosphate–acetonitrile eluent, the packed bed of either manufacturer's column began to collapse, and the back pressure increased rapidly from its initial value of about 1000 p.s.i. At this point resolution was severely degraded, and attempts to salvage the columns by adding additional packing material were unsuccessful. The lifetime of the Regis column was decreased even further when the flow direction was reversed regularly, as recommended by the manufacturer.

A linear response between tracer peak heights and injection volume of a solution with a 5-ppm concentration of each tracer was noted up to an injection volume of 250 μl (Fig. 7). With injection volumes greater than 100 μl, however, the early-eluting peaks which followed the solvent front, and/or the Cl^-/NO_3^- peak (Fig. 5) increased in width to the point that they sometimes interfered with tracer quantifi-

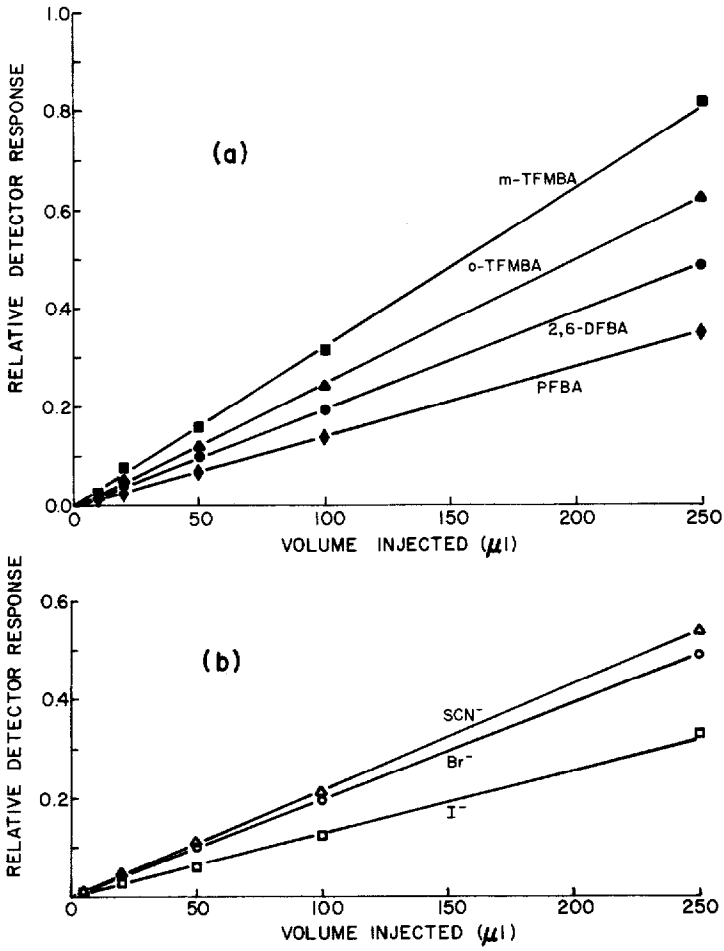

Fig. 7. Relationship of detector response to injection volume for the organic (a) and inorganic (b) tracer anions.

cation. For routine analyses, therefore, injection volumes were limited to 100 μl or less.

The analytical procedure described above has proven rapid and accurate for analysis of soil extracts obtained under a variety of conditions ranging from direct extraction of solution from unsaturated soil in the field, to laboratory extraction of bulk soil samples. Avoiding timeconsuming clean-up and pre-concentration procedures has allowed increased sample throughput and reduced errors by minimizing sample handling. Due to decreases in column efficiency over time, however, column replacement has been required after several months of continuous use.

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