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Determination of hexazinone and its metabolites in groundwater by capillary electrophoresis

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Abstract

A micellar electrokinetic chromatographic method was developed to separate and quantify hexazinone and metabolites C, A1, E, B and D in groundwater. Hexazinone and its metabolites were extracted from water using Supelclean ENVI-Carb solid-phase extraction tubes. Quantitation was performed using UV photodiode detection at 220, 225, 230 and 247 nm. Intra-assay and inter-assay reproducibility studies run at 0.5, 1.0, 2.0 and 5.0 ppb indicated the procedure was reproducible. Groundwater samples collected from US Geological Survey monitoring wells were analyzed for hexazinone and its metabolites by CE. A comparison was made between CE and an established HPLC method of the hexazinone and metabolite B. The linear regression for hexazinone was $y=1.007x+0.219$ with a correlation coefficient of 0.96 while the linear regression for metabolite B was $y=1.100x-0.057$ with a correlation coefficient of 0.91. © 1998 Elsevier Science B.V.

Keywords: Water analysis; Environmental analysis; Hexazinone; Triazines; Pesticides

1. Introduction

Hexazinone is a triazine dione herbicide which has been registered by the Environmental Protection Agency (EPA) for use on alfalfa, pasture and range grasses, pineapples, sugarcane and blueberries [1]. Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione] has a high water solubility and low soil absorption [2] which allows it to move easily into the groundwater. Contamination of groundwater has been reported in Hawaii (0.06–0.72 ppb), Florida (0.12–2.90 ppb), Maine (0.2–29 ppb) and North Carolina (0.74–34 ppb) with all amounts below the EPA's lifetime health advisory level of 210 ppb [1].

Methods to determine hexazinone in groundwater have focused on using either isocratic or gradient reversed-phase high-performance liquid chromatography (HPLC) [3,4] or enzyme linked immunosorbent assays (ELISA) [5,6]. Few methods have been published regarding triazine pesticides determination by CE [7–10]. No method to date has been published for hexazinone and its metabolites. Isocratic HPLC procedures can at best quantify hexazinone and metabolite B while gradient HPLC methods can determine hexazinone and many of the metabolites. ELISA techniques are very good but cannot distinguish between hexazinone and metabolites due to cross-reactivity. Although both HPLC and ELISA are sensitive, there are problems with either complexity, organic solvent use, or cross-reactivity. These disadvantages have led to the development of a capillary electrophoretic (CE) method.

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The use of CE for pesticide analysis has increased significantly. CE is a very efficient separation technique that achieves high resolution. The greatest limitation to CE is the detection sensitivity, especially with UV detectors. This can be overcome by sample concentration techniques or by using capillaries with increased path-lengths in the detection window as was done in this paper. One mode of CE, micellar electrokinetic chromatography (MECC), was first introduced by Terabe et al. [11] and is based on the partitioning of compounds distributed between the aqueous and micellar phase [12], thus improving the separation of charged and neutral compounds. This paper describes a MECC method for the analysis of hexazinone and five of its metabolites in groundwater.

2. Experimental

2.1. Materials

All chemicals used were analytical grade. Sodium phosphate dibasic, sodium borate, sodium dodecyl sulfate (SDS) were purchased from Sigma (St. Louis, MO, USA) while the sodium hydroxide was from Fluka (Ronkonkoma, NY, USA). Methylene chloride (HPLC grade) was bought from Fisher (Fair Lawn, NJ, USA) and the HPLC methanol from EM Science (Gibbstown, NJ, USA). Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione]; metabolite C [3-(4-hydroxycyclohexyl) - 6 - (methylamino) - 1 - methyl-1,3,5-triazine-2,4(1H,3H)dione]; metabolite A1 [3-(*trans*-2-hydroxycyclohexyl)- 6-9 -dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione]; metabolite E [3-(4-hydroxy-cyclohexyl)-1-methyl-1,3,5-triazine-2,4,6(1H,3H,5H)trione]; metabolite B [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)dione] and metabolite D [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1H,3H,5H)trione] were gifts from DuPont de Nemours (Experimental Station, Wilmington, DE, USA). The purity of hexazinone and metabolites was 99%. Atrazine (99% pure) was purchased from Cresent (New York, NY, USA).

2.2. Standard preparation

Working standards of hexazinone (6.56 μg/ml), metabolite B (11.25 μg/ml), metabolite D (10.3 μg/ml), metabolite E (10 μg/ml), metabolite C (10.45 μg/ml), metabolite A1 (21 μg/ml) and atrazine (6 μg/ml) were prepared by dissolving appropriate amounts of stock standard in methanol.

2.3. Collection of groundwater

Water samples were from US Geological Survey (USGS) monitoring wells located at Pineo Ridge in Cherryfield, ME, USA. Each well was purged with three well volumes with a Redi-Flo2 (Grundfos Pumps Corporation, Clovis, CA, USA) pumping system prior to collection in a 1-liter glass jar. All samples were stored in the dark at 4°C until time of analysis.

2.4. Sample extraction

A liter of water was placed into an Erlenmeyer flask along with 80 μl of atrazine. Atrazine was used to measure relative retention times due to migration time variability. A Supelclean ENVI-Carb solid-phase extraction tube (Supelco, Bellefonte, PA, USA) containing 500 mg of packing was activated with 5 ml of methanol followed by 5 ml of HPLC grade water. The liter of water was passed through the extraction tube under vacuum and then vacuum dried for 40 min before eluting the solid-phase tube with 3 ml of methylene chloride. A 7-ml glass vial was used to collect the eluent. The methylene chloride fraction was evaporated to dryness under nitrogen with the residue being reconstituted in 1 ml of HPLC water by sonicating for 30 s. Finally, the sample was filtered through a 0.2-μm filter before adding a 100-μl aliquot to the injection vial. Samples were dissolved in water to promote stacking.

2.5. CE analysis

Quantitation of hexazinone and metabolites was performed on a Hewlett-Packard (Avondale, PA, USA) 3D CE capillary electrophoretic system equipped with a photodiode array detector and an extended light path capillary. At the beginning of

each day the capillary was conditioned by flushing for 20 min with 1.0 *M* NaOH, 20 min with 0.1 *M* NaOH, 2 min with distilled water and 60 min with running buffer (50 m*M* SDS, 12 m*M* sodium phosphate, 10 m*M* sodium borate with 15% methanol). Prior to injection the capillary was flushed for 2 min with 0.1 *M* NaOH and then 3 min with running buffer. After each run the capillary was flushed with distilled water for 1 min.

The wavelengths monitored were 247 nm for hexazinone and metabolite A1, 230 nm for metabolite C, 225 nm for metabolites B, D and atrazine and 220 nm for metabolite E. The capillary column had an I.D. of 75 μm with a bubble factor of 2.7 and a total length of 48.5 cm and an effective length 40 cm.

Rinsing, sample introduction and separation were all controlled by a HP Vectra XM2 with CHEMSTATION software. Sample introduction into the system was performed hydrodynamically for 12 s at 60 mbar. The system was ramped from time 0 at 25 μA to 55 μA for the first minute and then kept constant at 55 μA using positive polarity. Current was determined by generating an Ohms plot for the running buffer. The capillary temperature was maintained at 35°C and the sample carousel at 25.8°C. Peak heights were used for all quantitations.

2.6. Fortification studies

To ascertain the percent recovery, 1-liter samples were spiked with hexazinone and metabolites C, A1, B, D, and E at concentrations of 0.5, 1.0, 2.0 and 5.0 ppb, respectively. At each concentration level five separate samples were extracted and analyzed.

2.7. Reproducibility studies

To determine within-day reproducibility water samples (1 liter) were spiked at 0.5, 1.0, 2.0 and 5.0 ppb with hexazinone and metabolites C, A1, B, D, and E. The samples were injected a total of ten times in one day. Between-day reproducibility was determined by preparing four water samples (1 liter) which were spiked at levels of 0.5, 1.0, 2.0, and 5.0 ppb. These were concentrated and analyzed on five separate days.

2.8. Linearity studies

From stock standards of hexazinone and metabolites A1, C, E, B and D various working standards were prepared for linearity testing. The concentrations ranged from 0.38 ppm to 13.1 ppm. All metabolites and hexazinone were found to be linear when comparing response to peak height.

3. Results and discussion

3.1. The effects of SDS and methanol on separation

Hexazinone has seven known metabolites (Fig. 1). With the addition of SDS five of the metabolites were separated from hexazinone (Fig. 2) and without

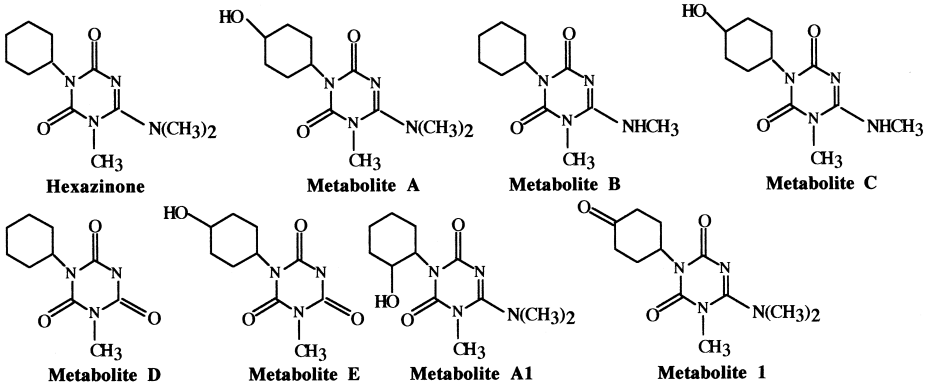

Fig. 1. Chemical structures of hexazinone and metabolites.

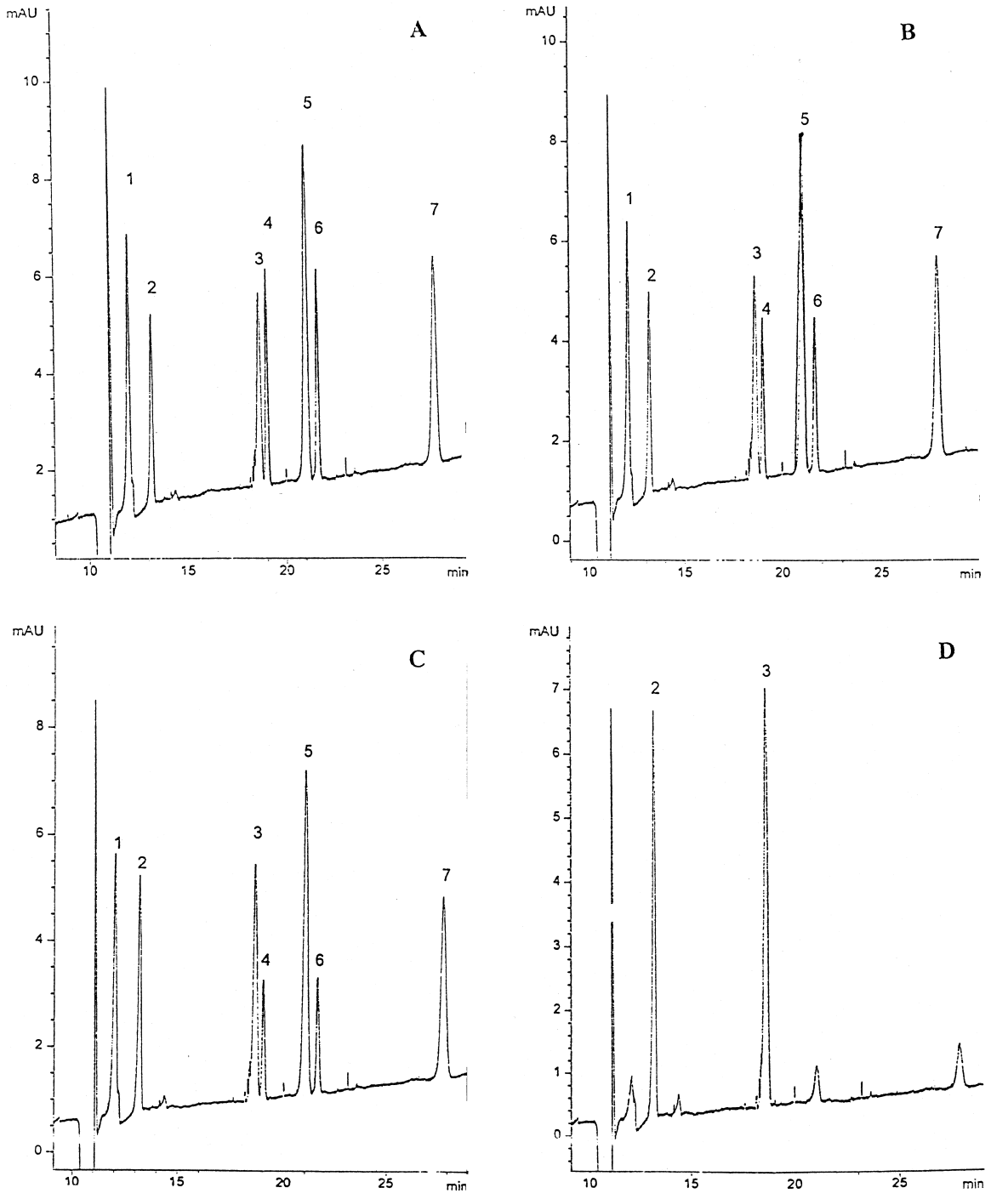

Fig. 2. Electropherogram of the separation of hexazinone and metabolites. A=220 nm; B=225 nm; C=230 nm; and D=247 nm. Peaks: 1=metabolite C; 2=metabolite A1; 3=hexazinone; 4=metabolite E; 5=metabolite B; 6=metabolite D; 7=atrazine. Analysis conditions; 48.5 cm×75 μm I.D. capillary column; injection hydrodynamic (12 s at 60 mbar); 50 m*M* SDS, 12 m*M* sodium phosphate, 10 m*M* sodium borate and 15% methanol (pH 9.0) time 0 at 25 μA to 55 μA for first minute and then constant at 55 μA.

it no separation occurred. However, metabolite A coeluted with metabolite C and metabolite 1 coeluted with hexazinone. This is not surprising because of the structural similarities (Fig. 1), but co-elution is a minimal problem in this instance. For example, metabolite C and A can be monitored at different wavelengths (C at 230 nm and A at 247 nm). As for metabolite 1 and hexazinone, even though the monitoring wavelength is the same (247 nm), one would not expect to see metabolite 1 in water.

The addition of SDS to the running buffer may also coat the capillary wall causing shifts in migration times. With this method migration time shifts occurred especially after buffer replenishment. The inlet and outlet vials were replenished after every five injections. After replenishment a standard which contained hexazinone, atrazine and the metabolites of interest was injected. Atrazine was added to water samples to determine the relative retention times of the compounds. This was another way to identify peaks in conjunction with spectral data. The relative differences for hexazinone (0.69) and its metabolites (A1, 0.41; C, 0.51; E, 0.72; B, 0.77 and D, 0.81) were very constant despite migration time changes.

While SDS was used for the initial separation, addition of 15% methanol to the running buffer was employed to achieve baseline resolution of hexazinone and five metabolites (Fig. 2) by decreasing the electroosmotic flow which increases the migration time for the compounds. With only 10% methanol added to the running buffer there was little resolution between hexazinone and metabolite E as well as between metabolites B and D. Thus, addition of 15% methanol produced the best results.

3.2. Quantitation

An external standard along with peak heights was used to calculate concentrations. The internal standard was tried but did not prove to be any better than using an external standard. Thus, the only function for adding atrazine was for ascertaining relative retention times. Peak height was employed over peak area because of the migration time variability which causes peak area changes. Peak area increases as the migration time increases since solutes which have a lower velocity tend to remain in the detection window for a longer period of time when compared to solutes with a higher velocity and this can artificially inflate values.

3.3. Fortification studies

Table 1 summarizes the results of fortifying water samples at concentrations of 0.5, 1.0, 2.0 and 5.0 ppb. Percent recoveries ranged from 79 to 100 for hexazinone; none detected to 54 for metabolite C; 75 to 91 for metabolite A1; 76 to 93 for metabolite E; 72 to 88 for metabolite B and 81 to 120 for metabolite D. The only recovery problem was with metabolite C which was most likely due to instability of the compound or poor adherence to the solid-phase. The relative standard deviation (R.S.D.) for samples spiked at 0.5 and 1.0 ppb tended to be higher than for samples spiked at 2.0 to 5.0 ppb. This points out the major disadvantage of CE. When dealing with low concentration samples CE may have problems in detecting these levels.

3.4. Reproducibility studies

Table 2 shows results for within-day and between-day reproducibility. For intra-assay results the R.S.D. values were very low ranging from 0.6 to 12% for a water sample spiked at each concentration level. The inter-assay R.S.D. values varied from 3.2 to 23.3% which are much higher than the within-day assays. The greatest increases in the R.S.D. values are within the 0.5 and 1.0 ppb samples. As mentioned above this is a sensitivity problem but still the results are acceptable at such a low level. If the sample was reconstituted in 500 μl instead of 1000

Table 1
Percent recovery of fortified water samples

Compound	Fortification level (ppb)			
	0.5	1.0	2.0	5.0
Hexazinone	100 (9.8)[a]	79 (25)	87 (8.6)	95 (11)
Metabolite C	ND[b]	52 (22)	30 (21)	54 (23)
Metabolite A1	85 (12)	75 (18)	85 (6.6)	91 (9.4)
Metabolite E	93 (9.9)	76 (18)	75 (15)	81 (16)
Metabolite B	75 (25)	80 (10)	72 (13)	88 (13)
Metabolite D	120 (12)	81 (23)	96 (11)	85 (20)

[a] R.S.D. (%) values based on the extraction of five separate spiked water samples.
[b] ND=none detected at a detection limit of 0.25 ppb.

Table 2
Reproducibility of hexazinone and metabolites in spiked groundwater

Compound	R.S.D. (%)		
	Spike (ppb)	Intra-assay[a]	Inter-assay[b]
Hexazinone	0.5	9.9	17
Hexazinone	1.0	5.8	22
Hexazinone	2.0	3.8	8.4
Hexazinone	5.0	1.0	3.2
Metabolite C	0.5	-	-
Metabolite C	1.0	-	-
Metabolite C	2.0	12	17
Metabolite C	5.0	2.4	3.2
Metabolite A1	0.5	9.3	17
Metabolite A1	1.0	2.6	23
Metabolite A1	2.0	3.1	7.6
Metabolite A1	5.0	2.5	9.3
Metabolite E	0.5	7.1	9.3
Metabolite E	1.0	9.7	19
Metabolite E	2.0	9.1	8.2
Metabolite E	5.0	3.2	6.3
Metabolite B	0.5	8.8	15
Metabolite B	1.0	5.8	23
Metabolite B	2.0	2.0	9.3
Metabolite B	5.0	0.6	3.6
Metabolite D	0.5	5.5	16
Metabolite D	1.0	6.0	15
Metabolite D	2.0	3.3	5.8
Metabolite D	5.0	3.4	13

[a] Intra-assay R.S.D. valuess based on ten determinations in one day.
[b] Inter-assay R.S.D. values based on determinations performed on five different days.

μl the sample would become more concentrated and the R.S.D.s would most likely be decreased. Also, metabolite C could not be detected at concentrations of 0.5 and 1.0 ppb but with using less solvent for reconstitution it may be possible to detect metabolite C at these lower levels.

3.5. Correlation studies

A comparison was made between an approved HPLC method and CE for the quantitation of hexazinone and metabolite B. Forty-seven water samples were analyzed for hexazinone and forty-three for metabolite B by both techniques.

The regression equation for hexazinone was $y=1.007x+0.219$ with a correlation coefficient of 0.96 (Fig. 3). The samples ranged from none detected to 10.25 ppb. The regression equation for metabolite B was $y=1.100x-0.057$ with a pearsons correlation of 0.91 (Fig. 4). The samples ranged from none detected to 1.74 ppb. Looking at the slope of both equations, it can be seen that there is no bias between hexazinone values run by both techniques and only a slightly higher bias for metabolite B by HPLC over CE. All five metabolites have been found in groundwater samples in Maine with B being the most widespread.

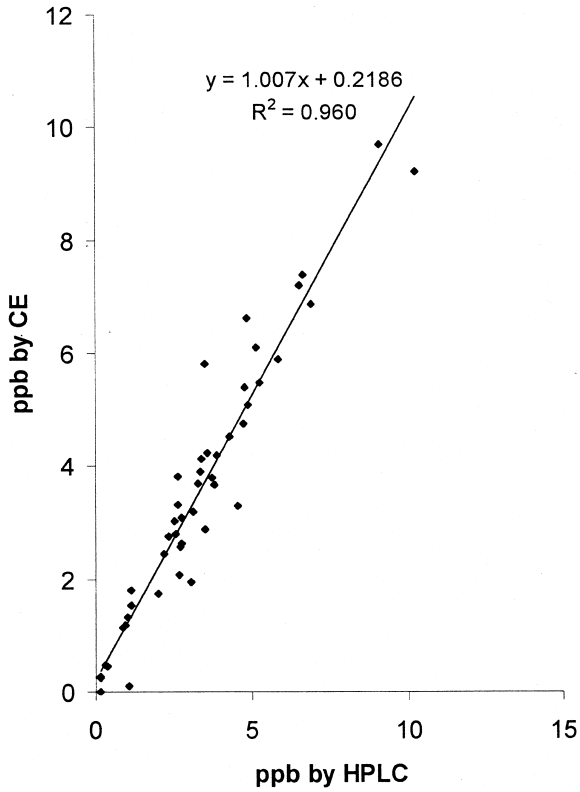

Fig. 3. Correlation of 47 groundwater samples analyzed by HPLC and CE for hexazinone.

3.6. Preliminary results for surface water

Analyzing surface water samples for hexazinone by HPLC has been a problem because of humic acid which can interfere with the analysis. However, there appears to be no problem with humic acid when analyzing hexazinone in surface water by CE. A

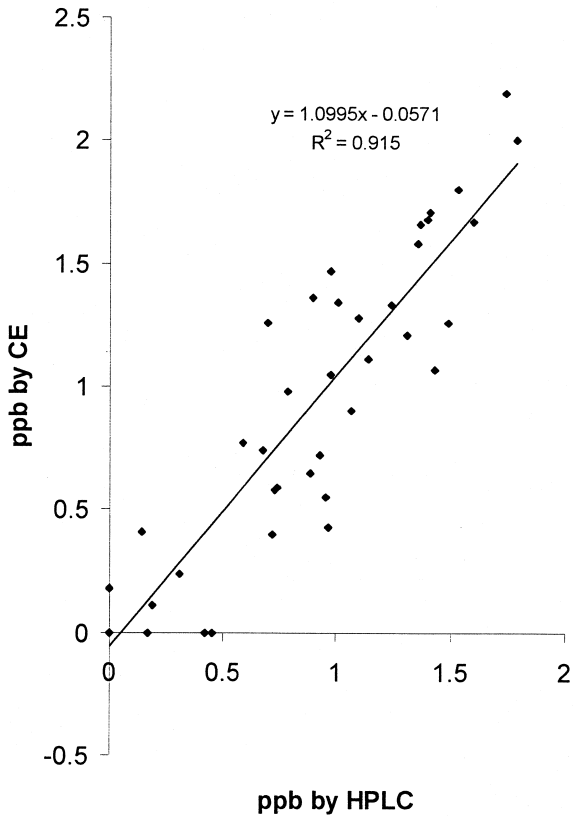

Fig. 4. Correlation of 43 groundwater samples analyzed by HPLC and CE for metabolite B.

comparison of a few water samples analyzed by HPLC and CE were in good agreement.

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