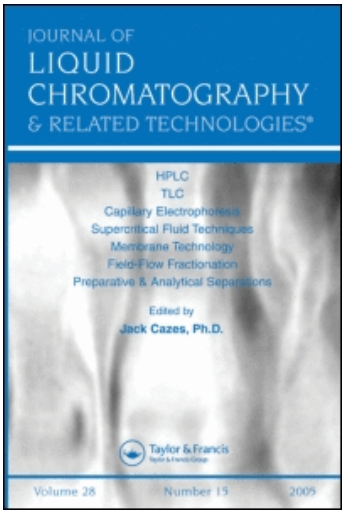

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ANALYSIS OF DIETHYLENETRIAMINE IN WATER AND SOIL AT PPB LEVELS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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ABSTRACT

A fast and sensitive method for the determination of diethylenetriamine in water and soil by High Performance Liquid Chromatography (HPLC) with Fluorescence Detection is described. Diethylenetriamine is converted to its fluorescamine derivatives through pre-column derivatization and is separated by an isocratic reverse phase chromatographic system (Nucleosil C18) with acetonitrile and borate buffer at pH 8 as the mobile phase. Water samples at concentrations of diethylenetriamine from 20 - 100 μg/L were derivatized with fluorescamine and chromatographed directly through direct aqueous injections. Recoveries averaged 94 $\pm$ 7% for a population of nine samples. Soil samples at concentrations of diethylenetriamine from 0.24 - 1.0 μg/g were extracted with $CaCl_2$ solution (2 M) followed by derivatization with fluorescamine. Derivatized soil extracts were then analyzed by HPLC. Recoveries averaged 21 $\pm$ 2% for a population of nine samples.

INTRODUCTION

Diethylenetriamine is commonly used as a chemical intermediate for reactive polyamide resins, fatty aminoamides, fatty imidazolines. It is also used as a solvent for sulfur, acid gases, various resins, and dyes. During its manufacture and commercial use, diethylenetriamine can enter the environment through emissions in air or in waste water. Because of its chemical and physical properties, diethylenetriamine

is expected to leach into the ground once it is released on to land. To date, the environmental fate of diethylenetriamine is still unknown largely due to the lack of an adequate analytical methodology. To study the environmental fate of diethylenetriamine, a rapid and sensitive analytical method is essential in order to identify and quantify diethylenetriamine at relevant environmental concentrations.

Fluorescamine, an important fluorogenic reagent, has been used extensively to label primary and secondary amines, amino acids, peptides, and proteins[1,2]. It also has been used to derivatize aliphatic diamines and polyamines through both pre- and post-column derivatization[3,4]. This non-fluorescent reagent reacts rapidly with primary amines in an aqueous environment to form pyrrolinones which fluoresce at 475-500 nm upon excitation at approximately 390 nm[5]. The resulting fluorescence is proportional to the amine concentration and can be used for quantitation. The derivatization reaction and the hydrolysis of excess reagent generally take place at pH $\geq$ 7 and at room temperature. The reaction rate depends strongly on the pH, the nature of the organic co-solvent, and the buffer selected. Diethylenetriamine contains two primary and one secondary amine groups that are susceptible to fluorescamine derivatization. This paper describes a quick and highly sensitive method for the determination of diethylenetriamine in water and soil as its fluorescamine derivative by HPLC with fluorescence detection.

MATERIALS AND METHODS

Chemicals:

Diethylenetriamine (lot # 00722J2) was obtained from Aldrich (Milwaukee, Wisconsin) with a chemical purity of 99%. Fluorescamine (lot # 08808E2) was also obtained from Aldrich with a chemical purity of 98%. Calcium chloride and boric acid (crystal) were obtained from Mallinckrodt (Paris, Kentucky) and were analytical grade. All other chemicals were obtained from commercial sources and were at a minimum reagent grade. All solvents were HPLC grade.

Water and soil:

Water recovery samples were prepared in deionized water collected in the state of Kentucky. Soil recovery samples were prepared in soil also collected in the state of Kentucky. The textual classification of the soil was clay.

Recovery sample preparation and fluorescamine derivative formation:

1. Water. Water recovery samples were prepared in deionized water. They were fortified with a stock solution of diethylenetriamine prepared in methanol. The fortification levels produced were 20.4, 61.2 and 102 μg/L (three replicates at each concentration). An additional three deionized water samples were left unfortified to be utilized as control samples. To 1.00 mL of sample solution was added 20 μL of dilute NaOH solution (0.1 N). Exactly 100 μL of fluorescamine stock solution (5 mg/mL prepared in

acetone) was then added to the mixture at room temperature. Rapid addition and mixing were essential to achieve optimal fluorescence. An aliquot of the reaction mixture was injected directly onto the HPLC column.

2. *Soil.* Soil recovery samples were prepared in Kentucky soil. Approximately 2 g of soil was weighed into a glass centrifuge tube and fortified with a stock solution of diethylenetriamine prepared in $CaCl_2$ (2M). The fortification levels produced were approximately 0.24, 0.50 and 1.0 μg/g (three replicates at each concentration). An additional three soil samples were left unfortified to be utilized as control samples. Each soil sample was extracted with 4.00 mL of $CaCl_2$ solution (2 M) by vortexing vigorously for approximately 3 minutes. The extract was separated via centrifugation for approximately 10 minutes at 2000 rpm. To exactly 1.00 mL of soil extract was added 20 μL of dilute NaOH solution (0.1 N). Exactly 400 μL of fluorescamine stock solution (2 mg/mL prepared in methanol) was then added to the mixture at room temperature. Rapid addition and mixing were essential to achieve optimal fluorescence. An aliquot of the reaction mixture was injected directly onto the HPLC column.

High Performance Liquid Chromatography (HPLC):

HPLC was performed on an isocratic reverse phase system with fluorescence detection. Instruments included a Hewlett Packard 1050 solvent pump, a Hewlett Packard 1050 autosampler, a Waters 470 fluorescence detector, and a Hewlett Packard 3396A integrator. A Nucleosil C18 (10 μm, 250 X 4.6 mm) column was used to separate diethylenetriamine-fluorescamine derivatives. The HPLC mobile phase was 50% methanol and 50% borate buffer (0.1 M, pH 8) at 1 mL/minutes for water samples, and 40% methanol and 60% borate buffer at 1.8 mL/minutes for soil samples. The borate buffer was prepared by titrating 0.1 M boric acid with 1 N sodium hydroxide. Fluorescence detection (excitation: 390 nm, emission: 475 nm) was used to monitor the eluted derivatives. The injection volume was 20 μL for all samples.

Quantitation:

The quantification of diethylenetriamine was achieved using an external calibration method. External calibration standards were prepared in deionized water and $CaCl_2$ solution (2 M) for water samples and soil samples, respectively, using stock solutions of diethylenetriamine prepared in methanol. The concentrations of the standards were 20, 40, 60, 80 and 100 μg/L. Each standard was subjected to derivatization with fluorescamine following procedures described above. Two complete sets of standards were analyzed with each sample set, one prior to analysis of the samples and one immediately following the samples. A standard curve was constructed by plotting the peak area (for water samples) or peak height (for soil samples) of diethylenetriamine-fluorescamine derivative versus the concentration (μg/L) of the standard injected using linear regression. The correlation coefficient, slope, y-intercept and minimum detectable limits were calculated. The concentrations of diethylenetriamine in each sample were

determined using the linear regression and the diethylenetriamine-fluorescamine derivative peak area or height of the samples.

For soil samples, since external calibration standards were prepared in pure $CaCl_2$ solution, the matrix effect on detector responses was examined in order to obtain accurate quantification. An experiment was conducted to determine the differences in detector response between a sample in pure $CaCl_2$ and a sample in soil extract ($CaCl_2$ extract). In this experiment, two 100 μg/L diethylenetriamine samples were prepared, one in pure $CaCl_2$ solution (2 M) and one in control soil extract ($CaCl_2$ extract). These two samples were derivatized with fluorescamine and analyzed by HPLC simultaneously. Four repetitive HPLC injections were performed for each sample alternately. The mean peak height of each sample was calculated. A detector response factor of 0.667 was calculated as the ratio of the peak height of the sample prepared in soil extract to the one prepared in pure $CaCl_2$. This detector response factor was used to correct the method recovery.

RESULTS AND DISCUSSION

Under slightly basic conditions (pH 8), the derivatization reaction between fluorescamine and diethylenetriamine in water resulted in at least three fluorescent derivatives, presumably as products of both the primary amino-group and the secondary amino-group. The separation of these derivatives was achieved using an isocratic reverse phase C18 (Nucleosil) column with a borate buffered (pH 8) mobile phase. Under the derivatization conditions, a major fluorescent derivative was formed and reached its highest fluorescent intensity almost instantaneously. This derivative (retention time ~ 3.7 minutes) was found to be stable for at least few hours and used for quantitation. Identification of the three major derivatives was beyond the scope of this work and was not pursued. Prior to achieving these experimental conditions, various experiments were conducted to optimize the derivatization reaction and the separation of various fluorescent products. The derivatization reactions were conducted at various pHs (pH 6, 7, 8 and 9) and in various buffers (NaOH, borate buffer, and phosphate buffer). It was found that the rate of formation as well as the intensity of the fluorescent derivatives increased with increasing pH. Although these fluorescent products were most intense at pH 9, the degradation (presumably via hydrolysis) of these products at pH 9 was significantly faster than at pH 8. Running the reaction at pH 8 was, therefore, more desirable. The effect of different buffers on the reaction rate was also examined. Borate buffer (pH 8) was preferable than phosphate buffer (pH 8). The reaction in phosphate buffer resulted in several fluorescent products with similar intensities, while a single predominant product was formed in borate buffer. Adjusting pH with a small amount of dilute NaOH solution produced similar results as to using borate buffer at pH 8. Instead of using borate buffer at pH 8, NaOH was used to adjust the pH of water samples as well as soil extracts in order to minimize the dilution of analyte. The water method was validated by fortification of deionized water with diethylenetriamine at nominal concentrations ranging from 100 -20 μg/L with a mean recovery of 94 $\pm$ 7%. The limit of quantitation was defined as one-half of the signal response of the lowest concentration calibration standards. Based on this

TABLE 1

Analytical Results for the Recovery of Diethylenetriamine From Water and Soil.

Sample Type	Concentration Fortified (μg/L or μg/g)	Concentration Recovered (μg/L or μg/g)	Recovery %	Number of Replicates	Overall Mean $\pm$ STD (n=9)
Water	20.4	18.5 $\pm$ 1	91 $\pm$ 5%	3	94.7 $\pm$ 7%
Water	61.2	57.2 $\pm$ 4	93 $\pm$ 6%	3	
Water	102	104 $\pm$ 4	102 $\pm$ 4%	3	
Water Control	NA	< 13.5	NA	3	
Soil	0.246	0.0337 $\pm$ 0.002	13.7 $\pm$ 0.8 %	3	13.8 $\pm$ 1%
Soil	0.499	0.0638 $\pm$ 0.002	12.8 $\pm$ 0.5%	3	21 $\pm$ 2%*
Soil	0.996	0.150 $\pm$ 0.002	15.0 $\pm$ 0.4%	3	
Soil Control	NA	<0.0186	NA	3	

* Corrected for detector response factor

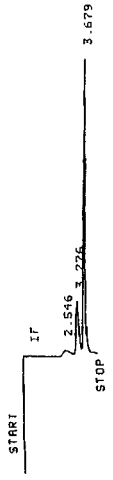

Figure 1. An HPLC chromatogram of a water sample at 102 μg/L using the reverse phase C18 system. (Peak of interest = 3.679 minutes)

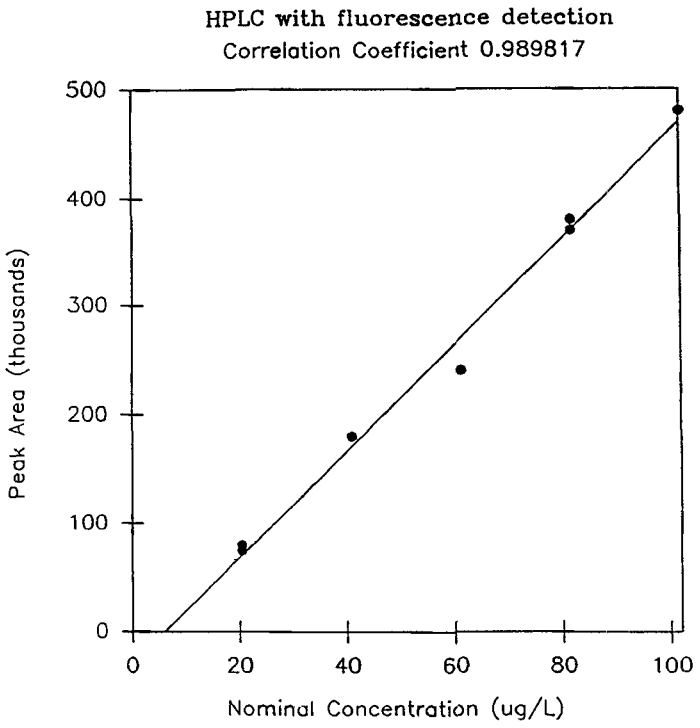

Figure 2. An external calibration curve for water method.

definition, a limit of quantitation for this method was calculated to be 13.5 μg/L. However, based on the signal-to-noise ratio, detector sensitivities, and injection volumes, a ten-fold lower quantitation limit (~ 1 μg/L) could easily be achieved. The recovery data for this water method is presented in Table 1. A representative chromatogram showing the analysis of diethylenetriamine at 102 μg/L is shown in Figure 1. A typical linear regression analysis for calibration standards is presented in Figure 2.

Development of a soil method presented much greater challenges owing to the difficulties in extracting diethylenetriamine from a soil matrix. The strong adsorption of diethylenetriamine to soil has been studied extensively[6]. Diethylenetriamine has a high water solubility and a relatively low vapor pressure. It contains two primary and one secondary amine groups, with pK_a values of 9.4 and 10.1. These functional groups will be partially protonated at pH ranges found in most soils and ground water. The ionic or electrostatic interactions between amines and the charged soil surface were considered the predominant mechanism in regard to soil adsorption[7]. Because of these interactions, the mineral content and the cation exchange capacity of a soil are also considered as important parameters influencing soil

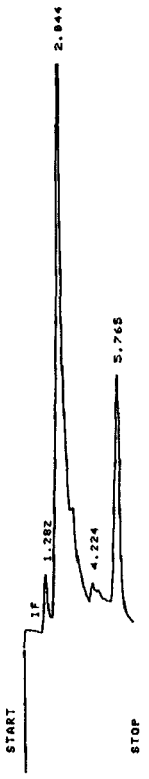

Figure 3. **An HPLC chromatogram of a soil sample at 0.98 μg/L using the reverse phase C18 system. (Peak of interest = 5.765 minutes)**

adsorption[8]. Various solvents were used to extract diethylenetriamine from soil during the method development. They included: $CaCl_2$ (1 M and 2 M), KCl (1 M and 2 M), NH_4OH (3.4%), HCl (0.1 M and 1 M), sodium borate buffer (0.1 M, pH 8), water, and mixtures of acetonitrile, methanol, and water. Among these solvents, $CaCl_2$ at a 2 M concentration proved to be the most promising solvent. The extraction procedures were also examined extensively. It was found that extended shaking (e.g. 2 hours, overnight and three days) did not improve extraction efficiencies, indicating that equilibrium was achieved after vigorously vortexing for 3 minutes. To achieve a lower quantitation limit, experiments were also conducted to optimize the solvent-to-soil ratio. Higher ratios would produce higher extraction efficiency but analytes would be more dilute if no concentration steps were involved. The optimal ratio was determined to be 2-to-1 for this method.

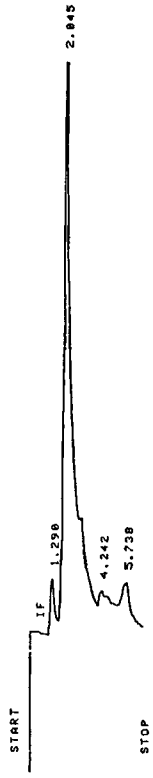

Figure 4. An HPLC chromatogram of a control soil sample. (Peak of interest = 5.738 minutes)

Similar to water samples, at least three fluorescent derivatives (with one major) were formed under the derivatization conditions. These three derivatives, however, could not be separated under the HPLC conditions described above. The co-elutions observed were due to the high ionic strength of the sample matrix (2 M $CaCl_2$). This matrix effect was significantly decreased when the injection volume was at or below 2 μL. Unfortunately, the sensitivity required for this method could not be achieved using a 2 μL injection volume. Various LC conditions were tested in an attempt to resolve these co-eluting peaks. These included various gradient elutions, various stationary phases (C18, C8, CN, NH_2, phenyl, etc.), many different buffered mobile phases at different pHs (pH 3.5, 5, 6, 7, 8, and 9), and with different organic modifiers (acetonitrile, methanol, THF, etc.). None of the conditions could successfully resolve these co-eluting peaks. The isocratic HPLC condition, slightly modified from the one used in the water method, was therefore chosen. As a result, the quantitation of diethylenetriamine in soil extract

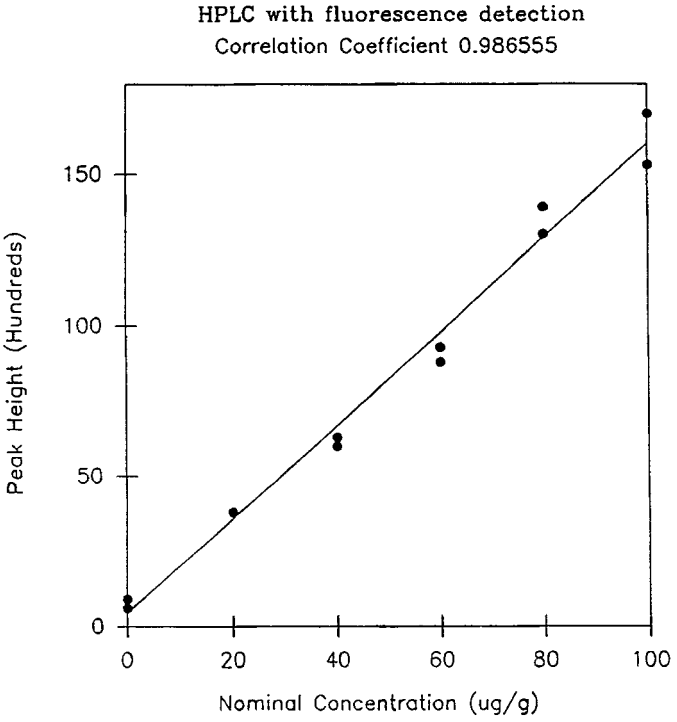

Figure 5. An external calibration curve for soil method.

was based on the peak height of the three co-eluting components. One of the three co-eluting components was also present in blank $CaCl_2$ solution which resulted in a relatively high intercept for the calibration curve. The fluorescent derivative peak (retention time ~ 5.7 minutes) reached its maximum within 20 minutes after the mixing with fluorescamine and was stable for a few hours. This peak could therefore be used for quantitation. The decision to use methanol to prepare the fluorescamine stock solutions was made after the observation that the use of acetone resulted in high background fluorescence in soil extracts. The use of methanol minimized the background interference, and therefore, maximized the sensitivity for diethylenetriamine.

The soil method was validated by fortification of matrix soil with diethylenetriamine at nominal concentrations ranging from 1.0 - 0.24 μg/g with a mean recovery of 13.8 $\pm$ 1.1% using external calibration standards prepared in pure $CaCl_2$ solution. The mean absolute method recovery, after correction for the detector response factor, was 21% $\pm$ 2%. The limit of quantitation was 0.13 μg/g after correction for method recovery (13.8%). Analytical results for the recovery of diethylenetriamine from

the matrix soil are presented in Table I. Representative chromatograms of a fortified soil sample and a soil control sample are shown in Figures 3 and 4, respectively. A typical linear regression analysis for diethylenetriamine standards is presented in Figure 5. These results demonstrated that diethylenetriamine was extremely difficult to extract once it was applied to soil. Although the method recovery of diethylenetriamine from soil was low (21%), the precision of the method was very good with a relative standard deviation of 8% for a population of 9 samples.

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