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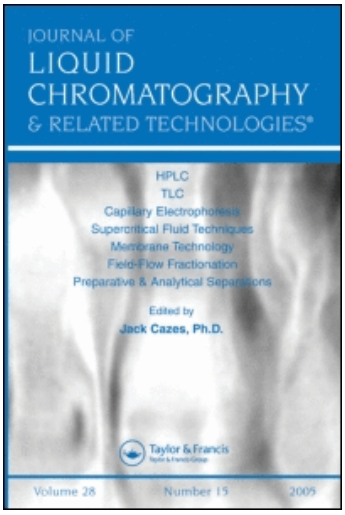

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Derivatization of Glycols, Hydroxyamines, and Polyols at Trace Levels with 3,5-Dinitrobenzoyl Chloride Utilizing Aqueous to Nonaqueous Phase Transfer on a Reverse Phase Cartridge

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DERIVATIZATION OF GLYCOLS, HYDROXYAMINES, AND POLYOLS AT TRACE LEVELS WITH 3,5-DINITROBENZOYL CHLORIDE UTILIZING AQUEOUS TO NONAQUEOUS PHASE TRANSFER ON A REVERSE PHASE CARTRIDGE

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ABSTRACT

A method is described for the trace analysis of glycols, polyols, and hydroxyamines in aqueous solutions. The samples are concentrated on an appropriate Sep Pak™ and dried with a stream of extra dry grade nitrogen followed by elution with a dried solvent. This simple but novel procedure permits rapid derivatization in a nonaqueous environment thus eliminating problems associated with the hydrolysis of the derivatization reagent.

INTRODUCTION

Various methods have been described for the analysis of glycols, polyols, and hydroxyamines. Analysis of these types of

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materials by gas chromatography and liquid chromatography is especially desireable due to the inherent strengths of these techniques and the present widespread availability of these instruments. Nevertheless, analysis of these types of compounds at trace levels in aqueous solutions has posed two major problems for both techniques. The first problem lies in the separation, elution, and detection problems particular to each instrument. For gas chromatographic analysis, low volatility compounded by high polarity often leads to totally retained or skewed peaks. For compounds possessing active hydrogens such as herein described, derivatization via silylation (1) has proven to be an effective means of compound modification thereby making gas chromatography amenable.

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In the case of liquid chromatography, these compounds present different obstacles now due to the lack of a chromophore for ultraviolet or fluorescence detection. Refractive index detection can be rather insensitive for trace level analysis and also mandates isocratic elution. As a result liquid chromatographers have commonly used precolumn derivatization techniques successfully employing reagents with high molar absorptivities (2) such as phenyl isocyanate, 3,5-dinitrobenzoyl chloride (DNBC), and dansyl chloride.

The second major problem is a direct consequence of measuring trace levels of the analyte in aqueous solutions. The derivatization reagents readily employed to overcome chromatographic deficiencies are invariably limited to their use in anhydrous

systems since they readily hydrolize rendering the reagents ineffective. For instance, the silylation reagents readily hydrolize with water forming hexamethyldisiloxane (3,4). Since the reaction with water is stoichiometric, successful silylation of the analyte requires large amounts of the silylation reagent. Although this approach has been successful, trace level determination (<100 ppm) is impractical due to the excessive amounts of reagent required. In a similar manner for HPLC analysis, derivatization with DNBC (or analog derivatization reagents) has been successful when excess levels of DNBC are used to react with the water and the analyte (5,6). Determinations as low as 100 ppm were obtained for various alcohols when extra steps were taken for water reduction via molecular sieves and phase transfer. In any case, the presence of water, thereby requiring excessive amounts of derivatization reagent to stoichiometrically react with the water, has prevented trace analysis of these types of materials.

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Other means of concentration have included extraction and washing of the underivatized or derivatized analyte (5,7). These methods have proved to be inaccurate for analytical purposes in many cases due to partitioning of the analyte between the aqueous and the nonaqueous phases. This can be especially true in surfactant, hydrophilic systems and/or due to the hydrophilic nature of the analyte itself.

A general method is reported here where glycols, polyols, hydroxyamines, and similar compounds can be analyzed at trace levels in aqueous solutions. The aqueous solution containing the

analyte is passed through a Sep Pak^{tm} where the analyte is concentrated. The Sep Pak^{tm} cartridge is then purged with extra dry grade nitrogen totally drying the system. This simple yet novel step permits elution of the analyte with a dry solvent for subsequent derivatization without concern for problems associated with hydrolization of the derivatization reagent. Trace levels of various glycols etc. can be analyzed rapidly. Examples are given with the focus made on liquid chromatographic analysis with DNBC derivatives. Problems using other techniques such as gas chromatography and/or other derivatives requiring anhydrous conditions are not anticipated.

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MATERIALS AND METHODS

Chromatography Systems

Separations were performed on two HPLC systems. The first was a Waters HPLC with dual 510 pumps, temperature control module with oven set at 60^{o}C, automated gradient controller and LKB 2140 photodiode array detector with LKB software driven on an IBM-XT. The column was a prepacked 4.6 mm x 25 cm LC-8-DB from Supelco (Bellefonte, Pa.) protected with a guard column. The second system was a Perkin-Elmer 3B HPLC with an LC-100 oven set at 60^{o}C and a Model LC-75 UV detector. The column was a prepacked 4.6 mm x 25 cm LC-8 from Altex (Berkely, Ca) protected with a guard column. Analyses were performed using a mobile phase flow rate of 1 ml/min. Absorbance was monitored at 233 nm. Gradient analyses were used generally starting with 50% acetonitrile/water to a final 100%

acetonitrile concentration. Gradients were optimized for the specific analysis performed.

Materials

Hydroxyamines (mono-, di-, and triethanolamine) were obtained as >99% pure from Olin Chemicals (Stamford, Conn.). The butyl monoglycol ether ($CH_3CH_2CH_2CH_2$-OCH_2CH_2OH) was obtained from EM Science (Cherry Hill, N.J.). Triethylamine, erythritol, and pentaerythritol were obtained from J.T. Baker (Phillipsburg, N.J.). Triethylene glycol monobutyl ether (butoxytriglycol), $CH_3(CH_2)_3$-$(OCH_2CH_2)_3$-OH, was obtained courtesy of Dearborn Chemical (Lake Zurich, Il).

Derivatization grade acetonitrile (Regis, Morton Grove, Il.) was dried overnight (20-50g) in a 60 ml amber bottle containing 5A molecular sieves (Alltech, Deerfield, Il.). The sieves had been previously washed with acetonitrile to reduce dust and then reactivated with heating at 200+°C. The 3,5-dinitrobenzoyl chloride (Regis) was added to the dried acetonitrile to obtain a 5,000 ppm DNBC/acetonitrile solution. The reverse phase cartridges or Sep Paks™ (Sep Paks are a trademark of Waters Associates), C-18, CN, Diol, and NH_2, were purchased from Waters (Milford, Mass.). Extra dry grade nitrogen was obtained locally with a specification of <3 ppm water. Syringes used for dispensing the aqueous solutions were either Hamilton for <500 ul amounts or 3 cc polypropylene syringes (Becton-Dickinson, Rutherford, N.J.) with luer-lok tips for >500 ul.

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Methods

Samples ranging from 0.2 ppm to 2000 ppm were made up in aqueous solution using deionized water (Calco, Rosemont, Il.) polished with a deactivated charcoal tank. The Sep Paks^{tm} were prepared by washing with 3 ml of acetonitrile followed by 3 ml of water. Excess water was then removed by gently forcing 3-5 ml of air through the Sep Pak^{tm} with a clean dry 3 cc syringe. An aliquot of the aqueous sample was syringe deposited on the prepared Sep Pak^{tm} and then again gently purged with 3-5 ml of air to remove excess water.

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For drying of the Sep Pak^{tm} a short length of 1/4" i.d., 3/8" o.d. tygon tubing was firmly inserted into the barrel end of a 3 cc polypropylene syringe while also being connected to the regulator of the extra dry nitrogen tank. The Sep Pak^{tm} was then secured to the luer-lok tip of the polypropylene syringe and purged for 30 minutes at a rate of 1.3 liters per minute. The Sep Pak^{tm} was then detached from the syringe upon which the 5000 ppm DNBC/acetonitrile solution was passed through the dried Sep Pak^{tm} (A glass syringe with teflon tipped plunger is recommended.). A 1g weighed amount of the DNBC/acetonitrile solution was collected in a 3.7 ml vial since this amount was found to be sufficient to elute the analyte. A teflon lined septum was secured to the vial and 3 ul of triethylamine was added at which point cloudiness in the headspace and occasional faint purple mixing lines are sometimes observed. The sample was then heated for 12 minutes at 70^{o}C. A 5 or 10 ul aliquot of the sample was then injected into the chromatograph for analysis.

Potential Problems

Due to the nature of the acetonitrile to absorb atmospheric moisture, the DNBC/acetonitrile solution bottle should not be left open thus preventing hydrolysis of the DNBC. New DNBC/acetonitrile should be made up on a weekly basis to insure the efficacy of the reagent.

The amount of triethylamine added to the sample should be approximately stoichiometrically equivalent to the amount of DNBC present. A lesser amount reduces the rate of derivatization whereas an excess does not appear to have any beneficial or adverse effects. The nitrogen used to dry the Sep Pak^{tm} should be as dry as possible. Any water introduced at this point will be eluted into the reaction vessel and cause hydrolysis of the DNBC. To further insure dryness, it is suggested that a gas purifier be placed between the regulator and the syringe adaptor.

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RESULTS AND DISCUSSION

Although many different types of analyses are presently available for the qualitative and quantitative analysis of alcoholic compounds, there still remains much investigation to be made for these types of materials at trace levels in aqueous solutions. For instance, earlier work (3,5,8) indicated that silylation or derivatization with DNBC can be made at dilute levels but the methodology required further dilution of the analyte by 40 to 100 fold seriously impeding practical trace level analysis. As a result, several concentration methods have been investigated such

as dehydration via reaction with dimethoxypropane (9) and evaporative techniques (10) but the authors have found them to be ineffective with the model compounds chosen.

The model compounds consisted of butyl monoglycol ether, triethyleneglycol monobutyl ether, the hydroxyamines (mono-, di-, and triethanolamine), and erythritol. They were chosen primarily because of our own particular interest and because of the diversity represented. In addition, these materials are extremely soluble in water and present a formidable test to the proposed methodology.

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The proposed method incorporates the use of C-18 or diol reverse phase cartridges for two purposes. First, the C-18 (or diol hereafter) cartridge acts as an extractive medium from which the analyte can be stripped from the aqueous solution. Second, it provides a retention bed for the analyte whereby the aqueous solution can be purged from the bed with dry nitrogen. Elution of the compounds of interest from the C-18 cartridge is then made with the acetonitrile-DNBC solution thus providing phase transfer of the analyte into a reactive medium (with triethylamine as the catalyst) producing the DNBC derivatives.

Reproduciblity, linearity, and the % recovery of the material from the reverse phase cartridges were tested. For the compound butyl monoglycol ether, samples were made at 10, 100, 500, and 1000 ppm. Different aliquots were deposited by syringe on the C-18 cartridges such that each contained equimolar amounts of the analyte. After elution and derivatization, 5 ul was injected into the liquid chromatograph. Excellent reproducibility was found with

TABLE 1

Reproducibility Study of Glycol Monobutyl Ether.

ppm of Sample	ml through Sep Pak^{tm}	Absorbance
10	10	0.202
100	1	0.193
500	0.2	0.188
1000	0.1	0.200

the results given in Table 1. A chromatogram of glycol monobutyl ether-DNBC derivative is shown in Figure 1.

A 200 ppm sample of triethyleneglycol monobutyl ether was then made up and different size aliquots were deposited on the C-18 cartridge. Again linear results for the DNBC-esters were obtained (See Figure 2). Two additional points were made (40 ul of a 2000 ppm sample and 2.5 ml of a 40 ppm sample) and the absorbance plotted on the graph at a location equivalent to the ul amounts of a 200 ppm sample. These extra points indicated a wide lattitude of sample capacity on the Sep Pak^{tm} with acceptable results. The 20 ppm sample was run on a different day without the benefit of correction by an internal standard or calibration curve.

Recovery efficiency from the C-18 cartridge was also tested for triethyleneglycol monobutyl ether. A standard solution of 10,000 ppm was made up in acetonitrile. From this standard 10 ul was directly derivatized with DNBC and chromatographed. (It was assumed that with excess derivatization reagent and optimum reaction conditions that the reaction is carried to completion.)

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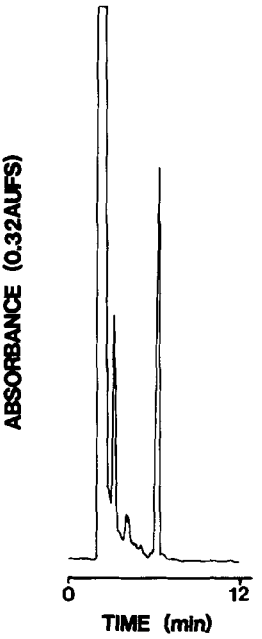

FIGURE 1. Chromatogram of the DNBC-ester of glycol monobutyl ether with an original concentration of 10 ppm in aqueous solution.

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TABLE 2

Recovery Efficiency of Triethyleneglycol Monobutyl Ether

Solution	Absorbance	Efficiency
Standard Solution	0.149	---
1 ml @ 100 ppm	0.152	102%
2 ml @ 100 ppm	0.299	100%
2 ml @ 100 ppm	0.317	106%
3 ml @ 100 ppm	0.447	100%
3 ml @ 100 ppm	0.466	104%
3 ml @ 100 ppm	0.457	102%

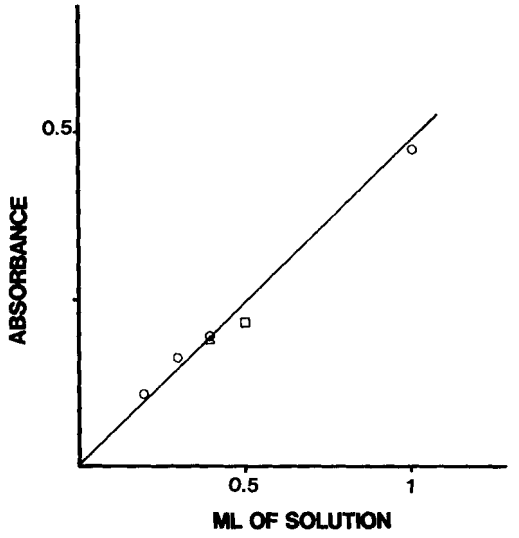

FIGURE 2. Plot of absorbance (DNBC-ester) vs. ml of solution forced through the Sep Pak^{tm} for 200 ppm triethylene-glycol monobutyl ether in aqueous solution. The two extra points, △ and □, represent 40 ul of a 2000 ppm sample and 2.5 ml of a 40 ppm sample respectively, and are plotted at a location equivalent to the ul amount of a 200 ppm sample.

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This served as a reference point to determine whether deposition and elution of the analyte for the aqueous samples on the C-18 cartridge was an efficient process. For the samples found in Table 2, aliquots of the standard solution were placed in a vial, stripped of acetonitrile by gently blowing with dry nitrogen and then diluted to 100 ppm with deionized water. Different size aliquots of the aqueous solution were then syringe deposited on the Sep Pak^{tm} and derivatized using the proposed methodology.

Two points can be realized from Table 2. First, the efficiencies indicate excellent deposition and elution of the

analyte on and from the C-18 cartridge. Efficiencies above 100% are attributed to random error whose source is undoubtedly the single determination made on the standard solution. Second, reproducibility within the multiple determinations and the group overall are excellent, indicating the method to be a valid means of analysis for these types of compounds. This data cannot be directly compared or plotted with the data shown in Figure 2 since the data were compiled on different instruments and different gradients thus producing slightly different peak shapes.

The hydroxyamine (mono-, di-, and triethanolamine) were then examined. A standard solution of approximately 3,300 ppm of each ethanolamine was made up in acetonitrile. A 3 ppm aqueous solution was made in a similar manner as before (i.e. by stripping the acetonitrile and diluting with deionized water). Up to 3 ml samples were deposited on the C-18 Sep Pak^{tm}, dried, eluted, and derivatized with the data given in Figure 3. These results show excellent linearity for all three ethanolamines. A chromatogram of the three ethanolamine-DNBC derivatives is shown in Figure 4.

Efficiency determinations again were made by absorbance comparisons to a standard sample which was directly derivatized. The derivatization efficiencies of the three ethanolamines were indistinguishable from each other and averaged about 90% for aqueous sample aliquots up to 3 ml placed on the Sep Pak^{tm}.

Finally, the compound, erythritol, was studied. Samples varying from 1.25 to 125 ppm were made up in aqueous solution. Identical 1 ml aliquots were placed on diol Sep Paks^{tm}, dried, and

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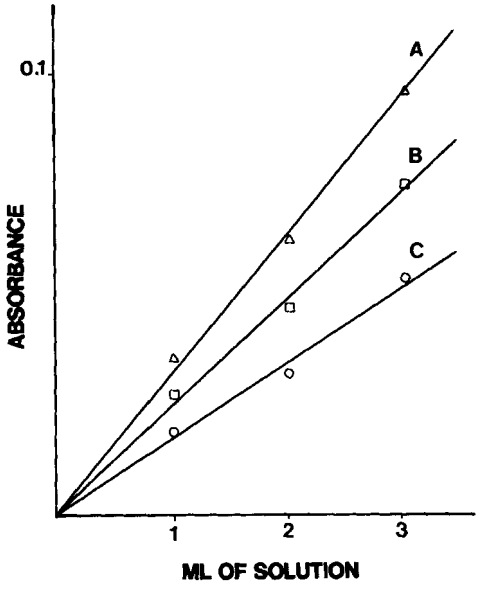

FIGURE 3. Plot of absorbance (DNBC-ester) vs ml of solution forced through Sep Pak^{tm} for the 3 ethanolamines; A - monoethanolamine, B - diethanolamine, C - triethanolamine, all at approximately 3 ppm in aqueous solution.

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eluted with DNBC/acetonitrile. Linear results were obtained for the DNBC-esters with the data shown in Figure 5. Efficiences were also determined as previously outlined and found to range from 40-50%. A second efficiency test was devised which consisted of collection of the eluted water phase from the Sep Pak^{tm}. This eluent was placed on a second diol Sep Pak^{tm} and the general derivatization scheme was followed for both Sep Paks^{tm}. Comparison of the absorbance of the DNBC-erythritol peaks from the two Sep Paks^{tm} confirmed that only approximately 50% of the erythritol was being retained while the remainder was being eluted with aqueous mobile phase. It is important to note that despite substantial

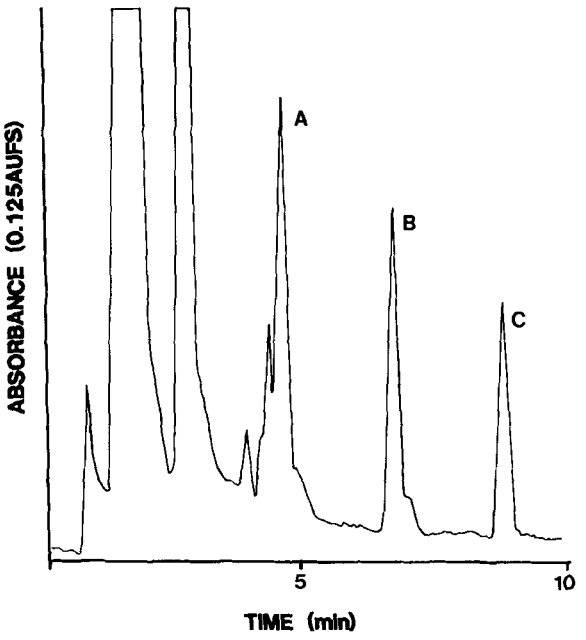

FIGURE 4. Chromatogram of the three ethanolamines; A - monoethanolamine, B - diethanolamine, C - triethanolamine.

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partitioning between the two phases (diol and aqueous), that linear results are still obtained when passing a limited fixed amount of aqueous sample through the Sep Pak^{tm}. It is not anticipated that using substantially larger volumes would still yield linearity or greatly increase the mass of the analyte left on the retention bed.

SCOPE AND LIMITATIONS

In conjunction with the erythritol, pentaerythritol was studied with similar conclusions. The sole limiting aspect found in this study was thus the inability of the reverse phase

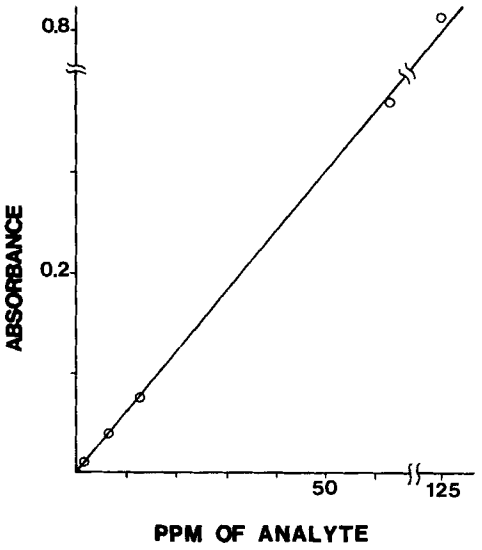

FIGURE 5. Calibration curve for the DNBC-ester of erythritol. One ml samples ranging from 1.25 to 125 ppm in aqueous solution were forced through diol Sep Paks^{tm} and derivatized according to the method. Injection volumes of 5 ul were made into the liquid chromatograph.

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cartridges to effectively retain 90+% of this category of compounds on the retention bed. Other Sep Paks^{tm} were tried (CN, NH_2, Si, and C-18) generally yielding disappointing results. The C-18 cartidge gave results almost equivalent to the diol. Nevertheless, when using a fixed volume (1 ml) of aqueous solution, linear results can be obtained. Future investigation in this area is potentially important since this classification encompasses compounds such as sugars and other polyols.

Two solutions to this problem are obvious. First, there in fact may be other types of reverse phase cartridges available or soon available that will provide adequate retention of the polyols.

A second solution would be to use larger cartridge bed volumes. The Sep Pak^{tm} may be thought of as extraction beds such that increasing the bed volume increases the amount of analyte retained. This was shown in the erythritol data whereby each Sep Pak^{tm} reduced the analyte in the aqueous solution by approximately 50%. The larger column bed can then be dried and the analyte derivatized. When economically necessary the cartridges can be reused by flushing with acetonitrile or other appropriate solvent.

Conversely, it should be stated that this method is perfectly amenable when analyzing solutions polyol solutions containing concentrations greater than 10 ppms. By simply decreasing the volume of the aqueous solution forced through the Sep Pak^{tm} to $\leq$100 ul, recovery efficiencies can be increased to an acceptable level. Despite loss of sensitivity due to dilution (10 fold or greater), adequate signal to noise yet exists due to multiple derivatization sites.

For the other types of materials the method presently shows great promise for the following reasons. First, materials such as the glycols and ethanolamines can be moderately concentrated, phase transferred and easily derivatized. The excellent sensitivity (0.1 AU for 3 ppm monoethanolamine, 0.202 AU for 10 ppm glycol monobutyl ether etc.) indicate that levels to and below 1 ppm are certainly attainable using this procedure. Further decreases in the detection limits are possible by use of other derivatives including those producing fluorescence. This is anticipated since the phase transfer of the analyte to a nonaqueous solvent provides a

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compatible solvent system for most types of derivatization. In addition, elution of the analyte from the Sep Pak^{tm} is not limited to acetonitrile (nor is it necessary to elute with the derivatization reagent incorporated into the solvent) but may be optimized for other types of derivatization provided that the solvent is adequately dried and will elute the compounds of interest from the Sep Pak^{tm}.

For samples containing analyte in concentrations substantially higher than 100 ppm, the volume of aqueous solution deposited on the Sep Pak^{tm} should be reduced in order to maintain an adequate excess of the derivatization reagent. Although the method was initially devised for low level analysis, it has been shown to be applicable for derivatization of the described compounds with DNBC at all levels down to sub ppm levels in the aqueous solutions.

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REFERENCES

1. Pierce, A.E., "Silylation of Organic Compounds". Pierce Chemical Co. Rockford Il. 1979.

2. Lawrence, J.F. and Frei, R.W., "Chemical Derivatization in Liquid Chromatography". Elsevier, Amsterdam. 1976.

3. Valdez, D., J.Chromatogr.Sci. 23, 128, 1985.

4. Brittain, G.D., Sullivan, E.S., and Schewe, L.R., "Recent Advances in Gas Chromatography". I.I. Domsky and J.A. Perry, eds. Marcell Dekker, New York. 1971. pp 223-229.

5. Valdez, D. and Reier, J.C., J.Chromatogr.Sci. Accepted for publication.

6. Wellons, S.L. and Carey, M.A., J.Chromatogr. 154, 219, 1978.

7. Carey, M.A. and Persinger, H.E., J.Chromatogr.Sci. 10, 537, 1972.

8. Valdez, D. and Iler, H.D., J.Amer.Oil.Chem.Soc. 63, 119, 1986.

9. Erley, D.S., Anal.Chem. 29, 1564, 1957.

10. Leenheer, J.A., "Water Analysis Vol.III Organic Species". R.A. Minear and L.H. Keith, eds. Academic Press, New York. 1984. pp 84-166.

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