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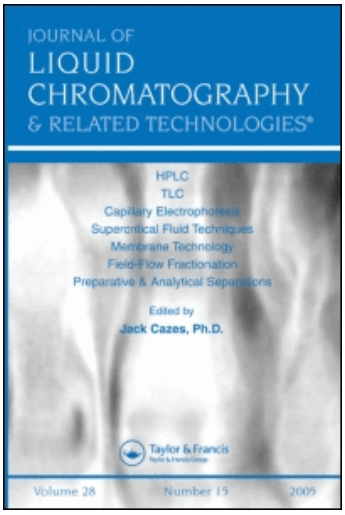

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TRACE ANALYSIS OF SODIUM AZIDE IN AN ORGANIC MATRIX

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TRACE ANALYSIS OF SODIUM AZIDE IN AN ORGANIC MATRIX

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ABSTRACT

A method for analysis of residual azide in an organic matrix (triazole derivative) is described. The azide was separated from the matrix using a solid phase extraction cartridge, and analyzed using reversed-phase chromatography with direct UV detection. The analysis of azide was used as a limit test for a level of 20 ppm relative to the weight of the organic matrix. Thermodynamic studies of the chromatographic system suggested that the interaction between azide and the stationary phase was hydrophobic in nature.

INTRODUCTION

Sodium azide is used for a variety of technological applications including agriculture, bio-medical sciences, the automotive industry, and in the production of explosives. In agricultural applications sodium azide is used as a herbicide, nematocide, and rot control inhibitor. [NIOSH (1992): NIOSH Recommenda-

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tions for Occupational Safety and Health: Compendium of policy documents and statements. DHHS (NIOSH) publication No. 92-100. Cincinnati, OH, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention; NIOSH.]

This compound is also known to be a potent vasodilator, which allows it to be utilized for blood pressure control.[1-3] In the medical sciences it is also used as a preservative and a diluent in automatic blood cell counters. Other important biological functions of sodium azide include inhibition of mitochondria metabolism,[4] regulation of the intercellular reactive species,[5] and scavenging of hydroxyl radical species.[6]

Sodium azide is an important reagent in fine organic, inorganic, and polymer synthesis.[6,8-12] Additionally, sodium azide is employed in the production of hydrazoic acid, which is needed for production of heavy metal azides used in shell detonators in the explosives.[13] Finally, sodium azide is a principal gas-generating reagent for propulsion of airbags commonly used in the automotive industry.[2]

On the other hand, azide salts are known to produce mutations, be highly toxic, and carcinogenic.[14,-17] They induce cell damage, neuronal injury, and block oxidative metabolism.[2, 15,18]

Rapidly growing production and increased occupational and domestic exposure to sodium azide dictates a necessity to develop fast, sensitive, and rugged methods for analysis of this ultimately dangerous compound in complex organic matrices. The NIOSH recommended exposure limits (REL's) are ceiling limits of 0.1 parts per million (ppm) hydrazoic acid vapor and 0.3 mg/m^3 sodium azide[19].[i] A number of methods have been described in the literature for the analysis of azide in various matrices. The analysis of azide has been performed using titrimetry,[20,21] spectrophotometry,[22,23] derivatization followed by HPLC,[24-28] ion chromatography,[29] and capillary electrophoresis.[30] Although these methods are adequate for the applications described, they typically require special treatment of the sample, such as oxidation with nitrite, conversion of azide into volatile hydrazoic acid followed by trapping it in a gas scrubber, or transformation of azide into dintrobenzoyl derivative, etc.

These special treatments may effect the method sensitivity, lead to matrix interferences, and increase the time and cost of analysis. A few methods of direct azide analysis were described in literature.[31,32] The current paper describes a fast, and simple method for residual azide analysis based on solid phase extraction to avoid matrix interferences, which is followed by HPLC analysis with direct UV-detection. The analysis of azide was used as a limit test for a level of 20 ppm relative to the weight of the organic matrix which corresponds to 0.2 ppm in solution.

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EXPERIMENTAL

Reagents

Concentrated sulfuric acid was purchased from Fisher (Springfield, NJ). Deionzied water was purchased from Aldrich (Milwaukee, WI). Azide was purchased from Sigma (St. Louis. MO) and triazole 4-*N,N*-dimethylaminomethyl-5-formyl-1,2,3-triazole was produced by the Process Research Department (Merck Research Laboratories, Rahway, NJ).

Chromatographic System

The HPLC system used in the present study was a Hewlett Packard (Paulo-Alto, CA) HP1100 model equipped with a temperature controlled column compartment. A strong cation exchange column, PRP-X300, was purchased from Hamilton (Reno, NV). The mobile phase for the elution of the azide from the chromatographic column, unless specified, consisted of an aqueous solution of 0.1% of sulfuric acid.

All separations were performed at a flow rate of 1.5 mL/min with a UV detector at 210 nm. The resulting chromatograms were processed using Turbo-Chrom software (Perkin Elmer, Wellesley, MA).

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Solid Phase Extraction

Prior to the azide analysis, four solid phase cartridges, OnGuard H (Dionex, Sunnyvale, CA), were assembled in series and were conditioned by passing through 10 mL of deionized water followed by 10 mL of sample solution. To analyze the samples, 100 mg of sample was dissolved in a 10 mL solution of 0.1% (1 mL acid in 1L water) sulfuric acid. The first 5 mL was discarded while the rest was retained for the analysis. An aliquot of 20 μL was then injected into the HPLC system.

RESULTS AND DISCUSSION

4-*N,N*-dimethylaminomethyl-5-formyl-1,2,3-triazole (here and after triazole aldehyde) (Fig. 1) is a key raw material in the synthesis of an active pharmaceutical ingredient. Azide is a key reagent in the synthesis of triazole aldehyde. The level of azide should be controlled carefully because of potential contamination in the final drug product.

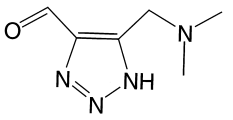

Figure 1. Chemical structure of 4-*N,N*-dimethylaminomethyl-5-formyl-1,2,3-triazole.

The column used for the azide analysis was a strong cation exchange polymer column, PRP-X300. The stationary phase has a negative charge on the surface and the ionization state of the ionic groups does not change so long as the pH of the mobile phase is two pH units greater than the pK_a of the SO_3^- groups. The effect of the pH becomes important if an ion-interaction mechanism is involved between the analyte and the strong cation exchange resin. In order to optimize the method, the first step was to establish the types of interactions that occur between the azide molecules and the polymeric stationary phase. In order to study such interactions two studies were undertaken. First, the concentration of sulfuric acid in the mobile phase was varied in order to study its effect on the analyte retention. Second, the effect of temperature on the retention factor, ***k′***, of the azide was studied and was used to determine the thermodynamic parameters of the interaction.

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Effect of Sulfuric Acid Concentration

The concentration of sulfuric acid was varied from 2 mM to 300 mM. For each concentration three injections were performed. The %RSD for the triplicate injections was less than 0.2%. Under these acidic conditions, the azide is in the form of hydrazoic acid ($pK_a = 4.69$).[33] It is possible that, at the mobile phase conditions employed, a dipole moment is induced on hydrazoic acid, which results in interaction with sulfate ions. This complex is more hydrophobic in nature. Indeed, an increase in ***k′*** was observed with the increase in concentration of the sulfuric acid (Fig. 2). The increase in ***k′*** of the hydrazoic acid complex is a result of interaction between the associate and the polymeric backbone.

Influence of Temperature on k' of the Azide

It is known that, the capacity factor of a solute is related to the change in partial molar free energy incurred during the transfer of solute between the mobile phase and the stationary phase and it is represented by the equation:

$$\ln k' = -(\Delta G^o/RT) + \ln \Phi \qquad (1)$$

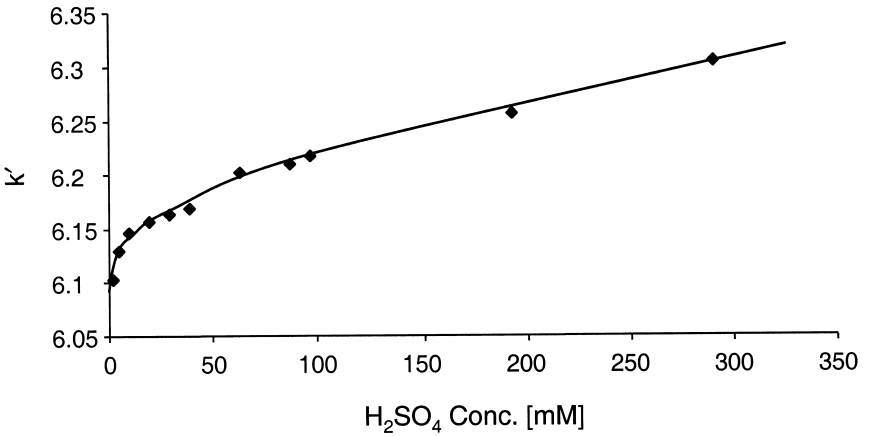

Figure 2. Influence of sulfuric acid concentration on k' of hydrazoic acid.

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where Φ represents the phases ratio. The free energy can be broken down into enthalpic and entropic term to give the van't Hoff equation:

$$\ln k' = -(\Delta H^{o}/RT) + \Delta S^{o}/R + \ln \Phi \qquad (2)$$

According to Eq. 2 a plot of ln k' vs. (1/T) should be linear, with a slope of $-(\Delta H°/R)$ and an intercept of $(\Delta S°/R + \ln \Phi)$.

The temperature of the column was increased from 5°C to 40°C in five degrees increment. At each temperature the analyte was injected five times into the HPLC system. The %RSD for the injections at each temperature was less than 0.1%.

The van't Hoff plot is presented in Fig. 3. The results indicated an overall high negative enthalpy of -3268 cal.mol^{-1} and a negative entropic term of –7.3. Such results suggest an adsorption process (e.g. hydrophobic interactions between the hydrazoic acid complex and the polymeric backbone of the stationary phase). If these interactions were electrostatic in nature both the entropic and enthalpic terms would be positive[34] (entropy-enthalpy compensation). Under these circumstances, the ***k'*** should increase with the increase in temperature, a phenomenon which was not observed in our studies. As a consequence, it was suggested that the interaction between the hydrazoic acid complex and the PRP-X300 stationary phase is predominately hydrophobic.

Azide Analysis

The pK_a of triazole aldehyde is around 9.5.[35] Under the mobile phase conditions, the compound will have a strong positive charge due to the protonation of

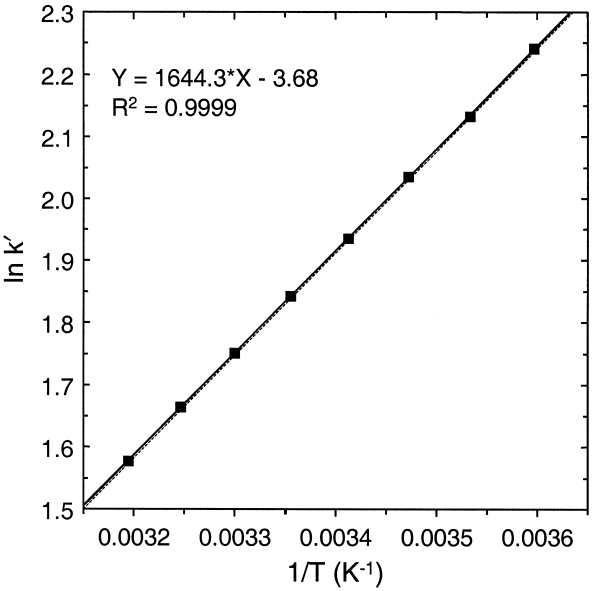

Figure 3. The van't Hoff plot of ***k'*** for hydrazoic acid.

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both the tertiary nitrogen and the triazole ring. As a consequence, the compound will be strongly retained on a cation exchange column. In the initial experiments where the samples of triazole aldehyde were spiked with sodium azide, retention of approximately 8.5 minutes was obtained for azide, while the triazole aldehyde showed a broad peak, which started eluting at 30 minutes continuing over 90 minutes. To shorten the analysis time, a solid phase extraction step was introduced in the analysis. The solid phase extractors consisted of cartridges packed with strong cation exchange resin (OnGuard H, which contained 16% cross-linked styrene-based sulfonic acid resin) expected to retain the triazole aldehyde. Three solutions were prepared corresponding to concentrations of azide of 0.0002, 0.001, and 0.1 mg/mL, respectively. In each of the azide solutions 10 mg/mL of triazole aldehyde was dissolved. These triazole aldehyde solutions were passed through four cartridges in series. The resulting solutions were analyzed by HPLC and the results were compared to the samples not passed through the solid phase extractors. The data obtained from these experiments are presented in Table 1. The recovery was calculated by the following equation.

$$\% \text{ Recovery} = \frac{\text{Area counts of Azide from solution passed through cartridge}}{\text{Area counts of Azide from solution not passed through cartridge}} \text{ X } 100$$

An acceptable recovery ranging from ~83% to ~97% was obtained. Additionally, a plot of column 2 vs. column 3 of Table 1 led to a straight line with

Table 1. Recovery of Azide from the Solid Phase Extractor

Azide Concentration (mg/mL)	Area Counts Before SPE	Area Counts After SPE	Recovery (%)
0.0002	978	841	86
0.001	4525	3722	83
0.1	369912	360399	97

a slope of 0.975, indicating that the SPE cartridge did not contribute to the loss of recovery. The method, as described, was used as a limit test for the analysis of azide at a level of 20 ppm azide in the triazole aldehyde. According to the International Committee for Harmonization (ICH), a limit test can be used, provided that the signal/noise (S/N) is at the limit of detection (S/N = 3). Thus, a sample of triazole aldehyde was spiked at a level of 20 ppm and subjected to the analysis in twelve replicates. The results are presented in Table 2.

The data of Table 2 indicate that the method can be used as a limit test for the analysis of azide in triazole aldehyde. Figure 4 shows the chromatograms of a sample of triazole aldehyde as is and spiked with an equivalent amount of 20 ppm azide.

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Table 2. Reproducibility of S/N for 20 ppm Azide Spiked in Triazole Aldehyde

Injection Number	S/N
1	4.1
2	4.4
3	5.1
4	6.1
5	4.3
6	4.1
7	3.0
8	4.9
9	3.6
10	3.7
11	5.8
12	5.7
Average	4.6
SD	0.95
%RSD	20.7

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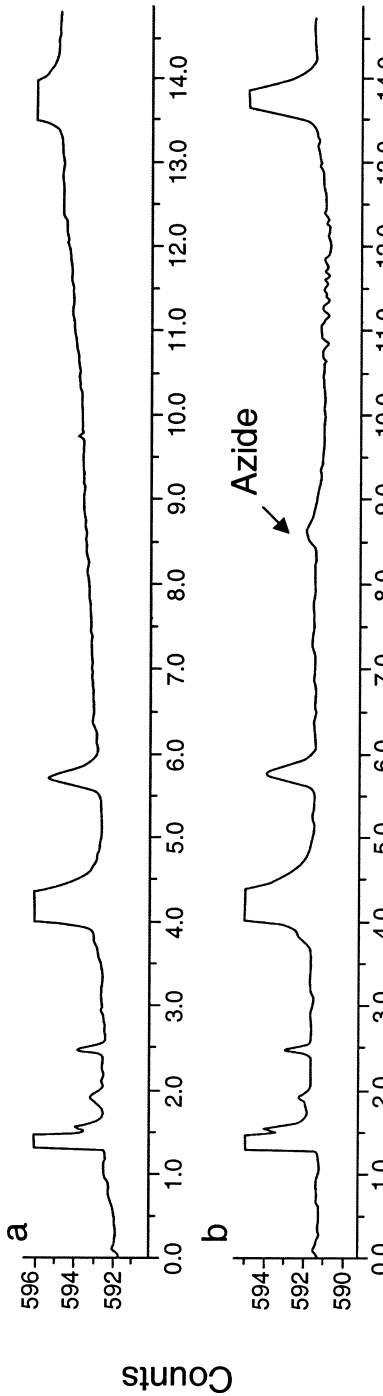

Figure 4. Chromatograms of a triazole aldehyde sample "as is" (a and b) and sample spiked with a corresponding amount of 20 ppm azide (c and d). (For the chromatographic conditions see Experimental Section.)

CONCLUSIONS

The interaction between the azide (hydrazoic acid) and the strong cation exchanger was assumed to be hydrophobic, while the interaction between the triazole aldehyde with this stationary phase was electrostatic. The results obtained from the azide analysis were supported by the thermodynamic and the sulfuric acid concentration studies. The analysis of azide in triazole aldehyde was used as a limit test with a S/N of 4.6 at a level of 20 ppm.

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