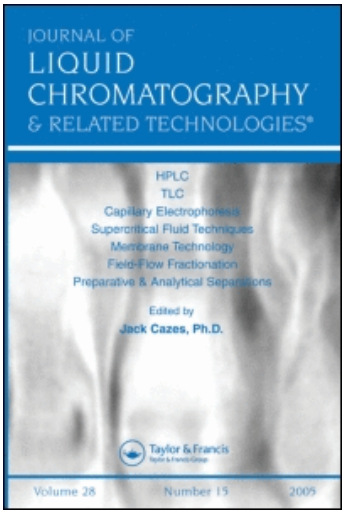

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Liquid Chromatographic Determination of Azide as the 3,5-Dinitrobenzoyl Derivative

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LIQUID CHROMATOGRAPHIC DETERMINATION OF AZIDE AS THE 3,5-DINITROBENZOYL DERIVATIVE

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ABSTRACT

A method for the determination of azide after conversion to 3,5-dinitrobenzoyl azide has been developed. The derivatization reaction is fast (3 min.), quantitative, and yields a product with strong ultraviolet absorption. The derivatization reaction mixture is separated by high performance liquid chromatography so that the azide derivative can be easily quantitated. The detection limit of the method is 10 ng NaN_3/mL. The total analysis time is 20 minutes per sample.

INTRODUCTION

Sodium azide has been used in pesticides, herbicides, soil fumigants, wood preservatives, and antihypertensive drugs. More recently, it has been proposed for use as a gas generant (N_2) in inflatable systems for vehicle occupant restraint in collisions. For the latter use, interest has been focused on formulations in which all azide is decomposed, to minimize any health effects of azide. Thus, a method was needed for determining small quantities of azide in the presence of several inorganic and organic reaction products. The products were both solids and gases, collected in vehicles and in specially designed chambers (tanks).

Spectrophotometric and titrimetric methods for determining sodium azide or azide ions in aqueous solutions are quite numerous (1-3), but not satisfactory for the determination of microgram amounts, either alone or in the presence of other substances. Recently, a method for the determination of azide by ion chromatography with microgram sensitivity has been published (4). Unfortunately, this method is subject to interferences by bromide and adipate ions (present in some inflatable restraint systems), and the sensitivity is limited to about 0.1 μg NaN_3/ml.

This paper describes a rapid, sensitive method for determining azide based on its reaction in slightly acidic aqueous solution with 3,5-dinitrobenzoyl chloride to form 3,5-dinitrobenzoyl azide. An aliquot of the reaction mixture is injected onto a high performance liquid chromatograph (HPLC) where the products are separated on a reversed-phase column, and the azide derivative is detected with an ultraviolet (UV) detector at concentrations as low as 10 ng NaN_3/ml of sample. Total analysis time is 20 minutes per sample.

EXPERIMENTAL

Apparatus. A duPont Model 830 HPLC (duPont Instruments, Wilmington, DE) with a fixed wavelength (254 nm) UV detector was used. A duPont Model 833 flow controller was used to control the mobile phase flow rate at 1.0 ml/min. A pneumatically-actuated (Valco) valve (Houston, TX) with a 27-μl loop was used for sample injection.

Reagents. The mobile phase was 50 % acetonitrile and 50 % water (v/v) prepared using "distilled-in-glass" grade acetonitrile from Burdick and Jackson (Muskegon, MI) and deionized water. The 3,5-dinitrobenzoyl chloride was obtained from Aldrich Chemical (Milwaukee, WI) and was used as received. The chromatographic column was 25 cm x 4.6 mm Zorbax ODS (duPont Instruments) preceeded by a 4 cm x 3.2 mm RP-18 microparticulate precolumn (Altex Div., Beckman Instruments, Berkeley, CA).

Confirmation of the Derivative Identity. A few milligrams of 3,5-dinitrobenzoyl azide was prepared using the procedure of Munch-Peterson (5). The identity of the 3,5-dinitrobenzoyl azide in samples was confirmed by comparing the retention time to that of this known standard. In addition, in one sample, the constituent associated with the 3,5-dinitrobenzoyl azide peak was collected, the solvent was evaporated, and the residue was analyzed by high resolution mass spectrometery using an AEI MS-30 mass spectrometer. The characteristic fragmentation pattern matched that of the standard and positively identified the peak as 3,5-dinitrobenzoyl azide.

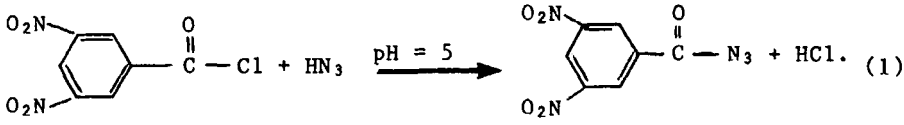
Procedure. Samples from several sources were obtained during the testing of experimental inflatable restraint systems. Air samples were collected by passing known volumes of air through midget impingers containing 0.02 N Na_2CO_3 or 0.04 N KOH solutions. The washings of tank tests (0.04 N or 0.1 N KOH) were used as received. Known quantities of solid samples were extracted at room temperature using known volumes of 0.04 N KOH to dissolve the soluble azide. Whatman filter papers were wetted with 0.02 N Na_2CO_3, used for wipe tests, and then placed in beakers containing sufficient 0.02 N Na_2CO_3 to cover the paper. Animal body fluid samples, diluted with normal saline solution, were used as received.

A 5-ml volume of one of the above prepared solutions was pipeted into a graduated, 12-ml test tube (Kontes K-569300, Vineland, NJ). (If a sample volume of less than 5.0 ml was used, sufficient 0.02 N Na_2CO_3 was added to bring the volume to 5.0 ml.) Two ml of acetonitrile and 5 drops of Bromthymol Blue indicator (0.1 g in 3.2 ml of 0.2 N NaOH diluted to 100 ml) were added. After adding 0.2 N HCl dropwise until the indicator undergoes the color change from blue to yellow, one more drop of acid was added (pH = 5). Then 50 µl of a solution of 1 g 3,5-dinitrobenzoyl chloride in 10 ml acetonitrile was added, the test tube was stoppered, shaken for

several seconds, and allowed to sit for three minutes. The final volume of the test tube was noted, and an aliquot was withdrawn for injection on the liquid chromatograph. Quantitation was accomplished by comparing the peak height for the unknown to that obtained for standards. Correction was made for the total volume in the test tube due to different amount of 0.2 N HCl being required to neutralize the sample solution and the standards. (Alternatively, all of the preparations can be brought to the same final volume by the addition of acetonitrile.)

RESULTS AND DISCUSSION

One reagent that forms derivatives that strongly absorb UV radiation is 3,5-dinitrobenzoyl chloride, which reacts in basic solution with alcohols and amines to form the 3,5-dinitrobenzoates and amides, respectively, (6). However, the nucleophilicity of the azide ion is also quite strong, and it can react with 3,5-dinitrobenzoyl chloride,



(1)

We have utilized this reaction to form a UV-absorbing derivative of azide prior to the determination by HPLC.

The chromatographic separation of the derivatization reaction mixture is accomplished without prior clean-up using reversed-phase liquid chromatography on Zorbax-ODS with 50/50 acetonitrile/water as mobile phase at a flow rate of 1.0 ml/mm. A typical chromatogram is shown in Fig. 1. As noted there, the first product that elutes is 3,5-dinitrobenzoic acid, which is the product of hydrolysis of the excess reagent. Three other peaks in the chromatogram are due to impurities in the reagent and do not interfere with 3,5-

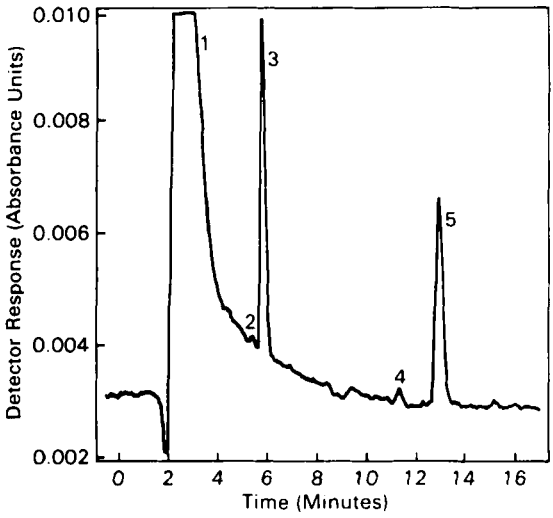


Figure 1. Chromatogram of reaction product of 0.31 μg NaN_3/ml (in impinger solution) with 3,5-dinitrobenzoyl chloride. 27-μl injection. UV detector x 0.01 absorbance units full scale. Peak 1--3,5-dinitrobenzoic acid. Peaks 2,3,4--impurities in reagent. Peak 5--3,5-dinitrobenzoyl azide.

dinitrobenzoyl azide, which elutes in about 13 minutes under these conditions.

Since hydroxide ion is a stronger nucleophile than azide, the derivatization reaction is sensitive to pH. Our results indicate that at pH $\geq$ 6.5 the azide derivative forms, but is subject to basic hydrolysis so that the amount of derivative decreases (half-life of about 2 hours at pH7). In acid solutions, pH < 3.0, the yield of azide is not quantitative, probably due to acid hydrolysis. At pH between 3.0 and 5.5 the azide derivative forms quantitatively and is quite stable, exhibiting only a 20 % loss in peak height after 10 hours. In our procedure samples are chromatographed immediately after reaction, so slow hydrolysis is not a problem. The use of Bromthymol Blue as an indicator for adjustment of the pH before the

addition of reagent is a unique and convenient way to handle samples of different pH. The indicator elutes from the HPLC column in the void volume and thus does not interfere.

The possibility of interference by other strong nucleophiles has also been investigated. Two types of interference are possible: positive interference in which the interfering specie reacts with the derivatizing reagent to yield a specie with the same retention time as the azide derivative, and a negative interference in which the interfering specie reacts with the derivatizing reagent to yield a specie with a retention time different from the azide derivative, but consumes sufficient reagent so that the reaction with azide is incomplete. The results of our study of potential interferences are shown in Table 1.

When the potential interference (except aniline) is present at eight times the azide concentration, the recovery of azide is 100 ± 3 %, which is within the precision level of the technique. It appears that thiocyanate, iodide, and methanol, although they are strong nucleophiles, do not react under the pH conditions that we have developed for the determination of azide. Acetate and ferric

TABLE 1. Effect of Potential Interferences on the Determination of Azide

Sample	Potetential Interference	Amount	Recovery of Azide, %
2.5 μg NaN_3	SCN^-	20 μg	100.0
"	I^-	20 μg	98.5
"	CH_3OH	2000 μg	100.3
"	CH_3COO^-	20 μg	100.8
"	Fe^{+3}	20 μg	101.2
"	$C_6H_5NH_2$	2 μg	97.3
"	"	20 μg	79.2
"	"	200 μg	22.0
"	"	2000 μg	5.6

TABLE 2. Typical Results for the Determination of Sodium Azide in Various Sample Matrices

Sample	Source	µg NaN_3/ml Added	µg NaN_3/ml Found	Spike Recovered (%)
1	Impinger Solution	---	< 0.01	
1A	Impinger Solution	0.30	0.30	100
2	Impinger Solution	--	5.66	
2A	Impinger Solution	3.0	8.63	99
3	Tank Wash	--	0.50	
3A	Tank Wash	0.67	1.18	101
4	Tank Wash	--	0.28	
4A	Tank Wash	0.67	0.94	99
5	Extracted Solid	--	0.51	
5A	Extracted Solid	0.30	0.80	97
6	Extracted Solid	--	2.18	
6A	Extracted Solid	1.00	3.20	102
7	Wipe Test	--	0.12	
7A	Wipe Test	0.30	0.42	100
8	Wipe Test	--	1.08	
8A	Wipe Test	3.0	4.04	99
9	Rabbit Blood Plasma	--	< 0.01	
9A	Rabbit Blood Plasma	0.30	0.31	103
10	Rabbit Blood Plasma	--	< 0.01	
10A	Rabbit Blood Plasma	5.0	4.85	97

ions, which interfere with the spectrophotometric methods for azide, do not interfere using the HPLC technique. However, aniline does exhibit negative interference (as might be expected from Eq. 1) by consuming reagent and yielding the 3,5-dinitrobenzoyl amide with a retention time of 15 minutes. At large excesses of aniline, insufficient derivatizing reagent is left to react completely with azide. Although amines do interfere, we have not noted any problems with the method, even with body fluid samples where amines may be present. Presumably their concentration is sufficiently low or they are less reactive than aniline, so they are not a problem.

Some typical results obtained with this method are shown in Table 2. The suffix "A" has been used to designate a spike sample, e.g., sample 1A was taken from the same source as sample 1 - an impinger solution used to collect air samples - but 0.3 μg NaN_3/ml was added to the impinger solution. Since no other methods are available for the determination of azide at this low level in this variety of matrices, this standard addition technique has been used to check the validity of the method.

The method has been in use for two years with no apparent problems. The reproducibility of the method is ± 3 % for repeat analyses of the same solution at the 0.3 μg NaN_3/ml level. The detection limit of the method, defined as the quantity of sodium azide originally present to give a 3,5-dinitrobenzoyl azide peak height twice the noise level, is 10 ng NaN_3/ml. This limit could be improved by the use of a larger injection loop or the use of a variable wavelength UV detector so that a wavelength closer to the maximum absorbance of the derivative (240 nm) could be utilized.

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