Journal of Chromatography, 464 (1989) 208–212
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 073

Note

Liquid chromatographic determination of hydrazine, carbohydrazide and thiocarbohydrazide in aqueous solutions

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(Received October 17th, 1988)

Hydrazine and carbohydrazide [$(NH_2NH)_2CO$] are used extensively as water additives in steam generating plants[1,2]. Thiocarbohydrazide [$(NH_2NH)_2CS$] is used as a metal complexing agent and analytical reagent, especially in the determination of vicinal diols in carbohydrates and proteins[3–7]. Additionally, carbohydrazide and thiocarbohydrazide are used as intermediates in the synthesis of various heterocyclic structures[8,9].

Gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods for the determination of hydrazine, with and without derivatization, have been published[10,11]. The most sensitive method reported to date involves extraction followed by HPLC and electrochemical detection with a pretreated electrode[12]. Another report described derivatization with salicylaldehyde followed by extraction and normal-phase HPLC[13]. In this work, the derivatization method of Matsui *et al.*[14] has been extended to include the concurrent determination of hydrazine and carbohydrazide in aqueous samples.

As hydrazine and carbohydrazide are water-treatment agents, their determination in aqueous solution is advantageous. Similarly, thiocarbohydrazide as a metal complexant and as an analytical reagent requires an assay method for aqueous samples. We know of no reports that have described an HPLC method for either carbohydrazide or thiocarbohydrazide. This paper describes a procedure for the determination of all three compounds in aqueous samples. The procedure avoids extraction steps and the use of specialized detectors while still maintaining the ability to assay samples in the low parts per million range.

EXPERIMENTAL

Apparatus

A Phase Separations (Queensferry, U.K.) 15 cm × 4.6 mm I.D. ODS-1 (5 μm) column was used in a Varian Model 5020 liquid chromatograph equipped with a column oven and a Rheodyne (Cotati, CA, U.S.A.) Model 7125 loop injector with a 10-μl loop. Peak areas were measured electronically with a Spectra-Physics (Darmstadt, F.R.G.) Model 4290 recording integrator. Analytes were detected by ultraviolet spectrophotometry using a Varian Model UV-100 variable-wavelength instrument.

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Reagents

HPLC-grade acetonitrile, synthesis-grade hydrazine hydrate [64% (w/w) hydrazine], synthesis-grade benzaldehyde and extra-pure grade potassium dihydrogenphosphate were supplied by Merck (Darmstadt, F.R.G.) and analytical-reagent grade ethanol by Frutarom (Haifa, Israel). Water was deionized and glass distilled prior to use. Buffer (0.1 *M*) was prepared by adding 13.6 g of potassium dihydrogenphosphate to a 1-l volumetric flask, diluting to mark with water, adjusting the pH to 7.0 with a few drops of dilute potassium hydroxide solution and then filtering through a 4.5-μm filter. The mobile phase was acetonitrile– buffer (45:55, v/v) and was filtered prior to use. Standards of the benzaldehyde derivates of hydrazine, carbohydrazide and thiocarbohydrazide were supplied by the Organic Chemistry Department (Makhteshim, Beer-Sheva, Israel). A stock solution of all three standards was prepared fresh daily by weighing exactly about 50 mg of each into the same 50-ml volumetric flask and diluting to the mark with acetonitrile. Dilutions of the stock solution were made as required and benzaldehyde reagent was prepared as a 15% (w/v) solution in ethanol.

Procedure

The flow-rate was 2.0 ml/min at a column oven setting of 40°C and the column was equilibrated for 20 min. Standard solution was injected and, if necessary, the eluent composition and compound concentration adjusted such that the capacity factor (k') of benzalazine was 14.6 $\pm$ 5% and the absorption was within the desired range. Stock solutions were prepared in duplicate and diluted such that the standard response was $\pm$ 10% of the sample response. Analytes were detected at 310 nm at a detector time constant of 0.5 s and 10 μl of solution were injected.

For samples with an analyte concentration expected to be at or above the 1 ppm range, accurately weigh, in duplicate, about 2 g of sample into a 25-ml volumetric flask, add 1 ml of benazaldehyde solution and place the flask in a 70°C water-bath for 30 min. Cool to the room temperature, add acetonitrile to the mark and make further dilutions of this solution as necessary with acetonitrile. Adjust the concentrations such that the peak areas of the standard and sample are within the linear detector range and agree to within $\pm$ 10%. Inject standard solutions until duplicate injections agree to within $\pm$ 5% at concentrations from 0.1 to about 10 ppm and then start the analytical sequence. Calculate the concentration of analytes in sample as follows:

$$\text{Concentration of analyte (\%)} = \frac{(\text{area/g sample})(\text{concentration of hydrazine standard})}{(\text{area/g standard}) \cdot F}$$

where area/g is the integration area divided by weight and the factor F is the molecular weight of the derivative/molecular weight of underivatized material. The values of F are hydrazine 6.56, carbohydrazide 2.95 and thiocarbohydrazide 2.66.

Samples containing low concentrations of hydrazine must be maintained in an oxygen-free atmosphere prior to derivatization. Failure to exclude oxygen leads to low results for hydrazine but not for carbohydrazide.

RESULTS AND DISCUSSION

The chromatographic separations were complete, as shown in Fig. 1. In this system unreacted benzaldehyde eluted first, followed in order by the benzaldehyde derivatives of carbohydrazide, thiocarbohydrazide and hydrazine. The capacity factors were 1.9, 4.1, 5.6 and 14.6, respectively. Absorption maxima in the UV spectra of carbohydrazide derivatives were noted at 300 and 319 nm whereas a maximum at 301 nm was reported for the hydrazine derivative[10]. An effective compromise was found by setting the detector wavelength at 310 nm for all three derivatives. This wavelength was superior to 254 nm because at 310 nm the detection limit was at least one order of magnitude better for all three compounds. The detection limits (signal-to-noise ratio > 3) found under these conditions were 17, 16 and 32 ppb for the hydrazine, carbohydrazide and thiocarbohydrazide derivatives, respectively (Fig. 2). The recoveries of hydrazine, carbohydrazide and thiocarbohydrazide (triplicate determinations) were 101, 106 and 104% respectively, when solutions containing 0.1% of each were reacted with an excess of benzaldehyde. When five samples, each containing 40 ppb of carbo-

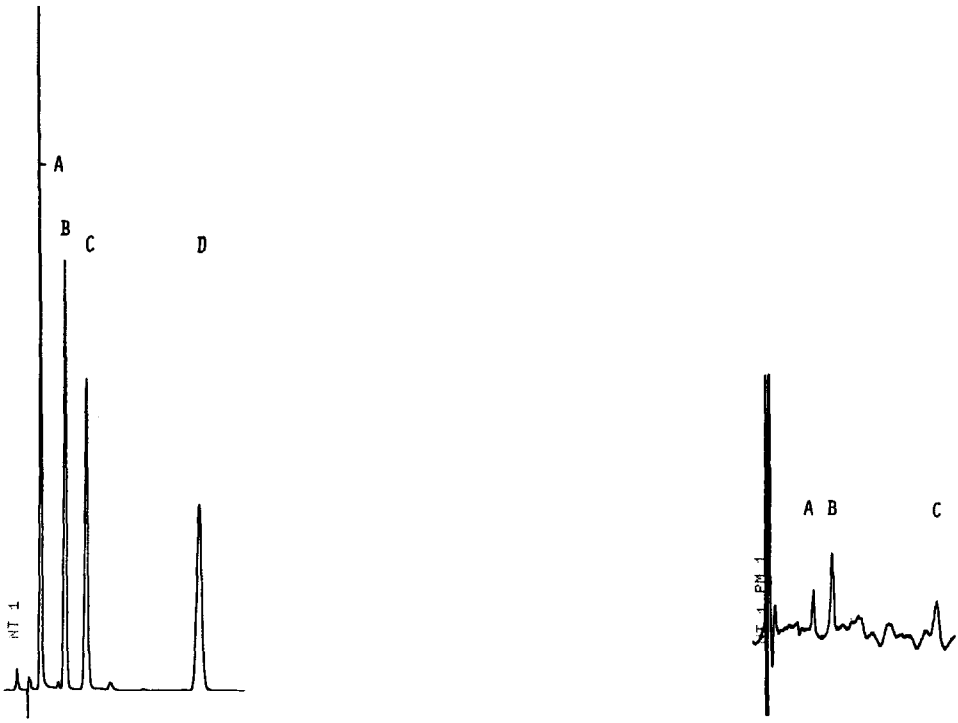

Fig. 1. Chromatogram showing resolution of benzaldehyde (A), carbohydrazide derivative (B), thiocarbohydrazide derivative (C) and benzalazine (D) using a 15-cm OSD-1 column; 10-μl aliquots from samples containing the benzaldehyde derivatives equivalent to 39 ppm of carbohydrazide, 79 ppm of thiocarbohydrazide and 33 ppm of hydrazine were injected with a detector wavelength of 310 nm.

Fig. 2. Detection of (A) carbohydrazide, (B) thiocarbohydrazide and (C) hydrazine in samples containing 16, 32 and 17 ppb, respectively, when 10 μl of the benzaldehyde derivatives were injected, with a detector wavelength of 310 nm (0.004 a.u.f.s.).

TABLE I

STUDY OF DETECTOR RESPONSE FOR BENZALDEHYDE DERIVATIVES OF HYDRAZINE, CARBOHYDRAZIDE AND THIOCARBOHYDRAZIDE

Benzaldehyde derivative of	*Solution[a] concentration (ppm)*	*Response[b] (area/g)*
Hydrazine	0.026	730
	0.052	800
	0.132	804
	13.3	742
Carbohydrazide	0.026	327
	0.052	296
	0.13	321
	13.2	310
Thiocarbohydrazide	0.064	396
	0.128	341
	0.316	328
	32	304

[a] 10 μl of solution injected.
[b] Average of duplicate injections at each concentration.

hydrazide, were analysed the average recovery was 83% with a relative standard deviation of 12%. Six samples containing about 0.4 ppm each of hydrazine were analysed by this method. The average recovery was 87% with a relative standard deviation of 5%.

When preparing or handling samples containing low concentrations of hydrazine, it is essential to prevent oxygen from contacting the sample prior to derivatization. A reaction time of 30 min at a bath temperature of 70°C was used for the derivatization of the analytes, which was in agreement with the derivatization conditions for hydrazine reported previously[14]. The benzaldehyde derivatives were preferred to the corresponding salicylaldehyde derivatives owing to their better solubilities in the solvents used.

A study of the linear response range of the detector for the three derivatives was performed and covered four orders of magnitudes (Table I). All the derivatives exhibited a linear response over three orders of magnitude of concentration from the 0.1 ppm level upward when 10 μl of sample were injected. Linear regression analysis on the points in the linear range, with at least four points for each compound, afforded a correlation coefficient for the resulting lines of at least 0.999 for all the derivatives.

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