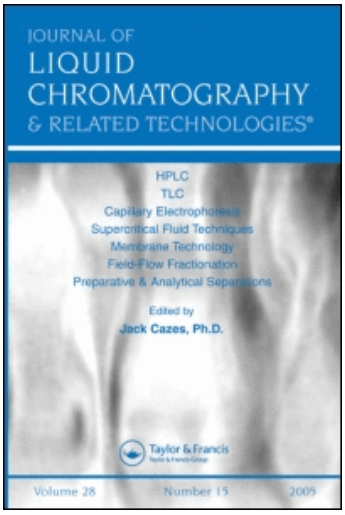

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A SELECTIVE DETERMINATION OF AZIDE BY ION-INTERACTION REVERSED-PHASE HPLC

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ABSTRACT

A method is presented for the analysis of sodium azide , based on the use of ion-interaction reversed-phase HPLC chromatography. A C-18 reversed-phase is the stationary phase and octylammonium ortho-phosphate at different pH values is the interaction reagent. Spectrophotometric detection at 230 nm is employed.

The analysis is free from interference by acetate, carbonate, chloride, fluoride, sulphite, hydrazine, hydroxylamine, nitrate, bromide, iodide, sulphide, thiocianate and nitrite.

A good correlation (r^2 = 0.9782) is obtained between peak area and concentration in the range between 1 and 250 ppb. Samples of tap water spiked with sodium azide (in the range within 25 and 250 ppb) gave per cent average recovery of 98%. The method sensitivity, expressed as signal to noise ratio equal to 3, is 50 ppb when the pH of the interaction reagent is equal to 3.0, 30 ppb for pH 6.4 and 10 ppb at pH 8.0.

INTRODUCTION

Sodium azide is a toxical environment pollutant. Due to its antiseptic properties, it has been widely used as a biostatic and antimicrobial agent [1] in water treatments until properties of canceromutagenis have been recognized [2,3]. Sodium azide is nowadays still used in diagnostic preparations as antiseptic [4] or in agriculture as nematocide [2]. Some azides find application in industry as detonators and as getters in electrical discharge tubes, as well as in anticorrosion studies and in the production of foam rubber[5].

Due to its mutagenic properties, suitable analytical methods for azide trace determination are required. Different methods are proposed in literature, based on: oxidation of azide with Ce(IV) and spectrophotometric evaluation of the unreacted Ce(IV)[6], spectrophotometric evaluation through the formation of iron(III)azide without [7] or with [8] previous distillation to hydrazoic acid, oxidation with Ce(IV) and gas-chromatographic evaluation with thermal conductivity detection[2] of the nitrogen evolved. Sodium azide content was also determined through potentiometric titrations with nitrite as the titrant : the end-point was evaluated by dicyanobis(1,10-phenanthroline)iron(II) dihydrate as the indicator[9] or by a nitrogen-oxide potentiometric sensor[10]. By an ion-chromatography method performed at pH 3.5 [1] detection limits of 2 ppm interference-free from chloride, nitrite, bromide, nitrate were obtained. By ion-pair HPLC chromatography with spectrophotometric detection sensitivity of 0.1 ppm were reached [11], but interference

was not checked. Other HPLC methods for azide determination in wine make use of pre-column derivatization reactions with 3,5-dinitrobenzoyl chloride [12,13]. A recent paper presents a new potentiometric gas-sensor which allows a selective determination of azide with sensitivity of 0.8 ppm[5].

In this paper we present a method, based on the use of the ion-interaction HPLC chromatography, which offers advantages of selectivity together with sensitivity as low as 10 ppb of N_3^-. The determination can be performed directly on the solution to be analyzed and does not require any other treatment.

EXPERIMENTAL

Apparatus.

Analyses were carried out with a Merk-Hitachi Lichrograph chromatograph Model L-6200, equipped with a two-channel Merck-Hitachi model D-2500 Chromato-integrator, interfaced with a Merck-Hitachi UV-Vis detector model L-4200 and a L-3720 Merck-Hitachi conductivity detector.

A Metrohom 654 pH-meter equipped with a combined glass-calomel electrode was employed for pH measurements and a Hitachi mod.150-20 spectrophotometer for absorbance measurements.

Chemicals and Reagents.

Ultrapure water from Millipore Milli-Q was used for the preparation of solutions. Sodium nitrite, sodium nitrate, sodium

sulphite, sodium iodide, sodium bromide and hydrazine were Merck reagents. Octylamine, sodium azide, hydroxylamine, sodium sulphide, sodium chloride, potassium carbonate and hydrogen peroxide 30% puriss. were Fluka analytical grade chemicals. Potassium thiocyanate and acetic acid were C.Erba chemicals.

Chromatographic conditions.

A 5 μ m ODS-2 Spherisorb Phase Separation column fully end-capped and with a carbon load of 12% (0.5 mM/g) was used, together with a guard pre-column Lichrospher RP-18 (5 μ m).

The solutions to be used as the mobile phase were prepared by adding to the weighed amount of octylamine (to prepare a 5mM solution) the required amount of orthophosphoric acid up to obtain the desired pH value.The solutions so prepared contained therefore the same (5mM) analytical concentration of octylamine and different total analytical concentrations of o-phosphoric acid.

The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal was obtained; a minimum of 1 hour was necessary. After use, the column was washed and regenerated by flowing a 50/50 v/v water/methanol mixture (0.5 ml/min for 2 hours).

RESULTS AND DISCUSSION

The technique of ion-interaction reversed-phase HPLC chromatography already used in this laboratory [14-17] for separations

of anions and amines makes use of a reversed-phase C-18 stationary phase. The interaction reagent is an alkylammonium salt and represents the only component of the mobile phase which, flowing under isocratic conditions, determines a modification of the stationary phase surface. Our data agree with the hypothesis that the lipophilic alkylammonium ion is adsorbed on the C-18 alkyl chain giving rise to a primary positevely-charged layer on the surface. Through electrostatic forces, the interaction reagent anion is bound as well, giving rise to an electrical double layer adsorbed onto the stationary phase surface. According to Bidlingmeyer [18], the moiety adsorbed is likely an ion-pair which at the same time is in equilibrium with its ions flowing in the mobile phase.

The original reversed-phase stationary phase is modified into a new stationary phase which is able to retain both anions and cations. Retention of anions and protonated amines proceeds through the formation and adsorption onto the surface of new ion-pairs formed respectively with the alkylammonium ion and the anion of the interaction reagent. This holds if experimental pH conditions are chosen at which amines are protonated and anions dissociated.

On the basis of these considerations, for the analysis of sodiumazide (sodium hydrazoate) as anion N_3^-, the pH value of the mobile phase has to be chosen as a function of the pK_a value of hydrazoic acid ($pK_a = 4.74$).

As concerns detection, spectrophotometry permits more favourable sensitivities to be gained, with respect to the conductometric one . Figure 1 reports the spectrum of absorbance as a function of wavelength for a solution of sodium azide 2.5 ppm and

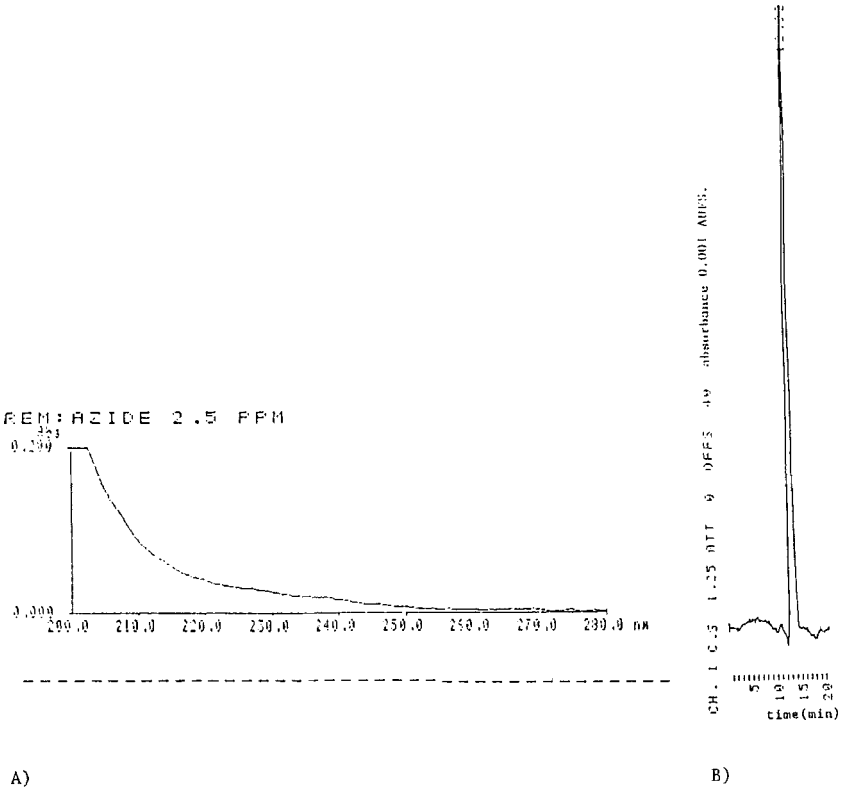

Figure 1.

A) Absorbance spectrum as a function of λ (nm) .
B) Azide 0.50 ppm. Stationary Phase : Phase Separation Spherisorb ODS-2, RP-18 (250 x 4.6 mm), endcapped, 5 μm.
Ion interaction reagent : 0.0050 mol/l octylamine ortho-phosphate.Flow-rate 0.5 ml/min. 100 μl injected. Spectrophotometric detection: 230 nm.

table I lists the molar absorptivity values evaluated in the range (210-260 nm) corresponding to the maximum of absorbance.

As the interaction reagent, octylammmonium orthophosphate was chosen . It does not absorb in this range of wavelength. Some experiments were also performed at 254 nm with octylammonium

TABLE I.
Sodium Azide. Molar Absorptivity at typical wavelenghts.

Wavelenghts (nm).	Molar Absorptivity (l mol $^{-1}$ cm $^{-1}$).
210.0	4108 ± 260
220.0	1894 ± 201
230.0	1191 ± 65
240.0	789 ± 52
250.0	350 ± 31
260.0	211 ± 20

salicylate as the interaction reagent and indirect spectrophotometric detection of azide : the method could be useful for azide qualitative analysis but sensitivity is less than that obtained with octylammonium o-phosphate.

Nothwithstanding azide absorbance shows its maximum value at 210 nm (figure 1) a wavelength of 230 nm was chosen as a compromise to guarantee good sensitivity together with a generally lower matrix effect. Figure I B reports a chromatogram recorded for a standard 0.50 ppm solution of sodium azide under the conditions described .

Two series of chromatographic measurements were performed for pH values of the mobile phase equal to 6.4 and 8.0, at which the molar fractions α of the azide dissociated form are respectively equal to 0.979 and 0.999. Some experiments were also performed at pH 3.0, in order to check if at pH values at which the dissociated fraction is very low (α = 0.002) , retention of azide is still

TABLE II.

Capacity factors, expressed as $K = (t_R-t_0)/t_0$, evaluated for typical analytes. t_R = retention time, t_0 = dead time

Stationary Phase : Phase Separation Spherisorb ODS-2, RP-18 (250 x 4.6 mm), endcapped, 5 μm.

Ion interaction reagent : 0.0050 mol/l octylamine ortho-phosphate.

Flow-rate 1.0 ml/min. 100 μl injected. Spectrophotometric detection: 230 nm.

	pH 3.0 t_0 = 3.47	pH 6.4 t_0 = 3.84	pH 8.0 t_0 = 3.39
Azide	0.36	0.94	0.69
Nitrate		1.30	
Nitrite	1.08	1.15	0.60
Iodate		1.65	
Bromate		0.69	
Thiocyanate		4.62	
Sulfide		> 7.0	

possible. These experiments showed the following interesting results:

a) azide is retained also at pH=3 even if with less favourable sensitivity than at higher pH values

b) sensitivity depends on pH

c) also retention depends on pH, as shown in table II, which reports the capacity factors for the pH values investigated.

This behaviour was shown also by other investigated anions and a study is in progress devoted to a systematic study of the role played on retention by the pH value of the mobile phase. The different retention times obtained for the different pH values can be ascribed to effects due to a different composition of the moiety adsorbed onto the stationary phase surface.

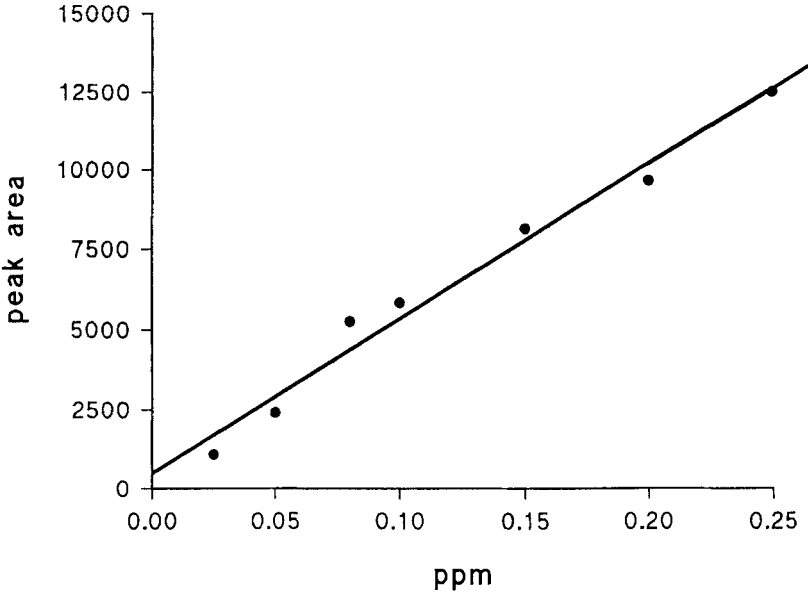

Figure 2.

Plot of peak areas vs. standard azide concentration (ppm).

As concerns sensitivity, evaluated as signal to noise ratio equal to 3 , this, as expected, directly depends on the molar fraction of the dissociated species, being 50 ppb for pH 3.0 , 30 ppb for pH 6.4 and 10 ppb for pH 8.0 . Notwithstanding sensitivity is more favourable at pH 8.0, the experiments were performed at pH 6.4 which is near to the most of aqueous solutions : in this way no pH adjustment is required .

Reproducibility of measurements was always within 3%. A calibration plot performed in the concentration range within 0.001 and 0.250 ppm showed (figure 2) a very good correlation (r^2= 0.9782)

between peak area (y) and concentration (x). The curve was fitted by the equation : $y = (479.7 \pm 467.4) + (48736.6 \pm 3253.2)\, x$.

Interferences.

As previously mentioned, qualitative and quantitative analysis of azide is required in different waters and the interference induced by the most common components must be considered.

It can be said that ion-interaction reagent chromatography behaves , when dealing with real matrices, as a partially selective technique , because only species able to form ion-pairs under the pH working conditions can be retained. No interference therefore comes from metals as well as from neutral, colloidal or high molecular-mass species.

Other species do not interfere because they do not absorb at 230 nm.

Other components, at last, are retained but show well-separated retention times with respect to azide.

In detail, the determination of azide is free from interference by the following species, which are usually indicated as the principal potential interferents :

acetate,carbonate,chloride,fluoride, sulphite, which do not absorb at 230 nm,

hydrazine and hydroxylamine, which at pH=6.4 do not give rise to protonated species and are not retained.

nitrates, bromide , iodide , sulphide, thiocyanate , which are retained under the used experimental conditions but are characterized by different capacity factors with respect to azide.

Figure 3 shows, as an example, the separation obtained for a mixture containing azide in the presence of bromide , nitrate , iodide and thiocyanate.

Nitrite . Nitrite is often indicated as one of the major interferent species in azide analysis. Also in our conditions when working at pH= 6.4 , nitrite and azide show comparable capacity factors and both are characterized by conductivity and by absorbance at 230 nm. On the other hand, it must be underlined that the mutual interference of these species is not due to resolution problems but to identification ones. Nitrite and azide in fact can not cohexist in aqueous solution, because they interact according to the reaction:

$N_3^- + NO_2^- \quad === \quad N_2O + N_2$ This is a fast reaction[9], on which the potentiometric titration of azide with nitrite as the titrant agent is based [9,10].

If azide is added to a solution containing nitrite, depending on the relative concentrations, the solution will contain azide or nitrite. The method here proposed performed at pH 6.4 cannot distinguish between the presence of the two analytes. Two ways are therefore proposed for a preliminar qualitative analysis. One makes use of a previous reaction with alkaline 20% hydrogen peroxide solution, as suggested by other authors [5]. Hydrogen peroxide does not affect azide concentration and at the same time oxidizes nitrite to nitrate, which in turn does not interfere. The method is efficaceous, provided that very pure hydrogen peroxide is used.

Another method which can be used in order to distinguish between the presence of nitrite or azide in a solution is to perform a

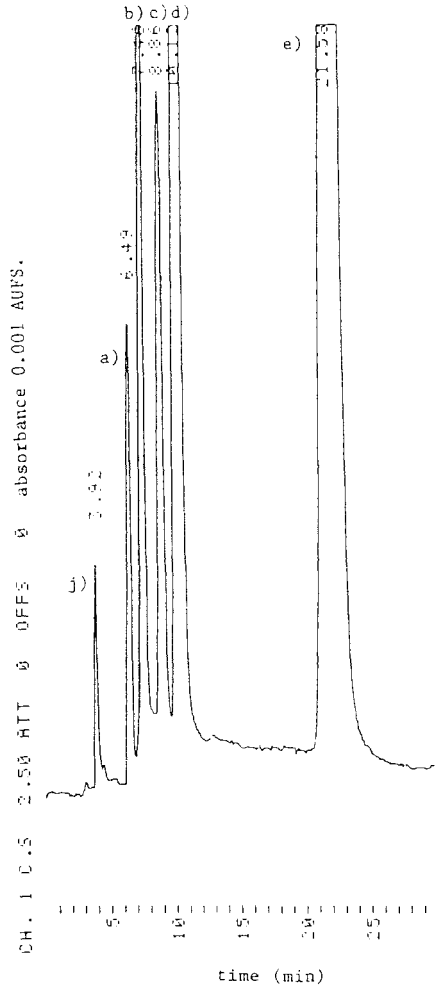

Figure 3.

Separation of a mixture of: a) bromide (40.00 ppm), b) azide (1.00 ppm), c) nitrate (0.50 ppm), d) iodide (0.50 ppm), e) thiocyanate (4.00 ppm) j) injection peak.
Stationary Phase : Phase Separation Spherisorb ODS-2, RP-18 (250x4.6 mm), endcapped, 5 μm.
Ion interaction reagent : 0.0050 mol/l octylamine ortho-phosphate.Flow-rate 1.0 ml/min. 100 μl injected. Spectrophotometric detection: 230 nm.

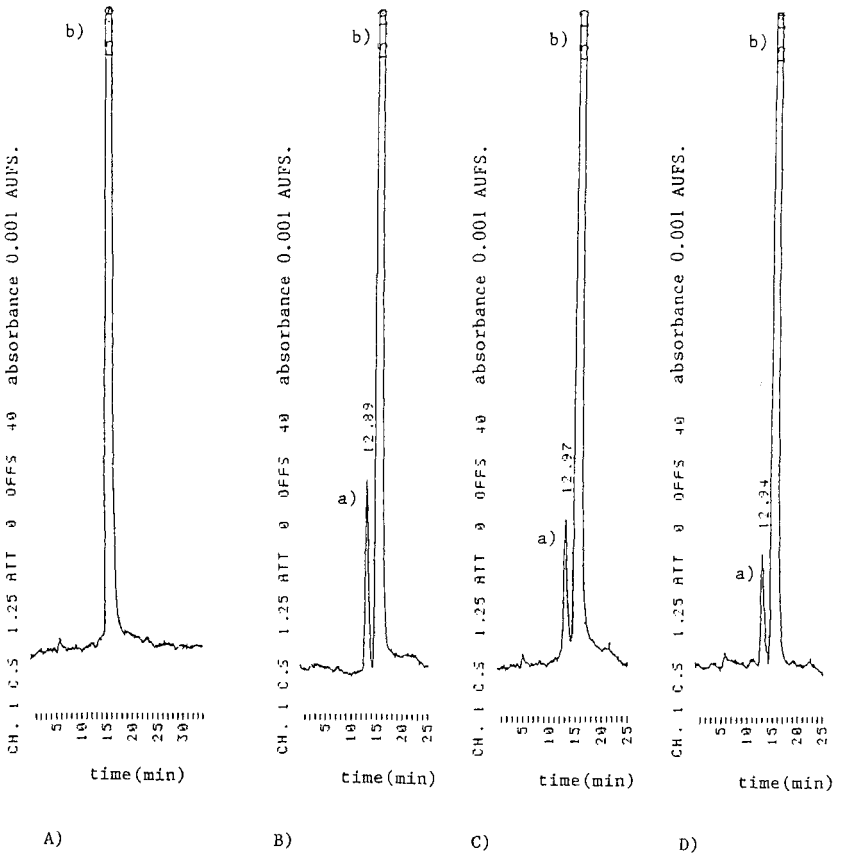

Figure 4.

Chromatograms of: a) tap water, b) tap water spiked with 0.25 ppm of azide, c) tap water spiked with 0.20 ppm of azide, d) tap water spiked with 0.15 ppm of azide.
Peaks : a) azide, b) nitrate.
Stationary Phase : Phase Separation Spherisorb ODS-2, RP-18 (250 x 4.6 mm), endcapped, 5 μm.
Ion interaction reagent : 0.0050 mol/l octylamine ortho-phosphate.Flow-rate 0.5 ml/min. 100 μl injected. Spectrophotometric detection: 230 nm.

TABLE III.
Sodium azide recovery in spiked samples of tap water.

Added (ppb)	Found (ppb) Average value.	Recovery %
250	238	95.2
200	183	91.5
150	154	102.7
100	110	110.0
80	90	112.5
50	46	92.0
25	21	84.0

chromatographic run by using as the interaction reagent octylamine ortho-phosphate at pH 3.0 . Under these conditions , as reported in table II, the two analytes show very different retention times.

Analysis of sodium azide in tap-water

The method was applied in the analysis of tap water.Figure 4 a) shows the chromatogram recorded for a sample of tap water, filtered through 0.20 μ m , using octylamine orto-phosphate at pH 6.4 as the mobile phase and spectrophotometric detection at 230 nm. The water does not contain azide or nitrite and , under these conditions, the only peak of nitrate is shown. The water was then spiked with different amounts of azide ranging from 25 to 250 ppb. Each measurement was done in triplicate. As an example, in figure 4 typical chromatograms obtained for water spiked respectively with 0.25, 0.20 and 0.15 ppm are reported. The quantitative analysis was then performed by the use of the standard calibration plot and by the

standard addition methods and the average results are reported in table III together with the concentration expected and the percent yield. Average recoveries of 98 % are obtained.

As a conclusion, the ion-interaction chromatographic method here described for the analysis of sodium azide offers advantages of sensitivity and selectivity. The method furthermore is rapid and does not require any derivatization step or particular pretreatments of the sample.

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