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Analysis of N-nitrosamines by high-performance liquid chromatography with post-column photohydrolysis and colorimetric detection

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Abstract

N-Nitrosamines eluted from reversed-phase HPLC were quantitatively photohydrolysed in a UV photoreactor in aqueous solution to give the nitrite ion which could be determined colorimetrically with the Griess reagent. The chromatographic behavior of N-nitroso compounds (including 19 volatile dialkyl and 7 non-volatile N-nitrosamines) was studied on three octadecylsilane columns. The capacity factor varies linearly with the number of carbons atom of the *n*-dialkyl chains. N-nitrosamines bearing di-*n*-alkyl chains with the same number of carbon atoms could be separated with a highly polar mobile phase. The yield of photohydrolysis depends upon pH and time of exposure under UV light. The response was shown to be linear in the 0–200 ng range with a limit of detection of 8 pmoles injected for N-dialkyl nitrosamines. This limit was 20 pmoles for N-nitrosamines bearing two phenyl groups. Although N-nitrosamines could be detected at 230 nm without post-column reaction, such a reaction enhances the specificity of detection in biological matrices such as gastric juice or alcoholic beverages.

Keywords: Photohydrolysis; Detectors, LC; N-nitrosamines

1. Introduction

N-Nitrosamines form a large class of genotoxic chemical carcinogens which occur in the human diet and environment [1]. They can be also formed endogenously in the human body, especially intragastrically [2]. Much interest is therefore directed toward quantitation of the various N-nitroso organic compounds (NOC) that occur in different matrices, such as diet, beverages [3] and gastric juice [4]. Since carcinogenic N-nitroso compounds formed in the stomach have been suggested to be involved in gastric cancer [4], the present study was aimed to develop an analytical method for the separation and quantitation of volatile and non-volatile N-nitrosamines. Until now, N-nitrosamines were determined either as total NOC by chemical denitrosation leading to nitric oxide which can be detected by chemiluminescence using thermal energy analysis (TEA) [5,6]. Such a detector is a modified chemiluminescence detector which uses thermal cleavage of the N–N bond to produce a nitrogen oxide radical. The

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nitrogen oxide is then reacted with ozone to produce excited nitrogen dioxide which emits a photon on decay that is detected and amplified by a photomultiplier tube. Volatile N-nitrosamines, e.g. nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR), are easily analyzed by gas chromatography coupled with TEA [2,7]. There are, however, a large number of polar nitroso compounds not generally amenable to direct analysis by GC, either because of their low volatility or their thermal instability [3]. Reversed-phase HPLC seems to be the method of choice for the analysis of apolar and polar NOCs. Attempts to combine HPLC with chemiluminescence detection [8] gave inconsistent results with respect to both sensitivity and resolution. However, Conboy and Hotchkiss [9] described an efficient photolytic interface between HPLC and TEA detector. The chromatographic effluent was introduced into a glass coil irradiated with ultraviolet light with a purge stream of helium. Nitrogen oxide produced by photolysis was carried by helium through three cold traps to the reaction chamber of the TEA. Although this system allows good selectivity and sensitivity for non-volatile N-nitrosamines, the interface is not very simple to use because it was home-made. In addition, the TEA equipment is expensive. This study describes an improved method for the separation and detection of volatile and non-volatile N-nitrosamines, that overcomes these limitations. Based upon an earlier procedure that allowed specific detection of N-nitrosamines by HPLC with colorimetric detection by a Griess-type [10] reagent of the nitrite generated by cleavage of nitroso compounds by either dilute acid at 90°C [11] or post-column photohydrolysis [12], it combines the advantages of reversed-phase HPLC with the simplicity of UV detection through a commercially available photoreactor.

2. Materials and methods

2.1. Chemicals

N-Nitrosamines (Table 1) were purchased from Sigma (St Louis, MO, USA) or NCI Chemical (Kansas City, MO, USA). N-Methylamyl amine (NMAA) was a gift from Pr. Mirvish (University of Nebraska, Omaha, NE, USA). The standard mixture according to the Environmental Protection Agency (EPA method 8270) was from Sigma. Other chemicals used were of analytical reagent or HPLC grade (Merck, Darmstadt, Germany). Chem Elut columns (Varian, France) were used for solid–liquid extraction of alcoholic beverages.

2.2. HPLC detection

Chromatographic separations were carried out on three octadecylsilane phases: Ultraspher ODS 15× 0.46 cm (Beckman, CA, USA), Lichrospher C_{18}, 25×0.4 cm (Merck) or Nucleosil C_{18}, 25×0.46 cm (Machery-Nagel, Düren, Germany) all with 5 μm particle diameter. The mobile phase consisted of an A/B mixture with variable composition; solvent A was water with 1% (v/v) H_3PO_4 or glacial acetic acid and 5% acetonitrile, and solvent B was acetonitrile with 5% of water containing 1% acid; the mobile phase was delivered at a flow-rate of 0.8 ml/min by a gradient pump (L6200A, Merck). The N-nitrosamines HPLC eluates were photohydrolysed in a photoreactor (Knauer, Berlin, Germany) in which a knitted teflon tubing coil of 6 m×0.30 mm I.D. is placed around a low-pressure UV-lamp emitting at 254 nm, 1.2 W capacity (Fig. 1). The resulting nitric oxide is transformed in aqueous medium to the nitrite ion which is detected spectrophotometrically at 546 nm (UV-L4250, Merck) by post-column formation of an azodye with the Griess reagent [10]. This reagent consisted of one part 0.1% (w/v) naphthylethylenediamine dihydrochloride in distilled water plus 1 part 1% sulfanilamide in 5% concentrated orthophosphoric acid, the two parts being mixed together each day. The effluent from the photolysis coil was mixed with Griess reagent pumped by an isocratic HPLC pump (L6000, Merck) at 0.8 ml/min in a low dead volume (10 μl) mixing tee (Lee Visco Jet micro-mixer). The resulting mixture was pumped through a teflon tubing coil of 1 m×0.30 mm I.D. immersed in a water bath at 60°C and then into the flow cell of the spectrophotometric detector by a connection teflon tube of 0.5 m×0.3 mm I.D. HPLC peaks were processed with a microcomputer with a PC-integration software package (Kontron, Basel, Switzerland). When detecting N-nitrosamines by UV at 230 nm the column outlet was directly connected to detector.

Table 1
Structure of 26 reference N-nitrosamines used

R	R′	Abbreviation		M_r	$\epsilon(\lambda)^a$ (UV)
VNA: volatile nitrosamines (R,R′)–N–N=O					
Methyl	Methyl	NDMA	C2H2N2O	74.08	ε (232)=5900
Methyl	Ethyl	NMEA	C3H8N2O	88.1	n.a.
Ethyl	Ethyl	NDEA	C4H10N2O	102.1	ε (233)=6500
Methyl	Propyl	NMPA	C4H10N2O	102.1	n.a.
Methyl	Butyl	NMBA	C5H12N2O	116,2	n.a.
Methyl	*tert.*-Butyl	NMtBA	C5H12N2O	116.2	ε (226)=5830
Ethyl	Propyl	NEPA	C5H12N2O	116.2	ε (232)=7760
Propyl	Propyl	NDPA	C6H14N2O	130.2	ε (233)=7585
Ethyl	Butyl	NEBA	C6H14N2O	130.2	n.a.
Propyl	Butyl	NPBA	C7H16N2O	144.2	n.a.
Butyl	Butyl	NDBA	C8H18N2O	158.2	ε (235)=7080
Isobutyl	Isobutyl	NDiBA	C8H18N2O	158.2	ε (237)=7030
Methyl	Amyl	NMAA	C6H14N2O	130.2	n.a.
Amyl	Amyl	NDAA	C10H22N2O	186.3	ε (235)=7330
Methyl	Benzyl	NMBzA	C8H10N2O	150.2	ε (231)=9600
Phenyl	Phenyl	NDPhA	C12H10N2O	198.2	ε (230)=34100
Methyl	Phenetyl	NMPhEtA	C9H12N2O	164.21	ε (230)=8040
	Piperidine	NPIP	C5H10N2O	114.2	ε (238)=4200
	Morpholine	NMORP	C4H8N2O2	116.1	ε (237)=7940
NVNC: non-volatile nitroso compounds					
Methyl	CH2COOH	NSarco	C3H6N2O3	118.1	ε (233)=6175
	Nornicotine	NNor	C9H11N3O	177.2	n.a.
	Piperazine	NPiperaz	C4H8N3O	114.1	n.a.
	Pyrrolidine	NPYR	C4H8N2O3	100.2	ε (230)=8130
	Proline	NPRO	C5H8N2O3	144.1	ε (236)=6500
Methyl	Guanidine	NMGua	C5H5N5O3	147.1	n.a.
N-Methyl	1-(3-Pyridyl)-1-butanone	NNK	C10H13N3O2	207.2	ε (230)=16400

[a] $\epsilon(\lambda)$=molecular extinction coefficient (M^{-1} cm^{-1}).
n.a.=not available.
Data available from technical literature of suppliers.

The capacity factor (k') was calculated from the solute retention time (t_R) and dead time (t_o) according to the equation: $k'=(t_R-t_o)/t_o$.

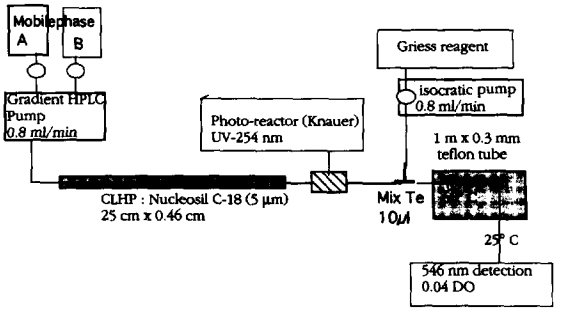

Fig. 1. Diagram of the HPLC–UV detector interface.

2.3. Determination of N-nitrosamines in different matrices

2.3.1. Gastric juice

Gastric juice samples were obtained by endoscopy. After measurement of pH, NDPA was added as internal standard to a 5-ml aliquot of gastric juice. Samples were extracted three times for 30 min with 10 ml of pestipur dichloromethane (SDS, Peypin, France) in an agitator. The volume of combined dichloromethane extracts was reduced to 0.5 ml in a Kuderna–Danish evaporator at 55°C under stirring, and the sample further reduced to dryness under a slow stream of nitrogen at 4°C. The dried residue was dissolved into HPLC mobile phase. In-vitro nitrosation of a 5-ml aliquot of gastric juice, pH

adjusted to 1.5 with 1 *M* HCl, was carried out with one part 160 m*M* $NaNO_2$ for 60 min at 37°C in darkness. N-nitrosation was stopped with one volume of 176 m*M* sulfamic acid [13]. N-nitrosamines were extracted with dichloromethane as described above. HPLC analysis was performed on a Nucleosil C_{18}, 25×0.46 cm, analytical column; mobile phase consisted of solvent A–solvent B (80:20, v/v) for 10 min, then linearly modified to 40:60 (v/v) in 10 min and to 10:90 (v/v) in 20 min and maintained for 20 min. Solvent A contained 1% acetic acid (v/v)

2.3.2. Alcoholic beverages (beer)

A volume of 50 ml added with 100 ng NMPA [7] was adsorbed by a Chem-Elut column according to the procedure recommended by the supplier (Varian, France). N-Nitrosamines were eluted with 5× 30 ml dichloromethane. These eluates were reduced to 0.5 ml in a Kuderna–Danish evaporator. HPLC analysis was performed as described above.

3. Results and discussion

3.1. Sensitivity of detection

The principle according to which the photohydrolysis detector works is the following: irradiation of an aqueous solution of N-nitroso compounds with UV light leads to cleavage of the N–NO bond to give nitric oxide NO˙. The NO˙ species is rapidly oxidized to nitrogen dioxide $NO_2^{\cdot}$ by oxygen. The $NO_2^{\cdot}$ species, after combination with another molecule of $NO_2^{\cdot}$ to give N_2O_4 or with NO˙ to give N_2O_3, is hydrolysed to nitrite ion NO_2^- [12]. This ion is then determined via its acid-catalyst diazotation of sulfanilamide, followed by coupling with N-1-naphthylethylenediamine di-HCl to give an azodye, which has a maximum absorbance at about 541 nm [10,14]. The aim of this work was to adapt this procedure to HPLC.

The procedure was validated by recording the visible spectrum of N-nitrosodiethylamine separated by HPLC, photohydrolysed in the photoreactor then mixed with Griess reagent. The maximum absorbance was set at 546 nm (Fig. 2C). Using the post-column reactor, the N-nitrosamines studied were nearly completely destroyed during the residence time (ca. 0.5 min at 0.8 ml/min flow-rate) of the column effluent in the photolysis coil. This was demonstrated by monitoring the effluent from the photolysis coil at 230 nm with the lamp of photoreactor on (Fig. 2B). Up to 90% of the nitrosamines were destroyed in the photoreactor, whatever the quantity injected between 5–30 ng. Attempts to increase the yield of hydrolysis were not successful.

Two calibration curves were recorded for two N-nitrosamines, i.e. the *n*-dialkyl NDPA and the

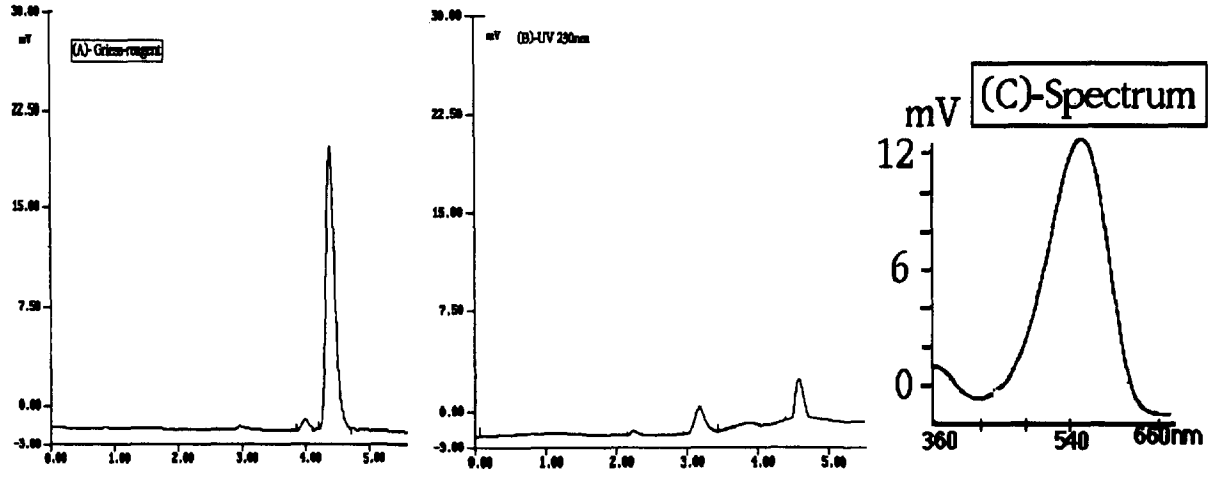

Fig. 2. Visible spectrum (C) of 200 ng N-nitrosodiethylamine (NDEA) analyzed by HPLC. Column, Lichrospher C_{18} 25×0.4 cm; mobile phase, A–B (75:25, v/v) at 0.8 ml/min, A and B containing 1% H_3PO_4 (v/v). HPLC eluates were photohydrolyzed in a photoreactor and then reacted with Griess reagent at 60°C (A) or detected directly at 230 nm without addition of Griess reagent (B).

n-diphenyl NDPhA (Fig. 3), either with UV detection at 230 nm or with visible detection at 546 nm after photohydrolysis and Griess colorimetric reaction. The HPLC mobile phase contained either H_3PO_4 (pH≅1.5) or CH_3COOH (pH≅2.5). Response was linear from 1 to 200 ng injected whatever the detection mode. In both cases, UV detection at 230 nm was the most sensitive, especially with the nitrosamine bearing two phenyl rings. The limits of detection, calculated as three times the signal-to-noise ratio were found to be 0.5 and 1 ng injected, i.e. 3.8 and 7.6 pmoles of NDPA, in UV and visible detection mode, respectively.

Table 2 shows the peak areas per nmole injected according to the two detection modes. In the UV detection mode, the nitrosamine bearing two phenyl rings gave a response factor two times higher than the *n*-dialkyl compound. Conversely, N-nitrosodipropylamine gave a better response than N-nitrosodiphenylamine when post-column reaction based upon Griess reaction was carried out. This response was enhanced when the mobile phase contained acetic acid instead of orthophosphoric acid. Such a result suggests that the stoichiometry of conversion of nitroso compounds into nitrite ion by photohydrolysis depends upon the nature of the nitroso derivatives. In order to establish the stoichiometry of the post-column reaction, nine N-nitrosamines of the 8270 EPA mixture were injected on a Nucleosil C_{18} column as shown in Fig. 4. As shown, the nine compounds of the test mixture gave a quite similar response with UV and visible detection after post-column reaction, except for NDPhA. Furthermore, the figure shows the ability of HPLC to separate volatile (NDMA, NMORP, NMEA, NDEA, NPIP and NDPA) and non-volatile NOCs such as NPYR. As four N-nitrosamines, i.e. NDMA, NDEA, NDBA and NPYR, were detected by GC–TEA in gastric juice [2], the HPLC procedure should be also able to separate and quantitate these nitrosamines. Indeed, although the detection limit was estimated to be about 8 pmoles injected, i.e. ten times higher than that reported for group determination of NOCs by TEA [6], such a detection limit could be easily reached by concentrating the biological sample (see below). Fig. 4 shows that the retention times of nine N-nitrosamines were about 1.1 min longer in Griess-reagent detection than in UV detection; this difference was due to the dead volume of the photoreactor and the reactor placed between the column outlet and the detector inlet. Despite the 7 m of microbore tubing of the post-column photolysis coil and mixing coils, no noticeable loss of chromatographic resolution occurred when UV detection (Fig. 4A) was replaced by photohydrolysis (Fig. 4B).

The maximum sensitivity of detection for N-nitro-

Table 2
Surface units (mV×min) per nmole injected of N-nitrosodipropylamine (NDPA) and N-nitrosodiphenylamine (NDPhA) detected by UV at 230 nm or at 546 nm with Griess reagent after post-column reaction

	UV 230 nm	Griess reagent with H_3PO_4	Griess reagent with CH_3COOH
NDPA	1.8±0.1	1.1±0.1	1.8±0.1
NDPhA	3.4±0.07	0.8±0.1	0.8±0.1

HPLC mobile phase contained either H_3PO_4 or CH_3COOH as described in Materials and Methods.

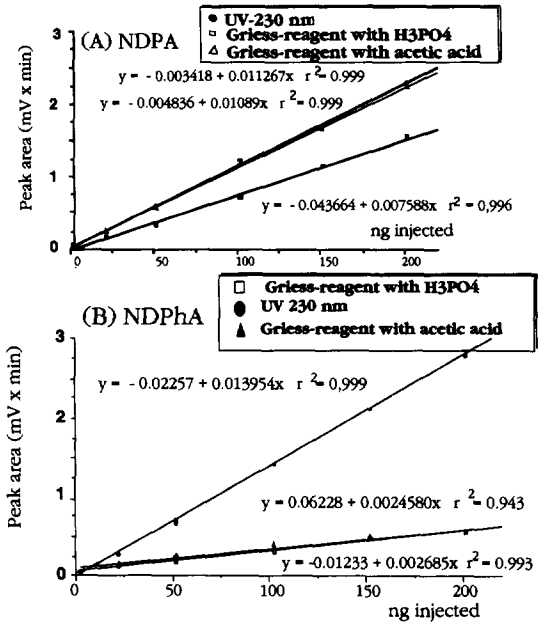

Fig. 3. Calibration curves of N-nitrosodipropylamine [NDPA (A)] and N-nitrosodiphenylamine [NDPhA (B)], either by UV detection at 230 nm (●) or by Griess reagent after photohydrolysis. Analytical HPLC conditions: Lichrospher C_{18} column, 25×0.4 cm; mobile phase, A–B (25:75, v/v) as described in Materials and Methods containing either 1% H_3PO_4 (□) or 1% CH_3COOH (▲) (v/v).

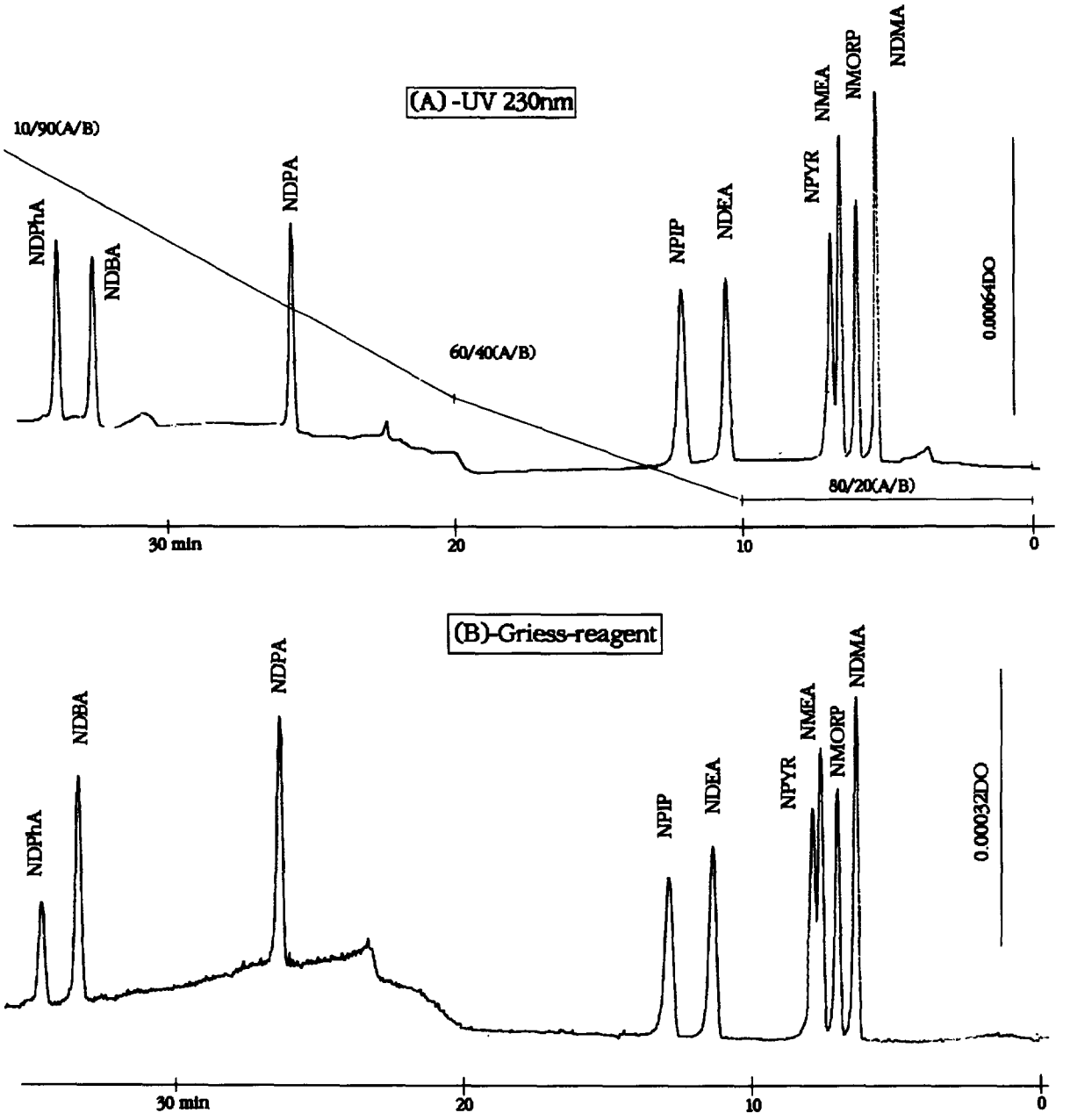

Fig. 4. HPLC profile of EPA 8270 mixture containing 9 N-nitrosamines (for nomenclature, see Table 1), 10 ng injected. Detection mode was either with Griess reagent (B) or UV 230 nm (A) as described in Materials and methods. HPLC conditions: Nucleosil C_{18} column 25×0.46 cm, 5 μm particle diameter; HPLC gradient program as described in Fig. 3A (see Materials and methods for A and B mobile phases).

samines was quite similar whatever the mode of detection (Fig. 3 and Fig. 4), except for NDPhA, despite the fact that the absorbance of the azodye (λ_{max} 546 nm; ϵ=37100 M^{-1} cm^{-1} [13]) is about 5–6 times higher than that of the corresponding N-nitrosamines (λ_{max}=230–235 nm; ϵ=4000–8000

M^{-1} cm^{-1}; see Table 1). It is apparent therefore that stoichiometric conversion of N-nitrosamines to nitrite ion and then to azodye is not achieved. In order to increase this response, attempts were carried out by modifying the organic solvent of the HPLC mobile phase (methanol instead of acetonitrile) or the acid incorporated in the mobile phase (acetic acid instead of orthophosphoric acid). Reduced flow-rates of the HPLC mobile phase (0.5 up to 1.2 ml/min) increased the yield of cleaved N-nitrosamines by increasing the time of exposure under UV light (Fig. 5), the exposure time varying between 20 and 60 s. As the flow-rate of the HPLC mobile phase cannot be decreased too much without loss of efficiency, the optimum was set at 0.8 ml/min. Furthermore, as the pH optimum of the Griess reaction has been shown to be about 1.5–2 [9], the pH of photohydrolysis reaction was tested by adding either H_3PO_4 or CH_3COOH to aqueous phase. Fig. 3 and Table 2 shows that acetic acid was the most efficient and increased markedly the sensitivity of all NOCs tested.

3.2. Chromatographic behavior of N-nitrosamines

Fig. 6 shows that for di-*n*-alkyl N-nitrosamines, the capacity factor k', as logarithmic values, varies linearly with the number of carbon atoms of the di-*n*-alkyl chains. Compounds bearing branched alkyl chains, such as NDiBA or NMtBA, were less retained than their linear homologs, such as NDBA and NMBA or NEPA, respectively. Furthermore, N-nitrosamines containing aromatic rings, such as NMBzA or NDPhA, were also less retained than the corresponding N-nitrosamines with alkyl chains, such as NDBA or NDHA (N-nitrosodihexylamine, not studied, but expected to have a retention time greater than NDAA).

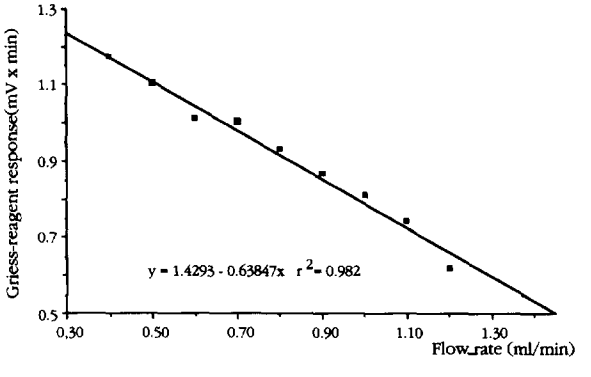

Fig. 5. Peak area (mV×min) vs. flow-rates of 0.3 nmol of N-nitrosodipropylamine detected by post-column reaction. HPLC conditions: Nucleosil C_{18}, 25×0.4 cm at variable flow-rates of A–B mixture (25:75, v/v).

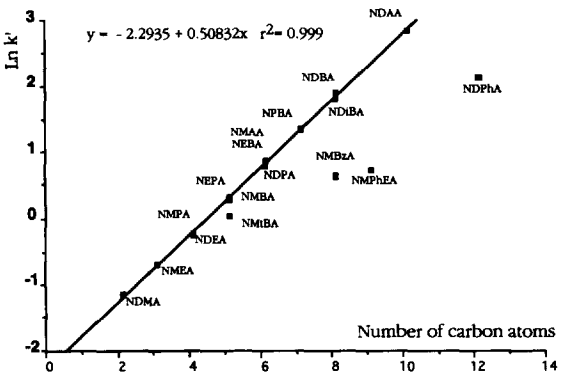

Fig. 6. Variation in capacity factor (k') of seventeen N-nitrosamines versus number of carbon atoms of alkyl or aryl substituents. HPLC conditions: Lichrospher C_{18}, 25×0.4 mm; mobile phase, A–B (60:40, v/v) at a flow-rate of 0.8 ml/min; the aqueous phase contained 1% (v/v) acetic acid.

As expected, N-nitrosamines bearing di-*n*-alkyl chains having the same number of carbon atoms, like NDPA, NEBA and NMAA, were not resolved under HPLC isocratic conditions reported in Fig. 6. The use of a more polar mobile phase containing up to 90% of aqueous phase A brought a dramatic enhancement of resolution of these compounds, as shown in Fig. 7A. Moreover, NEBA and NMAA peaks showed shoulders due to partial separation of their Z- and E-isomers [14] of dissymmetrical N-dialkylnitrosamines. Fig. 7B shows the potentialities of separation of Z/E-isomers of three methylalkyl N-nitrosamines. The ratio between E/Z-isomers at equilibrium in the mobile phase containing 1% acetic acid was about 80:20. Their interconversion rate following a first order kinetics was $2 \cdot 10^{-4}$ min^{-1}. The separation of Z/E-isomers of dissymmetrical isomers by HPLC increased with their dissymmetrical character. Furthermore, GC was unable to separate such isomers because the rate of interconversion increased with temperature [15].

Table 3 reports the capacity factors of 10 N-nitrosamines, including polar compounds such as

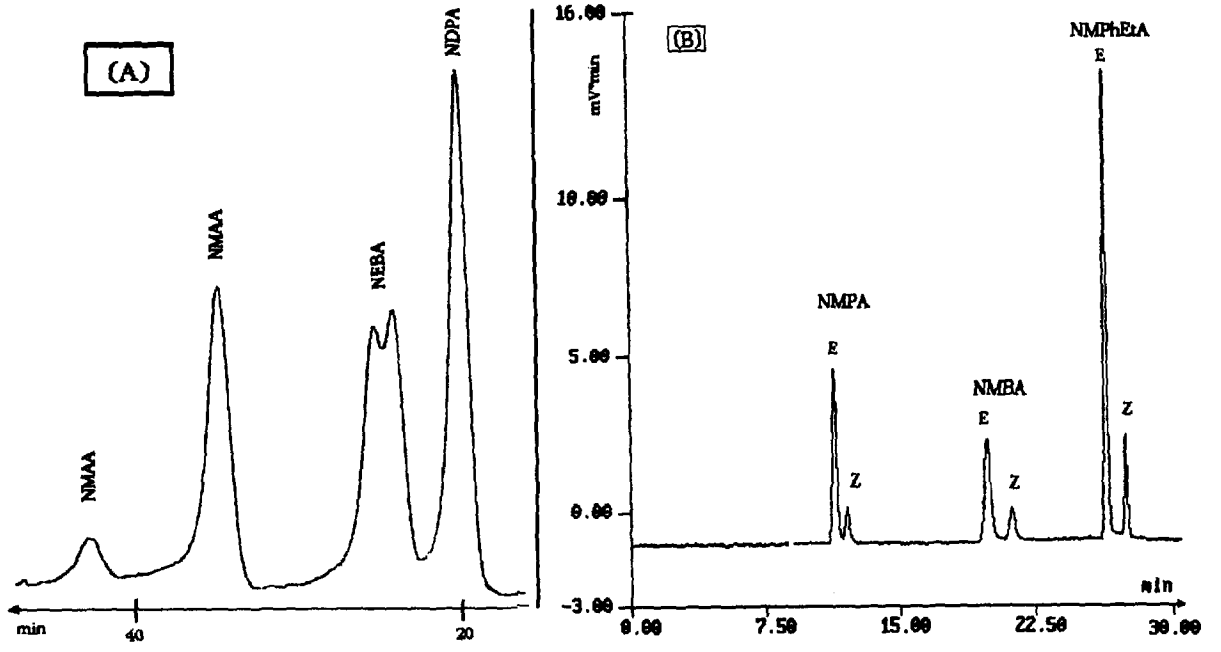

Fig. 7. Potentialities of HPLC. (A) Separation of three N-nitrosamines bearing two *n*-alkyl chains with a total of six carbon atoms. NDPA: N-nitroso dipropylamine; NEBA: N-nitroso ethylpropylamine; NMAA: N-nitroso methylamylamine. HPLC conditions: Lichrospher C_{18}, 25×0.4 cm; mobile phase, A–B (90:10) at a flow-rate of 0.8 ml/min. (B) Separation of Z/E-isomers of three N-methylalkyl nitrosamines (NMPA: N-nitroso methylpropylamine; NMBA: N-nitroso methylbutylamine; NMPhEtA: N-nitroso methylphenylethyl amine). HPLC conditions: Nucleosil C_{18}, 25×0.46 cm; mobile phase, A–B (80:20, v/v) for 10 min, then modified to 40:60 in 10 min and to 10:90 (v/v) in 20 min. Detection by Griess reagent at 546 nm. The aqueous phase contained 1% (v/v) acetic acid.

Table 3
Capacity factor (k') of polar N-nitrosamines or three octadecylsilane columns

Compound	Lichrospher C_{18}	Nucleosil C_{18}	Ultraspher C_{18}
N-Sarcosine	0.349	0.138	0.154
N-Proline	0.417	0.177	0.184
NDMA	0.593	0.278	0.276
N-Morpholine	0.766	0.415	0.396
N-Pyrrolidine	0.953	0.526	0.488
N-MGuanidine	1.383	0.902	0.833
N-Piperazine	2.363	1.583	1.433
NNK	1.062	0.472	0.388
N-Piperidine	2.4	1.61	1.447
N-Nornicotine	3.563	0.409	1.358

Mobile phase, A–B (80:20, v/v) at 0.8 ml/min; A=water with 1% CH_3COOH and 5% acetonitrile (v/v), B=acetonitrile with 5% water containing 1% acetic acid (v/v)

N-sarcosine or N-proline. As expected, such compounds were less retained by the apolar stationary phases than apolar compounds.

3.3. Applications

Gastric juice

Gastric juices were analyzed by HPLC–photohydrolysis–Griess reaction after extraction of N-nitrosamines with methylene chloride. HPLC profiles (Fig. 8) demonstrated the detection of N-nitrosamines, especially NDMA and NMOR. Specificity of detection was ascertained by the lack of HPLC peaks when the photoreactor was switched off (results not shown).

Application of the HPLC-specific detection for the analysis of N-nitrosamines in dichloromethane extracts of nitrosated gastric juice revealed the presence of numerous N-nitrosamines (Fig. 8B). Many of

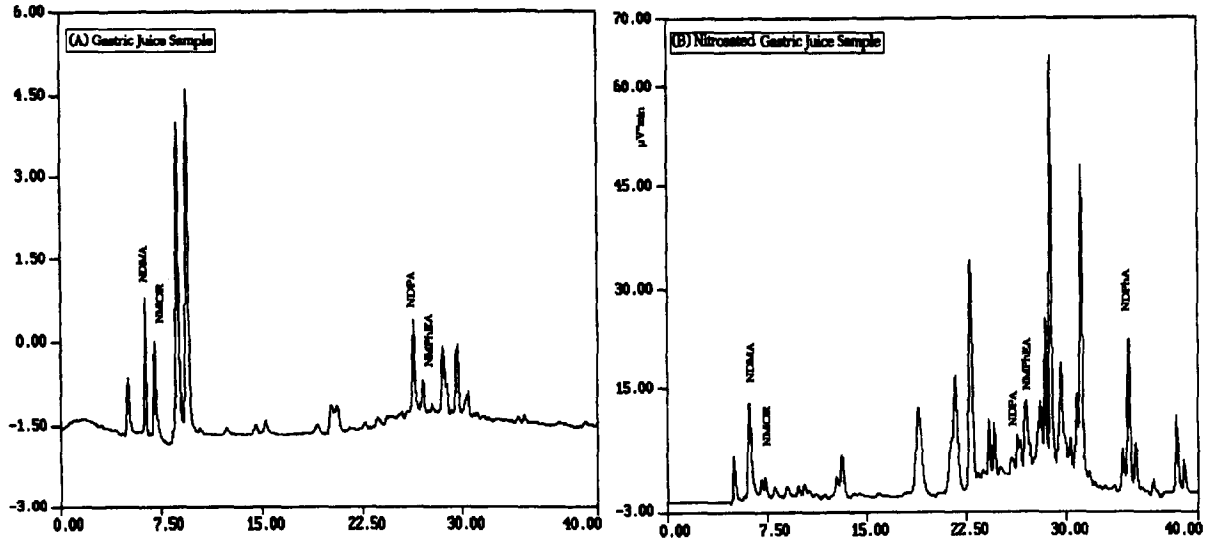

Fig. 8. HPLC chromatograms of organic extracts of gastric juices directly extracted by CH_2Cl_2 (A) or after N-nitrosation by nitrite (B). HPLC conditions as described in Materials and Methods. HPLC profiles were obtained by injection of 1.25 ml of gastric juice.

them are not identified yet. This result confirms that gastric juice contains endogenous amines that are nitrosable by nitrite ion at acidic pH [2].

Alcoholic beverages

HPLC-specific detection was applied to the identification of N-nitrosamines extractable by methylene chloride from beers (Fig. 9). Numerous N-nitrosamines were identified on the basis of their retention times and their specific detection by Griess reagent after photohydrolysis.

In conclusion, the HPLC–photohydrolysis–colorimetric method offers a powerful analytical tool for trace analysis of N-nitrosamines that are found in body fluids and food extracts. Although the thermal energy analysis is a highly sensitive detection method for nitrosamines separated by GC, the HPLC procedure allows a highly specific analysis of polar N-nitrosamines with good sensitivity. The limit of detection estimated to be 1 ng injected was quite similar to that determined by TEA, coupled to HPLC through a UV photoreactor and three cold traps [9]. This limit was higher than those for volatile N-nitrosamines which typically have thresholds of detection lower than 0.5 ng when analyzed by GC–TEA. However the sensitivity of the simple method described in this study, coupled to the 10–100 fold increase in injection volume of HPLC versus GC, results in an overall sensitivity for HPLC that is greater than that of GC–TEA. Finally, HPLC presents the possibilities of coupling to other detectors such as fluorescence [16] or chemiluminescence [17] for N-nitrosamine analysis.

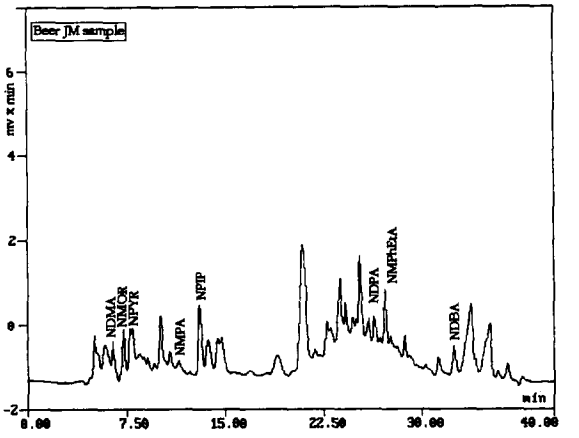

Fig. 9. HPLC profile of N-nitrosamines extracted by CH_2Cl_2 from 50 ml beer. HPLC conditions: see Fig. 8. An aliquot of 12.5 ml of beer was injected.

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