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Gas Chromatographic Determination of *N*-Nitrosamines in Beverages Following Automatic Solid-Phase Extraction

BEATRIZ JURADO-SÁNCHEZ,[†] EVARISTO BALLESTEROS,[*,‡] AND MERCEDES GALLEGO[†]

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071 Córdoba, Spain, and Department of Physical and Analytical Chemistry, E.P.S. of Linares, University of Jaén, E-23700 Linares, Jaén, Spain

A semiautomatic method for the determination of seven *N*-nitrosamines in beverages by gas chromatography with nitrogen–phosphorus detection is proposed. Beverage samples are aspirated into a solid-phase extraction module for preconcentration and cleanup. The influence of the experimental conditions was examined by using various sorbents among which LiChrolut EN was found to provide quantitative elution and the highest preconcentration factors of all. The proposed method is sensitive, with limits of detection between 7 and 33 ng/kg, and precise, with relative standard deviations from 4.3% to 6.0%. The recoveries of *N*-nitrosamines from beverage samples spiked with 0.5 or 1 μg/kg concentrations of these compounds ranged from 95% to 102%. The method was successfully applied to the determination of residues of the studied *N*-nitrosamines in beverages including beer, wine, liquor, whisky, cognac, rum, vodka, grape juice, cider, tonic water, and soft drinks. The analytes were only detected in beer samples, positives being confirmed by gas chromatography coupled with impact ionization mass spectrometry.

KEYWORDS: ***N*-Nitrosamines; beverages; continuous solid-phase extraction; GC-NPD; GC-MS**

INTRODUCTION

N-Nitrosamines (NAms), which can be present in the environment and in a wide variety of food products, exhibit mutagenic, carcinogenic, and teratogenic activities. More than 40 animal species including amphibians, fish, birds, mice, and primates are susceptible to NAm-induced carcinogenesis. The toxicological properties of these compounds are explained by their ability to form potent electrophilic alkylating agents, which can react with the nucleofilic site of ADN, ARN, and proteins. These reactions can induce mutations and hence initiate carcinogenesis. In food, the most important route of contamination of NAms is the use of nitrite and nitrate as curing agents in cured meat, cheese, or pickled fish products. The second common mechanism of contamination is through the drying of foods in air that has been directly heated by an open flame, which is the major cause of NAms in malt, beer, scotch whiskey and other dried foods. Other routes of NAms are from NAm-bearing materials that come into contact in the food or from some microorganism present in fermented food which reduces nitrate to nitrite, lowering the pH and producing substances that can catalyze nitrosation (*1*).

* Corresponding author. Tel: +34953648560. Fax: +34953648560. E-mail: eballes@ujaen.es.
[†] University of Córdoba.
[‡] University of Jaén.

Regulations limiting the levels of NAms in food are very rare, and the regulations imposed depend very much on the country. Examples of maximum contaminant levels for NAms in beer and bacon are 5 μg/kg in the United States, 0.5 μg/kg of NAms for beer in Italy, Switzerland, and Germany, and 2–15 μg/kg for meat, beer, and smoked products in Russia (*1*).

A number of extraction and cleanup procedures for the determination NAms in food have been described, including liquid–liquid extraction with dichloromethane for alcoholic beverages and solid samples (*2*); other solvent extraction methods employ a dry Celite column (*3, 4*) or low-temperature vacuum distillation (*5, 6*). These methods are time consuming, labor intensive, may result in some analyte losses, and require the use of larges volumes of toxic solvents. To prevent these problems, other sample preparation methods including supercritical-fluid extraction (*7*), solid-phase microextraction (*8*), and solid-phase extraction (SPE) with the use of different types of sorbents such as Chem Elut (*9*), Extrelut and Florisil (*10*), or activated carbon (*11*) have been proposed. The last method is useful in the selective removal of interferences of food matrix, provides fairly clean extracts, and reduces the use of environmentally toxic solvents; however, it uses extensive manipulations, thereby increasing some hazards through uncontrolled evaporation losses. In this context, the use of continuous SPE systems for sorption and elution of NAms prior to their separation/determination is crucial, avoiding losses during

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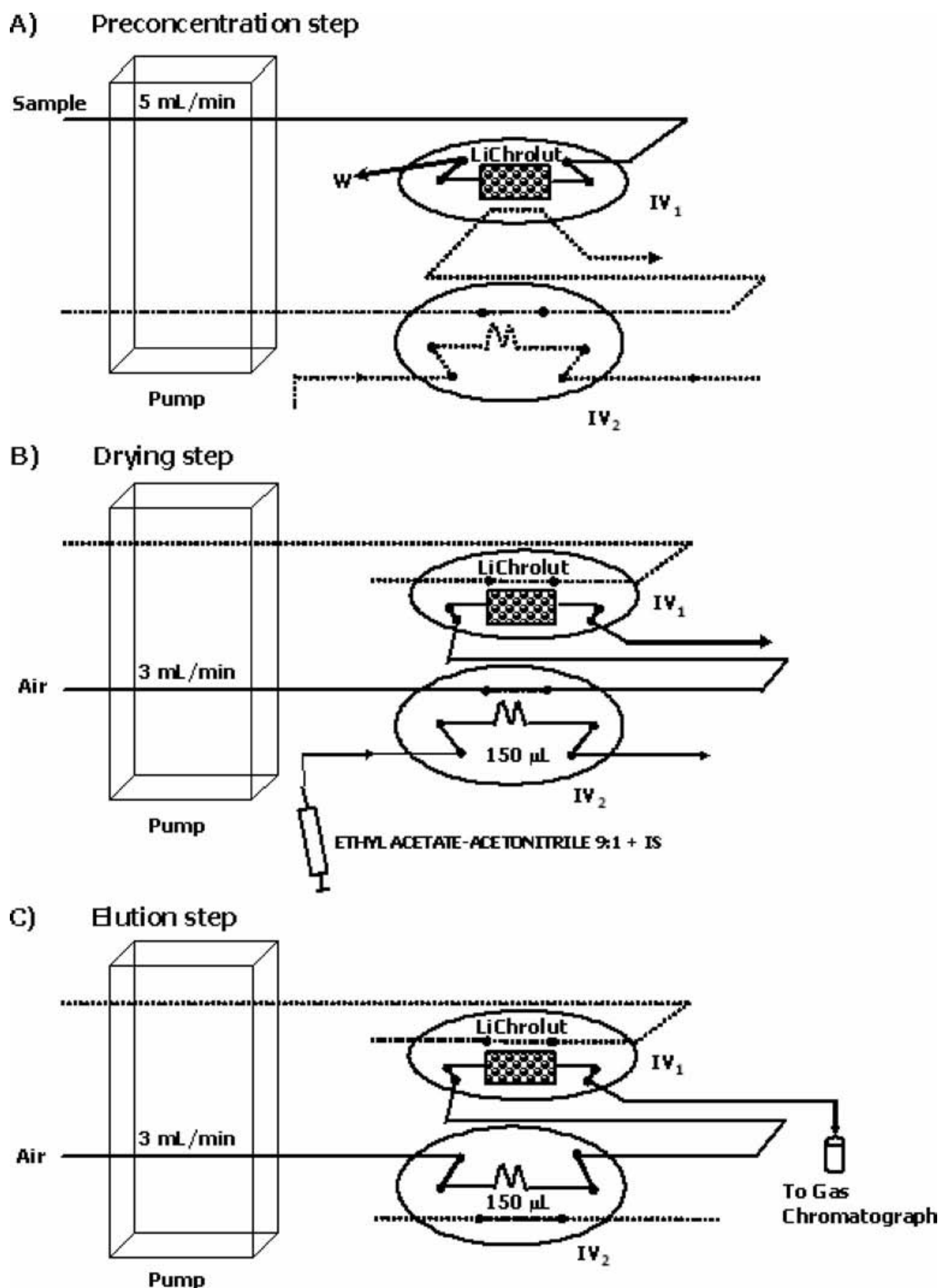

Figure 1. FI manifold for the online preconcentration of *N*-nitrosamines and their off-line determination by gas chromatography. Abbreviations: IV, injection valve; W, waste; IS, internal standard.

concentration and alterations in the chemical composition of the analytes. Thus, Luque-Pérez et al. have developed a continuous method for the determination of NAms in solid food samples, which is based on the continuous preconcentration of these analytes from a filtered solution (proceeding from stirring and heating of solid sample in water) in an alumine-A column, followed by their photochemical degradation and spectrophotometric detection after derivatization with a Griess-type reagent (*12*). In another continuous method, NAms in water are preconcentrated by an automated SPE extraction system and determined by HPLC using a chemiluminescence detector (*13*).

The determination of NAms in beverages has been carried out by gas chromatography using nitrogen–phosphorus detection (NPD) (*8*), thermal energy analyzer (*14, 15*), or mass spectrometry (MS) detection (*16, 17*). Liquid chromatography with fluorescence (*2*), chemiluminiscence (*13*), photoconductivic (*18*), amperometric (*19*), mass spectrometric (*20*), or colorimetric detection (*21*) has also been employed for this purpose. Recently, micellar electrokinetic chromatography with diode array detection has also been used for the determination of NAms in sausages (*7*).

Recently, our group has developed a screening and confirmation method for the determination of NAms in water using an online column integrated in the continuous system and gas chromatography with different types of detectors (*22*). This paper reports an improved version of the previous method as an elegant alternative to existing procedures for the determination of NAms in beverages. The two principal aims of this work were to simplify the sample treatment and to increase the sensitivity and selectivity with respect to such alternatives. We

Table 1. Chemical and Flow Variables Selected for the Proposed SPE Method

variable	optimum range (selected value)
sample pH	6–8 (7.4)
ionic strength, M KNO_3	0–3
amount of sorbent (mg)	35–80 (75)
amount of ethanol (%)	0–20
breakthrough volume (mL)	10–300 (250)
sample flow rate (mL/min)	0.4–5.5 (5.0)
eluent volume (μL)	100–175 (150)
air flow rate (mL/min)	2–4 (3)

examined the interferences of beverage matrices on the sorption of NAms on a LiChrolut EN packed microcolumn prior to their determination by gas chromatography with nitrogen–phosphorus or mass spectrometry detection.

MATERIALS AND METHODS

Safety. All products were handled with care, using efficient ventilating hoods, wearing latex gloves, and avoiding inhalation or skin contact, as NAms are potential or actual carcinogens.

Apparatus. Analyses were performed on an Agilent 6890 series gas chromatograph equipped with a NPD detector and 30 m × 0.25 mm i.d., 0.25 μm HP-5MS (cross-linked 5% phenylmethylpolysiloxane) fused silica column (Supelco, Madrid, Spain) and controlled by a computer running Agilent Chemstation software (Agilent Technologies, Madrid, Spain). Helium, at a flow rate of 1.0 mL/min, was used as carrier gas. The injection port and detector temperatures were kept at 250 and 300 °C, respectively. The GC oven was programmed from the initial temperature of 40 °C (3 min), ramped at a rate of 10 °C/min to 100 °C (1 min), and at 15 °C/min to the final temperature, 250 °C, which was maintained for 2 min. Sample injection was done in the splitless mode, using an injection volume of 1 μL. Nitrogen was used as the make-up gas.

Confirmatory assays were carried out on a Fisons gas chromatograph–mass spectrometer (GC8000/MD800; Thermo Quest, Madrid, Spain) controlled by a computer running MASSLAB software (ThermoQuest). The chromatographic column and temperature program were the same to those used with NPD, using ultrapure helium (6.0 grade, Air-Liquide, Seville, Spain) as carrier gas at 1 mL/min. The injection port and transfer line temperatures were maintained at 200 and 300 °C, respectively, throughout the experiments. The ion source temperature was 200 °C for the 70 eV electron impact mode. Mass spectra were recorded from m/z 42 to m/z 268. Samples (1 μL) were injected in the split mode (1:20 ratio).

The proposed continuous SPE system consisted of a Gilson Minipuls-3 peristaltic pump (Villiers-le-Bel, France) fitted with poly(vinyl chloride) pumping tubes, two Rheodyne 5041 injection valves (Cotati, CA), PTFE tubing of 0.5 mm i.d., commercially available connectors, and a PTFE (8 cm × 3 mm i.d.) laboratory-made sorption column containing 75 mg of LiChrolut EN sorbent. Because of its small particle size (40–120 μm), the LiChrolut EN sorbent is very prone to compaction when soaked in the flow system. In order to avoid abrupt changes in column compactness that might stop the solution flow and dislodge the system connections, each LiChrolut EN segment (1.0 cm long) was separated by one segment of an inert material (e.g., PTFE beads, segments of ~0.5 cm length).

Chemical and Standard Solutions. All chemicals and sorbents were of analytical grade or better. *N*-Nitrosodiphenylamine (NDPA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopiperidine (NPIP), *N*-nitrosomorpholine (NMOR), *N*-nitrosopyrrolidine (NPIR), *N*-nitrosodibutylamine (NDBA), and *N*-nitrosodimethylamine (NDMA) were purchased by Sigma-Aldrich (Madrid, Spain). Bromophos-methyl, 2-*tert*-butyl-4-methylphenol, and triphenyl phosphate were provided by Riedel-de-Haën (Seelze, Germany). Sodium aluminosilicate was supplied by Fluka (Madrid, Spain). Acetonitrile, ethyl acetate, methanol, dichloromethane, ethanol, 2-propanol, and diethyl ether were supplied by Merck (Darmstadt, Germany). Saccharose, glucose, tannic acid, and gallic acid were obtained from Fluka. LiChrolut EN (polystyrene divinylbenzene, superficial area ca. 900 m^2/g) was purchased from Merck. High-purity deionized water purified with a Milli-Q analytical reagent grade water purification system (Milli-Q A10 academic gradient system) was used for preparation of reagents and standards.

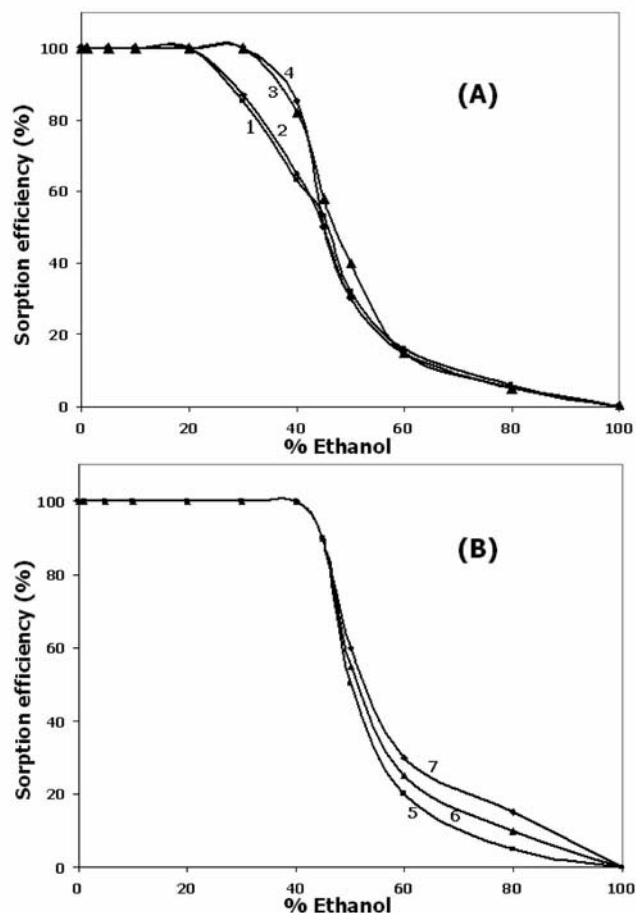

Figure 2. Influence of the percentage of ethanol in the sample on the *N*-nitrosamine retention: (1) *N*-nitrosodimethylamine, (2) *N*-nitrosodiethylamine, (3) *N*-nitrosodibutylamine, (4) *N*-nitrosodiphenylamine, (5) *N*-nitrosopyrrolidine, (6) *N*-nitrosopiperidine, and (7) *N*-nitrosomorpholine.

Standard stock solutions containing 10 g/L individual NAms were prepared in methanol and stored in glass-stopped bottles at −4 °C in the dark. Solutions of variable concentration were prepared daily by diluting the stock in Milli-Q water at pH 7.4. A mixture of ethyl acetate–acetonitrile (9:1), containing 10 mg/L bromophos-methyl (NPD) or 1 mg/L 2-*tert*-butyl-4-methylphenol (MS) as internal standard, was used as eluent and daily prepared before use.

Sample Preparation. All samples (liquor, wine, beer, whiskey, cognac, rum, vodka, grape juice, cider, tonic water, and soft drinks) were purchased at local markets in Spain. In the laboratory the samples were stored at 4 °C, and the seal of each bottle was broken before its analysis. If any sample (e.g., wine and grape samples) required filtering, this was passed through a 0.45 μm membrane filter (mixed cellulose esters; Millipore Ibérica, Spain) before its analysis. For beverages containing more than 20% (v/v) alcohol, 125 mL of these samples was diluted with 125 mL of Milli-Q water. For all samples the pH were adjusted to 7.4 with an appropriate amount of dilute $NaHCO_3$.

Analytical Method. The SPE system used for the determination of NAms in beverages is depicted in **Figure 1**. In the preconcentration step, 250 mL of a sample or a standard solution at pH 7.4 (adjusted with $NaHCO_3$) containing 0.04–25 μg/kg NAms was passed through the sorbent column (located in the loop of injection valve IV_1) at 5 mL/min. Retention of NAms was instantaneous, and the sample matrix was sent to waste (position in bold lines in **Figure 1**). In the drying step, IV_1 was switched and the sorbent column dried for 5 min with an air stream at 3 mL/min inserted via the carrier line of the second valve (IV_2); simultaneously the loop of IV_2 (150 μL) was filled with the eluent

Table 2. Analytical Figures of Merit of the Proposed Solid-Phase Extraction Method for Determination of *N*-Nitrosamines in Beverages

	NDMA	NDEA	NPIR	NPIP	NMOR	NDBA	NDPA
retention time (min)	5.01	6.68	9.70	10.41	9.91	12.85	17.43
detection limit[a] (μg/kg)	0.029	0.007	0.017	0.025	0.015	0.033	0.031
linear range[a] (μg/kg)	0.10–25	0.04–25	0.07–25	0.08–25	0.07–25	0.13–25	0.12–25
precision[a,b] (RSD, %)	4.3	4.5	5.2	4.7	5.0	4.8	6.0
m/z[c]	42, 43, *74*	42, 44, *102*	41, 42, *100*	*42*, 55, 114	*56*, 86, 116	57, *84*, 158	46, 167, *168*

[a] Sample volume, 250 mL. [b] RSD: relative standard deviation ($n = 11$) for 0.5 μg/kg. [c] *m/z* values used for confirmation by GC-MS: in italics, the base peaks used for quantitation of M^+ ions.

Table 3. Percent Recovery (±SD, $n = 3$) of *N*-Nitrosamines Added to Beverage Samples

	NDMA		NDEA		NPIR		NMOR		NPIP		NDBA		NDPA	
	0.5 μg/kg	1 μg/kg	0.5 μg/kg	1 μg/kg	0.5 μg/kg	1 μg/kg	0.5 μg/kg	1 μg/kg	0.5 μg/kg	1 μg/kg	0.5 μg/kg	1 μg/kg	0.5 μg/kg	1 μg/kg
red wine	95(6)	95(6)	101(5)	97(5)	100(6)	99(6)	99(7)	100(7)	97(6)	99(5)	100(5)	99(5)	101(5)	98(5)
rosé wine	96(6)	101(6)	97(6)	101(6)	96(5)	99(5)	99(6)	102(6)	100(6)	99(6)	97(6)	102(5)	99(5)	102(5)
white wine	96(7)	96(5)	95(5)	100(5)	102(7)	98(6)	100(7)	102(7)	96(7)	100(7)	95(5)	97(5)	102(7)	98(5)
sparkling wine	100(7)	100(6)	96(5)	98(5)	101(7)	100(6)	101(6)	98(5)	98(7)	101(6)	95(6)	99(5)	98(6)	101(6)
grape juice	98(6)	102(6)	99(8)	99(5)	100(6)	101(6)	102(7)	97(6)	99(6)	102(6)	102(8)	100(7)	95(5)	96(5)
cider	100(5)	101(5)	100(7)	98(6)	99(5)	100(6)	101(5)	99(5)	98(6)	101(6)	95(5)	99(5)	101(8)	101(5)
tonic water	102(7)	102(5)	95(7)	102(7)	97(6)	95(5)	95(6)	96(6)	100(8)	98(6)	101(7)	98(6)	99(6)	102(6)
liquor	100(6)	101(5)	95(6)	99(6)	99(5)	96(5)	97(7)	100(6)	101(7)	99(7)	100(7)	101(5)	96(5)	99(5)
nonalcoholic liquor	100(7)	98(5)	97(6)	102(6)	97(7)	102(5)	100(7)	102(6)	95(6)	99(5)	96(6)	102(6)	101(5)	98(5)
soft drink 1	100(5)	98(6)	101(7)	99(6)	100(7)	101(6)	98(6)	96(6)	102(5)	96(5)	100(5)	98(5)	100(7)	102(7)
soft drink 2	102(7)	99(6)	96(6)	100(6)	99(7)	97(5)	97(5)	96(5)	100(5)	99(5)	101(6)	95(6)	99(6)	101(5)
whiskey[a]	95(6)	98(6)	97(6)	101(5)	98(6)	96(6)	100(7)	102(7)	99(7)	101(6)	98(7)	101(7)	96(5)	99(5)
rum[a]	95(6)	99(5)	99(6)	99(6)	98(8)	102(6)	99(7)	101(7)	100(6)	97(5)	96(6)	100(6)	100(6)	95(5)
gin[a]	95(7)	101(6)	95(6)	98(5)	101(5)	96(5)	100(7)	96(6)	99(7)	101(7)	95(6)	101(6)	96(5)	100(5)
vodka[a]	95(7)	101(6)	100(7)	97(7)	102(6)	95(5)	99(5)	98(5)	101(8)	98(7)	102(7)	100(6)	101(6)	95(5)
nonalcoholic beer	102(8)	100(5)	98(7)	102(6)	99(5)	95(5)	100(6)	101(5)	97(6)	102(5)	101(7)	96(6)	101(7)	97(5)
alcoholic beer	100(7)	102(6)	95(6)	99(5)	95(7)	101(7)	97(5)	100(5)	96(6)	100(6)	95(6)	98(6)	99(7)	102(7)

[a] Sample diluted twice with water.

Table 4. *N*-Nitrosamines Found in Beverages (±SD, $n = 3$)

sample	ethanol (%)	NAm found	concn found (μg/kg)
red wine	11	none	
rosé wine	11	none	
white wine	11	none	
sparkling wine	10.5	none	
grape juice	0	none	
cider	4	none	
tonic water	0	none	
nonalcoholic liquor	0	none	
liquor	17	none	
soft drink 1	0	none	
soft drink 2	0	none	
whiskey	40	none	
rum	37.5	none	
gin	37.5	none	
vodka	37.5	none	
nonalcoholic beer 1	0	*N*-nitrosopiperidine	0.22 ± 0.01
nonalcoholic beer 2	<0.1	*N*-nitrosodimethylamine	0.40 ± 0.02
		N-nitrosopiperidine	0.39 ± 0.02
alcoholic beer 1	5.4	*N*-nitrosodimethylamine	0.14 ± 0.01
		N-nitrosopiperidine	0.15 ± 0.01
alcoholic beer 2	4.2	*N*-nitrosodimethylamine	0.56 ± 0.03
		N-nitrosopiperidine	0.29 ± 0.02

containing the IS (10 mg/L bromophos-methyl in ethyl acetate–acetonitrile, 9:1) by means of a syringe. In the elution step, IV_2 was switched, and the loop contents (150 μL) were injected into the same air stream used in the drying step to elute adsorbed NAms (in the opposite direction of the sample aspiration). The whole organic extract was collected in an Eppendorf vial containing sodium aluminosilicate (desiccator), and a 1 μL aliquot was manually injected into the gas chromatograph for analysis. Between samples, the sorbent column was cleaned, without removal from the continuous SPE system, with 450 μL of acetonitrile and conditioned with 5 mL of Milli-Q water. Under these conditions, the sorbent column was serviceable for at least 3 months.

RESULTS AND DISCUSSION

Optimization of the Preconcentration/Elution Process. In previous work, we developed a flow system for the preconcentration of these NAms in water by gas chromatography. LiChrolut EN and 9:1 ethyl acetate–acetonitrile proved the most efficient sorbent and eluent, respectively, for the intended purpose. In order to examine the influence of the sample matrix, in this work we initially used the previously optimized system (*22*) to study the performance of the sorbent and eluent by using an uncontaminated alcoholic beverage (white wine) and various nonalcoholic (soft drink) beverages that were fortified with a 1 μg/kg concentration of each of seven NAms at pH 7.4 as adjusted with $NaHCO_3$. The highest sorption for all analytes was obtained by using the same chemicals and flow variables as with water samples and also the same solid-phase extraction system. By way of summary, the optimum value of each variable is listed in **Table 1**.

Because the ethanol content in the alcoholic beverages studied ranged from 11.0% for wine to 40.0% for whiskey, establishing its influence was essential as the alcohol might affect retention of the analytes onto the sorbent column. Initially, such an influence was examined by using standard solutions prepared in water–ethanol medium containing a 1 μg/kg concentration of each NAm and variable proportions of ethanol from 0% to 100%. As can be seen from **Figure 2**, ethanol had no effect on retention in proportions up to 20% for NDMA and NDEA and 30–40% for the other NAms. Higher ethanol concentrations, however, resulted in dramatically decreased retention of NAms on the sorbent. This can be ascribed to the particular mechanism of sorption, which involves the partitioning of moderately polar organic compounds (NAms) from a polar phase (water) into a polymeric sorbent (LiChrolut EN) via a polar interaction such as hydrogen bonding between the amino nitrogen in the NAms

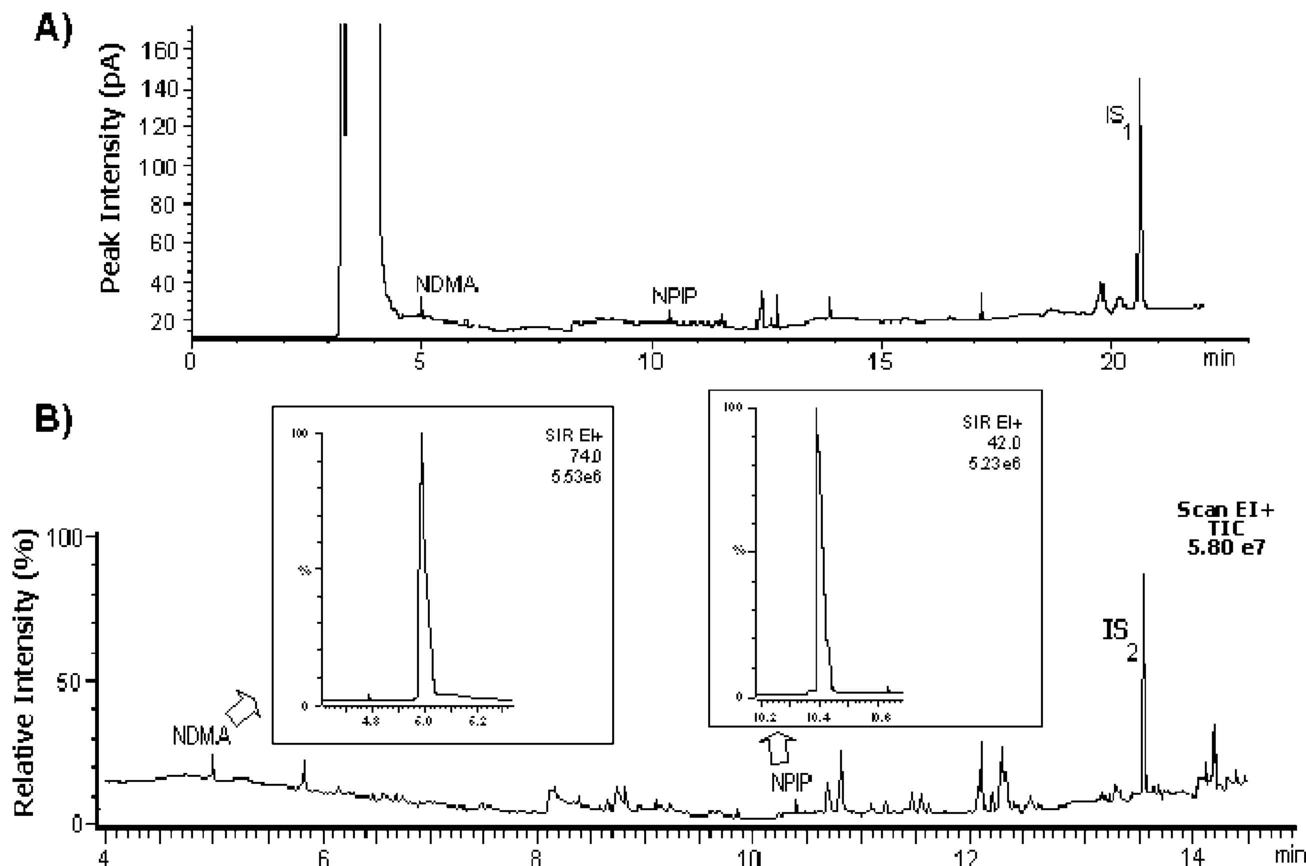

Figure 3. Gas chromatogram obtained following the preconcentration of 250 mL of an alcoholic beer with NPD (A) and MS detection (B). Peaks: NDMA, *N*-nitrosodimethylamine; NPIP, *N*-nitrosopiperidine; IS_1, bromophos-methyl (internal standard); IS_2, 2-*tert*-butyl-4-methylphenol (internal standard).

and the underlying sorbent surface. When the aqueous sample contains a high concentration of ethanol (viz., one above 20%), the solvent breaks the bonds and effectively solubilizes NAms, thereby dramatically reducing sorption of the analytes (see **Figure 2**). In order to avoid this problem, samples containing high proportions of ethanol (e.g., spirituous drinks) were diluted 1:1 with Milli-Q water so as to bring the ethanol content within the tolerated range (**Figure 2**). This had no adverse effect on sensitivity as the method afforded the preconcentration of up to 300 mL of sample and elution with 150 μL aliquots to obtain a preconcentration ratio of 2000.

The effect of common substances potentially present in alcoholic and nonalcoholic beverages was assessed by determining each NAm at a 1 μg/kg concentration. The interferences studied included tannic and gallic acids, glucose, ascorbic acid, citric acid, lactic acid, sorbate, glycerol, phosphate, and various metals. Tannins are one of the main constituents of beer and wine. The word "tannin" is used to designate a heterogeneous mixture of polyphenolic substances of vegetable origin and high molecular weights that are partly responsible for wine and beer flavor. We first examined the effect of tannic and gallic acids on the retention of NAms. No interference from either at a concentration of 2 g/kg, which is higher than those typically present in the studied beverages, was detected.

Because glucose is used as a sweetener in many beverages, we also examined its influence, using it in proportions of 15 g/kg. On the basis of the results, the effect of the presence of glucose on the retention of NAms was negligible. Finally, other compounds typically present in beverages including cations (Fe, Cu, Ca, Mg, Na, and K), ascorbic acid, citric acid, lactic acid, sorbate, glycerol, and phosphates were studied at concentrations of 200 mg/kg. No interference was detected as the likely result of none of the previous substances being retained on the sorbent column.

Sensitivity and Validation of the Method. When an internal standard is used, the chromatographic signals obtained are corrected because it allows the use of relative areas (the ratio of analyte peak area to internal standard peak area); in addition, this results in substantially improved precision. Three organic compounds (viz., bromophos-methyl, 2-*tert*-butyl-4-methylphenol, and triphenyl phosphate) were assessed as internal standards for addition to the eluent. Because triphenyl phosphate was partially retained on the sorbent and the other two compounds were not retained at all, bromophos-methyl and 2-*tert*-butyl-4-methylphenol were selected for further testing.

All NAms studied exhibited good gas chromatographic responses. Analytical curves for NAm standards were obtained by processing a sample volume of 250 mL of Milli-Q water containing variable concentrations of the analytes in the SPE system of **Figure 1**. The curves were constructed by plotting the analyte-to-IS peak area ratio against the analyte concentration. The linear range, detection limit, and precision (as relative standard deviation) for the determination of *N*-nitrosamines by GC-NPD, and the *m/z* values used for GC-MS confirmation, are shown in **Table 2**. The regression coefficients were greater than 0.994 in all cases. Detection limits were calculated as three times the standard deviation of background noise divided by the slope of each calibration graph. The repeatability of the proposed method as relative standard deviation for a standard mixture containing a 0.5 μg/kg concentration of each NAm ranged from 4.3% to 6.0% (n = 11, within day).

The validation of the proposed method was assessed by analyzing uncontaminated beverage samples spiked with 0.5 or 1 μg/kg standard mixture of the studied compounds as no certified reference materials for the beverages were available. Each sample was analyzed in triplicate ($n = 3$). The average recoveries thus obtained are listed in **Table 3**. All compounds were accurately identified and the average recoveries (95–102%) acceptable for all matrices. Therefore, matrix interferences were reduced or completely suppressed during the cleanup step in the SPE module.

Analysis of Beverages. The proposed method was applied to the determination of seven *N*-nitrosamines in alcoholic and nonalcoholic beverages including wines (red, rosé, white, and sparkling), grape juice, beers (with and without alcohol), cider, tonic water, liquor, nonalcoholic liquor, soft drinks, whiskey, rum, gin, and vodka.

Wine and grape juice samples were passed through filters of 0.45 μm pore size in order to remove particulates. Samples were analyzed by using the analytical procedure described under Materials and Methods. Spirituous beverages were diluted twice before analysis. In all cases, quantitation was done by NPD, and positive findings were confirmed by MS. Only beer samples were found to contain any NAms at detectable levels. The results are listed in **Table 4**. As can be seen, the beer samples contained *N*-nitrosopiperidine and/or *N*-nitrosodimethylamine at low concentrations; all other species were either not detected or present at levels below their limits of quantitation. Several studies of NAms in malt and beer have suggested that malt is the main source of NDMA contamination, which occurs as a result of the kilning process when the air is heated directly in an open flame (*1*). **Figure 3A** shows the chromatogram for an alcoholic beer sample containing the previous two NAms as obtained by NPD. **Figure 3B** illustrates the confirmatory chromatogram obtained with MS detection. As expected, there were no matrix interferences.

In conclusion and as a summary, the proposed method affords the simultaneous determination of seven NAms in beverages. By contrast, most existing methods can only determine one (NDMA or NPIR) (*3, 4, 6, 16, 17, 19*) or two *N*-nitrosamines (NDMA and NDEA or NDMA and NPIR) (*11, 18*) in beer; a few allow several *N*-nitrosamines to be determined in alcoholic beverages (*14, 20, 21*).

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