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Gas Chromatographic Determination of *N*-Nitrosamines, Aromatic Amines, and Melamine in Milk and Dairy Products Using an Automatic Solid-Phase Extraction System

Beatriz Jurado-Sánchez,[†] Evaristo Ballesteros,[*,‡] and Mercedes Gallego[*,†]

[†]Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071-Córdoba, Spain

[‡]Department of Physical and Analytical Chemistry, EPS of Linares, University of Jaén, E-23700 Linares, Jaén, Spain

ABSTRACT: A reliable analytical method was presented for the simultaneous determination of six *N*-nitrosamines, nine aromatic amines, and melamine in milk and dairy products using gas chromatography coupled with mass spectrometry. The sample treatment includes the precipitation of proteins with acetonitrile, centrifugation, solvent changeover by evaporation, and continuous solid-phase extraction for cleanup and preconcentration purposes. Samples (5 g) containing 0.15−500 ng of each amine were analyzed, and low detection limits (15−130 ng/kg) were achieved. Recoveries for milk and dairy products samples spiked with 1, 10, and 50 μg/kg ranged from 92% to 101%, with intraday and interday relative standard deviation values below 7.5%. The method was successfully applied to determine amine residues in several milk types (human breast, cow, and goat) and dairy products.

KEYWORDS: *N*-Nitrosamines, aromatic amines, melamine, milk, dairy products, continuous solid-phase extraction, gas chromatography−mass spectrometry

INTRODUCTION

The concern about the presence of toxic compounds such as *N*-nitrosamines (NAms), aromatic amines (AAs), and melamine in dairy products has increased in recent years. Carcinogenic NAms are formed in foodstuffs by chemical reactions between nitrosatable secondary amines such as the naturally occurring dimethylamine, nitrosating agents formed from nitrites, or nitrates that are used as preservatives, or nitrogen oxides from combustion gases in direct heating, or drying food processes.[1−3] The migration of AAs from adhesives, dye on food packages, and cooking utensils is the main source of these compounds in foodstuffs.[4,5] Melamine, which is used in the manufacture of amino resins and plastics, can also be added illegally to pet food and milk products to enhance apparent protein content.[6] Although melamine has low acute toxicity, illegal large-dose adulteration in routine dairy products can result in urinary calculi, acute renal failure, and even infant death.[7] Therefore, the high toxicity and exposure of AAs and melamine explain the strict regulation established by American and European rulings.[8−10]

The above food safety issues related to amine contamination led to the need for rapid and reliable analytical methods capable of detecting the target analytes at levels indicated by regulatory authorities. *N*-Nitrosamines have been determined in milk and other food samples by gas chromatography (GC) with thermal energy analyzers,[11−13] nitrogen−phosphorus[14] or mass spectrometry (MS) detection,[14,15] and by liquid chromatography (LC) with fluorescence detection.[16] With regard to aromatic amines and melamine in food, different methods have been proposed using GC−MS.[4,7,17−22] Strong polar stationary phases (such as a polyethylene glycol column) should be used for the direct detection of melamine because weak polar phases are not compatible due to band broadening and peak tailing. LC with diode array,[23,24] ultraviolet absorption,[25] or MS detection[4,26−28] and electrophoretic methods with different detectors[5,6,29,30] have also been reported to determine aromatic amines and melamine in milk products.

Milk and dairy products are complex food matrices given the presence of many compounds and the suspension caused by proteins; thus the previous step involving protein precipitation with an appropriate solvent, centrifugation, supernatant filtration, and preconcentration and/or purification steps is mandatory prior to amine determination.[18] Recent approaches in sample treatment have been directed toward techniques such as solid-phase microextraction,[15] hollow fiber sorptive extraction,[19] and solid-phase extraction (SPE).[13,18,20,21,23,27]

It is important to highlight that no methods have been developed to date for the simultaneous determination of *N*-nitrosamines, aromatic amines, and melamine in dairy products. Therefore, the main purpose of this work was to develop an accurate and reliable analytical methodology to determine these amines in milk and dairy products according to the safety levels established by American and European guidelines. The research was also intended to use a solid-phase extraction system for cleanup/preconcentration purposes. The high efficiency of the cleanup system provides good blanks and avoids the coextractive interferences.

MATERIALS AND METHODS

Chemicals and Standards. *N*-Nitrosamines, aromatic amines, and melamine (Figure 1) were obtained from Sigma−Aldrich (Madrid, Spain; purity, 99%). Methanol, acetonitrile, and ethyl acetate (chromatographic grade), trichloroacetic acid, sodium hydrogencarbonate, LiChrolut EN (particle size, 40−120 μm), and 2-*tert*-butyl-4-methylphenol (2TB4MP, internal standard) were obtained from Merck (Darmstadt, Germany).

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Ultrapure water was obtained using a Milli-Q purification system from Millipore (Bedford, MA).

Stock solutions of NAms, AAs, and melamine (1 g/L) were prepared in methanol and stored in a refrigerator at 4 °C. Working solution mixtures were prepared daily by diluting with a 0.1 M $NaHCO_3$ solution at pH ∼8. A mixture of ethyl acetate−acetonitrile (9:1) containing 1 mg/L of 2TB4MP was employed as eluent.

Instruments and Apparatus. All analyses were carried out on a Focus gas chromatograph coupled to a DSQ II mass spectrometer equipped with an AI/AS 3000 autosampler (Thermo Electron SA, Madrid, Spain) and controlled by a computer running XCalibur software (Thermo Electron SA, Madrid, Spain). The transfer line and source temperature were kept at 200 °C. The mass spectrometer worked in the electron impact mode (70 eV) by scanning from 40 to 250 amu to obtain full spectra of the target analytes or by selected ion-monitoring (SIM) for the quantification of the analytes. The ions used in the SIM mode are listed in Table 1. Quantification relied on the base peak ion (T), and three additional qualifier ions (Q1, Q2, and Q3) were used to confirm the identity of the analytes and peak purity. Each peak was checked for identity and proper integration; also, at least one of its three ion ratios (Q1/T, Q2/T, Q3/T) should be within ±30% of those for the pure standards to be considered positive. The injector was maintained at 250 °C, and all injections were done in the split mode (1:20 ratio). The time for solvent delay was set at 3 min. A SP-2380 column [30 m × 0.25 mm × 0.25 μm poly (90% biscyanopropyl/10% cyanopropylphenyl siloxane); Supelco, Madrid, Spain] was used with helium (purity 6.0) as the carrier gas at 1 mL/min. The oven temperature was programmed as follows: the initial temperature (90 °C) was increased at 9 °C/min to 180 °C and then at 15 °C/min to 250 °C, held for 2 min. The total GC run time was ∼17 min.

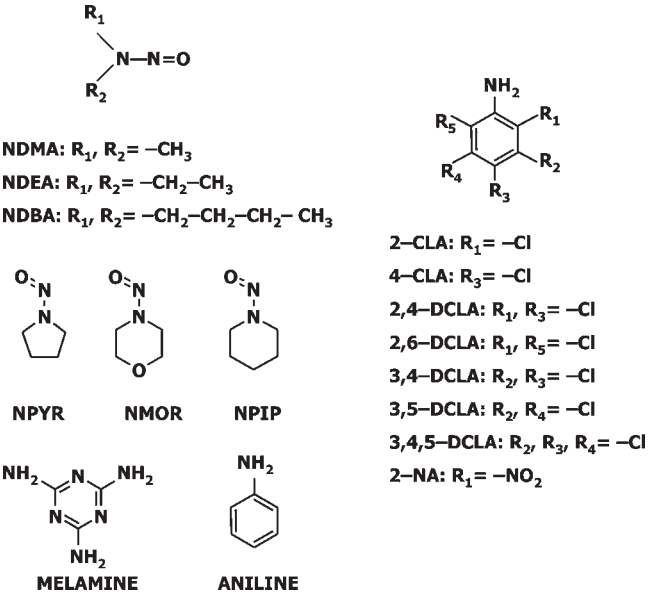

Figure 1. Chemical structures of *N*-nitrosamines, aromatic amines, and melamine. For compound abbreviations, see Table 1.

The SPE system was assembled from a Gilson Minipuls-3 peristaltic pump (Villiers-le-Bel, France) fitted with poly(vinylchloride) tubes and two Rheodyne (Cotati, CA) 5041 injection valves. A PTFE laboratory-made sorbent column packed with 75 mg of LiChrolut EN as described elsewhere was also employed.[31] The column was made from PTFE tubing (8 cm × 3 mm i.d.), and small glass wool plugs were placed above and below the sorbent bed to prevent material losses. Because of its small particle size, this sorbent is very prone to compaction when soaked in the flow system. To avoid abrupt changes in column compactness that might stop the solution flow and dislodge the system connections, each LiChrolut EN segment (1 cm long) was separated by another of inert material (e.g., PTFE beads, segments ∼0.5 cm long). The sorbent column was conditioned with 0.5 mL of acetonitrile and 1 mL of purified water, which rendered it serviceable for at least 1 month. A laboratory-made PTFE filter (3 cm × 3 mm i.d.) packed with glass wool was used to pass the aqueous phase into the SPE unit; the filter was replaced after 20−30 analyses.

Sample Preparation. Milk, cheese, yogurt, and cream samples were purchased from local markets. Raw cow milk samples were obtained from various farms using standard commercial breeding protocols (machine-milked of stainless steel and rubber). Human milk samples were kindly

Table 1. Analytical Figures of Merit of the Proposed Method and Mass Values Used for MS Detection

			precision RSD (%)[a]		
compound	linear range (μg/kg)	LOD (ng/kg)	within-day	between-day	m/z[b]
N-nitrosodimethylamine (NDMA)	0.15−100	45	3.6	5.4	30, 42, 43, **74**
N-nitrosodiethylamine (NDEA)	0.06−100	20	3.0	4.4	42, 44, 56, **102**
N-nitrosopyrrolidine (NPYR)	0.20−100	65	4.6	6.9	41, 42, 88, **100**
N-nitrosomorpholine (NMOR)	0.15−100	50	4.7	6.9	42, **56**, 86, 116
N-nitrosopiperidine (NPIP)	0.08−100	25	6.2	7.1	**42**, 55, 56, 114
N-nitrosodibutylamine (NDBA)	0.20−100	60	3.7	5.6	57, **84**, 115, 158
aniline (A)	0.05−100	15	3.8	5.6	65, 66, 92, **93**
2-chloroaniline (2-CLA)	0.03−100	10	6.0	6.9	65, 93, **127**,129
4-chloroaniline (4-CLA)	0.15−100	45	6.3	7.2	65, 93, **127**,129
2,4-dichloroaniline (2,4-DCLA)	0.30−100	95	5.2	7.1	63, 90, **161**, 163
2,6-dichloroaniline (2,6-DCLA)	0.30−100	85	4.7	7.1	63, 90, **161**, 163
3,4-dichloroaniline (3,4-DCLA)	0.40−100	125	6.1	7.2	63, 90, **161**, 163
3,5-dichloroaniline (3,5-DCLA)	0.45−100	130	6.1	7.5	63, 90, **161**, 163
2,4,5-trichloroaniline (2,4,5-TCLA)	0.15−100	50	5.8	6.8	193, **195**, 197, 199
2-nitroaniline (2-NA)	0.30−100	95	4.8	7.2	65, 80, 92, **138**
melamine	0.07−100	20	3.5	5.1	43, 68, 85, **126**

[a] RSD, relative standard deviation (n = 11) for 1 μg/kg. [b] m/z values (base peaks for quantification are boldfaced); m/z for IS (2TB4MP): 91, 121, **149**, 164.

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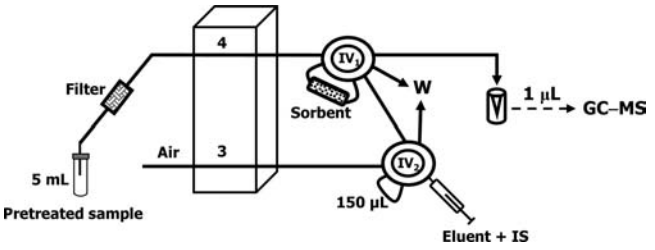

Figure 2. Continuous SPE unit for the cleanup/preconcentration of *N*-nitrosamines, aromatic amines, and melamine in milk and dairy products. IV, injection valve; W, waste; IS, internal standard; GC−MS, gas chromatograph with mass spectrometric detector.

donated by healthy lactating volunteers (40−60 mL). None of the donors reported occupational exposure to amines. In the laboratory, raw and human milk samples were frozen and stored in the dark until analysis (within 2 weeks of collection). The other samples were refrigerated at 4 °C until analysis (within 48 h). The frozen samples were thawed and mixed well; 100−250 g amounts of cheese samples were grated using a household cheese grater. A 5 g amount of each homogenized sample (milk, cream, yogurt, and cheese) was mixed with 5 mL of acetonitrile and vortexed for 1 min. The mixture was then centrifuged (5000 rpm) at 4 °C for 5 min; next, the supernatant was evaporated to ∼100 μL under a gentle stream of ultrahigh-purity N_2 (flow rate ca. 100 mL/min at room temperature) and redissolved to 5 mL with a 0.1 M $NaHCO_3$ solution at pH ∼8 (the optimum interval of sample pH was 6.5−8.5). For powdered milk, 1 g was dissolved in 5 mL of purified water according to the manufacturer's instructions, and the solution was processed like all other samples.

Analytical Procedure. The continuous SPE unit used for the cleanup/preconcentration of amines from evaporated/redissolved samples is depicted in Figure 2. In the preconcentration step, a volume of 5 mL of aqueous sample at pH ∼8 containing 0.15−500 ng of each amine was continuously filtered (to prevent suspended particles from reaching the continuous unit) and passed through the sorbent column, located in the loop of injection valve IV_1, at 4 mL/min. Amines were immediately retained, and the sample matrix was sent to waste. Next, IV_1 was switched and the sorbent column dried for 2 min with an air stream at 3 mL/min; simultaneously, the loop of IV_2 (150 μL) was filled with eluent [(9:1) ethyl acetate/acetonitrile containing 1 mg/L 2TB4MP as internal standard] by means of a syringe. In the elution step, IV_2 was switched to pass 150 μL of eluent, carried through the column by the air stream in the opposite direction of sample aspiration. The whole organic extract was collected in a conical glass insert (0.3 mL) inside a 2 mL amber glass GC vial, which was tightly sealed. Finally, extract aliquots of 1 μL were injected into the GC−MS instrument for analysis. Under these conditions, the sample-to-sample extraction time was quite short (ca. 5 min).

RESULTS AND DISCUSSION

Optimization of the Extraction Conditions. Milk and dairy products are complex emulsions that also contain a suspension of multiple components including proteins, fats, casein micelles, and lactose. Molecules such as protein micelles enclose water and other compounds, presenting a complex matrix that challenges the detection of trace amounts of contaminants. Moreover, amines might associate via nonspecific interactions to proteins, and the precipitating agent should also be able to efficiently dissociate it from the sample matrix.[32] For these reasons, precipitation and separation of the proteins is necessary prior to the determination of the amines. To precipitate proteins,

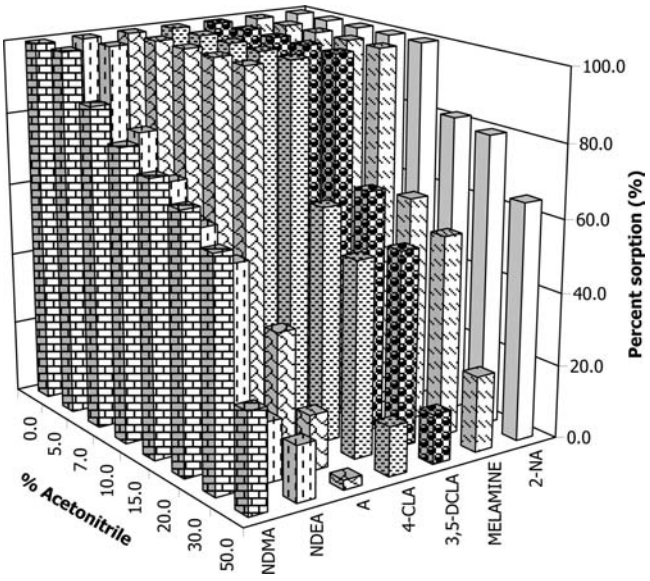

Figure 3. Influence of the percentage of acetonitrile in the aqueous sample (%, v/v) on the retention of the amines in the sorbent column. For the compound abbreviations, see Table 1.

several authors have employed aqueous solutions of trichloroacetic acid (TCA),[18,26,27,29] perchloric acid,[25] acetonitrile,[23,28] or methanol.[20] To precipitate proteins, we assayed different solutions: 1% TCA, 0.1 M $HClO_4$, methanol, and acetonitrile. First, preliminary experiments were conducted by adding 5 mL of each precipitating reagent to 5 g of various uncontaminated milk samples (whole, semi-skimmed, and skimmed), or those of a cream or a cheese. After mixing, the mixture was centrifuged for 10 min at 5000 rpm; efficient protein precipitation was only achieved with the solutions of 1% TCA or acetonitrile. Second, the extraction efficiency of both precipitating reagents was assessed by using 5 g of uncontaminated samples (milk, cream, and cheese), which were spiked with 5 μg/kg of each amine and then processes as described above. After centrifugation, amines were extracted from each supernatant with 5 mL of ethyl acetate, and 1 μL aliquots were injected into the GC−MS instrument. Extraction efficiencies were calculated by assigning 100% to ethyl acetate standards containing the same amount of amine spiked to the samples (5 μg/kg) that were directly injected into the GC−MS instrument. The highest extraction efficiency for all amines (∼95%) was obtained for acetonitrile (1% TCA solution provided ∼75%), which demonstrated that this solvent favors the precipitation of the proteins and their subsequent separation by centrifugation. Acetonitrile was finally selected to precipitate proteins from milk and dairy products. We also examined the effects of centrifugation-related variables such as rate, time, and temperature over the ranges 2000−5000 rpm, 1−10 min, and 2−25 °C, respectively. Centrifugation at 5000 rpm at ∼4 °C for 5 min resulted in the optimum separation of the supernatant containing the amines from precipitated milk proteins and facilitates the removal of the lipid fraction of these types of samples.[17]

To improve both the selectivity and the sensitivity of the method, we used a cleanup/preconcentration system described elsewhere to preconcentrate amines in water samples,[31] in which a mixture of ethyl acetate−acetonitrile (9:1, v/v) was the most effective eluent. In the present work, the supernatant (containing the amines) obtained after centrifugation contains a high volume

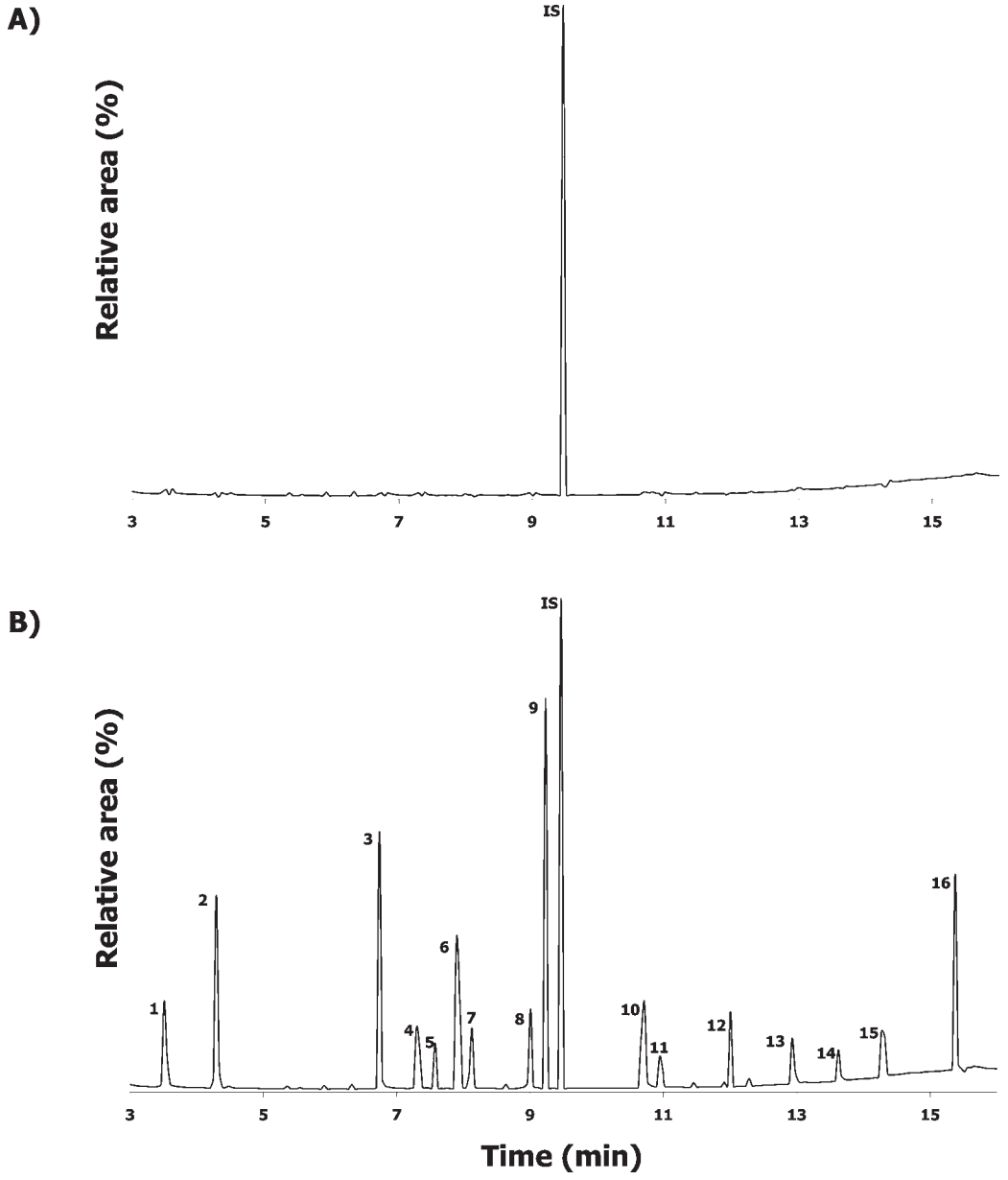

Figure 4. GC−MS chromatograms in SIM mode for an uncontaminated whole goat-milk sample (A) and the same sample spiked with 2 μg/kg of each amine (B). Peaks: (1) NDMA; (2) NDEA; (3) A; (4) NDBA; (5) 2,4-DCLA; (6) NPIP; (7) NPYR; (8) NMOR; (9) 2-CLA; (10) 4-CLA; (11) 3,5-DCLA; (12) 2,4,5-TCLA; (13) 2,6-DCLA; (14) 3,4-DCLA; (15) 2-NA; (16) melamine; (IS) internal standard. For compound abbreviations, see Table 1.

of acetonitrile (~50%), and therefore its effect was examined on the retention of the amines into a sorbent column packed with 75 mg of LiChrolut EN (using a system similar to that depicted in Figure 1). For this purpose, several standard solutions, containing 1 μg/kg of each amine prepared in various mixtures of acetonitrile−water from 0 to 50% (v/v) at pH ~8 (adjusted with diluted $NaHCO_3$), were aspirated through the sorbent column. The behavior is different as well for the NAms and for the AAs and melamine, as can be seen in Figure 3 for seven representative analytes (two NAms, four aromatic amines, and melamine); acetonitrile had no negative effect on the retention of the amines up to 5% for NAms and 15% for AAs and melamine. The greater increase in the acetonitrile percentage in the aqueous sample decreased the retention of amines on the sorbent column. This can be ascribed to the mechanism of amine sorption, which involves the partitioning of the amines from a polar phase (water) into a nonpolar phase such as a polymeric sorbent (LiChrolut EN) via hydrogen bonding and $\pi-\pi$ interactions between the analytes and the underlying sorbent surface. When the aqueous sample contains a high percentage of acetonitrile (higher than 5% for NAms or 15% for AAs and melamine), this solvent breaks the bonds and effectively solubilizes the amines, thereby dramatically decreasing the sorption of the analytes. To avoid this problem, the solvent changeover of the supernatant was mandatory. The supernatant was carefully evaporated at room temperature under a gentle stream of ultrahigh-purity N_2 for ca. 10 min to a final volume of ~100 μL and redissolved to 5 mL with a 0.1 M $NaHCO_3$ solution at pH ~8.0, and then aspirated into the continuous cleanup/preconcentration SPE unit.

Table 2. Determination of Amines in Milk and Dairy Products by Proposed GC−MS Method (±SD, $n = 3$)[a]

sample	compound	concentration found (μg/kg)	sample	compound	concentration found (μg/kg)
raw cow-milk 1−6	nd[b]		human milk 1−4	nd	
whole cow-milk 1	NDBA	0.51 ± 0.04	powdered cow-milk 1[c]	NDMA	1.6 ± 0.1
	A	1.8 ± 0.1		4-CLA	1.8 ± 0.1
	4-CLA	0.80 ± 0.06		melamine	3.6 ± 0.2
whole cow-milk 2−6	nd		powdered cow-milk 2[c]	NPIP	0.51 ± 0.04
				melamine	2.5 ± 0.1
semi-skimmed cow-milk 1	A	0.32 ± 0.02	cheese 1	NDEA	0.62 ± 0.04
				3,5-DCLA	1.1 ± 0.1
semi-skimmed cow-milk 2−4	nd		cheese 2	NPIP	0.25 ± 0.02
skimmed cow-milk 1−4	nd		cheese 3−5	nd	
whole goat-milk	nd		yogurt 1−4	nd	
semi-skimmed goat-milk	NDBA	0.69 ± 0.04	cream 1−4	nd	

[a] To 5 g of sample ($n = 3$). [b] Not detected. [c] To 1 g of sample ($n = 3$).

Method Validation. Linear range, analyte detectability, and precision of the proposed GC−MS method were studied under optimal experimental conditions (see Table 1). Calibration curves were constructed by using uncontaminated cow milk samples (5 g) spiked with 0.15−500 ng of each amine (NAms, AAs, and melamine) and processed as described in the Sample Preparation and Analytical Procedure sections. The equations for the standard curves were obtained by plotting the analyte to internal standard peak area ratios against the amount of amine. Regression coefficients were over 0.997 in all cases. Limits of detection (LODs) were determined as the analyte concentration that provides a chromatographic peak equal to 3 times the regression standard deviation, $S_{y/x}$, divided by the slope of each calibration graph, and ranging between 10 and 130 ng/kg. The lower limit of the linear range corresponds to the limit of quantification (LOQ), which is 3.3 × LOD.

The precision of the proposed method, as relative standard deviation (RSD), was calculated by measuring 11 uncontaminated milk samples spiked with 1, 10, and 50 μg/kg of each target compounds. A comparative study of within-day and between-day precision was conducted, the latter over 7 days, at three analyte concentration levels; the former parameter was found to range from 2.8% to 6.2% and the latter from 4.1% to 7.5%. Finally, a recovery study was conducted using uncontaminated samples (whole goat, semi-skimmed, skimmed, and powdered cow milks, cream, cheese, and yogurt) spiked at three different concentrations of each amine (1, 10, and 50 μg/kg) and analyzed in triplicate. The average recoveries ranged from 92% to 101%, which testifies to the applicability of the proposed method in these complex matrices. By way of example, Figure 4 shows the GC−MS chromatograms for an uncontaminated whole goat-milk sample as blank milk (A) and the same sample spiked with a 2 μg/kg concentration of each studied amine (B). As can be seen, good chromatographic resolution for the 16 amines was obtained without the need to derivatize them. Also, the chromatograms contained few significant peaks due to sample matrix, which testifies to the high efficiency of the cleanup step in the proposed method.

Analysis of Milk and Dairy Products. The proposed method was applied to the determination of six *N*-nitrosamines, nine aromatic amines, and melamine in human breast and raw cow milks, commercialized milks (whole, semi-skimmed, skimmed, and powdered), and dairy products (cheese, yogurt, and cream). After manual centrifugation/solvent changeover and continuous SPE for cleanup/preconcentration of the amines, the extract was injected into the GC−MS instrument for analysis.

Preliminary freeze−thaw stability tests were conducted to asses the stability of the analytes in milk and yogurt samples at a storage temperature of −20 °C. An amount of 1 kg of whole cow milk or yogurt spiked with 1 and 50 μg/kg concentrations of each analyte was split into 50 g portions and frozen at −20 °C; by exception, one portion was analyzed (in triplicate) as described above. All other portions were subjected to the same analytical procedure in triplicate every 2 days for 1 month following thawing 1 h before preparation. Freezing the samples under these conditions was found to suppress any adverse effect of the matrix on the analyte stability; in fact, the results were similar, within the error range for the method (RSD < 8%), to those for the unfrozen sample.

The results of the analysis of milk and dairy products are listed in Table 2. As can be seen, four NAms were detected, NDBA in two milk samples (one whole cow-milk and one semi-skimmed goat milk), and NDMA, NDEA, or NPIP in two powdered cow-milk and two cheese samples at concentrations ranging from 0.25 to 1.6 μg/kg, which were similar to those reported in the literature.[1,2] The formation of these NAms can be attributed to the addition of preservatives (nitrites and nitrates) during the manufacture of these products or to the nitrogen oxides that come into direct contact with food during the drying process. Aromatic amines such as aniline, 4-chloroaniline, or 3,5-dichloroaniline, which can migrate into food from composite food packaging bags, were detected in two cow milks, one powdered milk, and one cheese samples at concentrations lower than the recommended European-regulation level (20 μg/kg total amines).[8] Melamine, which may appear as a contaminant through migration from food-contact materials, was found in the two powdered cow milk samples analyzed at similar concentrations (average value, 3 μg/kg), which were much lower than the maximum allowed (2.5 mg/kg) by the European legislation for foods imported into the EU from China.[9] These concentrations were also lower than those reported in the literature (0.01−20.7 mg/kg) for powdered milk and dairy products.[18,19,22] Xu et al. found melamine at concentration above 6 g/kg in one adulterated milk sample, which is terrible due to how dangerous it is.[18]

The developed GC−MS method was also applied for the determination of NAms, AAs, and melamine in four human breast-milk samples. The analysis of these samples is a noninvasive procedure

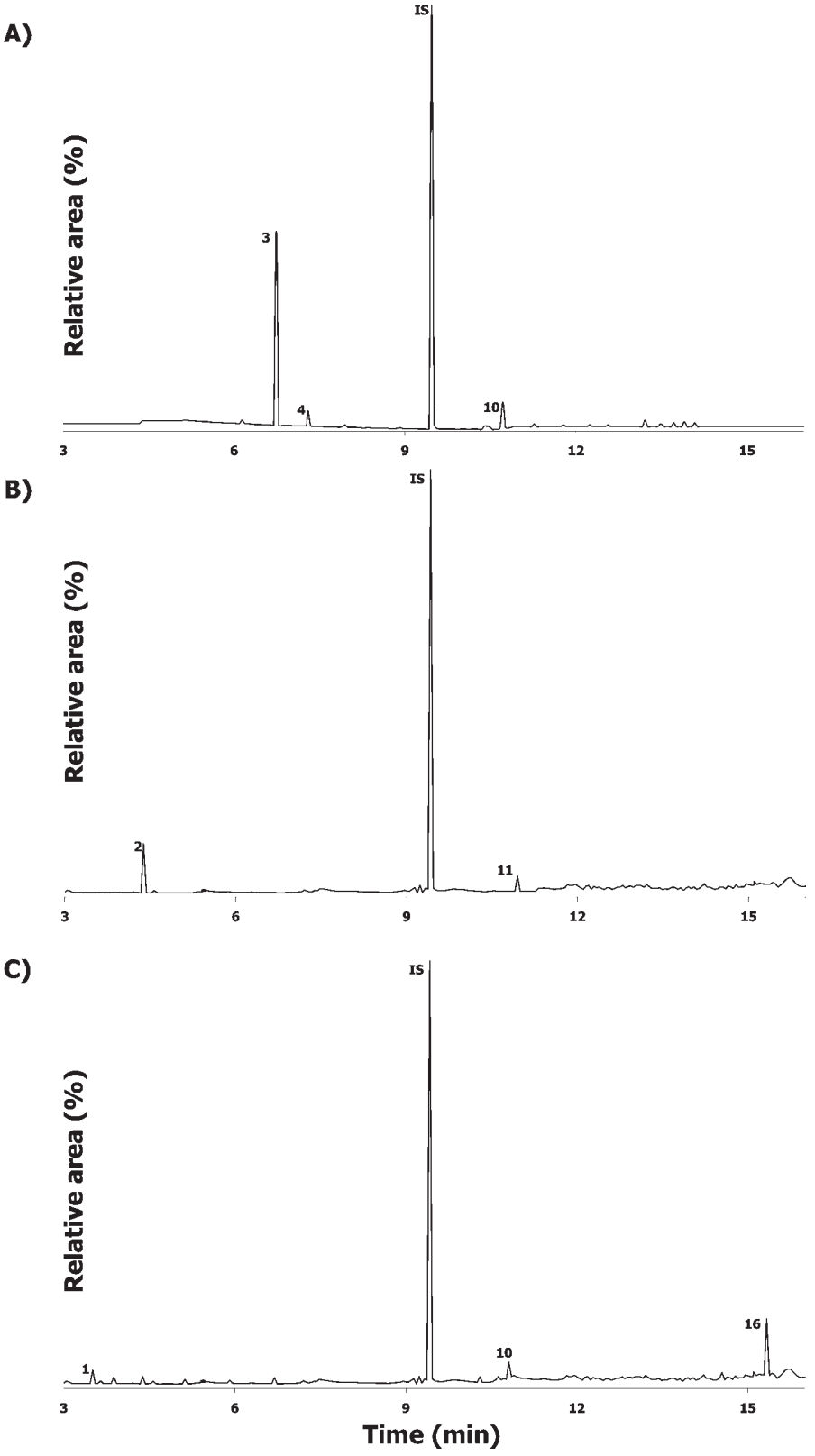

Figure 5. Chromatograms in SIM mode for amines obtained in the analysis of 5 g of whole cow-milk 1 (A) and cheese 1 (B), and ~1 g of powdered cow-milk 1 (C) (see Table 2). For peak identification, see Figure 4.

for evaluating exposure to these compounds. In this context, some AAs including aniline have been detected in milk from nonexposed lactating women at levels between 0.05 and 5.2 μg/kg.[17]

In the present study, fortunately none of the analytes studied were found in the human breast-milk samples analyzed nor were they present at levels below the LOD of the method. By way of

example, Figure 5 shows the chromatograms obtained from the analysis of whole cow-milk 1 (A), cheese 1 (B), and powdered cow-milk 1 (C). Finally, no interference from the milk and dairy product matrices was observed, probably because coextractives were eliminated during the continuous cleanup step as a consequence of not being retained on the sorbent column or because they were not eluted with ethyl acetate:acetonitrile.

In conclusion, the proposed method allows the simultaneous determination of six *N*-nitrosamines, nine aromatic amines, and melamine in a wide variety of milk and dairy product samples. However, the overall procedure includes several steps such as the precipitation of proteins, centrifugation, and solvent changeover to obtain an aqueous extract, which makes it compatible with an SPE unit for cleanup/preconcentration purposes. In this context, all of the methods that have been found in the literature individually determined NAms, AAs, or melamine except the method described by Wang and Chen[5] that simultaneously determined melamine and three aromatic amines (aniline, 2,4-diaminotoluene, and 4,4′-diaminophenylmethane) but by using several steps (centrifugation, ultrasonication, evaporation of the supernatant by rotary evaporator, redisolution, and filtration before determination by capillary electrophoresis). The sensitivity of the proposed method was adequate to detect *N*-nitrosamines, aromatic amines, and melamine under the safety limits proposed by the US FDA and European authorities[8−10] with LODs ranging between 10 and 130 ng/kg. These values are better than those provided by other methods to determine individual amine groups, NAms (LOD, 300 ng/kg),[13] AAs (LOD, 270−3000 ng/L in aqueous food),[4] or melamine in milk and dairy products (LOD: 10×10^3 ng/kg,[18] 10×10^6 ng/kg,[20] and 18×10^3 ng/kg[27]).

ABBREVIATIONS USED

A, aniline; 2-CLA, 2-chloroaniline; 4-CLA, 4-chloroaniline; 2,4-DCLA, 2,4-dichloroaniline; 2,6-DCLA, 2,6-dichloroaniline; 3,4-DCLA, 3,4-dichloroaniline; 3,5-DCLA, 3,5-dichloroaniline; GC−MS, gas chromatography−mass spectrometry; LC, liquid chromatography; IS, internal standard; IV, injection valve; LOD, limit of detection; 2-NA, 2-nitroaniline; NDBA, *N*-nitrosodibutylamine; NDEA, *N*-nitrosodiethylamine; NDMA, *N*-nitrosodimethylamine; NMOR, *N*-nitrosomorpholine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; RSD, relative standard deviation; SIM, selected ion monitoring; SPE, solid-phase extraction; 2,4,5-TCLA, 2,4,5-trichloroaniline; 2TB4MP, 2-*tert*-butyl-4-methylphenol; TCA, trichloroacetic acid.

SAFETY

Amines are known or suspected carcinogens, so they were handled with care, using fume hoods, wearing latex gloves, and avoiding inhalation or skin contact.

AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +34-953-648-560. E-mail: eballes@ujaen.es (E.B.); mercedes.gallego@uco.es (M.G.).

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