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Determination of N-nitrosodimethylamine in beer by gas chromatography–stable isotope dilution chemical ionization mass spectrometry

Marco Longo*, Claudia Lionetti, Aldo Cavallaro

Presidio Multizonale di Igiene e Prevenzione, Via Juvara 22, 20129 Milan, Italy

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Abstract

A sensitive method for the determination of N-nitrosodimethylamine in beer samples is described. The analyte was isolated by distillation and subsequent extraction from the distillate using dichloromethane. Analysis was carried out by gas chromatography combined with positive-ion chemical ionization mass spectrometry. Quantification was performed by stable isotope dilution using [$^2H_6$]-N-nitrosodimethylamine as the internal standard. The measurements exhibited excellent linearity in the range 0.10–4.00 μg kg^{-1} ($r = 0.99999$) and a detection limit of 0.04 μg kg^{-1} was achieved. The between-sample relative standard deviation of the method was 0.73% ($n = 5$) at a concentration level of 0.86 μg kg^{-1}. The recovery of the compound was 78 ± 2.1%. The proposed procedure seems to be a good alternative to the frequently used gas chromatography–thermal energy analysis approach for the determination of N-nitrosodimethylamine.

1. Introduction

The carcinogenic action of N-nitrosodimethylamine (NDMA) has been reported since 1956 [1]. The reaction of nitrogen oxides with alkaloids usually present in germinated malt during the drying process has been established as the primary source of NDMA contamination in beer and other malt beverages [2,3]. Österdahl [4] showed that beer consumption contributes greatly to the average daily intake of NDMA in some countries, even though low levels of contamination (low-ppb scale) are normally detected in beer samples. Therefore, the measurement of small amounts of NDMA in beer is a matter of great concern.

A number of methods for the determination of NDMA and other volatile nitrosamines in foodstuffs and beverages have been developed, based mainly on gas chromatography (GC) coupled with thermal energy analysis (TEA) [5–8]. TEA is a highly sensitive and selective technique for N-nitroso compounds, even though it also responds to some C-nitroso and C-nitro compounds [9]. Unfortunately, owing to its relatively high cost and limited versatility, a TEA detector

* Corresponding author.

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is not an allowable investment for many laboratories. On the other hand, the application of mass spectrometry (MS) as a detection technique for GC has expanded widely in recent years, owing to the increasing importance of GC–MS in analytical chemistry.

GC–MS has been applied extensively in order to confirm the presence of NDMA in samples previously tested by means of other techniques [10–13]. Methods based on GC–MS have sometimes been used for the quantification of NDMA in various matrices [14]. In 1976, Gaffield et al. [15] first reported the higher levels of sensitivity to NDMA achievable by chemical ionization (CI) MS in comparison with electron impact ionization. Actually, most of the ion current in CI is generally carried by the protonated molecular ion $[M+H]^+$ and few fragment ions are observed.

In this paper, a method alternative to TEA detection based on GC–isotope dilution (ID) CI-MS is described. A performance study was carried out in order to assess whether the method was suitable for the determination of NDMA in beer samples in terms of specificity, detection limit, precision and recovery. A careful choice of the GC operating conditions had to be made to avoid interferences from matrix-characteristic compounds giving an ion corresponding to a mass/charge ratio (m/z) of 75. GC optimization trials were performed until a chromatographic resolution sufficient to preclude any potential co-elution was obtained.

2. Experimental

2.1. Chemicals

NMDA and the deuterated analogue $[^2H_6]$NDMA were obtained from Aldrich (Milwaukee, WI, USA) and Cambridge Isotope Laboratories (Woburn, MA, USA), respectively. Methanol and dichloromethane (both of special-reagent grade) and isoamyl alcohol (analytical-reagent grade) were supplied by Carlo Erba (Milan, Italy). All other chemicals were of analytical-reagent grade and were obtained from local sources.

2.2. Sample preparation

Sample preparation was accomplished according to a modified procedure based on the official method II adopted by the Association of Official Analytical Chemists [7,16].

A 200.0-g beer sample was weighed directly into a 500-ml distillation flask containing 40 g of sodium chloride, then 1 ml of 10% sulfamic acid in water was added in order to prevent the artifactual formation of NDMA. The sample was spiked with 50 μl of a standard solution containing 10 μg ml^{-1} $[^2H_6]$NDMA (internal standard) in isoamyl alcohol. The flask was then connected to a distillation apparatus and the distillate (about 75 ml) was collected in a 100-ml flask placed in an ice-bath. The distillate was extracted with three 35-ml aliquots of dichloromethane in a 250-ml separating funnel. The extract was dried by passing it through a glass-wool-plugged chromatographic column containing 30 g of anhydrous sodium sulfate. The dried extract was collected directly in a 250-ml Kuderna–Danish (K–D) evaporator flask (Supelco, Bellefonte, PA, USA) assembled with a K–D concentrator tube (Supelco). After addition of a boiling chip to the K–D flask, a three-ball Snyder column (Supelco) was connected to the K–D evaporator and the extract was evaporated to about 4 ml by heating the tube at 60°C in a water-bath. The final concentration step was carried out by disconnecting the concentrator tube from the flask and attaching the three-ball Snyder column directly to the tube, after addition of another boiling chip. The extract was finally concentrated to 1.0–1.3 ml. The final sample extract volume was accurately measured by means of a precision syringe (Hamilton, Reno, NV, USA). Aliquots of 1 μl beer extract were injected three times successively into the GC–MS system.

2.3. GC–MS analysis

Analyses were performed using an HP5890A gas chromatograph coupled to an HP5989A quadrupole mass spectrometer (Hewlett–Packard, Palo Alto, CA, USA) equipped with a high-energy detector (HED) and operated in the

positive-ion CI mode. Samples were injected via an on-column injector onto a CPWax 52CB (Chrompack, Middleburg, Netherlands) fused-silica capillary column (25 m × 0.25 mm I.D.; 0.2 μm film thickness) connected to a deactivated fused-silica tube (1.5 m × 0.32 mm I.D.) used as a precolumn. Helium was used as the carrier gas at a column head pressure of 50 kPa.

The initial oven temperature was 35°C for 1 min followed by a programme rate of 70°C min^{-1} to 55°C, a 7-min isothermal step, a programme rate of 3°C min^{-1} to 70°C, then a programme rate of 20°C min^{-1} to 180°C. The source temperature was 200°C and the filament emission current and electron energy were 300 μA and 150 eV, respectively. Methane was used as the reagent gas (1.0 Torr source pressure; 1 Torr = 133.322 Pa) for chemical ionization. The spectrometer axis and ion abundance were tuned using perfluorotributylamine as the calibration gas. Quantification was performed by selected-ion monitoring (SIM) of the $[M+H]^+$ ion of NDMA (m/z = 75.0) and [$^2H_6$]NDMA (m/z = 81.0); the dwell time was 0.100 s per ion.

2.4. Calibration

Standard solutions of NDMA in methanol were prepared at concentrations of 0.2 and 2.0 μg ml^{-1}. Four aliquots of 200.0 g of NDMA-free beer were spiked with 50 μl of internal standard solution (2.5 μg kg^{-1}) and appropriate amounts of the NDMA solutions in order to achieve final concentrations of 0.10, 0.25, 0.50 and 4.00 μg kg^{-1}. A blank beer sample was prepared in a similar way by spiking 200.0 g of NDMA-free beer only with 50 μl of internal standard solution. These calibration samples were prepared and injected into the GC–MS system according to the procedure described above.

3. Results and discussion

3.1. Selectivity

A great lack of specificity in SIM of ions at m/z 75.0 and m/z 81.0 was experienced, as expected. Actually, a number of compounds present in the samples tested gave rise to signals relative to these ions, especially m/z 75.0. Nevertheless, no interfering peaks were observed at the retention time of NDMA in preliminary trials and more than 40 analyses of real samples. Further, the peak shapes of NDMA and its deuterated analogue were compared and checked for symmetry and exact matching (Fig. 1).

3.2. Linearity

A five-level calibration graph was obtained by plotting the peak-area ratios m/z 75.0 and 81.0 versus the known NDMA-to-[$^2H_6$]NDMA amount ratios for the calibration samples and performing least-square regression analysis [17]. The equation obtained showed an excellent linear fit ($r = 0.99999$) and a very low standard error ($s_{y/x} = 5.2642 \cdot 10^{-3}$). The slope ($b$), the intercept ($a$) and the corresponding confidence limits, calculated for three degrees of freedom at the 95% confidence level ($t = 3.18$), were $b = 0.966 \pm 0.0123$ and $a = 0.000362 \pm 0.00909$. It is noteworthy that the confidence interval for the intercept includes the theoretical value of 0.

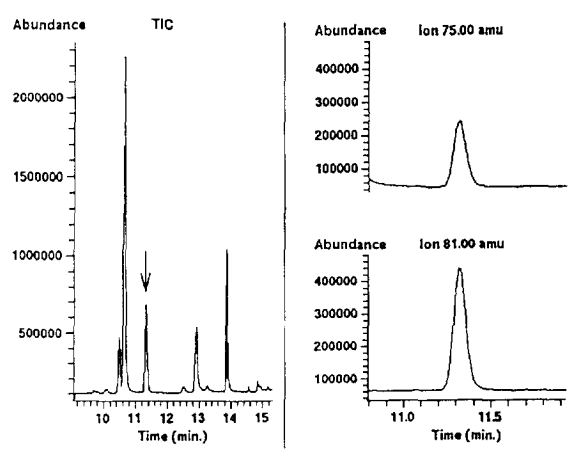

Fig. 1. Total ion chromatogram (TIC) and SIM traces relative to the protonated molecular ion of NDMA (m/z 75.0) and [$^2H_6$]NDMA (m/z 81.0) from a beer sample containing 1.36 μg kg^{-1} NDMA (peak indicated by the arrow in the TIC).

3.3. Limit of detection

The calculated limit of detection [17], defined as the lowest analyte amount yielding a signal equal to the blank signal plus three standard deviations of the blank, was about 0.04 $\mu g\ kg^{-1}$ (8 pg injected). This limit is at least comparable to those usually achievable by means of GC–TEA.

3.4. Precision and analysis of variance

Five 1-μl aliquots of a beer test sample containing 0.86 $\mu g\ kg^{-1}$ of NDMA were analysed on five different days to assess the precision of the method. Each aliquot was injected three times successively into the GC–MS system. The relative standard deviation (R.S.D.) was 0.73%. This value is much lower than that determined for similar concentration levels of NDMA reported previously using methods based on GC–TEA [5,7]. The results obtained were also submitted to a one-way analysis of variance to estimate the contribution of the sample preparation process (random-effect factor; between-sample variation) and the GC–MS measurement (residual value; within-sample variation) to the overall error of the method. The results are summarized in Table 1. A one-tailed F-test was performed to evaluate whether the between-sample mean square was significantly greater than the within-sample mean square. The calculated value of F (2.613) was lower than the critical value of F tabulated at the 95% confidence level ($F_{4,10} = 3.480$), hence there was no evidence of systematic error in the sample preparation step at the concentration level considered.

3.5. Recovery

It is known that precision and accuracy of ID-MS methods are largely insensitive to the recovery rate because all sources of variation are greatly reduced owing to the use of a stable isotopomer as the internal standard. Nevertheless, low recovery rates reduce the sensitivity and could have an indirect adverse effect on the precision of the measurement. Recoveries (R) were calculated by means of the following equation:

$$R\,(\%) = (A/A_{std}) \cdot V \cdot 100$$

Table 1
Results of five replicate analyses of a beer test sample

Aliquot No.	Measurement No. ($\mu g\ kg^{-1}$)			Mean ($\mu g\ kg^{-1}$)	R.S.D. (%) ($n = 3$)
	1	2	3		
1	0.85692	0.86043	0.86008	0.85914	0.23
2	0.85531	0.85804	0.86251	0.85862	0.42
3	0.87123	0.86863	0.85512	0.86499	1.00
4	0.87702	0.86066	0.87823	0.87197	1.13
5	0.84963	0.86088	0.85927	0.85659	0.71
Sample mean:	0.86226				
R.S.D. ($n = 5$)	0.73%				

Determination of the variance components

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-ratio
Between-sample	4	$4.7049 \cdot 10^{-4}$	$1.1762 \cdot 10^{-4}$	2.613
Within-sample	10	$4.5014 \cdot 10^{-4}$	$4.5014 \cdot 10^{-5}$	
Total	14	$9.2063 \cdot 10^{-4}$		

where $A = [^2H_6]$NDMA peak area after a 1-μl injection of test sample solution, $A_{std} = [^2H_6]$NDMA peak area after a 1-μl injection of a standard solution containing 500 ng ml^{-1} of labelled compound in dichloromethane–isoamyl alcohol (95:5) and V = final volume of beer extract collected (ml). The average recovery (mean of five determinations ± confidence limits) was 78 ± 2.1% ($p = 0.05$).

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