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Analytical Methods

Solid-phase microextraction of *N*-nitrosodimethylamine in beer

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Abstract

N-nitrosodimethylamine (NDMA) is a highly active carcinogen that has been detected in trace levels in beers. Solid-phase microextraction (SPME) was used to develop a method for the extraction of NDMA from beer using headspace sampling and gas chromatography with mass spectrometry detection. Polydimethylsiloxane/divinylbenzene (PDMS–DVB) fibres were used to evaluate the influence of equilibrium time, ionic strength, extraction time and temperature by means of a factorial design. The method was validated calculating the linearity, reproducibility, limit of detection and limit of quantification. The method was applied to the quantitative analysis of NDMA combining the standard addition method with an internal standard method.

Keywords: NDMA; Beer; SPME

1. Introduction

After the first report of toxicity and carcinogenicity of *N*-nitrosodimethyalmine (NDMA) published by Magee and Barnes (1956) there has been a great interest in the toxicology of this compound. Extensive studies have shown that more than 15 species of laboratory animals, including some primates, were susceptible to its carcinogenic action (Preussmann & Stewart, 1984).

NDMA has been detected in cured meats, fried bacon, seafood, dried milk products and beer (Tricker & Preussmann, 1991). The presence of NDMA in beer was first reported in 1979 (Goff & Fine, 1979; Spiegelhalder, Eisenbrand, & Preussmann, 1979) and has been considered a more serious matter than other foods due to initial estimates that suggested that of all foods or beverages ingested by man, beer contributed the most to the daily intake of NDMA through diet (Spiegelhalder, Eisenbrand, & Preussmann, 1980).

Examination of beer from different sources revealed that malt was the main source of NDMA contamination in beer (McWeeny, 1983). NDMA is formed during the direct-fire kilning of barley malt. Nitrogen oxides formed by combustion of ambient nitrogen come into direct contact with precursors like hordenine and gramine. These alkaloids are formed in malt roots during germination. Mechanisms for NDMA formation during nitrosation of gramine and hordenine have been suggested (Mangino & Scanlan, 1985; McWeeny, 1983).

Extraction methods for NDMA from beer typically used liquid–liquid extraction (Frommberger, 1989; Izquierdo-Pulido, Barbour, & Scanlan, 1996; Sen, Seaman, Bergeron, & Brousseau, 1996; Yin, Ding, & Liu, 1982) and solid-phase extraction (Yurchenko & Mölder, 2005). These methods are time consuming and require large amounts of toxic solvents and samples.

Solid-phase microextraction (SPME) is a simple technique, first described by Arthur and Pawliszyn (1990), which eliminates the need for solvents. Extraction and concentration occur simultaneously in a selective fibre. The fused silica fibre is coated with a thin film of polymeric stationary phase. The film concentrates the analyte on its

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surface during the absorption. The process has two steps: partition of analyte between the coating and the sample matrix, followed by desorption of the concentrated extract into the analytical instrument.

The focus of this work was to develop a method for the determination of NDMA in beer using solid-phase microextraction with headspace (HS) sampling and gas chromatography with mass spectrometry detection (GC–MS). HS sampling with polydimethylsiloxane/divinylbenzene fibres were used for the analysis according to preliminary experiments and previous works that showed their advantages over other types of sampling and fibres (Andrade, Reyes, & Rath, 2005; Grebel, Young, & Suffet, 2006).

2. Materials and methods

2.1. Reagents

All the reagents met the minimum analytical grade standard. The standard solution of NDMA was obtained from Sigma–Aldrich (Toluca, México) and was diluted to 1000 μg/L in methanol. Working solutions used in further studies were prepared by diluting different amounts of the global standard solution with distilled water to the required concentrations, and subsequently stored at 4 °C.

2.2. Gas chromatography

A gas chromatograph coupled with a mass spectrometer (Perkin–Elmer, model Clarus 500) was used to determine NDMA. An AT-WAX capillary column (30 m long, 0.25 mm I. D. and 0.5 μm film thickness) obtained from Alltech (Toluca, México) was used for the GC separation with the following oven temperature program: 100 °C (3-min hold), heating to 140 °C at 40 °C min^{-1} (1-min hold) and heating to 160 °C at 5 °C min^{-1} (3-min hold). Detection was done in the selective ion research (SIM) mode (at m/z of 74). The helium carrier gas flow-rate was 1.2 mL min^{-1}.

2.3. Sample preparation

All beer samples were purchased locally and stored at 4 °C before analysis. CO_2 was removed by transferring the sample to a large flask and shaking, gently at first and then vigorously, maintaining the beer temperature at 20–25 °C.

Optimization of the response was carried out with samples containing 5 mL of beer and 4 mL of a standard solution (40 μg/L). Sodium chloride was added to the sample vial when required and enough water was used to obtain a final volume of 10 mL.

2.4. Solid-phase microextraction

Fused silica fibres, coated with polydimethylsiloxane–divinylbenzene (PDMS–DVB, 65 μm) were used with a manual SPME holder (Supelco, Toluca, México). Before the first use, the fibre was conditioned according to the supplier's instructions.

Samples were poured into open-top vials with PTFE/silicone septum (15 mL, clear glass) and the vials were then placed in a heater at 30 °C to establish the equilibrium between the headspace and sample prior to the exposure of the fibre to the headspace. After a set extraction time, the fibre was withdrawn into the needle and then introduced into the injection port of the GC for thermal desorption at 225 °C, in the split mode, for 3 min.

2.5. Experimental design

Four parameters were evaluated in this work: equilibrium time, extraction temperature, ionic strength and extraction time. To test the influence of these factors and their interactions in the response (evaluated in terms of the ratio between the peak area of the NDMA and the peak area of the internal standard), a factorial design (2^4) was chosen. Low and high values of the variables (codified as −1 and +1) were selected according to preliminary experiments and are listed in Table 1.

2.6. Identification

Following a modification of the procedure reported by Yurchenko and Mölder (2005), the NDMA in the beer samples was identified. Retention times and mass spectra acquired from a beer sample and from a 20 mg/L standard solution (extracted and analyzed under the same conditions) were compared.

2.7. Quantitative analysis

When a mass spectrometer is used for quantitation very good results can be obtained by the combination of the internal standard and standard addition methods. Many possible sources of error (e.g., variable temperature, inconsistent desorption time, variable volume, etc.) are naturally accounted for, as both the standard and the analyte are extracted and analyzed under the same conditions (Pawliszyn, 1997).

Four vials containing 5 mL of beer and 1–4 mL of a standard solution (80 μg/L) were used for the analysis. NaCl (2 g) and enough water to obtain a final volume of

Table 1
Factor levels in the designs for NDMA SPME optimization

Variable	Levels	
	+1	−1
Extraction time (min)	45	15
Salt content (% m/v)	20	0
Extraction temperature (°C)	55	35
Equilibrium time (h)	12	24

10 mL were added to the vial. Analysis was carried out under optimum conditions.

Data are referenced to an unknown neighboring peak that was used as the internal standard. This procedure eliminates the dependency on sample size and provides better quantitation than the area normalization method (Matisova, Krupcik, Cellar, & Garaj, 1984).

3. Results and discussion

3.1. Identification

As can be seen in Fig. 1, several compounds present in the beer gave rise to signals relative to the ion at *m/z* 74. Nevertheless, no interfering peaks were observed at the retention time of either the NDMA (4.9 min) or the internal standard (5.4 min). Comparison of the mass spectra showed a good agreement between the relative abundance of the ions in the NDMA standard spectrum and those obtained in the sample spectrum.

3.2. Optimization of the response

From the statistical analysis of the experimental design, it was possible to determine that equilibrium time was the variable that affected the most the response, followed by extraction time and temperature. The addition of salt had a positive effect on the response; therefore, further experiments were carried out with the highest value of this variable.

Fig. 1a and b shows that the fibre selectivity toward the NDMA is strongly affected by the equilibrium time. In fact, there was no signal from the NDMA for equilibrium times shorter than 12 h. Beer contains many volatile compounds that are initially in the gas phase; therefore, their signal is higher at short equilibrium times. The higher the equilibrium time, the better the selectivity toward the NDMA.

To observe the dependence of the amount of NDMA extracted as a function of time, an equilibrium–time profile curve was constructed. Fig. 2 shows that at least 200 h is required to reach the equilibrium point. Compounds with low diffusion coefficients and low volatility have long equilibration times (Pawliszyn, 1997).

Because SPME has maximum sensitivity when the equilibrium point is reached, the working equilibrium time was fixed in 200 h. Temperature and extraction time were then optimized. A central composite design was constructed with three central points. Four additional observations were obtained in triplicate at (time = 0, temperature = $\pm$ 1.414) and (time = $\pm$ 1.414, temperature = 0).

Fig. 3 shows the estimated response surface for NDMA. This graph enables to find the combination of factor levels that maximize the amount of NDMA extracted using the HS-SPME technique on the experimental region.

The optimum conditions for the extraction were: temperature = 42 °C, extraction time = 40 min, salt content = 20%, equilibrium time = 200 h.

3.3. Method evaluation

Optimum conditions were used to evaluate the performance of the SPME method calculating the limit of detection (LOD), limit of quantification (LOQ), reproducibility, and linearity.

A linear regression analysis of the standard addition method with five concentration levels was performed, in triplicate. The linearity was satisfactory where the correlation coefficient equaled 0.996.

The LOD and LOQ were calculated using low concentration spikes and calculating the relative standard deviation (RSD) of the determination. LOD is then defined as three times the RSD obtained for the analyte concentration and the LOQ is defined as 10 times the RSD. The mean value for LOD was 0.8830 and 2.9435 μg/L for the LOQ.

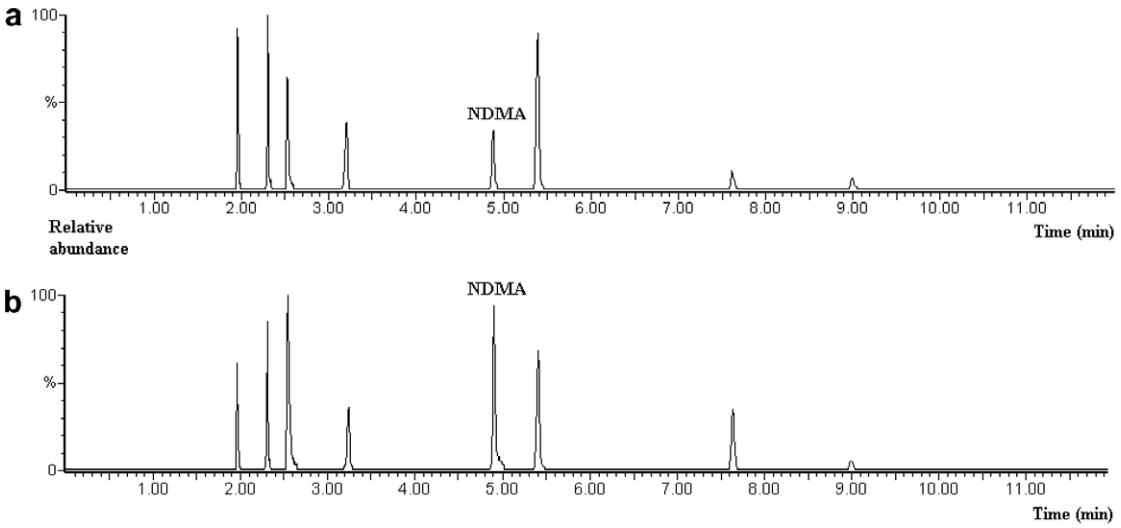

Fig. 1. Chromatogram of a beer sample at *m/z* 74. (a) Equilibrium time = 24 h. (b) Equilibrium time = 100 h.

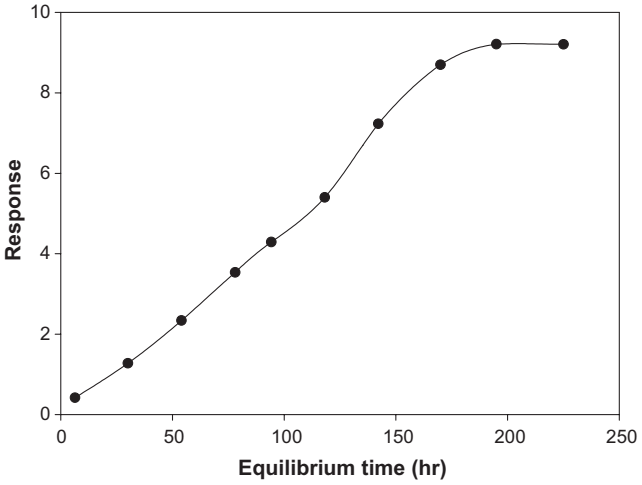

Fig. 2. Effect of equilibrium time on the extraction of NDMA from beer at 30 °C.

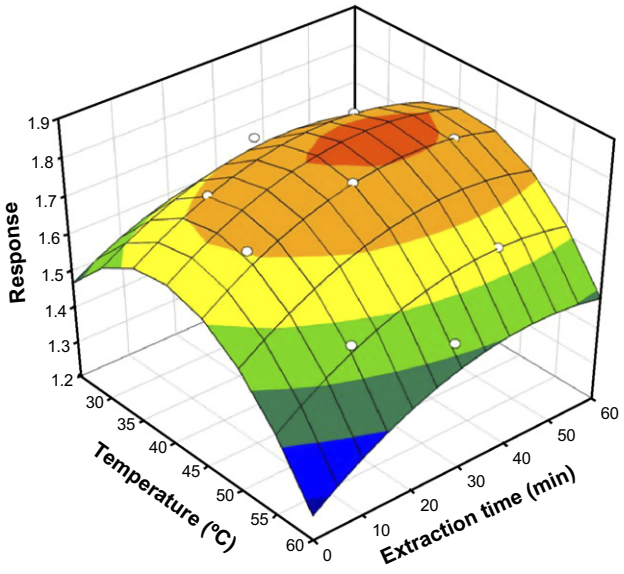

Fig. 3. Estimated response surface for the solid-phase microextraction of NDMA from beer.

Reproducibility of the optimized method was expressed as the relative standard deviation determined by five analyses of the same beer sample. The mean value was 4.6%.

3.4. Application of the HS-SPME-GC–MS method to beer samples

After setting the optimized conditions, determination of NDMA in the beer samples was carried out, in triplicate, by adding known amounts of the standard. A four-level graph was obtained and the concentration of the NDMA in the sample was calculated by extrapolation, performing least-square regression analysis. NDMA concentration in beer samples ranged from <LOD to 7.4 μg/L, with a mean value of 4.2 μg/L. A typical curve is shown in Fig. 4.

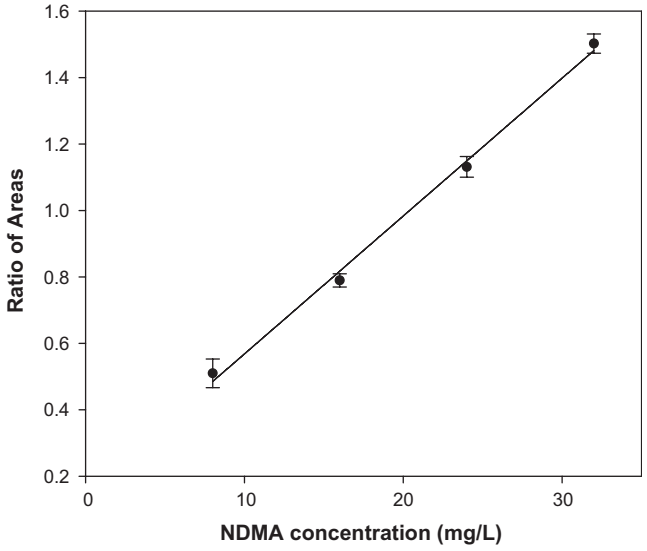

Fig. 4. Standard addition plot for NDMA in beer. The points plotted are the means ± standard deviation.

4. Conclusions

The feasibility of HS-SPME with GC–MS detection for the analysis of NDMA in beer has been demonstrated. Optimum conditions for quantitation were set according to factorial designs that proved to be a good approach to testing the influence of the variables in the response.

Equilibrium time was the most significant variable. The long period of time required to reach the equilibrium point suggests that the overall extraction rate of the NDMA is controlled by the diffusion in the boundary layer of liquid and gas phases.

The developed method was successfully applied to determine the levels of NDMA in some local beers giving excellent LOD and LOQ. The content of NDMA in one of the analyzed samples was higher than the limit of 5 μg/L established by the FDA. Some methods for the reduction of NDMA in beer have been suggested (McWeeny, 1983). Lowering the pH of the barely before kilning, the removal of the rootlets (in which amines are known to be concentrated) and the use of indirect-fired kilning are just some examples.

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