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# *N*-nitrosodimethylamine analysis in Estonian beer using positive-ion chemical ionization with gas chromatography mass spectrometry

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**Abstract**

*N*-nitrosodimethylamine (NDMA) is a potent animal carcinogen that has been detected in trace levels in beers. A total of 264 beer samples were analyzed for their NDMA content. For cleaning of the sample the two-step solid-phase extraction with Extrelut and Florisil sorbents were used. NDMA was separated by gas chromatography and detected by positive-ion chemical ionization using ammonia as reagent gas. The HP 6890 Plus GC/HP 5973 MSD with positive-ion chemical ionization option was used in the selected ion-monitoring mode. The limit of detection for NDMA using this method was 0.15 ppb with about 70–80% recovery. Of 158 Estonian beers analyzed during 2003–2004, the average NDMA level was found to be 0.20 ppb. Of 106 imported beer samples the average NDMA level was found to be 0.21 ppb.


*Keywords:* *N*-nitrosodimethylamine; Positive-ion chemical ionization; Gas chromatography; Mass spectrometry; Beer

## 1. Introduction

It is well known that a beer may contain trace amounts of *N*-nitrosodimethylamine (NDMA), a highly active carcinogen. NDMA can be formed during food processing, preservation, and preparation from precursor compounds already present in, or added to, the specific food items (Tricker & Preussmann, 1991a, 1991b). Most malt beverages, including beer and most brands of whiskey, regardless of origin, contain NDMA. The presence of NDMA in beer was first reported in 1977 (Sen, Seaman, & McPherson, 1980).

Reaction of oxides of nitrogen with certain alkaloids during the direct-fire drying step of the malting process has been established as the major pathway for the formation of *N*-nitrosamines in beer (Billedau, Miller, & Thompson, 1988). Previous investigations have reported NDMA levels in beer derived from the barley malt. Alkaloids (hordenine and gramine) formed in malt roots during germination may act as amine precursors in nitrosation (Fig. 1).

The NDMA concentration in malt depends on the type of the drying technique. High combustion temperatures (usually from 1500 to 1800 °C) yield high NDMA concentration. When green malt was dried with direct-fired kilns, NDMA concentrations of 15–80 ppb in pale malt and 80–320 ppb in dark malt were detected. Green malt is usually dried with hot air produced by burning coal in a stove and the temperature of the air is usually lower than 100 °C so the concentrations of nitrogen oxides in the air may be lower. In addition, the malt germination time was very short, the germ and root of the malt were usually very small and sometimes were not visible to the naked eye. This reduce the quantities of alkaloids in malt. Lower concentrations of nitrogen oxides

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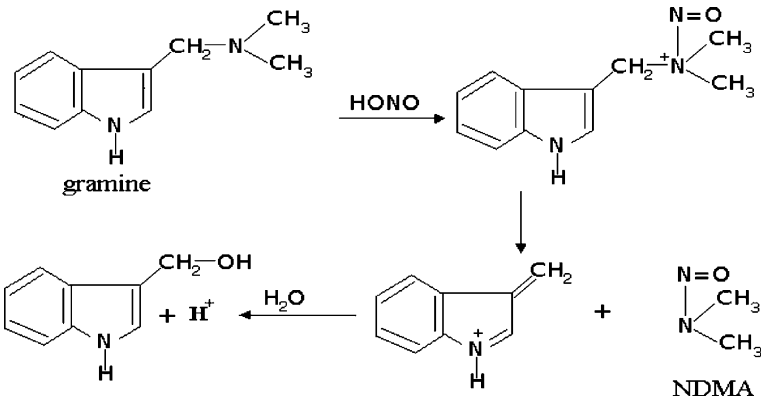


Fig. 1. NDMA formation in beer from gramine (Rubenchik, 1990).

in the air and alkaloids in the malt may thus prevail, reducing the formation of NDMA in malt (Song & Fu, 1988).

The limiting concentrations for two *N*-nitrosamines have been established in Estonia: the maximum allowed content of gross total NDMA and *N*-nitrosodiethylamine (NDEA) in beer is 3 μg/kg (The decision of the government of republic, 2000). The purpose of the present study was to measure the content of NDMA in Estonian beers and compare with those of imported beers using the method of sample preparation performed in our previous work and applying mass spectrometry for determination of NDMA in beer.

## 2. Method

### 2.1. Chemicals

For the sample preparation were purchased methanol from J.T. Baker (Holland), dichloromethane from Sigma–Aldrich (USA), hexane from Rathburn (Scotland), 0.1 N NaOH solution from Chemapo (Czechoslovakia), Extrelut from Merck (Germany) and Florisil 100/200 from Alltech (Belgium). As internal standard *N*-nitrosodi-*N*-propylamine (NDPA) solution 100 μg/mL in methanol from Ultra Scientific was used. NDMA in methanol was commercial product from Aldrich. Mixtures were stored at −20 °C and analyzed at room temperature. Helium (99.9996%) was used for gas chromatographic (GC) analysis.

### 2.2. Sample preparation

All beer samples were purchased from the local market and stored at 4 °C before the analysis. Two solid-phase extraction steps with Extrelut (Kieselguhr) and Florisil (85% $SiO_2$, 15.5% MgO, 0.5% $Na_2SO_4$) sorbents were used for the sample cleaning. The beer sample (25.0 ± 1.0 ml in 100 mL glass beaker) was mixed with 0.1 N NaOH (6 mL).

As the first step, about 6 g of Extrelut was placed at the bottom of the glass column (30 cm × 1.5 cm) and wetted with 20 mL hexane/dichloromethane 40:60 (v:v). After that the sample was eluted with two 20 mL portions of hexane/dichloromethane solution. The eluate was collected in a 50 mL concentrator flask and evaporated in water bath at 60 °C. As the second step, about 1 g Florisil was placed at the bottom of the Florisil cartridge (6.5 cm × 1.3 cm) and wetted with 6 mL dichloromethane/methanol 95:5 (v:v) and eluted with 6 mL dichloromethane/methanol solution. The solution was evaporated at 60 °C to 1 mL. Thereupon 200 μL of internal standard (NDPA, 1 μg/mL) was added. The prepared solution was transferred to the GC injector vial. Extractions were performed in duplicate (Jurtchenko, Tenno, Mölder, & Reinik, 2002).

### 2.3. Gas chromatography with mass selective detector (MSD)

GC analysis was carried out using Hewlett-Packard Model 6890 gas chromatograph equipped with a split/splitless injector. 2 μL of the sample solution was injected into the gas chromatograph using pulsed splitless injection in selected ion monitoring mode. Detection was done by a Hewlett–Packard MSD 5973 MSD mass spectrometer using a positive-ion chemical ionization. Positive-ion chemical ionization mass spectrometry was performed with ammonia as reagent gas.

Sample portions were injected into a chromatograph column (30 m DB-1701 MS; 0.25 mm i.d., 0.25 μm film thickness) containing 14% cyanopropylphenyl and 86% methyl polysiloxane. For the gas chromatography separation of the NDMA oven programme started at 45 °C (held 3 min), set at 50 °C/min from 45 to 180 °C, set at 10 °C/min from 180 to 220 °C and held isothermally at 220 °C for 0.5 min; the velocity of He carrier gas (99.9996%) was 1 ml/min.

### 2.4. Calculation method

Recovery is proportion of the amount of analyte present in the sample or added to the analytical portion of the test material that is extracted and presented for measurement. In this work recovery of NDMA was investigated by a standard addition experiment. Beer was fortified with appropriate volumes of standard solutions in methanol to get recoveries at the levels 0.1–1 ppb. For cleaning of the sample the two-step solid-phase extraction with Extrelut and Florisil sorbents were used. The sample was analyzed by GC–MS and recovery of NDMA was calculated by Eq. (1) (Eurachem Guide, 1998):

$$\text{Rec, } \% = [(C_1 - C_2)/C_3] \times 100, \quad (1)$$

where $C_1$ is the concentration determined in the fortified sample, $C_2$ is the concentration determined in the unfortified sample and $C_3$ is the concentration of fortification.

The recovery measurements of NDMA in beer samples are given in Table 1. Recovery with standard deviation is reported as mean of six independent measurements. Fig. 2 presents a chromatogram of a beer sample spiked with a standard solution containing NDMA and NDPA (internal standard) as an example.

Table 1
Recovery (%) of NDMA with standard deviation in different concentrations of a spiked beer sample (two-step solid-phase extraction method used in present work)

| Concentration of NDMA added (ppb) | Recovery (%) |
|---|---|
| 0.1 | 62 ± 1.5 |
| 0.25 | 74 ± 2.1 |
| 0.5 | 68 ± 1.5 |
| 0.75 | 71 ± 1.7 |
| 1 | 76 ± 1.9 |

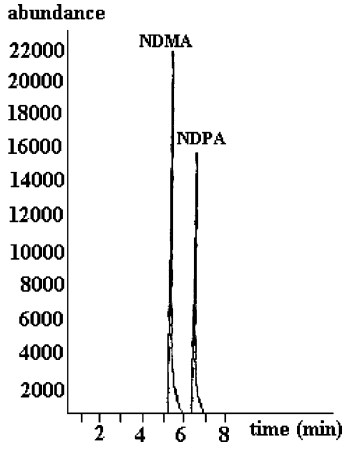


Fig. 2. GC-MS ion chromatogram of beer sample after analytical cleanup. Final extract of beer spiked with NDMA and NDPA.

The concentration of NDMA $C_{NDMA}$ (μg/mL) detected by GC was calculated using the following equation (Eisenbrand et al., 1983):

$$C_{NDMA} = C_{NDPA}(A_{NDMA}/A_{NDPA}), \quad (2)$$

where $C_{NDPA}$ is the concentration of the internal standard (NDPA) solution in the sample, $A_{NDMA}$ is designates the area of NDMA peak and $A_{NDPA}$ is the area of the internal standard peak.

The content of NDMA X (μg/l) in a sample was calculated as follows (Gough & Webb, 1980):

$$X = 10^5 \times C_{NDMA} V/V_2 \text{Rec}, \quad (3)$$

where $C_{NDMA}$ stands for NDMA concentration by GC detection, $V_2$ is the volume of the sample (mL), $V$ is the volume of the final concentrated extract (mL) and Rec – recovery of NDMA (%). The peaks were integrated by a Hewlett-Packard 6890 Gas Chromatograph.

### 2.5. Calibration

To calibrate the GC-MSD spectra of NDMA standard solutions were prepared which cover the concentration range 0.1–50 ng/ml. The concentration of NDPA solution was 10 ng/ml. The ratio of the chromatogram peak area of the NDMA ($A_{NDMA}$) to the peak area of the internal standard ($A_{NDPA}$) in beer was calculated. This calibration curve enables to calculate NDMA concentration using the GC-MS peak area measurements. The squared correlation coefficient $R^2$ was found to be 0.9998 for NDMA.

### 2.6. Limit of detection and limit of quantitation of N-nitrosodimethylamine

To demonstrate the method was under analytical control the limit of detection (LOD), the limit of quantitation (LOQ) and recovery experiments were performed. The LOD and the LOQ has been established using spiked samples. The LOD is the threshold concentration below which identification is unreliable.

The value of the LOD is given by the equation,

$$\text{LOD} = X_{bl} + KS_{bl}, \quad (4)$$

where $X_{bl}$ is the mean of the blank measures and $S_{bl}$ the standard deviation of the blank measures and $K$ is a numerical factor chosen according to the confidence level desired.

The LOQ is given by the equation

$$\text{LOQ} = K_Q \text{SD}, \quad (5)$$

where SD is the standard deviation at that point and $K_Q$ is the multiplier whose reciprocal equals the selected quantifying relative standard deviation. The IUPAC default value for $K_Q$ is 10 (The Nordic Committee of Food Analysis, 1996).

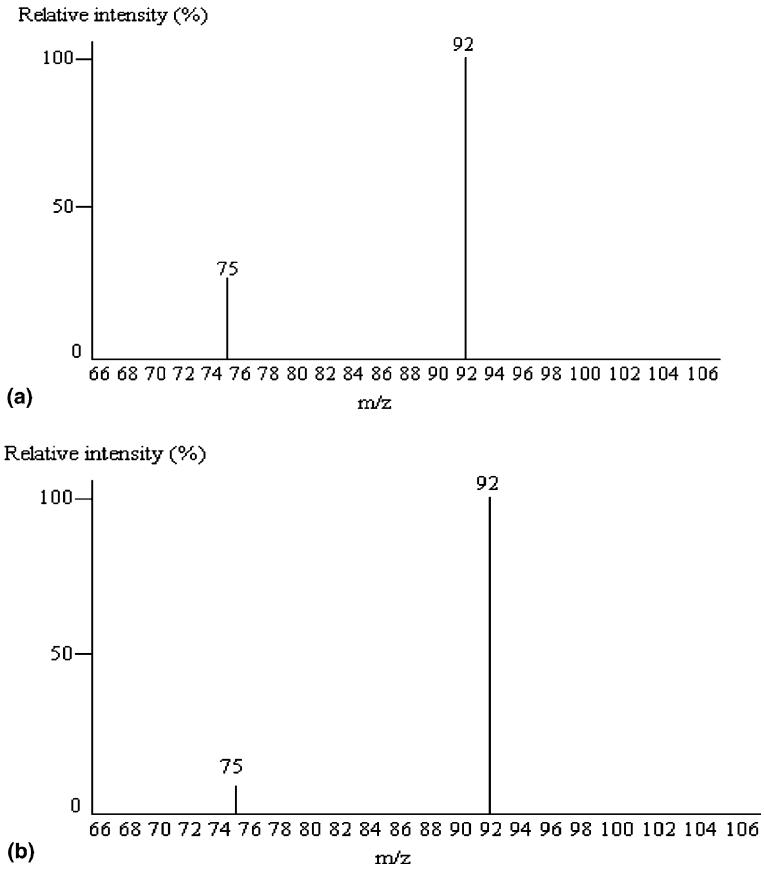


Fig. 3. Positive-ion chemical ionization mass spectra of NDMA standard (a) and NDMA from beer sample (b).

The standard deviation (SD) was calculated by Eq. (6):

$$\mathrm{SD} = \sqrt{\frac{\sum_{k=1}^{n}(\bar{x} - x_k)^2}{n-1}}, \tag{6}$$

where $n$ is the number of data points, $\bar{x}$ is the average concentration in the sample (ppb), $x_k$ is the concentration in the sample (ppb).

In this work the LOD and the LOQ of NDMA were calculated by Eq. (4) and (5), and were 0.15 and 0.3 ppb, respectively.

The reproducibility of results, which was determined by analyzing a solution at the 2.5 ppb level, was satisfactory with relative standard deviations (RSD) of 1–13%. The recovery of NDMA was 74% with standard deviation 0.22 and relative standard deviation 9.1 in a spiked beer sample. The relative standard deviation is expressed in percent and is obtained by multiplying the standard deviation by 100 and dividing this product by the average.

## 3. Discussion

Sample preparation methods used are based on vacuum distillation and steam distillation (Andrzejewski, Havery, & Fazio, 1981; Scanlan, Barbour, & Hotchkiss, 1980; Sen, Seaman, & Tessier, 1982; Spiegelhalder, Ei-

Table 2
Stability of NDMA in beer

| Sample | NDMA level (ppb) found by analysis | | | | |
|---|---|---|---|---|---|
| | Before storage | After storage | | | |
| | | 4 °C | 20 °C | 25 °C | 30 °C |
| Saku Hele | 0.43 | 0.40 | 0.37 | 0.31 | 0.24 |
| Saku Tume | 0.99 | 0.95 | 0.89 | 0.81 | 0.75 |
| Alexander | 0.77 | 0.74 | 0.78 | 0.71 | 0.63 |
| Double Bock | 0.91 | 0.88 | 0.86 | 0.81 | 0.71 |
| Saku Originaal with 5 ppb NDMA | 4.80 | 4.70 | 4.40 | 4.30 | 4.10 |

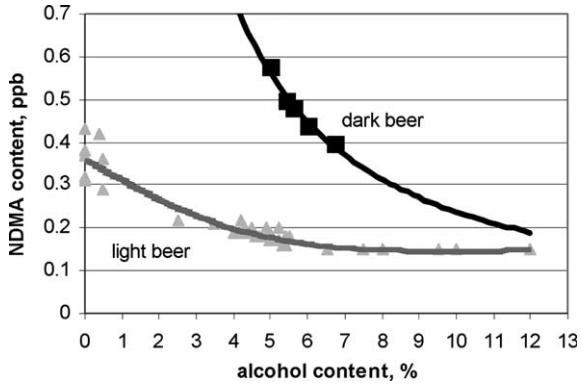


Fig. 4. The graphs showing percent of alcohol versus NDMA content in light and dark Estonian beer.

senbrand, & Preussmann, 1979), mineral oil distillation of solid samples (Tricker & Preussmann, 1991a, 1991b), direct extraction with dichloromethane, celite column extraction method (Hotchkiss, Havery, & Fazio, 1981; Sen et al., 1980; Sen, Seaman, Begeson, & Brousseau, 1996), solid-phase extraction with Extrelut sorbent (Osterdahl, 1988; Raoul, Gremaud, Biaudet, & Tureski, 1997; Tateo & Roundbehler, 1983).

Analytical methods to detect volatile *N*-nitrosamines in beer are based on GC (Fine, 1983; Frommberger, 1989; Kubacki, Havery, & Fazio, 1989; Osterdahl, 1988; Scanlan et al., 1980; Sen et al., 1996; Song & Fu, 1988; Spiegelhalder, Eisenbrand, & Preussmann, 1983; Tateo & Roundbehler, 1983; Tricker & Preussmann, 1991a, 1991b) and gas-liquid chromatography (Castegnaro, Pignatelli, & Walker, 1974; Goff & Fine, 1979; Sen et al., 1980; Sen & Seaman, 1981; Sen et al., 1982) with Thermal Energy Analyzer or gas chromatography high resolution mass spectrometry (Goff & Fine, 1979; Sen et al., 1980; Yin, Ding, & Liu, 1982).

Thermal Energy Analyzer as a selective detector for NDMA has been succesfully interfaced to gas chromatograph. In these publications the authors evaluated the LOD for GC as 0.05 μg/l (Leppänen & Ronkainen, 1982), 0.1 ppb (Kubacki et al., 1989; Scanlan et al., 1980; Song & Fu, 1988), 0.2 ppb (Frommberger, 1989) and 0.3 ppb (Andrzejewski et al., 1981). Unfortunately, these authors do not mention what kind of NDMA they studied. With a gas chromatography high resolution mass

Table 3
Measurements of NDMA level (ppb) in beers analyzed during 1991–2004

| Country of origin | 1991–1992 (Sen et al., 1996) | | | 1994 (Sen et al., 1996) | | | 2003–2004 (present work) | | |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | Mean | Range | *n* | Mean | Range | *n* | Mean | Range |
| Austria | 2 | 0.41 | 0.34–0.48 | | | | 6 | 0.33 | <0.15–0.54 |
| Australia | 5 | 0.27 | 0.13–0.52 | | | | | | |
| Belgium | 13 | 1.02 | <0.1–2.1 | 4 | 0.07 | <0.1–0.15 | | | |
| Brazil | 1 | 0.21 | | | | | | | |
| China | 2 | 0.27 | 0.1–0.45 | 1 | <0.1 | | | | |
| Czechoslovakia | 1 | 0.25 | | | | | 18 | 0.22 | <0.15–1.10 |
| Denmark | 1 | 0.1 | | 2 | 0.08 | <0.1–0.16 | 7 | 0.19 | <0.15–0.54 |
| England | 12 | 0.5 | 0.3–0.69 | 1 | <0.1 | | 6 | <0.15 | <0.15–0.60 |
| France | 4 | 0.64 | 0.32–1.29 | 2 | <0.1 | | | | |
| Germany | 16 | 0.5 | 0.21–0.82 | 2 | 0.05 | <0.1–0.11 | 20 | 0.23 | <0.15–0.65 |
| The Netherlands | 4 | 0.26 | 0.19–0.34 | 2 | 0.24 | 0.18–0.31 | 14 | 0.17 | <0.15–0.44 |
| Ireland | 3 | 0.05 | <0.1–0.15 | 1 | <0.1 | | 3 | 0.29 | <0.15–0.64 |
| Israel | 1 | 0.32 | | | | | | | |
| Italy | 2 | 1.05 | 0.35–1.75 | 1 | <0.1 | | | | |
| Jamaica | 3 | 0.43 | 0.41–0.45 | 1 | <0.1 | | | | |
| Japan | 4 | 0.33 | 0.27–0.35 | 1 | <0.1 | | | | |
| Kenya | 1 | <0.1 | | 1 | <0.1 | | | | |
| Mexico | 17 | 2.1 | <0.1–9.1 | 8 | <0.1 | | | | |
| Norway | 1 | 0.48 | | | | | | | |
| New Zealand | 2 | <0.1 | | 1 | <0.1 | | | | |
| Peru | 1 | 0.18 | | | | | | | |
| Philippines | 2 | 0.19 | 0.15–0.23 | | 0.45 | <0.1–0.9 | | | |
| Poland | | | | 2 | <0.1 | | | | |
| Portugal | 1 | 0.31 | | 1 | | | | | |
| Russia | 1 | 0.26 | | | | | 11 | 0.27 | <0.15–0.64 |
| Scotland | 2 | 0.22 | <0.1–0.45 | | | | | | |
| Trinidad | 1 | <0.1 | | | | | | | |
| Thailand | 1 | 0.3 | | 1 | <0.1 | | | | |
| United States | 1 | 0.7 | | 4 | 0.86 | <0.1–3.2 | | | |
| Yugoslavia | 1 | <0.1 | | | | | | | |
| Estonia | | | | | | | 158 | 0.20 | <0.15–1.31 |

Table 4
NDMA content in light imported beer

| Country of origin | Sample | Brewery | Alcohol content (%) | Number of samples | NDMA content (ppb) | |
|---|---|---|---|---|---|---|
| | | | | | Mean | Range |
| Czech | Staronpramen | Prazske Pivovary A.S. | 4 | 5 | 0.34 | <0.15–0.64 |
| | Starobrno Lezak Svetly | Starobrno A.S. | 5 | 3 | <0.15 | <0.15 |
| | Budweiser | Budweiser Budvar | 5 | 5 | 0.26 | <0.15–1.10 |
| | Kozel | Velke Popovice A.S. | 5 | 5 | 0.28 | <0.15–1.02 |
| Ukraine | Obolon Light | JSC Obolon | 4.5 | 3 | <0.15 | <0.15 |
| Denmark | Faxe free | Faxe Bryggeri A/S | 0 | 3 | 0.37 | 0.26–0.54 |
| | Carlsberg Beer | Carlsberg Breweries A/S | 5 | 4 | <0.15 | <0.15 |
| England | Cains | The Robert Cain & Co | 5 | 3 | 0.18 | <0.15–0.60 |
| | Foster's Lager Beer | Scottish Courage Ltd. | 5 | 3 | <0.15 | <0.15 |
| Russia | Kronverk Nevskoje | Vena Brewery | 0 | 3 | 0.37 | 0.20–0.64 |
| | Baltika 0 | JSC Baltika Brewery | 0 | 3 | 0.32 | 0.22–0.63 |
| | Baltika 8 | JSC Baltika Brewery | 5.3 | 3 | 0.2 | <0.15–0.32 |
| | Starii Melnik | Knyaz Rurik | 5.5 | 5 | 0.18 | <0.15–0.55 |
| Ireland | Guiness Draught Stout | Guiness Son & Company Ltd. | 4.2 | 3 | 0.29 | <0.15–0.64 |
| Finland | Koff | OY Sinebrychoff AB | 4.5 | 5 | 0.25 | <0.15–0.50 |
| | Lapin Kulta Premium | Hartwall PLC | 4.5 | 5 | 0.22 | 0.18–0.61 |
| Germany | Clausthaler Classic | Binding-Brauerei AG | 0 | 3 | 0.43 | 0.30–0.65 |
| | Beck's | Brauerei Beck & Co. | 0 | 3 | 0.32 | 0.25–0.44 |
| | Beck's | Brauerei Beck & Co. | 5 | 3 | 0.17 | <0.15–0.34 |
| | Holsten | Holsten Brauerei AG | 0 | 3 | 0.31 | 0.25–0.50 |
| | Holsten Premium | Holsten Brauerei AG | 5 | 5 | 0.17 | <0.15–0.21 |
| | Edelweiss Weisbier | Edelweiss | 5.5 | 3 | <0.15 | <0.15 |
| The Netherlands | Buckler | Heineken Brouwerijen B.V. | 0.5 | 3 | 0.29 | 0.22–0.44 |
| | Heineken Lager Beer | Heineken Brouwerijen B.V. | 5 | 3 | <0.15 | <0.15 |
| | Amstel Beer | Amstel Brouwerij B.V. | 5 | 5 | 0.19 | <0.15–0.44 |
| | Oranjeboon Premium | Brouwerij De Oranjeboon | 5 | 3 | 0.21 | 0.16–0.40 |
| Austria | Schlossgold | Brau Union Österreich AG | 0.4 | 3 | 0.42 | 0.25–0.54 |
| | Zipfer | Brauerei Zipf | 5.4 | 3 | 0.23 | <0.15–0.50 |
| Spain | San Miguel | San Miguel | 5.4 | 3 | 0.17 | <0.15–0.30 |

spectrometry the LOD of 0.1 ppb (Sen et al., 1980), 0.2 μg/l (Goff & Fine, 1979) and 0.5 μg/l (Yin et al., 1982) was reached.

In this work NDMA was separated by GC and detected by positive-ion chemical ionization using ammonia as reagent gas. The results obtained by an Extrelut–Florisil extraction method for the determination of NDMA in beer and ale were compared with those obtained by Extrelut extraction method (Osterdahl, 1988). In both cases the determination was done by GC using a mass selective detector. A comparison of these methods indicate that our two-step solid-phase extraction method has 10% better recovery. The advantage of this method is rapidity, effectiveness and simplicity.

NDMA was the only *N*-nitrosamine found in Estonian beer. In this work a method for separation and identification of NDMA in beer was developed. Fig. 3(a) shows the mass spectrum acquired from the injection of 50 ng NDMA standard and the spectrum of NDMA standard. Fig. 3(b) the spectrum of NDMA obtained when 2 μL of a cleaned-up beer sample was injected. No additional ions of significant relative abundance were detected in the spectrum of the extract, indicating that there was no interference from compounds having the same or similar retention time. Comparison of the two spectra showed the good agreement between the relative abundance of the ions in the sample spectrum and those obtained in the spectrum of the standard.

As usually, molecules where proton affinity is lower than the value for ammonia (i.e. PA (M) <PA ($NH_3$)) give mass spectra with $(M+NH_4)^+$ as the base peak

Table 5
NDMA content in different types of Estonian beer

| Sample | Type of beer | Alcohol content (%) | Number of samples | NDMA content (μg/l) | |
|---|---|---|---|---|---|
| | | | | Mean | Range |
| *Saku Brewery Ltd.* (Saku Õlletehas AS, Harju maakond, 75501 Saku) | | | | | |
| Saku Originaal | Light | 0.5 | 3 | 0.36 | <0.15–0.40 |
| Saku Originaal Lights | Light | 3.5 | 3 | 0.21 | 0.18–0.39 |
| Presidendi Pilsner | Light | 4.2 | 3 | 0.22 | <0.15–0.32 |
| Saku Pilsner | Light | 4.4 | 3 | 0.19 | <0.15–0.35 |
| Saku On Ice Tequila | Light | 4.5 | 3 | 0.2 | <0.15–0.30 |
| Saku Originaal Premium | Light | 4.6 | 3 | 0.18 | <0.15–0.30 |
| Saku On Ice | Light | 5 | 3 | 0.17 | <0.15–0.24 |
| Saku On Ice Laimiga | Light | 5 | 3 | 0.18 | <0.15–0.23 |
| Sack bei Reval | Light | 5 | 3 | 0.19 | <0.15–0.26 |
| Saku Hele | Light | 5.2 | 4 | 0.2 | 0.21–0.43 |
| Rock | Light | 5.3 | 3 | 0.16 | <0.15–0.20 |
| Saku Strong | Light | 6.5 | 3 | <0.15 | <0.15–0.37 |
| Saku Tume | Dark | 6.7 | 3 | 0.4 | 0.16–0.52 |
| Hard Rock | Light | 7.5 | 3 | <0.15 | <0.15–0.19 |
| Saku Porter | Porter | 7.5 | 3 | 0.57 | <0.15–1.18 |
| Sorts | Light | 8 | 3 | <0.15 | <0.15–0.17 |
| Presidendi 8 | Light | 8 | 3 | <0.15 | <0.15 |
| Presidendi 10 | Light | 10 | 3 | <0.15 | <0.15 |
| *Tartu Brewery* (AS Tartu Õlletehas, Tähtvere 56/62, 50050 Tartu) | | | | | |
| A. Le Coq Pilsner | Light | 4.2 | 3 | 0.19 | <0.15–0.54 |
| A. Le Coq Premium | Light | 4.7 | 4 | 0.18 | <0.15–0.52 |
| Albert Le Coq | Light | 4.9 | 3 | 0.2 | <0.15–0.33 |
| A. Le Coq Premium Extra | Light | 5 | 3 | 0.18 | <0.15–0.24 |
| Alexander | Light | 5.2 | 3 | 0.17 | <0.15–0.37 |
| Disel | Light | 5.2 | 3 | 0.17 | 0.16–0.20 |
| A. Le Coq Football | Light | 5.4 | 3 | 0.16 | <0.15–0.42 |
| A. Le Coq Black Beer | Dark | 5.4 | 3 | 0.5 | 0.35–0.60 |
| A. Le Coq Black Lights | Light | 5.4 | 3 | 0.18 | <0.15–0.27 |
| A. Le Coq Porter | Porter | 6.5 | 3 | 0.59 | 0.20–1.14 |
| Turbo Disel | Light | 7.5 | 3 | <0.15 | <0.15 |
| A. Le Coq JÕuluporter | Porter | 7.5 | 3 | 0.54 | 0.27–1.31 |
| Double Bock | Light | 8 | 4 | <0.15 | <0.15–0.31 |
| Saaremaa X | Light | 10 | 3 | <0.15 | <0.15–0.23 |
| *Viru Brewery* (AS Viru Õlu, Lääne-Virumaa, 45301 Harjala) | | | | | |
| Frederik alcohol free | Light | 0 | 3 | 0.38 | 0.27–0.36 |
| Shandy | Light | 2.5 | 3 | 0.22 | <0.15–0.40 |
| Frederik Pilsner | Light | 4.3 | 3 | 0.2 | <0.15–0.30 |
| Frederik Premium | Light | 4.6 | 3 | 0.18 | <0.15–0.25 |
| Talve olu | Dark | 5 | 3 | 0.58 | 0.26–0.80 |
| Jõulu Beer | Dark | 5.6 | 3 | 0.48 | 0.22–0.68 |
| Frederik Tosin | Light | 12 | 3 | <0.15 | <0.15–0.18 |
| Frederik Porter | Porter | 6.7 | 3 | 0.56 | <0.15–1.88 |
| Bear Beer | Light | 7.5 | 4 | <0.15 | <0.15–0.43 |
| Tugev | Light | 7.5 | 3 | <0.15 | <0.15 |
| *Pärnu Brewery* (Pärnu Õlu AS, Suur-Jõe 10/12, 80042 Pärnu) | | | | | |
| Kuningas | Light | 4.6 | 4 | 0.18 | <0.15–0.29 |
| Viiking | Light | 5.5 | 3 | 0.18 | <0.15–0.20 |
| Ziguli | Light | 4 | 3 | 0.19 | <0.15–0.33 |
| Ryklio Alus | Light | 9.5 | 3 | <0.15 | <0.15 |
| *Saaremaa Brewery* (AS Saaremaa Õlletehas, Pihtla tee 30, 93815 Kuressaare) | | | | | |
| Saaremaa Oma õlu | Light | 4.5 | 3 | 0.2 | <0.15–0.34 |
| Saaremaa | Light | 4.9 | 3 | 0.19 | <0.15–0.26 |
| Saaremaa Tömmu | Dark | 6 | 3 | 0.44 | 0.20–0.60 |
| *Nigula Brewery* (AS Nigula Õlu, Lääne-Viru maakond, 44001 Viru-Nigula) | | | | | |
| Gran Capitan | Light | 4.7 | 3 | 0.18 | <0.15–0.33 |
| Kõva Laks | Light | 7.5 | 3 | <0.15 | <0.15 |

and a much smaller peak for $(M+H)^+$ adduct. Ammonia as a reagent gas of relatively "soft" nature in mass spectrometry analysis shows milder fragmentation and is a convenient reagent gas for NDMA. Positive-ion chemical ionization with ammonia as reagent gas is the most selective technique to distinguish NDMA based on relative abundances of their adduct ions.

During transport, beer samples may be exposed for short durations to higher temperatures. To determine the effect of such exposure on the NDMA level in beer, the following experiment was carried out. Bottles of some different beer were analyzed. The samples were stored at 4, 20, 25 and 30 °C in the dark and usually analyzed after two weeks (Table 2). Samples at 4 °C showed no detectable changes, but a loss of NDMA was observed in samples kept at 20, 25 and 30 °C.

It is well known that NDMA formed in the process of beer production. It was found that NDMA could be formed during the kilning of malt (Mangino & Scanlan, 1985). The brewers try to change the malting process to reduce the NDMA formation (Spiegelhalder et al., 1983).

As shown in (Mangino & Scanlan, 1985) some other pathways for NDMA formation could be expected. One possible source of dimethylamine (DMA) in beer is hordenine and gramine, which is formed in the barley kernels during malting. NDMA could be formed from DMA during the brewing process, if traces of nitrosating agents were available at that stage; nitrite could be formed from nitrate during processing or nitrogen oxides might be present. Indeed, the DMA content in beer ranges between 200 and 300 μg/kg and is higher in dark than in pale beers (Hrdlicka, Dyr, & Kubickova, 1964). The possible importance of DMA in the malt as a precursor for NDMA in beer is underlined by the evidence that DMA concentrations in dark lager, dark strong lager and dark ale are relatively high and correspond with higher NDMA values (Spiegelhalder et al., 1983).

The concentration of NDMA depends on the percent of alcohol, since ethanol is an inhibitor of nitrosation. In beer NDMA inhibit to the alkyl nitrite (Rubenchik, 1990). Fig. 4 the graphs show percent of alcohol in beer versus NDMA content in light and dark Estonian beer. One can see from the graphs that in dark beer the level of NDMA is higher than in light beer with the same percent of alcohol.

In the studies of foreign beers carried out during 1991–1992 and 1994, the respective averages were 0.71 ppb ($n=106$; range, <0.1–9.1 ppb) and 0.15 ppb ($n=36$; range <0.1–3.2 ppb), see Table 3. We repeated some of these measurements during 2003–2004 using our two-step extraction method (Tables 3 and 4) and supplemented it by extensive measurements of NDMA content in Estonian beer (Table 5).

## 4. Results and conclusions

NDMA was found in 158 samples of Estonian beer at level ranging from <0.15 to 1.31 μg/l with an average of 0.20 μg/l. In 106 imported beers, NDMA ranged in the similar way from <0.15 to 1.10 μg/l with an average of 0.21 μg/l. The highest NDMA level in domestic beer were found in porter (0.57 ppb, the average of 12 samples) and in dark beer (0.48 ppb, the average of 15 samples).

NDMA concentration depends on percent of alcohol in beer. The highest concentration was observed in alcohol-free beer. Small beer (<5 vol%) contained higher level of NDMA than strong beers (>5 vol%). At alcohol percent around six, the NDMA concentration reaches the LOD.

In the dark beer the level of NDMA is higher than in light beer with the same percent of alcohol.

The results show that the temperature of storage has a significant effect on the concentration of NDMA.

A two-step solid-phase extraction method was developed. A comparison with other methods of determination of NDMA in beer indicates that our method has at least 10% better recovery. The limit of detection was calculated to be 0.15 ppb and the limit of quantitation 0.3 ppb.

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