LabWorks APPNOTE

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Determination of N-Nitrosamines in Foods Using Solventless Extraction Techniques

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Abstract

N-nitrosamines, a class of compounds commonly found in foods and beverages, have long been a health concern due to their carcinogenic risks [1]. These compounds are typically formed during food preparation and processing through the reaction of amines with nitrosating compounds. The conventional method of extracting N-nitrosamines utilizes a liquid-liquid extraction with harmful solvents like methylene chloride, a practice that is about to face a significant challenge with the impending EPA ban [2]. This study demonstrates a safer and more efficient solventless extraction approach. Dynamic headspace (DHS) was used to extract N-nitrosamines from a diverse range of sample types.

Introduction

N-nitrosamines are a class of compounds commonly found in foods and beverages. These compounds are highly carcinogenic. N-nitrosamines are formed through the reaction of amines with nitrites when foods or beverages are processed. For example, they are commonly found in cured meats due to the use of nitrite as a curing agent. Nitrate-based additives are used in cheese-making to prevent bloating during ripening and can result in N-nitrosamine formation. They can also be formed during the malt drying process and are passed into beer during brewing. In the US, acceptable nitrosamine levels in beer are five ppb [3]. However, no other US or European regulations exist on N-nitrosamines in foods and beverages.

Most methods for isolating N-nitrosamines, including liquid-liquid and solid-phase extraction techniques, are time-consuming and require large amounts of samples and organic solvents, usually dichloromethane [4]. While a few studies have utilized solid phase microextraction (SPME) as a solventless and automated approach, it has limitations [4]. SPME can produce misleading results due to the fiber phase not effectively extracting essential compounds, the analytes being too soluble in the matrix to partition out effectively, and/or competition effects. Additionally, SPME can have higher detection limits due to a small phase volume and limited capacity.

This study used dynamic headspace (DHS) as an automated, solventless means of extracting N-nitrosamines from food and beverage products. The DHS extraction technique involves purging the headspace above a sample and concentrating the volatiles onto a sorbent-filled trap. Because DHS is a non-equilibrium technique, more volatiles are driven into the headspace, resulting in improved recovery and extremely low detection limits.

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Experimental

Instrumentation

GERSTEL LabWorks Platform with DHS on Agilent 8890 GC/5977B Inert Plus MSD shown in Figure 1.

Figure 1: GERSTEL LabWorks Platform with DHS option

Analysis Conditions LabWorks Platform DHS

Trap	Tenax TA®
DHS	25 °C trap temperature 50 °C incubation temperature 750 mL volume 50 mL/min flow
TDU 2	Solvent vent/dry purge (1 min) 40 °C (1 min); 720 °C/min; 280 °C (3 min)
CIS 4	Glass bead-filled liner 10:1 split -120 °C; 12 °C/s; 275 °C (3 min)

Analysis Conditions Agilent 8890 GC

Column	30 m Rxi-5MS (Restek) d_i = 0.25 mm, d_f = 0.25 µm
Pneumatics	He, P_i = 6.78 psi Constant Flow 1 mL/min
Oven	35 °C; 10 °C/min; 280 °C (2 min)

Analysis Conditions Agilent 5977B MSD

Mode	SIM/Scan
Scan	40-300 m/z
SIM	see Table 1
Source Temp	230 °C
Quad Temp	150 °C

Table 1: SIM parameters for N-nitrosamine determination.

Analyte	Retention Time [min]	Segment	Quant. Ion [m/z]	Qual. Ion [m/z]
NDMA	3.467	1	74	42
NEMA	4.461	2	88	42
NDEA	5.451	3	102	42
NPYR	8.068	4	100	41
NDPA	8.127	4	130	70
NMOR	8.135	4	116	56
NPIP	8.755	5	114	42
NDBA	10.967	6	116	84
NDPhA	15.665	7	169	168

Standard/Sample Description

A 2000 µg/mL method 8270 B Nitrosamines mix in dichloromethane was purchased from AccuStandard (Part # M-8270-03-AL). The mix contains N-nitrosodimethylamine (NDMA), N-nitrosoethylmethylamine (NEMA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), N-nitrosodipropylamine (NDPA), N-nitrosomorpholine (NMOR), N-nitrosopiperidine (NPIP), N-nitrosodibutylamine (NDBA), and N-nitrosodiphenylamine (NDPhA). 1000 µg/mL standards of N-nitrosodimethylamine-d_6 (NDMA-d_6) and N-nitrosodi-n-propylamine-d_{14} (NDPA-d_{14}) in dichloromethane were purchased from Restek (Part # 33910 and 33911). Food samples were purchased at a local store.

Standard Preparation

A 1 µL aliquot of calibration and internal standard were spiked into a 10 mL screw-capped vial for a working calibration range of 0.5-50 ng. NDMA-d_6 and NDPA-d_{14} served as internal standards spiked at 25 ng. The standards were incubated at 50 °C for 2 minutes and then extracted for 15 minutes at 50 mL/min for a total trap volume of 750 mL. The analytes were trapped at 25 °C on a Tenax® TA-packed tube.

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Sample Preparation

The bacon was cooked in a frying pan before analysis, and all other samples were used as is. Approximately 1.0 g of sample was placed in a 10 mL screw-capped vial. Each sample was spiked with 25 ng of NDMA-d_6 and NDPA-d_{14}. The samples were incubated at 50 °C for 2 minutes and then extracted for 15 minutes at 50 mL/min for a total trap volume of 750 mL. The analytes were trapped at 25 °C on a Tenax® TA-packed tube.

Standard/Sample Introduction

The tubes were desorbed in solvent vent mode with 50 mL/min helium flow at 280 °C for 3 minutes. Analytes were trapped in the CIS 4 inlet at -120 °C on a glass bead-filled liner. When desorption was complete, analytes were transferred to the column in split (10:1) mode by heating the inlet rapidly to 280 °C for 3 minutes.

Results and Discussion

Figure 2 shows a representative chromatogram of the 10 ng calibration standard.

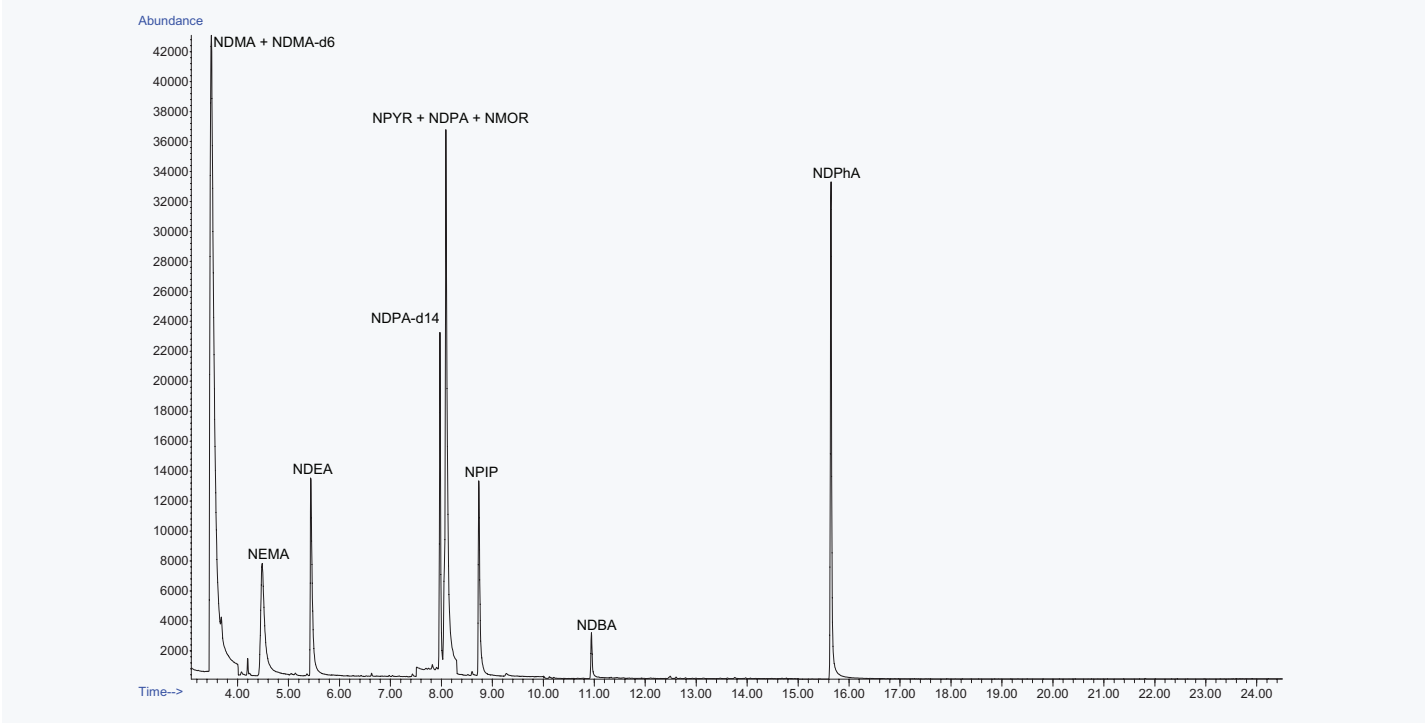

Figure 2: Representative chromatogram of the 10 ng N-nitrosamine calibration standard.

A five-point linear calibration curve was established for each compound. Linearity was excellent across the calibration range of 0.5-50 ng on tube. Correlation coefficients (R^2) were greater than 0.99 for every analyte, as shown in Table 2. The detection limit (DL) for all analytes was below 0.2 ng/tube, also shown in Table 2.

Table 2: Linearity and detection limit for target N-nitrosamines.

Analyte	R^2	DL (ng/tube)
NDMA	0.998	0.091
NEMA	0.998	0.099
NDEA	0.999	0.104
NPYR	0.999	0.067
NDPA	0.998	0.052
NMOR	0.999	0.065
NPIP	0.999	0.064
NDBA	0.993	0.096
NDPhA	0.999	0.191

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Table 3 shows the average concentration (ng/g) of N-nitrosamines determined in the food samples. NDMA, NEMA, NDEA, NPYR, NPIP, and NDBA were detected in one or more samples, many of which have been previously detected in these food types [5]. NDMA and NDBA were detected at the highest concentrations in the samples at approximately 3 ng/g. The beef jerky and cheese 2 samples contained the most N-nitrosamines, with four in each. Reproducibility was also assessed with triplicate measurements of each food type. The relative standard deviation was less than 20% for all analytes measured, as shown in Table 4.

Table 3: Concentrations (ng/g) of N-nitrosamines in food samples.

Analyte	Bacon	Beef Jerky	Cheese 1	Cheese 2
NDMA	3.57	-	-	3.47
NEMA	0.10	0.81	-	0.02
NDEA	-	0.22	0.85	0.25
NPYR	-	0.52	-	-
NPIP	-	-	0.59	0.39
NDBA	3.07	2.81	-	-

Table 4: Reproducibility (RSD) of N-nitrosamines in food samples.

Analyte	Bacon	Beef Jerky	Cheese 1	Cheese 2
NDMA	18.01	-	-	16.99
NEMA	7.49	11.44	-	1.86
NDEA	-	5.74	4.91	4.06
NPYR	-	2.48	-	-
NPIP	-	-	12.56	7.15
NDBA	1.03	5.35	-	-

Conclusions

This study successfully demonstrates the application of DHS as an efficient and solventless methodology for extracting N-nitrosamines from various food products. This offers a significant advantage over traditional liquid-liquid extraction techniques, which require harmful solvents like methylene chloride. DHS provides an automated, environmentally friendly means to extract various sample types with low concentrations of these harmful compounds.

References

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