

# Application News

## No. AD-0057

### GC-MS/MS

## High Sensitivity Detection and Quantification of Trace Levels of *N*-Nitrosamines in Beers by GC-MS/MS Method

Most of *N*-nitrosamines are known to be carcinogenic and mutagenic. Consumption of nitrosamines, e.g., *N*-nitrosodimethylamine (NDMA), was reported to be a cause of gastric cancer, liver cancer, glioma and blood disorder. *N*-nitrosamines could be formed by reaction between amine and nitrite under heating conditions in food processing. For example, they were found in nitrite-treated meat and malt-derived beverages like beer at trace level. The presence of NDMA in malt and beer was first reported in 1974 [1]. Its concentration in malt depends on the drying techniques used. According to the US and EU regulation, the amount of nitrosamines in beer must be controlled to the acceptable levels, typically at 0.2 ~ 5.0 ppb depending on the country [2]. The main *N*-nitrosamine that is monitored in malt and beer is NDMA. Here, we report a new GC-MS/MS method using Multiple Reaction Monitoring (MRM) mode for simultaneous detection and quantification of six *N*-nitrosamines including NDMA for enhanced selectivity and sensitivity from the potential matrix interferences in beer samples.

### Experimental

#### Preparation of Calibrants and Samples

Six *N*-nitrosamines, namely *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR) and *N*-nitrosopiperidine (NPiP) were selected in this study. *N*-nitrosodipropylamine (NDPA) was used as internal standard (IS). A nitrosamines mixed standard stock solution (10 ppm) was prepared using dichloromethane as solvent. Subsequently, a series of calibrants each spiked with 50.0 ppb IS (NDPA) ranging from 0.1 to 50.0 ppb was prepared. The beer samples were prepared based on the modified AOAC Official Method 982.11, 2000 [3].

#### Instrument and Analytical Conditions

GC-MS: GCMS-TQ8030  
 Auto injector: AOC-20i+s  
 Column: Stabilwax DB (Restek Corp.)  
 30 m L. x 0.25 mm I.D. x 0.25µm δf  
 Workstation: GCMS solution Version 4.01

Table 1: GC-MS/MS analytical conditions

GC	
Injection Temp.	200°C
Column Temp.	50°C (2 min), 20°C/min ~210°C (15 min)
Injection Mode	Pulse splitless (300 kPa for 1 min)
Carrier Gas	He
Linear Velocity	40.0 cm/sec
Purge Flow	3 mL/min
Injection Volume	4 mL

#### MS/MS

Ion Source Temp.	200°C
Interface Temp.	210°C
CID gas	Ar
Solvent cut Time	4 min
Event time	0.30 sec
Acquisition Mode	MRM

### Results and Discussion

#### Method Development

Figure 1 shows the Total Ion Chromatogram (TIC) of the six *N*-nitrosamines and internal standard (IS) in full scan mode. Based on the spectra of eluted peaks, precursor ions were selected for MS/MS product ion scan analysis as well as MRM optimization. As an example, figure 2 shows the full scan spectrum of NDEA and its product ion scans ( $m/z$  102) with two different collision energies (4 and 14 V).

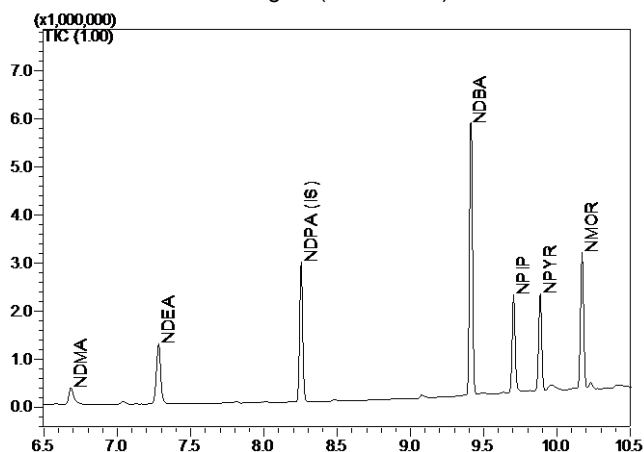


Figure 1: TIC of scan data for *N*-nitrosamines and internal standard NDPA in GC-MS mode

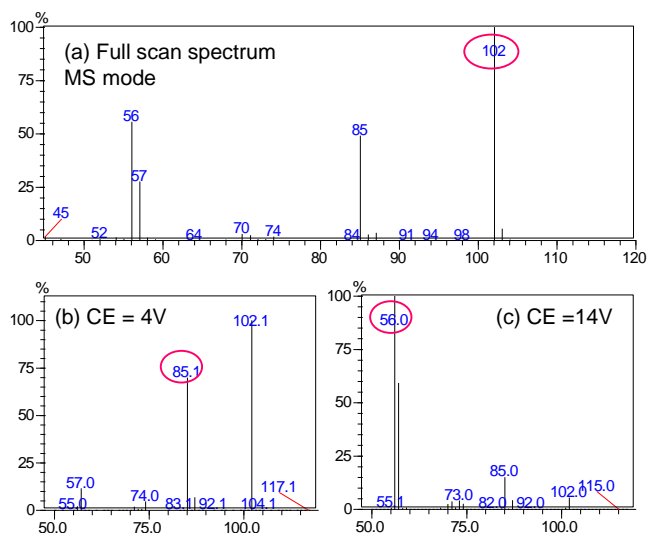


Figure 2: NDE full scan MS spectrum (a) and product ion scan of precursor ion m/z102 with different collision energy (b, c).

MRM optimization of the six *N*-nitrosamines studied were carried out systemically to obtain the optimized collision energy (CE) for two transitions. The MRM transitions and CE are compiled into Table 2. The MRM transition with higher intensity was used as the quantitative ion and the other one as qualitative ion for confirmation.

### Method Performance Evaluation

A MRM quantification method was set up based on the MRM transitions in Table 2. Linear calibration curves with internal standard (IS) were established for the six *N*-nitrosamines as shown in Figure 3. The linearity with correlation coefficient ( $R^2$ ) greater than 0.999 across the calibration range of 0.1 ppb – 50.0 ppb was obtained.

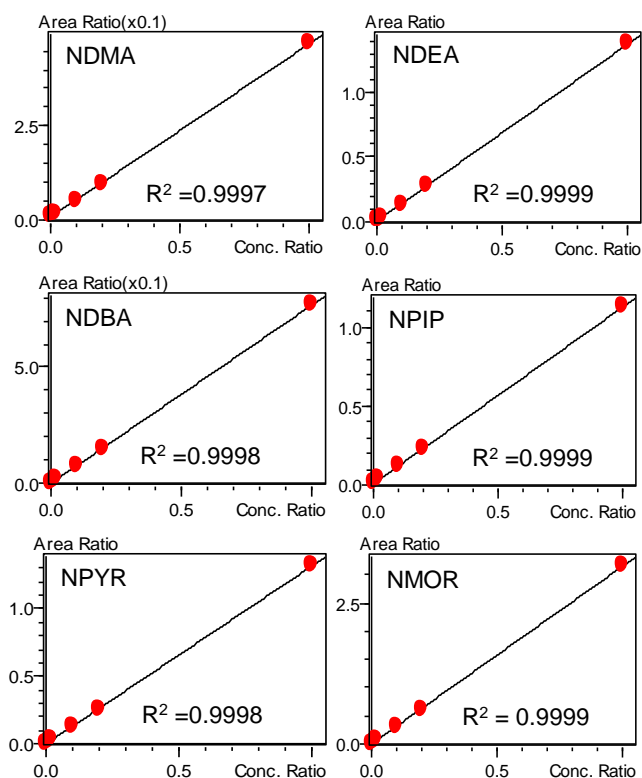


Figure 3: MRM calibration curves of six *N*-nitrosamines from 0.1, 0.5, 1.0, 5.0, 10.0 and 50.0 ppb with IS.

Table 2: MRM parameters of six *N*-nitrosamines and IS on GCMS-TQ8030 triple quadrupole

ID	Name	RT (min)	Quantitative Ion		Qualitative Ion	
			Transition	CE	Transition	CE
1	NDMA	6.685	74.1>42.0	11	74.1>30.0	11
2	NDE	7.282	102.1>85.1	4	102.1>56.0	14
3	NDPA (IS)	8.257	130.2>113.1	4	130.2>88.1	4
4	NDBA	9.412	158.2>99.1	8		
5	NPIP	9.708	114.1>97.1	8	114.1>84.1	8
6	NPYR	9.886	100.1>70.0	7	100.1>55.0	8
7	NMOR	10.172	116.1>86.0	6	116.1>56.0	11

Figure 4 shows the MRM peaks of the *N*-nitrosamines at 0.1 ppb level. The repeatability, LOD and LOQ were evaluated at the concentration level 0.5 ppb and tabulated in Table 3. The peak area %RSD for all the targeted analytes were below 5%, except for NPYR. The overlay mass chromatograms in Figure 5 demonstrated the peak area consistency of NMOR graphically for five consecutive injections.

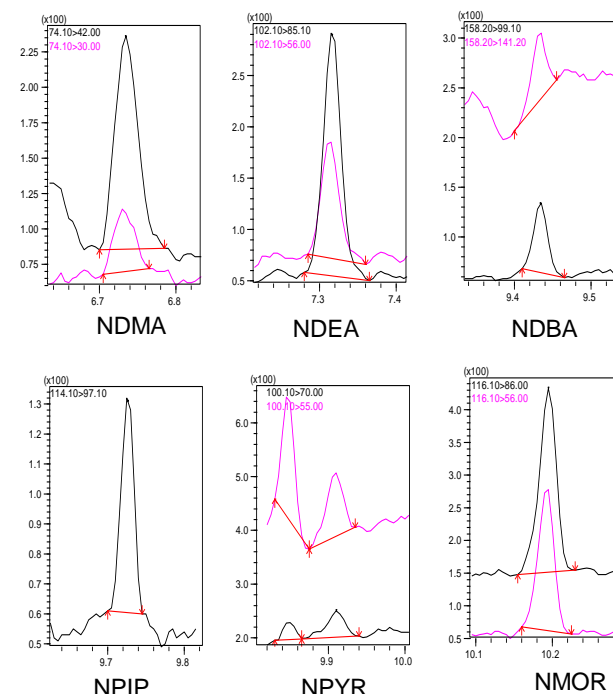


Figure 4: MRM peaks of six *N*-nitrosamines of 0.1 ppb on GCMS-TQ8030

Table 3: Performance evaluation of MRM quantification method of *N*-nitrosamines with 0.5 ppb level (n=5)

ID	Compound Name	%RSD	S/N	LOD* (ppb)	LOQ** (ppb)
1	NDMA	4.63	3.12	0.48	1.6
2	NDEA	2.76	33.77	0.04	0.15
4	NDBA	2.78	19.17	0.08	0.26
5	NPIP	4.77	16.58	0.09	0.3
6	NPYR	6.33	3.22	0.47	1.55
7	NMOR	1.5	62.44	0.02	0.08

\* S/N = 3; \*\* S/N = 10

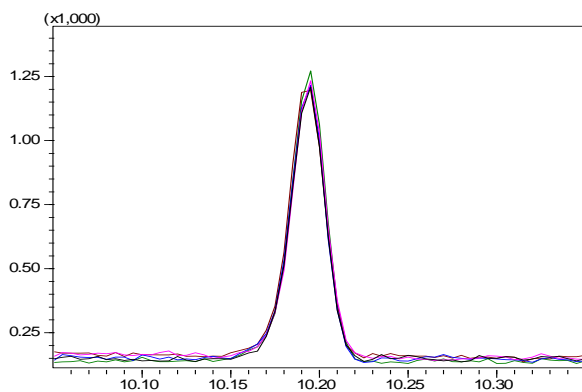


Figure 5: MRM chromatograms of NMOR at 0.5 ppb, five consecutive injections (n=5)

### N-nitrosamines in Beer Sample

The GC-MS/MS method was established for the application of beer samples. The investigated beer samples were pre-concentrated 25 times based on preparation method described earlier. Figure 6 shows the TICs of MRM data for the spiked beer extract. It was found that some trace amount of NDBA, NPYR and NMOR were present in the sample blank (unspiked beer extract) as summarized in Table 3. However, there is no safety concern as it is far below the regulation limit. The recovery of the N-nitrosamines was calculated with the post spiked beer sample extract to be within  $\pm 12\%$ .

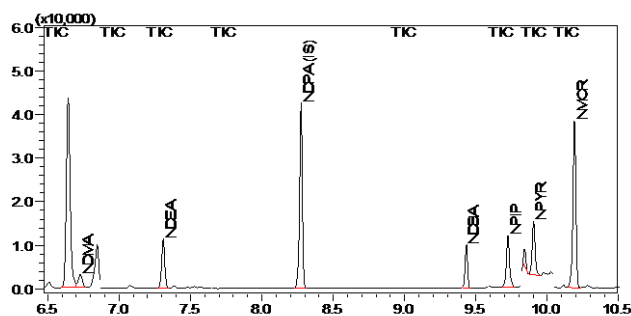


Figure 6: TICs of MRM data for spiked beer extract with added 50.0 ppb IS

Table 4: Analysis result and recovery of N-nitrosamines in beer sample

Compound	<sup>1</sup> Spiked beer extract (ppb)	<sup>2</sup> Recovery (%)	<sup>3</sup> Concentration in beer (ppb)
NDMA	9.6	96	Not Detected
NDEA	8.1	81	Not Detected
NDBA	9.6	93	0.012
NPIP	10.4	104	Not Detected
NPYR	10.6	102	0.016
NMOR	11.3	111	0.008

Notes:

<sup>1</sup> Mixed standard 10.0 ppb was spiked in beer extract

<sup>2</sup> Recovery = (Spiked beer extract – Sample blank / 10) \* 100

<sup>3</sup> Concentration in beer (ppb) = Sample blank / 25

### Conclusions

A highly sensitive and selective GC-MS/MS method using Shimadzu GCMS-TQ8030 was developed for N-nitrosamines analysis. With proper sample preparation and MRM data acquisition mode, the system has been used successfully to determine trace-level of six N-nitrosamines in beer sample which are lower than the limit maximum required by regulation.

### References

- [1] Sen, N. P., Seaman, S., McPherson, M. Nitrosamines in alcoholic beverages, Journal of Food Safety, 2, 13-18 (1980)
- [2] Brewing Quality Control Manual Series <http://www.alcbevtesting.com/wp-content/uploads/2012/01/BDASQualityManualSeriesPt2MaltJune2012>
- [3] AOAC Official Method 982.11, 2000

