PO-CON1322E

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Method Development for Trace Level Detection of N-Nitrosamines in Beer by GC-MS/MS

ASMS 2013 TP738

Lai Chin Loo[1], Cynthia Melanie Lahey[1], Gee Siang Ling[1], Sheot Harn Chan[2], Fang Yan Li[2], Yai Fong Chew[2], Shao Hua Chia[3]

[1]Shimadzu (Asia Pacific) Pte Ltd, Singapore

[2]Food Safety Laboratory, Food Safety Division, Applied Science Group, Health Sciences Authority, Singapore

[3]Department of Chemistry, National University of Singapore, Singapore

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1. Introduction

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.

2. Instrument & Analytical Conditions

Instrument

GC-MS : GCMS-TQ8030
Auto injector : AOC-20i+s

GC

Injection Temp.	: 200°C
Column	: Stabilwax DB (Restek Corp.) 30 mL. * 0.25 mm I.D. , δf = 0.25 µm
Column Temp.	: 50°C (2 min) at 20°C/min – 210°C (15 min)
Injection Mode	: Pulse Splitless (300 kPa for 1 min)
Carrier Gas	: He (constant linear velocity mode)
Linear Velocity	: 40 cm/sec
Purge Flow	: 3 mL/min
Injection Volume	: 4 µL

MS

Ion Source Temp.	: 200°C
Interface Temp.	: 210°C
Acquisition Mode	: MRM
CID Gas	: Ar
Solvent Cut Time	: 4 min
Event Time	: 0.30 sec
Acquisition Mode	: MRM

3. Standards and Sample Preparation

Preparation of Calibrants and Samples

Six *N*-nitrosamines, namely *N*-nitrosodimethylamin (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].

4. Result and Discussion

Method Development

Fig. 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, Fig. 2 shows the full scan spectrum of NDEA and its product ion scans (*m/z* 102) with two different collision energies (4V and 14 V).

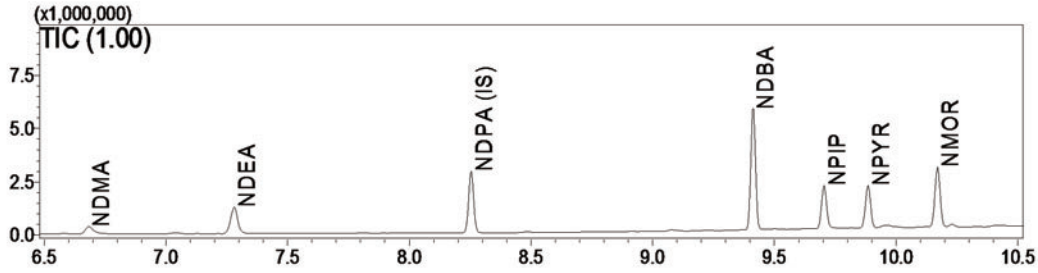

Fig. 1 TIC of scan data for *N*-nitrosamines and internal standard NDPA in GC-MS mode

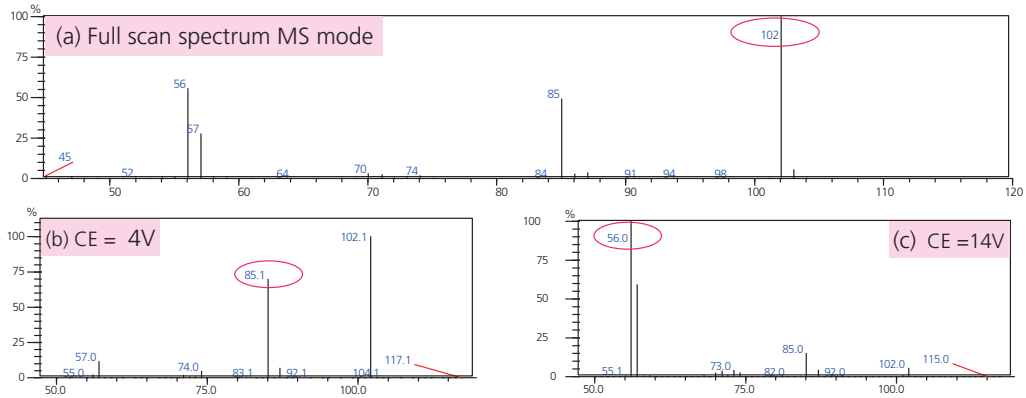

Fig. 2 NDEA full scan MS spectrum (a) and product ion scan of precursor ion *m/z* 102 with different collision energy (b, c)

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 1. The MRM transition with higher intensity was used as the quantitative ion and the other one as qualitative ion for confirmation.

Method Development for Trace Level Detection of N-Nitrosamines in Beer by GC-MS/MS

Method Performance Evaluation

A MRM quantification method was set up based on the MRM transitions in Table 1.
Linear calibration curves with internal standard (IS) were established for the six *N*-nitrosamines as shown in Fig. 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.

Table 1 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	NDEA	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

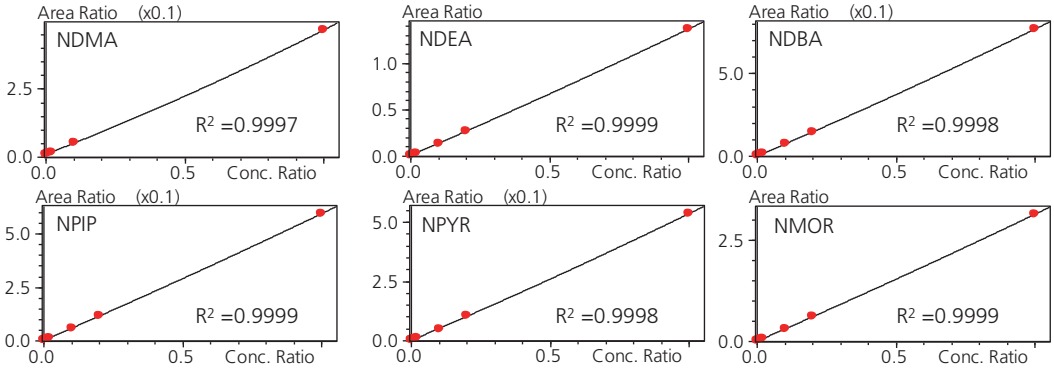

Fig. 3 MRM calibration curves of six *N*-nitrosamines from 0.1, 0.5,1.0,5.0,10.0 and 50.0 ppb with IS

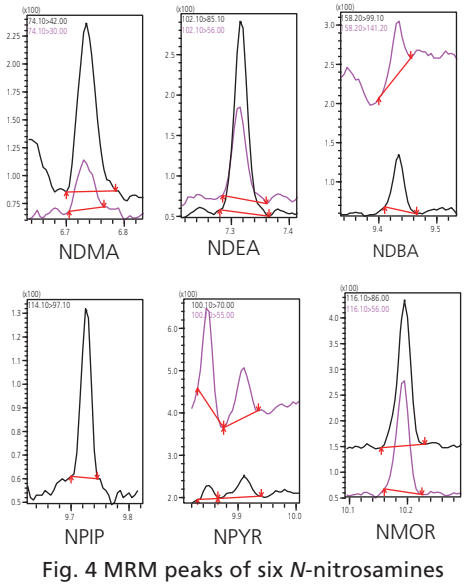

Fig. 4 MRM peaks of six *N*-nitrosamines of 0.1 ppb on GCMS-TQ8030

Table 2 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
3	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

Table 3 Analysis result and recovery of *N*-nitrosamines in beer sample

Compound	[1]Spiked beer extract (ppb)	[2]Recovery (%)	[3]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:
[1] Mixed standard 10.0 ppb was spiked in beer extract
[2] Recovery = (Spiked beer extract – Sample blank /10) * 100
[3] Concentration in beer (ppb) = Sample blank / 25

Method Development for Trace Level Detection of N-Nitrosamines in Beer by GC-MS/MS

Fig. 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 2. The peak area %RSD for all the target analytes were below 5%, except for NPYR. The overlay mass chromatograms in Fig. 5 demonstrated the peak area consistency of NMOR graphically for five consecutive injections.

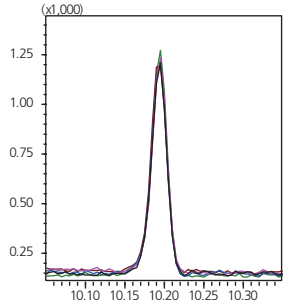

Fig. 5 MRM chromatograms of NMOR at 0.5 ppb, (n=5)

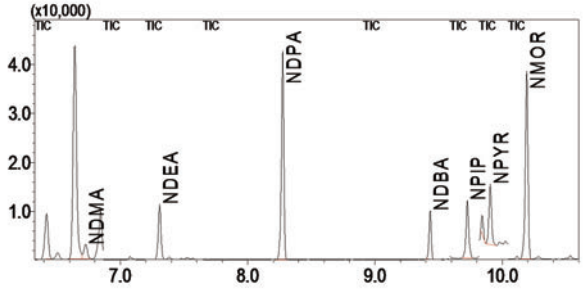

Fig. 6 TICs of MRM data for spiked beer extract with added 50.0 ppb IS

N-nitrosamines in Beer Sample

The GC-MS/MS method was established for application of beer samples. The investigated beer samples were pre-concentrated 25 times based on preparation method described earlier. Fig. 6 shows the TICs of MRM data for the spiked beer extract. It was found that some minute amount of NDBA, NPYR and NMOR were present in the sample blank (un-spiked 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 ±12 %.

5. Conclusion

A highly sensitive and selective GC-MS/MS method using Shimadzu GCMS-TQ8030 was developed for *N*-nitrosamines analysis. Along with proper sample preparation and MRM transitions data acquisition mode, the system is capable of determining trace-level of *N*-nitrosamines in beer sample beyond the required regulation. Hence, good linearity, repeatability and acceptable recoveries were obtained for all targeted analytes.

6. References

[1] [Sen, N. P., Seaman, S., McPherson, M. (1980), Nitrosamines in alcoholic beverages. Journal of Food Safety, 2, 13–18. (Last accessed 14 September 2012).
[2] Brewing Quality Control Manual Series http://www.alcbevtesting.com/wpcontent/uploads/2012/01/BDASQualityManualSeriesPt2MaltJune2012.pdf, (Last accessed 15 September 2012).
[3] AOAC Official Method 982.11, 2000 .

First Edition: June, 2013

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