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Application News

GCMS-TQ™8050 NX HS-20 NX Trap

Ethylene Oxide and 2-Chloroethanol Analysis in Instant Noodles by Dynamic Headspace and GC-MS/MS

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User Benefits

- Simple sample preparation procedure, direct sample analysis
- Sample recovery within 70 % to 130 % without matrix-matched calibration curves

■ Introduction

Ethylene oxide (EO) has been utilized as a fumigant pesticide due to its effectiveness in controlling pests. However, one of the risks associated with using EO is its potential to cause cancer if inhaled[1]. In addition to being a carcinogen, it can also cause eye and skin irritation from short-term exposure. The metabolite form of EO is 2-Chloroethanol (2-CE) and it is also being monitored since it may exhibit similar toxicity effect as EO [1]. In recent years, there have been recalls of instant noodle products due to the presence of EO. According to the European Commission, the maximum residual level (MRL) for composite products such as noodles should not exceed 0.02 mg/kg [2].

In this application notes, a method using dynamic headspace coupled with GC-MS/MS has been optimized to quantify both EO and 2-CE in instant noodles, with reference to Analysis of Ethylene Oxide (EO) & 2-Chloro Ethanol (2-CE) Application Databook [3]. The sample extraction was done directly by the headspace autosampler. Internal standard calibration curves were utilized to achieve good recovery for both EO and 2-CE (within the range of 70 % to 130 %) at a concentration of 10 ng/g, without the need for matrix-matched calibration curves.

■ Experimental

Analysis condition

GCMS-TQ8050 NX with HS-20 NX Trap (Shimadzu Corporation, Japan) were used in this work (Figure 1). GCMS-TQ8050 NX is equipped with a highly efficient detector and patented ion source technology for ultra trace analysis, providing the best sensitivity requirement for this application. The HS-20 NX Trap is capable in performing both static and dynamic headspace modes. In this study, dynamic headspace (which uses absorbent trap) was employed to enhance sensitivity (Figure 2).

Analytical conditions used in this application is tabulated in Table 1.

Figure 1: Picture of HS-20 NX Trap autosampler with GCMS-TQ™8050 NX.

Table 1: Dynamic headspace and GC-MS/MS analytical conditions for analysis of EO and 2-CE analysis in instant noodles.

Headspace Autosampler Parameter (HS-20 NX, Trap Model)	
Oven Temp.	70 °C
Sample Line Temp.	110 °C
Transfer Line Temp.	130 °C
Trap Cooling Temp.	-20 °C
Trap Desorb. Temp.	200 °C
Trap Equilib. Temp.	-20 °C
Shaking Level	5
Multi Injection Count	3
Pressurizing Gas Pressure	105 kPa (Helium)
Equilibrating Time	5.0 min
Pressurizing Time	0.3 min
Pressure Equilib. Time	0.0 min
Load Time	0.3 min
Load Equilib. Time	0.0 min
Dry Purge Time	0.0 min
Injection time	10.0 min
Needle Flush Time	10.0 min
GC Cycle Time	25.0 min
GC Parameter	
Carrier Gas	Helium
Injection Mode	Split mode, split ratio 15
Flow Control Mode	Linear Velocity, 40.2 cm/s
Purge Flow	3 mL/min
Column	SH-I-624 Sil MS (60 m x 0.25 mm ID x 1.4 µm df) [P/N: 227-36076-01]
Column Oven Temperature Program	35 °C (hold time: 4 min) → rate: 50 °C/min to 240 °C → rate: 20 °C/min to 300 °C (hold time: 3.9 min)
MS/MS Parameter	
Ion Source Temp.	250 °C
Interface Temp.	235 °C
Solvent Cut Time	3 min
MRM transition* (Collision Energy)	
EO	44>29 (6), 44>28 (6), 44>14 (18)
EO-d4	48>20 (6), 48>30 (5), 48>16 (20)
2-CE	80>31 (6), 80>44 (5), 82>31 (6)
2-CE-d4	84>33 (5), 86>33 (5)

*Some MRM transitions may not work well in certain matrices. User may need to select the best MRM transitions accordingly.

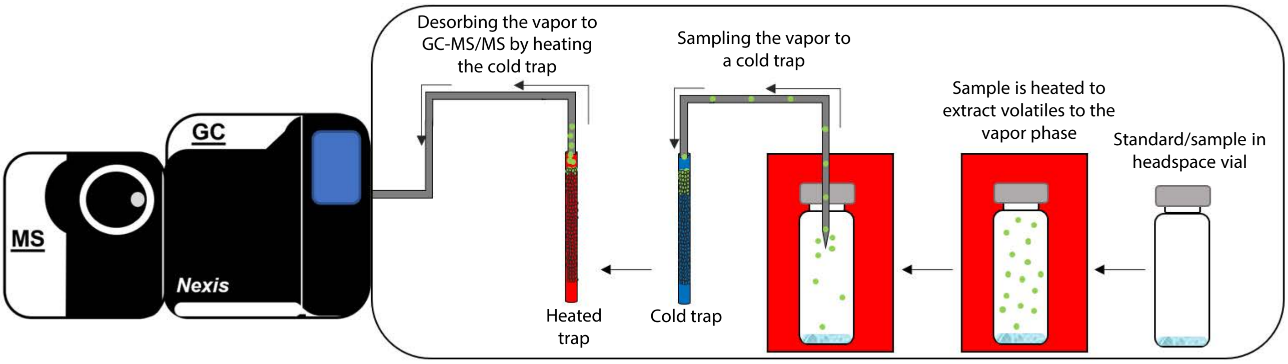

Figure 2: Dynamic headspace workflow, volatile analytes were concentrated in cold trap before being release into GCMSMS for analysis.

Standard and sample preparations

Internal standards, ethylene oxide-d4 and 2-chloroethanol-d4, were purchased from Cambridge Isotope Laboratories. Magnesium sulfate ($MgSO_4$), ethylene oxide and 2-chloroethanol were purchased from Merck. Acetonitrile was purchased from Kanto Chemical.

For the preparation of Calibration Standard Stock Solutions, EO and 2-CE were diluted with acetonitrile to final concentrations of 100, 200, 500, 1000, and 2000 pg/µL, while the internal standards (EO-d4 and 2-CE-d4) were added at a concentration of 500 pg/µL in each stock solution. A separate Internal Standard Stock Solution was prepared, containing 500 pg/µL of EO-d4 and 2-CE-d4 in acetonitrile, for spiking into the sample.

To construct a calibration curve, 5 µL of each calibration standard stock solution was aliquoted into separate 20-mL headspace vials. The vials were then promptly sealed with a crimp cap and loaded onto the headspace autosampler for analysis. Assuming 0.1 g of matrix was present in each headspace vial, the final concentration of EO and 2-CE in the matrix inside the headspace vial would be 5, 10, 25, 50, and 100 ng/g, with the internal standard at 25 ng/g each.

For sample preparation, 0.1 g of the sample was weighed into a 20-mL headspace vial. Then, 5 µL of Internal Standard Stock Solution (500 pg/µL) was spiked into the sample. The vial was promptly sealed with a crimp cap and loaded onto the headspace autosampler for analysis. For samples that contained water, an additional step of adding 50 mg of MgSO4 was performed before sealing the vial.

For recovery study, 0.1 g of the sample was weighed into a 20-mL headspace vial. Then, 5 µL of one of the Calibration Standard Stock Solutions, containing 200 pg/µL EO and 2-CE, as well as 500 pg/µL EO-d4 and 2-CE-d4 in acetonitrile, was spiked into the sample. The vial was promptly sealed with a crimp cap and loaded onto the headspace autosampler for sample analysis. The final concentration of EO and 2-CE in the sample inside the headspace vial will be 10 ng/g. For samples that contained water, 50 mg of MgSO4 was added before sealing the vial.

■ Results

Sensitivity, Repeatability and Linearity

Figure 3 shows 5-point internal standard calibration curves (5, 10, 25, 50 and 100 ng/g) and MRM mass chromatograms of EO, 2-CE and internal standards. The R^2 value for both compounds were greater than 0.999, demonstrating the capability of both the instruments and method to achieve excellent linearity within the range of 5 ng/g to 100 ng/g. Both compounds met the limit of quantitation (LOQ) criteria at 5 ng/g, as they exhibited signal-to-noise ratios (S/N) greater than 10 at this concentration. The %RSD (n=5) of the area count for both compounds at 5 ng/g were less than 10 % (Table 2).

Table 2: Area count repeatability results (n=5) and signal-to-noise ratios of EO and 2-CE at 5 ng/g.

No.	Compound Name		Injection 1	Injection 2	Injection 3	Injection 4	Injection 5	% RSD
1	EO	Area count	12773	11961	11473	12045	11328	4.8
		S/N	65	60	68	107	79	
2	2-CE	Area count	5109	5306	5251	5652	4568	7.6
		S/N	30	102	22	46	44	

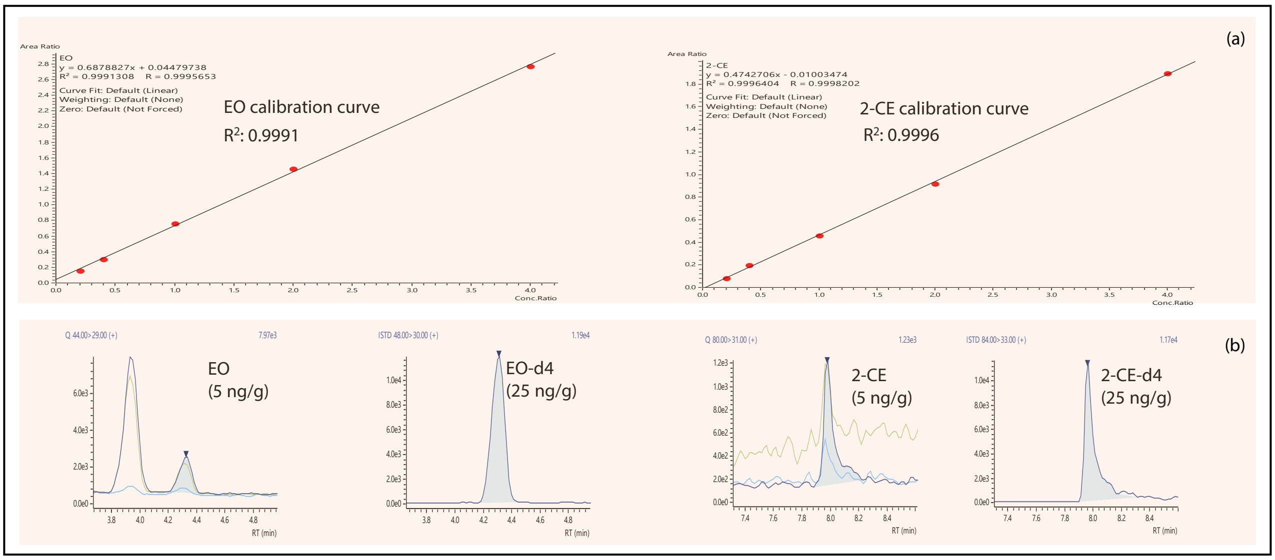

Figure 3: (a) Five-point calibration curves of EO and 2-CE; **(b)** MRM mass chromatograms of EO, EO-d4, 2-CE and 2-CE-d4.

Table 3a: EO and 2-CE concentration and recovery results in Instant Noodle A.

Sample in Instant Noodle A		Conc Result (ng/g)		% Recovery	
		EO	**2-CE**	**EO**	**2-CE**
Noodle A	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	9.737	11.454	97	115
Seasoning Powder A	**Unspiked**	N.D.*	**3.142 (<LOQ)**		
	10 ng/g spiked	10.602	12.969	106	98**
Seasoning Oil A	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	9.003	11.662	90	117

Table 3b: EO and 2-CE concentration and recovery results in Instant Noodle B.

Sample in Instant Noodle B		Conc Result (ng/g)		% Recovery	
		EO	**2-CE**	**EO**	**2-CE**
Noodle B	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	9.044	10.092	90	101
Seasoning Powder B	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	7.905	12.009	79	120
Dried Vegetable B	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	8.159	10.946	82	109
Seasoning Oil B	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	8.595	9.923	86	99
Sauce B1	**Unspiked**	N.D.*	**5.668**		
	10 ng/g spiked	9.834	13.984	98	83**
Sauce B2	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	7.185	10.850	72	109

Table 3c: EO and 2-CE concentration and recovery results in Instant Noodle C.

Sample in Instant Noodle C		Conc Result (ng/g)		% Recovery	
		EO	**2-CE**	**EO**	**2-CE**
Noodle C	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	9.246	12.170	92	122
Seasoning Powder C	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	8.051	11.360	81	114
Dried Vegetable C	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	8.206	11.816	82	118

Table 3d: EO and 2-CE concentration and recovery results in Instant Noodle D.

Sample in Instant Noodle D		Conc Result (ng/g)		% Recovery	
		EO	**2-CE**	**EO**	**2-CE**
Noodle D	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	8.832	10.952	88	110
Seasoning Powder D	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	7.926	11.078	79	111
Dried Vegetable D	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	9.085	11.089	91	111
Seasoning Oil D	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	8.888	10.931	89	109
Sauce D1	**Unspiked**	N.D.*	**4.340 (<LOQ)**		
	10 ng/g spiked	11.210	12.818	112	85**
Sauce D2	**Unspiked**	N.D.*	N.D.*		
	10 ng/g spiked	8.588	9.665	86	97

*Not Detected

**Percentage recovery was calculated after subtracting spiked concentration result from unspiked result.

Quantitation and Recovery Results

Four different types of instant noodles were purchased from a local supermarket. The ingredients in the instant noodles, such as noodle, sauce, seasoning powder and seasoning oil, were analyzed separately. Each sample was then spiked to achieve a final concentration of 10 ng/g in order to assess its recovery. The average results (n=2) and percentage recovery of the spiked samples were recorded in Table 3a-3d. For samples that yielded positive results, the spiked concentration result was subtracted from the unspiked result before determining the percentage recovery.

The experiment achieved recovery results within the range of 70 % to 130 % for all the samples. This indicates that the combination of dynamic headspace with GC-MS/MS and an internal standard is suitable for the analysis of EO and 2-CE. These results demonstrate that the use of matrix-matched calibration may not be necessary for certain samples. However, in cases where the recovery falls outside the 70 % to 130 % range due to challenging matrix interferences, widening the percentage recovery range or employing matrix-matched calibration curves could be considered to improved the result. For samples with severe matrix interferences, a different sample preparation approach such as manual extraction and clean-up, followed by injecting the solution into the GC-MS/MS using liquid injection mode, might be necessary, instead of dynamic headspace.

It is important to note that a high water content in a sample can negatively affect the peak shape and sensitivity of 2-CE. The addition of $MgSO_4$ helps to mitigate this issue by reducing peak broadening, resulting in increased intensity for 2-CE (Figure 4). Therefore, it is recommended to add $MgSO_4$ for samples that contain water. As demonstrated in Table 4, the addition of $MgSO_4$ does not impact the recovery of both EO and 2-CE.

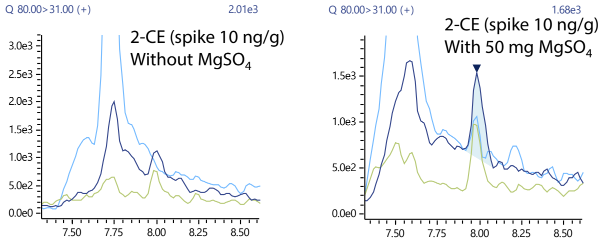

Figure 4: MRM mass chromatogram comparison between same sample (Sauce) that had been spiked with 10 ng/g but, one sample does not contain $MgSO_4$ while another contain 50 mg of $MgSO_4$.

Table 4: EO and 2-CE concentration results in spiked $MgSO_4$ (10 ng/g).

Magnesium sulphate	**Conc Result (ng/g)**		**% Recovery**	
	EO	**2-CE**	**EO**	**2-CE**
50 mg $MgSO_4$ (10 ng/g spiked)	9.021	9.958	90	100

■ Conclusion

An internal standard method was successfully developed for the analysis of EO and 2-CE in instant noodles using the HS-20 NX Trap with GCMS-TQ8050 NX. The dynamic headspace technique was employed, which reduced the hassle of sample preparation. Although matrix-matched calibration curves were not used, excellent linearity (R^2 value > 0.999) and recovery within 70 % to 130 % were achieved for all the samples analyzed in this experiment.

■ References

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3. Shimadzu Application Databook SAIP-GC001-APDB001, *Analysis of Ethylene Oxide (EO) & 2-Chloro Ethanol (2-CE) Application Databook,* accessed 18 December 2023 https://www.an.shimadzu.com/rs/119-VAD-470/images/EtO%20Analysis.pdf

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