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GC-MS – Chromatography – Food Safety

Analysis of Ethylene Oxide in Ramen (Instant Noodle) by GC-MS/MS

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Abstract

Ethylene oxide is typically used as a fumigant pesticide to reduce microbial or bacterial contamination. However, it is banned in the European Union (EU) because of its carcinogenic and mutagenic properties. In 2020, Belgium was the first country to raise the alarm about the presence of ethylene oxide in imported sesame seeds. Since then, ethylene oxide has also been found in various food additives, including locust bean gum, which is a thickening agent or stabilizer. These ingredients are commonly used in formulations and can be found in products such as flour, cereals, ice cream, chocolate, biscuits, bread, or cheese. As these items are sold by major brands and retailers, it has led to thousands of products being affected and taken off the shelves.

With the surge in demand for ethylene oxide detection worldwide, there is an increased need for greater laboratory capacity and relevant analysis methods to ensure food safety. In this Technology Brief, Shimadzu introduces an analysis of ethylene oxide with QuEChERS and GCMS-TQ8050 NX, which is equipped with AOC-20i/AOC-20s Plus for automation capabilities to meet the requirement for higher throughput and faster turnaround time.

The analysis of ethylene oxide has become critically important due to the recent food scandal in Europe

Keywords:
Ethylene Oxide, Food Safety, GCMS, GCMS-TQ8050 NX

Highlights

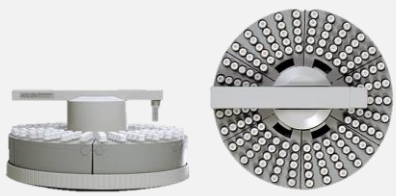
- *Shimadzu's GCMS-TQ8050NX, paired with the AOC-20i/AOC-20s Plus, can reliably analyze EO and 2-CE*
- *Excellent reproducibility of RSD below 5% is attained for sample analysis*
- *Good recovery rates are achieved for EO (98.9%) and 2-CE (106.3%) in complex food matrices*

Technologies Featured

GCMS-TQ8050 NX

AOC-20i Plus Injector

AOC-20s Plus Autosampler



1. INTRODUCTION

In September 2020, the EU conducted strict import customs inspections on sesame seeds imported from India after a case in which ethylene oxide (EO), an unapproved substance, was detected in sesame seeds from India at an EU residue tolerance level of 0.05 mg/kg.[1]

The Ministry of Food and Drug Safety and State Administration (MFDS) of South Korea was also alerted to the detection of 2-chloroethanol (2-CE), a metabolite of EO, in ramen instant noodles exported to Europe in August 2021.[2] The ministry then established provisional 2-CE standards for food products and took follow-up measures, such as issuing an "inspection order" to vendors that detected 2-CE.

EO is a colorless, sweet-smelling substance that is approved as a fumigant for controlling microorganisms and pests, including bacterial pathogens, mainly in Canada and the United States. However, the EU has banned insecticides because they can induce mutagenesis and are carcinogenic.[3] In addition, 2-CE is a metabolite produced when EO reacts with nucleophiles such as chlorides. Thus, the EU currently defines EO residue as the sum of EO and 2-CE.

In this regard, the EU Reference Laboratories for Residual of Pesticides-Single Residue Method (EURL-SRM) provides a method for the analysis of EO and 2-CE in sesame seeds. In this test method, common samples are extracted by the QuEChERS method and dry samples with high oil content are extracted by the QuOil method. Both EO and 2-CE are analyzed simultaneously without separate hydrolysis. Furthermore, to solve the problem whereby the extraction solvents – acetonitrile and residual water – may negatively affect the GC column and the MS filament, the method proposed the use of programmable temperature vaporization (PTV) injector, which is an inlet capable of temperature programming.

This Technology Brief introduces a solution that can be applied to the analysis of residual EO and 2-CE in ramen instant noodle using GC-MS/MS equipped with PTV based on EURL-SRM (Analysis Method for EO and 2-CE in Sesame[1]).

2. EXPERIMENT

2.1 Experiment Setup

Analytical Method

For the target compounds EO and 2-CE, EO-d_4 and 2-CE-d_4 were used as the internal standards respectively to create a calibration curve using the internal standard method. The final concentrations of EO and 2-CE matrix-matched standard solutions were prepared at 2.5, 5, 10, 25, and 50 ng/mL levels. The internal standards were added with a final concentration of 10 ng/mL each.

Sample Preparation Method

Samples of ramen instant noodles and their respective stock powders were homogenized. A resultant 5 g of the homogenates were subjected to QuEChERS extraction and cleanup as shown in Figure 1. The QuEChERS extracts were subsequently analyzed by GC-MS/MS according to the conditions in Table 1 and 2.

Gas chromatography system	Nexis GC-2030	**PTV system injection temperature**	90 ºC (0.8 min) → 230 ºC/min → 250 ºC (10 min)
Column	DB-624 (60 m x 0.25 mm,1.4 µm)	**Mass spectrometry system**	GCMS-TQ8050 NX
Carrier gas	He (99.999%)	**Ionization mode**	EI
Column flow	1.5 mL/min	**Interface temperature**	320 ºC
Injection mode	Split (4:1)	**Ion source temperature**	230 ºC
Flow control	Linear velocity (31.3 cm/sec)	**Acquisition mode**	MRM
Oven temperature	45 ºC (2 min) → 25 ºC/min → 250 ºC/min (5 min)		
Injection volume	1 µL		

Table 1. Analysis Conditions for GC-MS/MS

Target Compound	Quantifier Ion (*m/z*)	CE (V)	Qualifier Ion 1 (*m/z*)	CE (V)	Qualifier Ion 2 (*m/z*)	CE (V)
EO	44.00>29.00	5	44.00>28.00	5	44.00>14.00	20
EO-d_4	48.00>30.00	5	48.00>16.00	20	-	-
2-CE	80.00>31.00	5	80.00>43.00	5	82.00>31.00	5
2-CE-d_4	84.00>33.00	5	86.00>33.00	5	-	-

Table 2. Analytical conditions for EO, EO-d_4, 2-CE and 2-CE-d_4

QuEChERS Extraction (EN 15662)

Homogenization of sample using dry ice

5 g ± 0.02 g of sample homogenates

Extraction of sample using a mechanical shaker
(10 mL of acetonitrile containing 5% v/v of water)

1) In the case of oils (5 min)
2) In the case of finely comminuted material (15 min)
3) In the case of intact seeds or laboratory-homogenized material (15-30 min)

Add the QuEChERS buffer-partitioning salts mixture and shake for 1-2 minutes

Add internal standard (1 µg/mL,100 µL)

Centrifuge at >3000 g

Clean-up using dispersive SPE with C_{18} / PSA / $MgSO_4$ (25 / 25 / 150 mg/mL extract)

Transfer the extract to the GC vials

GC-MS/MS measurement

Figure 1. QuEChERS method flow chart

3. RESULTS AND DISCUSSION

3.1. Performance Evaluation

The coefficient of determination of the calibration curves for EO and 2-CE were confirmed at R^2 >0.999 (Figure 2), where the chromatograms for the 50 ng/mL levels of EO and 2-CE are shown in Figure 3.

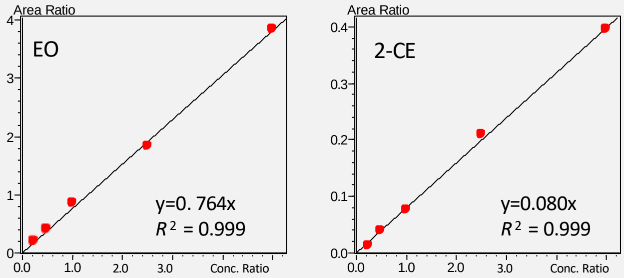


Figure 2. Calibration curves for EO and 2-CE

As shown in Table 3, the average accuracy and precision of EO (%RSD, n = 7) were 101.0% and 4.9%, respectively, and that of 2-CE (%RSD, n = 7) were 109.2% and 3.7%, respectively.

	EO		2-CE	
No.	Concentration (ng/mL)	Accuracy (%)	Concentration (ng/mL)	Accuracy (%)
1	5.3	105.6	5.4	108.8
2	5.0	99.0	5.3	105.7
3	5.4	108.3	5.4	107.7
4	5.0	99.2	5.3	106.2
5	4.9	98.9	5.5	109.6
6	4.7	93.4	5.4	108.6
7	5.2	103.5	5.9	117.7
Average	5.1	101.0	5.5	109.2
%RSD	4.9		3.7	

Table 3. Results of precision measurements (n = 7)

For the recovery test, a final concentration of 25 ng/mL EO and 2-CE were respectively spiked into the ramen instant noodle samples. The recovery test was evaluated four times. As shown in Table 4, the average recovery rate of EO ranged from 94.0-103.6%, and that of 2-CE ranged from 101.8-109.6%. The %RSD of EO and 2-CE were 4.7% and 3.4%, respectively.

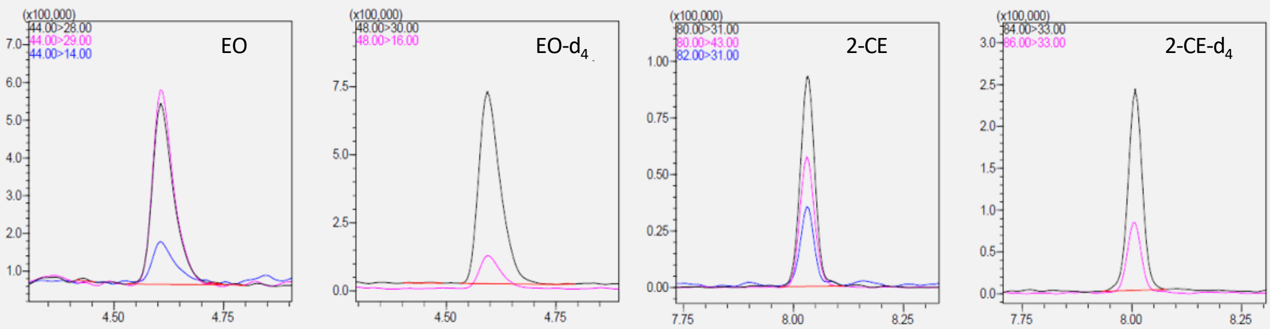


Figure 3. Chromatograms of EO, 2-CE, EO-d_4 and 2-CE-d_4 (standard 50 ng/mL)

	EO		2-CE	
No.	Spiked concentration (ng/mL)	Recovery Rate (%)	Spiked concentration (ng/mL)	Recovery Rate (%)
1	25.9	103.6	25.5	101.8
2	23.5	94.0	27.4	109.6
3	25.5	102.0	27.2	108.7
4	24.0	95.9	26.3	105.0
Average	24.7	98.9	26.6	106.3
%RSD	4.7		3.4	

Table 4. Evaluation results of recovery rate studies (n = 4)

4. CONCLUSION

Using Shimadzu GCMS-TQ8050 NX, this Technology Brief examined the analysis method of residual EO and 2-CE in ramen instant noodles based on EURL-SRM (Analysis method of EO and 2-CE in sesame[1]). The coefficient of determination (R^2) of the calibration curves for EO and 2-CE were >0.999. In the recovery test, the average recovery rates were confirmed at 98.9% and 106.3%, respectively for EO and 2-CE, while the %RSD were 4.7% and 3.4%, correspondingly.

5. REFERENCES

[1] EURL-SRM - Analytical Observations Report, Analysis of Ethylene Oxide and its Metabolite 2-Chloroethanol by the QuOil or the QuEChERS Method and GC-MS/MS, 2020

[2] MFDS (Ministry of Food and Drug Safety and National Administration) press release, test results of Ramen 2-Chloroethanol (2-CE) announced, 2021

[3] Thomas Bessaire *et al.*, Analysis of ethylene oxide in ice creams manufactured with contaminated carob bean gum (E410), Food additives & contaminants: Part A, **2021**, *38*(12), 2116-2127

READ MORE

GCMS-TQ8050 NX

Featuring a new, highly efficient detector and three types of noise-reduction technologies, the GCMS-TQ8050 NX can perform unprecedented quantitative analyses of ultra-trace amounts down to the femtogram level. Moreover, its ultra-high sensitivity and high mass resolution open up a whole new realm of quantitative analysis, with higher cost savings and greater uptime for maximized productivity.

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The AOC-20 Series are multifunctional robotic autosamplers that provide powerful support to improve the reliability of the analytical operations. The AOC-20 Series includes features such as effective solvent flushing (introducing air or solvent into the syringe before sampling) for samples that tend to remain in the syringe, continuous injection during analysis to improve the sensitivity of trace components, as well as pretreatment functions such as adding reaction reagents. With these advanced features, the AOC-20 Series offer unrivaled reliability and efficiency for the users.

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