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Quantitation of Ethylene Oxide (EtO) and 2-Choloro Ethanol(2-CE) in sesame seeds by using dynamic headspace GC-MS/MS

2022 AOAC Annual Meeting

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1. Overview

EtO is one of the most widely produced chemicals worldwide. It is colorless, odorless, flammable gaseous cyclic ether. Boiling point of EtO is 10.4 °C. It has very strong antibacterial property. Due to its small size, it shows a high diffusivity and strong penetrating properties and is thus very effective in the disinfestation or disinfection of dry food commodities. EtO is almost 10 times more effective than other fumigant such as methyl bromide and phosphine.

EtO is highly carcinogenic, mutagenic & genotoxic impurity for living being and hence it is very important to quantitate EtO in food matrices.

Figure 1: Shimadzu GCMS-TQ8050 NX with AOC-20i/ AOC-20s & HS-20 NX

2. Introduction

The European Chemical Agency (ECHA) has classified EtO in category 1B as regards carcinogenicity, mutagenicity and reproductive toxicity, and in category 3 as regards the acute toxicity . The US National Institute of Health (NIH) classified EtO as "known to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in humans, including epidemiological studies and studies on mechanisms of carcinogenesis." The US Environmental Protection Agency (EPA) has concluded that EtO is carcinogenic to humans by the inhalation route of exposure.

Considering carcinogenicity and no acceptable threshold for exposure, no Acceptable Daily Intake (ADI) was established for EtO. 2-CE and 2-bromoethanol are also considered weakly genotoxic and potentially carcinogenic. Given the inconclusive toxicological picture of 2-CE, it was decided by regulatory authorities to follow the precaution approach and consider 2-CE equally toxic to EtO. EU-MRLS (Maximum Residue Levels as per European Commission) for EtO & 2-CE are summarized in Table 1.

Table 1: EU-MRLs for EO & 2-CE

No.	Products	EU-MRLS for EtO & 2-CE
1	Teas, cocoa & spices	0.10 mg/kg
2	Nuts, oil fruits & oilseeds	0.05 mg/kg
3	Fruits, vegetables, sugar plants, fungi & pulses	0.02 mg/kg
4	Cereals & products of animal origin	0.02 mg/kg
5	Apicultural products	0.05 mg/kg

Commodities relevant for residues of EtO/2-CE are primarily spices, oilseeds and nuts. When it comes to such commodities (with high lipid content and low water content), testing laboratories widely employ below extraction methods,

A)QuEChERS-Method(EN 15662) Or
B)QuOil method (CEN/TS 17062:2019 modified)

Extracted solutions from above methods were analyzed by using GC-MS or GC-MS/MS equipped with liquid sampler. But different matrices required clean up reagent optimization and this could have varied effect on extraction efficiency.

To overcome these difficulties, we have developed and optimized three different dynamic headspace methods where GCMS-TQ8050 NX with HS-20 NX (Figure 1) is used for the analysis of EtO & 2-CE.

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3. Methods

3-1. HSGC-MS/MS analysis

Individual Certified Reference Standard (CRS) for EtO & 2-CE were procured from Sigma Aldrich. A mixture of EtO and 2-CE standards (2 ppm) was prepared by using CRS and analyzed in scan mode for identification. Steps such as precursor ion selection and MRM optimization at different Collision Energies (CE) were performed. Method with segmented MRM and optimum CE energies was generated. Instrument parameters are given in Table 2, whereas optimized MRM transitions of EtO & 2-CE are given in Table 3.

3-2. Analytical conditions

Table 2. Instrument parameters for HSGC-MS/MS

GCMS System	: GCMS-TQ8050 NX with HS-20 NX		
Chromatography Parameters			
Column	: RTX-VMS 60 m, 0.45 mm I.D., 2.55 µm df (S/N: 1242817)		
Injection Mode	: Split		
Flow Control Mode	: Column Flow		
Carrier Gas	: Helium		
Column Flow	: 3.0 mL/min		
Linear Velocity	: 44.0 cm/s		
Temp. Program	Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
	-	35	5.0
	20	235	5.0
Ionization Mode	: Electron Ionization (EI)		
Interface Temp.	: 230 °C		
Ion Source Temp.	: 230 °C		
Headspace parameters			
Oven Temp.	: 115 °C		
Sample Line Temp.	: 120 °C		
Transfer Line Temp.	: 130 °C		
Trap Cooling Temp.	: -10 °C		
Trap Desorption Temp.	: 280 °C		
Mult Inj. Count (MIC)	: For method 1 - MIC -1 : For method 2 - MIC -10 : For method 3 - MIC - 1		
Pressurizing Gas Pressure	: 192 kPa		

Table 3: MRM transitions for EtO & 2-CE

Peak ID	Compound	Principal	CE-1	Qualifier	CE-2	Qualifier	CE-3
1	EtO	44>29	6	44>28	6	44>14	18
2	2-CE	80>31	6	80>44	5	82>31	6

3. Methods

3-3. Sample preparation

Extraction of EtO & 2-CE from seasame seeds

5000 mg of sesame seeds sample + 5000 uL of diluent (Acetonitrile), mixed well & vortex for 15 to 20 minutes
Centrifuge for 5 min at 5000 rpm at 10° C.
Removed 100 uL from above solution, transferred it into 20 mL HS vial
Proceed for the analysis by using GC-MS/MS equipped with dynamic headspace sampler

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4. Results

To overcome difficulties of liquid injection mode, we have developed and optimized three different dynamic headspace methods. Brief about all three headspace methods is given in Table 4.

Table 4: Brief about all headspace methods

Method Details	Compounds	Sample preparation (%)	Advantage
Method 1	EtO & 2-CE	100	Single method for EtO & 2-CE
Method 2	2-CE	10	Trace level quantitation of 2-CE
Method 3	EtO	100	Trace level quantitation of EtO

Figure 2, 3, 4 & 5 depicts the calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO & 2-CE (Representative chromatograms) as per method 1, 2 & 3

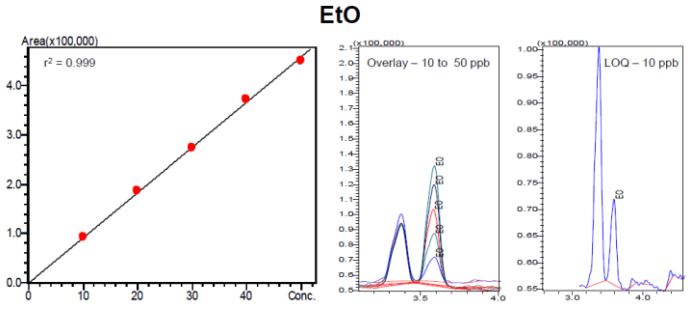

Figure 2: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO as per Method 1

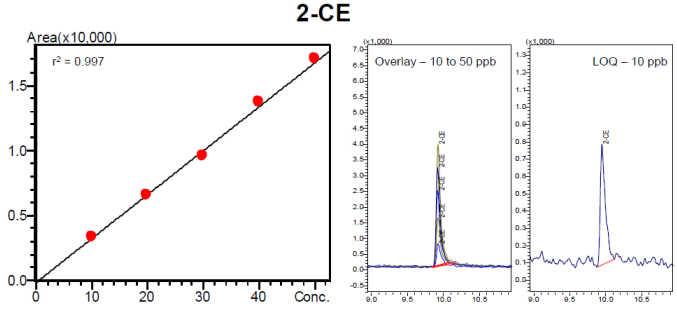

Figure 3: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE as per Method 1

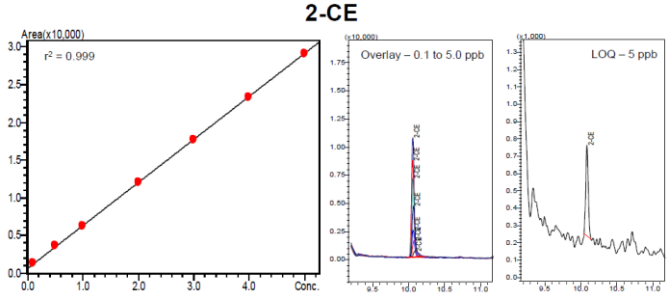

Figure 4: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE as per method 2

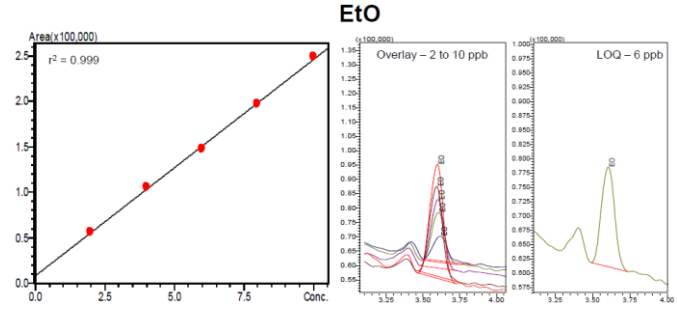

Figure 5: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO as per method 3

4. Results

Precision : Summary of precision standard solutions is shown in Table 5

Table 5: Summary for precision (n=6)

Details	Method 1		Method 2	Method 3
	EtO	2-CE	2-CE	EtO
LOQ level	10 ppb	10 ppb	5 ppb	6 ppb
% RSD (n=6)	2.1	4.9	9.1	1.7
S/N	16	57	53	26
Highest level	50 ppb	50 ppb	50 ppb	10 ppb
% RSD (n=6)	2.2	4.1	3.7	1.4
S/N	110	152	410	44

Linearity : Summary of calibration standard is shown in Table 6

Table 6: Summary for linearity (n=3 for each level)

Details	Method 1	Method 2	Method 3
	EtO & 2-CE	2-CE	EtO
Linearity levels (On column)	10,20,30,40 & 50 ppb	0.1,0.5,1.0,2.0, 3.0,4.0 & 5.0 ppb	2,4,6,8 & 10 ppb
r^2 (n=3)	EtO - 0.99950 2-CE - 0.99785	0.99974	0.99906

Accuracy : Summary of accuracy is shown in Table 7

Table 7: Summary for accuracy (n=3 for each level)

Details	Method 1		Method 2	Method 3
	EtO	2-CE	2-CE	EtO
Spiked LOQ conc.	10 ppb	10 ppb	5 ppb	6 ppb
Avg of % recovery	91%	121%	102%	82%
% RSD (n=3)	1.9	2.0	1.3	1.0

Details	Method 1		Method 2	Method 3
	EtO	2-CE	2-CE	EtO
Spiked highest conc.	50 ppb	50 ppb	50 ppb	10 ppb
Avg of % recovery	91%	101%	100%	90%
% RSD (n=3)	3.0	2.6	2.2	1.4

4. Results

Data obtained from all three headspace methods is well compared with each other, & summary of the results were given in Table 8.

Table 8: Comparison of results

Details	Method 1		Method 2	Method 3
	EtO	2-CE	2-CE	EtO
LOQ level conc.	10 ppb	10 ppb	5 ppb	6 ppb
% RSD (n=6)	2.1	4.9	9.1	1.7
Linearity levels (On Column)	10,20,30,40 & 50 ppb		0.1,0.5,1.0,2.0,3.0,4.0 & 5.0 ppb	2,4,6,8 & 10 ppb
r^2 (n=3 of each level)	0.99950	0.99785	0.99974	0.99906
Spiked LOQ level	10 ppb	10 ppb	5 ppb	6 ppb
Avg of % recovery	91%	121%	102%	82%
Spiked highest level	50 ppb	50 ppb	50 ppb	10 ppb
Avg of % recovery	91%	101%	100%	90%
Lowest conc.	10 ppb	10 ppb	0.1 ppb	2 ppb
Sample preparation	20-25 min		20-25 min	20-25 min
Cost	Cleanup reagent/QuEChERS-Not Required			
Regulatory compliance	Meets EU-MRLs			

Merits of headspace injection method

- Dynamic headspace has an edge over liquid injection technique in terms of sample preparation,less matrix interference & trace level quantitation
- EtO and 2-CE can be measured in single run with 10 ppb LOQ conc. by using Method 1, Where as 2-CE can be measured with 5 ppb LOQ conc. by using Method-2 & EtO can be measured with 6 ppb LOQ conc. by using Method-3
- No clean up reagents or extraction salts are used and hence no additional sample preparation which minimizes errors

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5. Conclusion

- Trace level quantification of EtO & 2-CE impurities in sesame seeds was successfully performed by using Shimadzu GCMS-TQ8050 NX with HS-20 NX dynamic headspace sampler.
- For EtO & 2-CE analysis, dynamic headspace mode outperforms the current regulatory limits, delivering multifold times more sensitivity compared to other injection techniques.
- Shimadzu GCMS-TQ8050 NX features a new highly efficient detector and superior noise reduction technology that enhance sensitivity and enables quantitation of EtO & 2-CE even at trace levels.

6. References

[1] EURL-SRM – Analytical Observation Report, Version 1.1 (December 2020)

First Edition: August, 2022

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