SHIMADZU
Excellence in Science

SAIP-GC001-APDB001

Solutions for

Analysis of Ethylene Oxide (EtO) & 2-Chloro Ethanol (2-CE)

Application Databook

Ayurveda
& Herbal

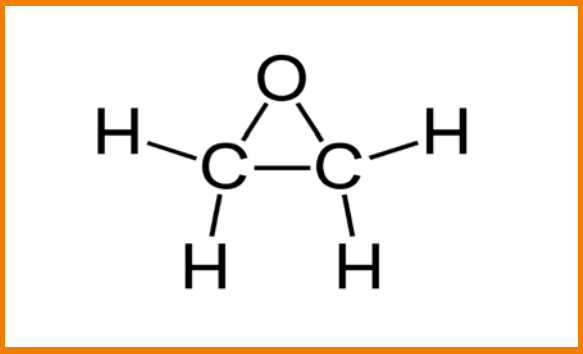
Agricultural
(Oil Seeds)

Medical
Devices/ Pharma

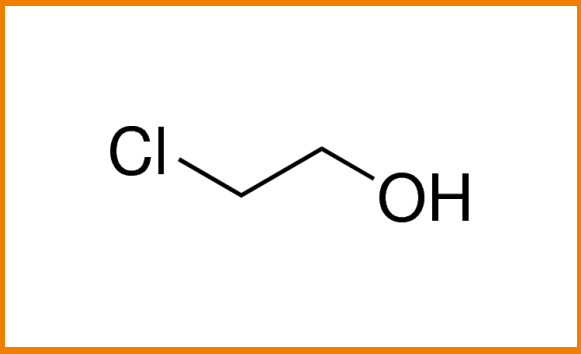
Spices

Processed
Foods

Chemical Structure of EtO & 2-CE



Ethylene Oxide (EtO)
(Molecular Formula – C_2H_4O)



2-Chloroethanol (2-CE)
(Molecular Formula – C_2H_5ClO)

Significance of quantitation of EtO & 2-CE at trace level in various food & other matrices

Even though Ethylene oxide (EtO / EO) is well-known to be toxic compound with carcinogenic and mutagenic concerns, it has been widely used for fumigation in the Food industry for effectively reducing or eliminating microbiological contamination with bacteria / fungi. Once in contact with food, EtO undergoes various reactions within the matrix, further producing reaction products such as ethylene glycol, 2-chloroethanol (2-CE) and 2-bromoethanol, which are also toxic in nature. Hence, the use of EtO for food fumigation has been phased out in many countries worldwide, due to toxicological concerns. In the EU, the use of EtO for the disinfection of foodstuffs, e.g. in storage areas, is not permitted (ECHA, 2020). EU has proposed separate maximum residual limits (MRLs) for EtO and its primary metabolite 2-CE in different food and agriculture commodities ranging from 0.02 to 0.1 mg/kg (Commission Regulation (EU) 2015/868).

The recent recall of food products exported to the EU due to non-compliance with EU regulations, has highlighted the importance of quantitation of EtO & 2-CE residues in food.

To ensure the quality and safety of food products, the European Rapid Alert System for Food and Feed (RASFF) prohibits the sales of goods exceeding the MRL values of 0.05 mg/kg (or 50 ppb) for the sum of EtO and 2-CE.

Reference: EURL-SRM - Analytical Observations Report

EU-MRLs of EtO & 2-CE for different products are

No.	Product	EU-MRLs
1	Teas, cocoa and spices	0.10* mg/kg
2	Nuts, oil fruits and oilseeds	0.05* mg/kg
3	Fruits, vegetables, sugar plants, fungi and pulses	0.02* mg/kg
4	Cereals and products of animal origin	0.02* mg/kg
5	Apicultural products	0.05* mg/kg

Different Methods As Per EURL-SRM Report

Method Reference	Components		Internal Standard	Liquid Mode		Mode
EURL-SRM Method	EtO	2-CE	Optional	QuEChERS-Method (EN 15662)	QuOil-Method (CEN/TS 17062:2019)	Liquid
Tadeo/Bononi Method	-	2-CE	-	EtO to 2-CE – Conversion Method		Liquid
-	-	2-BE*	-	EtO to 2-BE – Conversion Method		Liquid
Woodrow Method	EtO	-	-	-	-	Headspace
Ayoub Method	EtO	-	-	-	-	SPME

*2-BE: 2-Bromoethanol

Analysis of EtO & 2-CE in different matrices

No.	Product	Matrix Covered
1.0	Agricultural (Oil seeds)	Sesame Seed
2.0	Spices	Chilli Powder
		Chicken Masala
		Kitchen Masala
3.0	Ayurveda & Herbal	Amala Powder
		Ashwagandha Powder
		Boswellia Serrata Powder
4.0	Processed Foods	Instant Noodles
5.0	Medical Samples / Devices	Intraocular Lens
		Medical Devices

Shimadzu solutions

- **EtO & 2-CE in single run**
  1) Liquid Injection Method – with split (SPL) mode ☑
  2) Dynamic Headspace Method – with split mode (Solvent Extraction) ☑
  3) Dynamic Headspace Method – with split mode (As such in HS vial) ☑

- **Conversion method EtO to 2-CE**
  1) 2-CE is analyzed by Liquid Injection – with split ☑
  2) 2-CE is analyzed by Dynamic Headspace – with split ☑

Shimadzu's solution for analysis of EtO & 2-CE (Different Techniques / Instruments)

No.	Matrix Covered	Technique	Instrument
1.1	Sesame Seeds	Liquid Injection (EtO & 2-CE)	GCMS-TQ8050 NX with AOC-20i/s
		Dynamic Headspace (EtO & 2-CE) - Solvent extraction	GCMS-TQ8050 NX with HS-20 NX
1.2		Dynamic Headspace (EtO & 2-CE) - Direct sample in HS vial	GCMS-TQ8040 NX with HS-20 NX
1.3		Liquid Injection (2-CE) -Conversion Method	GCMS-TQ8040 NX with AOC-20i/s
		Dynamic Headspace Injection (2-CE) – Conversion Method	GCMS-TQ8040 NX with HS-20 NX
2.1	Chilli Powder	Dynamic Headspace (EtO & 2-CE) - Solvent extraction	GCMS-TQ8040 NX with AOC-30i
		Dynamic Headspace (EtO & 2-CE) - Solvent extraction	GCMS-TQ8050 NX with AOC-20i/s
2.2		Dynamic Headspace (EtO & 2-CE) - Direct sample in HS vial	GCMS-TQ8050 NX with HS-20 NX
2.3	Chicken Masala	Dynamic Headspace (EtO & 2-CE) - Solvent extraction	GCMS-TQ8050 NX with HS-20 NX
2.4	Kitchen Masala	Dynamic Headspace (EtO & 2-CE) - Solvent extraction	GCMS-TQ8050 NX with HS-20 NX
3.1	Amala Powder	Dynamic Headspace (EtO & 2-CE) - Solvent extraction	GCMS-TQ8050 NX with HS-20 NX
3.2	Ashwagandha Powder	Dynamic Headspace (EtO & 2-CE) - Solvent extraction	GCMS-TQ8050 NX with HS-20 NX
3.3	Boswellia Serrata Powder	Liquid Injection (EtO & 2-CE)	GCMS-TQ8050 NX with AOC-20i/s
		Dynamic Headspace (EtO & 2-CE) - Solvent extraction	GCMS-TQ8050 NX with HS-20 NX
4.1.	Noodles	Liquid Injection (EtO & 2-CE)	GCMS-TQ8050 NX with AOC-20i/s
5.1	Intraocular Lens	Static Headspace (EtO)	GCMS-QP2010 Ultra with HS-20
5.2	Medical Device	Liquid Injection / Static Headspace (EtO)	Nexis GC-2030 with AOC-20i/HS-20

1.1 Sesame Seeds Analysis - (Application Note)

EtO & 2-CE Analysis in Sesame Seeds

- This section deals with various approaches, extraction process & different instrument techniques used for the analysis of EtO and 2-CE in sesame seeds sample

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
EURL-SRM Analytical Observation Report (EtO & 2-CE)	Liquid	SPL	AOC-20i/s - GCMS-TQ8050 NX
Headspace – Method 1st (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX
Headspace – Method 2nd (2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX
Headspace – Method 3rd (EtO)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX

SCAN & DOWNLOAD

Published Application Note

- **Headspace** – Method 1st (EtO & 2-CE): Solvent extraction followed by the analysis with dynamic headspace mode. For simultaneous analysis of EtO & 2-CE in different matrices, above mentioned method is used most of the time.

Trace level quantitation of Ethylene Oxide (EtO) and 2-Choloroethanol (2-CE) in sesame seeds by using various GCMS/MS techniques with their own merits and demerits

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. 2-CE is prominent metabolite of EtO and is equally hazardous compound. EU-MRLS (Maximum Residue Levels as per European Commission) for EtO & 2-CE are different for different commodities. Out of many matrices EU-MRL for cereals, pulses & vegetables are the lowest and that is 0.02 mg/kg. Considering carcinogenicity and no acceptable threshold for exposure, no Acceptable Daily Intake (ADI) was established for EtO & 2-CE and hence it is very important to quantitate EtO & 2-CE, as low as possible in the food matrices.

With reference to EURL-SRM Analytical Observation Report, Shimadzu have successfully developed and validated methods for trace level quantification of EtO & 2-CE impurities in sesame seeds by using Shimadzu GCMS-TQ8050 NX with AOC-20i and AOC-20s liquid auto sampler / HS-20 NX headspace sampler (Dynamic)

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Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8050 NX, HS-20 NX; AOC™-20i / AOC-20s

Trace level quantitation of Ethylene Oxide (EtO) and 2-Chloroethanol (2-CE) in sesame seeds by using various GCMS/MS techniques with their own merits and demerits

Sanket Chiplunkar, Aseem Wagle, Rahul Dwivedi, Sujit Patil, Durvesh Sawant, Prashant Hase, Nitish Suryawanshi, Jitendra Kelkar, Pratap Rasam and Dheeraj Handique
Shimadzu Analytical (India) Pvt. Ltd.

User Benefits

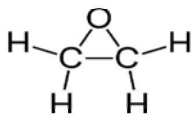
- Easy quantitation of EtO and 2-CE at 5 times lower than MRLs, in single run without derivatization / conversion
- Dynamic HS method involves less sample preparation, less contamination, less interference with low maintenance.
- Lower LOQs achieved for EtO and 2-CE using dynamic HS when measured in isolation

■ Introduction

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 and genotoxic impurity for living being and hence it is very important to quantitate EtO in food matrices.

EtO,2-CE & their metabolites : EtO fumigation has been initiated in India and other developing countries as a counter measure for reducing the incidences of sesame seed contaminations with salmonella and other fecal bacteria. After fumigation of food commodities with EtO, evaporation & the reactions with matrix constituents are the main dissipation pathways of EtO in food.
Once in contact with the food, EtO undergoes various reactions within the matrix and generate various reaction products, include ethylene glycol, diethylene glycol, dioxan, 2-bromoethanol (known as ethylene bromohydrin) & 2-CE (known as ethylene chlorohydrin). Also, EtO directly reacts with matrix components, such as amino acids, purines and fatty acids forming hydroxyethyl adducts.
2-CE is the most prominent reaction product of EtO. 2-CE is also an extremely hazardous substance. In matrix, 2-CE, undergoes reactions with fatty acids forming 2-CE esters.
EtO,2-CE (Figure 1) & their various reaction products are only removed at a limited extend, during aeration and many of them can serve as markers for EtO-fumigations.

Ethylene Oxide (EtO)
(Molecular Formula – C_2H_4O)



2-Chloroethanol (2-CE)
(Molecular Formula – C_2H_5ClO)

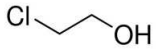


Figure 1: Structure of EtO & 2-CE

Toxicity/Regulations/Method : 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.
In 2008. regulatory authorities decided to introduce a joint residue definition for the two components: "Sum of ethylene oxide & 2- chloroethanol expressed as ethylene oxide" & this residue definition is still valid today.
EU-MRLS (Maximum Residue Levels as per European Commission) for EtO & 2-CE are summarized in Table 1.

Table 1: EU-MRLS for EtO & 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. 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 and AOC-20i/ AOC-20s (Figure 2) is used for the analysis of EtO & 2-CE.

Figure 2: GCMS-TQ™8050 NX with HS-20 NX & AOC™-20i / AOC™ -20s

■ Experimental

A mixture of EtO and 2-CE standards (2 ppm) was analyzed using 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.

The optimized MRM transitions of EtO & 2-CE standards are given in Table 2.

Table 2: MRM transitions of EtO & 2-CE

MRM Transitions						
Details	MRM-1	CE	MRM-2	CE	MRM-3	CE
EtO	44>29	6	44>28	6	44>14	18
2-CE	80>31	6	80>44	5	82>31	6

■ Method

Brief about liquid injection method is given in Table 3.

Table 3: Brief of liquid injection method

Method Details	Name of the compounds	Mode
Method-1	EtO & 2-CE in single method	Liquid Injection

Brief about all three headspace methods is given in Table 4.

Table 4: Brief of headspace methods

Method Details	Name of the compounds	Mode
Method-2	EtO & 2-CE in single method	Headspace Injection
Method-3	Only 2-CE	
Method-4	Only EtO	

Brief about analytical conditions for liquid injection & headspace injection are given in Table 5.

Table 5: Analytical conditions

GCMS System	:	GCMS-TQ8050 NX		
Liquid Sampler	:	AOC-20i and AOC-20s		
Headspace Sampler	:	HS-20 NX (Dynamic Headspace)		
Gas Chromatography Parameters				
Capillary Column	:	RTX-VMS (60 m X 0.45 mm ID x 2.55 um df)		
Injection Mode	:	Split		
Flow Control Mode	:	Column Flow		
Carrier Gas	:	Helium		
Column Flow	:	3.00 mL/min		
Linear Velocity	:	44.0 cm/s		
Purge Flow	:	3.0 mL/min		
Split Ratio	:	5 (For liquid injection method)		
Diluent	:	Acetonitrile		
Temp. Program	:	Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
			35.0	5.00
		20	235.0	5.00
MS Parameters				
Ionization Mode	:	EI		
Ion Source Temp.	:	230 °C		
Interface Temp.	:	230 °C		
CID Gas	:	Argon		
CID Gas pressure	:	200 kPa		
Tunning	:	High sensitivity		

Headspace parameters & split ratio				
Method	:	2	3	4
Oven Temp.	:	115 °C	110 °C	115 °C
Sample Line Temp.	:	120 °C	120 °C	120° C
Transfer Line Temp.	:	130 °C	130 °C	130 °C
Trap Cooling Temp.	:	-10 °C	-10 °C	-10 °C
Trap Desorb Temp.	:	280 °C	260 °C	280 °C
Trap Equilib. Temp.	:	-10 °C	-10 °C	-10 °C
Shaking Level	:	5	5	5
Multi Inj. Count	:	1	10	1
Pressurizing Gas Pressure (kPa)	:	192	192	192
Equilibrating Time (min)	:	15	15	15
Pressurizing Time (min)	:	0.5	0.5	0.5
Pressure Equilib. Time (min)	:	0.1	0.1	0.1
Load Time (min)	:	0.5	0.5	0.5
Load Equilib. Time (min)	:	0.1	0.1	0.1
Dry Purge Time (min)	:	0	0	0
Injection Time (min)	:	10	15	10
Needle Flush Time (min)	:	10	15	10
GC Cycle Time (min)	:	35	35	35
Split Ratio	:	20	5	20
Total Flow (mL)	:	66	21	66
Trap Tube	:	Tenax TA		
Vial Cap With Septa	**:**	**P/N - 226-84523-11**		
20 mL Headspace Vial	**:**	**P/N - 226-84520-02**		

***For this application use above-mentioned vials & caps with septa**

■ Liquid Injection (Method-1)

(Analysis of EtO & 2-CE in sesame seeds by liquid injection)

■ Sample Analysis

Extraction of EtO & 2-CE from sesame seeds for liquid injection

5000 mg of sesame seeds sample + 10000 uL of diluent (Acetonitrile), mixed well & vortex for 15 minutes

↓

Centrifuge for 5 min at 5000 rpm at 10 °C.

↓

Removed 5000 uL of supernant from above solution, transferred it into 15 mL of Tarson tube

↓

Add cleanup reagent and vortex for 5 minutes

↓

Centrifuge for 5 min at 5000 rpm at 10 °C.

↓

Removed supernant from above solution (matrix blank) and proceed for the analysis by using GC-MS/MS equipped with liquid injector

The optimized extraction and GC-MS/MS method was used for part method validation (As per ICH guidelines).

■ Linearity Solutions

Linearity standard stocks were prepared as mentioned in Table 6.

Table 6: Linearity standard stock solution preparations

Linearity Levels	Linearity stock Conc. in (ppb)	Volume taken from stock (µL)	Volume of diluent (µL)	Conc. in (ppb)
Level - 1	1000	125	9875	12.5
Level - 2		250	9750	25
Level - 3		500	9500	50
Level - 4		1250	8750	125
Level - 5		2500	7500	250

■ Matrix Match Linearity Solutions

Matrix match linearity standard solutions were prepared as mentioned in Table 7.

Table 7: Matrix match linearity standard solution preparations

Linearity Levels	Linearity level Conc. in (ppb)	Volume taken from linearity levels (µL)	Volume of matrix blank (µL)	Conc. in (ppb)
MM Level - 1	12.5	200	800	2.5
MM Level - 2	25	200	800	5
MM Level - 3	50	200	800	10
MM Level - 4	125	200	800	25
MM Level - 5	250	200	800	50

MM = Matrix Match

Note: In recovery study of liquid injection method, post spiked matrix matched calibration standards were used to calculate concentration of EtO & 2-CE in prespike sesame seeds samples.

■ Spiked Recovery Test

Weigh 5000 mg (± 10%) of sesame seeds and add respective uL of linearity standard stock solution. Further add diluent to make up volume of 10000 uL followed by above extraction procedure.

Figure 3 & 4 depicts the calibration curve, overlay of linearity standards & LOQ level chromatograms of EtO & 2-CE for Method-1.

EtO

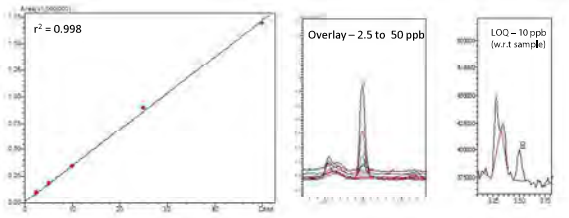


Figure 3: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO

2-CE

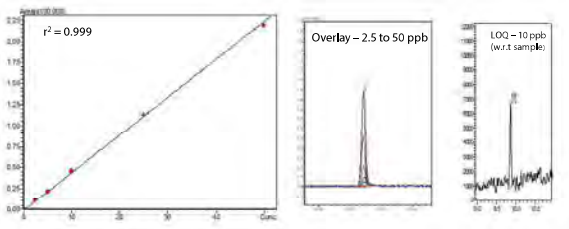


Figure 4: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE

■ Validation Parameters

Linearity :

Summary of calibration standard is shown in Table 8.

Table 8: Summary for linearity (n=3 for each level)

Method =>	Method-1	
	EtO	2-CE
Linearity levels (On column)	2.5,5,10,25 & 50 ppb	
r^2 (n=3)	0.99889	0.99917

r^2 = coefficient of determination

Precision :

Summary of precision standard solutions is shown in Table 9.

Table 9: Summary for precision (n=6)

Method =>	Method-1	
	EtO	2-CE
LOQ level conc.	10 ppb	10 ppb
% RSD (n=6)	7.7	9.4
S/N	14	34

RSD = Relative Standard Deviation

S/N = Signal to Nosie ratio

Accuracy:

Summary of accuracy is shown in Table 10.

Table 10: Summary for accuracy (n=3 for each level)

Method =>	Method-1	
	EtO	2-CE
Spiked LOQ conc.	10 ppb	10 ppb
Avg of % recovery (n=3)	73%	85%
% RSD (n=3)	8.8	4.9
Spiked middle conc.	20 ppb	20 ppb
Avg of % recovery (n=3)	79%	96%
% RSD (n=3)	6.3	2.8
Spiked highest conc.	50 ppb	50 ppb
Avg of % recovery (n=3)	85%	98%
% RSD (n=3)	2.3	2.9

Merits of liquid injection method

- EtO and 2-CE can be measured in single run with 10 ppb LOQ conc.
- No additional accessory is required, and no additional sample preparation (i.e., derivatization) is required.
- Non-derivatised method avoids possibility of incomplete derivatization or errors in sample preparation.

Demerits of liquid injection method

- Depending on type of the matrix, liquid injection method may require development of sample clean up procedure, to remove matrix interference or matrix effect.
- Even after proper clean up, chances of introduction of matrix in injection system are high which reduces life of consumables like column, liner, septum etc. and increases requirement of maintenance.
- High matrix effect may lead to prepare matrix match calibration and which leads to additional sample preparation.
- Matrix may have interferences when heated at high temperature in injection port leading to false quantitation.

■ Dynamic Headspace Injection (Method-2)

(Analysis of EtO & 2-CE in sesame seeds by single method)

■ Linearity Solutions

Standard solutions for linearity were prepared as mentioned in Table 11.

Table 11: Linearity standard solution preparations

Linearity Levels	Linearity stock Conc. in (ppb)	Volume taken from stock (µL)	Volume of diluent (µL)	Conc. in (ppb)
Level - 1	100	1000	9000	10
Level - 2		2000	8000	20
Level - 3		3000	7000	30
Level - 4		4000	6000	40
Level - 5		5000	5000	50

100 uL from above solution, transferred it into 20 mL HS vial and analysed as per optimized method.

■ Sample Analysis

Extraction of EtO & 2-CE from sesame seeds

1000 mg of sesame seeds sample + 1000 uL of diluent (Acetonitrile), mixed well & vortex for 15 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

■ Spiked Recovery Test

Weigh 1000 mg (± 10%) of sesame seeds and further add 1000 uL of respective linearity standard solution followed by above extraction procedure.

Figure 5 & 6 depicts the calibration curve, overlay of linearity standards & LOQ level chromatograms of EtO & 2-CE for Method-2.

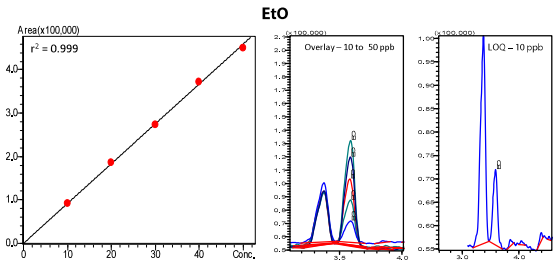


Figure 5: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO

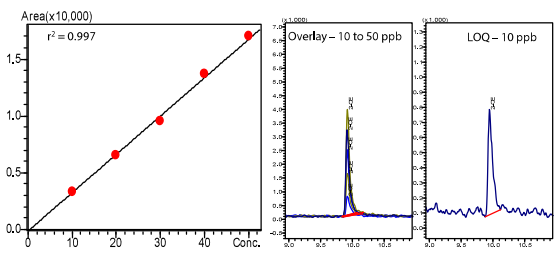


Figure 6: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE

■ Dynamic Headspace Injection (Method-3)

(Isolation method for analysis of only 2-CE in sesame seeds)

■ Linearity Solutions

Standard solutions for linearity were prepared as mentioned in Table 12.

Table 12: Linearity standard solution preparations

Linearity Levels	Linearity stock Conc. in (ppb)	Volume taken from stock (µL)	Volume of diluent (µL)	Conc. in (ppb)
Level - 1	10	100	9900	0.1
Level - 2		500	9500	0.5
Level - 3		1000	9000	1.0
Level - 4		2000	8000	2.0
Level - 5		3000	7000	3.0
Level – 6		4000	6000	4.0
Level - 7		5000	5000	5.0

■ Sample Analysis

Extraction of 2-CE from sesame seeds

100 mg of sesame seeds sample + 1000 uL of diluent (Acetonitrile), mixed well & vortex for 15 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

■ Spiked Recovery Test

Weigh 100 mg (± 10%) of sesame seeds and further add 1000 uL of respective linearity standard solution followed by above extraction procedure.

Figure 7 depicts the calibration curve, overlay of linearity standards & LOQ level chromatogram of 2-CE for Method-3.

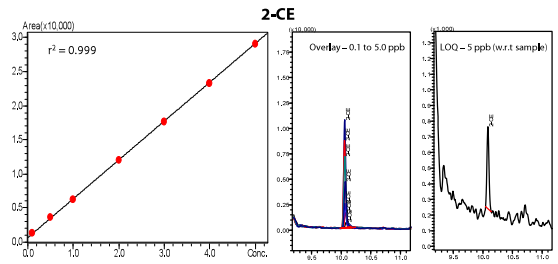


Figure 7: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE

■ Dynamic Headspace Injection (Method-4)

(Isolation method for analysis of only EtO in sesame seeds)

■ Linearity Solutions

Standard solutions for linearity were prepared as mentioned in Table 13.

Table 13: Linearity standard solution preparations

Linearity Levels	Linearity stock Conc. in (ppb)	Volume taken from stock (µL)	Volume of diluent (µL)	Conc. in (ppb)
Level - 1	100	200	9800	2
Level - 2		400	9600	4
Level - 3		600	9400	6
Level - 4		800	9200	8
Level - 5		1000	9000	10

1000 uL from above solution, transferred it into 20 mL HS vial and analysed as per optimized method.

■ Sample Analysis

Extraction of EtO from sesame seeds

5000 mg of sesame seeds sample + 5000 uL of diluent (Acetonitrile), mixed well & vortex for 15 minutes

↓

Centrifuge for 5 min at 5000 rpm at 10°C.

↓

Removed 1000 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

■ Spiked Recovery Test

Weigh 5000 mg (± 10%) of sesame seeds and further add 5000 uL of respective linearity standard solution followed by above extraction procedure.

Figure 8 depicts the calibration curve, overlay of linearity standards & LOQ level chromatogram of EtO for Method-4.

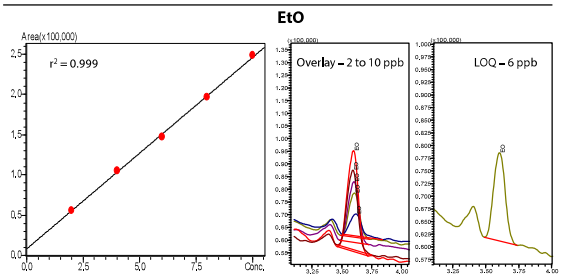


Figure 8: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO

■ Validation Parameters

Linearity :

Summary of calibration standard is shown in Table 14.

Table 14: Summary for linearity (n=3 for each level)

Method =>	Method-2	Method-3	Method-4
	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	0.99974	0.99906
	2-CE - 0.99785		

Precision :

Summary of precision standard solutions is shown in Table 15.

Table 15: Summary for precision (n=6)

Method =>	Method-2		Method-3	Method-4
	EtO	2-CE	2-CE	EtO
LOQ level conc.	10 ppb	10 ppb	5.0 ppb	6 ppb
% RSD (n=6)	2.1	4.9	9.1	1.7
S/N	16	57	53	26
Middle level conc.	30 ppb	30 ppb	30 ppb	-
% RSD (n=6)	2.1	2.6	4.1	-
S/N	38	99	197	-
Highest level conc.	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

Accuracy:

Summary of accuracy is shown in Table 16.

Table 16: Summary for accuracy (n=3 for each level)

Method =>	Method-2		Method-3	Method-4
	EtO	2-CE	2-CE	EtO
Spiked LOQ conc.	10 ppb	10 ppb	5 ppb	6 ppb
Avg of % recovery (n=3)	91%	121%	102%	82%
% RSD (n=3)	1.9	2.0	1.3	1.0
Spiked middle conc.	30 ppb	30 ppb	30 ppb	-
Avg of % recovery (n=3)	88%	113%	98%	-
% RSD (n=3)	5.9	1.3	1.6	-
Spiked highest conc.	50 ppb	50 ppb	50 ppb	10 ppb
Avg of % recovery (n=3)	91%	101%	100%	90%
% RSD (n=3)	3.0	2.6	2.2	1.4

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 a single run with 10 ppb LOQ conc. by using Method-2, whereas 2-CE can be measured with 5 ppb LOQ conc. by using Method-3 and EtO can be measured with 6 ppb LOQ conc. by using Method-4.
- No clean up reagents or extraction salts are used and hence no additional sample preparation which minimizes errors.

Demerits of headspace injection method

- Dynamic headspace is an additional accessory.

Data obtained from both mode of analysis (liquid & headspace) is well compared with each other, & summary of results were given in Table 17.

Table 17: Summary for comparison data

Method =>	Liquid Injection Method		Headspace Injection Method			
	Method-1		Method-2		Method-3	Method-4
	EtO	2-CE	EtO	2-CE	2-CE	EtO
LOQ level conc. (on column)	5 ppb	5 ppb	10 ppb	10 ppb	0.5 ppb	6 ppb
LOQ level conc. (w.r.t sample)	10 ppb	10 ppb	10 ppb	10 ppb	5 ppb	6 ppb
% RSD (n=6)	7.7	9.4	2.1	4.9	9.1	1.7
Linearity levels (on column)	2.5,5,10,25 & 50 ppb		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
Linearity levels (w.r.t sample)	5,10,20,50 & 100 ppb		10,20,30,40 & 50 ppb		1,5,10,20,30, 40 & 50 ppb	2,4,6,8 & 10 ppb
r^2 (n=3 of each level)	0.99889	0.99917	0.99950	0.99785	0.99974	0.99906
Spiked LOQ level (on column)	5 ppb	5 ppb	10 ppb	10 ppb	0.5 ppb	6 ppb
Spiked LOQ level (w.r.t sample)	10 ppb	10 ppb	10 ppb	10 ppb	5 ppb	6 ppb
Avg of % recovery (n=3)	73%	85%	91%	121%	102%	82%
Spiked highest level (on column)	25 ppb	25 ppb	50 ppb	50 ppb	5 ppb	10 ppb
Spiked highest level (w.r.t sample)	50 ppb	50 ppb	50 ppb	50 ppb	50 ppb	10 ppb
Avg of % recovery (n=3)	85%	98%	91%	101%	100%	90%
Lowest conc. (on column)	2.5 ppb	2.5 ppb	10 ppb	10 ppb	0.1 ppb	2 ppb
Lowest conc. (w.r.t sample)	5 ppb	5 ppb	10 ppb	10 ppb	1 ppb	2 ppb
Sample preparation time	35-40 min		20-25 min		20-25 min	20-25 min
Sample preparation conc.	50%		100%		10%	100%
Cost	Cleanup reagent/ QuEChERS-Required		Cleanup reagent/QuEChERS-Not Required			
Regulatory compliance	Meets EU-MRLs		Meets EU-MRLs			

■ Results

- Trace level quantification of EtO & 2-CE impurities in sesame seeds was successfully performed by using Shimadzu GCMS-TQ8050 NX with AOC-20i and AOC-20s liquid auto sampler / HS-20 NX headspace sampler (Dynamic).
- Shimadzu's GCMS-TQ8050 NX with AOC-20i / AOC-20s liquid autosampler & HS-20 NX dynamic headspace sampler is complete tool for the analysis of EtO & 2-CE.

■ Conclusion

- For EtO & 2-CE analysis, dynamic headspace mode outperforms the current regulatory limits. Dynamic headspace has an edge over liquid injection technique in terms of sample preparation, less matrix interference and precise quantitation.
- 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.

SHIMADZU

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1.2 Sesame Seeds Analysis - Direct Sample Method

EtO & 2-CE Analysis in Sesame Seeds (Direct Sample In Headspace Vial)

- As such sesame seed sample preparation into headspace vial for trace level quantitation of EtO & 2-CE. In this approach, sesame seeds sample was directly taken into headspace vial and analyzed for its EtO & 2-CE content.
- Shimadzu have successfully part validated this method by using Shimadzu's GCMS-TQ8040 NX with HS-20 NX headspace sampler

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
As such sample in headspace vial (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8040 NX

System Description

GCMS System	GCMS-TQ8040 NX
Headspace Sampler	HS-20 NX (Dynamic Headspace)
Capillary Column	SH-RTX-VMS (60 m X 0.25 mm ID x 1.4 um df)
Ionization Mode	Electron Ionization (EI)
Sample Information	EtO & 2-CE in sesame seeds
Sample Preparation	100 % (As such sample in HS vial) + 1 uL Acetonitrile (Diluent)
Spiked Sample Preparation	100 mg Sesame Seeds + 1 uL of 1 ppm Standard
	100 mg Sesame Seeds + 1 uL of 2 ppm Standard
	100 mg Sesame Seeds + 1 uL of 3 ppm Standard

Benefits

- As such sample preparation in headspace vial (without solvent extraction / without any conversion or derivatization process)
- Very less sample preparation time
- Very cost effective
- Shimadzu's optimized solution of dynamic headspace sampler for the analysis of EtO & 2-CE outperforming the current regulatory limits.

1.2 Sesame Seeds Analysis - Direct Sample Method

Chromatography Parameters				
GCMS System	:	GCMS-TQ8040 NX		
Liquid Sampler	:	-		
Headspace Sampler	:	HS-20 NX (Dynamic Headspace)		
Gas Chromatography Parameters				
Capillary Column	:	SH-VMS (60 m X 0.25 mm ID x 1.4 um df)		
Injection Mode	:	Split		
Flow Control Mode	:	Column Flow		
Carrier Gas	:	Helium		
Column Flow	:	2.00 mL/min		
Linear Velocity	:	36.0 cm/s		
Purge Flow	:	3.0 mL/min		
Split Ratio	:	10		
Diluent	:	Acetonitrile		
Temp. Program		Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
			35.0	5.00
		30	235.0	0.33
MS Parameters				
Ionization Mode	:	EI		
Ion Source Temp.	:	250 °C		
Interface Temp.	:	240 °C		
CID Gas	:	Argon		
CID Gas pressure	:	200 kPa		

Headspace parameters		
Method	:	Dynamic HS
Oven Temp.	:	110 °C
Sample Line Temp.	:	120 °C
Transfer Line Temp.	:	130 °C
Trap Cooling Temp.	:	-20 °C
Trap Desorb Temp.	:	280 °C
Trap Equilib. Temp.	:	-20 °C
Shaking Level	:	5
Multi Inj. Count	:	1
Pressurizing Gas Pressure (kPa)	:	192
Equilibrating Time (min)	:	15
Pressurizing Time (min)	:	0.5
Pressure Equilib. Time (min)	:	0.0
Load Time (min)	:	0.5
Load Equilib. Time (min)	:	0.0
Dry Purge Time (min)	:	0.0
Injection Time (min)	:	10
Needle Flush Time (min)	:	10
GC Cycle Time (min)	:	30
Split Ratio	:	10
Total Flow (mL)	:	25
Trap Tube	:	Tenax Ta

Representative Data

Direct Sample Method - Analysis of EtO & 2-CE (Dynamic Headspace)

EtO

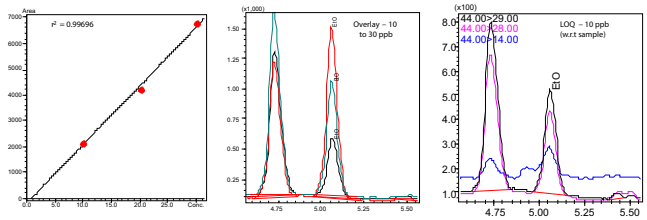


Figure 1: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution

2-CE

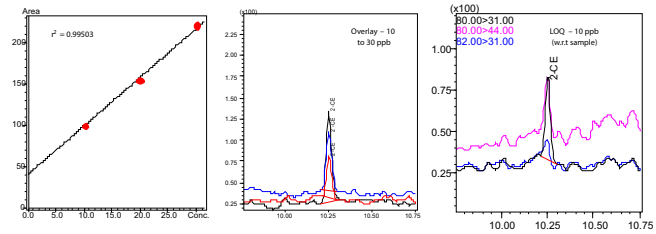


Figure 2: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution

Results :

Summary of results is shown in Table 1

Method =>	Dynamic Headspace	
	EtO	2-CE
Linearity levels (On column)	10,20 & 30 ppb	10,20 & 30 ppb
Linearity levels (w.r.t sample)	10,20 & 30 ppb	10,20 & 30 ppb
r^2 (n=3)	0.99696	0.99503
Level 1 - conc. (On column)	10 ppb	10 ppb
Level 1 - conc. (w.r.t sample)	10 ppb	10 ppb
% RSD (n=3)	2.2	10.1
S/N	26	28
Level 2 - conc. (On column)	20 ppb	20 ppb
Level 2 - conc. (w.r.t sample)	20 ppb	20 ppb
% RSD (n=3)	2.7	11.7
Level 3 - conc. (On column)	30 ppb	30 ppb
Level 3 - conc. (w.r.t sample)	30 ppb	30 ppb
% RSD (n=3)	9.7	14.1
Prespike 10 ppb – 1st (Recovery)	97 %	101 %
Prespike 10 ppb – 2nd (Recovery)	105 %	91 %

1.3 Sesame Seeds Analysis - Conversion Method

EtO & 2-CE Analysis in Sesame Seeds (Conversion)

- With reference to EURL-SRM Analytical Observation Report, Shimadzu have successfully optimized & part validated conversion method (EtO=>2-CE) for the trace level quantification of 2-CE in sesame seeds by using Shimadzu GCMS-TQ8040 NX with AOC-20i and AOC-20s (Liquid) / HS-20 NX (dynamic headspace)

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
EtO to 2-CE Conversion	Liquid	SPL	AOC-20i/s- GCMS-TQ8040 NX
EtO to 2-CE Conversion	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8040 NX

System Description

GCMS System	GCMS-TQ8040 NX
Liquid Sampler	AOC-20i and AOC-20s
Headspace Sampler	HS-20 NX (Dynamic Headspace)
Capillary Column	SH-Stabilwax DA (60 m X 0.25 mm ID x 0.25 um df)
Ionization Mode	Electron Ionization (EI)
Sample Information	2-CE in sesame seeds (Conversion method)
Sample Preparation	20 % for Liquid / Dynamic Headspace
Reference Method	EURL-SRM Analytical Observation report

Benefits

- With reference to EURL-SRM report, Shimadzu have optimized conversion method for trace level quantification of 2-CE.
  A) EtO to 2-CE conversion – Analysed by liquid injection
  B) EtO to 2-CE conversion – Analysed by dynamic headspace injection
- Shimadzu's GCMS-TQ8040 NX with AOC-20i / AOC-20s liquid autosampler & HS-20 NX dynamic headspace sampler is well compatible for the analysis of EtO & 2-CE

Sample Analysis

Conversion of EtO to 2-CE in sesame seeds and analysed by liquid injection

2 g of uncrushed sample + 6 mL of 0.02 N H2SO4 + 1 mL saturated NaCL solution, mixed well & vortex for 5 minutes

↓

Sonicate at 50 °C for 3 hrs

↓

Cool solution in refrigerator at -20 °C for 30 minutes

↓

In above solution add 4 mL water + 10 mL Ethyl acetate (Vortex for 1 minutes)

↓

Then add 10 g of sodium sulfate and vortex it for 1 min

↓

Centrifuge it at 5000 RPM for 5 minutes

↓

1 mL supernant solution+ 25 mg of PSA +250 mg of sodium sulfate & vortex for 5 minutes

↓

Filter through 0.22 um syringe filter

↓

Inject on GCMS/MS through liquid & dynamic headspace injection technique

- For accuracy study – Respective standard stock solution of EtO was prespike and further proceed as mentioned in sample analysis.
- Et0 => 2-CE conversion factor (0.55) was applied in calculation
- For linearity – Respective level of 2-CE standard solution was post spiked in matrix blank solution and further calibration curve was plotted.

1.3 Sesame Seeds Analysis - Conversion Method

Chromatography Parameters				
GCMS System	:	GCMS-TQ8040 NX		
Liquid Sampler	:	AOC-20i and AOC-20s		
Headspace Sampler	:	HS-20 NX (Dynamic Headspace)		
Gas Chromatography Parameters				
Capillary Column	:	SH-Stabilwax DA (60 m X 0.25 mm ID x 0.25 um df)		
Injection Mode	:	Split		
Flow Control Mode	:	Column Flow		
Carrier Gas	:	Helium		
Column Flow	:	1.00 mL/min		
Linear Velocity	:	25.8 cm/s		
Purge Flow	:	3.0 mL/min		
Split Ratio	:	1 (For liquid injection method)		
Injector Port Temp	:	230.0 °C		
Temp. Program		Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
			60.0	2.00
		20	235.0	9.25
MS Parameters				
Ionization Mode	:	EI		
Ion Source Temp.	:	230 °C		
Interface Temp.	:	230 °C		
CID Gas	:	Argon		
CID Gas pressure	:	200 kPa		

Headspace parameters		
Method	:	Dynamic HS
Oven Temp.	:	110 °C
Sample Line Temp.	:	170 °C
Transfer Line Temp.	:	180 °C
Trap Cooling Temp.	:	-10 °C
Trap Desorb Temp.	:	280 °C
Trap Equilib. Temp.	:	25°C
Shaking Level	:	5
Multi Inj. Count	:	10
Pressurizing Gas Pressure (kPa)	:	192
Equilibrating Time (min)	:	15
Pressurizing Time (min)	:	0.5
Pressure Equilib. Time (min)	:	0.1
Load Time (min)	:	0.5
Load Equilib. Time (min)	:	0.1
Dry Purge Time (min)	:	1
Injection Time (min)	:	5
Needle Flush Time (min)	:	15
GC Cycle Time (min)	:	30
Split Ratio	:	15
Total Flow (mL)	:	19
Trap Tube	:	Tenax Ta

Representative Data

Conversion Method – EtO =>2-CE (Liquid & Dynamic)

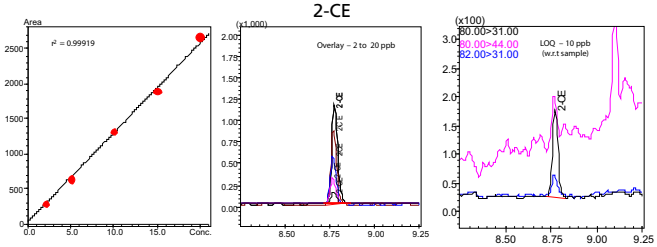


Figure 1: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE (Liquid)

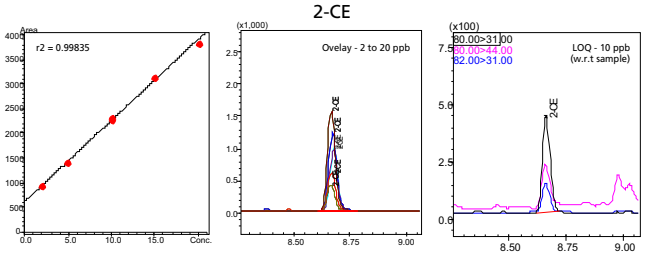


Figure 2: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE (Dynamic)

Results :

Summary of results is shown in Table 1

Method =>	Liquid	Dynamic HS
	2-CE	2-CE
Linearity levels (On column)	2,5,10,15 & 20 ppb	2,5,10,15 & 20 ppb
Linearity levels (w.r.t sample)	10,25,50,75 &100 ppb	10,25,50,75 &100 ppb
r^2 (n=3)	0.99919	0.99835
LOQ level conc. (On column)	2 ppb	2 ppb
LOQ level conc. (w.r.t sample)	10 ppb	10 ppb
% RSD (n=6)	7.6	3.8
S/N	16	44
Spiked LOQ level (On column)	2 ppb	2 ppb
Spiked LOQ level (w.r.t sample)	10 ppb	10 ppb
Avg of % recovery (n=3)	101 %	95 %

2.1 Chilli Powder Analysis (As Per Application Note Method)

EtO & 2-CE Analysis in Chilli Powder

- This section deals with various approaches, extraction process & different instrument techniques used for the analysis of EtO and 2-CE in chilli powder sample

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
EURL-SRM Analytical Observation Report (EtO & 2-CE)	Liquid	SPL	AOC-20i/s - GCMS-TQ8040 NX
	Liquid	SPL	AOC-20i/s - GCMS-TQ8040 NX
	(Analysis by GCMS-TQ8050 NX is to show comparison, only)		
Headspace – Method 1st (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX

System Description

GCMS System	GCMS-TQ8040 NX
Liquid Sampler	AOC-30i
GCMS System	GCMS-TQ8050 NX
Liquid Sampler	AOC-20i/s
Headspace Sampler	HS-20 NX (Dynamic Headspace)
Capillary Column	RTX-VMS (60 m X 0.45 mm ID x 2.55 um df)
Ionization Mode	Electron Ionization (EI)
Sample Information	EtO & 2-CE in chilli powder
Reference Method	EURL-SRM Analytical Observation report

* Chromatographic conditions & sample preparation method were same as mentioned in application note.

Benefits

- Shimadzu have optimized liquid injection method and developed dynamic headspace methods for the analysis of EtO & 2-CE in chilli powder matrix. Both these methods outperforms the current regulatory limits.
- Shimadzu's GCMS-TQ8040 NX / GCMS-TQ8050 NX with liquid autosampler & dynamic headspace sampler is complete tool for the analysis of EtO & 2-CE

2.1 Chilli Powder Analysis (As Per Application Note Method)

Representative Data

Solvent Extraction Method for EtO & 2-CE (Liquid)

EtO

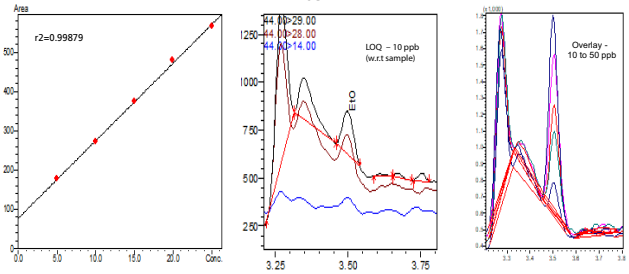


Figure 1: Calibration curve, chromatogram of LOQ solution & overlay of linearity standards for EtO

2-CE

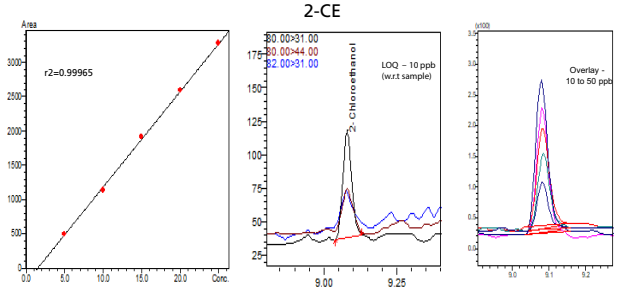


Figure 2: Calibration curve, chromatogram of LOQ solution & overlay of linearity standards for 2-CE

Results :

Linearity :

Summary of calibration standard is shown in Table 1

Table 1 : Summary for linearity (n=3 for each level)

Method =>	Liquid	
	EtO	2-CE
Linearity levels (On column)	5,10,15,20 & 25 ppb	
Linearity levels (w.r.t sample)	10,20,30,40 & 50 ppb	
r^2 (n=3)	0.99879	0.99965

Precision :

Summary of precision standard is shown in Table 2

Table 2: Summary of precision standard (n=6)

Method =>	Liquid	
	EtO	2-CE
LOQ level conc. (On column)	5 ppb	5 ppb
LOQ level conc. (w.r.t sample)	10 ppb	10 ppb
% RSD (n=6)	9.3	3.9
S/N	15	79
Highest level conc. (On column)	25 ppb	25 ppb
Highest level conc. (w.r.t sample)	50 ppb	50 ppb
% RSD (n=6)	5.6	3.9

Accuracy

Summary of accuracy is shown in Table 3

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

Method =>	Liquid	
	EtO	2-CE
Spiked LOQ level conc. (On column)	5 ppb	5 ppb
Spiked LOQ level conc. (w.r.t sample)	10 ppb	10 ppb
Avg of % recovery (n=3)	98 %	103 %
Spiked 2nd level conc. (On column)	10 ppb	10 ppb
Spiked 2nd level conc. (w.r.t sample)	20 ppb	20 ppb
Avg of % recovery (n=3)	96 %	118 %
Spiked 3rd level conc. (On column)	15 ppb	15 ppb
Spiked 3rd level conc. (w.r.t sample)	30 ppb	30 ppb
Avg of % recovery (n=3)	99 %	107 %

Comparison:

Summary of comparison for two instrument is shown in Table 4

Table 4: Summary for comparison

Method => Liquid	GCMS-TQ8040	GCMS-TQ8050
	EtO	EtO
LOQ level conc. (On column)	5 ppb	2.5 ppb
LOQ level conc. (w.r.t sample)	10 ppb	5 ppb
% RSD (n=6)	9.3	8.7
S/N	15	32
	2-CE	2-CE
LOQ level conc. (On column)	5 ppb	2.5 ppb
LOQ level conc. (w.r.t sample)	10 ppb	5 ppb
% RSD (n=6)	3.9	5.0
S/N	79	70

2.1 Chilli Powder Analysis (As Per Application Note Method)

Representative Data

Solvent Extraction Method for EtO & 2-CE (Dynamic)

EtO

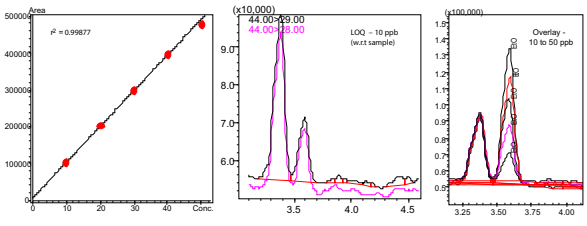


Figure 3: Calibration curve, chromatogram of LOQ solution & overlay of linearity standard for EtO

2-CE

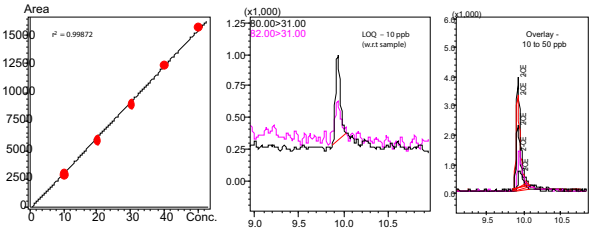


Figure 4: Calibration curve, chromatogram of LOQ solution & overlay of linearity standard for 2-CE

Linearity :

Summary of calibration standard is shown in Table 5

Table 5 : Summary for linearity (n=3 for each level)

Method =>	Liquid	
	EtO	2-CE
Linearity levels (On column)	10,20,30,40 & 50 ppb	
Linearity levels (w.r.t sample)	10,20,30,40 & 50 ppb	
r^2 (n=3)	0.99877	0.99872

Precision :

Summary of precision standard solutions is shown in Table 6

Table 6 : Summary for precision (n=6)

Method =>	Liquid	
	EtO	2-CE
LOQ level conc. (On column)	10 ppb	10 ppb
LOQ level conc. (w.r.t sample)	10 ppb	10 ppb
% RSD (n=6)	2.5	9.7
S/N	15	29

Accuracy:

Summary of accuracy is shown in Table 7

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

Method =>	Dynamic Headspace	
	EtO	2-CE
Spiked LOQ level conc. (On column)	10 ppb	10 ppb
Spiked LOQ level conc. (w.r.t sample)	10 ppb	10 ppb
Avg of % recovery (n=3)	90 %	93 %
Spiked 2nd level conc. (On column)	30 ppb	30 ppb
Spiked 2nd level conc. (w.r.t sample)	30 ppb	30 ppb
Avg of % recovery (n=3)	87 %	114 %
Spiked 3rd level conc. (On column)	50 ppb	50 ppb
Spiked 3rd level conc. (w.r.t sample)	50 ppb	50 ppb
Avg of % recovery (n=3)	71 %	105 %

2.2 Chilli Powder Analysis - Direct Sample Method

EtO & 2-CE Analysis In Chilli Powder (Direct Sample In Headspace Vial)

- As such chilli powder sample preparation into headspace vial for trace level quantitation of EtO & 2-CE. In this approach, chilli powder sample was directly taken into headspace vial and analyzed for its EtO & 2-CE content.
- Shimadzu have successfully part validated this method by using Shimadzu's GCMS-TQ8040 NX with HS-20 NX headspace sampler

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
As such sample in headspace vial (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8040 NX

System Description

GCMS System	GCMS-TQ8040 NX
Headspace Sampler	HS-20 NX (Dynamic Headspace)
Capillary Column	SH-RTX-VMS (60 m X 0.25 mm ID x 1.4 um df)
Ionization Mode	Electron Ionization (EI)
Sample Information	EtO & 2-CE in chilli powder
Sample Preparation	100 % (As such sample in HS vial) + 1 uL of Acetonitrile (Diluent)
Spiked Sample Preparation	100 mg chilli powder + 1 uL of 1 ppm standard
	100 mg chilli powder + 1 uL of 2 ppm standard
	100 mg chilli powder + 1 uL of 3 ppm standard
	100 mg chilli powder + 1 uL of 4 ppm standard
	100 mg chilli powder + 1 uL of 5 ppm standard

Benefits

- As such sample preparation in headspace vial (without solvent extraction / without any conversion or derivatization process)
- Very less sample preparation time
- Highly cost effective
- Shimadzu's optimized solution of dynamic headspace sampler for the analysis of EtO & 2-CE outperforming the current regulatory limits

2.2 Chilli Powder Analysis - Direct Sample Method

Chromatography Parameters				
GCMS System	:	GCMS-TQ8040 NX		
Liquid Sampler	:	-		
Headspace Sampler	:	HS-20 NX (Dynamic Headspace)		
Gas Chromatography Parameters				
Capillary Column	:	SH-VMS (60 m X 0.25 mm ID x 1.4 um df)		
Injection Mode	:	Split		
Flow Control Mode	:	Column Flow		
Carrier Gas	:	Helium		
Column Flow	:	2.00 mL/min		
Linear Velocity	:	36.0 cm/s		
Purge Flow	:	3.0 mL/min		
Split Ratio	:	8		
Diluent	:	Acetonitrile		
Temp. Program		Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
			35.0	5.00
		40	235.0	5.00
MS Parameters				
Ionization Mode	:	EI		
Ion Source Temp.	:	250 °C		
Interface Temp.	:	240 °C		
CID Gas	:	Argon		
CID Gas pressure	:	200 kPa		

Headspace parameters		
Method	:	Dynamic HS
Oven Temp.	:	70 °C
Sample Line Temp.	:	110 °C
Transfer Line Temp.	:	120 °C
Trap Cooling Temp.	:	-20 °C
Trap Desorb Temp.	:	280 °C
Trap Equilib. Temp.	:	-20 °C
Shaking Level	:	5
Multi Inj. Count	:	3
Pressurizing Gas Pressure (kPa)	:	192
Equilibrating Time (min)	:	15
Pressurizing Time (min)	:	0.5
Pressure Equilib. Time (min)	:	0.1
Load Time (min)	:	0.5
Load Equilib. Time (min)	:	0.1
Dry Purge Time (min)	:	0.0
Injection Time (min)	:	10
Needle Flush Time (min)	:	10
GC Cycle Time (min)	:	28
Split Ratio	:	8
Total Flow (mL)	:	21
Trap Tube	:	Tenax Ta

2.2 Chilli Powder Analysis - Direct Sample Method

Representative Data

Direct Sample Method - Analysis of EtO & 2-CE (Dynamic Headspace)

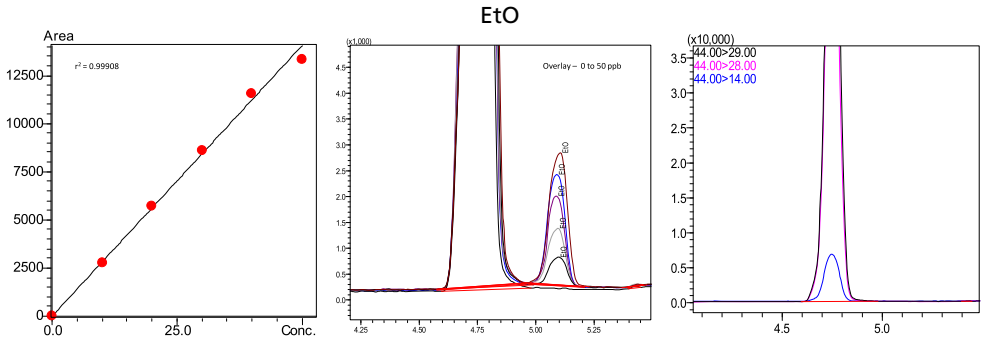


Figure 1: Calibration curve, overlay of linearity standards & chromatogram of sample solution

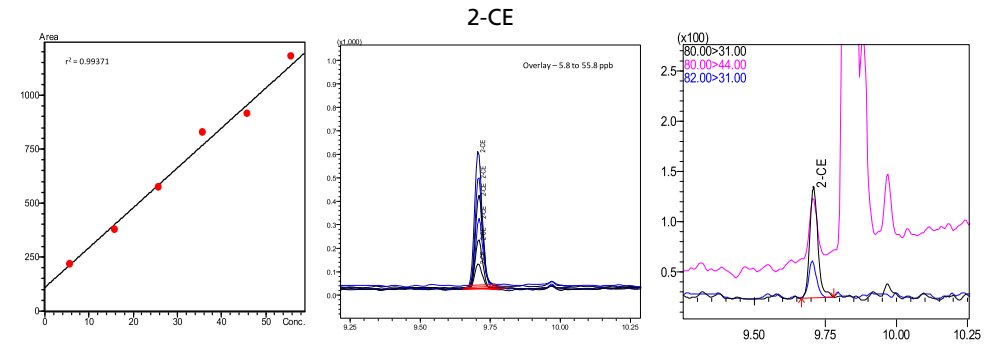


Figure 2: Calibration curve, overlay of linearity standards & chromatogram of sample solution

Results :

Summary of results is shown in Table 1

Table 1 : Summary of results

Method =>	Dynamic Headspace	
	EtO	2-CE
Linearity levels (On column)	10,20,30,40 & 50 ppb	10,20,30,40 & 50 ppb
Linearity levels (w.r.t sample)	10,20,30,40 & 50 ppb	10,20,30,40 & 50 ppb
r^2 (n=3)	0.99908	0.99371
Level 1 - conc. (On column)	10 ppb	10 ppb
Level 1 - conc. (w.r.t sample)	10 ppb	10 ppb
% RSD (n=3)	12.9	3.2
S/N	42	97
Sample (Conc. observed)	-	5.8 ppb
Pre spiked 10 ppb (Conc. observed)	10.8 ppb	17.3 ppb
Avg of % recovery (n=3)	108 %	109 %

2.3 & 2.4 Other Spices - Dynamic Headspace Method

EtO & 2-CE Analysis In Other Spices (Extracted solution In Headspace Vial)

2.3 Chicken Masala

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
Headspace – Method 1st (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX

2.4 Kitchen Masala

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
Headspace – Method 1st (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX

System Description

GCMS System	GCMS-TQ8050 NX
Headspace Sampler	HS-20 NX (Dynamic Headspace)
Capillary Column	RTX-VMS (60 m X 0.45 mm ID x 2.55 um df)
Ionization Mode	Electron Ionization (EI)
Sample Information	EtO & 2-CE in Other Spices
Reference Method	EURL-SRM Analytical Observation report

* Chromatographic conditions & sample preparation method were same as mentioned in application note.

Benefits

- Shimadzu have developed dynamic headspace methods for the analysis of EtO & 2-CE in spices matrix. This method outperforms the current regulatory limits.
- Shimadzu's GCMS-TQ8040 NX / GCMS-TQ8050 NX with liquid autosampler & dynamic headspace sampler is complete tool for the analysis of EtO & 2-CE.

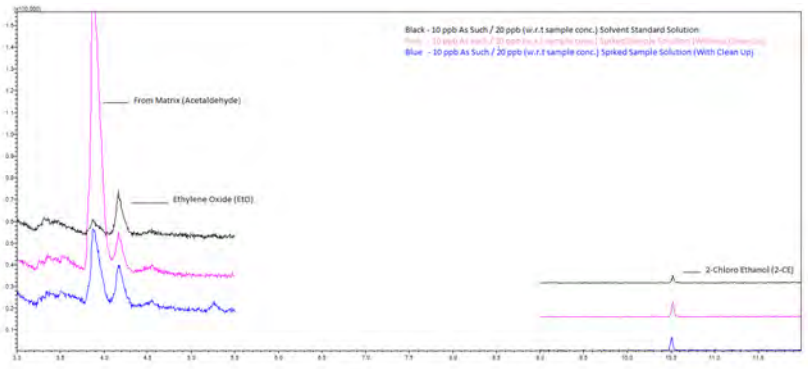
How to reduce acetaldehyde

- Spices matrix have very high amount of acetaldehyde, and which may interfere with EtO in analysis.
- So, to reduce acetaldehyde concentration from extracted sample solution, PSA is used as clean up reagent
- Amount (25 mg – 100 mg) optimization is require as per the type of matrix

2.3 & 2.4 Other Spices - Dynamic Headspace Method

- Generally clan up reagent is used in liquid injection method
- But here we used clean up reagent to reduce acetaldehyde concentration from sample solution.
- Because of the reduction in conc. of acetaldehyde, recovery for EtO is well improved and is almost 100%

Effect of clean up reagent => Reduces acetaldehyde conc. in sample solution



Representative Data

Solvent Extraction Method for EtO & 2-CE (Dynamic)

EtO

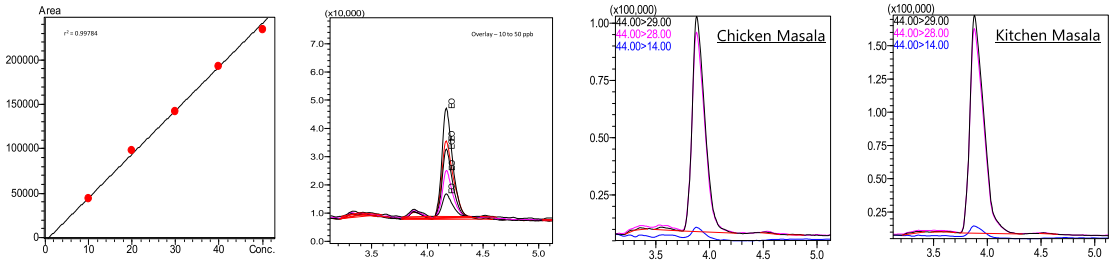


Figure 1 : Calibration curve, overlay of linearity standards & sample chromatogram for EtO (Dynamic)

2-CE

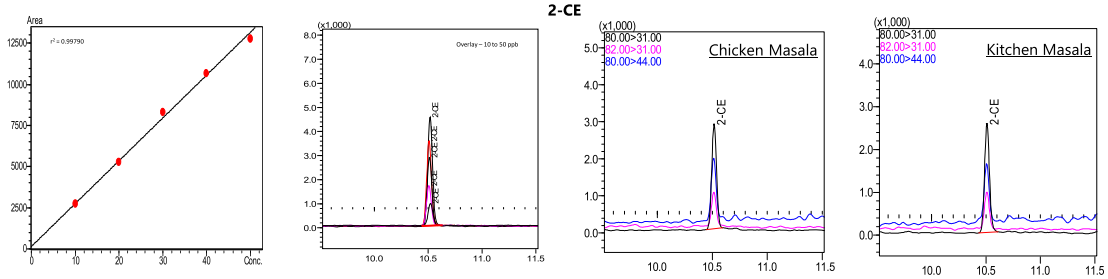


Figure 2 : Calibration curve, overlay of linearity standards & sample chromatogram for 2-CE (Dynamic)

Chicken Masala :

Linearity :

Summary of calibration standard is shown in Table 1

Table 1 : Summary of solvent standard linearity (n=1 for each level)

Method =>	Dynamic Headspace	
	EtO	2-CE
Linearity levels (On column)	10,20,30,40 & 50 ppb	
Linearity levels (w.r.t sample)	10,20,30,40 & 50 ppb	
r^2 (n=3)	0.99784	0.99790

Sample Analysis:

Summary of sample analysis is shown in Table 2

Table 2 : Summary for sample analysis

Method =>	Dynamic Headspace	
	EtO	2-CE
Sample (Conc. observed)	-	28 ppb
Pre spiked 20 ppb (Conc. observed)	21 ppb	45 ppb
% recovery	105 %	94 %

Kitchen Masala :

Linearity :

Summary of calibration standard is shown in Table 3

Table 3 : Summary of solvent standard linearity (n=1 for each level)

Method =>	Dynamic Headspace	
	EtO	2-CE
Linearity levels (On column)	10,20,30,40 & 50 ppb	
Linearity levels (w.r.t sample)	10,20,30,40 & 50 ppb	
r^2 (n=3)	0.99784	0.99790

Sample Analysis:

Summary of sample analysis is shown in Table 4

Table 4 : Summary for sample analysis

Method =>	Dynamic Headspace	
	EtO	2-CE
Sample (Conc. observed)	-	26 ppb
Pre spiked 20 ppb (Conc. observed)	18 ppb	42 ppb
% recovery	90 %	91 %

3.1, 3.2 & 3.3 Ayurveda Samples (Liquid & Dynamic Headspace)
Amala, Ashwagandha, Boswellia Serrata Powder

EtO & 2-CE Analysis In Ayurveda / Herbal Samples (Extracted solution In Headspace Vial)

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
3.1 Amala Powder Sample			
Headspace – Method 1st (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
3.2 Ashwagandha Powder Sample			
Headspace – Method 1st (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX

- This section deals with various approaches, extraction process & different instrument techniques used for the analysis of EtO and 2-CE in Boswellia Serrata powder sample

APPROACH	MODE OF ANALYSIS	INJECTOR	INSTRUMENT TECHNIQUE
3.3 Boswellia Serrata Powder Sample			
Headspace – Method 1st (EtO & 2-CE)	Dynamic Headspace	SPL	HS-20 NX - GCMS-TQ8050 NX
EURL-SRM Analytical Observation Report (EtO & 2-CE)	Liquid	SPL	AOC-20i/s - GCMS-TQ8050 NX

System Description

GCMS System	GCMS-TQ8050 NX
Headspace Sampler	HS-20 NX (Dynamic Headspace)
Capillary Column	RTX-VMS (60 m X 0.45 mm ID x 2.55 um df)
Ionization Mode	Electron Ionization (EI)
Sample Information	EtO & 2-CE in Ayurveda & Herbal Samples

Benefits

- Shimadzu have developed dynamic headspace methods for the analysis of EtO & 2-CE in spices matrix. This method outperforms the current regulatory limits.
- Shimadzu's GCMS-TQ8040 NX / GCMS-TQ8050 NX with liquid autosampler & dynamic headspace sampler is complete tool for the analysis of EtO & 2-CE.

3.1, 3.2 & 3.3 Ayurveda Samples (Liquid & Dynamic Headspace)

Amala, Ashwagandha, Boswellia Serrata Powder

Liquid Injection Parameters				
GCMS System	:	GCMS-TQ8050 NX		
Liquid Sampler	:	AOC-20i		
Headspace Sampler	:	HS-20 NX (Dynamic Headspace)		
Gas Chromatography Parameters				
Capillary Column	:	SH-VMS (60 m X 0.45 mm ID x 2.55 um df)		
Injection Mode	:	Split		
Flow Control Mode	:	Column Flow		
Carrier Gas	:	Helium		
Column Flow	:	3.00 mL/min		
Linear Velocity	:	44.0 cm/s		
Purge Flow	:	3.0 mL/min		
Split Ratio	:	5 (For liquid injection method)		
Diluent	:	Acetonitrile		
Temp. Program		Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
			35.0	5.00
		20	235.0	0.0
MS Parameters				
Ionization Mode	:	EI		
Ion Source Temp.	:	230 °C		
Interface Temp.	:	230 °C		
CID Gas	:	Argon		
CID Gas pressure	:	200 kPa		

Headspace parameters		
Method	:	Dynamic HS
Oven Temp.	:	115 °C
Sample Line Temp.	:	120 °C
Transfer Line Temp.	:	130 °C
Trap Cooling Temp.	:	-10 °C
Trap Desorb Temp.	:	280 °C
Trap Equilib. Temp.	:	25 °C
Shaking Level	:	5
Multi Inj. Count	:	1
Pressurizing Gas Pressure (kPa)	:	192
Equilibrating Time (min)	:	15
Pressurizing Time (min)	:	0.5
Pressure Equilib. Time (min)	:	0.1
Load Time (min)	:	0.5
Load Equilib. Time (min)	:	0.1
Dry Purge Time (min)	:	0.5
Injection Time (min)	:	10
Needle Flush Time (min)	:	10
GC Cycle Time (min)	:	30
Split Ratio	:	20
Total Flow (mL)	:	66
Trap Tube	:	Tenax Ta

Representative Data

Dynamic Headspace Injection (EtO & 2-CE)

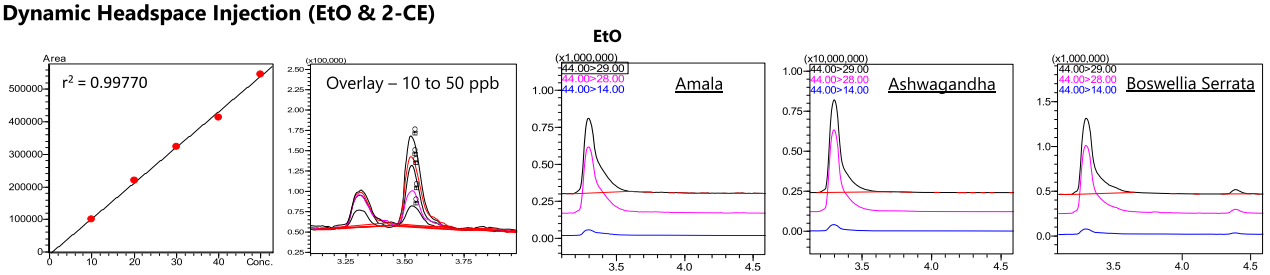


Figure 1 : Calibration curve, overlay of linearity standards & chromatograms of powder samples of Amala, Ashwagandha & Boswellia Serrata for EtO

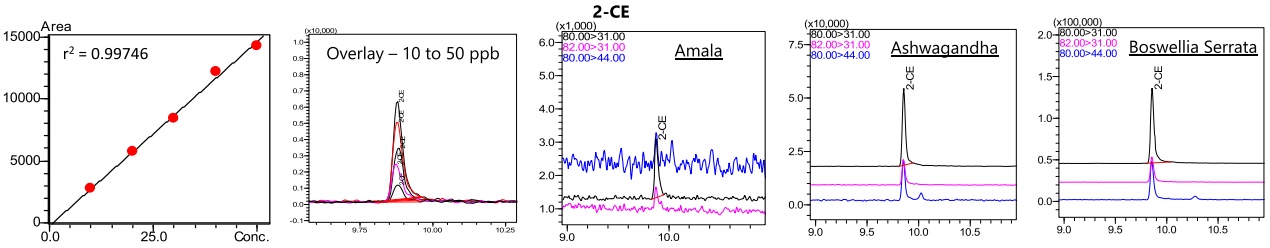


Figure 2 : Calibration curve, overlay of linearity standards & chromatograms of powder samples of Amala, Ashwagandha & Boswellia Serrata for 2-CE

Results :

Summary of results is shown in Table 1

Method =>	Dynamic Headspace	
	EtO	2-CE
Linearity levels (On column)	10,20,30,40 & 50 ppb	
Linearity levels (w.r.t sample)	10,20,30,40 & 50 ppb	
r^2 (n=1)	0.99770	0.99746
Sample Details	**Conc. of EtO**	**Conc. of 2-CE**
Amala Powder (n=2)	Below LOQ	16 ppb
Ashwagandha Powder (n=2)	Below LOQ	253 ppb
Boswellia Serrata Powder (n=2)	Below LOQ	1613 ppb

- Boswellia Serrata sample was analysed with both techniques (liquid & dynamic headspace) to show equivalency study
- Data is summarized as below

Summary of results: Liquid Injection Mode Vs Dynamic Headspace Mode

No.	Name	Boswellia Serrata Sample	
		Liquid Mode	Dynamic Mode
		Aug Conc. (ppb)	Aug Conc. (ppb)
1	EQ	Below LOQ	Below LOQ
2	2-CE	1397	1613

TECHNOLOGY BRIEF | NO. MST-216

GC-MS – Chromatography – Food Safety

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

Written by:

Jang Jin

Ok Hang Ji

Cho Kang Hee

Hong Young Min

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

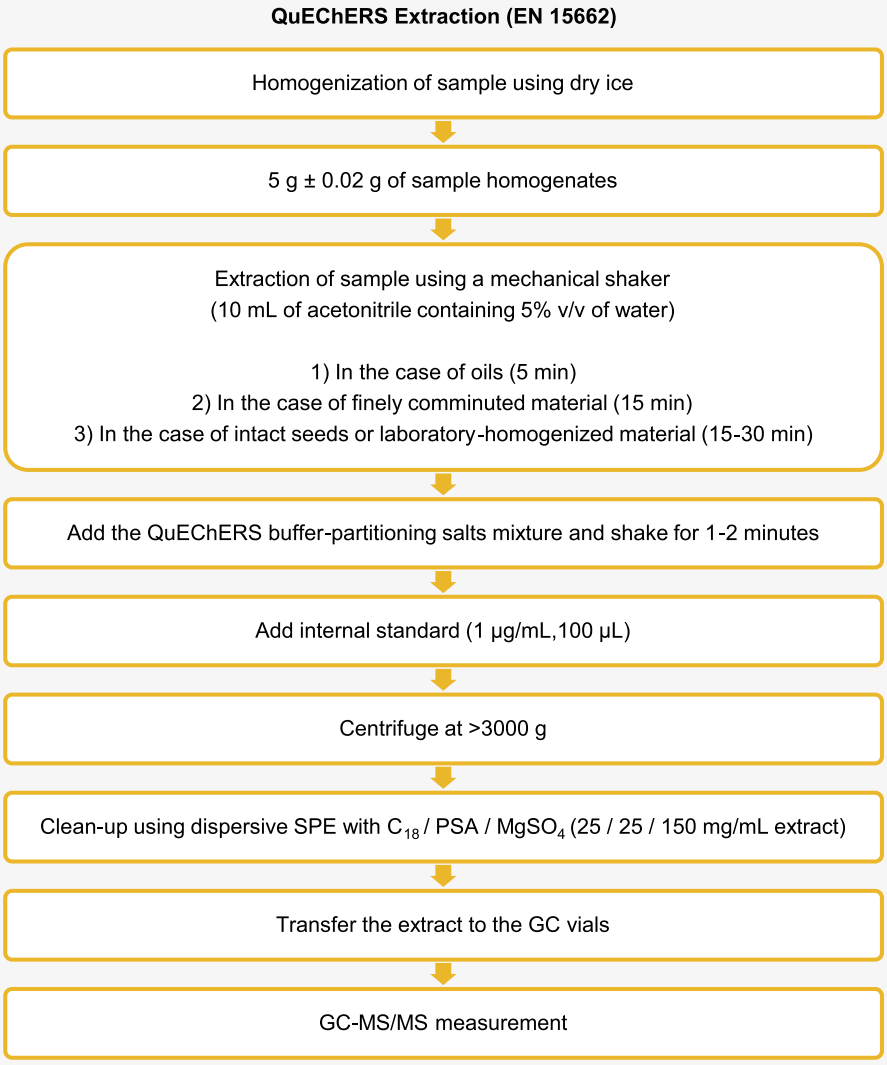


Figure 1. QuEChERS method flow chart

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.

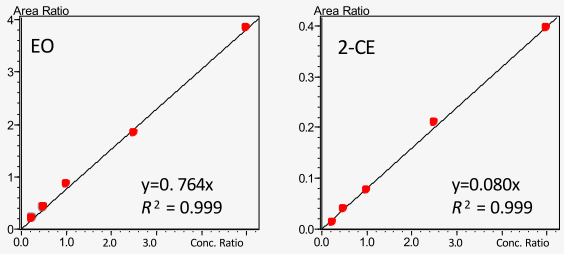


Figure 2. Calibration curves for EO and 2-CE

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.

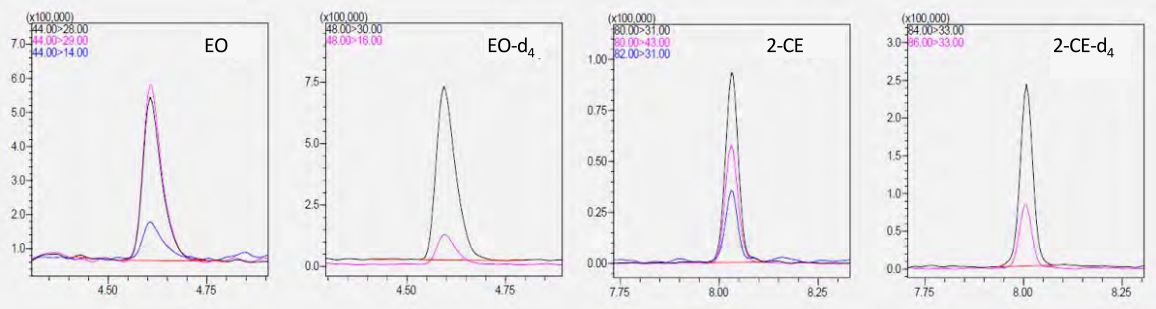


Figure 3. Chromatograms of EO, 2-CE, EO-d_4 and 2-CE-d_4 (standard 50 ng/mL)

	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

Analysis of residual Ethylene Oxide in Intraocular lenses using HS-GCMS

ASMS 2013 ThP11-200

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Analysis of residual Ethylene Oxide in Intraocular lenses using HS-GCMS

1. Introduction

It is imperative to employ effective agents to disinfect and sterilize instruments and equipments, used for patient care and healthcare staff. There are many methods of disinfection and it is very important to confirm the residues of disinfectant in finished product. In this poster we discuss the quantitation of Ethylene Oxide (EtO) as disinfectant residue in intraocular lenses. Ethylene Oxide sterilization is mainly used to sterilize medical and pharmaceutical products that cannot support conventional high temperature steam sterilization, such as devices that incorporate electronic components, plastic containers and intraocular lenses[1]. Boiling point of pure EtO is 10.73°C at atmospheric pressure (The molecular structure of EtO is given in Fig. 1). It is highly volatile and can be easily removed from product. As it is highly volatile there are less chances of finding it in product as an impurity. EtO is a potential carcinogen. Since it is still used in sterilization of medical products, it is necessary to quantify EtO precisely at very low level. Occupational Safety and Health Administration (OSHA) has set the permissible exposure limit for EtO as 1.0 ppm[2]. The objective of this study is to quantify EtO at very low concentration as the intraocular lenses are permanently implant in human eye. (Picture of intraocular lens is given in Fig. 2). The analysis is carried out by using Shimadzu Headspace-Gas chromatograph - Mass Spectrometer (GCMS-QP2010 Ultra coupled with HS-20).

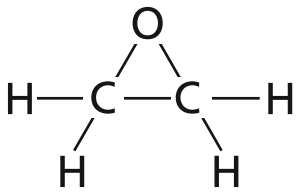

Fig. 1 Structure of Ethylene Oxide

Fig. 2 Intraocular lens

2. Method of Analysis

2-1: Extraction of Ethylene Oxide from Intraocular Lenses

Intraocular lenses were procured from local medical store. Standard stock solution of EtO (500 ppm in Dimethyl sulfoxide) was procured from Sigma Aldrich. Dimethyl sulfoxide was used as diluent for further sample and standard solution preparations. HS-GCMS technique was used for quantitation of EtO at very low concentration. Solutions were prepared as follows,

1) Blank Solution – 5 mL diluent was added to headspace vial and was crimped tightly with automated crimper.
2) Standard Solutions – 5 mL of each Std. EtO in the range of 2.5 to 3000 ppb was added to headspace vial and was crimped tightly with automated crimper.
3) Sample Solution – A piece of intraocular lens was transferred to headspace vial, 5 mL diluent was added and vial was crimped tightly with automated crimper

Partial method validation was carried out by performing Reproducibility, Linearity, LOD-LOQ determination and Recovery study. For validation, solutions of different concentrations were prepared using standard stock solution of EtO (500 ppm) as mentioned in Table 1.

Table 1 Method validation parameters

Parameter	Concentration
Reproducibility	1000 ppb
Linearity	5 levels – 100 ppb – 3000 ppb
Accuracy/Recovery	3 levels – 100 ppb – 500 ppb
Precision at LOQ level	2.5 ppb

Analysis of residual Ethylene Oxide in Intraocular lenses using HS-GCMS

2-2. HS-GCMS Analytical Conditions

Samples were analyzed using HS-20 coupled with GCMS-QP2010 Ultra (Fig. 3) as per the conditions given in Table 2.

Fig. 3 HS-20 coupled with GCMS-QP2010 Ultra byShimadzu

Table 2 HS-GCMS analytical parameters

Headspace parameters			
Mode	: Loop		
Oven Temp	: 100°C		
Sample Line Temp	: 110°C		
Transfer Line Temp	: 120°C		
Equilibrating Time	: 30.0 min		
Pressurizing Time	: 1.0 min		
Pressure Equilib. Time	: 0.10 min		
Load Time	: 0.50 min		
Load Equilib. Time	: 0.10 min		
Injection Time	: 1.0 min		
Needle Flush Time	: 5.0 min		
GC Cycle Time	: 26.0 min		
Chromatographic parameters			
Column	: Rtx-624 (60 m × 0.53 mm × 3.00 µm)		
Injection Mode	: Split		
Split Ratio	: 5.0		
Carrier Gas	: Helium		
Flow Control Mode	: Linear Velocity		
Linear Velocity	: 59.8 cm/sec		
Pressure	: 12.1 kPa		
Column Flow	: 5.50 mL/min		
Total Flow	: 33.0 mL/min		
Total Run Time	: 16.0 min		
Column Oven Temp	: Rate °C /min	Temperature °C	Hold time (min)
	30.0	40.0	5.0
		220.0	5.0
Mass Spectrometry parameters			
Ion Source Temp	: 200°C		
Interface Temp	: 220°C		
Ionization Mode	: EI		
Event Time	: 0.30 sec		
Mode	: SIM		
m/z	: 29, 43 and 44		
Start Time	: 2.0 min		
End Time	: 4.0 min		

Analysis of residual Ethylene Oxide in Intraocular lenses using HS-GCMS

3. Results

3-1. Fragmentation of Ethylene Oxide

According to fragmentation of Ethylene Oxide, molecular ion peak was *m/z*-44 with base peak at *m/z*-29, which was used for quantitation where as fragment ion with *m/z*-43 and *m/z*-44 were used as reference ions. Mass chromatograms of 1000 ppb EtO standard solution with *m/z* 29,43 and 44 are shown in Fig. 4, Mass spectrum of EtO is shown in Fig. 5. Validation data is summarized in Table 3. Fig. 6 and 7 shows, overlay mass chromatograms for *m/z*-29 at different concentrations and calibration curve for linearity levels, respectively.

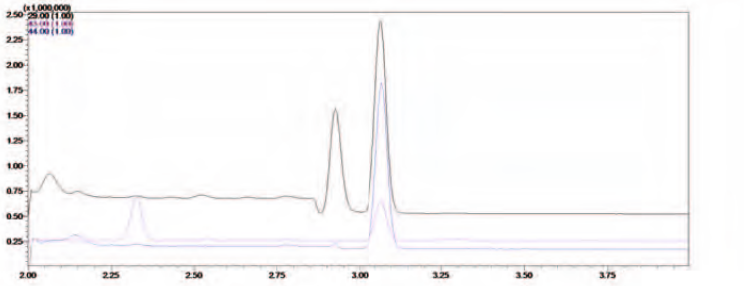

Fig. 4 Mass chromatograms of 1000 ppb Ethylene Oxide standard solution

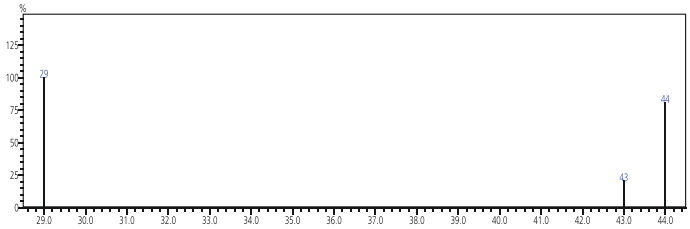

Fig. 5 Mass spectrum of Ethylene Oxide

3-2. Summary of validation results

Table 3 Summary of results for validation parameter

ID	Compound Name	Parameter	Concentration	Result
1	Ethylene Oxide	Reproducibility	1000 ppb	%RSD is 0.8 for area (n=6)
2		Linearity	100 ppb - 3000 ppb	Correlation Coefficient is 0.9999*
3		LOD	2.5 ppb - 10 ppb	0.8 ppb**
4		LOQ		2.5 ppb**
5		Precision at LOQ	2.5 ppb	Average of S/N ratio is 16 (n=6)
				%RSD is 9.6 for area (n=6)

*Linearity level– 100 ppb, 250 ppb, 500 ppb,1000 ppb and 3000 ppb. For linearity, refer Fig. 6 and Fig. 7.

**As per software calculations.

Analysis of residual Ethylene Oxide in Intraocular lenses using HS-GCMS

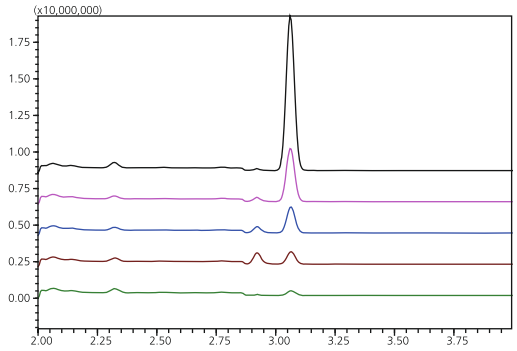

Fig. 6 Linearity levels overlay mass chromatogram

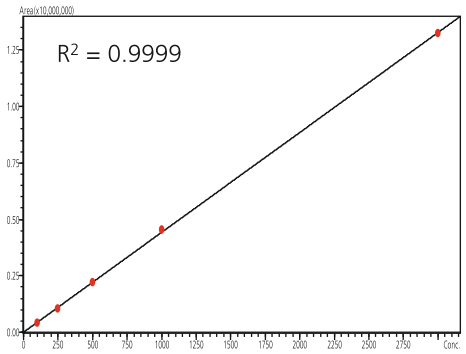

Fig. 7 Linearity levels calibration curve

3-3. Quantification of Ethylene Oxide in intraocular lens sample

Analysis of intraocular lens samples was done as per the given method. Recovery studies were carried out by spiking 100 ppb, 250 ppb and 500 ppb of standard solutions in sample of intraocular lenses. Fig. 8 gives overlay mass chromatogram of spiked and unspiked samples. Table 4, gives the summary of results.

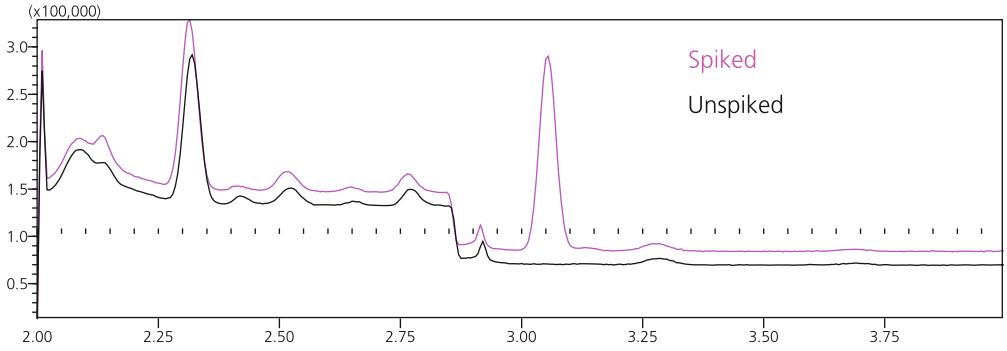

Fig. 8 Spiked and unspiked sample solution overlay mass chromatograms

Table 4 Summary of results for sample analysis

ID	Sample Name	Parameter	Concentration	Result
1	Unspiked Sample	Precision	NA	Below LOQ level
2	Intraocular lens samples spiked with different linearity levels standards	Recovery	100 ppb spiked	Recovery - 87%
			250 ppb spiked	Recovery - 92%
			500 ppb spiked	Recovery - 97%

Analysis of residual Ethylene Oxide in Intraocular lenses using HS-GCMS

4. Conclusion

- HS-GCMS method was developed for quantitation of residual EtO present in intraocular lenses sample. Part method validation was performed successfully. Results obtained for Reproducibility, Linearity, LOQ and Recovery studies were well within limit, as per ICH guidelines[3].
- With "Low Carryover" – The characteristics feature of HS-20 headspace, reproducibility even at very low concentration level could be achieved easily.
- High speed scan rate 20,000 u/sec is the characteristic feature of GCMS-QP2010 Ultra mass spectrometer, useful for quantitation of residual EtO at very low level (ppb level) with high sensitivity.

5. References

[1] "Biological Evaluation of Medical Devices-Part 7: Ethylene Oxide Sterilization Residuals," ANSI/AAMI/ISO 10993-7, Arlington, VA, Association for the Advancement of Medical Instrumentation, 1995.
[2] Potter, Wayne "OSHA Method No. 30, Ethylene Oxide ", OSHA Analytical Laboratory, Salt Lake City, Utah 84115, August 1981.
[3] ICH guidelines Q2(R1), Validation of Analytical Procedure Text And Methodology, 2005

First Edition: June, 2013

SHIMADZU

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GC Nexis™ GC -2030

Application News

Analysis of Residual Ethylene Oxide in Medical Devices by Gas Chromatography

N. Iwasa

User Benefits

- The simultaneous analysis of ethylene oxide (EO), ethylene chlorohydrin (ECH), and ethylene glycol (EG), which are residues by EOG sterilization, is possible.
- The requirements of gas chromatograph measurements for EO and ECH described in JIS T 0993-7: 2012 were satisfied.

■ Introduction

Ethylene oxide gas (EOG) is a flammable and colorless gas commonly used for medical device sterilization. Its permitted maximum residual levels are set by a range of international and local organizations, including the International Organization for Standardization (i.e., ISO 10993-7:2008) and Japanese Industrial Standards (i.e., JIS T 0993-7:2012).

EOG sterilization produces not only ethylene oxide (EO) residues, but also secondary compounds such as ethylene chlorohydrin (ECH) and ethylene glycol (EG) during the sterilization process. In these standards, allowable limits are specified for EO and ECH. Extraction can be either exhaustive or simulated-use. Exhaustive extraction entails a solvent extraction and allows a choice between the following two instrument configurations: the gas chromatograph (GC) and the headspace (HS) -GC.

In this article, the simultaneous analysis of EO, ECH, and EG by GC was performed with reference to JIS T 0993-7:2012, assuming a simulated-use or exhaustive extraction with water as an extraction solvent.

■ Preparation of Standard Solution

As the standard stock solution, a 100 µg/mL EO solution (P/N: 1021-31309, manufactured by GL Sciences Inc.) and a 500 µg/mL mixed solution of ECH and EG prepared by our division were prepared.

Four calibrator points were prepared by diluting the 500 µg/mL mixture of ECH and EG with water to final concentrations of 1, 5, 10, and 25 µg/mL, and cooling in a refrigerator. Then, a suitable amount of a 100 µg/mL EO solution cooled in a refrigerator was added to each concentration of the ECH and EG mixed solution to prepare 4 levels of mixed standard solution (1, 5, 10, and 25 µg/mL mixed standard solution of EO, ECH, and EG).

Table 1 summarizes the preparation methods for standard solutions.

※ Given that EO is easily volatilized, mixed solutions of ECH and EG were prepared, and then an EO solution was added to make the final solution. In addition, in order to suppress an evaporative loss of EO, it should be noted that 1.5 mL vials were used for preparation, and all the above-mentioned solutions and lab apparatus used to handle those solutions were kept at a sub-ambient temperature during the preparation.

Table 1 Preparation Method of Standard Solution

Standard concentrations (µg/mL)	500 µg/mL ECH・EG mixed solution (µL)	Distilled water (µL)	100 µg/mL EO solution (µL)
1	3	1482	15
5	15	1410	75
10	30	1320	150
25	75	1050	375

■ Analysis Conditions

In this experiment, the analysis conditions were established with reference to JIS T 0993-7:2012 using the gas chromatograph Nexis GC-2030. The instrument configuration and analysis conditions for this experiment are listed in Table 2.

Table 2 Instrument Configuration and Analysis Conditions

Model	: Nexis GC-2030 + AOC-20i Plus
Detector	: FID-2030 flame ionization detector
Analytical Column	: SH-Stabilwax™ (30 m × 0.53 mm I.D., d.f.= 1.00 µm)
Column Temperature	: 60 °C (3 min) – 20 °C/min – 200 °C (10 min) Total 20 min
Injection Temperature	: 250 °C
Injection Mode	: Split
Split Ratio	: 3
Carrier Gas	: N_2
Carrier Gas Controller	: Constant Linear Velocity
Linear Velocity	: 40 cm/sec
Detector Temperature	: 250 °C
Detector Gas	: H_2 32 mL/min, Air 200 mL/min
Make up Gas	: N_2 24 mL/min
Injection Volume	: 0.5 µL
Syringe	: Elastic Syringe, AOC (P/N: 221-49548) *1

*1 Using an elastic syringe for AOC (P/N: 221-49548) equipped with a plunger made of titanium enables stable sample introduction.

In this analysis, 20 mg of deactivated glass wool (P/N: 221-48600) were packed into a split glass insert (P/N: 221-41444-84) at a position 20 mm from the top. By increasing the amount of wool compared to the default amount and placing the wool slightly above the default position, the peak shape was stabilized and reproducibility was improved.

■ Measurement Evaluation

JIS T 0993-7:2012 contains the following statements with respect to system requirements of EO and ECH measurement.

※ This standard does not specify allowable limits for EG in medical devices .

- Resolution between the peak adjacent to EO or ECH be not less than 2.0
- Tailing factor for EO and ECH be not more than 1.8
- Relative deviation of the standard curve (RSD) does not exceed 5 % for the range of standards used
- %RSD of the EO and ECH peak area does not exceed 5% for the range of the standards used
- Correlation coefficient of the calibration curve be greater than 0.95.

■ Chromatogram and Calibration Curve of Standard Solution

In this article, we performed a simultaneous analysis using the mixture of EO, ECH, and EG standard solution, and confirmed whether the requirements mentioned above were satisfied for EO and ECH. Similar reference data are provided for EG.

The chromatograms of the EO, ECH, and EG standard solutions are shown in Fig. 1, the enlarged chromatograms and calibration curves of EO, ECH, and EG are shown in Figs. 2, 3, and 4, and the detailed analytical results are summarized in Tables 3, 4, and 5 respectively.

From the results of the standard solutions, the requirements for EO and ECH were satisfied, and good analytical results were obtained.

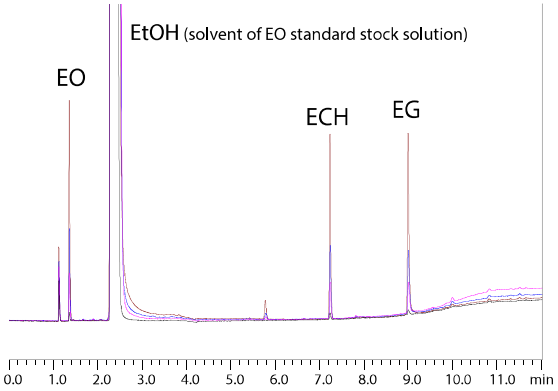

Fig. 1 Standard Chromatograms (0~12 min)

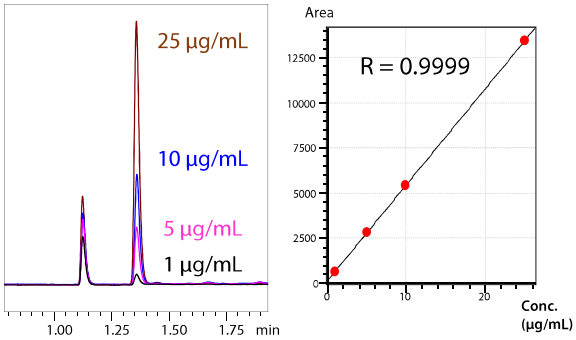

Fig. 2 Chromatogram and Calibration Curve of EO

Table 3 Analytical Results of EO (n=6) *3

Concentration (µg/mL)	1	5	10	25
Mean area value	653	2833	5426	13401
Area value %RSD	3.279	0.828	0.919	0.477
Resolution	6.304	6.520	6.508	6.411
Tailing factor	1.471	1.369	1.357	1.385
Limit of detection (µg/mL)*2	0.106	0.113	0.122	0.122
Limit of quantification (µg/mL)*2	0.355	0.377	0.407	0.407
S/N	27.9	136.8	245.7	642.5

*2 The limit of detection and the lower limit of quantification were calculated at S/N=3 and S/N=10, respectively.
*3 The chromatograms and analytical results are for reference purposes only and should not be regarded as guaranteed values.

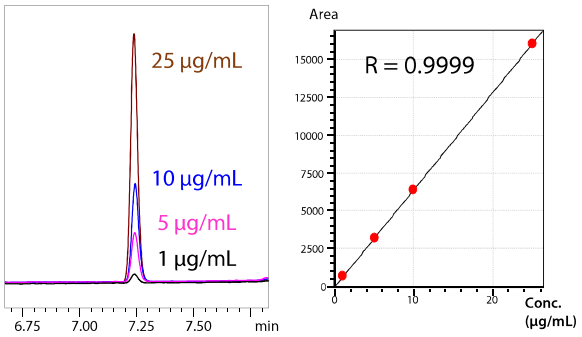

Fig. 3 Chromatogram and Calibration Curve of ECH

Table 4 Analytical Results of ECH (n=6) *3

Concentration (µg/mL)	1	5	10	25
Mean area value	632	3197	6384	16027
Area value %RSD	1.390	0.723	0.644	0.341
Resolution	65.77	23.34	23.18	23.21
Tailing factor	1.080	1.097	1.102	1.103
Limit of detection (µg/mL)*2	0.158	0.148	0.153	0.144
Limit of quantification (µg/mL)*2	0.527	0.494	0.511	0.479
S/N	19.6	103.2	196.0	545.9

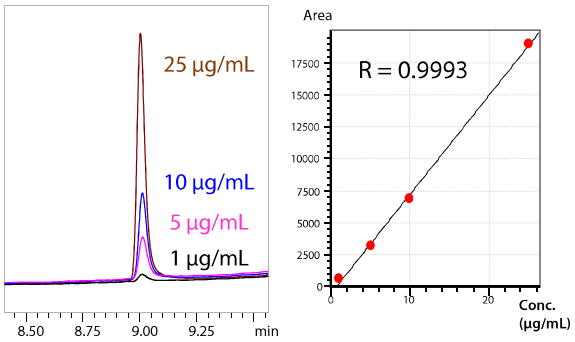

Fig. 4 Chromatogram and Calibration Curve of EG

Table 5 Analytical Results of EG (n=6) *3

Concentration (µg/mL)	1	5	10	25
Mean area value	578	3214	6884	19000
Area value %RSD	5.814	1.176	1.463	1.125
Resolution	21.73	27.86	28.36	29.03
Tailing factor	1.229	1.380	1.428	1.465
Limit of detection (µg/mL)*2	0.285	0.172	0.165	0.145
Limit of quantification (µg/mL)*2	0.952	0.575	0.550	0.484
S/N	15.4	85.3	174.2	543.0

※ EG is highly adsorptive and easily remains in syringes and inserts. The number of solvent washes was increased, and blank analysis was interposed between different samples.

■ Conclusion

Simultaneous analysis of EO, ECH, and EG residues by EOG sterilization was conducted by GC in reference to JIS T 0993-7:2012 and ISO 10993-7:2008. The Shimadzu Nexis GC-2030 satisfied the system requirements and is considered an excellent instrument for measuring residue in medical devices by EOG sterilization.

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Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/

01-00139-EN First Edition: Apr. 2021

Application News

GC Nexis™ GC-2030

Analysis of Residual Ethylene Oxide in Medical Devices by Headspace Gas Chromatography (Water Extraction)

N. Iwasa

User Benefits

- Regarding the JIS T 0993-7: 2012, the analysis conditions of residual ethylene oxide (EO) in medical devices were established by headspace gas analysis of solvent extract using water as an extraction solvent.
- The use of water as an extraction solvent has made it possible to perform environmentally friendly, safe, and inexpensive analyses.

Introduction

Ethylene oxide gas (EOG) is a flammable and colorless gas commonly used in medical device sterilization. Its permitted maximum residual levels are set by a range of international and local organizations, including the International Organization for Standardization (i.e., ISO 10993-7:2008) and Japanese Industrial Standards (i.e., JIS T 0993-7:2012).

In these standards, extraction can be either exhaustive or simulated-use. The exhaustive extraction entails a solvent extraction using both gas chromatography (GC) and headspace (HS). The choice of extraction solvent depends on the sample and its intended use. The use of water as the extraction solvent is attracting attention as an environmentally friendly analysis.

In this article, extraction of residual EO using water as extraction solvent by the HS-GC was performed in reference to the JIS and ISO section K.4.4 "Exhaustive Extraction with Ethanol Followed by Headspace Gas Analysis of the Ethanol Extract."

Instrument Configuration and Analytical Conditions

In this experiment, the headspace gas sampler HS-20 was connected to the Nexis GC-2030 for effective sample introduction. The analytical conditions for GC and HS were in reference to JIS T 0993-7:2012 as listed in Tables 1 and 2.

Table 1 GC Analytical Conditions

Model	: Nexis GC-2030
Detector	: FID-2030 flame ionization detector
Headspace Sampler	: HS-20
Analytical Column	: SH-Stabilwax™ (30 m × 0.53 mm I.D., d.f.= 2.00 µm)
Column Temperature	: 40 °C (5 min) – 30 °C/min – 200 °C (20 min) Total 30.33 min
Injection Mode	: Split
Split Ratio	: 20
Carrier Gas Controller	: Constant Linear Velocity
Linear Velocity	: 30 cm/sec (N_2)
Detector Temperature	: 250 °C
Detector Gas	: H_2 32 mL/min, Air 200 mL/min
Make up Gas	: N_2 24 mL/min
Injection Volume	: 1 mL

Table 2 HS-20 Analytical Conditions

Oven Temperature	: 70 °C
Sample Line Temperature	: 75 °C
Transfer Line Temperature	: 75 °C
Vial Volume	: 10 mL
Vial Shaking Level	: 3
Vial Equilibrating Time*1	: Standard) 30 min Sample) 180 min
Vial Pressurizating Time	: 1 min
Vial Pressure	: 100 kPa
Loading Time	: 1 min
Needle Flush Time	: 8 min

*1 The vial equivalating time listed in the Table 2 is an example only and varies depending on the type of samples.

Preparation of Standards and Samples

The standards and the samples used in this experiment were prepared in reference to JIS T 0993-7:2012.

For the standards, a 100 µg/mL EO solution and a 100 µg/mL propylene oxide (PO) internal standard solution were prepared. Five calibrator points were prepared by diluting the 100 µg/mL EO stock solution with water to 0.4, 0.8, 1.2, 1.6, and 2.0 µg/mL. Each calibrator solution also contained PO internal standard at 0.5 µg/mL. For a calibration curve, 5 mL of a calibrator solution was aliquoted into a 10 mL HS vial and hermetically sealed before analysis.

For the samples, EOG-sterilized bandage and suction catheter were selected to represent sheet and tube types of samples, respectively. The extraction solution was prepared by diluting the 100 µg/mL PO stock solution with water to 0.5 µg/mL. The bandage was cut into 10 mm square pieces while the suction catheter was trimmed into 5 mm long pieces. Ca. 0.5 g of sample pieces were placed in a 10 mL HS vial along with 5 mL of the 0.5 µg/mL PO extraction solution and hermetically sealed for analysis.

※ It should be noted that all the above-mentioned solutions and lab apparatus (e.g., volumetric flasks) used to handle those solutions were kept at a sub-ambient temperature during the preparation to suppress an evaporative loss of EO.

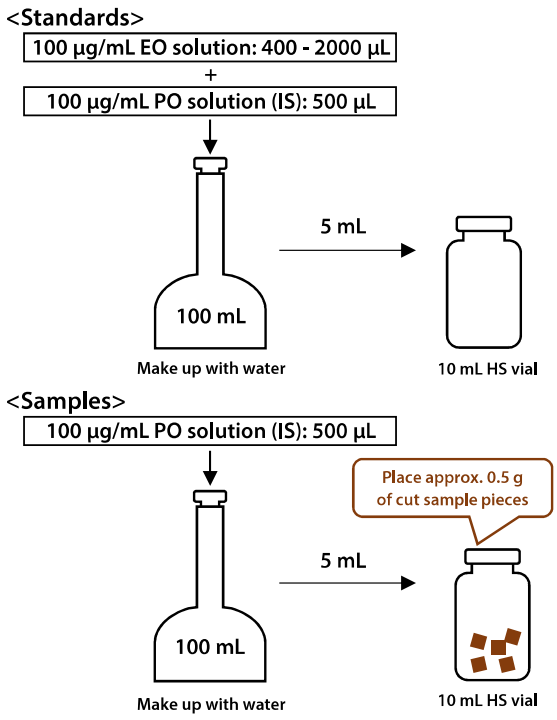

Fig. 1 Sample Preparation Method

■ System Requirements Test (Water Extraction)

JIS T 0993-7:2012 contains the following statements with respect to system requirements.

- Resolution between EO and PO be not less than 2.0
- Tailing factor for EO be not more than 1.8
- Relative deviation of the standard curve (RSD) does not exceed 5 % for the range of standards used
- %RSD of the EO peak area does not exceed 5% for the range of the standards used
- Correlation coefficient of the calibration curve be greater than 0.95.

The results obtained in this experiment satisfied all the above five criteria.

The detailed analytical results are summarized in Table 3. The chromatograms and a calibration curve are shown below in Figs. 2 and 3, respectively.

※ In the case of water extraction, the possibility of conversion of EO to ethylene glycol (EG) or ethylene chlorohydrin (ECH) should be evaluated, but not evaluated in this article. For simultaneous analysis of EO, ECH, and EG, please refer to Application News 01-00139-EN.

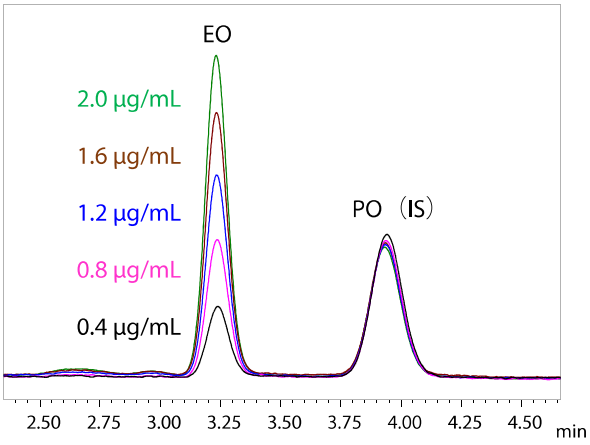

Fig. 2 Standard Chromatograms

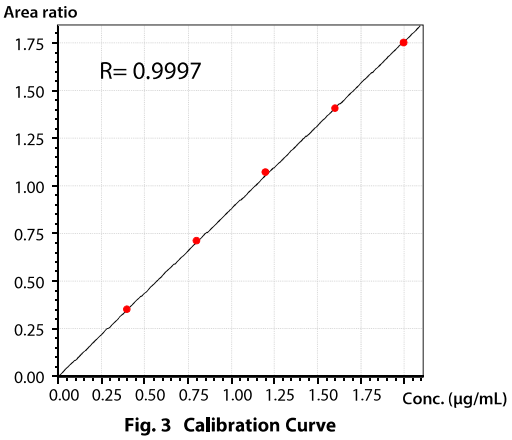

Fig. 3 Calibration Curve

Table 3 System Requirements Test Results (n=6) *3

Concentration (µg/mL)	0.4	0.8	1.2	1.6	2.0
Mean area value	3197	6178	9025	11803	14058
Area value %RSD	3.341	0.965	0.950	0.474	4.683
Mean area ratio	0.359	0.717	1.079	1.402	1.735
Area ratio %RSD	2.200	1.402	0.842	0.747	1.268
Resolution	3.325	3.325	3.328	3.330	3.324
Tailing factor	1.095	1.072	1.063	1.060	1.058
Limit of detection (µg/mL)*2	0.030	0.030	0.031	0.030	0.033
Limit of quantification (µg/mL)*2	0.099	0.100	0.105	0.100	0.110

*2 The limit of detection and the lower limit of quantification were calculated at S/N=3 and S/N=10, respectively.
*3 The chromatograms and quantitative results are for reference purposes only and should not be regarded as guaranteed values.

■ Sample Results (Water Extraction)

Fig. 4 are the overlaid chromatograms of the bandage and the suction catheter. The quantitative results are listed in Table 4.

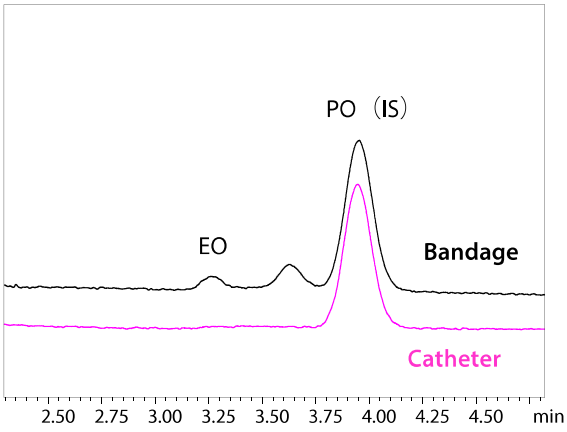

Fig. 4 Sample Chromatograms

Table 4 Quantitative Values of EO in 0.5 g of Samples (µg/0.5 g)*3

	Bandage	Catheter
Data 1	0.480	ND
Data 2	0.400	ND
Data 3	0.425	ND
Mean	0.435	—

■ Conclusion

In reference to JIS T 0993-7:2012 and ISO 10993-7:2008, quantitation of residual ethylene oxide in bandages and suction catheters was conducted by HS-GC using water as extraction solvent.

The Shimadzu GC-2030 + HS-20 system satisfied the system requirements and is considered an excellent instrument for measuring residual ethylene oxide in a medical device.

For ethanol extraction results, please refer to Application News No. G 336.

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Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/

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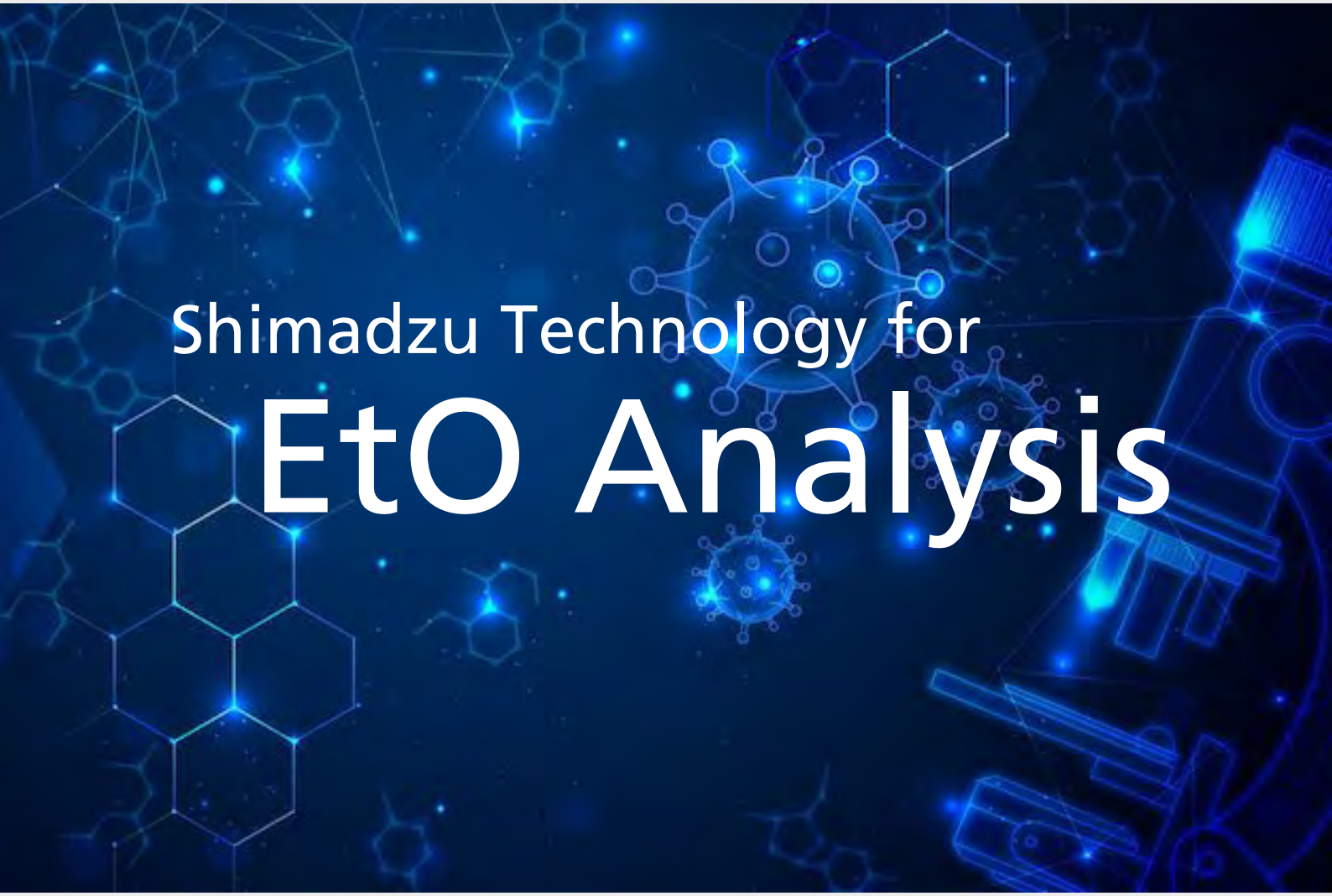
Shimadzu Technology for
EtO Analysis

GCMS-TQ8050 NX

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Highlighted Features

Triple Quadrupole Gas Chromatograph Mass Spectrometer with Smart Technologies

- Fully Automatic MRM Optimization
- Smart MRM for quick & easy method development
- Simultaneous analysis of more than 400 compounds in single run
- Design based on Shimadzu's UFMS Technology (Ultra Fast Mass Spectrometer)
    1. UF Sweeper® - High speed Collision cell with minimum dwell time of less than 0.5 ms achieving high speed MRM 800 transitions / sec
    2. UF Scanning - High speed calculation process enables 20,000 amu/sec scan speed
    3. UF Sensitivity - Patented ASSP™ ensures high sensitivity during high speed scanning with improved sensitivity upto 5 folds that observed in high scan speed systems.
    4. UF Quad - Ultra fast response quadrupole mass filter
- Shimadzu's proprietary, high-efficiency off-axis ion optics & low noise off axis detector offers unmatched sensitivity
- Secondary electron multiplier with patented Overdrive Lens and conversion dynode. Noise from outside the detector was reduced by installing a shield in the secondary electron multiplier.

GCMS-TQ8050 NX

- Sensitivity with EI MRM sensitivity S/N ratio of 100fg OFN is 40,000 :1 is the World's best in the industry.
- EI MRM IDL (Instrument Detection Limit): 0.5fg OFN, statistically derived at 99 % confidence level from the area precision of eight sequential splitless injections of 1 µL, 2 fg/µL OFN standard
- Fast GCMS capability with micro-bore column of 0.1mn ID for better resolution and higher throughput
- Ultra fast clean vacuum by air-cooled dual inlet TMP with differential pumping capacity of total 360 ltrs/sec for Helium
- Fast Scan/MRM measurements provide a wealth of qualitative and quantitative information
- Unique pesticide database with Auto / SMART MRM
    1. Method files can be automatically created without analyzing pesticides
    2. Automatic creation of method files with optimal dwell time during Scan/MRM measurement
- AART function automatically adjusts compound and MRM retention times for most accurate qualitative and quantitative analysis
- Front-opening ion source chamber makes maintenance fast and easy
- “Twin Line MS System” offers unique Dual Column Single MS capability for seamless switching of applications without venting MS
- Advanced Flow Technology (AFT) offers advanced chromatography capabilities – Heart-Cut with Multi Dean's Switch, Detector Splitter, Detector Switcher, Dual Oven Multi-Dimensional GC-GCMS System available as option
- Compatible with Shimadzu's unique Direct Insertion Probe (Solid Probe) with ease of operation
- Easy switch over to single quadrupole GCMS
- Same software operability & file compatibility with single quad GCMS
- Flexible platform with EI, CI and NCI ionization modes and precisely by changeover of EI to CI mode to identify and accurately measure the molecular weights of the analytes without any hassle.
- Unique application packages available as option with database for Pesticides, Flavor & Fragrance Compounds, Metabolites, Toxicology Compounds in addition to commercial databases like NIST 2020, Wiley etc.

GCMS-TQ8040 NX

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    3. UF Sensitivity - Patented ASSP™ ensures high sensitivity during high speed scanning .
    4. UF Quad - Ultra fast response quadrupole mass filter
- Shimadzu's proprietary, high-efficiency off-axis ion optics & low noise off axis detector offers unmatched sensitivity
- Patented Ion source design enhances sensitivity
- Dual filament design for higher productivity
- Fast GCMS capability with micro-bore column of 0.1 mn ID for better resolution and higher throughput

GCMS-TQ8040 NX

- Ultra-fast clean vacuum by air cooled dual inlet TMP with differential pumping capacity of total 360 ltrs/sec for Helium
- Pre-detector overdrive lens system reduces neutral noise ensuring low background
- Variety of measurement modes provide enhanced selectivity and method flexibility
- Fast Scan/MRM measurements provide a wealth of qualitative and quantitative information
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- Easy sTop' injection port reduces maintenance downtime
- Ecology mode reduces operating cost & environmental impact
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Nexis GC-2030

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Highlighted Features

- The Next Industry Standard GC – Nexis
- Designed with the analyst in mind: making routine analysis convenient with innovative “ClickTek” technology – “Install capillary columns & change liners without using any tools”
- Information at your finger-tips : Intuitive graphical icons and mobile device monitoring
- World's highest FID sensitivity 1.2 pgC/s (dodecane)
- Enhanced sensitivity for all detectors to support wide range of challenging applications
- Wide Range of Injectors, like Split/Splitless; PTV; OCI, WBI (Direct Injection) for complete analytical flexibility

Nexis GC-2030

- Excellent reproducibility with intelligent automatic flow controller
- Fast GC capability using Hydrogen as a carrier gas and suitable even for ultra-narrow bore columns up to 0.05mm id.
- Hydrogen sensor for additional safety
- Exceptional versatility in configuration to match any customized applications needs
- Unique advanced functions improves energy efficiency and reduces operating cost
- Easy transfer of existing methods with flexible injection modes – Constant Flow with Split or Splitless Injection; constant Pressure Split or Splitless, High Pressure Injection and Shimadzu's innovative Constant Linear Velocity Mode to achieve best column separation efficiency
- LabSolutions workstation software offers both easy operability and extensive functionality with guaranteed compliance for 21 CFR part 11 in Client / Server Network or stand-alone platforms
- Choice of wide range of accessories like Headspace Sampler, Auto injector/sampler, Pyrolizer, Purge & Trap, Thermal Desorption etc.
- Choice of 3 analytical flow lines with simultaneous control of three injectors and four detectors offers best versatility when used with multiple accessories
- Automation enhanced to next level with Eco functions – Day and time programming; System self-check; Automatic notification on consumables replacement; Carrier gas usage optimisation & low power consumption while idling.electron multiplier.

Headspace sampler HS-20 NX

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Highlighted Features

- Valve, Loop and Trap based headspace system with innovative design by direct interface with GC/GCMS
- Higher reproducibility through unique vial heating in oven, minimizes heat loss and increases thermal stability
- Unique isolation gas flow to achieve ultra-low carryover <0.0001% for maximum data reliability
- Super short transfer line for excellent repeatability.
- Available as Loop (Static) and Trap (Static + Dynamic) model and switch to perform ultra-sensitive analysis anytime using trap mode analysis
- 90 vial sample capacity for high throughput with 5 stage vial mixer/shaker and 12 vial incubator for overlap analysis to save valuable analysis time
- Interface Temperature 350°C and Vial Temperature 300°C, extends analysis from low to high boiling points compounds
- Totally inert sample flow line with Sulfinert® 1 ml sample loop as standard
- Electronic carrier gas control through AFC and vial pressurization with APC for unmatched accuracy and RT reproducibility
- Complete all vial leak check, vial pressure monitors and release function for safe operation
- Complete compliance with USP Residual Solvent requirements
- Software capabilities include Multiple Headspace Extraction (MHE) and Method Development Mode (MDM)
- Supported by Shimadzu LabSolution GC Software with 21 CFR part 11 compliance
- Easy operator maintenance for replacement of column, sample loop and needle

Auto-injector AOC-30i

Click here

Highlighted Features

Reliable Automatic Operation

- High Reproducibility

  AOC-30 automates the analysis, reduces an operator's workload, and enables continuous analysis with a high degree of accuracy that cannot be achieved by manual operation. Shimadzu's unique injection method achieves high reproducibility while preventing septum damage and liner contamination.

Space-saving Efficiency

- Configurable for Analysis Purposes

  The single tower system provides automated analysis of up to 30 samples, covering a wide range of analysis needs, and is recommended as the first selection. Injectors and samplers can be added to increase analysis capacity.

Built-in Pro Tips

ANALYTICAL INTELLIGENCE

- Sampler Navigator, Built-in Injection Expertise
- Injection may seem trivial, but in reality it is a very complex process that requires a lot of optimization. The Sampler Navigator reduces the guesswork involved by letting you choose from a carefully curated list of optimized methods, meticulously prepared by experts in gas chromatography. Get up and running with a single click.

AOC-20i/AOC-20s

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Highlighted Features

Auto Injector/Auto Sampler

- The AOC-20 Series is the perfect solution for automated analyses. Its proven reliability and reproducibility allows the user to take full advantage of the GC or GC/MS system capabilities.

The AOC-20 provides the highest degree of reliability.

- Reproducibility and reliability are what make an auto injector valuable to a laboratory. Reproducibility can be adversely affected not only by less than optimum injection conditions, but through sample evaporation as well. Reliability is a measure of how well the instrument faithfully reproduces injection of the sample. The AOC-20i ensures that both of these needs are met.

Flexible sample handling of up to 150 vials, greatly enhances laboratory productivity.

- The AOC-20s Auto Sampler carousel and robotic arm provide for sample transport to the AOC-20i Auto Injector using 1.5 mL and 4.0 mL vials. The system is smart too! It can tell the difference between the vial types by reading the information from the vial tray in use. The AOC-20i/AOC-20s is a powerful automation tool for the GC laboratory that allows the user to take full advantage of the GC system's capabilities.

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