

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

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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 disinfection or disinfestation 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.

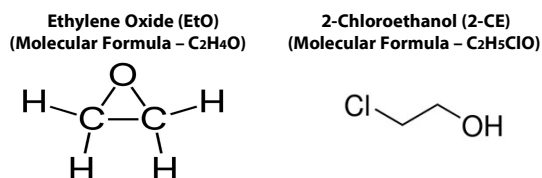


Figure 1: Structure of EtO & 2-CE

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		
Tuning	:	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 µL of diluent (Acetonitrile), mixed well & vortex for 15 minutes

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

Removed 5000 µL 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 ($\pm 10\%$) of sesame seeds and add respective μL of linearity standard stock solution. Further add diluent to make up volume of 10000 μL 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.

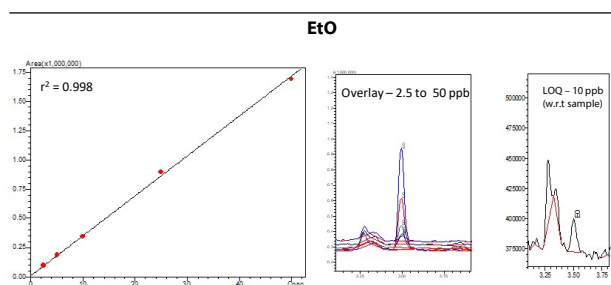


Figure 3: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO

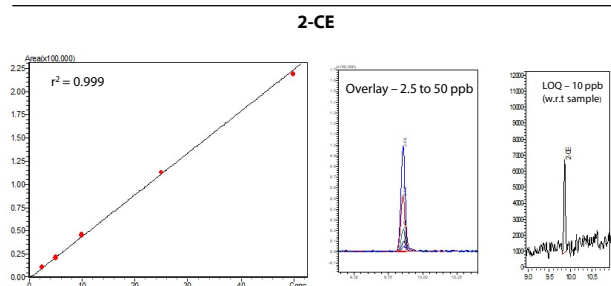


Figure 4: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE

■ 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 Noise 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 μL 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 μL of diluent (Acetonitrile), mixed well & vortex for 15 minutes

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

Removed 100 μL 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 ($\pm 10\%$) of sesame seeds and further add 1000 μL 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.

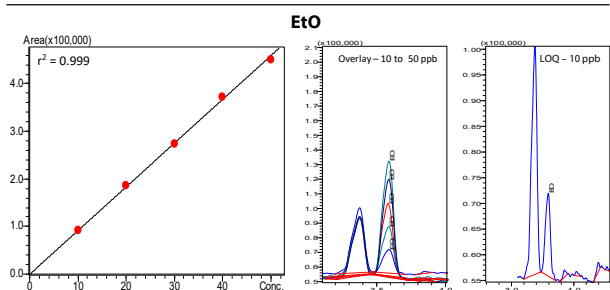


Figure 5: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO

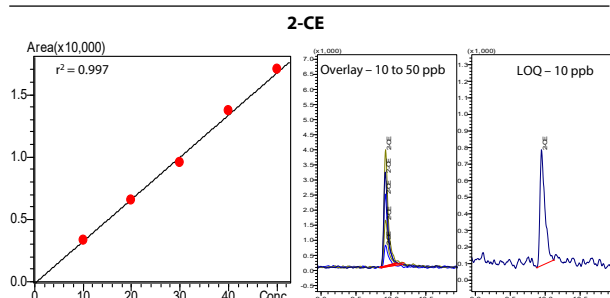


Figure 6: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE

■ 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 μL of diluent (Acetonitrile), mixed well & vortex for 15 minutes

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

Removed 100 μL 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 ($\pm 10\%$) of sesame seeds and further add 1000 μL 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.

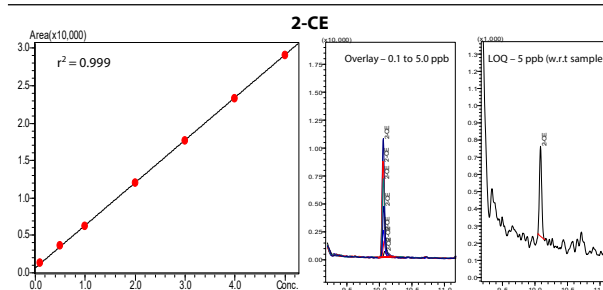


Figure 7: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for 2-CE

■ 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 μL 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 μL of diluent (Acetonitrile), mixed well & vortex for 15 minutes

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

Removed 1000 μL 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 ($\pm 10\%$) of sesame seeds and further add 5000 μL 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.

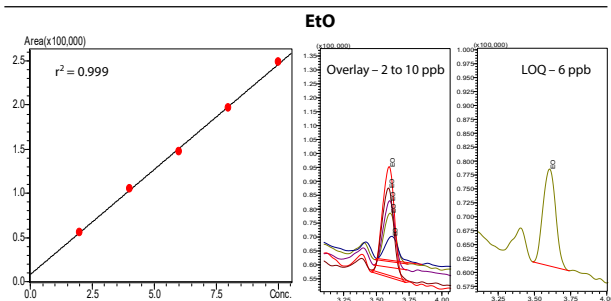


Figure 8: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for EtO

■ 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.

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Some products may be updated to newer models.



➤ GCMS-TQ™8050 NX

Triple Quadrupole Gas Chromatograph
Mass Spectrometer



➤ HS-20 NX series

Headspace Sampler



➤ AOC-20i/AOC-20s

Auto Injector/Auto Sampler for GC/GC-
MS

Related Solutions

➤ Food Contamination

➤ Price Inquiry

➤ Product Inquiry

➤ Technical Service /
Support Inquiry

➤ Other Inquiry