Approaches to Analyzing Ethylene Oxide and Its Derivatives in Sesame Seeds and Other Food Commodities Using Triple Quadrupole GC/MS/MS

Consumables Workflow Ordering Guide

Agilent

Ethylene oxide is used by the spice industry to prevent microbial contaminants such as Salmonella and *E. coli*, reduce bacterial loads, yeast and mold, coliforms, and other pathogens. It is employed instead of high temperature processes that may damage certain products such as herbs, spices, and seeds.

Ethylene oxide (EtO) reacts with food matrix to form 2-chloroethanol (2-CE or Ethylene chlorohydrin) and residues of EtO and 2-CE maybe found in foodstuffs that have been fumigated. Its presence in sesame seeds from India prompted a spate of product recalls across Europe, including bread and bagels and recently products containing locust bean gum (E410), a thickening agent used in ice-creams, chocolates, biscuits, bread, and crackers.[1,2]

While acute (short-term) effects of ethylene oxide consist of central nervous system depression and irritation of the eyes and mucous membranes, chronic (long-term) exposure can cause damage to the brain and nervous system. There is also some evidence linking ethylene oxide exposure to reproductive effects and increased risk of lymphoid cancer and, for females, breast cancer. Both the International Agency Research of Cancer (IARC) and the EPA have classified ethylene oxide as a category-1 carcinogen.[3]

While the maximum residual limits (MRLs) proposed by the EPA for ethylene oxide are 7 mg/kg,[4] the European Union (EU) has set the MRLs for both EtO and its primary metabolite 2-CE to 0.1 mg/kg for spices, and 0.05 mg/kg for nuts, oil fruits and oil seeds (Commission Regulation (EU) 2015/868).[5] The demand for ethylene oxide analysis has increased significantly worldwide since 2020.

Getting started with ethylene oxide and ethylene chlorohydrin analysis in foods

Ethylene oxide and its derivatives are analyzed by GC-MS or GC-MS/MS. There are number of methods, for the analysis of EtO or the sum of EtO and 2-CE, that utilize different approaches, including conversion of 2-CE to EtO under alkaline conditions or conversion of EtO to 2-CE under acidic conditions.

Sample preparation is an essential step for all the methods developed thus far. The Korean Ministry of Food and Drug Safety (MFDS) describes a method that uses QuEChERS for extraction and conversion of the EtO to 2-Bromoethanol (2-BE) via bromination followed by GC/MS quantification of 2-BE and 2-CE (Table 1).[6]

Table 1. GC-MS Analysis condition on an Agilent 7890B/7010B used by Korean Ministry of Food and Drug Safety (MFDS).[6] Under these conditions, the retention time of 2-CE: 6.4 min, 2-BE: 7.5 min.

GC-MS Conditions	
Column	DB-WAX (30 m × 0.25 mm, 0.5 µm) or equivalent
Mobile Phase Gas and Flow Rate	Helium, 1.0 mL/min
Injection Part Temperature	220 °C
Oven Temperature	80 °C - 2 min 200 °C - 16 °C/min - 2 min
Detector Temperature	260 °C
Ionization	Electron impact (EI), 70 eV
Injection Mode	Pulsed split mode (3:1) or equivalent method
Injection Volume	2 µL

In December 2020, EU Reference Laboratories (EURL) for Residues of Pesticides recommended a single-residue method (SRM) for the analysis of EtO and 2-CE in sesame seeds that uses QuEChERS extraction followed by GC/MS/MS analysis.[7]

Agilent scientists have developed various methods that meet the stringent EU requirements

1. EURL-SRM method for simultaneous detection of EtO and 2-CE

An improved EURL-SRM method for simultaneous measurement of EtO and 2-CE in commercial samples of sesame, curcuma, garlic powder, spices and herbs using QuEChERS.[8]

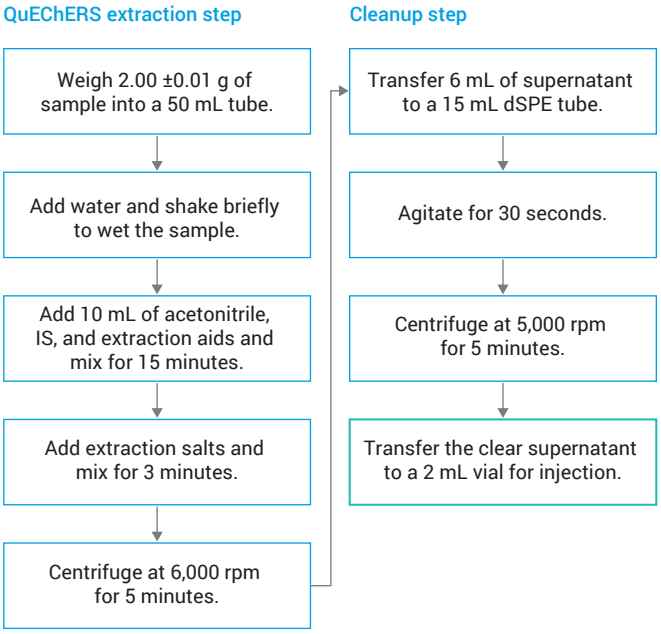

Figure 1. QuEChERS workflow for the extraction and cleanup of samples.[8]

Table 2. Recovery and relative standard deviation (RSD) of EtO and 2-CE in sesame and curcuma samples spiked at different levels.[8]

Matrix	Spike Level (mg/kg)	Recovery EtO (%)		Recovery 2-CE (%)	
		Average	RSD % (n = 3)	Average	RSD % (n = 3)
Sesame	0.05	100.1	9.1	97.9	6.3
	0.2	84.5	7.6	92.5	8.4
	0.5	92.0	6.9	88.8	2.7
Curcuma	0.05	100.6	16.4	106.2	4.4
	0.2	94.5	8.5	105.8	9.9
	0.5	92.5	5.2	94.4	4.3

Improvements that were made to the EURL-SRM method include:

a. An automated liner exchange option and an integrated precolumn back flush to protect the injector and analytical column from damage, and the detector from matrix contamination.

b. 'Detector OFF' time event in the MS method, to protect the filament from the solvent acetonitrile, which coelutes between EtO and 2-CE.

c. A cooled injection system (CIS) using a PTV-type inlet for reproducible and accurate injections.

Table 3. GC method parameters.[8]

Parameter	Value
Model	Agilent 8890 Gas Chromatograph
Injector	Gerstel CIS 4 with Automated Liner Exchange (ALEX) option
Injector Temperature	90 °C (0.8 min), 12 °C/s to 250 °C (14.3 min)
Injection Volume	2 µL; split 1:4
Liner Type	Glass wool (Gerstel p/n 010850-010-00)
Precolumn	5 m FS
Analytical Column	Agilent J&W HP-VOC GC, 30 m × 0.20 mm, 1.12 µm (p/n 19091R-303)
Carrier Gas	Helium
Analytical Column Flow	1 mL/min
Oven Gradient	45 °C (2 min), 50 °C/min to 220 °C (10 min)
Transfer Line Temperature	280 °C

Table 4. GC/TQ method parameters.[8]

Parameter	Value	
Model	Agilent 7010 triple quadrupole GC/MS	
Source Temperature	230 °C	
Quadrupole Temperature	150 °C	
Collision Gas Flow	1.5 mL/min (N_2)	
Quench Gas Flow	2.25 mL/min (He)	
Time Events	0 min – detector ON 2.95 min – detector OFF 3.6 min – detector ON	
MRM Transitions and Retention Times	ETO-D4 (2.56 min):	48 → 16 (CE 40) 48 → 30 (CE 5)
	ETO (2.57 min):	44 → 29 (CE 5) 44 → 28 (CE 5)
	2-CE-D4 (4.47 min):	44 → 15 (CE 5) 6 → 33 (CE 5)
	82-CE (4.48 min):	84 → 33 (CE 5) 80 → 44 (CE 0) 80 → 31 (CE 5) 80 → 43 (CE 0)

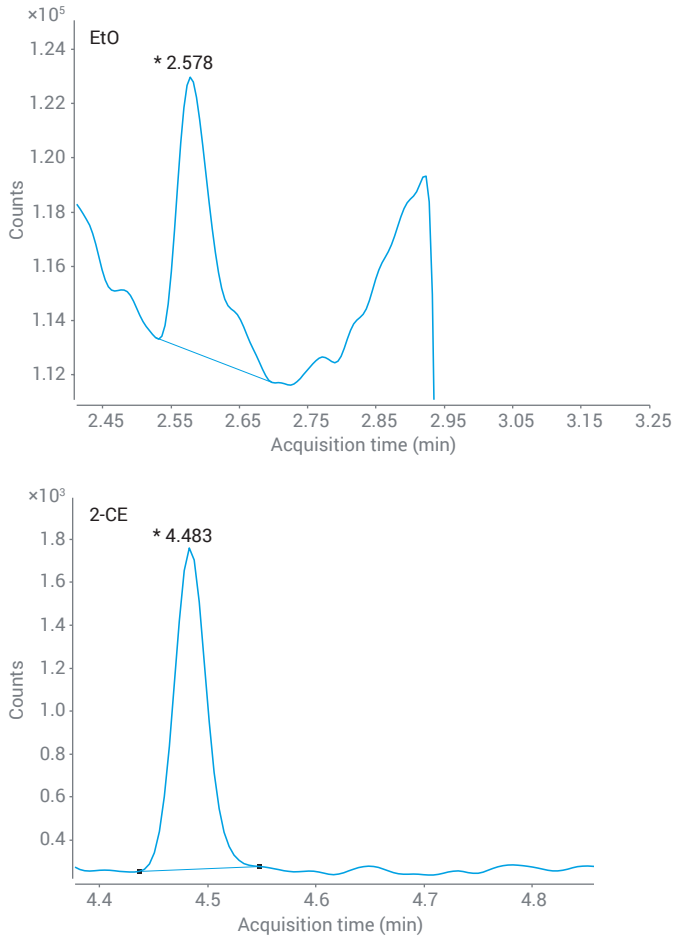

Figure 2. Chromatograms of EtO (MRM transition 44 → 29) at the 5 ng/mL level and 2-CE (MRM transition 80 → 44) at the 1 ng/mL level.[8]

2. Method for detecting 2-CE

An accurate and rugged method for detecting 2-CE as a marker of fumigation of sesame seeds was developed with an LOQ of 10 ng/g, which complies with the MRL set by EU at 50 ng/g. Here the EtO residue in the sample was converted to 2-CE during sample preparation.[9]

Table 5. Recovery in sesame seed sample using procedure in Figure 1.[9]

Compound Name	Spiking Amount (ng/g)	Observed Amount (ng/g)	Final Amount (ng/g)	Recovery (%)
Ethylene Chlorohydrin	10	10.078	10.078	100.8
	50	50.036	50.036	100.1
Ethylene Oxide	10	14.96	8.228	82.3

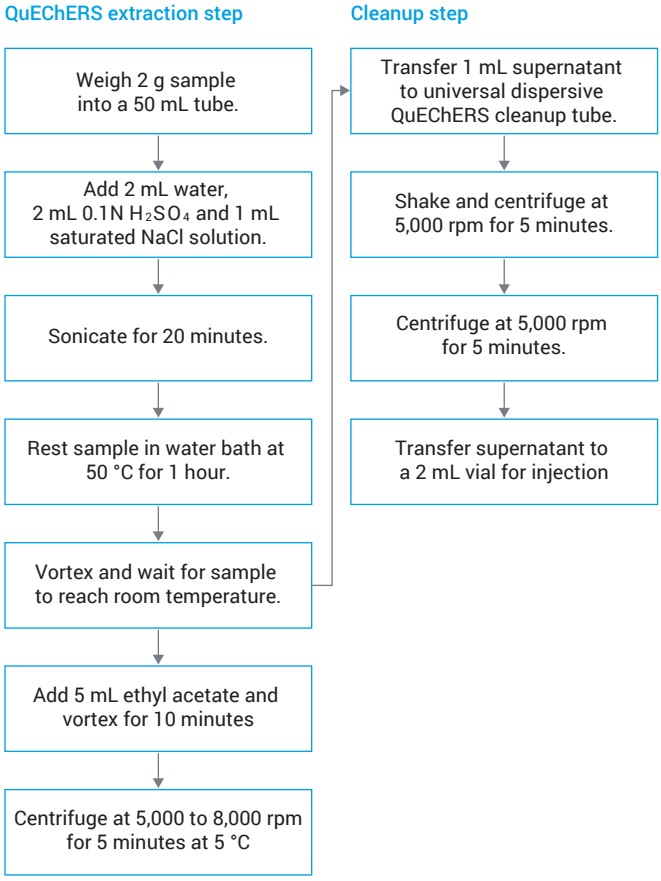

Figure 3. QuEChERS workflow for the extraction and cleanup of samples.[9]

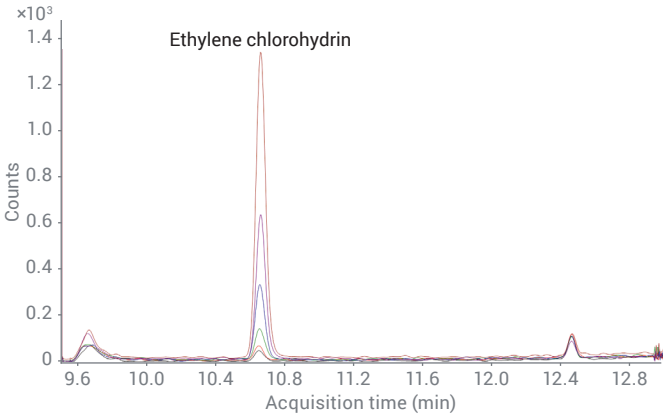

Figure 4. TIC MRM overlay of various concentrations of ethylene chlorohydrin in matrix ranging from 5 ppb to 200 ppb using method parameters described in Reference 9.

3. Headspace method for simultaneous detection of EtO and 2-CE

This method for simultaneous measurement of EtO and 2-CE in sesame seeds and black pepper avoids the sample preparation step completely by using a Headspace sampler for sample introduction onto a GC/MS/MS. The LOQ of the method was demonstrated at 5 ng/g for EO and 2-CE for sesame seeds and black pepper samples.[10]

Table 6. Headspace sampler and GC/MS/MS parameters.[10]

HS Parameters	
Model	Agilent CTC PAL3 Headspace Sampler 120 cm
Incubation Temperature	140 °C
Incubation Time	20 min
Syringe Temperature	150 °C
Agitation During Extraction	Yes; 250 rpm
Injection Volume	2.0 mL
Injection Flow Rate	30 mL/min
GC Parameters	
Inlet Temperature	250 °C
Inlet Liner	Agilent inlet liner, direct, 1.5 mm id (p/n 18740-80200)
Inlet Septa	Agilent inlet septa, 11 mm (p/n 8010-0239)
Column	Agilent J&W DB-VRX (p/n 122-1564)
Column Flow	1 mL/min
Split Ratio	10
Oven Program	35 °C for 4 min 15 °C/min to 150 °C, hold 1 min 40 °C/min to 240 °C, hold 7 min
Transfer Line Temperature	240 °C
Collision Gas	Argon, 0.5 mL/min
Quench Gas	Helium, 2.25 mL/min
Column Nuts	Agilent column nuts, self-tightening (p/n G3440-81011 and G3440-81013)
MS Parameters	
Ion Source Temperature	230 °C
Q1 and Q1 Temperature	150 °C
Solvent Delay	4.5 min
MRM Transitions for ETO	44 → 29 (CE:5) 44 → 28 (CE:5) 44 → 14 (CE:20)
MRM Transitions for ECH	80 → 31 (CE:5) 80 → 43 (CE:5) 82 → 31 (CE:5)
Gain Factor	20

References

1. www.foodsafetynews.com/2020/09/multi-country-recalls-due-to-ethylene-oxide-insesame-seeds/
2. www.foodqualityandsafety.com/article/eu-recalls-thousands-of-food-products-overethylene-oxide-concerns/
3. Tateo, F.; Bononi, M. Determination of Ethylene Chlorohydrin as Marker of Spices Fumigation with Ethylene Oxide. *Journal of Food Composition and Analysis*, **2006**, *19*, 83-87.
4. www.atsdr.cdc.gov/toxprofiles/tp137.pdf
5. Regulation (EU) 2015/868 of 26 May 2015 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 2,4,5-T, barban, binapacryl, bromophos-ethyl, camphechlor (toxaphene), chlorbufam, chloroxuron, chlozolinate, DNOC, diallate, dinoseb, dinoterb, dioxathion, ethylene oxide, fentin acetate, fentin hydroxide, flucycloxuron, flucythrinate, formothion, mecarbam, methacrifos, monolinuron, phenothrin, propham, pyrazophos, quinalphos, resmethrin, tecnazene and vinclozolin in or on certain products. *Off. J. Eur. Union L.*, **2015**, *145*, 1-71.
6. Korean Ministry of Food and Drug Safety (MFDS) - Ethylene Oxide and 2-chloroethanol Test Method in Food.
7. EURL-SRM-Analytical Observation Report: Analysis of Ethylene Oxide and its Metabolite 2-Chloroethanol by the QuOil or the QuEChERS Method and GC-MS/MS. December **2020**.
8. Simultaneous Analysis of Ethylene Oxide and 2-Chloroethanol in Sesame Seeds and Other Food Commodities: Challenges and Solutions, 5994-4942EN.
9. Estimation of Ethylene Oxide and Ethylene Chlorohydrin in Sesame Seeds Using Agilent 8890 GC and 7000D Triple Quadrupole MS System 5994-3805EN.
10. Estimation of Ethylene Oxide and Ethylene Chlorohydrin in Foodstuffs by HS-GC/MS/MS, 5994-5378EN.

Easy selection and ordering information

This guide lists all the columns and supplies you will need for ethylene oxide analysis using an appropriately setup system. To order items listed in the tables below from the Agilent online store, add items to your Favorite Products list by clicking on the MyList # header links. You can then enter the quantities for the products you need, add the products to your cart and proceed to checkout. Your list will remain under Favorite Products for your use with future orders.

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MyList 1: Consumables recommended for EtO:2-CE analysis EURL_SRM[7] for GC-MS/MS systems with standard inlet and injection port.

Description	Part No.
Sample Preparation	
Centrifuge Tubes and cap, 50 mL, 25/pk	5610-2049
QuEChERS extraction kit, EN 15662 method, 50/pk	5982-5650
Ceramic Homogenizers, 50 mL tubes, 100/pk	5982-9313
QuEChERS Dispersive Kit, Fruits and Vegetables with Fats and Waxes, EN method, 15 mL, 50/pk	5982-5156
Ceramic Homogenizers, 15 mL tubes, 100/pk (optional)	5982-9312
Captiva Econofilter, polypropylene, Nylon membrane, 25 mm, 0.2 mm, 1000/pk	5190-5271
Captiva Disposable Syringe, 5 mL, 100/pk	9301-6476
Columns & Accessories	
J&W HP-VOC GC Column, 30 m, 0.20 mm, 1.12 µm	19091R-303
Fused silica tubing, deactivated, 0.25 mm, 5 m	CP802505
Ultimate Union Kit, deactivated	G3182-61580
Inlet/non-MSD self-tightening nut (with collar)	G3440-81011
MSD self-tightening nut (with collar)	G3440-81013
Inlet Supplies	
Inlet liner, Ultra Inert, split, low pressure drop, glass wool	5190-2295
Inlet liner, universal, Ultra Inert, mid-frit, 870 µL, 4 mm, 1/pk*	5190-5105
BTO Inlet Septa, 11 mm, 50/pk	5183-4757
Ultra Inert Gold Seal, with washer, 10/pk	5190-6145
Blue Line 10uL PTFE tipped plunger syringe (fixed needle, 23-26/42/cone)	G4513-80203
Ferrule, 0.4 mm id, 15% graphite/85% Vespel, 10/pk	5181-3323
Sample Containment	
2 mL Amber screw top vials with write-on spot with blue screw caps, PTFE/Silicone septa, 100/pk	5190-2280
Cap, screw, blue PTFE/red Silicone septa, 100/pk	5182-0717
Standards	
Ethylene oxide in Methanol, 1000 µg/mL	NV-245-1
2-Chloroethanol in Methanol, 1000 µg/mL	EPA-1207-1
InfinityLab Ultrapure LC/MS water	5191-4498
InfinityLab Ultrapure LC/MS acetonitrile	5191-4496
MS Supplies	
EI Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	G7005-60061

* *Glass fritted liners are alternatives to glass wool. They provide the barrier and volatilization site without the risk of wool breakage or liner movement.*

MyList 2: Consumables recommended for EtO:2-CE analysis EURL using improved GC-MS/MS system setup with Gerstel CIS4 with automated liner exchange (ALEX) option and cooled injection system using PV-type inlet (Reference 5994-4942).

Description	Part No.
Sample Preparation	
Centrifuge Tubes and cap, 50 mL, 25/pk	5610-2049
QuEChERS extraction kit, EN 15662 method, 50/pk	5982-5650
Ceramic Homogenizers, 50 mL tubes, 100/pk	5982-9313
QuEChERS Dispersive Kit, Fruits and Vegetables with Fats and Waxes, EN method, 15 mL, 50/pk	5982-5156
Ceramic Homogenizers, 15 mL tubes, 100/pk (optional)	5982-9312
Captiva Econofilter, polypropylene, Nylon membrane, 25 mm, 0.2 µm, 1000/pk	5190-5271
Captiva Disposable Syringe, 5 mL, 100/pk	9301-6476
Columns & Accessories	
J&W HP-VOC GC Column, 30 m, 0.20 mm, 1.12 µm	19091R-303
Fused silica tubing, deactivated, 0.25 mm, 5 m	CP802505
Ultimate Union Kit, deactivated	G3182-61580
Blue Line 10 µL PTFE tipped plunger syringe (fixed needle, 23-26/42/cone)	G4513-80203
Sample Containment	
2 mL Amber screw top vials with write-on spot with blue screw caps, PTFE/Silicone septa, 100/pk	5190-2280
Cap, screw, blue PTFE/red Silicone septa, 100/pk	5182-0717
Standards	
Ethylene oxide in methanol, 1000 µg/mL	NV-245-1
2-Chloroethanol in methanol, 1000 µg/mL	EPA-1207-1
InfinityLab Ultrapure LC/MS water	5191-4498
InfinityLab Ultrapure LC/MS acetonitrile	5191-4496
MS Supplies	
EI Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	G7005-60061

* *Note: Gerstel CIS4 with automated liner exchange (ALEX) uses Glass wool liner (Gerstel p/n 010850-010-00).*

MyList 3: Consumables recommended for analyzing 2-CE, as a marker of EtO fumigation (Reference 5994-3805).

Description	Part No.
Sample Preparation	
Centrifuge Tubes and cap, 50 mL, 25/pk	5610-2049
QuEChERS Dispersive Kit, Universal, 2 mL, 100/pk	5982-0028
Columns & Accessories	
Agilent VF-624ms, 60 m x 0.25 mm x 1.4 µm	CP9103
Fused silica tubing, deactivated, 0.25 mm, 5 m	CP802505
Ultimate Union Kit, deactivated	G3182-61580
Inlet/non-MSD self-tightening nut (with collar)	G3440-81011
MSD self-tightening nut (with collar)	G3440-81013
Inlet supplies	
Inlet liner, Ultra Inert, splitless, single taper, glass wool, 1/pk	5190-2293
Splitless, UI, Fritted Liner, Low, 870 µl, 4 mm, 1/pk*	5190-5112
BTO Inlet Septa, 11 mm, 50/pk	5183-4757
Ultra-Inert Gold Seal, with washer, 10/pk	5190-6145
Blue Line 10uL PTFE tipped plunger syringe (fixed needle, 23-26/42/cone)	G4513-80203
Ferrule, 0.4 mm id, 15% graphite/85% Vespel, 10/pk	5181-3323
Sample Containment	
2 mL Amber screw top vials with write-on spot with blue screw caps, PTFE/Silicone septa, 100/pk	5190-2280
Cap, screw, blue PTFE/red Silicone septa, 100/pk	5182-0717
Standards	
Ethylene oxide in methanol, 1000 µg/mL	NV-245-1
2-Chloroethanol in methanol, 1000 µg/mL	EPA-1207-1
InfinityLab Ultrapure LC/MS water	5191-4498
MS Supplies	
EI Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	G7005-60061

* *Glass fritted liners are alternatives to glass wool. They provide the barrier and volatilization site without the risk of wool breakage or liner movement.*

MyList 4: Consumables recommended for EtO:2CE analysis using GC-MS/MS with Headspace sampler (Reference: 5994-5378).

Description	Part No.
Columns & Accessories	
Agilent J&W DB-VRX, 60 m, 0.25 mm, 1.40 µm	122-1564
Inlet/non-MSD self-tightening nut (with collar)	G3440-81011
MSD self-tightening nut (with collar)	G3440-81013
Inlet Supplies	
Agilent inlet liner, direct, 1.5 mm id for gas samples, head-space	18740-80200
Agilent Inlet septa, 11 mm, Non-stick Long Life, 50/pk	8010-0239
Ferrule, 0.4 mm id, 15% graphite/85% Vespel, 10/pk	5181-3323
Ultra-Inert Gold Seal, with washer, 10/pk	5190-6145
Sample Containment	
Vial, crimp top, headspace, amber, flat bottom, certified, 20 mL, 23 x 75 mm, 100/pk, vial size: 22.75 x 75 mm (20 mm cap)	5067-0226
Cap, crimp, headspace, aluminum, PTFE/silicone septa, 20 mm, 100/pk, cap size: 20 mm	5183-4477
Standards	
Ethylene oxide in methanol, 1000 µg/mL	NV-245-1
2-Chloroethanol in methanol, 1000 µg/mL	EPA-1207-1
InfinityLab Ultrapure LC/MS water	5191-4498
Headspace Syringe	
Agilent syringe for CTC headspace, PAL3 type, 2.5 mL, PTFE-tip plunger, 23/56/side hole	G7378-80101
MS Supplies	
EI Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	G7005-60061

MyList 5: Consumables recommended for 2BE:2CE analysis according to Korean MFDS.[6]

Description	Part No.
Columns & Accessories	
Centrifuge Tubes and cap, 50 mL, 25/pk	5610-2049
QuEChERS extraction salt packets, no centrifuge tubes, 50/pk	5982-6650
QuEChERS Dispersive Kit, 2 mL, 100/pk	5982-5121
Ceramic Homogenizers, 50 mL tubes, 100/pk	5982-9313
Captiva Econofilter, polypropylene, Nylon membrane, 25 mm, 0.2 µm, 1000/pk	5190-5271
Captiva Disposable Syringe, 5 mL, 100/pk	9301-6476
Columns & Accessories	
Agilent J&W DB-WAX UI column, 30 m, 0.25mm, 0.5 µm	122-7033UI
Inlet/non-MSD self-tightening nut (with collar)	G3440-81011
MSD self-tightening nut (with collar)	G3440-81013
Inlet Supplies	
Inlet liner, Ultra Inert, split, low pressure-drop, glass wool, 1/pk	5190-2295
Inlet liner, universal, Ultra Inert, mid-frit, 870 µL, 4 mm, 1/pk*	5190-5105
BTO Inlet Septa, 11 mm, 50/pk	5183-4757
Ultra Inert Gold Seal, with washer, 10/pk	5190-6145
Blue Line 10 µL PTFE tipped plunger syringe (fixed needle, 23-26/42/cone)	G4513-80203
Ferrule, 0.4 mm id, 15% graphite/85% Vespel, 10/pk	5181-3323
Sample Containment	
2 mL Amber screw top vials with write-on spot with blue screw caps, PTFE/Silicone septa, 100/pk	5190-2280
Cap, screw, blue PTFE/red Silicone septa, 100/pk	5182-0717
Standards	
Ethylene oxide in methanol, 1000 µg/mL	NV-245-1
2-Chloroethanol in methanol, 1000 µg/mL	EPA-1207-1
InfinityLab Ultrapure LC/MS water	5191-4498
InfinityLab Ultrapure LC/MS acetonitrile	5191-4496
MS Supplies	
EI Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	G7005-60061

* *Glass fritted liners are alternatives to glass wool. They provide the barrier and volatilization site without the risk of wool breakage or liner movement.*

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