Ethylene Oxide & 2-Chloroethanol analysis in Foods using Triple Quadrupole GC/MS/MS

Consumables Workflow Ordering Guide

Agilent

Ethylene Oxide & Ethylene Chlorohydrin in Foods by GC/TQ

Ethylene oxide (EO) 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 (EO) reacts with food matrix to form 2-chloroethanol (2-CE or Ethylene chlorohydrin) and residues of EO and 2-CE maybe found in foodstuffs that have been fumigated. Its presence in sesame seeds from India prompted a spate of product recalls, including bread and bagels, across Europe.[1]

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. EPA has concluded that ethylene oxide is carcinogenic to humans by the inhalation route of exposure.[2]

The European Union (EU) has set the maximum residual limits (MRL) for both EO and its primary metabolite 2-CE in various food and agriculture commodities ranging from 0.02 to 0.1 mg/kg (Commission Regulation (EU) 2015/868). The MRLs proposed by the EPA for ethylene oxide are 7 mg/kg.[3]

In October 2020, the EU Commission issued the Implementing Regulation (EU) 2020/1540[4] requiring 50% of sesame originating from India to be tested for ethylene oxide and ethylene chlorohydrin. Since the autumn of 2020, the demand for ethylene oxide analysis has increased significantly worldwide. While the initial focus was on sesame imported from India and products made from it, it has become apparent that ethylene oxide can be a relevant issue in various value chains. Recently products that contained locust bean gum (E410), a thickening agent used in ice-creams, chocolates, biscuits, bread, and crackers, were in the focus and the subject of many public recalls.[5]

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[6-10] available that utilize different approaches:

1. Determination of 2-CE and EO, with distinction between 2-CE and EO.
2. Determination of 2-CE with EO. In this case the result is expressed as the sum of EO and 2-CE.
3. Utilizing 2-CE as a marker to confirm the use of ethylene oxide. In this case, the ethylene oxide residue in the sample is converted to 2-CE, to estimate the actual EO initially present in the sample.
4. Utilizing 2-Bromoethanol (2-BE) as a marker to confirm the use of ethylene oxide. In this case, the ethylene oxide residue in the sample is converted to 2-BE through bromination followed by GC/MS quantitation of 2-BE and 2-CE.

Depending on the method used the sample preparation steps can be time consuming. The procedure described in EN-15662, mentioned by the EU Reference Labs for single residue methods (EURL SRM), can be adjusted for cereals, spices, and other dry commodities with low lipid content. This procedure is suitable for the analysis of both EO and 2-CE as these compounds are polar and show little tendency to partition into a separated lipid phase. Tables 1 and 2 show typical conditions and details of the EURL-SRM method.

Table 1. The EU Reference Lab Method parameters.[7]

Column	J&W HP-VOC GC Column, 30 m, 0.20 mm, 1.10 µm, Part Number:19091R-303
Pre-column	Fused Silica Tubing, Deactivated, Diameter: 0.25 mm, Agilent J&W, Length: 3 m
Mobile Phase / Flow	Helium, 1 mL constant flow
Injection mode	Split 1:4
Injection volume	2 µL
Injection temp. program	Start at 90 °C and hold for 0.8 min, ramp with 12°C/s to 250 °C, hold 10 min,
Oven temp. program	Start: 45 °C, hold 2 min, ramp to 150 °C reached at 4.1 min; ramp to 280°C reached at 5.1 min, hold till 16 min
MS Parameters	Mass axis tune mode: LowMass Ionisation mode: EI Transfer line temperature: 250°C; Ion source temperature: 270 °C

Table 2. EURL-SRM method MRM details of EO, 2-CE, and their respective deuterated forms (2-CE-D_4 and EO-D_4) using method parameters in Table 1.[7]

Name of Compound	Retention Time (min)	Parent (m/z)	Daughter (m/z)	CE
Ethylene oxide	2.57	44	14	20
		44	28	5
		44	29	5
Ethylene oxide D_4	2.57	48	16	20
		48	30	5
2-Chloroethanol	4.61	80	31	5
		80	43	5
		82	31	5
2-Chloroethanol D_4	4.61	84	33	5
		86	33	5

Table 3.GC-MS Analysis condition on an Agilent 7890B/7010B used by Korean Ministry of Food and Drug Safety (MFDS).[9] 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

Alternate time-saving method that meets EPA and EU requirements by converting Ethylene Oxide to Ethylene Chlorohydrin

Agilent scientists recently developed an accurate and rugged method[10] that is easier to implement with fewer time-consuming steps. During sample preparation, the ethylene oxide residue in the sample is converted to ethylene chlorohydrin, which is followed by liquid-liquid extraction (LLE) with ethyl acetate, and cleaned up before injecting into GC/TQ. The LOQ of the method was demonstrated at the 10 ng/g level satisfying regulatory requirements of MRL set at 50 ng/g by EU as well as the EPA. The results were repeatable across six replicates and recovery was satisfactory at the 10 ng/g spiked concentration of ethylene oxide and ethylene chlorohydrin in sesame seed samples (Table 4). The ethylene chlorohydrin peak at this level was easily distinguished from baseline and matrix with signal-to-noise ratios >2.9 (Figures 1 & 2).

1. Weigh 2 g of sample into 50 mL centrifuge tube.
2. Add 2 mL of water, 2 mL of 0.1 N H_2SO_4, and 1 mL of saturated sodium chloride solution.
3. Sonicate for 20 minutes.
4. Rest sample in water bath at 50 °C for 1 hour.
5. Vortex and wait until the sample reaches room temperature.
6. Add 5 mL ethyl acetate and vortex for 10 minutes.
7. Centrifuge at 8,000 rpm for 5 minutes at 5 °C. [Note: 5,000 rpm works as well]
8. Take 1 mL of supernatant and add it to a dispersive QuEChERS cleanup tube (universal) (part number 5982-0028).
9. Shake and centrifuge at 5,000 rpm for 5 minutes.
10. Collect supernatant in a vial and inject into the GC/MS/MS.

Table 4. Recovery in sesame seed sample.[10]

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

The method (Table 5 & 6) demonstrated in this work is useful for detecting ethylene chlorohydrin as a marker of fumigation of sesame seeds. Ethylene oxide was analyzed using the 8890 GC system coupled with the 7000D triple quadrupole MS. The LOQ was 10 ng/g, which complies with the MRL set by EU at 50 ng/g (Figure 1 & 2).

Table 5. GC method for detecting 2-chlorethanol as a marker of fumigation of sesame seeds.[10]

GC Conditions	
Column	Agilent VF-624 ms, 60 m × 0.25 mm, 1.4 µm (p/n CP9103)
Inlet	Agilent Multimode Inlet 5190-2293, splitless liner Injection volume: 2 µL
Injection Mode	Pulsed Splitless, 25 psi until 0.8 min, purge flow of 40 mL/min at 1.25 min
Inlet Temperature	250 °C
Oven	60 °C for 2 min, at 10 °C/min to 150 °C, at 40 °C/min to 250 °C, hold 20 min
Carrier Gas	99.9995% Helium at 1.0 mL/ min, constant flow mode

Table 6. MS method MS conditions for detecting 2-chloroethanol.[10]

MSD Conditions	
Quadrupole Temperature	150 °C
Ion Source Temperature	EI 280 °C
Transfer Line Temperature	250 °C
MRM Transitions for Ethylene Chlorohydrin	82 & 31 (CE: 5) 80 & 43 (CE: 5) 80 & 31 (CE: 5)
EMV Mode	Gain factor: 10
Dwell Time for Each Transition	75
Solvent Delay	9.5

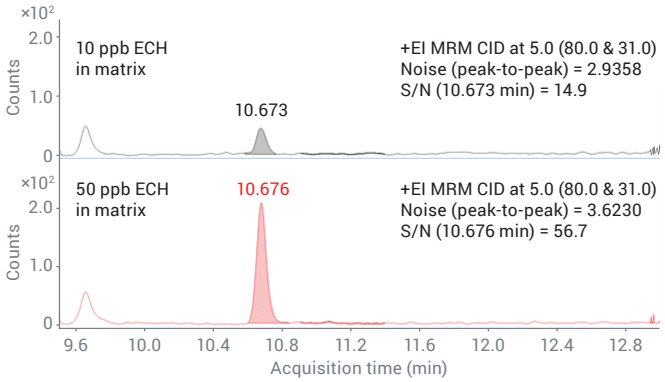

Figure 1. Sensitivity of ethylene chlorohydrin: MRM chromatograms of 10 ng/g spike and 50 ng/g spike using method parameters described in Tables 5 & 6.[10]

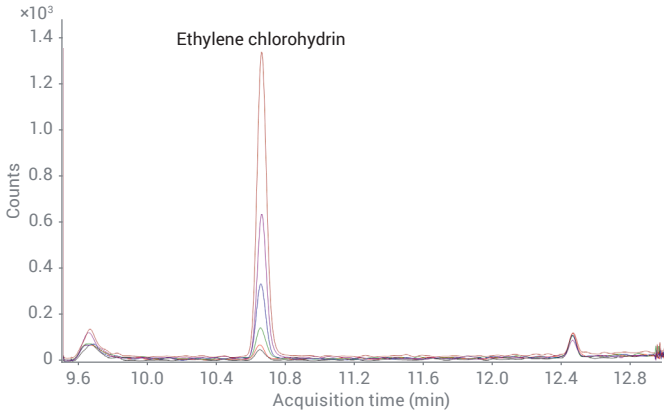

Figure 2. TIC MRM overlay of various concentrations of ethylene chlorohydrin in matrix ranging from 5 ppb to 200 ppb using method parameters described in Tables 5 & 6.[10]

References

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MyList 1: Columns and supplies for method converting Ethylene Oxide to 2-Chloroethanol, as described in 5994-3805.[10]

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-624 ms - 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 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
MS Supplies	
EI Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	G7005-60061

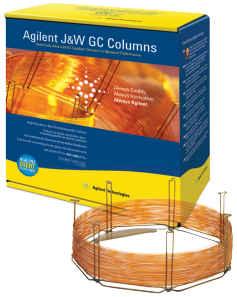
MyList 2: Columns and supplies for the EU reference Lab EURL-SRM method.[7]

Description	Part No.
Sample Preparation	
Centrifuge Tubes and cap, 50 mL, 25/pk	5610-2049
QuEChERS dispersive Kit, Universal, 2 mL, 100/pk	5982-5121
QuEChERS dispersive Kit, Universal, 2 mL, with Ceramic homogenizers, 100/pk	5982-5121CH
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	
HP-VOC, 30 m, 0.20 mm, 1.10 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	
2mL 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 3: Columns and supplies for Korean MFDS method.[9]

Description	Part No.
Sample Preparation	
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 mm, 1000/pk	5190-5271
Captiva Disposable Syringe, 5 mL, 100/pk	9301-6476
Columns & accessories	
Agilent J&W DB-WAX UI column, 30 m, 0.25 mm, 0.5 mm	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 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	
2mL 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



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