

Estimation of Ethylene Oxide and Ethylene Chlorohydrin in Sesame Seeds Using Agilent 8890 GC and 7000D Triple Quadropole MS System

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Abstract

This application note demonstrates the use of the Agilent 8890 GC system coupled with the Agilent 7000D GC/MS triple quadrupole mass spectrometer to detect and quantify ethylene oxide and ethylene chlorohydrin in sesame seed samples. The method provides the highest confidence in results for routine analysis for the food industry, whether involved in production, processing, storage, or commercial testing of sesame seed samples or for academic purposes. 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. A limit of quantitation (LOQ) of 10 ppb has been demonstrated in matrix.

Ethylene oxide is used to sterilize oilseeds and spices during storage. Residues of ethylene oxide and its derivative, ethylene chlorohydrin (produced by reactions during storage) may be found in these foodstuffs.¹ Ethylene chlorohydrin can be used as a suitable marker to confirm the use of ethylene oxide for fumigation. The ethylene chlorohydrin can be evaluated in sesame seeds by a simple GC/TQ analytical method. This evaluation also provides the estimation of actual ethylene oxide present in the sample initially by using a conversion factor.

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

Experimental

Chemicals required

- 0.1 N sulfuric acid (0.981 g sulfuric acid dissolved in 100 mL water)
- Saturated sodium chloride solution in water
- Water (Millipore, Milli-Q)
- Ethyl acetate (HPLC grade)
- Agilent QuEChERS dispersive cleanup kit (part number 5982-0028)
- Ethylene oxide reference standard (Sigma-Aldrich part number CRM48891)
- Ethylene chlorohydrin reference standard (Merck part number 8.00945.0100)

Apparatus required

An Agilent 8890 GC system equipped with an MMI inlet configured with postcolumn backflush option and a 7000D triple quadrupole MS, an ultrasonic bath, a water bath, a cold centrifuge, a table-top centrifuge, and a vortex mixer were used in this study. The procedure was as follows:

1. Weigh 2 g of sample into 50 mL centrifuge tube.
2. Add 2 mL of water, 2 mL of 0.1 N H₂SO₄, 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.
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 1. GC method.

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 2. MS method MS conditions.

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

Results and discussion

With the above method, the LOQ was estimated to be at 10 ng/g for ethylene chlorohydrin in sesame seed samples as at this level, the peak is easily distinguished from baseline and matrix with signal to noise ratios >2.9. Figure 1 highlights the quantifier and qualifier EICs at LOQ level spiking. This LOQ satisfies the needs of customers and regulatory requirements of MRL set at 50 ng/g by EU. Figure 2 demonstrates the signal-to-noise for 10 ppb and 50 ppb level matrix standards.

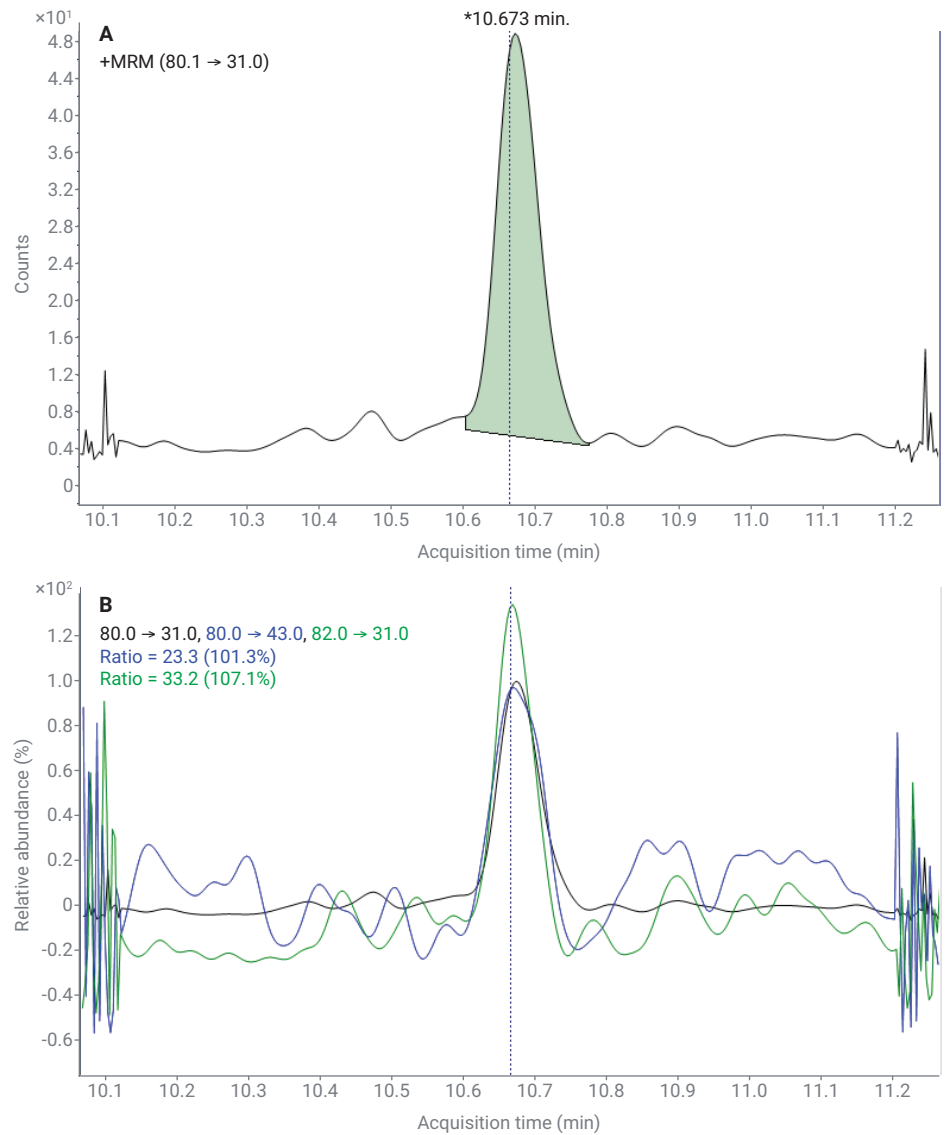


Figure 1. A quantifier and two qualifier peaks of ethylene chlorohydrin at the 10 ng/g spike level.

Calibration and linearity

A prespiked matrix linearity plot was generated for response (peak area) across concentration levels from 5 to 200 ng/g (Figure 4). Calibration was performed at six levels: 5, 10, 20, 50, 100, and 200 ng/g. Good linearity with $R^2 > 0.998$ was observed.

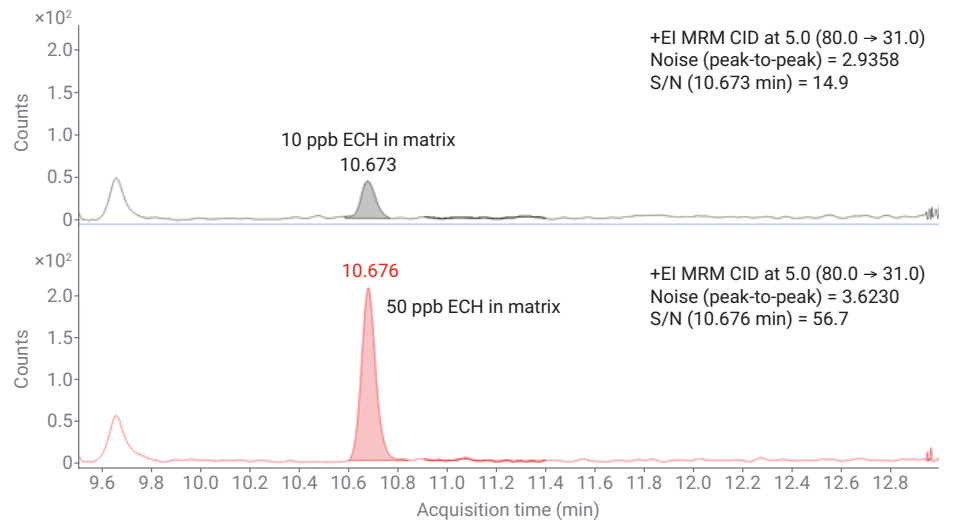


Figure 2. Sensitivity of ethylene chlorohydrin: MRM chromatograms of 10 ng/g spike and 50 ng/g spike.

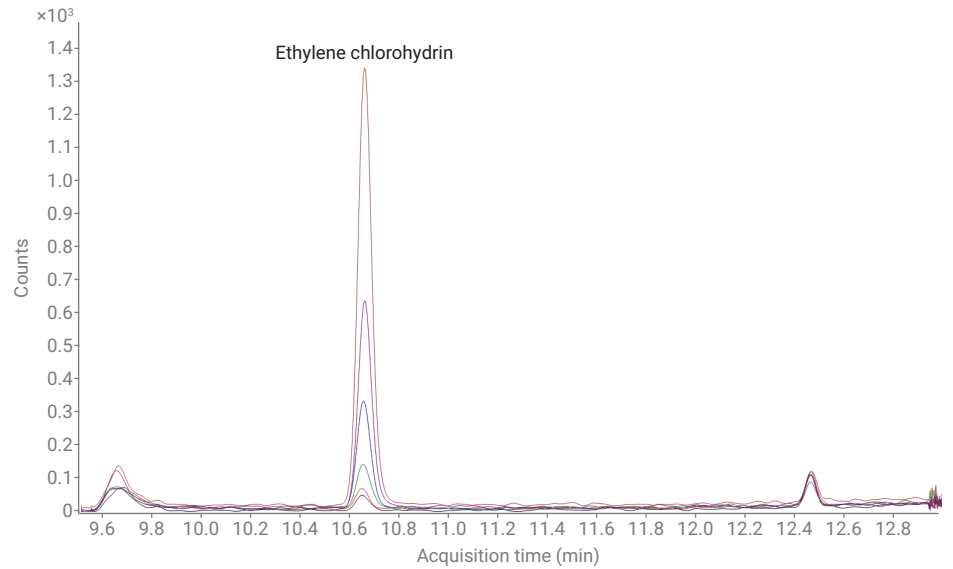


Figure 3. TIC MRM overlay of various concentrations of ethylene chlorohydrin in matrix ranging from 5 ppb to 200 ppb.

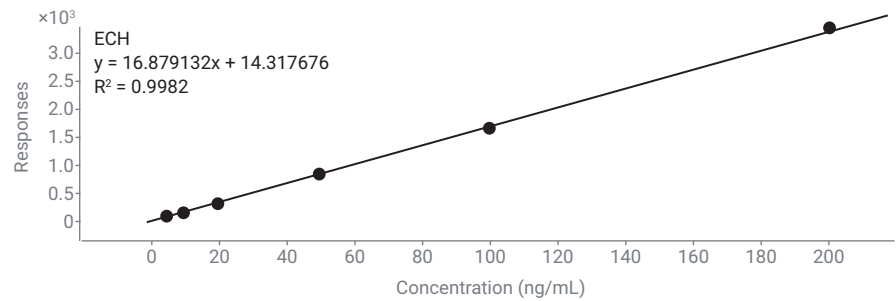


Figure 4. Calibration plot for ethylene chlorohydrin matrix-matched standards.

Repeatability

Repeatability of elution was demonstrated by injecting an ethylene chlorohydrin standard in matrix with a 50 ppb concentration. Relative standard deviation on peak areas of ethylene chlorohydrin calculated based on six replicate injections of 50 ppb matrix standard was 1.73%, as shown in Table 3.

Quantitation in sesame seed samples

The discussed method was extended to the sesame seed sample, which was purchased from a local market for the analysis and recovery study.

Recovery study

As shown in Table 4, no peak corresponding to ethylene chlorohydrin was found in the blank matrix of sesame seed. The recoveries of ethylene chlorohydrin and ethylene oxide from the real-world sesame seed sample were calculated using the spiking levels of 10 and 50 ppb for ethylene chlorohydrin and 10 ppb for ethylene oxide.

Three spike studies were performed as follows:

1. Ethylene chlorohydrin was spiked in the sesame seed sample at the 10 ppb level.
2. Ethylene chlorohydrin was spiked in the sesame seed sample at the 50 ppb level.
3. Ethylene oxide was spiked in the sesame seed sample at the 10 ppb level. This was done to check the applicability of the method for estimating the ethylene oxide content in sample. (A conversion factor of 0.55 was used as a multiplier to calculate the results for the ethylene oxide spiking sample).² The obtained results for percent recovery are discussed in Table 5.

Table 3. Percentage RSD (CV) for ethylene chlorohydrin for the 50 ppb matrix-matched standard.

Area Inj-1	Area Inj-2	Area Inj-3	Area Inj-4	Area Inj-5	Area Inj-6	%RSD
821	804	811	829	842	810	1.73

Table 4. Quantitation summary for calibration (5 to 200 ppb), spike recovery (10 and 50 ppb), and repeatability exercise (50 ppb).

Sample Name	Compound	Sample Type	RT	Response	Final Conc. (ng/g)
Matrix Blank_sesame seed	Ethylene Chlorohydrin	Sample			ND
Matrix calibration-1_5 ppb	Ethylene Chlorohydrin	Calibration	10.646	115	5.97
Matrix calibration-2_10 ppb	Ethylene Chlorohydrin	Calibration	10.650	165	8.9
Matrix calibration-3_20 ppb	Ethylene Chlorohydrin	Calibration	10.654	324	18.32
Matrix calibration-4_50 ppb	Ethylene Chlorohydrin	Calibration	10.654	856	49.85
Matrix calibration-5_100 ppb	Ethylene Chlorohydrin	Calibration	10.657	1,676	98.46
Matrix calibration-6_200 ppb	Ethylene Chlorohydrin	Calibration	10.657	3,449	203.5
Sesame_ECH SPK_10 ppb	Ethylene Chlorohydrin	Sample	10.673	184	10.08
Sesame_ECH SPK_50 ppb	Ethylene Chlorohydrin	Sample	10.676	859	50.04
Sesame_ETO SPK_10 ppb	Ethylene Chlorohydrin	Sample	10.673	267	8.23
50 ppb spk replicate-1	Ethylene Chlorohydrin	Sample	10.699	821	47.8
51 ppb spk replicate-2	Ethylene Chlorohydrin	Sample	10.707	804	46.79
52 ppb spk replicate-3	Ethylene Chlorohydrin	Sample	10.714	811	47.2
53 ppb spk replicate-4	Ethylene Chlorohydrin	Sample	10.718	829	48.29
54 ppb spk replicate-5	Ethylene Chlorohydrin	Sample	10.737	842	49.05
55 ppb spk replicate-6	Ethylene Chlorohydrin	Sample	10.733	810	47.14

Table 5. Recovery in sesame seed sample.

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

Conclusion

An accurate and rugged method was developed for analysis of ethylene oxide and ethylene chlorohydrin in sesame seeds. The sample preparation method uses easy and fewer time-consuming steps. The LOQ of the method is demonstrated at the 10 ng/g level in samples. Repeatable results were found for six replicates of spiked samples. Satisfactory recovery was found at the 10 ng/g spiked concentration of ethylene oxide and ethylene chlorohydrin in sesame seed samples. Thus, the method demonstrated in this study proves useful for the routine analysis of sesame seed samples fumigated with ethylene oxide under the established regulatory limits.

References

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