

Estimation of Ethylene Oxide and 2-Chloroethanol in Spices and Oilseeds Using QuEChERS Extraction and GC/MS/MS

Agilent 8890/7010 triple quadrupole GC/MS system



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Abstract

This application note demonstrates the use of an Agilent 8890 GC system coupled with an Agilent 7010 GC/MS triple quadrupole mass spectrometry system to detect and quantify ethylene oxide (EtO) and 2-chloroethanol (2-CE) simultaneously in foodstuffs such as flaxseed, cumin powder, and red chili powder. Sample preparation was done using QuEChERS extraction and a dispersive cleanup process followed by injection into GC/MS/MS through liquid injection. A limit of quantification (LOQ) of 10 ppb for both compounds was achieved in matrix. Average recoveries ranged from 75 to 86% for both compounds.

Introduction

EtO is used to sterilize foods to eliminate insects and bacteria, such as salmonella. Ethylene chlorohydrin, or 2-CE, is a derivative produced by the reaction of EtO with chlorine ions present in foodstuff. Use of EtO is banned in the European Union (EU) due to its carcinogenic and toxic properties.

Previously, methods for analysis of EtO (EtO and 2-CE) have been developed that include acidic conversion of EtO to 2-CE. These methods are time-consuming, labor-intensive, and require large quantities of harmful solvents. Due to the volatile nature of EtO, sample preparation is crucial. In December 2020, the EU Reference Laboratories (EURL) for Residues of Pesticides recommended a single-residue method for the analysis of EtO and 2-CE in sesame seeds using QuEChERS extraction followed by GC/MS/MS analysis.

The method adopted in this work demonstrates the use of an automated liquid sampler for sample introduction to the 8890 GC system coupled with a 7010 GC/MS triple quadrupole mass spectrometry system.

Table 1. GC/TQ parameters.

Parameter	Value
GC/MS/MS Method Parameters	
GC	Agilent 8890 GC with G4513 autosampler
Mass Spectrometer	Agilent 7010 triple quadrupole mass spectrometry system
Analytical Column	Agilent J&W DB-VRX (60 m x 0.25 mm, 1.4 µm)
Column Flow	Helium: 1.0 mL/min, constant flow
Injection Mode	Pulsed split (4:1)
Injection Volume	2 µL
Injection Program	Starts at 90 °C (hold for 0.8 min), ramped at 450 °C/min to 250 °C, hold for 10 min
Oven Program	Starts at 40 °C (hold for 1.0 min), ramped at 10 °C/min to 160 °C, and then at 30 °C/min to 245 °C, hold for 5 min
MS Parameters	Ionization mode: EI; Ion source temperature: 230 °C; Quadrupole temperature (Q1 and Q2): 150 °C
MRM Transitions	
EtO	44 & 14 (CE:20) 44 & 28 (CE:5) 44 & 29 (CE:5)
2-CE	80 & 31 (CE:5) 80 & 43 (CE:5) 82 & 31 (CE:5)

Experimental

Standard preparation

Due to the high volatility of EtO, its standard solutions were prepared at a low temperature (< 10 °C). As a diluent, acetonitrile was placed in a freezer (at -20 °C) for at least 15 minutes before use. The cold analytical standard solutions were diluted with cold acetonitrile to prepare the stock standard solutions of EtO and 2-CE, which had a concentration of 1 mg/mL each.

The stock standard solution was further diluted with acetonitrile to obtain a working standard solution which had a concentration of 10 µg/ml for EtO and 2-CE. All the calibration standards (2.0, 5.0, 10.0, 20.0, 50.0, 100, and 200 ng/mL) were freshly prepared by diluting the stock solution with acetonitrile. Matrix-matched standards of flaxseeds, cumin powder, and red chili powder were prepared by post-spiking the requisite amount of solution to extracts of each matrix. Before analysis, all stock solutions were preserved at a temperature of -20 °C to avoid degradation.

Sample preparation

Homogenized samples of flaxseed, cumin powder, and red chili powder were processed using the QuEChERS extraction procedure according to the EN 15662 procedure (Figure 1). Approximately 2.00 ± 0.01 g of each sample was weighed in 50 mL centrifuge tubes. Ten mL of cold water was added to the centrifuge tubes followed by capping and vortex mixing for 1 minute to ensure sample hydration, followed by the addition of 10.0 mL of cold acetonitrile along with two ceramic homogenizers to improve the extraction efficiency. The centrifuge tubes were capped and shaken for 10 minutes. The QuEChERS extraction salt (4 g of $MgSO_4$, 1 g of NaCl, 1 g of sodium citrate, and 0.5 g of disodium citrate sesquihydrate) was added, and the tubes were shaken for another 3 minutes. The samples were then centrifuged for 5 minutes at 6,000 rpm. The upper acetonitrile layer (6.0 mL) was transferred to QuEChERS Dispersive Kit 15 mL tubes (150 mg of PSA, 150 mg of C18EC, and 900 mg $MgSO_4$). The tubes were vortexed for 30 seconds followed by centrifugation at 5,000 rpm for 5 minutes. After centrifugation, the supernatant was transferred into GC vials for analysis.

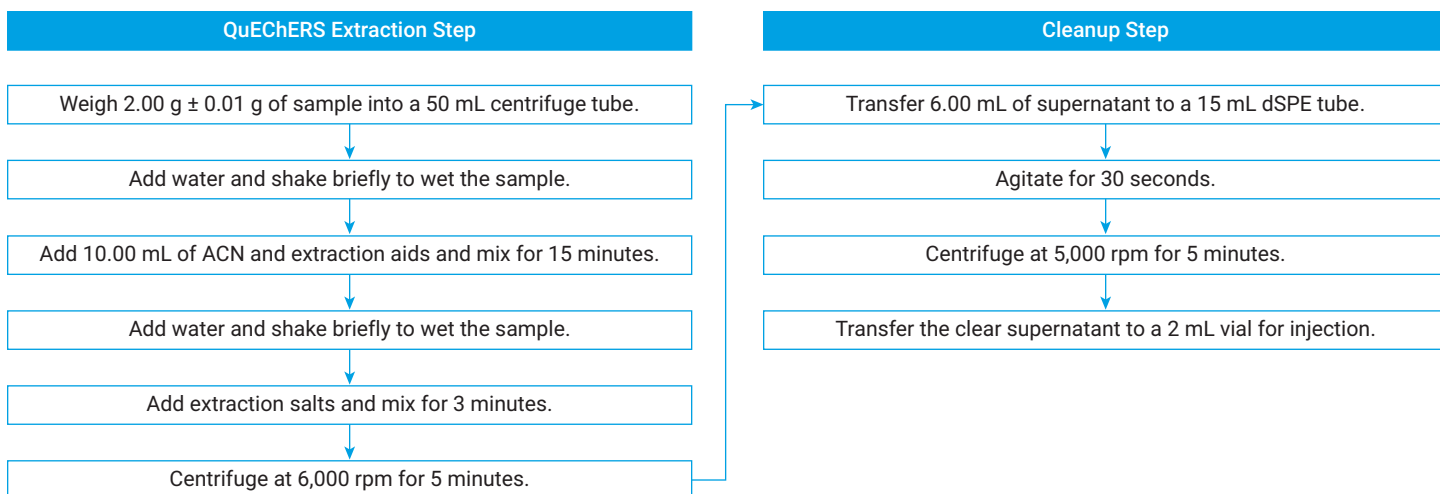


Figure 1. QuEChERS workflow for the extraction and cleanup of samples.

Results and discussion

Calibration

Matrix calibrations were performed for EtO and 2-CE at concentrations of 2, 5, 10, 20, 50, and 100 ng/mL in solvent. Similar calibrations were performed in post-spike matrix extracts for flaxseed, cumin powder, and red chili powder. Excellent values for $R^2 > 0.99$ were obtained.

Figures 8 and 9 show linearity of EtO and 2-CE respectively in acetonitrile. Figures 10 and 11 show linearity of EtO and 2-CE respectively in a flaxseed matrix.

Standard area repeatability

A repeatable elution was obtained by injecting a 10 ppb concentration of EtO and 2-CE in a matrix extract. As shown in Table 2, a % RSD data of EtO and 2-CE is calculated from peak areas of 6 replicate injections of 10 ppb matrix standards in flaxseed, cumin powder, and red chili powder.

Recovery

EtO and 2-CE were spiked in samples of flaxseed, cumin powder, and red chili powder at concentration levels of 20 and 50 ng/g. Acceptable recoveries were obtained by quantification through post-spike matrix-based calibration. Results are highlighted in Table 3.

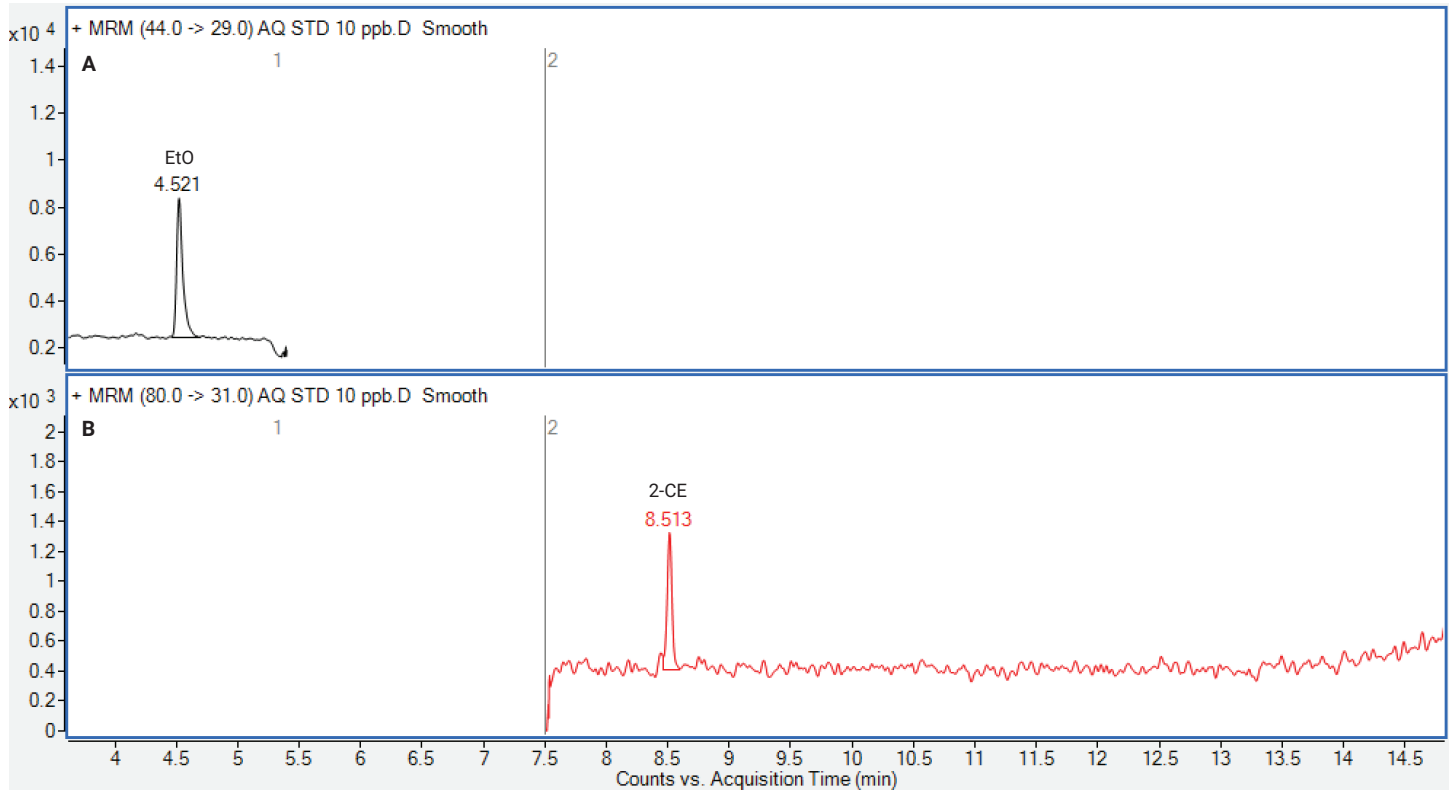


Figure 2. Chromatograms for (A) EtO at the 10 ng/mL level and (B) 2-CE at the 10 ng/mL level.

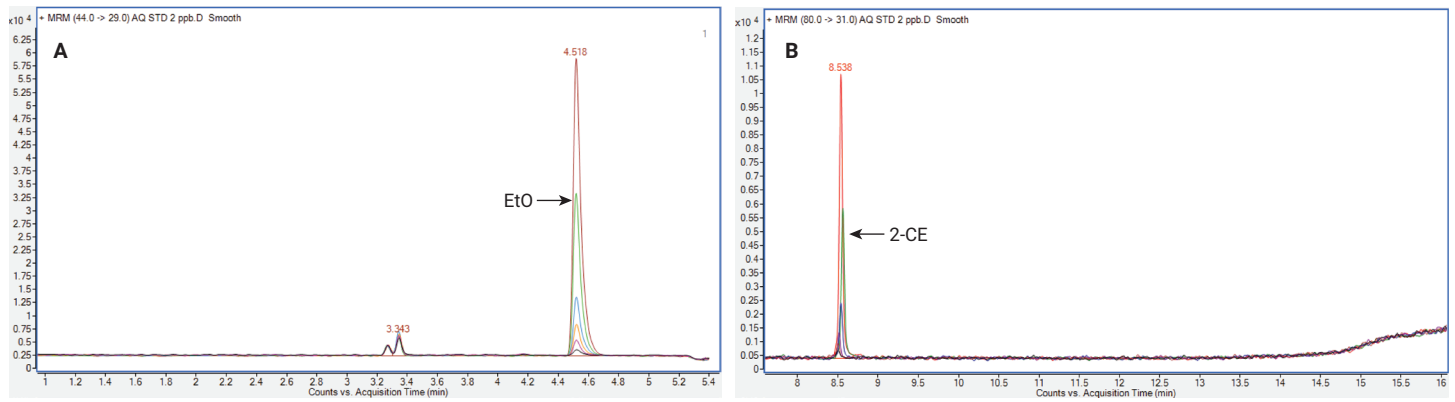


Figure 3. Overlaid chromatograms from a set of calibration standards from 2 ng/mL to 100 ng/mL; (A) EtO and (B) 2-CE.

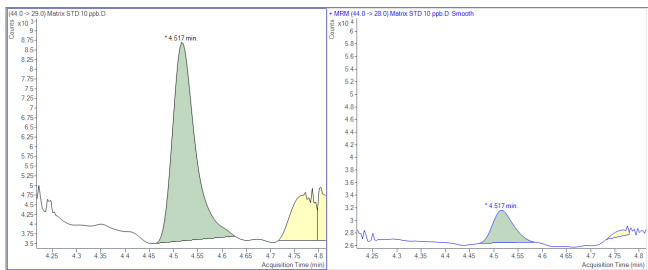


Figure 4. Quantifier and qualifier peaks of EtO at 10 ng/g matrix standard in flaxseed.

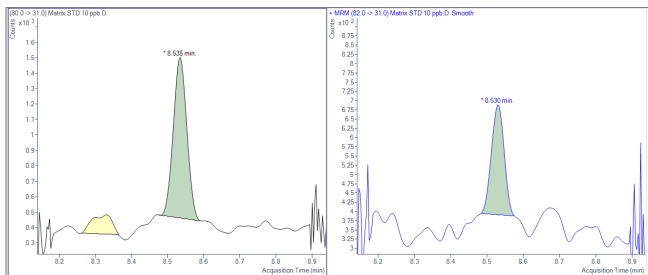


Figure 5. Quantifier and qualifier peaks of 2-CE at 10 ng/g matrix standard in flaxseed.

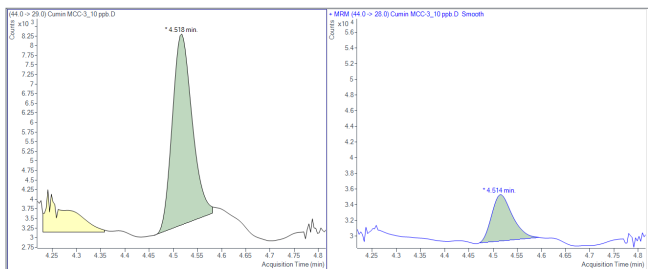


Figure 6. Quantifier and qualifier peaks of EtO at 10 ng/g matrix standard in cumin powder.

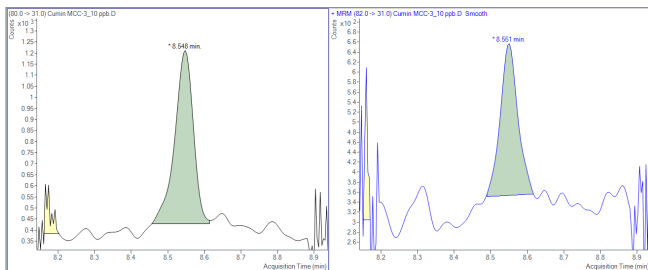


Figure 7. Quantifier and qualifier peaks of 2-CE at 10 ng/g matrix standard in cumin powder.

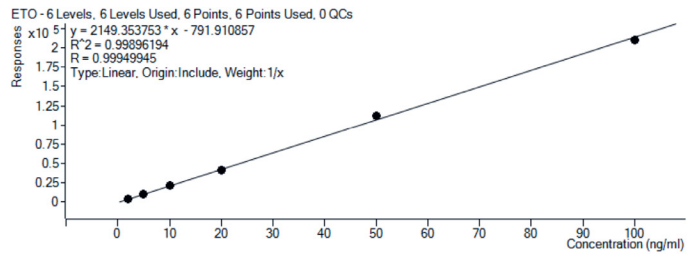


Figure 8. Calibration curve for EtO in acetonitrile ($R^2 > 0.998$).

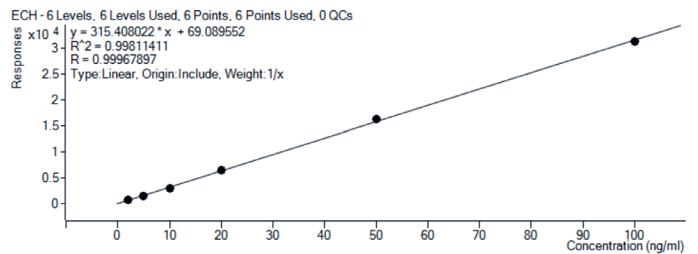


Figure 9. Calibration curve for 2-CE in acetonitrile ($R^2 > 0.998$).

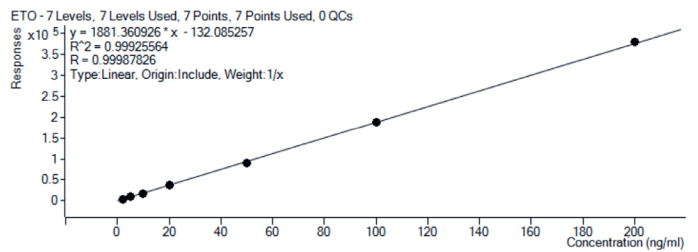


Figure 10. Calibration curve for EtO in flaxseed ($R^2 > 0.999$).

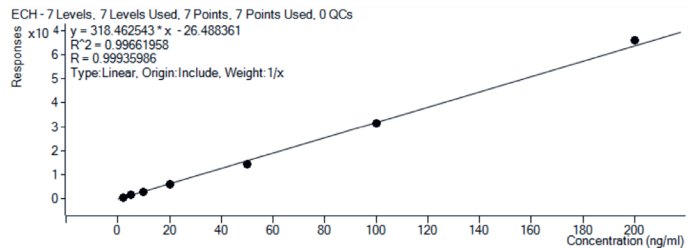


Figure 11. Calibration curve for 2-CE in flaxseed ($R^2 > 0.996$).

Table 2. Standard area repeatability for 6 replicates of 10 ppb matrix standard in flaxseed, cumin powder, and red chili powder.

Number of Injections (Replicates)	EtO (Peak Area)			2-CE (Peak Area)		
	Flaxseed	Cumin Powder	Chili Powder	Flaxseed	Cumin Powder	Chili Powder
	10 ng/mL					
Injection 1	2,266	2,193	3,135	1,065	893	735
Injection 2	2,264	2,205	3,445	1,049	1,035	910
Injection 3	2,545	2,179	3,425	1,129	1,125	880
Injection 4	2,562	2,162	3,440	993	1,048	755
Injection 5	2,392	2,228	3,385	1,236	1,168	845
Injection 6	2,526	2,135	3,335	1,100	979	785
Mean	2,426	2,184	3,361	1,095	1,041	818
SD	138.32	32.76	118	83.04	99.01	70.68
RSD (%)	5.70	1.50	3.51	7.58	9.51	8.64

Table 3. Percent Recovery of EtO and 2-CE in flaxseed and cumin powder spiked at 20 and 50 ng/g levels, respectively.

Food Matrix	EtO			2-CE		
	Spiked Concentration (ng/g)	Average Obtained Concentration (ng/g)	Recovery (%)	Spiked Concentration (ng/g)	Average Obtained Concentration (ng/g)	Recovery (%)
Flaxseed	20	15.12	75.6	20	15.84	79.2
Cumin Powder	50	39.02	78.0	50	43.29	86.6
Chili Powder	50	38.52	77.04	50	42.47	84.9

Conclusion

An accurate and rugged method was developed which meets the requirements of the EURL for a single-residue method that uses QuEChERS extraction followed by GC/MS/MS analysis for the analysis of EtO and 2-CE in flaxseed, cumin powder, and red chili powder. The LOQ of the method was demonstrated at 10 ng/g for all the tested matrices. Repeatable results are found for 6 successive replicates of matrix-based standards at 10 ng/g concentration levels for EtO and 2-CE. Excellent recoveries were obtained for both EtO and 2-CE in all tested matrices at 20 and 50 ng/g spiked concentration levels. Thus, this study demonstrates the usefulness of the developed method for the routine analysis of food samples for EtO and 2-CE at trace levels.

References

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