Application Note

Food and Beverage Testing

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Simultaneous Analysis of Ethylene Oxide and 2-Chloroethanol in Sesame Seeds and Other Food Commodities: Challenges and Solutions

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Abstract

The presence of ethylene oxide (EtO) and 2-chloroethanol (2-CE) in sesame seeds and other food commodities is an emerging food-safety concern. The first notification about the presence of EtO in sesame seeds occurred in August 2020, and since then over 800 new notifications have been published on the European Rapid Alert Food and Feed Safety (RASFF) portal. To ensure that foods are safe for consumption, food industry and enforcement agencies need reliable and robust methods to detect EtO and 2-CE in foods at levels below the EU established maximum residue limits (MRLs). The EU Reference Laboratories for Residues of Pesticides have proposed a method for the determination of these contaminants that uses Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) sample preparation followed by gas chromatography/tandem mass spectrometry (GC/MS/MS) analysis. However, laboratories encounter several challenges when it comes to applying this method to the analysis of EtO and 2-CE in food products. The high volatility of EtO requires the use of a dedicated column to ensure its separation from potential acetaldehyde interferences. Second, QuEChERS extracts often contain high amounts of nonvolatile material that can accumulate in the GC inlet liner and column inlet, affecting the accuracy and robustness of the analysis. Additionally, the analytical column can deteriorate due to the injection of samples containing high-boiling-point compounds. This application note describes an optimized and robust analytical method for the quantification of EtO and 2-CE in sesame and curcuma that uses a dedicated setup based on an Agilent 8890 Gas Chromatograph (GC) system, an Agilent 7010 Triple Quadrupole GC/MS (GC/TQ), and a Gerstel MPS sampler with the Automated Liner Exchange option. This option reduces downtime related to inlet maintenance, column exchange, and MS source cleaning compared to the proposed EU method.

Introduction

Ethylene oxide (EtO) is a disinfectant that was banned in the EU as a pesticide in 1991 due to its classification as a category-1 carcinogen by the International Agency Research of Cancer (IARC).[1] In September 2020, Belgium added a notification on the EU Rapid Alert System for Food and Feed (RASFF) concerning EtO residues in sesame seeds originating from India. The levels detected substantially exceeded the maximum residue level (MRL) of 0.05 mg/kg for sesame seeds set by Regulation (EU) 2015/868.[2] The notification resulted in increased testing and controls, leading to withdrawals and recalls of a significant number of conventional and organic products in many EU Member States. By February of 2022, over 700 notifications related to the occurrence of EtO, mostly in seeds (particularly sesame seeds) and spices, have been registered on the RASFF portal. The presence of unauthorized levels of EtO and its metabolite 2-chloroethanol (2-CE) is probably due to its use for fumigation to control insects and microorganisms (fungi and bacteria) in dry food products such as herbs, spices, nuts, and oily seeds. After contact with food products, EtO either interacts with the major matrix constituents leading to the formation of several metabolites, including 2-CE, or dissipates through evaporation. Because of the toxicity of EtO and 2-CE, a joint residue definition for the two components was introduced in 2008 in Regulation (EC) No 149/2008.[3] In 2015, the EU-MRLs for spices were lowered to 0.1 mg/kg. At the same time, the MRLs for nuts, oil fruits, and oilseeds were lowered to 0.05 mg/kg.[2] To enforce these regulations, accurate analysis methods for these contaminants in food are essential.

Various methods for the analysis of EtO, or the sum of EtO and 2-CE, have been published. Some of these methods are based on the conversion of 2-CE to EtO under alkaline conditions, an approach that was later optimized and used as an official standard in Germany. Other methods are based on the conversion of the EtO to 2-CE under acidic conditions, followed by extraction of 2-CE with ethyl acetate and analysis by GC/MS. However, these methods are time consuming, labor intensive, and require the use of large quantities of harmful solvents. Sample preparation is essential in the analysis of EtO and 2-CE. Therefore, in December 2020, EU Reference Laboratories for Residues of Pesticides recommended a single-residue method for the analysis of EtO and 2-CE in sesame seeds that uses QuEChERS extraction followed by GC/MS/MS analysis.[4] QuEChERS-based GC methods generally involve three steps:

1. Extraction using an organic solvent and partitioning salts,
2. Sample cleanup with adsorbent materials (dispersive sorbents), and
3. GC/MS/MS analysis.

However, food and feed matrices are known to be extremely complex and contain many interfering compounds that can lead to ion suppression effects, coelution, and severe contamination of the analytical instrumentation from the injector to the detector. In this application note, several improvements to the published EU Reference Laboratories for Residues of Pesticides method are proposed, including implementation of an automated liner exchange option. The option provides more accurate results by allowing unattended exchange of programmed-temperature-vaporization (PTV) inlet liners during an extended analytical sequence, which prevents accumulation of the high amounts of nonvolatile material present in QuEChERS extracts. Also, the use of precolumn backflushing protects the analytical column, resulting in increased robustness and higher productivity.

Materials and methods

Chemicals and reagents

HPLC-S gradient grade acetonitrile was from Biosolve. Reverse-osmosis water was prepared using a Millipore water purification system. Samples were generously provided by a research partner or purchased in a local supermarket. EtO in methanol at 50 mg/mL was acquired from Sigma-Aldrich. 2-CE was also purchased from Sigma-Aldrich (part number 23000). 2-CE-D4 was purchased from TechLab (part number 117067-62-6). EtO-D4 was synthesized in-house from 2-CE-D4 under alkaline conditions as described in the Recommended Single Residue Method.[3] The purity and concentration of the ETO-D4 was tested against an EtO standard using GC-flame ionization detection (FID).

Sample preparation overview

Food material: Samples were homogenized using a liquid-nitrogen-cooled grinder to avoid loss of EtO and 2-CE.

Extraction: QuEChERS extraction kit, EN 15662 method (part number 5982-5650)

Dispersive SPE cleanup: QuEChERS Dispersive Kit, Fruits and Vegetables with Fats and Waxes, EN method (150 mg PSA, 150 mg C18EC, 900 mg $MgSO_4$) (part number 5982-5156)

QuEChERS extraction

QuEChERS extraction was performed according to the EN 15662 procedure as shown in Figure 1. Briefly, sample (2 ±0.01 g) was weighed in 50 mL centrifuge tubes and spiked as required. Water (10 mL) was added to the centrifuge tubes followed by capping and vortexing for 1 minute or until the sample was homogeneous. After the samples were thoroughly wetted, acetonitrile (10 mL) and 20 µL of internal standard (EtO-D4 and 2-CE-D4 10 µg/mL) were added to the centrifuge tubes along with two ceramic homogenizers (part number 5982-9313) to improve the extraction efficiency of the target compounds. The centrifuge tubes were then capped and placed in a rotary shaker for 15 minutes. The tubes were then removed, the QuEChERS extraction salts (4 g of $MgSO_4$,1 g of NaCl, 1 g of NaCitrate, and 0.5 g of disodium citrate sesquihydrate) were added, and the tubes were shaken on the rotary shaker for another 3 minutes. The samples were then centrifuged for 5 minutes at 6,000 rpm, resulting in phase separation between the aqueous and organic solvents. Following centrifugation, the upper acetonitrile layer (6 mL) was transferred to QuEChERS Dispersive Kit 15 mL tubes (150 mg of PSA, 150 mg of C18EC, and 900 mg of $MgSO_4$). The tubes were vortexed for 30 seconds followed by centrifugation at 5,000 rpm for 5 minutes. After centrifugation, cleaned extracts were transferred to a 2 mL GC vial for analysis.

GC and GC/TQ instrument conditions

The GC and GC/TQ conditions and method parameters are listed in Tables 1 and 2, respectively.

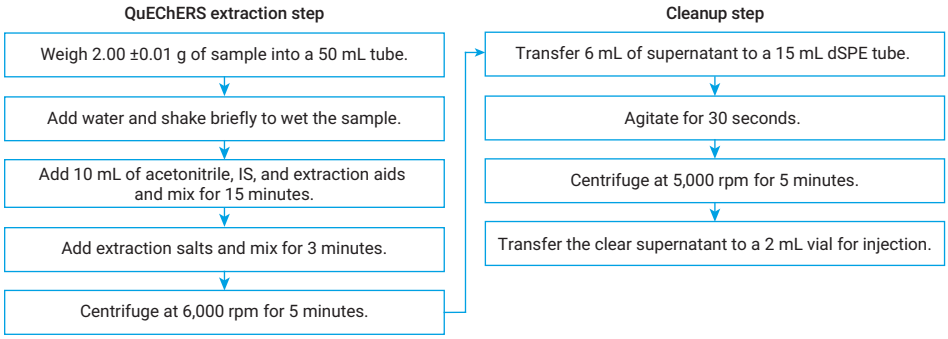

Figure 1. QuEChERS workflow for the extraction and cleanup of samples.

Table 1. GC method parameters.

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 2. GC/TQ method parameters.

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) 44 → 15 (CE 5) 2-CE-D4 (4.47 min): 86 → 33 (CE 5) 84 → 33 (CE 5) 2-CE (4.48 min): 80 → 44 (CE 0) 80 → 31 (CE 5) 80 → 43 (CE 0)

Calibration standard preparation

Stock standard solutions were prepared by mixing the individual stock standard solutions. Working standard solution was created by making a dilution of the stock solutions to 50 µg/mL EtO and 84.2 µg/mL 2-CE in acetonitrile that was stored at –18 °C until use. For calibration curve development, standards were mixed and prepared fresh in acetonitrile in the range between 1 to 100 ng/mL and were used within one day. Matrix-matched standards were prepared at 10, 40, and 100 ng/mL extract levels by spiking the QuEChERS extracts of the blank sesame and curcuma samples. Internal standards were spiked into all samples (20 µL of a 10 µg/mL solution) and taken through the entire QuEChERS workflow.

Results and discussion

Chromatography

Both EtO and 2-CE may occur in food samples. The 2-CE metabolite predominates, mainly because it is less volatile than EtO. As a result, simultaneous determination of both analytes in a single method is recommended. In addition, a dedicated method for quantification of EtO and 2-CE is proposed, rather than integrating the analytes into an existing multiresidue pesticides method. Specific to the described method, the solvent (acetonitrile) elutes between EtO and 2-CE, meaning that care should be taken to protect the filament by programing a "Detector OFF" time event in the MS method between 2.95 and 3.6 minutes. Also, optimal injection conditions are needed to ensure a good peak shape for the EtO. The cooled injection system (CIS), a PTV-type inlet, was used to perform cold split injection of samples. Samples were introduced into the liner without the intermediate evaporation step that is typical of classical split/splitless injectors. Sample transfer from the liner into the analytical column thus started the moment the injector was heated. The well-controlled evaporation process allowed for reproducible and accurate injection, optimizing results for the target analytes. Figure 2 shows the chromatograms for EtO at the 5 ng/mL level (equivalent to 25 µg/kg sample level) and 2-CE at the 1 ng/mL level (equivalent to 5 µg/kg sample level), which are well below the EU-MRL levels for spices and sesame seeds.

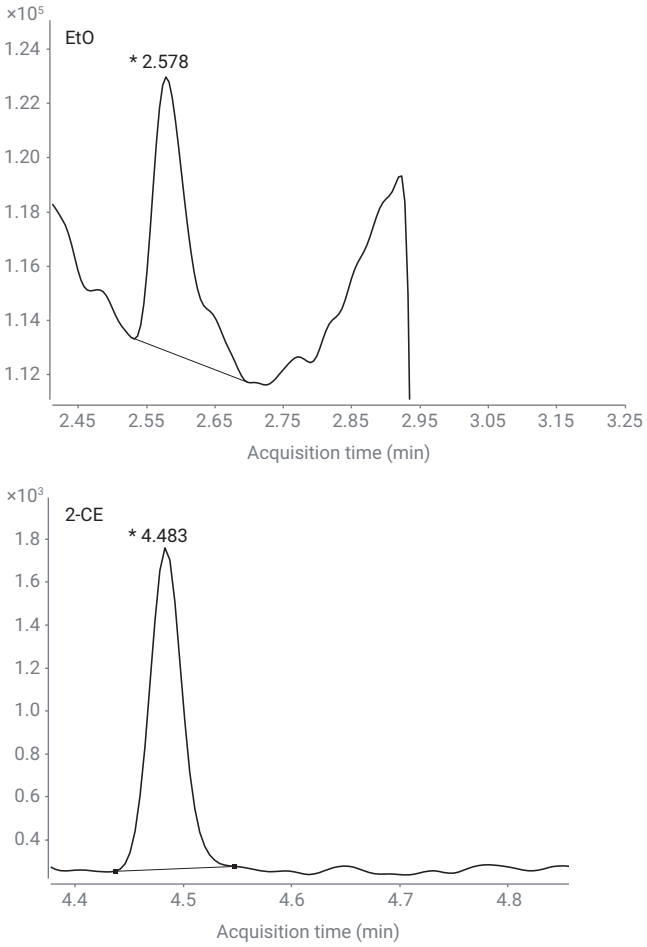

Figure 2. Chromatograms of EtO (MRM transition 44 → 29) at the 5 ng/mL level and 2-CE (MRM transition 80 → 44) at the 1 ng/mL level.

Acetaldehyde is often present in food products, particularly in those that are fatty. Because acetaldehyde and EtO have very similar mass spectra and retention indices, there are essentially no MRM transitions available to select for EtO if it coelutes with acetaldehyde. Therefore, it is essential to prevent their coelution during analysis. To test the method for this concern, acetaldehyde, EtO, and a mixture of acetaldehyde and EtO were injected to confirm chromatographic resolution on the HP-VOC column used. As shown in Figure 3, acetaldehyde eluted just before EtO and baseline separation was achieved.

Food matrices are extremely complex and contain many interfering compounds. Considering the nonselective character of the QuEChERS extraction method, the extracts obtained are relatively dirty and could contain trace amounts of water. The matrix compounds can accumulate in the injector, damage the analytical column, and contaminate the detector. Therefore, the method introduced two levels of matrix protection with:

1. Automated Liner Exchange option to allow unattended liner exchange, and
2. Integrated precolumn backflushing to ensure that high-boiling-point compounds do not reach the analytical column, increasing column lifetime and minimizing downtime related to detector maintenance.

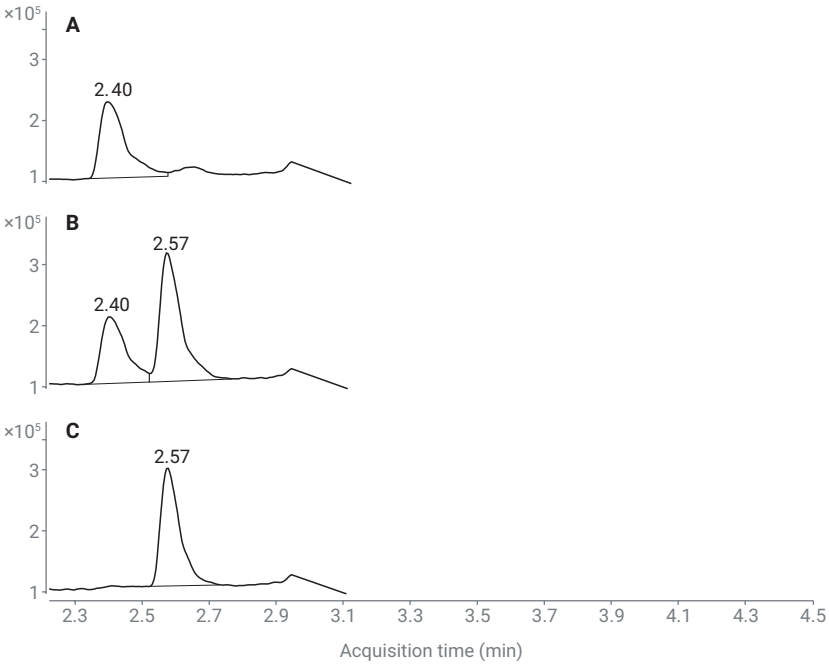

Figure 3. Chromatograms of the acetaldehyde (A), acetaldehyde and EtO mixture (B), and EtO (C) showing baseline separation of the compounds.

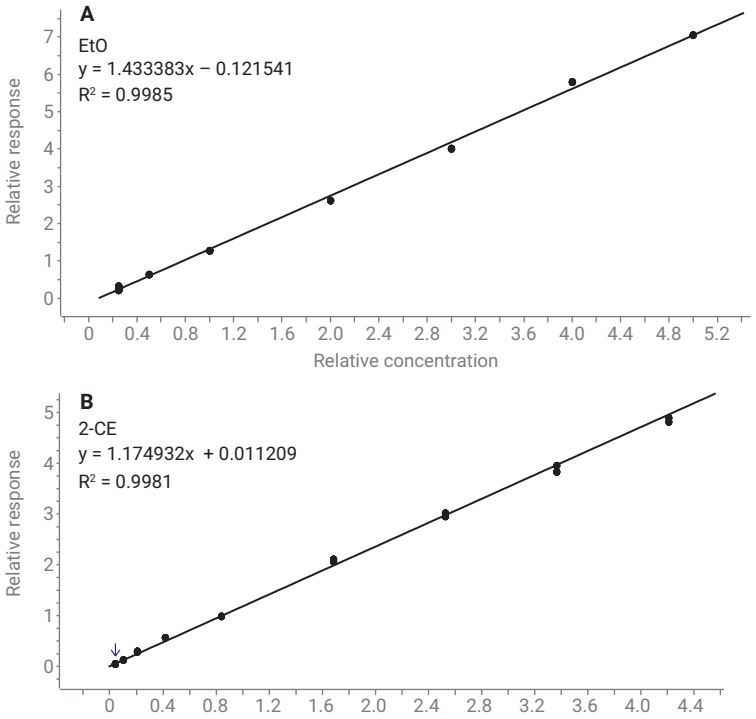

Figure 4. Calibration curves for EtO (A) and 2-CE (B).

Method performance

EtO was quantified using calibration curves ranging from 5 to 100 ng/mL, with linear fit, no weighting, and without including the origin. 2-CE was quantified using calibration curves ranging from 0.84 to 84 ng/mL, with linear fit, 1/x weighing, and without including the origin. The R^2 value was greater than 0.99 for both target compounds (Figure 4) indicating excellent fit.

To determine the chromatographic stability of the method, extracts of sesame seed and curcuma were spiked with EtO and 2-CE to 10, 40, and 100 ng/mL, and injected repeatedly from the same vial into the same liner. Table 3 shows the absolute area and recovery for EtO and 2-CE spiked into the sample extracts at 40 ng/mL. A small decrease in the absolute area of EtO in sesame, and especially curcuma, extract was observed. The decrease can be explained by evaporation of EtO from the vial, which was pierced several times while stored in the sample tray at room temperature. Despite this concern, the repeatability of the recovery was 5 to 6%, indicating that the deuterated internal standard allowed correction for the loss. Though less evaporation loss of 2-CE was expected because it is less volatile than EtO, due to liner activity there was about a 40% reduction in the absolute area between the first and fifth injection.

Some peak distortion was observed after injection, particularly for extracts containing interfering compounds such as curcuma. This distortion demonstrated that injection of relatively dirty QuEChERS extracts affects accurate analysis of 2-CE. Therefore, use of the Automated Liner Exchange (ALEX) option for the analysis of EtO and especially 2-CE is recommended. Using the ALEX option, the liners were exchanged after 20 injections (as a user-defined time event in the sequence), minimizing accumulation of nonvolatile material from the extracts in the liner that could adversely affect method accuracy. Also, the ALEX option allowed unattended exchange of PTV inlet liners during extended analytical sequences, minimizing downtime related to manual inlet maintenance while increasing instrument robustness and productivity for routine analyses.

To prevent high-boiling-point compounds from reaching the analytical column and contaminating the MS ion source, the precolumn backflushing option (using a purged Ultimate union) was used. Shown in Figure 5, the full-scan analysis of a curcuma extract performed without backflushing demonstrates the complexity of the injected food samples, particularly for compounds eluting after 5 minutes (which is after elution of the target compounds). Without precolumn backflushing, all the injected matrix reaches the analytical column

Table 3. Absolute area and recovery of the EtO and 2-CE from sample extracts injected repeatedly on the same liner.

	EtO				2-CE			
	Sesame, 40 ng/mL		Curcuma, 40 ng/mL		Sesame, 40 ng/mL		Curcuma, 40 ng/mL	
	Area	Recovery (%)	Area	Recovery (%)	Area	Recovery (%)	Area	Recovery (%)
Injection 1	230,904	87.2	258,262	92.9	56,959	100.65	66,763	97.81
Injection 2	240,094	98.7	227,977	84.7	51,690	103.81	51,575	96.67
Injection 3	241,489	101.5	185,710	78.7	43,990	103.92	44,970	98.12
Injection 4	221,448	95.8	221,869	85.5	37,175	101.54	38,618	98.70
Injection 5	207,740	94.9	178,201	81.7	34,618	104.89	40,934	95.73
Average	228,335	95.6	214,403	84.7	44,886	102.9	48,572	97.7
RSD	6.2	**5.6**	15.3	**6.3**	21.0	**1.7**	23.2	**1.2**

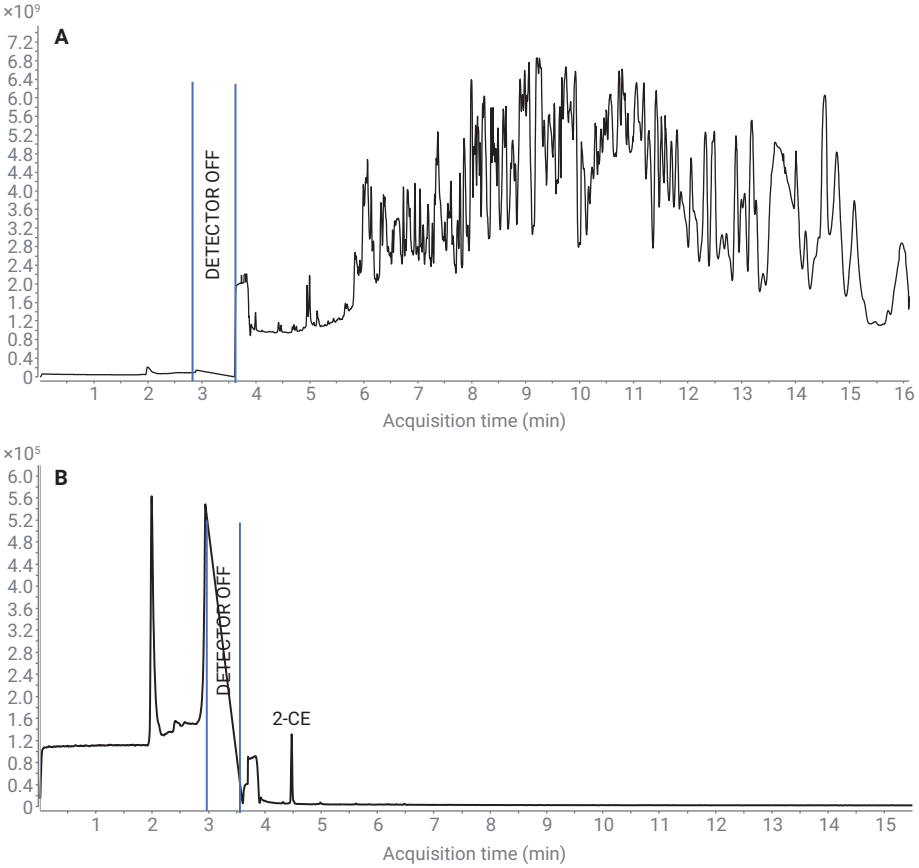

Figure 5. Chromatographic profile of the curcuma extract analyzed in scan mode without backflushing (A), and with backflushing (B).

and ultimately the MS ion source. When backflushing begins immediately after the transfer of the target analytes to the analytical column, the sample matrix has little to no influence on the column, allowing for analysis of more samples before maintenance is required compared to a conventional setup.

The sample preparation method described in this application note was validated by performing recovery experiments in triplicate at three different concentrations: 0.05, 0.2, and the 0.5 mg/kg sample-based level. The recovery was between 84.5 to 100.6% for EtO and 88.8 to 106.2% for 2-CE in the sesame and curcuma samples (Table 4).

Representative food samples collected in a local supermarket were analyzed using the method (Table 5). None of the commercial samples tested positive for EtO, probably because the samples, some of which had been thermally treated, suffered losses during storage or the thermal treatment. However, in border-control programs for example, under the correct sampling and storage conditions, significant amounts of EtO would be detected. All of the samples tested positive for 2-CE, some at levels above the MRL values for these commodities (i.e. Sesame 1, Curcuma 1, and plant material). Method accuracy was tested using a sesame reference material (kindly provided by a research partner). The value obtained was close to the average reported value of 4,660 µg/kg (27.3% CV).

Table 4. Recovery and relative standard deviation (RSD) of EtO and 2-CE in sesame and curcuma samples spiked at different levels.

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

Table 5. Amounts of EtO and 2-CE detected in commercial samples.

Matrix	EtO	2-CE	Sum EtO and 2-CE*
	µg/kg	µg/kg	µg/kg
Sesame, 1	n/d	224.7	122.9
Sesame, 2	n/d	75.7	41.4
Sesame, Reference Material	n/d	4,581**	2,505
Curcuma, 1	n/d	199.7	109.2
Curcuma, 2	n/d	12.7	6.9
Fresh Curcuma	n/d	2.3	1.3
Garlic Powder	n/d	9.2	5.0
Ginger Powder	n/d	27.7	15.2
Plant Material	n/d	9,653	5,280
Spices Mixture	n/d	31.2	17.1
Herbs Mixture	n/d	29.2	16.0

* Sum EtO and 2-CE = conc EtO + (concentration 2-CE in sample × 0.55)
(conversion factor based on conversion of molecular weights: 44/80 = 0.55)

** 4,660 µg/kg (27.3% CV)

Conclusion

The presented method meets the requirements of the EU Reference Laboratories for Residues of Pesticides single-residue method that uses QuEChERS extraction followed by GC/MS/MS analysis for determination of EtO and 2-CE sesame seeds. Recoveries were within 85 to 106%, with good repeatability for both EtO and 2-CE. LOQs for sesame and curcuma (representative of the spices food category), were lower than the currently established MRLs. Results were stable for both sesame and curcuma samples, with RSDs for triplicate extractions and analyses found to be well below 20% for the recovery experiments. The sample preparation and optimized GC/MS/MS setup based on the 8890 GC system, 7010B GC/TQ, and Gerstel MPS sampler with the Automated Liner Exchange option provided reliable results and excellent performance, demonstrating that the method can be used to quantify EtO and 2-CE in sesame and spice samples at the EU-regulated levels. The method has the advantages of high sensitivity, selectivity, accuracy, and sample throughput due to reduced downtime for inlet maintenance, analytical column exchange, and MS ion source cleaning.

References

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