Application note | 001186

Food analysis

Analysis of ethylene oxide and 2-chloroethanol residues in food using GC-MS/MS

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Keywords

Food, ethylene oxide, gas chromatography, pesticides, single-residue method, triple quadrupole

Goal

The aim of this application note is to demonstrate the utility of the Thermo Scientific™ TRACE™ 1610 GC system and the Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS for the analysis of ethylene oxide and 2-chloroethanol residues in food samples.

Introduction

Ethylene oxide (EO) is a colorless and flammable gas with a broad spectrum of applications, including the preservation of dry food products, such as seeds, milled cereals, spices, herbs, nuts, milk powder, and raisins. However, consumption of EO can negatively impact human health as it is a mutagenic and carcinogenic compound with additional adverse effects on the central nervous system and mucous membranes.[1,2] Thus, residues of EO and its degradation products therefore need to be monitored closely. The importance of the EO analysis is highlighted by the high number of notifications of EO detection in food published in the Rapid Alert System for Food and Feed (RASFF). From January 1 to April 30 in this year alone, 96 alerts have been registered for the detection of EO in food.[3]

Ethylene oxide poses challenges for analysts as a small and highly volatile molecule with a boiling point of only 10.7 °C. This means that special precautions must be taken during sample preparation to avoid analyte losses through evaporation. In addition, EO is weakly retained on GC columns and elutes just after the void time. EO is a reactive compound and easily forms reaction products (e.g., 2-chloroethanol, 2-bromoethanol

and ethylene glycol) within the sample matrix. The residue definition of EO according to Reg. (EU) 2015/868 includes two compounds, ethylene oxide and 2-chloroethanol (2CE), where the sum of EO and 2CE expressed as EO is required to be reported. The maximum residue level (MRL) depends on the commodity and ranges from 0.02 to 0.1 mg/kg.[4,5]

High sensitivity is a prerequisite to achieve the required limits of quantification for EO and its degradation products. However, analytical testing laboratories also require a robust and reliable system to test large numbers of samples without the need to perform maintenance on either the GC (i.e., exchange of the liner, trimming of the analytical column) or the mass spectrometer (cleaning and/or re-tuning of the ion source).

This application note demonstrates sensitivity, accuracy, linearity, and selectivity of the ethylene oxide residue analysis. An extended robustness study was also performed to show the stability of the analytical method. To meet regulatory compliance, the methodology follows European Commission quality control guidance document "Analytical quality control and method validation procedures for pesticide residues analysis in food and feed" (Document N° SANTE/11312/2021).[6]

Experimental

Sample preparation

Samples were extracted with aqueous acetonitrile according to a modified QuOil protocol.[7] The sample extracts were provided by SGS Institut Fresenius (Berlin, Germany). Figure 1 shows the sample preparation. The list of the matrices can be found in Figure 6.

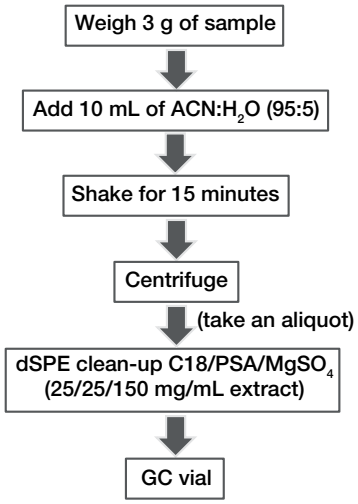

Figure 1. Sample preparation method

GC-MS method

The analysis was performed using a TRACE 1610 GC system coupled to a TSQ 9610 triple quadrupole GC-MS/MS equipped with a Thermo Scientific™ Advance Electron Ionization (AEI) source. The samples were injected using a Thermo Scientific™ TriPlus™ RSH autosampler. To avoid gradual evaporation of EO from the standards and samples during the unattended analysis, all vials were stored in the cooled drawer available for the TriPlus RSH autosampler that allows chilling of the sample vials to 7 °C.

Table 1A. GC parameters

TRACE 1610 GC parameters	
Injector	
Injector type	Thermo Scientific™ iConnect™ Programmable Temperature Vaporizing (PTV)
Injection volume [µL]	1
Liner	Siltek baffle (P/N 453T2120)
Operating mode	Split
Split flow [mL/min]	5
Split ratio	5
Purge flow [mL/min]	5
Vacuum compensation	On
Temperature [°C]	90
PTV temperature program	
Injection time [min]	0.80
Transfer rate [°C/s]	12
Transfer temperature [°C]	250
Transfer time [min]	10
Oven	
Guard column	Thermo Scientific™ GuardGOLD™ Capillary (5 m × 0.25 mm) (P/N 26050-0525)
Analytical column	Thermo Scientific™ TraceGOLD™ TG-624SilMS (30 m × 0.25 mm × 1.40 µm) (P/N 26059-3320)
Carrier gas	He
Carrier gas flow [mL/min]	1
Oven temperature program	
Temperature 1 [°C]	45
Hold [min]	2
Rate [°C/min]	50
Temperature 2 [°C]	150
Hold [min]	0
Rate [°C/min]	100
Temperature 3 [°C]	300
Hold [min]	10.4

Table 1B. MS parameters

TSQ 9610 triple quadrupole GC-MS/MS parameters	
Transfer line temperature [°C]	280
Ion source temperature [°C]	270
Ion transitions and collision energies	
Ethylene oxide	44 → 14 (20 eV)
Ethylene oxide	44 → 29 (5 eV)
2-chloroethanol	80 → 31 (5 eV)
2-chloroethanol	80 → 43 (5 eV)
2-chloroethanol-d_4	84 → 33 (5 eV)
2-chloroethanol-d_4	86 → 33 (5 eV)
Collision gas	Argon

Results and discussion

Sensitivity

A reduction of the injected amount of sample is often beneficial, as it reduces the impact of complex sample matrices on the analytical system, although a larger injection volume can increase sensitivity. Due to the sensitivity of the AEI ion source and a careful method optimization, it was possible to achieve optimal limits of quantitation for both evaluated compounds with an injection of only 1 µL of sample extract. The reduced injection volume also minimizes the total amount of matrix injected and the necessity to perform system maintenance.

Figure 2 shows the ion transitions for EO and 2CE when injecting a standard solution with a concentration of 0.002 mg/L. Because of the dilution factor of the sample preparation method (x 3.3), the concentration of 0.002 mg/L in the final extract corresponds to a concentration of about 0.007 mg/kg in the sample. As can be seen in the figure, all the transitions are characterized by an acceptable signal-to-noise ratio. Also, the ion ratios are stable and follow the DG SANTE guideline criteria,[6] i.e., the variability does not exceed 30%. The expected ion ratio for EO is 7%, thus the acceptable range is from 4.9% to 9.1%, whereas the ion ratio of 2CE is 100% and all the results from 70% to 130% fulfill the DG SANTE criteria. The ion ratio details can be found in Table 2 and Table 3.

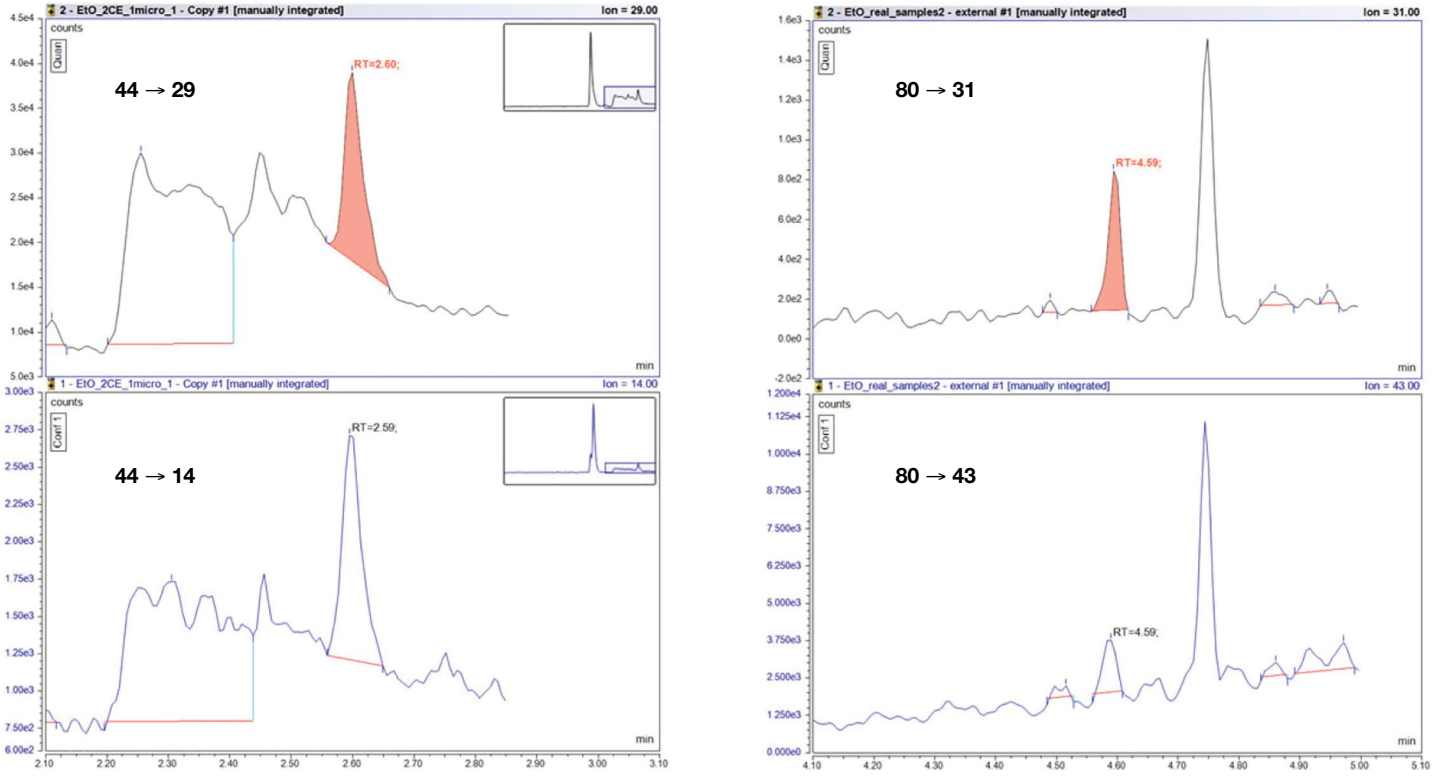

Figure 2. SRM chromatograms of ethylene oxide (left) and 2-chloroethanol (right) showing 0.33 pg on-column for both. Both standards are at 0.002 mg/L.

Table 2. Linearity details of ethylene oxide

Theoretical conc. [mg/L]	Peak area [counts · min]	Calculated conc. [mg/L]	Deviation of back-calculated conc. [%]	Ion ratio [%]
0.002	249	0.002	18	7.52
0.005	831	0.005	7	6.86
0.010	1499	0.009	-13	7.50
0.100	16883	0.087	-13	7.17
1.000	199845	1.025	0	6.78
5.000	982577	5.013	0	6.53

Table 3. Linearity details of 2-chloroethanol

Theoretical conc. [mg/L]	Peak area [counts · min]	Calculated conc. [mg/L]	Deviation of back-calculated conc. [%]	Ion ratio [%]
0.002	16	0.002	7	111
0.005	56	0.006	13	117
0.010	98	0.009	-7	91
0.100	1049	0.093	-7	106
1.000	10531	0.926	-7	105
5.000	57839	5.081	2	98

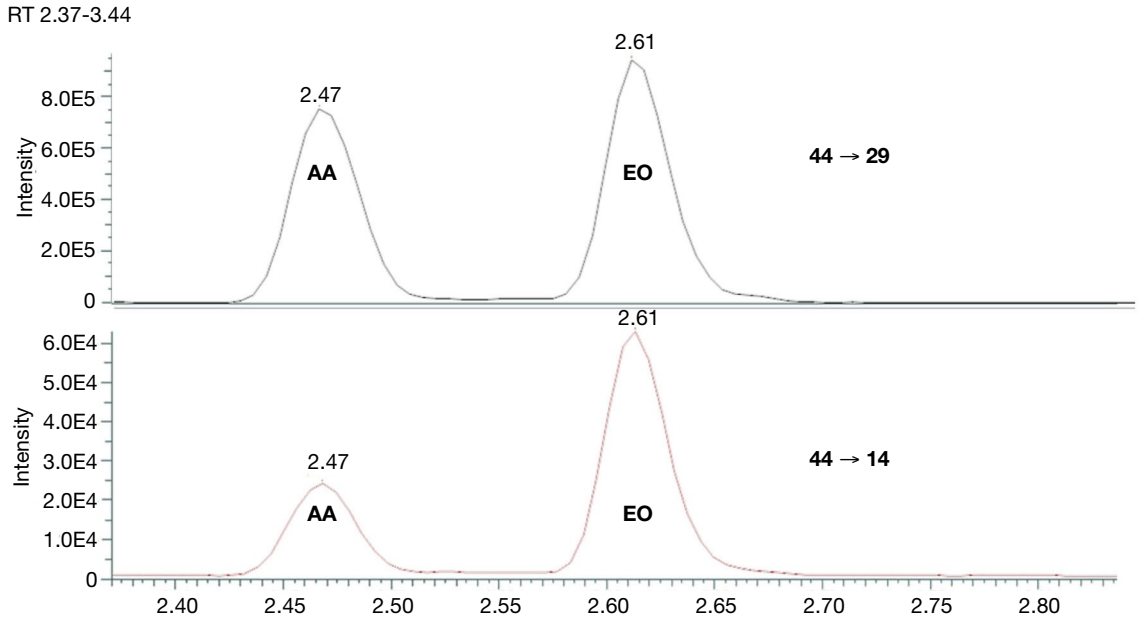

Figure 3. Separation between acetaldehyde (AA) and ethylene oxide (EO)

Selectivity

Selectivity is a very challenging aspect in the analysis of EO, specifically for EO itself. Due to the low molecular weight EO and its fragmentation products (the precursor ion is characterized by *m/z* 44 and the monitored product ions are *m/z* 29 and *m/z* 14), analysts face challenges with interferences caused by the presence of other compounds. In the case of non-selective transitions, the chromatographic separation becomes a crucial factor.

The most common interference in the analysis of EO is acetaldehyde (AA). This compound shares the same transitions as EO. If chromatographic separation is not achieved, coelution of AA and EO can lead to an overestimation of the reported EO concentration. In the worst-case scenario, the presence of AA could strongly alter the ion ratio of EO and produce a false negative result.

Using the TraceGOLD TG-624 column, designed towards the separation of volatile analytes, a retention time difference over 0.1 min was achieved between EO and AA (Figure 3). Thus, there was no risk for interferences on EO caused by the potential presence of AA.

Linearity

The linearity of the method was investigated in the concentration range from 0.002 mg/L to 5 mg/L, which corresponds to a range from 0.007 mg/kg to 16.5 mg/kg in the sample. Such a broad linear range improves the overall laboratory throughput as samples that contain high concentrations of the analytes do not have to be diluted and reinjected. The extended linear range is made possible by the Thermo Scientific™ XLXR™ detector that is standard across the TSQ 9610 GC-MS/MS product range. To obtain the best adjustment to data points and minimize the deviation of the back-calculated concentrations, a weighting factor 1/x was applied and the curve was not forced through the origin.

According to the DG SANTE guidelines, a calibration point can be included into the calibration range if the deviation of its back-calculated concentration from the true concentration is not higher than ± 20%.[6] All calibrations points in the experiment were within this criterion, demonstrating excellent linearity for the applied method (Figures 4 and 5). The detailed values of the back-calculated concentrations and the deviation to the true value of the standards are shown in Tables 2 and 3.

Sample analysis

The ability of the method to deliver improved sensitivity and accuracy in real food samples was examined. A batch of 10 samples, covering a wide range of typical foodstuffs tested for the potential presence of EO, was injected and quantified. For both compounds, EO and 2CE, an external calibration curve was applied. During the extraction, the samples were spiked with deuterated 2-chloroethanol (2CE-4D), so that for 2CE an internal standard also could be used for calibration.

A summary of the quantified results can be found in Figure 6. In all samples, no detectable amounts of EO were found. However, 2CE was detected in every sample in various concentrations ranging from 0.005 mg/kg to 0.12 mg/kg. The figure contains reference concentrations obtained in a laboratory accredited under ISO/IEC 17025:2005. Excellent agreement was achieved between the results of both laboratories. The biggest difference was observed between the internal standard calibration result and the reference value for the locust bean gum sample, which was 0.007 mg/kg and less than 20% of the reference concentration. It should also be noticed that the results obtained with the internal standard were in excellent agreement with those obtained with the external calibration curve. Thus, both quantitation approaches can be recommended for real sample analysis.

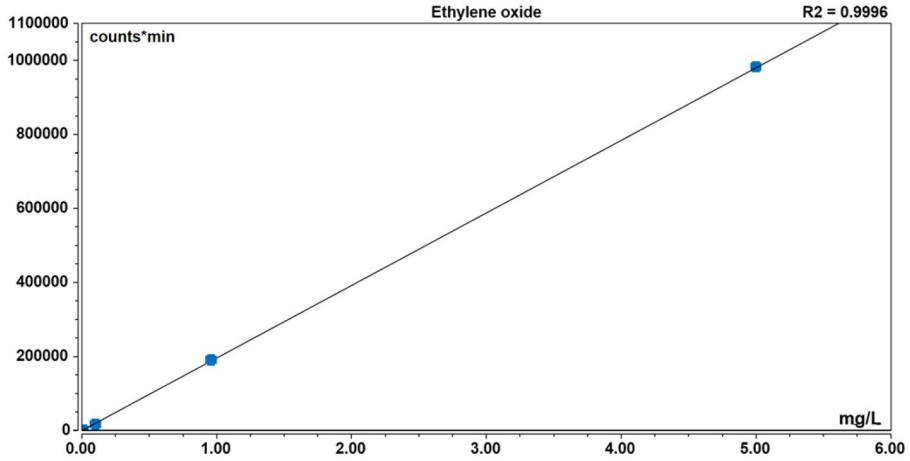

Figure 4. Ethylene oxide calibration curve in the range of 0.002–5 mg/L, corresponding to 0.007–16.5 mg/kg in the sample

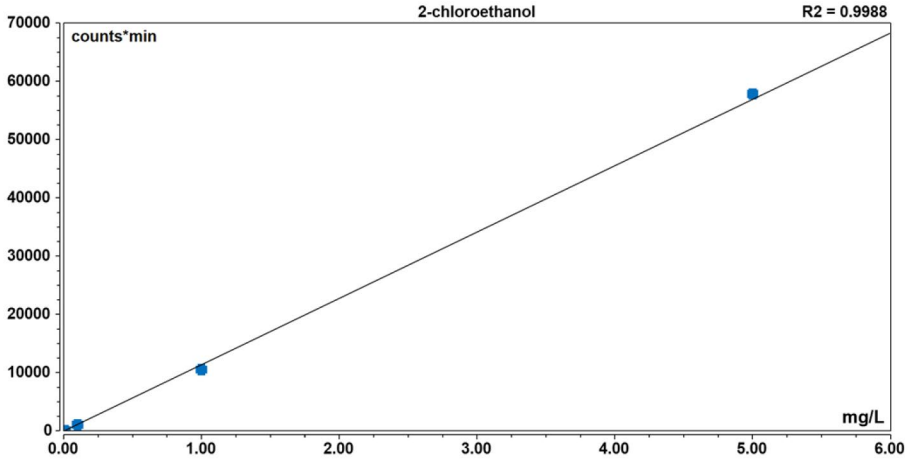

Figure 5. 2-chloroethanol calibration curve in the range of 0.002–5 mg/L, corresponding to 0.007–16.5 mg/kg in the sample

Robustness

The ability to run extended sequences, containing hundreds of samples, is an important aspect for productivity of analytical testing laboratories. Complex food matrices may affect all parts of the chromatographic system) and the ion source, leading to a need for maintenance. Ultimately, the contamination can lead to poor chromatographic performance, retention time shifts, variable peak areas, and degraded peak shapes. To evaluate the robustness of the TSQ 9610 GC-MS/MS, a sequence containing ten sample extracts was injected continuously for three days, resulting in a total number of 230 subsequent injections.

During the extended sequence, the system was interrupted only once, to change the septum. No other maintenance or tuning was performed. To evaluate the robustness, the peak characteristics of the isotopically labeled internal standard 2CE-4D were evaluated, as this compound was present in all samples. Figure 7 shows that the system´s response for 2CE-4D was stable, with no indications of peak shape deterioration. The relative standard deviation of the peak area was calculated to be ±8.8%, whereas the retention time deviated no more than 0.01 min, meeting the requirement of 0.1 min of the DG SANTE document.

Examples of peak shape obtained for sesame seed from the beginning (injection 6) and from the end (injection 226) of the sequence are depicted in Figure 8 and show no deterioration in peak shape, although the liner showed visible residues of the sample matrix upon inspection after completion of the sequence. However, thanks to the high sensitivity of the AEI ionization source, the injection volume could be reduced, allowing the overall robustness of the method to be significantly increased.

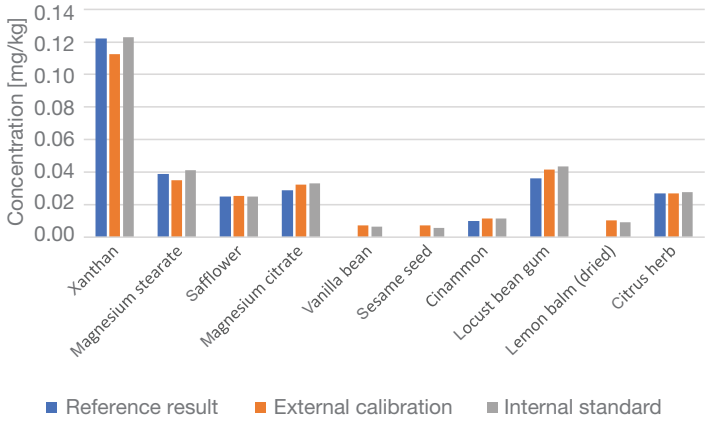

Figure 6. Real samples quantitation results. Since no ethylene oxide residue was found, the graphic contains only 2-chloroethanol results.

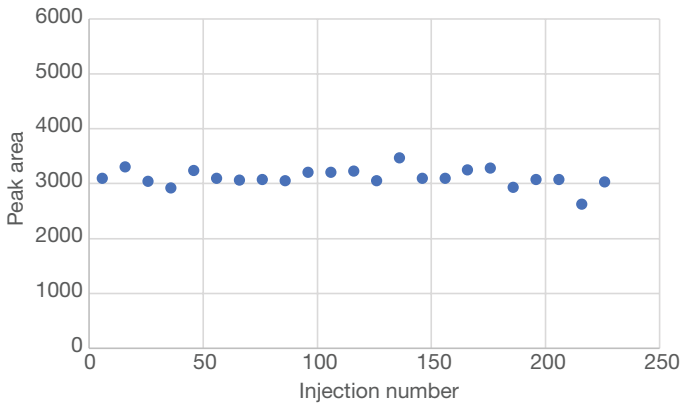

Figure 7. Summary of the robustness test, showing the response of the 2CE-4D standard in every 10th injection of the sequence (total number of injections: 230)

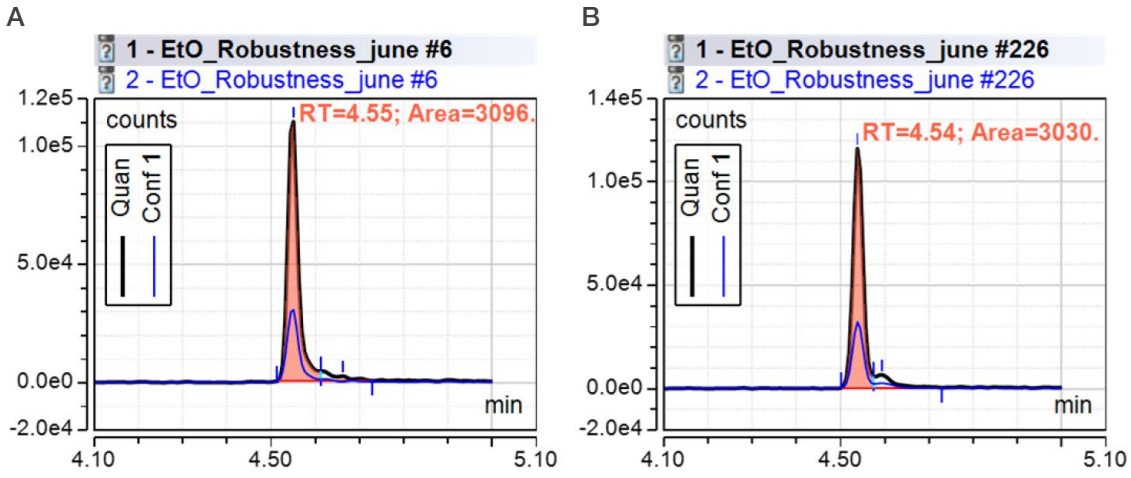

Figure 8. Chromatogram of 2CE-4D in sesame seed sample. (A) Beginning of the sequence; (B) end of the sequence

Conclusion

This application note demonstrates the superior performance of the TSQ 9610 GC-MS/MS system together with the AEI source for the analysis of ethylene oxide and 2-chloroethanol residues in food samples.

- Chromatography: the chromatographic method provided a very good retention of the analytes and separation from matrix interferences.
- The quantitation at MRL was easily achieved with an injection volume of 1 µL, which demonstrates excellent sensitivity of the instrument.
- The XLXR detector facilitates quantitation in a broad range on concentration showing good linearity between 0.007 and 16.5 mg/kg in the samples.
- Robustness: the system provided stable results during a 3-days long unattended sequence.

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