GC-MS/MS

Reliable analysis of ethylene oxide and 2-chloroethanol in food samples using gas chromatography mass spectrometry

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Abstract

Purpose: The aim of this study 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.

Methods: Standards in acetonitrile and QuOil extracts of real samples were analyzed with the TSQ 9610 triple quadrupole GC-MS/MS.

Results: The TSQ 9610 showed an excellent sensitivity, linearity, and robustness.

Introduction

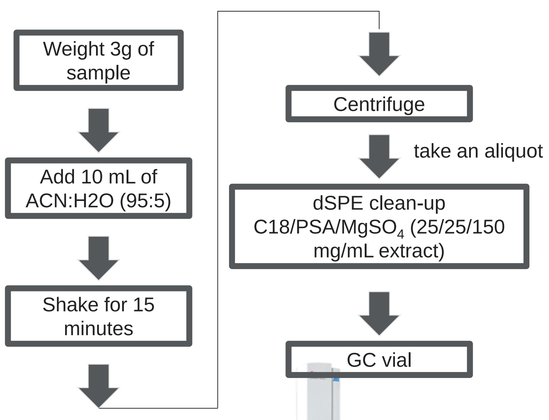
Ethylene oxide (EO) is a substance of a broad spectrum of applications. One of them is preservation of dry food products, such as seeds, milled cereals, spices, herbs, nuts, milk powder, raisins, etc. Upon consumption, ethylene oxide can negatively affect human health, with potential adverse effects on the central nervous system, mucous membranes, and mutagenic and carcinogenic effects. Residues of EO and its derivative products therefore need to be monitored closely.

Ethylene oxide is a challenging analyte, as the molecule is small and highly volatile with a boiling point of only 10.7 ˚ C. This means that special precautions have to be taken during the preparation of the sample to avoid analyte losses through evaporation. In the chromatographic column, the molecule is retained very weakly and elutes just after the void time. Furthermore, EO is converted into 2-chloroethanol (2CE), 2-bromoethanol and ethylene glycol through chemical reactions with the substances present in the commodity. The residue definition of EO according to Reg. (EU) 2015/868 includes two compounds - ethylene oxide and 2-chloroethanol (2CE), requiring top report the sum of EO and 2CE expressed as EO. The MRLs depend on the commodity, and they range from 0.02 to 0.1 mg/kg. Whilst high sensitivity is a prerequisite on the instrumental side to achieve the required limits of quantification limits for EO and its degradation products, 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 gas chromatography or the mass spectrometer side.

Materials and methods

Sample preparation

Figure 1. Sample preparation method.



Test Method(s)

Table 1. GC parameters

Thermo Scientific™TRACE™ 1610 GC Parameters	
Injector	
Injector type	iConnect™ Programmable Temperature Vaporizing (PTV)
Operating mode	Split
Split flow [ml/min]	5
Split ratio	5
Purge flow [ml/min]	5
Vacuum compensation	On
Temperature [˚ C]	90
PTV Ramp Settings	
Injection Time [min]	0.80
Transfer Rate [˚ C/s]	12
Transfer Temperature [˚ C]	250
Transfer Time [min]	10
Oven	
Guard column	GuardGOLD™ Capillary Columns (5m x 0.25mm)
Analytical column	TraceGOLD™ TG-624SilMS (30m x 0.25mm x 1.40um)
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 2. MS parameters

Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS Parameters	
Transfer line temperature [˚ C]	250
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 D4	84 → 33 (5 eV)
2-chloroethanol D4	84 → 33 (5 eV)

Data analysis

The data acquisition and processing were carried out with Thermo Scientific Chromeleon 7.3.1 Chromatography Data System (CDS).

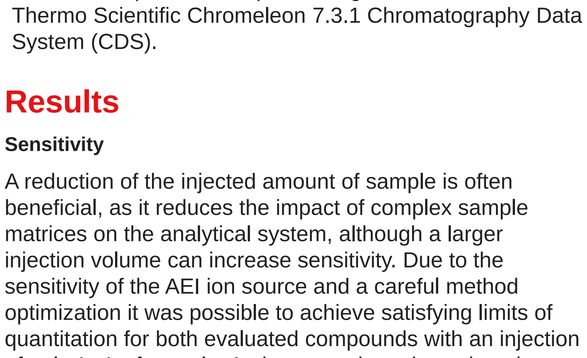
Results

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 satisfying limits of quantitation for both evaluated compounds with an injection of only 1 µL of sample. At the same time, the reduced injection volume helps to reduce the necessity of the system maintenance.

Figure 1 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 (x3.3), the concentration of 0.002 mg/L in the final extract would corresponds to a concentration of about 0.007 mg/kg in the sample. As can be seen on the figure, all the transitions are characterized by a high signal to noise ratio. Also, the ion ratios are stable and follows the DG SANTE guideline criteria6, i.e., the variability does not exceed 30%. The expected ion ration 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.

Figure 1. Ion transitions of ethylene oxide (left) and 2-chloroethanol (right). Both standards at 0.002 mg/L.



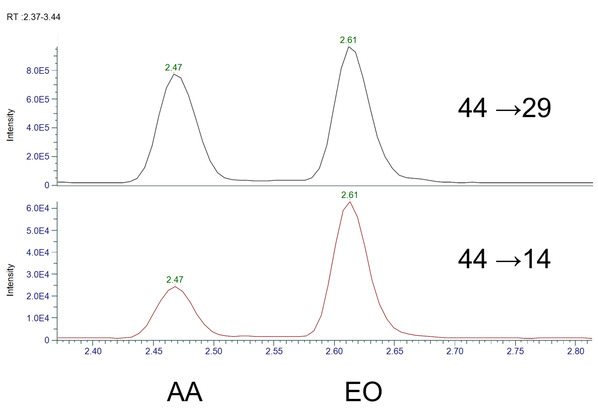
Sensitivity

Selectivity is a very challenging aspect of the analysis, specifically for EO itself. Because of the low molecular weight of the compound 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), there could be problems with interferences caused by presence of other compounds. In the case of non-selective transitions, the chromatographic separation becomes a crucial factor.

For EO, one of the most common interfering compounds is acetaldehyde (AA). This compound has the same transitions as EO. If chromatographic separation was not achieved, co-elution of AA and EO could lead to an overestimation of the EO concentration, and hence the reporting of a potentially incorrect result. In the worst-case scenario, the presence of AA could strongly affect the EO ion ratio and produce a false negative result.

Using the superior chromatographic resolution of the TG-624, a column dedicated to the separation of volatile analytes the difference in the retention times between EO and AA was over 0.1 min (see Figure 2). Thus, there was no risk for interferences on EO caused by the potential presence of AA.

Figure 2. Separation between acetaldehyde (AA) and ethylene oxide (EO)



Linearity

The linearity of the method was investigated in the concentration range from 0.002 mg/L to 5mg/L, which corresponded 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. This linearity is made possible by the XLXR detection system that is standard across the TSQ 9610 product range.

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 the calibrations points in the experiment were within this criterion, demonstrating excellent linearity for the method. Figures 3 and 4 show the calibration curves for EO and 2CE, respectively. The detailed values of the back calculated concentrations and the deviation to the true value of are shown in Table 3 and 4.

Table 3. Linearity details of ethylene oxide

Theoretical concentration	Peak area	Calculated concentration	Deviation of back calculated concentration	Ion ratio
[mg/L]	[counts · min]	[mg/L]	[%]	[%]
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 4. Linearity details of 2-chloroethanol

Theoretical concentration	Peak area	Calculated concentration	Deviation of back calculated concentration	Ion ratio
[mg/L]	[counts · min]	[mg/L]	[%]	[%]
0.002	16	0.002	7	111
0.005	56	0.006	133	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. Ethylene oxide calibration curve in the range of 0.002 - 5 mg/L, what corresponds to 0.007 – 16.5 mg/kg in the sample.

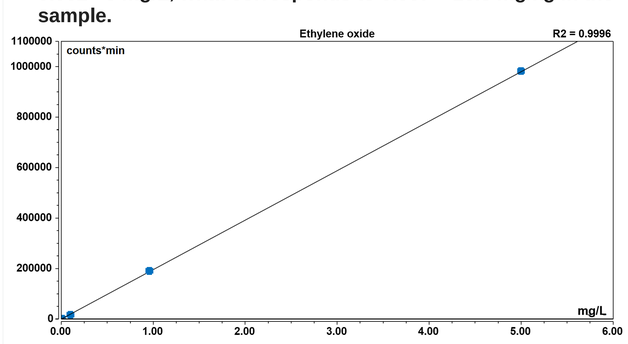
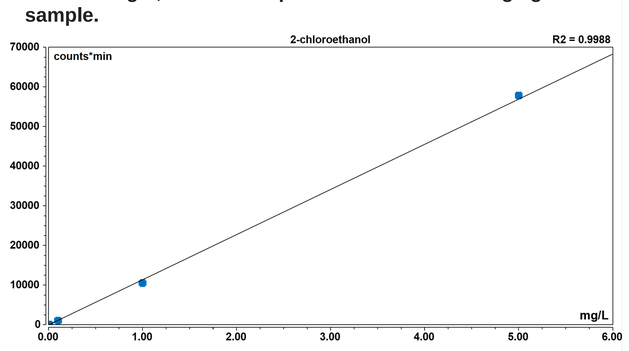


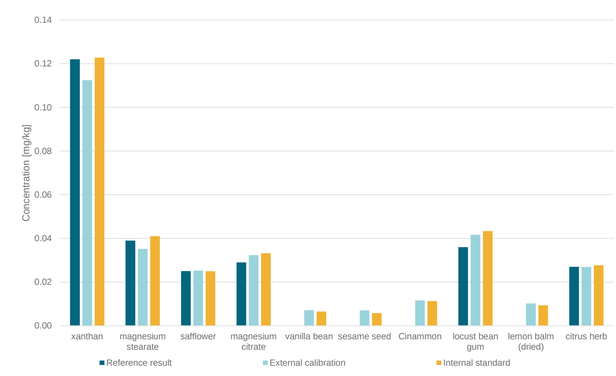
Figure 4. 2-chloroethanol calibration curve in the range of 0.002 - 5 mg/L, what corresponds to 0.007 – 16.5 mg/kg in the sample.



Samples analysis

To test the methods performance to deliver improved sensitivity and accuracy also in real food samples, 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-chlorethanol, so that for 2CE also an internal standard could be used for calibration. A summary of the quantitation can be found in Figure 5. In all analyzed samples, no detectable amounts of EO were found. However, 2CE was detected as a common degradation product. The figure contains reference concentrations obtained in a laboratory accredited under ISO/IEC 17025:2005. Excellent agreement was obtained between the results of both laboratories. The biggest difference between the internal standard result and the reference value was observed for the locust bean gum sample, and it was equal to 0.007 m/kg, which was less than 20% of the reference concentration. It should be also noticed that the results obtained with the internal standard were in agreement with those obtained with the external calibration curve. Thus, both quantitation approaches can be recommended for the real sample analysis.

Figure 5. Real samples quantitation results. Since no ethylene oxide residue was found, the graphic contains only 2-chloroethanol results



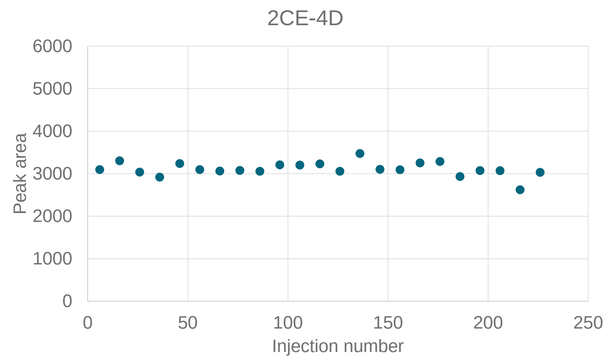
Robustness

The ability to run extended sequences, containing hundreds of samples, is an important point for analytical testing laboratories. The food matrices may affect all parts of the chromatographic system, for example, the liner, the ion source, the column, hence leading to a need for maintenance. Ultimately, the contamination would lead to poor chromatographic performance, retention time shifts, variable peak areas and degraded peak shapes. A sequence containing the ten samples was injected continuously for three days, resulting in a total number of 230 subsequent injections.

During the runtime of the sequence, the system was not interrupted, and no maintenance or tuning was performed. To evaluate the robustness, the peak characteristics of the isotopically labeled 2-chloroethanol were evaluated, as this compound was present in all samples. Figure 6 and show that the system´s response was stable, with no indications on degradation of the quality of the chromatographic separation. The relative standard deviation of the peak area was calculated to be ± 8.8%, whereas the retention time deviated 0.01 min, which was much less than 0.1min allowed by the DG SANTE document.

Examples of peaks obtained for sesame seed from the beginning (injection 6) and from the end (injection 226) of the sequence are depicted in Figure 7 and show that the peak shape remained identical, although the liner showed visible residues of the sample matrix upon inspection at the end of the sequence. However, the thanks to the high sensitivity of the TSQ 9610 triple quadrupole mass spectrometer and the AEI ionization source, the injection volume could be reduced, so that the overall robustness of the method is significantly increased.

Figure 6. Summary of the robustness test. Response of 2CE-4D standard in every 10th injection of the sequence (total number of injections: 230)



Conclusions

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

- Chromatography: the chromatographic method provided a very good retention of the analytes and separation from the matrix interferences
- Sensitivity: the quantitation at MRL was easily achieved, even with 1 µL injection volume
- Linearity: the XLXR detector facilitates quantitation in a broad range on concentration
- Robustness: the system provided stable results during a 3-days long unattended sequence

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