Application Note 281

Fully automated cryogen-free analysis of ethylene oxide and 2-chloroethanol in contaminated sesame seeds using headspace–trap with multi-step enrichment (MSE®) GC–MS

High levels of ethylene oxide (EtO) and 2-chloroethanol (2-CE) present in sesame seeds and other agricultural products have led to multiple product recalls throughout the European Union (EU). Here, we show headspace–trap with multi-step enrichment (MSE) on the Centri® platform to quantitatively determine the contaminants at the required 0.05 mg/kg (50 ppb) reporting threshold or maximum residue limit (MRL) without extensive and manual sample preparation. Excellent chromatographic performance is shown with the linearities of ethylene oxide and 2-chloroethanol at R^2 = 0.9983 and R^2 = 0.9995, respectively, within a single GC–MS analysis, and reproducibility with relative standard deviation (RSD) ≤ 5%. We also demonstrate the enhancement in sensitivity provided by this technique to go beyond the regulation, reaching lower levels of detection for reliable determination.

Introduction

Ethylene oxide (EtO) is used in many regions around the world as a fumigant to eliminate insects in seasonings, spices and foodstuffs; due to its strained three-membered ring structure, EtO is highly reactive, leading to effective bactericidal, fungicidal and sporicidal disinfection.[1] However, its use is banned in the EU due to its highly toxic properties as a carcinogen, mutagen and reproductive toxicant.[2] Since August 2020, European countries have flagged concerns of alarming levels of EtO present in various exports of sesame seeds, triggering global recalls of food products. The high reactivity of EtO means that it is prone to degradation, and so produces 2-chloroethanol (2-CE) as a by-product, which is also a toxic chemical restricted by the EU. This has led to regulation of both compounds – they have a combined maximum residue limit (MRL) of 0.05 mg/kg (50 ppb).[3]

Current methods for sample preparation include lengthy, cumbersome and solvent-heavy extraction procedures. They are manual, can be prone to human error and have expensive disposal costs associated with the harmful solvents used. Solvents used for extraction of target analytes may also extract unwanted components that enter the analytical system during sample injection and can cause contamination of subsequent analyses. To detect these lower concentrations without the need for complex sample preparation, headspace–trap (HS–trap) was investigated as a viable option for volatile contaminant detection to ensure imported foodstuffs meet safety requirements.

In this study, HS–trap with multi-step enrichment (MSE) (Figure 1) coupled with gas chromatography–mass spectrometry (GC–MS) was investigated and is shown to provide efficient extraction of EtO and 2-CE from sesame seeds without the need for solvents, whilst providing much cleaner sample extracts and meeting the regulatory requirement of 0.05 mg/kg.

The key enabling innovation at the heart of Centri is a multi-sorbent-bed focusing trap. A combination of different strength sorbents in the trap and cryogen-free cooling to -30˚C delivered excellent retention and preconcentration of both EtO and 2-CE. Exploiting this technology, multiple extracts were taken from the sample vial and loaded onto the same trap prior to GC injection – known as multi-step enrichment (MSE). Sample preconcentration (using MSE) and subsequent rapid injection to the GC in a narrow band of vapour provided sharp chromatographic peaks and so improved the sensitivity achieved. This was particularly important for the analysis of EtO, as its high volatility can hinder peak shape if headspace extraction is performed without a secondary refocusing step.

Automation of HS–trap with MSE on the Centri sample extraction and enrichment platform significantly improved productivity for this application *via* the sample overlap functionality known as 'prep-ahead'. The unique ability to isolate the headspace injection to trap from the carrier flow to the GC column using a low-volume inert valve means that the next sample can be incubated and extracted to the trap while the previously injected sample undergoes GC–MS analysis, reducing the overall analysis time of every sample.

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Experimental

Samples:

Uncontaminated sesame seeds were obtained from a local supermarket and compared with contaminated seeds sourced from a sesame seed batch that failed to pass EU entry criteria. Whole seeds (2 g) were added to 20-mL headspace vials. Vials containing contaminated seeds were capped rapidly due to the high volatility of EtO. All seeds were stored in a refrigerator prior to preparation.

Calibration standards:

Standards of EtO and 2-CE were combined into a stock solution (in hexane) at a concentration of 1000 ng/µL. From this solution, five calibration standards were prepared in hexane between 25 and 500 ng/µL. Introducing 1 µL of each standard to a 20-mL headspace vial with 2 g of uncontaminated sesame seeds equalled a calibration from 0.013–0.25 mg/kg (13–250 ng/g of seeds). All standards were stored in a refrigerator.

Extraction and preconcentration:

Instrument: Centri (Markes International)

Headspace–trap:

Incubation:	70°C (10 min) with agitation at 300 rpm
Extraction volume:	5 mL
Injection:	200°C (2 min)
Enrichment:	Three extractions from the same sample vial, with a three-minute sampling delay between repeat extractions (total 15 mL extracted for analysis)

Background to Centri®

Markes International's Centri system for GC–MS is the first sample extraction and enrichment platform to offer high-sensitivity unattended sampling and preconcentration of VOCs and SVOCs in solid, liquid and gaseous samples.

Centri allows full automation of sampling using HiSorb™ high-capacity sorptive extraction, headspace(–trap), SPME(–trap), and tube-based thermal desorption. Leading robotics and analyte-trapping technologies are used to improve sample throughput and maximise sensitivity for a range of applications – including profiling of foods, beverages and fragranced products, environmental monitoring, clinical investigations and forensic analysis.

In addition, Centri allows samples from any injection mode to be split and re-collected onto clean sorbent tubes, avoiding the need to repeat lengthy sample extraction procedures and improving security for valuable samples, amongst many other benefits.

For more on Centri, visit www.markes.com.

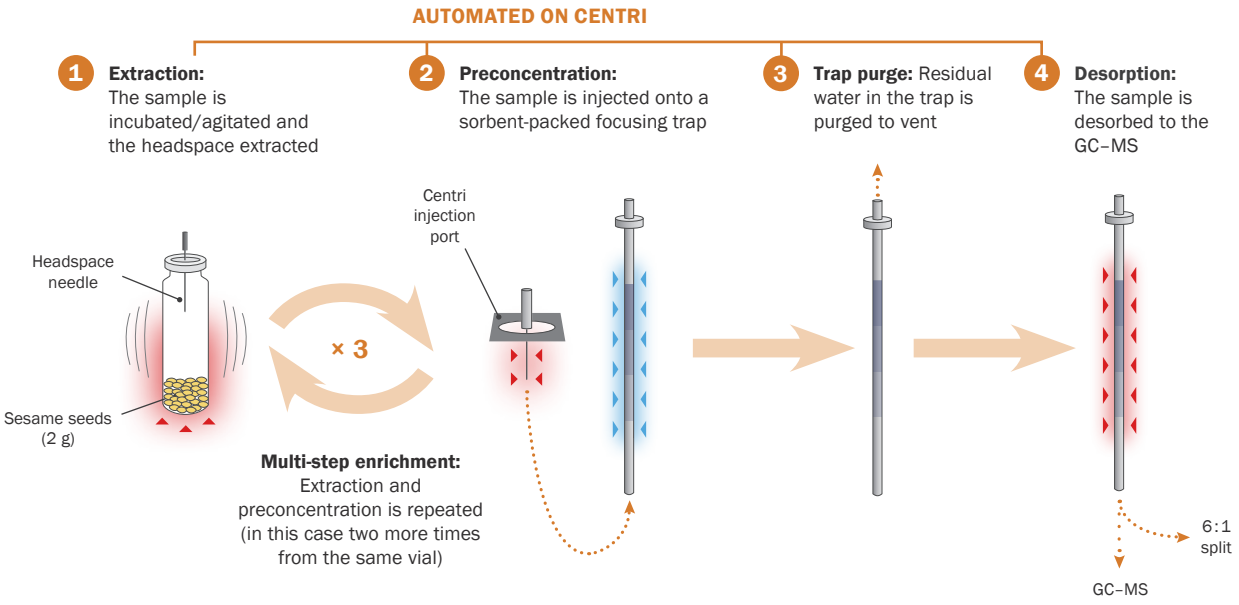

Figure 1: Headspace–trap with multi-step enrichment on the Centri platform.

Preconcentration:

Flow path:	180°C
Focusing trap:	U-T23ET0-2S
Purge flow:	20 mL/min for 1 min
Trap low:	-30°C
Trap high:	250°C (3 min)
Outlet split:	10 mL/min (6:1)

GC:

Column type:	MEGA®-624, 60 m × 320 µm × 1.8 µm
Column flow:	2 mL/min (constant flow)
Oven program:	35°C (5 min), then 10°C/min to 100°C (1 min), then 20°C/min to 230°C (5 min)

MS:

Transfer line:	230°C
Ion source:	250°C
Mass range:	29–300 m/z

Results and discussion

1. Sample preparation and analysis

All sesame seed samples were prepared as described in the experimental section before being placed on the autosampler tray of the Centri platform and analysed using HS–trap with MSE coupled with GC–MS. Using a syringe-based headspace method, a significant reduction in sample preparation and analysis time was observed compared to other commercially available techniques. This method is ideal for extraction of EtO and 2-CE because:

- Samples can be placed in a closed container (vial) and then onto an autosampler for direct extraction, including incubation and agitation steps.
- The fully automated sampling capability removes complex and manual preparation steps (*e.g.*, solvent extraction and/or derivatisation). This is especially the case with current methods (EURL-SRM[4] and BVL L 53.00-1[5]) used for extraction of EtO and 2-CE, where the manual workload and laboratory space required are significant and vulnerable to multiple opportunities for human error.
- It improves the health and safety aspect of this analysis by removing harmful derivatisation agents (*e.g.*, hydrobromic acid) used in some sample preparation methods as well as potentially dangerous cryogenic cooling liquids, which are required to trap very volatile compounds such as EtO.

Figure 2 displays a total ion chromatogram (TIC) from analysis of the contaminated seeds and extracted ion chromatograms (EIC) of the target contaminants EtO and 2-CE.

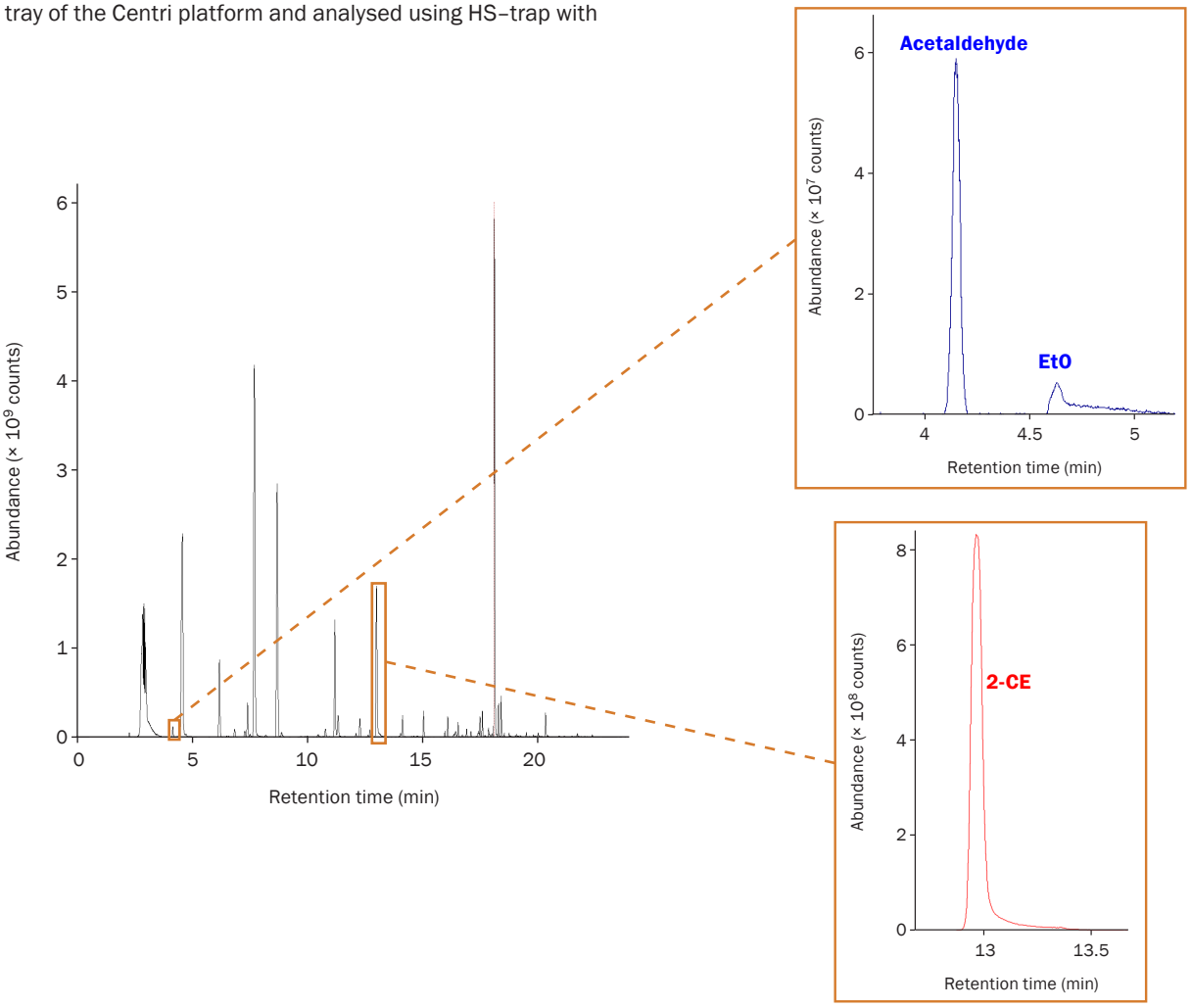

Figure 2: TIC for the contaminated seeds with EICs of target contaminants EtO (m/z ion 44) and 2-CE (m/z ion 31).

2. Calibration, reproducibility and method detection limits

A five-point calibration of EtO is shown in Figure 3 and the EtO concentration of the contaminated seeds is highlighted (gold circle). The R^2 value (0.9983) indicates excellent linearity achieved across the calibration range for EtO and the relative standard deviation (RSD) values of five replicates at 0.05 mg/kg verified reproducibility for EtO at 5% (Figure 4).

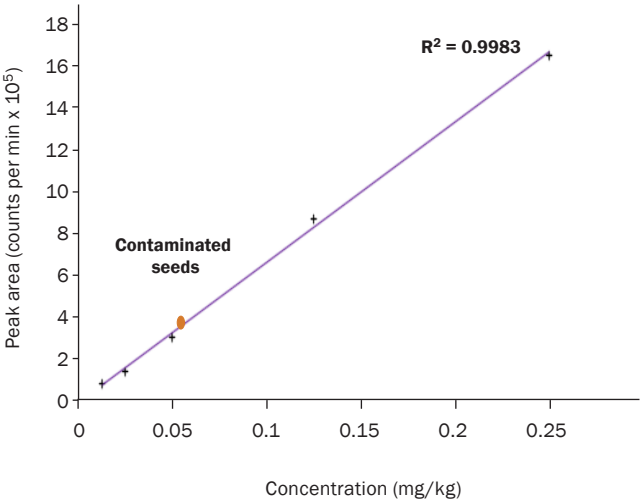

Figure 3: Calibration curve for EtO with a concentration range of 0.013–0.250 mg/kg. Excellent linearity is indicated with an R^2 value of 0.9983. The concentration of EtO present in the contaminated seeds is shown by the gold circle (0.055 mg/kg).

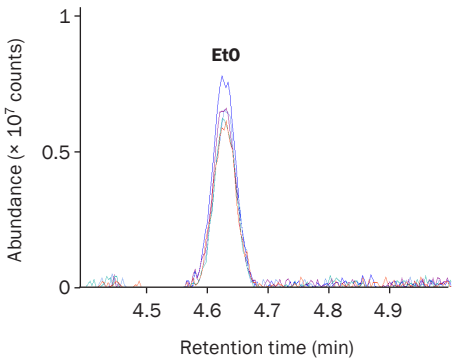

Figure 4: An EIC overlay (m/z ion 44) of five replicate spiked samples at a concentration of 0.05 mg/kg EtO, demonstrating excellent reproducibility with a relative standard deviation of 5%.

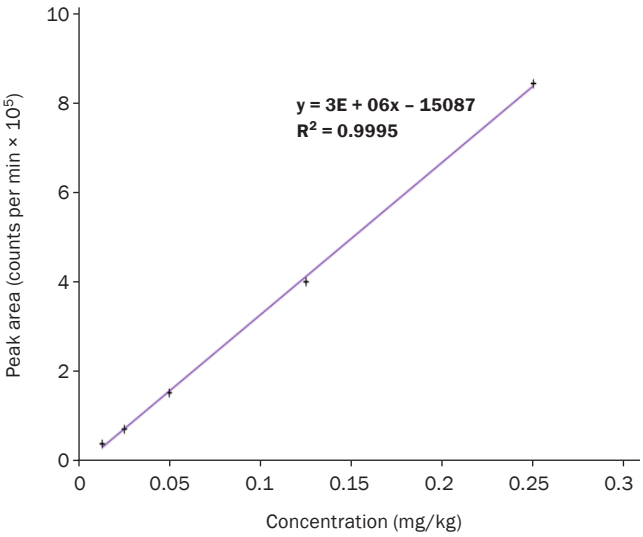

Figure 5: Calibration curve for 2-CE with a concentration range of 0.013–0.250 mg/kg showing excellent linearity indicated with an R^2 value of 0.9995.

Figure 5 shows the calibration curve for 2-CE. This was also confirmed to have excellent linearity (R^2 = 0.9995) and reproducibility (RSD value of 4% from five replicates at 0.05 mg/kg (Figure 6)). 2-CE exceeded the concentration of the calibration curve in the contaminated seeds so the concentration reported – 16 mg/kg – was calculated using the equation of the line.

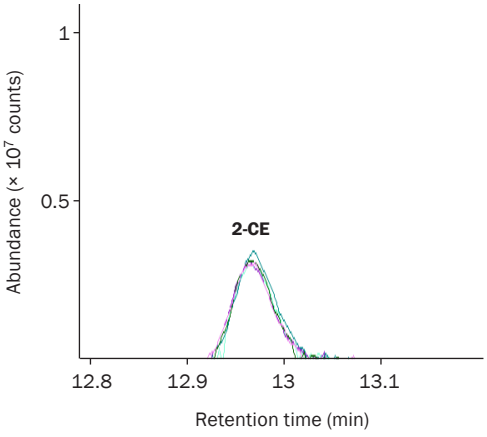

Figure 6: EIC overlay (m/z ion 31) of five replicate spiked samples at a concentration of 0.05 mg/kg 2-CE, demonstrating excellent reproducibility with a relative standard deviation of 4%.

Method detection limits (MDLs) were calculated to determine the minimum measured concentration of both target analytes that could be identified with 99% confidence. (Method detection limits provide an indication of the detection limit of the analytical method using the instrumentation in question.)

Five replicates at a calibration level of 0.05 mg/kg (five times the estimated MDL) were used to calculate the standard deviation (S_s), followed by a statistical calculation to determine the MDL:

MDLs = $t(n-1, 1 - \infty = 0.99)S_s$

where:

MDLs = method detection limits based on spiked samples,

$t(n-1, 1 - \infty = 0.99)$ = the Student's *t*-value appropriate for a single-tailed 99th percentile *t* statistic and a standard deviation estimate with $n-1$ degrees of freedom,

S_s = sample standard deviation of the replicate spiked sample analyses.

For EtO and 2-CE respectively, the standard deviations (Ss) were calculated as 3.053 x 10^{-3} and 2.034 x 10^{-3}, and the Student's *t*-value for five replicates with 99% confidence was 3.747. Therefore, the resulting MDLs for EtO and 2-CE were 0.011 and 0.008 mg/kg, respectively.

3. Contaminated seeds

Extracting EtO creates multiple challenges because of its low boiling point and high permeability; its highly strained epoxide ring means it can easily convert to more stable compounds, specifically in this case, to 2-CE. EtO may also react with hypochlorite (present in chlorinated water) to form 2-CE when the sesame seeds are soaked or washed during the farming process or in the field during irrigation.[6]

The concentration of 2-CE in the contaminated seeds was well over the maximum residue limit (Figure 7), indicating that this batch of sesame seeds was correctly rejected by the EU governing body.

Both individual target compounds in the contaminated seeds exceeded the maximum residue limit, as shown in Table 1. As the limit is quantified by combining both concentrations, this indicates that the contamination of these compounds was at an alarming level in terms of human consumption.

Contaminant	Average peak area (n = 5)	RSD%	Concentration (µg/kg)	Concentration in EtO equivalent (mg/kg)
EtO	367812	7	55	0.055
2-CE	54582834	3	15873*	8.760*
EtO + 2-CE	—			~8.8*

Table 1: The calculated concentrations of EtO and 2-CE in the contaminated seeds and a combined concentration.
*This is an extrapolated concentration due to the peak area being outside the calibration range. Conversion factor of 2-CE to EtO is (2-CE concentration) x 0.55 based on conversion of molecular weights (EtO = 44, 2-CE = 80, therefore 44/80 = 0.55).

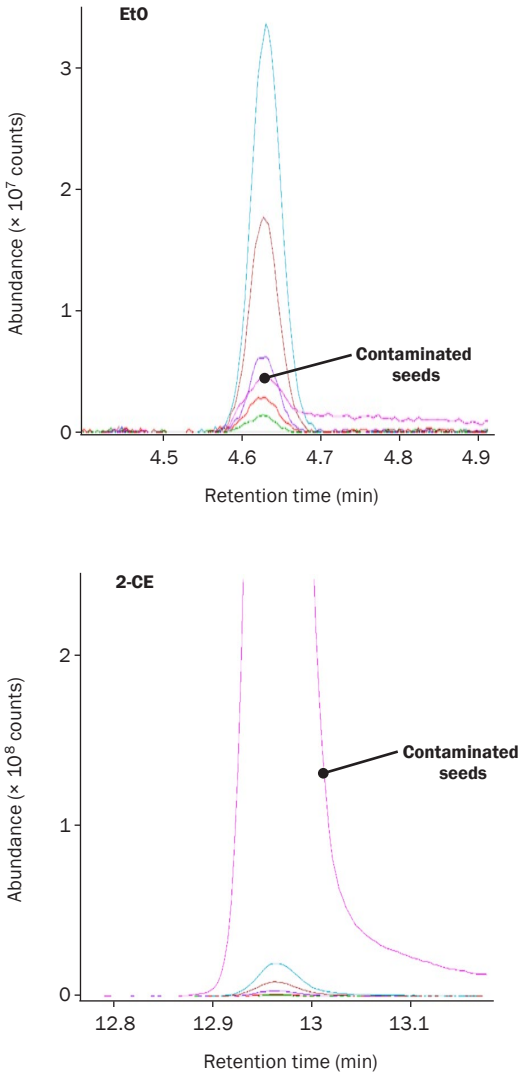

Figure 7: EIC overlay of calibration points ranging from 0.013–0.250 mg/kg with the contaminated seeds (pink) for EtO (top) and 2-CE (bottom).

It is worth noting that another contaminant extracted from the contaminated seeds was acetaldehyde, a toxic chemical with possible carcinogenic effects. Acetaldehyde is isomeric with EtO and was present at higher levels than EtO in all contaminated seeds analysed here, indicating potential partial conversion to acetaldehyde. In Figure 8, clear separation between EtO and acetaldehyde is shown under the analytical parameters used in this study. This is important to consider when optimising analytical methods because the mass spectrometer cannot distinguish between the resulting mass spectra of both components. Acetaldehyde is also a banned toxic compound for food contact under article 5(2)(c)(i): components of active and intelligent materials of Regulation 450/2009/E, suggesting that it should also be monitored in sesame seeds and foodstuffs relating to EtO contamination.[7]

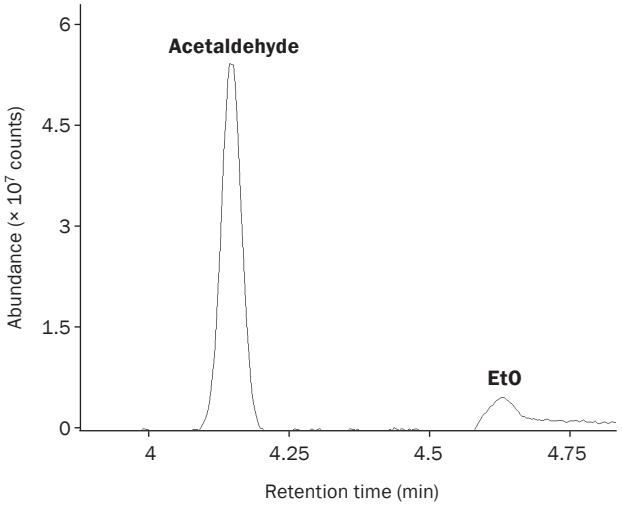

Figure 8: Clear separation between the peaks of EtO (right) and its toxic isomer acetaldehyde (left).

Conclusions

In this study, we have shown that HS–trap with MSE coupled with GC–MS is an efficient extraction technique for the analysis of EtO and 2-CE from sesame seeds that meets the EU regulatory requirement of 0.05 mg/kg. An enhancement in sensitivity was demonstrated by the lower limits of detection reached when the Centri platform allowed quantitative analysis to 0.013 mg/kg with excellent linearity (R^2 values > 0.99) and reproducibility (RSD values ≤ 5%) for both EtO and 2-CE.

Key enabling features of the Centri system include:

- The focusing trap:
  - Cryogen-free trapping: Multiple sorbents of increasing strength combined with a trapping temperature of -30˚C provide sharp chromatographic peaks, which is especially important for EtO.
  - Large-volume preconcentration: Exploiting the trap, larger than conventional headspace volumes (up to 5 mL) can be extracted from the sample vial, increasing the amount of each analyte extracted for detection.
  - Multi-step enrichment (MSE): This unique instrument capability further extends analyte preconcentration. Multiple extractions are loaded onto the trap for a single GC analysis. This enhances peak response further and correspondingly lowers detection limits, ideal for targeted analysis of trace-level analytes.
- Automation:
  - Enables a ‘hands-free’ workflow for the analyst, increasing productivity and eliminating areas vulnerable to human error.
  - Removes complex and manual preparation steps (*e.g.*, solvent extraction and/or derivatisation).
  - Provides excellent reproducibility, improving confidence in results.
  - Improves health and safety by removing harmful derivatisation agents and potentially dangerous cryogenic cooling liquids.

Further improvements to sample preparation could include cold-milling sesame seeds (whole seeds were used in this study) prior to weighing into a vial to increase the release of 2-CE. Testing the analytical method to evaluate trueness of results (*e.g.*, using a validated proficiency test scheme) would provide further confidence in the data.

Lastly, analyte detection in this study was carried out using a single-quadrupole mass spectrometer. Other commercially available mass spectrometers, such as triple-quadrupole or time-of-flight, could enhance the sensitivity of detection.

References

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Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.