SOLVENT-FREE SAMPLE PREPARATION FOR RESIDUAL FUMIGANTS ON FOODSTUFFS: MULTI-STEP ENRICHMENT-HEADSPACE-TRAP WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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INTRODUCTION

Fumigants, such as **ethylene oxide (EtO)** and **2-chloroethanol (2-CE)**, are biocidal aerosols used to control pests (Figure 1). Often, fumigants are applied prior to storage or transport of foodstuffs, but this may leave residues that later negatively affect consumer health. This has prompted increased scrutiny by regulatory bodies, with the European Union (EU) stipulating maximum residue limits (MRLs) for a range of commonly used fumigants. For example, levels of ethylene oxide above the EU MRL of 0.05 mg/kg have led to a number of product recalls, at great cost to suppliers.[1]

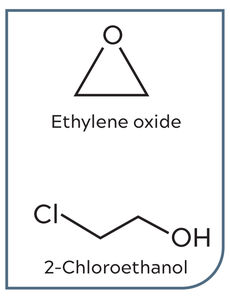

Figure 1: Chemical structures of ethylene oxide and 2-chloroethanol.

Consequently, there is a need for a robust, efficient and high-throughput method for extracting and analysing fumigants from foodstuffs. Traditionally, fumigant analysis has relied on liquid-liquid extraction methods such as QuEChERS along with gas chromatography-mass spectrometry (GC-MS).[2] However, these methods require extensive manual sample preparation, introduce a 'dirty' extract including many non-target compounds to the GC and generate significantly large volumes of environmentally damaging solvent waste.

We present a fully automated, solvent-free, environmentally-conscious technique in which static headspace methodology is enhanced by incorporation of a cryogen-free focusing trap.

WORKFLOW AND EXPERIMENTAL

Method development began with EtO using sesame seeds as an example matrix. EtO and its degradation product 2-CE are usually regulated together, since one is commonly derived from the other (Table 1). Therefore, both were included within the method development steps.

Fumigant	Abbreviation	Formula	Molecular weight
Ethylene oxide	EtO	C_2H_4O	44.05
2-Chloroethanol	2-CE	C_2H_5ClO	80.51

Table 1: Compounds used in method development, with abbreviations, chemical formulae and molecular weights.

Workflow

Sesame seeds were analysed whole. To prepare a sample, seeds (2 g) were weighed directly into a sample vial and a benzene-d_6 internal standard (1 µL at 100 µg/mL) was added before the vial was sealed (Figure 2). Fast procedures are key during the sample preparation steps when analysing EtO. Due to its high volatility, it is easily lost, making laborious sample preparation methods often unreliable.

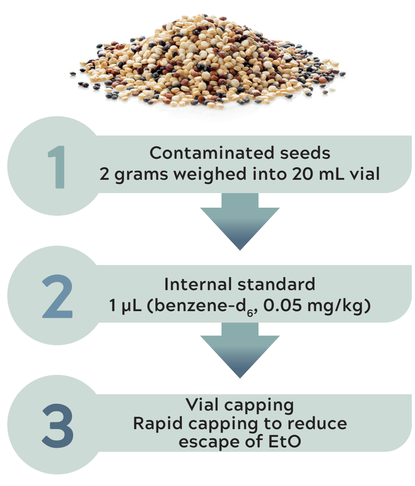

Figure 2: Sample preparation workflow for the headspace sampling in vial.

Validation

For method development and validation, fumigantfree matrices were used and 1 µL analytical standard containing diluted EtO and 2-CE at known concentrations was spiked onto the matrix in addition to benzene-d_6. We also assessed sesame seeds that had been rejected by border checks due to alarming levels of EtO contamination. To these, we added benzene-d_6 only.

Sample extraction was automated on the Centri® extraction and enrichment platform, using Centri's focusing trap. Downstream analysis was performed by GC-MS. Throughput was high at one sample every 30 minutes, or 48 samples a day per Centri platform.

MULTI-STEP ENRICHMENT–HEADSPACE–TRAP

Direct headspace is a straightforward technique in which the gas above a sample is extracted by syringe and injected directly to the GC column. Unlike QuEChERS, this requires no environmentally hazardous solvent to extract the analytes; however, its sensitivity is limited by the small volume of headspace that can be injected while maintaining good chromatography. We overcame this challenge by developing multi-step enrichment-headspace-trap (MSE®-HS-trap), as shown in Figure 3. Here, the analytes are preconcentrated on a multi-sorbent focusing trap after headspace extraction, prior to injection to the GC system.

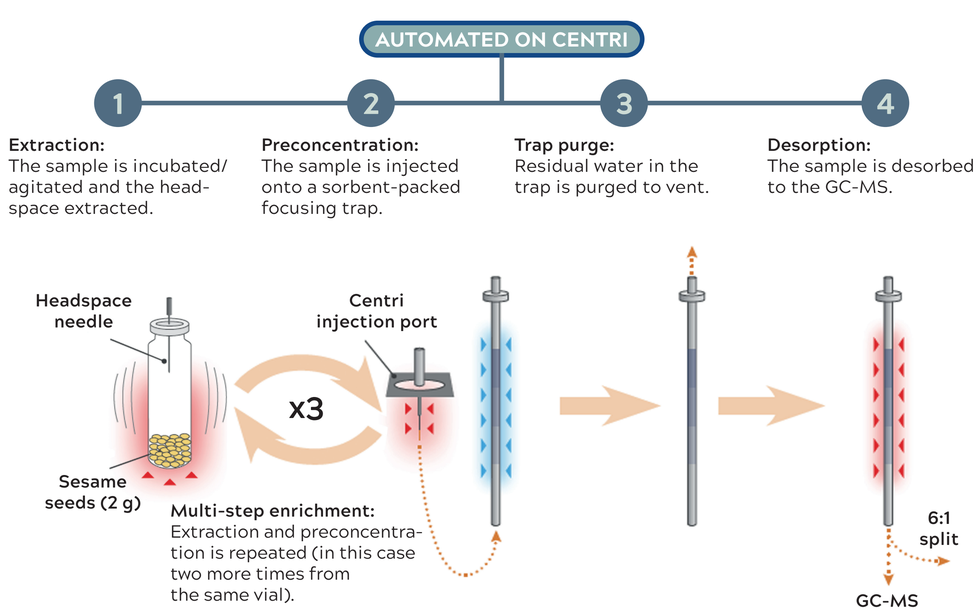

Figure 3: MSE-HS-trap workflow on the Centri platform.

Experimental

Sample incubation was performed at 70°C with agitation at 300 rpm (Figure 3 - Step 1). After 10 minutes, 5 mL from the headspace was transferred by syringe to a focusing trap, which was electrically cooled to -30°C (Step 2). Steps 1 and 2 were repeated twice more with three minutes between each extraction (to re-establish headspace equilibrium) such that analytes from a total of three extractions were concentrated on the trap. After trap purging to remove interferences such as water (Step 3), the flow of gas through the trap was reversed and the trap was rapidly heated to desorb analytes and transfer them to the GC column in a narrow band (Step 4). Thus, by decoupling headspace extraction from GC injection with the focusing trap, we greatly enhance extraction efficiency and hence sensitivity compared with direct headspace extraction.

METHOD VALIDATION

As shown in Table 2, the method was robust, quantitative and highly sensitive, with a minimum detection limit (MDL) far below the EU MRL of 0.05 mg/kg for each of the monitored compounds.

	Linearity (R^2)	Reproducibility (RSD%)	Sensitivity (MDL mg/kg)
EtO value	0.9983	5	0.011
2-CE value	0.9995	4	0.008

Table 2: Method validation statistics for analytes on sesame seeds. Linearity was determined from five calibration levels at 0.013-0.25 mg/kg. Relative standard deviation (RSD) was calculated from five replicates at 0.05 mg/kg. MDLs were determined from the standard deviation of the calculated concentration of these five replicates, multiplied by Student's t-value for 99% confidence.

ANALYSIS OF REAL SAMPLES

We assessed three replicates of sesame seeds known to be contaminated with EtO, and quantified EtO at an average value of 0.055 mg/kg - above the EU MRL of 0.05 mg/kg (Figure 4). Levels of 2-CE were very high, well above the highest calibration level used. By extrapolation of the calibration line, we calculated the 2-CE concentration to be 15.873 mg/kg. Thus, MSE-HS-trap is suitable for the detection of fumigants in real food samples, though wider calibration ranges than used here may be required when analysing heavily contaminated samples.

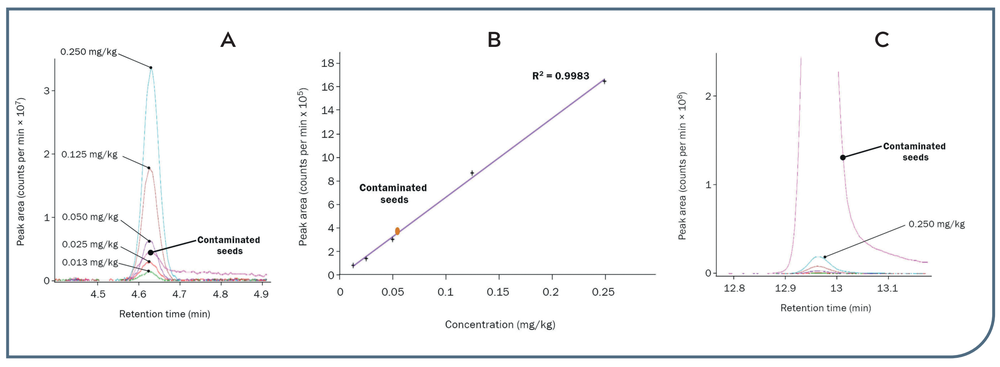

Figure 4: Analysis of a real sesame seed sample contaminated with EtO and 2-CE. The EtO contamination peak is clear with a good peak shape (**A**), and the concentration was readily quantified (**B**). 2-CE was present in very high concentrations, well above the highest calibration level used (**C**).

SIMULTANEOUS ANALYSIS OF MULTIPLE FUMIGANTS

In further work, we have extended the method to other environmentally harmful and potentially hazardous fumigants (see right). We have successfully extracted all listed fumigants with MSE-HS-trap and are in the process of adjusting conditions for optimum extraction efficiency. Furthermore, we intend to investigate fumigant extraction from a variety of food matrices.

- Bromoethane
- Bromomethane
- Carbon tetrachloride
- 1,2-Dibromoethane
- Hexane
- 1,2-Dichloroethane
- Ethyl formate
- Ethylene oxide
- Propylene oxide

CONCLUSIONS

- MSE-HS-trap is a fully automated, high-throughput method for the simultaneous detection of fumigant residues on foodstuffs.
- 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.
- The method does not generate solvent waste and is therefore substantially greener than other technologies such as QuEChERS.
- The method is robust, quantitative and highly sensitive, and able to detect fumigants at concentrations well below regulatory limits.
- Removes complex and manual preparation steps (e.g., solvent extraction and/or derivatisation).

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