Ethylene oxide in the sights of food safety inspectors

In large numbers of sesame seed shipments, especially from India, EU food inspectors have found residues of the highly toxic compound ethylene oxide, leading to food product recalls with associated public information campaigns. Many years ago, the fumigant was widely used as a disinfectant for foodstuffs, but its use for food and feed has been strictly prohibited in the EU since 1991. To safeguard the food supply, fast, safe, and reliable analysis methods are clearly needed.

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Ethylene oxide (EO) is a sweetish smelling, colorless, highly flammable gas, which in many countries outside the European Union EU is still in use as a biocide to eliminate insects, bacteria, and fungi from the food supply. The fumigant is especially used for dry foodstuffs, such as herbs, spices, nuts, or oily seeds. In the EU, the use of EO to treat food has been prohibited since 1991 due to its carcinogenic and genotoxic properties. Nevertheless, the EU commission's Rapid Alert System for Food and Feed (RASFF) has received a large and increasing number of notifications relating to EO contaminated foodstuffs found at EU entry points since 2020, initially mainly regarding sesame seed shipments from India. The determined EO amounts exceeded maximum residue level (MRL) of 0.05 mg/kg, specified in EU Commission Regulation 2015/868 [1], by a wide margin leading to further increased monitoring and, in turn, a significant number of product recalls in various EU member states. Incidentally, recalls were issued both for conventional and organic food products and the list of afflicted food products and of countries of origin has grown with the number of samples taken. The US Food and Drug Administration and Canadian authorities specify tolerance limits of 7 ppm for Ethylene oxide and 940 ppm for 2-CE for a range of spices, dried herbs, and vegetables.

2-CE, also known as ethylene chlorohydrin, counts among the most toxic halogenated organic compounds. Incidentally, 2-CE seems to be the dominating compound in foods treated with EO. This is widely attributed to the extreme reactivity and volatility of EO.

The active ingredient and its metabolite

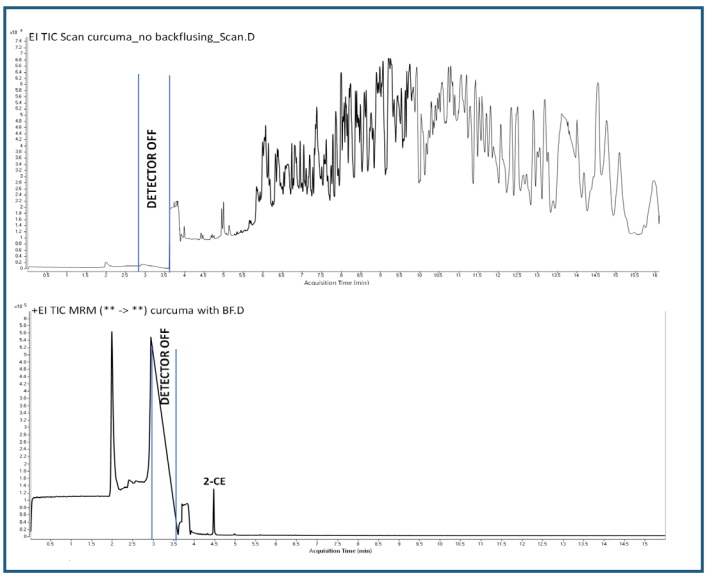
To safeguard a healthy food supply, industry and food safety inspectors need a proper toolkit of highly sensitive analysis methods. They must be able to reliably determine ethylene oxide (EO) and its main metabolite 2-chloroethanol (2-CE), at concentration levels well below the specified MRLs (see above). Literature searches reveal different methods used to determine EO or, in some cases, the sum of EO and 2-CE. Some methods, such as the official German method (§ 64 LFGB, L53.00-1) rely on converting 2-CE to EO under alkaline conditions and then converting the formed EO to 2-Iodoethanol (2-IE), which is in turn determined by GC-ECD or, more frequently nowadays, by GC-MS. Other methods rely on converting EO to 2-CE under acidic conditions followed by extraction of the resulting 2-CE

with ethyl acetate and GC-MS determination. In December 2020, the EU Community Reference Laboratory for Single Residue Methods in Stuttgart, Germany (https://pesticides.cvuas.de/) proposed a combination of a QuEChERS sample preparation method and GC-MS/MS determination [2].

Meeting the challenge

When analyzing food products for EO and 2-CE, there are challenges to overcome. The very volatile EO, for example, requires a special GC column to avoid co-elution with acetaldehyde, frequently found in fatty foods. To sharpen the challenge, acetaldehyde and EO not only have similar retention indices, but also similar mass spectra. At the other end of the molecular size spectrum, even after QuEChERS cleanup, food extracts contain large amounts of nonvolatile matrix material. Such matrix residue accumulates in the GC inlet liner impacting analyte recovery and thereby method accuracy and ruggedness. When nonvolatile material is then transferred to the GC column, it accumulates and impacts separation performance before eventually contaminating the MS ion source.

At the RIC Technologies laboratories, we took up the challenge of developing and validating an automated method for the determination of EO and 2-CE in matrix laden QuEChERS extracts while reducing or eliminating instrument downtime for cleaning and maintenance. The analysis method upon which our solution is based on was developed by the EU Community Reference Laboratory for Single Residue Methods at CVUA Stuttgart, Germany (https://pesticides.cvuas.de/). The method performs quantitative determination of EO and its main metabolite 2-CE in foodstuffs, specifically in sesame seeds and curcuma. It was found that to obtain sufficient method ruggedness, residue from the sample matrix must not be allowed to accumulate in the GC inlet liner, GC column or reach the MS ion source. The automated system we used consisted of an Agilent 8890 GC, Agilent 7010 Triple Quadrupol MS and a GERSTEL MultiPurpose Sampler (MPS) fitted with the Automated Liner Exchange (ALEX) option. ALEX removes and replaces dirty liners at user defined intervals. In addition, the analytical column was protected by integrating a pre-column backflush option, which improved ruggedness and reduced instrument downtime for maintenance.



GC-MS traces of curcuma extracts analyzed in scan mode without backflush (upper trace) and in MS/MS mode with backflush (lower trace). Source: Tatiana Cucu / RIC Technologies

A look at the analysis details

Method development was performed using EO and 2-CE standard solutions, the method validation was performed based on real samples that had been mechanically homogenized and spiked with deuterated standards. Quantification was performed using deuterated EO and 2-CE as internal standards. The QuEChERS extraction was performed manually according to DIN EN 15662.

To demonstrate chromatographic stability, extracts of sesame seeds and curcuma were spiked with EO and 2-CE resulting in concentrations of 10, 40, and 100 ng/mL. These were repeatedly injected from the same vial to the same GC inlet liner. A moderate reduction was observed in the EO peak areas for the sesame extract injections, slightly more for the curcuma extract injections. This was explained by evaporative loss of EO from the sample, which was kept at ambient

temperature over a period during which multiple septum punctures were performed to aspirate the samples. Nevertheless, repeatability was a respectable 5-6 %, indicating that the loss was adequately compensated for using internal standards in the calculation. Unexpectedly, the less volatile 2-CE also exhibited some loss over the period. The 40 % reduction in absolute 2-CE peak area seen between the first and fifth injection was clearly caused by increasing activity in the GC inlet liner. Further, peak distortion was observed after injection of samples with particularly high matrix loads, for example in the case of curcuma extracts. The injection of relatively "dirty" QuEChERS extracts influenced the recovery of 2-CE prompting the decision to use Automated Liner Exchange (ALEX) in the EO and 2-CE analysis system. Using the ALEX option, clean liners were inserted after 20 injections (user defined) to ensure that extracted nonvolatile matrix material couldn't accumulate to a degree that would influence 2-CE recovery and compromise the quality of the analysis results. Further, installing a pre-column backflush option clearly prevented a large amount of high boiling material from reaching the GC column and by extension the MS ionization source. When the backflush option was activated, MS source contamination was negligible. If pre-column backflush was activated shortly after the target analytes had reached the analytical column, the sample would have no or almost no impact on the analytical column, extending column life and protecting the MS ion source from contamination that would otherwise impact system stability and require down time for cleaning.

Succesful ethylene oxide determination

The described method for determination of ethylene oxide and 2-chloroethanol was validated by performing recovery experiments in triplicate at three different concentrations (0.05, 0.2, and 0.5 mg/kg) in actual samples. Recoveries ranged from 84.5 to 100.6 % for EO and from 88.8 to 106.2 % for 2-CE in sesame seed and curcuma samples. To demonstrate the practical usefulness of the method, representative food samples that had been purchased in a local food store were analyzed.

Result: None of the samples tested positive for EO, but all tested positive for 2-CE, some even exceeding the MRL levels. The precision of the developed method was tested using sesame seed reference samples kindly provided by a partner laboratory and the established value was close to the reported average of 4660 µg/kg (27.3 % CV). Recovery was between 85 and 106 %, with good repeatability for both EO and 2-CE. Concerning the sensitivity, the developed method achieves LOQs for sesame and curcuma (representative for food category spices) below the currently valid MRLs. Results were stable both for sesame and for curcuma samples, RSDs for triplicate combined extractions and analyses for the recovery experiments were well below 20 %. The sample preparation and the optimized GC-MS/MS method reliably delivered results and performance that would indicate the usefulness of the method for the quantitative determination of EO and 2-CE down to well below the regulated concentrations for sesame and spice samples. The process delivers high sensitivity, selectivity, precision, and especially high sample throughput with minimal downtime for cleaning and maintenance of the inlet system, replacement of GC columns, and MS ion source cleaning.

REFERENCES:

[1] Regulation (EU) 2015/868 of 26 May 2015 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 2,4,5-T, barban, binapacryl, bromophos-ethyl, camphechlor (toxaphene), chlorbufam, chloroxuron, chlozolinate, DNOC, di-allate, dinoseb, dinoterb, dioxathion, ethylene oxide, fentin acetate, fentin hydroxide, flucycloxuron, flucythrinate, formothion, mecarbam, methacrifos, monolinuron, phenothrin, propham, pyrazophos, quinalphos, resmethrin, tecnazene and vinclozolin in or on certain products. Off. J. Eur. Union L 145/1 – 71 (2015).

[2] EURL-SRM-Analytical Observation Report: Analysis of Ethylene Oxide and its metabolite 2-Chloroethanol by the QuOil or the QuEChERS method and GC-MS/MS. Dezember 2020. Link: https://www.eurl-pesticides.eu/library/docs/srm/EurlSrm_Observation_EO_V1.pdf

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