Determination of Ethylene Oxide in Selected Food

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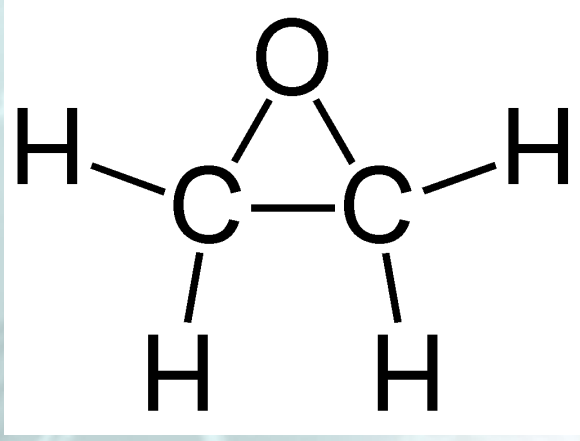
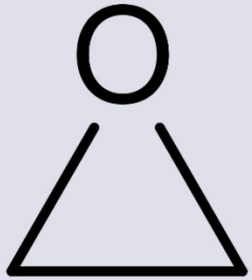
What is Ethylene Oxide?

Ethylene oxide or EO: *Three-membered ring organic compound with chemical formula C_2H_4O.*



Ethylene Oxide

Ethylene oxide (EO)	
IUPAC name	Oxirane 1,2-Epoxyethane Oxacyclopropane
CAS	75-21-8
Molecular formula	C_2H_4O
Molar mass	44.052 g/mol



Properties

- Colourless
- Highly flammable
- Very reactive gas
- Boiling point 10.4 °C

Ethylene Oxide Applications

Raw material in large-scale chemical production

- Ethylene glycol (antifreeze, solvents, polyester)
- Polyethylene glycol (cosmetics, pharmaceuticals, lubricants)
- Glycol ethers (brake fluids, lacquers and paints)
- Ethanolamines (soap and detergents)

Direct use (≈ 0.05% EO global production)

- Preservatives
- Fumigants
- Sterilization of medical devices

Why has the use of Ethylene Oxide been banned in food industry?

Toxicity:

IARC: Group 1 carcinogen (carcinogenic to humans)

U.S. EPA: Carcinogenic to humans by the inhalation route of exposure + evidence supporting mutagenicity

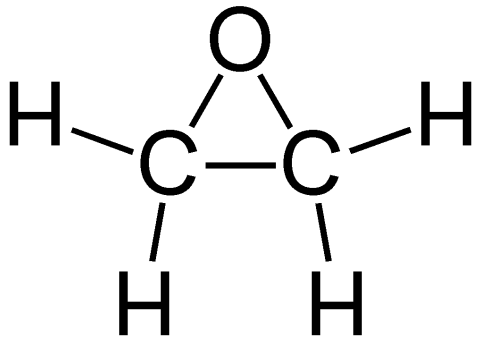
EU: Category 1B mutagen, 1B carcinogen, and 1B toxic compound for reproduction and other effects

The Problem: EO as Food Fumigant

Emerging contaminant ethylene oxide residues in food

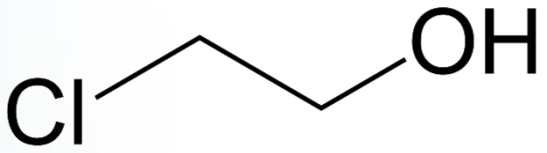
Ethylene oxide

- Free EO is quickly removed under aerated conditions
- Slower decrease in non-aerated and sealed products



2-Chloroethanol (2-CE or Ethylene Chlorohydrin)

- Formed in EO fumigated products in the presence of chloride ions
- Less volatile (limited removal by aeration)
- Marker residue for EO use



Surge in demand for EO analysis, why?

Ethylene Oxide NOT APPROVED AS FOOD FUMIGANT since 1991 in EU

MRL (mg/kg)	COMMODITY GROUP
0.02	Fruit, fresh and frozen Vegetables, fresh and frozen, except herbs and edible flowers Pulses Cereals Sugar plants Products of animal origin, except for honey and other apiculture products
0.05	Tree nuts Oilseeds and oil fruits Herbs and edible flowers Honey and other apiculture products
0.1	Teas, coffee, herbal infusions, cocoa, and carobs Spices

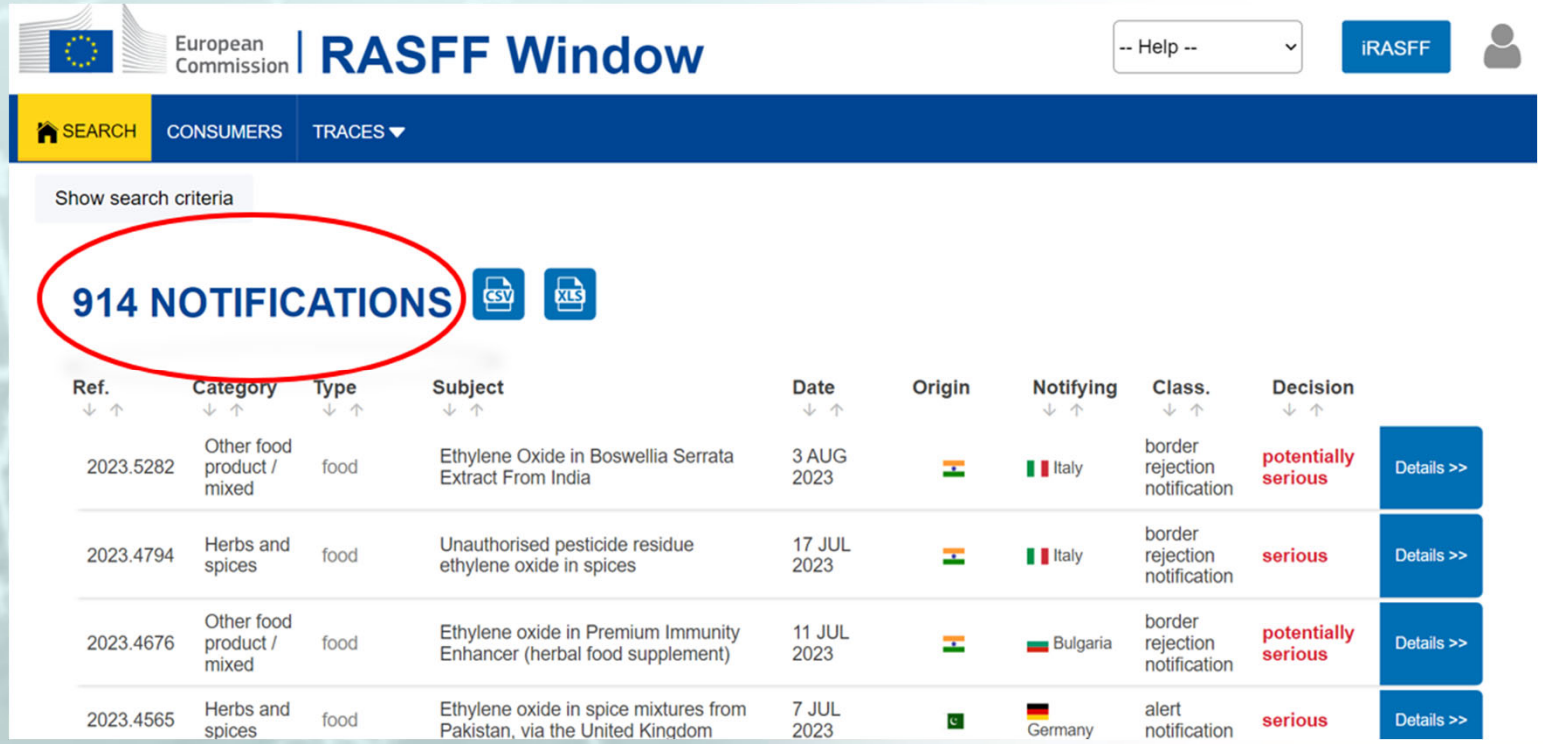
Residue definition

Ethylene oxide (sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide)

Latest Development

RASFF (Rapid Alert System for Food and Feed)

- Sep 9, 2020: Ethylene oxide residues (approx. 30 mg/kg) in sesame seeds from India
- Follow-up actions and additional notifications for sesame seeds and related products, leading to withdrawals/recalls in the EU



Ref.	Category	Type	Subject	Date	Origin	Notifying	Class.	Decision	
2023.5282	Other food product / mixed	food	Ethylene Oxide in Boswellia Serrata Extract From India	3 AUG 2023		Italy	border rejection notification	potentially serious	Details >>
2023.4794	Herbs and spices	food	Unauthorised pesticide residue ethylene oxide in spices	17 JUL 2023		Italy	border rejection notification	serious	Details >>
2023.4676	Other food product / mixed	food	Ethylene oxide in Premium Immunity Enhancer (herbal food supplement)	11 JUL 2023		Bulgaria	border rejection notification	potentially serious	Details >>
2023.4565	Herbs and spices	food	Ethylene oxide in spice mixtures from Pakistan, via the United Kingdom	7 JUL 2023		Germany	alert notification	serious	Details >>

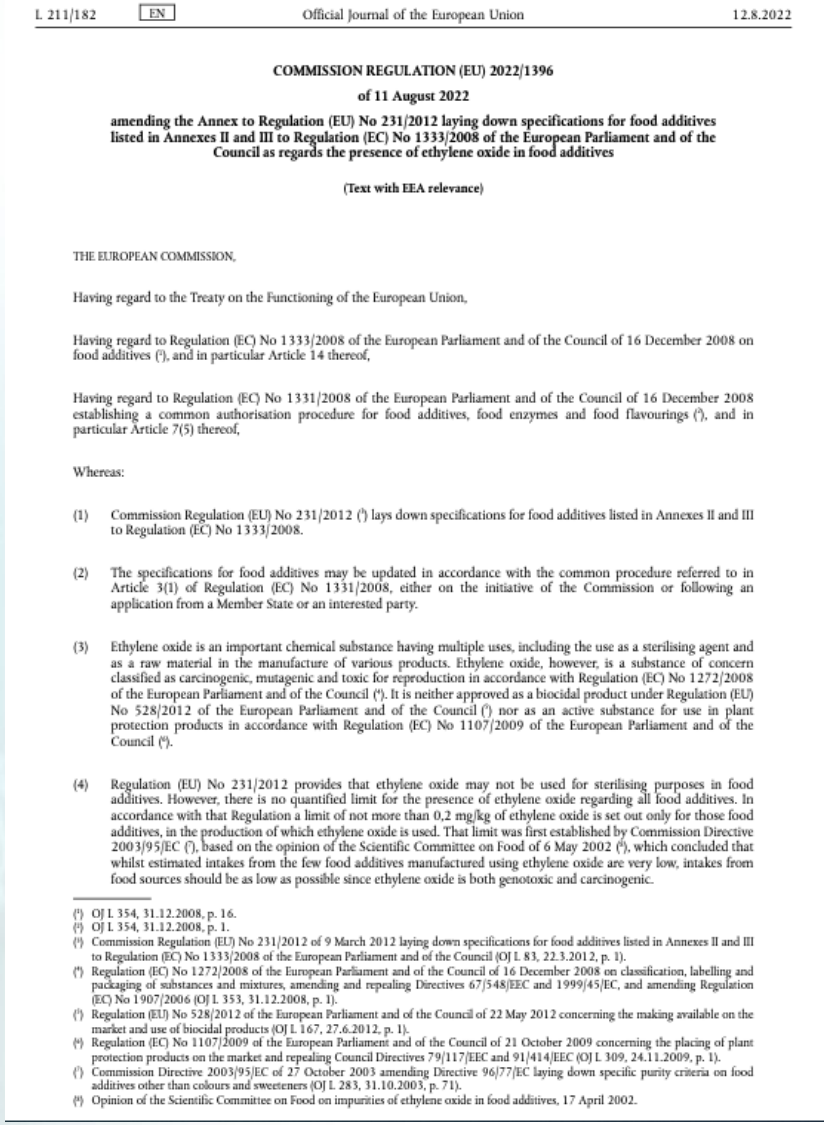
Latest Development

FOOD ADDITIVES

Amendment of COMMISSION REGULATION (EU) No 231/2012 (Effective from 1 Sep 2022)

Specifications for food additives (e.g. thickening agents)

- Bans use of ethylene oxide for sterilization of food additives
- Specifies EO limit of 0.1 mg/kg for additives



L 211/182 EN Official Journal of the European Union 12.8.2022

COMMISSION REGULATION (EU) 2022/1396

of 11 August 2022

amending the Annex to Regulation (EU) No 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the presence of ethylene oxide in food additives

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (1), and in particular Article 14 thereof,

Having regard to Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings (2), and in particular Article 7(5) thereof,

Whereas:

(1) Commission Regulation (EU) No 231/2012 (3) lays down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008.

(2) The specifications for food additives may be updated in accordance with the common procedure referred to in Article 3(1) of Regulation (EC) No 1331/2008, either on the initiative of the Commission or following an application from a Member State or an interested party.

(3) Ethylene oxide is an important chemical substance having multiple uses, including the use as a sterilising agent and as a raw material in the manufacture of various products. Ethylene oxide, however, is a substance of concern classified as carcinogenic, mutagenic and toxic for reproduction in accordance with Regulation (EC) No 1272/2008 of the European Parliament and of the Council (4). It is neither approved as a biocidal product under Regulation (EU) No 528/2012 of the European Parliament and of the Council (5) nor as an active substance for use in plant protection products in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council (6).

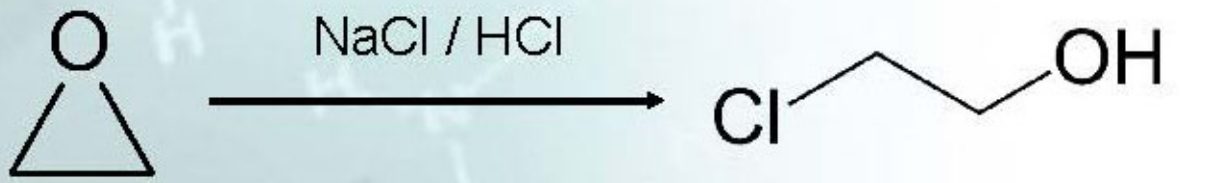
(4) Regulation (EU) No 231/2012 provides that ethylene oxide may not be used for sterilising purposes in food additives. However, there is no quantified limit for the presence of ethylene oxide regarding all food additives. In accordance with that Regulation a limit of not more than 0,2 mg/kg of ethylene oxide is set out only for those food additives, in the production of which ethylene oxide is used. That limit was first established by Commission Directive 2003/95/EC (7), based on the opinion of the Scientific Committee on Food of 6 May 2002 (8), which concluded that whilst estimated intakes from the few food additives manufactured using ethylene oxide are very low, intakes from food sources should be as low as possible since ethylene oxide is both genotoxic and carcinogenic.

(1) OJ L 354, 31.12.2008, p. 16.
(2) OJ L 354, 31.12.2008, p. 1.
(3) Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council (OJ L 83, 22.3.2012, p. 1).
(4) Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (OJ L 353, 31.12.2008, p. 1).
(5) Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products (OJ L 167, 27.6.2012, p. 1).
(6) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC (OJ L 309, 24.11.2009, p. 1).
(7) Commission Directive 2003/95/EC of 27 October 2003 amending Directive 96/77/EC laying down specific purity criteria on food additives other than colours and sweeteners (OJ L 283, 31.10.2003, p. 71).
(8) Opinion of the Scientific Committee on Food on impurities of ethylene oxide in food additives, 17 April 2002.

Analytical methods in EO analysis

Gas Chromatography - Tandem Mass Spectrometry (GC-MS/MS) is the most widely used analytical technique used for determination and quantitation of EO in various commodities

Method 1
Determination of 2-CE
(Total EO analysis)

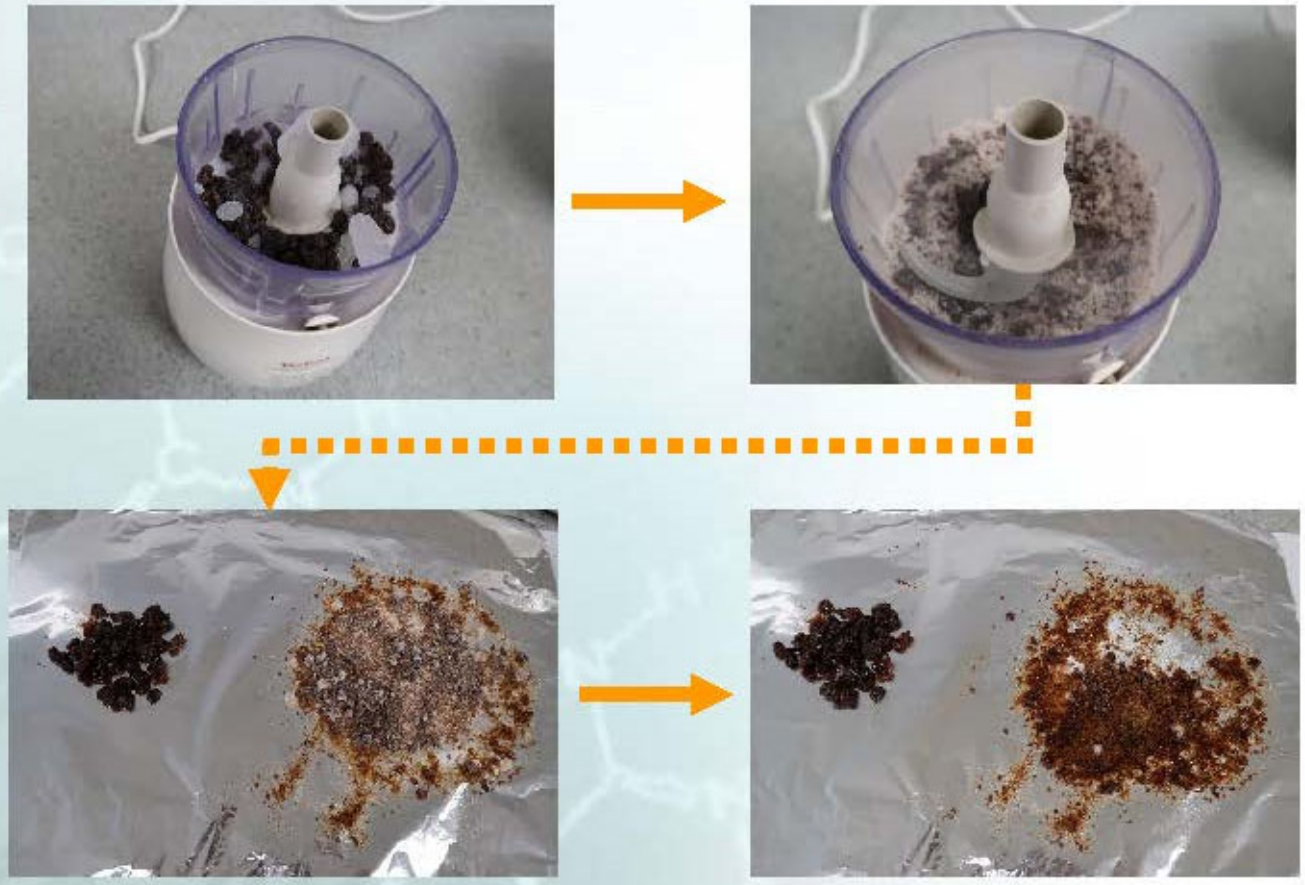


EO (sum) = 2-CE x 0.55

Residue definition

Ethylene oxide (sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide)

Sample Preparation



Homogenization

(Sample to dry ice = ca. 5:1)

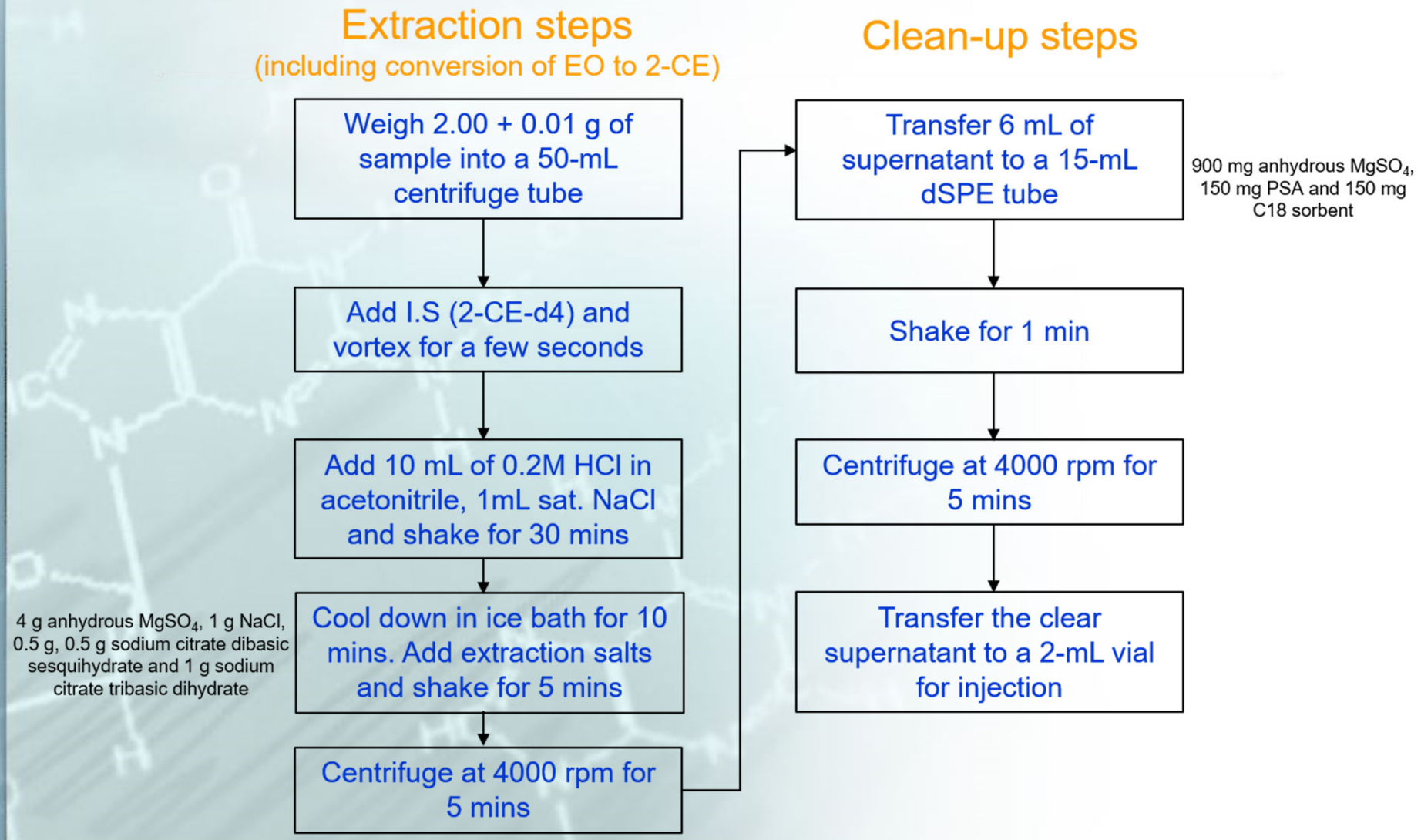
Sample Preparation

Sample crushing and mixing

Extraction Aid

Stainless steel balls

Workflow for sample extraction and clean-up

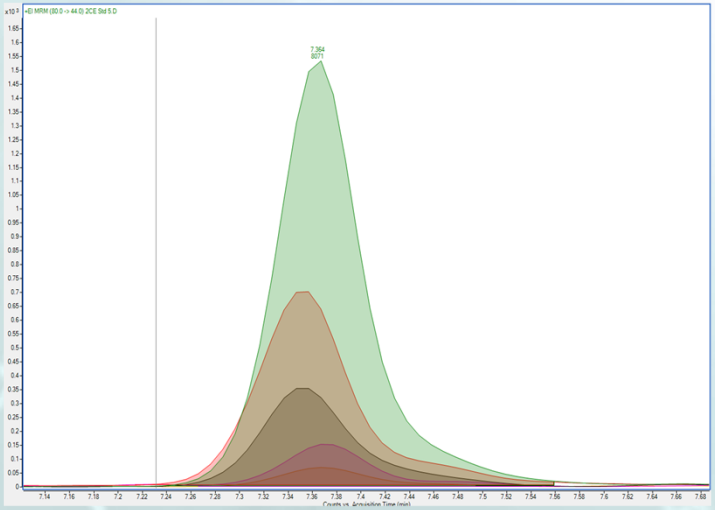


Instrumental Analysis

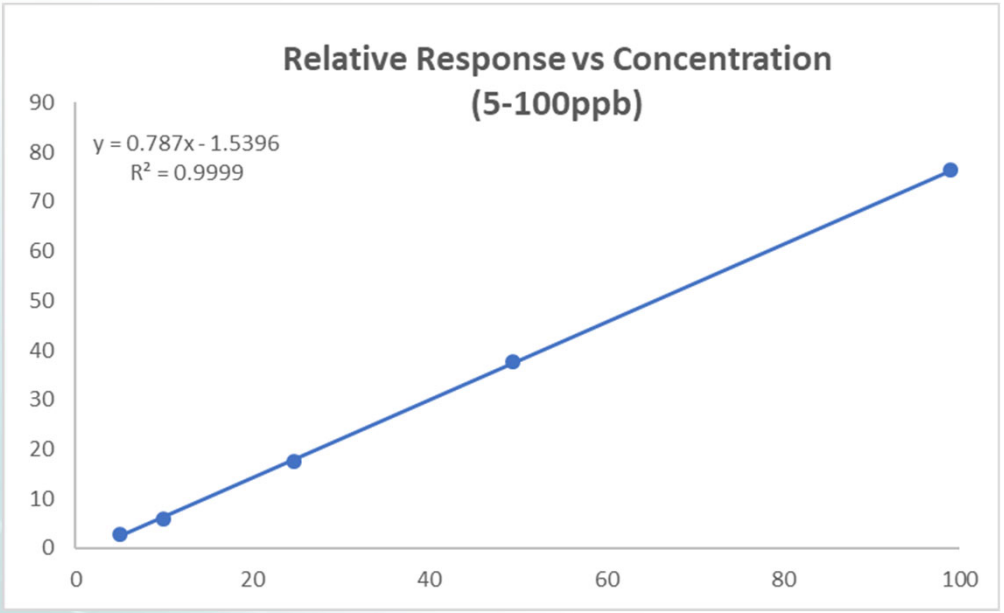
GC conditions	
Column	DB-624 UI (60 m x 0.25 mm, 1.4 µm)
Inlet	Programmable Temperature Vaporizer (PTV) (Injection volume 1 µL)
Injection mode	Splitless (3 min)
Inlet temp	100 °C (0.8 min), ramp with 12 °C/s to 250 °C (10 min)
Oven gradient	60 °C (2 min) ramp with 50 °C/min to 150 °C (0 min) ramp with 40 °C/min to 250 °C (16 min)
Carries gas	Helium, 1 mL/min

MSD conditions		
Ionization mode	Electron impact (EI)	
Ion source temp	270 °C	
Transfer line temp	250 °C	
MRM transitions	2-CE	80 --> 31 (CE 5) 82 --> 31 (CE 5) 80 --> 43 (CE 5) 80 --> 44 (CE 1)
	2-CE-d4	84 --> 33 (CE 5)

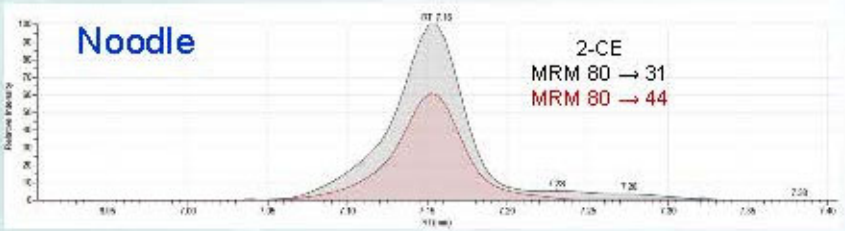
Linearity

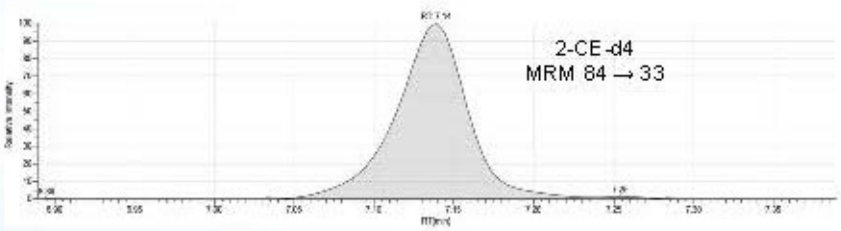


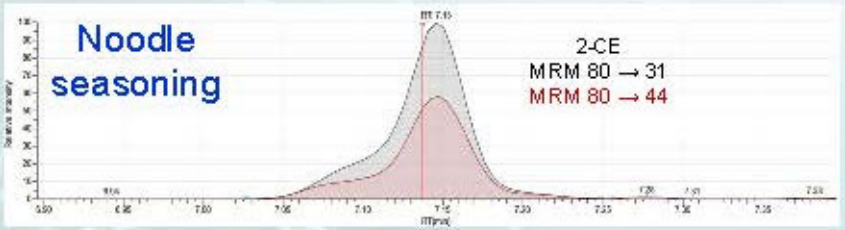
MRM overlay of various concentrations of 2-CE ranging from 5 to 100 ppb

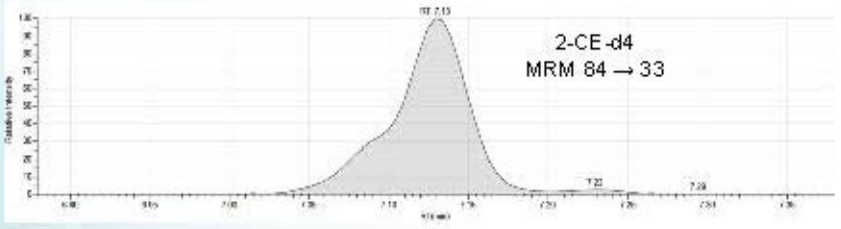


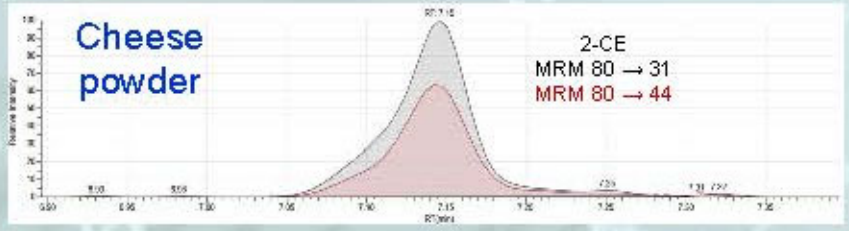
Chromatograms of 2-CE at 0.1 mg/kg in complex matrices

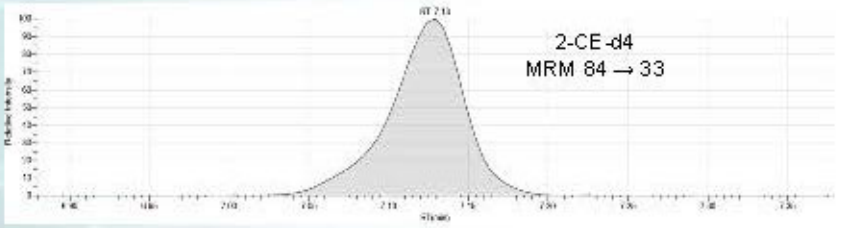


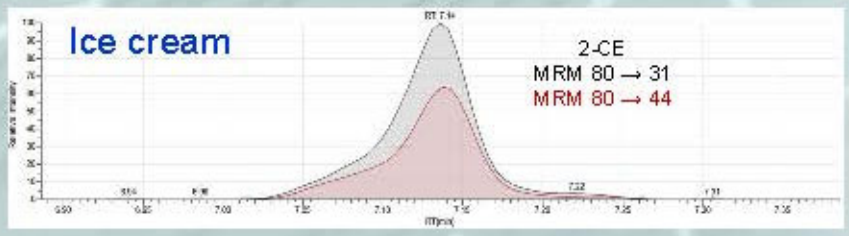


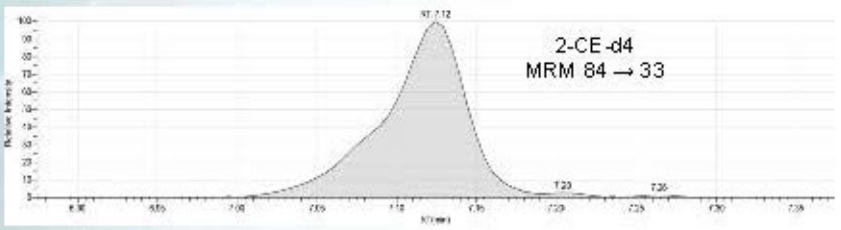








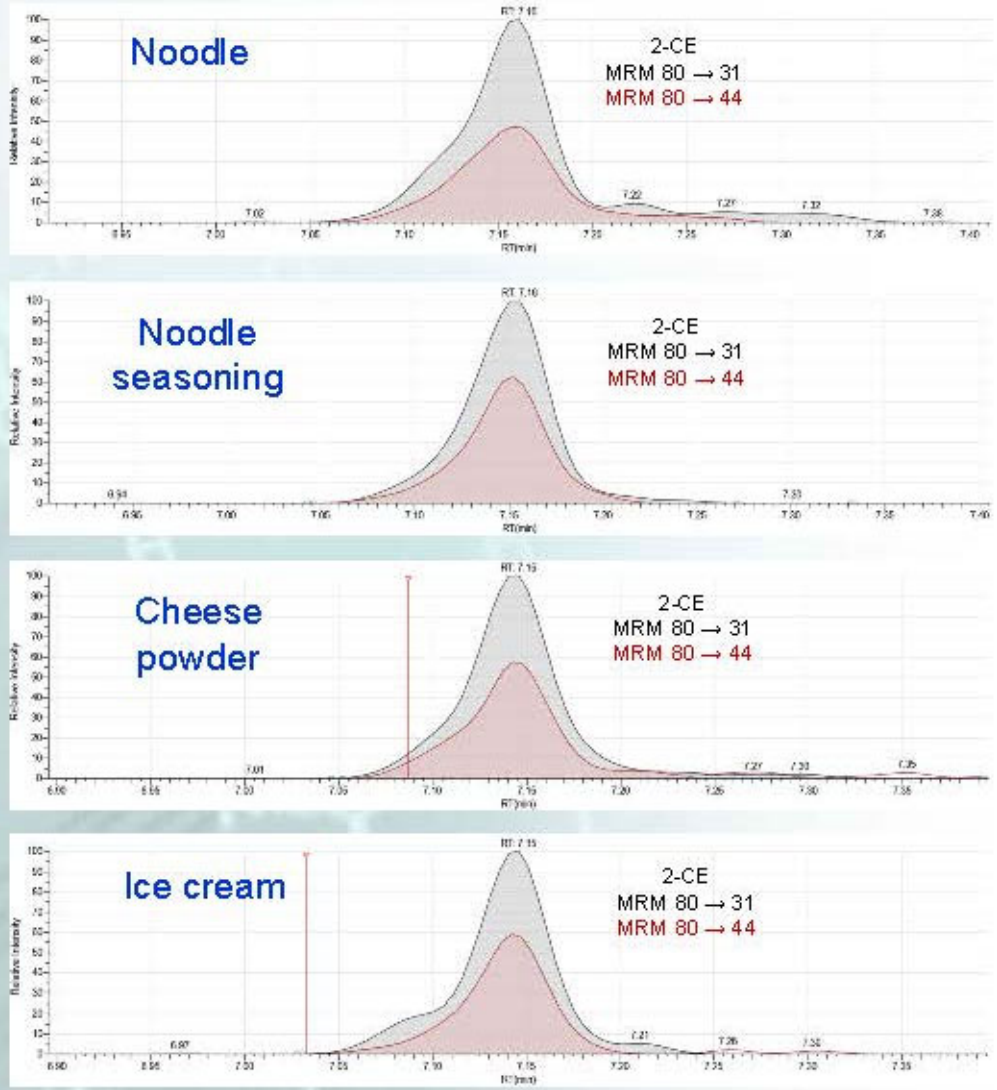




Recovery Study

Matrix	Spike Level (mg/kg)	Recovery of 2-CE (%)		Recovery of EO (%)
		Average	RSD % (n=3)	
Noodle	0.1	93.8	9.2	73.9
Noodle seasoning	0.1	95.9	11.7	75.6
Cheese powder	0.1	95.4	6.9	79.6
Ice cream	0.1	100.4	8.2	77.9

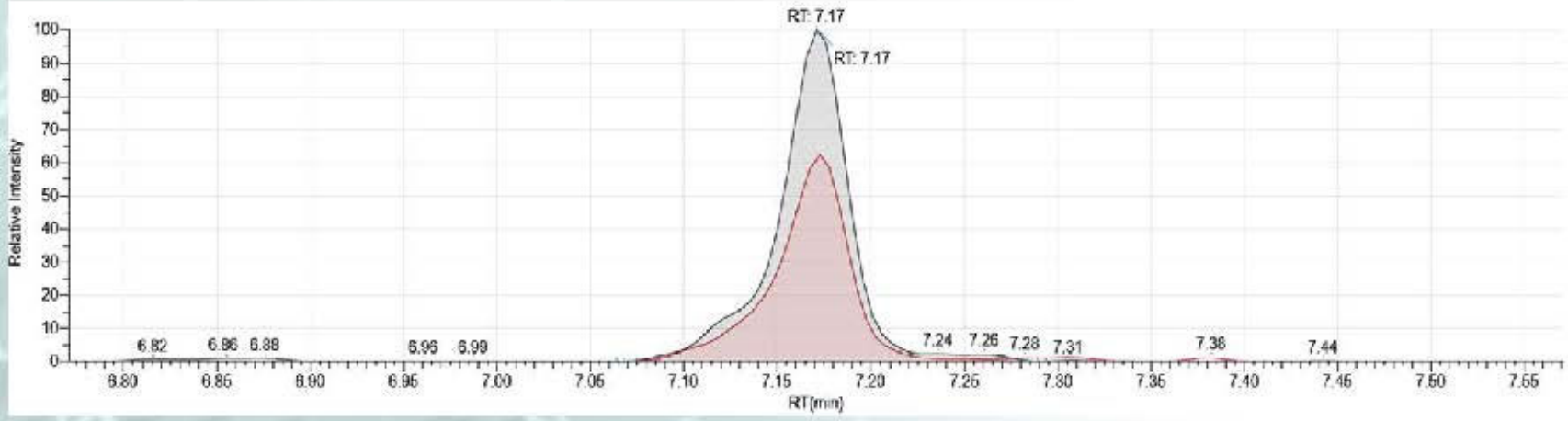
Sensitivity of 2-CE
MRM chromatograms of 0.05 mg/kg spike



Fapas® – Food Chemistry Proficiency Test Report 19368

Ethylene Oxide in Spice (cumin)

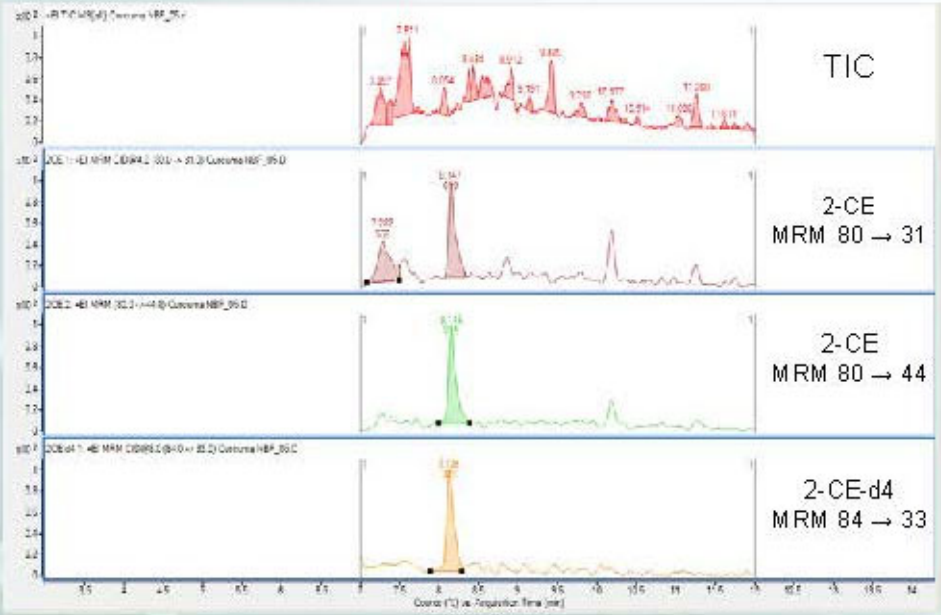
May-June 2023

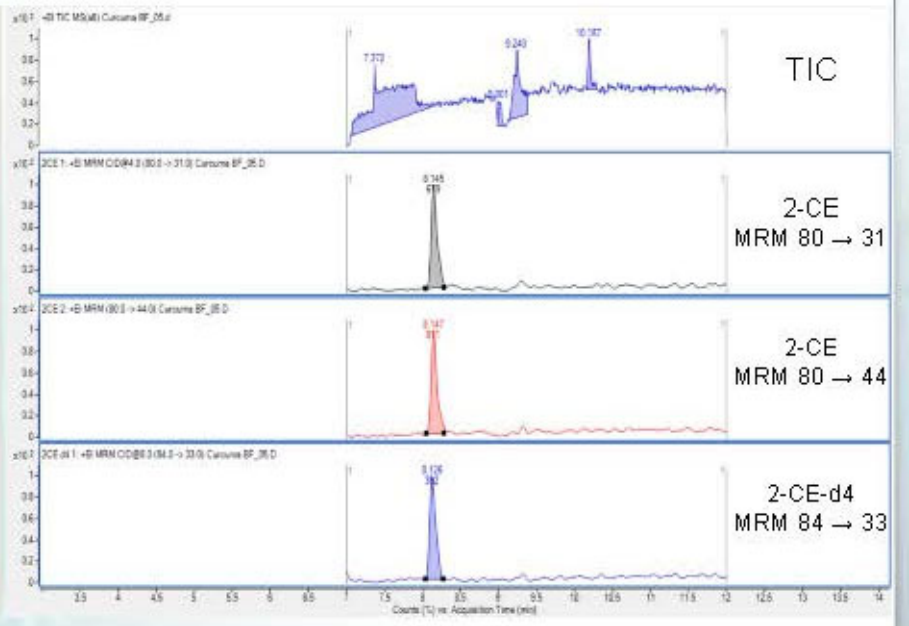


Laboratory result: 461 µg/kg (n =2)

[Assigned value: 474 ± 169.6 µg/kg]

Curcuma sample
(Pre-column Backflushing)





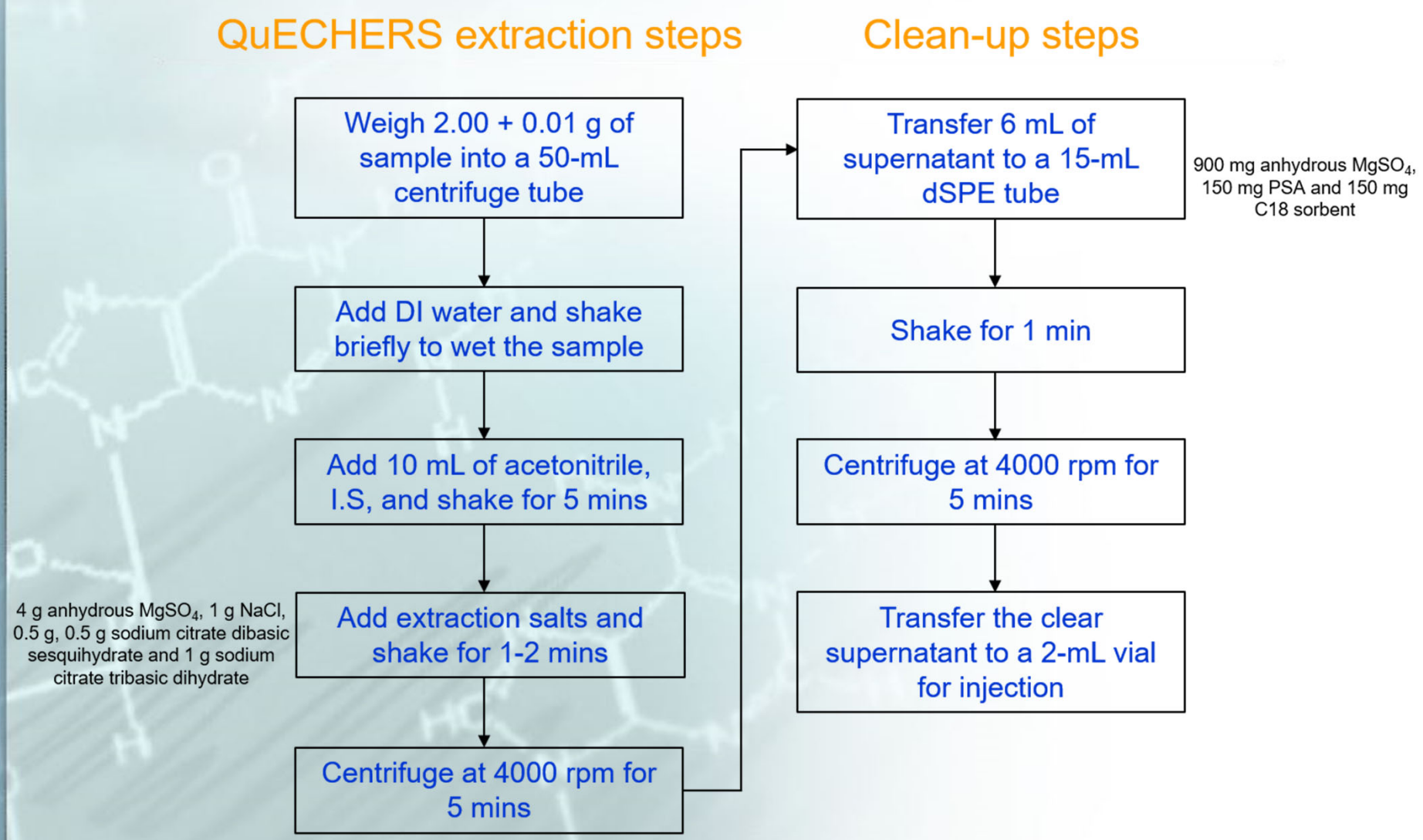
Without Backflushing

Backflushing
Pre-column = 5 m
Backflush time = 5 min

Method 2
Simultaneous detection of EO and 2-CE

Ref.: EURL-SRM “Analysis of Ethylene Oxide and its Metabolite 2-Chloroethanol by the QuOil or the QuEChERS Method and GC-MS/MS”, Version 1.1 (December 2020)

QuECHERS workflow for sample extraction and clean-up

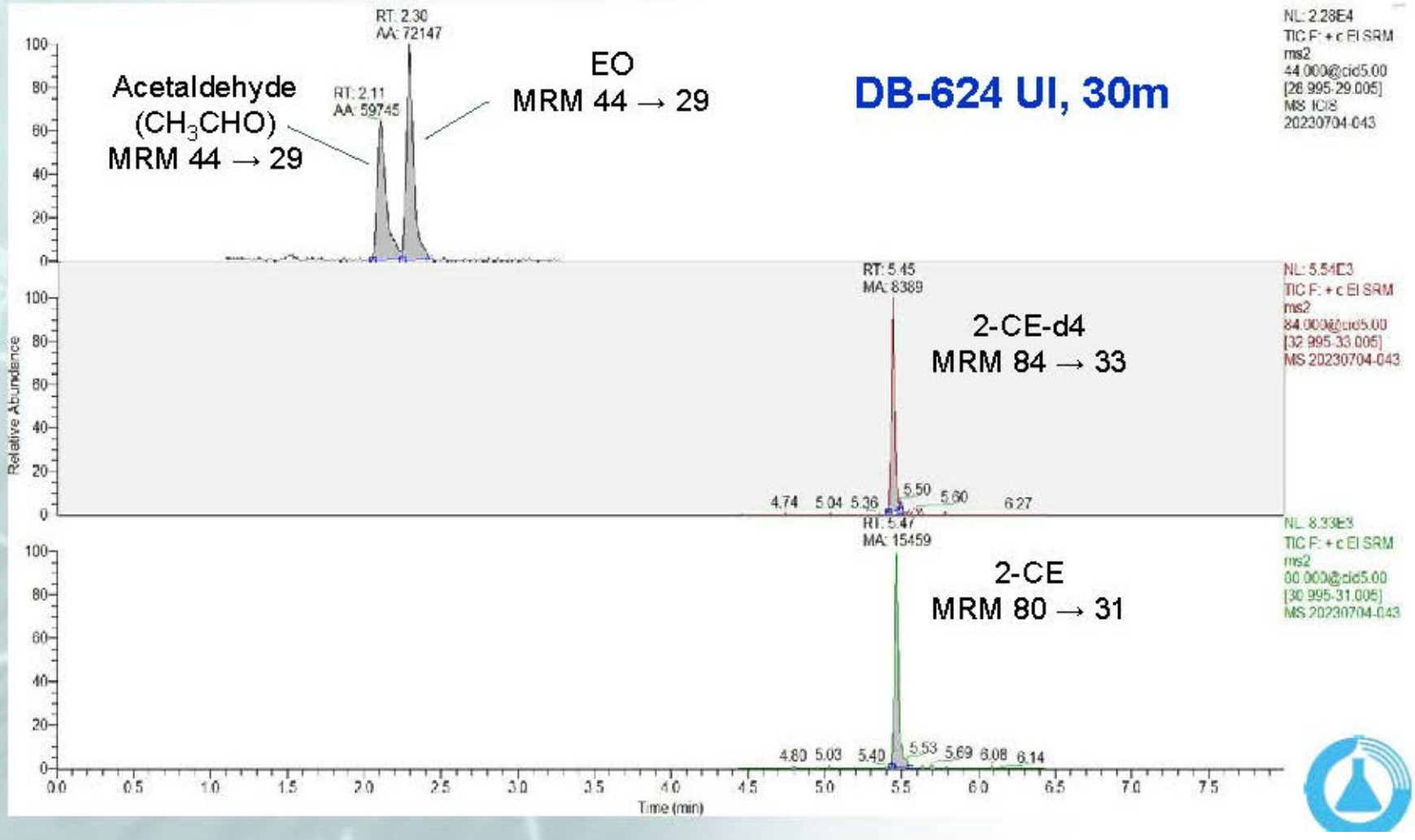


Instrumental Analysis

GC conditions	
Column	DB-624 UI (30 m or 60 m x 0.25 mm, 1.4 µm)
Inlet	Programmable Temperature Vaporizer (PTV) (Injection volume 2 µL)
Injection mode	Split (1:5)
Inlet temp	90 °C (0.8 min), ramp with 12 °C/s to 250 °C (10 min)
Oven gradient	45 °C (2 min) ramp with 25 °C/min to 250 °C (5 min)
Carries gas	Helium, 1 mL/min

MSD conditions		
Ionization mode	Electron impact (EI)	
Ion source temp	270 °C	
Transfer line temp	250 °C	
MRM transitions	EO	44 --> 29 (CE 5) 44 --> 28 (CE 5)
	2-CE	80 --> 31 (CE 5) 82 --> 31 (CE 5) 80 --> 43 (CE 5) 80 --> 44 (CE 1)
	2-CE-d4	84 --> 33 (CE 5)

Chromatograms of EO and 2-CE at 100 ng/ml standard solution



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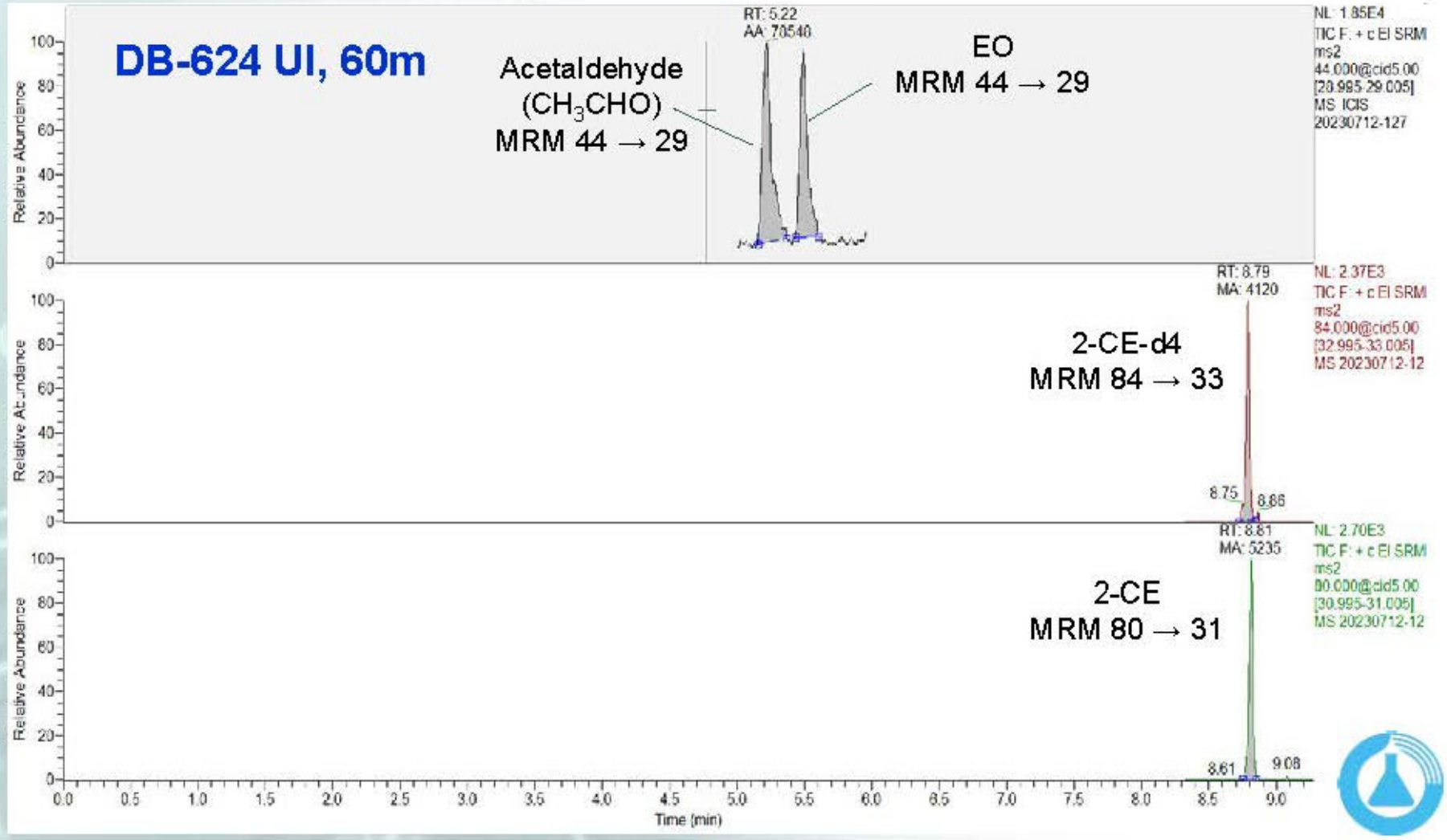

Notes for Consideration

- Take necessary precaution in handling highly volatile EO standard solutions
- Sample vial tray equipped with cooling to sub-ambient temperature capabilities prior to GC injection
- Smarter choice for GC column to prevent EO from other matrix interfering compounds


Thank You!