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Method of Test of Ethylene Oxide and its Reaction Product, 2-Chloroethanol, in Foods

1. Scope

This method is applicable for the determination of ethylene oxide and its reaction product, 2-chloroethanol(Note 1), in guar gum, carob gum (locust bean gum), empty capsules for food used, sesame, foods with high oil/fat content, spice plants and herbs, and ice cream.

Note 1: Ethylene oxide is a flammable gas, and it will rapidly react with chloride or chloride ions from the environment to yield 2-chloroethanol. Therefore, this method is to determine the total amount of ethylene oxide and 2-chloroethanol expressed as ethylene oxide.

2. Method

After extraction and purification, ethylene oxide and 2-chloroethanol are determined by gas chromatography/tandem mass spectrometry (GC-MS/MS).

2.1. Equipment

2.1.1. Gas chromatograph/tandem mass spectrometer

2.1.1.1. Ion source: electron ionization, EI.

2.1.1.2. Column: DB-624 UI capillary column, 1.4 μm, 0.25 mm × 60 m, or an equivalent product.

2.1.2. Vortex mixer.

2.1.3. High speed dispersing device: SPEX SamplePrep 2010 GenoGrinder®, > 1000 rpm, or other mechanical shaker.

2.1.4. Centrifuge: centrifugal force > 5000 ×g, temperature control < 10°C.

2.1.5. Grinder.

2.2. Chemicals

Acetonitrile, HPLC grade;
Sodium chloride, AR grade;
Sodium citrate, AR grade;
Disodium hydrogen citrate, AR grade;
Magnesium sulfate anhydrous, AR grade;
Primary secondary amine (PSA), AR grade;
Octadecysilane, end-capped (C18 EC), AR grade;
Deionized water, HPLC quality, resistivity ≥ 18 MΩ•cm (at 25°C);
Ethylene oxide, 1000 μg/mL in toluene, reference standard;
2-Chloroethanol, reference standard;

2-Chloroethanol-d_4, internal standard.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Membrane filter: 0.22 μm, PTFE.

2.3.3. Volumetric flask: 1 mL and 10 mL.

2.3.4. Ceramic homogenizer:
Bond Elut QuEChERS P/N 5982-9312, or an equivalent product.

2.3.5. Extraction powder(note 2): containing 4 g of magnesium sulfate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate and 0.5 g of disodium hydrogen citrate.

2.3.6. Clean-up centrifuge tube I(note 2): containing 150 mg of PSA and 900 mg of magnesium sulfate anhydrous, 6 mL, used for guar gum, carob gum (locust bean gum) and empty capsules for food used.

2.3.7. Clean-up centrifuge tube II(Note 2): containing 150 mg of PSA, 900 mg of magnesium sulfate anhydrous and 150 mg of C18, 6 mL, used for ice cream, sesame and foods with high fat/oil content.

2.3.8. Clean-up centrifuge tube III(Note 2): containing 150 mg of PSA, 855 mg of magnesium sulfate anhydrous and 45 mg of GCB, 6 mL, used for spice plants and herbs with high pigment content.

Note 2: Commercial clean-up kits can be used as needed.

2.4. 90% acetonitrile

Mix 450 mL of acetonitrile and 50 mL of deionized water.

2.5. Internal standard solution preparation

Transfer about 10 mg of 2-chloroethanol-d_4 internal standard accurately weighed into a 10-mL volumetric flask, dissolve and dilute to volume with acetonitrile as the internal standard stock solution. Store in a freezer in the dark. When to use, dilute appropriate amount of the internal standard stock solution with acetonitrile to 50 μg/mL as the internal standard solution.

2.6. Standard solution preparation

Accurately transfer 10 μL of ethylene oxide reference standard to a 1-mL volumetric flask, and dilute to volume with acetonitrile; transfer 10 mg of 2-chloroethanol reference standard accurately weighed to a 10-mL volumetric flask, dissolve and dilute to volume with acetonitrile as the standard stock solutions. Store in a freezer in the dark. When to use, take appropriate amount

of ethylene oxide standard stock solution, and dilute with acetonitrile to 0.1-1.0 μg/mL; mix appropriate amount of 2-chloroethanol standard stock solution and the internal standard solution, and dilute with acetonitrile to 0.02-0.2 μg/mL (containing 0.05 μg/mL internal standard) as the standard solutions.

2.7. Sample solution preparation

2.7.1 Guar gum and carob gum (locust bean gum)

Transfer about 2 g of the well-mixed sample accurately weighed into a centrifuge tube, add 10 mL of 90% acetonitrile, 10 μL of the internal standard solution and 1 granule of a ceramic homogenizer. Shake at 1000 rpm by the high speed dispersing device for 2 min, and centrifuge at 5000 ×g for 3 min at 10°C. Transfer 6 mL of the supernatant to a clean-up centrifuge tube I, shake at 1000 rpm by the high speed dispersing device for 1 min, and centrifuge at 5000 ×g for 3 min at 10°C. Take the supernatant, and filter with a membrane filter. Take the filtrate as the sample solution.

2.7.2 Empty capsules for food used

Homogenize the sample with dry ice. After the dry ice sublimating completely in the sample, transfer about 2 g of the hydroxypropyl methylcellulose (HPMC) capsule sample or the gelatin capsule sample accurately weighed into a centrifuge tube, add 10 mL of acetonitrile for the HPMC capsule sample (add 10 mL of 90% acetonitrile for the gelatin capsule sample), 10 μL of the internal standard solution and 1 granule of a ceramic homogenizer. Shake at 1000 rpm by the high speed dispersing device for 2 min, and centrifuge at 5000 ×g for 3 min at 10°C. Transfer 5 mL of the supernatant to a clean-up centrifuge tube I, shake at 1000 rpm by the high speed dispersing device for 1 min, and centrifuge at 5000 ×g for 3 min at 10°C. Take the supernatant, and filter with a membrane filter. Take the filtrate as the sample solution.

2.7.3 Sesame and foods with high fat/oil content

Transfer about 2 g of the well-mixed sample accurately weighed into a centrifuge tube, add 10 mL of 90% acetonitrile, 10 μL of the internal standard solution and 1 granule of a ceramic homogenizer. Shake at 1000 rpm by the high speed dispersing device for 30 min, and centrifuge at 5000 ×g for 3 min at 10°C. Transfer 6 mL of the supernatant to a clean-up

centrifuge tube II, shake at 1000 rpm by the high speed dispersing device for 1 min, and centrifuge at 5000 ×g for 3 min at 10°C. Take the supernatant, and filter with a membrane filter. Take the filtrate as the sample solution.

2.7.4 Spice plants and herbs with high pigment content

Transfer about 2 g of the well-mixed sample accurately weighed into a centrifuge tube, add 10 mL of 90% acetonitrile, 10 μL of the internal standard solution and 1 granule of a ceramic homogenizer. Shake at 1000 rpm by the high speed dispersing device for 10 min, and centrifuge at 5000 ×g for 3 min at 10°C. Transfer 6 mL of the supernatant to a clean-up centrifuge tube III, shake at 1000 rpm by the high speed dispersing device for 1 min, and centrifuge at 5000 ×g for 3 min at 10°C. Take the supernatant, and filter with a membrane filter. Take the filtrate as the sample solution.

2.7.5 Ice cream and foods with high water content

Transfer about 10 g of the well-mixed sample accurately weighed into a centrifuge tube, add 10 mL of acetonitrile, 10 μL of the internal standard solution and 1 granule of a ceramic homogenizer, and mix well. Add the extraction powder, cap the centrifuge tube, shake vigorously several times by hands to prevent coagulation of salt, and then shake at 1000 rpm by the high speed dispersing device for 1 min. Centrifuge at 5000 ×g for 3 min at 10°C and transfer 6 mL of the supernatant to a clean-up centrifuge tube II, shake at 1000 rpm by the high speed dispersing device for 1 min, and centrifuge at 5000 ×g for 3 min at 10°C. Take the supernatant, and filter with a membrane filter. Take the filtrate as the sample solution.

2.8. Standard curve preparation

Accurately inject 2 μL of the standard solutions into GC-MS/MS separately, and operate according to the following conditions. Establish the standard curve of ethylene oxide by peak areas of ethylene oxide vs. the added concentrations (0.1-1.0 μg/mL), and that of 2-chloroethanol by the ratios of the peak area of 2-chloroethanol to that of the internal standard vs. the added concentrations (0.02-0.2 μg/mL).

GC-MS/MS operating conditions(Note 3):

Column: DB-624 UI capillary column, 1.4 μm, 0.25 mm × 60 m.

Column temperature:
initial temperature: 40°C, hold for 5 min;
temperature rising rate: 30°C/min;
final temperature: 240°C, hold for 9 min.
Carrier gas flow rate: helium, 1 mL/min.
Injector temperature: 220°C.
Injection mode: split, 3:1.
Injection volume: 2 μL.
Ion source: EI, 70 eV.
Ion source temperature: 230°C.
Detection mode: multiple reaction monitoring (MRM). Detection ion pair and collision energy are shown as follows.

Analyte	Ion pair Precursor ion (*m/z*) > product ion (*m/z*)	Collision energy (eV)
Ethylene oxide	44 > 29*	5
2-Chloroethanol	80 > 44*	0
	80 > 31	4
2-Chloroethanol-d_4 (I.S.)	84 > 33	5

*Quantitative ion pair.

Note 3: All the parameters can be adjusted depending on the instrument used if the above conditions are not applicable.

2.9. Identification and quantification

Accurately inject 2 μL of the sample solution and the standard solutions into GC-MS/MS separately, and operate according to the conditions in section 2.8. Identify analytes based on the retention time and the relative ion intensities (Note 4,5). Calculate the amount of ethylene oxide (sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide) in the sample by the following formula:

$$\text{The amount of ethylene oxide in the sample (mg/kg)} = \frac{\sum[C \times V \times F]}{M}$$

Where,

C：the concentration of ethylene oxide or 2-chloroethanol in the sample solution calculated by the standard curve (μg/mL)

V：the volume of 90% acetonitrile or acetonitrile for sample extraction (10 mL)

M：the weight of the sample (g)

F：the conversion factor of ethylene oxide

ethylene oxide: 1

2-chloroethanol: 0.55

Note 4: Relative ion intensities are calculated by peaks areas of quantitative ions divided by peak areas of qualitative ions (≤ 100%). Maximum permitted tolerances of relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20～50	± 25
> 10～20	± 30
≤ 10	± 50

Note 5: Identify ethylene oxide based on the retention time as it has only one detected ion pair. However, when ethylene oxide is detected, 2-chloroethanol should be detected at the same time.

Remark

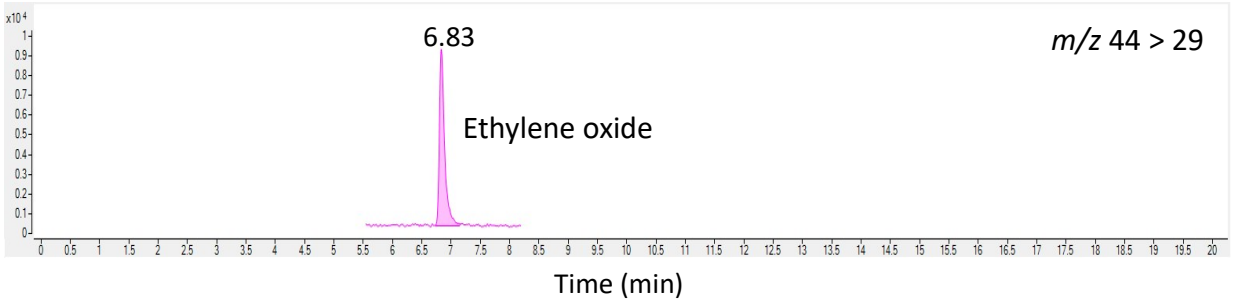
1. Limit of quantification (LOQ) for ethylene oxide (sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide) is 0.1 mg/kg.
2. Traces of 2-chloroethanol may be present in the environment. If there is any doubt concerning the analytical results, comprehensively judge the result based on source investigation or supporting information.
3. Acetaldehyde and ethylene oxide are isomers, and acetaldehyde may naturally exist in some samples, so the chromatographic conditions used should separate these two compounds.
4. Due to the diversity of food types and compositions, if the matrix effect significantly affects the quantitative result of ethylene oxide, the matrix-matched calibration curve or the standard addition method can be used for quantification.
5. Further validation should be performed when interference compounds appear in samples.

Reference

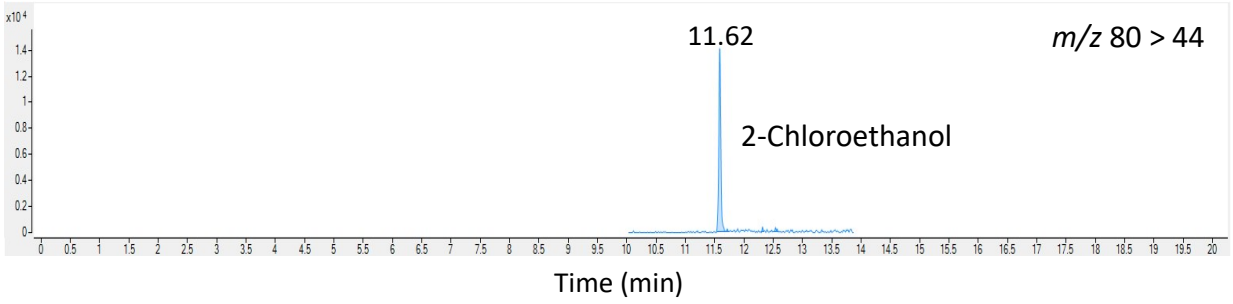
1. EU Reference Laboratory for Pesticides Requiring Single Residue Methods. 2020. Analysis of ethylene oxide and its metabolite 2-chloroethanol by the QuOil or the QuEChERS method and GC-MS/MS. EURL-SRM Analytical Observations Report (version 1.1).
2. The Commission of the European Communities. 2002. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Commission Decision 2002/657/EC.
[https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002D0657-20020817&from=EN]

Reference chromatograms

(A) Ethylene oxide



(B) 2-Chloroethanol



(C) 2-Chloroethanol-d_4

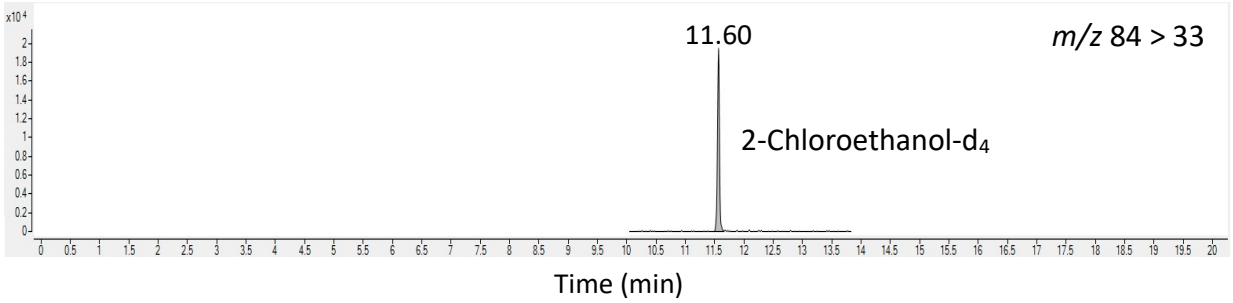


Figure. MRM chromatograms of ethylene oxide (A), 2-chloroethanol standard (B) and 2-chloroethanol-d_4 internal standard (C) analyzed by GC-MS/MS.