

4209 Determination of Ethylene Oxide for Pharmaceutical Packaging Materials and Containers

This method applies to the determination of residual ethylene oxide in pharmaceutical packaging materials and containers sterilized by ethylene oxide.

In this method, the ethylene oxide contained in test samples is extracted with water at a certain temperature and the content of ethylene oxide is determined by head-space gas chromatography.

Carry out the method for gas chromatography <0521>.

Column

Capillary columns available to meet the separation requirements can be used.

System Suitability

(1) The number of theoretical plates of the column is generally not less than 5000, calculated with reference to the peak of ethylene oxide.

(2) In the chromatogram, the resolution factor between the peaks of ethylene oxide and its neighbor substance shall be greater than 1.5.

(3) The relative standard deviation (RSD) of the peak areas of ethylene oxide for 5 replicate injections of the reference solution shall not be greater than 10%.

(4) The detection limit or quantification limit of the instrument shall not be lower than the limit.

Preparation of test solution

The preparation of the test solution shall be carried out immediately after sampling. The preparation method of test solution is as follows:

Method 1: Cut the test sample into pieces not exceeding 5mm, weigh 1.0g, place in a 20ml headspace vial, then add accurately 5ml of water, then seal immediately.

Method 2: Fill the container type test sample to the labeled filling volume with water, then maintain at 37 ± 1 for 1 hour to obtain the test solution. Measure accurately 5ml of the test solution into a 20ml headspace vial, then seal immediately.

Determination

Method 1 is preferably applied for testing. When the results of *method 1* do not comply with the requirements, *method 2* shall be used for retest or determination.

Method 1 (External standard method)

Chromatographic conditions: Use a capillary column (30m×0.25mm×1.4μm) coated with 6% cyanopropylphenyl-94% dimethylpolysiloxane as the stationary phase or an equivalent chromatographic column with the similar polarity coating; the initial temperature of column is 50, maintain the temperature for 10 minutes, the temperature of the injection port is 200 and that of the FID detector is 250, use high purity nitrogen or high purity helium as the carrier gas with a flow rate of 1.5ml/min.

Preparation of reference solution: The concentration of the reference solution shall be established based on the actual content of the ethylene oxide in the test solution.

40 In general, the chromatographic peak area of the reference solution should not exceed
41 twice of the corresponding chromatographic the peak area in the test solution. Dissolve
42 a quantity of ethylene oxide CRS in water, and dilute quantitatively and stepwise to
43 obtain the desired concentration of reference solution. Measure accurately 5ml of the
44 reference solution into a 20ml headspace vial, then seal immediately.

45 **Determination:** Equilibrate the reference solution and test solution at $60^{\circ}\text{C}\pm 1^{\circ}\text{C}$
46 for 40 minutes. Inject separately 1ml of the gaseous phase, and determine the peak area
47 of ethylene oxide. Perform separately a minimum of 2 parallel measurements of the
48 reference solution and test solution. Calculate the concentration of ethylene oxide in
49 test solution with respect to the peak area obtained in the chromatogram by external
50 standard method. Calculate the content of residual ethylene oxide in the test sample by
51 the following expressions.

52 Calculate the residual ethylene oxide by the following expression when using
53 method 1 for the preparation of test solution:

$$54 \quad X = \frac{5c}{m}$$

55 where X is the residual ethylene oxide in the test sample, $\mu\text{g/g}$;

56 5 is the volume of the test solution, ml;

57 c is the concentration of ethylene oxide in the test solution, $\mu\text{g/ml}$;

58 m is the weight of the test sample.

59 Calculate the residual ethylene oxide by the following expression when using
60 method 2 for the preparation of test solution:

$$61 \quad X = Vc$$

62 where X is the residual ethylene oxide in each test sample, μg ;

63 c is the concentration of ethylene oxide in the test solution, $\mu\text{g/ml}$;

64 **Method 2 (Calibration Curve Method)**

65 **Chromatographic conditions:** Refer to *method 1*.

66 **Preparation of a series of reference solutions:** The concentration range of the
67 series of reference solutions shall include the concentration of residual ethylene oxide
68 in the test sample. Dissolve a quantity of ethylene oxide CRS in water, and dilute
69 quantitatively and stepwise to produce a series of reference solutions with a containing
70 concentration of $0.4\sim 20\mu\text{g/ml}$. A minimum of 5 concentrations shall be included (if
71 necessary, the linear range can be adjusted based on the actual situation of the sample),
72 and the linear coefficient r is not less than 0.995. Measure accurately 5ml of each of the
73 reference solutions into a 20ml headspace vial, then seal immediately.

74 **Determination:** Equilibrate the reference solution and test solution at $60^{\circ}\text{C}\pm 1^{\circ}\text{C}$
75 for 40 minutes. Inject separately 1ml of the gaseous phase into the chromatogram, and
76 determine the peak area of ethylene oxide. Perform separately a minimum of 2 parallel
77 measurements of the reference solution and test solution. Calculate the regression
78 equation by using the concentration of reference solutions as the abscissa and the peak
79 areas correspondingly as the ordinate. Calculate the ethylene oxide content in the test
80 solution using the calibration curve, and calculate the content of residual ethylene oxide

81 in the sample by the same expressions as *method 1*.

82 **Preparation of reference solution:** The concentration of the reference solution
83 shall be such that accurate characterization can be achieved. Dissolve a quantity of
84 ethylene oxide CRS in water, and dilute quantitatively and stepwise to obtain the
85 desired concentration of reference solution. Measure accurately 5ml of the reference
86 solution into a 20ml headspace vial, then seal immediately.

87 **Determination:** Equilibrate the reference solution and test solution at $60^{\circ}\text{C}\pm 1^{\circ}\text{C}$
88 for 40 minutes. Inject separately 1ml of the gaseous phase into the chromatogram, and
89 record the mass spectrum. Compare the obtained mass spectrum with the standard mass
90 spectrum in the NIST library, and qualitatively determine whether it is ethylene oxide
91 based on the consistency with the retention time of the reference substance.

92 **[Notes]** (1) The portion of the test sample with a high residual amount of ethylene
93 oxide residue is preferably taken for the preparation of test solution, such as polymer
94 materials. In general, the residual amount of ethylene oxide in metals and glass is
95 relatively small.

96 (2) If manual injection is used in the assay, the injector shall be preheated to the
97 same temperature as the reference solution and test solution.

98 (3) The chromatographic conditions used shall ensure complete separation of
99 impurities and ethylene oxide in the test sample, such as ensuring complete separation
100 of ethylene oxide and acetaldehyde.

101 (4) If there are interference peaks in the chromatogram of the test solution or it can
102 not be determined whether it is the target substance when measured by *method 1* and
103 *method 2*, the following gas chromatography-mass spectrometry method can be used
104 as a reference for qualitative verification of ethylene oxide. For qualitative purpose, the
105 sampling amount can be appropriately increased if necessary.

106 Recommended chromatographic conditions: Use a capillary column
107 (60m \times 0.25mm \times 1.4 μm) coated with 6% cyanopropylphenyl-94%
108 dimethylpolysiloxane as the stationary phase or an equivalent chromatographic column
109 with the similar polarity coating; the initial temperature of column is 35 $^{\circ}\text{C}$, maintain the
110 temperature for 10 minutes, use high purity helium as the carrier gas with a flow rate
111 of 1.0ml/min, the temperature of the injection port is 200 $^{\circ}\text{C}$, use mass spectrometry as
112 detector.

113 Recommended mass spectrometry conditions: Use electron impact ionization (EI)
114 as the ion source, the ionization intensity is 70eV, use full scan mode for determination
115 with a mass range of 29~300m/z, the solvent delay time is 5 minutes.

起草单位: 山东省医疗器械和药品包装检验研究院

联系电话: 0531-82682912

参与单位: 上海市食品药品包装材料测试所、深圳市食品药品检验研究院