GCMTI RD-2:2020

Determination of Borneol in Chinese Medicinal Oil for External Use by Gas Chromatography

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Safety precautions: This method involves the use of hazardous materials. It is the user's responsibility to apply appropriate precaution when handling such materials. Use eye and hand protection and where necessary carry out the work in a fume cupboard.

1   Introduction

This method specifies the procedures for the determination of borneol, either natural borneol or synthetic borneol, in Chinese medicinal oil for external use. The sample is extracted and diluted with ethanol. Borneol[1] is qualitatively and quantitatively determined using gas chromatography with flame ionization detector (GC-FID) with internal standard calibration. [2]

This method is applicable to Chinese medicinal oil containing borneol with the lowest applicable level as 10 mg/g.

2   Reagents

Use reagents of analytical grade or equivalent unless otherwise specified.

2.1 Ethanol

2.2 Helium, at least 99.999 %

2.3 Internal standard, naphthalene, at least 95 %

2.4 Borneol reference standard, at least 95 %

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[1]   The method could not be used to distinguish between natural borneol (*d*-borneol) and synthetic borneol (isomers of borneol).

[2]   The method is intended to provide a reliable analytical method that can be used as quality control method for Chinese medicinal oil containing turpentine oil, eucalyptus oil, camphor, menthol, methyl salicylate and/or borneol as major active ingredients. It is the user's responsibility to assess the suitability of the Chinese medicinal oil products when adopting this method, especially whether other ingredient(s) or excipient(s) contain any of the 6 chemical markers of choice as well as the existence of other herbal material(s)/herbal material extract(s).

2.5 Standard stock solution, Std-Stock (10000 mg/L)

Weigh accurately about 100 mg of borneol reference standard (2.4) in a 10-mL volumetric flask (3.2) and make up to the mark with ethanol (2.1).

(There was no sign of degradation of Std-Stock when stored properly in room temperature for a period of 3 months.)

2.6 Naphthalene internal standard solution, Std-IS (10000 mg/L)

Weigh accurately about 100 mg of naphthalene (2.3) in a 10-mL volumetric flask (3.2) and make up to the mark with ethanol (2.1).

2.7 Standard intermediate solution, Std-Int (400 mg/L)

Pipette 0.8 mL of Std-Stock (2.5) in a 20-mL volumetric flask (3.2) and make up to the mark with ethanol (2.1).

2.8 Calibration standard solutions, Std-AS

Pipette 0.2 mL of Std-IS (2.6) and 5 different volumes of Std-Int (2.7) in separate 10-mL volumetric flasks, dilute with ethanol to produce a series of 5 calibration standard solutions to cover a calibration range within 20 - 160 mg/L for borneol and 200 mg/L for naphthalene.

3 Apparatus

3.1 Analytical balance, capable of weighing to 0.1 mg

3.2 Volumetric flasks, 10 mL and 20 mL

3.3 Pipettes

3.4 Ultrasonic bath

3.5 PTFE membrane filters, 0.45 µm

3.6 Fused silica capillary column, with polyethylene glycol stationary phase, 30 m x 0.25 mm x 0.25 µm, or with (5%-Phenyl)-methylpolysiloxane stationary phase, 60 m x 0.25 mm x 0.25 µm

3.7 Gas chromatograph, equipped with flame ionization detector (GC-FID)

4   Procedure

4.1   Sample preparations

Weigh accurately about 0.1 g of sample into a 10-mL volumetric flask, dissolve (with the aid of ultrasonic bath if necessary) and make up to the mark with ethanol. Make further dilution with ethanol if necessary. Pipette 2 mL of the resulting solution and 0.2 mL of Std-IS (2.6) to a 10-mL volumetric flask and make up to the mark with ethanol. Filter through a 0.45um PTFE filter to obtain the test solution.

4.2   Gas chromatographic analysis

Set up the GC-FID system according to manufacturer's manuals. Inject the calibration standard solutions (2.8) and test solutions (4.1) to the GC-FID system with the following conditions using either one column:

Injection volume:1 µL
Injection mode: Split mode, split ratio 50:1
Column flow rate: 1.5 mL/min
Temperature program:

Column (3.6)	Polyethylene glycol stationary phase (30 m x 0.25 mm x 0.25 µm)	(5%-Phenyl)-methylpolysiloxane stationary phase [3] (60 m x 0.25 mm x 0.25 µm)
Temperature programme	40℃ for 15 min, then 20℃/min to 190℃ for 4 min (Total run time 26.5 min)	65℃ for 5 min, 1℃/min to 80℃ for 0 min, 20℃/min to 190℃ for 3 min (Total run time 28.5 min)

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[3]   Although interference peak was observed for pCms containing Peppermint oil (薄荷油) when using column of low polarity (i.e. (5%-Phenyl)-methylpolysiloxane stationary phase) for analysis, borneol in pCms containing Peppermint oil could be quantified by using column of high polarity (i.e. Polyethylene glycol stationary phase).

5 Calibration

Plot the peak area ratio (PAR) of borneol to naphthalene against the corresponding concentrations of Std-AS to obtain a 5-point calibration curve. Obtain the slope (m), y-intercept (I) and the square of correlation coefficient (r^2) from the calibration curve.

where

$$\text{Peak area ratio (PAR)} = \frac{\text{Peak area of borneol}}{\text{Peak area of internal standard}}$$

6 Identification and calculation

Calculate the relative retention time (RRT) of borneol by using the following equation –

$$\text{RRT} = \frac{\text{Retention time (RT) of borneol peak}}{\text{Retention time (RT) of naphthalene peak}}$$

Identify borneol peak in the chromatogram of the test solution by comparing the RRT with those in the chromatogram of the Std-AS. The RRT of borneol peak in the chromatogram of the test solution and the Std-AS should not differ by more than 0.5 %.

Calculate the concentration (in mg/L) of borneol in the test solution by using the following equation –

$$\text{Concentration of borneol} = \frac{\text{PAR} - \text{I}}{\text{m}}$$

where

        PAR = the peak area ratio of borneol in the test solution,

        I   = the y-intercept of the 5-point calibration curve,

        m   = the slope of the 5-point calibration curve.

Calculate the content of borneol (in mg/g) in the sample by using the following equation –

$$\text{Content (mg/g) of borneol in sample} = \frac{\text{C x V x D}}{1000 \text{ x W}}$$

where

        C = the concentration, in mg/L, of borneol in the test solution,

D = dilution factor,

V = the final make-up volume, in mL, of the test solution,

W = the weight, in g, of the sample used for the preparation of the test solution.

7 Reference

Commission, C. P. (2015). *Pharmacopoeia of the People's Republic of China* (Vol. Volume 1). Beijing: China Medical Science Press.

E. Gonzalez-Penas, M. Lopez-alvares, F. Martinez de Narvajas, A. Ursua Simultaneous. (2000). GC Determination of Turpentine, Camphor, Menthol and Methyl Salicylate in a Topical Analgesic Formulation (Dologex). *Chromatographia*(52), pp. 245-248.

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Annex

(normative)

External standard calibration

The concentration of borneol in the test solution can also be determined using external standard calibration instead of internal standard calibration. If external standard calibration is applied, Std-AS and test solution without addition of Std-IS could be used.

Plot the peak area (A) of borneol against the corresponding concentrations of Std-AS to obtain a 5-point calibration curve. Obtain the slope (m), y-intercept (I) and the square of correlation coefficient (r^2) from the calibration curve.

Calculate the concentration (in mg/L) of borneol in the test solution by using the following equation –

$$\text{Concentration of borneol} = \frac{A - I}{m}$$

where

      A   = the peak area of borneol in the test solution,

      I   = the y-intercept of the 5-point calibration curve,

      m   = the slope of the 5-point calibration curve.