GCMTI RD-1:2019

Determination of α-Pinene, Eucalyptol, Camphor, Menthol and Methyl Salicylate 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 5 chemical markers (α-pinene, eucalyptol, camphor, menthol and methyl salicylate) commonly found in Chinese medicinal oil for external use. The sample is extracted and diluted with ethanol. The chemical markers are qualitatively and quantitatively determined using gas chromatography with flame ionization detector (GC-FID) with internal standard calibration. [1]

This method is applicable to Chinese medicinal oil containing any of the 5 markers with the lowest applicable level as listed in the following table.

Marker	Lowest applicable level (mg/g)
α-Pinene	50
Eucalyptol	25
Camphor	50
Menthol	125
Methyl salicylate	125

2   Reagents

Use reagents of analytical grade or equivalent unless otherwise specified.

2.1 Ethanol

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[1]   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, and/or methyl salicylate 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 5 chemical markers of choice as well as the existence of other herbal material(s)/herbal material extract(s).

2.2 Helium, at least 99.999 %

2.3 Internal standard, naphthalene, at least 95 %

2.4 Reference standards, α-pinene, eucalyptol, camphor, menthol and methyl salicylate, at least 95 %

2.5 Individual standard stock solutions, Std-Stock (20000 mg/L)

Weigh accurately about 200 mg of individual reference standards (2.4) in 10-mL volumetric flasks (3.2) respectively and make up to the mark with ethanol (2.1).

(There were no signs of degradation of Std-Stock when stored properly in room temperature for a period of 6 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 Mixed standard intermediate solution, Std-Int (2000 mg/L for α-pinene and camphor; 1000 mg/L for eucalyptol; 5000 mg/L for menthol and methyl salicylate)

Pipette 2 mL of α-pinene and camphor Std-Stock, 1 mL of eucalyptol Std-Stock, 5 mL of menthol and methyl salicylate Std-Stock in a 20-mL volumetric flask (3.2) and make up to the mark with ethanol (2.1).

2.8 Mixed calibration standard solution, Std-AS

Pipette 0.2 mL of Std-IS (2.6) and 5 different volume 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 100 - 800 mg/L for α-pinene and camphor, 50 - 400 mg/L for eucalyptol, 250 - 2000 mg/L for menthol and methyl salicylate, 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 (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)

5   Calibration

Plot the peak area ratio (PAR) of α-pinene, eucalyptol, camphor, menthol and methyl salicylate to naphthalene against the corresponding concentrations of Std-AS to obtain 5-point calibration curve for each analyte. Obtain the slope (m), y-intercept (I) and the square of correlation coefficient (r^2) from the calibration curve.

where

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

6   Identification and calculation

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

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

Identify α-pinene, eucalyptol, camphor, menthol and methyl salicylate peaks in the chromatogram of the test solution by comparing their RRT with those in the chromatogram of the Std-AS. The RRT of α-pinene, eucalyptol, camphor, menthol and methyl salicylate peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 0.5 %.

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

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

where

PAR = the peak area ratio of the analyte 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 the analyte (in mg/g) in the sample by using the following equation –

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

where

C = the concentration, in mg/L, of the analyte 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.

国家食品药品监督管理局 国家药品标准. *活络油*. WS3-868(Z-166) – 2005 (Z) – 2009.

Annex

(normative)

External standard calibration

The concentration of analyte 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 α-pinene, eucalyptol, camphor, menthol and methyl salicylate against the corresponding concentrations of Std-AS to obtain 5-point calibration curve for each standard. 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 the analyte in the test solution by using the following equation –

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

where

> A = the peak area of the analyte in the test solution,
> I = the y-intercept of the 5-point calibration curve,
> m = the slope of the 5-point calibration curve.