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GCMTI method publications

Identification of Cinnamaldehyde, Citronellal, Eugenol, Linalool, Linalyl Acetate and Thymol 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 identification of 6 chemical markers (cinnamaldehyde, citronellal, eugenol, linalool, linalyl acetate and thymol) commonly found in Chinese medicinal oil for external use. The sample is extracted and diluted with ethanol. The chemical markers are qualitatively determined using gas chromatography – mass spectrometry (GC-MS) or gas chromatography – tandem mass spectrometry (GC-MS/MS). [1)]

This method is applicable to Chinese medicinal oil containing any of the 6 markers with the lowest applicable level as 5 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 Reference standards, cinnamaldehdye, citronellal, eugenol, linalool, linalyl acetate and thymol, at least 95 %

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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 with active ingredients containing one or more of the 6 chemical markers, such as cinnamon oil, citronella oil, clove oil, lavender oil and thyme oil. 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).

2.4 Individual standard stock solution I, Std-Stock I (10000 mg/L)

Weigh accurately about 100 mg of each of citronellal, eugenol, linalool, linalyl acetate and thymol reference standards (2.3) 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 I solutions when stored properly in room temperature for a period of 3 months.)

2.5 Individual standard stock solution II, Std-Stock II (2000 mg/L)

Weigh accurately about 20 mg of cinnamaldehyde reference standard (2.3) in a 10-mL volumetric flask (3.2) and make up to the mark with ethanol (2.1).

(Std-Stock II shall be freshly prepared.)

2.6 Mixed standard intermediate solution I, Std-Int I (500 mg/L)

Pipette 1 mL of each of citronellal, eugenol, linalool, linalyl acetate and thymol Std-Stock I and 5 mL of cinnamaldehyde Std-Stock II in a 20-mL volumetric flask (3.2) and make up to the mark with ethanol (2.1).

2.7 Mixed standard intermediate solution II, Std-Int II (10 mg/L)

Pipette 0.2 mL of Std-Int I (2.6) into a 10-mL volumetric flask (3.2) and make up to the mark with ethanol (2.1).

2.8 Control point standard solution, Std-Ctrl (0.5 mg/L)

Pipette 0.5 mL of Std-Int II (2.7) in a 10-mL volumetric flask (3.2) and make up to the mark with ethanol (2.1).

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

3.7 Gas chromatograph, equipped with mass selective detector (GC-MS) or tandem mass spectrometer (GC-MS/MS)

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 0.1 mL of the resulting solution 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 system according to manufacturer's manuals. Inject the Std-Ctrl (2.8) and test solution (4.1) to the GC-MS or GC-MS/MS system with the following conditions:

Injection volume: 1 μL
Injection mode: Split mode, split ratio 50:1
Column flow rate: 1.0 mL/min
Temperature program: 60℃ for 10 min, 15℃/min to 180℃ for 6 min, 40℃/min to 220℃ for 3 min (Total run time 28 min)

For GC-MS,

Marker	SIM ions (m/z)		
	Qualifier ion 1	Qualifier ion 2	Qualifier ion 3
Cinnamaldehyde	131	132	103
Citronellal	69	41	95
Eugenol	164	149	103
Linalool	71	93	80
Linalyl acetate	93	80	121

Thymol	135	150	91

For GC-MS/MS,

Marker	MRM transition		CE (eV)
	Precursor ion (m/z)	Daughter ion (m/z)	
Cinnamaldehyde	131	77	25
	131	103	10
Citronellal	95	55	15
	109	67	5
Eugenol	164	149	10
	164	131	10
Linalool	121	93	5
	121	77	20
Linalyl acetate	136	93	10
	136	121	10
Thymol	135	91	15
	150	135	10

5 Identification

The target analyte in sample is identified by comparing the RT, the signal response and relative abundances of the selected ions or ion pairs found in test solution with the control levels established by triplicate injections of Std-Ctrl. The followings shall be met for positive identification:

5.1 The deviation of RT (%) of target analyte found in sample shall not differ by 2% compared with the average RT of that analyte in Std-Ctrl.

5.2 The signal response of base peak of the selected ions or ion pairs of the target analyte found in sample is equal to or greater than 70% of the average signal response of that analyte in Std-Ctrl, and the signal-to-noise ratio of detected peak is greater than 5:1.

5.3 For GC-MS, compare the relative abundances of qualifier ions of target analyte found in sample with those obtained from standard solution. The relative intensities shall meet the following tolerances.

Relative intensity (% of base peak)	Maximum permitted tolerance
> 50%	± 10%
> 20 to 50%	± 15%
> 10 to 20%	± 20%
≤ 10%	± 50%

Note: Relative abundances of qualifier ions of standard solution refers to the average relative abundances of the triplicate injections of Std-Ctrl

5.4 For GC-MS/MS, compare the relative abundances of MRM ion pairs in sample with those obtained from standard solution. The relative intensities shall meet the following tolerances.

Relative intensity (% of base peak)	Maximum permitted tolerance
> 50%	± 20%
> 20 to 50%	± 25%
> 10 to 20%	± 30%
≤ 10%	± 50%

Note: Relative abundances of MRM ion pairs of standard refers to the average relative abundances of the triplicate injections of Std-Ctrl

6 Reference

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

国家食品药品监督管理局 国家葯品标准. 活絡油. WS3-868(Z-166) – 2005 (Z) – 2009.

Analytical Method to Quantify 57 Suspected Allergens (and Isomers) in Ready to Inject Fragrance Materials by Gas Chromatography and Mass spectrometry, The International Fragrance Association (2016).

經濟部標準檢驗局臺南分局《以氣相層析法檢測香茅油中香茅醛含量之探討》。2016 年 12 月。

李英、劉麗等：<頂空氣相色譜-質譜聯用法測定蠟燭中香茅油的含量>，《香料香精化妝品》，第 3 期(2005 年 6 月)，頁 22-24。

A. Cutillas, A Carrasco et al, Thyme Essential Oils from Spain: Aromatic Profile Ascertained by GC-MS, and Their Antioxidant, Antilipoxygenase and Antimicrobial Activities, Journal of Food and Drug Analysis, 26, 529-544 (2018).

Determination of 59 Potential Allergens in Perfumes by Twin-line Fast GCMSMS, Application News No. SCA_280_089, Shimadzu Europa GmbH (2018).

Commission Decision 2002/657/EC, Official Journal of the European Communities, L 221, 8-36 (2002).