GCMTI RD-2:2021

Determination of Menthol in Pei Pa Koa by Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography- Mass Spectrometry (GC-MS)

GCMTI method publications

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Safety Precaution: *This procedure involves carcinogenic chemicals, corrosive chemicals and flammable solvents. Apply precautions when handling such chemicals, for example: use eye and hand protection and where necessary carry out the work in a fume cupboard to avoid inhalation of vapour.*

1. Introduction

1.1. Pei Pa Koa is a prevalent proprietary Chinese medicine in China and Hong Kong. It is used for the relief of sore throat, coughs, hoarseness and aphonia. The formulations and production procedures of Pei Pa Koa are varied with manufacturers, commonly it was made through a procedure by continuously decocting the Chinese herbal medicines including the fritillary bulb (Bulbus fritillariae cirrhosae, 川貝母), loquat leaf (Eriobotrya japonica, 枇杷葉), pomelo peel (Citrus maxima, 化橘紅), chinese bellflower root (Platycodon grandiflorum, 桔梗), bitter apricot kernel (Prunus armeniaca, 苦杏仁), licorice root (Glycyrrhiza uralensis, 甘草) and Menthol (薄荷) , followed by addition of syrup and honey base in ethanol. The common chemical markers in these Chinese herbal medicines are as follows:

Name of Chinese Herbal Medicines	Name of Common Chemical markers
Bulbus fritillariae cirrhosae (川貝母)	Peimisine
Eriobotrya japonica (枇杷葉)	Oleanolic acid and ursolic acid
Citrus maxima (化橘紅)	Naringin
Platycodon grandiflorum (桔梗)	Platycodin D
Prunus armeniaca (苦杏仁)	Amygdalin
Glycyrrhiza uralensis (甘草)	Liquiritin and Glycyrrhizic acid
Menthol (薄荷)	Menthol

1.2. This method specifies the procedures for the determination of the menthol in Pei Pa Koa sample.

1.3. The sample is diluted with solvent. The chemical markers are qualitatively and/or quantitatively determined by Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography- Mass Spectrometry (GC-MS).

2. Reagents

Note: All reagents used should be of analytical reagent grade or equivalent unless otherwise specified.

2.1. GC-FID standard solution preparation

2.1.1. Individual menthol and naphthalene (internal standard) stock standard solutions (~10000 μg/mL)

Prepare individual stock standard solutions by weighing accurately about 100 mg of menthol and naphthalene into two separate 10-mL volumetric flasks respectively, dissolve and make up to the graduated mark with ethanol.

2.1.2. Intermediate menthol and naphthalene standard solution (~1000 μg/mL)

Prepare the intermediate standard solution by transferring 1 mL of individual stock standard solution in two separate 10-mL volumetric flask respectively and make up to the graduated mark with ethanol.

2.1.3. Calibration standards, CS1 – CS5

2.1.3.1. A series of calibration standard solutions of ~10, 20, 50, 100, 200 μg/mL are prepared by transferring an appropriate amount of intermediate menthol standard solution into 10-mL volumetric flasks and make up with ethanol. Suggested volumes of standard solutions used for the preparation are listed below.

Calibration standards	**Volume of intermediate menthol standard solution (mL)**	**Final Volume (mL)**	**Concentration of menthol working standard solution (μg/mL)**
CS1	0.1	10	10
CS2	0.2	10	20
CS3	0.5	10	50
CS4	1	10	100
CS5	2	10	200

2.1.3.2. Subsequently, pipette 1 mL of the resultant solution and 0.05 mL of intermediate naphthalene standard solution into a GC vial.

Calibration standards	Volume of menthol working standard solution (mL)	Volume of naphtha-ene solution (mL)	Final Volume in GC vial (mL)	Conc. of menthol (μg/mL)	Conc. of naphtha-ene (μg/mL)
CS1	1	0.05	1.05	~10	~50
CS2	1	0.05	1.05	~20	~50
CS3	1	0.05	1.05	~50	~50
CS4	1	0.05	1.05	~100	~50
CS5	1	0.05	1.05	~200	~50

2.1.4. Menthol stock ICV solution (~10000 μg/mL)

Prepare the menthol stock ICV solution by weighing accurately about 100 mg of menthol into a 10-mL volumetric flask, dissolve and make up to the graduated mark with ethanol.

2.1.5. Menthol intermediate ICV standard solution (~1000 μg/mL)

Prepare the menthol intermediate ICV standard solution by transferring 1 mL of menthol stock ICV solution into a 10-mL volumetric flask and make up to the graduated mark with ethanol.

2.1.6. ICV standard solution (~100 μg/mL)

Prepare the ICV standard solution by transferring 1 mL of menthol intermediate ICV standard solution into a 10-mL volumetric flask and make up to the graduated mark with ethanol. Subsequently, pipette 1 mL of the resultant solution and 0.05 mL of intermediate naphthalene standard solution into a GC vial, make up to the graduated mark with ethanol.

Standard	Volume of menthol standard solution (mL)	Volume of naphtha-ene solution (mL)	Final Volume in GC vial (mL)	Conc. of menthol (μg/mL)	Conc. of naphtha-ene (μg/mL)
ICV standard solution	1	0.05	1.05	~100	~50

2.1.7. Spike standard solution (~1000 μg/mL)

Prepare the spike standard solutions by transferring 1 mL of menthol stock ICV solution into a 10-mL volumetric flask and make up to the graduated mark with ethanol.

2.2. GC-MS standard solution preparation

2.2.1. Individual menthol and naphthalene (internal standard) stock standard solutions (~1000 μg/mL)

Prepare individual stock standard solutions by weighing accurately about 10 mg of menthol and naphthalene into two separate 10-mL volumetric flasks respectively, dissolve and make up to the graduated mark with ethanol.

2.2.2. Intermediate menthol standard solution (~10 μg/mL)

Prepare the intermediate menthol standard solution by transferring 0.1 mL of individual menthol stock standard solution in a 10-mL volumetric flask and make up to the graduated mark with ethanol.

2.2.3. Intermediate naphthalene standard solution (~5 μg/mL)

Prepare the intermediate naphthalene standard solution by transferring 0.05 mL of individual naphthalene stock standard solution in a 10-mL volumetric flask and make up to the graduated mark with ethanol.

2.2.4. Calibration standards, CS1 – CS5

2.2.4.1. A series of calibration standard solutions of ~100, 250, 750, 1500, 3000 ng/mL are prepared by transferring an appropriate amount of intermediate menthol standard solution into 10-mL volumetric flasks and make up with ethanol. Suggested volumes of standard solutions used for the preparation are listed below.

Calibration standards	Volume of intermediate	Final Volume (mL)	Concentration of menthol

	menthol standard solution (mL)		**working standard solution (ng/mL)**
CS1	0.1	10	100
CS2	0.25	10	250
CS3	0.75	10	750
CS4	1.5	10	1500
CS5	3.0	10	3000

2.2.4.2. Subsequently, pipette 1 mL of the resultant solution and 0.05 mL of intermediate naphthalene standard solution into a GC vial.

Calibration standards	**Volume of menthol working standard solution (mL)**	**Volume of naphthal-ene solution (mL)**	**Final Volume in GC vial (mL)**	**Conc. of menthol (ng/mL)**	**Conc. of naphthal-ene (ng/mL)**
CS1	1	0.05	1.05	~100	~250
CS2	1	0.05	1.05	~250	~250
CS3	1	0.05	1.05	~750	~250
CS4	1	0.05	1.05	~1500	~250
CS5	1	0.05	1.05	~3000	~250

2.2.5. Menthol stock ICV solution (~1000 μg/mL)

Prepare the menthol stock ICV solution by weighing accurately about 10 mg of menthol into a 10-mL volumetric flask, dissolve and make up to the graduated mark with ethanol.

2.2.6. Menthol intermediate ICV standard solution (~10 μg/mL)

Prepare the menthol intermediate ICV standard solution by transferring 0.1 mL of menthol stock ICV solution into a 10-mL volumetric flask and make up to the graduated mark with ethanol.

2.2.7. ICV standard solution (~1500 ng/mL)

Prepare the ICV standard solution by transferring 1.5 mL of menthol intermediate ICV standard solution into a 10-mL volumetric flask and make up to the graduated mark with ethanol. Subsequently, pipette 1 mL of the resultant solution and 0.05 mL of intermediate naphthalene standard solution into a GC vial make up to the graduated mark with ethanol.

Standards	Volume of menthol standard solution (mL)	Volume of naphthalene solution (mL)	Final Volume in GC vial (mL)	Concentration of menthol (ng/mL)	Concentration of naphthalene (ng/mL)
ICV standard solution	1	0.05	1.05	~1500	~250

2.2.8. Spike standard solution (~120 µg/mL)

Prepare the spike standard solutions by transferring 1.2 mL of menthol stock ICV solution into a 10-mL volumetric flask and make up to the graduated mark with ethanol.

2.3. Distilled absolute ethanol, 99.5%.

2.4. Milli-Q water.

2.5. Solvent blank, absolute ethanol, 99.5%.

3. Apparatus

All glassware shall be rinsed with acetone and washed with detergent solution as soon as practicable after use. After detergent whing, glassware shall be rinsed immediately, firstly with acetone and then with water. The water rinse shall be followed by another two more rinses with acetone, respectively.

3.1. Volumetric flasks, 10-mL.

3.2. Auto pipettes, 300-µL, 1000-µL and 10000-µL.

3.3. Centrifuge tube, 15-mL.

3.4. Freezer, -20 °C.

3.5. Analytical balance, capable of weighing to 0.1 mg.

3.6. Ultrasonic bath.

3.7. PTFE membrane filters, 0.45 µm.

4. Procedures

4.1. GC-FID analysis

4.1.1. Sample preparation

4.1.1.1. Weigh accurately about 1.0 g of Pei Pa Koa sample into a 15-mL centrifuge tube.

4.1.1.2. Add 0.5 mL of Milli-Q water into the centrifuge tube. The sample is mixed by vortexing the centrifuge tube for 1 minute.

4.1.1.3. Add 9.5 mL of absolute ethanol into the centrifuge tube. The sample mixture is then sonicated in an ultrasonic bath for 20 minutes.

4.1.1.4. Store the sample mixture solution at -20 °C freezer for 30 minutes.

4.1.1.5. The sample mixture solution is centrifuged at 4000 rpm for 15 minutes. The supernatant solution is then filtered by 0.45μm PTFE membrane filter.

4.1.1.6. Pipette 1 mL of the sample solution into a GC vial. Add 50 μL of naphthalene intermediate solution (as internal standard) into the same GC vial.

4.1.1.7. Mix well prior to GC-FID analysis.

4.1.1.8 Inject 3 successive solvent blanks after each injection of sample.

4.1.2. GC-FID analysis

4.1.2.1. Operate the GC-FID system in accordance with the instrument manual. Analyse the samples with the following suggested conditions. It may be necessary to modify the operation conditions for optimum signal output.

4.1.2.2. Suggested GC conditions:

GC column	:	Restek Stabilwax-MS, 30 m x 0.25 mm x 0.25 µm or equivalent
Injector temperature	:	260℃
Injection volume	:	1 µL
Injection mode	:	Split mode, split ratio 20:1
Carrier gas	:	He, 99.999% purity
Column flow rate	:	1.5 mL/min
Temperature program	:	60 °C, hold 1 min; Ramp to 190°C at 15 °C /min, hold 0 min; Ramp to 240 °C at 45 °C /min, hold 3 mins;

4.1.2.3. Suggested FID conditions:

FID detector temperature	:	260 °C
Air flow	:	350 mL/min
Hydrogen flow	:	35 mL/min
Makeup gas flow	:	40 mL/min

4.2. GC-MS analysis

4.2.1. Sample preparation

4.2.1.1. Weigh accurately about 0.2 g of Pei Pa Koa sample into a 15-mL centrifuge tube.

4.2.1.2. Add 0.5 mL of Milli-Q water into the centrifuge tube. The sample is mixed by vortexing the centrifuge tube for 1 minute.

4.2.1.3. Add 9.5 mL of absolute ethanol into the centrifuge tube. The sample mixture is then sonicated in the ultrasonic bath for 20 minutes.

4.2.1.4. Store the sample mixture solution at -20 °C freezer for 30 minutes.

4.2.1.5. The sample mixture solution is centrifuged at 4000 rpm for 15 minutes. The supernatant solution is then filtered by 0.45µm PTFE membrane filter.

4.2.1.6. Transfer 2 mL of the sample solution into a 10-mL volumetric flask and make up to the graduated mark with ethanol.

4.2.1.7. Pipette 1 mL of the sample solution into a GC vial. Add 50 µL of naphthalene intermediate solution (as internal standard) into the same GC vial.

4.2.1.8. Mix well prior to GC-MS analysis.

4.2.2. GC-MS analysis

4.2.2.1 Operate the GC-MS system in accordance with the instrument manual. Analyse the samples with the following suggested conditions. It may be necessary to modify the operation conditions for optimum signal output.

4.2.2.2. Suggested GC conditions:

GC column	:	Restek Stabilwax-MS, 30 m x 0.25 mm x 0.25 µm or equivalent
Injector temperature	:	260℃
Injection volume	:	1 µL
Injection mode	:	Split mode, split ratio 20:1
Carrier gas	:	He, 99.999% purity
Column flow rate	:	1.0 mL/min
Temperature program	:	60 °C, hold 1 min; Ramp to 190°C at 15 °C /min, hold 0 min; Ramp to 240 °C at 45 °C /min, hold 3 mins;

4.2.2.3. Suggested MS conditions:

Ion source temperature	:	200°C
Transfer line temperature	:	220°C
Ionization mode	:	Electron impact (EI)

	Diagnostic ions (m/z)		
Target	**Quantification**	**Qualifier ion**	**Qualifier ion**

analytes	ion (Q1)	(R1)	(R2)
Menthol	81	71	95
Naphthalene	128	-	-

5. Calculation / result interpretation

5.1. Identification requirements

5.1.1. For GC-FID and GC-MS analysis, identification is based on comparing the relative retention time (RRT) of menthol in sample with that of calibration standard. The deviation of RRT of sample peak shall not differ by 0.5 %.

5.1.2. For GC-MS analysis, compare the relative abundances of qualifier ions to quantification ion (R1/Q1 and R2/Q1) of target analyte found in sample with those obtained from standard solution. The relative intensities shall meet the following tolerances.

Relative intensity to the base peak (%)	% Allowable deviation
> 50 %	± 10%
> 20 to 50 %	± 15%
> 10 to 20 %	± 20%
≤ 10 %	± 50%

5.2. Calibration curve

5.2.1. Plot the peak area ratios (menthol/naphthalene) against concentrations (in µg/mL for GC-FID; ng/mL for GC-MS) for the calibration standard solutions. Obtain the slope, y-intercept and the correlation coefficient (r) from the calibration curve.

5.2.2. Calculate the deviation of each calibration level using the following equation:

$$\text{Deviation of calibration level (\%)} = \frac{C - C_{theo}}{C_{theo}} \times 100\%$$

where C = Conc. of standard from the calibration

curve (in μg/mL for GC-FID; ng/mL for GC-MS) and

C_{theo} = Theoretical conc. of the calibration standard (in μg/mL for GC-ID; ng/mL for GC-MS)

The value C shall be given by:

$$\mathrm{C(ng\ or\ \mu g/mL)} = \frac{A - Y}{M}$$

where A = Peak area ratio of standard;
Y = Y-intercept of the calibration curve; and
M = Slope of the calibration curve.

5.3. Calculate the content of analyte in the sample using the following equation:

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

where C = Conc. of analyte from calibration curve (in μg/mL for GC-FID; ng/mL for GC-MS);
V = Final volume (mL);
D = Dilution factor; and
W = Sample weight (g).

5.4. Calculate the average deviation from the mean (ADM) of the pair of duplicate samples using the following equation:

$$\text{ADM } (\%) = \frac{D_1 - (D_1 + D_2)/2}{(D_1 + D_2)/2} \times 100\%$$

where D1 = Value of sample 1 and
D2 = Value of sample 2.

6. Reference

6.1. Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China Volume 1, 2015 ed. China Medical Science Press.

6.2. “Quantifying Uncertainty in Analytical Measurement”, Eurachem / CITAC Guide CG4, 3rd Edition, 2012.