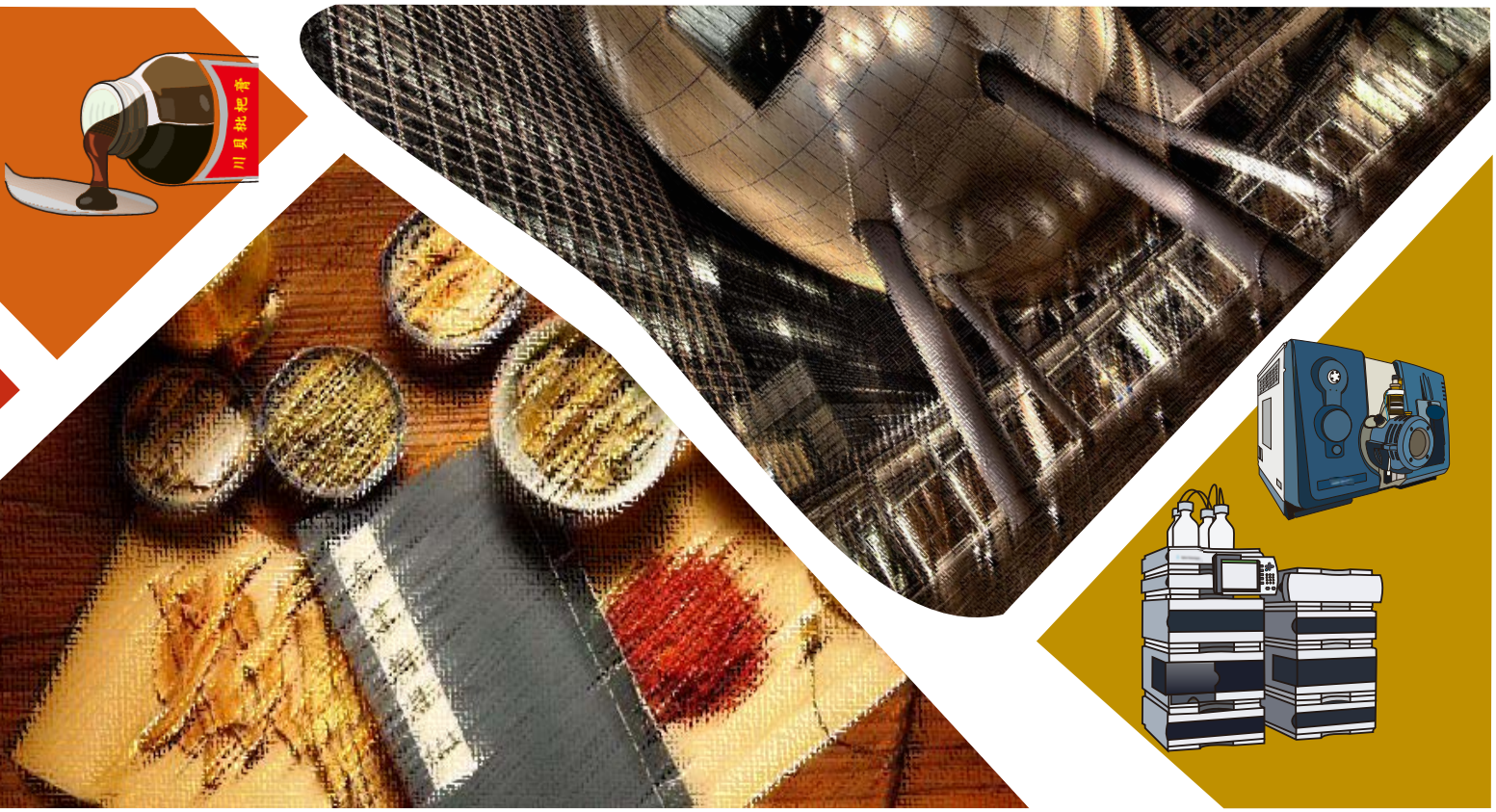
GCMTI RD-1:2021

Determination of Platycodin D, Amygdalin and Peimisine in Pei Pa Koa by High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS)

GCMTI method publications



Determination of Platycodin D, Amygdalin and Peimisine in Pei Pa Koa by High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS)

Safety Precaution: *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

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 different manufacturers, commonly it was made through a continuous procedure by decocting the Chinese herbal medicines including the fritillary bulb (Bulbus fritillariae cirrhosae, 川貝母), loquat leaf Eriobotryae Folium, 枇杷葉), pomelo peel (Citri Grandis Exocarpium, 化橘紅), chinese bellflower root (Platycodon grandiflorum, 桔梗), bitter apricot kernel (Armeniacae Semen Amarum, 苦杏仁), licorice root (Glycyrrhizae Radix Et Rhizoma, 甘草), 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
Eriobotryae Folium (枇杷葉)	Oleanolic acid and ursolic acid
Citri Grandis Exocarpium (化橘紅)	Naringin
Platycodon grandiflorum (桔梗)	Platycodin D
Armeniacae Semen Amarum (苦杏仁)	Amygdalin
Glycyrrhizae Radix Et Rhizoma (甘草)	Liquiritin and Glycyrrhizic acid

1.2. This method specifies the procedures for the determination of the Platycodin D, Amygdalin and Peimisine in Pei Pa Koa sample.

1.3. The sample is diluted with solvent. The chemical markers are qualitatively and/or quantitatively determined by High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS).

2. Reagents

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

2.1. Individual Stock standard solution (~1000 μg/mL)

Prepare Individual Stock standard solution by weighing about 5 mg of Platycodin D, Amygdalin and Peimisine respectively into three individual 5-mL volumetric flask, dissolve and make up to the graduated mark with methanol.

2.2. Mixed intermediate standard solution I (~10 μg/mL)

Prepare the mixed intermediate standard solution I by transferring three 100-μL of individual stock standard solution in a 10-mL volumetric flask and make up to the graduated mark with dilution solvent (Clause 2.15.).

2.3. Mixed intermediate standard solution II (~250 ng/mL)

Prepare the mixed intermediate standard solution II by transferring 250-μL of mixed intermediate standard solution I into a 10-mL volumetric flask and make up to the graduated mark with dilution solvent (Clause 2.15.).

2.4. Mixed working standard solution (calibration standards, CS1 - CS5)

A series of calibration standard solutions of ~0.5, 1, 2, 5, 10 ng/mL are prepared by transferring an appropriate amount of intermediate standard solution II or CS5 standard solution into 10-mL volumetric flasks and make up with dilution solvent (Clause 2.15.). Suggested volumes of standard solutions used for the preparation are listed below.

Standards	**Volume of Mixed Intermediate standard solution II (mL)**	**Volume of CS5 (mL)**	**Final Volume (mL)**
CS1		0.5	10
CS2		1	10
CS3		2	10
CS4	0.2		10
CS5	0.4		10

2.5. Stock ICV solution (~1000 μg/mL)

Prepare the Stock ICV solution by weighing about 5 mg of Platycodin D, Amygdalin and Peimisine from second source respectively into three individual 5-mL volumetric flask, dissolve and make up to the graduated mark with methanol.

2.6. Mixed intermediate ICV standard solution I (~10 μg/mL)

Prepare the mixed intermediate ICV standard solution I by transferring 100-μL of individual Stock ICV solution into a 10-mL volumetric flask and make up to the graduated mark with dilution solvent (Clause 2.15.).

2.7. Mixed intermediate ICV standard solution II (~250 ng/mL)

Prepare the mixed intermediate ICV standard solution II by transferring 250-μL of mixed intermediate standard solution I into a 10-mL volumetric flask and make up to the graduated mark with dilution solvent (Clause 2.15.).

2.8. Mixed ICV standard solution (~5 ng/mL)

Prepare the mixed ICV standard solution by transferring 200-μL of mixed intermediate ICV standard solution II into a10-mL volumetric flask and make up to the graduated mark with dilution solvent (Clause 2.15.).

2.9. Intermediate spike standard solutions

Prepare the intermediate spike standard solutions by transferring appropriate amount of individual stock standard solution into separate volumetric flasks (10-mL or 5-mL) and make up to the graduated mark with dilution solvent (Clause 2.15.). Suggested volumes of standard solutions used for the preparation are listed below.

Analytes	**Volume of individual stock standard solution (mL)**	**Final Volume (mL)**	**Concentration (μg/mL)**
Platycodin D	0.1	10	10
Amygdalin	0.25	5	50
Peimisine	0.1	10	10

2.10. Spike standard solution

Prepare the spike standard solution by transferring appropriate amount of intermediate spike standard solutions respectively into a 10-mL volumetric flask and make up to the graduated mark with dilution solvent (Clause 2.15.). Suggested volumes of standard solutions used for the preparation are listed below.

Analytes	Volume of intermediate spike standard solution (mL)	Final Volume (mL)	Concentration (ng/mL)
Platycodin D	0.25	10	250
Amygdalin	0.5		2500
Peimisine	0.2		200

2.11. Acetonitrile, LC-MS grade. .

2.12. Methanol, LC-MS grade.

2.13. Milli-Q Water.

2.14. Formic acid, analytical grade.

2.15. Dilution solvent

Acetonitrile : 0.02% Formic acid (2:8)

3. Apparatus

All glassware shall be rinsed with absolute ethanol and washed with detergent solution as soon as practicable after use. After detergent washing, glassware shall be rinsed immediately, firstly with water and then with acetone.

3.1. Volumetric flasks, 5-mL and 10-mL.

3.2. Auto Pipettes, 300-µL, 1000-µL and 5000-µL.

3.3. Analytical balance, capable of weighing to 0.01 mg.

3.4. Ultrasonic bath.

3.5. Centrifuge tube, 15-mL.

3.6. PTFE membrane filters, 0.45 µm.

4. Procedures

4.1. Sample preparation

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

4.1.2. Pipette 10-mL of dilution solvent (Clause 2.15.) into the centrifuge tube.

4.1.3. The sample mixture in the centrifuge tube is sonicated in the ultrasonic bath for 10 minutes.

4.1.4. The sample solution was filtered with a 0.45μm PTFE membrane and diluted with appropriate amount of dilution solvent prior to LC-MS/MS analysis.

4.1.5. For Platytcodin D and Peimisine analysis, the suggested dilution factor are 5-fold, respectively.

4.1.6. For Amygdalin analysis, the suggested dilution factor is50-fold.

4.2. HPLC-MS/MS analysis

4.2.1 Operate the HPLC-MS/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 resolution and signal output.

4.2.2. Suggested HPLC conditions:

HPLC system		Dionex UltiMate 3000 HPLC system			
Column	:	GL Science Intersil ODS-4, 2.1 X 250 mm, 5μm			
Column temperature	:	25 ºC			
Autosampler temperature	:	20 ºC			
Flow rate	:	0.3 mL/min			
Injection volume	:	5 μL			
Mobile Phase		A: 0.02% formic acid			
		B:Acetronitrile			
Gradient	:	Time(min)	A %	B %	Flow
		0.0	80	20	0.3
		4.0	80	20	0.3
		9.0	70	30	0.3
		14.0	50	50	0.3
		16.0	5	95	0.3
		18.0	5	95	0.3
		18.1	80	20	0.3
		22.0	80	20	0.3

4.2.3. Suggested MS conditions:

MS/MS system		AB SCIEX 6500+ system
Ionization mode	:	Electrospray ionization (ESI) +ve and–ve mode
Source temperature	:	500 ºC
Ion source gas 1 (GS1)	:	60
Ion source gas 2 (GS2)	:	70
Curtain gas (CUR)		20
Collision gas (CAD)		9
Scan Type	:	MRM
Ion spray Voltage		-4500V (-ve mode) and 5500V (+ve mode)

4.2.4. Suggested MRM acquisition conditions for the analysis of Platycodin D, Amygdalin and Peimisine:

Analytes	**MRM transitions**		**Dwell time msec**	**DP**	**EP**	**CE**	**CXP**
Platycodin D	1223.6 → 469.2	Quantifying MRM	100	-265	-10	-74	-25
	1223.6 → 681.4	Qualifying MRM	100	-265	-10	-79	-25
Amygdalin	456.2 → 323.1	Quantifying MRM	100	-100	-10	-18	-11
	456.2 → 179.1	Qualifying MRM	100	-100	-10	--22	-11
Peimisine	428.3 → 114.1	Quantifying MRM	100	110	10	39	15
	428.3 → 410.3	Qualifying MRM	100	110	10	39	15

5. Calculation / Result interpretation

5.1. Identification requirements

5.1.1. Targeted marker in sample is identified by comparison of the retention time of the detected peak with that of the standard. The retention time (RT) of the marker shall not differ from that of the standard by more than 5 %.

5.1.2. Calculate the relative abundance of ions (preferably including the quasimolecular ion) for
(i) at least two MRM ions for LC-MS/MS analyses; or
(ii) at least a pair of diagnostic ions for instrument capable of measuring accurate mass.

5.1.3. The relative abundance of MRM/diagnostic ions shall meet the tolerance for the positive identification of the marker (with reference to that of the standard solution or that of the average of the standard solutions):

Relative intensity to the base peak (%)	**% Allowable deviation LC**
> 50%	±20%
> 20 to 50%	±25%
> 10 to 20%	±30%
≤ 10%	±50%

5.2. Calibration curve

5.2.1. Plot the peak area against concentration (in mg/L) for each standard. Obtain the slope, y-intercept and the square of correlation coefficient (r^2) 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 = Concentration of standard obtained from the calibration curve (ng/mL)
C_{theo} = Theoretical concentration of the calibration standard (ng/mL)

The value C shall be given by:

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

where A = Peak area of standard
Y = y-intercept of the calibration curve
M = Slope of the calibration curve

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

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

where C = Concentration of marker obtained from calibration curve (ng/mL)
V = Final volume (mL)
D = Dilution factor
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

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.