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Determination of Psoralen and Isopsoralen in Proprietary Chinese Medicines containing Psoraleae and Ginseng by High Performance Liquid Chromatograph-Diode Array Detector (HPLC-DAD)

GCMTI Method Publications

Determination of Psoralen and Isopsoralen in Proprietary Chinese Medicines containing Psoraleae and Ginseng by High Performance Liquid Chromatograph-Diode Array Detector (HPLC-DAD)[1]

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. Proprietary Chinese medicines (pCm) containing psoraleae (補骨脂) and ginseng (人參) for nourishing and Yang invigorating are commonly found in Hong Kong. Nevertheless, analysis of the chemical markers of psoraleae and ginseng is a great challenge since it is susceptible to interference from matrix and other chemical components.

1.2. This method describes the procedures for qualitative and quantitative determination of the chemical markers of psoralae, namely psoralen and isopsoralen, in pCm containing psoraleae and ginseng by high performance liquid chromatograph-diode array detector (HPLC-DAD).

2. Reagents

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

2.1. Methanol, LC-MS grade.

2.2. Milli-Q water.

2.3. Phosphoric acid, purity ≥85%.

2.4. Psoralen, CAS no.: 66-97-7.

2.5. Isopsoralen, CAS no.: 523-50-2.

2.6. 0.05% (v/v) Phosphoric acid buffer solution

Use 0.5 mL of phosphoric acid (Clause 2.3) and make up to 1 L with water (Clause 2.2).

[1] This method intends to provide a reliable analytical method that can be used as a quality control method for determining the targeted chemical marker(s) in the corresponding pCm product(s). It is the user's responsibility to assess the suitability of testing their pCm products when adopting this method.

2.7. Extraction solvent

Methanol : water (7:3 v/v).

2.8. Preparation of standard solutions

2.8.1. Individual stock standard solutions (ca. 1000 µg/mL)

Weigh accurately about 10 mg of psoralen (Clause 2.4) and isopsoralen (Clause 2.5) into separate 10-mL volumetric flasks, dissolve and make up to the graduation mark with methanol (Clause 2.1), respectively.

2.8.2. Mixed intermediate standard solution (ca. 10 µg/mL)

Prepare mixed intermediate standard solution by transferring 0.1 mL of each individual stock standard solution into a 10-mL volumetric flask and make up to the graduation mark with extraction solvent (Clause 2.7).

2.8.3. Calibration standard solutions, CS1 – CS5

A series of calibration standard solutions are prepared by transferring appropriate amounts of mixed intermediate standard solution into 10-mL volumetric flasks and make up to the graduation mark with extraction solvent (Clause 2.7). Suggested volumes of standard solution used for the preparation are listed in the table below.

Calibration standards	Volume of mixed intermediate standard solution (mL)	Final Volume (mL)	Concentration of Psoralen and Isopsoralen (µg/mL)
CS1	0.25	10	0.25
CS2	0.50	10	0.50
CS3	1.00	10	1.00
CS4	3.00	10	3.00
CS5	5.00	10	5.00

2.8.4. Individual stock initial calibration verification (ICV) standard solutions (ca. 1000 µg/mL)

Prepare individual stock ICV standard solutions, from source different from that of the calibration standards. Weigh accurately about 10 mg of psoralen and isopsoralen into separate 10-mL volumetric flasks, dissolve and make up to the graduation mark with methanol (Clause 2.1), respectively.

2.8.5. Mixed intermediate ICV standard solution (ca. 10 µg/mL)

Prepare mixed intermediate ICV standard solution by transferring accurately 0.1 mL of each individual stock ICV standard solution into a 10-mL volumetric flask and make up to the graduation mark with extraction solvent (Clause 2.7).

2.8.6. ICV working standard solution (ca. 1 μg/mL)

Prepare ICV working standard solution by transferring 1 mL of mixed intermediate ICV standard solution into a 10-mL volumetric flask and make up to the graduation mark with extraction solvent (Clause 2.7).

2.8.7. Spike standard solutions (ca. 1000 μg/mL)

Refer to individual stock standard solutions (Clause 2.8.1).

3. Apparatus

All glassware shall be rinsed with acetone 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 twice.

3.1. Grinder or blender.

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

3.3. Volumetric flasks, 10-mL and 25-mL.

3.4. Auto pipettes, 100-μL, 300-μL and 1000-μL.

3.5. Centrifuge with rotation speed of at least 4000 rpm.

3.6. Centrifuge tubes, 15-mL.

3.7. Vortex mixer.

3.8. Ultrasonic bath.

3.9. PTFE membrane filters, 0.45 μm.

3.10. LC glass vials.

3.11. LC column: Hypersil GOLD^{TM} C18 Selectivity 3 μm, 2.1 mm × 100 mm, Thermo Scientific^{TM} or equivalent.

3.12. High Performance Liquid Chromatograph-Diode Array Detector (HPLC-DAD) system.

4. Procedures

4.1. Sample preparation

4.1.1. Grind and homogenize solid pCm samples using grinder or blender before analysis.

4.1.2. Weigh accurately about 0.25 g of pCm sample into a 15-mL centrifuge tube.

4.1.3. Add 10 mL of extraction solvent (Clause 2.7) into the centrifuge tube. Vortex the sample mixture in the centrifuge tube for 1 minute.

4.1.4. Sonicate the sample mixture in an ultrasonic bath for 20 minutes at room temperature.

4.1.5. Centrifuge the sample solution at 4000 rpm for 10 minutes. Carefully transfer the supernatant solution to a 25-mL volumetric flask.

4.1.6. Repeat clauses 4.1.3 to 4.1.5 twice with 5 mL of extraction solvent (Clause 2.7). Collect all supernatants in the same 25-mL volumetric flask and make up to graduation mark with extraction solvent (Clause 2.7).

4.1.7. Filter the sample solution with 0.45 µm PTFE membrane filter into a LC glass vial. The solution is ready for HPLC-DAD analysis.

Remark:
Dilute the sample solution with extraction solvent (Clause 2.7) if the concentration of analyte*(s) is not within the calibration range.*

4.2. HPLC-DAD analysis

4.2.1. Operate the HPLC-DAD system in accordance with the instrument manual. Carry out analysis with the conditions as suggested below. It may be necessary to modify the operation conditions for optimal signal output. Record the actual experimental conditions in the worksheet.

4.2.2. Suggested HPLC-DAD conditions:

HPLC system	:	Waters Alliance e2695 HPLC system or equivalent performance
Column	:	Thermo Scientific™ Hypersil GOLD™ C18 selectivity, 3 µm, 2.1 mm × 100 mm or equivalent
Column temperature	:	35 ºC
Flow rate	:	0.3 mL/min
Injection volume	:	5 µL
Mobile phase	:	A: 0.05 % Phosphoric acid solution (Clause 2.6) B: MeOH
Gradient	:	Time (min) A% B% 0.0 70 30

13.0	70	30
13.1	5	95
18.0	5	95
18.1	70	30
25.0	70	30

DAD wavelength : 245nm

4.2.3. Calibrate the HPLC-DAD system using at least 5 calibration standards (Clause 2.8.3).

4.2.4. Perform HPLC-DAD analysis for method blank(s), sample(s), sample duplicate(s), spike sample(s) and relevant check standard solution(s) according to the quality control plan as established in the laboratory.

5. Calculation / result interpretation

5.1. Identification requirement

For HPLC-DAD analysis, identify the target analyte in the sample by comparison of the retention time of the detected peak (RT_{sample}) with that of the average retention time (RT) of the calibration standards. The RT_{sample} shall not differ from that of the average RT of calibration standards by more than 5% for positive identification.

5.2. Establish the calibration curve by plotting the peak area against the concentration of analyte in the calibration standards in linear calibration mode.

5.3. Calculate the concentration of analyte in the sample, in µg/g, using the following equation:

$$\text{Concentration of analyte (µg/g)} = \frac{C \times V \times D}{W}$$

where C = Conc. of analyte obtained from calibration curve (in µg/mL)
V = Final volume (mL)
D = Dilution factor
W = Sample weight (g)

6. Reference

6.1. Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China Volume 1, 2020 ed. China Medical Science Press.

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

6.3. V. J. Barwick and S. L. R. Ellision, "VAM Project 3.2.1 Development and Harmonisation of Measurement Uncertainty Principles Part (d): Protocol for Uncertainty Evaluation from Validation data", LGC/VAM/1998/088 Version 5.1, January 2000.