GCMTI RD-1:2023

Determination of Salvianolic Acid B in Baifeng Wan by High Performance Liquid Chromatograph-Diode Array Detector (HPLC-DAD)

GCMTI method publications

Determination of Salvianolic Acid B in Baifeng Wan 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. Baifeng Wan is a prevalent proprietary Chinese medicine (pCm) in China and Hong Kong. It is commonly used for treating various diseases caused by blood deficiency or gynaecological disorders. Ancient Chinese medicines bibliography as well as Chinese Pharmacopeia (CP) have documented the major ingredients for the prescriptions. Nevertheless, in Hong Kong market there are numerous modified formulations of Baifeng Wan products with varying compositions. Among others, Chinese herbal medicines such as Ginseng Radix Et Rhizoma (人參), Angelicae Sinensis Radix (當歸), Chuanxiong Rhizoma (川芎), Cyperi Rhizoma (香附), Paeoniae Radix Alba (白芍), Rehmanniae Radix (地黃), Astragali Radix (黃芪), Salviae Miltiorrhizae Radix Et Rhizoma (丹參) and Glycyrrhizae Radix Et Rhizoma (甘草) are commonly found in different brands of Baifeng Wan products. The corresponding chemical markers are as follows:

Chinese Herbal Medicines	Common Chemical Markers
Ginseng Radix Et Rhizoma (人參)	Ginsenosides
Angelicae Sinensis Radix (當歸)	Z-Ligustilide
Chuanxiong Rhizoma (川芎)	Z-Ligustilide
Cyperi Rhizoma (香附)	α-cyperone
Paeoniae Radix Alba (白芍)	Paeoniflorin
Rehmanniae Radix (地黃)	Rehmannioside
Astragali Radix (黃芪)	Astragaloside IV
Salviae Miltiorrhizae Radix Et Rhizoma (丹參)	Tanshinone and salvianolic acid B
Glycyrrhizae Radix Et Rhizoma (甘草)	Liquiritin

[1] This method is intended to provide a reliable analytical method that can be used as 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 application to their pCm products when adopting this method.

1.2. This method specifies the procedures for qualitative and/or quantitative determination of salvianolic acid B in Baifeng Wan sample 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. Acetonitrile, LC-MS grade.

2.3. Milli-Q water.

2.4. Formic acid, LC-MS grade.

2.5. Salvianolic acid B, CAS. No.: 121521-90-2.

2.6. Extraction solvent

Methanol : water (1:1 v/v).

2.7. 0.4% Formic acid solution

Add 4 mL of formic acid in a 1-L measuring cylinder and make up to the mark with water (Clause 2.3.).

2.8. Preparation of standard solutions

2.8.1. Stock standard solution (ca. 1000 μg/mL)

Weigh accurately about 10 mg of salvianolic acid B into a 10-mL volumetric flask, dissolve and make up to the graduated mark with methanol.

2.8.2. Intermediate standard solution (ca. 30 μg/mL)

Prepare intermediate standard solution by transferring 0.3 mL of stock standard solution into a 10-mL volumetric flask and make up to the graduated mark with extraction solvent (Clause 2.6.).

2.8.3. Calibration standard solutions, CS1 – CS5

A series of calibration standard solutions are prepared by transferring an appropriate amount of intermediate standard solution into 10-mL volumetric flasks and make up with extraction solvent (Clause 2.6.). Suggested volumes of standard solution used for the preparation are listed in the table below.

Calibration standards	Volume of intermediate standard solution (mL)	Final volume (mL)	Conc. of salvianolic acid B (μg/mL)
CS1	0.5	10	1.5
CS2	0.8	10	2.4
CS3	1.0	10	3.0
CS4	2.0	10	6.0
CS5	5.0	10	15

2.8.4. Stock initial calibration verification (ICV) standard solution (ca. 1000 μg/mL)

Prepare stock ICV standard solution, from source different from that of the calibration standard. Weigh accurately about 10 mg of salvianolic acid B into a 10-mL volumetric flask, dissolve and make up to the graduated mark with methanol.

2.8.5. Intermediate ICV standard solution (ca. 30 μg/mL)

Prepare intermediate ICV standard solution by transferring accurately 0.3 mL of stock ICV standard solution into a 10-mL volumetric flask and make up to the graduated mark with extraction solvent (Clause 2.6.).

2.8.6. ICV working standard solution (ca. 3 μg/mL)

Prepare ICV working standard solution by transferring 1 mL of intermediate ICV standard solution into a 10-mL volumetric flask and make up to the graduated mark with extraction solvent (Clause 2.6.).

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

Refer to stock standard solution (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 acetone and then with water. The water rinse shall be followed by another two more rinses with acetone, respectively.

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, 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 polypropylene (PP) vials.

3.11. LC column: GL Sciences Inertsil ODS-4, 5 μm, 2.1 mm x 250 mm 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 samples using grinder or blender before analysis.

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

4.1.3. Add 10 mL of extraction solvent (Clause 2.6.) 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 2.6.). Collect all supernatant in the same 25-mL volumetric flask and make up to mark with extraction solvent (Clause 2.6.).

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

Remark: Further dilute the sample solution with extraction solvent (Clause 2.6.) 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	:	GL Sciences Inertsil ODS-4, 5 μm, 2.1mm×250 mm, or equivalent
Column temperature	:	25 ºC
Flow rate	:	0.2 mL/min
Injection volume	:	10 μL
Mobile phase	:	A: 0.4 % formic acid solution (Clause 2.7.) B: Acetonitrile

Gradient :	Time (min)	A%	B%
	0.0	80	20
	15.0	70	30
	30.0	20	80
	35.0	15	85
	40.0	15	85
	40.5	80	20
	50.0	80	20

DAD wavelength	:	286 nm

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 requirements

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 from calibration standards by more than 5%.

5.2. Establish the calibration curve by plotting the peak area against the concentration

of analyte 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; and
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.