



**Determination of Ginsenosides in Compound Chinese Medicine Granules
by HPLC-DAD¹**

Safety precautions: 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 vapor.

1. Introduction

- 1.1. Compound Chinese medicine granules are one of the proprietary Chinese medicines (pCm) sold in a variety of formulations in Hong Kong. Most of them are formulated in accordance with ancient Chinese medicine bibliographies. In general, they are produced by pulverising, decorating, filtering and concentrating a series of Chinese herbal medicines into a blend of water-soluble powders.
- 1.2. This method utilising the technique of high-performance liquid chromatography (HPLC) coupled with a diode array detector (DAD) is developed to analyse 10 formulations with Radix Ginseng (人參) as the principal or assistant drug, or as a major component. The 10 formulations are listed below.

Item	Formulation	Ancient Chinese Medicine Bibliography	Validated Analytes			
			Re	Rg1	Rf	Rb1
1.	聖愈湯	醫宗金鑑	✓	✓	✓	✓
2.	舉元煎	景嶽全書	✓	✓	✓	✓
3.	理中湯	傷寒論	✓	✓	✓	✓
4.	人參養榮湯	三因極一病證方論	✓	✓	---	✓
5.	大建中湯	金匱要略	✓	✓	✓	✓
6.	六君子湯	醫學正傳	✓	✓	✓	✓
7.	附子理中湯	三因極一病證方論	✓	---	---	---
8.	人參敗毒散	太平惠民和劑局方	✓	✓	✓	✓
9.	麥門冬湯	金匱要略	✓	✓	✓	✓
10.	溫經湯	金匱要略	✓	---	---	✓

- 1.3. This method has been validated for qualitative and/or quantitative determination of four analytes, viz. ginsenoside Re (Re), ginsenoside Rg1 (Rg1), ginsenoside Rf (Rf) and ginsenoside Rb1 (Rb1) in compound Chinese medicine granules using HPLC. The validated working range is 1–50 µg/mL in solution, which corresponds to 50–2500 µg/g in the original sample.



2. Reagents

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

2.1. Water, Milli Q.

2.2. Methanol (MeOH), LC-MS grade.

2.3. Ethanol (EtOH), absolute grade.

2.4. Acetonitrile (ACN), LC-MS grade.

2.5. 50 % MeOH Solution

Mix ~500 mL of MeOH and ~500 mL of water in a 1-L measuring cylinder.

2.6. 95 % ACN Solution

Mix ~950 mL of ACN and ~50 mL of water in a 1-L measuring cylinder.

2.7. 70 % ACN Solution

Mix ~700 mL of ACN and ~300 mL of water in a 1-L measuring cylinder.

2.8. Re, CAS. No.: 52286-59-6.

2.9. Rg1, CAS. No.: 22427-39-0.

2.10. Rf, CAS. No.: 52286-58-5.

2.11. Rb1, CAS. No.: 41753-43-9.

2.12. Standard Solutions

2.12.1. Individual Stock Standard Solutions (~2000 µg/mL)

Weigh accurately ~20 mg of Re, Rg1, Rf and Rb1 into separate 10-mL volumetric flasks respectively. Dissolve and make up to the graduation mark with MeOH.

2.12.2. Mixed Intermediate Standard Solution (INT, ~100 µg/mL)

Pipette 0.5 mL each from the individual stock standard solutions into a 10-mL volumetric flask. Make up to the graduation mark with MeOH.



2.13. Calibration Standard Solutions

Freshly prepare at least six calibration standard solutions from INT. Make up with 50 % MeOH solution in 10-mL volumetric flasks. Suggested concentrations of calibration standard solutions are listed below.

Calibration Standard Solution	Vol. of INT (mL)	Final Vol. (mL)	Final Conc. of Re, Rg1, Rf and Rb1 (µg/mL)
CS1	0.1	10.0	1
CS2	0.5	10.0	5
CS3	1.0	10.0	10
CS4	2.0	10.0	20
CS5	3.0	10.0	30
CS6	5.0	10.0	50

Remark: Other concentrations may be used as appropriate. Other sequences of dilution may be employed to achieve the required concentrations. Record the details in the data sheets.

2.14. Initial Calibration Verification (ICV) Standard Solutions

2.14.1. Stock ICV Standard Solutions (~2000 µg/mL)

Prepare stock ICV standard solutions according to Clause 2.12.1 using an independent source of reference materials (another brand or another batch of standard with same brand as appropriate).

2.14.2. Mixed Intermediate ICV Standard Solution (ICV, ~100 µg/mL)

Pipette 0.5 mL of the stock ICV standard solutions into a 10-mL volumetric flask. Make up to the graduation mark with MeOH.

2.14.3. ICV Working Standard Solution (~10 or 20 µg/mL)

Pipette 1.0 or 2.0 mL of ICV into a 10-mL volumetric flask. Make up to the graduation mark with 50 % MeOH solution.

2.15. Calibration Check Standard

Calibration check standard shall be the CS3 or CS4 of the calibration standard solutions, viz. ~10 or 20 µg/mL.



2.16. Method Blank

The method blank shall be carried out through the complete sample preparation procedures as per Clauses 4.1.3 to 4.3 and shall contain the same amount of reagents as the sample solutions.

3. Apparatus

***Note:** 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. Volumetric flasks, 10- and 25-mL.
- 3.3. Measuring cylinder, 1-L.
- 3.4. Analytical balance, capable of weighing to 0.01 mg.
- 3.5. Autopipettes, 300-, 1000- μ L and 10-mL.
- 3.6. Centrifuge tubes, 15-mL with polypropylene screw cap.
- 3.7. Ultrasonic bath.
- 3.8. Vortex mixer.
- 3.9. High speed centrifuge with rotation speed settable at least 4000 rpm.
- 3.10. Freezer, capable of operating at $\sim -20^{\circ}\text{C}$.
- 3.11. Glass test tubes.
- 3.12. Centrifugal evaporator or equivalent performance.
- 3.13. Aminopropyl (NH_2) SPE cartridges, 55- to 105- μm , 6-mL SPE column, containing 500 mg sorbent or equivalent.
- 3.14. PTFE membrane filters, 0.20- μm or equivalent.
- 3.15. LC glass vials.
- 3.16. HPLC column, Symmetry C_{18} Column (4.6×250 mm, 5 μm) or equivalent.
- 3.17. HPLC Alliance System or System with equivalent performance.



4. Procedures

4.1. Sample Preparation

- 4.1.1. Grind and homogenise solid samples using grinder or blender before analysis, if necessary.
- 4.1.2. Accurately weigh ~0.5 g of the sample into a 15-mL screw cap centrifuge tube.
- 4.1.3. Add 10 mL of 50 % MeOH solution into the centrifuge tube. Vortex the sample mixture in the centrifuge tube for ~1 min.
- 4.1.4. Sonicate the sample mixture in an ultrasonic bath for ~20 min at room temperature.
- 4.1.5. Centrifuge the sample mixture at ~4000 rpm for ~10 min to settle the residue. Transfer the supernatant solution to a 25-mL volumetric flask.
- 4.1.6. Repeat Clauses 4.1.3 to 4.1.5 twice with 4 – 5 mL of 50 % MeOH solution.
- 4.1.7. Collect all supernatant in the same 25-mL volumetric flask and make up to the mark with 50 % MeOH solution.
- 4.1.8. Transfer 2 mL of the sample solution to a new 15-mL centrifuge tube.
- 4.1.9. Pipette 6 mL of EtOH into the centrifuge tube and vortex for ~30 s.
- 4.1.10. Store the sample solution (Clause 4.1.9) in the freezer at ~-20 °C for ~10 min.
- 4.1.11. Centrifuge the sample solution at ~4000 rpm for ~10 min to settle the dispersing solids or top-floating layer.
- 4.1.12. Pipette 4 mL of supernatant to a glass test tube.
- 4.1.13. Evaporate the supernatant to dryness at ~80 °C by centrifugal evaporator, and then reconstitute with 1 mL of 50 % MeOH solution.
- 4.1.14. Pipette 0.5 mL of EtOH and then 8.5 mL of ACN into the glass test tube.
- 4.1.15. Vortex the sample solution for ~30 s.



4.2. Sample Cleanup

4.2.1. Condition a NH₂ SPE cartridge with ~5 mL of ACN. Equilibrate with 5 mL of **95 % ACN solution**. The eluant is discarded.

4.2.2. Totally transfer the sample solution as per Clause 4.1.15 to the NH₂ SPE cartridge. The eluant is discarded.

4.2.3. Rinse the glass test tube with 5 mL of **95 % ACN solution** and then totally transfer to the NH₂ SPE cartridge. The eluant is discarded.

4.2.4. Further add 5 mL of **70 % ACN solution** to the NH₂ SPE cartridge and collect the eluant in a new glass test tube.

4.2.5. Evaporate the eluant to dryness at ~80 °C by centrifugal evaporator, and then reconstitute with 1 mL of 50 % MeOH solution.

4.2.6. Filter the sample solution with 0.20-µm PTFE filter disc and collect the filtrate into a LC glass vial.

Remark: Further dilute the sample solution with 50 % MeOH solution if the concentration of analyte(s) is not within the calibration range.

4.3. Analysis of HPLC-DAD

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

4.3.2. Suggested HPLC-DAD conditions:

HPLC System	:	Alliance System or equivalent performance		
Column	:	Symmetry C ₁₈ Column (4.6 × 250 mm, 5 µm) or equivalent		
Mobile Phase A	:	Water		
Mobile Phase B	:	ACN		
Elution Program	:	Time (min)	A (%)	B (%)
		0.0	95	5
		8.0	95	5
		15.0	85	15
		25.0	80	20
		40.0	80	20
		70.0	78	22
		95.0	65	35
		100.0	15	85
		101.0	2	98



		116.0	2	98
		116.5	95	5
		125.0	95	5
Flow Rate	:	0.8 mL/min		
Injection Volume	:	10 µL		
Reported Retention Time	:	~55.0 min for Re; ~53.0 min for Rg1; ~91.5 min for Rf; and ~95.5 min for Rb1		
Column Temperature	:	25 °C		
DAD wavelength	:	203 nm		

5. Calculation/Result Interpretation

5.1. Identification Requirement

Identify the analyte(s) in the sample by comparison of the retention time of the detected peak (RT_{sample}) with that of the average retention time (RT) from calibration standards. The RT_{sample} shall not differ from that of the average RT from calibration standards by more than 5 % for positive identification.

5.2. Establish the calibration curve by plotting the peak area against the concentration for each analyte in linear calibration mode. Obtain the slope, y-intercept and the coefficient of determination (r^2) from the calibration curve.

5.3. Calculate the concentration of analyte in sample (µg/g) as follows:

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

where C = Concentration of analyte obtained from calibration curve (µg/mL);

V = Final volume (mL);

D = Dilution factor; and

W = Sample weight (g).

6. Reference

6.1. Chinese Pharmacopoeia Commission. (2025). *Pharmacopoeia of the People's Republic of China*. China Medical Science Press.

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

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