Rapid quantification of Chinese medicine Zuo Jin Pill using rapid resolution liquid chromatography

Application Note

Traditional Chinese Medicine

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Abstract

This Application Note describes the development of a rapid resolution LC method for the separation of the Chinese herbal prescription Zuo Jin Pill into its six major constituents: magnoflorine, jatrorrhizine, coptisine, palmatine, berberine and evodiamine. The method uses an Agilent 1200 Series Rapid Resolution Liquid Chromatography (RRLC) system coupled with an Agilent 1200 Series Diode Array Detector (DAD).

The separation was carried out on an Agilent ZORBAX Eclipse Plus C_{18} column (4.6 × 150 mm, 1.8 µm). The linear gradient was eluted with the mobile phase of an acetonitrile – acetate buffer. All calibration curves showed good linearity (r^2 >0.9998) within the test range 1.2-1200 µg/ml. The results demonstrate that this analytical method is simple, sensitive and reliable for rapidly analyzing six major bioactive compounds in ZJPs. It is useful for the comprehensive evaluation of the quality of this traditional Chinese Medicine (TCM).

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Introduction

Zuo Jin Pill (ZJP), an ancient traditional Chinese medicine (TCM), is widely adopted in China for the clinical treatment of hypochondric and costal pain, stomach ache, acid reflux, nausea and upset. Recent pharmacological studies showed that it also inhibits gastric secretions and provides antiflammatory, antifungal and analgesic benefits.[1] Coptis (*coptidis rhizoma*) and evodia (*evodiae fructus*) are the basic components of this Chinese herbal preparation.[2] Coptis dispells heat, dries dampness, purges fever and removes toxins. Evodia warms the middle torso, dispels cold, causes vitality to descend and controls pain.[3] The herb couples are much simpler than complicated formulas in composition and retain the basic therapeutic features.

The pharmacologically-active ingredients of coptis are a number of protoberberine alkaloids,[4] and those of evodia are indolequinoline and quinolone alkaloids.[5] Both of these herb drugs have been analyzed well by many different methods. Several studies have been established for simultaneously assaying the two herbs.[6-8] However, current methods need long analysis times, and incorporate complicated sample preparations and solvent systems.

In this Application Note, a method using a rapid resolution liquid chromatography (RRLC) coupled with a diode array detector (DAD) was developed for the simultaneous quantification of the main constituents present in various ZJPs. In addition, a comparative study was made between the performance of RRLC and high performance liquid chromatography (HPLC) techniques. During this test, six compounds in the formula, including magnoflorine, jatrorrhizine, coptisine, palmatine, berberine and evodiamine were analyzed.

Experimental

Equipment

The rapid resolution LC (RRLC) method was developed on an Agilent 1200 Series RRLC system with the following modules:

- Agilent 1200 Series Binary Pump SL with vacuum degasser
- Agilent 1200 Series High Performance Autosampler SL
- Agilent 1200 Series Thermostatted Column Compartment SL
- Agilent 1200 Series Diode Array Detector SL, with micro flow cell (2-µL volume, 3-mm path length)
- Agilent ChemStation B.03.02 for data acquisition and evaluation
- Agilent ZORBAX Eclipse Plus C18 Rapid Resolution High Throughput (RRHT) column (4.6 × 150 mm, 1.8 µm.)

Chemicals and materials

HPLC grade acetonitrile, acetic acid, and ammoniom acetate were purchased from Merck (Darmstadt, Germany). Pure water was obtained from a Millipore pure water system. All solvents and samples were filtered through 0.22-µm membrane filters before analysis.

The reference standards of jatrorrhizine, coptisine, palmatine, and berberine were purchased from Shanghai Winherb Medical Science Co., Ltd (Shanghai, China). Magnoflorine was purchased from Shanghai Tauto Biotech Co., Ltd (Shanghai, China). Evodiamine was purchased from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All standards were 98% pure or better.

Nine batches of ZJP were collected from different pharmaceutical companies in China. Sample number 10 was prepared by our laboratory according to China Pharmacopoeia.

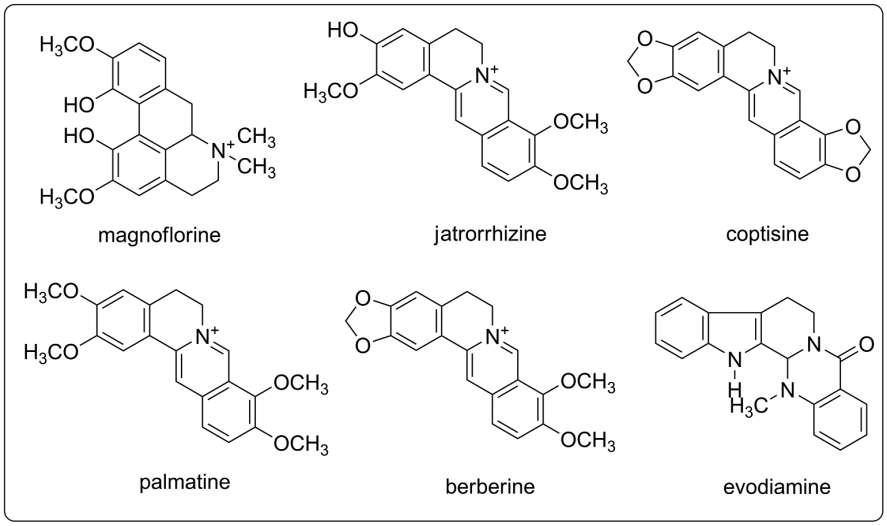

Figure 1
Structures of the constituents in ZJP.

Standard solutions and sample preparation

A series of 1200 µg/mL standard solutions were prepared in methanol. A calibration curve was formed from analysis of the diluted solutions. The stock solution was used to prepare the reference compound mixture solutions. All the solutions were stored at 4 °C in a refrigerator.

The ultrasonic extraction was performed by mixing 0.25 g of pulverized sample and 25 mL of methanol with 1% 1 M hydrochloric acid into a 25-mL volumetric flask. The flask was then sonicated for 1 hour at room temperature. The extract was adjusted to volume. All samples were filtered prior to use through a 0.22-µm nylon membrane filter.

Analytical method

Mobile phase	A = 50 mM ammonium acetate and 0.2% acetic acid, B = acetonitrile
Flow rate	1.2 mL/min
Gradient	0-3 min, 10%-20% B; 3-9 min, 20%-35% B; 9-15 min, 35%-100% B; 15-18 min, 100%B
Column temperature	35 °C
DAD wavelength	254 nm, 40 Hz, 4 nm slit, save all spectrum
Injection volume	5 µL, room temperature, no needle wash.

Results and discussion

Optimization of the chromatographic conditions

The RRLC conditions were optimized to improve detection. Reversed phase RRLC with gradient elution was used according to the literature reports.[6-8] The concentrations of ammonium acetate and acetic acid in the mobile phase were also optimized. The results showed that the addition of 0.2% acetic acid and 50 mM ammonium acetate in the mobile phase provided the best resolution, peak shape, and signal-to-noise ratio. The solvent system described here was easier to develop than that of mobile phase systems described in previous studies.[6-8]

Optimization of the extraction conditions

The most important parameters controlling extraction yield were determined to be the choice of solvent, extraction volume, and the extraction time. In order to optimize these parameters, the first set of analyses was performed using different volumes of methanol and ethanol solvent, while altering the concentration of hydrochloric acid. The results showed that 25 mL of methanol with 1% M hydrochloric acid was the most effective solvent, providing the best response. The analyses were then performed using different extraction times, such as 30, 60, and 90 min. The results of these tests showed that 60 min was sufficient to almost completely extract six compounds.

Comparison of HPLC and RRLC

The ZJP sample was analyzed with the RRLC system, and with the HPLC system (Figure 2). Comparable separations were achieved in 70 min on the HPLC, and 18 min with the RRLC method. The shorter run time of the RRLC (25% of that on the HPLC) was due to the analytical column packed with a 1.8 µm stationary phase, which allowed higher pressures and flow rates. Because of its savings in analysis time and solvent, as well as its high performance and efficiency, it was determined that RRLC is the best choice for the analysis of a complex system such as Chinese herbal prescriptions.

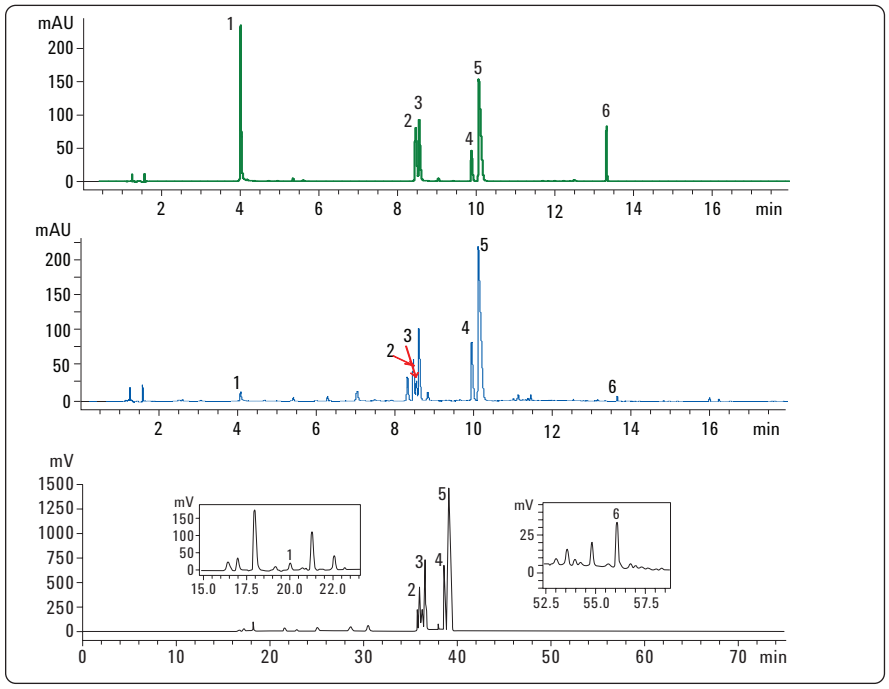

Figure 2

(a, b) RRLC-UV chromatograms of (a) six mixed bioactive markers and (b) ZJP (S4), and (c) HPLC-UV chromatogram of ZJP with the detection at 254 nm: (1) magnoflorine, (2) jatrorrhizine, (3) coptisine, (4) palmatine, (5) berberine, (6) evodiamine.

RRLC method validation

The linearity calibration curves were developed from the analyses of six different standard concentrations, in triplicate of chemical markers. The calibration curves and correlation coefficients were determined using a liner regression model. The limits of detection (LOD) were meaured with a signal-to-noise ratio (S/N) of about 3. The limits of quantification (LOQ) were measured with the signal-to-noise ratio (S/N) of about 10. The correlation coefficients ($r^2 > 0.9998$), LOD (1.4 - 12 ng), and LOQ (4.8 - 30 ng) are shown in Table 1. These resultes confirm good linear correlation and high sensitivity for these chromatographic conditions.

Analytes	Linear range (µg/ml)	Regressive equation*	r^2 (n=6)	LOD (ng)	LOQ (ng)
Magnoflorine	20-1200	y=0.23633x-1.44875	0.9999	12	30
Jatrorrhizine	3.4-425	y=1.20016x-2.96349	0.9999	2.04	6.8
Coptisine	3.28-477.5	y=1.48691x-4.96470	0.9998	1.97	6.56
Palmatine	2.6-325	y=0.93747x-2.73943	0.9998	1.56	5.2
Berberine	1.92-1200	y=1.29790x-8.90206	0.9998	1.92	5.76
Evodiamine	2.4-300	y=0.99174x-2.04918	0.9998	1.4	4.8

* In the regression equation y = ax + b, y refers to the peak area (A), x is the concentration of the reference (µg/mL), and r^2 is the correlation coefficient of the equation.

Table 1
Linear regression equation analysis in the determination of the six compounds.

The mixture standard solution was analyzed under the optimal conditions injected five times in one day for intra-day variation, then injected five times per day for three consecutive days for inter-day variation. The precision and accuracy of these analyses were calculated. The intra-day precision was within 0.32%, while the inter-day precision was within 0.63%. The accuracies were all between 98.6 and 100.78%. Five different solutions from the same sample were analyzed to verify the repeatability of the method. Results of this test showed the relative standard deviation (RSD) of repeatability to be less than 1.59%. Using standard addition, the recovery of this method was established with three different concentration levels (0.8, 1.0, and 1.2 times the matrix concentration) added to the reference standard. Three samples, at each concentration level, were made for the analyses. The solutions were extracted and quantified according to the optimized method, and the mean recoveries calculated for each. The mean recovery results were between 99.01 and 100.14%, with RSD less than 2.47% for ten components. These results indicate a satisfactory assay, with acceptable precision, accuracy, repeatability, and recovery (Table 2).

Compound	Intra-day (n=6)		Inter-day (n=3)		Repeatability(n=5)	Recovery	
	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	RSD (%)	Mean recovery (%)	RSD (%)
Magnoflorine	100.60	0.21	99.47	0.63	0.74	99.67	1.50
Jatrorrhizine	98.60	0.22	99.78	0.44	0.57	99.01	1.28
Coptisine	99.46	0.16	100.26	0.32	0.95	100.14	2.47
Palmatine	99.372	0.32	100.53	0.33	1.51	99.97	2.04
Berberine	99.05	0.11	99.98	0.32	1.59	100.11	1.92
Evodiamine	100.78	0.25	99.83	0.43	1.40	100.06	1.80

Table 2
Statistical results of precision, accuracy, repeatability and recovery of the six compounds.

Sample analysis

Once developed, the method was applied to the analysis of 10 batches of ZJP sample. The samples were tested to verify the presence of six marker compounds in the sample. The peaks were identified by comparing the retention times and UV spectra to those obtained from authentic samples. The quantitative analytical results indicated that the six-alkaloid contents in Chinese herbal preparations can vary significantly, depending upon the origin of the sample (Table 3). Multiple constituents are responsible for the therapeutic effects of Chinese herbal preparations, and the concentrations of these constituents may affect these therapeutic benefits.

Sample No.	Content of each compound in 10 samples (mg/g)					
	Magnoflorine	Jatrorrhizine	Coptisine	Palmatine	Berberine	Evodiamine
1	14.32	3.88	4.07	19.27	60.22	1.19
2	16.35	3.90	4.15	19.82	61.56	1.45
3	14.28	11.75	5.06	25.24	84.77	1.31
4	14.53	11.80	5.06	24.85	84.44	1.42
5	12.93	8.15	4.48	21.82	73.66	0.85
6	12.08	7.82	4.32	20.99	71.71	0.79
7	12.55	8.00	4.45	21.42	73.25	0.84
8	13.22	8.33	4.58	22.16	74.83	0.77
9	15.76	14.57	5.53	29.79	100.96	0.78
10	11.70	10.89	3.37	19.44	0.34	-

Table 3
Contents (mg/g) of the six compounds in the 10 samples.

Conclusion

A rapid RRLC-DAD method was established for the comprehensive analysis of six compounds in ZJP. Validation tests performed in this study indicated that the developed method was sensitive, reliable and rapid. All calibration curves showed good linearity (r^2 >0.9998) within the test range of 1.2-1200 µg/mL. The detection limits and quantification limits were in the ranges of 1.4-12 ng and 4.8-30 ng, respectively. The described method was successfully applied to the simultaneous separation of the complex constituents in ZJP, and resulted in a shorter time and better fit for effective quality control of this Chinese herbal prescription.

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