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Determination of Reserpine and Yohimbine in *Rauvolfia* by High-Performance Liquid Chromatography

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A new direct high performance liquid chromatography (HPLC) analytical method for the determination of reserpine and yohimbine in *Rauvolfia* has been developed. The reserpine and yohimbine was separated on a Zorbax stable bound (2.1 mm × 50 mm, 1.8 mm) C_{18} column with 50 % acetonitrile (containing 0.01 mol L^{-1} 1,2-ethylenediamine) as the mobile phase and detected with UV detector at 280 nm. This method provides good reproducibility and sensitivity for the quantification of reserpine and yohimbine. The relative standard derivation of overall intra-day variations were less than 2 % and the relative standard derivation of inter-day variations were less than 2.5 %. The standard recoveries (three different concentrations of markers: 0.1, 0.5 and 2.0 mg) were ranged from 97-102 %.

Key Words: Reserpine, Yohimbine, High performance liquid chromato-graphy, *Rauvolfia*.

INTRODUCTION

Rauvolfia (also spelled *Rauwolfia*) is a genus of evergreen trees and shrubs in the milkweed family, Apocynaceae. The approximately 85 species in the genus can mainly be found in tropical regions[1,2]. In Mainland China, this plant mainly distributed in Shimao, Xisuangbanna, Honghe, Dehong prefectures of Yunnan province[3].

Various chemical and pharmacological studies have demonstrated that the major biologically active ingredients present in *Rauvolfia* are alkaloids, including ajmaline, deserpidine, rescinnamine, serpentinine and yohimbine[4-6]. The reserpine and yohimbine (Fig. 1) are widely used as antihypertensive drug. It had drastic psychological side effects and has been replaced as a first-line antihypertensive drug by other compounds that lack such adverse effects, although combination drugs that include it are still available in some countries as second-line antihypertensive drugs. Therefore, the determination of reserpine and yohimbine in *Rauvolfia* is important for the quality controls of this herb medicine.

HPLC is an analytical method with the advantage of direct detection and simple

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operation for reserpine and yohimbine determination. Several works on the HPLC methods for the determination of reserpine and yohimbine were reported[7-11]. However, these methods usually need a tedious samples preparation or long time for chromatographic separation. In this paper, a simple and sensitive HPLC analytical method for the determination of reserpine and yohimbine in *Rauvolfia* using Zorbax stable bound rapid analysis column was developed. The reserpine and yohimbine can achieve a baseline separation within 3.5 min. Compared to the previous literatures, this is one of the most rapid methods to separation reserpine and yohimbine.

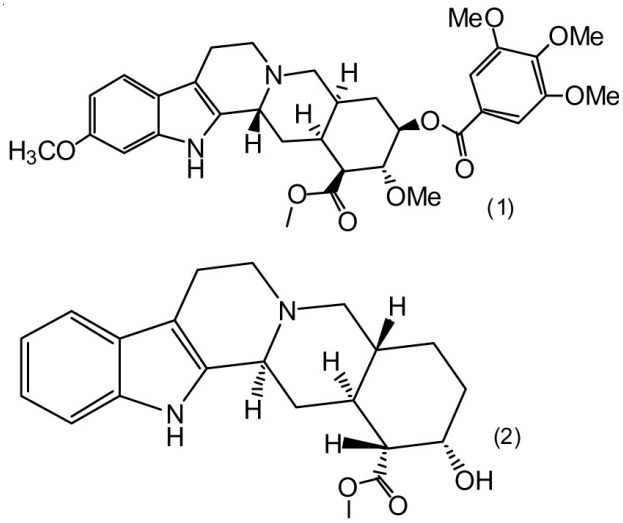

Fig. 1. Structure of reserpine (**1**) and yohimbine (**2**)

EXPERIMENTAL

The samples analyzed are dried root of *Rauvolfia*. The samples were collected in Shimao, Xisuangbanna, Dehong and Honghe Prefecture, Yunnan Province, P.R. China. The samples were identified by Prof. Xi-Wen Li, Kunming Institue of Botany. For each sample, at least 0.5 kg of herbal samples were dried at room temperature for constant weight and pulverized to 80 mesh.

The HPLC analysis was performed on a Shimadzu 10A HPLC system equipped with photodiode array detector and autosampler (Shimadzu Corporation, Japan). A Zorbax stable bound column (4.6 mm × 50 mm, 1.8 mm) (Agilent Technologies Inc, USA) was utilized.

HPLC grade acetonitrile (mobile phase), methanol and chloroform (for sample preparation) were provided by Fisher Scientific Inc. The ultrapure water used was obtained from a Milli-Q50 SP water system (Millipore Inc, USA). The mobile phase used is 50 % acetonitrile (containing 0.01 mol L^{-1} 1,2-ethylenediamine) at flow rate of 0.6 mL min^{-1}. The detect wavelength is 280 nm. The sample injection volume is

5 μL.

Preparation of sample: 1 g of dried powders sample was extracted with 50 mL of methanol and chloroform (1:1) by reflux at 60 °C for 0.5 h. The extracts were cooled and diluted to the 50 mL with chloroform. Then, 1 mL of the solution was concentrated to dryness under reduced pressure. The residue was dissolved in 1 mL of methanol. This solution was filtered through a 0.45 μm syringe filter and afford to HPLC analysis.

Preparation of standard solution: To prepare standard solutions, an accurately weighed amount of reserpine and yohimbine were dissolved in methanol for HPLC. Five concentrations were chosen, with the range 0.8-120 μg mL^{-1}, respectively.

RESULTS AND DISCUSSION

Optimal of chromatographic separation: Optimal chromatographic condition was obtained after testing different mobile phase systems with C_{18} column. 50 % acetonitrile (containing 0.01 mol L^{-1} 1,2-ethylenediamine) was found to be the best separation. Therefore, 50 % acetonitrile (containing 0.01 mol L^{-1} 1,2-ethylenediamine) was selected as mobile phase in this experience.

To shorten the chromatographic separation time, a Zorbax stable bound rapid analysis column (2.1 mm × 50 mm, 1.8 mm) was used in this experiment. With this rapid analysis column, the reserpine and yohimbine were separated completely within 3.5 min (Fig. 2). Compared to the previous literature[7-11], more than 70 % separation time was saved.

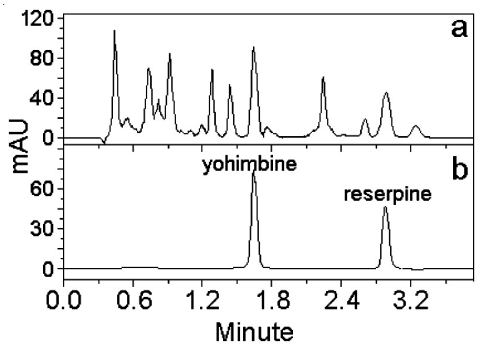

Fig. 2. Chromatogram of *Rauvolfia* sample (a) and standard sample (b)

According to the ultravoilet spectroscopy obtained by photodiode array detector, reserpine and yohimbine both have maximum absorbance at 280 nm. Thus, 280 nm was selected as detect wavelength.

Calibration graphs: Under the optimum conditions, the regression equations of reserpine and yohimbine were established based on the standard samples injected and their peak area. The limits of detection are calculated by the ratio of signal to noise (S/N = 3). The results were shown in Table-1. The reproducibility of this method was also examined for 10 μg mL^{-1} of reserpine and yohimbine. The relative standard deviations (n = 9) were shown in Table-1.

TABLE-1
REGRESSION EQUATION, COEFFICIENT AND DETECT LIMIT

Components	Regression equation C (μg mL^{-1})	Linearity range (μg mL^{-1})	Coefficient	Detect limits (μg mL^{-1})	RSD % (n = 9)
Reserpine	$A = 2.36 \times 10^5 C + 687$	1.0-120	r = 0.9994	0.3	1.10
Yohimbine	$A = 2.86 \times 10^5 C + 862$	0.8-150	r = 0.9997	0.2	0.94

Method recovery and precision: The recovery test was carried out by adding reserpine and yohimbine to the samples (three different concentrations of markers: 0.1, 0.5 and 2.0 mg). The sample was prepared as above "preparation of sample" procedure and injected for HPLC analysis to calculate the amount of the reserpine and yohimbine founded. The results shown that the recoveries (n = 5) were ranged from 97-102 %. This method is high recovery.

The measurements of intra- and inter-day variability (determination of the same samples for seven times) were utilized to determine the precision of the developed method. The results shown that the relative standard derivation of overall intra-day variations were less than 2 % and the relative standard derivation of inter-day variations were less than 2.5 %. This method is high precision.

Analysis of pentacyclic reserpine and yohimbine in samples: This method was subsequently applied to determination of the reserpine and yohimbine in different *Rauvolfia* varieties samples. The contents of reserpine and yohimbine are summarized in Table-2.

TABLE-2
DETERMINATION RESULTS (mg/g) OF THE RESERPINE AND YOHIMBINE IN *Rauvolfia* VARIETIES

Components	Samples (mg/g)				RSD (%) (n = 5)	Recovery (%) (n = 5)
	R. vomitoria (Shimao)	*R. yannanensis* (Tsiang Xisuangbanna)	*R. latifrons* (Tsiang Honghe)	*R. vomitoria* (Dehong)		
Reserpine	0.582	0.3637	0.496	0.749	2.2	97
Yohimbine	0.641	0.745	0.586	0.822	2.3	103

Conclusion

In this manuscript, a Zorbax stable bound C_{18} (2.1 mm × 50 mm, 1.8 μm) rapid analysis columns was used. The reserpine and yohimbine can achieve baseline separation with 3.5 min on this column. Compared to the normal column, 70 % of separation time was saved. It is one of the most rapid methods for chromatographic analysis of reserpine and yohimbine. The sample preparation for this method is simple. The reserpine and yohimbine were extracted from the samples with solvent, and can directly afford to HPLC analysis. This preparation does not need a complex purification procedure. In conclusion, this method is rapid, high sensitive and provides good reproducibility and accurateness for the quantification of reserpine and yohimbine in *Rauvolfia*.

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