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Research Article

QUANTITATIVE DETECTION OF RESERPINE IN *RAUWOLFIA SERPENTINA* USING HPTLC

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ABSTRACT

Rauwolfia serpentina is medicinally famous herb in Ayurvedic and western system of medicine. Reserpine is an indole alkaloid and is important constituent of *Rauwolfia* which is reported to posses anti hypertensive and tranquilizing activity. In the present study High Performance Thin Layer Chromatography has been developed for detection, monitoring and quantification of Reserpine in *Rauwolfia* and its preparations, which was found to be rapid and accurate. The method proposed was precise, sensitive, specific and reproducible with an average recovery of 98.78%. The limit of quantification was observed to be 40ng, and C.V% <2.3%.

Keywords: Rauwolfia serpentina, Reserpine, HPTLC, quantitative monitoring.

INTRODUCTION

The *Rauwolfia serpentina* Benth (family: Apocynaceae) is a medicinally famous herb in Ayurveda, Siddha, Unani and Western system of medicines[1]. Several alkaloids have been isolated from root bark of this plant including reserpine, ajmaline, ajmalicine, yohimbine, etc. The plant is extensively used in the treatment of insanity and snake bite[8]. The root extract of this plant is very useful in disorders of gastro intestinal tract viz., diarrhea, dysentery, cholera and colic[1].

Reserpine is an Indole alkaloid chemically it is (3β, 16β, 17α, 18β, 20α) – 11, 17 – dimethoxy – 18 [(3, 4, 5 – trimethoxybenzoyl) – oxyl] yohimban – 16 – carboxylic acid methyl ester or 3, 4, 5 – trimethoxybenzoyl methyl reserpate[2], used in lowering blood pressure[7-8], as tranquilizer[7-8] etc. Many methods like UV spectroscopy[2], HPLC[2], HPTLC[2], gas chromatography[5], voltametry[5], polarography[5], room temperature phosphometry[5] and spectrofluorimetry[5], are available for the determination of Reserpine in pharmaceutical preparations either in bulk, dosage forms or in biological fluids.

Many of these methods can not be used for the determination of reserpine in extracts due to the interference of other constituents of plant. In the present study we are reporting a HPTLC method for detection, monitoring and quantification of reserpine in *Rauwolfia species.* Method validation data is also presented.

MATERIALS AND METHODS

Whole plant of *Rauwolfia serpentina* were collected from Trissur (Kerala), Shimoga (Karnataka), were identified and authenticated by Dr. Jawahar C Raveendra, Botanist, Foundation for Revitalization of Local Health Tradition, Bangalore.

All the solvents used were of AR (Analytical Reagent) grade. The reference standard of Reserpine was purchased from Natural Remedies Pvt. Ltd. Bangalore.

Chromatographic conditions

Instrument: HPTLC system equipped with a sample applicator device Camag Linomat 5. Camag twin trough chamber, Camag TLC scanner and integration software (Wincats)

HPTLC Plate: Silica gel GF254 (Merck) 20 X 10 cm

Mobile Phase: Chloroform: Toluene: Ethyl acetate: Diethylamine (7:7:4:1)

Wavelength: 268nm.

Standard preparation

A 40µg/ml solution of Reserpine reference standard was prepared in methanol.

Sample preparation

Roots were excised from the plants, washed with running tap water and dried in an oven at not more than 60 ^{0}C. They were size reduced and about 100mg of powdered drug was accurately weighed and was treated with 1ml of ammonia solution left aside for 10min. 10ml methanol was added to the above mixture, refluxed on water bath for 10 min and filtered. The methanolic extract was concentrated, dissolved in 1ml methanol, filtered and used for further analysis.

Procedure

The TLC plate was activated by placing in an oven at the temperature of 110 ^{0}C for 20 min. the plate was spotted with test and standard preparation maintaining a distance of 8mm from the edge of TLC plate. It was developed upto 75mm in the twin trough chamber using mobile phase, dried in an oven and subjected for TLC scanning at 268nm.

RESULTS AND DISCUSSION

Under the chromatographic conditions described above, the Rf value of Reserpine was about 0.43. The Chromatogram of standard Reserpine and that of Reserpine in *Rauwolfia serpentina* is shown in figure 1 and 2 respectively.

The calibration curves were linear in the range of 500 to 900ng (Figure 3). Spectral comparison of reference standard and reserpine in sample (Figure 4) revealed the better resolution of reserpine from other constituents of *Rauwolfia*. The method is suitable for reliable quantification of reserpine which may be above or equal to 40 ng.

The inter day coefficient of variation for analysis of different samples was about 2.2% (table 1). Percent recovery was studied by adding the different known amounts of standard reserpine to the sample before sample preparation. The average recovery was found to be 98.78% (table 2).

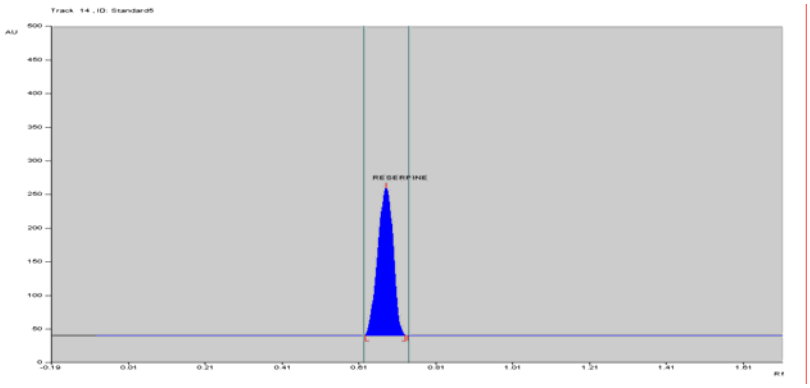

Fig. 1: A Typical HPTLC chromatogram of Reserpine standard

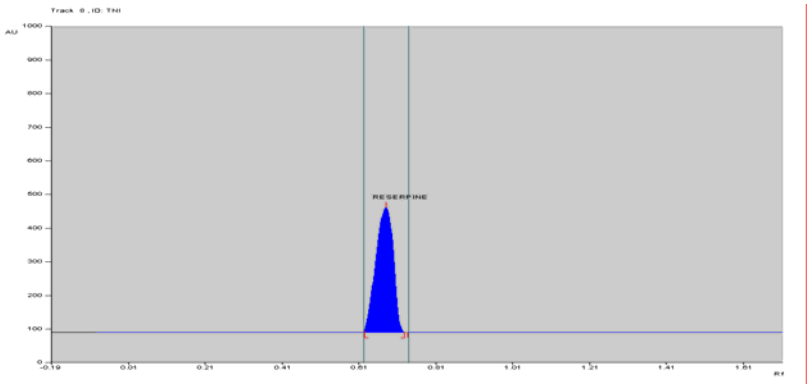

Fig. 2: A Typical HPTLC chromatogram of Reserpine in *Rauwolfia serpentina*

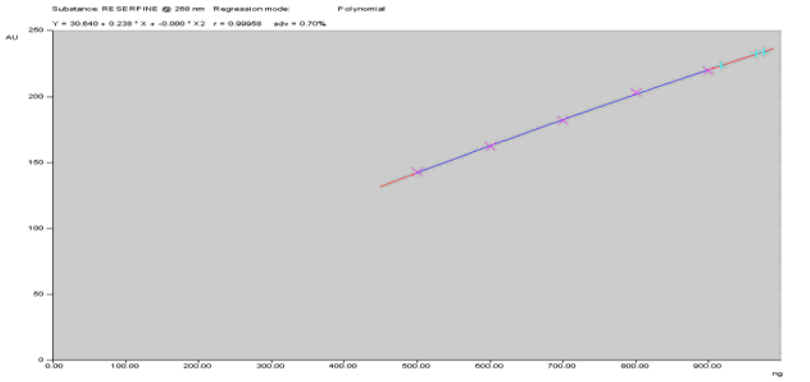

Fig. 3: A Typical calibration curve for Reserpine by HPTLC

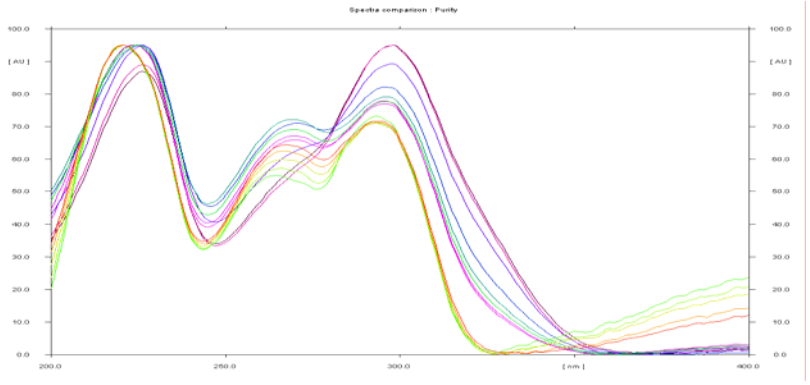

Fig. 4: Spectra comparison of Reserpine reference standard with the Reserpine in sample (scanning wavelength 200 – 400nm)

Table 1: Reserpine: Method Validation Data - Ruggedness

Sample	Reserpine (%w/w)		Mean	S.D	C.V (%)
	Day1	Day2			
1	0.046	0.047	0.0465	0.0005	1.5206
2	0.044	0.042	0.043	0.001	3.2888
3	0.045	0.043	0.044	0.001	3.2141
4	0.051	0.053	0.052	0.001	2.7196
5	0.05	0.048	0.049	0.001	2.8861
6	0.073	0.074	0.0735	0.0005	0.9620
7	0.083	0.085	0.084	0.001	1.6835

Table 2: Reserpine: Method Validation Data – Recovery

Sr no.	Amount of Rauwolfia Root Powder Taken (mg)	Amount of Reserpine (A) Represents	Amount Of Reserpine Std (mg) Added To (A)	Total Reserpine Taken (mg)	Total Reserpine Found (mg)	Recovery (%)
	(A)	(B)	(C)	(D)=(B)+(C)	(E)	(E)/(D) X 100
1	100	0.073	0.04	0.113	0.111531	98.7
2	99	0.072	0.036	0.108	0.106488	98.6
3	105	0.076	0.032	0.108	0.106812	98.9
4	110	0.08	0.044	0.124	0.122884	99.1
5	95	0.069	0.048	0.117	0.115128	98.4
6	90	0.065	0.052	0.117	0.11583	99.0
7	107	0.078	0.024	0.102	0.100164	98.2
8	85	0.062	0.02	0.082	0.081508	99.4

The same method could be applied for herbal preparations containing *Rauwolfia* and may give satisfactory results.

Thus, this newly developed HPTLC method is quick and reliable for quantitative monitoring of reserpine in *Rauwolfia* species and herbal preparations containing *Rauwolfia.*

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