HPTLC method validation of reserpine in *Rauwolfia serpentina* – A High Value medicinal Plant

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Abstract: *Rauwolfia serpentina* medicinal value well known in various system of medicine all over the world. 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 quantification of Reserpine in *Rauwolfia* and its allied preparations, which was found to be rapid and accurate. The method proposed was highly precise, sensitive, specific and reproducible with an average recovery of 78%. The limit of quantification was observed to be 112 ng.
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Keywords : *Rauwolfia serpentina*, reserpine, HPTLC, Validations, indole alkaloid

Introduction:

The *Rauwolfia serpentin*a Benth (family: Apocynaceae) is a medicinally famous herb in Ayurveda, Siddha, Unani and Western system of medicines (Qureshi et al., 2009). Almost, 50 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 (Kokate et al., 2003)). The root extract of this plant is very useful in disorders of gastro intestinal tract viz., diarrhea, dysentery, cholera and colic1, which are also used to treat hypertension and breast cancer (Von Poser et al., 1990; Stanford et al., 1986). Reserpine is an Indole alkaloid chemically it is yohimban – 16 – carboxylic acid methyl ester or 3, 4, 5 – trimethoxybenzoyl methyl reserpate (Sunday et al., 2007), used in lowering blood pressure (Kokate et al., 2003), as tranquilizer etc. (Kokate et al., 2003), Many methods like UV spectroscopy, HPLC, HPTLC (Sunday et al., 2007)), gas chromatography, voltametry, polarography (Dhruv et al., 2004) room temperature phosphometry and spectrofluorimetry (Dhruv et al, 2004) 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. International organizations like ISO, AOAC and IUPAC have published guidelines for method validation techniques Keeping in view of the above , present study we are reporting a HPTLC Method validation data for reserpine in *Rauwolfia* species.

MATERIALS AND METHODS:

Whole plant of *Rauwolfia serpentina* were collected from Haldwani district Nainital (Uttrakhand), and species were authenticated by Botanical Survey of India northern circle Dehardun Uttarakhand (India), the voucher specimens have been kept in the Institute (Centre for Aromatic Plants, Selaqui, Dehradun, Uttarakhand, India) All the solvents used were of AR (Analytical Reagent) grade. The reference standard of Reserpine was procured from Sigma Aldrich USA.

Chromatographic conditions

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 The plate was developed in Solvent system Chloroform: Methanol: Ammonia M:05:0.01) in previously saturated twin through chamber.

Standard preparation

A 10 mg of Reserpine reference standard was prepared in acetonitrile.

Preparation of Sample

15 gm dried root powder of *Rauwolfia serpentina* was extracted 3 times for 30 min in 20 ml methanol at 50^0C. The extract were evaporated dryness under vacuum and the residue was dissolved in 100 ml of 0.01 1M HCL. The filtered solution was adjusted to pH 6 with 0.01M NaOH and filter through 0.5 ìl filter membrane the filtrate was used as sample solutions. Sample 1 ml of sample taken for HPTLC analysis.

Procedure

The TLC plate was activated by placing in an oven at the temperature of 110 0C 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

Quantitave analysis of researpine by reverse phase High performance layer liquid Chromatography. The HPLC method was validating by determining linearity, peak purity and limits of detection and quantifications for qualitative purpose the method was evaluating by taking in to account of retention factor , precision, and selectivity for the standards higher retention factor time repeatability was apparent from RSD value below 1.2% for both the standard and sample peak purity was studies for reserpine limit of detection (LOD) and Quantification (LOQ) were evaluated for quantitative purpose (Table-1). The value of LOD and LOQ showed table (Table-1).

Method precision

Five replicate samples of a single batch of reserpine were performed and analyzed by the proposed HPTLC method: the result shown in next table. The % RSD calculated indicate that the methods has an acceptable level of precision. (Acceptance criteria & RSD_7.0).

System suitability: Five replicate injecting of standard solution were made into the HPTLC system as per proposed method. The result alone with % RSD of area counts rese rpine the result acceptance criteria %RSD_2.

Accuracy (Recovery): A known amount of reserpine powder @.10 mg/ml) final volume in applies 15 ìl and spiked with known amount of resperine at two different levels in triplicate D.0mg, 8.0mg). The samples were analyzed as per proposed method.

Robustness:

Change in wavelength using scammer 3 in wavelength 278, 280, 282, 284 nm is injected standard and sample of *Rauwolfia serpentina* powder and calculated reserpine content as below study – concludes that method is suitable in 278, 280, 282, 284 nm. change mobile phase composition of chloroform 9.5 : 0.5 methanol : 0.1 ammonia, and chloroform 9.9:, 0.1 methanol :0.1 ammonia study concluded that Rf change in Chloroform : Methanol : Ammonia 9.9 : 0.1 : 0.01. Chloroform 9.5: 0.5 methanol: 0.1 ammonia mobile phase imply for reserpine analysis. Results Indicated that the method has acceptable level of recovery (Acceptance criteria/ Recovery should below the range 70%-110%. Under the chromatographic conditions described above, the Rf value 0. 48, 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 200 ng to 1200 ng (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 112 ng. 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 78.78%.

Conclusion:

Case study developed techniques implying the method was suitable for quantifications of these compounds R^2 Values for the compounds >0.99 confirming the linearity of the method. The method can therefore be regarded as suitable for quality control and standardization of reserpine fingerprints. 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*

Table-1 Retention times correlation coefficients and linear range from regression analysis and limit of Quantification (LOQ) and detection (LOD).

Compounds	Rf ^{n}	R^2	Linear range in ng	LOD	LOQ
Reserpine	0.48±0.033	0.9970	200-1200	112 ng	376 ng

n – 10, Number of sample applied

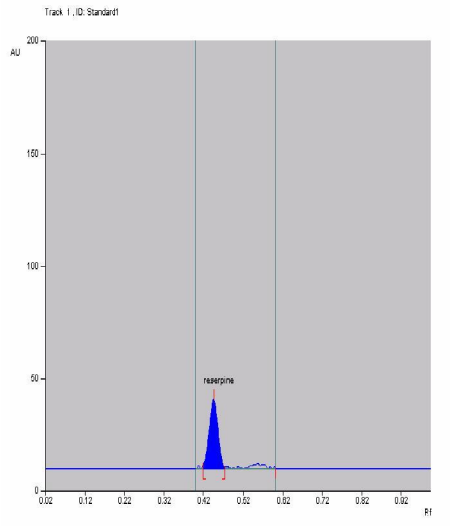

Fig: 2(a) Standard Peak

Figure 1. Peak of Standard

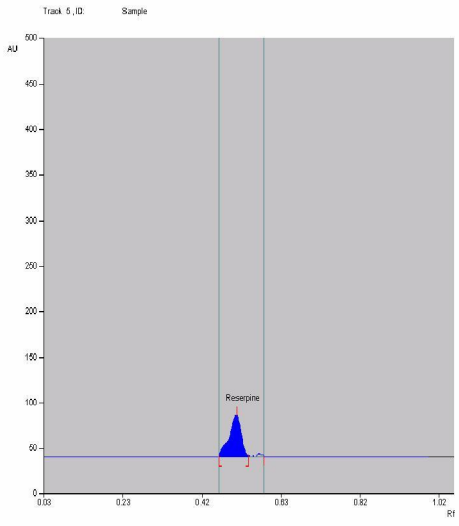

Fig: 2(b) Sample Peak

Figure 2. Peak of Sample

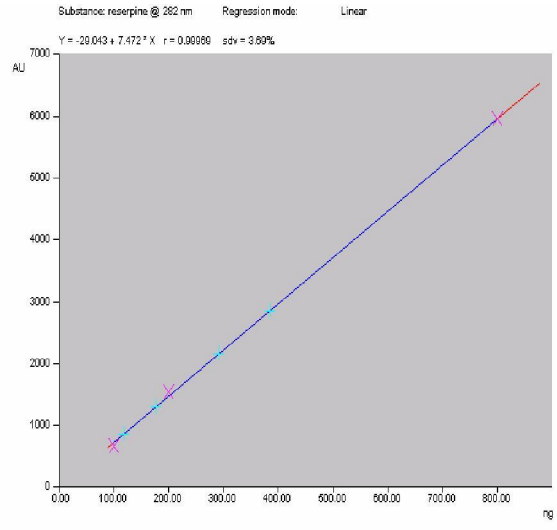

Figure 3. Standard Curve of Reserpine

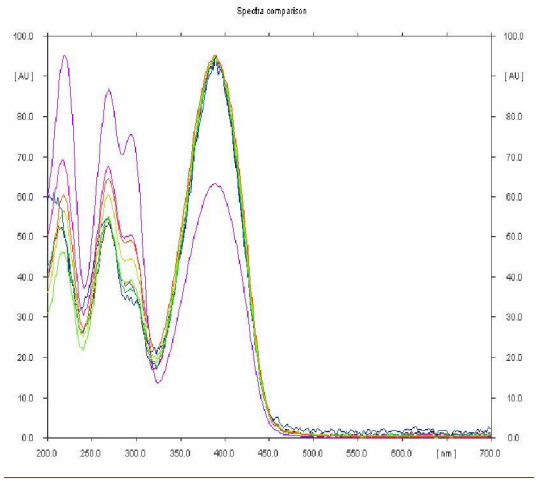

Figure 4. Spectral Comparison standard and sample

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