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Quantitative Estimation of Reserpine in Different Parts of *R. serpentina* and *R. tetraphylla* by Using HPTLC

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ABSTRACT

Due to its high demand over the world market the genuine plant (i.e R.serpentina Linn. benth. exkurz) is almost on he track of extinction and in future can be categorized as an endangered species. Therefore in our study it was attempted to search reserpine from other parts R. serpentina and R. tetraphylla. The analytical data revealed that 72% of reserpine present in R. Serpentina is found in its root where as 25% and 3% of reserpine are present in stem and leaf respectively. Similarly in case of R. tetraphylla the distribution of reserpine are 74%, 22% and 4% in root, stem and leaf respectively. So other parts of both the species can be explored for the isolation of bioactive reserpine and the commercial plant R. Serpentina can be minimized from over exploitations and extinction.

Key words: HPTLC, *Reserpine, R. serpentine, R. tetraphylla.*

INTRDUCTION

Several Rauwolfia species in India are known to possess ethno medicinal and folklore claims[1]. Rauwolfia species are very important due to their traditional medicinal use such as insanity, edema, Rheumatic pain, Epilepsy, Snake and Scorpio bite, Purgative, Sedative, Anthemilatic, relief cough, anti diarrhea and some intestinal disease due to the presence of Reserpine. Reserpine is also important in modern medicine system to treat a number of diseases like hypertension, neuropsychiatry disorder and as tranquilizer. Reserpine was the first herbal constituent included in modern medicine system. Further, due to its high demand over the world market the genuine plant (i.e *R.serpentina* linn. benth. exkurz) is almost on he track of extinction

and in future can be catagorised as an endangered species. There fore it is necessary to search the contents of Reserpine from other parts of different species.

Literature survey reveals that UV [2, 3], HPTLC [4], GC[5] and HPLC[6] methods are reported for the determination of Reserpine in pharmaceutical formulations as well as roots of different variety of Rauwolfia but no method as yet is reported for determination of Reserpine in different parts of *R. serpentina* and *R. tetraphylla* using HPTLC.

MATERIAL AND METHOD

Collection of plant material

The fresh plant material *R.serpentina linn* and *R. tetraphylla* was collected from Birla Institute of Technology campus, Mesera, Ranchi, in the month of July and August. The leaves, stems and root of *R. serpentine* and *R. tetraphylla* and other Parts were authenticated by Dr. S. Jha, Dept of Pharmaceutical sciences, B.I.T Mesra, Ranchi.

The part of the plant of such as root, stem and leaf are separated and cut into small pieces to accelerate shade drying, after drying the prepared course powder was subject to extraction.

Instrument and Chemicals

CAMAG (Switzerland) HPTLC system equipped with Linomat IV sample applicator was used for the application of the samples and the standard. Twin TLC chambers (20×10 cm^2) were used for developing the plates. CAMAG TLC Scanner III with CATS software was used for scanning the TLC plates. Standard Reserpine was procured from Sigma Aldrich, Banglore. Other chemicals used in the experiment were of analytical grade and procured from Nice Chemical Ltd. Cochin, India.

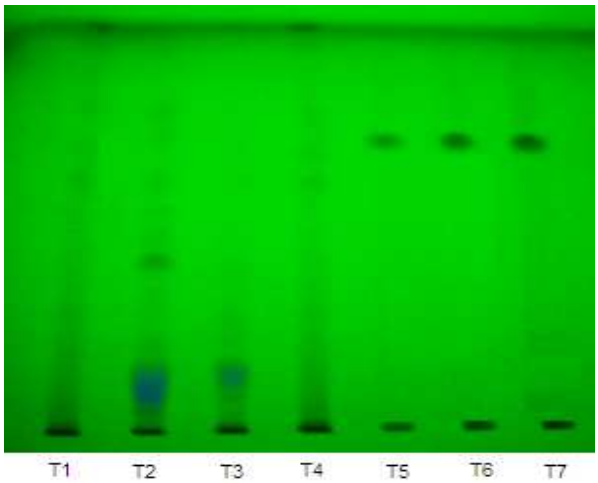


Fig 1: Finger print of methanolic extract of Different parts of R. tetraphylla and R. serpentine.

Track 1: R. tetraphylla (Stem); Track 2: R. serpentina (Stem); Track 3: R. tetraphylla (Leaf); Track 4: R. serpentina (Leaf); Track 5: R. tetraphylla (Root); Track 6: R. serpentina (Root); Track 7: Standard Reserpine; Solvent System: Toluene: Ethylacetate: diethylamine (7:2:1).

HPTLC Conditions
Stationary Phase: Silica gel 60 F254 (Merck) precoated TLC plates.
Mobile Phase: Toluene: Ethylacetate: diethylamine (7:2:1).
Saturation : 10min.
Wavelength : 200nm
Lamp : Deuterium.
Measurement Type : Remmission
Measurement Mode : Absorption
Optical Filter : Second order

Method of extraction
100gm powder drug of root, stem and leaf of *R. serpentina* and *R. tetraphylla* was taken in a 500 ml beaker separately. Sufficient amount of methanol was added and then boiled on a water bath with continuous stirring for half an hour and then filter through a filter paper, collect the filtrate and the residue was again subjected to decoction with sufficient methanol and boiled on a water bath. This process was continued for 3 to 4 times. Finally all the filtrates were collected and evaporated to dryness on water bath. 1g of alcoholic extract of root, stem and leaf of *R. serpentina* and *R. tetraphylla* were dissolved in alcohol separately and made up to the mark in 100ml volumetric flask separately.

Standard Solution
A stock solution of Reserpine was prepared by dissolving 10mg of Reserpine in methanol and made up to 10ml in a 10ml volumetric flask.

Linearity of Detector Response
In order to establish linearity, 5mcL to 25mcL of standard solutions of Reserpine containing 5mcg to 25mcg of standard reserpine were applied on 20×10 cm precoated TLC plates which were prewashed with methanol, as 6mm bands by using Camag Linomat IV sample applicators. The TLC chamber was saturated with the mobile phase for 30min. The plate was developed in a solvent system of Toluene: Ethylacetate: diethylamine (7:2:1) upto a distance of 8.0cm above the applied band at a tempeature of 27 ± 2^0 C. After devlopment the plate was removed and dried under a current of hot air. The purple coloured spot of reserpine was observed at R_f =0.69 and the plate was scanned at a wavelength of 200nm using Camag TLC scanner. The peak area of reserpine were recorded for each concentration. The calibration curve of reserpine was obtained by plotting a graph of peak area of reserpine verses concentration of reserpine. A linear regression was applied without forcing the intercept at X=0, while plotting the graph. A linear calibration curve was obtained in the range of 5 mcg to 25 mcg with a standad deviation of 0.85%.

Assay of Reserpine
Five mcL each of standard reserpine and the extracts of different parts of *R. tetraphylla* and *R. serpentina* were applied as 6mm bands by using Camag Linomat IV sample applicatos.The standard solution of the chemical marker (Standard reserpine) was also applied. The plates were devloped in Toluene: Ethylacetate: diethylamine (7:2:1) in CAMAG twin trough. The devloped plate was dried and then scanned at 200nm. UV spectrum scan of the various spots was also

carried out. The amount of reserpine present in each of the sample extracts were calculated by using regression equation (Table 1).

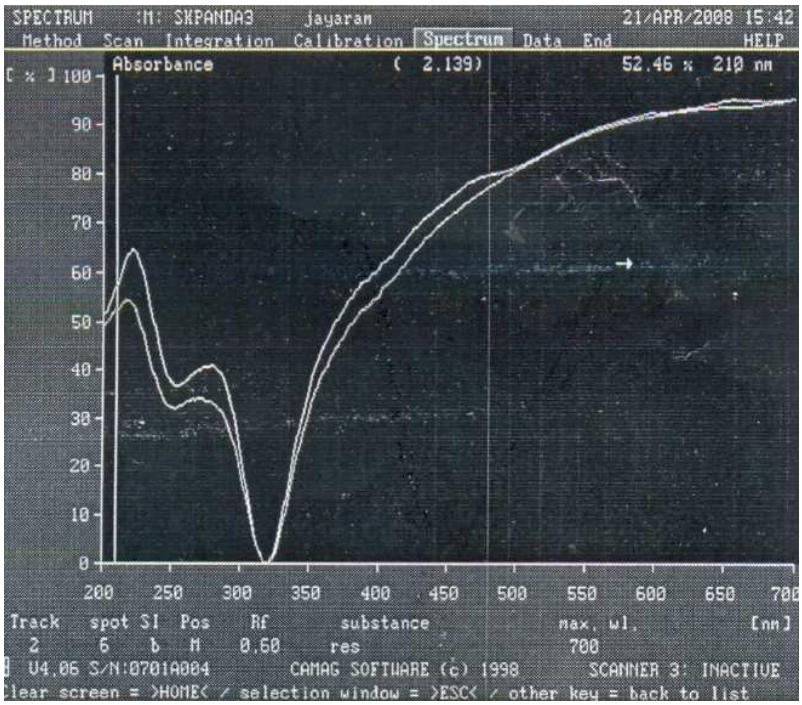


Fig 2: UV spectrum of reserpine

Table 1 : Assay of reserpine

Parts	Root (%w/w)	Stem(%w/w)	Leaf(%w/w)
R. Serpentina	0.456	0.191	0.062
R. tetraphylla	0.205	0.102	0.016

Method Validation

The method was validated as per ICH guidelines [7] for linearity, precision, limit of detection, limit of quantification, accuracy and specificity.

Accuracy

The accuracy of the method was assessed by performing recovery study by the addition of standard reserpine to the preanalysed extract of different parts of *R. Serpentina* and *R. tetraphylla*. The percent recoveries and the average percent recoveries were calculated for each (Table 2).

Table 2: Recovery study of reserpine

Plant	Parts	Amount of reserpine present in the test sample (mcg)	Reserpine added(mcg)	Amount found (mcg)	% Recovery
R. Serpentina	Root	6.5	10	16.44	99.63
	Stem	3.95	10	13.77	98.7
	Leaf	1.2	10	11.12	99.28
R. tetraphylla	Root	4.3	10	14.12	98.74
	Stem	1.41	10	11.18	97.98
	Leaf	0.64	10	10.43	98.02

Precision

Instrumental precision was checked by repeated scanning (n = 3) of the same spot of reserpine (20 µg) and expressed as relative standard deviation (% RSD). The repeatability of the method was affirmed by analyzing 20 µg of reserpine individually on TLC plate (n = 5) and expressed as % RSD (Table 3).

Table 3: Summary of validation parameters

Parameters	Results
Linearity	
Range(mcg/spot)	5-25
Equation	y = 387.95x + 120.06
Correlation coefficient (r)	0.9986
Standard deviation (%)	0.85
Precision (%RSD)	
Intra day (n = 3)	
Repeatability of Samples	0.94
Repeatability of peak area	1.42
Inter day (n = 3)	
Repeatability of Samples	0.84
Repeatability of peak area	1.12
Limit of detection (LOD, ng/spot)	42
Limit of quantification (LOQ, ng/spot)	124
Robustness	Robust

Linearity

The calibration curve for the estimation of OZ was constructed in the range 5-25mcg/spot and was checked for its linearity.

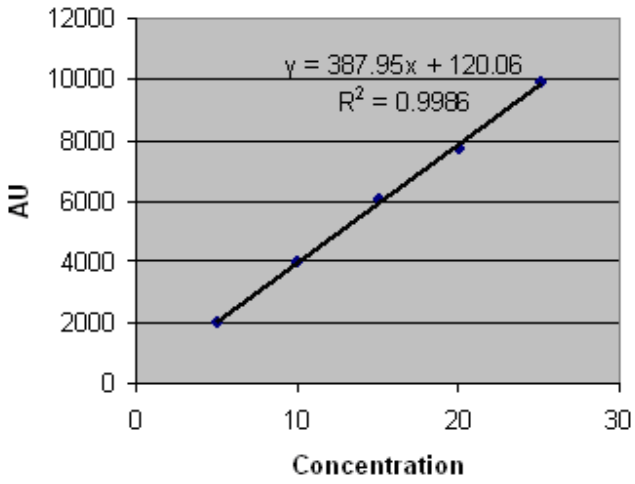


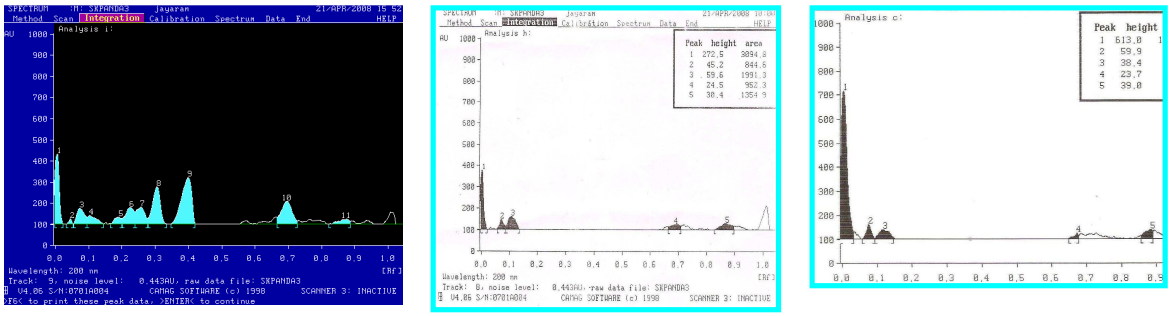
Fig 3: Linearity of standard reserpine

Limit of detection and quantitation (LOD and LOQ)

Limit of detection and quantitation was determined based upon signal-to-noise ratio.

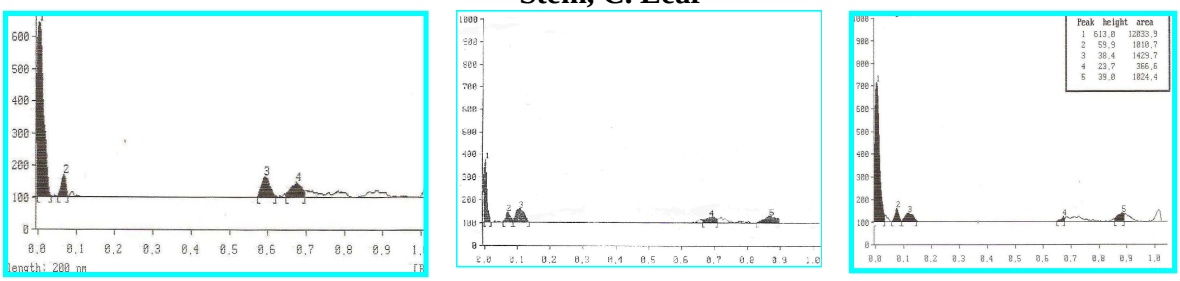
Robustness

Robustness of the method was determined by performing small variations in mobile phase ratio (i.e., small variations in volume), height of plate development and TLC tank saturation time. The results indicated insignificant differences in assay and thus indicative of a robust method.



A B C

Fig 4: Chromatograms of methanolic extract of different parts of R. serpentine; A. Root, B. Stem, C. Leaf



A B C

Fig 5: Chromatograms of methanolic extract of different parts of R. tetraphylla; A. Root, B. Stem, C. Leaf

RESULT AND DISCUSSION

A best TLC profile of methanolic extact (Fig 1) of different parts of *R. tetraphylla* and *R. Serpentina* along with reserpine standard was observed in the mobile phase of Toluene: Ethylacetate: diethylamine (7:2:1), in which the R_f value for reserpine was found to be 0.69. The identification of spot of reserpine in the sample solutions were confirmed by superimposing the UV absorption spectra of the peak (Rf 0.69) with that of chemical maker reserpine (Fig 2).

The detector responce of reserpine was found to be linearly dependent on the amount of reserpine against area under the curve as shown in fig 3. The equation of best fitting line was y = 387.95x + 120.06. The correlation coefficient obtained form the linearity was 0.9986. The chromatogram of methanolic extacts of different pats of *R. Serpentina* and *R. tetraphylla* are shown in fig 4 and 5. From the peaks coesponding to resepine the peak area of different extracts of *R. Serpentina* and *R. tetraphylla* were calculated. The amount of reserpine pesent in root, stem and leaf of *R. Serpentina* was found to be 0.456, 0.191and 0.062 %w/w of the extracts. For *R. tetraphylla* the amount of reserpine was found to be 0.205, 0.102 and 0.016 %w/w of the extracts in root, stem and leaf respectively. So it was found that 72% of reserpine present in *R. Serpentina* is found in its root where as 25% and 3% of reserpine are present in stem and root respectively. Similarly in case of *R. tetraphylla* the distribution of reserpine are 74%, 22% and 4% respectively. LOD was found to be 42 ng per spot. LOQ was calculated as 3.34 times of LOD i.e., 140.28 ng per spot but experimentally LOQ was found to be 124 ng per spot. The method was evaluated for pecision and repeatability in terms of % CV.

The value for repeatability of Samples and repeatability of peak area for intra day and inter day was found to be less than 2, which is indicative of a precise method. The mean recovery was found to be 98.72 indicating the reproducibility of the method. The method exhibited good robustness because the changes made in the chromatographic conditions did not influence the analytical results.

CONCLUSION

The proposed HPTLC method developed for the quantitative determination of reserpine in different parts of *R. Serpentina* and *R. Tetraphylla* was found to be simple, precise and accurate. The roots of this species are mainly explored rather than other parts. The analytical HPTLC data revealed that Reserpine is present in leaf, stem and root of both the species. So other parts of both the species can be explored for the isolation of bioactive reserpine. Further the commercial plant *R. Serpentina* can be minimized from over exploitations and extinction.

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