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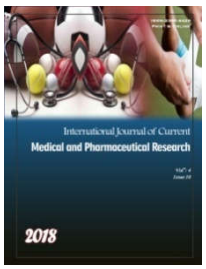

ASSESSMENT OF RESERPINE CONTENT BY HPTLC IN SOME AYURVEDIC FORMULATIONS CONTAINING *SARPAGANDHA* (*RAUWOLFIA SERPENTINA*)

Alok K. Hazra[1*]., Anindita Pal[2] and Tapas Kumar Sur[3]

[1]Quality Testing Laboratory, IRDM Faculty Centre, RKMVERI, Narendrapur, Kolkata
[2]School of Pharmacy, Techno India University, Kolkata
[3]Institute of Postgraduate Medical Education & Research, Kolkata

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ABSTRACT

Sarpagandha (Rauwolfia serpentina) is one of the most popular Vedic medicinal herbs used from 1000 BC to till date for reducing high blood pressure related ailments. It's a long journey of *Sarpagandha* from British India to the Western communities. Last 75 years intensive knowledge on *Sarpagandha* confirmed reserpine, an indole alkaloid is the most responsible component for its bioactivities. Reserpine also reported for severe adverse reactions, if not properly used. After that, several attempts were made to identify reserpine in formulations. In this context, a simplified valid method with precision instrument was developed for identification, quantification and standardization of herbal formulation containing *Sarpagandha* as an ingredient. Following Indian Ayurvedic Pharmacopeia's guidelines and advanced technological concepts three forms of Ayurvedic composition, namely *Sarpagandha Churna* (powder), *Sarpagandha Ghana Vati* (tablet) and *Sarpagandha Mishran* (tab) was studied. The present study, describes the easy procedure to determine reserpine content preciously in single herb or formulation. This study will be helpful not only for pharmaceuticals for standardization of formulation containing *Sarpagandha* as an ingredient and reserpine as bioactive constituents, but also be helpful for Ayurvedic practitioner to determine the dosage regimen for personalized medicine.

INTRODUCTION

Sarpagandha or *Rauwolfia serpentina* L. Benth Kurz is a small, woody, perennial shrub and is believed as one of the best-known medicinal plants in the world.[1] The plant was mentioned in the manuscripts of Indian system of medicine (Ayurveda) long ago since 1000 BC.[2] Traditional medical practitioners reckon the root of Sarpagandha for reducing high blood pressure and for treating various neurological symptomatic disorders including anxiety, psychosis, schizophrenia, epilepsy and insomnia.[3-7] For ages, it has been used in many parts of the world as an antidote against bite of poisonous snakes and reptiles.[8] The name Sarpagandha has been aptly derived for its special usage. The first modern paper on Sarpagandha was published in 1931 by Sen and Bose.[9] In 1949, Vakil published the first report of the antihypertensive effect of Sarpagandha.[10] Reserpine is the most important indole alkaloid present in root, stem and leaves of *R. serpentina*. It was first chemically isolated and identified by Muller and his co-workers in 1952 as methyl 18β-hydroxy-11,17α-dimethoxy-3β,20α-yohimban-16β-carboxylate-3,4,5-trimethoxybenzoate.[11] There are reports that 72% of reserpine is present in its root, whereas 25% and 3% of reserpine are present in stem and leaf respectively.[5,12-13] It has also been suggested that reserpine irreversibly blocks the vesicular monoamine transporter (VMAT), which usually transports free norepinephrine (NE), serotonine (5-HT) and dopamine (DA) from cytoplasm of presynaptic nerve terminal into storage vesicles and these neurotransmitters are metabolized by MAO (as well as by COMT) in the cytoplasm and consequently never reach the synapse.[14-17] A Cochrane Database Review has been undertaken to investigate the dose-related effects of reserpine on blood pressure, heart rate, and withdrawals due to adverse effects.[18] Besides its unique anti-hypertensive action, there are several reports that prolonged use and adequately higher dose of reserpine (above 0.5 mg/day) created serious adverse actions like, lethargy, sedation, psychiatric depression, hypotension, nausea, bradycardia, bronchospasm and withdrawal psychosis.[17]

Although in practice of Ayurvedic medicine, Sarpagandha is still popularly used either single herb as *Sarpagandha churna* or in classical formulations, like *Sarpagandha Ghana Vati,* and *Sarpagandha Mishran*.[19] Dose calculation of reserpine in terms of Sarpagandha used in different formulation is therefore essential for medical treatments and safety issues. But till date there are lack of simple valid method for quality control issues, particularly, standardization and quantification of reserpine

Corresponding author:* **Alok K. Hazra
Quality Testing Laboratory, IRDM Faculty Centre, RKMVERI, Narendrapur, Kolkata

present in Ayurvedic formulations.[20] Therefore, the objective of the present study was to identify and enumerate reserpine content wherever Sarpagandha has been used as ingredient in Ayurvedic formulations using high precision chromatographic techniques like, High Performance Thin Layer Chromatography (HPTLC).

MATERIALS AND METHODS

Test Drugs

Ayurvedic drugs *Sarpagandha Ghana Vati* (Baidyanath Ayurved Bhavan Pvt. Ltd, Kolkata) and *M-Sarpagandha Mishran* (IMPCL, Uttarakhand, India) were procured from local market and *Sarpagandha Churna* was provided by Quality Testing Laboratory, RKMVERI, Narendrapur, Kolkata. The compositions of test drug and formulations are given below:

Table 1 Description of test drug formulations

Test drug/Formulation	Ingredients	Quantity (%)
Sarpagandha Churna	Sarpagandha root powder	100
Sarpagandha Ghana Vati	Sarpagandha ghansatwa	50
	Khursaniajawain ghansatwa	10
	Jatamansi ghansatwa	5
	Bijay ghansatwa	5
	Pippalimool churna	25
	Excepients	5
M-Sarpagandha Mishran	Sarpagandha root churna	15.6
	Jatamansi root churna	15.6
	Vacha leaf churna	15.6
	Punarnava whole plant churna	15.6
	Brahmi whole plant churna	15.6
	Shankhapushpi whole plant churna	15.6
	Guduchi	3.4
	Excepients	3

Physico-chemical Properties

Determination of total ash: 1g of each test drug was incinerated in a crucible at 450^{0}C in a muffle furnace and cooled, weighed, and % of total ash was calculated.[21]

Determination of acid insoluble ash: Ash was further boiled for 5 min with 25 ml of 0.1 N HCl and filtered using ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and dried to a constant weight and finally, the percentage of acid insoluble ash was calculated.[21]

Extractive values: Extractive values of each test drug were determined using following methods:

Determination of alcohol soluble extractives: The powdered test drugs were macerated with 100 ml of alcohol in a closed flask for 24 h. It was then allowed to stand for 18 h and filtered. 25 ml of each filtrate was evaporated to dryness in a porcelain dish at 105^{0}C to constant weight. The percentage of alcohol soluble extractive was calculated.[21]

Determination of water soluble extractives: The powder test drugs were macerated with 100 ml of water in a close flask for 1 h. Then, it was boiled gently for another hour on water bath, cooled and weighed and the weight was re-adjusted. 25 ml of each filtrate was evaporated to dryness in a porcelain dish at 105^{0}C to constant weight. The percentage of water soluble extractive was calculated.[21]

Loss on drying: 1g of each drug was taken in a tarred glass bottle and heated at 105^{0}C in an oven till a constant weight. The percentage of moisture content was calculated.[21]

Extraction of Alkaloids

1g of each test powdered drug and 0.1g of Sarpagandha root powder were taken for extraction of reserpine. Each material was refluxed with 10 ml methanol containing 0.1M HCl in waterbath for an hour. Thereafter, sample solution was cooled, filtered and liquid-liquid separation was performed with n-hexane. The residual marc after hexane extraction was concentrated in reduced pressure and the residue was then dissolved in methanol-chloroform (98:2, v/v) in a 10 ml volumetric flask. Each sample solution was filtered through 0.22µm filter for HPTLC quantitative analysis.[22]

Estimation of reserpine by HPTLC

Alkaloid enriched test samples (as described earlier) were spotted in the form of bands with a Camag syringe (100µl) on precoated silica gel TLC plates (Merck, 60F_{254}, 10x10 cm) using automated Camag LinomatV applicator. Reserpine (SRL, India) was used as analytical standard for identification and calibration. Thereafter, the plate was developed in chloroform: toluene: ethyl acetate: diethylamine, (7:7:4:1 v/v). After development densitometric scanning was performed by Camag TLC Scanner3 at 268 nm and operated by WinCATS planar chromatography manager software.[23] The amount of reserpine present in test samples was calculated using standard calibration curve. ICH guidelines were followed for the method validation of the analytical procedures.[24]

Table 2 HPTLC parameters for Reserpine analysis

HPTLC Parameter	Reserpine
Calibration range (ng/spot)	4000-10000
Detection Wavelength (nm)	268
Mobile phase (Chloroform: Toluene: Ethyl acetate: Diethylamine, v/v)	7:7:4:1
R_f value	0.48
Regression equation	y = 595.6+ 412 x
Slope	412
Intercept	595.6
Correlation coefficient	0.9991

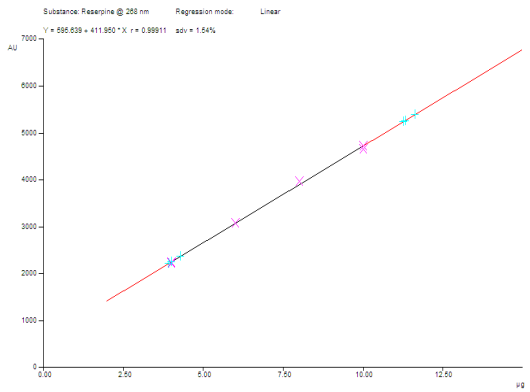

Fig 1 Standard calibration curve of reserpine

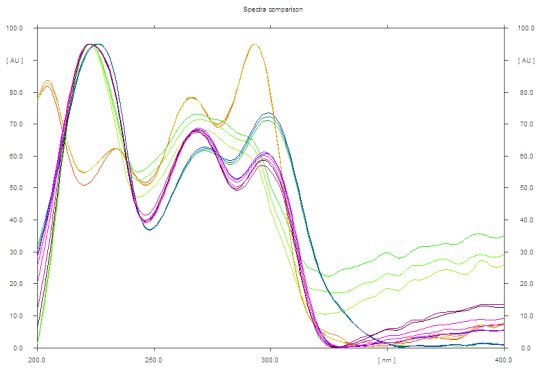

Fig 2 HPTLC Spectra of samples and standards

Table 3 Quality of Ayurvedic drugs containing *Sarpagandha*

Sample	Total Ash (%)	Acid insoluble ash (%)	Alcohol soluble extractive value (%)	Water soluble extractive value (%)	Loss on Drying (%)
Sarpagandha Churna (SC)	4.48 ± 0.18	0.12 ± 0.50	12.02 ± 0.38	56.41 ± 0.79	6.25 ± 0.53
Sarpagandha Ghana Vati (SGV)	25.29 ± 0.48	2.49 ± 0.29	28.24 ± 0.71	74.16 ± 0.86	5.26 ± 0.58
M-Sarpagandha Mishran (MSM)	14.61 ± 0.19	2.99 ± 0.56	16.14 ± 0.78	61.94 ± 0.78	5.55 ± 0.73

N=6; results are mean ± SEM;

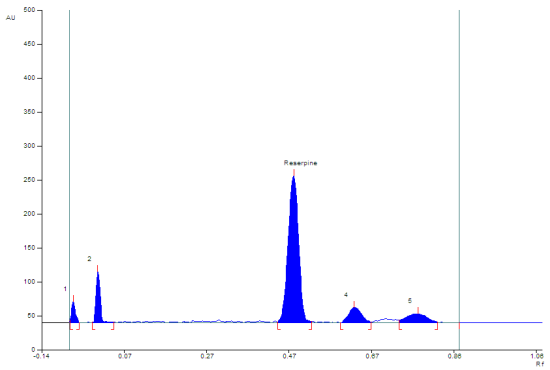

Fig 3 HPTLC of Reserpine standard

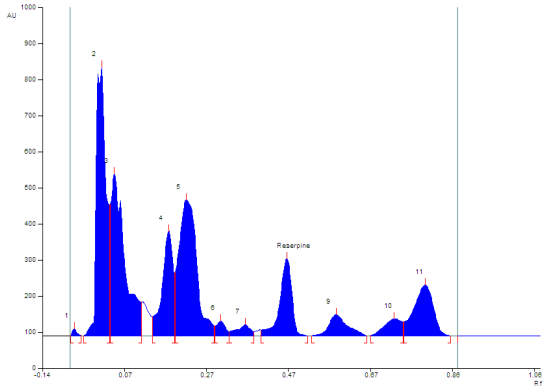

Fig 4 HPTLC of *Sarpagandha Churna*

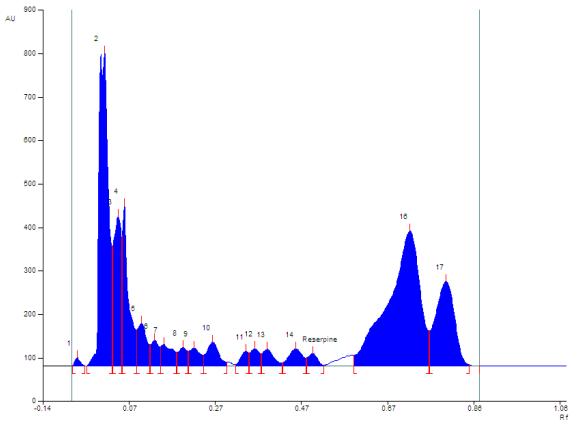

Fig 5 HPTLC of *Sapagandha Ghana Vati*

The method was validated for precision, repeatability and accuracy. The repeatability of the method was checked by repeated scanning of the same spot of reserpine (1 µg), six times and was expressed as co-efficient of variance (% CV). The variability of the method was studied by analyzing aliquots of reserpine on the same day and on different days and The outcome data were expressed as % CV. The recovery studies were done at three levels (50%, 100% and 150% addition). The percent recovery and average percent recovery was calculated for studying accuracy of method.

Statistical analysis

The results are presented as mean ± standard error of mean (SEM). The data are statistically analyzed using descriptive statistical methods.

RESULTS AND DISCUSSION

Assessment of Quality

The quality of the Ayurvedic drugs under investigation was performed according to Ayurvedic pharmacopoeia of India (API). The quality parameters for *churna* and *vati* drugs were chosen as described in API,[21] although particular drug was not described in pharmacopoeia. The ash content was highest in *Sarpagandha Ghana Vati* (SGV) whereas acid insoluble ash in *M-Sarpagandha Mishran* (MSM) was maximum than other two test drugs. Alcohol and water solubility was highest in SGV, whereas, loss on drying was nearly one percent higher in single herb powder (SC).

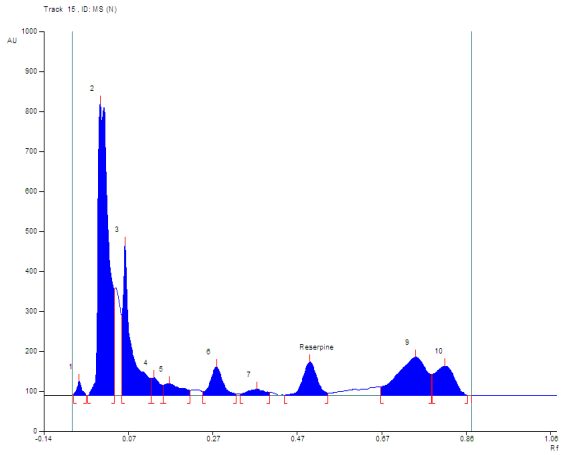

Fig 6 *M-Sarpagandha Mishran*

Assessment of Reserpine by HPTLC

Under the chromatographic conditions, the R_f value of reserpine is 0.48. The calibration curve for reserpine as standard is linear in the range of 4000-10000 ng shown in Fig 1. The regression coefficient of standard curve for analysis of different samples is 0.9991 and standard deviation is 1.54, shown in Table 2. A distinct identification of reserpine was observed from the spectral comparison of reference standard reserpine and samples wherein SC revealed almost similar absorption maxima characteristic to reserpine peak purity (Fig.

2). The chromatograms of standard reserpine, *Sarpagandha Churna*, *Sarpagandha Ghana Vati* and *M-Sarpagandha Mishran* are shown in Fig. 3-6 respectively having distinct features and clear resolution. The reserpine content in different samples is tabulated in Table 4.

Table 4 Reserpine content in Ayurvedic Drugs containing *Sarpagandha* by HPTLC

Sample name	Reserpine (mg/g)	Reserpine content in drug dosage form (mg/Tablet)
Sarpagandha Churna (SC)	18.99 ± 0.33	-
Sapagandha Ghana Vati (SGV)	1.50 ± 0.26	0.56[a]
M-Sarpagandha Mishran (MSM)	6.78 ± 0.32	1.69[b]

N=6; results are mean ± SEM; [a] average weight of each tablet of SGV is 375mg; [b] average weight of each tablet of MSM is 250mg

Sarpagandha contains many different phytochemicals including alkaloids. The most important alkaloid found in the plant is reserpine. The concentration of reserpine in the plant has been found to vary from 0.03% to 0.14% of the dry weight of the plant.[25] Other study confirmed reserpine content may be 0.9% to 6.65%.[26-27] In the present context, *Sarpagandha Churna* of root exhibited 1.89% reserpine, whereas two test formulations *Sarpagandha Ghana Vati* and *M-Sarpagandha Mishran* confirmed not more than 0.15% and 0.67% reserpine respectively. Obviously reserpine content may vary depending on the raw materials used in the formulation as well as preparation of drugs involving different process of manufacture. Dosage form of test drugs SGV and MSM is tablet weighing 375mg and 250mg respectively. The reserpine content in each tablet of SGV is 0.56mg and in each tablet of MSM is 1.69 mg. Moreover, HPTLC chromatograms exhibited *Sarpagandha Churna* has 11 peaks, *Sarpagandha Ghana Vati* has 17 peaks and *M-Sarpagandha Mishran* has 10 peaks that indicated fingerprint of test drugs. Individual peaks in a fingerprint chromatogram are usually depends on chemically distinct components. The concentration of reserpine is not only responsible for its therapeutic efficacies but also responsible for different symptomatic and non-symptomatic side effects.[28] Therefore, it is important to confirm the concentration of reserpine in the test drug dosage form and dosage regimen before prescribed it. In the treatment of hypertension or insomnia the prescribed dose of SGV is 1 tab/day and of MSM is 2 tabs/day. According to HPTLC analysis it is revealed that equivalent reserpine prescribed daily is 0.56mg of SGV and 3.4mg of MSM. The daily dose of reserpine in hypertensive patient is usually recommended globally below 0.5 mg/day or as directed by physician[16-17] and the maintenance dose is even lower than that. Higher doses create side effects that demerits its prescription. Based on this recommendation, SGV dosage regimen seems realistic than MSM. Therefore therapeutic regimen of *Sarpagandha* in hypertension should be checked and the dose should be calculated before prescribed to patients. The present study, describes the easy procedure to determine reserpine content precisely in single herb or formulation. This study will be helpful not only for standardization of formulation containing *Sarpagandha* as an ingredient and reserpine as bioactive constituents, but also be helpful for Ayurvedic practitioner to determine the dosage regimen for personalized medicine.

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