

# Comparative standardization study for determination of reserpine in *Rauwolfia serpentina* homoeopathic mother tinctures manufactured by different pharmaceutical industries using HPTLC as a check for quality control

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## Abstract

**Background:** *Rauwolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae) (Indian snakeroot), popularly known as *Sarpagandha* (Sanskrit), is used for the treatment of insanity, fever, snake bites, anxiety and in neuropsychiatric conditions. The antihypertensive actions of *Reserpine* are a result of its ability to deplete catecholamines (amongst other monoamine neurotransmitters) from peripheral sympathetic nerve endings which are normally involved in controlling heart rate, force of cardiac contraction and peripheral vascular resistance. **Objective:** Comparative study of *Reserpine* content in *R. serpentina* homoeopathic mother tinctures manufactured by different pharmaceutical industries and in-house mother tinctures applying high-performance thin-layer chromatography investigative techniques to facilitate the use of correct species. **Materials and Methods:** The authentic samples of roots of *R. serpentina* were supplied by Centre of Medicinal Plants Research in Homoeopathy, Emerald, Tamil Nadu, India. Authentic plant material was used to prepare the mother tincture (as per Homoeopathic Pharmacopoeia of India). *Reserpine* (C<sub>33</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>, M.P. 360°C, purity >99% w/w by high-performance liquid chromatography [HPLC]) was purchased from Sigma-Aldrich as a standard reference. The solvents for the study, namely, ethanol, HPLC water, toluene, ethyl acetate, diethylamine and chloroform were of analytical grade purity (MERCK Ltd.), used throughout. **Results:** Five samples of mother tinctures were used for the study, in-house mother tinctures (labelled: D and E) of *R. serpentina* shows a higher amount of *Reserpine* content than the marketed samples (labelled: A, B and C). **Conclusion:** It may be concluded that mother tinctures prepared by authentic plants showed the excess amount of *Reserpine* rather than that of mother tinctures procured from the market.

**Keywords:** High-performance thin-layer chromatography, Homoeopathic mother tincture, *Reserpine*

## INTRODUCTION

*Rauwolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae) is a plant whose roots are therapeutically used as a sedative, a hypnotic drug and in hypertension. *Reserpine* (an indole alkaloid) was isolated in 1952 from the dried root of *R. serpentina* (Indian snakeroot),<sup>[1]</sup> had been known as *Sarpagandha* (Hindi and Sanskrit), which is used for the treatment of insanity, fever, snake bites,<sup>[2]</sup> and in anxiety. The antihypertensive actions of *Reserpine* are a result of its ability to deplete catecholamines (amongst other monoamine neurotransmitters) from peripheral sympathetic nerve endings which are normally involved in controlling heart rate, force of cardiac contraction and peripheral vascular

resistance (Pharmacology, An Introduction: Pharmaceutical Sciences, Pharmacology, Edition 6, 2011, Henry Hitner and Barbara Nagle).

Its molecular structure was elucidated in 1953 and natural configuration published in 1955; the first total synthesis was accomplished by R. B. Woodward in 1958.<sup>[3]</sup> Santapau (1956)

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has studied botanical aspects of this plant. Kattel (1987) has reported phyllotaxical morphotypes. Variation of chemo-botanical characters in the indigenous collections of this plant was reported by Sethi *et al.* (1991).

*R. serpentina* mother tincture is used in Homoeopathy for treatment of blood pressure without any side effect, but in allopathic system of medicine, some side effects are reported such as nausea and vomiting, diarrhoea, shortness of breath, drowsiness, dizziness and headache.

The plant *R. serpentina* (L.) Benth ex Kurz (Apocynaceae) is a medicinally famous herb used in Ayurveda, Siddha, Unani, Homoeopathy and in Western systems of medicine.<sup>[3,4]</sup> There are different types of alkaloids present in *Rauwolfia* namely, ajmaline, ajmalinine, ajmalicine, serpentine and serpentinine.<sup>[5]</sup> *Reserpine*, *Yohimbine*,<sup>[6]</sup> rescinnamine, reserpine, rauwolfinine, renoxidine, rescinnamine, reserpiline, sarpagine, serpentinine, tetraphyllicine and 3-epi-a-yohimbine have also been reported. It also contains small amounts of phytosterol and fatty substances. The root of *R. serpentina* was found to possess 0.1% of the active principle, *Reserpine* (indole alkaloid).<sup>[7]</sup>

Number of factors relating to climate, altitude, rainfall and other conditions responsible for growth of plants affect the quality of active constituents present in a particular species even when it is grown in the same country. These conditions may produce major variations in the active constituents present in plants<sup>[8]</sup> and thus cause variation on the therapeutic efficacy. The understanding of how environmental factors affect the production of secondary metabolites will be of great importance for the conservation of medicinal plants and optimising field growth conditions for maximal recovery of active constituents. Resource availability theory suggests that the way a plant defends itself ultimately depends on resource availability and its intrinsic growth rate. This theory predicts that the rapidly growing plants in resource-rich habitats contain low levels of highly mobile secondary metabolites. Nitrogen is taken up early in the growing season in excess of the plant's need for growth. Excess nitrogen is available to be synthesised into N-based secondary metabolites.<sup>[9]</sup>

In Homoeopathy, mother tinctures( $\phi$ ) are defined as the original substance prepared with the aid of alcohol, directly from crude drug. They are the precursors of the corresponding potencies of the respective drug and the starting point for the production of homoeopathic medicines.<sup>[10]</sup> *R. serpentina* is one of the most important homoeopathic drugs being prescribed for various disorders including hypertension. Therefore, the present study is proposed to determine the quantity of the active constituent, *Reserpine* present in *R. serpentina* mother tinctures manufactured by different pharmaceutical industries were procured to ascertain whether there is uniformity or whether variation exists by applying high-performance thin-layer chromatography (HPTLC) technique studies.<sup>[10-12]</sup>

## MATERIALS AND METHODS

The roots of *R. serpentina* were collected by Center of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Tamil Nadu, and was authenticated by the staff of the Center of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Ooty. The voucher specimen has been deposited in the herbarium and in the laboratory of DDPR Central Research Institute for Homoeopathy, Noida, Uttar Pradesh, India, for future reference. Authentic plant material was used to prepare the Mother Tincture. Reserpine (C33H40N2O9, M. P. 360°C, purity >99% w/w by HPLC) was purchased from Sigma Aldrich. The solvents ethanol, HPLC water, toluene, ethyl acetate, diethyl amine, chloroform were of analytical grade purity (Merck Ltd.).

### Physicochemical studies

Moisture content was determined by loss on drying method. Total ash, water-soluble ash, foreign matter and acid-insoluble ash parameters were performed as per methods recommended in Homoeopathic Pharmacopeia of India.<sup>[13]</sup>

### Determination of physical constants (raw drug standardisation)

#### Loss on drying

Loss on drying is the loss of mass expressed as percentage w/w. The test for loss on drying determines both water and volatile matter in the crude drug by IR balance. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible.

An accurately weighed quantity of 2 g of powdered drug was taken in a porcelain dish. The porcelain dish was kept open in a vacuum oven, and the sample maintained at a constant temperature of 100°C. Then, it was cooled in a desiccator at room temperature. The procedure was repeated till constant weight on repeated weighing is observed. Percentage loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

### Determination of foreign matter

Weigh 100–500 g of the plant material under study and spread it out in a thin-layer. Inspect the sample with the unaided eye or with the use of a 6x lens and separate the foreign organic matter manually as completely as possible. Weigh the sorted foreign matter and determine the percentage of foreign matter from the weight of the drug taken.

### Ash value

Ash value is helpful in determining the quality and purity of a crude drug, especially in the powdered form. On incineration, crude drugs normally leave a quantity of ash as residue usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation

of non-dissipation of non-volatile elements. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

### Determination of total ash value

Accurately 2 g of the powdered drug in a silica crucible, previously ignited and weighed. Incinerate by gradually increasing the heat to temperatures not exceeding 450°C for 4 h, until free from carbon, crucible is cooled and weighed. Calculate the percentage of ash with reference to air-dried drug using the following formula.

$$\% \text{ Total ash value} = \frac{\text{Weight of crude drug taken}}{\text{Weight of total ash}} \times 100$$

### Determination of water-soluble ash value

The ash is boiled with 25 ml of water for 10 min. Filter and collect the insoluble matter on an ashless filter paper, wash with hot water and ignite in a crucible at a temperature not exceeding 450°C for 4 h. Cool in a dessicator and weigh. The difference in weight represents the weight of water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug using the following formula:

$$\% \text{ Water soluble ash value} = \frac{\text{Weight of total ash} - \text{Weight of water soluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

### Determination of acid-insoluble ash value

Boil the ash for 10 min with 25 ml of 2M HCl. Filter and collect the insoluble matter on ashless filter paper, wash with hot water and ignite in a crucible at a temperature not exceeding 450°C for 4 h. Cool in a dessicator and weigh. Calculate the percentage of acid-insoluble ash with reference to the air-dried drug using following formula:

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

The results obtained with reference to air-dried drug are tabulated and observations are recorded in Table 1.

### Phytochemical analysis

Phytochemical tests were conducted on the roots of *R. serpentina* to identify various phytochemicals present in the plant material (A.K. Gupta *et al.*, 2008).<sup>[14-16]</sup> The various tests conducted are given below and the observations are recorded in Table 2.

- Test for tannins (lead acetate test):** To the test solution, a few drops of 10% lead acetate were added. Precipitate was formed, indicates the presence of tannins
- Test for saponins (froth test):** A pinch of the dried powdered plant material was added to 2–3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicates the presence of saponin
- Test for triterpenoids (Salkowski's test):** To the test

solution, add a few drops of concentrated sulphuric acid, shake well and allow to stand for some time. Red colour appears in the lower layer indicating the presence of sterols and formation of yellow-coloured lower layer indicates the presence of triterpenoids

#### 4. Test for flavonoids:

- Sulphuric acid ( $\text{H}_2\text{SO}_4$  test): The test solution was treated with concentrated  $\text{H}_2\text{SO}_4$ , formation of orange colour indicates the presence of flavonoids

#### 5. Test for phenolic compounds (ferric chloride test):

To the test solution, few drops of ferric chloride test reagent were added. An intense green, purple, blue or black colour developed is taken as an evidence for the presence of tannins

#### 6. Tests for alkaloids:

- Dragendorff's test - To 2–3 ml of the filtrate, add a few drops of Dragendorff's reagent. Observe for formation of orange-brown precipitate
- Mayer's test - To 2–3 ml of the filtrate, add a few drops of Mayer's reagent. Observe for formation of precipitate
- Hager's test - To 2–3 ml of the filtrate, add a few drops of Hager's reagent. Observe for formation of yellow precipitate
- Wagner's test - To 2–3 ml of the filtrate, add a few drops of Wagner's reagent. Observe formation of reddish brown precipitate.

The results of phytochemical tests carried out are recorded in Table 2.

### Preparation of in-house mother tinctures

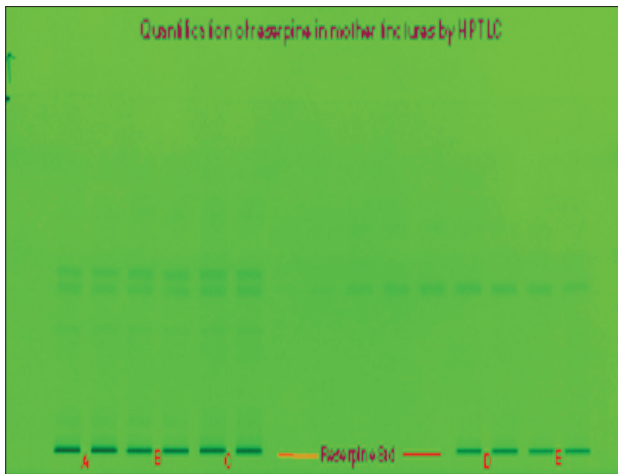
100 g of coarsely powdered root were taken, added 824 ml alcohol and 200 ml water to make 1000 ml of mother tincture using the percolation method (as per Homoeopathic

**Table 1: Test of raw material**

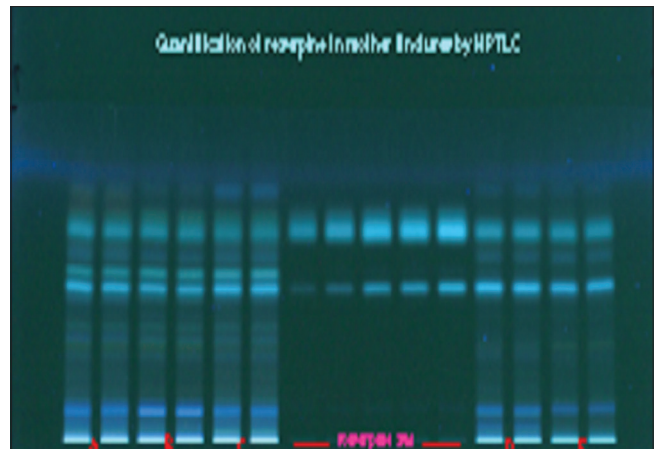
Name of test	Result (%)
Loss on drying	Not >8.13
Total ash	Not >4.09
Water-soluble ash	Not >2.88
Acid-insoluble ash	Not >0.85

**Table 2: Phytochemical tests**

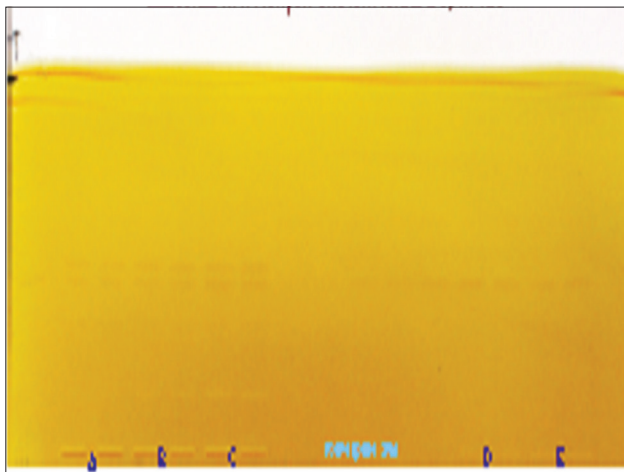
Name of phytochemical	Result
Tannins (lead acetate test)	Positive
Saponin (foam test)	Positive
Phenolic compounds ( $\text{FeCl}_3$ test)	Positive
Flavonoids (alkaline reagent test)	Positive
Triterpenoid (salkowski test)	Positive
Alkaloids	Positive
Mayer's test	Positive
Hager's test	Positive
Wagner's test	Positive
Dragend orff's test	Positive



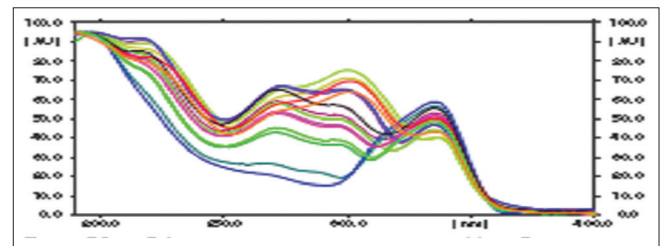
**Figure 1:** High-performance thin-layer chromatography fingerprints of A, B, C, D and E samples (*Rauwolfia serpentina*) under UV 254 nm



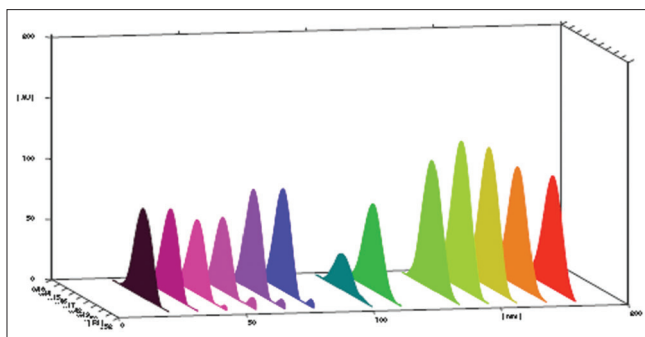
**Figure 2:** High-performance thin-layer chromatography fingerprints of A, B, C, D and E samples (*Rauwolfia serpentina*) under UV 366 nm



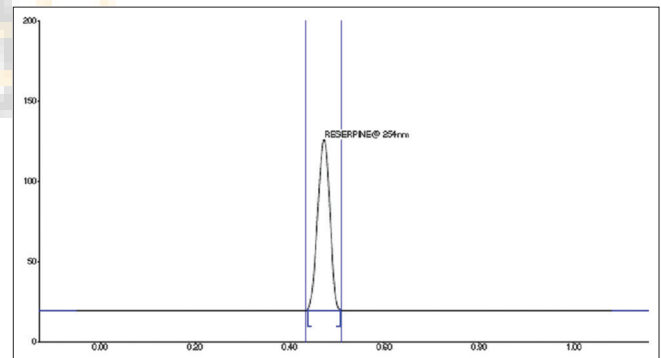
**Figure 3:** High-performance thin-layer chromatography fingerprints of A, B, C, D and E samples (*Rauwolfia serpentina*) after derivatisation



**Figure 4:** Overlay of absorption spectra of standard, in-house and market mother tinctures



**Figure 5:** Chromatogram of standard, in-house and market mother tinctures



**Figure 6:** Chromatogram of *Reserpine* ( $R_f = 0.44$ )

tincture (Banerjee, D.D. 2006, Augmented Textbook of Homoeopathic Pharmacy: B.Jain Publishers).<sup>[17]</sup>

**1. Organoleptic properties**

- Appearance: Clear liquid
- Colour: Yellowish brown
- Odour: Characteristic

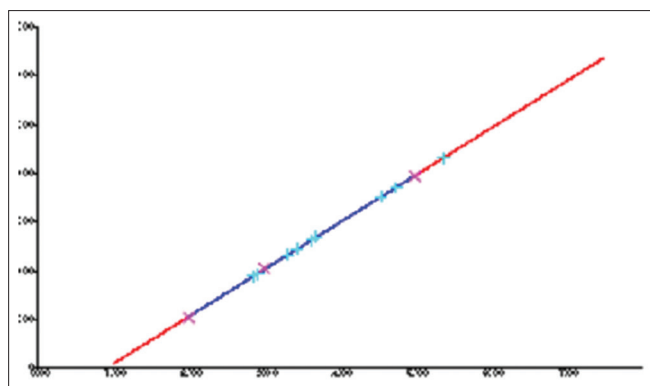
**2. Physicochemical properties**

- Sediments: Nil
- Weight per ml: 0.867–0.877 g
- Total solid: Not <1.0 percent w/v
- Alcohol content: 75.0–79.0 per cent v/v
- pH value: 5.9.

Pharmacopoeia of India).<sup>[17]</sup> This tincture was transferred to a suitable glass container and stored for further study.

**Standardisation of mother tincture**

Standardisation of mother tincture was conducted to identify the organoleptic and physicochemical properties of mother



**Figure 7:** Calibration curve of *Reserpine* in market samples and in-house mother tincture

### Quantification of *Reserpine* by high-performance thin-layer chromatography study

Quantification of *Reserpine* was done by HPTLC as mentioned below.

#### Preparation of standard *Reserpine*

Five milligrams of *Reserpine* was weighed in a 10 ml volumetric flask. To this, 5 ml chloroform and 5 ml ethanol were added to make final volume 0.5 µg/µl.

#### Preparation of sample

5 ml of mother tincture (about 4 g) was taken in 100 ml beaker, added 10 ml of distilled water and 0.2 ml concentrated hydrochloric acid; evaporated to dryness on water bath. Dissolved the residue in 2 ml of chloroform, methanol mixture (1:1) then carried out HPTLC analysis.

#### 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 × 10 cm.

Mobile Phase: Toluene-Ethyl Acetate-Diethylamine (7:2:1, v/v/v).

Wavelength: 254 nm.

#### Standardisation of in-house mother tincture

CAMAG HPTLC system comprising Linomat 5 as sample applicator and TLC Scanner controlled by winCATS software was used for quantitative evaluation. Stationary phase used was silica gel 60 F<sub>254</sub> and the mobile phase used was toluene-ethyl acetate-diethylamine (7:2:1, v/v/v). Samples and standard were applied as 8 mm bands with 6 mm distance between the tracks. Tank saturation and plate equilibrium were given with filter paper for 10 min. Ascending development for a distance of 80 mm in a Twin Trough Chamber was completed in approximately 15 min. Volume of standard mother tincture (ϕ) was first optimised at 4 µl for quantification. The λ max of *Reserpine* was found to be 254 nm after taking the spectra of the standard of *Reserpine*. Quantitative measurement in the absorbance mode was done at 254 nm using a slit dimension of 6.00 mm × 0.45 mm.

#### Linearity response

The volume of the in-house mother tincture was optimised to 2 µl for quantification. It was then simultaneously applied with different concentrations of standard *Reserpine*, i.e., 4, 6, 8 and 10 µl. The method was found to be linear with a regression of 0.99983, and a standard deviation of 1.67% and the amount of *Reserpine* was calculated in the mother tincture.

#### Standardisation of the in-house mother tinctures

Standardisations of the mother tinctures were done using HPTLC method. In-house mother tinctures were chromatographed simultaneously along with three other mother tinctures available from the market at 2 and 5 µl, respectively, on the same plate for comparison [Figures 1-3]. Multiwavelength (MWL) scan was done for finding the optimum wavelength for scanning. The optimum wavelength was found to be 254 nm. The entire plate was further scanned at this wavelength for quantification and spectral match [Figures 4 and 5]. Many fractions of in-house mother tinctures were matched with the help of its characteristic spectra with that of other marketed samples. Individual λ max of each fraction was also found with the help of spectral scanning, and then the plate was scanned with these selected wavelengths in MWL mode. The pattern of the peaks was compared for the in-house mother tinctures and marketed samples. It was observed that the response for various concentrations of standard *Reserpine* was linear in the range of 100–500 ng with a coefficient of variation of 0.99983 and a standard deviation of 1.67%. *Reserpine* was quantified and the amount was calculated in individual mother tinctures. With this method, all available mother tinctures were compared and the active principle was quantified [Figures 6 and 7].

#### Quantification of *Reserpine* in market samples and in-house mother tincture

##### Procedure

5 ml each of mother tincture taken for analysis, i.e. 5 ml of mother tincture weight is 4 g or 4000 mg, i.e., 5 ml = 4000 mg. Therefore, 1 ml = 800 mg or 1 µl = 0.800 µg

Hence, final concentration (2 ml) by dissolving chloroform and methanol mixture is (1:1, v/v) ratio. Sample applied on plate for sample A, B and C is 5 µl each and for sample for D and E is 2 µl.

So final concentration on plate of

- Sample A = 0.800 × 5 = 4000 µg
- Sample B = 0.800 × 5 = 4000 µg
- Sample C = 0.800 × 5 = 4000 µg
- Sample D = 0.800 × 2 = 1600 µg
- Sample E = 0.800 × 2 = 1600 µg.

#### Calculation of *Reserpine* content in sample A

- 4000 µg sample (A) on plate
- *Reserpine* content from calibration graph = 337.56 ng or 0.33756 µg.

#### Percentage of *Reserpine* in sample A

4000 µg = 0.33756 µg

$$\text{Therefore } 100 \mu\text{g} = \frac{0.33756 \times 100}{4000} = 0.0084\% \text{ of reserpine in sample A}$$

#### Calculation of *Reserpine* content in sample B

- 4000  $\mu\text{g}$  sample on plate
- *Reserpine* content from calibration graph = 288.57 ng or 0.28857  $\mu\text{g}$ .

#### Percentage of *Reserpine* content in sample B

$$4000 \mu\text{g} = 0.28857 \mu\text{g}.$$

$$\text{Therefore } 100 \mu\text{g} = \frac{0.28857 \times 100}{4000} = 0.0072\% \text{ of reserpine in sample B}$$

#### Calculation of *Reserpine* content in sample C

- 10,000  $\mu\text{g}$  sample (B) on plate
- *Reserpine* content from calibration graph = 366.26 ng or 0.36626  $\mu\text{g}$ .

#### Percentage of *Reserpine* content in sample C

$$4000 \mu\text{g} = 0.36626 \mu\text{g}$$

$$\text{Therefore } 100 \mu\text{g} = \frac{0.36626 \times 100}{4000} = 0.0091\% \text{ of reserpine in sample C}$$

#### Calculation of *Reserpine* content in sample D

- 1600  $\mu\text{g}$  sample on plate
- *Reserpine* content from calibration graph = 538.18 ng or 0.53818  $\mu\text{g}$ .

#### Percentage of *Reserpine* content in sample D

$$1600 \mu\text{g} = 0.53818 \mu\text{g}$$

$$\text{Therefore } 100 \mu\text{g} = \frac{0.53818 \times 100}{1600} = 0.5381\% \text{ of reserpine in sample D}$$

#### Calculation of *Reserpine* content in sample E

- 1600  $\mu\text{g}$  sample on plate
- *Reserpine* content from calibration graph = 465.11 ng or 0.46511  $\mu\text{g}$ .

#### Calculation of *Reserpine* content in sample E

$$1600 \mu\text{g} = 0.46511 \mu\text{g}$$

$$\text{Therefore } 100 \mu\text{g} = \frac{0.46511 \times 100}{1600} = 0.0290\% \text{ of reserpine in sample E}$$

The amount of *Reserpine* in *R. serpentina* in the in-house sample (D and E) and market sample mother tinctures (A, B and C) were calculated and presented in Table 3.

**Table 3: Content of *Reserpine***

Name of sample	Percentage of <i>Reserpine</i> content
A	0.0084
B	0.0072
C	0.0091
D	0.5381
E	0.0290

## DISCUSSION

Repeatability of the method was checked by scanning 15 tracks of 2  $\mu\text{l}$  volume in-house mother tincture. The coefficient of variation was found to be 0.0844. The percentage recovery of *Reserpine* was calculated using the above method. The average recovery values obtained were 96.6%–104.37%, which confirms that the method is validated. The HPTLC scanning of '*R. serpentina*' mother tincture ( $\phi$ ) obtained from manufacturer (A, B and C) and the in-house mother tinctures (D and E) had been done at 254 nm wavelength. The scanning report obtained after integration. From the results obtained after densitometry scanning, it was observed that the in-house mother tinctures (D and E) of *R. serpentina* shows a higher amount of *Reserpine* content than the marketed samples (A, B and C). It may be concluded that samples procured from the market are showing a lesser amount of *Reserpine* hence may not be up to the standard level. This quantification may lead to better quality checking of market samples which in turn will be responsible for better therapeutic efficacy.

## CONCLUSION

It is concluded that in-house prepared mother tincture showed excess greater amount of *Reserpine*, i.e., 0.5381% and 0.0290% (D and E) in comparison to marketed samples (A, B and C).

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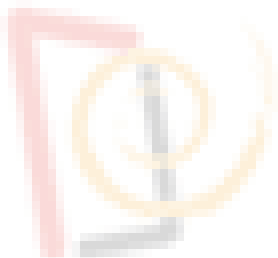
## Conflicts of interest

None declared.

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**एचपीटीएलसी का उपयोग करके विभिन्न औषध निर्माण (फार्मास्युटिकल) उद्योगों द्वारा निर्मित रोवोल्फ़िया सर्पेंटीना होम्योपैथिक मदर टिंचर्स में रेसरपीन का तुलनात्मक मानकीकरण अध्ययन |**

**सार**

**पृष्ठभूमि:** रोवोल्फ़िया सर्पेंटीना (इंडियन स्नेकरूट) जिसे सर्पगंधा के नाम से जाना जाता है, का पागलपन, ज्वर, सर्पदंश, चिंता और तंत्रिका मनोविज्ञान की स्थितियों के इलाज के लिए उपयोग किया जाता है। रेसरपीन का उच्चरक्तदाबरोधी (एंटीहाइपर टेसिव) असर पैरा सिंथेटिक नर्व एंडीग्स से कैटेकोलामाइन को समाप्त करने की इसकी क्षमता का एक परिणाम है जो आमतौर पर हृदय गति, हृदय संकुचन और परिधीय संवहनी प्रतिरोध के बल को नियंत्रित करने में सहायक होते हैं।

**उद्देश्य:** सही प्रजातियों के उपयोग की सुविधा के लिए एचपीटीएलसी का उपयोग करके विभिन्न औषध निर्माण उद्योगों द्वारा निर्मित आर. सर्पेंटीना होम्योपैथिक मदर टिंचर्स में रेसरपीन सामग्री का तुलनात्मक अध्ययन।

**सामग्री और विधि:** आर. सर्पेंटीना की जड़ें होम्योपैथी औषधीय पादप अनुसंधान केन्द्र (सीएमपीआरएच), एमराल्ड, ऊटी से एकत्रित की गईं। सिग्मा एल्डरिक से खरीदी गई रेसरपीन (सी33 एच40 एन2 ओ9, एम. वी. 3600 बए शुद्धता झ 99 प्रतिशत डब्लू/डब्लू एचपीएलसी द्वारा) की सहायता से मदर टिंचर का निर्माण किया गया। एथेनॉल, एचपीएलसी जल, टोलूइन, इथाइल एसीटेट, डाइइथाइल एमाइन, क्लोरोफॉर्म आदि विलायक विश्लेषणात्मक ग्रेड की शुद्धता (एमइआरसीके लिमिटेड) के थे।

**परिणाम:** डॉ. डी.पी. रस्तोगी केन्द्रीय होम्योपैथी अनुसंधान संस्थान, नोएडा में बनाये गये आर. सर्पेंटीना की मदर टिंचर्स (डी और ई), बाजार में उपलब्ध नमूनों (ए, बी और सी) की तुलना में रेसरपीन सामग्री की उच्च मात्रा को दर्शाते हैं।

**निष्कर्ष:** यह निष्कर्ष निकाला जा सकता है कि प्रामाणिक पादपों से तैयार मदर टिंचर्स ने बाजार से खरीदी गई मदर टिंचर्स की तुलना में अधिक मात्रा में रेसरपीन दिखाया।

### Vergleichende Standardisierungsstudie von Reserpin in *Rauwolfia serpentina* homöopathischen Mutter Tinkturen, die von verschiedenen pharmazeutischen Industrien unter Verwendung von HPTLC hergestellt wurden

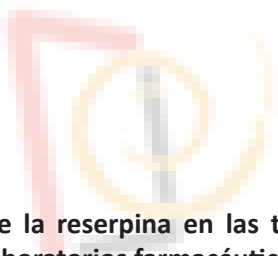
**Hintergrund:** *Rauwolfia serpentina* (Indian Snakeroot) im Volksmund bekannt als Sarpagandha und verwendet für die Behandlung von Wahnsinn, Fieber, Schlangenbisse, Angst und neuropsychiatrischen Bedingungen. Die antihypertensiven Wirkungen von Reserpin sind ein Ergebnis ihrer Fähigkeit, Katecholamine (unter anderen Monoamineneurotransmittern) aus peripheren sympathischen Nervenendigungen, die normalerweise an der Kontrolle der Herzfrequenz, der Kraft der Herzkontraktion und des peripheren Gefäßwiderstands beteiligt sind, abzubauen.

**Ziel:** Vergleichende Untersuchung des Reserpin-Gehalts in *R. serpentina* homöopathischen Mutter-Tinkturen, die von verschiedenen pharmazeutischen Industrien und im Haus Mutter-Tinkturen unter Verwendung von HPTLC hergestellt wurden, um die Verwendung von korrekten Spezies zu erleichtern.

**Materialien und Methoden:** Die Wurzeln der *R. serpentina* wurden vom Zentrum der Heilpflanzenforschung in der Homöopathie (CMPRH), Smaragd, Ooty gesammelt. Authentisches Pflanzenmaterial wurde verwendet, um die Mutter Tinktur vorzubereiten. Reserpin (C<sub>33</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>, M.P. 360 ° C, Reinheit > 99% G / G durch HPLC) wurde von Sigma Aldrich bezogen. Die Lösungsmittel Ethanol, HPLC Wasser, Toluol, Ethylacetat, Diethylamin, Chloroform waren von analytischer Reinheit (MERCK Ltd.).

**Ergebnisse:** Die im Labor von DDP, CRI (H) Noida (D und E) von *R. serpentina* hergestellten Mutter-Tinkturen zeigen eine höhere Menge an Reserpin-Gehalt im Vergleich zu Mutter-Tinkturen, die von verschiedenen pharmazeutischen Industrien (A, B und C) hergestellt wurden.

**Fazit:** Es kann gefolgert werden, dass Muttertinkturen, die von authentischen Pflanzen hergestellt wurden, die überschüssige Menge an Reserpin anstatt das zeigten Der aus dem Markt beschafften Muttertinkturen.



### Estudio de estandarización comparativa de la reserpina en las tinturas madre homeopáticas de *Rauwolfia serpentina* confeccionadas por diferentes laboratorios farmacéuticos utilizando la HPTLC

#### RESUMEN:

**Fundamento:** *Rauwolfia serpentina* (raíz de serpiente de la India) conocida popularmente como *Sarpagandha*, se ha utilizado en el tratamiento de locura, fiebre, mordeduras de serpiente, ansiedad y patologías neuropsiquiátricas. Los efectos antihipertensivos de la reserpina se deben a su capacidad de depleccionar las catecolaminas (entre otros neurotransmisores monoamínicos) de los terminales nerviosos simpáticos periféricos que normalmente intervienen en el control de la frecuencia cardíaca, la fuerza de contracción cardíaca y la resistencia vascular periférica.

**Objetivos:** Estudio comparativo del contenido en reserpina de las tinturas madres homeopáticas de *R. serpentina* confeccionadas por diferentes laboratorios farmacéuticos y de las tinturas madres internas mediante HPTLC (cromatografía de capa fina de alto rendimiento) para fomentar el uso de las especies correctas.

**Materials and Methods:** Las raíces de la *R. serpentina* se recogieron en el Centro de Investigación de Plantas Medicinales en Homeopatía (CMPRH), Emerald, Ooty. El material auténtico de la planta se utilizó para preparar la tintura madre. La reserpina (C<sub>33</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>, M.P. 360°C, pureza >99% p/p por HPLC) se adquirió en Sigma Aldrich. Los disolventes etanol, agua HPLC, tolueno, etilacetato, dietilamina y cloroformo eran de pureza de grado analítico (MERCK Ltd.).

**Resultados:** Las tinturas madre preparadas en el laboratorio de DDP, CRI(H) Noida (D y E) de *R. serpentina* mostraron una mayor cantidad de contenido en reserpina, en comparación con las tinturas madres fabricadas por diferentes laboratorios farmacéuticos (A, B y C).

**Conclusiones:** Cabe concluir que las tinturas madre preparadas con las plantas auténticas mostraron una mayor cantidad de reserpina que las tinturas madre adquiridas en el mercado.



## Etude comparative standardisée de la réserpine présente dans la teinture mère de *Rauwolfia serpentina* fabriquée par différentes industries pharmaceutiques utilisant le HPTLC

### RESUME:

**Contexte:** *Rauwolfia serpentina* (Indian snakeroot) dont le nom populaire est Sarpagandha est utilisé dans le traitement de la démence, pour des fièvres, après des morsures de serpents, pour l'anxiété et certaines pathologies psychiatriques. L'action antihypertensive de la réserpine résulte de sa capacité à réduire les cathécolamines ( parmi d'autres neurotransmetteurs) des terminaisons nerveuses périphériques sympathiques, qui normalement sont impliquées dans le contrôle du rythme cardiaque, de la contractilité cardiaque, et des résistances vasculaires périphériques

**Objectif:** L'étude comparative de la réserpine contenue dans la teinture mère de *R. serpentina* fabriquée par différentes industries pharmaceutiques et la teinture mère artisanale à l'aide du HPTLC pour s'assurer que les bonnes espèces sont utilisées.

**Méthodes et matériaux:** les racines de *R. serpentina* ont été récoltées au Center of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Ooty. Un matériel végétal authentifié a été utilisé pour préparer la teinture mère. La réserpine (C<sub>33</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>, M.P. 360°C, pureté >99% w/w par HPLC) a été achetée chez Sigma Aldrich. Les solvants, éthanol, HPLC, eau, toluène, éthyl acétate, diéthyl amine, chloroforme avaient un degré de pureté analytique (MERCK Ltd.).

**Résultats:** La teinture mère de *R. serpentina* préparée par le laboratoire du DDP, CRI(H) Noida (D and E) présente une quantité de réserpine plus importante que les autres teintures mères fabriquées par les différentes industries pharmaceutiques (A, B and C).

**Conclusion:** On peut en conclure que la teinture mère fabriquée à partir de la plante authentique présente de la réserpine en excès par rapport aux teintures mères présentes sur le marché.



使用高效薄層色譜法 (HPTLC)，去比較不同製藥廠生產的印度蛇根草順勢療法母酊，當中的蛇根碱標準水平

摘要：

**背景：**印度蛇根草 (*Rauwolfia serpentina*) 的通俗名稱為 Sarpagandha，它被用作處理精神錯亂、發燒、蛇咬、焦慮和神經精神病的情況。蛇根碱的抗高血壓作用，是來自它作用於周邊交感神經末梢，並（在其他單胺類神經遞質之中）耗盡兒茶酚胺的能力，通常會用於控制心跳率、心臟收縮力量和周邊血管阻力。

**目標：**使用 HPTLC，去比較不同製藥廠生產的印度蛇根草順勢療法母酊，以及機構內的母酊，當中的蛇根碱含量，並促進使用正確的品種。

**原料和方法：**從烏塔卡蒙德的翡翠湖的順勢療法藥用植物研究中心 (CMPRH) 取得印度蛇根草的根部。使用可靠的植物原料來製作母酊。由西格瑪奧德里奇公司中購買蛇根碱 (C<sub>33</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>，溶點：360°C，

**純度：**使用高效液相色譜法 (HPLC) 檢測，超過 99% 的重量比例)。溶劑，包括乙醇、HPLC 級水、甲苯、乙酸乙酯、二乙胺、三氯甲烷，都達到分析級純度 (MERCK Ltd.)。

**結果：**由諾伊達 DDP 順勢療法中央研究院 (DDP, CRI(H) Noida) 的實驗室製作的印度蛇根草母酊 (樣本 D 和 E)，比不同製藥廠所生產的母酊 (樣本 A、B 和 C)，有更高的蛇根碱含量。