



## Standardization of HPLC Method of Scopoletin in Different Extracts of *Convolvulus pluricaulis*

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### ABSTRACT

Different extract of *Convolvulus pluricaulis* choisy, (Methanol, hydro-alcohol (50%) and water) were prepared and tested of scopoletin. The maximum scopoletin content was observed in 50% ethanolic extract followed by methanol and water extracts. It was 0.1738%, 0.0932% and 0.0435% in hydro-alcoholic, Methanol and water extract respectively. A simple HPLC (High Performance Liquid Chromatography) was developed for the determination of Scopoletin in *Convolvulus pluricaulis* choisy. Shankpushpi is an astringent, hot aphrodisiac and a nervine tonic. It improves strength, digestive power, helpful in epilepsy, insomnia, heart disease and hematemesis. Analyte separation and quantification were achieved by high-performance liquid chromatography (HPLC) and UV detection at 366 nm. The method involves the use of C<sub>18</sub> column (Phenomenex, 250 mm × 4.6 mm, 5μm) with isocratic mixture of methanol and water containing 0.1% v/v formic acid in the ration of 30:70. Linearity was observed in the range of 20-100 ppm with correlation coefficient of 0.9961. Relative standard deviation of linearity of the method was found to be 1.29%. Detection limit was 5.0 ppm and quantification limit was 7.5 ppm. The repeatability of the method was found to be 0.71%. Recovery values from 99.10 to 100.1% indicate best accuracy of the method.

**Keywords:** *Convolvulus pluricaulis* Choisy, Liquid chromatography, Scopoletin, Standardization.

### INTRODUCTION

*Convolvulus pluricaulis* Choisy is a prostrate spreading perennial wild herb commonly found on sandy or rocky ground under xerophytic conditions in northern India. In India it is widely distributed in and grows on the waste land in the plains of Punjab, Bihar and Chhotanagpur. The leaves of Shankpushpi were used to treat chronic bronchitis and asthma. The root was used for childhood fever, and the oil stimulates the growth of hair. The whole herb was used medicinally in the form of a decoction with cumin and milk in fever, nervous debility, and loss of memory, syphilis and scrofula.

*Convolvulus pluricaulis* (CP) is a common plant in southern India where the whole plant is used in various formulae as a nervine tonic for improvement of memory and intellect. [1] The leaves and flowers possess hypotensive properties used for treating anxiety neurosis. [2] It is recommended as a brain tonic to promote intellect and memory, eliminate nervous disorders and to treat hypertension. [3]

It has been widely used in Ayurvedic medicine to treat nervous disorders, similar to the use of kava kava (*Piper methysticum*) and valerian (*Valeriana officinalis*) is prescribed by American herbalists. [4] It is only recently that Shankpushpi has been brought to American stores for medicinal use. Herbalists believe that Shankpushpi calms the nerves by regulating the body's production of the stress hormones, adrenaline and cortisol. [5]

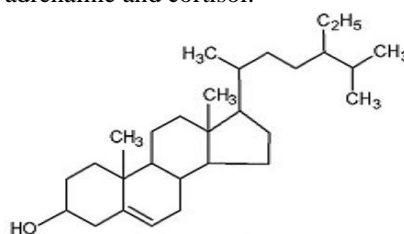


Fig. 1: Structure of scopoletin

The ethanolic extract of CP and its ethyl acetate and aqueous fractions were evaluated for their memory enhancing properties. Two doses (100 and 200 mg/kg/p.o.) of ethyl acetate and aqueous fractions of the ethanolic extract were administered in separate groups of animals. Both the doses of all the extracts, significantly improved learning and memory in rats. [6] An ethanolic extract of whole plant when

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administered to cholesterol fed gerbils, reduced serum cholesterol, LDL cholesterol triglycerides and phospholipids significantly after 90 days. [7] A methanolic extract of the whole plant produced alterations in the general behaviour pattern, reduction in spontaneous motor activity, hypothermia, potentiation of pentobarbitone sleeping time, reduction in exploratory behavioural pattern and suppression of aggressive behavior. [8]

A total water soluble fraction of the plant caused a marked and prolonged hypotension in dogs and inhibited the frog myocardium. [9-10] An ethanolic extract of the entire plant exerted a negative inotropic action on amphibian and mammalian myocardium. It also exerted spasmolytic activity on smooth muscles. [11]

The plant contains carbohydrate-D-glucose, maltose, rhamnose, sucrose, and starch. [12-16] It contains proteins, amino acids and the alkaloid shankhpushpina ( $C_{17}H_{25}NO_2$ ), having a melting point of 162-164°C. The most notable constituents are tropane alkaloids. Only convolvamine has been identified, but other alkaloids (convoline, convolidine, convolvine, confoline, convosine, etc) found in other species from this family are probably present. [15] The fresh plant contains volatile oils, fatty acids, fatty alcohols and hydrocarbons i.e. myristic acid (30.9%), palmitic acid (66.8%), linoleic acid (2.3%), and straight chain hydrocarbon hextriacontane. [12-13] The whole plant of *C. pluricaulis* contains scopoletin,  $\beta$ -sitosterol and ceryl alcohol. [12-13]

## MATERIAL AND METHODS

**Plant Material:** the whole plant material of *Convolvulus pluricaulis* was purchased from Tamil Nadu, India and identified by our Taxonomist. A voucher specimen has been maintained at R&D centre, Sanat Products Ltd., Sikandrabad, India. All other reagents were of HPLC grade or AR grade as per requirement. The active compound Scopoletin was purchased from ChromaDex (LGC Promochem, Bangalore, India).

### Chromatographic conditions and procedure

High Performance Liquid Chromatography (HPLC, Shimadzu, LC 2010A, Japan), Autosampler, UV-Detector was used for the analysis of Scopoletin. The data was acquired on the LC solution administrator data system (Japan). Phenomenex  $C_{18}$  column (250 mm  $\times$  4.6 mm, 5  $\mu$ m) (California, USA) and a isocratic mixture of methanol and water containing 0.1% v/v formic acid in the ration of 30: 70. The mobile phase was filtered through 0.45  $\mu$ m Millipore filter and degassed by sonication for 30 min. The flow rate was adjusted to 1.0 ml/min. Injection volume was adjusted to 20  $\mu$ l and detection was made at 366 nm.

### Preparation of standard solution

Standard solution of pure scopoletin was prepared by dissolving 2.0 mg in 20 ml (100 ppm) of methanol in a volumetric flask (stock solution). For the determination of limit of detection (LOD) and limit of quantification (LOQ), 2 ml of the stock solution was diluted to 10 ml (20 ppm), 4 ml of the same stock solution was diluted to 10 ml (40 ppm) and 6 ml of the same stock solution was diluted to 10 ml (60 ppm) for linearity study.

### Preparation of sample solution

Approx. 50 mg grinded powder of three extracted samples were taken and dissolved with 15 ml methanol separately. The samples were sonicated for 20 min. After sonication the volume was made up to 50 ml with HPLC grade methanol and filtered through 0.45  $\mu$ m membrane filter.

## Extraction procedure

The air- dried samples of *Convolvulus pluricaulis* were powdered and passed through 20 mesh sieve. The sieved material (100 g) was extracted with 400 ml methanol (99%), 50% alcohol and water separately at the temperature of 80-85°C for 1-2 hrs on a water bath. The material was filtered and marc was further refluxed three times with methanol, 50% alcohol and water separately. Following this all the extracts were pooled together, concentrated under vacuum using rota-vac (Heidolph, Schwalbach, Germany). Finally the material was air-dried after removal of the above solvents.

## Calibration curve

Five different concentrations of stock solution after dilution (20, 40, 60, 80, 100 ppm) with mobile phase were injected in triplicates. Regression equation and co-efficient of correlation ( $r^2$ ) was derived (Table 1).

## Validation of method

### Limits of detection (LOD) and quantification (LOQ)

For determination of the limit of detection (LOD) and limit of quantification (LOQ), different dilutions of the standard solution were analysed 6 times using mobile phase as a blank. The LOD and LOQ were determined on the basis of signal-to-noise ratio until the average responses were approximately three and ten times the responses of the blank respectively.

### Accuracy (recovery)

Accuracy of the method was ascertained by spiking the pre-analysed samples with known amount of standard solution (50%, 100%, and 150%). The average percentage recovery was estimated by applying values of peak area to the regression equations of the calibration graph. Three replicate samples of each concentration level were prepared.

### Method precision (repeatability)

The precision of the instruments was checked by repeatedly injecting and analyzing (n=6) standard solution 60 ppm. The results are reported in terms of relative standard deviation (RSD).

### Intermediate Precision (Reproducibility)

The interday and intraday precision of the proposed method were determined by analyzing standard solution at different concentrations (20, 40, 60, 80, 100 ppm) three times on the same day and on three different days. The results are reported in terms of RSD.

## RESULTS AND DISCUSSION

### Development of HPLC method

The method development and selection of a suitable mobile phase involved several trials because of the complexity of the chemical composition of the herbals and the affinity of the components towards various solvents. The proportions of the organic and aqueous phases were adjusted to obtain a rapid and simple assay method with reasonable run time, suitable retention time and sharp peak. Under optimized conditions HPLC with  $C_{18}$  column and UV detector at 366 nm using isocratic mixture of methanol and water as mobile phase gave well resolved symmetric peak for scopoletin. The total run time of scopoletin was found to be 30 minutes and the scopoletin appeared on chromatogram at 19.508, 19.583, 19.500 minutes in methanol Fig. 2 (b), hydro-alcoholic in Fig. 2 (c) and water extract in Fig. 2(d) respectively. The retention time of reference standard (scopoletin) was observed to be 19.579 minutes in Fig. 2 (a). This indicates that the present HPLC method is rapid; easy and convenient.

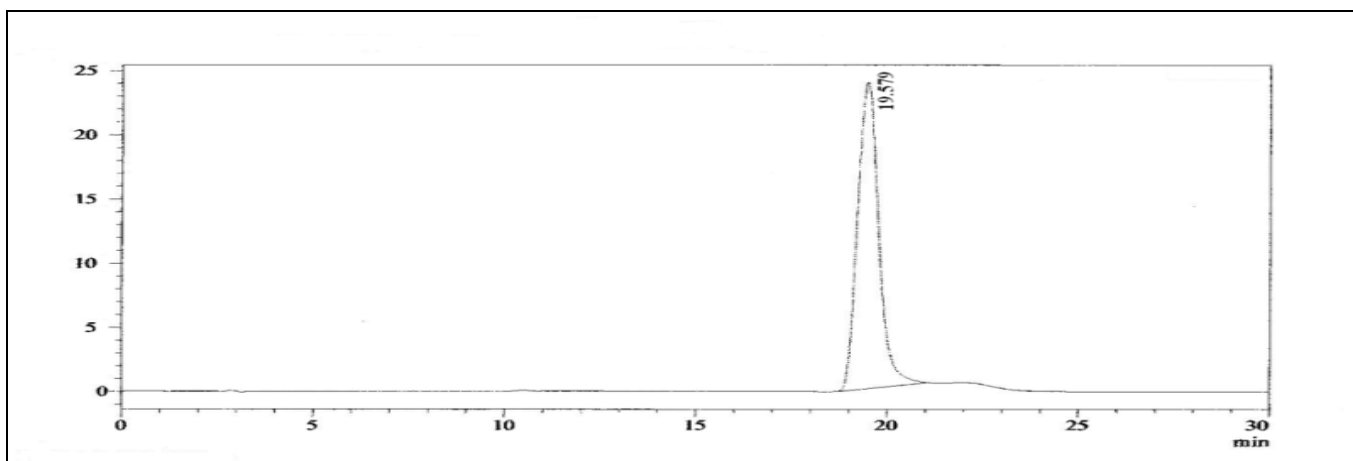


Fig. 2(a): HPLC Chromatogram of Reference Standard (Scopoletin)

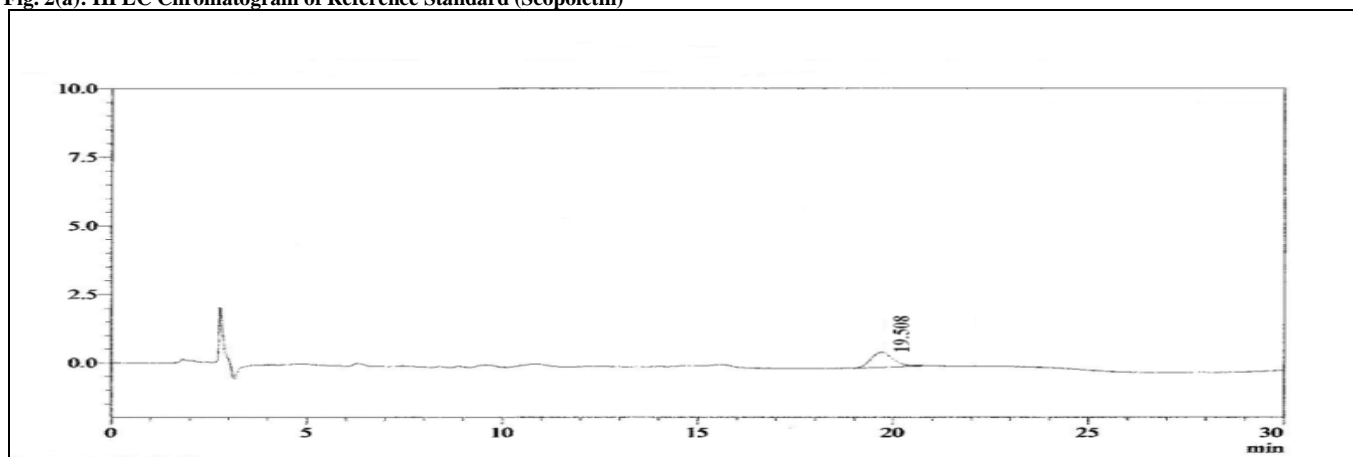


Fig. 2(b): HPLC Chromatogram of *Convolvulus pluricaulis* Choisy. (Methanol extract)

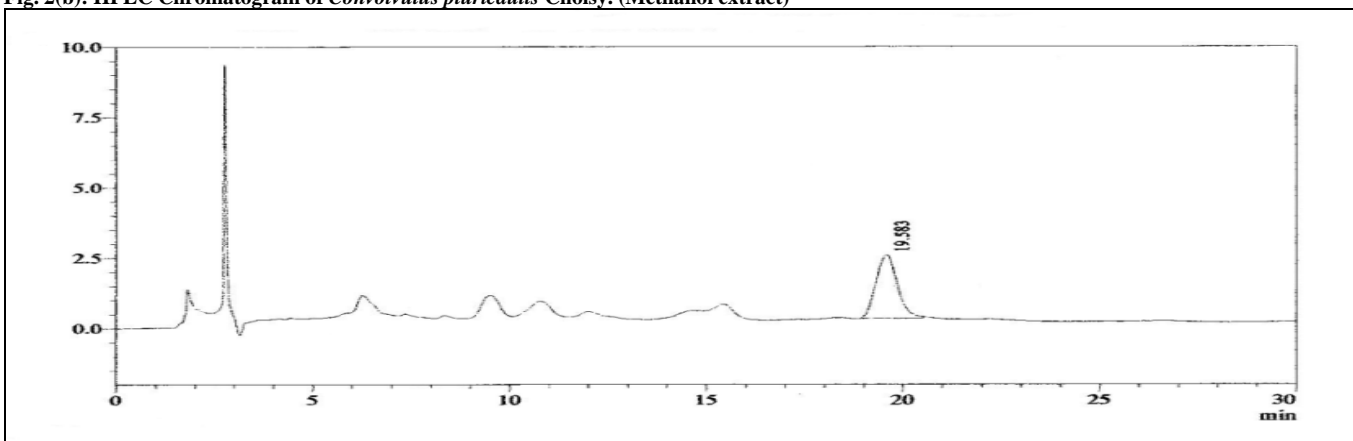


Fig. 2(c): HPLC Chromatogram of *Convolvulus pluricaulis* Choisy. (Hydro-alcoholic extract)

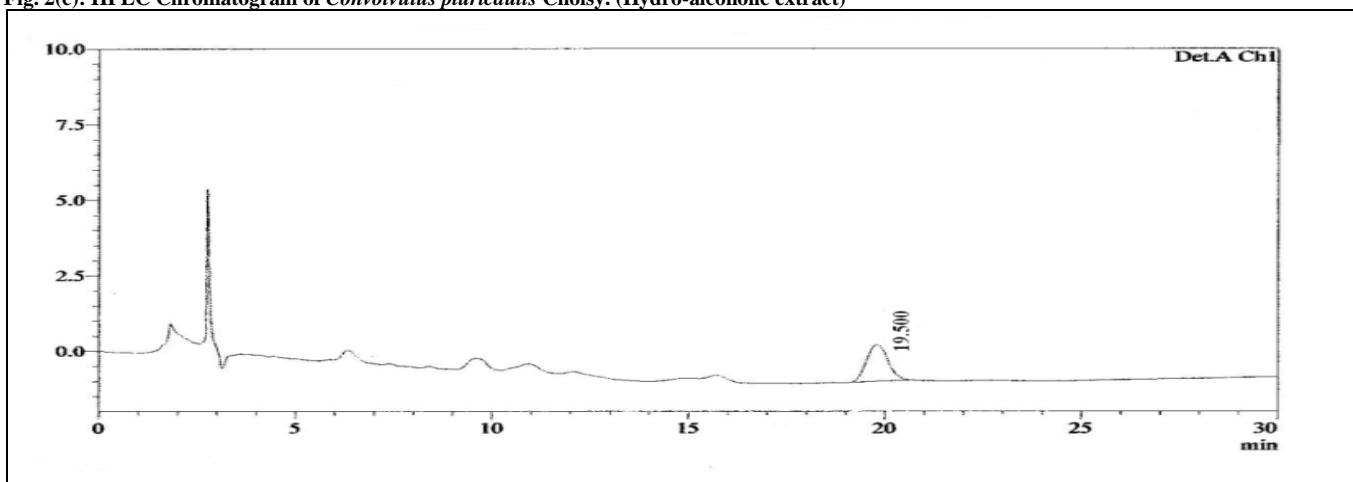


Fig. 2(d): HPLC Chromatogram of *Convolvulus pluricaulis* Choisy. (Water extract)

When the same drug solution was injected 6 times, the retention time of the peak was found to be same.

#### Validation of method

The calibration curve was prepared by plotting the peak area against standard concentration; it was found linear in the range of 20-100 ppm. The regression equation was found as  $y = 46474x + 30000$  with  $r^2$  of 0.9961, showing best linearity. The method was validated in terms of precision, repeatability, accuracy and other validation method parameters. The repeatability of the HPLC method and intermediate precisions for intra-day and inter-day variations are given in Table 1. The LOD value was found to be 5 ppm, which is the concentration that yields a signal-to-noise (S/N) ratio of 3:1. The LOQ value under the described conditions was 7.5 ppm with an S/N ratio of 10:1. This confirmed the sensitivity for quantification of compound. A recovery value from 99.10 to 100.1% indicates best accuracy of the method (Table 1).

#### HPLC analysis of scopoletin in different samples

Quantitative estimation of scopoletin in *Convolvulus pluricaulis* given in Table 2 revealed that the best resolution was found in hydro-alcoholic extract as compared to water and methanol extract. It was 0.1738% w/w in hydro-alcoholic extract, 0.0932% w/w in methanol extract and 0.0435% w/w in water extract.

**Table 1: Validation parameters of the developed HPLC method quantification of Scopoletin**

Validation Parameters	Results
Linearity range (ppm)	20-100 ppm
Correlation coefficient ( $r^2$ )	0.9961
Regression equation	$y = 46474x + 30000$
LOD (ppm)	5.0 ppm
LOQ (ppm)	7.5 ppm
Method precision (RSD %)	0.71
Intermediate precision (RSD %)	
Interday (%)	0.88
Intraday (%)	1.25
RSD % (Linearity of the method)	1.29

**Table 2: Scopoletin content in different extracts of *Convolvulus pluricaulis* Choisy**

Name of Extracts	Scopoletin content (%)
Hydro alcoholic Extract	0.1738%
Methanol Extract	0.0932%,
Water Extract	0.0435%

A method for analysis of *Convolvulus pluricaulis* Choisy using scopoletin as analytical marker was developed. The method was found to be simple, precise, specific, sensitive and accurate. It can be used for routine quality control analysis. The results also indicate that the maximum scopoletin content in *Convolvulus pluricaulis* was found in hydro-alcoholic extract as compared to methanol and water extract.

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