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# Development of a Validated HPTLC Method for Quantification of Scopoletin in *Ipomoea Turpethum* Root and its Market Formulations

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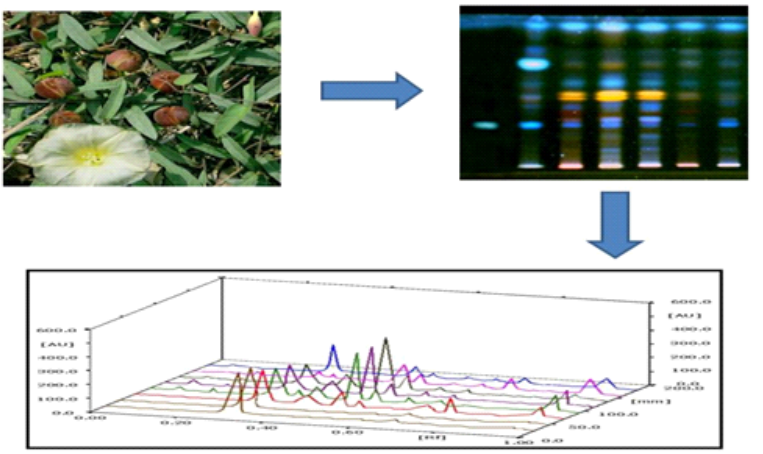
## Graphical Abstract



## Abstract

*Background: Ipomoea turpethum* R. Br.(F: Convolvulaceae)commonly known as Indian Jalap in English and Nisoth in Hindi has been commonly used as a purgative. In India under the name of Nisoth two drugs are available commercially, *Marsdeniatenacissima* as white Nisoth and *Ipomoea turpethum* as black Nisoth. The presence of a coumarin, scopoletin in *I. turpethum* distinguishes the two drugs available under the name of Nisoth.

*Purpose:* This paper is aimed to present a simple method of identification and quantification of scopoletin in *I. turpethum* and to check the presence of same in its different market formulations like Anand Churna (F-1), KayamChurna (F-2), Bhavnagari Churna (F-3), Manjistadi Churna (F-4), Abhayadi Modak (F-5) using HPTLC. Study design:

Simple and accurate HPTLC method is developed using scopoletin as a marker for chemo-profiling of *I. turpethum* and using it for evaluation of market formulations.

*Methods:* The chromatographic method for scopoletin was developed using silica gel 60 $F_{254}$ plates as stationary phase and toluene: ether (1: 1) with 10% glacial acetic acid as a mobile phase. The method was validated in terms of accuracy, precision, specificity, linearity, limit of detection (3:1) and limit of quantification (10:1).

*Results:* The chromatographic method gave good resolution of scopoletin, without any interference with the other compounds present in *I. turpethum* and its formulations. It was noted that amongst all the spots scopoletin resolved at $R_f$ 0.27, exhibiting characteristic blue fluorescence under UV light in *I. turpethum* andits different market formulations when scanned at 366 nm. Scopoletin content in *I. turpethum* root and its formulations was found to be equivalent. Among all formulations, the formulation F-5 (vati) contained less amount of scopoletin when compared to the actual amount of the *I. turpethum* incorporated. Thus, it may be concluded that in F-5 being vati, presence of excipients and method of preparation might have influenced the scopoletin content.

*Conclusion:* The method is simple, sensitive and precise. It can be used for the routine quality control testing of *I. turpethum* and to check presence of it in its different market formulations.

**Key words:** HPTLC, *Ipomoea turpethum* root, market formulations, scopoletin

## Introduction

*Ipomoea turpethum* (Linn.)R. Br. {Syn. *Operculina turpethum (*L.) Silva Manso, *Convolvulusturpethum* Linn. }, commonly known as Indian Jalap in English and black Nisoth in Hindi is valued in Ayurvedic system of medicine as a purgative [1-3]. Root is bitter, acrid, thermogenic, anthelmintic, expectorant, antipyretic and hepatic stimulant and are indicated in colic, constipation, anorexia, dropsy, vitiated conditions of vata, bronchitis, helminthiasis, inflammations, intermittent fever, abdominal tumors, obesity, gout, rheumatism, ulcers and jaundice (Varier, 1995, Anonymous, 2001, Nadkarni, 1954, Gupta and Ved, 2017). The plant is reported to possess purgative, anti-inflammatory, antibacterial, cytotoxic,

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antioxidant, hepatoprotective,antinephrotoxic and antidiabetic activities (Gupta and Ved, 2017). Major chemical constituents reported include $\alpha$- and $\beta$-turpethein, resin, turpethinic acids A, B, C, D and E, coumarin, $\beta$-sitosterol, betulin, lupeol, cycloartenol, lanosta-5-ene etc (Gupta and Ved, 2017).

The presence of coumarin, scopoletin distinguishes the root of *I. turpethum* from the root of its adulterant *Marsdeniatenacissima* (white nisoth).This paper describes difference in the TLC fingerprinting of root of *I. turpethum* and its different market formulations and identification of scopoletin amongst separated compounds that would serve as a tool for its quantification.

## Experimental

### Material

Roots of *I. turpethum* (Figure 1) andfive different market formulations Anand Churna (F-1), KayamChurna(F-2), Bhavnagari Churna(F-3), Manjistadi Churna(F-4), Abhayadi Modak (F-5) containing *I. turpethum* in varying amounts 20%, 6%, 11%, 4%, 80% respectively, were procured from the Jamnagar, Gujarat. All the samples were stored in a closed airtight container with appropriate labels.

**Herb** **Root**

**Figure 1: *Ipomoea turpethum*R. Br.**

### Chemicals and Reagents

Standard scopoletin was procured from Sigma Aldrich, India. All the solvents used were of chromatography grade and other chemicals used were of analytical (AR) grade.

### Extraction

5 g of powdered drug of *I. turpethum root* and its powdered formulations F-1 to F-5 were extracted separately in a soxhlet apparatus with 100 ml of methanol for 1 hour and the extract was evaporated under vacuum to yield semisolid residue. The residue in each case was separately treated with aqueous alkali 10% sodium hydroxide for 1 hour at room temperature. The non saponifiable matter was removed by treating with chloroform. Then the aqueous alkaline layer was acidified with 10% hydrochloric acid and extracted with chloroform. The chloroform extracts were separated and evaporated to dryness under vacuum to get 5.8%, 1.6%, 0.56%, 0.90%, 0.5% and 3.6%w/w for *I. turpethum* rootand its powdered formulations F-1 to F-5 respectively. The chloroform dried extracts were subjected to HPTLC analysis.

### Estimation of scopoletin by HPTLC method

### Chromatographic conditions

HPTLC was performed on 10 cm × 10 cm precoated silica gel 60 $F_{254}$ plates (E. Merck, Germany). Before chromatography the plates were pre-washed by methanol and activated at 60°C for 5 min. Samples were applied to the plates as bands 6 mm wide and 12.2 mm apart using Camag Linomat V applicator (Muttenz, Switzerland) fitted with a 100 microlitre syringe (Camag, Switzerland). Linear ascending development was performed in Camag twin-trough glass chamber (10 × 10 cm) with mobile phase vapour [toluene: ether (1: 1) saturated with 10% glacial acetic acid] at room temperature (25±2°C). Plate was dried and scanned in Camag TLC scanner using Win CATS software (version 1.4.3.6336) in absorption mode at 366 nm with slit dimensions 6.00 × 0.45 mm. The scanning speed was 20 mm/sec and source of radiation deuterium lamp.

The method was validated in terms of linearity, interday precision, intraday precision, repeatability, accuracy, and specificity. The limit of detection (LOD) was determined at a signal-to-noise ratio of 3:1 and limit of quantification (LOQ) at a signal-to-noise of 10:1.

International Conference on Harmonization (ICH) guideline was employed for validation of analytical method (ICH, 2005).

### Calibration curve

A stock solution (1 mg/ml) of scopoletin was prepared by dissolving accurately weighed 10 mg in 10 ml methanol in a volumetric flask. Standard solutions for calibration were prepared by dilution of the stock solution with methanol; the concentrations were such that amount of scopoletin ranges between 500 - 2500 ng. The correlation coefficient, slope intercepts and regression equation were also calculated to provide mathematical estimate degree of linearity. A calibration curve was derived by plotting peak area (Y axis) versus concentration (X axis).

**Quantification of scopoletin**

10 mg of dried chloroform extract of *I. turpethum* root and powdered formulations F-1 to F-5 was dissolved in 2 ml methanol in a volumetric flask. 10 µl of this solution was used for estimation of scopoletin. The peak area values of standard and samples were used to calculate the amount of scopoletin in the root and market formulations.

**Results and Discussion**

**Method Validation**

A series of standard solution was prepared from the stock solution, and amounts in the range 500 - 2500 ng for scopoletin were applied to the plates. A calibration curve was derived by plotting peak area (Y axis) versus concentration (X axis). The correlation coefficient, slope intercepts, and regression equation were also calculated to provide a mathematical estimate degree of linearity. The regression data obtained showed a good linear relationship. The data of inter-day precision, intra-day precision, repeatability of measurement, repeatability of application, LOD, and LOQ are given in Table 2. The method is specific for scopoletin because it resolved standard well in the presence of other phytoconstituents in *I. turpethum*.

**Quantification**

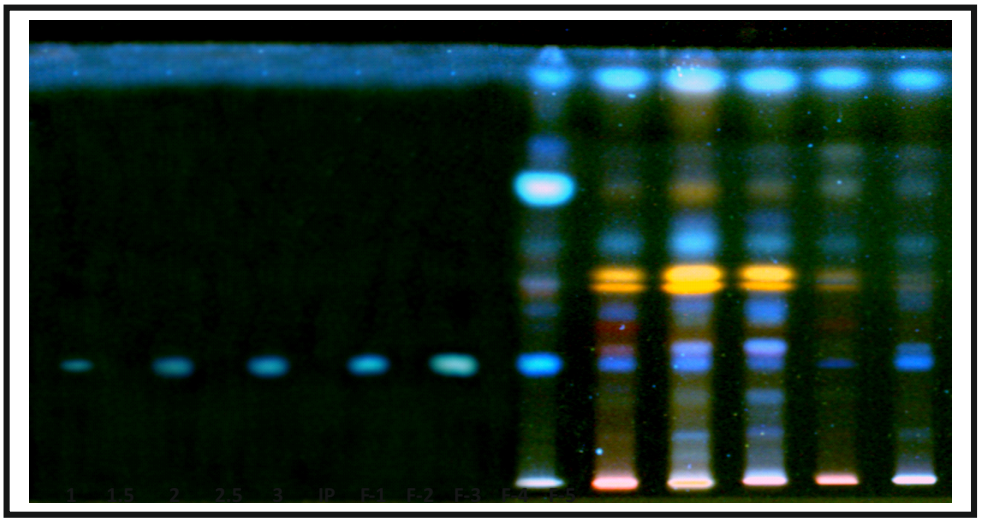
HPTLC studies revealed scopoletin at $R_f$ 0.27 (Figure 2). Its content in *I. turpethum* root and its formulations were found equivalent (Table 1). Among all formulations, the formulation F-5 (vati) contained less amount of scopoletin when compared with the actual amount of the *I. turpethum* incorporated. Thus, it may be concluded that in F-5 being vati, some stability influencing parameters might have influenced the scopoletin content.

**Table 1: Estimation of scopoletin in *I. turpethum* root and its market formulations**

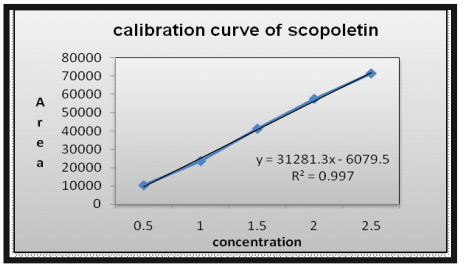
| Sample | Mean peak area ± S.D. (n=3) | Average amount of scopoletin (µg/spot) | Average %w/w of scopoletin in *I. turpethum* and its formulations F-1 to F-5 ± S.D. | % C.V. |
|---|---|---|---|---|
| Ipomoea extract | 54082.3 ± 397.08 | 1.92 | 0.223 ± 0.02 | 2.58 |
| F -1 | 34909.6 ± 1004.3 | 1.31 | 0.042 ± 0.05 | 1.87 |
| F -2 | 27415.3 ± 649.18 | 1.07 | 0.012 ± 0.02 | 1.96 |
| F -3 | 32110.0 ± 954.27 | 1.22 | 0.022 ± 0.01 | 2.15 |
| F -4 | 48674.6 ± 1273.4 | 1.75 | 0.126 ± 0.04 | 2.61 |
| F -5 | 19151.3 ± 586.14 | 0.80 | 0.008 ± 0.03 | 1.06 |

**Table 2: Summary of validation parameters of scopoletin**

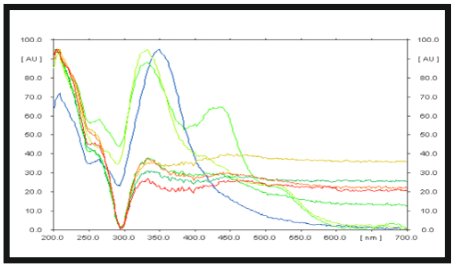
| Sr. No. | Parameters | Results |
|---|---|---|
| 1 | Linearity (R2) | **0.997** |
| 2 | Precision (% C.V.)<br>▪ Repeatability of Measurement<br>▪ Repeatability of Application<br>▪ Interday<br>▪ Intraday | <br>0.62<br>1.94<br>1.99-2.17%<br>1.25-2.03% |
| 3 | *Range* | [illegible] µg/spot |
| 4 | *Limit of Detection* | 0.038 µg/spot |
| 5 | *Limit of Quantification* | 0.11 µg/spot |
| 6 | Accuracy | 98.19 - 100% |
| 7 | Specificity | *Specific* |



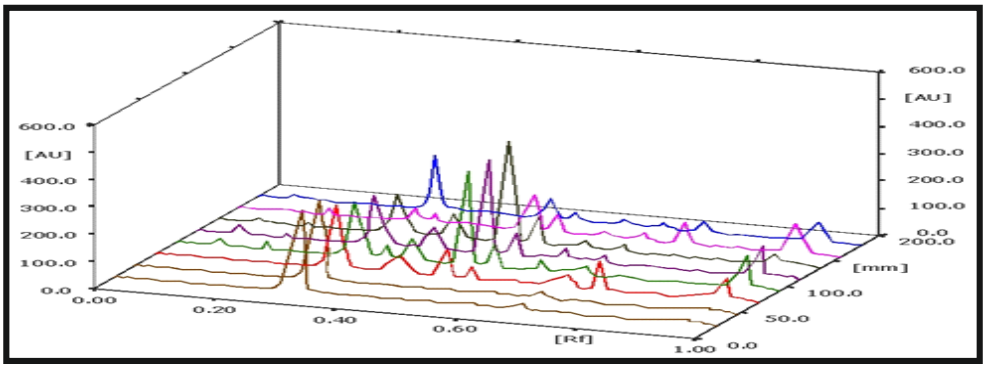
A. HPTLC chromatogram scanned at 366 nm




B. Standard Curve ofscopoletin



C. Spectral Comparison




D. Densitometric chromatogram scanned at 366 nm

**Figure 2: HPTLC study of scopoletin**

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