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# Simultaneous method development and validation of 1, 8-Cineole and Betulinic acid in Shati Vati - An Ayurvedic formulation by HPTLC

**Research Article**

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## Abstract

A simple, accurate and precise HPTLC method was developed and validated for simultaneous estimation of 1, 8-Cineole and Betulinic acid in Shati Vati - An Ayurvedic formulation by HPTLC. Shati Vati contains Shati rhizome powder have 1-8 cineole and Badar ripe fruit powder have Betulinic acid reported medicinal claims and uses. In the analytical method, toluene: ethyl acetate (93:07 v/v) was finalized as the mobile phase and a clear separation was achieved. The wavelength for 1-8 cineole and Betulinic acid was 665nm and 580nm respectively. The $R_f$ value of 1-8 cineole and betulinic acid was obtained 0.647 and 0.213 respectively. The developed analytical method was validated for Linearity, Accuracy, Precision, LOD, LOQ and Robustness as per ICH Q2 (R1) guideline. Results for the validation process were within the range of acceptance criteria of ICH guideline. HPTLC method have been applied successfully for estimation of 1-8 Cineole and Betulinic acid in Shati Vati an ayurvedic formulation.

**Key Words:** HPTLC, 1-8 Cineole, Betulinic acid, *Shati vati* an Ayurvedic formulation, ICH Q2 (R1) guideline, Method validation.

## Introduction

*Shati* and *Badar* both are the plants of Indian origin having medicinal therapeutic effect and provide health to the society. *Shati* drug consists of the dried transversely cut pieces of the rhizome of *Hedychium spicatum* Buch. -Ham. Ex J.E. Smith. *Shati* commonly known as *Kapurkachri* or botanically named *Hedychium spicatum* Buch. -Ham. from the Zingiberaceae family and a perennial, rhizomatous herb with fragrant with flowers; found largely in subtropical regions of western and Central Himalayas at 1500 to 2000 m altitude (1). *Badar d*rug consists of dried ripe fruit powder of *Ziziphus jujuba* Mill. *Badar* commonly known as *jujube, Bor* or botanically named *Ziziphus jujuba* Mill from Rhamnaceae family and an annual or biennial, hirsute deciduous, medium to large shrub, 5 to 10 m high; found in foothills of Himalayas, Deccan and Karnataka (2). *Shati Vati* an Ayurvedic formulation with *Shati* (3) (*Hedychium spicatum*) and *Badar* (4) *(Ziziphus jujube)* plants prescribed as anti-histaminic. *Shati Vati* is prescribed for Fever, cold and respiratory disorders. It was prepared in Parul institute of Ayurveda, Vadodara. Further study like clinical trials and evaluation parameters of *Shati Vati* is being carried out by the Kaumarbhritya(KB) department of Parul Institute of Ayurveda, Parul University. *Shati Vati* formulation contains *Shati* rhizome powder and *Badar* ripe fruit powder. Vati is prepared by Ayurvedic collage of Parul University, Kaumarbhritya (KB) Department as per ayurveda text book of Sharangadhar Samhita, Madhyam-khanda, Adhyay 7; 3 (5) by using different ingredients like; Shati rhizome powder, Badar ripe fruit powder, Sugar, Ghee and honey. Any drug may be of plant, animal or mineral origin may have varying properties as per their place of availability or climatic conditions.

Phytoconstituents of *Shati* and *Badar*:

***Shati*** (1)

Major constituents: 1-8 Cineole, β-caryophyllene

Other constituents: Linalool, elemol, sitosterol, α- and β- pinenes, limonene, sitosterol- β-D-glucoside, hedychenone, 7- hydroxyhedychenone.

***Badar*** (2)

Major constituents:Oleanolic acid

Other constituents:A natural polysaccharide of *ziziphus jujuba* L-arabinose and D- galactose, an acidic polysaccharide constituted by D- galacturonic acid, , L-rhamnose, L- arabinose, D-xylose, asimilobine, Betulinic acid, alphitolic acid, malic acid, betulonic acid, maslinic acid.

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**Aim and objectives**

Aim

Simultaneous Method Development and Validation of 1, 8-Cineole and Betulinic acid in Shati Vati - An Ayurvedic Formulation by HPTLC

Objectives

- To develop Simultaneous method of 1-8 cineole and betulinic acid in Novel Ayurvedic formulations.
- To Validate HPTLC method as per ICH guideline.

# Experimental / Material and Method

**Chemicals and Reagents collection**

1-8 Cineole and Betulinic acid were obtained from Natural remedies, Bengaluru, Karnataka and Yucca enterprises, Mumbai, Maharashtra respectively. Other solvents and chemicals were of analytical grade. Silica gel 60F254 TLC plates were purchased from Merck (Darmstadt, Germany).

**Table 1: Plant profile**

| Sr. no | Plant name | Latin name/ Botanical name | Family | Parts used | Std. Marker compound |
|---|---|---|---|---|---|
| 1 | Shati | *Hedychium spicatum* Buch-Ham. | Zingiberaceae | Rhizomes | 1-8 Cineole |
| 2 | Badar | *Ziziphus jujuba* Mill | Rhamnaceae | Dried ripe fruits | Betulinic acid |

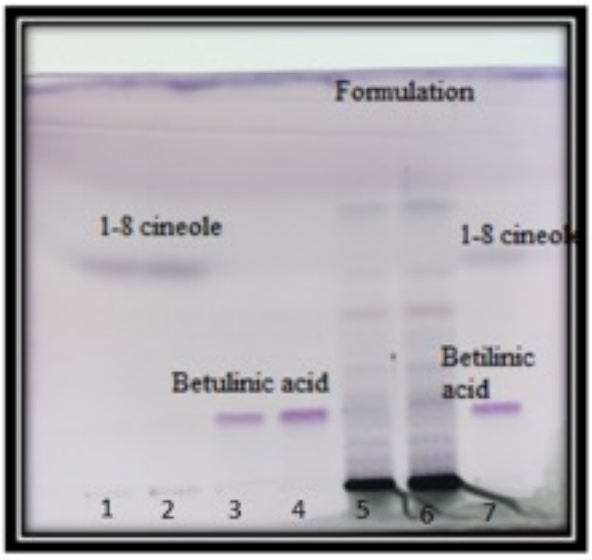
*Shati* plant consists of the dried transversely cut pieces of the rhizome of *Hedychium spicatum* Buch. -Ham. Ex J.E. Smith. *Shati* commonly known as *Kapurkachri* or botanically named *Hedychium spicatum* from the Zingiberaceae family . And *Badar d*rug consists of dried ripe fruit powder of *Ziziphus jujuba Mill*. *Badar* commonly known as *jujube, Bor* or botanically named *Ziziphus jujuba* Mill from Rhamnaceae family.

**HPTLC study**

Shati vati was subjected to HPTLC study at Parul university research center and the results with photo documentation after derivatization is depicted in Figures using LINOMAT 5 applicator, CAMAG visualization chamber and CAMAG scanner 4 with wincats software. A CAMAG TLC system were used for the analysis with CAMAG LINOMAT 5 an automatic TLC sample spotter and *CAMAG* glass chamber (20 X 10 cm and 10 X 10 cm). Chromatography was performed using silica gel 60F254 TLC plates (20 X 10 cm and 10 X 10 cm; layer thickness 250 µm) which was pre activated at 60 $^{o}$C for 5 min. Standard markers and sample (Formulation) were applied on the plate as 8 mm wide bands with an automatic TLC sampler under a flow of N2 gas, 10 mm from the bottom and 10 mm from the side and the space between two spots were 15 mm of the plate. The linear ascending development was carried out in a *CAMAG* twin trough chamber saturated with 20ml or 10ml mobile phase for 20 min at room temperature. The plates were developed up to 8 cm under chamber saturation conditions. Subsequently to the development, TLC plates were dried in current air with the help of a hair dryer. Derivatized the plate with the help of Anisaldehyde-sulphuric acid reagent (AS reagent) and after the post chromatographic derivatization, quantitative evaluations of the plates were performed with *CAMAG* scanner 3 (win CATS 4.0 integration software). After that plate scan through *CAMAG* TLC scanner (data resolution 100 µm step and scanning speed 20 mm/s). Standard stock solution of 1-8 cineole and Betulinic acid was 1000 µg/ml and working solution of 1-8 cineole and Betulinic acid was 100 µg/ml. HPTLC plate was applied with 1-8 cineole and Betulinic acid standard marker with different µl (see figure no 1) with linomat 5 applicator, was developed in Toluene: Ethyl acetate (93:07v/v) solvent system and the developed plates following drying were observed in CAMAG visualization chamber followed by scanning at 580nm and 665nm using CAMAG scanner 4. After derivatization, plate was examined for appearance of different bands at different Rf.

**Figure 1: Simultaneous method development with formulation**

Track 1: Standard marker 1-8 Cineole (1µl)
Track 2: Standard marker 1-8 Cineole (2 µl)
Track 3: Standard marker Betulinic acid (1µl)
Track 4: Standard marker Betulinic acid (2 µl)
Track 5: Formulation (Shati vati) (1 µl)
Track 6: Formulation (Shati vati) (2 µl)
Track 7: Simultaneous method (Standard marker 1-8 Cineole & Standard marker Betulinic acid) (2 µl)




**Solvent system- Toluene: ethyl acetate (93:07 v/v)**

**Table 2: Rf values of the standards @580 and @665**

| Sr. no | Markers | Rf @ 580 | Rf @ 665 |
|---|---|---|---|
| 1 | 1-8 Cineole | 0.643 | 0.647 |
| 2 | Betulinic acid | 0.219 | 0.213 |

**Method validation**

The developed analytical method was validated for Linearity, Accuracy, Precision, LOD, LOQ and Robustness as per ICH Q2 (R1) guideline.

**Figure 2: HPTLC chromatogram of standard mixture (1-8 cineole +Betulinic acid) Simultaneous method development @580**

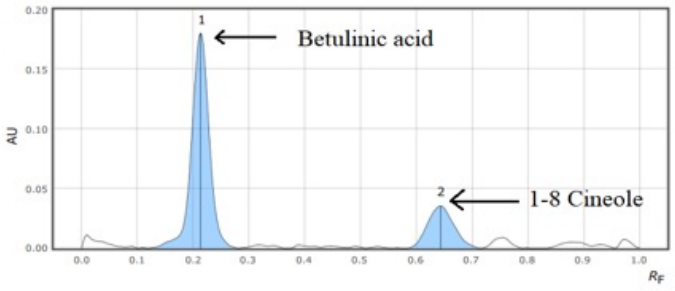
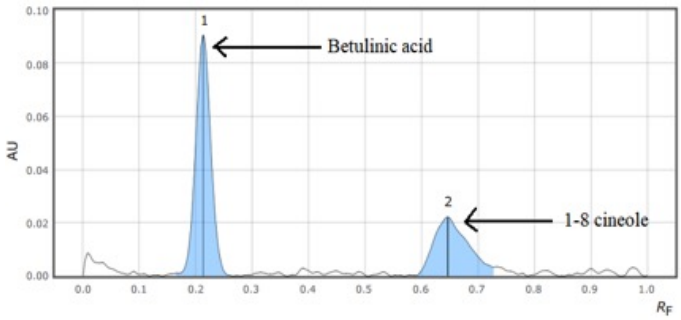



**Figure 3: HPTLC chromatogram of standard mixture (1- 8 cineole +Betulinic acid) Simultaneous method development @665**




## Results

**Table 3: Result table**

| Parameters | 1-8 Cineole | | Betulinic acid | |
|---|---|---|---|---|
| **Rf** | 0.64 | | 0.21 | |
| **Linearity range** | 200-700 ng/spot | | 200-700 ng/spot | |
| **Straight line equation** | y = 4E-06x + 0.0009 | | y = 1E-05x + 0.0022 | |
| **Correlation coefficient ($r^2$)** | 0.9754 | | 0.9895 | |
| **Repeatability (%RSD, n=6)** | 0.643755517 | | 0.898449732 | |
| **Interday precision (%RSD)** | **Concentration (ng/spot)** | **%RSD** | **Concentration (ng/spot)** | **%RSD** |
| | 200 | 0.58331 | 200 | 0.94789 |
| | 400 | 0.71623 | 400 | 0.97687 |
| | 600 | 1.03934 | 600 | 0.83774 |
| **Intraday precision (%RSD)** | **Concentration (ng/spot)** | **%RSD** | **Concentration (ng/spot)** | **%RSD** |
| | 200 | 0.58331 | 200 | 0.94789 |
| | 400 | 0.50760 | 400 | 0.57507 |
| | 600 | 0.57810 | 600 | 0.52185 |
| **Recovery study (%) ± SD** | **Level of % recovery** | **%RSD** | **Level of % recovery** | **%RSD** |
| | 80% | | 80% | 1.09858 |
| | 100% | 1.67991 | 100% | 1.25838 |
| | 120% | 1.32199 | 120% | 0.67424 |
| **Robustness** | **Change in mobile phase ratio** | **Change in saturation time** | **Change in mobile phase ratio** | **Change in saturation time** |
| | Toluene: Ethyl acetate (9.4:0.6 v/v) | 15 | Toluene: Ethyl acetate (9.4:0.6 v/v) | 15 |
| Result: | Rf =0.64 | Clear separation | Rf =0.21 | Clear separation |
| **LOD** | 8.25ng | | 0.99ng | |
| **LOQ** | 25ng | | 3ng | |

## Conclusion

HPTLC method was developed and validated as per the ICH guidelines for the estimation of 1-8 Cineole and Betulinic acid in Shati vati an ayurvedic formulation. By the trials of method development, it was concluded that the optimized method obtained simple, accurate, specific and precise. The performance of the developed method was validated according to the ICH guidelines for linearity, accuracy, precision, limit of detection, limit of quantification and robustness. The result obtained were satisfactory so the proposed method will give accurate and precise result when applied to the different ayurvedic and herbal products.

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