



**COMPARATIVE HPTLC FINGERPRINTING STUDY OF SHODHANA OF
VATSANABHA (*ACONITUM CHASMANTHUM* STAPF EX HOLMES) FROM TWO
DIFFERENT MEDIA**

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Article Received on 20/2/2023

Article Revised on 10/3/2023

Article Accepted on 30/3/2023

ABSTRACT

Introduction: *Ashuddha Vatsanabha (Aconitum Chasmanthum Stapf Ex Holmes)* is very toxic in nature. For therapeutic use, *Vatsanabha* can be use after *Shodhana* only. In this study, two different kind of media (*Gomutra* & *Godugdha*) were taken to purify *Ashuddha Vatsanabha*. Now, to see any changes after *Shodhana* as well as comparison of two media were done. For that, we have compare the HPTLC fingerprinting study to know the presence of compoments and comparison of more components. HPTLC is a sophisticated and automated form of TLC. With HPTLC, the analysis can be viewed using different wavelengths of light thereby providing a more complete picture of the plant than is typically observed with more specific types of analyses. **Material and Methods-** Principle of HPTLC, steps involved in procedure using specify techniques for chromatography. **Results and Observation-** After Derivatization, plate was examined visually for appearance of different bands at different R_f (R_f254 nm, R_f366 nm R_f 540 nm). **Discussion-** At different wavelength, tracks were having no. of spots. In all tracks, they showed the presence of components but some were having value showed the presence of same component. Some, were having different spots resulting in presence of another component also. **Conclusion-** *Gomutra Shodhita Vatsanabha* – GMSV Powder (Track 2) showed more components in respective of all wavelengths as comparison to *Godugdha Shodhita Vatsanabha* –GDSV Powder (Track 3). So, we can conclude that *Vatsanabha Shodhana* if done by *Gomutra* media can have more active constituents and other properties results in efficacy also than *Godugdha* media.

KEYWORDS: In this study, two different kind of media (*Gomutra* & *Godugdha*) were taken to purify *Ashuddha Vatsanabha*.

INTRODUCTION

Ashuddha Vatsanabha (Aconitum Chasmanthum Stapf Ex Holmes) is very toxic in nature. For therapeutic use, *Vatsanabha* can be use after *Shodhana* only. In this study, two different kind of media (*Gomutra* & *Godugdha*) were taken to purify *Ashuddha Vatsanabha*. Now, to see any changes after *Shodhana* as well as comparison of two media were done. For that, we have compare the HPTLC fingerprinting study to know the presence of compoments and comparison of more components. HPTLC is a sophisticated and automated form of TLC. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. With HPTLC, the analysis can be viewed using different wavelengths of light thereby providing a more complete

picture of the plant than is typically observed with more specific types of analyses.

MATERIAL AND METHODS

Principle of HPTLC

Principle remains the same as of TLC i.e. adsorption. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force). The component with more affinity towards stationary phase travels slower. Thus, the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

Steps involved in HPTLC

- Selection of chromatographic layer.

- Sample and standard preparation.
- Layer pre-washing, Layer pre-conditioning.
- Application of sample and standard.
- Chromatographic development.
- Detection of spots.
- Scanning.
- Documentation of chromatic plate.

Chromatographic conditions

Application mode : CAMAG Linomat 5-Applicator
 Filtering System : Whatman filter paper No.1
 Stationary Phase : MERCK-TLC/HPTLC
 Silica gel 60 F₂₅₄ on Aluminium sheets
 Space Between Band : 10mm
 Sample Application Volume : 5 µL and 10 µL
 Chamber Saturation Time : 30 minutes

Mobile Phase (MP) : Methanol
 Visualization : @254nm, @366nm and @540 nm (after derivatization)
 Spray reagent : AnisaldehydeSulphuric acid reagent
 Derivatization mode : CAMAG- Dio tank for about 1 minute
 Drying Mode, Temp. & Time : TLC Plate Heater Preheated at 100 ± 5 °C for 3 minutes

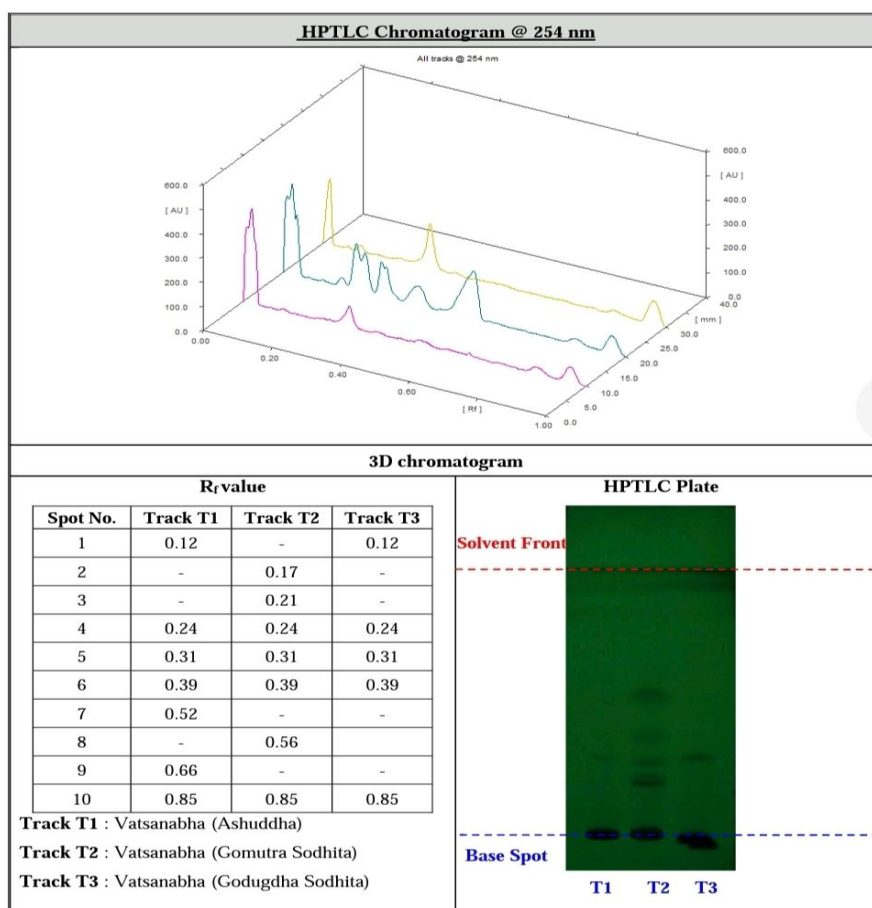
RESULTS AND OBSERVATION

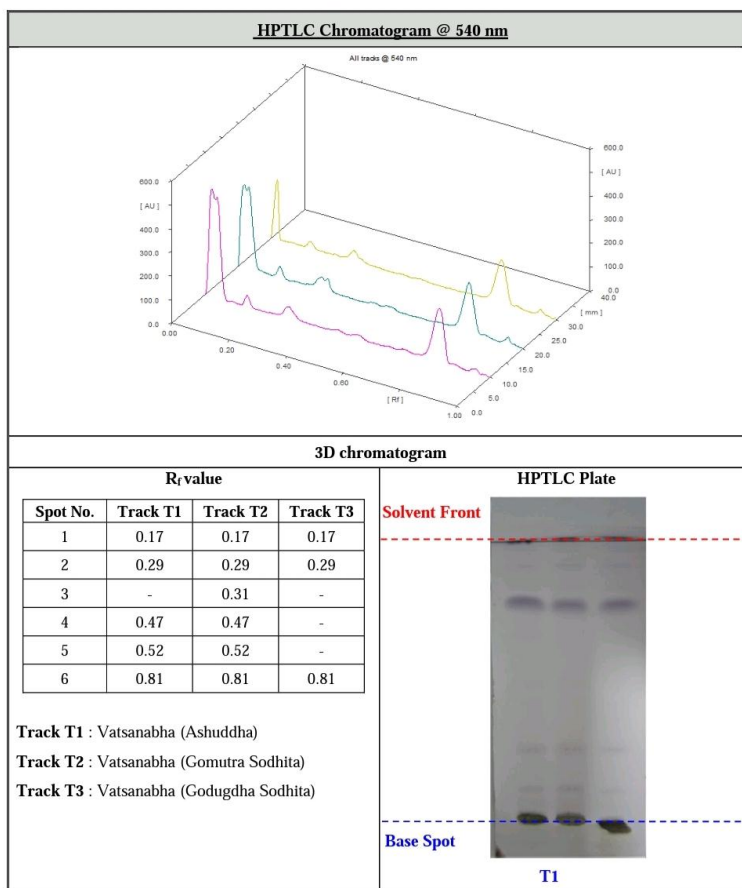
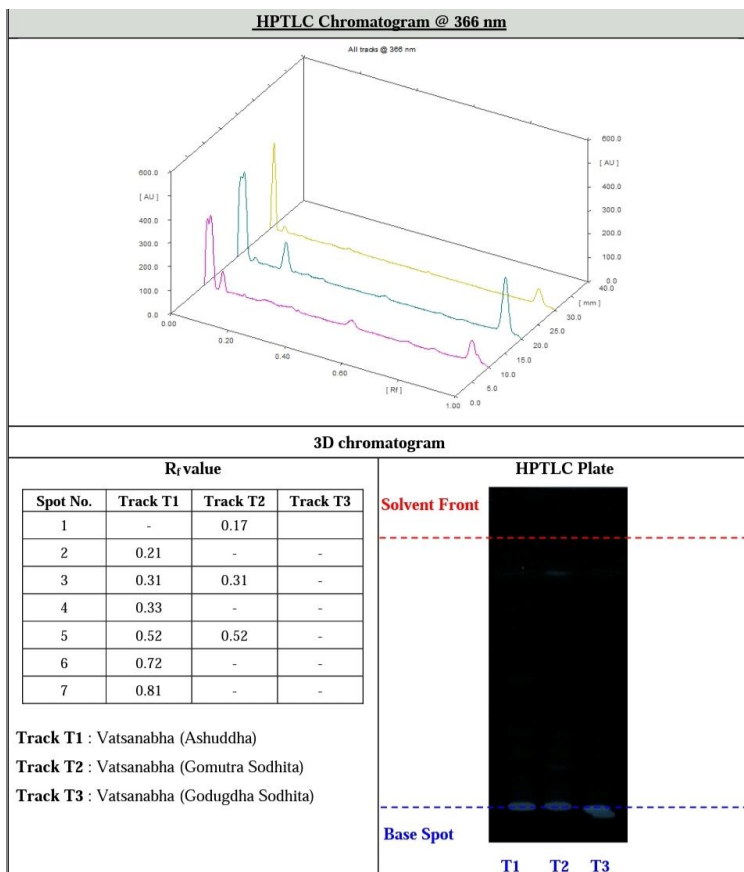
After Derivatization, plate was examined visually for appearance of different bands at different R_f (**R_f254 nm, R_f366 nm R_f 540 nm**).

Track 1- Vatsanabha Ashuddha - AV

Track 2- Gomutra Shodhita Vatsanabha - GMSV

Track 3- Godugdha Shodhita Vatsanabha - GDSV





DISCUSSION

HPTLC fingerprinting of *Ashuddha Vatsanabha* – AV Powder, *Gomutra Shodhita Vatsanabha* - GMSV Powder and *Godugdha Shodhit Vatsanabha* - GDSV Powder as comparative was done. The Various spots were done at R_f 254 nm, R_f 366 nm and R_f 540 nm respectively.

At R_f 254 nm, 07 spots were detected in AV Powder and GMSV Powder whereas in GDSV Powder 5 spots were detected. In all tracks, 4,5,6 and 10 no. spots were having same value showed the presence of same component. Same as in 1 spot no. AV Powder, GDSV Powder showed same component that was absent in GMSV Powder. Remaining Spots were having different constituents. But as comparison, GMSV -Track 2 showed more componets than GDSV -Track 3 at this wavelenth.

At R_f 366 nm, 6 spots were detected in AV Powder whereas 3 spots were detected in GMSV Powder. Absence of spot were showed in GDSV powder. Only 3 and 5 spot showed same in AV and GMSV powder. At this wavelength also GMSV -Track 2 showed more componets than GDSV -Track 3 at this wavelenth.

At R_f 540 nm, 5 spots in AV Powder, 6 spots in GMSV Powder whereas 3 spots in GDSV Powder were detected. In all tracks, spots no. 1, 2 and 6 were having same value showed the presence of same component. Same as spot no. 4 and 5 in AV Powder, GMSV Powder showed same component. Most of the spots were present in GMSV – Track 2 as comparison to both other tracks at this wavelength.

CONCLUSION

Gomutra Shodhit Vatsanabha – GMSV Powder (Track 2) showed more components in respective of all wavelengths as comparison to *Godugdha Shodhita Vatsanabha* –GDSV Powder (Track 3). So, we can conclude that *Vatsanabha Shodhana* if done by *Gomutra* media can have more active constituents and other properties results in efficacy also than *Godugdha* media.