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'HPTLC' an Important Tool for Quantification of Herbal Product: A Case Study

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Abstract: Large number of population prefer the Ayurvedic or herbal medicines to treat the various ailments. Herbal medicines are available in three forms as raw plant material or processed plant material or medicinal herbal product but mixtures have several chemical components. Therefore, it is very important to know the specific component having potential for treatment. For qualitative and quantitative analysis of the herbs and herbal drugs, High-performance thin layer chromatography (HPTLC) is one of the best techniques. Hence, a case study has performed to quantify vasicinone component from *Adhatoda zeylanica*.

Keywords: HPTLC, High performance thin layer chromatography, Adhatoda, Vasicinone, quantification, herbal drug.

Introduction:

Ayurveda is an important system of medicine trusted worldwide than synthetic[1]. Therefore, worlds one-fourth population is depend on ayurvedic medicine to treat variety of disease. Herbal drugs have number of chemical components in complex form. Due to complex nature of such drugs, it is tedious to get quality control parameter[2].

There are lot of factors affecting on quality of herbal drugs like time of collection, drying methods or most of the components are unknown etc.

To overcome such problems, in recent year many advances in chromatographic technique came in focus for quality control of complex herbal medicines[3]. High Performance Thin Layer Chromatography has become an important analytical technique to quantify the herbal drugs. This technique has advantages in quantification of analytes not only at micro and nanogram levels but

also cost effectiveness. Thus, this technique can be used as a tool in the quality control for extraction of active compounds[4]. The undertaken case study of *Adhatoda zeylanica*Medic. deals with the quantification of vasicinone componentby HPTLC.

Materials and Methods

Quantification of vasicinone was conducted by (NMIM'S SPTM, Shirpur Dhule) as described by Chaitali D and co-workers[5].

Collection of Plant material: The leaves of *Adhatoda* were collected and shade dried. These dried plant leaves were powdered and used for quantification.

Preparation of standard solution: Accurately weighed quantity of 2 mg of vasicinone was diluted in 5.0 ml with chloroform.

Preparation of plant extract: 2.0 gm of leaves powder was mixed in 50 ml of methanol and refluxed at 60 °C for 6 hrs for successive 4 days. Thereafter, the refluxed solution was filtered through disc filter i.e. 0.45 µ and collected in separate volumetric flaks for application on TLC plates.

Chromatographic conditions: Aliquot portions i.e. 6 µl of standard marker solution-c was applied on silica gel F_{254} TLC plates. Preferably methanol was used for satisfactory and well resolution peaks of vasicinone, with R_f values 0.81 ± 0.02.

The preliminary optimized chromatographic conditions on evaluation of various parameters were as follows:

Stationary phase	:	Silica Gel 60 F_{254} TLC plates
Thickness	:	200µm
Mobile Phase	:	Ethylacetate: Methanol: Water (6:3:0.2)
Mode of application	:	Band
Band width	:	6 mm
Sample volume	:	6 µl
Separation Tech	:	Ascending

Development of chamber	:	Twin through glass chamber, 10x10 cm
Saturation time	:	30 min with mobile phase and spotted plate
Migration distance	:	80 mm
Detection	:	UV Densitometric scanning
Scanning mode	:	Absorbance/ Reflectance
Scanning wavelength	:	274 nm
Slit Dimension	:	3 x 0.45 mm
Temperature	:	25 ± 3 °C

Detection and quantification:

Three bands of standard solution and three bands of leaf sample solution of equal volume i.e. 6 µl were applied on TLC plate and the plate was developed and scanned as per the final optimized chromatographic conditions. As the instrument gives directly the area of chromatogram in comparison to standard sample chromatogram. The concentration value was subsequently converted to percent amount viz. estimated by using following formula.

$$\% \text{ Estimated} = \frac{\text{Area value (sample)}}{\text{Area value of (standard)}} \times 100$$

Results and Discussion

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The quantification of vasicinone by HPTLC have evolved for extraction of *Adhatoda zeylanica*. It was found that 1.98mg/2gm of vasicinone as shown in figures.

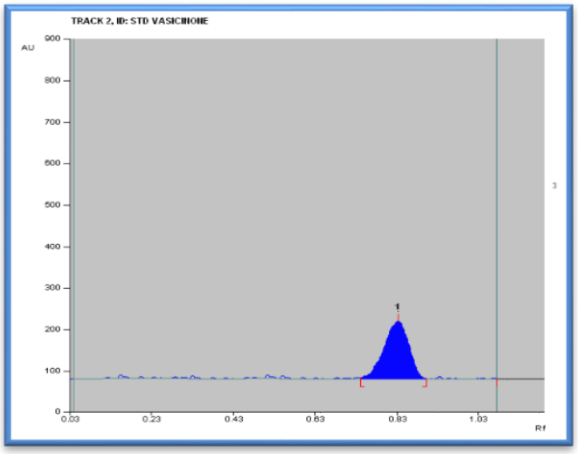

Fig. Standard chromatograph of vasicinone

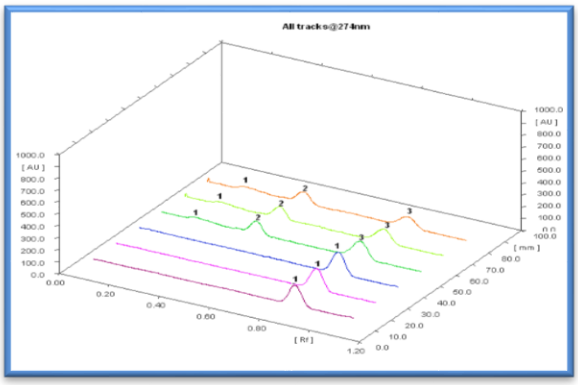

Fig. Leaf sample chromatograph 3D image (1-3 bands)

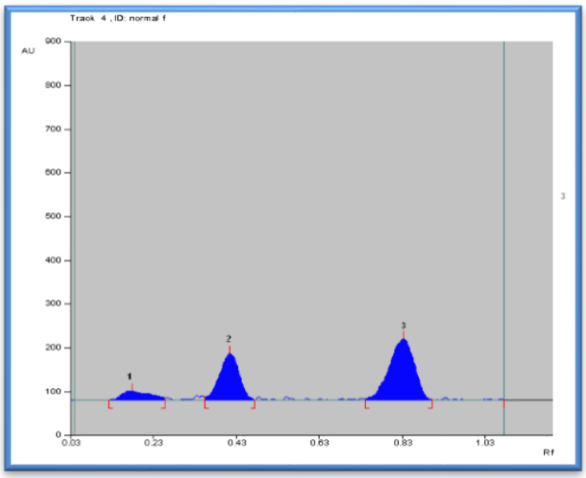

Fig. Leaf sample chromatograph (1-3 bands)

Discussion:

Suthar et. al., estimated the vasicinone contents quantitatively in *Adhatoda vasica* by HPTLC method. They developed and standardized a chromatographic HPTLC method for the quantification of alkaloids. They have also found that HPTLC method was simple, accurate and precise used for isolation of herbal drug component followed by characterization and confirmation by advanced spectroscopic methods[6].

Besides, Ganesen and Bhatt also reported the qualitative nature of some traditional crude drugs available in commercial market of Mumbai, India.They carried out tests for identification and quantification of these active principles by using TLC and HPTLC methods by using marker compounds[7].

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