www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Screening and standardization of *Adhatoda vasica* used as medicine in homoeopathy using HPTLC method

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ABSTRACT

A simple and accurate HPTLC method has been developed for quantification of *Adhatoda vasica* and finger printing of the in-housed mother tincture considered here to be a standard with that of different marketed samples available from manufacturers of homoeopathic medicines in India. This HPTLC method was quantitatively evaluated in terms of stability, repeatability, accuracy and calibration providing the utility in the analysis of the mother tincture.

Keywords: HPTLC; Mother Tincture; *Adhatoda vasica*; Fingerprint.

INTRODUCTION

Most of the modern medicines have originated from plant metabolites (Choudhari, 1996). There is a growing focus on the importance of medicinal plants in the traditional health care system (viz. Ayurveda, Unani, Homoeopathy, Yoga) in solving health care problems (Kapoor, 1990; Atal and Kapoor (1997); Ministry of Health and Family Welfare, 1996; De Mayo, 1959; Kadans, 1970; Kokate, 1988; Medicinal Plant in India, 1976; Nadkarni, 1954; Wallis 1967). Because of this awareness plant materials and herbal remedies derived from them represent substantial portion of the global market. Most of the developing countries have reviewed the traditional medical practices as an integral part of their culture so much so a large number of herbal remedies coming out to treat so many deadly ailments. But we are still facing problems for the standardization of herbal products and there are no specific prescribed provisions for Herbal Drugs in current Drug Legislation enforced in our country (Mukharjee et.al. 1998; Shastri, 1993). But in view of the growing interest in herbal medicines and photochemical, scientists are trying to develop methods for standardization of herbal drugs used in different formulation (Raina, 1993).

In order to obtain high quality products care should be taken right from proper identification of plants, seasons, area of collection, their isolation and purification process and rationalizing combination in case of polyherbal drugs (Chakravarthy, 1993; Narayana, 1993).

Due to lack of availability of suitable experimental and clinical models, the scientific validation and clinical effectiveness of most of the plant products becomes difficult. But in view of the growing interest in herbal medicines and phytochemicals, scientists are trying to develop methods for standardization of the herbal drugs used in different formulations. Though a number of scientific publications are available on various aspects of botanical, pharmacognostic, phytochemical and pharmacological investigations of plant material, no evidence is available by which if one investigates a particular plant material and process in a specific manner. So to develop a standard procedure for herbal product is highly necessary for the generation (Mukharjee et.al. 1999).

To identify and quantify active substances from herbal formulation product it is necessary to develop standard procedure with the use of latest technology. Though official treatise on homoeopathy is available in India it is obvious that various quality control parameters specified in these official books are not sufficient enough to fulfill the requirement as well as different regulations coming out from the homoeopathic medicines (Shanbhag and Jayraman (2008a); Shanbhag and Jayraman (2008b); Shanbhag and Jayraman (2007).

Adhatoda vasica is part of the *Acanthaceae* plant family. It is a small evergreen, sub-herbaceous bush which grows commonly in open plains, especially in the lower Himalayas (up to 1300 meters above sea level), India, Sri Lanka, Burma and Malaysia (Global Herbal Supplies [Accessed: 30 March 2009]).

Adhatoda vasica is traditionally used for respiratory tract ailments. It has been used in India as an antispasmodic for asthma and intermittent fever, also as an expectorant in cases of chronic bronchitis and

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Received on: 16-11-2009
Revised on: 17-07-2010
Accepted on: 19-07-2010

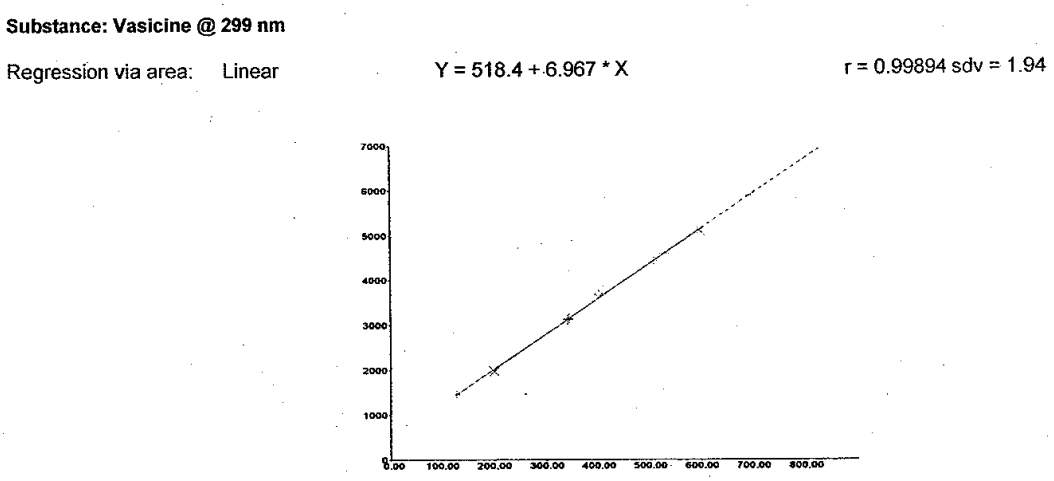

Figure 1: Calibration curve of Vasicine

phthisis. *Adhatoda* is beneficial in asthma due to both its antiasthmatic and expectorant properties (Padmawar A. [Accessed: 30 March 2009]).

Homeopathy is holistic system of therapy which works at reinforcing the body's own natural capacity to heal and achieving a gentle and lasting cure. Mother tinctures (MQ) are defined as the original tincture prepare with the aid of alcohol, directly from the crude drug. They are the precursors of the corresponding potencies of the respective drug and the starting point for the production of most homeopathic medicines. The in-housed standard mother tincture was strictly prepared as per the procedure laid down in Homeopathic Pharmacopoeia of India (HPI). The objective of this work is to make an in house standard mother tincture and compare it with different marketed samples using its fingerprint characteristics and to further quantify them with specific active principle of the known fraction (Verma and Vaid, 1995).

EXPERIMENTAL

Authentic plant (leaves) of *Adhatoda vasica* was used to prepare mother tincture. *Vasicine* (C_{11} $H_{12}N_2O$, m.p. 197°C-198°C, purity 99 % by HPLC) was purchased from Natural Remedies, Bangalore. The solvents used in this experiment were HPLC water, 99.9 % absolute ethanol, acetonitrile, 20% ammonia of Analytical grade purity (Merck Ltd.).

Preparation of Standard mother tincture

The dried leaves of plant coarsely powdered, 10 g of this powder was used and the requisite amount of alcohol and water was added as specified in HPI and the standard mother tincture was prepared by the percolation method. This tincture was transferred to suitable glass container and stored for further study (Sethi, 1996).

Preparation of Standard *Vasicine*

Weigh 5 mg *vasicine* in 5 mL volumetric flask and 5 mL ethanol was added (1µg/µL) in to it. Out of this, 1 mL of the standard solution was taken in another volumetric flask.

Standardization of standard other tincture

Camag HPTLC system comprising of Linomat 5 as sample applicator TLC Scanner 3 controlled by win CATS software version 1.3.4 was used for quantitative evaluation (Datta and Mukharjee, 1952). Stationary phase used for quantitative evaluation. Stationary phase used was Merck percolated TLC aluminum foil Silica Gel RP-18 F_{254} and the mobile phase used was Acetonitrile: Water: 20 % Ammonia (5:5:0.2) v/v. Samples and standard were applied at 8 mm bands with 6 mm distance between the tracks. Tank saturation was given with filter paper for 15 min. Ascending development for a distance of 80 mm in a twin trough chamber was completed in *ca*. 15 min. Volume of standard mother tincture was first optimized at 5 µL for fingerprinting. The λ_{max} of v*asicine* was found to be 300 nm after taking the spectra of the standard of *vasicine*. Quantitative measurement in the absorbance mode was at 225 nm using a slit dimension of 5.00 × 0.45 mm.

Linearity response

The volume of standard mother tincture was optimized to 5 µL for quantification. It was then simultaneously applied with different concentration of standard *vasicine*. The method was found to be linear with a regression of 0.99894 and a standard deviation of 1.94 % and amount of *vasicine* was calculated in the mother tincture.

Standardization of the standard mother tincture by fingerprint method

Standardization of the mother tincture was done by evaluating its fingerprint characteristics, using HPTLC method (Shanbhag, 1993; Raina, 1993). Standard mother tincture was chromatographed simultaneously along with four other mother tinctures available in market 5 µL on the same plate for comparison. Multi wavelength (MWL) scanning was done for finding the optimum wavelength. The optimum wavelength was

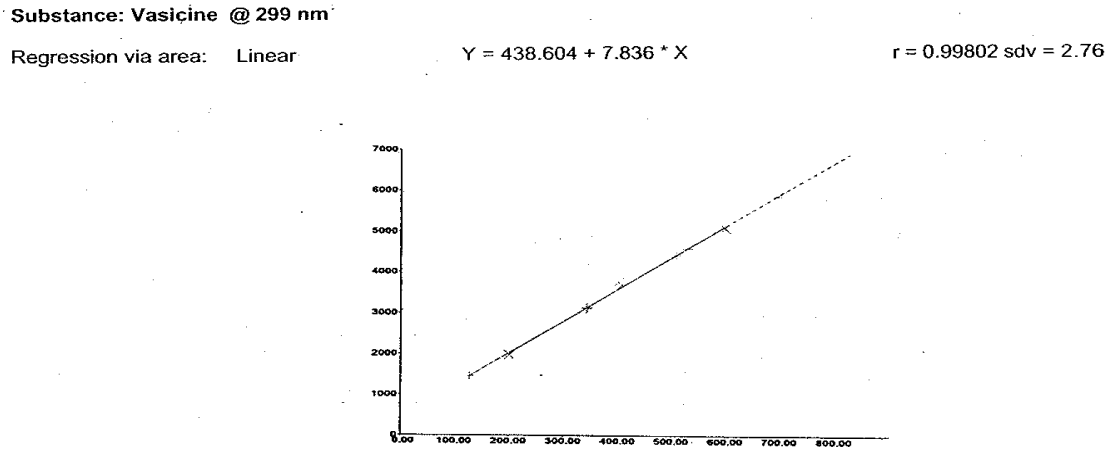

Figure 2: Calibration curve of Vasicine in marketed samples & Std. MQ

Table 1: Analysis of different *Adhatoda vasica* mother tincute at scanning wavelength 299 nm

Peak	A			A1			A2		
	R_f	Max. Ht.	Area %	R_f	Max. Ht.	Area %	R_f	Max. Ht.	Area %
1	0.09	162.5	34.07	0.07	374.7	57.98	0.10	340.9	95.25
2	0.20	24.8	5.15	0.21	225.8	32.81	0.22	16.0	3.40
3	0.33	41.0	13.29	0.36	34.4	3.95	0.27	14.7	1.36
4	0.48	15.0	2.25	0.46	19.4	1.21	-	-	-
5	0.72	141.0	45.24	0.54	38.1	3.35	-	-	-

Peak	A3			A4		
	R_f	Max. Ht.	Area %	R_f	Max. Ht.	Area %
1	0.08	319.2	53.23	0.07	186.6	61.95
2	0.21	57.5	10.07	0.20	58.6	22.17
3	0.38	29.7	4.66	0.39	30.2	11.02
4	0.70	125.9	30.58	0.77	18.8	4.87
5	-	-	-	-	-	-

found at 299 nm. The entire plate was further scanned at this wavelength for quantification and spectral match. Many fractions of standard mother tincture were matched with the help of its characteristic spectra with that of other marketed samples. Individual λ_{max} of each fraction was also found with the help of spectral scanning and then the plate was scanned with this selected wavelength in MWL mode. The pattern of the peaks was compared for the standard mother tincture and marketed samples (Table-1).

It was approved that the response for various concentrations of standard *vasicine* was linear in the range of 200 to 600 ng with a coefficient of variation of 0.99802 and standard deviation of 2.76 %. *Vasicine* was quantified and the amount was calculated in individual mother tinctures. With this method we compared all available mother tinctures and the active principle was also quantified. Thus the method can be said to be standardized.

Quantification of *Vasicine* in market samples and standard mother tincture

The amount of *vasicine* was calculated in standard mother tincture (A) and market samples (A1 to A4) and the results are tabulated in Table 2.

RESULTS AND DISCUSSION

The decomposition of the analyte during application or development was confirmed by two-dimensional chromatography. The chromatogram did not show any extra fraction. Repeatability of the method was checked by scanning 15 tracks of 5 µL volume standard mother tincture. The co-efficient of variation (CV) was found to be 0.465 %.

Table 2: Amount of vasicine in *Adhatoda vasica* mother tinctures

Sample	Wt. of *Vasicine* (mg) in 100 mL sample
A	18.31
A1	15.36
A2	8.8
A3	16.58
A4	11.72

Accuracy: The percentage recovery of *vasicine* values was calculated using the above method. The average recovery values obtained were 97.00 to 101.75 %, which confirms that the method is validation.

The HPTLC fingerprinting characteristic of *Adhatoda vasica* mother tinctures obtained from manufacturer (A1 to A4) and the in-house standard mother tincture (A) had been scanned at 299 nm wavelengths. From the results obtained after densitometric scanning, it was observed that the standard mother tinctures (A) of *Adhatoda vasica* shows 4 peaks. The marketed samples A1 shows 2 peaks, A2 shows 3 peaks, A3 shows 3 peaks and A4 shows 4 peaks.

CONCLUSION

Value of the four marketed tinctures (A1 to A4) was found to show minimum 3 different peaks with R_f values similar to standard mother tinctures (A) and they are similar within themselves. So from this study, it was confirmed that *Adhatoda vasica* tincture contain different components with R_f values (0.07-0.10, 0.20-0.22, 0.33-0.39, 0.46-0.48, 0.54, 0.70-0.77). These components must be considered to determine quality of any further sample of the same. The spectral analysis indicates that spectra with particular R_f values of various components (0.08, 0.21, 0.36, 0.46, 0.54, 0.77) have similar pattern within themselves. It may be concluded that samples procured from the market that are showing lesser peaks may not be up to the standard level.

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