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FUNDAMENTAL RESEARCH

Application of HPTLC in the Standardization of a Homoeopathic Mother Tincture of Nux Vomica*

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Abstract

In this study,we have chosen HPTLC (High Performance Thin Layer Chromatography) as a method of analysis to develop a standard procedure based on fingerprinting characteristics for the evaluation of homreopathic formulations. A simple and accurate HPTLC method has been developed for the quantification of brucine,one of the chief active chemical components of Nux vomica (along with strychnine),and the fingerprinting of the in-house mother tincture of Nux vomica, considered here to be a standard, with that of different marketed samples available from manufacturers of homreopathic medicines in India. This HPTLC method was quantitatively evaluated in terms of stability, repeatability, and accuracy.

Keywords : Standardization of homeopathic mother tinctures; HPTLC, as a tool for standardizing homeopathic mother tinctures; brucine, a measurable component of Nux vomica tincture; fingerprinting homeopathic tinctures with HPTLC.

Introduction

In order to obtain quality oriented herbal formulations, care should be taken right from the proper identification of plants, seasons and area of collection and their extraction and purification process. Despite the large amount of literature available, the standard procedures for quality control of plant materials with respect to their identification (be it pharmacognostic, phytochemical or biological activity) are not available. Though a number of scientific publications are available on various investigations of plant materials no evidence is available by which one investigates a particular plant material and process in a specified manner So to develop a standard procedure, identity of the raw materials as well as quality of the finished products by using various analytical techniques for herbal product is highly necessary for the generation.

Strychnos nux vomica is one of about 150 species of the genus Strychnos, in the family Strychnaceae. It grows as an evergreen tree with a fruit whose seeds are normally harvested at full maturity for their alkaloid content. It is used as a stomachic and stimulant to central nervous & respiratory system. About 1.5 to 5% of the total indole alkaloid that has therapeutic value is present in the seeds [1] [2]. The active principle brucine which is an indole alkaloid is present in the seed.

Homoeopathy is a holistic system of therapy which works at reinforcing the body's own natural capacity to heal and achieve a gentle and lasting cure. Mother tinctures (MQ) are defined as the original tincture prepared 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 homoeopathic medicines [3] . They contain a number of chemical entities. It is not possible to establish the chemical picture of an extract with a single chemical test. Though a series of chemical tests may establish the chemical picture and thus identity of a plant extract, it is not preferable be-cause of a poor selectivity and time factor involved. The alternative method that is available to establish a chemical picture is chromatographic analysis.

The method of chromatographic analysis affords the advantage of identifying the chemical entities present, which constitutes the chemical picture of a plant (herbal) extract and at the same time facilitates to quantify the extract. Chromatography is a technique by which the complex mixtures can be resolved into individual components. 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. This concept of standardisation may lead to a solution to the factors which are responsible for variation in the homoeopathic formulations.

Materials and Methods (Experimental)

Chemicals and Materials

Authentic seeds of Nux vomica were used to prepare the mother tincture. Brucine (C23H26N2O4 m.p. 178°C, purity 98% anhydrous) was purchased from Sigma-Aldrich. The solvents 99.9% absolute ethanol, HPLC water, toluene, ethyl acetate, diethyl amine were of Analytical Grade purity (MERCK Ltd.)

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Preparation of Standard Mother Tincture

The seed was coarsely powdered to prepare the standard mother tincture as specified in HPI and was used in this investigation [4].

Preparation of Standard Brucine

Standard brucine of concentration *O.lfJg/fJl* prepared in ethanol was used for our studies.

Standardisation of Standard Mother Tincture

Camag HPTLC [5] system comprising of Linomat 5 as sample applicator and TLC Scanner3 con-trolled by win CATS software version 1.3.4 was used for quantitative evaluation. Stationary phase used was MERCK precoated TLC Aluminium foil silica gel 60 F254 and the mobile phase used was Toluene-Ethyl acetate-Diethyl amine (7:2:1) v/v. Samples and standard were applied simultaneously on the same TLC plate and studied. Volume of standard MQ was first optimized at 4ì l for fingerprinting. The ë max of brucine was found after taking the spectrum of standard brucine [Fig. 1]. It was found to have two ë max one at 270 nm and the other at 305 nm. We have done this investigation in the absorbance mode at 270 nm [Fig. 2 & Fig. 3].

Linearity Response

The volume of the std. mother tincture was optimized to 5 ì l for quantification. It was then simultaneously applied with different concentration of standard brucine. The method was found to be linear with a polynomial regression of 0.99972 and a standard deviation of 1.09% and the amount of brucine was calculated in the mother tincture [Fig. 4] [Fig.5].

Standardisation of the std. mother tincture by fingerprint method

Standardisation[6] of the mother tincture was done by evaluating its fingerprint characteristics, using HPTLC method. Std. mother tincture was chromatographed simultaneously along with six other mother tinctures available in market at 5 ì l on the same plate for comparison [Table 1]. Multi wavelength (MWL) scan was done for finding the optimum wavelength for scanning. The optimum wavelength was found to be 270 nm. The entire plate was further scanned at this wavelength for quantitation and spectral match. Many fractions of std. mother tincture were matched with the help of its characteristic spectra with that of other marketed samples [Fig. 6]. Individual ë max of each fraction was also found with the help of spectral scanning and then the plate was scanned with these selected wavelengths in MWL mode. The pattern of the peaks was compared for the std. mother tincture and marketed samples.

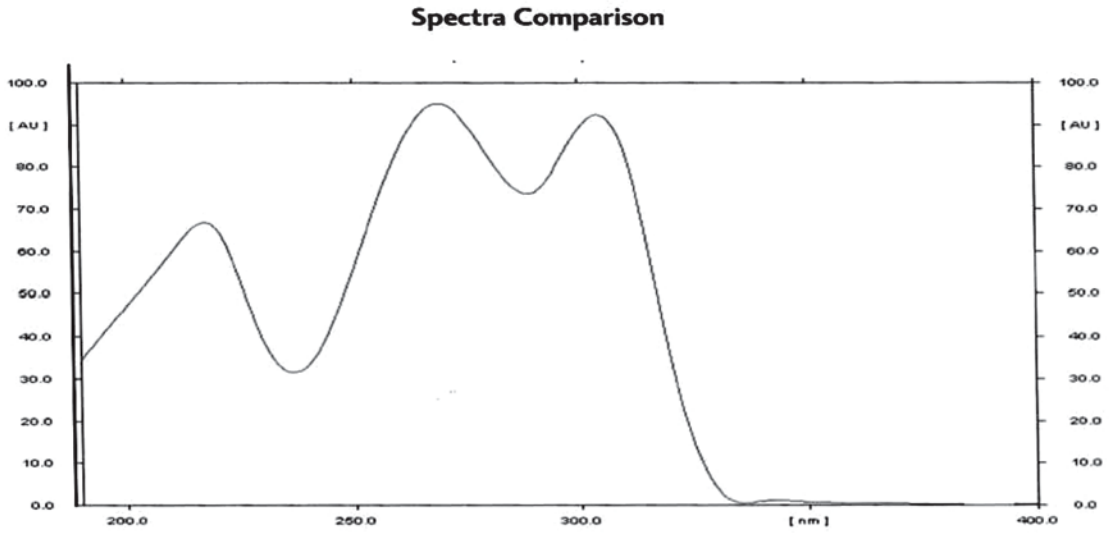

Figure 1: Absorption Spectrum of Standard Brucine

It was observed that the response for various concentrations of standard brucine was linear in the range of 200 ng to 1000 ng with a coefficient of variation of 0.99996 and a standard deviation of 0.52% [Fig.7] [Fig. 8] . Brucine was quantified & 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 standardised.

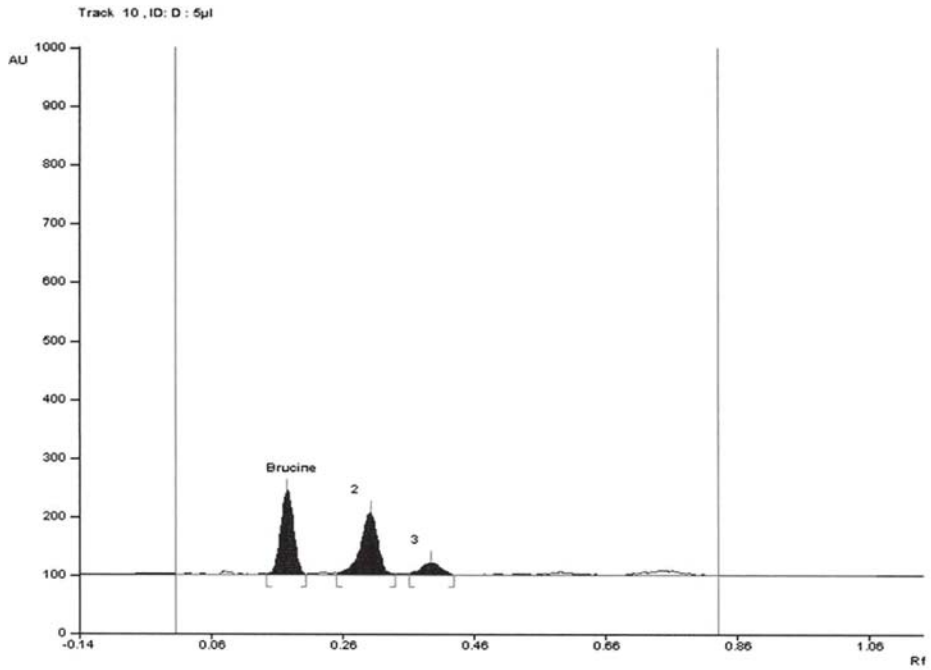

Figure 2: Chromatogram of Standard MQ

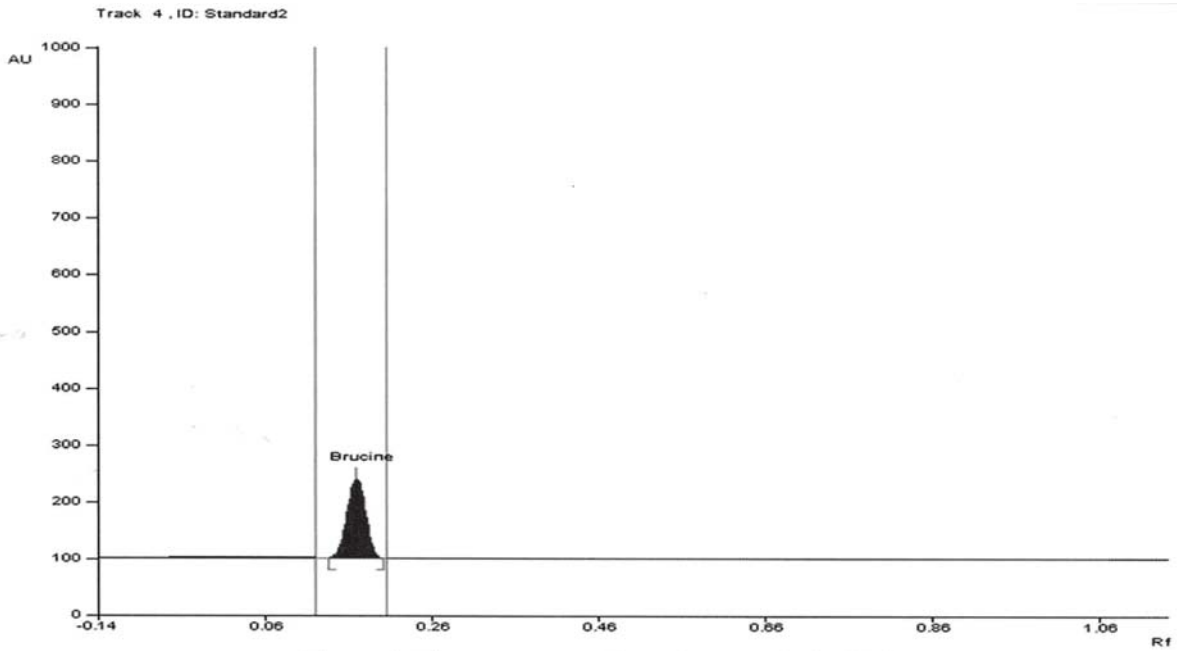

Figure 3: Chromatogram of Std. Brucine (Rf = 0.18)

Quantification of Brucine in Market Samples & Std. Mother Tincture

The amount of brucine was calculated in Std. mother tincture (D) and market samples (D1 to D6) and was found as given in Table 1

Accuracy

The percentage recovery of brucine was calculated using the above method. The average recovery values obtained were 100.17% to 102%, which confirms that

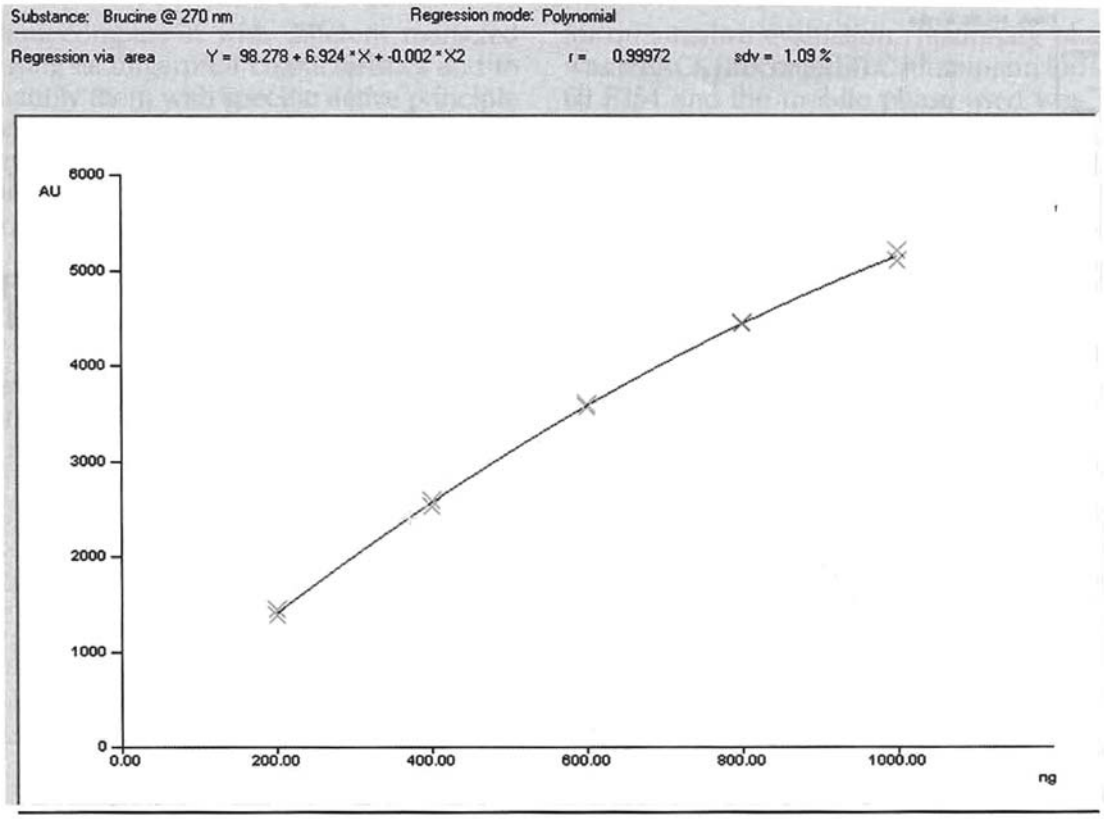

Figure 4: Calibration Curve of Brucine (area)

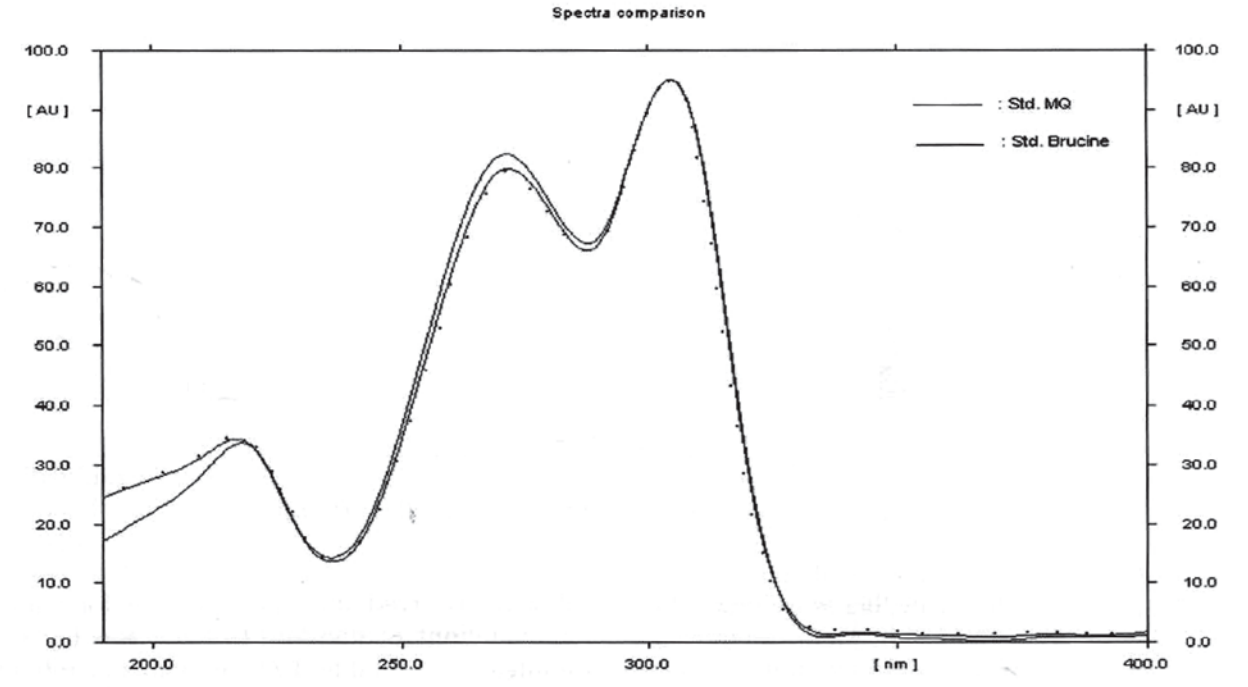

Figure 5: Overlay of Absorption Spectra of Std. Brucine and its Corres. Fraction in Std. MQ

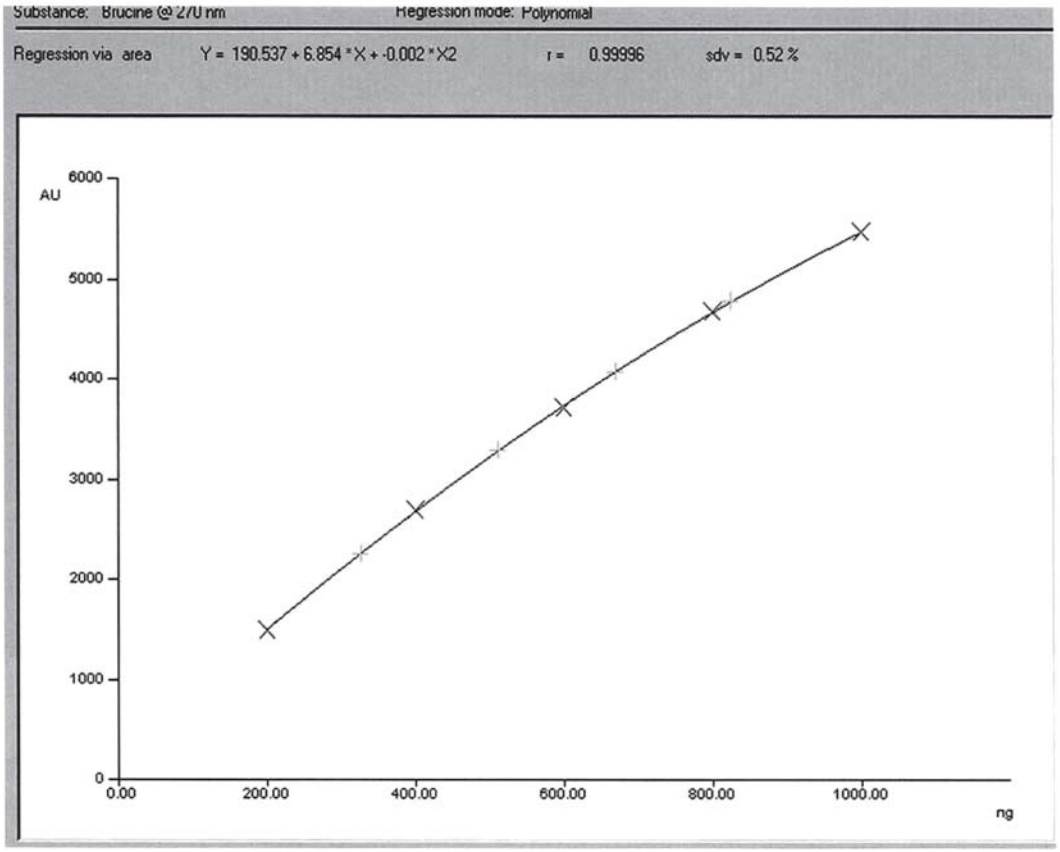

Figure 6: Calibration Curve of Brucine in Mktd Samples & Std. MQ (area)

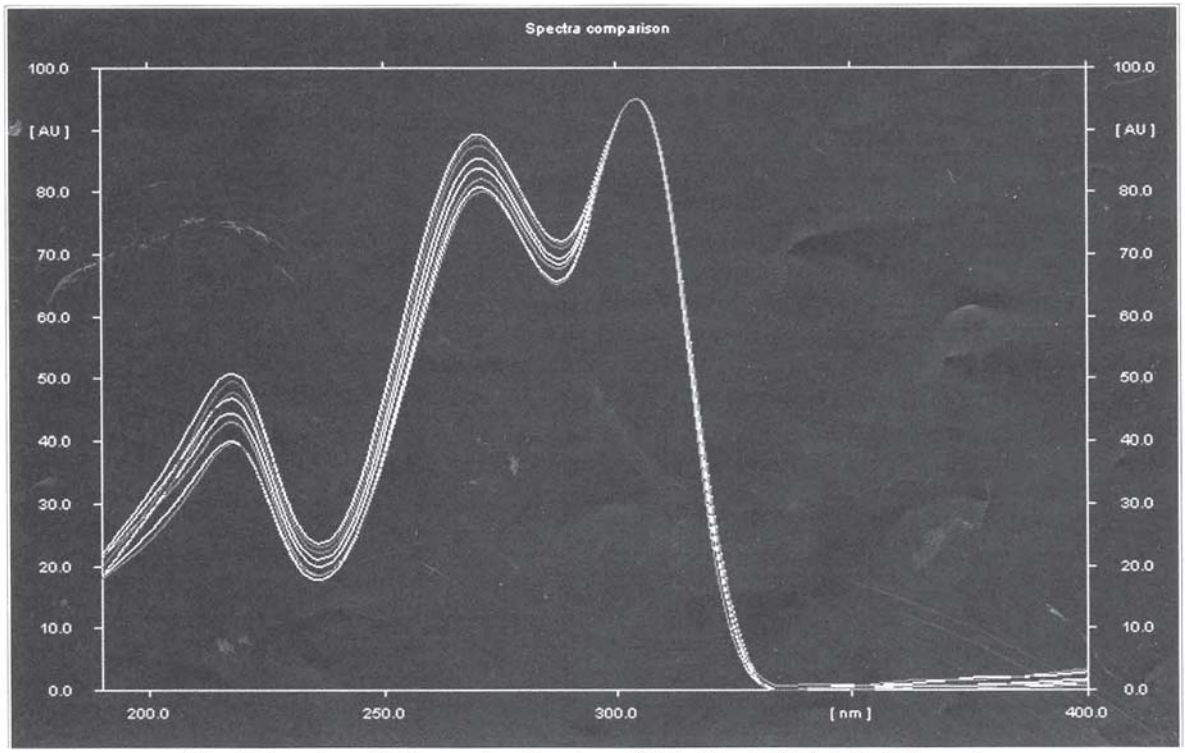

Fig. 7 Overlay of Absorption Spectra of Std, Std. MQ & Mktd. Samples

Curve identifying colors can not be reproduced here; however, these greyscale curves do at least permit a general comparison of the samples

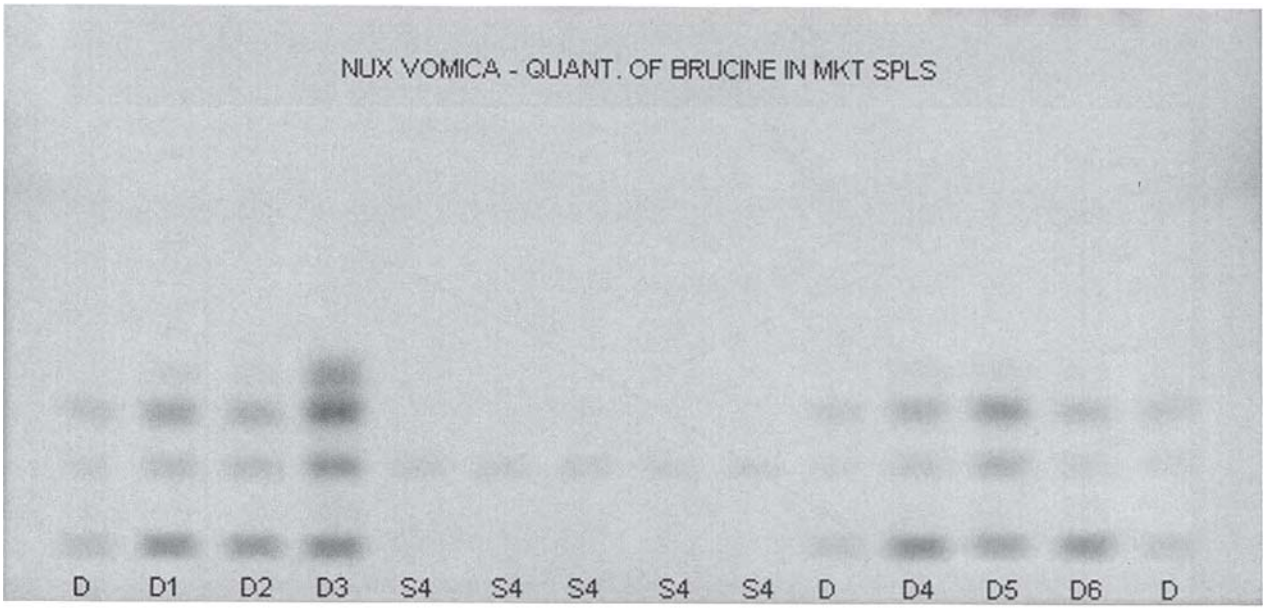

Figure 8 : Image under UV 254nm

the method is validated.

Results and Discussions

The scanning report as well as the fingerprint characters obtained after integration has been shown in Table 2.

Sr. No.	Name of sample	Wt. of Brucine in 100 ml sample
1.	D	6.5mg
2.	D1	13.4mg
3.	D2	16.5mg
4.	D3	25.06mg
5.	D4	22.74mg
6.	D5	25.72mg
7.	D6	10.24mg

Table 1 : The Amount of Brucine in Nux Vomica Mother Tinctures

From the results obtained after densitometric scanning, it was observed that the Std. MQ (D) of Nux vomica shows 3 peaks. The marketed samples D1 shows 4 peaks, D2 shows 3 peaks, D3 shows 3 peaks, D4 shows 4 peaks, D5 shows 4 peaks and D6 shows 4 peaks.

Value of the six marketed tinctures (D1 to D6) was found to show minimum 3 different peaks with Rf values similar to Std.MQ (D) & they are similar within themselves. So from this study it was confirmed that Nux vomica tincture contains different components with Rf values (0.16-0.18, 0.27-0.30, 0.31-0.32, 0.38-0.40,0.74). These components must be considered to determine quality of any further sample of the same. Also spectral analysis indicates that spectra with particular Rf values of various components (0.16,0.29,0.32,0.38,0.74) have similar pattern within themselves. It may be concluded that samples D1, D4,D5 and D6 each contained one component that was not present in our standard MQ. These 'extra' components could be contaminants, or they could derive from seeds that were harvested prematurely, or the manufacturers may have used a different Strychnos species or a different nux vomica variant than we used.

Peak	Rf	D		Peak	Rf	D1		Peak	Rf	D2	
		Max. Ht.	% area			Max. Ht.	% area			Max. Ht.	% area
1	0.17	144.3	45.12	1	0.16	247.2	40.39	1	0.16	285.9	47.38
2	0.30	107.0	44.46	2	0.29	194.1	44.08	2	0.29	211.5	45.62
3	0.39	21.4	10.42	3	0.38	35.3	8.47	3	0.38	31.2	6.99
				4	0.74	17.6	7.05				

Peak	Rf	D		Peak	Rf	D1		Peak	Rf	D2	
		Max. Ht.	% area			Max. Ht.	% area			Max. Ht.	% area
1	0.17	144.3	45.12	1	0.16	247.2	40.39	1	0.16	285.9	47.38
2	0.30	107.0	44.46	2	0.29	194.1	44.08	2	0.29	211.5	45.62
3	0.39	21.4	10.42	3	0.38	35.3	8.47	3	0.38	31.2	6.99
				4	0.74	17.6	7.05				

Peak	Rf	D6	
		Max. Ht.	% area
1	0.19	202.0	35.26
2	0.25	24.2	4.79
3	0.32	176.8	45.47
4	0.40	50.7	14.47

D : Standard mother tincture of Nux vomica prepared in our lab.
D1-D6: Six samples of Nux vomica from manufacturers Retention factor (Rf) corresponds to maximum peak height and is defined as the ratio of the distance from the point of application to the centre of the separated band and the dis-tance traveled by the solvent front from the point of application.

Table 2 : Analysis of different Nux Vomica mother tinctures at scanning wavelength 270 nm

Based on this approach our aim was to develop a standardised procedure to evaluate the mother tinc-tures for its accuracy, sensitivity and reproducibility. The above HPTLC method is very simple, powerful, rapid, reliable and cost effective with respect to the accuracy of the result based on both qualitative and quantitative analysis.

Conclusion

For homoeopathic formulations, development of standard procedure through HPTLC is a new approach which may lead to proper standardization of different homoeopathic tinctures based on fingerprinting characteristics. This investigation shows that these particular characteristics may be used as standardisation tool for Homoeopathic tinctures more effectively and most accurately and is utmost essential which could enable the society in general to have quality homoeopathic formulations in one hand and to gain a momentum in homoeopathic medicine in the other. However, these types of findings cannot rule out the need of further standardisation and evaluations of various homoeopathic formulations but definitely it may lead to a new way in the development of standard procedures for different homoeopathic mother tinctures as well as various other formulations.

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