Original Article

Application of HPTLC in Standardisation of Homoeopathic Mother Tincture

Rauwolfia serpentina and its Comparison with Products in Market

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Abstract

In this study, we have chosen HPTLC as a method of analysis to develop a standard procedure based on fingerprinting characteristics for the evaluation of homoeopathic formulations. A simple and accurate HPTLC method has been developed for the quantification of reserpine and fingerprinting of the in-house 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.

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Key words: HPTLC, Standardisation, Mother Tincture, reserpine, fingerprint, MWL, Spectra

INTRODUCTION

Rauwolfia serpentina is therapeutically used as a sedative, a hypnotic drug and in hypertension. About 0.1% of the active principle reserpine which is an indole alkaloid is present in the root. 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. The in-house standard mother tincture was strictly prepared as per the procedure laid down in the Homoeopathic 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.

MATERIALS AND METHODS

Chemicals and Materials:
Authentic dried roots of Rauwolfia serpentina from Bafco, Noida was used to prepare the mother tincture. Reserpine (C_{33}H_{40}N_{2}O_{9} m.p. 360°C, purity >97% w/w by TLC) was purchased from Natural Remedies, Bangalore. The solvents 99.9% absolute ethanol, HPLC water, toluene, ethyl acetate, diethyl amine, chloroform were of analytical grade purity (MERCK Ltd.)

Preparation of Standard Mother Tincture:
The dried root was coarsely powdered, 5 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.
Preparation of Standard Reserpine:
Five milligram of reserpine was weighed in a 10ml volumetric flask. To this 5 ml chloroform and 5 ml ethanol was added (0.5 µg/µl).

Standardisation of Standard Mother Tincture:
Camag HPTLC system comprising of Linomat 5 as sample applicator and TLC Scanner3 controlled by winCATS 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 as 8 mm bands with 6 mm distance between the tracks. Tank saturation and plate equilibrium was given with filter paper for 10 min. Ascending development for a distance of 80 mm in a twin trough chamber was completed in approximately 15 min. Volume of standard MQ was first optimized at 4µl for fingerprinting. The $\lambda_{max}$ of reserpine was found to be 225 nm after taking the spectra of the standard of reserpine [fig-1]. Quantitative measurement in the absorbance mode was done at 225 nm using a slit dimension of 6.00 x 0.45 mm [fig-2]

Linearity response:
The volume of the std. mother tincture was optimized to 2 µl for quantification. It was then simultaneously applied with different concentration of standard reserpine. The method was found to be linear with a regression of 0.99945 and a standard deviation of 1.82% and the amount of reserpine was calculated in the mother tincture [fig-4] [fig-5].

Standardisation of the std. mother tincture by fingerprint method:
Standardisation 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 2 µl 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 220 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 $\lambda_{max}$ 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. It was observed that the response for various concentrations of standard reserpine was linear in the range of 100 ng to 500 ng with a coefficient of variation of 0.99958 and a standard deviation of 1.79% [fig-7]. Reserpine 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 standardised.

Quantification of reserpine in market samples and Std. mother tincture:
The amount of reserpine was calculated in std. mother tincture (A) and market samples (A1 to A6) and was found as given in [TABLE-2].

RESULTS AND DISCUSSIONS
The decomposition of the analyte during application or development was confirmed by two-dimensional chromatography. The chromatogram did not show any extra fractions. Repeatability of the method was checked by scanning 15 tracks of 4 µl volume std. mother tincture. The coefficient of variation (CV) was found to be 0.454. The percentage recovery of reserpine was calculated using the above method. The average recovery values obtained were 96.6% to 104.37%, which confirms that the method is validated.

The HPTLC Fingerprinting characteristics of “Rauwolfia serpentina” mother tinctures obtained from manufacturer (A1 to A6) and the in-house std. MQ (A) had been scanned at 225 nm wavelength. The scanning report as well as the fingerprint characters obtained after integration has been shown in [TABLE-1]. From the results obtained after densitometric scanning, it was observed that the Std. MQ (A) of Rauwolfia serpentina shows 9 peaks. The marketed samples A1 shows 9 peaks, A2 shows 7 peaks, A3 shows 8 peaks, A4 shows 8 peaks, A5 shows 7 peaks and A6 shows 8 peaks.

Value of the six marketed tinctures (A1 to A6) was found to show minimum 7 different peaks with Rf values similar to
Figure 2: Chromatogram of std. MQ

Figure 3: Chromatogram of std. reserpine (Rf = 0.42)
std. MQ (A) and they are similar within themselves. So from this study it was confirmed that *Rauwolfia serpentina* tincture contains different components with Rf values (0.06, 0.09-0.10, 0.13-0.15, 0.18-0.19, 0.22, 0.26-0.27, 0.36, 0.41-0.43, 0.55, 0.66-0.68, 0.69). 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 (0.06, 0.13, 0.18, 0.26, 0.36, 0.42, 0.67) 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.

![Figure-4 calibration curve of reserpine (area)](image1)

**Figure-4** calibration curve of reserpine (area)

![Spectra comparison](image2)

**Figure-5** overlay of absorption spectra of std. reserpine and its corres. fraction in MQ
**TABLE-1:** ANALYSIS OF DIFFERENT RAUWOLFIA SERPENTINA MOTHER TINCTURES AT SCANNING WAVELENGTH 225 nm

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rf</th>
<th>A</th>
<th>Rf</th>
<th>A1</th>
<th>Rf</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Max. Ht.</td>
<td>% area</td>
<td>Max. Ht.</td>
<td>% area</td>
<td>Max. Ht.</td>
</tr>
<tr>
<td>1</td>
<td>0.06</td>
<td>39.9</td>
<td>5.18</td>
<td>1</td>
<td>0.06</td>
<td>29.7</td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
<td>15.5</td>
<td>2.32</td>
<td>2</td>
<td>0.09</td>
<td>189.1</td>
</tr>
<tr>
<td>3</td>
<td>0.13</td>
<td>15.7</td>
<td>3.28</td>
<td>3</td>
<td>0.15</td>
<td>33.2</td>
</tr>
<tr>
<td>4</td>
<td>0.18</td>
<td>58.1</td>
<td>14.29</td>
<td>4</td>
<td>0.18</td>
<td>28.8</td>
</tr>
<tr>
<td>5</td>
<td>0.26</td>
<td>74.8</td>
<td>20.85</td>
<td>5</td>
<td>0.22</td>
<td>34.6</td>
</tr>
<tr>
<td>6</td>
<td>0.36</td>
<td>56.6</td>
<td>14.84</td>
<td>6</td>
<td>0.26</td>
<td>38.8</td>
</tr>
<tr>
<td>7</td>
<td>0.42</td>
<td>78.2</td>
<td>22.83</td>
<td>7</td>
<td>0.36</td>
<td>21.8</td>
</tr>
<tr>
<td>8</td>
<td>0.55</td>
<td>10.1</td>
<td>3.04</td>
<td>8</td>
<td>0.43</td>
<td>25.4</td>
</tr>
<tr>
<td>9</td>
<td>0.67</td>
<td>42.7</td>
<td>13.38</td>
<td>9</td>
<td>0.68</td>
<td>15.4</td>
</tr>
</tbody>
</table>

**Figure-6** overlay of absorption spectra of std, std. MQ and mktd. Samples

**Figure-7** calibration curve of reserpine in mktd samples and std. MQ (area)
TABLE 2: THE AMOUNT OF RESERPINE IRAUWOLFIA SERPENTINA MOTHER TINCTURES

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of sample</th>
<th>Wt. of Reserpine in 100 ml sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>17.31 mg</td>
</tr>
<tr>
<td>2</td>
<td>A1</td>
<td>15.37 mg</td>
</tr>
<tr>
<td>3</td>
<td>A2</td>
<td>8.80 mg</td>
</tr>
<tr>
<td>4</td>
<td>A3</td>
<td>16.58 mg</td>
</tr>
<tr>
<td>5</td>
<td>A4</td>
<td>11.72 mg</td>
</tr>
<tr>
<td>6</td>
<td>A5</td>
<td>4.91 mg</td>
</tr>
<tr>
<td>7</td>
<td>A6</td>
<td>10.87 mg</td>
</tr>
</tbody>
</table>

Based on this approach our aim was to develop a standardised procedure to evaluate the mother tinctures for its accuracy, sensitivity and reproducibility. This standardisation may lead to a solution to the factors which are responsible for variation in the homoeopathic formulations. The above HPTLC method is powerful, rapid, reliable and cost effective with respect to the accuracy of the result based on both qualitative and quantitative analysis.

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REFERENCES


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