SCREENING AND STANDARDISATION OF TERMINALIA ARJUNA USED AS MEDICINE IN HOMOEOPATHY USING HPTLC METHOD

DAMODAR SHANBHAG, and AMIT KHANDAGALE*

Department of Chemistry, D. G. Ruparel College, Mahim, Mumbai - 400016, India
* Address for correspondence: amitnkhandagale@rediffmail.com

Received 15 August 2011; accepted 06 September 2011

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 Arjunolic acid 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.

KEY WORDS: HPTLC, Standardisation, Mother Tincture, Arjunolic Acid, Fingerprint, MWL, Spectra

INTRODUCTION:
The stem bark of Terminalia arjuna Linn. (family: Combretaceae), commonly known as Arjuna in Indian systems of medicine, is an important drug widely used in the preparations of Ayurvedic and Unani formulations used in cardioprotection. Terminalia arjuna stem bark is reported to contain different groups of chemical constituents, for example hydrolyzable tannins, triterpene acids, flavanoids, phenolics, and phyto sterols. Important triterpene acids are arjunein, arjunic acid, arjunolic acid, and arjungenin. Arjunolic acid (2, 3, 23-trihydroxyolean-12-en-28-oic acid; Figure 3.7) is used for its hypotensive effect and as an antioxidant, antiallergic, and antiasthmatic.

MATERIALS AND METHODS
Chemicals and Materials:
Authentic dried roots of Terminalia Arjuna from Bafco, Noida was used to prepare the mother tincture. Arjunolic Acid (C_{30}H_{48}O_{5}, m.p. 296°C, purity >99% w/w by TLC) was purchased from SPIC Pharma, Chennai. The solvents 99.9% absolute ethanol, HPLC water, toluene, ethyl acetate, diethyl amine, glacial acetic acid were of analytical grade purity (MERCK Ltd.).

Preparation of Standard Mother Tincture:
The dried bark was 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.

Preparation of Standard Arjunolic Acid:
Ten milligram of arjunolic acid was weighed in a 10 mg volumetric flask. To this 10 ml of ethanol was added.

Standardization 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 F_{254} and the mobile phase used was toluene-ethyl acetate-diethyl amine-glacial acetic acid.
(6.5 : 5.0 : 1.5 : 0.5) 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 5 µl for fingerprinting. The $\lambda_{\text{max}}$ of arjunolic acid was found to be 295 nm after taking the spectra of the standard of arjunolic acid [fig-1]. Quantitative measurement in the absorbance mode was done at 295 nm using a slit dimension of 6.00 x 0.45 mm.

**Fig. 1** Absorption spectrum of standard Arjunolic acid

**Linearity response:**
The volume of the std. mother tincture was optimized to 5 µl for quantification. It was then simultaneously applied with different concentration of standard arjunolic acid. The method was found to be linear with a regression of 0.99957 and a standard deviation of 1.69% and the amount of arjunolic acid was calculated in the mother tincture [fig-2].

**Fig. 2** Calibration curve of Arjunolic acid (area)

**Standardisation of the standard mother tincture by fingerprint method:**
Standardisation$^{11}$ 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 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 295 nm. The entire plate was further scanned at this wavelength for quantification 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-3]. Individual $\lambda_{\text{max}}$ 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 arjunolic acid was linear in the range of 200 ng to 1500 ng with a coefficient of variation of 0.99993 and a standard deviation of 0.58% [fig-4]. Arjunolic acid 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 arjunolic acid in market samples and standard mother tincture:**
The amount of arjunolic acid was calculated in standard mother tincture (A) and market samples (A1 to A4) and was
both qualitative and quantitative analysis. It may be concluded that values of various components (0.15, 0.31, 0.36, 0.40) have been calculated using the above method. The average recovery values obtained were 98.90% to 100.67%, which confirms that the method is validated.

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 co-efficient of variation (CV) was found to be 0.316. The percentage recovery of arjunolic acid was calculated using the above method. The average recovery values obtained were 98.90% to 100.67%, which confirms that the method is validated.

The HPTLC Fingerprinting characteristics of “Terminalia Arjuna” mother tinctures obtained from manufacturer (A1 to A4) and the in-house std. MQ (A) had been scanned at 295 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 Terminalia Arjuna shows 4 peaks. The marketed samples A1 shows 3 peaks, A2 shows 3 peaks, A3 shows 4 peaks, A4 shows 3 peaks. Value of the four marketed tinctures (A1 to A4) was found to show minimum 3 different peaks with Rf values similar to std. MQ (A) and they are similar within themselves. So from this study it was confirmed that Arjunolic Acid tincture contains different components with Rf values (0.11, 0.12, 0.15-0.16, 0.20-0.21-0.22, 0.35-0.36-0.37, 0.39-0.40-0.41, 0.43-0.44-0.45). 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 (0.15, 0.31, 0.36, 0.40) 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.

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.

REFERENCES


Table 1: Analysis of different terminalia arjuna mother tinctures at scanning wavelength 295 nm

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rf</th>
<th>Max. Ht.</th>
<th>% area</th>
<th>Rf</th>
<th>Max. Ht.</th>
<th>% area</th>
<th>Rf</th>
<th>Max. Ht.</th>
<th>% area</th>
<th>Rf</th>
<th>Max. Ht.</th>
<th>% area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.15</td>
<td>144.5</td>
<td>45.23</td>
<td>0.16</td>
<td>247.5</td>
<td>40.56</td>
<td>0.15</td>
<td>285.6</td>
<td>47.38</td>
<td>0.16</td>
<td>348.9</td>
<td>46.89</td>
</tr>
<tr>
<td>2</td>
<td>0.31</td>
<td>107.0</td>
<td>40.56</td>
<td>0.30</td>
<td>194.3</td>
<td>46.78</td>
<td>0.29</td>
<td>211.5</td>
<td>45.62</td>
<td>0.31</td>
<td>257.8</td>
<td>44.37</td>
</tr>
<tr>
<td>3</td>
<td>0.36</td>
<td>21.5</td>
<td>9.20</td>
<td>0.39</td>
<td>37.5</td>
<td>12.66</td>
<td>0.39</td>
<td>31.2</td>
<td>6.99</td>
<td>0.40</td>
<td>33.4</td>
<td>5.99</td>
</tr>
<tr>
<td>4</td>
<td>0.40</td>
<td>10.3</td>
<td>5.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

A – Standard mother tincture of Terminalia Arjuna prepared in our laboratory.
A1 – A4 – Four samples of Terminalia Arjuna tincture from manufacturer.
Rf corresponds to maximum peak height.

Table 2: Amount of Arjunolic acid in terminalia arjuna mother tinctures

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of sample</th>
<th>Wt. of Arjunolic Acid in 100 ml sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>12.54 mg</td>
</tr>
<tr>
<td>2</td>
<td>A1</td>
<td>16.86 mg</td>
</tr>
<tr>
<td>3</td>
<td>A2</td>
<td>15.67 mg</td>
</tr>
<tr>
<td>4</td>
<td>A3</td>
<td>22.45 mg</td>
</tr>
<tr>
<td>5</td>
<td>A4</td>
<td>10.67 mg</td>
</tr>
</tbody>
</table>

found as given in [TABLE-2].

Source of support: Nil; Conflict of interest: None declared