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Short Communication

Development and Validation of a Stability-Indicating HPTLC Method for Analysis of Arjunolic Acid in a Herbal Formulation

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Key Words

Arjunolic Acid
HPTLC
Terminalia arjuna
Stability indicating
Validation

1 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 preparation of Ayurvedic and Unani formulations used in cardioprotection [1, 2]. *T. arjuna* stem bark is reported to contain different groups of chemical constituents, for example hydrolyzable tannins [2, 3], triterpene acids, flavanoids, phenolics, and phyto sterols [3]. Important triterpene acids are arjunetin, arjunic acid, arjunolic acid, and arjungenin [4–6]. Arjunolic acid (2,3,23-trihydroxy-olean-12-en-28-oic acid; **Figure 1**) is used for its hypotensive effect and as an antioxidant, antiallergic, and antiasthmatic [6]. HPTLC and HPLC methods have been reported for analysis of arjunolic acid in the crude form [7–9] but the methods lack proper validation. Because no stability-indicating analytical method had been reported for quantification of arjunolic acid in pharmaceutical or herbal dosage forms it was thought worthwhile to develop a stability-indicating HPTLC method. The proposed method will be useful for standardization and quality control of formulations which contain arjunolic acid or arjuna as an ingredient.

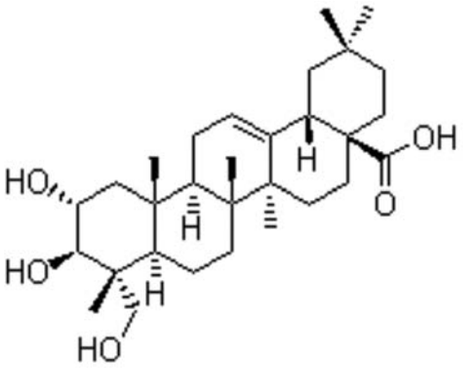

Figure 1

The structure of arjunolic acid.

2 Experimental

2.1 Chemicals and Reagents

Reference standard arjunolic acid (98.0%) was obtained as a gift from Sami Labs (Bangalore). Arjuna tablets were provided by Sri Sri Ayurveda, Bangalore. All chemicals and reagents used were of analytical grade and purchased from Merck Chemicals (India).

2.2 HPTLC

Chromatography was performed on 20 cm × 10 cm aluminum HPTLC plates coated with 0.2 μm layers of silica gel $60F_{254}$. The plates were pre-washed with methanol and activated at 60°C for 20 min before chromatography. Samples were applied as bands 4 mm wide, 7.4 mm apart by use of a CAMAG (Switzerland) Linomat V sample applicator fitted with a microlitre syringe. A constant application rate of 120 nL s^{-1} was used. Linear ascending development, to a distance of 80 mm, with chloroform–toluene–ethanol 4:4:1 (*v*/*v*) as mobile phase, was performed in a 20 cm × 10 cm twin-trough glass chamber. Before chromatography the chamber was saturated with mobile phase vapor for 15 min; 15 mL mobile phase was used for each development. After the development, plates were dried in a current of air by means of an air dryer, sprayed with a freshly prepared solution of anisaldehyde in sulfuric acid, then dried in hot air oven at 110°C for 5–7 min. Densitometric scanning at 600 nm was performed with a CAMAG TLC scanner III operated by winCats software. The source of radiation was a tungsten lamp, the slit dimensions were 4 mm × 0.45 mm, and the scanning speed was 10 mm s^{-1}.

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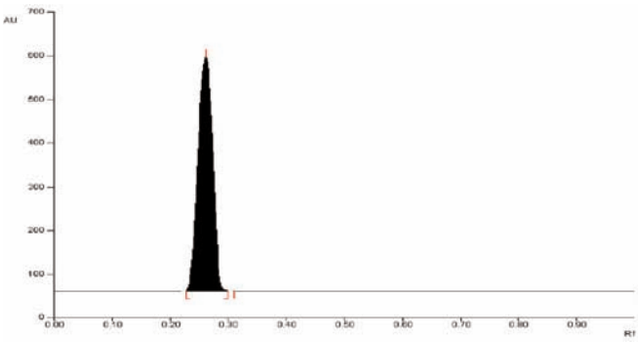

Figure 2

Chromatogram obtained at 600 nm from arjunolic acid (500 ng per band; R_F 0.28).

2.3 Method Validation

The method was validated in accordance with ICH guidelines [10], similar to other chromatographic methods established and validated in our laboratory [11–15], which are still in use for quality control of herbal drugs.

The precision of the method was assessed as method precision and intermediate precision. Inter-day and intra-day precision were assessed by analysis of samples at three different concentrations on the same day and on three different days, respectively. Inter-system and inter-analyst precision were assessed by repeating this procedure by use of a different system by the same manufacturer and by a different analyst, respectively.

The accuracy of the method was assessed by measurement of recovery. Pre-analyzed samples were spiked with standard at four different levels, 0, 50, 100, and 150% of the amount originally present and the mixtures were reanalyzed. The recovery experiment was conducted in triplicate.

The specificity of the method was ascertained by analyzing drug standard and sample. Peak purity for arjunolic acid was assessed by comparing spectra acquired at three different positions, i.e. peak start, peak apex, and peak end.

The robustness of the method was assessed at two levels (100 and 200 ng per band) by introducing small, deliberate changes in mobile phase composition, i.e. use of chloroform–toluene–ethanol 4:4:0.9 and 4:4:1.1 (*v*/*v*), and examining the effect on the result as RSD [%]. A low value of RSD indicates the method is robust.

The LOD and LOQ of arjunolic acid were determined as the amounts for which the signal-to-noise ratios (*S*/*N*) were 3 and 10, respectively.

2.4 Sample Preparation

Arjuna tablets were crushed and powdered. The powder (1 g) was heated under reflux with distilled water (20 mL) for 2 h then filtered. The filtrate was repeatedly extracted with 20 mL chloroform–ether 1:2 (*v*/*v*) until the extract became colorless. The organic extract thus obtained was pooled and evaporated to dryness by rotary evaporation below 50°C. The residue obtained was re-dissolved in chromatographic grade methanol (1.0 mL), which was applied in triplicate (2 µL each) to an HPTLC plate for quantification of arjunolic acid.

2.5 Forced Degradation

A stock solution was prepared containing 100 mg arjunolic acid in 100 mL methanol. This solution (1000 µg mL^{-1}) was used for further investigations. Forced degradation of arjunolic acid was carried out to provide an indication of the stability-indicating property and specificity of the method. To 10 mL of methanolic stock solution of arjunolic acid in a 100 mL volumetric flask:

– Methanolic 2.5 M HCl (10 mL) was added and the mixture was left to stand for 1 h at room temperature for completion of reaction (hydrochloric acid-induced degradation).

– Methanolic 2.5 M formic acid (10 mL) was added and the mixture was left to stand for 1 h at room temperature for completion of reaction (formic acid-induced degradation).

– Methanolic 1 M NaOH (10 mL) was added and the mixture was left to stand for 1 h at room temperature for completion of reaction (base-induced degradation).

– Hydrogen peroxide (H_2O_2; 15% *v*/*v*, 25 mL) was added and the mixture was left to stand for 1 h at room temperature (H_2O_2-induced degradation).

– Methanol (10 mL) was added and the solution was exposed to direct sunlight for 3 days (from 09:00 to 17:00 h at 30–35°C, total 24 h) on a wooden plank and on a terrace (photochemical degradation).

– Methanol (10 mL) was added and the solution was exposed to UV irradiation at 254 nm for 24 h in a UV chamber (UV-induced degradation).

After all the degradation studies, the volumes of samples were adjusted to 100 mL with methanol and 2 µL (200 ng per band) of the resulting solutions were applied to TLC plates in triplicate. The chromatograms were developed and scanned as described in Section 2.2, and peaks were obtained for the different degradation products and remaining arjunolic acid (out of 200 ng per band).

3 Results and Discussion

3.1 Optimization of the Mobile Phase

It was necessary to select a mobile phase which could be used for routine analysis of formulations containing arjunolic acid and for analysis of the degradation products of the compound. The method succeeded in fulfilling both requirements. Initially, mixtures of chloroform and toluene in different proportions were investigated as mobile phases. Although chloroform–toluene 1:1 (*v*/*v*) gave a well defined peak for arjunolic acid it failed to separate the degradation products from the active component. Chloroform–toluene–ethanol 4:4:1 (*v*/*v*) was found to give sharp and well defined peaks of the degradation products, and of arjunolic acid at R_F 0.28 (**Figure 2**).

3.2 Calibration

Different volumes of arjunolic acid stock solution of known concentration (100 µg mL^{-1} in methanol) were spotted in duplicate on a TLC plate to furnish 50, 100, 200, 300, 400, and 500 ng per band. The chromatogram was developed, sprayed with anisaldehyde sulfuric acid, dried, and scanned at 600 nm. Linear