High performance thin layer chromatographic method for quantification of gallic acid in *arjunarishta:* an ayurvedic formulation

Amit Kumar Sharma[1*], Anuj Agarwal[1], Ashok Bansal[2]

[1]Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad
[2]IFTM College of Pharmacy, Moradabad

ABSTRACT:

A sensitive, simple and accurate HPTLC method has been developed for the quantification of Gallic acid a polyphenols in *Arjunarishta*; an ayurvedic formulation. Chromatographic analysis was performed using alcoholic sample on silica gel 60F254 HPTLC plate, using a solvent system comprising of Acetonotrile: Water: Formic acid : Methanol, 7:5:1:1 (v/v/v/v) as mobile phase gives good separation of Gallic acid at R_f =0.38. Detection and quantification of Gallic acid was done by densitometric scanning at 254 nm. The calibration curve of Gallic acid was found to be linear (200 -1200 ng/spot) dependent on the concentration against area. The concentration of Gallic acid in the sample of *Arjunarishta,* by the proposed HPTLC method was found to be 0.08 µg. The proposed HPTLC method provided a good resolution of Gallic acid from other constituents present in the sample can be used for a routine quality control analysis and quantification of Gallic acid in *Arjunarishta*.

Keywords: *Arjunarishta*, Polyherbal, HPTLC, Gallic acid

INTRODUCTION:

The need of quality control for herbal medicines is due to the fact that the preparation of drug according to the ancient method has been reduced due to the commercialization of Ayurvedic pharmacy during past era **[1]**. Today there are additional requirements, distinct in nature, for modern routine quality control of botanical raw materials, in addition to physical tests and identification i.e. chemical composition. HPTLC method has emerged as an efficient tool for phytochemical evaluation of herbal drugs. The Govt. of India has adopted the "fingerprint" approach **[2-3]** for botanicals because it supports the traditional concept and is easy to practice at different levels of sophistication. The British Pharmacopoeia **[3]** has had an emphasis on using TLC and HPLC profiles to identify characteristic and active principles of herbal materials.

Arishtas are an important group of formulations used in Ayurveda. *Arjunarishta* (*Parthadyarishta*) is one of the ancient arishta and oral formulation prescribed in Ayurveda for cardiovascular disorders. The major plant ingredient of this formulation; *Terminalia arjuna* (Combretaceae) is a native plant of India and South East Asia and has been traditionally used as a cardioprotective agent. The main ingredient of this formulation (*Terminalia arjuna)* have found useful in cardiovascular disorders and in other diseases like; alleviating angina, congestive heart failure and respiratory oxy burst etc. [**4-6**]. Presence of glycosides are claimed to be cardiotonic. Since *Arjunarishta* is prepared by decoction, it is likely that non-polar constituents are not present in the formulation and the claimed cardioprotective activities might be due to polar constituents of *Terminalia arjuna*. Since *Arjunarishta* consist gallic-acid and no HPTLC method has been developed in literature for the quantification of Gallic acid in *Arjunarishta*. The present paper reports standardization of *Arjunarishta* by quantification of gallic-acid as a major

component by High Performance Thin Layer Chromatographic method which may be used as an alternative method for standardization of *Arjunarishta* using Gallic acid as a marker.

MATERIALS AND METHODS:

Instrument

Camag Linomat V Automatic Sample Spotter, Camag (Muttenz, Switzerland), Camag TLC Scanner III linked to winCATS software (Camag), Camag glass twin trough chamber (10 x 10 cm).

Materials

Silica gel 60F254 HPTLC plate (E. Merck, Baroda) precoated on aluminum sheet plates were employed. Acetonotrile, Formic acid, Methanol were used of AR grade. Standard Gallic acid was procured from Sigma Aldrich.

Preparation of standard solution

A stock solution of standard Gallic acid was prepared in 10 ml volumetric flask by dissolving 10 mg of accurately weighed Gallic acid standard, in about 5 ml of methanol, followed by sonication for 5 min and finally making the volume up to mark with methanol, to obtain working standard solution of Gallic acid, in the concentration range of 1000 µg/ml.

Preparation of sample solution:

1 ml of sample (formulation or decoction) was dried on rotavapor for half an hr to remove the alcohol. 5 ml of methanol was added to it and sonicated for 10 minutes and centrifuged at 3000 rpm to settle down the precipitated sugars. 1 ml of supernatant was passed through 0.45 µm filter (Millipore) and 5 µl was injected for quantification.

Chromatographic conditions [7-11]

Application: 5 µl of sample and 0.5 µl of Gallic acid standards were applied as band of 5 mm width.

Mobile phase: Acetonotrile: water: Formic acid: Methanol (7:5:1:1 v/v/v/v)

Saturation time: The chamber was saturated with mobile phase for 30-45 min.

Migration distance: 76 mm

Lamp: Deuterium

Wavelength of detection: 254 nm

Calibration of Gallic acid

The working standard solution of Gallic acid (1000µg/ml) was spotted to get different concentration ranging from 200–1200 ng/spot, using a micro syringe. The working standard solutions were spotted as sharp band of 5 mm width on precoated silica gel aluminum plate 60F254, (10cm x 10cm) with 200 µm thickness using an automatic sample applicator. The bands were applied at a distance of 10 mm from the bottom edge of the plate and the distance between the two bands was 10 mm. The plates were developed in the solvent system comprising of Acetonotrile: water: Formic acid: Methanol; 7:5:1:1 (v/v/v/v), in a Camag glass twin trough chamber at 25 ± 2°C,

up to a distance of 76 mm. After development the plates were dried by dryer for 5 min and densitometric scanning was performed on a Camag TLC scanner 3 in the reflectance absorbance mode at 254 nm. Peak areas of Gallic acid for each concentration were also recorded.

RESULT & DISCUSSION:

The well resolved densitometric chromatogram of maker compounds (Standard Gallic Acid) shows R_f value 0.38 (**Fig 1).** The method as described in the present study, utilize silica gel 60F254 TLC plate as stationary phase and Acetonotrile: water: Formic acid: Methanol (7:5:1:1 v/v/v/v) as mobile phase gives good separation of Gallic acid at R_f value similar to standard Gallic acid, from the other components present in sample (**Fig 2**). The TLC plate was visualized under UV light at 254 nm and the HPTLC photographed chromoplate is shown in **Fig 3**. The calibration curve of Gallic acid was found to be linear (200-1200 ng/spot) dependent on the concentration against area. The concentration of Gallic acid in the sample by the proposed HPTLC method was found to be 0.08 µg. Correlation coefficient 0.9966 indicates good linearity between concentration and peak area.

CONCLUSION:

The developed HPTLC method showed a good resolution of Gallic acid from other constituents present in the sample of *Arjunarishta*. The method can be used for a routine quality control analysis and quantitative determination of Gallic acid in *Arjunarishta*.

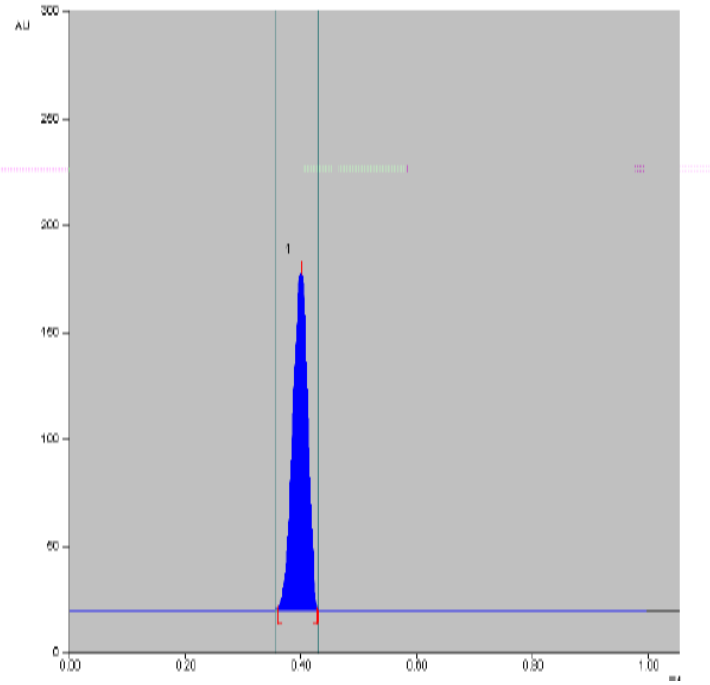


Figure 1: Densitometric chromatogram of Standard Gallic Acid.

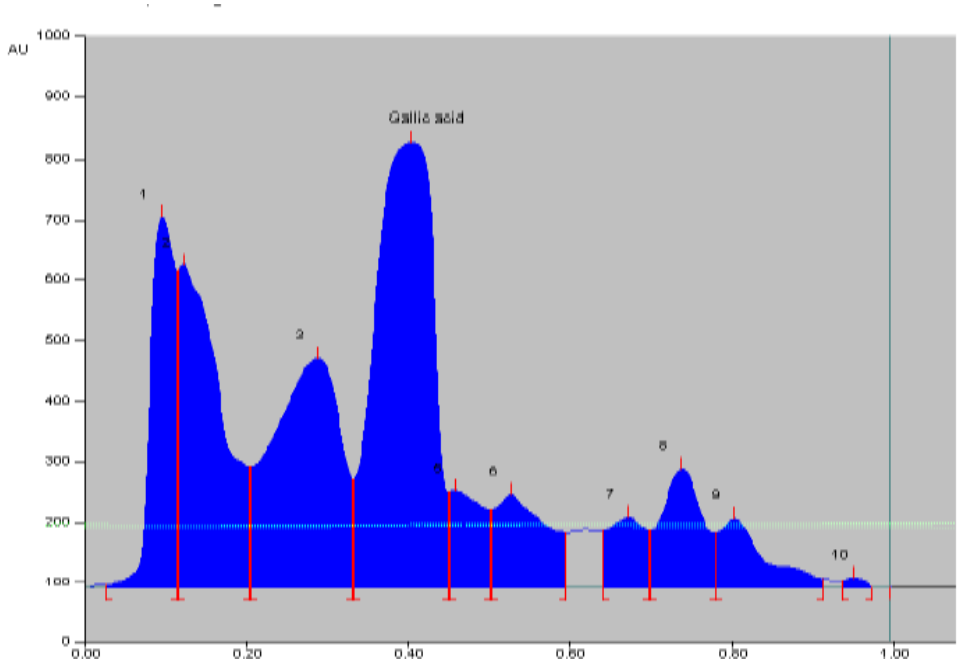
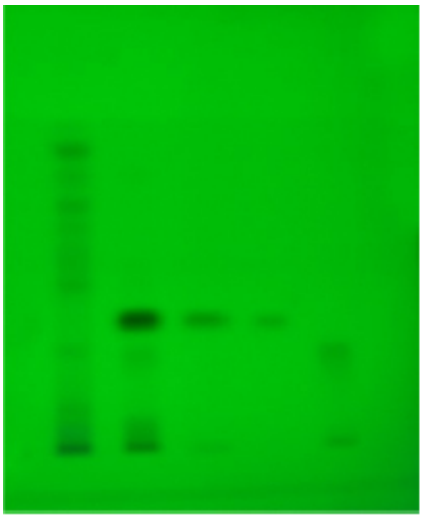


Figure 2: HPTLC finger print of *Arjunarishta*.



T1 T2 T3 T4 T5

Figure 3: Photograph chromoplate of extract and standard Gallic acid. Photograph chromoplate at 254.

Track 1: Arjunarishta

Track 2-5: Different concentrations of Gallic Acid

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