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Quantitative Estimation of Ascorbic Acid by HPTLC in different varieties of Amla

Chakraborthy GS

SVKM'S, NMIMS University, School of Pharmacy and Technology Management, Shirpur Campus, Shirpur, Maharashtra -425 405, India

Address for correspondence: *Dr. G. S. Chakraborthy; E-mail: phdgs77@indiatimes.com*

ABSTRACT

Medicinal plants provide good remedies for human diseases and play a vital role in our day-to-day lifes. *Phyllanthus emblica* Linn family- Euphorbiaceae, commonly known as amla, is an important household fruit. The anti-ascorbic agent Vitamin C is a very effective antioxidant constituent present in amla. It is considered to be the richest source of Vitamin C. The determination of ascorbic acid in this fruit was done by thin layer chromatography (TLC) and qualitative test. Thus, it was quantified using the High Performance Thin Layer Chromatography (HPTLC) method in different varieties of fruit collected from different geographical regions. The method was carried out in TLC precoated aluminium plates with silica gel 60 GF as stationary phase. The solvent system was Ethanol: Acetic Acid (9.5:0.5 v/v) with the Rf value of 0.76± 0.03). Quantitative analysis was carried out in the absorbance at 254 nm. The linearity regression analysis for the calibration showed r= 0.992 and 0.986 with respect to peak area and height in the concentration range of 0.5-5.0 g per spot. The highest content of ascorbic acid was found in the bigger variety collected from Shirpur. The method developed can be used for a routine analysis of ascorbic acid in crude drugs as well as in herbal and pharmaceutical dosage form containing amla as an ingredient.

Key words: Ascorbic acid, HPTLC, *Phyllanthus emblica*, Quantitative Analysis

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INTRODUCTION

Phyllanthus emblica Linn. (family-Euphorbiaceae), commonly known as amla, is an important household fruit. Amla is considered to be the richest source of Vitamin C. The fruits are sour, stomchich, laxative, and aphrodisiac in nature.[1] The root bark is useful in the treatment of ulcerative disease and as an astringent. The leaves are useful in the treatment of conjunctivitis and in dyspepsia.[2] *P. emblica* L. has been used for anti-inflammatory and antipyretic treatments by rural populations. Malays use a decoction of its leaves to treat fever.[3] In Indonesia, the pulp of the fruit is smeared on the head to dispel headaches and dizziness caused by excessive heat.[4]

High performance thin layer chromatography (HPTLC) is an important tool that can be used qualitatively as well as quantitatively for checking the purity and identity of crude drugs and also for quality control of finished products.[5] However, recent reviews show that the thin layer chromatography (TLC) and HPTLC techniques can be used to solve many qualitative and quantitative analytical problems in a wide range of fields including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology, and environmental analysis.[6,7] Ascorbic acid, commonly known as Vitamin C (antiscorbutic factor), plays a very important role in various oxidoreduction reactions. It is required for the maintenance of the integrity of collagen in animals, reduces resistance to infections,

and also provides a protective effect against cancer. Ascorbic acid is not synthesized in primates, therefore humans must obtain it from their diet and it is present in large quantities in various fruits and their juices.[8] In the present investigation, an attempt has therefore been made to develop a simple and reproducible HPTLC method for quantitative estimation of ascorbic acid in different varieties of amla collected from different places.

MATERIALS AND METHODS

Preparation of samples

Four different varieties of amla were collected from different regions such as the large variety of amla from Shirpur and Mumbai and the small variety of amla from Shirpur and Mumbai. Fresh fruits were crushed to remove seeds and 100 gms each were macerated with 150 ml of 50% methanol for 48 hours with occasional shaking. The extracts were separated and mark was again extracted twice with fresh 50% methanol. The extracts were pooled and concentrated in a rotatory vacuum evaporator to get 100 ml for each sample separately. One ml of each extract was further diluted to 5 ml with 50% methanol, which were used for quantitative estimation.

Standard Ascorbic acid solution

A 1 mg/ml solution of standard ascorbic acid (Sigma Aldrich) was made in 50% methanol for preparation of the standard curve.

Procedure

The five standard levels (1, 2, 3, 4, and 5 μg) of standard ascorbic acid were used for the calibration curve for which 1, 2, 3, 4, and 5 μl of standard solution was applied in duplicate on a TLC plate using a semi automatic Linomat V sample applicator. The chromatogram was developed for 15 mts, dried at room temperature, and scanned at 254 nm; average peak areas of two standards were calculated. The calibration curve of the standard drug concentration (X-axis) over the average peak height/area (Y-axis) was prepared to get a regression equation by Win Cats software, which was used for the estimation of ascorbic acid in amla.

Chromatographic conditions

Instrument: Camag HPTLC system, consisting of Linomat V spotting device and Scanner III with Win Cats 4 software
Stationary phase: TLC aluminum sheets silica gel 60 F_{254} pre coated layer (20 cm x 10 cm), thickness 0.2 mm., no. of tracks: 18, band length: 6 mm.

Mobile Phase: Ethanol: 10% glacial acetic acid (9.5: 0.5)
Development Chamber -Twin through chamber (20 x 10)
Distance run: 75 mm
Scanning wavelength - 256 nm

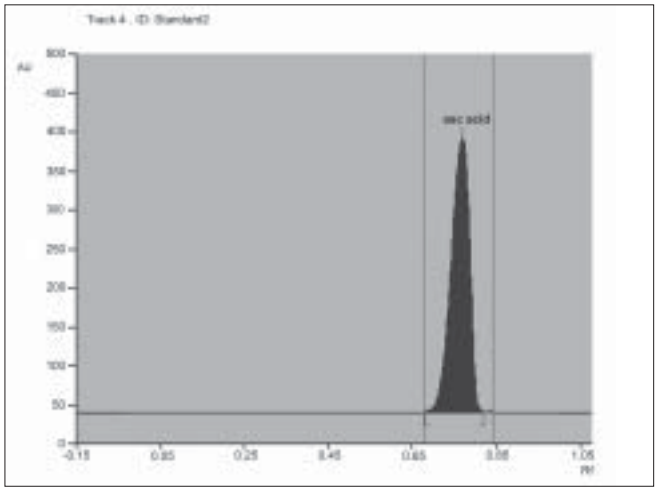

Figure 1: HPTLC Chromatogram of ascorbic acid

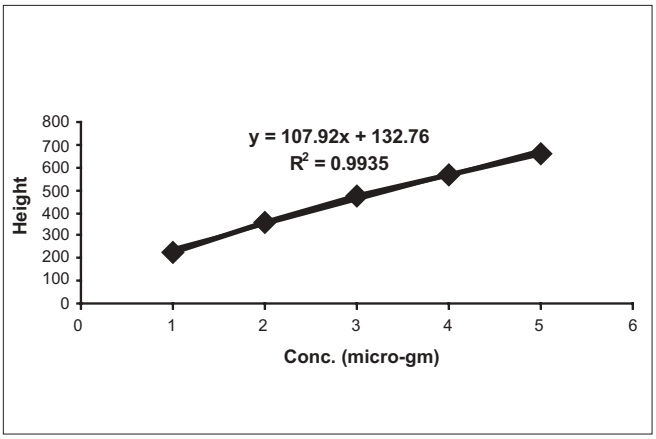

Figure 2: Standard curve of ascorbic acid with respect to height

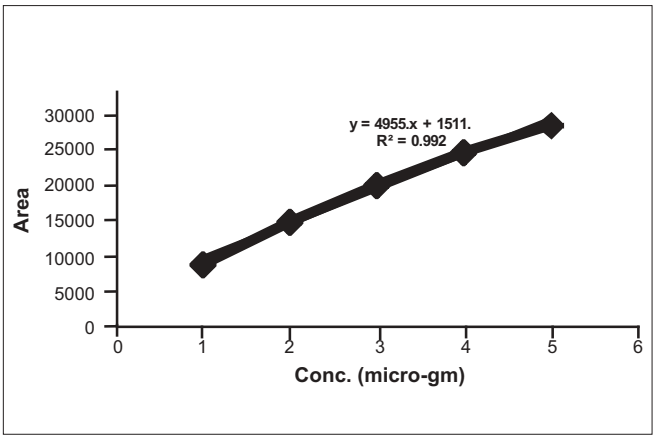

Figure 3: Standard curve of ascorbic acid with respect to area

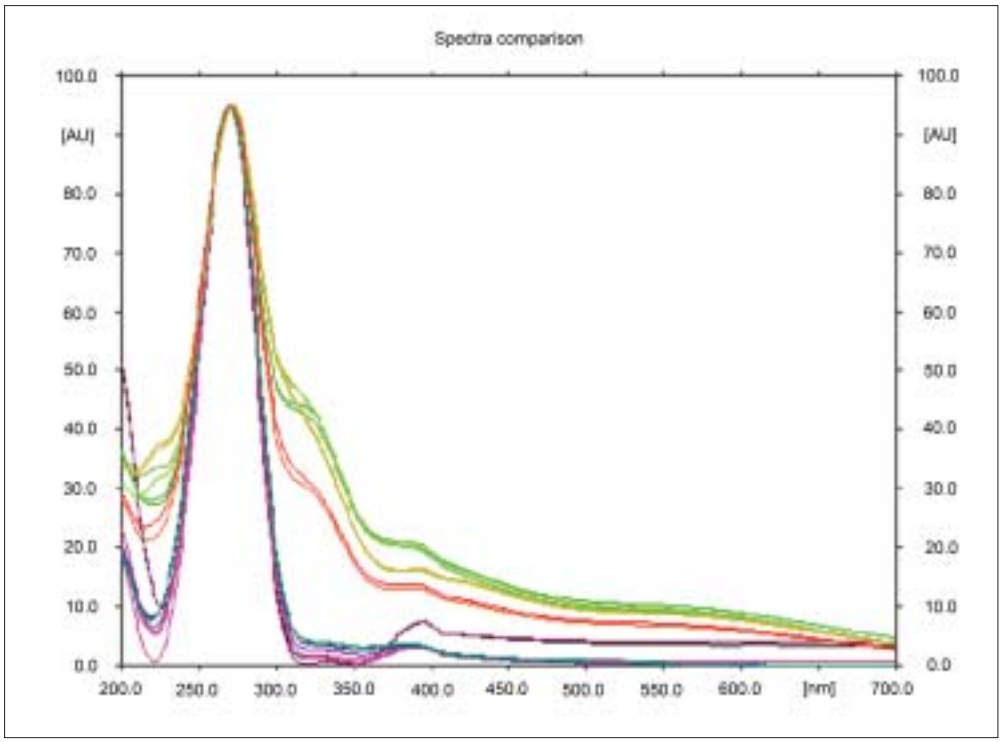

Figure 4: Superimposed UV spectra of ascorbic acid in standard and sample

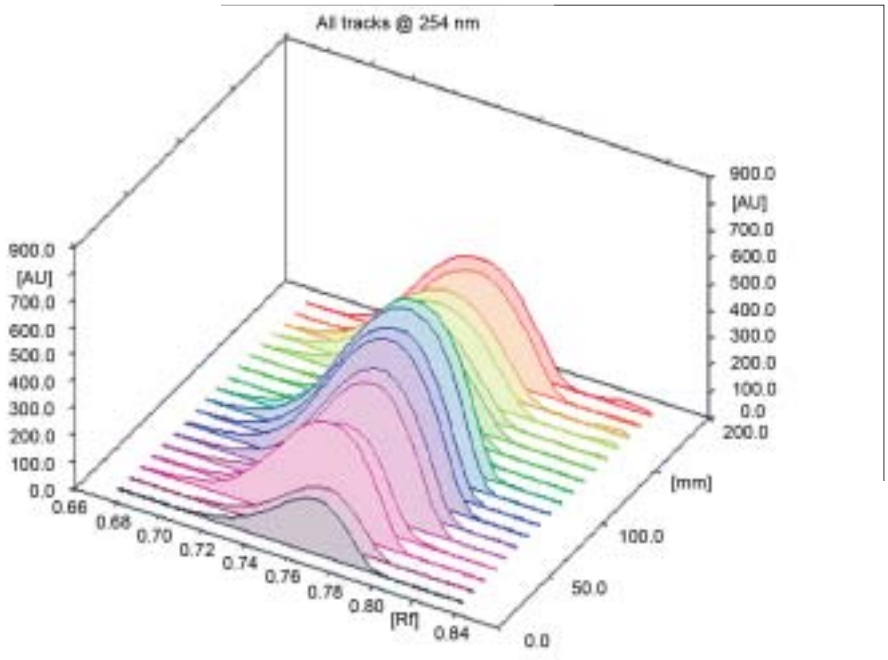

Figure 5: 3D view of all tracks

Slit dimension: 6.00 X 0.45 mm, Micro
Measurement mode - absorbance
Estimation of ascorbic acid in test sample

The mean peak height/area of duplicate samples was calculated and the content of ascorbic acid was quantified using the regression equation obtained from the standard curve.

RESULTS AND DISCUSSION

The standard ascorbic acid has an Rf value of 0.76 [Figure 1]. A good linear relationship (r^2 = 0.9925 and 0.9935 with respect to peak area and height, respectively) was observed between the concentration ranges of 1.0-5.0 μg [Figures 2 and 3]. The regression equation was found to be Y = 107.92x + 132.76 with respect to height and Y = 4955.6x

+ 1511.9 with respect to area, where Y is the peak height/area and X is the concentration of standard ascorbic acid. The highest content of ascorbic acid was found in the large variety of amla collected from Shirpur (607.5 mg/100 gm of fresh amla) using the present HPTLC method.

The UV spectrum of test samples is super imposable with that of standard ascorbic acid [Figure 4] indicating purity of peak. Simplicity, specificity, and sensitivity of the newly developed method makes it the apt choice for monitoring ascorbic acid content for standardization of raw materials at the time of formulating a preparation as well as for the quality control of the finished product.

CONCLUSION

It may be stated that the approach given for the standardization of amla using the HPTLC fingerprint method should be followed for standardization of all Unani and Ayurvedic compound- and single-drug formulations in which amla is an ingredient. The scientific, quality assessment parameters accepted by the World Health Organization (WHO) evolved in the present investigation will be helpful in checking the identity and quality and to detect adulterants/substitutes.

REFERENCES

1. Vaidyaratnam PS. Indian Medicinal Plants-A Compendium of 500 Species. Vol. 4. Orient Longman; 2006. p. 256.
2. Ben-Erik Van Wyk, Michael W. Medicinal plants of the world. Briza publications; 2004 . p. 320.
3. Burkill IH. A Dictionary of the Economic Products of the Malay Peninsula. Vol. 1. Kuala Lumpur: Ministry of Agriculture and Co-operatives; 1996.
4. Perry LM. Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. Cambridge: MIT Press; 1980. p. 149-50.
5. Sethi PD. Quantitative analysis of drugs in pharmaceutical formulations. New Delhi: CBS Publisher and distributors; 1997. p. 589.
6. Weins C, Hauck HH. Advances and developments in thin layer chromatography. LC-GC Int; 9, 1996. p. 710-7.
7. Kalász H, Bathori M. Present status and future perspectives of thin-layer chromatography. LC-GC Int; 10, 1997. p. 440-5.
8. Bruneton J. Pharmacognosy Phytochemistry Medicinal Plants. 2nd ed. France: Lavoisier Publishing Paris; 1999. p. 21.

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