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PARALLEL QUANTITATIVE ESTIMATION OF GALLIC ACID IN AQUEOUS EXTRACT OF *EMBLICA OFFICINALIS* AND POLY-HERBAL DOSAGE FORM (CAPSULE) BY HPTLC TO AUTHENTICATE THE RATIO OF THIS INGREDIENT DELIVERED TO THE FINAL FORMULATION

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Abstract:

The present study is carried out to examine and optimize the method to quantify the presence of Gallic acid, using HPTLC, in ingredient (aqueous extract of *Embllica officinalis*) as well as in a coded formulation containing extracts of four different herbs in equal quantity developed pharmaceutically in dosage form of capsules. In herbal industry HPTLC has made deeper inroads as method of choice for analysis and documentation of herbal drugs. In the present study the authors have taken utmost care to detect the ratio of marker (Gallic acid) in the aqueous extract of *Embllica officinalis* as well as in final compound formulation to correspond this ingredient's ratio in the final dosage form. The Chromatographic analysis was performed on CAMAG HPTLC System using Linomat IV applicator. Pre-coated TLC plates (Merck-code 1.05554.0007) Silica gel 60 F₂₅₄ were used for analysis. The mobile phase comprised of Toluene : Ethyl acetate: Formic acid::2:5:1.5 v/v and 254nm was detection wavelength. The regression equation showed good linearity in the range of 100-500µg/mL for Gallic acid ($R^2 > 0.998$) between the peak areas of each marker and concentration. The solvent front was run upto 9 cms. The gallic acid concentration in aqueous extract as well as in a coded formulation was found to be 7.83% and 1.59% respectively.

Keywords: *Embllica officinalis*, Gallic acid, HPTLC.

Introduction:

Embllica officinalis is effective in the treatment of amlapitta viz., peptic ulcer¹⁻². The fruits exhibit hypolipidaemic and antiatherosclerotic effects in the rabbits and rats³⁻⁴. The extract of amla also has antimicrobial properties⁵⁻⁶. Amla is an

antioxidant with free radical scavenging properties ⁷. Hepatoprotective ⁸, adaptogenic ⁹, antimutagenic ¹⁰, cytoprotective and antitumor ¹¹ antifungal¹², were also exhibited by Amla. The Gallic acid is the basis for the quality control of *Amla* and other plant-derived drugs from the herb. The main aim of the present study is to detect the ratio of marker Gallic acid in the aqueous extract of *Embllica officinalis* as well as in final compound formulation to correspond this ingredient's ratio in the final dosage form.

Materials and Methods:

Plant material and preparation of extract: The aqueous extract of dry fruit of *Embllica officinalis* (authenticated by voucher specimen (LIH No. 6934) was procured from an authentic supplier and the standard operating procedure to process it is as follows: The dry raw material was ground into coarse powder using a high-speed blender. This coarse powder was extracted with 60 liters of de-mineralized water by heating for 2 hours at 80 °C. Aqueous layer was decanted after cooling and the residual marc was extracted three times more using 45 liters of water every time. All the extracts were combined and filtered. Filtrate was concentrated under vacuum at 70-80 °C for 3 to 4 hours. Finally this extract was dried in vacuum tray drier at 70-80 °C for 14 to 16 hours. The dried extract was milled, sieved and packed in polythene bags for further use. The herb: extract ratio was found to be 25:10 and the yield was 40% on dried basis.

Chemicals and reagents

Gallic acid was procured from Natural Remedies Pvt. Ltd., Bangalore. acetonitrile, methanol, toluene, ethyl acetate, formic acid and double-distilled water was used in all experiments.

Sample preparation for HPTLC

Mobile phase: Toluene: Ethyl acetate: Formic acid: 2:5:1.5 v/v

Amla extract

150 mg of plant extract was taken and crushed in mortar pastel. From that, accurately weighed 100mg powder transferred to 25mL standard flask. Volume is made up to the mark with water: Acetonitrile : Acetic acid(90 : 10 : 0.2 v/v), sonicated for 10 min. It was filtered with 0.22μ filter to obtain sample stock solution.

Coded formulation: 20 capsules were taken, opened and crushed in mortar pastel. From that, accurately weighed 584mg powder transferred to 25mL standard flask. Volume is made up to the mark with water: Acetonitrile: Acetic acid (90: 10: 0.2 v/v), sonicated for 10 min. It was filtered with 0.22μ filter to obtain sample stock solution.

Experimental section for HPTLC:**Developing solvent system**

A number of solvent systems were tried, for extract, but the satisfactory resolution was obtained in the solvent Toluene :

Ethyl acetate: Formic acid(2:5:1.5 v/v)

Sample application

Application of bands of each extract was carried out (10mm in length and 10 μ l in quantity) using spray technique. Sample were applied on pre-coated silica gel 60 F₂₅₄ aluminium sheets (10 x 20 cm) with the help of Linomat IV applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 20 cm saturated with solvent Toluene: Ethyl acetate: Formic acid (2:5:1.5 v/v) for 15 min.

Detection of spots

The air-dried plates were viewed in ultraviolet radiation to mid day light. (Figure 1)The chromatograms were scanned by densitometer at 420 nm after spraying with anisaldehyde sulphuric acid The R_f values and finger print data were recorded by WIN CATS software.

Preparation of Calibration Curve for HPTLC: To prepare the calibration curve for HPTLC, standard stock solution (1000 μ g/mL) was prepared in methanol. Standard solutions of 100 μ g/ml 200 μ g/ml 300 μ g/ml 400 μ g/ml 500 μ g/ml 500 μ g/ml were prepared and 10 μ l each of these applied in 10mm band length on pre-coated silica gel 60 F₂₅₄ aluminium sheets (10 x 20 cm) with the help of Linomat IV applicator. The calibration curve was plotted. The details of R_f values and other HPTLC data is given the table 1, 2 and figures 1-9.

Table-1: Chromatographic Condition for HPTLC of Gallic acid.

Mobile Phase	Toluene: Ethyl acetate: Formic acid: 2:5:1.5 v/v
Stationary Phase	Pre-coated TLC plates (Merck-code 1.05554.0007) Silica gel 60 F ₂₅₄
Wavelength	254 nm
Solvent front run upto	9 cms
Application	CAMAG Linomat IV

Table 2: R_f values and other HPTLC data.

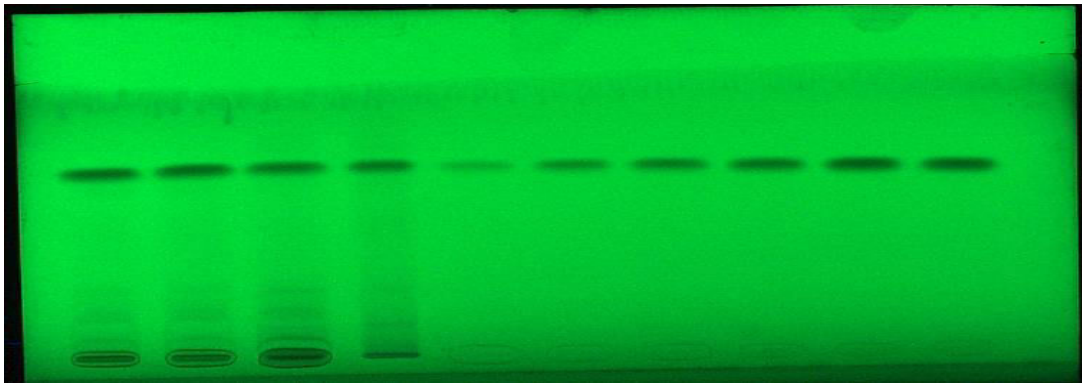
Track	Track id	Start R _f	Start height	Max R _f	Max height	Max %	End R _f	End height	Area	Area %	Assigned Substance
1	Amla	1.10	0.6	1.20	492.3	100.00	1.28	0.7	1508 3.0	100.00	Gallic acid
2	Amla	1.11	0.5	1.22	500.4	100.00	1.29	0.3	1557 1.3	100.00	Gallic acid
3	Coded(Mobile phase)	1.10	1.2	1.23	429.4	100.00	1.30	0.0	1287 6.7	100.00	Gallic acid
4	Coded(Mobile phase)	1.13	9.4	1.24	406.1	100.00	1.31	0.2	1182 8.6	100.00	Gallic acid
5	100µg	1.14	2.4	1.23	169.5	100.00	1.29	2.0	4441. 8	100.00	Gallic acid
6	200 µg	1.12	2.0	1.23	311.7	100.00	1.31	0.2	8695. 0	100.00	Gallic acid
7	300 µg	1.11	0.8	1.23	384.5	100.00	1.30	0.1	1140 1.6	100.00	Gallic acid
8	400 µg	1.12	3.2	1.22	445.1	100.00	1.30	0.1	1358 7.9	100.00	Gallic acid
9	500 µg	1.11	3.8	1.22	525.4	100.00	1.29	0.4	1717 8.7	100.00	Gallic acid
10	500 µg	1.10	1.2	1.21	512.9	100.00	1.29	0.6	1673 9.2	100.00	Gallic acid

Statistical analysis

Statistical calculations were carried out with the Microsoft Excel 2007 for Windows software package. Average, Sum, Standard Deviation (STDEV), Regression (RSQ) for Statistical Calculation, and Scattered Chart were used for Linearity; P values > 0.05 were considered to be significant.

Results

The amount of gallic acids in *Embllica officinalis* and in formulated capsules were analyzed using optimized chromatographic method. The standards of different strengths, samples & compound formulation were spotted on the HPTLC plate and peak areas were used for analysis of content by the regression equation. The developed mobile phase gave optimal separation, with well-defined and well-resolved sharp bands in standard, sample and formulation (Figure 1-9) at R_f 1.22-1.23 for gallic acid. The gallic acid in ingredient i.e. aqueous extract of *Embllica officinalis* was 7.83% w/w. The gallic acid in coded formulation of *Embllica officinalis* was 1.59% w/w.



Amla Amla Formu Formu 100µg/ml 200 µg/ml 300 µg/ml 400 µg/ml 500 µg/ml 500 µg/ml
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Fig. 1: HPTLC Chromatogram at 254nm.

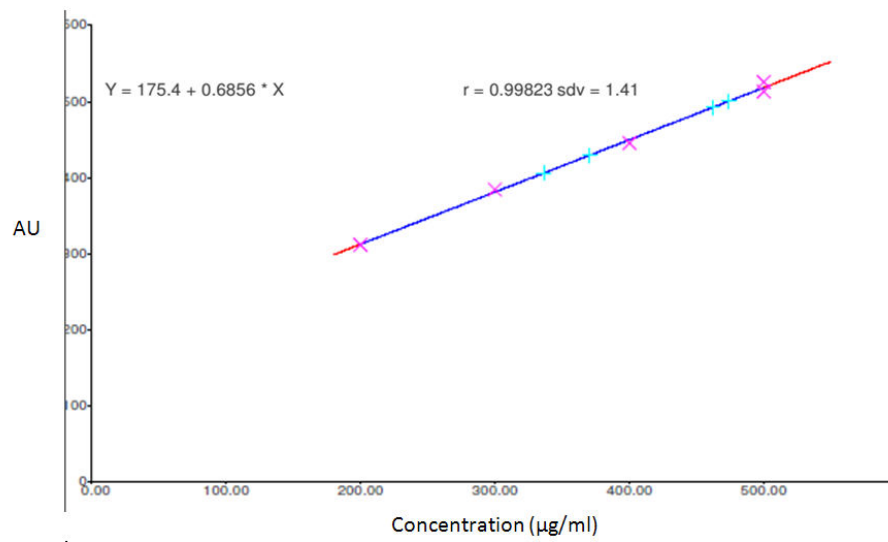


Fig. 2: HPTLC Calibration curve for Gallic acid.

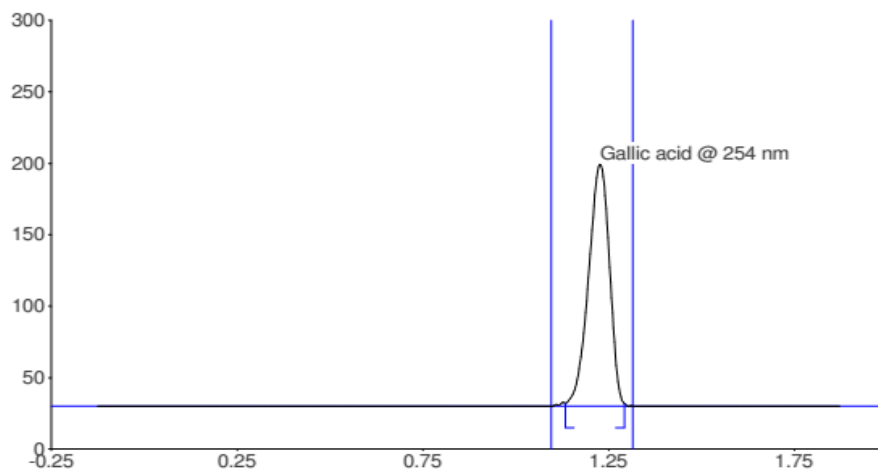


Fig. 3: HPTLC Chromatogram of Gallic acid 100µg/ml

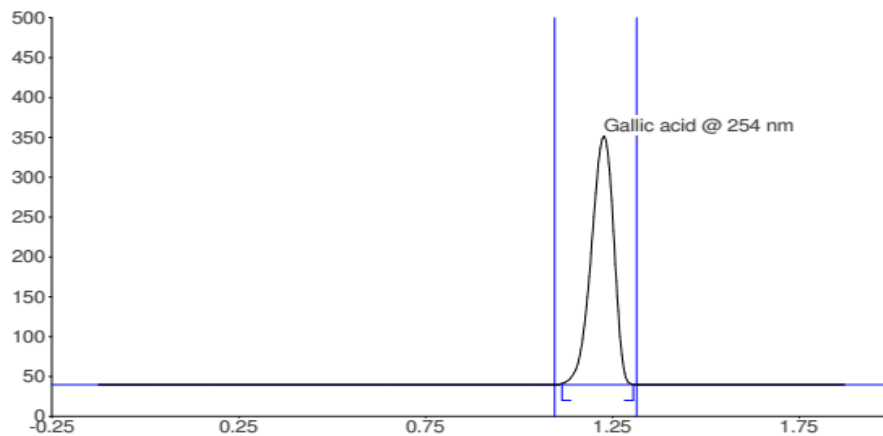


Fig. 4: HPTLC Chromatogram of Gallic acid 200µg/ ml

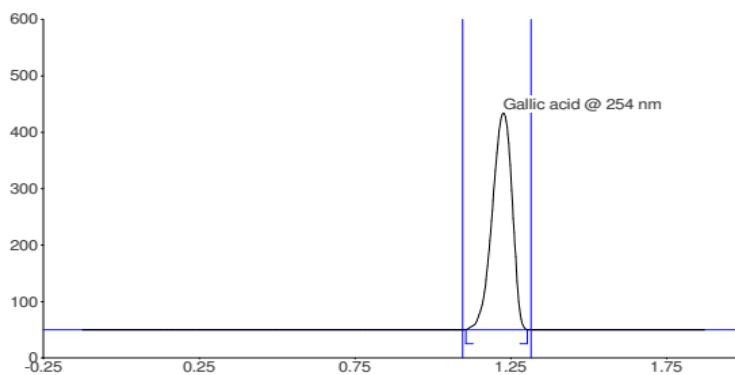


Fig. 5: HPTLC Chromatogram of Gallic acid 300µg/ ml.

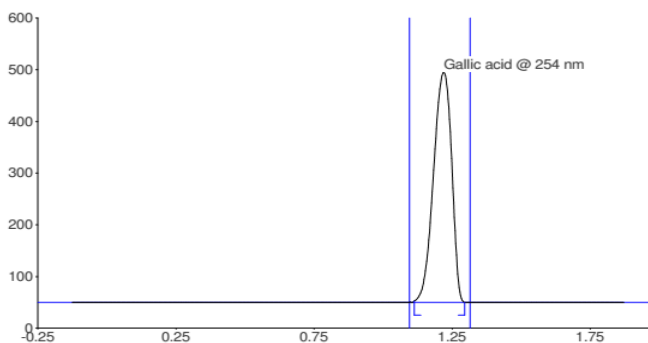


Fig. 6: HPTLC Chromatogram of Gallic acid 400µg/ ml

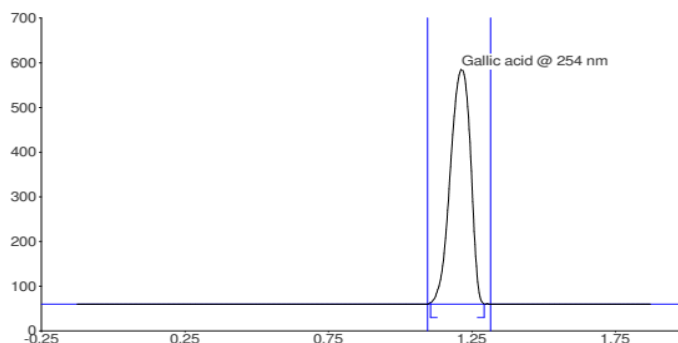
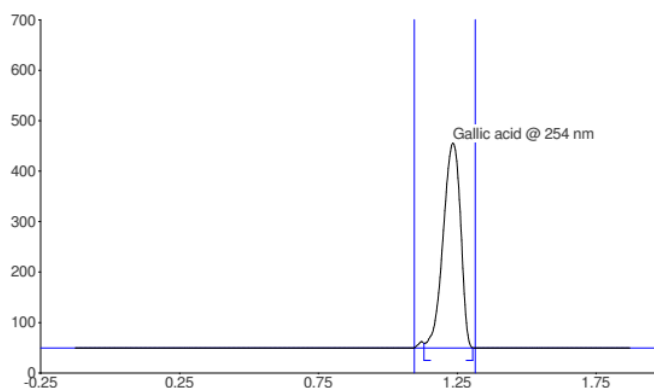
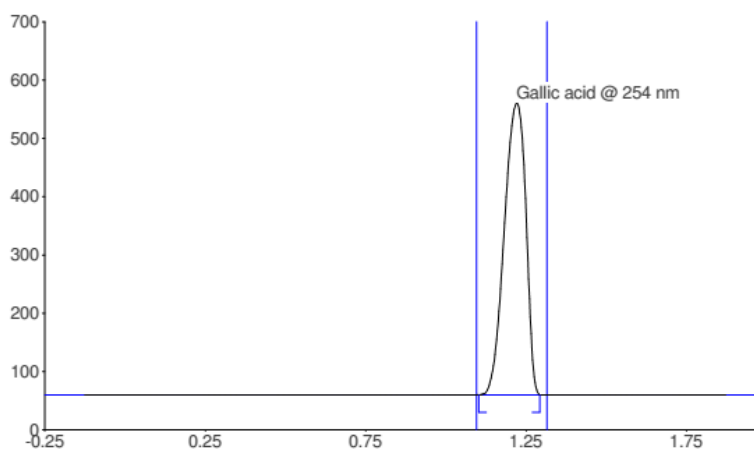


Fig. 7: HPTLC Chromatogram of Gallic acid 500µg/ ml**Fig. 8: HPTLC Chromatogram of Gallic acid in developed formulation.****Fig. 9: HPTLC Chromatogram of Gallic acid in Amla ext.****Discussion:**

The amount of Gallic acid present in *Emblica officinalis* raw drug and extract reported in several official pharmacopoeias viz.

Pharmacopoeias	Gallic acid in <i>Emblica officinalis</i> fruit
Ayurvedic Pharmacopoeia of India(API)[13]	NLT 0.8% raw drug & NLT 7.5 (Water extract)
Quality Standards of Indian Medicinal Plants(QSIMP) [14]	0.015-.022%(in fresh fruit)
Indian pharmacopoeia(IP)[15]	NLT 1.0 per cent w/w (dried fruit pericarp)

The gallic acid in ingredient i.e, aqueous extract of *Emblica officinalis* is found to be 7.83% w/w which complies with the API limit of NLT 5.5%[13]. As only this ingredient predominately contains Gallic acid in the poly-herbal formulation, the presence of Gallic acid band in HPTLC of finally coded formulation can surely be attributed to *Emblica officinalis*.

Conclusions

The gallic acid in aqueous extract of *Emblica officinalis* and coded formulation was found to be 7.83% w/w and 1.59% w/w respectively which reassures the fact that almost one fourth of the formulation consists of *Emblica officinalis*. This study also authenticates the presence of *Emblica officinalis* ingredient on the basis of gallic acid in the the pharmaceutically developed poly-herbal dosage form (capsule).

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