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NOTE

HPTLC Determination of Gallic Acid in Crude Drugs and Herbal Formulations

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A simple, selective, precise, accurate and cost effective high performance thin layer chromatographic (HPTLC) method for the analysis of gallic acid in some herbal formulations have been developed and validated. Aluminium TLC plate precoated with silica gel $60F_{254}$ was used as the stationary phase whereas ethyl acetate:formic acid (8.5:1.1, v/v) was used as the mobile phase. A compact and well resolved peak of gallic acid was observed by densitometric analysis in the absorbance mode at 272 nm. Calibration curve revealed a good linear relationship between the peak area and concentration. Validation of the developed method also carried out and found to be accurate, precise, specific and reproducible. The method proposed was further applied for estimation of gallic acid in the hydrolyzed samples of three Ayurvedic tablet formulations *viz.*, amla, haritaki and triphala. Hence, newly developed and validated HPTLC method for quantification of gallic acid may be useful in quality control and standardization of several herbal formulations and crude drugs containing phenolics and tannins.

Key Words: Gallic acid, HPTLC, Herbal formulation.

Gallic acid (3,4,5-trihydroxy benzoic acid) (Fig. 1) is an endogenous phenolic compound isolated from some fruits, berries, grapes, wine[1,2] tea leaves[3] and also in some hard wood species like oak trees, chestnut *etc.*[4,5]. It is a metabolite of propyl gallate and known to potentiate several pharmacological and biochemical pathways having strong antioxidant[6], anti-inflammatory[7], antimutagenic[8], anticancer activity[9] and cardio-protective[10] activity.

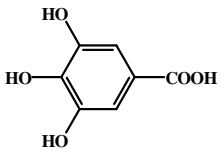

Fig. 1. Structure of gallic acid

Literature survey reveals that only few HPTLC methods using silica has been reported for determination of gallic acid in crude drugs[11-13] with poor range of linearity and validation parameters. Hence, the aim of present investigation is to develop a simple, economic, accurate, specific and reproducible HPTLC method for the determination of gallic acid in crude drugs and in herbal formulations.

HPTLC Instrumentation: The sample was spotted in the form of bands of width 4 mm using Camag 100 µL sample (Hamilton, Switzerland) syringe on pre-coated silica gel $60F_{254}$ aluminum plate (20 cm × 10 cm) using a Camag Linomat-V sample applicator (Switzerland). The plates were pre-washed by methanol and activated at 60 °C for 0.5 h prior to chromatography. The mobile phase consisted of ethyl acetate:formic acid (8.5:1.1, v/v) and 15 mL of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin through glass chamber, previously saturated with mobile phase for 15 min. The length of the chromatogram run was 80 mm. After the development, the plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III using deuterium lamp in absorbance mode at wavelength of 272 nm. The slit dimension was kept at 4.00 × 0.45 mm and 10 mm/s scanning speed was employed.

Calibration graph of gallic acid: A stock solution of standard gallic acid having a known concentration 1000 µg/mL was prepared in methanol. Different volumes of stock solution *i.e.* 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2 and 3 µL were applied in triplicate to get 100 ng to 3000 ng/spot on the HPTLC plate.

Analysis of the formulations: Ten tablets each were powdered and hydrolyzed in dilute HCl (10 %) in reflux condenser for 1 h, filtered and the filtrate was extracted with chloroform (thrice). The remaining acidic aqueous extract was taken and

evaporated to dryness in rotavapor. The residue thus obtained was dissolved in 10 mL of methanol and 2 µL of the filtered solution was applied on the TLC plate followed by development and scanning at 272 nm. The possibility of excipients interference in the analysis was studied.

Development and optimization of the mobile phase: TLC procedure was optimized for determining gallic acid in crude and herbal formulations. Initially, ethyl acetate:acetic acid in various ratios was tried. When ethyl acetate:formic acid (8.5:1.1, v/v) was used as mobile phase good resolution and well defined peak was observed at R_f value 0.89 ± 0.01, after chamber saturation for 15 min. prior to the chromatogram development at room temperature.

Calibration curve: Table-1 showed a good linear relationship over the concentration range 100-3000 ng per spot with respect to peak area.

TABLE-1
LINEAR REGRESSION DATA FOR THE CALIBRATION PLOT (n = 3)

Linearity range (ng/mL)	100-3000
Regression equation	442.573 + 6.426x
Correlation coefficient	0.9950
Slope ± SD	442.573 ± 0.57
Intercept ± SD	6.426 ± 0.46

Validation of the method: The proposed method was validated for accuracy (Table-2), repeatability (Table-3), precision (Table-4), roubtness (Table-5) and for LOD and LOQ using previously reported methods by laboratory[14-16].

TABLE-2
ACCURACY OF THE METHOD (n = 6)

Excess drug added to analyte (%)	Theoretical content (ng)	Amount of drug recovered (ng) ± SD	Recovery (%)
0	500	497.63 ± 3.48	99.52
50	750	748.59 ± 4.15	99.81
100	1000	1006.45 ± 5.95	100.64
150	1500	1493.55 ± 5.32	99.57

TABLE-3
REPEATABILITY OF THE METHOD (n = 6)

Concentration (ng/mL)	% RSD of area	% RSD of height	% RSD of R_f
100	1.4	1.07	1.13
400	0.23	1.16	1.3
500	0.44	0.93	0.65

TABLE-4
PRECISION OF THE METHOD (n = 6)

Concentration (ng/mL)	Inter-day precision (area) % RSD	Intra-day precision (area) % RSD	Inter-analyst precision (area) % RSD
100	1.68	1.38	1.43
400	0.60	0.36	0.72
500	0.69	0.52	0.75

TABLE-5
ROBUSTNESS OF THE METHOD

Mobile phase composition (ethyl acetate:formic acid)		% RSD of area	% RSD of R_f
Original (v/v)	Used (v/v)		
8.5: 1.1	8.7: 1.1	0.46	0.70
	9: 0.9	0.51	1.3
	8.5: 1.2	0.52	1.13

Limit of detection and limit of quantification of the method: The limit of detection (LOD) and limit of quantification (LOQ) were determined by signal to ratio method. For the proposed method, LOD and LOQ determined as 9.2 ng/spot and 30.5 ng/spot.

Analysis of the formulations: Three different tablets *i.e.* amla, haritaki and triphala were analyzed by the proposed method for the content of gallic acid. The sample chromatogram gave a single spot at R_f 0.85 ± 0.02. The peak obtained was pure and there was no interference from the excipients used in tablet formulation. The gallic acid contents in the tablets were range from 0.06-0.15 % w/w.

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