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Development of HPTLC fingerprinting method for estimation of gallic acid in polyherbal capsule

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Abstract

The aim of present study is to develop a simple, specific, accurate and reproducible HPTLC method for the quantitative estimation of gallic acid in raw and poly-herbal capsule dosage form The fingerprinting method was developed for raw materials *Eugenia jambolana* (bark), *Phyllanthus niruri* (whole plant) and laboratory batch polyherbal capsule via estimation of gallic acid by using HPTLC methods. The results indicate the presence of optimum level of gallic acid in raw as well as laboratory batch of prepared polyherbal capule.

Key-words: HPTLC, Gallic acid, Polyherbal capsule

Introduction

Traditional systems of medicine like Ayurveda, Unani and Siddha mainly use herbal formulations for the treatment of various ailments. These formulations require quantification of its constituents to ensure the quality of herbal drugs which should be maintained in each preparation batch to batch. The finger printing technique may be useful to the formulations for routine quality control test. In this study, highly sophisticated HPTLC.[1-3] Nowadays, HPTLC is applied to obtain fingerprint of herbal formulations. This technique involves accurate and precise instrumental application of the sample on adsorbent layer. It is designed to achieve much faster and better separation. Development of the chromatogram in equipments ensures distortion free and direct quantitative evaluation by means of densitometer. The sample fractions are quantified by scanning the chromatogram with a light beam in the visible or ultraviolet range of the spectrum and measuring the absorbance or fluorescence by diffuse reflectance.[4]

The present work was undertaken to evaluate the polyherbal capsule along with their two individual constituents.

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Material and Methods

Collection of herbs and their authentication

The plant parts viz., EJB: *Eugenia jambolana* (Bark), PNWP: *Phyllanthus niruri* (Whole plant) were collected in the months of July-September 2016 from the various local sites of Malwa region of Madhya Pradesh and identified & authenticated by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory

Preparation of formulation (Capsule) by wet granulation method

The formulation preparation began with trials by adding a different ratio of binders and selecting the quantity of lubricants and preservatives, and finally the procedure was optimized. Extracts were finely powdered (sieve 40), and mixed in the ratio as mentioned in Table and taken for the preparation of capsules by wet granulation technique using lactose solution as a binder. The wet mass was passed through sieve number 22 to obtain granules. The granules were dried at 45°C in a tray dryer. The granules were lubricated with magnesium stearate. Diluents and preservatives were added. After this, the granules from the optimized batch were filled in capsules colored yellow-red of size "0" in a capsule filling machine. The capsules were then deducted and transferred into poly bags, labeled, and the samples were evaluated as per the testing requirements. Each 500 mg of herbal capsule contained the extracts and lactose and excipients– quantity sufficient (q.s.)[5]

Development of HPTLC fingerprinting method for gallic acid[6-7]

The HPTLC fingerprinting method was developed for raw materials *Eugenia jambolana* (bark), *Phyllanthus niruri* (whole plant), polyherbal capsule via estimation of gallic acid.

Materials

All chemicals and reagents used were of analytical grade and were purchased from Hi-Media, India.

Instrumentation and chromatographic conditions

Spotting device: Linomat V Automatic Sample Spotter; CAMAG (Muttenz, Switzerland)
Syringe: 100 µL Hamilton (Bonaduz, Switzerland)
TLC chamber: Glass twin trough chamber (20 x 10 x 4 cm); CAMAG
Densitometer: TLC Scanner 3 linked to Win Cats software V.4.06; CAMAG
HPTLC plates: 10 x 10 cm, 0.2 mm thickness precoated with silica gel 60 F_{254}; E. Merck KgaA, Cat. no. 1.05548; (Darmstadt, Germany)
Experimental conditions: Temperature 25 ± 2 °C, relative humidity 40 %
Solvent system: Toluene: acetone: glacial acetic acid (6:2:2)
Detection wavelength: 270 nm
Slit dimension: 6.00 x 0.20 mm
Scanning speed: 20 mm/s & source of radiation deuterium lamp.

Calibration curve of gallic acid

A stock solution of gallic acid (100 µg/ml) was prepared in methanol. Different volumes of stock solution were spotted on the TLC plate to obtain concentrations of 300 - 900 ng spot^{-1} of gallic acid, respectively. The data of peak areas plotted against the corresponding concentrations were treated by least-square regression analysis method validation.

Method Validation

The method was validated for precision, accuracy, limit of detection, limit of quantification, robustness, ruggedness & specificity of sample application..

Precision

Repeatability of the sample application and measurement of peak area were carried out using six replicates of the same spot (600 ng spot^{-1} for gallic acid) and was expressed in terms of percent relative standard deviation (% R.S.D.). The intra- and inter-day variation for the determination of gallic acid was carried at three different concentration levels of 300, 600, 900 ng spot^{-1}.

Robustness of the method

By introducing small changes in the mobile phase composition, mobile phase volume, duration of mobile phase saturation and activation of pre washed TLC plates with methanol; the effects on the results were examined. Robustness of the method was done in triplicate at a concentration level of 600 ng spot^{-1} for gallic acid.

Limit of detection and limit of quantification

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times following the same method as explained in *Section* and the signal-to-noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting the known concentrations of gallic acid until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Recovery

The pre-analyzed samples were spiked with extra 50, 100 and 150 % of the standard gallic acid and the mixtures were reanalyzed by the proposed method. The experiment was conducted six times. This was done to check for the recovery of the gallic acid at different levels in the formulations.

Ruggedness

A solution of concentration 300 ng spot^{-1} was prepared and analyzed on day 0 and after 6, 12, 24, 48 and 72 h. Data were treated for % R.S.D. to assess ruggedness of the method.

Specificity

The specificity of the method was confirmed by analyzing the standard drugs and extract. The spot for gallic acid in the sample was confirmed by comparing the Rf values and spectra of the spot with that of the standard. The peak purity of the gallic acid was assessed by comparing the spectra at three different levels, viz. peak start (S), peak apex (M) and peak end (E) positions of the spot.

Estimation of gallic acid in raw materials and polyherbal capsule

The polyherbal capsule and raw materials *Eugenia jambolana* (bark), *Phyllanthus niruri* (whole plant), were extracted with 10 ml methanol : water (4:1 v/v) for 2 hr. The mixture was filtered through buchner funnel and the methanol was evaporated in a rotatory evaporator. The residual aqueous phase was acidified to pH 2 by addition of some drops of HCl (3N), and the volume was adjusted to 10 ml with distilled water. In order to isolate the gallic acid, another extraction by ethanol using a decanting bulb was carried out. The ethanol phase was dried by using anhydrous sodium sulphate then evaporated and residue was dissolved in 5 ml methanol.

Results and Discussion

The HPTLC fingerprinting method was developed for raw materials *Eugenia jambolana* (bark), *Phyllanthus niruri* (whole plant) and polyherbal capsule via estimation of gallic acid.
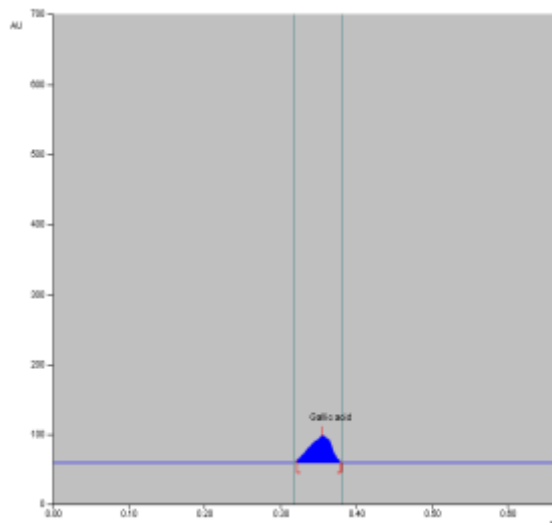

Fig. 1: HPTLC chromatogram of gallic acid

Table 1: Intra- and inter-day precision of HPTLC method (n=6)

Principle constituent	Amount (ng/spot)	Intra-day precision		Inter-day precision	
		S.D. of area	R.S.D. %	S.D. of area	R.S.D. %
Gallic acid	300	0.0404144	0.011196	0.0950437	0.02632
	600	0.0450924	0.006244	0.1662327	0.02301
	900	0.0450924	0.003922	0.2233830	0.01942

Table 2: % Recovery for gallic acid in tablet (n=6)

Excess drug added to the analyte (%)	Conc. found	SD	Recovery (%)	R.S.D. (%)
50	449.90	0.0321434	99.91	0.0071442
100	598.92	0.0264568	99.80	0.0044171
150	749.91	0.0251661	99.96	0.0033559

Table 3: Summary of validation parameters of HPTLC (Gallic Acid)

Parameters	Data of gallic acid
Linearity range	300-900 ng/spot
Correlation coefficient	0.998
Limit of detection	1.37291
Limit of quantitation	4.20234
Recovery (n=6)	99.88 %
Precision (R.S.D. %) Repeatability of application (n=6)	0.00638

Intra-day (n=6)	0.00711
Inter-day (n=6)	0.02292
Robustness	Robust
Specificity	Specific

Estimation of gallic acid in raw materials and polyherbal capsule

The polyherbal capsule and raw materials *Eugenia jambolana* (bark), *Phyllanthus niruri* (whole plant) were estimated for gallic acid content. The results are summarized in table 4.

Table 4: Content of gallic acid in raw materials and tablet

S. No.	Name	Gallic acid Content % (w/w)	Confidence level (95 %)
1.	*Eugenia jambolana*	3.18±0.291	±0.218
2.	*Phyllanthus niruri*	4.18±0.120	±0.516
3.	PHC	0.841±0.170	±0.387

References

1. Touchstone J.C., Practice of Thin Layer Chromatography, Published by John Wiley and Sons, Inc., USA, edn. 3 (1992).
2. ICH Q2A Text on Validation of Analytical Procedures, International Conference on Harmonization Tripartite Guidelines (1994).
3. ICH Q2B Text on validation of Analytical Procedures, Methodology International Conference on Harmonization, (1996)
4. Sethi PD, HPTLC – Quantitative analysis of pharmaceutical formulations, CBS Publishers and Distributors, New Delhi, 1996; pp. 1-30.
5. Divya Jyothi, Marina Koland, Sneh Priya, Jainey Puthenveetil James. Formulation of Herbal Capsule Containing *Trigonella Foenum-Graecum* Seed Extract for the Treatment of Diabete, J Young Pharm, 2017; 9(3): 352-356s .
6. Nagarsenkar Mangal S and Tayade Nitin G, Validated HPTLC method of analysis for artemether and its formulation, Journal of Pharmaceutical and Biomedical Analysis, 2007; 43; 839-844.
7. S. Mohamed Musthaba, M.T. Athar, Sanjula Baboota and Sayeed Ahmad. HPTLC Analysis of Neem Oil Extract in Herbal Dosage Form, Asian Journal of Chemistry; 2011, 23(1): 385-387.