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Research Article

QUANTIFICATION OF ELLAGIC ACID IN DADIMASHTAKA CHURNA BY HPTLC METHOD

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Abstract:

Herbal drugs, singularly and in combinations, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy. This creates a challenge in establishing quality control standards for raw materials and standardization of finished herbal drugs. Traditionally only a few markers of pharmacologically active constituents were employed to assess the quality and authenticity of complex herbal medicines. In the present study, an attempt has been made to develop a HPTLC (High Performance Thin Layer Chromatography) method for quantitative estimation of marker compound ellagic acid in laboratory prepared authentic formulation and in commercial formulation of Dadimashtaka Churna. As per Bhaisajaya Ratnavali it is used for the treatment of Diarrhoea. The key ingredient of Dadimashtaka churna is Pomegranate (Dadima). The two formulations were subjected to hydroalcoholic extractions using Soxhlet apparatus. Ellagic acid was quantified in the above two extracts by using HPTLC. It was observed that other constituent's presents in the formulation did not interfere with the peak of Ellagic acid. Thus, the solvent system of n-hexane and ethyl acetate is found to be an ideal mobile phase for separation of Ellagic acid. Standard ellagic showed a single peak in HPTLC chromatogram. The percentage recovery studies revealed a recovery of 99.4% w/w of ellagic acid, thus proving the accuracy and precision of the analysis. Since this method resolves and quantifies ellagic acid effectively, it can be used to quantify the concentration of ellagic acid in the herbal formulations.

Key Words: Dadimashtaka churna, Ellagic acid, Quantification, HPTLC, Validation.

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INTRODUCTION:

Herbal remedy has a long and an ultimate beneficial history and its helping numerous of the physical condition and the cure of the health disease of a huge population of the world. But, maintaining the quality is still getting suffered and remains challenging due to the changeability of chemical components which are involved in formulation of herbal drug. [1]. The herbal formulation or the conventional formulation of the established medicine is usually polyherbal. Each formulation contains 10 to 20 or even extra herbs: even a few have more than 50 herbs. For these formulations it's difficult to define the parameters for the quality control. Even the official standards are not defined for such medications [2]. Herbal drugs, singularly and in combinations, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy [3]. This creates a challenge in establishing quality control standards for raw materials and standardization of finished herbal drugs. Traditionally only а few markers of pharmacologically active constituents were employed to assess the quality and authenticity of complex herbal medicines [4]. Standardization is an important phase of creating and maintaining the quality and usefulness of Ayurvedic formulations or any multiple elements herbal formulations. One of the major barriers to the acceptance of the herbal product is the absence of standard quality profiles [5]. Chromatographic fingerprint examination of herbal drugs reports a comprehensive qualitative approach for the rationale of species authentication, evaluation of quality and ensuring the uniformity and constancy of herbal drugs and their associated products. The quantitative determination of constituents has been made easy by recent developments in analytical instrumentation [6] Dadimashtaka churna is a polyherbal traditional Ayurvedic formulation. The main ingredient of this polyherbal formulation is Punica granatum Linn. (Pomegranate). It is useful in treating Diarrhoea, amoebic dysentery, indigestion and loss of appetite. Prescribed dose is 4 to 6 grams along with warm water or rice soup twice daily after food [7]. In the following study a HPTLC method is developed for the quantification of Ellagic acid in the formulation.

MATERIALS AND METHODS:

Procurement of marketed formulations:

One Marketed formulation [MF] is procured from a registered Ayurvedic Pharmacy of Nashik.

In house preparation of antidiarrhoeal polyherbal formulation:

Following crude drugs were used in the preparation of in house formulation [IHF]. All ingredients were procured from local market of Jaipur, Rajasthan and were authenticated by department of Botany, University of Rajasthan, Jaipur, India.

Table 1 Composition of Dadimashataka churna

S.no	Ingredients	Amount in gram
1.	Punica grantum	160
2	Bambusa arundinacea	5
3	Cinnamomum zeylanicum	10
4	Cinnamomum tamala	10
5	Elettaria cardamomum	10
6	Mesua ferrea	10
7	Trachyspermum ammi	20
8	Coriandrum sativum	20
9	Cuminum cyminum	20
10	Piper longum	20
11	Piper longum root	20
12	Zingerabine officinale	20

PROCEDURE:

The ingredients were individually pulverized and sieved (80 mesh) to obtain respective fine powders. Powder of each ingredient was weighed separately and thoroughly mixed together as per the quantity mentioned in. The composite mixture was again sieved (80 mesh) to obtain a fine powder of the finished product. The finished product thus obtained in powder form was packed in sterilized polythene pouches, labelled and stored inside cool and dry place.[7].

EXTRACTION PROCESS:

MF and IHF were weighed about 100 g and transferred to round bottom flask of 500 ml flask. About 250 ml of hydroalcohol (70ml ethanol + 30ml distilled water) was added. The temperature of heating mental was maintained at 70 to 80° C. The cycles were repeated till the constituents are extracted completely, for colored components when solvent in siphon tube becomes colourless. The extract was filtered. The filtrate was distilled and the extract was vacuum dried at 40° C. [8]

High Performance Thin Layer Chromatography:

Equipments: A Camag HPTLC system comprising of Linomat V automatic sample applicator, Hamilton Syringe, Camag TLC Scanner-3, Camag Win CAT software, Camag Twin Troughplate

Chemicals: Standard Ellagic acid was purchased from Total herbs solutions Pvt. Ltd. (Mumbai, India). Analytical grade reagents; n-hexane, ethyl acetate and methanol (Merck Chemicals, India) were used. Stationary phase used was Silica gels $G_{60}F_{254}$,

20x10cm TLC plate were obtained from E. Merck Ltd (Mumbai, India).

Samples: MF-Marketed formulation and IHF- In-house formulation.

• **Preparation of standard Ellagic acid solution** A stock solution of Ellagic acid 1000ug/ml was prepared by dissolving 10mg of accurately weighed ellagic acid in methanol and making up the volume to 10 ml in volumetric flask covered with aluminum foil. The stock solution was further diluted with methanol to yield a concentration of 100µg/ml.

• Preparation of Sample solution

Sample solution of IHF and MF were prepared by dissolving 10 mg of the extract in methanol and making up the volume to 10 ml to get the concentration of 1000μ g/ml. The solution was filtered through whatman filter paper no. 41 and used for further chromatographic analysis.

• Preparation of Mobile Phase

Mobile Phase was prepared by mixing n- hexane and ethyl acetate in the proportion of 50:50 v/v.

• Chromatographic conditions

Stationary phase: HPTLC pre-coated silica gel G_{60} , F₂₅₄ (Merck) Thickness: 0.2 mm Mode of application: Band Band width: 6mm Separation technique: Ascending Temperature: $25 \pm 3^{\circ}$ Saturation time: 15 min with plate Migration distance: 80 mm Wavelength: 339 (CAMAG 3 Scanner)

• Procedure:

CAMAG-HPTLC system of Switzerland with a Linomat 5 sample applicator was used to obtain HPTLC fingerprinting. Different combinations of solvent systems were tried to obtain an excellent separation and sharp peaks for analysis. The investigations were performed in an air-conditioned room maintained at a temperature 22 °C and 55% humidity. Precoated silica gel HPTLC aluminum plates G₆₀F 254 plate (20 cm×20 cm, 0.2 mm thicknesses, 5-6 µm particle size, E-Merck, Germany) were used for chromatographic separation. The extract (2 µL) was spotted as bands of 6 mm width with the help of the auto sampler fitted with a 100 µL Hamilton syringe. The plates were prepared by using solvent system of n-hexane and ethyl acetate (50:50 v/v). The solvent system was transferred to CAMAG Twin Trough plate development chamber lined with filter paper and pre-saturated with mobile phase . The resulted plates were air dried and scanned. A spectro densitometer (Scanner 3. equipped with winCATS CAMAG) planar chromatography manager (version 1.3.0) software was employed for the densitometry measurements, spectra recording and data processing. The retention factor (Rf) value of each compound separated on plate and data of peak area of each band were recorded [9]. Chromatogram of Standard ellagic acid is given in Figure 2. Whearase chromatogram IHF and MF are shown in Figure 3 and 4 respectively.

• Calibration Curve for standard Ellagic acid

Standard solution of ellagic acid 100 µg mL⁻¹ is prepared and was applied duplicate in 4µL, 8µL, 12µL, 16µL, 20µL over the silica gel $G_{60}F_{254}$ plate [**Table 2**]. The plate was developed and analyzed. The plate was then developed using the optimized mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves [**Figure 1**]

• Validation of the proposed method:

The developed method was validated in terms of Linearity, Accuracy, and precision, Limit of detection, Limit of quantification, robustness and ruggedness. ICH guidelines were followed for the validation of the analytical methods developed. [10,11]

1. Linearity:

Standard stock of 100 μ g mL⁻¹ ellagic acid was prepared and diluted to appropriate concentrations for plotting the calibration curves. Five concentrations of the analyte solutions were analyzed in triplicate, and then the calibration curves were constructed by plotting the mean peak areas *versus* the concentration of each analyte. The data for linearity is given in **Table no.2**

2. Inter-day and intra-day precision:

The inter-day precision (RSD) was determined by analyzing standard solution of ellagic acid over the entire calibration range for three different days [**Table 3**]. The intra-day precision (RSD) was determined by analyzing standard solution of Ellagic acid over the entire calibration range for three times on the same day [**Table no.no. 4**].

3. Limit of detection:

The limit of detection (LOD) was determined using following formulae. LOD = 3.3(SD)/S Where, SD = Standard Deviation of response, S = avg. of the slope of the calibration curve. The minimum detectable limit was found to be in ng/spot for Ellagic acid.[Table no 5]

4. Limit of quantification:

Limit of quantification (LOQ) were determined using following formulae. LOQ = 10 (SD)/S, Where, SD = Standard Deviation of response, S = avg. of the slope of the calibration curve. The minimum quantified limit was found to be in ng/spot for Ellagic acid [Table no.5]

5. Specificity:

The peak purity of the ellagic acid was assessed by comparing the spectra at three different levels, viz. peak start, and peak apex and peak end positions of the spot.

6. Robustness of the method:

By introducing small changes in the mobile phase development distance, mobile phase volume and duration of mobile phase saturation, the effects on the results were examined. Robustness of the method was done three times at a concentration level of $5\mu g/spot$ and the % R.S.D. of peak area was calculated. Result is tabulated in **Table no. 6**.

7. Ruggedness of the method:

It expresses the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by spiking the standard three times with different analyst by using same equipment. Result is tabulated in **Table no.7**.

8. Accuracy:

The accuracy was determined by standard addition method. Accuracy of the method was tested by carrying out recovery studies at different spiked level by standard addition method. Standard ellagic solution was added at three different levels (80,100 and 120%). At each level three determinations were performed and results were calculated by the difference between the spiked and un-spiked sample analyzed under the same conditions. The Percentage recovery of ellagic acid were calculated at each level. Result is tabulated in **Table no.8 and 9**

The amount of ellagic acid in IHF and MF was tabulated in Table no.10

RESULT AND DISCUSSION:

Table 2 Linearity Data of Ellagic acid						
S.No	Parameters	Concentration (µg/spot)				
		4	8	12	16	20
1	Reading I	7121	14260	21105	28206	35008
2	Reading II	7260	14353	21209	28366	35250
3	Reading III	7306	14500	21601	28601	35300
4	Mean	7229	14371	21305	28391	35186
5	Standard Deviation	96.31	121.01	261.56	198.68	156.16
6	Relative standard deviation	1.33	0.84	1.23	0.70	0.44

Figure 1. Calibration curve of Ellagic acid



S.No	Compound	Marker Conc. (µg/Spot)	First day	Second day	Third day	Mean	Standard Deviation (SD)	Relative standard deviation (RSD)
1.	Ellagic acid	2	99.20	99.65	99.87	99.57	0.342	0.34
2.		7	98.65	97.62	98.35	98.197	0.526	0.54
3.		10	100.1	99.85	99.54	99.83	0.281	0.28

Table 3 Interday precision of Ellagic acid

Table 4 Intraday Precision of Ellagic acid

S.	Compound						Standard	
No.		Marker	First	Fourth	Eighth	Mean	Deviation	Relative
		Conc.	hour	hour	hour		(SD)	standard
		(µg/Spot)						deviation (RSD)
1.	Ellagic acid	2	99.89	98.65	99.31	99.283	0.620	0.62
2.		7	98.65	97.98	98.35	98.327	0.336	0.34
3.		10	100.10	100.26	99.98	100.113	0.140	0.14

Table 5 : Limit of detection and Quantification for Ellagic acid

S. No.	Concentration (ng/spot)	LOD	LOQ
1	2	0.00117	0.00355
2	7	0.00063	0.00192
3	10	0.00026	0.00080

Table 6 Robustness of the method for Ellagic acid

S No	Dovomotov	Peak area			Standard deviation	%
5. 1NO.	S.No. Parameter		II	III	of Peak Area	RSD
1.	Development distance of Ellagic acid (7.6, 8.0, 8.4 cm.)	3840.10	3897.21	3910.0	37.218	0.96
2.	Mobile phase volume of Ellagic acid (9.5, 10.0, 10.5 ml.)	3742.01	3758.0	3699.0	30.527	0.82
3.	Duration of mobile phase saturation of Ellagic acid. (19, 20, 21 min.	3897.10	3852.0	3877.0	22.594	0.58

Table 7 Ruggedness of the method for Ellagic acid

S.No.	Parameter	Peak Area		Standard	deviation	of	Peak	%	
		Ι	II	III	Area				RSD
1.	Analyst-I	3897.12	3985.20	3856.03	65.994				1.69
2.	Analyst-II	3862.20	3965.0	3898.56	52.128				1.33

S. No.	Level of Recovery	80%	100%	120%
		10	10	10
1.	Amount presents (µg.)	10	10	10
		10	10	10
	Amount of Std. added	8	10	12
2.	(ug.)	8	10	12
	(187	8	10	12
	Amount recovered	7.89	9.90	11.98
3.	(ug.)	8.10	9.82	11.90
		7.82	10.10	12.13
4.		98.625	99.0	99.83
	% Recovery	101.25	98.20	99.166
		97.75	101.0	101.083

Table 8 Recovery studies for Ellagic acid

Table 9. Statically validation of recovery for Ellagic acid

S.No.	Level of Recovery	Compound	% Recovery	Standard deviation	% RSD
1.	80	Ellagic acid	99.208	1.821	1.84
2.	100		99.4	1.442	1.45
3.	120		100.026	0.973	0.97







Figure 3 HPTLC Chromatogram of standard IHF



Figure 4 HPTLC Chromatogram of standard M	F
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Table 10 : Percentage of Ellagic acid in the formulations				
Sample No.	% Ellagic acid			
IHF	1.37 %			
MF	1.34%			

Calibration plot indicates that the response is linear function of concentration in the range 4-20 μ g for Ellagic acid. The correlation coefficient for Ellagic acid is 0.9999. Standard Ellagic acid shows a single peak in HPTLC chromatogram. The Rf value of the standard was 0.35. The measurement of peak area

three times inter-day and intra-day showed %R.S.D (< 2%) which suggest precision of the method .The low value of S.D and % R.S.D obtained after introducing small deliberate change in developed HPTLC method indicates the robustness of the method. Low % R.S.D values of between the peak

areas values proved the ruggedness of method indicating that Ellagic acid gives reproducible result for the proposed method. The result of recoveries studies were within acceptable limit indicating accuracy of method was good. It was observed that other constituent's presents in the formulation did not interfere with the peak of Ellagic acid .Therefore the method was specific. Thus, the solvent system of nhexane: ethyl acetate (50:50 v/v) was found to be an ideal mobile phase for separation of Ellagic acid The percentage of Ellagic acid found in IHF and MF are 1.37% and 1.34% respectively.

CONCLUSION:

The developed and validated HPTLC method is simple, precise, and accurate, and can be used for the quantification of Ellagic acid in herbal raw materials as well as in their formulations. Hence, these qualitycontrol parameters and the developed HPTLC methods may be measured as a means for assistance for scientific organizations and manufacturers in developing standards. The results obtained from the study could be utilized as a reference for setting limits in the routine standardization for the quality control and quality assurance of the compound formulations Ellagic acid can be used as an appropriate bio marker for standardization of this Ayurvedic compound formulations.

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REFERENCES:

1. Mosihuzzaman M. Choudhary MI. Protocols on safety, efficacy, standardization, and

documentation of herbal medicine. Pure Appl Chem 2008; 80(10):2195–2230.

- 2. Patwardhan B. Ayurveda: the designer medicine: a review of ethnopharmacology and bioprospective research. Indian Drugs 2000; 37(5):213-227.
- 3. Seitz U, Bonn G, Oefner P *et.al.*, Isotachophoretic analysis of flavonoids and phenol carboxylic acids of relevance to phytopharmaceutical industry. J Chromatogr 1991;559, 499-504.
- 4. O'Shea TJ .Capillary electrophoresis/electrochemistry. Curr Sep 1995;14(1): 18-23.
- 5. Elamthuruthy AT, Chah CR, Khan TA *et.al.*, Standardization of marketed Kumariasava – an Ayurvedic Aloe vera product. J Pharm Biomed Anal. 2005; 37; 937–941.
- Rózylo JK, Zabinska A, Matysiak J, Chromatogr. Sci. 2002; 40; 581.
- Siddhinandan Mishra , "Bhaishajya Ratnavali "Siddhiprada" Surbharti prakashan I edition .2006;259.
- Harborne JB. Phytochemical Methods: A guide to modern technique of plant Analysis. 3, London: Chapman & Hall; 1998;3:90
- 9. Sajeeth C.I, Manna P.K, R. Manavalan *et.al.*, Quantitative estimation of Gallic Acid, Rutin and Quercetin in certain herbal plants by HPTLC method, Der Chemica Sinica, 2010; 1 (2):80-85
- ICH Harmonised Tripartite Guideline. International Conference on Harmonisation guidelines on: Validation of Analytical Methods: Definitions and Terminology. Geneva 1994
- ICH Harmonised Tripartite Guideline. International Conference on Harmonisation quidelines on: Validation of Analytical Procedures: Methodology. Geneva.1996