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Determination Of Bergenin In *Caesalpinia Digyna* Rottler By RP-HPLC Method

M.J.N.Chandrasekar
Department of Pharmaceutical Chemistry,
J.S.S. College of Pharmacy,
Ootacamund, Tamilnadu, (INDIA)
Ph:+91 423 2443393; Fax: +91 423 2442937
E-mail: ncsekar_in@yahoo.com

R.Srinivasan[1], R.Gopinath[2], M.J.Nanjan[1], B.Suresh[1]
[1]Department of Pharmaceutical Chemistry, J.S.S. College of Pharmacy, Ootacamund, Tamilnadu (INDIA)
[2]Department of Pharmaceutical Analysis, J.S.S. College of Pharmacy, Ootacamund, Tamilnadu (INDIA)

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ABSTRACT

A simple, selective, rapid and precise reverse phase HPLC method has been developed for the standardization of *Caesalpinia digyna* root (Family: Leguminosae) using Bergenin as an analytical marker. The method was carried out on a Phenomenex Gemini C_{18} (25 cm x 4.6 mm i.d.,5 µ) column with a mobile phase consisting of Acetonitrile: Water (30:70 v/v) at a flow rate of 0.8 ml/min. Detection was carried out at 275 nm. Retention time of Bergenin was found to be 4.38.The calibration curve was linear in the range of 0.250 µg/ml to 2.5µg/ml of Bergenin and the correlation coefficient was 0.9999, indicating good linear dependence of peak area on concentration. The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation. The proposed method can be used for the standardization of Bergenin in *Caesalpinia digyna* root extract.

KEYWORDS

RP-HPLC;
Standardization;
Caesalpinia digyna;
Bergenin.

INTRODUCTION

Caesalpinia digyna is a large, scandent, prickly shrub or climber, growing wild in the scrub forests of the eastern Himalayas. The plant is one of the ingredients of an indigenous drug preparation, 'Geriforte', which has been used for curing senile purities with excellent results. The drug is also reported to exhibit antifatigue effect in rats[1-2]. The roots have marked astringent and antipyretic properties. They also have an intoxicating effect and are given internally in pthisis and scrofula[3]. The ethanol water extract of roots inhibits the growth of *Mycobacterium tuberculosis*[4]. Chemical investigations of the plant have shown the presence of Caesalpinine A, Cellallocinnine, Ellagic acid, Gallic acid, Pipecolic

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acid, Bergenin and Tannins[5-9]. Because of its widespread use in various geographic regions and to detect its adulteration with roots from younger trees and other lower quality roots, it is important to standardize the root of *Caesalpinia digyna*. No method of standardizing this potentially bioactive plant has been reported so far. We have Therefore, developed an RP-HPLC method for the standardization of its extract using Bergenin (Figure 1) as marker compound. The method was validated as per the ICH guidelines[10, 11].

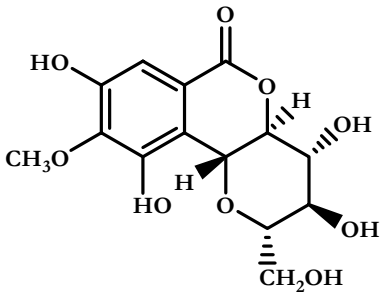

Figure 1: Structure of Bergenin

EXPERIMENTAL

2.1 Plant material

The root of *Caesalpinia digyna* was purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India and identified by Dr.D.Suresh Baburaj, Survey of Medicinal Plants and Collection Unit, Ootacamund, India. The root was cut into small pieces and ground to powder in a mill. The powder was stored in a closed vessel for further use.

2.2 Chemicals and Reagents

Acetonitrile HPLC grade was procured from E.merck (India) Ltd, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system.

2.3 Analytical marker

Bergenin was isolated from the root of *Caesalpinia digyna* and was characterized by spectral studies[12].

2.4 Standard and sample solutions

10 mg of Bergenin was dissolved in 5 ml of methanol in a 10 ml volumetric flask and the volume was made up to 10 ml with the same solvent (stock solution). Various concentrations were prepared from the stock solution. *Caesalpinia digyna* root powder (1 g) was extracted with 20 ml of methanol by heating for 15 min. The extract was filtered through whatman filter paper. The operation was repeated twice. The combined extract was evaporated to 10 ml and used for HPLC analysis.

2.5 Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector and Rheodyne 7725i injector with 50µl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Phenomenex Gemini C_{18} column (25cm x 4.6mm i.d., 5µ) was used for the separation. Mobile phase of a mixture of acetonitrile and water (30:70 v/v) was delivered at a flow rate of 0.8 ml/min with detection at 275 nm. Retention time of Bergenin was found to be 4.38. The mobile phase was filtered through a 0.2µ membrane filter and degassed. The injection volume was 50µl. Analysis was performed at ambient temperature.

RESULTS AND DISCUSSION

3.1 Chromatography

Standardization of Bergenin in *Caesalpinia digyna* root by RP-HPLC method was carried out using the optimized chromatographic conditions. Typical chromatogram of Bergenin and methanol extract of root is shown in figure 2 and figure 3, respectively. Detection was done at 275 nm. The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for six times and the mean peak area and mean peak area ratio of standard were calculated. The results are given in TABLE 1. The low RSD values are indicative of the high accuracy and precision of the method.

3.2 Method validation

3.2.1 Accuracy and precision

The accuracy of the method was determined by

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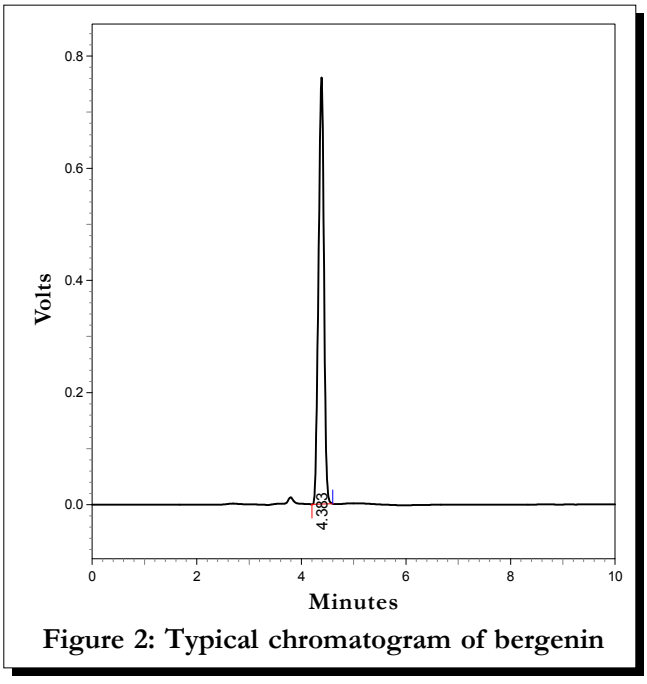

Figure 2: Typical chromatogram of bergenin

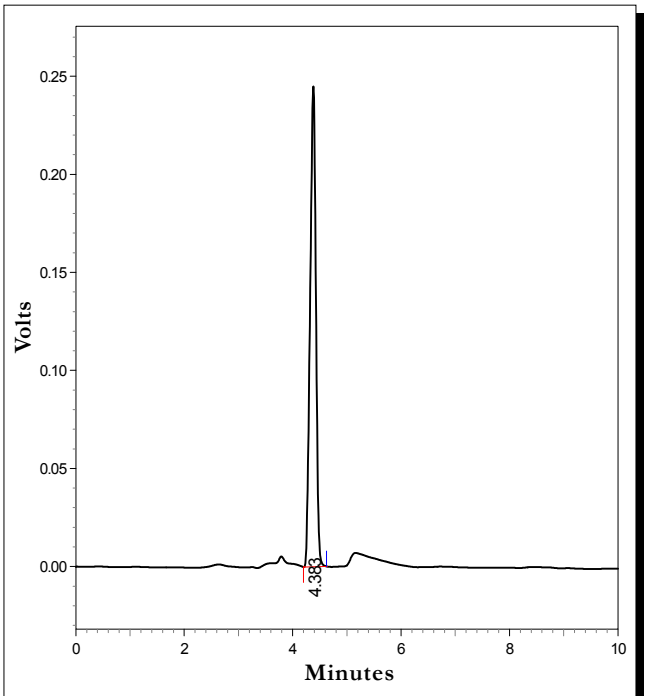

Figure 3: Chromatogram of bergenin in *Caesalpinia digyna*

recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in TABLE 2. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter-day and intra-day variations studies. In the intra day studies, six repeated injections of validation samples (0.25, 1.0 and 2.5 μg/ml) were made and the peak area and percentage C.V were calculated and presented in TABLE 3. In the inter day variation studies, six repeated injections of validation samples (0.25, 1.0 and 2.5 μg/ml) were made for three consecutive days and peak area and per-

TABLE 1: Results of HPLC analysis of Bergenin in the extract of *Caesalpinia digyna*

Plant extract	Constituent	Amount found by proposed method [%w/w]	RSD (%) (n=6)
Caesalpinia digyna root extract	Bergenin	0.430	0.51

TABLE 2: Results of recovery analysis

Plant extract	Amount of Bergenin Present in (ng) A	Amount of Bergenin Added to A (ng) B	Total Bergenin Taken (A+B) (ng) C	Total Bergenin Found (ng) D	% Recovery (D/C) x 100 (Mean)
Caesalpinia digyna root extract	430	250	680	675	99.44
		500	930	920	
		750	1180	1182	

TABLE 3: Results of intra and inter-day variability during validation of the proposed RP-HPLC method

Concentration (μg/ml)	Intra-day		Inter-day (n =6)	
	Accuracy (%)	Precision (C.V)	Accuracy (%)	Precision (C.V)
0.25	97.63	6.52	97.28	6.33
1.00	98.27	5.36	98.14	6.41
2.50	98.52	5.88	98.74	5.52

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centage C.V were calculated and presented in TABLE 3. From the data obtained, the developed RP-HPLC method was found to be precise.

3.2.2 Linearity and Range

The linearity of the method was determined at seven concentration levels ranging from 0.25 to 2.5 μg/ml. The calibration curve was constructed by plotting peak area against concentration of drugs. The slope and intercept value for calibration curve was y = 147664 x -169.29 (R^2=0.999). The results show that an excellent correlation exists between peak area and concentration of Bergenin within the concentration range indicated above. The calibration curve is shown in figure 4.

3.2.3 Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) (the smallest concentration of the analyte that gives a measurable response, signal to noise ratio of 3) and Limit of Quantification (LOQ) (the smallest concentration of the analyte, which gives response that can be accurately quantified, signal to noise ratio of 10) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD and LOQ were found to be 10 ng/ml and 30 ng/ml, respectively (TABLE 4).

TABLE 4: System suitability studies

S.No.	Parameters	Bergenin
1	Theoretical plate/meter	25712
3	Asymmetric factor	0.95
4	LOD (ng/ml)	10.00
5	LOQ (ng/ml)	30.00

3.2.4 Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C_{18}, Phenomenex LUNA C_{18} and Hichrom C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there are no marked changes in the chromatograms thus demonstrating

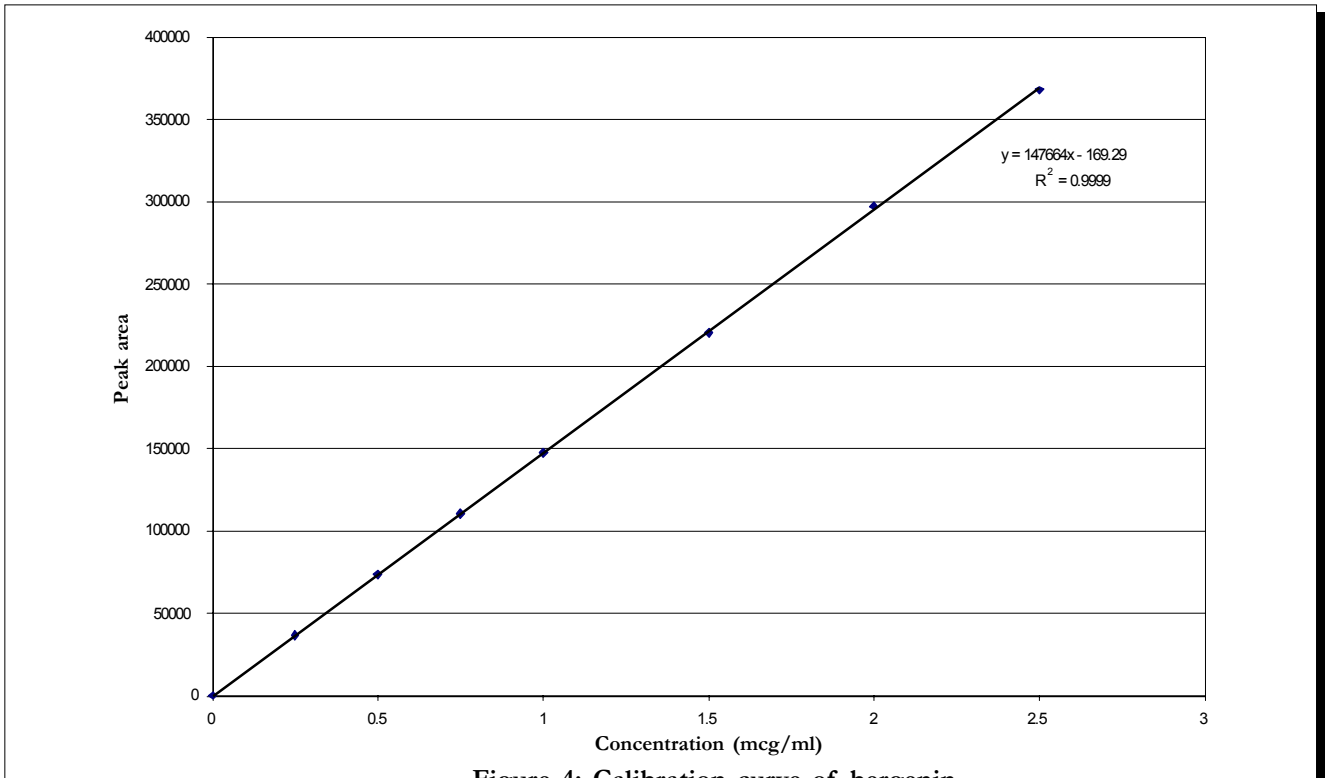

Figure 4: Calibration curve of bergenin

that the developed RP-HPLC method is rugged and robusted.

3.2.5 System suitability studies

The column efficiency and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of bergenin in *Caesalpinia digyna* root extract. System suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method (TABLE 4).

CONCLUSIONS

The proposed RP- HPLC method for standardization of bergenin in *Caesalpinia digyna* root extract is simple, rapid, accurate, precise, linear, rugged and robust. Hence the present RP-HPLC method is suitable for the standardization of bergenin in *Caesalpinia digyna* root extract.

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