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Short Communication

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HPLC Method for Determination of p-coumaric acid from the Medicinal Herb Leptadinia reticulata

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Abstract

The aim of present study was to develop and validate a simple, precise and rapid HPLC method for the quantification of p-coumaric acid in Leptadinia reticulata extractst. The analysis was performed by reverse-phase chromatography on an phenomenex C18 columns with isocratic elution of Methanol and 0.8%-Formic acid in water (6:4) at a flow rate of 1.0mL/min, a column temperature of 35°C, photodiode array detector detection at 326 nm. The method validated in terms of linearity accuracy precision LOD, LOQ and stability. The herb Leptidinia reticulata contains 0.17% p-coumaric acid. The linear range of method was 0.25-50µg/ml with correlation coefficient of 0.9993, the recovery was 98-99.4% and the relative standard deviation is 0.98 % (n=6). The developed method was found to be a relatively simple, precise and reproducible for the quantification of p-coumaric acid. The method does not employ any derivatization procedure and can be used as a quality control tool for the routine analysis of p-coumaric acid from an herb Leptadinia reticulata.

Key words: *Leptadinia reticulata*, p-coumaric acid, HPLC.

Introduction

Leptadinia reticulata is one of the well-known and frequently used herbal medicinal plant, which is an endangered species. It belongs to the Asclepiadaceae family and is considered to be a Rasayana drug and also referred to as Jivanti in ayurvedic texts and it thus used to vitalize nourish and rejuvenate the body [1]. The previously reported chemical constituents of L. reticulata, are stigmasterol, hentricontanole, α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin rutin, β-sistosterol, [2]. apigenin [3]. pragnaneglycosides reticulatin, deniculatin and leptaculatin which have been isolated from Arial parts of the plant [4]. Leptaden has been shown to provide effective treatment in case of deficient lactation and lack of lactation in humans [5] the phenolic acids of L. reticulata, including p-coumaric acid, have been confirmed that they possess a variety of biological activities.

HPLC coupled to various detectors has largely replaced spectroscopic methods in the qualitative and quantitative analysis of pharmaceutical and food products. HPLC for the determination of p-coumaric acid has been used in combination with detection using mass spectrometry (MS), PAD or UV absorption. Milena Nikolova et al, [6]. Reported HPLC method for determination of Phenolic acids in Amaryllidaceae species using Hypersil ODS RP18 column, the mobile phase

was comprised of solvent (A):25mM potassium dihydrogen phosphate adjusted to pH 2.98 by phosphoric acid; and solvent (B): methanol. Ke Liu et al, reported HPLC method developed for the determination of p-coumaric acid in rat plasma,using DiamonsilTM C_{18} column[7]. The mobile phase consisted of acetonitrile–water with 1% glacial acetic acid. Tianhong Zhang et al. [8] to develop a HPLC method for Simultaneous determination of phenolic acids in the herbsof Sumbucus chinensis Lindl using phenomenex C18 column with gradient elution using methanol-0.05 % formic acid in water as a mobile phase. Meng Z et al.[9] To develop HPLC method for the Pharmacokinetic study of p-coumaric acid in mouse after oral administration of extract of Ananas comosus L. leaves. Using a mobile phase of water-acetonitrile (82:18, v/v) and UV detection at 310 nm.

Previously reported HPLC methods for of p-coumaric acid, especially those in the older literature, have several disadvantages, including unsatisfactory separation times, poor resolution and gradient elution[10,11]. A detailed qualitative and quantitative analysis of L. reticulata have been carried out; however no previous studies have been reported on the quantification of p-coumaric acid of the medicinal herb L. reticulata. The aim of the present work was to develop a simple and rapid isocratic HPLC method for the determination of p-coumaric acid for routine analysis and quality control of medicinal herb L. reticulate.

Experimental

Material

The medicinal plant, Leptadinia reticulata was collected from Gandhi Krishi Vignan Kendra, Bengaluru; taxonomically identified and authenticated at DOS in Biotechnology, University of Mysore. The plant materials were washed and shade-dried for a day and then dried completely in a hot air oven at 38^0C. The plant material was powdered using rotary grinder and stored in airtight containers. Analytical grade solvents, Ethyl Acetate, Methanol and Formic Acid were obtained from Merck Ltd. (Mumbai India).

Standard preparations

Standard p-coumaric acid was weighed and dissolved in methanol to get 100µg/ml (stock solution); the stock solution was further diluted to different concentrations. This solution was used as working standard solution, for further chromatographic analysis.

Sample preparation

Plant powder (100g) was extracted in a soxhlet's apparatus with methanol (300 ml x 3), till the plant material became colorless. The combined methanolic extract was concentrated to dryness using rotary evaporator. The residue obtained was dissolved in 50ml of water and subjected to acid hydrolysis with 5% HCl (10 mL). The hydrolyzed solution was extracted with ethyl acetate (50 x 3) and combined organic layer was dried over anhydrous sodium sulfate. It was concentrated to dryness and the resultant residue was diluted with methanol for chromatographic analyses.

Apparatus and chromatographic conditions

A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10 AT VP pumps (Shimadzu) variable wave length programmable photodiode array detector SPD-M10A VP (Shimadzu) CTO – 10 AS VP column Oven (Shimadzu) SCL – 10 AVP system controller and reverse phase phenomenex C18 column (250 x 4.6mm, 5 µm) was used. The Mobile Phase component was methanol–0.8% formic acid in water in the ratio of 6:4, isocratic elution. The flow rate was maintained at 1ml/ min and injection volume was 20 µl. The wave length for detection was set at 326 nm and column temperature maintained at 35^oC.

Preparation of calibration curve

The stock solution of p-coumaric acid was diluted to six different concentrations (60-140%) and the calibration curve was prepared by plotting the standard concentrations against the peak response.

Results and Discussion

The mobile phase and column temperature conditions were optimized to obtain chromatograms with good separation and resolution within a short analysis time. It was found that the mobile phase containing formic acid resulted in good retention and separation of peaks and resulted in improved resolution using gradient elution. The mobile phase was optimized using various formic acid concentrations range from 0.1 to 1.2% formic acid in water and methanol eluent. The best eluting system was found to be methanol–1.0% formic acid in water as an efficient separation and good peak-shape was obtained. The effect of temperature on the peak separation was then investigated in the range of 30–40^{o}C with 5^{o}C difference between two consecutive analyses. The best peak separation and shape was found at 35ºC temperature with optimal flow rate of 1 mL/min. For each run injection volume was kept at 20 µL. The standard Retention time (RT-12.75) of p-coumaric acid is corresponding to the sample chromatogram (RT-12.69) of the L. reticulate extract along with other components. There was no interference from other component present in chromatogram. Standard p-coumaric and samples chromatogram of the medicinal herb L. reticulata is presented in figures 1 and 2.

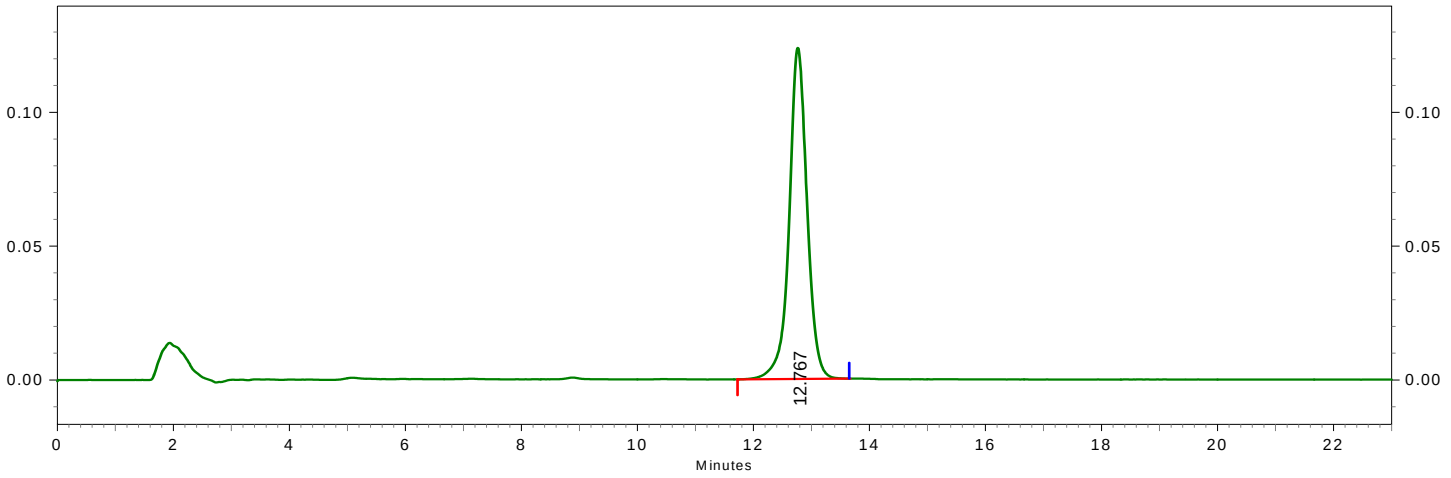

Figure 1: HPLC chromatogram of standard solution of p-coumaric acid.

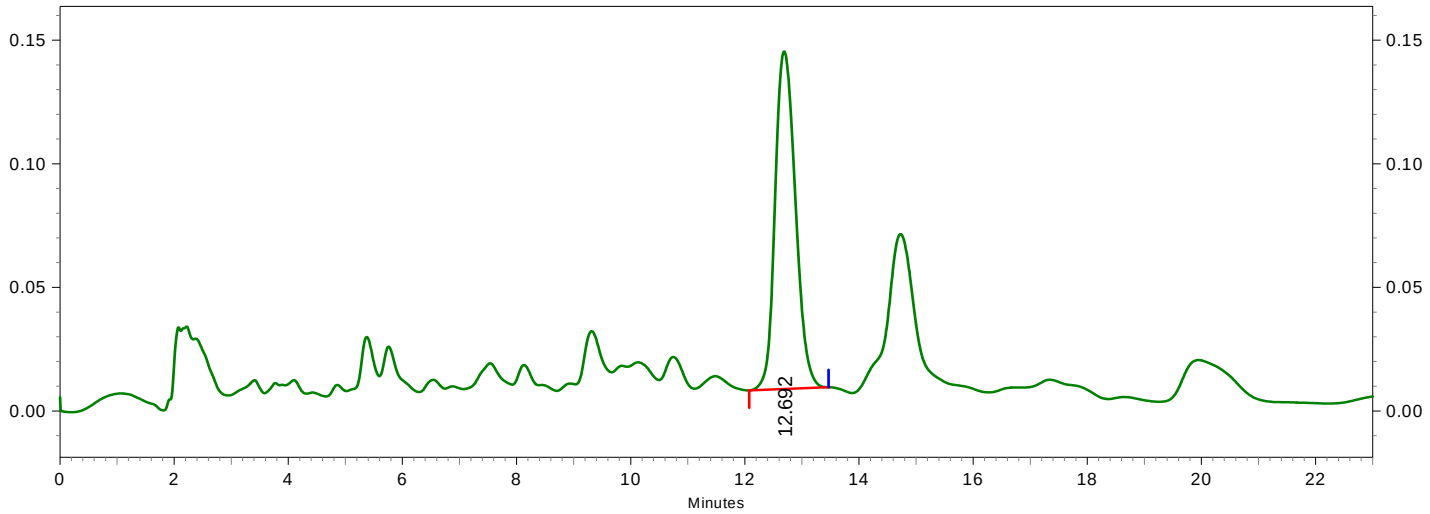

Figure 2: HPLC chromatogram of sample solution of Leptadinia reticulate.

Method validation

Linearity

The linearity of the peak area response verses concentration for a p-coumaric acid was studied between concentration ranges of 0.25-50µg/ml. The calibration curve constructed was evaluated by its correlation coefficient. The calibration equation from six replicates y=2.0679-0.9516 (r = 0.9993) demonstrated the linearity of the method, standard deviation of the slope and intercept for the calibration curve were 0.0179 and 0.0191 respectively.

Precision

The Precision of the analytical method was determined by intraday and interday, and percentage of RSD was found to be <0.71 and <0.82 respectively, which meet the acceptance criteria for the established method. The values are summarized in **Table 1**.

Table 1: Precision of p-coumaric acid.

Added µg /ml	INTRA DAY		INTER DAY	
	FUND± SD µg /ml	RSD of Precision	FUND± SD µg /ml	RSD of Precision
4	3.96± 0.316	0.53	3.92± 0.274	0.64
6	5.99± 1.451	0.84	5.95± 1.210	0.88
8	8.35± 1.104	0.76	8.17± 2.581	0.96

SD: standard deviation of six replicate determinations.
RSD: Relative standard deviation average of six replicate determinations.

Table 2: Accuracy and Recovery study of p-coumaric acid.

Added µg /ml	ACCURACY		RECOVERY
	FUND± SD µg /ml	RSD of Accuracy	In percentage
4	3.92± 1.530	0.74	98.0%
6	5.91± 1.296	0.39	98.5%
8	7.95± 0.659	0.24	99.4%

Table 3: Stability study of p-coumaric acid.

Added µg /ml	Room temperature (Recovery % ± SD)		Refrigerator temperature (Recovery % ± SD)		Frozen temperature (Recovery % ± SD)	
	24hr	72hr	24hr	72hr	24hr	72hr
2	98.0±3.76	97.9±4.18	98.7±4.25	98.4±3.97	98.2±3.47	97.5±3.93
20	101.9 ±2.85	101.2 ±3.98	102.3 ±3.59	102.1 ±4.42	101.4 ±2.56	102.4 ±4.23

Accuracy and Recovery study

Accuracy of the method was determined from recovery studies. The recovery was checked at three different concentrations (4, 6 and 8 µg /ml). The recovery experiment was performed by adding a known amount of pure sample to the pre-analyzed samples according to the procedure. The percent analytical recovery was calculated by comparing concentration obtained from the spiked sample with actual added concentration. The results of recoveries indicated that the method was accurate with overall recoveries for p-coumaric acid in the range of 98.0–99.4% .The values are summarized in Table 2.

Limit of detection and Limit of quantification

Limit of detection and Limit of quantification was evaluated by serial dilution of p-coumaric acid stock solution in order to obtain signal to noise ratios of 3:1 for limit of detection and 10:1 for limit of quantification. The LOD and LOQ values for analyte were found to be 0.05 and 0.10 µg /ml respectively.

Stability

The stability study indicated that the sample was quite stable when kept at room temperature and refrigeration temperature (4°C & -20°C) for 24hr (short time) and 72hr (long time). The results of the studies are given in **Table 3.** It was found that the percentage ratio are within the acceptance range of 90- 110%.

Conclusions

In this study a simple and isocratic HPLC-PDA method was developed and validated for the quantification of p-coumaric acid from the endangered medicinal herb Leptadinia reticulata is being reported for the time. . The experimental conditions, including the diluting solvent, mobile phase composition, column temperature and flow rate, were optimized to provide high-resolution and reproducible peaks.

The method was validated in compliance with the International Conference on Harmonisation (ICH) guidelines (ICH-Q2 (R1), 2005)[12] and is suitable for the determination of p-coumaric acid from the extracts Leptidina reticulata with excellent precision, accuracy and linearity. The method is isocratic with an uncomplicated mobile phase, and the sample preparation and assay procedure are simple and rapid. Therefore, we suggest that this method can be suitable for the quantification of p-coumaric acid in quality control labs. The method can also be extended to analysis of p-coumaric acid from any other source.

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