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Method development and validation for quantification of chlorogenic acid in *Coffea arabica* extract using high performance liquid chromatography

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ABSTRACT

Chlorogenic acid is commonly employed as a biomarker of Coffea arabica and responsible for some of important pharmacological activity. The quality control of chlorogenic acid in herbal product was important by using High Performance Liquid Chromatography (HPLC). An attempt was made to develop and validate an analytical method by using isocratic elution program with less solvent consumption and less run time for Coffea arabica extract using HPLC. The present study described a developed method used a Purospher reverse phase C_{18} end capped column (250× 4.6 millimeter; 5 micrometer) using an isocratic elution of potassium dihydrogen orthophosphate with acetonitrile as mobile phase with a flow rate of 1.0 ml/min and chlorogenic acid was monitored at 274 nm. Method has been successfully validated for linearity, specificity, precision, accuracy, range and ruggedness with good recoveries. Specificity of the method was assessed using PDA (Photo diode array) profile and peak purity was greater than 99%. Calibration curve exhibited good linear regression (r^2> 0.999) with in the test range and recovery was 96- 102%.

INTRODUCTION

Coffea arabica (L.) (family- Rubiaceae), commonly known as coffee. Coffee has been the most commercialized food product and most widely consumed beverage in the world for decades [1]. It is an evergreen, shrub or small tree up to 5 m tall with small glossy leaves originally found in the southwestern highlands of Ethiopia, Angola, Brazil, Costa Rica, Mexico and India [2]. The plant has been reported diverse biological activities which include antioxidant anti helmenthitic, antibacterial, antimicrobial, neuro generative, anti diabetic activities [3-9]. The chemistry of Coffea arabica has been extensively studied and main bioactive compounds present in green coffee are chlorogenic acid, caffeine, trigonelline. Chlorogenic acid is derived from esterification of trans- cinnamic acids such as caffeic, ferulic, p- coumaric and quinic acid [10-12]. It has been reported for various activities such as anti inflammatory, reduce liver inflammation, anti arthritic, anti obesity and neuroprotective [13-17]. Chlorogenic acid contains no chlorine and name comes from Greek language because green colored produced when it oxidized. Chlorogenic acid (Figure: 1) contain C16 chain with molecular weight of 354.31 and freely soluble in alcohol, acetone.

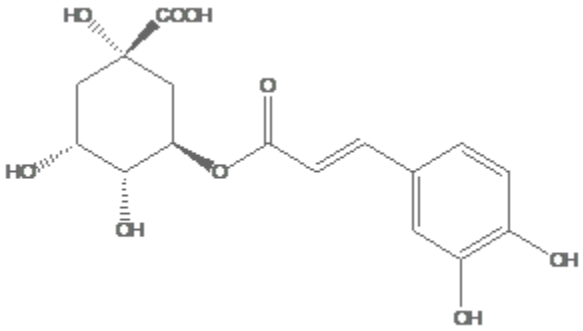

FIGURE. 1. Structure of standard compound - Chlorogenic acid

For the quantification of Coffea arabica extracts for the quantification of Coffea arabica extracts, we have chosen chlorogenic acid as analyte and HPLC as the technique which include method development and validation by consisting the parameter specificity, linearity and Range, accuracy, precision, Detection limit and quantitation limit, Robustness and System suitability.

MATERIALS AND METHODS

Plant Materials: Extracts of Coffea arabica were collected from different regions of India. The identity was confirmed by Department of Agronomy, R&D center. Natural Remedies Pvt. Ltd., Bangalore, India. The freshly collected extract samples were stored at 4ºC, protected from sun light and humidity before analysis.

Reagents and chemicals: Reference standard of chlorogenic acid (batch no CC2) was collected from Indofine chemicals. The purity of the standard compound was found to be 95%. HPLC grade acetonitrile (ACN) and potassium dihydrogen orthophosphate used in the study were obtained from Rankem. HPLC grade water was obtained from Sartorius Arium 611 water purification system. 0.45 micron (µ) Polyethersulfone (PES) membrane filter was obtained from Rankem.

HPLC Instrumentation and Conditions: The HPLC system (Shimadzu, 2010CHT) consisted of quaternary pump with vacuum degasser, thermostatted column compartment, autosampler and UV detector. A reverse phase end capped column (purospher C_{18}, (250× 4.6 millimeter; 5 micrometer) was used and the column temperature 30ºC. HPLC mobile phase (buffer) was prepared; potassium dihydrogen orthophosphate (1gm) was dissolved in 900 mL of HPLC water, volume was made up to 1000 mL with acetonitrile. The solution was filtered through a 0.45µm membrane filter and degassed in sonicator for 3 minutes. Mobile phase was run using isocratic elution with run time of 30 min. The flow rate was 1.0 mL/min and the injection volume was 20µL (micro liter). Chlorogenic acid was detected and analyzed at 274 nm.

Preparation of Standard Solutions. Stock solution of chlorogenic acid was prepared at 10 mg/ 50 mL (i.e. 0.2 milligram/milliliter) in phosphate buffer. The calibration curves were prepared using solutions of different concentrations ranging from 5 to 196 µg/ mL.

Sample preparation. Extracted material of plant Coffea arabica was used and an accurately weighed sample of extract (about100 milligram) was taken in 100 milliliter (mL) volumetric flask and dissolved in 50 mL of phosphate buffer. This was sonicated for 15-20 minute and volume was made up to 100 mL with phosphate buffer. Extract were filtered through 0.22 µm membrane filter before analysis.

Validation of the Method: The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines [18] and as the USP General Chapter <1225> Validation of compendial Procedures [19]. The accuracy of the developed method was evaluated in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting the serial dilutions of solutions of the standard and known concentrations. The LOD and LOQ were calculated based upon the signal-to noise ratio of more than 3 times for LOD and 10 times for LOQ respectively.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions. The analytical method described for the quantification of chlorogenic acid from Coffea arabica, therefore a separate HPLC method was developed for quantification of chlorogenic acid in different samples. During preliminary work reverse- phase HPLC column from different

manufacturers were tested in order to optimize the condition of separation. The best separation efficiency and peak shape were achieved on a reverse phase end capped (purospher C_{18}, (250× 4.6 millimeter; 5 micrometer). Optimal chromatographic separation of chlorogenic acid (Figure: 2) was achieved using isocratic elution of a mobile phase of a mobile phase containing phosphate buffer containing acetonotrile. Acetonitrile was preferred over alcohol type of solvent because it enhanced the separation as well as offered relative low back pressure. Selecting 274 nm as the detection wavelength, based on Ultra violet (UV) max for chlorogenic acid with no interference from other compounds presents in the samples.

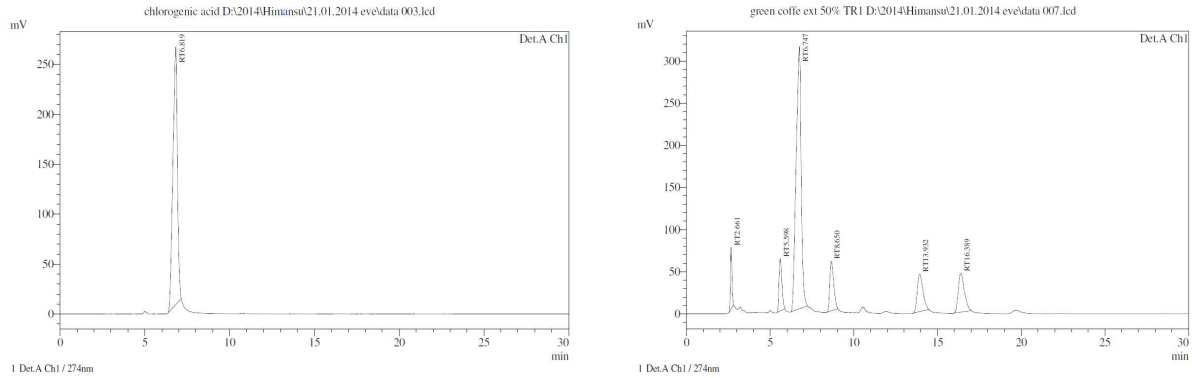

FIGURE. 2: Representative chromatogram of standard chlorogenic acid and Coffea arabica extract

Method Validation. The HPLC method was validated for precision, accuracy and linearity. The specificity was determined by injecting individual samples and ensuring no interference of the sample matrices. Linear calibration plots were obtained over six concentration levels (5- 196 µg/ mL). The results showed a linear correlation between the peak area and concentration (Table 2). The precision study used the plant sample GRC13005 P and followed and validated procedure for sample preparation. The precision of the assay was evaluated by carrying out the three independent analyses on two different days. The % Relative Standard Deviations (%RSD) of all three analyte was determined to be within the acceptable limit of 2.5%. Multiple injections illustrated that results were reproducible and had a low standard deviation (Table 1). The Relative standard deviation (RSD) of assay results for chlorogenic acid obtained in interday and intraday study was within 2.5% and exhibited 2.27% in intraday and 0.59% for interday confirmed the acceptable precision of developed method. The accuracy of the method was determined by spiking samples with a known amount of standard compound. The accuracy of the method was evaluated in triplicate at three concentration levels. The percentage recovery ranged was found to be 96 to 102 % for chlorogenic acid. (Table 2). The developed method exhibited acceptable performance in terms of sensitivity and baseline separation of marker compound without interference of sample matrix. New isocratic developed method for quantification of chlorogenic acid was rapid, less time, less solvent consuming with run time of 30 minute.

TABLE. 1: Results of system suitability test[a]

Parameter	Results
K’	5.547
A_s	1.319
N	14776
R_s	2.59
RRt	1.0
RSD (Rt)	0.10
RSD (AUC)	1.08

[a]Test performed as per the ICH; k’: capacity factor: A_s; symmetry factor;
N: no of theoretical plate: R_s: resolution; RSD (Rt): RSD of the retention time: RSD (AUC): RSD of the peak area.

Quantification of chlorogenic acid. Analysis of samples designated as GRC13005 P, GRCP1013 of Coffea arabica extracts. The samples were analyzed for quantification of chlorogenic acid by the developed HPLC method. The compound was identified by spiking the samples with standard solution of chlorogenic acid by comparison of UV spectra and retention time with standard. Content of chlorogenic acid in the extract samples were recorded as 25, 22 %w/w respectively by using PDA / UV detector.

TABLE. 2: Results of validation of HPLC method

Validation Parameter	Results
Specificity Peak purity (%)	>99
Linearity Conc. µg/ mL	4.89 to 195.6
R^2	0.9999
LOD µg/mL	2.95
LOQ µg/ mL	8.91
Precision Intraday precision RSD (%)	2.27
Interday precision RSD (%)	0.59
Accuracy: at the different concentration in triplicate (n=3) Recovery (%)	96-102

CONCLUSION

An HPLC method was developed and validated for quantification of chlorogenic acid obtained from extracts of Coffea arabica. Results from validation of the method exhibited good specificity, linearity, acuracy, precision and reproducibility. The method was found to be suitable for detection of extracts derived from Coffea arabica plant. Analysis of various samples used in in study indicated the usefullnes the method. from extracts. The reverse phase high performance liquid chromatographic method proves for the quantitative estimation of chlorogenic acid in the extracts of Coffea arabica

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