Analytical Method Development and Validation for the Simultaneous Estimation of Chlorogenic Acid and Caffeine in Bulk And its Capsule Dosage Form

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Abstract

Background: A simple RP-HPLC method for simultaneous estimation of Chlorogenic Acid and Caffeine in their Capsule dosage form has been developed. The separation was achieved by C18 (25 mm × 4.6 mm,5µm) Hypersil BDS column and Methanol: Water (pH 2.8) adjusted with OPA (45:55 v/v) as mobile phase, at a flow rate of 1 ml/min and injection volume 20 µL with PDA detection at 283 nm. Detection was carried out at 283 nm.

Result: Retention time of Chlorogenic Acid and Caffeine were found to be 3.382 min, 4.467 min respectively. This method was validated as per ICH (Q2 R1) guideline. Linearity observed for Chlorogenic Acid 1-5 µg/ml and Caffeine 0.5-2.5 µg/ml. The LOD of Chlorogenic Acid and Caffeine where found to be 0.426 and 0.182 and LOQ of Chlorogenic Acid and Caffeine where 1.321 and 0.545 respectively.

Conclusion: Established method was found to be accurate, precise and rapid for simultaneous estimation of Chlorogenic Acid and Caffeine in Capsule dosage form.

Keywords: Method Development and Validation, RP-HPLC, Chlorogenic acid, Caffeine, Accuracy, Precision.

Introduction

Herbal supplements are compounds derived naturally from plants' leaves, bark, roots, seeds, or flowers that can be used by people as medications [1,2]. For the treatment of numerous disorders, people use a wide range of plant extracts [2,3]. Products made from medicinal plants are called polyherbal preparations. These natural products are considered as safe. Herbal formulations which have reached widespread acceptability as therapeutic agents in India include nootropics, anti-diabetics, hepatoprotective agents and lipid lowering agents. However, Regarding the safety and effectiveness of these medications, there are numerous restrictions. [4]. Knowledge regarding the active ingredients of herbal preparations are not well defined, information on toxicity and adverse effects of these formulations are insufficient [5]. The development of analytical methods for poly herbal formulations which can certainly profile the phytochemical composition, including quantitative analyses of bioactive compounds and other constituents, is challenging to scientists [6].

For decades, coffee has been the most frequently consumed beverage and the most commercialised food item in the world. [7,9]. Coffee belongs to the family of Rubiaceae in the genus Coffea. 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. The plant has been reported for the biological activities which include antioxidant, neuro generative, anti helmenthitic, antibacterial, anti-diabetic activities, antimicrobial, anti-diabetic activities[8]. Chlorogenic acid, trigonelline, and caffeine are the primary bioactive chemicals found in green coffee, according to intensive research on the Coffea arabica. Chlorogenic acid is derived from esterification of trans- cinnamic acids such as caffeic, ferulic, p-coumaric and quinic acid. Chlorogenic acid (CGA) is one of the phenolic acid compound which are found in foods like tea and coffee. The primary property of phenolic compounds is their capability to trap free radicals, that causes the degenerative disease. Moreover, phenolic acid shows hepatoprotective properties that protect the liver from chemical or lipopolysaccharide induced injury. [9,10]. The chlorogenic acid can result from the altered metabolism of nutrients, including amino acids, glucose and fatty acids because of hypocholesterolemic influence. Some polyphenols are isolated from coffee, especially Chlorogenic acid are considered as well-known antioxidant agents. Many oxidative stress-related diseases, such as cancer, cardiovascular, ageing, and neurological diseases, are prevented by consuming foods high in chlorogenic acid. [7]. Chlorogenic acid contains no chlorine and name comes from Greek language because green coloured produced when it oxidized. Chlorogenic acid contain C16 chain with molecular weight of 354.31 and freely

soluble in alcohol, acetone. In comparison to roasted coffee, raw coffee has a substantially higher amount of chlorogenic acid. A review of the literature reveals that various analytical methods are available for the estimation of individual drugs. It includes UV spectrophotometry [9,10], HPLC [11-14], HPTLC [15,16] and LC–MS/MS [17-20]. Based on the literature survey it was found that there is no simple and RP-HPLC method available for simultaneous estimation of chlorogenic acid and caffeine in capsule dosage form.

Chemicals and Reagents

(a) Methanol- HPLC grade (Hi-media, India)

(b) Ortho phosphoric acid (Merck, India)

(c) HPLC grade water was prepared by using Milli Q water purification system.

Reference standards

a) Chlorogenic acid- purity > 99%, analytical standard

b) Caffeine- purity > 99%, analytical standard

Instruments

a) Micro Balance- Shimadzu (AX200)

b) pH meter- EUTECH Waterproof pHTestr 10

c) Ultra sonicator- Lifecare

d) Vaccum filtration pump- PCI Analysis (PCI 15)

e) HPLC- Shimadzu (LC 20 AD)

f) Column- Phenomenex luna 5µm C_{18} (250mm × 4.6mm, 5µm)

g) Injector- Rheodyne 7725i with 20µl loop

h) Detector- Photo Diode Array Detector.

Chromatographic conditions

Chromatographic separation was achieved at ambient condition with a mobile phase consisting of Methanol and Water (pH 2.8) adjusted with OPA in the ratio of 45: 55, respectively, and is degassed by using an ultrasonic bath. Isocratic elution was achieved on the C18 (250 × 4.6) mm column with a particle size of 5 µm. The flow rate was kept at 1.0 mL/min, and the injection volume was 20µl. Detection was achieved at 283nm.

Preparation of standard solution

The solution of standard was made by dissolving accurately weighed 10 mg of Chlorogenic acid and 10 mg of Caffeine in a 10 mL volumetric flask and make up the volume to 10 mL using methanol. The resulting solution was sonicated for 5 min to aid dissolution and then filtered through 0.22-µm syringe. From the above solution 1ml was transferred into 10ml of volumetric flask and made up with the mobile phase. Then aliquots of mixed solution was 0.3 ml, 0.4 ml and 0.5ml are transferred into a 10 ml volumetric flask to get the final concentration as 3, 4, 5 µg/ml respectively.

Preparation of sample solution

Accurately weighted and powdered 0.010 g capsule formulation was taken to 50 mL volumetric flask and volume was made up to 30 mL using water of chromatography grade. The resulting solution was sonicated for 15 min and then filtered through 0.22-µm syringe filter. With aliquots of 1ml, 1.5ml and 2ml to get a final concentration of 3, 4, 5µg/ml.

Analytical method development

Various steps involved in the method development were optimized for accurate and precise separation. Several parameters were studied to select optimal conditions for selection of chromatographic, selection of wavelength, selection of mobile phase, selection of method for separation and selection of detecting wavelength [21]. After taking several trials, the optimized chromatography method which shows optimum peak resolution and good symmetry for the simultaneous estimation of Chlorogenic acid and Caffeine is shown in Table 1.

Table:1 Chromatographic conditions

Parameters	Value
Column temperature	Ambient condition
Detection wavelength	283 nm
Flow rate	1 mL/min
Mobile phase	Methanol: Water (pH 2.8) adjusted with OPA
Mode of separation	Isocratic
Run time	10 mins

Validation of developed method

Linearity

The linearity response was determined by analysing 5 independent level of calibration curve in the range of 1-5 µg/mL (1, 2, 3, 4 & 5 µg/mL) for Chlorogenic acid and 0.5-2.5µg/mL (0.5, 1, 1.5, 2 & 2.5 µg/mL) for Caffeine. The linearity graph was plotted against peak area and concentration. Correlation coefficient and regression line equations for Chlorogenic acid and Caffeine were calculated.

Accuracy and precision

The accuracy of the method was executed at three different concentration levels of 50, 100 and 150% of the specific concentration of drugs. By calculating percentage recovery the accuracy of the method was evaluated. Repeatability was carried out by using five replicates at the assay concentration of Chlorogenic acid and Caffeine. Three different concentration levels of 50, 100, and 150% were used to conduct intra- and inter-day variations of chlorogenic acid and caffeine in triplicate. Results are expressed in RSD.

Specifcity

The Specificity of a method is to quantify the analyte response in the presence of additional excipients and contaminants. Specificity was established by comparing the chromatogram of the drug that was extracted from the tablet to that of the reference solution for excipients, impurities, and other degradants.

LOD and LOQ

The limit of detection (LOD) were calculated by using the formula LOD = 3.3 × Standard deviation/Slope and limit of quantification (LOQ) was calculated by LOQ = 10 × Standard deviation/Slope

Robustness

The robustness of an analytical technique is a ration of its ability to stay genuine by modest but purposeful changes in method parameters like flow rate, pH of Mobile phase and Mobile phase composition and the changes made in flow rate 0.9, 1, 1.1 mL/ min and Mobile phase ratio 43:57, 45:55 and 47:53.

Results

Optimized condition for method development

Various mobile phase compositions, different columns, pH, flow rate were investigated and the best result was achieved on Symmetry C_{18} (250 mm ×4.6 mm, 5µm) column with the mobile phase composition was Methanol: Water (pH 2.8) adjusted with OPA. The detection was carried out at 283 nm with 1.0 mL/min flow rate. The retention time of Chlorogenic acid is 3.385 and Caffeine is 4.473 respectively with a total run time of 10 min. The optimized chromatogram and optimized conditions are mentioned in Fig. 1.

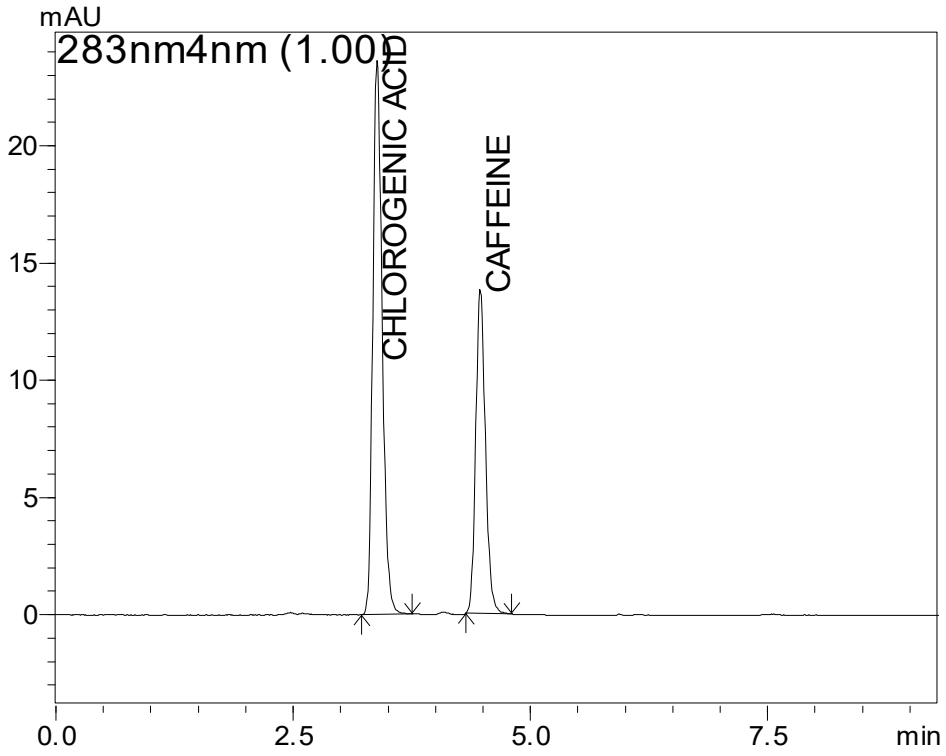

Fig. 1: Chromatogram for Chlorogenic and Caffeine Optimization

System suitability

The optimized method is acceptable as system suitability parameters are valid as it passed the criteria of acceptability, as shown in Table 2.

Table 2: System Suitability Parameters

Parameter	Chlorogenic acid	Caffeine
Retention time	3.38	4.47
Theoretical plates (*N*)	4787043	7810381
Resolution (Rs)	5.466	
Tailing factor (T_f)	1.275	1.275

Method validation

Linearity

The calibration curve for obtained for Chlorogenic acid and Caffeine was found to be linear in the range of 1-5µg/mL and 0.5-2.5 µg/mL and the correlation coefficient was found to be 0.999 and 0.9994 respectively. The calibration graph of both the drugs where shown in Fig. 2 and 3.

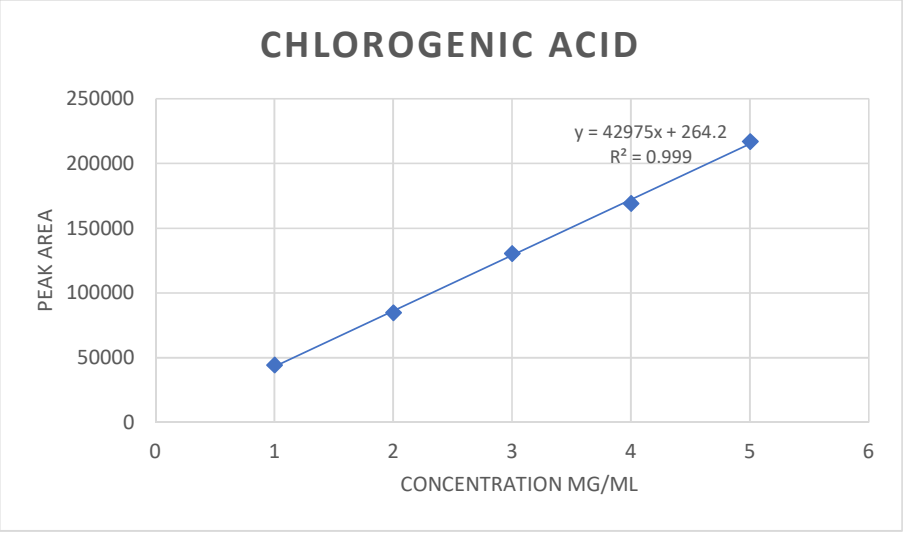

Fig. 2: Calibration curve of Chlorogenic acid

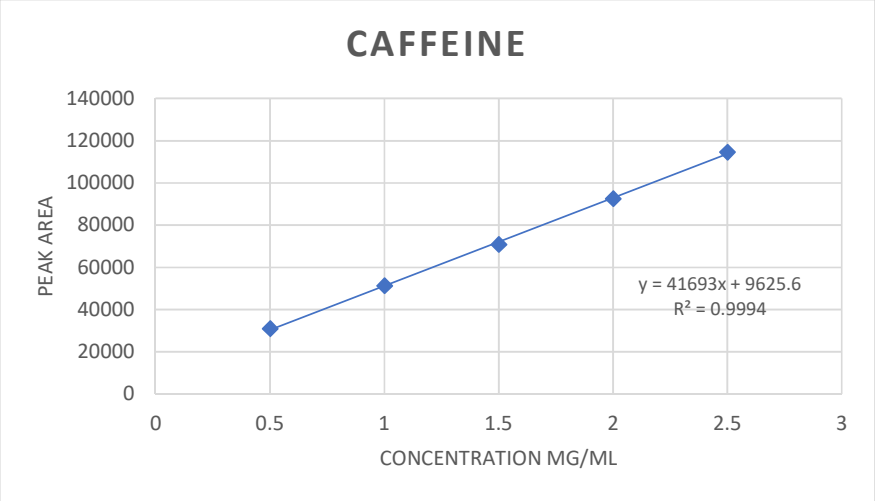

Fig. 3: Calibration curve of Caffeine

Accuracy

The accuracy of the method was determined by recovery experiments. This method is accurate as % Recovery was found in the range of 99.51–101.65% for Chlorogenic acid and 100.06–101.20% for Caffeine are shown in Tables 3.

Table 3: Accuracy (Recovery studies)

Drug	SpikeLevel (%)	Amount of drug added (µg/ml)	Amount of drug recovered (µg/ml)	Percentage Recovery*(%)	%RSD*
Chlorogenic acid	50	3	3.0324	101.08	0.19
	100	4	3.9324	98.31	0.15
	150	5	5.0434	100.86	0.11
Caffeine	50	1.5	1.4706	98.04	0.92
	100	2	1.9906	99.53	0.86
	150	2.5	2.5210	100.33	0.27

Precision

The precision of the method was determined by reproducibility and repeatability. Repeatability and intermediate precision where expressed in term of RSD. The area of drug peaks and % RSD of intra day and inter day were calculated. The results are shown in Table 4.

Table 4: Intra day and Inter day precision studies.

Parameters	**Peak area Chlorogenic acid**	**Peak area Caffeine**
Intraday	136729	586788
	136460	586876
	136578	586767
	126731	586726
	136719	586878
	136728	579834
Average*	136657	585644
%RSD	0.87	0.99
Inter day		
Day 1	77719	584788
Day 2	76760	596876
Day3	76578	586367
Day 4	78731	586326
Day 5	75719	586878
Day 6	76718	579834
Average*	77037	586844
%RSD	0.73	0.87

LOD and LOQ

The LOD is the smallest concentration of the analyte that give a measurable response and LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified that give a measurable response. LOD and LOQ for Chlorogenic acid was found to be 0.43 µg/mL and 1.35 µg/mL and Caffeine was found to be 0.18 µg/mL and

0.54 µg/mL respectively. Results are mentioned in Table 5.

Table 5: LOD and LOQ

Parameter	Chlorogenic acid	Caffeine
LOD	0.43µg/ml	0.18 µg/ml
LOQ	1.32 µg/ml	0.54 µg/ml

Robustness

Making a deliberate change in flow rate, mobile phase composition were taken place and RSD was found to be less than 2, specify that the method is robust. The observations, shown in Tables 6 suggest that the selected criteria remain unchanged by a slight variation of these parameter.

Table 6: Robustness Studies

Chromatographic Condition	Retention time for Chlorogenic acid	Retention time for Caffeine
Flow rate(ml/min)		
0.9	3.256	3.985
1	3.382	4.467
1.1	3.532	4.776
% Methanol Concentration		
43	4.967	5.461
45	4.467	3.382
47	3.464	3.904

Assay of Capsule dosage form

The amount of Chlorogenic acid and Caffeine in Capsule formulation was determined to be 42.5 percent and 36.2 percent respectively, in an assay of pharmaceutical Capsule dosage form operating the established and validated technique. The method was shown to be very precise, since the produced peaks did not interact with the standard peaks in any way. The method was confirmed to be reliable because the calculated percent RSD values were less than 1 percent. Result was shown in Fig 2.

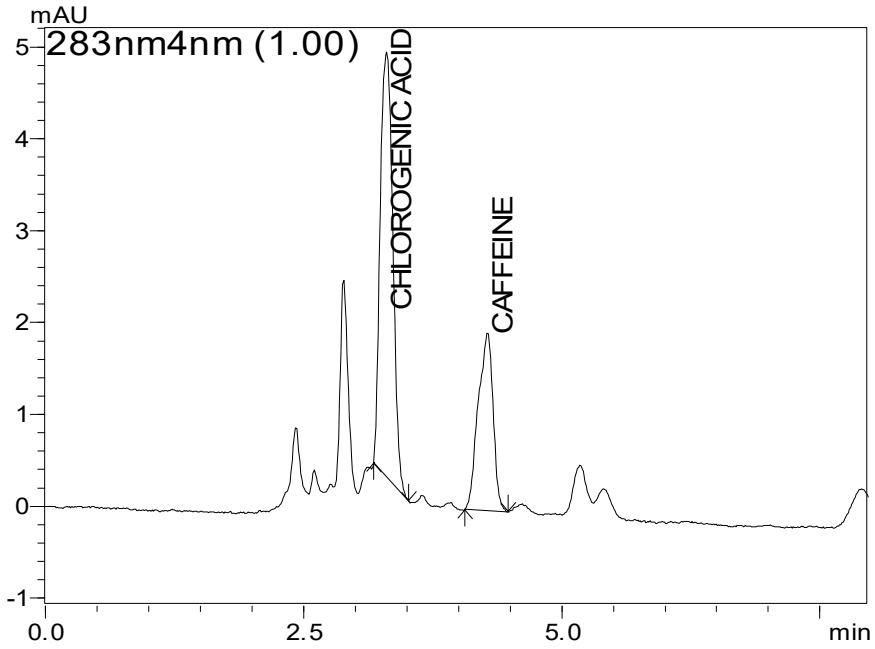

Fig 2: Chromatogram for Formulation of Chlorogenic acid and Caffeine

Discussion

The current approach was developed using Methanol: Water (pH 2.8) adjusted with OPA (45:55 v/v). The method developed was done by utilizing the minimal amount of organic solvents as the mobile phase which results in a more accurate and cost-effective method. In the present method, Chlorogenic acid and Caffeine were eluted with retention time 3.382 min and 4.467 min respectively. On the basis of literature review it was found that there is no method available for simultaneous estimation of Chlorogenic acid and Caffeine in combination.

Conclusion

A simple, accurate, precise and rapid RP-HPLC for simultaneous estimation of Chlorogenic Acid and Caffeine in Capsule dosage form. The method developed was validated according to their linearity, specificity, accuracy, precision, robustness and results were found within the acceptance limit as per ICH (Q2 R1) guideline. Analysis proves that the method is acceptable for the simultaneous determination of both drugs in pharmaceutical analysis and

routine quality control without any interference

Abbreviations

HPLC: High- performance Liquid chromatography; **ICH**: International Council for Harmonization; **LC-MS/MS**: Liquid chromatography mass spectrometry/mass spectrometry; **LOD**: Limit of detection; **LOQ**: Limit of quantification; **RP- HPLC**: Reversed-phase high-performance liquid chromatography; **RSD**: Relative standard deviation; **S:** Slope; **HPTLC**-High- performance Thin layer chromatography; **TLC**: Thin layer chromatography; **UV**: Ultraviolet.

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