

Enumerate chlorogenic acid in different forms of coffee by simple high performance thin layer chromatographic method

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Abstract

The health benefit of coffee consumption mainly depends on chlorogenic acid (CGA), one of its major active phenolic constituent. CGA have several biological properties like anticancer, antihypertensive, hepatoprotective, neuroprotective, renoprotective, antimicrobial etc. It is assumed that different forms of coffee contains different amount of CGA. In this regards, we developed and validated a simple and cost effective chromatographic technique to identify and quantify CGA in green coffee, roasted coffee and branded instant coffee available in Indian market.

Keywords: Coffee; Chlorogenic acid; HPTLC; UV-VIS spectroscopy

1. Introduction

Coffee is one of the most widely consumed beverages worldwide. So far, more than eighty coffee species (genus: *Coffea*, family: Rubiaceae) have been identified but only two are commercially important - *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee). 70% of global coffee market is dominant by Arabica coffee [1]. Green coffee beans are the unprocessed or unroasted seeds of coffee berries and rich in chlorogenic acid (CGA) and its related compound such as quinic acid, p-coumaric acid, ferulic acid and caffeic acid [2]. Besides CGA, coffee seeds contain caffeine, diterpenes, trigonelline etc. CGA is a phenolic compound with antioxidant activity and high bioavailability in human [3]. Extensive researches have been conducted to find out the health benefit of coffee consumption. Revuelta-Iniesta and Al-Dujaili (2014) reported that consumption of green coffee reduces hypertension by influencing 11 β -HSD1 enzyme activity in human [4]. CGA have anti-inflammatory, anticancer, antidiabetic, antimicrobial, hepatoprotective, neuroprotective, cardioprotective and lipid lowering properties [5-8]. Our study confirmed that CGA enriched green coffee treatment ameliorated renal injury [9]. Several comprehensive reviews have also been available on risk and benefit of coffee consumption [10,11].

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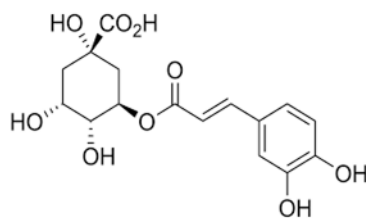


Figure 1 Empirical structure of Chlorogenic acid

Although, natural compounds of coffee seeds have been distorted or reduced during the process of decaffeination, stream treatment, roasting processed to produce instant coffee. Sophisticated analytical methods, like high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE) have been developed for the determination of non-volatile, volatile, polar and non-polar classes of compounds in coffee [12]. As *the health benefit of coffee consumption mainly depends on its major active constituent, namely on CGA and its derivatives*, hence standardization of coffee would like to be focused on the amount of CGA present in different form of coffee. In this regards, we developed and validated a simple and cost effect chromatographic technique to identify and quantify CGA in green coffee, roasted coffee and branded instant coffee available in Indian market.

2. Material and methods

2.1. Test samples preparation

Two different forms of coffee bean (seeds) - green and roasted coffee seed; and two branded instant coffee - Nescafe (Nestle, India) and BRU Coffee (Hindustan Unilever Ltd, India) were used. The green and roasted coffee bean seeds were made in coarse powder by electric grinder. The powder was merged in 60-40 methanol-water (v/v) at a solvent/solid ratio of 40 ml/g for 90 min at room temperature [13]. Two branded instant coffee, Nescafe and BRU Coffee were dissolved in 60% methanol water.

2.2. UV-vis spectroscopic analysis

All test samples were further diluted into different concentrations (100-500 µg/ml) and scan in spectrophotometer (UV-1800 Shimadzu, Japan) at 200-400λ [14]. The standard curve of chlorogenic acid (Sigma, USA) at different known concentration (5-9 mg/L) was performed and plotted as absorbance vs. concentration. The concentrations of CGA present in test samples were calculated against standard curve of CGA.

2.3. High performance thin layer chromatographic method

Presence of chlorogenic acid in test samples was determined using densitometric HPTLC technique. The reference standard chlorogenic acid (Sigma-Aldrich, USA) was dissolved in methanol at the concentration of 1 mg/ml and applied 200 ng, 300 ng, 400 ng, 500 ng, and 600 ng to prepare the standard calibration curve (peak height and area) by plotting peak areas versus concentration. The R_f of CGA was 0.35. The accuracy of method has been verified.

Table 1 Instrumental and chromatographic conditions.

Instrument	CAMAG Linomat 5	Slit dimensions	5 X 0.45 µm
Detector	CAMAG TLC Scanner 3	Brand length	8 mm
Soft-ware Analysis	winCATS Planar	Pre-dosage volume	0.2 µl
Calibration mode	Multi level	Application volume	2-10 µl
Evaluation mode	Peak height & Area	Lamp	D2
Stationary phase	HPTLC plates Silica gel 60 F 254 (E.MERCK KGaA)	Mobile phase	EA: DCM: FA: AA: DW= 10:2.5:1:1:1.1
Plate size	20.0 X 10.0 cm	Wavelength	330 nm

The test samples were spotted in the form of bands on a pre-coated silica gel plates (E. Merck, 60F₂₅₄, 20X10 cm) using CamagLinomat 5 applicator (Camag, USA). The plates were developed in ultrapure solvent system containing ethyl

acetate: dichloromethane: formic acid: acetic acid: water = 10:2.5:1:1:1.1 in Camag glass chamber and air dried for 5 min at 60°C. The densitometric scanning was performed on Camag TLC Scanner 3, operated by multi-level winCATS planar chromatography manager at absorbance 330 nm (Table 1). The amount of chlorogenic acid present in test samples were auto-generated against standard curve [15,16].

ICH guidelines were followed for the method validation of the analytical procedures [17]. The method was validated for precision, repeatability and accuracy. The repeatability of the method was checked by repeated scanning of the same spot of chlorogenic acid (300 ng), six times and was expressed as co-efficient of variance (% CV). The variability of the method was studied by analyzing aliquots of chlorogenic acid (as 200 ng, 400 ng and 600 ng) on the same day and on different days and the outcome data were expressed as % CV. The recovery studies were done at three levels (50%, 100% and 150% addition). The percent recovery and average percent recovery was calculated for studying accuracy of method.

2.4. Statistical analysis

Descriptive data were presented as mean and standard deviation. A p-value of less than 0.05 was considered statistically significant.

3. Results and discussion

Chlorogenic acid (CGA) was discovered in 1837 and International Union of Pure and Applied Chemistry (IUPAC) recommended 5-caffeoylquinic acid (5-CQA) or chlorogenic acid in the year 1908 [18,19]. The empirical structure of CGA is given in Fig. 1. Adequate amount of CGA is present in tobacco leaves, mulberry tree and coffee beans. It is found to be responsible for the astringent taste of coffee brews [20]. The biological activities of CGA are now well documented. During the process of decaffeination, roasting, extraction, blending and brewing some volatile constituents including CGA have been altered. The content of CGA (including their lactones) in coffee mostly vary from 26 mg to 1141 mg per 100 ml [21-22]. In general, considering the different existing brewing methods the values reported for major CGA in brews prepared at 6%-17.5% [23]. Thus it is essential to distinguish the amount of CGA present in different forms of coffee. Unfortunately, there are no simple and cost effective methods for the detection of CGA. Usually, HPLC, GC and capillary electrophoresis are used to assess CGA. In this context, two simple detection procedures for CGA in different forms of coffee samples were trying to establish.

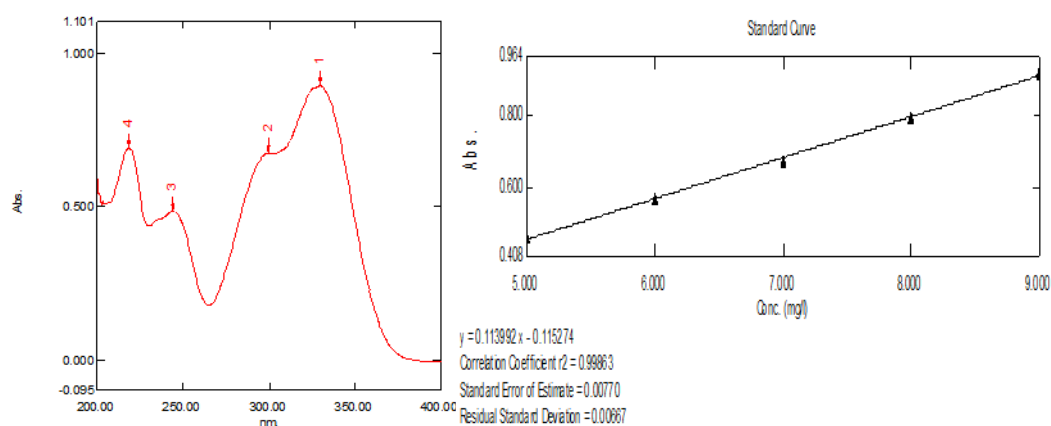


Figure 2 UV-VIS spectrum of CGA standard **Figure 3** UV-VIS standard curve of CGA

The UV-Vis spectrums of scanning of all four test samples are presented in Fig. 4. The content of CGA in test samples were derived from the standard curve, which is 5.3% in crud green coffee seed, 2.33% in roasted coffee seed, 31.16% in Nescafe and 20.53% in BRU coffee (Table 2).

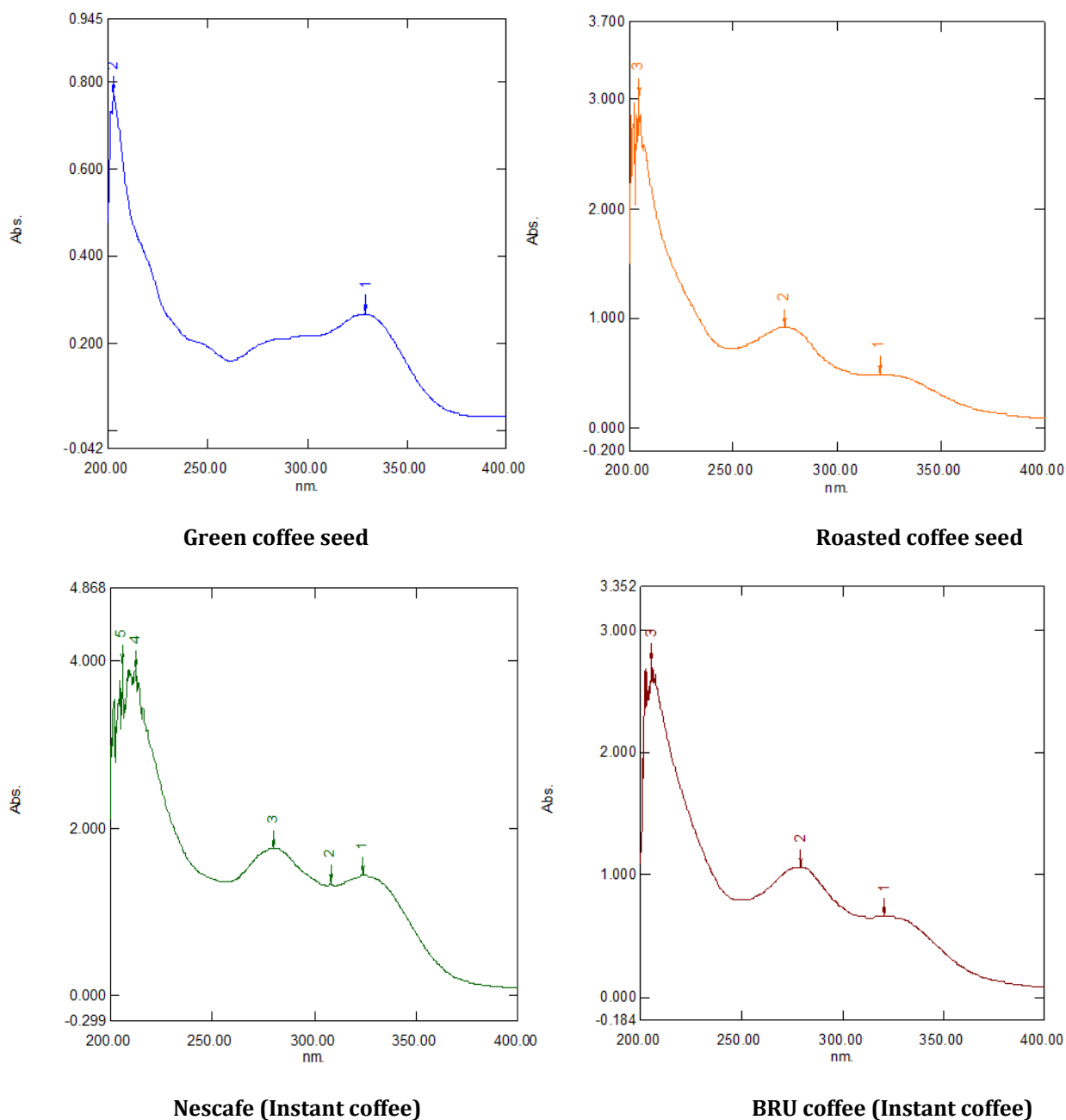


Figure 4 UV-Vis spectrum of coffee samples

The practice of HPTLC is well acceptable all over the world as it remains most flexible, reliable, reproducible and cost-effective separation technique for the analysis of food, botanicals and herbal drugs [16, 24].

In the present study, the HPTLC technique was optimized with a view to develop a stability indicating assay method [9]. The solvent system of the mobile phase having ethyl acetate: dichloromethane: formic acid: acetic acid: water (10:2.5:1:1:1.1, v/v) gave dense, compact and well separated spots of coffee samples at 330 nm. The limit detection for CGA and the limit of quantification was found to be 20ng/ml and 800µg/ml respectively. The R_f value of CGA was noted 0.35 (Fig. 5). The calibration curve of CGA was linear over the concentration range 100-600 ng per spot and regression was $r = 0.98858$ (Fig. 6).

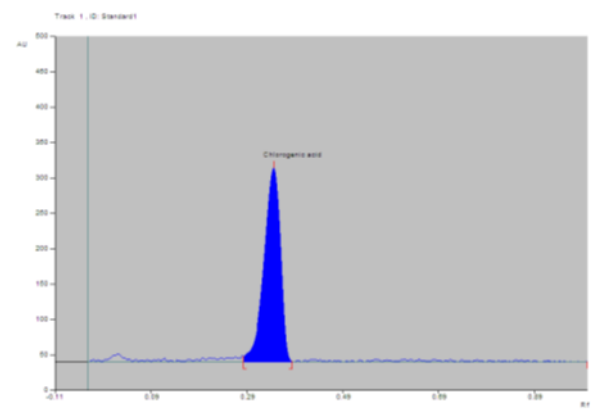


Figure 5 HPTLC chromatogram of CGA

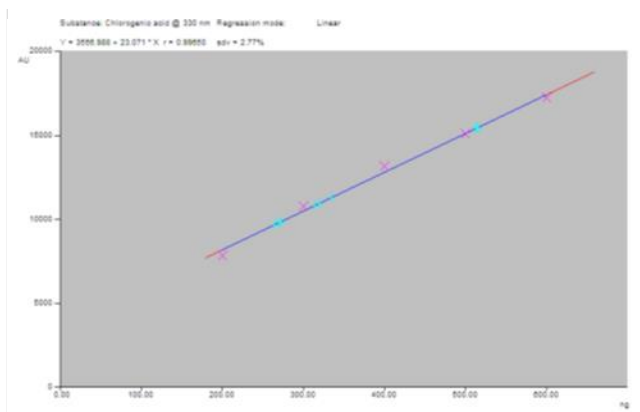


Figure 6 HPTLC standard curve of CGA

The presence of CGA in the HPTLC chromatograms of all test coffee samples was matched with known standard of CGA at R_f 0.35 when scan at 330 nm (Fig. 7-10). The HPTLC chromatogram of instant Nescafe represented well separated and prominent 11 characteristic bands, while instant BRU coffee showed only 8 less prominent bands, which clearly point out the nature of compounds other than CGA, are contrary in instant coffee samples. On the hand, nature of the compounds present in the HPTLC fingerprint of green coffee seeds was changed after roasting the coffee seeds that may be due to breakdown of CGA into derivatives or due to loss of moisture present on seeds. Table 2, represented the amount of CGA in different form of coffee samples. The ranges of CGA presents in different form of coffee samples were vary from 22.41 mg/g to 358.35 mg/g. The maximum amount of CGA was present in instant Nescafe (35.8%) followed by instant BRU coffee (22.9%), green coffee seed (6.45%) and roasted coffee seeds (2.24%).

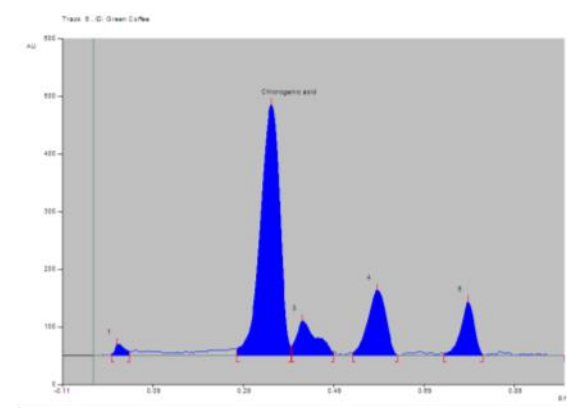


Figure 7 CGA in Green Coffee Seed

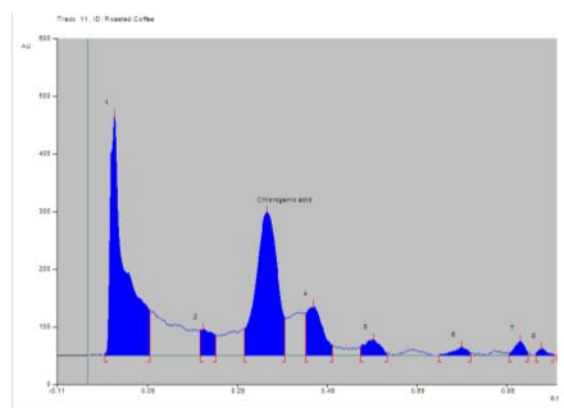


Figure 8 CGA in Roasted coffee seed

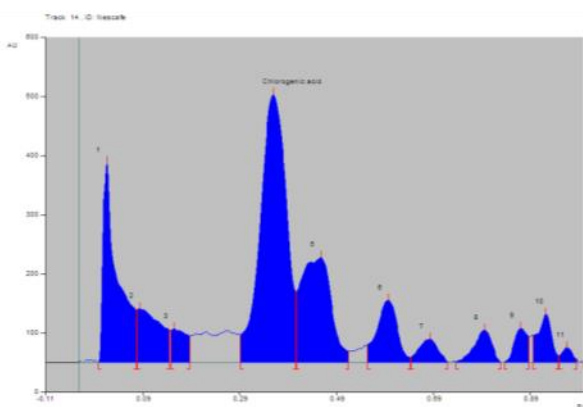


Figure 9 CGA in Nescafe (Instant coffee)

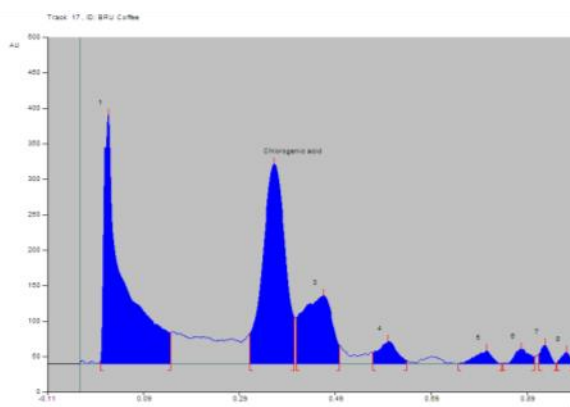


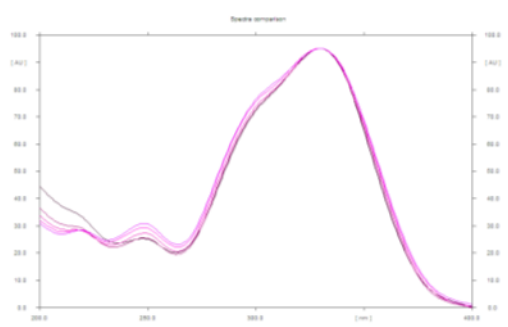
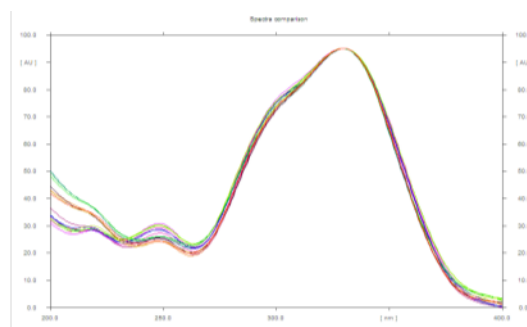
Figure 10 CGA in BRU Coffee (Instant coffee)

Table 2 Estimation of CGA by UV-Vis Spectroscopy and HPTLC.

	Chlorogenic acid (CGA)	
	UV-Vis Spectroscopy	HPTLC
Green coffee seed	53.08±1.04	64.58±0.17
Roasted coffee seed	23.29±1.01	22.41±0.32
Nescafe (Instant coffee)	311.62±3.26	358.35±3.61
BRU coffee (Instant coffee)	205.33±2.24	229.14±3.45

N=6 in each test; mean ± standard deviation; unit = mg/g

Furthermore, comparisons between two separate analytical methods *i.e.*, UV-Vis- spectral analysis and HPTLC clearly revealed that HPTLC method is more sensitive and reliable to detect CGA in coffee samples than other one.

**Figure 11** HPTLC spectrum of CGA standard**Figure 12** Integrated spectrum of CGA standard and test samples

Whilst reliability for detection of CGA by presently developed HPTLC method was confirm with the integrated spectra of all concentrations of standard CGA and different coffee samples (Fig 11-12). Thus, the method was found to be accurate, precise, suitable and cost effective for the estimation of chlorogenic acid (CGA) in any form of coffee samples.

4. Conclusion

This HPTLC method can be successfully employed for standardization and quantitative analysis of chlorogenic acid (CGA) in any forms of coffee as well as foods, herbs, phytochemicals and pharmaceuticals containing CGA.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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