RESEARCH NOTE
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A Simple HPLC Method for Determination of Caffeine Content in Tea and Coffee

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A simple HPLC-UV method was developed and validated for the determination of Caffeine content in tea and coffee samples of different local brands commercially available at Kathmandu and Kaski, Nepal. Water extracted caffeine was separated through c-18 column (ODS 5 µm, internal diameter 4.6 nm and length 150 mm) using methanol and water (40:60) as a mobile phase. The peak response time for caffeine was observed at 2.66 minutes using UV detector set at 275 nm. Method validation parameters viz. linearity, sensitivity (LOD & LOQ), repeatability and recovery were assessed for the detection of caffeine. Linear dynamic range was 2-10µg/ml with correlation coefficient (R^2) of 0.9928. The LOQ and LOD were found to be 0.7 and 0.2µg/ml respectively. Relative standard deviation for retention time for intraday repeatability was 0.9 and for inter-day 1.63%. Recovery was measured by spiking blank tea samples and the results of recovery were greater than 97%. The validated method was finally adapted to determinate caffeine content in Nepalese tea and coffee samples. The average caffeine contents in tea and coffee were found in the range of (2.5-3.53) % and (1.17-1.34) % on dry basis respectively.

Keywords: Extraction, Caffeine, HPLC, Method validation.

Introduction

Caffeine (1,3,7-trimethylxanthine) a purine alkaloid is the principle stimulating constituent in 60 plant species including tea, coffee, cocoa and so on (Francis, 1999). Besides tea and coffee, caffeine is also very widely consumed through a wide range of dietary products, like cocoa beverages, energy drinks, soft drinks etc. (Da Silva, 2011). Caffeine is a white, odorless powder with a slightly bitter taste. As a derivative of xanthine nucleus, caffeine has pharmacological property (Francis, 1999). It is a central nervous system and metabolic stimulant, and is used both recreationally and medically to reduce physical fatigue and to restore alertness when drowsiness occurs. It produces increased wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination (Sethuraman *et al.*, 2013). Caffeine is regarded as GRAS upto a level of 200 ppm (Da Silva, 2011). Caffeine does not accumulate in the body over the course of time and is normally excreted within several hours of consumption (Barone and Roberts, 1996). The average national tea consumption rate has been anticipated to be 3.5 g per person per day in Nepal (Rijal, 2014).

Variations in caffeine content in specific plant species result from varietal diversity, climatic changes in the growing areas, and horticultural techniques. In tea, youngest leaf has the highest concentration. Processing conditions also affect caffeine content (Hecimovic *et al*., 2011). High coffee roasting temperatures result in loss of caffeine in smaller amounts by sublimation (Ayelign and Sabally, 2013). There is a higher level of caffeine in tea than in coffee beans, but 200 cups of tea beverage are obtained per pound of tea leaves, whereas only about 40-60 cups of coffee are usually prepared per pound of coffee beans. Caffein content in coffee arabica, coffee robusta and instant coffee ranges from (0.58-1.7), (1.2-3.3), and (2.2-5.0) respectively, while in black, gree and instant tea its level ranges from (1.2-4.6), (1.0-2.4), (4.0-5.0) repsectively (Francis, 1999). The caffeine content in Nepalese tea and coffee shouldn't be less than 2 and 1 % on dry basis according to the mandatory standards set by Food Law, 1967 (DFTQC, 2012). This study was done with the objective of developing a validated method for routine analysis of caffein content in tea and coffee products available in Nepal.

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Materials and Methods

Sample collection

A total of 12 CTC samples, 7 green tea, 8 medium roasted coffee bean and 3 roasted coffee bean powder samples were collected from Kathmandu and Kaski district, Nepal. The coffee and tea samples were kept at room temperature throughout the analysis. All the glassware were properly cleaned, then rinsed with HPLC water before use. The chemicals and reagents used in this study were of analytical grade while the standard caffeine (Sigma-Aldrich) used in this study was traceable to National Institute of Science and Technology, US.

Moisture content determination

The moisture content of the tea and coffee samples was determined according to AOAC, 2005.

Sample preparation for caffeine determination

Caffeine was extraced in water according to Nhan and Phu (2012) with some modifications. Tea and coffee samples were first grounded to powder of 300±50 µ and about 0.3 g of grounded tea and coffee samples were weighed in 250 ml conical flasks. Then 200 ml of distilled water was added and placed over water bath (100^{o}C). Extraction was done for for half hour. Then the solution was cooled, volume maintained to 250 ml and filtered through Whatmann number 1 filter paper. 1 ml of the filtrate was pipetted into clean 10 ml volumetric flasks and made to the mark with HPLC water. Thus prepared sample was then filtered through microfilter (0.2µm) and filled into HPLC vials for analysis.

Preparation of stock and working solution

Caffeine stock solution of 100 ppm was prepared by accurately weighing 10 mg of pure caffeine (Sigma-Aldrich) and quantitatively transferring it into 100 ml volumetric flask and making it to the mark with the mobile phase. Working standards of 2, 4, 6, 8 and 10 ppm were prepared by serial dilution of the stock solution with hplc grade water. An external calibration curve of peak areas versus concentration of the standards was plotted . The caffeine content (ppm) of the various samples was calculated by interplotation within the regression equation of the best line of fit. After that the results were presented in percentage on dry basis.

Instrumentation

The following were the HPLC conditions;

- HPLC model 1514 (Simadzu Corporation)
- Zorbax Eclipse Plus 18 C Column, pore size 5µ, internal diameter 4.6nm and length 150 mm.
- Reverse phase – ODS
- Flow rate, 1 ml/min (constant),
- Column temperature at 40^{o}C
- UV detector set at 275 nm
- Mobile phase: Water: Methanol (60:40) both are HPLC grade
- Sample injection volume: 10 µl

Statistical analysis

Where applicable, results were expressed as Mean ± SD and analyzed statistically by using MS Excel 2007. For relevant calculations instrumental software Labsolutions.

Method Validation

As recommended by ICH, (2005), the validation characteristics considered in this study were linearity, range, limit of detection (LOQ), limit of quantification (LOD), repeatability and recovery. Five different standards caffeine solution from 2-10 ppm were taken to evaluate the plot of signal as a function of analyte concentration. For precision, the intraday and interday repeatability were performed taking 10 ppm standard solution for 6 determinations. The LOQ and LOD were determined by noting the signal to noise ratio comparing measured signals from samples with known concentrations. A signal to noise ratio between 3:1 and 10:1 was considered for LOD and LOQ. Recovery was tested by adding blank samples (decaffeinated tea) with different caffeine standard concentration and analyzing their content.

Results and Discussion

Before the validation parameters were conducted, identification test was conducted to discriminate between caffeine and compounds of closely related structures which are likely to be present. The discrimination procedure was confirmed by obtaining a positive result from tea & coffee products, blank and from standards. No significant background noise or peak interfered or coincided with the peak for caffeine.

Linearity and range

Five different concentrations of standard caffeine solution ranging from from 2 to 10 ppm were analyzed, which would fairly represent the available tea and coffee products. The calibration graph was generated using 10µl injection loop and the curve was established according to the response (peak area) and the concentration of caffeine in standard solutions. The results obtained showed a linear relationship. Each standard concentration response was the average of three determinations. The calibration graph (Figure 1)

shows a strong positive correlation between the instrumental signal and the concentration of the caffeine standards. The linearity studies showed that caffeine content was found to be linear in the concentration range of 2 to 10 ug/ml. The R^2 value was 0.9928.

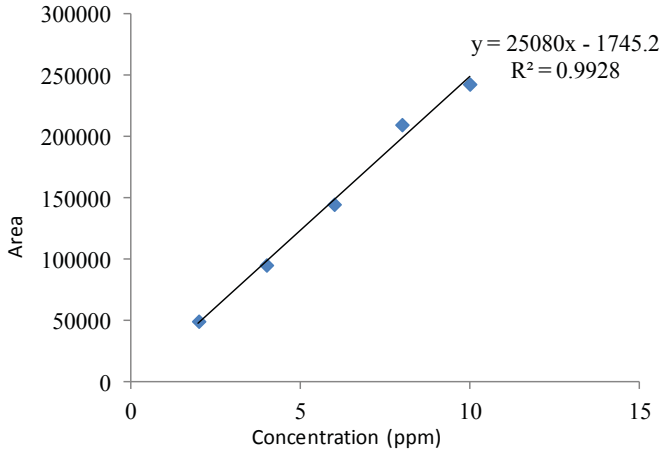

Figure 1. Calibration curve

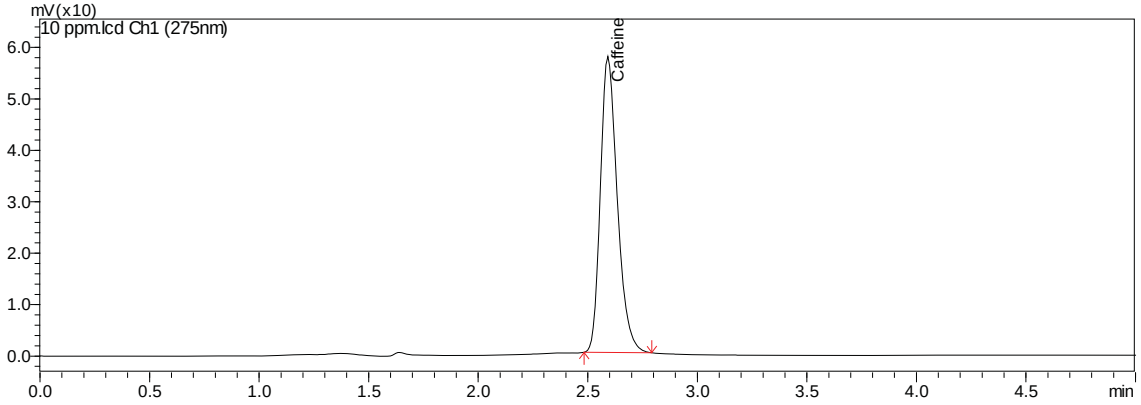

Figure 2. Chromatogram of caffeine at (2 ppm, Detector 275nm)

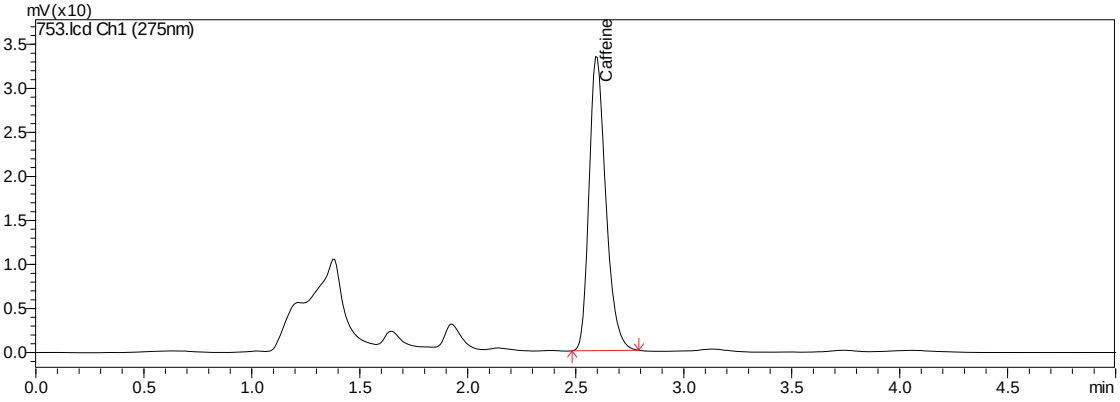

Figure 3. Chromatogram of caffeine in Tea Sample

Repeatability

The repeatability of the method was investigated by performing 6 repeated analysis of 1 standard solution (10 ppm) on the same day (for intra-day repeatability) and different day for inter day precision. The results showed that the % RSD for retention time and area were satisfactory for further analysis. The results of repeatability are shown in Table 1 and 2.

Table 1. Intra-day repeatability for 10 ppm

Number	Concentration of standard Sample (ppm)	Retention time (min)	Area
1	10	2.662	235470
2	10	2.651	233380
3	10	2.671	245310
4	10	2.607	240120
5	10	2.635	247110
6	10	2.664	234150
	Average	2.648	235040
	Std. Dev.	0.023	2686.217
	% RSD	0.903	1.1428

Table 2. Inter day repeatability for 10 ppm

Number	Standard sample concentration	Retention time	Area
1	10	2.662	234470
2	10	2.66	233410
3	10	2.71	242101
4	10	2.681	238350
5	10	2.659	240190
6	10	2.771	235130
	Average	2.690	237275.2
	Std. Dev.	0.044	3474.202
	% RSD	1.635	1.464208

Sensitivity

Sensitivity of the method was measured in terms of Limit of detection (LOD) and limit of Quantification (LOQ). The LOD and LOQ were determined by measuring the signal to noise ratio from standard low concentration of analyte comparing with baseline peak of blank sample. The instrumental software (Simatzu Lab Solutions Version 5.60SP2) detected signal to noise ratio 3:1 for the standard concentration of analyte caffeine at 0.2 ppm which was out limit of detection for this method. Similarly, LOQ was found to be 0.7 ppm at which the instrumental software assessed the signal to noise ratio of 10:1.

Recovery

The accuracy for the method was determined by spiking the blank samples (decaffeinated tea) at standard concentrations (1, 5 and 10 ppm) and analyzing their recoveries.

Table 3. Recoveries of caffeine

Blank No.	Concentration in the blank (mg)	Amount of spiked caffeine (mg)	Amount of caffeine found (mg)	Recovery %
1	0	1.27	1.24	97.6
2	0	2.52	2.49	98.8
3	0	3.81	3.75	98.4

Results of caffeine content in different tea and coffee samples

The caffeine content in tea and coffee samples were analyzed and converted into % dry basis (Table 5). The caffeine content in tea and coffee was not be less than 2 and 1% on dry basis respectively and this result was in consonance with the mandatory standards given by Nepal Government (DFTQC, 2012).

Table 5. Caffeine content (% dry basis) of tea and coffee samples

Brand no.	CTC Tea	roasted coffee beans	coffee powder	Green Tea
1	2.49±0.14	1.33±0.03	1.1±0.02	4.3±0.21
2	2.74±0.49	1.42±0.01	1.14±0.02	3.35±0.14
3	2.74±0.17	1.29±0.02	1.35±0.02	4.2±0.07
4	2.59±0.24	1.52±0.07	-	4.19±0.07
5	3.03±0.18	1.1±.11	-	2.57±0.16
6	3.12±0.24	1.23±0.07	-	2.34±0.05
7	2.99±.26	1.32±0.0	-	3.1±0.01
8	2.1±0.1	1.19±0.02	-	-
9	2.29±0.23	-	-	-
10	2.15±0.23	-	-	-
11	2.88±0.28	-	-	-
12	3.4±0.04	-	-	-

Note: Values are the mean ± standard deviation

Conclusion

Caffeine was analyzed in water extracts of tea and coffee samples. The retention time for caffeine was found to be 2.6 minutes. The different standard solutions taken for linearity showed a linear range with correlation coefficient (R^2) value of 0.9928. RSD for repeatability was satisfactory. The LOD and LOQ were found to be 0.7 and 0.2 ppm respectively. High recovery (97%) was found upon spiking of standard caffeine in blank samples. No significant matrix effect observed in the process of validation.

Thus validated method used for the quantification of caffeine in tea and coffee product samples collected from different places. Liquid chromatography permits a fast easy separation of caffeine from other substances such as tannic acid, caffeic acid and sugar. This method was recommended to the Central Food Laboratory, DFTQC for routine analysis of caffeine in tea and coffee.

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