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Rapid Differentiation of Tea through High - Performance Liquid Chromatography and Chemometric Analysis

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Abstract: ***A simple and fast high performance liquid chromatographic method for caffeine using an ODS column and a 50: 50 methanol: water mobile phase system was developed. Caffeine were separated by an methanol gradient within 30 min. The detection limit of the method was approximately 10 mg for all the compounds (by injecting 10 microL). Several green, black and instant teas were analysed using this method. By using the studied compounds as chemical descriptors, linear discriminant analysis was performed and complete differentiation of the green, black and instant teas was achieved.***

Keywords: chromatographic method, caffeine, ODS column, methanol - water mobile phase, separation, detection limit, tea analysis, chemometric analysis

1. Introduction

Caffeine (1, 3, 7 - trimethylxanthine) a purine alkaloid is the principle stimulating constituent in 60 plant species including tea, coffee, cocoa 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. 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 fl ow of thought, increased focus, and better general body coordination. 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 2.9 g per person per day in odisha). Variations in caffeine content in specifi c 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. High coffee roasting temperatures result in loss of caffeine in smaller amounts by sublimation. There is a higher level of caffeine in tea than in coffee beans, but 250 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.

2. Methodology

Procedure:

1.1 Equipment and glass ware

5.1.1 HPLC with UV Detector
5.1.2 C18 analytical column– 250mm X 4.6mm, 5µm
5.1.3 Syringe Filters –0.45 µm filters
5.1.4 Sonicator
5.1.5 Centrifuge
5.1.6 Balance
5.1.7 Measuring cylinder 1000 ml
5.1.8 Micro pipette
5.1.9 Ria vial
5.1.10 Vortex
5.1.11 Mobile phase Filtration Assembly

Reagents:

1.1.1 Methanol, HPLC grade
1.1.2 Caffeine Reference standard
1.1.3 Milli - Q - water

Mobile phase Preparation:

1.1.4 **Methanol: Water (50: 50 v/v):** Transfer 500 ml of water and 500 ml of Methanol into a 1000 ml volumetric flask. Mix and then Sonicate for 10 minutes.

1.2 Working Standard Solution:

1.2.1 Accurately weigh 10 ± 0.1 mg of Caffeine standard and transfer to 10 ml of volumetric flask. Mix well to dissolve completely with mobile phase, mark this flask as Standard Stock solution 1000 ppm. Prepare following dilutions ranging from 1 µg/ml to 50 µg/ml.

Stock Solution (µg/mL)	Volume taken from (mL)	Final volume (mL)	Final conc (µg/mL)
10	1	10	1
50	1	10	5
100	1	10	10
100	2.5	10	25
1000	0.5	10	50

1.3 HPLC conditions:

1.3.1 Use the following HPLC conditions as a guide:
1.3.1.1 Mobile Phase: Methanol: Water (50: 50)
1.3.1.2 Column: C18–Analytical Column 250mm X 4.6mm, 5µm
1.3.1.3 Column Temperature: 25°C
1.3.1.4 Flow rate: 1.0 mL/min
1.3.1.5 Detector Wavelength: 272nm
1.3.1.6 Injection Volume: 20µL

1.4 Sample preparation:

1.4.1 Mix well by shaking the sample to homogenize.
1.4.2 Take 0.5g of Sample into a 25ml volumetric flask.
1.4.3 Make up the volume with Mobile Phase i. e. Methanol: Water (50: 50)
1.4.4 Shake properly for 2 minute.
1.4.5 Transfer this solution to a 50ml centrifuge tube.
1.4.6 Centrifuge at 5500RPM for 5 minute to obtain a clear separation.
1.4.7 Filter through a 0.45µm filter and inject the sample in the HPLC system.
1.4.8 Prepare a Reagent Blank same as sample without sample.
1.4.9 Put the sample and standard in auto injection tray and analyze.

1.5 Sample Analysis and Calculations:

Calculate the concentrations of Caffeine as follows:

$$\text{Caffeine (mg/kg)} = \frac{\text{Area of Sample X Volume X DF X Standard conc X Purity}}{\text{Area of Standard X Sample Weight X 100}}$$

OR

$$\text{Caffeine (mg/kg)} = \frac{\text{Instrument Reading X Volume}}{\text{Sample Weight}}$$

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.

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.

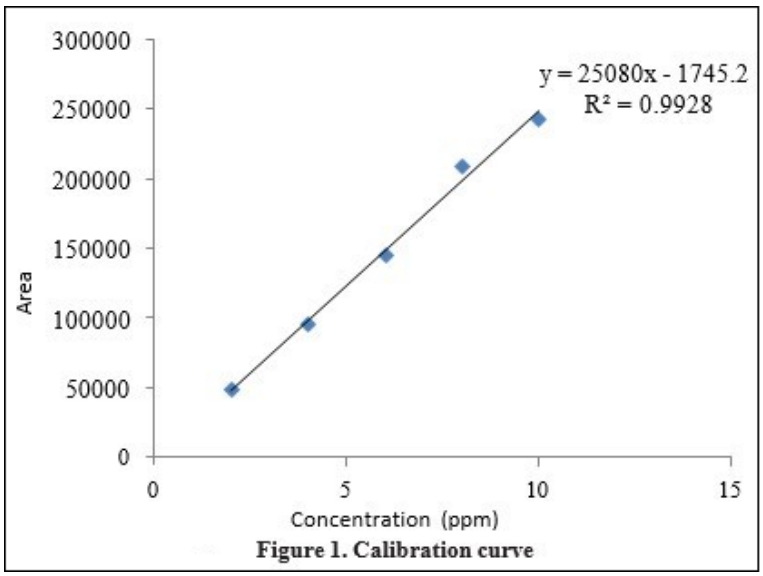
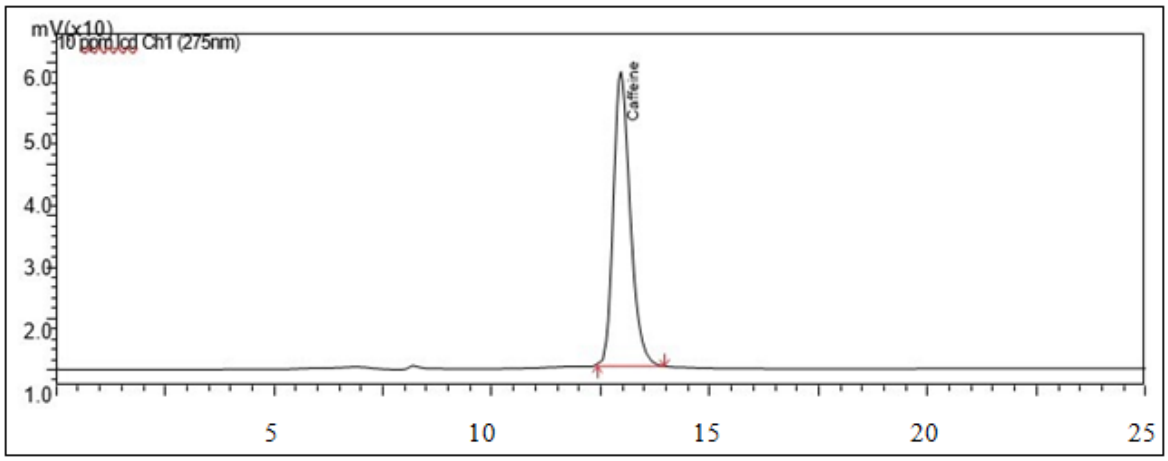


Figure 1. Calibration curve



Chromatogram of caffeine in Tea Sample

Intra - day repeatability for 10 ppm

Table 2: Inter day repeatability for 10 ppm

Concentration of standard		Retention time	
Number	Sample (ppm)	(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

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

3. Conclusion

Caffeine was analyzed in water extracts of tea and coffee samples. The retention time for caffeine was found to be 22 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. .

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