INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

Research Article

A COMPARATIVE STUDY ON CAFFEINE ESTIMATION IN COFFEE SAMPLES BY DIFFERENT METHODS

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Abstract

Caffeine content in test sample is determined by various analytical methods and its level is reported based on the proficiency of the method adopted. The international organization for standardization (ISO) has recommended two methods for the determination of caffeine content in coffee samples. The ISO 4052 determines caffeine content through UV spectrophotometry and ISO 20481 estimates caffeine content through high performance liquid chromatography (HPLC). In the present study, caffeine content in twenty two coffee samples was determined following both the ISO methods to assess the overall proficiency of these two methods. The results indicated that the caffeine level obtained by the ISO 20481 (HPLC method) was comparatively higher than those obtained by the ISO 4052 (UV spectrophotometry) in all the twenty two coffee samples. The overall deviation of caffeine level obtained by ISO 20481 (HPLC method) over the ISO 4052 (UV spectrophotometry) ranged from 0.07 to 0.71% on dry matter basis. However, statistical analysis of data (t-Test) indicated that there were no significant differences between the methods. Nonetheless, ISO 20481 (HPLC method) is seems to be simple, rapid and realistic in estimating caffeine content in coffee samples.
Keywords: Coffee, Caffeine, UV spectrophotometry, HPLC

Introduction

The stimulant effect of coffee makes it one of the most popular beverages in the world is due to the presence of caffeine (1, 3, 7- Trimethylxanthine).Although more than eighty coffee species have been identified worldwide, only two coffee species viz. *Coffee arabica* (known as arabica coffee) and *Coffea canephora* (popularly namedas robusta coffee) are economically important. Arabica and robusta coffees are different in their chemical composition including their caffeine level. Similarly, the caffeine level varies in filter and instant (soluble) coffee samples.Many analytical methods have been developed for the quality control of products containing caffeine: liquid chromatography-particle beam/electron ionization mass spectrophotometry (Castro et al., 2010), liquid chromatography- tandem mass spectrophotometry (Choi et al., 2013), near infra-red spectroscopy (Zang et al., 2013), near infra-red reflectance spectroscopy (Fox et al., 2013), gas chromatography (Sereshti and Samadi, 2014) and

fluorescence polarization immunoassays (Oberleitner et al., 2014).

The caffeine level in coffee samples is determined by various analytical methods. The International Organization for Standardization (ISO)has recommended two analytical methods viz., ISO 4052 (1983) and ISO 20481 (2008) for the determination of caffeine in a wide range of coffee samples. Quantitative analysis of caffeine in large of samples needs an accurate and rapid method. The complete extraction of caffeine from testsamples and removal of interfering substances varies from method to method. Therefore, choosing the right analytical method is essential for the accurate estimation of caffeine in test samples. There have been numerous reports on the estimation of caffeine in coffee samples following different analytical methods: high performance liquid chromatography (Silvarolla et al., 2000 and Wanyika et al., 2010),

gaschromatography (Sereshti and Samadi, 2014, Mc Cusker et al., 2003 and Mc Cusker et al., 2006) and spectrophotometry (Alpdogan et al., 2002). However, there are only limited reports available on the comparative study of caffeine estimation by different analytical methods for a given set of samples (Wanyika et al., 2010 and Alpdogan et al., 2002). To this end, a study was taken up to assess the overall proficiency of methods recommended by the ISO for the determination of caffeine content in coffee samples.

Experimental

Chemicals and caffeine stock preparation

All chemicals and solvents used were HPLC and analytical reagent grade unless otherwise indicated. Double distilled water was used throughout the experiment and double distilled water was further purified by Milli-Q system for HPLC instrumental analysis. Caffeine stock solution was prepared by dissolving appropriate quantity of caffeine either in warm water (HPLC method) or in chloroform (UV spectrophotometric method). Working standards of caffeine was prepared by from these stock solutions by diluting appropriately. Caffeine stock solution and working standards were prepared as and when required.

Sample details and preparation

Twenty two coffee samples comprising of eight unroasted coffee bean (6 arabica &2 robusta coffees),six roasted coffee powder, five instant (soluble)coffees and three decaffeinated coffee bean were chosen for this study. The unroasted and decaffeinated coffee bean samples were received from the coffee traders. The roasted coffee powder and instant (soluble) coffees were procured from the local super market during March 2014 and the details of the samples were shown in Table 1.

The unroasted and decaffeinated coffee bean samples were milled using a sample mill (Romer analytical sample mill, Singapore).The powdered sample was sieved over a 500 micron sieve plate. The powdered sample passed through 500 micron sieve was well mixed and required quantity of sample was taken for caffeine analysis. Other coffee types viz., roasted coffee powder and instant (soluble) coffees were also sieved and then used for caffeine analysis. The caffeine content of the sample was determined by following ISO 4052 (1983) and ISO 20481(2008) methods.

Analytical methods

Briefly, in ISO 4052 (1983) a known quantity of the sample was digested with 4N ammonium hydroxide for 2 minutes over a water bath set at 100^0 C. The digested sample was diluted to 100 ml using double distilled water and from this a known quantity of sample extract was loaded on to a glass chromatography column containing Celite 545 grade. The column was washed with 150 ml of diethyl ether (3 x 50 ml) followed by elution of caffeine from the Celite with 50 ml of chloroform. The maximum absorbance of the caffeine in chloroform solution was measured against the chloroform as blank using a Spectrophotometer (Secomam make UV–Visible Spectrophotometer, Germany). The caffeine concentration in test solution was calculated against reference caffeine solution at a concentration of 10ppm (10mg/L).

In ISO 20481(2008) method, a known quantity of coffee sample along with 5 gram of magnesium oxide was transferred to a 250ml capacity volumetric flask containing 200 ml of double distilled water. The sample mixture was incubated at 90^0 C for 20 minutes over a water bath and then made up to the mark with double distilled water. After cooling, the supernatant solution was passed through 0.2 micron nylon membrane filter under vacuum. Twenty microliter of filtrate was injected into HPLC instrument (Waters make, USA) equipped with a reverse phase C 18 column and UV detector tuned at 272 nm. Aqueous methanol (24%) was used as mobile phase to resolve the caffeine peak under isocratic elution mode. The caffeine level in test sample was calculated against a calibration curve prepared over a range of caffeine concentration ranging from 1 to 40 ppm. Linearity of the calibration curve was good as shown by the correlation coefficient (r^2) value of 0.993. Caffeine level in test sample was expressed as percentage of caffeine by mass (g/100g) on dry basis.

The moisture percentage in unroasted coffee bean, roasted coffee powder and instant (soluble) coffee was determined followingISO6673 (2003), IS 3077(1992a) and IS 2791 (1992b) respectively and expressed as %of moisture by mass. All the analytical results were expressed as mean ± SD of three parallel replications.

Results

The data on caffeine level in twenty two coffee samples obtained by ISO 20481 (2008) and ISO 4052 (1983) were presented in Table 1. Perusal of data indicated that caffeine level in unroasted arabica coffee bean samples ranged from 0.89 to 1.19% and from 1.01 to 1.46% as recorded by UV spectrophotometry and HPLC methods respectively. Similarly, the UV spectrophotometry and HPLC methods quantitated the caffeine level in the range of 1.95 to 2.1% and from 2.22 to 2.37% respectively in the unroasted robusta coffee bean samples. The caffeine level in decaffeinated coffee bean

Table 1. Comparison of caffeine content as determined by ultra violet spectrophotometric and high performance liquid chromatographic methods in coffee samples

Sample details	Caffeine content (%) - dry basis	
	UV Spectrophoto-metric method	HPLC method
A. Unroasted coffee bean samples		
Arabica coffee bean - A	0.89 ± 0.04	1.01 ± 0.10
Arabica coffee bean - B	0.93 ± 0.01	1.06 ± 0.08
Arabica coffee bean - C	1.12 ± 0.01	1.46 ± 0.00
Arabica coffee bean - D	1.19 ± 0.21	1.29 ± 0.08
Arabica coffee bean - E	0.99 ± 0.06	1.21 ± 0.01
Arabica coffee bean - F	1.12 ± 0.02	1.27 ± 0.05
Robusta coffee bean – G	2.10 ± 0.06	2.37 ± 0.21
Robusta coffee bean – H	1.95 ± 0.10	2.22 ± 0.01
B. Roasted coffee samples		
Pure soluble coffee(Narasus coffee)	4.21 ± 0.02	4.75 ± 0.05
Pure soluble coffee(BruGold coffee)	3.82 ± 0.04	4.36 ± 0.09
Pure soluble coffee(Nescafe coffee)	3.89 ± 0.01	4.27 ± 0.05
Soluble coffee: chicory(Bru coffee)	2.09 ± 0.03	2.70 ± 0.01
Soluble coffee: chicory(Bru coffee)	2.19 ± 0.20	2.90 ± 0.02
Pure roasted coffee powder (Coorg coffee)	1.54 ± 0.01	2.06 ± 0.03
Roasted coffee powder: chicory - Cothas coffee (70:30)	1.03 ± 0.01	1.45 ± 0.01
Roasted coffee powder &chicory - Leo coffee(70:30)	1.48 ± 0.08	1.94 ± 0.04
Roasted coffee powder &chicory – Alive coffee (70:30)	1.26 ± 0.01	1.72 ± 0.06
Roasted coffee powder &chicory – Leo coffee (60:40)	1.12 ± 0.03	1.42 ± 0.04
Roasted coffee powder &chicory –Mr. Bean coffee (53:47)	1.11 ± 0.07	1.40 ± 0.01
C. Decaffeinated coffee bean sample		
Sample – A	0.38 ± 0.03	0.45 ± 0.01
Sample - B	0.43 ± 0.03	0.50 ± 0.01
Sample - C	0.34 ± 0.03	0.41 ± 0.01

Statistical Analysis

Table 2 a. Statistical analysis (t-Test) of data on caffeine content determined by the UV spectrometry and HPLC method in eight unroasted coffee bean samples

	Variable 1	Variable 2
Mean	1.28625	1.48625
Variance	0.219855357	0.270026786
Observations	8	8
Pooled variance hypothesized mean difference	0	
df	14	
t stat	- 0.80821924	
P (T<=t) one tail	0.216242032	
t critical one tail	1.761310136	
P (T< =t) two tail	0.432484064	
t critical two tail	2.144786688	

Table 2 b. Statistical analysis (t-Test) of data on caffeine content determined by the UV spectrometry and HPLC method in eleven roasted coffee bean samples

	Variable 1	Variable 2
Mean	2.16	2.63554545
Variance	1.51	1.320487273
Observations	11	11
Pooled variance hypothesized mean difference	0	
df	20	
t stat	- 0.9	
P (T<=t) one tail	0.19	
t critical one tail	1.72	
P (T< =t) two tail	0.38	
t critical two tail	2.09	

and roasted coffee powder ranged from 0.34 to 0.43% &1.03 to 4.21% and 0.41 to 0.50% &1.4 to 4.75%as estimated by the UV spectrophotometry and HPLC methods respectively. In all the coffee samples tested under this study, the caffeine level obtained by HPLC method was comparatively higher than those obtained by UV spectrophotometry. The differences in the mean caffeine values obtained by HPLC versus the UV spectrophotometry methods ranged from 0.55% (in case of soluble coffees) to 0.2% (in case of green coffee beans).

Discussion

In the present study, the caffeine level recorded in arabica (0.89 - 1.46%) and robusta (1.95 - 2.37%) coffee bean samples was in line with the previously reported caffeine levels of 1 to 1.5% in Arabica and 2 to 3% in robusta coffee by Wilson (1999). Similarly, the caffeine level recorded in roasted coffee powder (1.03 to 2.06%), decaffeinated coffee bean samples (0.34 to 0.5%) and instant coffee powder (2.09 to 4.75 %) was also in good agreement with earlier findings by Smith (1985), Fujioka and Shibamoto (2008) and Smith(1985), respectively. Macrae (1985) reported that the solubility of caffeine in water increases rapidly with the temperature (19.23 gram caffeine/100 gram water at 80^0C versus 4.64g/100gram water at 40^0C). The HPLC method followed in the present study wherein the coffee samples are subjected to 90^0C for 20 minutes aiding virtual removal of all the caffeine molecules from the samples. Besides, in HPLC method, addition of magnesium oxide (MgO) facilitates the complete removal of interfering compounds and thus enhancing the chromatographic signal of caffeine resulting in the more accurate estimation of caffeine content.

Though there were apparent differences of caffeine values obtained between the methods, statistical analysis indicated that there were no significant differences between these two methods as the table "t" values were higher than the calculated "t" values in both unroasted and roasted coffee samples (Table 2 a,b). Similar to the present study, Alpdogan et al., (2002) estimated caffeine content in six samples comprising of cola, coffee and tea following derivative spectrophotometric method and compared it with HPLC method. They concluded that there was no significant difference between the mean caffeine values obtained by the two methods. On contrary, Wanyika et al., (2010) compared caffeine level in a variety of beverages collected from Kenyan market including coffee drink and reported that spectrophotometric method yielded higher caffeine value than HPLC method. A close examination of the analytical scheme followed in their study indicated that a mixture of hydrochloric and sulphuric acids was utilized for extracting caffeine from coffee samples. Nevertheless, they have concluded that estimating caffeine content by HPLC method is more realistic, as human consumption of coffee utilizes only hot water while brewing the coffee.

Conclusion

The amount of caffeine enters to the human system through drinking of coffee is prepared byhot wateras the extracting medium. Hence, it is always desirable to adopt a methodology in the laboratory which copycats the actual conditions prevailing across the consumer circle to assess the actual intake of caffeine through coffee. The HPLC method followed in the present study uses hot water for the extraction of caffeine from the coffee samples versus the use of strong ammonia solution in case of ultra violet spectrophotometric method. The ultra violet spectrophotometry method is

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lengthy and also excessively time consuming. Besides, it demands health deterring solvents like chloroform & diethyl ether which are not desirable in the milieu of environmental and personnel safety. Considering the above points, the HPLC method is seems to be simple, rapid and more realistic in estimating caffeine content in coffee samples.

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