Determination of Caffeine in Tea Products by an Improved High-Performance Liquid Chromatography Method

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At present, the commonly used HPLC method for the analysis of caffeine content in tea brews employs direct application of the samples on the column. This practice gradually reduces the efficiency of the column and shortens its life. In the modified method, the interfering tea pigments are effectively removed by passing the sample through a Sep-Pak C_{18} cartridge. Then its injection on a reversed phase μ-Bondapak C_{18} column employing acetonitrile and water (20:80 v/v) as mobile phase reduces the analysis time without affecting either the resolution of the peaks or the accuracy of caffeine determination. This method is shown to estimate accurately soluble caffeine contents in the brews of black tea, decaffeinated tea, and decaffeinated instant tea samples. Thus, the method is ideally suited for rapid routine anaylsis of black tea and its products.

Keywords: *Caffeine; Sep-Pak C_{18}; decaffeinated tea; HPLC; instant tea*

INTRODUCTION

Tea is a popular beverage. The stimulating effect of tea beverage is due to the presence of purine bases (caffeine, theobromine, and theophylline). These alkaloids of tea products are quantified by a number of methods. Caffeine is the major alkaloid of tea, present in the range of 3.0−4.0%. The most widely used method of determination, based on spectrophotometry (Ullah et al., 1987), gives higher values due to interfering compounds such as theobromine and theophylline. Kazi et al. (1985) estimated the caffeine from tea by refluxing the material with MgO, passing the filtrate through a heavy MgO column, and injecting the elute on a HPLC. Dulitzky et al. (1984) estimated the total caffeine and other purine alkaloids in coffee, tea, and cocoa by HPLC. This method involves refluxing the sample with water and MgO for 1 h, in a tector digestion tube (normally used in determining nitrogen), followed by extraction with solvent and subjection to HPLC. These methods are tedious, elaborate, and time-consuming and hence not suitable for routine anaysis. Also, Blauch et al. (1983), Liang et al. (1990), Timbie et al. (1978), and Watanabe et al. (1992) developed HPLC methods for caffeine estimation in cocoa, tea, and their products. These methods involve refluxtion of samples with water and aqueous alcohol, filtration, and HPLC. However, the HPLC method developed by Blauch et al. (1983) is a simple, accurate, and rapid for the determination of caffeine in coffee, tea, and instant cocoa beverages. Blauch's method involves sample preparation by dipping bagged tea in 177 mL of boiling water for 5 min. The following HPLC conditions were used: (1) HPLC Model M-45 solvent delivery system (Waters Associates, Milford, MA) and Model 7125 sample injector system (Rheodyne Inc., Berkeley, CA) with a 20 μL sample loop; (2) A Waters μ-Bondapak C_{18} column (3.9 mm × 30 cm); (3) mobile phase of acetonitrile and water (8:92 v/v); (4) Waters Model 450 variable wavelength detector at a sensitivity of 0.04 AUFS and a wavelength of 245 nm. However, in this method filtered brew was directly injected without removing the pigments, resulting in shortening of column life. All of the HPLC methods screened suffer from drawbacks of sample preparation and long analysis time and thus are not suitable for rapid analysis. Also, AOAC methods are reported to give artificially high values of caffeine in decaffeinated tea (Madison et al., 1976; Smyly et al., 1976). Accurate and quick methods are required for the determination of soluble caffeine in tea and tea products. The main objective of this study is to consolidate these methods and produce a quick and reproducible method for the routine analysis of caffeine in tea and its products.

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MATERIALS AND METHODS

Samples of black tea, decaffeinated tea, and decaffeinated instant tea were procured from the market.

A Sep-Pak C_{18} cartridge was obtained from Waters Associates, London, and a Millipore filter type FH (pore size o.5 μm) was from Millipore (India) Pvt. Ltd.

A Shimadzu HPLC solvent delivery system controller (Model LC-6A) with system controller (Model SCL-6A) was used. The injection system used a 20 μL sample loop. Detection was by a UV−visible spectrophotometer (SPD-6AV) set at a sensitivity of 0.08 AUFS and a wavelength of 276 nm. A μ-Bondapak C_{18} column (3.9 mm × 15 cm) was used to separate the caffeine. The data processor (Model CR- 4A Chromatopac) was set at a chart speed of 2.5 mm/min. The mobile phase consisted of acetonitrile and water (20:80 v/v) at a flow rate of 1 mL/min.

Reagents included methanol (GR), chloroform (GR), acetonitrile (GR), and double-distilled water (glass).

All solvents were obtained from Merck. Solvents were distilled and filtered through a 0.5 μm filter and degassed under vacuum prior to use.

Standard Caffeine. Stock solution was prepared by dissolving 80 mg of BDH grade caffeine in 100 mL of water. Working standard was prepared by diluting 10 mL of stock solution to 100 mL to give a concentration of 0.08 μg/μL.

Calibration Graph. Working standard solution (5−25 μL) was injected on the HPLC, and peak area responses were obtained. A standard graph for caffeine was prepared by plotting concentration versus area.

Sample Preparation. *(a) Black Tea/Decaffeinated Tea.* Two grams of tea was weighed into a 250 mL beaker, and 177 mL of boiling water was added. The tea was brewed for 6 min on a boiling water bath, with the temperature maintained at 80 °C. The brew was filtered through Whatman No. 44 filter

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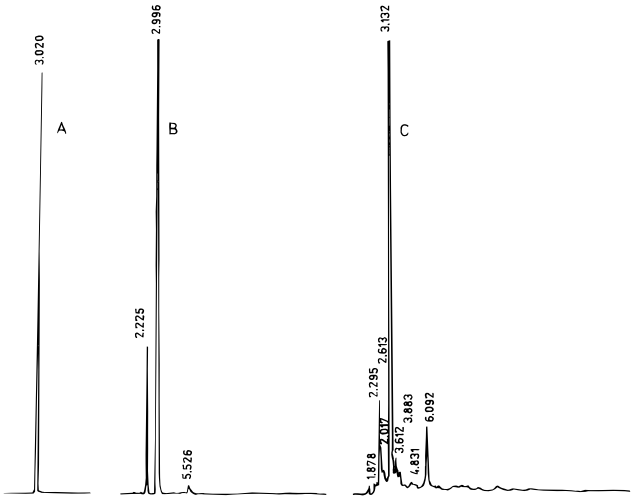

Figure 1. Chromatogram of caffeine: (A) caffeine standard solution of 8 mg/100 mL (RT = 3.023 min); (B) tea sample after Sep-Pak C_{18} cartridge treatment (RT = 2.996 min); (C) tea sample before Sep-Pak C_{18} cartridge treatment (RT = 3.132 min).

paper, and 2 mL of filtrate was subjected to the cleanup procedure as described below.

(b) Decaffeinated Instant Tea. Instant tea powder (0.5 g) was dissolved in 177 mL of boiling water; 2 mL was subjected to the cleanup procedure as described below.

Cleanup Procedure. A Sep-Pak C_{18} cartridge was prepared by first passing 2 mL of methanol by means of a glass syringe. Using an empty syringe, air was passed through the cartridge to expel any remaining methanol. Tea extract (1−2 mL) was then passed through the cartridge, and elute was rejected. Again, air was passed to expel any water. Caffeine was eluted from the cartridge with 6 mL of chloroform (drop by drop), followed by air, into an evaporating flask. The chloroform was removed on a water bath under vacuum. The residue in the flask was dissolved in water and made up to 4 mL. An aliquot (5−10 μL) of this solution was injected on the HPLC.

Preparation of Sep-Pak C_{18} for Reuse. After use, the cartridge was washed first with 35% (v/v) methanol in water (5 mL) followed by 80% (v/v) methanol in water (4 mL) and finally 2 mL of methanol for further use. By following the cleanup procedure carefully, >25 samples could be handled with one cartridge.

Caffeine Recovery Studies. Recovery studies involved passing 2 mL of caffeine stock solution through a Sep-Pak C_{18} cartridge. A small aliquot (5 μL) of the eluate was injected on the HPLC. The percentage of caffeine was calculated by comparing the values with the calibration curve.

Caffeine contents in black tea, decaffeinated tea, and decaffeinated instant tea brews were analyzed before (Blauch method) and after passing through the Sep-Pak C_{18} cartridge (modified method) (Figure 1).

Comparison of Methods. Caffeine values obtained by both the methods were analyzed by paired *t*-test for difference.

RESULTS AND DISCUSSION

The results of recovery studies of caffeine are presented in Table 1. The difference in the recovery of caffeine from the Sep-Pak C_{18} catridge was found only in the range of 0−2% even though the *t*-value is significant ($P < 0.05$). Considering the physiological effects on the quantity of caffeine consumed, it is important to estimate the caffeine content in a cup of tea and tea products, which is actually consumed, rather than the total caffeine content in tea. At present, HPLC is the best tool for the determinatin of caffeine in tea brew on a reversed phase μ-Bondapak C_{18} column using UV detector. The solubility of caffeine is very much related to temperature (Macrae, 1985). One gram of caffeine dissolves in 46 mL of cold water, in 5.5 mL of water at 80 °C, and in 1.5 mL of boiling water. The pigments present in the filtrate reduce the life of the

Table 1. Percent Recovery of Pure Caffeine by HPLC Method

replicate	Blauch's method without passing through Sep-Pak C_{18} cartridge	after passing through Sep-Pak C_{18} cartridge
1	100	100.00
2	100	98.75
3	100	98.00
4	100	99.00
5	100	100.00 [$t = 2.78$[a] (7 df)[b]]
6	100	99.90
7	100	98.90
8	100	99.70
mean	100	99.28
SD[c]	10	10.73

[a] Significant, $P < 0.05$. [b] df, degrees of freedom. [c] SD, standard deviation.

Table 2. Content (Percent) of Soluble Caffeine in Black Tea Samples

sample	Blauch's method	modified method
1	2.83	2.87
2	2.44	2.47
3	2.19	2.21
4	2.70	2.69 [$t = 0.53$NS[a] (9df)[b]]
5	2.33	2.30
6	2.06	2.00
7	2.81	2.79
8	2.71	2.79
9	2.89	2.83
10	2.41	2.43

[a] NS, not significant, $P > 0.05$. [b] df, degrees of freedom.

Table 3. Content (Percent) of Soluble Caffeine in Different Grades of Decaffeinated Tea Samples

tea sample	Blauch's method	modified method
decaffeinated leaf grade		
1	1.59	1.57
2	1.03	1.05
3	1.99	1.93
4	1.28	1.26
5	2.40	2.40
6	2.61	2.60
7	2.56	2.47 [$t = 0.70$NS[a] (13 df)[b]]
8	2.20	2.23
9	2.42	2.44
10	2.10	2.20
11	1.45	1.50
12	1.22	1.28
13	1.35	1.41
14	1.27	1.32
Fannings		
1	2.18	2.16
2	2.23	2.19
3	1.45	1.48
4	2.08	2.18
5	2.28	2.28 [$t = 2.23$NS (9 df)]
6	1.60	1.68
7	1.40	1.49
8	1.65	1.70
9	1.85	1.84
10	1.50	1.58
Golden Dust		
1	1.06	1.00
2	1.67	1.69 [$t = 0.56$NS (3 df)]
3	1.35	1.36
4	0.88	0.87
market samples		
1, Astor	0.23	0.23
2, Tetley	0.22	0.21
3, Bigelow	0.35	0.33 [$t = 0.12$NS (5 df)]
4, Salada	1.06	1.07
5, Milford	0.21	0.23
6, Lipton	0.24	0.23

[a] NS, not significant, $P > 0.05$. [b] df, degrees of freedom.

Table 4. Content (Percent) of Soluble Caffeine in Decaffeinated Instant Tea Samples

sample	Blauch's method	modified method
1	1.01	1.00
2	trace	trace
3	1.64	1.67
4	0.65	0.64
5	0.50	0.51
6	1.53	1.51 [$t = 0.98$NS[a] (12 df)[b]]
7	1.02	1.04
8	1.32	1.28
9	1.86	1.84
10	1.22	1.20
11	1.38	1.36
12	2.00	1.97
13	0.37	0.40

[a] NS, not significant, $P > 0.05$. [b] df, degrees of freedom.

column if they are not removed prior to injection on the HPLC. With Blauch's method, initially good results were obtained. It was observed that, after 100 injections, visible deterioration of the column efficiency occurred, noticeable by the appearance of peak broadening and shoulders on the peaks. It was observed that achievement of a good resolution of the caffeine peak was difficult due to the presence of interfering tea pigments in the extract. This problem was solved by standardizing a sample preparation procedure described earlier. This modified method gave good resolution and sharp peaks of caffeine without affecting the resolution or accuracy of determination. The approximate caffeine retention time of 3.0 min indicates the quickness of this method. Blauch's method and the present modified methods were screened for their resolution and accuracy in caffeine determination in samples of black tea, decaffeinated black tea, and decaffeinated instant tea. The results are given in Tables 2−4. The soluble caffeine estimated by both methods in black tea (Table 2), in decaffeinated tea, Golden Dust, and market tea samples (Table 3), and in instant tea (Table 4) compared well. Further, the caffeine in tea fannings estimated according to the present method was slightly higher, indicating better efficiency.

CONCLUSION

The modified method is ideally suitable for the rapid, routine analysis of a large number of tea samples. This study demonstrates the potential of using a Sep-Pak C_{18} cartridge for the purification of tea brew before injection on a HPLC μ-Bondapak C_{18} column. With this method good repeatability of the results is established. Also, the modified method increases the column life and reduces the analysis time. More than 25 samples can be prepared using a single Sep-Pak C_{18} cartridge by following the cleanup procedure carefully. The reversed phase μ-Bondapak C_{18} column resolution was good even after analysis of about 400 samples. A recovery of 98−100% caffeine was obtained from the Sep-Pak C_{18} cartridge.

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