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Extraction and Chromatographic Determination of Caffeine Contents in Commercial Beverages

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Abstract: High Pressure Liquid Chromatographic technique was employed to study caffeine contents in beverages. The samples were collected from local markets of Pakistan and determined its quality, quantity and suitability. A certified testing method for caffeine extraction was developed. Two extraction methods were used throughout this work solvent extraction and column extraction. The resulted extracts were concentrated then injected in HPLC equipped with ultra-violet detector. The quantitative results when compared with reference standard proved that column extraction is more selective then solvent extraction.

Key words: Caffeine, High Pressure Liquid Chromatography, column extraction, solvent extraction

INTRODUCTION

Caffeine is a stimulant commonly found in many foods, drinks and has a mild addictive effect on the body. Caffeine is known chemically as trimethylxanthine and the chemical formula is $C_8H_{10}N_4O_2$ (Burge and Raches, 2003). Caffeine is a white crystalline powder that tastes very bitter and occurs naturally in many plants, including coffee beans, tealeaves and cocoa nuts (Cabrera *et al.,* 2003). It is therefore found in a wide range of food products. Caffeine is added artificially to many others, including a variety of beverages.

Medically, caffeine is useful as a cardiac stimulant and also as a mild diuretic (it increases urine production). Recreationally, it is used to provide a boost of energy or a feeling of heightened alertness. It is often used to stay awake longer by college students and drivers. Many people feel, as they cannot function in the morning without a cup of coffee/tea to provide caffeine and the boost it gives them (Shirley *et al.,* 2003). The problem with caffeine is the longer-term effects, which tend to spiral. Once the adrenaline wears off, we face fatigue and depression. We take more caffeine to get the adrenaline going again because our body in a state of emergency all day long isn't very healthy and it also makes us jumpy and irritable (Schneider *et al.,* 2003). The most important long-term problem is the effect that caffeine has on sleep. Adenosine reception is important to sleep and especially to deep sleep. The half-life of caffeine in our body is about 6 h. That means that if we consume a big cup of coffee with 200 mg of caffeine in it at 3:00 PM, by 9:00 PM about 100 mg of that caffeine is still in our system (Allgeier *et al.,* 2003).

A review of the literature reveals that caffeine is an important factor in modifying the psychological state of its consumers under the present condition of usage. Caffeine is probably the most widely used drug and those who drink coffee, tea, cola or take OTC (Lin *et al.,* 2003). Caffeine containing drugs are all potential and susceptible candidates. Those of us who are normal can expect manifestations, which may be subtle at low doses, overt at high doses, with the possibility of being the victims of a habit, which results in tolerance and possible severe withdrawal symptoms. The pleasant stimulant feeling, which often occurs at low doses, may be replaced by psychological symptoms, which resemble anxiety and depressive neuroses at high doses (Pulitaival *et al.,* 2003). Those with more severe psychological problems may have their symptoms exaggerated with excessive caffeine usage, or such symptoms can actually be caused by excess. Diagnosis of such conditions must take caffeine usage into account.

The amount of caffeine in a cup of tea or coffee differs greatly, depending on the method of preparation. Caffeine's effect on our body, our nervous system, our mind, our psychology is no illusion (Lin *et al.,* 2003). With these findings we see that caffeine abuse is more prevalent than we may imagine. These facts should be brought to the attention of the medical community as well as the public in order that we may have the opportunity of being aware of the possible interactions between our environment and ourselves. Caffeine is a drug, which stimulates the central nervous system. In the amounts presently being consumed, it can cause insomnia, nervousness, irritability, anxiety and disturbances in the heart rate and rhythm. Cola like drinks account for 80

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to 90% of the caffeine added to foods today (Laasonen *et al.*, 2003). Its long-term effects on people are not clearly known.

All kinds of soft drinks are acidic, especially cola. Colas drinks make our bodies poor in oxygen (Lin *et al.*, 2003). Caffeine is one socially acceptable addictive drug found in tea and most soft drinks. This dangerous stimulant causes many problems for the body. These products with caffeine artificially stimulate the body and increase the heart rate. While this artificial stimulation temporarily arouses the intellect and fatigue seems to disappear, it is short lived. The excess stimulation depletes the body of vital energy as it struggles to deal with this poison that has entered its system. There are many effects from the consumption of caffeine, including increased incidence of bladder and stomach cancer, raised blood pressure, increased heart rate and it aggravates diabetes and damages the lining of the stomach.

MATERIALS AND METHODS

A variety of beverages samples were collected from local markets of Pakistan in different brands from the summer season 2004.

Reagents and apparatus: HPLC grades hexane, acetonitrile, methanol, ethanol, chloroform and water solvents were used for the extraction procedure. Anhydrous and granular sodium sulfate was used as dehydrating agent in the extraction process. Silica jel was used as separating material in column having a dimensions 36 in length and two inches internal dia. Caffeine was extracted by using silica jel as a separating agent and water: ethanol was used as a mobile phase. Estimation of caffeine contents was made by using High Pressure Liquid Chromatography (LC-9A, Shimadzu) with mobile phase 65:35 mixture of water methanol and acetonitrile run for 10 min. Caffeine elutes at approximately 1.1 min, the area of this peak is measured. Six replicates of each standard were taken and a plot of peak area VS concentration was made. Unknown samples were prepared. The results were expressed in mg.

PCSIR 17025 certified laboratory provided the reference standard material. Standard solutions of caffeine were prepared in deionized water ranging from 10-100 ppm. Ten concentrations of standards 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm were used for analysis passing through a 0.45 μ Teflon filter paper in a buchner filtration assembly under vacuum and degassed. Twenty microliter samples were injected manually through a syringe into the HPLC and flow rate of 1.5 mL min^{-1} was adjusted. Ten mililiter sample of commercial soft drinks and 100 mg of commercial tea sample was taken and treated in a similar fashion to the standard.

Extraction procedures: Two different methods were used to extract caffeine from beverages (Colas and Tea). Two different solvents with different polarities ethanol and chloroform were used. All of these solvents were of high purity HPLC grades.

Solvent extraction: A total 100 mL liquid sample of cola was transferred to separating funnel added the solvent (chloroform) and shake well for 2 h and then separated chloroform layer. The extracted samples were concentrated by turbo evaporator to 0.5 mL and then added 2 mL mobile phase. The solid tea sample of 100 mg was used and extracted with same fashion cited above.

Column extraction: The extraction procedure was carried out using glass column (36"length X 2" ID) and silica jel was used as adsorbent. A 100 mL sample of cola was taken and mixed with ethanol poured from the top of the column. Sample was concentrated in vacuum evaporator at 100°C and 2000 psi for 5 min. Tea samples 100 mg were taken, mixed with distilled water and boil for 30 min, cool it, diluted to 100 mL with same solvent. At the end 100 mL ethanol mixed with it and prepared with same way.

RESULTS AND DISCUSSION

The quantitative analysis simply is a comparison of retention data from the known and unknown samples (Eaimtrakarm *et al.*, 2003). Retention time in Table 1, 2, 3 and 4 were same and this proved the presence of caffeine in both samples (cola's and tea). In this study we succeeded to extract caffeine from cola and tea samples that were collected from local markets of Pakistan. The resulting program of each injection was compared in terms of the retention time. Peak area was directly proportional to the concentration of caffeine. Integration of each peak in comparing to the reference standard (99.8%) of caffeine. The area data of the chromatograms resulted from the CE and SE extraction reveals that there is broadens in both of them and this may be attributed to the hydrogen bonding was resulted polarity of the hydroxyl group, in the molecular structure of the ethanol. From data in Table 1 and 2, we concluded that the recovery of caffeine trough column extraction method was best as compared to solvent extraction method. The recovery was made by column extraction method in rang 96-99% and by solvent extraction method 86-90%.

The area in Table 2 and 3 were shown that column extraction method were more accurate and reliable then solvent extraction method. CE procedure used in industrial as will as R and D proposes. CE method consumed more time than SE. Length of the column is directly effect to the time and efficiency. In solvents extraction, chloroform is the best solvent for extraction of

Table 1: Solvent and column extraction of reference standard caffeine

	Solvent extraction			Column extraction		
Caffeine Conc. (ppm)	Retention time	Peak area	Recovery ppm	Retention time	Peak area	Recovery
10	1.488	30.8	8.55	1.486	34.8	9.55
20	1.487	61.4	17.35	1.485	66.34	19.35
30	1.489	93.01	26.01	1.484	96.34	29.01
40	1.486	122.90	38.01	1.483	125.40	39.01
50	1.485	153.00	45.05	1.485	160.23	48.05
60	1.487	185.01	55.02	1.488	191.28	58.02
70	1.488	216.35	64.06	1.487	228.34	68.06
80	1.488	248.06	73.05	1.487	257.16	79.05
90	1.488	276.39	81.61	1.489	288.54	88.61
100	1.487	308.09	90.32	1.486	328.21	98.32

Table 2: Solvent and column extraction of reference standard caffeine

	Solvent extraction			Column extraction		
Caffeine Conc. (ppm)	Retention time	Peak area	Recovery ppm	Retention time	Peak area	Recovery
10	1.480	30.8	6.35	1.485	32.50	9.32
20	1.481	61.4	15.20	1.484	64.45	18.52
30	1.483	93.01	21.03	1.483	96.09	28.52
40	1.480	122.90	34.02	1.485	128.23	38.65
50	1.480	153.00	45.32	1.485	159.25	48.32
60	1.482	185.01	52.63	1.486	192.15	59.02
70	1.484	216.35	61.25	1.485	222.03	68.54
80	1.485	248.06	71.23	1.484	256.09	79.03
90	1.484	276.39	80.52	1.484	284.50	88.21
100	1.485	308.09	91.65	1.482	320.61	98.02

Table 3: Caffeine contents in different cola's sample

	Solvent extraction			Column extraction		
Sample mg	Retention time	Peak area	Caffeine concentration ppm per 100	Retention time	Peak area	Caffeine concentration ppm per 100
30	1.485	23.28	22.70	1.480	25.18	25.79
50	1.483	45.44	24.35	1.482	51.40	27.01
70	1.481	73.01	23.82	1.483	76.01	26.52
90	1.480	92.90	22.83	1.480	101.90	24.72
110	1.482	120.00	23.85	1.484	126.00	25.35
130	1.482	141.01	23.89	1.481	152.01	26.39
150	1.483	166.35	24.35	1.480	174.35	27.32
170	1.484	188.06	22.38	1.482	201.06	25.34
190	1.482	216.39	23.85	1.483	226.30	26.35
210	1.483	238.09	22.45	1.480	251.03	24.45

Table 4: Caffeine contents in different tea sample

	Solvent extraction			Column extraction		
Sample mg	Retention time	Peak area	Caffeine concentration	Retention time	Peak area	Caffeine concentration
50	1.482	30.8	9.89	1.480	35.08	11.31
100	1.483	61.4	12.77	1.480	69.04	13.67
150	1.480	93.01	15.32	1.480	104.01	16.01
200	1.481	122.90	17.45	1.481	139.40	18.15
250	1.480	153.00	19.02	1.482	173.02	20.60
300	1.480	185.01	21.32	1.480	208.51	23.00
350	1.481	216.35	23.21	1.482	244.35	25.20
400	1.482	248.06	24.05	1.480	278.36	27.45
450	1.480	276.39	28.01	1.482	312.96	30.01
500	1.483	308.09	29.27	1.480	348.29	32.34

Each mentioned value is average of the six values

caffeine in commercial beverages, plants and other R and D purposes because caffeine is freely soluble in chloroform.

Table 5: Physical constituents in beverages (colas and tea)

Colas samples				Tea samples			
pH	TDS (ppm)	EC ($\mu S\ cm^{-1}$) at 25°C	Caffeine contents (12 Ounce Can)	pH	TDS (ppm)	EC ($\mu S\ cm^{-1}$) at 25°C	Caffeine contents (12 Ounce Can)
5.1	134	1345	60	5.7	364	532	70
4.9	137	1356	65	5.6	355	540	75
5.3	132	1403	71	5.5	325	610	78
5.2	148	1409	62	5.7	366	535	77
4.8	150	1356	58	5.4	365	525	76
4.6	160	1377	67	5.5	358	521	70
5.1	162	1385	65	5.3	348	605	80
4.8	158	1394	57	5.8	365	601	70
5.0	148	1401	72	5.5	352	532	73
4.7	156	1406	56	5.7	357	620	70

Caffeine was found in tea samples in range 70–75 mg per 12 ounce which were shown in Table 5. Colas contained caffeine contents in range of 57–72 mg per 12 ounce that were presented in Table 5. Americans alone drink 137 billion cups of coffee annually. That is over 50 tons of caffeine per day! Even more caffeine is consumed in the form of soda pops (Ling *et al.,* 2003). Tea contained more caffeine then cola's sample from Table 3 and 4. Comparing the colas, local colas topped out at 72 mg per 12 ounces; while the lowest was Diet Pepsi and Pepsi Light at 34 mg. Coca-Cola has 42 mg. While regular Pepsi-Cola has 65mg. Cola like drinks for 80 to 90% of the caffeine added to foods today (Sinues *et al.,* 2003). Physical parameter like pH, Conductivity and total dissolved solids in all commercial samples were found with in limits and were shown in Table 5.

CONCLUSIONS

Column extraction method and solvent extraction method were used for extraction of caffeine in both samples. Column extraction method was showed a good resolution as compared to the solvent extraction method and this method was used as industrial or research purposes. Caffeine contents were found more tea samples then cola's (soft drink). High Performance Liquid Chromatographic technique was employed for the determination of caffeine qualitatively and quantitatively. Electrical conductivity was found higher in cola's samples then tea. pH and TDS were found with in range.

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