

HPLC determination of caffeine in some beverages and pharmaceutical dosage forms available in Syrian market

Ayat Abboud^{1*}, Dima Aldiab

¹Pharmaceutical chemistry and drug quality control Department, Faculty of Pharmacy, Tishreen University, Lattakia, Syria,

²Analytical chemistry and food quality control Department, Faculty of Pharmacy, Tishreen University, Lattakia, Syria

*Corresponding author: E-Mail: ayatabboud@yahoo.fr

ABSTRACT

A reverse-phase high performance liquid chromatography method was validated for analysis of caffeine in various samples. The HPLC separation was achieved on a Hypersil C18 (250 mm×4.6 mm, 5 μ m) using a mobile phase of MeOH and water (40/60, v/v) at a flow rate of 1 ml min⁻¹ at room temperature and diode array detector set at 272 nm. The method produced linear response over the concentration range of 0.19-10 μ g.ml⁻¹. The method was found to be precise since the RSD% for intraday and inter day precision studies were less than 10%. LOD and LOQ were 0.023 μ g/mL and 0.065 μ g/mL, respectively, which enable to detect and quantify low amounts of CAF. The recovery of the method was 97.15 %. The selectivity of the method was accessed by CAF complete separation from Paracetamol and Aspirin. The method was applied for determination of CAF in beverages and dosage forms containing CAF available in Syrian market. Brands of coffee and each of black, green and mate tea yielded a CAF content ranged from 11.2 to 66.8 mg/serving. In soft drinks like cola, only 0.007-0.009 mg CAF/ ml was found. Percent content of CAF in tablets was found to be very close to the labeled amount (105% - 117.7%) except for sample D (61%).

KEY WORDS: HPLC, caffeine, validation, pharmaceutical, drinks.

1. INTRODUCTION

Caffeine (CAF) (1,3,7-trimethyl xanthine) is a natural occurring as well as a pharmacologically active substance (Textor, 2003) (Figure.1). CAF is an odorless white crystalline powder with very bitter taste (Burge and Raches, 2003). It is a mild central nervous system (CNS) stimulant (Svilaas, 2004) and the recommended daily dose for this effect is 200 mg/day (Paxeus and Schroder, 1996).

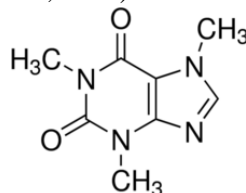


Figure.1. Chemical structures of CAF

CAF is considered the most frequently consumed dietary stimulant of CNS. CAF improves the mental performance (Wood, 1989; Battig and Welzl, 1993), as well as the motor performance, it provides energy, decrease fatigue (Glade, 2010), and leads to peripheral vasoconstriction and relaxation of smooth muscle (Komes, 2009). Other studies in rats indicate that CAF can induce dopamine release (Morgan, 1998; Solinas, 2002).

CAF is useful in limited doses. However, when high doses are consumed, the side effects on human body starts. High doses of CAF are associated with central nervous system disorders like addiction and anxiety (Nehlig, 1999), it can affect the cardiovascular system, increase poor liver function (Bispo, 2002), as well as it stimulates gastric secretion (Lang, 2010). A dose of 10 g is lethal, which is equivalent to about 100 cups of coffee (Paxeus and Schroder, 1996). CAF does not accumulate in the body and is normally excreted within several hours of consumption (Nour, 2008).

CAF enters the fetal circulation and leads to low birth weight as is used at pharmacological level (Chen, 2006), while some epidemiological studies referred to an association between its consumption and the risk of miscarriage (Signorello and Laughlin, 2004). Excessive consumption of CAF during lactation may cause irritability and wakefulness in a breast- fed baby (Chen, 2006).

In addition to the different effects of CAF on human health, CAF was used as indicator for contamination of domestic water because it is anthropogenic origin and it is detected in both waste and surface water (Sievers, 1977). The most common sources of CAF are coffee (Huck, 2005; Ashihara, 2008), cocoa beans, cola nuts and tealeaves. The worldwide consumption of products derived from these natural materials means that CAF is one of the most popular and commonly consumed drugs in the world (Kolayli, 2004). Besides the consumption of CAF as a part of our diets, it is used in many pharmaceutical preparations for its medicinal properties along with other drugs for headache, stimulation, and muscle relaxant.

There are different instrumental methods for the determination of CAF in plants, coffee, tea, soft drinks and pharmaceutical formulation. However, HPLC methods are the most common methods, especially when the samples are complex such as food and pharmaceutical preparation (Mumin, 2006; Viswanath, 2011; Sonali and Sandip 2012; Patil, 2012), while UV-Vis spectrophotometer method cannot be used directly for determination of CAF in complex samples such as coffee due to the matrix effect (Gebeyehu and Bikila, 2015).

In the present study, an isocratic reversed-phase (RP) HPLC method was validated and successfully applied to determine CAF levels in many beverages and pharmaceutical preparation.

Table.1. Description of studied tablets containing CAF

Drug Code	Labeled content (mg)				
	CAF	Paracetamol	Aspirin	Phenylepherine HCl	Orphenadrine Citrate
A	60	500	-	-	-
B	65	250	250	-	-
C	25	500	-	5	-
D	15	450	-	-	35

2. MATERIALS AND METHODS

Chemicals: MeOH HPLC grade were purchased from Merck. CAF, Paracetamol (Acetaminophen) and Aspirin was purchased from Sigma Aldrich.

Samples: Commercial ground and roasted coffees, black and green tea, mate tea, damask rose as well as carbonated soft drinks (cola) were obtained from local markets in Latakia. Many pharmaceutical preparations containing CAF were studied (table.1).

Apparatus: Chromatography was performed on a Jasco HPLC system consisting of a pump (Jasco Pu-2089 Plus), a Rheodyne 7725i injector (Cotati, CA, USA) with a 20 μ L loop and diode array detector (Jasco DAD-2070). Data acquisition and evaluation was performed using Borwin data acquisition software.

Chromatographic conditions: The chromatographic column was a BDS Hypersil C18 (250mm \times 4.6mm, length and internal diameter, particle size 5 μ m and pore size 100 \AA). The mobile phase consisted of water and methanol (60:40, v/v). The mobile phase was filtered through a 0.45 μ m filter and sonicated for 15 min. The injection volume was 20 μ l. The analysis was carried out under isocratic conditions using a flow rate of 1.0 mL/min. Chromatograms were recorded at 272 nm with a run time of 12 min.

Preparation of samples: For each sample of ground roasted coffee, black and green tea, mate tea and damask rose a total amount of 2 gram was weighed into a beaker and portions of 200 ml of freshly boiled water were added and left for three min at room temperature. The samples were then cooled in ice, filtered and diluted to proper concentration prior to analysis. Samples of carbonated soft drinks were sonicated for 20 min, filtered and then diluted prior to analysis.

To determine the content of CAF in pharmaceutical formulation, 20 tablets of each brand were accurately weighted and then finely powdered. A weight of powder equivalent to one tablet content was transferred into a 100 mL volumetric flask containing 50 mL of mobile phase; the solution was sonicated for 20 min and diluted up to the mark with mobile phase. Finally, the resulting solution was filtered and the obtained filtrate was used as sample stock solution. For HPLC system, the above stock solution was further diluted to get a sample concentration in range of 0.19 -10 μ g.mL⁻¹.

All experiments were carried out in duplicates and the results were expressed as means \pm standard error of three parallel replicates.

Preparation of standard CAF solutions: A standard stock solution containing CAF was prepared in mobile phase of 640 μ g.mL⁻¹. The standard solutions from 0.19 to 10 μ g.mL⁻¹ in mobile phase were made by a serial dilution of stock solution.

Method validation:

Linearity: Calibration curve was obtained by plotting the concentrations of CAF serial solutions versus peak area response. The regression equations were calculated from the calibration graphs.

Precision: The one-day precision of the assay method (intraday) was evaluated by carrying out six independent assays of standard CAF solution in one day and calculating RSD%. Similarly, the between-days precision (inter day) was evaluated in six consecutive days under the same experimental condition.

Recovery: Recovery study was performed by adding a known amount (20 mg) of CAF to 2g of grounded damask rose and then the sample was prepared and analyzed as mentioned previously.

Selectivity: The selectivity of the method was accessed by injecting a mixture containing CAF, Paracetamol and Aspirin.

Detection and Quantitation limits: Limits of detection (LOD) and quantitation (LOQ) were estimated by dilution a sample of coffee and a sample of pharmaceutical preparation. LOD was considered the lowest concentration

resulting in a signal-to-noise ratio of 3, while LOQ was the lowest concentration resulting in a signal-to-noise ratio of 10.

3. RESULTS AND DISCUSSION

The study aimed to validate an isocratic RP-HPLC method for analysis of CAF in terms of selectivity, linearity, precision, recovery, LOD and LOQ. The chromatographic method was successfully applied for determination of CAF in various sample including beverages and pharmaceutical preparations. Under the chosen HPLC conditions, CAF eluted after 5.75 minutes (figure.2).

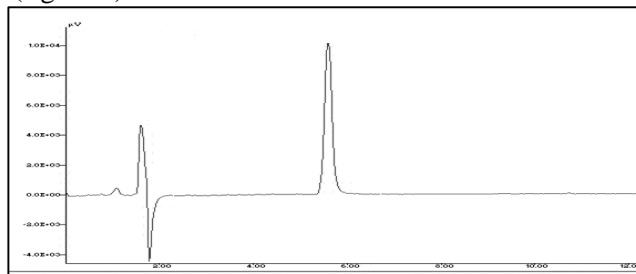


Figure.2. Chromatogram of standard solution of CAF

Method validation: The response of CAF was found to be linear in concentration range of 0.19 to 10 $\mu\text{g}\cdot\text{ml}^{-1}$. The linear regression equation was $y = 78129x - 11886$ with correlation coefficient of 0.9985 (figure.3).

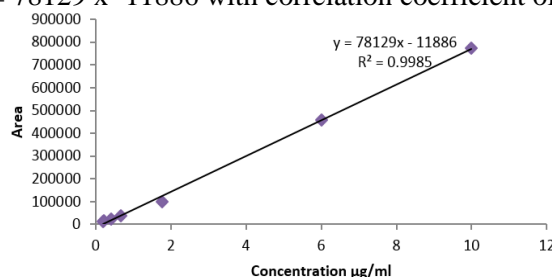


Figure.3. Standard curve of CAF

The method was found to be precise since the RSD% for intraday and inter day precision studies were less than 10% (Table.2).

Table.2. Data of precision study

CAF10 $\mu\text{g}/\text{ml}$	intra day		inter day	
	Retention time	Area	Retention time	Area
Mean	5.71	773254	5.75	784505
RSD %	0.23	2.74	1.99	4.33

LOD and LOQ were 0.023 $\mu\text{g}/\text{mL}$ and 0.065 $\mu\text{g}/\text{mL}$, respectively, which enable to detect and quantify low amounts of CAF in different samples. The recovery of the method was 97.15 %, where 19.43mg of CAF was found in 2g of grounded damask rose after the addition 20mg CAF. The selectivity of the method was accessed from the chromatogram where CAF was completely separated from Paracetamol (Retention time 3.9min) and Aspirin (Retention time 2.1min) with selectivity factor (α) of 1.8 and 11.4, respectively (figure.4).

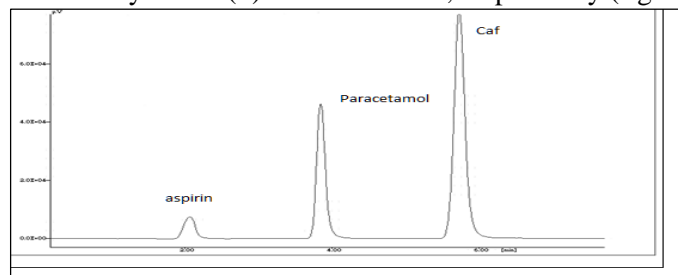


Figure.4. Chromatogram of mixture of Aspirin, Paracetamol and CAF under the chromatographic conditions

CAF content in beverages: Coffee, Tea and soft drinks are very commonly used beverages in all over the world. The CAF is the main stimulant occurred in these beverages. On average, 90% of adults consume CAF on a daily basis from the previous beverages (Patil, 2012). According to the method by which the herbal products are consumed in Syria, 2 gram of each of black, green, mate tea as well as damask rose are needed to prepare a cup (a serving of 200 ml), while 2 grams of coffee are needed for each cup of coffee (a serving of 75 ml). Thus, the study wanted to estimate the consumption of CAF in each serving of these beverages. CAF in studied beverages eluted after 5.75 minutes (figure.5). The amount of CAF ranged from none in damask rose to 66.8 mg/serving in black tea. The

results and details of CAF content per gram, per serving and the percentage of CAF in the studied samples are listed in table.3. Tea leaves contains till 3.5 % of CAF (Cabrera, 2003). The content of CAF in different tea varies depend on tea type, which is directly attributed to the processing conditions and leaf maturity. Generally, White tea, which is made from the youngest tea leaves, contained the highest CAF content (Chin, 2008; Komes, 2009), followed by black then green tea, while mate teas has the lowest content (Komes, 2009). In our study, the tea brands (black, green and mat) yielded a CAF content ranged from 11.2 to 66.8 mg/serving which are similar to those mentioned in some previous studies. Additionally, CAF content in black tea was higher than green tea as other studies (Chin, 2008). However, CAF content in mate tea was approximate to green tea, while other studies have found higher content of CAF in green tea compared to mate tea (Komes, 2009).

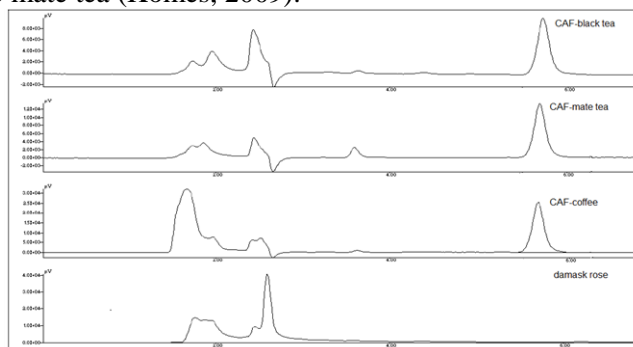


Figure.5. Chromatograms of black tea, mate tea, coffee and damask rose sample

Coffee has become one of the most widely consumed beverages throughout the world due to its pleasant taste, aroma and stimulant effect of CAF and health benefits (Gebeyehu and Bikila, 2015). It is not possible to determine CAF directly in coffee beans by conventional UV-Vis absorption measurement due to the spectral overlap of UV absorbing substances in the sample. Therefore, HPLC is a suitable method for this purpose (Chen, 2006). Coffee beans have about 1.1- 2.2% of CAF (Cabrera, 2003). Generally, home-prepared coffee contains 30 to 175 mg of CAF per 150 ml (Torequl Islami, 2016). This is in agreement with our results, where we found 39.4-60.8 mg of CAF per 150 ml (19.7-30.4 mg of CAF per serving of 75 ml as seen in table.3). Coffee content of CAF initially depends on the biological coffee plant species. For instance, the species *Coffea arabica* contains about half of the species *Coffea robusta* (Naegele, 2016). Other factors can affect CAF content like processing, storage, roasting process and temperature (Torequl Islami, 2016). It is necessary to mention that in our study no brand of coffee, tea, mate tea or damask rose referred to the content of CAF on product label, although it is desirable to consumers to have information about caffeine content in these products.

CAF is a part of the overall profile of soft drinks where is added as a flavoring agent (Nour, 2008), at concentration of nearly 0.1 mg/mL. The manufacturer justify their use to CAF to soft drinks by claiming that it enhances the aroma, although at this concentration only a small percentage of consumers notice its presence (Griffiths and Vernotica, 2000). However, our study found only 0.007-0.009 mg CAF per ml of cola, this means it 10-15 times lower than what it is generally used, additionally, no information about CAF content was found on product label.

Table.3. Content per gram, per serving and percentage of CAF in different samples

Sample	CAF content mg/g	CAF per serving 2g /200 ml	CAF per serving 2g /75 ml	CAF % (W/W)	CAF % (W/V)
black tea 1	16.5	33		1.65	
black tea 2	18.3	36.6		1.83	
black tea 3	33.4	66.8		3.34	
coffee 1	11.75	23.5	23.5	1.175	
coffee 2	9.85	19.7	19.7	0.985	
coffee 3	15.2	30.4	30.4	1.52	
green tea 1	5.6	11.2		0.56	
green tea 2	5.85	11.7		0.585	
green tea 3	6.64	13.28		0.664	
mate tea 1	6.72	13.44		0.672	
Mate tea 2	7.67	15.34		0.767	
damask rose 1	ND	ND		ND	
damask rose 2	ND	ND		ND	
cola 1		1.45			0.725
cola 2		1.84			0.92

CAF content in pharmaceutical preparation: There are about 2000 non-prescription and about 1000 prescription drugs containing CAF (Jeanne, 1987). Pharmaceutical companies offer different kinds of analgesic and non-steroidal anti-inflammatory drug mixtures for acute headache therapy, for example, mixing Paracetamol (Acetaminophen), Aspirin (acetylsalicylic acid), or Metamizol with CAF and Ergotamine (Mario and Gertrud, 2007). The consumption of over-the counter (OTC) analgesics like Aspirin or Acetaminophen in combination with CAF increases their activity about 40% depending on the specific type of pain involved (Sonali and Sandip, 2012).

In Syrian market, many multi-component tablet formulations available contain caffeine (CAF). In this study, four pharmaceutical preparations containing CAF were analyzed using the validated method, which shows that other drugs in combination with CAF do not interfere in the analysis (figure 6). Experimental results of % content of CAF in tablets were expressed as % of label claimed, was found to be very close to the labeled amount (105% - 117.7%) except for sample D (61%) (table.4).

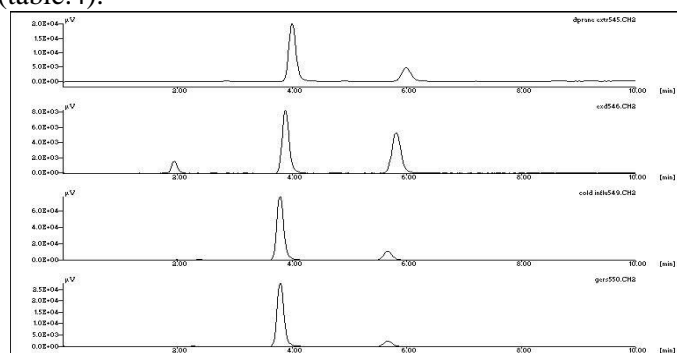


Figure.6. Chromatograms of studied pharmaceutical preparations containing caffeine

Table.4. Assay data of CAF in marketed drugs

Marketed drugs	A	B	C	D
labeled CAF content mg	60	65	25	15
Found CAF amount mg	63	76.54	26.3	9.16
CAF content Mean %	105%	117.76%	105.2%	61%

4. CONCLUSION

The results show that the HPLC method presented here can be considered suitable for the analytical determination of CAF in beverages and pharmaceutical tablets, being linear in the concentration range used, high selectivity, high precision and adequate accuracy at the concentrations studied.

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