QUANTIFICATION OF CAFFEINE IN SELECTED BEVERAGES VIA GAS CHROMATOGRAPHY-MASS SPECTROSCOPY

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ABSTRACT

Caffeine content of sugar free beverages (colas, coffee, energy drinks and herbal teas) was determined by gas chromatography-mass spectroscopy (GC-MS) without using any pre-separation or background correction techniques. The method is based on direct injection and standard addition prior to analysis. The results show that 5-hour energy (7390 mg/L) contains a much greater caffeine content than the other beverages investigated. The caffeine concentration (64-72 mg/L) found in soft drinks is in line with the United States Food and Drug Administration (US FDA) limit of caffeine content in carbonated beverages. Caffeine in herbal teas (3-49 mg/g) varies with the brand of tea. Decaffeinated coffee (1.5 mg/g) was found to contain about 94% less caffeine than caffeinated coffee (24 mg/g) whereas Lipton Decaffeinated Green Tea (3 mg/g) contained about 80% less caffeine than its caffeinated brand (13 mg/g). Non-caffeinated beverages were found to contain little or no caffeine. The wide range of caffeine concentrations in beverages makes it noteworthy for consumers to be aware of the caffeine content of beverages as high caffeine levels in the human body can lead to health implications.

Key words: Caffeine, Beverages, Direct injection, GC-MS.

INTRODUCTION

Caffeine consumption can be traced as far back as the mediaeval times in which chewing the leaves of plants containing caffeine promoted awareness and eased tiredness. Today, caffeine is consumed daily in chocolate, coffee, brewed tea and soft drinks[1,2]. According to statistics, the global average consumption of caffeine is between 80 and 400 mg per person per day[2]. In humans ingested caffeine is rapidly absorbed and metabolized by the liver, however, the majority of the ingested caffeine is converted into one or more metabolites. Thus, about 0.5% to 10% is excreted in urine and faeces[3,4].

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Although caffeine is found in more than 60 species of plants the presence of caffeine in environmental water is largely due to domestic wastewater discharge[5-7]. Researchers have maintained that a principal source of caffeine to domestic wastewater is likely to be the disposal of unconsumed coffee, tea and colas down household drains[8,9]. Interestingly, caffeine has been used as a chemical marker for wastewater contamination because of its unique characteristics. It is highly soluble in water (13 g/L and log k_{ow} = -0.07), insignificant volatility and it is very stable to hydrolytic degradation (half-life about 10 years) [10,11]. At pH conditions less than its pKa value of 10.4; caffeine is protonated. Researchers have maintained that the protonated form is more water soluble and less volatile than the neutral, unprotonated form[12,13]. However, bacterial strains (e.g. *Pseudomonas* and *Serratia*) and fungal strains (e.g. *Aspergillus* and *Penicillium*) have been reported to degrade caffeine[14].

Caffeine, an important constituent of a variety of beverages and food products is a paradox. It is one of the most powerful xanthine in its ability to increase alertness, put off sleep and to increase thinking capacity.[1] Furthermore, caffeine can be used medically as a vasodilator as well as a diuretic.[15] On the other hand, due to the increase number of cardiovascular disorders decaffeinated beverages and food products have been recommended as a result of the potential effects of chronic caffeine consumption on the cardiovascular system[15]. Caffeine consumption during pregnancy may increase the risk of abortion and alters fetal growth. In addition, high caffeine (150 mg caffeine/day) consumption may have some indirect effect on bone mineral density leading to osteoporosis[16]. To ensure safety, the US FDA has employed legislative guidelines stipulating a maximum of 200 ppm or 71 mg of caffeine per 12 oz. serving of caffeine in soft drinks[17,18].

Caffeine may also have environmental concerns; a study suggested that high concentrations of caffeine increase the effects of other environmental parameters on coral such as changes in ocean temperatures and pH, making them more likely to undergo bleaching[19]. Caffeine's effects on aquatic life such as fishes are not currently known as little research has been done but it has been found to affect the reproduction of mussels[20]. Hence, there is a need for individuals to be knowledgeable of the concentration of caffeine in commonly consumed beverages in order to help these individuals to properly manage their daily caffeine intake, prevent health problems and to reduce potential environmental effects.

Analytical techniques have been developed for determination of caffeine in beverages for quality control in food and beverage industries. These techniques include gas chromatography (GC), spectrophotometry, polarography and titrimetric methods[21]. However, these methods may have some disadvantages. Titrimetric methods may require a large volume of sample and may undergo interferences with redox reagents during analysis. The polargraphic method is time-consuming and suffers from the matrix effect caused by

chemical impurities in the food.[21] Spectrophotometric methods used in caffeine quantification are fast and simple, however, it is not reliable to use in samples with complex matrices because of background correction. To overcome the matrix effect often requires tedious pre-separation methods to remove possible interferences which may result in the loss of the desired analyte[21]. GC-MS is a very sensitive technique that is used today for the analysis of many organic compounds in aqueous media. One of its setbacks is that it is of a high cost but it has been proven to be very useful and reliable in the food industry and academic institutions.

The purpose of this study is to develop a direct and fundamental GC-MS method for the determination of caffeine in commonly consumed beverages without using pre-separation and matrix correction techniques. Colas, coffee, energy drinks and herbal teas were investigated because of their high caffeine content.

EXPERIMENTAL

Material and methods

Chemicals and materials

Natural caffeine 99.9% (Reagent Plus), chloroform (Chromaslov for HPLC), hydrochloric acid (HCl), methanol (Chromasolv) and sodium hydroxide (NaOH) were purchased from Sigma Aldrich (St. Louis, MO, USA). Caffeine surrogate internal standard (purity 99% $^{13}C_3$-labelled, 100 μg/mL) was obtained from Cambridge Isotope Laboratories, Inc (Andover, MA, USA).

Sample collection and preparation

All samples were purchased from the local markets in Barbados. Four types of beverages were analyzed for caffeine content namely carbonated sodas (cola), coffee, energy drinks and herbal teas.

Carbonated sodas and energy drinks

The colas (17 oz.) investigated included: Coke Light, Coke Zero, Diet Pepsi, Soda Water and Sprite Zero. Energy drinks examined included: Bullet (zero sugar, 8.4 oz.), Monster (absolute zero, 16 oz.), Red Bull (8.4 oz.) and 5-hour Energy (2 oz.).

Each sugar free sample, excluding Red Bull, was adjusted to pH 8 using 1 M NaOH and diluted to approximately 1 mg/L in methanol based on literature caffeine concentrations of each beverage. Of the resulting solution, 1 mL final volume was transferred to an amber

GC vial (Aligent). Diluted Red Bull (containing granulated beet sugar) was spiked with $^{13}C_3$ caffeine internal standard, and caffeine extracted by liquid-liquid extraction (LLE) using chloroform. The chloroform extracts were evaporated to dryness using a rotavapor rotary evaporator (Buchi Labortechnik AG, Switzerland; model RE-121) and reconstituted to a final volume of 1 mL in methanol and stored in amber vials. Reconstituted samples were stored in the refrigerator until analysis. All samples were prepared in triplicates. Procedural blanks were run periodically to check for caffeine contamination. A pH optimization of caffeine was studied for colas at pH 3 and 8.

Coffee and herbal teas

Colcafé Caffeinated and Decaffeinated Instant Coffee were studied. The following herbal teas were investigated: Lipton Green Tea, Lipton Decaffeinated Green Tea, Lipton 100% Natural Tea, Lipton Green Tea Lemon and Ginseng, Alvita Senna Leaf Caffeine Free Tea and STASH Peppermint Caffeine Free Tea. The weight of the dried ground tea leaves inside each single package ranged from 1.2-2.8 g.

Coffee (1.0 g) was homogenized with 100 mL of hot distilled water and thorough mixing. Whereas, 1 teabag was allowed to brew in 240 mL (8 oz. of water as recommended by the label) of hot distilled water for 15 mins. Both solutions were allowed to cool to room temperature, adjusted to pH 8 and diluted to provide approximately 1 mg/L in methanol based on literature caffeine concentrations of coffee and teas. Triplicate 1 mL volume of the finally prepared coffee and tea solutions in methanol were transferred to amber vials and analyzed via GC-MS as outlined below.

GC-MS analysis

Natural caffeine in beverages (exception to Red Bull) was quantified by standard addition of $^{13}C_3$ caffeine surrogate internal standard. The internal standard (1 mg/L) was added to the prepared samples prior to GC-MS analysis. Aliquots of 1 μL (Agilent Technologies 7693 autosampler) of the spiked samples were analyzed by GC-MS on an Agilent 7000A GC-MS triple quad model (USA). The samples were injected in splitless mode with the injector port temperature at 280°C. A 30 m x 0.25 mm i.d. DB-5MS (5% phenyl, 95% methylpolysiloxane) capillary column (Agilent, USA) with a 0.25 μm film thickness was used. Helium was used as the carrier gas at a constant flow of 1.2 mL/min. The column temperature program for the GC oven was as follows: initial temperature 70°C, maintained for 2 mins and then ramped at 20°C to 230°C, where it was held for 4 mins. Total run time was 10 mins. Natural caffeine (194 → 109) and its deuterated analogue (197 → 111) were characterized by multiple reaction monitoring (MRM). Both caffeine and its analogue produced a retention time of 6.4 ± 0.02 mins. The Red Bull sample was quantified by

internal standard calibration. The instrumental detection limit (LOD) and quantification limit (LOQ) were defined as the lowest analyte concentration that produced a peak with signal-to-noise ratio of 3 and 10, respectively[22].

RESULTS AND DISCUSSION

Table 1 shows results analysis of caffeine in coke at pH 3 and 8. The latter pH level gave a slightly higher caffeine concentration than the former. However, student's t-test analysis (95% confidence) revealed there was no significant difference between the caffeine concentration found in coke at pH 3 and 8. Researchers maintained that caffeine is found in its protonated state below pH 10.4.[12] Thus, this may be primary reason for the similar results at both pH levels investigated. Since pH 8 gave a slightly higher caffeine concentration than pH 3, the alkaline pH condition was used for analysis of beverages.

Table 1: pH optimization - Average caffeine concentration of coke zero at acidic and basic conditions (n = 3 replicates, LOQ = 0.1 ppb)

Beverage	pH	Caffeine (mg/L)	Relative standard deviation (%)
Coke zero	3	58	± 5.2
Coke zero	8	64	± 3.1

The caffeine concentration in caffeinated beverages was found in the order Diet Pepsi > Coke Light > Coke Zero, as reported in Fig. 1.
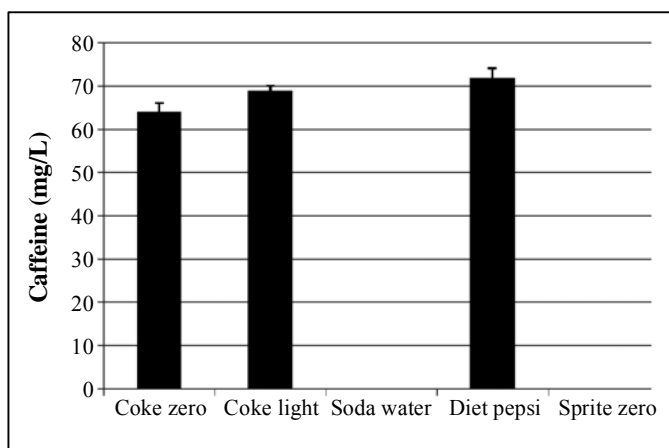

Fig. 1: Mean caffeine concentration in carbonated beverages (n = 3 replicates, LOQ = 0.1 ppb)

Soda water and Sprite Zero were found to have caffeine concentrations below the limit of quantification, 0.1 ppb. There was no significant difference (95% confidence) between the caffeine content of Coke Zero and Coke Light, however, a significant difference was found between Coke Zero and Diet Pepsi. Importantly, all carbonated beverages were found below the US FDA limit (100 mg per 17 oz. serving) of caffeine content in soft drinks.

Caffeine in coffee and herbal teas is reported in Table 2. Interestingly, Lipton Green Tea Lemon and Ginseng was found to contain about twice the caffeine content of regular coffee. This may be as a result of the type of coffee beans that was use in the manufacturing of the coffee as coffee beans are known to have varying caffeine content. Furthermore, the caffeine in herbal teas varies depending on the brand or type of plant used in its preparation.[23] The decaffeinated coffee was found to contain about 94% less caffeine than its caffeinated brand. This was expected as decaffeinated coffee removes about 94-98% of the caffeine originally present depending on the method of decaffeination[23]. Lipton Decaffeinated green tea was found to contain about 80% less caffeine than regular Lipton green tea. The difference in the levels of caffeine in decaffeinated coffee and tea in comparison to their caffeinated brands may be due to different methods of decaffeination. Caffeine free herbal teas contain negligible or no caffeine.

Table 2: Average caffeine concentration of coffee and herbal teas (n = 3 replicates, LOQ = 0.1 ppb)

Beverages	Weight (g)	Caffeine (mg/L)	Caffeine (mg/g)	Relative standard deviation (%)
Lipton green tea	1.9	99	13	± 7.3
Lipton decaffeinated green tea	1.5	21	3	± 9.8
Lipton 100% natural tea	2.8	87	8	± 2.8
Lipton green tea lemon and ginseng	1.5	305	49	± 8.5
Alvita senna leaf caffeine free tea	1.5	< LOQ	-	-
STASH Peppermint caffeine free tea	1.2	< LOQ	-	-
Colcafé instant coffee	1.0	236	24	± 4.3
Colcafé decaffeinated instant coffee	1.0	15	1.5	± 1.8

The caffeine content in energy drinks were found in the order 5- hour Energy > Red Bull > Bullet > Monster. There was a major difference in the caffeine concentration of

5-hour Energy compared to the other energy drinks as shown in Table 3. This may be as a result of 5-hour energy being consumed specifically for a maximum boost of energy resulting in improved alertness whereas the other energy drinks are commonly consumed socially. Moreover, it is caution not to consume 2 bottles of 5-hour energy daily as it may result in health implications such as tachycardia.

Caffeine was extracted from Red Bull via LLE as opposed to direct injection since it was the only sample that contained natural (beet) sugar. The sugar present in the sample has a tendency to block active sites in the GC column, which will in turn affect peak shape and resolution[24,25]. Thus, LLE and internal calibration was employed to overcome the matrix effect.

Table 3: Average caffeine concentration in energy drinks (n = 3 replicates, LOQ = 0.1 ppb)

Energy drinks	Caffeine (mg/L)	Relative standard deviation (%)
Bullet	171	± 2.9
Monster	135	± 3.7
Red bull	196	± 1.5
5-hour energy	7390	± 3.9

Although caffeine is a common source of energy that relieves tiredness it can also be detrimental to human health (e.g. cardiovascular disorders) and possibly the environment. Thus, it is quite important for individuals to be aware of their daily caffeine consumption. By the food industry listing the caffeine content of food and beverages on each product; it may be the most fitted alternative for individuals to properly manage their daily caffeine in-take.

CONCLUSION

The direct injection method developed for caffeine analysis of sugar free beverages via GC-MS is relative easy, fast and reliable. It does not require expensive solvents and reagents, thus it may be recommended for rapid, precise and sensitive quantification of caffeine in beverages. Among the beverages investigated 5-hour Energy contained by far the highest caffeine content. Energy drinks contained caffeine concentrations in the following order 5-hour Energy > Red Bull > Bullet > Monster. The caffeine content of carbonated beverages were found in the order Diet Pepsi > Coke Light > Coke Zero. Decaffeinated coffee and Lipton green tea contained about 94% and 80% less caffeine than their caffeinated brands, respectively. Caffeine in herbal teas varied depending on the brand of tea.

Interestingly, Lipton green tea with lemon and ginseng was found to have higher caffeine content than caffeinated coffee. Non-caffeinated beverages were found to contain insignificant caffeine.

Caffeine is commonly consumed because of its ability to increase alertness and thinking capacity, however, it is imperative for consumers to know the caffeine content of habitually consumed beverages as high caffeine consumption can have health implications.

ACKNOWLEDGEMENT

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