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Analytical methods for quality and quantity control of food supplements, containing caffeine

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Abstract: Many beverages such as soft drinks, coffee and tea contain the mild stimulant caffeine ($C_8H_{10}N_4O_2$). The caffeine content varies widely from about 100 µg/mL (100 ppm) in sodas to over 1000 µg/mL in certain types of coffee. For this reason the caffeine and the content in they need to be analyzed. A rapid and selective high-performance liquid chromatographic (HPLC) method is developed for the separation and determination of caffeine.

Keywords: Caffeine, HPLC, Quality, Control

1. Introduction

Recently the pharmaceutical and food industries produce a large number of supplements and products which contain caffeine. Xanthine derivate caffeine (CF), 1, 3, 7-trimethylxanthine, is widely found in the human diet. These compound naturally occurs in many food products such as tea, coffee, and cocoa beans, being the most abundant xanthine in chocolate (1, 2). Caffeine plays an important role in human nutrition and health maintenance. Due to the large consumption of caffeine and the production capacity requirements are constantly increasing. This paper reviews the methods for the quality and quantity control of food and supplements, containing caffeine (1, 2-10).

The main objective of this study is to produce a quick and reproducible method for the routine, simultaneous analyses of CF, in food, drinks, and herbal products (1, 11-19).

2. Material and Methods

2.1. Material

All solvents and reagents were of analytical grade unless indicated otherwise. The solutions were prepared with deionized water. The standards of CF, were obtained from Sigma. Acetonitrile and methanol (HPLC grade) were obtained from Sigma. Chloroform and tetrahydrofurane (THF) were HPLC grade, obtained from Sigma.

2.2. Real Samples

The natural products: black tea, green tea, Nescafe, coffee, cocoa powder.

Different energy drinks and beverages: Redbull, Pepsi, Coca Cola, Derby Cola, Shark, Pitbull, Party Cola.

2.3. Equipment

The chromatographic procedure was carried out using: Liquid chromatograph Shimadzu LC – 10 Advp equipped with 4.6 x 250 mm column Luna 5U C18 (2) 100 A, Phenomenex ODS with particle size 5 µm;

Detector SPD 10 AVvp – UV-VIS with fixed analytical wave lengths.

2.4. Chromatographic Conditions

Isocratic mobile phase, prepared by mixing of filtered and degassed Acetonitril / Phosphate buffer (35:65 v/v)

- 226 nm analytical wavelength;
- Column temperature 25 ^{0}C;
- Flow rate about 1.5 ml/min.

2.5. Procedure

In reversed phase HPLC water is considered a "weak" chromatographic solvent and organic solvents such as

methanol are considered "strong" chromatographic solvents. A typical methods development procedure for HPLC starts with elution using a strong solvent to elute all components in a mixture quickly. In a series of isocratic runs, weaker solvents are blended in with the strong solvent to achieve resolution of the components of interest (1-10,17,22).

2.6. Calibration Standards

Devise a plan for making a series of dilutions to prepare 4 calibration standards with concentrations from 20 to 100 µg/mL starting with the 1000 µg/mL caffeine standard. Use only your 10-mL and 25-mL volumetric flasks and HPLC grade water for the dilutions. Store each solution tightly capped in a labeled 15-mL vial (1).

The working standard solutions (0.2–10.0 µL) of CF were injected into the HPLC, and the peak area responses were obtained. A method of the external standard calibration was used. The separation of the standard mixture of CF using the method described is shown in Figure 1. The linear standard curves for CF were obtained separately by plotting concentration versus area.

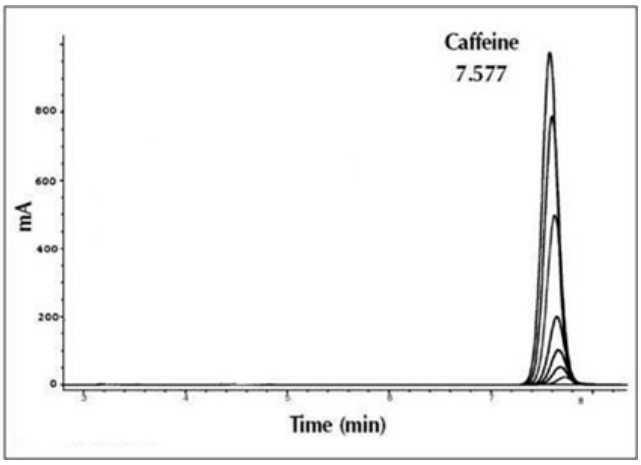

Figure 1. *Chromatograms of working stock solution*

2.7. Preparation of Caffeinated Beverage

Obtain a sample for which you wish to measure the caffeine content. If choose a carbonated beverage, your sample must be degassed before injection.

2.8. Analysis of Caffeine in Beverages

Reverse phase HPLC is used to determine the concentration of caffeine in coffee, tea, and soft drinks. The traditional method for the determination of caffeine is via extraction with spectrophotometric quantitation. Use of the liquid chromtography system permits a fast and easy separation of caffeine from other substances such as tannic acid, caffeic acid, and sucrose found in these beverages. Five standard solutions of caffeine are prepared and in injected into the HPLC. In addition, the beverages coffee, decaffeinated coffee, tea, and caffeine containing soft drinks are prepared as indicated in the following section and injected into the HPLC. From the resulting chromatograms, measurements of retention time, tR, and peak areas are made. If the flow rate and pump pressure are held constant throughout the entire experiment, tR may be used as a qualitative measure and the peak area as a quantitative measure. A calibration curve for peak area against concentration of the caffeine standards can then be employed to determine the concentration of caffeine in the four beverages. The solvent (mobile phase) in this experiment is 47% methanol / 53% water (1, 2, 5).

2.9. Sample Preparation and Extraction

The water extracts of a black tea (5.00 g) and green tea (5.00 g) were made by mixing for 30 min in hot water (200 mL, first boiled) in a thermal flask on the magnetic stirrer. The extracts were then filtered through a filter paper to remove the particulate matter. Ten milliliters of filtrate, adjusted to pH 8 with 0.1 M NaOH, were subjected to the cleanup procedure. The coffee powder samples were weighed (5 g) and extracted with boiling hot water (200 mL) by mixing in the thermal flask for 10 min on the magnetic stirrer. The extracts were then filtered through the filter paper to remove the particulate matter. Ten milliliters of filtrate, adjusted to pH 8 with 0.1M NaOH, were subjected to the cleanup procedure as described later. The cocoa powder (5 g), were filled up to 200 mL with water in a plastic container and extracted for 40 min at 45°C in the ultrasonic bath (1,5,15,18,19).

The extracts were then filtered through the filter paper to remove the particulate matter. Ten milliliters of filtrate, adjusted to pH 8 with 0.1M NaOH, were subjected to the clean-up procedure.

The samples of fizzy drinks were degassed for 25 min in an ultrasonic bath to release the CO2. Prior to the analysis, the samples were adjusted to pH 8 with 0.1M NaOH, filtered through a 0.20-µm nylon filter, and injected directly into the HPLC (1, 9).

3. Discussion

The development of the method was based on the experience obtained from the methods previously developed for the analysis of CF and some other compounds of interest (1–9). Of the columns tested (Hypersil ODS C18 100 ×4.6 mm, Cosmosil C18 150 × 4.6 mm, Cosmosil C18 250 × 4.6 mm, Phenomenex Luna 5 µm C8 150 × 4.6 mm, Phenomenex Luna 5 µm C8 250 × 4.6 mm, and Zorbax Eclipse XDB-5 µm C8 column 150 × 4.6 mm), it was only by using the Zorbax Eclipse XDB-5µm C8 column 150 × 4.6 mm that a good separation for CF.

The effect of the flow rate and the composition of the mobile phase on the retention time (tR), the peak width (W50), for CF were studied using the working standard solution.

The injection volume of the working standard solution was 5 µL, and the column temperature was at 25°C. The combination of 0.8 mL/min flow rate and water–THF (0.1 % THF in water, pH 8)–acetonitrile (90:10, v/v) as the mobile phase is selected as a compromise between the analyte retention time (sampling rate), separation efficiency (number of theoretical plates), and the consumption of solvents (1,25).

3.1. Validation of the HPLC Method

We studied the selectivity (different samples with different matrices) and linearity in the range of 0.5 to 300 mg/L (0.5, 1.0, 3.0, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0, 125.0, 150.0, 250.0, and 300.0mg/L). Also studied were the results of the assay, and limit of detection (LOD—signal to noise [S/N] ratio 3:1), and limit of quantitation. There was no interference in the HPLC results by the matrices ingredients in any of the tested samples, which indicates that the methods are selective (Fig. 2).

The accuracy of the method was determined by analyzing the solutions of the known concentrations (the working standard solutions) and comparing the measured and known values (1,2).

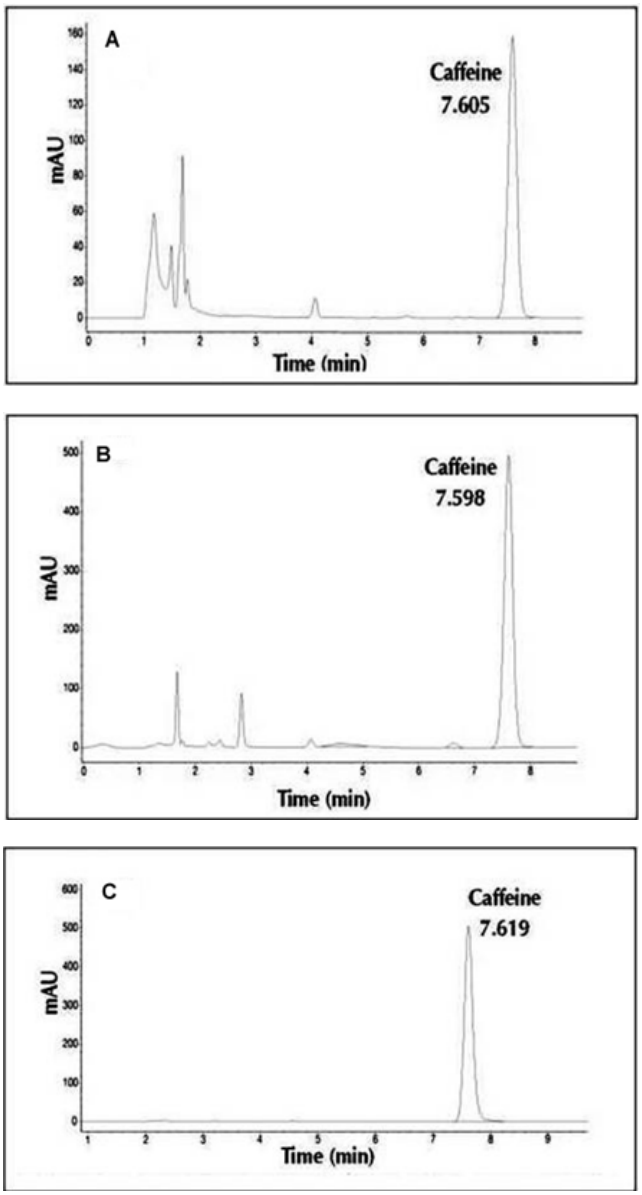

Figure 2. *Chromatograms of some real samples under the optimized conditions, at 273 nm. Mobile phase was water – THF (0.1% THF in water, pH8) – acetonitrile (90:10,v/v), flow rate was 0.8 mL/min, column temperature was 25°C: Coca cola (A), Red Bull (B), Pitbull(C).*

A repeatability test was performed to determine an intra-day variation in the peak's areas and retention times. The highest value for relative standard deviation (RSD) was 0.84% (n = 6), which indicates that repeatability of the method is acceptable.

An intermediate precision was evaluated over three days (inter-day repeatability) using the working solution. This solution (0.2–10.0 μL) was injected daily under the same conditions and the results were used for the repeatability study. The solution was stored at room temperature (25 ± 2°C) in sunlight (1,3,7,12, 18).

4. Results

The contents of CF obtained from the measurement of the numerous different samples of food, beverages, and natural products are shown in Table 1. These results show a strong correlation between the declared and determined values of CF for all analyzed samples, which implies a high efficacy and selectivity of the method used. The use of C8 column packing results in a better resolution, intensity, shape, and symmetry. The run time for the analysis is less than 8 min. The lowest concentration that can be quantitated (LOQ) with an acceptable accuracy and precision and it was 0.2 mg/L for CF respectively. Furthermore, the LOD defined as 0.07 mg of CF /L(1,20-25).

Table 1. *Application Results for Natural Products, Beverages, and Food Samples*

Beverage and food samples	Found values	Declared values
	CF	CF
Coca cola (mg/L)	106.7	≤ 150
Derby coca (mg/L)	112.0	≤ 150
Red bull (mg/L)	301.8	≤ 320
Pitbull (mg/L)	230.1	≤ 250
Party cola (mg/L)	101.2	≤ 150
Pepsi (mg/L)	118.0	≤ 150
Shark (mg/L)	349.0	≤ 350
Green tea (mg/100 g of sample)	1301.1	n.d.
Black tea (mg/100 g of sample)	989.7	n.d.
Nescaffe (mg/100 g of sample)	3582.1	n.d.
Cocoa (mg/100g of sample)	50.0	n.d.

* n.d. = not declared.

5. Conclusion

The present method was tested to measure the CF in food, beverages, and natural products. In this work a fast, accurate, and sensitive method was developed for the determination of CF in food, beverages, and natural products. The use of the SPE pretreatment for the samples and results of the recoveries for this procedure confirmed that there is no matrix effect, so the extracts can be assessed with a calibration curve set from the analytes aqueous standard. Finally, the data for sensitivity, accuracy, reproducibility, and high analysis frequency suggest that the proposed HPLC method could be used for a routine quality control of food, drinks, and herbal products.

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