Analytical validation and quantification of method for determination of the concentration of caffeine in decaffeinated products

LIMA, M. F. F. de[1]; FRANÇA. G. I. S.[1]; SOBRINHO, J. R. B. C.[1]; SOUSA, C. E. M. de[1]*.

[1]Centro Universitário Tabosa de Almeida, Avenida Portugal, 584, Bairro Universitário, CEP 55016-901, Caruaru, Pernambuco, Brasil.

e-mail: nandadelyma@gmail.com or eduardomiranda@nudfac.com.br

ABSTRACT

Address in this article the development and validation of analytical technique for quantification of caffeine by high performance liquid chromatography (HPLC-UV). The study aimed to determine the caffeine content and check the levels in decaffeinated coffees are within the standards determined by regulatory agencies. The chromatographic analysis was performed on a column C-18 reverse phase Phenomenex®, Gemini, mobile phase water/acetonitrile. The samples were extracted and delivered in acetonitrile and analyzed as compared to caffeine in chloroform standard (1 $mg.ml^{-1}$). The given technique presented the best conditions of chromatographic separation. The results provided by the validation demonstrated technique with very good linearity, precision and accuracy, In addition to simple, rapid implementation methodology, with lower costs and satisfactory results, reduced time without compromising the trustworthiness of the analysis.

Key words: caffeine; High Performance Liquid Chromatography (HPLC); Quantification; Validation.

1. Introduction

The caffeine (1,3,7-trimetil-1H-purino-2,6(3H,7H)-diona) is a xanthine with structural formula $C_8H_{10}N_4O_2$ and molecular mass of 198.19 g/mol1, presents a crystalline form, white and shiny appearance, characteristic of alkaloids group to which it belongs. Debugging of the substance in the human body is via the liver through the cytochrome P450 CYP1A2 system, consisting of demethylation of caffeine, producing three major active metabolites, 3.7-dimethylxanthine; 1.3- dimethylxanthine and in greater quantity, 1.7- dimethylxanthine. Its bioavailability via gastrointestinal is over 90% and plasma achieved peaks between 20 to 30 minutes after ingestion. Caffeine is present in the diet through the coffee, soft drinks, isotonic drinks, vitamin supplements and other produtos[1-4].

Figure 1.

The action in the body is directly in the excitable cell membranes ions and systemic actions in the central nervous system (CNS). Provides increased strength of muscular contraction, due to the effect of release of calcium ions from the sarcoplasmic reticulum of cells, preventing the re-uptake and the consequent increase of the free concentration. Performance in the sodium pump (Na^+/K^+), keeping the concentrations of potassium (K^+) high inside cells and low on your exterior, increasing cell excitability. In addition to inhibiting the Phosphodiesterase enzyme responsible for the degradation of the second messenger cyclic adenosine monophosphate (cAMP), increasing your time to half life. And, antagonism A1 receptors, adenosine, which also interact with the cAMP, causing an increase of this second Messenger and, in response, release of catecholamines, blood pressure, urine output and activation of CNS[5-7].

Numerous studies determine analytical methods for identification and quantification of caffeine. The techniques vary between the gravimetric methods, gas chromatography (GC), liquid chromatography (LC) coupled with mass spectrometry and infrared. Are efficient,

provide reliable results and few but highly laborious interfering, requiring the processing of large quantities of samples, high individual processing time or not available in the laboratories of analysis because of the high cost of equipment and reagents required in execution[8,9].

Methods with high performance liquid chromatography (HPLC-UV) are frequent in the literature of analysis of caffeine, nothing better effectiveness in the ultraviolet range. Despite the information available in the literature, it is remarkable the lack of analytical standard, processing time too long (> 10 min) and specific procedures of quantification. In this way, it is necessary to a method to quantify levels of caffeine in non-biological pathways to support studies of quality control of products[10-16].

This paper presents an analytical HPLC-UV method for the determination of the levels of caffeine in decaffeinated coffee. The technique uses a liquid-liquid extraction and analysed in the range $\mu m.mL^{-1}$. The study aims to determine the caffeine content and verify that the levels of caffeine in decaffeinated coffees available in the Brazilian market are within the standards determined by the regulatory agencies.

1. Materials and methods

2.1. Instrumentation

The procedure of high performance liquid chromatography (HPLC-UV) was carried out using a gas chromatograph system of binary pumps (LC 20AD), manual gun (SIL), an automatic transmitter, a system controller (CBM 20A) connected to the ultraviolet detection (SPD 20A) Shimadzu (Kyoto, Japan). For the sample extraction procedure of caffeine was used at Fanem centrifugal Excelsa II 206 BL (Brazil). The samples were stored in refrigerator Consul (São Paulo, Brazil) until analysis.

2.2. Chromatography conditions

The chromatographic analysis was performed on a C-18 reversed-phase column Phenomenex®, Gemini with a length of 150 mm, inner diameter of 4.6 mm e and 5 µm sized particles. The mobile phase was composed of water/acetonitrile (82:18 v/v) and pumped in 0.8 $mL.min^{-1}$. The procedure was performed in a temperature of 22° C and the detection in 285 nm wavelength.

2.3. Preparation of working solutions and quality control standards

The reserve solution of caffeine (1 $mg.mL^{-1}$) was prepared by dissolution of caffeine in chloroform (m/v). The calibration curve of caffeine was built using methanol at concentrations: 10, 25, 50, 75, 100 $\mu g.mL^{-1}$. Samples for precision and accuracy were also prepared the caffeine in chloroform standard (1 $mg.mL^{-1}$) and diluted in methanol, in concentration of 50 $\mu g.mL^{-1}$.

2.4. Sample preparation

The samples were prepared for a 0.25 g becker uploading, to which was added 0.5 mL of sulfuric acid (H_2SO_4) 2 M. Then added 5 mL of distilled water at a temperature of 70° C and was kept under stirring. The system was basified with 0.25 mL of potassium hydroxide (KOH) and kept stirring until it reaches room temperature. The samples received 4 mL of chloroform and were subjected to centrifugation at room temperature by 5° C at 2500 x g. The lower phase was separated and received 2 mL of chloroform and resubmitted the same spin conditions. The organic phase was transferred to becker and evaporated to dryness. The residue of the samples were redissolved in 2 mL acetonitrile/water (1:1 v/v) and packed in eppendorf for analysis.

2.5. Method validation

The quantification was based on the ratio between the area of the peak of samples of decaffeinated coffee and caffeine peak area of the standard. In determining the accuracy (relative standard deviation) and accuracy (error-related studies) samples were prepared from two analysts, with six applications each, analyzed on the same day (intra day precision and accuracy) and in another straight day (precision and accuracy inter day).

2.6. Method Application

The method was determined considering the best condition obtained from chromatographic procedure. The study used only for extraction each sample of decaffeinated coffee, the products have been listed 1, 2, 3, 4, 5 and 6, in random order, the names of the manufacturers were confidential for ethical reasons. Samples of the cafes after the extraction procedure were suspended in acetonitrile/water (1:1 v/v), and chilled to 8° C until analysis.

2.7. Statistical analysis

The values obtained from the analysis were calculated using the Lab-software Solutions (version 3.6). These data, plotted in Excel ® 2010, with the following statistical treatments: mean, standard deviation, coefficient of variation, and linear regression.

.3. Results and Discussion

3.1.HPLC chromatogram

Analyzing the results obtained in accordance with the experimental methodology applied, the caffeine in the samples presented good condition of separation of the other components of coffee and without interfering in all six samples. The retention time of caffeine was 4.578 minutes and the total time of race for each sample was 7.0 minutes, as you can see in Figure 1. In the literature it is reported the use of mobile phase consisting of methanol aqueous solution of phosphate buffer (< 0.1, > 0.01 $mol.L^{-1}$ and pH between 4.0 and 5.0), but the use of

aqueous solution of acetonitrile without buffer did not interfere in the quality of reading cromatográfica17-20. The peaks were well defined, in short time.

Figure 2.

3.2.Linearity

The linearity of the chromatographic analysis was assessed in various concentrations of caffeine, standard in the range of 10-100 $\mu g.mL^{-1}$. The results showed good linearity calibration curve, proven by the coefficient of determination (r^2) worth of 0.9965 and with great precision and accuracy, represented in Table 1. The detection limit was determined in 0.1 $\mu g.mL^{-1}$ and the limit of quantification was 0.35 $\mu g.mL^{-1}$.

Figure 3.

Table 1.

3.3.Precision and accuracy

In the study, the precisions were referred to with the relative standard deviation (RSD %) at concentrations of 50 $\mu g.mL^{-1}$, showing values <2%. The intraday precision, between the applicators 1 and 2, were of 0.48 % and 1.22 %, with an accuracy of 102.887 % and 100.790 %, respectively and day 2, 1.50 % and 0.64 % and an accuracy of 100.696 % and 99.591 %, respectively, according to the Table 2.

Table 2.

3.4.Quantitative study

Quantitative analysis of caffeine in products tested is described in Table 3. The concentration values were calculated from the area of caffeine in each sample. The average concentration found was 0.1887 $g.100.g^{-1}$ ± 10.7. All the values found within the range of less than 0.3 g.

$100.g^{-1}$, according to the Resolution of the Collegiate Board Brazilian (RDC) No. 277, September 22 2005, the National Agency of Sanitary Surveillance (ANVISA). The greatest concentration of caffeine was 0.2388 $g.100.g^{-1}$ and the minor by 0.0946 $g.100.g^{-1}$, in 1 and 6 samples, respectively.

Table 3.

4. Conclusion

Quantification of caffeine in decaffeinated coffee showed that all products presented below level 0.25 $g.100.g^{-1}$ represents that all products are analyzed with the determinations of the regulatory agencies of Brazil, which determines that the caffeine content of less than 0.3 $g.100.g^{-1}$. The results are ensured by the validation, which demonstrated that the technique developed presented very good linearity, precision and accuracy, as well as providing a simpler methodology for extraction and analysis, reduced run time of reading (7 minutes), without compromising the reliability of the results. The methodology developed has enabled the verification of a technique quickly and with lower costs of analysis and satisfactory results in the study of quantification of caffeine in decaffeinated coffees.

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6. References

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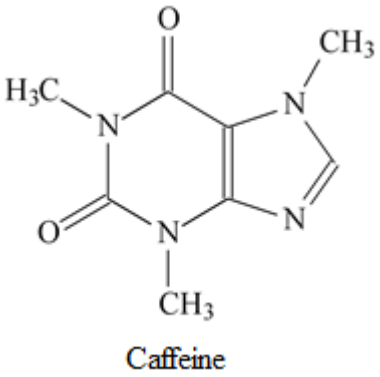
ANEXO – Imagens

Figure 1. Chemical structure of caffeine



Caffeine

Figure 2. Chromatogram with reading information from caffeine

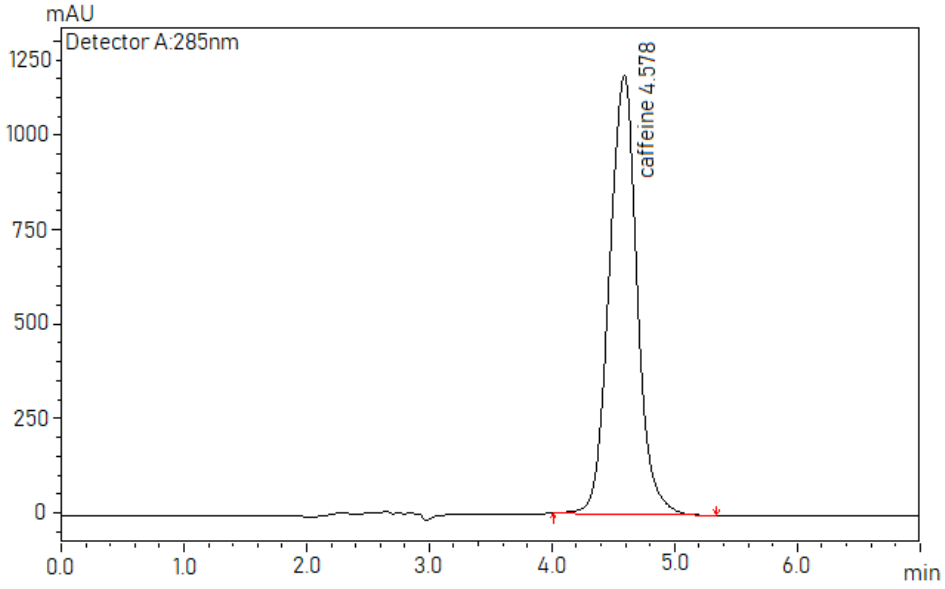


Figure 3. Concentration profile-caffeine after chromatographic reading area. Data on average. 6 samples.

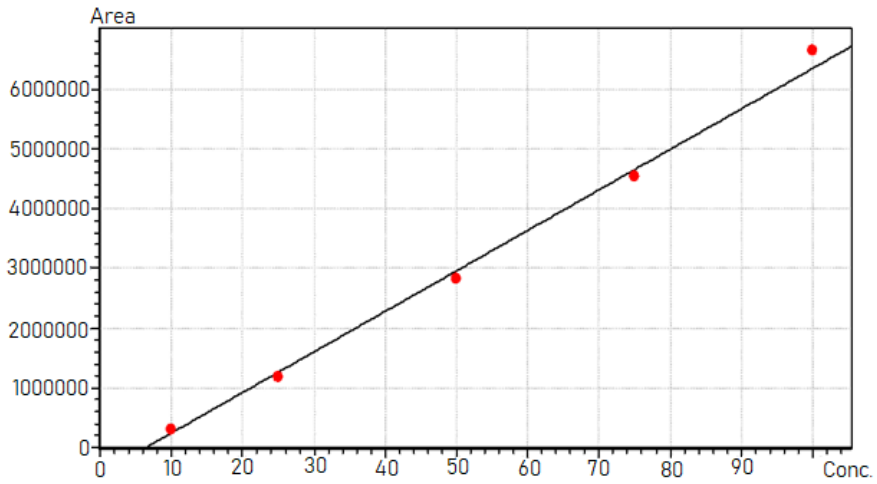


Table 1. Accuracy and precision of Linearity.

Spiked concentration ($\mu g\ mL^{-1}$)	Determinad concentration ($\mu g\ mL^{-1}$) (Mean ± SD) (n=3)	Precision (%)	Accuracy (%)
10	10.113± 0.255	2.52	101.13
25	24.961± 0.217	0.87	97.84
50	50.352± 0.605	1.20	100.70
75	77.553± 0.481	0.62	103.40
100	103.655± 1.095	1.06	103.65

Table 2: Precision and accuracy results of validation

Spiked conc. ($g.mL^{-1}$)	Intraday ($\mu g.mL^{-1}$) (n=6)			
	Day 1		Day 2	
	Applicator 1 Mean (R.S.D.)	Applicator 2 Mean (R.S.D.)	Applicator 1 Mean (R.S.D.)	Applicator 2 Mean (R.S.D.)

0.5	51.444 (0.48%)	50.395 (1.22%)	50.348 (1.50%)	49.796 (0.64%)
Acc. (R.E.)	102.887	100.790	100.696	99.591

Table 3: Concentrations of caffeine found in each sample

	Cafeína (n=4)	
Test data	**Conc. g/100g**	**Precision (R.S.D.) (%)**
Amostra 1	0.0946	6.935
Amostra 2	0.2371	4.103
Amostra 3	0.1964	8.937
Amostra 4	0.1679	5.566
Amostra 5	0.1972	3.768
Amostra 6	0.2388	3.480