IJPSR (2016), Vol. 7, Issue 6 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 January, 2016; received in revised form, 12 April, 2016; accepted, 04 May, 2016; published 01 June, 2016

A DERIVATIVE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF METHYLXANTHINES AND TANNINS IN GUARANA BULK (*PAULLINIA CUPANA*)

I. Roggia [1], S. Ziegler [2], I.B.M. Cruz [3], A.F. Ourique[1] and P. Gomes [*1, 2]

Programa de Pós Graduação em Nanociências, Centro Universitário Franciscano (UNIFRA) [1], Santa Maria/RS, Brazil.
Curso de Farmácia, Centro Universitário Franciscano (UNIFRA) [2], Santa Maria/RS, Brazil.
Programa de Pós-Graduação em Bioquímica Toxicológica, Universidade Federal de Santa Maria (UFSM)[3], Santa Maria, RS, Brazil.

Key words:

Methylxanthines; Tannins; *Paullinia Cupana;* Derivative Spectrophotometry.

Correspondence to Author:
Professor Patricia Gomes

Centro Universitário Franciscano,
Conjunto I, Rua dos Andradas, 1614,
Santa Maria/RS, 97010-032, Brazil.

Email:patriciagomes@unifra.br

ABSTRACT: A method using derivative UV spectrophotometry was developed and validated for the determination of methylxanthines and tannins in guarana bulk, using caffeine and catechin as markers. The spectra were obtained using first order derivatization, Δλ=20.000, and scale factor of 10. The quantification of actives was performed using wavelengths of 244 and 277 nm for catechin and caffeine, respectively. The parameters of specificity, linearity, precision, accuracy, robustness, limits of detection (LOD) and quantification (LOQ) were established to validate the method. The method was shown to be specific for quantification of methylxanthines and tannins, and linear over the concentration range 5–35 μg mL^{-1}, with a correlation coefficient of r=1(caffeine) and r=0.9993 (catechin). The results indicated good intra-day and inter-day precision with RSDs less than 2%. The method was accurate, exhibiting recoveries between 98 and 102%. The LODs were 0.184 μg mL^{-1} (caffeine) and 0.368 μg mL^{-1} (catechin), and the LOQs were 0.552 μg mL^{-1} (caffeine) and 1.104 μg mL^{-1} (catechin). Methylxanthines and tannins were quantified, and contents of 10 μg mL^{-1} and 20 μg mL^{-1}, respectively, were observed.

INTRODUCTION: *Paullinia cupana var. Sorbilis (Mart.) Ducke*, belongs to the family *Sapindaceae*, popularly known as guarana, and is a native fruit of the Brazilian Amazon, where it is widely cultivated[1, 2]. It is a versatile plant in the food industry, and is used in the preparation of energy drinks, soft drinks, and food supplements [3, 4].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.7(6).2334-42

Article can be accessed online on:
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (6).2334-42

It is also widely used in the pharmaceutical industry in the production of medicines, which are commercially available in various forms, such as teas prepared from fresh or dried plants, elixirs, drops, and capsules. Consequently, it is listed in the Brazilian Pharmacopoeia [5]. Furthermore, it is present in various cosmetic products due to its antioxidant and antimicrobial activity [6].

Guarana seeds contain large amounts of methylxanthines, including caffeine (CAF), theophylline, and theobromine, and tannins such as catechin (CAT) and epicatechin, as well as dimers composed of flavan-3-ol units (procyanidins B2, B3, and B4) [7]. Of these, caffeine and catechin (**Fig.1**) are the major components.

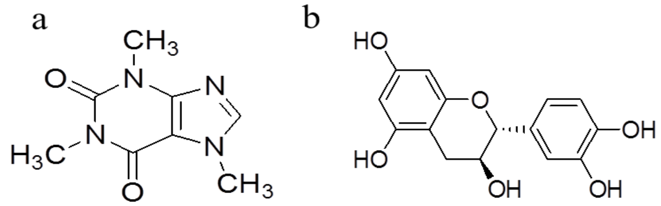

FIG. 1: CHEMICAL STRUCTURE OF (a) CAFFEINE (CAF) AND (b) CATECHIN (CAT)

These active compounds are responsible for several beneficial effects attributed to guarana, including antimicrobial [8, 9], antioxidant [8, 4], anticarcinogenic, antimutagenic [10], hypocholesterolemic [11], anti-inflammatory [12], and antiproliferative effects[10], as well as beneficial genomic effects [13] and memory improvement [14].

The literature provides different methods to identify and/or quantify methylxanthines and tannins in guarana samples, including spectrophotometric and chromatographic methods. In the Brazilian Pharmacopoeia [5], Antoneli-Ushirobira et al., [1], Pelozo et al., [15] and Sousa et al.,[2] describe methods using UV spectroscopy to quantify methylxanthines and UV-Vis spectroscopy to quantify tannins. We also found several quantification methods using high performance liquid chromatography (HPLC) [1, 2, 4, 7, 16, 17]. However, this technique requires expensive equipment and has a high cost of maintenance and operation[17].

In this context, UV spectrophotometry is a simple technique with low operating costs and is commonly used for quality control in the pharmaceutical industry, which requires fast and reliable results [18]. Its main limitation is its low selectivity [19]. When working with complex materials such as guarana, which has several active compounds, the lack of selectivity of UV spectroscopy makes it difficult to distinguish these compounds [2]. Furthermore, some visible spectrophotometric methods often require time-consuming pre-separation techniques to remove possible interferences from colored materials such as catechin, as described in the Brazilian Pharmacopoeia [5].

One of the simplest methods to overcome this limitation is derivatization of spectra. This operation allows the removal of spectral interference and leads to increased selectivity of the assay [20]. Thus, derivative spectrophotometry (UVD) is a tool to solve analytical problems encountered with conventional spectrophotometric methods [21]. The technique provides better separation of the bands, improved signal amplification, reduced noise compared to conventional UV, and has greater selectivity and specificity, often without the need for prior preparation of the sample [21, 22, 23].

Alpdogan et al., [24] developed a methodology for derivative spectrophotometry to determine caffeine in cola, coffee, and tea. For this, they used second-order (cola) and third-order (coffee and tea) spectra. In the second-order derivative spectrum, they used 246.7 nm and 234.1 nm for the quantitative determination of caffeine in cola. In the third-order derivative spectrum, they used 286.7 nm and 265 nm for coffee, and 286.7 nm and 300.3 nm for tea. They concluded that the method of derivative spectrophotometry was a relatively easy, fast, and cheap method for determining the caffeine content in cola, coffee, and tea.

Currently, there are no reports of UVD methods in the literature for the quantification of methylxanthines and tannins in guarana samples. Therefore, the aim of this study was to develop and validate an analytical method using UVD spectrophotometry for the quantification of methylxanthines and tannins in samples of guarana bulk using caffeine and catechin as markers, respectively.

MATERIALS AND METHODS:

Chemicals and reagents:

Acetonitrile and analytical-grade catechin and caffeine standards were purchase from Sigma-Aldrich (St. Louis, EUA). Methanol and trifluoroacetic acid (TFA) were acquired from J.T. Baker (Mexico City, Mexico). Acetone, sulfuric acid, ethanol, and ethyl acetate were acquired from Synth (São Paulo, Brazil). Chloroform and monobasic potassium phosphate were acquired from F Maia (São Paulo, Brazil). Sodium chloride and dibasic sodium phosphate were obtained from Nuclear (São Paulo, Brazil). Potassium chloride was obtained from the market Qhemis (São Paulo, Brazil), guarana bulk was kindly provided by EMBRAPA Oriental (Agropecuary Research Brazilian Enterprise) (Amazon – Maués, Brazil).

Experimental conditions:
In the method developed, the best solvent (1:1, v/v), and for solubilization of the active compounds (caffeine and catechin) and best method to extract the active guarana bulk were investigated. Furthermore, the spectrophotometric conditions evaluated were derivative order, lambda delta (Δλ), scale factor, and wavelength of maximum absorbance for active compounds. The experiments were conducted using a UV-1650 1650PC double-beam UV-Vis spectrophotometer (Shimadzu®, Japan) with 1 cm quartz cells (UV Probe 2.21®).

Active compound solubility (caffeine and catechin) were assessed in ethanol, methanol, deionized water, acetonitrile, acetone, chloroform, 2.5% sulfuric acid solution, ethyl acetate, methanol: deionized water (1:1, v/v), and phosphate buffers at pH 5.0, 7.4, and 9.0. Extraction methods and selectivity by spectrophotometry (200 to 400 nm) were tested to find the optimum wavelength and solvent for the quantification of active compounds.

Six different methods of extraction of caffeine and catechin from guarana samples were tested: (1) 15 min ultrasonication; (2) 30 min ultrasonication; (3) 45 min ultrasonication; (4) 60 min ultrasonication; (5) 15 min stirring and 15 min ultrasonication; (6) 15 min of stirring and 30 minultrasonication; (7) 30 min stirring and 30 min ultrasonication. A magnetic stirrer (IKA® basic HR 2, Brazil) and ultrasound bath (Unique® Brazil) were used. Spectra were derived, and caffeine and catechins were quantified, from predetermined parameters. The best conditions for absorption and extraction of caffeine and catechin were chosen to conduct the experiments.

Sample preparation:
The use of deionized water and ultrasonication for 45 min were chosen as the best conditions for extraction of caffeine and catechin from guarana bulk. These parameters were used for conducting the experiments. The sample preparation was performed by weighing 50 mg of guarana bulk into a 25mL volumetric flask, which was charged with deionized water (2000 µg mL^{-1}).

Samples were ultrasonicated for 45 min and then filtered through quantitative filter paper. Subsequently, 1.25 mL of this solution was transferred to a 10mL volumetric flask, which was charged with deionized water to a final concentration of 250 µg mL^{-1} of guarana [5]. After the scan, spectra in over the range 200–400 nm were plotted.

Spectra were plotted and derivatives were investigated by testing different orders of reaction (first, second, third, and fourth order), different values of Δλ (10.000, 20.000, 40.000, and 80.000), and a scale factor of 10.

Through the study of spectra recorded using different conditions, parameters chosen for the determination and quantification of caffeine and catechin specifically were chosen, and are shown in **Table 1**.

TABLE 1: PARAMETERS FOR SPECTROPHOTOMETRIC QUANTIFICATION OF CAFFEINE AND CATECHIN IN GUARANA BULK SAMPLES.

Parameter	Parameters Chosen
Derivative order	first-derivative
Δλ	20.000
Scale factor	10
wavelength	277 nm – caffeine 244 nm – catechin
Scan Speed	Medium

Method validation:
After the choice of optimal conditions, the method was validated using samples of guarana bulk. Caffeine and catechin were quantified, representing class of methylxanthines and tannins, respectively. The method was validated considering the following parameters: specificity, linearity, precision (repeatability and intermediate precision), robustness, accuracy, and detection and quantification limits according to the International Conference on Harmonization [25], Brazilian regulation RE 899/2003 of the Agência Nacional de Vigilância Sanitária [26], and the Food and Drug Administration [27].

Linearity: To construct the analytical curves (n = 3) 25 mg of both caffeine and catechin were combined and transferred to a 100 mLvolumetric flask, which was filled with deionized water and placed in ultrasound for 45 min to complete solubilization of the actives to yield a solution with a concentration of 250 µg mL^{-1}.

From this standard solution, the standard curves were constructed in concentrations from 5–35 µg mL^{-1}using deionized water as the solvent.

The absorbances were obtained using a UV spectrophotometer using the derivative method parameters described in **Table 1**.

The average analytical curve and its corresponding linear equation were determined by linear regression using the least squares method. Analyses were performed in triplicate for each condition. Results were statistically analyzed with Graph Pad Prism version 5.0 software (Graph Pad, EUA), using variance analysis (ANOVA).

Detection and quantification limits:

The detection (LOD) and quantification limits (LOQ) for markers were calculated based on standard deviation (SD) and the slope (S) of analytical curve based on Eqs. (1) and (2) [25, 26].

$$LOD = \frac{3.3\,x\,SD}{S} \quad (1)$$

$$LOQ = \frac{10\,x\,SD}{S} \quad (2)$$

Specificity:

The specificity of the method was assessed using catechin and caffeine standards. To prove the specificity of the method on quantification of caffeine and catechin in guarana samples, 15 mg of each active were weighed separately into 25mL volumetric flasks, which were then made up with deionized water and ultrasonicated for 15 min to produce 600 µg mL^{-1} solutions. Then, 0.25 mL was transferred to a 10 mLvolumetric flask, which was made up with deionized water to produce 15 µg mL^{-1} solutions. The same procedure was performed with mixed actives in the same volumetric flask.

Samples of guarana bulk were prepared and analyzed as described in section "Sample preparation". In order to assess possible interference in the quantification of caffeine and catechin, spectra were plotted from 200 to 400 nm.

Precision:

Precision of the method was evaluated by repeatability (inter-day precision) and intermediate precision (inter-day precision). Results were expressed as relative standard deviation (RSD).

The repeatability was evaluated through six repetitions of the determination of the 250 µg mL^{-1} guarana bulk sample, in the same day and under the same experimental conditions.

Intermediate precision was obtained by analyzing the 250 µg mL^{-1}sample on three different days by two different analysts. Samples were prepared and analyzed as described in section "Sample preparation".

Accuracy:

Accuracy was assessed by a standard addition method in which the percentage recovery was examined. The percentage recovery was expressed as relative standard deviation (RSD).

A 150 µg mL^{-1} standard solution of caffeine and catechin was prepared using deionized water as solvent and employing ultrasonication for 45 minutes.

For the sample containing guarana bulk, 50 mg of guarana bulk was transferred to a 25 mL volumetric flask and dissolved in deionized water, stirred using an ultrasonic bath for 45 min, and filtered through quantitative filter paper to produce a 2000 µg mL^{-1} solution.

Accuracy was determined by recovery analyses, adding known amounts of caffeine (12.5, 15.0, and 17.5 µg mL^{-1}) and catechin (25.0, 30.0 and 35 µg mL^{-1}), corresponding to 25, 50, and 75% of the initial concentration.

Robustness:

Robustness of the method was evaluated through small variations to the original method, such as wavelength (for caffeine 276 and 278 nm, and for catechin 243 and 245 nm), diluent (distilled water), filtration (a 0.45µm filter) and ultrasonication time

(30 and 60 min). Samples were prepared and analyzed as described in section "Sample preparation".

Analyses were performed in triplicate for each condition and active determined. Results were statistically analyzed with Graph Pad Prism version 5.0 software (Graph Pad, EUA), using analysis of variance (ANOVA) and Tukey post-test analysis.

RESULTS AND DISCUSSION:

Initially, in order to investigate the best solvent for solubility of caffeine and catechin, several analytical parameters were evaluated, and it was observed that the best solvent was water. Similarly, Antonelli-Ushirobira et al.,[1]. Physicochemically evaluated guarana seeds by different methods using UV spectrophotometry, and found that water had the best extraction capability for guarana actives compared to different mixtures of water and organic solvents, such as acetone, methanol, and ethanol.

The solubility of caffeine and catechin in water is also described in The Merck Index. Caffeine has good solubility in water, methanol, acetone, and chloroform, and low solubility in ether and benzene. Catechin has good solubility in hot water, alcohol, acetic acid, acetone, and is practically insoluble in benzene, chloroform, and petroleum ether [28].

The spectra (200–400 nm) show that caffeine and catechin have the same absorptivity wavelength, thus it is not possible to quantify them specifically by conventional spectrophotometry (**Fig. 2A**). Thus, we used UVD spectrophotometry for conducting the experiments (**Fig. 2B**)

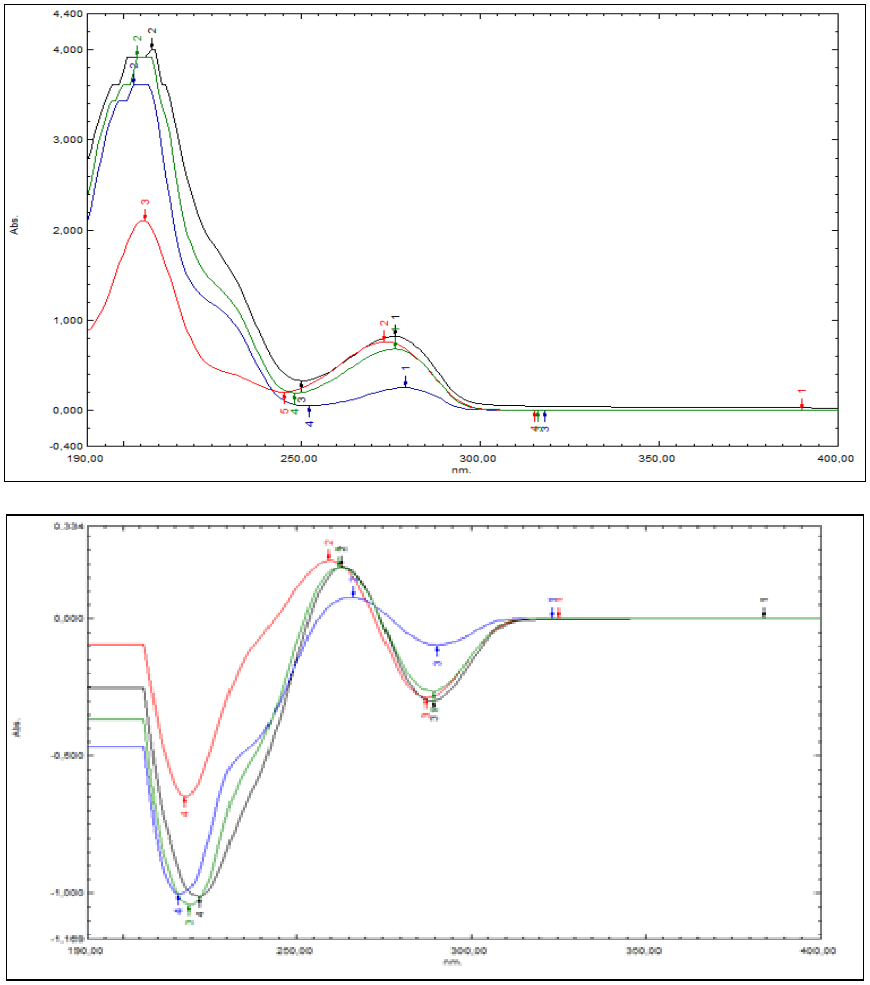

FIG. 2: SCANNING SPECTRA OF CAFFEINE (RED), CATECHIN (BLUE), CAFFEINE AND CATECHIN TOGETHER (GREEN) AND GUARANA (BLACK) NON-DERIVATIVE (2A) AND DERIVATIVE (FIRST-DERIVATIVE, ΔΛ 20.000, AND SCALE FACTOR 10) (2B).

As seen in **Fig.2A**, and as previously reported in the literature [21, 22, 29], one of the main problems with UV-Vis spectrophotometry when working with complex samples, such as guarana, is the superposition of bands (overlapping).

When this issue is seen, one alternative is to use UVD spectrophotometry. This can better individualize the components and eliminate the interference of one component over the other, thus allowing quantification of different actives in the same sample without the need of prior preparation.

After evaluation of various parameters, such as the derivative order (first, second, third, and fourth) and Δλ (10.000, 20.000, 40.000 and 80.000), it was observed that the best parameters for the quantification of caffeine and catechin was selectively using the first derivative, a Δλ of 20.000, and scale factor 10. When performed with other derivative orders (second, third, and fourth) and different Δλ, it was not possible to obtain well-resolved spectra. Using these parameters, wavelengths of 244 and 277 nm were chosen for quantification of catechin and caffeine, respectively.

The method was linear over a concentration range of 5–35 μg mL^{-1}. The linear equation was y =0.0093 (Abs μg mL^{-1}) x + 0.0004 (Abs) and y =0.0209 (Abs g mL^{-1}) x + 0.0192 (Abs), for caffeine and catechin, respectively. Data were analyzed by the least squares method (n=3) and showed an adequate correlation coefficient for caffeine (r=1) and catechin (r=0.9993). This indicates a strong linear relationship for both actives, as the correlation coefficient is greater than 0.99 [25, 26]. Thus, standard curves can be used for the interpolation of experimental values.

Data were statistically analyzed by ANOVA. Results of linearity obtained showed significant difference for $p<0.05$ for both caffeine and catechin, indicating good linearity for the method. The RSD was less than 5% for all concentrations.

The LODs were 0.184 μg mL^{-1} (caffeine) and 0.368μg mL^{-1} (catechin and LOQs were 0.552 μg mL^{-1} (caffeine) and 1.104 μg mL^{-1} (catechin).

The results for the precision of the method for quantitation of caffeine and catechin in guarana bulk samples are presented in **Table 2**. All RSD values are lower than 2.0%, indicating good intra-day and inter-day precision for caffeine and catechin, and demonstrating that the method is precise under the conditions set [25, 26].

TABLE 2: REPEATABILITY (INTRA-DAY) AND INTERMEDIATE PRECISION (INTER-DAY) OF THE METHOD FOR ASSAYING CAFFEINE AND CATECHIN AT CONCENTRATIONS OF 10 AND 20 mg ml^{-1}, RESPECTIVELY (n = 3).

Sample	Parameter	n	Concentration ± SD (μg mL^{-1})	Concentration ± SD (%)	RSD (%)
Caffeine	Intra-day	6	10.71 ± 0.068	107.10 ± 0.068	0.63
	Inter-day				
	1 day	3	10.89 ± 0.062	108.89 ± 0.062	0.57
	2 day	3	10.89 ± 0.062	108.90 ± 0.062	0.57
	3 day	3	10.92 ± 0.108	109.92 ± 0.108	0.98
	Day 1+2+3	9	10.90 ± 0.077	109.00 ± 0.077	0.71
Catechin	Intra-day	6	19.57 ± 0.111	97.85 ± 0.111	0.56
	Inter-day				
	1 day	3	19.59 ± 0.154	97.95 ± 0.154	0.79
	2 day	3	19.67 ± 0.221	98.35 ± 0.221	1.12
	3 day	3	19.75 ± 0.253	98.75 ± 0.253	1.28
	Day 1+2+3	9	19.67 ± 0.209	98.35 ± 0.209	1.06

The method proved accurate for the quantification of caffeine and catechin in samples of guarana bulk (**Table 3**), presenting mean recovery percentages of 100.51 and 99.81% for caffeine and catechin, respectively. The precision parameter must validated considering that guarana is a product of plant origin, and therefore consists of a complex matrix. Thus, the result obtained is in accordance with the literature, demonstrating a recovery of greater than 85% of the nominal value [30].

TABLE 3: ACCURACY OF THE METHOD TO ASSAY CAFFEINE AND CATECHIN (CONCENTRATIONS OF 10 AND 20 MG ML^{-1}, RESPECTIVELY).

Sample	Added ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	Recovery (%)	Mean ± SD (%)
Caffeine	12.50	13.18 ± 0.00	100.76 ± 0.00	100.51±1.16
	15.00	15.76 ± 0.00	101.52 ± 0.00	
	17.50	18.02 ± 0.19	99.24 ± 1.76	
Catechin	20.00	24.62 ± 0.03	101.61 ± 0.14	99.81±1.65
	30.00	28.99 ± 0.55	98.35 ± 2.86	
	35.00	34.20 ± 0.24	99.46 ± 1.24	

The developed method was robust for the determination and quantification of caffeine and catechin in the sample of guarana with respect to the parameters shown in the **Table 4**. The changed conditions were compared to the optimized conditions for the method, data were statistically analyzed, and there were no significant differences ($p>0.05$).

TABLE 4: RESULTS FOR THE ROBUSTNESS OF THE METHOD USED TO ASSAY CAFFEINE AND CATECHIN (CONCENTRATIONS OF 10 AND 20 MG ML^{-1}, RESPECTIVELY).

Sample	Conditions	Concentration ± DP ($\mu g\ mL^{-1}$)	RSD (%)
Caffeine	Recommended*	10.78 ± 0.164	1.52
	Filter use	10.89 ± 0.062	0.57
	Distilled water	10.96 ± 0.006	0.57
	Ultrasonic bath 30 min.	10.89 ± 0.062	0.57
	Ultrasonic bath 60 min.	10.89 ± 0.062	0.57
Catechin	Recommended*	19.05 ± 0.319	1.67
	Filter use	19.15 ± 0.120	0.63
	Distilled water	19.29 ± 0.100	0.52
	Ultrasonic bath 30 min.	19.37 ± 0.127	0.65
	Ultrasonic bath 60 min.	18.49 ± 0.138	0.75

*Recommended: Unfiltered sample, ultrasonic 45 minutes and dilution with deionized water

When the wavelength caffeine (277 nm) was changed to 276 or 278 nm (data not shown), the values found were statistically different ($p<0.05$). The same was observed for the catechin (244 nm) when changed to 243 or 245 nm. The non-robustness of the method with respect to change in wavelength can be justified due to the use of the cancellation-point or the zero crossing technique. This technique measures the absolute value of the amplitude of one component of the mixed absorption at the cancellation wavelength of the other component.

Therefore, it leads to considerable loss in sensitivity and accuracy of the method, requiring reading absorbance at wavelengths generally critical, or at a specific wavelength[22]. The quantification of actives in the guarana bulk demonstrated that it contained 10 $\mu g\ mL^{-1}$ (4.3%) of caffeine and 20 $\mu g\ mL^{-1}$ (7.8%) for catechin. These values are consistent with those given in the Brazilian Pharmacopeia [5].

The Brazilian Pharmacopoeia [5], reports using spectrophotometry in the UV and visible region for the quantification of methylxanthines and tannins, respectively, by quantification of caffeine at 271 nm and 691 nm in total tannins. This methodology shows the quantification of 5% methylxanthines (caffeine) and 4% tannin.

The method requires the use of acidic or basic solutions as extraction solvents. It also requires lengthy extractive processes, composed of various stages of heating and mixing of the solvent, mainly to quantify tannins spectrophotometrically in the visible region.

Another limitation on the quantification of tannins by visible absorption spectrophotometry (691 nm) is that the color of the reaction used to measure the absorbance maximum stability has 3 minutes after its preparation[2].

In this context, the derivative technique offers several advantages over conventional spectrophotometric methods. It allows better separation of the components of the mixture, reducing undesired interference, and thus increasing the sensitivity and selectivity of the analysis of samples containing more than one active.

Besides being applicable to the majority of binary and tertiary mixtures, the only requirement of this method is the selection of a wavelength, at which the absorbance of a compound cancels that for the other compounds [31].

CONCLUSIONS: The use of UVD spectrophotometry was instrumental in quantifying the caffeine and catechin in the sample of guarana bulk. This tool is presented as a method for solving the problems of spectral overlap of components. The great advantage of this technique is the possibility of minimizing the problems of interferences without the need for prior separation procedures.

The use of the spectrophotometric method developed for the determination of methylxanthines and tannins in guarana bulk proved to be simple, fast, and reliable by using first-order derivation, $\Delta\lambda = 20{,}000$, a scale factor of 10, and quantification of catechin at 244 nm and caffeine at 277 nm.

The methodology developed was validated according to national and international guidelines and was proven to be specific, linear, accurate, precise, and robust for the quantification of methylxanthines and tannins in guarana bulk, by quantification of caffeine and catechins as markers, respectively. It is a simple tool that can be used for routine quality control in laboratories.

ACKNOWLEDGEMENT: The authors would like to thank the CAPES/Brazil for the fellowships and the CNPq/Brazil (CNPq/JST project, MCTI/CNPq N° 09/2013) for the financial support.

CONFLICT OF INTEREST: The authors do not have any conflict of interest.

REFERENCES:

1. Antonelli-Ushirobira TM, Yamaguti E, Uhemura LM and Mello JCP: Controle de Qualidade de Amostras de *Paullinia cupana* H.B.K. var. *sorbilis*(Mart.) Ducke. Acta Farmaceutica Bonaerense 2004; 23:383-386.
2. Sousa SA, Alves SF, De Paula JAM, Fiuza TS, Paula JR and Bara MTF: Determinação de taninos e metilxantinas no guaraná em pó (*Paullinia cupana* Kunth, *Sapindaceae*) por cromatografia líquida de alta eficiência**.** Revista Brasileira de Farmacognosia 2010; 20:866-870.
3. Antonelli-Ushirobira TM, Kameshima EN, Gabriel M, Audi EA, Marques LC and Mello JCP: Acute and subchronic toxicological evaluation of the semipurified extract of seeds of guaraná (*Paullinia cupana*) in rodents. Food and Chemical Toxicology 2010; 48: 1817-1820.
4. Bittencourt LS, Machado DC, Machado MM, Dos Santos GFF, Algarve TD, Marinowic DR, Ribeiro EE, Soares FAA, Barbisan F, Athayde ML and Cruz IBM: The protective effects of guaraná extract (*Paullinia cupana*) on fibroblast NIH-3T3 cells exposed to sodium nitroprussid. Food and Chemical Toxicology 2013; 53:119-125.
5. Farmacopéia Brasileira. ed. Editora Atheneu, São Paulo, 2010.
6. Marchei E, Orsi D, Guarino C, Donato S, Pacifici R and Pichini S. Measurement of iodide and caffeine content in cellulite reduction cosmetic products sold in the European Market. Analytical Methods 2013; 5:376-383.
7. Klein T, Longheni R and Palazzo de Mello JC: Development of an analytical method using reversed-phase HPLC-PDA for a semipurified extract of *Paullinia cupana var. sorbilis (guaraná).* Talanta 2012; 88:502-506.
8. Basile A, Ferrara L, Pezza MD, Melle G, Sorbo S, Bassi P and Montesano D: Antibacterial and antioxidant activities of ethanol extract from *Paullinia cupana*. Mart. Journal of Ethnopharmacology 2005; 102:32–36.
9. Yamaggutti-Sasaki E, Ito LA, Canteli VCD, Ushirobira TMA, Ueda-Nakamura T, Dias Filho BP, Nakamura CV and Mello JCP: Antioxidant capacity and *in vitro*prevention of dental plaque formation by extracts and condensed tannins of *Paullinia cupana*.Molecules 2007; 12:1950-1963.
10. Portella RL, Barcelos RP, Da Rosa EJF, Ribeiro EE, Cruz IBM, Suleiman L and Soares FAA: Guaraná (*Paullinia cupana* Kunth) effects on LDL oxidation in elderly people:

an *in vitro* and *in vivo* study. Lipids in Health and disease 2013; 12:1-9.

11. Costa Krewer C, Suleiman L, Duarte MMMF, Ribeiro EE, Mostardeiro CP, Montano MAM, Da Rocha MIUM, Algarve TD, Bresciani G and Da Cruz IBM: Guaraná, a supplement rich in caffeine and catechin, modulates cytokines: evidence from human *in vitro* and *in vivo* protocols. European Food Research and Technology 2014; 239:49-57.
12. Hertz E, Cadoná FC, Machado AK, Azzolin V, Holmrich S, Assmann C, Ledur P, Ribeiro EE, De Souza Filho OC, Mânica-Cattani MF and Da Cruz IBM: Effect of *Paullinia cupana* on MCF-7 breast cancer cell response to chemotherapeutic drugs. Molecular and clinical oncology 2015; 3:37-43.
13. Machado AK, Cadoná FC, Azzolin VF, Dornelles EB, Barbisan F, Ribeiro, EE, Catani, MFM, Duarte MMMF, Saldanha JRP and Da Cruz IBM: Guaraná (*Paullinia cupana*) improves the proliferation and oxidative metabolism of senescent adipocyte stem cells derived from human lipoaspirates. Food Research International 2015; 67:426–433.
14. Kennedy DO, Haskelll CF, Wesnes KA and Scholey AB: Improved cognitive performance in human volunteers following administration of guaraná (*Paullinia cupana*) extract: comparison and interaction with Panax ginseng. Pharmacology Biochemistry and Behavior 2004; 79:401–411.
15. Pelozo MIG, Cardoso MLC and Mello JCP: Spectrophotometric determination of tannins and caffeine in preparations from *Paullinia cupana var. sorbilis.* Brazilian Archives of Biology and Technology 2008; 51:447-451.
16. Bispo MS, Veloso MCC, Pinheiro HLC, Oliveira RFS, Reis JON and Andrade JB: Simultaneous determination of caffeine, theobromine, and theophylline by high-performance liquid chromatography. Journal of Chromatographic Science 2002; 40:45-48.
17. Aragão NM, Veloso MCC, Bispo MS, Ferreira SLC and De Andrade JB: Multivariate optimization of the experimental conditions for determination of three methylxanthines by reversed-phase high-performance liquid chromatography. Talanta 2005; 67:1007–1013.
18. Skoog DA, Holler J and Nieman T: Princípios de Análise Instrumental. 5ª ed. Porto Alegre: Bookman, 2002.
19. Alves LDS, Rolim LA, Fontes DAF, Rolim-Neto PJ, Soares MFLR and Sobrinho JLS. Desenvolvimento de método analítico para quantificação de efabirenz por espectrofotometria no UV-VIS. Química Nova 2010; 33:1967-1972.
20. Karpinska J. (2012): Basic principles and analytical application of derivative spectrophotometry. Macro to nano spectroscopy, Dr. Jamal Uddin (Ed.), ISBN: 978-953-51-0664-7, In Tech, Available from: "http://www.intechopen.com / books / macro - to - nano-spectroscopy/ derivative spectrophotometry-principles-andanalytical-application - spectroscopy / derivative-spectrophotometry-principles-andanalytical-application.
21. Parmar S and Sharma A: Derivative UV-vis absorption spectra as an invigorated spectrophotometric method for spectral resolution and quantitative analysis: Theoretical aspects and analytical applications: A review. Trends in Analytical Chemistry 2016; 77: 44-53.
22. Donato EM, Canedo NAP, Adams AIH, Fröehlich PE and Bergold AM: Espectrofotometria derivada: uma contribuição prática para o desenvolvimento de métodos. Revista de Ciências Farmacêuticas Básica e Aplicada 2010; 31:125-130.
23. Saad AS, Attia AK, Alaraki MS and Elzanfaly ES: Comparative study on the selectivity of various spectrophotometric techniques for the determination of binary mixture of fenbendazole and rafoxanide. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2015; 150:682–690.
24. Alpdogan G, Karabina K and Sungur S: Derivative spectrophotometric determination of caffeine in some beverages. Turkish Journal of Chemistry 2002; 26:295-302.
25. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: Text and Methodology – Q2(R1). IFPMA: Geneva, 2005.
26. Brasil. Agência Nacional de Vigilância Sanitária. Ministério da Saúde. Guia para validação de métodos analíticos e bioanalíticos. Brasília, DF, 2003.
27. FDA. Food and Drug Administration. Analytical Procedures and Methods Validation for Drugs and Biologics: Guidance for Industry. Pharmaceutical Quality/CMC, 2015.
28. The Index Merck. O'Neil, MJ, ed. O Índice Merck: An Encyclopedia of Chemicals, Drugs, and Biologicals. Ed 14. New Jersey: Merck, 2006.
29. Tavares GD, Ishikawa G, Monteiro TF, Zanolini C, Kedor-Hackmann ÉRM, Boa-Chacra NA and Consiglieri VO: Derivative spectrophotometric method for determination of acyclovir in polymeric nanoparticles. Quimica Nova 2012; 35:203-206.
30. Brasil. Agência Nacional de Vigilância Sanitária. Ministério da Saúde. Consolidado de normas da COFID (Versão V). Orientações sobre registro de medicamentos fitoterápicos, produtos tradicionais fitoterápicos e medicamentos dinamizados, Brasília, DF, 2015.
31. Lotfy MH, Hegazi MA, Mowaka S and Mohamed SM: Validated spectrophotometric methods for simultaneous determination of omeprazole, tinidazole and doxycycline in their ternary mixture. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2016; 153:321–332.

How to cite this article:
Roggia I, Ziegler S, Cruz IBM, Ourique AF and Gomes P: A Derivative UV Spectrophotometric Method for the Determination of Methylxanthines and Tannins in Guarana Bulk (*Paullinia Cupana*). Int J Pharm Sci Res 2016; 7(6): 2334-42.doi: 10.13040/IJPSR.0975-8232.7(6).2334-42.