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Article

Dissolution test of herbal medicines containing *Paullinia cupana*: validation of methods for quantification and assessment of dissolution

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"Guaraná" (*Paullinia cupana*) is used as a physical activity enhancer and stimulator due to its methylxanthines and condensed tannins. The aim of this work was to evaluate the dissolution behavior of five herbal medicines in the form of capsules and tablets containing guaraná. Assay and dissolution methods were validated and results obtained allowed simultaneous marker quantification with precision, accuracy, selectivity and robustness. Findings showed that 100% of the herbal medicinal products analyzed did not provide satisfactory results concerning the presence of four markers, 60% had three markers (caffeine, catechin and epicatechin), while 40% had only caffeine at tested dosage forms. In addition, after 30 minutes, only capsule A showed at least 80% of the dissolved markers. In other capsules, marker dissolution did not exceed 60% whereas 60% of the samples had some characteristic pharmacotechnical problems. These results evidence the need for rigorous quality control to help ensure the therapeutic action of these drugs. To this end, dissolution studies are an essential tool for quality assurance of herbal medicines.

Uniterms: Herbal medicines/quality control. Herbal medicines/dissolution behavior. Medicinal plants. Guaraná/pharmacognosy. *Paullinia cupana*/pharmacognosy.

Guaraná (*Paullinia cupana*) é utilizado como revigorante e estimulante devido à presença de metilxantinas e taninos condensados. Este trabalho visou avaliar o comportamento de dissolução de cinco fitoterápicos, na forma de cápsulas e comprimidos, contendo guaraná. O método de quantificação e de dissolução foram validados e os resultados obtidos permitiram a quantificação dos marcadores simultaneamente, com precisão, exatidão, seletividade e robustez. Foi verificado que 100% dos fitoterápicos analisados encontravam em desacordo quanto à presença dos quatro marcadores, sendo que 60% apresentaram três marcadores (cafeína, catequina e epicatequina) e 40% apresentaram somente a cafeína. Além disso, após o tempo de 30 minutos de ensaio foi possível observar que somente a cápsula A apresentou pelo menos 80% dos marcadores dissolvidos. Nas demais cápsulas o comportamento de dissolução apresentado pelos marcadores não ultrapassou 60% e, além disso, 60% das amostras apresentaram alguns problemas farmacotécnicos característicos. Frente aos resultados obtidos torna-se evidente a necessidade de um rigoroso controle de qualidade que contribua para assegurar a ação terapêutica desses medicamentos e, nesse sentido, o estudo de dissolução constitui uma ferramenta essencial para a garantia de qualidade dos fitoterápicos.

Unitermos: Fitoterápicos/controle de qualidade. Fitoterápicos/comportamento de dissolução. Plantas medicinais. Guaraná/farmacognosia. *Paullinia cupana*/farmacognosia.

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INTRODUCTION

Paullinia cupana Kunth. ("Guaraná") is a plant of the Sapindaceae family, native to the Brazilian Amazon. Dried and slightly toasted seeds are the most commonly used parts (Rates, 2002). This plant is widely employed in Brazilian traditional medicine, has been used in industrial and homemade drinks as stimulating tonics and in cosmetic preparations. Moreover, the plant is indicated in cases of exhaustion, nervous depression and migraine treatment (Moraes, Micke, Tavares, 2003).

The seeds of "guaraná" are characterized by the presence of methylxanthines, mainly caffeine and theophylline plus theobromine in smaller quantities, as well as condensed tannins such as catechin, epicatechin, procyanidin B2, procyanidin B3 and procyanidin B4, which are dimeric units composed of flavan-3-ol (Antonelli-Ushirobira *et al*., 2007). The stimulating property attributed to "guaraná" is mostly due to the presence of caffeine, while the tannins are responsible for its astringent flavor. Some studies have demonstrated its antioxidant, antiviral, antibacterial and slimicide activities, besides some enzymatic inhibition (Yamaguti-Sasaki *et al.*, 2007).

For a herbal medicinal product to be safe and effective, among other factors, it requires rigorous quality control. In the case of solid oral dosage forms, certain parameters should be evaluated that involve an assessment of the dosage form, since some conditions and manufacturing processes may limit drug release and therefore its absorption, compromising pharmacological activity (Ansel, Popovich, Allen, 2000).

Due to their importance, dissolution tests are frequently used for synthetic drug quality control, but their use in natural product evaluation has not been widely adopted (Taglioli *et al.*, 2001), which is a problem (Bempong, Houghton, 1992; Taglioli *et al.*, 2001; Kressmann *et al.*, 2002; Westerhoff *et al.*, 2002; Sittichai *et al.*, 2007; Kratz *et al.*, 2008). A peculiarity of herbal medicines is the fact that in many cases the *in vivo/in vitro* biopharmaceutical characterization is hard due to their complex composition, high metabolism suffered by constituents in the plants itself, and because of the different, often difficult, methods for extraction involved (Emea, 2003), as well as for quantification of analytes in phytocomplex (Williamson, 2001).

In the present work, methods were developed and validated for both liquid chromatography as well as dissolution in order to evaluate the dissolution behavior of capsules and tablets of "guaraná" purchased from the local market, using the release of theophylline, caffeine, catechin and epicatechin, quantified by HPLC, as a parameter.

MATERIALS AND METHODS

Samples

The samples used were three brands of capsules and two of tablets containing "guaraná" randomly purchased from local shops (Goiânia-GO, Brazil).

The HPLC profile of an authentic sample of "guaraná" (dried fruit) identified by Prof Dr José Realino de Paula - FF/UFG, was performed to establish a specification for the analysis of the herbal medicines sold.

Quantification of markers of "guaraná" by HPLC

The method used for determination of methylxanthines (caffeine and theophylline) and tannins was based on that proposed by Saito *et al*. (2006), employed for the determination of epigallocatechin, epicatechin, catechin and caffeine in green tea samples.

A Varian® (Palo Alto, California, USA) liquid chromatograph Pro Star model equipped with a model Pro Star 410 automatic injection system, Pro Star 240 quaternary pump and model Pro Star 310 – UV-Visible detector, were used.

Chromatographic conditions were the following: RP 18, 250 x 4.6 mm, 5 µm (Varian) chromatographic column at isocratic elution mode, wavelength at 274 nm, injection volume of 20 µL (performed in duplicate), mobile phase flow of 1mL/min and mobile phase consisting of Water: Acetonitrile: Methanol: Ethyl acetate: Acetic acid (89: 6: 1: 3: 1). The standards used were caffeine anhydrous (99%), catechin hydrate (96%), epicatechin (90%) and teophylline anhydrous (99%), purchased from Sigma Aldrich.

To perform caffeine, theophylline, catechin and epicatechin assay, samples were prepared according to the Farmacopeia Brasileira (2003), except for the different solvent used for determination of methylxanthines (solution of hydrochloric acid 0.1 M). To prepare samples, the average weight of capsules and tablets was first determined according to the Farmacopeia Brasileira (1988). For the quantification of the markers, the equivalent of 250 mg of herbal content was weighed and transferred to a 100 mL volumetric flask. After being subjected to a mechanical horizontal shaker for 15 min with four portions of 20 mL of 0.1 M hydrochloric acid for extraction of methylxanthines and tannins, the volume of the flask was supplemented with 0.1 M hydrochloric acid. The sample was filtered using filter paper and injection was performed in duplicate for chromatography.

The calculation of the content of caffeine, theo-

phylline, catechin and epicatechin was performed using the following formula:

$$\% = \frac{SA \times SC \times DF \times 100}{SA' \times W}$$

In which: SA = sample area; SA' = standard area; SC = standard concentration (mg/mL); DF = dilution factor; W = sample weight in mg.

Chromatographic method validation

The validation of the analytical method for quantification of theophylline, caffeine, catechin and epicatechin in "guaraná" by HPLC was performed according to RE No. 899/2003 ANVISA (Brazil, 2003). The parameters evaluated were selectivity, linearity and range, precision (repeatability and intermediate), accuracy and robustness.

Selectivity was determined by analyzing the chromatographic profile (retention time and resolution) of the samples (capsules and tablets), standard compounds and solvent, HCl 0.1 M (blank) in order to verify possible interference.

The linearity was determined for each marker by injecting six different concentrations of the standard mixture into the HPLC apparatus. The solutions of the standards (theophylline, caffeine, catechin and epicatechin) were prepared in methanol, followed by serial dilution to achieve concentrations ranging from 0.5 μg/mL to 15.0 μg/mL.

Precision (repeatability) was determined by preparing and analyzing six replicates of the sample at 100% concentration. For this procedure, samples were prepared accordingly to the method described above. The results were expressed as relative standard deviation (RSD%).

Precision (intermediate) was determined by analyzing the sample in triplicate at 100% on two days and by two different analysts. For this procedure, samples were prepared accordingly to the method described above. The results were again expressed as relative standard deviation (RSD%).

Accuracy determination was undertaken by analyzing the sample in triplicate at the concentrations of 50, 100 and 150%, and was verified by the recovery procedure, i.e. verification of the differences between the averages of these values and the theoretical value found.

The robustness of the method was evaluated in triplicate using the following parameters: wavelength of the reading at 272 nm, 274 nm and 276 nm, and flow of the mobile phase at 0.8 mL/min, 1.0 mL/min and 1.2 mL/min. The result was expressed as relative standard deviation (RSD%).

Dissolution test

The analytical methodology used to evaluate dissolution followed United States Pharmacopeia (2007) specifications, which describe the general methodology for capsule and tablet dissolution tests. Tests were carried out on a Vankel VK 7000 Total Solution Dissolution device using USP apparatus 2 (paddle), HCl 0.1 M pH 1.2 medium, dissolution vessel volume of 900 mL, 37.5 ± 0.5 ºC temperature, stirring speed of 75 rpm, and sampling aliquots of 3 mL withdrawn at 0, 5, 10 and 30 minutes. Samples were automatically collected, filtrated and assayed by HPLC.

Validation of dissolution method

The dissolution test is classified according to its purpose in test category III, hence the need for validation assessment of the precision parameter by means of repeatability (Brazil, 2003).

The precision evaluated at the repeatability level was determined by analysis of six individual samples (six replicates) collected from dissolutor at a time of 30 minutes. The repeatability was expressed as relative standard deviation between samples (RSD%).

RESULTS AND DISCUSSION

Validation of HPLC method for quantification of markers for *P. cupana*

The selection of the wavelength of 274 nm was made by assessing the absorption spectra in the UV range of methylxanthines (theophylline and caffeine), catechin and epicatechin.

The choice of mobile phase pH was based on the pKa of the substances being eluted. The pKa of methylxanthines can vary from 8.4 to 13.9 and of catechins from 8.6 to 13.2 (Gennaro, 2004). The pH of the mobile phase must be below the ionization constant, because the increased ionized analyte raises its dissolution in the aqueous phase and reduces its retention time, since the ionic form passes through the column without retention (Ivanovic e*t al.*, 1995).

The HPLC method used was linear, selective, precise, accurate and robust for the four markers present in the "guaraná", namely, theophylline, caffeine, catechin and epicatechin.

The method of identification and quantification of methylxanthines, catechin and epicatechin by high performance liquid chromatography was selective because it is

capable of separating the markers, as shown in Figure 1. The data shows that the solution used for dilution of the markers does not interfere with the peak of the markers in the sample.

For the markers quantified, the method was linear with a value of $r^2 = 0.9992$ for theophylline ($y = 0.36074x + 1.11898$), 0.9979 for caffeine ($y = 0.37758x + 0$), 0.9982 for catechin ($y = 0.08182x + 0$), and 0.9990 for epicatechin ($y = 0.07145x + 0$).

The method was shown to be precise for quantification of the four markers, in both repeatability and intermediate precision, with RDS < 5% (Table I).

The method was shown to be accurate for theophylline, caffeine, catechin and epicatechin, with recovery values ranging from 95.18% to 104.11%.

The method was robust since it resisted slight wavelength variations (272, 274 and 276 nm); and mobile phase flow variations – 0.8; 1.0 and 1.2 mL/min for the theophylline, caffeine, catechin and epicatechin evaluated. RSD values were less than 5%.

Quantification of markers of *P. cupana*

A large variation in the percentage of the caffeine marker (1.32 to 6.51%) was observed among the five herbal samples analyzed, which was not related to the catechin and epicatechin (Table II, Figure 2). Given the stimulant activity of "guaraná" is mainly due to the presence of caffeine (Yamaguti-Sasaki *et al*., 2007), the non-uniformity in the

TABLE I - Precision of HPLC method for quantification of markers of *P. cupana*

Repeatability		
Marker	Mean Concentration/ RSD (μg/mL)	Mean %
Theophylline	0.2599 / 4.46	0.1039
Caffeine	14.55 / 1.74	5.82
Catechin	2.04 / 3.17	0.82
Epicatechin	1.84 / 4.01	0.73
Intermediate precision		
Theophylline	0.2297 /3.61	0.0919
Caffeine	14.70 / 1.85	5.88
Catechin	2.01 / 2.80	0.80
Epicatechin	1.91 / 4.16	0.77

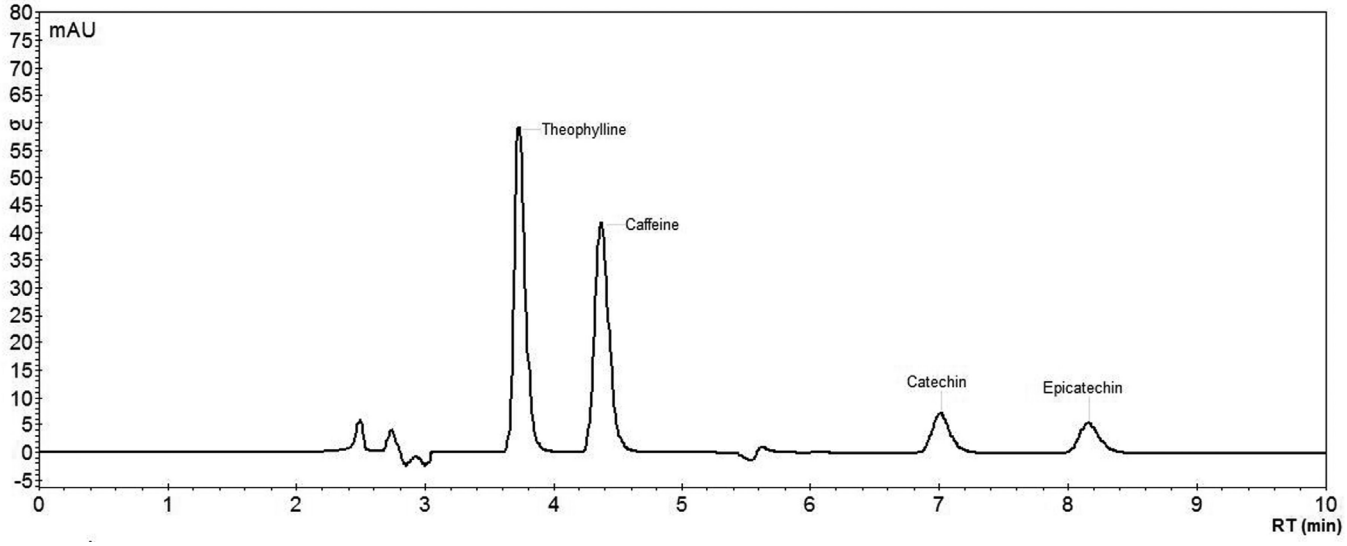

FIGURE 1 - Theophylline, caffeine, catechin and epicatechin standard solution chromatogram. Chromatographic conditions: water, acetonitrile, methanol, ethyl acetate and acetic acid (89:6:1:3:1) mobile phase; isocratic elution; flow of 1 mL/min; Varian® RP 18 250 x 4.6 mm, 5 μm column; and detector at 274 nm.

TABLE II - Percentage of markers in capsules and tablets of *P. cupana* analyzed

Samples	Markers			
	Caffeine	Theophylline	Catechin	Epicatechin
Capsule A	2.37 %	0.0	0.49%	0.49%
Capsule B	3.36%	0.0	0.60%	0.68%
Capsule C	6.51%	0.0	0.0	0.0
Tablet A	2.64%	0.0	0.0	0.0
Tablet B	1.32%	0.0	0.34%	0.41%

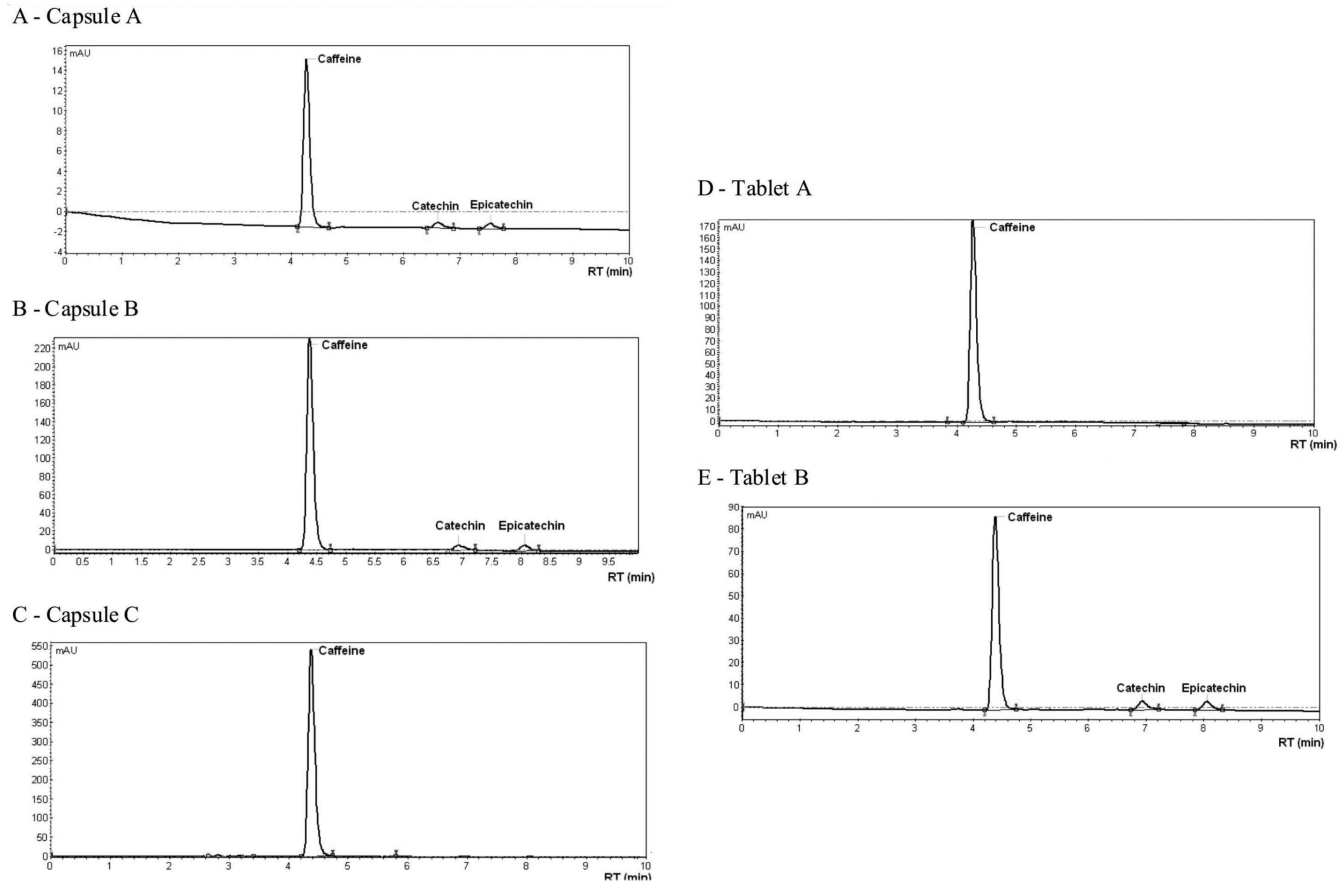

FIGURE 2 - Herbal medicine chromatogram. Chromatographic conditions: water, acetonitrile, methanol, ethyl acetate and acetic acid (89:6:1:3:1) mobile phase; isocratic elution; flow of 1 mL/min; Varian® RP 18 250 x 4.6 mm, 5 µm column; and detector at 274 nm. A- Capsule A, B - Capsule B, C- Capsule C, D – Tablet A, E – Tablet B.

concentration of this drug reveals a problem in relation to the dosage used and efficiency of this herbal medicine.

Thus, it is noteworthy that one of the great challenges faced by the botanical drugs market is the standardization of biological materials from natural sources. New technologies to modernize traditional herbs into mainstream pharmaceutical products are being evaluated with the goal of maximizing the opportunities and overcoming the challenges (Liu, Wang, 2008).

Validation of dissolution method:

The method was shown to be precise for the quantification of four markers, in repeatability precision, with aliquots being removed at the 30-minute timepoint, and RDS < 5%. For theophylline, the percentage was 0.0986, SD 0.0023 with RSD of 2.37. For caffeine, the percentage was 6.22, SD 0.08 and RSD 1.38. For catechin, the percentage was 0.88, SD 0.02 and RSD 3.04. Finally,

for epicatechin, the percentage was 0.72, SD 0.03 and RSD 4.39.

Dissolution test

None of the five samples of herbal medicines analyzed showed satisfactory results regarding dissolution profile and presence of the four proposed markers (Table III, Figure 3).

After 30 minutes, capsule A presented at least 80% of the markers dissolved (Figure 3A). In the other capsules, the dissolution profile presented by the markers, when present, did not exceed 60% (Figures 3B and C), probably due to formulation problems such as excipients and handling process used. Among the tablets, sample A reached 100% dissolution for caffeine, and was the only marker detected (Figure 3D). In tablet B, possibly due to pharmacotechnical problems, e.g. strength compression, its total disintegration, and consequently its total dissolution, did not occur, not releasing more than 40% of the markers assayed (Figure 3E).

The drug manufacturing processes and formulation components involved can influence dissolution and bioavailability. Tablets obtained by direct compression, dry or wet granulation, may display different *in vitro* and *in vivo* behavior. These factors directly affect dosage form disaggregation in gastrointestinal fluids, influencing dissolution and consequently, drug absorption (Storpirtis *et al.*, 1999).

The results of this study also demonstrated that among the samples, 60% had three markers present (caffeine, catechin and epicatechin) (Figures 3A, B and E) while 40% had only caffeine as a marker (Figures 3C and D). Since catechin, epicatechin and theophylline are markers of *P. cupana* (Farmacopeia Brasileira, 2003; Antonelli-Ushirobira *et al.*, 2007) its absence in some samples point to a quality problem. Furthermore, only the presence of caffeine in some herbal medicines analyzed is an important non-compliance. This result suggests the poor quality of these herbal medicines and possible adulteration by sophistication practices, for example, the addition of synthetic caffeine *P. cupana* powder. Sophistications practices are difficult to detect and occur usually by addition of synthetic or natural substances with structures similar to those of the active principle, medicinal plants or herbal products with low quantity of active principle, in order to fool quality control (Sharapin, 2000; Rocha, 2009). Moreover, for quality assurance purposes, desirable attributes include authenticity and purity. Authenticity relates to proving that the material is genuine and involves parameters including chemical analysis. Purity pertains to evaluating that there are no adulterants present in the plant material (Yadav, Dixit, 2008).

Another important aspect to highlight is that considering solid oral medicines can present major problems in relation to bioavailability, it is important to evaluate the dissolution of the drug from the dosage form by per-

TABLE III - Percentage of dissolution *versus* time for herbal medicines tested

	% Dissolution (mean)				
	time	theophylline (± SD)	caffeine (± SD)	catechin (± SD)	epicatechin (± SD)
Capsule A	5’	0.00	41.50 (± 1.95)	34.90 (± 0.47)	42.71 (± 0.51)
	10’	0.00	70.05 (± 1.76)	56.64 (± 0.35)	69.60 (± 0.40)
	30’	0.00	102.25 (± 2.07)	79.59 (± 0.51)	101.31 (± 0.48)
Capsule B	5’	0.00	19.60 (± 1.25)	15.17 (± 0.18)	21.50 (± 0.31)
	10’	0.00	34.92 (± 2.27)	26.72 (± 0.28)	37.37 (± 0.48)
	30’	0.00	59.88 (± 1.82)	42.90 (± 0.31)	55.84 (± 0.37)
Capsule C	5’	0.00	0.05 (± 0.01)	0.00	0.00
	10’	0.00	0.97 (± 0.07)	0.00	0.00
	30’	0.00	18.28 (± 4.44)	0.00	0.00
Tablet A	5’	0.00	59.86 (± 0.82)	0.00	0.00
	10’	0.00	99.96 (± 0.33)	0.00	0.00
	30’	0.00	106.49 (± 0.29)	0.00	0.00
Tablet B	5’	0.00	6.98 (± 0.15)	2.91 (± 0.03)	5.62 (± 0.11)
	10’	0.00	12.95 (± 0.15)	8.82 (± 0.08)	10.84 (± 0.05)
	30’	0.00	39.93 (± 0.47)	33.02 (± 0.13)	34.43 (± 0.17)

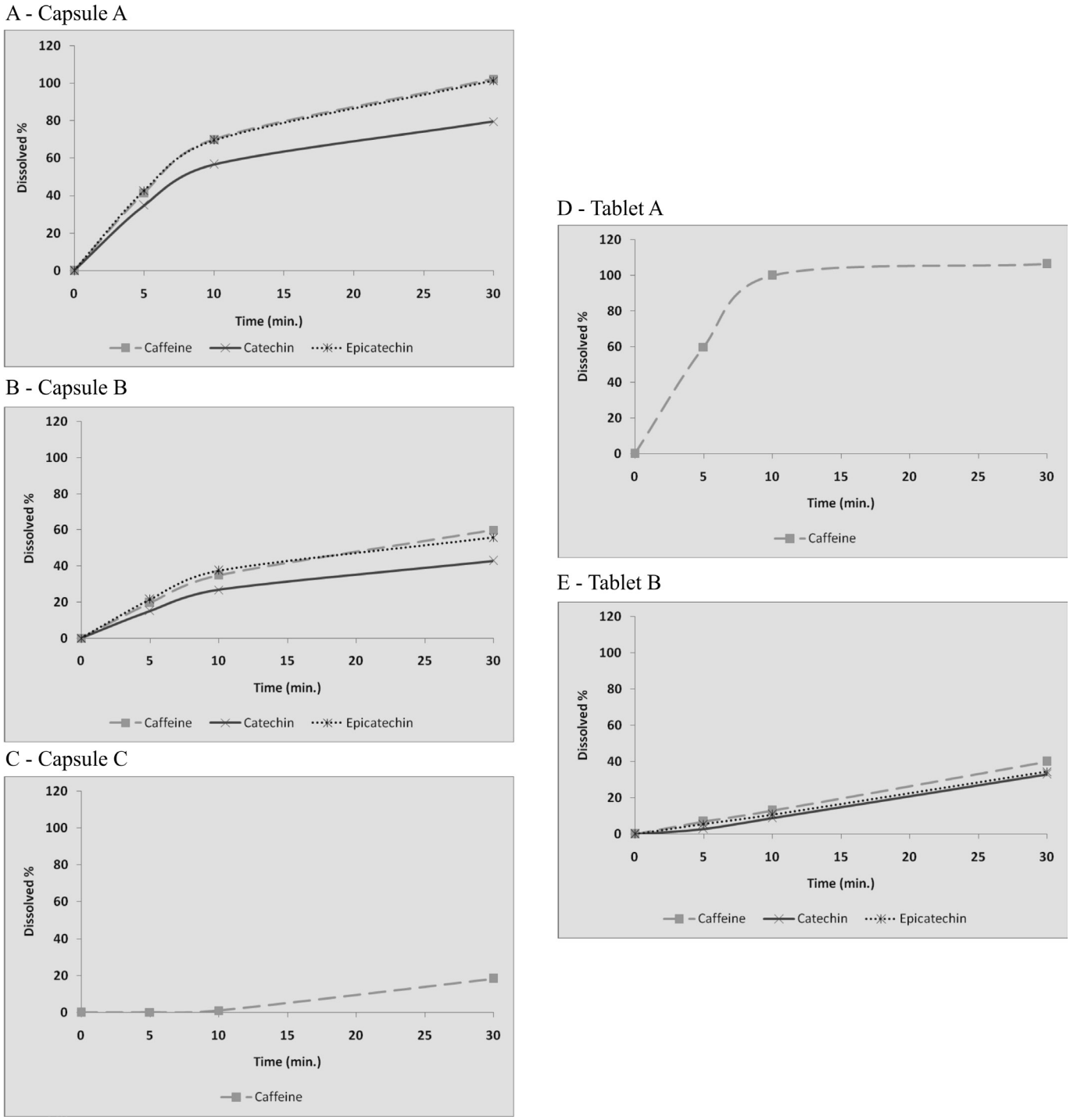

FIGURE 3 - Dissolution profile of herbal medicines of *P. cupana*, using apparatus 2 (paddle), HCl 0.1 M pH 1.2 medium, dissolution vessel volume of 900 mL, 37.5 ± 0.5 ºC temperature, stirring speed of 75 rpm, at 0, 5, 10 and 30 minutes. A- Capsule A, B - Capsule B, C- Capsule C, D – Tablet A, E – Tablet B.

forming in vitro tests that help to visualize the dissolution profile as a function of time.

Literature has demonstrated concerning data related to herbal medicinal product dissolution tests. A study performed with phytomedicines based on *Ginkgo biloba* demonstrated that 85% of the evaluated products failed to have the recommended chemical composition and therefore their use could not be indicated (Kratz *et al.*, 2008). In another study on *Ginkgo biloba* extract capsules and tablets, marked differences in dissolution behavior were

found, with values of 99% and 33% dissolution, on average, after 15 minutes and 1 hour, respectively (Kressmann *et al.*, 2002). The dissolution profile of *Senna* sp was less than 10% sennoside release from capsules containing dry extract, in a period of 60 minutes, in contrast to lyophilized *Senna* sp extract, which attained around 90% dissolution after the same period. Data on the dissolution test of *Passiflora* sp showed that capsules containing the crude extract presented 50% dissolution while lyophilized extract and other standardized extract reached around 100% for the same period (Taglioli *et al.*, 2001).

Considering the increasing consumption of herbal medicines, *P. cupana* included, there is a need for studies to ensure quality from raw material to finished product. Quality control is essential for the efficacy and safety of herbal products. Thus, dissolution studies are of great importance since they can contribute toward ensuring the effectiveness of herbal medicines.

CONCLUSION

The results showed that the quality of herbal medicines containing "guaraná" represents a serious public health problem, since 100% of the samples analyzed differed in terms of the presence of chemical markers of the drug both on the dissolution test as well as in relation to pharmacotechnical aspects.

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