

# The Importance of Dissolution Tests to Evaluate Quality of Dietary Supplements: Case Study of Controlled-Release Caffeine Capsules

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## ABSTRACT

Controlled-release capsules (named brand I and II) containing caffeine, available on the Brazilian and US market as dietary supplements, were assessed following the criteria described by the *United States Pharmacopeia*. The capsules were evaluated by average weight, caffeine content, disintegration, and dissolution tests. Test results for all capsules met the acceptance criteria for these tests, with the exception of the dissolution of brand I capsules. The release rate of an active pharmaceutical ingredient (API) from a dosage form, measured by a dissolution test, is one of the fundamental parameters leading to the formulation's feasibility. The dissolution test is not mandatory to approve a dietary supplement in Brazilian and US markets. This study highlights the importance of evaluating these products by means of a performance test, such as dissolution, using products containing caffeine as case study.

**KEYWORDS:** Caffeine, dietary supplements, controlled-release capsules, quality control, dissolution

## INTRODUCTION

The market size of global dietary supplements was valued at \$151.9 billion US dollars in 2021 and is expected to expand at a compound annual growth rate (CAGR) of 8.9% from 2022 to 2030. The increasing consumer knowledge of personal health and wellbeing is expected to be a factor for dietary supplements growth over the projection period (1). Caffeine is one of the most consumed stimulants in the world and is a frequent ingredient in dietary supplements. This compound has several properties (Fig. 1): it is a central nervous system stimulant, a diuretic, it decreases fatigue, it enhances mental focus and athletic performance and presents thermogenic effects (2). There is also evidence proposing that the consumption of caffeine appears to reduce caloric intake, contributing to weight loss (3).

The safety dose for caffeine recommended by the United States Food and Drug Administration (FDA) and National Health Surveillance Agency of Brazil (ANVISA) is around 400 mg/day for adults from all caffeine sources, such as coffee, tea, pills, and others (4, 5). High dosages (more

than 400 mg/day) of this compound can cause severe hypertension, arrhythmias, seizures, and even death. Individuals who are more sensitive may present adverse effects at lower dosages. The complete absorption of caffeine occurs in the small intestine, and it needs around 45 minutes to reach 99% bioavailability, with no substantial first pass effect (5).

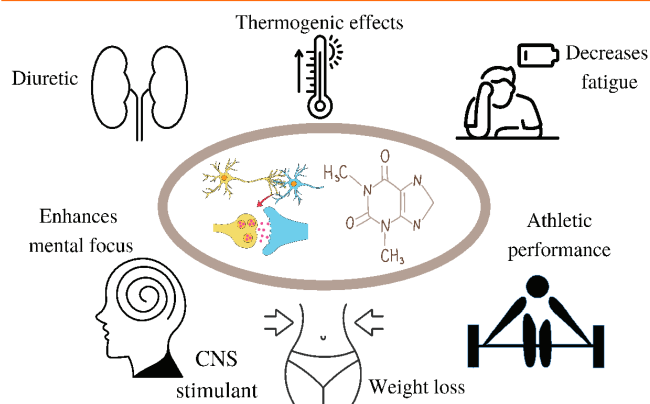


Figure 1. Properties of caffeine.

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The human body eliminates caffeine within a few hours, leading some people to take caffeinated beverages or supplements recurrently over time. To retain the stimulating effects of caffeine and avoid an overdose, sustained-release systems have been developed and introduced on the market (6). Different formulations of caffeine-controlled release products are currently available for purchase in the US and Brazil.

The oral bioavailability of an active pharmaceutical ingredient (API) regularly depends on its dissolution upon ingestion, absorption in the small intestine, and transport to its target site of action (7). Dissolution is a precondition for absorption and in vivo efficiency for almost all compounds given in oral solid dosage forms. API absorption depends on its dissolution and solubilization under physiological conditions, and the permeability across the gastrointestinal (GI) tract. Because of the critical nature of the first steps, in vitro dissolution is an important and necessary tool to predict the human biological response (8). For this reason, dissolution testing is required for quality control of solid dosage forms containing APIs (medicines), but it is not mandatory for dietary supplements.

Dissolution testing is an important instrument for characterizing the performance of oral solid dosage forms. Its significance is founded on the point that for an API to be effective, it must first be released from the product and dissolve in the GI fluids previously absorption (8).

The market of dietary supplements includes tablets, powders, and liquids. It is predicted that revenue from the tablet dosage form market will exceed \$100 billion US dollars (9). Capsules offer an alternative to tablets for oral delivery of therapeutic compounds. One advantage of capsules over tablets is flexibility to deliver not only solids but also non-aqueous liquids and semisolids as a unit dose solid dosage form. It can also be designed to delay the release of their contents into the GI tract, prolonging the therapeutic effect (10).

The aim of this work was to evaluate the quality of caffeine capsules available on the market as a modified-release formulation and to introduce the importance of performance tests such as dissolution for dietary supplements.

## MATERIALS AND METHODS

### Chemical Products and Capsules

The United States Pharmacopeia (USP) provided the reference standard used for caffeine. All solvents used were of analytical grade and purchased from Biotec

(Pinhais, PR, Brazil). Two different modified-release capsule brands were evaluated.

**Brand I:** Hard gelatin capsules containing caffeine microgranules (130 mg), water, tocopherol mix, safflower oil (500 mg), hydroxypropyl methylcellulose (HPMC), and silicon dioxide. According to the manufacturer, the delivery of caffeine occurs in two stages: 50% in the first hour and the remainder in the next 5 hours.

**Brand II:** Hard gelatin capsules containing caffeine beadlets (200 mg), sugar, gelatin, starch, food glaze, magnesium silicate, povidone, FD&C Yellow 6, and FD&C Red 40. According to the manufacturer, the release of caffeine occurs in a sustained manner for 8 hours.

The capsules are available in Brazil and the US. All analyses were conducted within the period of validity of the product.

### Average Weight

The methodology described in the USP chapter <2091> Weight Variation of Dietary Supplements was used. Twenty intact capsules were weighed individually using an analytical balance (Shimadzu AW220, São Paulo, Brazil), and the average weight was further calculated. The results were compared with the USP requirements (11).

### Caffeine Content

Ten capsules of each brand were opened and their contents (microgranules for brand I or beadlets for brand II) were removed, weighed, and crushed. An amount of the content equivalent to 50 mg of caffeine was weighed, transferred to 100-mL volumetric flask and the volume completed with distilled water. These flasks were shaken for 20 minutes in an ultrasonic bath and diluted as necessary. Caffeine content was determined by UV spectrophotometry at 237 nm using a UV spectrophotometer (Varian Cary 50). The UV spectrophotometric quantification method was adapted from Tan and colleagues and validated by linearity, precision, quantification and detection limits, and specificity (6).

The linear equation was achieved using weighed and diluted caffeine to obtain solutions in the range of 0.8–25.0 µg/mL. After analysis in a UV spectrophotometer at 237 nm, a chart provided the linear equation:  $y = 0.0523x - 0.006$  and  $r = 1$ , limit of quantification: 0.29 µg/mL, and limit of detection: 0.10 µg/mL.

### Disintegration Tests

Capsule disintegration tests were carried out in a USP disintegration apparatus (Nova Ética, 301-AC, São

Paulo, Brazil) in distilled water at 37 °C. The time for disintegration of each unit ( $n = 6$ ) was recorded (11).

### Dissolution Tests

Dissolution studies were performed using a Varian VK 7000 dissolution tester, equipped with USP apparatus 1 (basket). Samples ( $n = 6$ ) were analyzed in two different dissolution media: a) 900 mL of distilled water and b) 900 mL of 0.1 N hydrochloric (HCl) acid. Dissolution studies were conducted at 37 °C and stirring speed of 50 rpm. Samples were collected at defined time intervals for 480 min, filtered with a 45- $\mu\text{m}$  PVDF filter (Filtrilo), diluted to fit the equation curve, and quantified by UV spectrophotometry.

The dissolution efficiency was calculated by software DD Solver. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons test were employed to test the statistical significance regarding the dissolution efficiency of samples. Differences were considered significant for  $p < 0.05$  with a confidence level of 95%. The results were analyzed using Excel.

### Dissolution Kinetics

The results obtained from the dissolution tests were used to evaluate the dissolution kinetics of caffeine from the capsules. The straight-line equation and linear regression were used to determine the percentage of dissolved caffeine as a function of time. The kinetics models applied are described in Table 1, and the best model was selected based on coefficient of correlation analysis ( $R^2$ ) of linear regression (12–14).

Table 1. Dissolution Kinetic Models Applied to Caffeine Capsules

Model	Mathematical Equation	Release Mechanism
Zero order	$C = C_0 - K_0t$	Diffusion mechanism
First order	$\text{Log } C = \text{log } C_0 - K_1t/2.203$	Fick's first law, diffusion mechanism
Higuchi	$Q_0/Q_t = K_H t^{1/2}$	Diffusion medium-based mechanism in Fick's first law
Korsmeyer-Peppas	$Ct/C_\infty = K_k t^n$	Semi-empirical model, diffusion-based

## RESULTS AND DISCUSSION

### Average Weight, Caffeine Content, and Disintegration Time

The weight determination indicates if the units of a batch show weight homogeneity. The average weight of the capsules was  $832 \pm 5.5$  mg and  $520 \pm 2.0$  mg for brands I and II, respectively. Caffeine content was  $99.7 \pm 4.1\%$  and  $98.9 \pm 3.2\%$  for brands I and II, respectively. According to the USP, the requirements of average weight are met if

each of the individual weights is within the limits of 90–110% of the average weight. Capsules from both brands had variations in their weights within the specified limits, as well as presented a caffeine content in accordance with the amount stated on the label.

The time for disintegration of capsules was 5 minutes for both samples, indicating the rapid liberation of their content into the aqueous medium. Disintegration and dissolution tests are described in USP general chapter <2040> as a quality control tool to routinely assess the performance of dietary supplements, which states that hard-shell capsules must be completely disintegrated within 30 minutes (11). As both samples showed a shorter disintegration time, the caffeine release could be governed by the formulation of granules/beadlets and not by the capsule itself.

### Dissolution Test

The dissolution test is a performance assay applied to different pharmaceutical formulations to evaluate their drug release from the pharmaceutical form (15). Based on release profiles shown in Figure 2, brand I released less than 5% of the caffeine content in 8 hours in both media evaluated. Brand II showed a continued caffeine release over time, dissolving around 90% of caffeine content in 8 hours, in both water and acidic media.

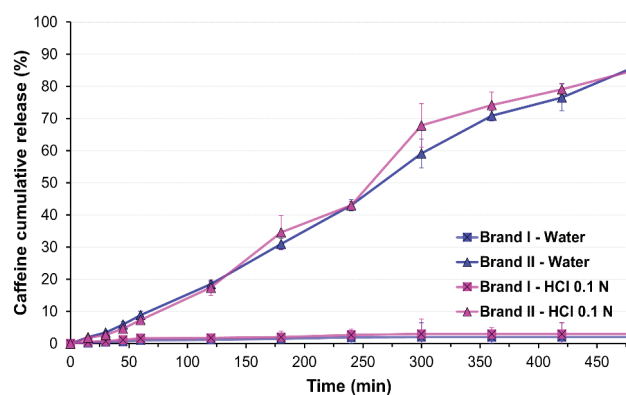


Figure 2. Dissolution profiles of controlled-release caffeine capsules.

The evaluation of dissolution is mandatory for medicines but is not required to register a product in the FDA or ANVISA as a dietary supplement. As there are no acceptance criteria to assess the performance of these products, we considered the release claimed by the brands on their labels. Brands I and II claimed to release the caffeine content over 5 and 8 hours, respectively. In controlled-release systems, the drug is released or activated at predetermined intervals or gradually released over a period of time, as was observed for brand

II release profiles (Fig. 2) (16). However, the low caffeine release of brand I indicates that the composition of the capsules interferes with the release mechanism.

Caffeine is a weak acid with pKa of 14.0 and lipophilicity (octanol-water partition coefficient, LogP) of 0.1 (17). Its water solubility is 11.0 mg/mL, being classified as class I (high solubility and high permeability) according to the Biopharmaceutical Classification System (18). The dosages of capsules used in the tests were 130 and 200 mg for brand I and II, respectively, and the volume of both media was 900 mL. This means that the sink condition (volume of solvent 5–10 times greater than the volume present in the saturated solution) was kept in the bulk solution during the test, so the low caffeine release from brand I was not due to saturation effects outside the pharmaceutical dosage.

Brand I was composed of different oils that covered the HPMC granules containing caffeine. The release of caffeine from the capsule was probably affected by the surrounding barrier formed by the oil, preventing the dissolution media from accessing the granules and the hydrophilic caffeine passing through the oil layer (Fig. 3). Controlled drug release from a hydrophilic matrix based on HPMC follows several types of physical phenomena, such as water, drug, and polymer chain diffusion, polymer swelling, and subsequent dissolution of drug and polymer. Even in the case of freely water-soluble molecules, such as diprophylline and theophiline, saturation solubility effects can occur within the dosage form (while providing sink conditions outside), impeding drug release (19).

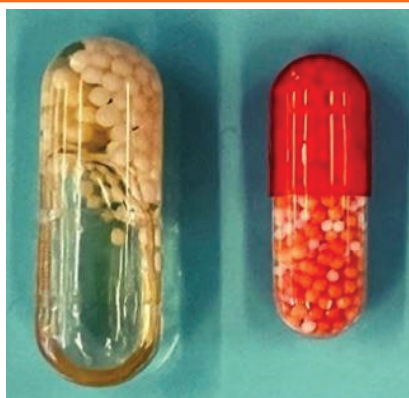


Figure 3. Images of capsules named Brand I and Brand II on left and right, respectively.

### Dissolution Kinetics

The quantitative interpretation of the values obtained from the dissolution tests was simplified by using mathematical models to describe the drug release from the pharmaceutical form (Table 2). Because brand I did not release at minimum 60% of caffeine, it was not possible to calculate the best model to describe the caffeine release (Table 2). For brand II, the best fit model was the Korsmeyer–Peppas equation. In this equation,  $M_t/M^\infty$  characterizes the fraction of permeated drug,  $t$  is time,  $K$  is the transport constant (dimension of  $\text{time}^{-1}$ ), and  $n$  is the transport exponent (dimensionless) (13, 20). The values of  $n$  calculated for brand II in water and HCl 0.1 N were 1.14 and 1.22, respectively. Values of  $n > 1$  are related to super case II kinetics, wherein multiple mechanisms are involved in drug release, such as diffusion, swelling, relaxation, and erosion (13, 20).

In addition, dissolution efficiency (DE) was employed to compare the dissolution profiles of the brand II capsules in different media (21). The time  $T$  in this study was 480 minutes (8 h). The DE values for brand II were similar in both media (Table 2).

The dissolution of an active ingredient administered in the solid state is a prerequisite for efficient and subsequent transport within the human body, which underscores the importance of dissolution tests for dietary supplements. Considering the high complexity of a component release from a controlled-release system, even the release of freely water-soluble molecules should not be taken for granted. In the case of caffeine, ineffective release of the active ingredient from the capsules during the time shown on the label could induce the patient to take more, contributing to possible toxicity and even lethality (i.e., most commonly via myocardial infarction or arrhythmia) if enough caffeine is consumed (22).

Gusev and colleagues evaluated the applicability of USP <2040> protocols for disintegration and dissolution testing of dietary supplements containing green tea available in the US market (23). The results indicated that in dissolution testing, for the release of epigallocatechin-3-gallate (EGCG), the most abundant of the green tea catechins, only 6 out of 20 dietary supplements were approved. These results raise concerns that EGCG was

Table 2. Dissolution Efficiency and Mathematical Model Parameters ( $R^2$ ) for Controlled-Release Caffeine Capsules

	DE (%)	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas
Brand II - Water	43.78 ± 2.24*	0.9944	0.9581	0.9287	0.9819	0.9970
Brand II - HCl 0.1 N	45.68 ± 3.18*	0.9802	0.9686	0.9225	0.9797	0.9892

\*Statistically similar ( $p < 0.05$ ) in different media for the same brand.



not released properly from green tea dosage forms of dietary supplements (23).

As the dietary supplement industry grows, the risk of interactions between prescription medications and dietary supplements may increase. In the US, approximately 80% of adults over 50 years take at least one prescription medicine, and more than 20% take at least five prescription medications, and more than half of these patients also use dietary supplements (9).

The brief case study of controlled-release capsules containing caffeine presented herein demonstrates a need to look at dietary supplements (in capsule or tablet form) with the same quality and safety criteria that the regulatory agencies use when assessing a medicine.

## CONCLUSIONS

Regulatory agencies such as ANVISA and the US FDA do not require the dissolution test for dietary supplements. Dissolution studies of two brands of controlled-release capsules containing caffeine indicated that one brand did not match the specification described on the label of the product. Differences in caffeine dissolution can lead to serious health problems from undesired intoxication or overdose, owing to absence of the desired effect. This case study raises an alert and supports the need to perform dissolution tests on products sold as dietary supplements in the form of tablets and capsules.

## DISCLOSURES

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