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ORIGINAL ARTICLE

New Formulation Containing Curcuminoids: Method Validation and Dissolution Profile

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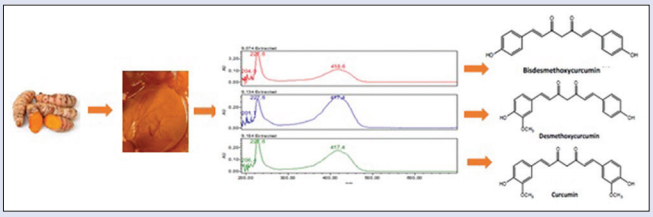
ABSTRACT

Background: *Curcuma longa*, popularly known as turmeric, contains secondary metabolites called curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) which are responsible for its therapeutic activity. **Objective:** The aim of this work was to obtain capsules using *C. longa* soft extract, to develop and validate a method for quantification of curcuminoids, and to study the dissolution profile of the capsules. **Materials and Methods:** The liquid extract was obtained through the percolation method and concentrated until it is characterized as soft extract. A high-performance liquid chromatography (HPLC) method was developed and validated for quantification of curcuminoids in the soft extract. The granule was obtained through wet granulation process and was encapsulated and the method for quantification of the markers was covalidated. **Results:** The soft extract showed 3.45% of bisdemethoxycurcumin, 3.03% of demethoxycurcumin, and 14.83% of curcumin, corresponding to 21.31% of total curcuminoids. The capsules had an average weight of 420 mg with approximately 21.56 mg of curcuminoids. The capsules showed better dissolution profile in neutral medium that simulates the pH of duodenum; however, in the stability test, only the variation found for bisdemethoxycurcumin was in compliance with the permitted. **Conclusions:** A soft extract of *C. longa* standardized in the three main curcuminoids was produced, as well as hard gelatinous capsules containing a dose of 21.56 mg of total curcuminoids. Thus, a new phytotherapeutic formulation was proposed, which proves to be an interesting strategy to carry out the active principles of *C. longa*.

Key words: Bisdemethoxycurcumin, curcumin, demethoxycurcumin, extract standardization

SUMMARY

- Method validation for quantification of curcuminoids in *Curcuma longa* soft extract
- New capsule formulation using *C. longa* soft extract as active principle
- Evaluation of capsules dissolution in acid and neutral medium.



Abbreviations used: HPLC: High-performance liquid chromatograph; LOD: Limit of detection; LOQ: Limit of quantification; PDA: Photodiode array detector; PTFE: Hydrophobic polytetrafluoroethylene; TLC: Thin-layer chromatography; UV: Ultraviolet

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INTRODUCTION

Curcuma longa L. (*Zingiberaceae)* is originated from Southeast Asia and distributed worldwide in tropical and subtropical regions.[1] Its rhizomes have been used for more than 5000 years by Ayurvedic medicine to treat inflammatory conditions without reports of adverse reactions.[1,2] The rhizomes of *C. longa* are characterized by the presence of secondary metabolites called curcuminoids, responsible for their therapeutic action.[3] Curcuminoids are yellowish pigments characteristic of this species, and among them, the following three compounds are highlighted: curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

A number of pharmacological activities have been described for *C. longa*, among them the anti-inflammatory, antioxidant, hepatoprotective, choleretic, digestive, and immunostimulatory.[4-6] These activities are mainly attributed to the curcuminoids and curcumin. The pharmacokinetics studies of curcumin show that in oral administration, it is poorly absorbed by the intestine and has low systemic bioavailability,[7] probably because when absorbed, it conjugates with glucuronide and sulfate in plasma.[8]

Due to so many activities proven in the literature and the poor bioavailability, several studies have tried to develop curcuminoids formulation for the treatment of disease, such as the development of dry extracts by the spray dryer technique, solid dispersions, or nanoparticulate formulations.[9-11] However, such techniques require expensive instrumentation, reagents, and equipment.

Plant extracts have been used in the development of formulations in the pharmaceutical industry as a source of active ingredient for herbal medicines.[12] However, the active ingredients present in the extracts are often isolated and used separately from its original plant matrix, which minimizes the therapeutic activity. Their activity or absorption may be increased when used together with the phytocomplex. In this context,

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it is interesting to develop a new and simple formulation using all the phytocomplexes present in the rhizomes of *C. longa*. The present work aimed to obtain capsules using *C. longa* soft extract as active principle, develop a method for quantification of curcuminoids in the soft extract and in the capsules, and determine its dissolution profile.

MATERIALS AND METHODS

Chemicals and standards

Methanol and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetic acid glacial was supplied by Sharlau (Sharlab, Spain) and ethanol by Vetec (Brazil). Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Standards of curcumin (≥65%), demethoxycurcumin (≥95.0%), and bisdemethoxycurcumin (≥95.0%) were from Sigma–Aldrich (St. Louis, MO, USA).

Plant material

The rhizomes of *C. longa* were obtained from the Cooperative of the Producers of Saffron of Mara Rosa (Cooperaçafrão) located in the city of Mara Rosa, Goiás, Brazil (14°00'59"S 49°10'38"W). The authenticity of the cultivated plant material was confirmed by a qualified professional. A voucher specimen (UB0029391) has been deposited in the Botanical Garden Research Institute of Rio de Janeiro, in Rio de Janeiro, Brazil. The rhizomes were washed in running water, sliced, and dried at 40°C in a circulating air oven until reaching a constant weight. Then, they were crushed in a knife mill and packed in plastic bags protected from light and moisture.

Fingerprint profile of the raw turmeric

The powder of *C. longa* rhizome was subjected to thin-layer chromatography (TLC). About 800 mg of powdered *C. longa* was extracted with 10 mL of methanol for 20 min in an ultrasonic bath and filtered on filter paper. One milligram of each standard (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) was weighed into a 5 mL flask, filling the volume with methanol, and the flasks were also sonicated for 20 min. An aliquot of rhizome extract and standards were applied in an aluminum chromatography plate impregnated with silica F254 nm (Merck, Darmstadt, Germany). The chromatography plate was placed on the chromatographic cell with mobile phase composed of chloroform-methanol (95:5 v/v). After elution, the chromatographic plate was dried at room temperature and read. The analysis was performed by comparing the standard bands with the bands of the powdered rhizome.

The presence of curcuminoids in the powdered *C. longa* rhizome was also determined by high-performance liquid chromatography (HPLC).[13-15] A Waters® HPLC Alliance with e2695 separation module, 2998 photodiode array detector (PDA), and *Empower 2.0* software from Waters Coorporation (Milford, MA, USA) were used. Chromatographic separations were performed using a Zorbax Eclipse® XDB-C18, Agilent® column (250 mm × 46 mm, 5 μm), and a Phenomenex C18 guard cartridge system (30 mm × 4 mm, 4 mm).

The mobile phase was eluted on a gradient flow of 1 mL/min, using 2% acetic acid in acetonitrile (A) and water (B), starting with 45% A; 15 min, 75% A; 20–23 min, 100% A; 28 min, 45% A; 30 min, detection wavelength of 420 nm, injection volume of 10 μL, and column temperature of 25°C. The mobile phase was filtered through a polyvinylidene difluoride membrane (0.45 mm, Merck) and degassed using an ultrasonic bath. Extract and analytical standard solutions were previously filtered through a 0.45 mm PTFE membrane (Millex).

Obtaining of *Curcuma longa* soft extract

The crude hydroalcoholic extract was obtained by the percolation method using a stainless-steel percolator (10 L capacity). Three kilograms of powdered *C. longa* and 10 L of 96% ethanol were put in contact for 24 h (maceration process), and subsequently, percolation started. The extract flow through the percolator was given freely. The crude extract was then concentrated at room temperature with the aid of a fan until solids content of more than 70% being characterized as a soft extract.[16] The soft extract was then stored under freezer at −17°C.

Validation method

The method was validated according to the Brazilian legislation[17] and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.[18] The analytical parameters of selectivity, linearity, range, precision (repeatability and intermediate precision), accuracy, and robustness were evaluated.

Before the validation, the chromatography system was evaluated to verify its capacity of providing reproducible results. This evaluation was carried out with the system suitability parameters, which is a set of tests to ensure that the equipment used is capable of generating acceptable, accurate and precise results. These parameters are the tailing factor (T), resolution, capacity factor (K), and number of theoretical plates (N), that were evaluated using the *Empower 2.0* software from Waters Coorporation (Milford, MA, USA).[19]

Selectivity

The selectivity of the method was evaluated by comparing the chromatograms of a blank (methanol) solution, the extract solution, and the standards. The ultraviolet (UV) spectral similarities of curcumin, demethoxycurcumin, and bisdemethoxycurcumin peaks in the standard and in the extract were also compared at 420 nm.

Linearity and range

The linearity was determined by calibration curves using the standards at six concentration levels of curcumin (13.3, 39.9, 66.5, 79.8, 106.4, and 133 μg/mL), demethoxycurcumin (6.6, 19.8, 33, 39.6, 52.8, and 66 μg/mL), and bisdemethoxycurcumin (3.3, 9.9, 16.5, 19.8, 26.4, and 33 μg/mL) diluted in methanol and obtained from HPLC analysis. Each point was prepared in triplicate and the equation of the standard linear regression and its coefficients (r) were calculated by the correlation between the peak areas and the concentration of the standards (Limit of detection [LOD] and limit of quantification [LOQ]).

The LOD and LOQ for each marker were calculated using the standard deviation between the linear coefficients (SDb) and the slope of the calibration curves (S), according to the Equations (1) and (2).

Equation (1) LOD = SDb. 3/S

Equation (2) LOQ = SDb. 10/S Precision

The precision of the extracted sample was evaluated at two levels, that is, repeatability (intraday) and intermediate precision (interday), expressed using the relative SD (RSD%) as criteria. The repeatability was evaluated by six injections of the test concentrations, comprising the central point of the standard linear range (500 μg/mL). The intermediate precision was verified by the same process, performed by different analysts on different days.

Accuracy

Accuracy was calculated by recovery analysis.[16] A known quantity of the three standards (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) was added to a known quantity of the soft

extract (soft extract + 3 standards). The standards and the extract solutions were individually analyzed first and after the addition of the standards to the extract solution. High, medium, and low concentration of the soft extract (240 µg/mL, 480 µg/mL, and 800 µg/mL) were used. The accuracy was calculated through the ratio between the average experimental concentration and theoretical concentration of the added standard according to Equation (3).

Equation (3) Accuracy = ([sample + standard] − sample without standard) 100%/Standard theoretical concentration

Robustness

The robustness of the method was verified by comparing the content of curcuminoids in the soft extract obtained from the original method of analysis and modified conditions. The following parameters were changed: mobile phase flow of 1 mL/min to 0.9 m/min and 1.1 mL/min, column oven temperature of 25°C to 26°C and 27°C, and the acetonitrile and acetic acid glacial manufacturer used in the mobile phase. The injections were performed in triplicate and the results were evaluated by the RSD calculation.

Capsules obtaining

The granule used to produce the capsules was obtained through the wet process, in which the soft extract was added to the adjuvants mixture containing microcrystalline cellulose, lactose monohydrate, and polyvinylpyrrolidone K-30. The mixture was dried in a circulating air oven (Alwis A-3000-5B) at 55°C for 2 h. The granule was then sized into a 1.5 mm mesh sieve and the lubricants magnesium stearate and magnesium silicate were added and homogenized. The proportion of the ingredients used in the production of the granule is described in Table 1.

The capsules were manually encapsulated using capsules size number 0. They were characterized by average weight, disintegration, content uniformity, total curcuminoids content per capsule, dissolution profile, and stability. To calculate the content of total curcuminoids in the granule, the analytical method was covalidated to guarantee that the adjuvants added to the formulation did not interfere with the quantification of the markers.

Covalidation of the analytical method for quantification of curcuminoids in the capsules

The covalidation of the method was performed following the Brazilian legislation,[17] evaluating the analytical parameters of linearity, selectivity, precision, and system suitability.

Capsules characterization-average weight determination

Twenty units of the filled capsules were weighed individually, then the contents of each capsule were withdrawn and the empty capsule was weighed. The weight of the contents of each unit (Wc) was calculated by the difference between the weight of the filled capsule and the empty capsule. The mean content weight (Wmc) was calculated by dividing the sum of the content weight of all units by 20. Thereafter, the variation weight of the content of each unit (Vc) was calculated in percentage according to the Equation (4):

Equation (4) $Vc(\%) = (Wc - Wmc/Wmc) \times 100$

Table 1: Proportion of ingredients used in the *Curcuma longa* granule

Ingredients	Proportion (%)
Curcuma longa soft extract	23.58
Microcrystalline cellulose	37.73
Lactose monohydrate	33.01
polyvinylpyrrolidone K-30	5.66
Magnesium stearate	3
Magnesium silicate	3

Disintegration test

The capsule disintegration test was performed using six capsules and purified water as the immersion liquid, maintained at 37 ± 1.0°C for 45 min on a disintegrator (Nova Etica 301 AC). At the end of the test, all units must be completely disintegrated.[16]

Content uniformity test

The content uniformity test was applied in 10 units of capsules, individually. From the ten obtained results, the average content (Ca), the SD, and the acceptance value (AV) were calculated by the Equation (5).

Equation (5) $AV = |M\text{-}Ca| + k.\ SD$

"*M*" is a reference value, which can be 98.5–101.5, depending on the average content (Ca) of the units. "*k*" *is* the acceptability constant, equal to 2.4 for 10 units tested in the first step, so the equation for this work will be $AV = |MCa| + 2.4$ SD The AV should not be more than 15.0.[16]

Dissolution tests

The dissolution tests of the capsules were conducted in 900 mL of medium maintained at 37 ± 0.5°C using United States Pharmacopeia (USP) apparatus I (basket) at 100 rpm (Hanson, Vision Elite 8, Chatsworth, CA, USA). Two dissolution mediums were tested to simulate the physiological conditions, an acid medium (HCl buffer pH 1.2) simulating the stomach and a neutral medium (phosphate buffer pH 6.8) simulating the duodenum, both prepared according to the United States Pharmacopeia.[20] As the curcuminoids have low solubility in aqueous media, 0.5% of the surfactant sodium lauryl sulfate was added to each dissolution medium.[21,22] Samples of 5 mL were collected manually at 5, 10, 20, 30, 45, 60, and 90 min and filtrated through 45 µm filter before injection. The tests were performed in triplicate for each medium. The content of curcuminoids in each aliquot was verified by the validated method and the dissolution profile curve was made by the percent dissolved on each point versus the collection time. The percentage dissolved was calculated considering the average obtained in the analysis of content uniformity.

To guarantee that the dissolution test is right and reproducible, it should be performed under sink condition. In this condition, the drug concentration in the dissolution medium should not exceed 10%–20% of saturation solubility of the drug, but it is applied to pure substances.[23,24] This condition was also verified in the dissolution study by dissolving one capsule in 180 mL of the dissolution medium (20% of the dissolution medium capacity), and the sink condition was obtained by the maximum dissolved concentration of each marker on 900 mL of the medium related to the area obtained for the 180 mL of the medium.

The dissolution efficiency (DE) was also calculated for each dissolution profile by the relation between the area under the curve (AUC) of the dissolution profile curve and the total area of rectangle (ATR), defined by ordinate at 100% dissolution and abscissa in 90 min,[25] as described in the Equation (6).

Equation (6) $DE\% = AUC/ATR \times 100$

The obtained results for DE% and the dissolved values for the markers on each dissolution media were compared by the F variance test and ANOVA, both with 95% confidence interval, using STATISTICA 7 program from StatSoft (Palo Alto, CA, USA).

Accelerated stability study for capsules

The accelerated stability study was performed on a climatic chamber (Nova Ética®, Série 400) at 40°C and 75% relative humidity during 90 days.[26] The capsules were added to identified flasks with threaded cap along with cotton to fill the voids. The markers were quantified at 0, 30, 60, and 90 days, and the curve of the content versus the storage time under forced conditions of degradation was plotted.

RESULTS AND DISCUSSION

The presence of curcuminoids in the powdered turmeric rhizomes was proved by its TLC and HPLC profile, as shown in Figures 1 and 2, respectively. The chromatogram of the powdered *C. longa* shows only the presence of the three curcuminoids because of the specificity of the method in detecting only these three markers.

The *C. longa* soft extract, obtained by the percolation process, presented a solid content of 78.94% after concentration and 3.45% of bisdemethoxycurcumin, 3.03% of demethoxycurcumin, and 14.83% of curcumin, corresponding to 21.31% of total curcuminoids. The percentage of curcuminoids obtained in this work, compared to other liquid extracts described in the literature, represents a high content of curcuminoids[13,27] and proves the advantage of soft extracts as higher markers content means higher biological activity.

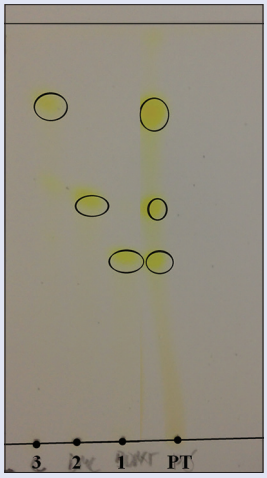

Figure 1: Thin-layer chromatography profile of curcuminoids standards and powdered *Curcuma longa*. 1: Bisdemethoxycurcumin, 2: Demethoxycurcumin, 3: Curcumin, PT: Powdered turmeric

Validation method-system suitability

The system suitability results are shown in Table 2. The results are in accordance with the established in the United States Pharmacopeia (USP) and by the Food and Drug Administration (FDA).[28] The results demonstrate that the developed method is suitable for the separation and quantification of curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

Selectivity

Figure 3a-c shows the chromatograms and UV spectrum (420 nm) of curcumin, demethoxycurcumin, and bisdemethoxycurcumin from the HPCL-PDA analysis of the *C. longa* soft extract and the curcuminoids standards. The chromatographic profile of the standards [Figure 3a], the soft extract [Figure 3b] and methanol [Figure 3d], and the UV spectral similarity of the extract and the standards shows the selectivity of the method. No interfering substances are observed in the curcuminoids retention times and the UV spectrum for the standards and the soft extract are identical.[29]

Linearity and range

The developed method presented to be linear as the linear regression coefficients (r), as shown in Table 3 for each marker, was higher than 0.99.[17] The calibration curve was linear in the range of 13.3–133 µg/mL for curcumin, 6.6–66 µg/mL for demethoxycurcumin, and 3.3–33 µg/mL for bisdemethoxycurcumin. The standard linear equation for curcumin was Y = 70254 × 22863 (n = 6, r = 0.9998), for demethoxycurcumin was Y = 139862 × 35088 (n = 6, r = 0.9998), and for bisdemethoxycurcumin was Y = 131723 × 41350 (n = 6, r = 0.9992).

Limit of detection and limit of quantification

The LOD value represents the lowest absolute concentration of the marker in the sample that can be detected but not necessarily quantified, which was 0.04 µg/mL for curcumin, 0.02 µg/mL for demethoxycurcumin, and

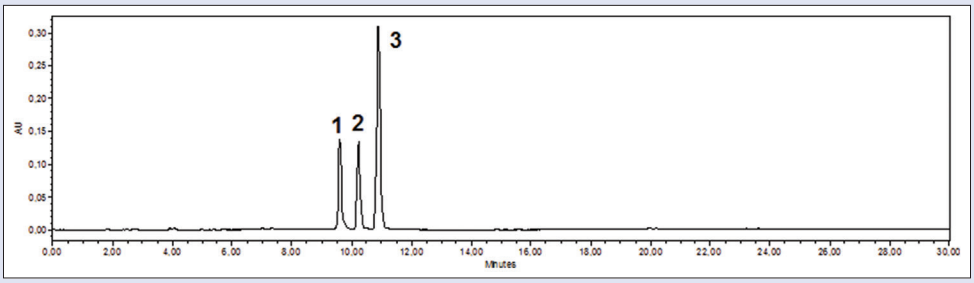

Figure 2: Powdered *Curcuma longa* chromatogram. 1: Bisdemethoxycurcumin, 2: Demethoxycurcumin, 3: Curcumin

Table 2: Values of system suitability for the *Curcuma longa* soft extract

Parameter	FDA*	Curcumin	Demethoxycurcumin	Bisdemethoxycurcumin
Resolution (R)	>2.0	3.30	3.32	10.49
Tailing factor (T)	≤2.0	1.12	1.12	1.15
Capacity factor (K)	>2	5.12	4.74	4.37
Number of theoretical plates (N)	>2000	44,661.67	42,544.35	40,579.63

*FDA: Food and Drug Administration recommendations

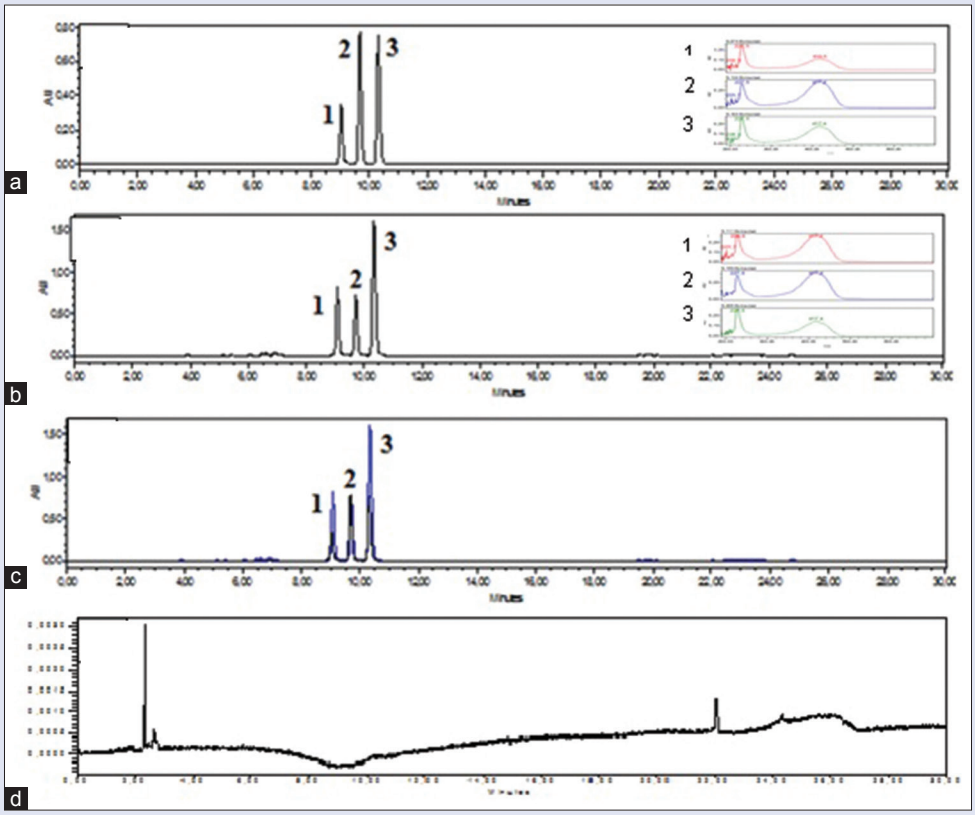

Figure 3: (a) Standards chromatograms; (b) *Curcuma longa* soft extract chromatograms; (c) chromatograms overlap of the standards (blue) and the *Curcuma longa* soft extract (black); and (d) methanol, followed by the ultraviolet spectra of curcuminoids (420 nm). 1: Bisdemethoxycurcumin, 2: Demethoxycurcumin, 3: Curcumin

Table 3: Results for the parameters of method validation of determination of curcuminoids

Parameters	Curcumin	Demethoxycurcumin	Bisdemethoxycurcumin
Linear regression coefficients (*r*)	0.9998	0.9998	0.9992
LOD (μg/mL)	0.04	0.02	0.07
LOQ (μg/mL)	0.13	0.06	0.22
Precision (RSD%)	2.75	3.27	3.07

LOD: Limit of detection; LOQ: Limit of quantification; RSD%: Relative standard deviation percentage

0.07 μg/mL for bisdemethoxycurcumin. The LOQ value represents the lowest amount of marker in a sample and which can be quantitatively determined with precision and accuracy, which was 0.13 μg/mL for curcumin, 0.13 μg/mL for demethoxycurcumin, and 0.22 μg/mL for bisdemethoxycurcumin [Table 3].

Precision

The results of method precision were calculated by the RSD for the interday and intraday precisions together. The RSD value obtained was <5% for each marker, among the six determinations as recommended by ANVISA.[17] The results show that successive measurements of the same method performed under the same conditions, in the same day, and by different analysts on different days are precise and with low deviations.

Accuracy

The average recovery (RSD %) from the accuracy of the method for the three markers are shown in Table 3. The recovery for curcumin ranged from 98.2% to 102.3% with an average of 100.4% and RSD of 3.3%; for demethoxycurcumin, it ranged from 97.7% to 101.4% with an average of 99.1% and RSD of 2.9%; and for bisdemethoxycurcumin, it ranged from 102.0% to 104.5% with an average of 103.2% and RSD of 2.4%. This test measures the amount the marker that is extracted and capable of being measured in the analytical portion of the test material.[30] According to the Brazilian legislation, the recovery range should be between 95% and 105%, and the RSD should be <5%.[17]

Robustness

The variations in the mobile phase flow, column oven temperature, and manufacturer of the acetonitrile and acetic acid glacial are used in the mobile phase, resulting in an RSD% value below 5% for the curcuminoids content, proving the robustness of the method [Table 3].

The robustness test is critical for transferring the analytical method for other laboratories because it assesses the ability of the method to resist small variations of the analytical parameters, indicating its confidence during normal use.[17,31]

Covalidation of the analytical method for quantification of curcuminoids in the capsules

The analytical method was covalidated to measure the curcuminoid content in the capsules since it was a different matrix than the one used in the validated method (*C. longa* soft extract). The method presented to be linear showing a linear regression coefficient (*r*) of 0.9984 for curcumin, 0.995 for demethoxycurcumin, and 0.9985 for bisdemethoxycurcumin. The method was also precise since the RSD value obtained was 6.92% for curcumin, 3.59% for demethoxycurcumin, and 2.51% for bisdemethoxycurcumin. According to the Brazilian legislation, as the herbal medicines are a complex matrix, it is admitted an RSD% value up to 15%,[32] demonstrating the precision of the method to measure the three markers.

Capsules characterization

The granule presented 6.27% of water content, corresponding to 93.73% of dry residue, and the total curcuminoids content in the granule was 5.01% (m/m).

The capsules disintegrated in 7 min and showed an average weight of 421.20 mg and maximum variation of 5.06%. The maximum permitted variation for average weight is 7.5% for capsules with weight >300 mg, and the maximum disintegration time is 45 min, according to the Brazilian legislation.[16]

The content uniformity test was evaluated calculating the acceptable value (AV), which must be <15.0 according to the Brazilian legislation. The AV value obtained for curcumin was 8.9, for demethoxycurcumin was 10.4, and for bisdemethoxycurcumin was 13.0. This test ensures that the dose of a given batch is in conformity with the declared dose.[16] All the three curcuminoids content was in the capsules are in conformity.

The total curcuminoids content in the capsules were 21.56 mg, obtained by the sum of the contents obtained in the content uniformity test. The recommended therapeutic dose of curcumin is 400–600 mg, 3 times a day.[1,33-35] As we are working with a soft-concentrated extract, compounds other than curcuminoids are present and concentrated in the extract. That is why a lower dose of approximately 20 mg curcuminoids was chosen for the capsules.

Dissolution tests

The sink condition results found for the acid medium (HCl buffer pH 1.2) were 37.27% for curcumin, 35.95% for demethoxycurcumin, and 34.71% for bisdemethoxycurcumin. For the neutral medium (phosphate buffer pH 6.8) the sink condition results were 29.56% for curcumin, 29.79% for demethoxycurcumin, and 28.93% for bisdemethoxycurcumin. The limit of maximum 20% in the sink condition is established for the pure substances,[24] in the present work, all the values found for this condition was higher because we are working with a complex matrix as active principle, and the values found show that they are not even close to the saturation concentration, ensuring the reproducibility of the method.

The Figure 4a and b demonstrates the dissolution profiles found for the acid and neutral medium, respectively. In acid medium, the percentage of curcuminoids dissolved did not reach 70% after 90 min of dissolution [Figure 4a], which can be explained due to their low solubility in acid medium.[36] In neutral medium, the complete dissolution of the three markers was observed in 30 min and kept until 90 min [Figure 4b] showing that there was no degradation of the markers until the end time.[37] This *in vitro* test allows predicting the performance of the release and dissolution or solubilization of a given drug *in vivo*.

The DE values found for the acid medium were 57.08% for curcumin, 63.23% for demethoxycurcumin, and 64.53% for bisdemthoxicurcumin. The values of DE found for the neutral medium were 105.52% for curcumin, 105.82% for demethoxycurcumin, and 107.76% for bisdemethoxycurcumin. The values obtained for each marker on each dissolution medium were compared using ANOVA test, proving that the acid medium had significantly lower DE ($P > 0.05$) than the neutral medium for the three markers, indicating that the neutral medium was

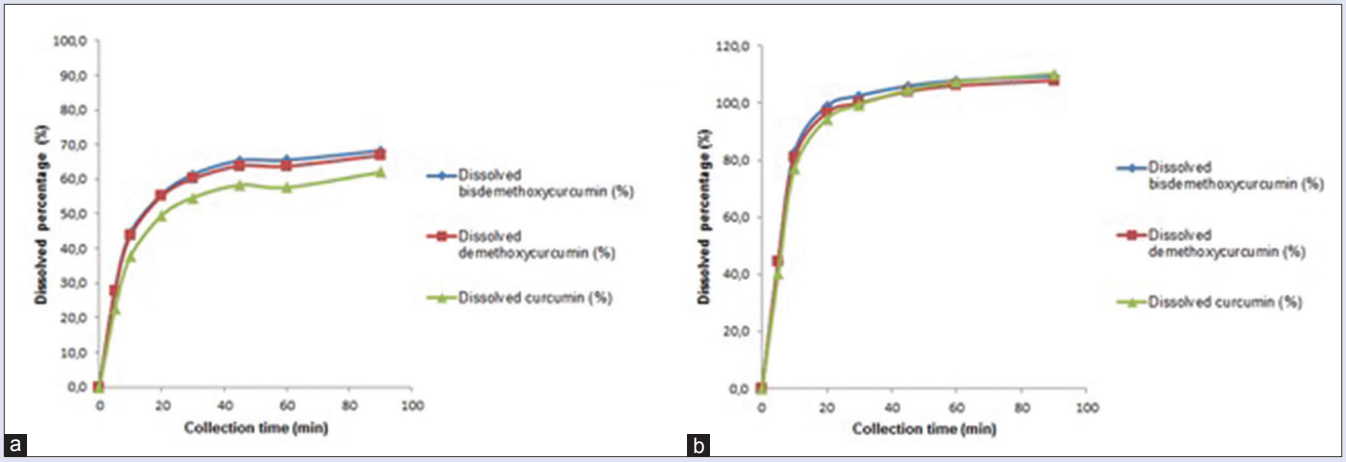

Figure 4: (a) Capsules dissolution profile in acid medium (HCl buffer pH 1.2); (b) Capsules dissolution profile in neutral (phosphate buffer pH 6.8)

Table 4: Results for the accelerated stability test of the capsules containing *Curcuma longa* soft extract

Time (days)	[ ] Bisdemethoxycurcumin mg/capsule	Variation	[ ] Demethoxycurcumin mg/capsule	Variation	[ ] Curcumin mg/capsule	Variation
0	0.94		0.78		3.09	
30	0.83	8.21	0.68	9.16	2.60	11.33
60	0.83	7.75	0.68	9.79	2.53	12.94
90	0.81	9.17	0.66	11.00	2.54	12.66

[ ]: Concentration

more efficient. The high DE in neutral pH medium may indicate a better absorption of these markers in the first portion of the intestine (duodenum). Monton *et al.*[38] had 100% of curcuminoids dissolved in 90 min using an HCl 0.05M + 0.8% of sodium lauryl sulfate medium for the dissolution of capsules containing *C. longa*-powdered rhizomes. In this work, some adjuvants were added to the formulation and the surfactant proportion in the mediums was lower; therefore, it can explain the lower dissolution in the acid medium.

Accelerated stability studies for the capsules

The results found for the accelerated stability studies are shown in Table 4. This study aimed to accelerate possible chemical degradation and/or physical changes of the pharmaceutical active under forced storage conditions.[26]

ANVISA recommends that the maximum variation compared to the time zero should be 10%. For the bisdemethoxycurcumin, the variation was lower than 10% during the 90 days of the experiment, proving to be stable. The demethoxycurcumin was stable until 60 days, after that it presents a variation higher than 10%. The curcumin had variation higher than 10% since the first 30 days evaluated, showing that it is easily degraded in this formulation. The stability results suggest that the bisdemethoxycurcumin can be used as an only marker in the formulation proposed because it did not degrade after 90 days. A deeper stability study of curcuminoids in formulations using *C. longa* soft extract as an active principle may be conducted.

CONCLUSIONS

Based on the obtained results for the validation parameters, it was developed a linear, precise, selective, accurate, and robust method for the quantification of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in the *C. longa* soft extract. The standardized soft extract was used as an active principle for hard gelatin capsules. The produced capsules presented better dissolution profile in the neutral medium that simulates the duodenum pH, which can indicate a higher absorption of these markers in this portion of the intestine. However, the stability test showed that only the bisdemethoxycurcumin is stable within 90 days in the proposed formulation, suggesting that it can be used as an only marker. There are no studies in the researched literature that uses *C. longa* soft extract as the active principle of a formulation. Studies regarding the *in vivo* pharmacokinetics of formulations containing soft extract are necessary, as well as studies regarding the stability of curcuminoids in this formulation.

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Conflicts of interest

There are no conflicts of interest.

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