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.

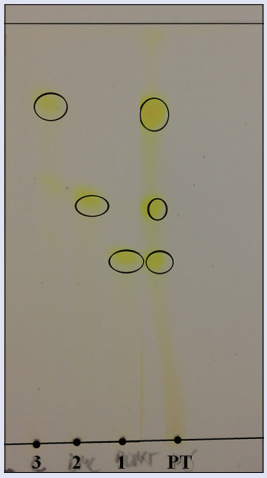

Figure 1: Thin-layer chromatography profile of curcuminoids standards and powdered *Curcuma longa*. 1: Bisdemethoxycurcumin, 2: Demethoxycurcumin, 3: Curcumin, PT: Powdered turmeric

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

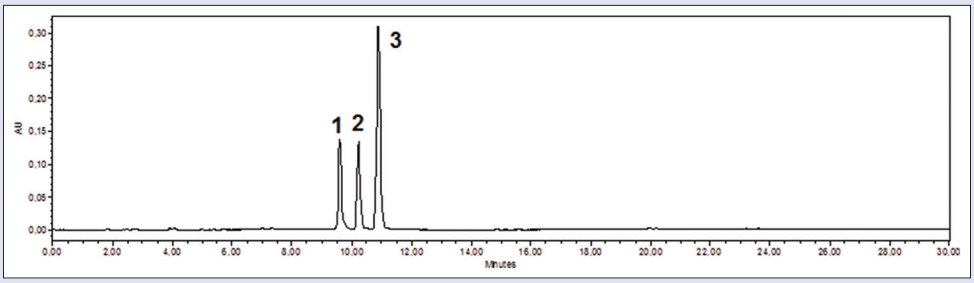

Figure 2: Powdered *Curcuma longa* chromatogram. 1: Bisdemethoxycurcumin, 2: Demethoxycurcumin, 3: Curcumin

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

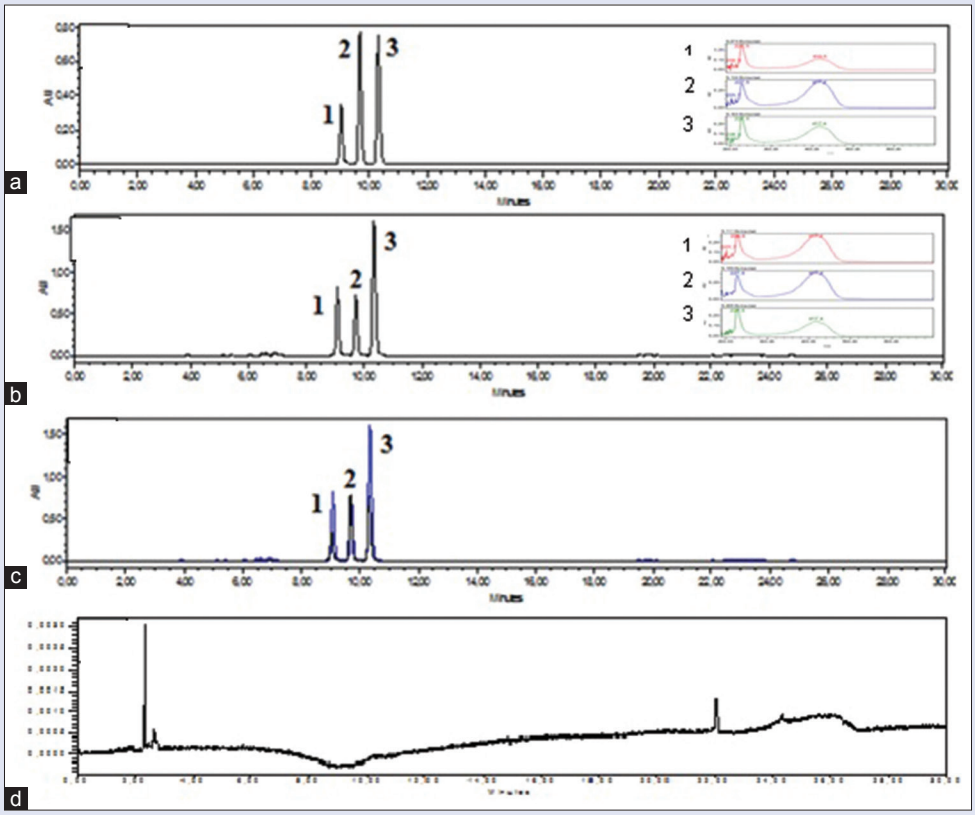

Figure 3: (a) Standards chromatograms; (b) *Curcuma longa* soft extract chromatograms; (c) chromatograms overlap of the standards (blue) and the *Curcuma longa* soft extract (black); and (d) methanol, followed by the ultraviolet spectra of curcuminoids (420 nm). 1: Bisdemethoxycurcumin, 2: Demethoxycurcumin,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

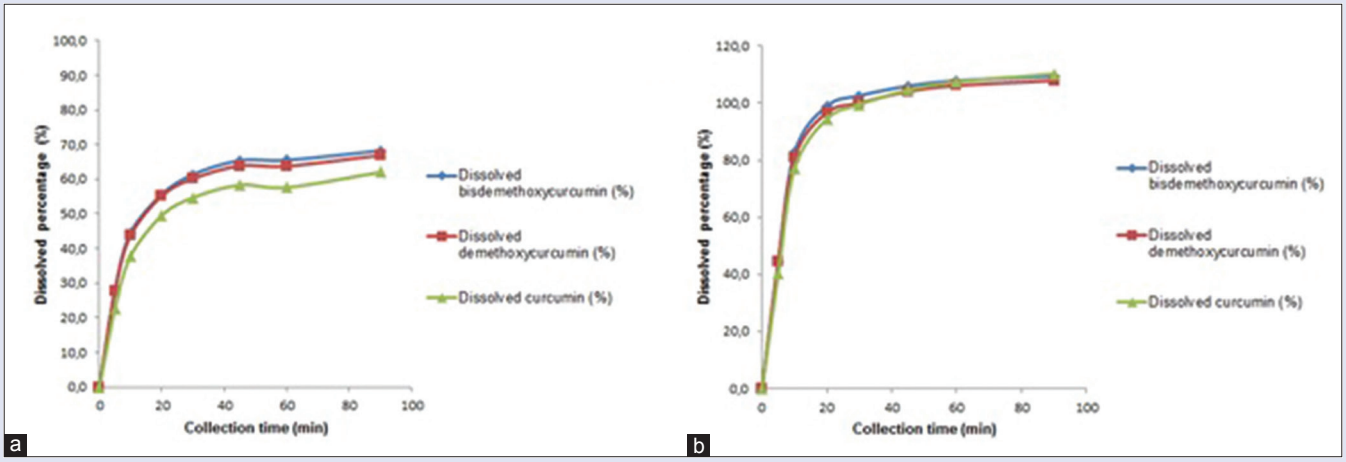

Figure 4: (a) Capsules dissolution profile in acid medium (HCl buffer pH 1.2); (b) Capsules dissolution profile in neutral (phosphate buffer pH 6.8)

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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