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Validation of Spectrophotometric Method for Quantification of Folic Acid in Capsules

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ABSTRACT

Introduction: Folic acid (FA) deficiency can be associated with various pathophysiological conditions, altering the homeostasis of the human organism. This study addresses the unique analytical challenges of FA quantification in hard capsules, a less-studied oral solid dosage form compared to tablets. The evaluation of quality, safety, and efficacy is essential to ensure that this product meets the characteristics described in the pharmacopoeia to guarantee biopharmaceutical and pharmacotherapeutic performance. This study aimed to develop and validate an analytical method for the quantification of FA in capsules using ultraviolet-visible absorption spectrophotometry. **Methods:** Starch and microcrystalline cellulose were selected as excipients for the preparation of capsules containing 5 mg of FA. Parameters such as selectivity and matrix effect, linearity, precision, accuracy, limit of detection (LD), limit of quantification (LQ), and robustness were evaluated according to RDC no. 166/2017. Later, weight determination, assay, content uniformity, and dissolution tests were conducted. **Results:** The method displayed high selectivity in pH 7.2 at 280 nm, with no matrix effect. Statistical treatment of the linearity (r = 0.9998, from 1.0–15 µg/mL) confirmed homoscedasticity of the data, showing a normal distribution (p > 0.05) and independence of residues. Precision, accuracy, LD (0.249 µg/mL), LQ (0.755 µg/mL), and robustness to wavelength and temperature variations were suitable. The capsules showed satisfactory results for weight determination (limits of variation of ± 10.0%, RSD (3%), and variation of theoretical content (96–101%). The validated analytical method demonstrated applicability in the quantification of FA encapsulated for the assay (96.3%), content uniformity (AV = 7.0), and dissolution tests (103%) using basket as apparatus, and phosphate buffer as dissolution medium. **Conclusion:** This method offers a cost-effective alternative for routine quality control of FA capsules, particularly in resource-limited settings. Though it is essential to validate the conditions used in the dissolution test.

KEYWORDS: Analytical method, spectrophotometry, quality control, folate, dissolution

INTRODUCTION

Folic acid (FA), also known as vitamin B9 or folate or vitamin M, can be naturally found in various foods such as spinach, beans, kale, oranges, soy, and beef liver, or it can be added to grain-derived foods (*1*). FA exhibits a fundamental neurotrophic property, playing a significant role in the differentiation, growth, and regeneration of the central nervous system. It has the potential to repair injuries and reduce the risk of neurological diseases, such as neural tube defects, spinal cord regression, Alzheimer's disease, and other neuropathies (*2*, *3*). Moreover, it is notably involved in the recovery of peripheral nerves, likely inducing Schwann cell proliferation, as well as migration and secretion of neural growth factors (*2*–*5*). It may also influence DNA synthesis and cell replication, particularly during embryogenesis and intrauterine growth, potentially impacting the gestation process (*5*, *6*).

FA deficiency can be associated with various patho-physiological conditions, such as congenital defects, impaired neonatal growth, cardiovascular diseases, inflammatory bowel disease, and even certain types of

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cancer (*7*). This association may be linked to poor vitamin absorption, increased catabolism, or heightened folate requirements, thereby altering the homeostatic state of FA in the body (*6*). Furthermore, it can lead to conditions such as megaloblastic anemia, mucositis, recurrent infections, hereditary folate malabsorption (HFM), and neurological deficits (*1, 6, 8, 9*).

In 2023, there was still no consensus regarding the exact Biopharmaceutics Classification System (BCS) classification of FA (*10*). According to Wu et al., 1 mg of FA was soluble in approximately 500 mL of aqueous medium at pH 3.0 (*11*). Considering the solubility criteria of *Brazilian Pharmacopeia* (FB7), FA can therefore be considered poorly soluble in aqueous medium adjusted to pH 3.0 (*12*). Hofsäss et al. evaluated the possibility of biowaivers for bioequivalence testing of generic immediate-release FA tablet formulations for registration purposes (*13*). According to these authors, FA absorption appears to be dose-dependent, with absorption reaching approximately 90% for doses below 320 µg, which can be achieved through a balanced diet, whereas for formulations containing 5 mg of the drug, absorption is significantly reduced and can drop to less than 50% (*13*). This classification underscores the need for sensitive analytical methods to detect low concentrations of dissolved FA, as well as robust dissolution testing to ensure adequate release, posing unique challenges for capsule formulations where shell dissolution may further influence bioavailability. Based on this, it was considered in this study that FA falls under BCS class 4, i.e., low solubility and low permeability.

FA is characterized as a yellow-orange crystalline powder; soluble in dilute solutions of alkaline hydroxides, carbonates, hydrochloric acid, and sulfuric acid; slightly soluble in cold water; and insoluble in ethyl alcohol, acetone, benzene, chloroform, and ether (*12, 14*). The assay of FA in tablets is performed using high-performance liquid chromatography equipped with an ultraviolet detector at 283 nm (HPLC-UV) (*12, 15*).

On the other hand, the literature includes studies on various active pharmaceutical ingredients quantified through previously validated spectrophotometric methods, applied to pharmaceuticals, foods, or plant-based products (*16–23*). There are also reports of FA quantification using UV-visible spectrophotometry in different matrices, such as salt, flours, and tablets (*24–28*). Despite these advancements, the application of UV-visible spectrophotometry to FA in hard capsules remains underexplored, particularly regarding excipient interactions and the dissolution behavior of FA in this dosage form, which may differ from tablets due to capsule shell disintegration dynamics. Added to this is the fact that this method is simple, fast, highly reproducible and accessible, with low operational costs, selectivity, sensitivity, satisfactory accuracy and precision (*26, 28–30*). Furthermore, it allows for the use of safer solvents, minimizing the production of waste from organic solvents and their environmental impacts, thereby complying with the 12 principles of green analytical chemistry proposed by Gałuszka et al. (*31*).

Compared to HPLC, the standard method for FA assay, UV-visible spectrophotometry offers lower operational costs and simpler instrumentation, making it accessible for small-scale laboratories. However, careful optimization is required to mitigate risks of interference, i.e., working range, pH, and temperature, and to ensure sensitivity for low-dose formulations like FA capsules (*23*). In this regard, a validation step is essential before applying the method.

This study aimed to develop and validate an analytical method for the quantification of FA in hard capsules using UV-visible absorption spectrophotometry, according to parameters by RDC no. 166, of July 24, 2017 (*32*). In addition, the validated method was applied to assess FA capsules via assay, content uniformity, and dissolution tests (*12, 33*).

METHODS

Materials and Reagents

The FA (batch 202009006) and microcrystalline cellulose (MCC) (PH-101 batch 210236) used in this study were obtained from Gemini Indústria de Insumos Farmacêuticos Ltda. (Anápolis, GO, Brazil). Corn starch (batch SE0449) was acquired from Fagron do Brasil Farmacêutica Ltda. (São Paulo, SP, Brazil). The hard gelatin capsules (no. 3, colorless, batch 7174903I) were purchased from Capsugel (Rio de Janeiro, RJ, Brazil). Purified water was supplied by the Gehaka reverse osmosis system (model OS10LXE, São Paulo, SP, Brazil). Buffer solutions were prepared at the time of use and according to the *Brazilian Pharmacopeia* (FB7) (*12*). All other reagents were of analytical grade.

Equipment

The following equipment was used in this study: FA2004C analytical balance (Jugo, Shanghai, China), A3 Tepron manual encapsulator (São Paulo, SP, Brazil), UDT-812GS dissolution apparatus (Logan Instruments Corp., Somerset, NJ, USA), and Nova Instruments UV-Vis 1600 spectrophotometer (Piracicaba, SP, Brazil). Thermo Scientific automatic pipettes (São Paulo, Brazil) and previously calibrated analytical glassware were also used.

Excipients Selection and Capsules Preparation

Considering the excipients present in solid oral formulations containing FA registered by Anvisa, corn starch and MCC (1:10) were selected as suitable diluents for filling the hard capsules (*34*).

Substances such as sodium lauryl sulfate (SLS) and lactose were excluded due to their potential to interfere with FA selectivity by altering its spectral properties and causing adverse events like gastrointestinal intolerance, respectively. Additionally, SLS may enhance FA solubility in a way that masks true dissolution behavior, while lactose could contribute to degradation in humid conditions (*35*, *36*).

Progressive dilution was carried out to obtain a random mixture, which was used to fill volumetrically 60 capsules (FA 5 mg plus excipients: 10.3 mg of starch, and 93.2 mg of MCC) (*26*, *37*). All products (n = 3) were stored in opaque high-density polyethylene containers, labeled, and kept at controlled ambient temperature (15–30 °C) and relative humidity (40–65%).

Analytical Validation

The analytical method was validated considering the parameters of selectivity and matrix effect, linearity, precision and accuracy, limit of detection, limit of quantification, and robustness (*32*).

Selectivity and Matrix Effect

FA can be spectrophotometrically detected in alkaline medium (*14*). In this regard, the stock solution (1000 µg/mL) was prepared using 0.1 mol/L sodium hydroxide (NaOH) as the solvent. Subsequently, three distinct solutions were prepared in triplicate at a concentration of 10 µg/mL using phosphate buffer pH 7.2 (PB 7.2), borate buffer pH 8.0, and purified water, which were subjected to scanning in the spectral region between 230 and 310 nm. Based on the highest intensity of the analytical response, PB 7.2 was chosen as the working solution.

Solutions containing corn starch, MCC, SLS, and lactose were prepared individually in PB 7.2, reaching 10 µg/mL, due to the nominal concentration of sample. After filtration using qualitative paper, the samples were subjected to spectral reading between 230 and 310 nm.

The matrix effect was evaluated by comparing the angular coefficients of the analytical curves constructed with FA Reference Standard (RS) in PB 7.2 and with the sample fortified with excipients (corn starch and MCC). The curves were established in the same manner as in the linearity for the same concentration levels in triplicate for FA concentrations of 1, 2.5, 5, 10, and 15 µg/mL. The measurements were taken at 280 nm. Parallelism of the lines indicates the absence of interference from the matrix components (*32*).

Linearity

The linearity of the method was evaluated at 280 nm on three distinct days using solutions prepared independently in triplicate. Initially, a stock solution of FA RS in 0.1 mol/L NaOH (1000 µg/mL) was prepared, which was then used to prepare solutions at concentrations of 1, 2.5, 5, 10, and 15 µg/mL using PB 7.2 as the solvent. PB 7.2 was used as the blank.

Precision and Accuracy

The precision and accuracy were evaluated using three points of the analytical curve: 1, 5, and 15 µg/mL. The FA solutions were prepared in triplicate on three distinct days, using PB 7.2 as the solvent. Relative standard deviation (RSD) and percentage recovery values were used to assess precision and accuracy, respectively.

Limit of detection (LD) and limit of quantification (LQ)

The LD and LQ values were determined according to RDC No. 166, of July 24, 2017 (*32*). In this study, LD and LQ were tested on three distinct days, in triplicate.

Robustness

The impact of variations in the analytical signal was evaluated to check the robustness of the proposed method. For this purpose, three variables were considered: wavelength (280 vs 283 nm), solvent (PB 7.2 vs water), and temperature (25 vs 30 °C). All solutions were prepared in triplicate (*32*).

Quality Assessment of Folic Acid (FA) Capsules

According to the *National Formulary of the Brazilian Pharmacopoeia*, three criteria are considered for approval: limits of variation (± 10.0% to mean < 300 mg), RSD (< 4%), and variation of theoretical content (VTC) within the range of 90–110% (*33*). Capsules (n = 10) were individually weighed, providing mean, standard deviation and RSD values. VTC values were determined from theoretical weight (156.5 mg) (*33*).

Assay

The sampling was based on the assay method as described in the monograph for FA tablets in accordance with FB7 (*12*). Twenty (n = 20) capsules were weighed individually and then emptied. The content of the capsules was homogenized, and an amount of powder equivalent to 20 mg of FA was transferred to a 100-mL volumetric flask, with the aid of 50 mL of PB 7.2. The mixture was homogenized, and the volume was completed with the same solvent, to obtain a solution of FA at 200 µg/mL.

After agitation and filtration through qualitative paper, 5 mL of the filtrate was transferred to a 100-mL volumetric flask using PB 7.2 as the solvent to obtain an FA working solution of 10.0 µg/mL. Subsequently, the readings were taken at 280 nm, using PB 7.2 as the blank. The amount of FA in the capsules was calculated from the obtained analytical curve. The test was performed in triplicate. It is expected to find no less than 90.0% and no more than 110.0% of the labeled amount of FA (*12*).

Content Uniformity

The content uniformity test was conducted with 10 units, individually weighed. The content of each capsule was transferred to separate 50-mL volumetric flasks (100 µg/mL), and the volume was completed with PB 7.2. After homogenization and filtration through qualitative paper, 1 mL of the filtrate was transferred to a 10-mL volumetric flask using PB 7.2 as the solvent to obtain the working solution of FA at 10 µg/mL. The measurements and the amount of FA in each tested unit were determined as described above. The acceptance value (AV) was calculated according to the *United States Pharmacopeia* (USP) (*15*).

Dissolution

In the present study, the dissolution test was conducted to evaluate the possibility of quantifying FA in capsules (n = 6) by spectrophotometry. The conditions described in FB7 for tablets were used with some adaptations (*12*).

A basket apparatus (apparatus type 1) was selected to accommodate capsule dissolution, i.e., to prevent floating or sticking issues that are common with paddles. PB 7.2 was chosen as the dissolution medium to align with the validated analytical method and because FA exhibits higher solubility in slightly alkaline media due to deprotonation of its carboxylic groups, thereby enhancing detection sensitivity compared with water, where solubility is limited.

For the dissolution test, 500 mL of PB 7.2 was used with light-protected vessels, and the apparatus was set to 50 rpm for 45 minutes. At the end of the specified time, 5 mL was collected from the middle zone, estimating a concentration of 10 µg/mL, after filtration. The absorbances were measured based on the spectrophotometric method developed and previously validated. The amount of dissolved FA in the medium was determined using the analytical curve. The allowed tolerance is at least 80% of the cumulative percentage of FA dissolved in relation to the declared dose (*12*, *14*).

Statistical Analysis

The Student's t-test was employed to assess the matrix effect by comparing the angular coefficients of analytical curves with and without excipients, with a significance level of 5% ($p < 0.05$).

Statistical treatment of linearity was performed by removing outliers using the Jackknife test, followed by verification of normality (Ryan-Joiner test) and independence of residuals (Durbin-Watson test).

Homoscedasticity of the data was verified by analyzing the residuals using the Kolmogorov-Smirnov test, where normal distribution was considered when $p > 0.05$. One-way analysis of variance (ANOVA) was applied to evaluate inter-day precision and robustness across variable conditions (wavelength, solvent, and temperature).

All values were expressed as arithmetic mean ± SD. Statistical data were obtained using GraphPadPrism (version 8.0.1, GraphPad Software Inc., CA, USA) and IBM SPSS (version 2.6.1) statistical software.

RESULTS AND DISCUSSION

Analytical Validation

In the present study, the proposed spectrophotometric method proved to be suitable for the quantification of FA in hard capsules, being selective, precise, accurate, and with adequate linearity in the established working range.

It is known that FA molecule exhibits solubility in alkaline hydroxide solutions and may show absorption peaks at 256nm (A_1^1 = 549a) and 283 nm (A_1^1 = 539a) (*12*, *14*, *15*). Based on this information, the spectral behavior of FA was verified in different media (Fig. 1). After spectral scanning, phosphate buffer pH 7.2 (PB 7.2) and wavelength of 280 nm were selected for detection and analysis, as the analytical response showed greater intensity.

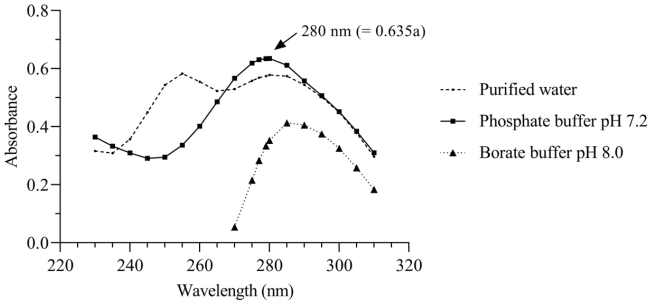


Figure 1. Ultraviolet spectrum of folic acid at 10 µg/mL (n = 3) obtained in purified water, phosphate buffer pH 7.2, and borate buffer pH 8.0 in the range of 230–310 nm.

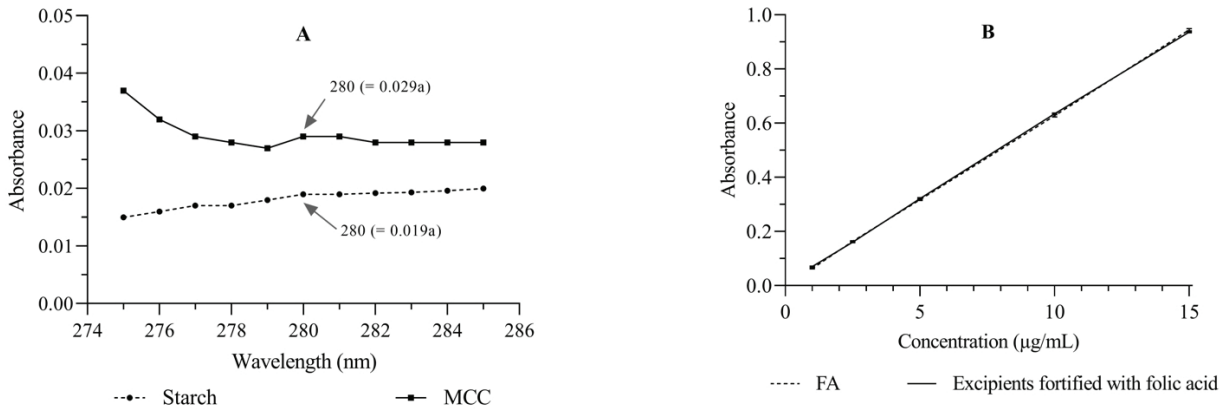


*Figure 2. Evaluation of selectivity and matrix effect verification. (**A**) Spectral scanning of excipients (corn starch and microcrystalline cellulose [MCC]) diluted in phosphate buffer pH 7.2, within the range of 275–285 nm. (**B**) Analytical curves of folic acid (FA) with or without excipients at 280 nm.*

Other studies of FA in different matrices using UV detection have reported working with solutions in the pH range of 7.0–8.0 and similar wavelengths (280–290 nm) (*25*, *26*, *38–40*). Modupe et al. evaluated the selectivity of a spectrophotometric method to quantify FA in fortified salt using three different wavelengths (256, 283, and 366 nm). Readings at 283 nm showed less interference from FA degradation products (p-amino benzoic acid glutamic acid and pterin-6-carboxylic acid) and other components of the selected matrix (*24*).

The minimal absorbance of corn starch (3.0%) and MCC (4.6%) at 280 nm can be attributed to their lack of chromophores absorbing in the UV range, unlike FA, which has a pteridine ring structure that contributes to its strong absorption. This ensures high selectivity for FA in the presence of these excipients (Fig. 2A). Data presented in Figure 2B reveal no matrix effect, because there is significant parallelism between the analytical curves ($p = 0.9892$).

As shown in Figure 3, the method demonstrated linearity within the established working range (1–15 µg/mL). The linear correlation coefficient (r) of the analytical curve was greater than 0.990, and its slope (a) in the one-way ANOVA test was significantly different from zero, with $p < 0.0001$ (*32*).

After statistical treatment of the linearity data, homoscedasticity was confirmed, showing a normal distribution ($p > 0.05$) and independence of residues (data not shown). Based on this, the ordinary least squares (OLS) model was adopted to fit and obtain the linear regression equation; i.e., to evaluate the linear association between variables using r and determination coefficient (R^2).

The proposed method showed adequate precision and accuracy, as the RSD was below 7%, and the recovery was between 80% and 120% in all analyses (Table 1) (*41*,*42*). Similar values were obtained in other studies using different analytical techniques (*38*).

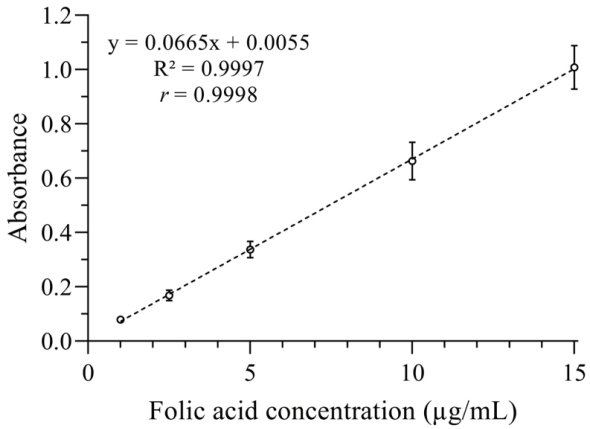


Figure 3. Analytical curve (n = 9) obtained from the method validation using phosphate buffer pH 7.2 at 280 nm. Data expressed as mean ± SD

Compared to HPLC, the standard method for FA assay, the proposed spectrophotometric method offers advantages in cost and simplicity (*12*, *15*). HPLC typically requires expensive equipment and organic solvents, with reported LD and LQ values for FA tablets around 0.5–1.0 µg/mL and 1.5–3.0 µg/mL, respectively (*39*). In contrast, our method achieved a lower LD (0.249 µg/mL) and LQ (0.755 µg/mL), indicating higher sensitivity, while reducing operational costs and solvent waste. However, HPLC may offer better specificity for complex matrices, suggesting that our method is best suited for routine quality control of capsules with minimal impurities. Furthermore, LD and LQ found are below the lowest concentration used in the

Table 1. Precision and Accuracy of Analytical Method for Quantification of Folic Acid in 280 nm Using Phosphate Buffer pH 7.2

Theoretical Concentration, µg/mL	Precision (Repeatability)											
	Day 1 (*n* = 3)			Day 2 (*n* = 3)			Day 3 (*n* = 3)					
	Conc. Obtained, µg/mL	RE %	RSD %	Conc. Obtained, µg/mL	RE %	RSD %	Conc. Obtained, µg/mL	RE %	RSD %	Conc. Obtained, µg/mL	RE %	RSD %
1	0.83	82.85	1.66	0.94	94.07	1.62	0.86	86.07	4.81	0.88	87.67	6.29
5	4.43	88.63	0.18	4.91	98.25	1.70	5.14	102.85	1.36	4.83	95.74	6.59
15	13.38	89.20	0.10	14.53	96.85	0.10	15.66	104.42	0.45	14.52	95.86	6.81

Conc.: concentration; RE: recovery; RSD: relative standard deviation.

analytical curve (1 µg/mL). Higher LD and LQ values for FA have been reported in different matrices, such as fortified salt (0.64–0.85 and 1.80–2.85 mg/L, respectively) and tablets (2.73 and 8.27 µg/mL, respectively) (*26*, *27*).

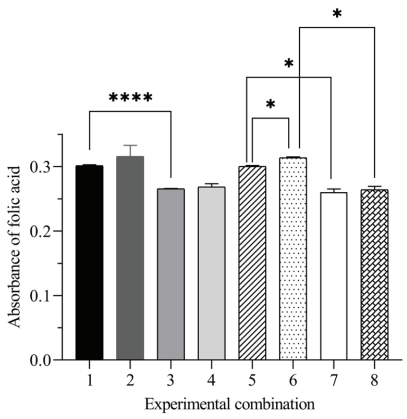
Recently, Omer et al. developed and validated two spectrophotometric methods for quantification of FA in tablets with satisfactory linearity (*43*). In the first method, an aqueous solution with sodium bicarbonate (0.1 mol/L) and three wavelengths (256, 283, and 366 nm) was used; LD was 1.46–2.44 µg/mL, LQ was 4.45–7.38 µg/mL, and the assay was approximately 104% FA. In the second method, a solution of sodium bicarbonate (0.1 mol/L) with hydrochloric acid (0.1 mol/L) at a wavelength of 295 nm was used, finding LD and LQ values of 1.53 and 4.66 µg/mL, respectively, and approximately 106% of FA for the assay (*43*). The authors reported that both methods can be used as a simple alternative to more complex methods for the assay of FA tablets (*43*).

Additionally, the proposed method showed robustness in two of three evaluated parameters: wavelength and temperature. When using water as solvent, a significant reduction in the analytical response was observed (Fig. 4). The reduced response in water highlights a key limitation, as FA's solubility decreases in neutral media, leading to aggregation or precipitation that lowers absorbance. This suggests that the method's applicability may be restricted to laboratories with access to pH-controlled buffers, potentially limiting its use in resource-constrained settings. Future studies could explore alternative buffers or pH adjustments to enhance robustness across solvents.

Ribeiro et al. evaluated other robustness parameters in different pharmaceutical formulations containing FA, including capsules (*26*). These authors concluded that derivative spectrophotometry is also robust in terms of stability and the effects of different sodium hydroxide brands and solvent concentrations (*26*).

Quality Assessment

Table 2 shows that the hard capsules obtained by the compounding process complied with the criteria of determination of weight, as well as content uniformity, dissolution, and assay tests (*12*, *33*). Cumulative release of FA was 103.1 ± 2.1%, which meets the pharmacopeial specification (*12*). This result corroborates with a study by Younis et al, who evaluated the dissolution of products containing FA in simulated intestinal fluid (*44*). These authors suggest that PB 7.2 likely enhances FA solubility compared to physiological conditions, where FA's solubility is limited due to protonation (e.g., gastric fluid, pH 1.2–3.0). This might overestimate the dissolution rate in vivo, highlighting the need for validation across a range of pH conditions to better predict bioavailability.



Variable	Nominal condition	Alternative condition	Combination							
			1	2	3	4	5	6	7	8
Wavelength (nm)	280 (A)	283 (a)	A	A	A	A	a	a	a	a
Solvent	Phosphate buffer pH 7.2 (B)	Water (b)	B	B	b	b	B	B	b	b
Temperature (ºC)	25 (C)	30 (c)	C	c	C	c	C	c	C	c

Figure 4. Assessment of the robustness in the analytical signal, considering three variables: wavelength (280 vs 283 nm), solvent (phosphate buffer pH 7.2 vs water), and temperature (25 vs 30 °C). Capital letters (A, B, C) correspond to the nominal conditions, and lowercase letters (a, b, c) correspond to the alternative conditions.
**Significant difference with p < 0.1; ****significant difference with p < 0.0001.*

Other studies in literature have also evaluated the dissolution of products containing FA. Đuriš et al. tested 17 mono and multi-component products with FA in various pharmaceutical forms (*45*). Among these, only 10 (59%) met the dissolution test requirements, including all hard capsules, whereas tablets and soft capsules (41%) failed to meet pharmacopeial criteria, which establishes a

Table 2. Results of Weight Determination, Content Uniformity, Dissolution, and Assay Tests of Folic Acid Capsules

	Weight Determination Test (*n* = 10)			Content Uniformity, % (*n* = 10)	Dissolution, % yield (*n* = 6)	Assay, mean % ± SD (*n* = 3)
	Limits of Variation* (mg)	RSD, %	VTC, %			
Specifications (*12*, *33*)	139.4–170.4	< 4	90–110	AV ≤ 15.0, n = 10 AV ≤ 25.0, n = 30	> 80	90.0–110.0
Sample	151.0–158.8	3	96–101	7.0%	101–106	96.3 ± 0.1

**Values from mean and standard deviation (154.9 ± 3.9 mg).*
AV: acceptance value; RSD: relative standard deviation; VTC: variation of the theoretical content based on theoretical weight of 156.5 mg.

tolerance of at least 80% (Q = 75% + 5%) of the stated FA amount at the end of the specified time (*45*).

Also, an increase in the release rate of FA was observed when subjected to dissolution testing in a basic medium compared with a more acid medium. This indicates a variation in FA solubility across different media, further supporting its solubility in slightly alkaline media (*14*, *45*). Some studies have suggested that FA solubility is limited in acidic pH due to the protonation of carboxylic groups. Conversely, in alkaline pH, FA becomes highly soluble. In summary, this occurs due to the deprotonation of carboxylic acid groups in the glutamic acid portion, resulting in the formation of carboxylic salts (*46*, *47*).

Matias et al. developed and validated a spectrophotometric method to quantify FA in tablets. The authors also evaluated the possibility of quantifying FA in the dissolution test using phosphate buffer pH 9.0 as a medium and compared this technique with the reference method (HPLC). Statistically, the proposed method has the same performance and can be applied for FA quantification, including the dissolution test, compared to HPLC (*25*). Several studies in the literature have found that the spectrophotometric method is equivalent to HPLC for the quantification of other therapeutic agents, yielding results comparable to the reference method with acceptable and satisfactory requirements. Thus, the method represents an alternative method for quality control (*18*).

Limitations of the current study include the lack of validation for the dissolution method employed, and PB 7.2 was used in all quality tests of hard capsules. Tests for FA tablets present different specifications than those used in this study (*12*, *15*). Our study is suitable for the spectrophotometric quantification of FA in hard capsules. Therefore, the quality of the hard capsules submitted to the validated method was satisfactory.

This study fills a critical gap in the literature by validating a spectrophotometric method for FA in hard capsules, a dosage form where capsule shell disintegration and excipient interactions (e.g., with corn starch and MCC) may uniquely affect analytical performance. Unlike tablets, capsules require careful consideration of shell dissolution, which our method accounts for by optimizing detection at pH 7.2, where FA solubility is maximized, ensuring accurate quantification despite these challenges.

CONCLUSION

The developed spectrophotometric method, using PB 7.2 as solvent and a wavelength of 280 nm for detection, proved to be suitable for quantification of FA in hard capsules. The method demonstrated selectivity, precision, accuracy, adequate linearity within the established working range, and robustness to wavelength and temperature variations. However, a lack of robustness to solvent changes (e.g., water) may limit the method's applicability in diverse settings, and the dissolution method requires validation to ensure reliable bioavailability assessments. The FA capsules analyzed by the validated method presented satisfactory quality and met the pharmacopoeial specifications required for immediate-release oral solid dosage forms. Future studies could also investigate the method's applicability to other FA dosage forms, such as tablets or soft capsules, and assess scalability for industrial quality control, potentially broadening its impact in pharmaceutical manufacturing. This validated method provides a cost-effective and environmentally friendly alternative to HPLC, with potential to enhance quality control of FA capsules in resource-limited settings, ensuring therapeutic efficacy for a critical nutrient.

DISCLOSURES

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