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Development and validation of a method for the determination of folic acid in different pharmaceutical formulations using derivative spectrophotometry

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Folic acid is a B complex water-soluble vitamin that is essential to humans, and its deficiency can cause problems including congenital malformations in the fetus as well as heart disease. Most countries affected by diseases associated with a lack of folic acid now supplement foods with the vitamin. There is therefore a need for the development of new analytical procedures able to determine folic acid present in different matrices. This work describes the development of zero order and first order derivative spectrophotometric methods for the determination of folic acid in different pharmaceutical formulations, using 0.1 mol L^{-1} NaOH as solvent. The methods are shown to be simple, selective, and robust. Good linearity was achieved, with correction coefficients ≥0.9996 and limits of detection and quantification ranging from 0.64 to 0.75 and from 1.80 to 2.85 mg L^{-1}, respectively. Recoveries of 98-104% were obtained in accuracy tests, and precision (as RSD) was between 0.2 and 4.8%. The methods can be used in routine analyses for quality control purposes, offering an alternative to the procedures already reported in the literature.

Uniterms: Folic acid/pharmaceutical formulations. Folic acid/pharmaceutical formulations/validation method. Folic acid/formulations/derivative spectrophotometry.

INTRODUCTION

Vitamins are a group of compounds essential for the normal development and growth of living beings. Folic acid (FA) and folates, which are different chemical forms of vitamin B$_9$, belong to the water-soluble B-group vitamins (Zare, Shishehbore, Nematollahi, 2011; Catharino, Godoy, Lima-Pallone, 2006; Nasser *et al.*, 2005). Folate is a generic term for compounds possessing vitamin activity similar to that of pteroylglutamic acid, and is the form of the vitamin naturally present in foods (Krishnaswamy, Nair, 2001). Folic acid is a synthetic form of folate, found in vitamin supplements and fortified foods (Bailey, 2000). The main dietary sources of folic acid are spinach, white beans, asparagus, dark-leaved vegetables, Brussels sprouts, soybean and its derivatives, oranges, and melons, amongst others (Nasser *et al.*, 2005; Deconinck

et al., 2011). An important feature of vitamins is that they are generally not synthesized by mammalian cells and must therefore be supplied in sufficient amounts in the diet (Aurora-Prado *et al.*, 2004).

At present, vitamin deficiency results mainly from poverty, food preferences, drug use, and chronic alcoholism, amongst other causes. If vitamin intake is insufficient, multivitamin preparations can be used in order to prevent vitamin deficiency and the associated physiological problems (Deconinck *et al.*, 2011; Aurora-Prado *et al.*, 2004).

The chemical name of FA is *N*-[4-[[(2-amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid (Deconinck *et al.*, 2011; Flores *et al.*, 2005; Nagaraja, Vasantha, Yathirajan, 2002). Its structure is shown in Figure 1. It is essential for the formation of red blood cells and is important for growth and the prevention of anemia (Zhao *et al.*, 2006). FA deficiency can lead to congenital malformations in the fetus (spina bifida, encephalocele, cleft palate, and hydrocephalus), as well as heart disease (Crane *et al.*, 1995; Czeize,

Correspondence: M. V. M. Ribeiro. Instituto de Química. Universidade Estadual Paulista – UNESP. Rua Prof. Francisco Degni 55 - Jardim Quitandinha - 14800-060 – Araraquara - SP, Brasil. E-mail: marcosvinicius1703@hotmail.com

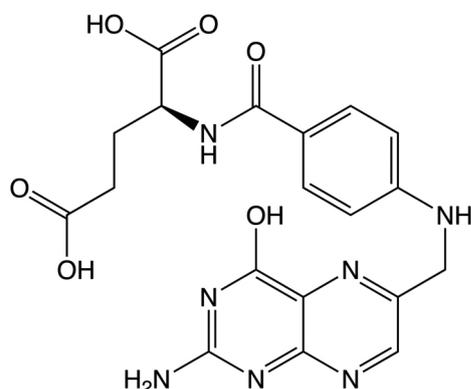Dudas, 1992; Oakley, Erickson, Adams, 1995; Santos, Pereira, 2007).

FIGURE 1 - Structure of the folic acid molecule.

To date, FA is the most important risk factor identified for neural tube defects (Santos, Pereira, 2007). The severity of these defects means that it is important to undertake genetic counseling, supplement diets with folic acid, and perform prenatal diagnosis of neural tube defects (Pacheco *et al.*, 2009). FA is required for normal growth, reproduction (during gestation and lactation), and antibody formation. It acts as a coenzyme in the metabolism of amino acids (glycine) and the synthesis of purines, pyrimidines, DNA, and RNA, and is vital for cell division and protein synthesis. Its deficiency can alter DNA synthesis and induce chromosomal changes (Santos, Pereira, 2007).

Folic acid is nontoxic, although there is some concern that high doses may mask pernicious anemia. This effect is only likely following ingestion of quantities greater than 5 mg. Medicines containing levels of folic acid above the recommended values can be harmful to consumers (Cozzolino, 2009). There is therefore a continuing need for the development of new analytical procedures for the determination of FA present in pharmaceutical formulations (Zhao *et al.*, 2006). In most countries affected by FA deficiency, foods are supplemented with the vitamin (Catharino, Godoy, 2001; Catharino, Visentainer, Godoy, 2003; Caselunghe, Lindeberg, 2000).

According to the Brazilian Pharmacopoeia (ANVISA, 2010), high performance liquid chromatography (HPLC) is recommended for the identification and quantification of FA in pharmaceutical formulations. Although HPLC is a well-established technology in the pharmaceutical field, offering sensitivity and specificity, its disadvantages include high operating costs, the need for large amounts of samples and solvents, and the generation of hazardous wastes such as organic solvents that are expensive to dispose of and can have significant environmental impacts (Deconinck *et al.*, 2011).

Methods reported in the literature for the determination of folic acid include HPLC with different detectors (Aurora-Prado *et al.*, 2004; Nelson, Sharpless, Sander, 2006; Chaudhary, Wang, Prabhu, 2010), electrophoresis (Zhao *et al.*, 2006), electrochemical methods (Vaze, Srivastava, 2007; Prasad *et al.*, 2010; Ensafi, Karimi-Maleh, 2010), flow injection analysis (Nie, He, Lu, 2000), and spectrophotometric methods (Pesce, Bodourian, 1986; Rao, Kanjilal, Mohan, 1978). The use of flow injection analysis and zero order spectrophotometry is problematic for the determination of folic acid in complex samples containing large amounts of interferents, because both techniques require prior extraction of the analyte, hence increasing the costs of routine analysis. However, the use of derivative spectrophotometry offers a simple and inexpensive solution for the determination of drugs in pharmaceutical formulations (Rêgo, Moura, Moita, 2010; Moura, Moita, 2012).

Derivative spectrophotometry has been widely used as a tool for the quality control of medicines, enabling the simultaneous determination of different drugs in multicomponent media. Derivation of the spectra enables the separation of superimposed signals and eliminates the background signal caused by the presence of other species in the sample. This technique can improve sensitivity and selectivity in the analysis of mixtures. Furthermore, it is accessible to most laboratories, since the procedure is simple, fast, and does not require prior extraction of the analyte from the sample (Donato *et al.*, 2010; Vidotti, Rollemberg, 2006).

According to the criteria of the Brazilian National Health Surveillance Agency (ANVISA, 2003), a method must be validated experimentally in order to ensure that it meets the requirements of the analytical applications, hence ensuring the reliability of the results. The parameters considered include specificity, linear range, precision, sensitivity, quantification limit, and accuracy (ICH, 2000; INMETRO, 2011). The objective of the present work was therefore to develop and validate a method for the quantification of folic acid in pharmaceutical formulations by derivative spectrophotometry, comparing the technique to existing methods described in the literature.

MATERIAL AND METHODS

Reagents

Standards and pharmaceuticals

A reference standard of folic acid (99.77%) was

obtained from Pharmanostra®. Samples of folic acid were studied in the form of tablets (5 mg) (Folacin®, lot #12097614), solution (400 mg L⁻¹) (Folacin®, lot #1106225), and capsules (5 mg) (lot #019239). The samples were purchased in drugstores and prescription pharmacies.

Evaluation of interferents employed excipients used in commercial pharmaceutical formulations. These were: lactose (Pharmanostra®), talc (Henrifarma®), magnesium stearate (Valdequímica®), microcrystalline cellulose (Pharmanostra®), silicon dioxide (Henrifarma®), titanium dioxide (Deg®), methylparaben (Fagron®), propylparaben (Pharmanostra®), and ascorbic acid (Impex).

Reagents

Evaluation of the solvents used employed three different brands of sodium hydroxide (Synth, 97.0%; Impex, 99.0%; and Vetec, 99.0%), sodium carbonate (Dinâmica, 99.5%), and hydrochloric acid (Merck, 37.0%). The solvent solutions were prepared using deionized water. All reagents used were analytical grade.

Instrumentation and treatment of the data

The spectrophotometric analyses were performed with a double beam Hitachi U-3000 spectrophotometer fitted with deuterium and tungsten lamps, using a scan range of 190-750 nm, scan speed of 300 nm min⁻¹, and slit width of 2.0 nm. Quartz cuvettes with optical path length of 1 cm were used. The samples were centrifuged using a New Instrument NI 1812 centrifuge. Mass measurements were made with a Denver Instrument APX 200 analytical balance.

Origin 7.5 software (OriginLab) was used for statistical treatment of the data and the construction of graphs. Derivation of the spectra was performed with Hitachi UV00 spectrophotometry software.

Analytical validation

Robustness

The robustness of the method was evaluated using the following parameters: stability of the folic acid solutions, brand of sodium hydroxide, and concentration of the solvent.

For evaluation of the stability of the solutions, stock solutions of 100 mg L⁻¹ folic acid were prepared in 0.1 mol L⁻¹ sodium hydroxide and 0.27 mol L⁻¹ sodium carbonate. The stock solutions were divided into three 250 mL flasks, one of which was transparent (allowing the passage of light) and the others amber (preventing the entry of light). The solution contained in the transparent

bottle was kept on the laboratory bench and was exposed to ambient light during the daytime, and the others were wrapped in aluminum foil. One of the two solutions that were protected from light was kept at an average ambient temperature of 27.0 ± 4.0 °C, while the other was refrigerated at an average temperature of 3.0 ± 2.0 °C. For the analyses, the stock solutions were diluted to 10 mg L⁻¹ and spectra were acquired daily during a period of 4 days, and subsequently every 5 days up to 30 days. The analysis of variance (ANOVA, p = 0.05) was used to evaluate the data obtained at the wavelength of maximum absorption, in the range studied, and at a further 14 points located around λ_{max}.

The study of different brands of sodium hydroxide was carried out using solutions of 10 mg L⁻¹ folic acid in 0.1 mol L⁻¹ solutions of sodium hydroxide obtained from the different manufacturers (Synth, Vetec, and Impex). All spectra were acquired in triplicate and the analysis of variance (ANOVA, p = 0.05) was used to evaluate the data obtained at the wavelength of maximum absorption and at a further 14 points located around λ_{max}.

The influence of the solvent concentration was evaluated using solutions of 10 mg L⁻¹ folic acid in different concentrations of sodium hydroxide: 0.05, 0.1, and 0.2 mol L⁻¹. All solutions were prepared in triplicate and then submitted to spectrophotometric analysis. The results were evaluated using the analysis of variance (ANOVA, p = 0.05), as described above.

Linearity

Analytical curves were constructed using six folic acid concentrations between 5 and 50 mg L⁻¹, prepared from a stock 100 mg L⁻¹ solution. Spectral scanning of the solutions was then performed to obtain the zero order spectra. The 1ˢᵗ, 2ⁿᵈ, 3ʳᵈ, and 4ᵗʰ order derivatives of the spectra were obtained, with Δλ varied between 1 and 10 nm, using the UV00 spectrophotometer software.

Detection and quantification limits

The limits of detection and quantification were calculated using the expressions LOD = 3 s/a and LOQ = 10 s/a, respectively, where s is the standard deviation of the blank signal and a is the slope (or angular coefficient) of the calibration curve (Moura, Moita, 2012). These calculations were performed using 21 blank spectra.

Selectivity

The selectivity of the method was assessed by analysis of the spectra of the excipients used in the pharmaceutical products prepared as tablets, capsules, and solution. The excipients were: lactose (50 to 250 mg

L^{-1}), talc (50 to 250 mg L^{-1}), magnesium stearate (50 to 250 mg L^{-1}), microcrystalline cellulose (50 to 250 mg L^{-1}), silicon dioxide (50 to 250 mg L^{-1}), titanium dioxide (50 to 150 mg L^{-1}), methylparaben (10 mg L^{-1}), propylparaben (10 mg L^{-1}), and ascorbic acid (100 mg L^{-1}). The spectra obtained were compared with the spectrum of a 10 mg L^{-1} folic acid standard solution.

Accuracy

Accuracy was assessed by addition and recovery tests using standards at three concentration levels (50, 100, and 150%). In the case of the Folacin® solution, a 100 mg L^{-1} standard solution of folic acid was prepared, and 0.5, 1.0, and 1.5 mL aliquots were transferred to three 10 mL volumetric flasks. The drug solution was then used to prepare a solution containing 200 mg L^{-1} of folic acid, and 0.5 mL aliquots were transferred to the flasks containing the folic acid standard solutions, prepared previously. The volumes were then completed with the solvent, resulting in final concentrations of 0, 5.0, 10.0, and 15.0 mg L^{-1} of folic acid standard and 10 mg L^{-1} of folic acid from the drug sample. All the procedures were performed in triplicate, using 0.1 mol L^{-1} NaOH as the solvent.

For the Folacin® tablet product, five tablets were crushed, and masses corresponding to 2.5 mg of folic acid were weighed out and transferred quantitatively to four 25 mL volumetric flasks, followed by addition of 2.5, 5.0, and 7.5 mL aliquots of a 500 mg L^{-1} folic acid standard solution. The volumes were then completed with the solvent, and the samples were centrifuged for 30 min at 3500 rpm, and then filtered with a quantitative filter paper to remove suspended material. Finally, 1 mL aliquots of each solution were transferred to 10 mL volumetric flasks and the volumes were completed with 0.1 mol L^{-1} NaOH to give final concentrations of 0, 5.0, 10.0, and 15.0 mg L^{-1} of standard and 10 mg L^{-1} of folic acid from the sample. These solutions were prepared in triplicate, and the accuracy was expressed in terms of the percentage recovery. The same procedure was used for the capsule form of the drug.

Precision

Evaluation was made of intra-day and inter-day precision. The drug samples were used to prepare 100 mg L^{-1} folic acid solutions, in triplicate. In the case of the tablet and capsule samples, the solutions were centrifuged for 30 min at 3500 rpm, and then filtered with quantitative filter paper to remove suspended material. The solutions were diluted to 10 mL in volumetric flasks to obtain (in triplicate) folic acid concentrations of 15.0, 25.0, and 35.0 mg L^{-1}. The results obtained were expressed in terms of relative standard deviations (RSD).

RESULTS AND DISCUSSION

According to the Brazilian Pharmacopoeia (BRASIL, 2010), folic acid is insoluble in water and organic solvents, but exhibits good solubility in hydroxides, carbonates, and hot hydrochloric acid. The literature reports methods using sodium hydroxide and sodium carbonate as solvents, with folic acid showing good solubility under these conditions (Flores *et al.*, 2005; Zhao *et al.*, 2006). This is due to reaction of the bases with the carboxylic groups present in the folic acid structure (Figure 1), resulting in the formation of carboxylic salts that are highly soluble in aqueous media. Under these conditions, the spectrum of the folic acid solution showed three well-defined bands of maximum absorption, at wavelengths of 255.5, 283.0, and 365.5 nm (Figure 2). In the case of hydrochloric acid, a low intensity band was observed and poor dissolution was achieved using acid concentrations of 1.0 and 3.0 mol L^{-1}.
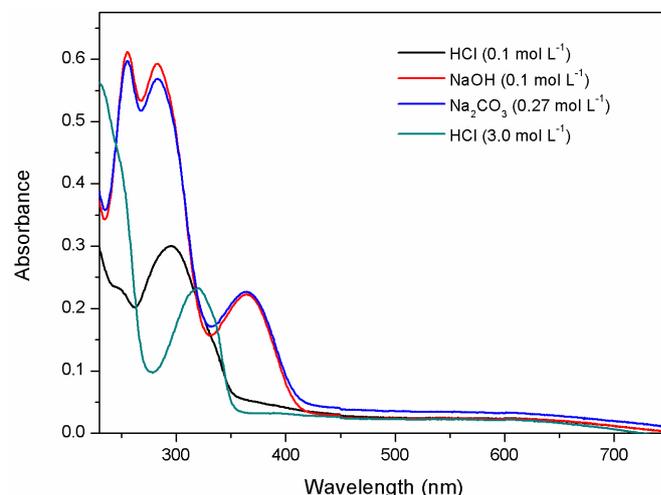

FIGURE 2 - Spectra obtained for 10 mg L^{-1} folic acid in the presence of different solvents.

Robustness of the method

Stability

The stability tests showed that in the presence of sodium hydroxide, the standard solution of folic acid was stable for up to 28 days at ambient temperature, in both the presence and absence of light (Figures 3 and 4). The analysis of variance (ANOVA, p = 0.05) performed for the wavelength range around λ_{max} showed that the absorbances were statistically the same during this period. The same behavior was observed for the refrigerated samples.

In the case of the standard solutions of folic acid in the presence of sodium carbonate, there was a marked

decrease in the absorbance value at a wavelength of 255.5 nm (Figure 3), which was due to photochemical degradation of the solution in the presence of light. This was also indicated by a progressive change in the color of the standard solution, from light yellow to dark yellow. In the absence of light, the behavior was similar to that observed using sodium hydroxide (Figure 4). These results were in agreement with the findings of other studies using HPLC (Catharino, Godoy, 2001; Catharino, Visentainer, Godoy, 2003). Sodium hydroxide was therefore selected as the solvent in subsequent experiments.
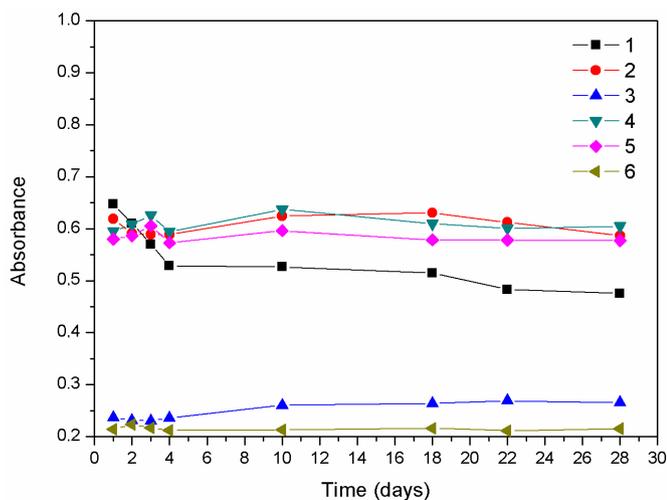

FIGURE 3 - Stability of 10 mg L^{-1} folic acid solutions in the presence of light, using 0.27 mol L^{-1} Na$_2$CO$_3$ (255.5 nm (1), 283.0 nm (2), 365.5 nm (3)) and 0.1 mol L^{-1} NaOH (255.5 nm (4), 283.0 nm (5), 365.5 nm (6)).
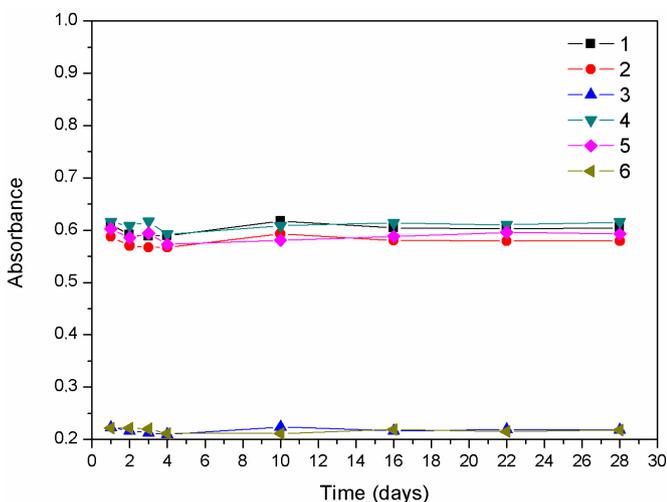

FIGURE 4 - Stability of 10 mg L^{-1} folic acid solutions in the absence of light, using 0.1 mol L^{-1} Na$_2$CO$_3$ (255.5 nm (1), 283.0 nm (2), 365.5 nm (3)) and 0.1 mol L^{-1} NaOH (255.5 nm (4), 283.0 nm (5), 365.5 nm (6)).

Influence of brand of sodium hydroxide

Evaluation of the folic acid spectra obtained using the different brands of sodium hydroxide showed that there was no significant difference (paired t-test, p = 0.05) between the measured absorbances for the Impex and Vetec brands, which could be explained by the fact that the degree of purity was 99.0% in both cases. However, when these spectra were compared with those obtained using sodium hydroxide from Synth, there was a difference in absorbance (ANOVA, p = 0.05), due to the lower degree of purity (97.0%) of the latter.

Influence of solvent concentration

No significant differences (ANOVA, p = 0.05) were observed between the folic acid absorbance spectra acquired for the samples with three different concentrations of sodium hydroxide (0.05, 0.1, and 0.2 mol L^{-1}). Since folic acid dissolved more rapidly in the presence of a higher concentration of the base, it was decided to use 0.1 mol L^{-1} sodium hydroxide, which provided good stability of the standard solution and lower consumption of the reagent, compared to use of the 0.2 mol L^{-1} solution.

Evaluation of selectivity

The excipients used in the preparation of the capsules and tablets showed poor solubility in the solvent used. Talc was easily precipitated, while titanium dioxide and magnesium stearate remained in suspension. At higher concentrations, the turbidity of the suspensions increased, resulting in higher analytical signals throughout the entire spectrum, due to scattering of the radiation. This interference could affect the precision and accuracy of the method, and was therefore avoided using centrifugation and filtration. The excipients were tested at different concentrations, because the manufacturers did not provide any information concerning the quantities used.

In the case of the excipients used in the solution formulation, there was superimposition of the zero order folic acid spectrum and the excipient spectra, because the absorbance maxima of the latter were very close to that of folic acid, which hindered its determination (Figure 5). The zero crossing technique was therefore used to eliminate this interference (Moura, Moita, 2012). The spectra of folic acid and the excipients were evaluated using four derivative orders and ten different Δλ. A significant folic acid signal was only obtained at a wavelength of 393.0 nm for the first derivative (D1) and for Δλ8, Δλ9, and Δλ10, with the excipients showing zero signals (Figure 6).
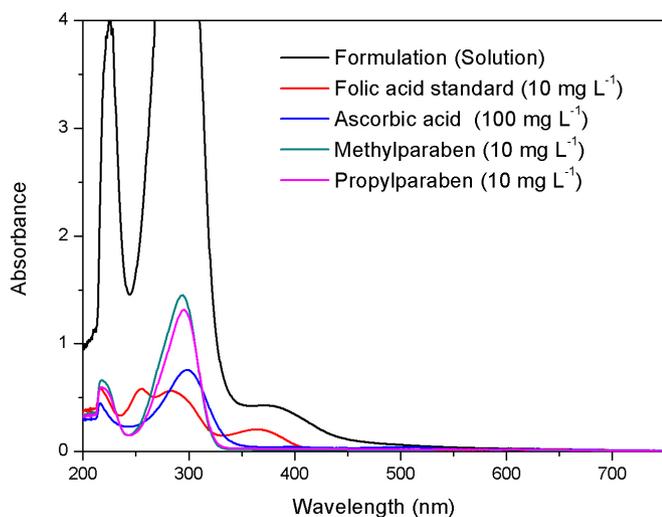
FIGURE 5 - Comparison of zero order spectra for 10 mg L^{-1} folic acid and the main excipients used in the solution formulation.
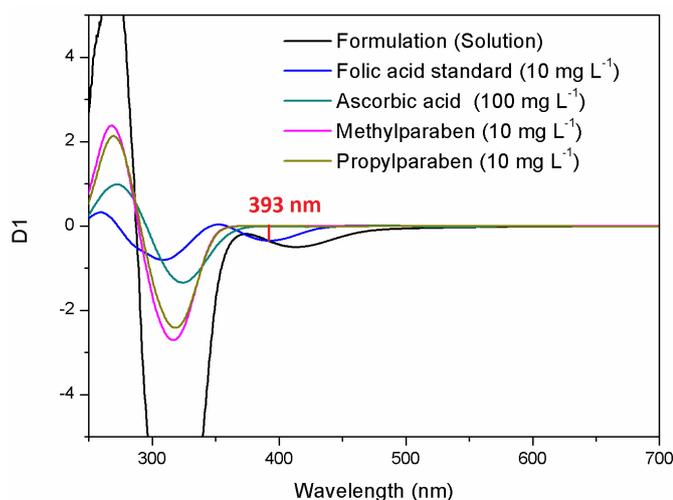

FIGURE 6 - Comparison of $\Delta\lambda10$ first derivative spectra for 10 mg L^{-1} folic acid and the main excipients used in the solution formulation.

Linearity and limits of detection and quantification

Table I shows the parameters of the calibration curves obtained for standard solutions of folic acid in 0.1 mol L^{-1} sodium hydroxide. The curves presented good slopes, intercepts near zero, and correlation coefficients that exceeded the values recommended by ANVISA (2003) (r = 0.99) and INMETRO (2011) (r = 0.9). The detection and quantification limits were in the ranges 0.64-0.85 and 1.80-2.85 mg L^{-1}, respectively, and were lower than the lowest concentration in the solutions analyzed (5 mg L^{-1}). The developed method therefore provided satisfactory sensitivity and linearity. The results were similar to those obtained previously using chromatographic (Aurora-Prado *et al.*, 2004; Zhao *et al.*, 2006; Nelson, Sharpless, Sander, 2006) and electrochemical techniques (Zare, Shishehbore, Nematollahi, 2011; Vaze, Srivastava, 2007; Ensafi, Karimi-Maleh, 2010) for the analysis of folic acid in different matrices.

Evaluation was made of differences in the analytical parameters at different $\Delta\lambda$, using the wavelength of 393.0 nm (D1), since in the case of the Folacin® solution, only the first derivative gave satisfactory results. It can be seen from the data provided in Table I that the results obtained for $\Delta\lambda10$ were similar to those found for $\Delta\lambda8$ and $\Delta\lambda9$, with only slight differences, since the increase of $\Delta\lambda$ provided better signal resolution by decreasing the noise present in the spectrum.

Accuracy of the method

The results of the accuracy tests (Table II), performed in triplicate using three concentration levels, showed that in most cases the recoveries ranged from 98 to 105%, within the limits established by ANVISA (80-120%). In

TABLE I - Analytical parameters for the quantification of folic acid

Method	λ (nm)	Linear equation	r	LOD (mg L^{-1})	LOQ (mg L^{-1})	LOL (mg L^{-1})
ZO	255.5	A=0.041+0.054C	0.9996	0.75	2.51	50
ZO	283.0	A=0.035+0.053C	0.9996	0.81	2.71	50
ZO	365.5	A=0.020+0.019C	0.9988	0.85	2.85	50
D1 ($\Delta\lambda8$)	393.0	D1=-0.004-0.032C	0.9998	0.67	1.80	50
D1 ($\Delta\lambda9$)	393.0	D1=-0.005-0.034C	0.9998	0.65	1.80	50
D1 ($\Delta\lambda10$)	393.0	D1=-0.005-0.035C	0.9998	0.64	1.78	50

ZO - Zero order, D1 - First order derivative, LOD - Limit of detection, LOQ - Limit of quantification, LOL - Limit of linearity, r - linear correlation coefficient.

tests with the Folacin® solution, the values obtained using the ZO method (365.5 nm) were in the range 90-94%, with RSD of up to 10.3%, due to the spectral overlap associated with the excipients used in the formulation (Figure 5). This difficulty was resolved using method D1 (393.0 nm) at different $\Delta\lambda$, resulting in recovery values in the range 100-104% (Table II).

Similar recoveries were obtained in previous studies using HPLC (94.3-105.8% and ≥95%) (Zhao *et al.*, 2006; Nelson, Sharpless, Sander, 2006), electrochemical methods (97.7-103.6%) (Beitollahi, Sheikhshoaie, 2011), and flow injection analysis (>95.3%) (Nie, He, Lu, 2000).

Precision of the method

Intra-day and inter-day precisions (Table III) were determined using three concentration levels. The data showed that the method provided high levels of precision when used by the same analyst on the same day and by

TABLE II – Results of accuracy tests for the samples at three concentration levels

Formulation	Method (λ, nm)	Added (mg L⁻¹)	Recovered (mg L⁻¹)	Recovery (%)	RSD (%)
Folacin® (solution)	ZO (365.5)	5.0	4.5	90	3.9
		10.0	9.4	94	10.3
		15.0	13.9	92	7.2
	D1 (393.0) $\Delta\lambda$10	5.0	4.9	98	3.1
		10.0	10.5	105	0.5
		15.0	15.2	101	2.0
	D1 (393.0) $\Delta\lambda$9	5.0	5.2	103	2.2
		10.0	10.5	105	3.2
		15.0	15.5	103	1.9
	D1 (393.0) $\Delta\lambda$8	5.0	5.0	100	3.5
		10.0	10.4	104	3.2
		15.0	15.3	102	2.0
Folacin® (tablet)	ZO (255.5)	5.0	5.1	103	4.8
		10.0	10.2	102	2.4
		15.0	15.0	100	1.2
	ZO (283.0)	5.0	5.1	102	4.9
		10.0	10.1	101	2.5
		15.0	14.9	99	1.4
	ZO (365.5)	5.0	5.0	100	5.0
		10.0	10.0	100	2.6
		15.0	14.7	98	2.6
	D1 (393.0) $\Delta\lambda$10	5.0	5.1	102	4.4
		10.0	10.1	101	1.6
		15.0	15.0	100	0.5
Capsules	ZO (255.5)	5.0	5.1	102	0.7
		10.0	10.4	104	1.5
		15.0	14.7	98	0.4
	ZO (283.0)	5.0	5.0	100	1.2
		10.0	10.4	104	1.5
		15.0	14.7	98	0.3
	ZO (365.5)	5.0	5.0	101	2.7
		10.0	10.4	104	1.4
		15.0	14.8	99	1.1
	D1 (393.0) $\Delta\lambda$10	5.0	5.2	104	1.2
		10.0	10.4	104	1.0
		15.0	14.7	98	0.9

ZO - Zero order, D1 - First order derivative, RSD - Relative standard deviation

different analysts on different days, with RSD values ranging from 0.2 to 4.8%, which were lower than the maximum allowed by ANVISA (5.0%). The lowest values were found for method D1 (393.0 nm), showing that use of the derivative improved the signal and eliminated interference.

The intra- and inter-day precisions were assessed by application of the F-test and the paired t-test (p = 0.05). The results revealed that for the three concentration levels studied, there were no statistically significant differences between the results obtained.

CONCLUSIONS

The methods developed for the determination of folic acid in pharmaceutical formulations were shown to be simple, selective, and robust in terms of stability and the effects of different brands of sodium hydroxide

TABLE III - Intra-day and inter-day precisions for the proposed methods

Formulation	Method (λ, nm)	Concentration level (mg L^{-1})	Intra-day precision		Inter-day precision
			RSD (%)	RSD (%)	RSD (%)
Folacin® (solution)	ZO (365.5)	15.0	1.3	4.7	4.1
		25.0	1.5	1.6	1.8
		35.0	4.3	0.4	2.7
	D1 (393.0) Δλ10	15.0	1.0	3.8	4.4
		25.0	0.4	2.4	0.5
		35.0	4.0	0.9	2.4
	D1 (393.0) Δλ9	15.0	1.9	4.3	4.7
		25.0	0.5	2.2	0.6
		35.0	3.4	0.8	1.1
	D1 (393.0) Δλ8	15.0	1.2	4.6	3.9
		25.0	1.0	2.2	0.8
		35.0	2.5	0.8	0.3
Folacin® (tablet)	ZO (255.5)	15.0	1.7	0.3	4.0
		25.0	1.3	0.6	0.8
		35.0	1.4	0.1	1.5
	ZO (283.0)	15.0	1.6	0.2	4.1
		25.0	1.4	0.5	0.9
		35.0	1.3	0.1	1.5
	ZO (365.5)	15.0	2.3	0.9	4.8
		25.0	1.8	1.6	2.5
		35.0	1.1	0.4	2.3
	D1 (393.0) Δλ10	15.0	1.5	0.6	3.8
		25.0	1.3	0.7	0.2
		35.0	1.5	0.1	0.7
Capsules	ZO (255.5)	15.0	1.4	0.9	1.4
		25.0	2.5	1.4	2.6
		35.0	1.2	1.7	2.7
	ZO (283.0)	15.0	1.2	0.7	1.4
		25.0	2.4	1.5	2.6
		35.0	1.2	1.7	2.7
	ZO (365.5)	15.0	1.8	1.5	1.3
		25.0	2.6	1.1	2.5
		35.0	1.3	1.9	2.6
	D1 (393.0) Δλ10	15.0	1.0	0.4	1.6
		25.0	2.3	1.6	2.9
		35.0	1.1	1.4	2.6

ZO - Zero order, D1 - First order derivative, RSD - Relative standard deviation.

and different solvent concentrations. Good linearity was achieved in the concentration range studied, together with satisfactory accuracy and precision. The proposed methods offer an alternative for use in the quality control of pharmaceutical formulations, being less complex and requiring smaller amounts of toxic reagents, compared to HPLC.

ACKNOWLEDGMENTS

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