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ECO-FRIENDLY AND MINIATURIZED ANALYTICAL METHOD FOR QUANTIFICATION OF RIFAXIMIN IN TABLETS

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Rifaximin is an oral antimicrobial, semisynthetic and nonabsorbable with minimal adverse effects that act locally in the gastrointestinal tract. Rifaximin does not have standardized methods of analysis for the tablets evaluation in official compendiums. The objective of this study is to develop and validate an analytical method for the quantification of rifaximin in tablets by spectrophotometry in the ultraviolet region, aiming at a miniaturized and eco-friendly method. The method was performed using 700 µL cuvette and purified water: ethanol (4:1, v/v) as diluent. The wavelength used was 233 nm and the range of concentrations was 4-14 µg mL-1. It was linear with correlation coefficient greater than 0.9999, precise with relative standard deviation equal 0.80%, 1.19% and 1.19% for intraday, interday and interanalyst precision, respectively, exact with average recovery of 100.56%, selective against the presence of interferents such as impurities, matrix compounds and solvents used and robust with the change of ethyl alcohol brand and proportion used as diluent. The method developed presents advantages as, minimum waste generation, reduction in amount of sample, standard and solvents used and reduction in time of analysis. Then this method can be used in routine analysis of rifaximin tablets as an alternative method, reliable, effective, really fast, low cost, eco-friendly and miniaturized. This study contemplates a current and innovative topic which is extremely important for the area of Quality Control of drugs and medicines and for the sustainable Green Analytical Chemistry.

Keywords: rifaximin, miniaturized method, spectrophotometry in the ultraviolet region, green analytical chemistry, quality control.

Introduction

Rifaximin (Figure 1A) is an antimicrobial, semisynthetic with broad spectrum of action nonabsorbable that acts locally in the gastrointestinal tract with minimal systemic adverse effects. It is a derivative of rifamycin and a structural analogue of rifampicin, that acts in RNA polimerase inhibiting bacterial RNA synthesis (1-3).

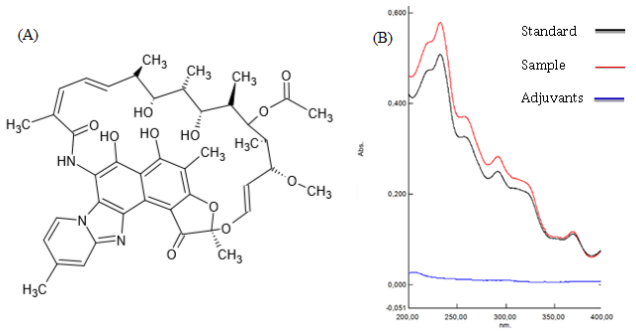

Figure 1. (A) Chemical structure of rifaximin (CAS 80621-81-4). (B) Sobreposition of standard, sample and adjuvants spectrums at concentration of 10 µg mL^{-1}.

The appearance of rifaximin is an orange, hygroscopic and crystalline powder that must be storage on places without light. Rifaximin is practically insoluble in water and soluble in acetone and methanol (4, 5).

This medicine is used for the treatment of diseases such as hepatic encephalopathy (6), travelers' diarrhea (7), irritable bowel syndrome (8), *Clostridium difficile* infections (9), ulcerative colitis (10), bacterial vaginosis (10) and acute diarrhea (11).

Despite rifaximin has been very used, it does not have monograph by spectrophotometric in ultraviolet region described in official compendiums (4, 5, 12-15).

Quality Control is very important for pharmaceutical products to confront the forgery and low quality, mainly for medicines because it ensures the safety and efficacy of the patient treatment, decreases bacterial resistance and promote the rational use of medicines (16). Furthermore, nowadays the use of eco-friendly methods that use green solvents has been appreciated in the analysis (17-18).

The concept of Green Chemistry started in 1990 and expanded quickly in Europe and the United States. The goal of Green Chemistry was to reduce pollution through the use of green solvents (19). Many companies are in agreement with this thought that also contemplates the idea of sustainability (20).

Eco-friendly analytical methods that decrease the use of toxic solvents and reduce the toxic waste generation are valid. This is extremely profitable for both environment and operator (21).

The objective of development of effective and reliable analytical method for the quality control is to provide reliable information about the nature and composition of the sample in analysis. Thus, several facts must be considered in the development of a new analytical method, for example, detection, reliability, separation of all compounds of interest, the time taken to carry out the analysis, low final cost and the use of green reagents (22-23).

The validation is important to ensure the quality and to show that the method is suited for the proposition intended and it is safety to be done (24).

The advantage of miniaturized system is low amount of sample, solvents and reagents, decrease time of analysis and mainly reduce the waste generation (25-27). In the literature two articles about spectrophotometric in ultraviolet region method were founded (28-29), however one of them uses toxic solvents like methanol and the other it is not miniaturized method as this research and therefore it does not contemplate the advantages mentioned above.

Therefore, the method proposed in this work intends the miniaturization, the use of eco-friendly solvents, low final

cost and to make the analysis faster. This is very interesting for industries and the environment.

Experiments

Materials

The reference standard was rifaximin, content 99.0%, acquired from the company NutraTech Development Limited – (China).
The pharmaceutical form was rifaximin tablets (labeled content 200 mg), lot 15718, under the trade name Flonorm ™, from Laboratory Gonher Farmaceutica LTDA.
The reagents tested in solubility and stability experiment were purified water (Milli-Q), ethyl alcohol (Synth®), sodium hydroxide solution (Synth®), hydrochloric acid solution (Qhemis®), phosphate buffer pH 3.0 and pH 6.0, borate buffer pH 9.0, and acetate buffer pH 3.0 with sodium lauryl sulfate 0.2%, all of them at concentration of 0.05 M.
The placebo was prepared mixing amounts of excipients usually present in tablets, following these proportions: 0.18% sodium carboxymethyl, 1.5% glyceryl palmitostearate, 1.0% colloidal anhydrous silica, 30% talc, 20% microcrystalline cellulose, 0.4% hypromellose, 1.4% titanium dioxide, 0.18% disodium edentate, 0.3% propylene glycol, 1.0% red iron oxide.

Equipment

The reference standard was weighed in analytical balance model H51 (Mettler Toledo®), the solutions was sonicated in ultrasound equipment Ultrasonic Cleaner (Unique®), the equipment used to analyze the solution was Spectrophotometer UV -1800 (Shimadzu®); and quartz cuvettes with capacity of 700 µL and 10 mm optical path.

Method Development

Solubility and Stability

The diluents tested in this experiment were purified water, solution with 10% and 20% of ethyl alcohol, sodium hydroxide solution with concentration 0.001 mol L^{-1}, hydrochloric acid solution with concentration 0.01 mol L^{-1}, phosphate buffer pH 3.0 and pH 6.0, borate buffer pH 9.0, and acetate buffer pH 3.0 with sodium lauryl sulfate 0.2%.
A solution was prepared with each diluent at final concentration of 200 µg mL^{-1} using a 25 mL amber volumetric flask. The scanning of all solutions was made after 0, 4, 6, 8 and 24 hours to check the solubility and stability of rifaximin in these diluents.

Ringbom Curve

The Ringbom curve was obtained by the determination of 33 different concentrations of standard rifaximin solution at the wavelength 233 nm.

0.00505 g of standard rifaximin was weighed and transferred to a 25 mL amber volumetric flask. 5 mL of ethyl alcohol was added and this solution was kept 15 minutes in ultrasound. After it, the volume was completing with purified water to obtain a final concentration of 200 µg mL^{-1}. It was filtered through filter paper and from this solution aliquots was transferred to a 2 mL amber volumetric flask, completing the volume with solution of purified water and 20% ethyl alcohol to get 33 concentrations from 0.2 to 200 µg mL^{-1}.

Sample weight

Twenty individually tablets were weighted and were macerated in a porcelain mortar and stocked to use it in validation parameters: selectivity and accuracy.

Method Validation

National and international guides were used in the validation of the method (24, 30-31).

Selectivity

The selectivity is the capacity of the method to identify or quantify the substance of interest, even in the presence of other compounds that can be in the sample (30).
In this method, the selectivity was confirmed by the sobreposition of the standard, sample, and adjuvants spectrum. Solutions of standard, sample and adjuvants were prepared at concentration of 100 µg mL^{-1}. For this, 0.00252 g of standard, 0.00466 g of sample e 0.00214 g of adjuvants (amount present in 0.00466 g of sample) were weighted and transferred to a 25 mL amber volumetric flask, added 5 mL of ethyl alcohol and kept in ultrasound equipment for 15 minutes. Then, completed the volume with purified water and filtered the solution through filter paper. Dilutions to10 µg mL^{-1} were made in 2 mL amber volumetric flask, completing the volume with solution of 20% of ethyl alcohol. The spectrums were obtained at 233 nm.

Linearity

From Ringbom curve, six points were chosen for the evaluation of the method linearity. It was prepared stock solution of standard at concentration of 100 µg mL^{-1} in the same way as detailed by the selectivity. Then, aliquots were transferred to a 2 mL volumetric flask and the volume was completed with solution of 20% of ethyl alcohol, in order to obtained solution at final concentrations of 4, 6, 8, 10, 12 and 14 µg mL^{-1}. The spectrum was obtained at 233 nm. This experiment was made in triplicate and in three different days.
The linearity was evaluated by three methods: equation of least square, Analysis of Variance (ANOVA) and residue analysis.

Precision

The precision evaluates the proximity between the results obtained by the intraday, interday, and interanalyst precisions (30).

Stock solutions of standard were prepared at concentration of 100 µg mL^{-1}. Aliquots from this solution were transferred to a 2 mL amber volumetric flask and the volume was completed with solution of 20% of ethyl alcohol. Six results were obtained in the same day and in different days to test the intraday and interday precision, respectively. The interanalyst precision was made by a second analyst, however under the same conditions and equipment.
The results were analyzed by relative standard deviation (RSD).

Robustness

The robustness evaluates small variations that can be performed in the analytic conditions of the method and it is important to describe this variations order to control them (30).
Five parameters (Table 1) were modified from the normal condition and the results were compared. The parameters changed were:
- Wavelength
- Ultrasound time
- Ethyl alcohol's brand
- Volumetric flask
- Proportion of ethyl alcohol

Table 1. Normal and changed conditions used in the robustness test and its statistical evaluation

Parameters	Normal Condition	Changed Condition	Test			
			Fcal	***Ftab***	***tcal***	***ttab***
Wavelength	233 nm	235 nm	4.51	19.00	6.09	2.78
Ultrasound time	15 minutes	10 minutes	4.26		5.87	
Ethyl alcohol's brand	Brand A	Brand B	6.81		1.68	
Volumetric flask	Amber	Colorless	4.00		4.98	
Proportion of ethyl alcohol	20%	18%	4.34		0.20	

Accuracy

The accuracy shows the closeness of agreement between individuals' values obtained in the method in relation to the real value (30).
Stock solutions of rifaximin standard and sample were prepared at concentration of 100 µg mL^{-1}. From each stock solution aliquots of 80 µL were transferred to a 2 mL amber volumetric flask and the volume was completing with purified water and 20% of ethyl alcohol obtaining a final concentration of 4 µg mL^{-1} in two amber volumetric flasks, one for the rifaximin standard and other for sample. After the aliquot of 80 µL of standard was transferred to a 2 mL amber volumetric flask and the contamination of sample was made by the addition of the 80 µL aliquot of standard obtaining final theoric concentration at 8 µg mL^{-1}. The aliquot of 80 µL was kept but the amount of standard auditioned was altered by 120 µL and after 160 µL obtained final theoric concentrations at 10 µg mL^{-1} and 12 µg mL^{-1}, respectively.
The contamined sample method was explained in the Table 2.

Table 2. Preparation of solutions used in the accuracy parameter and results obtained in the standard recovery test

	Volume added of sample rifaximin (µL) *	Volume added of standard rifaximin (µL)*	Final theoric concentration (µg mL^{-1})	Standard rifaximin recovered (µg mL^{-1})	Recovery (%)	Average recovery (%)	RSD (%)
Sample	80	-	4				
R1	80	80	8	4.02	100.63		
R2	80	120	10	6.02	100.33	100.56	0.21
R3	80	160	12	8.06	100.73		
Standard	-	80	4				

* at 100 µg mL^{-1} using volumetric flask of 2 mL

The accuracy is calculated by percentage of recuperation of the standard from amount known added in the sample through of the equation (1):

$$\% R = \left\{\frac{Cr-Ca}{Cp}\right\} X\ 100 \qquad (1)$$

Where:
Cr = concentration of sample solution added in the standard (µg mL^{-1})
Ca = concentration of sample solution
Cp = theoric concentration of standard solution

Results

Method Development

The sample weight from it was 369.79 g. The Figure 2A represents the weight values of twenty tablets and indicate by the limit maximum and minimum that all tablets are inside the value recommended by the Brazilian Pharmacopeia (12).

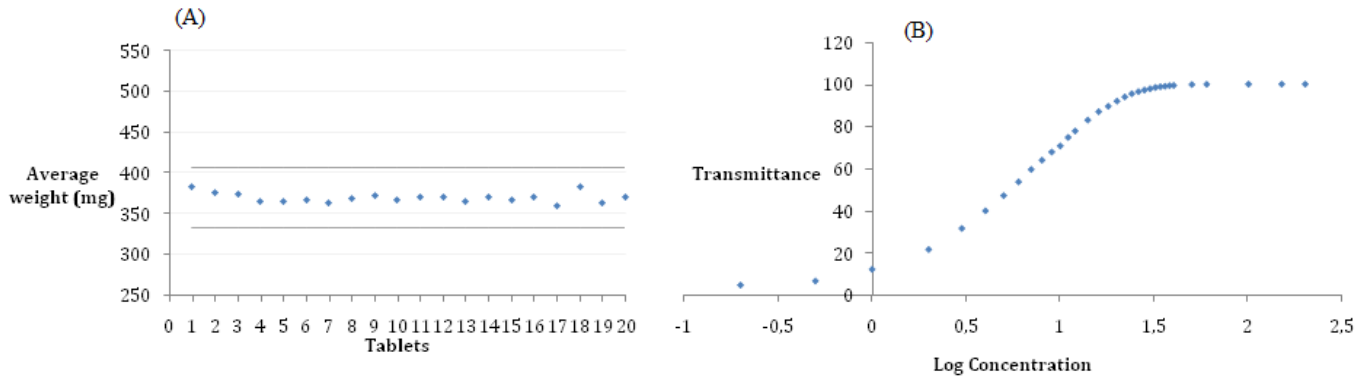

Figure 2. (A) Sample weight values of twenty tables analyzed and the minimum and maximum limits represented by the lines above and below, respectively. (B) Ringbom curve of rifaximin standard solution using purified water and 20% of ethyl alcohol.

The solution of purified water with 20% of ethyl alcohol was choose to be diluent for rifaximin, because rifaximin showed suitable solubility and stability in this diluent, as shown in Table 3.

Table 3. Absorbance values for all diluents tested in the experiment

Diluent	Concentration(µg mL^{-1})	λ	Absorbance values				
			0	4 h	6 h	8 h	24 h
Phosphate bufferpH 6.1	200	231	0.611	0.593	0.595	0.593	0.606
Phosphate buffer pH 3.0	200	231	0.545	0.555	0.559	0.539	0.501
Borate buffer pH 9.0	50	231	0.619	0.613	0.605	0.586	0.514
Acetate buffer pH 3.0+SLS0.2%	100	231	0.483	0.417	0.420	0.411	0.303
NaOH 0.001 mol L^{-1}	200	231	0.879	0.822	0.789	0.771	0.687
HCl 0.01 mol L^{-1}	200	231	0.551	0.569	0.572	0.575	0.576
Purified water + 10% ethyl alcohol	22	232	1.095	1.060	1.025	0.963	0.504
Purified water + 20% ethyl alcohol	22	232	1.137	1.130	1.150	1.129	1.165

ʎ= Wavelength; SLS= Sodium lauryl sulfate

The Ringbom curve was made using 33 points that varied from 0.2 µg mL^{-1} to 200 µg mL^{-1} obtained by spectrophotometry in the ultraviolet region as shown in Figure 2B.

From the Ringbom curve, six points were chosen to obtain the analytical curve utilized in the method validation. For the selection of the points were analyzed the linear association between them by the correlation coefficient (r) and the determination coefficient (R^2). Therefore, the concentrations of 4, 6, 8, 10, 12 and 14 µg mL^{-1} were appropriate once they had better linearity of response.

Method Validation

Selectivity

The Figure 1B shows the overlay of the standard, sample, and adjuvants spectrums using purified water and 20% of ethyl alcohol at concentration of 10 µg mL^{-1} obtained spectrophotometrically in the ultraviolet region.

Linearity

The linear regression equation (Figure 3A), where *y* is the absorbance values and *x* is the concentration values of rifaximin solutions, is $y = 0.0523\ x - 0.0057$ estimated by the method of least square, with coefficient of determination (R^2) equal to 0.9999 and correlation coefficient (r) equal to 0.9999 for the standard rifaximin.

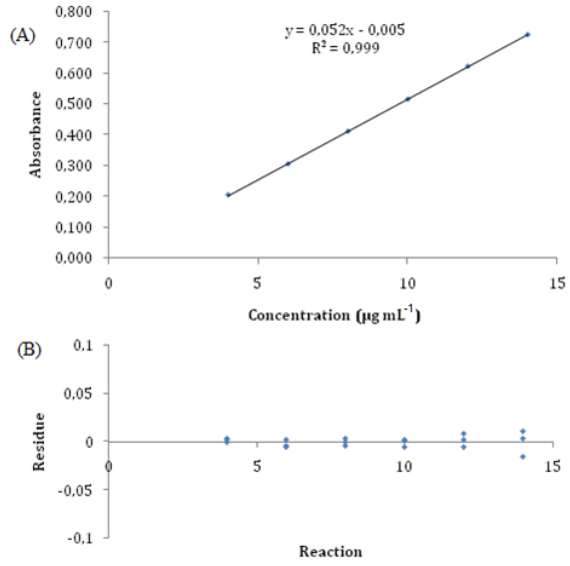

Figure 3. (A) Analytical curve of standard rifaximin using purified water and 20% of ethyl alcohol at concentration of 4, 6, 8, 10, 12 and 14 µg mL^{-1}, obtained by spectrophotometry in the ultraviolet region. (B) Residues graphic versus reaction of standard rifaximin solution.

The ANOVA (Table 4) was calculated through the values of the analytical curve.

Table 4. ANOVA of the absorbance values determined in the analytical curve of standard rifaximin by spectrophotometry in ultraviolet region

Variation source	DF	SS	Variance	F*calc*	F*tab* (0.05)
Among concentration	5	0.5735	0.114701	208.30*	3.11
Linear regression	1	0.5734	0.573434	1041.35*	4.75
Linearity deviation	4	0.0001	0.000018	0.03	3.26
Inside (waste)	12	0.0006	0.000046	-	-
TOTAL	17	0.5741	-	-	-

DF: degrees of freedom; SS: sum of squares.

The residues analysis, represented in Figure 3B, examines the quality of setting in regression and ANOVA and helps to determine if the common least square suppositions have been attended.

Precision

The values of absorbance and RSD for the standard rifaximin solution obtained in this parameter are represented in Table 5.

Table 5. Absorbance values of standard rifaximin solution obtained to the intraday, interday, and interanalyst precision parameters by spectrophotometry in the ultraviolet region

Test	Absorbance values						RSD (%)
	1	**2**	**3**	**4**	**5**	**6**	
Intraday	0.511	0.515	0.517	0.523	0.517	0.513	0.80 (n=6)
Interday	0.511	0.515	0.517	0.523	0.517	0.513	1.19 (n=12)
	0.521	0.518	0.517	0.505	0.527	0.525	
Interanalyst	0.521	0.518	0.517	0.505	0.527	0.525	1.19 (n=12)
	0.518	0.518	0.517	0.523	0.523	0.510	

Robustness

The values calculated through test F and test *t* for the variations realized in the robustness parameter are represented in the Table 1.

Accuracy

The values obtained through the recovery test by spectrophotometry in the ultraviolet region are described in Table 2.

Discussion

Selectivity

The selectivity was proved by the comparison of the absorbance values between the standard and sample rifaximin solutions. The sobreposition of the standard, sample, and adjuvants spectrum (Figure 1B) indicate that the adjuvants present in the sample composition do not influence the results obtained in the wavelength 233 nm. Therefore, the analytical method developed is selective to detection of rifaximin in the conditions described.

Linearity

The analytical curve demonstrates the proportionality between absorbance *versus* concentration. The linearity was emphasized by the correlation coefficient (r) equal 0.9999 and determination coefficient (R^2) equal 0.9999 and this result is in line with RDC 166 (2017) that specifically the correlation coefficient must be greater than 0.990 (30).
The ANOVA indicate (i) there is difference between the absorbance values of the different concentrations utilized as calculated F is greater than tabulated F; (ii) there is a linear regression between the six points because calculated F is greater than tabulated F; (iii) there is not linearity deviation, as calculated F is lower than tabulated F. All the values are represented in Table 1.
From de Gauss curve, that shows variability, one standard deviation is equivalent to 1.96 upwards or downwards. Taking residues graphic, it should be noted the variability between the points for the standard rifaximin solution is into the range -0.05 and +0.05, indicating that the variability between the points is accepted.
The residues graphic showed that the common least square suppositions and ANOVA were met. Then this method is linear and will produce estimation of non addicted coefficient with minimum variance.

Precision

According to the results obtained by the precision parameter, it should be noted the RSD values (RSD %), for the intraday, interday, and interanalyst precision are below 2%, therefore the method is precise.

Robustness

The test *t* show that *t* value is greater than *t* critical, or, *t* is significant and there is difference between the results for the alterations that were made at the wavelength, ultrasound time and volumetric flask utilized. So, changes in these parameters require caution for the develop method.
It is extremely important to make the analysis at wavelength of 233 nm because rifaxinin has maximum absorption in this wavelength, then a minimum variation in this wavelength could mean a risk for the final result.
Ultrasound time must be standardized in 15 minutes because below this time the rifaximin solubility could be compromised and may display inadequate results.
Rifaximin is a compound photosensitive that explains why there was difference between the results from amber and colorless volumetric flask.
Alterations made in the parameters ethyl alcohol brand and ethyl alcohol proportion do not present a great influence for the method developed, as the *t* value is lower than *t* critical or, *t* is not significant, then there is not difference between the results.

Even though the ethyl alcohol brand can be changed, the alteration made in proportion of ethyl alcohol did not have significant difference in the results, but the alteration was minimal, so caution must be taken in variation in this proportion, as in the development of the method the proportion of 10% of ethyl alcohol was tested but the results were unsatisfactory.

Accuracy

The values suggested by the Association of Official Analytical Chemists (AOAC) (31) such as acceptable to the recovery range were represented in Table 6.
For the analysis of pharmaceutical products, the accuracy specifications follow the range 98-102%. The value obtained for the recovery test was equal to 100.56% represented in Table 2, then there is evidence that the method developed is accurate.

Conclusion

The miniaturized and green method for the quantification of rifaximin in tablets by spectrophotometry in the ultraviolet region was developed and validated following the parameters: selectivity, linearity, precision, accuracy and robustness. This method presents advantages because it is fast, simple, requires fewer amounts of sample and solvents, generates minimum amount of waste and causes minimum prejudice to the environment and operator. Then, this method can be utilized in the routine analysis in the Quality Control more eco-friendly and sustainable.

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Conflict of interest

The authors report no conflicts of interest.

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