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SPECTROPHOTOMETRIC CHARACTERISTICS OF RIFAXIMIN AQUEOUS SOLUTION AND THE CONSEQUENCES FOR QUANTITATIVE ANALYSIS

BY

ELENA FASNIUC-PEREU[1], ANDREI-EUGEN BULGARIU[2] and LAURA BULGARIU[1,*]

[1]“Gheorghe Asachi” Technical University of Iaşi, “Cristofor Simionescu” Faculty of Chemical Engineering and Environment Protection, Department of Environmental Engineering and Management, Iaşi, Romania
[2]Emergency Hospital “Professor Doctor Nicolae Oblu” of Iaşi, Romania

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Abstract. Rifaximin is known as an oral antibiotic that can be used in a wide range of digestive diseases, but not only. Chemically, rifaximin is a yellow solid powder that can be relatively easily dissolved in methanol and which, because of its color, can be analyzed spectrophotometric. To be able to use such a simple method in the quantitative analysis of rifaximin, the most important spectrophotometric characteristics are required. In this study, these spectrophotometric characteristics (maximum wavelength (λ_{max}, nm), spectral band width ($\Delta\lambda_{1/2}$, nm), molar absorption coefficient (ε, $L \cdot mol^{-1} \cdot cm^{-1}$) are determined from VIS molecular spectra, recorded for aqueous solution containing a known concentration of rifaximin (4.22–16.88 $mg \cdot L^{-1}$) and different pH (1.0–7.0). Based on the obtained experimental results, the optimal conditions for the spectrophotometric analysis of rifaximin were established, namely: pH = 7.0 (distilled water or phosphate buffer) and λ_{max} = 444 nm. These conditions are suitable for the quantitative analysis of rifaximin with a detection limit of 0.0125-0.0179 $mg \cdot L^{-1}$ and a precision of 2.12-2.43%. The very good agreement between theoretical and experimental values for rifaximin concentration in tap and sea

*Corresponding author; *e-mail*: lbulg@ch.tuiasi.ro

water samples shows that this method is valid and can be used in quantitative analysis.

Keywords: rifaximin, spectrophotometric characteristics, aqueous solution, qualitative and quantitative analysis.

1. Introduction

From a pharmacological point of view, rifaximin is an antibiotic frequently prescribed in the treatment of digestive infections and hepatic encephalopathy, generated by numerous aerobic and anaerobic microorganisms (Rivkin and Gim, 2011; Kane and Ford, 2016). This wide use in the medical treatment of these diseases is mainly due to the fact that rifaximin is quite difficult to adsorb into the bloodstream, which means that the adverse effects of systemic exposure are minimal (Adachi and DuPont, 2006; Cheng *et al*., 2010). Therefore, numerous studies in the literature report the utility of rifaximin in medical research and beyond (Debbia *et al*., 2008; Mullen *et al*., 2014; Lopetuso *et al*., 2018). To carry out such studies, it is necessary to find a method for the quantitative analysis, which allows the easy, rapid, accurate and precise determination of rifaximin.

It is well known that VIS molecular absorption methods (spectrophotometric methods) fully fulfil all these conditions. In addition to ease of operation, these methods require inexpensive and easy-to-use equipment, small volumes of solution (1-2 mL), and can be used for the analysis of any organic or inorganic compound that is coloured (Deidda *et al*., 2018; El-Kholany *et al*., 2022). In such situations, the absorbance (A) is measured experimentally at difference concentrations, and the obtained values are used to obtain the calibration curve (Christian, 1994).

Rifaximin is a yellow powder that can be easily dissolved in methanol and easily diluted with distilled water. In this way, a coloured solution is obtained (in different shades of yellow), which can be directly analyzed spectrophotometrically. However, the yellow colour of rifaximin is most likely due to its functional groups that act as chromophores and can absorb radiation in the visible spectral domain (Toukabri *et al*., 2024).

But the chemical structure of these chromophores can be significantly influenced by the experimental conditions under which the rifaximim solution is prepared. For example, the variation of pH from acidic to the neutral range causes a significant change in the dissociation degree of the chromophoric functional groups. Consequently, the experimentally measured absorbance can vary within quite wide limits (depending on the solution pH), even if the rifaximin concentration in the analyzed solutions remains constant. These are the main reasons why, before being used in quantitative analysis, the optimal conditions under which the absorbance is to be measured experimentally must be

established. The selection of the optimal conditions, which ensure maximum sensitivity and accuracy in spectrophotometric analysis, is made by comparing the values of the characteristic parameters (λ_{max}, $\Delta\lambda_{1/2}$ and ε), determined in different experimental conditions.

In this study, the spectrophotometric characteristics of rifaximin was examined from VIS spectra recorded at different pH values (1.0, 2.0, 3.0, 4.0 and 7.0) in distilled water, and at pH of 7.0 in phosphate buffer solution. In each case the spectrophotometric parameters (λ_{max}, $\Delta\lambda_{1/2}$ and ε) were calculated, and the optimal conditions were selected. Under optimal conditions, the calibration curve was constructed, and the detection limit, linear dynamic range and sensibility of the spectrophotometric method were evaluated.

2. Materials and methods

All chemical reagents (rifaximin, K_2HPO_4, 1 N HNO_3, methanol) were of analytical degree and were used without further purification. Rifaximin solution with a concentration of 211 mg/L was prepared by dissolving an appropriate amount of solid rifaximin in methanol. The phosphate buffer solution (pH = 7.0) was prepared using the methodology described by Seracu (1989).

Aqueous solutions (25 mL) with constant concentration of rifaximin (16.88 mg/L) and different pH values were prepared for recording VIS spectra. pH values of 1.0, 2.0, 3.0 and 4.0 were obtained by adjusting the pH of distilled water with small volumes of 1N HNO_3 solution, while pH = 7.0 was considered the natural pH of distilled water or was obtained adding 5 mL of phosphate buffer solution. For all samples, VIS spectra (Digital VIS Spectrophotometer model YA1407020) were recorded in a 1 mL glass cell, measuring the absorbance of the solution against distilled water, in the spectral range between 340–610 nm. The qualitative spectrophotometric characteristics were determined for each recorded spectrum as follows:

(a) wavelength of maximum absorption: λ_{max} – graphically, and represents the wavelength corresponding to the highest experimental absorbance (A_{max});

(b) width of the spectral band: $\Delta\lambda_{1/2}$ – graphically, the range of wavelengths corresponding to half the maximum absorbance;

(c) molar absorption coefficient: ε – calculated from Lambert-Beer law as absorbance, measured in a 1 cm cell, for a 1 mol·L^{-1} rifaximin solution.

For quantitative spectrophotometric characterization, the calibration curves were recorded, measuring the absorbance at different rifaximin concentrations in the range 4.22 – 16.88 mg·L^{-1}, both in distilled water and in phosphate buffer of pH = 7.0. The limit of detection, limit of quantification, precision and sensibility (Christian, 1994; Dean, 1995) of the spectrophotometric method were determined using the experimental calibration curves. The

applicability of the proposed method was also evaluated using the recovery tests. Thus two series of samples were prepared. In the first series, in 25 mL flasks were added rifaximin (16.88 $mg \cdot L^{-1}$), 5 mL of phosphate buffer (pH = 7.0) and 15 mL of tap water and sea water. In the second series, the samples were prepared similarly, with the difference that was no phosphate buffer was added. In each case, the absorbance was measured experimentally at 444 nm, the concentration of rifaximin was calculated from the calibration curves, and compared with the theoretical values.

3. Results and discussion

Macroscopically, rifaximin is an orange powder that is hardly soluble in water, but quite easily soluble in methanol. Methanolic solutions of rifaximin are stable over time (at least 10 days), and due to their yellow colour they can be used for spectrophotometric measurements. Chemically, rifaximin is a heterocyclic macromolecule (Fig. 1), containing condensed aromatic nuclei and numerous hydroxyl, carbonyl and amino groups. The spatial distribution of these functional groups in the rifaximin molecule allow extensive delocalization of electrons from double bonds and, consequently, the appearance of chromophores, which are responsible for the presence of the yellow colour of rifaximin solutions. Therefore, the ionization degree of functional groups will significantly influence the colour of rifaximin solutions and their spectrophotometric characteristics.

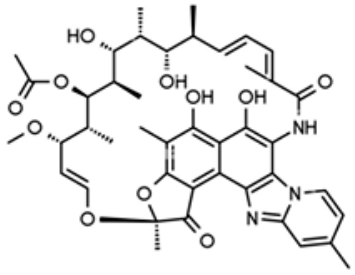
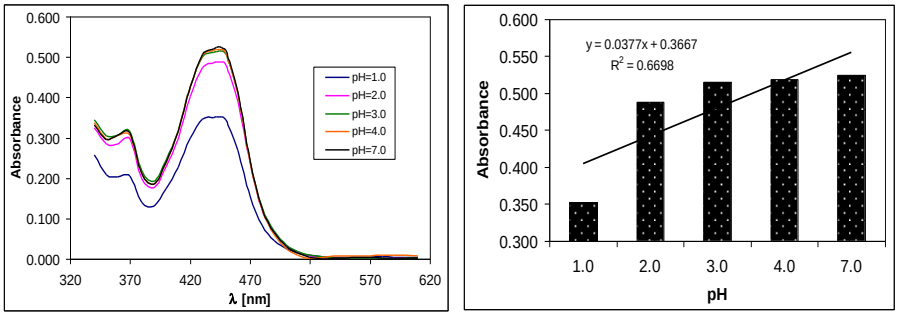


Fig. 1 – Chemical structure of rifaximin (Ouyang *et al*., 2020).

The ionization degree of functional groups is mainly influenced by the solution pH. In general, a low pH value (acid media) causes the protonation of functional groups (undissociated groups), while higher pH values (neutral media) leads to their ionization (Valcarcel *et al*., 2001; Calanni *et al*., 2014). Basic media is not suitable in this case as it would cause precipitation of rifaximin molecules due to low water solubility. Therefore, the first parameter for which the most suitable value must be determined in the solution pH.

In this study, the pH of rifaximin solutions was varied in the range of 1.0 – 7.0. For solutions with pH between 1.0 and 4.0, volumes of HNO_3 (1 N) were

added, while pH = 7.0 was considered the natural pH of distilled water. The VIS spectra recorded for the rifaximin solutions with different pH are shown in Fig. 2(a), and the spectrophotometric characteristics are summarized in Table 1.



(a) (b)

Fig. 2 – (a) VIS spectra of rifaximin solution at different pH. (b) Variation of absorbance measured at 444 nm for different pH.

Table 1

Qualitative spectrophotometric characteristics of VIS spectra recorded for rifaximin solutions at different pH ($c_{rifaximin}$ = 16.88 mg·L^{-1})

pH	λ_{max} [nm]	A_{max}	$\Delta\lambda_{1/2}$ [nm]	ε [L·mol^{-1}·cm^{-1}]
1.0	444	0.352	75	$1.639 \cdot 10^4$
2.0	444	0.488	71	$2.272 \cdot 10^4$
3.0	444	0.515	63	$2.398 \cdot 10^4$
4.0	444	0.519	62	$2.416 \cdot 10^4$
7.0	444	0.525	62	$2.444 \cdot 10^4$

As can be seen from Fig. 2(a), the VIS spectra of rifaximin have a single significant band, which has an absorption maximum (A_{max}) at 444 nm, for all pH values. The intensity of this absorption band increases with increasing pH, but this increase is much more pronounced at pH < 3.0, and less significant at pH > 3.0 (Fig. 2(b)). This change in A_{max} is most likely caused by the variation in the ionization degree of the rifaximin functional groups as the pH increases. In strong acid media (pH < 3.0), where most of the functional groups are protonated, the delocalization of electrons from multiple bonds is difficult, which leads to the decrease of the coloring intensity (A_{max} is low) of the chromophores. In slightly acid media (pH > 3.0), the ionization degree of the functional groups increases and consequently there is an increase in the color intensity (A_{max} is high, Fig. 2(b)).

In addition, the width of the spectral band ($\Delta\lambda_{1/2}$) decreases with increasing pH of rifaximin solution (Table 1), concomitantly with increasing molar absorption coefficient values (ε). The analysis of these results (Fig. 2 and Table 1) shows that for the spectrophotometric determination of rifaximin it is

recommended the use of aqueous solution with pH = 7.0, because in this case the sensitivity (ε [L·mol^{-1}·cm^{-1}]) and selectivity ($\Delta\lambda_{1/2}$ [nm]) of the experimental measurements have the highest values.

But, from an experimental point of view, maintaining a pH = 7.0 in distilled water is quite difficult to achieve, especially when the determination of rifaximin is made from solutions of complex composition, where secondary processes can occurs. The analytical approach in such situations is the use of buffer solutions, which maintain the pH at the desired value, regardless of the compositions of the solution to be analyzed. In this study, a phosphate buffer solution with pH = 7.0 was used, and the VIS spectra recorded in the absence and in presence of the buffer solution are illustrated in Fig. 3, and the calculated spectrophotometrically characteristics are presented in Table 2.

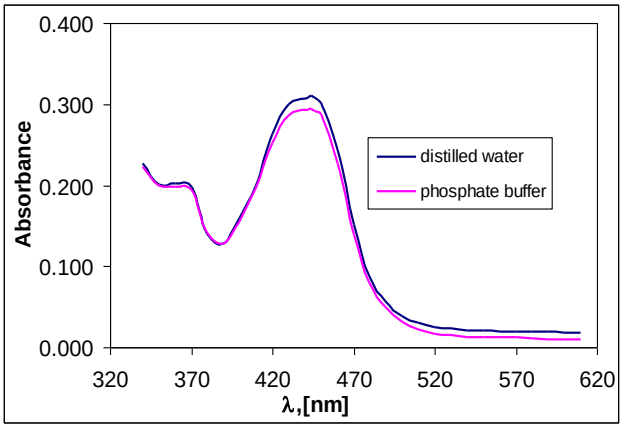


Fig. 3 – VIS spectra of rifaximin solution at pH = 7.0 in distilled water and phosphate buffer solution.

Table 2

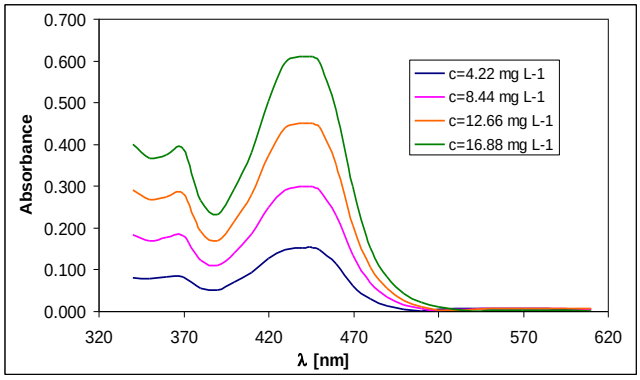
Qualitative spectrophotometric characteristics of VIS spectra recorded for rifaximin solutions at pH = 7.0 ($c_{rifaximin}$ = 8.44 mg·L^{-1})

Parameter	Distilled water	Phosphate buffer
λ_{max} [nm]	444	444
A_{max}	0.310	0.294
$\Delta\lambda_{1/2}$ [nm]	63	61
ε [L·mol^{-1}·cm^{-1}]	2.887 ·10^4	2.737 ·10^4

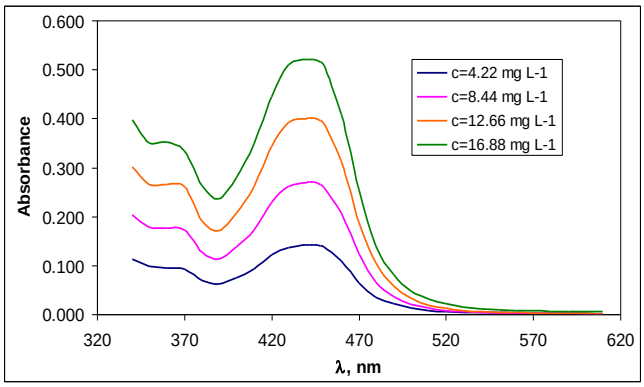
The VIS spectra recorded in this case (Fig. 3) and the values of the spectrophotometric characteristics (Table 2) show that both in distilled water and in phosphate buffer, the position of the absorption maximum (λ_{max}) and the colour purity ($\Delta\lambda_{1/2}$) do not change significantly. A rather small difference can be

observed in the case of parameters that are correlated with the sensibility of the spectrophotometric method (A_{max} and ε), although the difference between the values obtained in distilled water and in phosphate buffer does not exceed 6% (Table 3). Therefore, both media (distilled water and phosphate buffer) can be used for the quantitative determination of rifaximin, and their applicability was examined by plotting the calibration curves.

VIS spectra recorded for different concentrations of rifaximin at pH = 7.0 in distilled water and phosphate buffer are shown in Fig. 4. In both cases, the position of the absorption maximum is unchanged, and the intensity of the absorption bands increases with the increase in the concentration of rifaximin.



(a)

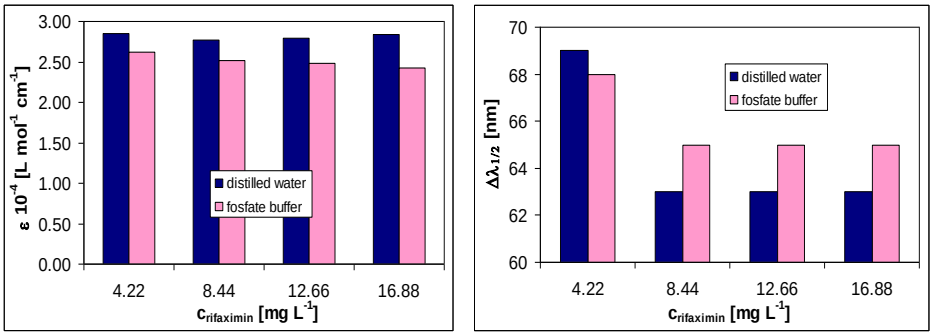


(b)

Fig. 4 – VIS spectra of rifaximin solution at pH = 7.0 and different concentrations in distilled water (a) and phosphate buffer (b).

The variation of molar absorption coefficients (ε [$L \cdot mol^{-1} \cdot cm^{-1}$]) and spectral band width ($\Delta\lambda_{1/2}$ [nm]) as a function of rifaximin concentration at pH = 7.0, in distilled water and phosphate buffer are illustrated in Fig. 5. As can be seen, the values of these spectrophotometric characteristics do not differ significantly in

the studied range of rifaximin concentration, although in the case of distilled water an increase in the values of ε by 17% can be observed, while $\Delta\lambda_{1/2}$ registers a decrease by 3%, compared to the case of phosphate buffer.



(a) (b)

Fig. 5 – Variation of molar absorption coefficients (ε) (a) and spectral band width ($\Delta\lambda_{1/2}$) (b) as a function of rifaximin concentration at pH = 7.0.

The calibration curves obtained at pH = 7.0, both in distilled water and in phosphate buffer are shown in Fig. 6, and the quantitative characteristics calculated from these plots are summarized in Table 3.

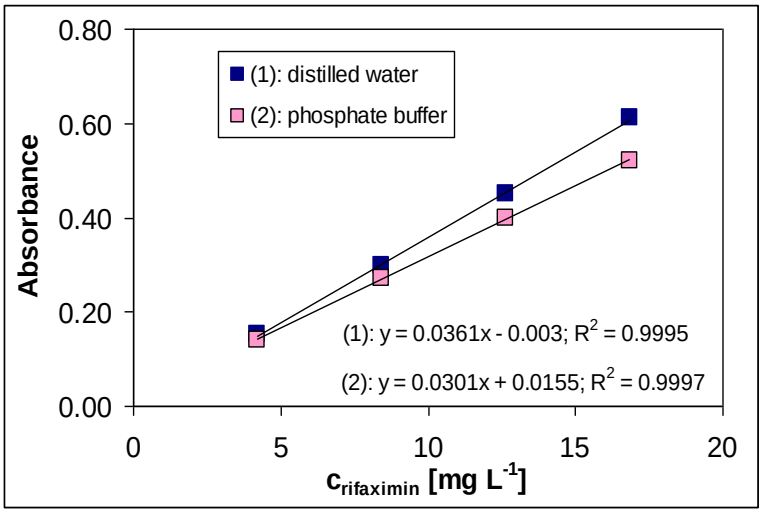


Fig. 6 – Calibration curves for quantitative analysis of rifaximin (λ = 444 nm, pH = 7.0).

Table 3
Quantitative characteristics for spectrophotometric determination of rifaximin at pH = 7.0

Parameter	Distilled water	Phosphate buffer
λ_{max}, [nm]	444	444
Linear concentration range, [mg·L^{-1}]	0.05 – 0.20	0.05 – 0.20
Regression coefficient	0.9995	0.9997
Calibration sensitivity, [L·mg^{-1}]	0.0361	0.0301
Limit of detection, [mg·L^{-1}]	0.0125	0.0178
Precision, [%]	2.12	2.43

The values of the parameters included in Table 3 indicate that the quantitative determination of rifaximin can be performed with similar performances both in distilled water and phosphate buffer (pH = 7.0). In both cases, the spectrophotometric determination of rifaximin has an acceptable linear concentration range, high sensitivity and precision and low detection limit (Table 3). Furthermore, the values of rifaximin concentration obtained from the calibration curves (experimental values) are in good agreement with the theoretical values, regardless of the type of water (tap water or sea water) used for sample preparation (Table 4), in absence and in presence of phosphate buffer (pH = 7.0). This shows that the constituents of water samples (common inorganic cations and anions) do not interfere in the spectrophotometric determination of rifaximin and highlights the practical applicability of this method in quantitative analysis.

Table 4
Theoretical and experimental values for rifampicin concentration in tap water and sea water samples

Sample	$C_{rifaximin}^{teor.}$, [mg·L^{-1}]	$C_{rifaximin}^{exp.}$, [mg·L^{-1}]	Δc, [mg·L^{-1}]
Distilled water			
Tap water	16.88	16.98	-0.101
Sea water	16.88	16.79	0.091
Phosphate buffer, pH=7.0			
Tap water	16.88	16.72	0.160
Sea water	16.88	16.79	0.091

However, when using this method for the analysis of complex samples, it is recommended to use phosphate buffer to maintain the pH at 7.0. When using un-buffered solution, the pH can change, and this significantly affects the sensitivity and accuracy of spectrophotometric method.

4. Conclusions

In this study, VIS spectra of rifaximin solution (16.88 mg·L^{-1}) were recorded at different pH (1.0–7.0), and the most important spectrophotometric characteristics (maximum wavelength (λ_{max} [nm]), spectral band width ($\Delta\lambda_{1/2}$ [nm]), molar absorption coefficient (ε [L·mol^{-1}·cm^{-1}])) were determined. The obtained values showed that the spectrophotometric determination of rifaximin occurs with the best sensitivity and selectivity at pH = 7.0 (the natural pH of distilled water). But experimentally, maintaining a pH = 7.0 in distilled water is quite difficult to achieve, especially when are analyzed solutions of complex compositions. Therefore, the use of buffer solutions is recommended, and in this case the phosphate buffer (pH = 7.0) was selected. The VIS spectra recorded for rifaximin solutions with known concentration (8.44 mg·L^{-1}) indicate that both media (distilled water and phosphate buffer) can be used for the quantitative determination of rifaximin, because the position of the absorption maximum (λ_{max}) and the colour purity ($\Delta\lambda_{1/2}$) do not change significantly. In order to test the applicability of this spectrophotometric method in quantitative analysis of rifaximin, the calibration curves were constructed (in distilled water and phosphate buffer, pH = 7.0). In both cases, the values of the quantitative characteristics have shown that the spectrophotometric method has an acceptable linear concentration range, high sensitivity and precision and low detection limit. In addition, the constituents of the water samples (tap water and sea water) do not interfere with the spectrophotometric determination of rifaximin. Therefore, this method can be used for the quantitative determination of rifaximin, but for the analysis of complex samples, it is recommended to use phosphate buffer to maintain the pH at 7.0.

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CARACTERISTICILE SPECTROFOTOMETRICE ALE SOLUŢIEI DE RIFAXIMINĂ ŞI CONSECINŢELE PENTRU ANALIZA CANTITATIVĂ

(Rezumat)

Rifaximina este cunoscută ca un antibiotic oral care poate fi utilizat într-o gamă largă de afecțiuni digestive, dar nu numai. Din punct de vedere chimic, rifaximina este o pulbere solidă, de culoare galbenă, care poate fi ușor dizolvată în metanol, şi care datorită culorii sale, poate fi analizată spectrofotometric. Pentru a putea folosi o metodă atât de simplă în analiza cantitativă a rifaximinei, este necesară cunoașterea celor mai importante caracteristici spectrofotometrice. În acest studiu, caracteristicile spectrofotometrice (lungimea de undă corespunzătoare maximului de absorbție (λ_{max}, nm), lățimea benzii spectrale ($\Delta\lambda_{1/2}$, nm) și coeficientul molar de absorbție (ε, $L \cdot mol^{-1} \cdot cm^{-1}$)) au fost determinate din spectrele VIS, înregistrate pentru o soluție apoasă care conține o concentrație cunoscută de rifaximină (4,22 – 16,88 $mg \cdot L^{-1}$) și pH diferit (1,0 – 7,0). Pe baza rezultatelor obținute au fost stabilite condițiile optime pentru analiza spectrofotometrică a rifaximinei, și anume pH = 7,0 (apă distilată sau tampon fosfat) și

λ_{max} = 444 nm. Aceste condiții sunt adecvate pentru analiza cantitativă, când determinarea rifaximinei se poate face cu o limită de detecție de 0,0125-0,0178 mg·L^{-1} și o precizie de 2,12-2,43%.Concordanța foarte bună dintre valorile teoretice și cele obținute experimental pentru concentrația de rifaximină din probele de apă de la robinet și de mare arată că această metodă este valabilă și poate fi utilizată în analiza cantitativă.