BULETINUL INSTITUTULUI POLITEHNIC DIN IAŞI
Publicat de
Universitatea Tehnică „Gheorghe Asachi” din Iaşi
Volumul 68 (72), Numărul 3, 2022
Secţia
CHIMIE şi INGINERIE CHIMICĂ

SPECTROPHOTOMETRIC DETERMINATION OF RIFAMPICIN IN AQUEOUS SOLUTION

BY

IUSTINA-DENISA TANASĂ[1], ANDREI-EUGEN BULGARIU[2], CĂTĂLINA SIRIŢEANU[1] and LAURA BULGARIU[1,*]

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

Received: June 6, 2022
Accepted for publication: July 15, 2022

Abstract. In this study, a simple spectrophotometric method was developed for the quantitative determination of rifampicin in aqueous solution. The method is based on the property of rifampicin to modify, in aqueous media at pH of 7.0, the degree of ionization of the functional groups. After ionization, a coloured compound is formed, which can be analysed spectrophotometrically. The visible spectra of the yellow compound were recorded at pH of 7.0, in different buffer solutions (phosphate, hexamethylentetraamine (HMT) and borax) and the qualitative characteristics were determined. The highest absorbance was obtained in phosphate buffer, at λ = 470 nm, against distilled water, and these conditions were selected for quantitative determinations. This procedure allows the quantitative analysis of rifampicin in a wide concentration range (0.01 – 0.16 mmol·L^{-1}), with a detection limit of 0.0002 mmol·L^{-1}. The very good correspondence between the calculated and experimental values of rifampicin concentration shows that this method is valid and can be used for quantitative analysis, at least for laboratory studies.

Keywords: spectrophotometric method, rifampicin, quantitative determination, aqueous solution.

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

1. Introduction

Rifampicin is an organic compound with low water solubility, which has antibiotic action, and is mainly used in the treatment of tuberculosis (Castaneda-Fernandez *et al*., 2022). Although this antibiotic is one of the first drug considered in the treatment of tuberculosis patients, its use more than two months is not recommended (Farokhi-Fard *et al*., 2019). This is because rifampicin, regardless of the administration form (oral or injectable) has severe hepatotoxic effects (Ramappa and Aithal, 2013). In order to minimize such secondary effects, finding new forms for rifampicin administration is still an open problem for which solutions are being sought (Li *et al*., 2008; Khadka *et al*., 2021). Consequently, it is necessary to develop a simple and rapid method for quantitative determination of this antibiotic that can be easily used at least in laboratory experiments.

Most often for the analysis of organic molecules with drug action, the recommended methods are chromatographic methods (Pereira *et al*., 2018; Goberna Bravo *et al*., 2020; Kotadiya and Patel, 2021). Whether it is gas chromatography (GC) or high-performance liquid chromatography (HPLC), the chromatographic methods ensure the determination of these types of molecules with high precision ($ng \cdot L^{-1}$), accuracy and selectivity (Brega *et al*., 1990; Kim *et al*., 2018). These are the most important reasons why chromatographic methods are imposed by most standards in the field of pharmaceutical quality control (Deidda *et al*., 2018).

However, in laboratory experiments, the use of chromatographic methods is not always the best choice. This is due to the fact that chromatographic methods require laborious experimental protocols, long working time and expensive laboratory equipment (Bulgariu, 2011). More suitable for such purposes are VIS molecular absorption methods (or spectrophotometric methods). Spectrophotometric methods are generally rapid, simple and inexpensive, and can be used for quantitative analysis in aqueous media or in organic solvents (Dean, 1995). The analytical performances of these methods are mainly determined by the colour intensity of analysed compound, and for their improvement, besides the rigorous selection of the experimental conditions (pH, buffer solution, maximum wavelength, etc.), it is often necessary to use a colour reagent (Ripan *et al*., 1963). In the case of rifampicin, it is not necessary to add a colour reagent. By adjusting pH to 7, the aqueous solution of rifampicin turns deep yellow due to the formation of a complex with charge transfer, which can be analysed spectrophotometrically.

In this study, the qualitative and quantitative characteristics of the yellow rifampicin complex obtained at pH 7.0 were examined, to highlight its usefulness in spectrophotometric determinations. The experiments were performed in three different buffer solutions (phosphate, HMT and borax), and the VIS spectra obtained in each case were analysed in detail. Based on the obtained results, a calibration curve was constructed, and the linear dynamic range, detection limit

and sensitivity of the method were determined. All these parameters are particularly useful in the quantitative analysis of this antibiotic.

2. Experimental

2.1. Materials

The chemical reagents: rifampicin, K_2HPO_4, hexamethylentetraamine (HMT), borax ($Na_2[B_4O_5(OH)_4]\cdot 8\,H_2O$), methanol (99%), NaOH (1N) and HNO_3 (1N) were of analytical degree and were used as received. The buffer solutions (phosphate, HMT and borax) were prepared according with the procedures reported by Seracu (1989). The stock solution of rifampicin (1.281 mmol·L^{-1}) was obtained by dissolving an exact amount of solid rifampicin (0.053 g) in 50 mL of methanol. All spectrophotometric measurements were performed using a Digital VIS Spectrophotometer model YA1407020, in 1.0 cm glass cells, against distilled water, at 21 ± 2°C.

2.2. Analytical procedure

The solutions used for VIS spectra recording were obtained by diluting 0.5 – 3.0 mL of stock solution of rifampicin (50 mg·L^{-1}) with distilled water. In each working solution, 5.0 mL of each buffer solution (phosphate, HMT and borax) with pH = 7.0 was added. In each flask was added distilled water to the mark and all solutions were mix. The absorbance was measured in VIS spectral domain (400 – 600 nm), in 1 cm glass cell, against distilled water. The qualitative analysis of each spectrum was done by determination of the following parameters (Christian, 1994):

(i) λ_{max} – the value of wavelength corresponding to the maximum absorbance;

(ii) $\Delta\lambda_{1/2}$ – the width of the spectral band, which represents the difference between the values of wavelengths corresponding to the half of the maximum absorbance;

(iii) ε - the molar absorption coefficient, which represents the absorbance of a solution of concentration 1 mol·L^{-1}, and is calculated form the Lambert-Beer law, according to the relation:

$$\varepsilon = \frac{A_{max}}{l \cdot c} \tag{1}$$

where: A_{max} is the maximum absorbance; l is the length of the optical path (1 cm) and c is the concentration of rifampicin (mol·L^{-1}).

The quantitative analysis of rifampicin was done using a prepared calibration curve. For the calibration curve, the absorbance was measured at 470 nm, in phosphate buffer solution (pH = 7.0), varying the concentration of rifampicin

between 0.01 and 0.16 mmol·L^{-1}. The rifampicin concentration in the analyzed samples was obtained from the regression equation of the calibration curve, and the experimental values were compared with the theoretical ones.

3. Results and discussion

Chemically, rifampicin is a heterocyclic organic compound (Fig. 1), which has the molecular weight of 823 g·mol^{-1}, and two ionizable groups with a corresponding pKa_1 of 1.8 and pKa_2 of 7.9 (Castaneda-Fernandez *et al*., 2022). Due to these chemical characteristics, at pH of 7.0 the heterocyclic nitrogen atom in the rifampicin molecule is still protonated (pH < pKa_2), and can interact electrostatically with anions in the aqueous solution to form a charge transfer complex with well-defined colour. In order for the stability of the formed complex not to be affected by the variation of the experimental conditions (the difference between pH and pKa_2 is quite small (0.9 units)), it is recommended the use buffer solutions, which allow to keep the pH at a constant value (Valcarcel *et al*., 2001).

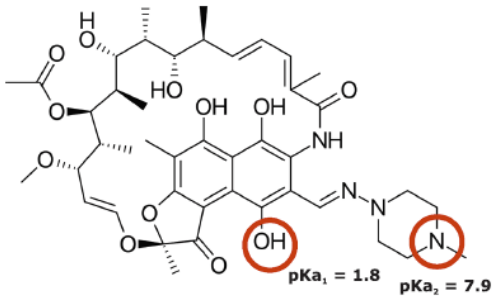

Fig. 1 – Chemical structure of rifampicin (Castaneda-Fernandez *et al*., 2022).

Three types of buffer solutions (with pH of 7.0) are the most commonly used for analytical purpose, namely phosphate buffer, HMT buffer and borax buffer. All these buffer solution are easy to prepare, stable over time and inexpensive. This is why these buffer solutions were selected for experimental studies.

The VIS spectra recorded for different concentrations of rifampicin at pH of 7.0 in phosphate buffer, HMT buffer and borax buffer are presented in Figs. 2-4, while the qualitative parameters (λ_{max}, $\Delta\lambda_{1/2}$ and ε) calculated for each case are summarized in Table 1.

As can be seen from Figs. 2-4 and Table 1, regardless of the nature of the buffer solution (phosphate, HMT or borax), at pH 7.0 rifampicin forms the same type of charge transfer complex that is yellow. This complex has a maximum absorption at 470 – 480 nm, measured against distilled water, and is stable over time (at least 72 hours).

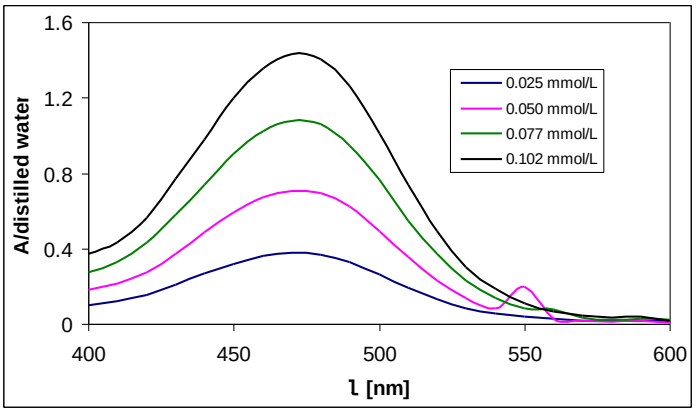

Fig. 2 – VIS spectra of rifampicin in phosphate buffer at pH of 7.0.

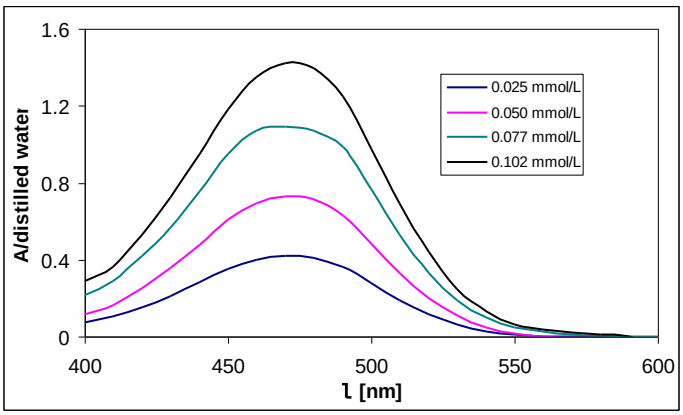

Fig. 3 – VIS spectra of rifampicin in HMT buffer at pH of 7.0.

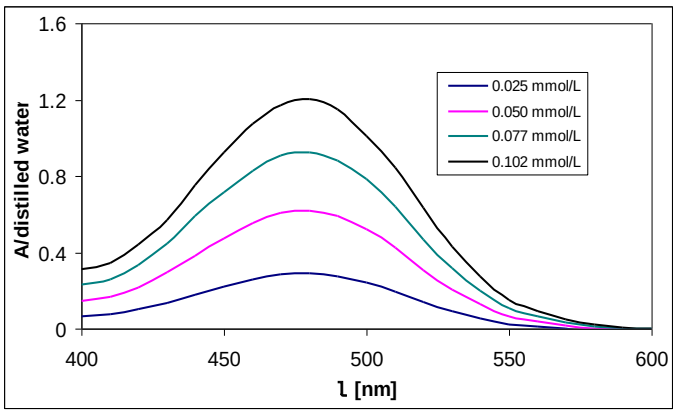

Fig. 4 – VIS spectra of rifampicin in borax buffer at pH of 7.0.

On the other hand, in the whole concentration range of rifampicin, the yellow complex does not change its composition, as evidenced by the close values of the molar absorption coefficient (ε), calculated for each case (see Table 1). Therefore, the chemical stability of the spectrophotometric complex is also ensured.

Table 1

Qualitative parameters of VIS spectra of rifampicin in the three buffer solutions (pH = 7.0)

Buffer solution	$c_{rifampicin}$ [mmol·L^{-1}]	ε [L·mol^{-1}·cm^{-1}]	λ_{max} [nm]	$\Delta\lambda_{1/2}$ [nm]
Phosphate	0.025	$1.50 \cdot 10^4$	470	80
	0.050	$1.45 \cdot 10^4$	470	82
	0.073	$1.46 \cdot 10^4$	470	84
	0.097	$1.46 \cdot 10^4$	470	82
HMT	0.025	$1.44 \cdot 10^4$	470	77
	0.051	$1.42 \cdot 10^4$	470	79
	0.076	$1.42 \cdot 10^4$	470	80
	0.102	$1.40 \cdot 10^4$	470	80
Borax	0.025	$1.12 \cdot 10^4$	480	86
	0.051	$1.20 \cdot 10^4$	480	90
	0.075	$1.20 \cdot 10^4$	480	90
	0.102	$1.17 \cdot 10^4$	480	89

However, the detailed analysis of the data presented in Table 1 shows that phosphate and HMT buffers are more suitable for use in the spectrophotometric determination of rifampicin than borax buffer. This is because in the case of phosphate and HMT buffers: (i) the values of the molar absorption coefficients are higher, which ensures a higher sensitivity, and (ii) the $\Delta\lambda_{1/2}$ values are lower, which makes the selectivity better. But because these differences are not significant, from a qualitative point of view, the three buffer solutions meet all the requirements and can be used for the quantitative determination of rifampicin by spectrophotometry.

Fig. 5 shows the dependence between the experimentally measured absorbance and the rifampicin concentration, at the wavelength corresponding to the absorption maximum (470 nm for phosphate and HMT buffer solutions and at 480 nm for borax buffer solution, respectively).

It can be seen from Fig. 5 that the most suitable buffer for quantitative determination of rifampicin is phosphate buffer, compared with HMT buffer and borax buffer. This selection is justified by: (i) the highest value of the regression coefficient (R^2), which suggests that there is a linear dependence between absorbance and concentration, according with Lambert-Beer law, and (ii) the

highest value of the slope regression line, which shows that in this case the method has the highest accuracy.

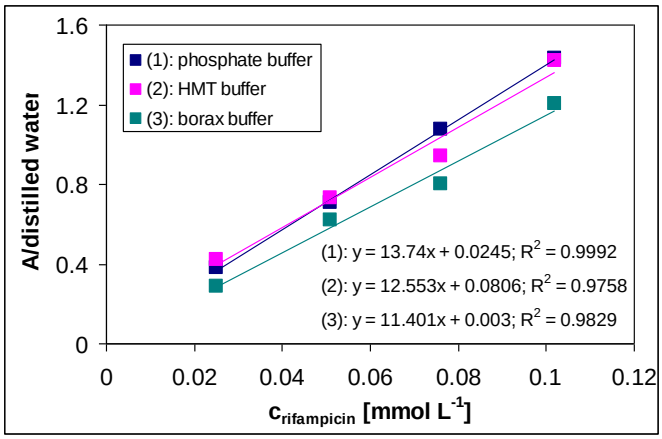

Fig. 5 – Experimental dependence between absorbance and rifampicin concentration for the three buffer solutions (pH = 7.0).

In order to check the applicability of phosphate buffer (pH = 7.0) in the quantitative analysis of rifampicin, the calibration curve was constructed using six etalon solutions. In each case, the absorbance was measured at 470 nm, against distilled water, and the obtained results are illustrated in Fig. 6, while the quantitative parameters used for the characterization of this method are summarized in Table 2.

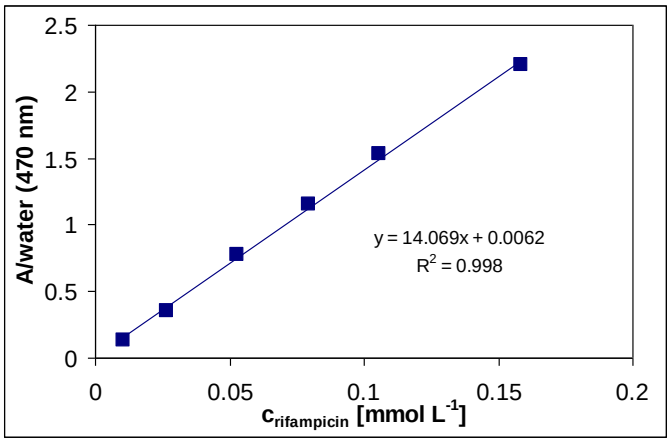

Fig. 6 – Calibration curve for quantitative analysis of rifampicin in phosphate buffer (pH = 7.0).

The values of quantitative parameters presented in Table 2 indicate that the determination of rifampicin in phosphate buffer (pH = 7.0) has high sensitivity and precision, and a reasonable linear concentration range. In addition, there is a good concordance between the concentration values calculated based on the calibration equation and the experimental ones, on entire linear concentration range (Table 3).

Table 2
Quantitative parameters for spectrophotometric determination of rifampicin in phosphate buffer (pH = 7.0)

Parameter	Determined value
λ_{max}, [nm]	470
Linear concentration range, [mmol·L^{-1}]	0.01 – 0.16
Regression coefficient	0.9980
Calibration sensitivity, [L·mmol^{-1}]	14.069
Limit of detection, [mmol·L^{-1}]	0.0002
RDS, [%]	1.38

Table 3
Calculated and experimental values of rifampicin concentration in phosphate buffer (pH = 7.0)

$C_{rifampicin}^{exp}$, [mmol·L^{-1}]	$C_{rifampicin}^{calc}$, [mmol·L^{-1}]	Δc, [%]
0.0105	0.0099	-1.16
0.0264	0.0244	-1.05
0.0528	0.0547	-0.91
0.0793	0.0814	-0.89
0.1057	0.1083	-0.87
0.1586	0.1558	-0.86

Therefore, all these aspects highlight the fact that this spectrophotometric method can be used in the quantitative analysis of this antibiotic, at least in laboratory studies.

3. Conclusions

In this study, the spectrophotometric determination of rifampicin in aqueous solution of pH 7.0 was examined in three different buffer solutions (phosphate buffer, HMT buffer and borax buffer). The obtained experimental results have shown that in all these three buffer solutions, a yellow complex of rifampicin is obtained. The VIS spectra recorded for each buffer solution allowed the determination of the qualitative spectrophotometric parameters (λ_{max}, ε and $\Delta\lambda_{1/2}$) and the selection of the most appropriate buffer solution. This is the

phosphate buffer where the maximum of absorption is obtained at 470 nm, and the values of ε and $\Delta\lambda_{1/2}$ ensure the highest sensitivity and selectivity of the rifampicin determination. In order to check the applicability of phosphate buffer (pH = 7.0) in the quantitative analysis of rifampicin, the calibration curve was constructed. The values of quantitative parameters indicate that the quantitative determination of rifampicin in phosphate buffer (pH = 7.0) has high sensitivity and precision, and a reasonable linear concentration range. Therefore, this spectrophotometric method can be used in the quantitative analysis of this antibiotic, at least in laboratory studies.

REFERENCES

Brega A., Prandini P., Amaglio C., Pafumi, *Determination of phenol, m-, o- and p-cresol, p-aminophenol and p-nitrophenol in urine by high-performance liquid chromatography*, J. Chromatogr. A **535**, 311-316 (1990).

Bulgariu L., *Instrumental methods of analysis* (in Romanian), Iaşi, Politehnium, (2011).

Castaneda-Fernandez C., Chavez-Santos R.M., Silva-Miranda M., Espitia-Pinzon C., Martinez R., Kozina A., *Optimization of rifampicin encapsulation in PLGA polymeric reservoirs*, Int. J. Pharm. **622**, 121844 (2022).

Christian G.D., *Analytical Chemistry*, New York, John Wiley & Sons, Inc., (1994).

Dean J.A., *Analytical Chemistry Handbook*, 3rd ed. New York, USA, McGrow-Hill Inc. (1995).

Deidda R., Orlandini S., Hubert P., Hubert C., *Risk-based approach for method development in pharmaceutical quality control context: A critical review*, J. Pharm. Biomed. Anal. **161**, 110-121 (2018).

Farokhi-Fard A., Golichenari B., Ghanbarlou M.M., Zanganeh S., Vaziri F., *Electroanalysis of isoniazid and rifampicin: Role of nanomaterial electrode modifiers*, Biosens. Bioelectron. **146**, 111731 (2019).

Goberna Bravo M.A., Durgbanshi A., Bose D., Mishra P., Albiol-Chiva J., Esteve-Romero J., Peris-Vicente J., *Quantification of rifampicin and rifabutin in plasma of tuberculosis patients by micellar liquid chromatography*, Microchem. J. **157**, 104865 (2020).

Khadka P., Sinha S., Tucker I.G., Dummer J., Hill P.C., Katare R., Das S.C., *Pharmacokinetics of rifampicin after repeated intra-tracheal administration of amorphous and crystalline powder formulations to Sprague Dawley rats*, Eur. J. Pharm. Biopharm. **162**, 1-11 (2021).

Kim C., Ryu H.D., Chung E.G., Kim Y., Lee J.K., *A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water: Sample preparation, liquid chromatography, and mass spectrometry*, J. Environ. Manag. **217**, 629-645 (2018).

Kotadiya R.M., Patel F.N., *Analytical Methods Practiced to Quantitation of Rifampicin: A Captious Survey*, Curr. Pharm. Anal. **17**(8), 983-999 (2021).

Li M., Rouaud O., Poncelet D., *Microencapsulation by solvent evaporation: State of the art for process engineering approaches*, Int. J. Pharm. **363**, 26-39 (2008).

Pereira M.N., Matos B.N., Gratieri T., Cunha-Filho M., Gelfuso G.M., *Development and validation of a simple chromatographic method for simultaneous determination of clindamycin phosphate and rifampicin in skin permeation studies*, J. Pharm. Biomed. Anal. **150**, 331-340 (2018).

Ramappa V., Aithal G.P., *Hepatotoxicity related to anti-tuberculosis drugs: mechanisms and management*. J. Clin. Exp. Hepatol. **3**(1), 37-49 (2013).

Ripan R., Popper E., Liteanu C., *Qualitative analytical chemistry (in Romanian)*. Fourth edition, Didactica si Pedagogica, Bucarest (1963).

Seracu D.I., *Îndreptar de Chimie analitică (in Romanian)*, Technică Publishing Hourse, Bucharest (1989).

Valcarcel M., Gomez-Hens, Rubio S., *Selectivity in analytical chemistry revisited*, TrAC Trends Anal. Chem., **20**(8), 386-393 (2001).

DETERMINAREA SPECTROFOTOMETRICĂ A RIFAMPICINEI DIN SOLUŢII APOASE

(Rezumat)

În acest studiu, a fost elaborată o metodă spectrofotometrică simplă pentru determinarea cantitativă a rifampicinei în soluție apoasă. Metoda se bazează pe proprietatea rifampicinei de a-și modifica, în medii apoase la pH de 7,0, gradul de ionizare al grupărilor funcționale. După ionizare, se formează un compus colorat, care poate fi analizat spectrofotometric. Spectrele în domeniu VIS ale compusului galben au fost înregistrate la pH de 7,0, în diferite soluții tampon (fosfat, hexametilentetraamină (HMT) și borax) și au fost determinate caracteristicile calitative. Cea mai mare absorbanță a fost obținută în tampon fosfat, la λ = 470 nm, față de apă distilată, iar aceste condiții au fost selectate pentru determinări cantitative. Această metodă permite analiza cantitativă a rifampicinei într-un interval larg de concentrații (0,01 – 0,16 mmol·L^{-1}), cu o limită de detecție de 0,0002 mmol·L^{-1}. Corespondența foarte bună dintre valorile calculate și experimentale ale concentrației de rifampicină arată că această metodă este valabilă și poate fi utilizată pentru analize cantitative, cel puțin în studii de laborator.