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DEVELOPMENT AND VALIDATION OF NEW SPECTROPHOTOMETRIC PROCEDURES FOR THE ANALYSIS OF ROXITHROMYCIN IN BULK AND DOSAGE FORMS

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ABSTRACT

Novel sensitive spectrophotometric techniques were developed and validated for a quantitative estimation of roxithromycin (ROX) in bulk and pharmaceuticals. In alkaline medium, procedure (A) was performed by ion-pairs formation between the studied macrolide and 1,2-naphthoquinone-4-sulphonate to formed orange - products with maxima absorption at 454 nm. While the second method (B) was carried out for coupling the amino group of the cited macrolide with phylloquinone using the charge transfer reaction to form coloured products with λ_{max} at 457 nm.
The methods presented good linearity in the range of concentrations between 1.0 - 28.8 µg/mL. Regression coefficients were 0.9997 and 0.9998 for methods (A) and (B), respectively, as well the detection limits were cited in 0.26-0.23 µg/mL. Validation measurements were rebuts, accurate and precise within %RSD values lower than 3.5%. These methods were successfully used to investigate roxithromycin quantitatively in tablets. Recoveries were 98.94–101.01% and no interferences were detected from excipients. Both procedures are rapid, simple and cost-effective and it can adopted quality control application of this antibiotic.

Keywords: roxithromycin, spectrophotometric determination, charge-transfer reactions, 1,2-naphthoquinone-4-sulphonate, phylloquinone, pharmaceutical formulations

INTRODUCTION

Roxithromycin, which called also Erythromycin 9-[O-(2-Methoxyethoxy) methyloxime] (Figure 1), is known as semi-synthetic 14-membered-ring of macrolide with a large antibacterial spectrum **(Young *et al.*, 1989)**. ROX affects bacterial protein synthesis, which is RNA-dependent, leading to bateriostatic inhibition of pathogens **(Parfitt, 2002)**. *In vivo*, ROX can treat many diseases like illness of respiratory tract as well the infections of soft tissueš which is due to some sensitive strains **(Donald 2002; Williams and Sefton, 1993)**. It can also replace penicillin and cephalosporin antibiotics, such as in the case of allergies **(Laurence *et al.*, 2018)**. In acidic conditions, ROX is stable better than erythromycin and consequently shows good pharmacokinetic parameters **(Omura and Tanaka, 1984)**. One of the advantages of this antibiotic in clinical applications is that it can be used in low doses **(Kirst and Sides, 1989; Markham and Faulds, 1994)**.

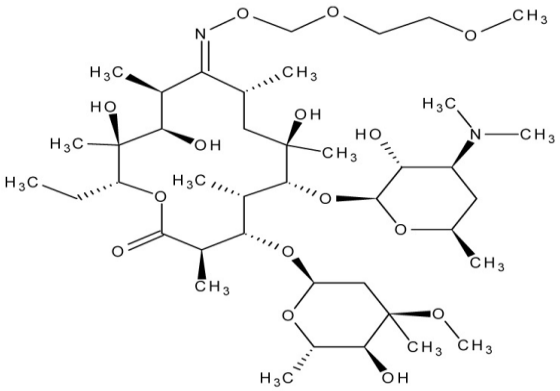

Figure 1 Chemical structure of ROX

Macrolides have been conventionally investigated in biological samples by bioassays **(Mahmoudi *et al.*, 2015; Grove and Randall 1955; Mahmoudi *et al.*, 2017; Horwitz 2000; Mahmoudi *et al.*, 2020)**. But compared to the current physico-chemical procedures, these tools cannot show good sensitive and/or selective, and sometimes they are laborious. In view of literature, ROX determination is basically performed using HPLC methods through electrochemical **(Pappa-Louisi *et al.*, 2001)**, spectrophotometric **(Macek *et al.*, 1999; Chepkwony *et al.*, 2001)**, fluorescence **(Sastre and Guchelaar 1998)** and mass spectrometric detection **(Yang and Carlson 2004; Miao and Metcalfe, 2003)**. European Pharmacopoeia (version 11.0) present LC-UV for ROX quantification with some recommendations like octadecylsilyl silica gel stationary phase and gradient elution **(European Pharmacopoeia, 2023)**. Other techniques have been also reported, such as thinlayer chromatography **(Tosti *et al.*, 2005)**, near infrared reflectance spectroscopy **(Feng *et al.*, 2006)**, flow injection chemiluminescence **(Song *et al.*, 2006)**, capillary zone electrophoresis **(Li *et al.*, 2002; *Wang et al., 2008).***
It was very noticeable that most of these reported methods covered ROX estimation in biological samples using expensive apparatuses which are usually unavailable in most of the pharmaceutical analysis laboratories. Hence, the use of such techniques for pharmaceutical applications of ROX is limited.
As spectrophotometry is usually available in laboratories and very easy to use for quality control assay, it becomes the most commonly recommended technique for the drugs analysis **(Görög 1994; Mahmoudi and Van Schepdael, 2024)**. Since macrolides have poor absorbance in UV, some spectrophotometric and fluorimetric methods were required aprior derivatization steps **(Sastry *et al.*, 1996; Khashaba, 2002)**. Consequently, spectrophotometric assay look like the best technique suitable to monitoring antibiotics and appears as the most advantageous. Thus, this context constrains to develop in an indispensable way a novel substitute spectrophotometric procedure for ROX estimation in its dosage forms.
Reaction of charge-transfer is extensively used to in develop spectrophotometric techniques in the aim of quality control of drugs **(Darwish *et al.*, 2013)**. Only some spectrophotometric procedures based on such reactions were described for determination of macrolides **(Ashour and Bayram, 2012; Mahmoudi and Van Schepdael, 2024)**. These methods usually used some automated instruments and therefore their applications are limited in many laboratories.
Moreover, these methods need to high analysis cost since it used considerable amount of solvents as organic modifier, which have toxic effects such as neurotoxic symptoms as well spontaneous abortion which affects some pregnants **(Ashour and Bayram, 2012)**, and of lymphohaemalopoietic cancer (leukemia and lymphomas) in both men and women **(Kristensen *et al.*, 2008)**.
Reducing human contact with toxic solvents is a major goal of analyst researchers, government agencies, the Organization of World Health, agencies of environmental protection and others. Nevertheless, research is being conducted to analyze new substitute methods to decrease the using up such solvents, which will be advantageous to ROX analysis.
Our actually work reports the development and validation of new spectrophotometric methods for quantitative estimation of ROX in tablets, due to their largest accessibility in common laboratories as well as its adequate sensitivity.

In this work, the assay was carried out using charge-transfer reactions between ROX amino groups and naphthoquinone agents. The approach presented here has two main advantages: (1) it allows many samples to be processed in a moderately short time and reduces the cost of analysis, (2) decrease in analysts' exposure to the harmful effects of toxic solvents.

MATERIAL AND METHODS

Apparatus

Spectrophotometer type of Lambda 365 UV-Visible (Perkin Elmer, USA) with quartz cells (l = 1 cm) have been employed for recording the absorption spectra. Balance model of Toledo (Woluwe) and Vortex mixer (Genesis) have been also used. In-house, Milli-Q system (Burgwedel, Germany) have been employed to produce pure water and then filtered via membranes of FH with 0.45 µm pore size.

Chemicals and pharmaceutical formulations

Roxithromycin standard, phylloquinone reagent (2-methyl-3-[(E)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione2-methyl-3-[(E)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione) and 1,2-naphthoquinone-4-sulphonate were purchased from Sigma-Aldrich (Steinheim, Germany). Roxid® tablets (Pharmalliance, Algeria) and Roxithromycine HUP® tablets (HUPP Pharma, Algeria) have been commercially sourced from the local market, these products were claimed to contain 150mg of the ROX standard.
Sodium hydroxide and salt sodium, both of analytical-reagent type, were obtained from Sigma–Aldrich (Steinheim, Germany). Additionally, isopropanol, acetone, ethanol and methanol were from the same source.

Preparation of standard solutions

A solution of stock for ROX standard was produced by measuring an appropriate quantity then dissolving it in methanol at a concentration of 100 µg/mL, and it was stored at 4°C. Daily solutions of ROX have been prepared using stock-solution by pouring suitable amount of methanol (MeOH) to achieve the desired concentrations within the range of linearity.

Preparation of tablet sample solutions

Preparation of tablet sample was made according to previously published protocols **(Mahmoudi, 2018; Mahmoudi *et al.*, 2018; Mahmoudi *et al.*, 2016)**. A number of tablets (10 tablets) have been finely ground into a powder. A powder portion weighing 150 mg was placed into 100-mL flasks with 20 mL of MeOH, and then mixture was thoroughly shaken for 20 minutes. Next, the solution was brought to volume using the same solvent, well shaken and passed through a membrane filter. A specific filtrate amounts were diluted using methanol to achieve the appropriate working concentrations within the range linearity (1.0-28.8 µg/mL).

Solutions of naphthoquinone

The Solution of NQ (0.2% w/v) was prepared as: a weigh of 0.2 g of NQ have been dissolved in pure water (NQS) or 75% ethanol (NQ1), then it poured into volumetric flask (100 mL) and diluted with the same solvent transfer to a 100 mL volumetric flask, dilute with the same solvent until to desired volume and shaken well. This solution should be newly prepared and kept away from the light.

General analytical procedure

A 1.0 mL solution of ROX (24 µg/mL) have been placed into volumetric flask of 10 mL with certain volume of NaOH solution (0.2M). Next, NQ solution of 0.2% (w/v) have been used followed by a volume of pure water until to the mark and then, let the reaction to carried on at ordinary temperature (25 °C) for 15-17 minutes. Final solutions absorbances have been recorded at 454 nm for ROX–NQS and at 457 nm for ROX–NQ1, using a reagent blank for reference.

Determination of molar ratio

Continuous variation approach of Job has been used **(Sawyer *et al.*, 1984)**, and solutions of ROX and each naphthoquinone at 2.5×10^{-3} M have been prepared, equimolary. Portions of ROX solutions with and each NQ have been composed with various matching relationships (0:10, 1:9, . . ., 9:1, 10:0). Derivatization reaction has been carried out at ordinary temperature, after that absorbance of the resulting colors has been measured and next plotted against the mole fraction of macrolide.

Method validation

These novel methods were validated in accordance with the guidelines set by the International Council on Harmonization and the United States Pharmacopeia **(ICH, 2005; USP, 2014)**. The parameters listed next were assessed: selectivities, linearities, limits of detection and quantification, precision, accuracy and robustness.

Methods comparison

The findings from this work were contrasted with reported bioassay and HPLC procedures **(Mahmoudi *et al.*, 2015; Chepkwony *et al.*, 2001)**. Statistical evaluation of the obtained results was performed based on Student's t test to determine whether there is a noteworthy difference between the procedures at a 5.0% significance level.

RESULTS AND DISCUSSION

Absorption spectra

At temperature of 25 °C, derivatization reaction of ROX with NQ has been permitted to occur, following by the plot of absorption spectra individually. When ROX and NQ mixture were combined, orange colored chromogen were formed and showing absorption maximum at 454 nm for ROX-NQS and 457 nm for ROX-NQ1, respectively. The observed band can attributed to a creation of ROX-NQ$^{\cdot}$ anion **(Darwish *et al.*, 2013; Mahmoudi and Van Schepdael, 2024)**, this is maybe created through the dissociation of initial donor-acceptor complex. The high ionization power contributed to polar solvent facilitated the dissociation of this complex, resulting in peaks observed in the absorption spectra of the formed product of ROX-NQ.

Charge-transfer reaction optimization

Charge-transfer reaction of ROX with NQ agents was optimized and the experimental conditions investigated and explored by systematically varying each variable while maintaining the others at a fixed level. To attain the best sensitivity, several factors were assessed, including the type of solvent, concentration of the alkaline media, and quantity of the NQ reagent, temperature and reaction time.

Solvent effect

Solvent is crucial part in derivatization reaction as it can effectively facilitate complete charge transfer and subsequently enable the dissociation and stabilization of resulting radical anion, and acts as molecule absorbing **(Ashour and Bayram, 2012; Mahmoudi and Van Schepdael, 2024)**.
Taking into account that the greater value of dielectric constant of solvent is favorable, a range of solvents including methanol, water, isopropanol, acetone and ethanol were investigated to evaluate mixture dissociation, complex construction, and to optimize sensitivity and complex stability.

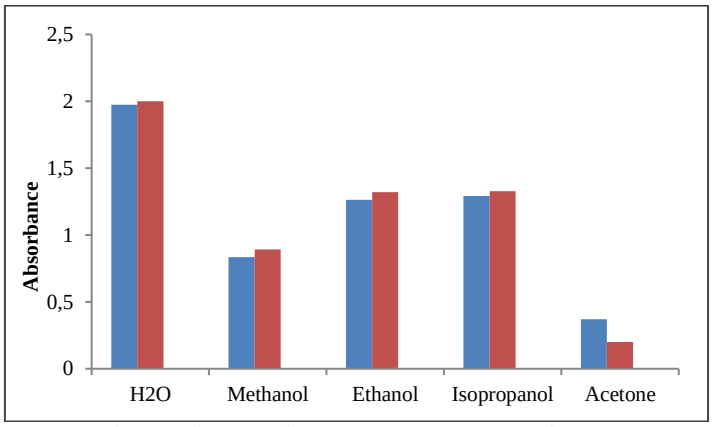

Figure 2 Influence of nature of solvent on the production of ROX–NQ, NQS (Bleu) and NQ1 (Red); NaOH 0.2 M: 1 mL; temperature: 25 °C; analysis time: 20 min

To evaluate the influence of nature of solvent, the derivatization process of ROX with NQ was proceed using the selected solvents. The highest sensitivity of the colored products compared to the reagent blank was observed at wavelengths of 454 and 457 nm. The results of this study (Figure 2) demonstrated that the water was found to be most suitable solvents and, the highest absorbance has been obtained when it was utilized, likely due to water's capacity to create stable hydrogen bond around some radical anion, along with the excellent solubility of NQ in water. Therefore, it has been used in next experiments to achieve maximum sensitivity for these two procedures. Else, it is important to note that the colorless reagent blank in this medium shows minimal absorbance at both wavelengths of 454 and 457 nm.

Influence of NQ reagent amount

In general, derivatization reaction depends on the quantity of derivative agent present in the reaction mixture and the associated equilibrium **(Darwish *et al.*, 2013; Ashour and Bayram, 2012)**, so the impact of NQ quantity on the reaction under investigation was analyzed. To determine the optimal amount of the NQ agent, different volumes of 0.2% (w/v) NQ, ranging from 0.25 to 2.5 mL were added to a ROX solution maintained at a constant concentration of 24 µg/mL. The obtained values are regrouped in Figure 3.
The results showed that the reaction was influenced by the reagent, and that increasing the quantity of NQ resulted in greater colored complex, reaching a maximum point. Consequently, color intensity and the absorbance were found to be maximum value for ROX by using 1.5 mL for both reagent NQS and NQ1. Therefore, we have chosen this value because of: (1) it provides sufficient NQ concentration to react with ROX, and (2) this level of NQ concentration ensures highly accurate readings. Beyond this concentration, the absorbance stay almost stable, and increasing the NQ volume further did not improve the detection sensitivity.

Effect of NaOH volumes

Alkaline medium was necessary for derivatization reaction of macrolides, while the obtained values exposed that ROX cannot react easily with NQ in acidic medium due to the because of the less density of electrons around the group of amine **(Ashour and Bayram, 2012; Mahmoudi and Van Schepdael, 2024)**. In alkaline medium, the complex absorbance is nearly zero, suggesting that ROX does not interact with NQ. It could be attributed to density of electrons of ROX, which is lower around amino group compared to alkaline conditions. To select the best environment, a reaction solution of ROX with NQ was prepared at different volumes of NaOH (0.2M) varying between 0.25 and 2.5 mL and then the reaction was proceeding.
The influence of different volumes of NaOH added to select the most suitable the concerned complexes is investigated by measuring the absorbance of reaction solutions in the visible region against a blank solution with 454 nm for ROX–NQS and 457 nm for ROX–NQ1 (Figure 4). Results showed that the optimum NaOH value which gave the best absorbance for all complexes is 1.0 mL by using the tow reagents NQS and NQ1, which means that the degree of derivatization was maximal. Consequently, this value was selected as optimal condition.

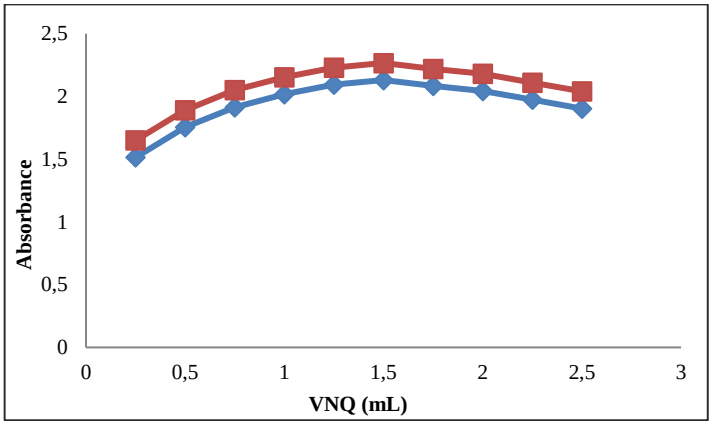

Figure 3 Influence of volume of 0.2% w/v NQ on the production of ROX–NQ, NQS (Bleu) and NQ1 (Red); NaOH 0.2 M: 1 mL; temperature: 25 °C; analysis time: 20 min

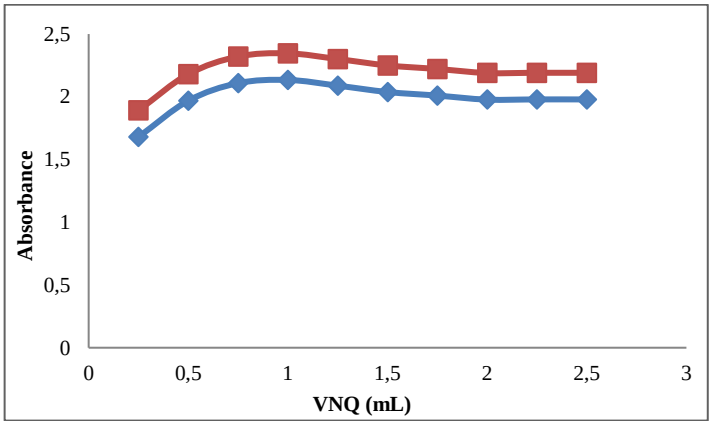

Figure 4 Influence of volume of NaOH on the production of ROX–NQ, NQS (Bleu) and NQ1 (Red); 0.2% NQ: 1 mL; temperature: 25 °C; analysis time: 20 min

Effect of time and temperature

Solutions of macrolide and the derivatization reagent were prepared and combined, allowing this reaction to carried out for different intervals of time ranging from 0 to 60 minutes at a temperature of 25 ± 1°C. The best time of reaction has been established by measuring the detection intensity of the formed products. By monitoring the color development, it was found that the optimum reaction time was 16 min and 17 min for ROX–NQS and ROX–NQ1, respectively. Complete color development was attained at these values and the complexes under consideration were formed instantaneously.
The temperature influence on ROX reaction with NQ was examined at various levels (25–80 °C), and the measured absorbance of the resulting complexes was analyzed at 454 nm for ROX–NQS and 457 nm for ROX–NQ1, respectively. Following an examination of temperature's impact, it was concluded that elevated temperatures (40−80°C) did not significantly influence the reaction. Consequently, all experiments were performed at ordinary temperature. The color produced continued to be stable at this for a period around 60 minutes. Therefore, a temperature of 25°C has been determined to be the perfect value for achieving highest color intensity.

Reaction mechanism and molar ratio

To determinate the molar ratio and stability constants of ROX and NQ a complex, continuous variation approach of Job was used **(Sawyer *et al.*, 1984; Rose, 1964)**. Different ratios of solutions of macrolide with derivative agent (equimolary) have been combined, and the absorbance of each mixture was measured under ideal conditions. As regrouped in Table 1, stability constant was expressed as log K, and the results were 3.91 ± 0.05 for ROX–NQS and 5.23 ± 0.02 for ROX –NQ1, respectively showing high stability of the complexes.

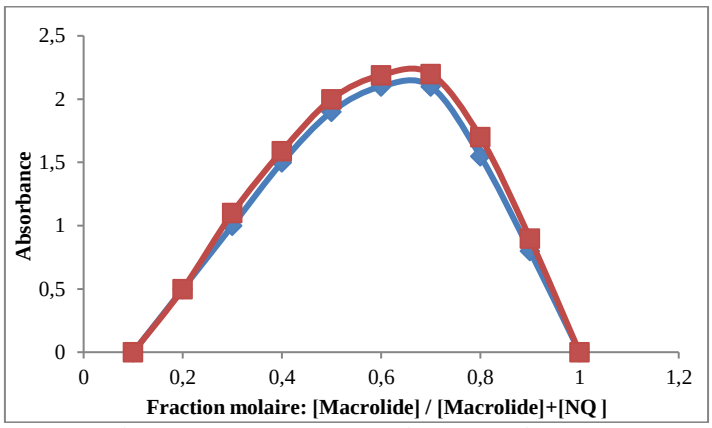

Figure 5 Job's application to charge-transfer reaction for roxithromycin – naphthoquinones, NQS (Bleu) and NQ1 (Red)

The findings as shown in Job's plot (Figure 5) suggested that the complexes can be produced at the ratio 1:2 of ROX: NQ. Formation mechanism of these complexes using macrolides has been discussed by Ashour and Bayram **(Ashour and Bayram, 2012; Mahmoudi and Van Schepdael, 2024)**. This pointed out that one mole of ROX interacted with two moles of each studied NQ. Given the existence of two atoms of nitrogen in the chemical structure of ROX (one in the dimethylamine group of sugar moieties and the other in the imine group), it was proposed that the reaction occurs as illustrated in Figure 6. A free electron from one atom of the nitrogen has been moved to the charge center of naphthoquinone.

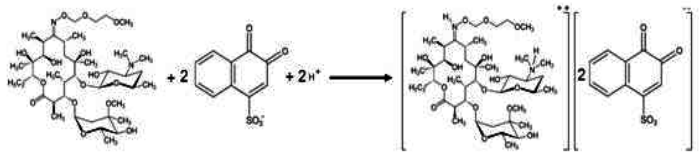

Figure 6 Proposed reaction mechanism pathway for the interaction between ROX and NQS.

Method validation

The validation of spectrophotometric techniques was estimated based on the followings: linearity, limit of detection (LOD), limit of quantification (LOQ), sensitivity, specificity, precision, and accuracy, using the optimal conditions.

Linearity and sensitivity

Using the optimized parameters, good linear curves were obtained for ROX solutions in the selected range of 1.0 - 28.8 µg/mL. The r values (r^2 = 0.9997 and

0.9998) were determined to be valid and noteworthy ($r^2 > 0.999$) by confidence intervals at 95%. The analysis data are shown in Table 1.
Limit of detection and limit of quantification have been evaluated at particular signal-to-noise ratios (S/N, 3:1 for LOQ and 10:1for LOD). The LOD values were 0.26-0.23 µg/mL and the LOQ values were 0.78-0.72 µg/mL for ROX–NQS and ROX–NQ1, respectively. These findings demonstrate that these techniques possess adequate sensitivity for quantifying drug substances at low concentrations (Table 1).

Table 1 Enhanced features of the suggested methods and data of regression for ROX

Factors	Measurements	
	NQS	NQ1
Color	Orange	Orange
λ_{max} (nm)	454	457
Formation time (min)	16	17
Logarithmic formation constants ($logK_f$)	3.91	5.23
Range of beers law (µg/mL)	1.0-28.8	1.0-28.8
Equation of regression (y)	y=0.0791x+0.2692	y=0.0874x+0.1079
Correlation coefficient(r^2)	0.9997	0.9998
Limit of detection (µg/ml)	0.26	0.23
Limit of quantification (µg/ml)	0.78	0.72
Molar absorptivity (L $mole^{-1}cm^{-1}$)	12,04	11,13
Sandell's sensitivity (µg cm^{-2})	0.0090	0.0081
Stability of colored species (h)	24	24
Mean% recovery ± S.D.	100.89	99.24

Precision and accuracy

Spectrophotometric assay precision was represented as relative standard deviation (RSD) and tested as repeatability (intra- day) and intermediate (inter-day) precisions **(Mahmoudi and Boukhechem, 2020; Mahmoudi, 2015)**. The obtained values of the precision measurements are presented in Table 2.
For repeatability, the RSD values of ROX at 24.0 mg/mL were 1.11 and 1.19% for both methods, respectively. The between days precision was performed at three concentration levels (80, 100, 120 %) and the results of the calculated % RSD remained below 3.36%.

Table 2 Assessment of intra-day and inter-day precisions and accuracy of ROX using the suggested methods.

NQ	Taken µg/mL	Intra-day			Inter-day(n=3)		
		Found [a] µg/mL	%RSD	%RE	Found [a] µg/mL	%RSD	%RE
NQS	28.8	29.0839	1.2637	0.9857	29.9039	1.9769	3.8329
	24.0	24.3510	08974	1.4623	24.4722	0.9985	1.9675
	19.2	19.3707	1.1578	0.8890	19.6486	3.3586	2.3364
NQ1	28.8	29.2816	0.9389	1.7204	29.3754	0.8914	2.0652
	24.0	24.2044	1.1241	0.8523	24.9317	3.0854	3.8541
	19.2	19.4178	1.4952	1.07522	19.5861	1.4987	1.8311

[a] Mean value of three tests
RE: relative error; RSD: relative standard deviation

Recovery study and relative error (RE) were the most used approaches to test accuracy **(Mahmoudi *et al.*, 2020).** It was investigated at three concentration levels (80-120%) for intra- and inter-day assays and the RE% was calculated (Table 2). RE% values of 0.85-3.85% for both methods were obtained. These results were well within the specified limit, confirming the accuracy of the methods. It can be concluded that this levels of precision and accuracy are appropriate to use in routine assay of ROX.

Robustness, selectivity and stability

The robustness was determined by assessing the impact of small yet important modifications in specific parameters that influence selectivity or quantitative outcomes **(Mahmoudi *et al.*, 2023; Mahmoudi, 2015)**. It was examined by evaluating the impact of NQ quantity, alkaline environment, and reaction duration on the method suitability and sensitivity of both methods. The averages of recoveries were 99.74 and 100.26 %, and the %RSD values were 0.97 and 1.10% for ROX–NQS and ROX–NQ1, respectively (Table 3). Therefore, it has been observed that the assay of ROX was robust under these conditions.

Table 3 Study of the robustness of ROX using spectrophotometric methods.

Recommended parameter	NQ	Factors	%Recoveries (%)	RSD (%)
t (min)	NQS	14	99.12	1.41
		18	101.48	1.05
	NQ1	15	101.01	0.94
		19	99.31	1.12
NQ (mL)	NQS	1.65	99.15	0.84
		1.85	100.84	0.98
	NQ1	1.65	101.21	1.33
		1.85	99.77	0.89
NaOH (mL)	NQS	0.90	98.55	1.14
		1.10	99.30	0.39
	NQ1	0.90	99.29	1.03
		1.10	100.97	1.26

To study the selectivity, placebo of Roxid® was used instead of ROX standard under the optimal conditions for both NQ derivatization an absorbance was measured at 454 and 457 nm. All the absorbances were near to zero (about 0.0001) and no significant effect on the responses was observed. It can be noted that none of the excipients significantly affected the relative detection intensity, confirming the method's specificity. The stability of ROX under operational conditions was checked and the finding showed that the antibiotic quantity was the same during the storage period.

Pharmaceutical application

Roxithromycine HUP® and Roxid® tablets are the commercial forms of ROX commonly available. They were analyzed by the developed and reported methods **(Chepkwony *et al.*, 2001)** and the achieved values were subsequently subjected to statistical comparison. results were then statistically compared. The averages of recovery of both ROX tablets were 99.89 % and 100.03 % for the new methodology of NQS and NQ1 reagents, respectively (Table 4). These averages of ROX in the tested tables were in good accordance with the labeled amounts and HPLC determinations (100.75%). According to statistical estimated of *t*- tests, no notable differences have been observed regarding to the calculated and theoretical results ($t_{exp}<t_{theo}$) for the tow techniques (developed and reported) at 95% confidence level.
The finding point out that the present methods were selective for ROX without interference from common excipients and demonstrated similar accuracy and precision to the reported method.

Table 4 Utilization of the suggested procedures for the analysis of ROX in pharmaceutical formulations.

Drug	Factor	Devlopped technique		Reported HPLC
		NQS	NQ1	
Roxithromycine HUP® tablets	%Recovery [a]	101.87	100.81	100.35
	%RSD	1.76	1.68	1.77
	t-value [b]	1.68	1.49	-
Roxid® tablets	%Recovery [a]	97.91	99.25	101.15
	%RSD	1.79	1.72	2.08
	t-value [b]	1.56	1.73	-

Comparison of methods

In this part, the analytical characteristics of the developed methods using NQs were compared with others previously published methods (HPLC and microbiological assays) for the analysis of ROX. Roxid® dosage form has been analyzed using these considered methods, while its recovery percentages were calculated and summarized in Table 5. The percentage contents were found to be 100.13, 99.65, 100.15 and 99.38% by the spectrophotometrical (NQS and NQ1), HPLC and

bioassay methods, respectively. The obtained values fell within the acceptable-range from 95 to 105%, demonstrating strong correlations linking the procedures examined.

Table 5 ROX determination in Roxid® dosage form using various methods.

Trials	% Recovery			
	UV		HPLC	bioassay
	NQS	NQ1		
1	99.19	100.14	98.99	97.08
2	100.85	98.94	100.54	98.71
3	99.38	99.56	99.57	101.86
4	101.01	100.81	100.63	99.08
5	100.24	98.83	101.02	100.18
Average of determinations	100.13	99.65	100.15	99.38

The proposed procedures offer comparable or superior linearities, outstanding recoveries, and agreeable run times compared to most existing methods. While some liquid chromatography (LC) techniques demonstrate greater sensitivity than the alternatives, they tend to be more expensive, complex, and needed additional expert analysts **(Mahmoudi *et al.*, 2015; Mahmoudi *et al.*, 2020)**. Moreover, LC consumed organic solvents higher in UV methods. However, bioassay has certain changeability as well it is appropriate for applications on drug dynamics **(Mahmoudi *et al.*, 2020; Baird and Hodges, 2000)**. However, this part of work established the prospect to correlate the current spectrophotometric finding with those derived from microbiological techniques. For drug security and best therapeutic efficacy, it is essential to integrate different routine techniques of drug analysis **(Mahmoudi *et al.*, 2020).**

These novel methods offer comparable recoveries, a broader range of linearity, and are more cost-effective and straightforward than the previously reported techniques. It can be seen that these methods were a good suitable alternative methods to analysis ROX antibiotic in its regular applications. Therefore, these procedures were developed is an innovative, easy, rapid, sensitive, reproducible, environment-friendly, and cost-effective approach for the detection of ROX in its dosage forms, being an alternative technique for routine quantitative determination.

CONCLUSION

This work reported outlines the development and validation of innovative techniques for the spectrophotometric investigation of ROX based on charge transfer reactions with NQ reagents, 1,2-naphthoquinone-4-sulphonate and phylloquinone using alkaline media and room temperature. These methods be subjected to fully validation in accordance with ICH Quality guidelines, and the results demonstrated that they were simple, accurate, specific, sensitive, and robust, exhibiting good linearity across a range of concentrations of 1.0 - 28.8 µg/mL, and they were devoid of influence from typical excipients and additives. The application of the developed techniques for the analysis of ROX in tablets has successfully demonstrated and the wide-ranging applicability of such new methodologies is confirmed by the satisfactory recoveries, which can prove the good agreement with the label claim. The procedures don't require any difficult sample preparation or crucial reaction conditions, and chemicals used in this approach are less expensive as well as more easily accessible. So these developed techniques can be utilized for can be used for the pharmaceutical analysis of the cited drug at ordinary laboratories.

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