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**NOVEL INDIRECT SPECTROPHOTOMETRIC APPROACHES FOR SPIRAMYCIN QUANTIFICATION IN DOSAGE FORMS USING NAPHTHOQUINONE DERIVATIVE REAGENTS**

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## NOVEL INDIRECT SPECTROPHOTOMETRIC APPROACHES FOR SPIRAMYCIN QUANTIFICATION IN DOSAGE FORMS USING NAPHTHOQUINONE DERIVATIVE REAGENTS

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**Abstract.** The development and validation of new spectrophotometric methods for the determination of spiramycin in pure and dosage forms have been described. Indirect analysis was performed *via* charge transfer reaction between spiramycin and naphthoquinone derivatives 1,2-naphthoquinone-4-sulphonate and phylloquinone in alkaline medium, resulting in formation of coloured complexes with maximum absorption at 453 and 456 nm, respectively. The reaction conditions, including solvent, reagent concentration, and reaction time, were optimized. Linear calibration graphs were plotted for the NQ-drug derivatives over the concentration range of 0.85–18.0 µg/mL and showed correlation coefficients of 0.9998, with a detection limit of 0.25 µg/mL and quantification limits about 0.75 µg/mL. The molar absorptivity and Sandell's sensitivity were 7.42–9.14×10<sup>4</sup> L×mole<sup>-1</sup>cm<sup>-1</sup> and 0.0092–0.0095 µg/cm<sup>2</sup>, respectively. Both methods demonstrated good precision, accuracy and robustness with % RSD below 3.1%. The methods were successfully applied for spiramycin quantification in tablet dosage forms. The suggested approaches are simple, effective, and sufficiently reliable to be employed as alternative quality-control methods.

**Keywords:** spectrophotometry, 1,2-naphthoquinone-4-sulphonate, phylloquinone, charge-transfer complex, validation.

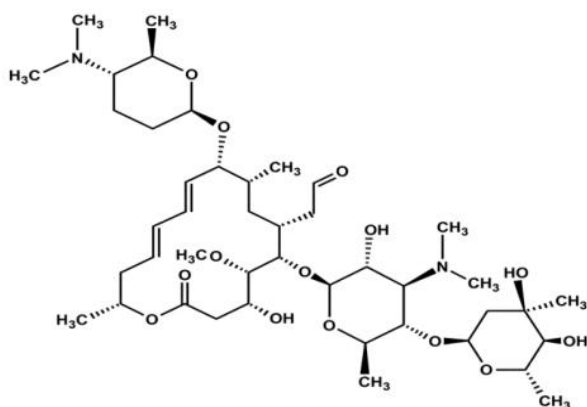
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### Introduction

Macrolide antibiotics, composed of a 12-, 14-, or 16-membered macrocyclic lactone ring, amino groups, and deoxy sugars, are derived from various *Streptomyces* species and/or obtained by semisynthetic processes. The 16-membered macrolides constitute a significant class of antibiotics, but have been overshadowed by their more famous 14-membered counterparts. They are mainly used in veterinary medicine and only a few of them have been introduced into clinical practice. The naturally occurring 16-membered macrolides currently used clinically include josamycin, spiramycin, tylosin, and midecamycin. These macrolide antibiotics are used for the treatment of infections caused by both Gram-positive and Gram-negative bacteria [1,2].

Spiramycin (SPI) is a 16-membered macrolide produced by fermentation of *Streptomyces ambofaciens*. The main components are spiramycins I, II and III, with spiramycin I being the major component (Figure 1).

Their chemical structures include a forosamine sugar at the C-9 position of the macrolactone ring and a disaccharide composed of mycaminose and mycarose sugars at the C-5 position [3]. Spiramycin, an antibiotic widely used for more than 30 years, is the treatment of choice for acquired toxoplasmosis during pregnancy [4]. Adverse effects associated with spiramycin include gastrointestinal disorders, allergic reactions and occasionally transient diffuse paresthesiae [1,5].



**Figure 1. Spiramycin chemical structure.**

Literature survey reveals that several methods for determination of spiramycin and other macrolides in bulk drugs, pharmaceutical formulations and biological fluids have been developed using physico-chemical techniques, including UV-Visible spectrophotometry [6], fluorescence spectroscopy [7], ratio spectroscopy [8], FTIR spectroscopy [9], and thin-layer chromatography [10]. In addition, a considerable numbers of methods have been reported using LC coupled with various detection techniques, like UV-Visible spectroscopy [11-13], electrochemical detection (ECD) [14] and mass spectrometry (MS) [15-18]. Microbiological assays can be used for screening purposes [19]; however, they often do not adequately discriminate among antibiotics. Nevertheless, most of the above methods suffer from several disadvantages, such as poor sensitivity, the requirement for expensive solvents and chemicals, tedious extraction procedures, measurements at shorter wavelengths, heating or cooling steps, complicated experimental setups. UV-Visible spectrophotometry, owing to its simplicity, cost-effectiveness, sensitivity, selectivity, and availability in most laboratories, remains competitive with chromatographic techniques.

In addition, indirect spectrophotometric methods have been reported for the analysis of macrolides using numerous chromophores agents, such as quinalizarin, 7,7,8,8-tetracyanoquinodimethane, alizarin, bromocresol green, eosin Y, rose Bengal, bromophenol blue, purpurin, and 2,4-dinitrophenyl hydrazine [20], 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole [21], 1,2-naphthoquinone-4-sulphonate (NQS) [21-25] and the 3-methylbenzothiazolin-2-one hydrazone/ferric chloride system [26]. Nonetheless, the use of naphthoquinone reagents for the spectrophotometric determination of spiramycin has not yet been reported.

Hence, the current study was undertaken to address this gap. Specifically, a novel methodology was applied to spiramycin, in which the incorporation of phylloquinone enhances sensitivity and improves analytical performance while offering a simpler protocol than existing methods.

In the present work, new accurate and sensitive spectrophotometric methods to assay of spiramycin in pure and dosage forms were developed and validated. The proposed techniques involved the formation of charge transfer complexes between SPI and naphthoquinone chromogenic agents 1,2-naphthoquinone-4-sulphonate and phylloquinone (NQ1). The methods are environmentally friendly, technically simple, time-saving and economical, while providing performance comparable to that of the reported bioassay and RP-HPLC methods.

## Experimental

### Materials and reagents

The reference standard of spiramycin (SPI) was sourced from Sigma-Aldrich (Steinheim, Germany), and SPI tablets were commercially purchased. Rovadal® tablets (Saida, Algeria) and Rovamycine® tablets (Saida, Algeria) were labeled to contain SPI as 1.5 MUI/tablet (450 mg). The 2-methyl-3-[(E)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione (phylloquinone) (NQ1) and 1,2-naphthoquinone-4-sulphonate (NQS) were obtained from the same source, as well as methanol, ethanol, isopropanol and acetone.

### Apparatus

A double-beam UV-Vis spectrophotometer, Lambda 365 model (PerkinElmer, USA), equipped with quartz cuvettes ( $l = 1$  cm) was used for absorbance measurements. A vortex mixer (Genesis) and an analytical balance (Toledo, Woluwe) were also used.

### Preparation of stock standard solutions

Accurately weighed amounts of standard SPI were dissolved in methanol to obtain a concentration of 25 µg/mL and stored in the dark as a stock solution. This stock solution was further diluted with methanol to obtain working solutions in the concentration ranges of 0.85–18.0 µg/mL.

### Tablets sample preparation

Tablets sample preparation followed a previously established protocol [27,28]. Initially, ten tablets were accurately weighed and ground into a fine powder. An amount of powder equivalent to the weight of one tablet was transferred into a 100 mL volumetric flask and

sonicated for 20 minutes with approximately 10 mL of methanol. The flask was then filled to the mark with the same solvent. After thorough mixing, the solution was filtered. An appropriate volume of the filtrate was then quantitatively diluted with methanol to obtain the desired working concentrations for the sample solutions.

#### **Preparation of naphthoquinones solutions**

To prepare 0.2% w/v naphthoquinone (NQ) solution, an accurately weighed amount of 0.2 g of NQS was dissolved in double distilled water (for NQS) or in ethanol 70% (for NQ1), transferred into a 100 mL volumetric flask, filled to the mark with the same solvent and mixed thoroughly to obtain a 0.2% w/v solution.

#### **Naphthoquinones derivatization**

A precisely measured volume of 1.0 mL of SPI solution, at the concentration of 15 µg/mL, was carefully transferred into a 10 mL volumetric flask (Final volume). Subsequently, 1.25 mL of 0.2 M NaOH solution and 1.0 mL of 0.2% (w/v) NQ solution (NQS or NQ1) were added. The reaction mixture was then brought to volume with distilled water. The preparations were allowed to stand at room temperature (25°C) for a predetermined period, after which absorbance measurements were recorded at wavelengths of 453 nm and 456 nm for SPI-NQS and SPI-NQ1, respectively, using a reagent blank consisting of water for NQS and 70% ethanol for NQ1. The naphthoquinone products were stable for 1.5 hours.

#### **Stoichiometric ratio**

In order to ascertain the stoichiometric ratio, Job's method of continuous variation [29] was employed, utilizing equimolar solutions of SPI and NQ at a concentration of  $2.08 \times 10^{-3}$  M. This methodology involved the preparation of a series of SPI and NQ solutions, as delineated in the general procedures, with different volume ratios (0:10, 1:9, ..., 9:1, 10:0). The absorbance of each solution was then plotted against the mole fraction of the drug.

#### **Validation of methods**

The proposed methods were validated according to the International Council for Harmonization (ICH) guidelines and standard references [30,32,34], assessing parameters such as linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ), robustness and relative recovery.

#### **Linearity, detection and quantification limits**

The absorbance values of the calibration solutions were recorded at the selected wavelengths, and regression analysis was performed using the least squares method.

The results included the slope, intercept, standard deviations of both parameters, and confidence intervals at a 95% confidence level at concentration ranges of 0.85–18.00 µg/mL. The calibration graphs were obtained by plotting the mean absorbance values against concentration. LOD and LOQ values were calculated using the following Eq.(1) and Eq.(2), respectively [30].

$$LOD = \frac{3.3 \times S}{b} \quad (1)$$

$$LOQ = \frac{10 \times S}{b} \quad (2)$$

where,  $S$  - the standard deviation of seven replicates ( $n=7$ ) for the absorbance of blanks;

$b$  - the slope of the calibration curve for each method. In addition, LOQ were estimated by successive dilution to the desired concentration level.

#### **Accuracy, precision and robustness**

The accuracy [31,32] was assessed through the recovery percentage of SPI solutions at three distinct concentration levels (80%, 100%, and 120%), analysed in triplicate ( $n=3$ ) on a single day (intra-day) as well as over three consecutive days (inter-day). Precision was estimated in a manner consistent with that of accuracy, with both precision and accuracy expressed as relative standard deviation (RSD) and relative error (RE), respectively. Furthermore, robustness was investigated to ascertain the impact of minor variations in the methodological conditions. The UV methods were scrutinized by altering key parameters, including the concentration of NQ, the alkaline medium, and the reaction time.

## **Results and discussion**

### **Methods development**

SPI in methanol usually showed an absorption peak at 232 nm, and, after derivatization with naphthoquinones (NQS and NQ1), orange-coloured complexes were formed. The overlain spectra of SPI-NQ recorded within the 200-800 nm wavelength range revealed that SPI-NQS exhibits absorbance maxima at 453 nm. Meanwhile, SPI-NQ1 absorbs at 456 nm and shows no overlapping absorbance peaks with potential excipients. These bands were probably formed by the dissociation of the original donor-acceptor complex [24]. Based on these observations both values were selected as the analytical wavelengths for the proposed methods.

### Optimization and derivatization studies

The optimization of the experimental conditions aimed at achieving maximum sensitivity and selectivity involved assessing various factors, including the nature of the solvent, the concentration of the NQ reagent, the concentration of the alkaline medium, as well as reaction time and temperature. This was done through evaluation of the reaction stoichiometry, calculation of the association constant and molar absorptivity in an aqueous medium, and verification of the proposed reaction mechanism.

#### Selection of Solvent and NQ volume

The nature of the solvent may facilitate complete charge transfer, enable complex dissociation, and stabilize the resulting radical anion, which is the absorbing species. In this study, the reaction was examined in various media, including water, methanol, ethanol, isopropanol, acetone, and acetonitrile. The literature suggests that solvents with high dielectric constants are generally more effective for this process [24].

Water and ethanol exhibit a higher dielectric constant; however, water provided the highest sensitivity. This is likely due to water's ability to form stable hydrogen bonds with the radical anion. Consequently, the high solubility of NQS in water and NQ1 in ethanol supported the selection of these solvents for subsequent experiments.

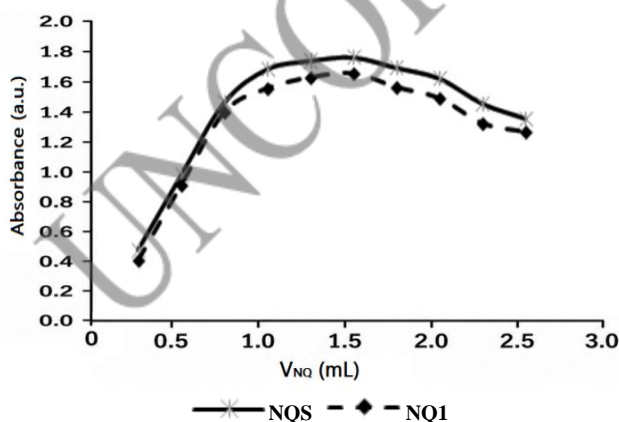
Thus, achieving the maximum conversion of the analyte into its absorbing species is governed by the molar excess of the reagent and the associated reaction equilibria. To optimize this parameter, the volume of the NQ reagent (NQS or NQ1) was evaluated over a range of 0.25–2.5 mL [0.2% (w/v)], while maintaining a constant SPI

concentration of 15  $\mu\text{g/mL}$  in an alkaline medium. As illustrated in Figure 2, the reaction yield was reagent-dependent, with the highest absorption achieved at 1.0 mL of 0.2% (w/v) NQ for both NQS and NQ1. This volume was established as the optimum for both Methods A and B, ensuring high analytical precision. Beyond this level, increasing the NQ volume had no beneficial effect on absorbance, which remained virtually constant, indicating that the reagent was no longer a limiting factor.

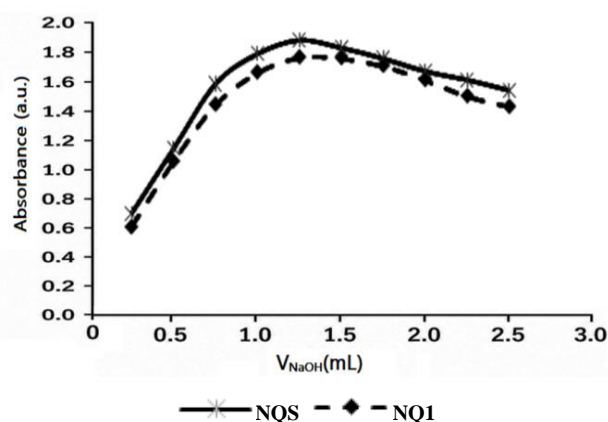
#### Alkalinity effects

The formation of an orange derivative, with maximum absorbance between 453 and 456 nm, *via* charge transfer reaction between SPI and NQ reagents (NQS/NQ1) is highly dependent on alkaline environment. Specifically, the reaction requires an alkaline medium to counteract the reduced electron density of the macrolide amino group under acidic conditions [24].

Screening of several alkaline agents (0.1–0.5 M), including borax, sodium bicarbonate, and disodium hydrogen phosphate, identified 0.2 M NaOH as the most effective medium. Other bases were discarded due to poor reproducibility, high background interference, or the formation of white colloidal precipitates upon dilution with organic solvents. As shown in Figure 3, optimization of the 0.2 M NaOH volume revealed that 1.25 mL provided the maximum absorbance and highest derivatization degree for both NQS and NQ1. The subsequent decline in absorbance at higher volumes suggests that an overabundance of hydroxide ions may inhibit the condensation process. Consequently, 1.25 mL (NQS/NQ1) was selected as the optimal volume.



**Figure 2.** Optimization of reagent volumes for SPI derivatization at 15  $\mu\text{g/mL}$  using NQS and NQ1 reagents. Conditions: 1.0 mL of 0.2 M NaOH, 20-minute at 25°C.



**Figure 3.** Optimization of alkalinity (NaOH volume) for the derivatization reaction of SPI (15  $\mu\text{g/mL}$ ) using NQS and NQ1 reagents. Conditions: 1.0 mL of NQ (0.2% w/v), 20-minute at 25°C.

### Optimal conditions for reaction temperature and time

To determine the optimal conditions for the reaction between SPI and NQ (NQS and NQ1) in an alkaline environment, the effects of temperature (25–75°C) and time (0–60 minutes) were investigated.

The study revealed that ambient laboratory temperature (25 ± 1°C) was ideal for achieving maximum sensitivity and stable orange-coloured complexes. Increasing the temperature beyond this range negatively affected the absorbance values, likely due to the instability of the resulting SPI-NQS and SPI-NQ1 derivatives. Consequently, 25 ± 1°C was selected as the optimal temperature for both methods.

The reaction progress was monitored at the respective absorption maxima at 453–456 nm for NQS and NQ1, respectively. The findings indicated that the reaction reached completion rapidly, with optimal colour development occurring after 17 minutes for NQS (Method A) and 19 minutes for NQ1 (Method B). Absorbance values increased steadily until the point-minute mark. Although a slight increase (up to 10%) was observed between 20 and 60 minutes, the initial completion times were selected to enhance method efficiency. Once formed under these optimal conditions, the coloured products remained stable for up to 1.5 hours.

### Stoichiometric ratio and reaction mechanism

The stoichiometric relationship between SPI and each NQ reagent was elucidated through Job's method of continuous variation [29]. Various equimolar solutions of the macrolide and the

reagent were carefully combined, and the absorbance of each resulting mixture was recorded. The Job's plots presented in Figure 4 reveal a drug-to-NQ ratio of 1:2. Notably, upon the introduction of NQ in quantities exceeding this ratio, a further increase in absorbance was observed. The  $\log(K_f)$  values were calculated to be 3.98 and 4.88 for Methods A and B, respectively, thereby confirming the considerable stability of the resulting complexes (Table 1).

In light of these findings, a reaction mechanism is proposed involving the transfer of two electrons one from each of the nitrogen atoms within a single spiramycin molecule (specifically from the dimethylamine groups) to the charge-deficient centre of two NQ molecules. In this instance, radical anions arise from the complete transfer of charge, as illustrated in the scheme presented in Figure 5.

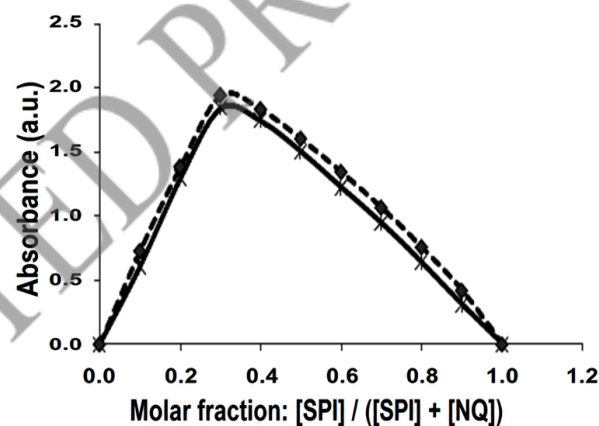


Figure 4. Stoichiometric determination of the SPI-NQ reaction via Job's method of continuous, NQS (—\*) and NQ1 (---♦---).

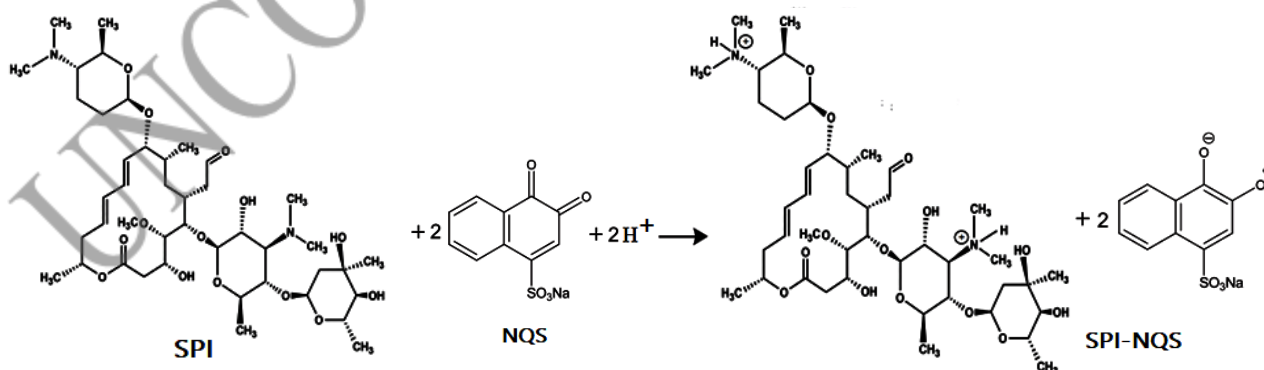


Figure 5. Possible mechanism of naphthoquinone-derivative obtained from the reaction of Spiramycin and NQS.

### Validation of methods

Validation of the proposed methods was conducted for parameters such as linearity, accuracy, precision, and robustness, in conformity with ICH guidelines and standard reference protocols [30,33].

#### Linearity, limits of detection (LOD) and quantification (LOQ)

The linearity of the proposed methods is evaluated by analysing standard solutions of SPI at different concentrations ranging from 0.85–18.0 µg/mL for SPI, in triplicates. The values of correlation coefficients are close to unity ( $R^2 > 0.9998$ ), indicating good linearity [30]. The regression equations are summarized in Table 1.

Sensitivity analysis for the SPI-NQS and SPI-NQ1 derivatives showed LOD values of 0.25–0.26 µg/mL and LOQ values of 0.75–0.78 µg/mL, reflecting the efficiency of the developed analytical procedures.

#### Accuracy and precision

The accuracy of the proposed methods was evaluated by determining SPI at three concentration levels corresponding to 80%, 100% and 120% of the working concentration. The accuracy results are presented in Table 1 and the % RSD values range from 0.7% to 2.3%. These values are satisfactory, since they are

lower than 5%, which is the maximum limit accepted by ICH [30]. To further ascertain the accuracy of the proposed methods, recovery studies were carried out by using the standard addition technique, yielding average recoveries in the range of 98.8%–101.2%.

The precision was evaluated by applying the proposed methods for determination of three different concentrations ( $n = 3$ ), which were analysed three times intra-daily for repeatability and inter-daily on three different days for intermediate precision. The precision results are summarized in Table 2. These methods demonstrated acceptable precision, with coefficients of variation (CV) ranging from 0.9 to 1.8% for intra-day assays and from 1.8 to 3.0% for inter-day assays. These values fall well within the < 5% threshold established by the recommended validation guidelines.

#### Stability, selectivity and robustness

The selectivity of the proposed methods was evaluated concurrently with accuracy by analysing pure SPI, and tablet formulations (including excipients and impurities) as well as samples subjected to photostability testing (photo-degradation products). The results confirmed high specificity, as no significant interference was observed.

Table 1  
Summary of optimized reaction parameters and linear regression statistics for SPI quantification.

Parameters	Value	
	NQS	NQ1
Colour	Orange	Orange
$\lambda_{max}$ (nm)	453	456
Reaction time (min)	17	19
Log formation constant (log $K_f$ )	3.98	4.88
Linear range (µg/mL)	0.85-18.0	0.85-18.0
Regression equation	$y = 0.1142x + 0.1527$	$y = 0.1213x - 0.0902$
Determination coefficient ( $R^2$ )	0.9998	0.9998
Limit of detection (µg/mL)	0.25	0.26
Limit of quantification (µg/mL)	0.75	0.78
Molar absorptivity $\times 10^4$ (L $\times$ mole $^{-1}$ $\times$ cm $^{-1}$ )	7.42	9.14
Sandell's sensitivity (µg $\times$ cm $^{-2}$ )	0.0092	0.0095

Table 2  
Precision and accuracy for SPI assessment: intra-day and inter-day variability.

NQ	Intra-day (n = 3)				Inter-day (n = 3)		
	Taken(added) µg/mL	Found µg/mL	%RSD	%RE	Found µg/mL	%RSD	%RE
NQS	18.0	18.27	1.3	1.5	18.32	2.2	1.8
	15.0	15.17	0.8	1.1	15.29	1.4	1.9
	12.0	12.22	1.7	1.8	12.26	0.7	2.2
NQ1	18.0	18.27	1.1	1.5	18.54	0.8	3.0
	15.0	15.14	1.7	0.9	15.28	2.1	1.9
	12.0	12.15	2.3	1.2	12.25	1.4	2.1

Additionally, photostability tests revealed that NQ derivatives remain stable for 1.5 hours under daylight conditions; however, absorbance decreases thereafter, leading to a complete molecular degradation within five hours. In contrast, the formed complexes exhibited good stability for up to 1.5 hours under normal laboratory conditions or when stored under refrigeration (4°C), maintaining a precision of %RSD < 3.1% (recovery average of 99.4%, n = 3).

The robustness of Methods A and B was confirmed by varying the reaction time, alkaline medium, and NQ concentration. The analytical results showed recoveries of 98.7–101.2% %, with %RSD values between 0.6% and 2.0% (Table 3). These findings indicate that the methods are unaffected by small fluctuations in experimental conditions. Since all calculated coefficients remained well below the 5.0% acceptance limit, the methods are proven to be robust for the routine analysis of SPI in pharmaceutical applications.

#### Analysis of pharmaceutical formulations

The proposed analytical methods were applied to determine the concentrations of the studied drug spiramycin (SPI) in pharmaceutical formulations. Specifically, commercial formulations containing 450 mg of spiramycin (Rovadal® and Rovamycine® tablets) were

obtained from local pharmacies to evaluate the methods' applicability.

The results, summarized in Tables 4 and 5, demonstrate high accuracy and precision. The average percentage content for the tablets ranged from 99.2% to 100.8%, showing excellent accordance with the declared values. To further validate the reliability of the findings, the results were statistically compared with those obtained using reported reference methods, including HPLC [34] and bioassay.

Table 3

Method robustness testing for SPI spectrophotometric measurements.					
Investigated condition		Spectrophotometric methods			
		Parameters (n = 3)	Recovery (%)	RSD (%)	
t (min)	NQS	18	100.4	1.9	
		16	99.5	0.8	
	NQ1	20	100.5	0.7	
		18	98.9	1.1	
NQ (mL)	NQS	0.9	99.7	1.3	
		1.1	99.4	0.6	
	NQ1	0.9	101.1	1.0	
		1.1	99.3	0.9	
	NaOH (mL)	NQS	1.35	100.6	1.4
			1.15	100.9	2.0
NQ1		1.35	99.8	1.7	
		1.15	98.9	1.2	

Table 4

Quantification of SPI in commercial tablets by spectrophotometric methods and HPLC.				
Samples	Parameters	Proposed methods		Reported HPLC <sup>c</sup>
		NQS	NQ1	
Rovadal® tablets	% Mean Content <sup>a</sup>	99.8	100.3	99.5
	%RSD	2.1	1.8	1.9
	<i>t</i> -value <sup>b</sup>	1.71	1.45	-
Rovamycine® tablets	% Mean Content <sup>a</sup>	100.8	99.2	100.4
	%RSD	2.3	2.0	2.1
	<i>t</i> -value <sup>b</sup>	1.82	1.58	-

<sup>a</sup>Value of five determinations (n = 5); <sup>b</sup>Theoretical tabulated value for *t* at 95% confidence (n = 5) is: *t* = 2.776; <sup>c</sup>Ref of [33].

Table 5

Application of the new methods to Rovamycine® tablets and comparison with different techniques.				
Samples	% Content <sup>b</sup>			
	Spectrophotometric methods		Bioassay	HPLC
	NQS	NQ1		
1	99.5	100.4	98.9	99.2
2	101.1	99.3	98.8	100.7
3	100.8	99.6	101.2	99.1
4	99.4	101.2	98.7	99.4
5	100.3	100.9	99.0	99.7
Average of determinations <sup>a</sup>	100.22	100.28	99.32	99.62

<sup>a</sup>Value of five determinations (n = 5); <sup>b</sup>Ref of [19] for Bioassay and Ref of [33] for HPLC.

At a 95% confidence level (with five degrees of freedom), the calculated *t*- values were lower than the theoretical tabulated values. This indicates no significant difference between the proposed and reference methods, confirming the feasibility and reliability of the new methodology.

The proposed methods proved to be highly specific and selective. Analysis confirmed that the results were virtually free from interferences from common excipients and coexisting substances typically found in pharmaceutical dosage forms.

#### **Comparative analysis of SPI determination in pharmaceutical tablets**

Spectrophotometric, bioassay and HPLC methods were applied to assay SPI in tablets. Findings indicate that the total recovery values are 100.22-100.28%, 99.32% and 99.62% using the three techniques, respectively. These results are consistent with previous studies [34] and fall within the acceptable pharmacopeial limits of 95–105% [35]. Table 5 displays the analytical results obtained by applying the proposed UV spectrophotometric methods for the assay of commercially available SPI tablets, clearly demonstrating the good precision of the methods and that the actual content of drugs are comparable to the label claim.

Beyond being more cost-effective, these novel methods represent a superior alternative for the determination of SPI content. The successful application of NQS method, HPLC, and bioassay to Rovamycine® tablets confirms their accuracy of the proposed procedures through the close agreement of the results obtained. This multi-method approach yields more comprehensive data than the direct spectrophotometric techniques reported in existing literature [27], highlighting the value of the developed procedures.

Compared with HPLC, the methods developed in this study are both simpler and more economical. Although HPLC is highly precise for quantification, it does not provide information on biological potency [33]. Despite the variability typically associated with microbiological tests, the findings confirm that spectrophotometric results correlate well with those obtained by bioassays. To ensure drug safety and efficacy, the use of complementary sensitive analytical methods is essential [31,32]. Therefore, the proposed techniques are practical for SPI quantification and represent a reliable alternative for standard quality control.

#### **Conclusions**

The proposed naphthoquinone derivative reagents (NQS and NQ1) allow the successful determination of spiramycin in pharmaceutical

dosage forms without the need for prior separation steps and without interference from formulation components. The findings of this study demonstrate that the proposed UV spectrophotometric methods provide simple, reliable, and cost-effective approach for the quantitative analysis of SPI in tablet formulations and showed good agreement with the label claim.

The two naphthoquinones-based methods showed good linearity ( $R^2= 0.9998$ ) over the concentration range of 0.85-18.0  $\mu\text{g/mL}$ , with LOD values of approximately 0.26  $\mu\text{g/mL}$  and LOQ values between 0.75 and 0.78  $\mu\text{g/mL}$ . The precision, accuracy and robustness results ( $\text{RSD} < 5\%$ ) confirm that these methods are precise and robust.

The proposed methods offer several advantages such as simplicity, sensitivity, specificity, versatility. They do not require sophisticated instrumentation or preliminary separation steps and can be readily applied in quality control laboratories for the determination of SPI in pharmaceutical formulations.

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