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DERIVATIZATION TECHNIQUES BASED ON CHARGE TRANSFER REACTIONS FOR SPECTROPHOTOMETRIC DETERMINATION OF JOSAMYCIN IN VARIOUS DOSAGE FORMS

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Abstract. New spectrophotometric methods have been developed for the determination of josamycin in pure and dosage forms based on charge transfer reactions. Method-A was based on the complexation reaction of 1,2-naphthoquinone-4-sulphonate with josamycin. Complex absorbance was measured at 454 nm. Method-B was developed by the charge transfer reaction of the amino group of josamycin with menadione. The formed orange products showed maximum absorbance at 458 nm. Lambert Beer's law was obeyed in the range of 1.0–28.8 μg/mL. The regression plots showed good linearity with determination coefficients of 0.9997. Molar absorptivity and Sandell's sensitivity were calculated, with a detection limit (LOD) down to 0.28 μg/mL and quantification limits (LOQ) of 0.85–0.89 μg/mL. The validity of procedures was tested for accuracy, precision, recovery and interference and the results were in accordance with ICH guidelines, with relative standard deviation (RSD %) values less than 5.0%. Determination results of josamycin in marketed formulations were in good agreement with the labelled quantities and without any interference from excipients, indicating that they can be used for quality control purposes.

Keywords: spectrophotometric analysis, josamycin, 1,2-naphthoquinone-4-sulphonate, menadione.

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Introduction

Macrolide antibiotics constitute an important class of antibacterial compounds isolated from culture broths of certain microorganisms. They are classified by the ring size of its central lactone ring (aglycone), to which amino and/or neutral sugar residues are appended. The predominant subclasses are based on 14- or 16-membered aglycone [1,2].

The 16-membered macrolides comprise two main families, tylosins and leucomycins, which are based on the substitution patterns of their aglycone units. Josamycin (JOS) is a member of leucomycin complex and a broad-spectrum antibiotic. It prevents bacterial growth by binding on bacterial 23S rRNA, in order to inhibiting protein translation and synthesis [1,3].

Josamycin, (3R,4R,5S,6R,8R,9R,10E,12E, 15R)-3-acetoxy-5-[O-2,6-dideoxy-4-O-isovaleryl-3-C-methyl-*α*-L-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-*β*-D-glucopyranosyl oxy]-6-formyl-methyl-9-hydroxy-4-methoxy-8,15-dimethyl-10,12-pentadecadien-15-olide, has been isolated from *Streptomyces narbonensis*. It is particularly indicated for the treatment of infections of the skin, respiratory tract, ear, nose and throat. It is used in human and veterinary practice. Like other macrolide products, JOS is a lipophilic molecule with ring of 16 atoms (Figure 1) [2,4].

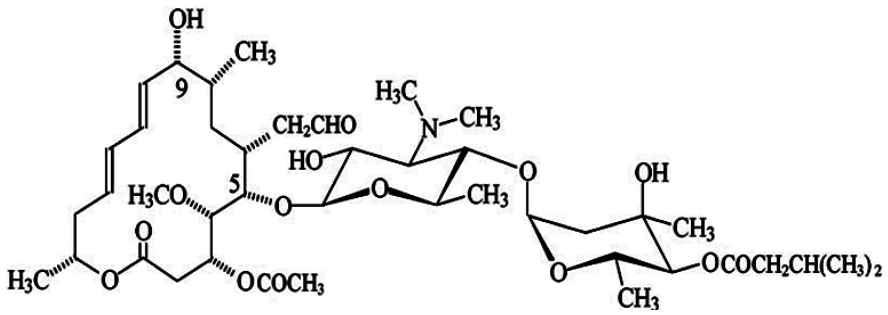

Figure 1. Chemical structure of josamycin.

JOS can be commercially produced by fermentation [3], but this process is not very selective and several related compounds are formed. Consequently, a selective method is required to quantify JOS in the presence of these molecules.

Numerous methods have been reported for the analysis of JOS in dosage forms and biological fluids using different techniques such as microbiological assay [5], thin layer chromatography [6], spectrophotometry [7], spectrofluorometry [8], voltammetry [9], and capillary electrophoresis [10-12]. High-performance liquid chromatography coupled to mass spectrometry [13-16], fluorescence detection [17], electrochemical detection [18] and UV detection [19,20] has also been described. Commonly, the majority of these methods require long experimental procedures for sample preparation and sophisticated and expensive equipment. Spectrophotometric techniques are often quite suitable because of their simplicity, low cost and its wide availability in laboratories.

Several spectrophotometric methods for macrolides analysis have been published which include the formation of coloured complexes using: quinalizarin, 7,7,8,8-tetracyanoquinoid methane, alizarin, bromocresol green, eosin Y, rose Bengal, bromophenol blue, purpurin [21], 2,4-dinitrophenyl hydrazine [5] and 3-methylbenzothiazolin-2-one hydrazone/ferric chloride system[22]. The 1,2-Naphthoquinone-4-sulphonate (NQS) has been used as a derivatization reagent for many studies [23,24]. However, the use of NQS reagent for the spectrophotometric estimation of JOS was not reported earlier. Thus, new alternative methods that can overcome the existing disadvantages of such determination are desired.

In this study, an attempt has been made to develop rapid, low-cost and repeatable spectrophotometric methods for the investigation of JOS in pure and pharmaceutical formulations. The chemical derivatization technique was based on the charge transfer reaction of the amino group in JOS with two different naphthoquinone reagents, 1,2-naphthoquinone-4-sulphonate and 2-methyl-naphthoquinone (NQ2), to form orange coloured products exhibiting absorption maxima at 454 and 458 nm, respectively. Of note, an important feature of the present work is that NQ2 derivatization was never employed for such a task before.

Experimental

Materials

JOS standard was provided by Sigma–Aldrich (Steinheim, Germany). Tablets containing JOS were obtained from commercial sources. Josacine® tablets (Astellas Pharma, France) were labelled to contain JOS as 500 mg/tablet. Josacine® oral suspension (Astellas Pharma, France) was labelled to contain JOS as 125 mg/5mL. These drugs were used without any purification step and the working solutions were prepared every day.

The 2-methyl-1,4-naphthoquinone (Menadione), 1,2-naphthoquinone-4-sulphonate, sodium salt and sodium hydroxide were of analytical-reagent grade from Sigma-Aldrich (Steinheim, Germany). Methanol, ethanol, isopropanol and acetone were of HPLC grade and also purchased from the same source.

Instrumentation

A double beam Lambda 365 UV-Visible spectrophotometer (Perkin Elmer, USA), and quartz cuvettes (l= 1 cm) were used for all absorbance measurements. An analytical balance model of Toledo (Woluwe, Belgium) and Vortex mixer (Genesis, Belgium) were used. Purified water was produced in-house using a Milli-Q system (Burgwedel, Germany) and filtered through FH membranes (0.45 μm).

Preparation of stock standard

JOS stock standard solutions (100 μg/mL) were prepared by dissolving an equivalent quantity of macrolide in methanol (10 mg/ 100 mL). The same solvent was used to prepare freshly solutions by an appropriate dilution. The solutions were prepared and used as working standards at concentrations of 1.0–28.8 μg/mL, serving in the assay as reference solutions [5].

Preparation of pharmaceutical samples

Josacine® tablets. The sample preparation was performed based on an already published protocol [25-27]. Ten tablets of Josacine® were weighed and then finely powdered. A weighed powder equivalent to 500 mg JOS was transferred into volumetric flasks of 100 mL containing some methanol (20 mL) and dissolved by ultrasonic bath for 20 min. Next, the solution was completed to 100 mL volume with the same solvent. The solution was mixed and filtered using a Millipore membrane filter. A precise volume of the filtrate was consequently diluted to obtain a sample solution of 1.0–28.8 μg/mL working concentration. This sample was evaluated in triplicate. The sample preparation process was performed twice.

Josacine® oral suspension

A weighed portion of the powder for oral suspension of JOS (125 mg) was introduced into a volumetric flask of 100 mL. Then, 20 mL of methanol was added. The mixture was then sonicated (5 min), adjusted to mark and mixed well. The dilution method was followed to obtain solutions having the same working concentrations range [5].

Naphthoquinones solutions

To prepare 0.2% w/v of NQ solution, 0.2 g of NQ was weighed and dissolved in distilled water (NQS) or in ethanol 70% (NQ2), transferred into 100mL standard volumetric flasks, diluted to the mark with the same solvent and mixed well. The solutions were freshly prepared and protected from light.

General derivatization procedure

An accurately measured 1.0 mL of JOS (24 µg/mL) was transferred into a 10 mL standard volumetric flask. Then optimal volume of 0.2 M NaOH solution was added followed by optimal volume of NQ solution. The reaction mixture was diluted to the mark with distilled water. The solutions were left at room temperature for certain time and the absorbance was measured at 454 nm and 458 nm for JOS–NQS and JOS–NQ2, respectively, against reagent blank.

Composition of formed complex

To determine the stoichiometric ratio, Job's method of continuous variation (Job, 1964) [28] was applied using equimolar solutions of JOS and NQ (2.5×10^{-3} M). In this method, solution series of JOS and NQ were prepared as described under the general procedures and comprising different proportions (0:10, 1:9, . . ., 9:1, 10:0). Then, the absorbance of each solution was plotted against the mole fraction of the drug.

Method validation

The developed procedures were validated according to ICH recommendations [29] for linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

Linearity and sensitivity

Stock standard solutions of JOS were diluted with methanol to prepare working solutions at concentrations range of 1.0–28.8 µg/mL. The solutions were derivatized as described under section of general derivatization procedure. The calibration graphs were obtained by plotting the absorbance average of each solution versus to the corresponding concentrations. Limit of detection (LOD) and limit of quantitation (LOQ) were estimated using the successive dilution method.

Accuracy and precision

Method accuracy [30] was estimated as percentages of JOS recoveries at three concentration levels (80%, 100% and 120%) in five replicates on the same day (intra-day) and on 3 consecutive days (inter-day).

Precision of methods [30] was evaluated using the selected conditions following the procedure described previously [31]. Precision and accuracy were calculated in terms of relative standard deviation (RSD) and relative error (RE), respectively.

The 10 mg JOS standard was introduced into 100 mL-flask and dissolved with methanol (20 mL), then the volume was completed with the same solvent. The mixed solution was consequently diluted (methanol) to obtain working concentrations of 19.2, 24.0 and 28.8 mg/mL.

Results and discussion

Selection of analytical wavelength

The absorption spectrum of JOS (24 µg/mL) was recorded over the range 200-800 nm using methanol as reference and showed an absorption peak with λ_{max} at 232 nm. JOS in methanol showed no absorbance in the 400–700 nm range. After derivatization, it was found that an orange complex was formed and the absorbance maximum appeared at 454 nm for JOS-NQS, while the reaction product formed with NQ2 was detected at 458 nm. Investigations were carried out to establish the most favourable conditions of complex formation between JOS and NQ for both methods.

Optimization studies

In the current study, derivatization procedures were optimized with a view to developing simple and sensitive methods for JOS determination based on spectrophotometry. The methods of steepest ascent were adopted for this assessment [23]. The method development was performed by varying one parameter of the experimental conditions at a time while keeping the others fixed and observing the effect produced on the absorbance of the coloured product. In an attempt to maximize the sensitivity and to achieve the optimum conditions, the effect of various parameters such as the solvent nature, NQ reagent concentration, alkaline medium concentration, reaction time and temperature was investigated.

Characterization of the reaction was carried out exploring JOS charge transfer reaction with NQ by the evaluation of the reaction stoichiometry. Calculation of the association constant, molar absorptivity (in water medium) and the verification of the proposed reaction mechanism were also estimated.

Effect of the solvent nature

The solvent plays an important role in some charge transfer reactions since it must be able to facilitate the total charge transfer, complex dissociation and stabilization of the radical anion formed, which is the absorbing species [23].

The effect of the solvent nature was examined through investigations in different polarity media, such as water, methanol, ethanol, isopropanol, acetone and acetonitrile. According to the literature, solvents with high dielectric constant are more effective to execute this task [21,23].

In the present case, with all the studied solvents, maximum absorbance against reagent blank of the orange solutions produced by the reaction between JOS and NQ was observed in water medium (Figure 2). This is probably related to the capacity of this solvent to form stable hydrogen bonds with the radical anion. Thus, taking into account its favourable dielectric constant and the high solubility of the NQ in water, it was chosen for all further measurements in order to obtain highest sensitivity for both methods. It is important to point out that the colourless reagent blank (NQ) in water medium exhibits negligible absorbance at 454 and 458 nm.

Effect of the NQ volume

The maximum conversion of the JOS into absorbing species depends on the amount of reagent available in the reaction solution and the equilibrium involved. So, the effect of NQ concentration was studied by varying the NQ volume over the range of 0.25–2.5 mL of 0.2% (w/v) NQ, while the JOS concentration was maintained constant at 24 µg/mL.

The results of this study are shown in Figure 3. The study revealed that the reaction was dependent on the reagent and increasing the concentration of NQ results in more product formation up to a maximum level after which the absorbance remained almost constant. A higher NQ volume had no beneficial effect on the absorption values. The highest absorbance was attained when the volume of 0.2% (w/v) NQ was 1.25 mL for both reagent NQS and NQ2 (Figure 3). Therefore, this volume of NQ was considered optimum.

Effect of the alkalinity

A condensation reaction of JOS with NQ in alkaline medium forms an orange colour product with maximum absorbance at 454 nm and 458 nm for JOS–NQS and JOS–NQ2, respectively. Similar to other macrolides, alkaline medium was necessary since the results revealed that JOS has difficulty to react with NQ in acidic media [23]. In this last media, product absorbance is close to 0, indicating that JOS does not react with NQ. This may be related to lower electron density at the level of the JOS amino group, in comparison with alkaline media.

In order to find the optimal medium for the quantitative determination of the studied macrolide, the reactions were examined in sodium hydroxide, disodium hydrogen phosphate, borax and sodium bicarbonate. All were prepared as aqueous solution in a concentration range of 0.1–0.6 M. Best results were obtained in case of sodium hydroxide (NaOH).

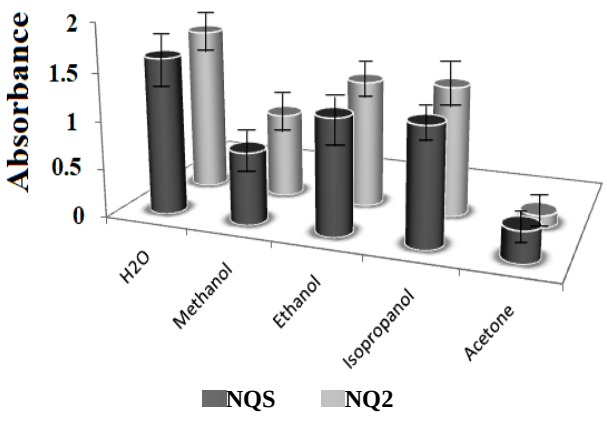

Figure 2. Solvent nature effect on the reaction of JOS (24 µg/mL) with NQS and NQ2; NaOH 0.2 M: 1 mL; temperature: 25°C; reaction time: 20 min.

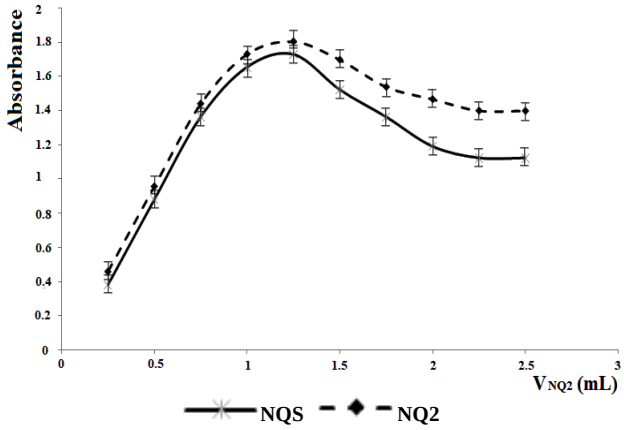

Figure 3. Effect of the NQ volume (0.2% w/v) on the reaction of JOS (24 µg/mL) with NQS and NQ2; NaOH 0.2 M: 1 mL; temperature: 25°C; reaction time: 20 min.

The influence of NaOH concentration on the absorbance of the reaction products was investigated by varying the NaOH volume in the range of 0.25–2.5 mL. In alkaline medium, absorbance increases rapidly up to 0.5–0.75 mL. Maximum absorbances were reached at volumes of 0.5 and 0.75 mL of NaOH for JOS–NQS and JOS–NQ2, respectively, which means that the derivatization degrees were maximal. Above these values, a decrease in the readings occurred (Figure 4). In order to keep the high sensitivity for the determination of JOS, these both values were selected for optimal experimental conditions.

Optimization of reaction temperature and time

The effect of temperature and time on the reaction of JOS with NQ in alkaline medium was studied at different values (25–80°C, 0–60 min) by observing the absorbance at 454 nm and 458 nm for JOS–NQS and JOS–NQ2, respectively.

The effect of temperature on the reaction was studied in the range 25–80°C. It was found that the reaction at ambient temperature went to completion in 20 min for both reagents. The results also revealed that increasing the temperature had a negative effect on the absorption values of the reaction solution, which might be due to the instability of the JOS–NQ derivative. Accordingly, ambient temperature (25°C) was found to be optimal for maximum colour development.

The influence of the time of reaction at this temperature on the colour intensity was investigated in the range 0–60 min. The experimental results show that the absorbance values increased from the beginning of the experiment up to 12 min and 15min for JOS–NQS and JOS–NQ2, respectively and the optimum reaction time was achieved. Therefore, these optimum times were selected in order to make the method time efficient. The coloured product was more or less stable up to 1 h at room temperature.

Stoichiometric relationship

The optimized conditions used for the assay were: 1.25 mL of NQ (0.2% w/v); 0.2 M NaOH 0.5 mL (NQS) and 0.75 mL (NQ2); reaction time 12 min (NQS) and 15 min (NQ2); temperature 25°C. Under these conditions, the stoichiometric ratio between JOS and each NQ in aqueous alkaline medium was studied by Job's method of continuous variation and mole-ratio method [28].

The symmetrical bell shape of Job's plot with the maximum situated at a molar ratio of 0.5, indicated that the stoichiometry of JOS:NQ is 1:1 (Figure 5). This would be consistent with the transfer of an electron from the free electron pair of the nitrogen atom present in the JOS molecule to the charge-deficient center of NQ [23]. The respective formation constants K_f of the formed complexes were calculated according to Amin, A.S. *et al.* [32] and were summarized in Table 1. The log(K_f) values were found to be 2.63 and 3.27 for JOS-NQS and JOS-NQ2 complexes, respectively, which confirms the good stability of the formed complexes.

Analytical method validation

The developed methods were validated according to the frequently recommended references and guidelines for validation of analytical procedures [29,33-36].

Linearity, limits of detection and quantification

The calibration curves showed linear responses over the range of concentrations used (1.0–28.8 µg/mL) for both methods and the determination coefficients were found to be 0.9997, which were close to unity (Table 1). LOD values were found to be 0.29 and 0.28 µg/mL for JOS–NQS and JOS–NQ2, respectively. On the other hand, LOQ values were estimated to be 0.89 (NQS) and 0.85 (NQ2), indicating high sensitivity of the developed methods.

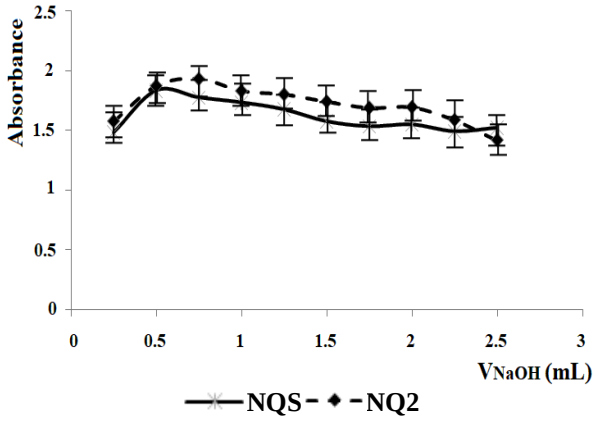

Figure 4. Effect of NaOH volume on the formation of JOS–NQ products, NQS and NQ2; 0.2% w/v NQ: 1 mL; temperature: 25°C; reaction time: 20 min.

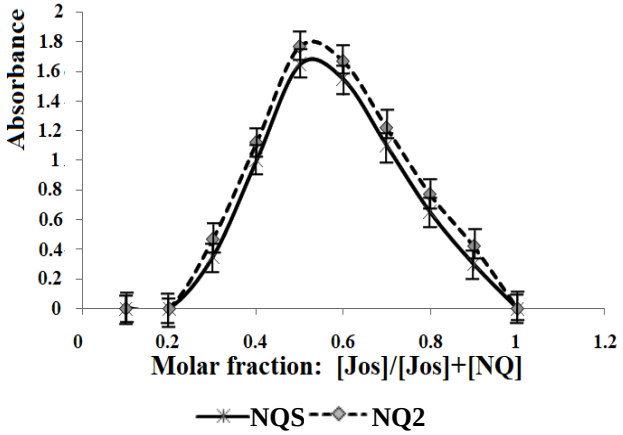

Figure 5. Application of Job's method to the reaction between JOS and naphthoquinones NQS and NQ2.

Accuracy and precision

Accuracy of the proposed methods was evaluated by recovery studies of JOS. The average recoveries were in the range 100.89%–103.28% for concentration levels 80, 100 and 120% of the working concentration of JOS. For the above three concentration levels % RSD were within 0.7–2.8% for both methods, which is satisfactory. The percent of RE values were found in the range of 0.9–2.0% for intra-day and 1.7–3.3% for inter-day assay (Table 2), which indicates that the accuracy and precision of the proposed methods are acceptable.

Stability, specificity and robustness

In this study, the effect of light and dark storage conditions was investigated and they gave variable influence on the stability of the coloured product. In daylight, the strong orange colour of the JOS–NQ derivative was observed for three hours. Then, the colour started to fade in the following two hours. After seven hours the solution became almost colourless. Later, the absorbance value decreased and finally became 0. This indicates the complete breakdown of the derivative molecules. While in dark conditions, a strong and stable orange colour was observed for five hours. After 8 hours, the solution became very light orange.

On the other hand, the stability of reagent solutions (JOS, NQ) was tested through both methods and the results indicated that there were no significant differences in concentrations of the complexes formed between JOS and both NQ reagents, upon reagent storage in the refrigerator (4°C) for 14 days, with a %RSD less than 5% (> 2.7%; n= 3), which indicates the good stability of the used solutions.

Robustness was evaluated by examining the influence of NQ concentration, alkaline medium and time of the reaction. The results of the robustness study varied very slightly (Table 3). The mean % recovery was 99.4 and 100.0% with % RSD values of 1.7 and 1.3% for JOS–NQS and JOS–NQ2, respectively. So, both methods can be considered robust enough to analyse JOS in dosage forms.

Derivative solutions of placebo gave an absorbance near to 0.0001 when measured at 454 and 458 nm for both methods. Therefore, it was revealed that the effect of the excipients on the absorbance was negligible, thus proving the method's specificity.

Table 1

Optimized characteristics of the proposed methods and regression dataof JOS.

Parameters	*Value*	
	NQS	*NQ2*
Colour	Orange	Orange
λ_{max}(nm)	454	458
Reaction time (min)	12	15
Logarithm of formation constants [log(K_f)]	2.63	3.27
Linear range (µg/mL)	1.0–28.8	1.0–28.8
Regression equation	y= 0.0752x+0.0337	y= 0.0789x+0.0379
Determination coefficient(r^2)	0.9997	0.9997
Limit of detection (µg/mL)	0.29	0.28
Limit of quantification (µg/mL)	0.89	0.85
Molar absorptivity (L/moL×cm)	6.90	7.12
Sandell's sensitivity (µg/cm^2)	0.0122	0.0117
% Recovery[a] (Pure JOS)	99.32	99.14
t-value[b,c]	1.61	1.50
F-value[b,c]	3.04	3.19

[a] Average of five experiments. [b] Theoretical value at 95% confidence limit and n= 5 for *F* is 6.26 and *t* is 2.776. [c] HPLC method [19].

Table 2

Determination of intra-day and inter-day accuracy and precision of JOS for the proposed methods.

		Intra-day			*Inter-day(n= 3)*		
NQ	*Taken, µg/mL*	*Found [a], µg/mL*	*% RSD*	*% RE*	*Found[a], µg/mL*	*% RSD*	*% RE*
NQS	28.8	29.1	2.8	1.0	29.3	2.1	1.9
	24.0	24.2	1.5	0.9	24.6	1.3	2.5
	19.2	19.6	0.9	2.0	19.8	0.9	3.1
NQ2	28.8	29.3	1.0	1.9	29.7	0.7	3.3
	24.0	24.3	1.6	1.1	24.4	2.6	1.7
	19.2	19.4	2.6	0.9	19.7	1.2	2.7

[a] Value of three determinations. RE: relative error; RSD: relative standard deviation.

Table 3

Robustness study of the spectrophotometric methods.

Investigated condition	*NQ*	*Parameters*	*Recovery (%)*	*RSD (%)*
t (min)	NQS	14	99.1	2.0
		10	101.4	0.9
	NQ2	13	100.1	1.8
		17	99.8	0.9
NQ (mL)	NQS	1.15	98.3	1.2
		1.35	98.0	0.9
	NQ2	1.15	101.3	1.4
		1.35	98.7	1.0
NaOH (mL)	NQS	0.40	98.7	2.0
		0.60	101.0	1.8
	NQ2	0.65	99.5	1.1
		0.85	100.3	1.7

Application to the pharmaceutical dosage forms

JOS dosage forms (Josacine® suspension and tablets) were subjected to the analysis by above described methods as well as with a selected HPLC method [19]. The obtained results were statistically compared with each other.

JOS tablets were found to contain 101.9% and 100.6% for NQS and NQ2 methods, respectively (Table 4). The contents of the oral suspension were estimated at 99.7% and 101.4% for both methods, respectively. The average content of JOS in the selected dosage forms was in good accordance with the declared values, indicating that the amount of drug in the samples met with the requirements (95–105%). Moreover, with respect to *t*-test, no significant differences were found between the proposed and the reported methods at 95% confidence level, this indicated similar accuracy and precision in the analysis of JOS. Furthermore, common excipients did not interfere with the assay, which demonstrated the feasibility and reliability of the present methods.

Comparison of different methods

In this study, microbiological assay, UV spectrophotometry and HPLC methods were used to assay JOS in tablets. The percentage contents were found to be 99.5%, 100.2% and 99.9% by bioassay, HPLC and UV methods, respectively (Table 5).

According to the obtained data, a good correlation between the studied techniques was observed. Using considered UV methods, results were comparable and within the acceptable limits of 95-105%. Furthermore, these methods are not more costly than the others and are suitable for JOS estimation studies.

JOS content in oral suspensions was determined by the UV method and microbiological assay [5], and the results indicate a satisfactory correlation. The linear curve equation was $y= 0.253x+0.870$ ($n= 12$; $R^2= 0.995$). It is noteworthy to mention that cross-referencing the developed procedures with another effectual method creates a possibility to provide more useful data.

Table 4

Application of the proposed method and HPLC to the determination of JOS in dosage forms.

Sample	*Parameters*	*Proposed method*		*HPLC method*
		NQS	*NQ2*	
Josacine® tablets	% Content[a]	101.9	100.6	98.9
	%RSD	1.9	2.0	2.0
	t-value[b]	1.64	1.58	-
Josacine® suspension	% Content[a]	99.7	101.4	101.1
	%RSD	2.0	2.2	2.0
	t-value[b]	1.72	1.53	-

[a]Value of five determinations (n= 5).

[b]Theoretical value for t at 95% confidence (n= 5) is: t= 2.776

Table 5

Analysis of JOS in tablets by the different used methods.

Sample	*% Content*			
	UVspectrophotometry		*HPLC*	*Microbiological Assay*
	NQS	*NQ2*		
1	99.3	101.1	98.0	99.0
2	100.2	99.1	101.6	97.3
3	99.0	98.7	99.0	101.3
4	101.4	100.7	100.7	98.9
5	98.0	101.5	101.8	101.1
Average of determinations	99.6	100.2	100.2	99.5

UV spectrophotometry methods are inexpensive and simple compared to other methods like HPLC. The most recent apparatus of UPLC (with or without -MS/MS) is quite expensive and demands considerable skill and expertise to assure success [37]. These last ones can be used for quantitative determination of antibiotics with high precision, but it cannot offer any accurate data about biological activity. However, the microbiological tests have certain changeability, but the findings of the present study confirmed the possibility to correlate the spectrophotometric results obtained from the developed methods with those from microbiological assay. To authenticate the quality of any drug, it is necessary to combine various performance and sensitive analytical methods, in order to match the quality and to predict the therapeutic efficacy as well as drug security. Hence, the studied colorimetric techniques are quite practical for quantitative determination of JOS antibiotic in its pharmaceutical preparations, being an adequate alternative technique for routine quality control of this drug.

Conclusions

This research describes two spectrophotometric methods based on charge transfer reactions for analysis of JOS in tablets and oral suspensions. Chemical derivatizations of JOS with both naphthoquinone reagents (NQS and NQ2) were enhanced in alkaline medium. The developed methods were precise, specific, sensitive and robust at a range of concentrations varying from 1.0 to 28.8 µg/mL. Content values showed good agreement with the label claim and proved the absence of interference by common additives and excipients. The proposed methods have the advantage of less expensive apparatus, simplicity, speed and flexibility. Moreover, they can be applied at room temperature and are suitable for routine quality control of this drug.

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