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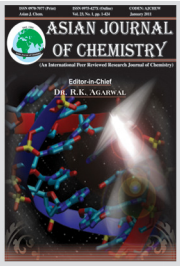

Determination of Josamycin and Ciprofloxacin in their Pharmaceutical Dosage Forms by Spectrophotometry

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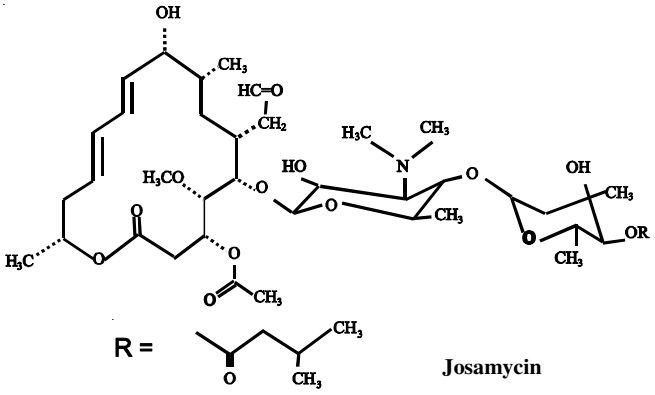
Two simple, rapid and sensitive spectrophotometric methods for the determination of josamycin and ciprofloxacin in their pharmaceutical dosage forms are described. Method (A) is based on the reaction of aldehyde group of josamycin with 2,4-dinitrophenyl hydrazine in methanolic hydrochloric acid to yield a yellow product which absorbs maxima at 411 nm, whereas method (B) depends on the reaction of ciprofloxacin with 1,2-naphthaquinone-4-sodium sulphonate in alkaline medium to form an orange coloured product measurable at 487 nm. The working conditions of both methods have been optimized and the reactions pathways were postulated. Regression analysis of Beer's law plots showed good correlation in the concentration ranges 10-160 and 5-120 µg/mL for josamycin and ciprofloxacin, respectively. Detection and quantification limits for both methods are calculated. The methods were successfully applied to the determination of both drugs in bulk drugs and their formulations. Statistical treatment of the experimental results indicates that the accuracy and precision of the methods are analytically acceptable. The validity of the methods was evaluated by parallel determinations by established procedures and by recovery studies.

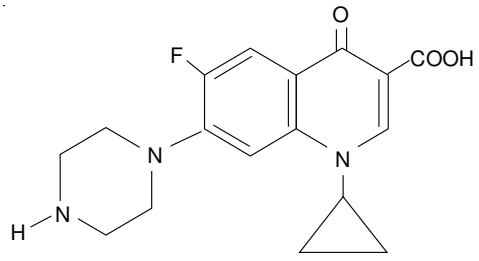
Key Words: Josamycin, Ciprofloxacin, 2,4-Dinitrophenyl hydrazine, 1,2-Napthaquinone-4-sodium sulphonate, Spectrophotometry.

INTRODUCTION

Josamycin is a macrolide antibiotic that is particularly indicated for the treatment of infections of the skin, respiratory tract, ear, nose and throat. Its important pharmacokinetic properties include accumulation in certain cells and an increase in blood plasma levels after repeated ingestion[1]. It also used as an alternative to penicillin-allergic patients[2]. Like other macrolide antibiotics, josamycin is a lipophilic molecule with a central lactone ring bearing 16 atoms to which several amino and sugars moieties are bound[2,3]. The structural formula of josamycin is shown in Fig. 1.



Josamycin



Ciprofloxacin

Fig. 1. Chemical structures of josamycin and ciprofloxacin

Ciprofloxacin is a quinolone antibacterial agent with fluorine at position 6 of naphthyridine ring. It was as a potent fluoroquinolone chemotherapeutic of the second-generation group of nalidixic acid derivatives of first commercially introduced in the 1980's. Due to the broad-spectrum effect and systemic matter of action, it is widely used both in human and veterinary medicine to treat infectious diseases, caused particularly by gram-negative and some gram-positive bacteria. The target of highly selective action of ciprofloxacin is bacterial DNA gyrase, a type of topoisomerase II[4]. The chemical structure of ciprofloxacin is shown in Fig. 1.

Due to the medicinal and therapeutic importance of josamycin and ciprofloxacin, there is much interest in their

determination for the purpose of pharmaceutical quality control. Several reports were initiated on josamycin determination both in formulations and in biological fluids, *viz.*, high performance liquid chromatography[5-9], high performance liquid chromatography/mass spectrometry[10,11], liquid chromatography[12,13], liquid chromatography/mass spectrometry[14-17], capillary electrophoresis[18,19], voltammetry[20-22], spectrophotometry[23], kinetic spectrophotometry[24,25] and turbidimetry[26].

Numerous analytical methods for the quantitative determination of ciprofloxacin in pharmaceutical preparations and biological samples have been referred and reviews have been published[27-29]. Recent reports for ciprofloxacin determination have mainly used in liquid chromatography[30], liquid chromatography/tandem mass spectrometry[31], magnetic separation followed by liquid chromatography/tandem mass spectrometry[32], high performance liquid chromatography[33-35], ultra high performance liquid chromatography/mass spectrometry[36,37], voltammetry[38], Rayleigh light scattering technique[39] and kinetic spectrophotometry[40,41].

Therefore, it was considered desirable to develop additional assay methods suitable for the rapid and reliable quality control of josamycin and ciprofloxacin pharmaceutical formulations. Spectrophotometric methods are considered the most widely used techniques. This is attributed to their inherent simplicity, low cost and wide availability in most quality control laboratories.

The aim of the present work is to develop rapid, simple, sensitive and selective spectrophotometric procedures for the determination of josamycin and ciprofloxacin in pure and in pharmaceutical dosage forms. The proposed methods are based on the reaction of aldehyde group of josamycin with 2,4-dinitrophenyl hydrazine reagent and the substitution reaction of ciprofloxacin with 1,2-naphthaquinone-4-sodium sulphonate reagent (NQS) in alkaline medium. The results obtained were satisfactorily accurate and precise.

EXPERIMENTAL

All the absorption spectral measurements were made using Ultrospec 2100 pro-88683 Biochrom UV-visible spectrometer (Cambridge, UK) with 1 cm matched quartz cells.

All chemicals were of analytical reagent grades, solvents were of spectroscopic grade and distilled water was used throughout.

Methanolic solution of 2,4-dinitrophenyl hydrazine, 0.3 % (Aldrich, UK) in 30 % concentrated hydrochloric acid, 11.47 M (BDH)/methanol (BDH) (v/v), aqueous solution of 1,2-naphthaquinone-4-sodium sulphonate, 0.7 % (BDH) and aqueous sodium hydroxide, 0.1 M (BDH) were used.

Reference standard samples of josamycin and ciprofloxacin hydrochloride were obtained from Saudi Arabian Japanese Pharmaceutical Co. Limited (SAJA) and used as received. Commercial dosage forms containing the studied drugs were obtained from the local markets.

Standard solutions: Standard stock solutions of pure josamycin and ciprofloxacin must be freshly prepared for corresponding methods.

For method (A): (1000 μg/mL) was prepared by dissolving 10 mg of pure josamycin in methanol in a 10 mL volumetric flask and diluted with the same solvent to the mark.

For method (B): (400 μg/mL) was prepared by dissolving 10 mg of pure ciprofloxacin hydrochloride in distilled water in a 25 mL volumetric flask and was further diluted with the same solvent to the mark.

Construction of calibration curves: Calibration curves were constructed according to the optimum conditions in Table-1.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS OF JOSAMYCIN AND CIPROFLOXACIN USING THE PROPOSED METHODS

Parameter	Method A (for josamycin)	Method B (for ciprofloxacin)
λ_{max} (nm)	411	487
Linearity range (μg/mL)	10-160	5-120
Detection limit (μg/mL)	2.4	1.2
Quantification limit (μg/mL)	8.0	4.0
*Regression equation:		
Slope (b)	0.0056	0.0161
Intercept (a)	0.0054	0.0090
Correlation coefficient (r)	0.9999	0.9999

*With respect to A = a + bC where C is concentration of drug in (μg/mL) and A is absorbance.

Method (A), determination of josamycin: In 10 mL volumetric flasks, different aliquots of stock josamycin solution were transferred to provide final concentration range 10-160 μg/mL. To each flask, 2 mL of 0.3 % 2,4-dinitrophenyl hydrazine reagent solution were added. Each solution was made up to the mark with 30 % HCl/CH_3OH solvent and the absorbances of the coloured solutions were measured against a reagent blank at 411 nm.

Method (B), determination of ciprofloxacin: In a series of 10 mL volumetric flasks, different aliquots of ciprofloxacin standard solution equivalent to 5-120 μg/mL were transferred. 0.5 mL of 0.7 % 1,2-naphthaquinone-4-sodium sulphonate reagent solution and 0.2 mL of 0.1 M NaOH aqueous solution were successively added. Each solution was made up to the mark with distilled water and the absorbances of the coloured solutions were measured against a reagent blank at 487 nm.

In either method, a calibration curve was prepared by plotting the absorbance as a function of concentration of drug solution. Alternatively, the corresponding regression equation was derived.

Procedure for pharmaceutical dosage forms

Method (A): Ten tablets were weighed and finely powdered. An amount of powder equivalent to 25 mg of josamycin was dissolved in 10 mL of methanol, filtered in a 25 mL volumetric flask then completed to volume with methanol and proceeded as described for method (A).

Method (B): An accurately weighed amount of the powder of 10 pulverized tablets equivalent to 10 mg of ciprofloxacin hydrochloride was dissolved in 10 mL of distilled water, filtered in a 25 mL volumetric flask and the volume was completed to the mark with distilled water, then it was proceeded as described for method (B).

In either method, the nominal content of tablets was calculated either from the previous plotted calibration graph or by using the regression equation.

RESULTS AND DISCUSSION

Method (A): 2,4-Dinitrophenyl hydrazine (DNPH) reacts with aldehyde group compounds in methanolic hydrochloric acid medium to form the yellow product 2,4-dinitrophenyl hydrazone[42]. Josamycin, like other macrolide antibiotics, has a central lactone ring to which several functional groups are bound. Among these groups is the aldehyde group which is responsible for the formation of the coloured reaction product 2,4-dinitrophenyl hydrazone with λ_{max} at 411 nm (Fig. 2). The reaction pathway is shown in **Scheme-I**.

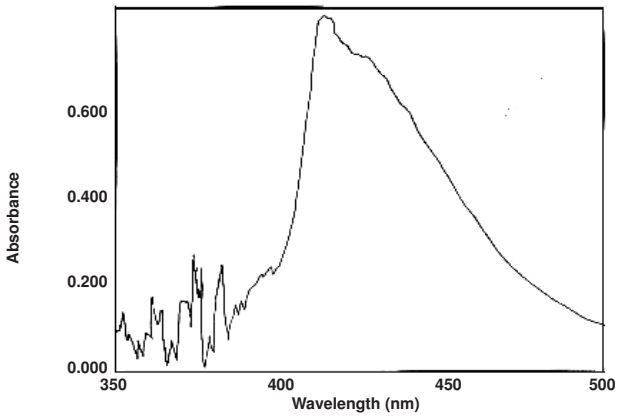
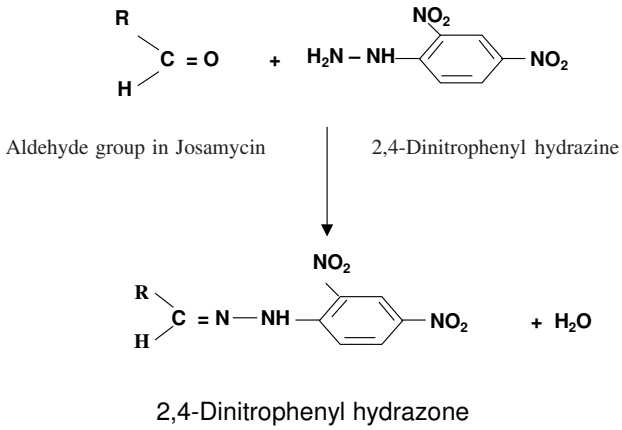


Fig. 2. Absorption spectrum of josamycin (40 µg/mL)/DNPH reaction product



Scheme-I: Mechanism of the reaction of josamycin and DNPH reagent

To optimize the conditions, parameters such as reagent concentration and time were investigated. The optimum conditions were established by varying one variable and observing the effect on the absorbance of the coloured product.

30 % HCl/CH_3OH (v/v) was found to be the solvent of choice for josamycin to affect its dissolution. Trials were made to dissolve the drug in HCl alone or using different ratios of HCl/CH_3OH. It was found that the solubility of josamycin in different concentrations of HCl was not completed, so methanol was added in different ratios. 30 % of HCl/CH_3OH was chosen as the suitable ratio which gives maximum absorbance of the reaction product. Maximum absorption at the relevant maxima was also obtained upon using 2 mL of 0.3 % (w/v) of DNPH reagent. Higher reagent concentrations did not affect the colour intensity.

Josamycin was capable of reaction with DNPH at ambient temperature and maximum colour intensity was obtained immediately and remained stable for up to 0.5 h.

Method (B): 1,2-Naphthaquinone-4-sodium sulphonate reagent (NQS) is used for the determination of aliphatic primary and secondary amines. Ciprofloxacin reacts with NQS reagent in NaOH medium. Replacement of the sulphonate group of the naphthaquinone sulphonic acid by the amino group on ciprofloxacin takes place to give the colour product, N-alkylaminonaphthaquinone which absorbs maxima at 487 nm (Fig. 3). **Scheme-II** shows the possible reaction pathway predicted from literature[42] and from results of the present work.

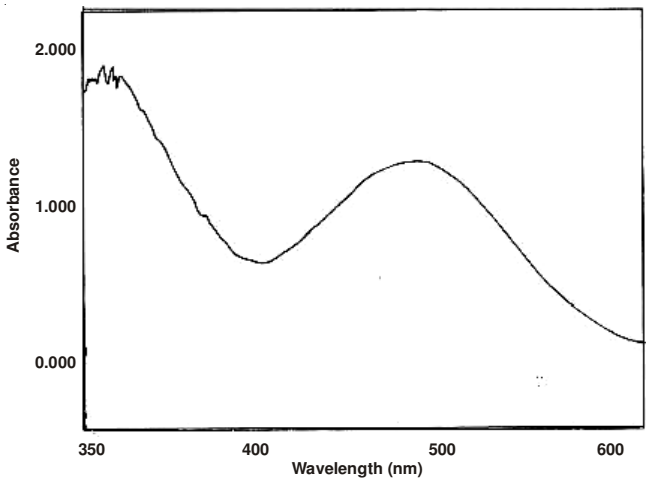
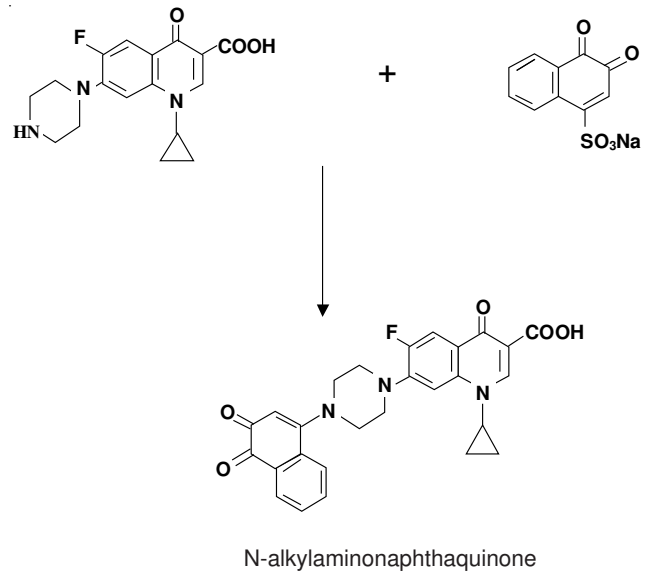


Fig. 3. Absorption spectrum of ciprofloxacin (80 µg/mL)/NQS reaction product



Scheme-II: Mechanism of the reaction of ciprofloxacin and NQS reagent

The absorptiometric properties of the coloured species as well as the influence of different parameters on the colour development are extensively studied to determine optimal conditions of the assay procedure. The reaction was studied as a function of the concentration of reagents, order of addition of reactants, time and stability.

The reaction is affected strongly in alkaline medium, where maximum colour intensity was obtained upon using

0.2 mL of 0.1 M NaOH. It was found that 0.5 mL of 0.7 % (w/v) aqueous NQS solution is optimal for maximum development of the orange colour.

After fixing the concentration parameters, a few other experiments were performed to ascertain the influence of the order of addition of reactants. The order, drug:NQS reagent: NaOH gave maximum absorbance and stability and hence the same order was followed throughout the investigation. Maximum absorption at 487 nm was obtained immediately at ambient temperature and the product remained stable for up to 0.5 h.

Validity: Under the experimental conditions described, Beer's law is obeyed for both methods over the concentration ranges given in Table-1. Regression equations and correlation coefficients obtained by the method of least squares are also compiled in Table-1. The limits of detection and quantification were calculated from the standard deviation of the absorbance measurements from a series of ten blank solutions for each method. The limits of detection (K = 3) and limits of quantification (K = 10) were established according to IUPAC definitions[43] and recorded in Table-1.

In order to determine the accuracy and precision of the methods, pure drug solutions containing different concentrations of each drug were prepared and analyzed applying the proposed procedure for each method. The analytical results obtained from this investigation are summarized in Table-2.

TABLE 2
DETERMINATION OF JOSAMYCIN AND CIPROFLOXACIN IN PURE AND PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED AND REFERENCE METHODS [Ref. 25,44]

Drug form	Recovery (%) ± SD	
	Proposed methods	Reference methods
Pure josamycin	100.7 ± 0.87* (n = 8)	100.0 ± 0.42* (n = 5)
t-value	1.71 (2.201)	–
F-value	4.22 (6.09)	–
Josaxin tablets** (500 mg josamycin/tablet)	99.4 ± 1.25 (n = 3)	100.3 ± 0.40 (n = 3)
t-value	1.171 (2.776)	–
F-value	9.75 (19.0)	–
Pure ciprofloxacin HCl	100.1 ± 0.59* (n = 9)	100.0 ± 0.51* (n = 4)
t-value	0.333(2.201)	–
F-value	1.346 (8.85)	–
Ciprobay tablets** (500 mg ciprofloxacin/tablet)	99.6 ± 0.69 (n = 3)	99.4 ± 0.52 (n = 4)
t-value	0.437 (2.571)	–
F-value	1.778 (9.55)	–

*Found (%) ± SD; **Product of Saudi Arabian Japanese Pharmaceutical Co., Figures in parentheses are the tabulated values of t and F at 95 % confidence limits.

Applications: To ascertain the reliability of the methods, the proposed methods for the determination of josamycin and ciprofloxacin were successfully applied to commercial tablets together with the reference methods[25,44]. To avoid the interferences from additives and excipients usually found in formulations and, the standard addition technique was adopted for both methods.

To a fixed amount drug in the formulation, pure drug was added at three different levels and the total was found by the proposed methods. The experiment was repeated three times for each level. The results of this study presented in Table-2 reveal that accuracy and precision of the methods were unaffected by the various co-formulated substances.

The results obtained were compared statistically by applying students t-test for accuracy and F-test for precision[45] with the reference spectrophotometric procedures. The results showed that the calculated t- and F- values were less than the tabulated values indicating that there was no significant difference between the proposed and the comparison methods.

Conclusion

Although josamycin and ciprofloxacin have been determined by a variety of techniques[5-41,44]. The methods described here are simple, rapid, convenient and do not require special working conditions unlike many other reported methods. The methods are advantageous when compared to many of the reported spectrophotometric methods. The procedures employ shorter reaction times, stable coloured species and inexpensive reagents. The determinations can be performed at room temperature and do not require heating step. The proposed methods can be used as alternative methods to the reported ones for the routine determination of josamycin and ciprofloxacin tablets. This encourages their successful use in routine analysis of these drugs in quality control laboratories.

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