SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF OFLOXACIN AND LEVOFLOXACIN IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT:
A new, simple, accurate and sensitive spectrophotometric method has been developed for the analysis of ofloxacin (OFL) and levofloxacin (LEV) in their pharmaceutical formulations, through oxidation of these drugs with a known excess of cerium (IV) sulphate in acidic medium and the residual oxidant is determined by treating with a fixed amount of methyl orange, then measuring the absorbance at 507 nm. The amount of cerium (IV) sulphate reacted corresponds to the drug concentration in the sample solution. Different variables affecting the reaction were carefully studied. Beer's law correlating the absorbance with concentration was obeyed in the range of 0.5—3.0 and 1.0—3.5 μg mL^{-1} for OFL and LEV respectively, with good correlation coefficient (0.99854 and 0.99931 for OFL and LEV respectively). The method was validated in terms of accuracy and precision and successfully applied to the determination of each drug in its pharmaceutical formulations. The results obtained by the proposed method were comparable with those obtained by reported method.

KEYWORDS: Fluoroquinolones; spectrophotometry; cerium (IV) sulphate; methyl orange dye; pharmaceutical formulations; oxidation.

INTRODUCTION

Ofloxacin (OFL) [(±)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7 H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid] and Levofloxacin (LEV) [S-isomer of racemic OFL] (Figure 1) belong to the fluoroquinolones class of antibiotics. They are synthetic broad spectrum antibacterial drugs that exhibit significant activity against both gram-positive and gram-negative bacteria. (Kaur et al. 2008)

Several analytical methods for quantitative determination of OFL and LEV in their pharmaceutical formulations were reported in the scientific literature. These include high performance liquid chromatography (HPLC) (Joshi 2002; Samanidou et al. 2003; Shervington et al. 2005; Santoro et al. 2006) capillary electrophoresis (CE) (Elbashir et al. 2008; Elbashir et al. 2008; Faria et al. 2006), atomic absorption spectrometry (Ragab and Amin 2004), spectrofluorimetry (Ocana-Gonzalez et al 2000 and Salem et al. 2007) and spectrophotometry (Salem 2005; Mishra and Yadav 2004; Garcia et al. 2005; El-Brashy et al. 2005; Ashour and Al-Khalil 2005) among others. Chromatographic methods have been extensively used and recommended. However these methods generally require complex and expensive equipment, provision for use and disposal of solvents, labor-intensive sample preparation procedures and personal skills in chromatographic techniques. Spectrophotometric methods are the most convenient technique because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories. Unfortunately, the spectrophotometric methods that have been reported for the determination of OFL and LEV in their pharmaceutical formulations were associated with some major disadvantages, such as lack of sensitivity, tedious extraction procedures, and time consumption. Indirect oxidative methods using Cerium Sulphate were applied for determination of some FQs (Adegoke and Balogun 2010; Basavaiah et al. 2006; Ebraheem et al. 2011), but there is no work in the literature reporting about the application of Ce(IV) for the determination of OFL and LEV. Thus, the present paper describes the optimization, validation, and application of UV-VIS spectrophotometric for the determination of the cited drugs through reaction with excess of cerium (IV) sulphate and the determination of the unreacted oxidant by treatment with methyl orange (MO).

The proposed method is practical and valuable for the routine application in quality control laboratories for the analysis of the two studied drugs.

MATERIAL AND METHODS

Instrumentation

All absorbance measurements were made with a double beam UV-1800 (SHIMADZU, Japan) ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells and Temperature Controller was used for all spectrophotometric measurements.

Chemicals and Reagents

All chemicals used were of analytical reagent grade. Chemicals (suppliers) were as follows: OFL (Hoechst AG, Frankfurt, Germany); LEV (Sigma Chem. Co., USA); Cerium(IV) sulphate (Loba-Chemie Indoaustranal Co., India);

methyl orange (Fluka Chemika Sigma-Aldrich); sulphuric acid (S.d.Fine Chem, Mumbai, India); Pharmaceutical formulations: The following available commercial preparations were analyzed: Optiflox eyedrop (Jamjoom Pharma, Saudi Arabia) labeled to contain 3 mg OFL per mL; Tavanic tablets (Aventis Pharma, Germany) labled to contain 500 mg LEV per tablet; Doubly distilled water was used to prepare all solutions.

Cerium (IV) Sulphate (250 μg mL^{-1}):

A 0.01 g mL^{-1} cerium (IV) sulphate stock solution was prepared by dissolving 0.5 g of the chemical in 1.0 M sulphuric acid and transferred into 50 mL volumetric flask and diluting to the mark with the same acid. Stock solution was diluted appropriately with 1 M sulphuric acid to yield 250 μg mL^{-1} cerium (IV) sulphate solution.

Methyl Orange (50 μg mL^{-1}):

A 500 μg mL^{-1} solution was first prepared by dissolving 50 mg of dye in water and diluting to the mark in a 100 mL calibrated flask and filtered. This was diluted 10-fold to obtain a working concentration of 50 μg mL^{-1}.

Sulphuric acid (5 M):

This was prepared by adding 274 mL of concentrated sulphuric acid to 726 mL water with cooling.

Preparation of stock and sample solutions

Stock Standard Solutions:

A stock standard solutions (1 mg mL^{-1}) of OFL and LEV, were prepared by accurately weighing 10 mg of pure sample of each drug, and transferring to a 10 mL volumetric flask, with addition of water to make up to volume. (In case of OFL was dissolved first in 0.5 mL of 0.1 M sodium hydroxide then the volume was completed to the mark with water).

OFL Sample Solution:

A 3.33 mL of optiflox eyedrop, was transferred into a 10 mL calibrated flask, 0.5 mL of 0.1 M sodium hydroxide was added and diluted to the volume with water, to obtain stock solution with a concentration of 1 mg mL^{-1}.

LEV Sample Solution:

The contents of 10 tablets of Tavanic were weighed, ground into a fine powder using mortar and pestle and mixed. An accurately weighed portion of the powder equivalent to one tablet, transferred into a 500 mL volumetric flask. The volume was made up to the mark with water. After 15min of mechanically shaking, the solution was filtrated through Whatman No. 42 filter paper; the filtrates were suitably diluted to obtain 1 mg mL^{-1} as a suitable concentration for the analysis.

General Procedure:

Aliquots of OFL and LEV stock solutions were added to 10 mL volumetric flasks to give final concentrations of 0.5–3.0 and 1.0–3.5 μg mL^{-1} respectively. To each flask were added 1 mL of sulphuric acid (5 M) and 1 mL of cerium (IV) sulphate solution (250 μg mL^{-1}). After mixing, flasks were allowed to stand at room temperature for 10 min with occasional swirling. Finally 1 mL of methyl orange solution (50 μg mL^{-1}) was added; the solution was diluted to the mark with water and mixed. After 5 minutes, the absorbance of each solution was measured at 507 nm against a reagent blank prepared in the same manner using 1 mL water instead of 1 mL methyl orange solution.

RESULTS AND DISCUSSION

The ability of cerium (IV) sulphate to oxidise OFL and LEV, and bleach the colour of methyl orange is the basis of the indirect spectrophotometric method developed here (Figure 2). In this method, the drugs were reacted with a measured excess of cerium (IV) sulphate in acidic medium and the unreacted oxidant was determined by reacting with methyl orange (MO) followed by absorbance measurement at 507 nm (Scheme 1). The absorbance increased linearly with increasing concentration of each drug, when increasing amounts of each drug were added to a fixed amount of cerium (IV) sulphate, consumed the latter and there occurred a concomitant fall in its concentration. When fixed amount of the dye was added to decreasing amounts of oxidant, a concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective λ_{max} with increasing concentration of each drug.

$$FQ + Ce(IV)_{excess} \rightarrow \text{FQ oxidation product} + Ce(III) + Ce(IV)_{unreacted}$$

$$Ce(IV)_{unreacted} + MO \rightarrow \text{oxidation product of MO} + \text{unreacted MO}$$

$$\updownarrow$$

measured spectrophotometrically at λ_{max}=507 nm

Scheme 1: *Reaction scheme of the indirect determination of OFL or LEV by oxidation with Ce(IV) sulphate.*

Optimization of reaction variables:

Preliminary experiments were performed to fix the optimum concentrations of the methyl orange dye that could be determined spectrophotometrically, and this was found to be 5 μg mL^{-1}. A cerium (IV) sulphate concentration of 25 μg mL^{-1} was found to destroy the red colour due to 5 μg mL^{-1} methyl orange in acidic conditions. Hence, different amounts of each drug were reacted with 1 mL of 250 μg mL^{-1} oxidant before determining the residual cerium (IV) sulphate as described under the respective procedure.

The effect of the order of reagents addition:

It was observed that regardless of each drug amount added, methyl orange was almost totally bleached if the reagents addition order was dye+drug+oxidant or dye+oxidant+drug. The reason of this observation is that the Ce(IV) sulphate didn't have enough time to oxidize the drug because it rapidly bleaches methyl orange. In conclusion, the drug and oxidant solutions respectively must be added first, their addition order doesn't influence the reaction and methyl orange have to be added after a given period of time during which the drug is totally oxidized by $Ce(SO_4)_2$.

The effect of time on the oxidation reaction:

It was observed that if methyl orange is added immediately to the solution containing each one of the drugs and cerium (IV) sulphate in acidic medium, the resulted solution is bleached rapidly and the absorbance is very low. This can be explained by the fact that the oxidation of the drug by cerium (IV) sulphate is a time developing reaction and thus the influence of the reaction time was studied. In this respect, quantitative reactions between the drugs and cerium (IV) sulphate have been let to react at different times before adding the indicator and measuring the absorbance. It was observed that the absorbance of these solutions increases with the time up to 10 minutes remaining then constant (Figure 3). Thus, for further measurements a reaction time of 10 minutes was selected for both OFL and LEV.

The standing time of 5 min was necessary for the bleaching of dye colour by the residual oxidant. The measured colour was stable for hours in the presence of reaction product.

The effect of sulphuric acid concentration:

The reaction was carried out in sulphuric acid medium. The highest absorption intensity was obtained at 5 M H_2SO_4 for OFL and LEV (Figure 4). One mL of acid was used in the assay procedure.

Method Validation:

Analytical Parameters of the Method:

A linear relation was found between absorbance at λ_{max} and concentration of OFL and LEV in the ranges shown in Table1. The calibration graphs are described by the equation:

$Y = a + b X$

(Where Y = absorbance, a = intercept, b = slope and X = concentration in µg mL^{-1}) obtained by the method of least squares. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to The International Conference of Harmonization (ICH) guidelines for validation of analytical procedures (ICH Guideline 2005). The following formula was used: LOD or LOQ= *k*SDa/b, where *k* = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The apparent molar absorptivity and Sandell sensitivity values together with the limits of detection and quantification compiled in Table 1 are indicative of the high sensitivity of the proposed method.

Accuracy and Precision:

The accuracy and precision of the method were evaluated by performing five replicate analyses on pure drug solutions at three different amount/ concentration levels (within the working ranges). The relative error (%), an indicator of accuracy did not exceed 0.91 and 1.82% for OFL and LEV respectively, and intra day precision which is also called the repeatability expressed in relative standard deviation (RSD) (%) was within 3.4 and 3.1 for OFL and LEV respectively, indicating the high accuracy and precision of the method. The results of this study are compiled in Table 2. The reproducibility of the method, also called the day-to-day precision, was assessed by performing replicate analyses on pure drug solutions at three levels over a period of five days preparing all solutions afresh each day. The day-to-day RSD values were less than 4.5% reflecting the usefulness of the method in routine analysis of the investigated drugs in quality control laboratories.

Recovery Studies of the proposed method:

The accuracy and precision of the method were further assessed by performing recovery experiments. To a fixed amount of each drug in the dosage form, pure drug was added at three different levels and the total was found by the proposed method. Each test was performed in triplicate. The percent recoveries of the added pure OFL and LEV were in the range of 98-102 and 99.7-101% respectively (Table 3) revealing good accuracies and non-interference from excipients and diluents. This was further confirmed by the fact that no more than the stoichiometric amount of cerium (IV) was consumed when the tablet extract/eyedrops solutions were treated with cerium (IV) under the described experimental conditions.

Application of the proposed method to analysis of OFL and LEV in dosage forms:

Commercial eyedrops and tablets containing OFL and LEV were successfully analysed by the proposed method. Co-formulated substances did not interfere. It is evident from the above-mentioned results that the proposed method gave satisfactory results with the two drugs. Thus their pharmaceutical dosage forms were subjected to the analysis of their OFL and LEV contents in each case by the proposed method. The label claim percentage was 98.68 and 100.40% for OFL and LEV respectively. This result was compared with that obtained from the official method [6] by statistical analysis with respect to the accuracy (by *t*-test) and precision (by *F*-test). No significant differences were found between

the calculated and theoretical values of *t*- and *F*-tests at 95% confidence level proving similar accuracy and precision in the determination of OFL and LEV by both methods (Table 4).

CONCLUSIONS

The present study described the successful development of new, simple, sensitive, selective, accurate and rapid spectrophotometric method for the accurate determination of OFL and LEV; each one in its dosage forms using cerium (IV) sulphate as the oxidimetric reagent.

The proposed method is superior to the previously reported spectrophotometric methods for the determination of OFL and LEV in terms of their simplicity. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. The other advantages include that, the method involve the measurement of stable coloured species, have shorter contact times and free from extraction and boiling step compared to many of the previously reported procedures. Therefore, the method is practical and valuable for routine analysis in quality control laboratories for analysis of each investigated drug.

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