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Validated methods for the determination of some antimicrobial drugs

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ABSTRACT

Two simple, sensitive and accurate methods were developed for the determination of some antimicrobial substances. The first method is based on the measurement of difference in absorbance (?A) of equimolar portions of gatifloxacin solution in 0.1 M HCl and in 0.1 M NaOH at 291 nm. Beer's law is obeyed over a concentration range of 1- 9 μg ml $^{-1}$ with mean recovery 99.03 ± 0.03. The second method is depended on the measurement of a native fluorescence of Ofloxacin upon excitation at 290 nm with emission bands having maxima at 488 nm and found to be proportional to the concentration range 0.2 -2 μg ml-1 for Ofloxacin. Regression analysis showed good regression coefficient. The proposed methods were successfully applied for the determination of studied drugs in bulk powder and pharmaceutical dosage forms with good accuracy and precision. The results were found to agree statistically with those obtained by the official methods. Furthermore, the methods were validated according to ICH regulations and also assessed by applying the standard addition technique.

KEYWORDS

Difference spectrophotometry (ΔA);
Spectroflourimetric;
Antimicrobial substances.

INTRODUCTION

Synthetic substances, which have been recently synthesized, seem to be exhibit high efficiency in inhibiting or even killing microorganisms. Such compounds are referred to as anti-microbial agents, for example, sulfonamides, quinolones and fluoroquinolones. Ofloxacin (OFX) is one of the second-generation quinolones. It is 9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3de]-1,4-benoxazine-6-carboxylic acid[1]. Gatifloxacin (GFX) is methylpiperazin-1-yl) - 4-oxo-quinoline-3-carboxylic acid.

Several methods for the determination of OFX in dosage form or in biological fluids have been reported. They include HPLC methods for determination of OFX alone or simultaneous determination with other drugs in several dosage forms[2-15] in whole blood[16], milk sample[17] and in human plasma[18, 19]. Also simultaneous determinations of OFX with other drugs by electrophoresis techniques have been developed in dosage form[20] and in biological fluid[21]. Similarly, a literature survey for GFX revealed that several techniques have been reported for the simultaneous determination of it with other corticosteroids in milk[22] and biological

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fluid[23]. Developed methods for the determination of GFX in dosage form or in biological fluids have been reported. They include HPLC methods for determination of GFX alone[25-28] or simultaneous determination with other drugs in several dosage forms[24]. Also determination of GFX using specroflourimetric[29, 30] and spectrophotometric techniques[31].

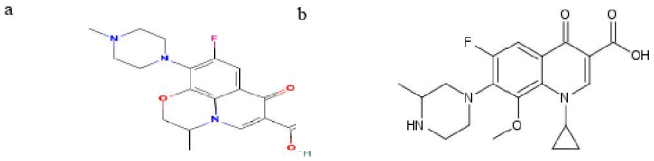

Figure 1 : Structural formula of (a) Ofloxacin and (b) Gatifloxacin

In the present work, rapid, sensitive and cost-effective two different methods, namely (?A) spectrphotometry and spectrfluorimetry, were described for determination of OFX and GFX in bulk powder and in pharmaceutical dosage forms with subsequent validation of the proposed methods.

EXPERIMENTAL

Apparatus

- Shimadzu UV-VIS spectrophotometer 2450 PC(Japan)
- Digital pH- meter, PW 9409 Pye Unicum
- Shimadzu spectrofluorimeter, RF- 1501, 150W xenon lamp (Japan) connected with printer EPSON LX-300.
- Cuvette, quartz of 1×1× 4.5 cm.

Materials

All chemicals, solvents and reagents were of analytical or HPLC grade.

- Ofloxacin (OFX) was kindly supplied by Allergan Co., Cairo, Egypt. Its purity was assessed according to British pharmacopoeia[1] and was found to be 99.88 ± 0.89[1].
- Gatifloxacin (GFX) was kindly supplied by NODCAR Egypt S.A.E, Cairo, A.R.E. Its purity was assessed according to the reported method[1] and was found to be 99.67 ± 0.03.
- Dexaflox eye drop: (Boehringer Ingelheim, Co. Cairo, Egypt), Batch. No: 803106. each 1 ml labeled to contain 3mg OFX and 1 mg Dexamethasone
- Zymar eye drop: batch No. 57455 (manufactured by Allergan, Waco, Texas, U.S.A.) each ml labeled to contain 3 mg GFX (as base).

Standard solutions

Stock standard solutions

For first method 4 μg ml^{-1} OFX solution and for the second method 10 μg ml^{-1} GFX solution were prepared in methanol.

Dosage forms solution

Dexaflox eye drop

One ml aliquot of the Dexaflox eye drop equivalent to 3 mg OFX was transferred into volumetric flask (100 ml) and the volume was completed with methanol. An aliquot of 1 ml was diluted to 10 ml in volumetric flask to produce final concentration 3 μg ml^{-1}. Appropriate dilutions of sample preparation were assayed as mentioned under procedure of calibration curves and the concentration of each component was calculated by using the corresponding regression equation.

Zymer eye drop

Shake well a bottle of Zymer eye drop, transfer about 1ml of the bottle to a 100 ml volumetric flask and complete the volume with methanol. Different aliquots (0.5-3 ml) equivalent to (15-90 μg) for GFX were transferred into 10 ml volumetric flasks and the volume was completed with methanol to get final concentrations ranged from 1.5- 9 μg ml^{-1}.

CALIBRATION PROCEDURE

Spectrofluorimetric method

Accurate aliquots of the prepared working standard solutions equivalent to (2-20 μg) were transferred into five volumetric flasks (10 ml) and the volume was completed with methanol. The fluorescence intensity was recorded for OFX against a blank at 290 nm excitation wavelength giving emission at 488 nm. Calibration curve was obtained by plotting the fluorescence intensity against the concentration and the regression equation was computed.

Difference spectrophotometric method

Different aliquots equivalent to (10- 90 μg) GFX were transferred into 10 ml volumetric flasks and the volume was completed using 0.1N HCl, each concentration was measured against an equimolar concentration of the drug in 0.1N NaOH as a blank at 291nm. Calibration curve was obtained by plotting the difference in absorbance ΔA versus the corresponding concentrations and the regression equation was computed for GFX.

RESULTS AND DISCUSSION

Spectrofluorimetric method

The present work describes a simple, sensitive and rapid direct spectrofluorimetric method for the determination of OFX, upon displayed emission band at 488 nm after excitation of its methanolic solution at 290 nm as shown in (Figure 2). Study of different factors such as study of different excitation wavelengths, solvent for dilution such as methanol, 0.1M HCl, Distilled water and 0.1M NaOH were carefully tested. Linear relationship was obtained over the concentration range of 0.2- 2 ng ml^{-1} for OFX and regression equations data were shown in TABLE 1.

Difference spectrophotometric method

The present work describes a simple and rapid direct spectrophotometric method for the determination of GFX. The acidic pH shows a hyperchromic and bathochromic shift as shown in (Figure 3). Linear relationship was obtained over the concentration range of 1- 9 μg ml^{-1} for GFX.

METHOD VALIDATION

The validation[32] of the methods were assessed by estimation of linearity, accuracy, LOD, LOQ, selectivity, intraday and inter day variations ± RSD as shown in (TABLE 1).

Linearity range

Under the experimental conditions, calibrations for OFX and GFX show linear relationship and regression equations data such as slopes, intercepts, regression coefficient and residual of sum square were shown in (TABLE 1).

Accuracy

It was determined by applying the proposed methods on at least five different concentrations within the linearity range for drug substance and pharmaceutical dosage forms. The percentage relative standard deviation revealed high accuracy (TABLE 1). Also these results were compared statistically[33, 34] with results of the official methods[1] where the calculated t- and F values less than the tabulated ones (TABLE 1).

(LOD) and (LOQ)

The signal: noise ratios of 3:1 and 10:1 were considered as LOD & LOQ and were found to be (0.06, 0.2 μg / ml) and (0.3, 1.1 μg / ml) for OFX and GFX, respectively, (TABLE 1) and revealed the high sensitivity of the proposed method.

Precision

For evaluation the intraday precision, results of three replicate analyses of three different concentrations were calculated on a single day. The interday precision was

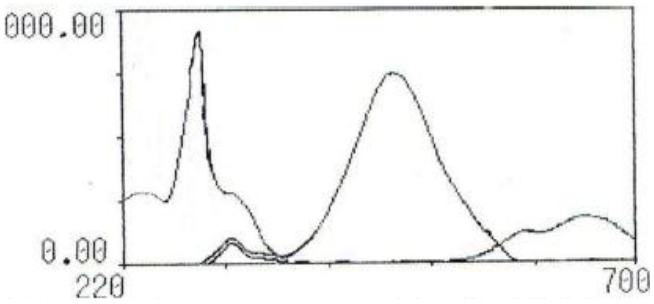

Figure 2 : Excitation and emission spectra of Ofloxacin (1 - 9 μg ml^{-1}) and blank solution

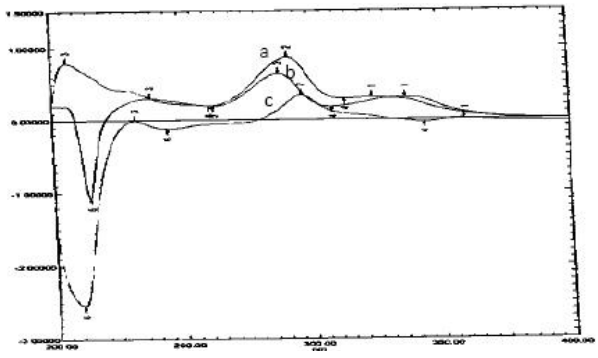

Figure 3 : Spectra of equimolar solution of Gatifloxacin in (a) 0.1 M HCl, (b) 0.1 M NaOH and (c) 0.1 M HCl against 0.1 M NaOH as a blank.

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TABLE 1 : Analytical and validation data for the determination of OFX and GFX by the proposed methods.

Parameters	Methods	
	Spectrfluorimetry	difference spectrophotometry
Wave length, λ (nm)	488 nm	291 nm
Linearity range (μg/ml)	0.2 - 2	1-9
Intercept	27.386	0.0144
SE of intercept	3.2	0.0009
Slope	447.19	0.0371
SE of Slope	2.6	0.0002
Regression coefficient (r^2)	0.9999	0.9999
Residual SS	66.3	0.00001
	Accuracy[(a)] mean ± RSD	
Drug substance	98.87 ± 0.37	99.03 ± 0.03
Dexaflox eye drop	98.94 ± 0.44	98.75 ± 0.32
Standard addition	98.65 ± 0.1	99.61 ± 0.22
	Precision[(b)] ±RSD	
Intra – day	± 0.43	± 0.18
Inter – day	± 0.51	± 0.22
Limit of detection (LOD)	0.06	0.3
Limit of quantification (LOQ)	0.2	1.1

[(a)] Mean of five different experiments
[(b)] Mean of nine different experiments

TABLE 2 : Statistical comparison of results of analysis of the cited drugs in drug substance powder.

Item	Spectro fluorimetric method	Official methods	Difference spectro photometric method	reported methods
Drug substance	OFX		GFX	
Mean[(a)]	98.88	98.99	99.03	99.05
SD	0.37	0.33	0.03	0.03
SE	0.07	0.07	0.01	0.006
Variance	0.14	0.11	0.0009	0.0009
t- (2.262)[b]	1.01		1.67	
F (6.26)[b]	1.27		1	

[(a)] Mean of five different experiments
[(b)] Theoretical values of t- and F at p= 0.05

calculated from the freshly prepared samples with the same concentration analyzed on three days. The percentage relative standard deviations (± RSD %) indicated the repeatability and reproducibility of the proposed method (TABLE 1).

Method validation of dosage forms

The validity of the proposed method was assessed by assay of the pharmaceutical dosage forms and applying the standard addition technique within the linearity range of the cited method and the results were shown in (TABLE 1) revealing that no interference from the additives.

CONCLUSION

The proposed methods are valid, simple and selective and could be used in quality control laboratories for the determination of the cited drugs in drug substance and pharmaceutical product where economy and saving time are essential.

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