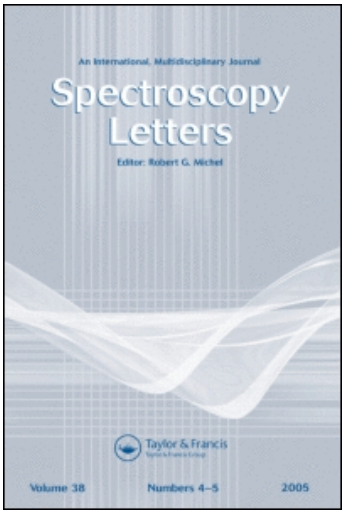

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Spectrophotometric and Spectrofluorimetric Determination of Ofloxacin

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SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC DETERMINATION OF OFLOXACIN

(**Keywords**: absorption spectrophotometry, derivative spectrophotometry, spectrofluorimetry, ofloxacin.)

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ABSTRACT

Two methods are presented for the analysis of ofloxacin. The first method is based on the application of A_{max}, ΔA, first and second derivative techniques for its determination in bulk powder, tablet form, and in urine. The use of absorbance and derivative maxima ratios as purity indices has been discussed. The second depends on the fluorescence characteristics of ofloxacin in acidic solutions. The spectrofluorimetric method is 10 times more sensitive than the spectrophotometric one. The accuracy and reproducibility of the methods were shown by the within day and between day coefficient of variation (less than 3%).

INTRODUCTION

Ofloxacin, (±)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxacine-6-carboxylic acid, is a new broad spectrum

fluorinated quinolone antibacterial agent[1]. Few methods have been revealed in literature for its determination. It has been quantitatively determined, mostly in biological fluids, by HPLC[2-6]. A colour based on the formation of ternary complex of ofloxacin with eosin and palladium II[7] has been used for its determination in pharmaceutical preparations. Ofloxacin has also been determined by TLC[8] and voltammetry[9].

It was felt useful to develop spectrophotometric and fluorimetric methods for the determination of ofloxacin in pharmaceutical preparations. It has been shown that the application of derivative techniques to spectrophotometry is very useful when there exists signal overlapping or interference[10-12].

The object of this work was to develop a spectrophotometric method for the analysis of ofloxacin in its dosage forms. The method is based on the application of the A_{max} method, the absorbance difference method (ΔA)[13,14] and first (D_1) and second (D_2) derivative techniques. The use of the absorbance and derivative maxima ratios[15] as purity indices has been discussed.

Ofloxacin in acidic solutions showed strong fluorescence which was adopted to develop a relatively more sensitive method of analysis.

EXPERIMENTAL

Apparatus

A Perkin-Elmer Model 550 S UV-VIS spectrophotometer and a Hitachi Model 561 recorder were used. The spectra of test and reference solutions were recorded in 1 cm quartz cells over the range of 370 to 200 nm. Suitable settings are: scan speed 120 nm/min; chart speed 60 mm/min; ordinate maximum and minimum was adjusted according to the magnitude of the D_1 and D_2 values; response time 10 sec. The fluorimetric measurements were carried out on a Perkin-Elmer Model 650-10S spectrofluorimeter equipped with 1 cm quartz cells and a Perkin Elmer Model 56 recorder. The instrument controlls were set as follows: sensitiveity range, 0.1; slit width, 10 nm for both excitation and emission; response and mode, normal.

Materials

All reagents and solvents used were analytical reagent grade. Tarivid tablets, Hochest Co., containing 200 mg ofloxacin.

Standard Solutions

Stock standard solution of ofloxacin containing 1 mg/ml was prepared in 0.1 N NaOH. Working standard

solutions for spectrophotometry (0.2 mg/ml) and for spectrofluorimetry (20 μg/ml) were used.

Calibration Graphs

1. For spectrophotometry: two equal portions of the working standard solution within the concentration range 0.2 - 1.0 mg were transferred into two separate sets of 100-ml volumetric flasks (a and b). Set (a) was completed to volume with 0.1 N HCl while set (b) was completed with 0.1 N NaOH. The zero order, D^0 and D^1 spectra of set (a) were recorded against 0.1 N HCl. For differential measurements, solution (a) was placed in sample cell and solution (b) in reference cell, and the delta absorbance ($\Delta A = A_{acid} - A_{alk.}$) was recorded.

2. For spectrofluorimetry: different portions of the working standard solution within the concentration range of 20 - 100 μg were diluted to 100 ml with 0.1 N H_2SO_4. The fluorescence emission spectra were recorded using λ_{ex}298 nm.

Content Uniformity Analysis of Tablets

A quantity of the powdered tablets, equivalent to 100 mg of ofloxacin, was transferred into a 100-ml volumetric flask using 0.1 N NaOH solution. The contents of the flask were shaken for about 30 min. and made up to volume and filtered. Two different portions

of the filtrate were diluted to contain 0.6 mg/ml and 60 µg/ml for spectrophotometric and spectrofluorimetric analysis, respectively. The above described procedures as mentioned under calibration graphs were applied.

Spiked Urine Analysis

Different portions of the working standard solution within the concentration range of 0.2 - 1.0 mg were transferred into separate 100-ml volumetric flasks. One ml urine was added to each flask and the volume was made with 0.1 N HCl. The procedure was continued as under calibration graphs for spectrophotometry using blank (1 ml urine diluted to 100 ml with 0.1 N HCl.

RESULTS AND DISCUSSION

The absorption, absorption-difference (ΔA), first and second derivative spectra of ofloxacin are shown in Fig. 1. Ofloxacin possesses two maxima in the UV region at 292 nm and 324 nm in 0.1 N HCl (Fig. 1a), where the first wavelength was used for its analysis (A_{max} method). Ofloxacin exhibited a hyperchromic effect when its alkaline solutions are rendered acidic, which has been used to apply a spectrophotometric method (ΔA) for its determination to eliminate interferences that are pH-insensitive. Therefore, the peak height at 298 nm when ofloxacin in 0.1 N HCl solution was measured

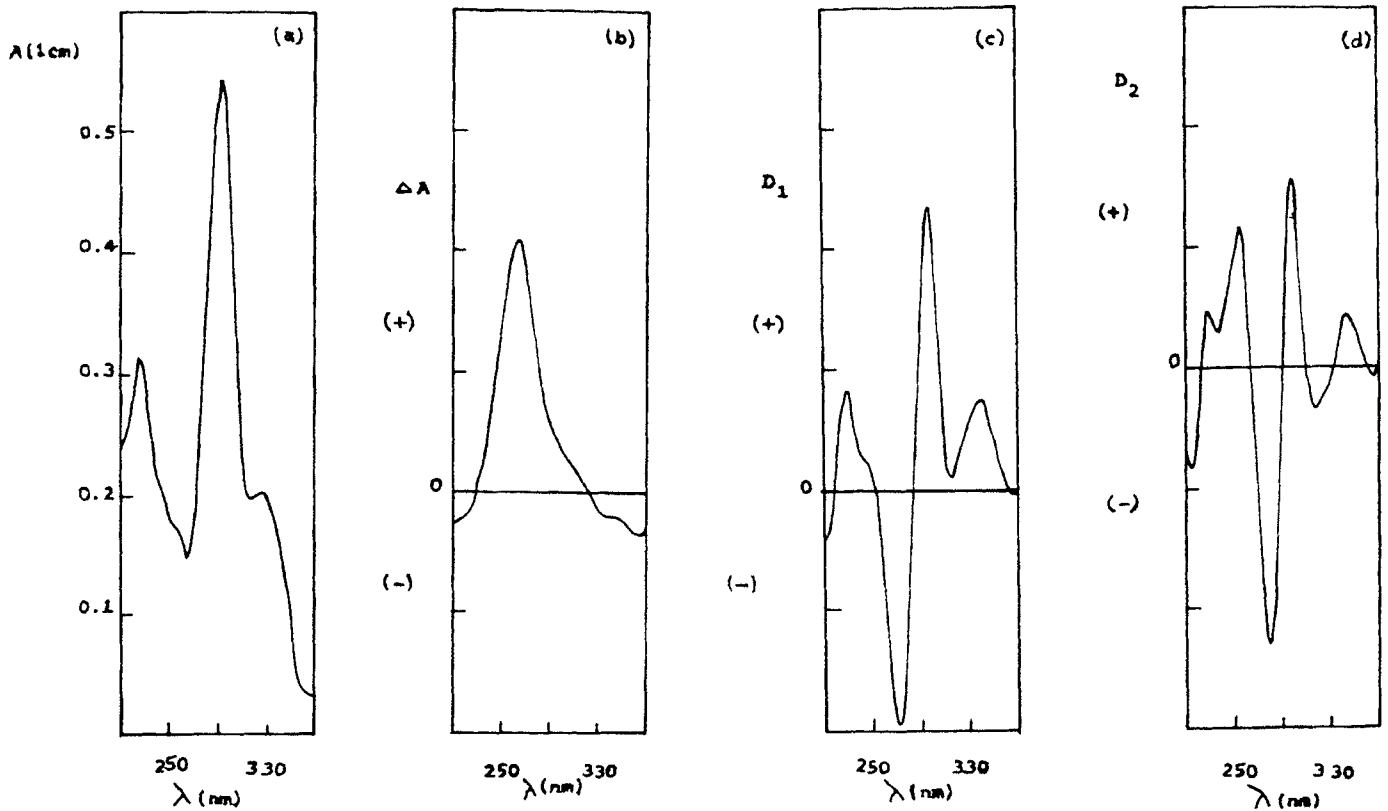

FIG 1. (a) Absorption, (b) absorption-difference (ΔA), (c) D_1 and (d) D_2 spectra of 0.6 mg/100 ml ofloxacin in 0.1 N HCl.

TABLE 1

Ratios of Absorption, First and Second Derivative Maxima for Different Concentration of Ofloxacin.

Concentration mg/100 ml	A292nm/A324nm	D1 295nm/D1 270nm	D2 280nm/D2 298nm
0.2	2.182	1.195	1.466
0.3	2.242	1.217	1.464
0.4	2.197	1.188	1.458
0.5	2.206	1.206	1.450
0.6	2.205	1.204	1.463
0.7	2.256	1.210	1.452
0.8	2.199	1.195	1.450
1.0	2.207	1.205	1.440
Mean	2.212	1.203	1.455
CV%	1.11%	0.77%	0.61%

against its solution in 0.1 N NaOH was used for its determination (Fig. 1b).

The first and second derivative spectra (Fig. 1c and d) possess positive and negative peaks in which the peak height at 295 nm and 280 nm were adopted for D_1 and D_2 measurements, respectively.

The ratios of the absorption, first and second derivative maxima were calculated and used for the detection of the presence of interferences[15]. The ratios of different concentrations of ofloxacin in 0.1 N

HCl were presented in Table 1. They were independent of concentration and were reasonably reproducible. The ratios are specific for each compound and can be used for its identification and quantitation[15]. The D_1 ratios were more accurate (Table 1) due to the more prominent peaks that enhances the spectral details which make the drug identification easier and the quantitation more sensitive.

Under the described experimental conditions, the graphs obtained by plotting A_{max}, ΔA, D_1 and D_2 values versus concentration in the range of 0.2 - 1.0 mg/100 ml showed linear relationships. The following linear regression equations were derived using the method of least squares:

$$A = 0.010 + 0.878\ C$$

$$\Delta A = 0.174 + 8.470\ C$$

$$D_1 = -0.005 + 9.675\ C$$

$$D_2 = -0.014 + 9.690\ C$$

where C is the concentration in mg/100 ml, A is the absorbance at 292 nm, ΔA, D_1 and D_2 are the peak height in cm at 298 nm, 295 nm, 280 nm, respectively. The corresponding correlation coefficients were 0.9998, 0.9998, 0.9997 and 0.9999, respectively.

Solutions of ofloxacin in 0.1 N H_2SO_4 exhibited a strong fluorescence at 512 nm (excitation wavelength 298 nm). A linear correlation was obtained between the

fluorescence (F) and concentration (C) in the range of 0.2 - 1.0 μg/ml. The linear regression equation derived using the method of least squares was as follows:

$$F_{512nm} = 0.55 + 106.25\ C$$

The correlation coefficient was 0.9999.

To prove the validity and the applicability of the proposed methods and the reproducibility of the obtained results, four replicate experiments of different concentrations of oflaxacin were carried out; the within day coefficients of variation are listed in Tables 2 and 3. The values of the between day coefficient of variation for different concentrations of ofloxacin obtained from experiments carried out over a period of five days are presented in Tables 2 and 3. These results indicate that the proposed methods are highly reproducible where the within day CV% was 1% and the between day CV% was less than 3%.

The proposed methods were applied to the determination of ofloxacin in tablet formulation. The results are shown in Table 4. Although the A_{max}, Δ A and derivative methods gave comparable results, the derivative technique may be preferable in case of interferences.

The spectrophotometric methods were used for the determination of ofloxacin in urine. The urine samples were spiked with it in the concentration range of 0.4 -

TABLE 2

Within Day and Between Day Precision Of Ofloxacin Analysis

Method	Theoretical concentration mg / 100 ml					
	0.2 mg / 100 ml		0.6 / 100 ml		1.0 mg / 100 ml	
	Found Concentration	CV%	Found Concentration	CV%	Found Concentration	CV%
A_{max}						
Within day CV%*	0.198	1.01	0.606	0.74	0.995	0.36
Between day CV%**	0.194	2.63	0.602	2.48	0.995	1.05
ΔA						
Within day CV%	0.201	1.04	0.601	0.63	1.000	0.59
Between day CV%	0.200	1.80	0.601	0.75	1.000	0.99
D_1						
Within day CV%	0.200	0.85	0.600	0.3	0.999	0.29
Between day CV%	0.204	1.52	0.614	1.14	0.998	0.85
D_2						
Within day CV%	0.202	0.54	0.600	0.37	0.996	0.28
Between day CV%	0.204	1.72	0.595	1.11	0.996	0.89

* Average of 4 experiments.
** Average of 5 experiments carried on 5 days.

TABLE 3

Within Day and Between Day Precision of Ofloxacin Analysis by the Spectrofluorimetric Method.

Theoretical Concentration µg/ml	Within Day Found Concentration	CV%*	Between Day Found Concentration	CV%**
0.2	0.196	0.51	0.201	2.49
0.6	0.603	0.43	0.604	1.52
1.0	0.995	0.17	0.991	1.18

* Average of 4 experiments.
** Average of 5 experiments carried on 5 days.

TABLE 4

Determination of Ofloxacin in Tablets and Urine.

Method	Sample Powder	Tablets	Urine
	Recovery*, %	(Mean + SD)	
A_{max}	99.7 + 1.4	100.8 + 1.48	100.8 + 0.81
Δ A	100.0 + 1.15	101.0 + 1.19	100.9 + 1.06
D_1	100.0 + 0.90	100.3 + 1.14	100.4 + 0.64
D_2	100.1 + 0.86	99.9 + 0.85	100.2 + 0.42
Fluorimetry	99.8 + 1.07	99.7 + 1.26	--

* Mean + standard deviation for 5 determinations.

1.0 mg/100 ml. The results obtained are summarized in Table 4. After good clean-up procedure ofloxacin can be determined in urine using the spectrofluorimetric method.

The proposed methods offer distinct advantages in rapidity, simplicity, and sensitivity and can be easily used in quality control laboratory for the analysis of ofloxacin in bulk material, tablet form and in urine. At the normal instrumetnal gain setting used, the spectrofluorimetric method is 10 times more sensitive than the spectrophotometric methods.

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