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DIFFERENCE ABSORBANCE SPECTROPHOTOMETRIC DETERMINATION OF OFLOXACIN AND ORNIDAZOLE IN DOSAGE FORM

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ABSTRACT

Describing the simple, precise and rapid method based on difference absorbance spectroscopic technique for the determination of Ofloxacin (OF) and ornidazole (OZ) in tablet dosage form. The projected method was based upon measuring the difference absorbance of OF at 293.4 nm (λ max of 0.1N HCl) in 0.1N NaOH vs 0.1N HCl solutions and other drug OZ difference absorbance in 0.1 N NaOH vs 0.1 N HCl at 277.9 nm (λ max of 0.1N HCl). The Beer's law was obeyed in the concentration range of 4-24 ug/mL and 5-30 µg/mL for OF and OZ respectively. The lower limits of detection (LOD) of OF and OZ are 0.31 and 0.35 µg/mL, respectively, while the lower limits of quantification (LOQ) of OF and OZ were 0.92 and 1.04 µg/ mL, respectively. The precision and accuracy of the method was acceptable; the relative standard deviations did not exceed 1.1% (n=6) and recovery was between 98.25% and 100.8% for OF and OZ, respectively. The proposed method was validated and successfully applied for the determination of both drugs in bulk powder, laboratory prepared mixture and commercial dosage forms such as tablets without interference from the commonly encountered excipients and additives.

Keywords: Ofloxacin, Ornidazole, Difference spectroscopy, Beer's law.

INTRODUCTION

Olfoxacin (OF) [1] is a synthetic antibiotic of second-generation fluoroquinolone drug. Chemically, it is (±)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (Fig.1).

In adults, Ofloxacin prescribed in treatment as antibacterial demonstrated for severe and life threatening diseases. It is a synthetic broad spectrum antibacterial agent official in BP [2], USP [3] and EP [4]. It is given for urinary tract infection, bronchitis, pneumonia, prostatitis, syphilis and infections of reproductive organs. It's mode of action done by blocking bacterial replication act through inhibiting DNA gyrase enzyme. Literature survey reveals UV spectrophotometric Derivative method [5], atomic absorption spectrometry [6], spectroflurometry, HPLC [7] and microbiological method [8] for its determination. The BP and USP recommended non aqueous titration for the raw material and HPLC methods for tablets. Because of importance of OF, several analytical techniques were developed for the determination in bulk, dosage and biological fluids.

Ornidazole (OZ) is a substituted imidazole derivative use in the treatment of antibacterial and antiprotozoal infections. Chemically it is 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole [9]. It is used in the treatment of Anaerobic infections both pre& post operatively, Bacterial vaginosis, Amoebic dysentery, Amoebic liver abscess, Hepatic and intestinal amoebiasis, Other protozoan infection like Giardiasis, Trichomoniasis. It is not official in any pharmacopeia. Literature survey shows that ornidazole is estimated by voltametry [10], spectrophotometric [11] and HPLC [12] methods for its determination in dosage forms and biological fluids.

Determination of drug using direct UV-Vis spectrophotmetric technique is subjected to estimate the

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compound free from interference from excipients used in formulation. Difference absorbance spectrometry is a useful analytical technique to interpret the quantitative information from spectra of the drug consists of drug component and for eliminating interference from the formulation matrix by using this technique.

The technique of difference absorbance spectrophotometric method is based upon the measurement of a absorbance difference (ΔA) which can be induce by changing the pH of solvent medium of two equimolar solutions of Ofloxacin and Ornidazole in basic solution against their acidic solution. The choice of the optimum wavelength is based on the fact that the contribution of each component exhibited maximum absorbance. Therefore, a measurement of absorbance from difference absorbance was carried out at λ max of 0.1N HCl wavelength i.e 293.4 nm for OF and 277.9 nm for OZ.

The combination of OF and OZ is commercially available in the market prescribed to control gastrointestinal infections caused by bacteria or amoebic infection and urinary tract infections due to susceptible uropathogens. Both drugs in tablet form can be analyzed by spectrophotometry [13], HPLC, capillary zone electrophoresis [14] analytical techniques. Literature survey reveals no difference absorbance UV-spectroscopic methods were reported for this combination in tablet dosage form. Difference spectroscopic method was a simple, rapid, precise and economic technique have been developed for the determination of OF and OZ in pharmaceutical dosage form.

MATERIALS AND METHODS

Instruments

A Perkin Elmer Double beam UV-visible spectrophotometer (Model Lambda 25) with 10-mm Matched quartz cells, bandwidth 1 nm was used for spectrum collection. The Systronics pH meter in combination with a calomel glass electrode was used. Pure drug samples of Ofloxacin and Ornidazole (Roorkee Research Laboratories., Roorkee, India) were used having 99 % and 99.8 % purity, respectively. Acidic solution (0.1N HCL) and basic solution (0.1 N NaOH) used as solvent for zero-crossing difference spectrophotometric analysis. All reagents used were of analytical grade and used without any further purification. Double distilled deionized water was prepared by Millipore system (Sartorius, USA) using 0.2 um membrane filter. Tablets were procured locally. Sartorious (BS-124S) made weighing balance was used for measuring minute quantities of drug.

Solutions

Stock solutions were prepared by dissolving 10 mg of each drug separately into two different 100 mL volumetric flasks (and vortex at 37°C for about 5 min if necessary) containing 0.1 N NaOH and 0.1N HCl. The flasks were made up to the volume with the respective solvent to obtain final concentration 100 ug/mL, respectively. The prepared solutions were protected from light throughout the study.

Procedure

Preparation of Calibration graph

Aliquots containing equivalent to 5-24 µg/mL OF and 5-30 µg/mL OZ were quantitatively transferred from the stock solutions of the two drugs into two separate sets of 10 mL test tubes. Each drug should consists of two series, one for acidic and another for basic. The first series of flasks were made up to the volume with 0.1 N NaOH and the absorption spectra were recorded against blank solution of 0.1N NaOH. The flasks of the second series were made up to the volume with 0.1 N HCl and the absorption spectra were recorded against blank solution of 0.1 N HCl. Difference absorbance (Δ*A*) between equimolar acidic solution and basic solution were measured by subtracting the of the second series (in 0.1 N NaOH) from the spectra of the first one (in 0.1 N HCl) for each concentration of both drugs. The absorbance of pure OF and OZ solutions were taken immediately, zero time after preparation. The (Δ*A*) values of the difference absorption at 293.4 nm for OF and 277.9 nm for OZ were plotted *vs.* concentration of each drug (µg/mL) to get the calibration graphs. On the other hand, the corresponding regression equations and statistical parameters were derived.

Determination of OF and OZ in synthetic solutions

The synthetic drug solutions were prepared by transferring appropriate aliquots from stock solution of the two drugs were transferred into a series of three 10 mL volumetric flasks to prepare three samples of equimolar solution of OF and OZ in 0.1 N HCl and 0.1 N NaOH separately to evaluate their results with the test solution in order to assess the specificity of the method for samples containing different concentrations of OF and OZ.

Assay procedure for tablets

Ten tablets were weighed and pulverized. From this sample equivalent to 20 mg of OF (Esoflox 200) and OZ (Ormed 500) was dissolved in minimum quantity of methanol and diluted up to 100mL with acidic and alkaline solutions stirred for about 10 min. The solution was filtered through Whatman filter paper no.41. Results of OF and OZ were computed from the graph.

Method Validation

The developed method was validated according to ICH guidelines (16). The following parameters were considered: specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision.

Linearity

Described method was linear over the range of 5-

30 ug/mL (OF) and 5-40 ug/ml (OZ). 5 point calibration curves were organized on different days. The results obtained were used to calculate the equation of the line by using least squares regression method. The linearity of calibration graphs and adherence to Beer's law were validated by the high value of the correlation coefficient.

Recovery Studies

To come across the accuracy of the proposed method, was checked by recovery study, by addition of standard drug solution to analyzed sample solution at three different concentration levels (80 %, 100 %, and 120 %) within the range of linearity for both drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 8 µg mL-1 of ofloxacin and 10 µg mL-1 of ornidazole.

Selectivity

A test was performed to determine the effect of matrix by analyzing the placebo blank and synthetic mixture containing OF. The composition: starch (10 mg), cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium (15 mg) and sodium alginate (10 mg) was prepared and its solution was also prepared according to method. The absorbance of the placebo solution in each was almost equal to the absorbance of the blank which revealed no interference. The drug was extracted and solution prepared as described for tablets. The solutions after appropriate dilution wherever necessary were analyzed following the recommended procedures. The absorbance resulting were nearly the same as those obtained for pure OF solutions of identical concentrations. This undeniably confirmed the non-interference of the inactive ingredients in the assay of drugs.

Precision of the Method

The reproducibility of the proposed method was determined by performing assay at different time intervals by repeating at least five times. To study intraday precision, method was repeated at different intervals in a day (morning, afternoon, evening), similarly method was repeated on five different days and average % RSD was calculated

Robustness and Ruggedness

Robustness of the methods was confirmed by altering the wavelength range and slit width, but results show modest inference when change with silt width, that these variables significantly cannot affected the absorbance of the drugs indicating the robustness of the methods. Ruggedness of the methods was analyzed by different analysts maintained the similar operational conditions using similar homogenous slot of drug aliquots.

The influence of other parameter scan speed was studied to optimize the signal of spectra to give good selectivity and higher sensitivity in detection.

Limit of quantification (LOQ) and limit of detection (LOD)

The (LOQ) was determined according to ICH recommendations (16) to establish the lowest concentration that can be measured, below which the calibration graph is non linear (LOQ=10 σ/S) where S is the slope and σis the standard deviation of the intercept of regression line of the calibration curve). The (LOD) was determined by evaluation by evaluating the lowest concentration of the analyte that can be detected (LOD=3.3 σ/S). The results of LOQ and LOD of OF and OZ by the proposed method were given in Table 1. The proposed method is sensitive as integrated by the high molar absorbtivity values of OF and OZ.

RESULTS AND DISCUSSION

The proposed method for determination of ofloxacin and ornidazole in dosage form were found to be accurate, simple and rapid. In this difference absorption of OF in 0.1N NaOH vs 0.1 N HCl maximum absorbance was obtained at 292 nm. Based on this information 293.4 nm was chosen for measuring the absorbance of OF, since the (ΔA) values of the OZ difference spectra were more optimal at this point and found linear for accurate measurements of different concentrations. Like the way, the difference spectra of OZ in 0.1N NaOH vs 0.1 N HCl showing maximum absorbance at 277.9 nm and used for OZ determination.

Table 1. Analytical data for the simultaneous determination of OF and OZ using the proposed method

Parameters	OF	OZ
Concentration	4-24 µg/mL	5-30 µg/mL
Correlation coefficient (r)	0.999	0.998
Molecular absorbtivity L mol-1 cm-1	1.25x104	2.1 x104
Slope	0.031	0.15
Intercept	0.058	0.043
%RSD	0.74	0.89
LOD (µg/mL)	0.21	0.35
LOQ (µg/mL)	0.62	1.04

Table 2. Application of the proposed method for estimation of OF and OZ in synthetic solutions

Drug determined	Amount present (µg/mL)	Amount found (µg/mL)	% Found	% RSD
OFL	4	3.98	99.5	0.79
	8	7.95	99.37	0.94
	12	11.99	99.91	0.96
	16	16.2	101.25	0.84
	20	20.1	100.5	0.99
	24	24.4	101.6	0.83
ORZ	5	4.98	99.6	0.85
	10	9.98	99.8	0.77
	15	15.05	100.3	0.65
	20	20.15	100.75	0.89
	25	25.3	101.2	0.55
	30	30.25	100.8	0.41

Table 3. Evaluation of the accuracy and precision data of the proposed method Intra-day Analysis

Amount present (µg/mL) n=5		Amount found (µg/mL) with CL		% Amount found		%RSD	
OF	**OZ**	**OF**	**OZ**	**OF**	**OZ**	**OF**	**OZ**
6	9	6.1±0.04	9.05±0.07	101.6	100.55	0.67	1.8
9	12	9.05± 0.02	11.90± 0.12	100.55	99.16	0.56	1.58
12	15	12.2± 0.08	15.4± 0.22	101.66	102.6	0.89	1.8

CL= confidence limits, RSD= Relative standard deviation

Inter-day Analysis

Amount present (µg/mL) n=5		Amount found (µg/mL) with CL		% Amount found		%RSD	
OF	**OZ**	**OF**	**OZ**	**OF**	**OZ**	**OF**	**OZ**
6	9	6.05± 0.02	9.2± 0.08	100.83	102.22	0.64	0.82
9	12	8.95± 0.03	11.95± 0.12	99.44	99.58	0.67	1.5
12	15	12.05± 0.07	15.1± 0.18	100.41	100.66	1.1	1.67

CL = confidence limits RSD= Relative standard deviation

Table 4. Assay results for the determination of OF and OZ in Esoflox 200® and Ormed 500 tablet by the proposed method

Brand Label	Label claim (mg/tablet)	% Label Claim Estimated	SD	Coefficient of Variance	Reference method % recovery 13
Esoflox 200	200	98.96	0.38	0.38	100.2 t=2.19 F=1.21
Ormed 500	500	102.6	0.28	0.287	101.6 t=1.38 F=4.7

*** Tabulated t value is 2.77**

Table 5. Recovery studies

Amount added to Final solution (µg/mL)		Amount found to Final solution (µg/mL)		% Recovery	
OF	**OZ**	**OF**	**OZ**	**OF**	**OZ**
6.4	8	6.4	7.9	100	98.75
8	10	8.1	10.1	101.25	101
9.6	12	9.5	12.2	98.95	101.6

***average of five determinations**

Fig 1. Structure of Ofloxacin

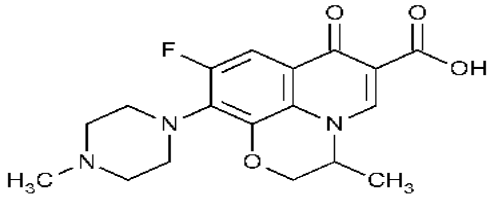


Fig 2. Structure of ornidazole

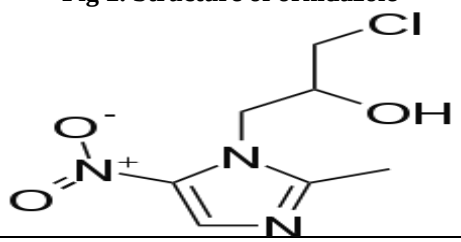


Fig 1. A) Ofloxacin in 0.1N HCl. B) Ofloxacin in 0.1N NaOH.

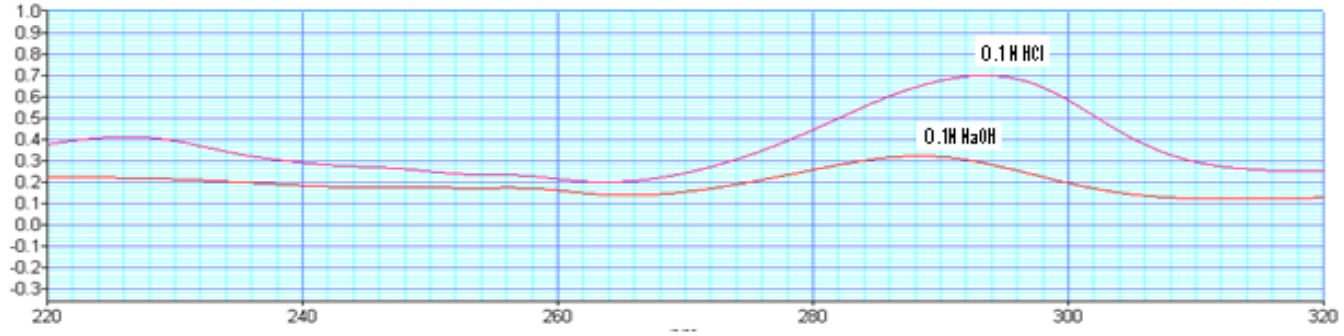
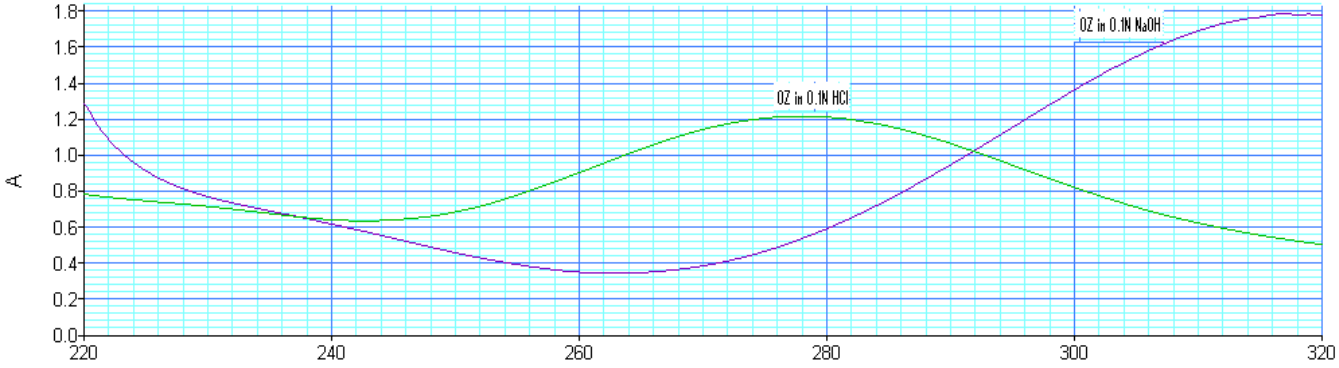


Fig 2. A) Ornidazole in 0.1N HCl. B) Ornidazole in 0.1N NaOH.



The influence of other parameter scan speed was studied to optimize the signal of spectra to give good selectivity and higher sensitivity in detection. Medium scan speed 480 nm/min was chosen throughout the work. Study of the time effect on the absorbance of OF and OZ was performed in 0.1 N NaOH *vs.* 0.1 N HCl. It was confirmed that absorbance varies with time. However, in this study it was used that, measuring the absorbance at zero time gave reproducible results with no need to wait for complete hydrolysis of the drug.

CONCLUSION

Because the chromatographic methods are more expensive, time consuming, and need more steps, the proposed difference absorbance method is adequate for routine analysis as an alternative technique, simple and cheap. Further, this technique offers a substitute approach to the enrichment of sensitivity and specificity in drug analysis. Experimentally no interference from tablet excipients was observed. The values of % RSD and correlation of coefficient for determination were found to be (%RSD 0.48-1.08) and correlation coefficient was 0.999 for both the drugs. The results of recovery studies for tablet were found to be in the range of 98-101 % for both drugs values are reported in Table 5. It can be easily and conveniently adopted for routine quality control analysis. The proposed method was accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic validated as per ICH guidelines.

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