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Simultaneous Determination of Ofloxacin and Flavoxate Hydrochloride by Absorption Ratio and Second Derivative UV Spectrophotometry

Mahesh Attimarad

Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa, KSA

ABSTRACT: The objective of this study was to develop simple, precise, accurate and sensitive UV spectrophotometric methods for the simultaneous determination of ofloxacin (OFX) and flavoxate HCl (FLX) in pharmaceutical formulations. The first method is based on absorption ratio method, by formation of Q absorbance equation at 289 nm (λ_{max} of OFX) and 322.4 nm (isoabsorptive point). The linearity range was found to be 1 to 30 $\mu\text{g/ml}$ for FLX and OFX. In the method-II second derivative absorption at 311.4 nm for OFX (zero crossing for FLX) and at 246.2 nm for FLX (zero crossing for OFX) was used for the determination of the drugs and the linearity range was found to be 2 to 30 $\mu\text{g/ml}$ for OFX and 2-75 $\mu\text{g/ml}$ for FLX. The accuracy and precision of the methods were determined and validated statistically. Both the methods showed good reproducibility and recovery with % RSD less than 1.5%. Both the methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of OFX and FLX in combined dosage form.

KEYWORDS: Spectrophotometry, second derivative, absorption ratio, ofloxacin, flavoxate HCl, Validation

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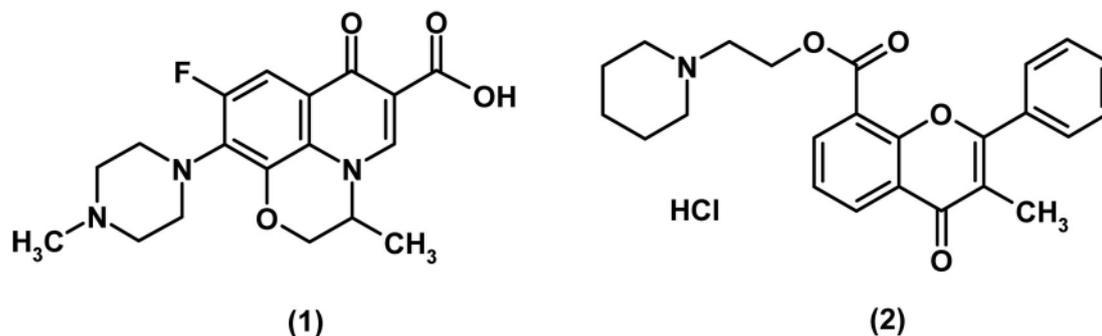
INTRODUCTION

Flavoxate hydrochloride (FLX), 3-methylflavone-8-carboxylic acid β -piperidinoethyl ester hydrochloride (Fig. 1) is strong smooth muscle relaxant, with selective action on the pelvic[1]. It is used for the symptomatic relief of pain, urinary frequency, and incontinence associated with inflammatory disorders of the urinary tract. It is also used for the relief of vesico-urethral spasms resulting from instrumentation or surgery[2]. The literature survey reveals that FLX was analyzed in its pharmaceutical preparations by Ultra-violet spectrophotometry[3,4] high performance liquid chromatography[5-8] and potentiometric determination in combination[10]. The official method for determination of FLX is

non-aqueous titration using perchloric acid as titrant in pure form and spectrophotometry in tablets [4]. Gilliland et. al., reported the LC-MS/MS determination of Flavoxate and 3-Methylflavone-8-Carboxylic acid (MFA) in Human NaF Plasma[11].

Ofloxacin (OFX) a synthetic fluoroquinolone, anti-infective agent has an expanded spectrum of activity and increased antibacterial potency compared with nonfluorinated quinolones [12]. Chemically it is 9-fluoro-2, 3 dihydro-3-methyl-10(4-methyl-1-piperazynyl-7-oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid (Fig.1). It acts by targeting bacterial DNA gyrase and topoisomerase IV. Extensive literature survey reveals that various analytical methods have been reported for the estimation of OFX in single and combination form such as spectrophotometric [13-15], conductometric [16], HPLC [17-22] and LC/MS/MS [23-24].

Corresponding Author:
Email: mattimarad@gmail.com

Figure 1: Structures of Ofloxacin (1) and Flavoxate HCl (2)

A solid pharmaceutical formulation containing Flavoxate HCl 200 mg and Ofloxacin 200 mg (film coated tablets) is available in the market for the treatment of urinary tract infections associated with dysuria, nocturia and suprapubic pain. For routine analysis a simple, rapid and cost effective analytical method is preferred. But, till date no single method is yet reported for simultaneous estimation of FLX and OFX. The objective of the present study was to develop simple, rapid, accurate, reproducible, validated and economical analytical methods for the simultaneous determination of OFX and FLX from pure and tablet formulation. Media used was double distilled water.

EXPERIMENTAL

Chemicals and Reagents

Double beam UV-visible spectrophotometer (SHIMADZU 1700), connected to computer and loaded with UV-probe software was used. For all absorbance measurements 1 cm matched quartz cuvettes were used. Shimadzu AUX220 balance was used for weighing the samples. Demineralized and double distilled water and Whatmann filter paper (no.41) were used throughout the experimental work. The sample of ofloxacin was procured from Micro Labs, India and flavoxate HCl from WINDLAS Biotech LTD, India. Multicomponent tablet ZENFLOX UTI (OFX 200 mg and FLX 200mg per tablet) marketed by Mankind Pharma LTD, New Delhi, India was purchased from local market.

Preparation of Standard Stock Solution

Standard stock solutions of OFX and FLX (200 µg/ml) were prepared by dissolving accurately weighed 20 mg of the each drugs in water using 100 ml volumetric flasks separately. The working

standard solutions of these drugs were obtained by diluting the stock solutions in water.

METHOD DEVELOPMENT

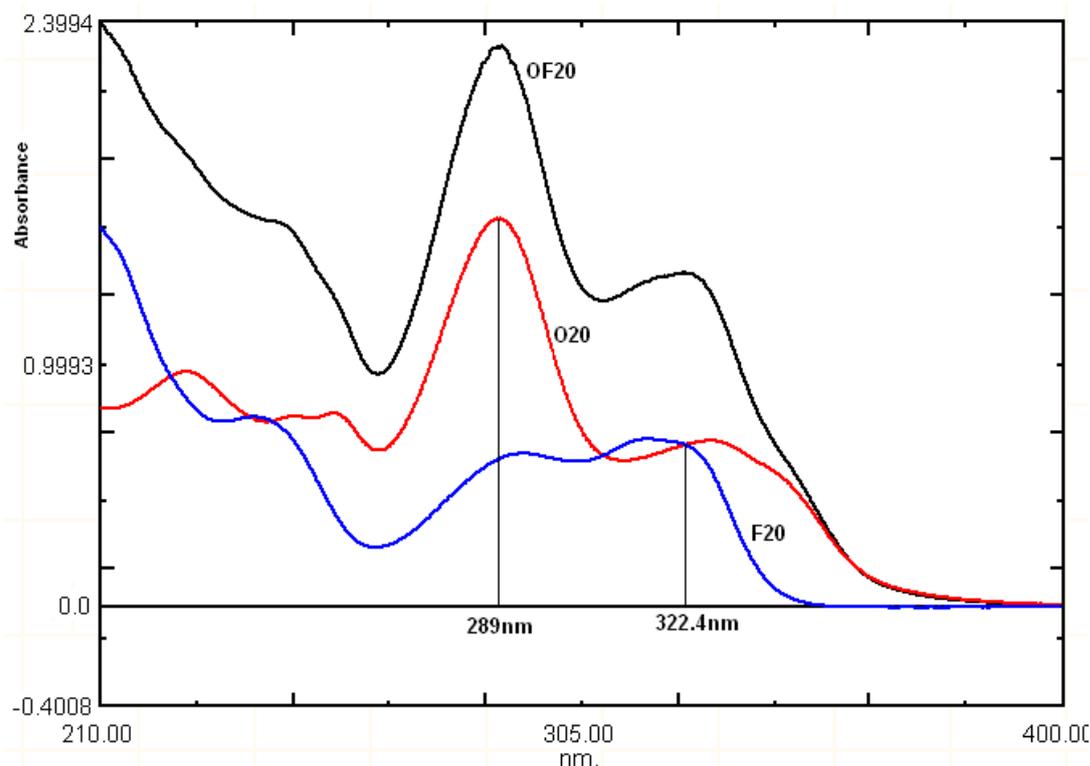
Method I (Q-Absorbance ratio method):

Q-Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and other being the max of one of the two compounds. From the stock solutions, working standard solutions of OFX (20 µg/ml) and FLX (20 µg/ml) were prepared by appropriate dilution and were scanned in the 200-400 nm UV range to determine the maximum absorbance (λ_{max}) and isoabsorptive point. OFX and FLX have λ_{max} at 289 nm and at 317.6 nm, respectively. Both the drugs were found to have same absorbance at 322.4 nm (isoabsorptive point). The wavelengths selected for analysis were 289 nm (λ_{max} of OFX) and 322.4 nm (isoabsorptive point) (Fig.2). A series of standard solutions ranging from 1-30 µg/mL of both the drugs OFX and FLX were prepared and the absorbance of solutions was recorded at 289 nm and 322.4 nm to plot a calibration curve of absorbance versus concentration. The calibration curves were found to be linear in the concentration range under study. Absorptivity values of OFX and FLX were determined at selected wavelengths and are presented in Table-1.

The concentrations of two drugs in mixture were calculated by using following equations:

$$C_{\text{OFX}} = \frac{0.641 - 1.06}{0.405 - 1.06} \times \frac{A_2}{33.214} \quad (1)$$

$$C_{\text{FLX}} = \frac{0.641 - 0.405}{1.06 - 0.405} \times \frac{A_2}{33.20} \quad (2)$$

Figure 2: UV absorption spectra of OFX(20 µg/ml, O20), FLX(20 µg/ml, F20) and combination (20 µg/ml each, OF20)**Table 1:** Absorptivity Values at 289 nm (λ_{max} of OFX) and 322.4 nm (isoabsorptive point)

	Absorptivity at 289 nm		Absorptivity at 322.4 nm	
	Ofloxacin	Flavoxate HCl	Ofloxacin	Flavoxate HCl
*Mean	$a_{x_1} = 81.944$	$a_{y_1} = 31.285$	$a_{x_2} = 33.214$	$a_{y_2} = 33.209$
\pm RSD	0.13	0.09	0.17	0.51

* Absorptivity values are the mean of six determinations. RSD. is relative standard deviation. a_{x_1} and a_{x_2} absorptivities of Ofloxacin at 289 nm and 322.4 nm, respectively; a_{y_1} and a_{y_2} absorptivities of Flavoxate HCl at 289 nm and 322.4 nm, respectively

where, A_2 is the absorbances of mixture at 322.4 nm at different concentrations of ofloxacin and flavoxate.

Method II (Second derivative Spectrophotometric method)

In the second order derivative method, solutions of 20 µg/ml of OFX and FLX were prepared separately. Both the solutions were scanned in the spectrum mode from 200 to 400 nm. The absorption spectra thus obtained were derivatized from first to fourth order. Second order derivative was selected

for analysis of both the drugs. The zero crossing wavelengths, 311.4 nm and 246.2 nm were selected for OFX and FLX, respectively. The calibration curves were constructed by scanning different concentration solutions and derivitizing the spectrum. The absorbances of OFX and FLX were measured at a 311.4 nm and 246.2 nm, respectively and working calibration curves of both the drugs were plotted separately. The concentration of individual drug present in the mixture was determined against calibration curve in quantitation mode.

Analysis of Pharmaceutical Dosage Forms

Twenty tablets were weighed; their average weight were determined and finely powdered. The correct amount of powder (equivalent to 20 mg of the drugs) (n=5) was transferred into 100ml volumetric flask and dissolved in 60 ml of water by stirring for 15 min. Than volume was made up to the mark with water. After filtration, an appropriate aliquots were subjected to above methods and the amount of OFX and FLX were determined.

Validation of the Methods

The developed methods were validated according to ICH guideline [25-26] for validation of analytical procedures in order to determine the linearity, selectivity, precision and accuracy for each analyte.

Linearity

Linearity of the proposed methods were determined by analyzing the solutions of OFX in the range of 1-30 µg/ml and FLX in the range of 1 to 75 µg/ml. The Limit of Detection (LOD) and Limit of Quantification (LOQ) was then established by evaluating the minimum level at which the solution of the drug can be readily quantified with accuracy. LOD and LOQ were calculated according to the $3.3\sigma/s$ and $10\sigma/s$ criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Accuracy

Accuracy of the method was determined by performing recovery studies by standard addition method in which pre-analyzed samples were taken (10 µg/ml) and standard drug was added at three different levels i.e. 50%, 100% and 150%. The total concentrations were determined by using the proposed methods. Each level was made in triplicate. The % recovery of the added pure drugs were calculated as $\% \text{ recovery} = [(C_t - C_s) / C_a] \times 100$, where C_t is the total drug concentration measured after standard addition; C_s drug concentration in the formulation sample; C_a drug concentration added to formulation.

Precision

Repeatability was assessed by preparing fresh 20 µg/ml solutions from independent stock solutions and measuring the absorbances at selected wavelengths (n=6). Intra-day and inter-day precision of the method were confirmed by repeating the

absorbance at 5µg/ml, 10 µg/ml, 15 µg/ml three times in a day and also on two different days.

RESULTS AND DISCUSSION

Two validated spectrophotometric methods were developed for the simultaneous estimation of OFX and FLX in tablet formulations. Both the drugs are soluble in water and showed good UV absorption, hence to develop simple, economical and nature friendly analytical methods, water has been selected as solvent.

The UV spectra of OFX and FLX 20 µg/ml each and their mixture were reproduced in Fig.2. The λ_{max} of OFX and FLX were found to be 289 nm and 317.6 nm, respectively. Three isoabsorptive points were found at 223.2 nm, 311.2 nm and 322.4 nm. In the synthetic mixture mean recovery and standard deviation were found to 100.85 ± 1.92 , 101.53 ± 1.66 and 99.24 ± 1.09 at 223.2 nm, 311.2 and 322.4 nm respectively. For constructing Q equation 322.4 nm was selected due to low standard deviation and good mean recovery and another wavelength was 289 nm. In the first method (absorption ratio) linear response obtained was in the concentration range of 1-30 µg/ml with correlation coefficient 0.9996 for OFX and 1-30 µg/ml with correlation coefficient 0.9998 for FLX (Table 2).

For second derivative spectroscopy, the spectra of the OFX and FLX obtained by scanning in water, were changed to second derivative spectra (Fig. 3). For the second derivative spectra 8 nm was found to be optimum $\Delta\lambda$, hence 8 nm was used. One maxima absorbance and one minima wavelengths were found for OFX at 311.4 nm and 334.7 nm respectively, where zero crossing for FLX (Fig. 3 and 4). In the synthetic mixture mean recovery and standard deviation were found to 100.51 ± 0.78 and 99.81 ± 1.68 at 311.4 nm and 334.7 nm respectively. Two minima wavelengths at 246.2 nm, 321.4 nm and three maxima wave lengths at 234.2 nm, 260.4 nm and 352.2 nm were found for FLX where zero crossing for OFX (Fig.3 and 5). In the synthetic mixture mean recovery and standard deviation were found to 100.95 ± 0.82 99.48 ± 1.89 , 101.8 ± 1.69 , 101.26 ± 1.99 and 101.25 ± 2.01 at 246.2 nm, 321.4 nm 234.2 nm, 260.4 nm and 352.2 nm respectively. The 311.4nm peak for OFL and 246.2 nm peak for FLX were selected for determination of drug in synthetic mixture and pharmaceutical formulation due to low standard deviation and good mean recovery.

Table 2: Validation parameters of both the methods

	Method I	Method II
Beer's law range		
Ofloxacin ($\mu\text{g/ml}$)	1-30	2-30
Flavoxate HCl ($\mu\text{g/ml}$)	1-30	2-75
Wavelength (nm)	289, 322.4	311.4, 246.2
Correlation Coeffi. (r^2)	0.9996, 0.9998	0.9991, 0.9997
Slope	0.081, 0.062	1.46×10^{-4} , 1.63×10^{-5}
Intercept	0.0015, 0.011	1.65×10^{-4} , 6.39×10^{-5}
LOD	0.32, 0.25	0.60, 0.31
LOQ	0.96, 0.76	1.82, 0.94
%RSD		
Repeatability	0.68, 0.79	0.95, 0.86
Intraday precision	0.40, 0.98	0.92, 0.89
Interday precision	1.26, 1.06	1.35, 1.18

In case of r^2 , slope, intercept, LOD, LOQ & %RSD value 1st is Ofloxacin & 2nd is Flavoxate HCl.

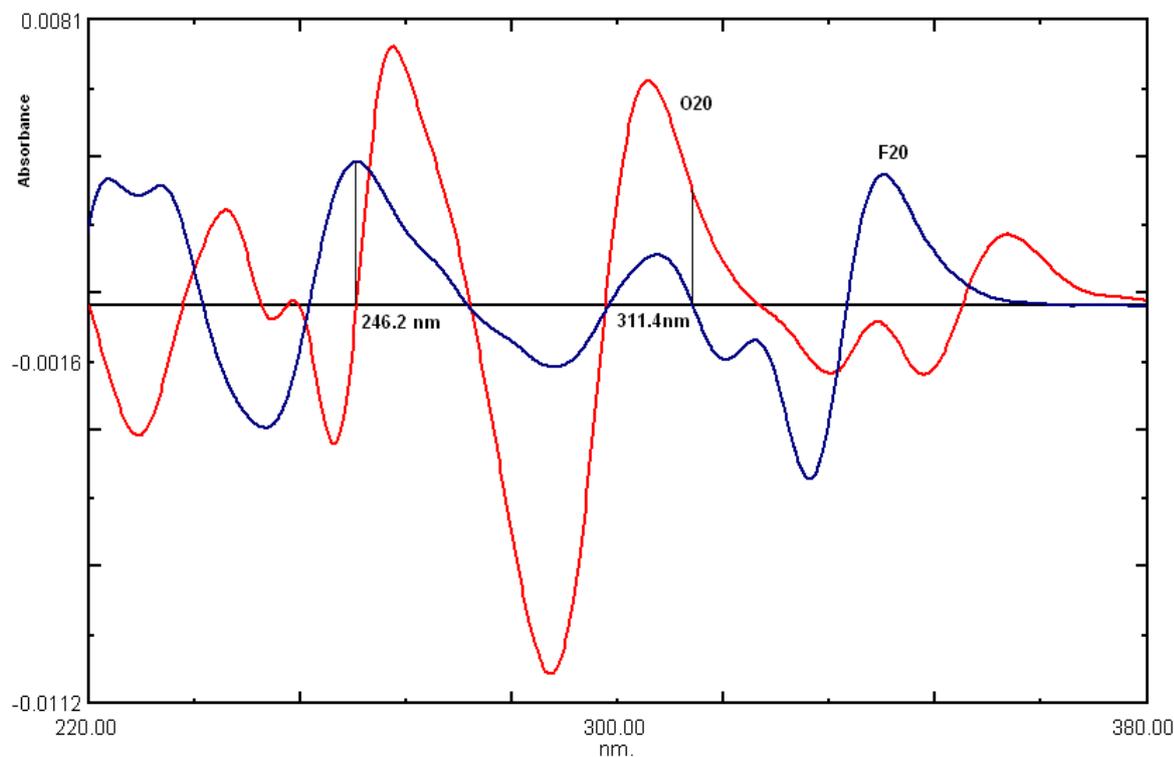
Figure 3: Second order derivative UV absorption spectra of OFX(20 $\mu\text{g/ml}$, O20), FLX(20 $\mu\text{g/ml}$, F20)

Figure 4: Second order derivative UV absorption spectra of OFX(2-30 $\mu\text{g/ml}$)

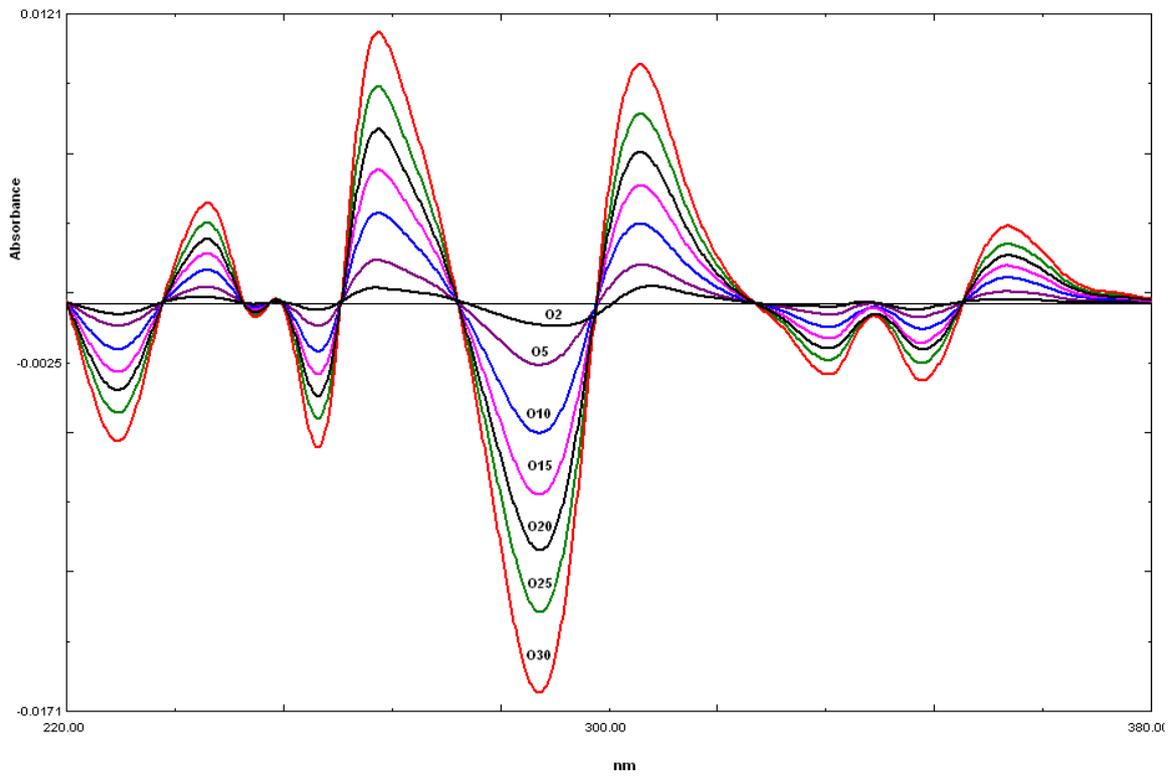


Figure 5: Second order derivative UV absorption spectra of FLX (2-75 $\mu\text{g/ml}$)

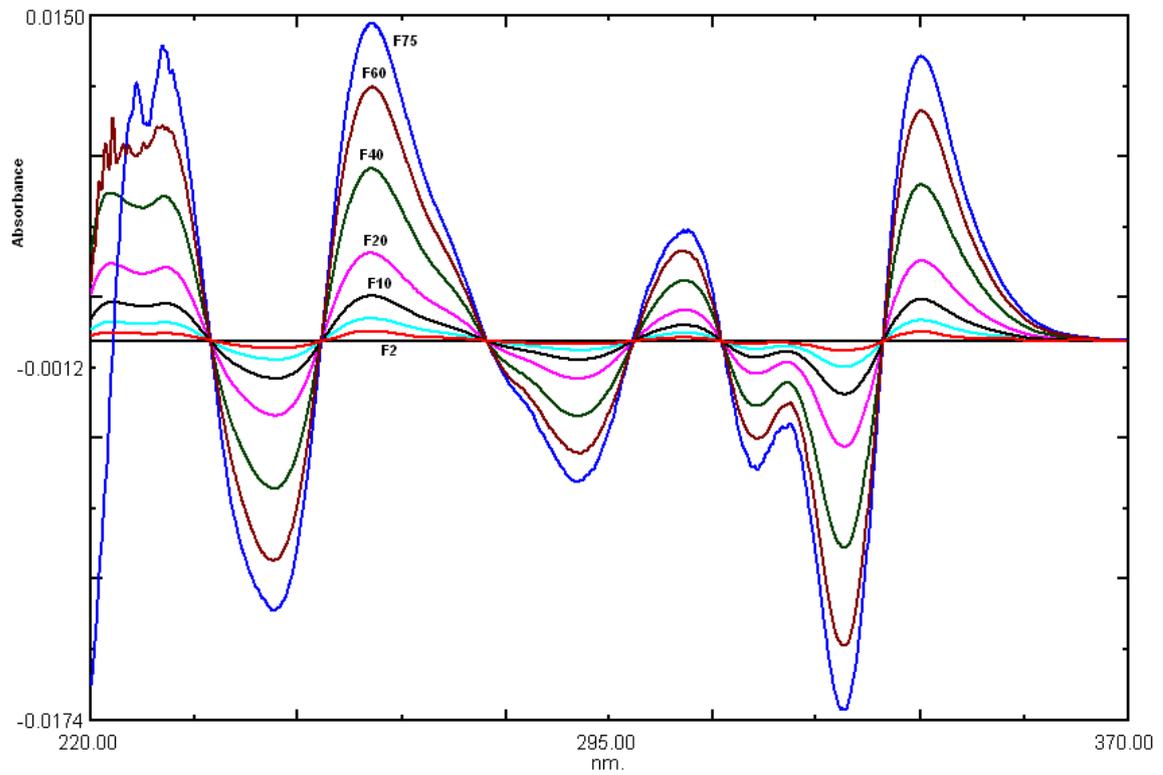


Table 3: Recovery studies

Sr. No.	Amount of Pure Drug Added ($\mu\text{g/ml}$)		% Recovery			
			Method I		Method II	
	OFX	FLX	OFX	FLX	OFX	FLX
1	5	5	101.39	98.97	99.12	99.60
2	10	10	99.54	101.31	100.58	101.16
3	15	15	101.28	99.23	99.23	100.97
		Avg.	100.736	99.653	99.643	100.576
		\pm RSD	1.02	0.86	0.818	0.847

Table 4: Results of Assay of tablets by Method I & II

Sr. No.	Wt. taken (mg each)	% Label Claim				
		Method I		Method II		
	OFX	OFX	FLX	OFX	FLX	
1	20.10	99.10	101.14	99.13	101.15	
2	20.00	101.9	101.32	99.16	100.70	
3	20.10	99.26	101.50	98.81	101.6	
4	20.10	100.54	100.52	99.61	101.91	
5	20.10	101.2	99.26	101.31	99.06	
		Avg.	100.34	100.748	99.604	100.884
		\pm RSD	1.121	0.89	0.998	1.112

Method linear response obtained was in the concentration range of 2-30 $\mu\text{g/ml}$ for OFX and 2 to 75 $\mu\text{g/ml}$ for FLX with correlation coefficient more

than 0.999. The LOD and LOQ for method I and method II for both the drugs were calculated (Table 2) and tested practically.

Accuracy

The excellent mean % recovery values, close to 100% and their low standard deviation (RSD 1.02%) represent high accuracy of the analytical methods. The validity and reliability of the proposed methods were further assessed by determining the mean percentage recovery at 50, 100 and 150 % level. The average % recovery ranges from 99.6% to 100.7% for OFX and FLX for both the methods and are presented in Table-3.

Precision

Precision was determined by studying repeatability and intermediate precision. Repeatability (% RSD) ranged from 0.68% to 0.95% for both the drugs in both the methods (Table 2). Repeatability results indicated the precision under the same operating conditions over a short interval of time and inter assay precision. The inter and intra-day variations (% RSD is less than 1.35%) for both drugs in both the methods. RSD values found for both the drugs in both the methods were well with the acceptable range indicating that these methods have excellent repeatability and intermediate precision.

Estimation of formulation

Solid pharmaceutical formulation (tablets) was analyzed and amount of OFX and FLX determined by proposed methods, the average % amount ranges from 99.604% to 100.884% as shown in Table-4 with standard deviation not more than 1.121%. Assay values of formulation was same as mentioned in the label claim indicating that the interference of excipient matrix is insignificant in estimation of OFX and FLX by proposed analytical methods.

CONCLUSION

A absorption ratio and second derivative spectrophotometric methods were developed and showed the advantages of being simple, economical, rapid, accurate, precise and nature friendly (water is the media). The sample recoveries from formulation were in good agreement with their respective label claims, which suggested non-interference of formulations excipients in the estimation. Moreover, the present methods are fast with respect to analysis time as compared to sophisticated chromatographic techniques. Hence can be used for the routine analysis of ofloxacin and flavoxate HCl in pharmaceutical formulations and for dissolution samples of formulations.

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