Stability Indicating Assay Method by UV Spectrophotometry for Estimation of Ofloxacin and Flavoxate Hydrochloride in Tablet Dosage Form

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Abstract - *The present research work discusses the development of a stability-indicating UV spectrophotometric method for the estimation of Ofloxacin and Flavoxate hydrochloride in tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) was found to be 266 nm for ofloxacin and 272 nm for flavoxate. Degradation studies of ofloxacin and flavoxate showed prominent degradation in acid hydrolysis.*

Key words - *Stability Indicating, Ofloxacin, Flavoxate, UV spectrophotometry.*

I. INTRODUCTION

Ofloxacin (OFL) is 9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazin-yl)-7-oxo-7H-pyridol[1,2,3-de]-1,4,-benzoxazine-6-carboxylic acid. It is a member of quinolone class of antibacterial drugs wherein the 1 and 8 positions are joined in the form of 1,4-oxazine ring. This quinolone is also widely distributed into most body fluids and tissues. Higher concentration of ofloxacin is achieved in CSF than can be obtained with ciprofloxacin. The oral bioavailability of ofloxacin is 95% to 100%. The amount of an administered dose of ofloxacin excreted in the urine in a 24 – 48 hour period range from 70% to 90%. There relatively little biliary excretion of this drug. The elimination half life of ofloxacin ranges from 4.5 to 7 hours. [1] Flavoxate is indicated in urinary frequency, urgency and dysuria associated with lower urinary tract infection. It is an anti-muscarinic, has high affinity for receptors in urinary bladder and salivary glands with additional smooth muscle relaxant and local anaesthetic properties. It is relatively selective for M_1/M_3 subtypes than for M_2. Because of vasicoselective action it is used for instability resulting in urinary frequency and urge incontinence. Anticholinergic side effects are common after oral dosing, but intravasical instillation increases bladder capacity with few side effects.[2]

The concept of analytical chemistry lies in the precise and accurate measurements. This determination requires highly sophisticated instruments and methods like HPLC, gas chromatography, HPTLC, spectrophotometry, fluorimetry etc. Instrumental methods are sensitive, accurate, precise and desirable for regular determination of drug in formulations, thereby is advantageous than the conventional volumetric methods. After literature survey, it was found that OFL was estimated independently and in combination with other drugs by several chromatographic [3-12], spectrometric[13] and flourimetric[14] method in pharmaceutical formulation and in biological samples. Similarly, FLA was estimated by HPLC[15-16], ultraviolet spectrophotometry[17], voltammetry[18], capillary electrophoresis [19] and potentiometric[20] determination techniques. In view of the need of analytical method in the quality control laboratories for routine analysis of OFL and FLA in formulations, attempts are being made to develop simple and accurate instrumental methods for simultaneous estimation of OFL and FLA.

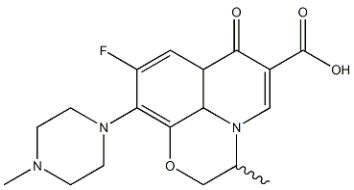


Figure 1: ***Structure of OFL***

The present work describes the development of a simple, precise, accurate and reproducible chromatographic method for the simultaneous estimation of OFL and FLA in pharmaceutical dosage form. The developed method was

validated in accordance with ICH Guidelines [22].

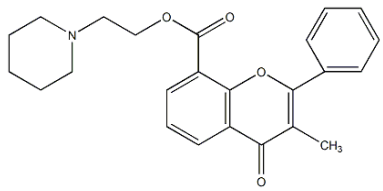


Figure 2: Structure of FLA

II MATERIAL AND METHODS

Reagents

OFL and FLA were purchased from Active Pharm Lab, Hyderabad. Hydrochloric acid, sodium hydroxide (AR Grade) and hydrogen peroxide (AR Grade) was purchased from Merck (Mumbai).

Instruments

UV-Visible double beam spectrophotometer (Jasco V-630), electronic balance (AX 200, Shimadzu), oven were utilized for present work.

Preparation of standard stock solutions

The stock solutions (100µg/ml) of OFL and FLA were prepared by dissolving accurately 10 mg of drug in sufficient quantity of methanol followed by sonication for 15 min and finally adjusted the volume up to 100 ml with methanol.

Selection of analytical wavelengths

Appropriate dilutions with methanol were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm and λ_{max} was determined for each drug.

Preparation of calibration curve

For each drug, appropriate aliquots were pipette out from standard stock solution into the series of 10 ml volumetric flask and the volume was made up to the mark with methanol to get concentration of 5-25 µg/ml of both OFL and FLA. Solutions of different concentrations for each drug were analyzed at their respective wavelengths and absorbance was recorded.

Simultaneous equation method

Two wavelengths selected for the method (266 nm and 272 nm) that are absorption maximas of OFL and FLA respectively in methanol. Standard stock solution of 100 µg/ml of both the drug was prepared separately in methanol. The stock solution of both drugs was further diluted separately with methanol to get series of standard solution of 5-25 µg/ml for both OFL and FLA. The absorbance was measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations.

$CX = A1ay2 – A2ay1 / ax1ay2 – ax2ay1$ . . Eq. 1

$CY = A1ax2 – A2ax1 / ay1ax2 – ay2ax1$ . . Eq. 2

Where, A1 and A2 are absorbances of mixture at 266 nm and 272 nm respectively, ax1 and ax2 are absorptivities of OFL at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of FLA at λ1 and λ2 respectively. Cx and Cy are concentrations of OFL and FLA respectively.

Preparation for marketed formulation assay

Marketed tablet containing 200 mg of OFL and 200 mg of FLA were used. Twenty tablets were weighed and average weight was calculated. The contents of tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 10 mg of OFL and 10 mg of FLA were transferred to 100 ml volumetric flask and dissolved in 25 ml of methanol solution by sonication for 10 min and volume was then adjusted up to 100 ml with methanol. The solution was filtered through Whatmann filter paper No. 41 and aliquot of 10µg/ml was diluted. The absorbance of sample solution was measured at selected wavelengths.

A. Method Validation

The analytical method was validated with respect to parameters such as linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, robustness, and recovery (ICH Q2(R1), 2005).

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve.

Accuracy

Recovery studies were carried out by applying the method to drug contents present in solid dosage form to which known amount of standard OFL and standard FLA was added at 80 %, 100 % and 120 % levels. The technique includes addition of standard drug solution to pre-analyzed sample solution. The recovery

study was performed three times at each level.

Precision

The precision of the method, as intra-day repeatability was evaluated by performing six independent assays of the test sample preparation and calculating the % RSD. The intermediate (interday) precision of the method was checked by performing same procedure on different days under the same experimental conditions.

LOD and LOQ

The LOD and LOQ of OFL and FLA were calculated by mathematical equation:

$$LOD = 3.3 \times \sigma / S$$

σ= Standard deviation of the response, S = slope of the calibration curve

$$LOQ = 10 \times \sigma / S$$

σ= Standard deviation of the response, S = slope of the calibration curve

B. Forced Degradation Studies

Forced degradation studies were performed on OFL and FLA to prove the stability indicating property of the method. The stress conditions employed for degradation study included light exposure, acid degradation, alkaline degradation, thermal degradation, oxidative degradation. (ICH Q1A (R2), 2003).

Acid degradation

Solution for acid degradation studies was prepared in methanol and 1 N hydrochloric acid for OFL & 0.1N hydrochloric acid for FLA. The resultant solutions were analysed after 3 hours.

Alkali degradation

Solution for alkaline degradation studies was prepared in methanol and 1.5N sodium hydroxide for OFL, & for FLA 1N sodium hydroxide was used. The resultant solutions were analysed after 3 hours.

Oxidative studies

In oxidative studies both drugs were treated with 6% H_2O_2, kept for three hours and then volume was made with methanol and the solutions were analysed.

Thermal degradation studies

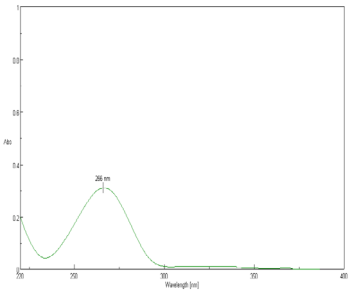
Both the drugs were exposed to dry heat of 50°C in a convection oven for 6 hours. The API powder was removed from the oven and powder equivalent to the 10 mg for both drugs were weighed and then solutions were prepared in methanol and analysed.

Photostability studies

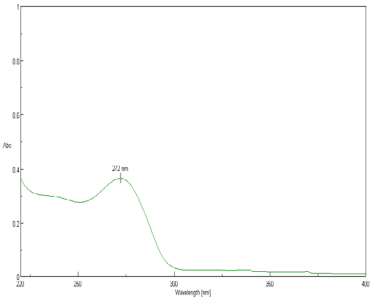
Both the drugs were exposed to the short wavelength (254 nm) of UV light for 10 hours. After that, powder equivalent to the 10 mg for both drugs was weighed and then solutions were prepared in methanol and analysed.

III RESULTS AND DISCUSSION

The UV scanning showed spectrum exhibiting λ_{max} of 266 nm and 272 nm for OFL and FLA respectively (**Figure 3**).



OFL



FLA

Figure 3 : *λ_{max} of OFL and FLA*

The linearity of the proposed method was investigated in the range of 5-25 µg/ml for both OFL and FLA respectively. Calibration curves showed a linear relationship between the absorbance and concentration.

Table 1 : Calibration curve parameter

PARAMETERS	OFL (266 nm)	FLA (272nm)
Slope*	0.0315	0.0363
Intercept*	0.0116	0.0224
Correlation coefficient*	0.9995	0.9991
Linearity range (µg/ml)	5-25 µg/ml	5-25 µg/ml

The line equation for OFL (y = 0.0315x - 0.0116) with r^2 of 0.9995 and for FLA (y = 0.0363x + 0.0224) with r^2 of 0.9991 was obtained (**Table 1, Figure 4**).

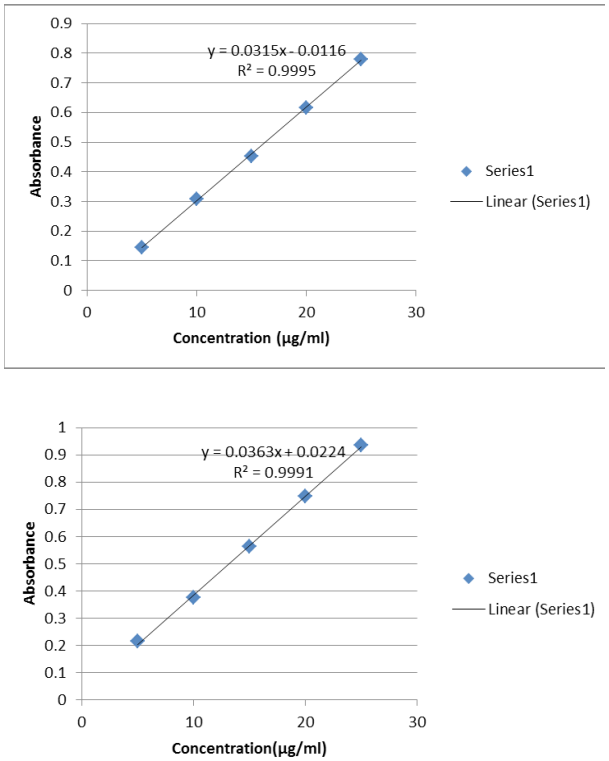


Figure 4: *Calibration curve of OFL and FLA*

The result of marketed formulation assay analysis was found within the prescribed limits. The results of tablet analysis are summarized in **Table 2**.

Table 2: Analysis of marketed formulation

Sr. No.	Amount Taken (ppm)		Amount Found (ppm)		*% Label Claim	
	OFL	FLA	OFL	FLA	OFL	FLA
1	10	10	10.23	10.04	102.3	100.4

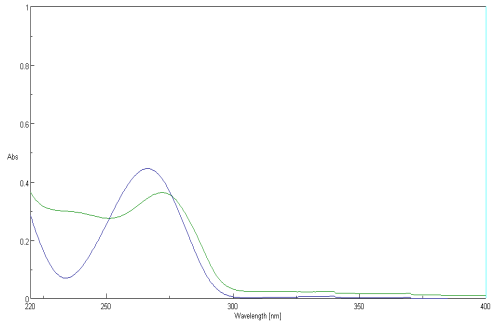


Figure 5: Overlaid spectra of OFL and FLA

The overlaid spectra of OFL and FLA exhibited λmax of 266 nm and 272 nm for OFL and FLA respectively which were quite clearly separated from each other (**Figure 5**).

The LOD of OFL and FLA were found to be 0.049 µg/ml and 0.0705 μg/ml and the LOQ of OFL and FLA were found to be 0.15 μg/ml and 0.21 µg/ml. Validation was performed as per ICH guidelines (ICH Q2(R1),2005) for Linearity, accuracy, precision, LOD and LOQ. The results of method validation parameters are summarized in **Table 3**. The result of forced degradation of OFL was summarized in **Table 4.** And the result of forced degradation of FLA was summarized in **Table 5.** The stability studies of OFL and FLA were conducted and the degradation characteristics were found to be much more prominent in acid hydrolysis in both OFL and FLA (**Figure 6**). The spectrum of alkali, oxidative, thermal and photo degradation studies are evident. (**Figure 7, 8, 9, 10)**

Table 3: Validation parameter for OFL and FLA

No	Parameters	OFL	FLA
1	Linearity	5-25 µg/ml	5-25 µg/ml
2	Accuracy (80%)	99.76%	99.66%
	Accuracy (100%)	99.75%	99.98%
	Accuracy (120%)	99.04%	99.93%
3	Intraday precision (%RSD)	0.11	0.08
4	Interday precision (%RSD)	0.37	0.25
5	LOD	0.049	0.0705
6	LOQ	0.15	0.21

Table 4 : Results of forced degradation study of OFL

Stress condition	Time	% Degradation
Acidic Degradation	3 hours	18.8
Alkali Degradation	3 hour	8.3
Oxidative Degradation	3 hour	2.7
Thermal Degradation	6 hours	1.6
Photo Degradation	10 hours	1.5

Table 5 : Results of forced degradation study of FLA

Stress condition	Time	% Degradation
Acid Degradation	3 hours	18.86
Alkali Degradation	3 hour	16.4
Oxidative Degradation	3 hour	5.40
Thermal Degradation	6 hours	1.8
Photo Degradation	10 hours	1.3

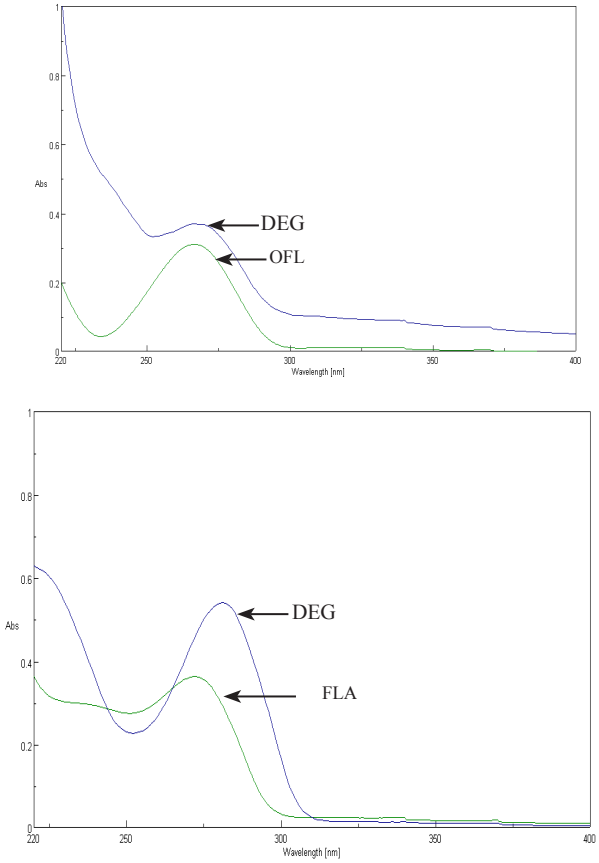


Figure 6: Acid degradation of OFL and FLA
DEG: Degradation Product, OFL: Ofloxacin, FLA: Flavoxate Hydrochloride

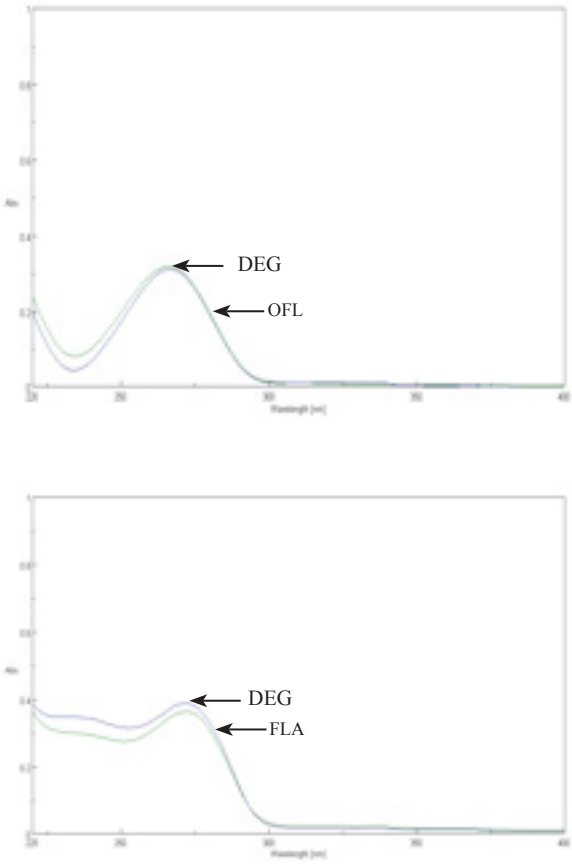
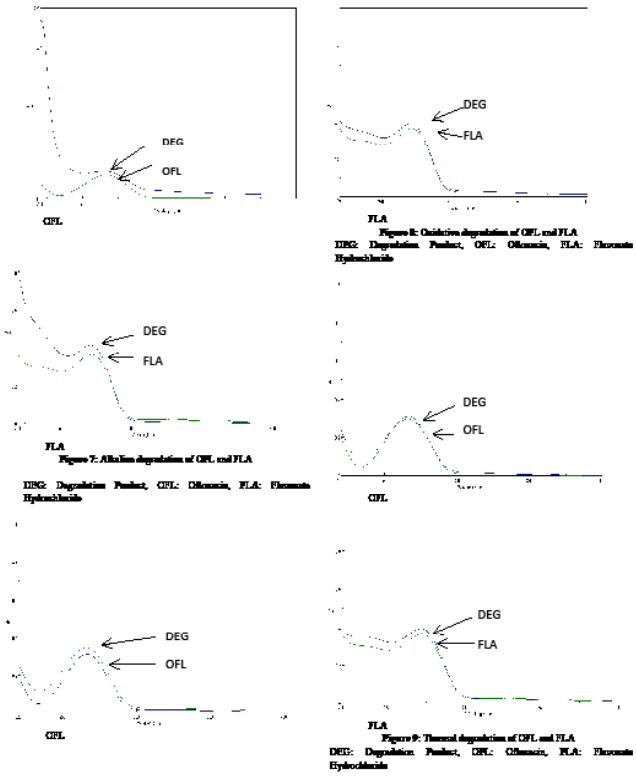


Figure 10: Photo degradation of OFL and FLA
DEG: Degradation Product, OFL: Ofloxacin, FLA: Flavoxate Hydrochloride



IV CONCLUSION

The proposed method is found to be simple, sensitive and reproducible and hence it can be used in routine analysis for simultaneous determination of stability indicating UV spectrophotometric method for OFL and FLA in bulk. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

ACKNOWLEDGMENT

The authors are thankful to the Management and Principal, P.E.S. Modern College of Pharmacy, Nigdi, Pune for providing the facilities to carry out the study.

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