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Development and validation of RP-HPLC method for the simultaneous estimation of Ofloxacin and Flavoxate HCl in combined dosage form.

Md. Azheruddin[1*], Jomol Joseph[1], Quddus Junaidy[1].

1. Department of Pharmaceutical Analysis & Quality Assurance, School of Pharmacy, Anurag Group of Institutions, Venkatapur, R.R Dist, Andhra Pradesh, India.

ABSTRACT

A simple, precise and sensitive reverse-phase high performance liquid chromatographic method was developed and validated for the simultaneous estimation of Ofloxacin and Flavoxate Hcl in pharmaceutical formulations. Chromatographic separation was performed on a High performance liquid chromatography equipped with auto sampler and UV detector. Good sensitivity for all analyte was observed with UV detection at wavelength of 301 nm, Separation was performed on a BDS Hypersil C18 (250 X 4.6mm) 5µm,using a mixture of 0.1% Triethyl Amine buffer pH 4 and Acetonitrile in the ratio of (40:60, v/v). The method results in excellent separation with good resolution between the two analytes. The within day variation between Ofloxacin and Flavoxate Hcl 1.72 and 1.97 % . The recovery was greater than 95 % with RSD less than 1.95 %. The method was validated according to ICH guidelines by performing linearity, accuracy, precision, limits of quantitation and selectivity. The results show the method is suitable for its intended use.

Keywords: Ofloxacin (OX), Flavoxate HCl (FX), HPLC, Simultaneous determination.

*Corresponding Author Email: azheruddin704@gmail.com

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INTRODUCTION

Chemically Ofloxacin 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 (Figure 1) and Flavoxate is 2-(1-piperidyl)ethyl 3-methyl-4-oxo-2-phenyl-chromene-8-carboxylate (Figure 2). Ofloxacin[1] is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. Flavoxate[2] acts as a direct antagonist at muscarinic acetylcholine receptors in cholinergically innervated organs. Its anticholinergic-parasympatholytic action reduces the tonus of smooth muscle in the bladder, effectively reducing the number of required voids, urge incontinence episodes, urge severity and improving retention, facilitating increased volume per void. Patients who had catheterization, urinary incontinence, & dysuria, in such cases there is all chances of bacterial infections like Escherichia coli, staphylococcus saprophyticus etc., in such cases Ofloxacin is given in combination with Flavoxate.

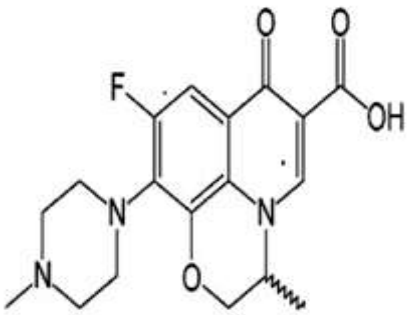

Figure 1: Structure of Ofloxacin

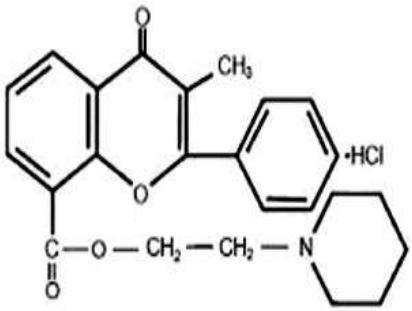

Figure 2: Structure of Flavoxate HCl

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 drugs in formulations, thereby is advantageous than the conventional volumetric methods. On the literature survey it was found that Ofloxacin was estimated independently and in combination with other drugs by several chromatographic[3-12], spectrometric[13] and flourimetric[14] methods in pharmaceutical formulations and in biological samples. Similarly Flavoxate was estimated by HPLC[15-16], Ultraviolet Spectrophotometry, Voltammetry[18], capillary electrophoresis[19] and potentiometric[20]

determination techniques.. And one analytical method was found for simultaneous estimation of Ofloxacin and Flavoxate in combination [21].

In view of the need analytical method in the quality control laboratories for routine analysis of Ofloxacin and Flavoxate in formulations, attempts are being made to develop simple and accurate instrumental methods for simultaneous estimation of Ofloxacin and Flavoxate and extend it for their determination in formulation and in laboratory prepared synthetic mixture. The present work describes the development of a simple, precise, accurate and reproducible chromatographic method for the simultaneous estimation of OX and FX in Pharmaceutical dosage form. The developed method was validated in accordance with ICH Guidelines.

MATERIALS AND METHODS

Instruments:

HPLC apparatus consisting of Shimadzu system , UV detector (set at 301nm), software LC Solutions and a injection volume with a 20 µL was used for development and evaluation of this method. Chromatographic separation was performed using a BDS Hypersil C18 (250X4.6)5µ with isocratic elution.

Chemicals and Reagents:

Analytical pure samples of OX and FX were provided by Macs Bio Pharma and AMIS Pharmaceuticals as gift samples respectively. Formulation, Zenflox- UTI (OX-200mg + FX200mg) manufactured by Mankind Pharma LTD, New Delhi, India, was purchased from a local pharmacy in Hyderabad.

The mobile phase consisted of a mixture of 0.1% Triethylamine buffer pH 4 and Acetonitrile in the ratio of (40:60, v/v) with flow rate as 1 ml/min. Wave length 301nm, Peak identity was confirmed by retention time comparison and the HPLC system was operated at room temperature.

Preparation of Mobile phase:

0.1 ml of Triethylamine was pippeted and dissolved it in to 100ml of HPLC water. The final pH 4.0 was adjusted using O-Phosphoric Acid. Finally composition of mobile phase was made to 0.1% Triethylamine pH 4: Acetonitrile (40:60). The prepared mobile phase was filter through 0.45µm membrane filter paper.

Preparation of standard solution:

Standard stock solution of Ofloxacin and Flavoxate HCl was prepared by dissolving 10mg of drug in 100ml of Mobile phase to get a concentration of 100µg/ml. From the above standard

stock solutions dilutions ranging from 100ng/ml – 1000ng/ml each of Ofloxacin and flavoxate HCl were prepared in 10ml volumetric flasks and the resultant dilutions were sonicated. Mobile phase was used as diluents

Preparation of Test solutions and Estimation of OX and FX in Formulation:

For analysis of commercial formulations 5 tablets (Zenflox UTI containing 200mg of OX and 200mg of FX) were weighed, powered and weight equivalent to 10mg of OX and 10mg of FX was taken and transferred into a volumetric flask and made upto 100ml with water, sonicated for 10min, filtered and further diluted with mobile phase to get the required concentration of respective drugs and the chromatograms were recorded at 301nm. Then the amount of drugs present in the formulation was calculated and the results are shown in (Table 5) along with %RSD values

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase combination were tried. A satisfactory separation and good peak symmetry was found in a mixture of 0.1% Triethylamine buffer pH 4 and Acetonitrile in the ratio of (40:60, v/v) at flow rate 1ml/min proved to be better than the other mixture in terms of resolution and peak shape. The optimum wavelength for detection was set at 301nm at which much better detector responses for both drug were obtained as seen from the UV spectra (Figure 3).

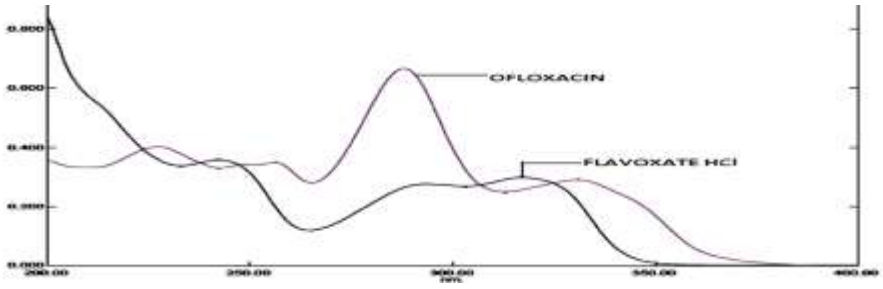

Figure 3: Over lay spectra of OX and FX

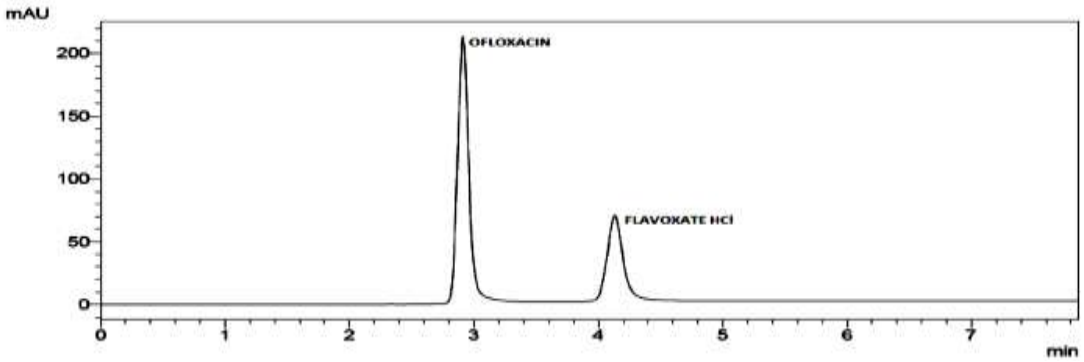

Figure 4: Chromatograms of Ofloxacin and Flavoxate at 301 nm

As shown in (Figure 4), the retention times were 3.10 for Ofloxacin and 4.61 for Flavoxate Hcl. The optical regression characteristics and validation parameters are showed in (Table 1).

VALIDATION OF THE PROPOSED METHOD

The developed chromatographic method for the simultaneous determination of Ofloxacin and Flavoxate Hcl was validated using ICH guidelines. Assessed validation parameters include linearity, limit of quantitation, selectivity, accuracy and repeatability.

Linearity:

Linearity of the proposed method was done by analyzing ten solutions in the range LOQ for both Ofloxacin and Flavoxate Hcl. Good linearity was observed over the range of 100-1000ng/ml for both Ofloxacin and Flavoxate Hcl. The calibration curve was made using concentration of the analytes versus peak area. The correlation coefficient from the linear regression analysis was calculated and found to be 0.997 and 0.997 for both the analytes. This indicates that there exists a good linear relationship between concentration of drugs and the peak area. The linear regression graphs of Ofloxacin in (Figures 5) and Flavoxate Hcl in (Figure 6) respectively.

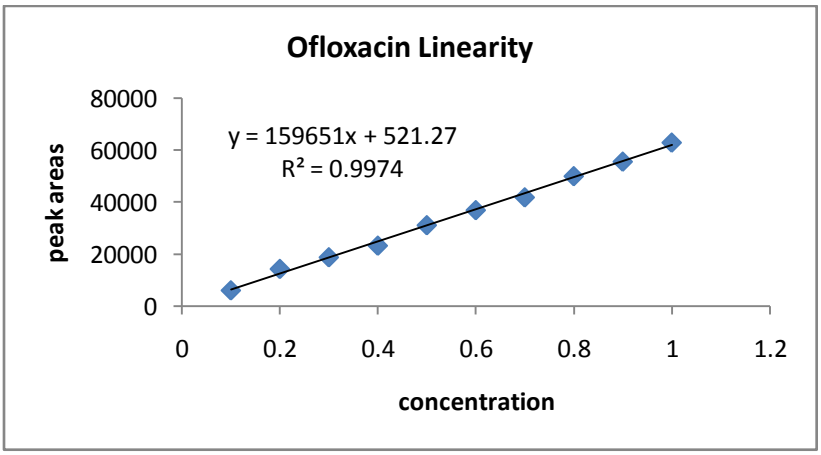

Figure 5: Calibration graph of Ofloxacin

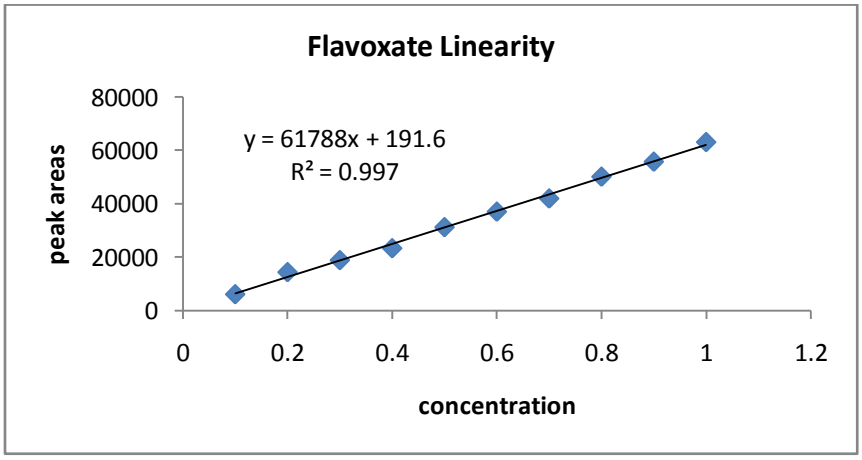

Figure 6: Calibration graph of Flavoxate HCl

Limit of Detection and Quantification:

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Two types of solutions i.e. blank and progressively decreasing concentrations of each analyte were prepared and analyzed. The limit of quantification (LOQ) was then established by evaluating the minimum level at which the analyte can be readily quantified with accuracy (signal to noise ratio of 10:1) and the limit of detection (LOD) was established by calculating the signal to noise ratio of 3 :1)

The limits of quantitation were found to be 0.07µg/ml and 0.09µg/ml for Ofloxacin and Flavoxate Hcl respectively. And the limit of detection was found to be 0.023µg/ml and 0.029µg/ml for Ofloxacin and Flavoxate HCl respectively. The values of which are shown in Table 1.

Table 1: Statistical data of calibration curve.

Parameter	OX	FX
Range	100-1000ng/ml	100-1000ng/ml
Slope	15962	6178
Intercept	587.9	191.6
R^2	0.997	0.997
Wavelength (nm)	301	301
Regression equation (y = mx +c)	Y=15962x + 587.9	Y = 6178x + 191.6
LOD(µg/ml)	0.023	0.029
LOQ(µg/ml)	0.07	0.09

R^2 = correlation coefficient

Precision:

The precision of the method was demonstrated by inter-day and intra- day variation studies. Intraday precision was determined by analyzing Ofloxacin and Flavoxate Hcl three times in the same day. Inter day precision was determined by analyzing both the drugs three successive days. The % RSD were found to be below 2.0 % for both Ofloxacin and Flavoxate Hcl (Table 2).

Table 2: Precision values of OX and FX

Drug	Concentration (ng/ml)	% RSD*	
		Intra Day	Inter Day
OX	400	0.210	0.5
	600	0.143	1.2
FX	400	0.800	1.9
	600	0.678	1.3

*= % RSD of 3 observations

Accuracy:

The accuracy of the method was performed by adding known amounts of Ofloxacin and

Flavoxate Hcl to placebo solution and then comparing the added amount with the observed amount. Two levels of solutions were made which correspond to 50 and 100% of the nominal analytical concentration. Each level was made in triplicate. The recovery range and the relative standard deviation for each of the analytes were found to be 101-95% and 96.15-99.5% respectively (Table 3).

Table 3: Recovery values of OX and FX

Drug	Amount added (mg) (%)	Amount recovered (mg)	% Recovery	% RSD*
OX	4 (100%)	4.04	101	0.452
	2(50%)	1.9	95	0.634
FX	4 (100%)	3.84	96.15	0.408
	2 (50%)	1.99	99.5	0.554

***= % RSD of 3 observations**

Robustness:

Robustness was carried by varying experimental parameters of proposed method. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition & flow rate. No significant change was observed. The values are shown in(Table 4).

Table 4: Robustness values of OX and FX

Factor	Value	Peak Area		%RSD	
		OX	FX	OX	FX
pH	3.8	1264103	577442	1.0	1.2
	4	1256438	589454		
	4.2	1238769	575789		
Mobile Phase	0.1% Triethyl Amine buffer pH 4 and Acetonitrile in the ratio of (38:62, v/v)	1537653	625424		
	0.1% Triethyl Amine buffer pH 4 and Acetonitrile in the ratio of (40:60, v/v)	1558786	629746	1.3	0.4
	0.1% Triethyl Amine buffer pH 4 and Acetonitrile in the ratio of (42:58, v/v)	1579945	630187		
Flow Rate (ml/min)	0.9	1429756	602357	1.5	1.5
	1.0	1455547	616784		
	1.1	1475345	620890		

System suitability testing:

Table 5: Analysis of formulation and its %RSD values.

Drug	Label claim (mg)	Amount found (mg)	%Label claim	%RSD*
OX	200	201.5	100.75	0.175
		201.0	100.5	
FX	200	203.0	101.5	0.349
		202.6	101.6	

**= %RSD of 3 observations.*

System suitability test parameters like Resolution, Retention Time, Theoretical plate and Tailing

factor are shown in (Table 6).

Table 6: System suitability parameters of RP-HPLC method

Parameter	Ofloxacin	Flavoxate Hcl
Retention time (min)	3.098	4.607
Theoretical plate	4246	2568
Tailing factor	1.05	1.26
Resolution (min)	1.6	

CONCLUSION

Simultaneous determination of Ofloxacin and Flavoxate Hcl in their pharmaceutical formulation using HPLC has been successfully achieved. The method is accurate and precise for reliable quality control evaluation of drugs with good accuracy and precision. From these values it is concluded that the new HPLC method is suitable for the simultaneous determination of these two components in their pharmaceutical formulations.

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