Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Original Article

STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF OFLOXACIN AND FLAVOXATE HYDROCHLORIDE

MAHENDRA P RATHOD, SHAILAJA B JADHAV*, PRIYANKA D SATPUTE

P. E. S.'s Modern College of Pharmacy, Nigdi, Pune 411044
Email: sbjadhav_pharma@yahoo.co.in

Received: 01 Apr 2015 Revised and Accepted: 01 Jul 2015

ABSTRACT

Objective: The objective of this study was to develop and validate a stability indicating reverse-phase HPLC method for simultaneous estimation of Ofloxacin and Flavoxate hydrochloride from their combination product.

Methods: The proposed RP-HPLC method was developed using inertsil C18, 5 µm, 250 mm × 4.6 mm column. The mobile phase used was a mixture of methanol and water in the proportion of 50:50 (v/v) with apparent pH adjusted to 4.9, and UV detection at 274 nm using a PDA detector and Empower-2 software. The flow rate was 1.0 ml/min. Ofloxacin, Flavoxate hydrochloride and their combination drug product were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analysed by the proposed method.

Results: With the optimized method, retention times of Ofloxacin and Flavoxate hydrochloride were found to be 4.3 and 2.98 respectively. Peak homogeneity data of Ofloxacin and Flavoxate hydrochloride peaks obtained using PDA detector, in the stressed sample chromatograms demonstrated the specificity of the method for their estimation in the presence of degradants. The described method was linear over a range of 10-60 µg/ml with regression coefficient of 0.9996 and 0.9998. The mean recoveries were 99.57% and 99.99% for Ofloxacin and Flavoxate hydrochloride, respectively.

Conclusion: Stress testing, which covered acid, alkali, peroxide, photolytic and thermal degradation was performed to prove the specificity of the proposed method and degradation, was achieved. The developed method was validated according to ICH guidelines and was found to be simple, precise and accurate with the prescribed values.

Keywords: Ofloxacin, Flavoxate hydrochloride, Stability-indicating method, HPLC.

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 (fig. 1) and Flavoxate is 2-(1-piperidyl)ethyl-3-methyl-4–oxo-2-phenyl-chromene-8-carboxylate (fig. 2). Ofloxacin 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 replicating one DNA double helix into two [1]. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian DNA.

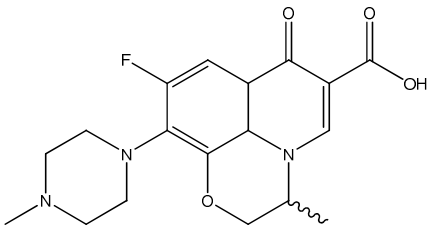

Fig. 1: Structure of Ofloxacin (OFX)

Flavoxate acts as a direct antagonist at muscarinic acetylcholine receptors in cholinergically innervated organ. 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, and dysuria, in such case there is all chances of bacterial like *Escherichia coli*, *Staphylococcus saprophyticus* etc, in such case Ofloxacin is given in combination with Flavoxate [2].

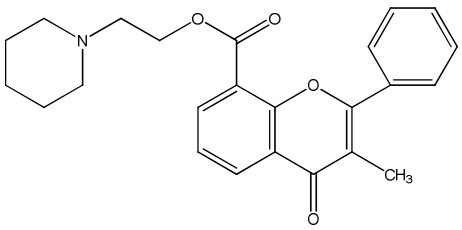

Fig. 2: Structure of Flavoxate (FLX)

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.

On the literature survey, it was found that Ofloxacin was estimated independently and in combination with other drugs by several Chromatographic [3-10], Spectrometric [11] and Fluorimetric [12] methods in pharmaceutical formulations and in biological samples. Similarly Flavoxate was estimated by Ultraviolet Spectrophotometry [13], HPLC [14-17], Voltammetry [18], and Capillary electrophoresis [19] determination techniques. One analytical method was found for stability indicating simultaneous estimation of Ofloxacin and Flavoxate HCl in combination [20].

In view of the need analytical method in the quality control laboratories for routine analysis of OFX (Ofloxacin) and FLX (Flavoxate hydrochloride) in formulations, attempts are being made to develop simple and accurate instrumental methods for simultaneous estimation of OFX and FLX, and extend it for their determination in formulation. The present work describes the development of a simple, precise, accurate and reproducible chromatographic method for the simultaneous estimation of OFX and

FLX in pharmaceutical dosage form. The developed method was validated in accordance with ICH Guidelines.

MATERIALS AND METHODS

Reagents

Ofloxacin and Flavoxate HCl were purchased from Active Pharm Lab., Hyderabad. HPLC grade methanol and water were purchased from Merck (Mumbai). Sodium Hydroxide (AR Grade) and Hydrogen peroxide (AR Grade) were purchased from Merck (Mumbai).

Instrumentation

Water HPLC system (Water 2690/5) was used for method development and validation. The HPLC system was equipped with a binary pump, a temperature controlled auto-sampler and a photo-diode array detector. The software used was Empower-2.

Chromatographic condition

The HPLC column used was Inertsil C-18 (250 × 4.6 mm, 5 µm particle size). Mobile phase comprised of a 50:50 (v/v) mixture of methanol and water. The pH of mobile phase was adjusted to 4.9 with ortho-phosphoric acid. 1 ml/min flow rate was used for method development and validation. The auto-sampler temperature was set at 25 °C and the detection wavelength was 274 nm and an injection volume of 20 µl was used.

Preparation of stock and standard solution

Accurately weighed quantity of OFX and FLX (equivalent to 10 mg each) was transferred into two separate 100 ml volumetric flasks. The drugs were dissolved in 50 ml of mobile phase with shaking and sonicated for 10 min, and then the volume was made up to the mark with the mobile phase to obtain standard stock solution of each drug of concentration 100 µg/ml.

Aliquots of standard stock solution of OFX and FLX were transferred using bulb pipettes into 10 ml volumetric flasks and, the solutions were made up to volume with mobile phase to yield final concentration of 10, 20, 30, 40, 50 and 60 µg/ml for both drugs.

Calibration curves for OFX and FLX

The marketed formulation contains OFX and FLX in a ratio of 1:1. Appropriate aliquots of OFX and FLX stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 10-60 µg/ml of OFX and FLX. The solutions were injected using a 20 µl fixed loop system and chromatograms were recorded.

Calibration curves were constructed by plotting average peak areas *versus* concentrations, and regression equations were computed for both the drugs (table 1).

Table 1: Linear regression data for calibration curves of OFX and FLX

Parameter	OFX	FLX
Linearity range (µg/ml)	10-60	10-60
Linear regression equation	y = 37719x+24070	y = 78704x+137863
r^2 (Correlation coefficient)	0.9996	0.9998
Slope	37719	78704
Intercept	24070	137863
SD	705811.8	1472597

Preparation of tablets for assay

Twenty Zenflox UTI tablets containing 200 mg each of OLX and FLX were weighed, crushed and mixed in a mortar and pestle for 20 min. A portion of powder equivalent to the 10 mg of OFX and FLX was accurately weighed and transferred to volumetric flask (100 ml) and, 50 ml of HPLC grade methanol was added to the flask. The volumetric flasks were sonicated for 10 min to effect complete dissolution of the OFX and FLX and the solutions were then made up to volume with HPLC grade water. Aliquots of the solutions were filtered through a 0.45 µm filter, and 1 ml of filtered solution was transferred to 10 ml volumetric flasks and made up to the volume with mobile phase, to yield concentration of each of the two drugs in the range of linearity previously described.

Validation of the method

The analytical method was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, recovery and robustness (table 2).

Table 2: Summary of validation parameters for OFX and FLX

Parameter	OFX	FLX
Linearity range (µg/ml)	10-60	10-6110
Correlation coefficient	0.9996	0.9998
LOD (µg/ml)	0.363	0.196
LOQ (µg/ml)	1.10	0.594
Recovery (%)	99.57	99.99
Precision (%RSD)	0.19	0.08
Inter day (n=3)		
Intraday (n=3)	0.09	0.05
Robustness	Robust	Robust
Retention Time	4.3	2.98
Tailing Factor	1.26	1.12
Resolution	7.5	

Linearity

The constructed calibration curves were linear over the concentration range of 10-60 µg/ml for both OFX and FLX. Peak areas of OFX and FLX were plotted versus their respective concentration and linear regression analysis performed on the resultant curves. Correlation coefficient R (with n=3) was found to be 0.9996 and 0.9998 for OFX and FLX respectively. Typically the regression equations were y = 37719x+24070 for OFX, and y = 78704x+137863 for FLX respectively.

LOD and LOQ

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of the 'y' intercept and 'slope' of the calibration curves was used. Following formula was used to calculate the LOD and LOQ values.

The Limit of Detection = 3.3 × SD/S

The Limit of Quantitation = 10 × SD/S

Where;

SD = standard deviation

S = slope of calibration curve

Precision

The intra-day and inter-day variability or precision data are summarized in table and was assessed by using the standard solution prepared to produce solution of 10 µg/ml concentration of each drug. Repeatability was investigated by injecting six replicate samples of each drug. Inter-day precision was assessed by injecting the same samples over three consecutive days.

Accuracy

Recovery studies were carried out at 80%, 100% and 120% level. The solutions were filtered through a 0.45 µm membrane filter paper and analyzed by RP-HPLC method. Recovery studies were carried out by applying the method to drug contents present in tablet dosage form to which known amount of standard OFX and standard FLX was added at 80%, 100% and 120% levels. The technique includes an addition of standard drug solution to pre-analysed sample solution. The recovery study was performed three times at each level.

Specificity

The specificity of the HPLC method was ascertained by analyzing standard drug and sample solutions. The retention times of OFX and FLX in the sample solution were confirmed not only by comparing with that of the respective standards but also using the diodarray. The chromatogram of tablet sample showed only two peaks at retention time of 4.3 and 2.98 mins for OFX and FLX respectively, indicating that there is no interference of the excipients in the tablet formulation.

Robustness test

The robustness of the study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. We have measured the effect of deliberate changes on peak area as well as on retention time. The factors chosen for this study were the flow rate, mobile phase composition and temperature.

Forced degradation study

In order to establish whether the analytical method was stability indicating, marketed formulation of OFX and FLX was stressed under various conditions to conduct forced degradation studies. As these drugs are freely soluble and stable in methanol, methanol was used as a solvent in all forced degradation studies.

Oxidative studies

In oxidative studies, marketed formulation was treated with 6% H_2O_2, kept for three hours then volume were made with methanol and the solution was analysed.

Acid degradation studies

Solutions for acid degradation studies, was prepared in methanol and 0.1 N hydrochloric acid. The resultant solutions were analysed after 3 hours.

Alkaline degradation studies

Solutions for alkaline degradation studies, was prepared in methanol and 1 N sodium hydroxide. The resultant solutions were analysed after 3 hours.

Thermal degradation studies

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

Photostability studies

Marketed formulation was powdered, and the powder was 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.

RESULTS AND DISCUSSION

An Inertsil C-18, 250 mm × 4.6 mm i.d. Column maintained at ambient temperature (25 °C) was used for the separation and method validation for the determination of OFX and FLX in Zenflox UTI tablets. The composition, pH and the flow rate of the mobile phase were changed to optimize the separation condition using stressed samples and the main related substances of the two compounds of interest.

The HPLC method was optimized to develop a stability indicating assay method for the quantitative determination of OFX and FLX from tablet dosage form. Initially methanol-water in varying ratios was tried as the mobile phase. The mobile phase consisting of methanol and water (50:50 v/v), pH adjusted to 4.9 with ortho-phosphoric acid gave good results. The flow rate of 1 ml/min was selected for use for further studies after several preliminary investigatory chromatographic runs. Under the described experimental condition, all the peaks were well defined and free from tailing. The effect of small deliberate changes in the mobile phase composition, pH and flow rate, was evaluated as a part of testing for method robustness.

The retention times of OFX and FLX were found to be 4.3 and 2.98 respectively (fig. 3).

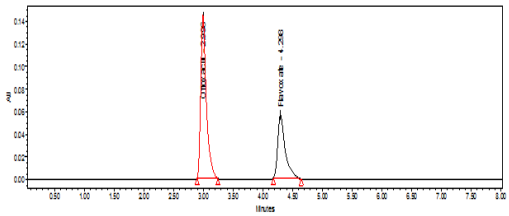

Fig. 3*:* Chromatogram of market formulation of OFX and FLX

All stressed samples tested in the solid state and in solution remained color less following testing and, OFX and FLX were found to be relatively stable following exposure to dry heat and the UV light. No degradation peaks were observed under photolytic and thermal degradation condition for OFX and FLX (fig. 4 and fig. 5).

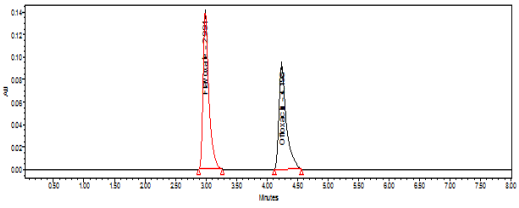

Fig. 4: Chromatogram of mixture of OFX and FLX under photolytic condition

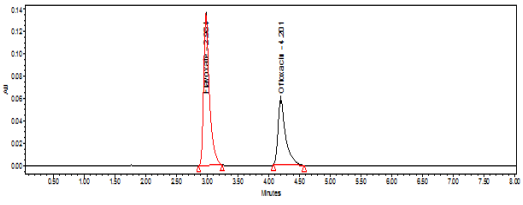

Fig. 5: Chromatogram of mixture of OFX and FLX under thermal condition

FLX was found to be more stable under alkaline condition rather than under acidic stress condition. FLX decomposed to 16.22%,

8.03%, 4.9% under acidic, alkaline and oxidative stress condition respectively. On the other hand, OFX decomposed to 10.23% and 3.1%, under alkaline and oxidative stress conditions respectively and no degradation was observed under acidic stress condition.

One additional peak was observed at 2.10 in the chromatogram of the mixture of OFX and FLX under acidic conditions.

Under alkaline conditions, two additional peaks were observed for the combination, one at 2.03 and other one at 3.24, whereas under oxidative conditions, three peaks were observed. (fig. 6 to fig. 8).

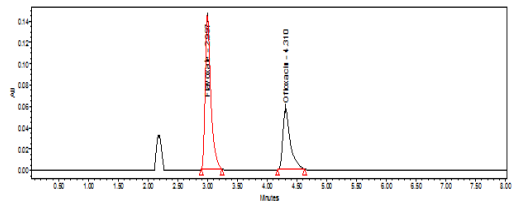

Fig. 6: Chromatogram of mixture of OFX and FLX degraded under acidic condition

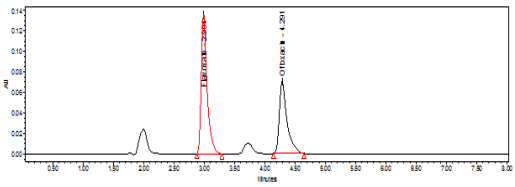

Fig. 7: Chromatogram of mixture of OFX and FLX degraded under alkaline conditions

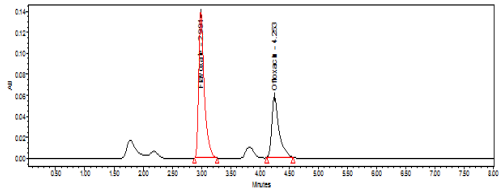

Fig. 8: Chromatogram of mixture of OFX and FLX degraded under oxidative condition

The forced degradation studies were summarized (table 3).

Table 3: Summary of degradation studies for OFX and FLX

S. No.	Parameter	% Degradation (±SD, n = 3)	
		OFX	FLX
1	Acidic Degradation	ND	16.22%±5.49
2	Alkali Degradation	10.23%±1.06	8.03%±3.74
3	Oxidative Degradation	3.1%±4.27	4.9%±2.11
4	Thermal Degradation	ND	ND
5	Photo Degradation	ND	ND

ND–No degradation observed

CONCLUSION

A simple, rapid, accurate and precise stability indicating HPLC analytical method has been developed and validated for the routine analysis of OFX and FLX. The results of stress testing undertaken according to the International Conference on Harmonization guidelines reveal that the method is selective and stability–indicating. The proposed method has the ability to separate these drugs from their degradation products, related substances or excipients found in tablet dosage forms.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Sato K, Inoue Y, Fujii T, Aoyama H, Mitsuhashi S. Antibacterial activity of ofloxacin and its mode of action. Infection 1986;14(4):S226-30.
2. Pedersen E. Studies on the effect and mode of action of flavoxate in human urinary bladder and sphincter. Urol Int 1977;32:202–8.
3. Ali MS. Simultaneous determination of ofloxacin, tetrahydrozoline hydrochloride, and prednisolone acetate by high-performance liquid chromatography. J Chrom Sci 2002;40(8):429-33.
4. Espinosa-Mansilla A. HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric (PIF) detection and multi-emission scanning: application to urine and serum. J Chromatogr B: Anal Technol Biomed Life Sci 2005;822:185-93.
5. Fabrea D. A reproducible, simple and sensitive HPLC assay for determination of ofloxacin in plasma and lung tissue: Application in pharmacokinetic studies. J Pharm Biomed Anal 1994;12(11):1463-9.
6. Groeneveld AJN, Brouwers JRBJ. Quantitative determination of ofloxacin, ciprofloxacin, norfloxacin and pefloxacin in serum by high pressure liquid chromatography. Pharm Weekblad 1986;8(1):79-84.
7. Hongyuan Y, Kyung HR. Direct determination of ofloxacin enantiomers in human urine by ligand exchange chromatography. J Liq Chromatogr Relat Technol 2007;30(9):1497–511.
8. Horie M, Saito K, Nose N, Nakazawa H. Simultaneous determination of benofloxacin, danofloxacin, enrofloxacin and ofloxacin in chicken tissue by high-performance liquid chromatography. J Chromatogr B: Biomed Appl 1994;653(1):69-76.
9. Ohkubo T, Kudo M, Sugawara K. Determination of ofloxacin in human serum by high performance liquid chromatography with column switching. J Chromatogr 1992;573(2):289-93.
10. Samanidou VF, Demetriou CE, Papadoyannis IN. Direct determination of four fluoroquinolones, enoxacin, norfloxacin, ofloxacin, & ciprofloxacin, in pharmaceuticals and blood serum by HPLC. Anal Bioanal Chem 2003;375(5):623-9.
11. Hopkala H, Kowalczuk D. Application tablets of derivative UV spectrophotometry for the determination of ciprofloxacin, norfloxacin and ofloxacin in tablets. Acta Pol Pharm 2000;57(1):3-13.
12. Feng, Yu-Lin, Dong, Chuan. Simultaneous determination of trace ofloxacin, ciprofloxacin, and sparfloxacin by micelle TLC–fluorimetry. J Chrom Sci 2004;42(9):474-7.
13. Zheng YJ. Ultra-violet spectrophotometric determination of flavoxate hydrochloride tablets. Yaowu-Fenxi-Zazhi 1993;13:339–40.
14. Wang YT, Wang TL, Zhang J. RP-IP-HPLC determination of flavoxate hydrochloride tablets. Yaowu-Fenxi-Zazhi 2002;22:202–5.
15. El-Gindy A, Abdel-Salam RA, Sallam S. High-performance liquid chromatographic determination of flavoxate hydrochloride and its hydrolysis product. Drug Dev Ind Pharm 2008;34:1311–22.
16. El-Gindy A, Sallam S, Abdel-Salam RA. High performance liquid chromatographic determination of 3-methylflavone-8-carboxylic acid, the main active metabolite of flavoxate hydrochloride in human urine. J Pharm Biomed Anal 2007;44:274–8.

17. Sheu MT, Yeh GC, Ke WT, Ho HO. Development of a high-performance liquid chromatographic method for bioequivalence study of flavoxate tablets. J Chromatogr B Biomed Sci Appl 2001;751:79–86.
18. Ghoneim MM, El-Attar MA, Razeq SA. Voltammetric quantitation at the mercury electrode of the anticholinergic drug flavoxate hydrochloride in bulk and in a pharmaceutical formulation. Central Eur J Chem 2007;5:496–507.
19. Zhang CX, Sun ZP, Ling DK, Zheng JS, Guo J, Li XY. Determination of 3-methylflavone-8-carboxylic acid, the main metabolite of flavoxate, in human urine by capillary electrophoresis with direct injection. J Chromatogr 1993;612:287–94.
20. Yunoos M, Sowjanya M, Pawan Kumar K, Ashok Kumar CH. Stability indicating RP-HPLC method for the simultaneous determination of ofloxacin and flavoxate in bulk and pharmaceutical formulation. J Chem Pharm Res 2014;6(9):381-8.