Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(9):381-388

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Stability indicating RP-HPLC method for the simultaneous determination of Ofloxacin and Flavoxate in bulk and pharmaceutical formulations

Mohammad Yunoos, M. Sowjanya*, K. Pavan Kumar and Ch. Ashok Kumar

Department of Pharmaceutical Analysis, Bapatla College of Pharmacy, Bapatla, Guntur Dist., Andhra Pradesh, India

---

ABSTRACT

A simple and precise stability indicating RP-HPLC method was developed and validated for the simultaneous determination of ofloxacin and flavoxate in pharmaceutical dosage forms. Chromatography was carried out on Inertsil ODS C18 (250mm x 4.6mm, 5 µ particle size) using a mobile phase of Phosphate buffer (KH_2PO_4): acetonitrile (52:48 % v/v) adjusted to pH 4.0 with 0.1% orthophosphoric acid at a flow rate of 1.0 ml/min. The analyte was monitored using PDA detector at 225 nm. The retention time was found to be 2.197 min and 3.514 min for Ofloxacin and Flavoxate respectively. Linearity was observed in the concentration range of 10-60 µg/ml for both Ofloxacin and Flavoxate with correlation coefficient of 0.999. The mean recoveries obtained for Ofloxacin and Flavoxate were found to be 99.96% and 99.93% respectively. Stress testing which covered acid, alkali hydrolysis, peroxide, photolytic and thermal degradation was performed to prove the specificity of the proposed method and degradation was achieved. The developed method has been statistically validated according to ICH guide lines and found to be simple, precise and accurate with the prescribed values. Thus the proposed method was successfully applied for the stability indicating simultaneous estimation of Ofloxacin and Flavoxate in routine quality control analysis in bulk and pharmaceutical formulations.

Keywords: Ofloxacin, Flavoxate, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Ofloxacin:
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. Ofloxacin is a broad-spectrum antibiotic which is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, which is an enzyme necessary to separate (mostly in prokaryotes, in bacteria in particular) replicated DNA, thereby inhibiting bacterial cell division. Ofloxacin is also considered to be contraindicated within the pediatric population, pregnancy, nursing mothers, patients with psychiatric illnesses and in patients with epilepsy or other seizure disorders [1].

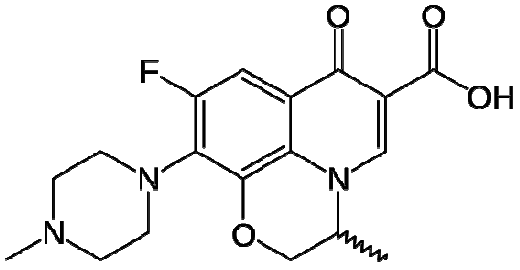

Fig.1: Structure of Ofloxacin

Flavoxate:
Chemically is 2-(1-piperidyl) ethyl 3-methyl-4-oxo-2-phenylchromene-8-carboxylate. Flavoxate 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. Flavoxate is contraindicated in patients who have any of the following obstructive conditions: pyloric or duodenal obstruction, obstructive intestinal lesions or ileus, achalasia, gastrointestinal hemorrhage and obstructive uropathies of the lower urinary tract [2].

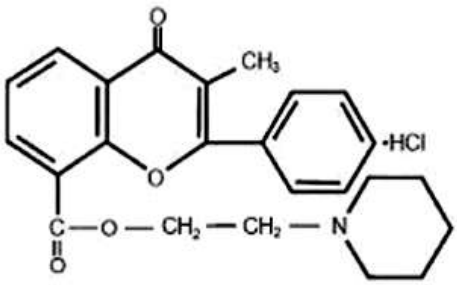

Fig.2: Structure of Flavoxate

Objective:
Literature review reveals that simultaneous estimation of ofloxacin and flavoxate by spectrophotometric methods [3, 4], HPLC methods in biological samples like human plasma and urine [5, 6], RP-HPLC method in pharmaceutical dosage form [7] and a few RP-HPLC methods were reported with other drugs in combination [8-10]. However there was no stability indicating method reported for these drugs. Hence the present study was aimed to develop a simple, fast, economical, selective, accurate, precise and sensitive Stability Indicating RPHPLC method for the simultaneous determination of Ofloxacin and Flavoxate in bulk and its Pharmaceutical dosage forms suitable for routine quality control analysis.

EXPERIMENTAL SECTION

Chemicals and solutions:
HPLC grade methanol and acetonitrile (Merck), HPLC grade Water, potassium dihydrogen orthophosphate and orthophosphoric acid was used for the analysis.

Instrumentation:
Quantitative HPLC was performed on Waters technologies 2695 series, PDA detector module equipped with auto injector with empower software. A reverse phase Inertsil ODS C18 (250mmx 4.6mm, particle size 5µm) analytical column was used. Weighing was done on shimadzu balance.

Chromatographic conditions:
Preliminary studies were conducted and trails were made for the method development. Separation and analysis was carried out on Inertsil ODS C18 (250mm x 4.6mm, 5 µ particle size) column. The optimized mobile phase consisting of phosphate buffer (potassium dihydogen ortho phosphate pH adjusted to 4.0 with 0.1% orthophosphoric acid) and acetonitrile in the ratio of 52:48 %v/v. Flow rate was maintained at 1.0 ml/min and run time for 6 min. Prior to sample injection, column was saturated with mobile phase for 40 min and injection volume was 10µl which was injected by auto sampler. The detection response was measured at 225 nm at ambient temperature. The optimized method parameters are given below in the table no 1.

Table -1: Optimization of Chromatographic conditions

Method Parameters	Optimized condition
Column	Inertsil ODS C 18 (250 x 4.6 mm×5µ)
Wavelength detection	225 nm
Mobile phase composition	Potassium dihydrogen ortho phosphate buffer (pH 4.0): acetonitrile (52: 48 % v/v)
Pump mode	Isocratic
Flow rate	1.0 ml/min
Injection volume	10 µl
Run time	6 min

Preparation of diluent:
Prepared by mixing equal volumes of acetonitrile and water in the ratio of 50:50% v/v.

Preparation of Standard solution:
Accurately weighed and transferred 10 mg of both Ofloxacine and Flavoxate reference standard in to the 25 ml volumetric flasks separately. Add 15 ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solutions, 1 ml was pipette out in to a 10 ml volumetric flask and then make up to the final volume with diluent to obtained concentration of 40µg/ml.

Preparation of sample solution:
20 tablets were weighed and calculated the average weight of each tablet .The powder equivalent to 200 mg of Ofloxacin and Flavoxate each (1.0452gm) was transferred into a 250ml volumetric flask, 200 ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution, 0.5ml was pipette out into a 10 ml volumetric flask and then made up to 10 ml with diluent to obtained concentration of 40µg/ml.

Method validation:
System suitability:
System suitability test should be carried out to verify that the analytical system is working properly and can give accurate and precise results. Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters were evaluated from tailing factor, retention times and theoretical plates of standard chromatograms. Standard solution preparations were injected five times into the chromatograph and retention times were recorded. The results obtained are tabulated in table no.2

Accuracy:
The accuracy of an analytical method is the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The study was performed by making three different standard concentrations at 50%, 100% and 150% levels of known amounts of both drugs. The accuracy of an analytical method should be established across its range. Finally, the final volume made up with diluent (KH_2PO_4: acetonitrile) and mixed well. The resulting mixtures were analyzed by the proposed HPLC method at 225 nm.

System Precision and Method precision:
The system precision was carried out to ensure that the analytical system is working properly Injected standard preparation six times into the HPLC and calculated the %RSD for both Ofloxacin and Flavoxate peaks. The results obtained are tabulated. The retention time and area of six determinations were measured and % RSD was calculated**.** In method precision, a homogenous sample of Ofloxacin and Flavoxate of a single batch were analyzed six times and % RSD was calculated.

Specificity:
Specificity is the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the Chromatograms obtained from the drug standards with that of obtained from the tablet solution .The retention times of the drug standards and the drug from sample solutions were same, so the method was specific without interference from excipients in the tablets.

Linearity:
The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different levels of standard solutions were Prepared and inject into the HPLC and the chromatograms were recorded.

Robustness and Ruggedness:
The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like mobile phase composition, flow rate and temperature which may differ but the responses were still within the specified limits of the assay. The standard solution, sample solution and sample solution spiked with impurities were injected into the chromatograph at varied conditions of flow ± 10%ml/min, mobile phase composition by ± 10% organic phase and temperature by ± 5ºC .

Forced degradation:
Stress testing of the drug substance can help identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and to develop and validate the stability indicating power of the procedures used.

Acid degradation studies:
To 1 ml of stock solution of Flavoxate and Ofloxacin, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C.The resultant solution was then diluted to obtain 40µg/ml solution each and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies:
To 1 ml of stock solution Flavoxate and Ofloxacin, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60ºC. The resultant solution was then diluted to obtain 40µg/ml solution each and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample

Hydrolytic studies:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. The resultant solution was then diluted to 40µg/ml solution each and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Peroxide studies:
To 1 ml of stock solution of Flavoxate and Oflaxacin, 1 ml of 20% H_2O_2 was added separately. The solutions were kept for 30 min at 60^0c. The resultant solution was diluted to obtain 40µg/ml solution each and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photolytic studies:
The photochemical stability of the drug was also studied by exposing the 40 µg/ml solution to UV light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. The resultant solution was diluted to obtain 40µg/ml solution each and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal studies:
The standard drug solution was placed in oven at 105°C for 6 hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40µg/ml solution each and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Solution Stability:
Standard and sample solutions were prepared as per the proposed method and injected into the chromatographic system at initial and 24hours by storing solutions at room temperature. Calculated the % difference in RSD at regular intervals.

RESULTS AND DISCUSSION

Ofloxacin and Flavoxate is not official in any pharmacopoeia and there is no stability indicating RP-HPLC method was reported for the estimation in pharmaceutical dosage forms. Hence author has planned to develop a validated stability indicating RP-HPLC method for the estimation of Ofloxacin and Flavoxate in pharmaceutical dosage form.
From this study, it was found that a Simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for the estimation of Ofloxacin and Flavoxate in pharmaceutical dosage form. Separation was done by using mobile phase composed of Phosphate buffer(KH_2PO_4) and acetonitrile in the ratio of 52:48 % v/v. Chromatographic separation were carried out on Inertsil ODS C18 column (250mm x 4.6mm,5µ particle size) at a flow rate 1.0 ml/min using PDA detection at 225 nm. The retention time of ofloxacin and flavoxate were found to be 2.197 min. and 3.514 min. respectively.

Linearity was evaluated in the concentration range of 10-60 µg/ml for both ofloxacin and flavoxate. The calibration curves of ofloxacin and flavoxate were described by the equation y=18584x+1690and y=7713x+938.8 with correlation coefficient of 0.999 as shown in figure.3 and 4. System suitability results as shown in table no.2. The %RSD in precision, accuracy and robustness studies were found to be less than 2.0%, indicating that the method is precise, accurate and robust. Accuracy data as shown in table no.3. Validation summary obtained from the marketed formulations are given in table no.4.

Table-2: System suitability

S.No	System suitability parameters	Ofloxacin	Flavoxate
1	Tailing factor(T_f)	1.33	1.67
2	Resolution (Rs)	5.24	
3	Retention time(Rt)	2.197	3.514
4	Theoretical plates(N)	2351	2939

Table- 3: Accuracy Results

Sample	Level	Peak area*	Mean % Recovery *± SD	% RSD
Ofloxacin	50%	369354	99.60 ±0.85	0.85
	100%	744988	100.45±0.53	0.53
	150%	1110534	99.83± 0.44	0.44
Flavoxate	50%	156973	99.53 ±1.18	1.19
	100%	314301	99.65 ±0.40	0.46
	150%	4766011	100.6 ±0.94	0.93

**Mean of three determinations*

Specificity:

The chromatograms of standard and sample were identical to each other as shown in figure. 3 and figure.4. The blank and placebo injections were also identical without any interference from the excipients

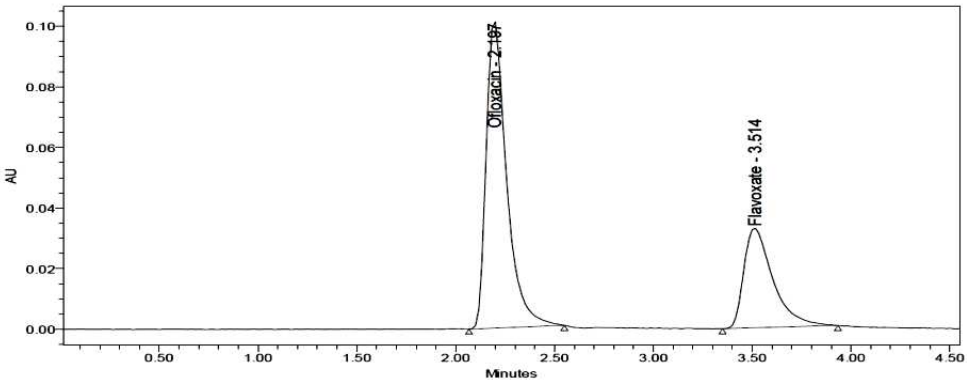

Fig.3: Chromatogram of standard solution

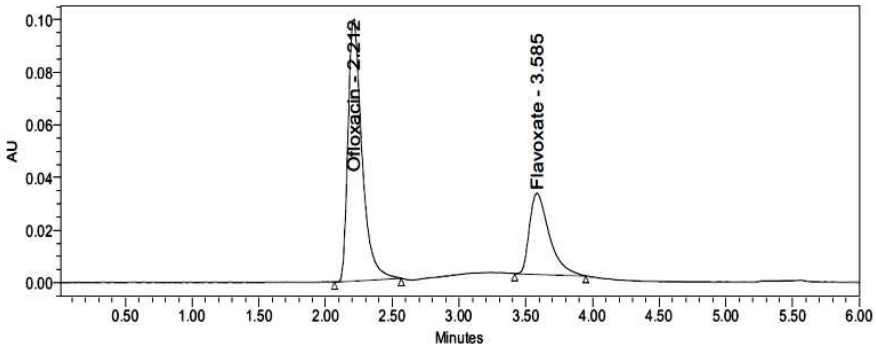

Fig.4: Chromatogram of sample solution

Table -4: Validation Parameters of the Proposed RP-HPLC Method

Parameter	Ofloxacin	Flavoxate
Regression equation	Y=18584x+1690	Y=7713x+938.8
Correlation coefficient	0.999	0.999
LOD (µg/ml)	0.30	0.40
LOQ (µg/ml)	0.91	1.22
System precision (% RSD)	0.60	1.90
Method precision(% RSD)	0.56	0.86
Assay	99.7-99.8%	99.8-100.4%

Linearity:
The calibration curve was found to be linear over the concentration range of 10 - 60 µg/ml (as shown in Fig 5 and Fig 6). The correlation coefficient was found to be 0.999 for both Ofloxacin and Flavoxate.

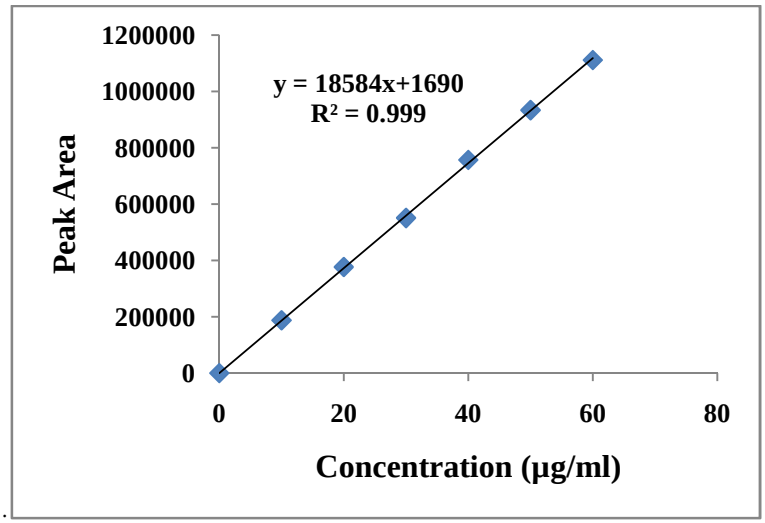

Fig.5: Calibration curve of Ofloxacin

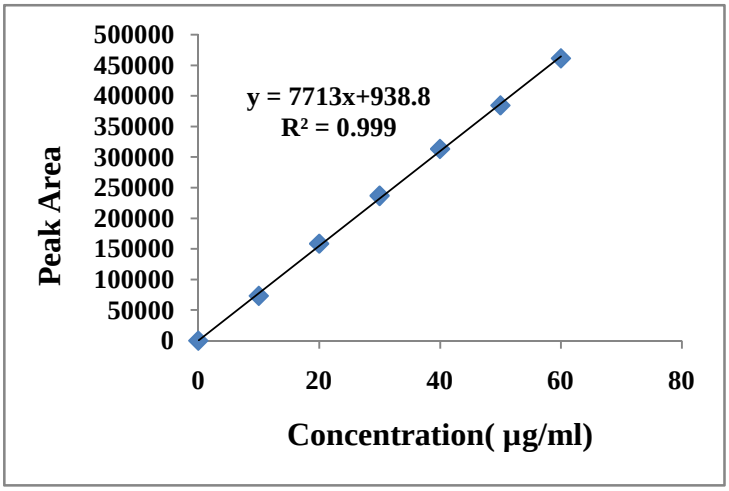

Fig.6: Calibration curve of Flavoxate

Robustness:
The developed method is robust with deliberate changes with variation of mobile phase composition, flow rate and temperature for Ofloxacin and Flavoxate. The results are given in table 5.

Table-5: Results of robustness study

S.No.	Parameter	Level	Ofloxacin			Flavoxate		
			Rt (min)	Peak area	Tailing factor	Rt (min)	Peak area	Tailing factor
1.	Flow rate (±0.1ml/min)	0.9	2.219	717915	1.67	3.603	255123	1.64
		1.1	2.216	719884	1.62	3.599	248287	1.65
2.	Mobile phase Composition (±10%v/v)	62:38	2.218	729911	1.65	3.602	234128	1.64
		42:58	2.218	723331	1.68	3.601	252973	1.65
3.	Temperature (±5°C)	25°C	2.218	750390	1.64	3.603	255600	1.62
		35°C	2.205	740141	1.64	3.575	316255	1.67

Degradation studies:

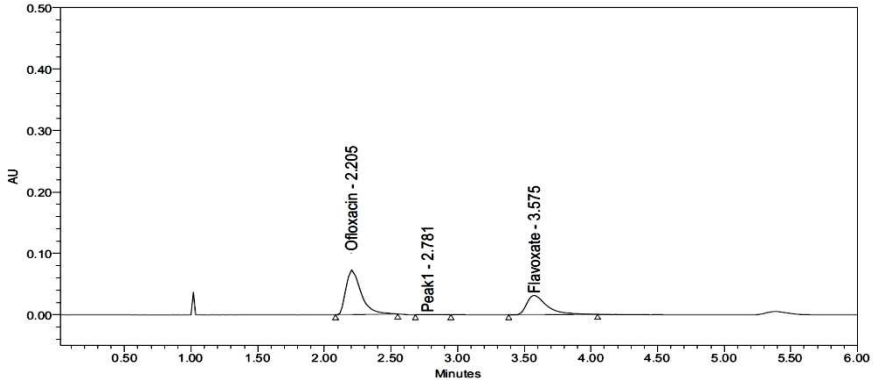

Fig.7: Chromatogram of Acid hydrolysis

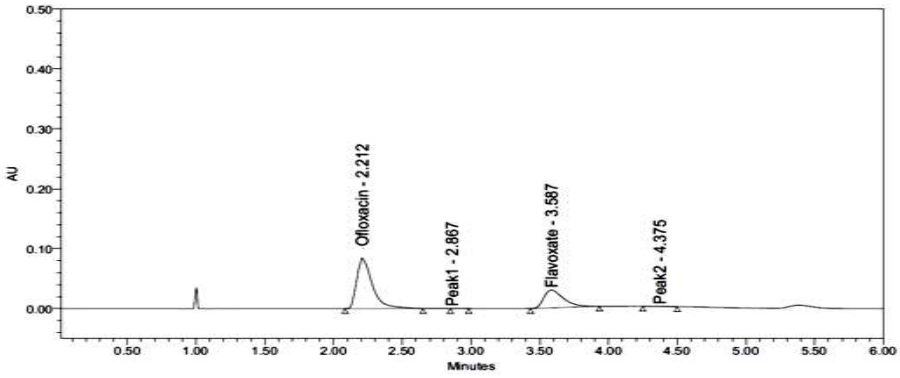

Fig.8: Chromatogram of Base Hydrolysis

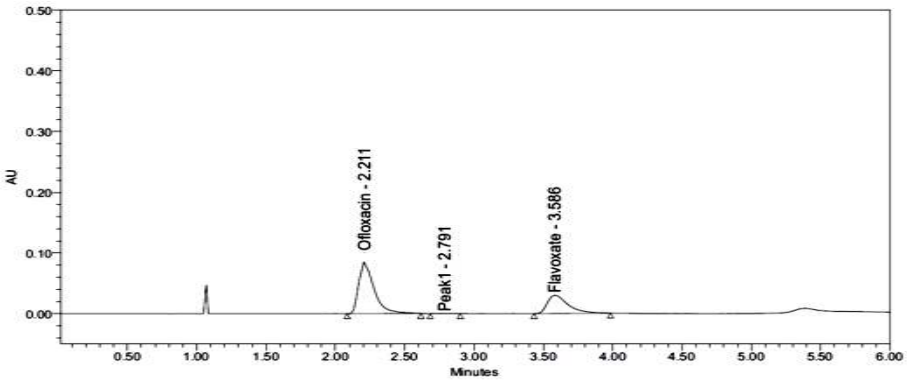

Fig.9: Chromatogram of Oxidation (peroxide)

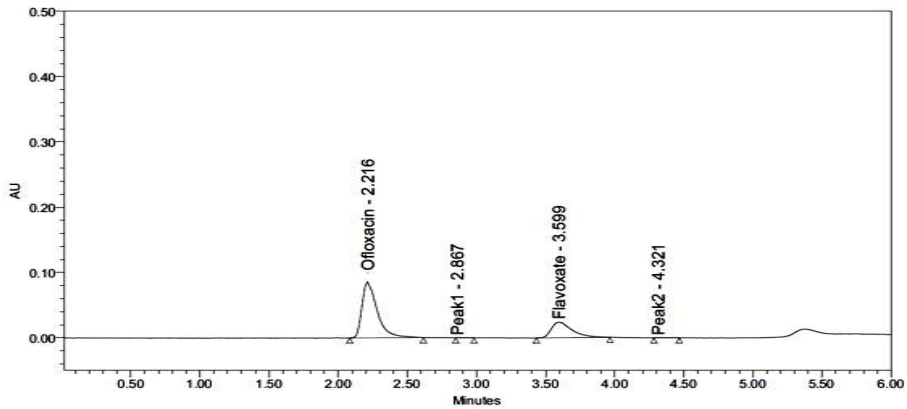

Fig.10: Chromatogram of Heat Exposure

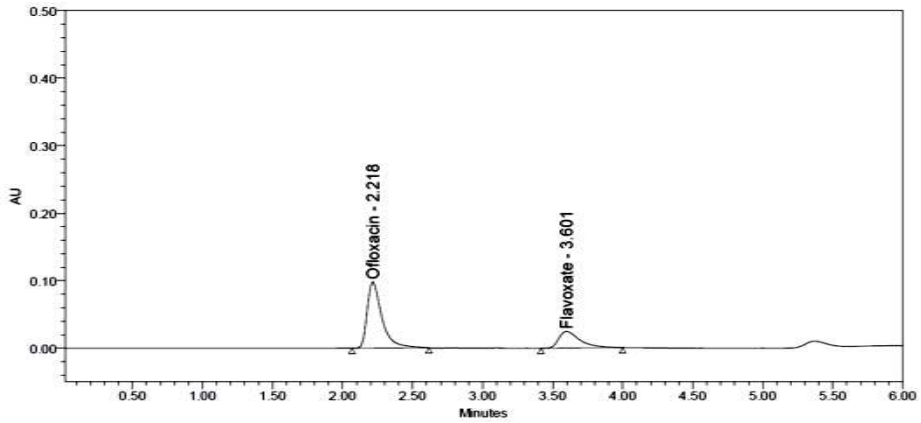

Fig.11: Chromatogram of UV Exposure:

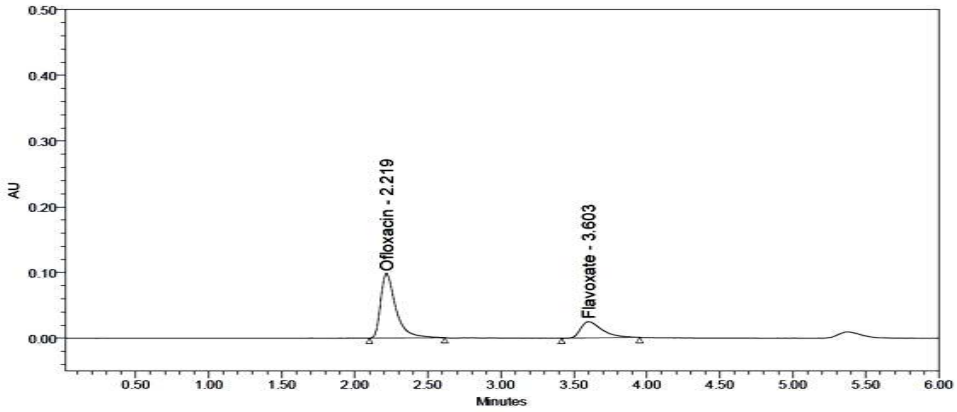

Fig.12: Chromatogram of neutral hydrolysis

CONCLUSION

From this study it is concluded that the proposed Stability Indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Ofloxacin and Flavoxate in bulk & Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

Acknowledgement
The author is grateful to Bapatla College of Pharmacy, Guntur dist., Andhra Pradesh, India for providing research facilities.

REFERENCES

[1] D Henry; W Ellison; J Sullivan; DL Mansfield; DJ Manger; GH Talbot. *Antimicrobial Agents and Chemotherapy*, **1998**, 42, 3012-3016.
[2] E Pedersen. *Urol Int*., **1977**, 32, 202–208.
[3] V Mahesh Attimarad. *Journal of Basics and clinical pharmacy*, **2010- 2011**, 2(1), 53.
[4] Jamol Joseph; Akiful Haque; VVLN Prasad; V Prakash Diwan. *Inventi:ppaqa*, **2013**, 1134 /13
[5] V Mahesh Attimarad; Sreeharsha; S Ramachandra Sett., *Journal of liquid chromatography and Related technologies*, **2011-2012**, 35(6), 768-777.
[6] Rania Nabin El Shaheny; Nahed Mahmoud El Enany. *Anal.methods*, **2014**, 6, 1001-1010.
[7] Md.Azheruddin; Jomal Joseph; Quddus Junaidy. *American.J. Pharma Tech Re.*, **2014**, 4(2), 337-348.
[8] MS Ali; M Ghori; A Saeed. *J of Chromatographic Science*, **2002**, 40(8), 429-33.
[9] A Espinosa-Mansilla; A Munoz de la Pena; GD Gonzalez; F Salinas *J of ChromatographyB*, **2005**, 822(1-2), 185-93.
[10] D Fabrea; F Bressollea; JM Kinowskia ; O Bouveta; F Paganinc; M Galtiera. *J Pharm Biomed Anal.*, **1994**, 12(11), 1463-1469.