



RESEARCH ARTICLE

SIMULTANEOUS DETERMINATION OF OFLOXACIN AND CEFIXIME IN COMBINED TABLET DOSAGE FORM BY HPLC AND ABSORBANCE CORRECTION METHOD

Arpit Patwari¹, Manan Dabhi¹, Ishwarsinh Rathod¹, Urvish Desai¹, Bhanubhai Suhagia^{2*} and Mukesh Sharma^{2,3}

¹Department of Quality Assurance, L.M. College of Pharmacy, Ahmedabad-380 009, Gujarat, India

²Faculty of Pharmacy, Dharmsinh Desai University, Nadiad-387 001, Gujarat, India

³Shah-Schulman Center for Surface Science and Nanotechnology, Dharmsinh Desai University, Nadiad-387 001, Gujarat, India

*E-mail: bnsuhagia@rediffmail.com

Tel.: +91 9427000135.

Received: Nov 01, 2014 / Revised: Nov 27, 2014 / Accepted: Nov 28, 2014

High performance liquid chromatography and absorbance correction methods were developed and applied for the simultaneous determination of ofloxacin and cefixime. Chromatographic separation was achieved on reversed-phase C18 column(25 cm × 4.6 mm id, 5 μm) in the isocratic mode using methanol-water-triethylamine (25:75:1.5, v/v/v), pH adjusted to 3.50 ± 0.05 with orthophosphoric acid as the mobile phase at a flow rate 0.9 ml/min. Quantitation was achieved with UV detection at 254 nm. In the proposed HPLC method, quantification was achieved over the concentration range of 5-30 and 5-30 μg/ml, with mean recoveries of 99.95±1.31 and 100.36±1.24% for ofloxacin and cefixime respectively. In the absorbance correction method, quantification was achieved over the concentration range of 5-25 μg/ml for both drugs, with mean recoveries of 101.23±0.85 and 100.53±1.40% for ofloxacin and cefixime respectively. Determination was performed at 262.5 and 345.5 nm for ofloxacin and cefixime. The proposed methods were successfully applied for the analysis of synthetic mixtures and pharmaceutical formulations of ofloxacin and cefixime without any interference from common excipients. The results obtained by applying the proposed methods were statistically compared by the Student's t-test.

Key words: Ofloxacin, Cefixime, Liquid chromatography, Absorbance correction method.

INTRODUCTION

Ofloxacin (OFX) chemically is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piprazinyl)-7-oxo-7H-pyrido [1,2,3-de] -1,4-benzoxazine-6-carboxylic acid. It is a broad spectrum antibacterial agent, belonging to the group of fluoroquinolones. Ofloxacin is active against a wide variety of gram-positive and gram-negative organism. It is used in the treatment of urinary tract infection, conjunctivitis, gonorrhoea, respiratory tract infection and skin infection. Cefixime (CEFI) chemically(6R,7R)-7-[2-(2-amino-4-thiazolyl) glyoxylamido]-8-oxo-3-vinyl-

5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid-7-(Z)-[O-carboxymethyl]oxime] trihydrate. Cefixime is an oral third generation cephalosporin antibiotic. It is used to treat gonorrhoea, tonsillitis, pharyngitis, urinary tract infection and acute bronchitis (Moffat *et al* 2004; Hardman and Limbird, 2006).

HPLC has been remained as a method of choice for determination of drugs alone or in combination with other drugs in the pharmaceutical formulations (Bhimavarapu *et al* 2011; Basaveswara Rao *et al* 2012a; 2012b; Chhabra and Banerjee, 2013). Literature survey

reveals that HPLC (Lehr and Damm, 1988; Kalta *et al* 2008; Kasabe *et al* 2009; Boopathy *et al* 2010), HPTLC (Argekar *et al* 1996; Eric-Jovanovic *et al* 1998; Gandhimathi *et al* 2008), LC-MS (Meng *et al* 2005), Capillary electrophoresis (Elbashir *et al* 2008; See *et al* 2009) and UV spectroscopy (Shankar *et al* 2001; Wankhede *et al* 2008; Bhusari and Chaple, 2009; Game and Sakarkar, 2011) method has been reported for determination of ofloxacin and cefixime alone or combination with other drugs in pharmaceutical formulations and in human plasma. Ofloxacin and Cefixime are formulated together in the form of combined tablet dosage form which is used in the treatment of conjunctivitis, keratitis and endophthalmitis. Ofloxacin and cefixime combination tablets are not official in any pharmacopoeia.

The main problem of spectrophotometric binary mixtures analysis is the simultaneous determination of two drugs without prior separation. No Isocratic HPLC method and spectrophotometric methods are available for simultaneous determination of ofloxacin and cefixime, so there was a need to develop accurate method for their determination in combination. The aim of this work is to determine both drugs concurrently by simple, rapid, and selective HPLC and absorbance correction methods which could be used for quality control and routine analysis.

MATERIALS AND METHODS

Instrumentation

All absorption spectra were recorded with a Shimadzu UV-1700 Pharmaspec, UV-Visible double-beam spectrophotometer with 1 cm quartz cuvettes (Shimadzu Corporation, Kyoto, Japan). The spectral bandwidth was 0.5 nm.

HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with a model series LC-10 AS pump, Rheodyne 7725i injector with a 100 μ l loop and SPD-10A UV-Visible detector. A Grace smart reversed-phase C18 column (Grace Discovery and Division; 5 μ m, 25 cm \times 4.6 mm id) was used as the stationary phase. Class CR10 software was used for data acquisition.

Reference substances, samples, reagents and chemicals

OFX powder was procured from Lincoln Pharma Pvt. Ltd. (Ahmedabad, India). CEFI powder was procured from Astron Research Limited, Ahmedabad, India).

For HPLC work, methanol (RFCL Limited, India),

phosphoric acid (RFCL Limited, India) and triethylamine (RFCL Ltd., India) were of HPLC grade. Triple distilled water was used. Cefi-O (Batch No. OHPN01) was manufactured by Piramal Healthcare Ltd. Zifi-O (Batch No. YNA0061) was manufactured by Alkums Drugs and Pharmaceutical Ltd. Each tablet claimed to contain 200 mg OFX and 200 mg CEFI (as free base).

Preparation of standard solutions

For HPLC method

Stock standard solutions OFX (1 mg/ml) and CEFI (as free base, 1 mg/ml) were prepared separately in methanol (protected from light due to photosensitivity).

The stock solutions were diluted with mobile phase to prepare final concentrations of 100 and 100 μ g/ml for OFX and CEFI respectively (Working standard solutions).

For Absorbance correction method

Stock standard solutions OFX (250 μ g/ml) and CEFI (as free base, 250 μ g/ml) were prepared separately in methanol (protected from light due to photosensitivity).

The stock solutions were diluted with methanol to prepare final concentrations of 50 and 50 μ g/ml for OFX and CEFI respectively (Working standard solutions).

HPLC method

A Grace Smart (250 mm \times 4.6 mm i.d, 5 μ m particle size) reversed-phase C18 column was used for separation and quantitation. The mobile phase consisted of methanol-water-triethylamine (25:75:1.5, v/v/v), pH adjusted to 3.50 \pm 0.05 with orthophosphoric acid.

The mobile phase was filtered through Millipore filter paper type HV (0.45 μ m) and degassed by sonication. A flow rate of 0.9 ml/min was maintained. The injection volume was 20 μ l. The detector was set at 254 nm.

Linearity

Aliquot equivalent to 0.5-3.0 ml of OFX working standard solution and 0.5-3.0 ml of CEFI working standard solution were transferred separately into 10 ml volumetric flasks from their respective working standard solution and completed to volume with mobile phase. Calibration graphs for both OFX and CEFI were obtained by plotting the peak area against concentration, and the corresponding regression equations were calculated.

Absorbance correction method

Linearity

Appropriate aliquots from the stock solutions of OFX and CEFI were used to prepare three different sets of dilutions. Series A, B and C as follows.

Series A consisted of different concentrations of OFX (5-25 $\mu\text{g/ml}$). Aliquot of OFX working standard solution (50 $\mu\text{g/ml}$) was transferred separately into a series of 10 ml volumetric flask, HCl (1 ml, 0.1 N) was added and diluted with methanol to obtain final concentration of 5-25 $\mu\text{g/ml}$.

Series B consisted of different concentrations of CEFI (5-25 $\mu\text{g/ml}$). Aliquot of CEFI working standard solution (50 $\mu\text{g/ml}$) was transferred separately into a series of 10 ml volumetric flask; HCl (1 ml, 0.1 N) was added and diluted with methanol to obtain final concentration of 5-25 $\mu\text{g/ml}$. Series C comprised of mixture of OFX and CEFI having varying concentrations of OFX (5-25 $\mu\text{g/ml}$) and CEFI (5-25 $\mu\text{g/ml}$).

Aliquot equivalent to 0.5-2.5 ml of OFX working standard solution and 0.5-2.5 ml of CEFI working standard solution were transferred into 10 ml volumetric flasks, HCl (1 ml, 0.1 N) was added and completed to volume with methanol. The absorbance of the solutions of series A and C were measured at 262.5 nm (λ_1) and 345.5 nm (λ_2) while absorbance of the solutions of series B was measured at 262.5 nm (λ_1).

The difference in absorbance between 262.5 nm and 345.5 nm is due to CEFI and this difference was plotted against CEFI concentration. The absorbance at 345.5 nm is due to OFX only and was plotted against OFX concentration.

Preparation of pharmaceutical samples

For HPLC method

To determine the content of OFX and CEFI in a combination tablet, twenty tablets were weighed, their mean weight was determined and finely powdered.

The weight of the tablet triturate equivalent to 20 mg of OFX was transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min, and diluted to 100 ml with methanol.

The resulting solution was filtered through whatmann filter paper No. 41. The filtered solution (2.5 ml) was taken in 50 ml volumetric flask and diluted up to mark with mobile phase to obtain final concentration of OFX (10 $\mu\text{g/ml}$) and CEFI (10 $\mu\text{g/ml}$) respectively.

For Absorbance correction method

To determine the content of OFX and CEFI in a combination tablet, twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 20 mg of OFX was transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min, and diluted to 100 ml with methanol. The resulting solution was filtered through Whatman filter paper No. 41. Solution (2.5 ml) was transferred into 50 ml volumetric flask; HCl (1 ml, 0.1 N) was added and diluted up to mark with methanol to obtain final concentration of OFX (10 $\mu\text{g/ml}$) and CEFI (10 $\mu\text{g/ml}$) respectively. The absorbance of final sample solution was measured at 262.5 nm and 345.5 nm. The amount of OFX and CEFI was computed using respective equation of straight line.

RESULTS AND DISCUSSION

HPLC method

The developed HPLC method has been applied for simultaneous determination of OFX and CEFI. To optimize the chromatographic conditions for the separation of OFX and CEFI, mobile phase composition, the effect of pH and wavelength of detection were investigated. During the method development work, Grace smart RP-C18 column (250 mm \times 4.6 mm i.d, 5 μm particle size) was used and gave the most suitable resolution. A satisfactory separation was obtained with mobile phase consisting of methanol-water-triethylamine (25:75:1.5, v/v/v), pH adjusted to 3.50 \pm 0.05 with orthophosphoric acid, with flow rate of 0.9 ml/min. Quantitation based on peak area was achieved with UV detection at 254 nm. The specificity of HPLC method is illustrated in **Figure 1**, which showed complete separation of compounds in mixtures. The retention time for CEFI and OFX were found to be 5.748 and 7.240 min respectively.

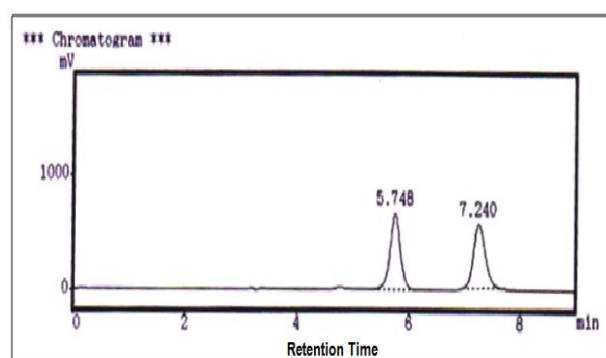


Fig. 1. Chromatogram of CEFI and OFX

External standard calibration method was applied for analysis of OFX and CEFI. A linear relation was obtained between peak area and the concentration of the drug in the range of 5-30 $\mu\text{g/ml}$ and 5-30 $\mu\text{g/ml}$ for OFX and CEFI respectively. The regression equations were computed to be:

$$Y = 322378X + 28658, r = 0.9999 \text{ (for Ofloxacin)}$$

$$Y = 341811X + 12998, r = 0.9999 \text{ (for Cefixime)}$$

where Y is the peak area and X is the concentration in $\mu\text{g/ml}$ and *r* is the correlation coefficient.

Absorbance correction method

Absorbance correction method uses the absorbance at two selected wavelengths. First wavelength (λ_1) at which minimum absorbance of interfering component and some absorbance of pure component of interest was observed. Second wavelength (λ_2) was the wavelength at which the absorbance of the interfering component was equal to absorbance of the interfering component at λ_1 . From the overlaid spectrum of OFX and CEFI in 0.1 N HCl (**Figure 2**), it was observed that CEFI has zero absorbance at 345.5 nm, whereas OFX has considerable absorbance. Thus, OFX was estimated directly at 345.5 nm with no

interference of CEFI.

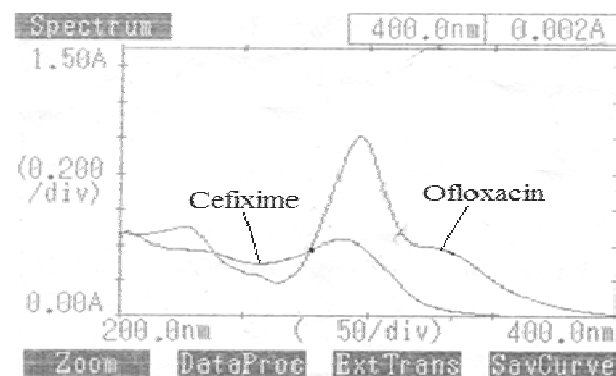


Fig. 2. Absorption spectra of Ofloxacin (10 $\mu\text{g/ml}$) and Cefixime (10 $\mu\text{g/ml}$) in methanol.

The difference in absorbance at these two wavelengths ($A_{262.5 \text{ nm}} - A_{345.5 \text{ nm}}$) is selected to remove the interference of OFX in measurement of CEFI at 262.5 nm and the difference in the absorbance is proportional to the concentration of CEFI in the mixture. Regression analysis for series A and C shows no difference in the equations of straight line and thus indicated that there is no interference of CEFI in the determination of OFX. Regression analysis for series B and C shows no difference in the equations of straight line and thus indicated that there is no interference of OFX in the determination of CEFI (**Table 1**).

Table 1. Regression equation of Series A, B and C in absorbance correction method

Series	Concentration ($\mu\text{g/ml}$)		Regression equation	Correlation coefficient
	OFX	CEFI		
A	5-25	0	$Y = 0.0204X + 0.0028$	0.9994
B	0	5-25	$Y = 0.0358X - 0.0041$	0.9999
C	5-25	5-25	^a $Y = 0.0202X + 0.0034$	0.9998
			^b $Y = 0.0351X - 0.0027$	0.9998

^aRegression equation for OFX; ^bRegression equation for CEFI

Analysis of pharmaceutical formulations

The proposed HPLC and absorbance correction methods were applied to the simultaneous determination of OFX and CEFI in Cefi-O and

Zifi-O tablets. Three replicated determinations were made. Satisfactory results were obtained for each compound in good agreement with label claims (**Table 2**).

Table 2. Determination of OFX and CEFI in pharmaceutical formulation by HPLC and Absorbance correction method

Sample ^a	HPLC method % Recovery \pm S.D. ^b		Absorbance correction method % Recovery \pm S.D. ^b	
	OFX	CEFI	OFX	CEFI
Cefi-O	101.39 \pm 1.24	101.88 \pm 1.13	102.67 \pm 0.49	101.64 \pm 1.34
Zifi-O	101.53 \pm 1.04	102.35 \pm 0.98	102.20 \pm 1.40	100.99 \pm 1.69

^aLabel claim: OFX (200 mg) and CEFI (200 mg); ^bAverage of three determinations

Validation of the methods

Linearity and range

The linearity of the HPLC and absorbance correction methods for the determination of OFX and CEFI was evaluated by analyzing a series of different concentrations of each drug. In this study six concentrations were chosen for each drug in HPLC method and five concentrations were chosen for each drug in Absorbance

correction method. Each concentration was repeated six times. The linearity of the calibration graphs was validated by the high value of the correlation coefficient. The calibration range was established through consideration of the practical range necessary, according to each drug concentration present in the pharmaceutical products to give accurate, precise and linear results (**Table 3**).

Table 3. Summary of validation parameter for the proposed methods

Parameters	HPLC method		Absorbance correction method	
	OFX	CEFI	OFX	CEFI
Linearity Range ($\mu\text{g/ml}$)	5-30	5-30	5-25	5-25
Slope	322378	341811	0.0204	0.0358
Intercept	28658	12998	0.0028	-0.0041
Correlation coefficient (r)	0.9999	0.9999	0.9994	0.9999
Repeatability RSD (%)	0.74	0.54	0.83	0.64
Intraday RSD (%)	0.40-0.79	0.81-0.95	0.42-0.95	0.47-1.04
Interday RSD (%)	1.44-2.28	1.17-1.98	0.88-2.10	1.10-2.18
Accuracy (% Recovery)	99.95 \pm 1.31	100.36 \pm 1.24	101.23 \pm 0.85	100.53 \pm 1.40
LOD ($\mu\text{g/ml}$)	0.48	0.28	0.80	0.31
LOQ ($\mu\text{g/ml}$)	1.47	0.87	2.44	0.94

Precision

Evaluation of precision estimates repeatability and intermediate precision were performed at three concentration levels for each drug on three different days.

The low values of relative standard deviation (% RSD) of the intraday and interday determinations. **Table 3** indicated that there was no statistically significant difference between the mean results obtained from one day to another.

Detection and quantitation limits

According to ICH recommendation (ICH, 2005), the approach based on the standard deviation of the response and slope was used for determining the limit of detection (LOD) and limit of quantitation (LOQ). The calculated values for

HPLC and absorbance correction method are given in **Table 3**.

Accuracy

The interference of excipients in the pharmaceutical formulations was studied in detail by proposed methods. To perform this study, standard addition method was applied to the pharmaceutical formulation containing these compounds. This study was performed by addition of known amounts of studied drugs to a known concentration of the commercial pharmaceutical product. The excellent recoveries of standard addition method (**Table 3**) prove the good precision and accuracy of the proposed methods. Consequently, the excipients in the studied pharmaceutical formulations do not interfere in the analysis of these compounds.

Table 4. Statistical comparison of developed methods using Student's t-test

Parameter	OFX		CEFI	
	Absorbance correction	HPLC	Absorbance correction	HPLC
Mean	101.31	102.11	102.43	101.46
Variance	3.04	0.97	0.22	1.06
T calc	-0.74		2.009	
t critical	2.015		2.015	
P (T<t) one tail	0.214		0.353	
T calc < t critical	Yes		Yes	

Statistical analysis

Assay results of two different methods were statistically compared using paired *t* test. The calculated *t*-values were less than the theoretical ones, indicating no significant difference in accuracy and precision of HPLC and Absorbance correction methods for estimation of ofloxacin and cefixime in their combined dosage form. Statistical comparison of developed HPLC method and Absorbance correction method is shown in **Table 4** using Student's *t*-test.

REFERENCES

- Argekar AP, Kapadia SU, Raj SV. Determination of some fluoroquinolones in pharmaceutical preparations by HPTLC method. *Indian Drugs* 1996;33(3);107-11.
- Basaveswara Rao MV, Prasanthi V, Sivanadh M, Venkata Rao G. Newer RP-HPLC method for the determination of doxazosin in human plasma and formulation. *Bull. Pharm. Res.* 2012;2(1):1-4.
- Basaveswara Rao MV, Nagendrakumar AVD, Sivanadh M, Venkata Rao G. Validated RP-HPLC method for the estimation of telmisartan in tablet formulation. *Bull. Pharm. Res.* 2012;2(2):50-5.
- Bhimavarapu R, Chitra KP, Meda H, Kanikanti D, Anne M, Gowthami N. Forced degradation study of paracetamol in tablet formulation using RP-HPLC. *Bull. Pharm. Res.* 2011;1(3):13-7.
- Bhusari KP, Chaple DR. Simultaneous spectrophotometric estimation of ofloxacin and ornidazole in tablet dosage form. *Asian J. Res. Chem.* 2009;2(1);60-2.
- Boopathy D, Prakash M, Mathew B, Suresh Kumar S, Perumal P. Method development and validation of simultaneous determination of Ofloxacin and Satranidazole in pharmaceutical dosage form by RP-HPLC. *Der Pharm. Lett.* 2010;2(2);22-6.
- Chhabra GS, Banerjee SK. Stability indicating assay method development and validation of dronedarone hydrochloride in its bulk form by RP-HPLC. *Bull. Pharm. Res.* 2013;3(2):58-65.
- Elbashir AA, Saad B, Ali ASM, Saleh MI, Aboul-Enein HY. Development and validation of a capillary zone electrophoresis method for the determination of ofloxacin in tablets. *J. Liq. Chrom. Rel. Technol.* 2008;31(18);2771-83. [DOI: 10.1080/10826070802388367]
- Eric-Jovanovic S, Agbaba D, Zivanov-Stakic D, Vladimirov S. HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms. *J. Pharm. Biomed. Anal.* 1998;18(4-5);893-8. [DOI: 10.1016/S0731-7085(98)00274-X]
- Game MD, Sakarkar DM. Simultaneous spectrophotometric estimation of nitazoxanide and ofloxacin in tablets. *Indian J. Pharm. Sci.* 2011;73(1):70-4. [DOI: 10.4103/0250-474X.89759]
- Gandhimathi M, Ravi TK, Shukla N. Validated high performance thin layer chromatography method for

CONCLUSION

The developed HPLC and absorbance correction methods are precise, specific, and accurate. Statistical analysis proved that the method is suitable for the analysis of OFX and CEFI as a bulk drug and in pharmaceutical formulation without any interference from the excipients. HPLC method provided a good resolution between OFX and CEFI within a short time. Absorbance correction method is simple, rapid, and more flexible than HPLC.

- simultaneous estimation of ofloxacin and ornidazole in tablet dosage form. *Indian J. Pharm. Sci.* 2008;68(6);838-40. [DOI: 10.4103/0250-474X.3103]
- Hardman JG, Limbird LE. Goodman and Gilman's The Pharmacologic Basis of Therapeutics, 11th edition, McGraw-Hill, New York: 2006.
- International Conference on Harmonization, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2 (R1), Geneva: 2005.
- Kalta RR, Sharma R, Chaturvedi SC. Simultaneous RP-HPLC determination of nitazoxanide and ofloxacin in combined tablet dosage form. *Indian J. Pharm. Sci.* 2008;70(4):491-4. [DOI: 10.4103/0250-474X.44600]
- Kasabe AJ, Shitole VV, Waghmare VV, Mohite V. Simultaneous estimation of metronidazole and ofloxacin in combined dosage forms by RP-HPLC Method. *Int. J. ChemTech. Res.* 2009;1(4);1244-50.
- Lehr KH, Damm P. Quantification of the enantiomers of ofloxacin in biological fluids by high-performance liquid chromatography. *J. Chromatogr.* 1988;425(1);153-61.
- Meng IF, Chen X, Zeng Y, Zhong D. Sensitive liquid chromatography-tandem mass spectrometry method for the determination of cefixime in human plasma: application to a pharmacokinetic study. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2005;819(2);277-82. [DOI: 10.1016/j.jchromb.2005.02.015]
- Moffat AC, Osselton MD, Widdop B. Clarke's Analysis of Drugs and Poisons, 3rd edition, Pharmaceutical Press, London: 2004.
- See KL, Elbashir AA, Saad B, Ali AS, Aboul-Enein HY. Simultaneous determination of ofloxacin and ornidazole in pharmaceutical preparation by capillary zone electrophoresis. *Biomed. Chromatogr.* 2009;23(12);1283-90. [DOI: 10.1002/bmc.1251]
- Shankar DG, Sushma K, Laxmi RV, Reddy MN, Murthy TK, Rao SY. UV and Visible spectrophotometric method for the determination of cefixime. *Indian Drugs* 2001;38(12): 617-9.
- Wankhede SB, Prakash A, Chitlange SS. Simultaneous spectrophotometric estimation of ofloxacin and satranidazole in tablet dosage form. *Asian J. Res. Chem.* 2008;1(1):9-11.
