International Journal of Research in Pharmaceutical sciences and Technology

Analytical method development and validation for simultaneous estimation of moxifloxacin hydrochloride and ketorolac tromethamine by using RP-HPLC

R. Raghavi*, D. Saravanan, A. Maheswaran, P. Divakar

Department of Pharmaceutical Analysis, Jaya College of Paramedical Sciences, College of Pharmacy, Chennai-602024, Tamil Nadu, India.

ABSTRACT

The main objectives of the present research were to develop the new method for the simultaneous estimation and validation of Moxifloxacin HCl and Ketorolac Tromethamine in pure form and in pharmaceutical dosage form by RP-HPLC. The chromatogram of Moxifloxacin HCl and Ketorolac Tromethamine was developed through column (Inertsil ODS C18), UV detection at 304 nm at a flow rate of 1.0 ml/min with Buffer (pH 4.0):Acetonitrile:Methanol (50:30:20) V/V as mobile phase. The method was validated by various validation parameters such as accuracy, precision, linearity, specificity as per the ICH guidelines. A linearity range of Moxifloxacin HCl and Ketorolac Tromethamine was found to be 60 to 140 µg/ml and 48 to 112 µg/ml respectively. The retention time of Moxifloxacin HCl and Ketorolac Tromethamine was found to be 2.07 min and 4.06 min respectively. % RSD of retention time and peak areas obtained in system precision for Moxifloxacin HCl was 0.21 and 0.80 respectively and for Ketorolac Tromethamine were 0.90 and 1.06 respectively. The % recovery of standard Moxifloxacin HCl and Ketorolac Tromethamine was found to be 100.18 to 100.08% and 99.97 to 99.93% respectively. The % recovery of Moxifloxacin HCl and Ketorolac Tromethamine in dosage form was found to be 98.73 to 100.92% and 98.10 to 100.77% respectively. This method was simple, accurate, precise and sensitive. Hence, the developed method was employed for the routine analysis of Tenofovir in pharmaceutical dosage form.

Keywords: Moxifloxacin HCl; Ketorolac Tromethamine Tenofovir; Estimation; Analytical method; HPLC

ISSN: 2581-9143
Research Article

Corresponding Author

Name: R. Raghavi
Email: raghavi9799@gmail.com

Article Info

Received on: 22-08-2020
Revised on: 02-09-2020
Accepted on: 25-09-2020

DOI: https://doi.org/10.33974/ijrpst.v2i1.234

INTRODUCTION

Moxifloxacin hydrochloride (MOX) is chemically 1-Cyclopropyl-6-fluoro-1, 4-dihydro-8- methoxy-7-[(4aS,7aS)-octahydro-6Hpyrrolo[3, 4- b]pyridin-6-yl]-4-oxo-3 quinolinecarboxylic acid hydrochloride (Figure 1), which is a synthetic fourth generation broad-spectrum fluoroquinolone antibiotic. MOX acts by inhibiting type II topoisomerase, topoisomerase IV and DNA gyrase that are involved in the DNA replication and, metabolism[1].

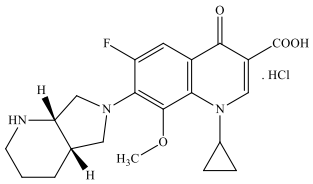

Figure 1: Structure of Moxifloxacin HCl

Ketorolac tromethamine (KET) is chemically (±)-5-benzoyl-2,3- dihydro-1H-pyrrolizine-1-carboxylic acid, a compound with 2-amino-2-(hydroxymethyl)-1,3- propanediol, which is a pyrrolizine carboxylic acid derivative (Figure 2). KET is nonsteroidal anti-inflammatory drug used to treat short, moderate and severe pain and produce severe side effects, gastric bleeding. The combination of MOX and KET is broadly used for the treatment of postoperative inflammation and infection in the cataract surgery[1].

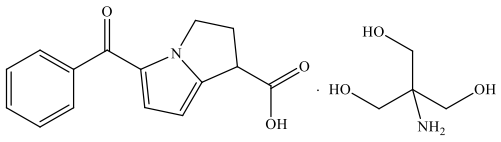

Figure 2: Structure of Ketorolac Tromethamine

Different methods such as estimation of MOX by UV spectrophotometry[1,2], estimation of MOX in

biological fluids by HPLC[3,4] and HPTLC[5] with other drugs were reported in the literature. Correspondingly, KET was determined by HPLC[6,7,8]. But, only some of the analytical methods were also reported for the simultaneous estimation of MOX and KET in a mixture by UV spectrophotometry[9], LC-MS[10], stability indicating RP-HPLC[11], RP-HPLC[12,13] and HPTLC method[13]. A lot of literature review did not reveal that any simple and economical method for the simultaneous determination of MOX and KET in combined dosage forms by RP-HPLC. Hence, the attempts were made to develop and validate the simple, sensitive, precise, and isocratic RP-HPLC method for the simultaneous estimation of both drugs in pharmaceutical formulations.

MATERIALS AND METHODS

Materials

Chemicals and reagents

Moxifloxacin HCl & Keterolac bulk drugs and Eye drops (MOXICIP KT) were obtained from gift samples and Cipla pharmaceuticals. HPLC grade Methanol, Acetonitrile and Water were purchased from Merck Specialty Pvt Ltd. Potassium Dihydrogen Phosphate was procured from Rankem Chemicals. All the other chemicals and reagents used were of analytical grade and purchased from Finar chemicals limited and Fisher Scientific India Pvt Ltd.

Instrumentation

High performance liquid chromatographic system (Cyberlab) consisting of a pump, Hamilton injector, **c**olumn **(**Zodiac ODS C18) equipped with UV-Visible detector and WS 100 workstation. Citizen, Digital Ultrasonic cleaner was used for sonication and Global DPH 500 pH meter was used for adjusting the pH of the buffer. Analytical Technologies UV-Visible Spectrophotometer was used for the detection and optimization of Wavelength.

Preparation of 0.05 M potassium Dihydrogen Orthophosphate buffer pH 4.0

3.4 gm of potassium dihydrogen phosphate was weighed and dissolved in 100 ml of water and volume was made up to 500 ml with water. Adjust the pH to 4.0 ± 0.02 using Acetonitrile. The buffer was filtered through 0.45µm filters to remove all fine particles and gases.

Method for selection of Wavelength

Preparation of standard stock solution

About 10 mg of Moxifloxacin HCl and 10 mg of Ketorolac Tromethamine were weighed into a 100 ml volumetric flask, to this 25 ml of methanol was added, sonicated for 10 minutes and the volume was made up to mark with the methanol. This solution was filtered through 0.45 µm filter.

Preparation of working standard solution

1 ml standard stock solution (100 µg/ml) was transferred into 10 ml volumetric flasks and the volume was made up to the mark with methanol (10 µg/ml).

Determination of detection Wavelength

10 ml of working standard solution of Moxifloxacin HCl and Ketorolac Tromethamine (10 µg/ml) in methanol was scanned using UV-Visible spectrophotometer between wavelength ranges from 190 to 400 nm against methanol as blank.

Preparation of mobile phase

Buffer (pH 4.0), Acetonitrile and Methanol were mixed in (50:30:20) V/V ration and the resulting solution was sonicated on a sonicator for 30 min, and then finally filtered through a 0.45µm membrane filter.

Method development

Preparation of Standard stock solution

Weighed accurately about 60 mg of Moxifloxacin HCl and 48 mg of Ketorolac Tromethamine in 100 ml of the volumetric flask, dissolved with 25 mL of the mobile phase and sonicated for 10 minutes. The volume of the flask was made up to the mark with the mobile phase and filtered through a 0.45µm membrane filter.

Preparation of working standard solution

5 ml standard stock solution was pipetted out and transferred into 50 ml volumetric flask and the volume of the flask was made up to the mark with the mobile phase.

Optimization of the method

10 ml of working standard solution was injected with a flow rate of 1 ml/min at 304 nm.

General procedure for construction of the calibration curve

Aliquots of (0.6-1.4ml) the standard drug stock solutions (600 µg/ml of Moxifloxacin HCl and 480 µg/ml of Ketorolac Tromethamine) were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with the mobile phase. This solution was sonicated, filtered and 10µl of this solution was injected into HPLC and analyzed. The calibration curve was constructed by plotting the peak area versus the respective drug concentrations of Moxifloxacin HCl (60-140µg/ml) and Ketorolac Tromethamine (48-112µg/ml).

Estimation of Moxifloxacin HCl and Ketorolac Tromethamine (Assay)

Preparation of Standard solution

About 5 mg of Moxifloxacin HCl and 4 mg of Ketorolac Tromethamine were weighed into a 100 ml

volumetric flask, to this 25ml of the mobile phase was added, sonicated for 10 minutes and the volume was made up with the mobile phase. The resulting solution was filtered through a 0.45µm membrane filter.

Preparation of Sample solution

5ml of 25 mg of Moxifloxacin HCl and 4ml of 20 mg of Ketorolac tromethamine transferred 25ml volumetric flask, 10 ml of the mobile was added, and then sonicated for 5 minutes. The volume of the flask was made up to 25 ml with mobile phase.

Preparation of working sample solution

Transferred 5.0 ml of stock solution into 50 ml of volumetric flask and made up to volume with the mobile phase.

HPLC method validation

Precision

Preparation of standard Moxifloxacin HCl solution: 5mg of Moxifloxacin HCl was taken in a 100 ml volumetric flask, dissolved in 25 ml of the mobile phase and sonicated for 10 minutes. The volume of the flask was made up to 100ml with the mobile phase and the resulting solution was filtered through a 0.45µm membrane filter. From this, 1ml of the solution was pipetted out and diluted to 10 ml with the mobile phase.

Preparation of standard Ketorolac Tromethamine solution: 4mg of Ketorolac Tromethamine was taken in a 100 ml volumetric flask, dissolved in 25 ml of the mobile phase and sonicated for 10 minutes. The volume of the flask was make up to 100ml with mobile phase and the resulting solution was filtered through a 0.45µm membrane filter. From this, 1ml of the solution was pipetted out and diluted to 10 ml with the mobile phase.

RESULTS AND DISCUSSIONS

Selection of Wavelength

The absorption curve shows characteristic absorption maxima at 297 nm for Moxifloxacin HCl (Figure 3), at 320 nm for Ketorolac Tromethamine (Figure 3) and 304 nm the same absorbance for both the drugs. Thus 304 nm was selected as the detector wavelength for the HPLC chromatographic method.

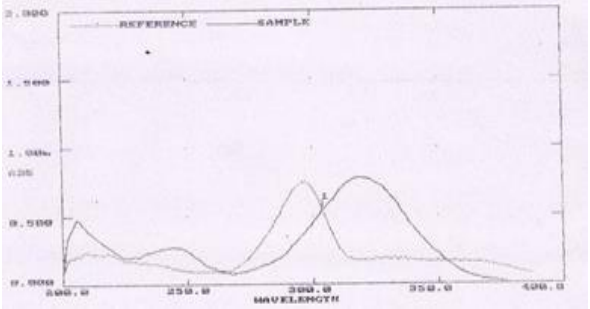

Figure 3: UV chromatogram of Moxifloxacin HCl and Ketorolac Tromethamine

Optimization method

The Moxifloxacin HCl peak was observed at 2.077 min with peak area 6172.055, and theoretical plates of 4263. The Ketorolac Tromethamine peak was observed at 4.067 min with peak area 4716.017 and theoretical plates of 5357 (Table 1). The peaks of Moxifloxacin HCl and Ketorolac Tromethamine were eluted properly (Figure 4). Hence this method was considered for further development.

Table 1: Results of Chromatogram of Moxifloxacin HCl and Ketorolac Tromethamine by using Phosphate buffer:Acetonitrile:Methanol (50:30:20) pH:4.0

S.NO	Name	RT	Height	Area	TP
1	Moxifloxacin HCl	2.077	615.190	6172.055	4263
2	Ketorolac Tromethamine	4.067	378.253	4716.017	5357

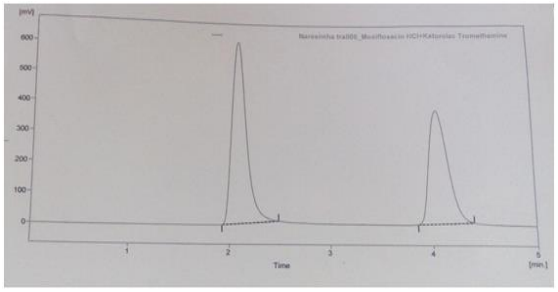

Figure 4: Chromatogram of Moxifloxacin HCl and Ketorolac Tromethamine by using Phosphate buffer:Acetonitrile:Methanol (50:30:20) pH:4.0

Linearity and range

A graph was plotted for Moxifloxacin HCl and Ketorolac Tromethamine against the concentrations of the solutions and the peak areas (Table 2 & Table 3). The correlation coefficient R^2 was determined and was found to be 0.9979 for Moxifloxacin HCl (Figure 5) and 0.9977 for Ketorolac Tromethamine (Figure 6).

Table 2: Linearity data of Moxifloxacin HCl and Ketorolac Tromethamine

S. No.	Moxifloxacin HCl		Ketorolac Tromethamine	
	µg/mL	Area	µg/mL	Area
1	60	4127.217	48	3132.14
2	80	5252.73	64	4001.261
3	100	6259.872	80	4809.099
4	120	7432.037	96	5733.133
5	140	8740.281	112	6772.281

Estimation of Moxifloxacin HCl and Ketorolac Tromethamine (Assay)

The peak area for Moxifloxacin HCl and Ketorolac Tromethamine were 6266.333 and 4775.51 (Table 4). The % purity of Moxifloxacin HCl and Ketorolac Tromethamine were 99.49 and 100.83 (Table 3).

Table 3: Statistical data of the regression equations for determination of Moxifloxacin HCl and Ketorolac Tromethamine

Parameter	Moxifloxacin HCl	Ketorolac Tromethamine
Linearity range (µg/mL)	60-140	48-112
Regression equation (y)	659.71+57.02x	383.51+56.32x
Intercept (b)	659.71	383.51
Slope (a)	57.02	56.32
Correlation coefficient (r)	0.9979	0.9977

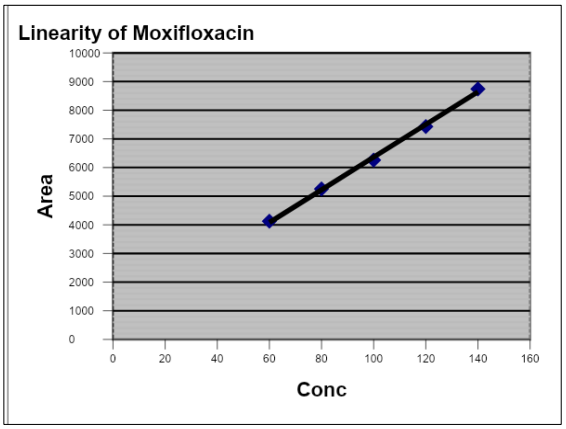

Figure 5: Graph for Linearity data of Moxifloxacin HCl

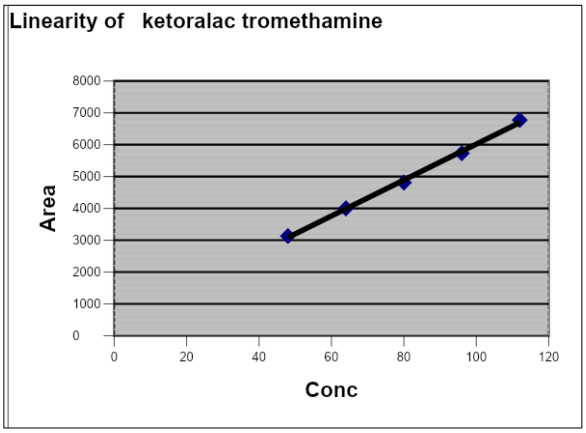

Figure 6: Graph for Linearity data of Ketorolac Tromethamine

Table 4: Overview Results of Assay

Specifications	Moxifloxacin HCl	Ketorolac Tromethamine
Standard peak area	6257.131	4775.456
	6240.905	4749.875
	6261.565	4810.414
	6265.919	4733.082
	6274.786	4800.199
Average	6260.061	4773.805
Sample peak area	6287.440	4737.976
	6274.464	4808.420
	6254.069	4781.101
	6274.786	4800.199
	6240.905	4749.875
	6266.333	4775.51
Amount of standard (in mg)	4.97	4.03
Amount of sample (in mg)	25.2	20.2
% purity of standard	98.6	99.8
% purity of sample	99.49	100.83

Table 5: Precision results of Moxifloxacin HCl and Ketorolac Tromethamine

S. No.	Moxifloxacin HCl		Ketorolac Tromethamine	
	Retention time	Peak area	Retention time	Peak area
1	2.077	6137.949	4.067	4716.017
2	2.077	6204.208	4.107	4712.087
3	2.070	6145.971	4.117	4705.468
4	2.073	6187.949	4.130	4724.778
5	2.083	6276.876	4.180	4838.002
6	2.077	6190.210	4.107	4760.061
Average	2.0762	6190.527	4.118	4742.736
Standard Deviation	0.0044	49.799	0.037	50.479
% RSD	0.21	0.80	0.90	1.06

Table 6: Accuracy results of standard Moxifloxacin HCl and Ketorolac Tromethamine

Concentration (µg/ml)	Peak area	Amount (µg)	Average amount (µg)	Standard deviation	% Recovery	% RSD
Moxifloxacin HCl						
80	5252.73	80.09	80.14	0.046	100.18	0.057
	5258.69	80.15				
	5241.56	80.18				
100	6259.872	100.05	100.09	0.045	100.09	0.045
	6260.865	100.09				
	6259.853	100.14				
120	7432.037	120.15	120.10	0.045	100.08	0.037
	7432.031	120.10				
	7433.027	120.06				
Ketorolac Tromethamine						
64	4001.261	63.91	63.98	0.076	99.97	0.119
	4001.256	64.06				
	4001.260	63.96				
80	4809.099	80.07	79.94	0.113	99.92	0.141
	4809.120	79.91				
	4809.156	79.85				
96	5733.133	96.02	95.93	0.095	99.93	0.099
	5733.245	95.83				
	5733.210	95.94				

Table 7: Accuracy results of standard Moxifloxacin HCl and Ketorolac Tromethamine by standard addition method

Concentration (µg/ml)	Peak area	Amount (µg)	Average Amount (µg)	Standard deviation	% Recovery	% RSD
Moxifloxacin HCl						
80+20	6792.409	98.73	98.73	0.057	98.73	0.058
	6403.692	98.76				
	6259.324	98.84				
100+20	7659.385	121.45	121.53	0.078	101.27	0.064
	7730.537	121.56				
	7432.037	121.60				
120+20	8689.774	141.26	141.29	0.031	100.92	0.022
	8821.788	141.32				
	8740.235	141.28				
Ketorolac Tromethamine						
64+16	5157.593	78.41	78.48	0.015	98.1	0.019
	4832.731	78.43				
	4729.099	78.44				
80+16	5829.489	96.75	96.98	0.21	101.02	0.217
	5926.363	97.15				
	5733.133	97.04				
96+16	6785.489	113.91	113.99	0.28	101.78	0.246
	6864.216	114.30				
	6772.281	113.76				

Hence % purity of both drugs was found to be within the limits (98-102 %).

System precision

The system precision was determined by analyzing the standard preparation of Moxifloxacin HCl and Ketorolac Tromethamine for six times. The chromatograms were recorded and the results were summarized in Table 5. % RSD of retention time and peak areas obtained for Moxifloxacin HCl was 0.21 and 0.80 respectively and for Ketorolac Tromethamine were 0.90 and 1.06 respectively.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD for this method was found to be 1.83µg/ml for Moxifloxacin HCl and 1.48µg/ml for Ketorolac Tromethamine respectively. The LOQ for this method was found to be 5.55µg/ml for Moxifloxacin Hcl and 4.49µg/ml for Ketorolac Tromethamine respectively.

Validation of the methods

Procedure for the standard: Samples of the standard were prepared and tested at three levels such as 80, 100, 120 µg/ml for Moxifloxacin HCl and 64, 80, 96 µg/ml Ketorolac Tromethamine by the proposed method. Samples of samples were prepared and tested at three levels such as 80, 96, 112 µg/ml by standard addition method, in which variable amounts of a previously analyzed portion of a standard drug were added to the sample (Table 6 and Table 7). The average percent recovery of standard and samples were found to be 93.96–100.7% and respectively, indicates the good accuracy of the method.

CONCLUSION

The proposed RP-HPLC (Reverse Phase High performance Liquid Chromatography) method has been evaluated for accuracy, precision and linearity. The method was found to be precise, accurate and linear over the linear concentration range. The analytical method validation of Moxifloxacin HCl and Ketorolac Tromethamine by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Moxifloxacin HCl and Ketorolac Tromethamine. The method was validated as per ICH guidelines. Therefore, this HPLC method can be used as routine analysis of these drugs in bulk, pharmaceutical formulations and also for stability studies.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENT

Authors are thankful to Management and Principal, Jaya College of Paramedical Sciences, College of Pharmacy, Chennai, Tamil Nadu, India for providing all the facilities to carry out the work.

REFERENCES

1. Sahu, S.K., Azam, A., & Banarjee, M. (2011). Spectrophotometric Estimation of Moxifloxacin in Bulk and its Pharmaceutical Formulations. Pharmacologyonline, 2, 491-502.
2. Dinesh, M.D., Atul, A.S., Sanjay, J.S. (2011). Quantitative determination of Moxifloxacin hydrochloride in bulk and ophthalmic solution by UV-spectrophotometry and first order derivative using area under curve. Scholars Research Library Der Pharmacia Lettre, 3(3), 453-456.
3. Najma, S., Mahwish, A., Sana, S., Somia, G., Muhammad, S.A., Moona, M.K. (2010). HPLC assay for moxifloxacin in bulk and pharmaceutical formulations and serum application to invitro metal interactions. Journal of Chinese Chemical Society, 57 (4A), 2010.
4. Xu, Y.H., Li, D., Liu, X.Y., Li, Y.Z., Li, J. (2010). HPLC assay with ultraviolet detection for Moxifloxacin; validation and application to a pharmacokinetic study in Chinese volunteers. Journal of Chromatoraphy B, 878(32), 3437-3441. https://doi.org/10.1016/j.jchromb.2010.10.024
5. Dhillon, V., & Chaudhary, A. K. (2010). A validated HPTLC method for estimation of moxifloxacin hydrochloride in tablets. Pharmaceutical methods, 1(1), 54–56. https://doi.org/10.4103/2229-4708.72232
6. Chaudhary, R.S., Gangwal, S.S., Jindal, K.C., Khanna, S. (1993). Reversed-phase high performance liquid chromatography of ketorolac and its applicationto bioequivalent studies in human serum. Journal of Chromatography B: Biomedical Sciences and Applications, 180–184. https://doi.org/10.1016/0378-4347(93)80240-5
7. Sunil, G., Jambulingam, M., Ananda Thangadurai, S., Kamalakannan, D., Sundaraganapathy, R., Jothimanivannan, C. (2017). Development and validation of Ketorolac Tromethamine in eye drop formulation by RP-HPLC method. Arabian Journal of Chemistry, 10(1), S928-S935. https://doi.org/10.1016/j.arabjc.2012.12.031
8. Wu, A. T., & Massey, I. J. (1990). Simultaneous determination of ketorolac and its hydroxylated metabolite in plasma by high-performance liquid chromatography. Journal of chromatography, 534, 241–246. https://doi.org/10.1016/s0378-4347(00)82169-6
9. Ghandi, L.R., Dewani, A.P., Bakal, R.L., Chandewar, A.V. (2011). Absorption ratio method for the estimation of moxifloxacin hcl & ketorolac tromethamine in their combined dosage form by uv-visible spectroscopy. International Journal Pharmaceutical Medicine, 3(7), 21- 26.
10. Raju, B., Ramesh, M., Borkar, R. M., Padiya, R., Banerjee, S. K., & Srinivas, R. (2012). Development and validation of liquid chromatography-mass spectrometric method for simultaneous determination of moxifloxacin and ketorolac in rat plasma: application to pharmacokinetic study. Biomedical chromatography : BMC, 26(11), 1341–1347. https://doi.org/10.1002/bmc.2701
11. Razzaq, Syed Naeem, Khan, Islam Ullah, Ashfaq, Muhammad, & Mariam, Irfana. (2012). Stability indicating HPLC method for simultaneous determination of moxifloxacin hydrochloride and ketorolac tromethamine in pharmaceutical formulations. Química Nova, 35(6), 1216-1221. https://doi.org/10.1590/S0100-40422012000600028

12. Patel, D., Patel, M., Patel, K. (2012). Simultaneous RP-HPLC Estimation of Moxifloxacin Hydrochloride and Ketorolac Tromethamine in Ophthalmic Dosage Forms. Asian Journal of Research In Chemistry, 697.

13. Begum, S., Bharathi, K., Vaddepally, L., Rani, G. (2014). A validated RP-HPLC method for simultaneous estimation of moxifloxacin hydrochloride and ketorolac tromethamine in ophthalmic dosage form. Der Pharmacia Lettre, 6. 335-341.