Journal of Pharmacreations

PharmaCreations

ISSN: 2348-6295

Pharmacreations \ Vol 9 \ Issue 2 \ Apr - Jun - 20222

Jouranl Home page: www. pharmareations.com

Research article Open Access

Analytical method development and validation forofloxacin and methazolamide by using RP-HPLC

K.Silpa*, A.Sai Shilpa, B.Sandya, M.Saritha, N.Seetharamulu, Shabista Fathima

Department of pharmaceutical Analysis and Quality assurance, Teegala Ram Reddy College of Pharmacy, Telangana, India.

Corresponding author: K. Silpa

ABSTRACT

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Ofloxacin and Methazolamide in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Ofloxacin and Methazolamide was done by RP-HPLC. The Phosphate buffer was pH 4.6 and the mobile phase was optimized which consists of MEOH : Phosphate buffer mixed in the ratio of 70:30 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µm,

Keywords: Methazolamidem, Ofloxacin,

INTRODUCTION

Ofloxacin acts on DNA gyrase and toposiomerase IV, enzymes which, like human topoisomerase, prevents the excessive supercoiling of DNA during replication or transcription. By inhibiting their function, the drug thereby inhibits normal cell division.

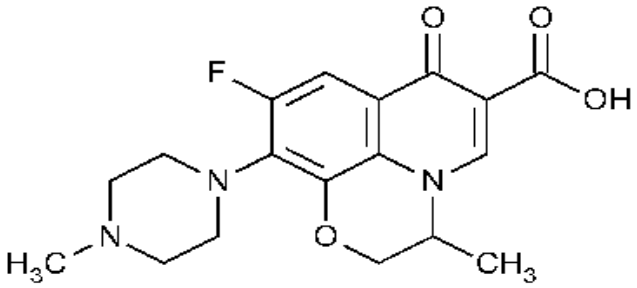


Fig 1: Structure Of oflaxacin

Methazolamide is a potent inhibitor of carbonic anhydrase. Inhibition of carbonic anhydrase in the ciliary processes of the eye decreases aqueous humor secretion, presumably by slowing the formation of bicarbonate ions with subsequent reduction in sodium and fluid transport.

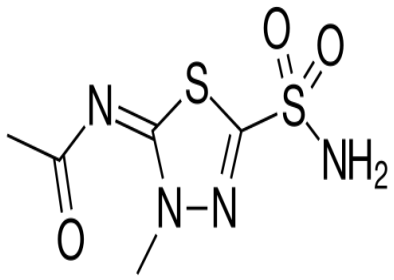


Fig 2: Structure of Methazolamide

MATERIALS AND METHODS

List of Instruments used

S.No.	Instrument	ModelNo.	Software	Manufacturer's name
1	HPLCAlliance PDADetector	Waters2695	Empower	Waters
2	UVdoublebeam spectrophotometer	UV3000	UVWin5	LabIndia
3	Digitalweighing balance	BSA224SCW	-	Satorius
4	pHmeter	AD102U	-	Lab India
5	Ultrasonicator	SE60US	-	-
6	Suctionpump	VE115N	-	-

List of Chemicals

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLCGrade
2	Methanol	Merck	HPLCGrade
3	Acetonitrile	Merck	HPLCGrade
4	Potassiumdihydrogenort	Merck	A.R
5	Ofloxacin& Methazolamide	-	-

Optimized chromatogram is obtained by following conditions

Column	:	Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent
Buffer pH	:	4.6
Mobile phase	:	70% Meoh : 30% phosphate buffer ph-4.6
Flow rate	:	1 ml per min
Wavelength	:	273 nm
Temperature	:	ambient.

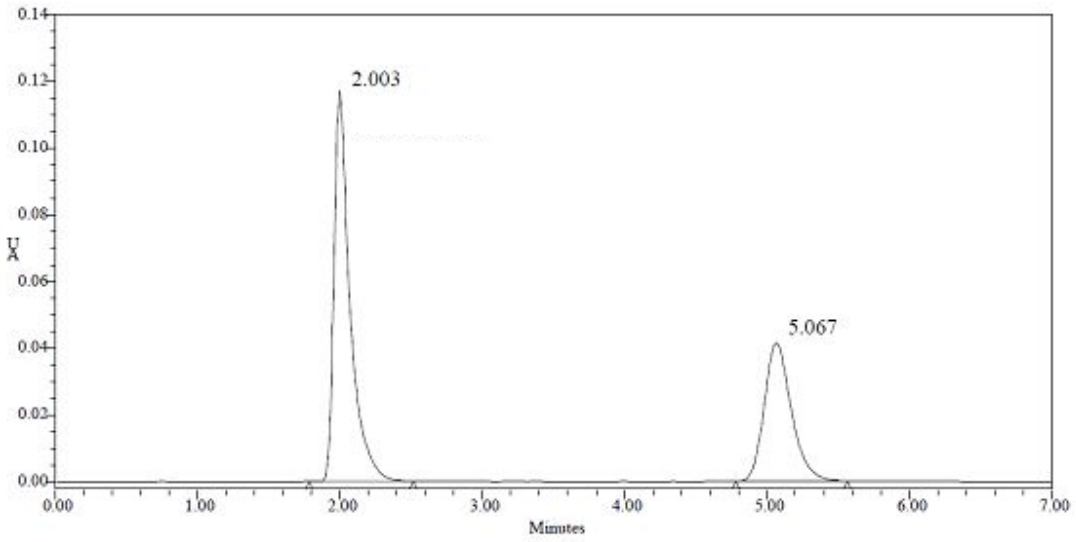


Fig 3: Chromatogram for Ofloxacin and Methazolamide

System Suitability

The system suitability of the method was checked by injecting five different preparations of the Ofloxacin and Methazolamide standard. The parameters of system suitability were checked.

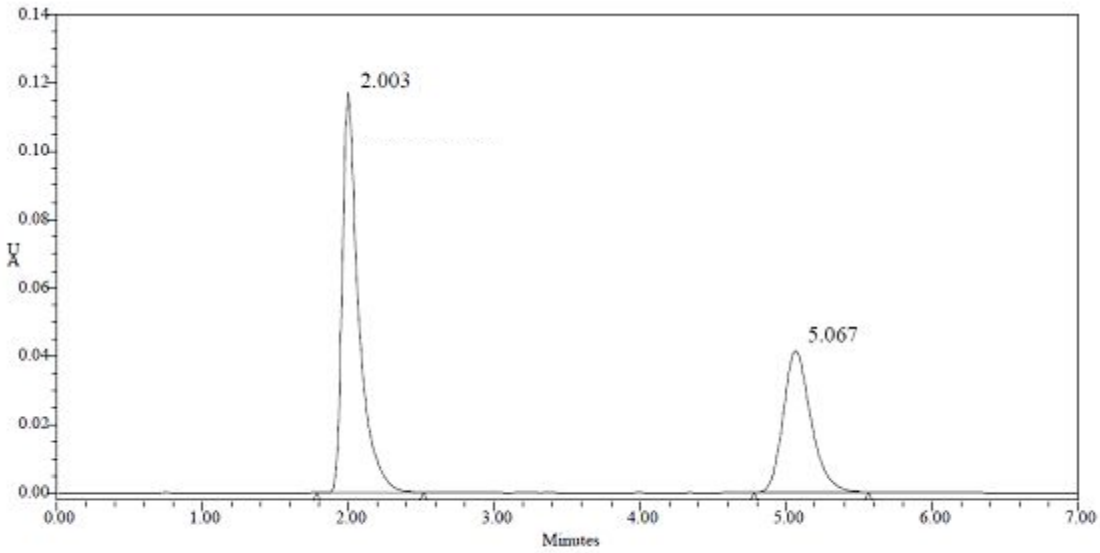


Fig 4: Chromatogram for system suitability

Table 1: Results of system suitability parameters for Ofloxacin and Methazolamide

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Ofloxacin	2.003	920101	116666		1.6	2711.8
2	Methazolamide	5.067	552058	41531	11.0	1.3	3428.2

Resolution between two drugs should not be less than 2. Theoretical plates should not be less than 2000. Tailing factor should not be less than 0.9 and not more than 2. It was found from above data that all the system suitability parameters for developed method were within the limit.

Precision

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table 2: Results of method precision for Ofloxacin

S. No	Sample area	Standard area	Percentage purity
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09
Average			**100.84**
%RSD			**0.304**

Table 3: Results of method precision for Methazolamide

S. No	Sample area	Standard area	Percentage purity
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09
Average			**101.24**
%RSD			**0.46**

%RSD for sample should be NMT 2. The %RSD for the standard solution is below 2, which is within the limits hence the method is precise.

Results of Intermediate precision for Methazolamide

%RSD of five different sample solutions should not be more than 2. The %RSD obtained is within the limit, hence the method is rugged.

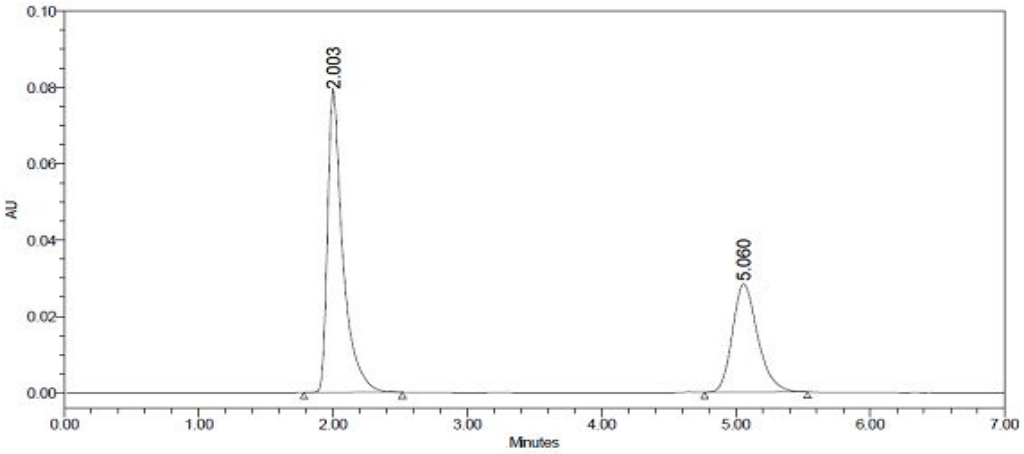
Table 4: Results of Accuracy

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		ARTE	**PIPE**	**ARTE**	**PIPE**	**ARTE**	**PIPE**
50%	**1**	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average Recovery					**99.7%**	**99.7%**
100%	**1**	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					**99.6%**	**99.8%**
150%	**1**	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					**99.7%**	**99.8%**

The percentage recovery at each level should be between (97-103%). The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence the method is accurate.

Linearity

The linearity range was found to lie from 25% to 125% and chromatograms are shown below.



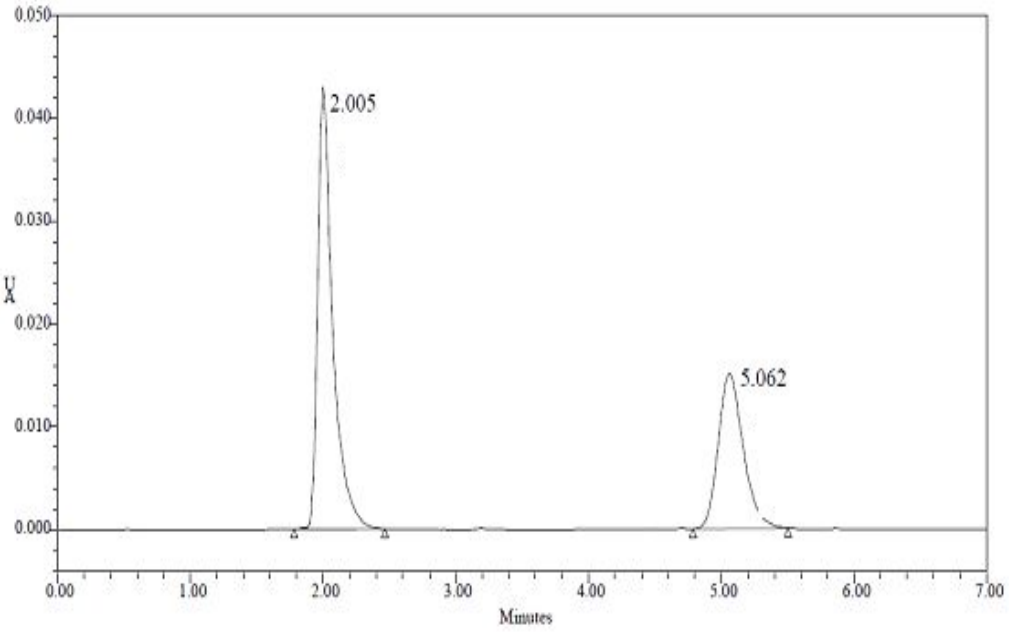
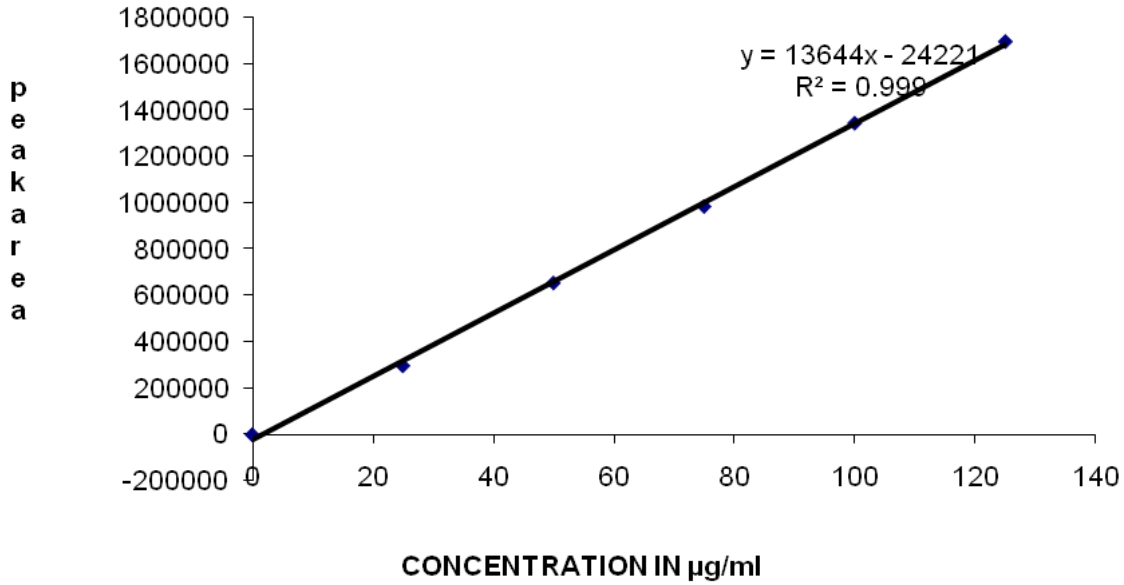


Table 5: Area of different concentration of Ofloxacin and Methazolamide

Concentration (µg/ml)	Peak area of ofloxacin	Peak area of Methazolamide
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614



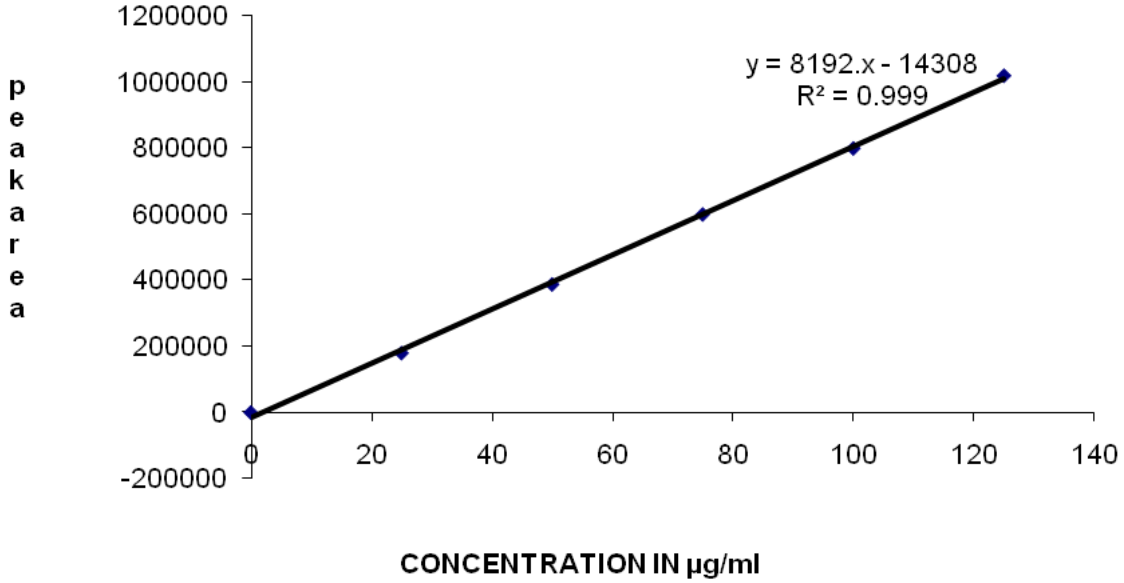


Table 6:Analytical performance parameters of Ofloxacin and Methazolamide

Parameters	Ofloxacin	Methazolamide
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearity was established in the range of 25 to 150µg/ml. The results obtained are within the limit.

ROBUSTNESS

The standard and samples of Ofloxacin and Methazolamide were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 7: Results for effect of variation in flow

S. No	peak area for Less flow (0.7 ml/min)		peak area for More flow (0.9 ml/min)	
	Ofloxacin	**Methazolamide**	**Ofloxacin**	**Methazolamide**
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	**986306**	**582223**	**976755**	**591667**
%RSD	**0.45**	**0.80**	**0.53**	**0.80**

Percentage RSD should not be more than 2. The %RSD obtained for change of flow rate, variation in mobile phase was found to be below 2, which is within the acceptance criteria. Hence the method is robust.

CONCLUSION

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Ofloxacin and Methazolamide in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Ofloxacin and Methazolamide was done by RP-HPLC. The Phosphate buffer was pH 4.6 and the mobile phase was optimized which consists of MEOH: Phosphate buffer mixed in the ratio of 70:30 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 273 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Ofloxacin and Methazolamide were found to be from 25-125 µg/ml. Linear regression coefficient was not more than 0.999.

REFERENCES

1. Rapid RP-HPLC method for simultaneous estimation of sparfloxacin, gatifloxacin, Methazolamideand TinidazoleAsian J. Pharm Res. 2011;1(4):119-25.
2. Maslarska Vania. rp-hplc method for simultaneous determination of metronidazole and ofloxacin in synthetic mixturemarch. Vols. 23-25. prague, czech republic; 2016.
3. JelamVazir, Minal Rohit, Dhananjay Meshram. Development and Validation of Spectroscopic Method for the Estimation Of Metronidazole And Norfloxacin In Syrup Dosage Form. 2015;4(5):655-668. Available from: https://storage.googleapis.com/journal-uploads/wjpps/article_issue/1430381550.pdf
4. SoumyaJyotiGhosh SoumendraDarbar, ParthaPratimChowdhury Shyama Prasad Chattopadhyay, MatishRanjanChakraborty. Simultaneous determination and validation of ofloxacin and methazolamide in combined dosage pharmaceutical formulation. Int J Pharm Tech Res. 2010;2(1):367-74.
5. Pande VV, Chandorkar JG. A sensitive method HPLC method on determination of 2-methyl 5 nitro imidazole and reaction mass of intermediates of ornidazole in ornidazole bulk manufacturing. Int J Pharm Tech Res. 2009;1(2):310-2.
6. Okeri Henry A, Ikhuoria M. Arhewoh. Analytical profile of the fluoruquiniloneantibacterials I. Ofloxacin. Afr J Biotechnol. 2008;7(6):670-80.
7. SonaliParamane LataKotapalli, Thomas Asha, Deshpande AD. Simultaneous RP-HPLC estimation of gatifloxacin and ornidazole in tablet dosage form. Indian J Pharm Sci. 2007;4(69):525-8.
8. RanjitSingh MukeshMaithani, Saraf Shailendra K. ShubiniSaraf and Ram C. Gupta Simultaneous estimation of ciprofloxacin hydrochloride, ofloxacin, tinidazole and ornidazole by RP HPLC. EurAsian J Anal Chem. 2009;4(2):161-7.