ejpmr, 2019,6(2), 627-630

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

NOVEL METHOD OF DEVELOPMENT AND SYNCHRONIZED VALIDATION OF SATRANIDAZOLE AND OFLOXACIN IN COMBINED DOSAGE FORM ASSISTED BY RP-HPLC

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Article Received on 10/12/2018 **Article Revised on 30/12/2018** **Article Accepted on 20/01/2019**

ABSTRACT

A Simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of satranidazole and ofloxacin. Chromatographic separation of these drugs were performed on Kromasil C_{18} column (250 x 4.6 mm, 5 µ) as stationary phase with a mobile phase comprising of 20 m M potassium dihydrogen phosphate: acetonitrile in the ratio of 60:40 (v/v) containing 0.1% glacial acetic acid at a flow rate of 1 mL/min and UV detection at 318 nm. The linearity of satranidazole and ofloxacin were in the range of 1.5 to 3.6 µg/mL and 1.0 to 2.4 µg/mL respectively. The recovery was calculated by standard addition method. The average recovery was found to be 100.63% and 100.02% for satranidazole and ofloxacin respectively. The proposed method was found to be accurate, precise and rapid for simultaneous determination of satranidazole and ofloxacin.

KEYWORDS: Ofloxacin, RP-HPLC, Satranidazole, Tablet.

INTRODUCTION

Satranidazole (SAT), is a novel nitroimidazole derivative. Chemically, it is 1-methylsulfonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone.[1] It is used as antiprotozoal and anitibacterial agent in the treatment of amoebiasis.

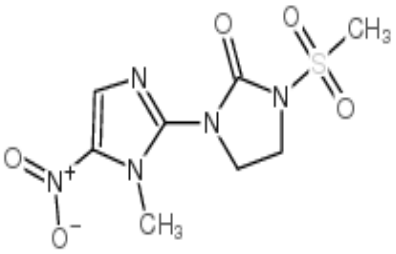


Fig.No.1. Satranidazole.

Ofloxacin (OFL) is a fluoroquinolone derivative. Chemically, it is (±)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido-[1,2,3-de]-1,4 benzoxazine-6- carboxylic acid.[2] It is mainly used as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases

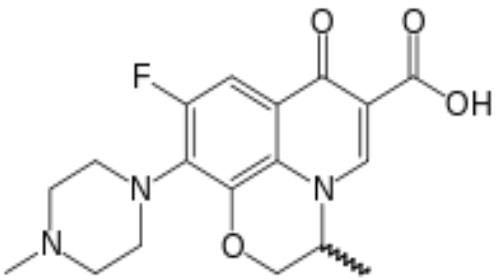


Fig.No.2. Ofloxacin.

Literature survey revealed that a number of methods have been reported for estimation of ofloxacin individually or in combination with other drugs[2, 3, 4] and satranidazole is estimated individualy.[5,6,7] However there is only one analytical method reported for this combination of drugs by using spectrophotometer.[8, 9] For best of our knowledge, no method has been reported for simultaneous determination of satranidazole and ofloxacin by HPLC. Hence, an attempt has been made to develop new RP-HPLC method for its simultaneous

estimation in pharmaceutical dosage form with good accuracy, precision and simplicity.[10, 11]

MATERIALS AND METHODS

Experimental

The formulations, satrogyl-*o* tablet (containing 300 mg of satranidazole and 200 mg ofloxacin; manufactured by Alkem Laboratories Limited, Baddi), were procured from pharmacies. Satranidazole working standard was obtained as a gift sample from Alkem laboratories, Ofloxacin from Zhejianh Kangyo Pharm. Co. Ltd. Acetonitrile and methanol were of HPLC grade purchased from E-Merck (India) Ltd. Glacial acetic acid used were of analytical grade purchased from Spectrochem Pvt. Ltd. Potassium dihydrogen *ortho* phosphate was of AR grade purchased from Qualigens Ltd.

Mobile Phase

A mixture of 55 volumes of mixed phosphate buffer and 45 volumes of acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Preparation of buffer

Weigh accurately about 1.625 gms of potassium dihydrogen phosphate and 0.3 gms of dipotassium hydrogen phosphate were dissolved in 1000ml of water.Sonicate it for 10minutes to remove gases.

Instrument

The analysis was carried on the integrated HPLC system (Agilent 1100 series, Germany) consisted of G1311A Quaternary pump with G1379A Degasser, G1329A Autosampler (1-100 µL) with G1330B Autosampler Thermostat, G1316A Column Compartment with temperature controller and G1314A VWD UV detector. Chromatograms were processed and the results were analyzed by using Chemstation software (Rev.10.01).

Optimized chromatographic condition

Analytical Column	:	Kromasil 100-5C18, (250 x 4.6 mm, 5 µm).
Mobile Phase	:	20 mM Potassium dihydrogen phosphate: Acetonitrile (60:40) containing 0.1% glacial acetic acid.
UV Detection	:	318 nm
Flow Rate	:	1.00 mL/min
Injection Volume	:	5 µL
Temperature	:	Ambient
Run Time	:	8.0 min.
Retention Time	:	Ofloxacin ~ 2.29 Satranidazole ~ 4.80

Standard solution preparation

Satranidazole and ofloxacin standard stock solution-I and II (1 mg/mL) were prepared by dissolving 10 mg of the drug in 10 mL methanol and further dilutions were prepared in mobile phase to obtain calibration standards in the concentration range of 1.5-3.6 µg/mL and 1.0-2.4 µg/mL respectively.

Linearity

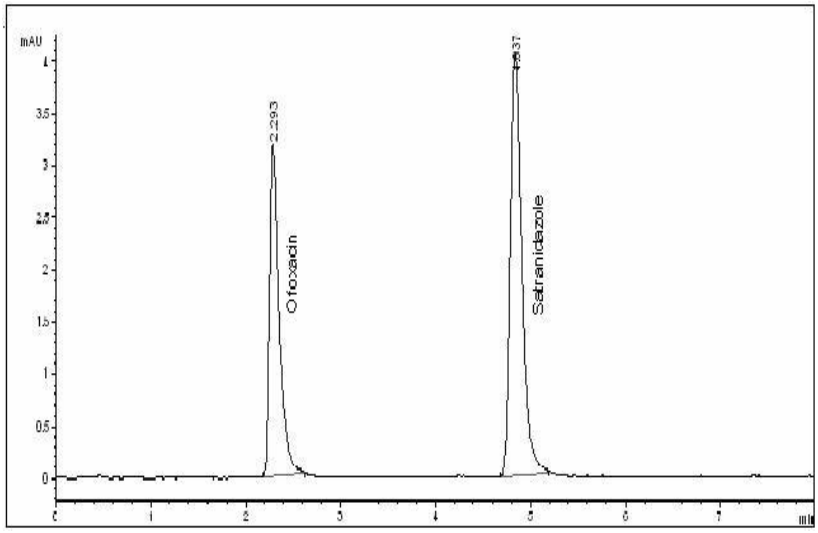
Eight different concentrations from 1.5-3.6 µg/mL for satranidazole and 1.0-2.4 µg/mL for ofloxacin were prepared for linearity studies. The responses were measured as peak areas and plotted against concentrations. Linear regression least square fit data obtained from the above calibration curve. The respective slopes (m), intercept (b) and correlation co-efficient (r) are also obtained.

Sample preparation

Twenty tablets were weighed and average weight was calculated. These tablets were powdered. Weight equivalent to the one tablet was taken in a 100 mL volumetric flask; dissolved in minimum amount of mobile phase and diluted up to the mark with the same. The solution was then filtered through a Whatmann filter paper no. 41 and filtrate was collected in the flask. 0.1 mL of the filtrate was diluted to 100 mL with mobile phase to get 3 µg/mL of satranidazole and 2 µg/mL of ofloxacin.

RESULT AND DISCUSSION

Typical HPLC chromatogram showing ofloxacin (2 µg/mL) and satranidazole (3 µg/mL).



Assay

From the above sample solution 5 µL was injected along with the same concentration of the standard solution under the optimized chromatographic conditions. The peak area values of satranidazole and ofloxacin were calculated. The peak area values of SAT and OFL present in that solution was then estimated using calibration curve method.

Results of assay experiment.

Drug	Labeled claim, mg	Amount found (n=6)	%RSD	%Assay
Satranidazole	300	300.05	1.45	100.02
Ofloxacin	200	203.61	2.07	101.80

Recovery

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation and to study the accuracy and precision of the method. Recovery experiment was performed by the standard addition method.[15] The recovery of the standard was studied at the three different levels *viz*. 80%, 100% and 120% of the estimated amount of drug. Each set of recovery of added standard was calculated. The results of recovery experiment are tabulated.

Recovery studies of Satranidazole and Ofloxacin

Drug	Original mount,	Added amount,	Total amount	%Recovery	%RSD
	mg	mg	found, mg		
	300	240	539.85	99.97	0.877
SAT	300	300	611.03	101.84	1.197
	300	360	660.57	100.09	1.222
	200	160	356.93	99.15	2.063
OFL	200	200	404.36	101.09	2.764
	200	240	439.24	99.83	3.685

(n= each value is average of three determination)

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

The limit of detection (LOD) and quantification (LOQ) were evaluated from calibration curves plotted in concentration ranges of 0.150-0.360 µg/mL for satranidazole and 0.100-0.240 µg/mL for ofloxacin, with formula LOD = 3.3 syx/S and LOQ = 10 syx/S (where syx = residual error and S= slope of the calibration curve). The LOD and LOQ for each drug were thus obtained. Thereafter, the standard drug solutions at each value of LOD and LOQ concentration were injected six times and % RSD of area of the replicate injections were calculated.

Ruggedness

Ruggedness tests were performed on HPLC assay of satranidazole and ofloxacin of pharmaceutical tablet and peak area response of standard solution. The effects of different chromatographic columns by different analysts on different days were evaluated on % assay of pharmaceutical tablet and peak area response of standard solutions. The % RSD not more than 3 indicting good ruggedness of the developed HPLC method.

Robustness

Robustness of the method was ascertained by evaluating the effect of deliberate change pH of the mobile phase by adding different volumes of glacial acetic acid,

proportion of organic solvent in mobile phase and flow rate. The pH of mobile phase was varied within a range of 0.2 unit of the optimize pH (4.0) by adding different volumes of glacial acetic acid. While proportion of organic solvent (acetonitrile) was varied in the range of ±2%. The mobile phase were employed, keeping the other chromatographic conditions constant, to evaluate the influence of pH and organic solvent on resolution between two drugs. Keeping other chromatographic conditions optimized different flow rates ± 0.1 mL/min were employed to check influence of flow rate on the proposed method.

Robustness study for Satranidazole and Ofloxacin.

	Volume of acetic acid			**Change in ACN**				**Change in**	
Studied		**added in MP**		**Composition**			**Flow rate, mL/min**		
Parameter	0.5%	(Ideal)	1.5%		(Ideal)			(Ideal)	
	pH:	1.0%		62:38	60:40	58:42	0.9	1.0	1.1
	4.20	pH: 4.00	pH: 3.80						
% Assay Satranidazole	100.56	100.03	101.36	99.20	100.64	100.18	100.18	100.51	99.45
% Assay Ofloxacin	101.20	100.52	101.68	100.26	100.48	101.50	100.85	100.25	101.28

Simple and selective LC method is described for the determination of Satranidazole and Ofloxacin in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumes of Methanol and 30 volumes of water and 30 volumes of Acetonitrile with detection of 270 nm. Linearity was observed in the range 100-300 µg/ml for Satranidazole (r^2 =0.997) and 150-450 µg /ml for Ofloxacin (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. It is most precise method which is indicated by the The method was found to be precise which is indicated by the repeatability analysis, with < 2 showing %RSD. The statistical data thus obtained proves validity of the methods and can be used for pharmaceutical dosage form routine analysis.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Satranidazole and ofloxacin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

ACKNOWLEDGEMENTS

I, Dr.Tribhuvan Singh thankful to management of Scient Institute of Pharmacy, Ibrahimpatnam, Hyderabad for providing necessary facilities to carry out the research work.

REFERENCES

1. WHO Model Formulary 2008 *(PDF). World Health Organization. 2009. p. 140.* Retrieved 8 December 2016.
2. Ofloxacin Use during Pregnancy | Drugs.com", *www.drugs.com*. Retrieved 28 December 2016.
3. *Fischer, Janos; Ganellin, C. Robin (2006).* Analogue-based Drug Discovery. *John Wiley & Sons. p. 500.*
4. "WHO Model List of Essential Medicines (19th List)" *(PDF). World Health Organization. April 2015*. Retrieved 8 December 2016.
5. "Ofloxacin". *International Drug Price Indicator Guide*. Retrieved 8 December 2016.
6. *Hamilton, Richart (2015). Tarascon Pocket Pharmacopoeia 2015 Deluxe Lab-Coat Edition. Jones & Bartlett Learning. p. 101.*
7. Ortho-McNeil-Janssen Pharmaceuticals, Inc. *(2008).* "FLOXIN Tablets (Ofloxacin Tablets)" *(Susan Blank; Julia Schillinger* "DOHMH ALERT #8: Fluoroquinolone-resistant gonorrhea, NYC". *USA: New York County Medical Society*. Retrieved July 22, 2009.
8. *Knapp JS, Fox KK, Trees DL, Whittington WL.* "Fluoroquinolone resistance in Neisseria gonorrhoeae". *Emerging Infect. Dis., 1997; 3(1): 33–39.*
9. *Dan M (April 2004).* "The use of fluoroquinolones in gonorrhoea: the increasing problem of resistance". *Expert Opin Pharmacother. 5(4): 829–54.*
10. Singh T, Dhiraj Kumar, Anurag M, Sandeep A, Kishore Kumar DVK. Development of RP-HPLC Method for simultaneous estimation of Cefadroxil and Ambroxol in pure and Pharmaceutical Dosage form. British Journal of Pharmaceutical Research. April 19, 2017; 15(6): 1-7.