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Simultaneous Estimation of Gatiloxacin, OrnidazolE and its Isomer by Reverse Phase High Performance Liquid Chromatography

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Abstract: A specific, accurate, precise and sensitive validated reverse phase liquid chromatographic (RP-HPLC) method has been developed for the simultaneous estimation of Gatifloxacin, Ornidazole and its isomer in bulk drug as well as tablet dosage form. Drugs were analysed on C_{18} column using mobile phase acetonitrile: water: triethylamine (pH 3.3) (30:70:0.1 v/v/v). A flow rate was maintained at 1.3 ml/min and detection was made at 302 nm. The retention time for Gatifloxacin, Ornidazole and its isomer was found to be 3.77, 4.91 and 5.60 min respectively. Proposed method was validated for accuracy, precision, linearity and range, ruggedness. Linearity of Gatifloxacin and Ornidazole was in the range of 2-40 µg/ml and 5-100 µg/ml respectively. Average percentage recoveries obtained for Gatifloxacin and Ornidazole were 100.78 % and 100.28 %.

Keywords: Gatifloxacin, Ornidazole, RP-HPLC, Validation.

Introduction

Chemically Gatifloxacin (Gati) is (±) -1-Cyclopropyl-6-fluro-1,4-dihydro-8-methoxy-7-(3-methyl-piperazinyl)-4-oxo-3-quinolone carboxylic acid sesquihydrate[1] and Ornidazole is [α-(chloromethyl-2-methyl-5-nitroimidazole]ethanol)[2]. Literature reveals that few Spectrophotometric [3,4,5], HPLC [6,7,8] and HPTLC [9,10,11] methods are reported for estimation of Gatifloxacin and Ornidazole alone and in combination. No method was found for simultaneous estimation of Gati, Orni and its isomer. So, in the present investigation an attempt has been made to develop accurate and precise RP-HPLC method for simultaneous estimation of Gati and Orni with its isomer in combination dosage form.

Experimental

Gatifloxacin (Gati) and Ornidazole (Orni) bulk powders were kindly gifted by Jaipur pharmaceuticals, Jaipur and Vapi Care Pharma Pvt. ltd, Vapi respectively. HPLC grade water and acetonitrile were procured from Loba Chemie Pvt. Ltd., Mumbai.

Instrumentation

Analysis of all the samples were performed using JASCO PU 1580 HPLC system consisted of a intelligent pump, a 1575 intelligent UV-Visible detector with precision loop injector (Rhenodyne, 20µl). The data was processed using Borwin 1.27 software. All the samples were filtered through whatman filter paper no. 41 and degassed by sonication for 15min.

Chromatographic condition
RP-HPLC method was developed on JASCO intersil C_{18}, 4.6 (i.d.) x 250 mm, 10µm column. A mobile phase containing acetonitrile: water: triethylamine (pH 3.3) (30:70:0.1 v/v/v), (pH adjusted with 5% orthophosphoric acid) was selected because it was found ideally resolves the peaks of Gati (Rt = 3.77 min), Orni and its isomer (Rt = 4.91, 5.60 min) as shown in Figure 1. Wavelength was selected at 302 nm by scanning standard solution of both the drugs over 200-400 nm wavelength. Measurements were made at the flow rate of 1.3 ml/min with injection volume 20µl.

Preparation of standard stock solution
Standard stock solution containing Gati and Orni were prepared individually by dissolving 100 mg of Gati and 250 mg of Orni in methanol. The final dilution of both the solution were made up to 100 ml with methanol to get stock solution containing 1000 µg/ml of Gati and 2500 µg/ml of Orni.

Calibration Curve
An aliquot portion of standard stock solution of Gati and Orni were further diluted with mobile phase to the series of concentration ranging from 0 – 40 µg/ml for Gati and 0 -100 µg/ml for Orni. The mobile phase was allowed to equilibrate with stationary phase until the steady baseline was obtained. Then each dilution of both the drug was injected and the peak area recorded. The calibration curve was constructed by plotting concentration of the drug against average peak area and regression equation was computed.

Validation of method
The developed method was validated in terms of accuracy, precision, linearity and range, ruggedness study.

Sample preparation
Twenty tablets (Gatri oz, Nicholas Piramal India Ltd.) were weighed and finely powdered. An accurately weighed quantity of powder equivalent to 100 mg of Gati (equivalent to 250 mg) was taken in 100 ml of volumetric flask and dissolved in methanol. Volume was made up to the mark by methanol. The solution was sonicated for 15 min. and then filtered through whatman filter paper no. 41. Further dilutions were made by mobile phase.

Results and Discussion
To develop a precise, accurate and suitable RP-HPLC method for the simultaneous estimation of Gati and Orni, different mobile phase were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The results obtained by the assay of marketed formulation are summarized in table no. 1. System suitability testes were carried as per USP and parameters are summarized in table no. 2.

Method Validation
Accuracy
The proposed HPLC method ascertained on the basis of recovery study performed by the standard addition method at 80%, 100% and 120% known amount of standard Gati and Orni were added to preanalysed samples and were subjected to the propose HPLC method. Results of the recovery studies were shown in table no. 3.

Precision
Precision of an analytical method is express as the S.D. and R.S.D. of the series of the measurements. It was ascertained by replicate estimation of marketed formulation (five times). The % R.S.D was found to be less than 2 % which proves that method is precise.

Linearity and Range
According to 80 % to 120% of the test concentration was taken and dilutions were made appropriately. The regression for Gati and Orni was found to be Y = 5985.1X and Y = 10389X with coefficient of correlation (r^2) 0.999 and 0.9995 respectively.

Ruggedness
Ruggedness was ascertained by carrying out the analysis for interday variation, intraday variation and different analyst. The results are summarized in table no 4.

Conclusion
The proposed method is simple, sensitive and reproducible and hence can be used in routine simultaneous estimation of Gati and Orni in bulk as well as in pharmaceutical preparation. Statistical analysis of the results has been carried out revealing high accuracy and precision. The RSD for all the parameters were found to be less than one, which indicates the validity of the method.

Table 1. Results of Gati and Orni in marketed formulation

Drug Name	% Amount Found	% RSD
Gati	100.05	0.62
Orni	100.62	0.65

Table 2. System Suitability Parameters

Parameters	Gati	Orni I	Orni II
Asymmetry	0.171	0.804	0.758
Retention time (min)	3.77	4.91	5.60
Capacity Factor	1.98	2.88	3.43
Selectivity	1.458	1.184	
Resolution	6.574	3.636	

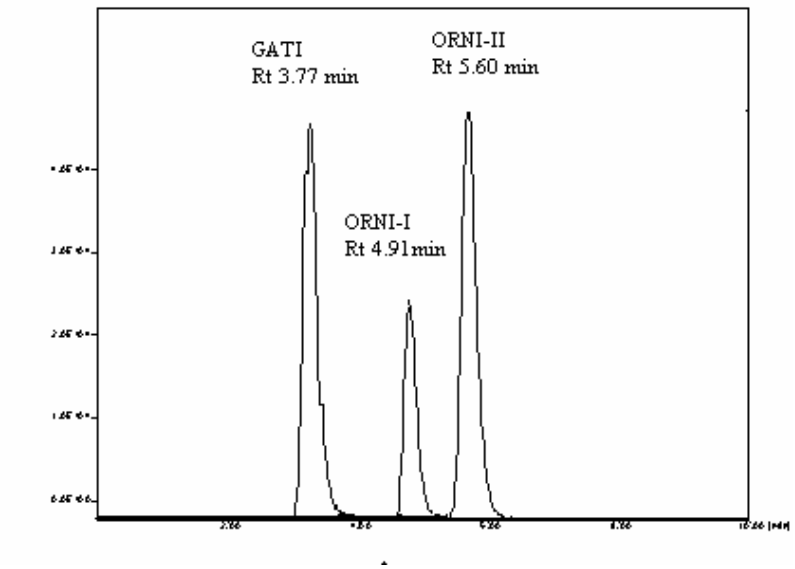
Table 3. Recovery data

Drug Name	% Recovery	% RSD
Gati	101.78	0.45
Orni	100.28	0.55

Table 4. Ruggedness parameters

Parameters	Label Claim ± SD	
Drug Name	Gati	Orni
Interday Variation	99.70 ± 0.53	99.59 ± 0.66
Intraday Variation	99.93 ± 0.54	99.78 ± 0.33
Different Analyst	100.42 ± 0.76	100.52 ± 0.88

Figure 1. Chromatogram of Gati, Orni and its isomer with Rt 3.77, 4.91and 5.60 min respectively.



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References

1. Blok J.H., Bcale J. M., Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry, Lippincott, 951-954.
2. Singh P., Mittal R., Sharma G.C., Singh s., Singh A., Profile of drug substances, excipients and related substances, Elsevier Inc, 126-130.
3. Basavaiah K., Kumar A., Sensitive spectrophotometric methods for quantitative determination of gatifloxacin in pharmaceutical formulation, J. Mex. Chem. Soc. 2007, 51 (2), 106-112.
4. Changchuan G., Deng D., Zhijiechu L. W., Wei J., Rapid determination of gatifloxacin in biological samples and pharmaceutical products using curopium sensitized fluoresecence spectroscopy, J. Biolumin. Chemilumin., 2008, 23 (1), 7-13.
5. Sivasubramanian L., Mutahukumaran A., Spectrophotometric determination of gatifloxacin in pharmaceutical formulation, Indian J Pharm Sci, 2006, 68 (5), 672-675.
6. Borner K. Hartwig H. Lode H., Determination of gatifloxacin in human serum and urine by HPLC, Chromatog. , 2000, 58 (1), 105-107.
7. Saleh D., Syed N., Hammani M., Development and validation of an HPLC method for the determination of gatifloxacin stability in human plasma, J Pharm and Bio med Anal., 2006, 41(1), 251-255.
8. Parmane S., Kothapalli L., Thomas A., Deshpande A., Simultaneous RP-HPLC estimation of gatifloxacin and Ornidazole in tablet dosage form, Indian J Pharm Sci, 2007, 69 (1), 525-528.
9. Soma S., Vidyasagan V., Narsaiah N., Anandkumar R., Validated HPLC method for determination of Ornidazole in human serum and urine, Indian J Pharm Sci, 2005, 67 (3), 302-306.
10. Shah S., Suhagia B. N., Baldaniya M., A simple, sensitive HPTLC method for the determination of gatifloxacin in tablet dosage forms, Indian J Pharm Sci, 2004, 66(3), 306-308.
11. Suhagia N., Shah S., Rathod I., Patel H., Shah D., Maroliya B., Determination of gatifloxacin and Ornidazole in tablet dosage forms by HPTLC, Anal. Sci., 2006, 22 (5), 743.

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