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Development and Validation of HPLC Method for the Simultaneous Estimation of Satranidazole and Gatifloxacin in Tablet Dosage Form

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Abstract: A simple, precise, accurate and precise reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of satranidazole and gatifloxacin simultaneously in combined dosage forms. A Lichrospher 100 C-18 and mobile phase comprises of Water: Acetonitrile: Triethylamine (75:25:0.35, v/v/v) were used for separation. Final pH was adjusted to 3.2 ± 0.02 with 10% v/v *o*-phosphoric acid. Measurements were made at the effluent flow rate of 1.0 ml/min with injection volume 20 µl and ultraviolet (UV) detection at 320 nm, as both components shows reasonable good response at this wavelength. The retention times of satranidazole and gatifloxacin were 6.0 min and 3.44 min, respectively. The method was validated in terms of linearity, accuracy, precision, robustness and specificity. Linearity of satranidazole and gatifloxacin was in the range of 1-70 µg/ml and 1-70 µg/ml, respectively. Average percentage recoveries obtained for satranidazole and gatifloxacin were 99.80 % and 100.20 %, respectively. The limit of detection and limit of quantification were found to be 0.3 and 1.0 µg/ml for satranidazole, respectively and for gatifloxacin were 0.5 and 1.0 µg/ml, respectively. The method is useful in the quality control of bulk manufacturing and pharmaceutical dosage forms.
Key words: Satranidazole; Gatifloxacin; RP-HPLC; Validation.

1. Introduction

Chemically satranidazole 3-(1-methyl-5-nitroimidazol-2-yl)-1-ethylsulfonyl) imidazolidin-2-one[1] and chemically gatifloxacin is 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline-carboxilic acid[2]. Literature survey reveals that few, spectrophotometric[3-5], HPLC[6-9] and HPTLC[10] for the estimation of satranidazole and gatifloxacin alone or in combination with other agents are reported. So far no HPLC method has been reported for the estimation of satranidazole and gatifloxacin in formulation.   In the present investigation an attempt has been made to develop accurate and precise HPLC method for the simultaneous estimation of satranidazole and gatifloxacin in combined dosage forms.

2. Experimental

2.1 Chemicals

Satranidazole (SAT) and Gatifloxacin (GAT) bulk powders were kindly gifted by Torrent research centre, Ahmedabad. Acetonitrile, Water used was of HPLC grade (Rankem). Triethylamine and *o*-phosphoric acid were of AR grade, S.D. Fine Chemicals Ltd., Mumbai. Combined tablets of Satranidazole and Gatifloxacin were prepared in the laboratory.

2.2 Instrumentation

A Merck - Hitachi Isocratic High Performance Liquid Chromatography with a Lichrospher 100 RP-180, C-18, 5 µm column having 250x4.0 mm internal diameter and equipped with Hitachi pump L – 7110, Rheodyne universal injector 77251 with injection volume 20 µl and Hitachi L - 7420 UV - Visible detector and monitored by Merck - Hitachi HSM software, was used.

2.3 Chromatographic condition

The chromatographic separations were performed using Lichrospher 100 C-18, 5 µm, 250 X 4.0 mm i.d. column, at ambient temperature. The mobile phase containing water: acetonitrile: triethylamine in the proportion of 75: 25: 0.35 (v/v/v) and the final pH adjusted to 3.25 ± 0.02 with 10% v/v *o*-phosphoric acid was selected because it was found ideally resolves the peaks of SAT (T_R=6.0) and GAT (T_R=4.0), respectively as shown in Fig. 1.

Wavelength was selected by scanning standard solutions of both drugs over 200 nm to 400 nm wavelengths. Measurements were made at the effluent flow rate of 1.0 ml/min with injection volume 20 μl and ultraviolet (UV) detection at 320 nm, as both components shows reasonable good response at this wavelength.

2.4 Preparation of mobile phase and standard solutions

The mobile phase was prepared by mixing water: acetonitrile: triethylamine in the proportion of 75:25:0.35 (v/v/v) and the final pH adjusted to 3.25 ± 0.02 with 10% v/v *o*-phosphoric acid. The mobile phase was filtered through nylon 0.45 μm, 47 mm membrane filter and was degassed before use. Accurately weighed SAT (25.0 mg) and GAT (25.0 mg) was transferred to a 25 ml volumetric flask, dissolved in and diluted to the mark with water. Ten ml aliquots each from stock solutions of SAT and GAT were transferred and mixed in 100 ml volumetric flask and volume was made up with mobile phase up to mark to get 100 μg/ml mixed standard stock solution. A synthetic mixture was prepared in the laboratory comprising of Satranidazole and Gatifloxacin in the proportion of 5:2(w/w)[11]

2.5 Validation of the method

The developed method was validated in terms of linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application.

3. Results and Discussion

3.1 Method Development

As per the value of Ka and solubility of both the compounds, various compositions of mobile phase with different pH ranges (2.75 to 7.0) were tried and best resolution was obtained with mobile phase consisting of water, acetonitrile and triethylamine in the proportion of 75:25:0.35 (v/v/v) with final pH adjusted 3.2 ± 0.02 with 10% v/v *o*-phosphoric acid. A satisfactory separation and peak symmetry for SAT and GAT were obtained with above mentioned mobile phase Quantification was achieved with UV detection at 320 nm based on peak area. The results of the validation and system suitability results are given in Table I.

3.2 Method Validation[12]

The proposed method has been validated for the simultaneous determination of SAT and GAT in bulk dosage form using following parameters.

3.2.1 Precision

Precision study was performed to find out intra-day and inter-day variations in the estimation of SAT and GAT of different concentrations, with the proposed method. Percentage relative standard deviation of all the parameters is less than 2% which indicates that the proposed method is repeatable. Results are shown in Table I.

3.2.2 Accuracy

This was carried out to check the recovery of the drugs at three different levels in the formulations i.e. multilevel recovery study. The pre analyzed samples were spiked with standard SAT and GAT and the mixtures were analyzed by proposed method. The experiment was repeated for five times (n=5). Results of recovery studies are shown in Table II.

3.2.3 Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for SAT and GAT were found to be 1-70 and 1-70 μg/ml, respectively. The regression equation for SAT and GAT were found to be y=21305x – 2998.2 and y=17380x - 36555 with coefficients of correlation, (r) 0.9998 and 0.9958, respectively.

3.2.4 Limit of Detection and the Limit of Quantification

The limit of detection and the limit of quantification of the drugs were calculated as in the text. The LOD and LOQ were found to be 0.3 and 1.0 μg/ml, respectively for SAT and 0.5 and 1.0 μg/ml, respectively for GAT. The LOD was considered as signal/noise ratio of 3:1 and LOQ as signal/noise ratio of 10:1.

3.2.5 The Assay

The assay value for the marketed formulation was found to be within the limits as listed in Table III. The low CV value indicated the suitability of the method for routine analysis of SAT and GAT in pharmaceutical dosage forms.

3.2.5 Specificity

The peak purity of SAT and GAT were assessed by comparing the retention time (T_R) of standard SAT and GAT. Good correlation was also found between the retention time of standards and sample of SAT and GAT.

Conclusion

The proposed method is simple, sensitive, precise, reproducible and accurate and hence can be used in routine for the simultaneous determination of SAT and GAT in bulk as well as in pharmaceutical preparations.

Acknowledgement

Table I: **Validation and System Suitability Studies**

Parameter	SAT	GAT
Linearity range(μg/ml)	1-70	1-70
Coefficient of correlation	0.9998	0.9958
Slope	21305	17380
Intercept	2998.2	36555
Limit of detection (μg/ml)	0.3	0.5
Limit of quantification (μg/ml)	1.0	1.0
Retention time (min)	6.0	3.44
Asymmetry factor	1.33	1.68
Tailing factor	1.66	1.70
Theoretical plate	2735.8	2560.87
Precision (% RSD) Inter-day (n=5) Intra-day (n=5)	1.16 1.33	1.22 1.22

Table II: Recovery Data for the Proposed Method (n=5)

Drug	Level	Amount of sample taken (μg/ml)	Amount of standard spiked (μg/ml)	% Recovery ± S.D (n=5)
SAT	I	25	10	100.17± 0.23
	II	25	25	99.72 ± 1.59
	III	25	35	99.53 ± 1.43
GAT	I	10	5	100.45 ± 1.25
	II	10	10	101.25 ± 1.09
	III	10	20	98.90 ± 1.48

Table III: Analysis of SAT and GAT Formulations by Proposed Method (n=5)

Formulations	Labeled amount (mg)		Amount found (mg)		% Assay ± % CV	
	SAT	GAT	SAT	GAT	SAT	GAT
Bulk powder	25	10	24.73	9.96	98.92 ± 1.65	99.64 ± 1.03

Fig. 1: A typical chromatogram for SAT (3.44 min) and GAT (6.0 min)
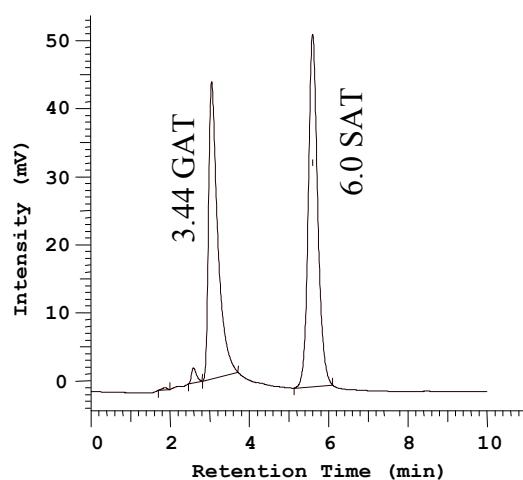
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