A validated RP-HPLC method for estimation of satranidazole in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, selective, rapid, precise and accurate reverse phase high pressure liquid chromatographic method has been developed for the estimation of Satranidazole from pharmaceutical formulation. The method was carried out on a HiQ SiL C18 (250 mm × 4.6 mm i.d.) column with mobile phase consisting of acetonitrile: TBASH, (70:30 v/v), at flow rate of 1.0 ml/min. Detection was carried out at 305 nm. Clonazepam was used as an internal standard. The retention time of Satranidazole and Clonazepam was 3.058, and 3757 min, respectively. The developed method was validated for of linearity, accuracy, precision, limit of detection and limit of quantitation. The proposed method can be used for estimation of these drugs in combined dosage forms. © 2009 Trade Science Inc. - INDIA

KEYWORDS

INTRODUCTION

Satranidazole (SAT), is a novel nitro-imidazole derivative. Chemically, it is 1-methylsulfonyl-3-(1-methyl-5-nitro-2- imidazolyl)-2-imidazolidinone[1]. It is used as antiprotozoal and antibacterial agent in the treatment of amoebiasis[2]. Tablet formulation containing 300 mg SAT is available in the market. Literature survey revealed that Satranidazole is estimated by HPTLC[3,4], and colorimetric methods[5-7] and in combination with Ofloxacin[8]. No HPLC method was reported for estimation of drug in tablet dosage form by using internal standard. The present work describes the development of a validated RP-HPLC method, which can quantify these components from a dosage form. The present RP-HPLC method was validated as per the ICH guidelines[9].

EXPERIMENTAL

Reagents and chemicals

Acetonitrile (HPLC grade) and water (HPLC grade) were procured from Merck specialties Pvt. Ltd., Mumbai. Tetra butyl ammonium hydrogen sulphate (TBASH) (L.R grade) was obtained from S.D.Fine-Chem Ltd; Mumbai. Working standard of Satranidazole and Clonazepam were obtained from Alkem laboratories pvt. Ltd Mumbai as gift samples.

Method development

Different mobile phases containing methanol, water, Acetonitrile, and different buffers in different proportions were tried and finally Acetonitrile: TBASH (70:30), was selected as an appropriate mobile phase which gave good resolution and acceptable peak pa-

Full Paper

TABLE 1: System suitability studies

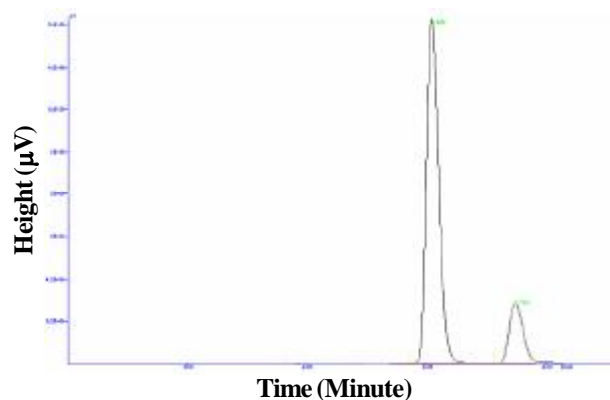| Sr. no.	Parameters	Satranidazole
1.	Theoretical plate/ meter	4123.77
2.	Resolution Factor	2.37
3.	Asymmetry	1.10
4.	LOD (µg/ml)	0.021
5.	LOQ (µg/ml)	0.085

Figure 1: Chromatogram of satranidazole (3.058 min), clonazepam (3.758 min) respectively
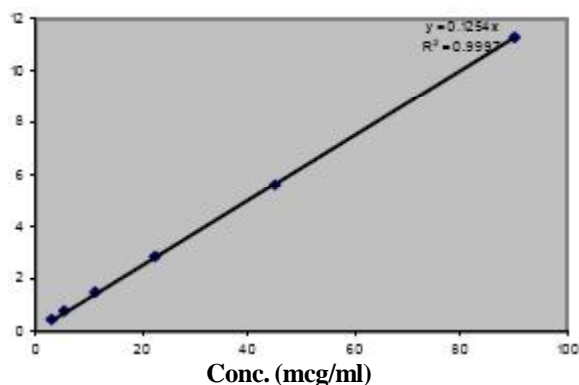

Figure 2: Calibration curve for satranidazole

rameters for Satranidazole.

System suitability studies

The resolution, number of theoretical plates and peak asymmetry were calculated for the standard solutions and is as shown in TABLE 1. The values obtained demonstrated the suitability of the system for the analysis of drug. The typical chromatogram of standard solution is as shown in figure 1.

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Jasco chromatographic system equipped with a Jasco PU-2080 plus HPLC pump, Jasco UV-2075 plus UV/VIS detector and Rheodyne injector with 20 µl loop

volume. HiQ SiL C18 (250mm × 4.6 mm i.d) was used for the separation; mobile phase of a mixture of Acetonitrile: TBASH (70:30 v/v), was delivered at a flow rate of 1 ml/min with detection at 305 nm. The mobile phase was filtered through a 0.2µ membrane filter and degassed. The injection volume was 20 µl; analysis was performed at ambient temperature.

Preparation of standard solutions

Standard stock solutions of strength 1.0 mg/ml of Satranidazole and 0.5 mg/ml Clonazepam were prepared separately using acetonitrile. From Standard stock solution of each drug, mixed standard solution was prepared in mobile phase to contain 15 µg/ml of Satranidazole and 5 µg/ml of Clonazepam an internal standard.

Calibration curve

Linearity of the system was investigated by serially diluting the stock solutions to give concentrations in the range of 2µg/ml to 30 µg/ml for Satranidazole. An aliquot (20 µl) was injected using mixture of Acetonitrile: TBAHS (70:30), as mobile phase. Calibration curves were obtained by plotting the response factor vs. concentration. The response factor is calculated as area of the drug peak divided by area of peak for internal standard. The calibration curve is as shown in figure 2 for Satranidazole. The equations of the regression line is
For Satranidazole y = 0.125X

Assay

Preparation of sample solutions

Twenty Tablets, each containing 300 mg Satranidazole were weighed and finely powdered. A quantity of powder equivalent to 25 mg Satranidazole was weighed and transferred to 25 ml volumetric flask. To this, acetonitrile was added and sonicated for 10 min; the volume was made up to 25 ml with acetonitrile to get solution of 1000 µg/ml. The solution was filtered using whatmann filter paper. From the filtrate appropriate dilutions were made to obtain concentration in the range of 2µg/ml to 30 µg/ml for Satranidazole. Clonazepam was added to each sample dilution at 5 µg/ml as internal standard.

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard so-

Full Paper

lution was injected and the chromatogram was recorded. The retention time of Satranidazole and Clonazepam was found to be 3.058min and 3.758 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The proposed method was found to be specific and no interference from common tablet excipients like lactose, starch etc was observed. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated. The assay was calculated from the equation of regression line for each drug. The percentage of individual drugs found in formulations was calculated and presented in TABLE 2. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations.

Method validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

Linearity and range

The linearity of the method was determined for the formulation at five concentration levels ranging from 2 to 30 µg/ml for Satranidazole. The equation for regression line was $y = 0.125X$ for Satranidazole .The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% and the percentage recovery calculated and presented in TABLE 2. Recovery was within the range of $100 \pm 2\%$ which indicates accuracy of the method.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, 3 repeated injections of standard and sample solutions were made in a day and the response factor of drug peaks and percentage RSD were calculated and presented in TABLE 3. In the inter day variation studies, 3 repeated injections of standard and sample solutions were made on 3 consecutive days and response factor of drugs peaks and percentage RSD were

TABLE 2: Results of assay of formulation and recovery studies

Sr. no.	% Mean recovery	S.D	% RSD
1	99.89	0.571	1.4
2	100.07	0.6	1.33
3	99.88	0.42	0.84

TABLE 3: Intraday and interday precision studies (System precision)

Concentration (µg/ml)	Satranidazole	
	% RSD	
	Interday	Intra day
3.00	0.16	0.22
5.625	0.29	0.3
11.25	0.2	0.27
22.5	0.32	0.25
45	0.45	0.37

RSD = Relative standard deviation (n = 3)

TABLE 4: Intraday and interday precision studies (Method precision)

Concentration (µg/ml)	Satranidazole	
	% RSD	
	Interday	Intra day
3.00	0.36	0.52
5.625	0.42	0.48
11.25	0.56	0.43
22.5	0.36	0.21
45	0.21	0.34

RSD = Relative standard deviation (n = 3)

calculated and presented in TABLE 3. The data obtained, %RSD not more than 1.5%, indicates that the developed RP-HPLC method is precise.

Limit of detection and limit of quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

LOD = (3.3 × standard deviation)/ Slope of calibration curve

The LOD for Satranidazole was found to be 0.021 µg/ml.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

LOQ = (10 × standard deviation) / Slope of calibration curve.

The LOQ was 0.085 µg/ml for Satranidazole.

Robustness

Robustness is checked by making slight deliberate change in the experimental procedures. In the present

Full Paper

method a deliberate change of room temperature and pH below 3 was made and the effects were noted. The method was found to be robust with respect to change in room temperatures.

RESULT AND DISCUSSION

The proposed method was found to be simple and linear in the concentration range of 2 to 30 μg/ml for Satranidazole. The method was found to be accurate and precise as indicated by recovery studies and % RSD not more than 1.5. Moreover LOD and LOQ for Satranidazole were found to be 0.021 μg/ml and 0.085 μg/ml. Thus the method is specific and sensitive.

CONCLUSION

The proposed RP-HPLC method for the estimation of Satranidazole in tablet dosage forms was found to be sensitive, accurate, precise, simple and rapid. Hence the present RP–HPLC method may be used for routine analysis of the raw materials and formulations.

ACKNOWLEDGMENTS

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