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# REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF SATRANIDAZOLE IN PHARMACEUTICAL FORMULATIONS

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**ABSTRACT**

A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the determination of satranidazole in pharmaceutical formulations. The method was carried out on a isocratic ODS - C18 (250 x 4.6mm i.d.,5 μ) column with a mobile phase consisting of Acetonitrile , 0.025M Ammonium phosphate buffer and 1.0% Ortho phosphoric acid in the ratio 65:35:5 v/v/v) at a flow rate 1.2mL/min. Detection was carried out at 318nm using UV lamp visible detector. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of satranidazole in pharmaceutical formulations.

**Key words:** Satranidazole, HPLC, Linearity, Validation.



## INTRODUCTION

Satranidazole{1-methylsulfonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone}[1](Fig.1) is nitroimidazole derivative widely used as antiprotozoal agent in the treatment of amoebiasis[2].Methods for the determination of satranidazole in pharmaceutical formulations and biological materials which have been reported previously included spectrophotometric[3-6],spectrofluorimetry,HPLC[7-9],HPTLC[10,11] and electron-capture gas chromatographic determination[12]. The main purpose of the present study was to establish a relatively simple, single - step, sensitive, validated and inexpensive RP HPLC method for the determination of satranidazole in pure form and in pharmaceutical dosage form, since most of the previous methods have been found to be relatively complicated and expensive. In the present investigation a new RP HPLC method has been reported for the estimation of satranidazole from pharmceutical dosage forms.

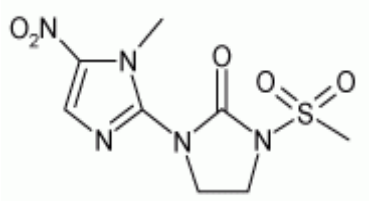



Fig.-1: Chemical structure of Satranidazole

## EXPERIMENTAL

### Reagents and chemicals

Acetonitrile HPLC grade was procured from E.Merck (India) Ltd,Mumbai. Ammonium phosphate AR grade and ortho phosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system.

**Apparatus and Chromatographic conditions**

Chromatographic separation was performed on a Shimadzu (LC 8200AHT) isocratic HPLC system equipped with isocratic liquid pump and UV- Visible spectrophotometric detector was used for the analysis .The data was recorded using window based single channel soft ware.The purity determination performed on a stainless steel column 250 mm long, 4.6 mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5 µm diameter (Use ODS,C18,5µ,250×4.6mm i.d) with the mobile phase containing of Acetonitrile , 0.025M Ammonium phosphate buffer and 1.0% Ortho phosphoric acid in the ratio 65:35:5 v/v/v at a flow rate 1.2mL/min at ambient temperature. Flow rate was kept at 1.2mL/min, and the elution was monitored at 318nm.

Table-1: Optimized chromatographic conditions

| Chromate graph | PEAK HPLC |
|---|---|
| Elution | Iso cratic |
| Mobile phase | Acetonitrile ,0.025M Ammonium phosphate buffer and 1 % ortho phosphoric acid in the ratio 65:35:5 |
| API Concentration | 10 µg/mL |
| Column | ODS C-18 RP ( 4.6 mm i.d x 250 mm) |
| Flow rate | 1.2 mL / min |
| Detection | UV at 318 nm |
| Injection volume | 20 µL |
| Temperature | Ambient |
| Retention time | 5.637 minutes |
| Run time | 9 minutes |
| Area | 100040.3 mAU |
| pH | 4.0 |
| Theoretical plates | 5531.62 |
| Pressure | 17-20 Mpa |
| Tailing factor | 1.29 |

Table-2: Calibration of the RP HPLC for the estimation of Satranidazole

| Concentration (µg.mL) | Area (mAU) |
|---|---|
| 5 | 52641.6 |
| 10 | 103797.8 |
| 15 | 136480.3 |
| 20 | 186931.6 |
| 25 | 213611.5 |
| 30 | 259584.3 |
| 35 | 305814.7 |
| 40 | 356160.3 |
| Regression equation<br>Slope (a) | Y = a X + b<br>8406.446 |
| Intercept (b) | 12732.73 |
| Correlation coefficient | 0.9998 |

Table-3: Precision data

| Day (Conc.= 10 µg/ml) | Precession Area Mean | R.S.D. |
|---|---|---|
| Day- 1 | 104018.5 | 0.733 |
| Day-2 | 132979.2 | 1.830 |

Averages of six determinations

Table-4: Results of analysis (Recovery studies) of tablet containing satranidazole

| Pharmaceutical formulation | Amount of Satranidazole | | % of recovery |
|---|---|---|---|
| | Labelled | Found | |
| Tablet – 1 | 300 mg | 302.328 mg | 100.776 % |

Average of three determinations

**Preparation of standard solution**

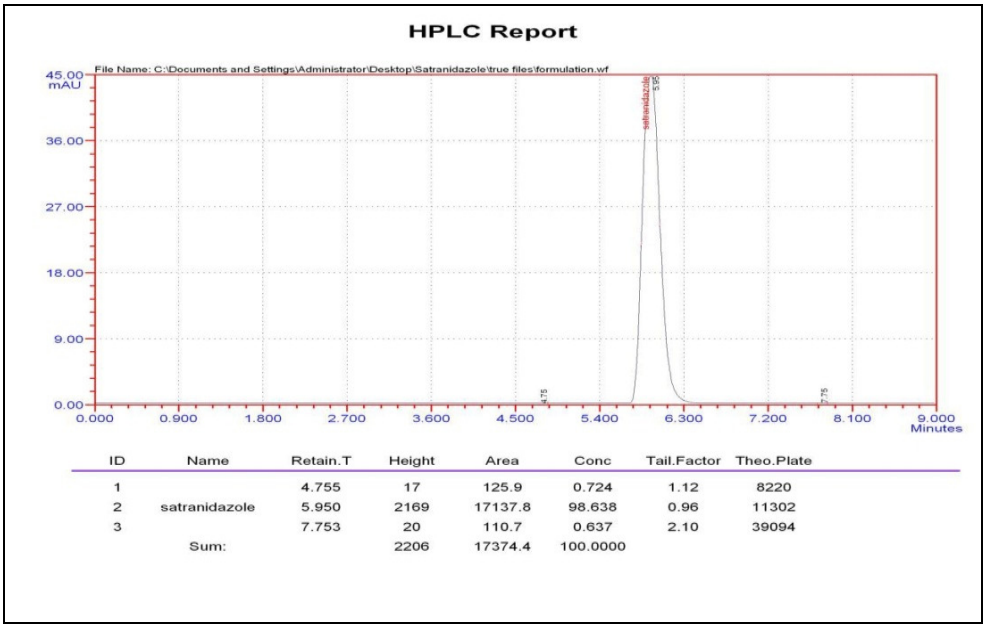
The standard stock solution of satranidazole was prepared by dissolving 25mg of the drug in 25mL of the methanol to get 1.0mg/mL solution. Final working standard solution of 50µg/mL of satranidazole was prepared by diluting 5.0mL solution of the above solution to 10mL with mobile phase (Acetonitrile, 0.025M Ammonium phosphate buffer and 1.0 % ortho phosphoric acid in the ratio 65:35:5 v/v/v). 5.0, 10.0, 15, 20 25, 30, 35, and 40µg/mL concentration of solutions were prepared and injected under operating chromatographic conditions. Calibration curves were constructed by plotting peak area versus concentration of satranidazole and the regression equation were calculated.

**Preparation of Marketed formulations**

In case of marketed formulations, two accurately weighed tablets were crushed to a fine powder and an amount equivalent to 10 mg of satranidazole was added into different 100 mL volumetric flasks and volume was made up with methanol. The samples were filtered through a 0.45-µm-membrane filter; different serial dilutions (5.0 - 40µg/mL) were made from this solution in 25mL volumetric flask and were injected for HPLC analysis.

**Assay method**

Under optimized chromatographic conditions as reported in **Table.1**, a steady baseline was recorded, when standard solution of satranidazole were injected and the chromatogram was recorded (**Fig.2**). The retention time of satranidazole was found to be 5.637 min respectively. This procedure was repeated for the sample solutions obtained from the marketed formulations. The response factor (peak area ratio of standard peak area) of the standard solution and sample solution were calculated.




| ID | Name | Retain.T | Height | Area | Conc | Tail.Factor | Theo.Plate |
|---|---|---|---|---|---|---|---|
| 1 | | 4.755 | 17 | 125.9 | 0.724 | 1.12 | 8220 |
| 2 | satranidazole | 5.950 | 2169 | 17137.8 | 98.638 | 0.96 | 11302 |
| 3 | | 7.753 | 20 | 110.7 | 0.637 | 2.10 | 39094 |
| | Sum: | | 2206 | 17374.4 | 100.0000 | | |

Fig .-2: HPLC Chromatogram of satranidazole

## RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop precise and stable assay method. the pure drugs satranidazole were run in different mobile phase compositions with different C18 columns (ODS,C18,5µ,250×4.6 mm) Phenomenex C18 column (25 cm x 4.6mm i.d., 5µ).The flow rate was also varied from 0.5 ml to 1.2mL.min. Finally, Isocratic C18 (ODS,C18,5µ,250×4.6 mm) with a mobile phase of a mixture of Acetonitrile, 0.025M Ammonium phosphate buffer and 1.0 % ortho phosphoric acid in the ratio 65:35:5 v/v/v at a flow rate of 1.2mL.min with a detection at 318nm gave sharp and symmetrical peak with retention time of 5.637 min for respectively. The typical chromatogram of sample solution of satranidazole is shown in Fig.2. Detection was done at 318nm. The peak area ratio of standard and sample solutions was calculated. The assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated. The percentage of satranidazole found in formulations, mean, standard deviation in formulations were 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 formulation.

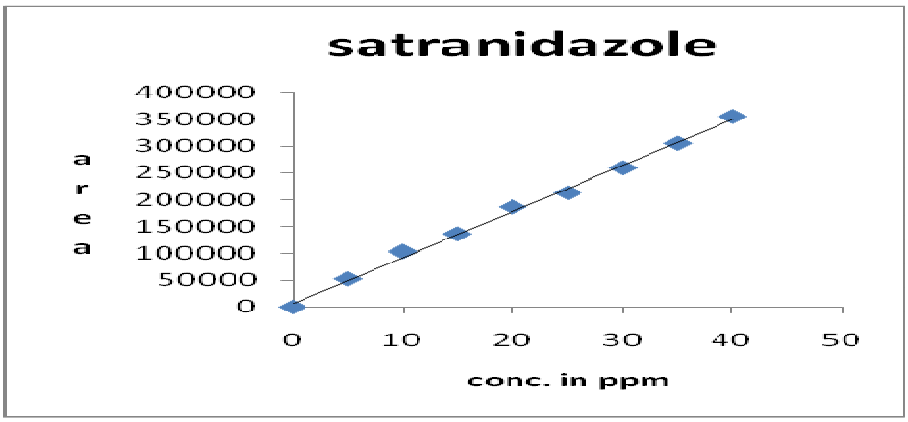



Fig.-3: Linearity of satranidazole

**Method Validation**

For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human use [ICH-1996] have recommended the accomplishment of accuracy tests, precision,specificity, linearity of the method.

**(a) Linearity and Range**

The linearity of the method was determined at eight concentration levels ranging from 5.0µg ml to 40.0µg.mL for satranidazole. The calibration curve was constructed by plotting response factor against various concentrations of satranidazole. The slope and intercept value for calibration curve of satranidazole was Y =8406.446x-12732.73 (R2=0.9998). The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above (Table.2). The calibration curve is shown in Fig. 3.

**(b)Limit of Detection and Limit of Quantification**

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for satranidazole was found to be 18.0ng.mL respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 65.0ng.mL for satranidazole, respectively.

**(c) Precision and Accuracy**

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response

factor of drug peaks and percentage RSD were calculated. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in Table.3. From the data obtained, the developed RP-HPLC method was found to be precise.

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in (Table.4). From the data obtained, the mean recovery was found to be 100.776 % for satranidazole, indicating very good reproducibility of the developed RP – HPLC method.

**(d)Ruggedness and Robustness**

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010AHT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C18, Phenomenex and Gemini C18. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

## CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of satranidazole in pharmaceutical dosage form is accurate, precise, linear, rugged, robust, simple and rapid. Hence the developed RP-HPLC method can be recommended for routine and quality control analysis of satranidazole.

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## REFERENCES

1. http://sci.com/scichem/jqp016/41841.html.
2. K.D. Tripathi , Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, **(5)** 754(2004).
3. S. Appala Raju, M. Shobha, S. Manjunath, *Asian Journal of Chemistry*, **14(1)**, 520 (2002).
4. B. H. M.Mruthyunjayaswamy, S. M. Mali Patil, S.Appala Raju, *Journal of Indian Council of Chemists*, **18(2)**, 60 (2001).
5. B. H. M.Mruthyunjayaswamy, S. M. Mali Patil, S. Appala Raju, *Journal of the Indian Chemical Society*, **80(9)**, 863 (2003).
6. S. B. Wankhede, A. Prakash, S. Chitlange, *Research Journal of Pharmacy and Technology*, **1(4)**, 441 (2008).
7. Sunder Natarajan, Bhanu Raman, *Asian Journal of Chemistry*, **20(3)**, 1833 (2008).
8. Sachin Rohidas Shinde, Suvarna Indravadan Bhoir, Namdev Shamrao Pawar, Ashok Mukund Bhagwat, Ajay Suresh Ghumatkar, *International Journal of PharmTech Research*, **2(3)**, 2032 (2010).
9. M. B.Patel, K. M. Patel, G. S.Patel, B. N. Suhagia, A. M. Prajapati, *Journal of Liquid Chromatography & Related Technologies*, **30(18)**, 2755 (2007).
10. Jogender Lalla, Purnima Hamrapurkar, R. Anu., Tarun Wadhwa, *Journal of Planar Chromatography-Modern TLC* , **16(6)**, 447 (2003).
11. M.B. Patel , K.M. Patel , G.S. Patel ,B.N. Suhagia and A.M. Prajapati ,*J. Liq Chromatogr Rel Technol.*(**30)** 2459 (2007).
    S.C. Bhatia, V.D. Shanbhag, *Journal of Chromatography, Biomedical Applications* , **305(2)**, 325 (1984).

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