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A NEW RP-HPLC METHOD FOR THE ESTIMATION OF SATRANIDAZOLE IN BULK DRUGS AND PHARMACEUTICAL FORMULATIONS

P. Rama Krishna Veni, K. Jyothi Kumari, B. Hari Babu*
Department of Chemistry, Acharya Nagarjuna University, Nagarjunanagar, Guntur-522510, Andhra Pradesh.
Email: dr.b.haribabu@gmail.com

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Abstract:

A new, simple, precise and accurate reverse phase HPLC method was developed for the estimation of satranidazole in bulk drugs and pharmaceutical formulations. The mobile phase used was 55% methanol, 30% acetonitrile and 15% of 1% orthophosphoric acid and the pH was maintained at 4.2. The resultant chromatogram obtained has a high resolution and low tailing factor (1.07). The linearity curve showed a correlation coefficient (r^2) of 0.999 for a wide range of drug concentration of 0.2-1.0 mg/ml. The method was also validated in respect of precision, accuracy and specificity.

Keywords: Satranidazole, Estimation, RP-HPLC.

Introduction:

Chemically Satranidazole (MF: $C_8H_{11}N_5O_5S$; MW: 289) (Fig.1) is 1-methyl sulphonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone and is soluble in methanol[1]. It is a nitroimidazole derivative possessing a C-N linkage at C_2 of the imidazole ring. It is more active towards anaerobes than many other nitroimidazoles. It shows activity against, common protozoa like *E. hystolytica, T.vaginalis* and *giardia*[2,3] and also acts as antibacterial agent in the treatment of amoebiasis[4]. In the present investigation an attempt was made to develop a prominent HPLC method for the estimation of SAT in both bulk drugs and pharmaceutical dosage forms.

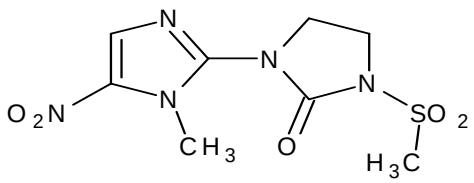


Fig.1. Structure of satranidazole [IUPAC Name: 1-(1-methyl-5-nitroimidazol-2-yl)-3-methylsulfonylimidazolidin-2-one]

2. Materials and Methods

2.1. Equipment

Analysis was carried out using PEAK 7000 isocratic HPLC with rheodyne manual sample injector with switch (77251) and the column used was Analytical column kromosil 100-5 C18.250x4.6mm. Electronic balance-ELB300, DIGISUN pH measurements.

2.2. Chemicals and reagents

Satranidazole reference standard was a kind gift of V.V.MED Laboratories, Hyderabad and the tablet formulation (Satrogyl-Alkem-300mg) used for testing the method was purchased from local market. The solvents used were Methanol and Acetonitrile of HPLC grade and phosphoric acid (GR) of Merck manufactures.

2.3. Optimized Chromatographic Conditions

Chromatographic analysis of the satranidazole was done using a Kromasil C18, (250x4.6mm, 5µm). The mobile phase composition used was MeOH (HPLC grade) 55%, Acetonitrile(HPLC grade) 30% and 1% orthophosphoric acid(GR)-15% was filtered through 0.5µ nylon membrane filter before use and the pH was at 4.2. The analysis was carried out in isocratic mode at a flow rate of 1ml/min. The detector wavelength is 280nm and the operating pressure is 15Mpa at room temperature. The injecting volume is 20µL and the total run time is 6min.

2.4. Preparation of standard solutions

Pure standards of satranidazole were used as external standards in the analysis. Different concentrations of the standards were used based on the range required to plot a suitable calibration curve. About 20mg of the standard satranidazole was accurately weighed and transferred into 10ml volumetric flask and made up to the mark by using sufficient mobile phase. The flasks containing standard solution was sonicated for 10 minutes to degas it. The standard solution was then filtered with 0.45µm membrane filter paper. Different concentrations of these standards were analyzed using the same chromatographic conditions as those of the target compounds and a calibration curve was generated.

2.5. Sample preparation

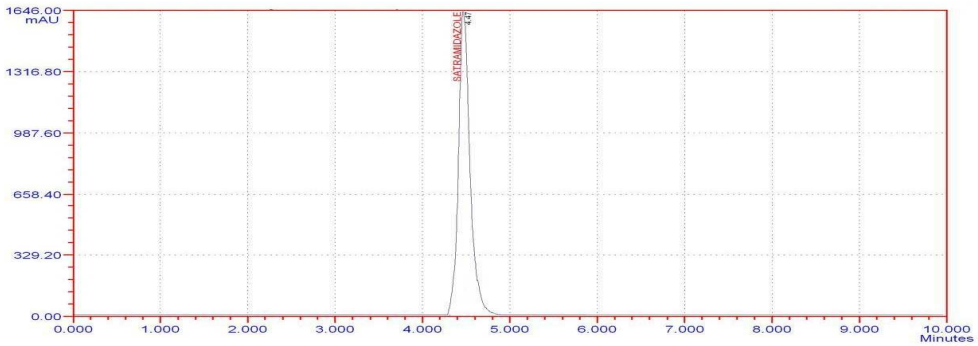
About 1mg of given commercial sample was taken into 10ml volumetric flask and added mobile phase to get 0.1ml/ml sample solution. The sample solution was then filtered with 0.45µm membrane sample filter.

2.6. Procedure for analysis

With the optimized chromatographic conditions set for satranidazole a study base line was recorded and stabilized for abut 30min. After the stabilization of base line successive aliquots of the sample solution were recorded, until the reproducibility of the peak areas was adequate. The sample was injected into the column at flow rate of 1ml/min.

3. Results and Discussion

A survey of literature revealed that SAT was estimated by using UV-Spectrophotometric method[5], Colorimetric method[6], HPLC[7], HPTLC[8,9] and RP-HPLC[10] methods. However, in the present investigation, a more reliable specific stability indicating RP-HPLC method was developed for estimation of SAT in bulk from and pharmaceutical formulation. The method was in good agreement in respect of linearity (0.999), accuracy (99.33%), precision, run time (6min) when compared with above mentioned HPLC methods[7-10]. The method was developed according to ICH guide lines. The standard chromatogram of the SAT was given in Fig. 2.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	SATRAMIDAZOLE	4.465	154858	1261668.8	100.000	1.07	5987
	Sum:		154858	1261668.8	100.0000		

Fig 2: standard chromatogram of the SAT

3.1. Method Validation

After the completion of HPLC method development the method was validated in terms of different parameters like specificity, linearity, precision, accuracy, LOD and LOQ.

3.1.1. Evaluation of linearity

The Linearity of the method was evaluated by analyzing different concentrations of the standard solutions. Satranidazole solutions of 0.2ppm, 0.4ppm, 0.6ppm, 0.8ppm, 1.0 ppm with standard 100% pure Satranidazole in the mobile phase was prepared and analyzed. After analysis the area of peaks were recorded and were reported in Table-1. It was found that there were no notable changes in the chromatograms for flow rate variation, column temperature variation and mobile phase variation for a wide range of drug concentration of 0.2-1.0 mg/ml. a plot was drawn by taking concentration on x-axis and area of the peaks on y-axis. It was fount that a straight line satisfying linearity condition i.e. the correlation coefficient (r^2=0.999, Fig. 3) of regression was found almost equal to 1.

Table-1: Linearity data of SAT for the developed method.

TEST-2	LINEARITY			
	S.NO	CONC ppm	AREA	INTERCEPT = -0.0122
	1	0.2	229120.3	
	2	0.4	426004.4	SLOPE = 1034364.65
	3	0.6	612444.4	
	4	0.8	856120.9	C.C = 0.99909
	5	1.0	1048426.7	

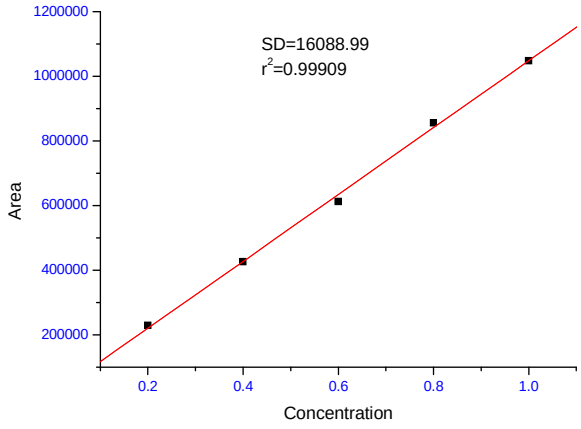


Fig 3: Linearity of satranidazole

3.1.2. Accuracy (% Recovery)

To study of the reliability, validity, suitability and accuracy of the method, recovery experiments were carried out for stranidazole. For this, the recovery studies were performed using standard addition method i.e. a known quantity of pure drug was added to the pre-analysed sample formulation. The results of these experiments were

given in Table 2. The % recovery of the drug in is calculated by using the formula given below. The recovery of the Satranidazole is 99.33%.

% recovery = [(b-a)/c] X100

Where a- The amount of drug found before the addition of standard drug.

b- The amount of drug found after the addition of standard drug.

c- The amount of standard drug added.

The values obtained (Table-2) above are more reliable and in good agreement in terms reliability, suitability and accuracy when compared to earlier methods[7-10].

Table 2: Accuracy data of the developed method.

TEST-3	ACCURACY			% ACCURACY
	Conc. 0.4 mg/ml	AREA	TH.PLATES	99.33
	STANDARD	426004.4	7115	
	SAMPLE	423164.7	7077.83	

3.1.3. Precision

A standard solution (1.2mg/ml) of drug substance was injected five times and corresponding peak areas were recorded. The % RSD found were less than 1%. The value of the %RSD obtained in intraday precision is 0.061(Table 3&4) and the inter day precision is 0.998. The values of %RSD within a day, day to day variation (<1%) proves that the method is precise.

Table 3: Intraday precision.

TEST.1	CONC 1.2 mg/ml			PRECISSION
Intraday	INJECTION	AREA	TH.P	%R.S.D = 0.061
	1	1547100.5	4534	
	2	1546076.1	4538	
	3	1545174.5	4545	
	4	1546921.5	4539	
	5	1547570.0	4529	
	6	1541501.5	4532	

Table 4: Inter-day precision.

TEST.1	CONC 1.2 mg/ml			PRECISSION
Inter-day	INJECTION	AREA	TH.P	%R.S.D = 0.998
	1	1508030.2		
	2	1520128.7		
	3	1539151.5		
	4	1534902.6		
	5	1534955.5		
	6	1551666.0		

3.1.4. Specificity of the method

The specificity of the method was determined by observing any interference encountered from the ingredients present in the formulations. The test results obtained were compared with that of test results those obtained for standard drug. In the present study, it was shown that those ingredients are not interfering with the proposed method.

3.1.5. Ruggedness

Inter-day variations were performed by using five replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on two different days over a period of one week.

3.1.6. Robustness and system suitability

Robustness of the method was carried out by varying two parameters slightly from the Optimized chromatographic conditions, such as flow rate, column temperature and mobile phase. It was found that there were no notable changes in the chromatograms for flow rate variation, column temperature variation and mobile phase variation. The robustness limit for the above parameter variations was well within the acceptable limit and is less than 2%. This shows that the method is having good system suitability under the given set of conditions.

3.1.7. Limit of Detection and limit of Quantification (LOD and LOQ)

The limit of Detection (LOD) and limit of Quantification (LOQ) (Table 5) of the developed method were determined by injecting increasingly low concentrations of the standard solutions by following the developed HPLC method. The LOD is the smallest concentration of the analyte which gives a measurable response. The LOD for Satranidazole was found to be 20 ng/ml. The LOQ is the smallest concentration of the analyte, which

gives response that can be absolutely quantified. The LOQ for Satranidazole was found to be 75ng/ml. The results of LOD and LOQ supported the sensitivity of the developed method.

Table 5: LOD and LOQ

Limit of detection (L. O. D.)	20ng/ml
Limit of quantification (L. O. Q)	75ng/ml

Conclusion

A more accurate, precise and convenient RP-HPLC method was developed and validated interms of linearity, accuracy, precition,etc. The proposed method is also applicable to the analysis of satranidazole in bulk drugs and pharmaceutical formulations.

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Corresponding Author:

B. Hari Babu*

Department of Chemistry,

Acharya Nagarjuna University,

Nagarjunanagar, Guntur-522510, Andhra Pradesh.

Email: dr.b.haribabu@gmail.com