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HPLC Determination of Satranidazole in Bulk and Pharmaceutical Dosage Forms

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A simple, fast, sensitive, specific, precise, accurate and rugged stability indicating reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of satranidazole in bulk material and in formulations. The stability indicating capability of the method was proven by subjecting the drugs to ICH stress conditions of alkaline and acidic hydrolysis, oxidation, photolysis and thermal degradation and resolution of the degradation products formed therein. The specificity was confirmed by spiking process related impurities of satranidazole and their separation from the main peak. The separation was obtained using a mobile phase of mixture of pH 3.0 buffer and acetonitrile in the ratio 600:400 with final pH of 3.6 on a ODS column (4.6 mm × 250 mm, 5 μ) with UV detection at 250 nm at 1 mL/min flow rate. For stress studies, a diode array detector was used. The elution of satranidazole was at 4.77 min. The linear dynamic range was 60 mcg/mL to 240 mcg/mL for satranidazole. Percentage recoveries for satranidazole was 99.64 %.

Key Words: Satranidazole, Degradation products, HPLC.

INTRODUCTION

Satranidazole is a broad spectrum 5-nitroimidazole derivative antiprotozoal, which is highly potent,well tolerated and clinically useful against common protozoa, twice as active as other nitroimidazoles against giardiasis and amoebiasis. It is rapidly absorbed and exhibits higher plasma and liver concentration than metronidazole. Chemically, it is 3-(1-methyl-5-nitroimidazol-2-yl)-1-(methylsulfonyl)imidazolidin-2-one (m.f. $C_8H_{11}N_5O_5S$; m.w. 289.26) (Fig. 1). It is not reported in any pharmacopoeias such as IP, USP and BP.

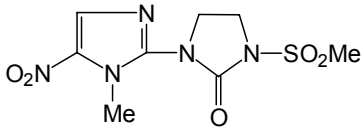


Fig. 1. 3-(1-Methyl-5-nitroimidazol-2-yl)-1-(methylsulfonyl)imidazolidin-2-one

Satranidazole tablets are a new anti-protozoal medication. Literature survey reveals that no HPLC methods, especially stability indicating assay methods, are reported for the estimation of satranidazole from bulk and tablet dosage forms. HPTLC[1] and spectrophotometric methods[2] are reported.

A validated stability indicating assay method[3], based on stress studies and availability of impurities, is essential and helps in successful development of a stable formulation.

In the present investigation, a specific stability indicating reverse phase HPLC method is described for estimation of the drug from the bulk and from tablet dosage forms. The drug was subjected to stress studies as per ICH guidelines[4,5] and the degradation products formed were separated from the main peaks The known impurities of satranidazole was also spiked and separated from the main peak of satranidazole. The specificity of the method in presence of excipients was also established The proposed validated stability indicating that the method can be used for routine as well as stability studies.

EXPERIMENTAL

The system used was a dionex HPLC equipped with a quaternary pump, a autosampler, a thermostated column compartment and a variable wavelength detector controlled by a Chromeleon software. For degradation studies. Quaternary pump and a diode array detector were used.

Working standard of satranidazole with potency of 99.87 % and impurities were obtained from Alkem Labs. Triethylamine and orthophosphoric acid of AR grade of SD Fine Chem were used. Acetonitrile and methanol of HPLC grade of qualigens were used. HPLC grade water was obtained using millipore water purification system.

Chromatographic conditions: The analysis was carried out on a quaternary HPLC system using a ODS column (4.6 mm × 250 mm, 5 μ) with UV detection at 250 nm at ambient room temperatures (30 °C) using a 20 μL injection volume.

Mobile phase: A mixture of pH 3.0 buffer (To 1000 mL of water, was added 1.6 mL orthophosphoric acid and adjusted to pH 3.0 using triethylamine) and acetonitrile in the ratio (600:400) was prepared, pH adjusted to 3.6 using orthophosphoric acid, filtered, degassed and used.

Solutions: Standard stock solutions were prepared by dissolving 30 mg of satranidazole working standard in 80 mL of mobile phase and diluting to 100 mL with the same. This was further diluted with mobile phase to get a solution of 150 mcg/mL of satranidazole.

Calibration: Five different concentrations (60-240 mcg/mL) of satranidazole were prepared for linearity studies. The responses were measured as peak areas and plotted against concentration. Linear regression

least squares fit data obtained from the above calibration curves are given in Table-1. The respective slopes (m), intercepts (b) and correlation co-efficient (r) are also indicated. The linearity plots are shown in Fig. 2.

TABLE-1
LINEARITY STUDY OF SATRANIDAZOLE

Concentration (µg/mL)	Area
60	795.4
90	1206.7
120	1598.0
150	1989.0
180	2388.7
240	3214.3
Slope	20.068
Intercept	7.657
Correlation co-efficient	0.9999

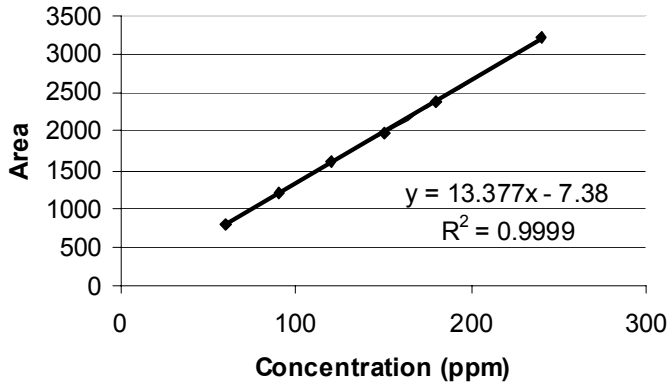
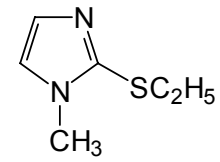


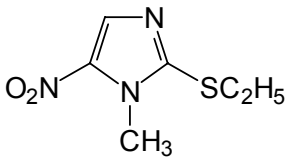
Fig. 2. Linearity curve of satranidazole

Estimation of satranidazole from tablets: 20 Tablets of the product under study were weighed, powdered and powder quantity equivalent to 150 mg of satranidazole was transferred to a dry 100 mL volumetric flask, sonicated with mobile phase and made to volume with mobile phase. This was further diluted with mobile phase to get final concentration of 150 mcg/mL of satranidazole and filtered. 20 µL each of standard and test preparation were injected into the chromatograph and the responses recorded.

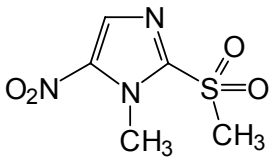
Specificity: The process impurities, *viz.*, impurity **1-4** (Fig. 3) were spiked to standard solution of satranidazole to confirm the specificity of the method. Impurity **4** does not exhibit any absorbance at the wavelength of interest.



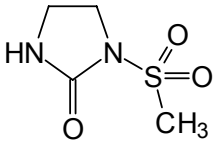
Impurity 1
2-(Ethylthio)-1-methyl-1H-imidazole



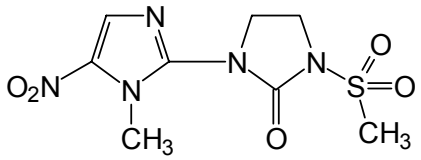
Impurity 2
2-(Ethylthio)-1-methyl-5-nitro-1H-imidazole



Impurity 3
1-Methyl-2-(methylsulfonyl)-5-nitro-1H-imidazole



Impurity 4
1-(Methylsulfonyl)imidazolin-2-one



Satranidazole
1-Methylsulfonyl-3-(1-methyl-5-nitroimidazole-2-yl)imidazolidin-2-one

Fig. 3. Chemical formula and structure of satranidazole and process impurities

Stress testing: To further confirm the stability indicating nature of the proposed analytical method, the drug of interest *viz.*, satranidazole were subjected to conditions of stress testing. The drugs were subjected to hydrolysis, both acid and base, using 5 N HCl and 1 N NaOH solution respectively, oxidation using 5 % H_2O_2 solution, heat and UV radiation.

The objective of the study was to generate the degradation products under various stress conditions and check the specificity of the method with respect to the degradation products. The stress conditions were varied both in terms of temperature and time from moderate to extreme to achieve the desired degradation. The results of the stress studies are tabulated under Tables 2 and 3.

TABLE-2
STRESS CONDITIONS USED FOR DEGRADATION STUDIES

Degradation mechanism	Medium	Conditions for satranidazole
Acid hydrolysis	5 N HCl	Ambient/48 h
Alkaline hydrolysis	1 N NaOH	Ambient/1 h
Oxidation	5 % H_2O_2	Ambient/48 h
Heat	–	100 ºC/48 h
Ultraviolet radiation	–	Ambient/48 h

TABLE-3
RESULTS OF THE STRESS STUDY FOR SATRANIDAZOLE

Degradation mechanism	Satranidazole main peak RT (min)	Degradation peak RTs (min)	Spectral purity of satranidazole
Acid hydrolysis	4.372	AH1 3.384	999.92
Alkaline hydrolysis	4.379	BH1 2.820	999.60
		BH2 3.020	
		BH3 3.390	
		BH4 4.850	
Oxidation	4.373	No peak	999.91
Heat	4.372	No peak	999.92
Ultraviolet radiation	4.372	No Peak	999.92

The spectral purity of the main peak was evaluated using photo diode array detector and chem station spectral purity software to ensure that the degradation peaks were well resolved from the main peak. From the above data, it is clear that the degradation products obtained under the stress conditions and the process impurities are well resolved from the main peak of satranidazole.

The method validation parameters such as precision, accuracy, linearity, limit of detection, limit of quantitation, robustness, ruggedness, solution stability, *etc.* were ascertained:

Precision: (a) System precision: The system precision was established by six replicate injections of the standard solution containing both the analytes of interest. The values of the relative standard deviation (0.05 % for satranidazole) lie well within the limits, indicating the injection repeatability of the method. (b) Method precision: The method precision was established by carrying out the analysis of the analytes six times using the proposed method. The values of the relative standard deviation (0.47 % for satranidazole) lie well within the limits, indicating the sample repeatability of the method.

Accuracy: The accuracy of the method was established using the standard addition method. The recovery of the added standards was found at

three different concentration levels for each drug ie 90 to 210 mcg/mL of satranidazole, in triplicate. The results of the recovery analysis are given in Table-4.

TABLE-4
RECOVERY STUDIES of SATRANIDAZOLE

Level (%)	Amount of standard added† (mg)	Amount of Standard recovered† (mg)	Recovery (%)
60	18.09	18.11	100.07
100	30.36	30.11	99.18
140	41.31	41.16	99.64

†Average of 3 values.

Specificity/selectivity: In addition to stress studies, to further confirm the specificity of method,all the possible excipients used in the manufacture of a typical tablet dosage form example starch, microcrystalline cellulose, lactose, talc, magnesium stearate, colloidal silicon dioxide, sodium starch glycollate were subjected to sample preparation as for tablets and then chromatographed. No interference of the placebo mixtures with the peak of satranidazole was observed.

Limit of detection: The linearity for satranidazole was performed from 0.3 to 45 mcg/mL. Linearity graph was plotted and correlation coefficient determined. Limit of detection (LOD) was predicted for satranidazole from the linearity curve using the formula.

$$LOD = 3.3 \times \text{Styex (residual standard deviation)/slope}$$

The limit of detection (LOD) for satranidazole was confirmed to be 0.5 mcg/mL.

Limit of quantitiation: The limit of quantification (LOQ) was predicted from the linearity curve using the formula.

$$LOQ = 10 \times \text{Styex (residual standard deviation)/slope}$$

Predicted LOQ value was verified by giving six replicate injections of standard solution at about predicted level and the precision at LOQ was confirmed.

The limit of quantitation (LOQ) for satranidazole were confirmed to be 1.5 mcg/mL.

Ruggedness (intermediate precision): The ruggedness of the method was demonstrated by analysis of the samples by two different chemists using two different sets of instruments. The RSD of the two sets of data indicates the ruggedness of the method. Further, the t-test was performed on the data and the difference was found to be not significant.

Robustness: The robustness of the method was determined as a measure of the analytical methods capability to be unaffected by small variations in method parameters.

The different variations are as given below:
Variation in flow rates by ± 0.2 mL/min
Variation in wavelength by ± 2 nm
Variation in temperature by ± 5 °C
Variation in composition of mobile phase by ± 5 % (in terms of organic component)
Variation in pH by ± 0.2 units

The results of the analysis of the samples under the conditions of the above variations indicated the nature of robustness of the method.

Stability of solution: The stability of the solutions under study were established by analyzing the solution after 24 h. The results indicate no significant change in the assay results of the same solution thus confirming the stability of the drug in the solvents used for the analysis.

System suitability tests: System suitability tests were carried out on six replicate injections of the standard solution containing satranidazole. The various chromatographic figures of merit such as relative standard deviation for replicate injections, theoretical plates per meter and tailing factor were obtained. The results are given in Table-5.

TABLE-5
SYSTEM SUITABILITY TESTS

Test	Results satranidazole	Acceptance criteria
Retention time	About 4.37 min	–
RSD of replicate injections	0.05 %	Not more than 2.0 %
Tailing factor	1.21	Not more than 1.5
Theoretical plates/metre	6302	Not less than 3000

RESULTS AND DISCUSSION

The stress studies were conducted so as to arrive at a stability indicating method capable of differentiating between the actives and the excipients as well as any degradation products, which may normally be generated during the shelf life of the product.The proposed method has been shown to be stability indicating in nature.

Satranidazole, a weak acid, is sparingly soluble in water with a pKa value of about 0.89. Initially, a octadecylsilane column was used with varying proportions of acetonitrile:water which gave tailing of satranidazole. Hence triethylamine was used to reduce the tailing. The pH of mobile phase was adjusted to 3.6 to obtain a better peak shape and better resolution.

The final composition of 600:400 ratio of pH 3.0 buffer and acetonitrile with final pH of 3.6 gave optimum resolution in shortest time of both actives and degradants.

The proposed mobile phase gave a good resolution of the main peak (resolution between satranidazole and its acid degradants, *viz.*, AH1 and base degradants BH3 and BH4 were not less than 2.0). Similarly, the resolution between the satranidazole peak and the process impurities was not less than 2.0. The peaks of satranidazole were found to be symmetrical and well defined, free from tailing (values of 1.19). The elution of satranidazole was at 4.37 min at a flow rate of 1.0 mL/min. The optimum wavelength for detection was found to be 250 nm, wherein most of the impurities and degradation products were detected. The linearity of the calibration curves indicate the suitability of the method over a wide range of concentration of the active (60 to 240 mcg/mL for satranidazole). The sensitivity of the method using the said method was found to be good.

The total run time of each analysis for stability studies is about 15 min, which is rapid and economical considering the stability indicating nature of the method.

Conclusion

The proposed analytical methodology is fast, specific, simple and rugged and stability indicating. Hence, it can be used for routine quality control analysis as well as stability studies of development of similar formulations.

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