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High-Performance Liquid Chromatographic Analysis of Nitroimidazole Derivative Satranidazole Using a Liquid Extraction Method

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Abstract: A simple, selective and precise liquid chromatographic method with UV detection (318 nm) was developed and validated for the estimation of a new anti protozoal agent Satranidazole in human plasma. Separation was achieved with an RP C_{18} column using a mixture of buffer and acetonitrile in the ratio of 60:40, containing 0.1% glacial acetic acid, at a flow rate of 1 ml/min. Tinidazole was used as an internal standard. Excellent linearity was observed over a range of 0.05-15.00 µg/ml. The analyte recovery from plasma solutions was more than 80%. The method was validated in terms of linearity, accuracy, precision, recovery and stability. This method was found to be simple, precise and reproducible and can be applied to use for the pharmacokinetic studies of Satranidazole in human volunteers.
Keywords: HPLC, Satranidazole, plasma.

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. Chemicaliy, 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). It is not yet reported in any pharmacopoeias such as IP, USP and BP.[1]

Literature survey revealed that few methods have been reported for individual estimation of Satranidazole using HPLC[1], HPTLC[2,3] and spectrophotometer[4,5]. However, there are two analytical methods: simultaneous equation and absorption ratio method[6] for the estimation of Ofloxacin and Satranidazole in a combined dosage formulation. Further, no method has been reported for the assay of Satranidazole in biological fluid. Thus the absence of suitable analytical method for the assay of Satranidazole in biological fluid, led us to develop a new method for its assay.

The objective of current study was to develop a simple, sensitive, rapid and accurate method suitable for pharmacokinetic studies on Satranidazole in human plasma.

Experimental

Chemical and Reagents

Satranidazole working standard was obtained as a gift sample from Alkem laboratories, Tinidazole from Aarti Drugs Ltd. Acetonitrile, Methanol, Dichloromethane and Diethyl ether were of HPLC grade purchased from E-Merck (India) Ltd. Acetic acid was of spectrochem Pvt. Ltd. Potassium

dihydrogen ortho phosphate and sodium hydroxide were of AR grade purchased from Qualigens Ltd. Boric acid and potassium chloride was obtained from S.D. Fine chemicals Ltd.

Instrumentation and Chromatographic Conditions

LC analysis was performed on Agilent-1100 series equipped with quaternary pump (G1311A), an auto sampler (G1329A) and UV detector (G1314A) operated with Chemstation software. The column used was Inertsil ODS 3V, (250 X 4.6 mm, 5 µm). The mobile phase used was 20 mM potassium dihydrogen ortho phosphate: acetonitrile (60:40) containing 0.1% acetic acid, at flow rate 1.0 mL/min. The injection volume used was 5 µL and both analytes were monitored by UV detector set at 318 nm.

Preparation of calibration standards and quality control samples

Satranidazole Stock solution-I and II (1mg/mL) were prepared by dissolving the drug in methanol and further dilutions were prepared in diluent (80:20, methanol:water, v/v) to obtain working standards in the concentration range of 0.05-15.00 µg/mL in human plasma for the calibration standards. Low, medium and high-concentration, quality control solutions (0.150, 7.507 and 12.01 µg/mL, respectively) were also prepared in diluent (methanol:water 80:20, v/v) from stock solution-II. Similarly stock solution of internal standard, Tinidazole was prepared in methanol and working internal standard solution in diluent.

Sample preparation

To 1 mL of plasma contained in a 15 mL centrifuge tubes, appropriate aliquots of the drug and fixed aliquot of (1.8 µg/mL) of internal standard, Tinidazole were added and tubes vortexed for 60 sec. After the addition of 1 mL alkaline borate buffer (pH-10) the tubes were vortexed for 60 sec. The analytes were extracted with 5 mL of dichloromethane: diethyl ether (3:2, v/v) by mixing for 60 sec, followed by centrifugation for 10 min at 3000 rpm. The lower organic layer was transferred to another test tube and evaporated to dryness under a stream of nitrogen at 45^0C. The residue was reconstituted in 200 µL mobile phase and 5 µL aliquot was injected onto the HPLC system.

Method Validation

Selectivity

Six randomly selected, drug free human plasma samples were processed by the similar extraction procedure and analyzed to determine endogenous plasma components that might contribute to the interference at the retention time of analyte and internal standard.

Calibration and linearity for validation

A standard curve of eight points was plotted. Standards were prepared by spiking the plasma sample with stock solution-I (1 mg/mL) to obtain the concentration levels of 0.05-15.00 µg/mL. Quality control samples were prepared at the concentration of 0.150 µg/mL (Low QC), 7.507 µg/mL (Medium QC) and 12.011 µg/mL for (High QC) by spiking the stock solution-II.

A calibration curve for the Satranidazole was constructed from a blank sample (plasma sample processed without an IS), a zero sample (plasma with IS) and eight non-zero samples covering the total range including lower limit of quantification. Linearity was assessed by the least square regression analysis with a weight factor of 1/conc. The correlation coefficient (r^2) was determined and the limit of acceptance was set at 0.99 or better. The acceptance criteria for each back calculated standard concentration was ± 15% deviation from nominal value except at LLOQ, it was set as ± 20%.[7]

Precision and accuracy

The accuracy and precision of the method was evaluated by replicate analysis of spiked quality control samples. The intra-day data were obtained by replicate analysis of QC plasma samples (n=6). The inter-day data were obtained by analyzing the same QC plasma samples over the period of three weeks. Mean standard deviations and relative standard deviations were calculated from QC values, acceptance limit was set less than 15% at any concentration study and used in the estimation of intra and inter day precision. Similarly, for accuracy, the mean value deviation upto ± 15% of the nominal concentration was considered acceptable.

Recovery

Recovery of satranidazole in plasma was evaluated by comparing the mean detector response at different quality control samples post-extracted drug free plasma at the corresponding concentrations. Similarly, the recovery of internal standard from plasma was evaluated.

Stability

Stability studies provide the data to show whether the drug in plasma or in solution under different experiment conditions, during sample handling and analysis, is stable.

The short term stock solution stability of the drug and the IS was evaluated by comparing with fresh stock at room temperature for 6 hours.

Auto sampler stability was determined by analyzing six aliquots each of low and high QC samples that were processed and reconstituted before storing at 4^0C for 24 hrs. Thereafter, samples were analyzed and concentrations were calculated by using calibration curve obtained by plotting data as freshly spiked samples.

The freeze-thaw stability was determined after three freeze and thaw cycles. During this experiment the

plasma drug concentrations were stored at intended storage temperature for 24 hours and then thawed, unassisted, at room temperature. Completely thawed the samples were refrozen for 12 to 24 hours under the same conditions. The freeze thaw cycle was repeated two more times and then the samples were analyzed on the third cycle. The plasma concentrations at low and high quality control samples were calculated by using values obtained from freshly spiked calibration curve. Similarly bench top stabilities, at room temperature, for 6 hours, of low and high quality control samples were calculated by using values obtained from freshly spiked calibration curve.

Table 1 : Intra-day and Inter-day Accuracy and precision of Satranidazole in Human Plasma

QC Sample	Nominal concentration (µg/mL)	Found concentration (µg/mL)	C.V. (%)	Accuracy (%)
Intra-day accuracy and precision				
LLOQ	0.050	0.051	2.806	102.2
LQC	0.150	0.166	1.145	110.5
MQC	7.507	8.010	2.020	106.7
HQC	12.01	12.70	0.474	105.7
Inter-day accuracy and precision				
LLOQ	0.050	0.053	4.873	105.5
LQC	0.150	0.162	2.666	108.0
MQC	7.507	7.941	2.561	105.8
HQC	12.01	12.73	0.661	105.9

Table 2: Stability data of Satranidazole in human plasma

Sample Concentration (µg/mL)	Found Concentration (µg/mL)	Precision (%)	Accuracy (%)
Short-term stability for 6 h in plasma			
0.150	0.158	0.467	105.2
12.01	12.64	2.216	105.2
Stability (after 3rd freeze thaw cycle)			
0.150	0.162	2.020	107.8
12.01	12.39	3.994	103.1
Autosampler stability for 24 h at 4^{0}C			
0.153	0.167	2.949	111.2
12.01	12.86	0.993	107.1

Figure 1: Typical chromatogram of the drug free Human Plasma

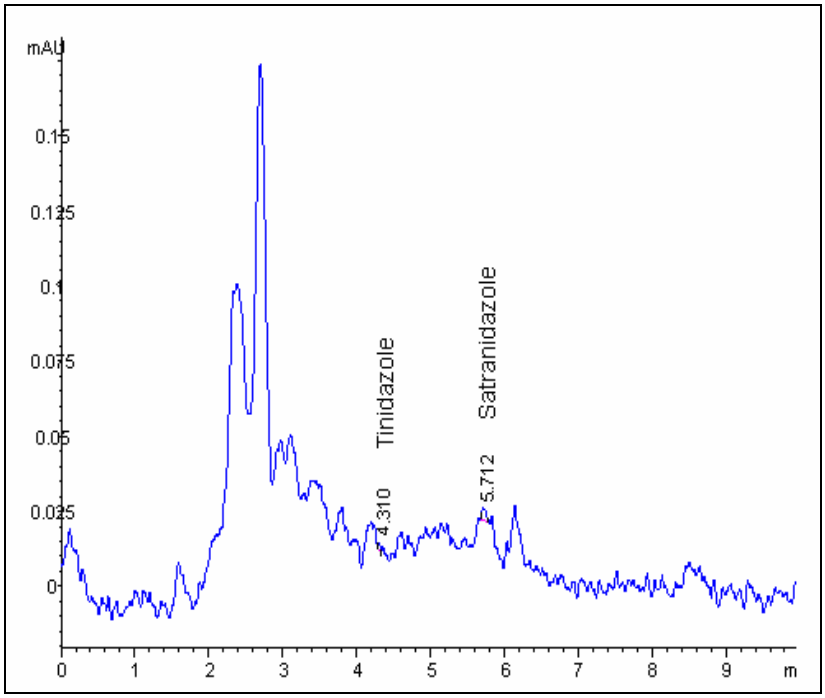


Figure 2 : Chromatogram of the extract from plasma spiked with Satranidazole (7.507 µg/mL) and Tinidazole (1.8µg/mL)

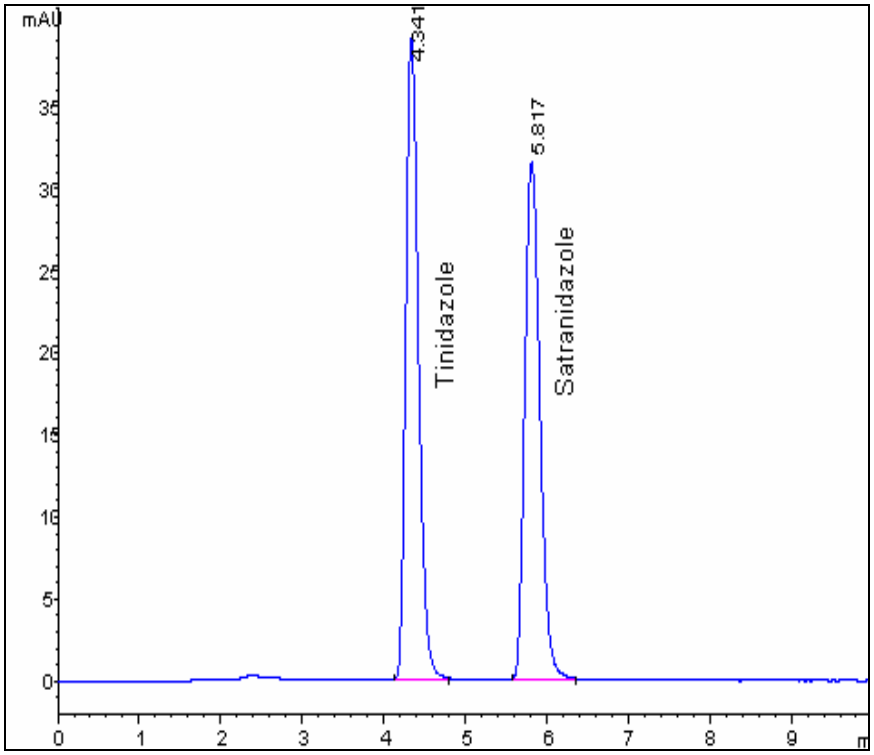
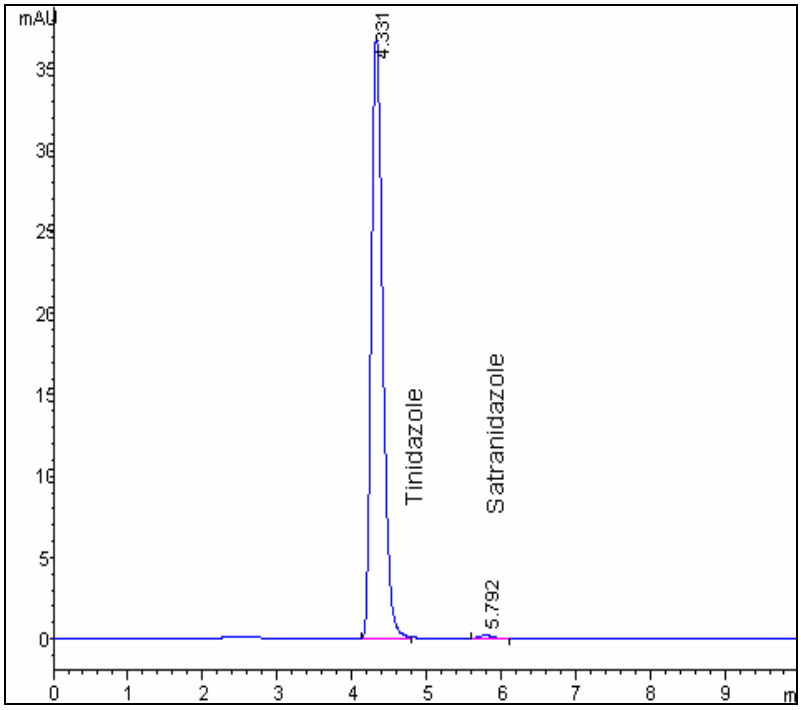


Figure 3 : Chromatogram of the extract from plasma spiked with Satranidazole (0.05 µg/mL) and Tinidazole (1.8µg/mL)



Results and Discussion

Selectivity

Selectivity of the method was confirmed by using six different randomly selected, drug free, plasma sources to determine the extent to which endogenous plasma components may contribute to interference at the retention time of analyte and internal standard. Figure 1 shows a typical HPLC chromatogram of extract from drug free plasma and Figure 2 shows the chromatogram of an extract of plasma sample at MQC level i.e. Satranidazole (7.507 µg/mL) and the Internal Standard (1.8 µg/mL). The internal standard and the analyte were eluted with retention times of 4.2 and 5.4 min respectively.

Linearity

The calibration curve was found to be linear within the range of concentration studied. An average of six individual measurements of the points used to prepare the calibration curve was calculated done at each concentration level (n=6). The measured precision of back calculated concentrations of calibration samples in human plasma ranged from 1.09-3.40% and accuracy ranged from 96.31-106.57%. The results reveal that the method has good reproducibility over a wide concentration range with an average recovery of 78.83%. Figure 3 shows a chromatogram of plasma at LLOQ level.

Precision and Accuracy

The intra-day and inter-day precision and accuracy data were within the acceptance range of CV ≤15% and nominal ±15% (≤ 20% for LLOQ QC) which is illustrated in Table 1.

Stability

Stock solution stability has accuracy value better than 95% throughout the storage period of 6 hours at 23 ± 2°C. From all stability data (Table 2) the results clearly indicates that the drug remains stable even after three successive Freeze/Thaw cycles, 6 hours at bench top and 24 hours in an autosampler tray at 4°C.

Conclusion

The present investigation successfully describes a simple, sensitive and selective bioanalytical method for the estimation of satranidazole in plasma. The method is validated and it satisfied the requirement of linearity, recovery, accuracy, precision and stability for a bioequivalence study. This method has a successful application for Pharmacokinetic assay.

Acknowledgements

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