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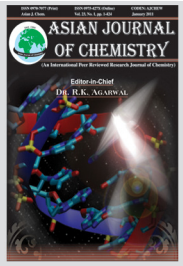

HPLC Determination of Satranidazole in Rat Plasma

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A rapid, simple and sensitive RP-HPLC method with diode array detection (DAD) was developed for determination of satranidazole from rat plasma. Separation was achieved on reverse phase C_{18} column (250 mm × 4.6 mm × 5 µm), using a mixture of methanol: 10 mM phosphate buffer pH 3 adjusted with orthophosphoric acid, in ratio of 30:70 (% v/v) at a flow rate of 1 mL/min with UV detection at 320 nm within 10 min. The plasma samples were prepared by simple single-step deproteinization with acetonitrile, yielding more than 80 % extraction recovery. The calibration curve was linear (correlation coefficient of 0.9968) in the concentration range of 1-60 µg/mL. The lowest limit of quantification (LLOQ) was 1 µg/mL. The intra-day precision was less than 15 % RSD. The present method was selective enough to analyze satranidazole in rat plasma and was successfully applied for estimating the pharmacokinetic parameters of satranidazole following oral administration of single 54 mg/kg satranidazole to rats.

Key Words: Satranidazole, RP-HPLC, Pharmacokinetics, Rat plasma.

INTRODUCTION

Satranidazole, 1-methanesulphonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone, is a nitroimidazole derivative. It is used as antiprotozoal and antibacterial agent in the treatment of amoebiasis[1]. Satranidazole possessing a C-N linkage at C_2 of the imidazole ring acts by extensive DNA damage characterized by helix destabilization and strand breakage[2]. Literature survey reveals few spectrophotometric[3-7], HPLC[8-10] and HPTLC[11-13] methods for the estimation of satranidazole alone or in combination with other agents. Few studies regarding the pharmacokinetic profiles of satranidazole have been described[14,15]. The aim of this study is to develop and validating a simple, rapid, sensitive and reproducible HPLC method for the determination of satranidazole in rat plasma suitable for the subsequent pharmacokinetic study after oral administration to rats.

EXPERIMENTAL

Satranidazole (purity > 98 %) was kindly provided by Alkem Labs Pvt. Ltd. Mumbai. Methanol and acetonitrile (ACN) were of chromatographic grade (Sisco Laboratories Pvt. Ltd, Mumbai). All other reagents were of analytical grade.

Twelve male Sprague-Dawley rats, weighing 250-300 g, were used. The rats were housed under controlled environmental conditions with a commercial food diet and water freely available. Animal experiments were carried out according to institutional guidelines for the care and the use of laboratory animals and approved by the animal ethics committee.

Preparation of stock solutions, standards and quality control samples: Stock solution of satranidazole (1000 µg/mL) was prepared by dissolving 10 mg of satranidazole in acetonitrile to make 10 mL. This was stored in an amber coloured glass volumetric flasks until being used. The standard stock solution of satranidazole was appropriately diluted with acetonitrile to get working standard solutions having concentrations 10, 20, 40, 60, 80, 100, 200, 400, 600 µg/mL. Rat plasma calibration standards of satranidazole were prepared by adding 20 µL of working solutions within the concentration range of 10-600 µg/mL into 200 µL of drug-free rat plasma. This gave the concentration of 1, 2, 4, 6, 8, 10, 20, 40, 60 µg/mL. This concentration range covered the plasma concentrations expected during the present experimental studies.

Quality control samples were prepared in the same way as that for calibration, by spiking blank plasma with satranidazole solution of known concentrations. Three different types of quality control samples were prepared - the low QC (LQC-1.5 µg/mL) sample, the medium QC (MQC-12.55 µg/mL) sample and the high QC (HQC-42.555 µg/mL) sample.

Sample preparation: Blood sample (2-3 mL) withdrawn was immediately centrifuged at 4500 rpm for 15 min at 4 ºC and then transferred into an Eppendorf tube and was kept at -20 ºC until analysis. 200 µL of blank plasma, calibration standards, quality control samples and plasma samples were

pretreated by acetonitrile (0.3 mL) for protein precipitation. After centrifugation at 10000 rpm for 10 min, the supernatant (20 µL) was injected directly into rheodyne injector for HPLC analysis.

Liquid chromatographic conditions: The HPLC system used consisted of binary system (Gradient mode) of pump (model JASCO; PU-2080 plus) with universal loop injector (Rheodyne 7725i) of injection capacity 20 µL. Detector consists of photodiode array detector MD-2010 plus, JASCO; Data was integrated using Jasco Chrompass version 1.8.6.1 and LC-Net II/ADC system. Chromatographic separation was achieved using the C_{18} (250 × 5 µm × 4.6 mm) Hypersil Gold, Thermo Scientific column, at ambient temperature.

Method validation

Selectivity: The selectivity of the method was evaluated by analyzing blank plasma samples from six rats. Each blank sample was tested for interference using the protein precipitation procedure and HPLC conditions discussed earlier. The blank response was compared with the peak area of the lowest limit of quantification (LLOQ) sample (Fig. 1).

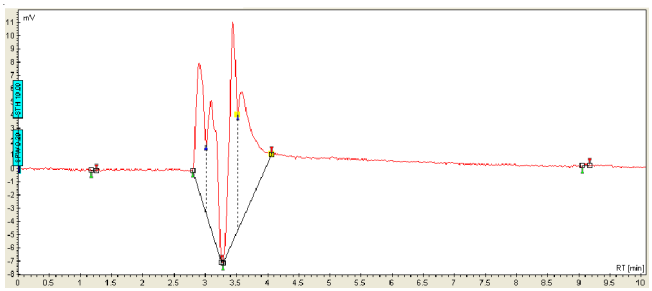

Fig. 1. Chromatogram of blank rat plasma

Linearity: Calibration curve of eight concentrations of satranidazole ranging from 1-60 µg/mL were assayed. Blank plasma samples were analyzed to confirm the absence of interferences but were not used to construct the calibration function.

Precision and accuracy: The precision of the assay was determined from the quality control plasma samples by replicate analyses of three concentration levels of satranidazole (1.5, 12.5 and 42.5 µg/mL). Intra-day precision and accuracy were determined by repeated analyses of the group of standards on one day (n = 3). The concentration of each sample was determined using the calibration curve prepared and analyzed on the same day.

Extraction recovery: The recovery of satranidazole was determined at limit of quantification, medium quality control and high quality control concentrations. Recoveries were calculated by comparing the analyte peak area obtained from plasma samples with those from the standard solutions at the same concentration.

Stability

Freeze and thaw stability: Quality control plasma samples at two concentration levels were stored at the freezing temperature for 24 h and thawed at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The samples were analyzed after a total of three such freeze thaw cycles.

Short-term temperature stability: Quality control plasma samples at two concentration levels, low quality control and high quality control were kept at room temperature for a period (6 h) that exceeded the routine preparation time of the samples (3-4 h).

Long-term stability: Quality control plasma samples at two concentration levels low quality control and high quality control kept at low temperature (-20 ºC) were studied for a period of 2 weeks.

Application to pharmacokinetic study: The developed HPLC assay method was used in the pharmacokinetic study after oral administration of satranidazole 54 mg/kg to rats. Twelve male Sprague-Dawley rats (250-300 g) were used. The rats were housed under controlled environmental conditions with a commercial food diet with free access to water. The rats were acclimatized to the facilities for three weeks and then fasted with free access to water for 12 h prior to experiment. Satranidazole suspension in 2 % acacia was delivered orally. The suspensions were made and vortexed for 1 min just before the administration.

About 0.5 mL blood samples via the retro-orbital venous plexus were collected in EDTA tubes from rats under ether anesthesia at 0.25, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, 24 h after administration of drug. The blood sample was immediately transferred to a micro-centrifuge tube and then centrifuged (4500 rpm, 15 min at 4 ºC) to obtain plasma as supernatant layer. It was separated without disturbing the buffy layer. The separated plasma samples were kept at -20 ºC until analysis. Frozen rat plasma samples were left on the bench to thaw naturally before analysis. Acetonitrile 0.3 mL was then added and vortexed for 1 min at high speed and then centrifuged at 10000 rpm for 10 min. The resultant supernatant (20 µL) was injected in to the HPLC system. To determine the pharmacokinetic parameters of satranidazole, the concentration-time data were analyzed by Graph Pad Prism Software (version 5.00).

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: Instead of acetonitrile and buffer used in analysis of satranidazole as reported in the literature[10,11], we chose methanol and buffer as mobile phase as methanol is cheaper than acetonitrile. Looking at the different chromatographic parameters during method development, the finally recommended mobile phase consisted of methanol:10 mM phosphate buffer adjusted to pH 3 with orthophosphoric acid, in ratio of 30:70 (v/v). The best resolution and sensitivity of the method was obtained at 320 nm and mobile phase flow rate of 1 mL/min. Typical chromatogram (Fig. 2) at the optimized condition gave sharp and symmetric peak with retention time 5.4 min and the HPLC run time was 10 min. Thus, the system became ready for next sample injection within a short time.

Method validation

Selectivity: The selectivity of the method was ensured at the lower limit of quantification (LLOQ) *i.e.*, 1 µg/mL of plasma. It was found that there is no significant area at retention time of satranidazole. It proves the chromatographic conditions for selectivity criteria.

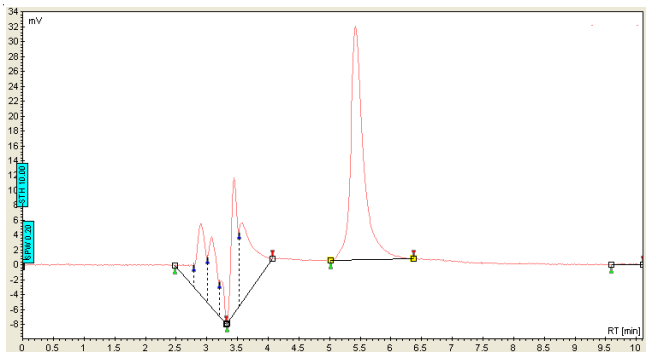

Fig. 2. Chromatogram of plasma spiked with drug

Linearity: By plotting the peak-area of satranidazole *versus* analyte concentration (µg/mL) in spiked plasma samples, the calibration curve was obtained in the range of 1-60 µg/mL. The correlation coefficient of this curve was r^2 0.9968. The percentage nominal values of the back calculated concentrations of calibration curve standards were between 85-115 % at all points except at the LLOQ, where they were between 80-120 %. It can be concluded that method meets this acceptance criteria.

Precision and accuracy: The intra-day accuracy and precision were expressed in terms of RSD %. At the low quality control, medium quality control and high quality control levels, the maximum intra-day percentage RSD values were 8.67, 6.40 and 8.42, respectively (Table-1). The percentage RSD was less than 15 %. It can be concluded that the method meets this acceptance criteria.

TABLE-1
RESULTS OF THE PRECISION AND ACCURACY OF BIOANALYTICAL METHOD FOR SATRANIDAZOLE

S. No.	Low quality control (1.5 µg/mL)	Medium quality control (12.5 µg/mL)	High quality control (42.5 µg/mL)
Set-1	1.43	11.23	38.43
	1.39	12.45	40.45
	1.63	12.67	45.21
Mean	1.48	12.12	41.36
RSD (%)	8.67	6.40	8.42
Set-2	1.56	12.91	37.10
	1.60	11.82	40.37
	1.39	13.19	42.28
Mean	1.52	12.64	39.92
RSD (%)	7.35	5.73	6.56

Extraction recovery: The amount of drug area obtained from unextracted samples was considered as 100 % and was compared with extracted area of drug from plasma samples (Table-2). It can be observed that the recovery of satranidazole was consistent over the three levels.

Stability studies

Freeze thaw stability: The samples were found to be stable for three freeze thaw cycles, with % RSD < 15 % and percentage nominal for low quality control within 80-120 % and high quality control within 85-115 % (Table-3).

Short-term stability: The samples were found to be stable at room temperature for 6 h. The RSD % was found to be < 15 % and % nominal within range (Table-4).

TABLE-2
RESULTS OF THE RECOVERY OF SATRANIDAZOLE FROM RAT PLASMA

Level	Target conc. (µg/mL)	Recovery (%)
Low quality control	1.5	80.00
Medium quality control	12.5	85.71
High quality control	42.5	88.88

TABLE-3
RESULTS FOR FREEZE THAW STABILITY OF SATRANIDAZOLE IN RAT PLASMA

S. No.	Freeze thaw stability		
	Sample	Back calcd. conc.	Nominal (%)
1	Low quality control (1.5 µg/mL)	1.51	100.54
2		1.66	110.72
3		1.43	95.45
Mean		1.53	
RSD (%)		7.61	
1	High quality control (42.5 µg/mL)	37.40	87.99
2		38.92	91.58
3		39.69	93.38
Mean		38.67	
RSD (%)		3.02	

TABLE-4
RESULTS FOR SHORT-TERM STABILITY OF SATRANIDAZOLE IN RAT PLASMA

S. No.	Short-term temperature stability		
	Sample	Back calcd. conc.	Nominal (%)
1	Low quality control (1.5 µg/mL)	1.4	93.33
2		1.53	102.00
3		1.67	111.33
Mean		1.53	–
RSD (%)		8.81	–
1	High quality control (42.5 µg/mL)	42.45	99.88
2		45.32	106.64
3		39.45	92.82
Mean		42.41	–
RSD (%)		6.92	–

Long-term stability: In long-term stability studies quality control samples were studied for two weeks and RSD % was found to be 7.07 % for low quality control and 4.65 % for high quality control and the percentage nominal within range (Table-5).

TABLE-5
RESULTS FOR LONG-TERM STABILITY OF SATRANIDAZOLE IN RAT PLASMA

S. No.	Long-term stability		
	Sample	Back calcd. conc.	Nominal (%)
1	Low quality control (1.5 µg/mL)	1.63	108.67
2		1.42	94.67
3		1.49	99.33
Mean		1.51	–
RSD (%)		7.07	–
1	High quality control (42.5 µg/mL)	44.65	105.06
2		43.98	103.48
3		40.89	96.21
Mean		43.17	–
RSD (%)		4.65	–

Application to pharmacokinetic study: The applicability of the assay method was demonstrated in pharmacokinetic study which was approved by local ethics committee. The plasma samples were collected up to 24 h after single oral dose of 54 mg/kg satranidazole. The representative chromatograms are given in Figs. 3 and 4. The satranidazole plasma concentration-time data was analyzed with the aid of program Graph Pad Prism (version 5.00).

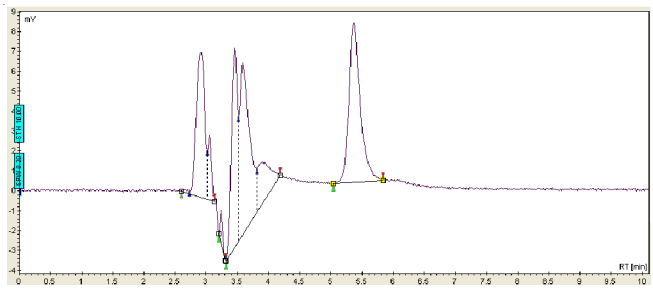

Fig. 3. Chromatogram of plasma collected from rat after oral administration of satranidazole at 1 h

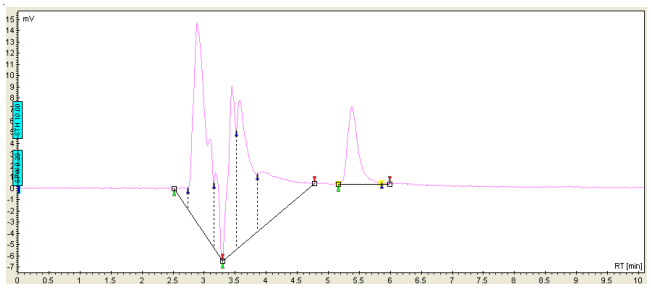

Fig. 4. Chromatogram of plasma collected from rat after oral administration of satranidazole at 18 h

The maximum plasma concentration (C_{max}) for satranidazole was found to be 47.55 mg/kg. The plasma levels reached their maximum (T_{max}) 2 h after administration. Area under concentration-time curve (AUC) measured from 0-24 h was 409.3 mg h/kg (Fig. 5).

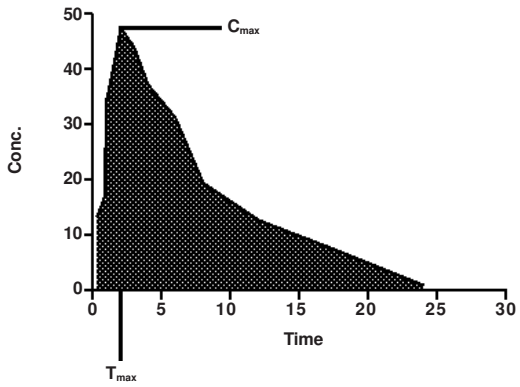

Fig. 5. Area under curve of satranidazole

Conclusion

To our best of knowledge, this is the first description of pharmacokinetics of satranidazole in rat plasma by HPLC-DAD method in the literature. The small amount of biological matrices required (0.2 mL per determination) makes this method suitable for routine analysis in preclinical pharmacokinetic studies and might be useful alternate to perform clinical pharmacokinetic studies in determination of satranidazole.

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