Send Orders for Reprints to reprints@benthamscience.ae

Current Pharmaceutical Analysis, 2015, *11,* 35-42

LC-MS/MS Assay for Quantification of a Novel Antitubercular Molecule S006-830: Pharmacokinetic and Plasma Protein Binding Studies in Rats

Mahendra Kumar Hidau[1,2], Yeshwant Singh[1#], Sudhir Shahi[1], Poojari Mounika[1] and Shio Kumar Singh*[1#]

[1]Pharmacokinetics & Metabolism Division, CSIR- Central Drug Research Institute, Lucknow, India-226031; [2]Academy of Scientific and Innovative Research, New Delhi, India

Abstract: A highly sensitive and selective LC-MS/MS assay was developed and validated for determination of a novel antitubercular compound S006-830 in rat plasma. The analyte and internal standard (IS) were extracted from plasma by a two step liquid–liquid extraction procedure using 2% isopropanol in n-hexane. Chromatographic separation was achieved on a Phenomenex, Luna C-18 column (3µm, 100mm x 2mm i.d.) under isocratic condition [92:8 (v/v); ACN (0.1% formic acid) : ammonium acetate buffer (10 mM, pH 4)] at a flow rate of 0.450 ml/min. The quantification was performed on Q-trap 5500 LC-MS/MS coupled with ekspert ultra LC 100-XL system (AB Sciex). The detection was performed in positive electrospray mode using multiple reaction monitoring. The precursor to production of ion transitions selected for quantification of S006-830 and IS was m/z 424.353/203.00 and 330.300/267.400 respectively. LC-MS/MS method was found sensitive and reproducible over a linearity range of 0.15-40 ng/ml. Recoveries of S006-830 from spiked plasma samples were consistent and found to be more than 70%. Further, the applicability of this method has been described by determining pharmacokinetic (PK) profile and plasma protein binding of S006-830 in rats. Irregular plasma-concentration time profile was observed. Oral PK profile of S006-830 at 50 mg/Kg demonstrated that mean (±SEM) $T_{1/2}$ and mean residence time were 8.30 ± 1.30 h and 8.44 ± 0.57 h, while C_{max} and $AUC_{0\text{-}last}$ were 1.94 ± 0.30 µg /ml and 6.25± 1.66 µg.h /ml respectively. Plasma protein binding for S006-830 was found to be 58.63 ± 3.4 %.

Keywords: Antitubercular, extraction, LC-MS/MS, method validation, pharmacokinetics, protein binding, recovery.

1. INTRODUCTION

Tuberculosis (TB) still remains a global health problem despite all efforts, although major progress has been made towards 2015 global targets set within the context of the Millennium Development Goals. According to the WHO report, in 2012, about 8.6 million people developed TB and 1.3 million died from this disease that included 320000 deaths among HIV-positive people [1, 2, 3]. As per WHO approved treatment strategy, the first line standard TB combination therapy involves Ioniazid, Rifampicin, Ethambutol and Pyrazinamide daily for two months followed by four months of Isoniazid and Rifampicin three times a week. This treatment for TB is still inadequate to address many challenges including development of drug resistance, which offers one of the major threats leading to treatment failure [4]. It is found that drug resistance emerges more frequently in those treatment regimens which use only one drug, compared to a combinational therapy which uses more than one drug having different mechanisms of action, leading to a multi factorial origin of drug resistance. In addition, a number of other factors like patients non-compliance, breaks in the therapy and antibiotic course etc. may equally and potentially attribute to the development of drug resistant *M.tuberculosis* strains [5].

To end the global TB epidemics, it is crucial to develop new TB diagnostics, medicines and vaccines. In this endeavor, Medicinal & Process Chemistry Division of Council of Scientific and Industrial Research- Central Drug Research Institute (CSIR- CDRI) has pipelined a new antitubercular molecule coded S006-830. It is a potent and novel Diisopropyl-(2-{4-[(4-methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy}-ethyl)-amine. S006-830 has bactericidal activity effectively comparable to Ethambutol and Pyrazinamide (work currently considered for publication elsewhere). S006-830 is found to be active against drug sensitive, single drug (Rifampicin) and multidrug (Isoniazid and Rifampicin) resistant clinical isolates of *M.tuberculosis*.

In the present study, a highly sensitive and selective high-performance liquid chromatography–tandem mass spectrometric method (LC-MS/MS) method was developed for the determination of S006-830 in Sprague Dawley (SD) rat plasma. The detection of S006-830 was performed in positive mode electro spray ionization in multiple reaction monitoring (MRM) mode using Q-trap 5500. This method was validated for its sensitivity, selectivity and reproducibility and was successfully applied to pharmacokinetic characterization and *in-vitro* plasma protein binding studies of S006-830 in SD rats.

*Address correspondence to this author at the Pharmacokinetics & Metabolism Division, CSIR- Central Drug Research Institute, Sector-10, Jankipuram Extension, Sitapur Road, Lucknow, India-226031; Tel: 91-522-2771940, 91-522-2771960; Fax: 91-522-2771941; E-mail: sk_singh@cdri.res.in, shiokumar@yahoo.com

[#]This author holds equal first authorship to the research work.

1875-676X/15 $58.00+.00 © 2015 Bentham Science Publishers

2. EXPERIMENTAL

2.1. Chemicals and Reagents

Pure reference standards (>99%) of S006-830 and α-artether [used as internal standard (IS)], (Fig. **1a, b**) were obtained from Medicinal & Process Chemistry Division, CSIR- CDRI, India. Acetonitrile (ACN), MS grade, ammonium acetate and glacial acetic acid used for acid buffer preparation were purchased from E. Merck Limited (Mumbai, India). Ultra pure water was obtained from a Milli-Q PLUS PF system. A 0.22µm cellulose membrane (Whatman International Ltd., Mailstone, England) was used for filtration of buffer. Dextran coated charcoal for plasma protein binding study was obtained from Sigma Chemicals (USA). Dulbecco's Phosphate buffered saline (DPBS) (Ca^{2+} and Mg^{2+} free) was purchased from HiMedia Lab., (Mumbai, India). All other chemicals and reagents (isopropanol, n-hexane, and formic acid) were of HPLC analytical reagent grade.

2.2. Method Development and Validation

2.2.1. Stock Solutions, Calibration and Quality Control Standards

The accurate amounts of S006-830 and α-arteether were weighed using calibrated Denver Instrument APX-60 balance. Stock solutions of both compounds (0.5 mg/ml) were prepared by dissolving 0.5 mg of respective compound in 1 ml of ACN. Working solutions of the compound S006-830 (0.018, 0.037, 0.075, 0.15, 0.31, 0.62, 1.25, 2.5 and 5 µg/ml) were prepared from stock solution. Analytical standards (AS) of S006-830 were prepared by parallel dilution technique in the range of 0.15, 0.31, 0.62, 1.25, 2.5, 5, 10, 20 and 40 ng/ml in ACN for the determination of linearity range. Quality control (QC) samples at different concentrations (0.30, 1, 15 and 30 ng/ml as lower limit of quantification (LLOQ), low quality control (LQC), medium quality control (MQC) and high quality control (HQC) respectively) were prepared separately in three replicates. Concentration of IS for all the samples was kept 4 ng/ml by spiking 5 µl of 0.8 µg/ml α-arteether except for AS and matrix blanks.

2.2.2. Sample Preparation

Drug free rat plasma was collected from healthy male SD rats provided by National Laboratory Animal Centre (NLAC), CDRI. The plasma was obtained by centrifuging the whole blood at 2000 g for 15 min. The supernatant was separated and stored at -80°C for further use. Plasma samples were extracted and processed by a two step liquid–liquid extraction procedure using 2% isopropanol in n-hexane as an extracting solvent. Calibration standard of S006-830 was prepared in the linearity range of 0.15 to 40 ng/ml. The matrix blank, control and standard QC plasma samples were prepared by spiking 8 µl of S006-830 from respective working stocks into 17 µl plasma and 5 µl IS (except for matrix blanks) and vortex mixed followed by addition of 970 µl ACN. This mixture was again vortex mixed for 4 min. and centrifuged at 3000 g for 15 min. Then organic layer was transferred (snap freezed aqueous layer in liquid nitrogen) into clean tubes, which was kept for complete drying in speed vac-concentrator. Remaining plasma of this step was re-extracted in a similar way. The dried residues were then reconstituted using 1 ml ACN. The resultant mixture was subjected to LC-MS/MS analysis for S006-830 determination.

2.2.3. Liquid Chromatography

A ekspert ultra LC 100-XL HPLC system (AB Sciex), consisting of flow control valves, vacuum degasser, ekspert 100 pump with ekspert 100-XL autosampler was used to deliver mobile phase [92:8 (v/v); ACN (0.1% formic acid) : ammonium acetate buffer (10 mM, pH 4)] at a flow rate of 0.450 ml/min. Mobile gas was degassed for 15 min using ultrasonic bath (Bransonic Cleaning Equipment Company, USA) prior to use. The chromatography was performed on a Phenomenex, Luna C-18 column (3 µm, 100 mm x 2 mm i.d.) with a C-18 guard column (Phenomenex, Luna C-18, 5 µm, 30 mm x 2 mm, i.d.) with each run taking 3 min. The column temperature was maintained at 40°C using ekspert 100 oven.

2.2.4. LC-MS/MS Analysis

The Q-trap 5500 LC-MS/MS (AB Sciex) mass spectrometer was used for sample analysis. Analyst 1.6 software (AB Sciex) was used for the control of equipment, sample acquisition and data analysis. Zero air was used as nebulizing gas (GS 1, 70 psi) and nitrogen as curtain gas (CUR, 35 psi). Declustering potential (DP) was optimized, while ion spray voltage, nebulizing and CUR conditions were used in default mode. Dwell time was 0.2 s and MS scan was performed in positive ion mode. In positive electrospray ionization (ESI) mode, signals were observed at 424.2 and 330.1 m/z as protonated molecular ions ($M + H^+$) for S006-830 and IS respectively (Fig. **2a, b**). These molecular ions were fur-

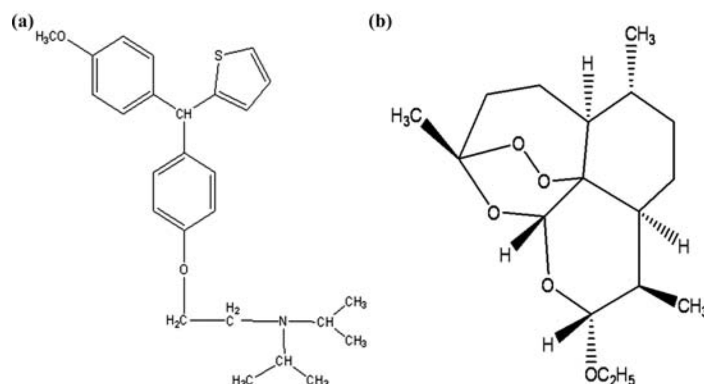

Fig. (1). Chemical Structure of S006-830 (a), IS α-arteether (b).

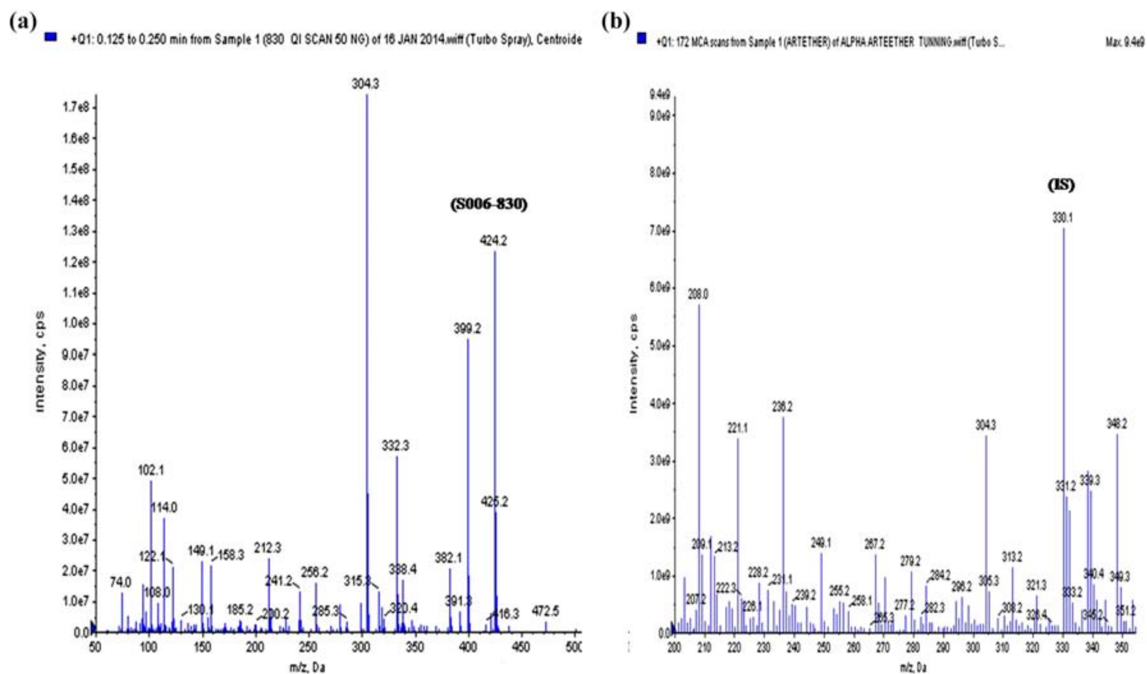

Fig. (2). Precursor ion spectra [M+H$^+$] of S006-830 (**a**) and α-arteether (**b**).

ther fragmented by collision activated dissociation (CAD), using nitrogen as collision gas. The product ion spectra have shown formation of characteristic product ions at m/z 203.0, 382.1, 128.1 for S006-830 and m/z 267.4, 163.2 for IS (Fig. **3a, b**). DP was optimized to 90 V. Flow injection analysis was used to optimize collision energies (CE). The CE optimized for m/z 424.353/203.00 was 40 eV and 16 eV for IS. The CUR and collision gas were optimized to 35 and 7 psi respectively. The ion source gas 1 (GS1) and ion source gas 2 (GS2) were optimized to 70 and 30 psi respectively. Entrance potential (EP) and collision cell exit potential (CXP) were optimized to 10 and 18 V respectively. The ion source potential was set at 5500 V and source gas temperature was optimized to 400ºC. The optimized operating conditions of MS/MS in MRM mode are summarized in Table **1**.

2.2.5. Method Validation

The method was validated in terms of recovery from plasma, specificity & sensitivity, accuracy & precision, intra & inter-batch variations and reproducibility. The inter & intra- day precision was determined by subjecting the data to one way analysis of variance (ANOVA) in terms of relative standard deviation (% RSD). The method was validated as per US FDA guidelines for industry on bioanalytical method

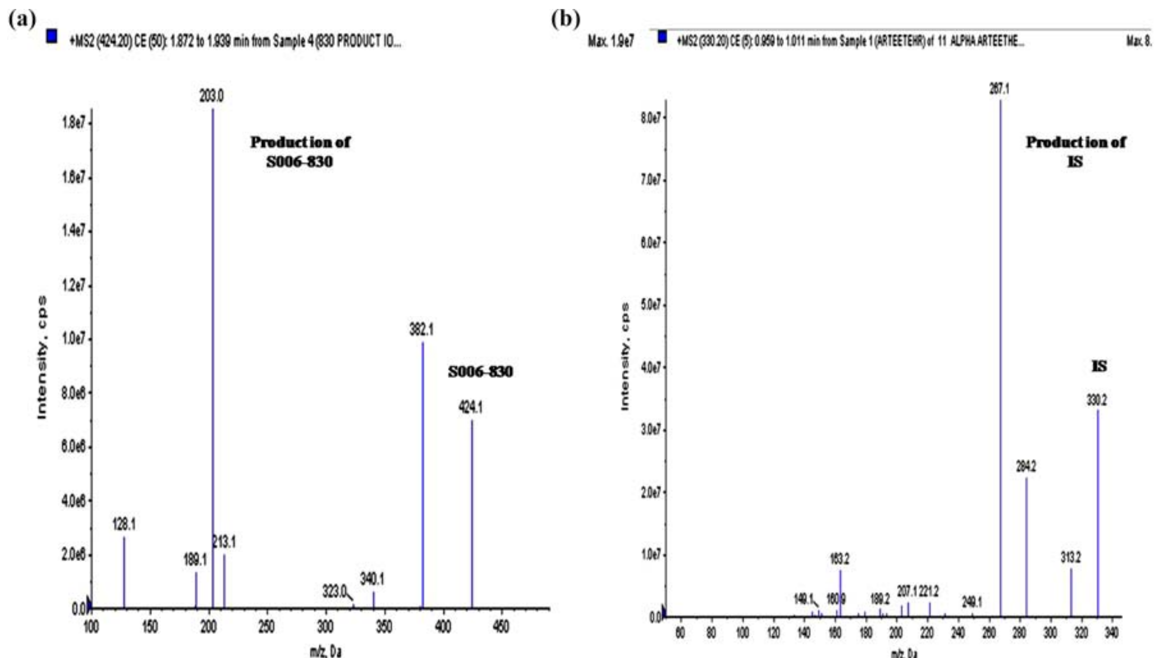

Fig. (3). Product ion spectra of S006-830 (**a**) and α-arteether (**b**).

Table 1. MRM conditions for S006-830 and IS.

Analyte	$[M\text{-}H]^+$	Product ion/s	Declustering potential (V)	Collision energy (eV)
S006-830	424.353	203.0	90	40
	424.353	382.0	90	28
	424.353	128.200	90	35
α-arteether (IS)	330.300	267.4	90	16

validation [6, 7]. The absolute extraction recovery of S006-830 was determined by comparing the peak area of extracted sample spiked with known amount of the analyte with the peak area of analytical standard at corresponding concentrations. The accuracy and precision studies and recoveries determination were carried out in quadruplets for 5 different days at LLQC, LQC, MQC and HQC. This method was extrapolated in SD rat plasma following partial validation.

2.2.6. Stability Studies

Bench top, freeze-thaw, dry residue and long term stability studies were performed to determine the stability of S006-830 in stock solution and plasma. The stability of S006-830 was evaluated in triplicates at four spiked plasma concentrations that corresponded to LLQC, LQC, MQC and HQC.

2.3. Application to Pharmacokinetic Study

2.3.1. Subjects

Healthy male SD rats (240-270 g) were obtained from NLAC of CDRI, India. Before the commencement of studies, animals were acclimatized for 3 days in proper ventilated polypropylene cases under standard laboratory conditions with regular 12 hr. light-dark cycle, temperature (22±2°C) and relative humidity (50±5%). Standard pellet laboratory chow and water were allowed ad lib. Guidelines approved by the “Animal Experimentation Ethics Committee, India” and Good laboratory practice (GLP) were followed throughout the animal experimentation. Pain to the animals was minimized by using ether anesthesia at all the times during study.

2.3.2. Dose Formulation and Administration

Fresh oral formulation of S006-830 was prepared in triple distilled water at 50 mg/Kg dose. The formulation was subjected to QC, stability and homogeneity test to ensure the strength before dosing. The volume factor for the formulations was 1 ml/Kg. The formulation was well stirred to ensure the content uniformity before dosing using a 20 G gavage needle.

2.3.3. Study Design and Sampling Procedure

The study was single oral dose designed to estimate PK of S006-830. The study was carried out in two groups of experimental animals (for sparse sampling, animals were divided into 2 groups: A & B. Blood samples were collected alternatively from these groups. Sample was first taken from group A animals followed by collection from B group and so on), each group comprising three animals (n=3). Subjects were given 50 mg/Kg of S006-830. Blood samples were collected from three different rats for each time points by sparse sampling approach. Blood samples for PK analysis were collected before dosing and at following time points 0.083, 0.33, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72 and 96 h post dose using. 150 µl blood was drawn per time point from respective animals, wherein two samples were collected from each rat in the study group, first sample being collected by cardiac puncture followed by terminal sampling from inferior vena cava. Thus the total volume of the blood withdrawn within 24 h by cardiac puncture was less than 5% of the blood volume [8]. Blood samples were centrifuged at 2500 g for 7 min to obtain plasma, which was stored at -70°C prior to LC-MS/MS analysis. Analysis was performed within 30 days of sample collection.

2.3.4. Statistical Analysis

The primary endpoints for these studies were area under the curve ($AUC_{0\text{-}last}$), maximum plasma concentration (C_{max}), time to attain C_{max} (T_{max}), elimination half life ($T_{1/2}$) and mean residence time (MRT) and bioavailability. These PK parameters were calculated by non-compartmental analysis using Winnonlin software (Version 1.5, Pharsight Corporation). Results were expressed as mean ± SEM.

2.4. Application to Plasma Protein Binding Study

The method was successfully applied to determine % plasma protein binding of S006-830 *in vitro* in SD rat plasma. Study was performed using dextran coated charcoal suspension, which was prepared by weighing 0.66 g of dextran-coated charcoal in 100 ml of DPBS (9.5 g L^{-1}) followed by continuous stirring with a magnetic stirrer at room temperature until the charcoal got suspended. This suspension was prepared at least 20 hours before use and stored at 5-10°C. The stored charcoal mixture was re-suspended before use [9, 10].

Study was carried out at 1 µg/ml spiked plasma concentration (n=3). The spiked plasma was allowed to equilibrate for 20 min before the start of experiment. Charcoal suspension (3.0 ml) was transferred into a 20 ml glass tube, centrifuged at 2500 g for 15 min at 27°C followed by the careful decant of supernatant. The spiked plasma (3.0 ml) was then added to the charcoal pellet with continuous stirring at 37°C. 100 µl samples were withdrawn serially at 0 min (immediately after the addition of spiked plasma to charcoal pallet), 0.083, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6 h in 0.5 ml polypropylene micro-centrifuge tubes and centrifuged immediately at 10,000 g for 3 min at 37°C. Supernatant was separated and immediately transferred into 1.5 ml micro-centrifuge tubes and stored at -70°C until analyzed.

3. RESULTS AND DISCUSSION

3.1. LC-MS/MS Optimization

The spectral responses of S006-830 and IS was evaluated with ESI mode both in positive and negative. In terms of sensitivity and intensity of the response, positive mode ESI was selected over negative mode ESI. A continuous flow analysis was carried out to obtain parent and product ion mass spectra of S006-830 and IS. Using the information discussed in section 2.2.4, a MRM method was developed for S006-830 quantification by optimizing the collision energies for different transitions. Following optimization, on the basis of the better sensitivity and reproducibility, transition m/z 424.353/203.00 was selected for S006-830 quantification (keeping all other transition as qualifiers), while transition m/z 330.300/267.400 for IS.

3.2. Sample Extraction and Recovery

In this study, we tried to develop an extraction procedure that meets the requirements of specificity, sensitivity and recovery. Attempts were made using single step protein precipitation, liquid-liquid extraction and solid phase extraction procedures [11-13]. Protein precipitation using ACN was not followed due to severe matrix suppression. Liquid-liquid extraction was carried out using hexane alone and different combinations of hexane (90-10, % v/v): isopropanol (1-3.5, % v/v). Finally, a two step liquid-liquid extraction procedure was developed and validated using 2% isopropanol in n-hexane as an extracting solvent due to better recoveries and reproducibility. Recoveries of S006-830 from spiked plasma samples were consistent and found to be more than 70%. These values are reported in the Table **2**.

3.3. Precision and Accuracy

The calibration range was set from 0.15-40 ng/ml. Calibration curves were fitted with linear models using $1/x^2$ weighting factors over this range. Least-square linear regression constants (r^2) were always ≥0.990 (n=3) (Fig. **4**). Intra and inter batch accuracy and precision for S006-830 was assessed at LLQC, LQC, MQC and HQC for five days. Concentrations of QC standards were calculated from each curve. The accuracy was calculated by comparing the average calculated concentrations to their % nominal values and the precision by the percent co-efficient of variation (%CV). The results from a 5-day precision and accuracy batches are listed in Table **3**. As per current US FDA regulatory guidelines, the standard back-calculated concentrations for lower limit of quantification (LLOQ) must be within ±20% of nominal concentrations and for all other standards back-calculated concentrations must be within ±15% of their nominal concentrations. The results were completely within the acceptance limits.

3.4. Specificity of Method and Matrix Effect

Specificity was established by evaluating the chromatograms acquired from the plasma spiked with S006-830 at LOQ to those obtained from blank plasma. No visible interference was obtained in the region of drug and the IS elution.

Table 2. % Recovery of spiked plasma samples at LLQC, LQC, MQC and HQC.

QC standard	Conc.(ng/ml)	% Recovery of S006-830			Mean ± SEM
		Day1	Day2	Day3	
LLQC	0.31	80.07	76.84	88.63	81.85±4.06
LQC	1	72.16	63.24	77.49	70.96±4.80
MQC	15	81.47	79.79	75.21	78.82±2.16
HQC	30	85.03	79.43	78.40	80.95±2.37

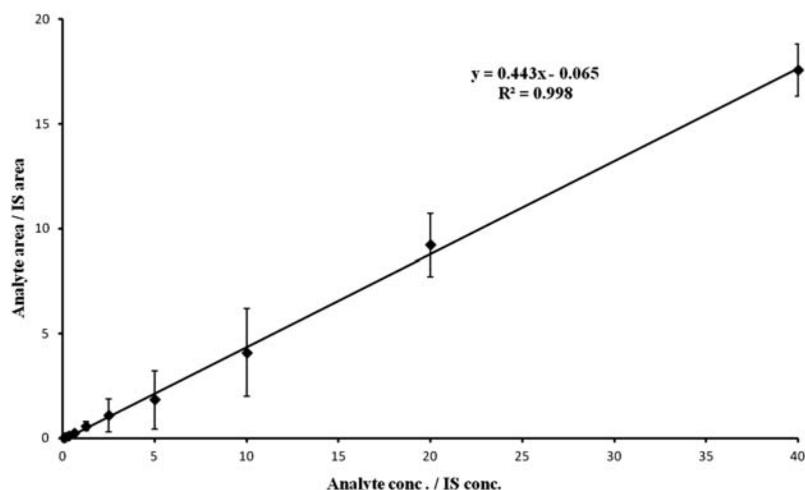

Fig. (4). Calibration curve of S006-830 in rat plasma (n=3).

Table 3. Intra and inter assay accuracy and precision for the S006-830 QC samples in the rat plasma.

QC standard	Nominal Conc. (ng/ml)	Accuracy (%) (n=4)		Precision (% CV) (n=4)	
		Intra-day	Inter-day	Intra-day	Inter-day
LLQC	0.31	-4.21	-4.60	0.76	0.80
LQC	1	-2.79	-1.84	3.04	2.36
MQC	15	6.42	1.94	1.26	6.97
HQC	30	2.47	3.12	4.54	7.99

The representative MRM chromatograms for blank plasma, plasma spiked with the S006-830, test sample after oral dosing and the IS are shown in Fig. (**5**). Further, to estimate any matrix effect, blank post extracted plasma samples (n=4 at LQC) were spiked with S006-830 and the chromatogram obtained was compared with the corresponding AS in terms of the peak areas [14]**.** The average peak area for AS samples was 1.49 ± 0.30 e^4, while for post extracted plasma samples spiked with analyte it was 1.58 ± 0.10 e^4. Statistical comparison of the areas revealed that there was no significant difference between the values indicating that no matrix effect existed. Thus extraction procedure and chromatographic conditions yielded a clean chromatogram for the S006-830.

3.5. Stability Studies Under Various Conditions

The freeze-thaw stability study was successfully conducted in rat plasma after three freeze-thaw cycles between -70°C and melting ice temperatures. Bench top stability for S006-830 was estimated by keeping the QC samples at room temperature for 8h. Dry residue stability was evaluated by extracting the QC samples by liquid- liquid extraction procedure as mentioned above and keeping the dry residues at -70°C for three consecutive days followed by reconstitution and sample analysis. Long term stability was conducted by keeping the QC samples for 30 days at -70°C followed by sample analysis. Stability data is shown in Table **4**.

3.6. Application to PK and çplasma Protein Binding Studies

The validated method was successfully applied to estimate pharmacokinetic parameters of S006-830 in male SD rats. Single oral dose study was conducted at 50 mg/Kg. Irregular plasma-concentration time profile was observed for S006-830. The possible reasons for such plasma concentra-

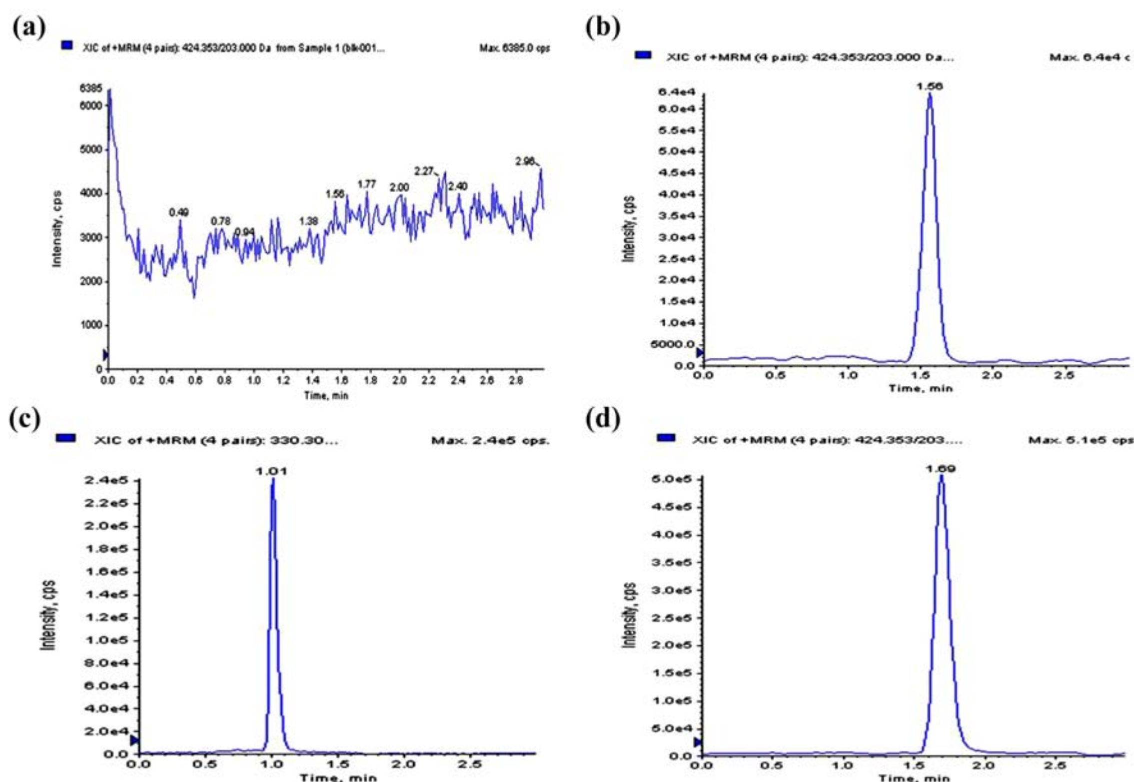

Fig. (5). MRM chromatograms: Blank extracted plasma (**a**), spiked S006-830 at 10 ng/ml (**b**), spiked IS at 4 ng/ml (**c**), test sample at 1 h post oral dose administration at 50 mg/Kg (**d**).

Table 4. Stability of S006-830 in rat plasma under various storage conditions (N=3).

Stability Storage conditions	Nominal Conc. (ng/ml)	Conc. recovered (mean ±SD) (ng/ml)	% Recovery#
Freeze-thaw	0.31	0.37 ± 0.06	119.3
	1	0.70 ± 0.20	70
	15	12.88 ± 2.31	85.86
	30	26.73 ± 5.24	89.1
Bench top stability	0.31	0.34 ± 0.03	109.6
	1	0.89 ± 0.30	89
	15	14.34 ± 4.21	95.6
	30	24.30 ± 6.00	81
Dry residue stability	0.31	0.33 ± 0.01	106
	1	0.78 ± 0.33	78
	15	11.98 ± 2.50	79.86
	30	24.10 ± 3.54	80.30
Long term stability	0.31	0.28 ± 0.01	90.32
	1	0.71 ± 0.26	71
	15	12.30 ± 5.20	82
	30	25.46 ± 7.25	84.86

Freeze thaw stability after 3cycles, Bench-top stability for 8 h at ambient temperature, Long term storage stability for 30 days at -60°C.
#Recovery is given as percent relative to spiked nominal concentration

tion-time profile may be in part attributed to the aqueous solubility of S006-830 due to variable dissolution environment, which might have resulted into precipitation of a fraction of dose in the absorptive regions of gastrointestinal tract [15].

Oral PK profile of S006-830 at 50 mg/Kg in healthy male rats is shown in Table **5**. The plasma concentration-time profile for oral dose S006-830 is shown in Fig. (**6**). After oral dosing of S006-830, it appeared that the absorption was fast as peak plasma concentration appeared at < 1 h post dose.

Table 5. Single oral dose pharmacokinetic parameters of S006-830 at 50 mg/Kg dose in SD Rats (n=3).

PK Parameters	Mean ± SD (50 mg/kg)
C_{max} (μg/ml)	1.94± 0.30
$T_{1/2}$(h)	8.30± 1.30
$AUC_{0\text{-}last}$ (μg.h/ml)	6.25± 1.66
Vz (L)	108.7± 32.17
Cl (L/h/Kg)	9.09± 2.99
MRT (h)	8.44± 0.57

Similarly, the method was successfully applied to determine plasma protein binding of S006-830 at 1μg/ml concentration (N=3). The method was based on charcoal adsorption kinetics that operates under non-equilibrium conditions. Association and dissociation of drugs with proteins is a dynamic phenomenon with charcoal acting as a sink for free drug removal. The percentage binding is then estimated from the decline of % drug remaining in the supernatant after the addition of charcoal. Percent S006-830 remaining in the su-

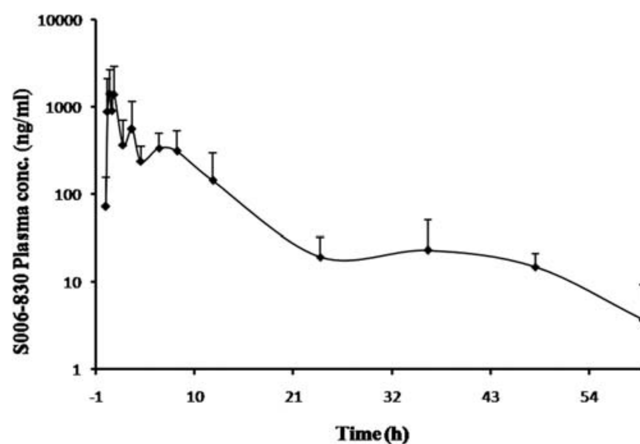

Fig. (6). Concentration–time profile of S006-830 after 50 mg/Kg oral dose in SD rats (mean ± SEM, n = 3).

pernatant plasma verses time was fitted to a two–compartment model, intravenous bolus non-linear regression program on WinNonlin 5.1. Plasma protein binding for S006-830 was found 58.63 ± 3.4 %.

CONCLUSION

A highly sensitive, selective, precise and straightforward LC–MS/MS bioanalytical method has been successfully developed and validated for determination of S006-830 in rat plasma with the lower limit of quantification 0.15 ng/ml and linearity was obtained over a concentration range 0.15-40 ng/ml. An efficient liquid-liquid extraction method for sample preparation was developed which yielded absolute recovery of more than 70%. Stability studies have shown that S006-830 exhibited good stability during storage and sample

processing under various conditions. Moreover, the plasma volume utilized for sample processing was quite small (25µl) reflecting a very high sensitivity of the developed method. This method was successfully applied for the single dose pharmacokinetic study of S006-830 following oral administration and plasma protein binding studies in SD rats. The developed and validated method may be applied in various preclinical regulatory and exploratory studies like toxicokinetic, multiple dose pharmacokinetics and drug-drug interaction studies in addition to others.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The Authors acknowledge the Director, CSIR-CDRI and CSIR for availing all the facilities required for this research work. Authors also acknowledge Medicinal & Process Chemistry Division for providing S006-830 for pharmacokinetic evaluation. CDRI communication no. is 8768.

REFERENCES

[1] Global Tuberculosis Report, WHO, 2013. http://apps.who.int/iris/bitstream/10665/91542/1/WHO_HTM_TB_2013.13_eng.pdf?ua=1

[2] Trends in Tuberculosis Morbidity and Mortality, American Lung Association Research and Health Education Epidemiology and Statistics Unit, April **2013**. http://www.lung.org/finding-cures/our-research/trend-reports/TB-Trend-Report.pdf

[3] Zaman, K., Tuberculosis: a global health problem. *J. Health Popul. Nutr.,* **2010**, *28*(2), 111-3.

[4] Ma, Z.; Lienhardt, C.; McIlleron, H.; Nunn, A.J.; Wang, X., Global tuberculosis drug development pipeline: the need and the reality. *Lancet,* **2010**, *375*(9731), 2100-9.

[5] Gandhi, N.R.; Nunn, P.; Dheda, K.; Schaaf, H.S.; Zignol, M.; van Soolingen, D.; Jensen, P.; Bayona, J. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet,* **2010**, *375*(9728), 1830-43.

[6] Furey, A.; Moriarty, M.; Bane, V.; Kinsella, B.; Lehane, M. Ion suppression; a critical review on causes, evaluation, prevention and applications. *Talanta,* **2013**, *115*, 104-22.

[7] Guidance for Industry, Bioanalytical Method Validation. http://www.regulations.gov. fda guidliens

[8] Sabarinath, S.; Madhusudanan, K.P.; Gupta, R.C. Pharmacokinetics of the diastereomers of arteether, a potent antimalarial drug, in rats. *Biophar. Drug Dispos.,* **2005**, *26*(6), 211-23.

[9] Srivastava, P.; Gupta, R. C. *In situ* absorption and protein binding characteristics of CDRI-85/92, an antiulcer pharmacophore. *Int. J. Pharm.,* **2003**, *257*(1-2), 97-102.

[10] Khurana, M.; Paliwal, J.K.; Kamboj, V.P.; Gupta, R.C., Binding of centchroman with human serum as determined by charcoal adsorption method. *Int. J. Pharm.,* **1999**, *192*(2), 109-14.

[11] Lachatre, F.; Marquet, P.; Ragot, S.; Gaulier, J.M.; Cardot, P.; Dupuy, J.L. Simultaneous determination of four anthracyclines and three metabolites in human serum by liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. B. Biomed. Sci. Appl.,* **2000**, *738*(2), 281-91.

[12] Fogli, S.; Danesi, R.; Innocenti, F.; Di Paolo, A.; Bocci, G.; Barbara, C.; Del Tacca, M. An improved HPLC method for therapeutic drug monitoring of daunorubicin, idarubicin, doxorubicin, epirubicin, and their 13-dihydro metabolites in human plasma. *Ther. Drug. Monit.,* **1999**, *21*(3), 367-75.

[13] Sottani, C.; Tranfo, G.; Bettinelli, M.; Faranda, P.; Spagnoli, M.; Minoia, C. Trace determination of anthracyclines in urine: a new high-performance liquid chromatography/tandem mass spectrometry method for assessing exposure of hospital personnel. *Rapid Commun. Mass. Spectrom.,* **2004**, *18*(20), 2426-36.

[14] Taylor, P. J., Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. *Clin. Biochem.,* **2005**, *38*(4), 328-34.

[15] Poelma, F.G.; Breas, R.; Tukker, J.J.; Crommelin, D.J. Intestinal absorption of drugs. The influence of mixed micelles on on the disappearance kinetics of drugs from the small intestine of the rat. *J. Pharm. Pharmacol.,* **1991**, *43*(5), 317-24.

Received: May 26, 2014 Revised: June 10, 2014 Accepted: August 08, 2014