Study of Pharmacokinetics and Tissue Distribution of BITS-17 in Rat Plasma and Tissue Homogenate Using a Validated LC Method

Addepalli Venkata Ramani*, Vadlamani Lakshmi Indira, SatyaVani, Sandeep Gill, Perumal Yogeeswari, and Dharmarajan Sriram

Abstract

Accurate, simple, sensitive, reproducible and specific liquid chromatographic method has been developed for estimation of BITS-17 in rat plasma and other biological tissues using β-naphthol as an internal standard (IS). The applicability of the validated method was tested to estimate the concentrations of BITS-17 (a novel anti-tubercular agent) in plasma and tissues that were harvested in a rat tissue distribution study. A liquid - liquid extraction technique was used to extract BITS-17 and internal standard from the plasma and various tissue homogenates. The main pharmacokinetic parameters obtained after intravenous administration were t½=14 ± 0.20 h, K0=0.06 ± 0.10 h⁻¹, mean plasma clearance=1.35 ± 0.16 mg/h and mean volume of distribution = 1.99 ± 0.49L. The systemic absorption was tested with two formulations was slow after oral administration and maximum bioavailability was 33.02%. The peak plasma concentration of both the formulations was found to be 1.31 ± 0.06 and 1.0 ± 0.12 mg/mL, respectively, which was much above the levels of minimum inhibitory concentrations of BITS-17. The concentrations of BITS-17 were found in various tissues but the levels in lung and brain signifies a good therapeutic target.

Keywords: BITS 17; β-naphthol; Rat plasma; Method validation; HPLC; Pharmacokinetics; Tissue distribution

Introduction

Tuberculosis (TB) is the leading cause of mortality among all infectious diseases worldwide and is responsible for over two million deaths annually [1]. One of the most effective and widely used anti-tuberculosis (TB) drugs is isoniazid (INH); a pro-drug activated via oxidation that forms an adduct with NAD(+) to inhibit NADH dependent targets of Mycobacterium tuberculosis (MTB), such as the enoyl-acyl carrier protein reductase (InhA). In our earlier work we developed a novel, highly effective, and fast acting anti-TB drugs with low toxicity profiles and performing activity against both drug-sensitive and drug resistant MTB [2]. The INH moiety was incorporated in the 1,3-thiazinan scaffold and was evaluated for invitro MTBactivity [3-7]. Compound N-(2-(4-(benzyloxy) phenyl)-4-oxo-1,3-thiazinan-3-yl) isonicotinamide (BITS 17) inhibited MTB with MIC of 0.12 µM and was three times more potent than INH was selected for further studies [2].

In this present study, a simple, robust and reproducible method has been developed and validated to estimate BITS-17 concentrations in rat plasma. This method meets the requirements and provides high degree of accuracy, sensitivity and specificity by simple extraction using high performance liquid chromatography and detection by ultraviolet -visible spectroscopy [8-11]. A tissue distribution study performed after oral and intravenous (i.v.) administration will provide the concentration of an NCE in various tissues relative to the circulatory levels of the NCE, and this aids in mapping tissue to plasma ratios during drug absorptive, distributive and elimination phases. The outcome of such key experiments will provide information regarding the propensity (or lack of it) to accumulate in tissue (s). By estimating the concentrations in plasma and other tissues, one can establish the tissue/plasma ratio, which will help the researcher to use plasma as a surrogate to assess the levels in other tissues during pharmacology, PK/ PD, toxicokinetic studies, etc. One of the protocols followed (following oral administration) is to collect both plasma and tissue samples, during the absorption phase, around Cmax (the time point at which maximum concentration of NCE is observed in plasma) and another time point during the elimination phase of the NCE. Nine tissues are harvested at the suggested time points and the concentration of NCE in each tissue will be analyzed to compute tissue/plasma ratios. It is tedious task for a bioanalyst to develop a method and validate it for the determination of an NCE in each tissue, especially when radio labeled NCE is not available. Therefore, we looked into the feasibility of applying a validated method (in plasma), without further modification, to measure NCE levels in the tissue samples. In the present paper, we are extending the applicability of a previously validated method in plasma to quantitate BITS-17 in various tissues. In addition, certain key validation experiments, such as accuracy, precision, matrix effect, recovery, stability studies (freeze-thaw, bench-top, in-injector and long-term), that were performed with each tissue matrix are reported.

Materials and Methods

Chemicals and reagents

Test substance BITS 17 (Figure 1) was synthesized at Drug research laboratory, BITS-PILANI Hyderabad Campus. β-naphthol, Phosphated buffer saline and Ammonium acetate were supplied by Sigma Chemicals. Acetonitrile (HPLC grade) and Methanol (HPLC grade) were supplied by Sigma-Aldrich. The reagents Glacial acetic acid and ethyl acetate were of HPLC grade.

Analytical method

Analysis of the anti tubercular drug was performed using a validated method.

*Corresponding author: Addepalli Venkata Ramani, Medicinal Chemistry & Tuberculosis Research Laboratory, Department of Pharmacy, Birla Institute of Technology & Science - Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad-500078, India, E-mail: ramanialaddepalli@yahoo.com

Received July 24, 2012; Accepted September 12, 2012; Published September 16, 2012

Citation: Ramani AV, Indira VL, SatyaVani, Gill S, Yogeeswari P, et al. (2012) Study of Pharmacokinetics and Tissue Distribution of BITS-17 in Rat Plasma and Tissue Homogenate Using a Validated LC Method. J Bioanal Biom 4: 079-084. doi:10.4172/1948-593X.1000067

Copyright: © 2012 Ramani AV, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
The recovery of BITS-17 and IS from plasma and various tissue homogenates was determined by comparing the responses of the analytes extracted from replicate QC samples (n=4) with the response of analyte from the neat samples at equivalent concentrations. Recoveries was determined at low and high quality control concentrations, whereas the recovery of the IS was determined at a single concentration. Recoveries extracted from replicate QC samples (n=4) with the response of plasma in bulk at appropriate concentrations (0.25, 0.4, 0.6, 0.8, 1, 2, 4 and 6 µg/ml) prepared by spiking 100 µL of blank plasma with 10 µL of standard stock solution and 10 µL of IS 200 µg/mL. Standard tissue samples were prepared in similar manner with appropriate blank tissues. The acceptance criterion for each back-calculated standard concentration was ± 15% deviation from the nominal value except at LLOQ, which was set at ± 20%.

Inter/intra assay precision and accuracy was determined by analyzing six replicates at four different QC levels as described above on four different days. The criteria for acceptability of the data included accuracy within ± 15% deviation (S.D) from the nominal values and a precision of within ± 15% relative standard deviation (R.S.D), except for LLOQ, where it should not exceed ± 20% of S.D.

The stability of BITS-17 and IS in the injection solvent was determined periodically by injecting replicate preparations of processed samples up to 24 h (in auto-sampler at 4°C) after the initial injection. The ratio of peak-areas of the BITS-17 to that of IS against the nominal concentration of calibration standards. Plasma samples were (0.25, 0.4, 0.6, 0.8, 1, 2, 4 and 6 µg/mL) prepared by spiking 100 µL of blank plasma with 10 µL of standard stock solution and 10 µL of IS 200 µg/mL. Standard tissue samples were prepared in similar manner with appropriate blank tissues. The acceptance criterion for each back-calculated standard concentration was ± 15% deviation from the nominal value except at LLOQ, which was set at ± 20%.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

Method validation

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.

The specificity and selectivity of the method was evaluated by analyzing rat plasma and tissue from six different batches of rat to demonstrate the potential interferences at LC peak region for the IS and analyte.
for animal use. The two oral formulations (50 mg/kg) constituted of a suspension of drug firstly, in sodium CMC and tween 80. Second formulation constituted of cremophore ELP, sodium CMC and SLS. The animals were fasted overnight (~14 h) and had free access to water throughout the experimental period. Animals were provided with standard diet 3 h post-dosing. The formulation for intravenous administration (10 mg/kg) was prepared with propylene glycol, cremophore ELP, N-methyl-pyrrolidone in normal saline. The rats were anaesthetized in ether and blood samples (~0.50 mL) were collected from retro-orbital plexus into microfuge tube (containing 20 L of saturated EDTA) at 0.5, 1, 1.5, 2, 3, 5, 8, 10 and 24 h post-dosing. Plasma was harvested by centrifuging the blood using micro centrifuge at 6000 rpm for 5 min. Rat plasma (100 µL) samples were spiked with IS and processed as described above.

Pharmacokinetic parameters were calculated by employing a non-compartmental analysis (Gibaldi and Perrier, 1982). The peak plasma concentrations (C_{max}) and the corresponding time (T_{max}) were directly obtained from the raw data. The area under the plasma concentration vs. time curve up to the last quantifiable time point, AUC (0-t) was obtained by a linear and log-linear trapezoidal summation. The AUC (0-t) extrapolated to infinity (i.e. AUC (0-∞)) by adding the quotient of Clast/Kel, where Clast represents the last measurable time concentration and Kel represents the apparent terminal rate constant. Kel was calculated by the linear regression of the log-transformed concentrations of the drug in the terminal phase [12-15]. The half-life (t½) of the terminal elimination phase was obtained using the relationship t½=0.693/Kel by non-compartmental method were evaluated by using Win Nonlin™ Enterprise, Version: 5.3 (Pharsight Corporation, Mountain View, CA).

Application to tissue distribution study

The tissue distribution study was performed in overnight (~12 h) fasted healthy male Wistar rats (n=6, weight range 200–240 g) following the approval from the ethics committee for animal use [16,17]. During the fasting time animals had free access to water. BITS-17 was administered to rats by oral administration at a dose of 50 mg/kg (in the form of a suspension, prepared using cremophore ELP, sodium CMC and SLS). At each time point, viz. 1 h (during the initial absorption phase), 2 h (around C_{max}) and 5 and 8 h (elimination phase), two rats were sacrificed and various tissues (heart, lung, liver, stomach, small intestine, large intestine and brain along with blood and plasma) were collected separately from each animal. Tissue homogenate samples (50 mL) were spiked with IS solution and processed as described in the ‘extraction procedure for biosamples’ section. The standard curve was generated using individual tissue homogenates. Along with respective samples, QC samples at low, medium and high concentrations spiked in respective blank tissue homogenates were assayed in duplicate and were randomly distributed among standard calibrators and unknown samples in the analytical run; not more than 33% of the QC samples were greater than ± 15% of the nominal concentration.

Figure 2: Typical chromatograms of an extract of blank plasma (A); Blank plasma spiked with BITS 17 and IS (B); Blank tissue (C); Blank tissue spiked with BITS 17 and IS (D); Rat liver tissue sample obtained after 2 h after 50 mg/kg oral dose of BITS 17(E); Rat lung tissue sample obtained after 2 h after 50 mg/kg oral dose of BITS 17(F)
Results
Optimization of chromatographic detection

Chromatographic conditions were optimized to achieve the good separation, high reproducibility of BITS-17 and IS from the endogenous interferences of the biological matrix. Wavelength of 315 nm was selected at the λ_{max} of BITS-17 as an optimum response of IS was also observed. Different buffer like di-potassium hydrogen phosphate, potassium di-hydrogen phosphate, ammonium acetate of different pH with different composition of acetonitrile was tried. Different column C18, C8 and cyano were also investigated. The optimum resolution, proper run time of the analyte, with good peak shape was achieved by using 10 mM of ammonium acetate (pH 4.5) and acetonitrile at a ratio of 68:32 with a Brownlee Analytical C18 column (4.6×150 mm, 3 µm, using 10 mM of ammonium acetate (pH 4.5) and acetonitrile at a ratio proper run time of the analyte, with good peak shape was achieved by using 10 mM of ammonium acetate (pH 4.5) and acetonitrile at a ratio of 68:32 with a Brownlee Analytical C18 column (4.6×150 mm, 3 µm, Perkin Elmer Corporation, U.S.A.). Robustness studies showed that a small variation in pH of the buffer in the mobile phase, C18 columns from different vendor like Thermo, Merck and Phenomenex did not affect the analysis much.

Validation parameters

Specificity and selectivity: A typical chromatogram for the biomatrix (free of analyte and IS) and spiked with analyte at LLOQ and IS are shown in the figure 2. No interfering peaks from endogenous compounds are observed at the retention times of analyte and IS. The retention time of BITS-17 and IS were 10.5 and 8.5 min, respectively. The total chromatographic run time was only 13 mins.

Recovery: The extraction recoveries of the BITS-17 from biomatrix were calculated by comparing the response of biological samples spiked with the analyte and extracted with a suitable solvent to that of standard solutions. The results of the comparison of neat standards vs biological samples extracted standards were estimated for BITS-17 at low, mid, high QC concentrations and peak area ratios (analyte/internal standard) were used for the calculations. The mean recovery of >91% was found in plasma and >75% in other tissue homogenates. The recovery of IS at 20.0 µg/mL was 96.5%.

Calibration curve: The calibration curve was prepared by selecting the best fit of peak-area ratios (peak area analyte/peak area IS) versus concentration, and fitted to the equation y=mx+c. The average regression (n=4) for BITS-17 was found to be >0.99. Calibration curve were obtained without weighing factor. The linearity range, regression equation, percent accuracy observed for the mean and back calculated concentrations for BITS-17 in each of the matrix is given in table 1.

Precision and accuracy: The method provides accuracy and precision for the determination of BITS-17. The accuracy, intra- and inter-assay precision was determined by analyzing six replicates of QC samples at four concentrations. Table 2 gives a summary of the recoveries for rat biological samples spiked with different concentrations of BITS-17. The intra-day precision ranged from 0.86 to 6.22 and inter day precision ranges from 0.71 to 4.83 for all the biological samples. The results demonstrated the reproducibility of the devised method.

Stability: The stability was predicted at LQC and HQC concentrations in a battery of stability tests viz., in-injector, bench-top, repeated three freeze/thaw cycles and at -80 ± 10°C for 20 days (Tables 3 and 4). These results indicate that the analyte was stable in-injector (24 h), bench top (room temperature for 10 h), after freeze thaw condition (3 cycles). The results were also proved that the analyte are stable for 20 days in biological samples.

Pharmacokinetic analysis: The concentration of BITS-17 in

### Table 1: Linear regression of peak areas and concentration for BITS 17 in biological samples.

| Sample       | Linearity range (µg/ml) | Slope | Intercept | r² | Accuracy range  |
|--------------|-------------------------|-------|-----------|----|-----------------|
| Plasma       | 0.25 - 6                | 0.273 | 0.0101    | 0.9995 | 99.69 - 101.90  |
| Blood        | 0.25 - 4                | 0.458 | 0.0226    | 0.9993 | 98.18 - 101.19  |
| Lung         | 0.4 - 4                 | 0.415 | 0.0210    | 0.9986 | 98.37 - 100.01  |
| Liver        | 0.5 - 5                 | 0.274 | 0.0510    | 0.9990 | 98.48 - 101.77  |
| Heart        | 0.4 - 6                 | 0.317 | 0.0060    | 0.9888 | 98.34 - 101.89  |
| Small intestine | 0.6 - 6                | 0.273 | 0.0541    | 0.9991 | 99.22 - 101.03  |
| Large intestine | 0.25 - 2               | 0.430 | 0.0218    | 0.9898 | 96.75 - 99.95   |
| Brain        | 0.25 - 2                | 0.408 | 0.0700    | 0.9991 | 99.33 - 99.82   |
| Stomach      | 0.25 - 2                | 0.521 | 0.0220    | 0.9994 | 97.92 - 101.19  |
a plasma sample was determined by the same method. Plasma concentration time data of the analyte were analyzed by non-compartmental method. The basic pharmacokinetic parameters assessed were half life (t½), elimination rate constant (Kel), mean plasma clearance (CL) and mean volume of distribution (Vd) as reported in Table 5. After a single i.v. bolus dose of 10 mg/kg body weight, BITS-17 had a t½ of 1.14 h with moderate clearance (22.48 mL/min/kg) and volume of distribution (1.99 L). Oral administration of a single dose of 50 mg/kg body weight the peak concentration was achieved in 1.31 ± 0.06 and 1.0 ± 0.12 µg/mL with formulation one and two, respectively. The plasma levels then declined rapidly with formulation one and the levels sustained with the second one (Figure 3). The oral bioavailability also increased from 16.7% to 33%.

The low systemic bioavailability of the compound can be attributed to its very poor aqueous solubility in the gastrointestinal fluids, therefore low and delayed absorption. The levels of BITS-17 are much above the MIC values.

Table 3: Stability data of BITS 17 quality controls in Liver, lungs, heart and brain.

| Biological Sample | Liver | Lung | Heart | Brain |
|------------------|-------|------|-------|-------|
|                  | Mean ± SD* | Accuracyb | Mean ± SD* | Accuracyb | Mean ± SD* | Accuracyb | Mean ± SD* | Accuracyb |
| Low QC           | (µg/ml) | (%)  | (µg/ml) | (%)  | (µg/ml) | (%)  | (µg/ml) | (%)  |
| Third freeze thaw | 0.699 ± 0.005 | 99.89 | 0.501 ± 0.008 | 100.11 | 0.502 ± 0.010 | 100.47 | 0.302 ± 0.007 | 100.75 |
| 10 h (bench top) | 0.704 ± 0.004 | 100.55 | 0.504 ± 0.008 | 100.78 | 0.506 ± 0.003 | 101.15 | 0.303 ± 0.007 | 100.93 |
| 24 h (in injector) | 0.702 ± 0.004 | 100.25 | 0.519 ± 0.008 | 103.87 | 0.498 ± 0.005 | 99.59 | 0.292 ± 0.009 | 97.43 |
| 20 days at -80°C | 0.695 ± 0.005 | 99.26 | 0.500 ± 0.006 | 100.05 | 5.05 ± 0.065 | 101.00 | 0.295 ± 0.005 | 98.19 |
| High QC          | (µg/ml) | (%)  | (µg/ml) | (%)  | (µg/ml) | (%)  | (µg/ml) | (%)  |
| Third freeze thaw | 5.009 ± 0.047 | 100.17 | 3.089 ± 0.002 | 102.96 | 5.089 ± 0.108 | 101.77 | 1.559 ± 0.019 | 103.92 |
| 10 h (bench top) | 5.084 ± 0.058 | 101.68 | 3.039 ± 0.042 | 101.31 | 5.085 ± 0.072 | 101.70 | 1.523 ± 0.029 | 101.51 |
| 24 h (in injector) | 5.094 ± 0.056 | 101.87 | 3.158 ± 0.160 | 105.27 | 4.922 ± 0.139 | 98.43 | 1.491 ± 0.005 | 99.37 |
| 20 days at -80°C | 5.066 ± 0.062 | 101.31 | 3.083 ± 0.021 | 102.76 | 5.050 ± 0.065 | 101.00 | 1.527 ± 0.042 | 101.80 |

QC, quality Control

*Back –Calculated plasma concentrations

b(Mean assayed concentration/ mean assayed concentration at 0 h i.e., fresh samples) x 100

Table 4: Stability data of BITS 17 quality controls in stomach, blood, large intestine, small intestine.

| Biological Sample | Stomach | Blood | Large intestine | Small intestine |
|------------------|---------|-------|----------------|----------------|
|                  | Mean ± SD* | Accuracyb | Mean ± SD* | Accuracyb | Mean ± SD* | Accuracyb | Mean ± SD* | Accuracyb |
| Low QC           | (µg/ml) | (%)  | (µg/ml) | (%)  | (µg/ml) | (%)  | (µg/ml) | (%)  |
| Third freeze thaw | 0.298 ± 0.006 | 99.21 | 0.304 ± 0.002 | 101.37 | 0.308 ± 0.010 | 102.79 | 0.699 ± 0.005 | 99.89 |
| 10 h (bench top) | 0.302 ± 0.004 | 100.60 | 0.297 ± 0.006 | 98.84 | 0.308 ± 0.008 | 102.63 | 0.704 ± 0.004 | 100.55 |
| 24 h (in injector) | 0.300 ± 0.005 | 99.94 | 0.300 ± 0.005 | 99.94 | 0.290 ± 0.007 | 98.57 | 0.678 ± 0.004 | 99.87 |
| 20 days at -80°C | 0.305 ± 0.003 | 101.63 | 0.295 ± 0.005 | 98.40 | 0.310 ± 0.005 | 103.40 | 0.695 ± 0.005 | 99.26 |
| High QC          | (µg/ml) | (%)  | (µg/ml) | (%)  | (µg/ml) | (%)  | (µg/ml) | (%)  |
| Third freeze thaw | 3.214 ± 0.056 | 107.11 | 3.095 ± 0.052 | 103.15 | 3.070 ± 0.052 | 102.32 | 5.009 ± 0.047 | 100.17 |
| 10 h (bench top) | 3.095 ± 0.113 | 103.15 | 3.046 ± 0.050 | 101.54 | 3.071 ± 0.063 | 102.36 | 5.084 ± 0.058 | 101.68 |
| 24 h (in injector) | 2.938 ± 0.047 | 97.93 | 2.938 ± 0.047 | 97.93 | 3.089 ± 0.051 | 102.96 | 5.052 ± 0.056 | 101.03 |
| 20 days at -80°C | 2.969 ± 0.046 | 98.96 | 3.028 ± 0.054 | 100.92 | 3.113 ± 0.049 | 103.77 | 5.066 ± 0.062 | 101.31 |

QC, quality Control

*Back –Calculated plasma concentrations

b(Mean assayed concentration/ mean assayed concentration at 0 h i.e., fresh samples) x 100

Table 5: Pharmacokinetic parameters of BITS 17 following oral and intravenous administration.

| Route of administration | Oral | Formulation 2 | Intravenous |
|-------------------------|------|---------------|-------------|
| Formulation 1          | 50   | 50            | 10          |
| AUC(0-t)/(µg.hr/mL)     | 5.87 ± 0.24 | 11.61 ± 0.28 | 7.50 ± 0.94 |
| Cmax/(µg/mL)            | 1.31 ± 0.06 | 1.0 ± 0.12    | 5.29 ± 1.41 |
| t1/2 (hr)               | 3.00 | -             | -           |
| Ke (1/hr)               | -    | -             | 0.62 ± 0.10 |
| t1/2 (hr)               | -    | -             | 1.14 ± 0.20 |
| Vl (L)                  | -    | -             | 1.99 ± 0.49 |
| Cl(mL/kg/min)           | -    | -             | 22.48 ± 0.16 |
| bioavailability (%)     | 16.7 |               | 33.02       |

t is 5 and 24 h for iv and oral, respectively
Tissue distribution analysis: There is a wide tissue distribution of BITS-17 in rats after oral administration, shown in figure 4. Drug concentrations in highly perfused tissues were highest after 2 hours post dosing except for stomach. Table 6 comprises of the tissue to plasma ratios, assessing the tissue–plasma ratio, the plasma could be used as a surrogate to determine the concentrations of BITS-17 in other tissues in a pharmacodynamic, efficacy and toxicokinetic study. Comparing the distribution profiles, the maximum concentration was in intestine as the drug has low absorption due to its poor solubility. Presence of drug concentrations significantly above MIC levels in brain till 5 hours and in lungs at 2 hours post administration signifies that brain and lungs could be good therapeutic targets for the drug.

References
1. Stephen D L, Alimuddin I Z (2011) Tuberculosis. The Lancet 378: 57-72.
2. Ramani AV, Monika A, Indira VL, Karyavardhi G, Venkatesh J, et al. (2012) Synthesis of highly potent novel anti-tubercular isoniazid analogues with preliminary pharmacokinetic evaluation. Bioorg Med Chem Lett 22: 2764-2767.
3. World Health Organization (2011) 2011/2012 Tuberculosis Global Facts.
4. Manjashetty TH, Yogeesswari P, Srim D (2011) Microwave assisted one-pot synthesis of highly potent novel isoniazid analogues. Bioorg Med Chem Lett 21: 2125-2128.
5. Pedro E, Silva AD, Ramosa DF, Bonacoorso HG, Iglesia AI, et al. (2008) Synthesis and in vitro antmycobacterial activity of 3-substituted 5-hydroxy-5-trifluor [chloro] methyl-4, 5-dihydro-1H-1-(isonicotinoyl) pyrazoles. Int J Antimicrob Agents 32: 139-144.
6. Srim D, Yogeesswari P, Madhu P (2006) Synthesis and in-vitro antitubercular activity of some 1-{(4-sub) phenyl} - 3-(4-{1-[((pyridine-4-carbonyl) hydrazono]ethyl phenyl) thiourea. Bioorg Med Chem Lett 16: 876-878.
7. Heam MJ, Cynamon MH (2004) Design and synthesis of antituberculars: preparation and evaluation against Mycobacterium tuberculosis of an isoniazid Schiff base. J Antimicrob Chemother 53: 185-191.
8. Food and Drug Administration of the United States, Guidance for industry Bioanalytical Method Validation (2011).
9. Ph Hubert, P Chiap, J Crommen, B Boulanger, E Chapuzel et al. (1999) The SFSTP guide on the validation of chromatographic methods for drug bioanalysis: from the Washington Conference to the laboratory. Analy Chim Acta 391: 135-148.
10. Bansal S, DeStefano (2007) Key Elements of Bioanalytical Method Validation for Small Molecules. The AAPS Journal 9: E109-E114.
11. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, et al. (2007) Workshop/Conference Report- Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays. The AAPS Journal 9: E30-E42.
12. Toutain PL, Bousquet-Melou A (2004) Plasma clearance. J Vet Pharmacol Ther 27: 415-425.
13. Toutain PL, Bousquet-Melou A (2004) Plasma terminal half-life. J Vet Pharmacol Ther 27: 427-439.
14. Toutain PL, Bousquet-Melou A (2004) Volumes of distribution. J Vet Pharmacol Ther 27: 441-453.
15. Toutain PL, Bousquet-Melou A (2004) Bioavailability and its assessment. J Vet Pharmacol Ther 27: 441-453.
16. Dhanikula AB, Singh DR, Panchagnula R (2005) In vivo pharmacokinetic and tissue distribution studies in mice of alternative formulations for local and systemic delivery of Paclitaxel: gel, film, prodrug, liposomes and micelles. Curr Drug Deliv 2: 35-44.
17. Lanao JM, Fraile MA (2005) Drug tissue distribution: study methods and therapeutic implications. Curr Pharm Des 11: 3829-3845.