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

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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, Kel=0.62 ± 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 C_{max} (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 validateit 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

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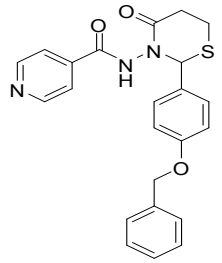

Figure 1: Structural representation of BITS 17.

HPLC technique as described below. Analyses were performed on a reverse phase HPLC (Perkin Elmer LC system equipped with Flexar quaternary pump along with peltier controlled Flexar auto-sampler and Flexar PDA) on Brownlee Analytical C_{18} column (4.6 × 150 mm, 3 µm, Perkin Elmer Corporation, U.S.A) column. The mobile phase used is a mixture of 0.01 M ammonium acetate (pH=4.5) and acetonitrile mixture (62:38,v/v). The detection wavelength was set at 315 nm. Chromatography separation was performed at room temperature and flow rate was maintained at 1 mL/min.

Preparation of stock solutions and calibration standards

Primary stock solutions of BITS- 17 and IS for preparation of standard and quality control (QC) samples were prepared by weighing separately. The primary stock solutions(1.0 mg/mL) of the analyte and IS were prepared in methanol and stored at -20°C, which were found to be stable for one month (data not shown).

Preparation of calibration and quality control samples

Appropriate dilutions were made in methanol for BITS-17 to produce working stock solutions of 2.5, 4.0, 6.0, 8.0, 10, 20, 40, 60 µg/ml on the day of analysis and these stocks were used to obtain a calibration curve (CC). Another set of working stock solutions was made in methanol (from primary stock) at 50, 9, 3 and 2.5 µg/mL, of which 50 and 9 µg/mL were used for QC high and QC medium respectively. Whereas working stock solutions at 3 and 2.5 µg/mL were used for QC low and lower limit of quantitation (LLOQ) of BITS 17, respectively. A working solution of IS (100 µg/mL) was also prepared in methanol. Calibration samples were prepared by spiking 100 µL of control rat plasma with the appropriate amount of analyte (10 µL) and IS (20 µL) on the day of analysis. Samples for the determination of recovery, precision and accuracy were prepared by spiking control rat plasma in bulk at appropriate concentrations (0.25, 0.3, 0.9 and 5 µg/mL as LLOQ, LQC, MQC and HQC for BITS 17). Aliquots of 100 µL volumes were taken into different tubes and depending on the nature of experiment, samples were stored at -80 ± 10°C until analysis.

Extraction procedure for biosamples

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 of 20.0 µg/mL.

Following removal of each tissue from the second group of dissected rat, the tissues were put into the physiological saline to exclude the remaining blood stain. Tissues were dried using fibreless tissue paper, weighed and cut into small pieces using a surgical blade. Tissue homogenate was made using phosphate buffer saline (pH 7.4) under ice with a homogenizer. All tissues were diluted five times with phosphate buffer saline.

Liquid - liquid extraction method was used for the extraction of BITS-17 in biosamples. To each of plasma and tissue samples, IS solution (20 µL) equivalent to 20 µg was added mixed for 15 sec on a cyclomixer; followed by extraction with 1.7 ml of suitable extraction solvent viz. plasma with ethyl acetate, stomach and blood with mixture of equivolume mixture of ethyl acetate and dichloromethane. The mixture was vortexed for 2 min and centrifugated for 4 min at 3200 rpm. The organic layer (1.4 mL) was separated and evaporated to dryness at 50°C using a gentle stream of. The residue was reconstituted in 150 µL of the reconstitution solvent (0.01M ammonium acetate: acetonitrile: 62:38, v/v) and 10 µL was injected onto HPLC system at 315 nm (λ_{max} of the analyte). From rest of the tissues the analyte and IS was recovered by simple protein precipitation process.

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 at for the IS and analyte.

Calibration curves were acquired by plotting the peak area ratio 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%.

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 and IS obtained at initial cycle were used as the reference to determine the relative stability of the BITS-17 at subsequent points. Stability of BITS-17 in the biomatrix after 10 h (bench top) exposure at ambient temperature (25 ± 2°C) was determined at two concentrations in six replicates. Freezer stability of the BITS 17 in biomatrix was assessed by analyzing the low and high QC samples stored at -80 ± 10°C for at least 30 days. The stability of BITS 17 in biomatrix following repeated three freeze-thaw cycles (stored at -80 ± 10°C between cycles) was assessed using QC samples spiked with analyte. Samples were processed as described above. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (i.e., ± 15% S.D) and precision (i.e., 15% R.S.D).

Application to pharmacokinetic study

Male Wistar rats, ~3 months of age and weighing between 200-250 g were used in this study following the approval from ethics committee

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-pyrollidone in normal saline. The rats were anaesthetized in ether and blood samples (~0.5.00 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.

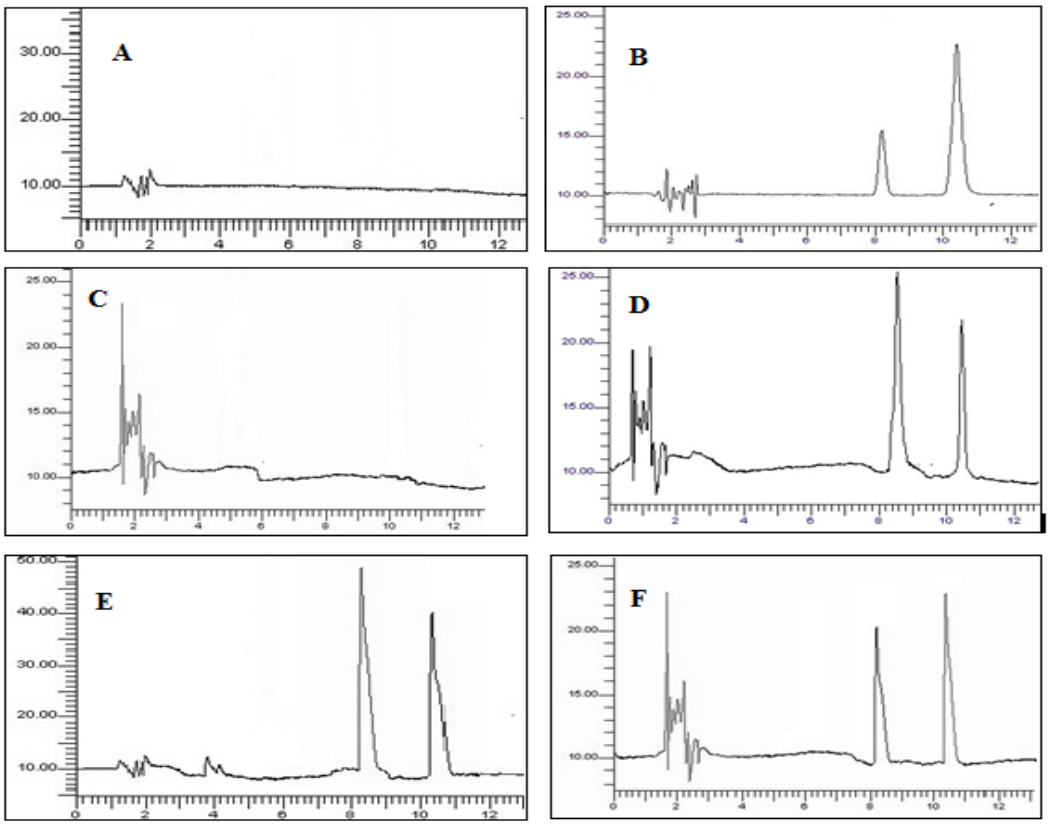

Figure 2: Typical chromatograms of an extract of blank plasma (A); Blank plasma spiked with BITS 17 and IS (B); Blank tissue (C); Blank tissue spiked with BITS 17 and IS (D); Rat liver tissue sample obtained after 2 h after 50 mg/kg oral dose of BITS 17(E); Rat lung tissue sample obtained after 2 h after 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 were 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 C_{18} 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 of equivalent concentration. 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 determining 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 intraday precision ranged from 0.86 to 6.22 and inter day precision ranges from 0.71 to 4.83for all the biological samples. The results demonstrated the reproducibility of the devised method.

Sample	Linearity range µg/ml	Slope	Intercept	r^2	Accuracy range
Plasma	0.25 - 6	0.273	0.0101	0.9995	99.59 - 101.90
Blood	0.25 - 4	0.458	0.0226	0.9993	98.18 - 101.19
Lung	0.4 - 4	0.415	0.0210	0.9999	99.37 - 100.01
Liver	0.6 - 5	0.274	0.0510	0.9990	99.48 - 101.77
Heart	0.4 - 6	0.317	0.0060	0.9988	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	0.9989	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

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

Biological sample	Nominal (µg/ml)		Intraday			Interday		
			Obtained (µg/ml)	Accuracy (%)	% CV	Obtained (µg/ml)	Accuracy (%)	% CV
Plasma	LLOQ	0.25	0.259	103.71	1.86	0.256	102.51	3.33
	LQC	0.30	0.318	105.89	2.97	0.312	104.07	3.61
	MQC	0.90	0.967	107.40	1.95	0.920	102.23	3.80
	HQC	5.00	4.792	95.84	1.48	4.987	100.00	3.05
Blood	LLOQ	0.25	0.248	99.15	0.90	0.251	100.21	1.22
	LQC	0.30	0.296	98.77	1.50	0.299	99.53	2.57
	MQC	0.70	0.698	99.69	0.91	0.700	100.07	0.94
	HQC	3.00	3.045	101.50	1.58	3.023	100.92	1.38
Lung	LLOQ	0.40	0.407	101.70	1.60	0.400	100.12	2.21
	LQC	0.50	0.500	100.06	1.25	0.502	100.36	1.15
	MQC	0.90	0.901	100.09	1.83	0.902	100.20	1.00
	HQC	3.00	3.022	100.73	1.68	3.044	101.40	1.39
Liver	LLOQ	0.60	0.618	103.08	1.23	0.604	100.59	1.92
	LQC	0.70	0.691	98.74	1.39	0.699	99.83	1.14
	MQC	1.50	1.502	100.11	1.42	1.501	100.03	0.71
	HQC	5.00	4.976	99.53	1.21	5.012	100.43	1.15
Heart	LLOQ	0.40	0.399	99.84	1.26	0.402	100.42	1.62
	LQC	0.50	0.492	98.46	1.44	0.496	99.23	2.14
	MQC	1.50	1.499	99.94	1.14	1.500	100.00	0.90
	HQC	5.00	4.922	98.44	2.04	4.949	99.21	1.57
Small intestine	LLOQ	0.60	0.608	101.39	1.25	0.592	98.67	2.95
	LQC	0.70	0.681	97.31	1.41	0.688	98.34	1.83
	MQC	1.50	1.493	99.55	1.43	1.493	99.51	1.16
	HQC	5.00	4.975	99.50	1.21	5.029	100.69	1.13
Large intestine	LLOQ	0.25	0.261	103.71	0.86	0.251	100.05	2.78
	LQC	0.30	0.309	103.01	1.94	0.301	100.26	2.98
	MQC	0.70	0.752	107.48	0.90	0.717	102.40	4.46
	HQC	3.00	3.071	103.66	1.25	3.038	101.61	1.78
Brain	LLOQ	0.25	0.238	95.29	1.30	0.246	98.23	2.88
	LQC	0.30	0.303	101.05	6.22	0.298	99.36	4.83
	MQC	0.70	0.697	99.60	1.75	0.699	99.87	2.16
	HQC	1.50	1.497	99.81	3.69	1.498	99.99	2.09
Stomach	LLOQ	0.25	0.248	99.15	0.90	0.250	99.97	1.61
	LQC	0.30	0.296	98.77	1.50	0.299	99.66	2.25
	MQC	0.70	0.698	99.69	0.91	0.701	100.11	0.93
	HQC	3.00	3.045	101.50	1.58	3.010	100.16	1.97

Accuracy (%), [(Obtained concentration/nominal concentration) x 100]; CV (%), coefficient of variation [(S.D./mean) x 100].

Table 2: Intraday and inter day precision and accuracy of BITS 17 from Biological samples.

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

Biological Sample	Liver		Lung		Heart		Brain	
	Mean ± SD[a]	Accuracy[b]	Mean ± SD[a]	Accuracy[b]	Mean ± SD[a]	Accuracy[b]	Mean ± SD[a]	Accuracy[b]
	(µg/ml)	(%)	(µg/ml)	(%)	(µg/ml)	(%)	(µg/ml)	(%)
Low QC								
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
24h (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 -80ºC	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								
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
10h (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
24h (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 -80ºC	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
[a]Back –Calculated plasma concentrations
[b](Mean assayed concentration/ mean assayed concentration at 0 h i.e., fresh samples) x 100

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

Biological Sample	Stomach		Blood		Large intestine		Small intestine	
	Mean ± SD[a]	Accuracy[b]	Mean ± SD[a]	Accuracy[b]	Mean ± SD[a]	Accuracy[b]	Mean ± SD[a]	Accuracy[b]
	(µg/ml)	(%)	(µg/ml)	(%)	(µg/ml)	(%)	(µg/ml)	(%)
Low QC								
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	99.14	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	96.57	0.678 ± 0.004	96.87
20 days at -80ºC	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								
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 ± .056	101.03
20 days at -80ºC	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
[a]Back –Calculated plasma concentrations
[b](Mean assayed concentration/ mean assayed concentration at 0 h i.e., fresh samples) x 100Parameters

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

	Route of administration		
	Oral		
	Formulation 1	Formulation 2	Intravenous
Dose (mg/kg)	50	50	10
$AUC^{(0-t)}$(µg.hr/mL)	5.87 ± 0.24	11.61 ± 0.28	7.50 ± 0.94
C_{max}/C_0(µg/mL)	1.31 ± 0.06	1.0 ± 0.12	5.29 ± 1.41
T_{max}(hr)	3.00	2.00	-
Kel (1/hr)	-	-	0.62 ± 0.10
$t_{1/2}$(hr)	-	-	1.14 ± 0.20
V_d(lit)	-	-	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

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

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.

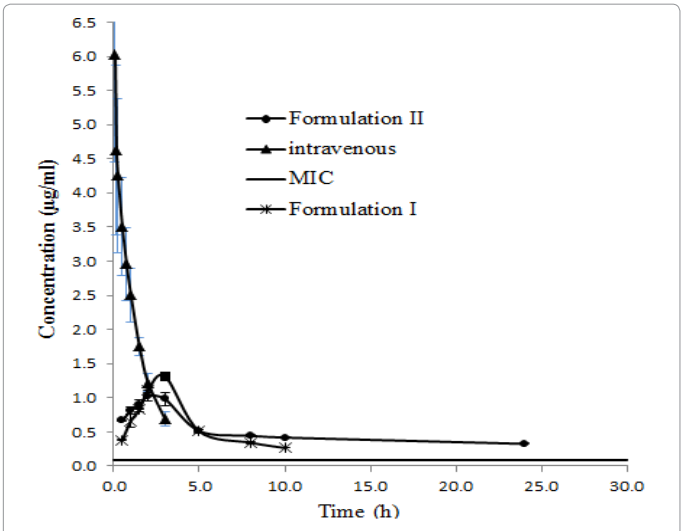

Figure 3: Mean plasma concentration – time curve of BITS 17 in ratsfollowing intravenous and oral administration (well above the MIC).

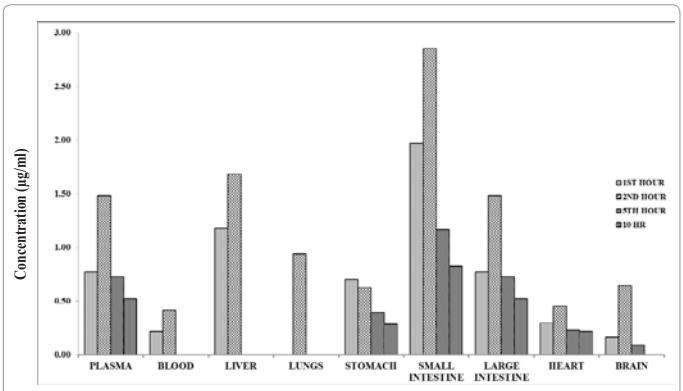

Figure 4: Tissuedistributions of BITS 17 in rats following oral administration (50 mg/kg).

Sample	1h	Ratio	2h	Ratio	5h	ratio	10h	ratio
Plasma	0.80	NA	1.50	NA	0.70	NA	0.50	NA
Blood	0.20	NA	0.40	NA	0.00	NA	0.00	NA
Lung	0.00	0.00	0.90	0.60	0.00	0.00	0.00	0.00
Liver	1.20	1.50	1.70	1.10	0.00	0.00	0.00	0.00
Heart	0.30	0.40	0.50	0.30	0.20	0.30	0.20	0.40
Small intestine	2.00	2.60	2.90	1.90	1.20	1.60	0.80	1.60
Large intestine	0.80	1.00	1.50	1.00	0.70	1.00	0.50	1.00
Brain	0.20	0.20	0.60	0.40	0.10	0.10	0.00	0.00
Stomach	0.70	0.90	0.60	0.40	0.40	0.50	0.30	0.50

Table 6: Biological sample concentrations (µg/ml) of BITS 17 at various time points along with ratios (tissue to plasma).

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.

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