Development and Validation of UHPLC/ESI-Q-TOF-MS Method for Terbutaline Estimations in Experimental Rodents: Stability Effects and Plasma Pharmacokinetics

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Abstract: An ultra high performance liquid chromatography-electrospray ionization-tandem mass spectrometric method (UHPLC/ESI-Q-TOF-MS) for the analysis of terbutaline (TB) in Wistar rat plasma has been developed and validated. The chromatographic separation was achieved on a Waters ACQUITY UPLC™ BEH C18 (100.0 mm×2.1 mm; 1.7 μm) column using isocratic mobile phase, consisting of 2 mM ammonium acetate and acetonitrile (90: 10; v/v), at a flow rate of 0.25 mL min⁻¹. The transitions occurred at m/z 226.19→152.12 for TB, and m/z 260.34→183.11 for the internal standard. The recovery of the analytes from Wistar rat plasma was optimized using liquid-liquid extraction technique (LLE) in ethyl acetate. The total run time was 3.0 min and the elution of TB occurred at 1.85±0.05 min. The linear dynamic range was established over the concentration range 1–1000 ng mL⁻¹ (r²; 0.9938±0.0005) for TB. The intra-assay and inter-assay accuracy in terms of % CV was in between 1.8–3.5. The lower limit of quantitation (LLOQ) for TB was 1.0 ng mL⁻¹. Analytes were stable under various conditions (in autosampler, during freeze–thaw, at room temperature, and under deep-freeze conditions). The developed method was successfully applied for pharmacokinetic profiling in rodents.

Keywords: Asthma, Bronchodilator, Ex-vivo Stability, In-vitro assay, Matrix effect, Pharmacokinetics, Terbutaline; UHPLC/ESI-Q-TOF-MS, Validation.

1. INTRODUCTION

Terbutaline (TB), an amphiphilic β-([(tert-buty lamino) methyl]-3,5-dihydroxy- benzyl alcohol is a synthetic β₂-adrenoceptor (β₂AR) stimulant that is widely used to treat bronchial asthma (BA), chronic obstructive pulmonary diseases (COPD) and related disorders [1, 2]. There has been a plethora of analytical methods reported for the quantitative estimation of TB in bulk, pharmaceutical formulations and biomatrices mainly based on high performance-liquid chromatography (HPLC) [3-11], liquid chromatography-tandem mass spectrometry (LC/MS/MS) [12, 13] and gas chromatography-mass spectrometry (GC-MS) [14, 15]. These published methods either involve cumbersome sample preparation steps or are insufficiently sensitive or precise to perform TB analysis. A validated HPLC method for the determination of TB and its possible degradation products has been reported in literature [16], utilizing Hypersil 100 C₁₈, 150×4.6 mm (5 μm) column, found inadequate sensitivity of the method due to high limit of quantitation (LOQ; 0.11 μg mL⁻¹) and limit of detection (LOD; 0.37 μg mL⁻¹) values. In our previous study, a stability-indicating high performance-thin layer chromatography (HPTLC) has been developed and validated for the direct estimation of TB and its potential degradants in bulk and submicronized dry powder inhalers

[17]. The low value of LOQ (28.35 ng spot⁻¹) and LOD (9.41 ng spot⁻¹) indicated adequate assay sensitivity of our reported method. Recently, a chiral liquid chromatography–tandem mass spectrometric method (LC–MS/MS) for the simultaneous analysis of bambuterol and its active metabolite terbutaline in Wistar rat plasma has been reported [18]. In this method, the chromatographic separation was achieved on a teicoplanin-containing Chirobiotic T chiral (250 mm×4.6 mm; 5 μm) column at a flow-rate of 0.4 mL min⁻¹, however, and has the disadvantage of being time-consuming because of longer retention time (Rₜ >15 min) and lengthy processing steps. All these studies have further emphasized the need to perform rapid and sensitive quality-control analysis of TB with shorter retention time and improved effects. An approach to chiral separation and analysis of TB enantiomers by chiral complexation and electrospray high-field asymmetric waveform ion mobility spectrometry coupled to mass spectrometry (ESI-FAIMS-MS) has been also reported in literature [19]. The limit of detection is 0.10% (−)-terbutaline in a sample of (+)-terbutaline, which is the limiting factor of the method. In a recent study, TB sulfonconjugates were examined in urinary samples by LC/ESI-MS/MS on Orbitrap mass spectrometer [20]. However, the method was not sensitive enough to detect TB in the urine after single dose administration. Therefore a hyphenated chromatographic technique with advanced feature is required to compensate the aforesaid loopholes. However, UHPLC is a novel chromatographic technique utilizing high linear velocities, which is based on concept using columns with smaller
packing (1.7-1.8 μm porous particles) and operated under high pressure (up to 15,000 psi). This is an extremely powerful approach which dramatically improves peak resolution, sensitivity and speed of analysis [18, 21]. In addition, time-of-flight mass spectrometry (Q-TOF-MS) allows the generation of mass information with higher accuracy and precision. UHPLC is specially designed to resist higher back-pressures, with the advantages of fast injection cycles, low injection volumes, negligible carryover and temperature control (4–40 °C), which collectively contributes to speedy and sensitive analysis [22]. Furthermore, acquity UHPLC columns contain hybrid X-Terra sorbent, which utilizes bridged ethylsiloxane/silica hybrid (BEH) structure, ensures the column stability under the high pressure and wide pH range (1–12) [22]. In addition to UHPLC, the use of orthogonal quadrupole time-of-flight mass spectrometry (Q-TOF-MS), with low and high collision-energy full scans acquisition simultaneously performed, offers more possibilities in screening and identification, resulting in valuable fragmentation information [17, 22, 23]. Consequently UHPLC/ESI-Q-TOF-MS has been proved to be a powerful hyphenated technique for bioanalytical investigation [23, 24].

In this paper, for the first time, a validated assay of TB by UHPLC/ESI-Q-TOF-MS was developed and successfully implicated for bioanalytical investigations. This method exhibited excellent performance with respect to high sensitivity and lesser retention time.

2. MATERIAL AND METHODS

2.1. Chemicals

Terbutaline (Assigned purity >99.5%; M.P. 248 ºC) was purchased from Netco Ltd., India. Propranolol hydrochloride as internal standard was a gift from Jubilant Clinsys Clinical Research Limited, Noida, India. HPLC-MS grade acetonitrile (Assigned purity: 99.9%) was purchased from Sigma-Aldrich, Germany. Solvents used for UHPLC were of gradient grade purity and bought from Merck (Darmstadt, Germany). MS grade ammonium acetate and ammonium formate were obtained from Fluka analytical, Sigma-Aldrich, Netherland. Formic acid (Assigned purity >98%) was commercially obtained from Fluka analytical, Germany. Deionized water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Blank plasma from Wistar rats was provided by Yash laboratories, Mumbai, India.

2.2. UHPLC Conditions

UHPLC was performed with a Waters ACQUITY UPLC™ system (Waters Corp., MA, USA) equipped with a binary solvent delivery system, an auto-sampler, column manager and a tunable MS detector (Synapt; Waters, Manchester, UK). Chromatographic separation was performed on a Waters ACQUITY UPLC™ BEH C18 (100.0 mm ×2.1 mm; 1.7 μm) column. The mobile phase for UHPLC analysis consisted of acetonitrile–2 mM ammonium acetate (1: 9; v/v), which was degassed. For isotopic elution, the flow rate of the mobile phase was kept at 0.25 mL min⁻¹ and 10 μL of sample solution was injected in each run. The total chromatographic run time was 3.0 min.

2.3. Q-TOF-MS Conditions

Mass spectrometry was performed on a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) mass spectrometer. The Q-TOF Premier™ was operated in V mode with resolution over 8500 mass with 1.0 min scan time, and 0.02 s inter-scan delay. Argon was employed as the collision gas at a pressure of 5.3 × 10⁻⁵ Torr. Quantitation was performed using Synapt Mass Spectrometry (Synapt MS) of the transitions of m/z 226.19→152.12 for TB and m/z 260.34→183.11 for IS (Propranolol) respectively with a scan time of 1.0 min scan time, and 0.02 s inter-scan per transition. The optimum values for compound-dependent parameters like trap collision energy (Trap CE) and transfer collision energy (Tran CE) were set to 13.2 and 11.9 V, respectively for fragmentation information. The accurate mass and composition for the precursor ions and for the fragment ions were calculated using the MassLynx V 4.1 software.

2.4. Quality Control (QC) Sample and Standard Preparation

The stock solution of 100 μg mL⁻¹ of TB was prepared by dissolving requisite amount in water sonicated at 44 kHz/250W for 20 min. Calibration curve (CC) standards consisting of a set of ten non-zero concentrations (A-J) were prepared by 5% aqueous analytes spiking in blank rat plasma (50 μL aqueous aliquots to 950 μL blank rat plasma) yielding concentration range from 1–1000 ng mL⁻¹ for TB. The final concentrations for each analyte were prepared to be 1, 2, 5, 10, 20, 50, 100, 250, 500, 750 and 1000 ng mL⁻¹. QC samples were prepared independently at three levels; 800 ng mL⁻¹ (HQC, high quality control), 402 ng mL⁻¹ (MQC, middle quality control) and 2 ng mL⁻¹ (LQC, low quality control). A 100 ng mL⁻¹, internal standard working solution was prepared by diluting the stock solution in methanol-water (50: 50 v/v). All the solutions were stored at 2–8 ºC until use.

2.5. Sample Preparation Protocol

All the solutions (CC standards, QC samples and unknown plasma samples) were freshly prepared before carrying out the experiments. The 200 μL aliquot of each samples were taken into glass tube, 50 μL of IS (100 ng mL⁻¹) was added in each sample, and further 500 μL of formic acid (5% w/v) was incorporated to mixture and vortexed at 300 rpm for 5 min). Finally, 10 μL of plasma was extracted with 1 mL of ethyl acetate, and the reaction mixture was evaporated to 900 μL. 50 μL of reconstitution solution (acetonitrile: ammonium acetate buffer; 10: 90, v/v) was then added and vortexing for 10 s at 300 rpm, the solution was transferred into the clean autosampler vials and 10 μL was injected into UHPLC/MS system for analysis.

2.6. Bioanalytical Method Validation

The method validation of TB in rodent plasma was performed according to USFDA guidelines [21]. The linearity of the method was determined by analysis of five standard plots containing ten non-zero concentrations. Peak area ratios of analyte/ IS were utilized for the construction of calibration curves, using weighted (1/x²) linear least squares
regression of the plasma concentrations and the measured peak area ratios. The lower limit of quantification (LLOQ) is the lowest concentration of the calibration curve, which could be measured with acceptable accuracy and precision. The LLOQ was determined based on the signal-to-noise ratio of 10:1. The extraction efficiency (recovery) of TB was performed at LQC, MQC and HQC levels. It was evaluated by comparing the mean area response of six replicates of extracted samples (spiked before extraction) to that of extracted drug free plasma samples (spiked after extraction) at each QC levels. The recovery of IS was similarly estimated. For determining the intra-day accuracy and precision, replicate analysis of plasma samples of TB was performed on the same day. The run consisted of a calibration curve and six replicates of LQC, MQC and HQC samples. The inter-day accuracy and precision were assessed by analysis of six precision and accuracy batches on three consecutive validation days. However, for evaluating the ruggedness of the method, one batch of precision and accuracy was run using a different column (same type) by a different analyst employing the same or another instrument. The six replicates were run for LQC, MQC and HQC samples.

2.7. Matrix Effect

To study the effect of matrix on analyte quantification 6 samples were prepared from 6 different batches of plasma at LQC and HQC levels and checked for the % accuracy and precision (%CV) in both the QC samples. This was assessed by comparing the back calculated value from the QC’s nominal concentration. After specified storage conditions, samples were processed and analyzed. The matrix effect was investigated by postextraction spike method. Peak area (A) of the analyte in spiked blank plasma with a known concentration (MQC) was compared with the corresponding peak area (B) obtained by direct injection of standard in the mobile phase. The ratio (A/B=100) is defined as the matrix effect.

2.8. Ex Vivo Stability

The stability of TB in rodent plasma was evaluated by analyzing six replicates of plasma samples at the concentrations of 2 ng mL⁻¹ (LQC) and 800 ng mL⁻¹ (HQC) which were exposed to different conditions (time and temperature). Percentage stability was determined as; % Stability = mean corrected response of stability stock/ mean response of comparison stock×100.

2.8.1. Long Term Stability

The long-term stability was assessed after storage of the standard spiked plasma samples at deep freeze (−80 °C) for one month. Six replicates of LQC and HQC were used for analysis.

2.8.2. Freeze Thaw Stability

The freeze/thaw stability in plasma was evaluated for three consecutive freeze–thaw cycles from −20 °C to room temperature (+25 °C). Six replicates of LQC and HQC were analyzed after undergoing three freeze-thaw cycles.

2.8.3. Bench Top Stability

Bench top stability was determined for 24 h storage in optimized conditions, using six sets each of LQC and HQC.

The QC samples were quantified against the freshly spiked calibration curve standards

2.8.4. Post Processing Stability

Short-term stability was determined after the exposure (of processed samples) at 10 °C for 24 h in autosampler using six sets each of LQC and HQC. After specified storage conditions, samples were processed and analyzed. The analytes are considered to be stable when the precisions are below 15% and the accuracies are in the range of 85–115% respectively for both levels [12].

2.9. Pharmacokinetic Investigation In Vivo

2.9.1. Experimental Animal

Wistar rats (n=6; 200–250 g, 8–10 weeks old) were provided from Experimental Central Animal House, Hamdard University and kept in an environmentally controlled room (Temperature: 25±2 °C, humidity: 60±5%, 12 h dark–light cycle) for at least 1 week before the experiments. Animals were fed on standard pelleted diet (Ashirwad Industries, Chandigarh, India) and water was provided ad libitum. The rats were fasted overnight before the day of the experiment.

2.9.2. Experimental Design Protocol

The animal protocol used in this study was approved by Hamdard University’s Institutional Animal Ethics Committee. Rats were fasted for 12 h with free access to water prior to the pharmacokinetic investigation. The bioanalytical method was implicated for quantitative estimation of TB in Wistar rat’s plasma after a single oral dose (5mg kg⁻¹ dissolved in distilled water, given by oral feeding tube). Using venous catheter, 1 mL of blood samples were withdrawn and procured in pre-heparinized glass tubes at different time intervals (0, 0.083, 0.166, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 16 h). Further, blood samples were centrifuged (2500×g; 10 min; 20 °C) to separate plasma fractions. The collected plasma samples were preserved for investigation at −80 °C until analysis. Concentration–time curves were established for each analyte and used for the determination of pharmacokinetic parameters such as peak plasma concentration (Cₘₐₓ), peak time (Tₘₐₓ), extent of absorption (AUC) and half-life (T½) by a non-compartmental analysis using PK Solutions Version 2.0; Summit Research Services, USA.

3. RESULTS AND DISCUSSION

3.1. UHPLC/ESI-Q-TOF-MS/MS Analysis

TB is a low molecular weight compound (MW; 226), containing a secondary amine in its structure. Due to the presence of basic nitrogen in the molecule, TB exhibited favorable sensitivity in positive ion mode detection. Optimum chromatographic separation of TB, was achieved by acetonitrile: ammonium acetate buffer (10: 90; v/v), with a flow rate of 0.25 mL min⁻¹. Baseline separation of both TB and IS was obtained within runtime of 3 mins, without any interference. Methanol, acetone and isopropyl alcohol have been also tried for mobile phase selection but they didn’t afford chromatographic separation. Although various buffer systems were studied but the fine peak with better signal response was observed for ammonium acetate buffer, due to
its volatile and compatible nature. The MS full scan spectra for TBS showed protonated precursor [M+H]+ ions at m/z 226.19 and product ion mass spectra at m/z 152.12 respectively (Figs. 1A and 1C). During direct infusion, the mass spectra of IS showed precursor ion peaks at m/z 260.34 as [M+H]+ ions and most abundant product ions at m/z 183.11 (Figs. 1B and 1D). The optimum collision energies employed were 13.0 and 16.3 eV for TB and IS, respectively. Quantification was done on the basis of main product ions. Identical capillary voltage of 3.2 KV was used for monitoring the precursor ions.

The most widely employed biological sample preparation methodologies currently are liquid–liquid extraction (LLE), protein precipitation (PPT), and solid-phase extraction (SPE). In the early stage of method development, a PPT method was employed to separate TB from plasma samples, but strong ion suppression from the endogenous substances in plasma occurred (at 1 min). Although it could be decreased by chromatographic separation, the run time would be sacrificed. Finally, liquid–liquid extraction (LLE) procedures were used to prepare TB plasma samples in our study. To obtain optimum recovery, four organic extraction solvents were evaluated including ethyl ether, ethyl acetate, ethyl ether–dichloromethane (2: 1; v/v) and n-hexane–dichloromethane (2: 1; v/v). It was found that ethyl acetate alone could yield the highest recovery (>80%) for TB and IS. A HPLC method reported for TB estimation by Daraghmeh et al. (2002) used Hypersil 100 C18 column which showed inadequate sensitivity and elaborated retention time [16]. For a LC–MS/MS method recently reported by Luo et al. (2010) had longer retention time (Rt > 15 min) and lengthy processing steps using teicoplanin-containing Chirobiotic T chiral column [18]. However, we used Waters ACQUITY UPLC™ BEH C18 column with 1.7 μm particle size which helped in separation and elution of both analytes, TB and IS at 1.85 (±0.05) and 2.40 (±0.08) min, respectively (Fig. 2). The elution of TB (100 ng mL⁻¹) occurred at 1.87 min (Fig. 2A) and IS (100 ng mL⁻¹) at 2.40 min (Fig. 2B). Chromatogram of blank plasma (extracted and reconstituted) is shown Fig. (2C); however TB spiked plasma sample (1 ng mL⁻¹) retained at 1.90 (Fig. 2D).

3.2. Bioanalytical Method Validation

3.2.1. Linearity

The calibration curves of TB were linear over the concentration range of 1–1000 ng mL⁻¹. The least squares regression analysis gave the linear equation y = 0.0749±0.0038X where y is the peak area ratio of the analyte to the IS and x is the concentration of the analyte. The accuracy and precision (%CV) observed for the calibration curve standards ranged from 93.34–102.06 % and 1.01–4.07, respectively. The LLOQ achieved was 1.0 ng mL⁻¹ for TB.

3.2.2. Recovery, Accuracy and Precision

No endogenous peak was observed at the retention time of the analyte and IS both for any of the batches. Representative chromatograms (Fig. 2C) of extracted blank plasma fortified with IS and blank plasma fortified with TB demonstrates the selectivity of the method. The mean recovery (n = 6) for TB at HQC, MQC and LQC levels was 91.59, 85.6 and 83.7 %, respectively. The recovery of internal standard was 100.1%. Precision (% CV) for intra-batch and inter-batch ranged from 1.82 to 3.55 % for all the QC levels. The detailed results are presented in Table 1. The accuracy results for intra-batch and inter-batch were within 94.50–99.35 % at all QC levels (Table 1).

3.2.3. Ruggedness

One complete precision and accuracy batch was processed and analyzed by different analysts using different col-

Fig. (1). Mass spectrum of: (A) TBS precursor ion (protonated precursor [M+H]+ ions at m/z 226.19); (B) IS precursor ion (protonated precursor [M+H]+ ions at m/z 260.34 as); (C) TBS product ion (major fragmented product ion at m/z 152.12); and (D) IS product ion (major fragmented product ion at m/z 183.11) showing fragmentation transitions.
umn and different sets of solutions. The mean accuracy ($n = 6$) for drug ranged from 99.0–100.13 and the precision ranged from 1.80–3.06 (Table 2).

### 3.3. Matrix Effect

Matrix effect is due to co-elution of some endogenous components present in biological samples. The matrix effect (A/B<100) for TB at LQC was 96.01 % ($n = 6$; % CV 3.28), however at HQC was 97.21 ($n = 6$; % CV 3.15). The CV (%) < 5 suggested that the method was free from matrix effect. No significant ion suppression or enhancement from plasma

### 3.4. Ex Vivo Stability

First time here we reported the stability evaluation of analytes, actually designed to cover anticipated conditions of
3.4. Stability Experiments

Table 3. *Ex Vivo* Stability Data

<table>
<thead>
<tr>
<th>Condition</th>
<th>LQC</th>
<th>HQC</th>
</tr>
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<tbody>
<tr>
<td>Long term stability; recovery (ng) after storage (−80 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial 1.89 ± 0.01</td>
<td>742.5 ± 10.02</td>
<td></td>
</tr>
<tr>
<td>1 month 1.81 ± 0.03 (95.23 %)</td>
<td>711.2 ± 12.51 (95.78%)</td>
<td></td>
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<tr>
<td>Freeze–thaw stability; recovery (ng) after freeze–thaw cycles (−20 °C to 25 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle 0 1.89 ± 0.01</td>
<td>742.50 ± 10.02</td>
<td></td>
</tr>
<tr>
<td>Cycle 1 1.87 ± 0.01 (98.94%)</td>
<td>740.11 ± 12.00 (99.76%)</td>
<td></td>
</tr>
<tr>
<td>Cycle 2 1.86 ± 0.01 (98.41%)</td>
<td>731.32 ± 10.17 (98.49%)</td>
<td></td>
</tr>
<tr>
<td>Cycle 3 1.85 ± 0.01 (97.88%)</td>
<td>712.61 ± 14.15 (95.97%)</td>
<td></td>
</tr>
<tr>
<td>Bench top stability; recovery (ng) at room temperature (25 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h 1.89 ± 0.01</td>
<td>742.51 ± 10.02</td>
<td></td>
</tr>
<tr>
<td>24 h 1.84 ± 0.01 (97.35%)</td>
<td>720.90 ± 8.25 (97.09%)</td>
<td></td>
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<tr>
<td>Post processing stability; recovery (ng) after storage in autosampler (10 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h 1.89 ± 0.01</td>
<td>742.51 ± 10.02</td>
<td></td>
</tr>
<tr>
<td>24 h 1.85 ± 0.02 (97.88%)</td>
<td>723.62 ± 11.05 (97.45%)</td>
<td></td>
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</table>

Values (Mean ± SD) are derived from 6 replicates. Figures in parenthesis represent analyte concentration (%) relative to time zero. Theoretical contents; LQC: 2 ng mL\(^{-1}\); MQC: 400 ng mL\(^{-1}\); HQC: 800 ng mL\(^{-1}\) and LLOQ: 1 ng mL\(^{-1}\).

Fig. (3). Plasma conc. vs. time curve of TB (5 mg/kg, oral) in Wistar rats. Each time point is mean±SD (n = 6).

handling of the experimental samples. Table 3, summarizes the results of stability experiments, which showed that TB was stable during all storage conditions (long–term, freeze–thaw, bench–top and post processing stability). The analytes stability in plasma sample was investigated at two QC levels (in LQC and HQC). The recovery of the analytes relative to that at time zero is reviewed. After 1 month of storage (Long-term stability) the recovery of TB was 95.23% (LQC), and 95.78% (HQC). After 1, 2 and 3 cycles of freeze–thaw (freeze–thaw stability), TB was recovered in the range of 97.88–98.94% (LQC) and 95.97–99.76% (HQC). After 24 h (bench–top stability) the recovery of TB was 97.35% (LQC), and 97.09% (HQC). The recovery (post processing stability) of TB was 97.88% (LQC) and 97.45% (HQC).

3.5. Pharmacokinetic Investigation *In Vivo*

The developed and validated UHPLC/ESI-Q-TOF-MS/MS method described above had been applied success-fully to the pharmacokinetic investigation of TB in Wistar male rats. Fig. (3) shows the profile of the mean TB plasma concentration versus time. Meanwhile, the main pharmacokinetic parameters for TB are presented in Table 4. The \( \text{AUC}_{0-\infty} \) (mean area under the plasma concentration–time curve) for TB was 735.10±102.33 h ng mL\(^{-1}\), whereas \( T_{0.5} \) (mean elimination half-life) was calculated as 8.34±1.42 h. \( C_{\text{max}} \) (mean peak plasma concentration) for TB was 258.00±15.32 ng mL\(^{-1}\) at \( T_{\text{max}} \) (mean peak time) of 1.00±0.18 h.

4. CONCLUSION

UHPLC with QTOF-MS offers improved quality data in terms of increased detection limits, and chromatographic resolution with greater sensitivity. In the present investigation a validated UHPLC/ESI-Q-TOF-MS/MS method for the determination of TB was optimized. This method was sensitive enough to monitor the low-dosage PK studies of TB in plasma. The advantages of our developed method over pre-
venous techniques are the short analysis time (3 min), high sensitivity (LLOQ: 1.0 ng mL⁻¹) and simple extraction procedure. The assay was successfully employed for pharmacokinetic studies in Wistar rat plasma with acceptable precision, adequate sensitivity and satisfied accuracy. It would be, furthermore, applicable for clinical studies.

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CONFLICT OF INTEREST

Authors have no conflict of interest in this paper. The research work was funded by Defense R & D Organization (DRDO), New Delhi, India.

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Mean values are derived from Fig.4 by using non-compartmental pharmacokinetic data analysis software (PK Solutions version 2.0TM, Summit Research Services, Montrose, USA).

Table 4. Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean Value (±SD)</th>
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<tr>
<td>AUC⁰⁻ sup (h.ng mL⁻¹)</td>
<td>735.10±102.33</td>
</tr>
<tr>
<td>C max (ng mL⁻¹)</td>
<td>258.00±15.32</td>
</tr>
<tr>
<td>T max (h)</td>
<td>1.00±0.18</td>
</tr>
<tr>
<td>T 0.5 (h)</td>
<td>8.34±1.42</td>
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</table>

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