Journal of Pharmaceutical Analysis 2013;3(6):434–439

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

www.elsevier.com/locate/jpa
www.sciencedirect.com

ORIGINAL ARTICLE

Optimization and validation of a fast RP–HPLC method for the determination of dobutamine in rat plasma: Pharmacokinetic studies in healthy rat subjects

Ramesh Thippani[a], Nageswara Rao Pothuraju[a,*], Nageswara Rao Ramisetti[b], Saida Shaik[b]

[a]*Department of Chemistry, National Institute of Technology, Warangal, India*
[b]*Analytical Chemistry Division, IICT, Hyderabad, AP, India*

Received 3 April 2013; accepted 27 July 2013
Available online 2 August 2013

KEYWORDS

Dobutamine;
RP–HPLC;
Validation;
Rat plasma;
Pharmacokinetics

Abstract A novel isocratic reverse phase high performance liquid chromatography (RP–HPLC) with photo diode array (PDA) detection method for the determination of dobutamine (DBT) in rat plasma was developed and validated after optimization of various chromatographic conditions and other experimental parameters. Homoveratrylamine was used as an internal standard. Methanol was used as the extracting solvent for the preparation of plasma samples. Samples were separated on a Symmetry C18 (250 mm × 4.6 mm i.d., 5 μm) analytical column. Acetonitrile and 15 mM potassium dihydrogen phosphate (pH 5.0 with 0.3% TEA) (20:80, v/v) was used. The column oven temperature was optimized at 35 °C and the flow rate was 0.8 mL/min. The detection wavelength was fixed at 230 nm for entire analysis. The calibration curve was found to be linear over the concentration range of 50–2000 ng/mL (r^2 = 0.9992). The limit of quantification (LOQ) of the method was 50 ng/mL. The % RSD values of accuracy and precision values for intra and inter days were < 15% at quality control (QC) concentrations. Recovery, stability and robustness were studied within the acceptable range according to ICH guidelines. The method was efficiently applied to a pharmacokinetic study in healthy Wistar rats.

*Corresponding author. Tel.: +91 870 2462662; fax: +91 870 2459547.
E-mail addresses: tippaniramesh.1@gmail.com (R. Thippani), pnr.nitw@gmail.com (N.R. Pothuraju)

Peer review under responsibility of Xi'an Jiaotong University.

Production and hosting by Elsevier

1. Introduction

Dobutamine (DBT) is an inotropic synthetic catecholamine [(*RS*)-4-(2-{[4-(4-hydroxyphenyl) butan-2yl] amino} ethyl) benzene-1, 2-diol] (Fig. 1A), which acts on adrenergic sympathetic receptor and is used to treat the majority of the patients with cardiogenic shock [1,2]. It is selective β_1 receptor blocker used in the treatment

2095-1779 © 2013 Xi'an Jiaotong University. Production and hosting by Elsevier B.V. Open access under CC BY-NC-ND license.
http://dx.doi.org/10.1016/j.jpha.2013.07.003

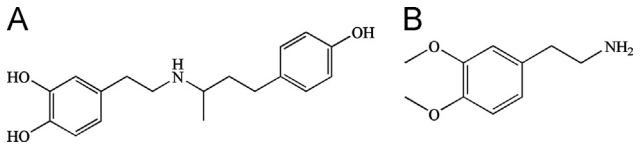

Fig. 1 Chemical structure of (A) dobutamine (DBT) and (B) homoveratrylamine (IS).

of several diseases of the cardiovascular system, especially hypertension and anxiety disorders [3]. This is available as both (+) and (−) racemic mixture, (+) isomer is a β_1 agonist and α_1 antagonist, while the (−) isomer is a α_1 agonist [4]. Racemic DBT has mild β_2 agonist activity [5]. This is a white crystalline powder and polaric nature so easily soluble in polar solvents like water, methanol etc. The melting point of DBT is 189 °C–191 °C. Homoveratrylamine [2-(3, 4-dimethoxyphenyl) ethylamine] (Fig. 1B) was used as an internal standard (IS) for determination of DBT in spiked rat plasma samples.

Several methods have been reported for the analyses of this compound, such as spectrophotometric assay [6], pH metric determination [7], chemiluminescence [8], HPLC assay with fluorimetric, electrochemical and UV detection [9–14] and solid phase extraction method [15].The present paper reports on the development of a new, validated, fast and sensitive method by using HPLC with PDA detection for the quantitative determination of DBT in rat plasma. Compared with old methods this is more sensitive, rapid and validated at very low level of quality control (QC) samples. This simple assay using only 100 µL of plasma has been successfully applied to the analysis of plasma samples in a preliminary pharmacokinetic study in healthy rats under a variety of ICH recommended test conditions [16].

2. Experimental

2.1. Chemicals and reagents

DBT (>99.8% purity) and Homoveratrylamine (IS >98.9% purity) were gift samples from Inogent Laboratories Limited, Hyderabad, India. HPLC grade acetonitrile and methanol (Sigma Aldrich, Mumbai, India) were used. Analytical reagent grade (AR) potassium dihydrogen orthophosphate, TEA and ortho phosphoric acid (SD Fine Chemicals, Mumbai, India) (99.5% purity) were used and water used was purified by Millipore synergy (Millipore, France).

2.2. Instrumentation

The HPLC system consisting of a quaternary LC-20AD pump, a SPD-M20A diode array detector, a SIL-20AC auto sampler, a DGU-20A5 degasser and CBM-20A communications bus module (all from Shimadzu, Kyoto, Japan) was used. The pH measurements were carried out by Elico, model LI 120, pH meter equipped with a combined glass–calomel electrode. The chromatographic and the integrated data were recorded using HP-Vectra (Hewlett Packard, Waldron, Germany) computer system using LC-Solution data acquiring software (Shimadzu, Kyoto, Japan). Separation and quantitation were made on a symmetry C18 column (250 mm × 4.6 mm i.d., 5 µm) (Waters, Chromatographie technik Technik, Germany).

2.3. Preparation of standard stock and working solutions

DBT and IS stock solutions (1 mg/mL) and the respective working standard solutions were prepared in methanol. DBT working standards at concentrations of 1, 2.5, 5, 10, 25, 35, and 40 µg/mL were prepared for calibration standards and used to spike the whole rat plasma. Similarly, IS working standards were prepared separately, for QC standards by diluting the stock solution in methanol. All the stock solutions and working standards were stored at 4 °C and brought to room temperature (20 °C) before use.

2.4. Preparation of calibration standards and QC samples in plasma

The plasma calibration standards were prepared by spiking 900 µL of blank plasma with 50 µL of IS and 50 µL of appropriate working standard solution containing 1, 2.5, 5, 10, 25, 35 and 40 µg/mL, to give final concentration at 50, 125, 250, 500, 1250, 1750 and 2000 ng/mL. The QC samples at concentrations of 50 ng/mL (LOQ), 100 ng/mL (low QC), 400 ng/mL (middle QC) and 1000 ng/mL (high QC) were prepared separately in similar manner.

2.5. Extraction efficiency

The extraction efficiency of DBT was determined by comparing the peak areas measured after analysis of spiked plasma sample with those found after direct injection of non-biological (unextracted) samples into the chromatographic system at the same concentration level. Different organic extraction solvents (6% TCA, acetonitrile, methanol and 0.1% formic acid) were tried in the experiment, and methanol was proved to be the most efficient solvent for extracting DBT from rat plasma and had a small variation in extraction recoveries over the concentration range. Spiked plasma sample was prepared in triplicate at single concentration of 400 ng/mL of DBT and 5 µg/mL of IS.

2.6. Method validation

The method was validated for linearity, selectivity, sensitivity, accuracy, precision, recovery, matrix effect, robustness and stability according to the US Food and Drug Administration (FDA) [17] and International Conference on Harmonization (ICH) guidelines for the validation of bioanalytical method [16,18]. The selectivity of this method was investigated by analyzing three individual rat blank plasma samples. Each blank sample was tested for interference using the present analytical method and compared with spiked sample whose concentration of the analyte was at the LLOQ. Calibration standard samples in rat plasma were prepared for three separate batches. Intra-day and inter-day precision and accuracy were evaluated by measurement of DBT in plasma in five replicates of QC samples at three different concentrations for three separate batches. The matrix effect was evaluated by adding known amounts of the analyte to pre–treated blank plasma samples, immediately before injection [19]. The standard solutions and the corresponding peak area ratios were calculated. The stability of the studied drug in rat plasma was assessed under different study conditions; i.e., initial condition, at –20 °C for one months (short term), at –20 °C for 3 months (long term) and freeze–thaw condition using QC samples.

2.7. *Animals and plasma sample preparation for pharmacokinetic study*

In vivo drug release was investigated in male Wistar rats. The rats (190–210 g) were procured from M/S. Mahavir enterprises, Hyderabad, India. The use of animals was approved by the 'Institutional Animal Ethical Committee' (University College of Pharmaceutical Sciences, Warangal, India), throughout the experimental period. The animals were housed under standard condition in cages at room temperature (20±2 °C) with 60–70% relative humidity and were exposed to 12/12 h light/dark cycle. They were fed with standard laboratory diet supplied by M/S. Rayans Biotechnologies Pvt. Ltd., Hyderabad, India. Food and water were allowed ad libitum during the experiment. After oral administration of a single dose of DBT (25 mg/kg) to healthy Wistar rats ($n=3$), 1 mL of blood samples were collected at 0, 1, 2, 4, 6, 8, 12 and 24 h time points into heparinized collection tubes. Whole blood samples were centrifuged for 20 min at 4000 rpm and stored at –20 °C. The stored plasma samples were allowed to thaw at room temperature (20 °C) before processing. An aliquot 100 µL of plasma sample was pipetted into a 10 mL polypropylene tube and 2 mL of methanol was added. The mixture was vortex mixed briefly and centrifuged at 4000 rpm for 15 min after standing for 5 min at room temperature; the supernatant was carefully transferred into vial and injected into HPLC system. Statistical analysis was performed using Microsoft Excel 2003 while pharmacokinetic software (Ramkin) based on non compartment model was used to calculate all the parameters of pharmacokinetics.

3. Results and discussion

3.1. *Chromatographic conditions*

To obtain the best chromatographic conditions, different columns, mobile phases with different pH values and organic modifier were tested to provide sufficient selectivity and sensitivity in short separation time. In the early stage of method development a short HPLC column X Bridge BEH C18 (50 mm × 30 mm i.d., 3.5 µm) was used with the mobile phase consisting of 15 mM potassium dihydrogen phosphate (pH 5.0, 0.3% TEA): acetonitrile (80:20) at flow rate of 0.5 mL/min and PDA detection at 230 nm. The retention time was very less for DBT and IS and both were not well separated. There was no matrix effect seen but the results were not reproducible probably due to the co–elution of both the analyte and IS. Hence the longer column Symmetry C18 (250 mm × 4.6 mm i.d., 5 µm) was used, using the same mobile phase at a flow rate of 0.8 mL/min. The results were reproducible and the chromatogram showed a good resolution between DBT and IS (Fig. 2). Homoveratrylamine was chosen as an IS because of its structural and chemical similarity to DBT, also both IS and DBT showed the same maximum wavelength at 230 nm.

3.2. *Optimization of conditions*

All the chromatographic parameters were optimized for better results. Various solvents (6% TCA, acetonitrile, methanol and 0.1% formic acid) were studied to optimize the extraction of DBT and IS from rat plasma. From the results, acceptable recoveries were obtained only with methanol. However, peaks were broad and more tailing in the case of extraction with 6% TCA or 0.1% formic acid. The reason might be the stronger solvent effect in the case of extraction solvents containing methanol and acetonitrile. Variation of pH plays an important role in the separation process and it was found that at higher (pH 8.0) and lower (pH 3.0) values the tailing of DBT peak was more and also resolution was poor between analyte and IS. pH (5.0) was chosen as the optimum value for resolution of drugs from endogenous biological substances, better peak shape and reasonable run time. At low concentration of triethyle amine (TEA) the resolution between analyte and IS was poor. At 0.3% (v/v) level of TEA at pH 5.0 the analyte and IS were well separated, so 0.3% TEA was chosen for further analysis. The effect of buffer

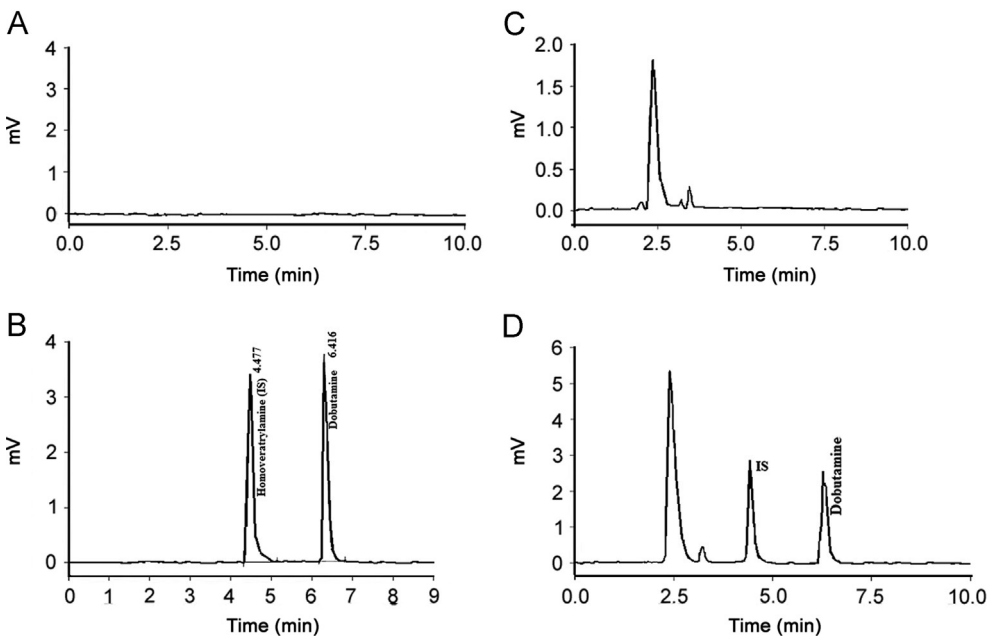

Fig. 2 Representative RP–HPLC chromatograms of: (A) the blank mobile phase, (B) the standard 1:1 mixture, (C) the blank rat plasma sample and (D) the blank plasma sample spiked with 500 ng/mL of analyte and IS.

concentration on the retention of DBT and IS was also studied. Initially, the retention times of the analyte and IS were comparatively longer with the mobile phases containing low concentration of potassium dihydrogen phosphate buffer. Finally, this problem was solved by using 15 mM potassium dihydrogen phosphate buffer with pH 5.0 (adjusted with ortho phosphoric acid). The mobile phase was pumped at various column oven temperatures in the range of 25–45 °C. Peak shapes and heights were improved and retention times were decreased with increasing temperature without affecting peak areas and resolution. So depending upon these parameters, 35 °C was selected to be the optimum temperature for the separation of these two analytes. By using above optimum conditions and mobile phase we found good peak shape and resolution of analyte and IS. Proposed chromatogram and the method were sufficiently specific to the drug. Intermediate precision was performed to confirm that separation was satisfactory under external conditions to the method.

3.3. Method validation

3.3.1. Linearity, selectivity and sensitivity

A calibration curve was plotted between peak area ratios of DBT to IS versus DBT concentration in plasma. The chromatographic responses (ratio of peak area of DBT to IS versus DBT concentration) were found to be linear over an analytical range of 50–2000 ng/mL with correlation coefficient (r^2) value of 0.9992, which showed reproducibility. The calibration curves were obtained by weighted linear regression (weighing factor $1/x^2$) using the Microsoft Excel 2003 software. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards. The lower limit of quantification (LLOQ), the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision for the analyte from the normal plasma sample, was 100 ng/mL. The limit of detection (LOD) and limit of quantification (LOQ) were determined at 3 and 10 times the baseline noise, respectively. The LOQ was defined by the lowest concentration that gave a signal-to-noise ratio equal to or greater than 10 whilst exhibiting an accuracy of $\leq 15\%$. The LOD and LOQ were found to be 15 ng/mL and 50 ng/mL. The selectivity of the method was established by the analysis of blank plasma spiked at the 1:1 ratio of DBT and IS. No significant interferences and matrix effect were observed at the retention times of DBT and IS in spiked and rat plasma samples.

3.3.2. Accuracy and precision

The intra- and inter-day accuracy and precision of DBT quality control samples [50 (LOQ), 100 (LQC), 400 (MQC) and 1000 (HQC) ng/mL] are shown in Table 1. The relative standard deviation (RSD) of intra-day assay of the drug was ranged from 4.8% to 8.9% and that of the inter-day assay was from 3.8% to 11.7%. Accurate data ranged from 99.8% to 103.6% for both the conditions, indicated that there was no interference from endogenous plasma components. RSD values of intra-day and inter-day precision were less than 11.7% (should be less than 15% according to ICH guidelines bioanalytical method validation), which revealed that the precision and accuracy at the LLOQ and at low, medium and high concentrations of DBT in plasma were within the acceptable limits.

Table 1 The intra and inter day precision, accuracy and matrix effect of DBT ($n=5$) in plasma.

Concentration (ng/mL)	Precision		Accuracy (%)	Matrix effect[a]
	Mean ± SD (ng/mL)	% RSD		
Intra-day				
50	50.2 ± 0.4	5.4	100.4	96.3
100	103.6 ± 2.7	8.9	103.6	97.2
400	399.4 ± 4.9	4.8	99.8	97.7
1000	1014.3 ± 3.3	7.6	101.4	98.4
Inter-day				
50	50.3 ± 0.3	3.8	100.6	98.9
100	99.8 ± 5.7	6.5	99.8	97.2
400	402.0 ± 0.9	11.7	100.5	95.6
1000	1032.0 ± 6.8	10.4	103.2	96.1

[a]Sample/standard peak area ratio

3.3.3. Recovery and matrix effect

Recovery was determined by comparing the detector response of the pre-extracted spiked sample with those of post-extraction spiked sample onto a blank matrix because the analyte and IS have the same matrix. The matrix effect could be considered the same for pre- and post-extraction spiked samples. The overall recovery of DBT at four QC concentrations (50, 100, 400 and 1000 ng/mL) was 98 ± 1.7%. The matrix effect was tested as described in Section 2.6, QC samples were injected to rats and collected the samples. After the pre-treatment and compared with QC standard solutions, the results obtained were quite good, since the sample/standard peak area ratios were always higher than 90% (Table 1).

3.3.4. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, deliberate variations in method parameters and provides an indication of its reliability during normal usage. An experimental design, the augmented Plackett–Burman, was applied to study the influence of the internal parameters (percentage of buffer composition, buffer concentration and pH). A Plackett–Burman design is an orthogonal two-level experimental design that can be used to fit linear models. The experiments were run randomly with a plasma sample spiked with 100 ng/mL DBT and IS. Plotting the scaled and centered coefficient plotted in Fig. 3 revealed that logarithmic transformations were necessary for optimizing the responses. The respective 95% confidence intervals are shown as error bars. Coefficients with 95% confidence intervals including zero were statistically insignificant. The results indicated that the method was robust as its performance was negligibly affected by minor changes in the parameters.

3.3.5. Stability

The result of the stability validation is presented in Table 2. The results revealed that the final concentration of the drug in each quality control sample at stability conditions i.e., freeze–thaw condition, initial condition, storing at –20 °C for one month

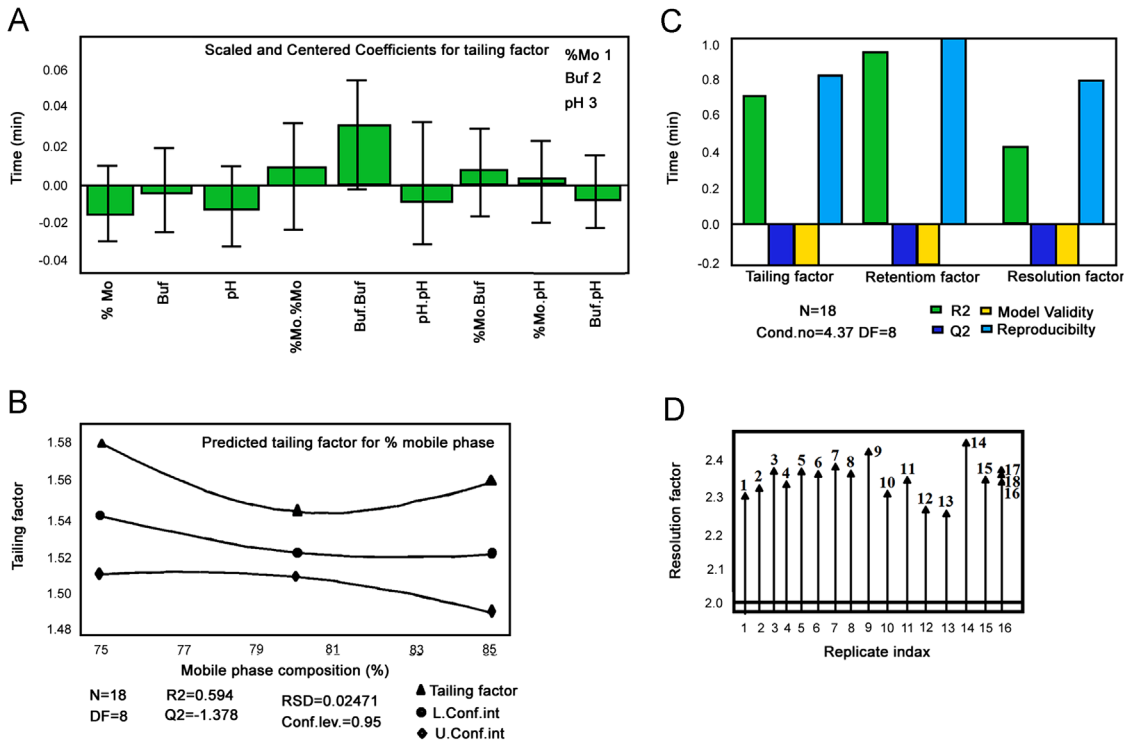

Fig. 3 Scaled and centered coefficients of resolution factor (Rs). (A) (1) Percentage mobile phase (% Mo), (2) Concentration of buffer in mM, (3) pH. (B) Tailing factor predicted for percentage modifier ($R^2=0.594$, $Q^2=-1.378$ and RSD = 0.02471), (C) Model validity and reproducibility chart. (D) Plot of replications for resolution factor with experimental number labels.

Table 2 Stability of DBT in plasma ($n=5$) under various storage conditions.

QC (ng/mL)	Remaining percentage[a] (mean ± SD)			
	Initial (at 20 °C)	1 month at −20 °C (short term)	3 months at −20 °C (long term)	Freeze–thaw condition
50	95.5 ± 0.2	100.2 ± 1.5	94.7 ± 3.2	98.6 ± 2.9
100	94.3 ± 2.9	99.5 ± 2.4	98.6 ± 2.4	99.0 ± 7.4
400	95.6 ± 0.7	101.8 ± 0.9	98.8 ± 2.8	98.8 ± 4.2
1000	96.7 ± 0.4	99.8 ± 1.7	97.3 ± 3.6	101.0 ± 8.6

[a]Remaining percentage = (concentration found)/(concentration added) × 100.

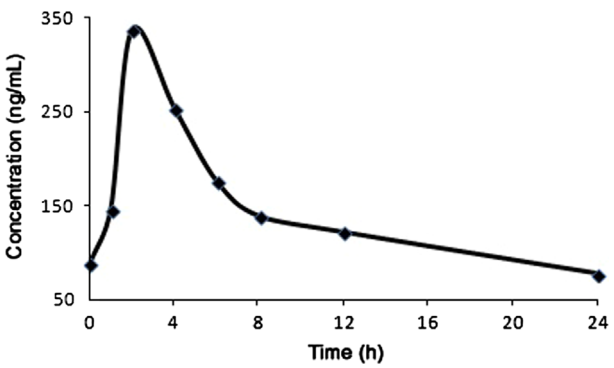

Fig. 4 Mean plasma concentration verses time profile of DBT.

(short term) and at –20 °C for 3 months (long term) was found to be similar with initial concentration. The SD value ($n=5$) of final concentration of drug after storing the samples in all the stability conditions was found to be less than 15%. The accuracy of stored samples was found to be nearly equivalent to 100%. Hence, it can be inferred that DBT was stable in rat plasma.

3.3.6. Application to pharmacokinetics in rats

The developed method was applied to quantify DBT concentration in pharmacokinetic study carried out on rats. The mean plasma concentrations versus time profile following a single oral administration of DBT to 3 rats is presented in Fig. 4. The pharmacokinetic study of DBT (ng/mL) was performed after administering a single 25 mg oral dose. The pharmacokinetic parameters (C_{max},

Table 3 Pharmacokinetic parameters of DBT in rats ($n=3$) after oral administration.

Parameter	Observed (mean ± SD)
C_{max} (ng/mL)	337 ± 12
T_{max} (h)	2.0 ± 0.02
$AUC_{0\to t}$ (ng mL/h)	34428 ± 915
$AUC_{0\to\infty}$ (ng mL/h)	35417 ± 1015
$T_{1/2}$ (h)	0.64 ± 0.17
K_{el} (h^{-1})	1.21 ± 0.13
$AUC_{0\to t}/AUC_{0\to\infty}$	0.972
MRT[a] (h)	6.47 ± 1.88
Clearance, Cl (L/h)	11.46 ± 3.21

[a]Mean residence time.

T_{max}, K_{el}, $t_{1/2}$, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$) of DBT in these all subjects were calculated and are shown in Table 3. Therefore, the terminal phase of DBT in the study was well characterized and the analytical assay was able to detect low concentrations at the end of the plasma concentration time profile. The present developed HPLC assay method could be successfully applied to the determination of DBT in several pharmacokinetic studies conducted in any institution.

4. Conclusion

A simple and rapid bioanalytical assay method has been developed and validated in plasma. The validated method was demonstrated to be accurate, precise, selective and sensitive. The method was found to be linear ($r^2=0.9992$) within the analytical range of 50–2000 ng/mL. A maximum recovery of drug from plasma was resulted using methanol as extracting solvent in comparison to other organic solvents. Compared with previously validated methods, the present assay method was carried out with extensive validation parameters as per ICH guidelines. The drug was stable in rat plasma and the developed method was applied to pharmacokinetic studies. In summary it can be suitable for use in all laboratory equipped with sophisticated or unsophisticated instruments.

Acknowledgment

The authors thank the Directors, National Institute of Technology, Warangal and Indian Institute of Chemical Technology, Hyderabad for providing research facilities and encouragement. Mr. Thippani Ramesh thanks MHRD, Government of India for providing financial assistance.

References

[1] E. Rivers, B. Nguyen, S. Havstad, et al., Early goal—directed therapy in the treatment of severe sepsis and septic shock, N. Engl. J. Med. 345 (2001) 1368–1377.
[2] M. Metra, S. Nodari, A. D'Aloia, et al., Beta-blocker therapy influences the hemodynamic response to inotropic agents in patients with heart failure. A randomized comparison of dobutamine and enoximone before and after chronic treatment with metoprolol or carvedilol, J. Am. Coll. Cardiol. 40 (2002) 1248–1258.
[3] K. Parker, L. Brunton, L.S. Goodman, et al., Goodman and Gilman's manual of pharmacology and therapeutics, McGraw-Hill Medical, 2008, p. 159 <http://www.mhprofessional.com/product.php?cat=116&isbn=0071593233>.
[4] R.R. Ruffolo, Review: the pharmacology of dobutamine, Am. J. Med. Sci. 294 (1987) 244–248.
[5] F.A. Tibayan, A.N. Chesnutt, H.G. Folkesson, et al., Dobutamine increases alveolar liquid clearance in ventilated rats by beta-2 receptor stimulation, Am. J. Respir. Crit. Care. Med. 156 (1997) 438–444.
[6] M.E. El-Kommos, Spectrophotometric determination of dobutamine hydrochloride using 3-methyl benzothiazolin-2-one hydrazone, Analyst 112 (1987) 101–103.
[7] L.L. Reis, M.A.S. Peterlini, M.L.G. Pedreira, et al., Pedreira hydrogen-ion potential of dobutamine hydrochloride solutions exposed to environmental conditions of neonatal intensive care units, J. Pediatr. (Rio J.) 85 (2009) 553–556.
[8] H. Liu, L. Zhang, J. Zhou, et al., Flow injection chemiluminescence determination of dobutamine hydrochloride injection using luminol–ferricyanide/ferrocyanide system, Anal. Chim. Acta 541 (2005) 125–129.
[9] G. Alberts, F. Boomsma, A.J. Man in't Veld, et al., Simultaneous determination of catecholamines and dobutamine in human plasma and urine by high-performance liquid chromatography with fluorimetric detection, J. Chromatogr. B 583 (1992) 236–240.
[10] R. Knoll, M. Brandl, Rapid and simple method for the routine determination of dobutamine in human plasma by high-performance liquid chromatography, J. Chromatogr. B 345 (1985) 425–429.
[11] H. Husseini, V. Mitrovie, M. Schlepper, et al., Rapid and sensitive assay of dobutamine in plasma by high-performance liquid chromatography and electrochemical detection, J. Chromatogr. B 620 (1993) 164–168.
[12] J. Zimmermann, R. Dennhardt, H.J. Gramm, et al., Measurement of plasma catecholamines by high-performance liquid chromatography with electrochemical detection in intensive care patients after dobutamine infusion, J. Chromatogr. B 567 (1991) 240–247.
[13] M. Yan, L.T. Webater, J.L. Blumer, et al., Kinetic interactions of dopamine and dobutamine with human catechol-o-methyltransferase and monoamine oxidase in vitro, J. Pharmacol. Exp. Ther. 301 (2002) 315–321.
[14] N. Patel, M. Taki, P. Tunstell, et al., Stability of dobutamine 500 mg in 50 ml syringes prepared using a Central Intravenous Additive Service, Eur. J. Hosp. Pharm. 19 (2012) 52–56.
[15] C. Leilour, T. Dine, M. Luyckxt, et al., Solid phase extraction and high performance liquid chromatographic determination of dobutamine in plasma of dialysed patients, Biomed. Chromatogr. 8 (1994) 309–312.
[16] ICH. Guideline Q2A, Text on validation of analytical procedures. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, 1994. ⟨http://www.ich.org⟩ (November 2012).
[17] U.S. Department of health and human services, food and drug administration, center for drug evaluation and research (CDER), guidance for industry, bioanalytical method validation, 2001. ⟨http://www.fda.gov⟩ (January 2013).
[18] T. Ramesh, P.N. Rao, Development and validation of a stability-indicating RP-HPLC assay method and stress degradation studies on dapiprazole, J. Chromatogr. Sci. 51 (9) (2013) 856–860.
[19] E. Chambers, D.M. Wagrowski-Diehl, Z. Lu, et al., Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses, J. Chromatogr. B 852 (2007) 22–34.