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Liquid chromatography–tandem mass spectrometry method for the quantification of dimebon in rat plasma and brain tissue

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ABSTRACT

A sensitive high-performance liquid chromatography–positive ion electrospray tandem mass spectrometry method was developed and validated for the quantification of dimebon in rat plasma and brain tissue. Following liquid–liquid extraction, the analyte was separated using a gradient mobile phase on a reversed phase column and analyzed by MS/MS in the multiple reaction monitoring mode using the respective $[M+H]^+$ ions, *m/z* 320–277 for dimebon and *m/z* 407–100 for the internal standard. The assay exhibited a linear dynamic range of 0.25–250 ng/mL for dimebon in rat plasma and brain tissue. Acceptable precision (<11%) and accuracy (100 ± 7%) were obtained for concentrations over the standard curve range. A run time of 2.5 min for each sample made it possible to analyze more than 250 samples per day. The method was successfully applied to quantify dimebon concentrations in a rodent pharmacokinetic study. Moreover, it can be believed that the assay method in rat plasma would facilitate the ease of adaptability of dimebon quantification in human plasma for clinical trials.

1. Introduction

Dimebon is used as a non-selective antihistamine in Russia since 1983 and it was withdrawn from the market with the advent of more selective treatments. Recently, dimebon has been proposed to be useful for treating neurodegenerative disorders [1,2]. Dimebon is an orally available drug that is used as novel drug in clinical testing for the treatment of Alzheimer's and Huntington's diseases – two progressive, devastating conditions with limited treatment options. Dimebon demonstrated significant positive effects in six-month randomized, double-blinded, placebo-controlled phase II trial of 183 patients with mild to moderate Alzheimer's disease [3,4]. The phase III trial of dimebon in Alzheimer's disease will soon be initiated [5]. Dimebon also demonstrated efficacy in phase II trial of patients with Huntington's disease [6].

Animal studies showing potential beneficial effects of dimebon on Alzheimer's disease models were shown in Russian research in 2000 [7]. Dimebon demonstrated cognition and memory-enhancing properties in the active avoidance test in rats treated with the neurotoxin AF64A, which selectively destroys cholinergic neurons [1]. Dimebon protected neurons in the cerebellum cell culture against the neurotoxic action of β-amyloid fragment (Aβ25–35, EC_{50} = 25 μM). *In vitro*, dimebon displayed Ca^{2+}-blocking properties (IC_{50} = 57 μM, on isolated rat ileum intestine) and pronounced anticholinesterase activity (IC_{50} = 7.9 and 42 μM for butyrylcholine esterase and acetylcholine esterase, respectively). Dimebon exhibited strong anti-NMDA activity in the prevention of NMDA-induced seizures in mice (EC_{50} = 42 ± 6 mg/kg, i.p.) [1]. It binds with moderate affinity to the human and rat 5-HT_6 receptor as well as the native rat 5-HT_6 receptor, and acts as an antagonist in functional cAMP assays [8].

Despite extremely encouraging results in clinical trials, the mechanisms responsible for beneficial actions of dimebon in Alzheimer's disease and Huntington's disease remain poorly understood. While dimebon has activity at two relevant and validated targets for Alzheimer's disease (AChE and NMDA) but interestingly, these are not postulated to be the reason for its efficacy in neurodegenerative diseases, rather, dimebon is thought to operate by a novel mitochondrial mechanism of action [9]. The rationale for this is that proteins found in the brain of Alzheimer's disease patients (β-amyloid/APP) are toxic to neurons and specifically, cause the opening of mitochondrial pores and eventually, neuronal death. By blocking these pores, dimebon keeps the mitochondrial function around normal and thus, prevents cognitive decline. Recently Wu et al. [10] reports that Ca^{2+} and the mitochondria stabilizing effects of dimebon may only in part be responsible for beneficial effects in human clinical trials. Schaffhauser et al. [8] demonstrates that inhibition of the 5-HT_6 receptor is an additional pharmacological activity of dimebon which may play an important role in the apparent clinical efficacy. Further evaluation of dimebon in

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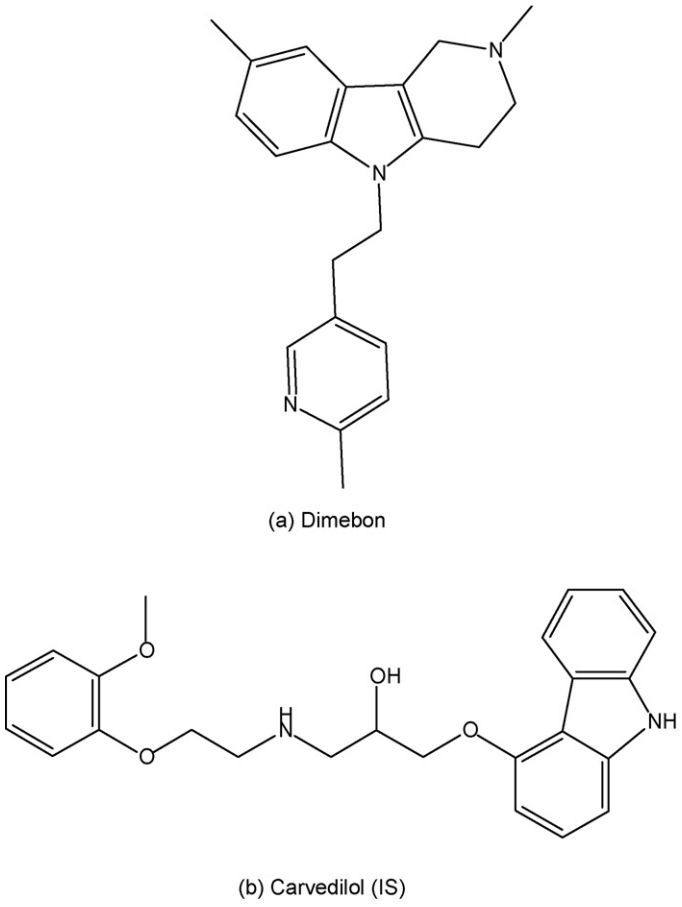

Fig. 1. Chemical structures for dimebon and the IS (carvedilol).

Alzheimer's disease and Huntington's disease whole animal models will be required in order to understand its mechanism of action. It is important to note that dimebon interacts with at least 17 additional targets [10] and that these interactions have not been fully characterized. Despite promising clinical effects in the treatment of mild to moderate Alzheimer's disease, new animal research shows that dimebon increases beta-amyloid (Aβ) levels remains something of a mystery that requires further research [11]. To support the animal models as well as the clinical trials of dimebon a sensitive analytical method to estimate the concentrations of dimebon in plasma/brain tissue is required. Till date no analytical method for the estimation of dimebon in plasma/brain tissue is available.

This paper describes a simple, selective, sensitive and reproducible triple quad mass spectrometric method with commercially available internal standard for the quantification of dimebon in rat plasma and brain tissue. Moreover, it can be believed that development of a method in rat plasma would facilitate the ease of adaptability of dimebon assay in human plasma for clinical trials.

2. Experimental

2.1. Chemicals

Dimebon (dimebolin hydrochloride) and carvedilol (internal standard) drug substances were obtained from R&D department of Suven Life Sciences Ltd., (Hyderabad, India) and Wockhardt Research Center (Aurangabad, India), respectively. Chemical structures are presented in Fig. 1. HPLC-grade LiChrosolv methanol and HPLC-grade LiChrosolv acetonitrile were purchased from Merck (Darmstadt, Germany). Ammonium acetate, formic acid, *tert*-butyl methyl ether, *n*-hexane and sodium hydroxide pellets were purchased from Merck (Worli, Mumbai, India). HPLC-grade water from Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

2.2. LC–MS/MS instrument and conditions

The HPLC SIL HTC system (Shimadzu Corporation, Kyoto, Japan) is equipped with LC-AD VP binary pump, a DGU20A5 degasser and a SIL-HTC auto sampler equipped with a CTO-10AS VP thermostated column oven. The chromatography was performed using column Polyhydroxyethyl A 100 × 2.1 mm, 5 μm; 100-A° (PolyLC Inc., Columbia, USA) at a temperature of 30 °C. The analyte was eluted by a gradient mobile phase system consisting of solvent A (10 mM ammonium acetate buffer, pH adjusted to 5.0 with diluted formic acid) and solvent B (acetonitrile). After sample injection, a combination of 70% solvent A and 30% solvent B was held for 0.6 min, then the solvent B was steeply changed to 85% until 1.0 min. The combination of 15% solvent A and 85% solvent B was held up to 2.0 min and then the solvent B was steeply reversed back to 30% from 2.0 to 2.2 min. Finally the combination of 70% solvent A and 30% solvent B held up to 2.5 min for equilibration of the column. The mobile phase was pumped at a flow-rate of 0.8 mL/min with a split ratio of load to waste 10:90.

Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument (MDS-SCIEX, Concord, Ontario, Canada) using multiple reaction monitoring (MRM). A turboionspray interface operating in positive ionization mode was used. Typical source conditions were as follows: the turbo-gas temperature was set at 250 °C, and the ion spray needle voltage was adjusted at 5500 V. The mass spectrometer was operated at unit resolution for both Q1 and Q3 in the MRM mode, with a dwell time of 200 ms per MRM channel. The precursor/product ion pairs monitored were *m*/*z* 320–277 for dimebon and *m*/*z* 407–100 for the internal standard (IS). Ion source gas 1 and gas 2 were set at 25 and 20 (arbitrary units), respectively; curtain gas was set at 12 (arbitrary units) and the collision gas was set at 6 (arbitrary units). The collision energy was set at 20 for dimebon and 40 for the IS, respectively. Data acquisition was performed with analyst 1.4.2 software (MDS-SCIEX, Concord, Ontario, Canada).

2.3. Sample preparation

Standard stock solutions of dimebon (1 mg/mL) and the IS (1 mg/mL) were separately prepared in methanol. Working solutions for calibration and controls were prepared daily by appropriate dilution in water–methanol (50:50, v/v; diluent). The IS working solution (1.0 μg/mL) was prepared daily by diluting its stock solution with diluent. Working solutions (0.5 mL) were added to drug-free rat plasma/brain tissue homogenate (24.5 mL) as a bulk, to obtain dimebon concentration levels of 0.25, 0.50, 1, 2, 5, 10, 25, 50, 100 and 250 ng/mL as a single batch at each concentration. Quality control (QC) samples were also prepared as a bulk on an independent weighing of standard drug, at concentrations of 0.25 (LLOQ), 0.75 (low), 100 (medium) and 200 ng/mL (high) as a single batch at each concentration. The calibration and control bulk samples were divided into aliquots in micro centrifuge tubes (Tarson, Kolkata, India; 2 mL) and stored in the freezer at <−50 °C until analysis.

A plasma sample (0.5 mL) was pipetted into a 15 mL glass tube and then 25 μL of the IS working solution (1.0 μg/mL) was added. After vortex mixing for 10 s, 3 mL aliquot of the extraction solvent, *tert*-butyl methyl ether:*n*-hexane (80:20, v/v), was added and the sample was vortex-mixed for 3 min. The organic layer (2.4 mL) was transferred to a glass tube and evaporated to dryness using an evaporator at 40 °C under a stream of nitrogen. Then the

dried extract was reconstituted in 200 μL of reconstitution solvent (10 mM ammonium acetate buffer, pH adjusted to 5.0 with diluted formic acid and acetonitrile, 50/50, v/v) and a 10-μL aliquot was injected into the chromatographic system.

In the extraction of dimebon from 20% brain tissue homogenate in water (0.5 mL), 100 μL of sodium hydroxide solution (0.1N) was added and vortex-mixed. Then further extraction procedure was similar to plasma sample extraction procedure described.

2.4. Bioanalytical method validation

A calibration curve was constructed from a blank sample (a plasma/brain tissue sample processed without the IS), a zero sample (a plasma/brain tissue sample processed with the IS) and 10 non-zero samples covering the total range 0.25–250 ng/mL, including the lower limit of quantification (LLOQ). The five calibration curves were generated using the analyte to the IS peak area ratios by weighted ($1/x^2$) least-squares linear regression on consecutive days. The acceptance criterion for a calibration curve was a correlation coefficient (r) of 0.99 or better, and that each back-calculated standard concentration must be within 15% deviation from the nominal value except at the LLOQ, for which the maximum acceptable deviation was set at 20%. At least 67% of non-zero standards were required to meet the above criteria, including acceptable LLOQ and upper limit of quantification.

The within-batch precision and accuracy were determined by analyzing four sets of QC samples (LLOQ, low, medium and high concentrations) each comprised of six replicates in a batch. The between-batch precision and accuracy were determined by analyzing such five different batches. The acceptance criteria for within- and between-batch precision were 20% or better for LLOQ and 15% or better for the other concentrations, and the accuracy were $100 \pm 20\%$ or better for LLOQ and $100 \pm 15\%$ or better for the other concentrations.

Recovery of dimebon from the extraction procedure was determined by a comparison of the peak area of dimebon in spiked plasma/brain tissue samples (six each of low, medium and high QCs) with the peak area of dimebon in samples prepared by spiking extracted drug-free plasma/brain tissue samples with the same amounts of dimebon at the step immediately prior to chromatog-

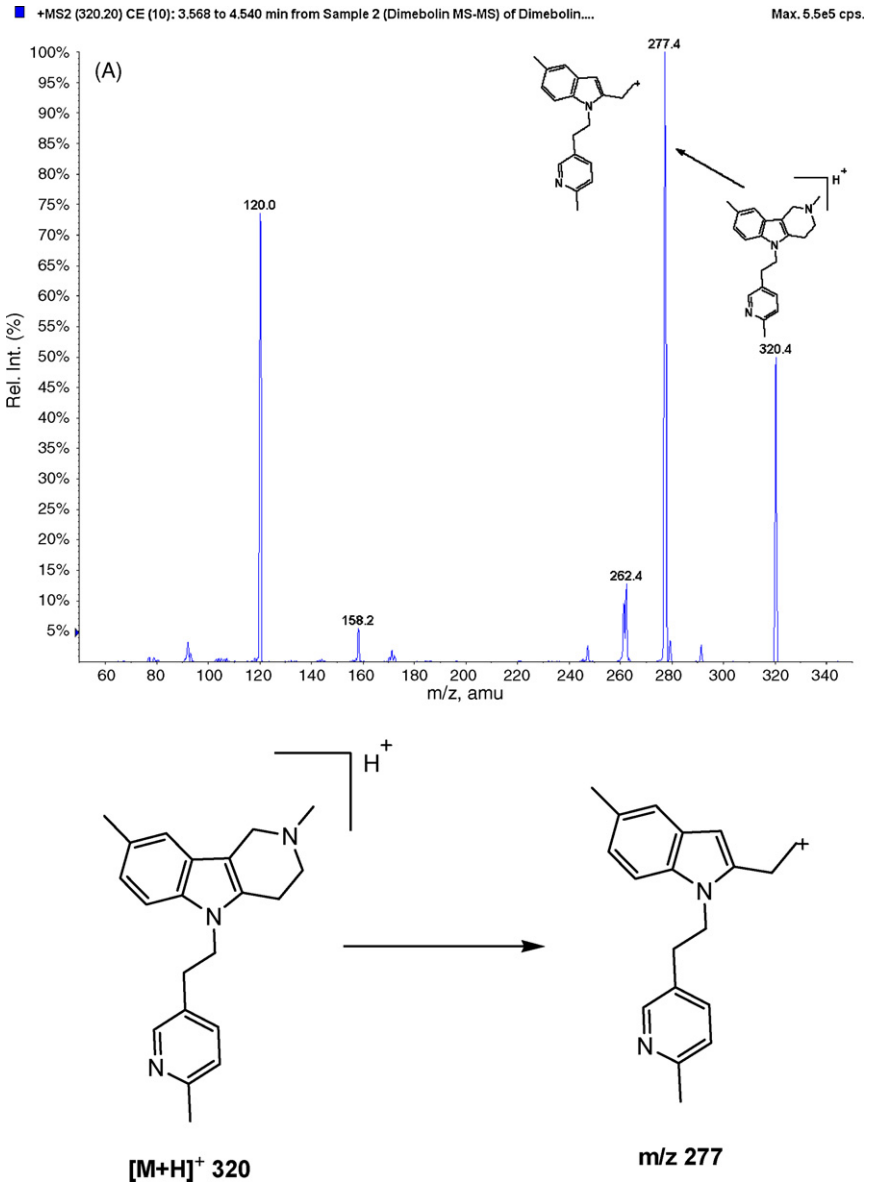

Fig. 2. Full-scan positive ion turboionspray product ion mass spectra and the proposed patterns of fragmentation of (A) dimebon and (B) carvedilol (internal standard).

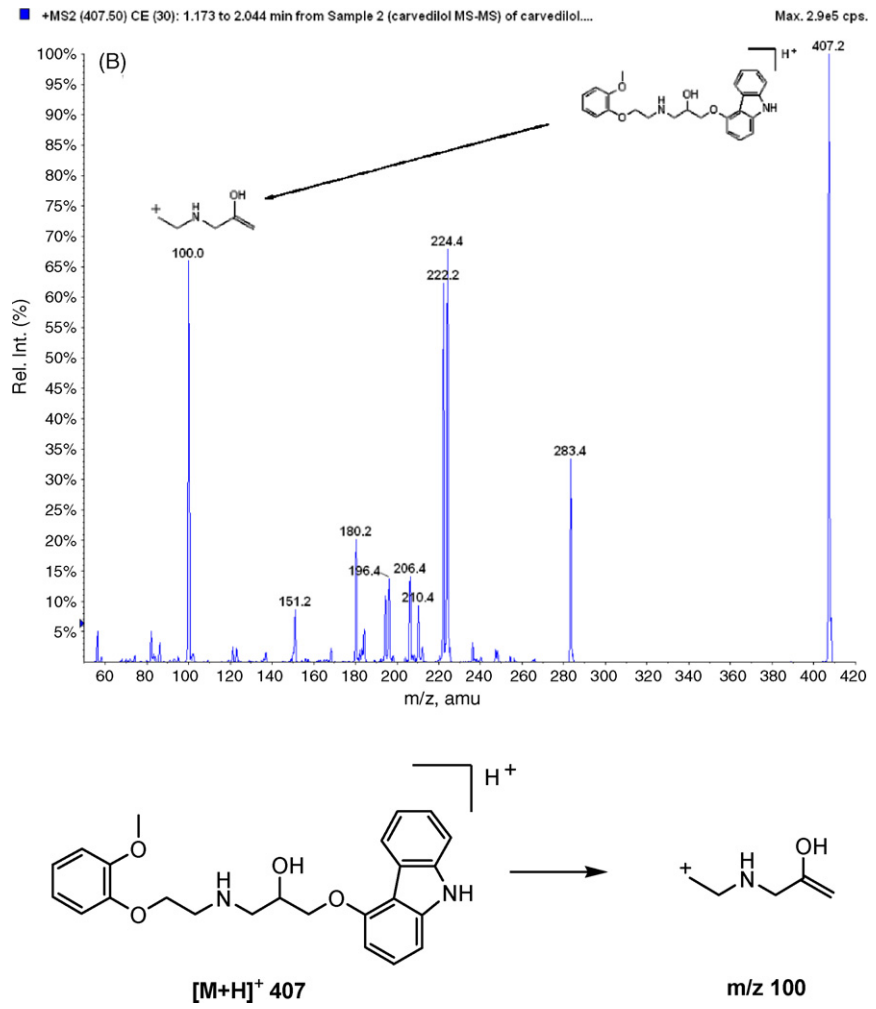

Fig. 2. (*Continued*).

raphy. Similarly, recovery of the IS was determined by comparing the mean peak areas of extracted medium QC samples ($n = 6$) to mean peak areas of the IS in samples prepared by spiking extracted drug-free plasma/brain tissue samples with the same amounts of the IS at the step immediately prior to chromatography.

The stability of the analyte and the IS in rat plasma/brain tissue under different temperature and timing conditions, as well as their stability in the stock solutions were evaluated (data not shown). QC samples were subjected to short-term room temperature conditions, long-term storage conditions (<−50 °C) and freeze–thaw stability studies. All the stability studies were conducted at two concentration levels (0.75 and 200 ng/mL as low and high QC values) with six replicates for each. All these stability samples were compared against freshly prepared samples.

3. Results and discussion

3.1. Mass spectrometry

In order to develop a method with the desired LLOQ (0.25 ng/mL), it was necessary to use MS–MS detection, as MS–MS methods provide improved limit of detection and selectivity. The inherent selectivity of MS–MS detection was also expected to be beneficial in developing a selective and sensitive method. $[M+H]^+$ was the predominant ion in the Q1 spectrum and was used as the precursor ion to obtain product ion spectra. The product ion mass spectra, and their proposed rationalizations in terms of fragmentation patterns of dimebon and the IS are illustrated in Fig. 2. The product ion mass spectrum of dimebon showed predominant fragment ions at *m*/*z* 277, 262, 158 and 120. Dimebon in the positive ionization mode shows a selective loss of the methyl-methyleneamine group, resulting in the product ion *m*/*z* 277 (Fig. 2A). The product ion mass spectrum of the IS showed the formation of characteristic product ions at *m*/*z* 283, 224, 222, 210, 206, 196, 180, 151 and 100. Fragmentation of the IS in the positive ionization mode shows the loss of 9H-carbazol-4-yloxy and 2-methoxy-phenoxy group, resulting in the product ion *m*/*z* 100 (Fig. 2B). The most sensitive mass transition was from *m*/*z* 320–277 for dimebon and *m*/*z* 407–100 for the IS.

3.2. Optimization of chromatographic conditions

Several different chromatographic columns, including Zorbax XDB®, YMC-Pack ODS-AQ®, Waters Atlantis® C_{18}, Chromolith Performance® and Polyhydroxyethyl A were tested to optimize the good peak shape and response. Series of experiments were conducted to select the best stationary and mobile phases that would give optimum peak shape and response. No sharp peak was found on Zorbax XDB® and YMC-Pack ODS-AQ® columns using different possible mobile phases. Peak tailing was found on Waters Atlantis® C_{18} and Chromolith Performance® columns using different possible mobile phases. A significantly good peak shape and response were obtained using Polyhydroxyethyl A column with gradient elution of ammonium acetate buffer and acetoni-

trile (Fig. 3). It was found that a mixture of 10 mM ammonium acetate buffer (pH adjusted to 5 with formic acid) and acetonitrile could achieve this purpose under gradient program and was finally adopted as the mobile phase. Because of high percentage of buffer, Polyhydroxyethyl A allowed good peak shape and retention. Polyhydroxyethyl A column is especially attractive in situations where very high levels of water are required in the mobile phase for adequate retention and for sharper peak shapes. Retention in the column is not well-understood but appears to be a combination of hydrophilic interaction, ion-exchange and some reversed-phase retention. After elution of the analyte and the IS, the gradient conditions were modified to attain equilibrium and also to avoid carryover effect. A flow-rate of 0.8 mL/min produced good peak shapes with retention times of 0.55 min for both the analyte and the IS and permitted a run time of 2.5 min.

The pH of the aqueous phase of the liquid chromatographic mobile phase influences both the chromatographic elution of the compounds and the formation of the $[M+H]^+$ molecular ions and is strongly related to their degree of ionization. The pK_a values of the analyte and the IS were calculated using the MarvinSketch/Swing 4.0.3 software. As both dimebon and carvedilol are neutral and basic compounds with pK_a values 6.8 and 8.7, respectively, the

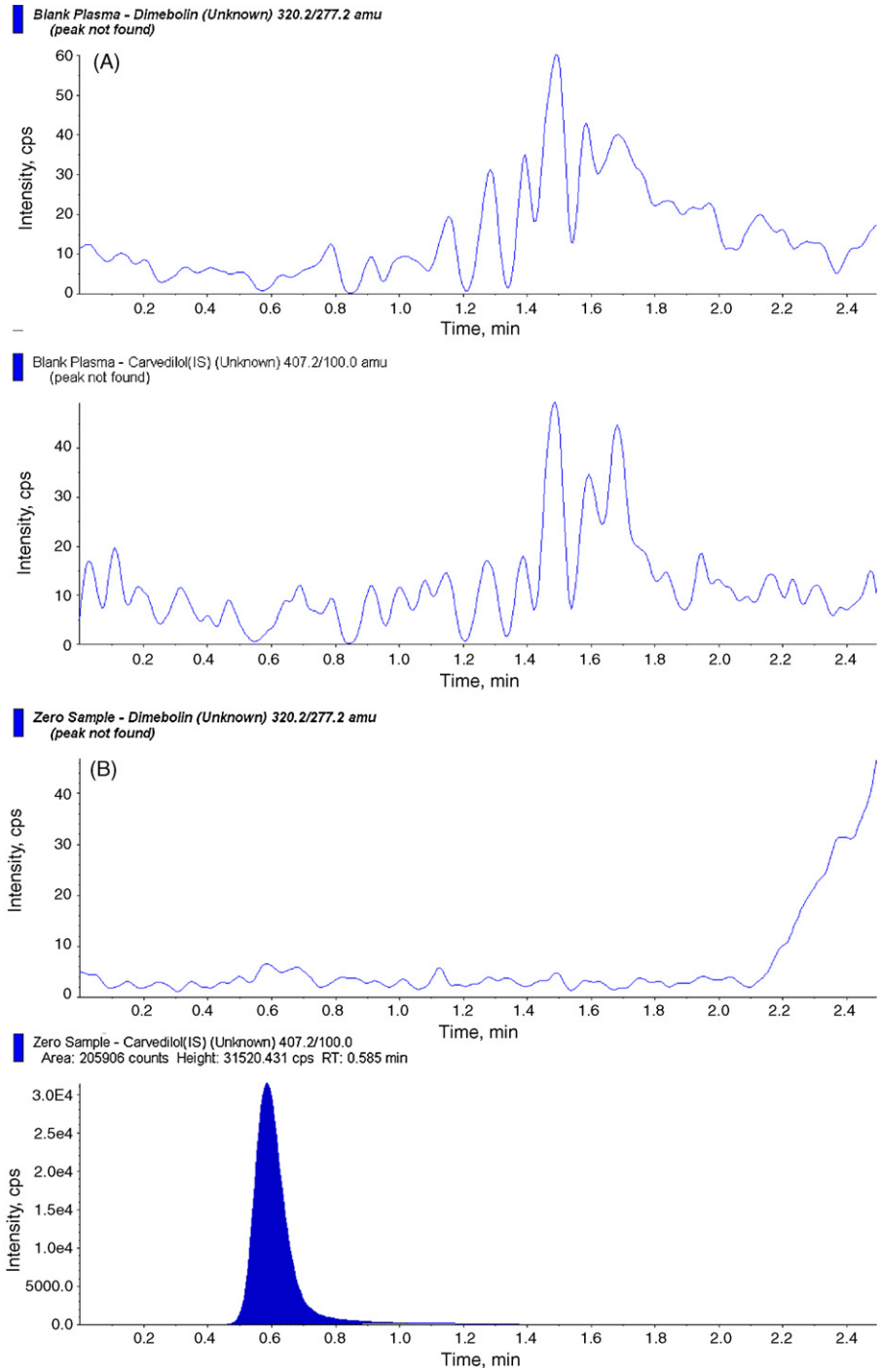

Fig. 3. MRM chromatograms in rat plasma for dimebon and IS resulting from analysis of: (A) blank (drug and the IS free) rat plasma; (B) zero sample (drug-free spiked with the IS) rat plasma; (C) 0.25 ng/mL (LLOQ) of dimebon spiked with the IS.

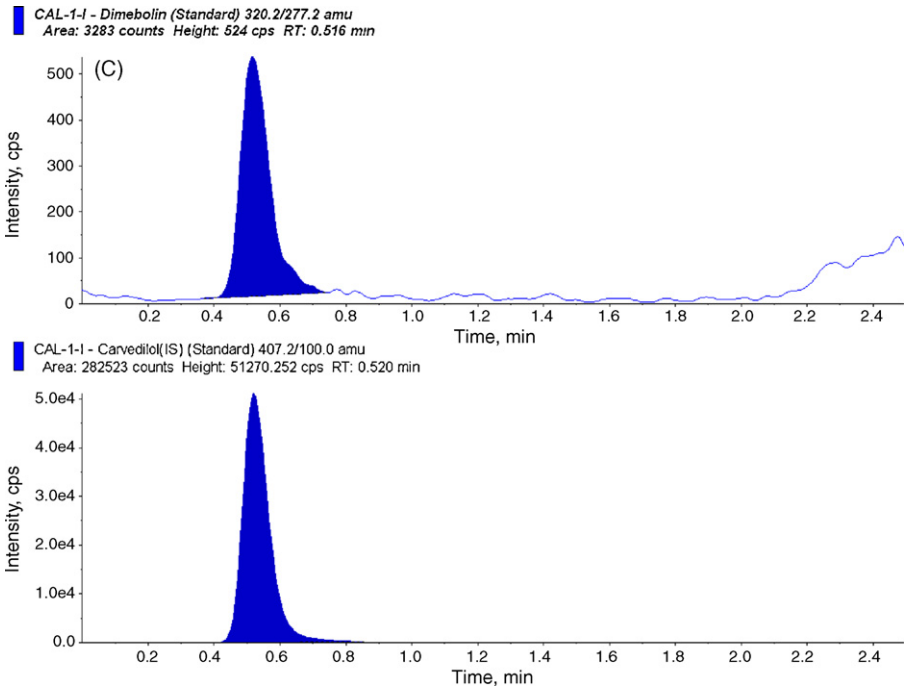

Fig. 3. (*Continued*).

use of slightly acidic solutions favors ionization of the analyte and its IS by protonation of their basic sites. Therefore, it was found that, positive ionization of the compounds in the electrospray ion source increases in acidic mobile phases. Hence the pH of ammonium acetate buffer was adjusted to 5.0 with formic acid to obtain high sensitivity and good peak shapes.

3.3. Optimization of extraction procedures

Liquid–liquid extraction (LLE) was used for the sample preparation in this work. Six organic solvents, *n*-hexane, ethyl acetate, diethyl ether, dichloromethane, chloroform, *tert*-butyl methyl ether and their mixtures in different combinations and ratios were evaluated. Finally, combination of *tert*-butyl methyl ether and *n*-hexane (80:20, v/v) was found to be optimal, which can produce a clean chromatogram for a blank plasma/brain tissue sample. The average absolute recoveries of dimebon from spiked plasma/brain tissue samples was 73.1 ± 2.6% and the recovery of the IS was 78.3 ± 2.4% at the concentration used in the assay (0.5 μg/mL). Recoveries of the analyte and the IS were good and it was consistent, precise and reproducible.

Choosing the appropriate internal standard is an important aspect to achieving acceptable method performance, especially with LC–MS/MS, where matrix effects can lead to poor analytical results. Ideally, an isotopically labeled internal standard for the analyte should be used, but it is not commercially available. Several compounds were investigated to find a suitable IS, and finally carvedilol was found to be suitable which has similar chromatographic properties to dimebon. Clean chromatograms were obtained and no significant direct interferences in the MRM channels at the relevant retention times were observed. However, in ESI, signal suppression or enhancement may occur due to co-eluting endogenous components from the sample matrix. All validation experiments in this assay were performed with matrixes obtained from pooled rat samples. As all data fall within 15% deviation (data not shown), it can be concluded that the degree of matrix effect was sufficiently low to produce acceptable data.

3.4. Assay performance and validation

The 10-point calibration curve was linear over the concentration range 0.25–250 ng/mL. The calibration model was selected based on the analysis of the data by linear regression with/without intercepts and weighting factors ($1/x$, $1/x^2$ and none). The best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x^2$ weighing factor, giving a mean linear regression equation for the calibration curve of:

$$y = 0.0351(\pm 0.0004)x + 0.0005(\pm 0.0007)$$

where y was the peak area ratio of the analyte to the IS and x was the concentration of the analyte. The mean correlation coefficient of the weighted calibration curve generated during the validation was 0.9992 ± 0.0001.

The selectivity of the method was examined by analyzing ($n = 6$) blank rat plasma extracts (Fig. 3A) and an extract spiked only with the IS (Fig. 3B). As shown in Fig. 3A, no significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free rat plasma at the retention time of the analyte. Similarly, Fig. 3B shows the absence of direct interference from the IS to the MRM channel of the analyte. Fig. 3C depicts a representative ion-chromatogram for the LLOQ (0.25 ng/mL) in rat plasma. Excellent sensitivity was observed for a 10-μL injection volume; the LLOQ corresponds to ca. 12.5 pg on-column. The mean response for the analyte peak at the assay sensitivity limit (0.25 ng/mL) was ≈10-fold greater than the mean response for the peak in six blank rat plasma samples at the retention time of the analyte. The between-batch precision at the LLOQ was 5.3%, and the between-batch accuracy was 100.2% (Table 1). The within-batch precision was 3.6% and the accuracy was 97.7% for dimebon.

The lowest and highest QC concentrations of dimebon ranged from 0.75 to 200 ng/mL in rat plasma. For the between-batch experiments the precision ranged from 4.8 to 10.5% and the accuracy from 93.7 to 104.8% (Table 1). For the within-batch experiments the precision and accuracy for the analyte met the acceptance criteria.

Table 1
Precision and accuracy of the method for determining dimebon concentrations in rat plasma samples.

Concentration added (ng/mL)	Between-batch ($n = 30$)			Within-batch ($n = 6$)		
	Concentration found (mean ± S.D.) (pg/mL)	Precision (%)	Accuracy (%)	Concentration found (mean ± S.D.) (pg/mL)	Precision (%)	Accuracy (%)
0.25	0.25 ± 0.01	5.3	100.2	0.24 ± 0.01	3.6	97.7
0.75	0.79 ± 0.08	9.8	104.8	0.77 ± 0.02	2.9	102.7
100	93.7 ± 9.8	10.5	93.7	96.3 ± 3.6	3.8	96.3
200	196.7 ± 9.4	4.8	98.3	196.2 ± 0.7	0.4	98.1

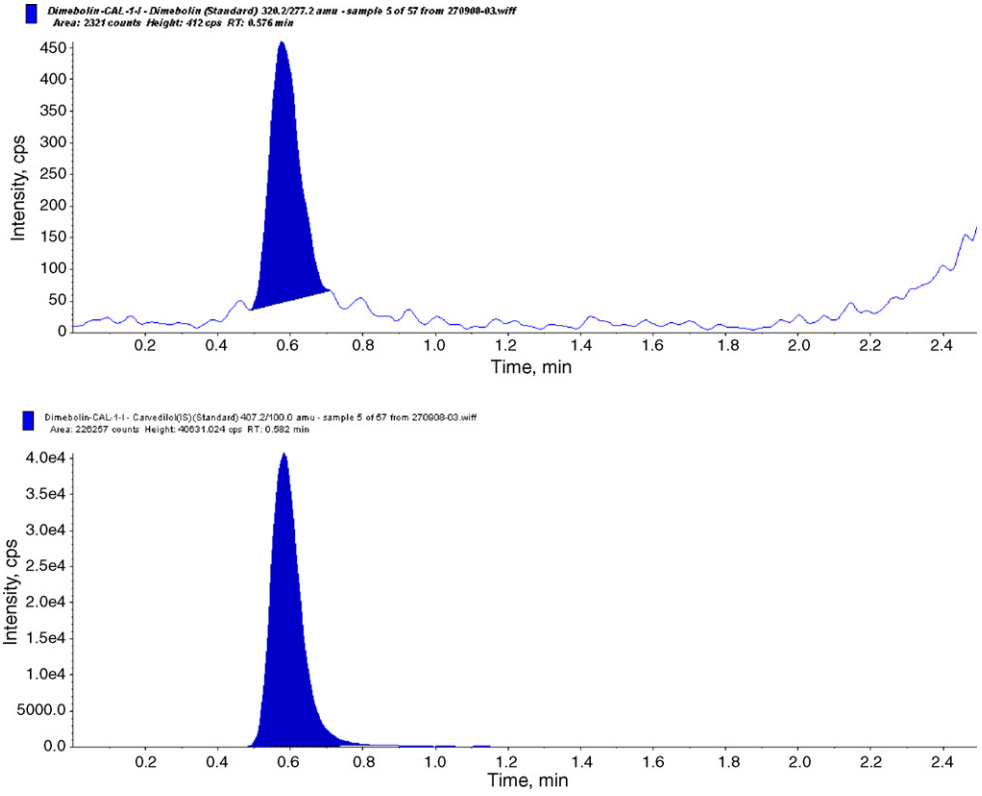

Fig. 4. MRM chromatograms in rat brain tissue for dimebon and IS resulting from analysis of 0.25 ng/mL (LLOQ) of dimebon spiked with the IS.

Similarly no significant direct interference in the blank brain tissue traces was observed from endogenous substances in drug-free rat brain tissue at the retention time of the analyte and no direct interference from the IS to the MRM channel of the analyte (data not shown). Fig. 4 depicts a representative ion-chromatogram for the LLOQ (0.25 ng/mL) in rat brain tissue. The between-batch precision at the LLOQ was 5.4%, and the between-batch accuracy was 98.0% (Table 2). The lowest and highest QC concentrations of dimebon ranged from 0.75 to 200 ng/mL in rat brain. For the between-batch experiments the precision ranged from 6.3 to 8.9% and the accuracy from 94.4 to 101.3% (Table 2). For the within-batch experiments the precision and accuracy for the analyte met the acceptance criteria.

3.5. Stability studies

All the stability data results of low and high QC samples of dimebon were within 10% deviation (data not shown). For short-term stability determination, stored plasma/brain tissue aliquots were thawed and kept at room temperature for a period of time exceeding that expected to be encountered during routine sample preparation (around 24 h). Samples were extracted and analyzed as described above and the results indicate reliable stability behavior under the experimental conditions of the regular analytical procedure. The stability of QC samples kept in the autosampler for 32 h was also assessed. The results indicate that solutions of the analyte and the IS can remain in the autosampler for at least 32 h

Table 2
Precision and accuracy of the method for determining dimebon concentrations in rat brain tissue homogenate samples.

Concentration added (ng/mL)	Between-batch ($n = 30$)			Within-batch ($n = 6$)		
	Concentration found (mean ± S.D.) (pg/mL)	Precision (%)	Accuracy (%)	Concentration found (mean ± S.D.) (pg/mL)	Precision (%)	Accuracy (%)
0.25	0.25 ± 0.01	5.4	98.0	0.25 ± 0.01	5.5	99.7
0.75	0.76 ± 0.08	8.9	101.3	0.75 ± 0.02	2.8	99.9
100	94.4 ± 7.0	7.5	94.4	94.3 ± 1.2	1.3	94.3
200	194.4 ± 12.3	6.3	97.2	197.5 ± 7.5	3.8	98.8

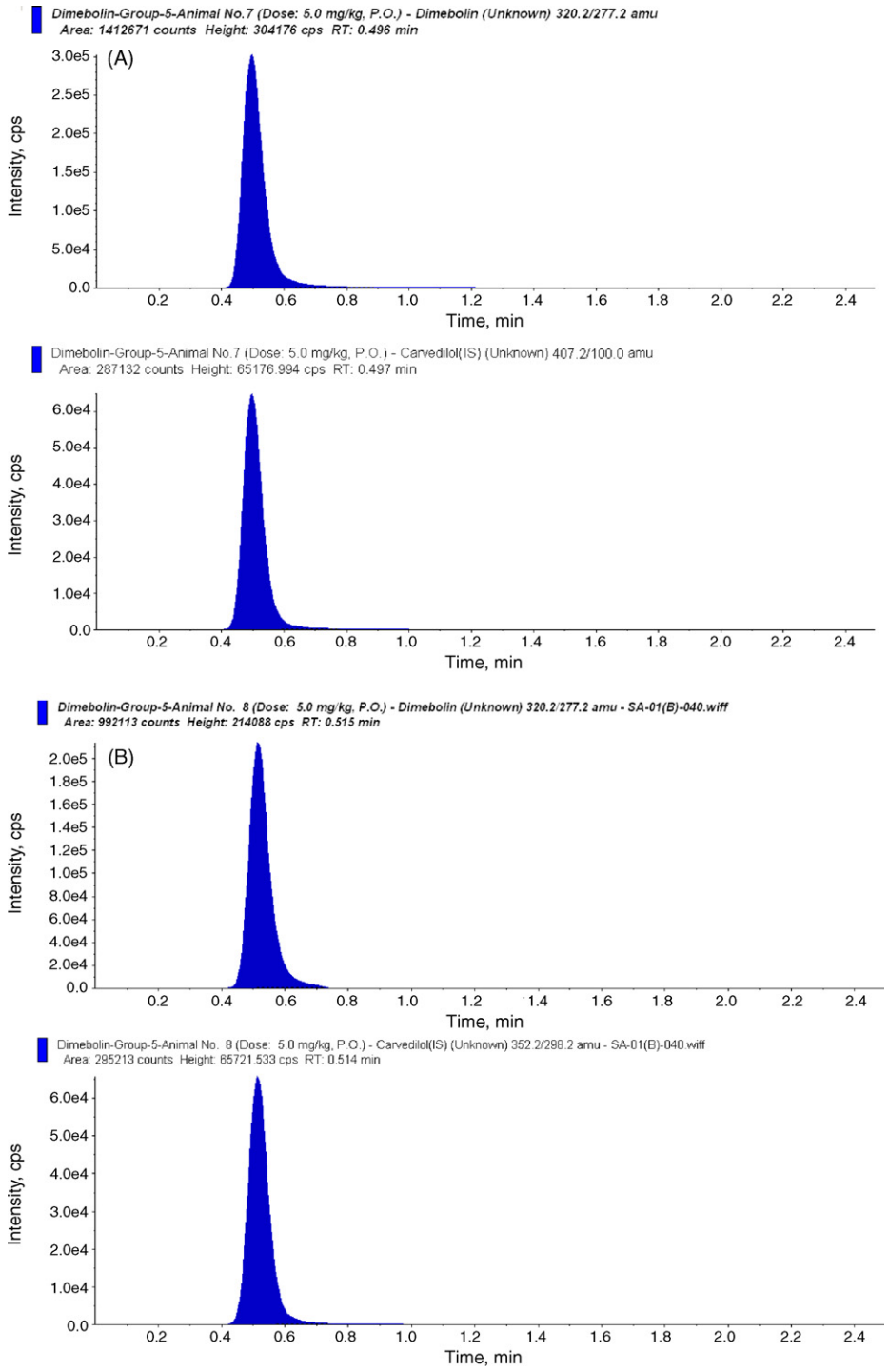

Fig. 5. Representative MRM chromatograms resulting from the analysis of (A) plasma and (B) brain tissue samples, after the oral administration of dimebon (5 mg/kg) to rats.

without showing significant loss in the quantified values, indicating that samples should be processed within this period of time.

The stability data of the analyte in plasma/brain tissue over three freeze–thaw cycles indicate that the analyte is stable in rat plasma/brain tissue for three freeze–thaw cycles, when stored at <−50 °C and thawed to room temperature.

The long-term stability data of the analyte in rat plasma/brain tissue stored for a period of 30 days at <−50 °C showed reliable stability behavior, as the mean of the results of the tested samples were within the acceptance criteria of ±15% of the initial values of the controls. These findings indicate that storage of the analyte in plasma/brain tissue samples at <−50 °C is adequate, and no stability-related problems would be expected during routine analyses for pharmacokinetic studies.

The stability of the stock solutions was tested and established at room temperature for 26 h and under refrigeration (~4 °C) for 30 days (data not shown). The results revealed optimum stability for the prepared stock solutions at least 24 h at room temperature. Therefore stock solutions are stable throughout the period intended for their daily use.

3.6. Application

To demonstrate the applicability of the LC–MS/MS method, a pharmacokinetic study was carried out in Wistar rats. The method was used to quantify concentrations of dimebon in the plasma and brain tissue samples of rats, which received oral administration of 5 mg/kg dose of dimebon. Plasma samples and brain tissue homogenates were frozen at <−50 °C until analyzed. The MRM chromatograms obtained for an extracted rat plasma and brain tissue sample are depicted in Fig. 5. During the study sample analysis, there were no batch rejections and reassays. This demonstrates the performance of the method in real sample analysis.

4. Conclusions

In summary, a method is described for the quantification of dimebon in rodent plasma/brain tissue by LC–MS/MS in positive electrospray ionization mode using carvedilol as internal standard and fully validated according to commonly accepted criteria. The current method has shown acceptable precision and adequate sensitivity for the quantification of dimebon in rat plasma/brain tissue samples obtained from rodent pharmacokinetic study. The desired sensitivity of dimebon was achieved with an LLOQ of 0.25 ng/mL. Many variables related to the electrospray reproducibility were optimized for both precision and sensitivity to obtain these results. The simplicity of the assay using rapid liquid–liquid extraction and characterizing by a turnover rate of 2.5 min per sample, make it an attractive procedure in high-throughput bioanalysis of dimebon. It can be believed that development of a method in rat plasma would facilitate the ease of adaptability of dimebon assay in human plasma for clinical trials.

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