Simple and sensitive LC-MS/MS-based assay for the quantification of dimemorfan in human plasma for use in a pharmacokinetic study

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ABSTRACT: Dimemorfan phosphate has been widely used for 40 years throughout the world for the treatment of coughs. This is the first report on the use of an LC-MS/MS-based assay for the determination of dimemorfan in human plasma using estazolam as an internal standard after one-step protein precipitation with acetonitrile. Chromatographic separation was achieved on a Phenomenex Luna C\textsubscript{18} column (3\,\mu m, 50 × 2.0 mm) using a fast gradient method, which involves water and methanol as the mobile phase (both containing 0.1\% formic acid). Dimemorfan and estazolam were detected with proton adducts at m/z values of 255.8 → 155.1 and 295.0 → 267.0, respectively, in the selected reaction monitoring positive mode. The linear dynamic range of the assay was 0.04–5.00\,ng/mL. The chromatographic run time for each plasma sample was <5 min. The method was proven to be accurate, precise, and repeatable. Finally, the developed method was successfully applied for the determination of dimemorfan in a pharmacokinetic study using healthy Chinese subjects. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: dimemorfan; LC-MS/MS; method validation; human plasma; pharmacokinetics

Introduction

Dimemorfan phosphate was first developed in Japan in 1974 under the trade name Astomin and then subsequently in Italy and Spain. Dimemorphan phosphate (\textit{d}-3-methyl-N-methylmorphinan phosphate, Fig. 1) directly acts on the cough center in the medulla, has an antitussive effect and is more potent than dextromethorphan and codeine. In addition, the adverse reactions associated with the utilization of dimemorphan phosphate are relatively mild, for example, it does not produce drug dependency (Ida, 1997). In addition, dimemorfan is considered a sigma-1 receptor antagonist and also affects the NMDA receptor and L-type calcium channels. These cause dimemorfan to have significant anti-inflammatory (Wang \textit{et al}, 2008), anticonvulsant (Shin \textit{et al}, 2004, 2005; Chou \textit{et al}, 1999) and anti-amnesic (Wang \textit{et al}, 2003) effects in rodents as well as preventing ischemic strokes and having neuroprotective potential (Shen \textit{et al}, 2008; Shin \textit{et al}, 2011). While healthy adult males were orally given 90\,mg of dimemorphan phosphate, peak concentrations of dimemorfan (C\textsubscript{max} 7.00–8.00\,ng/mL) were achieved 1–2 h after dosing, 60\% of the dosed drug was excreted in the urine in 24 h, and <2\% was excreted in its intact form (Ida, 1997). Dimemorfan was metabolized by hepatic CYP3A4 and CYP2D6 to form two major metabolites, including \textit{d}-3-hydroxymethyl-N-methylmorphinan and \textit{d}-3-methylmorphinan (Chou \textit{et al}, 2010). CYP3A plays a major role in the demethylation of dimemorfan (Chou \textit{et al}, 2005).

The reported analytical methods available for the determination of dimemorfan in samples are mainly based on GC/MS (Shigeru \textit{et al}, 1984; Hasegawa \textit{et al}, 2012). There are several disadvantages to these assays, including the following: (a) the utilization of solid-phase extraction to treat the samples, which can be costly and time consuming; (b) the LLOQ (lower limit of quantitation, 1.00–2.50\,ng/mL) fails to meet the requirements for use in a low-dose pharmacokinetic study; and (c) the long chromatographic run time (>8.75 min) limits the throughput required for use in a pharmacokinetic study. Therefore, we have developed a novel LC-MS/MS-based assay for dimemorfan, which is more sensitive, convenient and efficient than the GC/MS-based assays (Shigeru \textit{et al}, 1984; Hasegawa \textit{et al}, 2012). Finally, the newly validated LC-MS/MS method was successfully applied to the determination of dimemorphan phosphate in a pharmacokinetic study on healthy Chinese subjects.

Materials and experiments

Materials and reagents

Dimemorfan was obtained from the Sichuan GuoRui Pharmaceutical Co. Ltd (Sichuan, China). Estazolam (internal standard, IS) was obtained from the National Institutes for Food and Drug Control of China. All of the compounds were of 99.9\% purity. Acetonitrile and methanol of HPLC grade were purchased from...
Merck Co. Ltd (Germany). We purchased HPLC-grade formic acid from ROE Scientific Inc. (Newark, USA). Deionized water was prepared with a SMART ultra-pure water system (Shanghai Canrex Analytic Instrument Co. Ltd, Shanghai, China). All of the other chemicals and solvents were of analytical grade. Dimemorfan phosphate syrup (100 mL:0.25 g, Sichuan GuoRui Pharmaceutical Co. Ltd, China) was used in the pharmacokinetic study. Blank human plasma samples were collected from healthy volunteers and stored at −80°C.

Analytical procedure

**Preparation of stock solutions, calibration standards and quality control samples.** Two samples of dimemorfan (solid) were weighed separately by different individuals; one of them was used to prepare the stock standard solutions of the plasma calibration standards, and the other was used to prepare the stock standard solutions of the quality control (QC) plasma samples. Stock standard solutions of dimemorfan and the IS were prepared in methanol to achieve concentrations of 1.00 × 10⁶ ng/mL for dimemorfan and 50.00 ng/mL for the IS, and the stock standard solutions were stored at 4°C. Then appropriate dilutions of the stock solutions were made to produce working stock solutions (0.39–50.00 ng/mL) in methanol–water (1:1, v/v), which were used to prepare plasma calibration standards, and the other was used to prepare the stock standard solutions of the plasma QC samples. Stock standard solutions of the quality control (QC) plasma samples (0.08, 0.16, 0.31, 0.63, 1.25, 2.50 and 5.00 ng/mL). Quality control plasma samples (0.08, 0.16, 0.31 and 4.00 ng/mL) were also prepared using the same procedure.

**Sample preparation.** Plasma samples were prepared by protein precipitation. Aliquots of 20 μL of the IS (50.00 ng/mL) were added to 220 μL plasma samples and mixed. Then, a 400 μL volume of acetonitrile was added. After a thorough vortex mixing for 2 min and centrifugation at 15,700 g for 10 min, 20 μL aliquots of the extracted supernatant samples were immediately subjected to LC-MS/MS analysis.

**Instrumentation and LC-MS conditions**

**Chromatographic conditions.** The HPLC system used was a Shimadzu LC-20A chromatographic system, which included two LC-20 AD pumps, a SIL-20 ACHT autosampler, a CTO-20 AC column oven and a CBM-20 A system controller (Shimadzu, Japan). Chromatographic separation was achieved on a Phenomenex Luna C18 column (3 μm, 50 × 2.0 mm), which was protected by a guard column (5 μm, 4.0 × 3.0 mm i.d., Phenomenex, USA). The columns were maintained at 40°C, and 20 μL of each sample was subjected to LC-MS/MS analysis. The mobile phase was a mixture of water and methanol, both containing 0.1% formic acid at a flow rate of 0.2 mL/min. The fast gradient method, which enables the fast and sensitive measurement of drugs and metabolites in biological samples, was modified directly from the method reported by Hu et al. (2011, 2013). The fast gradient method is composed of the following steps: begin with a 95% water mobile phase and hold for 0.5 min; change to a 20% water mobile phase and hold for 1.5 min; increase the percentage of the water in the mobile phase from 20 to 95% in 0.01 min; and finally, stop the gradient at 4.5 min.

**Mass spectrometry conditions.** An AB Sciex API 4000 mass spectrometer (Toronto, Canada) with a Turbo V electrospray ionization source was used. Dimemorfan and the IS were detected in positive ion mode. The selected reaction monitoring transitions were performed at m/z values of 255.8 → 155.1 for dimemorfan and 295.0 → 267.0 for the IS. The declustering potential (DP), collision energy (CE), entrance potential and collision exit potential were 85, 47, 10 and 15 V for dimemorfan and the IS, respectively. The curtain gas, ion source gas 1 and ion source gas 2 pressures were 25, 45 and 55 psi, respectively. The ion spray voltage was set to 5500 V, and the source temperature was maintained at 500°C. AB Sciex Analyst software (version 1.4) was used to control the system and perform the data analysis.

**Method validation**

The assay performance was assessed with regard to the specificity, LLOQ (0.04 ng/mL), matrix effects, recovery, linearity, precision, accuracy and stability.

**Specificity.** The specificity of the method was measured by analyzing blank human plasma samples from six different donors, blank plasma spiked with the IS and a plasma sample collected at 12 h after a single oral administration of dimemorfan syrup to a volunteer.

**Matrix effects and extraction recoveries.** The matrix effects and extraction recoveries were determined at three QC levels (0.08, 1.25 and 4.00 ng/mL, n = 6) for dimemorfan and at 5.00 ng/mL for the IS. The matrix effects were measured by comparing the peak areas of the analyte (A) added to post-protein-precipitated blank plasma (from six different batches) with those of the pure standard solution containing equivalent amounts of the compound (B). The ratio (A/B × 100)% was used to indicate the matrix effects. Additionally, according to the European Medicines Agency, the variable coefficient (CV) of the IS-normalized matrix effects calculated from the six different batches of matrix should not be greater than 15% (European Medicines Agency, 2011).

The extraction efficiency was also measured by the equation (A′/B′ × 100)%, where A′ stands for the peak area of the analyte added to blank plasma and then pretreated by protein precipitation, and B′ stands for the analyte spiked with post-protein-precipitated blank plasma at equivalent concentrations.

**Linearity, carry-over effect and LLOQ.** The calibration curves (y = ax + b) were constructed by plotting the peak area ratios of dimemorfan to the IS vs the spiked concentrations of dimemorfan using a 1/x (where x is the concentration of the sample) weighted linear least-squares regression model. Each calibration curve included a blank sample, a zero sample and eight samples containing different concentrations of dimemorfan (n = 6). The blank and zero samples were used to check for interference, and analysis of a blank sample following the analysis of samples of various concentrations for the
calibration curve was also used to monitor the carry-over effects of dimemorfan and the IS. According to guidelines set by the China Food and Drug Administration and the US Food and Drug Administration, the LLOQ can be defined as the lowest concentration on the calibration curve, if the analyte response, accuracy and precision of LLOQ meet the requirements (China Food and Drug Administration, 2005; US Food and Drug Administration, 2001). The accepted criteria for the back-calculated standard concentrations is that they are within ±15% except for the LLOQ (0.040 ng/mL), which should be within ±20% (n = 6).

**Accuracy and precision.** The intra- and inter-day precisions were evaluated by various QC levels (0.08, 1.25, and 4.00 ng/mL, n = 6) on the same day and on three different days. The precision was assessed by the relative standard deviation (RSD, %), and the accuracy was calculated by comparing the calculated concentrations with the nominal concentrations. The precision (RSD) and accuracy (%) should be <15% and within 85–115%, respectively, and we prepared the six samples within the acceptable accuracy and precision for the LLOQ. The RSD and accuracy of the LLOQ (0.04 ng/mL) should be <20% and within 80–120% according to guidelines of the US Food and Drug Administration (2001).

**Stability.** The stability of the stock solutions of dimemorfan and the IS was tested for 6 h at room temperature and for 32 days at 4°C. The stability of dimemorfan in human plasma was estimated by analyzing replicates (n = 3) of plasma samples at the three QC levels (0.08, 1.25, and 4.00 ng/mL) under the following conditions: short-term stability after storage at room temperature (25°C) for 6 h; long-term storage stability after storage at −20°C for 6 days and −80°C for 289 days; and freeze–thaw stability through three freeze–thaw cycles (−80–25°C). The post-preparative stability was examined for 24 h at 20°C. All of their relative standard deviations should be within ±15%.

**Application to a pharmacokinetic study**

The validated method was used to determine the dimemorfan plasma concentrations in a randomized three-period single-dose crossover pharmacokinetic study with a wash-out period of 7 days that was approved by the Ethical Committee of Third Xiangya Hospital of Central South University. Informed consent was obtained before the initiation of the study. In this clinical study, six healthy Chinese male volunteers (20.8 ± 2.6 years and 61.3 ± 4.8 kg) and six female volunteers (21.7 ± 1.5 years and 52.5 ± 4.2 kg) were randomly assigned to three groups. The clinical dose is 20 mg (8 mL); we chose 40 mg (16 mL) as the maximum dose and 10 mg (4 mL) as the minimum dose. After an overnight fast (10 h), subjects took a single oral dose of 10, 20 or 40 mg of dimemorfan syrup (100 mL/0.25 g) with 250 mL of water (accurately measured). Then blood samples (5 mL) were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36 and 48 h after dosing. The heparinized blood was centrifuged at 2840g for 5 min, and the resulting plasma was stored at −80°C until analysis.

**Data analysis**

The Drug and Statistics Software (DAS, version 2.1, Mathematical Pharmacology Professional Committee of China) was applied to calculate the main pharmacokinetic parameters of dimemorfan by a non-compartmental model, and individual plasma concentration–time curves were constructed. Linear regression analysis was performed on log-transformed $C_{\text{max}}$ (maximum plasma concentration) and $\text{AUC}_{0-\infty} = \text{AUC}_{0-t} + C_t/k_t$, where AUC is the area under the plasma concentration–time curve and $k_t$ is the elimination rate constant) against the log-transformed dose, and the 95% CIs (confidence intervals) of the slopes were calculated to assess the dose-proportionality of the $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$. There is dose proportionality for the drug if the 95% CI of the slope includes the value 1.0. Statistical analyses were performed with SPSS software (version 19.0 for Windows; SPSS Inc., Chicago, IL, USA).

**Results and discussion**

**Sample preparation**

Protein precipitation was more convenient and time-saving than solid-phase extraction for sample preparation (Shigeru et al., 1984; Hasegawa et al., 2012). Therefore, a one-step protein precipitation was optimized for sample preparation. Acetonitrile was used as the protein precipitant because no matrix effects were observed. In contrast, matrix effects were found when methanol and trifluoroacetic acid were tested (data not shown).

**Method development**

The optimal mass spectrometric conditions for the detection of dimemorfan and the IS were achieved using the protonated molecular ions (M + H)$^+$ at $m/z$ values of 255.8 for dimemorfan and 295.0 for the IS in the Q1 full-scan mode. The specific product ions with $m/z$ values of 155.1 and 267.0 were selected as target ions for dimemorfan and the IS, respectively. After the optimization of the DP (1–200 V) and CE (5–130 V) values, the following parameters were used for detection: DP = 85 V and CE = 47 V for dimemorfan, and DP = 84 V and CE = 35 V for the IS (Fig. 2).

The peak shapes, retention behaviors and MS intensities of the analytes can be affected by the column, mobile phase, and LC conditions. Several C$_{18}$ columns including the Zorbax Eclipse XDB, Zorbax TC, Luna and AGT Venusil were tested, and the best results were obtained with a Phenomenex Luna C$_{18}$ column. Furthermore, various combinations of methanol, acetonitrile and buffers (formic acid and ammonium formate) were tested. We achieved an optimal mobile phase that consisted of water and methanol (both containing 0.1% formic acid). According to the studies by Hu et al. (2011, 2013), a very fast LC gradient was able to increase the sensitivity of the analytes by narrowing the peak widths and eluting the analytes at a high concentration of the organic component of the mobile phase. The fast LC gradient retains the analytes with a mobile phase containing 95% water, allowing the unretained polar matrix components to be eluted from the column (Hu et al., 2013). The analytes are then eluted from the column with a mobile phase containing a high proportion of organic solvent (methanol) (Hu et al., 2013). Taking advantage of this approach, very narrow peaks (as shown in Fig. 3) and high MS intensities were achieved for dimemorfan and the IS. The retention times of dimemorfan and the IS were 2.15 and 2.42 min, respectively, and their widths were 0.15 and 0.2 min, respectively. In addition, the LC run time was only 4.5 min for each sample.
Method validation

Specificity. Six blank plasma samples, plasma samples spiked with dimemorfan (LLOQ, 0.04 ng/mL) and the IS, and plasma samples \((n = 6)\) collected 12 h after a single oral administration of dimemorfan syrup were analyzed. Comparison of the chromatograms indicated that there was no endogenous interfering component at the retention times of dimemorfan and the IS (Fig. 3), suggesting that the specificity of the results was satisfactory.

Matrix effect and recovery

For the low (0.08 ng/mL), medium (1.25 ng/mL) and high (4.00 ng/mL) concentrations of the QC samples, the matrix effects of dimemorfan were 88.1, 88.2 and 87.3%, the matrix effects of dimemorfan normalized by the IS were 88.8, 90.1 and 83.8%, and the CVs (%) were 7.5, 6.5 and 12.7%, respectively. Furthermore, the RSD values of the matrix effects normalized by the IS were 4.9, 8.6 and 7.9%, respectively. The recoveries of dimemorphan at the concentrations of 0.08, 1.25 and 4.00 ng/mL were 109.3, 106.9 and 103.8%, and the recoveries of the IS were 101.4, 105.8 and 104.7%, respectively. The results indicated that the matrix effects and the recoveries of dimemorfan and the IS were acceptable, which suggests that the developed assay is reliable.

Linearity, carry-over effect and LLOQ. The method has good linearity in the concentration range of 0.04–5.00 ng/mL for dimemorfan in human plasma. The linear regression equation of the analyte calibration curve \((n = 6)\) was \(Y = (8.40 \pm 1.65) \times 10^{-3}X + (4.78 \pm 2.98) \times 10^{-4}\) \((r = 0.998 \pm 0.001)\). The average regression coefficient \((n = 6)\) was \(\geq 0.995\).
The peak areas of the blank samples injected immediately after the sample with the highest concentration were <5% of the LLOQ concentration. Furthermore, under the same conditions, there was no signal for the IS at the ion channel of the IS, which means that the carry-over effect was negligible (European Medicines Agency, 2011).

The LLOQs in previous reports were 1.00 and 2.50 ng/mL (Shigeru et al., 1984; Hasegawa et al., 2012). The LLOQ of this LC-MS/MS-based assay for the quantification of dimemorfan

### Table 1. Inter-day (n = 3) and intra-day (n = 6) precision and assay accuracy of quality control samples for the determination of dimemorfan at three concentrations (0.08, 1.25 and 4.00 ng/mL) in human plasma

<table>
<thead>
<tr>
<th>Spiked concentration (ng/mL)</th>
<th>Inter-day (n = 3)</th>
<th>Intra-day (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured concentration</td>
<td>Precision (RSD %)</td>
</tr>
<tr>
<td>0.08</td>
<td>0.08</td>
<td>5.4</td>
</tr>
<tr>
<td>1.25</td>
<td>1.28</td>
<td>2.3</td>
</tr>
<tr>
<td>4.00</td>
<td>4.30</td>
<td>5.0</td>
</tr>
</tbody>
</table>
method was 0.04 ng/mL, and the accuracy and precision were 103.7 and 9.2%. The analyte response at the LLOQ should be at least 5 times the response compared with the blank response (as shown in Fig. 3B), which was sufficient for the pharmacokinetic study of dimemorfan even at the lowest dosage level (10 mg).

**Precision and accuracy.** The intra- and inter-day assay accuracy and precision data are summarized in Table 1. The inter-day accuracy ranged from 98.5 to 107.5%, with the precision ranging from 2.3 to 5.4%. The intra-day accuracy ranged from 102.7 to 103.3%, and the precision ranged from 2.7 to 6.9%. It was evident that both the precision and accuracy of the method are satisfactory.

**Stability studies.** The dimemorfan and IS stock solutions were stable at room temperature for 6 h and at 4°C for 32 days. For the human plasma samples, there was no significant degradation at room temperature for 6 h, over three freeze–thaw cycles at −80°C, at −20°C for 6 days, or at −80°C for 289 days. The post-preparative samples were also stable in the autosampler maintained at 20°C for 24 h. The results of the stability tests are summarized in Table 2. The results were within the assay variability limits (±15%) throughout the entire experimental process.

**Dilution test**

Samples diluted from three high concentrations (5.00, 10.00 and 16.00 ng/mL) using blank human plasma were analyzed to study the dilution effects. The 5.00 ng/mL concentration was diluted by 2- and 4-fold, the 10.00 ng/mL concentration by 2-fold, and the 16.00 ng/mL concentration by 4-fold using blank human plasma.

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**Table 2.** Results of stability of dimemorfan under various conditions

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>6 h Room temperature (n=3)</th>
<th>289 days −80°C (n=3)</th>
<th>6 days 20°C (n=3)</th>
<th>Three cycles, freeze–thaw (n=3)</th>
<th>24 h post-preparative (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bias (%)</td>
<td>RSD (%)</td>
<td>Bias (%)</td>
<td>RSD (%)</td>
<td>Bias (%)</td>
</tr>
<tr>
<td>0.08</td>
<td>9.2</td>
<td>10.7</td>
<td>14.6</td>
<td>5.2</td>
<td>7.9</td>
</tr>
<tr>
<td>1.25</td>
<td>8.9</td>
<td>3.3</td>
<td>6.8</td>
<td>6.2</td>
<td>3.2</td>
</tr>
<tr>
<td>4.00</td>
<td>4.8</td>
<td>4.4</td>
<td>6.4</td>
<td>3.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

**Table 3.** Mean concentration, accuracy and RSD of samples diluted 5.00 ng/mL 2- and 4-fold, 10.00 ng/mL 2-fold, and 16.00 ng/mL 4-fold with human plasma blank (n=6)

<table>
<thead>
<tr>
<th>High concentration (ng/mL)</th>
<th>Magnification</th>
<th>Mean (ng/mL)</th>
<th>Accuracy (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.00</td>
<td>2-fold (n=6)</td>
<td>2.50</td>
<td>99.8</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>4-fold (n=6)</td>
<td>1.21</td>
<td>96.9</td>
<td>3.0</td>
</tr>
<tr>
<td>10.00</td>
<td>2-fold (n=6)</td>
<td>5.07</td>
<td>101.4</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>4-fold (n=6)</td>
<td>4.18</td>
<td>104.5</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**Figure 4.** Mean (SD) plasma concentration–time profiles of dimemorfan from 12 healthy Chinese volunteers after the oral administration of a single dose of 10, 20 or 40 mg of dimemorfan syrup. Each point represents the mean and standard deviation (SD).

**Table 4.** Pharmacokinetic parameters (mean ± SD) of dimemorfan after single oral administration of 10, 20 and 40 mg to healthy Chinese volunteers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>10 mg</th>
<th>20 mg</th>
<th>40 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>10.4 ± 2.6</td>
<td>11.0 ± 2.6</td>
<td>10.8 ± 2.3</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>2.8 ± 1.2</td>
<td>2.0 ± 0.6</td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td>$C_{max}$ (μg/L)</td>
<td>0.94 ± 0.44</td>
<td>2.19 ± 1.40</td>
<td>5.30 ± 4.83</td>
</tr>
<tr>
<td>$AUC_{0-1}$ (μg h/L)</td>
<td>11.06 ± 5.74</td>
<td>26.41 ± 18.08</td>
<td>53.78 ± 38.28</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (μg h/L)</td>
<td>12.14 ± 6.02</td>
<td>28.46 ± 20.21</td>
<td>57.26 ± 41.40</td>
</tr>
<tr>
<td>$V$ (L)</td>
<td>15,244 ± 7617</td>
<td>12,831 ± 1306</td>
<td>1445 ± 1668</td>
</tr>
<tr>
<td>$CL$ (L/h)</td>
<td>1189 ± 1009</td>
<td>1283 ± 1306</td>
<td>20,211 ± 20,953</td>
</tr>
</tbody>
</table>

$T_{1/2}$, Half-life; $T_{max}$, time to reach $C_{max}$; $C_{max}$, maximum plasma concentration; $AUC_{0-1}$, area under the plasma concentration–time curve; $V$, apparent volume of distribution; $CL$, clearance.
blank human plasma (n = 6). Their precisions were 3.3, 3.0, 7.1 and 3.6%, and their mean accuracies were 99.8, 96.9, 101.4 and 104.5%, respectively. These data (Table 3) indicated that samples whose concentrations were beyond the upper limit of the standard curves could be reanalyzed by simple dilution.

**Pharmacokinetic study**

The present method was applied to the analysis of plasma samples obtained from the single-dose pharmacokinetic study of dimemorfan syrup. Figure 4 depicts the mean plasma concentration–time profile of dimemorfan, and the pharmacokinetic parameters were calculated using DAS software (Table 4). $T_{\text{max}}$ was about 2 h and $C_{\text{max}}$ values were 0.94 ± 0.44, 2.19 ± 1.40 and 5.30 ± 4.83 for 10, 20 and 40 mg, respectively. The results of log–log regression analysis of $C_{\text{max}}, \text{AUC}_{0–\infty}$ and $\text{AUC}_{0–\infty}$ vs dose (Ln$C_{\text{max}}, \ln\text{AUC}_{0–\infty}$ and $\ln\text{AUC}_{0–\infty}$ vs lnDose) revealed slopes of 1.08 (95% CI, 0.64–1.51), 1.04 (95% CI, 0.56–1.52) and 1.00 (95% CI, 0.54–1.47), indicating a dose-proportional relationship within the 10–40 mg dosage range. The previous report (Ida, 1997) supported our results.

**Conclusions**

In summary, a simple, sensitive, specific and repeatable LC-MS/MS assay was developed and validated to quantify dimemorfan in human plasma. The current assay shows significant improvement in assay performance compared with the published GC/MS-based assays because of its simple sample preparation, short assay time and high sensitivity. Finally, the assay was successfully applied to a pharmacokinetic study of dimemorfan in humans.

**Acknowledgements**

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**References**


Chou YC, Liao JF, Chang WY, Lin MF and Chen CF. Binding of dimemorfan to sigma-1 receptor and its anticonvulsant and locomotor effects in mice, compared with dextromethorphan and dextrophan. *Brain Research* 1999; **821**: 516–519.


Shin EJ, Nah SY, Kim WK, Ko KH, Jhoo WK, Lim YK, Cha JY, Chen CF and Kim HC. The dextromethorphan analog dimemorfan attenuates kainate-induced seizures via sigma1 receptor activation: comparison with the effects of dextromethorphan. *British Journal of Pharmacology* 2005; **144**: 908–918.


