A simple and rapid liquid chromatography–tandem mass spectrometric method is developed and validated for the determination of gabapentin in human plasma. Metformin is used as an internal standard. The method utilizes protein precipitation with acetonitrile followed by separation on a C₈ column using 10 mM ammonium formate buffer pH 3.0 and acetonitrile as mobile phase. Detection was performed on a quadrupole mass spectrometer using an electrospray ionization interface in selected reaction monitoring mode. The method proves to be specific, sensitive, accurate, precise, and linear over the concentration range of 50–5000 ng/mL with correlation coefficients greater than 0.99. The lower limit of quantification for gabapentin is 50 ng/mL using a 200-µL plasma sample. Intra- and inter-day precisions are less than 8.4% whereas accuracies are within 10.2%. The method is successfully applied to a bioequivalence study of gabapentin in 24 healthy volunteers.

Introduction

Gabapentin is an antiepileptic drug structurally related to the neurotransmitter gamma-aminobutyric acid (GABA). Gabapentin is indicated as adjunctive therapy in the treatment of partial seizures with and without secondary generalization in adults with epilepsy and for the management of postherpetic neuralgia. The mechanism of action of gabapentin is not completely understood because it is neither a GABA agonist nor an inhibitor of GABA uptake or degradation. Gabapentin is absorbed with time to peak plasma concentration of 2–3 h. The drug is not protein-bound or metabolized. Gabapentin is mainly excreted by the kidney as unchanged drug with an elimination half-life of 5–7 h (1).

Several analytical methods have been reported for the determination of gabapentin in human plasma and biological samples. The methods are based on high-performance liquid chromatography (HPLC) with UV (2–4) or fluorescence detection (5–10), capillary electrophoresis (CE) (11–12), gas chromatography (GC) with flame ionization (13) or mass spectrometry (MS) (14–16), and liquid chromatography–tandem mass spectrometry (LC–MS–MS) (17–22). The HPLC and CE methods are time-consuming because they require derivatization of gabapentin to produce a detectable chromophore. The GC methods also require derivatization of gabapentin to improve volatility and avoid column interactions. For routine analysis, derivatization step increases the time of sample preparation and cost. LC–MS–MS is currently considered the method of choice for determining gabapentin levels in biological samples because the drug can be directly detected without derivatization, thus making sample preparation time shorter. The purpose of the present study was to develop and validate an LC–MS–MS method with simple sample preparation to determine gabapentin concentration in human plasma and to apply it to a bioequivalence study of 400 mg gabapentin capsule in 24 healthy volunteers.

Chemicals and Reagents

Gabapentin (Sandoz Pvt. Ltd., Thane, India) was obtained from Novartis Ltd. (Bangkok, Thailand). Metformin hydrochloride [internal standard (IS)] was supplied by Bureau of Drug and Narcotic (Nonthaburi, Thailand). Ammonium formate (Fluka, Neu-Ulm, Germany) and formic acid (Carlo Erba, Milano, Italy) were analytical-grade. HPLC-grade methanol and acetonitrile were obtained from Mallinckrodt (Paris, KY). Drug-free human plasma was purchased from the Thai Red Cross Society (Bangkok, Thailand).

Instrumentation and LC–MS–MS conditions

The LC–MS–MS was performed using a TSQ Quantum Ultra connected to a Finnigan Surveyor HPLC system (Thermo Electron Corporation, San Jose, CA). Separation was performed on an Acclaim 120 C₈ column (3 µm, 100 x 2.1 mm i.d., Dionex, Sunnyvale, CA), protected by a C₈ guard column (4 x 3.0 mm i.d., Phenomenex, Torrance, CA). The mobile phase was a mixture of 10 mM ammonium formate buffer pH 3.0 (adjusted with formic acid) and acetonitrile (40:60, v/v) at a flow rate of 0.2 mL/min. The column and autosampler tray temperatures were set at 30°C.
and 10°C, respectively. Detection was performed on a quadrupole mass spectrometer equipped with an electrospray ionization source operated in positive ion mode. The optimum MS conditions included a capillary temperature of 235°C, vaporizer temperature of 200°C, spray voltage of 4500 V, and collision energies of 12 eV for gabapentin and 14 eV for metformin. Quantification was performed using selected reaction monitoring (SRM) of the transitions of \( m/z \) 172 → 154 for gabapentin and \( m/z \) 130 → 113 for metformin. The scan time for each analyte was set to 0.2 s, and argon was used as the collision gas.

Preparation of calibration standards and quality control samples
Stock solutions (1000 µg/mL) of gabapentin and IS were separately prepared in methanol. Working solutions of gabapentin were prepared by diluting aliquots of stock solution with 50% methanol. Calibration standards were prepared by spiking 180 µL blank plasma with 20 µL gabapentin working solutions to produce final concentrations of 50, 100, 250, 500, 1000, 2500, 4000, and 5000 ng/mL. Quality control samples (QCs) of gabapentin in human plasma were prepared at lower limit of quantification (LLOQ), low (QCL), medium (QCM), and high (QCH) concentrations of 50, 150, 2000, and 4500 ng/mL, respectively.

Sample preparation
Plasma samples were deproteinized with acetonitrile. Briefly, 50 µL of IS solution (1500 ng/mL) and 500 µL of acetonitrile were added to a 200 µL aliquot of human plasma, vortex-mixed for 15 s, and centrifuged at 13,000 rpm for 5 min. An aliquot of 5 µL of the supernatant was injected into the LC–MS–MS system.

Method validation
The method was validated according to the currently accepted U.S. Food and Drug Administration bioanalytical method validation guidance (23) on selectivity, linearity, accuracy, precision, recovery, dilution integrity, matrix effect, and stability. Selectivity was evaluated using at least six different batches of blank plasma. Any endogenous peak found in blank plasma at the retention time of analyte should be less than 20% of the mean response of analyte in extracted LLOQ whereas that at the retention time of IS should be less than 5% of the mean response of IS.

Figure 1. Mass spectra of gabapentin and metformin (IS).

Figure 2. Typical chromatograms of blank human plasma (A); blank human plasma spiked with gabapentin at LLOQ (50 ng/mL) and metformin as IS (B); and plasma sample of a volunteer at 4 h after oral administration of 400 mg gabapentin (C).
in extracted LLOQ. The calibration curves were generated by plotting drug to IS peak area ratios against drug concentrations using weighted (1/concentration^2) least-squares linear regression. The intra-day accuracy and precision were determined using six replicates of QCs at four concentration levels (LLOQ, QCL, QCM, and QCH). The inter-day accuracy and precision were determined for three days. Accuracy expressed as relative error (RE) and precision expressed as coefficient of variation (CV) should be within 15%, except at LLOQ where it should be less than 20%. Recovery was evaluated using six replicates at QCL, QCM, and QCH by comparing mean peak areas of extracted QCs with those of extracted blank plasma post-fortified with QC working solutions. Matrix effect was assessed using six replicates of QCL and QCH in six different batches of blank plasma including normal, lipemic, and hemolyzed plasma. Accuracy and precision within 15% was considered “no matrix effect.” Dilution integrity was assessed by diluting samples having concentration exceeding the concentration of an upper limit of quantification (ULOQ) with blank plasma prior to extraction and assayed along with calibration curve and regular QC samples in one of the validation runs. Accuracy and precision of dilution integrity samples should be within 15%. Stability of gabapentin in plasma was evaluated for short-term and long-term storage after three freeze-thaw cycles and post-preparative stability. Stability of gabapentin in plasma was evaluated for three days. Accuracy expressed as relative error (RE) and precision expressed as coefficient of variation (CV) were determined for three days. Accuracy expressed as relative error (RE) and precision expressed as coefficient of variation (CV) should be within 15%, except at LLOQ where it should be less than 20%. Recovery was evaluated using six replicates at QCL, QCM, and QCH by comparing mean peak areas of extracted QCs with those of extracted blank plasma post-fortified with QC working solutions. Matrix effect was assessed using six replicates of QCL and QCH in six different batches of blank plasma including normal, lipemic, and hemolyzed plasma. Accuracy and precision within 15% was considered “no matrix effect.” Dilution integrity was assessed by diluting samples having concentration exceeding the concentration of an upper limit of quantification (ULOQ) with blank plasma prior to extraction and assayed along with calibration curve and regular QC samples in one of the validation runs. Accuracy and precision of dilution integrity samples should be within 15%. Stability of gabapentin in plasma was evaluated for short-term and long-term storage after three freeze-thaw cycles and post-preparative stability. Stability of gabapentin and IS stock solutions was also determined.

Results and Discussion

Selectivity
The mass spectra of gabapentin and IS are presented in Figure 1. Under the described chromatographic conditions, the retention times of gabapentin and IS were 1.61 and 1.54 min, respectively. No peak interferences were observed at the retention times of analyte and IS. Figure 2 shows chromatograms obtained from blank plasma, blank plasma spiked with analyte at LLOQ and IS, and plasma sample of a volunteer obtained 4 h after oral administration of 400 mg gabapentin capsule, indicating selectivity of the method for routine sample analysis.

Table I. Intra- and Inter-day Accuracy and Precision for Gabapentin Determination in Human Plasma

<table>
<thead>
<tr>
<th>Gabapentin concentration (ng/mL)</th>
<th>50.0</th>
<th>150.0</th>
<th>2000.0</th>
<th>4500.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-day (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>50.6 ± 3.1</td>
<td>153.9 ± 4.1</td>
<td>2086.4 ± 33.8</td>
<td>4501.3 ± 119.5</td>
</tr>
<tr>
<td>RE (%)</td>
<td>1.2</td>
<td>2.6</td>
<td>4.3</td>
<td>0.0</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.1</td>
<td>2.7</td>
<td>1.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>44.9 ± 1.3</td>
<td>140.6 ± 5.0</td>
<td>2076.7 ± 50.2</td>
<td>4395.2 ± 54.2</td>
</tr>
<tr>
<td>RE (%)</td>
<td>-10.2</td>
<td>-6.2</td>
<td>3.8</td>
<td>-3.3</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.9</td>
<td>3.6</td>
<td>2.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>49.5 ± 4.1</td>
<td>151.9 ± 6.5</td>
<td>2117.3 ± 34.7</td>
<td>4570.1 ± 117.5</td>
</tr>
<tr>
<td>RE (%)</td>
<td>-1.1</td>
<td>1.3</td>
<td>5.9</td>
<td>1.6</td>
</tr>
<tr>
<td>CV (%)</td>
<td>8.4</td>
<td>4.3</td>
<td>1.6</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Inter-day (n = 18)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>48.3 ± 3.8</td>
<td>148.8 ± 7.8</td>
<td>2093.4 ± 41.8</td>
<td>4488.9 ± 120.8</td>
</tr>
<tr>
<td>RE (%)</td>
<td>-3.4</td>
<td>-0.8</td>
<td>4.7</td>
<td>-0.2</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.9</td>
<td>5.2</td>
<td>2.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Linearity and sensitivity
Linear regression was performed with a weighting factor of 1/concentration^2. The method exhibited good linearity over a concentration range of 50–5000 ng/mL with correlation coefficients (r) greater than 0.99 as determined on three different days. Accuracy and precision at LLOQ were proved to be within acceptable limits. Previous study has reported LLOQ at 20 ng/mL using additional evaporation and concentration steps after protein precipitation as sample preparation method (22). Although the LLOQ of the present method was 50 ng/mL, it was able to determine gabapentin levels in actual plasma samples until 32 h after drug administration in bioequivalence studies. The resulting mean values of area under the curve (AUC) extrapolation to infinity were less than 4% (data not shown), indicating sufficient sensitivity of the method. In addition, the present method required a single sample extraction step, resulting in short analysis time and high sample throughput.

Accuracy and precision
As shown in Table I, the intra-day accuracy ranged from –10.2 to 5.9% whereas precision was less than 8.4%. The inter-day accuracy ranged from –3.4 to 4.7%, and precision was less than 7.9%. These results indicated that the method is accurate, reliable, and reproducible.

Recovery and matrix effect
Mean recoveries of gabapentin from plasma were 85.4%, 88.5%, and 92.4% for QCL, QCM, and QCH, respectively. The mean recovery of IS was 68.3%. Assessment of matrix effect was performed to ensure that accuracy and precision was not affected by different batches of plasma. The back-calculated concentrations of analyte in QCL and QCH showed accuracy within 1.9% and precision of less than 11.6%. These results were well within the acceptable limits, indicating no impact of the matrix effect on the analytical method.

Dilution integrity
The purpose of dilution integrity experiment was to demonstrate the validity of dilution procedure when performing routine analysis of study samples originally more than the ULOQ. Dilution integrity was tested by determining analyte concentrations in 2- and 10-fold dilution samples prepared from a drug spiked plasma sample at 1.5-times the ULOQ concentration (7500 ng/mL) in six replicates. Accuracy and precision of dilution integrity samples were within 5.6% and 3.4%, respectively.

Stability
Stability testing was conducted to evaluate analyte stability in stock solutions and in plasma samples under different conditions. Stock solution stability was performed by comparing peak area response of sample of analyte and IS with that of sample prepared from fresh stock solutions. Stock solutions of both analyte and IS kept at room temperature were stable for 5 h, and those stored at –20°C were stable for five months. Spiked plasma samples were stable for at least 5 h at room temperature, five months at –20°C,
and after three freeze-and-thaw cycles. Processed samples were stable for 22 h at 10 °C and after three freeze-and-thaw cycles. Processed samples were stable for 22 h at 10 °C in an autosampler. Accuracy was in the range of 96.3–103.3% for QCL and 92.4–101.3% for QCH. These results showed reliable stability behavior of gabapentin under various storage conditions.

Method application

The proposed and validated method was used to determine gabapentin concentrations in plasma samples of 24 healthy Thai volunteers who participated in a bioequivalence study of 400 mg gabapentin capsule consumption under fasting conditions. Blood samples (6 mL) were drawn at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 9, 12, 24, and 32 h after dosing. Plasma samples were separated and kept frozen at −20 °C until analysis. Figure 3 shows the mean plasma concentrations-time profiles of gabapentin. The data generated as parts of this study demonstrated suitability of this LC–MS–MS method to pharmacokinetic and bioequivalence studies.

Conclusion

A simple and rapid LC–MS–MS method for the determination of gabapentin in human plasma was developed and fully validated using a single step protein precipitation as sample preparation procedure. The method is accurate, precise, and linear over the concentration range of 50–5000 ng/mL. The present method was successfully applied to a bioequivalence study of 400 mg gabapentin capsules in 24 healthy Thai volunteers.

Acknowledgment

This work was supported in part by Novartis (Thailand) Limited.

References