Gemifloxacin, a broad-spectrum fluoroquinolone antibacterial agent of the fluoroquinolone class, is used to treat bacterial infections, including acute bacterial exacerbation of chronic bronchitis and community-acquired pneumonia. This study aimed to develop a simple and robust analysis of gemifloxacin in human plasma by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). The sample was prepared using simple protein precipitation procedures with acetonitrile and separated using the Gemini C18 column with a mobile phase (0.1% formic acid: 0.1% formic acid in acetonitrile = 78:22 (V/V)). Moxifloxacin was used as an internal standard. The mass spectrometer was operated in the positive ion mode using multiple reaction monitoring. Each precursor ion of gemifloxacin and moxifloxacin was monitored at m/z 390.1/402.1, and their product ions were monitored at m/z 372.3/384.4. The calibration curve showed linearity in 0.005–5 μg/mL with an appropriate correlation coefficient (≥0.99). The variation coefficient of intra- and interprecision values of gemifloxacin was <15%. The inter- and intracurvature values ranged from 85% to 115%, except for the lower limit of quantification (accuracy range: 80%–120%). The proposed method was performed with a simple preparation step, and moxifloxacin, which is easily accessible, was used as the internal standard. These results suggest that the present assay is a practical analytical method and, therefore, can be readily applied for analysis, including in pharmacokinetic studies and therapeutic drug monitoring of gemifloxacin.

1. Introduction

Gemifloxacin is a broad-spectrum fluoroquinolone antibacterial agent that demonstrates activity against a wide range of Gram-positive and Gram-negative microorganisms and is therefore used to treat infections, such as acute bacterial exacerbation of chronic bronchitis and community-acquired pneumonia [1]. However, some safety-related issues have been noted. The US Food and Drug Administration issued a warning that fluoroquinolone can exert adverse effects on the central nervous system, reduce blood sugar levels, and cause aortic aneurysms, among other adverse reactions [2, 3]. Fluoroquinolones demonstrate a concentration-dependent bactericidal effect, and the ratio of the peak concentration to the minimum inhibitory concentration (MIC) and the area under the curve to the MIC correlate with clinical efficacy [4]. A correlation between the fluoroquinolone concentration and the risk of adverse reactions has not been ruled out [5]. Therefore, pharmacokinetic data could be used for therapeutic drug monitoring to ensure efficacy and safety, and this necessitates the development of valid and simple quantification methods for fluoroquinolones, including gemifloxacin, which is used worldwide. Among the more widely used fluoroquinolones [6], several methods for determining ciprofloxacin [7–9], levofloxacin [10–13], or moxifloxacin concentrations in human biological fluids, including plasma, serum, and urine, have been reported [14–16].
Analytical methods for the determination of gemifloxacin concentrations include capillary electrophoresis [17], spectrofluorimetry [18], high-performance liquid chromatography (HPLC) [19–24], and high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) [25–27]. Among these, HPLC and LC-MS/MS are the preferred methods because of their inherent selectivity and high sensitivity. However, only a few previously reported HPLC and LC-MS/MS methods can quantify gemifloxacin in biological fluids, such as urine and serum or plasma [22, 25–27]. Moreover, previous methods of HPLC and LC-MS/MS necessitated sophisticated conditions, such as specific chromatographic columns [23] or elusive internal standards such as isotope-labeled gemifloxacin [25]. The major drawbacks include the tedious, time-consuming sample-preparation procedures, such as derivatization [18, 24] or extraction [18, 26] after the drying of the final extract and the reconstitution steps [22]. These methods not only necessitate lengthy sample-preparation procedures but also require longer chromatographic run times (≥4 minutes) [19, 20, 22–24, 26, 27].

This study was conducted with an aim to develop a novel, simple, rapid, and reliable LC-MS/MS-based method for determining human plasma concentrations of gemifloxacin and to validate the novel method in comparison with previously reported methods. The methods include simple sample preparation or an optimal internal standard, which has only been used in limited instrumental conditions or with complicated procedures as in the previously reported methods. The introduction of a simple protein precipitation using acetonitrile, in combination with a dilution step that involves ammonium acetate, is a powerful strategy to improve efficiency while using moxifloxacin as the internal standard, which eliminates the price pressure and availability issues associated with isotope-labeled substances and can enhance assay productivity and cost-effectiveness. The proposed method was validated according to the Guidance for Industry recommendations by the FDA, and the contribution of the method to the analytical determination of gemifloxacin in samples of biological origin was ascertained through its successful application in a pharmacokinetics study [28].

2. Materials and Methods

2.1. Study Design and Procedures. This open-label, parallel-arm clinical trial was conducted to evaluate the effect of renal impairment on the pharmacokinetics of the orally administered 320 mg Factive Tab®. The study was approved by the Institutional Review Board of Chonnam and Jeonbuk National University Hospital, Republic of Korea, and was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines.

Participants were enrolled based on the results of a detailed physical and laboratory examination and were grouped according to their renal function. Patients with evidence of uncontrolled or unstable comorbidities, including renal replacement therapy, concomitant medication use that could affect the pharmacokinetics of gemifloxacin, who were pregnant or nursing, or who had any other severe medical problem were excluded. Korean men, aged 20–70 years, with normal renal function (estimated glomerular filtration rate (eGFR) > 90 mL/min) or with varying degrees of renal impairment (15 ≤ eGFR < 90 mL/min) were enrolled. The participants were administered a single oral dose of 320 mg gemifloxacin in the fasting state. Serial blood (0 (predose) and afterdose 0.33, 0.67, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, and 48 hours) and urine (0 (predose) and afterdose 0–6, 6–12, 12–24, and 24–48 hour) samples were collected to determine the gemifloxacin concentrations. After sampling, blood samples were bottled in heparinized vacutainer tubes and centrifuged at 3000 rpm for 10 min at 4°C. The separated plasma supernatant was then stored at −70°C until the analysis. Safety was monitored throughout the study based on the assessment of adverse events, the results of laboratory tests, and the participants’ vital signs.

2.2. Reagents and Materials. Moxifloxacin, which was used as the internal standard in this study, and gemifloxacin were purchased from Sigma-Aldrich (St. Louis, MO, USA), and their chemical structures are shown in Figure 1 [29, 30]. The test formulation (Factive® 320 mg gemifloxacin tablet) was obtained from LG Life Sciences, Ltd. Seoul, Republic of Korea (lot no. FAT18508B). HPLC-grade acetonitrile and methanol were purchased from Honeywell International Inc. (Charlotte, NC, USA). All other reagents (e.g., ethanol, formic acid, ammonium acetate) were of analytical grade. Deionized water that was obtained from the ELGA Purelab ultrawater purification system (Woodridge, IL, USA) was used for all experiments in this study.

2.3. Instruments and Conditions. The LC-MS/MS system consisted of a Shimadzu Prominence HPLC (Shimadzu Co., Kyoto, Japan) liquid system that was equipped with a dual solvent pump LC-20AD, a SIL-20AC autosampler, and a CTO-20AC column oven that interfaced with an AB SCIEX API 4000 QTRAP mass spectrometer (AB Sciex, Redwood City, CA, USA).

Chromatographic separation was performed using a 5.0 μm, 50 × 2.0 mm Gemini C18 column (Phenomenex, Torrance, California, United States) that was maintained at 40°C. The mobile phase consisted of 0.1% formic acid and 0.1% formic acid in acetonitrile (78:22, v/v), and the flow rate was set at 0.3 mL/min. The autosampler cooler temperature was maintained at 4°C. The sample injection volume was 20 μL, and the total run time was 3 minutes. The analytes were quantified by a mass spectrometer equipped with a turbo ion spray (TIS), which was operated in the positive mode. The mass spectrometry process was set up in a multiple reaction monitoring (MRM) mode to monitor m/z transitions of 390.1 to 372.3 and 402.1 to 384.4 for gemifloxacin and the internal standard, respectively (Figure 2). The MS parameters for monitoring gemifloxacin and internal standard are as follows: spray voltage = 4,500 V; nebulizer gas 1 = 50 psi; drying gas 2 = 60 psi; source temperature = 500°C; and curtain gas = 20 psi.
2.4. Standard Solutions and Samples. The standard solution of gemifloxacin (1000 μg/mL) was prepared by dissolving gemifloxacin in 50% ethanol, and the working solution of gemifloxacin was prepared by dilutions of the stock solution in 50% methanol. Moxifloxacin (1000 μg/mL standard solution) was prepared by dissolving it in 0.1% formic acid. The standard samples for calibration were prepared with blank human plasma (90 μL) using a 10 μL aliquot of working solution (ten-fold to each target concentration) to obtain concentrations of 0.005, 0.02, 0.05, 0.2, 0.5, 2, and 5 μg/mL. The quality control (QC) samples, with concentrations of 0.02, 0.2, and 2 μg/mL, were prepared by spiking a 10 μL aliquot of working solution (ten-fold to each target concentration) in 90 μL blank plasma. All calibration standards and QC samples were prepared fresh every day.

2.5. Sample Preparation. The 100-μL calibration standard, QC samples, and study plasma samples were spiked with 10 μL internal standard solution (1 μg/mL of moxifloxacin in...
50% methanol with 0.1% formic acid), and 200 μL acetonitrile was added; the mixture was vortexed for 1 minute. The samples were sequentially centrifuged at 12,000 rpm for 2 minutes at 4°C, and the supernatant was diluted ten-fold with 20 mM ammonium acetate and 0.1% formic acid. An aliquot (20 μL) of this diluted solution was injected into the LC-MS/MS system for subsequent assay.

2.6. Validation of the Method. The test method was validated according to the recommendations of the bioanalytical method validation guidance for industry that were published by the U.S. Food and Drug Administration [28]. The validation of the method included the components of selectivity, linearity, accuracy, precision, matrix effects, carryover, and stability. The selectivity of the method was determined by comparing the chromatograms of blank human plasma to blank human plasma spiked with gemifloxacin at the lower limit of quantification (LLOQ). The chromatograms of the samples obtained using LC-MS/MS were analyzed to confirm the absence of any interference peaks around gemifloxacin under assay conditions.

The linearity was determined via the analysis of calibration standards at various concentrations. The calibration curve was analyzed via linear regression of the peak area ratios \( f = aC + b \) using a weighing factor \( 1/C \). According to each batch, \( f \) (the ratio of areas between gemifloxacin and the internal standard) was calculated and substituted in the calibration curve on that day to determine the actual concentration. The intraday accuracy and precision evaluations were performed by replicating the analyses of the QC samples on the same day. The interday accuracy and precision were evaluated by analyses of the QC samples on three days each. The run comprised three replicates each of low-, medium-, and high-QC samples and a set of calibrations. The intra- and interaccuracy and precision values were calculated. The acceptance criterion for accuracy and precision values in the intra- and interbatches was within ±15% for all QCs [28].

The matrix effect was evaluated by comparing the peak area of the spiked extract of the blank plasma to the peak area of the spiked solvent. Six individual blank plasma were deproteinized and prespecified amounts of gemifloxacin were spiked to the postdeproteinized plasma. Furthermore, the internal standard solution was prepared and analyzed in the same way as the analyte (gemifloxacin) solution. Finally, the internal standard spiking solution (ISTD) normalized matrix factor was calculated using the following equation:

\[
\text{ISTD normalized matrix factor} = \frac{\text{matrix factor of analyte}}{\text{matrix factor of ISTD}}
\]  

(1)

To investigate the carryover effect, the analysis of the blank plasmas after analyzing samples with analyte or the internal standard at the upper limit of quantification was performed three times. The stability experiments were performed using spiked plasma at low- and high-QC concentrations \( (n = 3) \) under the following conditions: short-term stability on the bench-top for 2 hours at room temperature, or after undergoing three freeze (−70°C) and thaw (room temperature) cycles, or based on the postpreparative stability in the autosampler at 4°C for 24 hours. The stability was assessed by calculating the difference among three batches.

3. Results and Discussion

3.1. Method Validation

3.1.1. Selectivity and Matrix Effect. The representative chromatograms of different samples are shown in Figure 3. The left panel demonstrates that the chromatograms of gemifloxacin in blank plasma, in blank plasma at LLOQ, and in the plasma sample derived from a participant at 0.67 h after administration of a single oral dose of 320 mg of gemifloxacin. No significant interference peak was observed with the quantitation of gemifloxacin at all retention times. As shown in Figure 3, the results suggest that the method demonstrated high specificity and could accurately quantify the concentration of gemifloxacin in human plasma. The ISTD normalized matrix factor ranged from 1.047 to 1.130 and the coefficient of variation (%) was 2.84. These results indicate that the individual differences in the components within the matrix did not interfere with the quantification.

3.1.2. Linearity, Accuracy, and Precision. The calibration curves of three different batches were generated under the aforementioned conditions. A seven-point calibration curve with standard solutions showed linearity over the concentration range of 0.005–5 μg/mL for gemifloxacin, with all correlation coefficients higher than 0.99. As shown in Table 1, the intra- and interaccuracy ranged from 85% to 115%, and the intra- and interday precision values of gemifloxacin were within ±15% for all QCs.

3.1.3. Carryover and Stability. The carryover effect was not observed at the retention times of gemifloxacin or the internal standard. The accuracy of stability at each level is within 15%, which shows good stability under various experimental conditions (Table 2).

3.2. Comparison of the Analytical Methods and Application to the Human Plasma Samples in the Study. Several analytical methods, in matrices such as plasma, serum, or whole blood, urine, or pharmaceutical formulations, for determining gemifloxacin concentrations have been reported and mainly include liquid chromatography with spectrophotometric detection (LC/UV) [19–22] or liquid chromatography methods with spectrofluorometric detection (LC/FD) [23, 24]. Furthermore, capillary electrophoresis [17] and derivatization have been used in combination with spectrophotometry [18]. The previously reported capillary electrophoresis, spectrofluorometry, LC/UV, and LC/FD approaches presented long run times, and necessitated sophisticated preparation procedures, such as extraction and evaporative drying, that required high sample volumes.

To date, only three previously reported methods to quantify gemifloxacin using LC-MS/MS have been reported [25–27]: one was developed for the determination of
gemifloxacin in human plasma [25]; one for that in rat tissues and serum [26]; and one for that in human urine [27]. The latter two could not be directly applied to human plasma samples as they were designated for other matrices, such as tissues, serum, and urine, and were characterized by either a higher sample volume (3.0 mL urine) or a laborious liquid

Figure 3: Representative chromatograms of gemifloxacin in (a) blank plasma; (b) blank plasma spiked with gemifloxacin at the lower limit of quantitation; and (c) plasma obtained from a participant at 0.67 h after the administration of a single oral dose of 320 mg gemifloxacin. Left panel: gemifloxacin; right panel: moxifloxacin (internal standard).

Table 1: Accuracy and precision values of the proposed method for determining gemifloxacin levels in human plasma samples (n = 3).

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>% Intraday Accuracy</th>
<th>Intraday Precision (% CV)</th>
<th>% Interday Accuracy</th>
<th>Interday Precision (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>98.67</td>
<td>10.65</td>
<td>102.98</td>
<td>2.58</td>
</tr>
<tr>
<td>0.2</td>
<td>105.50</td>
<td>1.71</td>
<td>108.97</td>
<td>8.01</td>
</tr>
<tr>
<td>2</td>
<td>92.50</td>
<td>3.90</td>
<td>101.63</td>
<td>5.51</td>
</tr>
</tbody>
</table>

CV, coefficient of variation.

Table 2: Stability of the proposed method for determining gemifloxacin levels in human plasma samples (n = 3).

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>Short-term Storage Stability</th>
<th>Freeze-thaw Stability</th>
<th>Postpreparative Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Accuracy</td>
<td>% CV</td>
<td>% Accuracy</td>
</tr>
<tr>
<td>0.02</td>
<td>92.66</td>
<td>13.43</td>
<td>107.63</td>
</tr>
<tr>
<td>2</td>
<td>97.23</td>
<td>0.59</td>
<td>101.82</td>
</tr>
</tbody>
</table>

CV, coefficient of variation.
<table>
<thead>
<tr>
<th>Method</th>
<th>Matrix</th>
<th>Internal standard</th>
<th>Sample volume (μL)</th>
<th>Sample preparation</th>
<th>Linearity range (µg/mL)</th>
<th>Run time (minute)</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary electrophoresis Elbashir 2008</td>
<td>Solution</td>
<td>Flumequine</td>
<td>NA</td>
<td>Filtration + dilution</td>
<td>5–50</td>
<td>NR</td>
<td>In vitro</td>
<td>[17]</td>
</tr>
<tr>
<td>Spectrofluorimetry Kepekci Tekkeli 2011</td>
<td>Human plasma</td>
<td>None</td>
<td>100</td>
<td>DEV + LLE</td>
<td>0.04–0.2</td>
<td>NR</td>
<td>In vitro</td>
<td>[18]</td>
</tr>
<tr>
<td>LC/UV Sultana 2011</td>
<td>Human serum</td>
<td>None</td>
<td>1000</td>
<td>PPT + filtration</td>
<td>0.1–1.2</td>
<td>NR</td>
<td>In vitro</td>
<td>[19]</td>
</tr>
<tr>
<td>LC/UV (PDA) Nageswara Rao 2011</td>
<td>Solution</td>
<td>None</td>
<td>NA</td>
<td>Dilution + filtration</td>
<td>0.1–200</td>
<td>NR</td>
<td>In vitro</td>
<td>[20]</td>
</tr>
<tr>
<td>LC/UV (DAD) Gumustas 2012</td>
<td>Solution</td>
<td>Granisetron</td>
<td>NA</td>
<td>Filtration + dilution</td>
<td>0.25–20</td>
<td>&lt;5</td>
<td>In vitro</td>
<td>[21]</td>
</tr>
<tr>
<td>LC/UV Mousavi 2018</td>
<td>Human plasma</td>
<td>Chloramphenicol</td>
<td>1500</td>
<td>PPT + Evaporation</td>
<td>0.003–5</td>
<td>18</td>
<td>320 mg single oral study</td>
<td>[22]</td>
</tr>
<tr>
<td>LC/FD Nageswara Rao 2012</td>
<td>Rat dried blood spots</td>
<td>Ciprofloxacin</td>
<td>30</td>
<td>Sonication in solution</td>
<td>0.025–5</td>
<td>10</td>
<td>20 mg/kg single oral experiment</td>
<td>[23]</td>
</tr>
<tr>
<td>LC/FD Onal 2021</td>
<td>Solution</td>
<td>None</td>
<td>100</td>
<td>DEV</td>
<td>0.01–0.2</td>
<td>6</td>
<td>In vitro</td>
<td>[24]</td>
</tr>
<tr>
<td>LC-MS/MS (current)</td>
<td>Human plasma</td>
<td>Moxifloxacin</td>
<td>100</td>
<td>PPT (5 min.)</td>
<td>0.005–5</td>
<td>3</td>
<td>320 mg single oral study</td>
<td>THIS WORK</td>
</tr>
<tr>
<td>LC-MS/MS Doyle 2000</td>
<td>Human plasma</td>
<td>[(13)CH3]Gemifloxacin</td>
<td>50</td>
<td>PPT (appr. 1 hour)</td>
<td>0.01–5</td>
<td>1.5</td>
<td>320 mg single oral study</td>
<td>[25]</td>
</tr>
<tr>
<td>LC-MS/MS Roy 2010</td>
<td>Rat tissue homogenate serum</td>
<td>Ciprofloxacin</td>
<td>200</td>
<td>LLE</td>
<td>0.125–5 ng/mL</td>
<td>12.5</td>
<td>200 mg/kg single oral experiment</td>
<td>[26]</td>
</tr>
<tr>
<td>LC-MS/MS Kadi 2013</td>
<td>Human urine</td>
<td>Ofloxacin</td>
<td>3000</td>
<td>Filtration + dilution</td>
<td>0.005–0.5</td>
<td>4</td>
<td>320 mg single oral study</td>
<td>[27]</td>
</tr>
</tbody>
</table>

LC/UV, liquid chromatography with spectrophotometric detection; PDA, photodiode array detector; DAD, diode array detector; LC/FD, liquid chromatography methods with spectrofluorometric detection (LC/ FD); LC-MS/MS, high-performance liquid chromatography-tandem mass spectrometry; NA, not applicable; NR, not reported; DEV, derivatization; LLE, liquid–liquid extraction; PPT, protein precipitation.
Gemifloxacin Concentration (µg/mL)  

**Figure 4:** Mean (±standard error) plasma concentration–time profiles of gemifloxacin that were derived based on the samples that were obtained from 13 participants after the administration of a single oral dose of 320 mg gemifloxacin.

extraction sample preparation with a longer run time. Previously, an LC-MS/MS method for determining gemifloxacin concentration in human plasma was reported, but this method included a time-consuming procedure that was associated with a tedious protein precipitation sample preparation (approximately 1 hour) and also used a difficult-to-obtain internal standard.

The novel assay for gemifloxacin in human plasma that we developed is simple and fast (protein precipitation of plasma and a 3-min run-time), reliable, selective, accurate, and employs an isocratic LC-MS/MS with high throughput. The protein in the samples was precipitated with acetonitrile, which is more simplistic and easier (approximately 5 minutes) than those that were reported previously. The precipitated samples were diluted with ammonium acetate solution before injection into the LC-MS/MS systems, which enabled the attainment of a good peak without the addition of salt to the mobile phase or the use of a methanol-containing mobile phase that could result in a relatively long chromatographic run time [26]. Before applying the ammonium acetate method in a developing process, the other two split peaks for gemifloxacin were presented in a mixture after the protein precipitation procedure, which was run with the same LC-MS/MS conditions (data not shown). Instead of isotope-labeled gemifloxacin, moxifloxacin was used as the internal standard in the gemifloxacin analysis, as moxifloxacin has similar chemical properties as gemifloxacin and is adequate as an internal standard for gemifloxacin, as evidenced by stable signals and peaks in the absence of a matrix effect.

This assay shows specificity, stability, and high interday accuracy (101.63–102.98%) and precision (2.58–5.51%) in low-, medium-, and high-QC samples as well as excellent linearity (0.005–5 µg/mL). No significant matrix effect or carryover effect was observed. The validation data meets the criteria of the FDA bioanalytical method validation guideline [28]. Only one LC-MS/MS method for analysis of gemifloxacin in human plasma has been reported previously. Compared to the present method, the reported assay has comparable sensitivity, linearity (0.01–5 µg/mL), double the LLOQ compared to the present one), accuracy, precision, and stability; however, validation experiments for matrix and carryover effects were not performed [25].

The principal advantage of the present method described here is the simultaneous achievement of efficiency, simplicity, and validity with a low sample volume (100 µL) within a short run time of 3 minutes. This minimal sample clean-up and short run time make this method suitable for routine clinical settings or the application to a large number of pharmacokinetic samples without errors and time-consuming preparation.

The comparison of the current LC-MS/MS assay to some of the previously reported methods from the literature is summarized in Table 3. This analytical method was successfully applied for determining gemifloxacin levels in 169 plasma samples derived from 13 Korean participants who received a single oral dose of 320 mg gemifloxacin in the pharmacokinetic study. Gemifloxacin concentrations in the plasma samples of this study ranged from 0.00538 to 3.44 µg/mL. The mean concentration–time profiles of gemifloxacin in plasma samples obtained from participants after administration of a single oral dose of 320 mg gemifloxacin formulation are shown in Figure 4. Similarly, the method could be feasible for samples from urine without an extra filtration step following an appropriate validation (concentrations in the urine samples in this study ranged from 1.78 to 338 µg/mL).

4. Conclusions

The present study described a novel method for the quantitative determination of gemifloxacin in human plasma. The proposed LC-MS/MS method is practical and robust and allows the determination of gemifloxacin levels in human plasma samples within a short 3-min test duration. The assay was successfully validated in terms of linearity, accuracy, precision, carryover and matrix effects, and stability. The plasma samples from a clinical pharmacokinetic study in human were analyzed to evaluate the applicability of this assay. These results suggest that this novel method for the quantitation of gemifloxacin could be applied for the analytical determination of gemifloxacin in samples of biological origin. The proposed methodology constitutes an easily accessible alternative to the already existing methods that are used for routine analysis of the gemifloxacin in analyses, such as a pharmacokinetic study and therapeutic drug monitoring.

Data Availability

The data used in of this study are included within the article, and further information is available upon reasonable request to the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest with regard to the publication of this article.
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