Development of HPLC Method for the Determination of Buspirone in Rat Plasma Using Fluorescence Detection and Its Application to a Pharmacokinetic Study

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A simple and sensitive analytical method for the quantitative determination of buspirone in rat plasma by HPLC with fluorescence detection was developed and validated using naproxen as an internal standard. A relatively small-volume (150 μL) aliquot of rat plasma sample was prepared by a simple deproteinization procedure using acetonitrile as a precipitating organic solvent. Chromatographic separation was performed using Kinetex® C8 column with an isocratic mobile phase consisting of acetonitrile and 10-mM potassium phosphate buffer (pH 6.0) at a flow rate of 1.0 mL/min. The eluent was monitored by fluorescence detector at a wavelength pair of 237/380 nm (excitation/emission). The linearity was established at 20.0–5000 ng/mL, and the limit of detection was 6.51 ng/mL. The precision (≤14.6%), accuracy (89.2–108%), and stability (89.1–101%) were within acceptable ranges. The newly developed method was successfully applied to intravenous and oral pharmacokinetic studies of buspirone in rats.

Key words  buspirone; HPLC; fluorescence detection; rat plasma; pharmacokinetics

Buspirone (Fig. 1; 8-[4-(4-pyrimidin-2-ylpiperazin-1-yl)butyl]-8-azaspiro[4,5]decane-7,9-dione), an azapirone, is an antianxiety agent that has dopaminergic, noradrenergic, and serotonin-modulating activity. It is chemically unrelated to benzodiazepines or barbiturates, and it exerts a pharmacologically unique anxiolytic effect without sedative, muscle relaxant, and anticonvulsant properties. Oral buspirone formulation (e.g., Buspar®) is currently used in the treatment of anxiety disorders and the short-term relief of the symptoms of anxiety. Orally administered buspirone is rapidly absorbed but it undergoes first-pass metabolism, resulting in a low bioavailability of less than 5%. Buspirone is metabolized primarily by CYP450 3A4-mediated oxidation. However, little information is available regarding important pharmacokinetic issues that include the exact reason for the low bioavailability, kinetics of organ clearance, and mechanisms of elimination of buspirone in animals or humans. Therefore, development of analytical tools for quantification of buspirone in various biological fluids will contribute to the progress of mechanistic pharmacokinetic and pharmacological studies of buspirone in a pre-clinical or clinical setting. A few analytical methods have been reported for the determination of buspirone in human plasma by HPLC with ultraviolet spectrophotometry (UV) method and HPLC with tandem mass spectrometry (HPLC-MS/MS) method and in rabbit serum by HPLC-UV method. However, these methods have several limitations such as a relatively large sample volume (i.e., 500 μL) and costly and/or laborious sample preparation procedures that include a liquid–liquid or solid phase extraction. Moreover, mass spectrometry requires relatively expensive equipment and highly skilled technical expertise, which may not always be feasible for most laboratories in resource-limited settings. Previously, the reported plasma concentration versus time profiles of buspirone in rats using a HPLC-UV method but did not provide full bioanalytical method validation data, and the sensitivity of the method also needs to be further improved. In general, fluorescence spectroscopy (FL) analysis can offer better selectivity and sensitivity than UV analysis in HPLC method, and buspirone contains a fluorophore, i.e., aromatic ring. Thus, in the current situation, a simple and sensitive HPLC-FL method can be a viable alternative to the previously reported HPLC-MS/MS and HPLC-UV methods for bioanalytical measurement of buspirone. To the best of our knowledge, there have been no studies in which a fully validated HPLC-FL method was developed for the determination of buspirone in animal or human biological samples.

In the present study, we developed a simple and sensitive HPLC-FL analytical method for the determination of buspirone in rat plasma. The newly developed method was comprehensively validated using naproxen (Fig. 1; (+)-(S)-2-(6-methoxynaphthalen-2-yl)propanoic acid) as an internal standard (IS). Then, the intravenous and oral pharmacokinetic profiles of buspirone were investigated in rats.

Experimental

Materials  Buspirone (purity ≥99%), naproxen (purity ≥98%), and potassium phosphate monobasic were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Acetonitrile and methanol of HPLC grade were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, U.S.A.). Water of HPLC grade was purchased from Honeywell International Inc. (Muskegon, MI, U.S.A.). All other reagents were of analytical grade and used without further purification.

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Animals Male Sprague-Dawley rats (7–9 weeks old; 230–300 g) were purchased from Orient Bio Co. (Pyeongtaek, Republic of Korea). The rats were retained in a clean room (Animal Center for Pharmaceutical Research, Seoul National University, Seoul, Republic of Korea) at a temperature of 20–23°C with 12-h light (07:00–19:00) and dark (19:00–07:00) cycles, and a relative humidity of 50±5%. They were housed in metabolic cages (Tecniplast USA Inc., West Chester, PA, U.S.A.) under filtered, pathogen-free air, with food (Agribbrands Purina Canada Inc., Levis, QC, Canada) and water available ad libitum. The present rat study was approved by the Institutional Animal Care and Use Committee of Seoul National University (date of approval: 22/07/2013; approval number: SNU-130722-1).

Chromatographic Conditions The HPLC-FL system consisted of the Waters e2695 Separations Module equipped with the Waters 2475 multi λ fluorescence detector (Waters Co., Milford, MA, U.S.A.). Chromatographic separation was performed using a Kinetex® C8 column (250×4.6 mm, 5 µm, 100 Å; Phenomenex, Torrance, CA, U.S.A.) coupled to a HPLC guard column (SecurityGuard HPLC Cartridge System, Phenomenex) packed with the same stationary phase at room temperature. The isocratic mobile phase consisted of potassium phosphate buffer (pH 6.0, 10 mM) and acetonitrile (70:30, v/v). The flow rate was maintained at 1.0 mL/min, and the total run time was 13 min. The fluorescence excitation and emission wavelengths for analytes were 237 and 703 nm, respectively. The column cleaning and regeneration were performed according to the manufacturer’s protocols. The column was serially flushed with the following solvents mixtures (50 mL for each step): methanol–water (5:95, v/v) for buffer removal, methanol–water (95:5, v/v), tetrahydrofuran, methanol–water (95:5, v/v), and methanol–water (5:95, v/v). In addition, the guard column was used to protect the analytical column from possible impurities in plasma samples.

Standard Samples and Quality Control (QC) Samples Stock solutions of buspirone and IS (1.0 mg/mL in methanol) were prepared. The stock solution of buspirone was serially diluted with the mobile phase to prepare working standard solution at a concentration range from 200 to 5000 ng/mL. The working solution of IS was prepared with acetonitrile at a concentration of 100 ng/mL. All working solutions were stored at −20°C. The standard samples for calibration were prepared by spiking the blank rat plasma with each working standard solution, thereby yielding final plasma concentrations of 500, 2000, 1000, 500, 200, 100, 50, and 20.0 ng/mL. The QC samples were prepared using different stock solutions of buspirone in the same way as the standard samples. The concentrations of QC samples were 2500 (high; HQC), 250 (middle; MQC), 60.0 (low; LQC), and 20.0 ng/mL (lower limit of quantification; LLOQ).

Sample Preparation A 150 µL aliquot of plasma sample (i.e., blank plasma, standard samples, or QC samples) was deproteinized with a 500 µL aliquot of acetonitrile containing IS (100 ng/mL). After vortex-mixing for 5 min followed by centrifugation at 16000×g for 5 min, a 600 µL aliquot of the supernatant was transferred to a clean 1.7 mL microtube and allowed to evaporate under a gentle nitrogen gas stream. The resultant dried residue was reconstituted with 60 µL of mobile phase, and a 20 µL aliquot was injected into the HPLC system.

Method Validation This bioanalytical method was validated for selectivity, linearity, sensitivity, accuracy, precision, extraction recovery, matrix effect, and stability. Validation runs were conducted on three consecutive days.

The selectivity of the method for buspirone was evaluated by comparing chromatograms of buspirone and IS in blank rat plasma, blank plasma spiked with buspirone and IS, and rat plasma sample obtained from the rat pharmacokinetic study. The existence of potential interferences at the acquisition windows of buspirone and IS was checked.

The linearity of the method was evaluated by increasing amounts of buspirone to a blank biological matrix. The calibration curves (n=4) were constructed by plotting the peak area ratios of buspirone to IS (y-axis) versus the nominal concentrations of buspirone (20.0–5000 ng/mL) in plasma (x-axis) using linear regression analysis.

The sensitivity of the method was evaluated by the LLOQ and limit of detection (LOD). The LLOQ was defined as the lowest concentration of buspirone in the calibration curves that could be quantitated (signal-to-noise (S/N) ratio of more than 10). Buspirone peak at the LLOQ level should be identifiable, discrete, and reproducible with acceptable precision (less than 20%) and accuracy (within 80–120%). The LOD was defined as the lowest concentration of buspirone that could be distinguished from the absence of analytes (blank) (S/N ratio of more than 3.3).

The accuracy and precision of the method were evaluated by the assessment of plasma samples spiked with 20 (LLOQ), 60 (low QC), 250 (middle QC), and 2500 (high QC) ng/mL buspirone in five replicates over three different validation days. Accuracy and precision were assessed by comparing the measured concentrations in the QC samples [five separately prepared sets measured on one day (intra-day), three different days (inter-day)] with the respective nominal concentrations, which are expressed as relative error (accuracy) and the respective coefficients of variation of the mean values (precision).

The extraction recovery of analytes (buspirone, IS) was evaluated by comparing analytical signals obtained from the extracted sample with those obtained from the post-extracted spiked sample. The matrix effect was assessed by comparing analytical signals obtained from the post-extracted spiked sample with those obtained from the non-extracted neat sample (diluted stock solution). Four replicates were evaluated at the LLOQ and three QC concentration levels.

The stability of buspirone in rat plasma was determined by comparing analytical signals obtained from plasma samples exposed to various handling and storage conditions with those obtained from freshly prepared plasma samples. Bench-top stability was determined after exposure of the spiked plasma samples to room temperature for 3 h. Freeze-thaw stability was assessed after three complete freeze-thaw cycles (−20°C to room temperature) on consecutive days. Long-term stability was evaluated after storage of the spiked plasma samples at −20°C for 4 weeks. Post-preparative stability (autosampler stability) was assessed after exposure of the extracted samples at 25°C for 24 h in the autosampler. Five replicates were evaluated at the LLOQ and three QC concentration levels.

Application to a Pharmacokinetic Study in Rats Before the experiment, 6 rats were fasted overnight with access to water. The femoral artery and vein of the rats were cannulated with a polyethylene tube (Becton Dickinson Diagnostics,
Sparks, MD, U.S.A.) under anesthesia with Zoletil (20 mg/kg, intramuscular injection) (Vibrac, Carros, France). Rats were given a single intravenous dose \((\text{via the femoral vein; } 5 \text{ mg/kg})\) or an oral dose (using a feeding tube; 20 mg/kg) of buspirone (dissolved in normal saline). An approximately 250 µL aliquot of blood samples was collected \(\text{via the femoral artery at predetermined time points (0, 1, 5, 15, 30, 45, 60, 90, 120, 180 \text{ min for the intravenous study; } 0, 2, 5, 10, 15, 20, 30, 45, 60, 90, 120 \text{ min for the oral study). An approximately 250 µL aliquot of heparinized normal saline (20 U/mL) was used to flush the cannula immediately after each blood sampling to prevent blood clotting. After centrifugation of the blood sample at 16000×g for 3 min, a 150 µL aliquot of plasma sample was stored at \(-20^\circ\text{C}\) until HPLC analysis.

**Data Analysis** The analytical data acquisition and processing were performed using Empower TM 2 Software (Version 6.20.00.00; Waters Co.). All chromatograms were evaluated by the internal standard method and peak-area ratios of the analyte over IS were used for calculation (least squares regression, \(1/x\) weighting, \(x=\text{concentration}\)). Non-compartmental analysis (WinNonlin, version 3.1, NCA200 and 201; Certara USA Inc., Princeton, NJ, U.S.A.) was performed to calculate the following pharmacokinetic parameters: the total area under the plasma concentration versus time curve from time zero to infinity (AUC); the total area under the first moment of plasma concentration versus time curve \((\text{AUMC})\); the time-averaged total body plasma clearance \((\text{CL})\); the terminal half-life \((t_{1/2})\); and the apparent volume of distribution at steady state \((V_{ss}, \text{calculated as dose×AUMC/AUC}^\circ\). For comparison, the extent of absolute oral bioavailability \((F; \text{expressed as percent of dose administered})\) was calculated by dividing the dose-normalized \(\text{AUC}\) after oral administration by the dose-normalized \(\text{AUC}\) after intravenous injection. The peak plasma concentration \((C_{\text{max}})\) and time to reach \(C_{\text{max}}(T_{\text{max}})\) were read directly from the experimental data. Unless indicated otherwise, all data were expressed as the mean±standard deviation (S.D.), except for median (ranges) for \(T_{\text{max}}\), and they were rounded to three significant figures.

**Results and Discussion**

### Optimization of Chromatographic Conditions and Sample Preparation Procedures

The maximum excitation wavelength of buspirone (237 nm) was selected based on a previous study that reported spectrophotometric determination of buspirone in pharmaceutical dosage forms. Then, to optimize emission wavelength, fluorescence intensity of buspirone (1 µg/mL; dissolved in pH 6.0 mobile phase) was measured at various emission wavelength ranging from 370 to 390 nm. As a result, an emission wavelength of 380 nm was found to provide the highest fluorescence intensity (data not shown). Therefore, we selected the 237/380 nm (excitation/emission) wavelength pair in this study.

To select a stationary phase for the HPLC analysis, we tested several HPLC columns including Luna® CN column, Kinetex® C18 column, and Kinetex® C8 column (250×4.6 mm, 5 µm; Phenomenex). As a result, the Kinetex® C8 column was found to achieve higher peak resolution and intensity with better chromatographic separation than the other columns tested (data not shown), and therefore, it was selected as a stationary phase in the present study. Based on the previously reported HPLC-UV methods, an acetonitrile–phosphate buffer system was employed as a mobile phase for the HPLC analysis. The mobile phase composition was then optimized with respect to the content of acetonitrile (30–40%) and the pH of the mobile phase (pH 5.0–7.0). As a result, 30% acetonitrile content and pH 6.0 were found to achieve the best chromatographic separation and peak resolution, and therefore, these conditions were selected for the present HPLC-FL method.

**Figure 1**

**Chemical Structures of Buspirone and Naproxen (IS)**
a simple and inexpensive sample preparation method, compared with the liquid–liquid or solid phase extraction. In our preliminary study, several precipitating organic solvents such as methanol, acetonitrile, trichloroacetic acid, and their mixture were tested. Among these organic solvents, acetonitrile was found to yield the highest recovery and lowest matrix effect for the analytes with a high speed centrifugation at 16000×g for a short precipitation time of 5 min (data not shown).

Fig. 2. Representative Chromatograms of Buspirone and IS in Rat Plasma
Blank rat plasma (A); blank rat plasma spiked with buspirone (250 ng/mL, MQC) and IS (B); plasma sample collected at 1 min after the intravenous administration of buspirone solution at a dose of 5 mg/kg in rats, where calculated concentration of buspirone was 3560 ng/mL (C); plasma sample collected at 5 min after the oral administration of buspirone solution at a dose of 20 mg/kg in rats, where calculated concentration of buspirone was 2120 ng/mL (D); plasma sample collected at 120 min after the intravenous administration of buspirone solution at a dose of 5 mg/kg in rats, where calculated concentration of buspirone was 66.8 ng/mL (E).
Selectivity, Linearity, Sensitivity, Precision, and Accuracy  As shown in the representative HPLC chromatograms of blank rat plasma, blank rat plasma spiked with buspirone and IS, and rat plasma sample obtained from the intravenous and oral pharmacokinetic studies (Fig. 2), the buspirone and IS peaks did not overlap with peaks from endogenous substances in blank rat plasma. This result suggests that the present analytical method achieved sufficient selectivity without endogenous plasma interferences at the retention times of analytes. The calibration curves (peak area ratio versus concentration) for buspirone were linear over the range of 20–5000 ng/mL in rat plasma samples. The fitted equations for the calibration curves were as follows: y=(0.000165±0.0000130)x−(0.000767±0.001816), where y represents the ratio of the peak area of buspirone to that of IS, and x represents the plasma concentration of buspirone. The correlation coefficients \( r^2 \) were over 0.998, indicating a good linearity of the method. The LOD and LLOQ values for buspirone were determined to be 6.51 and 20.0 ng/mL, respectively. The intra-day and inter-day precision and accuracy of the method were determined for buspirone at the LLOQ and three QC levels, and they are summarized in Table 1. The intra-day and inter-day precision was 14.6% or less, and the accuracy of the method ranged from 89.2 to 108%. These results indicate that the values are within the acceptable range, and the present method is accurate, precise, and reproducible.

Recently, we reported the determination of buspirone concentration in other rat pharmacokinetic studies using a HPLC-UV method.\(^{12,15}\) However, in these previous studies, full bioanalytical method validation data were not provided, and chromatographic conditions and sample preparation procedures were randomly selected without optimization. Moreover, the sensitivity of our previous methods (LOQ=100 ng/mL) was not sufficient for lower-dose preclinical study, which needed further improvement. Generally, FL analysis is known to be more sensitive and selective than UV analysis, primarily needing further improvement. Generally, FL analysis is known to be more sensitive and selective than UV analysis, primarily due to its higher specificity and lower intensity of background. However, this may not be the case for pharmacokinetic studies using small laboratory animals. Moreover, the previous method employed liquid–liquid extraction technique which is known to be labor-consuming and highly sensitive to operational conditions and require unsafe solvents and large sample volumes, compared with the simple deproteinization procedure used in this study.\(^{20}\) Therefore, our present HPLC-FL method can be a feasible alternative to the previous HPLC-UV method regarding bioanalytical measurement of buspirone in rats.

Recovery, Matrix Effect, and Stability  The recovery and matrix effect of the method were determined for buspirone at the LLOQ and three QC levels and for IS at 100 ng/mL, and they are summarized in Table 2. The mean recovery of buspirone ranged from 94.8 to 111% with coefficients of variation (CV) values less than 8.57%. The mean matrix effect for buspirone ranged from 92.7 to 99.6% with CV values less than 11.6%. The stability of buspirone was tested under several normal laboratory conditions used in the present HPLC-FL method. The bench-top stability, autosampler stability, freeze-thaw stability, and long-term stability were determined for buspirone at the LLOQ and three QC levels. As shown in Table 3, the bias in the concentration was within ±15% of the nominal value; the mean remaining fraction of buspirone ranged from 89.1 to 101.1% with CV values less than 7.83%. These results indicate that the current sample preparation procedure offers high extraction recovery and minimal matrix effect, and that buspirone is stable under handling and storage conditions.

Application to a Pharmacokinetic Study in Rats  Plasma concentration versus time profiles of buspirone after intravenous administration at a dose of 5 mg/kg and oral administration at a dose of 20 mg/kg in rats are shown in Fig. 3, and relevant pharmacokinetic parameters are listed in Table 4. In both the intravenous and oral studies, the linear terminal phases of the concentration versus time curves were clearly identi-

| Table 1. Intra- and Inter-Day Precision and Accuracy of Buspirone in Rat Plasma (\( n=5 \)) |
|---------------------------------|-----------------|------------------|
| **Nominal concentration** (ng/mL) | **Precision (%)** | **Accuracy (%)** |
|                                 | **Intra-day** | **Inter-day** |
| LLOQ (20.0)                  | 14.6          | 13.9           |
| LQC (60.0)                   | 6.56          | 6.71           |
| MQC (250)                    | 3.99          | 11.4           |
| HQC (2500)                   | 9.35          | 7.19           |
| LLOQ (20.0)                  | 14.6          | 13.9           |
| LQC (60.0)                   | 6.56          | 6.71           |
| MQC (250)                    | 3.99          | 11.4           |
| HQC (2500)                   | 9.35          | 7.19           |

Table 2. Recovery and Matrix Effect of Buspirone and IS in Rat Plasma (\( n=4 \))

<table>
<thead>
<tr>
<th><strong>Nominal concentration (ng/mL)</strong></th>
<th><strong>Recovery (%)</strong></th>
<th><strong>Matrix effect (%)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>LLOQ (20.0)</td>
<td>102±8.74</td>
<td>97.8±12.1</td>
</tr>
<tr>
<td>LQC (60.0)</td>
<td>99.0±4.04</td>
<td>94.2±4.15</td>
</tr>
<tr>
<td>MQC (250)</td>
<td>111±0.964</td>
<td>92.7±2.99</td>
</tr>
<tr>
<td>HQC (2500)</td>
<td>94.8±4.10</td>
<td>94.6±11.0</td>
</tr>
<tr>
<td>IS (Naproxen, 100)</td>
<td>92.0±1.73</td>
<td>99.6±3.75</td>
</tr>
</tbody>
</table>

Table 3. Stability (%) of Buspirone in Rat Plasma (\( n=5 \))

<table>
<thead>
<tr>
<th><strong>Nominal concentration (ng/mL)</strong></th>
<th><strong>Bench-top(^a)</strong></th>
<th><strong>Autosampler(^b)</strong></th>
<th><strong>Freeze-thaw(^c)</strong></th>
<th><strong>Long-term(^d)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>LLOQ (20.0)</td>
<td>86.1±8.66</td>
<td>99.4±13.2</td>
<td>95.2±14.7</td>
<td>97.3±10.4</td>
</tr>
<tr>
<td>LQC (60.0)</td>
<td>93.9±11.8</td>
<td>109±9.47</td>
<td>113±5.76</td>
<td>97.8±5.97</td>
</tr>
<tr>
<td>MQC (250)</td>
<td>97.0±4.39</td>
<td>107±3.42</td>
<td>106±6.18</td>
<td>101±2.49</td>
</tr>
<tr>
<td>HQC (2500)</td>
<td>103±8.36</td>
<td>114±2.48</td>
<td>112±6.06</td>
<td>92.6±7.02</td>
</tr>
</tbody>
</table>

\(^a\) Room temperature for 3 h. \(^b\) 25°C for 24 h in the autosampler. \(^c\) Three freezing and thawing cycles. \(^d\) –20°C for 30 d.
then they decreased in a multi-exponential manner. The ter-

tonate increased within a very short time period (5–10 min) and

buspirone is rapidly absorbed after oral dosing, resulting in a

and incomplete in rats. A previous clinical study reported that

bioavailability (\(F\)) was observed to be high (97.9 mL/min/kg and 6220 mL/kg, respectively), in-

CL and \(V_{ss}\) were observed to be high (97.9 mL/min/kg and 6220 mL/kg, respectively), in-

mean values for \(T_{1/2}\) and \(T_{max}\) of less than 1 h and its

\(V_{ss}\) is 5300 mL/kg, 6) which

coincides well with the present rat data (Table 4). However, the CL, oral \(t_{1/2}\), and \(F\) of buspirone were considerably differ-

between clinical literature values 6) and the present rat data as follows: 28.3 versus 97.9 mL/min/kg, 191 versus 65.9 min, and 4 versus 17.5%, respectively.

It was reported that buspirone is metabolized primarily by human CYP3A4 and rat Cyp3a, and its active metabolites include 1-pyrimidinyl piperazine and 6'-hydroxy buspi-

rone. 5,24–26) To roughly identify the presence of buspirone me-

tabolites in rats, representative chromatograms of rat plasma

samples obtained from the present rat pharmacokinetic study were compared with those of the QC samples (Figs. 2B–D). In the chromatograms of rat plasma samples collected at 1 min after intravenous dosing (Fig. 2C) and 5 min after oral dosing (Fig. 2D), a few new peaks were observed at earlier retention times (1.9 min for M1 peak; 5.6 min for M2 peak; 6.3 min for M3 peak) than buspirone (9.7 min). Since a non-polar reversed-

phase C8 column was used, these new peaks may indicate some polar metabolites of buspirone. However, rigorous iden-
tification of buspirone metabolites may be beyond the scope of our present results, which merits further investigation.

Conclusion

In the present study, we developed and validated an HPLC-

FL method using a simple deproteinization method for the efficient quantification of buspirone in rat plasma. The chro-

matographic conditions and sample preparation procedures for the proposed method were optimized with respect to the fluo-

rescence wavelength, HPLC column, mobile phase composi-

tion, and deproteinization processes. The bioanalytical method described in this study had acceptable selectivity, linearity, sensitivity, accuracy, and precision for the quantitative deter-

mination of buspirone in rat plasma. Results of the method were reproducible, and degradation of the analyte was not observed during the analytical process. Moreover, the newly
developed method was successfully applied to rat pharmacoki-

netic studies of buspirone. To the best of our knowledge, this is the first work to develop a simple and validated HPLC-

FL method for the determination of buspirone in rat plasma, which will be useful in relevant pre-clinical pharmacoki-

netic studies. It is also plausible that the present bioanalytical

method can potentially be suitable for clinical use after partial

modification and validation.

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Conflict of Interest

The authors declare no conflict of interest.

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