Development and validation of a simple, sensitive and accurate LC-MS/MS method for the determination of guanfacine, a selective $\alpha_{2A}$-adrenergic receptor agonist, in plasma and its application to a pharmacokinetic study

Xiaonan Li, Ning Li, Xiaolin Sun, Wei Yang, Yu Dai, Jie Xu, Wei Zhang, Chunfeng Wang, Suilou Wang and Xijing Chen*

ABSTRACT: A simple, practical, accurate and sensitive liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was developed and fully validated for the quantitation of guanfacine in beagle dog plasma. After protein precipitation by acetonitrile, the analytes were separated on a C18 chromatographic column by methanol and water containing 0.1% (v/v) formic acid with a gradient elution. The subsequent detection utilized a mass spectrometry under positive ion mode with multiple reaction monitoring of guanfacine and enalaprilat (internal standard) at $m/z$ 246.2 $\rightarrow$ 159.0 and $m/z$ 349.2 $\rightarrow$ 205.9, respectively. Good linearity was obtained over the concentration range of 0.1–20 ng/mL for guanfacine in dog plasma and the lower limit of quantification of this method was 0.1 ng/mL. The intra- and inter-day precisions were <10.8% relative standard deviation with an accuracy of 92.9–108.4%. The matrix effects ranged from 89.4 to 100.7% and extraction recoveries were >90%. Stability studies showed that both analytes were stable during sample preparation and analysis. The established method was successfully applied to an in vivo pharmacokinetic study in beagle dogs after a single oral dose of 4 mg guanfacine extended-release tablets. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: guanfacine; LC-MS/MS; pharmacokinetics; beagle dogs

Introduction

Guanfacine, N-amidino-2-(2,6-dichlorophenyl) acetamide, is a selective $\alpha_{2A}$-adrenergic receptor agonist. It is centrally acting as antihypertensive drug which can decrease the release of presynaptic norepinephrine neuron by directly stimulating $\alpha_{2A}$-adrenergic receptors in the central nervous system, resulting in a slight reduction in arterial blood pressure and heart rate (van Zwieten et al., 1983, 1984; Sorkin and Heel, 1986). There has been a long history of guanfacine use to treat moderate to severe hypertension.

Recently, new applications of guanfacine have been discovered and investigated. In addition to treating hypertension, it is also useful for the treatment of attention-deficit hyperactivity disorder (ADHD) as well as several other related disorders (Hunt et al., 1995; Scanhill et al., 2001; Arnsten, 2010; Arnsten and Jin, 2012). However, its hypertensive effects are a disadvantage when treating ADHD in children and adolescents.

As a result, a new oral formulation of guanfacine, its extended-release tablets (GER) has been approved recently by the US Food and Drug Administration, mainly for the treatment of ADHD in in children and adolescents. Compared with the general preparation, the dosing interval of GER is greatly prolonged and the side effects can be minimized because of its sustained and slow release effect, which can result in steady plasma concentrations over a long period of time. Recent findings have revealed that GER causes no impairment in cognitive tasks or daytime sleepiness, and no sedation was observed when treating ADHD (Kollins et al., 2011). When prescribed alone or as adjunctive therapy to psychostimulants, GER could significantly improve ADHD symptoms with less influence on blood pressure, pulse rate and electrocardiogram (Spencer et al., 2009; Sallee et al., 2012; Signorovitch et al., 2012; Cruz, 2010; Childress, 2012; Connor et al., 2010). Thus, GER is not only safer but also more helpful for improving compliance, tolerability and controlling symptoms.

Considering these significant advantages of GER over the general preparation, the evaluation of its release and pharmacokinetic behavior is of particular clinical importance. However, owing to its low administration dosage, slow release and widely distribution into tissues throughout the body, the plasma
concentration of GER is difficult to determine (Biederman et al., 2008; Swearingen et al., 2007). Therefore, highly sensitive and accurate analytical methods are urgently required.

In earlier publications, guanfacine has been determined in biological fluids by electron-capture gas–liquid chromatography (lower limit of quantification, LLOQ, 0.5 ng/mL), gas chromatography–mass spectrometry (lower limit of detection, LOD, 50 ng/mL) and HPLC-MS (LOD, 5 ng/mL) (Guerrut et al., 1979; Haglock et al., 2008; Wolf et al., 2011). As for the pharmacokinetic (PK) study of GER, these methods showed disadvantages such as low sensitivity and relatively low extraction recovery, and a complicated and time-consuming sample pretreatment process.

More recently, new sampling techniques like dried blood spots (DBS) and dried plasma spots have been adopted for guanfacine determination. The DBS assay (Li et al., 2011) showed relatively high sensitivity, but compared with traditional sampling methods, this kind of method involves too many steps, making it more cumbersome, time-consuming and labor-intensive. The dried plasma spot assay (Li et al., 2012) was also limited by its inadequate sensitivity (LLOQ was 0.25 ng/mL) for the present PK study of GER and involves even more complicated processes than DBS.

Therefore, the primary objective of the present study was to develop a more practical and reliable liquid chromatography–electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method for the determination of guanfacine in beagle dog plasma after oral administration of GER. To the knowledge of the authors, it is the most sensitive LC-MS/MS method using a simple traditional sampling method (wet plasma samples) for the PK study of GER in beagle dogs.

Experimental
Regents and chemicals
The reference standard of guanfacine (purity > 99.9%, Fig. 1) and GER (purity > 99.4%) were provided by Henan Zhongshuai Medical Technology Development Co. Ltd (Zhengzhou, China). The reference standard of enalaprilat (purity > 99.0%, Fig. 1) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile of HPLC grade were purchased from Tedia Company Inc. (Fairfield, OH, USA). Formic acid and other reagents (analytical grade) were obtained from Nanjing Chemical Reagent Co. Ltd (Nanjing, China). Water used during the whole experiment was distilled and deionized.

Instrumentation
The HPLC system (Shimadzu LC-20 AD series) consisted of a binary pump, a degasser, a SIL-20 AC series autosampler and a CTO-20A series column oven (Kyoto, Japan). The detector was an ABI Sciex API4000 triple quadrupole mass spectrometer equipped with a turbo ion spray interface (Foster City, CA, USA). The analyst software version 1.5.1 pack-age was used to control the LC-MS/MS system for data acquisition and analysis (Applied Biosystems Sciex, USA).

LC-MS/MS conditions
The chromatographic separation was performed on a VP-ODS C18 column (150 × 2.0 mm, i.d. 5 μm; Shimadzu, Kyoto, Japan) which was maintained at 40 °C. The mobile phase consisted of solvent A (0.1% formic acid in distilled deionized water) and solvent B (methanol) was pumped at a flow rate of 0.35 mL/min with a gradient elution. The gradient profile involved 0–1.00 min, 20% B; a linear increase to 90% B within 0.5 min; 90% B for 2.5 min; a linear decrease to 20% B over 0.5 min; 20% B for 1.5 min; 6.00 min, stop. The total run time was 6 min. The autosampler temperature was kept at 5 °C and the injection volume was 10 μL.

The separated analytes were detected by an API 4000 triple quadrupole mass spectrometer with a turbo ion spray interface. A positive multiple-reaction monitoring (MRM) mode was applied for the detection of ion transitions at m/z 246.2 → 159.0 [M + H]+ for guanfacine and m/z 349.2 → 205.9 [M + H]+ for internal standard (IS), respectively. The optimized MS/MS parameters were as follows: dwell time, 200 ms; ion spray voltage, 5.5 kV; curtain gas, gas 1 and gas 2 (all nitrogen), 15, 40 and 35 units, respectively; source temperature, 400 °C; collision-activated disso-ciation, 4 units; declustering potential, 68 V for both guanfacine and IS; collision energy, 42.8 eV for m/z 246.2 → 159.0 (guanfacine) and 25.7 eV for m/z 349.2 → 205.9 (IS).

Preparation of standards and quality control samples
The stock solutions of guanfacine (1 mg/mL) in methanol were diluted with methanol to obtain a series of standard solutions (0.1, 0.3, 1, 3, 10 and 20 ng/mL). Aliquots of 200 μL of these standard solutions were evaporated to dryness by a centrifugal thickener (50 °C). The residue was reconstituted with methanol–water–formic acid (20:80:0.08, v/v/v) solution, vortex-mixed for 2 min and centrifuged (4 °C) at 16,000 g for 10 min, then 200 μL of blank plasma, which was freshly prepared from beagle dogs by centrifugation at 4000 g for 10 min, was added to the residue of each standard solution to achieve final calibration standards at the concentration of 0.1, 0.3, 1, 3, 10 and 20 ng/mL.

The IS standard solution (2 ng/mL) was obtained by diluting the stock solution of enalaprilat (1 mg/mL) with acetonitrile. Quality control (QC) samples (0.3, 3 and 16 ng/mL) were prepared in the same way as calibration standards. All of the prepared standards and QC samples were stored at 4 °C until analysis.

Sample pretreatment
Aliquots of 200 μL sample plasma and 800 μL of IS acetonitrile solution (2 ng/mL) were added to 1.5 mL polyethylene tubes. After deproteinization by vortex-mixing for 2 min and centrifugation (4 °C) at 16,000 g for 10 min, aliquots of 800 μL supernatant were transferred and evaporated to dryness by the centrifugal thickener (50 °C). The residue was reconstituted by 100 μL of methanol–water–formic acid (20:80:0.08, v/v/v) solution, vortex-mixed for 1 min and then centrifuged (4 °C) at 16,000 g for 10 min. Finally, 10 μL of the supernatant was injected onto the LC-MS/MS system for quantitative analysis.

Method validation
A full validation of the developed method was performed according to US Food and Drug Administration (2001) guidelines for bioanalytical method validation. The specificity of this method was evaluated by analyzing blank plasma samples obtained from six different beagle dogs. The linearity was assessed by five calibration curves which were constructed by plotting measured peak area ratios of guanfacine and IS (represented by y) to nominal concentrations of guanfacine (repre-sented by c) with a weight of 1/c. The LLOQ was defined as the minimum concentration of plasma samples that could be accurately
Determination of guanfacine in beagle dog plasma

quantified by this method (relative standard deviation, RSD < 20%). The matrix effects were evaluated by comparing the peak areas of the analytes in post-extracted blank plasma samples spiked with guanfacine at three QC levels (n = 5) with that of the same concentration pure standard solutions directly dried and reconstituted with the mobile phase. The precision and accuracy were evaluated by determining QC samples (0.3, 3 and 16 ng/mL). To analyze intra-day precision and accuracy, five replicates of each QC sample at low, medium and high concentration levels were analyzed on the same day. The assay was performed in three consecutive days to evaluate inter-day precision and accuracy. The absolute recovery was determined by comparing the peak areas of analytes in extracted plasma samples at three QC concentrations (n = 5) with those of the pure standards without extraction. The stability of the stock solution prepared in methanol was studied after maintenance for nearly one month at 4 °C. The stability of guanfacine in plasma was evaluated by analyzing QC samples (n = 5) after short-term (pre-processed samples stored at room temperature for 4 h) and long-term storage (stored at −20 °C for 20 days). The stabilities of QC samples after three freeze (−20 °C) and thaw (room temperature) cycles and post-preparative stability (post-processed samples stored at 4 °C for 24 h) were also analyzed.

Application of the method to a pharmacokinetic study in beagle dogs

Six healthy beagle dogs, three male and three female (9.53 ± 0.75 kg) were purchased from Shanghai Xin’gang Laboratory Animal Center (Shanghai, China). They were kept in separate cages under controlled conditions and given natural light with free access to clean water and standard pellet feed. The animal study was carried out according to the guidance of the Animal Ethics Committee in China Pharmaceutical University (Nanjing, China).

Each beagle dog was orally given one guanfacine extended release tablet (containing 4 mg of guanfacine). Blood samples were collected from the forelimb vein into heparinized tubes before dosing and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 14 h after dosing. Plasma samples were then obtained by centrifugation at 4000g for 10 min and kept at −20 °C until analysis.

All of the pharmacokinetic parameters were calculated by noncompartmental analysis using Drug and statistics software (DAS 2.1.1 version, Mathematical Pharmacology Professional Committee of China).

Results and discussion

Method development and optimization

Electrospray ionization (ESI) was adopted in this method because of its advantages in molecular weight confirmation, good selectivity for ionized and polar substances and especially high sensitivity, which is very necessary for the determination of guanfacine in plasma. In order to optimize the MS/MS conditions, standard solutions (1 μg/mL) of guanfacine and IS were directly injected onto the mass spectrometer. A full scan of the analytes was performed in both negative and positive mode. The ion intensity of guanfacine in positive mode was higher than that in negative mode. This is probably because of its alkaline guanidine group, which is prone to form positive ions. As a matter of experience, positive-scan mode is usually the preferred choice for basic compounds. Thus, positive-scan mode was selected and further optimized. A Q1 mass full scan (parent ion confirmation) was the first step, during which the values of declustering potential, ion spray voltage, source temperature, curtain gas, gas 1 and gas 2 were optimized to achieve the highest stability and ion response for molecular ions. As a result, the [M + H]+ ions of guanfacine and IS were m/z 246.2 and m/z 349.2, respectively. Then the product ion scan was carried out, and the molecular ions of guanfacine and IS were used to obtain their product ions with optimized collision energy values. Finally, the mass spectrometric fragmentations were m/z 159.0 for guanfacine and m/z 205.9 for IS, respectively. As the final step, MRM mode was performed to further confirm the selectivity, sensitivity and stability of the ion transitions at m/z 246.2 → 159.0 for guanfacine and m/z 349.2 → 205.9 for IS. Both ion transitions showed high and stable signal responses when collision-activated dissociation was set to 4 units. The full-scan product ion mass spectra of guanfacine and IS under positive MRM mode are provided in Fig. 2.

In order to achieve satisfactory chromatographic resolution, proper retention time and perfect peak shapes, the chromatographic conditions were carefully optimized. When selecting the mobile phase, methanol was a higher priority than acetonitrile because the solubility of guanfacine in methanol was better and the analytes could be thoroughly eluted. Since the detection was operated under positive mode, formic acid was added to provide protons and further increase the peak response of the analytes. The final composition of the mobile phase was methanol and water containing 0.1% formic acid. The Shimadzu VP-ODS C18 column tended to be the most efficient because it could give the best peak shapes, better resolution and proper retention times. To further improve the elution efficiency and peak shape, a simple and short gradient elution program was set up. A column temperature of 40 °C and a flow rate of 0.35 mL/min were both optimal to achieve shorter retention times and sharp peaks. Under the current optimized LC-MS/MS conditions, the analytes were all well separated and detected with high sensitivity and selectivity.

Selection of IS

Enalaprilat, an antihypertensive drug, was selected as the internal standard for this method. It had similar chromatographic

![Figure 2](wileyonlinelibrary.com/journal/bmc)
behavior and ESI response to guanfacine. In addition, the extraction recovery of enalaprilat was satisfactory and it was stable during the whole analytical process.

Sample preparation
As mentioned above, low plasma concentration of guanfacine has been reported in several publications (Biederman et al., 2008; Swearingen et al., 2007). Therefore, it is critical to develop a sample pretreatment method that can not only concentrate the plasma concentration of guanfacine but also provide high extraction recovery. During the optimization process of sample preparation, LLE and protein precipitation were tested and compared. Since guanfacine can easily dissolve in water, water-soluble impurities in plasma may have considerable effects on its determination. For the same reason, LLE was not suitable for the extraction of guanfacine from plasma because of its low extraction efficiency for water-soluble substances. However, direct injection after protein precipitation was not sensitive enough since plasma samples were diluted many-fold after precipitation. Thus, a concentration procedure involving drying and reconstitution of the supernatant obtained by protein precipitation was adopted. In this situation, the addition of beagle plasma could be reduced. Moreover, without being afraid of affecting sensitivity, addition of the precipitator could be increased to make sure that plasma proteins were completely precipitated and make the post-extracted plasma samples cleaner, which is not only good for the instruments but also significantly reduces the interference on determination. After several attempts and optimization, a sample preparation method with high recovery was determined, as described in detail in the section ‘Sample pretreatment’.

Assay validation
Specificity. Representative chromatograms of blank plasma, blank plasma spiked with guanfacine at the LLOQ (0.1 ng/mL) and plasma sample obtained at 10 h after oral administration were shown in Fig. 3. The results showed that the analytes were well separated and the retention times of guanfacine and IS were about 3.47 and 3.66 min, respectively. Compared with spiked plasma, analytical results of blank plasma samples from six different individuals showed no effect of interference from endogenous substances in plasma on the peaks of both analytes.

Linearity and LLOQ. The linearity of this method was assessed by the calibration curves determined on five separate days. The method showed good linearity over the concentration range of 0.1–20 ng/mL with the correlation coefficients (r) >0.994. The typical regression equation was y = 0.141c + 0.00296 (r = 0.9970) and the corresponding calibration curve is shown in Fig. 4. This method was sensitive enough to investigate the pharmacokinetic characteristics of GER when the LLOQ was defined as 0.1 ng/mL using only 200 μL of beagle plasma; the precision and accuracy values at LLOQ were all within the acceptable range.

Precision and accuracy. The precision and accuracy results of QC samples are listed in Table 1. The RSD values of intra-day and inter-day precision were all <10.8%. The accuracy results were ranged from 93.7 to 108.4% for intra-day accuracy and from 92.9 to 101.0% for inter-day accuracy. These results demonstrated that the developed method is quite reproducible and accurate.

Matrix effects and recovery. As shown in Table 2, the mean values of matrix effects for guanfacine at low, medium and high QC concentrations were 99.1, 100.7 and 89.4%, respectively. The mean recovery results were all >99.0% at three QC levels for guanfacine. The mean matrix effects and recovery for IS determined at 2 ng/mL were 98.7 and 101.7%, respectively. The results suggested that there was no ion suppression or enhancement by the plasma matrix on detection of the analytes.
Stability. The stability of guanfacine was evaluated under various conditions, and RSD values of all the measured concentrations did not exceed the acceptable range of 80–120% at low QC level and 85–115% at medium and high QC levels. The results (Table 3) showed that guanfacine could maintain its stability during the whole analytical process.

Application in pharmacokinetic study

The well-established LC-MS/MS method was successfully applied to determine the plasma concentration of guanfacine as well as to investigate the pharmacokinetic characteristics of GER after oral administration in beagle dogs. The mean plasma concentration–time profile of guanfacine is shown in Fig. 5. As can be seen in Table 4, the main pharmacokinetic parameters were calculated and listed. The mean maximum plasma concentration ($C_{\text{max}}$) of guanfacine was 5.72 ± 1.78 ng/mL and achieved at a time ($T_{\text{max}}$) of 3.08 ± 1.63 h after dosing. The mean areas under the concentration–time curve from 0 to 14 h ($\text{AUC}_{[0-14\ h]}$) and from 0 h to infinity ($\text{AUC}_{[0-\infty]}$) were 42.02 ± 16.88 and 44.14 ± 17.19 ng/mL h, respectively. The mean elimination half-life ($t_{1/2}$) was 2.60 ± 1.43 h. The apparent volume of distribution ($V_{z}/F$) and apparent oral clearance ($CL_{z}/F$) were 395.52 ± 302.96 L and 100.84 ± 33.62 L/h, respectively. The results showed that the plasma concentration of guanfacine after oral administration of GER was gently increased to $C_{\text{max}}$ without large fluctuations, and maintained steady around $C_{\text{max}}$ from 2 to 4 h, then slowly decreased to about 0.416 ± 0.36 ng/mL within 14 h.

Table 1. Precision and accuracy results for the assay of guanfacine in beagle plasma

<table>
<thead>
<tr>
<th>Nominal concentration (ng/mL)</th>
<th>Intra-day ($n = 5$)</th>
<th>Inter-day ($n = 15$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated concentration (mean ± SD, ng/mL)</td>
<td>Precision (RSD, %)</td>
</tr>
<tr>
<td>0.3</td>
<td>0.325 ± 0.010</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>2.810 ± 0.304</td>
<td>10.8</td>
</tr>
<tr>
<td>16</td>
<td>15.940 ± 0.971</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Table 2. Matrix effects and extraction recovery for the determination of guanfacine and IS in beagle plasma ($n = 5$)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Nominal concentration (ng/mL)</th>
<th>Matrix effects (mean ± SD, %)</th>
<th>Extraction recovery (mean ± SD, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanfacine</td>
<td>0.3</td>
<td>99.1 ± 4.6</td>
<td>103.9 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>100.7 ± 12.4</td>
<td>99.0 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>89.4 ± 3.7</td>
<td>107.3 ± 5.4</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td>2</td>
<td>98.7 ± 8.8</td>
<td>101.7 ± 8.8</td>
</tr>
</tbody>
</table>

Table 3. Stability of guanfacine in beagle plasma at three QC levels ($n = 5$)

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Nominal concentration (ng/mL)</th>
<th>Calculated concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Pre-preparative</td>
<td>0.3</td>
<td>0.30</td>
</tr>
<tr>
<td>stability</td>
<td>3</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>15.12</td>
</tr>
<tr>
<td>Post-preparative</td>
<td>0.3</td>
<td>0.29</td>
</tr>
<tr>
<td>stability</td>
<td>3</td>
<td>2.94</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>14.68</td>
</tr>
<tr>
<td>Long-term storage</td>
<td>0.3</td>
<td>0.32</td>
</tr>
<tr>
<td>stability</td>
<td>3</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>15.38</td>
</tr>
<tr>
<td>Three freeze</td>
<td>0.3</td>
<td>0.30</td>
</tr>
<tr>
<td>and thaw cycles</td>
<td>3</td>
<td>3.14</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>15.94</td>
</tr>
</tbody>
</table>

Figure 5. Mean plasma concentration–time profile of guanfacine following a single oral administration of 4 mg GER to beagle dogs (Data are mean ± SD, $n = 6$).

Table 4. Main pharmacokinetic parameters of guanfacine following a single oral dose of 4 mg guanfacine extended release tablets to beagle dogs ($n = 6$)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>5.72 ± 1.78</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>3.08 ± 1.63</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>2.60 ± 1.43</td>
</tr>
<tr>
<td>$\text{AUC}_{[0-14\ h]}$ (ng/mL h)</td>
<td>42.02 ± 16.88</td>
</tr>
<tr>
<td>$\text{AUC}_{[0-\infty]}$ (ng/mL h)</td>
<td>44.14 ± 17.19</td>
</tr>
<tr>
<td>$CL_{z}/F$ (L/h)</td>
<td>100.84 ± 33.62</td>
</tr>
<tr>
<td>$V_{z}/F$ (L)</td>
<td>395.52 ± 302.96</td>
</tr>
</tbody>
</table>
GER in beagle dogs showed relative shorter $T_{\text{max}}$ and higher $C_{\text{max}}$ than in human, thus the absorption of GER was a little faster in dogs. The shorter $t_{1/2}$ in dogs demonstrated that the elimination of GER was also faster than in human (Swearingen et al., 2007; Roesch et al., 2013). The reason why the main pharmacokinetic parameters of GER in dogs differ from those in human is probably species differences.

**Conclusion**

A practical, accurate and sensitive LC-ESI-MS/MS method with good reproducibility and high absolute recovery was established and fully validated for the determination of guanfacine in plasma. This method has been successfully applied to the pharmacokinetic study of GER after single oral administration to beagle dogs. The results showed that the developed method was a preferred choice to guarantee accuracy and reliability as well as to save time and effort. This method is very suitable for rapid and high-throughput PK studies of GER.

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