Implementation of a reference-scaled average bioequivalence approach for highly variable generic drug products of agomelatine in Chinese subjects

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Received 30 June 2015; received in revised form 20 August 2015; accepted 12 October 2015

KEY WORDS
Reference-scaled average bioequivalence; Agomelatine; 3-Hydroxy-agomelatine; 7-Desmethyl-agomelatine; Chinese subjects; High variability; Generic drug

Abstract The aim of this study was to apply the reference-scaled average bioequivalence (RSABE) approach to evaluate the bioequivalence of 2 formulations of agomelatine, and to investigate the pharmacokinetic properties of agomelatine in Chinese healthy male subjects. This was performed in a single-dose, randomized-sequence, open-label, four-way crossover study with a one-day washout period between doses. Healthy Chinese males were randomly assigned to receive 25 mg of either the test or reference formulation. The formulations were considered bioequivalent if 90% confidence intervals (CIs) for the log-transformed ratios and ratio of geometric means (GMR) of AUC and Cmax of agomelatine were within the predetermined bioequivalence range based on RSABE method. Results showed that both of the 90% CIs for the log-transformed ratios of AUC and Cmax of 7-desmethyl-agomelatine and 3-hydroxy-agomelatine were within the predetermined bioequivalence range. The 90% CIs for natural log-transformed ratios of Cmax, AUC0–t and AUC0–∞ of agomelatine (104.42–139.86, 101.33–123.83 and 97.90–117.94) were within the RSABE acceptance limits, and 3-hydroxy-agomelatine (105.55–123.03, 101.95–109.10 and 101.72–108.70) and 7-desmethyl-agomelatine (104.50–125.23, 102.36–111.50 and 101.62–110.64) were within the FDA bioequivalence definition intervals (0.80–1.25 for AUC and
ties are attributed to the extensive melatonin.7. The absolute oral bioavailability of this drug is low (approximately 23.9%) than placebo (50.0%).10,11 Agomelatine improves sleep quality and reduced waking after sleep onset in depressed patients.12 A therapeutic dose (25 mg once daily) preserves vigilance and memory in healthy volunteers.5,6 Due to the risk of common liver enzyme elevation and rare serious liver complications, routine laboratory monitoring of liver function is recommended periodically throughout treatment.9

The existing data on agomelatine metabolism, bioavailability and pharmacokinetics in Caucasians indicate that the absorption of agomelatine is rapid, with the median \( T_{\text{max}} \) 0.75–1.5 h and almost complete with at least 80% intestinal absorption.10,11 However, the absolute oral bioavailability of this drug is low (approximately 3%–4%) and highly variable (estimated to 104%). These properties are attributed to the extensive first pass metabolism of agomelatine.2

A systemically active generic drug is considered to be bioequivalent to the reference-listed drug if the rate and extent of absorption of the two products do not show a significant difference.12 The US Food and Drug Administration (FDA) uses peak drug concentrations (\( C_{\text{max}} \)) in plasma or other appropriate biological fluid as an index of drug rate of absorption and the area under the drug plasma concentration versus time curve (AUC) as an index of a drug’s extent of absorption.13 Due to the highly variable features (highly variable drugs are defined as those for which within-subject variability \([\text{CV}(<)]\) in bioequivalence measures is 30% or greater), a standard number of subjects (e.g., 18–24) may not be able to demonstrate the bioequivalence of generic products or their corresponding reference product using a two-way crossover design. Although agomelatine pilot data are published for Caucasians, they may not be applicable to the bioequivalence in other populations due to ethnic differences. Pei et al.14 investigated the intra-individual variability of agomelatine in healthy Chinese volunteers. Results showed that the intra-subject variability in \( AUC_{0-24} \) (CV = 43.52%) and \( C_{\text{max}} \) (CV = 78.34%). Wang et al.15 evaluated the inter-individual variability in AUC and \( C_{\text{max}} \) of agomelatine tablets in Chinese healthy male subjects and found inter-individual CVs of \( C_{\text{max}} \), \( AUC_{0-24} \) and \( AUC_{0-\infty} \) to be 102.20%, 131.74% and 130.59%, respectively. The intra-individual CVs of \( C_{\text{max}} \), \( AUC_{0-24} \) and \( AUC_{0-\infty} \) were 84.34%, 49.61% and 50.83%, respectively. In preliminary experiments with a four-way crossover method, the within-subjects variability of AUC and \( C_{\text{max}} \) of agomelatine were 53% and 70%, respectively. Comparable values for 3-hydroxy-agomelatine were 21.2% and 37.8%, and for 7-desmethyl-agomelatine were 42.6% and 61.4%. These results showed that although the within-subject CV of agomelatine could be reduced with a four-way crossover method, it was still difficult to evaluate the bioequivalence. Song et al.16 found no differences in agomelatine pharmacokinetics between the rs2069514 GG homozygotes (\( n = 35 \)) and the rs2069514 AG allele (\( n = 35 \)) in all subjects, suggesting that the rs762551, rs2470890 and rs2472304 genetic polymorphisms might be associated with the marked inter-individual variability of agomelatine.

The topic of bioequivalence evaluation of highly variable drugs is one that has been intensely debated in many recent articles, conferences and meetings.17 The FDA observed that studies of highly variable drugs generally used more subjects than studies of lower variability.18 For the highly variable drug agomelatine, excessively large sample sizes would be required by a standard bioequivalence study, but the FDA discourages unnecessary human testing. These observations raise questions about the appropriate sample sizes for bioequivalence studies of drug products for which high variability does not appear to impact safety and efficacy. An additional concern is that the large sample sizes needed for bioequivalence studies of highly variable drugs may deter the development of new generic products.19,20 A final concern is that a highly variable reference product may not be shown to be bioequivalent to itself in a crossover study using a relatively modest number of subjects (e.g., 18–40).21 The commonly-accepted method for statistical analysis of bioequivalence data is the average bioequivalence (ABE) approach. Bioequivalence is established when the difference between the logarithmic means occur between preset regulatory limits, as shown below:

\[
\left( \mu_T - \mu_R \right)^2 \leq \theta_\alpha^2 \tag{1}
\]

where \( \mu_T \) is the population average response of the log-transformed measure for the test (T) formulation, \( \mu_R \) is the population average response of the log-transformed measure for the reference (R) formulation, and \( \theta_\alpha \) is equal to ln 1.25. So the limits are:

\[ \ln 0.8 \leq (\mu_T - \mu_R) \leq \ln 1.25 \tag{2} \]

On one hand, only the average means of main pharmacokinetic parameters (e.g., AUC and \( C_{\text{max}} \)) are taken into consideration in ABE method, and the individual variations of pharmacokinetic parameters are not considered. Thus, the two formulations showed ABE does not guarantee individuals’ bioequivalence (IBE). On another hand, the bioequivalence criteria for the ABE method are identical for both low variability and high variability drugs.

For a time, the FDA worked toward implementing an individual bioequivalence (IBE) approach for studies submitted to New Drug Applications (NDAs) and Abbreviated New Drug Application (ANDAs, for generic drugs). It was argued that requiring drug products to meet an IBE rather than an ABE standard would improve formulation switchability.22,23 The proposed criteria for acceptable IBE included the comparison of test and reference means, comparison of within-subject variances, assessment subject-by-formulation interactions, and ability to scale the bioequivalence limits if within-subject variability of the reference...
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product exceeded predetermined values. Under IBE, the inequality used to determine if two products are bioequivalent is as follows:

\[
(\mu_T - \mu_R)^2 + \sigma_{DR}^2 + (\sigma_{WR}^2 - \sigma_{WR}^2) \leq \theta_1
\]

where \( \sigma_{DR}^2 \) is the population subject-by-formulation interaction variance component, \( \sigma_{WR}^2 \) is the population within-subject variance of the test formulation, \( \sigma_{WR}^2 \) is the population within-subject variance of the reference formulation, and \( \theta_1 \) is the bioequivalence limit for IBE. From 1999 to 2001, at the FDA's request, the pharmaceutical industry applied the IBE study design and analysis to NDAs and ANDAs for modified-release drug products. The IBE was used to evaluate the bioequivalence of modified-release drug products because it was thought that, due to the relative complexity of modified-release formulations, the likelihood was greatest of detecting subject-by-formulation interactions with these types of drug products. However, analysis of these data failed to detect the presence of clinically significant subject-by-formulation interactions.

To lower the sample size required for bioequivalence studies of highly variable drugs, the FDA and European Medicines Agency (EMA) have recommended the RSABE approach, whereby the bioequivalence acceptance limits are scaled to the variability of a reference product.

The RSABE for both AUC and \( C_{\text{max}} \) is evaluated as below:

\[
(\mu_T - \mu_R)^2 \leq \theta_S
\]

where \( \mu_T \) is the population average response of the log-transformed measure for the test (T) formulation, \( \mu_R \) is the population average response of the log-transformed measure for the reference (R) formulation, and \( \theta_S \) is the bioequivalence limit for RSABE. Therefore, the aim of this study was to investigate the pharmacokinetic properties of agomelatine and the bioequivalence of a test agomelatine tablet (Chongqing FuAn Pharmaceutical Group Qingyutang Pharmaceutical Co., Ltd., Chongqing, China; lot No. 130301) and a reference agomelatine tablet (Servier, French; lot No. 893158) to obtain regulatory approval for the test formulation. In this study, we used the RSABE method to evaluate the bioequivalence of two formulations with parent agomelatine for the first time in healthy Chinese male subjects.

2. Materials and methods

2.1. Study design and procedures

A single-dose, randomized-sequence, open-label, four-way crossover study was conducted at the phase I Clinical Research Unit of the Third Xiangya Hospital of Central South University (Changsha, China) from November 2012 to April 2014. The study (Chinese National Registry Code: 2013L00911) was performed in accordance with the 2008 version of the World Medical Association Declaration of Helsinki, the International Conference on Harmonization Guideline for Good Clinical Practice, and the local regulatory guidelines of the State Food and Drug Administration (SFDA) of People's Republic of China. The study protocol, protocol amendment, and informed-consent form were approved by the independent ethics and research committee at the Third Xiangya Hospital of Central South University prior to the initiation of the study. Before undergoing any study procedure, all participants provided written consent after they had been informed of the study's purpose, nature, procedures, and risks by clinical investigators.

A computer-generated random number table of SPSS 17.0 was applied to assign subjects in a ratio of 1:1 to receive a single 25-mg dose of (administered with 250 mL of tap water at room temperature) either the test or the reference formulation of agomelatine. Voluntees were admitted into the phase I clinical research unit at 9:00 p.m. the day before study and fasted 10 h before each drug administration. Neither caffeine-containing nor alcoholic beverages were allowed until 24 h after dosing. Smoking was forbidden during the same interval after the dose administration. As the half-life of agomelatine is approximately 1–2 h, a one day washout period was used following administration of the initial formulation, after which the alternate formulation was administered. The design scheme of the study is summarized in Table 1.

2.2. Subjects

Formulations were considered bioequivalent if the 90% CIs for the log-transformed ratios and ratio of geometric means (GMR) of AUC and \( C_{\text{max}} \) of agomelatine were within the predetermined bioequivalence range based on RSABE method. Both the 90% CIs
for the log-transformed ratios of AUC and $C_{\text{max}}$ of 7-desmethyl-agomelatine and 3-hydroxy-agomelatine were within the bioequivalence range of ABE method. The sample size was calculated by the within-subject variability (37.8%) of 3-hydroxy-agomelatine from pre-experiment as follows:

$$n = \left[ (t_a + t_b)\sigma_d / \delta \right]^2$$  \hspace{1cm} (8)

where $t_a$ is $t$ value of the $a$ inspection standards, $t_b$ is the type II error rate, $\delta$ is the requirements of discrimination, and $\sigma_d$ is the within-subject variability. As $t_a$=1.6449, $t_b$=1.2816, $\delta$=0.2, $\sigma_d$=0.378, the sample size used was $n$=31.

Based on the above, a minimum of 32 subjects were required. Taking into account the test management and lost cases, 44 subjects were enrolled in the four-way crossover study.

Forty-four healthy Chinese male volunteers aged 18–40 years with body mass indices (BMI) between 19 and 25 kg/m$^2$ were assessed for inclusion in the study. As females can be influenced by additional variables such as menstruation and pregnancy, the guidelines of the Chinese State Food and Drug Administration (SFDA) generally recommend selecting healthy males for bioequivalence studies. Subjects were judged to be eligible for the study when no clinically significant abnormal findings existed on a complete medical examination. The exam included medical history, physical examination, 12-lead electrocardiogram, hematology, blood biochemistry and urinalysis.

2.3. Blood sampling

Blood samples (5 mL) were collected from a suitable forearm vein into anticoagulant tube by an indwelling catheter at the following time point: 0 (before administration), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0 and 8.0 h after drug administration. After washout and administration of the alternate formulation, blood samples were drawn and analyzed in the same way.

2.4. Tolerability assessments

Subjects were carefully monitored by vital signs (sitting blood pressure, heart rate, breathing rate, and oral body temperature), clinical laboratory tests (hematology, blood biochemistry, and urinalysis), 12-lead ECGs, and physical examinations at baseline and at the end of each study period. National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0 was used to describe and grade all toxicities and adverse events. The relationship of adverse events to study drug was documented by the investigator as unrelated or unlikely, possibly, probably, or definitely related.

2.5. Pharmacokinetic evaluations

An LC–MS/MS validated method for the simultaneous determinations of agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine concentrations in human plasma. The analytes were quantified by use of phenacetin as the internal standard. The plasma sample clean-up procedure was performed by liquid-liquid extraction. Aliquots (5 µL) were injected onto the analytical column (Phenomenex ODS3, 150 mm × 4.6 mm, 5 µm, USA). The mobile phase consisted of methanol and formic acid aqueous solution (20%) within 5 mmol/L ammonium formate (70:30, v/v) was delivered with a flow rate of 0.8 mL/min, with a run time of approximately 7 min. Positively charged ions, created at atmospheric pressure, were transferred to an Agilent 6460 triple-quadrupole LC–MS (Agilent, USA). The transitions for agomelatine were selected from $m/z$ 244.1 → 185.1, 3-hydroxy-agomelatine from $m/z$ 260.1 → 201.1, 7-desmethyl-agomelatine from $m/z$ 230.1 → 171.1, and the internal standard from $m/z$ 180.1 → 110.1.

2.6. Pharmacokinetic analysis

A non-compartmental analysis was used to determine the pharmacokinetic parameters using WinNonlin 6.1. $C_{\text{max}}$ and $T_{\text{max}}$ were obtained directly from the plasma concentration-time curves. The AUC$_{0-\infty}$ was calculated according to the trapezoidal rule. AUC$_{0-\infty}$ was calculated as follows:

$$\text{AUC}_{0-\infty} = \text{AUC}_{0-t} + C_t / k_e$$ \hspace{1cm} (9)

where $C_t$ was the last measured concentration at time $t$, and $k_e$ was the terminal elimination rate constant estimated by log-linear regression analysis of data visually assessed to be a terminal log-linear phase. At least 3 points were used for estimation of $k_e$. The apparent terminal elimination $t_{1/2}$ was calculated as follows:

$$t_{1/2} = 0.693 / k_e$$ \hspace{1cm} (10)

Intra-individual variability for the considered pharmacokinetic parameters was assessed by CV(%).
Bioequivalence limits, upper, lower = \(e^{\pm 2.326 \sigma_{\text{ln}}/n^{0.5}}\)  \(\times\) (11)

For 3-hydroxy-agomelatine and 7-desmethyl-agomelatine, the test/reference ratios of AUC were within the predetermined bioequivalence range of 0.80 to 1.25 and \(C_{\text{max}}\) ratios were within 0.75–1.33, according to the guidelines of the SFDA of the China.

The bioequivalence assessment of the parent drug agomelatine was an essential goal of the present study. Evaluation of the bioequivalence of the two metabolites was considered as possibly supportive evidence for the bioequivalence of the parent drug.

3. Results

A total of 44 male subjects were enrolled in the study. Index, mean (range): age, 22.8 (2.5) years (range, 19–28 years); height, 170 (10) cm (range, 157–181 cm); weight, 60.5 (6.3) kg (range, 51–74 kg); BMI, 20.7 (1.6) kg/m² (range, 19.0–24.0 kg/m²). Each subject received the test formulation and the reference formulation twice, respectively. All volunteers completed the study.

3.1. Tolerability

There were no protocol violations or serious adverse events observed in the study. Twenty subjects experienced a total of 37 mild adverse events in this four-way crossover study. The most frequently recorded were somnolence (17), dizziness (6), insomnia (6), epigastric pain (1). Somnolence, dizziness and insomnia were considered to be definitely related to the study treatment, and epigastric pain was considered to be probably related to the study medication. There were no withdrawals from the study due to adverse events.

3.2. Method validation

The calibration curves for agomelatine, 7-desmethyl-agomelatine and 3-hydroxy-agomelatine were linear over the concentration ranges of 0.0457–100 μg/L, 0.1372–300 μg/L and 0.4572–1000 μg/L in human plasma, respectively. The mean regression equation of the calibration curve for agomelatine was \(Y = 0.1188X - 0.0005 \ (r^2 = 0.9962)\), for 7-desmethyl-agomelatine was \(Y = 0.0734X - 0.0003 \ (r^2 = 0.9975)\), and for 3-hydroxy-agomelatine is \(Y = 0.0543X - 0.0007 \ (r^2 = 0.9978)\) with lower limits of quantitation being 0.0457, 0.1372 and 0.4572 μg/L, respectively. Precision values were all <15%, and accuracy was between 85% and 115%. Technically, the assay for the determination of agomelatine and its metabolites from human plasma was highly reproducible, sensitive, and accurate method.

3.3. Pharmacokinetic properties

Following single 25-mg oral doses of the test and reference formulations, the mean plasma concentration–time curve of agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine are shown in Fig. 1A–C, respectively. Mean pharmacokinetic parameters (AUC\(_{0-\infty}\), AUC\(_{0-\infty}\), \(C_{\text{max}}\), T\(_{\text{max}}\), and t\(_{1/2}\)) and CV(%) are summarized in Table 2.

For the parent agomelatine, no period or sequence effects were detected for any pharmacokinetics properties on ANOVA. A significant subject effect was observed for AUC\(_{0-\infty}\), AUC\(_{0-\infty}\), \(C_{\text{max}}\). There were no significant differences between the two formulations in regard to AUC\(_{0-\infty}\), AUC\(_{0-\infty}\), \(C_{\text{max}}\) or t\(_{1/2}\) by two 1-side t tests, with the exception of T\(_{\text{max}}\) (1.44 [0.75] h for the test formulation and 1.22 [0.86] h for the reference formulation (P<0.05 by Mann–Whitney U test). For the metabolite 3-hydroxy-agomelatine and 7-desmethyl-agomelatine, no period, formulation, or sequence effects were observed for any pharmacokinetic properties by ANOVA, and there were no significant differences between the two formulations in AUC\(_{0-\infty}\), AUC\(_{0-\infty}\), \(C_{\text{max}}\) by two 1-side t test or in T\(_{\text{max}}\) by Mann–Whitney U test.

3.4. Bioequivalence evaluation

The 90% CIs of the ratios (T/R) for the log-transformed AUC\(_{0-\infty}\), AUC\(_{0-\infty}\), \(C_{\text{max}}\) are listed in Table 3. There were no significant differences between the test and reference formulations. The 90%
The median values of $T_{\text{max}}$ for 3-hydroxy-agomelatine and 7-desmethyl-agomelatine confirmed the rapid disappearance of the parent compound which was comparable between the two formulations.

The FDA has recommended the RSABE approach to evaluate the bioequivalence of highly variable drugs (e.g., agomelatine). Accordingly, the acceptance limits for such a study is to be scaled to the variability of the reference formulation. In the present study, we used the RSABE approach to assess the bioequivalence of two formulations of parent compounds for the first time in Chinese healthy male subjects. The standard criteria were used to evaluate the bioequivalence of the test formulation and the reference formulation, along with studies of the metabolites 3-hydroxy-agomelatine and 7-desmethyl-agomelatine.

The aim of this study was to apply the RSABE approach to evaluate the bioequivalence of 2 formulations of agomelatine, a drug with highly variable kinetics, and to investigate the pharmacokinetic properties of agomelatine in Chinese healthy male subjects. There are a few reports in the literature on the pharmacokinetics of agomelatine in Chinese population. Pei et al.\textsuperscript{14} investigated the CV(%) of agomelatine in 16 Chinese healthy male volunteers and showed significant ethnic differences between Chinese and Caucasian subjects in $C_{\text{max}}$ and $AUC_{0-t}$ whereas no ethnic differences in $T_{\text{max}}$ or $t_{1/2}$ were found. Less obvious first-pass effects in Chinese subjects may partially account for why both $C_{\text{max}}$ and $AUC$ of Chinese males were much higher than those of Caucasian males. In this study, the median (SD) agomelatine and its metabolites $AUC_{0-t}$, $T_{\text{max}}$, and $C_{\text{max}}$ for Chinese subjects (summarized in Table 3) are presented for the first time.

The 90% CIs of the test/reference ratios of $C_{\text{max}}$, $AUC_{0-t}$, $AUC_{0-\infty}$ for agomelatine and metabolites were all located within RASBE and the standard criteria range, respectively. The %CV of the main pharmacokinetic parameters of agomelatine and metabolites varied greatly. The large inter-subject variability in pharmacokinetic behavior observed in our study was consistent with the previous literature in other populations.\textsuperscript{7} Agomelatine is rapidly absorbed from the gastrointestinal tract and immediately transported to the liver, where it is extensively metabolized by cytochrome P450 (CYP) isoenzymes CYP1A1, CYP1A2 and CYP2C9\textsuperscript{9,10}. 7-Desmethyl-agomelatine and 3-hydroxy-agomelatine were identified as the two metabolites of agomelatine, which have less activity than the parent drug, and no significant differences of absorption and metabolism were found among agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine for the two formulations in 44 subjects.

### Table 2 Pharamacokinetic parameters and CV (%) of agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine after a single 25-mg oral dose of a test or a reference formulation of agomelatine 25-mg tablet in healthy fasted Chinese adult males.

| Parameter          | Agomelatine | 3-Hydroxy-agomelatine | 7-Desmethyl-agomelatine |
|--------------------|-------------|-----------------------|------------------------|
|                    | Test        | Reference             | Test                   | Reference             |
| $AUC_{0-\infty}$ (µg·h/L) | 8.59 (10.17) | 7.99 (10.15)          | 88.30 (31.81)          | 84.44 (32.49)         |
| CV (%)             | 54.1        | 60.1                  | 14.4                   | 23.7                  |
| $AUC_{0-\infty}$ (µg·h/L) | 8.72 (10.16) | 8.31 (10.23)          | 89.78 (32.27)          | 86.11 (33.06)         |
| CV (%)             | 52.6        | 54.7                  | 14.4                   | 23.4                  |
| $C_{\text{max}}$ (µg·h/L) | 7.55 (10.11) | 5.74 (6.91)           | 50.09 (25.45)          | 43.30 (22.45)         |
| CV (%)             | 84.4        | 80.0                  | 43.9                   | 42.0                  |
| $T_{\text{max}}$ (h) | 1.14 (0.75) | 1.22 (0.86)           | 1.13 (0.72)            | 1.25 (0.81)           |
| $t_{1/2}$ (h)      | 1.24 (1.40) | 1.58 (1.32)           | 1.24 (0.24)            | 1.29 (0.26)           |

Data are expressed as Mean (SD), unless otherwise specified; $n=44$.

### Table 3 Comparison of 90% CIs of natural log(ln)-transformed parameters of agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine for a test or reference formulation of agomelatine 25-mg tablet after a single 25-mg oral dose in healthy fasted Chinese adult males ($n=44$).

| Parameter          | Ratio | 90% CI | Power |
|--------------------|-------|--------|-------|
| ln$C_{\text{max}}$ | 1.21  | 1.04–1.40 | 0.808 |
| ln$AUC_{0-t}$      | 1.12  | 1.01–1.24 | 0.978 |
| ln$AUC_{0-\infty}$| 1.07  | 0.98–1.18 | 0.989 |
| ln$AUC_{3-\text{Hydroxy-agomelatine}}$ | 1.14 | 1.06–1.23 | 0.999 |
| ln$AUC_{7-\text{Desmethyl-agomelatine}}$ | 1.05 | 1.02–1.10 | 1.000 |

CIs for natural log-transformed ratios of $C_{\text{max}}$, $AUC_{0-t}$, $AUC_{0-\infty}$ of agomelatine (104.42–139.86, 101.33–123.83, and 97.90–117.94, respectively) were within the RASBE acceptance limits (8.99%–204.13%, 59.48%–139.86%, and 61.38%–162.91% for $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$, respectively). The metabolites 3-hydroxy-agomelatine and 7-desmethyl-agomelatine were within the predetermined regulatory 90% CI ranges for bioequivalence (80%–125% for $AUC_{0-t}$ and $AUC_{0-\infty}$, and 75–133% for $C_{\text{max}}$ for the T/R ratio).

### 4. Discussion

According to US FDA guidelines,\textsuperscript{35} only the parent compound released from the formulation rather than the metabolite is generally recommended for bioequivalence studies. However, when a metabolite contributes meaningfully to the drug's pharmacologic effects or when a parent compound is difficult to analyze in plasma, metabolite quantitation is also recommended. Although the pharmacokinetic parameters of agomelatine itself are the most essential criteria for bioequivalence evaluation, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine were assessed in the present study to provide supporting evidence.
The present study had several limitations that should be considered. The pharmacokinetic data of this study were obtained only from Chinese healthy males who were administered a single dose. Therefore, the pharmacokinetics might be different in other targeted populations or after other dosage regimens.

5. Conclusions

The RSABE approach was successfully applied to evaluate the bioequivalence of two formulations of the highly variable drug agomelatine in Chinese male volunteers. This study found that the test and reference formulations of agomelatine 25-mg tablet met the regulatory definition.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81102499), Hunan Science and Technology Project (No. 2011SK3261), the Fundamental Research Funds for the Central Universities of Central South University (No. 2014zzts313). The authors are also grateful for the support from Chongqing FuAn Pharmaceutical Group Qingyutang Pharmaceutical Co., Ltd.

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