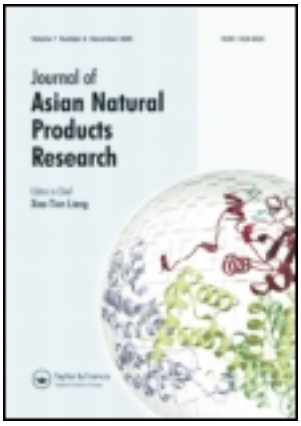


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# Pharmacokinetics, tissue distribution, and excretion of buagafuran in rats

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The pharmacokinetics, tissue distribution, and excretion of buagafuran (BF, 4-butyl-α-agarofuran), a promising antianxiety drug isolated from Gharu-wood (*Aquilaria agallocha* Roxb), were investigated in rats. BF plasma concentration was determined in rats after oral and intravenous doses by GC-TOF-MS. BF showed nonlinear pharmacokinetics after oral and intravenous administration of 4, 16, and 64 mg/kg. The $AUC_{0-\infty}$ and $C_{max}$ did not increase proportionally with doses, indicating the saturation in absorption kinetics of BF in rats after oral dosage. BF absorption was extremely poor with an absolute bioavailability below 9.5%. After oral administration of $^{3}$H-BF (4 mg/kg) to rats, radioactivity was well distributed to the tissues examined. The highest radioactivity was found in gastrointestinal tract, followed by liver and kidney. Radioactivity in brain, as a target organ, was about 20–40% of that in plasma at all time points. Total mean percent recovery of radioactive dose was about 80% in rats (51.2% in urine; 28.7% in feces). Bile elimination was also the major excretion route of BF, and 45.4% of the radioactive dose was recovered in bile.

**Keywords:** buagafuran; pharmacokinetics; tissue distribution; excretion; GC-TOF-MS



## 1. Introduction

Buagafuran (BF, 4-butyl-α-agarofuran, Figure 1, previously named as AF-5) is a derivative of sesquiterpenoid agarofuran isolated from the traditional Chinese medicine Gharu-wood (*Aquilaria agallocha* Roxb), which has attracted much interest due to its structural diversities and broad spectrum of biological activities, including sedative, hypnotic, appetite suppressive, antiemetic, and antibacterial activities [1–5]. BF was proved to have significant antianxiety activity with a higher potency and a lower toxicity compared with diazepam and buspirone, assessed in the elevated plus-maze test, open field test, light/dark test, tail-suspension test, and operant conditioned response test [6–9]. The antianxiety mechanism of BF was related to the modulation of central monoamine neurotransmitters, and it could significantly reduce the level of dopamine in homogenates of striatum, midbrain, and cortex to alleviate anxiety [10].

Despite extensive researches on the pharmacological activities of BF, little is known about its pharmacokinetics. The aim of this study is to determine the pharmacokinetic profile of BF in rats after administration of various doses by different routes and to examine the compound tissue distribution and excretion after single oral dosing of $^{3}$H-BF. The result obtained in the present study should be helpful for the formulation and dosage

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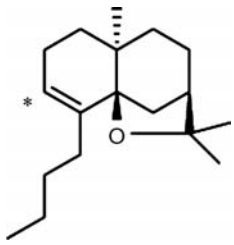

Figure 1. Chemical structure of BF ($^{*3}$H-labeled position).

regimens design, and for the better understanding of its pharmacological activities.

## 2. Results

BF plasma concentration was quantified based on a validated GC-TOF-MS method (published in *Journal of Pharmaceutical and Biomedical Analysis*) with minor modification. The chromatography of blank plasma, BF quality control (40 ng/ml), and rat plasma after an oral dosing of BF (16 mg/kg) are shown in Figure 2. The extracted ion traces *m/z* 262.22 (BF) and *m/z* 266.25 (BF-$d_4$) were used for the quantification. No other endogenous compound in rat plasma produced the ions at *m/z* 262.22 and 266.25. The intraday and interday variability of the assay for plasma is listed in Table 1. It demonstrated that the GC-TOF-MS method for the determination of BF was reliable and reproducible, since both % coefficient of variation (CV) and % bias were below 15% for all estimated concentration of BF. The stability of BF during the determination was assessed under a variety of conditions. Analysis of these samples consistently afforded values that were nearly identical to those of freshly prepared quality control (QC) samples, thus confirming the overall stability of BF in plasma under frozen storage, assay processing, and freeze–thaw conditions.

BF plasma concentration after intravenous administration rapidly declined. Mean (±SD) concentration-time profiles for BF after intravenous administration are shown in Figure 3. Pharmacokinetic parameters determined are presented in Table 2. $C_{max}$ values proportionally increased with dose, but AUC was disproportionately high relative to the dosage change. $V_d$ values remarkably increased from 3.8 to 40.5 L, and $t_{1/2}$ was prolonged from 0.7 to 6.6 h with increasing dose. The clearance (CL) was not significantly changed from 2.3 to 4.3 L/h.

BF appeared rapidly in plasma after oral administration. Figure 4 shows the plasma concentration-time profiles for BF after a single oral dose at 4, 16, and 64 mg/kg. Table 3 summarizes the pharmacokinetic parameters of BF oral doses in rats. Interestingly, $T_{max}$ was significantly prolonged with increasing dose, with values of 0.17, 0.33, and 0.67 h for 4, 16, and 64 mg/kg, respectively. The findings could be due to decreased solubility and/or delayed absorption of BF at higher oral doses. In addition, $C_{max}$ values for 16 and 64 mg/kg oral doses were not significantly different, with values of 127.6 and 109.9 ng/ml. AUC did not increase proportionally with dose, with values of 20.9, 137.5, and 239.8 ng h/ml for 4, 16, and 64 mg/kg, respectively. The CL and $V_z$ values for oral dose of 64 mg/kg remarkably increased compared with lower dose. The absolute bioavailability calculated for 4, 16, and 64 mg/kg was 9.5, 8.1, and 6.6%, respectively.

$^{3}$H-BF (4 mg/kg) was absorbed and distributed into 15 tissues examined at 0.5 h after oral administration in female and male rats. Tissue level of radioactivity is shown in Table 4. At the absorption stage, the highest level of radioactivity was found in gastrointestinal tract at 0.5 h time point, which was likely to be a result of oral administration, followed by liver and kidney. The radioactivity in brain, as a target organ, was about 20% of that in plasma at the same time point. Tissue to plasma ratio in spleen, lung, large intestine, heart, muscle, testes, fat, metra, and ovaries was relatively lower over the

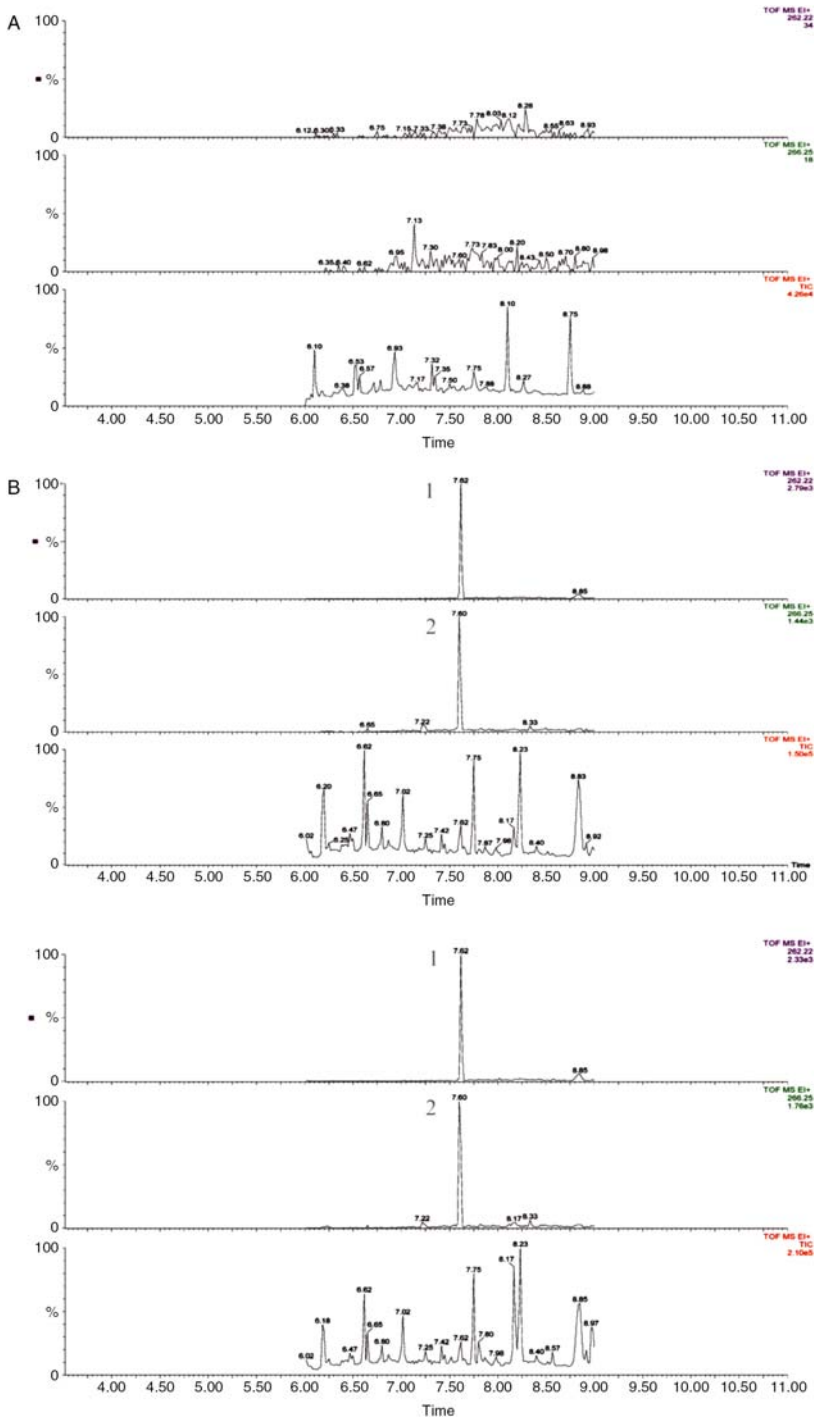


Figure 2. Total ion chromatography, extracted ion chromatography of the blank plasma (A), the plasma spiked with BF and BF-$d_4$ (IS) (B), and dosed plasma (C). Peak 1 is BF (*m/z* 262.2 Da) and Peak 2 is BF-$d_4$ (*m/z* 266.2).

Table 1. Intra- and inter-day precision and accuracy of GC-TOF-MS analysis of BF in rat plasma.

| | Intraday | | | Interday | | |
|---|---|---|---|---|---|---|
| Concentrations (ng/ml) | Mean ± SD ($n = 5$) | Precision (% CV) | Accuracy (% bias) | Mean ± SD ($n = 5$) | Precision (% CV) | Accuracy (% bias) |
| 12.5 | 11.6 ± 0.4 | 3.5 | −7.2 | 12.9 ± 0.9 | 6.9 | 3.2 |
| 50 | 52.2 ± 1.2 | 2.3 | 4.4 | 51.5 ± 3.9 | 7.7 | 3.0 |
| 200 | 200.6 ± 13.9 | 6.9 | 0.3 | 204.8 ± 9.9 | 4.8 | 2.4 |

range of 0.34–0.91. By 2.5 h postdose, tissue-to-plasma ratio was increased with time in most tissues examined. In particular, tissue-to-plasma ratio in brain was observed up to 41%. At all three time points, the metra and ovaries had higher concentration than the testes between genders.

The cumulative excretion of drug-related radioactivity of $^{3}$H-BF after single oral administration is given in Table 5. Approximately, 51.2% of $^{3}$H-BF dose was recovered in urine and 28.7% in feces. Excretion of $^{3}$H-BF and its radioactive metabolites appeared to be complete by 288 h after dosing in rats. Biliary excretion of drug-related radioactivity also was the major route, and 45.4% of dose was recovered in bile by 96 h (Figure 5).

## 3. Discussion

After a single oral dose of 4, 16, and 64 mg/kg, BF showed nonlinear pharmacokinetics in rats. The AUC and $C_{max}$ did not increase proportionally with doses, mostly due to the increased CL or decreased oral absorption of BF at high dose. The low bioavailability of BF after oral dosing may be attributed to several reasons: (1) low aqueous solubility of the compound in the gastrointestinal tract, (2) active transporter-mediated efflux in gastrointestinal membrane, and/or (3) presystemic degradation or metabolism of the compound in intestinal lumen before absorption. Our studies *in vitro* found that BF was the substrates of both *p*-glycoprotein and CYP3A. *P*-glycoprotein can pump BF to lumen and block its

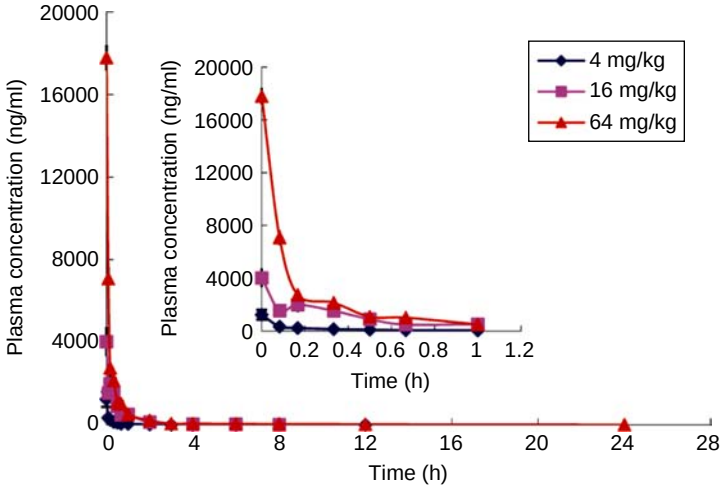


Figure 3. Plasma concentration-time profiles of BF after a single intravenous dose at 4, 16, and 64 mg/kg in rats.

Table 2. Pharmacokinetic parameters of BF in rats following a single intravenous dose at 4, 16, and 64 mg/kg ($n = 5$).

| Parameter | Dose | | |
|---|---|---|---|
| | 4 mg/kg | 16 mg/kg | 64 mg/kg |
| $AUC_{0-\infty}$ (ng h/ml) | 220.8 | 1697.5 | 3624.0 |
| $C_{max}$ (ng/ml) | 1243.0 | 4036.6 | 17,785.5 |
| $T_{1/2}$ (h) | 0.7 | 1.2 | 6.6 |
| CL (L/h) | 4.3 | 2.3 | 4.2 |
| $V_d$ (L) | 4.3 | 3.8 | 40.5 |



absorption, then enhance the metabolism by intestinal CYP3A in rat recirculating perfusion model. Verapamil and cyclosporine A, as *p*-glycoprotein inhibitors, markedly improve the absorption of BF [11]. The poor bioavailability of BF may be partly due to the interplay of *p*-glycoprotein/CYP3A on the absorption of BF in intestinal lumen.

$^{3}$H-BF distributed preferentially into well perfused organs such as liver and kidney at an early time point (0.5 h) and was also detectable in most of tissues after oral administration at all times points. $^{3}$H-BF failed to accumulate in fat depots even though it is highly lipophilic. In addition, tissue-to-plasma ratio in the liver at all time points tested clearly suggested that BF had a very high affinity for liver with the highest concentration even after 5 h. The presence of a high accumulation of radioactivity in the liver was indicative of involvement of enterohepatic circulation in the rats. Enterohepatic circulation prolonged the presence of BF in the systemic circulation and exposed organs systems such as the liver and the gastrointestinal tract to high concentration of BF.

Moreover, radioactivity in brain, as a target organ, was about 20–40% of that in plasma at all time points. This observation was justified and desirable, considering that brain is the compound's site of action. The result demonstrated that BF may be considered as a potential drug for the treatment of anxiety disorders.

In conclusion, BF was rapidly absorbed after oral administration,

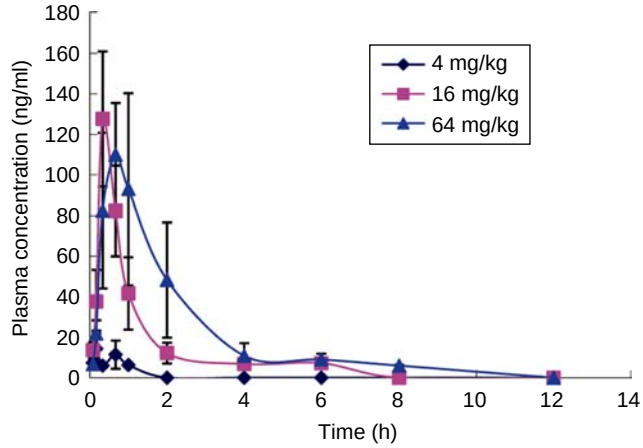


Figure 4. Plasma concentration-time profiles of BF after a single oral dose at 4, 16, and 64 mg/kg in rats.

Table 3. Pharmacokinetic parameters of BF in rats following a single oral dose at 4, 16, and 64 mg/kg ($n = 5$).

| | Dose | | |
|---|---|---|---|
| Parameter | 4 mg/kg | 16 mg/kg | 64 mg/kg |
| $AUC_{0-\infty}$ (ng h/ml) | 20.9 | 137.5 | 239.8 |
| $C_{max}$ (ng/ml) | 14.3 | 127.6 | 109.9 |
| $T_{max}$ (h) | 0.17 | 0.33 | 0.67 |
| $T_{1/2}$ (h) | 1.3 | 1.6 | 1.8 |
| MRT (h) | 1.9 | 2.1 | 2.5 |
| CL (L/h) | 25.7 | 27.9 | 64.1 |
| $V_d$ (L) | 63.4 | 65.3 | 164.2 |
| Bioavailability (%) | 9.5 | 8.1 | 6.6 |

Note: MRT, mean resistance time.



showing saturation in the absorption kinetics in rats at high dose. The tissue-to-plasma ratio presented by the compound indicated that a reasonable amount of drug reached the target tissue, which was important for pharmacodynamic effect reported in animals. This present investigation on BF pharmacokinetics, tissue distribution, and excretion will provide valuable information to clinic, which is essential for understanding the safety and efficacy of this drug.

## 4. Materials and methods

### *4.1 Chemicals and reagents*

BF (purity > 99.8%), internal standard (IS, BF-$d_4$), and $^3$H-BF were synthesized and provided by the Departments of Medicinal Chemistry (Institute of Materia Medica, Chinese Academy of Medical Sciences). $^3$H-BF had a specific activity of 12.5 mCi/mmol with radiochemical purity >95%. Hexane and other reagents were obtained from Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing,

Table 4. Radioequivalents (μg Eq/g) in tissues of rats at 0.5, 2.5, and 5 h after oral administration of $^3$H-BF (4 mg/kg).

| | Radioequivalents (μg Eq/g) | | |
|---|---|---|---|
| Tissue | 0.5 h | 2.5 h | 5 h |
| Liver | 6.38 ± 5.14 (6.79) | 2.93 ± 1.17 (7.91) | 2.80 ± 0.88 (7.77) |
| Kidney | 3.21 ± 2.11 (3.41) | 1.90 ± 0.93 (5.15) | 1.46 ± 0.54 (4.05) |
| Stomach | 6.45 ± 4.18 (6.86) | 1.02 ± 0.56 (2.76) | 0.57 ± 0.38 (1.58) |
| Small intestine | 4.46 ± 4.04 (4.74) | 1.27 ± 0.68 (3.43) | 0.40 ± 0.13 (1.12) |
| Spleen | 0.68 ± 0.39 (0.72) | 0.45 ± 0.28 (1.22) | 0.34 ± 0.14 (0.94) |
| Lung | 0.86 ± 0.50 (0.91) | 0.38 ± 0.12 (1.01) | 0.46 ± 0.20 (1.28) |
| Large intestine | 0.56 ± 0.32 (0.60) | 0.33 ± 0.12 (0.89) | 0.22 ± 0.13 (0.61) |
| Heart | 0.75 ± 0.37 (0.79) | 0.32 ± 0.14 (0.87) | 0.33 ± 0.11 (0.91) |
| Muscle | 0.62 ± 0.34 (0.66) | 0.25 ± 0.08 (0.68) | 0.41 ± 0.19 (1.15) |
| Testes | 0.32 ± 0.18 (0.34) | 0.24 ± 0.07 (0.65) | 0.33 ± 0.12 (0.93) |
| Fat | 0.51 ± 0.26 (0.54) | 0.24 ± 0.09 (0.64) | 0.32 ± 0.05 (0.88) |
| Brain | 0.19 ± 0.07 (0.20) | 0.15 ± 0.14 (0.41) | 0.09 ± 0.02 (0.24) |
| Metra | 0.60 ± 0.20 (0.64) | 0.56 ± 0.26 (1.52) | 0.52 ± 0.23 (1.44) |
| Ovaries | 0.85 ± 0.49 (0.91) | 0.61 ± 0.28 (1.64) | 0.52 ± 0.13 (1.45) |
| Blood | 0.94 ± 0.64 | 0.37 ± 0.19 | 0.36 ± 0.13 |

Note: Values in parentheses represent tissue to plasma ratio.

Table 5. Percentage (mean ± SD) of radioactivity excreted in urine and feces of rats administered oral H-BF at 4 mg/kg.

| Collection period (h) | $^{3}$H-excreted in urine (%) | $^{3}$H-excreted in feces (%) | Total $^{3}$H-excreted (%) |
|---|---|---|---|
| 0–24 | 42.30 ± 8.97 | 8.04 ± 6.67 | 50.34 ± 9.02 |
| 24–48 | 4.92 ± 1.26 | 7.24 ± 3.06 | 12.16 ± 3.11 |
| 48–72 | 2.18 ± 0.56 | 8.71 ± 2.66 | 10.89 ± 2.74 |
| 72–96 | 0.40 ± 0.10 | 1.91 ± 0.59 | 2.31 ± 0.62 |
| 96–120 | 0.66 ± 0.17 | 1.75 ± 0.37 | 2.41 ± 0.45 |
| 120–144 | 0.16 ± 0.04 | 0.39 ± 0.08 | 0.55 ± 0.10 |
| 144–168 | 0.22 ± 0.06 | 0.29 ± 0.05 | 0.51 ± 0.07 |
| 168–192 | 0.08 ± 0.02 | 0.27 ± 0.05 | 0.35 ± 0.06 |
| 192–216 | 0.10 ± 0.03 | 0.05 ± 0.01 | 0.15 ± 0.04 |
| 216–240 | 0.10 ± 0.03 | 0.02 ± 0.01 | 0.12 ± 0.03 |
| 240–264 | 0.08 ± 0.02 | 0.01 ± 0.004 | 0.09 ± 0.02 |
| 264–288 | 0.04 ± 0.01 | 0.01 ± 0.002 | 0.05 ± 0.01 |
| 0–288 | 51.24 ± 13.01 | 28.71 ± 5.10 | 79.95 ± 13.52 |



China). All the reagents were of analytical grade.

### 4.2 Animals

Male and female Wistar rats weighing 220–250 g in the study were provided by Animal Center of Chinese Academy of Medical Sciences [certificate No. SCXK (Beijing) 2000-0006]. All animals were maintained at a controlled temperature (24 ± 2°C) and a regular 12 h light/dark cycle. Food and water were freely available. The protocols for animal studies were approved by the Institute Animal Care and Welfare Committee.

### 4.3 Pharmacokinetics and bioavailability study using BF

#### 4.3.1 Drug administration and blood sampling

The rats were fasted 12 h before receiving BF and fed 4 h after administration. The oral dosing solutions used for all animal studies were prepared by suspending the

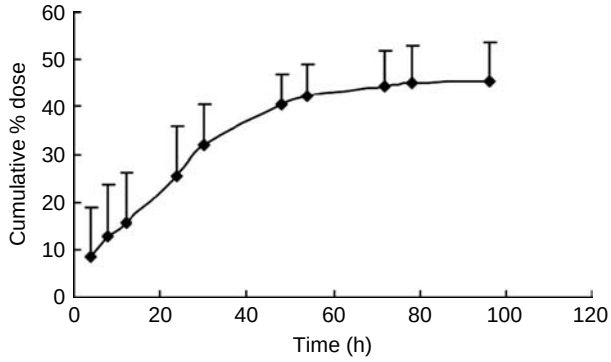


Figure 5. Elimination of radioactivity in bile after oral administration (4 mg/kg, $n = 5$).

required amounts of BF in 0.5% sodium carboxymethyl cellulose. Plasma pharmacokinetics of BF was studied in rats after a single oral dose of BF at 4, 16, and 64 mg/kg. Blood samples were collected via the femoral vein into heparinized syringes at 0.08, 0.17, 0.33, 0.67, 1, 2, 3, 4, 6, 8, 12, and 24 h after dosing. For intravenous dosing, BF was solubilized in PEG400/saline (1:1, v/v) and was injected via the tail vein of rats. After a single intravenous dose of 4, 16, and 64 mg/kg, blood samples were collected via the femoral vein at 0, 0.17, 0.5, 0.67, 1, 2, 3, 4, 6, 8, 12, and 24 h. Plasma was prepared by centrifugation of blood at 4000 rpm for 5 min and stored at $-20$°C.

#### 4.3.2 *Samples preparation*

An aliquot (1 ml) of plasma sample was spiked with an IS ($d_4$-BF) and mixed with 4 ml hexane to extract by vortex for 2 min. The upper organic portion was obtained by centrifugation at 3000 rpm for 5 min and evaporated to dryness under nitrogen at 40°C. The residue was dissolved in 20 μl of hexane, and 1 μl aliquot of hexane was injected into the GC-TOF-MS system.

#### 4.3.3 *Analytical methodology*

GC-TOF-MS analysis was performed on an Agilent 6890 GC apparatus (Agilent Corp., Santa Clara, CA, USA) equipped with a time of flight mass spectrometry (Micro-Mass, Manchester, UK). A HP-1 capillary column (25 m × 0.2 mm × 0.11 μm) was employed with helium as a carrier gas. The oven temperature program was set as follows: initial temperature, 70°C for 1 min; gradient of 30°C/min until 160°C; gradient of 10°C/min until 210°C; gradient of 20°C/min until 250°C; and hold time, 2 min. The injector temperature was 240°C and an injection volume of 1 μl was employed in the splitless mode. The TOF-MS was operated using electron impact ionization mode and the electron collision energy was 70 eV. An ion source temperature of 200°C, an interface temperature of 200°C, and detector voltage of 2.7 kV were used. The full scan range of the sample for the qualitative analysis was from *m/z* 50 to 500, and then the quantification was the extracted ion chromatograms from the full scans. The extracted ion traces *m/z* 262.22 (BF) and *m/z* 266.25 (IS) were used for quantification. The raw data were analyzed by MassLyns V4.0 software [12]. In the bioanalytical method validation, at least five QC samples of three different concentrations of BF were processed and injected on a single day (intraday) and at different days (interday) for evaluating the precision. The variability of BF determination as the % of CV should be $\leq 15\%$ at all the concentration. Accuracy was expressed as % of bias which should be within limits of $\pm 15\%$ at all concentrations of BF. The freeze–thaw stability of BF in plasma samples was determined over three freeze-thaw cycles. In each freeze-thaw cycle, the samples were frozen and stored at $-20$°C for 24 h, then thawed at ambient temperature. To assess long-term stability, the plasma samples were stored at $-20$°C for 10 days. For the short-term stability, fresh plasma samples were kept at room temperature for 12 h before extraction.

### 4.4 *Disposition study using $^3$H-BF*

#### 4.4.1 *Drug administration*

The dosing solution was prepared by combining $^3$H-BF with unlabeled BF in absolute alcohol. The mixture was dried in rotate film evaporator, and then suspended in 0.5% sodium carboxymethyl cellulose. Rats were administered by a single oral dose of $^3$H-BF at 4 mg/kg (240 μCi/kg).

#### 4.4.2 *Excretion study*

Five rats were housed in individual metabolism cages for the collection of

urine and feces after oral administration of $^{3}$H-BF at 4 mg/kg. Urine and feces samples were collected at 24 h intervals over a 12-day period postdose. The excreta were stored frozen at −20°C for the counting of total radioactivity.

The cannulas were surgically inserted into the bile duct of the rats. Five rats were given $^{3}$H-BF at a dose of 4 mg/kg. Bile was collected from the rats up to 96 h postdose. Bile samples were stored at −20°C until they were analyzed for total radioactivity.

#### 4.4.3 *Tissue distribution*

Thirty male and female rats were administered with a single oral dose of $^{3}$H-BF at 4 mg/kg. Ten male and female rats each were sacrificed and various tissues including liver, kidney, stomach, small intestine, spleen, lung, blood, large intestine, heart, muscle, testes, fat, brain, metra, and ovaries of each rat were promptly removed at 0.5, 2.5, and 5 h for the determination of radioactivity. One additional rat was sacrificed predose to provide control tissues for analysis. Liver, kidney, stomach, small intestine, lung, large intestine, muscle, fat, testes, and brain were homogenized with a probe homogenizer, and duplicate aliquots (about 0.2 g) were analyzed. Other tissue samples were cut into small pieces, and the whole samples were analyzed in duplicate.

#### 4.4.4 *Measurement of radioactivity*

An aliquot of blood, urine, bile (100 μl), fecal, and tissue homogenate (about 0.1 g) prepared as described above was put into a vial. The samples were digested and decolorized with perchloric acid/hydrogen peroxide. After digestion, scintillation cocktail was added to the vial, and counted using a P.E.1414 Liquid Scintillation Counter (Perkin-Elmer Bioscience, Waltham, MA, USA). Tissue levels of radioactivity were expressed as microgram equivalents per gram of tissue and were calculated by dividing the dpm per gram of tissue by the specific activity of the $^{3}$H-BF administered. The percentage of dose excreted in urine, feces, or bile was calculated as dpm in urine, feces, or bile divided by total dpm dose × 100.

### 4.5 *Pharmacokinetics calculations and statistics*

Pharmacokinetic analysis of BF in plasma was performed using noncompartmental and compartmental methods via the proprietary drug and statistics computer software package (Version 2.0, Anhui Province Center for Drug Clinical Evaluation, Wuhushi, China).

The bioavailability ($F_{po}$) of BF after intravenous and oral administration was calculated by the following equation:

$$F_{po} = (\mathrm{AUC}_{po} \times \mathrm{Dose}_{iv}/\mathrm{AUC}_{iv} \times \mathrm{Dose}_{po}) \times 100\%.$$

## Acknowledgements

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