Biopharmaceutical Evaluation of a Solid Dispersion System Containing Sibutramine Freebase

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To increase the solubility of sibutramine freebase, the solid dispersion was prepared using a fluid-bed granulator. The solid dispersion containing sibutramine freebase was characterized by differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD). After filling the sibutramine solid dispersion in the gelatin hard capsule, we performed *in vitro* dissolution test, the stability test under accelerated conditions and pharmacokinetic study in beagle dogs. The DSC and XRD data showed that sibutramine solid dispersion would be amorphous state. The dissolution rate of sibutramine solid dispersion was significantly increased about 70% than sibutramine freebase. The stability of sibutramine solid dispersion capsules was equivalent or above to commercial product of sibutramine. In beagle dogs, the sibutramine solid dispersion showed equivalent pharmacokinetic behavior with commercial product of sibutramine hydrochloride. In conclusion, the solid dispersion system provided a possible way to overcome the low solubility of sibutramine freebase, and the sibutramine solid dispersion can be a bioequivalent with the commercial product in humans.

Key Words : Solid dispersion, Sibutramine freebase, Solubility, Pharmacokinetic behavior

Introduction

Sibutramine is a serotonin and noradrenaline re-uptake inhibitor that is relatively ineffective as an antidepressant but has a pro-satiety effect.[1] It has a small thermogenic effect by limiting the decline in metabolic rate that normally occurs with weight loss.[2-4] Sibutramine undergoes extensive first-pass metabolism, mainly by hepatic cytochrome p450 3A4 enzymes, to active primary (M1) and secondary (M2) amine metabolites, which are more potent than the parent compound.[4-6] The drug exists in an oil state at room temperature because of its low melting point, so it is difficult to handle pharmaceutically. Also, the drug has poor solubility in water. In order to increase the solubility and improve the handling of sibutramine freebase, a hydrochloride monohydrate salt form has been developed by Abbott, the original production company. It was made available commercially in a capsule dosage form under the brand name of Meridia® or Redultil®.[7]

Recently, sibutramine freebase was crystallized in a solid powder form due to the development of crystallization technology. Therefore it is not necessary to be in salt form for easy handling. However, unlike the hydrochloride salt form, sibutramine freebase itself is not soluble in water, so it did not show the same *in vitro* dissolution profiles as the brand drug.

Generally, the change of the salts form may affect the biological properties of the drug as well as the solubility profiles and dissolution rates.[8,9] Therefore, it is necessary to overcome this poor dissolution characteristic. Solid dispersions of many poorly water soluble drugs with hydrophilic carrier matrix have been formulated for improving drug dissolution rate. Moreover, solid dispersions may improve the bioavailability of poorly water soluble drugs by increasing the drug dissolution rate and their saturation solubility in the gastrointestinal fluids.[10-13]

The purpose of this study was to develop the solid dispersion of sibutramine freebase having high dissolution characteristics. For the formulation study, a suitable solubilizing agent and excipients were selected and a finalized formulation containing sibutramine freebase was taken for an pharmacokinetic study with the commercial product in beagle dogs. This study was also focused on the characterization of the physico-chemical properties of sibutramine solid dispersion and the evaluation of the stability and the pharmacokinetic behaviors in beagle dogs for the prediction of bioequivalence in humans.

Experimental Procedures

Materials. Sibutramine freebase was purchased from Cipla (Mumbai, India), TPGS (tocopheryl polyethylene glycol 1000 succinate) was obtained from Eastman (Kingsport, USA), Cremophor RH 40 and Poloxamer 407 (Lutrol F147) were obtained from BASF (Ludwigshafen, Germany) and Gelucire 44/14 was purchased from Gattefosse (Saint-Priest, France). Citric acid and other reagents were purchased from Sigma-Aldrich Company (St. Louis, USA).

Animals. Healthy 6-7 months old beagle dogs (male, 6.9 ± 0.4 kg) were purchased from Chemon Korea Inc. (Yongin, Korea). Twelve dogs were used in this study and split into two groups of six. The animals were kept in an environmentally controlled breeding room for a week before the

start of the experiments. Each dog was fasted for 14 h prior to each study day, but water was allowed ad libitum.

Formulation study. Nonionic surfactants, which were in solid or semi-solid state at room temperature, were used to make the solid dispersion for screening of solubilizing agents. TPGS, Poloxamer 407, Cremophor RH 40, Gelucire 44/14 were used in the screening test for selection of the optimal solubilizing agent. Each surfactant was mixed homogeneously with the drug at a ratio of 1:1, and the solid dispersion was prepared by addition of hydroxypropylmethylcellulose (HPMC) and mannitol to the above drug and surfactant mixture. Then the capsule filler Ludipress® and a lubricant magnesium stearate were added during blending stage. Also, the effect of the acidifier (citric acid) to the formulations was evaluated because sibutramine dissolves more easily in a low pH medium.

Preparation of sibutramine solid dispersion. The solid dispersion containing sibutramine freebase was made through a solvent evaporation method and co-melt method. The methods were compared with each other in terms of the dissolution behavior of sibutramine. First, the co-melt method was used to make a solid dispersion by simultaneously melting the drug and surfactant at about 50 ± 2 °C, then HPMC was added to form the solid dispersion as the temperature was reduced. Second, the solvent evaporation method was used to make a solid dispersion by the spraying of an ethanol/water solution containing sibutramine freebase and surfactant to HPMC in a fluid-bed granulator. After the solid dispersion was prepared, other excipients, mentioned above, were added and mixed, and then each sibutramine solid dispersion formulation equivalent to 8.37 mg of sibutramine freebase was filled into gelatin hard capsules.

Differential scanning calorimetry (DSC). The DSC thermograms were recorded using a DSC (Mettler-Toledo, DSC822e). Approximately 2 to 5 mg of each sample was heated in an aluminum pan with lid from −30 to 150 °C at a scanning rate of 10 °C/min under a stream of nitrogen.

Powder X-ray diffraction (XRD). Powder X-ray diffraction patterns were recorded using a powder X-ray diffractometer (Mac Science, Japan, Model: M18XHF-SRA) under the following conditions: target Cu; filter Ni; voltage 40 kV; current 300 mA; receiving slit 0.15 millimeters. The data were collected in the continuous scan mode using a step size of 0.02° at 2θ/s. The scanned range was 3-50°.

***In vitro* dissolution study.** The dissolution test was performed in a Dissolution Apparatus II (Vankel VK7020, Varian) according to Korea Pharmacopoeia 8th edition (KP VIII) dissolution procedure. Each formulation of a commercial product as reference drug, sibutramine freebase capsule, and sibutramine freebase solid dispersion capsule (n = 6) were put into a sinker. The sinker containing formulations was placed in 900 mL of dissolution media (pH 1.2, pH 4.0, pH 6.8 and water) at 37 ± 0.5 °C with paddle speed of 50 rpm. After the dilution of dissolution sample with methanol, the concentration of sibutramine was determined by HPLC.

Accelerated stability test. The sibutramine solid dispersion capsules were packed in press- through package blisters, and stored in an incubator maintained at 40 °C and 75% RH with the commercial product for comparative stability. At appropriate time intervals, samples were withdrawn and examined. The content of sibutramine and impurities were determined by HPLC.

HPLC analysis. A Gemini C_{18} (4.6 × 250 mm, 5 μm, Phenomenex, Torrance, USA) column was used to analyze levels of sibutramine. The composition of mobile phase was water: acetonitrile = 64:36 (v/v). The flow rate was 1.5 mL/min, detection wavelength was 229 nm and injection volume was 10 μL.

***In vivo* absorption study in beagle dogs.** Six beagle dogs were orally administered one gelatin capsule containing 10 mg of sibutramine hydrochloride, equivalent to 8.37 mg of sibutramine freebase, as reference drug or sibutramine solid dispersion with sibutramine freebase of 8.37 mg. 200 μL of each blood sample was collected at designated time intervals of 0.33, 0.67, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72 h and it was centrifuged for 10 min at 4,000 rpm. The plasma was kept immediately at -20 °C until HPLC/MS/MS analysis.

Pharmacokinetic analysis. C_{max} values (maximum plasma concentrations), T_{max} times (times to C_{max}), AUC (area under the concentration-time curve), K_{el} (apparent elimination rate constant) and $t_{1/2}$ (half-life) of sibutramine, M1 and M2 were calculated by BA Calc 2002 program (KFDA, Korea). Results were expressed as mean±S.D. Statistical comparisons between groups were conducted by paired Student's t-test with the significance level of $P<0.05$.

Blood sample analysis. Plasma concentrations of sibutramine, metabolite M1 and metabolite M2 were analyzed using a validated high-performance liquid chromatographic-mass spectrometric (HPLC/MS/MS) method with a slight modification of the earlier method.[14-16] Briefly, 200 μL of plasma and 10 μL of internal standard solution prepared with amlodipine at a concentration of 5 μg/mL were added to glass tube. To this, 20 μL 0.1 M NaOH and 1200 μL of hexane:ethylacetate (90:10) organic solvent were added. After vigorous vortex mixing for 5 min, the mixture was centrifuged at 3000 rpm for 3 min and the organic phase was transferred to a clean glass tube and evaporated to dryness under a flow of nitrogen gas. The dry residue was reconstituted with 120 μL of 50% acetonitile, and a 10 μL aliquot of this reconstituted solution was injected onto the HPLC/MS/MS system.

HPLC/MS/MS analysis condition. HPLC/MS/MS system included a Shieseido Capcell Pak UG C18 column (5 μm, 2.0 × 150 mm, Shiseido Co., Tokyo, Japan). The mobile phase consisted of 50% acetonitrile containing 0.1% formic acid. The mass spectrometer with an electrospray source was run in the positive ion mode, and m/z 280.4, 266.4, 252.3 and 409 were monitored for sibutramine, M1, M2, and internal standard, respectively. Linear calibration curves were acquired in the ranges from 0.15 to 160 ng/mL for sibutramine, M1 and M2.

Results and Discussion

Formulation study. The effect of various nonionic surfac-

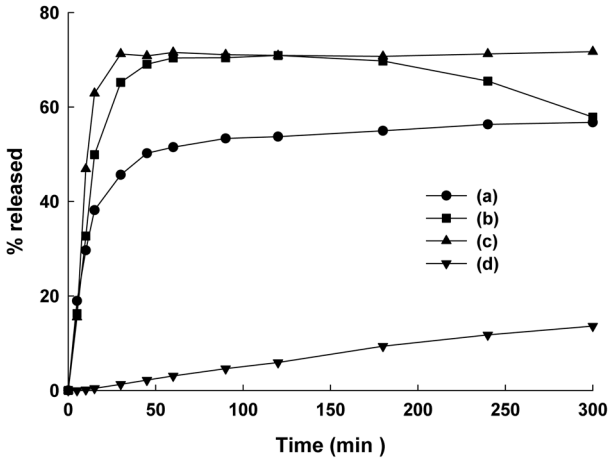

Figure 1. The dissolution profiles of sibutramine from (a) reference drug, (b) sibutramine solid dispersion containing poloxamer, (c) sibutramine solid dispersion containing poloxamer and citric acid and (d) sibutramine freebase raw material at the dissolution media pH 6.8.

tants such as Poloxamer 407, Cremophor RH40, Gelucire 44/14 and TPGS on the dissolution profiles of sibutramine freebase from solid dispersions was examined. The dissolution rate of sibutramine freebase was significantly enhanced by all surfactants tested. Among the surfactants, Poloxamer 407 was most effective at increasing the dissolution rate of sibutramine in all dissolution media (buffers of pH 1.2, 4.0, 6.8 and water) and the dissolution increasing effect was more significant in the dissolution medium of pH 6.8 and water. Figure 1 shows the effect of citric acid on the dissolution profile of sibutramine. Initial sibutramine release was faster when citric acid was incorporated in the solid dispersion formulation due largely to its solubility enhancing effect. No difference was found in the dissolution profile of sibutramine contained in the solid dispersion formuations prepared by either co-melt or solvent evaporation method.

Dissolution profiles of sibutramine solid dispersion. The composition of optimized final formulation determined from the formulation study was sibutramine freebase solid dispersion that was composed of sibutramine 8.37 mg, Poloxamer 407 8.83 mg, citric acid 5.8 mg, hydroxypropylmethylcellulose 8 mg, mannitol 87 mg, silicone dioxide 13 mg, sodium carboxymethylcellulose 3 mg, Ludipress® 106 mg and magnesium stearate 2 mg. Dissolution studies were performed for the commercial product, sibutramine freebase and sibutramine solid dispersion formulations. Figure 2 shows the dissolution profiles of sibutramine obtained in dissolution media having various pH values. The released sibutramine from sibutramine freebase capsule exhibited the pH-dependent and incomplete dissolution behavior. In buffers of pH 1.2 and 4.0, the released amounts of sibutramine from sibutramine freebase capsules were 101.1% and 103.4% within 60 min, whereas those were 11.7% and 17.3% in buffer of pH 6.8 and water, respectively. Solid dispersions of sibutramine freebase were prepared using surfactant and HPMC to enhance the dissolution rate. The released amounts of sibutramine from solid dispersion capsules were significantly higher than that of sibutramine from the sibutramine freebase capsules especially in buffer pH 6.8 and water media. The released sibutramine from solid dispersion capsules was also 8 to 10% higher than that from the commercial capsule.

Differential scanning calorimetry (DSC). The sibutramine in solid dispersion exhibited a reduction in endothermal peak height compared to pure drug alone. As shown in Figure 3, the DSC peak of solid dispersion was smaller and broader than the sibutramine freebase peak. Moreover,

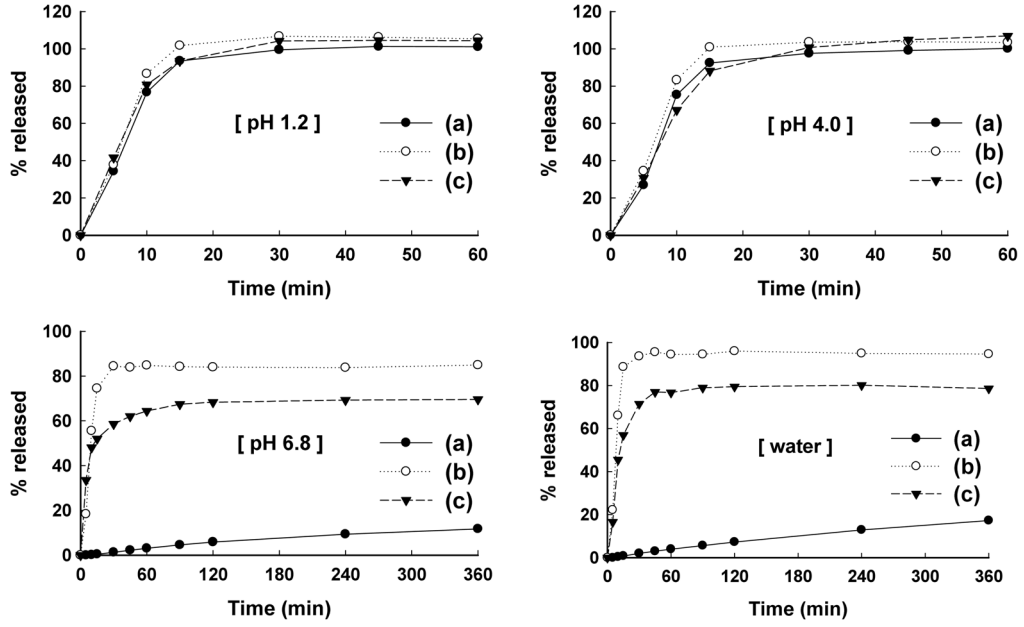

Figure 2. Comparative dissolution profiles of sibutramine from (a) sibutramine freebase drug, (b) sibutramine solid dispersion drug and (c) reference drug at various pH media.

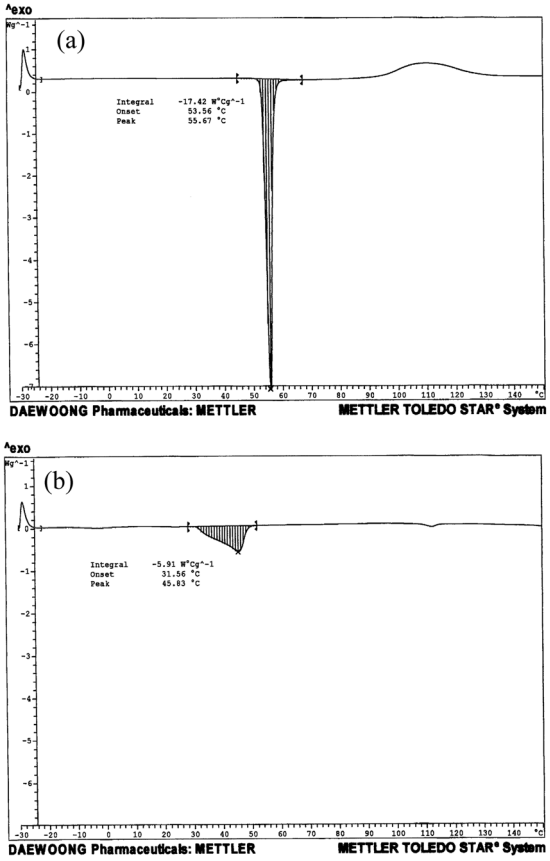

Figure 3. DSC graphs of (a) sibutramine freebase (crystalline raw material) and (b) sibutramine solid dispersion.

the peak was shifted away from the sibutramine freebase peak. From these results, it was presumed that sibutramine solid dispersion existed as non-crystalline form and there was interaction between sibutramine and carrier such as poloxamer 407 (Fig. 3).

Powder X-ray diffraction (XRD). The X-ray diffraction patterns for the sibutramine freebase and the solid dispersion are depicted in Figure 4. The sibutramine freebase gave a diffraction peak corresponded to a separate crystalline drug phase. Solid dispersion prepared by co-melt method showed the absence of diffraction peak of sibutramine freebase pointing to a transition of sibutramine freebase from a crystalline to an amorphous state as a consequence of the preparation procedure. Both the DSC and X-ray results confirmed that sibutramine freebase is present as an amorphous state in solid dispersion.

Accelerated stability test. At accelerated conditions under 40 °C, 75% RH, the sibutramine solid dispersion capsules exhibited slight but insignificant fall within the assay value. Total impurities were just increased up to 0.36% after storage for 6 months as shown in Figure 5. These results indicated that the sibutramine solid dispersion capsules would be very stable formulation for sibutramine freebase.

***In vivo* evaluation in beagle dogs.** HPLC/MS/MS chromatograms of sibutramine, metabolite M1, M2 and

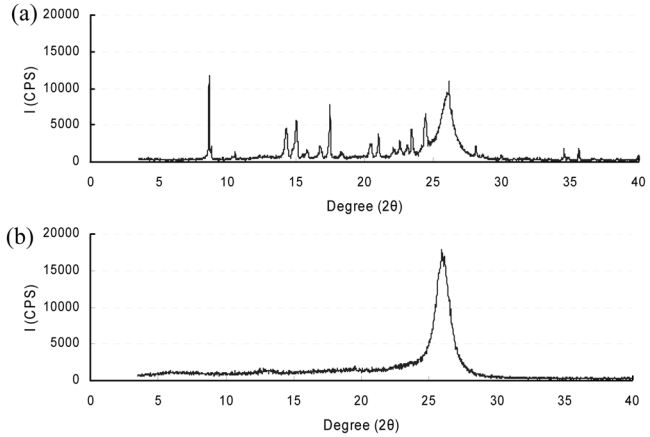

Figure 4. XRD graphs of (a) sibutramine freebase (crystalline raw material) and (b) sibutramine solid dispersion.

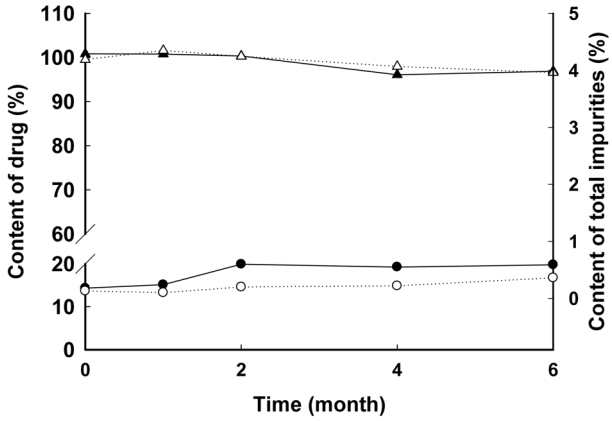

Figure 5. Stability of sibutramine solid dispersion capsules and commercial products in an accelerated condition of 40 °C and 75% RH. Sibutramine content of commercial product (▲) and solid dispersion capsule (△). Total impurities of commercial product (●) and solid dispersion capsule (○).

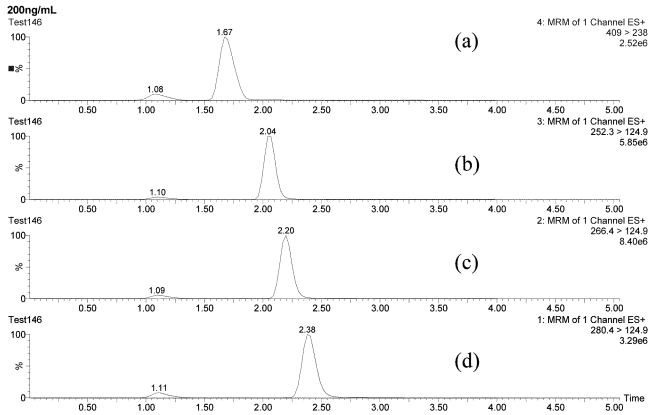

Figure 6. HPLC/MS/MS chromatograms of (a) amlodipine as internal standard, (b) metabolite M2, (c) metabolite M1 and (d) sibutramine spiked in beagle dog plasma.

amlodipine as internal standard were shown in Figure 6. Acceptable linearity was observed over the concentration range 0.05-200 ng/mL plasma (r^2 = 0.995 for sibutramine; r^2 = 0.997 for M1; r^2 = 0.997 for M2). Figure 7 shows the mean

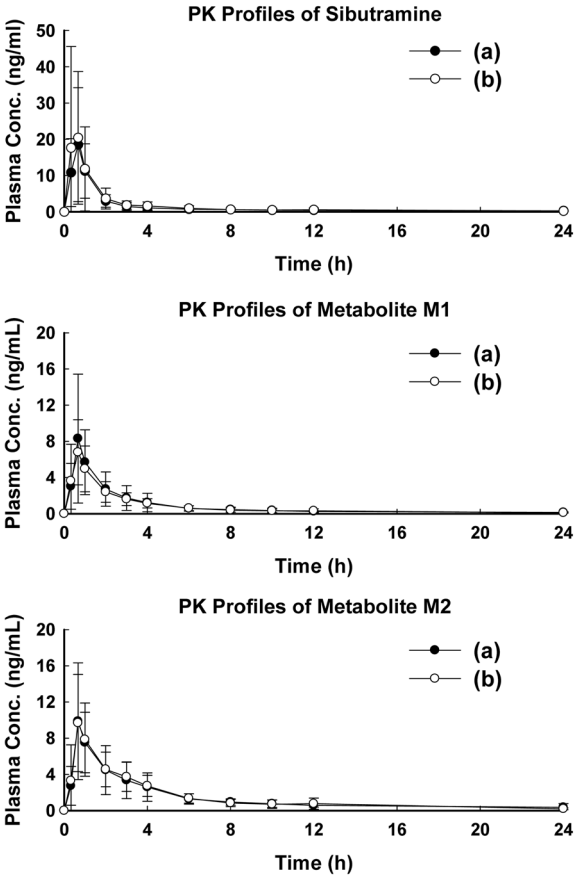

Figure 7. The blood concentration of sibutramine and its active metabolite M1 and M2 from (a) reference drug and (b) test drug in beagle dogs.

plasma-concentration time profiles of sibutramine, M1 and M2 metabolites of sibutramine that resulted after oral administration of either the commercial product (reference drug) and sibutramine solid dispersion formulations (test drug) to each of the six beagle dogs. The pharmacokinetic parameters of sibutramine, M1 and M2 calculated from the plasma concentration curves are summarized in Table 1. The results show that the absorption of sibutramine, M1 and M2 was fast as it appears in plasma samples withdrawn after 0.33 h in all dogs from both reference and test drugs. There were no significant differences in plasma concentrations of each observed time points between the reference and test drug. For sibutramine, the mean (S.D.) C_{max} for the reference and test drugs were 21.26 (14.95) and 27.80 (28.39) ng/mL, respectively; T_{max} occurred at 0.58 (0.21) and 0.61 (0.47) h. The $AUC_{0\text{-}72hr}$ values were 39.80 (20.98) ng·h/mL for the reference drug and 44.58 (31.72) ng·h/mL for the test drug. The $t_{l/2}$ were 36.99 (18.95) and 35.64 (13.53) h for the reference and test drugs. None of the differences in the pharmacokinetic properties of the 2 formulations were statistically significant. For M1 metabolite, the mean (S.D.) C_{max} for the reference and test drugs were 8.70 (6.95) and 7.12 (3.66) ng/mL, respectively; T_{max} occurred at 0.67 (0.14) and 0.75 (0.40) h. The $AUC_{0\text{-}72hr}$ values were 20.43 (13.06) ng·h/mL for the reference drug and 19.29 (8.19) ng·h/mL for the test drug. The $t_{l/2}$ were 13.44 (4.81) and 23.58 (22.57) h for the reference and test drugs. None of the differences in the pharmacokinetic properties of the 2 formulations were statistically significant. For M2 metabolite, the mean (S.D.) C_{max} for the reference and test drugs were 10.38 (6.08) and 10.28 (4.94) ng/mL, respectively; T_{max} occurred at 0.73 (0.13) and 0.92 (0.67) h. The $AUC_{0\text{-}72hr}$ values were 35.82 (17.70) ng·h/mL for the reference drug and 34.09 (13.47) ng·h/mL for the test drug. The $t_{l/2}$ values were 12.65 (9.65) and 9.29 (4.92) h for the reference and test drugs. None of the differences in the pharmacokinetic properties of the two formulations were statistically significant. It was found that the bioavailability of solid dispersion of sibutramine was equivalent to the reference drug.

Conclusions

Poloxamer 407 and citric acid were a good excipient to increase the solubility of sibutramine freebase. The preparation process of sibutramine freebase was very simple and would be applied to commercial manufacture. *In vitro* dissolution studies revealed that the release of sibutramine from solid dispersion formulations was higher than that of sibutramine freebase capsule and was equal to the commercial product. The sibutramine solid dispersion capsule was stable at accelerated conditions for 6 months. The pharmacokinetic parameters of sibutramine, M1 and M2 between sibutramine freebase solid dispersion and commercial product, were statistically equal in beagle dogs. With these results, the solid dispersion system provided a possible way to overcome the low solubility of sibutramine freebase, and the sibutramine solid dispersion can be a bioequivalent to the commercial product in humans.

Table 1. Pharmacokinetic parameters after administration of reference and test (sibutramine solid dispersion) drugs. Values are average ± S.D.

		$AUC_{0\text{-}72hr}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	K_e (h^{-1})
Reference	Sibutramine	39.80 ± 20.98	21.26 ± 14.95	0.58 ± 0.21	36.99 ± 18.95	0.03 ± 0.05
	M1	20.43 ± 13.06	8.70 ± 6.95	0.67 ± 0.14	13.44 ± 4.81	0.06 ± 0.02
	M2	35.82 ± 17.70	10.38 ± 6.08	0.73 ± 0.13	12.65 ± 9.65	0.08 ± 0.05
Test	Sibutramine	44.58 ± 31.72	27.80 ± 28.39	0.61 ± 0.47	35.64 ± 13.53	0.02 ± 0.01
	M1	19.29 ± 8.19	7.12 ± 3.66	0.75 ± 0.40	23.58 ± 22.57	0.06 ± 0.05
	M2	34.09 ± 13.47	10.28 ± 4.94	0.92 ± 0.67	9.29 ± 4.92	0.09 ± 0.04

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