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# HPLC determination and pharmacokinetics of sustained-release bupropion tablets in dogs

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**Abstract**

The pharmacokinetics and bioequivalency of a newly developed sustained-release bupropion tablet was studied in six dogs after single oral administration and compared with a regular tablet (RT) in randomized two-period crossover design. A sensitive and rapid HPLC method was developed and validated for the quantitative determination of bupropion in dog plasma. The compound and the internal standard (I.S.) (hydroxyethylfludiazepam) were extracted from the plasma samples by liquid–liquid extraction. The extracts were analyzed by a reversed-phase HPLC with 50 mmol/l phosphate buffer (pH 5.5)–methanol (45:55, v/v) as the eluent. The assay was specific for bupropion. The calibration curves were linear in the range between 1 and 750 ng/ml. The validated lower limit of quantification was 1 ng/ml. The overall precision (expressed as R.S.D.) of quality controls were within 15%. The method was successfully applied to the bioequivalency study of bupropion in the two formulations. The $C_{max}$ of sustained-release tablet (ST) was significantly lower than that of the RT and the $T_{max}$ was significantly longer than that of the RT ($P < 0.05$). The relative bioavailability of the ST was (99.1 ± 1.51)%, the results of ANOVA and two one sided tests indicated that the new ST exhibited good sustained release properties and was bioequivalent to the RT.


*Keywords:* HPLC; Bupropion; Pharmacokinetics; Bioequivalency; Sustained release tablet; Dogs

## 1. Introduction

Bupropion hydrochloride (DL-2-tert-butylamino-3′-chloropropiophenonehydrochlo ride, Fig. 1A) is a structurally novel antidepressant drug with neurochemical properties different from those of the commonly used tricyclic antidepressants [1]. Bupropion was reported to act primarily via a noradrenergic mechanism but also exhibit some dopaminergic activity [2,3]. Animal studies showed bupropion to be relatively free of sympathomimetic, sympatholytic, cholinolytic or cardiovascular effects [4]. Bupropion has some important

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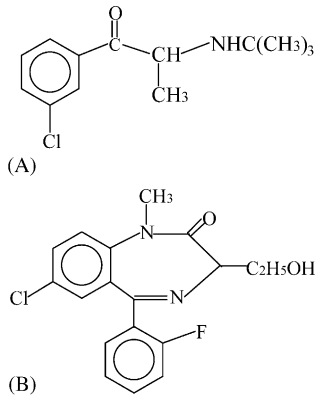


Fig. 1. Chemical structures of bupropion (A) and I.S. (B).

clinical advantages over the widely used tricyclic antidepressants [5]. A sustained-release formulation would further improve its safety profile and allow for a more convenient dosing regimen compared with the regular immediate-release formulations.

Bupropion is extensively metabolized in the body, with less than 1% of the oral dose excreted unchanged in man [6–8]. The absolute bioavailability of oral formulation of bupropion is low. High sensitivity and specificity are, therefore, required for methods used in pharmacokinetic studies, particularly in the case of sustained-release formulations.

Early approaches to determine plasma bupropion included gas chromatography [9], absorption spectrometry [10], fluorimetry [11], which were either insufficiently sensitive or cumbersome in their application to large numbers of samples. Radioimmunoassay (RIA), was sensitive enough for quantitation bupropion in biological fluids, but it was subject to interference by metabolites, the removal of which was necessary before assay [12]. An HPLC method with a detection limit of 50 ng/ml was reported for the determination of plasma bupropion [13], and another improved one with dual-wavelength UV detection could simultaneously measure bupropion and its metabolites [14]. An LC/MS/MS assay was also applied to the determination of bupropion [15]. In this study, a rapid, simple and sensitive HPLC method was developed for the pharmacokinetics study of bupropion in the newly-developed sustained-release tablet (ST). The validation of the HPLC method, the time-profile of plasma concentrations of bupropion in dogs and the, pharmacokinetics and statistical moment parameters and bioequivalency of the ST were evaluated and compared with those of regular immediate-release formulation.

## 2. Experimental

### 2.1. Chemicals

Bupropion regular immediate-release tablets (RT) (75 mg/tablet, No. 000412) and ST (150 mg/tablet, No. 010211) were supplied by Shenyang HuaTai Research Institute (Shenyang, China). Internal standard (I.S., hydroxyethylfludiazepam) for HPLC analysis was provided by the Institute for Drug Control of Liaoning Province. Methanol was of HPLC grade, isoamyl alcohol, *n*-heptane and some other reagents of analytical grade were from Yuwang Reagent Company (Shandong, China).

### 2.2. Calibration standards and quality control samples

Stock solution of Bupropion hydrochloride and I.S. were prepared with methanol. Working solutions were prepared by diluting stock solutions with redistilled water. All solutions were stored at 4 °C until analysis. Calibration standards of concentration range from 1 to 750 ng/ml were prepared by spiking 0.5 ml of blank plasma with 50 μl of the appropriate working solution. The three calibration standards were independently prepared. QC samples were prepared in bulk at the concentration of 1, 100, 500 ng/ml and stored at −20 °C.

### 2.3. HPLC system

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10AT pump, an SPD-10A UV detector set at 254 nm. The analyte was determined at room temperature on an analytical column (Diamonsil™ C18, 150 mm × 4.6 mm,

I.D., 5 μm). The mobile phase was a mixture of 50 mmol/l sodium phosphate buffer (pH 5.5)–methanol (45:55, v/v) and pumped at a flow-rate of 1.4 ml/min.

### 2.4. Sample preparation

To a 0.5 ml plasma spiked with 50 μl of 1.0 μg/ml hydroxyethylfludiazepam (as I.S.), 0.5 ml of 0.5 mol/l sodium carbonate buffer (pH 10.8) and 5 ml of 1.5% (v/v) isoamyl alcohol in *n*-heptane were added. The mixture was vortexted for 20 s, mechanically shaken for 20 min and centrifuged at 3000 r/min for 10 min. The organic layer was then transferred to a 10 ml tube containing 200 μl of 0.1 mol/l hydrochloric acid. After vortexting for 20 s, mechanical shaking for 20 min and centrifuging at 3000 r/min for 10 min, the organic layer was removed by aspiration, the acid layer was evaporated to dryness under nitrogen stream in a 40 °C water bath. The residue was redissolved in 200 μl of mobile phase and 20 μl of the solution was injected into the HPLC system.

### 2.5. Assay validation

The extraction recoveries of bupropion and I.S. were determined by comparing the peak area of extracted samples to those of nonprocessed standard solutions at same concentration.

Calibration curves were constructed with plasma standards spiked with 1, 3, 20, 100, 300, 500, 750 ng/ml of bupropion. Linear regression analysis of the peak area ratio (analyte/I.S.) versus concentration was performed using 1/(concentration)/(concentration) as weight factor. During the method validation, calibration standards were independently prepared and measured on 3 consecutive days.

The intra-day and inter-day precision and accuracy were determined based on pentuplicate measurements of QC samples at low, middle and high concentrations (1, 100, 500 ng/ml). All samples were spiked with bupropion on day 1, and then analyzed on three different days. The bupropion concentration in QC samples was calculated from the linear regression equation obtained on the same day.

### 2.6. Pharmacokinetic study

A randomized two-period crossover design for was carried out. Six-month-old male beagle LRE-strain dogs weighing approximately 10 kg were used. Dogs were fasted overnight, dosed without feeding and divided into two groups (A and B) at random. Each dog of group A was administered orally with four bupropion immediate-release tablets, blood was collected using a heparinized tube through a forelimb cephalic vein before administration and at 10, 20, 30, 45 min; 1.0, 1.5, 2.0, 4.0, 7.0, 10, 13, 24 h after administration. Each dog of group B was administered orally with two bupropion ST, blood was collected before administration and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10, 13, 24 h after administration. The dose of each dog in both groups was 300 mg. Blood samples were centrifuged at 3000 r/min for 10 min to separate plasma, which was stored at −20 °C until analysis. The plasma samples were thawed at room temperature just before assaying.

### 2.7. Statistical analysis

Plasma drug concentration–time data from the study were analyzed by using the 3P87 computer program and the bioequivalency was evaluated by SPSS computer program which were both edited and published by Chinese Pharmacology Association. The area under the plasma concentration–time curve (AUC) and half-life on terminal phase ($t_{1/2}$) were calculated from plasma drug concentration–time curve. The $AUC_{0-t}$ was calculated with the trapezoidal method and the $AUC_{t-\infty}$ was calculated from the concentration of last point divided by elimination slope. The maximal plasma concentration ($C_{max}$) and the time required to reach the maximal plasma concentration ($T_{max}$) were recorded directly from the measured data.

## 3. Results and discussion

### 3.1. Specificity

Representive chromatograms of blank plasma sample, plasma spiked with 1 ng/ml of bupropion,

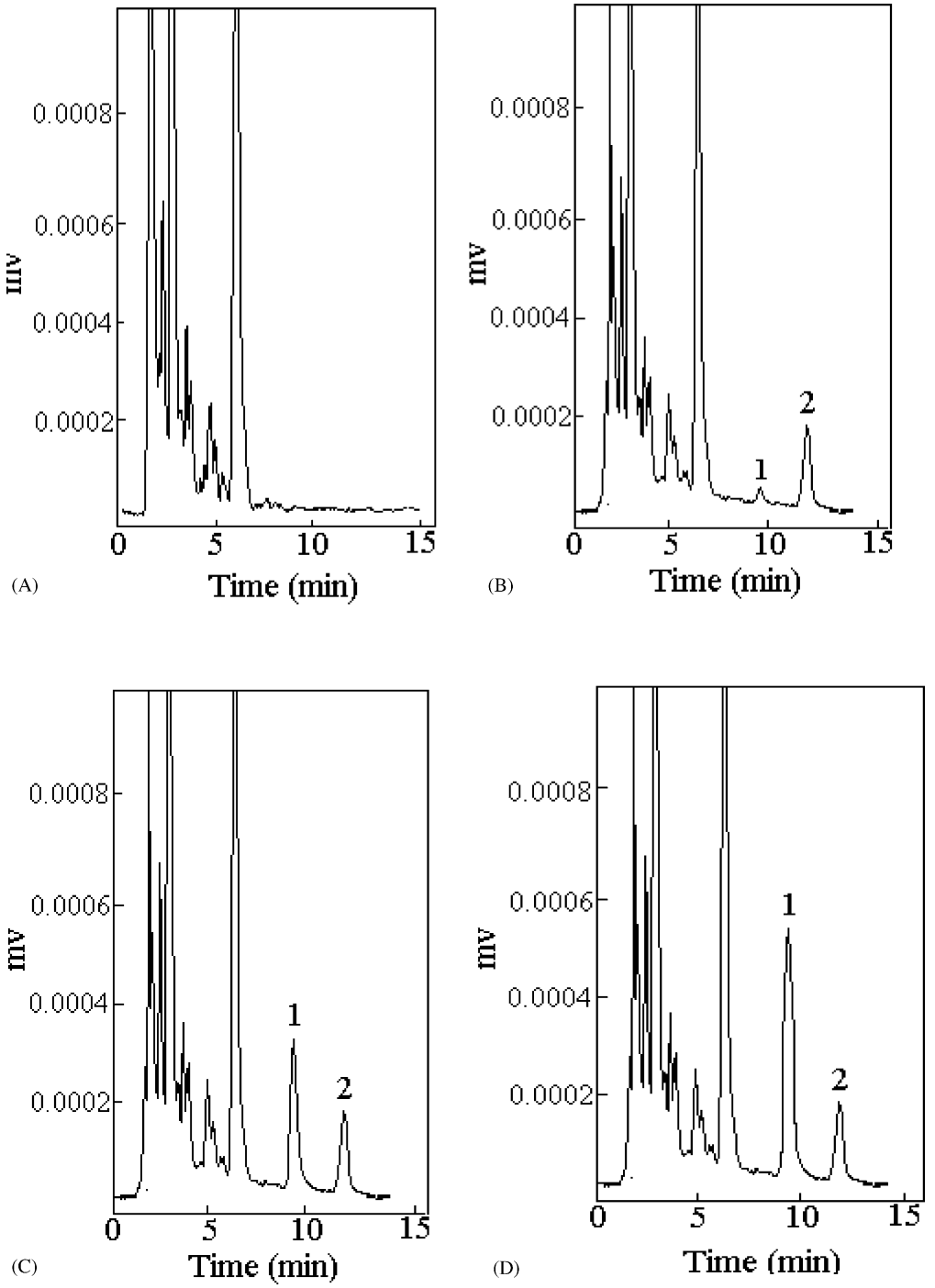


Fig. 2. Typical HPLC chromatograms for measurement of bupropion in dog plasma samples: (A) blank plasma; (B) plasma sample containing 1 ng/ml of bupropion; (C) plasma sample containing 100 ng/ml of bupropion; (D) plasma sample at 6 h after administration of ST of bupropion at a dose of 300 mg. Peaks: (1) bupropion; (2) I.S. (I.S.).

plasma spiked with 100 ng/ml of bupropion and plasma sample at 6 h after administration of ST of bupropion at a dose of 300 mg are presented in Fig. 2. Retention times of bupropion and I.S. were approximately 9.2 and 11.5 min, respectively. There was no interfering peak from endogenous substances in the blank plasma.

### 3.2. Linearity and range

Calibration standards containing 1–750 ng/ml were prepared from working solutions of bupropion and blank plasma. The calibration curve was constructed by plotting the peak area ratio of bupropion to I.S. against the bupropion concentration in plasma. A weighted linear regression method with a weight factor of $1/c^2$ was employed. The data for three measurements of the calibration curve are listed in Table 1, which showed a good linearity within the examined concentration range. The LOQ for bupropion was $0.99 \pm 0.14$ ng/ml in plasma at a signal to noise ratio of 10.0.

### 3.3. Extraction recovery

The extraction recovery was calculated in plasma samples ($n = 3$) spiked with bupropion standard at a concentration of 20 ng/ml. The extraction recovery of I.S. at concentration of 50 ng/ml was investigated similarly. Ephedrine hydrochloride, whose structure was the most similar to that of bupropion, was initially selected for the international standard, however, its retention time was under 5 min, and it was subject to interference by the endogenous substances in plasma. Other substances such as diazepam, clonazepam and flurazepam were tested but had low extraction recoveries (16.7, 22.4 and 28.5%, respectively), though their HPLC retention behaviors were similar to that of bupropion under the HPLC conditions. Different extraction reagents such as diethyl ether and ethyl acetate were tested, but little improvement was obtained for their recoveries. Only hydroxyethylfludiazepam exhibited an extraction recovery similar to that of bupropion and was separated well from the analyte in the HPLC eluting within 13 min. It was, therefore, used as the I.S. in this assay. The mean recovery of 87.3 and 84.2% was obtained for bupropion and I.S., respectively.

### 3.4. Precision and accuracy

The intra- and inter-day precision and accuracy of the developed method were evaluated with five replicates of samples at concentration of 1, 100, 500 ng/ml, and on three different days. The results are presented in Table 2.

### 3.5. Pharmacokinetic study

The pharmacokinetic study was performed in six dogs after administration of bupropion at a single oral dose of 300 mg. Plasma concentration–time curves of bupropion after oral administration of RT and ST are shown in Fig. 3. The mean values of pharmacokinetic parameters and statistical moment parameters of RT and ST are summarized in Table 3.

The $C_{max}$ of ST was significantly lower than that of RT and the $T_{max}$ was significantly longer ($P < 0.05$), which suggested that the new ST possessed good sustained release properties. The pharmacokinetics parameters were transformed to log data in the SPSS program automatically before ANOVA and two one sided tests. The results showed the

Table 1
The linearity of calibration curves of bupropion concentration in dog plasma[a]

| | Run | Slope $\times 10^{-2}$ | Intercept $\times 10^{-2}$ | Correlation coefficient |
|---|---|---|---|---|
| Bupropion | 1 | 0.97 | 4.62 | 0.9986 |
| | 2 | 1.31 | 3.36 | 0.9987 |
| | 3 | 1.34 | 6.65 | 0.9993 |

[a] Peak area ratio = slope × concentration of bupropion + intercept.

Table 2
The accuracy and precision of the method (mean ± S.D., $n = 5$)

| Concentration (ng/ml) | Within-day C (ng/ml) | Within-day R.S.D. (%) | Between-day C (ng/ml) | Between-day R.S.D. (%) |
|---|---|---|---|---|
| 1 | 0.99 ± 0.13 | 10 | 0.99 ± 0.14 | 12 |
| 100 | 95.33 ± 3.74 | 3.9 | 101.19 ± 11.77 | 12 |
| 500 | 488.39 ± 13.42 | 2.7 | 493.19 ± 37.36 | 7.6 |

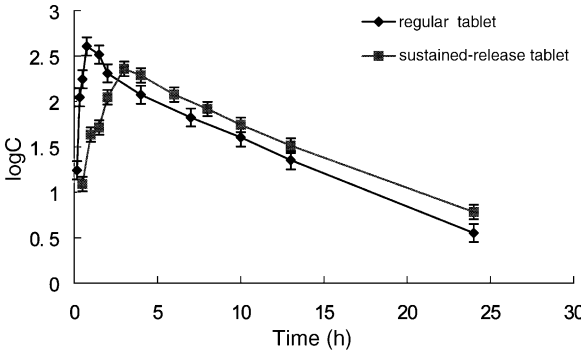


Fig. 3. The mean plasma concentration–time curve of bupropion after oral administration of RT and ST to dogs (dose: 300 mg).

Table 3
The mean values of main pharmacokinetic parameters and statistical moment parameters of bupropion after single oral dose of ST and RT (mean ± S.D., $n = 6$)

| Parameter | RT | ST |
|---|---|---|
| $C_{max}$ (ng/ml) | 404.8 ± 50.50 | 238.1 ± 29.1 |
| $T_{max}$ (h) | 1.00 ± 0.39 | 3.17 ± 0.41 |
| $t_{1/2Ke}$ (h) | 4.15 ± 1.10 | 4.55 ± 0.75 |
| $AUC_{(0\rightarrow 24)}$ ((ng/ml) h) | 1482.2 ± 207.0 | 1454.0 ± 297.3 |
| $AUC_{(0\rightarrow \infty)}$ ((ng/ml) h) | 1506.1 ± 194.9 | 1492.3 ± 294.5 |
| Lag time (h) | 0.15 ± 0.02 | 0.35 ± 0.04 |
| CL (mg/h/(ng/ml)) | 0.21 ± 0.03 | 0.25 ± 0.04 |
| Vd (mg/(ng/ml)) | 1.08 ± 0.21 | 1.32 ± 0.27 |
| MRT (h) | 5.26 ± 0.16 | 7.50 ± 0.22 |
| VRT ($h^2$) | 29.43 ± 0.79 | 33.34 ± 0.98 |

relative bioavailability of the ST was (99.1 ± 1.51)%, the 90% confidence intervals of the AUC after the log transformation of the data was 92.61–106.0% of the reference formulation, which indicated the new ST was bioequivalent to RT.

## 4. Conclusion

The HPLC method presented here fulfils the general requirement for bioanalytical assays [16] and is suitable for the pharmacokinetic study of bupropion during preclinical drug development. The newly-developed ST of bupropion with markedly lower peak plasma concentrations could improve its safety profile and allow for a more convenient dosing regimen.

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