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Application of HPLC Method Using Normal Phase Column in a Comparative Pharmacokinetic Study of Two Sulpiride Tablet Formulations

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ABSTRACT

An HPLC method using normal phase column eluted with an aqueous solvent and detected by fluorescence was applied to analyze sulpiride concentrations in plasma samples obtained from a comparative pharmacokinetic study. This comparative study was conducted to determine the bioequivalence of two tablet products (Dogmatyl and Sulpin) containing sulpiride on 12 normal healthy Chinese male volunteers in a single-dose, two-period, two-sequence, two-treatment crossover design. The pharmacokinetic parameters, $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$, and C_{max}, were calculated from plasma data and compared using the SAS General Linear Model computer program. A two one-sided *t* distribution test was also performed, as well as the 90% confidence interval method, to determine the mean difference of these three pharmacokinetic parameters. The results suggest that these two sulpiride tablet products are bioequivalent when orally administered in a 400 mg single dose of two tablets.

Key words: sulpiride, normal phase column, bioequivalence, HPLC

INTRODUCTION

Sulpiride, 5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy benzamide, possesses antipsychotic, antidepressive and antiulcer effects. It has a peculiar affinity for the D_2 and D_4 brain dopamine receptors with a low frequency of extrapyramidal side effects[(1)]. The recommended oral dose of sulpiride in the treatment of schizophrenia is 200 to 400 mg twice daily with a gradual increase based on clinical response to a maximum of 1200 mg daily[(1)]. Dose reductions are recommended in patients with renal impairment[(2)]. Sulpiride also exhibits neuroleptic and thymoleptic properties being used in mental disorders as a behavior regulator in the psychopathology of senescence, in depression and in schizophrenia, with a first dose of 200 mg and a daily dose increment of 200 mg to a maximum of 800 mg. It is also used at doses of 50 to 150 mg in the treatment of gastric or duodenal ulcers, in the treatment of the irritable colon due to psychosomatic stress, and in various vertigo syndromes.

Sulpiride is slowly and poorly absorbed from the gastrointestinal tract, with peak serum levels occurring in 2 to 6 hours; its bioavailability is approximately 27%[(3)]. Sulpiride does not appear to be metabolized, showing that 70 to 90% of an intravenous dose and 15 to 25% of an oral dose is excreted unchanged in the urine. A high percentage of an oral dose of sulpiride has been recovered in feces; the terminal half-life of sulpiride is 6 to 10 hours[(4)]. The dose proportionality study demonstrated that sulpiride followed a linear disposition kinetic when administration of sulpiride between dose 100 to 200 mg[(5)].

In healthy subjects, the plasma concentration of unchanged drug vs. time and urinary excretion rate vs. time, following intravenous administration of sulpiride 100 mg, were consistent with a two compartment open model. The apparent elimination half-life was approximately 5.9 hours and the volume of distribution at steady-state approximately 0.859 L/Kg. About 93.1 ± 6.6 % of the administered dose was recovered unchanged in urine. Comparison of total clearance (89.8 mL/min), renal clearance (83.0 mL/min), and renal clearance of unbound drug (97.6 mL/min) indicate that sulpiride is mainly excreted by the renal route[(5)]. The absorption of sulpiride was relatively slow with very large interindividual variations in the rate and the extent of absorption[(6)].

Due to its poor bioavailability and large individual variation, a study is needed to prove that generic products of sulpiride are bioequivalent to the innovative product and are clinically beneficial. The aim of the present study was to examine comparative pharmacokinetics of two tablet formulations of sulpiride (Dogmatyl® vs. Sulpin®) in healthy Chinese males with an improved and simplified HPLC method.

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MATERIALS AND METHODS

I. *Drug and Reagents*

The innovative Dogmatyl® 200-mg tablet (lot no. 269) was obtained from Fujisawa Pharmaceutical Co., Taoyuan, Taiwan. Sulpin® 200-mg tablet (lot no. R870901T), made by Sin-Tong Chemical Industrial Co. Ltd. (Taoyuan, Taiwan), was used as the test product. The standard sulpiride and internal standard, metoclopramide, were both purchased from Sigma Chemical (St. Louis, MO, USA). All other reagents used were reagent or pharmaceutical grade.

II. *Physical Characterizations*

The potency, uniformity, and dissolution test of sulpiride in these two tablet formulations (Dogmatyl® and Sulpin®) were assayed according to the pharmacopoeia specifications (USP XXIII). Test results demonstrated that the potency (n=20) was 99.57% and 96.27% for Dogmatyl® and Sulpin®, respectively. The content uniformity of dosage units (n=10) were 99.37±0.85% and 96.07±0.86% for Dogmatyl® and Sulpin®, respectively. The results of the potency and uniformity data of these two tablet formulations both met the criteria of the pharmacopoeia specifications (potency: 90-110%; uniformity: 85-115%). Three dissolution media, including 0.1N HCl solution, pH4.5 acetate buffer, and pH6.8 phosphate buffer solutions were employed to compare the dissolution profiles of these two products using Dissolution Apparatus II (stirring rate = 50 rpm; temperature = 37˚C; n = 6). The closeness of profiles was statistically determined by comparison of the f_2 value following the guidelines of SUPAC IR[7].

III. *Assay Method*

The preparation and extraction method of plasma samples have been reported[8] and are summarized as follows. Plasma sample (1 mL) was spiked with 0.1 mL internal standard (metoclopramide, 1.5 μg/mL in methanol) solution and 0.1 mL NaOH solution (1N). After vortex mixing thoroughly for 5 s, the mixture was extracted with 6 mL of ethylacetate/dichloromethane (5:1 v/v), then vortex mixing for 5 min, and centrifuged at 4000 rpm for 10 min. The supernatant (organic phase) is transferred to another clean glass tube and evaporated under a stream of nitrogen gas at 40˚C until completely dry. Next, 0.2 mL of mobile phase was added to dissolve the residue, and 0.1ÉnmL was injected automatically into the HPLC system for analysis.

The HPLC system consists of a pump (Jasco PU-980 Intelligent HPLC Pump, Tokyo, Japan) and an autosampler (Jasco AS-950-10 Intelligent Sampler, Tokyo, Japan). A 250×4-mm (id) normal phase column (LiChrospher Si 60, Merck, Germany) with a particle size of 5 μm was employed. The mobile phase consisted of triethylamine solution (0.5%, pH 4.0), methanol and acetonitrile in the proportion of 10:5:85 (v/v). The flow rate was set at 1.8 mL/min. The eluent was detected with a fluorescence detector (JASCO FP-920 Fluorescence Detector, Tokyo, Japan) at the wavelength of 300 nm for excitation and 365 nm for emission, respectively. The HPLC system was controlled by a PC workstation installed with Borwin computer software (JMBS developments, France). The HPLC method was validated and reported in the same previous study[8]. High precision and accuracy with a minimal interference and the peak of highly symmetry were demonstrated. The low limit of quantitation was 20 ng/mL with a coefficient of variation less than 20%. A linear range was found to be from 20 ng/mL to 1500 ng/mL. This HPLC method was validated with the precision for inter-day and intra-day run being 0.36% to 8.01% and 0.29% to 5.25%, respectively and the accuracy (relative error of mean, %) for inter-day and intra-day run being -1.58% to 5.02% and -2.14% to 5.21% respectively.

IV. *Subjects*

The protocol of the bioequivalence study was approved by the Internal Review Board of Taipei Medical University Hospital. A total of 12 healthy male subjects participated in this study after signing a consent form. The subjects had a mean ± SD age of 22 ± 2 years (20-25 years), body weight of 66 ± 6 kg (55-78 kg), and height of 172 ± 5 cm (165-184 cm). Subjects with a history of drug allergies or idiosyncrasies, renal or hepatic impairment, or drug or alcohol abuse were excluded. Subjects who used medications of any kind within 2 weeks of the start or during the study were also excluded.

V. *Study Design*

The study was conducted in a crossover design with 12 subjects receiving a 400 mg single dose (two 200 mg tablets) of Dogmatyl® and Sulpin®. Each subject was requested to fast for at least 10 hours overnight the day before and 4 hours after each treatment. A single dose consisting of two R (Dogmatyl®) or T (Sulpin®) tablets was randomly given to each subject with 200 mL of water. The washout period between two periods was 1 week.

Heparinized venous blood samples (about 10 mL) were collected by means of an indwelling venous cannula of the cubital vein on the profiling day according to the predetermined time schedule, which included a blank sample just prior to dosing and then at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36, 48, 52, and 60 hours after drug administration. Any deviation from the stated sampling times was recorded. Plasma was immediately separated by centrifugation at 3000 rpm for 10 min, then was transferred to labeled tubes, and stored at -25 ˚C until assay.

VI. *Pharmacokinetic Data Analysis*

All pharmacokinetic variables were calculated by noncompartmental methods. C_{max} and T_{max} were obtained directly from the concentration-time curve data. The area under the concentration-time curve from time zero (predose) to time of

Journal of Food and Drug Analysis, Vol. 10, No. 1, 2002

last quantifiable concentration ($AUC_{0\text{-}last}$) was calculated using the linear trapezoidal method, and Cl/F is equal to (dose/$AUC_{0\text{-}inf}$). The terminal rate constant, β, was calculated by applying a log-linear regression analysis to at least the last three time points. $T_{1/2}$ is the terminal half-life. MRT is the mean residence time of the drug. $AUMC_{0\text{-}last}$ is the area under the moment-versus-time-curve to the last sample point and is determined using the linear trapezoidal method.

VII. *Statistical Analysis*

A two-way ANOVA performed with the SAS General Linear Models Procedure at a significance level of 0.05 was carried out. The test (T) and reference (R) treatments of each study were compared with respect to relevant pharmacokinetic variables using an analysis of variance with subject, treatment, and period effects with the raw data. Point estimates and 90% confidence intervals for the "T/R" mean ratios of these raw data were calculated. Whenever there was no statistically significant difference, statistic power to detect at least a 20% difference between products was checked. Bioequivalence of the test treatment to the reference treatment was assessed on the basis of the confidence intervals for the "T/R" mean ratios of these raw variables in relation to the bioequivalence range of 80%-120% for the raw data.

RESULTS AND DISCUSSION

Figure 1 displays the individual and the mean of sulpiride plasma concentration-time profile in 12 volunteers for the Dogmatyl® and Sulpin®. The pharmacokinetic parameters were calculated correspondingly and statistical analysis results for two formulations of sulpiride tablets were delineated in Table 1. The ratios (mean ± SD) of $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$, and C_{max} of the test drug (Sulpin®) to the reference drug (Dogmatyl®) are 0.98 ± 0.11, 0.98 ± 0.10 and 1.00 ± 0.10, respectively. There was no significant difference ($p > 0.05$) in bioavailability between the two products as indicated by these three parameters. The 90% confidence intervals of the mean difference were in a range of 94.16-104.31%, 93.97-103.85%, and 94.56-103.53% for $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$, and C_{max}, respectively. The 90% confidence interval of the mean difference for these three pharmacokinetic parameters fell within the range of 80%-120%. The same results of statistical analysis were obtained using the two one-sided *t* distribution method. The values of statistical power to compare mean ratios of $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$ and C_{max} between two products were close to 1.0000. The results of ANOVA test of three pharmacokinetic parameters show that the factor of the subject was the only one determined to be significant. There was no statistically significant group, period, or treatment effect on these three pharmacokinetic parameters in this crossover design.

Other pharmacokinetic parameters, such as β, T_{max}, $T_{1/2}$, $MRT_{0\text{-}inf}$, and CL/F, for the two products are shown in Table 2. Insignificant difference was found for those parameters between these two products. The mean $AUC_{last\text{-}inf}$ was less than 3.83% and 3.76% for Dogmatyl and Sulpin tablets. This indicates that the estimation of AUC is more reliable with less extent of extrapolation as a consequence of the designed time period for sampling.

The dissolution profiles in different media of the two tablet formulations are shown in Figure 2, which demonstrates that f_2 values were 66.90, 94.27, and 85.60 for disso-

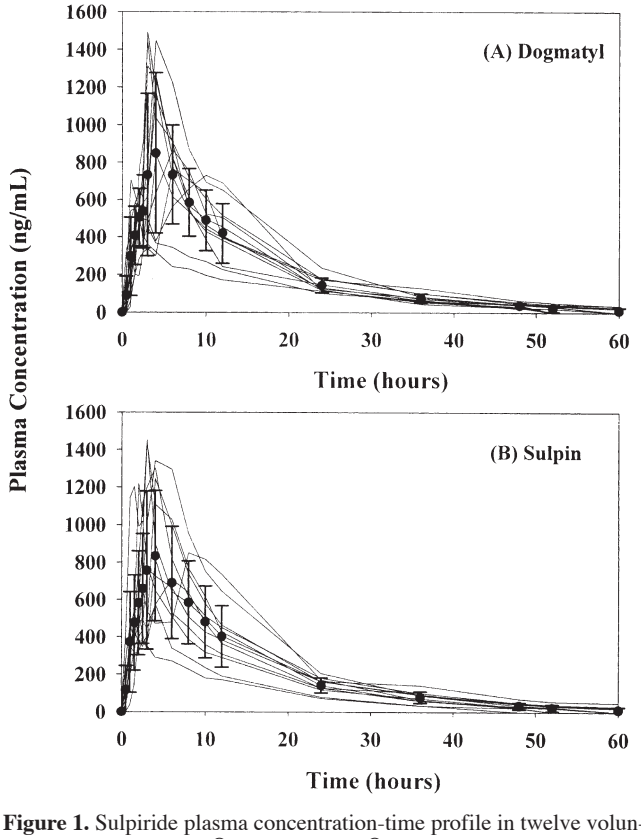

Figure 1. Sulpiride plasma concentration-time profile in twelve volunteers for (A) Dogmatyl® and (B) Sulpin® products.

Table 1. Pharmacokinetic parameters of BE study for sulpiride tablet formulations and statistical analysis

Parameter	Dogmatyl Mean (SD)	Sulpin Mean (SD)	Ratio (T/R) Mean (SD)	Statistical Analysis		
				F Value (Pr>F)	90% C.I.	Power
$AUC_{0\text{-}last}$ (ng*h/mL)	12360.62 (3242.15)	12266.45 (3779.49)	0.98 (0.11)	0.07 (0.7910)	94.16-104.31	1.0000
$AUC_{0\text{-}inf}$ (ng*h/mL)	12843.39 (3321.76)	12703.24 (3819.78)	0.98 (0.10)	0.16 (0.6975)	93.97-103.85	1.0000
C_{max} (ng/mL)	1030.10 (363.80)	1020.29 (340.22)	1.00 (0.10)	0.15 (0.7084)	94.56-103.53	1.0000

lution in the media of 0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer solutions, respectively. An f_2 value between 50 and 100 suggests the two dissolution profiles are similar. Since the dissolution profiles of the two products were similar and bioequivalence was claimed for these two products, a possible correlation between *in vitro* dissolution and *in vivo* bioequivalence might exist.

Comparisons of pharmacokinetic parameters were made between those obtained in this study and those reported by Chen *et. al.*[9], using the same single dose of 400 mg for two tablet formulations (Dogmatyl® vs. Sulpiride®) on Chinese male volunteers. It indicates that T_{max} (1.50 ± 0.63 vs 4.21 ± 2.27 hr) obtained in Chen' study is much shorter than that obtained in this study for the same formulation of Dogmatyl®, whereas C_{max} (1.468 ± 0.631 vs 1.030 ± 0.364 μg/mL) is higher in Chen's study. $T_{1/2}$ (8.396 ± 1.953 vs 11.98 ± 2.12 hr) and β (0.087 ± 0.021 vs 0.0595 ± 0.0104 hr^{-1}) for the terminal phase obtained by these two studies are also quite different. However, $AUC_{0-\infty}$ (13.56 ± 9.09 vs 12.84 ± 3.32 μg.h/mL) of these two studies is at a comparable level.

According to T_{max}, it can be realized that the absorption rate of sulpiride in this study was slower than that reported by Chen *et al*. In turn, a lower C_{max} resulted with a slower absorption rate. However, AUC form zero to infinite was maintained at a similar level, indicating that the bioavailability of sulpiride for these two studies could reach the same extent after extrapolation to the infinite time. Correspondingly, the percentage of extrapolation of AUC after the last sampling point was lower for this study (3.83 ± 1.27% vs 22.09 ± 10.78%).

On the other hand, the terminal half-life for these two products based on the regression on the data point in the terminal phase (11.98 ± 2.12 hr for Dogmatyl® and 11.49 ± 2.64 hr for Sulpin®) found no significant difference statistically, ($p = 0.5049$), but were substantially longer than those that have been reported as 5.3 hr (3.7-7.1 hr) for intravenous administration of 100 mg[3], 6.39 ± 1.74 hr for oral administration of 400 mg capsule[6], 9.9 ± 1.7 hr for oral administration of 200 mg tablet[10], and 7.17 ± 1.09 hr for intramuscular administration of 100 mg[5]. A longer time period of sampling during the terminal phase might be attributed to some extent. It was also reported by Wiesel et al. that the half-lives for two subjects were 11.0 and 13.9 hr when the three-compartment model instead of the two-compartment model was applied[3]. Therefore, the longer half-life obtained in this study is probably a result of being able to detect drug concentrations at the terminal phase in longer periods of sampling time. Alternatively, whether or not the ethnic difference plays a role in this matter might be worthy for further study.

CONCLUSIONS

The bioequivalence study of two commercial sulpiride

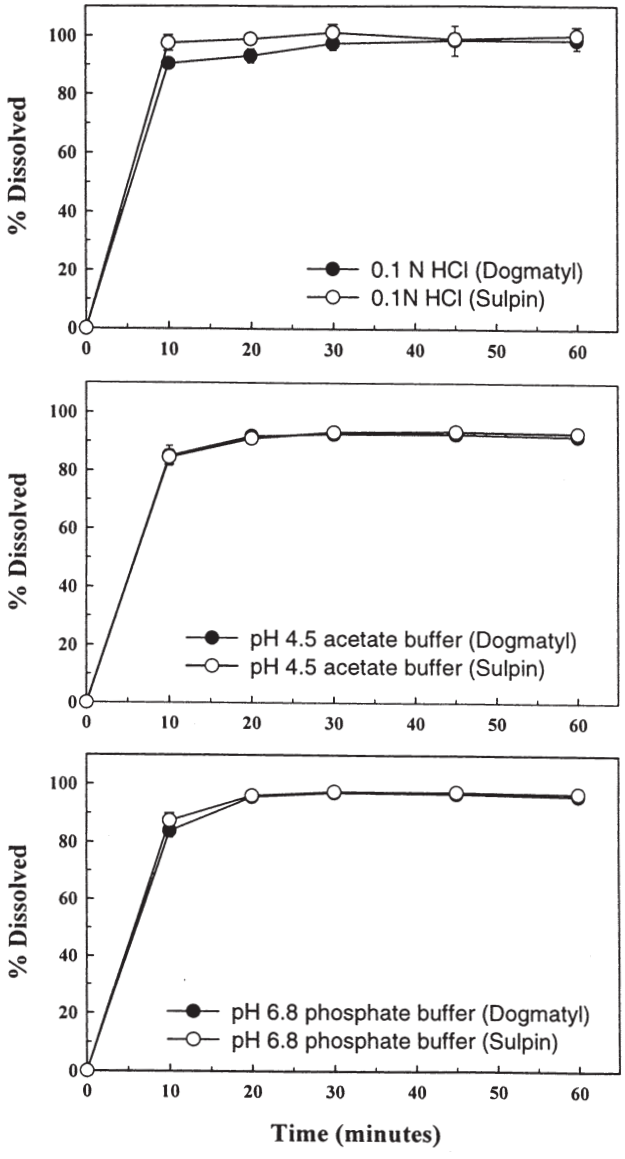

Figure 2. The dissolution profiles in different medium of the two tablet formulations. (A) 0.1 N HCl, (B) pH 4.5 acetate buffer, (C) pH 6.8 phosphate buffer.

Table 2. Pharmacokinetic parameters of sulpiride tablet formulations other than pivotal parameters

Parameter	Dogmatyl		Sulpin	
	Mean (SD)	CV (%)	Mean (SD)	CV (%)
β (hr-1)	0.0595 (0.0104)	17.42	0.0633 (0.0144)	22.73
T_{max} (hr)	4.21 (2.27)	53.96	3.83 (1.79)	46.64
$T_{1/2}$ (hr)	11.98 (2.12)	17.70	11.49 (2.64)	22.95
$MRT_{0\text{-}inf}$ (hr)	15.98 (2.72)	17.01	15.35 (2.29)	14.89
CL/F (L/hr)	33.54 (10.61)	31.63	35.31 (14.57)	41.26
$AUC_{last\text{-}inf}$ (%)	3.83 (1.27)	33.13	3.76 (1.99)	52.83

200 mg tablets (Dogmatyl® vs. Sulpin®) with a 400 mg single-dosed of two tablets oral administration in 12 healthy, normal male volunteers was conducted. The statistical analysis results based on comparisons of three pharmacokinetic parameters ($AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$ and C_{max}) showed that these two tablet products appear to be bioequivalent.

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應用正相矽膠管柱的高效液相層析法於二種 Sulpiride錠片處方的藥物動力學比較

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摘　要

應用一種配合水性溶媒移動相之正相矽膠管柱以螢光偵測的改良式高效液相層析法於比較兩種市售Sulpiride錠片處方（Dogmatyl和Sulpin）的口服藥物動力學。利用十二位健康華人男性自願者，以單劑量，二週期，二個次序、二種交叉試驗設計比較二個產品的相等性試驗。Sulpiride的血漿濃度分析也經簡化為以液相萃取鹼化的血漿樣品，再以此經確效的高效液相層析法檢測。由血漿數據計算的主要藥動學參數包括$AUC_{0\text{-}last}$、$AUC_{0\text{-}inf}$、與C_{max}，並利用SAS電腦軟體進行統計分析比較。Two one-sided t distribution和90%可信賴度區間用來比較三個主要參數的平均差異性。結果顯示當口服使用二種單劑量400毫克的二顆錠片產品具有生體相等性。

關鍵詞：正相矽膠管柱，Sulpiride，生體相等性，高效液相層析法