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Development of a high-performance liquid chromatographic method for bioanalytical applications with sulpiride

Ming-Chuan Huang[a], Hsiu-O Ho[a], Geng-Cheng Yeh[b], Wen-Ting Ke[c], Li-Chin Lin[d], T.-M. Bruce Hsu[d], Ching-Cheng Kao[a], Ming-Thau Sheu[a,*]

[a]*Graduate Institute of Pharmaceutical Sciences, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan, ROC*
[b]*Department of Pediatrics, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan, ROC*
[c]*Taiwan Biopharmtech Co., Ltd., Taipei, Taiwan, ROC*
[d]*Sin-Tong Chemical Industrial Co., Ltd., Taoyuan, Taiwan, ROC*

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Abstract

An improved HPLC method using a silica gel column with fluorescence detection (excitation at 300 nm and emission at 365 nm) was developed for the determination of sulpiride concentrations in plasma. Analysis of sulpiride in plasma samples was simplified by a one-step liquid–liquid extraction after alkaline treatment of only 1 ml of plasma. The low limit of quantitation was 20 ng/ml with a coefficient of variation of less than 20%. A linear range was found from 20 to 1500 ng/ml. This HPLC method was validated with the precision for inter-day and intra-day runs being 0.36–8.01% and 0.29–5.25%, respectively, and the accuracy (standard deviation of mean, SD) for inter-day and intra-day runs being −1.58 to 5.02% and −2.14 to 5.21%, respectively. Bioequivalence of the two products was evaluated in 12 normal healthy male volunteers in a single-dose, two-period, two-sequence, two-treatment cross-over study. Sulpiride plasma concentrations were analyzed with this validated HPLC method. Results demonstrated that the two tablet formulations of sulpiride appear to be bioequivalent.

Keywords: Silica column; Reversed phase eluents; Bioequivalence; Sulpiride

1. Introduction

Sulpiride, 5-(aminosulfonyl)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide, possesses antipsychotic, antidepressive, and antiulcer effects. It has 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 200–400 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 and is 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 50–150 mg in the treatment of gastric or duodenal ulcers, in the

*Corresponding author. Tel.: +886-2-2377-1942; fax: +886-2-2377-1942.
E-mail address: mingsheu@tmu.edu.tw (M.-T. Sheu).

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treatment of 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 2–6 h; its bioavailability is ~27% [3]. Sulpiride does not appear to be metabolized, showing that 70–90% of an intravenous dose and 15–25% 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 elimination half-life of sulpiride is 6–10 h [4]. A dose proportionality study demonstrated that sulpiride follows a linear disposition kinetic when administered at doses between 100 and 400 mg [5].

A number of methods are available for analysis of sulpiride in biological fluids, including spectroflurometric techniques [6], gas chromatography (GC) [7,8], high-performance liquid chromatographic (HPLC) methods with UV detection [9,10], and HPLC with fluorescence detection [11,12]. These colorimetric, spectrophotometric, and spectroflurometric techniques are not sensitive or specific enough for clinical studies. Methylated derivatization is required for GC analysis to detect sulpiride as a single peak [7,8]. Although HPLC methods are routinely used, a large amount of plasma or a complicated extraction procedure is often necessary [9,12]. Because of these factors, a more sensitive and simpler method, using solid-phase extraction and HPLC with fluorescence detection has been reported [13]. However, this method includes a solid-extraction procedure to enhance drug recovery, back-extraction to eliminate interfering peaks presumably derived from the SepPak C_{18} cartridge, and a constant column temperature to produce sharp and symmetrical elution peaks. Single use of a cartridge is also recommended because recovery of the drug significantly decreased with repeated use of a cartridge.

An interesting application of silica columns not only offers a powerful and versatile approach to the analysis of a wide range of basic drugs and their metabolites with simple methanol-buffer eluents [14], but provides hydrophilic interaction chromatography for separating hydrophilic peptides [15,16] with the use of an acetonitrile–water mixed solution containing 0.1% trifluoroacetic acid. In contrast to ion-pair and reversed-phase systems, most compounds show excellent symmetry, with symmetry factors better than 1.2. The column also shows high efficiency of $n=50\,000$ plates/m ($h=4$) at a flow-rate of ~1 ml/min. Together these factors compensate somewhat for the narrow retention time range and help ensure good resolution of complex mixtures [14]. The aim of the present study was to further improve the HPLC method of assaying sulpiride using a silica gel column with a fluorescence detector for a bioequivalence study.

2. Experimental

2.1. Drugs and reagents

Standard sulpiride and the internal standard, metoclopramide, were both purchased from Sigma (St. Louis, MO, USA). Methanol and acetonitrile for liquid chromatography were HPLC grade obtained from Merck (Germany). All other reagents used were reagent grade or better. Dogmatyl 200-mg tablets (lot no. 269) were obtained from the innovative Fujisawa Pharmaceutical (Taoyuan, Taiwan). Sulpin 200-mg tablets (lot no. R870901T) made by Sin-Tong Chemical Industrial (Taoyuan, Taiwan) were used as the test product.

2.2. Instrumentation

A high-performance liquid chromatographic system equipped with a pump (Jasco PU-980 Intelligent HPLC Pump, Tokyo, Japan) and an autosampler (Jasco AS-950-10 Intelligent Sampler) were used. A 250×4 mm (I.D.) silica column (LiChrospher Si 60, Merck, Germany) with a particle size of 5 μm was employed. The mobile phase consisted of a triethylamine solution (0.5%, pH 4.0), methanol, and acetonitrile in the proportion of 10:5:85 (v/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) at a wavelength of 300 nm for excitation and 365 nm for emission. The HPLC system was controlled with Borwin computer software (JMBS Developments, France) installed on a PC workstation.

2.3. Internal standard solution and sample preparation

Plasma sample preparation and the extraction method are described step by step as follows. The plasma sample (1 ml) was spiked with 0.1 ml of an internal standard (metoclopramide, 1.5 μg/ml in methanol) solution and 0.1 ml of a NaOH solution (1 N). After vortex mixing thoroughly for 5 s, the mixture was extracted with 6 ml of ethylacetate/dichloromethane (5:1, v/v), then vortex-mixed for 5 min, and centrifuged at 2950 *g* for another 10 min. The supernatant (organic phase) was transferred to another clean glass tube and evaporated under a stream of nitrogen gas at 40°C until completely dry. Then, 0.2 ml of the mobile phase was added to dissolve the residue, and 0.1 ml was automatically injected into the HPLC system for analysis.

2.4. Quantification and calibration curve preparation

To examine the linearity of the assay, calibration curves for sulpiride at concentrations ranging from 20 to 1500 ng/ml in plasma were prepared. Standard plasma samples containing sulpiride at concentrations of 20, 50, 100, 500, 1000, and 1500 ng/ml were added with 150 ng metoclopramide and extracted and analyzed as described above. The peak area ratio (PAR) of sulpiride to metoclopramide was measured, and a calibration curve was obtained from the least-squares linear regression of the PAR versus spiked concentrations. The regression line was used to calculate concentrations of sulpiride in the unknown plasma samples based on the PAR.

2.5. Validation of the assay method

Several pre-dose human plasma samples from different subjects were tested for the presence of interfering compounds. The intra- and inter-assay coefficients of variation and standard deviations of the mean were used to validate the precision and accuracy of the assay by determining standard samples of sulpiride in plasma. For inter-day validation, six sets of control samples at six different concentrations (20–1500 ng/ml) were evaluated on 6 different days (six standard curves were constructed). For intra-day validation, six sets of controls at six different drug concentrations were assayed with one standard curve on the same run.

2.6. Blood sample collection and processing

The bioequivalence study (approved by the Ethics Committee of Taipei Medical University Hospital) was a cross-over design with 12 subjects receiving a 400-mg single dose (two 200-mg tablets) of Dogmatyl (R) or Sulpin (T). After dosing, heparized venous blood samples (~10 ml) were collected by means of an indwelling venous cannula of the cubital vein according to a 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 h after dosing. Plasma was separated immediately by centrifugation at 1690 *g* for 10 min, was then transferred to suitably labeled tubes, and stored at −25°C.

3. Results and discussion

It has been reported that the recovery of sulpiride using dichloromethane and *ter*-butyl methyl ether was ~10%. When ethyl acetate was used as the extraction solvent, the recovery exceeded 50%, but another decomposed peak appeared on the chromatogram [13]. This problem was overcome by using a solid-phase extraction procedure and back extraction with 0.01 *M* hydrochloride in the same study. However, after several trials in this study, it was found that one-step liquid–liquid extraction of sulpiride in plasma after the alkaline treatment followed by chromatographic elution using a silica gel column could resolve the problem. A mixed solvent of ethyl acetate and dichloromethane (5:1) was demonstrated to be suitable as the extraction solvent with minimal interference.

A silica gel column was initially preserved in hexane. Before analysis, serial reconditioning was conducted by eluting with solvents of gradually increasing polarity from ethyl acetate, dichloromethane, acetonitrile, methanol, and then finally water (the volume for each solvent was ~100 ml). Thereafter, this silica gel column can use reversed-phase eluents. The addition of triethylamine (TEA) or an

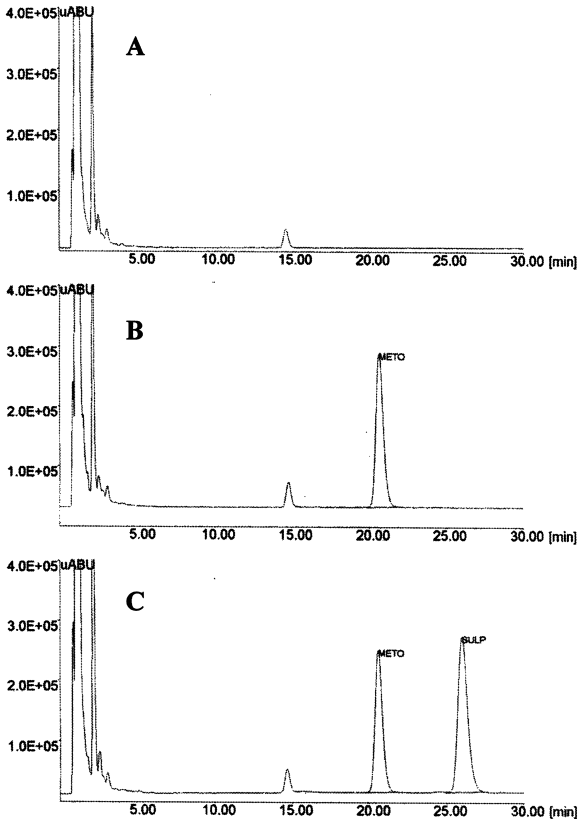

Fig. 1. HPLC chromatograms. (A) blank plasma; (B) the internal standard, metoclopramide; (C) metoclopramide and sulpiride.

equivalent in the mobile phase acts as a masking agent for free silanol groups on the silica gel to minimize the interaction between basic drugs and the silica gel, which generally causes tailing of peaks and also results in longer retention times. In this study, it was found that the addition of TEA at a level elucidated in the Experimental section was suitable for the purpose.

Fig. 1 shows typical HPLC chromatograms of sample analysis. No interfering peaks were observed for drug-free human plasma. The retention times of sulpiride and metoclopramide were around 20 and 25 min, respectively. Good separation and baselines with low background were observed. The peaks of interest were well resolved, and there was no interference from endogenous plasma substances. Also, the symmetry of both peaks (sulpiride and metoclopramide) was clearly indicated even though the retention times for both peaks were longer than 20 min.

Using the peak area of the sulpiride sample at the same injection amount without extraction as 100%, it was found that the recovery of sulpiride added to human plasma was almost quantitative (at least 50%). This indicates that the method is reproducible and suitable for the analysis of plasma samples.

The inter-day and intra-day validations for assaying sulpiride in plasma samples are shown in Tables 1 and 2. The coefficients of variation (C.V.) of inter-

Table 1
Precision and accuracy of intra-day validation

Concentration (ng/ml)	20	50	100	500	1000	1500
Y1*	20.71	52.62	106.23	496.24	980.11	1513.74
Y2	20.23	48.45	104.66	502.14	985.98	1507.89
Y3	20.61	53.14	103.92	503.28	971.95	1517.60
Y4	19.15	50.36	104.55	501.47	985.56	1508.63
Y5	21.93	50.76	104.68	504.28	969.64	1517.72
Y6	22.06	53.36	107.19	493.04	978.43	1516.53
Mean	20.78	51.45	105.21	500.08	978.61	1513.69
SD	1.09	1.93	1.24	4.44	6.78	4.45
C.V. (%)	5.25	3.74	1.18	0.89	0.69	0.29
Rel. err. (%)	3.91	2.90	5.21	0.01	−2.14	0.91

*, number of replication; C.V., coefficient of variation; Rel. err., relative error of the mean; SD, standard deviation.

Table 2
Precision and accuracy of inter-day validation

Concentration (ng/ml)	20	50	100	500	1000	1500
Y1*	22.20	54.22	106.22	493.61	977.37	1517.31
Y2	21.70	54.59	103.61	494.98	981.46	1513.49
Y3	21.86	53.06	103.78	492.04	991.51	1508.45
Y4	19.73	46.94	105.98	506.42	980.83	1511.05
Y5	17.84	46.77	104.31	504.44	994.12	1501.65
Y6	20.71	52.62	106.23	496.24	980.11	1513.74
Mean	20.67	51.37	105.02	497.96	984.23	1510.95
SD	1.66	3.57	1.25	5.99	6.84	5.43
C.V. (%)	8.01	6.95	1.19	1.20	0.70	0.36
Rel. err. (%)	3.37	2.73	5.02	−0.41	−1.58	0.73

*, number of replication; C.V., coefficient of variation; Rel. err., relative error of the mean; SD, standard deviation.

day and intra-day assays were 0.36–8.01% and 0.29–5.25%, respectively, indicating that the analysis has good precision. The relative errors of the mean (REM) were −1.58 to 5.02% and −2.14 to 5.21% for inter-day and intra-day assays, respectively, depicting the high accuracy of the analysis.

The linearity of the calibration curve of sulpiride (Figs. 2 and 3) was well correlated ($r^2>0.999$) within a range of 20–1500 ng/ml. All data show the excellent reproducibility of the sample analysis. Since the coefficient of variation for 20 ng/ml was less than 20%, it was set as the limit of quantitation (LOQ). The coefficients of variation for the remaining standard concentrations were all less than 15%, which complies with the requirements of assay validation. Preferably, only 1 ml of plasma sample is necessary to have such a low limit of quantitation. Expectedly, the limit of detection (LOD) was even lower than one-third of LOQ.

This method was applied to a bioequivalence study of two sulpiride tablet formulations. The absolute relative errors of the mean in each sample analysis run for QC samples were between −4.57 and 4.99%, indicating that the stability of the drug in plasma during storage periods was acceptable. Fig. 4 displays similar bioavailabilities with the mean of sulpiride plasma concentration–time profiles for 12 volunteers for the test and reference products. The pivotal pharmacokinetic parameters were calculated correspondingly. The mean±SD ratios of AUC_{0-last}, AUC_{0-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 the ranges of 94.16–104.31%, 93.97–103.85%, and 94.56–103.53% for AUC_{0-last},

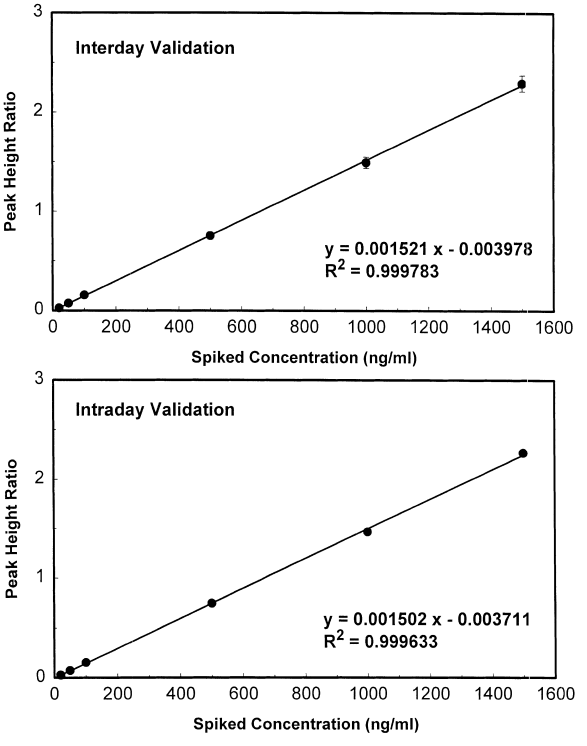

Fig. 2. Typical calibration curve for the assay of sulpiride plasma concentrations.

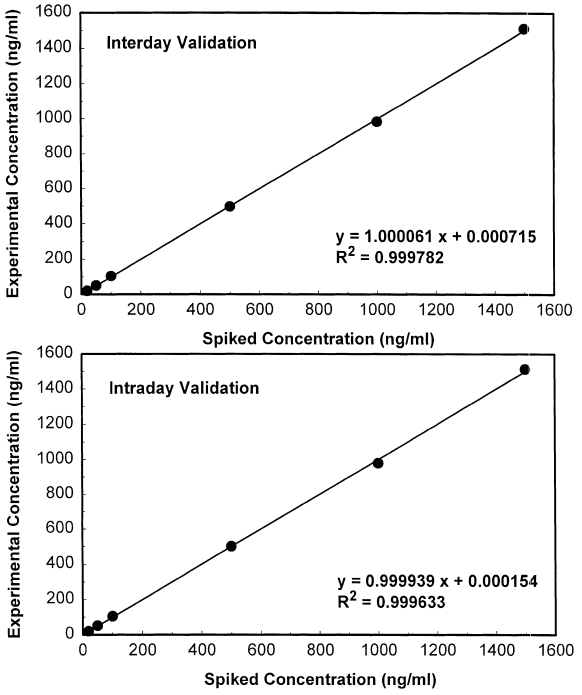

Fig. 3. Validation of HPLC assays for sulpiride plasma concentrations.

AUC_{0-inf} and C_{max}, respectively. The 90% confidence intervals of the mean difference for these three pivotal parameters fell within the range of 80–120%. The same results were obtained for statistical analyses using the two one-sided *t* distribution methods.

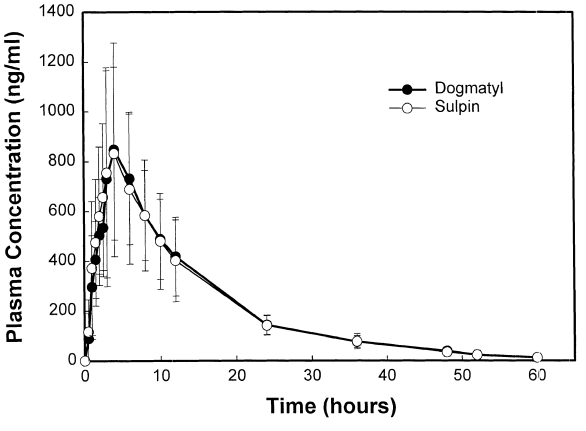

Fig. 4. Sulpiride plasma concentration–time profile in 12 volunteers for the test and reference products.

The values of the statistical power to compare mean ratios of AUC_{0-last}, AUC_{0-inf} and C_{max} between the two products were close to 1.00. The results of ANOVA of the three pivotal parameters show that only the factor of the subject was determined to be significant. There was no group, period, or treatment effect on these three pivotal parameters in this crossover design.

4. Conclusions

In conclusion, the use of a silica column for assaying sulpiride concentrations in plasma is simple and provides good performance with the HPLC method. High precision and accuracy with minimal interference and peaks with highly symmetry were demonstrated. A one-step liquid–liquid extraction further provides a simple and practical way to process plasma samples containing sulpiride with almost quantitative recovery. This method was successfully applied to a bioequivalence study of two commercial 200-mg sulpiride tablets (Dogmatyl vs. Sulpin) with 400-mg single dose of two tablets orally administered in 12 healthy, normal male volunteers. The statistical analysis results based on comparisons of three pivotal parameters (AUC_{0-last}, AUC_{0-inf} and C_{max}) showed that these two tablet products appear to be bioequivalent.

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