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Short communication

High performance liquid chromatographic determination, pharmacokinetic and comparative bioavailability studies of cisapride

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Abstract

A sensitive and specific reversed phase HPLC method was developed to quantitate plasma levels of cisapride in order to conduct comparative bioavailability studies. The drug and internal standard was extracted from plasma with heptane–isoamyl alcohol (95:5 v/v) and back extracted with sulfuric acid. The acidic layer was then re-extracted with the same extracting solvent. The separated organic layer was evaporated to dryness under nitrogen and the residue reconstituted with acetonitrile. Analysis was performed on a C-8 Sil-X-10 HPLC column, with a mobile phase of acetonitrile, water, and triethylamine (75:25:0.01) and UV detection at 215 nm. The standard curve covering the concentration range 5–160 ng/ml was linear ($r^2 = 0.9992$), relative errors were within $\pm 10\%$ and the CV% ranged from 1.34 to 11.82. The in vivo study was carried out in 12 healthy volunteers according to a single dose, two-sequence, cross over randomized design. The bioavailability was compared using the total area under the plasma level versus time curve (AUC_{0-34}, $AUC_{0-\infty}$), peak plasma concentration (C_{max}) and time to C_{max} (T_{max}). No statistically significant difference was found between the $AUC_{0-\infty}$ or C_{max} values of the test (cisapride) and reference (Propulsid®). It was, therefore, concluded that the generic cisapride was bioequivalent with the innovator formulation.

Keywords: Cisapride; HPLC; Pharmacokinetics; Bioavailability

1. Introduction

Bioavailability issues have been an increasing concern to drug regulatory authorities once assessing the safety and efficacy of drug products. The increasing number of synonym drug products requires special attention in terms of bioavailability issues. Local drug regulatory authorities have, therefore, issued guidelines to ensure adequate bioavailability studies in new drug applications for synonym drugs. Cisapride (Fig. 1A), a substituted piperidinyl benzamide chemically related to metoclopramide, is a novel gastrokinetic drug, devoid of antidopaminergic and direct cholinergic effects [5,6]. Cisapride is rapidly absorbed from the gastro-intestinal tract and undergoes first pass

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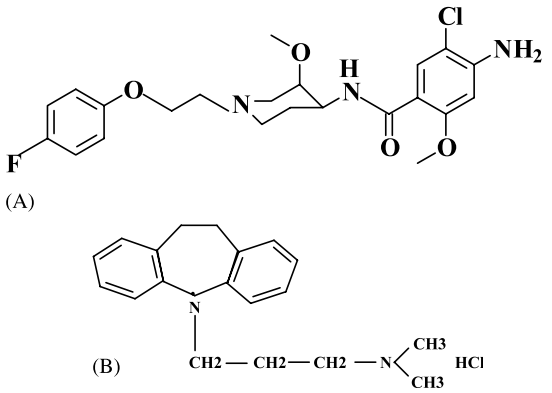

Fig. 1. Chemical structure of (A) cisapride, (B) clomipramine.

metabolism in the liver and gut wall. The main metabolic pathways are oxidative N-dealkylation, producing the major metabolite norcisapride, and aromatic hydroxylation. More than 90% of a dose is excreted as metabolites in the urine and feces in approximately equal amounts. Cisapride is about 98% bound to plasma proteins [7,8]. Following a single oral dose of cisapride tablet preparation low plasma concentrations are achieved and, therefore, a sensitive HPLC assay is required to determine the plasma levels. Thus far, a number of HPLC methods have been published [1–4]. However, the limit of quantitation reported in these studies is insufficient to determine the low plasma concentrations achieved following routine doses of this drug. The objectives of this study were, therefore, to establish a sensitive, specific and reliable HPLC assay to quantify plasma cisapride concentrations and to examine the comparative bioavailability of a 10-mg tablet of cisapride with that of the innovator Propulsid® as the reference.

2. Material and methods

2.1. Reagents and solutions

Cisapride was obtained from Jensson Life Sciences Products Division (Beerse, Belgium), the internal standard, clomipramine, from Sigma (St. Louis, MO), acetonitrile from Fluka (Germany), heptane, isoamylalcohol, sulfuric acid, ammonia from Merck (Germany). All reagents and solutions were either HPLC or analytical grade.

2.2. Study products

Cisapride tablets, 10 mg (batch no: B-7801001) were from Sobahan manufacturing, Iran and Propulsid® tablets, 10 mg (batch no: 98H28/996) were from Jensson pharmaceutical company, Belgium.

2.3. Chromatographic conditions

A reversed phase HPLC method was developed to quantitate plasma levels of cisapride. The apparatus used was a Jasco HPLC system (Japan), consisting of a model 980-PU intelligent solvent delivery pump, 7125-rheodyne injector, a computerized system controller (with the Borwin software), a UV-975 detector. Chromatographic separation was performed using a Perkin Elmer C-8 Sil-X-10 (250 mm × 4.6 mm) HPLC column. The mobile phase consisted of 75% acetonitrile, 25% water, and 0.01% triethylamine. The apparent pH* of the mixed solvent system was adjusted to 4 ± 0.1 with a dilute solution of orthophosphoric acid. The aqueous phase was eluted at a flow rate of 1 ml/min, and effluent was monitored at 215 nm, at attenuation of 0.0005 and gain $\times 10$. Quantitation was achieved by measurement of the peak area ratios of the drug to the internal standard.

2.4. Sample preparation

To 2 ml of plasma in a 15-ml test tube, 0.5 ml NaOH (1 M), 50 μl of internal standard solution (10 μg/ml) was added and extracted twice with 3 ml of extracting solvent (heptane–isoamyl alcohol, 95:5 v/v) vortexed for 2 min and centrifuged at $2000 \times g$ for 3 min. Upper layers were separated in a tube and back extracted with 3 ml of 0.05 M sulfuric acid. The organic layer was removed and the remaining acidic layer was then rendered alkaline with 150 μl of concentrated ammonia and re-extracted with 4 ml of extracting solvent. The separated organic layer was evaporated to dryness under a stream of nitrogen at room

temperature. The residue was reconstituted with 150 μl of acetonitrile and a 100 μl aliquot was injected to HPLC.

2.5. Calibration procedure

To 2 ml of blank plasma, 100 μl of cisapride standard solutions at concentrations of 3.2, 1.6, 0.8, 0.4, 0.2, 0.01 μg/ml and 50 μl of internal standard at fixed concentration of 10 μg/ml was added to obtain cisapride standard concentrations ranging from 5 to 160 ng/ml.

All calibration samples were taken through the extraction procedure. The calibration curve was plotted using peak ratios of cisapride to internal standard versus various cisapride concentrations. Final sample concentrations were calculated by determining the peak area ratio of cisapride related to internal standard and comparing the ratio with the standard curve, obtained after analysis of calibration samples.

2.6. Extraction efficiency

Recoveries of cisapride from spiked samples were determined by comparing the peak areas obtained by extraction of freshly prepared plasma at concentration of 5–160 ng/ml with those found by direct injection of an aqueous standard solution at equivalent concentration.

2.7. Precision

2.7.1. Within-day variability

The within-day variability of the assay was determined by repeated analysis of quality control samples at concentrations ranging from 5 to 160 ng/ml on the same day.

2.7.2. Between-day variability

The between-day variability of the assay was determined by repeated analysis of quality control samples at concentrations ranging from 5 to 160 ng/ml on 3 different days.

2.8. In vivo study design

The ethics committee on human studies of the Isfahan University of Medical Sciences approved the study. Twelve healthy adult male volunteers aged between 21 and 30 years and weighing from 58 to 90 kg participated in the study. The study was performed at Al-Zahra university hospital. On the basis of medical history, clinical examinations and laboratory tests including hematology, blood biochemistry, and urine analysis, no subject had a history or evidence of hepatic, renal, gastrointestinal or hematological deviations, or any acute or chronic diseases or drug allergy. The subjects were instructed to abstain from taking any medication and xanthin containing foods for at least 2 weeks prior to and during the study period. No milk or dairy products were served during the study. Informed consent was obtained from the subjects after explaining the nature and purpose of the

Table 1
The extraction efficiency of cisapride in plasma samples

Concentration (ng/ml)	Number of experiments			Mean	S.D.	CV%
	1	2	3			
5	95.64	112.25	73.70	73.70	93.86	15.79
10	99.07	98.40	87.10	87.10	94.85	5.49
20	79.65	102.27	63.66	63.66	81.86	15.84
40	104.47	73.74	75.77	75.77	84.66	14.03
80	93.97	82.73	79.49	79.49	85.40	6.21
160	83.86	95.34	83.86	83.86	87.68	5.41

study. The protocol used was the conventional, two-way, split group crossover study with six subjects in each of the treatment group. In the first trial period, after an overnight fast, subjects were given a single dose of two 10-mg tablets of either formulation (reference or test product) in a randomized fashion with 200 ml of water. Food and drinks (other than water, which was allowed after 2 h) were not allowed until 4 h after ingestion of the tablets. Lunch and dinner were served at 5 and 12 h after dosing to all volunteers. Approximately 10 ml blood samples were drawn into heparinized tubes through an indwelling cannula before (0 h) and at 0.5, 1, 1.5, 2, 3, 5, 7, 9, 12, 15, 24, 30, and 34 h after dosing. The blood samples were centrifuged at 3000 rpm for 15 min, plasma was separated and kept frozen at $-20\,^{\circ}C$ in coded glass tubes. It has been reported that cisapride is stable in frozen plasma ($-20\,^{\circ}C$) for 9 months, in plasma stored at room temperature ($20\pm3\,^{\circ}C$), and refrigerator ($4\,^{\circ}C$) for 6 h [3,4,9]. After a period of 7 days the study was repeated in the same manner to complete the crossover design.

2.9. Pharmacokinetic analysis

Estimation and calculation of pharmacokinetic parameters were performed using MS Excel software. The data obtained were reanalyzed using Win nonlin 1. The maximum Cisapride concentration (C_{max}) and the corresponding peak time (T_{max}) were determined by the inspection of the individual drug plasma concentration–time profiles. The elimination rate constant (K_{el}) was obtained from the least square fitted terminal log-linear portion of the plasma concentration–time profile. The elimination half-life ($T_{1/2}$) was calculated as $0.693/K_{el}$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated by equation $AUC_{0-t}+C_t/K_{el}$ where C_t is the last measurable concentration. The apparent volume of distribution normalized for F was estimated as $Dose/AUC_{0-\infty}K_{el}$.

2.10. Statistical analysis

For the purpose of bioequivalence analysis, AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were considered as primary variables. Two-way ANOVA for crossover design was used to assess the effect of formulations, periods, sequences, and subjects on these parameters. A difference between two related

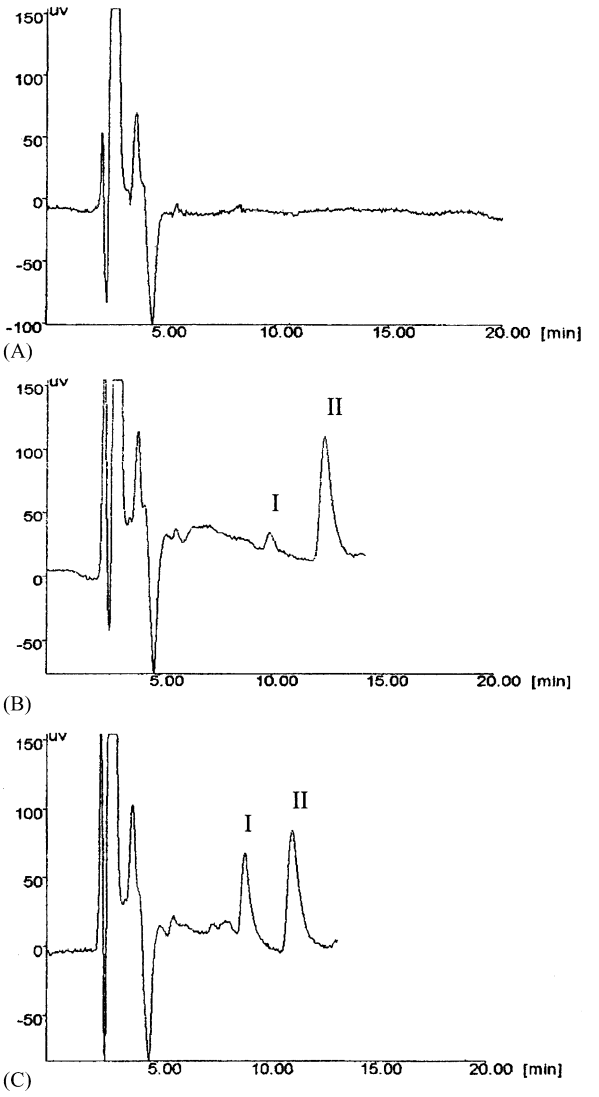

Fig. 2. Chromatograms of blank human plasma (A), control plasma spiked with cisapride (I) and the internal standard (II) (B), and plasma from a healthy subject after ingestion of cisapride tablet (C).

Table 2
Within-day variability of the HPLC method for determining concentrations of cisapride in plasma

Concentration (ng/ml)	Day		
	Within day variation		
	1	2	3
5	5.4±0.5	5±0.45	6.1±0.39
10	10.9±0.3	10.6±0.45	10.2±0.58
20	19.1±2.3	18.6±2.8	24.6±1.8
40	40.1±3.3	38.4±3.3	39.3±3.58
80	71.6±4.5	75.5±3.9	80.5±6.5
120	115±2.7	120±3.8	115.7±3.7
160	150.4±7.4	163.5±6.5	151.2±9.2

parameters was considered statistically significant for a P-value equal to or less than 0.05. The 90% confidence interval of the ratio of pharmacokinetic parameters of test to reference products as well as those of logarithmically transformed were also estimated [10–12]. All statistical analysis was performed using SPSS 10.

3. Result and discussion

The extractability of cisapride, a lipophilic ($\log P = 3.96$) weak base ($pK_a = 7.83$), from plasma was calculated from comparison directly injected standards and standards subjected to extraction procedure. The optimum compromise between chromatographic purity and extraction recovery was obtained at pH 13 using heptane–isoamyl alcohol (95:5, v/v). The assay recovery over the 5–160 ng/ml plasma concentration ranges, is presented in Table 1.

Fig. 2 shows some chromatograms of blank human plasma (A), control plasma spiked with cisapride and the internal standard (B) and plasma from a healthy subject after intake of cisapride (C). All samples were spiked with the internal standard at a concentration of 250 ng/ml. All chromatograms are free interferences at the retention times of cisapride or internal standard, and both compound eluted as completely resolved peaks.

Table 3
Between-days variability of the HPLC assay used for the determination of plasma cisapride concentrations

Concentration (ng/ml)	Experiments									Mean	S.D	C.V%	Error%
	First day			Second day			Third day						
	1	2	3	4	5	6	7	8	9				
5	5.4	5.0	6.1	4.8	5.8	4.2	5.6	5.6	6.3	5.38	0.80	14.82	7.6
10	10.9	10.6	10.2	9.7	12.2	11.8	8.9	7.2	12.9	10.47	1.39	13.24	4.7
20	19.1	18.6	24.6	20.1	18.7	19.2	21.6	20.7	18.8	19.86	0.75	3.77	0.7
40	30.9	38.4	39.3	40.1	39.3	33.9	46.0	34.9	37.6	38.62	3.38	8.76	3.5
80	71.6	75.5	80.5	79.9	77.9	75.7	91.2	84.4	113.2	87.05	2.13	2.45	8.8
120	115.0	120.0	115.7	123.4	121.3	120.2	112.2	124.7	120.8	120.43	1.61	1.34	0.4
160	150.4	163.5	151.2	160.7	173.2	157.6	157.5	182.6	155.2	164.46	8.21	4.99	2.8

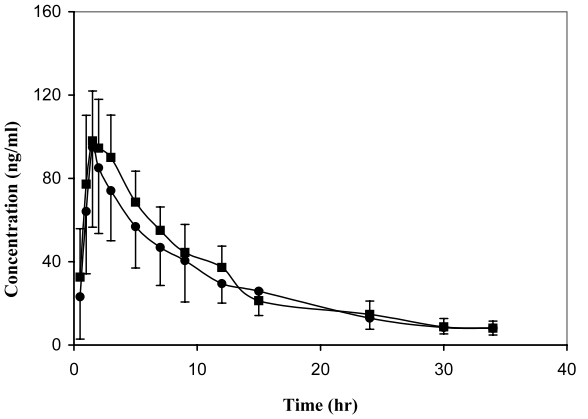

Fig. 3. The mean plasma cisapride levels vs. time profiles following ingestion of a single dose two 10-mg tablet for test and reference products (Cisapride® Propulsid®) to 12 healthy volunteers. Data is shown as mean ± S.D., test ●, and reference ■.

Linear relationships were found when the peak area ratios of cisapride to the internal standard were plotted versus the cisapride plasma concentrations (5–160 ng/ml). The standard curve, covering a 5–160 ng/ml concentration range, was linear ($r^2 = 0.9992$). Relative errors were within ±10% and the CV% ranged from 1.34 to 14.82. The limit of quantitation (sensitivity) and detection limit of the assay was found to be less than 5 and 1 ng/ml, respectively. The results of within- and between-day variability are presented in Tables 2 and 3. According to CV% and Error% obtained. These data indicate that the method is reproducible within day and between days.

Cisapride was well tolerated by the subjects; unexpected incidents that could have influenced the outcome of the study did not occur. All volunteers who started the study continued to the end and were discharged in good health. Both formulations were absorbed from the gastrointestinal tract and cisapride was measurable at the first sampling time (0.5 h) in nearly all volunteers. The mean concentration–time profile for the two formulations is shown in Fig. 3. All the calculated pharmacokinetic parameters were in good agreement with the previously reported values [4,13–28]. The statistical analyses of pharmacokinetic parameters for the brands of cisapride tablets are summarized in Tables 4 and 5.

The parameters T_{max} and $AUC_{0-\infty}$ corresponds to the respective rate and extent of drug absorption, while C_{max} is related to both of these two processes [29], with all three measures being useful for comparing the bioavailability of the two preparations.

The AUC_{0-t} and $AUC_{0-\infty}$ for the two products were not statistically different ($P > 0.05$), suggesting that the plasma profiles generated by Propulsid® were comparable to those produced by cisapride manufactured by Sobhan Company. ANOVA for these parameters, after log-transformation of data, showed no statistically significant difference between the two formulations. ANOVA did not reveal any considerable differences in periods, formulations, or sequences ($P > 0.05$). Ninety percent confidence intervals of the ratio of the $AUC_{0-\infty}$ of the two formulations (0.81–1.02) were found to be within the FDA acceptable range of 80–125%.

For bioequivalance evaluation, the C_{max} values of two products were also analyzed with the ANOVA procedure, indicating no statistical difference between two formulations. Furthermore, there was no significant difference with regards to periods and sequence ($P > 0.05$). The ninety percent confidence interval of the ratio of C_{max} of the two formulations was 0.85–1.15, which lies within the FDA acceptable range of 80–125% (Table 6). However, a statistically significant difference was observed between the T_{max} values ($P < 0.05$) of the two products, although the slight difference may not be therapeutically significant or important.

The numerical value of the Pharmacokinetic parameters k_{el}, $T_{1/2}$ V_d/F are given in Table 5. No statistically significant differences were observed between the values of the two products for any of the three parameters. In addition, the values were comparable to those reported previously [29].

In summary, the developed assay is sensitive enough to be used in pharmacokinetic, pharmacodynamics and bioequivalance studies on cisapride. Based on estimated pharmacokinetic parameters and statistical analyses, it was found that the cisapride tablets manufactured by Sobhan

Table 4
Primary pharmacokinetic parameters of the two brands of cisapride

Subjects	Cisapride			Propulsid®		
	C_{max}[a] (ng/ml)	T_{max}[b] (h)	$AUC_{0-\infty}$[c] (h ng/ml)	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-\infty}$ (h ng/ml)
A	128.27	2	1599.0	122.25	1	1212.9
B	85.32	1.5	804.6	122.67	1	1155.1
D	56.93	2	657.0	63.83	3	899.9
E	96.69	3	1152.3	136.99	2	1508.9
F	138.59	1.5	1204.0	110.38	2	1178.1
G	59.04	1.5	1043.2	87.11	3	811.9
H	171.31	1.5	903.7	118.98	3	1094.7
I	123.35	1.5	1264.8	131.51	3	1600.0
J	153.10	2	1134.7	97.43	3	1084.7
K	94.64	1	1168.0	121.15	1	1375.4
L	78.87	2	1018.4	63.79	2	923.2
M	92.38	2	1202.3	123.24	2	1629.0
Mean	106.54	1.79	1096.0	108.28	2.13	1206.2
Std	36.33	0.50	240.8	24.86	0.86	272.0

[a] Maximum cisapride plasma concentration.
[b] Time to maximum concentration.
[c] Area under the plasma cisapride concentration curve.

Table 5
Pharmacokinetic parameters of the two brands of cisapride

Subjects	Cisapride			Propulsid®		
	K_{el}[a] (h)	V_d/f[b] (l/kg)	$T_{1/2}$[c]	K_{el} (h)	V_d/f (l/kg)	$T_{1/2}$
A	0.077	0.16	9.05	0.067	0.25	10.41
B	0.066	0.38	10.53	0.060	0.29	11.49
D	0.063	0.49	11.05	0.070	0.32	9.97
E	0.038	0.45	18.05	0.077	0.17	8.98
F	0.102	0.16	6.79	0.076	0.22	9.16
G	0.065	0.30	10.69	0.083	0.30	8.32
H	0.061	0.36	11.31	0.104	0.18	6.64
I	0.083	0.19	8.35	0.063	0.20	11.02
J	0.074	0.24	9.43	0.075	0.25	9.25
K	0.051	0.34	13.70	0.054	0.27	12.86
L	0.085	0.23	8.12	0.057	0.38	12.14
M	0.046	0.36	14.94	0.039	0.31	17.72
Mean	0.068	0.30	10.10	0.069	0.26	10.66
Std	0.018	0.11	3.19	0.016	0.06	2.81

[a] Elimination rate constant.
[b] Volume of distribution/fraction of cisapride absorbed.
[c] Elimination half-life.

company were bioequivalent to Propulsid®, manufactured by Jensson company, and that both products can be considered equally effective in medical practice. The estimated pharmacokinetic parameters found to be in accordance with previous reports.

Table 6
Ninety percent confidence interval of primary pharmacokinetic parameters

Pharmacokinetic parameters	90% confidence intervals
AUC_{0-34}	0.82–1.03
AUC_{0-8}	0.83–1.04
C_{max}	0.85–1.15
T_{max}	0.75–1.22
Log-transformed Pharmacokinetic parameters	90% confidence intervals
AUC_{0-34}	0.97–1.00
AUC_{0-8}	0.97–1.00
C_{max}	0.96–1.02

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References

[1] Z. Desta, N.V. Soukhova, A. Morocho, J. Park, S.K. Mahal, D.A. Flockhart, J. Chromatogr. B 744 (2000) 263–272.
[2] M.A. Campanero, B. Calahorra, E. Garcia-Quetglas, J. Honorato, J.J. Carballal, Chromatographia 47 (1998) 537–541.
[3] Y. Preechagoon, B.G. Charles, J. Chromatogr. B 670 (1995) 139–143.
[4] R. Woestenborghs, W. Lorreyne, F. Vanrompaey, J. Heykants, J. Chromatogr. 424 (1988) 195–200.
[5] P.L. Munson, Principles of Pharmacology, Basic Concepts and Clinical Application, Chapman & Hall, 1995, pp. 1097–1101.
[6] J.E.F. Reynolds, MARTINDALE, the Extra Pharmacopoeia, 30th ed., The Royal Pharmaceutical Society, 1995, p. 1213.
[7] R.W. McCallum, C. Prakash, D.M. Campoli-Richards, K.L. Goa, Drugs 36 (1988) 625–681.
[8] D.W. Sifton, Physician Desk Reference (PDR), 53rd ed., Montvale, Medical Economics Company, 1999, pp. 1430–1432.
[9] M.A. Campanero, G. Calahorra, E. Garacia-Quetglas, G. Honorato, J.J. Carballal, Chromatographia 47 (9) (1998) 537–547.
[10] W.J. Westlake, J. Pharm. Sci. 61 (8) (1972) 1340–1341.
[11] M.D. Rockville, FDA guidance on statistical procedures for bioequivalence studies using a standard two-treatment crossover designs, Division of Bioequivalence, Office of Generic Drugs, Center for Drug Evaluation and Research, Food and Drug Administration, 1992.
[12] S. Chow, Drug Inf. J. 33 (1999) 435–444.
[13] M.C. Condado, A. Malave, M.A. Dorantes-Hernandez, F. Rathinavelu, J. Assoc. Off. Anal. Chem. Int. 84 (2001), 1, 9–12.
[14] P.B. Rao, S.D. Duhri, M. Sundaresan, M.A. Dave, Indian Drugs 34 (9) (1997) 505–507.
[15] M.T. Maya, C.R. Domingos, M.T. Guerreiro, A.P. Filipe, J.A. Morais, Eur. J. Drug Metab. Pharmacokinet. 23 (3) (1998) 377–381.
[16] S. Cisternino, J. Schlatter, J.L. Saulnier, J. Chromatogr. B 714 (1998) 395–398.
[17] H.M. Lee, S.J. Choi, C.K. Jeong, Y.S. Kim, K.C. Lee, H.S. Lee, J. Chromatogr. B 727 (1999) 213–217.
[18] H. Zhou, J. Herron, C. Clyde, P. Lee, W. Mechlinski, L. Pesco-Koplowitz, Clin. Ther. 20 (2) (1998) 292–298.
[19] E.M. Offman, D.J. Freeman, G.K. Dresser, C. Munoz, J.R. Bend, D.G. Bailey, Clin. Pharm. Ther. 70 (2001) 17–23.
[20] Q. Zhao, M.A. Wojcik, J.L. Parier, L. Pesco-Koplowitz, Pharmacotherapy 21 (2) (2001) 149–157.
[21] L. Ereshefsky, D. Dugan, Depress. Anxiety 1 (Suppl. 1) (2000) 30–44.
[22] E.L. Michalets, C.R. Williams, Clin. Pharmacokinet. 39 (1) (2000) 49–75.
[23] Z. Desta, N. Soukhova, S.K. Mahal, D.A. Flockhart, Metab. Inhib. Stud. 28 (7) (2000) 789–800.
[24] M.T. Maya, C.R. Domingos, M.T. Guerreiro, A.P. Filipe, J.A. Morais, Eur. J. Drug Metab. Pharmacokinet. 23 (3) (1998) 377–381.
[25] T. Yamamoto, K. Takano, M. Sanaka, Y. Kuyama, M. Yamanaka, Y. Koike, S. Mineshita, Int. J. Clin. Pharmacol. Ther. 36 (8) (1998) 432–434.
[26] J.A. Barone, L.M. Jessen, J.L. Colaizzi, R.H. Bierman, Ann. Pharmacother. 28 (4) (1994) 488–500.
[27] K. Lauritsen, L.S. Laursen, J. Rask-Madsen, Clin. Pharmacokinet. 19 (2) (1990) 94–125.
[28] J.A. Barone, Y.C. Huang, R.H. Bierman, J.L. Colaizzi, J.F. Long, D.A. Kerr, A. Van Peer, R. Woestenborghs, J. Heykants, Clin. Pharm. 6 (8) (1987) 640–645.
[29] K.H. Yuen, K.K. Peh, K.L. Chan, W.T. Toh, Drug Dev. Ind. Pharm. 24 (10) (1998) 955–999.