Journal of Food and Drug Analysis, Vol. 10, No. 1, 2002, Pages 13-17 藥物食品分析 第十卷 第一期

Pharmacokinetics and Bioequivalent Study of Generic Fluoxetine Capsules Preparation

RYH-NAN PAN[1], TING-HSIEN CHEN[2], CHRISTINE SHU-HUI HUANG[2] AND CHENG-HUEI HSIONG[2]*

[1.] *Taiwan Police Collegev, #153 Shing-Lung Rd., Section 3, Taipei, Taiwan, Republic of China*

[2.] *Pharmaceutical Research Institute, National Defense Medical Center, P.O. Box 90048-512, Taipei, Taiwan 100, Republic of China*

(Received: August 27, 2001; Accepted: November 21, 2001)

ABSTRACT

The pharmacokinetics and relative bioavailability of fluoxetine capsules manufactured by two different pharmaceutical factories were carried out. A multiple oral doses ((20 mg/cap) × 2/day × 13 day)of fluoxetine was administered in 8 healthy young Chinese males in a completely double-blind cross-over design with a two week washout period between each dose. Plasma samples were obtained before (three minimum concentrations) and at various appropriate intervals after last dosing up to 72 hours. The plasma concentrations were then analyzed by a HPLC method. The limit of quantitation of this HPLC method was 5 ng/mL. The coefficients of variation of the within-day and between-day calibration curves (n = 6) range from 5 ng/mL to 500 ng/mL were less than 16 %, and the accuracy of this method was also verified. Values for the area under the plasma concentration-time curve at steady state (AUC), peak concentration (C_{max}), time to peak concentration (T_{max}), elimination rate constant, half-life, oral clearance were estimated and compared for each preparation. By ANOVA, power analysis, 90% confidence interval, and two one-sided tests, PROZAC and FLUOXETINE can be considered bioequivalent.

Key words: fluoxetine, relative bioavailability, pharmacokinetics, HPLC

INTRODUCTION

Fluoxetine is a bicyclical derivative of phenylpropylamine. It is the most widely used selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitor (SSRIs), and is prescribed for a variety of psychopathological conditions including mood and eating disorders, obsessive-compulsive disorders, depression in the elderly and dysthymia[(1-9)]. Food does not appear to affect the systemic bioavailability of fluoxetine, although it may delay its absorption as a consequence. Thus, fluoxetine may be administered with or without food[(1,5)]. Absolute bioavailability of oral fluoxetine in dogs is about 72% of the intravenous dose[(1)]. In humans, following a single oral 40 mg dose, peak plasma concentrations of fluoxetine from 15 to 55 ng/mL are observed after 6 to 8 hours[(8)].

Over the concentration range from 200 to 1,000 ng/mL, approximately 94.5% of fluoxetine is bound *in vitro* to human serum protein, including albumin and α_1-acid-glycoprotein. The interaction between fluoxetine and other highly protein-bound drugs has not been fully evaluated, but may be important[(8-9)].

Fluoxetine is extensively metabolized in the liver to norfluoxetine and a number of other, unidentified metabolites. The only identified active metabolite, norfluoxetine, is formed by demethylation of fluoxetine. In animal models, norfluoxetine's potency and serotonin as an uptake blocker are essentially equivalent to fluoxetine's. The primary route of elimination appears to be hepatic metabolism to inactive metabolites excreted by the kidneys[(5-9)].

* Author for correspondence. Tel:02-87923100 ext. 18845; Fax:02-87923163; E-mail:chhsiong@ndmctsgh.edu.tw

The relatively slow elimination of fluoxetine (elimination half-life of 7 to 9 days), assures significant accumulation of these active species in chronic use. After 30 days of dosing at 40 mg/day, plasma concentrations of fluoxetine in the range of 91 to 302 ng/mL and plasma concentrations of norfluoxetine in the range of 72 to 258 ng/mL have been observed. Plasma concentrations of fluoxetine were higher than those predicted by single-dose studies, presumably because fluoxetine's metabolism is not proportional to dose. Norfluoxetine, however, appears to have linear pharmacokinetics. Its mean terminal half-life after a single dose was 8.6 days and after multiple dosing was 9.3 days. Thus, even if patients are given a fixed dose, steady state plasma concentrations are only achieved after continuous dosing for weeks. Nevertheless, plasma concentrations do not appear to increase without limit. Specifically, patients receiving fluoxetine at doses of 40-80 mg/day over periods as long as 3 years exhibited, on average, plasma concentrations similar to those seen among patients treated for 4 or 5 weeks[(1-9)].

The purpose of this study was to evaluate the pharmacokinetic property and bioavailability of two fluoxetine capsules from different pharmaceutical factories. (PROZAC, Eli Lily Product Co., U.S.A., FLUOXETINE, Tung-Yang Chemical Co., Ltd., R.O.C.)

MATERIALS AND METHODS

I. *Instrumentation*

The HPLC set was equipped with a pump (Kratos-400, USA), an automatic sampler (SIC Autosampler 23, Japan), a variable wavelength UV detector and an integrator (SIC

chromatocorder 12, Japan). Separations were performed under 40˚C controlled by a heater (Kratos PCR 520, USA) on a reverse phase C8 (4.6 mm×250 mm) column. In this experiment, 180 μL protriptyline (10 μg/mL) was chosen to be the internal standard. The assays for protriptyline and fluoxetine were performed by using mobile phase CH_3CN/0.067 M KH_2PO_4 (PH = 3.0) (70/30), flow rate 0.8 mL/min and an UV detector wavelength of 226 nm at 0.005 AUFS throughout the assay. The dissolution test was performed using the Jasco automatic dissolution apparatus (Japan) and a self-designed programmer, which can fully automatically change the dissolution medium continuously[10-12].

II. *Drug*

PROZAC (20 mg/cap., Dista Product Eli Lily Co., U.S.A.) and FLUOXETINE (20 mg/cap., Tung-Yang Chemical Co., R.O.C.) were purchased and obtained from the manufacturer. Analysis of these two preparations by HPLC method showed that PROZAC and FLUOXETINE contained 95.97% and 101.1% of the labeled amount respectively.

III. *Dissolution Test*

The *in vitro* dissolution rate in pH values of 1.2, 3.6 and 7.5 media for each dosage form was determined according to the general method of USP XXI, rotating basket method (50 rpm; replication = 6). A self-designed fully automatic continuously changing pH dissolution medium system was used. The amount of dissolved drug at two-minute intervals was determined spectrophotometrically (λmax = 226 nm).

IV. *Subject*

The Subjects involved in this study were 8 healthy young Chinese males (Table 2) whose body weights ranged from 55 to 74 kg (mean ± SD: 64.13 ± 5.80) and ages ranged from 19 to 22 years (mean ± SD: 20.63 ± 0.86). All subjects were in good physical condition as determined by complete physical and clinical examinations before the study. These subjects were instructed to abstain from any drugs for at least 2 weeks prior to and during the study. Subjects with a history of drug or alcohol abuse or drug sensitivity were excluded to each subject. The study was explained and informed consent was obtained.

Table 1. Precision and accuracy of fluoxetine in plasma determined by the HPLC method

Known conc. (ng/mL)	Conc. found (ng/mL)	Coefficient of variation (%)	Accuracy (%mean deviation)
within-day(n=6)			
5	4.85 ± 0.74	15.18	-2.68
10	9.93 ± 0.68	6.88	-0.53
20	19.36 ± 1.43	7.36	-3.78
50	51.02 ± 1.79	3.51	0.36
100	101.66 ± 4.33	4.26	0.15
200	204.10 ± 6.79	3.33	0.08
500	510.62 ± 10.42	2.04	0.15
between-day(n=6)			
5	4.92 ± 0.17	3.40	-0.93
10	10.40 ± 0.35	3.38	4.07
20	19.24 ± 1.12	5.81	-3.96
50	48.93 ± 1.22	2.49	-2.54
100	102.79 ± 1.48	1.44	2.31
200	202.55 ± 2.60	1.29	0.79
500	503.95 ± 13.23	2.63	0.26

Table 2. Sex, age, and height of each subject

Subject	Sex	Age(years)	Weight(kg)	Height(cm)
1	M	22	63	172
2	M	20	74	175
3	M	21	55	167
4	M	21	64	172
5	M	21	65	170
6	M	21	70	170
7	M	20	57	168
8	M	19	65	175
Mean	–	20.63	64.13	171.13
SD	–	0.86	5.80	2.76

V. *Study Design*

A multiple dose of fluoxetine (20 mg) was given on a b.i.d. schedule and with 200 mL water for 13 days. All 8 subjects received each formulation according to randomized crossover design with at least a two-week washout period, between each treatment. Before drug administration, 30 mL of blank blood was withdrawn for the construction of the individuals' calibration curve. Following drug administration, a 10 mL blood sample was collected from a forearm vein at day 12 and 13 before each dose and at day 14 after the final dose, at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48 and 72 hr. Plasma samples were stored at -30˚C until subsequent assay.

VI. *Assay Method*

To 3.0 mL of plasma sample, 0.5 mL saturated Na_2CO_3 solution was added. Then 0.18 mL of 10 μg/mL protriptyline solution was added as an internal standard. The mixture was then vortexed for a few seconds to ensure adequate mixing followed by adding 10 mL hexane: isoamyl alcohol (99:1). The mixture was vortexed for 1 min then centrifuged (1080×g) for 30 min to express the organic layer. The aqueous lower layer was discarded. The organic layer (8.0 mL) was transferred into another tube for back-extraction with 0.6 mL 0.05% phosphoric acid. The tube was vortex-mixed for 1 min and centrifuged for 10 min. The upper organic layer was discarded and the acidic solution was transferred to a clean vial for HPLC analysis. The assay for fluoxetine was performed by using a reverse phase C8 column and mobile phase CH_3CN / 0.067 M KH_2PO_4 (pH = 3.0) (70/30). A flow rate of 0.8 mL/min, UV detector wavelength of 226 nm at 0.005 AUFS, a column temperature of 40˚C and an injecting

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

volume of 200 μL were used throughout the assay. Under the described assay conditions, retention times of protriptyline and fluoxetine were 8 and 18 min., respectively. In order to validate the accuracy and precision of the assay method, between-day and within-day standard curves for the plasma samples were constructed (n = 6). Standard solutions were also determined. Furthermore, every subject had his own calibration curve using his own blank plasma.

VII. *Pharmacokinetic Analysis*

At steady state, the area under the plasma concentration-time curve ($AUC_{0\text{-}12}$) was calculated from time 0 to 12 hours using the trapezoidal rule. The AUC from zero time to infinity (AUC_∞) included a terminal slope correction factor, C_n/β where C_n was the last measured concentration-time curve point, and β was estimated from the slope of the terminal log-linear phase of the semilog plot of concentration versus time. The elimination half-life ($T_{1/2}$) was equal to -0.693/β. The maximum plasma concentration (C_{max}), and time to maximum plasma concentration (T_{max}) were observed from the measured plasma concentration following drug administration.

Pharmacokinetic parameters, such as apparent volume of distribution (Vd/F) and the total plasma oral clearance (Cl_0) were calculated according to the following formula:

$$Cl_0 = \frac{Cl}{F} = \frac{D}{AUC_{0-12}}$$

Where F was fraction absorbed of drug, and D was the dose administered, Cl was IV clearance[13].

VIII. *Statistical Analysis*

Analysis of Variance (ANOVA), 90% confidence intervals, power analysis and two one-sided tests were used to make statistical evaluations of pharmacokinetic data and the assessment of bioequivalence.

RESULTS AND DISCUSSION

Under the described assay conditions, linearity was observed in plasma standard curves over a range of 5-500 ng/mL. Plasma within-day and between-day standard curves had correlation coefficients greater than 0.99. The limit of quantitation was 5 ng/mL in plasma. From the results shown in Table 1, for within-day analysis, the coefficient of variation were range from 2.04 to 15.18% and the deviation from expected concentration, as a measurement of accuracy, ranged from -3.78% to +0.36%, for between-day analysis, the coefficient of variation were all within 10% and the deviation from expected concentration ranged from -3.96% to +4.07%. These results indicated that the method was precise and accurate.

From the results of *in vitro* dissolution studies, prozac and fluoxetine had very similar releasing profiles in various media. Under all conditions, prozac and fluoxetine dissolved completely within two hours (Figure 1- 4).

During long term administration steady-state plasma fluoxetine concentrations were achieved within 2 to 4 weeks[1]. The steady state was further approved in our study after comparing the pre-dosing concentrations of fluoxetine (Cmin) of day 12, 13, and 14. No significant difference was found between Cmin of day of 12, 13, and 14 by ANOVA (Table 3).

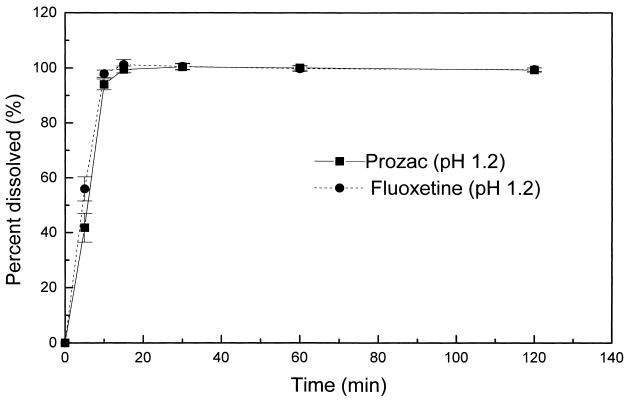

Figure 1. Dissolution profiles of Prozac and Fluoxetine capsules in pH 1.2 dissolution medium.

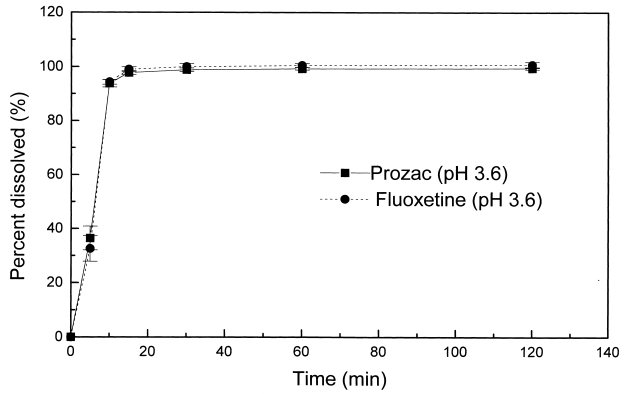

Figure 2. Dissolution profiles of Prozac and Fluoxetine capsules in pH 3.6 dissolution medium.

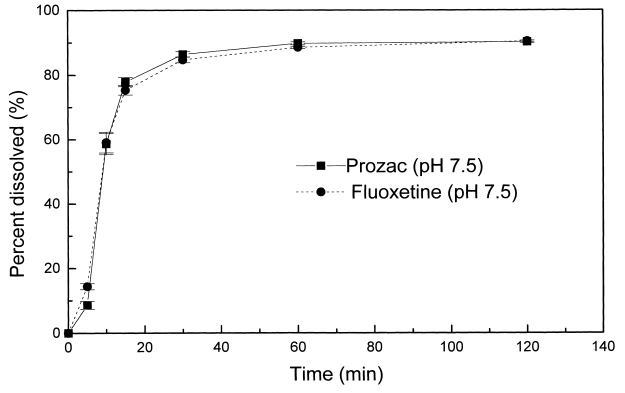

Figure 3. Dissolution profiles of Prozac and Fluoxetine capsules in pH 7.5 dissolution medium.

Table 3. Pharmacokinetic parameters following 40 mg of oral dose of fluoxetine

Parameters	Prozac	Fluoxetine	90% confidence Interval (%)
AUC_{0-12} ($\mu g \cdot hr/mL$)[a]	10.10 ± 2.08	9.77 ± 2.94	85.8 – 101.5
AUC_{∞} ($\mu g \cdot hr/mL$)	23.33 ± 9.70	22.52 ± 9.63	–
C_{max} (ng/mL)[a]	218.09 ± 41.52	194.82 ± 49.53	83.0 – 95.6
T_{max} (hr)	3.6 ± 1.4	4.8 ± 2.3	–
Cl/F (L/hr)[a]	0.98 ± 0.36	1.24 ± 1.09	–
V/F (L)[a]	108.22 ± 20.24	121.75 ± 45.26	–
$T_{1/2}$ (hr)[a]	83.0 ± 22.4	87.2 ± 37.2	–
Cmin of day 12 (ng/mL)[b]	175.81 ± 83.01	163.37 ± 78.98	–
Cmin of day 13 (ng/mL)[b]	195.99 ± 99.83	173.94 ± 69.94	–
Cmin of day 14 (ng/mL)[b]	196.23 ± 120.01	177.21 ± 100.92	–

[a] No significant difference ($p>0.05$, power >0.8) between the two drugs by ANOVA.
[b] No significant difference ($p>0.05$) between Cmin of day 12, 13 and 14 by ANOVA.

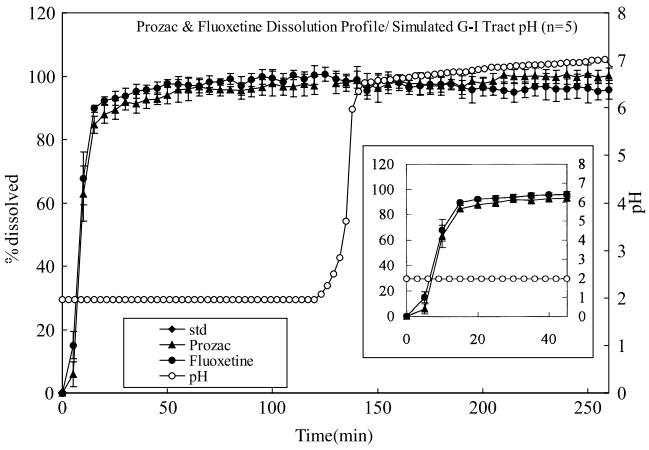

Figure 4. Dissolution profiles of Prozac and Fluoxetine capsules in continuously changing pH dissolution medium.

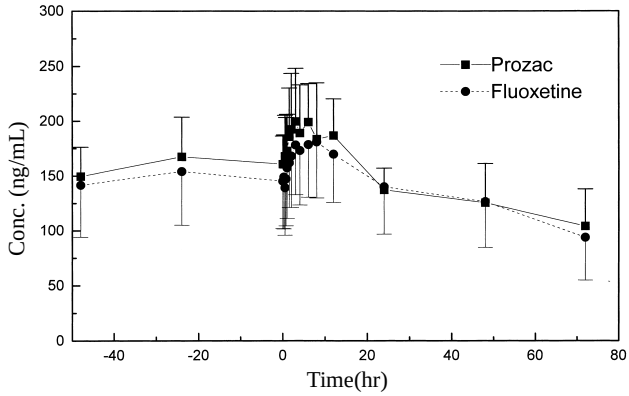

Figure 5. Plasma concentration-time profile after oral administration of 40 mg/day capsules of Prozac and Fluoxetine (Mean ± S.E.).

The mean plasma concentration-time profiles for each preparation are shown in Figure 5. No significant difference in fluoxetine plasma concentration was observed between the two preparations. On the basis of AUC_{0-12} and therapeutic duration, they were not significantly different with power larger than 0.9. The maximum plasma concentration for prozac and fluoxetine were non-significantly different with approximate power of 1.0. The pharmacokinetic parameters for each preparation are listed in Table 3. After applying ANOVA to analyze the pharmacokinetic parameters, neither apparent volume of distribution, elimination half-life, nor oral clearance differed significantly between treatments. By comparing the overall oral clearance of these two preparations with those of each subject, the result showed that intra-subject variation was not significant.

There were no significant age and body weight differences among the volunteers involved in this study. Since double-blind randomized crossover design was used, the interference of inter-subject variation on the test of two one-sided tests was minimized. From the pharmacokinetic data such as oral clearance, half-life, apparent volume of distribution between proprietary and generic products, intra-subject variation was acceptable.

Considering the statistical errors, which may be caused by ANOVA, power analysis, 90% confidence interval and two one-sided tests were applied as indicators for the purpose of assessing bioequivalence. Regardless of whether ANOVA, 90% confidence interval, power analysis or two one sided tests were used, prozac and fluoxetine were bioequivalent.

A comparison of single-dose versus steady-state pharmacokinetics in men showed that $T_{1/2}$ was longer (3.45 vs. 1.9 days) and Cl was lower (0.98 vs. 35.5 L/hr) after multiple-dose administration than after single-dose administration. This change in pharmacokinetic leads to a larger AUC at steady state than is observed after a single dose[1,14].

In conclusion, based on the present results, AUC_{0-12} and C_{max} were not significantly different with power equal to 1.0. Also, according to the results of two one-sided tests, we can conclude that prozac and fluoxetine are bioequivalent.

REFERENCES

1. Altamura, A. C., Moro, A. R. and Percudani, M. 1994. Clinical pharmacokinetics of fluoxetine. Clinical Pharmacokinet 26: 201-214.
2. Lemberger, L., Bergestrom, R. F., Wolen, R. L., Farid, N. A., Enas, G. G. and Aronoff, G. R. 1985. Fluoxetine: clinical pharmacology and physiologic disposition. J. Clin. Psychiatry 46: 14-19.

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

3. Beasley, C. M., Bosomworth, J. C. and Wernicke, J. F. 1990. Fluoxetine: relationships among dose, response, adverse events, and plasma concentrations in the treatment of depression. Psychopharmacology Bulletin 26: 18-24.
4. Goodnick, P. J. 1991. Pharmacokinetics of second generation antidepressants: Fluoxetine. Psychopharmacology Bulletin 27: 503-512.
5. Bergestrom, R. F., Lemberger, L., Farid, N. A. and Wolen, R. L. 1988. Clinical pharmacology and pharmacokinetics of fluoxetine: a review. British Journal of Psychiatry 153: suppl. 3 47-50.
6. Richelson, E. 1984. The newer antidepressants: structure, pharmacokinetics, pharmacodynamics, and proposed mechanisms of action. Psychopharmacology Bulletin 20: 213-223.
7. Benfield, P., Heel, R. C. and Lewis, S. P. 1986. Fluoxetine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficiency in depressive illness. Drug 32: 481-508.
8. Aronoff, G. R., Bergstrom, R. F., Pottratz, S. T., Sloan, R. S., Wolen, R. L. and Lemberger, L. 1984. Fluoxetine kinetics and protein binding in normal and impaired renal function. Clin. Pharmacol Ther. 36: 138-144.
9. Keck, P. E. and McElroy, S. L. 1992. Ratio of plasma fluoxetine to norfluoxetine concentrations and associated sedation. J. Clin. Psychiatry 53: 127-129.
10. Thomarre, P., Wang, K., Meersch-Mougeot, V. D. and Diquet, B. 1992. Sensitive micromethod for column liquid chromatographic determination of fluoxetine and norfluoxetine in human plasma. Journal of Chromatography 583: 217-221.
11. Gupta, R. N. and Steiner, M. 1990. Determination of fluoxetine and norfluoxetine in serum by liquid chromatography with fluorescence detection. Journal of Liquid 13: 3785-3797.
12. Wong, S. H. Y., Dellafera, S. S. and Fernandes, R. 1990. Determination of fluoxetine and nor fluoxetine by high-performance liquid chromatography. Journal of Chromatography 499: 601-608.
13. Shargel, L. and Yu, A. B. C. 1993. Applied Biopharmaceutics and Pharmacokinetics. 3rd ed. pp. 353-374. Appleton & Lange. Connecticut, U. S. A.
14. Gilman, A. G., Rall, T. W., Nies, A. S. and Taylor, P. 1991. The Pharmacological Basis of Therapeutics. 8th ed. pp.1681. Pergamon Press. New York, U. S. A.

Fluoxetine 藥動學及兩種口服膠囊生體相等性研究

潘日南[1]　陳鼎憲[2]　黃淑惠[2]　熊正輝[2*]

[1] 台灣警察專科學校　消防安全科　台北市興隆路三段153號
[2] 國防醫學院　藥品製造研究所　台北市民權東路六段161號

（收稿：August 27 , 2001 ；接受： November 21, 2001）

摘　要

此試驗由二家不同藥廠所提供之Fluoxetine膠囊進行之藥物動力學及相對生體可用率試驗。Fluoxetine 20mg口服劑量每天2次重複給與13天於8位健康年輕男性進行之雙盲交叉試驗。給與每一劑量投藥間隔二星期。收集試驗前及試驗後適當時間之血液檢品直至72小時。藥物血漿濃度利用高效能液相層析儀進行分析。定量極限為5 ng/mL。同日間分析及異日間分析CV值均小於18%也證明此分析方法之準確度。計算穩定狀態之AUC, C_{max}, T_{max}，排除速率常數，半衰期值，並經統計學上之比較。發現無論變異數分析，power分析，90%信賴區間，and two one-sided tests結果，PROZAC和FLUOXETINE具生物相等性。

關鍵詞：fluoxetine，相對生體可用率，藥物動力學，HPLC