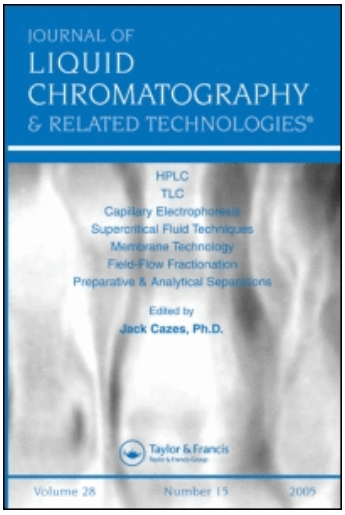

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SIMPLE DETERMINATION OF FLUOXETINE AND NORFLUOXETINE IN HUMAN SERUM AND URINE BY DABSYLATION FOLLOWED BY HPLC WITH VISIBLE LIGHT DETECTION

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SIMPLE DETERMINATION OF FLUOXETINE AND NORFLUOXETINE IN HUMAN SERUM AND URINE BY DABSYLATION FOLLOWED BY HPLC WITH VISIBLE LIGHT DETECTION

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□ *Fluoxetine (FLX), a selective serotonin reuptake inhibitor used as an antidepressant agent, is mainly* N*-demethylated to norfluoxetine (NFLX)* in vivo. *In this study, FLX and NFLX levels in human serum and urine (each 100 μL) were simultaneously analyzed by isocratic HPLC on a C_{30} column with visible light detection at 450 nm after pre-column derivatization with 4-(dimethylamino)azobenzene-4′-sulfonyl chloride. After basic extraction of the samples into pentane, derivatization with 4-(dimethylamino)azobenzene-4′-sulfonyl chloride was conducted in borate buffer (pH 8.0) at 70° C and 3 min. Protriptyline was utilized as an internal standard. Regression equations for FLX hydrochloride and NFLX hydrochloride in human serum both showed good linearity in the range of 0.02–0.5 μg/mL with the detection limit of 0.01 μg/mL. The corresponding values for FLX hydrochloride and NFLX hydrochloride in human urine were 0.05–0.5 μg/mL with the detection limit of 0.03 μg/mL, and 0.02–0.5 μg/mL with the detection limit of 0.01 μg/mL, respectively. The coefficients of variation were less than 14.1% with good recovery. This method is expected to be useful for simple and sensitive routine determination of FLX and NFLX in human serum and urine.*

Keywords 4-(dimethylamino)azobenzene-4′-sulfonyl chloride, C_{30} column, fluoxetine, HPLC-visible light detection, norfluoxetine

INTRODUCTION

Fluoxetine (FLX), (±)-*N*-methyl-γ-[4-(trifluoromethyl)phenoxy]benzene propanamine, is a highly selective serotonin reuptake inhibitor[1,2] and has become the most commonly prescribed antidepressant drug worldwide since its approval in 1987.[3] *In vivo*, FLX predominantly undergoes *N*-demethylation to norfluoxetine (NFLX), which has similar activity to FLX.[1,4,5] Both FLX and NFLX have long elimination half-lives, ranging

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from 1 to 6 days and from 5 to 6 days, respectively; about 11% of the dose is excreted as unchanged FLX and about 7% as NFLX.[6,7] Many analytical procedures have been described for the simultaneous determination of FLX and NFLX in human serum and urine.

Almost all recent assays are based on either high-performance liquid chromatography (HPLC) or gas chromatography (GC). Most of the methods involve HPLC using fluorescence[8–11] or mass spectrometric detectors.[12] GC methods have also been developed using ECD,[13] NPD,[14] and MS.[15] However, these methods either need rather large sample volumes and a long derivatization time or require specialist equipment and expensive reagents. Furthermore, instruments for routine determination of psychotropic drugs usually employ an ultraviolet detector.[16–23]

The aim of this study was to develop a simple, sensitive, and economical assay of FLX and NFLX that would be suitable for pharmacokinetic studies, therapeutic drug monitoring, and so forth. We succeeded in developing an isocratic HPLC assay of FLX and NFLX in human serum and urine (each 100 μL) with visible light detection after pre-column derivatization with 4-(dimethylamino)azobenzene-4′-sulfonyl chloride (Dabsyl-Cl), which is generally used to form derivatives of compounds containing primary and secondary amine groups.[24–26] Protriptyline was chosen as an inexpensive internal standard (IS). The reaction scheme is presented in Figure 1.

EXPERIMENTAL

Reagents

Dabsyl-Cl, FLX hydrochloride, NFLX hydrochloride, protriptyline hydrochloride, amoxapine, maprotiline hydrochloride, nortriptyline hydrochloride, human serum, and acetonitrile were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Human urine in the form of freeze-dried powder (normal, quantitative urine control) was obtained from Bio-Rad Laboratories (Irvine, CA, U.S.A). Fluvoxamine maleate, paroxetine hydrochloride, and general reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Equipment

The HPLC system comprised a Model LC-10AT*vp* pump (Shimadzu, Kyoto, Japan), a Rheodyne valve (Cotati, CA, U.S.A.) with a 100 μL loop, and a model SPD-10A*vp* UV/Vis detector (Shimadzu) operating at 450 nm. A C_{30} column (Develosil C30-UG-3, Nomura Chemical Co., Aichi, Japan) of 150 × 3.0 mm i.d., containing 5 μm particles, was used.

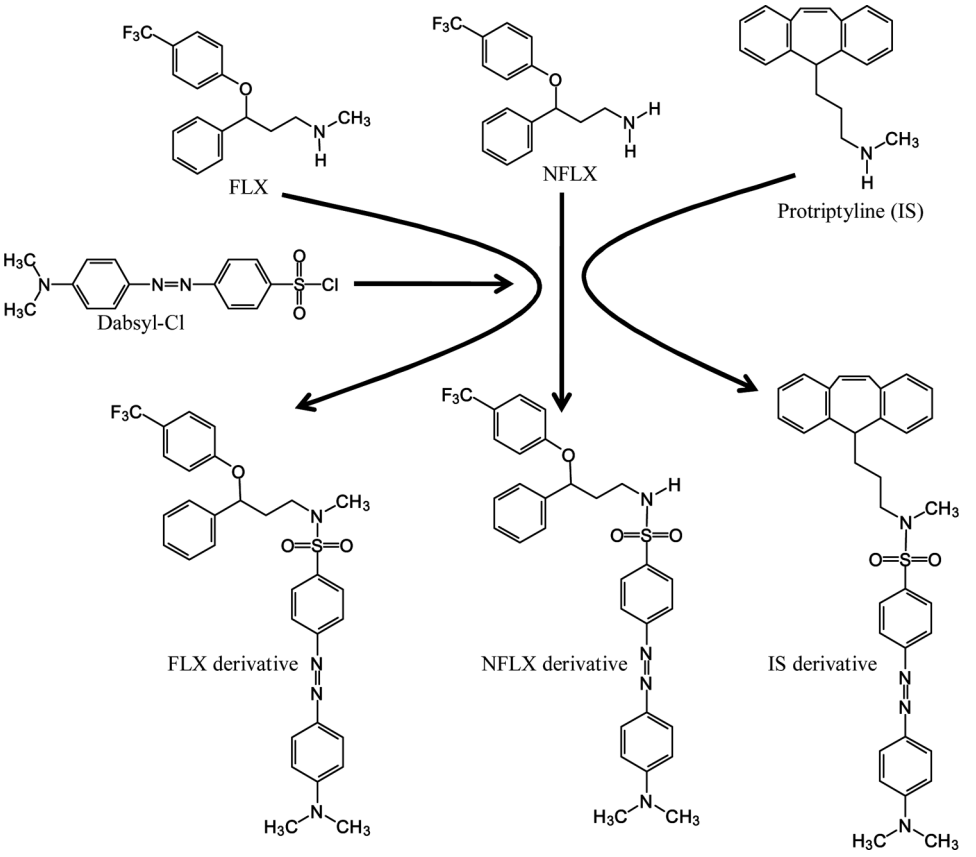

FIGURE 1 Reaction scheme of FLX, NFLX, and IS with Dabsyl-Cl.

Quantification of the peaks was performed using a Chromatopac Model C-R8A integrator (Shimadzu). The mobile phase was prepared by the addition of acetonitrile (740 mL) to a solution of trifluoroacetic acid (0.1 v/v%) in 260 mL of water. The samples were eluted from the column at room temperature, at a flow rate of 0.64 mL/min.

Extraction from Human Serum and Urine

An aliquot of 100 μL of sample was rendered alkaline by the addition of NaOH (1 M, 100 μL). Protriptyline hydrochloride solution in water (0.3 μg/mL, 100 μL) was added as an IS to prepare standard curves. The mixture was agitated for 1 min and extracted with pentane (3 mL, twice). The pentane phases were combined and evaporated, and derivatization was performed as follows.

Derivatization

Borate buffer (0.1 M) was adjusted to pH 8.0 by the addition of NaOH (1 M). Borate buffer (300 μL) was added to the extract. Dabsyl-Cl solution in acetonitrile (0.3 mg/mL, 100 μL) was added and the mixture was agitated, and then allowed to stand for 3 min at 70°C. Next, it was set on ice for 1 min, and an aliquot (100 μL) was injected into the HPLC system.

Calibration Curves

Various amounts of a solution of FLX hydrochloride and NFLX hydrochloride (1 mg/mL) in water were added to human biological fluids to give FLX hydrochloride and NFLX hydrochloride concentrations of 0, 0.02, 0.05, 0.1, 0.2, and 0.5 μg/mL. All samples were extracted and analyzed using the procedures described previously. Calibration curves based on the peak area ratios of FLX hydrochloride and NFLX hydrochloride to the IS were constructed.

RESULTS AND DISCUSSION

Reaction Time Course and pH Dependency

For the time course study, the reaction time was set at 3, 5, 10, 15, or 20 min. A sample (100 μL) of FLX hydrochloride and NFLX hydrochloride (each 0.5 μg/mL) mixed solution in water was added to borate buffer (0.1 M, 300 μL, pH 8.0), and derivatized with Dabsyl-Cl (0.3 mg/mL in acetonitrile, 100 μL). The derivatization reaction reached a plateau at 3 min, and, thereafter, the peak areas of the derivatives tended to decrease (Figure 2).

Next, pH dependency (pH 7.5 to 9.5) was examined at the derivatization time of 3 min. Peak areas of all derivatives were found at a maximum at pH 8.0. Thus, the derivatization time of 3 min at pH 8.0 was selected (Figure 3).

Chromatogram

Figure 4 shows typical chromatograms obtained from (A-1) drug-free serum; (A-2) serum spiked with FLX hydrochloride (0.5 μg/mL), NFLX hydrochloride (0.5 μg/mL), and IS (0.3 μg/mL); (B-1) drug-free urine; and (B-2) urine spiked with FLX hydrochloride (0.5 μg/mL), NFLX hydrochloride (0.5 μg/mL), and IS (0.3 μg/mL). The retention times of NFLX,

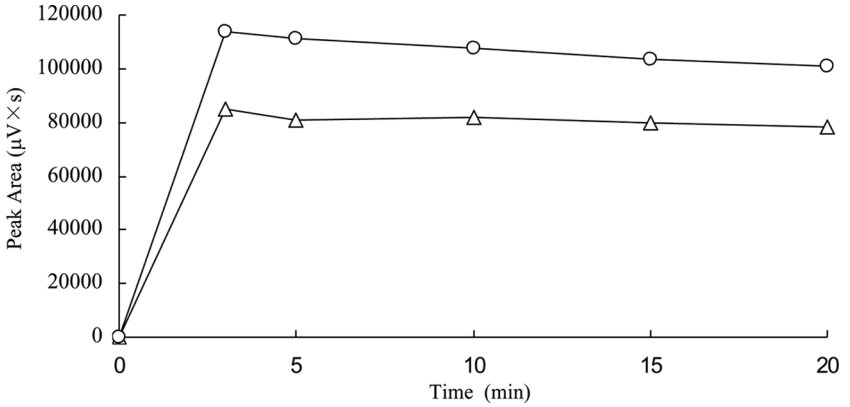

FIGURE 2 Time courses of the formation of FLX (○) and NFLX (△) derivatives with Dabsyl-Cl. Standard samples (each 0.5 μg/mL) were allowed to react with Dabsyl-Cl in borate buffer at pH 8.0. (○), Dabsyl-FLX ; (△), Dabsyl-NFLX.

FLX, and IS derivatives were 14.4, 25.8, and 29.4 min, respectively. The running time was 33 min.

Comparison of C_{30} Column with Other Columns

FLX hydrochloride and NFLX hydrochloride in water (each 0.5 μg/mL) were derivatized with Dabsyl-Cl and analyzed on C_4-MS, C_8-MS, C_{18}-MS-II, C_{22}-AR-II, and Cholester columns (Nacalai Tesque, Kyoto,

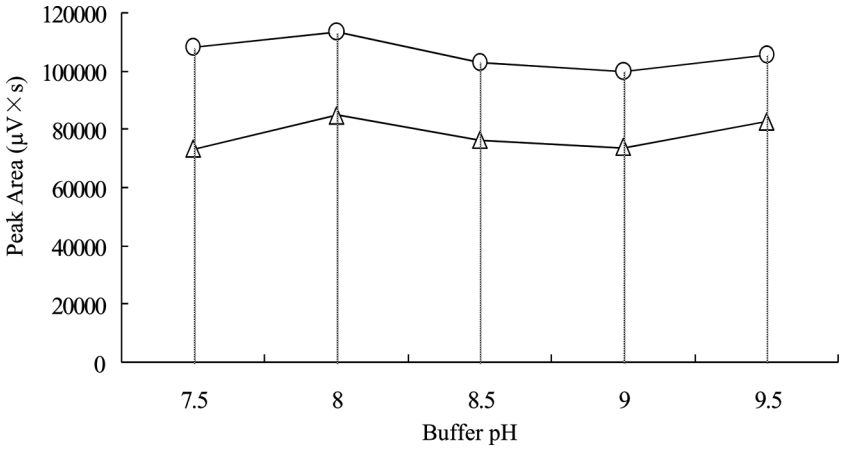

FIGURE 3 pH Dependency of the formation of FLX and NFLX derivatives with Dabsyl-Cl. Standard samples (each 0.5 μg/mL) were allowed to react with Dabsyl-Cl for 3 min in various borate buffers. (○), Dabsyl-FLX; (△), Dabsyl-NFLX.

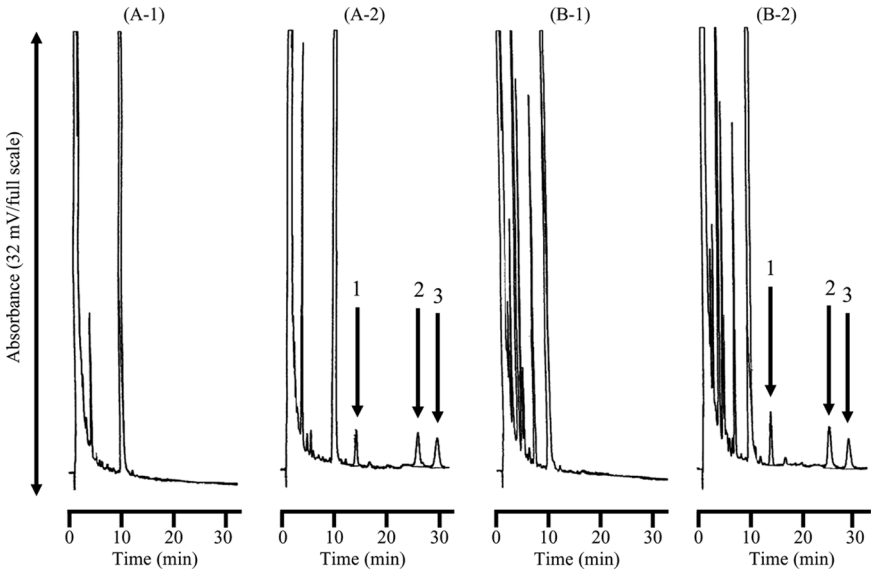

FIGURE 4 Typical chromatograms of FLX, NFLX, and IS derivatives with Dabsyl-Cl in human serum (A) and urine (B) using a C_{30} column. (A-1) and (B-1), Blank; (A-2) and (B-2), Samples spiked with FLX hydrochloride (0.5 μg/mL), NFLX hydrochloride (0.5 μg/mL), and IS (0.3 μg/mL). Peaks: 1 = NFLX derivative, 2 = FLX derivative, and 3 = IS derivative.

Japan) using the same conditions as described previously for the C_{30} column (data not shown). Our preliminary studies indicated that all these columns except C_{30} showed a blank large peak at about 10 min, overlapping the NFLX derivative peak. Only C_{30} showed no interference with the peaks of interest. Therefore, we adopted the C_{30} column for simultaneous determination of FLX and NFLX.

Standard Curves and Detection Limits of FLX and NFLX

Standard curves of FLX and NFLX were constructed by plotting integrated peak area ratios of FLX hydrochloride and NFLX hydrochloride to IS vs. FLX hydrochloride and NFLX hydrochloride concentration (Table 1). In human serum samples, linear relationships were obtained for FLX hydrochloride ($y = 2.035x + 0.0106$, concentrations ranging from 0.02 to 0.5 μg/mL, $r = 0.9979$) and NFLX hydrochloride ($y = 1.979x + 0.0099$, concentrations ranging from 0.02 to 0.5 μg/mL, $r = 0.9981$). The lower limits of detection for FLX hydrochloride and NFLX hydrochloride were 0.01 μg/mL (0.029 μM, 0.0089 μg/mL as FLX, and 0.030 μM, 0.0089 μg/mL as NFLX) (signal-to-noise ratio of 3:1). In human urine samples, linear relationships were obtained for FLX hydrochloride ($y = 2.251x + 0.0337$, concentrations ranging from 0.05 to 0.5 μg/mL,

TABLE 1 Linear Correlation Parameters of FLX Hydrochloride and NFLX Hydrochloride

	Slope	Intercept	Concentration Range	*r*
Serum				
FLX hydrochloride	2.035	+0.0106	0.02 to 0.5 μg/mL	0.9979
NFLX hydrochloride	1.979	+0.0099	0.02 to 0.5 μg/mL	0.9981
Urine				
FLX hydrochloride	2.251	+0.0337	0.05 to 0.5 μg/mL	0.9992
NFLX hydrochloride	1.820	+0.0096	0.02 to 0.5 μg/mL	0.9989

$r = 0.9992$) and NFLX hydrochloride ($y = 1.820x + 0.0096$, concentrations ranging from 0.02 to 0.5 μg/mL, $r = 0.9989$). The lower limits of detection for FLX hydrochloride and NFLX hydrochloride were 0.03 μg/mL (0.087 μM, 0.027 μg/mL as FLX), 0.01 μg/mL (0.030 μM, 0.0089 μg/mL as NFLX), respectively, (signal-to-noise ratio of 3:1).

We previously developed an HPLC method utilizing fluorescence detection of FLX and NFLX after pre-column derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole[10] as a fluorescence labeling agent, and we also reported separation of (*R,S*)-FLX enantiomers following the formation of diastereomer derivatives with (*R*)-(+)-4-nitro-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole.[11] Regarding sensitivity of FLX determination in human serum, the present method is half as sensitive as those methods, while the sensitivity of FLX determination in human urine is one-third of that in one reference,[11] but twice that in another reference.[10] As for the sensitivity of NFLX determination in human serum, the present method is one-fifth as sensitive as that in one reference,[10] while for human urine, it is four times that in the same reference.[10] The time course of FLX and NFLX levels in urine in a volunteer orally given 20 mg of FLX ranged from 0.01 to 0.12 μg/mL.[27] In a volunteer chronically treated with Prozac Weekly® (90 mg week^{-1}), an enteric-coated FLX hydrochloride that delays release into the bloodstream,[6] first morning urine samples were taken for 7 days following administration, and the concentrations of FLX and NFLX were in the ranges of 0.52 to 1.42 μg/mL and 0.45 to 1.92 μg/mL, respectively. Therefore, our method may be particularly useful for kinetic studies in human urine.

Precision and Accuracy

Precision and accuracy for intra-day and inter-day assays of FLX and NFLX derivatives are shown in Tables 2 and 3. For the intra- and inter-day assays using human serum samples, the range of standard deviation for FLX hydrochloride and NFLX hydrochloride was within 4.2% to 9.1%. The recoveries of FLX hydrochloride and NFLX hydrochloride were within

TABLE 2 Intra- and Inter-Day Assay Reproducibility for Determination of FLX Hydrochloride and NFLX Hydrochloride in Human Serum

Concentration (μg/mL)	Measured (μg/mL) (Mean ± S.D., $n = 4$)	C.V. (%)	Recovery (%)
Intra-day assay			
FLX hydrochloride			
0.02	0.0204 ± 0.0011	5.4	102.0
0.05	0.0506 ± 0.0025	4.9	101.2
0.5	0.499 ± 0.021	4.2	99.8
NFLX hydrochloride			
0.02	0.0195 ± 0.00094	4.8	97.5
0.05	0.0492 ± 0.0029	5.9	98.4
0.5	0.504 ± 0.035	6.9	100.8
Inter-day assay			
FLX hydrochloride			
0.02	0.0196 ± 0.00131	6.7	98.0
0.05	0.0501 ± 0.0027	5.4	100.2
0.5	0.493 ± 0.021	4.3	98.6
NFLX hydrochloride			
0.02	0.0188 ± 0.00103	5.5	94.0
0.05	0.0509 ± 0.0045	8.8	101.8
0.5	0.495 ± 0.045	9.1	99.0

94.0% to 102.0%. For the intra- and inter-day assays using human urine samples, the range of standard deviation for FLX hydrochloride and NFLX hydrochloride was within 6.2% to 14.1%. The recoveries of FLX hydrochloride and NFLX hydrochloride were within 99.8% to 110.0%. The assay exhibited satisfactory precision and accuracy.

TABLE 3 Intra- and Inter-Day Assay Reproducibility for Determination of FLX Hydrochloride and NFLX Hydrochloride in Human Urine

Concentration (μg/mL)	Measured (μg/mL) (Mean ± S.D., $n = 4$)	C.V. (%)	Recovery (%)
Intra-day assay			
FLX hydrochloride			
0.05	0.0523 ± 0.0034	6.5	104.6
0.5	0.502 ± 0.031	6.2	100.4
NFLX hydrochloride			
0.02	0.0210 ± 0.0017	8.1	105.0
0.05	0.0511 ± 0.0047	9.2	102.2
0.5	0.499 ± 0.039	7.8	99.8
Inter-day assay			
FLX hydrochloride			
0.05	0.0542 ± 0.0039	7.2	108.4
0.5	0.502 ± 0.038	7.6	100.4
NFLX hydrochloride			
0.02	0.0220 ± 0.0024	10.9	110.0
0.05	0.0545 ± 0.0077	14.1	109.0
0.5	0.502 ± 0.051	10.2	100.4

TABLE 4 Interference by Other Antidepressants

Derivatives	Relative Retention Time
Amoxapine	0.24
Fluvoxamine	0.48
NFLX	0.49
Paroxetine	0.71
FLX	0.87
IS	1.00
Nortriptyline	1.18
Maprotiline	1.24

Interference

As shown in Table 4, the retention times of other antidepressant derivatives were investigated. The relative retention times of amoxapine, fluvoxamine, paroxetine, nortriptyline, and maprotiline derivatives were 0.24, 0.48, 0.71, 1.18, and 1.24, respectively. The peak of the fluvoxamine derivative almost wholly overlapped the NFLX derivative peak.

CONCLUSION

The present method was developed to provide a rapid, simple, easy-to-perform, and inexpensive procedure for simultaneous quantification of FLX and NFLX in very small volume (100 μL) samples from human beings. The use of a C_{30} column obviated interference with the peaks of interest. This method provides lower limits of detection for FLX and NFLX in human urine compared with our previously reported method.[10] Validation data (precision, accuracy, and recovery) were satisfactory for simultaneous FLX and NFLX determination. Among related compounds examined, the fluvoxamine derivative interfered with the NFLX derivative peak. The established method is expected to be particularly suitable for routine therapeutic drug monitoring, especially analysis of urinary excretion, of FLX and NFLX during treatment for depression in patients not receiving fluvoxamine.

REFERENCES

1. Schmidt, M. J.; Fuller, R. W.; Wong, D. T. Fluoxetine, a Highly Selective Serotonin Reuptake Inhibitor: A Review of Preclinical Studies. *Br. J. Psychiatry Suppl.* **1988**, *3*, 40–46.
2. Ciraulo, D. A.; Shader, R. I. Fluoxetine Drug-Drug Interactions. *J. Clin Psycho-Pharmacol.* **1990**, *10*, 213–217.
3. Wong, D. T.; Bymaster, F. P.; Engleman, E. A. Prozac (Fluoxetine, Lilly110140), the First Selective Serotonin Uptake Inhibitor and an Antidepressant Drug: Twenty Years Since Its First Publication. *Life Sci.* **1995**, *57*, 411–441.

4. Wong, D. T.; Bymaster, F. P.; Reid, L. R.; Mayle, D. A.; Krushinski, J. H.; Robertson, D. W. Norfluoxetine Enantiomers as Inhibitors of Serotonin Uptake in Rat Brain. *Neuropsychopharmacology* **1993**, *8*, 337–344.
5. Baumann, P. Pharmacokinetic-Pharmacodynamic Relationship of the Selective Serotonin Reuptake Inhibitors. *Clin. Pharmacokinet.* **1996**, *31*, 444–469.
6. Unceta, N.; Gómez-Caballero, A.; Sánchez, A.; Millán, S.; Sampedro, M. C.; Goicolea, M. A.; Sallés, J.; Barrio, R. J. Simultaneous Determination of Citalopram, Fluoxetine and Their Main Metabolites in Human Urine Samples by Solid-Phase Microextraction Coupled with High-Performance Liquid Chromatography. *J. Pharm. Biomed. Anal.* **2008**, *46*, 763–770.
7. Altamura, A. C.; Moro, A. R.; Percudani, M. Clinical Pharmacokinetics of Fluoxetine. *Clin. Pharmacokinet.* **1994**, *26*, 201–214.
8. Suckow, R. F.; Zhang, M. F.; Cooper, T. B. Sensitive and Selective Liquid-Chromatographic Assay of Fluoxetine and Norfluoxetine in Plasma with Fluorescence Detection After Precolumn Derivatization. *Clin. Chem.* **1992**, *38*, 1756–1761.
9. de Freitas, D. F.; Porto, C. E.; Vieira, E. P.; de Siqueira, M. E. Three-Phase, Liquid-Phase Microextraction Combined with High Performance Liquid Chromatography-Fluorescence Detection for the Simultaneous Determination of Fluoxetine and Norfluoxetine in Human Plasma. *J. Pharm. Biomed. Anal.* **2009**, *51*, 170–177.
10. Higashi, Y.; Gao, R.; Fujii, Y. Determination of Fluoxetine and Norfluoxetine in Human Serum and Urine by HPLC Using a Cholester Column with Fluorescence Detection. *J. Liq. Chromatogr. Rel. Technol.* **2009**, *32*, 1141–1151.
11. Higashi, Y.; Gao, R.; Fujii, Y. Determination of Fluoxetine Enantiomers in Human Serum and Urine by Isocratic HPLC and Pre-Column Fluorescence Derivatization with (*R*)-(+)-4-nitro-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole. *J. Liq. Chromatogr. Rel. Technol.* **2010**, *33*, 481–490.
12. Juan, H.; Zhiling, Z.; Huande, L. Simultaneous Determination of Fluoxetine, Citalopram, Paroxetine, Venlafaxine in Plasma by High Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry (HPLC-MS/ESI). *J. Chromatogr. B* **2005**, *820*, 33–39.
13. Lantz, R. J.; Farid, K. Z.; Koons, J.; Tenbarge, J. B.; Bopp, R. J. Determination of Fluoxetine and Norfluoxetine In Human Plasma by Capillary Gas Chromatography with Electron-Capture Detection. *J. Chromatogr.* **1993**, *614*, 175–179.
14. Ulrich, S. Direct Stereoselective Assay of Fluoxetine and Norfluoxetine Enantiomers in Human Plasma or Serum by Two-Dimensional Gas-Liquid Chromatography with Nitrogen-Phosphorus Selective Detection. *J. Chromatogr. B* **2003**, *783*, 481–90.
15. Crifasi, J. A.; Le, N. X.; Long, C. Simultaneous Identification and Quantitation of Fluoxetine and Its Metabolite, Norfluoxetine, in Biological Samples by GC-MS. *J. Anal. Toxicol.* **1997**, *21*, 415–419.
16. Li, K. M.; Thompson, M. R.; McGregor, I. S. Rapid Quantitation of Fluoxetine and Norfluoxetine in Serum by Micro-Disc Solid-Phase Extraction with High-Performance Liquid Chromatography-Ultraviolet Absorbance Detection. *J. Chromatogr. B* **2004**, *804*, 319–326.
17. Llerena, A.; Dorado, P.; Berecz, R.; González, A.; Jesús Norberto, M.; de la Rubia, A.; Cáceres, M. Determination of Fluoxetine and Norfluoxetine in Human Plasma by High-Performance Liquid Chromatography with Ultraviolet Detection in Psychiatric Patients. *J. Chromatogr. B* **2003**, *783*, 25–31.
18. Qrsulak, P. J.; Kenney, J. T.; Debus, J. R.; Crowley, G.; Wittman, P. D. Determination of the Antidepressant Fluoxetine and Its Metabolite Norfluoxetine in Serum by Reversed-Phase HPLC, with Ultraviolet Detection. *Chin. Chem.* **1988**, *34*, 1875–1878.
19. Alvarez, J. C.; Bothua, D.; Collignon, I.; Advenier, C.; Spreux-Varoquaux, O. Determination of Fluoxetine and Its Metabolite Norfluoxetine in Serum and Brain Areas Using High-Performance Liquid Chromatography with Ultraviolet Detection. *J. Chromatogr. B* **1998**, *707*, 175–180.
20. Maya, M. T.; Domingos, C. R.; Guerreiro, M. T.; Morais, J. A. Determination of the Antidepressant Fluoxetine in Human Plasma by LC with UV Detection. *J. Pharm. Biomed. Anal.* **2000**, *23*, 989–996.
21. Binsumait, I. A.; Hadidi, K. A.; Raghib, S. A. Stability of Fluoxetine in Stored Plasma, Aqueous, and Methanolic Solutions Determined by HPLC with UV Detection. *Pharmazie* **2001**, *56*, 311–313.
22. Tournel, G.; Houdret, N.; Hedouin, V.; Deveau, M.; Gosset, D.; Lhermitte, M. High-Performance Liquid Chromatographic Method to Screen and Quantitate Seven Selective Serotonin Reuptake Inhibitors in Human Serum. *J. Chromatogr. B* **2001**, *761*, 147–158.

23. Cruz-Vera, M.; Lucena, R.; Cárdenas, S.; Valcárcel, M. Combined Use of Carbon Nanotubes and Ionic Liquid to Improve the Determination of Antidepressants in Urine Samples by Liquid Chromatography. *Anal. Bioanal. Chem.* **2008**, *391*, 1139–1145.
24. Stocchi, V.; Piccoli, G.; Magnani, M.; Palma, F.; Biagiarelli, B.; Cucchiarini, L. Reversed-Phase High-Performance Liquid Chromatography Separation of Dimethylaminoazobenzene Sulfonyl- and Dimethylaminoazobenzene Thiohydantoin-Amino Acid Derivatives for Amino Acid Analysis and Microsequencing Studies at the Picomole Level. *Anal. Biochem.* **1989**, *178*, 107–117.
25. Doorn, L.; Jansen, E. H.; Van Leeuwen, F. X. Comparison of High-Performance Liquid Chromatographic Detection Methods for Thyronine and Tyrosine Residues in Toxicological Studies of the Thyroid. *J. Chromatogr.* **1991**, *553*, 135–142.
26. Knecht, R.; Chang, J. Y. Liquid Chromatographic Determination of Amino Acids After Gas-Phase Hydrolysis and Derivatization with (Dimethylamino)Azobenzenesulfonyl Chloride. *Anal. Chem.* **1986**, *58*, 2375–2379.
27. Flores, J. R.; Nevado, J. J.; Peñalvo, G. C.; Diez, N. M. Development and Validation Method for Determination of Fluoxetine and Its Main Metabolite Norfluoxetine by Nonaqueous Capillary Electrophoresis in Human Urine. *Talanta* **2005**, *65*, 163–171.