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Determination of fluvoxamine and its metabolite fluvoxamino acid by liquid–liquid extraction and column-switching high-performance liquid chromatography

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Abstract

This study describes a new simultaneous determination of fluvoxamine and fluvoxamino acid by automated column-switching high-performance liquid chromatography. The test compounds were extracted from 1.5 ml of plasma using chloroform–toluene (15:85, v/v), and the extract was injected into a hydrophilic metaacrylate polymer column for clean-up and a C18 analytical column for separation. The mobile phase for separation consisted of phosphate buffer (0.02 M, pH 4.6), acetonitrile and perchloric acid (60%) (62.4:37.5:0.1, v/v/v) and was delivered at a flow rate of 0.6 ml/min. The peak was detected using a UV detector set at 254 nm. The method was validated for the concentration range 0.8–153.6 ng/ml for fluvoxamine and 0.6–115.2 ng/ml for fluvoxamino acid, and their good linearity ($r > 0.998$) were confirmed. Intra-day coefficient variations (CVs) for fluvoxamine and fluvoxamino acid were less than 6.6 and 6.0%, respectively. Inter-day CVs for corresponding compounds were 6.3 and 6.5%, respectively. Relative errors ranged from −18 to 9% and mean recoveries were 96–100%. The limit of quantification was 1.2 and 0.9 ng/ml for fluvoxamine and fluvoxamino acid, respectively. This method shows successful application for pharmacokinetic studies and therapeutic drug monitoring.

Keywords: Fluvoxamine; Fluvoxamino acid; HPLC; Column-switching

1. Introduction

Fluvoxamine, a selective serotonin (5-HT) reuptake inhibitor, shows similar therapeutic efficacy to tricyclic antidepressants, but fewer anticholinergic and cardiovascular side effects [1]. Nausea is the most common side effect of fluvoxamine [1]. Fluvoxamine undergoes extensive metabolism, and the major metabolite in human urine is fluvoxamino acid [2]. A clinical trial showed that the steady-state plasma concentration of fluvoxamino acid was correlated with the therapeutic outcome [3]. It appears that fluvoxamino acid as well as fluvoxamine contributes to the antidepressant effect during fluvoxamine treatment, and the combined steady-state plasma concentration correlates well with the therapeutic outcome. On the other hand, the fluvoxamino acid/fluvoxamine ratio was significantly different between CYP2D6 genotypes [4]. From not only a clinical point of view but also a pharmacokinetic point of view, therefore, monitoring of both fluvoxamine and fluvoxamino acid is clinically relevant.

Several HPLC methods with ultraviolet detection for the determination of fluvoxamine concentration have been widely reported [5–18]. Although simultaneous determination of antidepressants including fluvoxamine has been reported [16], potential active metabolite has not been included in these studies. Of these reports, only a report by Ohkubo et al. [16] described determination of steady-state plasma concentration of fluvoxamine and fluvoxamino acid in de-

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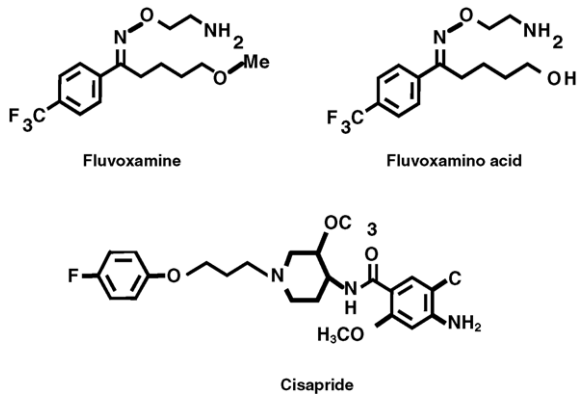

Fig. 1. Chemical structure analogues of fluvoxamine, fluvoxamino acid and cisapride.

pressed patients. However, because preclinical pharmacokinetic data showed C_{max} of 10–15 ng/ml after a single oral dose (50 mg) of fluvoxamine [19], the quantification limits (10 ng/ml) of both compounds in their report [16] are too high to obtain pharmacokinetic parameters of these compounds. In the present study, therefore, we describe an automated column-switching HPLC with ultraviolet detection for determination of fluvoxamine and fluvoxamino acid in plasma using a simple liquid–liquid extraction. The sensitive system allows measurement of plasma concentrations of fluvoxamine and fluvoxamino acid up to 36 and 12 h, respectively, after an oral administration of 50 mg fluvoxamine in pharmacokinetic study.

2. Experimental

2.1. Chemicals

Fluvoxamine and fluvoxamino acid (Fig. 1) were synthesized by Wako Pure Chemical Industries (Osaka, Japan). The structures of these compounds were confirmed using NMR spectra and FAB–MS. The purity of these materials was more than 99.5%. Cisapride as internal standard (IS) (Fig. 1) was kindly provided by Yoshitomi Pharmaceutical (Osaka, Japan). Potassium phosphate monobasic, acetonitrile, perchloric acid, toluene and chloroform were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Drug solutions

Stock solutions of fluvoxamine, fluvoxamino acid and IS for generating standard curves were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 76.8, 57.6 and 1000 μg/ml, respectively. High-concentration working standard solutions of fluvoxamine (768 ng/ml), fluvoxamino acid (576 ng/ml) and IS (10 μg/ml) were obtained by 100 times dilution of each stock solution with purified water. Low-concentration working standard solution of fluvoxamine (96 ng/ml) and fluvoxamino acid (72 ng/ml) were obtained by further diluting the high-concentration working standard solution eight times with purified water. Stock solutions were stable at 4 °C for at least 3 months. Drug-free plasma from healthy donors was used for validation studies. Calibration curves were prepared by spiking 12.5, 25, 50 μl of low-concentration working standard solutions and 12.5, 25, 50, 100, 150, 200, 300 μl of high-concentration working standard solutions in 1.5 ml of blank plasma (final volume) to yield the following final concentrations: 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, 76.8, 102.4 and 153.6 ng/ml for fluvoxamine and 0.6, 1.2, 2.4, 4.8, 9.6, 19.2, 38.4, 57.6, 76.8 and 115.2 ng/ml for fluvoxamino acid. Standard curves were prepared daily and constructed by linear regression analysis of the compounds/internal standard peak-height ratio versus the respective concentration of fluvoxamine and fluvoxamino acid. Stock solution of each compound was separately prepared for quality controls in the same manner as for standard curves. Working plasma solutions were obtained by dilution of new stock solutions 1000 times (360 μg/ml for fluvoxamine and 270 μg/ml for fluvoxamino acid) with blank plasma to yield 360 ng/ml for fluvoxamine and 270 ng/ml for fluvoxamino acid. Quality control samples were obtained by spiking 5–400 μl of working plasma solutions in 1.5 ml of blank plasma (final volume) to yield the final concentrations range of 1.2, 24 and 96 ng/ml for fluvoxamine and 0.9, 18 and 72 ng/ml for fluvoxamino acid, and kept at −20 °C until analysis. All standard curves were checked using these quality control samples.

2.3. Sample collections

A tablet containing 50 mg of fluvoxamine (Luvox®, Fujisawa Pharmaceutical, Osaka, Japan) was orally administered to each of 10 healthy volunteers. Their mean (range) age was 31 years (25–36 years) and body weight was 64 kg (54–85 kg). Blood samples were obtained before and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36 and 48 h after the dosing. Blood samples were collected in heparinized tubes and centrifuged immediately at 2500 × *g* for 10 min. The plasma was stored at −20 °C until analysis. The study protocol was approved by Ethical Committee of Hirosaki University School of Medicine and Ethical Committee of Yamagata University School of Medicine and a written informed consent was given from each subject.

2.4. Apparatus

The column-switching HPLC system consisted of two Shimadzu LC-10AT high-pressure pumps (for eluent A and B), a Shimadzu CTO-10A column oven and a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), a Shimadzu SPD-10AVP (Kyoto, Japan), a Tosoh multiple autovalve PT-8000, and a Tosoh autosampler AS-8020 (500 μL injection volume) (Tokyo, Japan). A TSK gel

PW precolumn (a hydrophilic metaacrylate polymer column) for sample clean-up (column I; 35 mm × 4.6 mm ID, particle size 10 μm; Tosoh, Tokyo, Japan) and a C18 STR ODS-II column as an analytical column (column II; 150 mm × 4.6 mm ID, particle size 5 μm; Shinwa Chemical Industry, Kyoto, Japan) were used.

2.5. Extraction procedure

IS (cisapride) 80 μL of 10 μg/ml, 1.0 ml NaOH (0.25 M) were added to 1.5 ml of plasma. The tubes were vortex-mixed for 10 s and 5 ml of toluene–chloroform (85:15, v/v) was added as extraction solvent. After 10 min of shaking, the mixture was centrifuged at 1700 × *g* for 10 min at 4 °C, and the organic phase was evaporated in vacuo at 45 °C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved with 750 μL of eluent A and used as an extract.

2.6. Chromatographic condition

Column-switching chromatographic condition was set based on our previous report [20]. A 0.5 ml portion of the extract was automatically injected into the HPLC system. Column-switching time schedule was set based on retention time of fluvoxamino acid in column I (about 12 min), retention time of achievement of maximal recovery of fluvoxamine from column I to column II (about 17 min) and retention time of appearance of interference peaks (about 20 min). From 0 to 11.5 min after the sample injection, fluvoxamine, fluvoxamino acid and IS were separated from the interfering substances present in the extract on column I with a mobile phase (eluent A) of phosphate buffer (0.02 M, pH 4.6), acetonitrile and perchloric acid (60%) (92.4:7.5:0.1, v/v/v). Between 11.5 and 18.0 min after the injection, two analytes and IS retained on column I were eluted with a mobile phase (eluent B) of phosphate buffer (0.02 M, pH 4.6), acetonitrile and perchloric acid (60%) (62.4:37.5:0.1, v/v/v), and effluent from column I was switched to column II. Then fluvoxamine and fluvoxamino acid were separated on column II by eluting with eluent B (between 18.0 and 35.0 min). The flow rates of eluents A and B were 1.2 and 0.6 mL/min, respectively. The temperatures of column I and II were about 25 (room temperatures) and 30 °C, respectively. The peak was detected using a UV detector set at 254 nm. The peak area was used for the quantification of these two compounds.

3. Results and discussion

3.1. Chromatography

Effects of constitution ratio of perchloric acid (0, 0.1, 0.25 and 1.0%) in mobile phase on peak separation of fluvoxamine, fluvoxamino acid and IS were investigated, and 0.1% perchloric acid obtained optimal separation of these compounds.

Table 1
Extraction recovery of analytes from plasma (n =6)

Analyte	Concentration added (ng/mL)	Recovery (%)	CV (%)
Fluvoxamine	1.2	94.8	0.77
	24.0	94.8	0.66
	96.0	94.9	0.71
Fluvoxamino acid	0.9	96.4	0.91
	18.0	96.7	0.90
	72.0	96.4	0.64

A mobile phase with the same pH was adjusted with phospholic acid as with 0.1% perchloric acid did not lead to separation of any these compounds, suggesting a specific effect of perchloric acid but not pH on this method. The chromatogram of an extracted blank plasma sample is shown in Fig. 2A. A representative chromatogram of an extracted blank plasma sample spiked with working aqueous solution containing fluvoxamine and fluvoxamino acid and cisapride (internal standard) is shown in Fig. 2B (2.4 and 1.6 ng/ml, respectively) and C (96 and 72 ng/ml, respectively). The chromatograms of extracted plasma samples obtained from a patient receiving fluvoxamine 100 mg/day did not show interference peaks (Fig. 2D). All compounds were well separated from each other and from the front of the solvent peaks. Plasma concentrations were 41.7 ng/ml for fluvoxamine and 44.8 ng/ml for fluvoxamino acid.

3.2. Recovery and linearity

Absolute recovery from plasma was calculated by comparing the peak areas of pure standards prepared in purified water, and injected directly into the analytical column with those of extracted plasma samples containing the same amount of the test compound (n = 6 each). Recoveries and their CV values were determined at three different concentrations ranging from 1.2 to 96 ng/ml for fluvoxamine and from 0.9 to 72 ng/ml for fluvoxamino acid. Recoveries and their CV values were 94–96% with less than 1% for both compounds (Table 1). Calibration curves were linear over the concentrations range from 0.8 to 153.6 ng/ml (r > 0.998) for fluvoxamine and from 0.6 to 115.2 ng/ml for fluvoxamino acid (r > 0.999).

3.3. Sensitivity

Chloroform, toluene, *n*-hexane, heptane, isopropylether, diethylether and ethyl acetate and their combination were investigated. The combination of toluene–chloroform (85:15, v/v) was the highest sensitivity among these organic solvents because of flat baseline. The limit of detection was defined as analyte responses that were at least three times the response compared to blank response. The limits of detection of fluvoxamine and fluvoxamino acid were 0.4 and 0.3 ng/ml, respectively. The lowest standard on the calibration curve was defined as the limit of quantification as analyte peaks by which both compounds in blank plasma were identifiable,

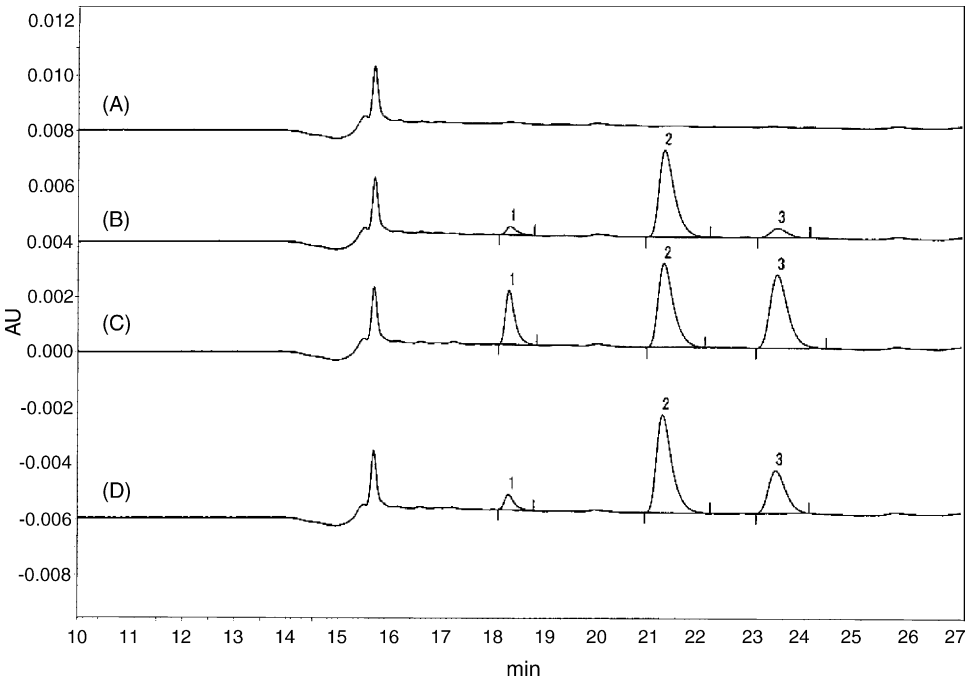

Fig. 2. Representative chromatogram of extracts of blank plasma (A) and extracts of plasma spiked with 2.4 ng/ml for fluvoxamine and 1.6 ng/ml for fluvoxamino acid (B) and 96 ng/ml for fluvoxamine and 72 ng/ml for fluvoxamino acid (C) and plasma from a patient (D). The peak 1 corresponds with fluvoxamino acid; peak 2 with cisapride; peak 3 with fluvoxamine.

discrete and reproducible with a precision of 20% and accuracy of 80–125%. The limits of quantification were 1.2 ng/ml for fluvoxamine and 0.9 ng/ml for fluvoxamino acid.

3.4. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with three different concentrations of two compounds. Intra- and inter-day precisions were assessed by analyzing six quality control samples at each concentration on the same day and by analyzing a quality control for 6 days, respectively. These extracts underwent the same column-switching procedure. Intra-day coefficient variations (CVs) for fluvoxamine and fluvoxamino acid were less than 6.6 and 6.0%, respectively. Inter-day CVs for corresponding compounds were 6.3 and 6.5%, respectively (Table 2). Accuracy was expressed as mean percent error (relative error) [(measured plasma concentration − spiked concentration in plasma)/spiked concentration in plasma] × 100 (%) of each quality control plasma sample, while precision was quantitated by calculating intra- and inter CV values.

Table 2
Precision (CV) and accuracy (relative error) for determination of analytes in spiked plasma ($n = 6$)

Analyte	Concentration added (ng/mL)	Intra-day		Inter-day	
		CV (%)	Relative error (%)	CV (%)	Relative error (%)
Fluvoxamine	1.2	6.6	−5.8	6.3	−10.0
	24.0	2.1	−3.8	3.7	−5.1
	96.0	0.8	−1.3	1.0	−2.3
Fluvoxamino acid	0.9	6.0	−7.8	6.5	−10.9
	18.0	2.1	−5.6	2.8	−5.5
	72.0	1.1	−1.3	1.2	−2.4

3.5. Drug concentrations in human plasma

Fig. 3 shows the mean plasma concentration versus time curves of fluvoxamine and fluvoxamino acid after an oral administration of fluvoxamine (50 mg) in 10 subjects. The pharmacokinetic parameters of fluvoxamine and fluvoxamino acid are summarized in Table 3.

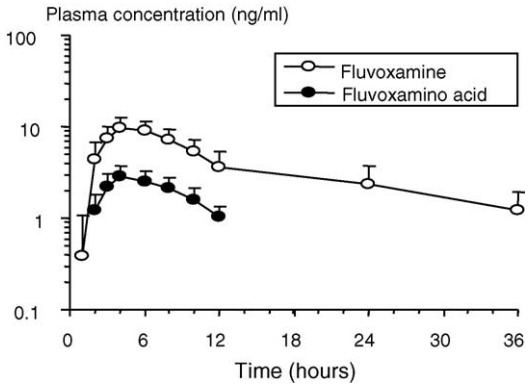

Fig. 3. Mean plasma concentration–time curves of fluvoxamine and fluvoxamino acid from 0.5 to 36 h in 10 healthy volunteers after a single-oral dose of fluvoxamine 50 mg. Error bars indicate standard deviation.

Table 3
Pharmacokinetic parameters of fluvoxamine and fluvoxamino acid after a oral 50 mg dose of fluvoxamine in 10 healthy volunteers

Parameters	Mean ± S.D.
Fluvoxamine	
C_{max} (ng/mL)	10.2 ± 2.7
T_{max} (h)	4.5 ± 1.0
$T_{1/2}$ (h)	14.1 ± 3.0
AUC(0–36) (ng h/mL)	131 ± 44
AUC(0–∞) (ng h/mL)	158 ± 64
Fluvoxamino acid	
C_{max} (ng/mL)	2.9 ± 0.8
T_{max} (h)	4.0 ± 0.8
AUC(0–12) (ng h/mL)	21.1 ± 5.9

AUC, area under plasma concentration–time curve; C_{max}, peak concentration; T_{max}, time to C_{max}; $T_{1/2}$, elimination half-life.

The sensitivity in the present simple HPLC method was superior to a previous method using HPLC analysis [16], enabling the monitoring of plasma concentrations of fluvoxamine up to 36 h and fluvoxamino acid up to 12 h after an administration of fluvoxamine 50 mg in all volunteers. Consequently, precise pharmacokinetic parameters were obtained from plasma concentration of fluvoxamine from 12 to 36 h after administration in elimination phase. These results can apply to clinical pharmacokinetic studies in patients receiving fluvoxamine treatment.

4. Conclusion

The new HPLC procedure described for simultaneous determination of fluvoxamine and fluvoxamino acid is suitable for routine analysis even though it is a little time consuming. Satisfactory validation data were achieved for linearity, precision and recovery. The limit of quantification obtained allows measurement of not only therapeutic concentration of fluvoxamine but also pharmacokinetic study using healthy volunteers.

References

[1] M.I. Wilde, G.L. Plosker, P. Benfield, Drugs 46 (1993) 895–924.
[2] H. Overmars, P.M. Scherpenisse, L.C. Post, Eur. J. Drug Metab. Pharmacokinet. 8 (1983) 269–280.
[3] G. Gerstenberg, T. Aoshima, T. Fukasawa, K. Yoshida, H. Takahashi, H. Higuchi, Y. Murata, R. Shimoyama, T. Ohkubo, T. Shimizu, K. Otani, Psychopharmacology 167 (2003) 443–448.
[4] G. Gerstenberg, T. Aoshima, T. Fukasawa, K. Yoshida, H. Takahashi, H. Higuchi, Y. Murata, R. Shimoyama, T. Ohkubo, T. Shimizu, K. Otani, Ther. Drug Monit. 25 (2003) 463–468.
[5] G.J. de Jong, J. Chromatogr. 183 (1880) 203–211.
[6] J. Pommery, M. Lhermitte, Biomed. Chromatogr. 3 (1989) 177–179.
[7] V. Van der Meersch-Mougeot, B. Diquet, J. Chromatogr. 567 (1991) 441–449.
[8] R.H. Pullen, A.A. Fatmi, J. Chromatogr. 574 (1992) 101–107.
[9] S. Hartter, H. Wetzel, C. Hiemke, Clin. Chem. 38 (1992) 2082–2086.
[10] S.H. Wong, H.R. Kranzler, S. DellaFera, R. Fernandes, Biomed. Chromatogr. 8 (1994) 278–282.
[11] L. Palego, D. Marazziti, L. Biondi, G. Giannaccini, N. Sarno, A. Armani, A. Lucacchini, G.B. Cassano, L. Dell'Osso, Ther. Drug Monit. 22 (2000) 190–194.
[12] A. Lucca, G. Gentilini, S. Lopez-Silva, A. Soldarini, Ther. Drug Monit. 22 (2000) 271–276.
[13] R. Skibinski, G. Misztal, Acta Pol. Pharm. 58 (2001) 97–100.
[14] G. Tournel, N. Houdret, V. Hedouin, M. Deveau, D. Gosset, M. Lhermitte, J. Chromatogr. B 761 (2001) 147–158.
[15] L. Labat, M. Deveaux, P. Dallet, J.P. Dubost, J. Chromatogr. B 773 (2002) 17–23.
[16] T. Ohkubo, R. Shimoyama, K. Otani, K. Yoshida, H. Higuchi, T. Shimizu, Anal. Sci. 19 (2003) 859–864.
[17] C. Duverneuil, G.L. de la Grandmaison, P. de Mazancourt, J.C. Alvarez, Ther. Drug Monit. 25 (2003) 565–573.
[18] K. Titier, N. Castaing, E. Scotto-Gomez, F. Pehourcq, N. Moore, M. Molimard, Ther. Drug Monit. 25 (2003) 581–587.
[19] J. van Harten, Clin. Pharmacokinet. 24 (1993) 203–220.
[20] N. Yasui-Furukori, Y. Inoue, T. Tateishi, J. Chromatogr. B 789 (2003) 239–245.