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Determination of a new atypical antipsychotic agent perospirone and its metabolite in human plasma by automated column-switching high-performance liquid chromatography

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Abstract

A simple and sensitive column-switching high-performance liquid chromatographic (HPLC) method with fluorescence detection is described for the quantification of perospirone, a serotonin and dopamine antagonist, and its metabolite ID-15036 in human plasma. The test compounds were extracted from 2 ml of plasma using chloroform–hexane (30:70, v/v) and the extract was injected into a column I (TSK-PW precolumn, 10 μm, 35×4.6 mm I.D.) for clean-up and column II (C_{18} STR ODS-II analytical column, 5 μm, 150×4.6 mm I.D.) for separation. The peak was detected using a fluorescence detector set at Ex 315 nm and Em 405 nm, and the total time for a chromatographic separation was ~30 min. The method was validated for the concentration range from 0.1 to 100 ng/ml. Mean recoveries were 97% for perospirone and 96% for ID-15036. Intra- and inter-day relative standard deviations were less than 2.8 and 5.3% for perospirone and 2.4 and 4.4% for ID-15036, respectively, at the concentration range from 0.3 to 30 ng/ml. This method shows good specificity with respect to commonly prescribed psychotropic drugs, and it could be successfully applied for pharmacokinetic studies and therapeutic drug monitoring.
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Keywords: Perospirone

1. Introduction

Perospirone (*cis*-*N*-[4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]butyl]cyclohexane-1,2-dicarboximide; Fig. 1) is a new serotonin 5-HT_2 and dopamine D_2 antagonist [1]. It has been reported that perospirone is effective in the treatment of positive and negative symptoms in schizophrenia, and is well tolerated compared with haloperidol treatment [2]. Based on these findings, perospirone is regarded as one of the atypical antipsychotic agents such as risperidone, clozapine, olanzapine and quetiapine.

Preclinical in vitro studies with human liver microsomes and recombinantly expressed microsomes have suggested that perospirone undergoes hydroxylation, *N*-dealkylation and *S*-oxidation, which are catalyzed by CYP1A1, 2C8, 2D6 and 3A4

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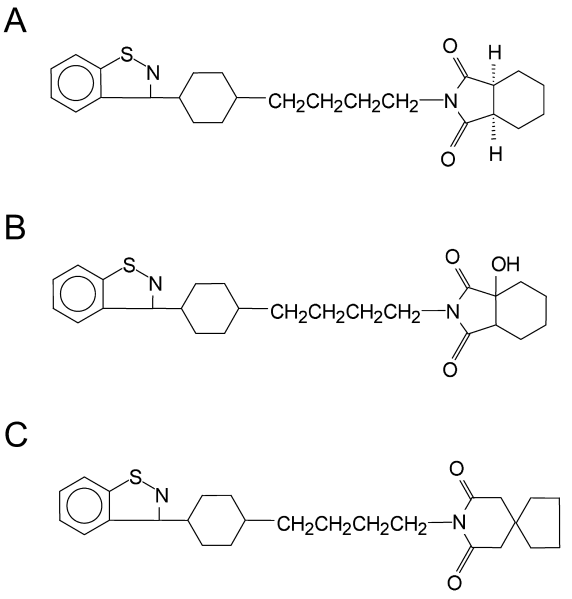

Fig. 1. Chemical structures of perospirone (A), ID-15036 (B) and tiospirone (C).

[3]. Of 17 metabolites of perospirone, only ID-15036 (Fig. 1) has pharmacologically active (antiserotonergic) property, although it is weaker than the parent compound (personal communication, Sumitomo Pharmaceutical, Osaka, Japan). In addition, the plasma concentration of ID-15036 is much higher than that of perospirone after single and repeated oral dose of perospirone [4]. Therefore, it is clinically important for a pharmacokinetic and pharmacodynamic study to detect not only perospirone but also ID-15036 in human plasma.

To our knowledge, however, there is no report of a simple HPLC method for these compounds, although preclinical pharmacokinetic studies were performed using liquid chromatography–mass–mass spectrometry (personal communication, Sumitomo Pharmaceutical, Osaka, Japan), which was sensitive, but very expensive. In the present study, we describe a new and sensitive column-switching HPLC method for determination of perospirone and its metabolite ID-15036 in plasma using liquid–liquid extraction. The assay fulfils the requirements for use in therapeutic drug monitoring.

2. Experimental

2.1. Chemicals

Perospirone and its metabolite ID-15036 and tiospirone (Fig. 1), the internal standard (I.S.), were kindly provided by Sumitomo Pharmaceutical (Osaka, Japan). The purity of these materials was greater than 99.5%. Potassium phosphate monobasic, acetonitrile, perchloric acid, *n*-hexane, 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 perospirone, metabolite ID-15036 and I.S. for generating standard curves were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 2.0 mg/ml. High working standard solutions of perospirone, ID-15036 and I.S. (2.0 μg/ml) were obtained by diluting each stock solution 1000 times with purified water. Middle (200 ng/ml) and low (20 ng/ml) working standard solutions of perospirone and ID-15036 were obtained by further diluting each working standard solution 10 and 100 times, respectively, 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 10–80 μl of low working solutions (20 ng/ml), 16–63 μl of medium working solutions (200 ng/ml), and 12.5–100 μl of high working solutions (2.0 μg/ml) in 2 ml of blank plasma (final volume) to yield final concentrations 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.3, 12.5, 25, 37.5, 50, 75 and 100 ng/ml for each analysis. Standard curves were prepared daily and constructed by linear regression analysis of the compounds/internal standard peak-height ratio versus the respective concentration of perospirone or ID-15036. Stock solutions of perospirone and ID-15036 were separately prepared for quality controls in the same manner as for standard curves. Working plasma solutions were obtained by diluting stock solutions 6667 times with blank plasma (300 ng/ml). Quality

control samples were obtained by spiking 2–200 μl of working plasma solutions in 2 ml of blank plasma (final volume) to yield final concentrations of 0.3, 3 and 30 ng/ml, and stored at −20 °C until analysis. All standard curves were checked using these quality control samples.

2.3. Sample collection

Repeated oral doses (32 mg) of perospirone for at least 4 weeks were given to three schizophrenic patients and blood was obtained before dosing and at 0.5, 1, 2, 3, 4, 6 and 8 h after dosing. Blood samples were collected in heparinized tubes and centrifuged immediately at 1700 *g* for 10 min. The plasma was stored at −20 °C until analysis. This study was approved by the Ethical Committee of Hirosaki University Hospital and written informed consent was given by each patient and her family.

2.4. Apparatus

The column-switching HPLC system (Fig. 2) consisted of two Shimadzu LC-10AT high-pressure pumps (for eluents A and B), a Shimadzu CTO-10A column oven, a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), a Shimadzu RF-10AXL (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; 35×4.6 mm I.D., particle size 10 μm; Tosoh, Tokyo, Japan) and a C_{18} STR ODS-II column as an analytical column (column II; 150×4.6 mm I.D., particle size 5 μm; Shinwa Chemical Industry, Kyoto, Japan) were used.

2.5. Extraction procedure

First 100 μl of 250 ng/ml I.S. (tiospirone) and 0.5 ml NaOH (0.001 *M*) were added to 2 ml of plasma and 0.5 ml of water. The tubes were vortex-mixed for 10 s and 5 ml of *n*-hexane–chloroform (70:30, 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 conditions

Column-switching chromatographic conditions were set based on our previous report [5]. A 0.5-ml portion of the extract was automatically injected into

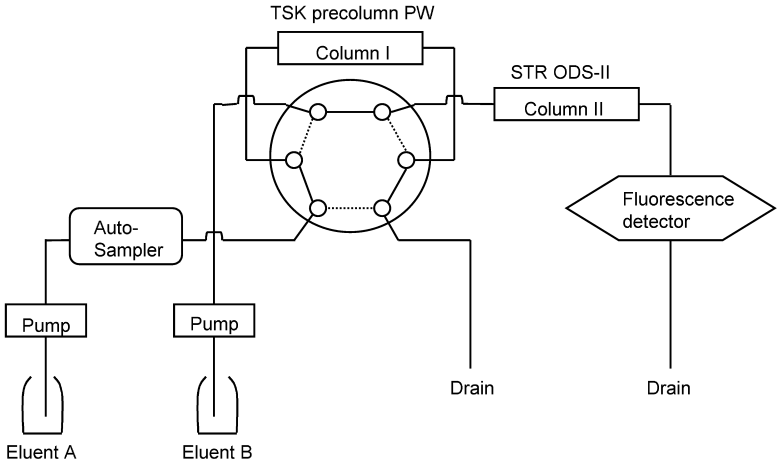

Fig. 2. Flow diagram of the column-switching HPLC system.

Table 1
Time program for the column-switching HPLC

Time after injection (min)	Eluent A or B	
	Column I	Column II
0.0–13.5	A	B
13.5–17.0	B	B
17.0–35.0	A	B

the HPLC system. The column-switching system was operated according to the time program depicted in Table 1. From 0 to 13.5 min after the sample injection perospirone, ID-15036 and tiospirone were separated from the interfering substances present in the extract on column I with a mobile phase (eluent A) of phosphate buffer (0.05 *M*, pH 4.6), perchloric acid (6 *M*) and acetonitrile (94.9:0.5:4.6, v/v). Between 13.5 and 17.0 min after the injection, perospirone, ID-15036 and tiospirone retained on column I were eluted with a mobile phase (eluent B) of phosphate buffer (0.05 *M*, pH 4.6), perchloric acid (6 *M*) and acetonitrile (58:0.5:41.5, v/v), and effluent from column I was switched to column II. Then perospirone, ID-15036 and tiospirone were separated on column II by eluting with eluent B (between 17.0 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 ~25 °C (room temperature) and 30 °C, respectively. The fluorescence detector was set at an excitation wavelength of 315 nm and an emission wavelength of 405 nm. The peak height was used for the quantification of perospirone and ID-15036.

3. Results and discussion

3.1. Chromatography

A representative chromatogram of an unextracted working aqueous solution containing perospirone, ID-15036 and tiospirone (internal standard) is shown in Fig. 3A. The chromatogram of an extracted blank plasma sample is shown in Fig. 3B, while the chromatogram of an extracted sample spiked with 2.0 ng/ml of perospirone and ID-15036 and I.S. is shown in Fig. 3C. Both compounds were well

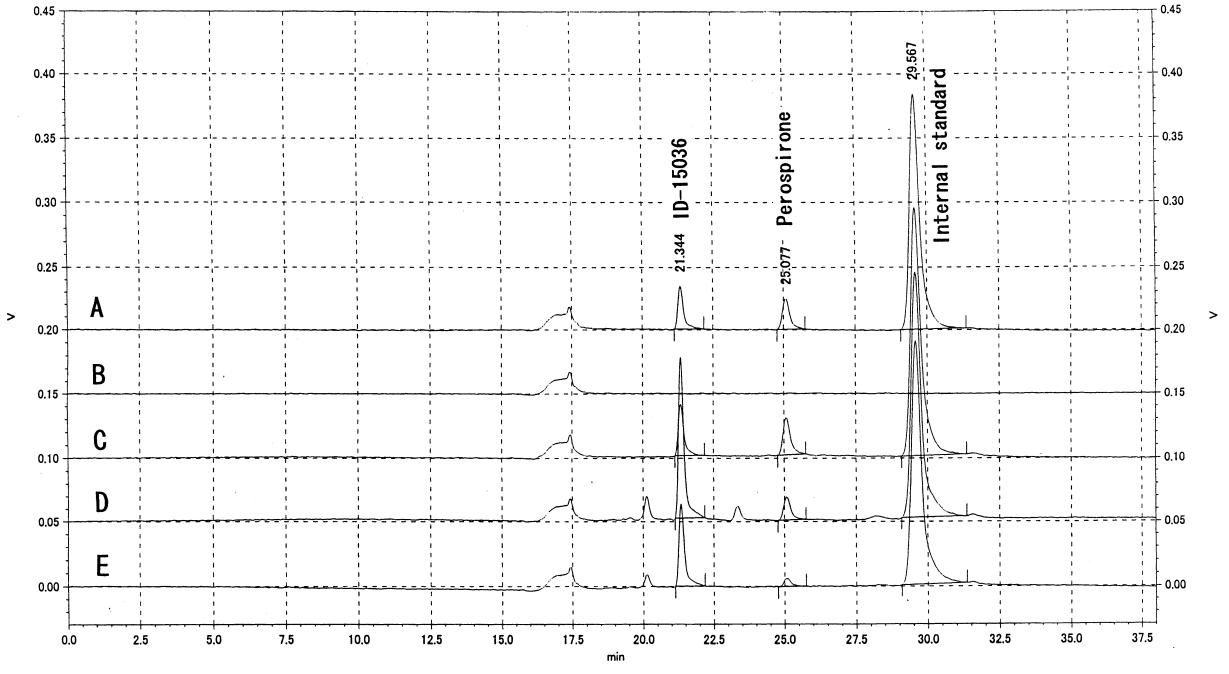

Fig. 3. Chromatograms of references without extraction (2 ng) (A) and extracts from blank pooled human plasma (B), plasma spiked with analytes (each a concentration of 2 ng/ml) (C), and a plasma sample of patient 2 (D) and patient 3 (E).

separated from each other and from the front of the solvent peaks. The chromatograms of extracted plasma samples obtained from two patients receiving 32 mg/day perospirone did not show interference peaks (Fig. 3D,E). Plasma concentrations of perospirone and ID-15036 were 1.2 and 6.4 ng/ml in patient 1 and 0.4 and 3.3 ng/ml in patient 2, respectively.

3.2. Recovery and linearity

Recovery from plasma was calculated by comparing the peak heights 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 C.V. values were determined at six different concentrations ranging from 0.3 to 30 ng/ml (Table 2). Calibration curves were linear over the concentration ranges from 0.1 to 100 ng/ml ($r=0.9999$ for perospirone and $r=0.9998$ for ID-15036) (Table 3).

3.3. Sensitivity

The limit of detection for each compound was defined as analyte responses are at least three times the response compared to blank response. The lowest standard on the calibration curve was defined as the limit of quantification as analyte peaks for both compounds were identifiable, discrete, and reproducible with a precision of 20% and accuracy of 80–120%. The limits of detection and quantification were 0.06 and 0.1 ng/ml for both perospirone and ID-15036.

3.4. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with three different concentrations of perospirone and ID-15036. Intra- and inter-day precisions were assessed by analyzing six quality control samples at each concentration on the same day and mean values of two quality controls for 6 days, respectively. These extracts underwent the same column-switching procedure. Intra- and inter-day relative standard deviations were less than 2.8 and 5.3% for perospirone and 2.4 and 4.4% for ID-15036, respectively, in the concentration range of 0.3–30 ng/ml (Table 4). Accuracy was expected as percent error (relative error) [(measured concentration−spiked concentration)/spiked concentration]×100 (%), while precision was quantitated by calculating intra- and inter C.V. values.

Table 2
Extraction recovery of the two analytes from plasma

Analyte	Concentration added (ng/ml)	Recovery (%) ($n=6$)	C.V. (%) ($n=6$)
Perospirone	0.3	96.8	0.9
	3	96.8	0.7
	30	96.9	0.8
ID-15036	0.3	95.4	1.0
	3	95.7	0.9
	30	95.5	0.9

3.5. Specificity

Potential interference from co-administered drugs was investigated by determining their retention times in this system. No peaks were observed until 60 min after injections of extracts with nitrazepam, diazepam, alprazolam, chlorpromazine, levomeprom-

Table 3
Individual and mean values for slope, intercepts and correlation coefficients of five calibration curves for perospirone and ID-15036

Analyte	Curve	Slope	Intercept	r
Perospirone	1	0.0374	0.0003	0.9999
	2	0.0408	−0.0028	0.9999
	3	0.0375	−0.0013	0.9999
	4	0.0373	0.0002	0.9999
	5	0.0410	−0.0021	0.9998
	Mean	0.0388	−0.0011	
	SD	0.0017	0.0012	
	SE	0.0008	0.0005	
ID-15036	1	0.0620	0.0418	0.9997
	2	0.0645	0.0254	0.9998
	3	0.0665	0.0242	0.9998
	4	0.0636	0.0292	0.9998
	5	0.0670	0.0364	0.9998
	Mean	0.0647	0.0314	
	SD	0.0018	0.0067	
	SE	0.0008	0.0030	

Table 4
Precision (C.V.) and accuracy (relative error) for determination of two analytes in spiked plasma ($n=6$)

Analyte	Concentration added (ng/ml)	Within-day		Between-day	
		C.V. (%)	Relative error (%)	C.V. (%)	Relative error (%)
Perospirone	0.3	2.27	6.67	5.31	−3.33
	3	2.01	1.33	4.16	1.33
	30	0.85	−1.17	2.49	0.74
ID-15036	0.3	2.46	1.11	4.43	−1.00
	3	1.93	1.00	3.39	−1.43
	30	1.28	−3.26	2.27	−0.59

azine haloperidol, bromperidol or risperidone. However, their metabolites were not investigated.

3.6. Drug concentrations in human plasma

Fig. 4 shows concentration versus time curves obtained after oral administration of four tablets of perospirone (8 mg) to three schizophrenic patients in two equally divided doses (8 a.m. and 8 p.m.). Pharmacokinetic parameters of perospirone and ID-15036 in patients treated with 32 mg/day of perospirone are shown in Table 5. Plasma concentration of ID-15036 was higher than that of perospirone in all three patients. Accordingly, AUC from 0 to 8 h of ID-15036 was ~8.5 times higher than perospirone. Perospirone was not detectable in plasma in two patients before perospirone dosing (12 h after perospirone dosing) at steady state (trough level). No interfering peaks were observed, despite the fact that many various drugs were co-administered with perospirone, e.g. flunitrazepam, quazepam, biperiden, etc.

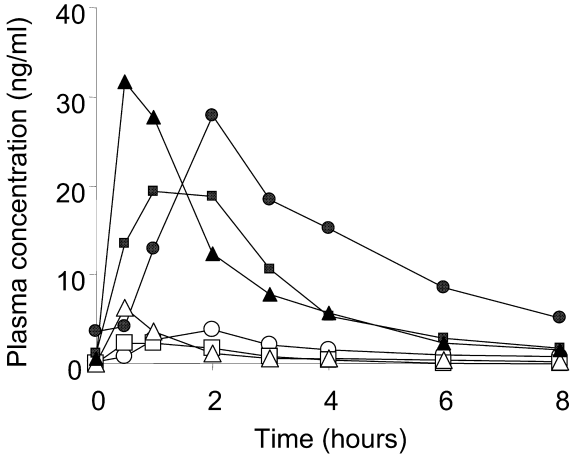

Fig. 4. Plasma concentration–time curves of perospirone and ID-15036 from 0 to 8 h in three schizophrenic patients receiving perospirone 32 mg/day. Open and solid symbols indicate data for perospirone and ID-15036, respectively. Circles, squares and triangles indicate values of patients 1, 2 and 3, respectively.

Table 5
Clinical profile and pharmacokinetic parameters of perospirone and ID-15036 in three schizophrenic patients receiving perospirone 32 mg/day

	Patient 1	Patient 2	Patient 3
Age (years)	55	30	41
Gender	F	F	F
Body weight (kg)	87	48	60
Perospirone			
C_{max} (ng/ml)	3.7	2.3	6.3
t_{max} (h)	2	0.75	0.5
AUC(0–8) (ng*h/ml)	13.2	5.8	9.2
V_{SS} (l)	4178	4621	3451
$t_{1/2}$ (h)	3.3	0.8	3.1
ID-15036			
C_{max} (ng/ml)	27.9	19.3	31.7
t_{max} (h)	2	1	0.5
AUC(0–8) (ng*h/ml)	103.9	66.3	71.3

AUC, area under plasma concentration-time curve; C_{max}, peak concentration; t_{max}, time to C_{max}; $t_{1/2}$, elimination half-life; V_{ss}, steady-state volume of distribution.

4. Conclusion

The HPLC procedure described for perospirone and ID-15036 determination 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 therapeutic concentration of perospirone and ID-15036.

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