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Short Communication

Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection

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ABSTRACT

A method for the determination of risperidone and its active metabolite 9-hydroxyrisperidone in human plasma has been developed. The procedure involved a multi-step liquid–liquid extraction with an internal standard. The parent drug and its metabolite were separated on a cyano column used in the reversed-phase model. The coulometric detection allows quantification in the range 2–100 ng/ml. The precision, accuracy and specificity have been checked, and show that the method is reliable for clinical studies.

INTRODUCTION

Risperidone (I, R64766) or 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one is a new antipsychotic with both serotonin-S2 and dopamine-D2 antagonistic properties [1–3]. In humans and experimental animals it undergoes hydroxylation at the tetrahydropyridopyrimidine moiety, yielding 9-hydroxyrisperidone (II, R76477) as the major metabolite [4] with a pharmacological activity similar to that of the parent compound [5].

Two radioimmunoassay (RIA) methods have been developed by Woestenborghs *et al.* [6]. The first needs an extraction step for the determination of the unchanged drug. The second measures only the sum of risperidone and 9-hydroxyrisperidone by direct RIA. Detection limits were *ca.* 0.1 ng/ml plasma. A high-performance liquid chromatographic (HPLC) method with UV detection has also been published, but it is suitable only for experimental animals and healthy volunteers, because of poor specificity when poly-medicated patients are under study. The aim of this study was to develop a more specific method for the determination of I and II. This method should be useful for pharmacokinetic studies and drug monitoring, especially for psychiatric patients and for hospital laboratories that are not equipped for RIA.

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EXPERIMENTAL

Chemicals

Risperidone, 9-hydroxyrisperidone and methylrisperidone (III, R68808, internal standard) were supplied by courtesy of Janssen Pharmaceutica (Beerse, Belgium). Their chemical structures are given in Fig. 1. Aacetonitrile, methanol, dichloromethane (LiChrosolv, HPLC grade) and *n*-hexane (for residue analysis) were purchased from Merck (Nogent sur Marne, France). Sulphuric acid (0.05 *M*), ammonia (28%), sodium borate, potassium dihydrogenphosphate and sodium hydroxide (10 *M*) (RP Normapur) were obtained from Prolabo (Paris, France). Water was purified on a Milli-Q Plus system (Millipore, Saint Quentin en Yvelines, France).

Apparatus

All chromatographic experiments were performed using a 510 HPLC pump (Waters, Saint Quentin en Yvelines, France) connected to a SSI LP-21 pulse dampener (Cunow, Cergy-Pontoise, France) with a Kontron 460 autosampler (Kontron, Saint Quentin en Yvelines, France). The 5100 A Coulochem electrochemical detector (Cunow) was equipped with a 5020 guard cell and a 5011 analytical cell.

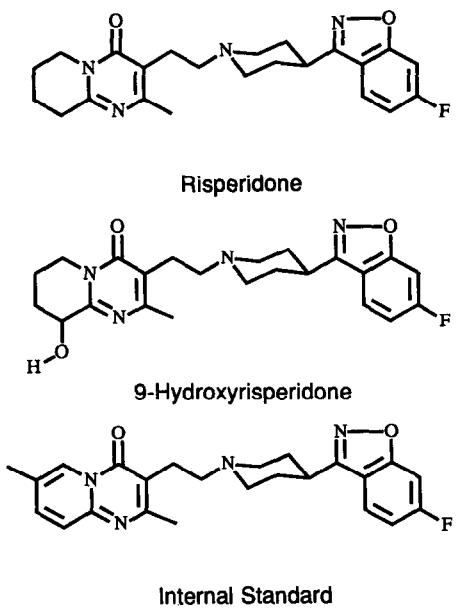

Fig. 1. Structures of risperidone, 9-hydroxyrisperidone and the internal standard.

The applied potential of the 5020 guard cell was 1.00 V, and that of the 5011 analytical cell was 0.55 V for detector 1 and 0.80 V for detector 2. The chromatographic signal was monitored at detector 2.

The analytical column (250 mm × 4.6 mm I.D.) was packed with Ultrasphere (5 μm particle size) coated with cyano groups (Beckman, Gagny, France) and the guard column (30 mm × 4.6 mm I.D.) with Spherisorb cyano (5 μm particle size) (Brownlee Labs., Touzart et Matignon, Vitry sur Seine, France).

Isocratic elution was performed with acetonitrile–0.05 *M* potassium dihydrogenphosphate (adjusted to pH 6.5 with 28% ammonia) (60:40, v/v), which was filtered through a 0.2-μm regenerated cellulosis membrane (Schleicher et Schuell, Ecquevilly, France) and degassed with a constant stream of helium. The flow-rate was 1 ml/min.

Preparation of standard curves

Stock standard solutions were prepared in methanol at a concentration of 400 μg/ml for I, II and III and stored at −24°C. Working standard solutions were freshly prepared every week by successive dilutions of the stock solutions (2–100 ng per 0.1 ml) and stored at 4°C. A standard curve for the determination of I and II in human plasma was made with spiked plasma at the following concentrations: 0, 2, 5, 10, 20, 50 and 100 ng/ml. The internal standard concentration was set at 20 ng/ml. All calibration samples were taken through the extraction procedure described below.

Extraction procedure

To 1 ml of plasma in glass tubes were added 0.1 ml (20 ng) of working internal standard solution and 1 ml of 0.05 *M* borate buffer (pH 10). The tube was briefly shaken on a vortex-mixer, then the plasma sample was poured on an Extrelut 3 column (Merck). After 5 min, the column was eluted with 15 ml of hexane–dichloromethane

(50:50, v/v). The organic layer was collected in a 15-ml glass tube, then 3 ml of 0.05 *M* H_2SO_4 were added and the contents mixed for 10 min. The mixture was centrifuged (10 min, 3000 *g*), and the organic layer discarded. The acid layer was washed with 6 ml of hexane–dichloromethane (50:50, v/v) for 5 min. The mixture was centrifuged and the organic layer discarded. The acid layer was made basic with 0.15 ml of 28% ammonia and re-extracted twice with 3 ml of the same hexane–dichloromethane mixture. The combined organic layers were then evaporated to dryness under a gentle stream of nitrogen in a dry block heater set at 60°C. The residue was dissolved in 0.1 ml of the elution solvent, and 0.02-ml aliquots were injected into the HPLC column.

RESULTS AND DISCUSSION

Fig. 2 shows the hydrodynamic voltammograms of I, II and III. These show that, in theory, using a potential higher than 0.8 V should improve the sensitivity, but in practice the signal-to-noise ratio was no better. Hence, extensive care must be taken over the purity of the reagents and the cleanliness of glassware and apparatus as reported by Hariharan *et al.* [8], who used coulometric detection to determine plasma haloperidol and reduced haloperidol. The glassware was soaked with 3% Mucasol (Poly-labo, Strasbourg, France), sonicated for 30 min, rinsed several times with ultrapure water and methanol, and then air-dried. Glassware (Chromacol 0.2 CTV, Touzart et Matignon) used for the automatic injector was rinsed with methanol twice, shaken on a vortex-mixer and air-dried. The detector cells must be cleaned every two weeks with 6 *M* HNO_3 in order to maintain their sensitivity.

The mobile phase was recycled, and renewed once a week. After two weeks of use, the analytical column was regenerated with acetonitrile–water (40:60, v/v) adjusted to pH 3 with 1 *M* H_2SO_4. A Spherisorb cyano column was also tested, but it needed to be more frequently regenerated.

Under these conditions, the signal was suitably noise-free and without "ghost peaks" when the analytical cell was set at 0.8 V. Fig. 3 shows chromatograms of blank plasma (A), reference plasma spiked with risperidone and 9-hydroxyrisperidone (B and C) and plasma from a patient after intake of risperidone (D). Retention times were 7.8, 11.2 and 14.3 min for I, II and II, respectively. The specificity of the method was tested by injecting some commonly used psychiatric drugs (Table I). A 20-ng amount of each was injected into the HPLC system and, after extraction, the only one that interfered in the assay was pipamperone.

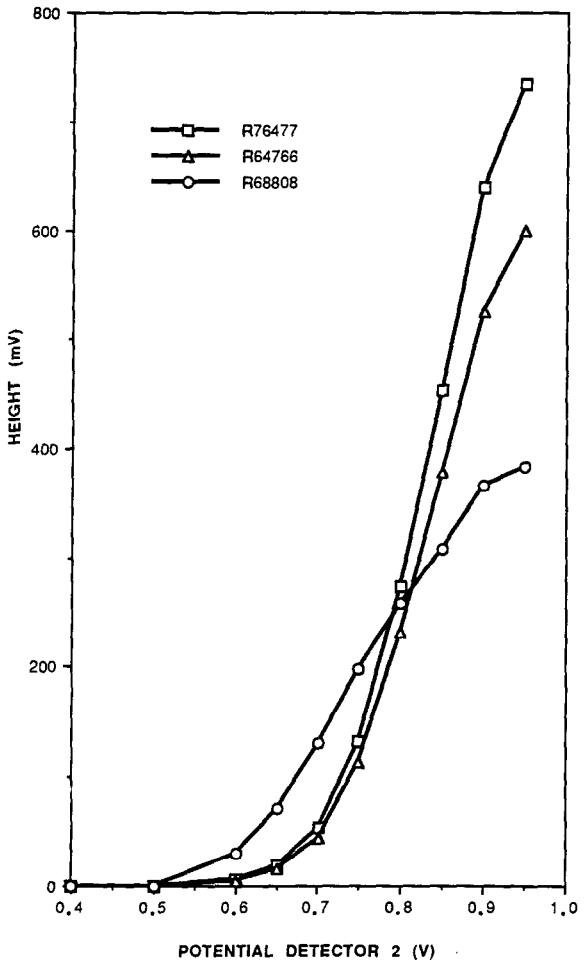

Fig. 2. Hydrodynamic voltammograms of risperidone (R64766), 9-hydroxyrisperidone (R76477) and methylrisperidone (R68808). Each point represents the mean of two injections (10 ng per 20 μl). Chromatographic conditions are as described in Experimental, except that detector 1 was set at 0.0 V.

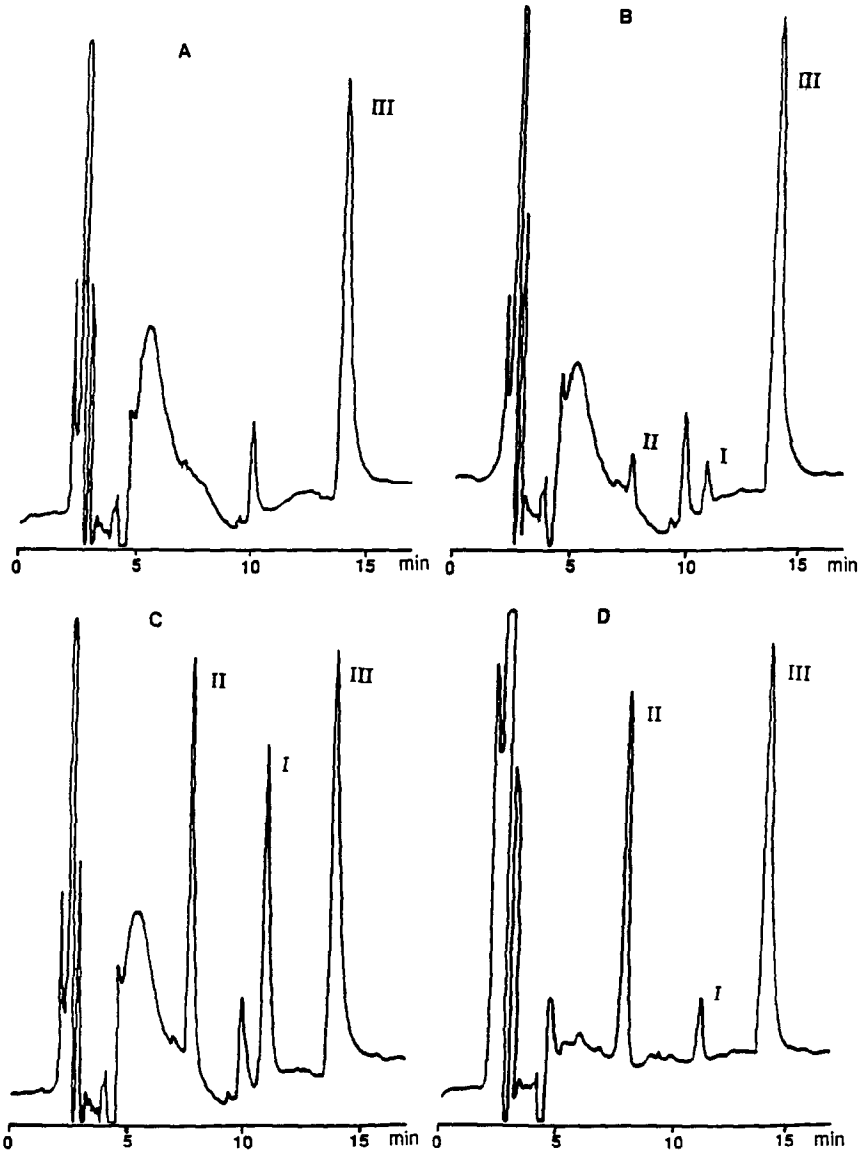

Fig. 3. Representative chromatograms for (A) blank plasma with internal standard (20 ng), (B) plasma spiked with 2 ng each of I and II, and 20 ng of internal standard, (C) plasma spiked with 20 ng each of I, II and internal standard, and (D) plasma sample from a patient, containing 2.6 ng of risperidone and 17.9 ng of 9-hydroxyrisperidone. Peaks: I = risperidone; II = 9-hydroxyrisperidone; III = methylrisperidone (internal standard).

The mean recoveries for I and II, using the extraction method described above, calculated at three different concentrations (5, 20 and 100 ng/ml) were 78.2 ± 2.9 and 69.0 ± 4.0% (mean ± S.D.), respectively (Table II). The linearity, precision and accuracy of the method (Table III) were evaluated from a calibration curve in the range 2–100 ng/ml (four replicate extracted plasma for each concentration) by quadratic regression analysis of the peak-height ratio (peak height of each compound/I.S. peak height) *versus* the concentration. Typical equations were $y = -0.228x^2 +$

TABLE I

RETENTION TIMES OF SELECTED DRUGS

Drugs were determined by analysing 1 ml of plasma sample spiked with a minimum of 20 ng for each compound.

Drug	Retention time (min)
Haloperidol	21.1
Reduced haloperidol	14.9
Droperidol	10.9
Pipamperone	9.7
Desipramine	16.7
Imipramine	19.7
Clomipramine	28.5
Bromazepam	N.D.[a]
Diazepam	N.D.
Triazolam	N.D.
Estazolam	N.D.
Alprazolam	N.D.
Oxazepam	N.D.
Flunitrazepam	6.8
Carbamazepine	N.D.
Cyamemazine	8.3
Chlorpromazine	26.4
Trihexyphenidyl	20.1
Chlorazepate	N.D.
Ethylbenzatropine	N.D.
Valproic acid	N.D.
Diphenylhydantoïn	N.D.
Phenobarbital	N.D.

[a] N.D. = not detected.

$14.210x - 0.154$ ($r = 0.9997$) for I, and $y = -0.215x^2 + 12.760x + 0.052$ ($r = 0.9998$) for II.

The inter-day accuracy (Table IV) was evaluated over a period of two months with spiked plasma samples, and ranged from 85.6 to 119.2% and 92.1 to 103.8% for I and II, respectively.

Our HPLC method has been compared (Table V) with the RIA method developed by Woestenborghs *et al.* [6], which measures the total concentration of I and II. We used plasma from patients ($n = 25$) who received between 0 and 16 mg of risperidone. No interference was detected and the accuracy (HPLC *versus* RIA assay) was 96.9 ± 15.9%.

CONCLUSIONS

Although this method involves the use of a coulometric detector and a cyano column, it is selective for the determination of I and II; ODS columns give more analytical interferences. It proved to be accurate and sensitive, and coulometric detection improves the limit of quantitation by a factor of 5 compared with UV detection. This method is able to quantify the two subtances with a good precision in the range 2–100 ng/ml and is well adapted for the monitoring of plasma levels in psychiatric patients.

TABLE II

EXTRACTION RECOVERY OF THE THREE ANALYTES FROM PLASMA

Analyte	Concentration added (ng/ml)	Recovery, (mean ± S.D., $n = 5$) (%)
Risperidone	5	79.6 ± 1.6
	20	76.5 ± 3.6
	100	77.0 ± 3.0
9-Hydroxyrisperidone	5	72.5 ± 3.5
	20	68.4 ± 3.3
	100	65.2 ± 1.9
Methylrisperidone	20	68.3 ± 5.3

TABLE III

LINEARITY, ACCURACY AND PRECISION OF THE METHOD

Concentration added (ng/ml)	Calculated concentration (mean ± S.D., $n = 4$) (ng/ml)	Coefficient of variation (%)	Relative error (%)
Risperidone			
2	1.67 ± 0.14	8.4	−16.3
5	5.22 ± 0.63	12.1	4.4
10	9.80 ± 0.36	3.7	−2.5
20	20.50 ± 0.59	2.9	2.4
50	49.80 ± 0.78	1.6	−0.4
100	100.00 ± 1.68	1.7	0.0
Hydroxyrisperidone			
2	1.94 ± 0.16	8.1	−3.0
5	5.03 ± 0.62	12.3	0.7
10	10.00 ± 0.44	4.4	0.0
20	20.11 ± 0.90	4.5	0.5
50	49.91 ± 0.76	1.5	−0.2
100	100.01 ± 0.49	0.5	0.0

TABLE IV

INTER-DAY ACCURACY OF THE METHOD FOR THE DETERMINATION OF I AND II IN PLASMA ON THREE DIFFERENT DAYS

Control sample (ng/ml)	Analyte	Concentration found (mean ± S.D.) (ng/ml)	Found / added (%)
83.3	I	71.3 ± 2.3	85.6
	II	79.1 ± 4.5	95.0
62.5	I	54.9 ± 5.5	87.8
	II	59.0 ± 4.8	94.4
41.6	I	36.0 ± 2.7	86.5
	II	38.3 ± 2.7	92.1
20.8	I	18.8 ± 1.0	90.4
	II	19.7 ± 1.5	94.7
10.4	I	9.7 ± 0.8	93.3
	II	10.1 ± 0.8	97.1
5.2	I	5.1 ± 0.3	98.0
	II	5.1 ± 0.1	98.0
2.6	I	3.1 ± 0.2	119.2
	II	2.7 ± 0.6	103.8

TABLE V

CORRELATION BETWEEN RIA ASSAY AND HPLC METHOD

Patient No.[a]	RIA, I + II (ng/ml)	HPLC, I + II (ng/ml)	HPLC/RIA (%)
1	15.2	14.6	96.0
2	70.1	63.6	90.7
5	39.2	30.3	77.3
10	72.3	79.6	110.0
14	37.6	30.5	81.1
15	40.4	39.4	97.5
16	36.8	34.7	94.3
19	26.4	24.5	92.8
20	77.2	108.3	140.3
21	62.8	55.9	89.0
27	232	206	88.8
28	230	214	93.0
29	250	235	94.0
33	26.2	20.5	78.2
38	6.88	N.D.[b]	–
48	38.9	39.1	100.5
51	22.1	29.3	132.5
54	71.1	66.0	93.0
61	N.D.	N.D.	–
77	41.1	32.2	78.3
84	9.4	11.4	121.3
85	22.7	21.1	93.0
87	66.4	63.1	95.0
111	94.6	96.7	102.2
122	105.0	95.3	90.8

[a] Patients received between 0 and 16 mg of risperidone daily.
[b] N.D. = not detected.

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