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Determination of risperidone and 9-hydroxyrisperidone in human plasma by liquid chromatography: application to the evaluation of CYP2D6 drug interactions

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Abstract

A high-pressure liquid chromatography with ultra-violet detection method for the simultaneous determination of risperidone and 9-hydroxyrisperidone in plasma after liquid–liquid extraction has been developed. The limit of quantitation was 5 nmol/L, and the inter-day coefficient of variation was less than 8% for both compounds. The mean recoveries of risperidone and 9-hydroxyrisperidone added to plasma were 96.8 and 99.4%, with an intra-day coefficient of variation of under 5 and 6%, respectively. Studies of analytical interference showed that the most commonly co-administered antidepressants and benzodiazepines did not interfere. The method was used for the determination of the plasma concentrations of a schizophrenic patient treated daily with an oral dose of 4.5 mg risperidone. The patient suffered severe extrapyramidal side-effects after adding risperidone to his previous medication of haloperidol and levomepromazine. The risperidone plasma concentration was well above the average (182 nmol/L), which suggests that a pharmacokinetic interaction occurred, presumably due to inhibition of the enzyme CYP2D6.
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1. Introduction

Risperidone is an antipsychotic agent chemically classified as a benzisoxazole derivative. It is a selective monoaminergic antagonist with high affinity for serotonergic 5-HT_2 and dopaminergic D_2 receptors [1]. Clinical trials in psychotic patients have shown risperidone to be effective in the treatment of the positive, negative, and affective symptoms of schizophrenia [2]. Furthermore, risperidone therapy is associated with reduced extrapyramidal symptoms. Patients with chronic schizophrenia receiving risperidone treatment require 10 times less antiparkinsonian medication compared with those receiving haloperidol treatment [3]. Recently, the

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FDA approved the use of risperidone for the treatment of residual schizophrenia, and a large prospective clinical study comparing risperidone with haloperidol has demonstrated that patients treated with risperidone have a lower risk of relapse than those treated with haloperidol [4]. Due to the favorable clinical effects of risperidone a substantial increase has occurred in its use during the last few years, as demonstrated by the pharmacoepidemiological data from Extremadura (Spain) and Hungary [5] (Table 1). The data show a similar sharp increase in the use of risperidone at both locations.

In humans, risperidone is metabolized mainly in the liver. The main metabolite of risperidone, 9-hydroxyrisperidone (Fig. 1), has a similar pharmacological activity as the parent compound, and the serum concentration of the active moiety is thus the sum of the serum concentrations of risperidone and 9-hydroxyrisperidone [6,7].

Risperidone is metabolized to 9-hydroxyrisperidone by the polymorphic cytochrome P450 CYP2D6 enzyme [7]. Thus, inter-individual variability in plasma levels of the parent drug or metabolite can occur. Therefore, plasma level monitoring may help to determine the required dose of the drug.

The aim of the present study was to validate a specific method for the quantitative determination of risperidone and 9-hydroxyrisperidone in plasma, excluding interference from commonly used psychotropic drugs and their metabolites in order to make the method suitable for routine therapeutic drug monitoring. A case report of a possible interaction between risperidone and haloperidol causing severe side-effects is also reported.

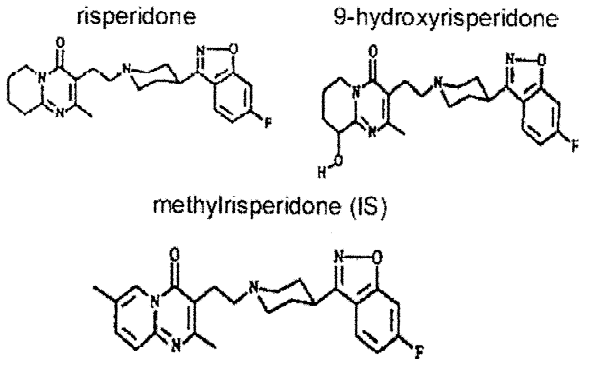

Fig. 1. Molecular structure of risperidone and its main metabolite.

2. Experimental

2.1. Chemicals and reagents

Risperidone, 9-hydroxyrisperidone and methylrisperidone (internal standard, I.S.) were donated by Janssen Research Products (Lammerdries, Belgium). HPLC-grade heptane, acetonitrile, isoamyl-alcohol, potassium dihydrogenphosphate, dimethyloctylamine (DMOA), potassium hydroxide, acetic acid and hydrochloric acid were obtained from Merck (Darmstadt, Germany). Diisopropyl ether and sodium hydroxide were from Panreac (Barcelona, Spain). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Standard solutions

Stock standard solutions were prepared in hydrochloric acid at a concentration of 1 mmol/L for risperidone and 9-hydroxyrisperidone and 200 μg/

Table 1
Use of risperidone in Extremadura (an autonomous community of Spain with 1.1 million inhabitants) and in Hungary (10.1 million inhabitants) [5]. Values are calculated according to the WHO standard measure of the defined daily dose (DDD)/1000 inhabitants/day and the percentage risperidone use of total antipsychotic drug sales for outpatients [19]

Risperidone	1993	1994	1995	1996	1997	1998	1999	2000
Extremadura (Spain)								
DDD/1000 inhabitants/day	0	0.03	0.22	0.41	0.55	0.67	0.71	0.84
Percent of total antipsychotics	0	0.6	4.6	8.2	10.3	11.9	12.2	13.5
Hungary								
DDD/1000 inhabitants/day	0	0	0.01	0.13	0.26	0.29	0.43	0.56
Percent of total antipsychotics	0	0	0.4	5.4	9.0	9.3	11.8	13.3

mL for methylrisperidone. Working standard solutions were prepared by diluting the stock solutions with methanol (10 μmol/L for risperidone and 9-hydroxyrisperidone and 5 μg/mL for the I.S.). These solutions were stable when stored for up to 6 months at −20 °C. A standard curve for the determination of risperidone and 9-hydroxyrisperidone in human plasma was prepared with spiked plasma at concentrations of 10, 20, 40, 80 and 160 nmol/L. Samples at all concentrations were extracted according to the procedure described below.

2.3. Instruments and chromatographic conditions

The liquid chromatographic system consisted of a Beckman Model 110B pump, a Beckman 166 programmable detector module set at a wavelength of 278 nm, and a Rheodyne Model 7725i injector (loop volume 20 μL), coupled to a 386 PC with Beckman Gold software V. 3.2 (Beckman Instruments, Fullertone, CA, USA). The analytical column (250×4.6 mm I.D., 3 μm) was packed with Hypersil ODS coated with C_{18} groups (Sugelabor, Madrid, Spain). The mobile phase was a mixture of acetonitrile (28%), water (72%), 5.44 g/L KH_2PO_4 (40 m*M*) and 400 μL DMOA. Before analysis, the mobile phase was filtered through a 0.22 μm filter (Millipore, USA), and then degassed ultrasonically for 15 min. The flow-rate was 0.8 mL/min at ambient temperature.

2.4. Assay procedure

Sample preparation was carried out by liquid–liquid extraction. A 1 mL aliquot of human plasma was pipetted into a 10 mL polypropylene tube with 1 mL of sodium hydroxide (0.5 *M*). The plasma was extracted with 4 mL of isoamyl alcohol in diisopropyl ether (3:97, v/v) after the addition of 50 μL of the I.S. (5 μg/mL). The tubes were capped, shaken vertically for 10 min and then centrifuged for 10 min at 3000 *g*. The aqueous layer was then collected in a 10 mL centrifuge tube, and 175 μL of acetic acid (25 m*M*) was added and the contents mixed for 5 min. The mixture was centrifuged for 5 min at 3000 *g*, the organic layer discarded and then 500 μL of heptane was added. The heptane layer was aspirated and evaporated in a gentle stream of nitrogen. Sample aliquots of 30 μL volume were injected into the HPLC apparatus.

2.5. Calibration graphs

From the recorded peak heights, the ratios of drugs to internal standard were calculated. The results obtained from spiked plasma samples containing known amounts of drug were calculated on the basis of linear regression analysis.

2.6. Recovery and assay validation

The recovery was calculated by comparing the peak heights after injection of risperidone and 9-hydroxyrisperidone dissolved in mobile phase with the peak heights obtained after extraction of the same amount of compounds from plasma. Sample extraction and subsequent HPLC analysis were carried out as described previously. The intra-assay and inter-day precision and accuracy were evaluated by analyzing blank plasma spiked with different amounts of 9-hydroxyrisperidone and risperidone, and by calculating their concentrations from a standard curve produced on the same day. The inter-day assay variance was determined by analysing 1 mL aliquots of spiked plasma samples containing risperidone and 9-hydroxyrisperidone on different days. Plasma samples from patients were stored at −20 °C until analysis. The risperidone and 9-hydroxyrisperidone solutions were stable for up to 6 months.

2.7. Application—case report

A 21-year-old patient with moderate mental retardation had been treated with the typical antipsychotic drugs haloperidol (5.5 mg/day) and levomepromazine (250 mg/day) for years. After adding 4.5 mg of risperidone to his medication, severe extrapyramidal symptoms developed within days.

To analyze the cause of the sudden appearance of the treatment-related extrapyramidal side-effects, therapeutic drug monitoring of risperidone was carried out. Blood samples from the patient were collected by venipuncture into heparinized tubes in the morning before breakfast and before the morning dose. One tube containing 5 mL blood was immediately centrifuged (1900 *g*) at ambient temperature,

and the separated plasma layer was stored at −20 °C until analysis. A second tube containing 5 mL blood was stored at −20 °C until genotyping analysis. The *CYP2D6* genotype was determined using a previously published method [8].

3. Results

3.1. Chromatography and recovery

Fig. 2 shows chromatograms of blank plasma from a healthy drug-free blood donor spiked with 9-hydroxyrisperidone, risperidone and the internal standard and plasma from a patient after intake of 4.5 mg of risperidone. The retention times were 3.6, 5.0, and 8.2 min for spiked samples of 9-hydroxyrisperidone, risperidone and I.S., respectively. The chromatographic run time was less than 10 min.

The standard curves for risperidone and 9-hydroxyrisperidone were linear over the range 10–160 nmol/L (r=0.99) for both compounds. The mean recoveries for risperidone and 9-hydroxyrisperidone calculated at three different concentrations (20, 40 and 80 nmol/L) were 96.8 and 99.4%, respectively (Table 2).

3.2. Precision and accuracy

The method was found to be reproducible, as indicated by the low values obtained for the coefficients of variation (<8%) for risperidone and

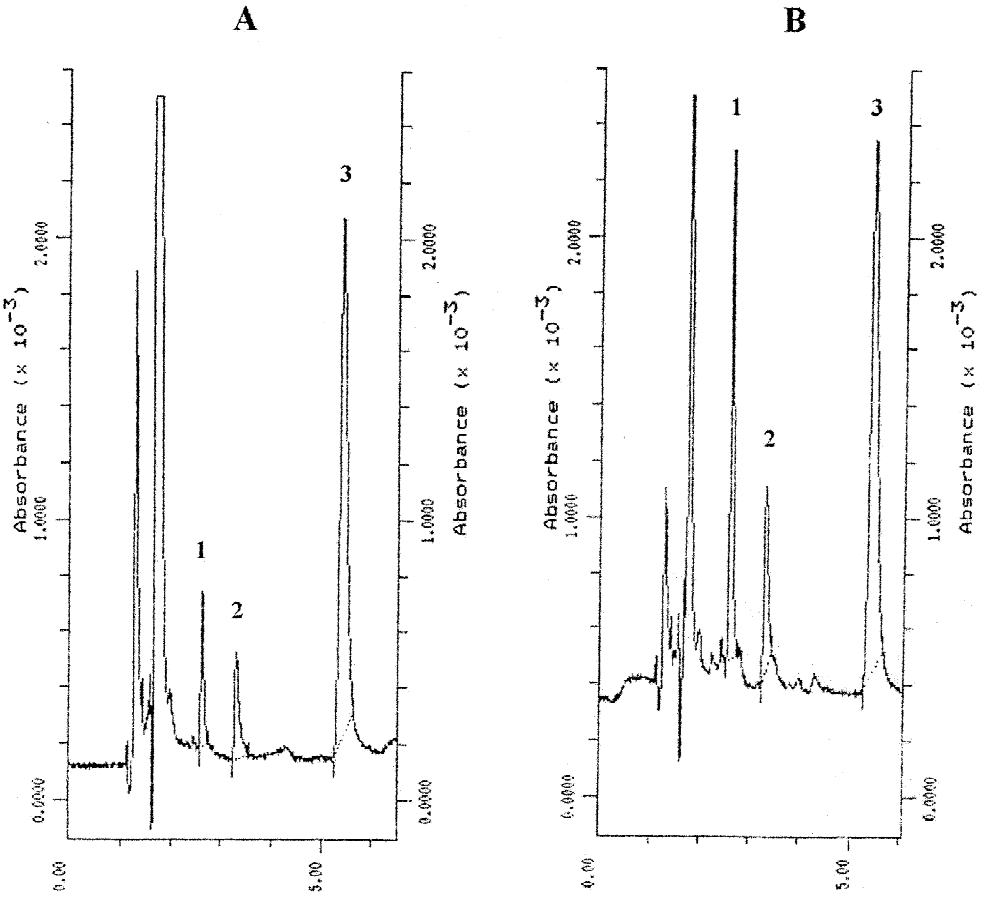

Fig. 2. (A) Plasma blank spiked with 40 nmol/L 9-hydroxyrisperidone, risperidone and the internal standard (methylrisperidone). (B) Plasma from a patient treated with risperidone. Peaks: 1=9-hydroxyrisperidone, 2=risperidone, 3=methylrisperidone (internal standard).

Table 2
Recovery of the analytical method (n=11)

Conc. (nmol/L)	Recovery (%) (mean±SD)	
	Risperidone	9-Hydroxyrisperidone
20	91.0±3.3	96.7±6.5
40	102.8±6.2	104.5±1.3
80	96.6±5.2	97.1±5.0

9-hydroxyrisperidone (Table 3). The lower limits of quantitation were 5 nmol/L for both risperidone and 9-hydroxyrisperidone.

3.3. Analysis for interference

No endogenous substances interfered with the detection of 9-hydroxyrisperidone, risperidone or I.S.

Table 3
Accuracy, intra-assay reproducibility and precision (n=8)

	Conc. added (nmol/L)	Accuracy (%)	C.V. (%)	
			Intra-assay	Inter-day
Risperidone	20	89.8	4.6	8.0
	40	103.4	2.9	6.0
	80	97.5	1.5	5.3
9-Hydroxy-risperidone	20	91.8	6.5	6.8
	40	101.6	1.6	1.2
	80	98.4	1.2	5.1

Table 4
Chromatographic interference with risperidone determination: retention times of selected psychotropic drugs

Drug	Retention time (min)	Drug	Retention time (min)
Clorazepate	1.0.	Fluvoxamine	10.0
Alprazolam	2.6	Chlorpromazine	n.d.
Midazolam	2.6	Flurazepam	n.d.
Lorazepam	2.7	Haloperidol	n.d.
Imipramine	3.7	Lithium	n.d.
Zopiclone	3.8	Perphenazine	n.d.
Chlometiazol	3.9	Phenobarbital	n.d.
Levopromazine	3.9	Pimozide	n.d.
Fluoxetine	4.2	Pinazepam	n.d.
Prometazine	4.8	Sulpiride	n.d.
Amitryptiline	5.2	Tiapridal	n.d.
Clomipramine	5.9	Triazolam	n.d.
Thioridazine	6.4	Trifluoperazine	n.d.
Zolpidem	6.6	Trimipramine	n.d.
Maprotiline	7.4	Valpromide	n.d.

n.d., not detectable.

at their retention times. The specificity of the method was tested by injecting 42 commonly used psychiatric drugs. Numerous antidepressants, anxiolytic and neuroleptic drugs did not interfere with the method (Table 4).

3.4. Case study

The present method was applied to the routine therapeutic drug monitoring for risperidone and 9-hydroxyrisperidone in psychiatric patients [9]. The method was also used to determine the plasma concentration of risperidone in the reported case of a patient treated daily with 4.5 mg. The plasma levels of the described patient were 182.2 and 42.3 nmol/L for risperidone and 9-hydroxyrisperidone, respectively. The ratio of risperidone to 9-hydroxyrisperidone was 4.31. The active moiety (sum of risperidone plus metabolite) was 224.5 nmol/L. The *CYP2D6* genotype of the patient was **1/*4*, thus the patient was classified as heterozygous (one active *CYP2D6* gene).

4. Discussion

The aim of the present study was to develop a specific method for the quantitative determination of risperidone and 9-hydroxyrisperidone in plasma samples. The method was designed to be rapid, specific and easy to perform. The principal advantage of the method presented here relative to other published methods [10,11] is the use of liquid–liquid sample preparation as part of the chromatographic procedure.

Antipsychotic polytherapy is relatively common among psychiatric patients [12]. Commonly a patient may receive different antipsychotic and antidepressant drugs concomitantly. The present method is capable of separating the most common psychotropic drugs used in clinical practice (42 tested drugs), as well as endogenous interfering substances.

The described liquid–liquid extraction method is less expensive than solid-phase sample preparation [10,11]. Methylrisperidone was used as internal standard, similar to previous studies [13–15], and is a more suitable internal standard than haloperidol [16] or clozapine [17] due to the fact that psychiatric

patients may frequently be treated simultaneously with several antipsychotics [12], as the patient described here.

By using a rather laborious extraction procedure, Woestenborghs et al. [13] obtained recoveries of risperidone and 9-hydroxyrisperidone of 75 and 70%, respectively. Our method, with extraction recoveries of 96.8% for risperidone and 99.4% for 9-hydroxyrisperidone, is more efficient that Olesen's method [16], for which extraction recoveries were 60 and 45%, respectively.

The intra-assay and inter-day variations of risperidone and 9-hydroxyrisperidone (<8%) were found to be better than for most other reported methods [13,17]. This method is able to quantify the two substances with good precision in the range 10–160 nmol/L and is well adapted for the monitoring of plasma levels in psychiatric patients.

Since risperidone is metabolized by CYP2D6 [7], and this enzyme is also involved in the metabolism of several other clinically used psychotropic and other drugs (such as thioridazine, haloperidol, tricyclic antidepressants, beta-blockers, etc.) [18], pharmacokinetic interactions due to competitive inhibition of the enzyme may occur. Furthermore, the activity of the CYP2D6 enzyme is genetically impaired in around 7% of Caucasians [18], thus, in this group of patients, the disposition of risperidone and other drugs metabolized by this enzyme is impaired.

The case of the patient described here points out the importance of pharmacokinetic interactions in antipsychotic drug treatment. The risperidone plasma concentration of the patient was higher than the average range [9]. Furthermore, the risperidone/9-hydroxyrisperidone ratio was 4.31, substantially higher than the usual value for patients receiving risperidone monotherapy. The risperidone/9-hydroxyrisperidone ratio is correlated to the CYP2D6 enzyme activity as measured by debrisoquine hydroxylation, and in patients receiving concomittant treatment with known strong inhibitors of CYP2D6 the ratio is higher (0.2 ± 0.3 versus 0.5 ± 0.4 [9]). The present data suggest that the severe extrapyramidal symptoms of the patient may have been the result of a drug interaction with haloperidol and levomepromazine causing strong CYP2D6 enzyme inhibition. Our conclusion is further supported by the CYP2D6 genotyping analysis of the patient, which revealed that the patient was a heterozygote for the *CYP2D6* gene, thus enzyme activity was not completely impaired by the genotypically inherited poor metabolizer status.

In conclusion, in the case of severe side-effects or therapeutical inefficacy, therapeutic plasma concentration monitoring of risperidone with the present method may help to determine CYP2D6 inhibition due to genetically impaired CYP2D6 activity or drug interaction.

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