ELSEVIER

Journal of Chromatography B, 772 (2002) 373–378

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Simplified high-performance liquid chromatographic method for determination of risperidone and 9-hydroxyrisperidone in plasma after overdose

K. Titier, E. Déridet, E. Cardone, A. Abouelfath, N. Moore*

Department of Pharmacology and Toxicology, EA 525, University Victor Segalen, Place Amélie Raba-Léon, 33076 Bordeaux Cedex, France

Received 2 October 2001; received in revised form 22 January 2002; accepted 6 February 2002

Abstract

For toxicological purposes, a HPLC assay was developed for the simultaneous determination of risperidone and 9-hydroxyrisperidone in human plasma. After a single-step liquid–liquid extraction, both compounds were separated on a C_{18} column and measured at 280 nm. A good inter-assay accuracy (<116%) was achieved with inter-assay precision less than 12%. Quantification limits were 10 ng/ml. This rapid method (run time <5 min) is currently used for poison management. © 2002 Published by Elsevier Science B.V.

Keywords: Risperidone; 9-Hydroxyrisperidone

1. Introduction

Risperidone (RSP), a benzisoxasole derivative, is one of the newer high-potency antipsychotic agents with 5-HT2 and D2 receptor blocking capacity [1]. These compounds are rapidly taking a large market share for the treatment of schizophrenia and other psychoses. RSP is effective in the treatment of positive as well as negative symptoms of schizophrenia. It has a limited risk for causing extrapyramidal side effects at therapeutically effective doses. Its main metabolite, 9-hydroxyrisperidone (OH-RSP), has an antipsychotic activity profile similar to that of the parent drug [2]. Suicide and suicide attempts are very frequent in the user population for antipsychotics [3,4], and several intoxications involving risperidone have already been published [5–7]. Consequently, it is essential to use a simple and rapid method for the determination of risperidone and its metabolite in case of poisoning.

The published high-performance liquid chromatography (HPLC) methods for both RSP and OH-RSP analyses in biological fluids included UV [8–12] or electrochemical detection [13–16]. Among those different techniques, some require solid-phase extraction [9,11,16] or multi-step liquid–liquid extraction [12,15] which is not easy to run for routine application. On an other hand, the use of another psychotic drug as internal standard described in some HPLC techniques [8,10] could be detrimental for the management of multiple drug intoxications.

*Corresponding author. Tel.: +33-5-5757-1560; fax: +33-5-5624-5889.

E-mail address: nicholas.moore@pharmaco.u-bordeaux2.fr (N. Moore).

1570-0232/02/$ – see front matter © 2002 Published by Elsevier Science B.V.
PII: S1570-0232(02)00091-0

The aim of this study was to establish a simple, rapid and accurate HPLC method for the measurement of risperidone and hydroxyrisperidone in plasma after overdose. The assay requires a small sample volume, involves a single step liquid extraction with a specific internal standard (I.S.; methylrisperidone) and a short chromatographic run. The method described is especially adapted for RSP poisoning cases leading to concentrations generally above the therapeutic range (1–28 ng/ml) [17].

2. Experimental

2.1. Chemicals

RSP (Fig. 1) was purchased from Sigma (St. Quentin Fallavier, France). OH-RSP and I.S., methylrisperidone (R068808), were generously donated by Janssen Foundation (Beerse, Belgium). All reagents used for the assay were of HPLC or analytical grade. Water was deionized and purified by a Milli-Q system (Millipore, Bedford, MA, USA). Heparinized healthy human volunteers plasma was purchased from Etablissement de Transfusion Sanguine d'Aquitaine (ETSA, Bordeaux, France).

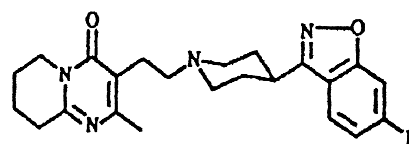
2.2. Equipment

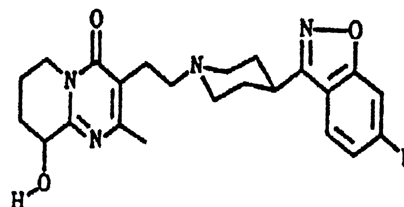
The chromatographic apparatus (ThermoQuest, San Jose, CA, USA) was equipped with a constant flow pump M 590, a Model 1000 ultraviolet detector and a Datajet integrator.

The chromatographic separation was performed at room temperature on a Novapack C_{18} analytical column (Waters) (15 cm×3.9 mm; 5 μm particle size).

The mobile phase consisted of acetonitrile–phosphate buffer, pH 3.8 (0.1 *M*) (68:32, v/v). The mobile phase was filtered through a 0.5 μm filter and degassed before use. The flow-rate was maintained at 1 ml/min.

The compounds were chromatographed at 280 nm within 5 min. The unknown concentrations of RSP and OH-RSP were quantified using linear regression of response (drug/I.S. peak height ratio) versus RSP or OH-RSP concentrations.





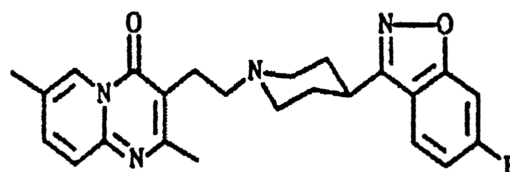


Fig. 1. Chemical structures of risperidone, 9-hydroxyrisperidone and internal standard.

2.3. Standard solutions

Stock solutions of RSP, OH-RSP and I.S. were prepared at a concentration of 1 mg/ml in methanol and stored at −20 °C. They were stable for at least 3 months. The internal standard stock solution was diluted daily in bidistilled water to yield a 3 μg/ml working solution.

Plasma standards containing known amounts of risperidone and 9-hydroxyrisperidone were prepared by spiking plasma to yield concentrations of 10, 20, 50, 100, 150 and 200 ng/ml. Plasma quality controls spiked with 10, 30, 70, 120 and 170 ng/ml were prepared to test the accuracy and the precision of the method.

2.4. Sample preparation

To 200 μl of plasma was added 50 μl of I.S. (3 μg/ml) and 500 μl of borate buffer, pH 10. The mixture was extracted in 5 ml of pentane–dichloromethane (70:30, v/v) by rotative shaking during 10 min. After centrifugation, the organic layer was evaporated to dryness under nitrogen. The residue was dissolved in 100 μl of mobile phase and 80 μl was injected into the chromatograph.

2.5. Recovery, precision, accuracy

2.5.1. Recovery

Extraction recoveries from human plasma were determined by comparison of HPLC responses from extracted samples containing known amounts of RSP and OH-RSP (30, 70, 120 ng/ml) to those from unextracted and directly injected standards, spiked with the same amounts.

2.5.2. Precision

The precision of the developed method was determined by analysis of five quality control samples containing 10, 30, 70, 120 and 170 ng/ml of risperidone and its metabolite. Each of the quality control samples was replicated ($n=6$) and analysed on 4 consecutive days. Subsequently, the mean of each set of the concentrations and the percent deviation of the quality control samples were calculated. One-way analysis of variance (ANOVA), with the day of analysis as variable of classification, was used to calculate the inter- and intra-assay variation.

The following formulas were used in order to calculate the inter-assay precision and intra-day precision, respectively:

$$\frac{[(\text{Day mean square} - \text{error mean square})/n]^{1/2}}{\text{Grand mean}} \cdot 100\%$$

$$\frac{(\text{Error mean square})^{1/2}}{\text{Grand mean}} \cdot 100\%$$

The day mean square, the error mean square and grand mean square are expressions originating from ANOVA. n is the number of replicates within each day (six) for each concentration. If the error mean square is higher than the day mean square the inter-assay precision is regarded as zero. This signifies that no significant additional variation is observed as a result of performing the assay in different runs.

2.5.3. Accuracy

Accuracy was measured as the % difference from theoretical according to the equation:

$$\text{Accuracy (\%)} = (\text{concentration}_{\text{measured}}/\text{concentration}_{\text{theoretical}}) \cdot 100$$

3. Results and discussion

Under the chromatographic conditions, RSP and OH-RSP were sufficiently resolved from endogenous plasma and tissue compounds (Fig. 2) with the three compounds chromatographed within 5 min.

3.1. Precision, accuracy and linearity

The results obtained for precision and accuracy are listed in Table 1 and Table 2. The method showed a intra-assay precision ranging from 2.02 to 11.06% and from 2.10 to 11.17% for RSP and OH-RSP, respectively. The inter-assay precisions ranged from 2.37 to 10.69% and from 1.84 to 11.69% for RSP

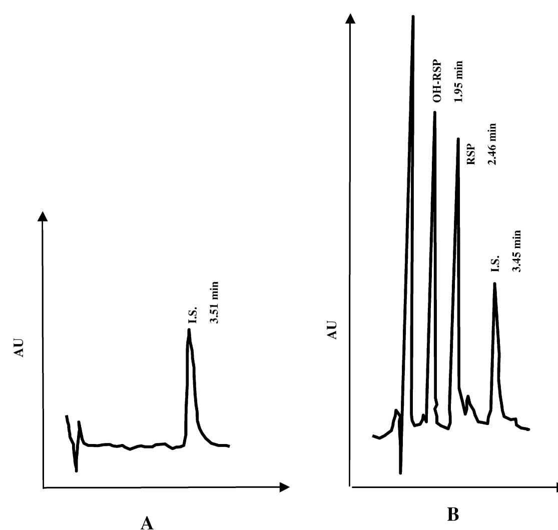


Fig. 2. Representative chromatograms of (A) blank plasma with internal standard (I.S.); (B) plasma spiked with 200 ng/ml of risperidone (RSP) and hydroxyrisperidone (OH-RSP).

Table 1
Precision and accuracy of the determination of risperidone and 9-hydroxyrisperidone in spiked plasma

Concentration (ng/ml)	Intra-assay precision (%)	Inter-assay precision (%)	Accuracy (%)
Risperidone			
10	11.06	7.19	113
30	8.36	10.69	111
70	5.87	2.37	109
120	2.71	5.43	108
170	2.02	4.09	108
9-Hydroxyrisperidone			
10	11.17	5.13	97
30	8.89	11.69	116
70	6.56	9.79	109
120	2.10	1.84	110
170	4.20	7.05	109

and OH-RSP, respectively. The inter-assay accuracies were better than 113 and 116% for RSP and OH-RSP, respectively. The lower limit of quantification was 10 ng/ml for both compounds.

From four calibration curves (Fig. 3) constructed with six unique calibration points ranging from 10 to 200 ng/ml a good correlation coefficient was found for risperidone [$r^2=0.97$; $y=(80\pm3.0)\cdot10^{-4}x+(0.024\pm0.034)$; $y=(\text{mean slope}\pm\text{SD})x+(\text{mean intercept}\pm\text{SD})$] and its metabolite [$r^2=0.99$; $y=(100\pm2.4)\cdot10^{-4}x+(0.074\pm0.027)$].

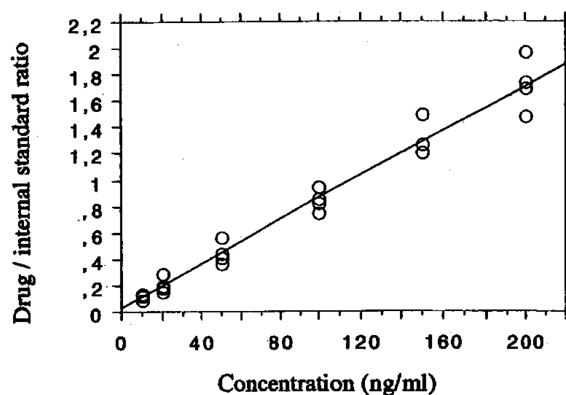


Fig. 3. Risperidone standard curve (generated over 4 separate days). Mean $r^2=0.97$; slope (mean±SD)=$(80\pm3.0)\cdot10^{-4}$; intercept (mean±SD)=0.024±0.034.

3.2. Extraction efficiency

The recoveries of risperidone were (mean±RSD, $n=4$) 87.4±6.3, 84.4±3.4, 83.5±9.3% for 70, 120 and 170 ng/ml human plasma quality controls (QCs). For the metabolite data were 85.8±7.6, 79.3±3.3, 76.9±9.9%, respectively. The recovery of I.S. was 96.5±3.5%.

3.3. Stability

To determine the influence of temperature on the stability of drugs, QC samples spiked with RSP and

Table 2
Data of the determination of risperidone and 9-hydroxyrisperidone in spiked plasma

	Day 1		Day 2		Day 3		Day 4	
	Mean±SD ($n=6$)	Accuracy (%)	Mean±SD ($n=6$)	Accuracy (%)	Mean±SD ($n=6$)	Accuracy (%)	Mean±SD ($n=6$)	Accuracy (%)
Risperidone (ng/ml)								
10	10.4±0.5	104	12.3±1.6	123	10.7±1.1	106	12.0±1.5	120
30	34.5±0.5	115	33.75±4.1	113	29.8±2.0	99	30.67±1.2	102
70	77.3±4.8	110	77.5±3.4	111	75.6±6.3	108	73.4±2.5	105
120	133.0±1.1	111	128.0±4.9	107	119.8±3.8	100	136.3±3.5	114
170	187.0±4.7	110	181.0±2.1	107	179.6±1.8	106	189.8±4.8	112
9-Hydroxyrisperidone (ng/ml)								
10	9.0±0.5	91	10.4±1.5	110	9.3±0.7	109	10.1±1.3	110
30	34.9±2.3	116	34.0±2.6	113	32.67±1.2	101	32.2±2.5	102
70	81.0±5.7	116	83.8±0.4	120	72.5±8.3	104	64.0±3.2	105
120	146.7±1.0	122	138.8±2.8	116	125.5±4.4	104	118.2±3.4	114
170	207.5±7.1	122	198.8±2.8	117	173.3±7.7	102	159.8±11.1	94

OH-RSP were stored under different conditions: at −20 °C during 32 days; at +4 °C during 72 h; at +20 °C during 24 h. No decomposition of RSP and OH-RSP was noted in the frozen samples over 1 month. Storage for 72 h at +4 °C produced no significant decrease of RSP and OH-RSP concentrations. Storage at room temperature for 24 h showed good stability of both compounds, with RSD and bias values less than 10%. Both compounds appeared to be stable after three repeated freeze–thaw cycles, as evidenced by the RSD of less than 10% and the accuracy bias of less than 17%.

3.4. Specificity

Potential interferences with determinations were investigated by assaying different blank plasma spiked with drugs commonly found in voluntary drug intoxications. No significant chromatographic interference was found with tested tricyclic antidepressants (amitriptyline, clomipramine, imipramine), selective serotonin reuptake inhibitors (fluoxetine, paroxetine), benzodiazepines (diazepam, bromazepam, zopiclone, flunitrazepam) and neuroleptics (haloperidol, clozapine).

3.5. Clinical case

In voluntary drug intoxications, the ingested dose and the beginning of intoxication are often unknown. Nevertheless, the expected concentrations are generally above the therapeutic concentration. The therapeutic concentrations of risperidone are in the range of 1–28 ng/ml [17]. By using the described method we were able to manage a few cases of risperidone poisoning. A typical patient chromatogram is shown in Fig. 4. This patient also ingested clonazepam and paroxetine. Consequently, in order to verify the specificity of detection, an absorption spectrum was carried out on the chromatographic peaks of RSP and OH-RSP obtained from this drug overdose patient. No interference was noted. The risperidone concentration was markedly above the therapeutic concentration (317 and 146 ng/ml for risperidone and its metabolite, respectively).

In conclusion, the HPLC procedure described is specific and easy to perform, leading to determination of plasma risperidone and hydroxyrisperidone concentrations. The limit of quantitation and short duration of this assay are particularly adapted to the management of acute RSP intoxications.

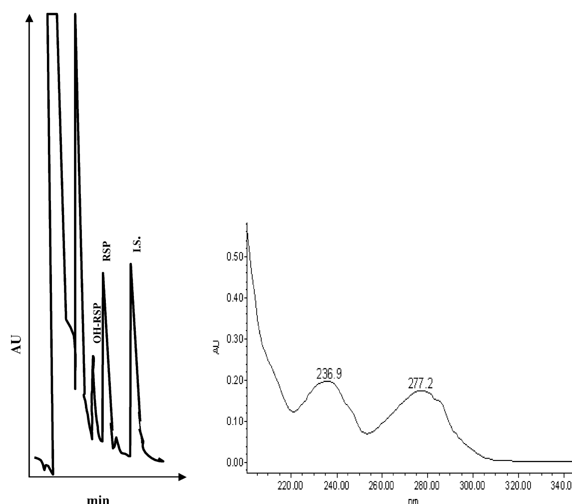


Fig. 4. Chromatogram of patient sample (diluted 1:1, v/v, in blank plasma) at 280 nm. Specific absorption spectrum of risperidone (RSP) performed on photodiode array detector.

In this routine context, simplicity and rapidity are clear bonuses. The relevance of plasma risperidone monitoring in routine in clinical practice is uncertain. Its determination in overdose would help quantify the dose taken, and alert to large overdoses which may pose a cardiac risk.

References

[1] E. Richelson, J. Clin. Psychiatry 60 (1999) 5.
[2] J.S. Markowitz, C.S. Brown, T.R. Moore, Ann. Pharmacother. 33 (1999) 73.
[3] C. Montout, F. Casadebaig, R. Lagnaoui, H. Verdoux, A. Philippe, B. Bégaud, N. Moore, Schizophr. Res., in press. Manuscript reference SCHRES 1611. Still waiting publication date, since August 2001.
[4] E.C. Harris, B. Barraclough, Br. J. Psychiatry 170 (1997) 205.
[5] D.S. Ravin, J.W. Levenson, Ann. Pharmacother. 31 (1997) 867.
[6] A.C. Springfield, E. Bodiford, J. Anal. Toxicol. 20 (1996) 202.
[7] G. Laroussinie, M. Zenut, M. Beal, C. Bernard, J. Lavarenne, Therapie 52 (1997) 155.
[8] A. Avenoso, G. Facciola, M. Salemi, E. Spina, J. Chromatogr. 746 (2000) 173.

[9] M.C. Price, D.W. Hoffman, Ther. Drug Monit. 19 (1997) 333.
[10] O.V. Olesen, K. Linnet, J. Chromatogr. 698 (1997) 209.
[11] T. Nagasaki, T. Ohkubo, K. Sugawara, N. Yasui, H. Furukori, S. Kaneko, J. Pharm. Biomed. Anal. 19 (1999) 595.
[12] R. Woestenborghs, W. Lorreyne, F. Van Rompaey, J. Heykants, J. Chromatogr. 583 (1992) 223.
[13] M. Aravagiri, S.R. Marder, T. Van Putten, K.K. Midha, J. Pharm. Sci. 82 (1993) 447.
[14] M. Aravagiri, S.R. Marder, D. Wirshing, W.C. Wirshing, Pharmacopsychiatry 31 (1998) 102.
[15] J.P. Le Moing, S. Edouard, J.C. Levron, J. Chromatogr. 614 (1993) 333.
[16] D.S. Schatz, A. Saria, Pharmacology 60 (2000) 51.
[17] A.E. Balant-Gorgia, M. Gex-Fabry, C. Genet, L.P. Balant, Ther. Drug Monit. 21 (1999) 105.