A SIMPLE AND SENSITIVE METHOD FOR THERAPEUTIC MONITORING OF RISPERIDONE AND 9-HYDROXYRISPERIDONE IN HUMAN PLASMA/SERUM

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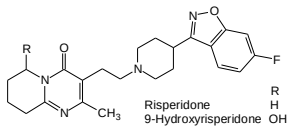
Introduction

Risperidone (Risperdal, Janssen-Cilag/Organon), is an atypical antipsychotic introduced in 1993 that is licensed in the UK for the treatment of schizophrenia. Risperidone is metabolized to 9-hydroxyrisperidone (9-OH-risperidone), which has a similar pharmacological profile to that of the parent compound and accumulates in plasma on chronic dosing. Some studies have demonstrated significant potential for drug-drug interactions with risperidone [1], hence therapeutic monitoring may help not only in assessing compliance with oral dosage, but also in dose optimization.

Early methods for risperidone/9-OH-risperidone generally lacked sensitivity and specificity [2,3]. Other more recent methods either use HPLC-MS [4] or involve extensive sample preparation.



We have developed a relatively simple and rapid method for risperidone/9-OH-risperidone assay in human plasma/serum. The limit of accurate measurement is 2 µg/L for both risperidone/9-OH-risperidone, using a 1.0 mL sample. Hence the method has sufficient sensitivity to measure the concentrations of these compounds attained during low-dose risperidone treatment.

Materials and methods

Risperidone and 9-OH-risperidone were gifts from Janssen (Beerse, Belgium). The internal standard (benzimidazole), Tris (tris[hydroxymethyl]aminomethane) and newborn calf serum were from Sigma-Aldrich and ammonium perchlorate from Fluka. Coarsley filtered human serum was from Scipac (Sittingbourne, Kent). Methanol and methyl *tert*.-butyl ether (MTBE) were HPLC grade.

HPLC: PU-1580 intelligent pump, AS-950 autosampler and AS-1550 UV detector (280 nm) (all Jasco, UK). Data collection was via an Atlas chromatography data system (Thermo, UK). The analytical column (125 x 4.6 mm i.d.) contained Phenosphere SCX silica (5 µm aps) (Phenomenex, Cheshire, UK). The eluent was 35 mmol/L ammonium perchlorate in methanol adjusted to apparent pH 5.0 (flow-rate of 1.0 mL/min).

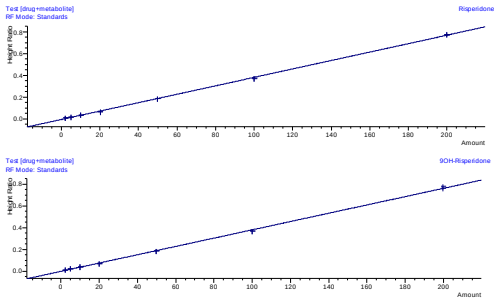
Calibration solutions were prepared in newborn calf serum by appropriate dilution of stock risperidone and 9-OH-risperidone working solutions (10 and 1 mg/L free base in methanol) to give concentrations of 2, 5, 10, 20, 50, 100 and 200 µg/L both analytes. Internal quality control (IQC) solutions were similary prepared in human serum by dilution of separate stock solutions to give 6, 15 and 70 µg/L for both analytes. Calibration standards and IQCs were stored at -20°C.

Samples were measured in duplicate. Internal standard solution, aqueous benzimidazole (2 mg/L, 50 µL) and Tris buffer (2 mol/L, pH 10.6, 200 µL) were added to plasma/serum (1.0 mL). After extraction into MTBE by vortex-mixing (30 s) and centrifugation (1000 g, 4 min), the extract was evaporated to dryness. The residue was reconstituted in methanol and a portion (100 µL) was analysed by HPLC.

Results and Discussion

The calibration graphs for risperidone and 9-OH-risperidone were linear over the calibration range (r > 0.99, Figure 1). The recovery of risperidone and 9-OH-risperidone from calf serum and human plasma/serum were compared by replicate analyses (n=6) of calibration solutions spiked in calf serum and human plasma/serum. There was no significant difference in the extraction efficiency of the analytes from calf and human serum/plasma (paired t-test, $p<0.05$).

Figure 1. Typical calibration graphs of risperidone and 9-hydroxyrisperidone



Intra- and inter-assay precision were evaluated at three different concentrations (n = 5) (Tables 1 & 2). Individual batch analyses were accepted if the IQC results were within ± 10 % of the nominal values, or ± 15 % for the lowest IQC.

Table 1. Intra-assay precision at three concentrations (n = 5)

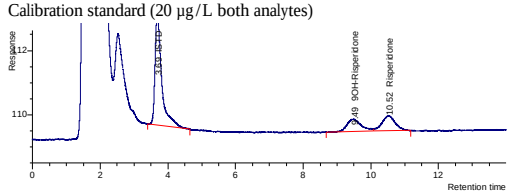
Analyte	Nominal (µg/L)	Measured (µg/L)	RSD (%)
Risperidone	6	6	9
	15	17	8
	70	67	6
9-OH-Risperidone	6	8	12
	15	17	5
	70	68	6

Table 2. Inter-assay precision at three concentrations (n = 6)

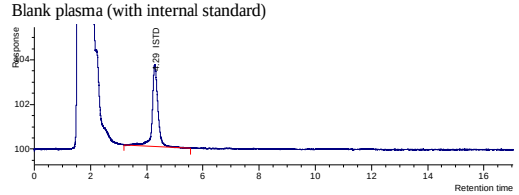
Analyte	Nominal (µg/L)	Measured (µg/L)	RSD (%)
Risperidone	6	7	8
	15	17	7
	70	76	2
9-OH-Risperidone	6	6	15
	15	17	6
	70	80	3

In clinical samples (n = 55, prescribed dose 2-8 mg/d risperidone) the median (10th and 90th percentiles) risperidone and 9-OH-risperidone were 15 (5-66) and 28 (8-59) µg/L, respectively. Typical chromatograms are shown below:

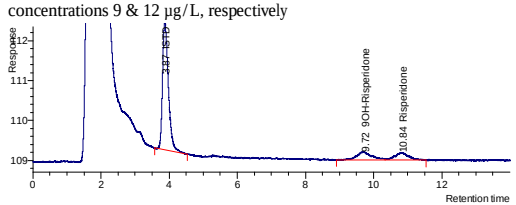
Calibration standard (20 µg/L both analytes)



Blank plasma (with internal standard)



Patient plasma sample (dose 2 mg/d risperidone); risperidone and 9-OH-risperidone concentrations 9 & 12 µg/L, respectively



Conclusions

The method described is simple, economical, and selective with no interferences from commonly co-prescribed psychoactive drugs, and has adequate sensitivity to measure risperidone and 9-OH-risperidone concentrations in plasma/serum from patients prescribed low dose risperidone.

References

1. Leysen JE, et al. J Pharmacol Exp Ther. 1988;247:661-70.
2. Price MC, Hoffman DW. Ther Drug Monit 1997;19:333-7.
3. Nagasaki T, et al. J Pharm Biomed Anal 1999;19:595-601.
4. Kratzsch C, et al. J Mass Spectrom 2003;38:283-95.