A SIMPLE AND SENSITIVE METHOD FOR THERAPEUTIC MONITORING OF AMISULPRIDE IN HUMAN PLASMA/SERUM

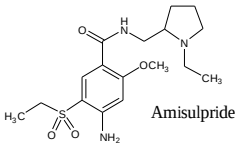
Connally G[a], Morgan PE[b], Spencer EP[b], Flanagan RJ[b]
[a]Department of Chemistry, Loughborough, , Leicestershire LE11 3TU, UK
[b]Medical Toxicology Unit, Guy's & St. Thomas' Hospital Trust, London SE14 5ER, UK

Guy's and St Thomas' Hospital NHS Trust

Introduction

Amisulpride (Solian, Sanofi-Synthelabo) is a second generation(atypical) antipsychotic. It is a dopamine antagonist with high selectivity for dopamine D_2 and D_3 receptors [1]. Low dose (50-300 mg/d) treatment is used in patients with predominantly negative symptoms of schizophrenia when it seems to facilitate dopaminergic transmission [1,2]. For patients suffering from acute episodes of schizophrenia with predominantly positive symptoms or from chronic schizophrenia with positive and negative symptoms, higher doses (400-1200 mg/d) are given [1,3]. Corresponding to the the two dose ranges, two plasma threshold concentrations associated with improved treatment efficacy (28-92 µg/L at low-dose and >153 µg/L at high dose) have been suggested [4]. The two main metabolites of amisulpride are both inactive, with much of the drug being excreted unchanged in urine.

We have developed a simple and sensitive method for the measurement of total amisulpride [(-)S- and (+)R-amisulpride] in human plasma/serum. The limit of accurate measurement is 2 µg/L with a 200 µL sample.



Amisulpride

Materials and methods

Amisulpride was a gift from Sanofi-Synthelabo (UK). The internal standard (benzimidazole), Tris (tris[hydroxymethyl]aminomethane) and newborn calf serum were from Sigma-Aldrich, and ammonium perchlorate was from Fluka. Coarsely filtered human serum was from Scipac (Sittingbourne, Kent). Methanol and methyl *tert.*-butyl ether (MTBE) were HPLC grade. Water was deionised.

HPLC system: PU-1580 intelligent pump, AS-950 autosampler, and FP-1520 fluorescence detector (excitation: 270 nm, emission: 350 nm) (all Jasco, UK), and Atlas chromatography data system (Thermo, UK). The pre-column and analytical column, stainless steel tubes (10 and 125 x 4.6 mm i.d., respectively) contained Waters Spherisorb® S5SCX sulphopropyl-modified silica (5 µm aps) (both Hichrom, UK). The eluent was methanol containing 0.2 % perchloric acid (70 %) (flow-rate 1.0 mL/min).

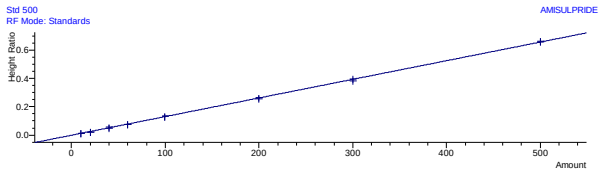
Calibration solutions were prepared in newborn calf serum by appropriate dilution of stock amisulpride solution (100 mg/L free base in methanol) to give concentrations of 10, 20, 40, 60, 100, 150, 200, 300, 500 µg/L amisulpride. Internal quality control (IQC) solutions were similarly prepared in human serum by dilution of separate stock solution to give 25, 75 and 250 µg/L amisulpride. Calibration standards and IQCs were stored at -20°C.

Sample preparation : Internal standard solution, aqueous benzimidazole (2 mg/L, 25 µL) and Tris buffer (2 mol/L, pH 10.6, 100 µL) were added to plasma/serum (200 µL). After extraction into MTBE (600 µL) by mixing (30 s) and centrifugation (1000 g, 4 min), the extract was evaporated to dryness and the residue reconstituted in methanol. A portion (100 µL) of the methanolic extract was analyzed.

Results and Discussion

Using the conditions described, the calibration graph was linear over the calibration range (r > 0.99). The amisulpride response was in fact linear up to 2000 µg/L.

Typical calibration graph for amisulpride



Intra- and inter-assay precision (RSD) were less than 10 % when assessed at three different concentrations (n = 6). Individual batch analyses were accepted if the IQC results were within ± 10 % of the nominal values, or ± 15 % for the lowest IQC.

Intra-assay precision at three concentrations (n=6)

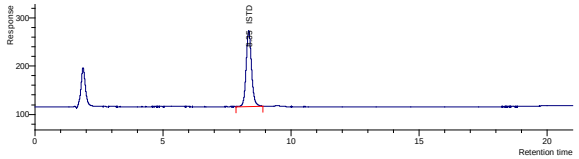
Nominal (µg/L)	Measured (µg/L)	RSD (%)
25	24	9.9
75	63	7.2
250	246	3.6

Inter-assay precision and accuracy at three concentrations (n=6)

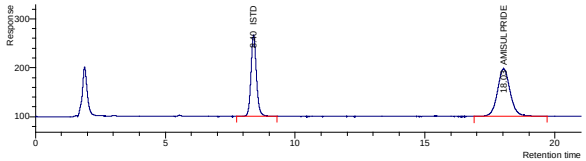
Nominal (µg/L)	Measured (µg/L)	RSD (%)	Accuracy (%)
25	25	3.2	101
75	64	4.1	99
250	240	4.9	96

The extraction of amisulpride from calf serum and human plasma/serum showed no significant difference (paired t-test, p > 0.05). In clinical samples (n = 23, median amisulpride dose 1200 mg/d, range 200-1200 mg/d) the median (10th and 90th percentiles) amisulpride concentration was 340 (127-892) µg/L. Representative chromatograms are shown below:

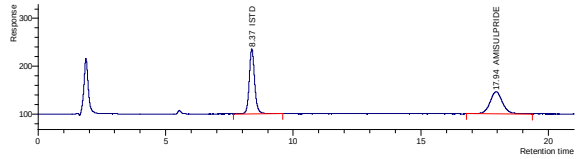
Plasma blank (with internal standard)



Calibration standard (200 µg/L amisulpride)



Patient sample (Dose 1200 mg/d; [Amisulpride]= 290 µg/L)



Conclusions

The method described is simple, economical, relatively rapid, requires a small sample volume. No inteference from commonly co-prescribed psychoactive drugs has been encountered. The method has sufficient sensitivity to measure amisulpride in plasma/serum from patients prescribed low doses of the drug.

References

1. Coukell AJ, Spencer CM, Benfield P. *CNS Drugs* 1996; **6**:237-256.
2. Loo H, Poirier-Littre M-F, Theron M, Rein W, Fleurot O. *Brit J Psychaitr* 1997; **170**:18-22.
3. Moller, HJ. *Acta Psychiatr Scand* 2000; **101**:17-22.
4. Xiberas X, Martinot J-L, Mallet L, Artiges E, Canal M, Loc'h C, Maziere B, Paillere-Martinot, M-L. *J Clin Psychopharmacol* 2001; **21**:207-214.