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Journal of Chromatography B, 784 (2003) 405–410
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Automated determination of amisulpride by liquid chromatography with column switching and spectrophotometric detection

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Received 14 August 2001; received in revised form 7 October 2002; accepted 10 October 2002

Abstract

A fully automated chromatographic method including on-line blood serum or plasma clean-up, isocratic high-performance liquid chromatography (HPLC) and spectrophotometric detection was developed for quantitative analysis of the new antipsychotic drug amisulpride. After injection of serum or plasma onto the HPLC system and clean-up on a pre-column (10×4.0 mm I.D.) filled with Silica CN 20 μm (pore size 10 nm) by an eluent consisting of 8% acetonitrile in deionized water, the chromatographic separation was performed on Lichrospher CN (5 μm; 250×4.6 mm I.D.) by an eluent consisting of 50% acetonitrile and 50% aqueous potassium phosphate buffer (0.008 *M*, pH 6.4). The UV detector was set at 254 nm. The limit of quantification was about 10 μg/l. The method revealed linearity between 10 and 600 μg/l (correlation coefficients R^2>0.9996). The inter-assay reproducibility (coefficient of variation) of quality control samples was between 2.8 and 11.3%. Inaccuracy was between −0.6 and +9.1%. The performance of daily calibration standards revealed an imprecision always below 15% and maximum inaccuracy of 7.7%. The method can be applied to therapeutic drug monitoring as well as pharmacokinetic studies of amisulpride.

Keywords: Column switching; Amisulpride

1. Introduction

Amisulpride is a substituted benzamide that has been introduced in the clinic as an atypical antipsychotic drug to treat psychotic syndromes [1]. It antagonizes dopamine D_2 and D_3 receptors [2]. In high doses, the drug blocks postsynaptic dopamine receptor-mediated effects similar to typical antipsychotics whereas in low doses it appears to antagonize predominantly presynaptic receptors and thus facilitate dopaminergic transmission [2]. Accordingly, two daily dose ranges have been defined: 50–300 mg to treat patients with predominantly negative symptoms of schizophrenia and 400–800 mg for patients with positive symptoms [1,3].

A recent positron emission tomography (PET) imaging study [4] reported a curvilinear relationship between doses and plasma concentrations of amisulpride and the dopamine receptor binding whereby plasma concentrations were related more accurately

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1570-0232/02/$ – see front matter
PII: S1570-0232(02)00808-5

with receptor binding than doses. Similar to the two dose ranges, two plasma ranges were found. Plasma concentrations below 92 ng/ml were associated with low striatal binding but sufficiently high to block extrastriatal dopamine receptors, whereas at plasma concentrations above 153 ng/ml marked binding to both extrastriatal and striatal regions, was found.

Amisulpride is a racemic mixture of two enantiomers, (−)*S*- and (+)*R*-amisulpride. A study of Castelli et al. 2001 [4] showed that (–)*S*-amisulpride is the active enantiomer of (±)-*RS*-amisulpride with regard to the ability to bind to dopamine D_2 and dopamine D_3 receptors. (−)*S*-amisulpride was found to be 20–40 times more potent than (+)*R*-enantiomer but had about the same activity as the racemate. It seems thus sufficient to analyse the racemate.

The high interindividual variability of amispulpride levels in blood at a given dose [5,6] and the good relation between drug levels in blood and dopamine receptor occupancy in brain [5], make it useful to monitor blood levels of patients treated with amisulpride. High-performance liquid chromatography (HPLC) methods with either UV [5–9], fluorescence [9,10] or mass spectrometric [11] detection, gas chromatography [12], a radioreceptor assay [13] and a radioimmunoassay [6] have been reported. Also, a HPLC assay is published by Ascalone et al. 1996 [20] about the stereospecific determination of amisulpride in human plasma and urine. Under these conditions a separation between the (+)*R*- and (−)*S*- enantiomers of amisulpride was achieved. All chromatographic methods require off-line sample clean up by liquid–liquid or solid-phase extraction to remove matrix constituents before chromatographic analysis. Here we describe a fully automated HPLC method with column switching for on-line sample clean up that enables rapid quantitative analysis of amisulpride for drug monitoring and pharmacokinetic studies.

2. Experimental

2.1. Chemicals

Amisulpride (racemate, free base, Lot. Bo. 160764) was kindly supplied by Sanofi-Synthelabo (Berlin, Germany). Acetonitrile (HPLC grade), orthophosphoric acid and dipotassium hydrogenphosphate trihydrate (p.a.) were obtained from Merck (Darmstadt, Germany). Water was deionized and filtered through a Milli-Q water processing system (Millipore, Eschborn, Germany).

2.2. Standards

Stock solutions for either quality control (QC) samples or calibration (C) samples were prepared by dissolving 10 mg of pure substance in 10 ml methanol each. They were diluted with deionized water and mixed with drug free plasma from healthy volunteers to obtain calibration standards of free base at six different concentrations, 10, 25, 50, 100, 200 and 600 ng/ml amisulpride. QC-samples were prepared at four different concentrations, 20, 60, 150 and 400 ng/ml. All standards could be stored in the dark at −20 °C for several months without measurable decomposition.

2.3. Plasma or serum samples

Human plasma or serum samples were obtained from healthy non-treated volunteers or from patients treated with amisulpride. To analyze trough serum concentrations blood was collected in the morning immediately before the first daily dose of amisulpride.

For demonstrating the stability of standards and samples we measured two concentrations of amisulpride stored under different conditions. Stability of amisulpride is satisfactory under all conditions. For example, for amisulpride stored at −20 °C for 6 months we found a loss of only 4%.

2.4. Instrumentation

The HPLC system consisted of an Agilent 1100 Series with a binary pump, an autosampler, a thermostatted column compartment (containing an electric six-port switching valve coupled to the autosampler) and a variable wavelength detector set at 254 nm. All HPLC components were purchased from BioRad (München, Germany). Data acquisition and integration was performed by means of the HP ChemStation (Version A.06.01). The analytical column (250×4.6 mm I. D.) was packed with Li-

chrospher CN (5 μm particle size) by MZ-Analysentechnik (Mainz, Germany). The clean-up column (10×4,6 mm) was filled with 20 μm particles of Silica (pore size 10 nm) CN (MZ-Analysentechnik).

2.5. Chromatographic procedure

Sample clean-up and chromatographic separation were performed at room temperature.

2.5.1. 0–5 min

After recentrifugation of serum or plasma (10 000 *g* for 5 min), 100 μl of the supernatant were injected onto the clean-up column. Proteins and other interfering compounds were washed to waste by using deionized water containing 8% (v/v) acetonitrile at a flow-rate of 1.5 ml/min.

2.5.2. 5–10 min

After the electric six port valve had been switched at 5 min, the analytical run was started. The analytes to be determined were eluted onto the analytical column (back flush) and separated by the analytical mobile phase (second HPLC pump) consisting of 50% acetonitrile and 50% K_2HPO_4 (0.008 *M*), adjusted to pH 6.4 by H_3PO_4 at a flow-rate of 1.5 ml/min.

2.5.3. 10–20 min

Ten minutes after the start of the analytical run (at 10 min) the switching valve was reset.

The clean-up column was replaced after injection of at least 100 serum or plasma samples.

2.6. Interferences

To control for possible interferences with drugs that may be used in combination with amisulpride the suggested interfering compounds were prepared in blank plasma as described for QC- and C-samples of amisulpride.

2.7. Calculations

The peak heights obtained from spiked serum or plasma, containing known amounts of drugs, were subjected to linear regression analysis for the calculation of correlation coefficients, slopes and intercepts. Drug concentrations in samples containing unknown amounts of drug were calculated on the basis of the computed regression lines.

2.8. Precision, accuracy, recovery and limit of detection

Precision and accuracy were evaluated by nine replicate analyses of quality-control samples at three different days to evaluate inter-assay variability. Precision was calculated as coefficient of variation (C.V.), while accuracy was determined from the difference between nominal and determined concentrations. Recovery was analysed by comparing the peak-heights of five serum samples with 10 ng/ml and five serum samples with 600 ng/ml amisulpride after column-switching with five samples of the same amount injected directly onto the analytical column. For determining the limit of detection we compared the integrated signal of five blank samples between 12 and 14 min with that of five samples supplemented with 5 ng/ml amisulpride.

3. Results and discussion

Column-switching techniques are most useful for automated and rapid analysis of drugs in complex matrices [14–16]. In addition to a conventional isocratic HPLC system, a pre-column for sample clean-up, a second HPLC pump and a six-port switching valve are needed. The procedure can be easily automated and urgent samples analyzed within 1 h.

Here we injected human serum directly onto the HPLC system and analyzed the sample for amisulpride within less than 20 min on a CN analytical column (Fig. 1A–C). Inter-assay variabilities (precision) according to the analysis of QC-samples was between 2.8 and 11.3% (Table 1). The inaccuracies were between −0.6 and +9.1% (Table 1). The recovery for the samples with 10 ng/ml was 140% and for the samples with 600 ng/ml amisulpride 110%. The limit of detection was ascertained at 5 ng/ml with a signal-to-noise ratio >4.

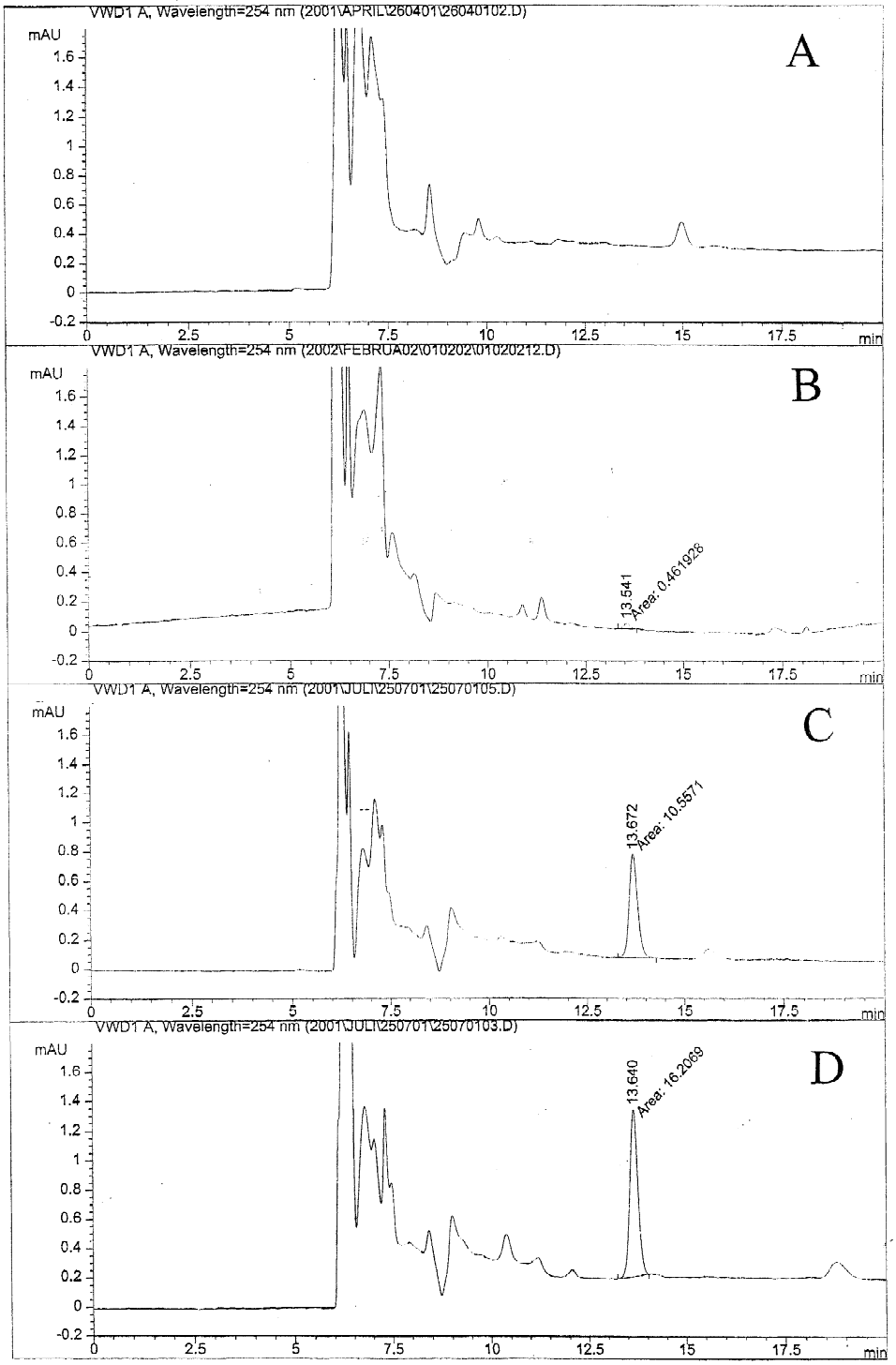

Fig. 1. Representative chromatograms of (A) a drug free serum; (B) a drug free serum supplemented with 10.00 μg/l amisulpride, (C) a drug free serum supplemented with 200.00 μg/l amisulpride (1); and (C) a chromatogram obtained after analysis of authentic human serum of a subject after ingestion of 600 mg (three doses of 200 mg) amisulpride/day (1, amisulpride 320.8 ng/l).

Table 1
Precision and accuracy of the amisulpride assay according to the analysis of quality control samples on different days

Analyte	Conc. added (ng/ml)	Mean (ng/ml)	Precision (%)	Inaccuracy (%)	*N*
Amisulpride	20.00	18.17	11.34	−9.14	7
	60.00	58.27	8.62	−2.89	9
	150.00	145.1	4.56	−3.24	9
	400.00	397.6	2.86	−0.59	9

A precision below 15% was considered acceptable for therapeutic drug monitoring [17], since it was in the range recommended for determination of antiepileptics according to legal requirements [17,18].

The method revealed linearity between 10.00 and 600.00 ng/ml with a correlation coefficients $R^2 \geq 0.9996$ [$y = 0.0026 \pm 0.0004$ (SD)$x + 0.0100 \pm 1.0209$ (SD)].

Testing standard solutions containing other psychotropic drugs that may be applied in combination with amisulpride, interferences could be observed with the neuroleptic clozapine and the antidepressant fluvoxamine (Table 2). This should be taken into account when these drugs are combined with amisulpride. Common therapeutic concentrations of risperidone and *O*-desmethylvenlafaxine are not expected to give an interfering signal.

The method described here offers the possibility to analyze samples automatically. All other chromatographic methods [5–13] use off-line sample clean-up. Chromatographic methods reported so far as well as radioreceptor or radioimmunoassays use batch wise analysis for efficient performance. This is not necessary for the procedure described here. The HPLC method allows measurement of single samples. A sample arriving in the morning can thus be processed the same day and reporting of results is possible within only 1 h in case of an emergency. Sufficiently rapid sample processing is as important as sufficient accuracy of the method for an effective therapeutic drug monitoring service [19]. The described method exhibits both, sufficient accuracy and sufficient speed to report valid results within an appropriate time schedule. HPLC with on-line column switching as described here is therefore useful for therapeutic drug monitoring of amisulpride and also for pharmacokinetic studies.

Table 2
List of tested interferences and retention time differences to amisulpride

Drug	Retention time (min) ± Amisulpride
Alprazolam	n.d.
Amisulpride	0.00
Amitriptyline	+4.6
Carbamazepine	n.d.
Citalopram	+2.5
Clomipramine	+5.7
Clozapine	**+0.2**
Desipramine	+4.0
Diazepam	n.d.
Dipiperone	n.d.
Fluoxetine	+3.57
Fluvoxamine	**+0.5**
Haloperidol	+2.6
Imipramine	+4.4
Lorazepam	n.d.
Maprotiline	+4.3
N-Desmethyl-venlafaxine	+1.0
Nefazodone	n.d.
Nordiazepam	n.d.
Norclozapine	n.d.
Nortryptiline	+4.0
O-Desmethyl-venlafaxine	−0.8
Oxazepam	n.d.
Paroxetine	n.d.
Risperidone	−0.34
Sertraline	n.d.
Temazepam	n.d.
Venlafaxine	+3.7
Zolpidem	n.d.

n.d., no detectable peak from 0 to 20 min. Relevant interferences are bold.

Acknowledgements

The authors thank Dr. F.-X. Eich and Sanofi-Synthelabo (Berlin, Germany) for generous gift of pure substance and financial support. The technical assistance of Anette Rieger-Gies helped us substantially to finish this work.

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