Journal of Chromatography, 416 (1987) 414–419
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3576

Note

Determination of a new benzamide, amisulpride, in human plasma by reversed-phase ion-pair high-performance liquid chromatography

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(First received October 31st, 1986; revised manuscript received January 4th, 1987)

Amisulpride, amino-4-N-(ethyl-1-pyrolidinyl-2)-methylethylsulphonyl-5-methoxy-2-benzamide, is a new substituted benzamide (Fig. 1) with antipsychotic properties, and some of the pharmacological characteristics of sulpiride. This class of neuroleptics is very interesting in psychiatry, because of low incidence of adverse reactions.

Various techniques have been proposed for determination of benzamides [1, 2], but at present there is no simple method for determination of amisulpride in biological fluids.

This note reports the development of a high-performance liquid chromatographic (HPLC) method with UV detection for the determination of amisulpride in body fluids. The proposed technique is simple, selective and sensitive.

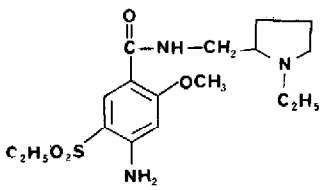
EXPERIMENTAL

Chromatographic system

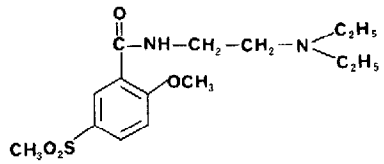
The HPLC system consisted of a Beckman 114M solvent-delivery system (Beckman Instruments, Berkeley, CA, U.S.A.) fitted with a Rheodyne 7125 sample valve equipped with a 50-μl loop, and a Waters LC spectrophotometer Lambda-Max Model 481 operated at 226 nm (Waters Assoc., Milford, MA, U.S.A.).

The column was an RP-18, 25 cm$\times$4.6 mm I.D., packed with 5-μm particles

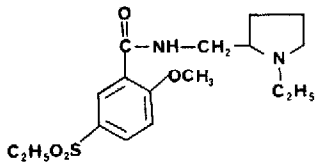
0378-4347/87/$03.50



AMISULPRIDE



TIAPRIDE



SULTOPRIDE

Fig. 1. Chemical formulae of amisulpride, tiapride and sultopride.

(Altex, ODS II, Beckman Instruments) and was used at room temperature. The mobile phase consisted of methanol–water–diethylamine (532:468:0.8) maintained at a flow-rate of 1 ml/min (ca. 200 bar pressure); the mobile phase was degassed for 5 min by sonication using a Bransonic sonicator.

Reagents

All reagents were of analytical grade. Methanol, water and chloroform were purchased from Merck (Darmstadt, F.R.G.); diethylamine and sodium hydroxide were obtained from Prolabo (Paris, France). All glassware was soaked in sulphochromic mixture (U.C.B, Belgium) for one day and rinsed thoroughly with doubly distilled water. Amisulpride, sultopride and tiapride bases were kindly supplied by Delagrange (Paris, France).

Extraction procedure

To 1 ml of human plasma were added 65 μl of a 10 μg/ml solution of internal standard (I.S.), 200 μl of 1 *M* sodium hydroxide and 10 ml of chloroform. The mixture was shaken for 30 min using an alternating agitator (Realis Labo, Villejuif, France). The solution was then centrifuged for 15 min at 2000 *g* at -10 °C. The lower organic phase was transferred to a clean nipple tube and then evaporated to dryness in a water-bath at 25 °C under a gentle stream of nitrogen. The residue was dissolved in 100 μl of the mobile phase, 50 μl of which were injected

into the chromatograph. The estimated extraction ratio was determined for three concentrations of amisulpride (50, 500 and 1250 ng/ml, five determinations for each point).

Calibration curves

The calibration curves were obtained by adding known amounts of amisulpride to human plasma. The final concentrations were 0, 50, 125, 250, 500 and 1000 ng/ml. These standards were extracted under the experimental conditions described above.

RESULTS

Fig. 2 shows the chromatograms obtained after extraction of blank and spiked plasmas with known amounts of amisulpride, tiapride and sultopride. Fig. 3 shows a chromatogram of plasma from a healthy volunteer (3 h after an oral administration of 100 mg of amisulpride).

The retention times for tiapride, sultopride and amisulpride are 6.4, 9.6 and 10.3 min, respectively [capacity ratios (k') 7, 11 and 11.9, respectively].

The calibration curve is linear over the range 0–1.25 μg/ml (correlation coefficient $r=0.999$). The equation of the curves for amisulpride is given by: $y=1.893x-0.03$ ($n=9$) (tiapride as I.S.) and $y=2.616x-0.02$ ($n=9$) (sultopride as I.S.), where y is the ratio of the peak heights of amisulpride and I.S. and x is the concentration of amisulpride in μg/ml. The estimated extraction ratio is 73%.

The within-day reproducibility of the method was checked at plasma concentrations of 50, 500 and 1000 ng/ml amisulpride. Ten determinations of each were done on the same day.

The day-to-day reproducibility was assessed at 50, 500 and 1000 ng/ml for amisulpride over a period of ten days. The results are given in Table I.

The accuracy, defined as the difference between the found and true values, is 93.5, 95 and 95.5% for concentrations of 0.05, 0.5 and 1 μg/ml of amisulpride, respectively.

When very low concentrations are expected, the amount of I.S. (20 μl instead of 65 μl) should be decreased in order to reach a high sensitivity of detection. In this case the detection limit (signal-to-noise ratio of 2) under the described experimental conditions is 5 ng/ml.

Different blank plasmas from humans were tested for the absence of interfering endogenous components. Drugs that might be used in association with a benzamide had been tested at 226 nm. All the substances tested were separated from amisulpride and I.S. (Table II). With the exception of nitrazepam, the extraction was very poor in any case.

DISCUSSION

As far as the I.S. is concerned, it appears that different compounds could be used: sultopride or tiapride (retention times, 9.6 and 6.4 min); but experimentally, depending on the batch of plasma used to prepare the calibration curves,

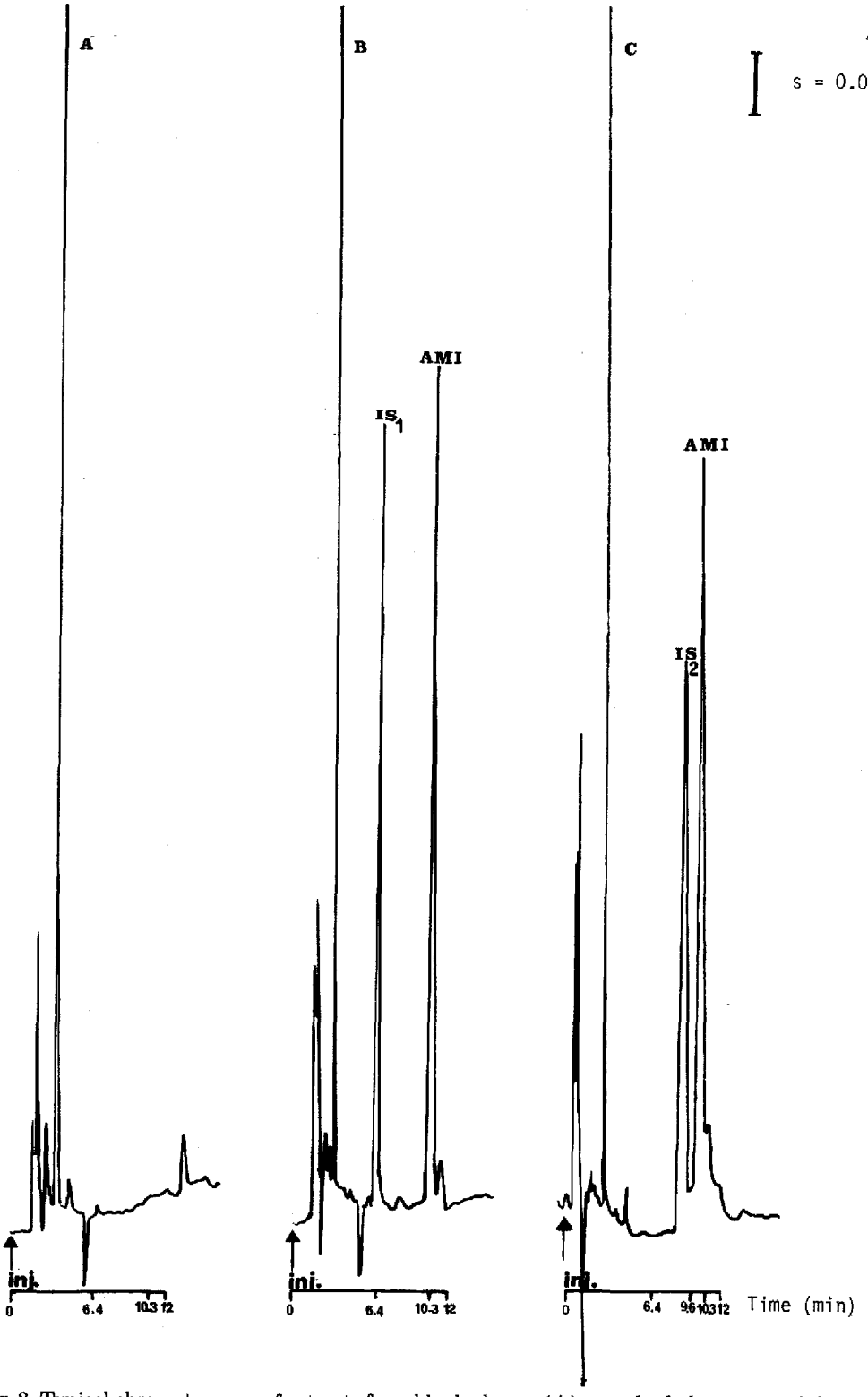


Fig. 2. Typical chromatograms of extracts from blank plasma (A), standard plasma containing 500 ng/ml amisulpride and 650 ng/ml tiapride (B) and standard plasma spiked with 500 ng/ml amisulpride and 650 ng/ml sultopride (C). Peaks: AMI = amisulpride; IS_1 = tiapride; IS_2 = sultopride.

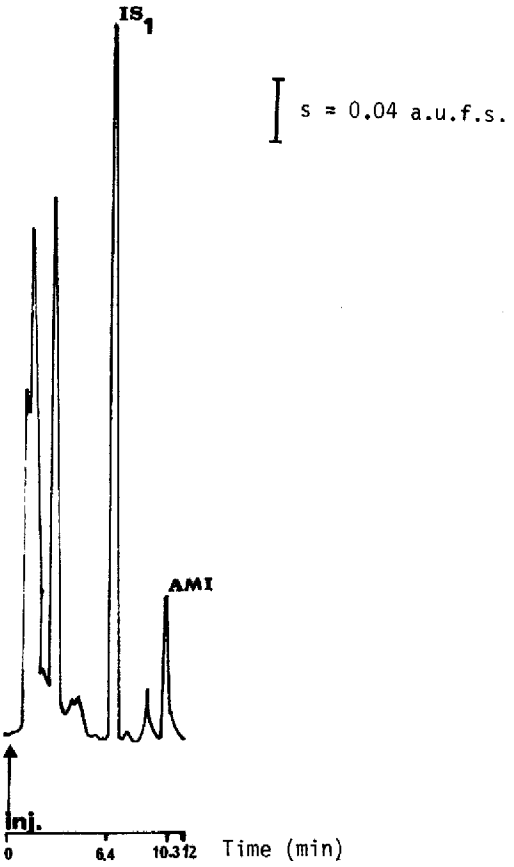


Fig. 3. Typical chromatogram obtained from a plasma extract containing 95 ng/ml amisulpride. Peaks: AMI = amisulpride; IS_1 = tiapride.

an additional peak is sometimes eluted with the same retention time as that of sultopride. This naturally leads to a wrong quantification for the I.S. Provided that the blank plasma is clear of this unknown component, then sultopride could

TABLE I

DAY-TO-DAY AND WITHIN-DAY REPRODUCIBILITY FROM ASSAYS PERFORMED, WITH TEN DETERMINATIONS AT EACH CONCENTRATION

Concentration of amisulpride (ng/ml)	Coefficient of variation (%)			
	Day-to-day		Within-day	
	Tiapride as I.S.	Sultopride as I.S.	Tiapride as I.S.	Sultopride as I.S.
50	3.40	6.80	7.75	1.77
500	3.05	3.45	2.65	3.20
1000	2.70	2.95	3.20	1.75

TABLE II

INTERFERENCE STUDIES OF SOME BENZAMIDES AND BENZODIAZEPINES

Drug	Retention time (min)	Capacity factor k'
Sulpiride	4.25	4.21
Tiapride	6.40	7.0
Sultopride	9.60	11.0
Amisulpride	10.3	11.9
Metoclopramide	>20	>24
Desmethyldiazepam	9.40	10.75
Nitrazepam	12.0	14.0
Flunitrazepam	13.0	15.25
Oxazepam	>20	>24

be an appropriate I.S. For all the determinations, precision and linearity were studied using both tiapride and sultopride as I.S, and the results were shown to be equivalent (Table II). The choice of tiapride as I.S. allows a quite good resolution ($R=0.975$), and also offers the opportunity of measuring any other more polar metabolite that might be chromatographed between amisulpride and tiapride.

In conclusion the method we propose here is very sensitive and relatively simple, with a high degree of precision, and can be applied either in drug monitoring or in human and animal pharmacokinetic trials.

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