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Short Communication

Determination of (+)-5-(2,3-dihydrobenzofuran-7-yl)-3-methyl-8-nitro-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol (NNC 01-0687), a novel dopamine D-1 receptor antagonist, in plasma by solid-phase extraction and high-performance liquid chromatography

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ABSTRACT

A fast and reliable method has been established for the determination of the dopamine D-1 receptor antagonist (+)-5-(2,3-dihydrobenzofuran-7-yl)-3-methyl-8-nitro-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol (NNC 01-0687) in plasma. A combination of reversed-phase extraction on C_{18} columns and straight-phase high-performance liquid chromatographic analysis with ultraviolet detection at 287 nm resulted in very clean chromatograms. The limit of quantitation was about 1 ng/ml of plasma. Validation of the method showed good selectivity, linearity, recovery, accuracy and precision. Several modifications of the method were possible with little or no influence on the assay.

INTRODUCTION

Since the discovery of the first selective dopamine D-1 antagonist, SCH 23390, in 1983 [1], the possibility of developing novel neuroleptic compounds with a selective dopamine D-1 profile has been investigated [2,3]. (+)-5-(2,3-Dihydrobenzofuran-7-yl)-3-methyl-8-nitro-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol (NNC 01-0687; compound I) is such a selective dopamine D-1 receptor antagonist [4–6] currently in clinical development as an antipsychotic agent. Pharmacokinetic studies call for specific and sensitive methods of determining drug concentrations in biological fluids (plasma, serum, urine, bile) and various tissue extracts. The assay described here for human plasma is fast, reliable and very robust. It is based on a combination of reversed-phase extraction and straight-phase high-performance liquid chromatography (HPLC) which results in very clean chromatograms. The retention of both analyte and internal standard, (+)-5-(2,3-dihydrobenzofuran-7-yl)-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol (NNC 01-0534, compound II, Fig. 1), can be selectively changed by minor changes in the mobile phase, making the assay very flexible. No or only minor modifications are necessary for assaying I in other matric-

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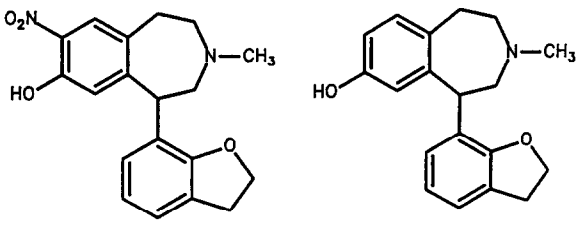

I II

Fig. 1. Structures of I (NNC 01-0687) and II (internal standard).

es. Several related benzazepines have successfully been analysed in our laboratory using the described method.

EXPERIMENTAL

Chemicals

Compounds I (as mono-maleate salt) and II were synthesized at Novo Nordisk (Bagsvaerd, Denmark). Demineralized water was further purified by passage through a Milli-Q water purification system (Millipore, Milford, MA, USA). All organic solvents were of HPLC-grade purity.

Instrumentation

The chromatographic system was from Waters/Millipore (Milford, MA, USA). It consisted of two Model 510 pumps, a WISP Model 712 autosampler, a Model 481 UV absorbance detector (at 287 nm), and a Maxima 860 data-collecting system installed on a Digital VAX computer. Separation was performed on a 100 mm × 4.6 mm I.D. MicroSpher 3-μm silica column (Chrompack, Middelburg, Netherlands). The mobile phase consisted of heptane–2-propanol–25% ammonia in water–water (90:10:0.1:0.1, v/v). A Labofuge GL from Heraeus Sepatech (Osterode, Germany) was used for sample centrifugation and a SVC 200H SpeedVac concentrator from Savant Instruments (Farmingdale, NY, USA) for evaporation of solvents.

Standards

Stock solutions of I and II [1 mg (base) per ml] were prepared in methanol. These solutions were stable for at least three months when stored at −18°C. Working solutions (0.010–10 μg/ml) were obtained by diluting stock solutions in water. Working solutions were only used on the day of preparation.

Extraction procedure

Heparinized plasma samples were extracted on disposable Bond-Elut C_{18} SPE columns (2.8 ml, 500 mg stationary phase) from Analytichem International (Harbour City, CA, USA). All elutions of extraction columns were done by centrifugation at approximately 700 *g*, except for the final wash with 50% methanol, which was done at 2900 *g*. The extraction columns were conditioned with 2 ml of acetonitrile followed by 2 × 2 ml of water. Aliquots of 1000 μl of plasma were mixed with 100 μl of internal standard solution (1–10 μg/ml, depending on the expected concentration range), 900 μl of water and 500 μl of acetonitrile. The mixtures were applied on the extraction columns which were subsequently washed with 2 × 2 ml of water and 2 ml of 50% methanol in water. Elution of the analytes was obtained by application of 2 × 1 ml of 1% ammonia in acetonitrile. Eluates were pooled and evaporated to dryness in the SpeedVac concentrator (2 h with heating). The extracts were redissolved in 150 μl of mobile phase, of which 100 μl were injected into the HPLC system.

Quantitation

Quantitation was based on electronically integrated peak areas using a series of spiked plasma samples covering the concentration range in the samples. The calibrators were extracted and analysed as described above. Peak-area ratio (I/II) was used as response factor.

RESULTS AND DISCUSSION

Selectivity

Representative HPLC chromatograms of extracted plasma samples are shown in Fig. 2. The extracts were generally very clean. Endogenous substances did not interfere with the analysis. Occasionaly small endogenous peaks occurred in the extracted plasma samples close to the ana-

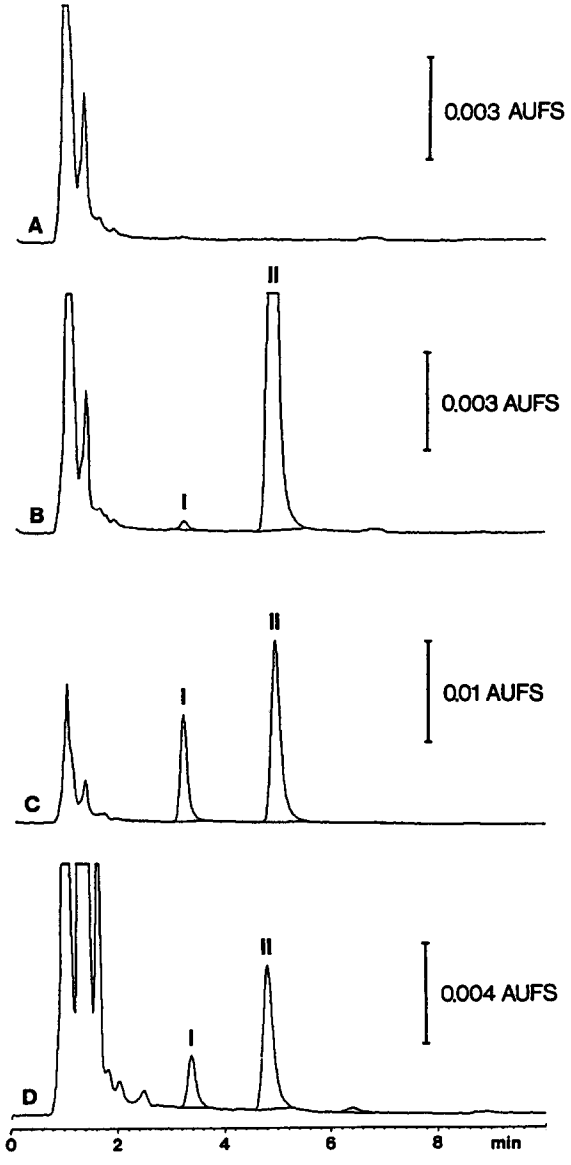

Fig. 2. Chromatograms of (A) blank human plasma, (B) human plasma spiked with 2.6 ng of I and 551 ng of II, (C) human plasma spiked with 102 ng of I and 551 ng of II and (D) human plasma from a volunteer taken 4 h after an oral dose of 11.2 mg of I. The estimated concentration of I was 25.1 ng/ml.

lytes, but additional washing of the extraction columns with 100% acetonitrile prior to elution effectively removed these peaks without elution of the analytes.

None of the identified metabolites of I interfered with the analysis. If the volume of 2-propanol in the mobile phase was increased to 30%, several metabolites could be assayed by the method — but then the parent compound co-eluted with endogenous compounds in the front of the chromatogram.

Linearity

The assay was found to be linear in the range 2 ng/ml to 2 μg/ml (one-way analysis of variance, $\alpha = 5\%$). However, when samples covered a wide concentration range, a reduced calibration curve (2–25 or 50 ng/ml) was used for quantitation in the low range in order to obtain more accurate results. Most correlation coefficients (r) were >0.999 using two × eight calibration standards between 2 ng/ml and 2 μg/ml.

Recovery

Recovery of I and II was estimated as the ratio between the slopes of an extracted plasma calibration curve (peak area) and the corresponding directly injected calibration curve in mobile phase. The recoveries were 93% (I) and 98% (II). Both were constant throughout the concentration range (2 ng/ml to 2 μg/ml).

Limit of quantitation

The criteria used to estimate the limit of quantitation (LOQ) were a maximal coefficient of variation of 15% for six replicate assays and a mean deviation from the nominal concentration also less than 15%. LOQ was estimated to be approximately 1 ng/ml. For routine use, however, it was set at 2 ng/ml.

Accuracy and precision

Within-assay accuracy and precision were estimated by analysing six spiked plasma pools with nominal concentrations of I between 1 and 410 ng/ml. Six 1-ml aliquots of each pool were analysed. The concentration was determined using appropriate calibration curves.

Between-assay accuracy and precision were estimated by analysing three plasma pools spiked with different concentrations of I. On six occasions a 1-ml aliquot of each pool was assayed. Separate calibration curves were included in each

TABLE I

WITHIN-ASSAY AND BETWEEN-ASSAY ACCURACY AND PRECISION

Concentration added (ng/ml)	Estimated concentration (mean ± S.D., n = 6) (ng/ml)	Coefficient of variation (%)	Percentage of nominal concentration
Within-assay			
1.0	1.0 ± 0.2	15.1	100
2.0	2.0 ± 0.1	2.3	102
3.1	3.0 ± 0.3	10.9	98
4.1	4.2 ± 0.3	8.2	102
41	45 ± 1.0	2.2	109
410	442 ± 8.4	1.9	108
Between-assay			
4.1	4.1 ± 0.7	16.2	100
41	42 ± 3.1	7.3	103
410	425 ± 14.6	3.4	104

assay. The results are summarized in Table I. Only minor deviations from nominal concentration were observed. Generally, the between-assay coefficient of variation was approximately twice the corresponding within-assay variation.

Ruggedness

Extensive ruggedness testing proved the method to be very flexible. Several modifications were possible without affecting the estimated sample concentrations. Repetitive freezing–thawing of plasma prior to analysis was studied. One to four cycles had no effect on the results. Ongoing stability studies have proved frozen plasma samples (−18°C) to be stable for several months.

The influence of plasma volume used in the assay was investigated by analysing aliquots of 100, 300 and 1000 μl of plasma spiked with equal amounts of I. The different volumes gave identical peak-area ratios, showing that different volumes can be assayed with a common calibration curve.

Both the volume of water and the volume of acetonitrile added to the assay mixture could be varied by ±50% without change in the estimated sample concentration. Various modifications of the washing procedure for the extraction columns were tested. Additional washing (2 ml) with water, acetonitrile and 50% methanol in water was possible. If the methanol concentration was increased to 60%, elution of I occurred. The ammonia concentration in the elution solvent could be varied from 0.25 to 1.5% without affecting the recovery. The 2-h evaporation of solvent could be replaced by overnight evaporation (without heating).

Extracted samples were stable for at least one week prior to HPLC analysis when stored at −18°C.

Even small variations in the mobile phase could cause marked changes in the retention time of both analyte and internal standard. As the two compounds were not equally sensitive to different changes, this could be used advantageously to change the selectivity and resolution of the HPLC system. Also, the temperature of the analytical column could be used for this purpose, as the retention time of II was unaffected by variations in the column temperature (25–40°C).

The performance of the analytical column was very stable. Several columns have lasted for more than a year with more than 5000 samples injected. Regeneration was possible by flushing the degraded column with 100 ml of mobile phase–chloroform (1:1, v/v).

CONCLUSION

The method described in the present paper has a sufficient quantitation limit, high recovery, good accuracy and precision. It is fast and indifferent to several variations in the assay methodology. It has been used for drug assay in several preclinical and clinical studies (to be published).

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REFERENCES

1 J. Hyttel, *Eur. J. Pharmacol.*, 91 (1983) 153.
2 A. V. Christensen, J. Arnt, J. Hyttel, J. J. Larsen and O. Svendsen, *Life Sci.*, 34 (1984) 1529.
3 M. Abou-Gharbia and J. A. Moyer, in J. McCall (Editor), *Annual Reports in Medicinal Chemistry*, Academic Press, New York, 1989, p. 1.
4 P. H. Andersen and E. B. Nielsen, *Drug News Perspect.* 4 (1991) 150.
5 E. B. Nielsen and P. H. Andersen, *Eur. J. Pharmacol.*, in press.
6 P. H. Andersen, F. G. Grønvald, R. Hohlweg, L. B. Hansen, E. Guddal, C. Braestrup and E. B. Nielsen, *Eur. J. Pharmacol.*, in press.