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Short Communication

High-performance liquid chromatographic assay of α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol (BMY 14802), a potential antipsychotic drug, in monkey and rat plasma

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ABSTRACT

A high-performance liquid chromatographic–fluorescence method was developed for the quantitative analysis of BMY-14802 (I) in monkey and rat plasma. After the addition of the internal standard (BMY-14853 I.S.), 250 μl of plasma were made basic by the addition of 2 ml of saturated sodium carbonate buffer. Compound I and the I.S. were then extracted into 5 ml of methyl *tert.*-butyl ether. The organic phase was evaporated and the resulting residue was reconstituted in mobile phase. Final separation and quantitation of I was achieved on an octadecyl column with a 0.05 *M* potassium phospate acetonitrile triethylamine–85% phosphoric acid (650:350:0.1:0.05, v/v) mobile phase. Fluorescence detection was used to monitor the eluent at an excitation wavelength of 240 nm and an emission wavelength of 400 nm. The limit of detection was 0.5 ng/ml. The standard curve was linear over the range 5.0–1000 ng/ml. Intra-assay and inter-assay precision values were less than 4.0% relative standard deviation and accuracy was within 12% of nominal values. Compound I was shown to be stable in monkey and rat plasma for at least six months when stored at −20°C.

INTRODUCTION

BMY-14802, α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol (I, Fig. 1), is a new antipsychotic agent currently under development at Bristol-Myers Squibb Pharmaceutical Research Institute. It has been suggested that I may be an atypical neuroleptic in as much as it is devoid of the cataleptic effects associated with known butyrophenones and other typical neuroleptics, *e.g.*, haloperidol [1–3]. The two suspected metabolites of I, MJ-14676 [5-fluoro-2-(1-piperazinyl)pyrimidine, II, Fig. 1], and the ketone metabolite, MJ-14786 [1-(4-fluorophenyl)-4-[4-(5-fluoro-2-pyrimidinyl)-1-piperazinyl]butanone, III, Fig. 1], were investigated during the course of this work. The metabolic significance of inter-

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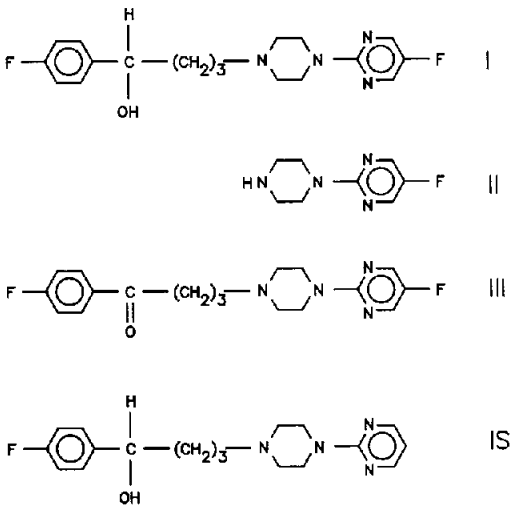

Fig. 1. Structures of I, II, III and internal standard (IS).

conversion between the hydroxy and ketone forms of this class of compound is currently under investigation [4,5].

The method presented herein determines compound I by reversed-phase high-performance liquid chromatography (HPLC) using fluorescence detection with an excitation wavelength of 240 nm and an emission wavelength of 400 nm. The monofluoro analogue of I, α-(4-fluorophenyl)-4-(2-pyrimidinyl)-1-piperazine butanol (BMY-14853, Fig. 1), is used as an internal standard (I.S.). This HPLC assay method has been used in the analysis of plasma samples from non-clinical and toxicologic pharmacokinetic studies.

EXPERIMENTAL

Reagents and standards

Compound I ($C_{18}H_{22}F_2N_4O$·HCl, MW 384.90), compound II ($C_8H_{11}FN_4$·HCl, MW 218.7), compound III ($C_{18}H_{20}N_4OF_2$·HCl, MW 382.84) and I.S. ($C_{18}H_{23}FN_4O$·HCl, MW 366.9) were obtained from the Department of CNS Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute. Acetonitrile, optima grade, monobasic potassium phosphate, sodium carbonate monohydrate and 85% phosphoric acid were all obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Triethylamine (TEA) was from Eastman Kodak (Rochester, NY, U.S.A.). Methyl *tert.*-butyl ether was from Burdick and Jackson (Muskegon, MI, U.S.A.). Deionized water was obtained from a Milli-Q® filtering system (Millipore, Milford, MA, U.S.A.). Rat and monkey plasma was EDTA-treated and obtained from Cocalico Biologicals (Reamstown, PA, U.S.A.).

Chromatographic system

Separation and quantitation of I was achieved on an Apex octadecyl column (150 mm × 4.6 mm I.D., 5 μm) (Jones Chromatography, Littleton, CO, U.S.A.). The precolumn was from Upchurch Scientific (Oak Harbor, WA, U.S.A.) and was packed with Pellicular ODS, 37–53 μm (Whatman, Clifton, NJ, U.S.A.). The HPLC system consisted of an M-45 pump connected to a WISP 710B autoinjector (Waters Assoc., Milford, MA, U.S.A.) and a Spectroflow 980 fluorescence detector (Kratos, Ramsey, NJ, U.S.A.). Peaks were detected by monitoring the eluent at an excitation wavelength of 240 nm and an emission wavelength of 400 nm. A Spectra-Physics Model 4270 integrator (San Jose, CA, U.S.A.) was utilized to monitor chromatographic performance. The data management system used to acquire and process data has been previously described [6]. Specificity work was accomplished using an HP ChemStation with diode-array detector, HP 1040 M, and programmable fluorescence detector, HP 1046 A, all from Hewlett-Packard (Palo Alto, CA, U.S.A.). The mobile phase was isocratic and consisted of 0.05 *M* potassium phosphate–acetonitrile–TEA–85% phosphoric acid (650:350:0.1:0.05, v/v), pH 5.0.

Standard curves

A stock solution of compound I was prepared in rat or monkey plasma. The standard curve was prepared by serial dilution of the stock solution with control plasma (rat or monkey) to give nominal concentrations of 5, 10, 100, 200, 400, 600 and 800 ng/ml. Standards were assayed in duplicate. A working internal standard solution was prepared in Milli-Q water at a concentration of approximately 1.0 μg/ml.

Plasma extraction procedure

A 0.25-ml aliquot of plasma sample was transferred to a 125 mm × 16 mm screw-cap tube which contained 100 μl of working standard solution and 2.0 ml of saturated sodium carbonate solution (114 g/l). The contents of the tube were vortex-mixed for about 10 s. A 5-ml volume of methyl *tert.*-butyl ether was added, the tube capped, and the sample extracted for 15–30 min on a Rotorack® (Fisher Scientific, Fair Lawn, NJ, U.S.A.). The tubes were then spun for 10 min at 1000 *g* in an IEC Centra-7 centrifuge (Internal Equipment, Needham Heights, MA, U.S.A.). The lower aqueous phase was frozen in finely crushed dry-ice and the organic layer was decanted into a fresh evaporation tube (100 mm × 13 mm). The tubes were placed in a water bath, and the organic phase was evaporated to dryness under a gentle stream of nitrogen at 40°C (N-EVAP®, Organomation Assoc., South Berlin, MA, U.S.A.). The residue remaining in the tubes was reconstituted in 125 μl of mobile phase. The tubes were vortexed the contents transferred to glass limited-volume inserts in WISP vials, and 15–50 μl were injected onto the column.

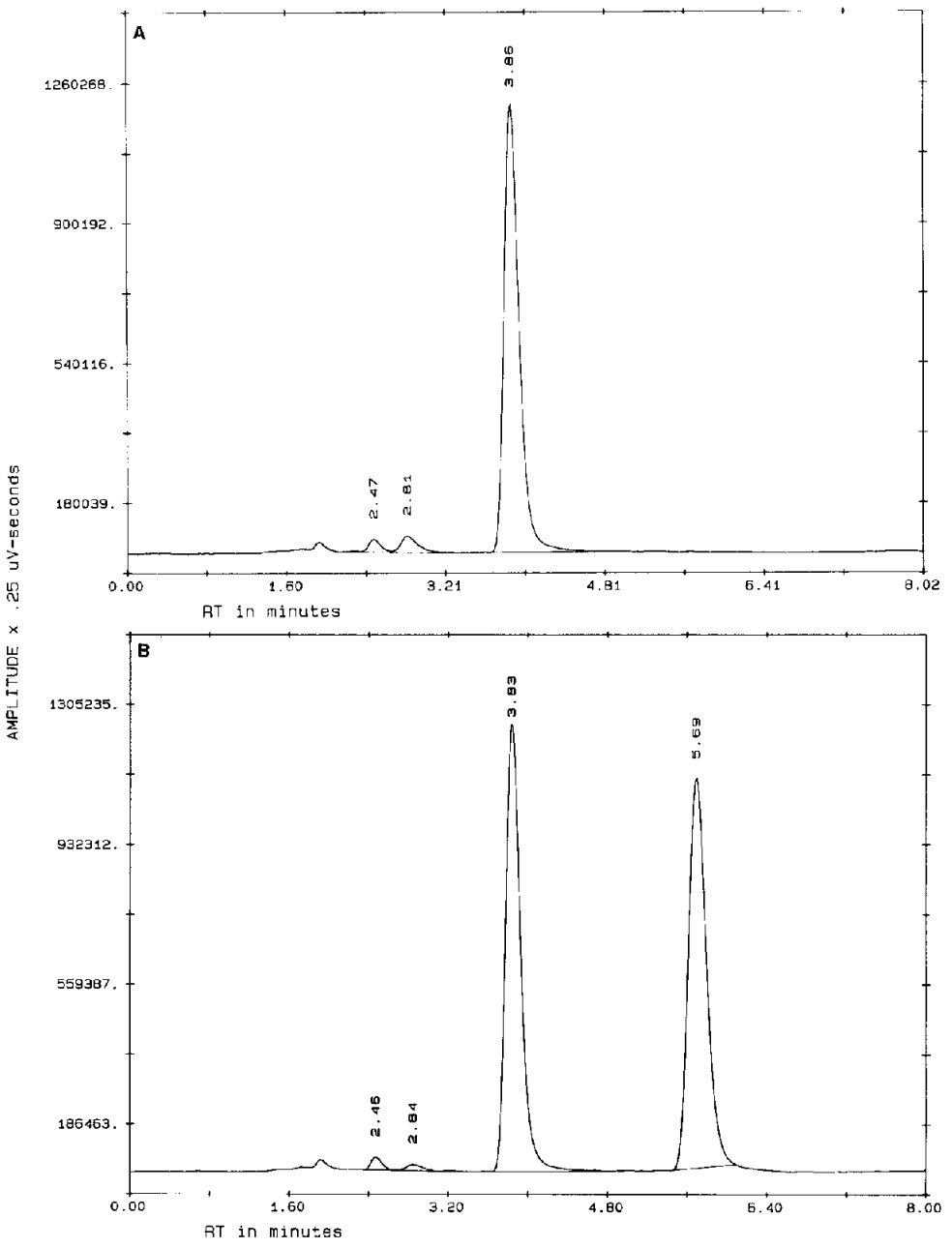

Fig. 2. Typical chromatograms for I and internal standard in rat plasma. (A) Blank rat plasma containing 396 ng/ml internal standard; (B) rat plasma containing 256 ng/ml I and 396 ng/ml internal standard.

RESULTS AND DISCUSSION

Chromatograms of extracted control rat plasma standards (blank and 256 ng/ml) are shown in Fig. 2. No interfering peaks were present at the retention time for I in the blank samples. Comparable chromatograms were obtained for extracted monkey plasma standards. An example of plasma concentration–time profiles for I and III in a rat after oral administration of a single dose of 50 mg/kg I is shown in Fig. 3. Plasma concentrations of III are approximately ten-fold lower than I.

Assay validation

Intra-assay and inter-assay precision and accuracy values were established at two concentrations in each matrix (40 and 810 ng/ml I in monkey and rat plasma). The mean calibration curve for I was defined by the equation $y = 0.0051\ x - 0.0044$ and was linear from 5.0 to 1000 ng/ml I in plasma. The mean correlation coefficient was 0.999. Intra-assay precision values were within 2.0% relative standard deviation (R.S.D.) and accuracy samples deviated less than 12% from nominal values. Inter-assay precision values were within 4% R.S.D. and accuracy samples deviated less than 12% from nominal values (Table I).

The results of limit of detection/lower limit of quantitation experiments are summarized in Table II. The lower limit of quantitation (LLQ) for I was set at 5.0 ng/ml based on accuracy and precision values. Overall recovery was based on the ratio of the slopes of extracted *versus* non-extracted (prepared in mobile phase) standard curves. The extraction recovery of I from monkey and rat plasma was 100%.

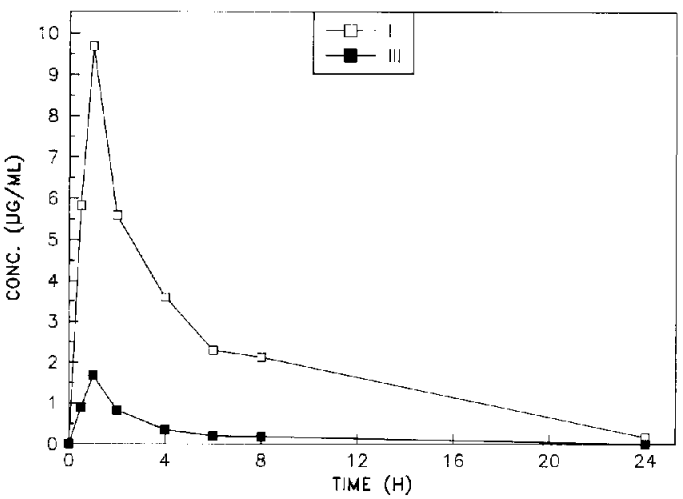

Fig. 3. Plasma concentration time profiles of I and III in rat after oral administration of a single dose of 50 mg/kg I.

TABLE I

ACCURACY AND PRECISION DATA OF I IN PLASMA ($n=5$)

Mean observed concentration (ng/ml)	Inter-assay		Intra-assay	
	Precision (% R.S.D.)	Accuracy (% deviation)	Precision (% R.S.D.)	Accuracy (% deviation)
Rat				
40.26	3.8	0.6	1.1	0.4–4.6
813.65	2.1	0.4	1.6	0.2–3.0
Monkey				
44.26	0.7	10.7	1.9	9.2–11.5
847.77	1.4	4.7	1.8	2.8 6.0

Selectivity

Selectivity was assessed with respect to separation of peaks of interest from endogenous materials in the matrix, separation from potential metabolites and peak purity determination. This work was accomplished primarily through the use of the diode-array detector, HP ChemStation and associated software [7].

Peak purity was assessed by two methods. In the first method, spectral scans were examined on the upslope, apex and downslope of the given peak of interest. The spectra were then overlaid and peak purity assessed (0–100% pure). In the second method, pure compound was chromatographed and a reference spectrum was generated. The reference spectra were compared to all integrated peaks in the chromatogram. The "match value" indicated a degree of similarity between the spectra (*i.e.*, 0 = no match, to 1000 = exact match) and the identity of the peak. The peak at the retention time of I was found to be homogeneous and matched with the reference spectrum of I in both monkey and rat plasma.

TABLE II

LOWER LIMIT OF QUANTITATION FOR I IN RAT PLASMA ($n=10$)

Nominal concentration (ng/ml)	Measured concentration (ng/ml)	Precision (% R.S.D.)	Accuracy (% deviation)	Mean peak area	Mean signal-to-noise ratio
0	–	–	–	6628.72	–
0.5	0.33	25.7	34.5	13353.80	2
1.0	0.64	7.1	35.8	18499.50	3
5.0	4.56	5.9	8.8	84676.70	13

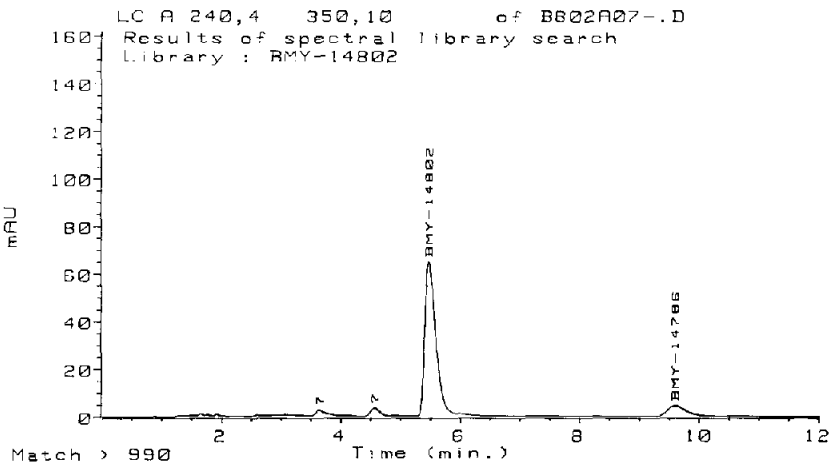

Fig. 4. Chromatogram of a 2-h plasma sample extract from a female rat after 21 days of dosing with 200 mg/kg I per day. This chromatogram was then compared to spectra generated from the pure compounds. Spectral matches were obtained for I (BMY-14802) and III (MJ-14786).

Plasma samples from a male and female monkey dosed with 240 mg/kg I per day and a male and female rat dosed with 200 mg/kg I per day were used to check for separation from potential metabolites. Peaks eluted with retention times of approximately 5 and 9.5 min in both monkey and rat plasma samples which corresponded to the retention times of I and III, respectively. A spectral match (*versus* pure I and III spectra) of >990 was observed in both male and female rat plasma samples (Fig. 4). The female rat plasma sample was spiked with III to see if III coeluted with the peak at 9.5 min. The peak was significantly amplified compared to the unspiked chromatogram. These results putatively identified the peak at 9.5 min in the dosed rat sample as compound III, the ketone metabolite of I. There was clearly a peak at the retention time of III in monkey plasma; peak purity and peak match values, however, were not nearly as good as those obtained in the rat (712 *versus* 994). Compound II was not sufficiently resolved in rat and monkey plasma for its analysis by the above methods.

CONCLUSION

The assay procedure described herein for the quantitation of I in rat and monkey plasma is sensitive, selective, reproducible, accurate and precise. The validated standard curve range is 5–1000 ng/ml. The lower limits of detection and quantitation are 0.5 and 5.0 ng/ml, respectively. The assay procedure yields a matrix and that is free of endogenous and metabolite interfering peaks at the retention times of I and internal standard. This assay is suitable for the quantification of I and its ketone metabolite (III) in monkey and rat plasma. The assay method has been successfully used to analyze non-clinical study samples.

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