Journal of Chromatography, 497 (1989) 342–348
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4982

Note

Determination of saterinone in plasma by high-performance liquid chromatography

MARTIN RUDOLPH

Beiersdorf AG, R&D Pharma, Biochemical Department, Unnastrasse 48, D-2000 Hamburg 20 (F.R.G.)

(First received May 25th, 1989; revised manuscript received July 27th, 1989)

Saterinone, 1-[(4-cyano-1,2-dihydro-6-methyl-2-oxopyridin-5-yl)phenoxy]-3-[4-(2-methoxyphenyl)piperazin-1-yl]propan-2-ol (I, Fig. 1), is a novel drug for the treatment of chronic cardiac insufficiency with a dual action profile. The vasodilating properties of I are combined with a positive inotropic effect [1,2].

A sensitive method is needed for pharmacokinetic studies in both humans and animals. The high-performance liquid chromatographic (HPLC) method described here is simple and rapid and allows the determination of I in plasma in the low nanogram range. The method involves liquid–liquid extraction after

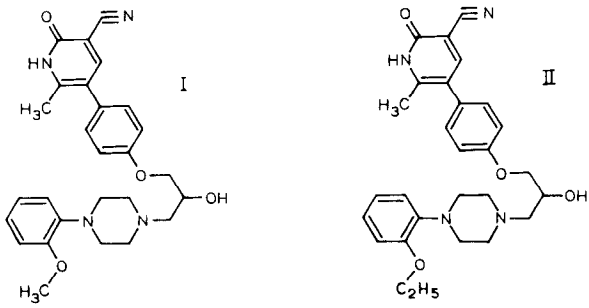

Fig. 1. Structures of saterinone (I) and the internal standard (II).

0378-4347/89/$03.50

addition of an internal standard (II, Fig. 1), re-extraction and separation by HPLC with fluorimetric detection.

EXPERIMENTAL

Reagents

All chemicals were of analytical-reagent grade unless indicated otherwise. Tetrahydrofurane (ChromAR) was obtained from Promochem (Wesel, F.R.G.). Perchloric acid (70%), phosphoric acid (85%), sodium hydroxide solution (1 *M*), dichloromethane, acetonitrile (LiChrosolv) and *tert.*-butyl methyl ether for residue analysis were supplied by E. Merck (Darmstadt, F.R.G.). Sodium 1-pentanesulphonate was supplied by Sigma (Deisenhofen, F.R.G.). All aqueous solutions were prepared with water purified on a Bion exchanger (Pierce, Rodgau, F.R.G.). Drug-free human plasma was obtained from healthy volunteers. I and II were synthesized in our laboratories.

Equipment

Screw-capped glass vessels (12 and 8 ml) with PTFE gaskets (Schott, Mainz, F.R.G.), an Evapotec vortex evaporator (Haake Buchler, Saddle Brook, NJ, U.S.A.) and a Vortex Reax-2000 mixer (Heidolph, Kelheim, F.R.G.) were used. Other equipment was of the usual laboratory standard.

Apparatus and HPLC conditions

The HPLC system consisted of a Model 510 pump, a WISP 712 autosampler (both from Waters Assoc., Eschborn, F.R.G.), a Model F-1000 fluorescence detector and a Model D-2000 integrator (both from E. Merck). A Polyencap-A (5 μm) column (125 mm$\times$4 mm I.D.) (Bischoff Analysentechnik, Leonberg, F.R.G.) with a 20 mm$\times$4 mm I.D. Spherisorb ODS II (5 μm) pre-column was used.

The mobile phase was prepared by dissolving 1.92 g of sodium 1-pentanesulphonate in 500 ml of water and diluting with 300 ml of 0.01 *M* perchloric acid. After addition of 300 ml of acetonitrile and 50 ml of tetrahydrofuran, the solution was mixed and filtered through a 0.2-μm PTFE membrane before use. The flow-rate was 0.75 ml/min, the injection volume was 50 μl and the retention times were 3.1 min for I and 4.4 min for II.

The fluorimeter was set at an excitation wavelength of 345 nm and an emission wavelength of 435 nm.

Preparation of internal standard solution

A 27.79-mg amount of II·HCl·H_2O was weighed into a 250-ml volumetric flask and dissolved in and diluted to volume with methanol. A 10-ml volume of this solution was diluted with 90 ml of water, giving a concentration of 50 ng in 50 μl.

Analytical procedure

For analysis, 1 ml of plasma was pipetted into a 12-ml screw-capped glass vessel, 50 μl of the internal standard solution (equivalent to 50 ng of II) were added and the samples were shaken on a vortex-mixer for 10 s. A 50-μl volume of 1 *M* sodium hydroxide solution and 3 ml of dichloromethane were added and the solution was shaken for 15 min (250 strokes per min).

The mixture was centrifuged at 2850 *g* for 10 min and the lower organic phase was transferred into an 8-ml screw-capped glass vessel and evaporated to dryness. The residue was dissolved in 0.5 ml of *tert.*-butyl methyl ether on a vortex mixer for 10 s and 100 μl of 0.01 *M* perchloric acid were added. The mixture was shaken on a vortex-mixer for 1 min and centrifuged at 2850 *g* for 5 min. The lower aqueous phase was transferred into an autosampler vial and 50 μl were injected into the HPLC system.

Calculations

The peak heights for I and II were determined and their ratios calculated. The concentrations of the samples in ng/ml were taken from a calibration graph of peak-height ratios for different concentrations of I to II plotted against concentration of I in ng/ml.

Recovery

The recovery of I and II from plasma was investigated. Before each work-up, 1 ml of plasma was spiked with 50 μl of an aqueous solution of the compound. The recovery could be determined by comparing the analytical results after work-up with those for the standard without work-up. Four analyses were performed for each concentration. The mean (±S.D.) recovery of I was 80.4±1.8% and for II 87.8±3.3%.

RESULTS AND DISCUSSION

Specificity

Blank value. For plasma samples containing neither I nor II that were treated according to the described method, no significant peaks with the retention times of either substance were found. A chromatogram of a purified plasma sample spiked with I and II prior to analysis is shown in Fig. 2.

Verification. To verify the results of the validation procedure, a second HPLC method was used (Fig. 3). The values obtained for the determination of the linearity, accuracy and precision were in the calculated range of the results obtained with the original method, which demonstrates that there is no falsification of the results from interfering substances.

Precision

The precision is described by the variations for samples spiked with 25 ng/

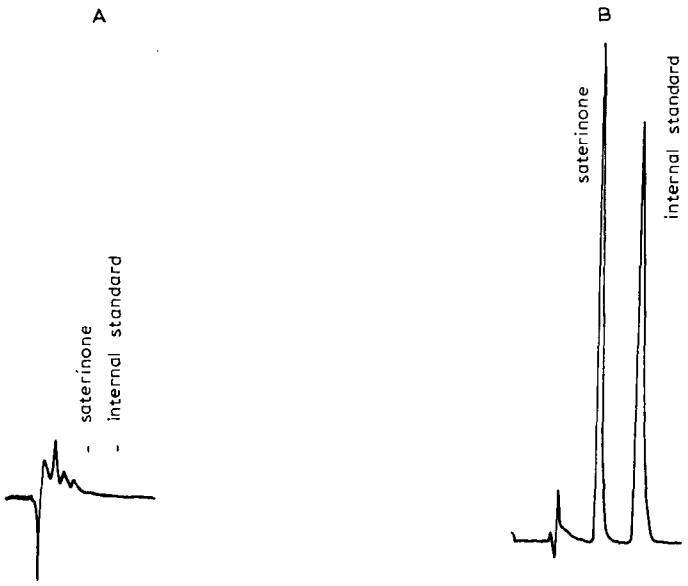

Fig. 2. HPLC of (A) a blank after work-up of 1 ml of drug-free plasma and (B) a plasma sample containing 32 ng/ml saterinone and 20 ng/ml internal standard.

ml I each after work-up. The mean coefficient of variation for samples analysed on one day represents the within-assay variability and that for samples analysed on different days represents the between-assay variability. The coefficients of variation obtained with the proposed method were 1.7% between assay and 1.0–2.0% within assay.

Reproducibility

The day-to-day reproducibility was verified by preparing four calibration graphs. Two analyses were performed for each concentration. Comparable results indicated that the analytical method was reproducible with the described precision.

Linearity

A concentration range of 0.25–100 ng/ml was chosen for the spiked plasma samples, with individual concentrations of 0.25, 0.75, 1, 5, 10, 25, 50 and 100 ng/ml. Each analysis was performed four times. The parameters for the resulting calibration graphs are given in Table I. The calibration graph was linear for the chosen concentration range.

Accuracy

The values for the spiked plasma samples were calculated from the calibration graph. Four analyses were performed for each concentration (Table I). For the mean deviation the median value was more useful, because of the greater

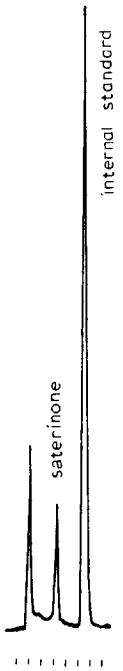

Fig. 3. HPLC of a plasma sample spiked with 5 ng/ml saterinone and 20 ng/ml internal standard after determination with the verification method.

TABLE I

DATA FOR LINEARITY AND ACCURACY ($n=4$)

Concentration added (ng/ml)	Concentration found (ng/ml)	Deviation from added amount (%)
0.25	0.26 ± 0.06	+4.0
0.75	0.59 ± 0.08	−21.3
1.0	1.10 ± 0.11	+10.0
5.0	5.03 ± 0.04	+0.6
10.0	9.65 ± 0.19	−3.5
25.0	25.86 ± 0.57	+3.4
50.0	49.47 ± 0.52	−1.1
100.0	100.05 ± 1.57	+0.1
	x_{median}	+0.4

y-Intercept	−0.012
Slope	39.32
Correlation coefficient	0.9998

deviations in the low concentration ranges. Further, the slope of the calibration graph was an indication of the accuracy. The amounts determined were correct if the slope was close to unity. On plotting spiked amounts versus the determined values the calculated slope was 1.0003. The accuracy of the method was therefore confirmed, because of the small deviation ($x_{median}=0.4\%$) and the closeness of the slope to unity.

Detection and determination limits

The above limits were determined following the procedure described in ref. 3. The standard deviation of the blank and of the sample containing the smallest added amount for the calibration graph were used, together with the slope of the calibration graph as a measure of the sensitivity, to calculate the detection limit, which was found to be 0.18 ng/ml.

The limit of determination was the lowest concentration in the spiking experiments meeting the requirements of being greater than or equal to the detection limit and the coefficient of variation being less than or equal to 20%. As the losses during work-up were always taken into account by addition of the internal standard and calculation of the concentrations from the ratios (I/II), the requirement that the recovery must be greater than 70% was fulfilled. For the developed method, this yielded a limit of determination of 0.75 ng/ml.

Stability

The stability of stock solutions of I and II in methanol and water was studied over a period of three months. When stored in the cold (4°C), no decomposition was observed. The plasma samples were stored at −85°C. The same results were obtained over a period of ten weeks with the accuracy given above.

CONCLUSIONS

A sensitive and selective HPLC method for the determination of I in plasma (serum) samples has been developed. It was demonstrated by an alternative separation method that there were no interferences. Because of the rapid work-up, a large number of samples could be analysed daily for pharmacokinetic studies. Further investigations have been carried out that indicate that other biological fluids such as urine or bile could also be analysed by this method.

ACKNOWLEDGEMENTS

The author thanks Dr. W. Diembeck for his critical evaluation of the manuscript and Mr. G. Schmiedel for excellent technical assistance.

REFERENCES

1 B.I. Armah, E. Hofferber and W. Stenzel, Drug Res., 9 (1988) 1287.

2 B.I. Armah, E. Hofferber and P. Jacobitz, Drug Res., 9 (1988) 1303.
3 Statistical Evaluation of Analytical Methods and Results XI-1 to XI-14, Mitteilung VI der Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Vorratsschutzmittel, Methodensammlung der Arbeitsgruppe Analytik, 6th Delivery, Verlag Chemie, Weinheim, 1985.