Journal of Chromatography, 413 (1987) 370–375
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3394

Note

Highly sensitive determination of 2-hydroxy-N-{3-[*m*-(1-piperidinylmethyl)phenoxy]propyl}acetamide (desacetyl TZU-0460) in rat plasma by high-performance liquid chromatography

SATOSHI IWAMURA* and KUNIO TSUKAMOTO

Pharmacological Research Department, Teikoku Hormone Mfg. Co., Ltd., 1604 Shimosakunobe, Takatsu-ku, Kawasaki (Japan)

(First received June 30th, 1986; revised manuscript received August 22nd, 1986)

2-Acetoxy-N-{3-[*m*-(1-piperidinylmethyl)phenoxy]propyl}acetamide hydrochloride (TZU-0460, I, Fig. 1) is a novel histamine H_2-receptor antagonist [1,2] developed by Teikoku Hormone.

It has been reported that the molecules of I in the plasma and tissues were found to be mostly deacetylated to the corresponding compound, desacetyl I (II, Fig. 1) [3]. Compound II has been determined in biological samples by gas chromatography—mass spectrometry (GC—MS) [4].

This paper describes a reversed-phase high-performance liquid chromatography (HPLC) procedure with UV detection at 198 nm for the analysis of II in rat plasma. This method is simple and accurate with good reproducibility and high sensitivity.

EXPERIMENTAL

Reagents

Compound I, $C_{19}H_{28}N_2O_4 \cdot HCl$ (mol.wt. 384.90), and the internal standard, N-{3-[*m*-(1-piperidinylmethyl)phenoxy]propyl}acetamide (TZU-9357, III, Fig. 1), $C_{17}H_{26}N_2O_2$ (mol.wt. 290.40), were synthesized in the chemical research laboratory of Teikoku Hormone. Acetonitrile of HPLC grade was purchased from Nakarai (Kyoto, Japan). Deionized water was filtered through a 0.45-μm microfilter (Fuji Photo Film, Tokyo, Japan), and the filtrate was used in this experiment. Bond-Elut® C_{18} (volume 3 ml) was purchased from Analytichem International (Harbor, CA, U.S.A.).

0378-4347/87/$03.50

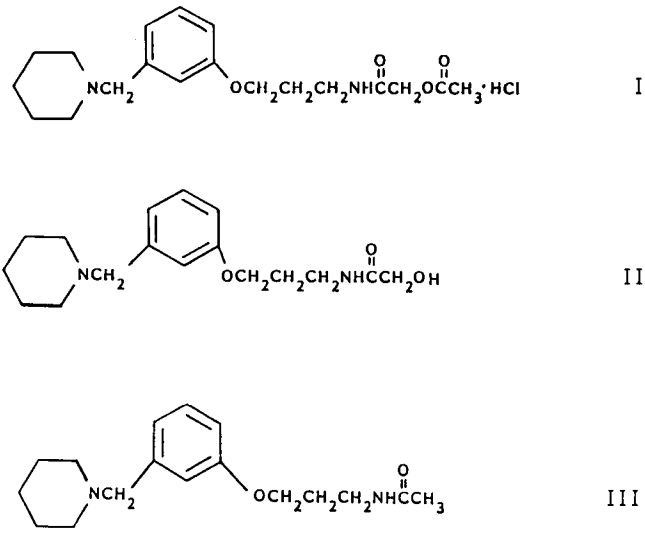

Fig. 1. Chemical structures of I, desacetyl I (II) and the internal standard (III).

Buffer solutions

The buffer solutions were made up as follows: pH 6.5 buffer, 0.001 *M* ammonium monohydrogen phosphate solution, was adjusted to pH 6.5 with 0.001 *M* ammonium dihydrogen phosphate solution; pH 6.0 buffer, 0.1% acetic acid solution, was adjusted to pH 6.0 with triethylamine; pH 5.4 buffer, 0.01% phosphoric acid solution, was adjusted to pH 5.4 with triethylamine.

Chromatographic conditions

Chromatography was performed with a Model 6000A pump and U6K universal injector (Waters Assoc., Milford, MA, U.S.A.) equipped with a Model S-301A, a variable-wavelength UV absorbance detector (Soma Photochemical Industry, Japan) operated at 198 nm. The analytical column was a stainless-steel tube (150×4 mm I.D.) filled with 5-μm Nucleosil C_{18} (Macherey-Nagel, Düren, F.R.G.) by the balanced-density slurry-packing technique and was used under ambient conditions. The mobile phase, acetonitrile—buffer, pH 5.4 (50:500) was degassed and then passed at a flow-rate of 1.0 ml/min. Retention times were 12.9 min for II and 18.8 min for the internal standard, III. Data were calculated using a Shimadzu CR-2AX (Shimadzu, Kyoto, Japan). GC—MS was performed as previously reported [3].

Sample preparations

Calibration standards. To 1 ml of drug-free rat plasma were added 200 ng of III, and 5, 10, 50, 100 and 250 ng of II, respectively. Calibration curves were prepared by plotting the relationship between the peak-height ratios of II to III and the amounts of II.

Column extraction of plasma. A 100-μl volume of III solution (2 μg/ml) and 2 ml of deionized water were added to 25–500 μl of plasma in an 8-ml glass tube.

After vortex mixing for 20 s, the solution was applied to a Bond-Elut C_{18} column, activated with 6 ml of methanol and 6 ml of deionized water. The column was then washed with 3 ml of deionized water and 6 ml of acetonitrile—buffer, pH 6.5 (20:80). Compounds II and III were then eluted with 2 ml of acetonitrile—buffer, pH 6.0 (15:85). The eluent was evaporated in vacuo, the residue was dissolved with 50 μl of the HPLC mobile phase and 5–10 μl of this solution were injected onto the chromatograph.

Recovery

The recovery of II was determined by comparison with the HPLC responses of known amounts of samples on the column.

Collection of rat plasma

Compound I (30 mg/kg) was dissolved in a saline solution and was orally administered at a volume of 5 ml/kg to male Wistar rats ($n=7$) weighing 260–340 g that had been fasted for 24 h. Blood was collected from the abdominal aorta under light anaesthesia with diethyl ether and placed in heparinized tubes. Plasma was separated by centrifugation and stored at $-20\,^{\circ}C$ until analysed.

Calculation of half-life ($t_{1/2}$) and the area under the plasma concentration curve (AUC)

Plasma concentrations were plotted semilogarithmically as a function of time, and the apparent elimination rate constant (k) was estimated from the slope of the terminal linear segment of such plots by the method of linear least-squares regression analysis. The plasma half-life was obtained by dividing 0.693 by k. The AUC value was calculated using the trapezoidal rule. The area from the last data point to infinity was calculated by dividing the plasma concentration to the last point by k.

RESULTS AND DISCUSSION

The C_{18} silica minicolumn eluent was monitored at 198 nm, since this wavelength corresponds to the maximum UV absorbance of II in HPLC solvent (Fig. 2). Fig. 3 shows typical chromatograms of extracts of (a) drug-free rat plasma and (b) plasma after oral adminsitration of I. Two peaks, II and III, were not influenced by impurities in the rat plasma. These results were obtained only by HPLC of the sample obtained by the procedure described in column extraction of plasma. Various purification methods were studied, and it was concluded that this procedure is very effective for the removal of impurities with good recovery of the materials examined.

The calibration curve was prepared by plotting the relationship between the peak-height ratio (y) of II to III and the amount of II ($x=5$–250 ng) in each sample. The calibration curve was linear ($y=0.008589x+0.0062$), as shown by the correlation coefficients (r) of 0.9978 ($n=16$).

Recovery of II from drug-free rat plasma by the C_{18} silica minicolumn was 90.5% at a concentration fo 200 ng/ml. Compound II was added to drug-free rat

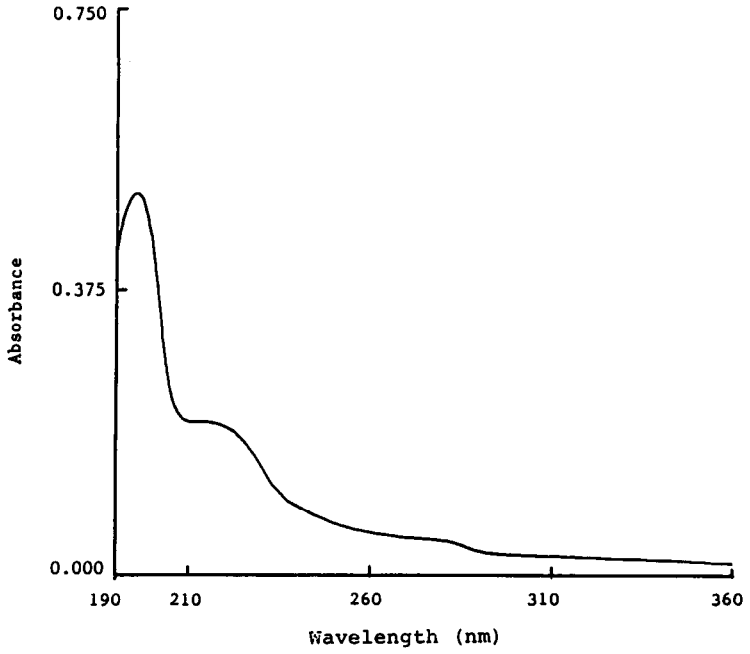

Fig. 2. UV spectrum of II (2 μg/ml) in the HPLC solvent.

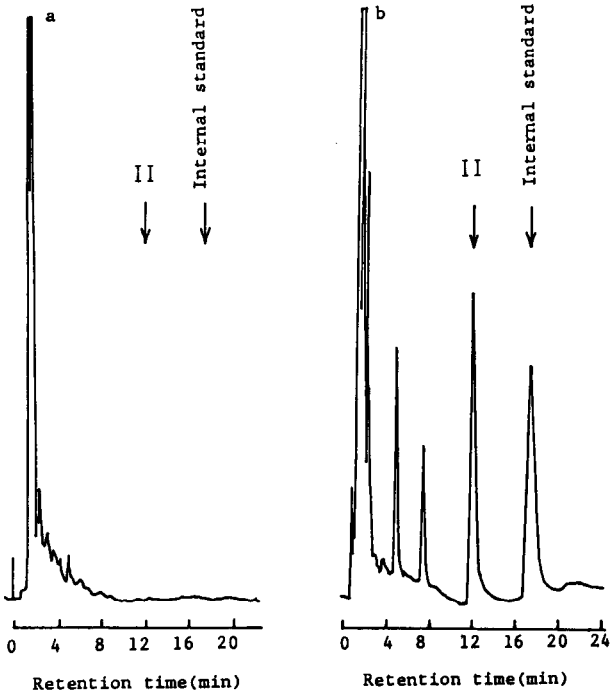

Fig. 3. Chromatograms of extracts of rat plasma. (a) Drug-free plasma; (b) II in plasma (148 ng per 50 μl of plasma) 1 h after a single oral administration of I (30 mg/kg); peak II is retained at 12.9 min.

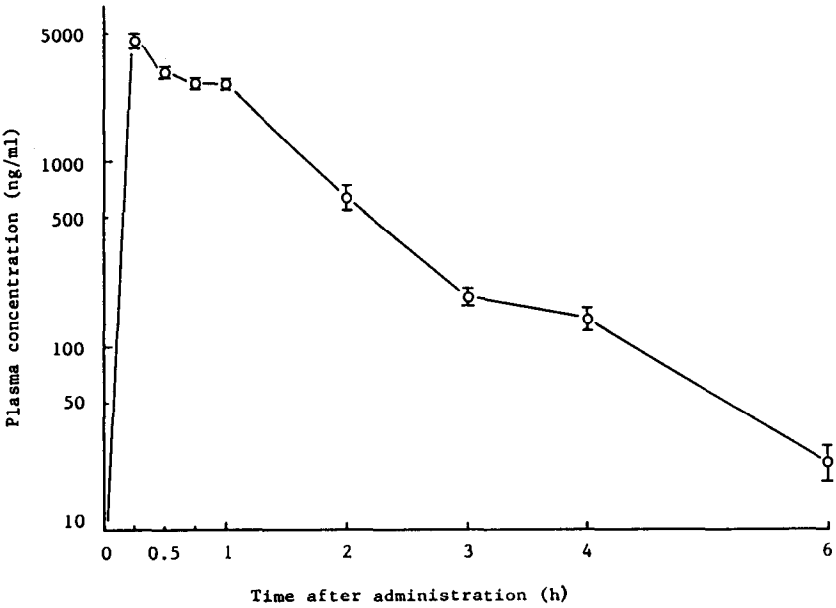

Fig. 4. Plasma concentrations of II after a single oral administration of I (30 mg/kg) to male rats ($n=7$). Each point represents the mean ± S.E.

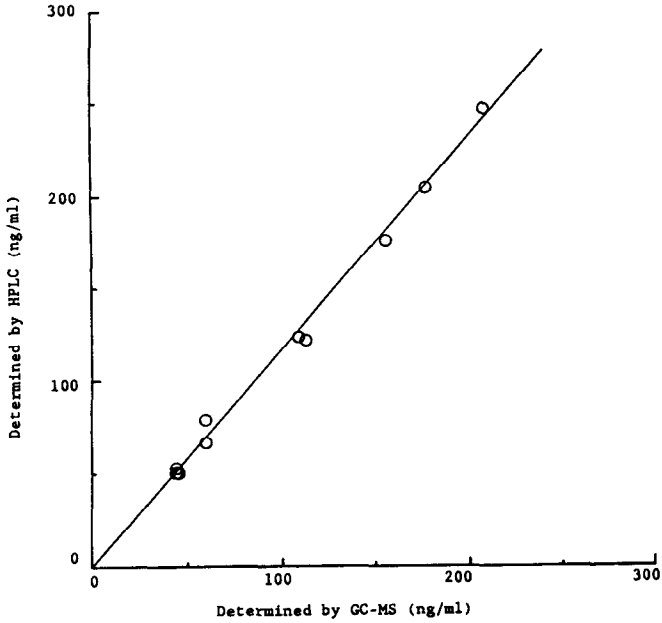

Fig. 5. Correlation of the HPLC method with the GC—MS method for the determination of II in rat plasma.

plasma at two concentrations and then analysed in replicate ($n=4$). The coefficients of variation at concentrations of 10 and 250 ng/ml were 8.47 and 1.26%, respectively. These results indicate good recovery and reproducibility. Under these

conditions, the detection limit for II was 5 ng/ml (500 pg injected).

Fig. 4 shows the plasma concentration of II after a single oral administration of I (30 mg/kg). The value of $t_{1/2}$ of II was 0.74 h, the maximum concentration was 4575 ng/ml at 0.25 h and $AUC_{0-\infty}$ was 5288 ng·h/ml. These results were similar to those determined by the GC—MS method [4].

In an attempt to gain additional information on the accuracy of the method, rat plasma samples were analysed by this method and by the original GC—MS technique. The correlation between the results obtained from the two methods was good (Fig. 5; $r=0.9944$, $n=15$). Therefore, this HPLC method is extremely sensitive and specific for the determination of II in plasma samples.

ACKNOWLEDGEMENTS

We thank Dr. Hiromu Mori and Dr. Masaaki Tarutani for their generous support of this study, and Dr. Kenyu Shibata for the synthesis of the internal standard compounds. We are also grateful for the excellent technical assistance of Mrs. Hiromi Mori.

REFERENCES

1 M. Tarutani, H. Sakuma, K. Shiratsuchi and M. Mieda, Arzneim.-Forsch., 35 (1985) 703.
2 M. Tarutani, H. Sakuma, K. Shiratsuchi and M. Mieda, Arzneim.-Forsch., 35 (1985) 844.
3 S. Honma, R. Akutsu, S. Iwamura, Y. Kawabe and K. Tsukamoto, Pharmacometrics, 30 (1985) 329.
4 S. Iwamura, S. Honma, R. Akutsu, Y. Kawabe and K. Tsukamoto, Pharmacometrics, 30 (1985) 299.