Journal of Chromatography, 226 (1981) 510—513
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1023

Note

Determination of methyclothiazide in human plasma by high-performance liquid chromatography

CRAIG A. HARTMAN, NORBERT KUCHARCZYK*, R. DUANE SOFIA and JAMES L. PERHACH, Jr.

Department of Biochemistry, Wallace Laboratories, Division of Carter-Wallace, Inc., Cranbury, NJ 08512 (U.S.A.)

(Received April 24th, 1981)

The oral diuretic—antihypertensive agent, methyclothiazide (MCT), 6-chloro-3-chloromethyl-3,4-dihydro-2-methyl-7-sulfamoyl-1,2,4-benzothiadiazine 1,1-dioxide, Aquatensen® (Wallace Laboratories, Cranbury, NJ, U.S.A.), is a member of the thiazide family of drugs. No assay method for methyclothiazide in human plasma has been published to date, but this report describes a high-performance liquid chromatography (HPLC) procedure developed in our laboratories, for the determination of the drug in human plasma.

EXPERIMENTAL

Materials

All reagents were of analytical grade. Aqueous solutions were prepared using deionized water (Milli-Q-Water System, Millipore Corp., Bedford, MA, U.S.A.). Glass-distilled methanol (Burdick and Jackson Labs., Muskegon, MI, U.S.A.) was used for HPLC. Methyclothiazide (MCT) Lot No. JC1755 was from Wallace Laboratories (Division of Carter-Wallace, Inc., Cranbury, NJ, U.S.A.). Glass-distilled ethyl acetate (Burdick and Jackson Labs.) was used for plasma extraction. Acetophenetidine (phenacetin) and anhydrous magnesium sulfate were obtained from Sigma (St. Louis, MO, U.S.A.). Sodium hydroxide pellets were obtained from Mallinckrodt (Paris, KT, U.S.A.).

High-performance liquid chromatography

The chromatograph was a modular instrument, equipped with two Model 6000A pumps, a Model 720 system controller, a Model 710B "WISP" autosampler (all from Waters Assoc., Milford, MA, U.S.A.), a Model 770 variable-wave-

0378-4347/81/0000—0000/$02.50

length spectrophotometric detector and a Model LC 250/3 column oven, both from Kratos (Division of Schoeffel Instruments, Westwood, NJ, U.S.A.). A 10-μm μBondapak C_{18} column (Waters Assoc.) was used; the mobile phase was methanol—water (35:65). The column oven temperature was 35°C. A flow-rate of 1.5 ml/min was used yielding an operating pressure of approximately 1500 p.s.i. The spectrophotometric detector had an 8-μl cell volume and was operated at a wavelength of 225 nm.

Standards

A standard solution of MCT was prepared in methanol (1.0 mg/ml) and stored at 4°C. This solution was then diluted as necessary to prepare the appropriate plasma standards for each drug assay run. The internal standard of acetophenetidine (phenacetin) was also prepared in methanol (8 μg/ml) and stored at 4°C. Peak area ratios of MCT to phenacetin were determined for plasma standards.

Sample preparation procedure

To 2 ml of plasma (or standard) were added 2 ml of 0.1 *M* sodium hydroxide and 8 ml of ethyl acetate. The mixture was vigorously stirred for 30 sec on a Vortex Genie Mixer (Scientific Products, Evanston, IL, U.S.A.) and centrifuged for 10 min at 10°C and 2000 *g*.

The organic layer was drawn off and passed through a 35 mm × 6 mm column of anhydrous magnesium sulfate. The column was rinsed with an additional 2 ml of ethyl acetate. The extraction mixture was evaporated to dryness under nitrogen in a 50°C water-bath. The residue was redissolved in 100 μl of methanol, containing the internal standard phenacetin (8 μg/ml). The sampler injected a 20-μl volume onto the column of the high-performance liquid chromatograph.

Reproducibility and recovery

Reproducibility was determined for the concentration range of 5.0, 10.0, 20.0, 50.0, and 100.0 ng/ml MCT in plasma by quadruplicate analyses of samples at each concentration. Drug recovery from plasma after sample preparation was determined by comparison of the peak height ratios with those obtained from methanol solutions containing known concentrations of MCT.

RESULTS AND DISCUSSION

A linear relationship between the peak height ratio and plasma concentration of MCT exists in the range 5—100 ng/ml. The correlation coefficient r^2 is 0.9987.

The precision (reproducibility) of this method was determined by quadruplicate analyses of standard samples at each concentration. The results (Table I) show that the precision, expressed as the coefficient of variation (C.V.), was 8.3% or better for the concentration range 5—100 ng/ml.

The accuracy, calculated as the relative mean error*, was 4.7% or better for

*Relative mean error $= \frac{\text{absolute value of theoretical} - \text{determined value}}{\text{theoretical value}} \times 100.$

TABLE I

PRECISION (C.V.) AND ACCURACY (M.E.) OF THE DETERMINATION OF METHYCLOTHIAZIDE IN HUMAN PLASMA IN THE RANGE 5—100 ng/ml

Theoretical concentration of MCT (ng/ml)	Calculated (mean ± S.D.)	C.V. (%)	M.E.* (%)
100	98.8 ± 4.5	4.5	1.2
50	52.5 ± 3.6	6.8	4.7
20	19.8 ± 1.2	6.1	1.0
10	10.0 ± 0.6	5.9	0.5
5	4.0 ± 0.3	8.3	25.8

*M.E. = relative mean error; see text for definition.

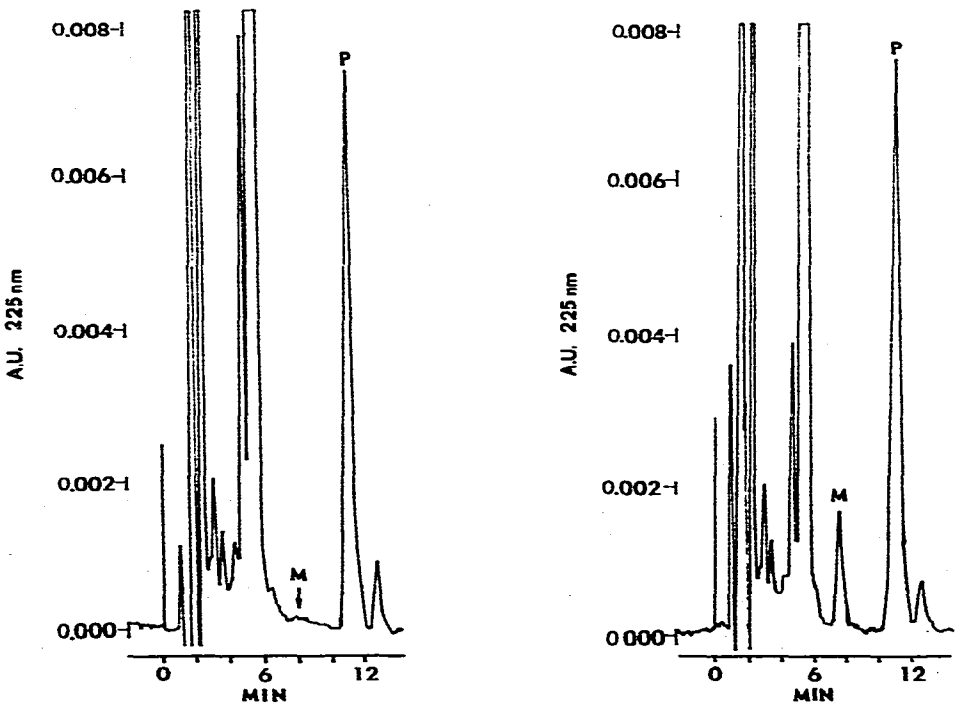

Fig. 1. Chromatogram of plasma from a patient before dosing with MCT (M). Phenacetin (P) as internal standard. Column, μBondapak C_{18}; mobile phase, methanol—water (35 : 65).

Fig. 2. Chromatogram of plasma from a patient after a single oral dose of 10 mg of MCT (M). See legend to Fig. 1 for further details.

the concentration range 10—100 ng/ml and 25.8% for the 5 ng/ml concentration. The accuracy is more commonly expressed as recovery, which for our method was 99.0—104.9% for the concentration range 10—100 ng/ml and 79.5% for the 5 ng/ml concentration.

In order to obtain a realistic estimate of the sensitivity of the assay, the limit of detection [1] was calculated on the basis of the peak height ratio value for

zero concentration as estimated from linear regression and the standard deviation for the lowest plasma concentration used. The limit of detection was found to be 1.5 ng/ml.

Complete (baseline) resolution of MCT and the internal standard phenacetin from endogenous plasma substances was considered a prerequisite for a good assay and was achieved using the mobile phase composition described (see Fig. 1). An example of an analysis of plasma obtained from a patient 3 h after an oral dose of 10 mg of MCT (two Aquatensen® tablets) is shown in Fig. 2. The determined concentration was 24 ng/ml.

Thiazides are used as step 1 therapy in the treatment of hypertension. More severe hypertension involves concomitant administration of other antihypertensive agents. We have determined that some of the common agents used, namely propranolol, prazosin, clonidine and methyldopa, do not interfere with the determination of MCT when these drugs were added at a concentration of 1 mg/ml to the sample and each sample carried through the procedure described in this paper.

The time needed for analysis was 75 min for sample preparation and 15 min for chromatographic analysis. In the automated mode many samples can be prepared within a few hours and with the automatic sampler capacity of 48 samples all samples can be analyzed in a 12-h overnight run.

CONCLUSION

An automated HPLC assay has been developed that is sufficiently sensitive, accurate and precise for the routine clinical monitoring of plasma levels of methyclothiazide. The presence of propranolol, prazosin, clonidine or methyldopa in plasma does not interfere with this assay.

REFERENCE

1 R. Gabriels, Anal. Chem., 42 (1970) 1439.