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Note

Determination of metazosin in biological fluids by reversed-phase high-performance liquid chromatography with fluorescence detection

P. STEHLÍK[a,*] and H. HOUBOVÁ

Research Institute for Pharmacy and Biochemistry, Kouřimská 17, 130 60 Prague 3 (Czechoslovakia)

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Metazosin (MET), 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-methoxypropionyl)piperazine, is an original Czechoslovak compound with an apparent effect as an antihypertensive agent. It is analogous to prazosin (PRZ) both chemically and (potentially) pharmacologically, so PRZ can be used as an internal standard in the determination of MET by methods similar to that for PRZ. The methods of determination of PRZ are based on the extraction of alkaline biological fluids with an organic solvent (diethyl ether, chloroform, ethyl acetate); after centrifugation, evaporation and reconstitution the sample is injected into the chromatograph [1]; or after re-extraction of the organic phase with inorganic acid, part of acidic layer is injected [2]; or after re-extraction of acidic phase (after alkalization) with organic solvent and after centrifugation, evaporation and reconstitution, the sample is injected [3]. Other methods are based on deproteinization of plasma followed by centrifugation and injection of the supernatant [4,5]. The deproteinization methods are the simplest, but the blank samples are not quite clear. Therefore we used a method with one-step extraction. This method is quick and it gives quite clear blank samples. The analytical method has not been aimed at maximal limit of detection, because the plasma levels of MET are high.

[a]Address for correspondence: 331 51 Kaznějov 516, Czechoslovakia.

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EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a Model SP 8770 pump (Spectra Physics, San Jose, CA, U.S.A.), a Model 7125 injector (Rheodyne, Cotati, CA, U.S.A.), a Model FS 970 fluorescence detector (Kratos, Ramsey, NJ, U.S.A.) and a Model SP 4100 integrator (Spectra Physics, San Jose, CA, U.S.A.). Separation was carried out on a 250 mm×4.6 mm I.D. Supelcosil LC-18 column (5 μm particle size) with and LC-18 guard column (50 mm×4.6 mm I.D., 40 μm particle size). The column and guard column were supplied from Supelco (Bellefonte, PA, U.S.A.). Extraction was performed on a microshaker ML-1 (Premed, Warsaw, Poland).

Materials

MET and PRZ were supplied from Research Institute for Pharmacy and Biochemistry (Prague, Czechoslovakia). Triethylamine and dichloromethane were purchased from Fluka (Buchs, Switzerland). Phosphoric acid, diethyl ether, sodium hydroxide and methanol were supplied from Lachema (Brno, Czechoslovakia) in p.a. quality. Diethyl ether was chemically cleaned and distilled. Methanol was rectified. Water was redistilled. The extraction solvent was diethyl ether–dichloromethane (3:1, v/v).

Extraction

A 10-μl volume of the internal standard solution (5 μg PRZ per ml of 30% methanol) were measured into a 10-ml glass centrifuge tube to which 100 μl of plasma (urine), 50 μl of 2 *M* sodium hydroxide and 2 ml of extraction solvent were added. The tube was stoppered and vortex-mixed for 1 min. The mixture was centrifuged for 5 min at 2500 *g*. The organic layer was transferred to a clean, dry conical tube and evaporated to dryness. The residue was reconstituted with 200 μl of the mobile phase, and 20 μl were injected into the chromatograph.

Calibration curves

A 10-μl volume of the standard solution of MET (0.2, 1, 2.5, 5, 10, or 20 μg per ml of 30% methanol, i.e. 20, 100, 250, 500, 1000, or 2000 ng/ml of plasma) and 10 μl of the internal standard solution (5 μg per ml of 30% methanol) were measured into a 10-ml glass centrifuge tube, and other procedures were performed as described above.

Chromatographic conditions

The mobile phase was methanol–triethylamine–phosphoric acid–water (45:0.5:0.55:53.95, v/v). The flow-rate was 1.0 ml/min. The temperature of the column and guard column was maintained at 40°C. The excitation wave-

length of the fluorescence detector was fixed at 340 nm (entrance filter 7.54) and an emission filter 370 nm was used. The time constant was fixed at 4 s. The sensitivity was maintained at 6.60. We have worked with range 0.2–1 μA.

RESULTS AND DISCUSSION

Under the conditions described the retention times of MET and PRZ are 5.4 and 7.3 min, respectively, and the capacity factors are 1.000 and 1.074, respectively. Fig. 1 shows chromatograms of the blank plasma sample and the calibration plasma sample at the point 500 ng MET per ml plasma. All blank plasma samples were quite clear. Similar results were obtained with urine samples.

The intra-day precision data are shown in Table I. The mean calibration curve is C (ng/ml of plasma) = 2.9 + 397.1 (area MET/PRZ) with correlation

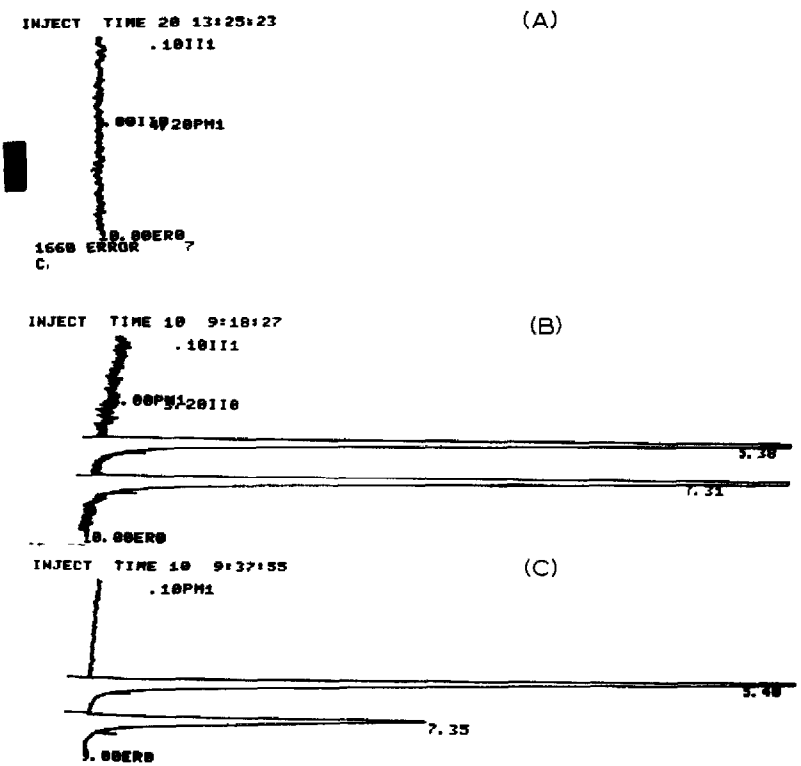

Fig. 1. Chromatograms of (A) a blank plasma sample, (B) a plasma sample spiked with 500 ng of MET per ml of plasma and 500 ng of PRZ per ml of plasma, and (C) a plasma sample of a healthy volunteer, taken 4 h after oral administration of 5 mg of MET.

TABLE I

INTRA-DAY PRECISION FOR MET IN PLASMA

Amount added (ng/ml)	Amount found (mean ± S.D. $n=7$) (ng/ml)	Coefficient of variation (%)
20	20.3 ± 1.7	8.4
100	100.1 ± 5.6	5.6
250	251.8 ± 7.7	3.1
500	499.3 ± 13.6	2.7
1000	1000.0 ± 14.0	1.4
2000	2000.0 ± 62.2	3.1
Mean		4.1

TABLE II

DAY-TO-DAY PRECISION FOR MET IN PLASMA

Amount added (ng/ml)	Amount found (mean ± S.D., $n=3$) (ng/ml)	Coefficient of variation (%)
20	20.7 ± 1.9	9.5
100	100.3 ± 7.6	7.6
250	249.2 ± 11.5	4.6
500	498.3 ± 7.6	1.5
1000	1003.3 ± 15.2	1.5
2000	1967.0 ± 23.1	1.2
Mean		7.0

TABLE III

RECOVERY OF MET

Values are taken at the calibration point 500 ng/ml, and expressed as the ratio of the peak height of the plasma sample to that of the standard solution

Material	Amount added (ng/ml)	Recovery (mean ± S.D., $n=3$) (%)	Coefficient of variation (%)
Plasma	500	96 ± 3	3.1
Urine	500	95 ± 4	4.2

coefficient $r=0.9994$. For urine the equation is C (ng/ml of urine) $=3.4+396.7$(area MET/PRZ) with correlation coefficient $r=0.9988$. In both cases the calibration curves are linear over the concentration range studied, and it is possible to use one-point calibration with the equation C (ng/

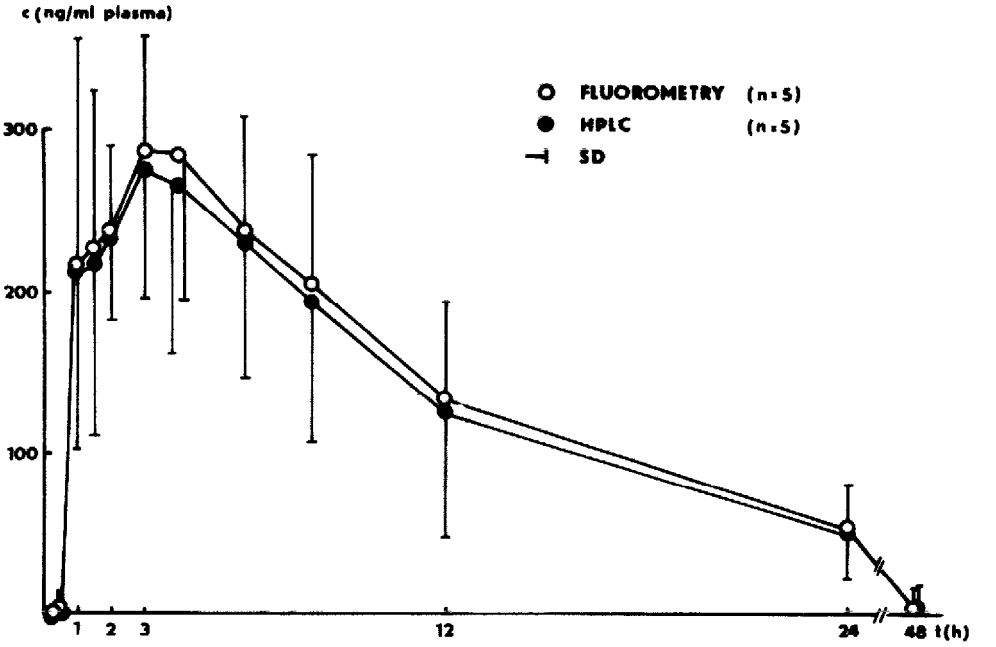

Fig. 2. Plasma concentration-time profile of MET after oral administration of 5 mg of MET.

ml) = 400 (area MET/PRZ). The mean coefficient of variation is 4%. Table II lists the day-to-day precision of one calibration curve over three days. The recovery of MET is shown in Table III: from plasma it is 96 ± 3% and from urine 95 ± 4%. MET is stable in frozen plasma samples (−18°C) for at least two months (99 ± 4%). The limit of detection (at a signal-to-noise ratio of 2) with the range 0.2 μA is 5 ng MET per ml plasma.

With regard to further phases of clinical examination we tested the method for interference from other drugs by injecting standard drug solutions (10 μg per ml of 50% methanol). For quinine and quinidine the concentration was 1 μg/ml. The following drugs were tested: quinine, quinidine, diclofenac, lonazolac, amidepin, flobufen, amitriptyline, prothiaden, naftidrofuryl, labetalol, propranolol, metoprolol, desacetyltrimepranol, mepamil, verapamil, furosemide, chlorthalidone, pelentan, trimetazon, diazepam and dorsiflex. Only quinine and quinidine showed any interference. Their peaks occur at 5.1 min, and at higher concentrations they are able to interfere the analysis of MET/PRZ.

The concentration–time curve for the drug is shown in Fig. 2, as determined both by the present high-performance liquid chromatographic method and by spectrofluorimetry [6].

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