*Journal of Chromatography*, 434 (1988) 187–190
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4458

## Note

# Simultaneous liquid chromatographic determination of norepinephrine, 3,4-dihydroxyphenylethyleneglycol, 3,4-dihydroxyphenylalanine and 3,4-dihydroxyphenylacetic acid in human plasma

ANNE PREMEL-CABIC and PIERRE ALLAIN*

*Laboratoire de Pharmacologie, Centre Hospitalier Universitaire, Rue Larrey, 49033 Angers Cedex (France)*

(First received May 10th, 1988; revised manuscript received August 23rd, 1988)

Liquid chromatography (LC) with electrochemical detection (ED) is very useful for the determination of catecholamines in plasma after extraction on alumina. Since the separation on alumina is specific for the catechol nucleus, the deaminated metabolites 3,4-dihydroxyphenylethyleneglycol (DHPG), 3,4-dihydroxymandelic acid (DHMA), 3,4-dihydroxyphenylacetic acid (DOPAC) and the catecholamine precursor 3,4-dihydroxyphenylalanine (DOPA) are also adsorbed. Their simultaneous analysis after elution from alumina is possible by LC by optimization of the mobile phase. Such a method was described by Rossetti et al. [1] for the simultaneous assay of catecholamines, DOPA and DOPAC, and more recently by Eisenhofer et al. [2] for the simultaneous assay of catecholamines, DHPG, DOPA and DOPAC. Radioenzymatic methods have been widely used for measurement of DOPA [3] and DHPG [4–6] in plasma.

However, few data about the concentrations of DHPG, DOPA and DOPAC in plasma from healthy subjects are yet available. Here we describe an LC–ED procedure that we used for the simultaneous assay of norepinephrine (NE), DHPG, DOPA and DOPAC in the plasma of 36 healthy subjects.

## EXPERIMENTAL

### *Reagents*

Epinephrine (E), dopamine (DA), NE, DHPG, DHMA, DOPA and DOPAC were obtained from Sigma (St. Louis, MO, U.S.A.). 3,4-Dihydroxybenzylamine

0378-4347/88/$03.50 

(DHBA) was purchased from Aldrich (Milwaukee, WI, U.S.A.) and heptanesulphonate was from Eastman Organic Chemicals (Rochester, NY, U.S.A.). Alumina (neutral activity, grade I; Woelm, Eschwege, F.R.G.) was prepared as recommended by Anton and Sayre [7] and stored in a desiccator. All other chemicals were of analytical grade and were used without further pretreatment.

Stock solutions of NE, E, DA, DHPG, DHMA, DOPA, DOPAC and DHBA were prepared separately at a concentration of 100 mg/l in 10 mmol/l hydrochloric acid. A working standard mixture containing (per litre) 25 μg of NE, 50 μg of DHPG, 100 μg of DOPA and DOPAC was made in 10 mmol/l hydrochloric acid. The stock solution of DHBA was diluted with 10 mmol/l hydrochloric acid to give a 25 μg/l working internal standard solution. Stock solutions were stable at 4 °C as for one month.

*Sample preparation*

In a 1.5-ml polypropylene conical tube were mixed 1 ml of plasma, 50 μl of 10 g/l sodium metabisulphite solution and 50 μl of 50 g/l EDTA solution. To this were added 50 μl of working internal standard solution, 50 μl of 3 mol/l Tris buffer (pH 8.5) and 15–20 mg of alumina. The tube was shaken for 5 min, then the supernatant was aspirated and discarded. The alumina was washed three times with 1.4-ml portions of water. The wash was aspirated, and 100 μl of 0.1 mol/l perchloric acid were added. The tube was shaken for 5 min, then centrifuged at 10 000 *g*. The supernatant liquid was transferred to an autosampler vial, and 40 μl were injected into the LC system.

*Chromatographic and detection conditions*

The LC system included a Model Constametric III pump (Laboratory Data Control, Paris, France), a home-made column compartment, a WISP 710 B autosampler (Waters Assoc., Milford, MA, U.S.A.), a Model LC-3 amperometric detector (Bioanalytical Systems, West Lafayette, IN, U.S.A.) and a Model 3390 A integrator (Hewlett-Packard, Les Ulis, France). The analytical reversed-phase column (250 mm × 4.6 mm I.D.) was packed with Spherisorb 5 octadecylsilane (Laboratory Data Control). The mobile phase (pH 2.5) contained (per litre) 0.1 mol of potassium dihydrogenphosphate, 0.05 mol of phosphoric acid, 0.3 mmol of EDTA and 1 mmol of heptanesulphonate. The flow-rate was 1.1 ml/min and the column temperature was 30 °C. The amperometric detector was operated at 0.70 V vs. an Ag/AgCl reference electrode.

*Blood samples*

Plasma concentrations of NE, DHPG, DOPA and DOPAC were determined in 36 healthy volunteers, ages (mean ± S.D.) 45.5 ± 14.9 years. Blood samples were drawn in the morning after an overnight fast in sitting subjects. Samples (10 ml) were collected in heparinized tubes by venepuncture. Blood was immediately centrifuged and plasma frozen at −30 °C. Analyses were performed within four weeks.

## RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of a standard solution of NE, E, DA, DHPG, DHMA, DOPA, DOPAC and DHBA and of an extract of a plasma sample. All the products were well separated. However, in plasma samples, DHMA was often eluted with unknown substances, which made its measurement impossible. Uric acid, the major contaminant from human plasma, did not interfere with the peaks of interest. After repeated use of the column, the addition of more ion-pairing agent was necessary to maintain good resolution. The detection limit of the method was ca. 50 ng/l for NE and DHPG, 70 ng/l for E, 100 ng/l for DA, 150 ng/l for DOPA and 230 ng/l for DOPAC. This sensitivity was not sufficient to quantify endogenous concentrations of free E and DA. The overall absolute analytical recovery calculated for the concentration range of the calibration curve was 74%

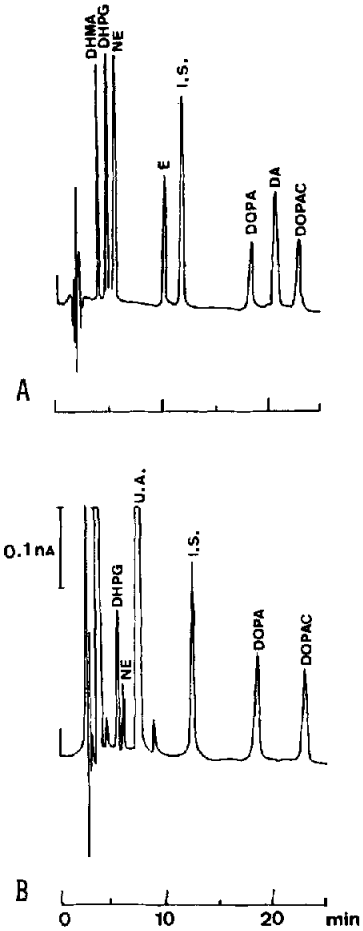


Fig. 1. Chromatograms obtained after injection of (A) 40 $\mu$l of a standard mixture containing 7500 ng/l of each reference and internal standard (I.S.) and (B) 40 $\mu$l of an extract of a plasma sample containing 880 ng/l DHPG, 400 ng/l NE, 1920 ng/l DOPA and 2500 ng/l DOPAC (U.A. = uric acid).

for NE, 53% for DHPG, 58% for DOPA, 23% for DOPAC and 73% for the internal standard (DHBA). The linearity was good up to 1000 ng/l for NE, 2000 ng/l for DHPG and 4000 ng/l for DOPA and DOPAC. The intra-assay coefficient of variation ($n=8$) was 4.3% for NE, 6.7% for DHPG, 4.3% for DOPA and 7.2% for DOPAC.

From plasma of 36 healthy subjects, we obtained the following values (mean $\pm$ S.D.): NE, 410 $\pm$ 140 ng/l; DHPG, 1060 $\pm$ 290 ng/l; DOPA, 1870 $\pm$ 460 ng/l and DOPAC, 2610 $\pm$ 770 ng/l. The comparative high mean concentration of NE could be explained by the sitting position of the subjects prior to and during the blood sampling. Unlike plasma NE, plasma DHPG, DOPA and DOPAC are not affected by the posture [2,4]. Our mean concentrations of DHPG and DOPAC are in good agreement with those reported by Kopin [8] in a review of the literature and our mean value for DOPA is consistent with previous reports [3, 9].

In conclusion, LC–ED is a simple, rapid and accurate method for simultaneous determination of NE, DHPG, DOPA and DOPAC in plasma. It should be useful for the study of disease- and drug-induced variations of these compounds in plasma.

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