Journal of Chromatography, 380 (1986) 229—231
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

CHROMBIO. 3126

Letter to the Editor

High-performance liquid chromatographic determination of urinary catecholamines by direct pre-column fluorescence derivatization with 1,2-diphenylethylenediamine

Sir,

In a previous publication, we reported on the use of 1,2-diphenylethylenediamine (DPE) as a sensitive and selective fluorogenic reagent for catecholamines (CAs: epinephrine, E; norepinephrine, NE; dopamine, DA) [1]. This reaction is accelerated by water-miscible organic solvents, and it has been applied to the pre-column fluorescence derivatization of urinary CAs in aqueous ethanol [2]. This method requires clean-up of urine samples by weak cation-exchange chromatography.

Recently, we have found that in aqueous acetonitrile DPE reacts with CAs more selectively and the reaction has been applied to the determination of CAs in human plasma [3]. The purpose of this study was to investigate the use of this reaction for the high-performance liquid chromatographic (HPLC) determination of human urinary CAs without sample clean-up, using isoproterenol (IP) as an internal standard.

Apparatus, sample collection and HPLC conditions were the same as described previously [2, 3]. To 1.0 ml of a mixture of 0.5 *M* potassium chloride–acetonitrile (2:3), 10 μl each of urine, 0.5 nmol/ml IP solution and 75 m*M* potassium hexacyanoferrate(III) and 100 μl of 0.1 *M* DPE solution were successively added. The mixture was allowed to stand at 37°C for 40 min, and a 100-μl aliquot was injected into the chromatograph. Since the concentration of E in human urine is usually the lowest among the three CAs, the fluorescence of the HPLC eluates was monitored using the excitation and emission maxima of the DPE derivative of E (350 and 480 nm, respectively).

Fig. 1 shows a typical chromatogram of a sample of human urine. The CA peaks (peaks 1—3) were identified by the previously described technique [3]. Peak 5 was identified as 3,4-dihydroxyphenylacetic acid (DOPAC) by the same technique. Most of the early eluting peaks (6 in Fig. 1) were also observed, even when the DPE solution was omitted in the procedure; they are due to natively fluorescent compounds present in urine.

The DPE reaction in aqueous acetonitrile is selective for CAs and thus

0378-4347/86/$03.50 © 1986 Elsevier Science Publishers B.V.

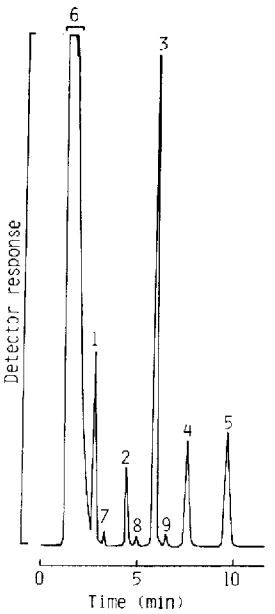

Fig. 1. Chromatogram of a sample of human urine. Sample volume: 10 μl. Peaks (concentrations in nmol/ml in parentheses): 1 = NE (0.81); 2 = E (0.30); 3 = DA (32.55); 4 = IP (0.50); 5 = DOPAC; 6—9 = unidentified.

permits a direct derivatization of CAs in urine. Other conditions for the derivatization reaction were as described previously [2, 3].

In order to estimate the validity of the recommended procedure, CAs (nmol) in 24-h urine from 26 healthy persons determined by this procedure (x) were compared with those obtained by the procedure with sample clean-up using a Toyopak SP (a cation exchanger, sulphopropyl resin; Toyo Soda, Tokyo, Japan) cartridge [3] (y). The data showed a good correlation: $y = 0.982x + 5.09$ ($r = 0.998$) for NE, $y = 1.002x - 0.14$ ($r = 0.999$) for E and $y = 0.993x + 6.57$ ($r = 0.997$) for DA.

Linear relationships were obtained between the ratios of the peak heights of CAs to that of IP and the amounts of CAs added in the range of 0.01–2.0 nmol each to 10 μl of urine. The detection limits for NE, E and DA were 6 pmol per 10 μl of urine (5 fmol per 100-μl injection volume) each (at a signal-to-noise ratio of 3). The within-day coefficients of variation for NE, E and DA ($n = 10$) were 3.0, 3.6 and 2.4% at mean concentrations of 137, 88 and 783 nmol/ml of a control urine, respectively.

The amounts of free CAs (nmol) in 24-h urines from 26 healthy persons (21—53 years old) assayed by the recommended procedure were 303 ± 125 for NE, 80 ± 41 for E and 1540 ± 548 for DA (mean ± S.D.). These values are in good agreement with those reported previously [2, 4].

Faculty of Pharmaceutical Sciences,
Kyushu University 62, Maidashi, Higashi-ku,
Fukuoka 812 (Japan)

HITOSHI NOHTA
AKANE MITSUI
YOSUKE OHKURA*

1 H. Nohta, A. Mitsui and Y. Ohkura, Anal. Chim. Acta, 165 (1984) 171.
2 H. Nohta, A. Mitsui and Y. Ohkura, Bunseki Kagaku, 33 (1984) E263.
3 A. Mitsui, H. Nohta and Y. Ohkura, J. Chromatogr., 344 (1985) 61.
4 T.P. Moyer, N.-S. Jiang, G.M. Tyce and S.G. Sheps, Clin. Chem., 25 (1979) 256.

(First received December 17th, 1985; revised manuscript received February 17th, 1986)