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Note

Direct clean-up and analysis of urinary catecholamines

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Catecholamines play an important role as neurotransmitters in the central nervous system, in the regulation of blood pressure or for the detection of catecholamine-secreting tumours, e.g. phaeochromocytoma [1, 2].

The development of high-performance liquid chromatographic (HPLC) techniques during the last decade has shown that this method is particularly useful for the determination of catecholamines in physiological fluids. With respect to the chemical properties of the catecholamines and their metabolites different modes of HPLC such as reversed-phase, reversed-phase ion-pair, ion-exchange including either electrochemical detection or fluorescence detection after pre- or postcolumn derivatization have been exploited [3, 4].

For the quantitation of catecholamines in physiological fluids preliminary sample clean-up steps still present a crucial factor for routine clinical analysis. Liquid—solid extraction methods, e.g. the use of cation exchangers, acid-washed aluminium oxide or the use of boric acid affinity gels either in the column under low-pressure conditions or in the batch mode are in common use [3, 4].

Recently Neidhart et al. [5] introduced a column-switching technique consisting of an aluminium oxide precolumn and a cation-exchange analytical column which allows the direct analysis of epinephrine and norepinephrine in urine with fluorimetric detection after postcolumn derivatization. Hansson et al. [6] developed a column-switching technique with a boronic acid—silica supported precolumn and a reversed-phase C_{18} analytical column allowing the electrochemical detection of 3,4-dihydroxyphenylacetic acid.

By use of a column-switching technique we introduced a combined boric acid high-performance liquid affinity chromatography (HPLAC)—RP-C_{18} HPLC method for the on-line clean-up and analysis of ribonucleosides in

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physiological fluids [7, 8]. First results in catecholamine research with on-line ion-pair reversed-phase chromatography of spiked urines by ultraviolet detection showed that the column-switching technique is applicable for analysis of these biogenic amines [9]. Because, under these conditions no reproducible retention times were achieved, we have now developed a method for the on-line clean-up and analysis of the principal urinary catecholamines epinephrine (E), norepinephrine (NE) and dopamine (DA) including a boric acid HPLAC—cation-exchange HPLC column-switching technique with electrochemical detection. This provides a powerful improvement compared to the system introduced by Hansson et al. [6] for the analysis of just one of the dopamine catabolites.

EXPERIMENTAL

Chemicals

Norepinephrine (NE), epinephrine (E) and dopamine (DA) were purchased from Aldrich (Steinheim, F.R.G.). In all buffer preparations double-distilled water and salts from E. Merck (Darmstadt, F.R.G.) of the purest grade available were used.

Urine treatment

Fresh human urine (500 μl) was membrane-filtered (0.2 μm; Schleicher & Schüll, Dassel, F.R.G.) and an aliquot of 100 μl was applied to the high-performance liquid affinity chromatography column (HPLAC column 1). To check for possible interfering compounds a blank urine was prepared from another 500-μl aliquot by adjusting the pH to 10 with concentrated ammonia to destroy endogenous free catecholamines. After standing for 5 h at 25°C an aliquot of this pretreated urine was analysed.

Chromatography

HPLAC column 1 was filled with laboratory-prepared phenylboronic acid-substituted silica [10] in a stainless-steel column (50 × 4 mm I.D.). Column 2 was filled with a cation-exchange material (Nucleosil 10 SA, 10 μm; 250 × 4 mm I.D.; Macherey & Nagel, Düren, F.R.G.).

Column 2 was isocratically eluted with 0.45 mol/l formic acid adjusted to pH 3.0; flow was 2.0 ml/min. The elution of column 1 is described in the results section. Under these chromatographic conditions the following retention times (min) were found: NE (6.71), E (10.16) and DA (12.59). Electrochemical detection was carried out with an electric potential set at +0.75 V.

HPLC apparatus

The HPLC equipment consisted of two Altex Model 110 A pumps (Altex, U.S.A.) controlled by a Model 420 microprocessor and a Rheodyne Model 7125 loop injector for sample introduction. Detection was performed with an electrochemical detector (ELCD 656 and 641 VA detector; Metrohm, Switzerland) with a glassy carbon electrode and Ag/AgCl reference electrode. Areas under the peaks were integrated with a Hewlett-Packard Model 3390 integrator (Hewlett-Packard, Frankfurt, F.R.G.).

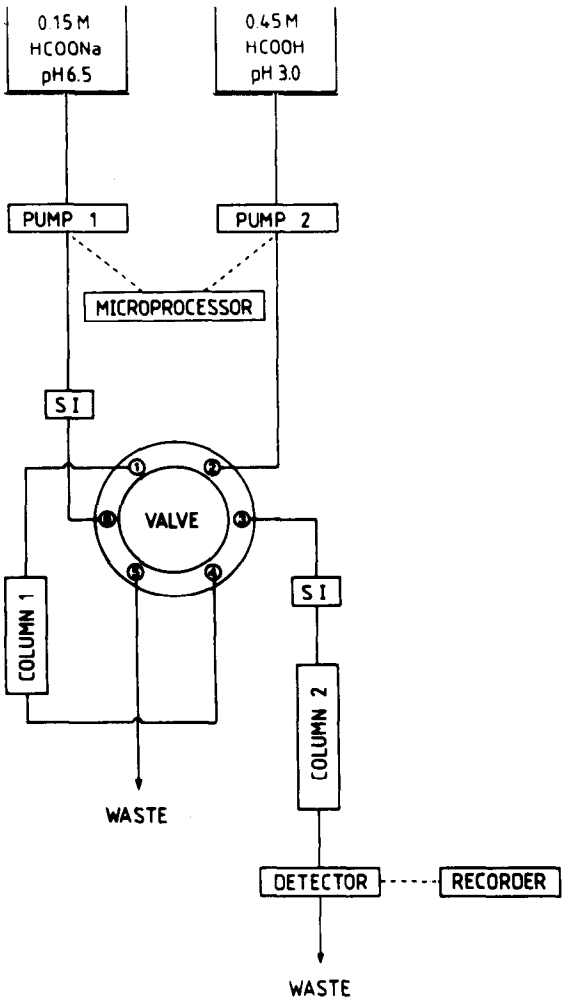

Fig. 1. On-line system set-up.

For the direct on-line analysis the gradient system was additionally equipped with a second Rheodyne 7125 loop injector (SI) and a Rheodyne Model 7010 six-port valve which were incorporated as shown schematically in Fig. 1.

RESULTS AND DISCUSSION

For the direct clean-up and analysis of catecholamines in urine the HPLAC column 1 was equilibrated for 2 min in valve position "Load" (Fig. 2) with 0.15 mol/l sodium formate pH 6.5. After sample injection (synthetic mixture or urine) column 1 was washed with the same buffer for 3.30 min at 1.0 ml/min. During that time catecholamines were selectively retarded on the HPLAC column whereas the sample matrix was discharged. After this clean-up step the valve was switched to "Inject" and thereby connected in series in front of column 2 (Fig. 2). The group-specifically bound catecholamines on column 1 were then eluted under acidic conditions (0.45 mol/l formic acid

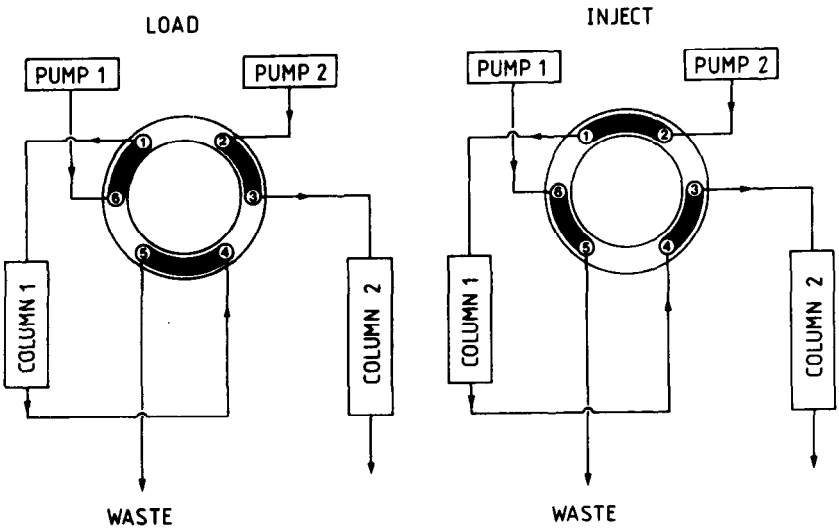

Fig. 2. Switching-valve positions.

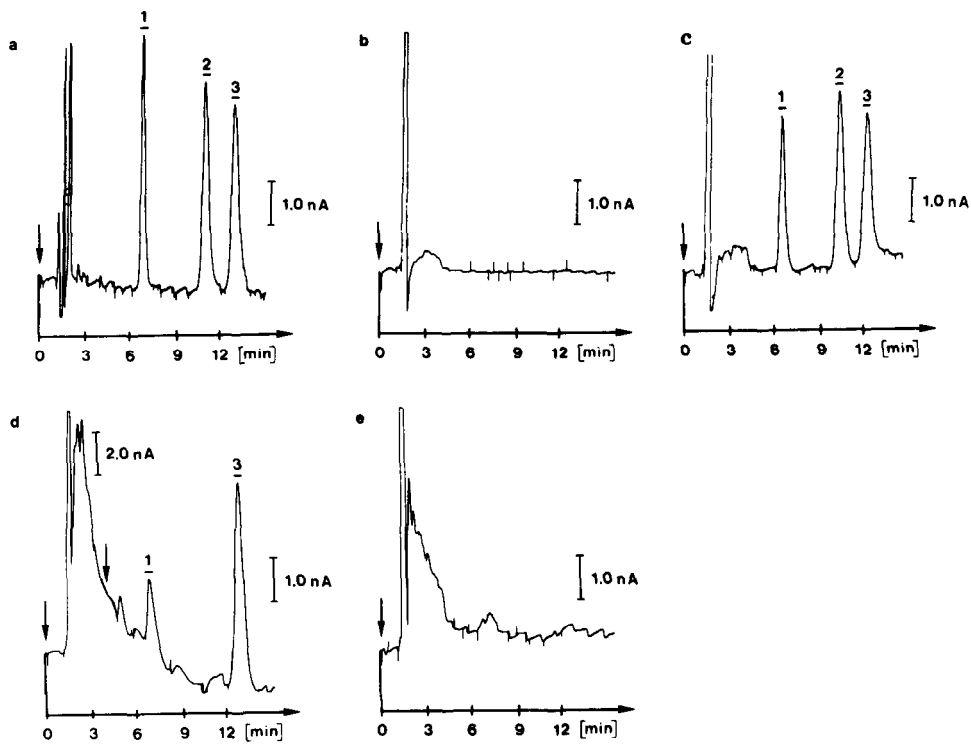

Fig. 3. (a) Off-line cation-exchange HPLC analysis of a synthetic mixture of 50 pmol each of the catecholamines NE (1), E (2) and DA (3). (b) On-line blank run. (c) On-line HPLAC—cation-exchange HPLC of a sample identical to that in (a). (d) On-line analysis of 100 μl of membrane-filtered native human urine. The arrow indicates change in sensitivity. (e) On-line analysis of 50 μl of urine as in (d) after destruction of endogenous catecholamines.

pH 3.0) in a small volume through positions 2—1—4—3 of the valve and transferred to the top of column 2 over a period of 1.10 min at 2.0 ml/min. This elution buffer was also used for the subsequent isocratic analytical separation. The valve was then switched back into position "Load" and elution of column 2 could be carried out.

A comparison of the analysis of E, NE and DA on the cation-exchange column (Fig. 3a) with the on-line procedure (Fig. 3c) shows that the latter one does not essentially affect band broadening and resolution of the catecholamines investigated. The column-switching technique also does not influence the electrochemical detection under the conditions applied (Fig. 3b). The method described allows the direct analysis of the principal catecholamines in native urine in a quarter of an hour (Fig. 3d). As shown in Fig. 3e for the analysis of a urine blank, possible interfering peaks are not detected. This allows the quantitative determination of the compounds of interest.

To monitor the accuracy of the overall chromatographic system, the matrix-dependent and -independent recovery of catecholamines was determined. For matrix-independent recovery synthetic mixtures of catecholamines were applied directly to the cation-exchange column 2 and identical mixtures subsequently analysed by the on-line HPLAC—cation-exchange HPLC system. For matrix-dependent recovery the amount of catecholamines present in a control urine was determined by the external standard method. The control urine was then spiked with defined amounts of catecholamines and analysed anew. Results are summarized in Tables I and II.

TABLE I

MATRIX-INDEPENDENT RECOVERY OF CATECHOLAMINES

Catecholamine	Recovery* (%)	R.S.D.** (%)
Norepinephrine	98.9	0.6
Epinephrine	101.4	2.3
Dopamine	97.8	1.0

*Each value is an average of three runs.
**Relative standard deviation.

TABLE II

MATRIX-DEPENDENT RECOVERY OF CATECHOLAMINES ADDED TO CONTROL URINE

Catecholamine	Catecholamine amount* (ng)			Average recovery (%)
	Urine	Spike	Urine + spike (found)	
Norepinephrine	2.5 ± 0.1	8.2 ± 0.2	10.1 ± 0.8	94.7
Epinephrine	—	10.0 ± 0.3	9.9 ± 0.4	99.0
Dopamine	7.7 ± 0.9	8.1 ± 0.5	15.5 ± 0.1	98.1

*Each value is an average of three runs ± standard deviations.

The recovery of catecholamines is very high, due to the fact that sample clean-up and analysis can be carried out under acidic conditions and error-prone evaporation and redissolution steps are avoided.

The method described consists of only a few working steps and allows the direct and quantitative determination of the principal free urinary catecholamines NE, E and DA in a quarter of an hour.

This on-line HPLAC—HPLC system should represent the method of choice, as it is particularly suitable for automation and applicable for routine clinical analysis.

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