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Note

Application of a sodium gradient in dynamic cation-exchange systems for rapid analysis by high-performance liquid chromatography and electrochemical detection of urinary catecholamines after a single purification step with aluminium oxide

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Analysis of urinary catecholamines is of interest in various pathological states [1, 2]. Application of high-performance liquid chromatography (HPLC) has recently renewed interest in the analysis of urinary catecholamines, due to the selectivity and relative simplcity of this technique. Moyer et al. [3], for instance, reported that combined analysis of the catecholamines noradrenaline (NA), adrenaline (A), and dopamine (DA) using HPLC is superior to analysis of metanephrines in detecting pheochromocytoma.

Several methods for the analysis of urinary catecholamines using reversed-phase HPLC have been described in the literature. The abundance of interfering substances in urine necessitates purification of samples before chromatography. Although used by most authors, adsorption onto aluminium oxide has been shown not to be specific enough to be used as a single purification step. Therefore, additional extraction of urine with organic solvents [4] or additional chromatographic procedures like cation-exchange chromatography [5], or boric acid-gel chromatography [3] had to be used.

In this paper we report that rapid analysis of adrenaline, noradrenaline and dopamine in urine after a single purification step with aluminium oxide is pos-

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sible when a dynamic cation-exchange system is used and a sodium gradient at pH 7 is applied.

EXPERIMENTAL

Apparatus

The analyses were performed on a Hewlett-Packard 1084 B chromatograph with an electrochemical detector (Bioanalytical Systems, LC-4 controller and TL-5 cell). The detector was operated at +0.6 V against an Ag/AgCl reference electrode. Prepacked reversed-phase columns were used. An analytical column, 15 cm × 4.5 mm I.D., packed with Hypersil ODS, 5 μm particle size, was obtained from Chrompack (Middelburg, The Netherlands), and in some experiments combined with a 3 cm × 4.6 mm I.D. precolumn, packed with LiChrosorb C_{18}, 10 μm particle size (Brownlee Labs., Santa Clara, CA, U.S.A.). The columns were loaded with sodium dodecyl sulphate (SDS) by pumping through 50 ml of 1% (w/v) SDS in distilled water. After this, columns were equilibrated until the retention of catecholamines was constant, usually with 100 ml of eluent or less.

Materials

Aluminium oxide (Al_2O_3) was obtained from BDH (activity II; BDH, Poole, Great Britain) and prepared according to the method of Weil-Malherbe [6]. All other chemicals were reagent grade. Water was demineralized and distilled in an all-glass apparatus.

Stock solutions (ca. 10—50 ppm) of standards were prepared in 0.05 *M* $HClO_4$ with 0.05% (w/v) tetrasodium EDTA and 0.05% (w/v) $Na_2S_2O_5$ and stored at 4°C for two weeks or less.

Sample preparation

Urine was collected over 0.5 g of disodium EDTA and 0.5 g of $Na_2S_2O_5$ and stored at —70°C. Standard solutions were prepared by diluting 50—200 μl of stock solutions to 25 ml with 0.05 *M* phosphate buffer pH 6.8.

To 0.7 g of aluminium oxide 10 ml of 0.1 *M* Tris buffer pH 8.4 and 0.2 ml of 1 *M* sodium hydroxide were added in a stoppered glass tube. The tube was shaken on a Vortex mixer and the supernatant aspirated off. To 25 ml of urine or of a standard solution were added: ca. 10 mmol of the internal standard dihydroxybenzylamine (DHBA, 100 μl), 2.5 ml of 1 *M* Tris buffer pH 8.4, 1.25 ml of 10% (w/v) disodium EDTA and 0.25 ml of 5% (w/v) $Na_2S_2O_5$. The sample was transferred to the tube containing Al_2O_3. The pH was adjusted to 8.4—8.6 with 5 *M* and 1 *M* sodium hydroxide. The tube was shaken gently for 5 min. After the Al_2O_3 had settled, the supernatant was aspirated off and the Al_2O_3 was washed three times with 10 ml of 0.05% (w/v) disodium EDTA. The catecholamines were eluted with 5 ml of 0.1 *M* $HClO_4$ containing 0.05% (w/v) disodium EDTA and 0.05% (w/v) $Na_2S_2O_5$; 50 or 100 μl of this eluent were injected into the HPLC system.

Chromatographic conditions

The flow-rate was adjusted to 2 ml/min and the temperature of the column

compartment and of the eluents was 30°C. The eluents contained 0.01—0.1 *M* Na_2HPO_4, 0—10% propanol, 0—250 mg/l SDS, and 1.34 mmol of disodium EDTA as specified in the legends to the figures. Addition of EDTA to the eluent eliminated difficulties apparently caused by metal ions from the stainless steel of the apparatus [7]. The pH of the eluent was adjusted with $HClO_4$.

RESULTS AND DISCUSSION

For the analysis of A, NA and DA in Al_2O_3 extracts of urine, use was made of dynamic cation exchange with SDS as anionic surfactant as previously described [7]. It has been shown how the retention of cations in such dynamic cation-exchange systems can be varied by means of the organic modifier concentration, SDS and counterion concentration and pH of the mobile phase [7, 8].

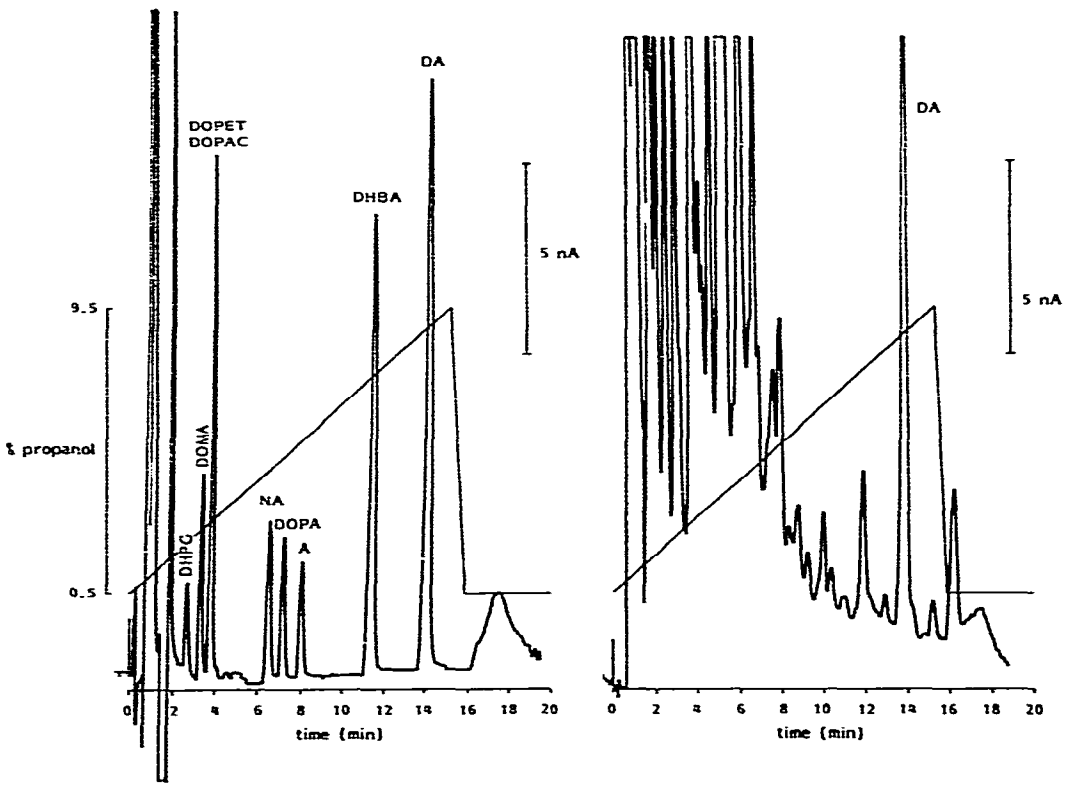

Fig. 1. Application of a propanol gradient for the separation of catecholamines from standard solution or urine, prepurified on Al_2O_3. For preparation of urine samples, see Experimental. The eluent contained 0.05 *M* Na_2HPO_4 and 1.34 mmol/l disodium EDTA; the pH was adjusted to 3 with $HClO_4$. The gradient was 0.5—9.5% propanol and 12.5—237.5 mg/l SDS. Flow-rate was 2 ml/min, the temperature of the eluent and column 30°C. The electrochemical detector was operated at +0.6 V (vs. Ag/AgCl). Abbreviations: DA = dopamine, DHBA = dihydroxybenzylamine, A = adrenaline, NA = noradrenaline, DOPA = dihydroxyphenylalanine, DOPAC = dihydroxyphenylacetic acid, DOMA = dihydroxymandelic acid, DOPET = dihydroxyphenylethanol, DHPG = dihydroxyphenylglycol.

To obtain selective elution of NA, A and DA within a reasonable time, a propanol gradient was applied. Fig. 1, however, shows that many urine constituents are present in the Al_2O_3 extract, preventing the determination of NA and A. It was observed that a considerably cleaner chromatogram was obtained when a high propanol content (4%) was used for the entire chromatography. To obtain a sufficient retention of NA and A with 4% propanol a low sodium concentration can be used. Since under these conditions the k' of DA is very large, the application of a sodium gradient is obvious.

Fig. 2 shows chromatograms of the same standard mixture and urinary Al_2O_3 extract as in Fig. 1, but now obtained by applying a sodium gradient at fixed propanol (4%) content. As can be seen, NA, A, and DA are eluted free of interfering substances, although interfering peaks are close to NA and A and close to the retention time of the internal standard DHBA.

Due to the low pH of the eluent, one can expect that the remaining interferences are acidic compounds. In that case their retention can be drastically reduced by increasing the pH of the eluent. The favourable effect of increasing pH on the background of the urinary Al_2O_3 extract is shown in Fig. 3, which is the same experiment as in Fig. 2 but now at pH 7. The application

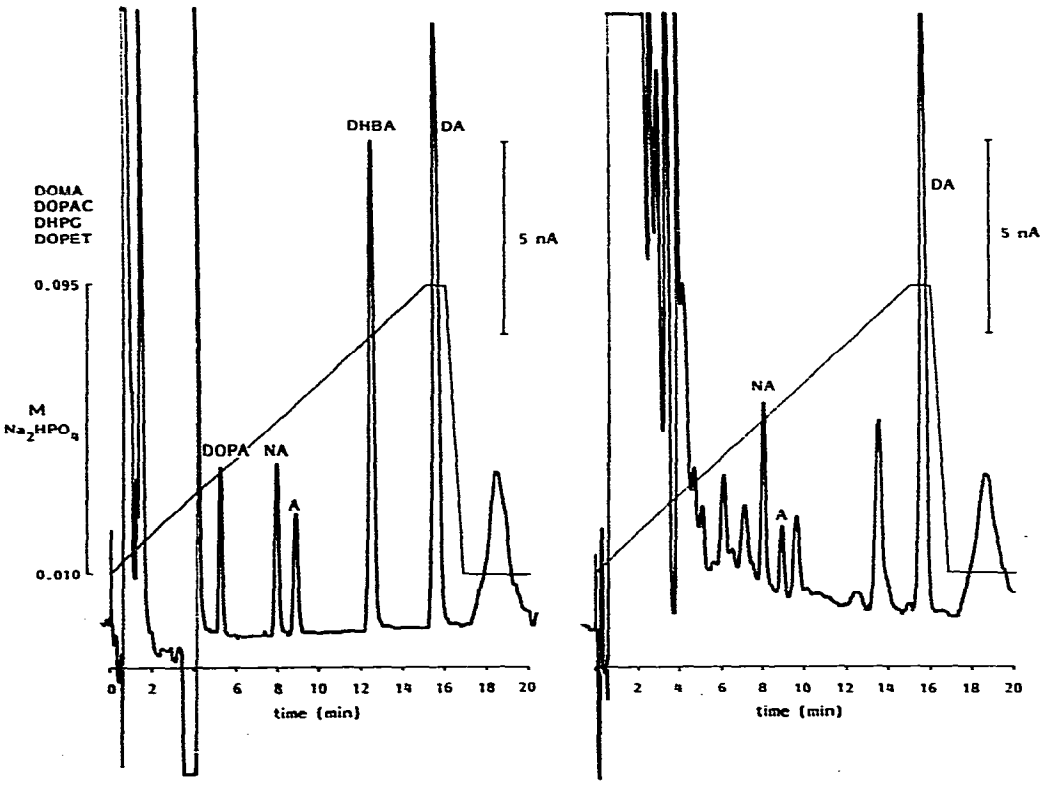

Fig. 2. Application of a sodium gradient for the separation of catecholamines from standard solution or urine, prepurified on Al_2O_3. Same samples as in Fig. 1. The eluent contained 4% propanol, 100 mg/l SDS, 1.34 mmol/l disodium EDTA, and the pH was adjusted to 3 with $HClO_4$. Flow-rate was 2 ml/min, the temperature of the eluent and column 30°C. The electrochemical detector was operated at +0.6 V (vs. Ag/AgCl). For abbreviations see Fig. 1.

of a Na^+ gradient with 4% propanol at pH 7 was found to be the best choice for the analysis of A, NA, and DA directly in Al_2O_3 extracts of urine. The reproducibility of the retention time was determined to be 0.6% (C.V., n=10) for all components, and 3—4 min elution (at 2 ml/min) with the original eluent was sufficient to re-equilibrate the column for the next analysis. The column used to record Figs. 1—3 was one year old and its plate number was ca. 3500 at the time of recording.

The shift of the baseline of the electrochemical detector due to the Na^+ gradient (and also for the propanol gradient) was surprisingly small and of the order of 0.25 nA. This marginally influences the analysis since the expected peak heights of the catecholamines extracted from urine ranges between 5 and 25 nA (see also Fig. 3).

Another favourable effect was noticed when comparing the response of the detector for the catecholamines at pH 3 and 7. It was found that the response of NA, DA, and DHBA (internal standard) was constant in the pH range 3—7 but increased for A by a factor of 1.5 in the pH range 4.5—7.

The electrochemical detector used is suitable for routine use. The cell was cleaned with methanol only after several hundred analyses and polished only every few months.

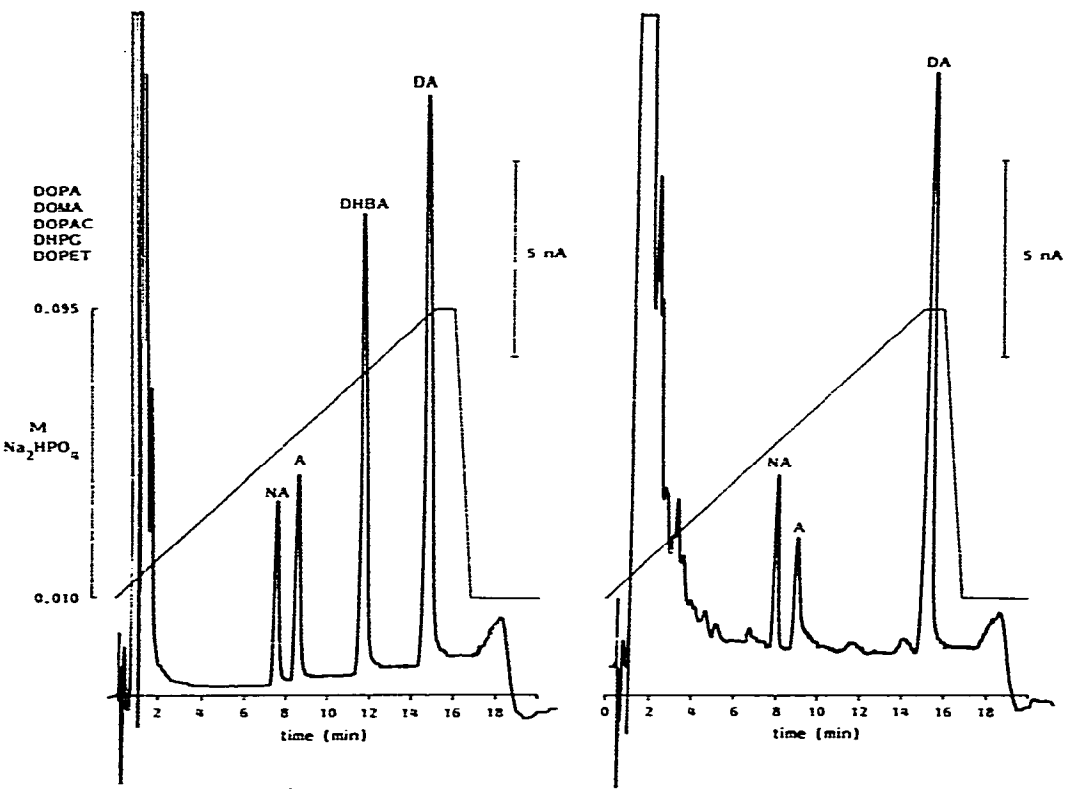

Fig. 3. Application of a sodium gradient for the separation of catecholamines from standard solution or urine, prepurified on Al_2O_3. Same experiment as in Fig. 2, but now at pH 7. For further details see legend to Fig. 2. For abbreviations see Fig. 1.

In conclusion, dynamic cation-exchange chromatography when applying a Na^+ gradient allows a simple and rapid analysis of the three major urinary catecholamines, since sample clean-up can be restricted to a single step using Al_2O_3. The present experiments show the first application of a sodium gradient as an useful chromatographic parameter in combination with electrochemical detection.

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