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Short Communication

Separation of catecholamines by high-performance liquid chromatography on dynamically-modified silica*

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Introduction

The determination of catecholamines in urine is of clinical interest due to their importance in the diagnosis of various tumour diseases. Their determination in low-dosage pharmaceutical preparations is also of practical importance. For this purpose the use of high-performance liquid chromatography (HPLC) using fluorimetric or electrochemical detection has been proposed by various authors using either ion-exchange (e.g. [1–2]) or reversed-phase column materials (e.g. [3–6]). Quantitative determination of catecholamines by HPLC in pharmaceutical preparations has also been reported (e.g. [7–10]).

In recent years the use of HPLC on bare silica dynamically modified by the adsorption of cetyltrimethylammonium (CTMA) ions to its surface has been demonstrated as an alternative to reversed-phase chromatography on chemically bonded phases [11–13]. It has been demonstrated that this approach permits the standardization of reversed-phase separations by avoiding the brand-to-brand variations in selectivity observed with bonded-phase separations [14–15].

The aim of the present investigation was to investigate the possibility of using the dynamic modification approach in separating the labile catecholamines in pharmaceutical preparations as well as in biological samples.

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Experimental

Chemicals

Adrenaline bitartrate, noradrenaline bitartrate and dopamine hydrochloride were of pharmacopoeial quality. 3,4-Dihydroxybenzylamine hydrochloride was obtained from Sigma (St Louis, MO, USA). Acetonitrile (HPLC grade S) was from Rathburn (Walkerburn, UK). All other reagents were of analytical grade from E. Merck (Darmstadt, FRG).

Preparations

Two pharmaceutical preparations of the following composition (expressed as mg/ml) were analyzed: (A) Lidocaine hydrochloride 5, noradrenaline 0.005, sodium metabisulfite 0.6, methylparaben 1 and sodium chloride 8; (B) Bupivacaine hydrochloride 2.5, adrenaline 0.005, sodium metabisulfite 0.5 and sodium chloride 8.

Chromatography

A liquid chromatograph consisting of a Kontron Model 410 LC pump, a Pye Unicam LC-UV spectrophotometer detector operated at 280 nm or a Metrohm Model 656/641 electrochemical detector, and a Rheodyne Model 7125 injection valve equipped with a 20-μl loop were used. Chromatograms were recorded on a Kipp & Zonen Model BD-8 recorder and retention data were measured and processed by a Hewlett-Packard Model 3353A laboratory data system. All experiments were performed on columns of 120 or 250 × 4.6 mm i.d. (Knauer, Oberursl, FRG) packed with LiChrospher SI 100 5-μm (E. Merck). The eluents were mixtures of acetonitrile or methanol, water and 0.2 M potassium phosphate buffer of various pH values and with the addition of 2.5 mM CTMA bromide. The pH values stated are those of the undiluted buffers and not those of the final eluents. The buffers were prepared from a solution of potassium dihydrogen phosphate titrated with 5 M potassium hydroxide to the prescribed pH value and diluted with water to a final concentration of 0.2 M. The columns were equilibrated by conditioning overnight. During chromatography the analytical column was guarded by a silica pre-column (100 × 8 mm i.d.) packed with LiChroprep SI 60 and inserted between the pump and the injection valve. After each experiment the column was restored to its initial state by rinsing with methanol–0.1 M nitric acid (1:1, v/v) and finally with methanol.

The amount of CTMA adsorbed onto the LiChrospher SI 100 column was determined by the elution method previously described [16].

Test and standard solutions

Solutions for the calibration curve were in the range 4 ng/ml–8.8 mg/ml of each of adrenaline bitartrate, noradrenaline bitartrate, dopamine hydrochloride and 3,4-dihydroxybenzylamine hydrochloride in 80% v/v methanol.

Standard solutions for the quantitative determination of adrenaline or noradrenaline in pharmaceutical preparations contained 0.5 μg/ml of adrenaline or noradrenaline in 80% v/v methanol.

Pharmaceutical preparations were diluted (1 + 9) with 80% v/v methanol.

Urine samples were preserved by the addition of hydrochloric acid to 0.05 M and stored at −20°C. For analysis 0.5 ml was diluted with 0.5 ml of methanol.

Spiked urine samples were prepared from 0.5 ml of urine, 0.4 ml of methanol, and

0.100 ml of a solution containing 1.75×10^{-4}% m/v of each of the three catecholamines and of 3,4-dihydroxybenzylamine as an internal standard.

Results and Discussion

The HPLC method was developed using a solution containing 0.1% m/v of each of the three catecholamines and of 3,4-dihydroxybenzylamine and utilizing UV-detection. Several parameters will influence the retention and selectivity in a chromatographic system based on the dynamic modification approach [13]. The initial eluent composition was based on a standard system reported earlier [13]; acetonitrile was later substituted for methanol, resulting in a mixture of acetonitrile–water–0.2 M potassium phosphate (pH 7.5) (50:45:5 v/v/v). Acetonitrile was chosen as the organic modifier in the light of previous findings that it is often preferable to methanol in the separation of amines by reversed-phase chromatography [17].

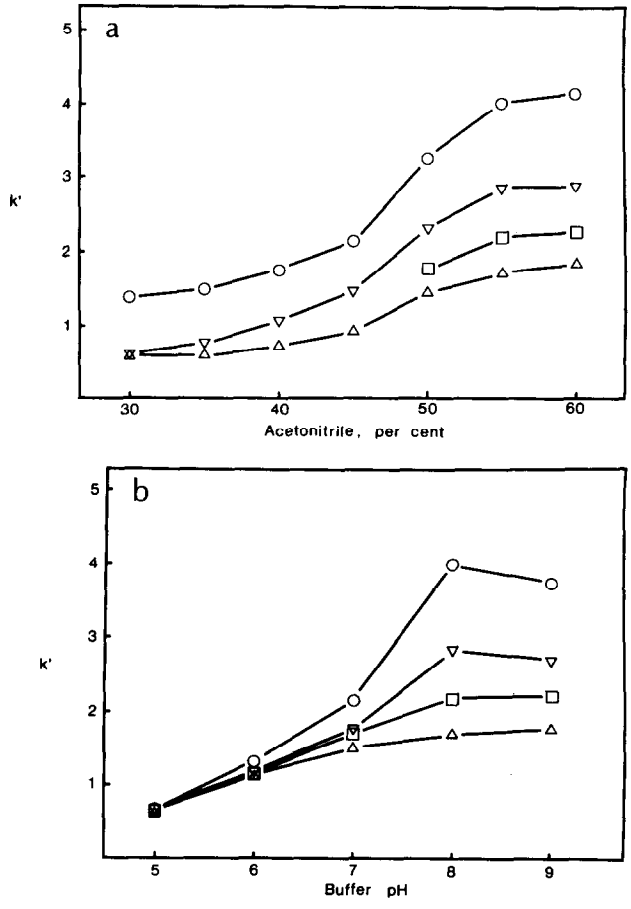

Figure 1
Influence of acetonitrile concentration (a) and of buffer pH (b) on retention. Key to solutes: ○, adrenaline; ▽, dopamine; □, 3,4-dihydroxybenzylamine; △, noradrenaline. Column: LiChrospher SI 100 (120 × 4.6 mm i.d.). Eluents: (a) acetonitrile–water mixtures containing 5% of 0.2 M potassium phosphate buffer pH 8.0 and 2.5 mM CTMA; (b) acetonitrile–water–0.2 M potassium phosphate buffer (55:40:5 v/v/v) at various pH values containing 2.5 mM CTMA.

To optimize the system the influence of the acetonitrile concentration and of the buffer pH on the separation was investigated as shown in Fig. 1. The influence of the organic modifier concentration on the retention of the four test substances is opposite to the effect observed for a reversed-phase partition retention mechanism, as is the case with non-ionic and anionic compounds [13, 14]. It has previously been found that even with cationic compounds reversed-phase partition is the most important retention mechanism, ion-exchange being possible only for very hydrophobic cations due to the strong affinity of the CTMA ions for ionized silanol groups [18]. For such polar compounds as the catecholamines, however, a more complex mixed mechanism of a different nature is responsible for the retention. Further studies are needed to elucidate the details of this mechanism.

The optimal eluent parameters were chosen from Figs 1a and 1b as 55% v/v acetonitrile and pH 8. When analysing mixtures of catecholamines at lower concentrations using this eluent it was found that deterioration of the catecholamine peaks took place, resulting both in poor peak shape and reduced response factor. As shown in Fig. 2a total deterioration is observed when reducing the concentration of catecholamines in the test solution from 0.1% to 0.001% w/v. It was found possible to avoid this problem by substituting methanol for acetonitrile as the organic modifier as shown in Fig. 2b. It was found necessary, however, to adjust the modifier concentration and the buffer pH to achieve equivalent separation. The deterioration of the peaks may be attributable either to trace amounts of metals present in the silica material, or to adsorption; methanol is apparently able to protect the catecholamines from this effect.

To enhance detection sensitivity for the analysis of real samples, electrochemical detection was utilized. The voltage used in detecting catecholamines is most often 600 mV or more [5]. It was found that by reducing it to 400 mV less than 5% reduction in response for catecholamines was observed. At the same time, however, the response to interfering compounds in urine was reduced to a degree which made it possible to avoid the clean-up stage which is normally essential [1–6]. Of importance in simplifying the analysis is also the fact that all the non-ionic and anionic compounds which are retained by reversed-phase partition only are eluted at short retention times, due to the high organic modifier concentration and the low amount of CTMA adsorbed (*ca* 0.1 mmol/g). Thus the most important effect of adding CTMA to the eluent is that it improves the peak shape of catecholamines while at the same time preventing the retention of cationic compounds by the ion-exchange mechanism. Chromatograms of urine samples with and without the addition of catecholamines and internal standard are shown in Fig. 3.

The HPLC method has been used for the quantitation of pharmaceutical preparations containing catecholamines in low concentrations. All excipients including preservatives such as methylparaben are eluted at the solvent front. When the preparations are analysed by reversed-phase chromatography on bonded phase materials, relatively low modifier concentrations are used and then methylparaben is strongly retained on the column. For quantitation external standardization was used. Linearity of detector response was established for each of the three catecholamines adrenaline, noradrenaline and dopamine, respectively, for injected amounts ranging from 0.80 to 80 ng ($n = 7$): $y = 8.46x - 12.6$ ($r = 0.9989$); $y = 10.3x - 17.8$ ($r = 0.9974$); and $y = 18.5x - 18.4$ ($r = 0.9994$). The precision of the method was investigated by analysing five individually prepared dilutions of preparations A and B containing 0.5 μg/ml of noradrenaline and adrenaline respectively (~ 10 ng injected) (Fig. 4). Relative standard deviations of 1.6% for noradrenaline and 2.1% for adrenaline were found.

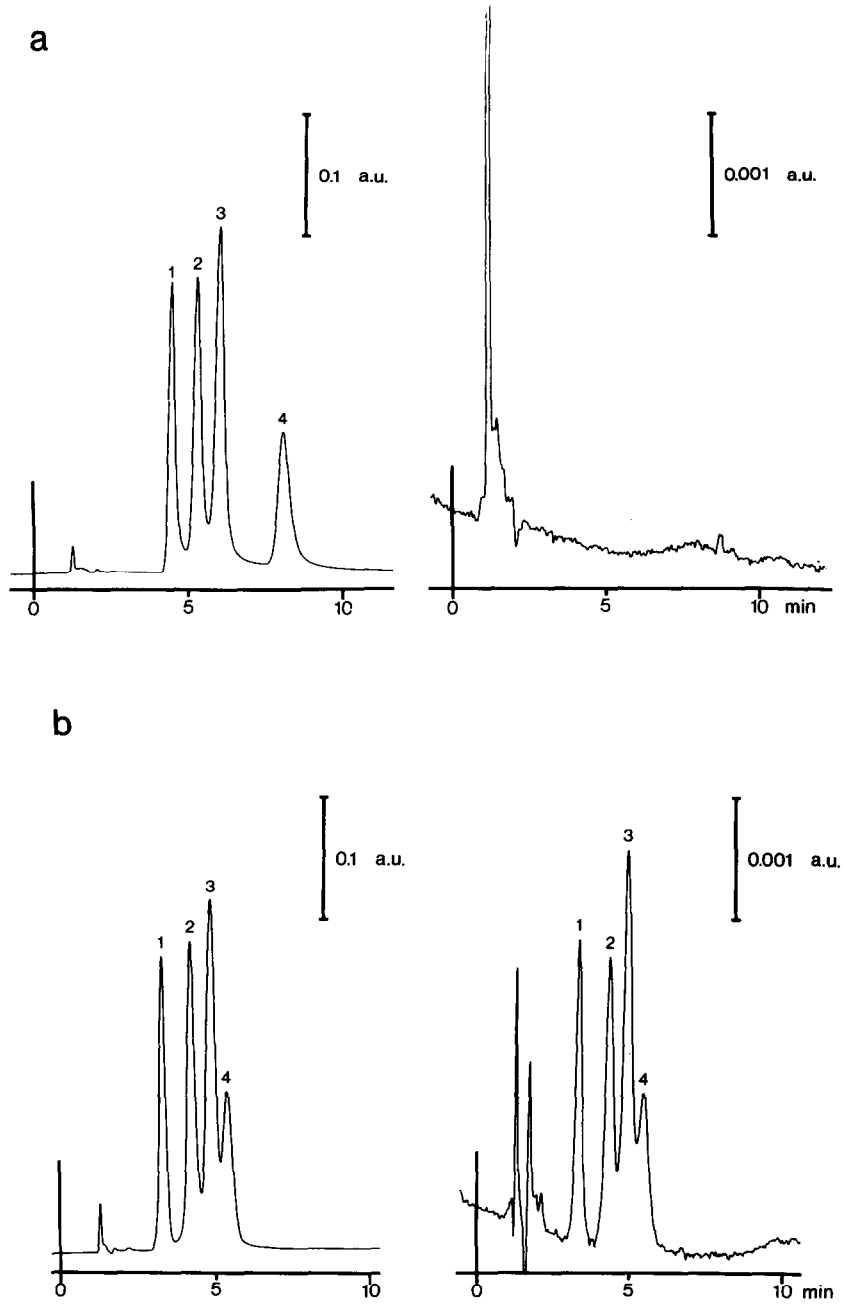

Figure 2
Influence of type of modifier on chromatographic behaviour for various amounts of catecholamines injected (20 μg or 0.2 μg of each of four catecholamines). Column: LiChrospher SI 100 (120 × 4.6 mm i.d.). Eluents: (a) acetonitrile–water–0.2 M potassium phosphate buffer pH 8.0 (55:40:5 v/v/v) containing 2.5 mM CTMA; (b) methanol–water–0.2 M potassium phosphate buffer pH 7.0 (80:15:5 v/v/v) containing 2.5 mM CTMA. Detection wavelength: 280 nm. Flow rate: 1 ml/min. Peaks: 1 = noradrenaline; 2 = 3,4-dihydroxybenzylamine; 3 = dopamine; 4 = adrenaline.

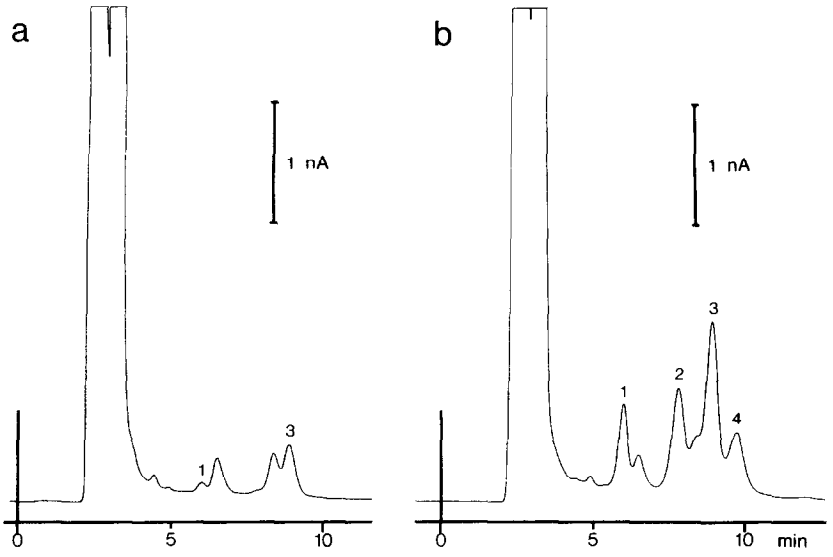

Figure 3
Chromatograms of urine samples with (b) and without (a) the addition of catecholamines and internal standard (3.5 μg of each injected). Column: LiChrospher SI 100 (250 × 4.6 mm i.d.). Eluent: methanol–water–0.2 M potassium phosphate buffer pH 7.0 (80:15:5 v/v/v). Polarization voltage: 400 mV. Flow rate: 1 ml/min. Peak identification as in Fig. 2.

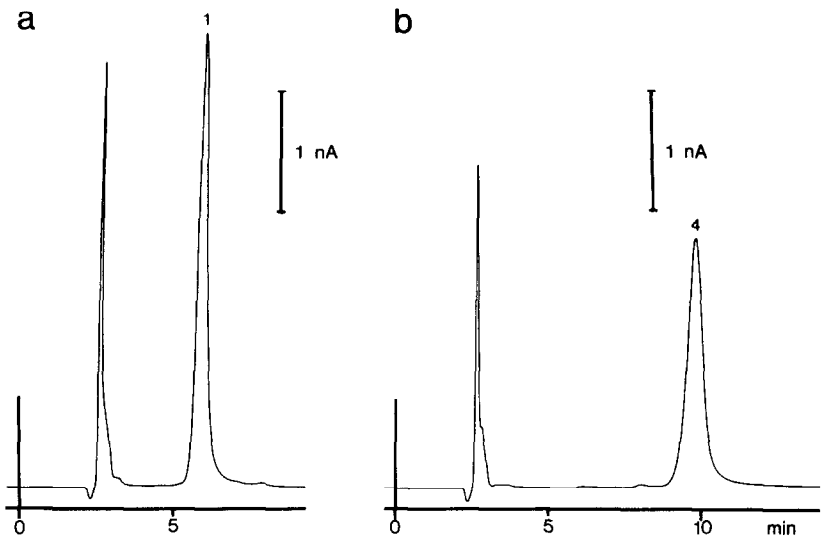

Figure 4
Chromatograms of pharmaceutical preparations A (a) and B (b). Chromatographic conditions and peak identification as in Fig. 3.

Conclusion

An HPLC method based on dynamically-modified silica has been elaborated and shown to be suitable for separating catecholamines. Using electrochemical detection it has been found possible to quantitate catecholamines in low-dosage pharmaceutical preparations. The separation of catecholamines in urine has also been demonstrated. In the analysis of pharmaceutical preparations the method has the advantages that other active ingredients, as well as excipients, are eluted at the solvent front, and that the selectivity is more reproducible than that for bonded reversed-phase materials.

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