Journal of Chromatography, 619 (1993) 111–115
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 7014

Short Communication

Determination of dopamine and its metabolites in microdialysates by capillary liquid chromatography with electrochemical detection

V. F. Ruban

Institute of Macromolecular Compounds, Russian Academy of Science, 199 004 St. Petersburg (Russia)

(First received November 16th, 1992; revised manuscript received May 26th, 1993)

ABSTRACT

A liquid chromatographic method with packed fused-silica capillary columns and amperometric detection for the determination of trace amounts of catecholamines in microdialysis samples has been developed. The analysis time for dopamine and the products of its metabolism does not exceed 10 min. The minimum detectable dopamine concentration in a 2-μl sample is 0.05 μg/l. The relative standard deviation in concentration determinations is ± 0.03 ($n = 10$). The small sample volume required and the speed of analysis make it possible to analyse biologically important compounds with a rapidly changing concentration.

INTRODUCTION

Microdialysis as a method of continuous sampling of biological fluids [1] may be combined with various analytical methods, including electrochemical methods (voltammetry, potentiometry), immunoassay, mass spectrometry, gas and liquid chromatography (LC), and capillary electrophoresis.

The use of chromatographic and electrophoretic separating methods makes it possible to monitor continuously the composition of biological samples. A problem for analytical determinations in dialysates is directly related to the minimum sample volume. Another important problem is the speed of analysis, which at a fixed microdialysis flow-rate also requires a decrease in the sample volume. The miniaturization of the separating system, combined with microdialysis, is necessary in many cases, in particular for the determination of trace concentrations of endogenous and exogenous substances.

Compared with conventional LC on 4.6 mm I.D. columns and microcolumn LC with 1 mm I.D. columns, capillary LC on fused-silica capillary columns with an I.D. of less than 0.5 mm, packed with fine-grain sorbent, leads to a corresponding decrease in flow-rates, sample and detection volumes down to the nanolitre level. Although this decrease usually cause a drop in the concentration sensitivity, the mass sensitivity of capillary LC is higher than that of conventional LC [2].

This paper shows some examples of the application of short flexible reversed-phase fused-silica capillary columns with an I.D. of 0.32 mm. These

0378-4347/93/$06.00

are used in combination with microdialysis for the determination of dopamine (DA) and the products of its metabolism in the extracellular space of the rat brain.

EXPERIMENTAL

Chromatography

The capillary chromatograph consisted of a pump with a 2-ml syringe (Scientific and Technical Corporation Nauchpribor, Oryol, Russia) and a modified Rheodyne 7125 injector with channels 0.2 mm in diameter (width). The injector was connected to a 65 mm × 0.32 mm I.D. capillary column packed by the slurry method [3] with 10-μm Separon C_{18} sorbent (Tessek, Prague, Czech Republic). The column outlet was connected to a "wall-jet" cell [4] of an electrochemical detector designed and constructed in our laboratory [5]. The working electrode was made of gold wire, 0.5 mm in diameter. The effective volume of the measuring cell was 20 nl. The time constant of the detector was 0.5 s. The detector signal was recorded with an OH 814/1 recording potentiometer (Radelkis, Budapest, Hungary).

The mobile phase (pH 4.0) contained 10.54 g/l citric acid (Reakhim, St. Petersburg, Russia), 6.56 g/l sodium acetate (Reakhim), 0.1 g/l EDTA (Serva, Heidelberg, Germany), 0.14 g/l sodium octylsulphonate (Diagnosticum, Moscow, Russia) and 7.5% acetonitrile (Kriochrom, St. Petersburg, Russia).

A syringe pump with a flow-rate of 2 μl/min was used for microdialysis [6]. U-shaped dialysis probes with the length of the dialysis part *ca.* 5 mm were used. The diameter of the dialysis tube was 200 μm and its permeability was 50 000 daltons. The probes were implanted into the brain striatum of rats of the Sprague–Dawley line; the implantation coordinates were: $A = 0.5$; $B = 2.5$; $H = 6.5$. The probe outlet was connected by a polyethylene capillary (0.21 mm I.D.) to the loop of the injection valve made of fused-silica capillary (250 μm × 0.1 mm I.D.). The calculated value of the loop volume was 2.0 μl.

Samples

The dialysis perfusion of the striatum was carried out with artificial cerebrospinal fluid (ACF), which contained 140 mmol of sodium chloride, 3.5 mmol of potassium chloride, 1.3 mmol of calcium chloride, 1 mmol of magnesium chloride and 5 mmol of 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES); the pH was 7.2.

The peak heights were calibrated for concentrations by using solutions of the corresponding catecholamines (Serva) in ACF. The calibration slope obtained by direct injection of a standard solution, and by injection of a sample after it had passed through the dialysis probe placed in standard solution, were compared. Hence, it is possible to evaluate more accurately the true concentration of catecholamines and the products of their metabolism in the extracellular space of the brain.

RESULTS AND DISCUSSION

The aim of this work was to investigate the chromatographic separation of catecholamines on packed fused-silica capillary columns by in-

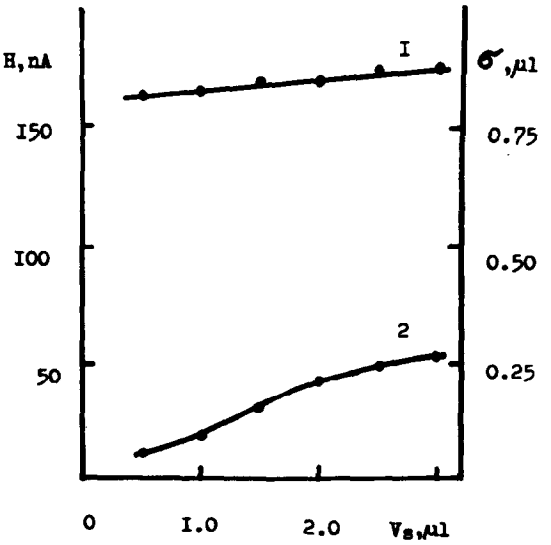

Fig. 1. (1) Dispersion volume (σ) and (2) height of the chromatographic peak for DA (*H*) *versus* volume of the ACF sample (V_s). Flow-rate, 10 μl/min.

jecting samples of submicrolitre volume. The change from 1 mm I.D. [7–9] to 0.32 mm I.D. columns at the same sample volume leads to a considerable increase in the concentration of eluted components. Fig. 1 shows that an increase in sample volume to values close to the free volume of the column leads to a systematic increase in the detector signal. Because, in this case, the dispersion of the chromatographic peak is low, it is possible to enhance the detection sensitivity by increasing the sample volume to values that exceed the optimum values by one or two orders of magnitude [10]. When a small sample volume is required in capillary LC, this is in good agreement with the fundamental property of microdialysis: the concentration of components diffusing from the biological medium into the dialysate greatly increases with decreasing flow-rate of perfusion.

The maximum sensitivity of catecholamine determination (Fig. 2) is observed at flow-rates higher than 5 μl/min. This value is six times higher than that corresponding to the greatest efficiency of a 0.32 mm I.D. column containing a sorbent with a mean particle diameter of 5 μm in the eluent used [11]. The rapid attainment of the maximum height of the chromatographic peak with increasing flow-rate is generally not characteristic of amperometric detection and, in our case, is due to a marked increase in peak spreading. Although the increase in the flow-rate shortens the analysis time, it simultaneously decreases the separating power of the column. The voltammograms for catecholamines on a gold working electrode in the range from +0.3 to +0.7 V are shown in Fig. 3. As a result of the low potential of the working electrode, the noise level is low. Hence, under

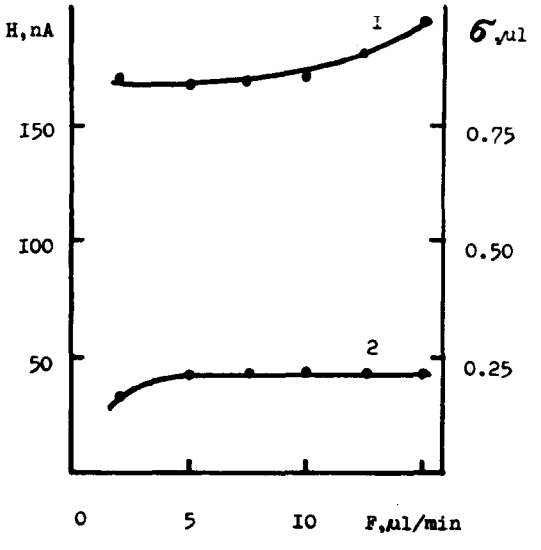

Fig. 2. (1) Dispersion volume (σ) and (2) height of the chromatographic peak (H) *versus* flow-rate (F). Volume of DA sample in ACF, 2 μl.

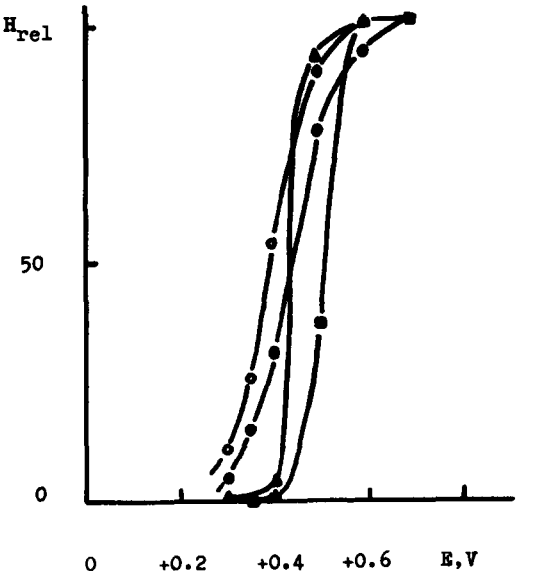

Fig. 3. Hydrodynamic voltammograms corresponding to the relative peak height (H_{rel}) for (○) DA, (▲) 5-HIAA, (●) DOPAC, and (■) HVA.

TABLE I

RELATIVE PEAK HEIGHTS OF DA AND ITS METABOLITES FOR EQUAL CONCENTRATION *VERSUS* WORKING ELECTRODE POTENTIAL, E

E (V)	DA/DOPAC	DA/5-HIAA	DA/HVA
+0.30	2.1	31	–
+0.35	1.9	25	–
+0.40	1.7	1.7	28
+0.50	1.1	1.0	0.8
+0.60	1.0	1.1	0.35
+0.70	1.0	1.1	0.34

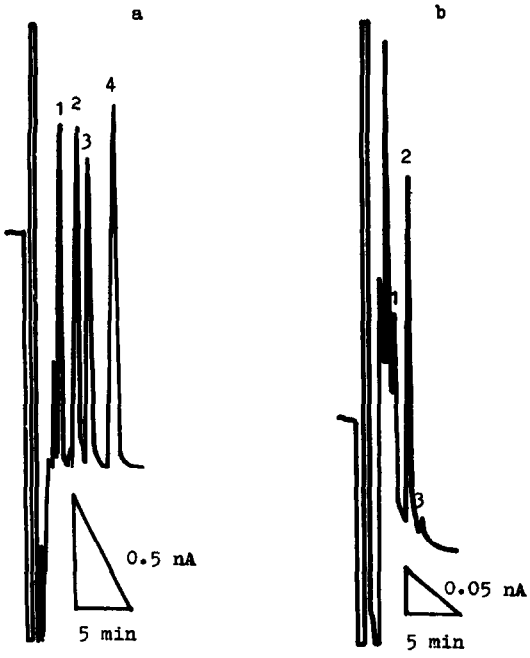

Fig. 4. Chromatograms of a model mixture of catecholamines at different potentials of the working electrode: (a) +0.7 V; (b) +0.3 V. Flow-rate, 8.3 μl/min. Peaks: 1 = DOPAC; 2 = DA; 3 = 5-HIAA; 4 = HVA. Concentration of each, 100 μg/l.

these conditions, a high signal-to-noise ratio is attained. Moreover, by varying the potential of the working electrode over this range, it is possible to increase greatly the selectivity of electrochemical detection (Table I).

The chromatograms of model mixtures of DA and the main products of its metabolism are shown in Fig. 4. When the potential of the working electrode was decreased from +0.7 V to +0.3 V, the heights of the chromatographic peaks decreased considerably but to different extents. For instance, the height of the DA peak is twice that of the 3,4-dihydroxyphenylacetic acid (DOPAC) peak and thirty times that of 5-hydroxyindolacetic acid (5-HIAA) peak. The peak of homovanillic acid (HVA) is completely eliminated. The variation of the potential makes it possible to increase greatly the selectivity of the chromatographic determination of DA against the background of its metabolites, because their concentration in the extracellular space can exceed the DA content by *ca.* 2 orders of magnitude.

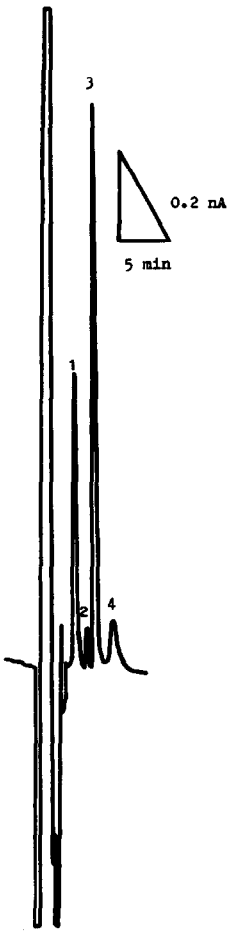

Fig. 5. Chromatogram of the sample of striatum dialysate. Peaks: 1 = DOPAC (47 μg/l); 2 = DA (6.3 μg/l); 3 = 5-HIAA (99 μg/l); 4 = HVA (2.5 μg/l). For conditions see Experimental and legend to Fig. 4a.

The calibration curves are linear for DA from 1 to 1000 μg/l, with a correlation coefficient of 0.998 for direct injection of standards and 0.996 after dialysis.

The chromatogram of catecholamines in the dialysate is shown in Fig. 5. The relative standard deviation in the determination of peak height ($n = 10$) was 0.03, and that in retention time, for example, for HVA is 0.008. The detection limit for DA in a 2-μl sample is 0.05 μg/l. The minimum detectable amount of DA at a signal-to-noise ratio of 2 is 0.5 fmol.

ACKNOWLEDGEMENT

I thank Dr. N. Saulskaja for help in carrying out the experiments and for discussing the paper.

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