CAPSULES
notes and applications from Bioanalytical Systems, Inc.

Microbore Catecholamines and Metabolites

Purpose

Determination of catecholamines and their metabolites in small volume samples such as those obtained from microdialysis.

The determination of catecholamines and their metabolites from brain microdialysates poses two technical problems: the small sample size generated by this sampling technique, and the need to elute an array of compounds in a reasonable period of time. Microbore columns can solve both these problems. Sample volumes for microbore columns are typically in the 1-10 µL range, which is less than the sample size usually collected during microdialysis. Microbore columns also provide rapid and efficient separations, allowing the determination of catecholamines and their metabolites in one isocratic run.

Here we present several catecholamine separations on a 150 x 1 mm SepStik column. To demonstrate the versatility of this column, we use three mobile phases, each with slightly different properties.

Existing Methods

Gas chromatography-mass spectrometry, radioimmunoassay, and liquid chromatography with fluorescence or electrochemical detection [1,2]. Microbore LCEC has become the method of choice.

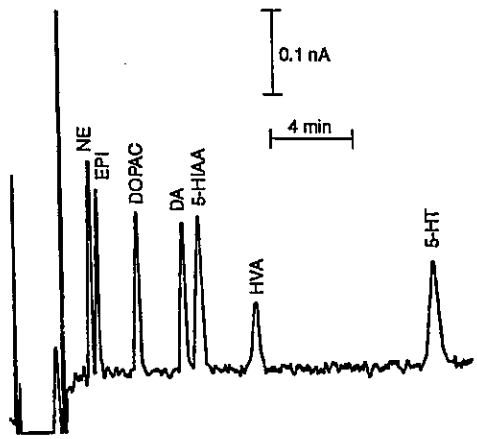

Figure 1. Separation of catecholamines and metabolites (5 pg each) using mobile phase 1. Injection volume was 5 mL. NE = norepinephrine, EPI = epinephrine, DOPAC = dihydroxyphenylacetic acid, DA = dopamine, 5-HIAA = 5-hydoxyindoleacetic acid, HVA = homovanillic acid, 5-HT = serotonin.

Conditions

System: BAS-200A or BAS-480 systems, equipped for microbore chromatography. A backpressure column (PN MF-8949) was installed before the injector, to bring overall system pressure up to an optimal range (2800-3400 PSI).
Electrode: Classic 3 mm glassy carbon (PN MF-1000)
Potential: + 750 mV vs. Ag/AgCl
Column: 5µM, C_{18}, 150 x 1 mm SepStik (PN MF-8912)
Temperature: 35° C
Flow Rate: 70 µL/min
Mobile Phase 1: 1L (14.5 mM NaH_2PO_4, 30 mM sodium citrate, 27 µM disodium-EDTA, 10 mM diethylamine•HCl, 2.2 mM 1-octanesulfonic acid, sodium salt), pH to 3.4 with H_3PO_4; 40 mL acetonitrile; 10 mL tetrahydrofuran.
Mobile Phase 2: 1L (14.5 mM NaH_2PO_4, 30 mM sodium citrate, 27 µM disodium-EDTA, 10 mM diethylamine•HCl, 1.95 mM 1-decanesulfonic acid, sodium salt), pH to 3.4 with H_3PO_4; 80 mL acetonitrile; 10 mL tetrahydrofuran.
Mobile Phase 3: 1L (25 mM NaH_2PO_4, 50 mM sodium citrate, 27 µM disodium-EDTA, 10 mM diethylamine•HCl, 2.2 mM 1-octanesulfonic acid, sodium salt), pH to 3.2 with H_3PO_4; 30 mL methanol; 22 mL dimethylacetamide.
Detection Limit: Varies with compound, but approximately 2-5 x 10^{-14} moles injected.

Notes

Separation of standards, using mobile phase 1, is shown in F1. This mobile phase produces an optimum separation of the catecholamines and their metabolites. Separation of a rat-brain dialysate is shown in F2. The same sample, spiked with standards, is shown in F3.

Mobile phase 2 exhibits selectivity for the catecholamines relative to the metabolites (F4). Note that 5-HIAA and DOPAC, which are typically present in large amounts (F3) are eluted early, so they do not interfere with the other analytes. This would be useful, for example, if dopamine were the only analyte of interest. In fact, dopamine can be eluted earlier,

with lower detection limits, by increasing the amount of organic solvent in the mobile phase.

Mobile phase 3 provides a similar separation to mobile phase 1 (F5 and F6). This mobile phase replaces the more noxious and volatile tetrahydrofuran with dimethylacetamide. Dimethylacetamide will selectively move dopamine and serotonin within the chromatogram. Increasing the amount of this solvent will reduce their retention times relative to the other peaks, and vice versa. We adjusted the amount of dimethylacetamide to center the dopamine peak between DOPAC and 5-HIAA (F5).

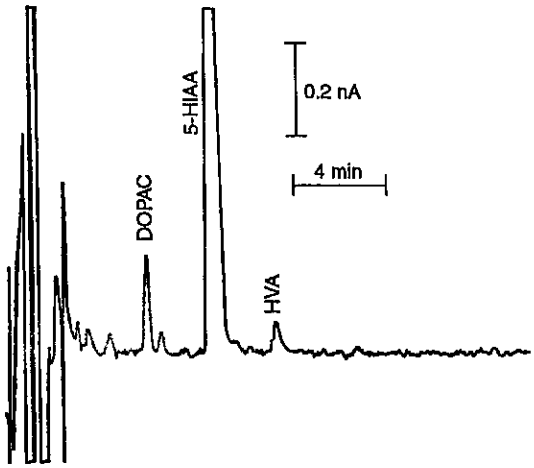

Figure 2. Rat-brain microdialysate, 5 µL injection, mobile phase 1.

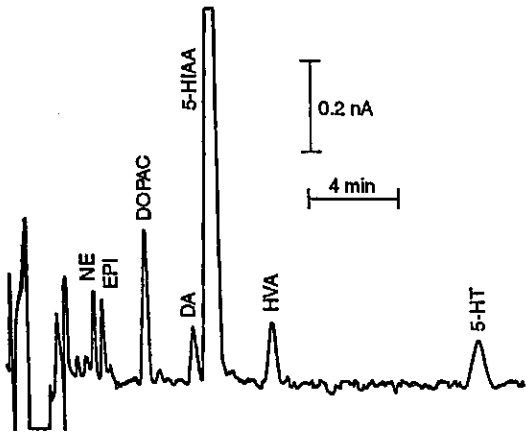

Figure 3. Same sample as in F2, but spiked with 3 pg standards.

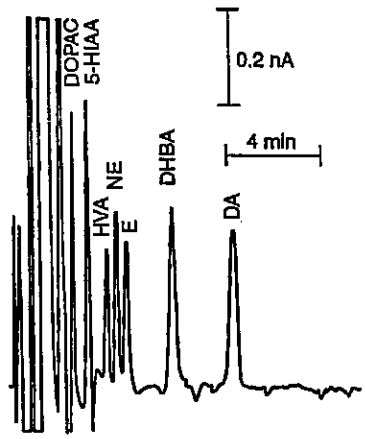

Figure 4. Separation of catecholamines and metabolites (10 pg each) using mobile phase 2. Injection volume was 20 µL.

References

1. *Cheng, F.-C., L.-L. Yang, F.-M. Chang, L.-G. Chia and J.-S. Kuo, J. Chromatogr., 582 (1992) 19-27.*

2. *Cheng, F.-C., J.-S. Kuo, Y. Shih, J.-S. Lai, D.-R. Ni and L.-G. Chia, J. Chromatogr. 615 (1993) 225-236*

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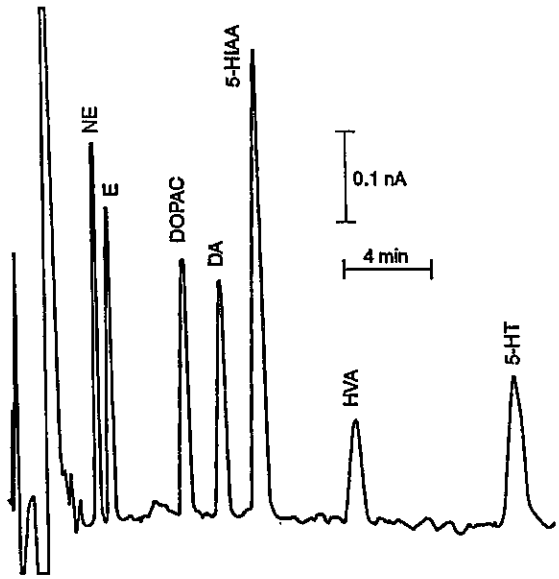

Figure 5. Separation of catecholamines and metabolites (10 pg each, except 20 pg 5-HIAA) using mobile phase 3. Injection volume was 5 µL.

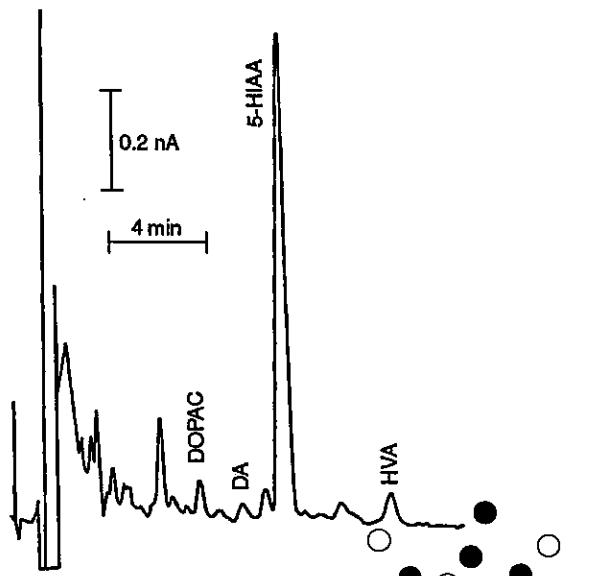

Figure 6. Rat brain microdialysate, 5 µL injection. Mobile phase 3.

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