CAPSULES
notes and applications from Bioanalytical Systems, Inc.
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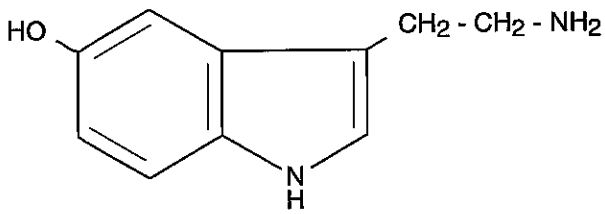
Determination of Serotonin in Rat Brain Microdialysate Using a UniJet Microbore Column and Radial-Flow Cell

Purpose

Determination of serotonin (5-hydroxytryptamine, 5-HT, F1) in rat brain microdialysates. These low concentration samples were separated and detected utilizing a UniJet microbore column and a radial-flow UniJet cell.



Existing Methods

Thin-layer chromatography, gas chromatography, radioimmunoassay, and liquid chromatography with fluorescence or electrochemical detection.

Conditions

Systems: BAS-200A or BAS-480 system equipped for microbore column chromatography.

Electrode: UniJet 3 mm glassy carbon electrode (PN MF-2061)

Potential: + 550 mV *vs.* Ag/AgCl

Column: 3 µM, C_{18}, 100 x 1 mm (PN MF-8949)

Flow rate: 100 µL/min

Mobile Phase 1: 1L (25 mM NaH_2PO_4, 50 mM sodium citrate, 27 µM disodium-EDTA, 10 mM diethylamine·HCl, 10 mM NaCl, 2 mM decane-sulfonic acid (sodium salt), pH to 3.2 with H_3PO_4); 175 mL acetonitrile.

Mobile Phase 2: 1L (0.1 M Monochloroacetic acid, 0.5 mM EDTA, 10 mM NaCl, 0.65 mM sodium octylsulfonic acid, pH to 3.1 with H_3PO_4); 100 mL acetonitrile.

Detection Limit: 0.25 pg 5-HT on column.

Notes

Typical chromatograms of 5-HT standard, microdialysate sample from rat brain and spiked microdialysate using mobile phase 1 are presented in F2. F3 shows the chromatograms using both mobile phase 1 and mobile phase 2 for determination of the same standard and microdialysate samples. A calibration curve of 5-HT is presented in F4. The concentration-time curves of 5-HT in microdialysate from rat brain are presented in F5.

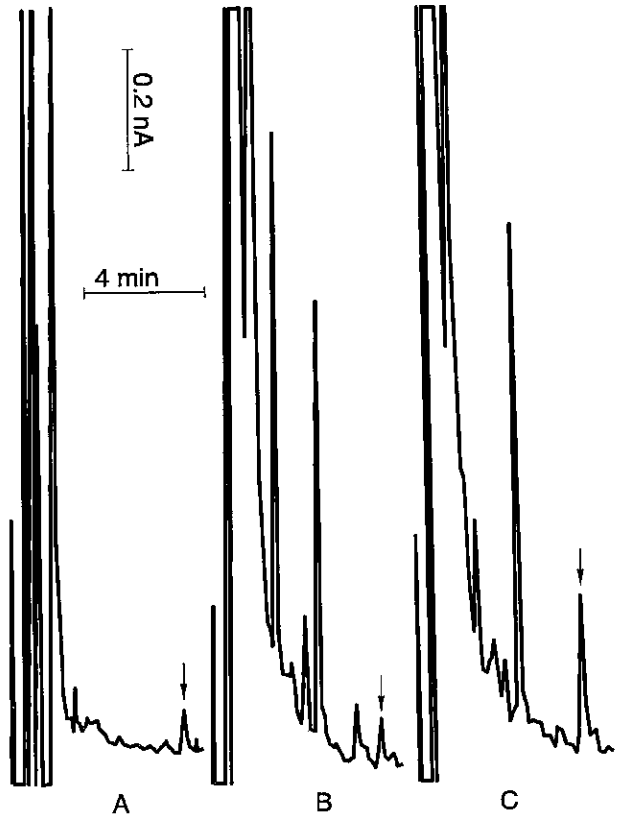


Figure 2. Chromatograms of 1 pg serotonin standard (A), microdialysate from rat striatum (B) and a mixture of serotonin standard and microdialysate from rat striatum (C) using mobile phase 1. 5 µL were injected. Conditions of microdialysis: CMA/12, 4 mm probe; 2 µL/min perfusion with Ringer's solution; 100 µL collection volume. Arrows show serotonin peak.

References

1. *Huang, T., Shoup, R.E. and Kissinger, P.T., Current Separations, 9 (1990) 139.*
2. *Bohs, C.E., Linhares, M.C. and Kissinger, P.T., Current Separations, 12 (1994) 181.*

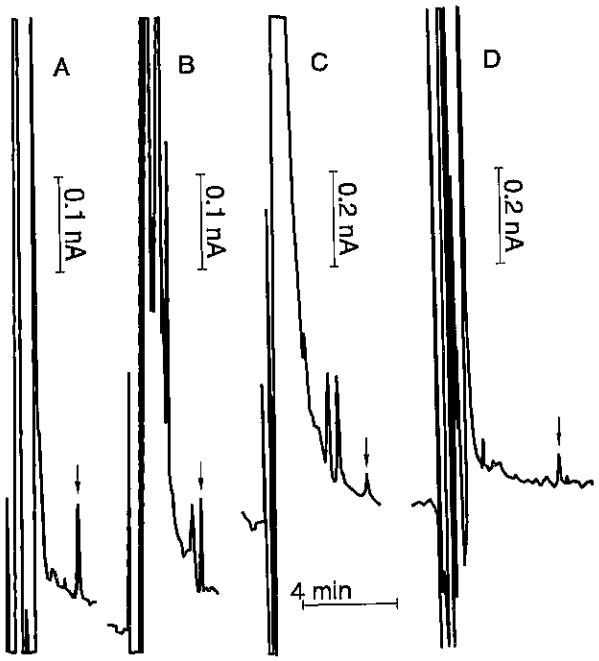


Figure 3. Chromatograms of 1 pg serotonin standards (A,D) and microdialysate samples from rat striatum (B, C) using mobile phase 1 (A and B) and mobile phase 2 (C and D). Microdialysis conditions same as in F1.

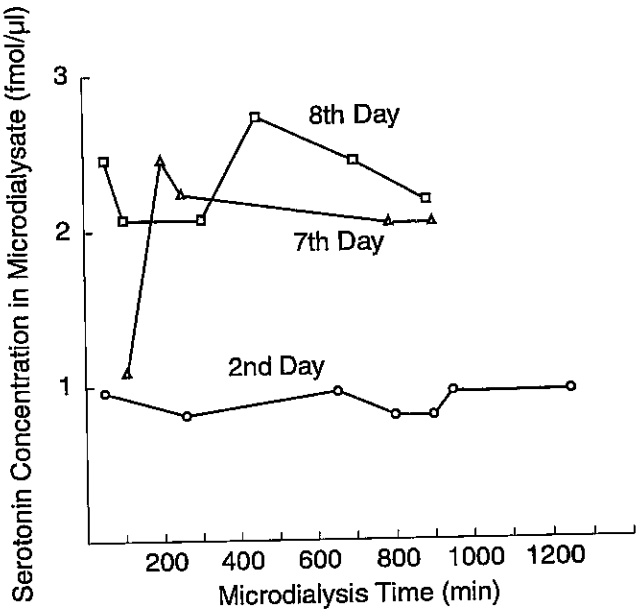


Figure 5. Concentration-time curve of serotonin in microdialysates from rat brain striatum. LCEC conditions same as in F1.

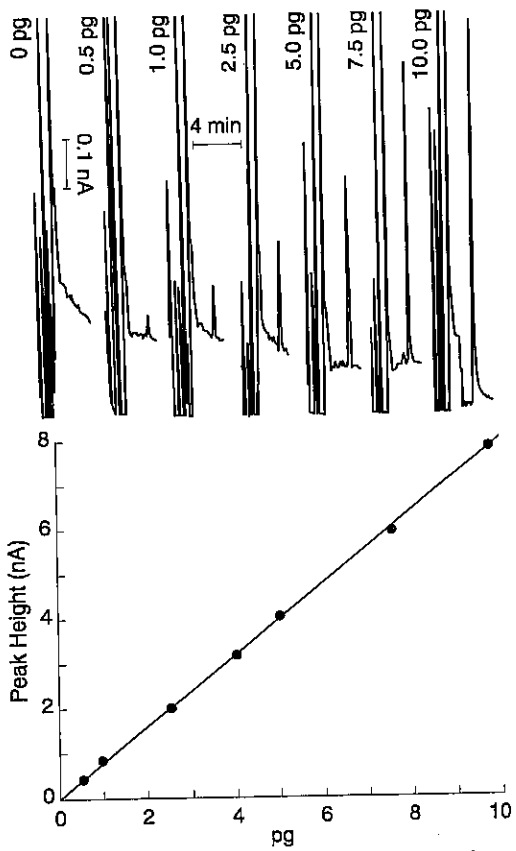


Figure 4. Calibration curve for serotonin using mobile phase 1.