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Rapid Determination of Dopamine and Serotonin in Microdialysis Perfusates with HPLC-ECD

INTRODUCTION

In vivo methods today are pushing toward greater temporal and spatial resolution. This means smaller samples from shorter collection times, using smaller probes in discrete brain regions. The result is more samples of lower concentration, which requires both high sensitivity and rapid chromatography.

Two approaches have evolved to address these needs: rapid off-line analysis to support very short collection times, or a balancing of collection and chromatographic times for direct on-line analysis.

This new method for rapid analysis accommodates both approaches without sacrificing sensitivity. It uses a low-volume amperometric detection approach to optimize speed, sensitivity, and reliability.

This work presents an example of how the method can be used to measure prefrontal cortical levels of dopamine (DA) and serotonin (5-HT) under basal and amphetamine stimulated conditions.

EQUIPMENT AND CONDITIONS

An isocratic high-performance liquid chromatography (HPLC) system similar to the Thermo Scientific Dionex UltiMate® 3000 system including a pump, autosampler, and Thermo Scientific ESA Coulochem® III detector

Coulochem III Organizer Module with Temperature Control (P/N 70-9121TA)

Liquid Chromatography

Column: Capcell Pak®: 1.5 × 50 mm, 3 µm, C18, MG100 (Shiseido Co., Ltd. P/N 88-90832)

Flow Rate: 0.20 mL/min

Temperature: 30 °C

Inj. Volume: 10 µL (partial loop)

Detector

Coulochem III (P/N 70-9143)

Analytical Cell (P/N 70-4131)

Guard Cell (P/N 55-0417)

Cell Potentials: Analytical Cell, E = +220 mV
Guard Cell, EGC = +275 mV

Mobile Phase

Sodium dihydrogen phosphate 150 mM, citric acid 4.76 mM, sodium dodecyl sulfate 3 mM, EDTA 50 µM, 10% (v/v) methanol, 15% (v/v) acetonitrile, pH=5.60 with sodium hydroxide (99.99%), semiconductor grade

Microdialysis

Probe: CMA11, 3 mm membrane length

Perfusion Flow Rate: 2.0 µL/min

Artificial Cerebrospinal Fluid (aCSF) Composition:

Sodium: 147 mM

Potassium: 2.7 mM

Calcium: 1.2 mM

Magnesium: 1.0 mM

Chloride: 150 mM

Phosphate: (pH 7.4 ± 2) 2 mM

Collection Period: 20 min into 10 µL 0.2 M perchloric acid (containing 0.2 µM EDTA)

Animal Model: Awake

Probe Coordinates: AP +3.2, LR +0.8, DV -5.0
(from Bregma)

RESULTS AND DISCUSSION

This method resolved both monoamines in under 4 min (Figure 1), showed excellent linearity (Figure 2), and extreme sensitivity (~50 fg on column). The method was capable of reliably and simultaneously measuring both monoamines under basal conditions (Figure 3).

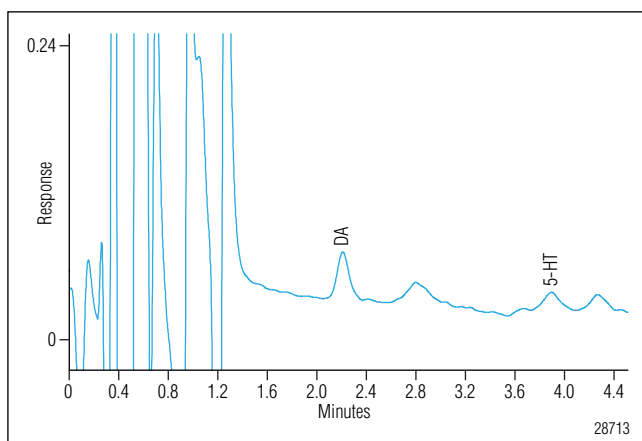


Figure 1. Chromatography of DA and 5-HT standards (200 fg on column) in aCSF.

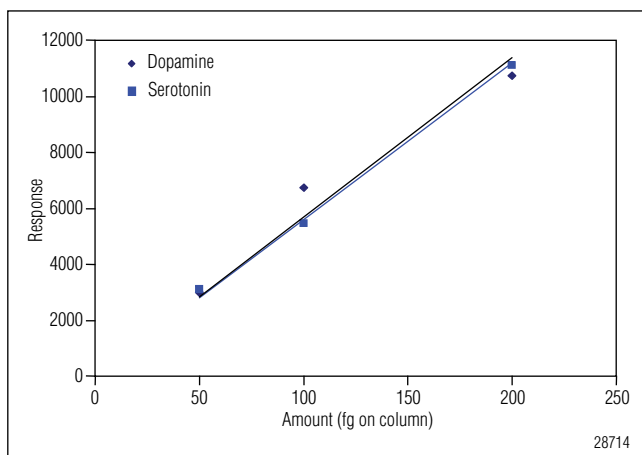


Figure 2. Linear curves for DA and 5-HT.

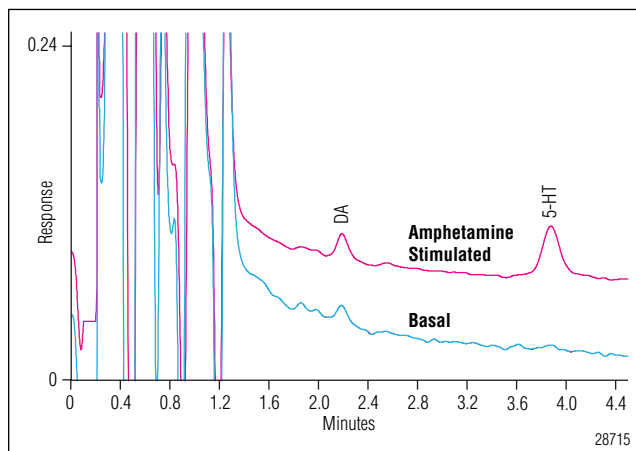


Figure 3. Microdialysis samples obtained from a 3 mm concentric microdialysis probe placed in the prefrontal cortex of an awake rat, before and after amphetamine (2 mg/kg i.p.) stimulation.

Stimulation with amphetamine (2 mg/kg i.p.) resulted in significant increases in 5-HT.

This method can be of great benefit to any researcher interested in studying the regional interplay of monoamine neurotransmitters during pharmacological manipulation or behavior.

ACKNOWLEDGEMENT

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