

Noradrenaline, Dopamine and Serotonin for Microdialysis

< Analytical Condition, Mobile Phase and Standard Solution >

Analytical Condition

HPLC-ECD system	HTEC-510 (ONLINE: EAS-20S, OFFLINE: M-510/514)
Separation Column	EICOMPAK CAX (φ2.0 x 200 mm)
Precolumn for mobile phase	PC-04-AC (φ4.0 x 5.0 mm)
Mobile Phase	70% 0.1 mol/L acetate ammonium buffer including 50 mg/L EDTA-2Na and 0.05 mol/L sodium sulfate, 30% methanol
Flow rate	250 µL/min
Column Temp.	35 °C
Working Electrode	WE-3G (Graphite)
Gasket	GS-25
Applied potential	+450 mV vs. Ag/AgCl
Time Constant	3.0 sec

* The EICOMPAK CAX and this mobile phase condition do not allow for the separation of NE and epinephrine. Only use this condition for samples which do not contain or contain minimum levels of epinephrine.

< Mobile Phase Preparation >

H ₂ O	Acetate ammonium	Acetic acid	Sodium sulfate	EDTA·2Na	Methanol
700 mL	5.189 g	164 µL	7.102 g	50 mg	300 mL

< Standard Chromatogram >

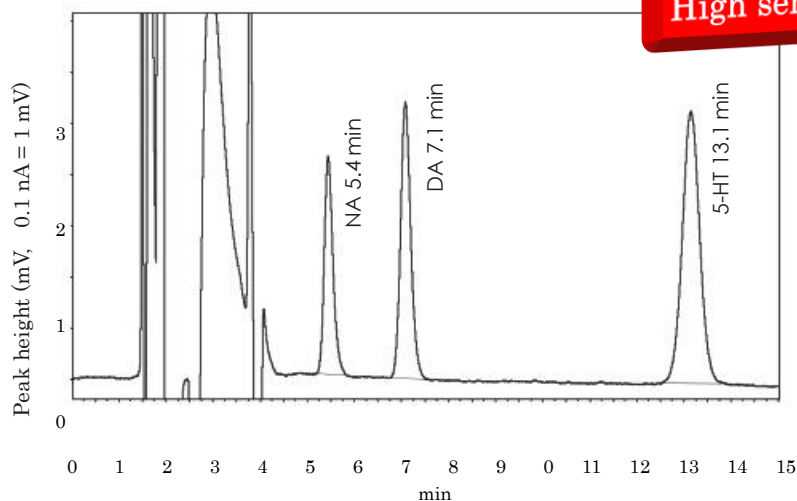


Fig. 1 Chromatogram obtained by 10 pg/10 µl injection of a standard solution of NA, DA and 5-HT

Lower Detection Limit

When using the EICOMPAK CAX on the Eicom HTEC-510, the lower detection limit for DA is 0.05 pg/injection and 0.1 pg/injection of both NA and 5-HT with a signal to noise ratio (S/N) of 3.

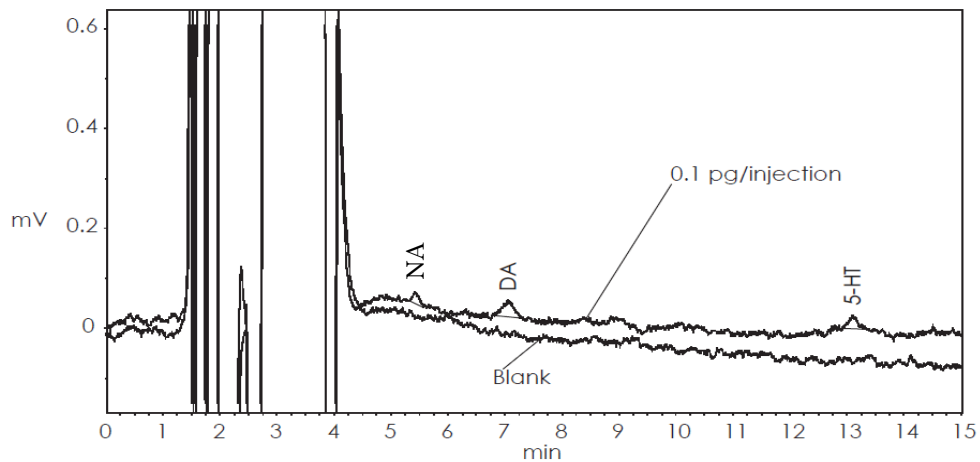


Fig. 2 Chromatogram obtained by a 0.1 pg on the column of a mixture of NA, DA and 5-HT and one obtained from a blank solution

< Brain Microdialysis Application >

Animal: Male Wister Rat, BW 240–270 g
Microdialysis Probe: Eicom A-I-4-03 (membrane length 3 mm)
Probe Location: Prefrontal Cortex (A = +2.5 mm, L = 0.6 mm, V = -3 mm)
Perfusate: Ringer's solution 1 μ l/min
Injection Volume: 1 μ l/min \times 15 min. via an automated online injector (Eicom EAS-20S)

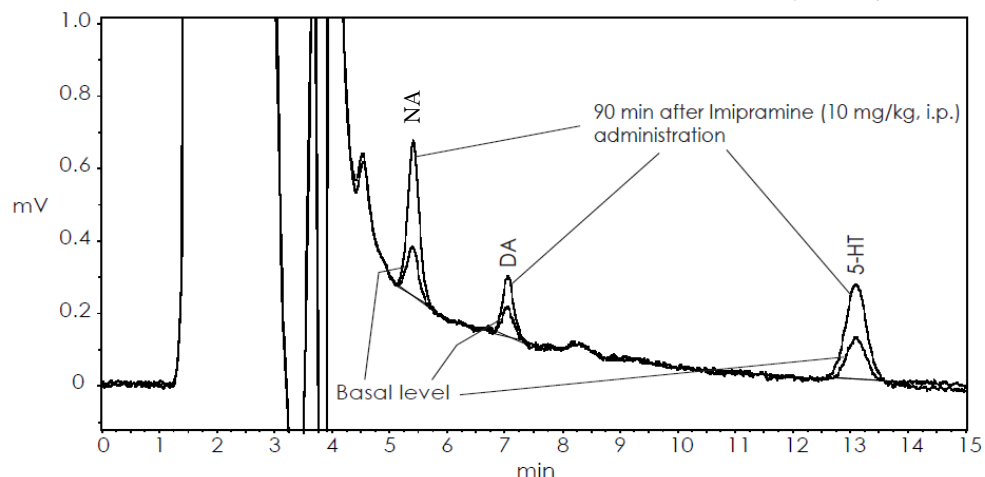


Fig. 3 Simultaneous analysis of NA, DA and 5-HT obtained from a

prefrontal cortex microdialysis sample using the EICOMPAK CAX

This figure shows the basal condition and the condition 90 min. after imipramine administration. Basal levels for NA, DA and 5-HT are 1.71, 0.54, 0.83 pg in 15 μ l respectively. Following drug administration NA, DA and 5-HT levels increased to 5.49, 1.09, 2.10 pg in 15 μ l, respectively.

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