



The Simultaneous Analysis of Norepinephrine, Dopamine & Serotonin in Microdialysis Samples

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In vivo brain-microdialysis users have been in search of a separation method for norepinephrine (NE, noradrenalin), dopamine (DA) and serotonin (5-HT) with both high sensitivity and specificity from a single brain microdialysate sample. Historically, it has been difficult to detect NE and 5-HT in the same chromatogram using a reverse phase column due to the difference in the retention times of these compounds. Until now, it has been necessary to split the sample and apply two different analytical methods in order to measure NE, DA and 5-HT with high specificity and sensitivity.

Now, Eicom introduces the EICOMPAK CAX (cation exchange mode, 2.0 × 200 mm) which allows for the detection of NE, DA and 5-HT from a single sample injection. This method can be applied using a single constant applied voltage

(DC mode) electrochemical detection. The required analysis time is only 15 min with a sensitivity of 50–100 fg/injection using Eicom's HTEC-500. The system requirements necessary to achieve optimal performance from the EICOMPAK CAX column has been incorporated into the HTEC-500.

Analytical Conditions

Analytical Instrument	Eicom HTEC-500
Separation Column	EICOMPAK CAX (2.0 id × 200 mm)
Column Temperature	35°C
Flow Rate	250 µl/min
Detecting Condition	+450 mV vs. Ag/AgCl
Working Electrode	Graphite (WE-3G) with Gasket #GS-25
Time Constant	3.0 sec
Data Acquisition	Eicom EPC-500

When using the EICOMPAK CAX on the Eicom HTEC-500, the lower limit of detection for DA is 50 fg/injection and 100 fg/injection for both NE and 5-HT with a S/N = 3. Using a 10 µl injection volume for the 10 pg/injection standard solution containing NE, DA and 5-HT, the CV% was 2.51, 2.63 and 2.98 respectively. When using a 50 µl loop, linearity is obtained within the injection volume range of 0–25 µl. The column can tolerate up to a 40 µl injection volume.

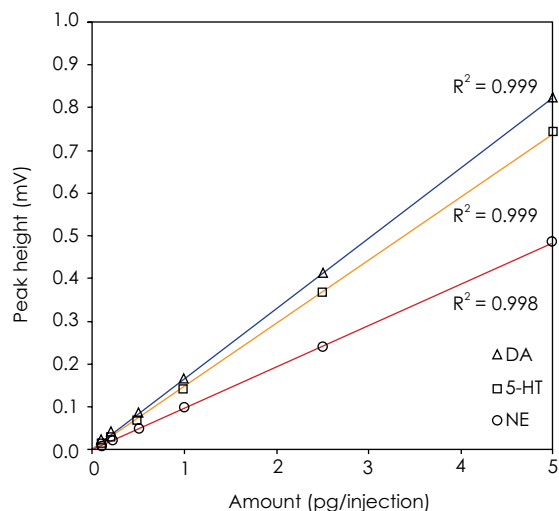


Fig. 1 Calibration curve for DA, NE and 5-HT in the range. 3 of 0.1-5 pg/injection with the Eicom HTEC-500.

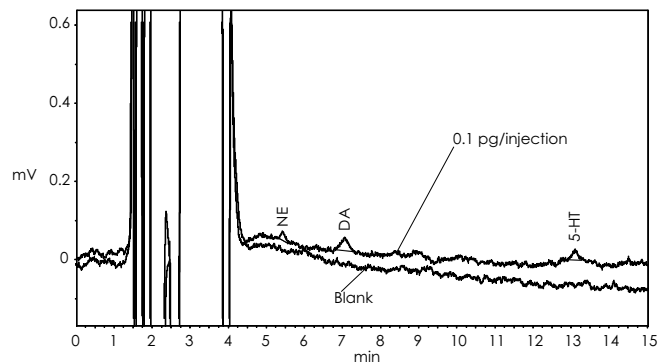


Fig. 2 Chromatogram obtained by a 100 fg/injection of a standard solution of NE, DA and 5-HT in 0.1 M phosphate buffer (pH3.5) and blank matrix.

Microdialysis Conditions

Animal	Freely moving 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 automated online injector (Eicom EAS-20)

This new cutting edge methodology is user friendly and easily applied to microdialysis samples. The high sensitivity and selectivity was exemplified by TTX perfusion. This methodology enhances the efficiency of monoamine neurotransmitter analysis and allows for greater sample resolution when conducting pharmacological and behavioral tests.

Eicom will provide a more detailed protocol including trouble shooting and mobile phase conditions. If higher sensitivity is required, Eicom also provides the PP-ODS column for the analysis of DA and 5-HT. The analysis time is only 5 min with a sensitivity level of 30 fg/injection. The EICOMPAK CAX with the current separation conditions does not allow the complete separation of NE and epinephrine. The difference in their retention time is only half a minute. Epinephrine levels in the brain are minimal and usually are below the sensitivity level. If detection of epinephrine levels is required, use EICOMPAK CA-50DS.

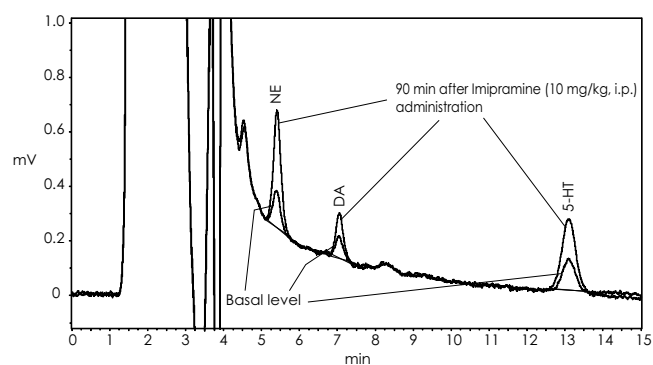


Fig. 3 Simultaneous analysis of NE, DA and 5-HT obtained from a prefrontal cortex microdialysis sample using the EICOMPAK CAX. This figure shows the basal condition and the condition 90 min. after imipramine administration. Basal levels for NE, DA and 5-HT are 1.71, 0.54, 0.83 pg in 15 μ l, respectively. Following drug administration NE, DA and 5-HT levels increased to 5.49, 1.09, 2.10 pg in 15 μ l, respectively.

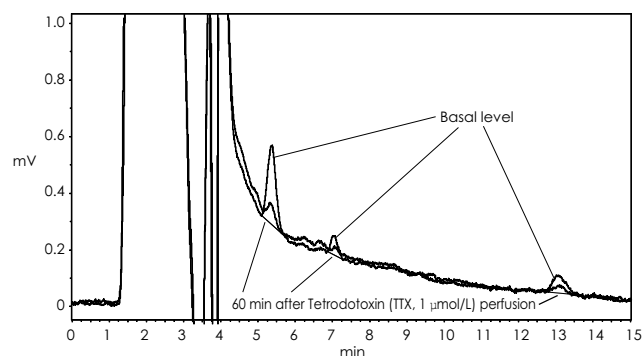


Fig. 4 Simultaneous analysis of NE, DA and 5-HT obtained from a prefrontal cortex microdialysis sample using the EICOMPAK CAX. This figure shows the basal condition and the condition 60 min. after perfusion with tetrodotoxin at 1 μ M. Basal levels for NE, DA and 5-HT are 5.11, 0.37, 0.34 pg in 15 μ l, respectively. Following drug administration NE, DA and 5-HT levels decreased to 0.62, 0.06, 0.19 pg in 15 μ l, respectively.