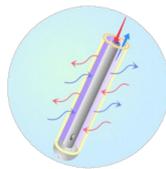


Fast analysis of Dopamine and Serotonin for high time resolution in microdialysis experiments

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Improving time resolution for measurements in brain microdialysates

Microdialysis of neurotransmitters in vivo has become an invaluable tool to study neurotransmission in the living brain. Extracellular fluid of the brain is sampled through a the semipermeable membrane with a microdialysis probe.



HPLC analysis requires fractionation of the sample stream, and the size of the fractions will affect time resolution. To accurately measure fast responses, a high time resolution is necessary as shown in Fig. 1

We developed a robust commercially available on-line solution to improve time resolution, as shown in this poster for DA and 5-HT

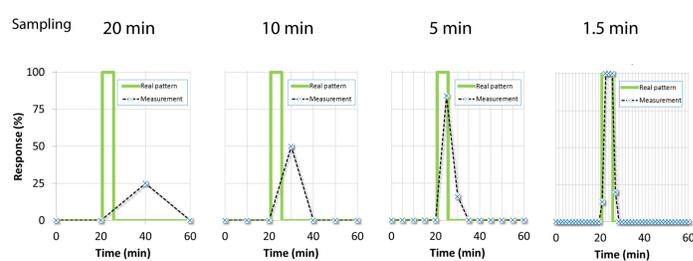


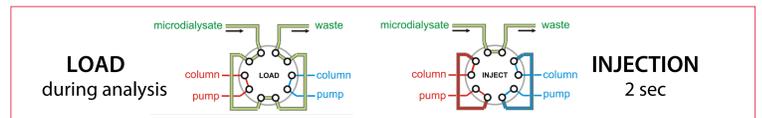
Fig. 1. Higher time resolution more accurately describes fast patterns.

On-line sampling of da and 5-ht for analysis in parallel channels

Time resolution is influenced by:

- perfusion rate (typically 1-2 $\mu\text{L}/\text{min}$)
- total analysis time to process a sample
- size and number of serial sample loops

Two sample loops \rightarrow factor 2 better time resolution.



The applied analysis of DA and 5-HT shows reproducible results (<2%RSD) and a detection limit of 100 picomole/L.

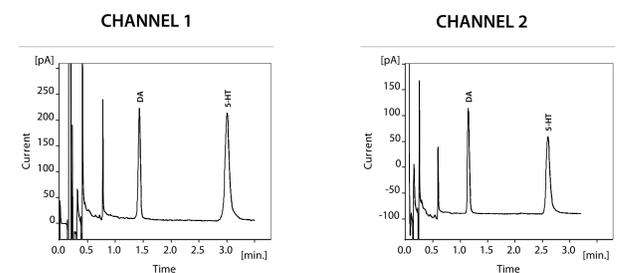


Fig. 2. Simultaneously recorded chromatograms of 10 nM DA and 5-HT standards collected during the previous analysis.

Method

The ALEXYS Neurotransmitter Analyzer is equipped with a 10 port valve to simultaneously analyze two different time samples that are collected in two serially installed sample loops.

Conditions for analysis of DA and 5-HT

LC	ALEXYS Neurotransmitter Analyzer with DECADE Elite and 10-port valve
Flow cell	2 mm glassy carbon SenCell, saltbridge reference, AST setting '1'
V _{injection}	1.5 μL per channel
Columns	UHPLC C18 column, 1.0x100 mm, 1.7 μm particles
Mobile phase	Acetate buffer pH 5.8, ion pairing agent, acetonitrile
Flow rate	175 $\mu\text{L}/\text{min}$ at 35 $^{\circ}\text{C}$; 280 $\mu\text{L}/\text{min}$ at 60 $^{\circ}\text{C}$ (pressure 480-490 bar in both cases)

Conditions of microdialysis

Perfusion solution 147 mM Na⁺, 1.2 mM Ca²⁺, 3 mM K⁺, 1.2 mM Mg²⁺, 152.4 mM Cl⁻ in water

Temperature influence on analysis time

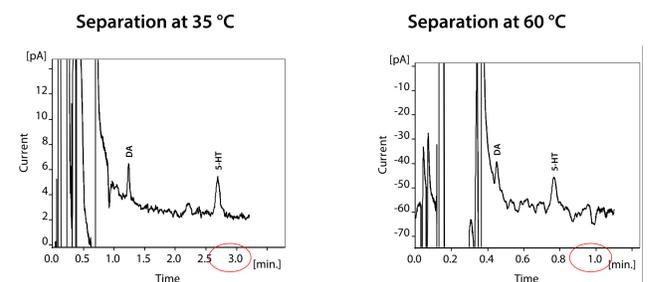


Fig. 3. The new DECADE Elite can be set to 60 $^{\circ}\text{C}$, and this facilitates even shorter analysis times. Shown with 100 pmole/L standards under conditions of equal backpressure.

On-line microdialysis experiment

The tip of the probe had been immersed in perfusion fluid and then transferred for 25 min into standard solution (10 nM DA and 5-HT in perfusion fluid). The response was continuously monitored before, during and after the transfer (Fig. 3).

With a time resolution of 1 data point per 1.8 minutes, fast responses are detected.

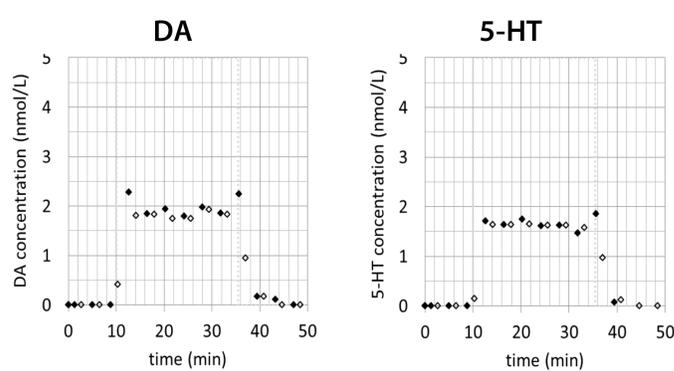


Fig. 4 Responses from sample loop 1 (closed) and 2 (open). The red line indicates the transfer of the probe from blank to solution containing 10 nM DA and 5-HT.

Conclusion

The ALEXYS UHPLC Neurotransmitter Analyzer is a dedicated system solution to sensitively measure neurotransmitters in small samples.

Sensitivity can now be combined with a time resolution of < 2 min in combination with on-line microdialysis as shown for the analysis of DA and 5-HT.

