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 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.

Time resolution is influenced by:
- perfusion rate (typically 1-2 μL/min)
- total analysis time to process a sample
- size and number of serial sample loops

On-line sampling with the ALEXYS Neurotransmitter Analyzer

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

The versatile UHPLC ALEXYS Neurotransmitter Analyzer is based on the DECADE Elite electrochemical detector (ECD) with SenCell. Separation and detection of dopamine and serotonin is achieved using two sub-2 μm particle columns in parallel. A fast isocratic LC-ECD method assures high time resolution for low level changes in neurotransmitter response.

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.

Two sample loops: > factor 2 better time resolution.

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, cartridge reference, AST setting "3"
- V<sub>inj</sub>: 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: 1.0 μL/min at 35 °C; 2.0 μL/min at 60 °C (pressure 600/600 bar in both cases)

Conditions of microdialysis

- Perfusion solution: 147 mM Na<sup>+</sup>, 1.2 mM Ca<sup>2+</sup>, 3 mM K<sup>+</sup>, 152.4 mM Cl<sup>-</sup> in water
- Solution flow rate: 1.0 μL/min through probe

Chromatograms from the analysis of DA and 5-HT

The method for quantification of DA and 5-HT shows reproducible results (>97%RSD) and a detection limit of 100 picoliter/L.

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 μM 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. UHPLC/ECD analysis of small samples (down to 1.5 μL) and detection limit down to 0.15 fmol.

CONCLUSION

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

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

www.AntecScientific.com

VISIT ANTEC AT BOOTH # 1936