Fast and sensitive detection of **DA, 5-HT** and **metabolites** using the **UHPLC** ALEXYS Neurotransmitter Analyzer

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ALEXYS Neurotransmitter Analyzer

Challenge: in vivo quantitation of neurotransmitters in microdialysis

- Small sample volume (<10 µL)
- Complex mixture of neurotransmitters
- Both low (pM range) and high (µM range) concentrations present in sample

Solution: ALEXYS Neurotransmitter Analyzer

- UHPLC columns (sub-2 µm particle size) for superior peak efficiencies and resolution at higher flow rates.
- AS110 UHPLC autosampler with customized injection program for efficient sample use
- DECADE Elite electrochemical detector in combination with amperometric flow cell (SenCell) for best possible sensitivity.

Fig. 1. ALEXYS® Neurotransmitter Analyzer (top) and SenCell (right).

In this poster we present an overview of several fast and sensitive methods for the analysis of neurotransmitters in microdialysate samples, demonstrating the versatility and performance of the Antec ALEXYS Neurotransmitters Analyzer.

Monoamines and Metabolites

- Ion pairing reversed phase separation
- Relatively long column to generate enough separation efficiency.
- For samples without interfering peaks; apply one of the presented alternatives if necessary

Conditions

LC	ALEXYS® Neurotransmitter Analyzer
Column	Acquity UHPLC BEH (Waters) 1.0 x 100 mm, 1.7 µm part. + prefilter
T_{oven}	37 °C
Mobile phase	Phosphate/citrate buffer (pH 3.0) 0.1 mM EDTA.Na2, 8 mM KCl 8% acetonitrile, 600 mg/L sodium 1-octanesulfonate
Flow rate	50 µL/min
Ecell	640 mV vs Ag/AgCl (ISAAC, 8 mM KCl)
Backpressure	about 265 bar
Icell	about 2 nA
LOD	0.2-0.4 fmol on column
cLOD	150-250 pmol/L ($V_{injection}$ = 1.5 µL)

In case a larger sample volume is available:
cLOD 40-80 pmol/L ($V_{injection}$ = 5 µL)

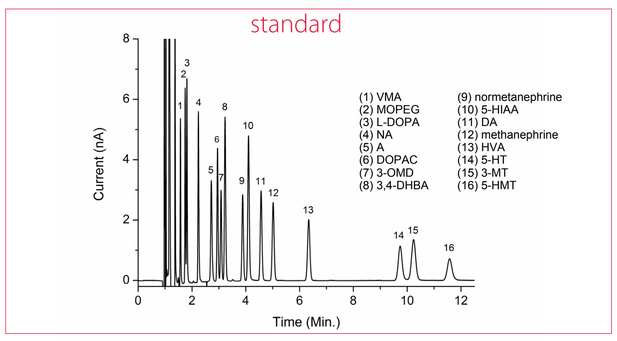

Fig. 2. Chromatogram of a 100 nmol/L standard of 16 neuro-transmitters and metabolites in perfusion fluid and 10 mmol/L acetic acid. Column efficiency >200.000 plates/m (except for A and 5-HMT). Resolution ≥ 1.4. Injection volume 2 µL

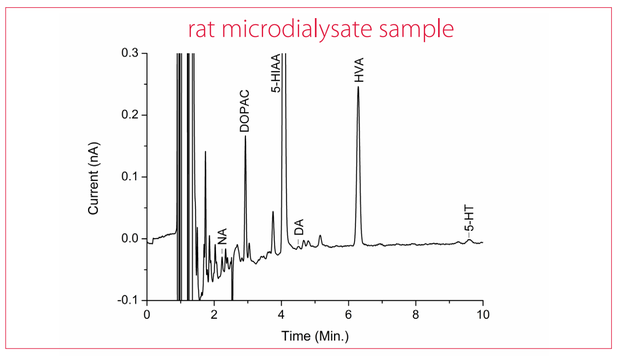

Fig. 3. Analysis of basal level rat prefrontal cortex dialysate. The concentration was calculated to be 0.4 nmol/L NA, 5.8 nmol/L DOPAC, 55.5 nmol/L 5-HIAA, 0.1 nmol/L DA, 10.7 nmol/L HVA and 0.9 nmol/L 5-HT. Injection volume: 2 µL. Sample kindly provided by Gerdien Korte-Bouws, Department of Psychopharmacology, University of Utrecht

Acidic Metabolites

- Reversed phase separation
- Selective mobile phase conditions
- Short analysis time

Conditions

LC	ALEXYS® Neurotransmitter Analyzer
Column	Acquity UHPLC BEH (Waters) 1.0 x 50 mm, 1.7 µm part. + prefilter
T_{oven}	37 °C
Mobile phase	Phosphate/citrate buffer (pH 3.0) 0.1 mM EDTA.Na_2, 10% acetonitrile no ion-pairing agent
Flow rate	175 µL/min
Ecell	800 mV vs Ag/AgCl (salt bridge, sat'd KCl)
Backpressure	about 450 bar
Icell	about 3.5 nA
LOD	0.2-0.4 fmol on column
cLOD	150-250 pmol/L ($V_{injection}$n = 1.5 µL)

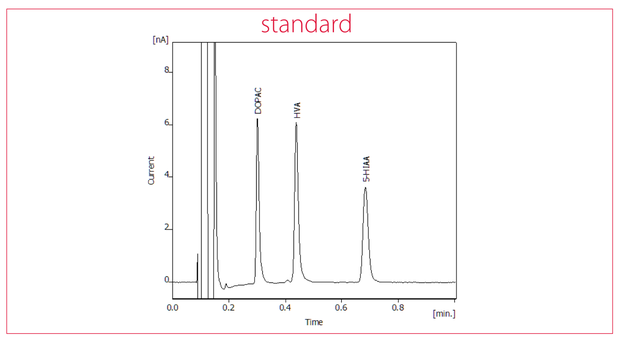

Fig. 4. Chromatogram of 100 nmol/L DOPAC, HVA and 5-HIAA in Ringer solution acidified with 10 mmol/L acetic ac-id. Injection volume: 1.5 µL.

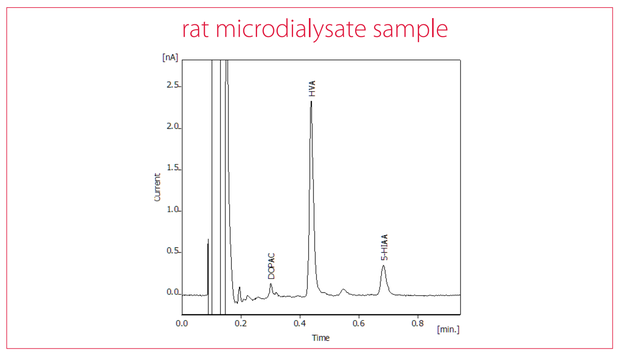

Fig. 5. Chromatogram of a basal level rat microdialysate sample from prefrontal cortex. The concentration was calcu-lated to be 4 nmol/L DOPAC, 41 nmol/L HVA and 11 nmol/L 5-HIAA. Injection volume: 1.5 µ L. Sample kindly provided by Gerdien Korte-Bouws, Department of Psychopharmacology, University of Utrecht

DA and 5-HT

- Ion pairing reversed phase separation
- Selective mobile phase conditions
- Short analysis time

Conditions

LC	ALEXYS® Neurotransmitter Analyzer
Column	Acquity UHPLC BEH (Waters) 1.0 x 50 mm, 1.7 µm part. + prefilter
T_{oven}	37 °C
Mobile phase	Acetate buffer (pH 5.8) 0.1 mM EDTA.Na2, 7.5% aceto nitrile 250 mg/L sodium 1-octanesulfonate
Flow rate	200 µL/min
Ecell	460 mV vs Ag/AgCl (salt bridge, sat'd KCl)
Backpressure	about 500 bar
Icell	about 0.2 nA
LOD	0.15 fmol on column
cLOD	100 pmol/L ($V_{injection}$ = 1.5 µL)

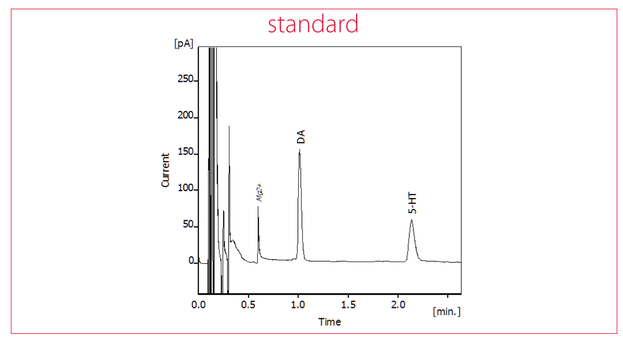

Fig. 6. Chromatogram of 10 nmol/L DA and 5-HT in Ring-er solution acidified with 10 mmol/L acetic acid. Injection volume: 1.5 µL.

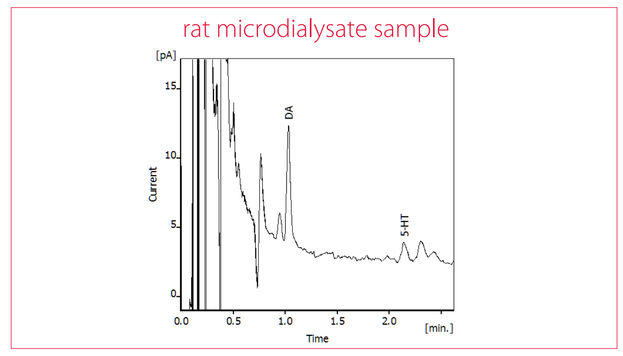

Fig. 7. Chromatogram of a basal level rat microdialysate sample. The concentration was calculated to be 0.7 nmol/L DA and 0.2 nmol/L 5-HT. Injection volume: 1.5 µL. Sample kindly provided by Jolien Schoors, University of Brussels (Belgium).

Alternative approach: dual channel analysis

- Simultaneously running two applications on a single sample
- Autosampler with 2 sampling loops in series to minimize sample consumption during injection cycle (Fig. 10)
- Increased efficiency and selectivity.
- Example of combinations:

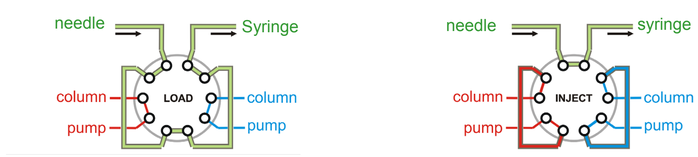

Fig. 10. Connections and flow path in a 10-port valve used for loading one sample onto two parallel UH-PLC-ECD channels with a single injection.

Conclusions

The Antec ALEXYS Neurotransmitters Analyzer is a dedicated and fully flexible analytical UHPLC-ECD system for analyzing different neurotransmitters in small samples:

- Optimized methods
- Small sample use
- Short total analysis time
- Excellent sensitivity
- Flexible system solution
- Saving rodents, as high quality data reduces quantity of animals needed

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