



Recent Developments in Neurotransmitter Analysis

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Neurochemistry challenges and solution

Microdialysis of neurotransmitters *in vivo* has become an invaluable tool to study neurotransmission in the living brain. Extracellular fluid of the brain is sampled via a microdialysis probe and fractions are collected for analysis of neurotransmitter concentrations using UHPLC with electrochemical detection (ECD).

We developed a solution to the challenges in microdialysate analysis:

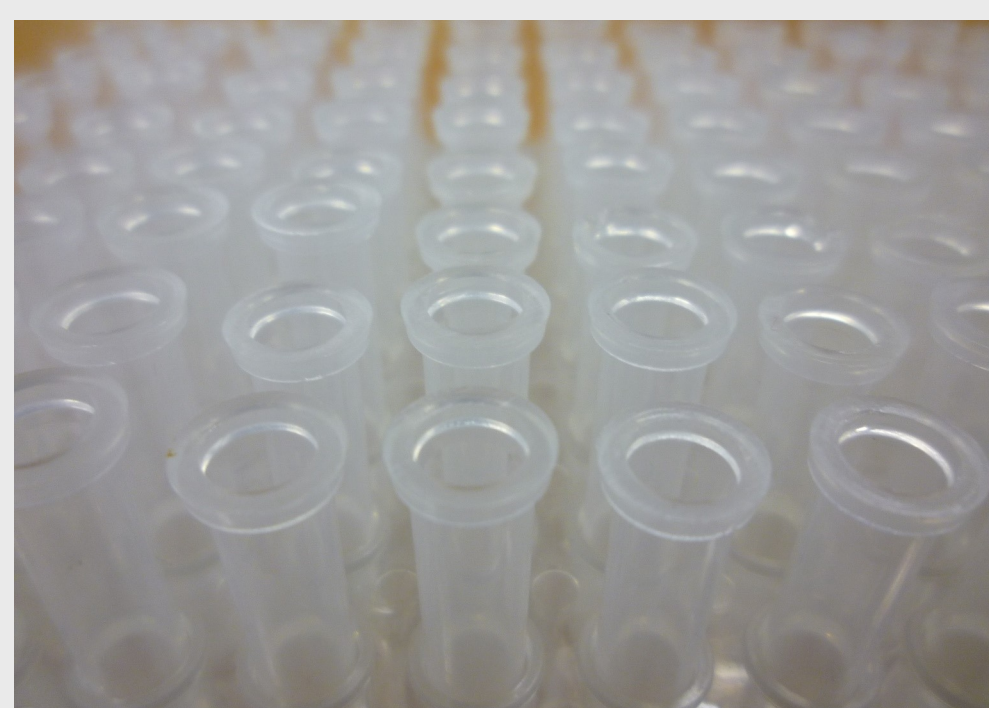
Small samples

Temporal resolution of microdialysis is limited by the mass sensitivity (LOD) of the analytical method. Perfusion flow rate and required temporal resolution define the size of each collected fraction.



Many samples

With the need for increased time resolution a growing number of samples is generated by collecting fractions at short time intervals. Often the experimental design requires replicates, resulting in even more samples.



The use of lab animals

Obtaining more information from less samples reduces the number of assays and lab animals involved.

Sub-nanomolar concentrations

A microdialysis sample contains neurotransmitters in low picomolar concentrations, as well as their metabolites and other components in significantly higher concentrations. This poses a further challenge to the separation power of the analytical system.



Solution: ALEXYS® Neurotransmitter Analyzer

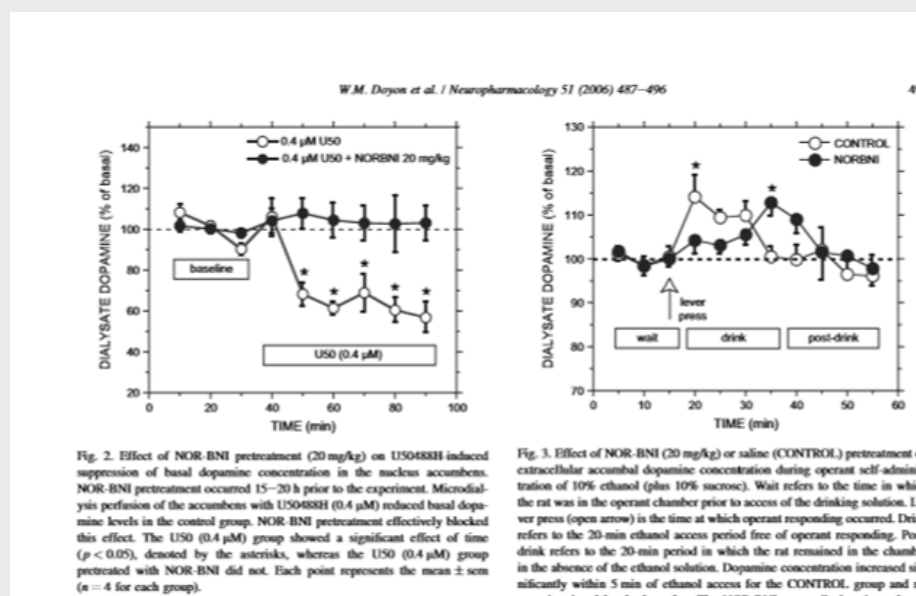
The Antec ALEXYS Neurotransmitters Analyzer is a dedicated and fully flexible UHPLC/ECD system for analyzing different neurotransmitters in small samples:

- Multi-component or target analysis
- Short analysis time
- Automated sample processing
- Efficient data generation



Recent developments

The recent development of the DECADE Elite (electrochemical detector) and the SenCell (electrochemical flow cell) combined with high efficiency separation columns made it possible to improve the methods for neurotransmitter analysis.



In this poster we present an overview of the methods for analyzing neurotransmitters in microdialysate samples with the Antec ALEXYS Neurotransmitters Analyzer.

Analysis of all monoamines and metabolites

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

Table 1. Repeatability of analysis of standards (5 µL). Unweighted linear regression using standards of 0.1, 0.2, 0.5, 1, 2 and 4 nmol/L. Limits of detection based on S/N-ratio of 3.

	Repeatability (RSD area, n=8)		Linearity (r)	Limit of detection	
	1 nmol/L	10 nmol/L		conc. (pmol/L)	(fmol)
NA	<3%	<1%	0.999	<50	<0.2
DOPAC	<3%	<1%	0.998	<100	<0.3
5-HIAA	<2%	<1%	0.997	<50	<0.2
DA	<3%	<1%	0.999	<50	<0.2
HVA	<4%	<1%	0.999	<50	<0.3
5-HT	<4%	<1%	0.998	<100	<0.5

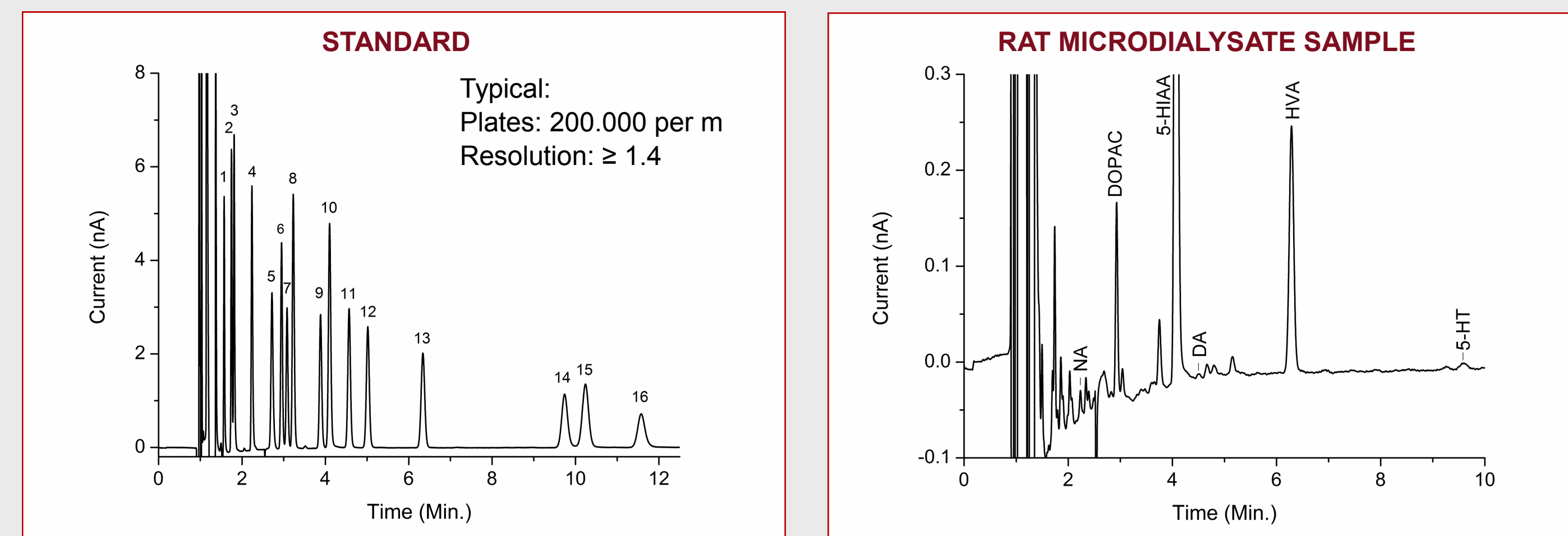


Fig. 1. **Left:** Chromatogram of a 100 nmol/L standard of 16 neurotransmitters and metabolites in perfusion fluid and 10 nmol/L acetic acid. Injection volume 2 µL: (1) VMA, (2) MOPEG, (3) L-DOPA, (4) NA, (5) A, (6) DOPAC, (7) 3-OMD, (8) 3,4-DHBA, (9) Normetanephrine, (10) 5-HIAA, (11) DA, (12) Methanephrine, (13) HVA, (14) 5-HT, (15) 3-MT and (16) 5-MHT. **Right:** Analysis of basal level rat prefrontal cortex dialysate. Concentrations were measured as 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. Both chromatograms based on injection volumes of 2 µL.



- Detection limit 0.15 fmol on column (100 pmol/L; 1.5 µL)

Analysis of ACh

Basal concentrations are measured in a sample of 10 µL using UHPLC/ECD with a post-column IMER.

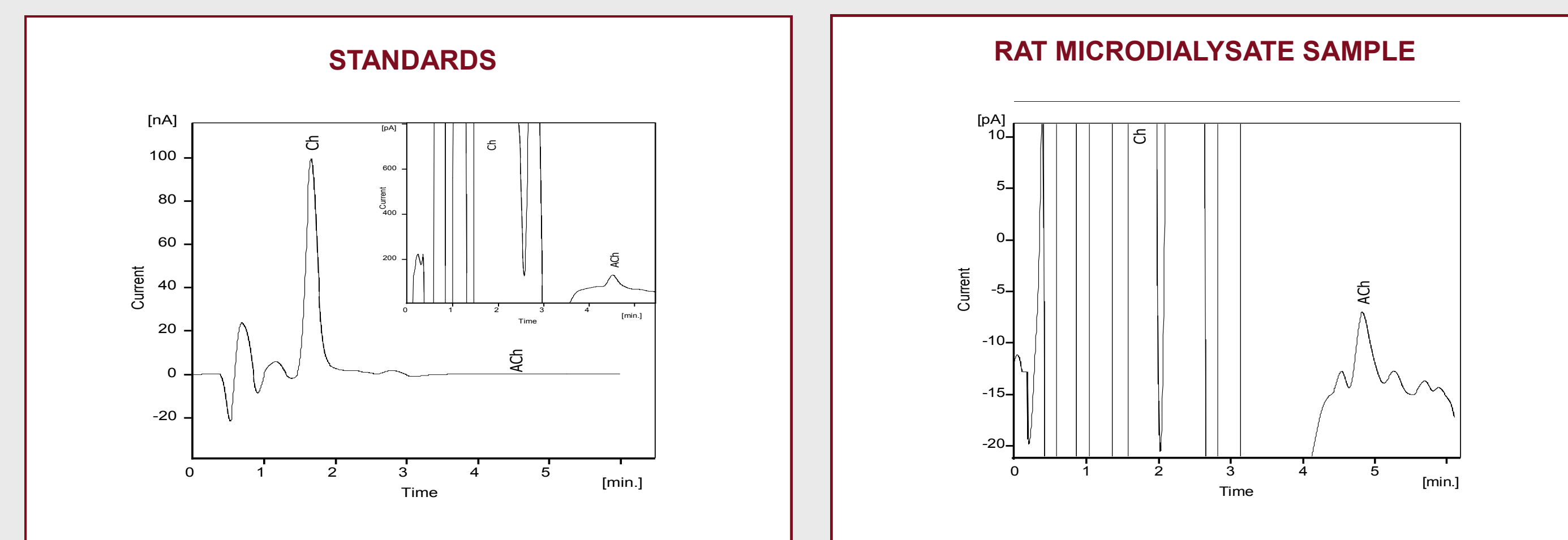
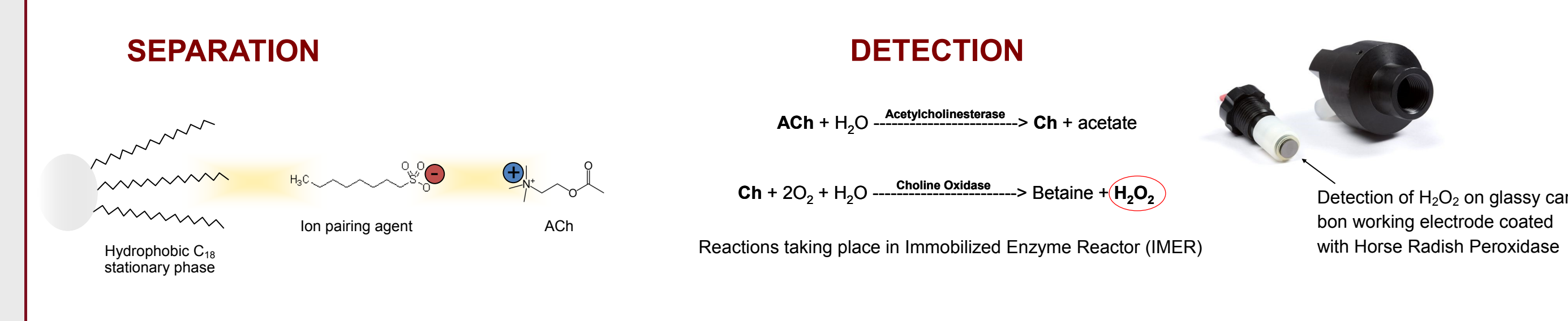


Fig. 5. **Left:** chromatogram of 2 µmol/L Choline and 2 nmol/L Acetylcholine in Ringer solution. The inset shows the same chromatogram, but zoomed in on the baseline. **Right:** Chromatogram of a basal level rat microdialysate sample. The acetylcholine concentration was calculated to be 1 nmol/L.

- Detection limit 3 fmol on column (0.3 nmol/L; 10 µL)

Dual channel analysis

Two independent and complementary analyses using a single injection. This set-up increases efficiency and selectivity.

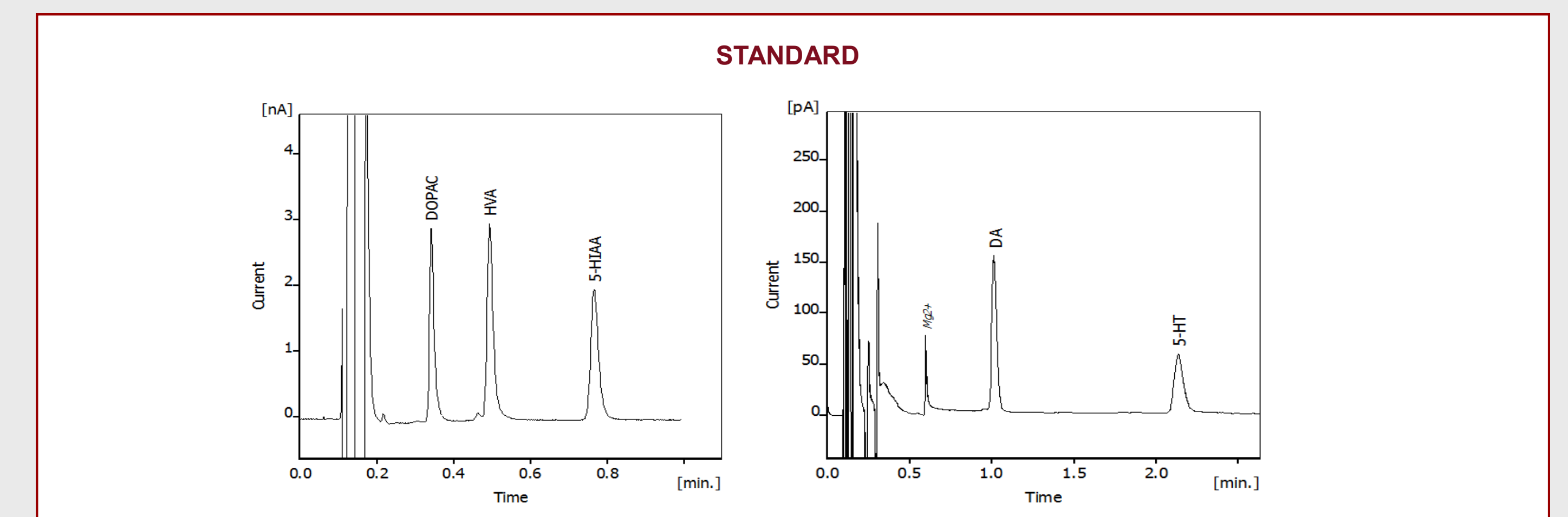
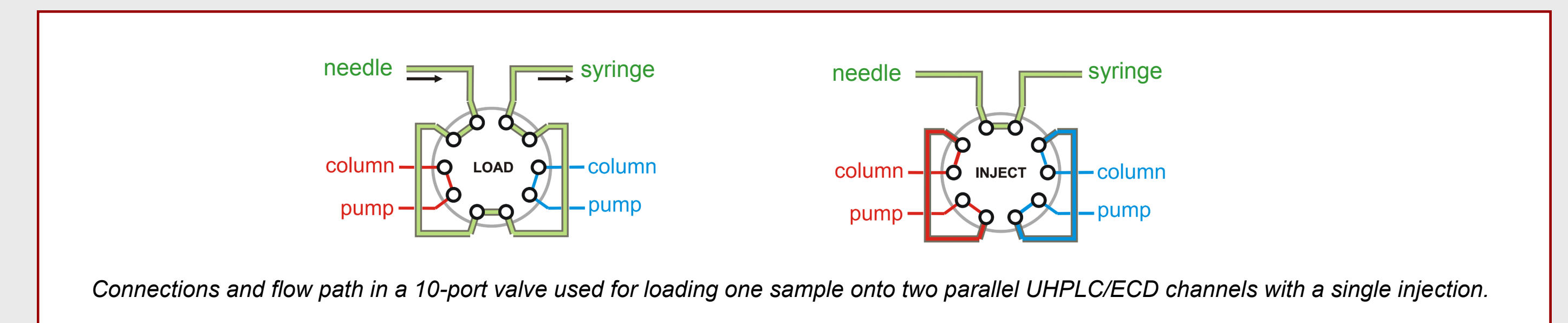


Fig. 3. Chromatograms simultaneously obtained from a single injection of a mix of 100 nmol/L acidic metabolites and 10 nmol/L monoamines in Ringer solution (containing Mg²⁺) acidified with 10 nmol/L acetic acid. Both chromatograms were separated and detected in parallel UHPLC/ECD lines that were running with different columns, mobile phases and detector settings. Chromatograms based on injections of 1.5 µL on column for each.

- Efficient sample processing with 2 complementary analyses in parallel.

Conclusions

The ALEXYS Neurotransmitter Analyzer is a dedicated UHPLC/ECD system for the analysis of microdialysis samples:

- **Superior detection sensitivity**
 - * LOD for monoamines down to 0.15 fmol (30 pmol/L; 5 µL)
- **Optimized for small sample volumes**
 - * Total sample use (incl. loop overflow) down to 3 µL
 - * High temporal resolution
- **Flexible system solution**
 - * Target or multi-component analysis
 - * One system, multiple neurotransmitter kits
- **Saving lab animals**
 - * Getting more information from less samples, reduces the number of assays and lab animals involved.

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