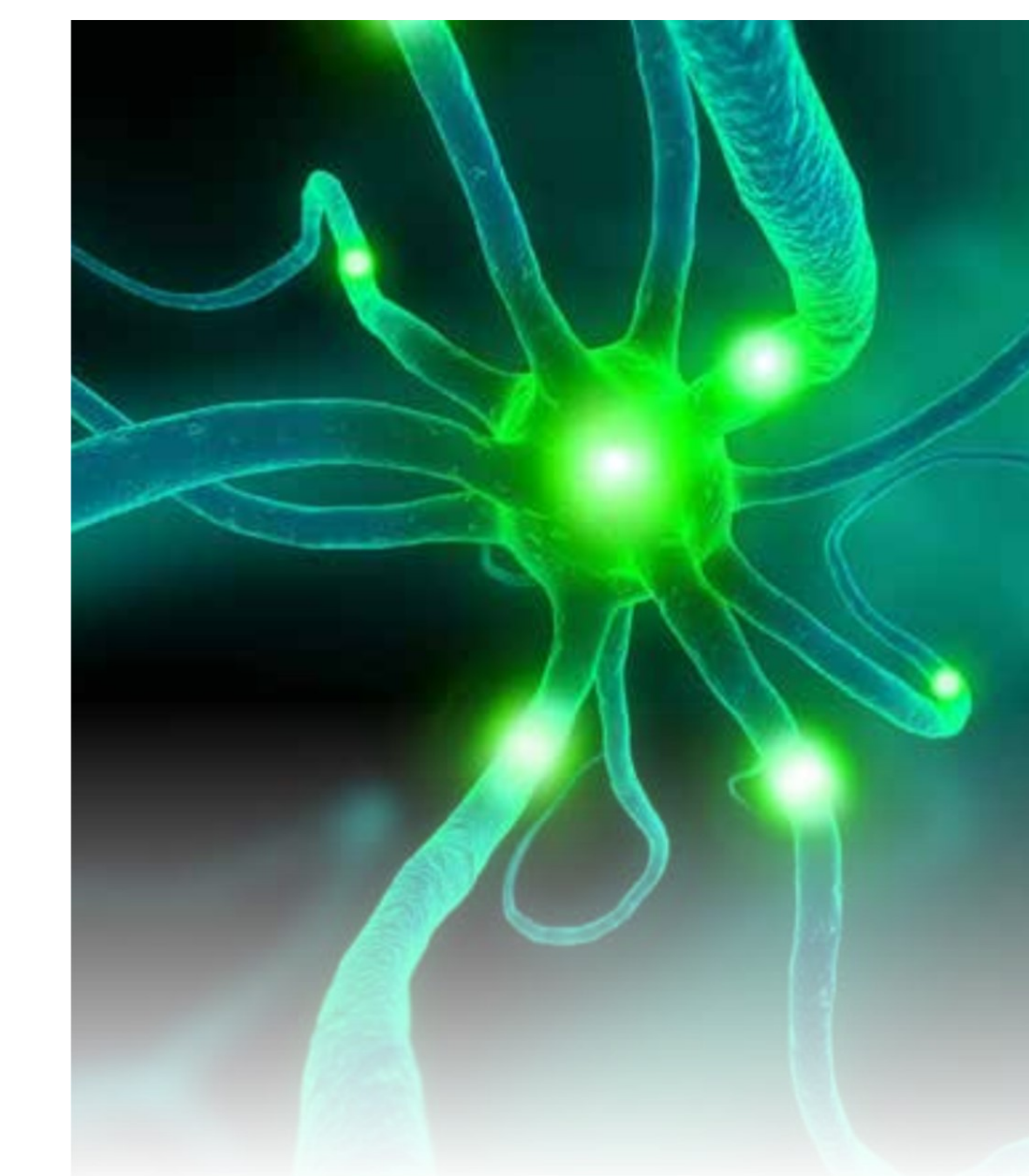# Fast and sensitive analysis of **acetylcholine**, **GABA**, **glutamate**, **monoamines** and **metabolites** using the ALEXYS Neurotransmitter Analyzer

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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 .

We developed a system approach based on UHPLC with electrochemical detection (ECD) to meet the challenges in microdialysate analysis, which are:

Small and many 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.

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 challenge to the separation power of the analytical system.

ALEXYS Neurotransmitter Analyzer

A new versatile UHPLC ALEXYS Neurotransmitter Analyzer based on the DECADE Elite detector with SenCell has been developed. This analyzer is a dedicated and fully flexible UHPLC/ECD system for analyzing different neurotransmitters in small samples.

Fig 1. ALEXYS Analyzer with DECADE Elite electrochemical detector and SenCell (right insert)

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 = 3.

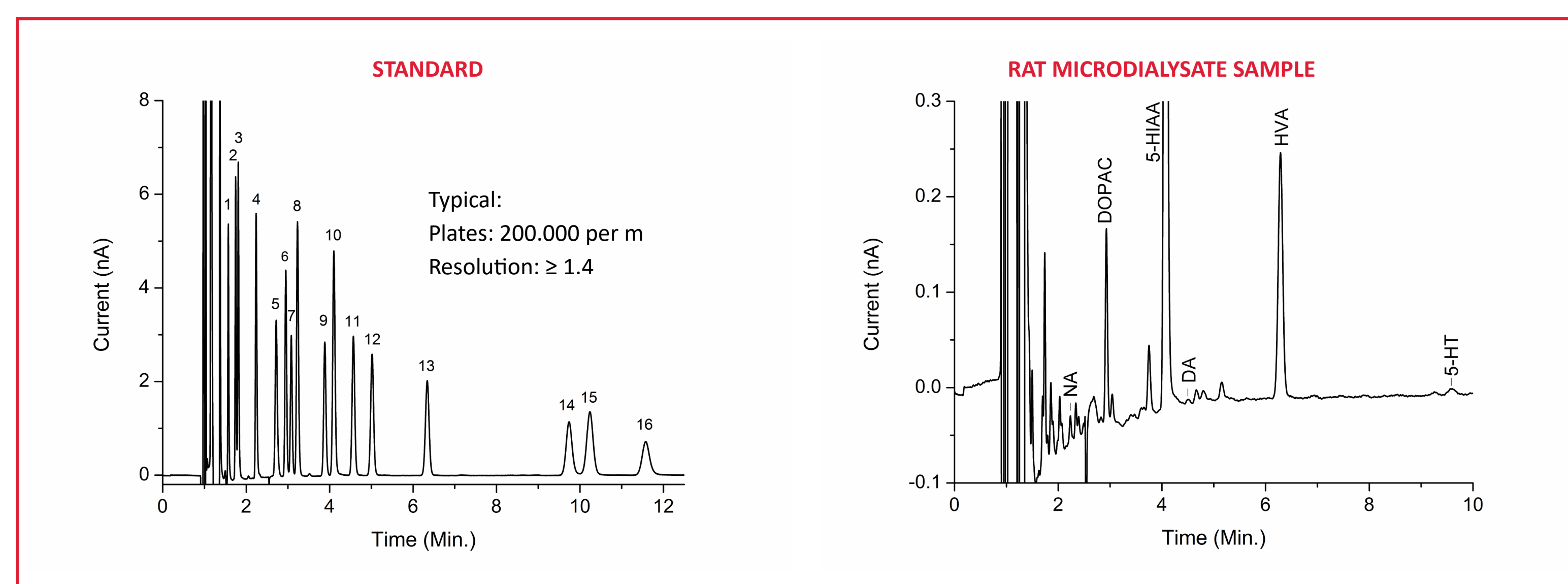|	Repeatability (RSD area; n=8)		Linearity (r)	Limit of detection	
	1 nmol/L	10 nmol/L		conc. (pmol/L)	On-column (fmol)
NA	<3%	<1%	0.999	<50	<0.2
DOPAC	<5%	<1%	0.998	<100	<0.3
5-HIAA	<2%	<1%	0.997	<50	<0.2
DA	<3%	<1%	0.999	<50	<0.2
HVA	<4%	<1%	0.999	<50	<0.3
5-HT	<4%	<1%	0.998	<100	<0.5

Fig 2. Left: Chromatogram of a 100 nmol/L standard of 16 neurotransmitters and metabolites in perfusion fluid and 10 mmol/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.

Analysis of Choline and Acetylcholine

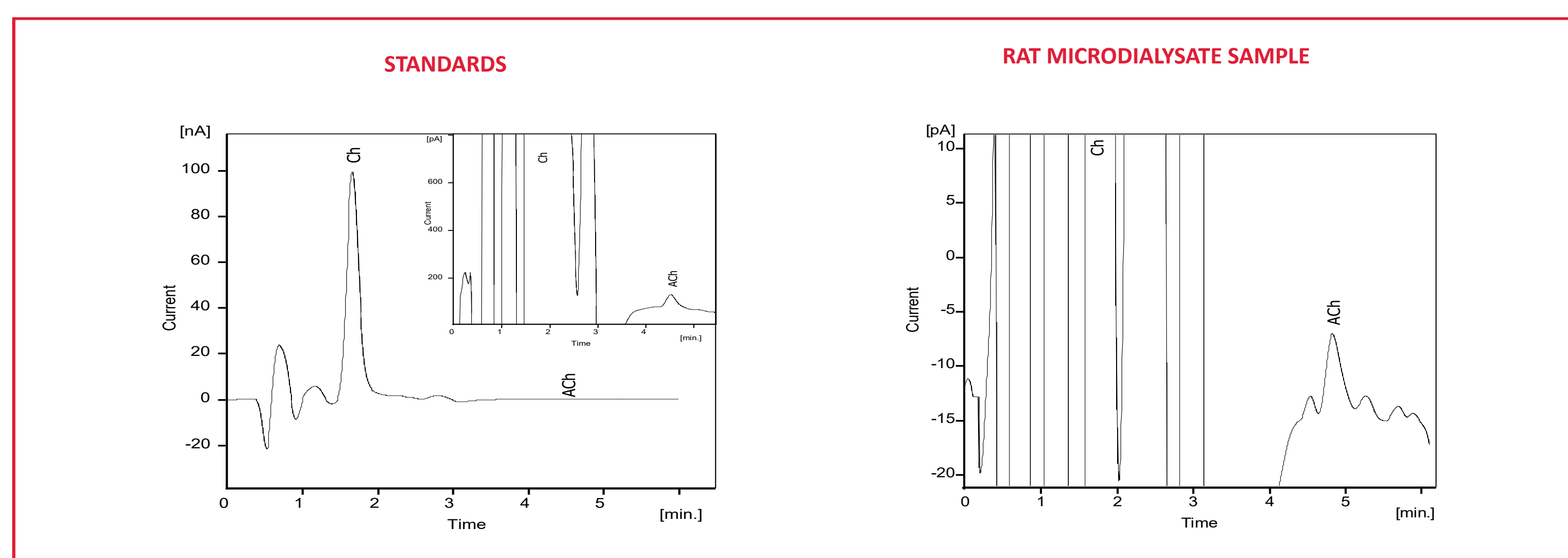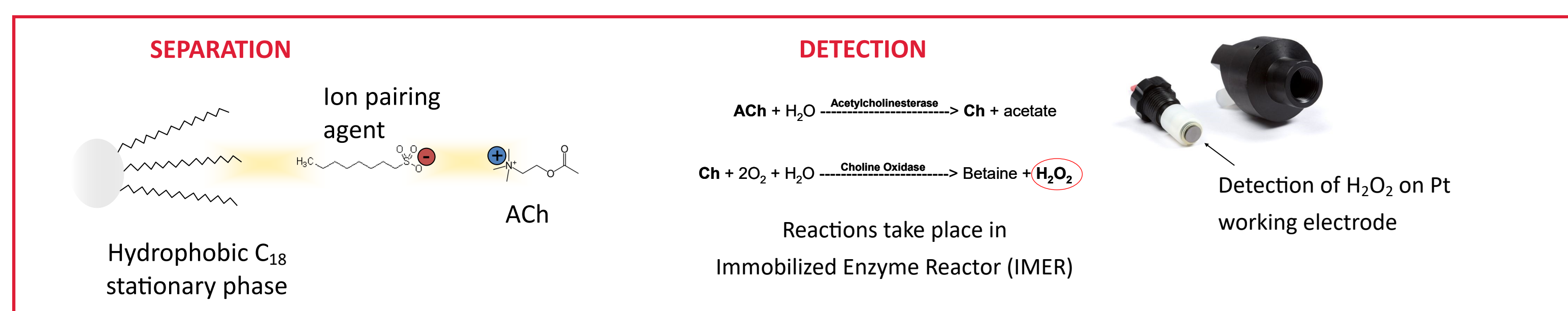Basal concentrations are measured in a sample of 10 µL using UHPLC/ECD with a post-column IMER.

Fig. 3. 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.

Analysis of GABA, Glu and other amino acids
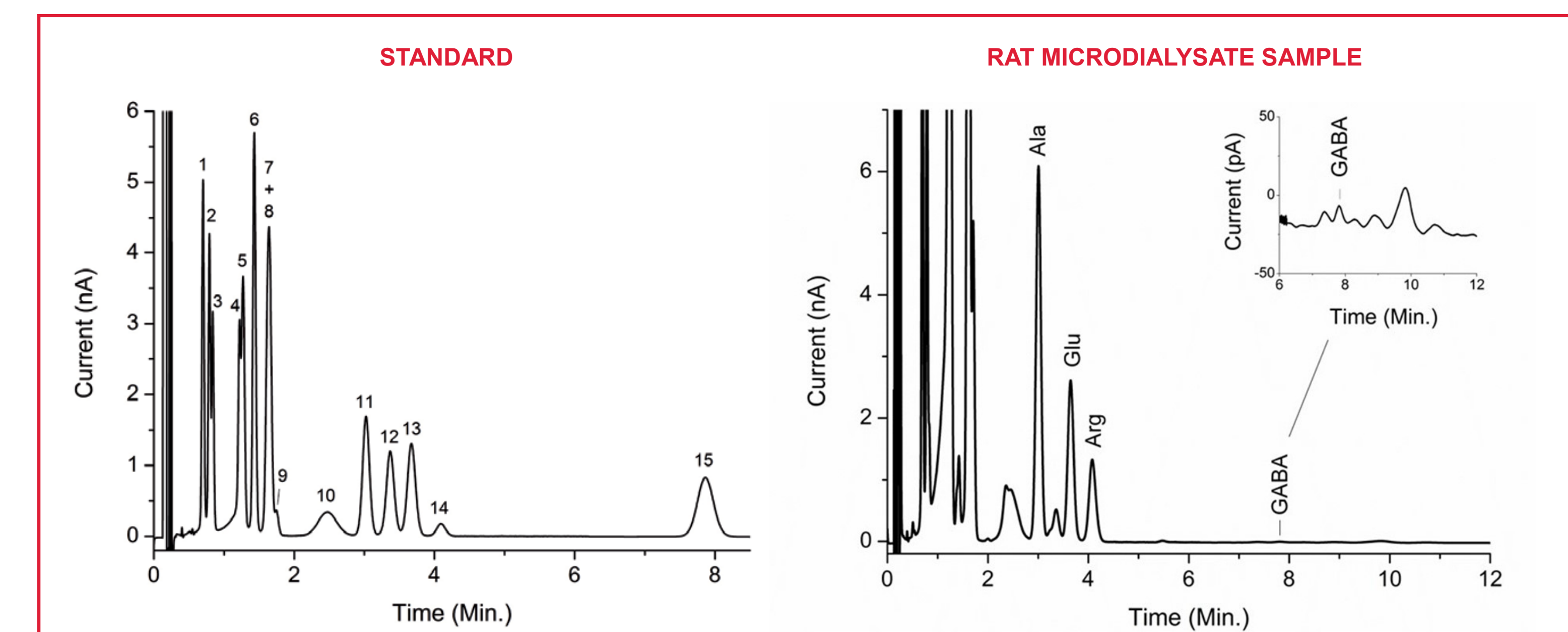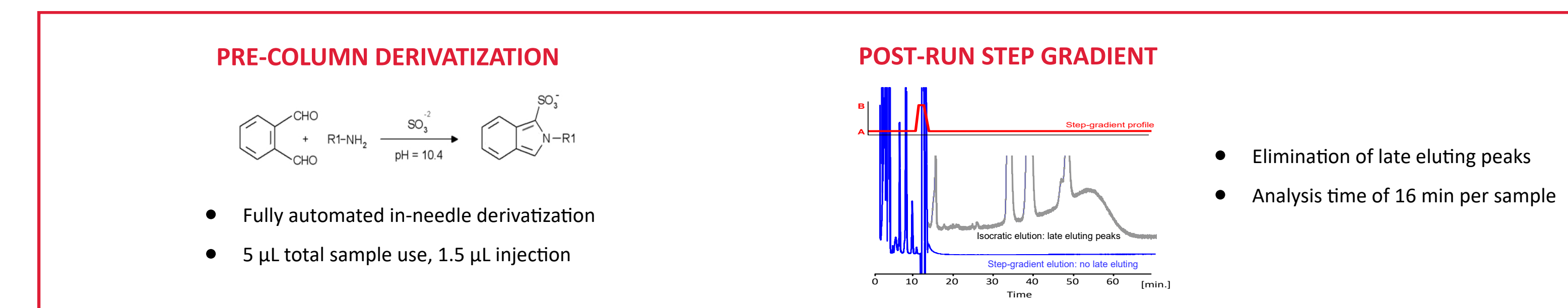
UHPLC/ECD analysis with a detection limit of 15 fmol.

Fig. 4. Left: Chromatogram of a 2.5 µmol/L standard of 14 amino acids in water, derivatized with ortho-phthalaldehyde (OPA): (1) Serine, (2) Taurine, (3) Asparagine, (4) OPA, (5) Glycine, (6) Histidine, (7) Aspartate, (8) Glutamine, (9) Cystine, (10) Trans 4-hydroxy-L-Proline, (11) Alanine, (12) Citrulline , (13) Glutamate, (14) Arginine and (15) GABA . Right: Chromatogram of a rat prefrontal cortex dialysate sample. Insert: zoom-in on GABA peak.

Summary

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

- **Superior detection sensitivity**
  - ✳ LOD for monoamines down to 0.15 fmol (30 pmol/L; 5 µL)

- **Optimized for small sample volumes**
  - ✳ High temporal resolution

- **Flexible system solution**
  - ✳ Target or multi-component analysis
  - ✳ One system, multiple neurotransmitter applications

- **Reducing the need for lab animals**
  - ✳ Getting more information from less and fewer samples helps to reduce the number of lab animals that have to be involved.

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