

New ALEXYS[®] Analyzers for Monoamines and GABA/Glutamate Analysis in Microdialysates

Antec[®]
Proven Performance!

*M. EYSBERG, H.-J. BROUWER, L. M. VAN HEERWAARDEN, N. J. REINHOUD
Antec, Industrieweg 12, 2382NV Zoeterwoude, The Netherlands

Fast and sensitive analysis of Monoamines and Metabolites

The new ALEXYS[®] Micro LC analyzer allows the use of capillary LC columns and higher back pressures for fast and sensitive analysis of monoamines and their metabolites. The hardware is optimised to prevent dead volumes by using a special injection valve, small ID connecting tubing of short length, and a small volume electrochemical cell. The maximum backpressure of this system is 700 bar.

In this poster, preliminary results are presented about the development of a single column solution for the fast analysis of monoamines. The new Analyser is based on a novel ALEXYS UHPLC hardware (pump and autosampler) and the new NeuroSep columns.



NeuroSep[™] columns (Antec) with ID of 1.0 and 0.5 mm, 5 and 15 cm length.

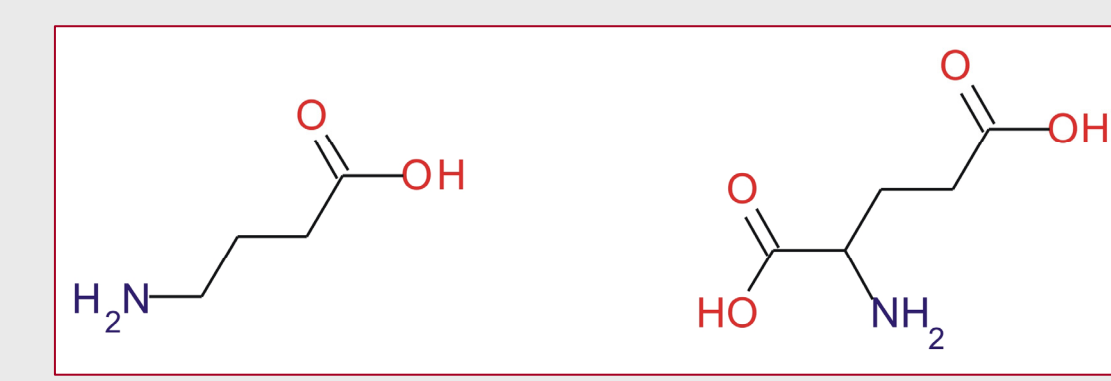
ALEXYS[®] Monoamines Analyzer with ultra low dead volume and maximum system pressure of 700 bar.



Fast and sensitive analysis of GABA and Glutamate

Ultra-High Performance Liquid Chromatography (UHPLC) is a rapidly growing separation technique based on the application of LC columns with sub-2 μm particles operating at higher linear velocities and high back pressures. UHPLC offers advantages in chromatographic resolution, analysis speed, and sensitivity over conventional HPLC systems. The combination of Electrochemical Detection (ECD) with UHPLC can be a powerful solution to increase the sample throughput & sensitivity of neurotransmitter analysis in microdialysates and brain homogenates.

In this poster preliminary results are presented about the development of a user-friendly UHPLC solution for the fast analysis of GABA and Glutamate. The new analyzer is based on novel ALEXYS[®] UHPLC hardware (pump and autosampler). Separation and detection is achieved using a single sub-2 particle column and automated pre-column derivatization with o-phthalaldehyde (OPA) / sulphite [1,2], respectively.



Structure of GABA (left) and Glutamate (right).

ALEXYS[®] GABA/Glu Analyzer equipped with the new LC 110S pump and the AS 110 micro autosampler with UHPLC injector



Fast analysis

For fast analysis, the flow rate was increased up to reaching 80% of the maximum system backpressure. The analysis time could thus be reduced from 25 min to less than 7 min. Due to the features of the NeuroSep column, this was achieved while keeping good column performance (>100.000 plates/m, and peak asymmetry around 1.0).

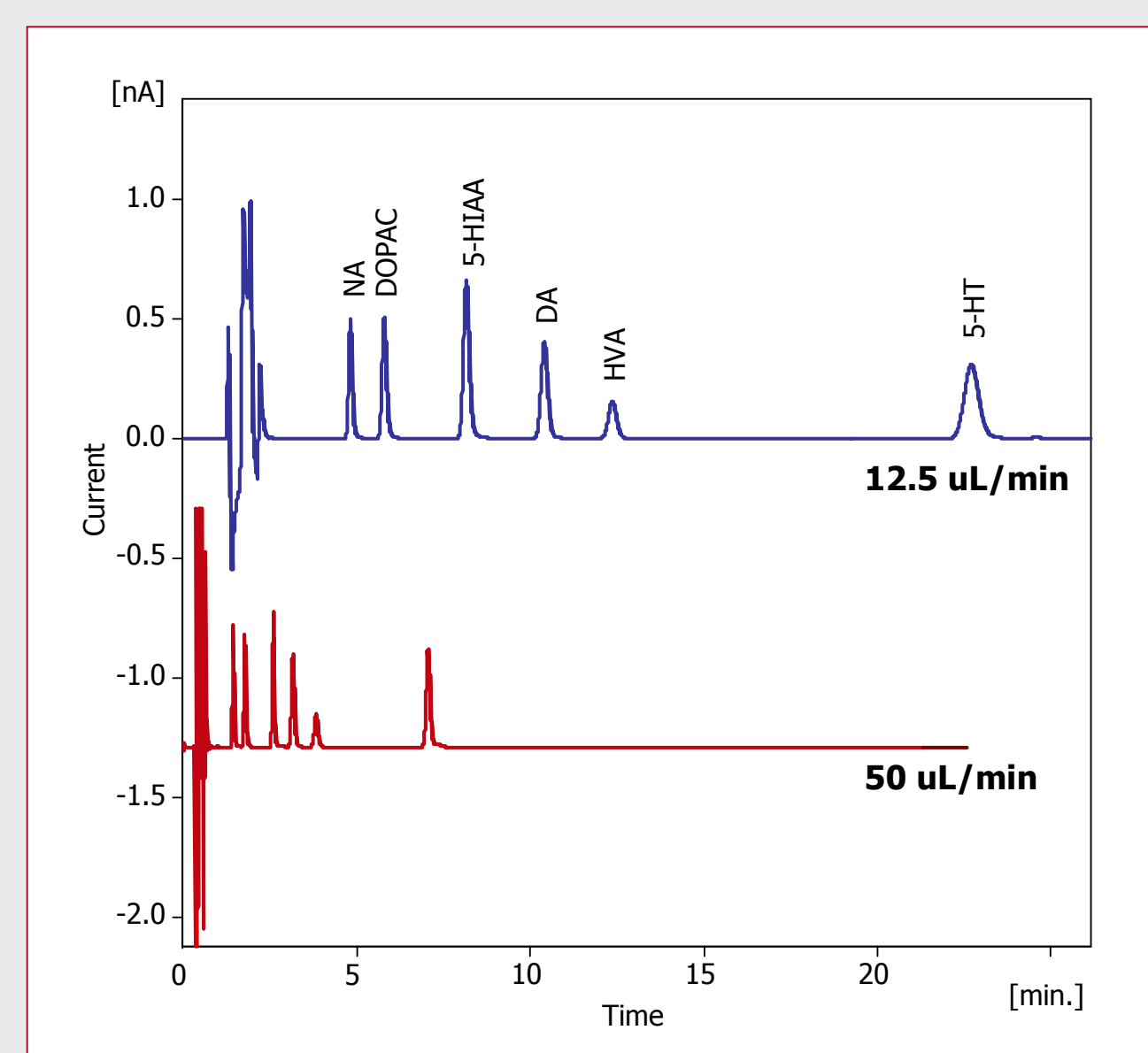


Fig. 1. Chromatograms of 2 μL injections of 100 nM standards mix, analysed at different flow rates. All other conditions were identical between these analyses.

Small sample use

To facilitate the analysis of samples of 5 μL or less, we used:

- Autosampler AS110 with micro fluidic pathway
- User defined injection program
- Direct injections from microdialysis vials

Performance evaluation

The system performance was tested under optimised conditions using microdialysis vials filled with samples of only 6 μL . The total sample use was 5 μL , from which 4 μL was actually injected on column. If necessary, this can be minimised further. Using such small sample volumes, the following results were achieved:

- RSD < 0.3% for retention time (100 nM, n=8)
- RSD < 1.0% for peak area (100 nM, n=8)
- Correlation coefficient > 0.999 (20-100 nM)
- On-column detection limits of 0.6 fmol
- cLOD of about 150 pmole/L, 4 μL injections

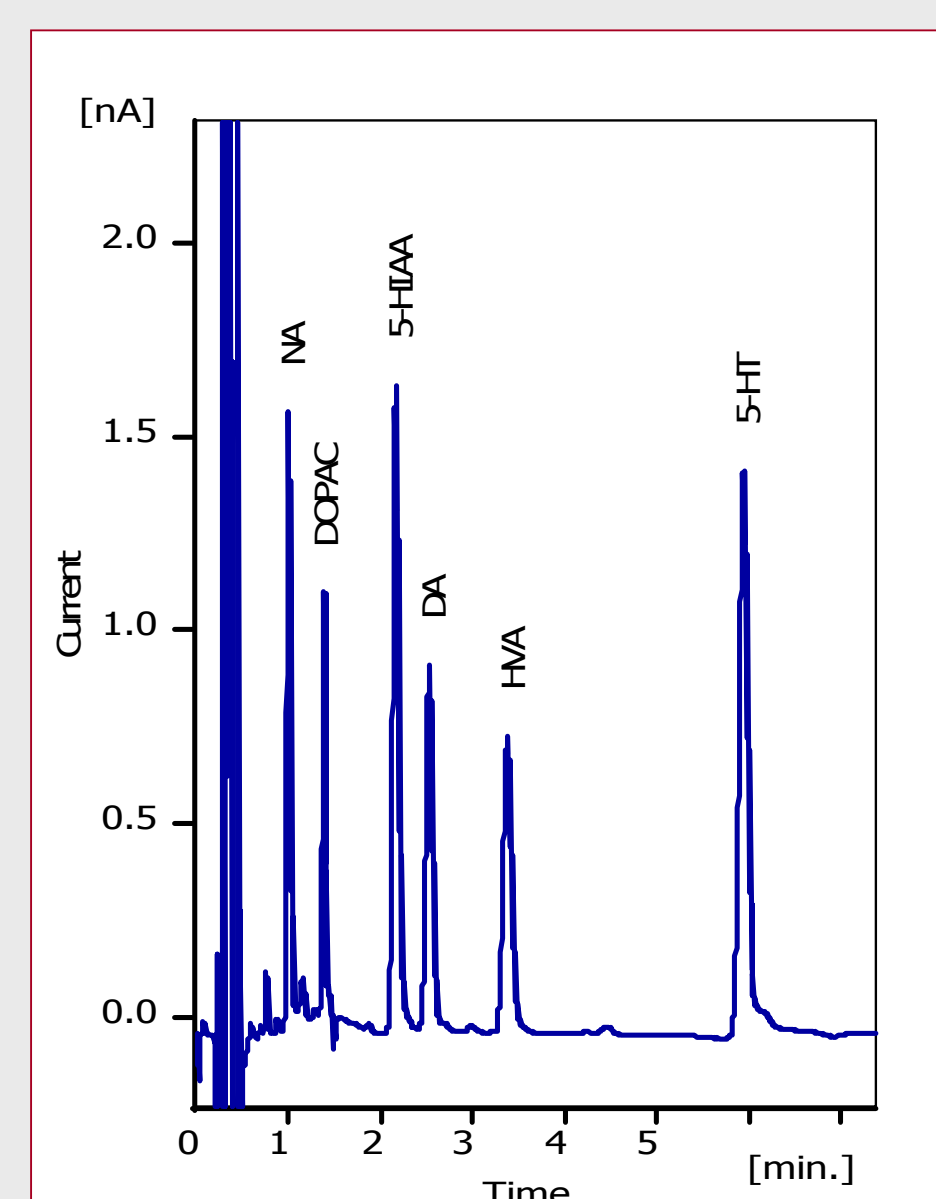


Fig. 2. Chromatogram of 100 nM standards in Ringer solution + 10 mM acetic acid.

Analysis of Microdialysates

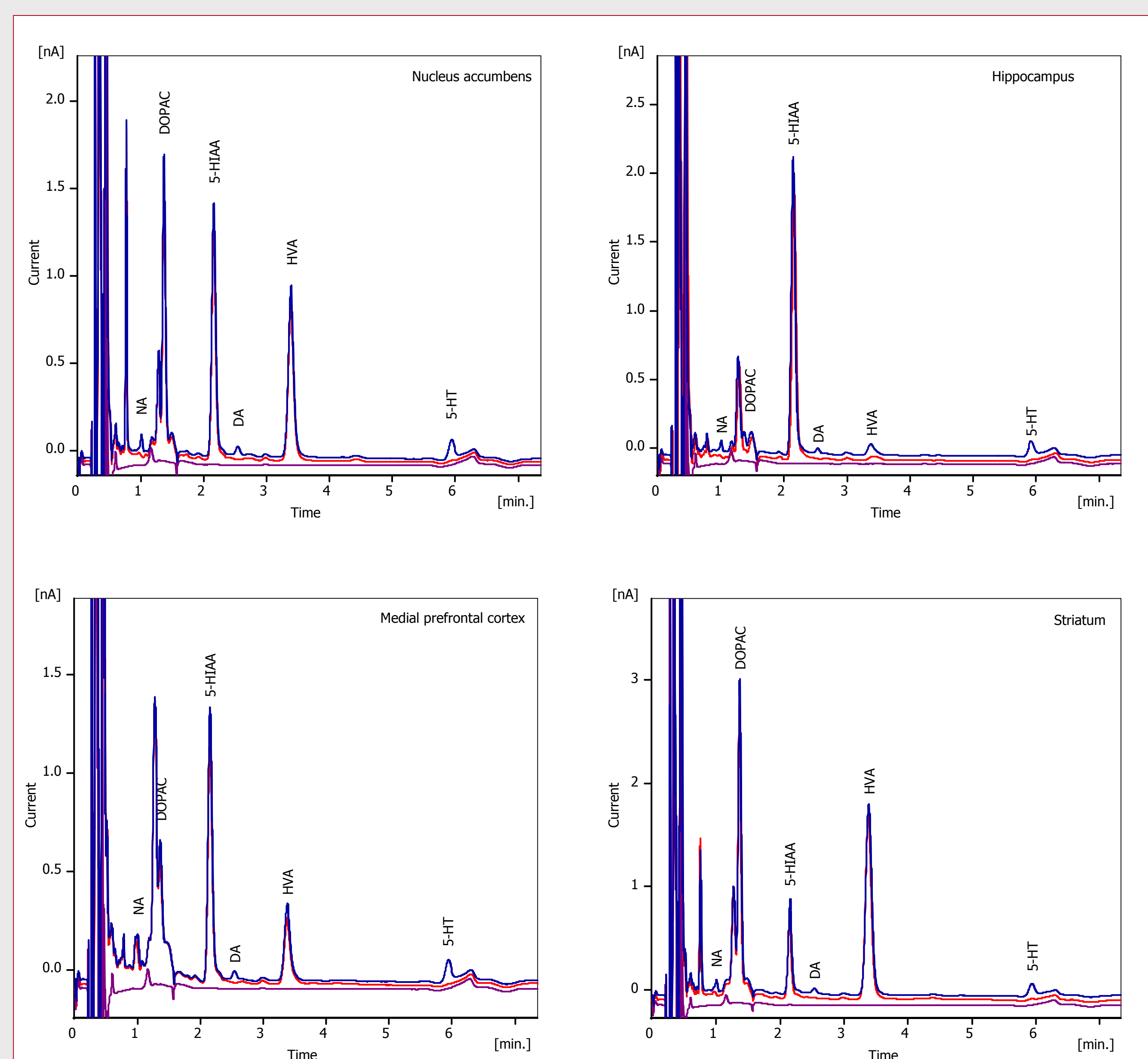


Figure 3. Analysis of blank perfusion fluid (purple trace), basal level (red trace) and 10 nM spiked (blue trace) rat microdialysates from different brain regions: Nucleus Accumbens, Striatum, Hippocampus and Medial Prefrontal Cortex. The samples were obtained by dialysis of 8 test animals for 16 hours at a flow rate of 2 $\mu\text{L}/\text{min}$ using perfusion fluid consisting of 147 mM NaCl, 4.0 mM KCl, 1.2 mM MgCl₂ and 0.7 mM CaCl₂. After a sterility check, all samples (per brain region) were pooled and frozen at -80°C until analysis. Samples were provided by Mr. Niels Leguit, Abbot Healthcare Products B.V., Weesp, the Netherlands.

Conclusion

Fast and sensitive analysis of biogenic amines and metabolites is demonstrated using the new ALEXYS Monoamines Analyzer with 0.5 mm ID NeuroSep[™] Capillary columns.

- Detection limits about 150 pmole/L
- Sample use is 5 μL out of a total sample volume of 6 μL
- Biogenic amines and acidic metabolites analyzed in less than 7 minutes

Method

LC: ALEXYS[®] GABA/Glu analyzer
Column: NeuroSep C₁₈, 50 x 2.1 mm ID, 1.8 μm (Antec)
Flow cell: VT-03 with 2 mm GC electrode and Salt Bridge reference
V_{injection}: 5 μL (sample use 13 μL)
T_{oven}: 45 °C (column + flow cell)
Flow rate: 1000 $\mu\text{L}/\text{min}$
Pressure: 590 bar
E_{cell}: 800 mV vs. Ag/AgCl

Automated OPA derivatization was achieved using a user-defined injection program in the AS 110 micro autosampler (mixing ratio sample:reagent was 5:1).

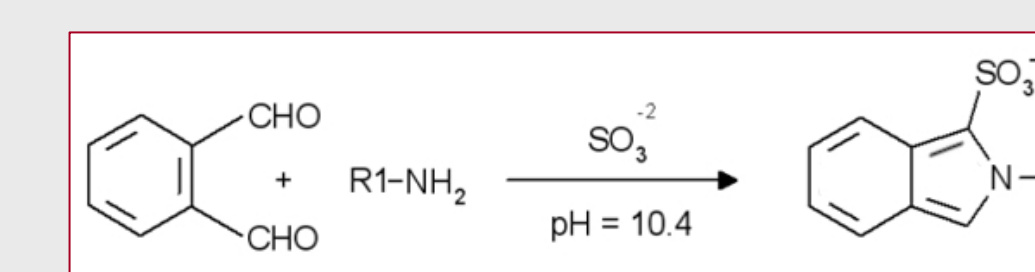


Figure 4. Reaction scheme of the derivatization of primary alkyl amines with o-phthalaldehyde and sulphite into an electrochemically-active isoindole sulfonate.

Selectivity

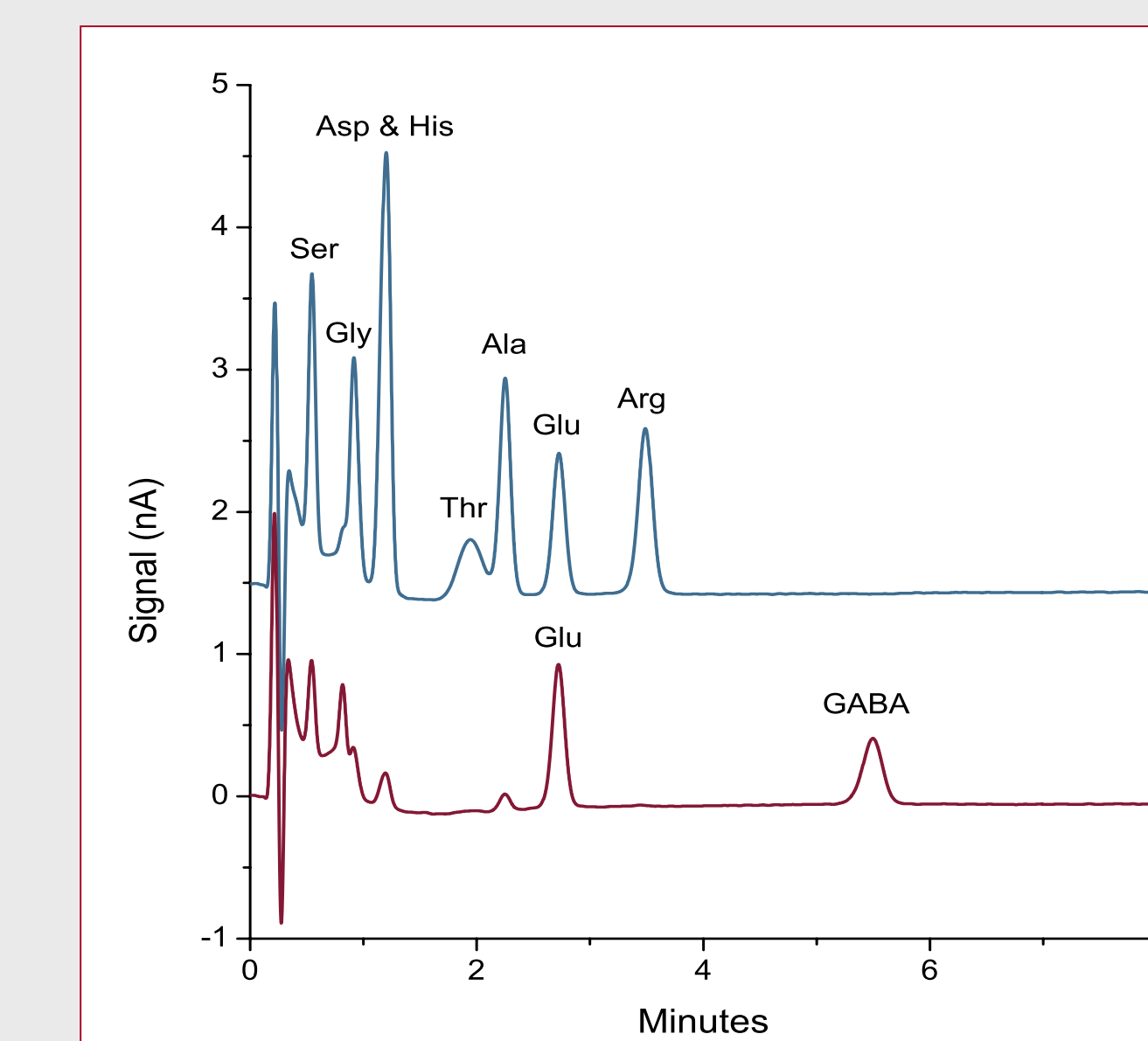


Figure 5. Chromatogram (red) of 500 nM Glu and GABA in perfusion fluid. Chromatogram (blue) of an amino acid mix (Sigma-Aldrich, AAS18-10X1ML). Note: GABA not present in amino acid mix.

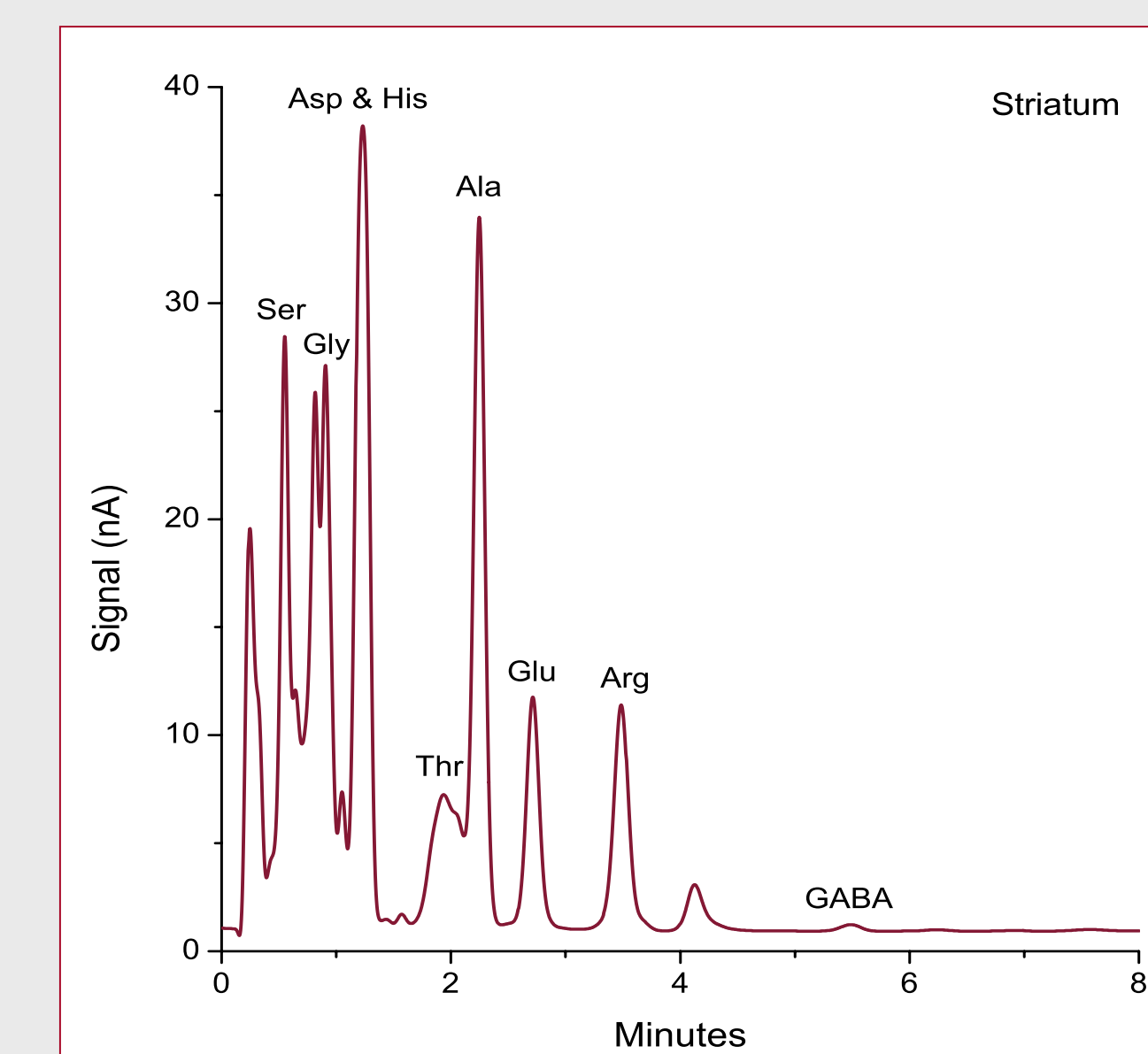


Figure 6. Identification of amino acids in pooled rat striatum dialysate.

Table 1. LC performance data, based on 500 nM standards

Name	Ret. time (min)	Capacity	Asymmetry	Eff/I [t.p./m]
Glu	2.67	19.1	1.03	51798
GABA	5.69	41.8	0.93	89787

Limit of detection (LOD, s/n=3) and linearity

Concentration LOD < 10 nM for both GABA and Glu (5 μL injection, sample use 13 μL)
On-column LOD < 50 fmol (GABA)
Correlation coefficient > 0.999 in the range of 0.1-1 μM Glu and 10-100 nM GABA.

Analysis of Microdialysates

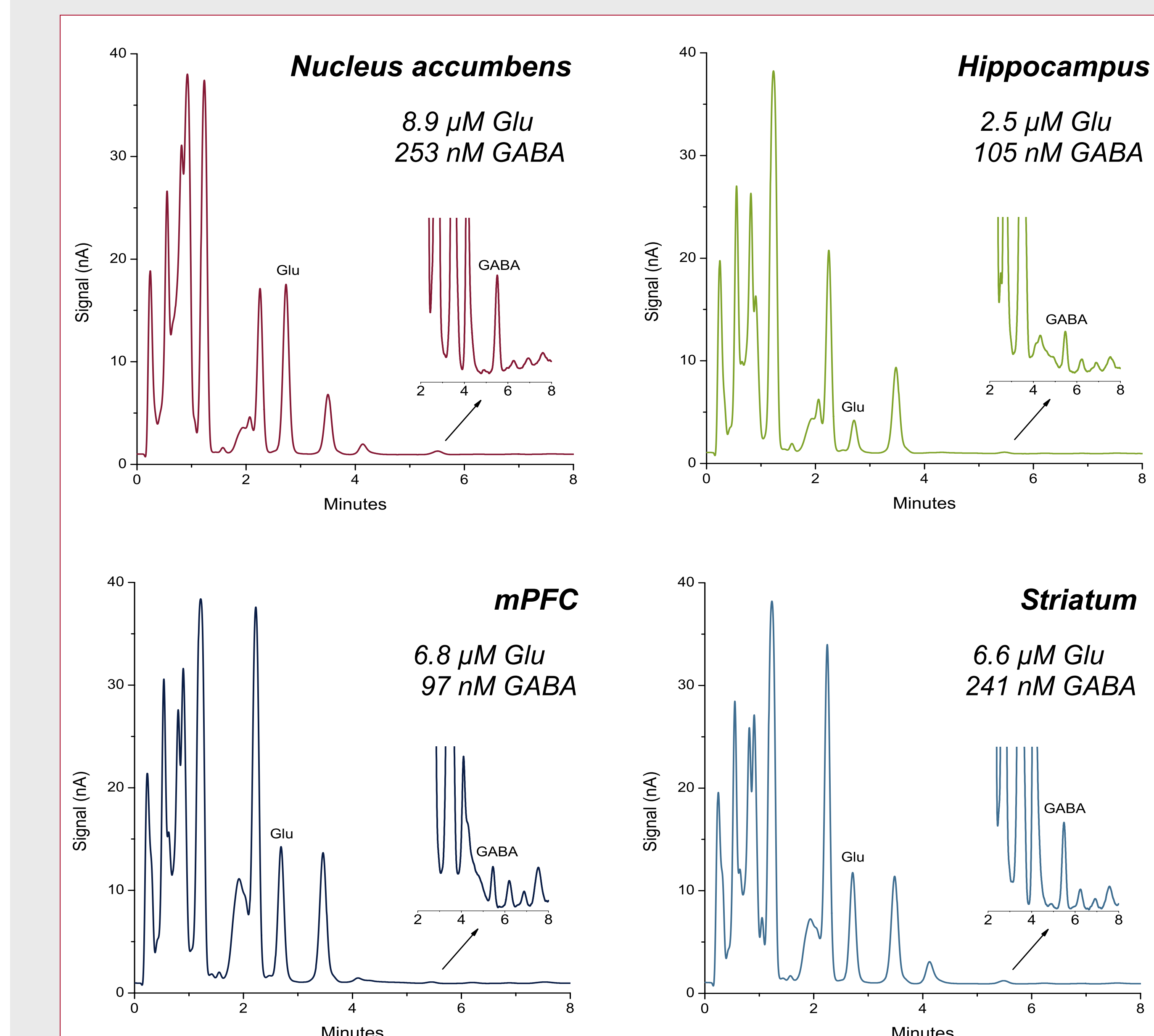


Figure 7. Analysis of basal level GABA and Glutamate in pooled rat microdialysate from different brain regions (Nucleus Accumbens, Striatum, Hippocampus and Medial Prefrontal Cortex), with the measured concentrations of GABA and Glutamate inside the chromatogram. The samples were obtained by dialysis of 8 test animals for 16 hours at a flow rate of 2 $\mu\text{L}/\text{min}$ using perfusion fluid consisting of 147 mM NaCl, 4.0 mM KCl, 1.2 mM MgCl₂ and 0.7 mM CaCl₂. After a sterility check, all samples (per brain region) were pooled and frozen at -80°C until analysis. Samples were provided by Mr. Niels Leguit, Abbot Healthcare Products B.V., Weesp, the Netherlands.

Conclusions

Fast and sensitive analysis of GABA and Glu can be achieved using the ALEXYS GABA and Glutamate Analyzer[™] - UHPLC in combination with a sub-2 micron C₁₈ UHPLC column. The elution of GABA and Glu within 7 min with good selectivity and resolution (Glu sufficiently resolved from interfering amino acids) is a considerable improvement compared to conventional methods, illustrating the potential of UHPLC-ECD with respect to high sample throughput.

Literature

- [1] W.A. Jacobs, J. Chromatography, 392 (1987) 435-441
- [2] S. Smith, T. Sharp, J. Chromatography B, 652 (2), (1994) 228-233