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Introduction

For the analysis of γ -aminobutyric acid (GABA) and Glutamate (Glu) in brain dialysates an automated pre-column derivatisation with ortho-phthalaldehyde (OPA) and sulphite has been used. Analysis of the oxidizable derivatives showed a large difference in retention times. Therefore, an ALEXYS micro LC-EC system with DCC and column switching has been used (Fig. 2). With this approach glutamate is resolved from the chromatographic front using a 5 + 15 cm column in series. When glutamate arrives on the second column, a second pump takes over column 2 and the valve switches column 1 to cell 2. As a result the retention time of the late eluting GABA peak is reduced considerably.

Characteristics of this method:

- Glu is resolved from the chromatographic front
- The retention time of late eluting GABA is reduced considerably
- Total analysis time: 25 min per run including OPA derivatisation
- Automated OPA derivatisation for better reproducibility

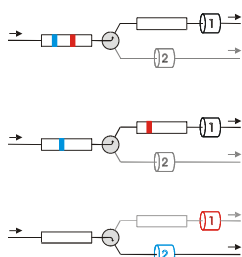


Fig. 1. Glutamate (red) is resolved from the front of the chromatogram and detected in cell 1. The run time of late eluting GABA (blue) is shortened by column switching followed by detection in cell 2.

Method

HPLC ALEXYS GABA/Glu Analyzer (p/n 180.0070A)
Flow cells 2 x VT-03 with 2mm GC electrode and salt bridge reference
 $V_{injection}$ 20 μ L sample + 2 μ L reagent, inject 5 μ L
 T_{oven} 35 $^{\circ}$ C for column and flow cell
Flow rate 200 μ L/min (both pumps)
Range 50 nA/V
ADF™ 0.05 Hz

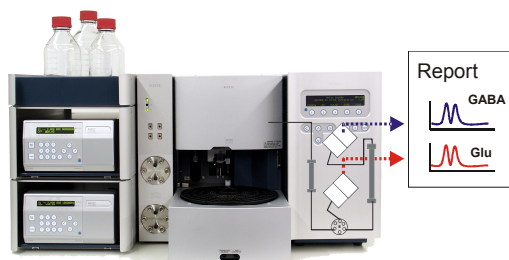


Fig. 2. ALEXYS GABA/Glu Analyzer.

Reproducibility

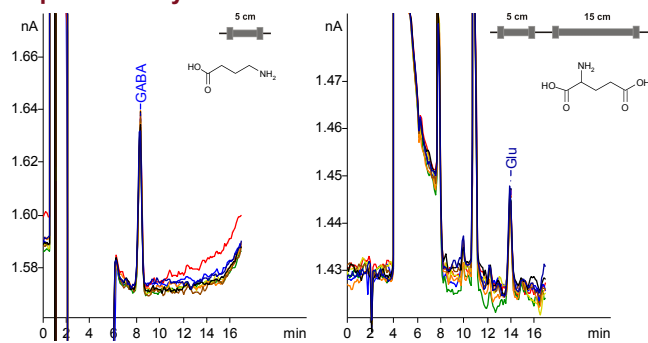


Figure 3. Overlay of 8 chromatograms of 50 nM GABA (left) and Glu (right) standard.

Table 3. Reproducibility (n=8) of 50 nM GABA and 500 nM Glu.

concentration		Retention, min		Height, nA		Area, nA*sec	
		mean	%RSD	mean	%RSD	mean	%RSD
Glu	500 nM	13.95	0.03	0.200	1.1	3.69	1.7
GABA	50 nM	8.31	0.15	0.067	1.7	1.28	2.5

Detection limit and linearity

Detection limit (s/n=3) is 6 nM for Glu, and 3 nM for GABA (5 μ L injections). RSD in peak heights for 10 nM GABA is 5%. The correlation coefficient for a calibration line between 10 – 80 nM and 100 – 800nM was better than 0.999 for peak height and area.

Analysis of microdialysate

A pooled microdialysate from the basal lateral amygdala was analysed and a concentration of 0.93 μ M Glu and 23 nM GABA was measured (Fig. 4).

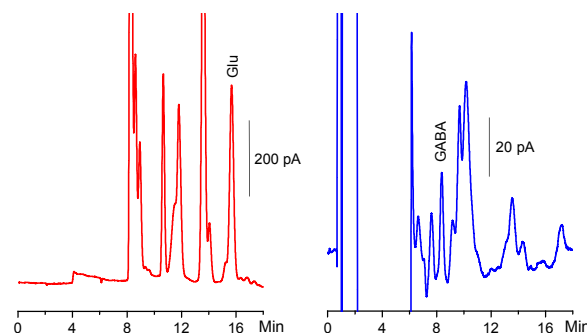


Figure 4. Analysis of dialysate from Basal Lateral Amygdala.

Conclusion

The ALEXYS GABA/Glu Analyzer is a dedicated LC-EC system for the trace analysis of GABA and Glu in microdialysates. The system combines good chromatographic performance with ease of use. The method includes a fully automated derivatisation and column switching step and shows good linearity, reproducibility and detection limits.