Automated analysis of **γ-aminobutyric acid (GABA)** and **glutamate** in microdialysate samples using pre-column OPA-sulfite reagent, UHPLC separation and electrochemical detection

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Introduction

GABA-Glu analysis in microdialysates

A method for the analysis of γ-aminobutyric acid (GABA) and glutamate (Glu) in freely moving test animals using microdialysis is presented. The quantification of these neurotransmitter amino acids in microdialysate samples is challenging due to the following conditions:

- Small sample volumes (typically 5- 20 µL)
- Low GABA concentration levels (nanomolar range)
- High concentration levels of Glu and other amino acids (micromolar range)

The analytical method of choice that can handle all these requirements is microbore ultra-high performance liquid chromatography with electrochemical detection (UHPLC-ECD) in combination with automated pre-column OPA-sulfite derivatization and a post-run column flush. The detection limit of about 5 nmol/L for GABA, obtained with this method, is low enough to quantify the low basal levels of GABA present in microdialysis samples as well as accommodate the measurement of a decrease in those levels. This is shown with a couple of example chromatograms of rat dorsal hippocampus samples from a study that evaluated a new model for the study of prenatal insults [1].

A new model for the study of prenatal insults

Prenatal insults such as stress or infection are known to increase susceptibility to psychotic disorders like schizophrenia and bipolar disorder in adult life. One of the pathways that is involved is the kynurenine pathway, through the increase of kynurenic acid (KYNA) levels. KYNA is considered a neuromodulator that decreases the extracellular levels of the neurotransmitters Glu and GABA in the brain. Hypofunction of glutamatergic signaling is causally linked to neurodevelopmental disorders. By feeding pregnant Wistar dams with chow laced with kynurenine, fetal brain KYNA elevation is stimulated, and GABA and Glu levels are decreased in adulthood. In this poster we highlight some example chromatograms from this study.

Materials & methods

Method for analysis of GABA-Glu in microdialysates

The fast and sensitive method for GABA and Glu analysis is based on the use of the versatile ALEXYS Neurotransmitter Analyzer (Fig. 1), which is a UHPLC-ECD system equipped with the DECADE Elite detector and SenCell. The highlights of the method (Table 1) are listed below:

- Automated odorless in-needle OPA-sulfite derivatization (Fig. 2).
- Small sample use per analysis: 9 µL (injection volume 5 µL)
- Fast and efficient separation using sub-2 µm particle column
- A short total runtime of 20 min by applying a fourfold increased flow rate.
- Post separation step-gradient to eliminate late eluting components (Fig. 2)
- Detection limit of about 5 nM GABA (5 µL injection based on 9 µL sample use)
- Reproducible (RSD area<2%) and linear (r>0.998) responses in the relevant concentration ranges

Table 1 - Conditions for GABA-Glu analysis

HPLC system	ALEXYS Neurotransmitter Analyzer (Antec Scientific)
Temperature	40 °C (separation and detection)
Reversed phase UHPLC separation	
Column	Acquity UPLC HSS T3, 1x50 mm, 1.8 µm (Waters™)
Pre-column filter	Acquity in-line filter kit (Waters™)
Mobile phase A (separation)	50 mM phosphoric acid, 50 mM citric acid, 0.1 mM EDTA.Na_2 set to pH 3.25 with 50% NaOH solution, 5% acetonitrile
Mobile phase B (post-run flush)	Same as mobile phase A, but with 60% acetonitrile
Flow rate	200 µL/mL
Mobile phase gradient	0-10 and 12-20 min: 100% A 10-12 min: 5% A, 95% B
Backpressure	About 400 bar
$V_{injection}$	5 µL full loop fill, as part of the automated sample pre-column derivatization
Electrochemical detection	
Detector	DECADE Elite (Antec Scientific)
Flow cell	SenCell™ with 2 mm GC working electrode and Ag/AgCl reference electrode, AST 1
Potential (E_{cell})	850 mV vs. Ag/AgCl
Range	50 nA for Glu detection; 1 nA for GABA detection
ADF™	0.5 Hz

Fig 1.ALEXYS Neurotransmitter Analyzer.

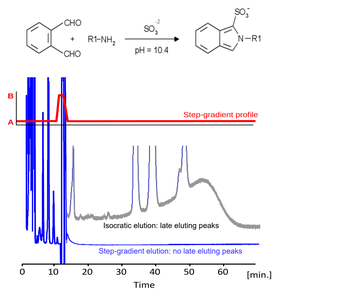

Fig. 2 *Top:* derivatization reaction with OPA/sulphite (detectable with ECD). *Bottom:* step gradient profile and clean-up of late eluting peaks.

EKyn model

A schematic representation of the experimental EKyn model is shown in figure 3.

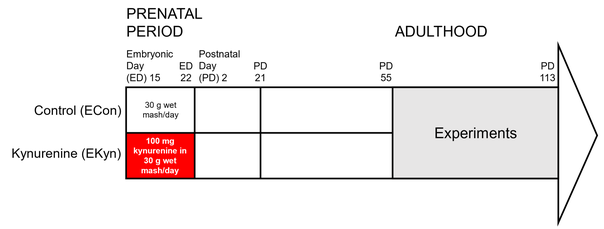

Fig. 3 Pregnant Wistar dams were daily fed 30 g of rodent chow (ECon) or 30 g of rodent chow laced with 100 mg kynurenine (EKyn) from embryonic day (ED) 15 to ED 22. Upon birth, dams received normal rodent chow ad libitum. Offspring were weaned at postnatal day (PD) 21, pair-housed by sex and used in experiments when they reached adulthood at PD 56.

Animals:

Adult Wistar rats were obtained from Charles River Laboratories. Offspring used in experiments were housed in a facility fully accredited by the American Association for the Accreditation of Laboratory Animal Care. Animals were kept on a 12/12 h light/dark cycle. All protocols were approved by the Institutional Animal care and Use Committee and were in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals.*

Microdialysis:

- CNS probe (SciPro Scientific, 2 mm PES membrane/14 mm shaft, 6 kd) in guide cannula (SciPro Scientific, 14 mm shaft, 1.0 mm OD) over the dorsal hippocampus (AP: -3.4, LM: +1.5, DV: -1.5).
- Perfusion: Ringer solution (147 mM NaCl, 4 mM KCl, 1.4 mM $CaCl_2$) at a flow rate of 1.0 µL/min.
- Sample collection: continuous hour long fractions, stored under -80°C until analysis.

Results

Decreases in extracellular glutamate levels were found in the dorsal hippocampus of EKyn male and female offspring, while decreased GABA levels were present only in males during the dark phase. The example chromatograms from the microdialysate samples clearly show the signals of GABA and Glu for the basal levels in a rat from the control group (ECon) as well as the treated group (EKyn).

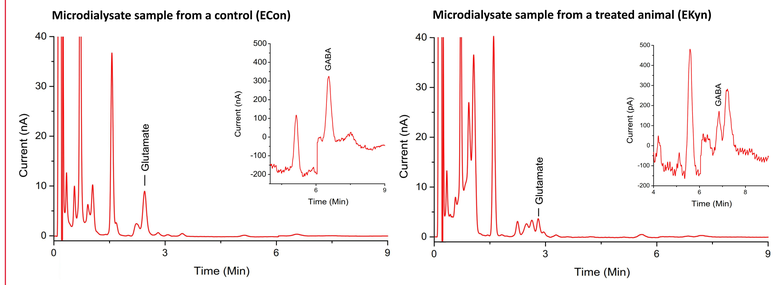

Fig. 4 Chromatograms from the analysis of GABA and Glu in basal level male rat dorsal hippocampus (HC) microdialysate samples, collected in the dark phase. Left: sample from the control group (ECon). Right: sample from the treated group (EKyn) Inserts: zoom-in on GABA peak. Analytical conditions according to Table 1.

Table 2 - Concentration of Glutamate & GABA in rat dorsal hippocampus collected in the dark phase (figure 4)

Sample	*Brain region*	*Concentration Glutamate (µmol/L)*	*Concentration GABA (nmol/L)*
ECon	HC	1.9	102
EKyn	HC	0.9	30

Conclusions

A fast and sensitive UHPLC-ECD method is presented for the analysis of the amino acid neurotransmitters GABA and Glu in microdialysates using the ALEXYS Neurotransmitter Analyzer. The system combines good chromatographic performance with ease of use. The method combines an improved fully automated derivatization and a post-separation step-gradient for elimination of late eluting components to increase sample throughput.

References

1. C. J. Wright, K. M. Rentschler, N. T. J. Wagner, A. M. Lewis, S. Beggiato, A. Pocivavsek, *Frontiers in Psychiatry,* **12** (2021), 1-12

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