On-line Monitoring of Monoamines and Metabolites in Microdialysates with Improved Time Resolution

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INTRODUCTION

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 subsequently analyzed online or offline. HPLC in combination with electrochemical detection is the method of choice to separate and quantify brain neurotransmitters and metabolites in these samples. The indolamines, catecholamines, and metabolites are electrochemically active and are detectable with high sensitivity without the need for derivatization. In the offline approach the collected samples are fractionated and analyzed with HPLC-ECD using an auto sampler. In the online approach the microdialysis stream is collected in a sampling valve and directly analyzed in a HPLC-ECD system. The online method has some distinct advantages:

- **Zero sample loss**
- **No sample degradation**
- **Direct feedback**

The Antec ALEXYS® Analyzers are specifically designed to measure low neurotransmitter levels in very small sample volumes. Typical detection limits for NA, DA and 5-HT are better than 100 pmol/L (< 0.5 fmol).
In this poster we present two ALEXYS®OMD Analyzers for online measurement of *in vivo* microdialysates which cover different needs:

- **ALEXYS® OMD Analyzer, Monoamines & Metabolites**: optimized for in vivo experiments were the quantitation of both monoamines and their acid metabolites is required with high selectivity and detection sensitivity.
- **ALEXYS® OMD Analyzer, Time Resolution**: optimized for in vivo experiments were a better temporal resolution is required to measure fast neurological responses.

ALEXYS® OMD ANALYZER, MONOAMINES & METABOLITES

Features:

- Measurement of all monoamines & metabolites
- 14-port valve, 2 loops
- Optimized analysis on a parallel dual channel HPLC-ECD system

Measuring the response of as many as possible neurotransmitters & metabolites within 10 - 15 min is challenging. The selectivity of the chromatography is limited and therefor two optimized LC channels are used. A sample splitter and a 14-port valve with two loops are used to collect the microdialysis samples on line and inject them in 12 min intervals. After injection, the next sample is collected without any loss of sample/data.

Figure 1. Left side: ALEXYS® OMD Analyzer for on-line in vivo microdialysis. Right side: Dual loop sampling valve for parallel analysis of Monoamines and Metabolites. During HPLC analysis the subsequent sample is loaded in the loop (no loss of sample/data).

ON-LINE MICRODIALYSIS

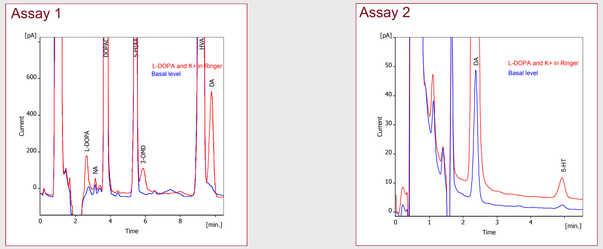

*Figure 2. Chromatograms of rat brain microdialysis recorded with the ALEXYS OMD Monoamines analyzer. The **blue** traces show the basal levels and the **red** traces were recorded after stimulation of the brain with L-DOPA and K+ in the microdialysis solution.*

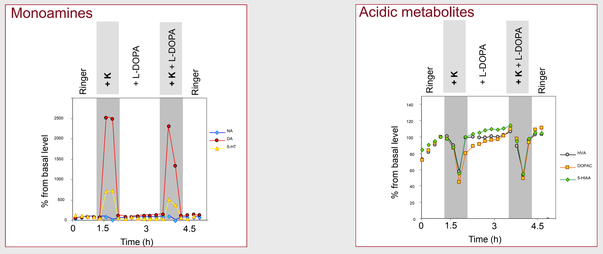

Figure 3. Response plots obtained from in-vivo microdialysis of rat brain and response to different additives in the microdialysis Ringer solution. Data obtained with the ALEXYS OMD analyzer, monoamines & Metabolites.

ALEXYS® OMD ANALYZER, TIME RESOLUTION

Features:

- Measurement of 2 monoamines (DA/5-HT or NA/DA) every 3 minutes
- 14-port valve, 3 loops
- 3 identical parallel LC channels

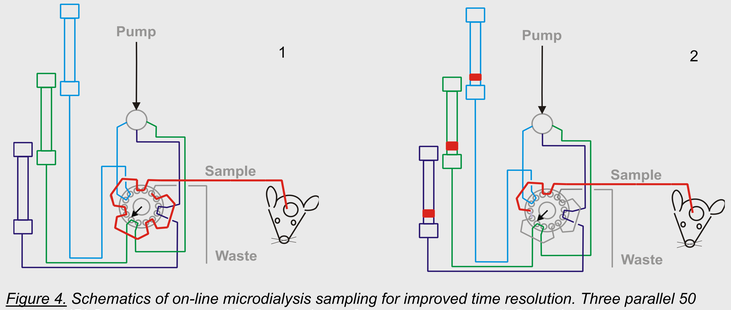

Figure 4. Schematics of on-line microdialysis sampling for improved time resolution. Three parallel 50 x 1 mm HPLC columns are used for fast analysis of neurotransmitters. (1) Collection of sample in three different sample loops connected in series. (2) Simultaneous injection of sample from the sample loops into the three parallel LC channels.

Conventional sampling interval of 10 to 20 minutes (1 µL/min dialysis flow rate) are not sufficient to accurately describe fast neurochemical responses. To improve time resolution, a 14-port valve with 3 loops connected in series is used. The content of the loops is simultaneously injected on 3 parallel LC channels and during the analysis time the next three samples are collected in the three loops. With this particular set-up a time resolution of 3 minutes was achieved as demonstrated in the figures below.

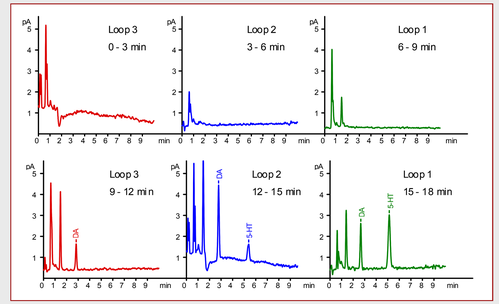

Figure 5. Chromatograms of two subsequent analyses with three columns in parallel. The first traces (top left) show the start, without peaks. After 9 minutes the first peaks

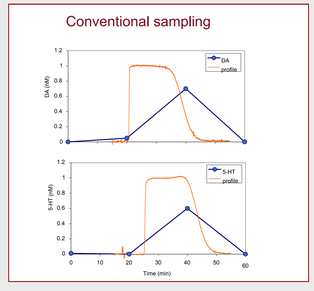

Figure 6. Conventional sampling, resulting in data collection once every 20 min.

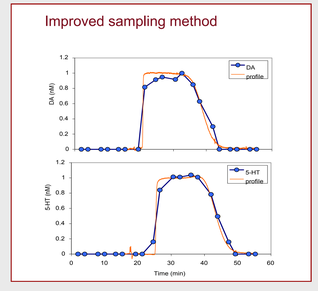

Figure 7. New sampling method with 3 min time resolution for accurate description of neurochemical response.

CONCLUSIONS

The advantage of on-line analyses of neurotransmitters is:

- **Improved time resolution & sample throughput,** by using parallel assays (≤ 3 minutes sampling rate)
- **Efficient system solution**, all monoamines and large scope of metabolites are detected
- **Rodents saving**, as much information from one rat as possible

The ALEXYS® OMD Analyzers are dedicated and optimized solutions for online analysis of in vivo microdialysis samples!

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