Recent Developments in Neurotransmitter Analysis

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Antec® Proven Performance!

Objective

A number of dedicated solutions for the analysis of neurotransmitters in micro-dialysates are presented, with a special focus on analyzing small volume samples without making compromises on sensitivity. Additionally, to collect as much as possible information from the small sample to save rodents.

Introduction

For many years, HPLC with electrochemical detection has been the method of choice for neurotransmitter analysis. In brain research the use of microdialysis has become an invaluable tool to collect neurotransmitters. In these types of samples, the neurotransmitter levels are often below the nanomolar and sometimes even below picomolar concentration range. Available sample volumes are usually small (1-5 µL) due to the low microdialysis flow rate (1 µL/min) and the time resolution required. Therefore, these types of samples put a high demand on the analytical method and hardware. We show different system solution for specific combinations of demands. Typical detection limits of 20-100 pmol/L are obtained using 5ul injection volume (0.1 - 0.5 fmol on column).

Instrumentation and Features

The different ALEXYS® neurotransmitter analyzers we have developed are all dedicated to meet the challenges that microdialysate samples pose (small sample volume and low concentrations of neurotransmitters). However, these different analyzers are further specialized to meet specific requirements. All systems are based on the Antec DECADE II™ detector with either Dual or Triple Cell Control (DCC or TCC) for signal detection.

ALEXYS Monoamines Analyzer

- All monoamines & metabolites
- One injection, two chromatograms, fully flexible
- Small sample volumes < 5 µL

ALEXYS OMD Monoamines Analyzer

- All monoamines & metabolites
- 14-port valve, 2 loops, no sample loss
- Immediate results

ALEXYS OMD Time Resolution Analyzer

- 1 rat, 14-port valve, 3 loops
- Increased sampling frequency
- 3 µL loops = 3 minutes samples

Separation and Detection

For separation of the components of interest, a suitable combination of column (type Antec ALF C18 column) and mobile phase composition was used. A suitable working potential was set over the amperometric flow cell (µ-VT03 wall-jet cell).

ALEXYS Monoamines analyzer

Analysis of fractionated and stored samples

By means of a dual loop 10-port valve a single injection is loaded on two LC-flow paths simultaneously. This concept was applied to the analysis of neurotransmitters and their metabolites to:

- Resolve NA from the front peak using ion-pair chromatography in flow path 1.
- Improve sensitivity for 5-HT and DA by shortening the analysis time in flow path 2.
- Apply optimized ECD settings to each flow path to obtain the best sensitivity.
- Minimise sample loss (filling two sample loops during one injection event).
- Increase sample throughput.

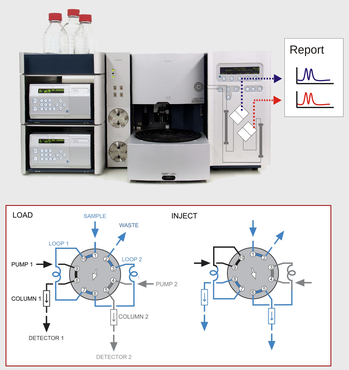

Figure 1: Top: ALEXYS Monoamines Analyzer. Bottom: Schematic configuration of sampling 2 parallel systems with a 10-port valve. Left side: load position, right side: inject position.

Chromatograms of mouse prefrontal cortex microdialysate

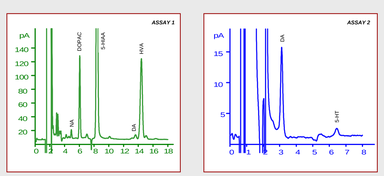

Figure 2: Analysis of mouse prefrontal cortex microdialysate. With courtesy of Mrs. Gerdien Korte-Bouws, Psychopharmacology, University of Utrecht, The Netherlands. Assay 1 was optimised for analysis of NA and metabolites (acidic mobile phase). Assay 2 was optimised for best sensitivity of DA and 5-HT, and short run time. Both chromatograms from the same sample, analysed simultaneously!

Chromatograms of rat prefrontal cortex microdialysate

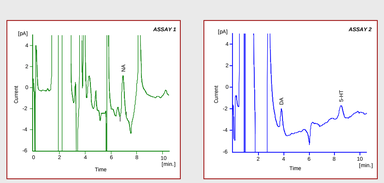

Figure 3: Analysis of rat prefrontal cortex microdialysate. Assay 1 was optimised for analysis of NA (detector settings selective for NA, and mobile phase with pH 6.0 - thus no metabolites in the chromatogram). Assay 2 was optimised for best sensitivity of DA and 5-HT, and short run time. Both chromatograms from the same sample, analysed simultaneously!

ALEXYS OMD Monoamines analyzer

On-line analysis of microdialysate

By means of a sample stream splitter, the microdialysate flow is online connected to a 14-port valve where the split streams are collected in two sample loops. At injection, the two identical samples are loaded on two independent LC-flow paths simultaneously that run different assays. The analysis time is used to load the next sample in the sample loops. This concept was applied to the analysis of neurotransmitters and their metabolites in an on-line version to:

- Obtain data about as many as possible components after a stimulus.
- Resolve NA and L-DOPA from the front peak and measurement of the acidic metabolites.
- Increase sensitivity for 5-HT and DA by shortening the analysis time.
- Apply optimized ECD settings to each individual LC-flow path.
- Obtain direct feedback about the processes in the brain and the course of the experiment.

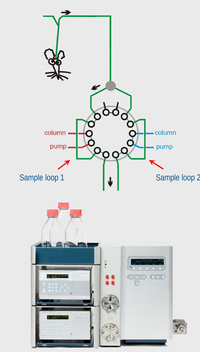

Figure 5: On-line microdialysis sampling for obtaining as much as possible data from each injection. Analysis and loading of subsequent sample occurs simultaneously.

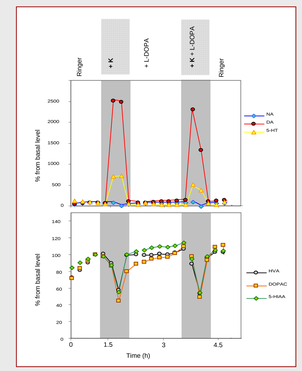

Figure 6: Response plots obtained from microdialysis of rat brain and response to different additives in the microdialysate Ringer solution. Data obtained with ALEXYS OMD Monoamines analyzer.

Chromatograms of standards

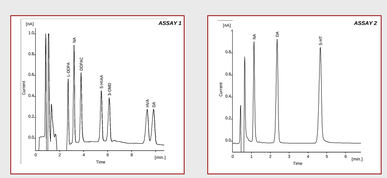

Figure 7: Analysis of standard mix of neurotransmitters and acidic metabolites (100 nM) using the ALEXYS OMD Monoamines analyzer.
One sample is simultaneously analysed with 2 different assays for optimal sensitivity.

Chromatograms of on-line microdialysate experiment with rat brain

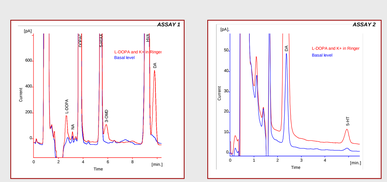

Figure 8: Chromatograms of rat brain microdialysate recorded with the ALEXYS OMD Monoamines analyzer. The blue traces show the basal levels and the red traces were recorded after stimulation of the brain with L-DOPA and K+ in the microdialysate solution.

ALEXYS Time Resolution analyzer

On-line analysis of microdialysate

Conventional sampling of once every 20 minutes is not enough to accurately describe fast neurochemical responses. To improve time resolution, a 14-port valve with 3 loops connected in series is used to directly collect the microdialysate. The content of these loops is then simultaneously injected on 3 HPLC flow paths that run the same analysis, and the analysis time is used to collect the following three samples. A DECADE II™ Triple Cell Control (TCC) detector acquires all signals. In this way, the time resolution can be improved to 3 minutes.

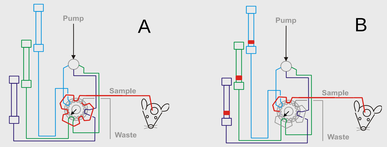

Figure 9A : On-line microdialysis sampling for improved time resolution. Collection of sample in three sample loops. Figure 9B: Simultaneous injection of sample in three parallel 50 x 1 mm ID LC columns for fast analysis of neurotransmitters.

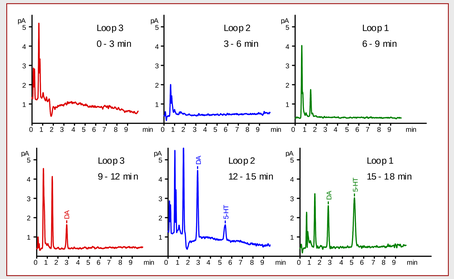

Fig. 10. Chromatograms of two subsequent analyses with three columns in parallel. The first traces (top left) show the start, without peaks. After 9 minutes the first peaks appear.

Conventional sampling

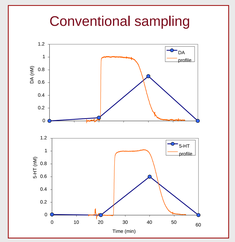

Fig. 11. Conventional sampling, resulting in data once every 20 min.

Improved sampling method

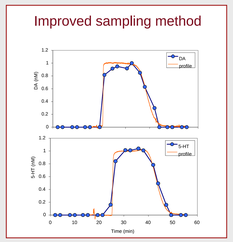

Fig. 12. New sampling method with 3 min time resolution for more accurate description of neurochemical response.

Conclusions

ALEXYS monoamine analyzer using a multi-channel approach is a proven concept for neurotransmitter analysis.

Unique features of the ALEXYS® Analyzers are:

- **Fast analysis times,** without compromising chromatographic resolution.
- **Superior detection sensitivity**, optimized conditions for each channel separately.
- **Optimized for small samples,** using customized injection programming.
- **Improved time resolution,** by using a parallel assays and on-line microdialysis
- **Efficient system solution**, rodents saving!