Antec Scientific

Application Note

Neuroscience

ALEXYS Analyzer for Highest Sensitivity in Neurotransmitter Analysis

Monoamines and Metabolites
Noradrenaline
Dopamine
Serotonin
5-hydroxyindole acetic acid (5-HIAA)
3,4-dihydroxyphenylacetic acid (DOPAC)
homovanillic acid (HVA)

OPA derivatized amines and amino acids
GABA and Glutamate
Histamine (LNAAs)
4-aminobutyrate (GABA)
Glutamate (Glu)
LNAAs

Choline and Acetylcholine
Choline (Ch)
Acetylcholine (ACh)

Markers for oxidative stress
3-nitro-L-Tyrosine
8-OH-DPAT

Glutathione and other thiols

Neurotransmitter analysis and On-line Microdialysis (OMD)

- **Immediate feedback on the microdialysis experiment**
- **High time resolution**
- **High throughput, up to 3 simultaneous experiments**
- **Multi component analysis in parallel**

Summary

The ALEXYS Analyzer OMD is a modular system for HPLC with electrochemical detection of neurotransmitters. This application note shows the applicability of the system to measure various biogenic amines in on-line microdialysis (OMD) which is a continuously flowing microdialysis sampling setup. Instead of collecting samples and putting them in an autosampler, the sample is collected in a sample loop and injected into the system for immediate analysis. During analysis the next sample is already being collected in the loop and the process repeats continuously. Different set-ups are highlighted for improved time resolution, high throughput, and a parallel setup for multicomponent analysis (biogenic amines and metabolites).

Introduction

Microdialysis is a well-established technique for selectively sampling specific components from living tissue. In-vivo microdialysis is applied to analyze neurotransmitters in specific brain regions [1-4]. The principle is based on a semi-permeable probe being inserted in tissue, and continuously perfused with artificial cerebrospinal fluid (aCSF). Small molecules that pass through the dialysis membrane enter the perfusion fluid which is collected for further analysis (Fig. 1). The monoamine neurotransmitters are present in low concentrations, and they are analyzed with HPLC/ECD using the ALEXYS Analyzer for OMD sampling (Fig. 2).

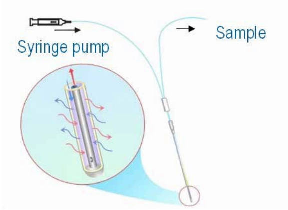


Figure 1: Principle of microdialysis. Perfusion fluid is pumped through a hollow fiber, small molecules pass through the semi-permeable membrane.

Figure 2: ALEXYS Analyzer for on-line microdialysis sampling equipped with a valve (Time Resolution and single channel version)

Several configurations are available

- *On-line microdialysis* - for repeatedly analyzing the microdialysis perfusion liquid. During HPLC analysis, the valve switches back to Load position and the succeeding sample is being collected. Time resolution is the time between subsequent injections and is usually determined by the HPLC analysis time. Data are readily generated, which allows direct evaluation of the course of the experiment.

- *Time resolution* - To monitor a relatively fast change in neurotransmitter levels, the number of data points has to be sufficient to cover the investigated pattern (Fig. 3). Instead of one injection loop, 3 injection loops are filled in series and simultaneously injected and analyzed in 3 parallel HPLC channels. This improves time resolution with a factor 3. To be able to use smaller sample fractions it is even more important to apply a technique with sufficient sensitivity.

- *Two parallel applications* - The option of the ALEXYS system to run two independent analyses in parallel makes it possible to apply for example the analysis of metabolites and the analysis of monoamines to the sample stream. This increases the amount of information that is obtained from each individual experiment.

- *High throughput* - A single ALEXYS system has the option to run up to 3 identical analyses in parallel, while keeping the same small footprint of a single system. Up to 3 independent in-vivo experiments can be performed at the same time, thus making efficient use of bench space and resources.

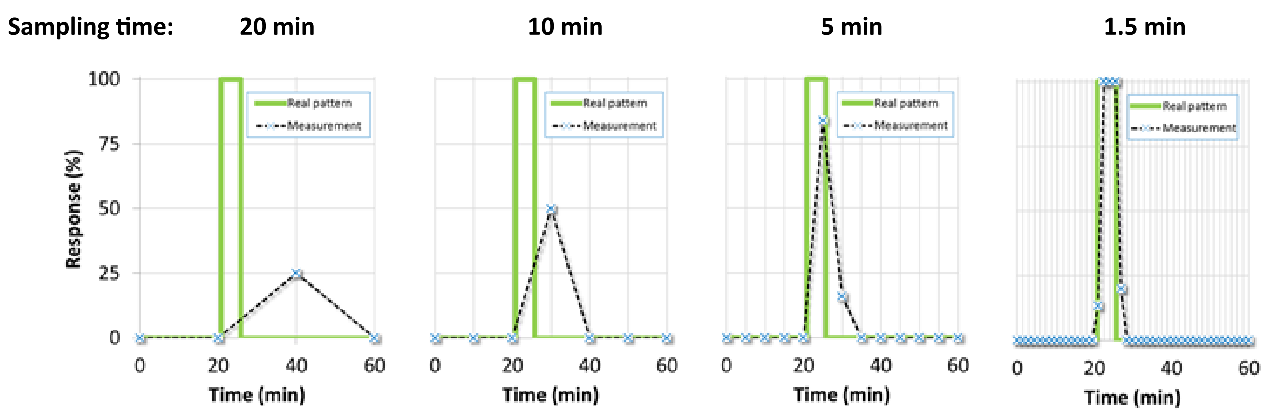


Figure 3: Increasing time resolution more accurately describes a fast in vivo response (green line).

This note highlights each configuration with data obtained with the ALEXYS Analyzer for OMD (Fig. 2). Details on HPLC/ECD settings are in Antec Scientific application notes [4]. The ALEXYS Analyzer for OMD can also be equipped with an autosampler for off-line analysis of monoamines, metabolites, GABA and glutamate, or acetylcholine.

Materials and method

ALEXYS Analyzer for on-line LC/EC analysis of monoamines

The ALEXYS Analyzer for OMD consists of the P6.1L pump with integrated degasser, the DECADE Elite EC detector, and Clarity data acquisition software. For on-line coupling to a microdialysis experiment, additional hardware parts are an electrical injector and the application of specific columns and SenCell flow cell. The analysis of monoamines and acidic metabolites is described in detail elsewhere [3, 4].

On-line microdialysis hardware

The microdialysis sample stream is connected to a 6 port valve on an electrical injector (Fig. 4). The sample loop has to match the total analysis time and the volume of sample that is generated in that time. As an example, if the total analysis time is 8 min, and the microdialysis flow is set to 2 µL/min, then the sample size that is available every 8 min is 16 µL. In this set-up, a sample stream is fractionated and analyzed with a single LC/EC method. The time resolution is governed by the time it takes to perform the analysis of a sample.

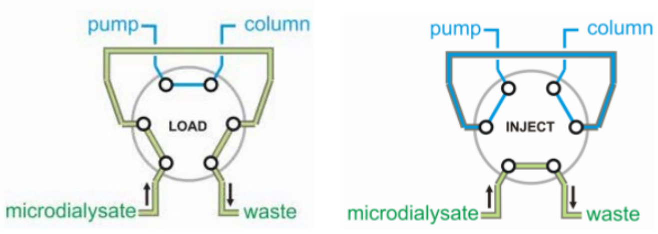


Figure 4: Connections at 2-positions 6-port valve for on-line microdialysis sampling.

Time resolution hardware

To increase time resolution, the microdialysis tubing is connected to an electrical injector with a 14-port valve (Fig. 5). Instead of one sample loop, the valve is mounted with 3 identical custom fit sample loops connected in series. The

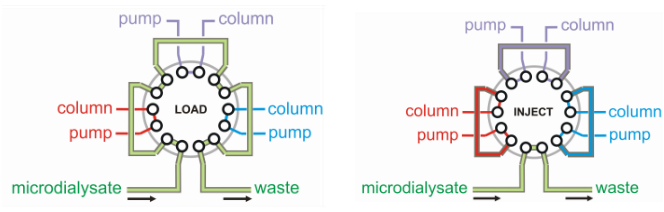


Figure 5: Connections at 2-positions 14-port valve for high time resolution with on-line microdialysis sampling.

sample collected in each of these sample loops gets a unique time stamp in the data plot. During the time of analysis the next sample is being collected. . Given the flow rate and analysis time, a loop volume of 5 µL each is chosen.

At every injection, 3 samples will be simultaneously processed in 3 identical parallel running LC/EC flow paths. For this, a special DECADE Elite with 3 cell controls ('TCC') is used. Three identical analytical columns and flow cells are necessary, but only one pump will suffice when using a flow splitter.

High throughput hardware

In case several identical in-vivo experiments are done with the same LC/EC analysis, the ALEXYS system can be equipped with multiple parallel channels to handle up to 4 on-line setups, while maintaining a small footprint. For this set-up, each microdialysis path is connected to an electrical injector with a

6 port valve with one sampling loop (Fig. 4). Each sample loop is connected to an LC/EC flow path containing identical columns and flow cells. At every injection, the samples are processed simultaneously. A special DECADE Elite with multi-cell control has to be used: up to 4 flow cells can be handled with the QCC version, but this detector is also available in versions that handle 3 (TCC) or 2 (DCC) flow cells. One pump with a flow splitter is applied.

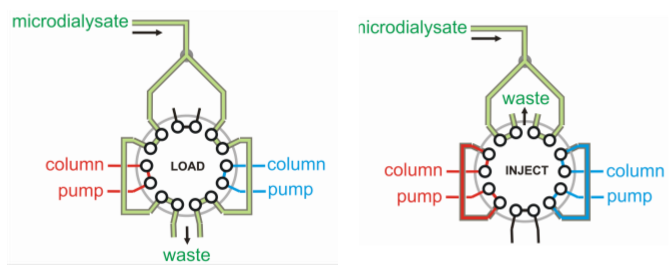


Figure 6: Connections at 2-positions 14-port valve for running 2 independent parallel analyses using on-line microdialysis sampling.

Dual channel applications for the same sample

The microdialysis flow path is split in two equal streams and collected in two sample loops that are connected to an electrical injector with a 14 port valve (Fig. 6). Both samples are analyzed with two different parallel LC/EC channels. For example, the analysis of acidic metabolites can be combined with the sensitive analysis of monoamines, thus increasing the amount of data measured in each sample. For this to work, two independent LC/EC flow paths have to be set up in the ALEXYS system. This means equipping the system with 2 sets of pulse dampers, pumps, columns, flow cells, and a single DECADE Elite with Dual Cell Control (DCC) option. In this set-up, the time resolution is governed by the time it takes to finish both analyses. Also here, the total volume of the two sample loops should match the volume of sample that is collected between every injection.

Calibration

To calibrate a system, a standard with known concentration has to be analyzed. In the on-line microdialysis set-up there are three ways to do this, each with its merits and limitations:

- *Backwards aspiration complete loop filling* - Disconnect the microdialysis tubing from the valve and connect a piece of tubing with a plastic syringe attached to the end (Fig. 7). Instead of connecting/disconnecting regularly and creating wear/tear, a manual micro switch like the CMA 110 Liquid Switch is advised. By pulling the plastic syringe, the liquid will move in reversed direction through the sampling loops. The outlet line must be used to aspirate calibration standards. It has to be taken into consideration that the efficiency of filling the loops is 100% in this way, which may not be the case when running the microdialysis set-up. The efficiency of loop filling during an experiment can be measured by comparing the response of a standard that is inserted in the loops by the backward aspiration method, and the response to the same standard, after inserting it in the glass syringe and inserting it automatically into to the sample loops under the same conditions of microdialysis sampling. For any combination of microdialysis flow rate and time between injections, loop filling efficiency will be a fixed value throughout the experiment. Note that high loop filling efficiency is important to have enough sensitivity.

- *Insertion by syringe pump* - Disconnect the microdialysis probe from the tubing and fill the syringe with the standard solution instead of perfusion fluid (Fig. 8). Set the flow rate at the same value as used for the experiment with probe and repeat 3 or 4 analyses until signals are reproducible. This way of injecting takes into account the efficiency of the loop filling, but it takes more time compared to the backwards aspiration method as it is also necessary to flush and clean the lines and syringe afterwards to prevent carry-over.

- *Probing* - In this set-up the glass syringe is filled with perfusion fluid, a probe is connected to the flow path and its tip immersed in a standard solution (Fig. 9). The measured responses in a stabilized microdialysis set-up give values that take into account the loop filling efficiency as well as the probe efficiency. Actual probe efficiency can be calculated when comparing with the values as obtained from the insertion by syringe pump method (see Fig. 10 for an example).

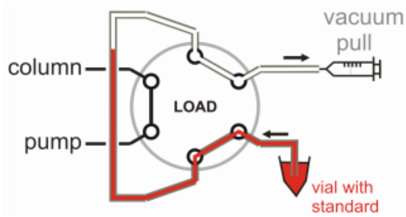


Figure 7: Schematic representation of 'backward aspiration' injection method on a sampling valve.

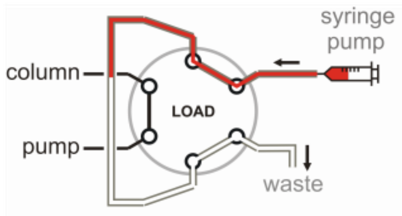


Figure 8: Schematic representation of 'insertion by syringe pump' injection method on a sampling valve

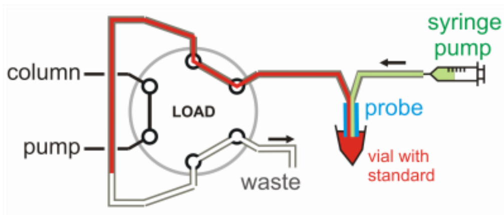


Figure 9: Schematic representation of the 'probing' method in an on-line microdialysis set-up

It may be clear that all these methods result in values that characterize the system. For daily calibration however, the easiest and quickest method to apply is the backwards aspiration method. Loop filling efficiency is a fixed parameter for a specific set of microdialysis flow rate, loop size and analysis time, so this can also be taken into account as a correction factor for quantifications once it is measured.

Results and discussion

On-line microdialysis

Direct coupling of microdialysis with the ALEXYS Analyzer for OMD results in immediate feedback of the microdialysis experiment. Parameters that need to be coordinated for on-line microdialysis, that differ from a standard off-line approach using an autosampler, are the sample loop size and microdialysis flow rate. The total analysis time and microdialysis flow rate define the total available sample volume for each analysis. This volume has to be collected in the sample loop and injected on column. It has to be taken into account that the loadability of a column can be a limiting factor when analysis times are long. To decrease the sample size in such case, the microdialysis flow rate can be adjusted to a lower flow rate. This will increase probe efficiency (dialysis recovery) as a side effect.

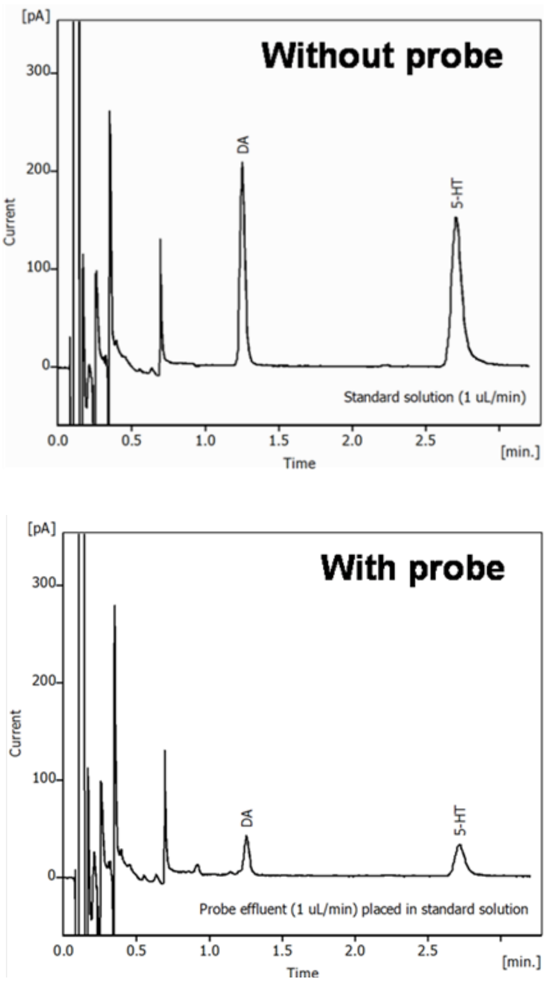


Figure 10: Two example chromatograms used for calculation of probe efficiency. A standard is directly analyzed or sampled through a probe in an online microdialysis coupled ALEXYS system. Probe efficiency was calculated to be 15-20% in this case.

If the total analysis time is for example 8 min for a certain application, the total available sample volume will be 16 µL with the combination of 2 µL/min microdialysis flow rate. For a microbore column, this is a rather large volume. If the microdialysis flow rate is decreased down to for example 0.8 µL/min, then the sample volume is almost 5 µL which is more compatible with microbore HPLC.

For applications running under high pressures that require stainless steel sample loops, the available pre-cut volumes are 1 µL, 1.5 µL, 2 µL, 5 µL and 10 µL. The applications that run under pressures where PEEK tubing can be used, sample loops can be made with a tubing cutter. The minimum length of tubing necessary for a sample loop is 10 cm, which corresponds with 1.2 µL when using tubing with an ID of 125 µm. In all cases it is advisable to calibrate the loop volume: tolerances of the materials affect the effective loop volume.

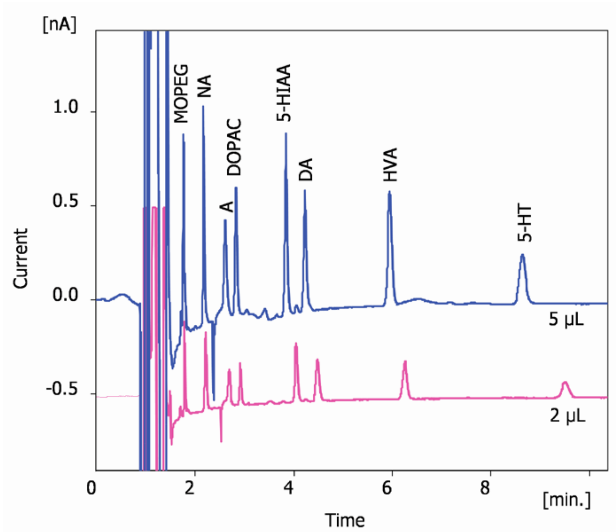
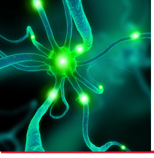


Figure 7: Effect of injection volume on chromatograms of 10 nmol/L standards in Ringers solution with 10 mmol/L acetic acid. Conditions as in Table 1, but with µVT-03 flow cell and ISAAC ref. electrode vs 8 mmol/L KCl (640 mV).



Time resolution

To show the applicability of the high Time Resolution version of the ALEXYS system, an artificial on-line coupled sample stream with fast changes in neurotransmitter levels was generated for testing. Sample loop filling efficiency was measured for every loop, and the system was calibrated with standards. The example data shown in Fig. 11 were generated with a 9 min analysis of DA and 5-HT, sample loops of 3 µL each and a microdialysis flow rate of 1 µL/min. Applying analyses with shorter total analysis times will of course result in even higher time resolutions. In principle, time resolution is the analysis time divided by 3 with this set-up.

Two parallel applications

The example data in Fig. 12 shows results from a real experiment with an in-vivo brain microdialysis experiment on-line coupled to the ALEXYS system. The two analyses that were applied to the sample stream were the analysis of acidic metabolites and the analysis of monoamines.

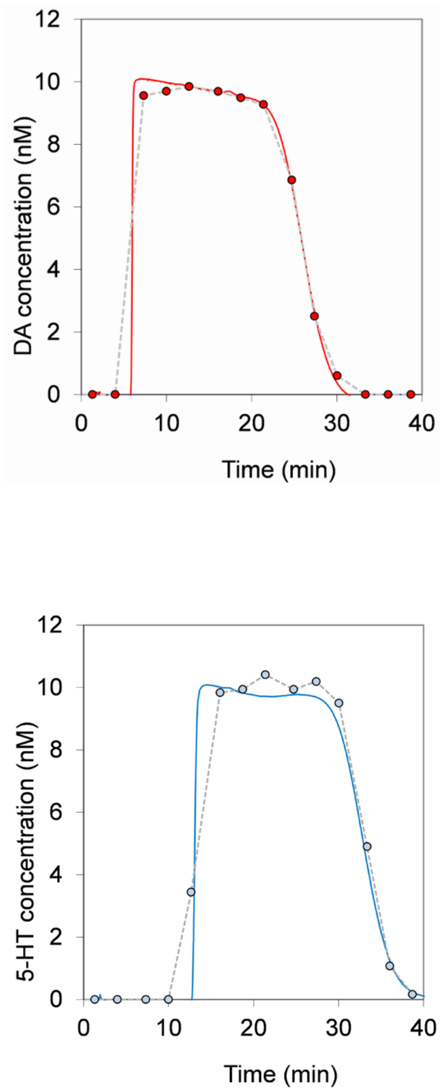


Figure 11: Fast changes in DA and 5-HT levels (red and blue colored profiles) are accurately measured with the ALEXYS Analyzer for OMD with High Time resolution option (dots and dashes).

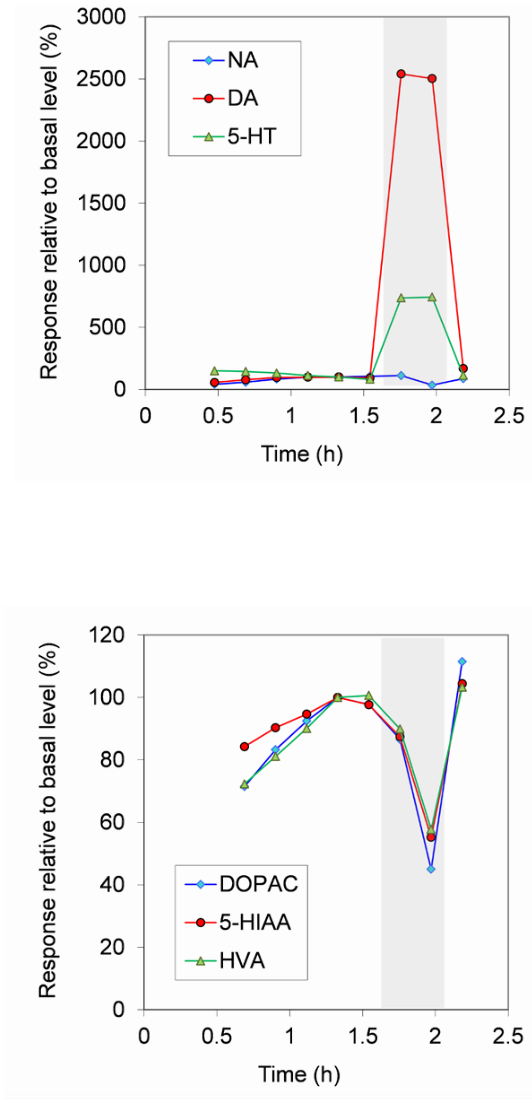


Figure 12: Response plots as obtained from an on-line coupled in-vivo brain microdialysis experiment. A sample split set-up was applied for parallel analyses with the ALEXYS Analyzer for OMD (applications for monoamines and acidic metabolites). The grey area in the graph indicates the time span when the perfusion fluid contained a high potassium concentration to induce a response.

High throughput

The example data for the high throughput version of the ALEXYS system is shown with the analysis of dopamine (Fig. 12). These data were recorded simultaneously from three individual on-line coupled microdialysis experiments using only one ALEXYS system.

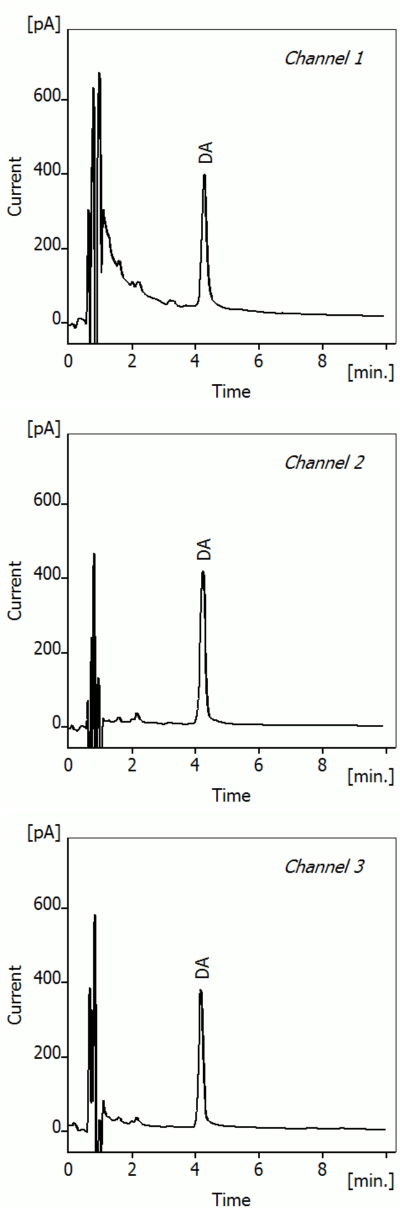


Figure 13: Chromatograms of 10 nmol/L DA in Ringer solution recorded simultaneously for 3 parallel running on-line coupled microdialysis experiments using the ALEXYS Analyzer for OMD with High Throughput option. Sample loops of 5 µL were used for each channel, and perfusion fluid was collected at 1 µL/min.

References

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Conclusion

The on-line coupling of a brain microdialysis experiment to the ALEXYS Analyzer is shown to generate data with different features depending on the set-up version. The single-channel version will give direct feedback of the course of the experiment. The high-throughput version can efficiently process 3 experiments at the same time. The high time resolution version generates data with a 3 x higher time resolution compared to the single-channel set-up. The dual application version can simultaneously apply two different analyses on the same sample stream.

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Additional versions of ALEXYS Analyzer for OMD. Left: High Throughput version, where three valves and one pump with a flow splitter are used for three HPLC channels running in parallel. Right: Parallel Channel version, where 2 pumps are needed for the two independent analyses running in parallel. The Time Resolution and single channel version are depicted in Fig. 2.

Ordering information

ALEXYS OMD Time Resolution - DA/5-HT	
180.0095W	ALEXYS electrical inj. TCC base
180.0512WB	Add-on parts for OMD Time Resolution
116.4120 (3x)	SenCell with 2 mm GC WE and sb REF
250.1136 (3x)	NeuroSep 105, 50 x 1 mm, 3um, C18-3

ALEXYS OMD High Throughput 3 channels - DA/5-HT	
180.0095W	ALEXYS electrical inj. TCC base
180.0510WB	Add-on parts for OMD High Throughput
116.4120 (3x)	SenCell with 2 mm GC WE and sb REF
250.1136 (3x)	NeuroSep 105, 50 x 1 mm, 3um, C18-3

ALEXYS OMD 2 parallel analyses - monoamines and metabolites	
180.0094W	ALEXYS electrical inj. DCC base
180.0516WB	Add-on parts for parallel channel OMD
116.4120 (2x)	SenCell with 2 mm GC WE and sb REF
250.1137 (2x)	NeuroSep 115, 150 x 1 mm, 3um, C18-3

ALEXYS OMD - DA/5-HT	
180.0093W	ALEXYS electrical inj. SCC base
180.0514WB	Add-on parts for single channel OMD
116.4120	SenCell with 2 mm GC WE and sb REF
250.1136	NeuroSep 105, 50 x 1 mm, 3um, C18-3

Ask us for alternatives in case you need to analyze a different set of components.

Antec Scientific (USA)
info@AntecScientific.com
www.AntecScientific.com
T 888 572 0012

Antec Scientific (worldwide)
info@AntecScientific.com
www.AntecScientific.com
T +31 71 5813333

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