New ALEXYS® Neurotransmitter Analyzer using UHPLC

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NEUROTRANSMITTER ANALYSIS IN BRAIN MICRODIALYSATES

Measuring neurotransmitters in brain microdialysates remains an analytical challenge, despite significant improvements over the years.
With the dedicated ALEXYS Neurotransmitter Analyzer we want to accomplish:

- Small sample analysis (1 - 10 uL)
  - the smaller the sample the better the time resolution in microdialysis
- Sensitivity
  - Monoamines down to 0.05 fmol, 33 pmol/L
  - GABA down to 15 fmol, 10 nmol/L
  - Acetylcholine down to 3 fmol, 0.3 nmol/L
- Multi component analysis
  - Get as much information as possible from a sample, thus decreasing the need for test animals
- Speed of analysis
  - Short analysis time using UHPLC where possible

We want it all and we want it fast

To achieve multi-component analysis requires optimization of chromatography. Having an excellent detection sensitivity is useless if peaks are not separated. However, achieving multi-component analysis with 1 hour run times is also unacceptable. Therefore in an optimized systems all 4 requirements must be met.

UHPLC

The development of separation columns with smaller particles (sub-2 micron) has opened the possibility to increase flow rates without significant loss of separation efficiency.

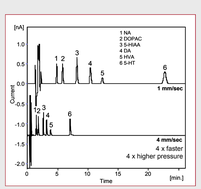
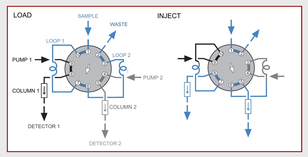


Figure 2. Increasing the flow rate in the analysis decreases total analysis time.

PARALLEL ANALYSIS

When using a special HPLC set-up with 2 instead of 1 assay, more components of interest can be detected (multi-component analysis).

The ALEXYS Neurotransmitter Analyzer is equipped with a 10 port valve that can be set-up with two loops to simultaneously inject a single sample on two different/optimized independent (U)HPLC assays.



SMALL SAMPLE USE

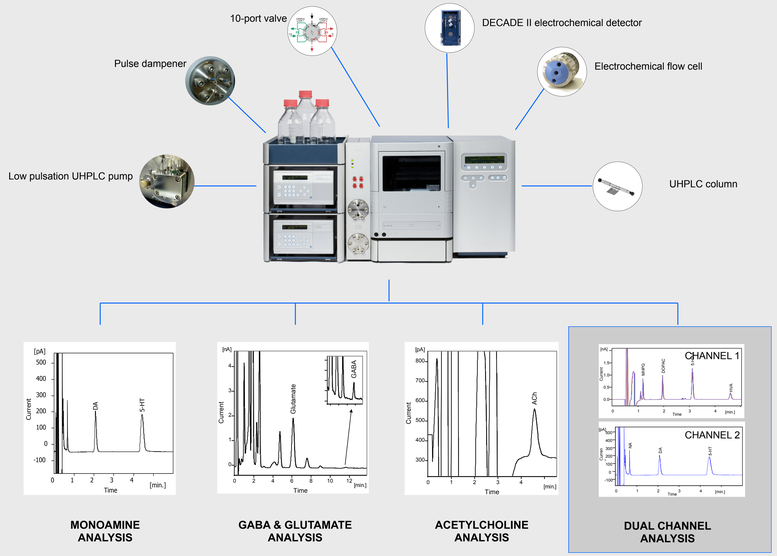
The ALEXYS Neurotransmitter Analyzer is a flexible system with optimized features to handle small samples.

The approaches to handle small samples are:

I Optimized injection programs present in the autosampler for efficient small sample use.

II Online coupled microdialysis experiments
- No autosampler necessary
- Immediate results

ALEXYS NEUROTRANSMITTER ANALYZER



METHOD EVALUATION

The performance ALEXYS Neurotransmitter Analyzer in dual channel analysis mode is shown using the combination of the assays for monoamines and the assay for acidic metabolites:

Conditions

LC	ALEXYS® Neurotransmitter Analyzer	
T_{oven}	38 °C (separation and detection)	
$V_{Injection}$	5 µL per channel (sample use 15 µL only, using specially developed injection program)	
	Channel 1	**Channel 2**
For components	*MHPG, DOPAC, 5-HIAA, HVA*	*NA, DA and 5-HT*
Column	Acquity UHPLC HSS T3 (Waters) 1.0x100 mm, 1.7 um	Acquity UHPLC HSS T3 (Waters) 1.0x100 mm, 1.7 um
Mobile phase	Phosphate buffer (pH 3.0), ACN	Acetic acid buffer (pH 5.5), ion pairing agent, ACN
Flow rate	100 µL/min	100 uL/min
Flow cell	SenCell 2 mm GC, sb, spacing 'I'	SenCell 2 mm GC, sb, spacing 'I'
Backpressure	about 420 bar	about 420 bar

CONCLUSION

The ALEXYS Neurotransmitter Analyzer is dedicated to sensitively measure different neurotransmitters in small samples. The special parallel analysis mode option efficiently results in data about more components of interest as it simultaneously applies two assays on each sample.

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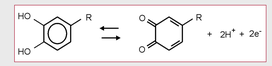
ELECTROCHEMICAL DETECTION

Electrochemical detection (ECD) is a sensitive and selective detection method. The principle is based on detecting the electron transfer when a component of interest is oxidized (or reduced). For the analysis of monoamines, ECD is still the method of choice due to it's sensitivity and selectivity.



MICRODIALYSATE SAMPLE

In this example, the acidic metabolites were measured in parallel with the assay for monoamine levels, which are typically present in far lower concentrations. A single injection thus results in two chromatograms, which are recorded under different and optimized HPLC-ECD settings.

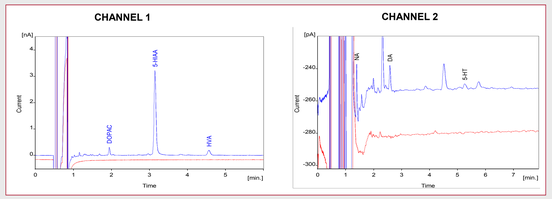


Figure 3. Overlay of chromatograms resulting from dual channel parallel analysis from a single injection of basal level rat Prefrontal Cortex (PFC) microdialysate (blue curve) and blank (red curve). Samples kindly provided by Mrs. Gerdien Korte-Bouws, Department of Psychopharmacology, University of Utrecht, The Netherlands.

Table 1. Calculated levels of monoamines and metabolites from the sample shown in Figure 3

	Concentration (nM)
Acidic metabolites	
DOPAC	2.6
5-HIAA	36.1
HVA	7.8
Monoamines	
NA	0.24
DA	0.21
5-HT	0.12

LINEARITY

The calibration plots of both assays resulted in correlation coefficients >0.998 for all components.

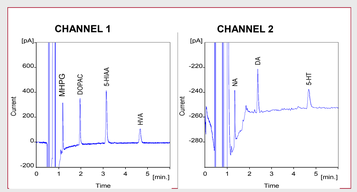


Figure 4. Overlay of blank and standard mix of 5 nM acidic metabolites and 0.5 nM monoamines in Ringer solution with 10 mM acetic acid.

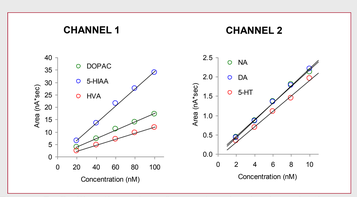


Figure 5. Calibration plots from standard mixes in the range of 20-100 nM acidic metabolites and 2-10 nM monoamines in Ringer solution with 10 mM acetic acid.

REPEATABILITY

The repeatability in both assays is <2% RSD for a mid-range concentration. This is also valid when using 10 uL instead of 15 uL of sample

Table 2. Relative Standard Deviation (RSD) and averaged values obtained from 8 chromatograms of 100 nM acidic metabolites and 10 nM monoamines in Ringer solution with 10 mM acetic acid.

	Tr		Area		Height	
	Avg (min)	RSD (%)	Avg (nA*sec)	RSD (%)	Avg (nA)	RSD (%)
Assay 1						
MHPG	1.2	0.45	9.7	1.2	7.0	0.5
DOPAC	2.0	0.37	17	1.9	7.1	1.9
5-HIAA	3.2	0.28	34	1.2	8.8	1.2
HVA	4.7	0.25	12	0.3	2.2	0.3
Assay 2						
NA	1.3	0.49	2.1	1.3	0.49	0.8
DA	2.4	0.32	2.2	0.3	0.70	0.3
5-HT	4.7	0.22	2.0	0.8	0.36	0.7

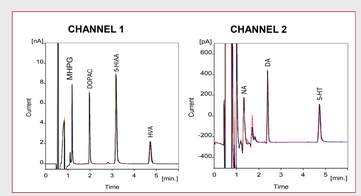


Figure 6. Overlay of 8 chromatograms per assay from standard mixes of 100 nM acidic metabolites and 10 nM monoamines in Ringer with 10 mM acetic acid.