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

Monoamines and Acidic Metabolites

- **ALEXYS Neurotransmitter Analyzer**
- **Analysis of NA, DA, 5-HT, HVA, 5-HIAA, DOPAC**
- **Analysis of tyramine, tyrosine, octopamine, and tryptophan**
- **LOD: 0.1 - 0.5 fmol on-column (below 100 pmol/L in less than 10 µL sample)**

Summary

The ALEXYS Neurotransmitter Analyzer using UHPLC columns and electrochemical detection (ECD) has been applied for the analysis of neurotransmitters in microdialysis samples, cerebrospinal fluid (CSF) and brain tissue homogenates. HPLC and ECD settings are optimized for different target compounds with respect to selectivity and detection sensitivity. The system applies a DECADE Elite ECD with a SenCell, a powerful combination for the best possible detection limits. The AS110 autosampler facilitates micro volume sample handling (few microliters), in a dedicated injection method. Detection limits are in the range of 0.1 - 0.5 fmol on column (below 100 pmol/L in less than 10 µL sample) and repeatability is better than 2% RSD for most components.

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 fractions are collected for further analysis. HPLC in combination with electrochemical detection is often used to analyze neurotransmitters and metabolites [1-3]. The indolamines, monoamines (catecholamines), and metabolites are electrochemically active and are detectable with high sensitivity without the need for derivatization. The amino acid neurotransmitters and choline and acetylcholine can be detected using the same instrumentation (Figure 1).

Method requirements for analysis of neurotransmitters in microdialysis samples are challenging with respect to selectivity, sensitivity, and available sample volume. There is a growing interest for collecting smaller fractions as this results in a better temporal resolution of the microdialysis experiment. Typical flow rates in microdialysis are 1 2 µL/min, decreasing the fraction size to a few microliters would enable a temporal resolution of a few minutes. The concentrations of NA, DA, and 5-HT in microdialysis fractions can be below 100 pmol/L. In combination with a small sample volume of a few microliters, this requires an extremely low limit of detection down to the range of 0.1 – 0.5 fmol on column [1-8].

The concentrations of the metabolites DOPAC, HVA and 5-HIAA are usually considerably higher (about 100 – 1000 times), which places another challenge on the analytical method. The peak resolution should be sufficient to enable quantification of the minor peaks next to the major metabolite peaks. Finally, the analysis should be completed within an acceptable run time. This is challenging because of differences in polarity of the substances of interest.

Over the years, many papers have appeared on improving the speed of separations or analyzing small sample volumes at low detection limits [1-8]. In this application note a scalable, fast UHPLC method for noradrenaline (NA), dopamine (DA) and serotonin (5-HT), and their metabolites homovanillic acid (HVA), 5-hydroxyindole acetic acid (5-HIAA), and 3,4-dihydroxyphenylacetic acid (DOPAC) is presented. In addition, separation and optimized detection settings for insect neurotransmitters tyramine, tyrosine, octopamine, and tryptophan are given as well.

Method

ALEXYS Neurotransmitter Analyzer

The ALEXYS Neurotransmitter Analyzer (Antec, Zoeterwoude, the Netherlands) for analysis of monoamines and metabolites consists of a P6.1L pump with degasser, a DECADE Elite electrochemical detector, Clarity chromatography software of DataApex (Prague, The Czech Republic) and an AS 110 autosampler (other injector options are a manual injector and on-line coupling to a microdialysis experiment). A SenCell flow cell with a 2 mm glassy carbon working electrode and a sub-2µm particle 50 or 100 mm length 1.0 mm ID separation column are bundled in the additional application specific 'ALEXYS Monoamine kit' (see ordering info). Other kits are available as well, such as kits for acetylcholine, GABA, and glutamate [3, 4].

Figure 1: ALEXYS Neurotransmitter Analyzer with AS110 autosampler and DECADE Elite.

Sample preparation

Before injection, sample preparation should be applied to produce a sample that is relatively free of interferences to prevent damage like clogging of the system or column. Another consideration to treat a sample is to prevent degradation of the components of interest if it will not be analyzed immediately after collection. These are the treatment advises for different samples:

- *Microdialysate samples* - These samples are relatively clean and can be injected in the system without the need for filtering or other treatment. However, to prevent degradation of the monoamines, acidification with or without an anti-oxidant is most often applied to the sample [5, 6]

- *Brain homogenate samples* - Preparation of a sample from brain tissue usually consists of homogenization in a dilution

of perchloric acid, followed by a centrifugation step to remove debris [5, 7].

- *Cerebrospinal fluid* - These sample are relatively more complex compared to microdialysis samples, and in literature it can be found that such samples either are not processed before injection, or they are acidified and centrifuged [8], or acid/anti-oxidant mix added before injection [5]. We highly recommend to apply at least a centrifugation or filtration step before injection to remove particles: sub-2 micron columns have a higher risk of clogging compared to the larger particle columns as used in older research.

- *Blood and urine* (*in clinical analysis)* - For the analysis of catecholamines in blood or urine, complete SPE work-up kits are commercially available (e.g. at Chromsystems or BioRad). Such samples, however, have a clinical/diagnostics background, and the details are covered in another Antec application note [9].

Injection

A dedicated and reproducible injection program has been developed for the AS110 autosampler that efficiently handles small samples of only a few microliters. The details are described elsewhere [10], in short the injection program works without 'loop overfill' that is usually applied in full loop injections. It efficiently transports only 2 µL in addition to the injection volume between air bubbles to the loop without 'wasting' any additional sample.

Another mode of injection is the direct coupling of microdialysis to the ALEXYS using an electric valve. In principle, the continuous flow runs through an injection valve and at regular intervals a sample is injected. The analyses described in this application note can be applied to such on-line microdialysis setup. Details about this set-up have been described elsewhere [11].

Separation

In the eighties of last century, a lot of research was done to develop and optimize the analysis of catecholamines, the precursors and metabolites, but this field is still progressing

until today, see for example references [12 - 20]. Monoamines have a positive charge at pH<7, and they can gain retention on

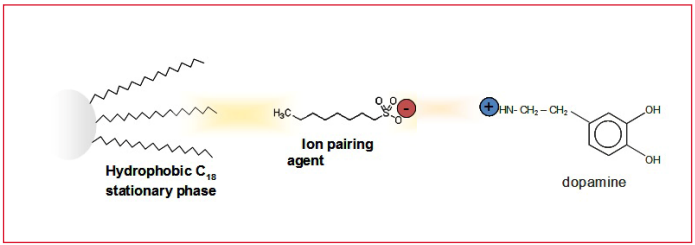


Figure 2: Schematic representation of ion-pairing principle for HPLC separation of monoamines on C18 particles

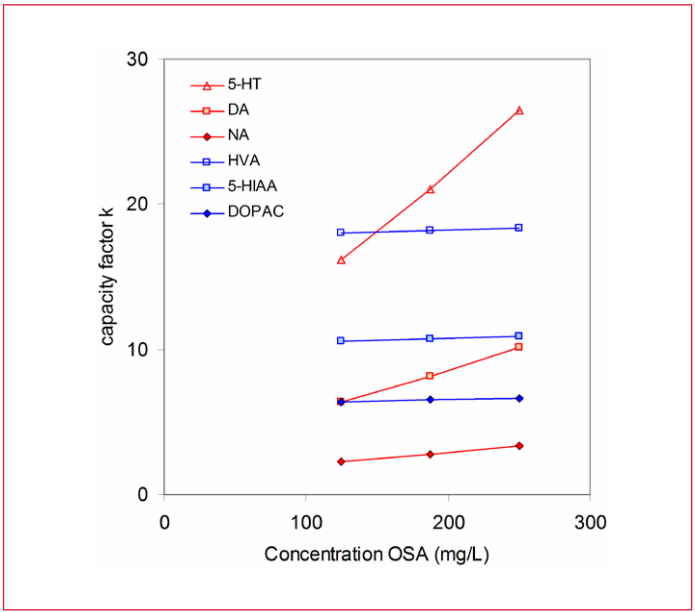


Figure 3: Effect of the ion-pairing agent octane sulfonic acid sodium salt (OSA) on retention behavior of monoamines (red) and acidic metabolites (blue).

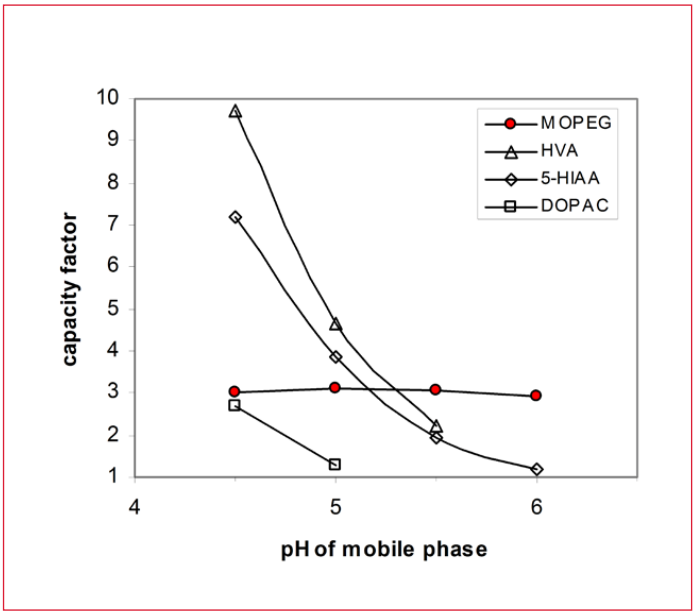


Figure 4: Effect of pH on retention behavior of molecules with a carboxyl group. For reference, the red dots indicate the retention of a molecule without a carboxyl group.

a (neutrally charged) reversed phase column when ion-pairing agent is added to the mobile phase (Fig. 2). The monoamine retention times respond to the concentration of ionpairing agent in the mobile phase (Fig. 3).

The acidic metabolites have a carboxyl group with a pKa value of 4.7. They are best retained on reversed phase columns when applying a mobile phase with acidic pH. When applying a neutral pH, the negative charge of the carboxyl group makes them elute in the solvent front. The pH of the mobile phase therefore strongly affects the separation and retention times of acidic metabolites (Fig. 4.).

Detection

Monoamines and acidic metabolites are electrochemically detectable on a glassy carbon working electrode. A number of excellent papers are available reporting voltammetric behavior of relevant biogenic amines and metabolites [12 - 14]. Nagao and Tanimura [14] classified the biogenic amines in four groups depending on their electrochemical behavior in a mobile phase at pH 3.6 and flow cell with glassy carbon and Ag/AgCl electrodes. The four groups are: catechol compounds such as the catecholamines, DOPAC and DOPA (E½ = 380-500 mV), indoles such as 5-HT and 5-HIAA (E½ = 480-520 mV), vanillic compounds such as VMA, HVA and MHPG (E½ = 640-680 mV) and monohydroxyphenols such as tryptophan and tyrosine (E½ = 870 mV). It should be noted that these given values are affected by pH (shift of about 60 mV for every pH unit), mobile phase composition and differences in glassy carbon working electrode materials. It may be clear that the working potential has to be set as low as possible to ensure selectivity, but high enough to generate a clear response for the specific component(s) of interest. The working potential can also be used as a tool to enhance selectivity of the method:

- If there is only interest in the analysis of DA (and/or NA), but not 5-HT, then the working potential can be set to a lower value compared to the setting suggested in the settings table. In such case, 5-HT (and many other components) will not generate a signal.

- For detection of the monohydroxyphenols, a relatively high potential is necessary.

Electrode activation

It is important to realize that a new or freshly polished electrode can behave differently from an electrode that is in use for a longer time. A flow cell can build up a 'history' which can result in a chromatogram with different relative peak heights compared to a new cell. However, flow cells can often be 'reinitialized' by applying an electrochemical pulse. The HPLC is not changed, the pump is on and the usual mobile phase is applied. The detector is set to PULSE mode for about 10 min with pulse settings E1=+1.0V, E2=-1.0V, t1=1000ms, t2=1000ms, t3=0 and ts=20ms. After 10 minutes the detector is set to DC mode at the detection potential [21]. The background current should drop below 25 nA in less than 30 min. This activation procedure can be programmed in the DECADE Elite detector and Clarity software for automated application. The pulse mode is not available in the SDC or Lite versions of the detector.

Results and discussion

Method optimization for analysis of neurotransmitters was carried out in two steps. Firstly, the HPLC separation was optimized with special attention to injection volume, selectivity and total analysis time. In a second step the working potential and detection settings were optimized for best detection sensitivity. It is not always required to measure all neurotransmitters and metabolites together, therefore several methods are described for different selections of target substances. Small differences in detection potential or mobile phase composition can have a considerable effect on assay validation parameters. All the presented applications show good performance with repeatability of signal (n=6) better than 2% RSD for peak area, and correlation coefficients better than 0.998.

Analysis of monoamines and acidic metabolites and some other related components

For the 'single shot' analysis of the monoamines NA, DA and 5-HT, and the acidic metabolites DOPAC, 5-HIAA and HVA, the settings in Table 1 were used to obtain a chromatogram as given in Figure 5. Ten other components of interest were added to the standard mix as well to demonstrate the separation performance. The column efficiency is better than 200.000 plates/m for most substances, resolution is >1.4 and total elution time is <12 min.

The theoretical maximum loadability of a microbore column with about 200.000 plates/m is in the range of 0.5-3 µL for peaks between 2-12 min assuming no more than 5% contribution by injection dispersion to the total column band broadening [22]. The combination of C18 column material and

a mobile phase with ion pairing agent and a few percent organic solvent seems to extend the loadability of the column: for the peaks between 3-12 min the plate numbers and asymmetry were not affected up to 5 µL injections, while the earlier peaks only showed peak broadening above 2.5 µL injections. To avoid any unwanted peak broadening, the advice for the application settings presented in Table 1 is to inject 2 µL on column. An injection volume of 5 µL, is a bit of a trade-off, it results in a decreased peak efficiency but peaks are higher and thus a better sensitivity (Figure 7). Sensitivity of the analysis of monoamines and acidic metabolites was checked with 5 µL full loop injections and showed a detection limit of 0.2-0.4 fmol on column (40-80 pmol/L for 5 µL injections).

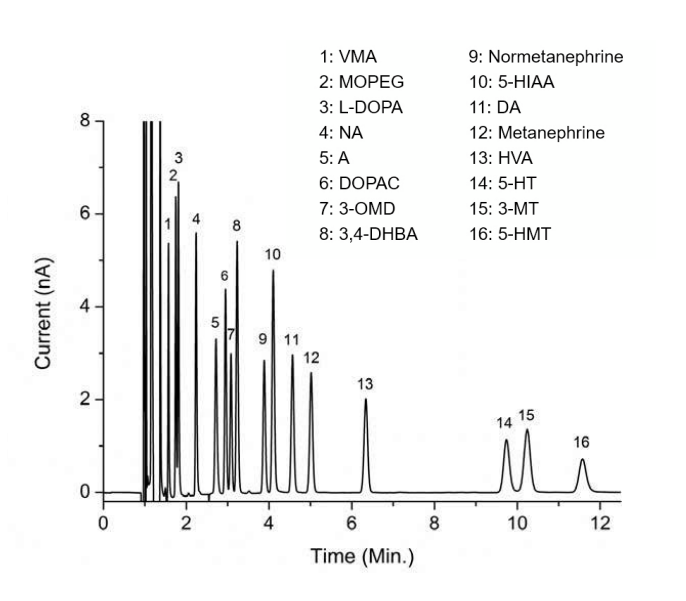


Figure 5: Chromatogram of 100 nmol/L standards in Ringer solution with 10 mmol/L acetic acid. Injection volume 2 µL. Conditions as in Table 1.

Table 1

LC-ECD settings for analysis of all monoamines and their acidic metabolites

UHPLC	ALEXYS Neurotransmitter Analyzer
Column	Acquity UPLC BEH C18, 1.7µm, 1 x 100 mm (Waters)
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase	100 mM phosphoric acid, 100 mM citric acid, 0.1 mM EDTA.Na_2 set to pH 3.0, 600 mg/L octanesulfonic acid sodium salt, 8% acetonitrile Refresh at least every 3 days.
Flow rate	50 µL/mL
Temperature	37 °C for separation and detection
Backpressure	About 250 bar
$V_{injection}$	2 µL (up to 5 µL max)
Injection method	Dedicated user program with minimal sample use
Needle wash	Water (refresh weekly)
Pump piston wash	15% isopropanol in water (refresh weekly)
Flow cell	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 1
Ecell	800 mV vs. salt bridge reference electrode
Range	1 nA/V for near-LOD signals; 50 nA/V for higher signals
ADF	0.5 Hz
I-cell	About 3 nA

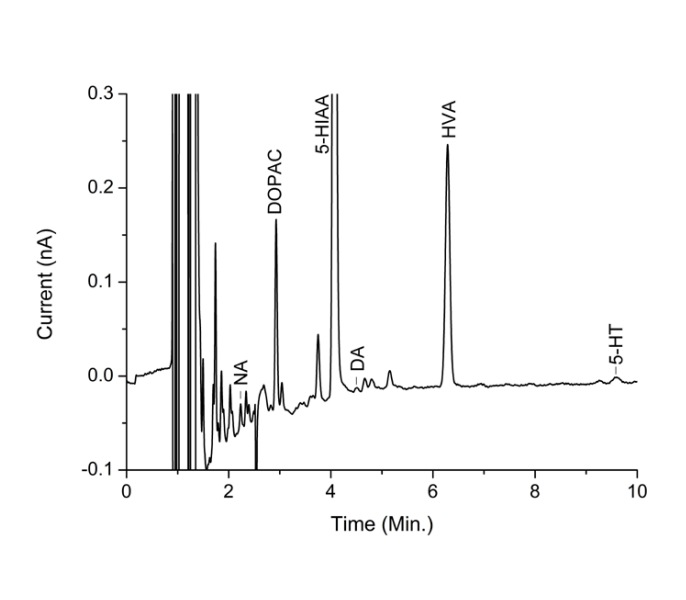


Figure 6: Chromatogram of rat prefrontal Cortex microdialysate (after administration NA re-uptake inhibitor). Sample kindly provided by Gerdien Korte-Bouws, Department of Phychopharmacology, University of Utrecht. Injection volume 2 µL. Conditions as in Table 1, but with µVT-03 flow cell and ISAAC reference electrode vs 8 mmol/L KCl (640 mV).

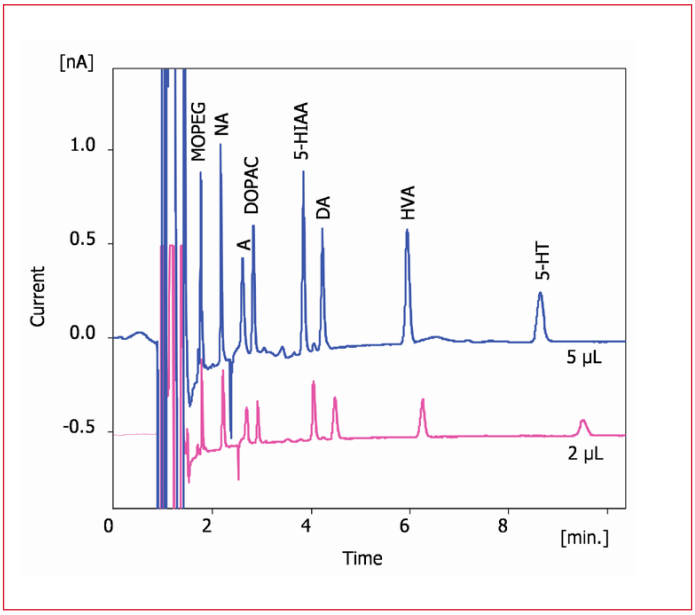


Figure 7: Effect of injection volume on chromatograms of 10 nmol/L standards in Ringers solution with 10 mmol/L acetic acid. Conditions as in Table 1, but with µVT-03 flow cell and ISAAC ref. electrode vs 8 mmol/L KCl (640 mV).

A microdialysate sample was analyzed with the conditions from Table 1 to show the applicability of the method (Figure 6). Concentrations of the monoamines was calculated to be in the range of 0.1-0.9 nmol/L, which is near the detection limit. The selectivity and sensitivity of the method is sufficient to analyze these samples.

For (more) complex samples, or generally spoken in case the selectivity is not sufficient, a so called DCC (dual cell control) setup is advised. In such setup a dual channel HPLC system is used with different HPLC conditions for both channels. One sample is simultaneously injected with a dual loop valve and analyzed under different conditions as described elsewhere [23].

Analysis of acidic metabolites

For the analysis of the acidic metabolites DOPAC, 5-HIAA and HVA, the settings in Table 2 can generate a chromatogram as given in Figure 8. As the mobile phase does not contain ionpairing agent, the monoamines will not appear in the chromatogram as they will elute as part of the solvent front. Sensitivity of the analysis of acidic metabolites was checked with 2 µL full loop injections and showed a detection limit of about 0.2 nmol/L.

Two different microdialysis samples were analyzed to show the applicability of the method (Figure 9). Concentrations of the acidic metabolites were calculated to be in the range of 4-240 nmol/L, which is well above the detection limit of the application. It should be noted that there are brain region specific peaks eluting after the last peak of interest. Analysis time of 1.5 min instead of 1 min may have to be applied to make sure that such peaks do not show up in the following

Table 2

LC-ECD settings for analysis of acidic metabolites HVA, DOPAC and 5-HIAA

UHPLC	ALEXYS Neurotransmitter Analyzer
Column	Acquity UPLC BEH C18, 1.7µm, 1 x 50 mm (Waters)
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase	100 mM phosphoric acid, 100 mM citric acid, 0.1 mM $EDTA.Na_2$ set to pH 3.0, 10% acetonitrile. Refresh at least every 3 days
Flow rate	175 µL/mL
Temperature	37 °C for separation and detection
Backpressure	About 450 bar
$V_{injection}$	1 µL
Injection method	Dedicated user program with minimal sample use
Needle wash	Water (refresh weekly)
Pump piston wash	15% isopropanol in water (refresh weekly)
Flow cell	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 1
Ecell	800 mV vs. salt bridge reference electrode
Range	1 nA/V for near-LOD signals; 50 nA/V for higher signals
ADF	Opt. raw - 0.5 Hz
I-cell	About 3 nA

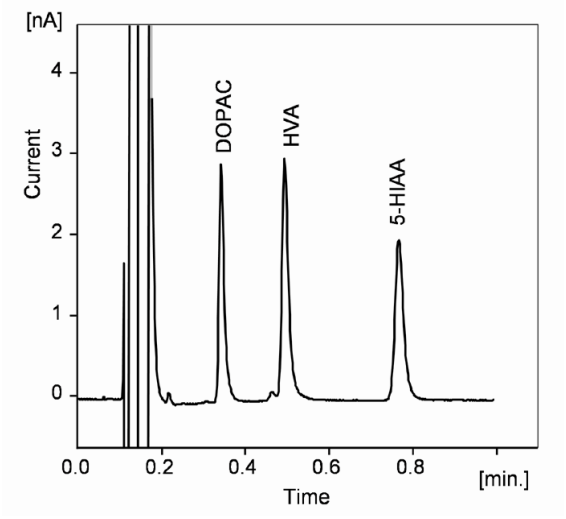


Figure 8: Chromatogram of 100 nmol/L DOPAC, HVA and 5-HIAA in Ringer solution with 10 mmol/L acetic acid. Injection volume 1 µL. Conditions as in Table 2

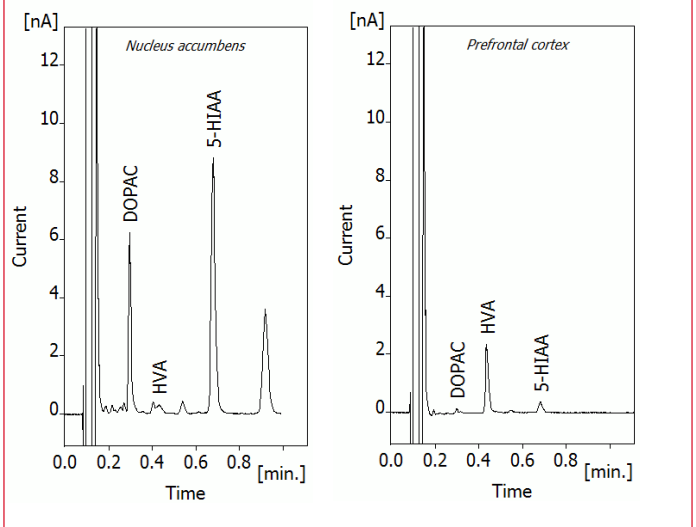
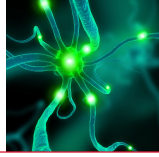


Figure 9: Chromatograms of rat brain microdialysate from prefrontal cortex and nucleus accumbens. Samples kindly provided by Gerdien Korte-Bouws, Department of Phychopharmacology, University of Utrecht. Injection volume 2 µL. Conditions as in Table 2, but with flow rate of 0.2 mL/min.



chromatogram. In case the sample shows a need for more separation, the acetonitrile concentration in the mobile phase can be lowered from 10% to 5% (which would double the analysis time to 2 min).

Analysis of NA, DA and 5-HT

For the selective analysis of monoamines, the acidic metabolites can be moved out of the chromatogram by increasing the pH of the mobile phase. Applying the mobile phase composition as given in Table 3, resulted in chromatograms as in Figure 10 and Figure 11. Detection limit is 0.1 nmol/L for NA and DA, and 0.3 nmol/L for 5-HT using an injection volume of 5 µL. Better detection limits for 5-HT are feasible if more selective settings would be applied (see below).

A microdialysate sample was analyzed to show the applicability of the method (Figure 11). In this chromatogram, concentrations of the monoamines were calculated to be 0.3 nmol/L NA, 1.6 nmol/L DA and 0.8 nmol/L 5-HT.

Target analysis of NA/DA or DA/5-HT

Analysis of NA and DA is accomplished by applying the conditions as given in in Table 3, but lowering the working potential to about 300 mV. At such low potential a number of peaks (incl. 5-HT) will not be visible anymore resulting in a rather 'clean' chromatogram. This clearly shows how the working potential can improve the selectivity for this analysis.

Table 3

LC-ECD settings for analysis of NA, DA and 5-HT

UHPLC	ALEXYS Neurotransmitter Analyzer
Column	Acquity UPLC BEH C18, 1.7µm, 1 x 100 mm (Waters)
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase	100 mM citric acid, 100 mM phosphoric acid, pH 6.0, 0.1 mM EDTA.Na_2 , 950 mg/L OSA, 5% acetonitrile. Refresh at least every 3 days.
Flow rate	75 µL/mL
Temperature	42 °C for separation and detection
Backpressure	About 370 bar
$V_{injection}$	2 µL
Injection method	Dedicated user program with minimal sample use
Needle wash	Water (refresh weekly)
Pump piston wash	15% isopropanol in water (refresh weekly)
Flow cell	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 1
Ecell	460 mV vs. salt bridge reference electrode
Range	1 nA/V for near-LOD signals; 50 nA/V for higher signals
ADF	Opt. 0.5 Hz
I-cell	About 0.5 nA

For analysis of DA and 5-HT, the acidic metabolites are selectively moved to the unretained front in the chromatogram by increasing the pH of the mobile phase. DA and 5-HT are both sufficiently retained and easily separated even when a much shorter column is used. A shorter column results in elution of

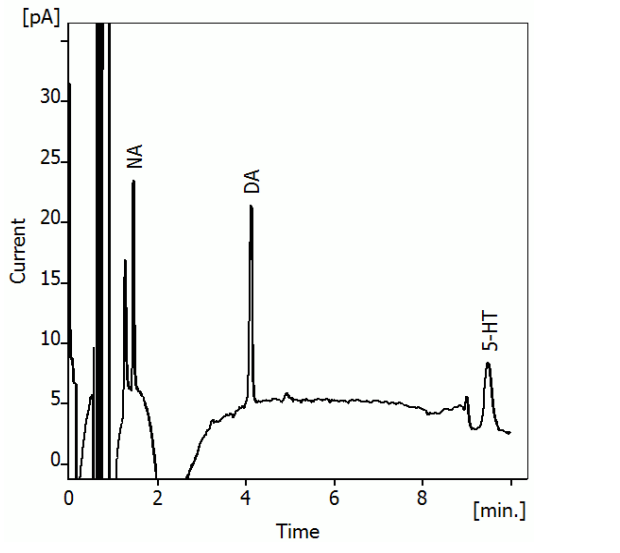


Figure 10: Chromatogram of 2 nmol/L standard of NA, DA and 5-HT in Ringer solution with 10 mmol/L acetic acid. Injection volume 5 µL. Conditions as in Table 3, but with the use of a µVT-03 flow cell.

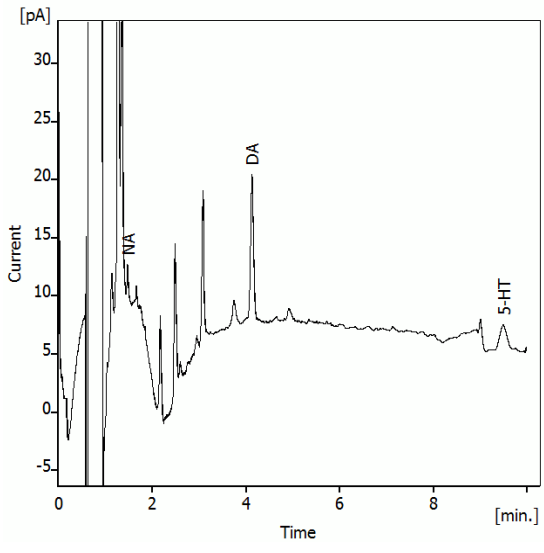


Figure 11: Chromatogram of rat brain Nuccleus accumbens dialysate, acidified during collection with acetic acid (10 mmol/L final concentration). Injection volume 5 µL. Conditions as in Table 3, but with the use of a µVT-03 flow cell.

NA in the solvent front, but more important it has less on-column dilution, which results in smaller peak volumes and therefore better sensitivity compared to using the longer column.

Sensitivity of the analysis of DA and 5-HT was checked with 1.5 µL injections (and 3 µL total sample use) and showed a detection limit of 100 pmol/L (0.15 fmol on column).

A microdialysate sample was analyzed with the conditions from Table 4 to show the applicability of the method (Figure 13). Concentrations of the monoamines were calculated of be 0.7 nmol/L DA and 0.2 nmol/L 5-HT.

Target analysis of DA or 5-HT only

For selective analysis of DA or 5-HT only, the working potential is an important parameter as can be seen in Figure 14. In case of DA analysis, lowering the working potential makes other peaks less/not visible. As a result fast run times are feasible with excellent detection sensitivity. A very short total analysis time of only 1 min is feasible in case there is only the need to measure 5-HT (Figure 15). The mobile phase composition and short column make all the other components elute in the solvent front. Sensitivity of the analysis of DA and 5-HT was checked with 1.5 µL injections (and 3 µL total sample use) and showed a detection limit of 100 pmol/L (0.15 fmol on column). A microdialysis sample was analyzed with the conditions from Table 5 to show the applicability of the method (Figure 16). Concentrations of the monoamines were calculated of be 0.7 nmol/L DA and 0.2 nmol/L 5-HT.

Table 4

LC-ECD settings for analysis of DA and 5-HT

UHPLC	ALEXYS Neurotransmitter Analyzer
Column	Acquity UPLC BEH C18, 1.7µm, 1 x 50 mm (Waters)
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase	200 mM acetic acid, 0.1 mM EDTA.Na_2, pH 5.8, 250 mg/L sodium 1-decanesulfonate, 7.5 % acetonitrile Refresh at least every 3 days.
Flow rate	175 µL/mL
Temperature	35 °C for separation and detection
Backpressure	About 470 bar
$V_{injection}$	2 µL
Injection method	Dedicated user program with minimal sample use
Needle wash	Water (refresh weekly)
Pump piston wash	15% isopropanol in water (refresh weekly)
Flow cell	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 1
Ecell	460 mV vs. salt bridge reference electrode
Range	1 nA/V
ADF	Opt. raw - 0.5 Hz
I-cell	About 0.2 nA

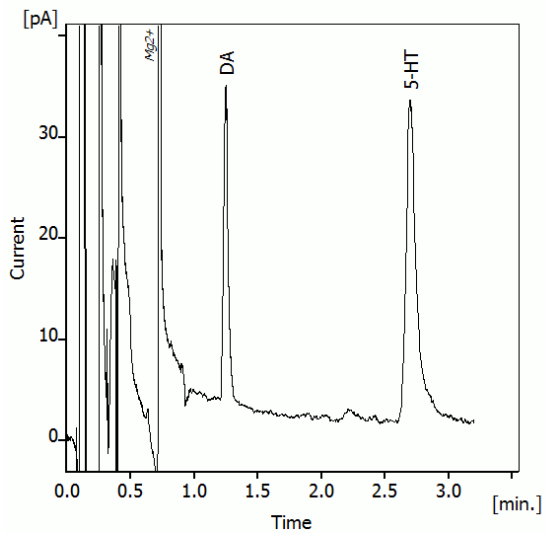


Figure 12: Chromatogram of 1 nmol/L DA and 5-HT in Ringer solution (which contains among others 1.2 mmol/L Mg2+) with 10 mmol/L acetic acid. Injection volume 1.5 µL. Conditions as in Table 4.

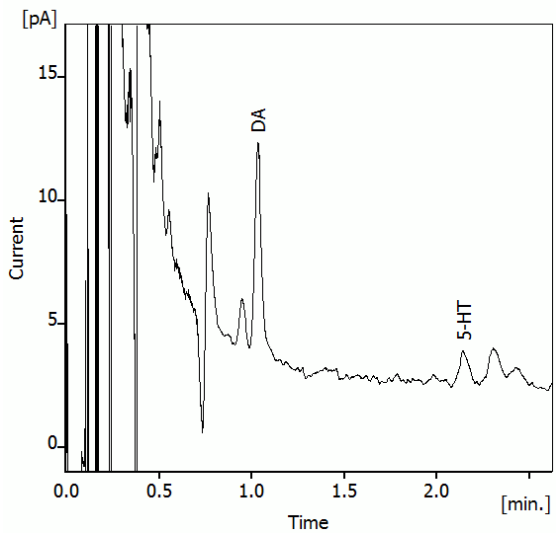
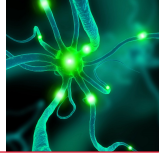


Figure 13: Chromatogram of rat brain basal level microdialysate sample with preservative mix (containing citric acid, EDTA and acetic acid). Sample kindly provided by Jolien van Schoors, Department of Pharmaceutical Chemistry and Drug Analysis, Vrije Universiteit Brussel. Injection volume 1.5 µL. Conditions as in Table 4.



Analysis of tyramine, tyrosine, octopamine, tryptophan

The analysis of the monohydroxyphenols require a higher working potential for their detection compared to the previously described components. Using the conditions as given in Table 2, they are not detectable. Only after increasing the potential with about 0.2 V did these components show a signal in the chromatogram (Figure 17).

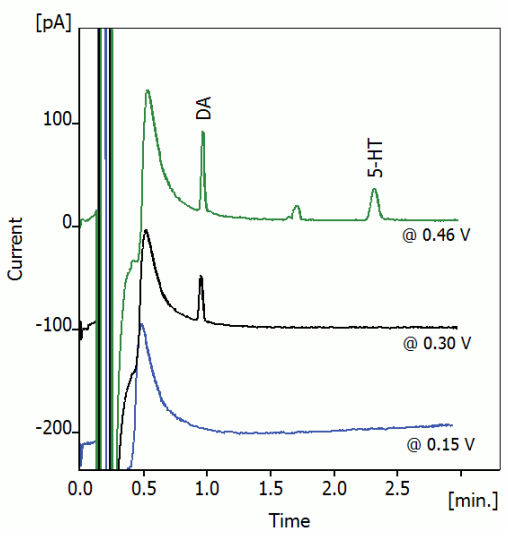


Figure 14: Chromatogram of 1 nmol/L DA and 5-HT in Ringer solution with 10 mmol/L acetic acid, analyzed with different working potentials . Injection volume 1.5 µL. Conditions as in Table 5, but with flow rate of 150 µL/min (pressure about 400 bar) and 250 mg/L octane sulfonic acid sodium salt in the mobile phase.

Table 5

LC-ECD settings for analysis 5-HT

UHPLC	ALEXYS Neurotransmitter Analyzer
Column	Acquity UPLC BEH C18, 1.7µm, 1 x 50 mm (Waters)
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase	100 mM citric acid, 100 mM phosphoric acid, 0.1 mM EDTA.Na_2, pH 6.0, 5 % acetonitrile, 25 mg/L octane sulfonic acid, sodium salt. Refresh at least every 3 days.
Flow rate	100 µL/mL
Temperature	37 °C for separation and detection
Backpressure	About 270 bar
$V_{injection}$	2 µL
Injection method	Dedicated user program with minimal sample use
Needle wash	Water (refresh weekly)
Pump piston wash	15% isopropanol in water (refresh weekly)
Flow cell	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 1
Ecell	460 mV vs. salt bridge reference electrode
Range	1 nA/V
ADF	Opt. raw - 0.5 Hz
I-cell	About 0.7 nA

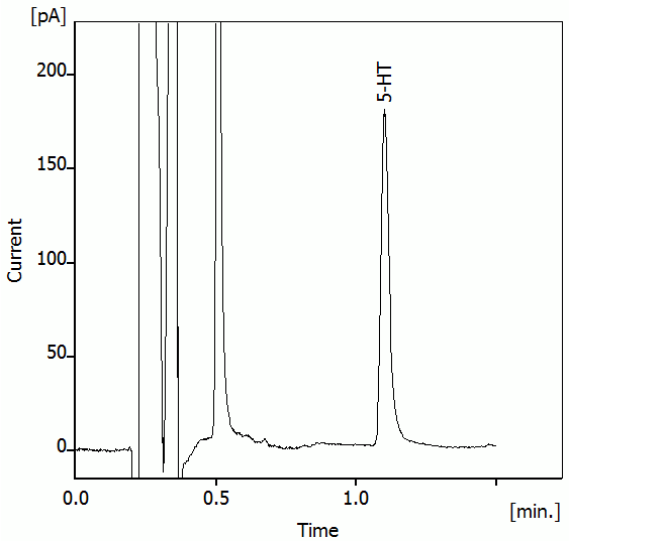


Figure 15: Chromatogram of 10 nmol/L 5-HT in Ringer solution with 10 mmol/L acetic acid. Injection volume 1.5 µL. Conditions as in Table 5.

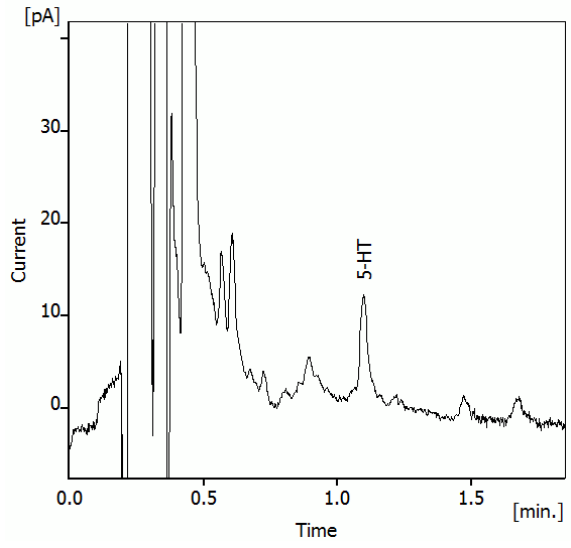


Figure 16: Chromatograms of rat brain microdialysate from prefrontal cortex. Samples kindly provided by Gerdien Korte-Bouws, Department of Phychopharmacology, University of Utrecht. Injection volume 1.5 µL. Conditions as in Table 5.

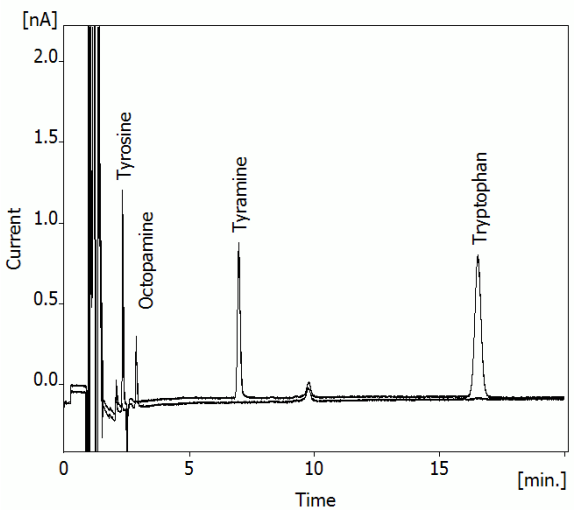


Figure 17: Chromatograms of 100 nmol/L standards in Ringers solution with 10 mmol/L acetic acid. Conditions as in Table 1, but with µVT-03 flow cell and ISAAC reference electrode vs 8 mmol/L KCl (850 mV).

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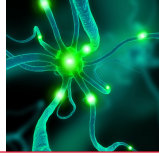

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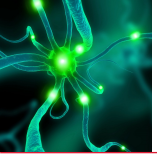
Conclusion

The ALEXYS Neurotransmitter Analyzer is a dedicated platform for fast analysis of monoamines and their metabolites in small samples with excellent detection limits. The system applies UHPLC with a DECADE Elite detector, an amperometric SenCell, and a dedicated autosampler for handling micro volumes. Settings for different sets of target compounds are presented with excellent sensitivity and repeatability, as well as applicability to real samples. Detection limits are in the range of 0.1 - 0.5 fmol on column (below 100 pmol/L in less than 10 µL sample) and repeatability is better than 2% RSD for most components.



Ordering information

ALEXYS Neurotransmitter Analyzer for analysis of monoamines	
180.0091UW	ALEXYS Neurotransmitters SCC base
116.4120	SenCell with 2 mm GC WE and sb REF
250.1165*	Acquity UPLC in-line filter kit + 6 frits (205000343)
250.1166*	Acquity UPLC BEH C18, 1.7µm,1 x 50 mm (186002344)
250.1163*	Acquity UPLC BEH C18, 1.7µm,1 x 100 mm (186002346)

*) Columns are products of Waters Corporation (Milford, USA). The Waters part numbers are given between parenthesis for reordering purposes.

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