Analysis of Monoamines and Metabolites, GABA and Glutamate, and Acetylcholine using UHPLC-ECD

Reinhoud N.J.*, Brouwer H.J, Van Heerwaarden L.M., Eysberg M.
Antec, Industrieweg 12, 2382 NV Zoeterwoude, The Netherlands

ALEXYS® Neurotransmitter Analyzer

ALEXYS® Neurotransmitter Analyzer

Assays for Monoamines and metabolites, GABA and Glutamate, and Acetylcholine have been developed on the ALEXYS Neurotransmitter Analyzer using UHPLC with electrochemical detection.

The challenges to analyze these samples are:

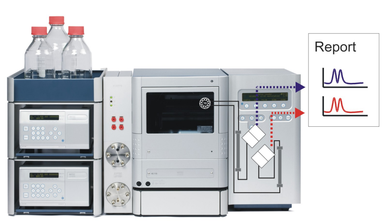
- small volume (<15 uL)
- complex mixture of neurotransmitters
- Both low (picomolar) and high (micromolar) concentrations present in sample

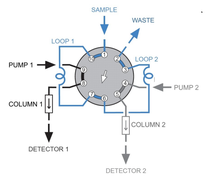
The ALEXYS Neurotransmitter analyzer is dedicated to meet these challenges

- UHPLC columns with sub-2 micron particles for high separation efficiency at increased flow rates (shortens analysis time)
- Autosampler with user defined injection programming to minimize sample use
- DECADE II electrochemical detector with amperometric flow cell for best possible sensitivity

Monoamines and metabolites

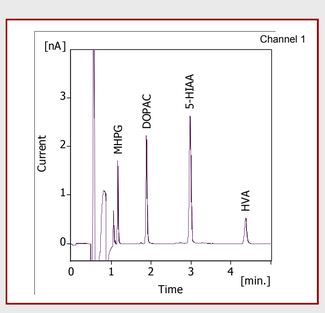
SINGLE or DUAL CHANNEL ANALYSIS





- Single channel, or dual for complex samples
- A 10-port valve with two loops in series to minimize sample consumption during injection cycle
- Simultaneous analysis of single sample with any two isocratic applications, for example:
  Channel 1 -Metabolites
  Channel 2 -monoamines
- E.g. detection limit 0.3 fmol DA and 5-HT (50 pM, 5 µL)
- Required sample volume >10 µL (or >5 uL with smaller sample loops)

Chromatograms of monoamines and metabolites: 5 min



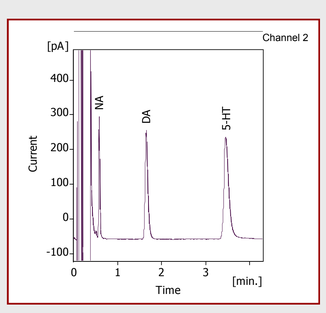
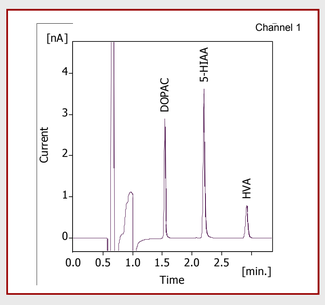


Figure 1. Mix of 100 nM metabolites and 10 nM NA, DA and 5-HT in acidified modified Ringer (no Mg^{2+}) solution, analysed in 5 minutes on the basis of a single injection.

Chromatograms of monoamines and metabolites: 3 min



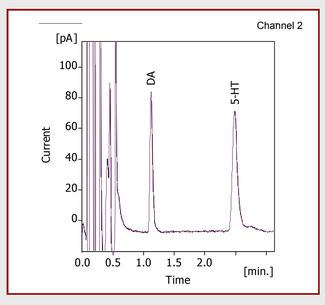
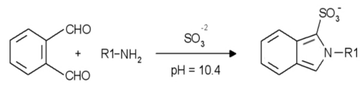


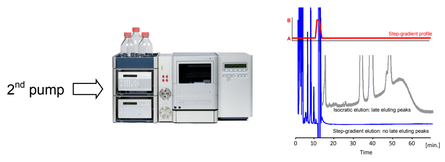
Figure 2. Mix of 100 nM metabolites and 2 nM DA and 5-HT in acidified Ringer solution, analysed in 3 minutes on the basis of a single injection.

GABA and glutamate

PRECOLUMN DERIVATIZATION



STEP GRADIENT ELUTION FOR BASELINE CLEAN-UP



No late eluting peaks

next analysis can be started after 16 min

- Automated in-needle pre-column derivatisation of amino acids with OPA/sulphite reagent (odorless) into stabile and electrochemically-active isoindol sulfonates.
- Step-gradient concept to remove late eluting peaks from the baseline thus reducing the total analysis time.
- Analysis time < 20 minutes (including derivatization)
- Detection limit GABA > 10 nM
- Required sample volume: >9 µL

Chromatograms of rat Nucleus Accumbens microdialysate

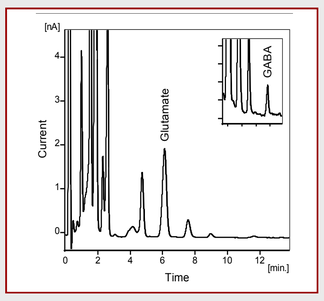


Figure 3. Analysis of basal level GABA (250 nM) and glutamate (8.9 µM) in pooled rat microdialysate from the Nucleus Accumbens. Samples were provided by Mr. Niels Leguit, Abbot Healthcare Products B.V., Weesp, the Netherlands.

Other amino acids

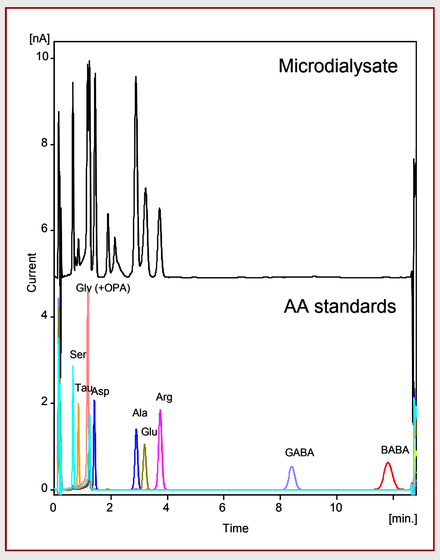
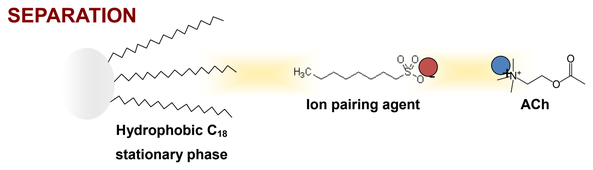


Figure 4. Chromatograms of microdialysate sample and amino acid standards

Acetylcholine

SEPARATION

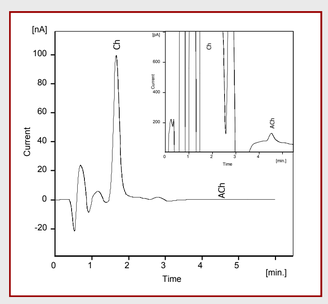


DETECTION

Detection of H_2O_2 on glassy carbon working electrode coated with Horse Radish Peroxidase

- Fast ion-pairing separation on C_{18} UHPLC column followed by selective Post-column Immobilized Enzyme Reactor (IMER) for the conversion of ACh and Ch into electro-chemically detectable H_2O_2.
- FLEXCELL™ in combination with Glassy carbon electrode with Horseradish Peroxidase (HRP) coating for better sensitivity, detection limits and baseline stability.
- Analysis time < 6 minutes
- Detection limit 3 fmol on column (0.3 nM/10 µL)
- Required sample volume >10 µL

Analysis of standard and basal level rat brain microdialysate



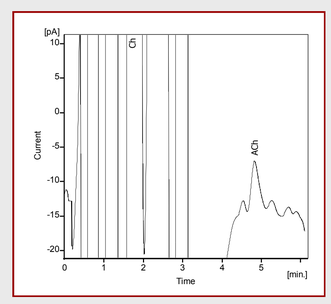


Fig. 5. Left: chromatogram of 2 umole/L Choline and 2 nmole/L Acetylcholine in Ringer solution. The inset shows the same chromatogram, but zoomed in on the baseline. Right: Chromatogram of a basal level rat microdialysate sample. The acetylcholine concentration was calculated to be 1 nmole/L.

Conclusions

The ALEXYS® Neurotransmitter analyzer is a versatile UHPLC system to measure neurotransmitters.

- Superior detection sensitivity
- Optimized for small sample volumes
- One analyzer for various neurotransmitter assays