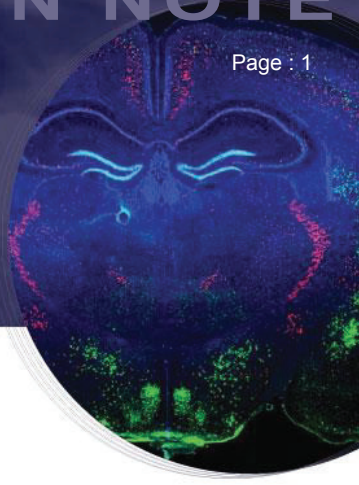
COMPILATION OF NEUROSCIENCE APPLICATIONS



THE SMARTEST LC-EC APPLICATIONS FOR NEUROSCIENCE ANALYSIS EVER MASTERMINDED

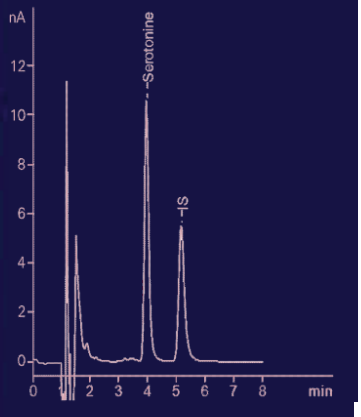
Monoamines and the metabolites
- *Noradrenalin*
- *Dopamine*
- *Serotonin*
- *5-hydroxyindole acetic acid (5-HIAA)*
- *3,4-dihydroxyphenylacetic acid (DOPAC)*
- *homovanillic acid (HVA)*

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

Choline and Acetylcholine
- *Choline (Ch)*
- *Acetylcholine (ACh)*

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

Glutathione and other thiols



INTRODUCTION

Unlike many other detectors for HPLC, amperometric electrochemical detection (ECD) can be miniaturized without losing detection sensitivity. Generally accepted benefits of micro HPLC are the improvement in performance (less peak dilution) and the reduced solvent consumption. But most interesting feature of miniaturized HPLC-ECD is the ability to analyze small samples while maintaining an excellent signal to noise ratio, as both signal and noise decrease with miniaturized electrodes. It is this feature that made HPLC with electrochemical detection the method of choice for neurotransmitter analysis, especially in combination with microdialysis sampling

A number of applications is presented resulting from the work of a few of the many users of Antec equipment. Results may depend on details that are not published here.

- ALEXYS Analyzers in Neuroscience
- Optimized for performance
- Dedicated system solutions

Summary

A selection is presented of different application notes from the work of a few of our many users. These notes demonstrate the versatility of our analyzers in different experimental conditions.

Contents

213-005	MHPG and noradrenaline
213-001	5-HT and 5-HIAA
213-003	GABA and glutamate
211-001	Amino Acid Neurotransmitters
213-002	DOPAC, DA, HVA and 5-HT
213-015	DOPAC, DA, 5-HIAA, HVA and 5HT
217-005	Ecstasy: MDMA and MDA
217-009	Enantioselective analysis of 8-OH-DPAT
211-002	L-tyrosine and 3-nitro-L-tyrosine

Fig. 1. One of the dedicated ALEXYS Analyzers.

MHPG & noradrenaline in rat preoptic area dialysate

Introduction

Analysis of the catecholamines in brain, peripheral tissues and body fluids has resulted in a more than basic understanding of the normal and disturbed peripheral sympathetic and central nervous systems in man and experimental animals. Quantitative analysis of the various metabolites has been at least equally important for the understanding of the neurodynamics of catecholamines.
Each of the catecholamines can be metabolised by the enzymes catechol-O-methyltransferase (COMT) and monoamineoxidase (MAO). Major metabolites of adrenaline are metanephrine and vanillylmandelic acid (VMA). Dopamine metabolites include homovanillic acid (HVA) and 3-methoxytyramine (3-MT). Noradrenaline metabolites include normetanephrine, VMA and 3-methoxy-4-hydroxyphenylglycol (MHPG). MHPG is considered to be (almost) exclusively of central nervous system origin.
This application describes the determination of noradrenaline and its metabolite MHPG after microdialysis sampling in rat preoptic area.

Method

Microdialysis sampling is accomplished by implanting a small dialysis membrane into living tissue. The membrane is integrated into a probe which is flushed by an isotonic perfusion fluid, at a constant flow rate. The difference between the concentration of a chemical in the tissue and concentration in the perfusion fluid creates a concentration gradient which drives this analyte across the dialyzing membrane. Since diffusion is bi-directional, the same device may either deliver or sample chemicals in the targeted tissue.

In combination with microdialysis the DECADE offers the possibility of fully automated analysis with on-line sample injection. A dialysis probe is on-line connected with an automated injector in the DECADE. In the 'auto mode' dialysates are analysed by automatically switching the injection valve and starting the data system.

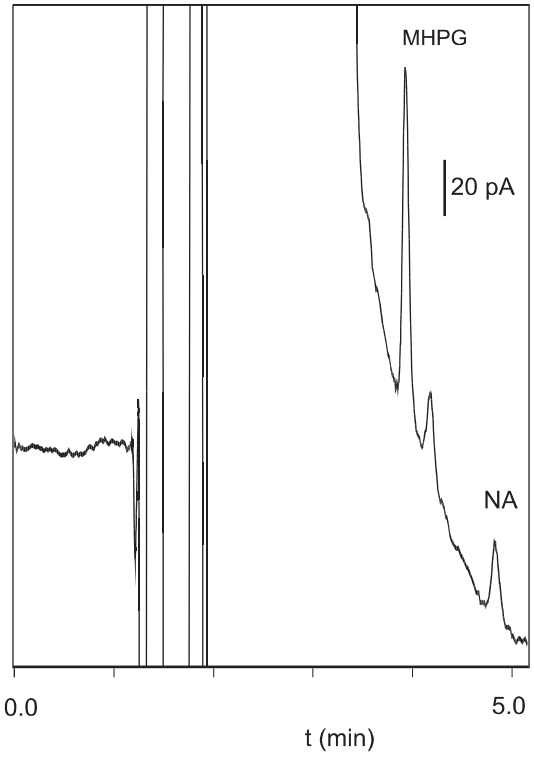


Fig. 1. Analysis of rat preoptic area dialysate. Concentrations (amounts) are 10 nM (318 fmol) MHPG and 0.36 nM (11 fmol) NA. Courtesy: Astrid Linthorst, Max Planck Inst. Psychiat., Clin. Inst., Munich, Germany.

Table 1

Conditions	
Column	Supelco LC-18-DB, 150 x 4.6 mm, 3 µm
Cell	SenCell* with 2 mm glassy carbon electrode and Ag/AgCl salt bridge REF, AST 2
Flow rate	1.0 ml/min
Mobile phase	75 mM NaH2PO4, 0.1 mM EDTA, 0.55 mM OSA, pH 4.95, 7.5% methanol
Sample	Rat preoptic area dialysate, 50 µl inj. (30 µl sample + 20 µl, 25 mM HAc, off-line sampling)
Temperature	35 °C
E-cell	650 mV (vs. Ag/AgCl, sat'd)

* original data obtained with VT-03 with 3 mm glassy carbon electrode and Ag/AgCl salt bridge REF

Recommendation

Collected microdialysis samples are best analysed using the ALEXYS Monoamines Analyzer which is also available for online microdialysis (OMD). The OMD version has an electric valve instead of an autosampler.

PART NUMBERS AND CONFIGURATION	
180.0088B	ALEXYS Monoamines Analyzer
180.0081B	ALEXYS OMD Monoamines Analyzer

5-HT and 5-HIAA in rat hippocampal dialysate

Introduction

5-Hydroxytryptamine (5-HT, serotonin) is synthesized from the amino acid tryptophan (Fig. 1) via 5-hydroxytryptophan and is metabolised to 5-hydroxyindoleacetic acid (5-HIAA). Physiological actions of 5-HT include the control of circadian rhythms, sleep regulation, sex drive, and thermoregulation. It has influence on melatonin synthesis and on aldosterone regulation.
Furthermore, it is involved in psychiatric disorders such as depression, autism and schizophrenia. The identification and quantification of 5-HT and metabolites is of great importance in the recognition and treatment of these disorders.
Conditions are given for highly sensitive determination of 5-HT and 5-HIAA after microdialysis sampling in rat hippocampus.

Method

Microdialysis sampling is accomplished by implanting a small dialysis membrane into living tissue. The membrane is integrated into a probe which is flushed by an isotonic perfusion fluid, at a constant flow rate. The difference between the concentration of a chemical in the tissue and concentration in the perfusion fluid creates a concentration gradient which drives this analyte across the dialyzing membrane. Since diffusion is bi-directional, the same device may either deliver or sample chemicals in the targeted tissue.
The dialysates are collected and analysed off-line. Dialysates are "clean", protein-free samples requiring no pre-treatment prior to analysis.

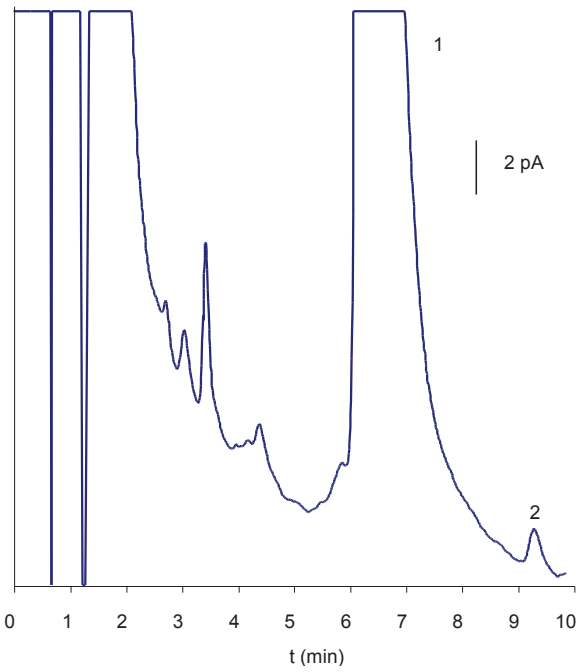


Fig. 1. Analysis of rat hippocampal dialysate, without using a (re)uptake inhibitor. Concentrations (amounts) are (1) 5-HIAA 185 nmol/l (5.5 pmol) and (2) 5-HT 154 pmol/l (4.6 fmol). Courtesy: Astrid Linthorst, Max Planck Inst. Psychiat., Clin. Inst., Munich, Germany. Ref.: Endocrinology 135 (1994) 520 - 532.

Table 2

Conditions	
Column	Supelco LC-18-DB, 3 µm, 150 x 4.6 mm
Cell	SenCell* with 2 mm glassy carbon electrode and Ag/AgCl REF (salt bridge), AST 2
Flow rate	1.0 ml/min
Mobile phase	75 mM NaH2PO4, 0.1 mM EDTA, 0.2 mM OSA, pH 4.30, 18% methanol
Sample	Rat hippocampal dialysate, 50 µl inj. (30 µl sample + 20 µl 25 mM HAc)
Temperature	Ambient
E-cell	600 mV (vs. Ag/AgCl)

*Original data obtained with VT-03 flow cell with 3 mm glassy carbon electrode and Ag/ AgCl REF (salt bridge)

Recommendation
Collected microdialysis samples are best analysed using the ALEXYS Monoamines Analyzer which is also available for online microdialysis (OMD). The OMD version has an electric valve in stead of an autosampler.

PART NUMBERS AND CONFIGURATION	
180.0088B	ALEXYS Monoamines Analyzer
180.0081B	ALEXYS OMD Monoamines Analyzer

L-tyrosine and 3-nitro-L-tyrosine

Introduction

Nitric oxide (NO) is produced in the endothelial cells and neurons by nitric oxide synthetase and plays an important role in humans under many physiological and pathological conditions. It is known to function as an endothelium derived vascular relaxing factor or to be involved in the signal transduction in the brain. Recently NO itself or an oxidant derived from NO were proposed to be cytotoxic. NO contains an unpaired electron that can combine with free radicals such as superoxide (O_2^-) and NO and O_2^- produce a strong oxidant, peroxynitrite ($ONOO^-$) in vivo. $ONOO^-$ is supposed to be involved in several process that lead to oxidative stress and chronic ischemic injury of the brain. Therefore a sensitive detection method is required for this unstable molecule in human material. Peroxynitrite has been reported to react with L-tyrosine to produce 3-nitro-L-tyrosine (NO_2-Tyr), which appears to be a suitable marker for ONOO- mediated tissue damage. In this application NO_2-Tyr standards are determined with a detection limit of 0.5 nmol/L.

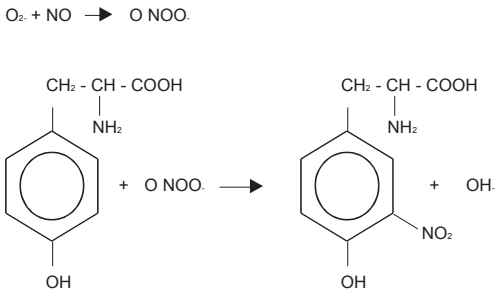


Fig. 1 Synthesis of 3-nitro-L-tyrosine from L-tyrosine and peroxynitrite.

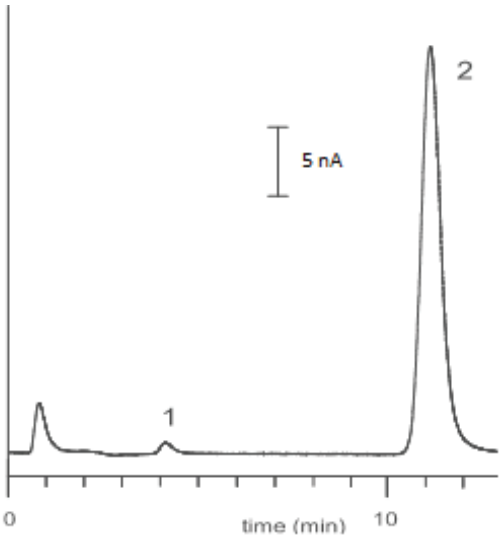


Fig. 2 Analysis of 6.5 µmol/l L-tyrosine (1) and 4.3 µmol/l 3-nitro-L-tyrosine (2) standards using a working potential of 600 mV in combination with a reactor potential of -850 mV.

Method

Amperometric detection at 600 mV in combination with a reactor potential of -850 mV (Fig. 2) results in the best detection sensitivity for NO_2-Tyr standards. Increasing the detection potential to 1000 mV results in an improved signal for tyrosine.

Reference

W. Maruyama, Y. Hashizume, K. Matsubara and M. Naoi, J. Chromatogr. B, 676 (1996) 153-158.

Table 9

Conditions	
Column	C18, 50 x 1 mm, 5 µm
Flow rate	0.05 ml/min
Mobile phase	H3PO4 50 mM, citric acid 50 mM, pH=3.1 with KOH, 40 mg/l EDTA, 100 mg/l octane sulphonic acid (OSA), 5% methanol
Sample	10 µl injection
Temperature	30 °C
E-reactor	-850 mV (vs. HyREF)
E-cell	600 mV (vs. Ag/AgCl)

*SenCell with 2mm glassy carbon WE and ISAAC REF - add 2 mM KCl

Recommendation

Collected microdialysis samples are best analysed using the ALEXYS Nitrotyrosine Analyzer.

PART NUMBERS AND CONFIGURATION	
180.0072B	ALEXYS Nitrotyrosine Analyzer