COMPILATION OF NEUROSCIENCE APPLICATIONS

THE SMARTEST LC-EC APPLICATIONS FOR NEUROSCIENCE ANALYSIS EVER MASTERMINDED

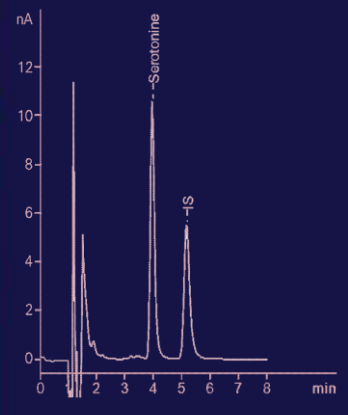
Monoamines and the metabolites
- *Noradrenalin*
- *Dopamine*
- *Serotonin*
- *5-hydroxyindole acetic acid (5-HIAA)*
- *3,4-dihydroxyphenylacetic acid (DOPAC)*
- *homovanillic acid (HVA)*

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

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.

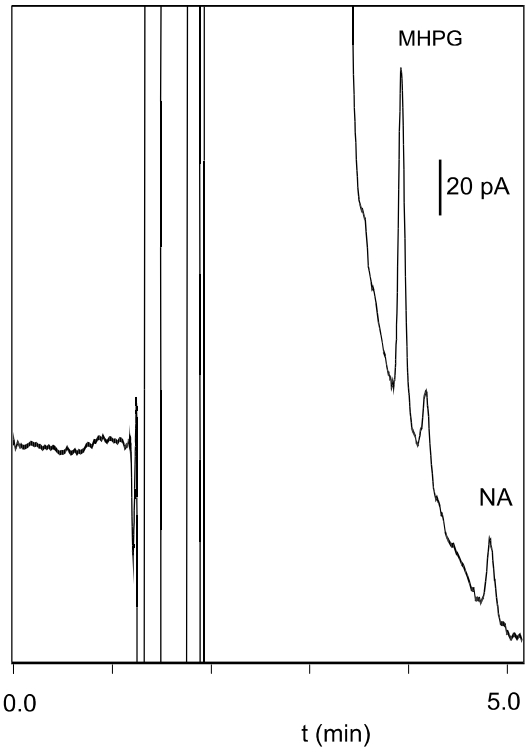


Fig. 1. Analysis of rat preoptic area dialysate. Concentrations (amounts) are 10 nM (318 fmol) MHPG and 0.36 nM (11 fmol) NA. Courtesy: Astrid Linthorst, Max Planck Inst. Psychiat., Clin. Inst., Munich, Germany.

Table 1

Conditions	
Column	Supelco LC-18-DB, 150 x 4.6 mm, 3 µm
Cell	VT-03 with 3 mm glassy carbon electrode and Ag/AgCl salt bridge REF
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)

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.

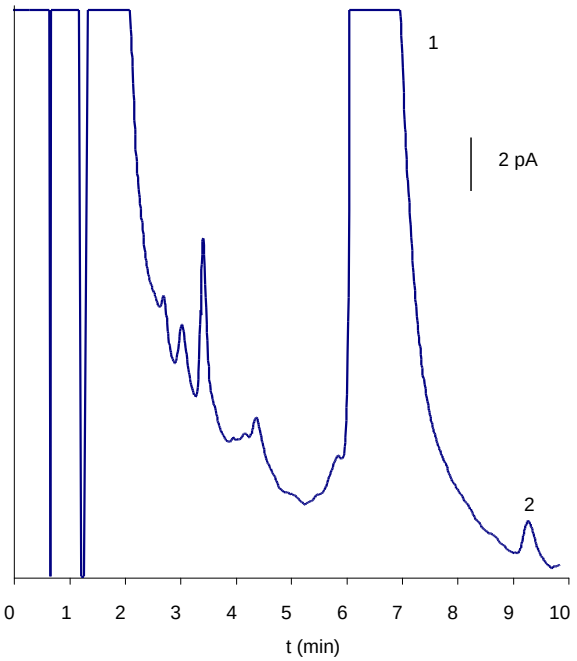


Fig. 1. Analysis of rat hippocampal dialysate, without using a (re)uptake inhibitor. Concentrations (amounts) are (1) 5-HIAA 185 nmol/l (5.5 pmol) and (2) 5-HT 154 pmol/l (4.6 fmol). Courtesy: Astrid Linthorst, Max Planck Inst. Psychiat., Clin. Inst., Munich, Germany. Ref.: Endocrinology 135 (1994) 520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

Table 2

Conditions	
Column	Supelco LC-18-DB, 3 µm, 150 x 4.6 mm
Cell	VT-03 flow cell with 3 mm glassy carbon electrode and Ag/AgCl REF (salt bridge)
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
Flow cell	VT-03 flow cell with GC working electrode
E-cell	600 mV (vs. Ag/AgCl)

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

GABA and glutamate in ventral hippocampal dialysate

Introduction

GABA and glutamate can be measured at extremely high sensitivity by LC-EC. A detection limit of 10 femtomol for GABA is reported [1]. Perfusate samples are collected and derivatised with ortho phthalaldehyde (OPA) and sodium sulphite. The linearity of the method is determined between 0.1 and 40 pmol on-column, regression analysis results in a correlation coefficient of 0.9995. The method combines sensitivity and reproducibility with ease of use. It permits measurement of amino acid neurotransmitter concentrations from extra-cellular fluid using microdialysis.

Method

The LC-EC parameters are given in Table I. The derivatisation reagent consisted of 22 mg OPA (Aldrich) dissolved in 0.5 ml sodium sulphite (1 mol/l) to which was added 0.5 ml absolute ethanol and 0.9 ml sodium tetraborate buffer 0.1 mol/l adjusted to pH 10.4 with 5 mol/l sodium hydroxide. The reagent was prepared daily and remained stable throughout the working day if kept in darkened vial.

The derivatisation reaction proceeded at room temperature. A volume of 0.4 µl of derivatization reagent were reacted with 20 µl microdialysate for 10 min in a polyethylene vial before injection onto the column.

A number of system peaks in Fig. 1 are due to the OPA reagent which is electroactive. Under the described conditions the resolution is sufficient for reproducible and sensitive analysis.

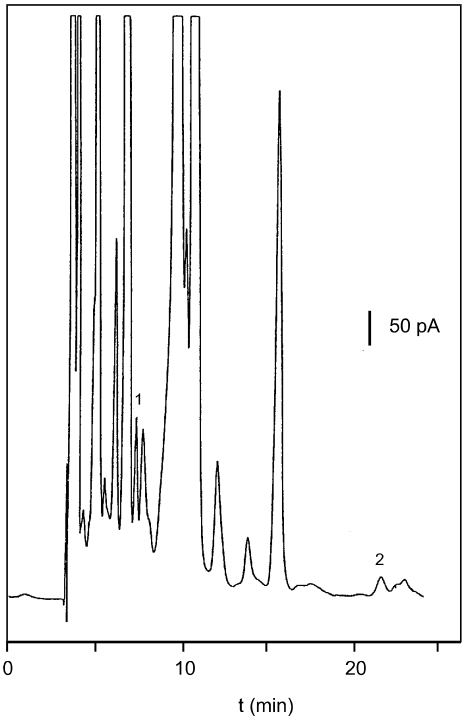


Fig. 1. Analysis of rat ventral hippocampal dialysate after OPA-sulphite derivatisation. Concentrations (amounts) are: 1. glutamate 3.6 nmol/l (72 fmol) and 2. GABA 1.7 nmol/l (34 fmol). Courtesy: Dr. C.A. Marsden, Dept. of Phys. and Pharm., Univ. of Nottingham Med. School, UK.

References

1. H.L. Rowley, K.F. Martin and C.A. Marsden; Journal of Neuroscience Methods 57 (1) (1995) 93-99.

Table 3

Conditions	
Column	Rainin Dynamax C18, 250 x 4.6 mm, 5 µm
Cell	VT-03 with 3 mm glassy carbon electrode and Ag/AgCl salt bridge REF
Flow rate	0.70 ml/min
Mobile phase	Phosphate buffer 100 mM, pH 4.5, EDTA 0.5 mM, 25% MeOH
Sample	20 µl inj., amino acids derivatised with OPA-sulphite
Temperature	ambient
E-cell	850 mV (vs. Ag/AgCl, sat'd)

Recommendation

Amino acid neurotransmitters are best analyzed using the GABA Glutamate analyzer which includes a fully automated derivatization step using a programmable autosampler.

PART NUMBERS AND CONFIGURATION	
180.0070B	ALEXYS GABA Glutamate Analyzer

Amino Acid Neurotransmitters

Introduction

Several approaches have been described for the determination of amino acids using electrochemical detection. Direct detection is possible using amperometric detection with a copper working electrode [1], and pulsed amperometric detection with a gold working electrode [2]. In both cases separation is achieved by anion chromatography at high pH.

Reversed phase separation followed by amperometric detection on a glassy carbon electrode is restricted to tyrosine (with phenol group), and tryptophan (with indole ring) [3]. Other amino acids lack electrochemical activity and derivatisation is required prior to detection.

In this application a derivatisation with ortho phthalaldehyde (OPA) and sodium sulphite is described. Detection limits down to 5 femtomol for glycine and 10 femtomol for GABA are reported [4]. The linearity of the method is determined between 0.1 and 40 pmol on-column. Regression analysis results in a correlation coefficient of 0.9995.

Method

The LC-EC parameters are given in Table I. The derivatization reagent consisted of 22 mg OPA (Aldrich) dissolved in 0.5 ml sodium sulphite (1 mol/l) to which was added 0.5 ml absolute ethanol and 0.9 ml sodium tetraborate buffer 0.1 mol/l adjusted to pH 10.4 with 5 mol/l sodium hydroxide. The reagent was prepared daily and remained stable throughout the working day if kept in a darkened vial. The reaction proceeded at room temperature. A volume of 20 µl of derivatization reagent was added to 1 ml amino acid standard and reacted for 10 min in a polyethylene vial before injection onto the column.

Table 4

Conditions	
Column	Rainin Dynamax C18, 250 x 4.6 mm, 5 µm
Cell	VT-03 flow cell with 3 mm glassy carbon electrode and Ag/AgCl REF (salt bridge)
Flow rate	0.70 ml/min
Mobile phase	Phosphate buffer 100 mM, pH 4.5, EDTA 0.5 mM, 25% MeOH
Temperature	ambient
E-cell	850 mV (vs. Ag/AgCl sat'd)

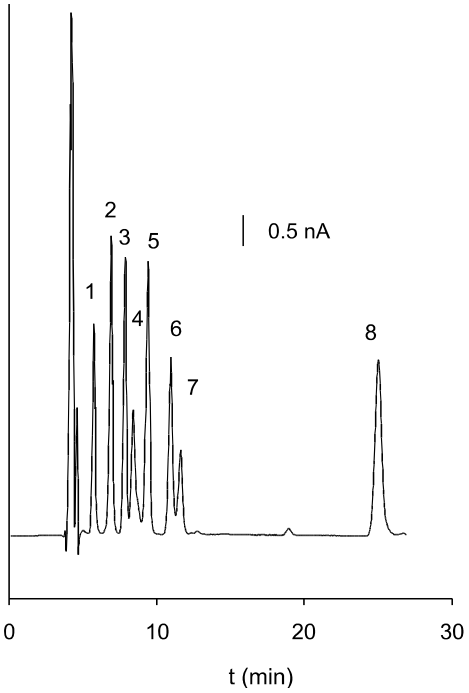


Fig. 1. Analysis of amino acid neurotransmitters after OPA-sulphite derivatisation. The amount injected is 20 pmol (1 µmol/l) for each compound (20 µL). Peaks are: serine (1), glycine (2), taurine (3), glutamate (4), arginine (5), alanine (6), OPA reagent (7) and GABA (8). Courtesy: Dr. C.A. Marsden, Dept. Physiol. and Pharmacol., Univ. of Nottingham Med. Sch., UK.

References

1. D.A. Martens and W.T. Frankenberger, J. Liquid Chromatogr. 15 (3) (1992) 423
2. P. Luo, F. Zhang, R.P. Baldwin, Anal. Chem. 63 (1991) 1702
3. P.R. Brown, Anal. Chem. 62 (1990) 995A
4. H.L. Rowley, K.F. Martin and C.A. Marsden, J. Neurosc. Methods 57 (1) (1995) 93

Recommendation

Amino acid neurotransmitters are best analyzed using the GABA Glutamate analyzer which includes a fully automated derivatization step using a programmable autosampler.

PART NUMBERS AND CONFIGURATION	
180.0070B	ALEXYS GABA Glutamate Analyzer

Cappilary LC of DA, DOPAC, HVA and 5-HT in Microdialysates

Introduction

Capillary LC is a challenging application area for electrochemical detection. In principle, miniaturisation in LC-EC is possible without loss of sensitivity. In fact, when certain requirements are met the sensitivity (S/N ratio) will get better when smaller working electrodes are used. Miniaturisation results in less signal, but the noise decreases even more, resulting in a better S/N ratio.
To achieve better detection sensitivity it is mandatory that the dead volume, due to injection valve, capillary connections and detection volume is minimised.
The detector in combination with a micro flow cell (0.7 mm WE diameter) has an excellent performance in capillary-LC (Fig. 1). The detection cell volume can be decreased down to 11 nl by using a thinner spacer. The detector can be equipped (option) with a built-in electrically actuated VALCO micro injector (60 to 500 nl injection volume). Both features are important in minimising the dead-volume.

Method

In combination with microdialysis the detector offers the possibility of automated analysis with on-line sample injection. A dialysis probe is on-line connected with an automated injector in the detector. In the 'auto mode' the injection valve is switched, the integrator is started and the dialysate can be analysed.
An important consideration for the use of capillary-LC in combination with microdialysis is the small injection volume that allows a high sample throughput. In standard LC the time resolution for a dialysis experiment is depending not only on the speed of analysis but also on the time needed to acquire enough dialysate to fill the injection loop.

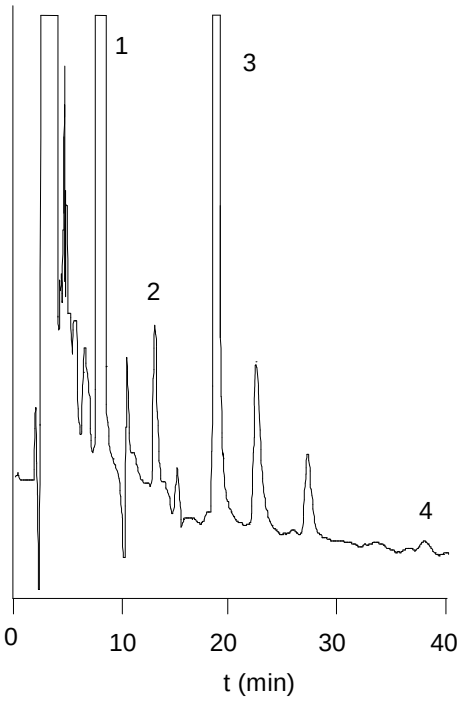


Fig. 1. Capillary LC of rat striatum dialysate. Concentrations and absolute amounts are: (1) Dopac 1 µmol/l (64 fmole), (2) DA 5 nmol/l (5 amole), (3) HVA 0.23 µmol/l (14 fmole) and (4) 5HT 3 nmol/l (0.18 fmole).

In capillary-LC only the analysis time will be the limiting factor. Using a 1 µl/min dialysis flow rate in combination with a 100 nl injection volume minimises the sampling time to only 6 seconds!

Table 5

Conditions	
Column	Fusica-C, C18, 3 µm, 150 x 0.32 mm, LC-Packings, Amsterdam, Netherlands
Cell	µVT-03 with 0.7 mm glassy carbon electrode and Ag/AgCl REF (salt bridge)
Flow rate	10 µl/min
Mobile phase	Phosphate buffer 50 mM, pH=3.7, 0.1 mM EDTA, 4 mM heptane sulphonate, 7% acetonitrile
Sample	Rat striatum dialysate, 60 nl injection
Temperature	30 °C
E cell	800 mV (vs. Ag/AgCl, std.)

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

Capillary LC of DOPAC, DA, 5-HIAA, HVA and 5HT

Introduction

Steroid hormones prime neural circuits for sexual behaviour by regulating enzymes, receptors or other proteins affecting neurotransmitter function. Dopamine (DA) activity in the medial preoptic area (MPOA) contributes to the control of male rat sexual behaviour. DA is released before and during copulation in three integrative neural systems. The nigrostriatal system enhances readiness to respond, the mesolimbic system promotes many appetitive behaviours, the MPOA contributes to sexual motivation, genital reflexes, and copulation.

A method is given for analysis of extracellular DA and its metabolites in male rats' MPOA. Detection limits down to 26 and 13 attomole for respectively DA and 5HT are reported [1].

Method

Microdialysis probes are implanted with the dialysis membrane extending into the MPOA area. The probes are perfused with Dulbecco's phosphate buffered saline with a Harvard '22' infusion pump. Samples are collected every 6 min. Because of the retention time of serotonin at 12 min, only every other sample was analysed, the remainder was frozen and analysed when necessary.

Fractions of 0.5 µl were analysed on a 5 x 0.32 mm column using electrochemical detection with an 11 nanoliter flow cell.

References

5. E.M. Hull, J. Du, D.S. Lorrain and L. Matuszewich, J. of Neurosc., 15 (1995) 7465 – 7471.

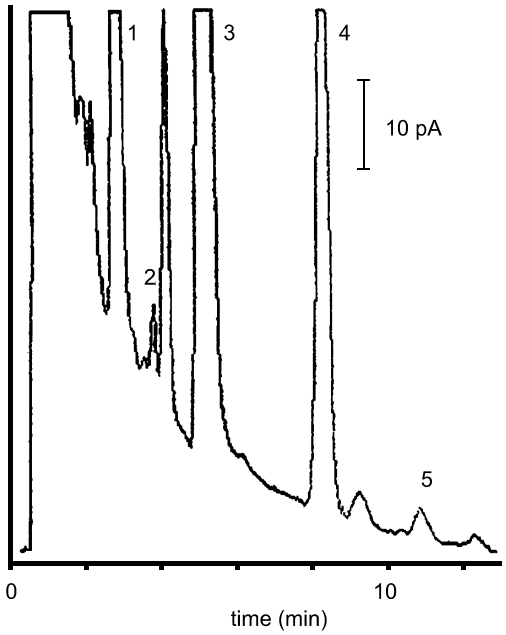


Fig. 1 Analysis of dopamine (DA), serotonin (5HT), and metabolites from medial preoptic area microdialysate. Peaks and amounts in femtomole: (1) DOPAC 18, (2) dopamine 1.6, (3) 5HIAA 60, (4) HVA 88.5, (5) 5HT 0.2. Courtesy: ref [1].

Table 6

Conditions	
Column	Fusica C18, 0.32 x 5 mm, 3 µm (LC Packings, Amsterdam)
Cell	µVT-03 with 0.7 mm glassy carbon electrode and Ag/AgCl REF (salt bridge)
Flow rate	8 µl/min (Accurate flow spitter)
Mobile phase	Citric acid 32 mM, sodium acetate 54 mM , pH 3.8 with HAc, EDTA 0.074 mM, octane sulphonic acid 0.215 mM, methanol 3%, THF 0.004%
Sample	Rat MPOA area dialysate
Injection	0.5 µl
Temperature	ambient
E-cell	700 mV (vs. Ag/AgCl, sat'd)

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

Ecstasy: MDMA and MDA in blood and brain

Introduction

Ecstasy (3,4-methylene dioxymethamphetamine, MDMA) is seen to be safe by many users but its metabolite (3,4-methylene di-oxyamphetamine, MDA) has been shown to have long-term neu-rotoxic effects. There is evidence that MDMA given to young rats on postnatal day 10 fails to produce the serotonergic neurotoxicity found in older rats, such as density reduction of 5-HT uptake sites, and also fails to produce acute changes in dopamine levels or hyperthermia. It remains to be determined why there are age-related differences in neurotoxic susceptibility to these drugs. It could reflect differences in neuronal development, oxidative status or drug metabolism.

In this application note the analysis of MDMA and MDA is de-scribed using HPLCECD of blood and brain samples after liquid-liquid extraction. The limit of detection was 0.2 pmole for both analytes.

Method

MDMA and MDA were analysed using HPLC with electrochemical detection (ECD). The n-ethyl derivative of MDMA (N-ethyl-3,4-methylene dioxyamphetamine, MDEA) was used as internal stan-dard.

Brain tissue samples were sonicated in 1 ml 0.1 mole/L perchloric acid containing 40 pmole MDEA, and centrifuged. The super-natant was stored at –80 °C until analysis.

Whole blood was centrifuged and the plasma removed. 500 µl plasma was transferred to an Eppendorf tube, 200 µl 1 mole/L PCA containing 40 pmole/L MDEA was added and the mixture was centrifuged. The supernatant was removed and stored at –80 °C until analysis.

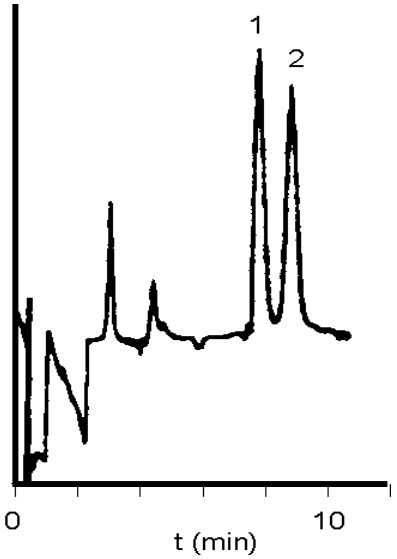


Fig. 1. Analysis of MDA (1) and MDMA (2) standards concentration: 1 µmole/l. Courtesy: ref [1].

Both brain and plasma supernatants were extracted using organic solvents. 200 µl 1 mole/L NaOH and 1 ml organic solvent (ethyl acetate/N-hexane/1-butanol, 10:10:1) were added to the super-natant. The mixture was vortexed for 60 s and then centrifuged for 2 min. The organic layer was transferred to an Eppendorf tube. 40 µl of 400 mmole/L acetic acid was added and the mixture vortexed for 60 s followed by centrifugation for 1 min. The acetic acid layer was transferred to a sample vial and traces of organic solvent were removed by a stream of nitrogen and warming the sample to 30 °C. Samples were analysed on the HPLC-ECD system.

References

1. Poster presentation: Measurement of MDMA and MDA in brain tissue by HPLC and electrochemical detection, A. Townsend, C.D. Forster, E. O'Shea, K.C.F. Fone, G.W. Bennet and C.A. Marsden.

Table 7

Conditions	
Column	HiChrom RPB, 2.1 x 100 mm, 3 µm
Cell	VT03 with 3mm glassy carbon WE and salt bridge REF
Flow rate	0.35 ml/min
Mobile phase	0.05M KH2PO4, 1mM EDTA, 3mM NaCl, 15% Methanol, pH=4
Sample	Brain and blood extracts
Injection	20 µl
Temperature	Ambient
E-cell	900 mV (vs. Ag/AgCl, sat'd)

Recommendation

Blood or brain tissue samples are best analysed using an ALEXYS Analyzer. Depending on available sample volume a standard or micro version should be used.

PART NUMBERS AND CONFIGURATION	
180.0037B	ALEXYS Cool, Micro
110.4115	VT-03 cell 2 mm GC sb

Enantioselective LC-EC analysis of 8-OH-DPAT in rat blood

Introduction

The 5HT1A receptor subtype appears to be a target for the treatment of depression and anxiety. Both the R- and S- eniantiomers of the potent 5HT1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) are pharmacologically active, show different pharmaco-kinetic and –dynamic profiles (1), may be inter-converted in vivo and hence it is important to be able to differentiate between these enantiomers. With standard column materials enantiomers are not or very poorly separated but the use of chiral columns may provide excellent separation profiles. In pharmacokinetic studies with rats numerous samples are needed and hence only quite small blood samples can be drawn. In addition, 8-OH-DPAT being highly potent, a low dose is needed so the sensitivity of the assay is a crucial factor (2).
8-OH-DPAT is relatively easy to oxidise at a low potential and therefore LCEC was applied to investigate the fate of 8-OH-DPAT in vivo (3). Only 50 µl blood samples were needed for a simultaneous determination of the R- and the S- moiety with a limit of detection of 0.5 ng/ml, corresponding with a concentration detection limit of 1.5 nM. It appeared that the R- and the S-enantiomer have a different elimination profile, pointing at a stereoselective mechanism.

Method

To 50 µl of hemolyzed blood 50 µl R-7-OH-DPAT (internal standard) and 2 ml acetonitrile were added and mixed. Then 25 µl 3M NaOH was added, mixed and centrifuged. The supernatant was transferred to a new tube and mixed with 3 ml dichloromethane and 750 µl borate buffer (pH 10).
The water phase was carefully removed and the organic phase evaporated to dryness, reconstituted in phosphate buffer, solid-phase extracted on NARC-2 columns, evaporated to dryness, re-dissolved in 100 µl water acetonitrile (80:20, v/v) and 50 µl injected into the LCEC system (3).

Table 8

Conditions	
Column	Chiralcel OD-R (Diacel, Tokyo, Japan), 250 x 4.6 mm, 10 µm
Cell	VT-03 flow cell with 3mm glassy carbon WE and ISAAC REF
Flow rate	1 ml/min
Mobile phase	50 mM phosphate (pH 5.5), acetonitrile (80:20, v/v), 5 mM KCl and 20 mg/L EDTA
Sample	hemolysed rat blood
Injection	50 µl
Temperature	30°C
E-cell	630 mV (vs. Ag/AgCl 5 mM, ISAAC)

Fig. 1 Analysis of rat blood 30 min after infusion of 3 mg/kg in 5 min. of R-8-OH-DPAT (2). Concentration: 183 ng/ml. Peak 1 is internal standard. Courtesy: ref. [3].

References

1. L.J. Cornfield, G. Lambert, L.E. Arvidsson, C. Mellin, J. Vallgarda, U. Hacksell and D.L. Nelson, Mol. Pharmacol. 39 (1991) 780
2. H.Yu and T. Lewander, Euro. Neuropsychopharmacol. 7 (1997) 165.
3. K.P. Zuideveld, N.Treijtel, P.H. Van Der Graaf and M. Danhof, J. Chrom. 738 (2000), 67-73.

Recommendation

Blood or brain tissue samples are best analysed using an ALEXYS Analyzer. Depending on available sample volume a standard or micro version should be used.

PART NUMBERS AND CONFIGURATION	
180.0037B	ALEXYS Cool, Micro
110.4115	VT-03 cell 2 mm GC sb

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.

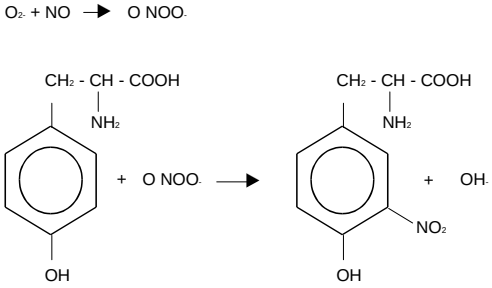


Fig. 1 Synthesis of 3-nitro-L-tyrosine from L-tyrosine and peroxynitrite.

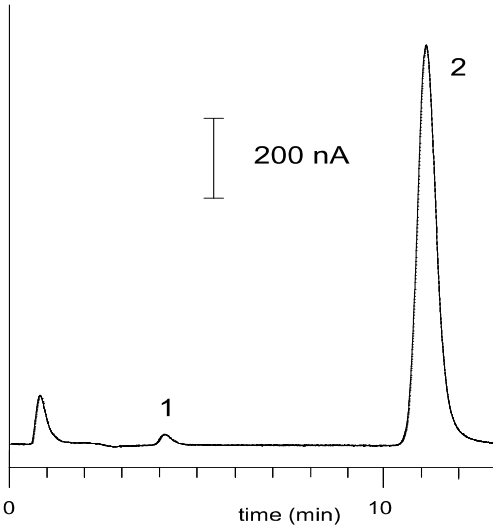


Fig. 2 Analysis of 6.5 µmol/l L-tyrosine (1) and 4.3 µmol/l 3-nitro-L-tyrosine (2) standards using a working potential of 600 mV in combination with a reactor potential of -850 mV.

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)

Recommendation

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

PART NUMBERS AND CONFIGURATION	
180.0072B	ALEXYS Nitrotyrosine Analyzer