COMPILATION OF PHARMACEUTICAL APPLICATIONS

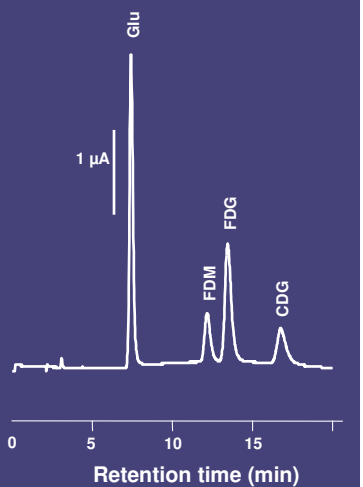
THE MOST RELIABLE LC-EC APPLICATIONS FOR PHARMACEUTICAL & BIOTECH ANALYSIS EVER FORMULATED

Aminoglycosides
- *Amikacin*
- *Framycetin Sulphate*
- *Gentamicin Sulphate*
- *Kanamycin Sulphate*
- *Lincomycin*
- *Neomycin*
- *Spectinomycin*
- *Tobramycin*

PET imaging tracer
- *FDG*

Macrolide antibiotics
- *Azithromycin*
- *Azaerythromycin*
- *Clarithromycin*
- *Erythromycin*
- *Roxithromycin*

Bioanalysis of pharmaceutics
- *Artemisinin*
- *Dihydro-artemisinin*
- *Artemether*
- *Etoposide*
- *8-OH-DPAT*
- *mesna BNP7787*
- *Vincristine*



INTRODUCTION

The QC testing of drug compounds is performed on a worldwide scale by pharmaceutical companies, contract research labs (CRO) and various other institutions. Regulatory requirements are broader and more stringent than ever before and will only increase to protect human and nature.

With the Current Good Manufacturing Practices (cGMPs) for human pharmaceuticals quality standards are set to insure product quality and safety. Adherence to the cGMP regulations assures the identity, strength, quality, and purity of drug products by requiring that manufacturers of medications adequately control manufacturing operations. Reproducible, reliable and robust methods of analysis play a vital role in regulatory compliance.

- ALEXYS Analyzers in Pharma and Biotech
- Optimized for performance
- Dedicated system solutions
- Reproducible & Robust

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. ALEXYS Analyzer.

Azithromycin in pharmaceutical dosage forms

Introduction

Azithromycin belongs to the macrolides and is an acid stable erythromycin derivative used for antibacterial infections. It has quite favourable pharmacokinetic properties and is highly effective against respiratory tract infections, sexually transmitted diseases and non-classical pathogens like Heliobacter pylori.
Azithromycin has been analysed in biological fluids and in pharmaceutical dosage forms by LC using different detection techniques. Among these, electrochemical detection (EC) excels because of its intrinsic sensitivity and precision. In some cases quite unusual LC-EC conditions (pH, modifier, column materials) have been applied. Also, in the official USP method two amperometric working electrodes in a serial arrangement have been prescribed. We have determined the LC and EC conditions allowing the use of standard reversed-phase column materials, relatively low modifier concentrations, a neutral pH and a single working electrode.

Method

Mobile phase conditions were optimised towards the use of a single working electrode, a low background current and a standard reversed phase column. It appeared that the conditions listed below fulfilled these requirements resulting in highly sensitive, selective and reproducible analyses.
Stock solutions of Azithromycin (USP) were made up in 100% in methanol to a final concentration of 200 µM. Standards for a calibration curve were obtained by diluting this solution 200, 2000 times with mobile phase.

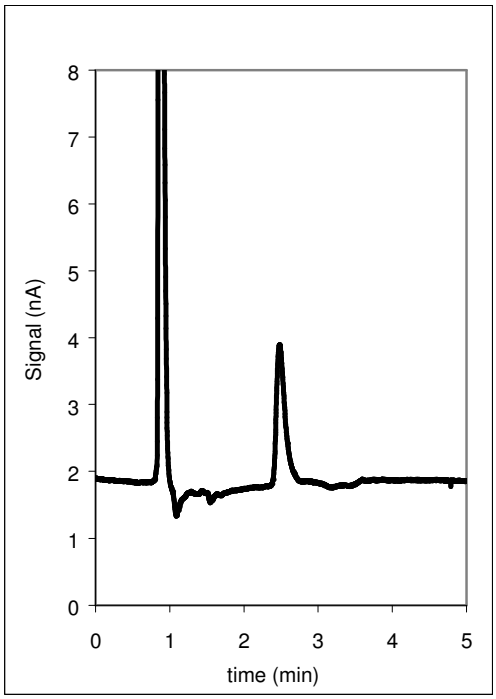


Figure 1 Analysis of 4 µmol/L azithromycin standard.

To analyse the azithromycin amount in a pharmaceutical preparation, Zithromax tablets (Pfizer) with a stated weight and content of 1,000 and 500 mg resp. were weighed and crushed to powder. A powder fraction 300 mg was dissolved in a final volume of 200 ml methanol in an ultrasonic bath at 30°C during 15 min. A 2 ml fraction of the suspension was diluted 5 times with methanol and 500 times further diluted with mobile phase, filtered over a Gelman nylon Acrodisk 0.2 µm filter and analysed

Table 1

Conditions	
Column	Waters Spherisorb ODS2 100 x 4.6 mm, 3 µm
Flow rate	1.0 mL/min
Sample	20 µl
Mobile phase	200 mL of 50 mmol/L phosphoric acid and 2 mmol/L KCl is set to pH 5.0 (by NaOH). Then 800 mL methanol is added (final concentration of 80 % MeOH). NB: At a higher pH value precipitation of buffer salts occurs.
Temperature	30 oC
E-cell	1200 mV vs. Ag/AgCl (ISAAC)
I-cell	about 60 nA

Recommendation

The advised configuration for this application is the ALEXYS Azithromycine Analyzer.

PART NUMBERS AND CONFIGURATION	
180.0086A	ALEXYS Azithromycin Analyzer

Formoterol in human urine and plasma

Introduction

Next to corticosteroid administration the use of b2-adrenergic drugs is quite common in asthma therapy and inhalation of these drugs gives acute relief in bronchoconstrictive situations. Formoterol is a typical example of the group of b2-adrenoreceptor agonists with interesting pharmacokinetic and -dynamic properties: high potency, rapid onset and long duration of action. There are two enantiomers of formoterol and the racemate is given although the RR-isomer is the active compound. Due to the high potency, quite small dosages are needed and hence extremely sensitive methods are required for formoterol analyses in bodily fluids. Like several other b2-adrenergic drugs formoterol is electrochemically active at a relatively low working potential, so the well-known sensitivity of LCEC provides an excellent tool to study the pharmacokinetics of formoterol in plasma and urine. Detection limits of 60 and 75 pmole/l were obtained for the RR and SS isomer respectively.

Method

A two-step extraction procedure is needed for a satisfactory clean-up of urine samples (1), whereas a one-step solid-phase extraction is sufficient for plasma (2). In case of urine, 100 µl of internal standard solution (RS isomer) was mixed with 100 µl of 250 mM phosphate (pH 8), added to 1 ml aliquot of urine sample, vortexed and 3 ml ethyl acetate was added. After tumbling and centrifugation the organic layer was removed and extracted on a preconditioned silica column with methanol. The eluate was evaporated to dryness under nitrogen, reconstituted in 100 µl mobile phase and injected onto the LCEC system. To separate the different isomers a chiral α1-acidglycoprotein column was used at ambient temperature.

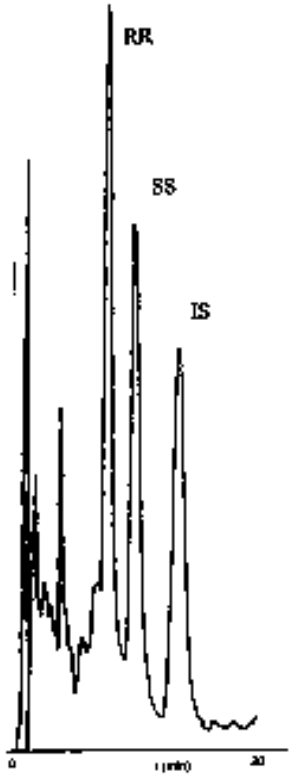


Fig. 1 Analysis of formoterol enantiomers (SS and RR) and its diastereoisomer as internal standard (IS). Concentrations in urine are 5.9 nmol/l each. Courtesy: ref. [1].

References

1. J.J. Butter, B.T.J. van den Berg, E.J.G. Portier, G. Kaiser and C.J. van Boxtel, J. Liq. Chrom & Rel. Technol. 19, (1996) 993 – 1005.
2. B.T.J. van den Berg, E.J.G. Portier, M. van den Berg, M.C.P. Braat and C.J. van Boxtel, Ther. Drug Monit. 16 (1994) 196 – 199.

Table 2

Conditions	
Column	α1-acidglycoprotein (AGP) column 100 x 4.0 mm, with guard 10 x 4.0 mm (Baker)
Flow rate	0.9 ml/min
Mobile phase	Phosphate buffer 50 mM, pH 7.0 with isopropanol (100 : 1.5, v/v), EDTA 75 µM
Sample	50 µl inj.
Temperature	ambient
I cell	1.3 nA
E-cell	630 mV (vs. Ag/AgCl sat'd)

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0035B	ALEXYS Analyzer – cooled
110.4105	VT03 flow cell, 3 mm GC, sb

Etoposide in biological matrices

Introduction

Cancer cells may become resistant to a variety of drugs with different structures or cellular targets, called multi-drug resistance. Particularly the blood-brain and the blood-cerebrospinal fluid (blood-CSF) barrier limit the access of drugs to the central nervous system and hence limit the effectiveness of anti-cancer drugs in CNS tumours.

In the current study etoposide has been used as a tool to investigate transport processes over the blood-CSF barrier in mice. It appears that Multidrug Resistance Related Protein 1 (MRP1) helps to limit distribution of xenobiotics like etoposide and converse multi-drug resistance (MDR). MRP 1 inhibitors as well as MDR-inhibitors can be used to improve the passage of anti-cancer drugs over the blood-CSF and the blood-brain barrier (1).

Due to the fact that etoposide is electrochemically active, the high sensitivity of LCEC could be fully exploited to study pharmacokinetic profiles of this drug in brains of MDR1a and MDR1b deficient mice (MRP1(+/+)) and MDR1a-MDR1b and MRP1 deficient mice (MRP1(-/-)).

Method

Etoposide was infused into the tail vein of MRP1(-/-) and MRP1(+/+) mice (60 mg/kg, 100 µl in 2 min). CSF was collected during a period of one hour after start of the infusion. Subsequently, the animals were decapitated and blood and brain were collected for etoposide analysis. Prior to analysis, CSF, brain tissue and plasma were stored at –30°C. All 3 matrices were analysed by LCEC but CSF without further pre-treatment. Plasma was diluted with phosphate buffer (50 mM, pH 6.0) and brain tissue was homogenised (0.2 g/ml phosphate buffer). Etoposide was extracted by means of diethylether/dichloromethane (2:1, v/v), evaporated to dryness, reconstituted in mobile phase and injected into the LCEC system.

Table 3

Conditions	
Column (1)	Spherisorb C18 100 x 0.8 mm ID, 5µm particles (CSF samples)
Flow rate (1)	40 µl/min
Mobile phase (1)	50 mM acetate (pH 3.8), 50 µM EDTA, acetonitril (3.8 : 1, v/v)
Column (2)	Alltech Econosil C18 250 x 4.6 mm ID, 10 µm particles (brain tissue and plasma samples)
Flow rate (2)	1.1 ml/min
Mobile phase (2)	50 mM acetate (pH 3.8), 50 µM EDTA, acetonitril (2 : 1, v/v)
Temperature	40°C
E-cell	900 mV (vs. Ag/AgCl sat'd)

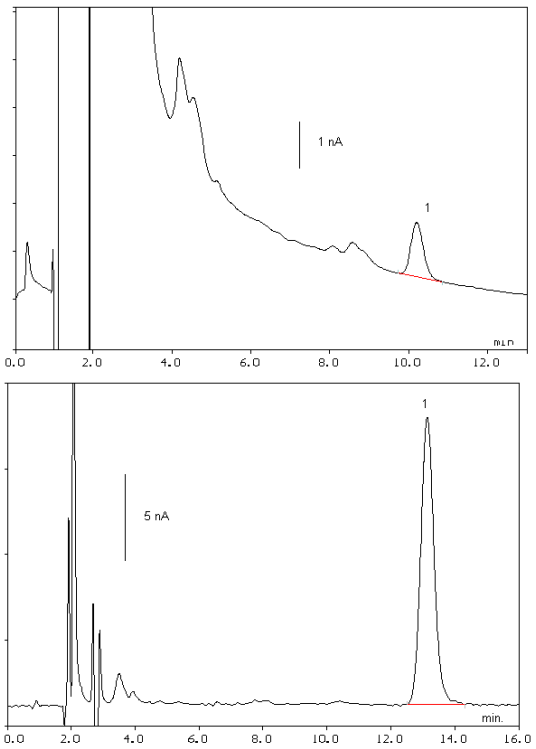


Fig. 1. Chromatogram of 4.2 µmol/l etoposide (1) in mouse plasma (bottom) and of 230 nmol/l in CSF (top). Courtesy: ref. [1].

References

1. J. Wijnholds, E.C.M. de Lange, G.L. Scheffer, D-J. van den Berg, C.A.A.M. Mol, M. van der Valk, A.H. Schinkel, R.J. Scheper, D.D. Breimer and P. Borst, J. Clin. Invest. 105, (2000), 279 – 285.

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0035B	ALEXYS Analyzer – cooled
110.4105	VT03 flow cell, 3 mm GC, sb

Artemisinin, dihydro-artemisinin and artemether in serum

Introduction

The artemisinin derivatives artemether and dihydro-artemisinin are considered to have potent anti-malarial properties. Several HPLC methods have been described for the analysis of these compounds in serum making use of reductive electrochemical detection [1-4]. It is well-known that reductive EC at relatively high potentials requires very efficient de-oxygenation, both of the mobile phase and sample to prevent interference of broad oxygen peaks in the chromatogram. It has been demonstrated that vacuum degassing falls short and more drastic measures must be taken to remove oxygen.

Although mercury as working electrode has been indicated to show the best selectivity in reductive LCEC, it has patently obvious disadvantages. Therefore, glassy carbon has been selected for the current application. It appears that the DECADE with VT-03 flow cell and glassy carbon working electrode gives excellent results regarding sensitivity (4 – 220 ng/ ml serum), selectivity and reproducibility, allowing strongly increased sample throughput with high accuracy [4].

Method

Both the mobile phase (60% acetate buffer, 0.1 M, pH 5) with 40% acetonitril (v/v) and the samples were thoroughly degassed with Helium of pro analyse quality (!). Back diffusion of oxygen was prevented by replacing all Teflon tubing through stainless steel except between column and flow cell where PEEK was applied. To prevent adsorption, all glass ware including the sample vials were silanised and subsequently rinsed with methanol. Serum samples with internal standard (25 µl of 1 µg/ml artemisinin) were extracted from 1 ml serum with 10 ml 1-chlorobutane/ethylacetate (9:1, v/v) evaporated to dryness under nitrogen, re-dissolved in 250 to 500 µl water/ethanol (50:50, v/v). After deoxygenation with Argon for 15 min, 100 µl was injected. Further LCEC conditions are given in table I.

LC.

Table 4

Conditions	
Column	Alltech Versa-Pack CN, 10 µm (300 x 4.6 mm ID)
Flow rate	1 ml/min
Mobile phase	60% acetate buffer 100 mM, pH 5.0, 40% ACN (v/v)
Sample	100 µl inj.
Temperature	30°C
I cell	20 to 50 nA
E-cell	-1.00 V (vs. Ag/AgCl sat'd)

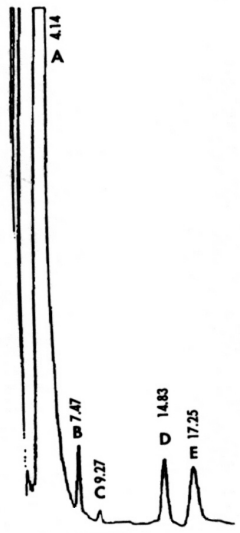


Fig. 1. Chromatogram of plasma spiked with 50 ng/ml artemether (E) and artemisinin (D). Other peaks are (A), a-dihydroartemisinin (B) and b-dihydroartemisinin (C). Courtesy: ref [4].

References

1. G. Edwards, Transactions of the Royal Society of Tropical Medicine and Hygiene 88 (1994) 37
2. V. Melendez, J.O. Peggin, T.G. Brewer and A.D. Theoharides, J. Pharm. Sci. 80 (1991) 132
3. N. Sandrenan, A.Sioufi, J. Godbillon, C. Netter, M. Donker and C. van Valkenburg, J. Chrom. (B) 691 (1997) 145.
4. M.A. van Agtmael, J.J. Butter, E.J.G. Portier and C.J. van Boxtel; Ther. Drug Monit. 20 (1998) 109-116

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0035B	ALEXYS Analyzer – cooled
110.4105	VT03 flow cell, 3 mm GC, sb

8-hydroxy-2'-deoxyguanosine in brain tissue DNA

Introduction

Oxidative damage to proteins and DNA occurs as a consequence of the generation of oxidants such as superoxide anions, hydrogen peroxide and hydroxyl radicals. 8-hydroxy-2'-deoxyguanosine (8-OH-dGuo) is considered to be a useful biomarker of oxidative DNA damage since its formation can also be induced by oxidative stress (1). The analysis of the pattern of DNA adduct formation is required for evaluation and comparison of the genotoxic response of chemicals in biological systems. The classical and quite expensive approach involves the use of radioactively labelled genotoxic agents. However, a number of DNA adducts appears to be electrochemically active (2). For this class of adducts the comparable, high sensitivity of LC-EC offers an excellent and inexpensive alternative. In this application note the method for 8-OH-dGuo is presented. Further details can be found in the paper of Te Koppele et al. (3).

Method

Human brain tissue DNA was obtained from clinically diagnosed Alzheimer's disease cases. After isolation and preparation of DNA samples, the DNA was hydrolysed and 50 µl aliquots were injected directly into the HPLC system. To quantitate normal DNA nucleosides and 8-OH-dGuo, isocratical separation occurred on a reverse phase column and the effluent was detected by UV and EC detection, connected in series, respectively. Reproducibility of the overall assay was validated with human and rat brain, processed as described above. The detection limit in these samples was 3 fmol per injection.

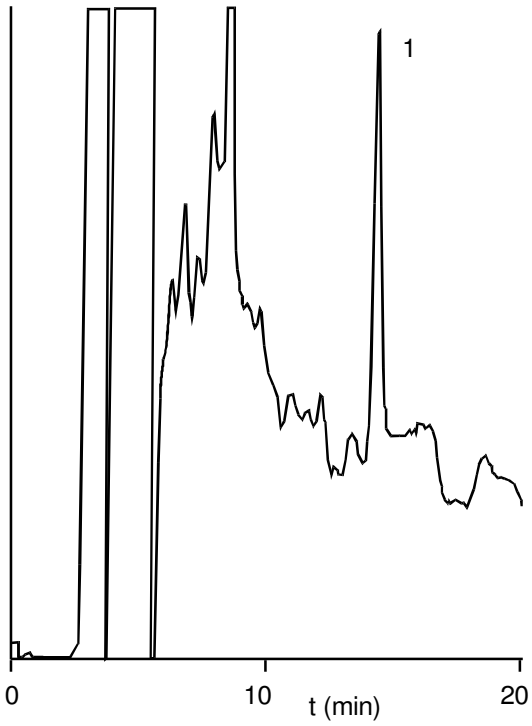


Fig. 1. Analysis of hydrolized DNA, isolated from human brain tissue. Peak 1 is 141 fmole 8-OH-dGuo. Courtesy: ref. [3].

References

1. R.A. Floyd and J.M. Carney; Ann. Neurol. Suppl. 32 (1992) S22-S27.
2. J.W. Park, C.K.Cundy and B.N. Ames; Carcinogenesis 10 (1989) 827-832.
3. J.M. te Koppele, P.J. Lucassen, A.N. Sakkee, J.G. van Asten, R. David, D.F. Swaab and C.F.A van Bezooijen; Neurobiology of Aging 17 (1996) 819-826.

Table 5

Conditions	
Column	Supelcosil LC-18-S, 250 x 4.6 mm, 5µm
Flow rate	1.0 ml/min
Mobile phase	50 mM citrate (pH=3.5) with 10% methanol (V/V)
Temperature	ambient
E-cell	0.70 V (vs. Ag/AgCl sat'd)

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0035B	ALEXYS Analyzer – cooled
110.4105	VT03 flow cell, 3 mm GC, sb

O^6-methylguanine in rat spleen DNA

Introduction

The analysis of the pattern of DNA adduct formation is required for evaluation and comparison of the genotoxic response of chemicals in biological systems. The classical and quite expensive approach involves the use of radioactively labelled genotoxic agents. However, a number of DNA adducts appears to be electrochemically active (Park et al., 1989). For this class of adducts the comparable, high sensitivity of LC-EC offers an excellent and inexpensive alternative. In this application note the method for O6-methylguanine is presented. Further details can be found in the paper of De Groot et al. (1994).

Method

Hydrolysed DNA, obtained from rat liver tissue, is injected on a strong cation exchange column (Partisil 10-SCX) and detected by UV absorption. The O6-methylguanine peak fraction is collected, freeze-dried, and twice lyophilised/re-dissolved in formic acid. Five µl of the formic acid solution is injected on the reversed phase column for LC-EC processing.

If the fraction collection step is omitted, strong interference may be expected from the relatively extremely high levels of guanine and adenine in the sample, leading to unfavourable detection conditions. With the present settings the detection limit is between 30 and 50 fmol on column.

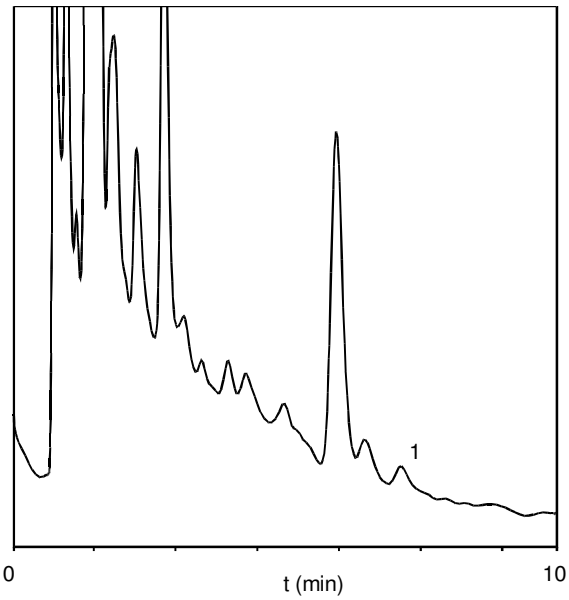


Fig. 2. Analysis of O6-MetGu (1) in rat spleen DNA. Concentration (amount) is 34 nmol/l (170 fmol). Courtesy: ref [1].

References

1. A.J.L. de Groot, J.G. Jansen, C.F.M. van Valkenburg and A.A. van Zeeland; Mutation Research 307 (1994) 61-66.
2. J.W. Park, C.K. Cundy and B.N. Ames; Carcinogenesis 10 (1989) 827-832.

Table 6

Conditions	
Column	Chrompack Hypersil ODS, 100 x 3 mm, 5µm
Flow rate	0.60 ml/min
Mobile phase	20 mM potassium phosphate (pH=6.0) with 5% methanol
Temperature	ambient
Sample	5 µl injection, purified and hydrolysed rat spleen DNA, treated with methylating agent
I-cell	20 nA
E-cell	1150 mV (vs. Ag/AgCl sat'd)

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0035B	ALEXYS Analyzer – cooled
110.4105	VT03 flow cell, 3 mm GC, sb

8-Hydroxydeoxyguanosine in lymphocyte DNA

Introduction

The analysis of the pattern of DNA adduct formation is required for evaluation and comparison of the genotoxic response of chemicals in biological systems. The classical and quite expensive approach involves the use of radioactively labelled genotoxic agents. However, a number of DNA adducts appears to be electrochemically active (Park et al., 1989). For this class of adducts the comparable, high sensitivity of LC-EC offers an excellent and inexpensive alternative. In this application note the method for 8-hydroxydeoxyguanosine is presented. Further details can be found in the paper of De Groot et al. (1994).

Method

Hydrolysed DNA, obtained from lymphocytes of a non smoking volunteer, is injected on a strong cation exchange column (Partisil 10-SCX) and detected by UV absorption. The 8-hydroxydeoxyguanosine peak fraction is freeze-dried, and twice lyophilised and re-dissolved in formic acid. Ten µl of the formic acid solution is injected on the reversed phase column for LC-EC processing.

If the fraction collection step is omitted, strong interference may be expected from the relatively extremely high levels of guanine and adenine in the sample, leading to unfavourable detection conditions. With the present settings the detection limit is between 30 and 50 fmol on column.

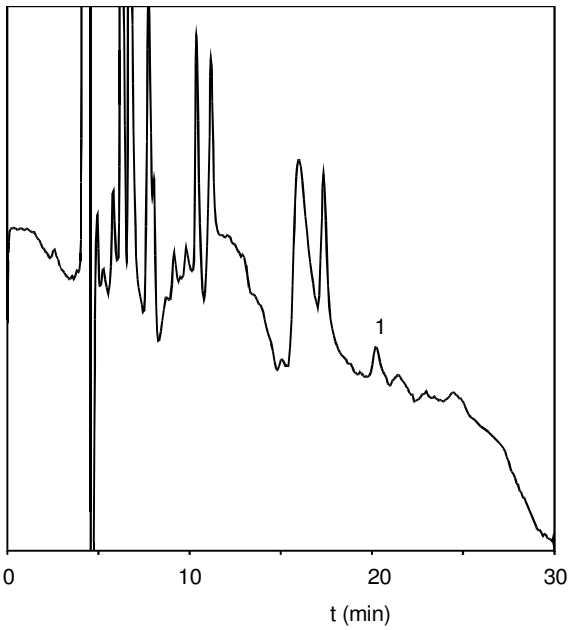


Fig. 2. Analysis of 8-OH-dGuo (1) in lymphocyte DNA of a non-smoking volunteer. Concentration (amount) is 4 nmol/l (40 fmol). Courtesy: ref [1].

References

1. A.J.L. de Groot, J.G. Jansen, C.F.M. van Valkenburg and A.A. van Zeeland; Mutation Research 307 (1994) 61-66.
2. J.W. Park, C.K. Cundy and B.N. Ames; Carcinogenesis 10 (1989) 827-832.

Table 7

Conditions	
Column	Supelcosil LC-18S, 250 x 4.6 mm, 5 µm
Flow rate	0.60 ml/min
Mobile phase	50 mM Citrate buffer, pH 3.5, EDTA 15 µM, 10% methanol
Sample	10 µl injection, enzymatically hydrolysed DNA from lymphocytes of a non-smoking volunteer
Temperature	ambient
E-cell	850 mV (vs. Ag/AgCl sat'd)
I-cell	4 nA

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0035B	ALEXYS Analyzer – cooled
110.4105	VT03 flow cell, 3 mm GC, sb

7-methylguanine in rat spleen DNA

Introduction

The analysis of the pattern of DNA adduct formation is required for evaluation and comparison of the genotoxic response of chemicals in biological systems. The classical and quite expensive approach involves the use of radioactively labelled genotoxic agents. However, a number of DNA adducts appears to be electrochemically active (Park et al., 1989). For this class of adducts the comparable, high sensitivity of LC-EC offers an excellent and inexpensive alternative. In this application note the method for 7-methylguanine is presented. Further details can be found in the paper of De Groot et al. (1994).

Method

Hydrolysed DNA, obtained from rat liver tissue, is injected on a strong cation exchange column (Partisil 10-SCX) and detected by UV absorption. The 7-methylguanine peak fraction is freeze-dried, and twice lyophilised/re-dissolved in formic acid. Five µl of the formic acid solution is injected on the reversed phase column for LC-EC processing.

If the fraction collection step is omitted, strong interference may be expected from the relatively extremely high levels of guanine and adenine in the sample, leading to unfavourable detection conditions. With the present settings the detection limit is between 30 and 50 fmol on column.

References

1. A.J.L. de Groot, J.G. Jansen, C.F.M. van Valkenburg and A.A. van Zeeland; Mutation Research 307 (1994) 61-66.
2. J.W. Park, C.K. Cundy and B.N. Ames; Carcinogenesis 10 (1989) 827-832.

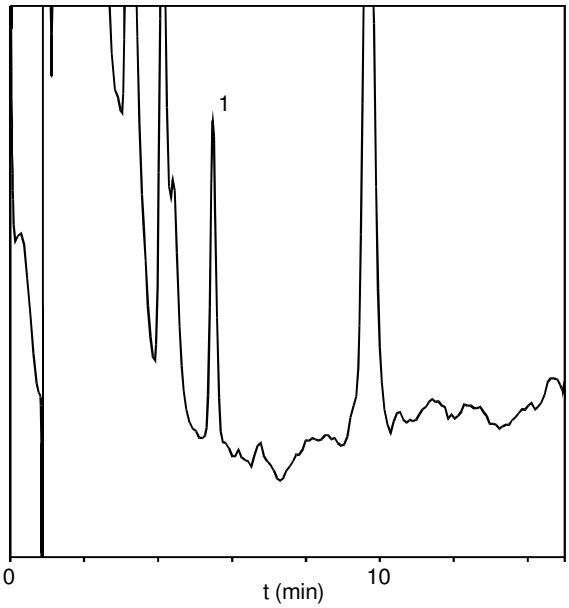


Fig. 2. Analysis of 7-MetGu (1) in rat spleen DNA. Concentration (amount) is 21 nmol/l (105 fmol).Courtesy: ref [1].

Table 8

Conditions	
Column	Chrompack Hypersil ODS, 100 x 3 mm, 5µm
Flow rate	0.60 ml/min
Mobile phase	20 mM potassium phosphate (pH=6.0) with 2% methanol
Temperature	ambient
Sample	5 µl injection, purified and hydrolysed rat spleen DNA, treated with methylating agent
I-cell	20 nA
E-cell	1150 mV (vs. Ag/AgCl sat'd)

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0035B	ALEXYS Analyzer – cooled
110.4105	VT03 flow cell, 3 mm GC, sb

Vitamin K in plasma using microbore LC-EC

Introduction

Vitamin K is a fat soluble vitamin involved in several biochemical processes as a cofactor in carboxylation reactions. An example is the clotting of the blood.
Vitamin K is necessary for the proper formation of the blood plasma protein prothrombin, the inactive precursor of thrombin. This enzyme converts the protein fibrinogen of blood plasma into fibrin, the insoluble, fibrous protein that holds blood clots together. The K vitamins are a group of several closely related molecules, consisting of a naphthoquinone skeleton with an isoprene side chain of differing length.

Method

Several assays have been described for the analysis of vitamin K. The K vitamins occur at very low concentrations in plasma (0.1 - 4 ng/ml). Therefore, a sensitive detection method such as EC detection is required. To improve the selectivity of the method, a 2 step sample pre-treatment is used consisting of a liquid-liquid extraction (LLE) followed by a solid phase extraction (SPE). Before EC detection the quinone moiety is converted into a quinol, by a post-column reactor flow cell.

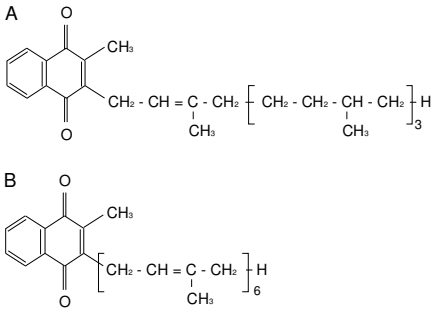


Fig. 1 Two major forms of vitamin K. (A) Vitamin K1 (phylloquinone) is found in plants. (B) Vitamin K2 (menaquinone-6) is found in animals.

Table 9

Conditions	
Column	Higgins Spherisorb ODS2 50 x 2.1 mm, 5 µm
Flow rate	0.2 ml/min
Mobile phase	0.1 M lithiumperchlorate in methanol with 4% water
Sample	plasma after LLE, SPE, evaporation and reconstitution in mobile phase
Temperature	30 °C
E-cell	reactor: -500 mV, detection: 300 mV (vs. Hy-REF)

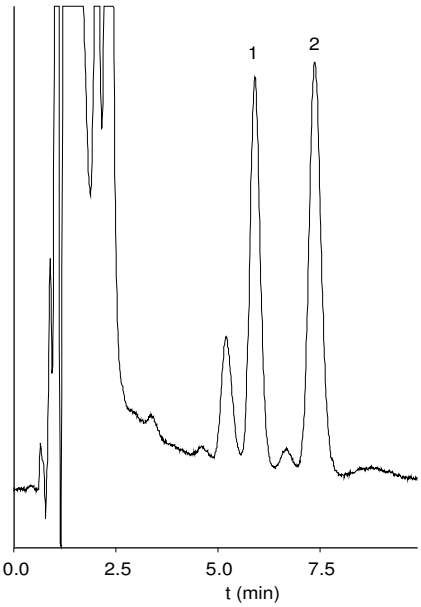


Fig. 2 Analysis of vitamin K1 (1) in plasma extract. Concentrations are 2.5 ng/ml for vitamin K1 and 5 ng/ml for internal standard 2,3 hydrophylloquinon (2).

References

1. M.J. Shearer, Adv. in Chromatogr. 21 (1983) 243 - 301
2. J.P. Langenberg and U.R. Tjaden, J. Chromatogr. 305 (1984) 61 - 72

Recommendation

The advised configuration for this application is the ALEXYS Vitamin K Analyzer using an additional cell for pre-reduction of the quinones.

PART NUMBERS AND CONFIGURATION	
180.0072B	ALEXYS Vitamin K Analyzer

Analysis of Q10, ubiquinols, vitamin E, and ß-carotene in human LDL

Introduction

Coenzyme Q10 is one of a number of naturally occurring ubiquinones that act as electron acceptors in mitochrondrial oxidative phosphorylation. The reduced forms, the ubiquinols, are strong anti-oxidants. Also, oxidation of LDL (low-density lipoproteins) is believed to play an important role in early atherosclerosis. According to this oxidation hypothesis LDL is protected against oxidative stress by i.a. the Q10 antioxidants, thereby slowing down the formation of modified LDL. More specifically, the potent lipophilic antioxidants α-TOH (tocoferol, vitamin E), QH2-10 (ubiquinol-10, the reduced form of ubiquinone-10 or Q10), β-carotene and lycopene are supposed to be the important factors in this protection process. There is no unanimity about the relative physiological importance of these compounds.
The analysis by liquid chromatography with oxidative electrochemical detection (LC-EC) is very attractive because of its sensitivity and selectivity for the reduced compounds. However, due to the high lipophilicity of the compounds of interest very high modifier concentrations are required in the mobile phase putting specific demands to the LC-EC analysis to maintain sufficient electrical conductivity of the mobile phase. The method described here provides the conditions required. Detailed data regarding the method are given in [1].

Method

Blood samples were collected into evacuated tubes containing K3-EDTA and placed on ice in the dark. Within 2 hours the plasma was separated by centrifugation. Subsequently, ultra-centrifugation was performed to isolate the LDL. Prior to extraction BHT (butylated hydroxytoluene) as antioxidant (250 µg/ml) was added and all treatments were done in a nitrogen atmosphere.

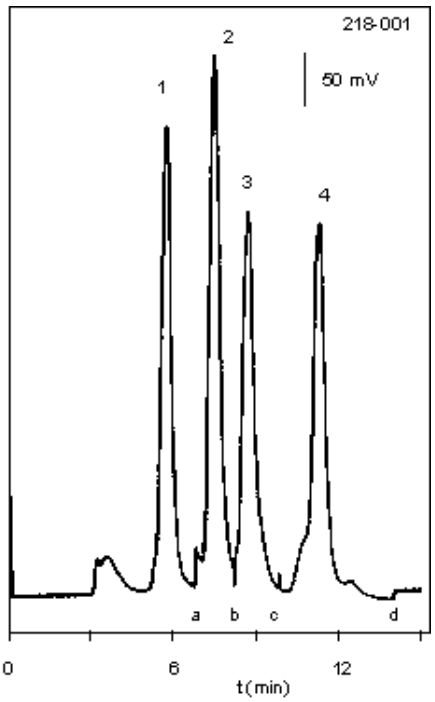


Fig. 1. Analysis of human LDL. Concentrations (amounts) are: 1. a-tocopherol (vitamin E) 3.6 µmol/l (72.9 pmol), 2. ubiquinol-9 0.77 µmol/l (15.5 pmol), 3. ubiquinol-10 0.21 µmol/l (4.2 pmol), and 4. b-carotene 0.11 µmol/l (2.2 pmol). The range is programmed at 10 (t=0), 1 (a), 0.5 (b), 1 (c) and 10 nA/V (d). Courtesy ref [1].

Samples (200 µl) were mixed with 2 ml methanol, 4 ml hexane was added, vortex mixed, centrifuged, the hexane layer collected and the procedure was repeated. The 2 hexane layers were pooled, dried with nitrogen, the residue stored at –20°C and dissolved in mobile phase just prior to analysis. See [1] for further details.

References

1. Yolanda B. de Rijke et al., Arterioscler. Thromb. Vasc. Biol. 17 (1997) 127-133

Table 10

Conditions	
Column	Inertsil ODS-2, 5 µm, 200 x 3 mm
Flow rate	0.35 mL/min
Sample	20 µl extracted from human plasma
Mobile phase	22.5% methanol, 77.5 % ethanol/isopropanol (95/5), 20 mM LiClO4
Temperature	30 °C for column and flow cell
E-cell	600 mV vs. Ag/AgCl (sat'd with LiCl)

Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0035B	ALEXYS Analyzer – cooled
110.4305	VT03 flow cell, 3 mm GC, HyREF

Analysis of Mesna and its pro-drug BNP7787

Introduction

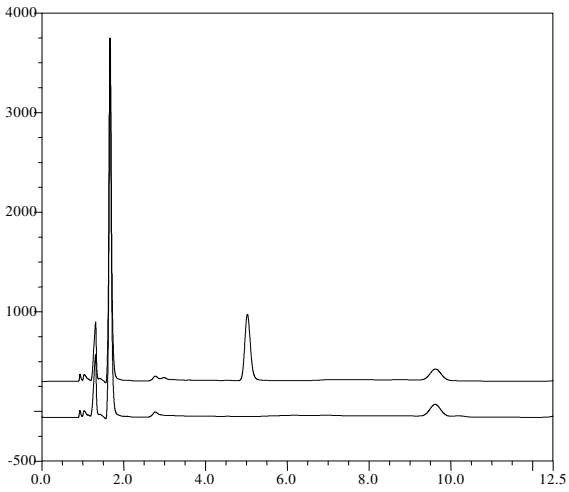
Cisplatin is an important and frequently used compound for the treatment of solid tumours. Administration of the disulfide BNP7787 is considered to protect against cisplatin-induced toxicities. The active compound is the metabolite mesna, which is selectively formed in kidney, intestine and bone marrow by enzymatic reduction (fig. 1). The high local concentrations of mesna can inactivate cisplatin by complex formation without interference with the anti-tumour efficacy of cisplatin.
LC-EC is preferred to determine mesna and its disulfide in plasma and urine (1,2). This provides a sensitive and selective determination due to the oxidation of the thiol group present in the mesna molecule. BNP7787 can be detected indirectly by reducing the disulfide to mesna first. The combination of an Au working electrode and a low working potential is responsible for the high sensitivity and selectivity (2). Hence, sample pre-treatment can be kept simple and can be limited to de-proteinisation of plasma or dilution with mobile phase in case of urine. The limits of detection for BNP7787 and mesna in plasma are 1.6 and 0.63 µM resp. and in urine 0.8 and 1.6 µM resp. (2). The current LC-EC method can be applied to determine the mesna and BNP7787 concentration in plasma and urine samples of patients treated with mesna or BNP7787 (2).

Method

One volume of EDTA plasma sample was added to 1 volume of 0.33 M H_2SO_4 and 1 volume (5 g/100 ml) of Na-hexametaphosphate. After centrifugation the supernatant was transferred and stored at –20°C until use. The urine samples were collected in bottles containing 1g/l EDTA and 0.2 M HCl. These samples were measured after a 50-fold dilution with mobile phase.

Table 11

Conditions	
Column	Phenomenex 5 ODS-4 100 x 4.6 mm with pellicular C18 pre-column
Flow rate	1 ml/min
Mobile phase	Na-citrate (0.1 M), Tetrabutylammonium dihydrogen phosphate (1 mM), cysteamine (0.1 µM), adjusted to pH 3.5 with 85% phosphoric acid
Sample	plasma after deproteinization or urine after dilution with mobile phase
Temperature	36°C
Ecell	0.85 V (vs. Ag/AgCl sat'd)



Representative chromatogram of a patient plasma sample collected before the start (A) and at the end of the 15-min i.v. infusion of 41 g/m^2 BNP7787 (B). The measured mesna concentration in the patient sample was 74.5 µM.

Calibration and QC samples were derived by spiking deproteinized plasma or urine of healthy volunteers in duplicate on the day of use. Pre-reduction of BNP7787 was done with Na-borohydride. Method validation was done by measuring recovery, lower limit of quantitation, linearity and within- and between-day accuracy and precision.
Cysteamine was added to the mobile phase to prevent possible adsorption of mesna to the stationary phase.

References

1. El-Yazigi et al. Ther. Drug Monit. 17 (1995) 153
2. M. Verschraagen et al., J. Chromatogr. 753 (2001) 293

Recommendation

The advised configuration for this application is the ALEXYS Disulfides Analyzer.

PART NUMBERS AND CONFIGURATION	
180.0069B	ALEXYS Disulfides Analyzer

Vincristine in human serum

Introduction

Vincristine is an anti-neoplastic Vinca alkaloid used in the treatment of various childhood and adult malignancies like acute lymphocytic leukemia, lymphoma, sarcoma and neuro-blastoma (1). Although vincristine administration is part of an established therapy for childhood malignancies, the pharma-cokinetic profile of this very potent cytotoxic drug was not well known. This is probably due to limitations in the analytical tools available to measure vincristine (2). To study pharmacokinetics in pediatric patients a highly selective and sensitive method is required. It appears that vincristine can be analysed by LCEC under favourable conditions with high sensitivity (1 µg/L) but in the procedure a tedious sample pre-treatment was required (3). In the current study a simple and rapid LCEC method for determining vincristine in serum is described. High reproduci-bility (C.V. better than 5%) and sensitivity (0.3 µg/L) is re-ported, fully meeting the requirements for clinical pharmacoki-netic studies in children.

Method

Blood samples were centrifuged and the serum (1.2 ml) was stored at –80°C in the dark. Immediately after thawing samples were processed for analysis. Internal standard (100 µl of 2.0 mg/L vinblastine in water) was added, mixed and the mixture was centrifuged during 5 min at 1,500 g. After centrifugation 1.0 ml was injected onto the C18 pre-concentration column and washed during 10 min with water/methanol. After 10 min the solvent selector valve was switched and the mobile phase was back-flushed through this column leading to desorption and transport to the C18 analytical column. The solvent selec-tor valve was switched back after 5 min. Both columns were re-conditioned for 10 min and the compounds of interest were eluted within 8 min.
Note: the system settings described in (4) have been further optimised (see also Table I).

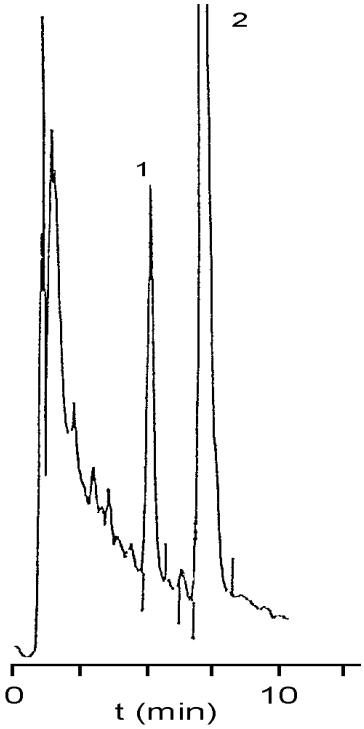


Fig. 1. Analysis of plasma of a patient treated with 1.5 mg/m2 vincristine. Concentrations: 8.3 ng/ml vincristine (1) and 20.4 ng/ml vinblastine (2, IS). Range: 100 nA full scale. Courtesy: ref. [4].

References

1. W.R. Crom, A.M. Glynn-Barnhart, J.H. Rodman, M.E. Teresi, R.E. Kavanagh, M.L. Christensen, M.V. Relling and W.E. Evans, Clin. Pharmacokin. 12 (1987) 168.
2. V.S. Sethi, S.S. Burton and D.V. Jackson, Cancer Chemother. Pharmacol. 4 (1980) 183.
3. D.E.M.M. Vendrig, J. Teeuwsen and J.J.M. Holthuis, J. Chromat. 424 (1988) 83
4. H. Bloemhof, K.N. van Dijk, S.S.N. de Graaf, D.E.M.M. Vendrig and D.R.A. Uges, J. Chromat. 572 (1991) 171 –179.

Table 12

Conditions	
Preconc. Column (1)	Chrompack C18 ODS (100 x 4.6 mm)
Guard column (2)	Whatman ODS 30 µm (100 x 3 mm)
Flow rate (1 and 2)	1 mL/min
Sample	1 mL
Mobile phase (1)	water/methanol (95 : 20, v/v)
Mobile phase (2)	methanol/acetonitrile/25 mM phosphate pH 7.0, 50:15:35, v/v/v
Temperature	30 °C for column and flow cell
E-cell	0.70 V (vs. Ag/AgCl sat'd)

Recommendation

The advised configuration for this application is an ALEXYS Analyzer using an autosampler with 10-PV, two pumps and sample cooling option.

PART NUMBERS AND CONFIGURATION	
180.0090B	ALEXYS Analyzer for Vincristine
110.4105	VT03 flow cell, 3 mm GC, sb