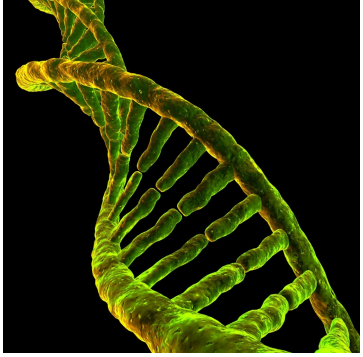
Application Note

Clinical & Diagnostic



The most selective LC-EC applications for Clinical & Diagnostics analysis

Catecholamines
Serotonin
Metanephrines
VMA
HVA
5-HIAA

PET imaging tracer
Fluorodeoxyglucose (FDG)
FDG impurities

Sulfides
Homocysteine
Glutathione
Disulfides

Vitamins, minerals
A, C, D, E, and K
Iodide
Q10
Ubiquinols

DNA adducts
8-hydroxy-2'-deoxyguanosine
O^6-methylguanine
8-Hydroxydeoxyguanosine
7-Methylguanine

DNA adduct analysis

- **ALEXYS Analyzers for analysis of DNA adducts**
- **Optimized for performance**
- **Dedicated system solutions**
- **Reproducible and robust**

Oxidative damage to proteins and DNA occurs as a consequence of the generation of oxidants such as superoxide anions, hydrogen peroxide and hydroxyl radicals. For example, 8-hydroxy-2'-deoxyguanosine (8-OH-dGuo) is considered to be a useful biomarker of oxidative DNA damage since its formation can 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 evaluation approach involves the use of radioactively labelled genotoxic agents. An excellent and inexpensive analytical alternative is LC separation followed by electrochemical detection as a number of DNA adducts appears to be electrochemically active (2), for example 8-hydroxy-2'-deoxyguanosine, O^6-methylguanine and 7-methylguanine for which example chromatograms are shown in this note.

This note shows a selection of example chromatograms from the work of a few of our many detector users to support the applicability of the electrochemical approach and the use of our detectors in particular.

Methods

This section shows a summary of methods and is followed by example chromatograms for various DNA adducts in different samples.

Method summary according to reference [3]:

Human brain tissue DNA was obtained from clinically diagnosed Alzheimer's disease cases. After isolation and preparation of DNA samples, the DNA was hydrolyzed, and 50 µL aliquots were injected directly into the HPLC system. To quantitate normal DNA nucleosides and 8-OH-dGuo, isocratic separation was done on a reverse phase column and the effluent was analyzed with serially connected UV and EC detectors. The assay was validated with human and rat brain samples, and the detection limit in these samples was 3 fmol per injection on column. More details related to the method and the research can be found in the paper of Te Koppele *et al.* (3).

Method summary according to reference [4]:

A sample clean-up step was applied before analysis. Hydrolyzed DNA, obtained from various sample sources was injected on a strong cation exchange column (Partisil 10-SCX) and analyzed by UV absorption. The relevant peak fraction was collected, freeze-dried, and twice lyophilized/re-dissolved. If the fraction collection step is omitted, strong interference may be expected from the relatively high levels of guanine and adenine in the sample, leading to unfavorable detection conditions. When using the sample clean-up step before the analysis, the detection limit is between 30 and 50 fmol on column.

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

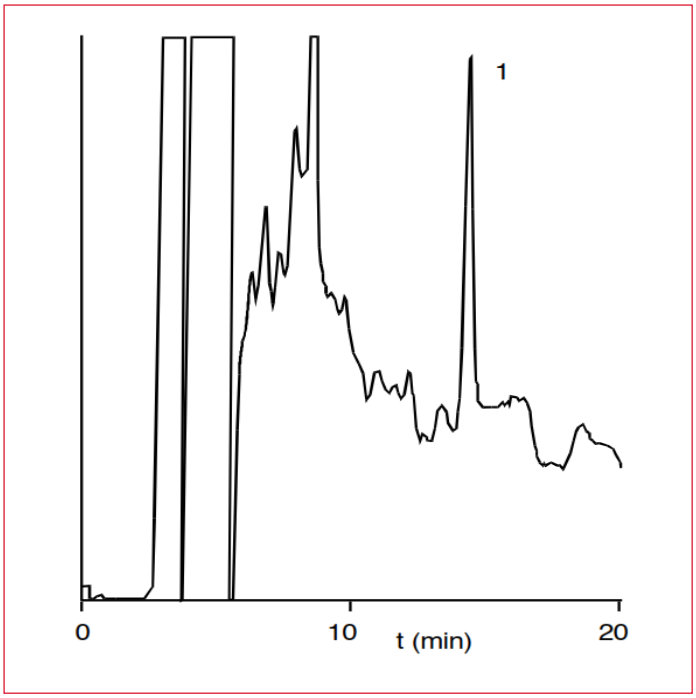


Figure 1: Analysis of hydrolyzed DNA, isolated from human brain tissue. Peak 1 is 141 fmole 8-OH-dGuo. Courtesy: ref. [3].

Table 1

LC-ECD conditions	
Columns	Supelcosil™ LC-18 HPLC Column, 5 µm particle size, L x I.D. 25 cm x 4.6 mm (MilliporeSigma)
Mobile phase (MP)	50 mM citrate, pH 3.5, 10% methanol (v/v)
Flow rate	1 mL/min
Temperature	Ambient
E_{cell}	0.70 V on glassy carbon (vs. Ag/AgCl sat'd)

O^6-methylguanine in rat liver DNA

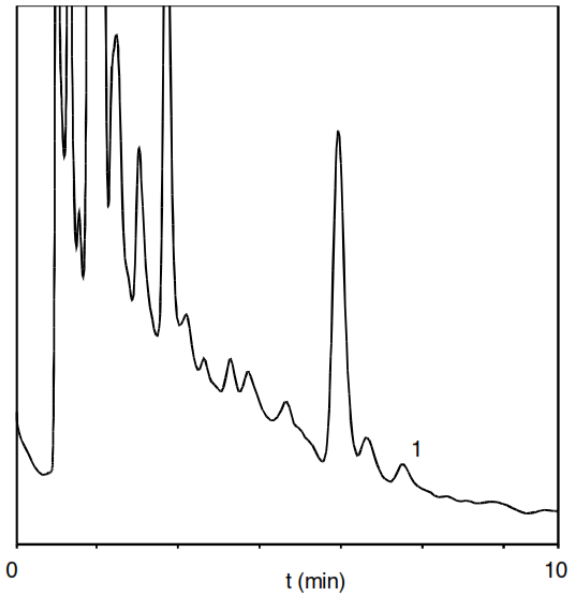


Figure 2: Chromatogram of purified and hydrolyzed rat liver DNA, treated with methylating agent. Peak 1 is 34 nmol/L (170 fmol) O^6-MetGu.
Injection volume: 5 µL. Courtesy: ref [4]

Table 2

LC-ECD conditions	
Column	Hypersil™ ODS, 5 µm particle size, L x I.D. 10 cm x 3 mm (Chrompac)
Mobile phase (MP)	20 mM potassium phosphate (pH=6.0), 5% MeOH
Flow rate	0.6 mL/min
Temperature	Ambient
E_{cell}	1.15 V on glassy carbon (vs. Ag/AgCl sat'd)
I_{cell}	About 20 nA

8-Hydroxy-deoxyguanosine in lymphocyte DNA

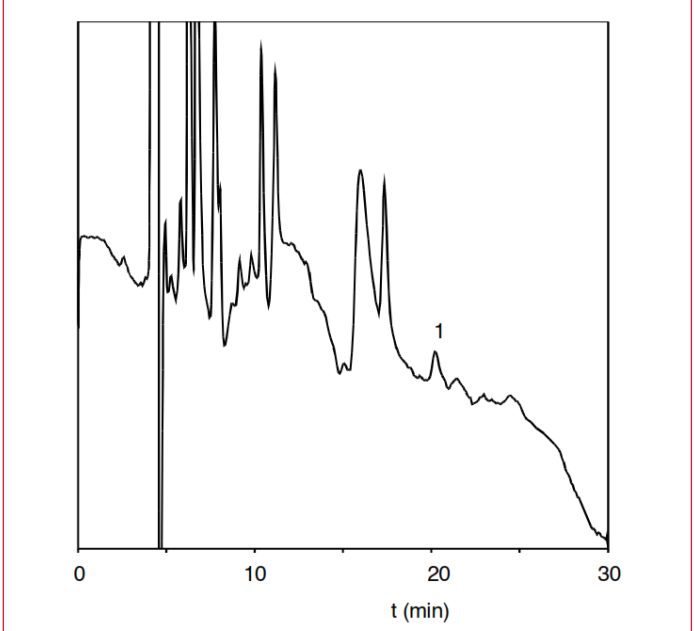


Figure 3: Chromatogram of purified and hydrolyzed lymphocyte DNA of a non-smoking volunteer. Peak 1 is 4 nmol/l (40 fmol) 8-OH-dGuo.
Injection volume: 10 µL. Condition see Table 3. Courtesy: ref [4]

Table 3

LC-ECD conditions	
Columns	SUPELCOSIL™ LC-18 HPLC Column, 5 µm particle size, L x I.D. 25 cm x 4.6 mm (MilliporeSigma)
Mobile phase (MP)	50 mM citrate, pH 3.5, 15 µM EDTA,10% methanol
Flow rate	1 mL/min
Temperature	Ambient
E_{cell}	0.85 V on glassy carbon (vs. Ag/AgCl sat'd)
I_{cell}	About 4 nA

7-methylguanine in rat spleen DNA

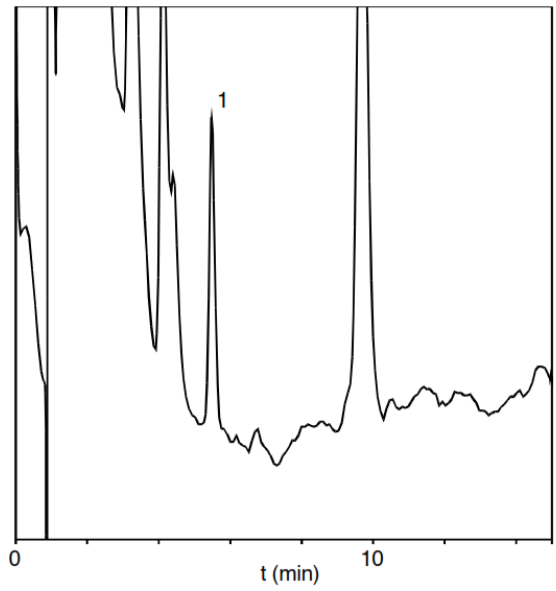


Figure 4: Chromatogram of purified and hydrolyzed rat spleen DNA treated with methylating agent. Peak 1 is 21 nmol/l (105 fmol) 7-MetGu. Injection volume: 5 µL. Conditions see Table 4. Courtesy: ref [4]

Table 4

LC-ECD conditions	
Column	Hypersil™ ODS, 5 µm particle size, L x I.D. 10 cm x 3 mm (Chrompac)
Mobile phase (MP)	20 mM potassium phosphate (pH=6.0), 2% MeOH
Flow rate	0.6 mL/min
Temperature	Ambient
E_{cell}	1.15 V on glassy carbon (vs. Ag/AgCl sat'd)
I_{cell}	About 20 nA

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.
4. A.J.L. de Groot, J.G. Jansen, C.F.M. van Valkenburg and A.A. van Zeeland; Mutation Research 307 (1994) 61-66.

Recommended LC-ECD hardware

The advised analytical configuration for this application is the ALEXYS Analyzer with ECD and SenCell with glassy carbon electrode and saltbridge reference (UV detector is optionally available), and an autosampler with sample cooling option.

Figure 5: Recommended instrument configuration for the applications: the ALEXYS Analyzer.
The system consists of a P6.1L pump with integrated degasser, an AS110 autosampler, and the DECADE Elite electrochemical detector.

Ordering information

Detector only	
176.0035A	DECADE Elite SCC electrochemical detector
116.4120	SenCell 2 mm GC sb
Recommended ALEXYS analyzer + parts	
180.0035W	ALEXYS Cool base
116.4120	SenCell 2 mm GC sb
Software	
195.0035#	Clarity CDS single instr. incl LC, AS module

#) optional: Antec ECD drivers for use with Chromeleon CDS , OpenLAB CDS or OpenLAB Chemstation CDS are available.

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