

# Analysis of a Promutagenic Exocyclic DNA Adduct in Human Urine by High Performance Liquid Chromatography APCI Tandem Mass Spectrometry

*A.-M. Hoberg and H. E. Poulsen*

Dept. Clinical Pharmacology Q.7642, Rigshospitalet, DK-2100 Copenhagen Ø, Denmark

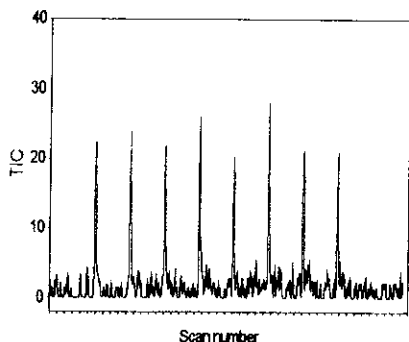
## Introduction

The hunt for specific biomarkers to monitor and assess the importance of oxidative stress in humans has been greatly amplified over the past decade. Adducts from chemically modified DNA are being pointed out as suitable biomarkers due to their implication in the carcinogenesis pathway. Exocyclic DNA adducts have been reported as being potent promutagenic species and probably indicative of endogenous oxidative damage. Aldehyde products from lipid peroxidation of  $\omega$ -6 unsaturated fatty acids, are thought to be important precursors forming 5- or 6-member rings with nucleic acids characteristic for etheno-adducts and the malondialdehyde adduct on deoxyguanosine, M<sub>1</sub>GdR. Modified genomic DNA is a measure for the steady-state level from formation and repair/removal. Free modified nucleosides excreted in urine are a measure for the total body induced damage under steady state condition. We present a method incorporating on-line purification, detection and quantification of cyclic DNA adducts in crude bio-extracts with prospects for rapid high throughput analysis.

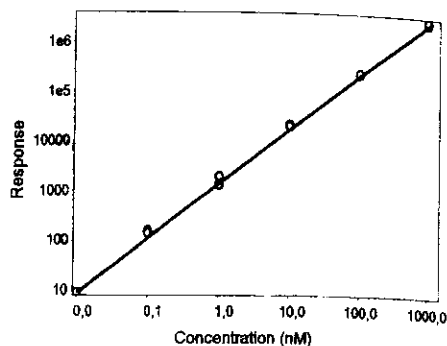
## Experimental & Discussion

A key problem in assaying excreted etheno adducts is their low level in conjunction with the complexity of urine. We have developed an assay for determinations of the etheno adduct 1,N<sup>6</sup>-ethenodeoxyadenosine ( $\epsilon$ dA) in urine using high performance liquid chromatography in combination with tandem mass spectrometry. The method is based on on-line purification and concentration with use of column switching valves, two HPLC columns and direct injection of raw urine.  $\epsilon$ dA was analysed using atmospheric pressure chemical ionisation (APCI) in the positive ion mode with multiple reaction monitoring (MRM) in a triple quadrupole mass spectrometer. Quantitation was performed on the characteristic transition  $m/z$  276.3(MH<sup>+</sup>)  $\rightarrow$  160.3(BH<sub>2</sub><sup>+</sup>). Promising results have been obtained with  $\epsilon$ dA standard with regard to sensitivity. Figure 1 show eight repeated injections of 10  $\mu$ L 0.1 nM  $\epsilon$ dA standard indicating the limit of detection is below

1 fmole. The standard curve is displayed in Figure 2 revealing an excellent response linearity over several magnitudes without the use of internal standard.

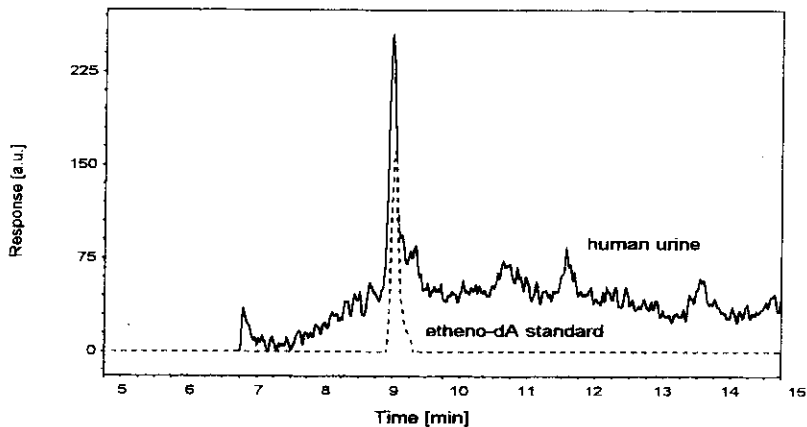


**Figure 1** Injections of 1 fmole  $\epsilon$ DA



**Figure 2** Standard curve  $\epsilon$ DA

Human spot urine was collected from a healthy volunteer and analysed day-fresh. Figure 3 show the chromatogram obtained from injection of 100  $\mu$ L urine. The compound eluting after 9 minutes could be identified as  $\epsilon$ DA after comparing with standard. The peak area indicate the  $\epsilon$ DA concentration was about 150 pM in the spot urine.



**Figure 3** LC-MSMS chromatograms of  $\epsilon$ DA standard and human urine

To the best of our knowledge, this is the first report on  $\epsilon$ DA measured in human urine by use of LC-MSMS. Time consumed per sample was 25 minutes in total which in principle would enable analysis of >50 samples/24 hours. The use of internal standard will be required for precise quantitative measurements.