ELSEVIER

Journal of Chromatography B, 697 (1997) 243–249

JOURNAL OF CHROMATOGRAPHY B

Short communication

# Detection of 8-hydroxydeoxyguanosine in K562 human hematopoietic cells by high-performance capillary electrophoresis

Oliver Ullrich, Tilman Grune*

*Clinics of Physical Medicine and Rehabilitation, Medical Faculty (Charité), Humboldt University Berlin, Hessische Strasse 3-4, 10115 Berlin, Germany*

**Abstract**

A method for the detection of 8-hydroxydeoxyguanosine by high-performance capillary electrophoresis (HPCE) was developed. Separations were performed in an uncoated silica capillary (44 cm×75 μm I.D.) with a P/ACE system with diode-array detector. The separation of purine deoxynucleosides and 8-hydroxydeoxyguanosine was optimized with regard to pH, temperature, applied potential and hydrodynamic injection time. Optimum conditions were 20 m*M* borate buffer (pH 9.5), 25°C, 25 kV, 20 s load and detection at 254 nm. This method allowed the detection of 8-hydroxydeoxyguanosine in the presence of a $10^5$-fold higher amount of deoxyguanosine. Isolated nuclei from K562 human hematopoietic cells were treated with 15 m*M* hydrogen peroxide for 2 h. The nuclei were extensively dialyzed and DNA was isolated, enzymatically hydrolyzed to the deoxynucleosides and analyzed by HPCE. DNA from hydrogen peroxide treated nuclei had a 4-fold higher content of 8-hydroxydeoxyguanosine than untreated controls. HPCE analysis of 8-hydroxydeoxyguanosine is fast and simple. Furthermore, it requires a very small sample volume, which makes it useful for biomedical and clinical applications.


*Keywords:* 8-Hydroxydeoxyguanosine

## 1. Introduction

8-Hydroxydeoxyguanosine is one of the major oxidatively modified DNA base products and reported to be formed by hydroxyl radicals, singlet oxygen or photodynamic action [1,2]. 8-Hydroxydeoxyguanosine (8-OH-dG) in DNA induces targeted G:C-to-T:A transversions unless repaired prior to DNA replication in vitro [3] and in vivo [4–6]. The 8-OH-dG-level is a commonly used biomarker of oxidative DNA damage and therefore suitable for in vitro testing of the genotoxicity of various substances.

8-OH-dG is usually detected by high-performance liquid chromatography (HPLC) with electrochemical detection [7,8]. High-performance capillary electrophoresis (HPCE) is a rapidly growing separation technique which extends the application range of conventional electrophoresis to small molecules. HPCE is highly efficient (number of theoretical plates $N>10^5$) and requires only very small sample volumes (1 to 50 nl). Thus HPCE could be very useful for detection of 8-OH-dG in biological samples available in small amounts [9,10]. HPCE analysis may be useful for many biomedical and clinical applications, because of the automated instrumentation and usually simple sample pretreatment. This study is a first approach to the development of an

*Corresponding author.


*PII* S0378-4347(97)00151-5

HPCE method for detection of 8-hydroxy-deoxyguanosine in isolated DNA samples.

## 2. Experimental

### 2.1. Materials

Deoxynucleoside reference standards for CE were purchased from Boehringer Mannheim (Mannheim, Germany), 8-hydroxydeoxyguanosine, DNase I, snake venom phosphodiesterase and alkaline phosphatase were from Sigma (St. Louis, MO, USA).

### 2.2. Instrumentation

For all electrophoretic separations, a Beckman P/ACE 5510 instrument with a P/ACE diode array detector (Beckman Instruments, Fullerton, CA, USA) was used. Analyses were performed in an uncoated silica capillary of 44 cm×75 μm I.D. UV absorbance detection was performed in a range from 200 to 300 nm.

### 2.3. Sample preparation

Nuclei from $5\times10^7$ K562 human hematopoietic cells (ATCC CCL 243) were prepared by the method of Hymer and Kuff [11]. Nuclei were incubated with 0 and 15 m*M* hydrogen peroxide, respectively, in 10 m*M* phosphate buffer (pH 7.2) for 2 h. The hydrogen peroxide was removed by extensive dialysis at 4°C in 10 m*M* phosphate buffer with 10 m*M* KCl (pH 7.2).

DNA was isolated using a kit from Boehringer Mannheim [12] which avoids phenol/chloroform extraction, since it is known to induce 8-OH-dG formation in DNA by subsequent exposure to the air [13]. DNA samples (60 μg) were dissolved in Tris–HCl (40 m*M*, pH 8.5), containing 10 m*M* $MgCl_2$ and digested to free nucleosides by incubation overnight at 37°C with a mixture of DNase I (200 mU/μg DNA), snake venom phosphodiesterase (10 mU/μg DNA) and alkaline phosphatase (10 mU/μg DNA). Proteins were removed by centrifugation of the trichloroacetic acid precipitates. The supernatant was neutralized and then extracted three times with diethyl ether [14].

### 2.4. Separation conditions

A 20 m*M* borate buffer was used because of the pH range [23]. The 20 m*M* concentration permitted to minimize Joule heating effects. The separation was optimized with respect to pH, temperature, applied potential, injection mode (pressure or voltage injection) and injection time. The final optimized conditions used for analysis were pH 9.5, 25°C, 25 kV and 20 s pressure injection. Deoxyguanosine and 8-hydroxyguanosine content was quantified by external calibration with known standards. Identification of desoxynucleosides were performed by coelution with standards.

## 3. Results and discussion

Our aim was to develop separation conditions under which both 8-hydroxy-deoxyguanosine and deoxyguanosine are well separated, since in biological samples, the concentration of deoxyguanosine is about $10^4$–$10^5$-fold higher than that of 8-hydroxy-deoxyguanosine [15].

### 3.1. Optimization of separation conditions (Fig. 1)

#### 3.1.1. Influence of pH

The influence of pH on the separation was investigated in the pH range of 7.0 to 12.0. With increasing pH the migration time of 8-hydroxydeoxyguanosine, deoxyguanosine and deoxycytidine increased, while the migration time of deoxyadenosine and deoxythymidine decreased. The pH had no influence on the order of migration. The number of theoretical plates (*N*) achieved a plateau at pH 9.0, and was in the range of 95 000–130 000 for 8-OH-dG, dG and dC. Short migration times, good efficiency of separation and a maximum difference in the migration time between 8-OH-dG and dG was achieved at pH 9.5. Although the highest number of theoretical plates was obtained at pH 9.0, the difference in the migration time between 8-OH-dG and dG, an essential condition for the separation of biological samples, was higher at pH 9.5.

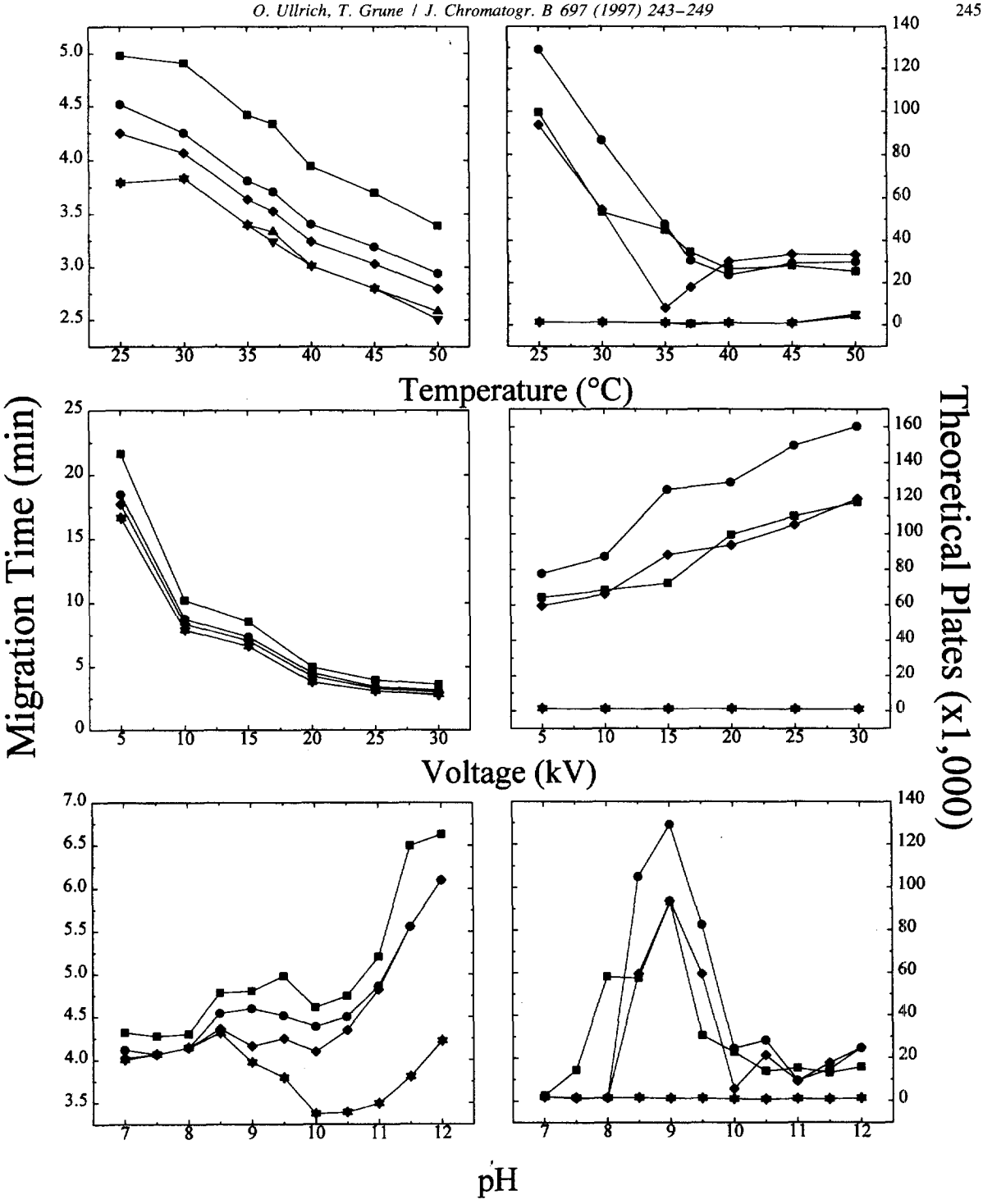


Fig. 1. Optimization of deoxynucleoside separation by capillary electrophoresis with respect to temperature, voltage and pH. The figure demonstrates the migration time (left hand side) and the separation efficiency (right hand site) of deoxynucleosides and 8-hydroxy-deoxyguanosine. If not indicated otherwise, the conditions were: 20 m*M* borate buffer pH 9.5, 25°C, 20 kV, (capillary: uncoated silica, 44 cm×75 μm I.D.). The symbols represent: (■) 8-OH-dG, (●) dG, (▲) dA, (▼) dT and (♦) dC.

*3.1.2. Temperature effects*

Temperature effects on the separation at pH 9.5 were investigated in the range 25–50°C. Higher temperatures caused faster migration times, because as the temperature increases the viscosity decreases and the electroosmotic flow increases as well. The separation efficiency decreases drastically with increasing temperature from 25 to 35°C, while from 35°C to 50°C it remains at a very low level. The best separation was achieved as 25°C.

*3.1.3. Voltage dependence*

Increased voltage decreases drastically the migration time and thus the number of theoretical plates is increased. Short separation times give the highest efficiencies, since diffusion is the most important cause of band broadening. Joule heating limits the optimization of the separation by increasing the voltage. We achieved the best separation using 25 or 30 kV.

*3.2. Optimized conditions*

The optimal conditions for the separation of dG and 8-OH-dG separations were: 20 m*M* borate buffer, pH 9.5, 25°C, 25 kV. The influence of the hydrodynamic injection time on the sensitivity and efficiency of separation was tested between 5 s and 40 s. The optimal injection time was 20 s. Fig. 2 shows the electropherogram of a standard solution separated under these conditions. Using optimized conditions, the reproducibility of migration times was investigated for the standard solution. The run-to-run-reproducibility of 8-OH-dG was 100±4.1% (3.42±0.14 min, $n=10$) and the day-to-day reproducibility was 100±11.8% (3.41±0.40 min, $n=10$).

*3.3. Detection and quantification limit*

The minimal quantitable concentration of 8-OH-dG in an equimolar mixture of all nucleosides was in the range of 0.1 μ*M* (LLQ). To mimic biological conditions, 8-OH-dG was analyzed together with a $10^4$-fold higher concentration (Fig. 3) of dG with a quantitation limit of about 0.2 μ*M* (LLQ). The lower limit of quantitation (LLQ) was determined as the lowest concentration which can be analysed with a within-day CV lower 20%. The LLD was 0.05 or 0.1 μ*M* in the case of equimolar concentrations or using $10^4$-fold higher amounts of the other nucleosides, respectively. The lower limit of detection (LLD) was estimated at a signal-to-noise ratio of three.

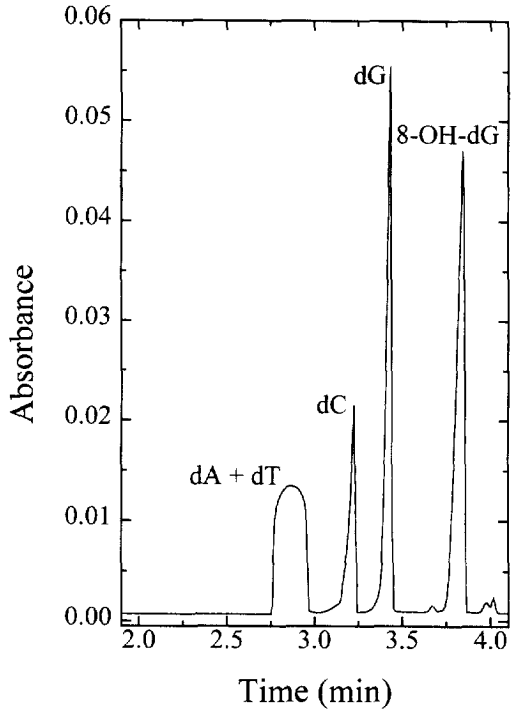


Fig. 2. Electropherogram of a standard solution of 8-hydroxydeoxyguanosine and deoxynucleosides (10 μ*M*). Separation conditions: 20 m*M* borate buffer, pH 9.5, 25°C, 25 kV, 20 s hydrodynamic injection. The concentration of the nucleosides was 10 μ*M*.

*3.4. Analysis of DNA from K562 cells*

The electropherogram of a diethyl ether-extracted standard solution of deoxynucleosides and 8-hydroxydeoxyguanosine is shown in Fig. 4. The migration times were about 0.6 to 0.7 min higher in comparison to the non-extracted standard, due to the changes in the sample constituents. The elution profile of a hydrolysate of isolated DNA from hydrogen peroxide treated nuclei from K562 cells is shown in Fig. 5. The average recoveries of dG and 8-OH-dG after diethyl ether-extraction were 73% and 71%, respectively. The method allows the separation of 8-hydroxydeoxyguanosine from the

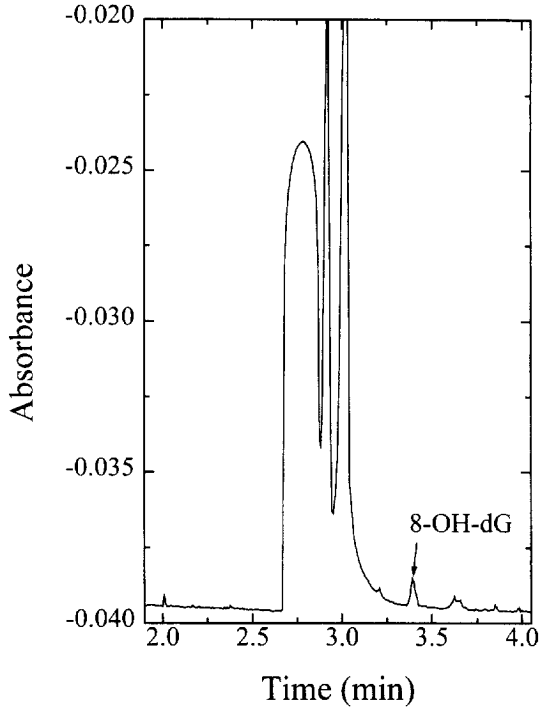


Fig. 3. Electropherogram of a solution of 0.5 μ*M* 8-hydroxy-deoxyguanosine and 500 m*M* deoxynucleosides. Separation conditions are as in Fig. 2 except for the nucleoside concentration.

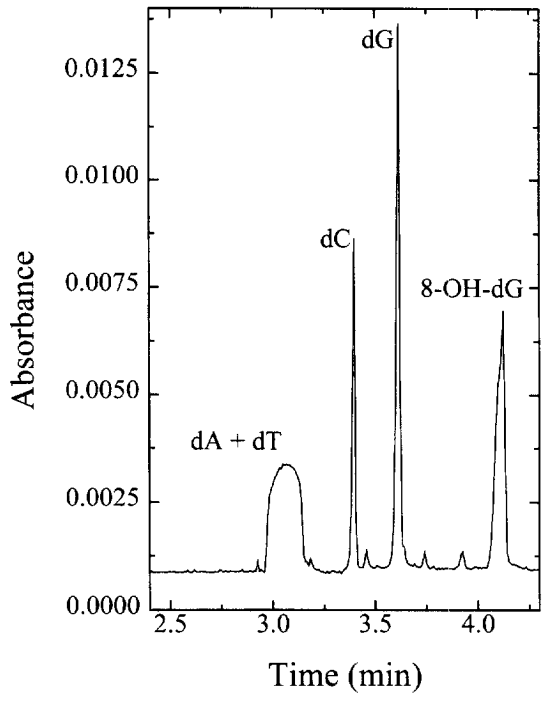


Fig. 4. Electropherogram of diethyl ether extracts from a standard solution of 8-hydroxy-deoxyguanosine and deoxynucleosides (10 μ*M*). Separation conditions are as in Fig. 2.

other naturally occurring DNA nucleosides. The deoxynucleosides dA and dT are not separated, whereas the peaks of dG and dC are well resolved.

The 8-OH-dG content of untreated DNA isolated from nuclei of K562 cells was ($5.5\times10^{-5}\pm1.6\times10^{-5}$) mol 8-OH-dG/mol dG and increases to ($22.9\times10^{-5}\pm4.3\times10^{-5}$) mol 8-OH-dG/mol dG in hydrogen peroxide treated nuclei (Data are given as mean±S.D., $n=4$.) The basal level of 8-OH-dG in this experiment was about 10-fold higher than the in vivo level, which has been reported for many cells types from $10^{-4}$ to $10^{-5}$ mol 8-OH-dG/mol dG [16]. One explanation of increased basal levels of 8-OH-dG in cancer cells, which has been observed for invasive ductal breast carcinoma [17], HeLa cells [18], squamous cell carcinoma [19] and adenocarcinoma of the lung [20], hepatocellular carcinoma [21], renal cell carcinoma [22], mucinous stomach carcinoma [20], ovary serous cystadenocarcinoma [20], is the hypothesis of 'persistent oxidative stress in cancer' [16]. The concept of persistent oxidative stress in cancer cells could explain in part the characteristics of tumor biology, such as activated transcription factors, proto-oncogenes, genetic instability and chemotherapy-resistance [16]. Our results show that K562 human chronic myelogenous leukemia cells also have a high basal level of oxidatively damaged DNA. Furthermore, oxidative damage during the isolation of the nuclei and further sample preparation, despite avoiding phenol/chloroform extraction, could contribute to a high 8-OH-dG level in the controls. The DNA of hydrogen peroxide treated nuclei contains about 4-fold higher concentration of 8-OH-dG than the untreated cells. Hydroxyl radicals may be formed from hydrogen peroxide by Fenton reaction with transition metal ions in the nucleus. The amount of 8-OH-dG in K562 cells after and without hydrogen peroxide treatment is in the same absolute and relative range measured in HeLa cells by Plummer and Faux [18].

The optimized separation conditions for biological samles of 8-OH-dG in DNA are comparable to those

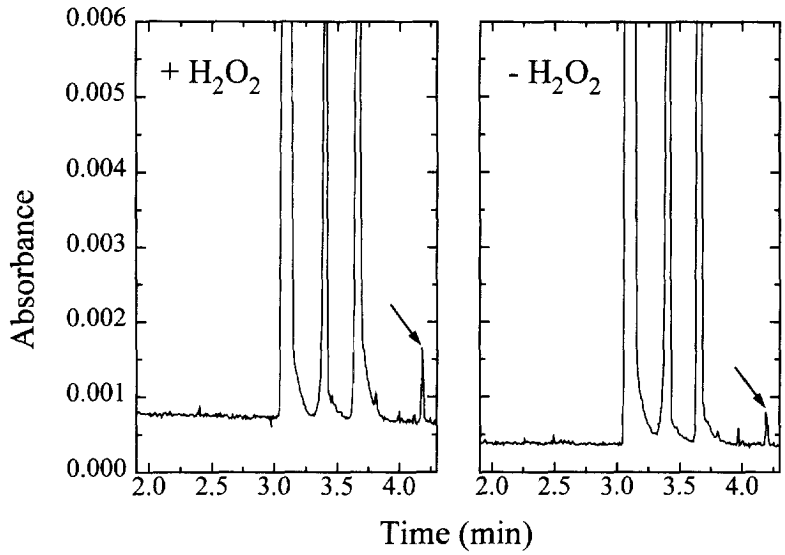


Fig. 5. Electropherogram of diethyl ether-extracts from enzymatically digested DNA isolated from nuclei of K562 human hematopoietic cells treated or non-treated with hydrogen peroxide. Separation conditions as in Fig. 2. The arrow indicates the 8-OH-dG peak.

used for purine base and purine nucleoside separation described earlier [23]. A great advantage of the method presented here, is that it is useful for the measurement of minor bases in DNA such as 8-OH-dG. Therefore, the HPCE method for the detection of the biomarker 8-OH-dG could be useful for studies of the role of oxidative stress in DNA damage and its significance in aging and cancer. Because of the automated and fast sample analysis, requiring only a very small sample volume, the HPCE assay for 8-OH-dG could be useful in clinical applications in the future, e.g. screening detection of DNA-damage in the human genome in reproductive and environmental medicine.

## 4. List of abbreviations

| | |
|---|---|
| dA | deoxyadenosine |
| dC | deoxycytidine |
| dG | deoxyguanosine |
| HPCE | high-performance capillary electrophoresis |
| 8-OH-dG | 8-hydroxydeoxyguanosine |
| dT | deoxythymidine |

## Acknowledgments

The work of OU was supported by a grant of the Ernst Schering Research Foundation and to TG from the DFG (1240/3-1) and the Charité Research Fond.

## References

[1] H. Kasai, S. Nishimura, Nucleic Acid Res. 12 (1984) 2137–2145.
[2] H. Kasai, Z. Yamaizumi, M. Berger, J. Cadet, J. Am. Chem. Soc. 114 (1992) 9692–9694.
[3] S. Shibutani, M. Takeshita, A.P. Grollmann, Nature 349 (1991) 431–434.
[4] M.L. Wood, M. Dizdaroglu, E. Gajewski, J.M. Essigmann, Biochemistry 29 (1990) 7024–7032.
[5] M. Moriya, C. Ou, V. Bodepudi, F. Johnson, M. Takeshita, A.P. Grollmann, Mutat. Res. 254 (1991) 281–288.
[6] K.C. Cheng, D.S. Cahill, D.H. Kasai, S. Nishimura, L.A. Loeb, J. Biol. Chem. 267 (1992) 166–172.
[7] R.A. Floyd, J.J. Watson, P.K. Wong, D.H. Altmiller, R.C. Rickard, Free Rad. Res. Commun. 1 (1986) 163–172.
[8] J.W. Park, K.C. Cundy, B.N. Ames, Carcinogenesis 10 (1989) 827–832.
[9] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298–1302.
[10] W. Kuhr, Anal. Chem. 62 (1990) 403R–414R.

[11] W.C. Hymer, E.L. Kuff, J. Histochem. Cytochem. 12 (1964) 359–363.
[12] J.C. Colosi, B.A. Schaal, Nucleic Acids Res. 21 (1993) 1051–1052.
[13] H.G. Claycamp, Carcinogenesis 13 (1992) 1289–1292.
[14] T. Grune and D. Perrett, in A. Sahota and M. Taylor (Editors), Purine and Pyrimidine Metabolism in Man VIII, Plenum Press, New York, 1995, p. 805.
[15] T. Takeuchi, M. Nakajima, Y. Ohta, K. Mure, T. Takeshita, K. Morimoto, Carcinogenesis 15 (1994) 1519–1523.
[16] S. Toyokuni, O. Keisei, J. Yodoi, H. Hiai, FEBS Lett. 358 (1995) 1–3.
[17] D.C. Malins, R. Haimanot, Cancer Res. 51 (1991) 5430–5432.
[18] S.M. Plummer, S.P. Faux, Carcinogenesis 15 (1994) 449–453.
[19] P. Jaruga, T. Zastawny, J. Budzbon, J. Skokowski, M. Dizdaroglu, R. Olinski, FEBS Lett. 341 (1994) 59–64.
[20] R. Olinski, T. Zastawny, J. Budzbon, J. Skokowski, W. Zegarski, M. Dizdaroglu, FEBS Lett. 309 (1992) 193–198.
[21] R. Shimoda, M. Nagashima, M. Sakomoto, N. Yamaguchi, S. Hirohashi, J. Yokota, H. Kasai, Cancer Res. 54 (1994) 3171–3172.
[22] K. Okamoto, S. Toyokuni, K. Uchida, O. Ogawa, J. Takenawa, Y. Kakehi, H. Kinoshita, Y. Hattori-Nakakuki, H. Hiai, O. Yoshida, Int. J. Cancer. 58 (1991) 794–798.
[23] T. Grune, G.A. Ross, H. Schmidt, W. Siems, D. Perrett, J. Chromatogr. 636 (1993) 105–111.