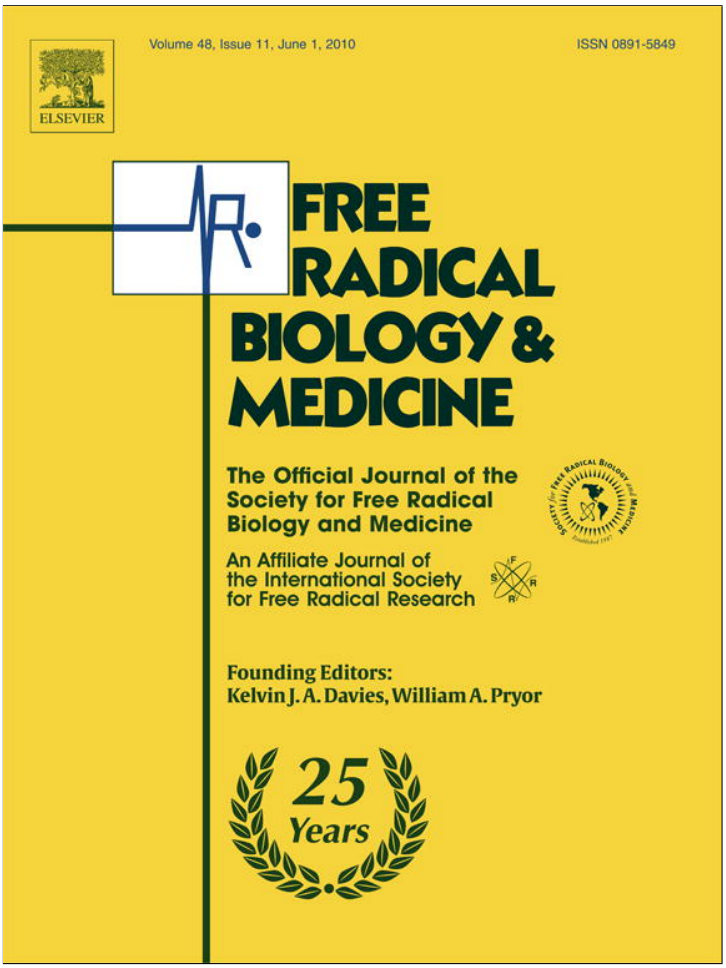

Free Radical Biology & Medicine 48 (2010) 1457–1459

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Commentary

Measurement of oxidatively generated base damage in cellular DNA and urine

Jean Cadet[a,b,*], Henrik Poulsen[c,d,e,*]

[a] Institut Nanosciences et Cryogénie, CEA/Grenoble, F-38054 Grenoble Cedex 9, France
[b] Department of Nuclear Medicine and Health Science, University of Sherbrooke, Quebec, Canada J1H 5N4
[c] Laboratory of Clinical Pharmacology Q7642, H:S Rigshospitalet, Tagensvej 20, DK-2100, Copenhagen, Denmark
[d] Department of Clinical Pharmacology, Bispebjerg Hospital, DK-2200 Copenhagen, Denmark
[e] Faculty of Health Sciences, University of Copenhagen, DK-2200, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 28 February 2010
Accepted 5 March 2010
Available online 12 March 2010

Many efforts have been devoted during the last 40 years to the measurement of oxidized bases and nucleosides in cellular DNA and fluids such as urine and plasma [1,2]. This still constitutes a challenging analytical issue due at least partly to the complexity of the lesion pattern, the difficulties of detecting low amounts of oxidatively formed DNA damage, typically within the range of a few lesions per 10^7 to 10^9 normal bases and the elevated risk of artefactual oxidation [3]. The first attempts to assess the formation of oxidatively damage to cellular DNA were made at the beginning of the 70's with the measurement of 5,6-dihydroxy-5,6-dihydrothymine ("thymine glycol"), one of the main $^{\bullet}$OH and one-electron oxidation products of cellular DNA [1], using an indirect approach based on its $NaBH_4$ reduction into 2-methylglycerol [4]. However the insertion of ^{3}H or ^{14}C thymine bases in cellular DNA prior to exposure to oxidizing has led through self-radiolysis processes to the overestimation of thymine glycol damage [5]. Related artefactual oxidation process has been also shown to occur more recently in ^{32}P-postlabeling assays that were designed for monitoring 8-oxo-7,8-dihydroguanine (8-oxoGua), thus requiring a tedious HPLC pre-purification of 8-oxo-7,8-dihydro-2′-deoxyguanosine 3′-monophosphate enzymatically released from DNA [6]. One may also mention that so far immunoassays that have been developed for measuring 8-oxoGua and other oxidized bases including thymine glycol and adenine N1-oxide have not led to conclusive data [3,7]. This is mostly due, as it has been shown for 8-oxoGua, to the fact that either polyclonal or monoclonal antibodies raised against the latter oxidized base exhibit a relatively high cross-reactivity with the overwhelming guanine bases that is about of 10^{-4} [7] This is likely to explain the lack of accurate detection of oxidized bases in DNA whose levels are at least two orders of magnitude lower!

The introduction of the gas-chromatography-mass spectrometry (GC-MS) method in the mid 80's through the pioneering work of Dizdaroglu [8] has provided a strong impetus to the measurement of oxidatively generated base damage to cellular DNA. More than 15 oxidized pyrimidine and purine bases have been reported to be measured in cellular DNA by GC-MS. However, major discrepancies were observed between the reported yields of 8-oxoGua measured by CG-MS and HPLC-electrochemical detection (ECD) respectively [9]. The latter method was introduced in 1986 by Floyd et al. [10] consecutively to the identification of 8-oxoGua as a major $^{\bullet}$OH-mediated oxidation product of guanine [11]. The main origin of the observed higher yield of 8-oxoGua by at least two orders of magnitude in GC-MS analysis with respect to HPLC-ECD measurement was explained in terms of spurious oxidation of the overwhelming normal guanine bases. The average yield of artifactual oxidation was estimated to be about 10^{-4} during the derivatization step which is required for making the base lesions volatile for the GC analysis [for a review, see 12 and references therein]. Similar artefactual oxidation reactions were found to occur for several oxidized purine and pyrimidine bases [12], thus requiring a pre-purification of the modified bases by HPLC or their specific release from DNA by DNA repair glycosylases in order to avoid the presence of non modified bases [13]. A second matter of concern that is indirectly associated with the GC-MS assay deals with the lack of stability of several modifications including 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde) under the hot acid formic treatment that is required for the release of the free bases from DNA [14]. A third contributing factor to the overestimated values of measured oxidized bases that is shared by all chromatographic methods has been more recently identified. This has been shown to be accounted for by adventitious oxidation reactions to DNA during the extraction step and the subsequent work-up.

More specific and quantitative measurement of oxidatively damaged DNA nucleosides in cellular DNA is now achieved with the availability of HPLC associated with electrospray ionization tandem mass spectrometry (ESI-MS/MS) that involves ionization of the DNA components in either the positive or negative mode [15–18]. The detection and quantitation of the DNA lesions are achieved in a highly accurate way using the multiple reaction monitoring (MRM) mode and the isotopic dilution technique. This represents so far the best available assay for monitoring the formation of oxidized nucleosides and bases when the level of modifications is within the sub-femtomole range. However it should be stressed that HPLC with a

* Corresponding authors. J. Cadet is to be contacted at Institut Nanosciences et Cryogénie, CEA/Grenoble, F-38054 Grenoble Cedex 9, France. H. Poulsen, Laboratory of Clinical Pharmacology Q7642, H:S Rigshospitalet, Tagensvej 20, DK-2100, Copenhagen, Denmark.
E-mail addresses: jean.cadet@cea.fr (J. Cadet), hepo@rh.hosp.dk (H. Poulsen).

0891-5849/$ – see front matter
doi:10.1016/j.freeradbiomed.2010.03.004

single quadrupole (HPLC-MS) which is about 50-fold less sensitive than HPLC-MS/MS is not suitable for measuring frequency of lesions within the range of a few modifications per 10^7 to 10^9 normal nucleosides. This is due to the presence of erratic fragments arising from the ionization of impurities, thus leading to inconclusive data as illustrated by the overestimated values of radiation-induced yields of 8-oxo-7,8-dihydro-2′-deoxyadenosine (8-oxodAdo) and 8-oxo-7,8-dihydroguanosine (8-oxodGuo) by HPLC-MS [19,20] with respect to HPLC-MS/MS measurements [1]. This remark applies as well to the high values of spiroiminodihydantoin nucleosides [21] and (5′*R*)-5′,8-cyclo-2′-deoxyadenosine [22] that were detected by HPLC-MS analysis upon exposure of cellular DNA to one-electron oxidant and ionizing radiation respectively. It may be added that accurate measurement of oxidatively generated damage with a frequency lower than 1 lesion per 10^9 nucleosides may necessitate the use of MS^3 detection. This has been shown to be the case for the measurement of tandem base modifications between vicinal guanine and thymine [23] and guanine and cytosine [24] respectively.

It may be added that a general consensus now exists on chromatographic methods that are available for the measurement of 8-oxodGuo in cellular DNA thank to the cooperative efforts of the 25 laboratories that have participated in the European Standard Committee on Oxidative DNA Damage (ESCODD network) [25,26]. DNA extraction methods have been assessed and compared leading to recommended protocols [27,28]. It has been shown that the extent of spurious oxidation of the guanine bases was inversely proportional to the amount of extracted DNA [29], requiring at least 30 μg of the latter compound in order to minimize the occurrence of artifactual oxidation. It should however be noted that a novel HPLC-MS/MS assay has recently become available allowing a reduced contribution of artefactual oxidation of guanine during DNA extraction [30]. This should be however validated by further comparison with existing protocols. Therefore it appears that the versatile HPLC-MS/MS technique and to a lesser extent the robust HPLC-ECD method whose application is however restricted to the detection of electroactive lesions including 8-oxodGo, 8-oxodAdo, FapyGua, FapyAde and 5-hydroxysubstituted derivatives of 2′-deoxycytidine and 2-‘deoxyuridine represent the methods of choice for measuring oxidatively generated damage to cellular DNA at least under conditions of acute exposure to oxidizing agents. It may be added that low variations in the frequency of oxidized bases typically when generated upon exposure to low chronic exposure to oxidizing agents are better accounted by enzymic assays. The latter assays involved initial incubation of released DNA with DNA repair glycosylases and subsequent detection of the DNA strand breaks thus generated by either the comet assay [31] or the alkaline elution technique [32].

We would like to stress the importance of formalized comparison schemes between methods that include inter-laboratory comparison of identical samples. The progress provided by the ESCODD [25,26] initiative coupled with the methodological advancement by single laboratories could not have been achieved by a single group. With regard to measurement of oxidized DNA lesions in urine a similar initiative has been established [33]. It may be stressed as discussed in this issue [34] that HPLC-MS/MS measurement of 8-oxodGuo appears much more accurate and quantitative than the immunodetection assay [35] that suffers from a lack of specificity due to interference with urine components including urea [36,37]. It is expected that the HPLC-MS/MS method which has been applied to the measurement of 8-oxoGua, 8-oxodGuo, 8-oxo-7,8-dihydroguanosine, 8-oxodAdo and FapyGua [38–45] would be extended to other oxidized nucleosides thank to the versatility of the tandem mass spectrometry technique. These include 5,6-dihydroxy-5,6-dihydrothymidine, 5-(hydroxymethyl)-2′-deoxyuridine and 5-formyl-2′-deoxyuridine that have been shown to be generated either by $^{\bullet}OH$ or one-electron oxidation in cellular DNA [1].

Acknowledgments

JC acknowledges partial support received from Marie Curie Research Training Network under contract MRTN-CT-2003-50586 (CLUSTOXDNA) and COST Action CM0603 on “Free Radicals in Chemical Biology” (CHEMBIORADICAL)

References

[1] Cadet, J.; Douki, T.; Ravanat, J. -L. Oxidatively generated damage to the guanine moiety of DNA: Mechanistic aspects and formation in cells. *Acc. Chem. Res.* **41:** 1075–1083; 2008.
[2] Cooke, M. S.; Olinski, R.; Loft, S. members of the European Standards Committee on Urinary (DNA) Lesion Analysis (ESCULA). Measurement and meaning of oxidatively modified DNA lesions in urine. *Cancer Epidemiol. Biomark. Prev.* **17:** 3–14; 2008.
[3] Cadet, J.; Douki, T.; Frelon, S.; Sauvaigo, S.; Pouget, J. -P.; Ravanat, J. -L. Assessment of oxidative base damage to isolated and cellular DNA by HPLC-MS/MS measurement. *Free Radic. Biol. Med.* **33:**441–449; 2002.
[4] Hariharan, P. V.; Cerutti, P. A. Formation and repair of gamma-ray induced thymine damage in Micrococcus radiodurans. *J. Mol. Biol.* **66:**65–81; 1972.
[5] Cadet, J.; Berger, M. Radiation-induced degradation of the purine bases within DNA and realted model compounds. *Int. J. Radiat. Biol.* **47:**127–143; 1985.
[6] Möller, L.; Hofer, T. [^{32}P]ATP mediates formation of 8-hydroxy-2′-deoxyguanosine from 2′-deoxyguanosine, a possible problem in the 32P-postlabeling assay. *Carcinogenesis* **18:**2415–2419; 1997.
[7] Yin, B.; Whyatt, R. M.; Perer, F. P.; Randall, M. C.; Cooper, T. B.; Santella, R. M. Determination of 8-hydroxydeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA. *Free Radic. Biol. Med.* **18:**1023–1032; 1995.
[8] Dizdaroglu, M. Gas chromatography-mass spectrometry of free radical-induced products of pyrimidines and purines in DNA. *Methods Enzymol.* **193:**842–857; 1990.
[9] Halliwell, B.; Dizdaroglu, M. The measurement of oxidative damage to DNA by HPLC and GC-MS techniques. *Free Radic. Res. Commun.* **16:**75–87; 1992.
[10] Floyd, R. A.; Watson, J. J.; Wong, P. K.; Altmiller, D. H.; Rickard, R. C. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanism of formation. *Free Radic. Res. Commun.* **1:**163–172; 1986.
[11] Kasai, H.; Nishimura, S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res.* **12:**2137–2145; 1984.
[12] Cadet, J.; Douki, T.; Ravanat, J. -L. Artifacts associated with the measurement of oxidized DNA bases. *Environ. Health Perspect.* **105:**1034–1039; 1997.
[13] Jaruga, P.; Speina, E.; Gackowski, D.; Tudek, B.; Olinski, R. Endogenous oxidative DNA base modifications analysed with repair enzymes and GC/MS technique. *Nucleic Acids Res.* **28:**E16; 2000.
[14] Douki, T.; Martini, R.; Ravanat, J. L.; Tureski, R. J.; Cadet, J. Measurement of 2, 6-diamino-4-hydroxy-5-formamidopyrimidine and 8-oxo-7, 8-dihydroguanine in isolated DNA exposed to gamma radiation in aqueous solution. *Carcinogenesis* **18:** 2385–2391; 1997.
[15] Ravanat, J. L.; Duretz, B.; Guiller, A.; Douki, T.; Cadet, J. Isotope dilution high-performance liquid chromatography-electrospray tandem mass spectrometry assay for the measurement of 8-oxo-7, 8-dihydro-2′-deoxyguanosine in biological samples. *J. Chromatogr. B* **715:**349–356; 1998.
[16] Frelon, S.; Douki, T.; Ravanat, J. -L.; Pouget, J. -P.; Tornabene, C.; Cadet, J. High-performance liquid chromatography-tandem mass spectrometry measurement of radiation-induced base damage to isolated and cellular DNA. *Chem. Res. Toxicol.* **13:**1002–1010; 2000.
[17] Glintborg, B.; Weimann, A.; Kensler, T. W.; Poulsen, H. E. Oltipraz chemoprevention trial in Qidong, People's Republic of China:unaltered oxidative biomarkers. *Free Radic. Biol. Med.* **41:**1010–1014; 2006.
[18] Singh, R.; Teichert, F.; Verschoyle, R. D.; Kaur, B.; Vives, M.; Sharma, R. A.; Steward, W. P.; Gescher, A. J.; Farmer, P. B. Simultaneous determination of 8-oxo-2′-deoxyguanosine and 8-oxo-2′-deoxyadenosine in DNA using online column-switching liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **23:**151–160; 2009.
[19] Tuo, J.; Jaruga, P.; Rodriguez, H.; Dizdaroglu, M.; Bohr, V. A. The cockayne syndrome group B gene product is involved in cellular repair of 8-hydroxyadenine in DNA. *J. Biol. Chem.* **277:**30832–30837; 2002.
[20] Tuo, J.; Jaruga, P.; Rodriguez, H.; Bohr, V. A.; Dizdaroglu, M. Primary fibroblasts of Cockayne syndrome patients are defective in cellular repair of 8-hydroxyguanine and 8-hydroxyadenine resulting from oxidative stress. *FASEB J.* **17:**668–674; 2003.
[21] Hailer, M. K.; Slade, P. G.; Martin, B. D.; Sugden, K. D. Nei deficient *Escherichia coli* are sensitive to chromate and accumulate the oxidized guanine lesion spiroiminodihydantoin. *Chem. Res. Toxicol.* **18:**1378–1383; 2005.
[22] D'Errico, M.; Parlanti, E.; Teson, M.; Bernades de Jesus, B. M.; Degan, P.; Calcagnile, A.; Jaruga, P.; Bjørås, M.; Crescenzi, M.; Pedrini, A. M.; Egly, J. -M.; Zambruno, G.; Stefanini, M.; Dizdaroglu, M.; Dogliotti, E. New functions of XPC in the protection of human skin cells from oxidative damage. *EMBO J.* **25:**4305–4315; 2006.
[23] Jiang, Y.; Hong, H.; Cao, H.; Wang, Y. In vivo formation and in vitro replication of a guanine-thymine intrastrand cross-link lesion. *Biochemistry* **46:**12757–12763; 2007.
[24] Hong, H.; Cao, H.; Wang, Y. Formation and genotoxicity of a guanine-cytosine intrastrand cross-link lesion in vivo. *Nucleic Acids Res.* **35:**7118–7127; 2007.

[25] Collins, A. R.; Cadet, J.; Möller, L.; Poulsen, H.; Viña, J. Are we sure we know how to measure 8-oxo-7, 8-dihydroguanine in DNA from human cells? *Arch. Biochem. Biophys.* **423:**57–65; 2004.
[26] Gedik, C. M.; Collins, A. R., ESCODD Establishing the background level of base oxidation in human lymphocyte DNA: results of an inter-laboratory validation study. *FASEB J.* **19:**82–84; 2005.
[27] Ravanat, J. -L.; Douki, T.; Duez, P.; Gremaud, E.; Herbert, K.; Hofer, T.; Lassere, L.; Saint-Pierre, C.; Favier, A.; Cadet, J. Cellular background of 8-oxo-7, 8-dihydro-2′-deoxyguanosine: an isotope based method to evaluate artefactual oxidation of DNA during its extraction and subsequent work-up. *Carcinogenesis* **23:** 1911–1918; 2002.
[28] Chao, M. R.; Yen, C. C.; Hu, C. W. Prevention of artifactual oxidation in determination of cellular 8-oxo-7, 8-dihydro-2′-deoxyguanosine by isotope-dilution LC-MS/MS with automated solid-phase extraction. *Free Radic. Biol. Med.* **44:**464–473; 2008.
[29] Badouard, C.; Ménézo, Y.; Panteix, G.; Ravanat, J. -L.; Douki, T.; Favier, A. Determination of new types of DNA lesions in human sperm. *Zygote* **16:**9–13; 2008.
[30] Mangal, D.; Vudathala, D.; Park, J. -H.; Lee, S. H.; Penning, T. M.; Blair, I. A. Analysis of 7, 8-dihydro-8-oxo-2′-deoxyguanosine in cellular DNA during oxidative stress. *Chem. Res. Toxicol.* **22:**788–797; 2009.
[31] Azqueta, A.; Shaposhnikov, S.; Collins, A. R. DNA oxidation: Investigating it skey role in environmental mutagenesis with the comet assay. *Mutat. Res.* **674:**101–108; 2009.
[32] Pflaum, M.; Will, O.; Epe, B. Determination of steady-state levels of oxidative DNA base modifications in mammalian cells by means of repair endonucleases. *Carcinogenesis* **18:**2225–2231; 1997.
[33] Cooke, M. S.; Olinski, R.; Loft, S. Members of the European Standards Committee on Urinary(DNA) Lesion Analysis (ESCULA). Measurement and meaning of oxidatively modified DNA lesions in urine. *Cancer Epidemiol. Biomark. Prev.* **17:**3–14; 2008.
[34] Garratt, L. W.; Mistry, V.; Singh, R.; Sandhu, J.; Sheil, B.; Cooke, M. S.; Sly, P. D. Interpretation of urinary 8-oxo-7, 8-dihydro-2′-deoxyguanosine is adversely affected by methodological inaccuracies when using a commercial ELISA. *Free Radic. Biol. Chem.* **48:**1460–1464; 2010.
[35] Toyokuni, S.; Tanaka, T.; Hattori, Y.; Nishiyama, Y.; Yoshida, A.; Uchida, K.; Hiai, H.; Ochi, H.; Osawa, T. Quantitative immunohistochemical determination of 8-hydroxy-2′-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. *Lab. Invest.* **76:**365–374; 1997.
[36] Song, M. -F.; Li, Y. -S.; Ootsuyama, Y.; Kasai, H.; Kawai, K.; Ohta, M.; Eguchi, Y.; Yamato, M.; Matsumoto, Y.; Yoshida, R.; Ogawa, Y. Urea, the most abundant component in urine, cross-reacts with a commercial 8-OHdG ELISA kit and contributes to over-estimation of urinary 8-OHdG. *Free Radic. Biol. Med.* **47:**41–46; 2009.
[37] Cooke, M. A commentary on "Urea, the most abundant component in urine, cross-reacts with a commercial 8-OHdG ELISA kit and contributes to overestimation of urinary 8-OHdG". What is ELISA detecting? *Free Radic. Biol. Med.* **47:** 30-31; 2009.
[38] Weimann, A.; Belling, D.; Poulsen, H. E. Measurement of 8-oxo-2′-deoxyguanosine and 8-oxo-2′-deoxyadenosine in DNA and human urine by high performance liquid chromatography electrospray tandem mass spectrometry. *Free Radic. Biol. Med.* **30:**757–764; 2001.
[39] Weimann, A.; Belling, D.; Poulsen, H. E. Quantification of 8-oxo-guanine and guanine at the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. *Nucleic Acids Res.* **30** (EZ); 2002.
[40] Sabatini, L.; Barbieri, A.; Tosi, M.; Roda, A.; Violante, F. S. A method for routine quantitation of urinary 8-hydroxy-2′-deoxyguanosine based on solid-phase extraction and micro-high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **19:** 147–152; 2005.
[41] Hu, C. W.; Wang, C. J.; Chang, L. W.; Chao, M. R. Clinical-scale high throughput analysis of urinary 8-oxo-7, 8-dihydro-2′-deoxyguanosine by isotope-dilution liquid chromatography-tandem mass spectrometry with on-line solid-phase extraction. *Clin. Chem.* **52:**1381–1388; 2006.
[42] Malayappan, B.; Garrett, T. J.; Segal, M.; Leeuwenburgh, C. Urinary analysis of 8-oxoguanine, 8-oxoguanosine, fapyguanine and 8-oxo-2′-deoxyguanosine by high-performance liquid chromatography-electrospray tandem mass spectrometry as a measure of oxidative stress. *J. Chromatogr. A* **1167:**54–62; 2007.
[43] Harri, M.; Kasai, H.; Mori, T.; Tornaeus, J.; Savela, K.; Peltonen, K. Analysis of 8-hydroxy-2′-deoxyguanosine in urine using high performance liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. B* **853:**242–246; 2007.
[44] Evans, M. D.; Singh, R.; Mistry, V.; Sandhu, K.; Farmer, P. B.; Cooke, M. S. Analysis of urinary 8-oxo-7, 8-dihydro-purine -2′-deoxytibonucleosides by LC-MS/MS and improved ELISA. *Free Radic. Res.* **42:**831–840; 2008.
[45] Crow, B.; Bishop, M.; Kovalcik, K.; Norton, D.; George, J.; Bralley, J. A. A simple and cost effective method for the quantification of 8-hydroxy-2′-deoxyguanosine from urine using liquid chromatography tandem mass spectrometry. *Biomed. Chromatogr.* **22:**394–401; 2008.