Research Paper

A serially coupled stationary phase method for the determination of urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine by liquid chromatography ion trap tandem mass spectrometry

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ARTICLE INFO

ABSTRACT

Oxidative attack to DNA is of particular interest since DNA modifications can lead to heritable mutations. The most studied product of DNA oxidation is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). While 8-oxodG determination in blood and tissue cells is prone to artifacts, its measurement in urine employing liquid chromatography tandem mass spectrometry (LC-MS/MS) has gained more and more interest for increased reliability. LC-MS/MS can be affected by matrix effects and this is particularly true when ion trap is used as MS analyzer, due to ion accumulation in the trap and related space charge effect. In the present work, we have developed a LC-MS/MS method where the combination of cation exchange and reverse phase solid phases resulted in LC separation optimization. This together with the employment of an isotopically labeled internal standard, allowed the usage of ion trap LC-MS/MS, typically not employed for quantitative measurement in biological samples, for the measurement of 8-oxodG in urine samples from control populations.

Four different urine matrices were employed for method validation. Limit of quantitation was set at least at 0.5 ng/ml. While analyzing urine samples from healthy volunteers, 8-oxodG levels reported as ng/ml were statistically different comparing males with females ($p < 0.05$, Mann Whitney test); while comparing results normalized for creatinine no statistical significant difference was found. Mean urinary 8-oxodG level found in healthy volunteers was 1.16 ± 0.46 nmol/mmol creatinine.

The present method by enhancing at best the chromatographic performances allows the usage of ion trap LC-MS/MS for the measurement of 8-oxodG in urine samples from control populations.

Introduction

Normal cellular activity and exposure to oxidizing agents can lead to the formation of reactive oxygen species (ROS). Oxidative stress, the condition of oxidative imbalance implicated in many human diseases, appears whenever ROS are produced in excess or not adequately detoxified. ROS can attack biological macromolecules and the oxidative attack to DNA, in particular, is of interest since DNA modifications can lead to heritable mutations. Due to its low redox potential, guanine is one of the main targets of ROS in DNA (and RNA). 8-Oxo-7,8-dihydroguanine is the most abundant product of DNA oxidation, while 8-oxo-7,8-dihydro-2′-deoxyguanosine is the most studied one, due both to the availability of detection methods and its mutagenic potential [1,2]. The presence of oxidative guanine derivatives in the DNA molecules, resulting in base mispairing with adenine in place of cytosine, may be mutagenic [3]. Determination of 8-oxodG in blood cells or tissues is, in most cases, poorly reproducible and overestimated due to artifactual oxidation during nucleic acid extraction and manipulation [1,4]. It has to be reported, nevertheless, that recently the Standard Committee on Oxidative DNA Damage (ESCODD network) has published recommended protocols to keep at minimum artifactual DNA oxidation [5,6], but still sample collection is not so straightforward. During the last two decades the measurement of

8-oxodG in urine has gained more and more interest, due to the non-invasive method of sample collection, 8-oxodG long-term stability in urine [7,8] and no artifactual DNA oxidation [9]. Another important advantage of measuring 8-oxodG in urine resides in the lack of sample manipulation, especially when employing LC-MS/MS as detection technique. Recently, a series of recommendations on urinary 8-oxodG determination, resulting from a large inter-laboratory study have been published [10]. On the other hand, data interpretation remains an incompletely resolved issue: urinary levels of oxidized derivatives of nucleic acids, in fact, not only depend on their formation upon oxidative attack, but also on antioxidant defenses, on the extent of nucleic acid turnover and on the efficiency of the repair systems, with Nudix hydrolase mut T homologue 1 (MTH1) taking care of oxidized 2′-deoxyribonuleotide repairing, while nucleotide excision (NER) and probably nucleotide incision (NIR) repair systems, repairing the DNA molecule [11].

It has to be noted that the rationale behind the choice of studying the oxidized nucleoside 8-oxodG and not the oxidized base itself is related to the characteristic of the former of not being influenced by the diet and so that urinary 8-oxodG is thought to derive only from body's cells, although this is still an open issue [2].

LC-MS/MS is a powerful tool for the measurement of biomarkers in biological samples. Even if the powerfulness of this analytical technique is universally recognized, it is also known that LC-MS/MS can be affected by matrix effects, since biological samples, like urine, are complex matrices containing many compounds that can interfere with the target measurement [1,2,4,12]. This is particularly true for LC-MS/MS systems where ion trap is used as MS analyzer, due to ion accumulation in the trap that lead to space charge effect [13]. Time mass analyzers as ion traps, chosen usually for the more affordable price compared to others, do not perform as well if compared to spatial mass analyzers (e.g. triple quadrupoles), when multiple reaction monitoring (MRM) is performed [14,15].

In a previous work we developed a method where the combination of surface-activated ionization (SACI) source with electrospray ionization (ESI), together with cation-exchange chromatography, improving ion trap performances by the reduction of matrix effects, enabled the measurement of 8-oxodG in diluted urine [12]. In the present work, we went further in method development and employed a different approach to liquid chromatography. Through the combination of cation exchange and reverse phase solid phases we significantly improved chromatographic separation. This resulted in the possibility of increasing the concentration of urine samples injected in the MS ion trap without any further modification. By this approach, together with the employment of ESI ionization and the addition of an isotopically labeled internal standard, we have significantly improved the analytical sensitivity, decreasing 6-fold the limit of quantitation (LOQ) (from 3 ng/ml to at least 0.5 ng/ml).

Here are presented the results of method validation and the data resulting from the measurement of 8-oxodG urinary levels in a group of healthy volunteers.

Materials and methods

Reagents

8-oxodG, dimethyl sulfoxide (DMSO), formic acid, hydrogen peroxide, ascorbic acid, ammonium hydroxide and potassium phosphate monobasic (KH_2PO_4) were purchased from Sigma-Aldrich (Milan, Italy). HPLC grade water and methanol were purchased from Carlo Erba (Milan, Italy). Sep-Pak Vac 1 cc C18 SPE cartridges were purchased from Waters (Milford, MA, USA).

$^{15}[N_5]2′$-deoxyguanosine ($^{15}[N_5]2$-dG) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). $^{15}[N_5]8$-oxodG was synthesized through the oxidation of $^{15}[N_5]2$-dG according to the method published by Hu et al. [16] upon minor modifications as published by Andreoli et al. [1]. As reported in the synthesis method, the yield of oxidized product is not fixed and can represent, at best, the 30% of the original $^{15}[N_5]2$-dG. For this reason a constant amount of internal standard was employed in all experiments, but the reported concentration (25 ng/ml) is purely theoretic. Aliquots of stock solutions of 8-oxodG (5 mM in DMSO) and internal standard (IS) (theoretic 56.25 μM in 0.1 M KH_2PO_4/methanol, 85/15 v/v) were stored at −80 and −20 °C, respectively, until usage.

Chromatography

A serial stationary phase LC method was developed by connecting three LC columns as follows: first a Biobasic SCX 50×2.1 mm, 5 μm, then an Aquasil C18 150×2.1 mm, 5 μm and finally an Aquasil C18 100×2.1 mm, 3 μm. A Biobasic SCX 10×2.1 mm, 5 μm precolumn was also employed. All columns were purchased from Thermo Scientific (Thermo Scientific, San Jose, CA, USA). Chromatography was performed on an Ultimate 3000 HPLC (DIONEX, Germering, Germany). HPLC gradient was set at a flow rate of 150 μl/min using as eluents: (A) 0.5% (v/v) formic acid/H_2O and (B) 0.5% (v/v) formic acid in methanol. To prevent contamination of the mass spectrometer, during the first 15 min and the last 25 min the eluate was diverted to the waste as it contained a large amount of polar compounds derived from the urine samples.

The columns were equilibrated for 3 min with 100% solution A, then the percentage of eluent B was linearly incremented to 40% in the following 17 min. Solution B percentage was further increased to 80% from minute 20 to 22 and kept at 80% for 7.5 min. The system returned to 100% of solution A in 30 s and columns were re-equilibrated with it until the end of run (total run, 50 min).

Mass spectrometry

Mass spectra were acquired using a HCT Ultra ion trap spectrometer equipped with an electrospray ion source (Bruker Daltonics, Bremen, Germany). Mass spectrometry was carried out by the program Esquire Control (V6.2) and LC-MS was controlled by Hystar (V3.2) (Bruker Daltonics, Bremen, Germany).

The spectrometer was operated in the positive mode with a scan range from 100 to 400 m/z. MS parameter optimization was obtained by directly infusing in the MS 14 μg/ml of 8-oxodG. Optimized conditions were as follows: nebulizer pressure 25 psi, dry gas 9 L/min, dry temperature 350 °C, capillary voltage of 4500 V with an end plate of −500 V.

MRM scans were performed by analyzing the following ion transitions: transition of the precursor ion at 284 m/z to the product ion at 168 m/z for 8-oxodG, and transition from the parent ion at 289 m/z to the product ion at 173 m/z for the internal standard ($^{15}[N_5]8$-oxodG). Ion isolation width was set to 2 m/z with a fragmentation amplitude of 0.6 V.

Standards, internal quality controls (IQCs) and urine samples were injected at least twice.

Standard, IQC and sample preparation

Working solutions (water) for 8-oxodG and internal standard (IS) were prepared weekly. Further dilutions were made fresh daily. Four different urine matrices were tested for the preparation of standard curves and IQC. In particular, a stock solution containing urine matrix and IS was prepared upon usage, it was divided in

equal amounts and adequate quantities of standard and/or water were added to build up 8-point calibration curves (range 0–20 ng/ml) and 2 level IQC (2.5, 5 ng/ml).

Urine samples derived from 49 healthy volunteers were collected, thoroughly mixed and stored in aliquots at −80 °C. After thawing and mixing, IS water solution was added to each urine sample, centrifuged and injected in the LC-MS. Final dilution of urine was 1:2 in samples, standards or IQC. The study was approved by the Department of Laboratory Medicine review board and all enrolled subjects gave written informed consent to participate in the study.

Creatinine measurement

Quantitative measurement of urinary creatinine was performed on an Architect ci8200 (Abbott Diagnostics Roma, Italy) by an enzymatic method where creatinine is quantified through sarcosine/sarcosine oxidase reaction with the formation of a colored compound characterized by an absorption maximum at 548 nm [17].

Data analysis

MS data were processed by the employment of DataAnalysis (V4.0 SP4) and concentrations calculated by QuantAnalysis (V2.0 SP4) (Bruker Daltonics, Breme, Germany). Excel (Microsoft, USA) was employed for data elaboration and non-parametric statistic calculations (Mann Whitney test) were performed by the employment of GraphPad Prism (V5.04) (GraphPad Software, Inc. La Jolla, CA, USA).

Results

Method validation

Water solutions of 8-oxodG (10 ng/ml) and of ^{15}[N$_5$]8-oxodG IS (theoretic concentration 25 ng/ml; see Materials and methods) were employed to determine the retention time (rt) of both compounds, that resulted 20.66 min ± 0.04. and 20.67 min ± 0.05 (mean of 10 replicates). By repeating the analysis of the same solutions over a period of 5 months, we found a 1 min shift of the rt. Further experiments were performed using human urine matrix in order to obtain results closer to real biological samples, with overlapping rt compared to water solutions. Fig. 1 shows the MS/MS scan and the extracted ion chromatogram (EIC) of 2 ng/ml 8-oxodG added to urine matrix diluted to 1:2 in the presence of ^{15}[N$_5$]8-oxodG IS (theoretic concentration 25 ng/ml). The EIC and the mass spectrum of 8-oxodG product ion (167.9 m/z), together with the EIC and the mass spectrum of ^{15}[N$_5$]8-oxodG IS product ion (172.9 m/z), are reported.

8-oxodG is an endogenous component of human urine. To evaluate the influence of urine matrix on 8-oxodG determination, four different urine samples were employed for the preparation of standard curves by adding to each matrix increasing amounts of 8-oxodG standard (0, 0.5, 1, 2, 3, 4, 10 and 20 ng/ml) and a constant amount of IS (estimated as 25 ng/ml). Standard curves derived from matrix 1 did not include the 20 ng/ml point (Fig. 2). Each matrix was employed to build up from four to seven distinct standard curves, run in separate experiments. Results are reported as 8-oxodG peak area/IS peak area. Good linearity and reproducibility was reached thorough all the concentration range with all matrices (R^2 ranging from 0.9895 to 0.9992). Table 1 shows, in detail, the comparison between mean target/IS area ratios for matrix alone compared to matrix with the addition of 0.5 ng/ml 8-oxodG for all the 4 different matrices tested. Each data-point

was repeated at least 5 times in different analytical sets. Both the CV% of matrix alone and of the 0.5 ng/ml 8-oxodG point was close to 20% in most samples. However, in one case (matrix 3) about 8% CV% in the 0.5 ng/ml point was observed, together with lower endogenous 8-oxodG/IS peak area ratio. Therefore 0.5 ng/ml was defined as the limit of quantification of the method (LOQ), since the CV% was largely < 20% [18]. This result was also confirmed by the consecutive analysis of 15 replicates of a 0.5 ng/ml standard in urine (data not shown). Matrix 3 was selected for the preparation of standard curves employed for the analysis of urine samples from healthy volunteers.

The same four urine matrices were employed for the production of IQC samples. Two sets of IQCs were prepared by the addition of 2.5 (level 1) or 5 ng/ml (level 2) of 8-oxodG in the presence of IS. As reported in Table 2, recovery ranged from 86.8 to 103.6% for level 1 and from 98.8 to 115.4% for level 2, depending on the urine matrix selected. Overall recovery (weighted mean of all matrixes) was 96.8% for level 1 and 104.8% for level 2. The number of between-day IQC repeats ranged from 5 to 11, with a total amount of repeats of 25 for 2.5 ng/ml and 30 for 5 ng/ml 8-oxodG. Overall CV% (weighted mean) was 14.79% for level 1 (range: 11.97–16.98) and 11.61% for level 2 (range: 6.74–16.84). When considering matrix 3 (selected to be employed when dosing 8-oxodG in healthy volunteers) recovery and CV% were 94.1% and 13.17% for level 1 and 98.8% and 9.42% for level 2. After this initial evaluation, IQCs were run in each analytical session.

Urine samples

Forty-nine healthy volunteers (25 females, 24 males) aged 21–65, mean 41.14 ± 11.89 years (females, 40.6 ± 11.82; males, 41.54 ± 12.21) were enrolled. Aliquots of urine samples from each volunteer were frozen immediately at −80 °C and thawed just before analysis. Before the injection in the LC-MS system, a constant amount of internal standard was added to each sample, that was injected at least two times during the analytical session. In addition, most samples were measured in at least two different analytical sessions. In Fig. 3 is represented the LC-MS/MS analysis of a sample where 8-oxodG/IS peak area ratio was 0.74 and the corresponding 8-oxodG concentration was 2.3 ng/ml (mean of three analytical sessions). Considering that an 8-oxodG peak area/IS peak area ratio of 0.3224 corresponds to 0.5 ng/ml 8-oxodG (the LOQ of the present method), all samples with 8-oxodG/IS peak area ratio < 0.30 were considered as lower than LOQ and were excluded from statistical analyses.

8-oxodG peak area/IS peak area ratios were differently distributed in males and females (Table 3), and significantly higher mean levels were detected in males compared to females ($p < 0.01$). This difference was also observed when 8-oxodG levels were expressed as ng/ml ($p < 0.05$). By contrast, when 8-oxodG concentration was reported as μg/g creatinine, no statistically significant difference was found according to gender. As summarized in Table 3 in fact, the different 8-oxodG levels found between males and females were related to different urine concentrations, as indicated by the detection of statistically higher creatinine levels in males. The mean level of urinary 8-oxodG in the whole population was 1.16 ± 0.46 nmol/mmol creatinine (weighted mean ± SD), 1.12 ± 0.4 in females and 1.19 ± 0.51 in males (Table 3).

The number of samples < LOQ was higher in females (6/25) than in males (1/24): indeed, 8-oxodG area/IS area ratios < 0.30 (< LOQ) were found only in samples with creatinine level < 0.3 g/L. 8-OxodG was detectable in six out of these seven samples when the analysis was repeated employing undiluted urine (data not shown).
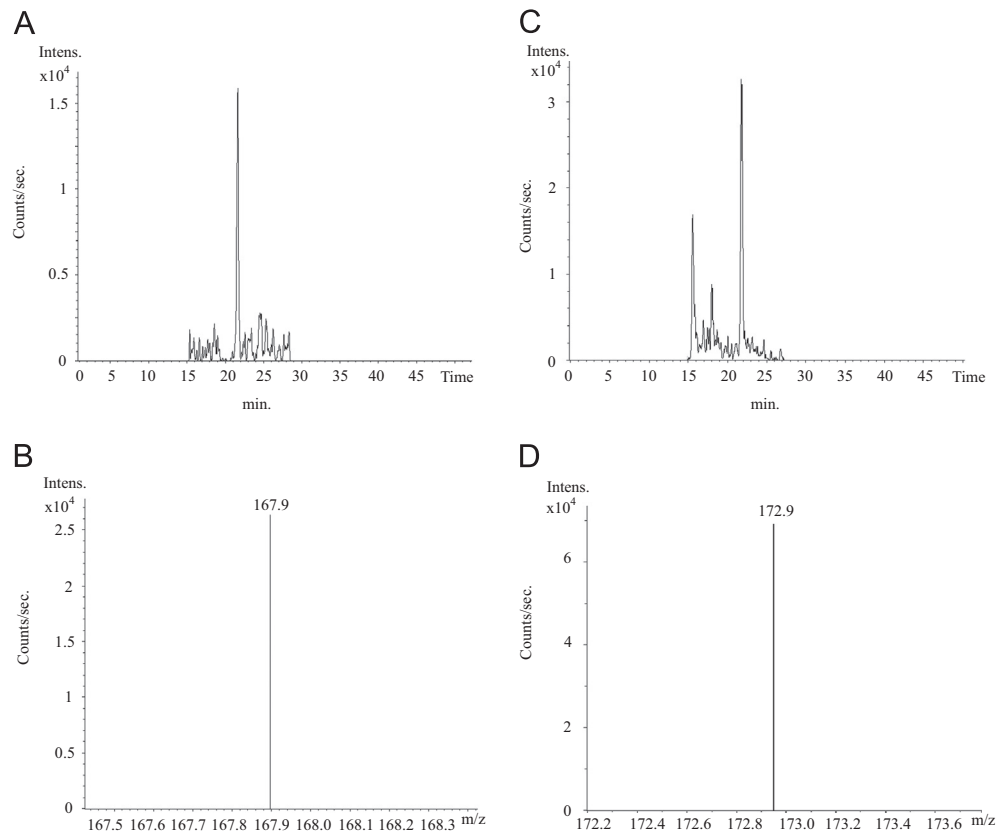
Fig. 1. Extracted ion chromatogram (EIC) and MS/MS scan of 2 ng/ml 8-oxodG and of ^{15}[N$_5$]8-oxodG (theoretic concentration 25 ng/ml) added to urine matrix (matrix 3 diluted 1:2). Panel A: EIC of 8-oxodG product ion. Retention time (rt) 21.7 min., 167.9 ± 0.2 m/z (284 m/z parent ion); smoothed (3.04 Gauss, 1 cycle). Panel B: MS/MS mass spectrum of 8-oxodG product ion (167.9 m/z). Panel C: EIC of ^{15}[N$_5$]8-oxodG product ion. rt 21.8 min., 172.9 ± 0.2 m/z (289 m/z parent ion) smoothed (3.03 Gauss, 1 cycle). Panel D: MS/MS mass spectrum of ^{15}[N$_5$]8-oxodG product ion (172.9 m/z).
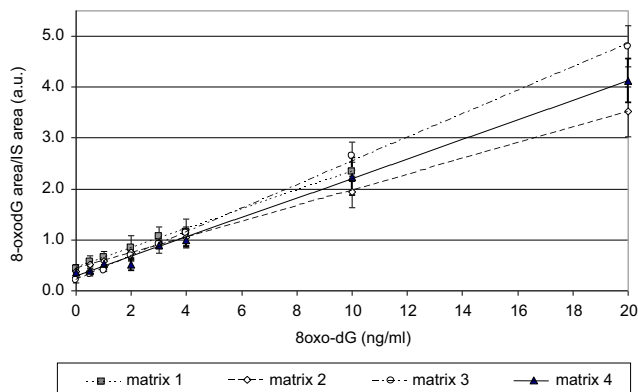

Fig. 2. Comparison of 8-oxodG standard in different urine matrixes (1:2 dilution). Results reported as 8-oxodG peak area/IS peak area. Each matrix was employed to build up from four to seven distinct standard curves. Data are shown as mean ± standard deviation (SD). Matrix 1 did not include the standard point at 20 ng/ml.

Table 1

Mean ratio 8-oxodG area/IS area in different urine matrices (1:2 dilution) ± 0.5 ng/ml 8-oxodG.

Urine matrix	Added 8-oxodG (ng/ml)	8-oxodG area/IS area (average)	SD	CV%	Repeats
1	0	0.4324	0.087	20.19	5
1	0.5	0.5667	0.125	22.04	5
2	0	0.3853	0.069	17.89	7
2	0.5	0.5121	0.086	16.78	6
3	0	0.2060	0.052	25.02	6
3	0.5	0.3224	0.027	8.36	5
4	0	0.3683	0.084	22.87	5
4	0.5	0.3976	0.082	20.70	5

CV%: between-day coefficient of variation.

Discussion

In the present paper we report the development of a LC-MS/MS method based on the combination of different HPLC solid phases (namely cation exchange and reverse phase) and a formic acid–water/methanol gradient. This approach resulted in increased efficiency of HPLC separation that allowed reliable quantitative measurement of 8-oxodG in urine samples by the MRM technique, employing an ion trap MS spectrometer. It is well known that the ion trap detectors work best in full scan analysis than in MRM, and that matrix effect, always an issue in LC/MS, is even more problematic in ion traps due to ion accumulation combined with space charge effect [13-15]. In a previously published work we combined surface-activated ionization source with ESI, together with cation-exchange chromatography, to improve ion trap performances. This strategy enabled the reliable measure of 8-oxodG in 1:10 diluted urine samples [12]. However, due to the low amount of endogenous 8-oxodG in urine from healthy subjects, this method was not sensitive enough to allow the detection of the analyte in several cases (data not shown). Here enhanced liquid chromatography performances resulted in the possibility of significantly decreasing sample dilution to a factor 1:2 and of using a regular ESI ion source. We also improved method accuracy by employing an isotopically labeled internal standard (chemically oxidized ^{15}[N$_5$]8-oxodG). This approach, together with the normalization of results by creatinine levels, is in line with the requirements resulting from a large inter-laboratory study published recently [10].

Table 2

Two level internal quality controls prepared in different urine matrixes diluted 1:2. Accuracy and precision reported for each single matrix and for overall system.

ICQ level 1 (2.5 ng/ml)*	8-OxodG/IS (peak area)*	ng/ml observed	Recovery	No. of repeats	CV%
Matrix 1	0.95 ± 0.16	2.59 ± 0.31	103.6	5	11.97
Matrix 2	0.75 ± 0.12	2.17 ± 0.37	86.8	4	16.98
Matrix 3	0.73 ± 0.10	2.35 ± 0.31	94.1	6	13.17
Matrix 4	0.80 ± 0.10	2.48 ± 0.40	99.2	10	16.29
All matrixes	0.80 ± 0.11	2.42 ± 0.36	96.8	25	14.79
ICQ level 2 (5 ng/ml)*	8-OxodG/IS (peak area)*	ng/ml observed	Recovery	No. of repeats	CV%
Matrix 1	1.42 ± 0.34	5.77 ± 0.39	115.4	6	6.74
Matrix 2	1.25 ± 0.19	5.14 ± 0.48	102.8	7	9.44
Matrix 3	1.32 ± 0.17	4.94 ± 0.47	98.8	6	9.42
Matrix 4	1.37 ± 0.21	5.18 ± 0.87	103.6	11	16.84
All matrixes	1.34 ± 0.22	5.24 ± 0.60	104.8	30	11.61

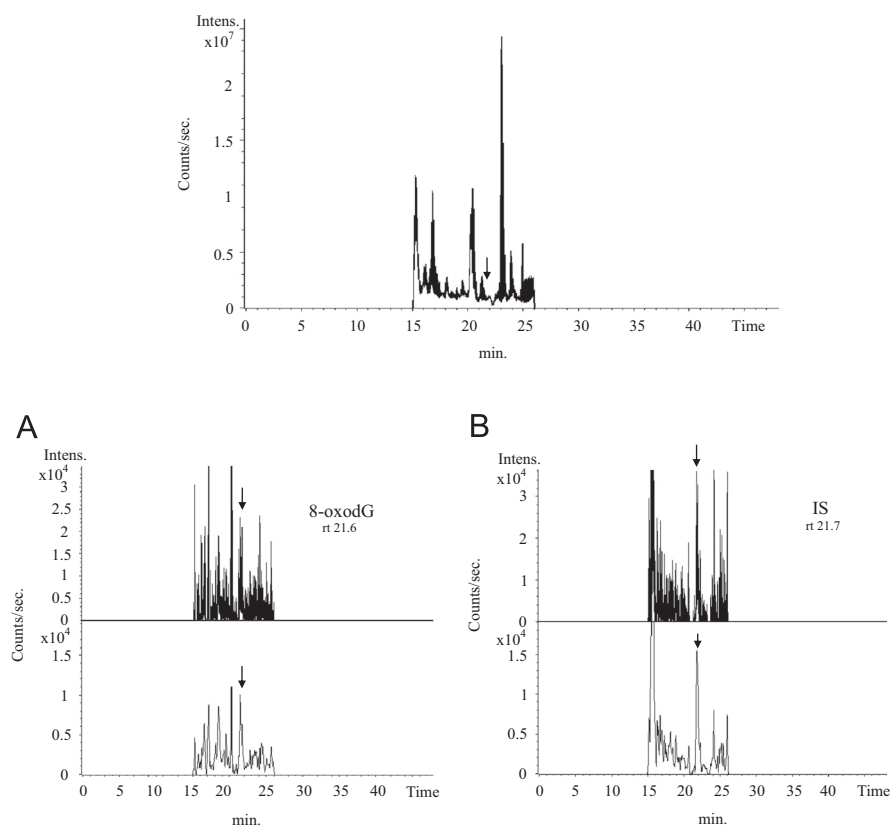* Data reported as mean $\pm$ standard deviation.

Fig. 3. MS/MS analysis of a urine sample. 8-OxodG/IS peak area ratio=0.74 and correspondent 8-oxodG concentration=2.3 ng/ml (mean of 3 analytical sessions). Upper panel: TIC+ all MSn. 8-OxodG chromatographic peak is indicated by an arrow. Lower panels: panel A, EIC of 8-oxodG product ion. Rt 21.6 min., 167.8 ± 0.2 m/z (284 m/z parent ion); upper box, unsmoothed; lower box, smoothed (3.01 Gauss, 1 cycle) arrow in correspondence of 8-oxodG product ion. Panel B: EIC of $^{15}[N_5]$8-oxodG product ion. Rt 21.7 min., 172.8 ± 0.2 m/z (289 m/z parent ion); upper box, unsmoothed; lower box, smoothed (3.01 Gauss, 1 cycle). an arrow in correspondence of IS product ion.

Table 3

Summary of results obtained in urine from healthy volunteers.

Gender	8-OxodG peak area/ IS peak area	8-OxodG ng/ml	Creatinine g/L	8-OxodG nmol/ mmol creatinine
Females (n=19)	0.593 ± 0.272	3.40 ± 2.33	0.99 ± 0.70	1.12 ± 0.40
Males (n=23)	0.789 ± 0.349	5.07 ± 2.98	1.64 ± 0.79	1.19 ± 0.51
	$p < 0.01$	$p < 0.05$	$p < 0.01$	$p = 0.39$

Results reported as mean $\pm$ SD. Samples with 8-oxodG < LOQ not included in statistical calculations (Mann Whitney test).

To analyze the influence of the urine matrix on the detection of 8-oxodG, we compared the results obtained from 4 independent urine samples spiked with increasing standard concentrations in the range 0.5–20 ng/ml. Results were linear in all the range of concentrations regardless of the matrix used. However, the LOQ was strictly dependent on the matrix employed for the generation of the calibration curve, and strongly influenced by its endogenous 8-oxodG content. Using a matrix with low endogenous 8-oxodG, LOQ could be fixed to at least 0.5 ng/ml (1.77 pmol/ml), a 6-fold increased level of sensitivity compared to the SACI/ESI-based method previously described [12]. The comparison with the sensitivity of previously published LC/MS methods for 8-oxodG

detection (all developed on triple quadrupole instruments) is difficult due to the different approaches used for LOQ determination. In some studies the chemically oxidized IS was employed [1,19]. This approach avoids the problem of endogenous 8-oxodG, but is flawed by the uncertain concentration of IS due to the variable efficiency of chemical oxidation necessary for the synthesis of the IS. In other studies, the method used for LOQ value determination is not clearly explained [20,21]. Nevertheless, reports describing 8-oxodG detection in urine by LC-MS/MS [1,19–21] showed LOQ values in the range of 0.7–3.3 pmol/ml, that are in line with the sensitivity of our method. It is also worth noting that our results were obtained without any need for sample processing and/or concentration, thus reducing the risk of artifactual sample oxidation. The precision of the method was satisfactory, with a CV% lower than 18% and less than 15% discrepancy from expected values regardless of the matrix used for the generation of the standard curve. These results are also comparable to those previously reported using triple quadrupole instruments [1,19].

When we examined urine samples from healthy volunteers, we observed a different distribution of 8-oxodG area/IS area ratios in males and females, with significantly higher mean ratios in males. This difference was also observed when 8-oxodG levels were considered in terms of concentration (ng/ml), but not when results were normalized for creatinine urinary content. In fact, the main difference found in males compared to females was related to different urine concentrations, with statistically higher creatinine levels in males. 8-OxodG area/IS area ratios < 0.30, corresponding to concentrations below LOQ, were found only in samples with creatinine level < 0.3 g/L, that were more frequent in females (6/25) than in males (1/24), but 8-oxodG resulted detectable in 6 out of 7 samples when reanalyzed undiluted. A single study found significant higher 8-oxodG creatinine-normalized levels in females compared to males [20], but other reports, including a recent large inter-laboratory study, confirmed that 8-oxodG levels are not gender-related after correction for creatinine content [10,19,21,22] underlining the need for creatinine correction for a reliable assessment of 8-oxodG in urine [10].

In addition, mean urinary 8-oxodG level detected in the present study (1.16 ± 0.46 nmol/mmol creatinine) is comparable with previously published data obtained by different LC-MS/MS methods [1,10,19,21,22]. The consistency between our results and previous reports obtained by the triple quadrupole MS/MS further supports the reliability of the multi-stationary phase LC-ion trap MS approach described in this report. Therefore the present method, by enhancing at best the chromatographic performances, allows the sensitive and accurate measurement of 8-oxodG in urine samples by ion trap LC-MS/MS, typically not employed for quantitative measurement in biological samples.

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