8-Hydroxy-2′-deoxyguanosine

Application Determination in urine

Analytical principle High performance liquid chromatography/ electrochemical detection

Completed in January 2002

Summary

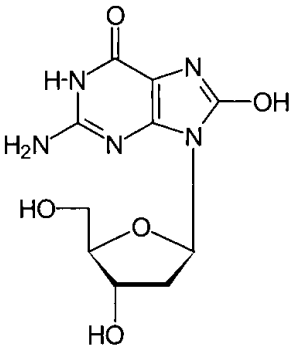
This method serves to quantify 8-hydroxy-2′-deoxyguanosine, which is formed from the reaction of various reactive oxygen species with human genetic material and is excreted in the urine after DNA repair.
8-Hydroxy-2′-deoxyguanosine (8-OHdG) is separated from acidified urine by liquid/ solid chromatography in sample preparation columns filled with phenyl-modified silica gel. After elution of the solid phase, 8-OHdG is separated by high performance liquid chromatography and quantified by means of an electrochemical detector. Calibration is performed using calibration standards that are prepared in pooled urine and are treated in the same manner as the samples to be analysed.

8-Hydroxy-2′-deoxyguanosine (8-OHdG)

Within-series imprecision:	Standard deviation (rel.)	s_w = 8.7% or 9.5%
	Prognostic range	u = 19.4% or 21.2%
	at a concentration of 10 µg or 100 µg 8-OHdG per litre urine	
	and where n = 10 determinations	
Between-day imprecision:	Standard deviation (rel.)	s_w = 9.3% or 11.2%
	Prognostic range	u = 20.6% or 24.8%
	at a concentration of 10 µg or 100 µg 8-OHdG per litre urine	
	and where n = 10 determinations	
Accuracy:	Recovery rate	r = 108% at 10 µg/L and 94% at 100 µg/L
Detection limit:	5 µg 8-OHdG per litre urine	

8-Hydroxy-2′-deoxyguanosine (8-OHdG)

Reactive oxygen species (ROS), such as singlet oxygen, ozone, hydrogen peroxide, superoxide radical anions and hydroxyl radicals, are formed by different *endogenous* processes (e. g. by incomplete reduction of oxygen in the respiratory chain) and *exogenous* processes (e. g. due to ionising radiation, inorganic and organic toxic substances and their metabolites).
If ROS react with the genetic material, some of the bases in the DNA strands may be oxidised. Covalent DNA adducts are formed.



8-Hydroxy-2′-deoxyguanosine [CAS number: 88847-89-6] (8-OHdG, Synonyms: 8-oxo-7,8-dihydro-2′-deoxyguanosine, 8-oxo-dG) is such a covalent modified nucleoside of the genetic material. Of the base adducts that are formed by reactions with ROS it is the oxidation product of DNA that occurs in the largest quantities.
Guanine modified in this manner then pairs with adenine instead of cytosine, and this leads to G:C → T:A transversions in bacterial and mammalian cells. However, ROS can also cause oxidation in the nucleotide pool of the cells. The deoxyribonucleoside-5′-triphosphate of 8-OHdG generated there is paired with adenine during DNA synthesis and after repair and renewed replication A:T → C:G transversions are caused [1].
Such DNA adducts may lead to mutations and cause the initiation of cancer cells if they are not repaired. Therefore they represent a marker for the initiation and possibly also for the progression in the multiple-step concept of carcinogenesis [2].
As already mentioned, ROS may also be formed endogenously during cell respiration. Therefore the organism has developed protective mechanisms to prevent damage to DNA or to repair it. ROS are intercepted by various enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (vitamin E, glutathione, ascorbate) antioxidants. In general, oxidative stress arises when the equilibrium between the oxidants and the endogenous antioxidants shifts in favour of the oxidants.
Moreover, cells exhibit a series of repair mechanisms to protect themselves against the genotoxic effects of 8-OHdG on the DNA strand. For instance *Escherichia coli* has three repair enzymes:

1) Formamidopyridine-DNA-glycosylase (Fpg also known as MutM, a glycosylase and AP-lyase) excises 8-hydroxyguanine (8-OHGua) when it is paired with cytosine [3, 4].
2) MutY (a monofunctional glycosylase) excises adenine from the 8-OHGua : adenine erroneous pairing [5].
3) MutT (a triphosphatase) hydrolyses 8-OHGua-triphosphate to the harmless monophosphate [1].

Fpg and MutY function on the principle of base excision repair (BER). During BER erroneous or abnormal DNA bases are excised from the DNA in the form of free modified bases by DNA glycosylases. Apuric or apyrimidic (AP) sites remain in the DNA. AP endonucleases subsequently remove the deoxyribose phosphate groups, and individual nucleotide gaps remain. These gaps are then filled again by DNA polymerases [6, 7]. Recently homologues of such enzymes have also been discovered in mammalian cells [8–12].
There is an additional single-step repair mechanism in humans (*nucleotide excision repair*) in which the entire modified nucleotide (e. g. 8-OHdG) is removed from the DNA [13].
Both 8-OHdG and 8-hydroxyguanine (8-OHGua) are repair products of the oxidised guanine base from DNA. 8-OHGua is the main excretion product of DNA repair in both the eukaryotes and the prokaryotes. At least 90% are excised in the form of 8-OHGua by the glycosylases [14].
Although 8-OHGua is excreted in higher concentrations than 8-OHdG, it is not suitable as a biomarker for oxidative stress because its excretion concentration is strongly influenced by individual nutritional habits. Rats, for example, excrete about 85% less 8-OHGua when fed on a diet without nucleic acids than when they have a "normal" diet. In contrast, the renal excretion of 8-OHdG is not significantly affected by diet in rats [15, 16]. In addition, as 8-OHdG is scarcely metabolised further in the organism and it is renally excreted, its determination is a suitable diagnostic tool for biological monitoring.
The mean quantity of 8-OHdG excreted by humans is approximately 60 ng/kg body weight or 1.5 to 4.5 µg/L urine per day [17]. Other publications report normal excretion in the range of between 2 and 15 µg per litre urine [18].
Sixty-nine test subjects (41 men, 28 women) of different ages were investigated at intervals of 4 to 8 weeks in six independent measurement series in 1996 [19] (Table 1). The main objective of the study was to establish the baseline value and to investigate the intraindividual and interindividual differences in 8-OHdG excretion. In addition, the influence of verifiable and measurable confounders such as age, gender, body mass index, smoking and passive smoking on 8-OHdG excretion was investigated.

Table 1. Investigation results on 8-OHdG excretion in urine

8-OHdG [μg/g creatinine]	n	Investigated groups of persons	Reference
2.52 ± 0.95	21	Non-exposed non-smokers	Tagesson et al. 1993 [18]
5.25 ± 1.88	5	Non-smokers exposed to diazo-dyes	
3.45 ± 1.45	9	Non-smokers exposed to asbestos	
3.98 ± 1.32	16	Workers in the rubber industry, non-smokers	
2.82 ± 1.12	20	Non-exposed smokers	
4.71 ± 2.2	25	Smokers exposed to diazo-dyes	
3.53 ± 1.40	21	Non-smokers exposed to asbestos	
3.3 ± 1.20	12	Workers in the rubber industry, non-smokers	
3.40 ± 1.22	65	Smokers and non-smokers exposed to benzene	Lagorio et al. 1994 [17]
28.41 ± 17.40	14	SCC (small-cell carcinoma) lung cancer patients	Erhola et al. 1997 [21]
19.77 ± 17.40	23	Non-SCC lung cancer patients	
19.38 ± 18.49	52	Control group	
6.75 ± 4.71	60	Healthy non-smokers	Germadnik et al. 1997 [19]
35.80 ± 16.40	81	Diabetics not dependent on insulin	Leinonen et al. 1997 [23]
24.28 ± 15.19	100	Control group	
10.8 (5.5–12.3)	27	Strong chromate reducers (5.4 (<1.0–7.3) μg Cr from erythrocytes/L blood)	Lewalter 1999 [24]
32.5 (10.2–71.3)	54	Weak chromate reducers (13.8 (5.2–26.8) μg Cr from erythrocytes/L blood)	
9.2 (<5.0–10.6)	50	Control group (1.1 (<1.0–2.0))	
5.88 ± 4.23	85	Children with Down's syndrome	Jovanovic et al. 1998 [25]
3.37 ± 2.6	81	Children in the control group (living in the same household)	
		Mean values in 24 h urine from 6 measurement series	Germadnik et al. 1997 [19]
4.85 ± 1.00	23	Smokers	
3.9 ± 1.03	22	Passive smokers	
3.96 ± 1.08	21	Non-smokers	
4.16 ± 1.13	40	Men	
4.4 ± 1.08	26	Women	
4.26 ± 1.10	67	Total	
3.93		**Medians of 8-OHdG in 24 h urine for investigations 1 to 6**	
3.30			
2.93		Investigation 1	
4.33		Investigation 2	
4.11		Investigation 3	
4.03		Investigation 4	
4.28		Investigation 5	
		Investigation 6	
		All investigations	

The mean values calculated from the 6 measurements showed that on average smokers excreted 25% more 8-OHdG than non-smokers or passive smokers. In contrast, no significant difference could be found between the baseline excretion of passive smokers and non-smokers.

Highly significant differences in the concentrations of 8-OHdG were determined between the individual examination dates. Based on the six independent measurement series an average interindividual VC of 48% (18 to 107%) and an average intraindividual VC of 36% in a range of 7.4 to 44.9 nmol/24 h were calculated for 8-OHdG excretion. Men excrete 27% more 8-OHdG in urine than women. However, these differences disappear when the values are based on the individual body weights. If the values were standardised on the creatinine excretion, then women excreted slightly more 8-OHdG than men.

The confounders smoking, gender, weight, size and age have a distinct influence on the 8-OHdG excretion per 24 h. However, if the values are based on the body weight, only smoking and age remain as dominant confounders.

The vitamin levels of each test person were also measured (vitamins A, C and E as well as β-carotene). Highly significant negative correlations for β-carotene and vitamin E with 8-OHdG excretion were found. No relationship between the vitamin A and C levels and 8-OHdG excretion was observed [19].

The determination of 8-OHdG in urine is very suitable for biological monitoring of groups of people who have been exposed over a long period to substances that are known to be mutagenic or carcinogenic and that are assumed to act by means of an ROS-related mechanism. These groups (e. g. workers in the azo-dye, rubber and asbestos industries [20], the chromate industry [24], or patients suffering from cancer who have recently undergone chemotherapy or radiotherapy [21]) exhibit considerably elevated concentrations of 8-OHdG in the urine compared with unexposed control groups (Table 1). Great fluctuations in 8-OHdG excretion were observed within a group. This can be attributed to the individual differences in tobacco consumption and age, and also to gender [19], diet [22], illnesses (e. g. diabetes mellitus [23]) and the genetic characteristics of the test persons.

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Analytical principle High performance liquid chromatography/ electrochemical detection

Completed in January 2002

Contents

1 General principles

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is separated from acidified urine by liquid/ solid chromatography in sample preparation columns filled with phenyl-modified silica gel. After elution of the solid phase, 8-OHdG is separated by high performance

liquid chromatography and quantified by means of an electrochemical detector. Calibration is performed using calibration standards that are prepared in pooled urine and are treated in the same manner as the samples to be analysed.

2 Equipment, chemicals and solutions

2.1 Equipment

HPLC system consisting of a gradient pump, a device for degassing the eluents, a column thermostat, an injection valve, an autosampler, an electrochemical detector (working electrode: graphite, reference electrode: Ag/AgCl, e. g. HP-ECD 1049 A from Agilent) and an integrator or a PC system for data evaluation.

High performance liquid chromatographic column:
Zorbax Bonus RP, length: 250 mm, inner diameter: 4.6 mm; particle diameter: 5 µm (e. g. Agilent Zorbax Bonus RP Order No.: 880668-901)

Test-tube shaker (Vortex mixer) (e. g. from IKA)

Adjustable pipette 10–100 µL (e. g. from Eppendorf)

Adjustable pipette 100–1000 µL (e. g. from Eppendorf)

Adjustable pipette 1000–5000 µL (e. g. from Eppendorf)

10 mL Polyethylene tubes

Sealable plastic bottles for sample storage

Volumetric flasks 20, 25, 100, 2000 mL

Laboratory centrifuge (e. g. from Hettich)

Microvials for the HPLC autosampler

2.2 Chemicals

Phenyl cartridge 3 mL, 500 mg (e. g. from Baker, Art. No. 7095-03, Bakerbond spe phenyl C_6H_5)

8-Hydroxy-2′-deoxyguanosine (e. g. from Sigma, H-5653)

Formic acid for trace analysis (e. g. Suprapur from Merck)

Acetonitrile (e. g. Lichrosolv from Merck)

Na_2SO_4 anhydrous p.a. (e. g. from Riedel-de Haën)

Methanol (e. g. from Merck)

Perchloric acid, p.a. (e. g. from Merck)

Deionised water (e. g. produced by means of Millipore® technology)

2.3 Solutions and conditioning

Eluent A (40% acetonitrile and 60% H_2O):
1200 mL water are placed in a 2 L volumetric flask. The volumetric flask is filled to its nominal volume with acetonitrile while swirling the contents at regular intervals.

Eluent B (0.1% formic acid):
Add 2 mL concentrated formic acid to 2000 mL water.

0.4 M Perchloric acid:
Approx. 50 mL water are placed in a 100 mL volumetric flask. 3.4 mL concentrated perchloric acid are added using a pipette, and the flask is filled to its nominal volume with water.

Conditioning of the phenyl cartridge:
The 3 mL phenyl cartridges (500 mg) are conditioned by introducing 2.5 mL methanol and then 2.5 mL water and allowing the solvents to run slowly through the cartridges. The cartridges are subsequently dried by suction.

Elution solution for the phenyl cartridges (pH 2):
Approx. 70 mL water are placed in a glass beaker. Formic acid is carefully added using a pipette while monitoring the pH by means of a pH meter and stirring until a pH value of 2 is reached. Then 40 mL methanol are added to 60 mL of this formic acid solution in a glass beaker, and the contents are thoroughly mixed.

2.4 Calibration standards

The calibration standards are prepared in pooled urine. Pooled urine is prepared from spontaneous urine samples collected in a suitable vessel. The samples are thoroughly mixed and stored at –18 °C until the standards and the control material are prepared.

Stock solution:
Approximately 10 mg 8-OHdG are weighed exactly into a 100 mL volumetric flask. The flask is subsequently filled to its nominal volume with water (100 mg/L).

Working solution A:
10 mL of the stock solution are pipetted into a 100 mL volumetric flask. The flask is subsequently filled to its nominal volume with water (10 mg/L).

Working solution B:
1.0 mL of the stock solution is pipetted into a 100 mL volumetric flask. The flask is subsequently filled to its nominal volume with water (1 mg/L).

Calibration standards:
Calibration standards in pooled urine are prepared in accordance with the following pipetting scheme. For this purpose the volumes of the relevant working solutions shown in Table 2 are each filled into a 50 mL volumetric flask, and then the flask is filled to its nominal volume with pooled urine. This material is divided into 6 mL

aliquots which are pipetted into 10 mL sealable polyethylene tubes and stored at approx. –18 °C. Calibration standards thus stored are stable for at least 2 months.

Table 2. Pipetting scheme for the preparation of calibration standards in pooled urine in 50 mL volumetric flasks

Volume of working solution A [µL]	Volume of working solution B [µL]	Concentration of the calibration standard [µg/L]
–	–	0
–	50	1 *
–	150	3 *
–	250	5
–	500	10
100	–	20
250	–	50
500	–	100

*) The detection limit is dependent on the quantity of urine used (see Section 9.3).

3 Specimen collection and sample preparation

The urine is collected in sealable plastic bottles. The urine samples should be processed as soon as possible, at the latest after 3 days of storage at 4 °C. If the samples cannot be processed immediately, the urine must be stored in the deep-freezer at approx. –20 °C until processing can be carried out. The stability of frozen urine samples was checked over a period of 2 months.

3.1 Sample preparation

Before analysis, the samples are thawed (if necessary) and thoroughly mixed. Then 5 mL urine are pipetted into a 20 mL test-tube, and the pH is adjusted to a value of 5 by adding 0.4 M $HClO_4$ (the pH value is monitored by means of a pH electrode; if the pH value is too low, 0.1 M NaOH must be added). The solution is subsequently transferred to a pre-treated phenyl cartridge. It is eluted using 9 mL water. The aqueous eluate is collected and transferred to a second *fresh* phenyl cartridge that has been conditioned as described above. Any eluate which drips out during this operation is discarded. Then the cartridge is eluted with 1 mL of a mixture of 60 parts of water at pH = 2 and 40 parts of methanol into an autosampler vial for the subsequent HPLC analysis.

4 Operational parameters

4.1 Operational parameters for high performance liquid chromatography

Separation column:	Material:	Steel
	Length:	250 mm
	Inner diameter:	4.6 mm
	Column packing:	Zorbax Bonus RP, 5 µm
Separation principle:	Reversed phase	
Temperature:	40 °C	
Detection:	Electrochemical detector (amperometry) Graphite working electrode Reference electrode with internal electrolytes (Ag/AgCl) Potential 700 mV	
Mobile phase:	Eluent A:	40% CH_3CN and 60% H_2O
	Eluent B:	0.1% formic acid
	(The eluents must be degassed before use!)	
Gradient:	11 min 96% B, 4% A 12 min 10% B, 90% A 15 min 10% B, 90% A 20 min 96% B, 4% A 25 min 96% B, 4% A	
Flow rate:	0.8 mL/min	
Injection volume:	50 µL	

Retention time of 8-OHdG: 9 minutes

All other parameters must be optimised in accordance with the manufacturer's instructions.
A retention time of 9.0 minutes was found for 8-hydroxy-2′-deoxyguanosine under the chromatographic conditions described here. This retention time serves only as a guideline. Users of the method must satisfy themselves of the separation power of the HPLC column they use and of the resulting retention behaviour of the substance.
Figure 1 shows the HPLC chromatograms of a native urine pool and several calibration standards in urine.

5 Analytical determination

In each case 50 µL of the urine samples processed as described in Section 3 are injected into the HPLC instrument. If the measured values are not within the linear range of the calibration curve, the urine samples are diluted with ultrapure water and processed anew.
A quality control sample is analysed with each analytical series.

6 Calibration

The calibration standards are processed in the same manner as the urine samples (Section 3.1) and analysed by means of high performance liquid chromatography with nitrogen-specific detection as described in Sections 4 and 5. In each case 50 µL of the processed calibration standards in urine are injected into the HPLC.
The calibration curve is obtained by plotting the integrated peak area of 8-OHdG as a function of the concentrations used. It is unnecessary to plot a complete calibration graph for every analytical series. It is sufficient to analyse one calibration standard for every analytical series. The ratio of the result obtained for this standard and the result for the equivalent standard in the complete calibration graph is calculated. Using this quotient, each result read off the calibration graph is corrected.
New calibration graphs should be plotted if systematic deviations are observed in the results of the precision check and if a new mobile phase or a new electrode on the detector is used.
Urine samples with an 8-OHdG concentration of between 5 µg/L and 50 µg/L can be measured without interference under the conditions described here and using this detector.

7 Calculation of the analytical result

The resulting peak areas of the analyte are used to read off the corresponding concentration of 8-OHdG in µg per litre urine from the relevant calibration curve. As a rule, the pooled urine used to prepare the calibration standards exhibits a background signal, and so the resulting calibration graph has to be shifted in parallel so that it passes through the zero point of the coordinates. The concentration of 8-OHdG in the urine used to prepare the calibration standards can be read off the intercept with the axis before the parallel shift is carried out.

8 Standardisation and quality control

Quality control of the analytical results is carried out as stipulated in the guidelines of the Bundesärztekammer (German Medical Association) [26, 27] and in the special preliminary remarks to this series. In order to check the precision of the method a urine control sample containing a constant concentration of 8-OHdG is analysed. As material for quality control is not commercially available, it must be prepared in the laboratory. It is advisable to use urine or pooled urine with a native concentration of 8-OHdG of approx. 10 µg/L, or to spike the urine of non-exposed persons with 10 µg/L. A six-month supply of this control material is prepared, divided into aliquots in 10 mL polyethylene tubes and stored in the deep-freezer. The theoretical value and the tolerance range for this quality control material are determined in the course of a pre-analytical period (one analysis of the control material on each of 20 different days) [28–30].

9 Evaluation of the method

9.1 Precision

Pooled urine spiked with 10 µg/L and 100 µg/L of 8-OHdG was processed and analysed as described in the previous sections to check the precision in the series. Ten replicate determinations of the urine samples yielded the precision in the series documented in Table 3.
In addition, the precision from day to day was determined. The same material was used as for the determination of the precision in the series. This urine was processed and analysed on each of 10 different days. The precision results are also shown in Table 3.

Table 3. Precision for the determination of 8-OHdG

	n	Concentration [µg/L]	Standard deviation (rel.) [%]	Prognostic range [%]
In the series	10	10	8.7	19.4
	10	100	9.5	21.2
From day to day	10	10	9.3	20.6
	10	100	11.2	24.8

9.2 Accuracy

Recovery experiments were carried out to test the accuracy of the method. Urine was spiked with 10 or 100 µg 8-OHdG per litre for this purpose. Each of these solutions was subsequently processed and analysed ten times in accordance with Section 3. The mean relative recovery rates were 108% (10 µg/L) and 94% (100 µg/L).

In addition, the losses due to processing were checked as a measure of the accuracy. For this purpose reference standards prepared in urine were processed and analysed. Aqueous standards with the same quantity of 8-OHdG were simultaneously prepared in the same manner as the calibration standards. These aqueous standards were injected into the HPLC and analysed without further treatment. The mean recovery rate of 86.6% (range 85–90%) at a concentration of 10 µg/L was found by comparison of the peak areas of the aqueous standards with the peak areas of the 10 processed calibration standards in urine. This means that an average of 13.4% of 8-OHdG is lost during processing of the urine samples.

9.3 Detection limits

Under the conditions given here the detection limit, calculated as three times the signal/noise ratio of the analytical background in the temporal vicinity of the analyte signal, is approximately 5 µg/L and is limited by the background noise. Many of the urine samples investigated by this method did not exhibit this background interference. In such cases it was possible to detect 8-OHdG concentrations as low as 1 µg/L with certainty.

9.4 Sources of error

Interfering peaks which impaired the quantification of 8-OHdG below a value of 5 µg/L were observed in the vicinity of the 8-OHdG peak in some of the analysed urine samples. 8-OHdG background levels are given as 2 to 15 µg/L urine or µg/g creatinine in the literature. During optimisation of the method it became apparent that the selected phenyl cartridge and the HPLC separation column had a decisive influence on the reproducibility, the separation of interfering peaks and the cleaning-up of the solutions for analysis. Therefore the phenyl cartridges should be checked by batch before use, and the quantity of elution solutions should be adjusted as required. As the method is carried out without an internal standard, it is essential to treat the samples in an identical manner.

It is absolutely necessary to ensure that the eluents are thoroughly degassed, preferably by means of helium or by using a degasser. In general, the baseline of the ECD must be readjusted after each separation run. Electronic compensation was carried out by the HPLC instrument used in this case. Apart from thorough cleansing of the graphite electrode no further cleaning steps are generally necessary. However, we expressly refer the reader to the appropriate instructions given by the manufacturer of the electrochemical detection cell.

10 Discussion of the method

As a rule, the analytical method presented here is capable of reliably and reproducibly determining 8-OHdG excreted in urine in the range of the lower background levels of 1 to 2 µg/L urine. However, interfering peaks were observed in some urine samples. Although the sample clean-up with phenyl cartridges largely eliminated the interference, this still limited the detection limit of the method to 5 µg/L.
The sample preparation by means of phenyl cartridges functions according to chromatographic principles. The analyte is separated from part of the urine matrix in the first phenyl cartridge. As the analyte is also slightly retained, the first column must be eluted with relatively large amounts of water (9 mL). The analyte is subsequently enriched on the second phenyl cartridge, and then it is eluted using only 1 mL of an acidic methanol/water mixture (pH 2). Thus the analyte can be enriched by a factor of 5. The quantities of elution solutions given here represent an optimum for the phenyl cartridges used by the author of the method. As the properties of the solid phase material can vary not only from one manufacturer to another but also between batches, the user of the method must satisfy himself of the separation power of the material used.
The examiner of the method established that the procedure is readily replicated and verified its reliability. The reliability criteria are excellent, especially in view of the fact that the method had to be carried out without using an internal standard. A suitable internal standard is not available at present. Up to 40 determinations of 8-OHdG can be carried out and evaluated per day by an experienced analyst.
As described, the sensitive and selective determination of 8-OHdG in urine was achieved using an HPLC with electrochemical detection (ECD). Compared with UV detection, this detection is more sensitive for 8-OHdG by a factor of approximately 1000 [31, 32], and moreover it is relatively inexpensive to purchase. However, before measurement is carried out using the electrochemical detector various cleaning steps are necessary due to the strong influence of the urine matrix (see also e. g. [17, 19, 20]). Recent research on the determination of 8-OHdG in urine also includes the use of the HPLC-MS technique (e. g. [33]). In this case clean-up of the sample may be unnecessary due to the use of column-combination techniques. The use of this type of detection is also possible with the sample processing described here, provided slight modifications are made. In this case 5 mL urine are passed through only one conditioned (see Section 2.3) phenyl cartridge, eluted with methanol/water (40 : 60, pH 2) and then measured. The mass transfer $283.9 \rightarrow 167.85$ in the positive electrospray mode is recorded. Deuterated 8-OHdG is recommended as the internal standard in the LC-MS/MS procedure. The interference which may sometimes occur in an ECD has not been observed when the LC-MS technique is used. Independent of the urine matrix, the detection limit of 1 µg 8-OHdG per litre urine required to evaluate the background level can be reliably achieved without interference.

Instruments used:
HPLC HP 1090 from Agilent with integrated autosampler, electrochemical detector HP-ECD 1049 A, evaluation software: HP Chemstation for LC Rev. A.0603[509].

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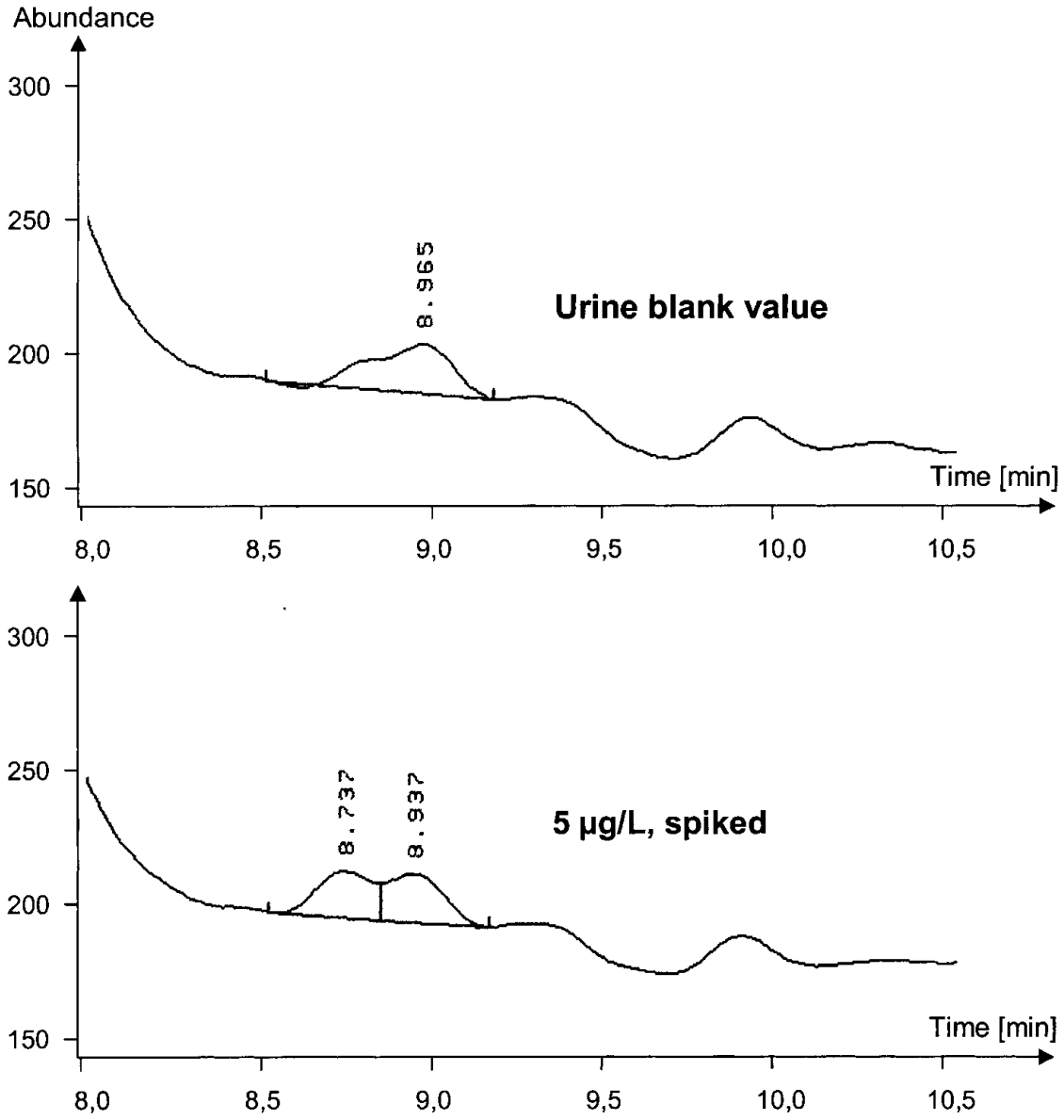

Fig. 1. Example chromatograms of a processed sample of pooled urine

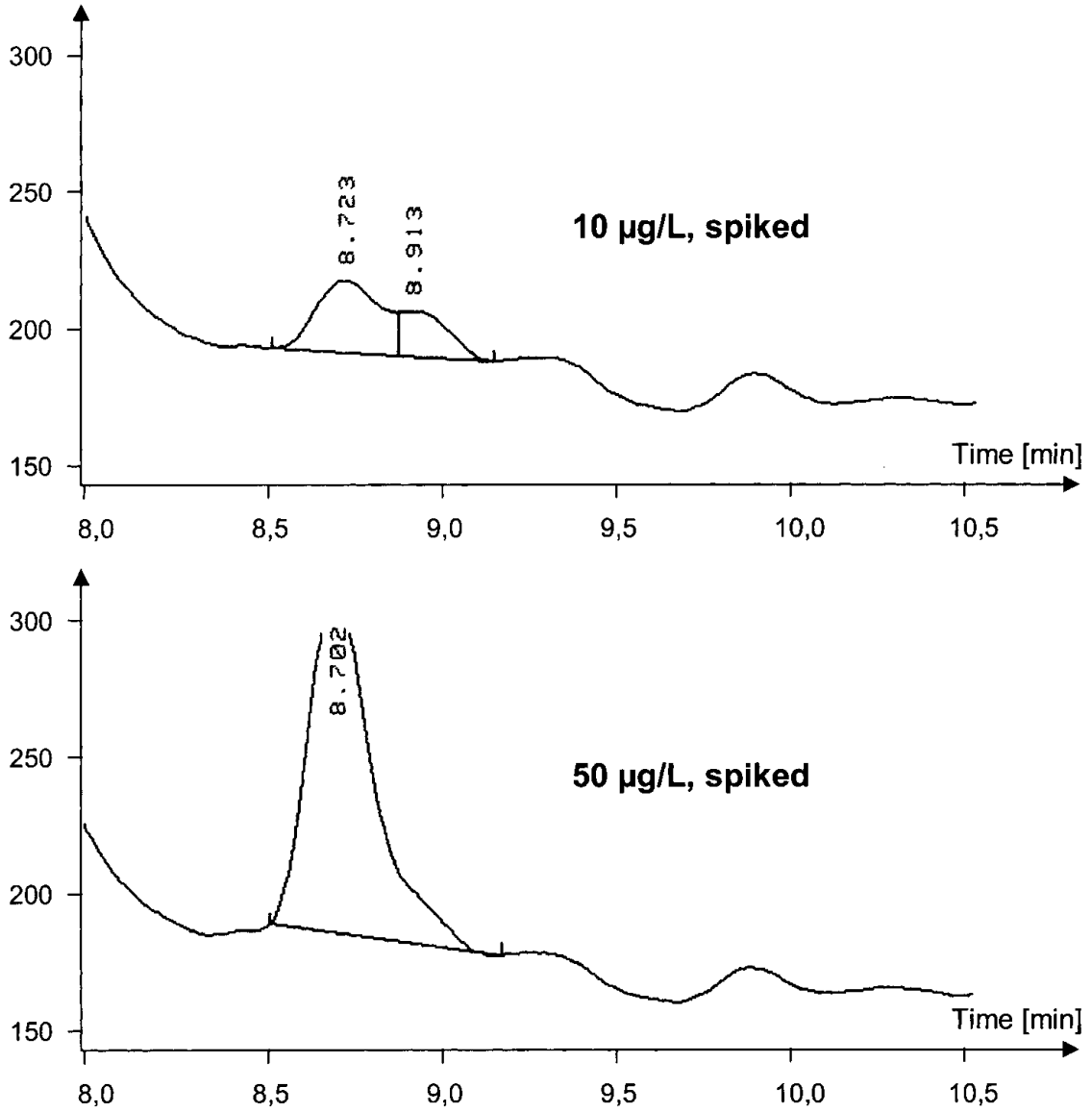

Fig. 1 (continued)