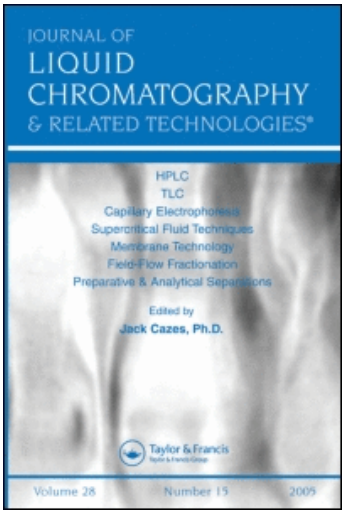

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Detection of Trace Amounts of 8-Hydroxy-2'-Deoxyguanosine in Commercial 2'-Deoxyguanosine by Means of HPLC Analysis and Electrochemical Analysis

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DETECTION OF TRACE AMOUNTS OF 8-HYDROXY-2'-DEOXYGUANOSINE IN COMMERCIAL 2'-DEOXYGUANOSINE BY MEANS OF HPLC ANALYSIS AND ELECTRO-CHEMICAL ANALYSIS

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ABSTRACT

A commercial sample of 2'-deoxyguanosine (dG) was submitted to reversed phase HPLC analysis. The column effluent was analyzed by UV detection set at 293 nm coupled to an electrochemical detector at 600 mV oxidation potential. When both detectors were set at their highest sensitivity, the UV detector did not show the presence of the 8-hydroxy-2'-deoxyguanosine but the electrochemical detector was capable of detecting this oxidatively modified 2'-deoxyguanosine. When planning in vitro experiments with 2'-deoxyguanosine in order to detect the oxidatively modified nucleoside it is necessary to assess the presence of this C8-hydroxylated derivative in the commercial product.

INTRODUCTION

The determination of 8-hydroxy-2'-deoxyguanosine by high pressure liquid chromatography is a promising technique for the detection of oxidatively

modified guanosine nucleosides from DNA exposed to oxygen radical forming (carcinogenic) agents (1,2). When planning to perform experiments with 2'-deoxyguanosine in order to detect any oxidatively modification after treatment with oxygen radical forming agents, it is necessary to assess whether the C-8 hydroxylated derivative of dG is present - even in small amounts - as the degree of C-8 hydroxylation might be very low. We were therefore interested whether the commercial dG-sample is free of any oxidative byproducts. We employed reversed phase HPLC coupled to ultraviolet (UV) and electrochemical (EC) detection.

MATERIALS

High pressure liquid chromatography

Use was made of a Spectra Physics SP 8000B liquid chromatograph equipped with an injection valve (Valco), a sample loop of 20 µl, and a Hibar Lichrosorb reversed phase column (dimensions: 4.0 mm ID x 25 cm length) packed with 7 µm particles. The eluting solvent was 2.5% methanol in 10 mM ammonium acetate pH 5.3 set at a flow rate of 1.5 ml/min. The oven temperature was 40°C. The column effluent was directed to a Spectra Physics SP 8400 ultraviolet/visible wavelength detector set at 293 nm and to an electrochemical detector from Pye Unicam PU 4022 set at 600 mV oxidation. As the electrochemical detector is very sensitive to electrostatic electricity and temperature variations when set at its highest sensitivity, the detector cell was installed in an insulating box in which the inner surface was covered with aluminium foil (Figure 1).

Chemicals

2'-Deoxyguanosine was purchased from Boerhinger-Mannheim. L-Ascorbic acid and perhydrol (30 % hydrogen peroxide) were from Merck. Methanol and water were HPLC grade from Alltech Europe.

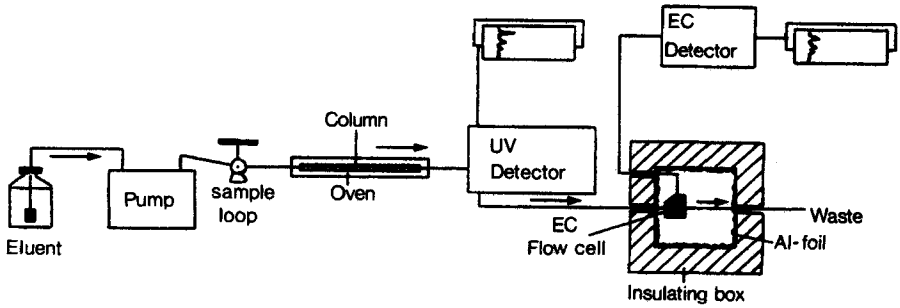

Figure 1. Schematic drawing of the HPLC apparatus used for the detection of trace amounts of 8-hydroxy-2'-deoxyguanosine in commercial 2'-deoxyguanosine.

METHODS

Synthesis of 8-hydroxy-2'-deoxyguanosine

To 0.6 mg of 2'-deoxyguanosine, 2.5 mg of L-ascorbic acid was added in an Eppendorf tube (1.5 ml). Both compounds were dissolved in 1 ml of the HPLC eluent. To the solution, 10 µl of perhydrol was added and the reaction started by vigorous stirring. The tube was wrapped in aluminium foil and kept at 37°C. After 1 hour, 20 µl of the reaction mixture was applied on the reversed phase column and analyzed by HPLC/UV/EC.

Analysis of commercial 2'-deoxyguanosine for the presence of 8-hydroxy-2'-deoxyguanosine

Approximately 0.6 mg of 2'-deoxyguanosine was dissolved in 1 ml of the HPLC eluent. From this solution 20 µl (app. 50 nmol dG) was applied on the reversed phase column.

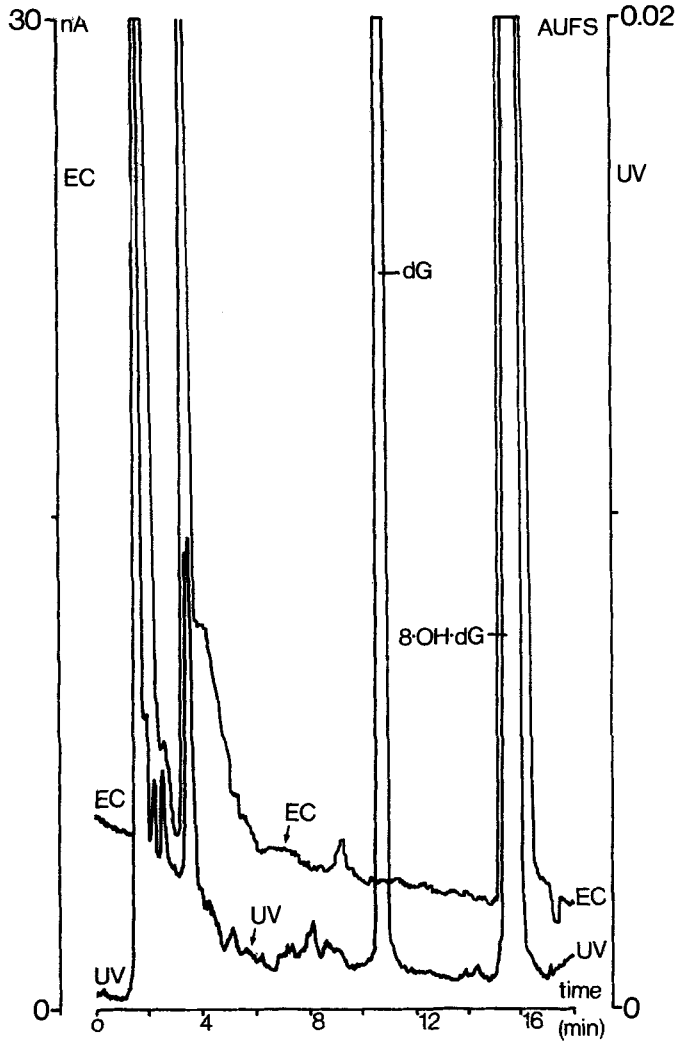

Figure 2. HPLC chromatogram of the reaction mixture of 2'-deoxyguanosine (dG) with L-ascorbic acid and hydrogen peroxide employing ultraviolet detection (R 0.02, recorder 10 mV) and electrochemical detection (30 nA, recorder 1 V). The dG peak is only detected by the UV detector, while 8-hydroxy-2'-deoxyguanosine (8-OH-dG) is also detected by the EC detector. Chromatographic conditions as in text.

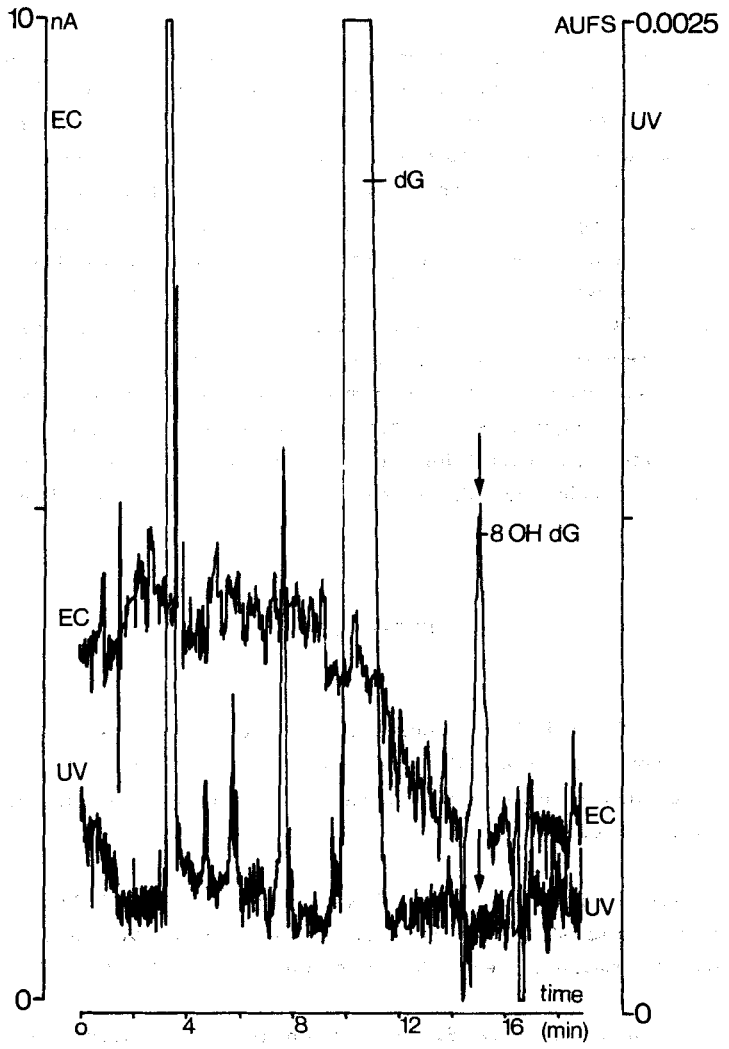

Figure 3. HPLC chromatogram of a commercial sample of 2'-deoxyguanosine (app. 50 nmol dG) dissolved in the HPLC eluent. Both detector systems are set at their highest sensitivity: UV detector: R:0.0025, recorder 10 mV); electrochemical detector: 10 nA at an amplification of tenfold the normal detector output, recorder 2 V). The small peak on the EC detector plot represents 8-hydroxy-2'-deoxyguanosine (8-OH-dG). Chromatographic conditions as in text.

RESULTS AND DISCUSSION

After reaction of 2'-deoxyguanosine in the presence of L-ascorbic acid and hydrogen peroxide, a peak appears with a retention time of about 15.5 minutes. This peak represents the C-8 hydroxyl derivative of 2'-deoxyguanosine as has already been described elsewhere (2,3). No 2'-deoxyguanosine is detected by the EC detector (Figure 2). When 20 µl of the solution of the commercial sample of 2'-deoxyguanosine in 1 ml eluent is analyzed by HPLC/UV, no peak is observed at a retention time of 15.5 minutes even at the highest sensitivity of the ultra violet detector (Figure 3).
However, the EC detector plot shows the presence of a small peak that appears at a retention time of that shown by 8-hydroxyl-2'-deoxyguanosine, The identity was confirmed by cochromatography as the C-8 hydroxylated derivative of 2'-deoxyguanosine. This small peak is not caused by sample loop contamination as several injections of the same sample or scrupulous cleaning of the sample loop, did not show any decrease in peak height.

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