ANALYSIS OF BIOMARKERS OF OXIDATIVE STRESS IN URINE USING UPLC-MS/MS AND HPLC-ECD

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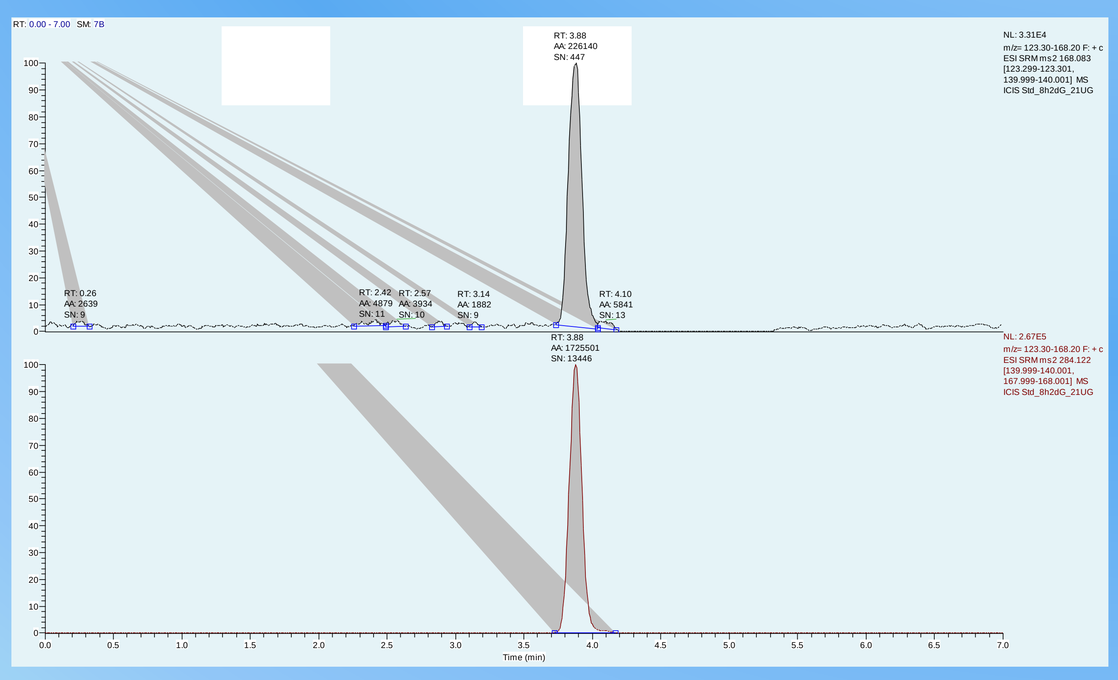
INTRODUCTION:

The aim of the study was to optimize UPLC-MS/MS for routine determination of biomarkers of oxidative stress related to DNA damage. As a biomarker of oxidative damage of DNA was used 8-OH-2-deoxy-quanosine (8-OHdG). It is believed that 8-OHdG is also a suitable marker of various inflammatory and precancerous conditions and can be used to predict early illnesses associated with unhealthy lifestyle [1,2]. Levels of 8-OHdG were monitored in real urine samples.

ANALYSIS OF BIOMARKERS USING UPLC-MS/MS:

Quantitative determinations of 8-OHdG were carried out using UPLC Ultimate 3000 with mass spectrometer TSQ Access Max, (Thermo Fisher Scientific, USA). The chromatographic separation was achieved using a Kinetex® column with size: 50 × 2.1 mm, F5 stationary phase with core-shell particles, particle size 1.7µm, 100 Å (Phenomenex, USA) protected with Ascentis® Express C18 Guard Column with 2µm particles in-line filtr (Supelco, Germany).
Mobile phase A consisted of water (pH 3 using LC-MS purity acetic acid) and methanol (using formic acid 0.2 mM) in the ratio 90/10 (v/v). Mobile phase B consisted the mixture of acetonitrile and methanol with the ratio 70/30 (v/v).
Analytical separation was realized under solvent gradient with 7 min total time per one run. The temperature of column space was maintained at 40 °C, a flow rate was set at 0.5 mL/min and 5 µl of sample was injected. After optimization of separation conditions, the method was applied to biological material – urine (Fig.1, 2).
Analytical samples were prepared by simple dilution with mobile phase A, centrifuged and filtered using 0.22 µm Nylon membrane filters adapted for syringe. Urine samples were provided by one healthy volunteer on every day collection.

Fig.1: SRM of 8-OHdG standard in 2.1 µg/ml concentration

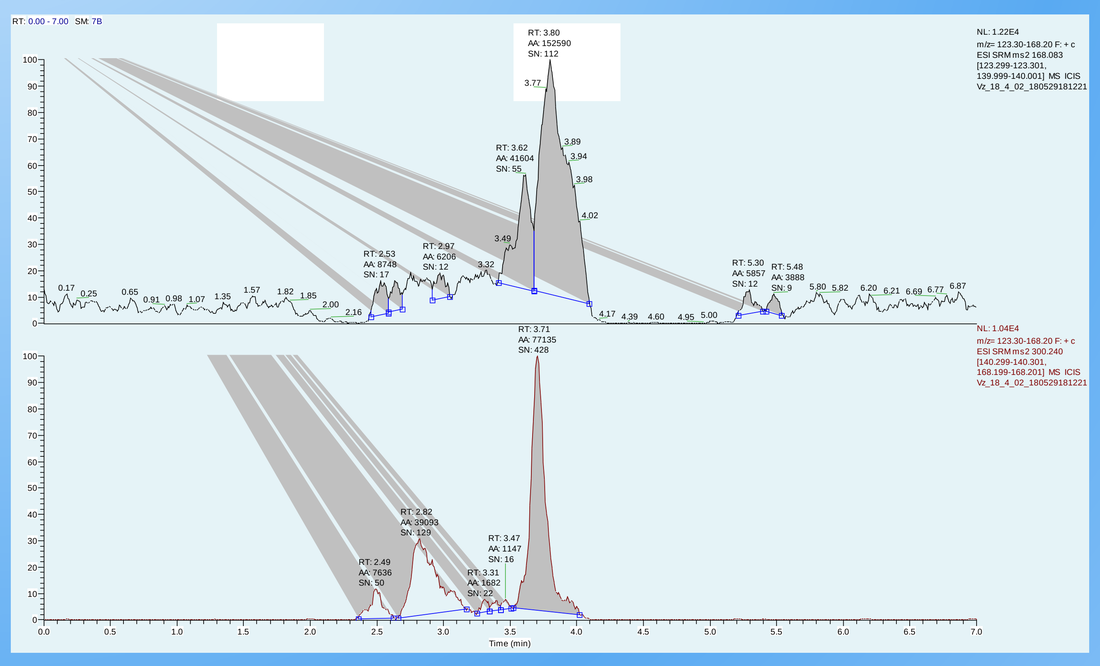


Results were compared with creatinine concentration in every urine samples for the correction at diuresis. HITACHI COBAS enzymatique method (Roche) was used for quantitative determination of creatinine in urine samples.

ANALYSIS OF BIOMARKERS USING HPLC-ECD:

The HPLC-ECD separations were performed using an ESA 582 isocratic pump (Thermo Fisher Scientific, formerly ESA Inc., USA) with a pulse damper and a Rheodyne manual injector equipped with a 10 loop (Rheodyne, Cotati, CA, USA). A Coulochem III (ESA Inc.) dual-channel coulometric detector was used for the detection, with parameter settings as follows: screening potential E1 = +100 mV (*vs* Pd/H_2), analytical potential E2 = +450 mV (*vs* Pd/H_2), gain 500 nA/V.
A Nucleodur Gravity C8 5 µm, 250 mm × 2.1 mm I.D. HPLC column (Macherey-Nagel, Düren, Germany), with a corresponding guard column, was used for the separations. The mobile phase consisted of 25 mM potassium hydrogen phosphate pH 6,2 (set-up with phosphoric acid, filtered through a 0,22 µm nylon filter) and methanol, 96/4 (v/v). Flow-rate 0,25 ml/min was used. To remove proteins and other high-molecular-weight impurities, the urine samples were centrifuged (7200x*G*) through a Microcon YM-10 filter (Millipore Merck, Darmstadt, Germany) and diluted with the mobile phase (1/3, v/v) prior to HPLC analysis. The calibration for 8-OHdG was done within the range of 4.10^{-8} - 8.10^{-7} mol/l (11 – 226 µg/l)

Fig.2: SRM of 8-OHdG in real urine



RESULTS:

The levels of 8-OHdG in urine samples (n=26) varied from 0,013 to 0,111 µg/ml with corresponding values in creatinine-normalized samples required from 0,0125 to 0,245 µg 8-OHdG/g creatinine (median = 0,065 µg/ml, 0,109 µg 8-OHdG/g creatinine respectively. The main advantage of this method is easy and rapid performance with the preservation of original ability to simultaneously monitor the effect of oxidative stress damage to DNA in patients with mainly cancer diseases. Our MS results were compared with HPLC-ECD measurements.

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REFERENCES:

[1] R. Rozalski, D. Gackowski, A. Siomek-Gorecka, M. Starczak, M. Modrzejewska, Z. Banaszkiewicz, R. Olinski, Biomarkers 20 (2015) 287-291.
[2] P.G. Cocate, A.J. Natali, A. de Oliveira, G.Z. Longo, R.D.G. Alfenas, M.D.G. Peluzio, E.C. dos Santos, J.M. Buthers, L.L. de Oliveira, H.H.M. Hermsdorff, Nutrition 30 (2014) 660-665.