

Long Term Monitoring of 8-Hydroxy-2'-Deoxyguanosine and 8-Hydroxyguanosine in Urine as Indicators of Oxidative Stress Using UHPLC-QqQ



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INTRODUCTION

The oxidative stress resulting from either overproduction of the hydroxyl free radicals or inadequate antioxidant defenses is thought to precede a number of diseases including cancer, cardiovascular, diabetes and neurodegenerative disorders. Currently urinary levels of 8-Hydroxy-2'-Deoxyguanosine are being studied as a biomarker for estimating oxidative stress. Samples of urine of one volunteer were stored at -18 °C. Samples of 0.5 ml of urine were diluted by 0.5 ml of 0.2 mM formic acid in methanol, centrifugated at 10000 rpm for 10 minutes, filtrated through 0.22 µm Nylon filter and 20 µl was injected. UHPLC-QqQ analyses were carried out in Ultimate 3000 system combined with TSQ Acces Max. Kinetex F5 column (50 x 2.1mm, 1.7 µm) with precolumn Zorbax Eclipse XDB-C18 (4,6 x 5 mm, 1.8 µm) was used for chromatography separation. Mobile phase A consists in formic acid (0.1 % v/v). Mobile phase B consists in methanol (0.1% v/v). Mass spectrometric detection was based on MRM transitions and HESI; collision gas pressure; 1,5 mTorr cycle time 0.5 s; capillary temperature in source 325 °C; vaporizer temperature 350 °C; auxiliary gas pressure 45 arb units; ion sweet gas pressure 2 arb units; positive polarity spray voltage 3500 V; discharge current 4 µA. MRM transitions of 8-Hydroxy-2'-Deoxyguanosine: 284.122 (parent ion), 168.000 (quantifier ion), 140.000 (qualifier ion). MRM transitions of 8-Hydroxyguanosine: 300.240 (parent ion), 168.200 (quantifier ion), 140.300 (qualifier ion). The resulted concentrations of biomarkers were standardized at urine creatinine values. Medians of 8-Hydroxy-2'-Deoxyguanosine, 8-Hydroxyguanosine were 12.07 ng/g, 50.45 ng/g, respectively.

SAMPLE PREPARATION

- Storage of samples at -18°C before sample preparation

Sample Preparation Process

- dilution of urine (0.5 mL) in 0.2mM formic acid in methanol (0.5 mL)
- centrifugation (10, 000 RPM, 10 min)
- filtration through microfilters (nylon, 0.22 µm)

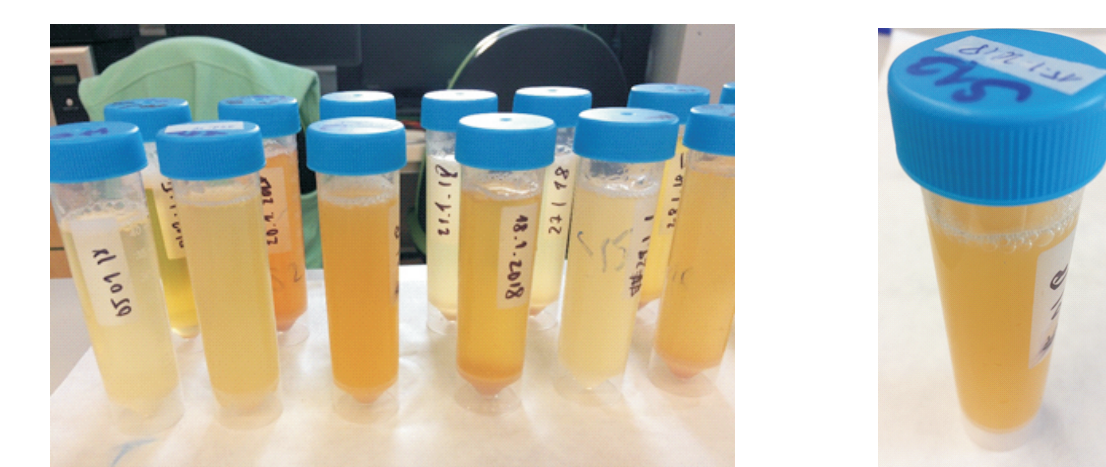


Fig.1: Urine samples

Instrumentation

- UHPLC-QqQ analyses were carried out in Ultimate 3000 system combined with TSQ Acces Max.

HPLC Conditions

- Kinetex F5 column**
- 50 x 2.1mm, 1.7 µm (Phenomenex)
- Precolumn Zorbax Eclipse XDB- C18**
- 4.6 x 5 mm, 1.8 µm (Agilent)

Mobile phase

- Solvent A - Formic Acid (0.1% in water)
- Solvent B - Formic Acid (0.1% in methanol)

Gradient elution

Time [min]	A [%]	B [%]
0	100	0
0.1	100	0
0.2	65	35
0.3	55	45
0.4	2	98
0.7	0	100
3	0	100
4	95	5
5	95	5
6	100	0
7	100	0

- Flow rate 0.50 mL/min
- Injection volume 20 µL

MS Parameters

- Ionization Mode ESI⁺
- Cycle Time 0.5 s
- Collision Gas Pressure: 1,5 mTorr
- Capillary Temperature in Source: 325 °C
- Vaporizer Temperature: 350 °C
- Auxiliary Gas Pressure: 45 arb units
- Ion Sweet Gas Pressure: 2 arb units
- Polarity Spray voltage: 3500 V
- discharge current 4 µA
- Scan mode SRM

Scan monitoring reactions and CID for analytes

Analyte	Parent mass	Product mass	Collision energy (eV)	Tube Lens
8-OH-2-deoxy-guanosine	284.1	140.0	48	48
	284.1	168.0	15	48
8-OH-guanosine	300.2	140.3	28	50
	300.2	168.2	16	50

LOD/LOQ

	8-OH-guanosine (ng/ml)	8-OH-2'-deoxy-guanosine (ng/ml)
LOD	2.2	3.7
LOQ	2.5	4.3

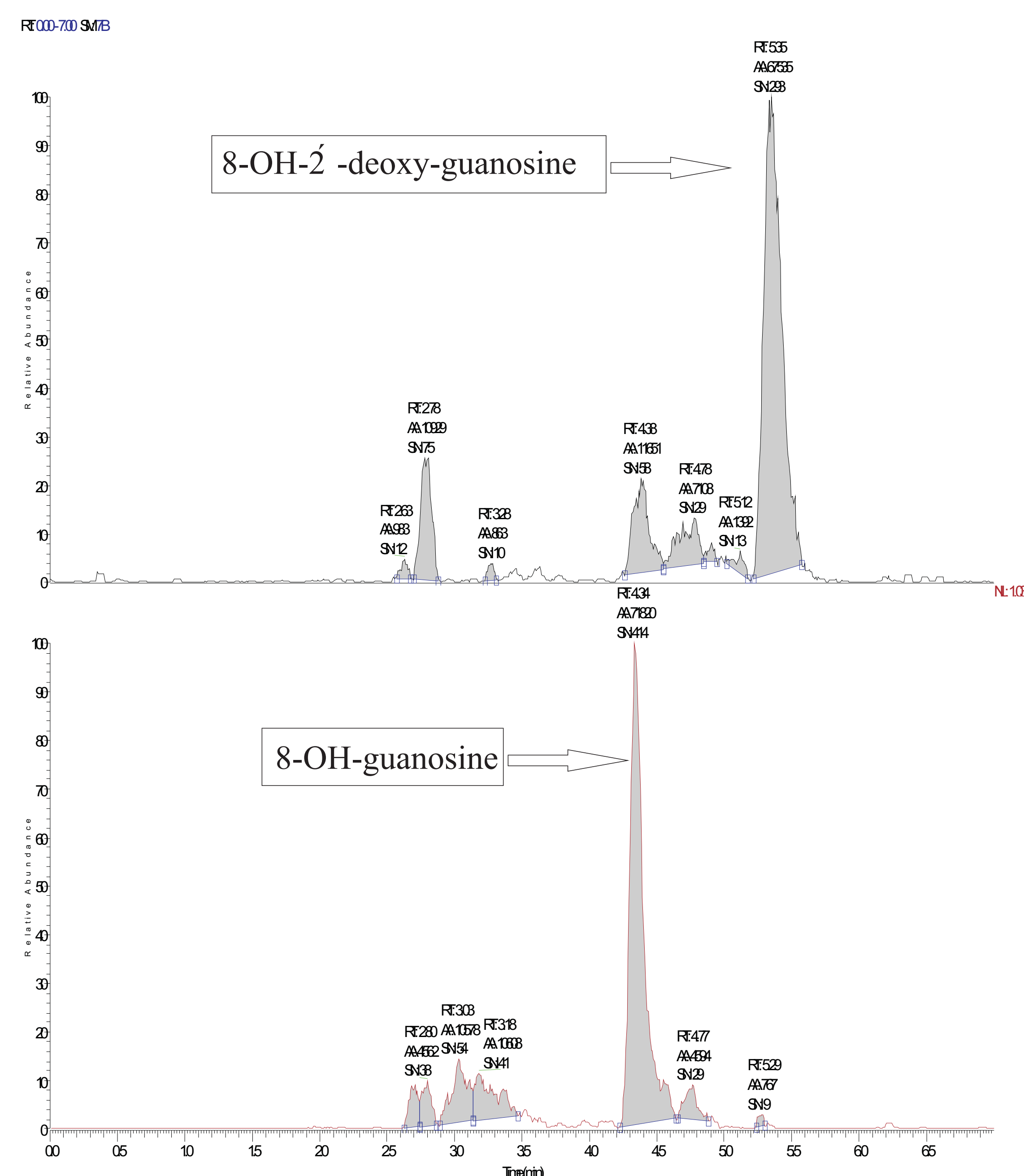
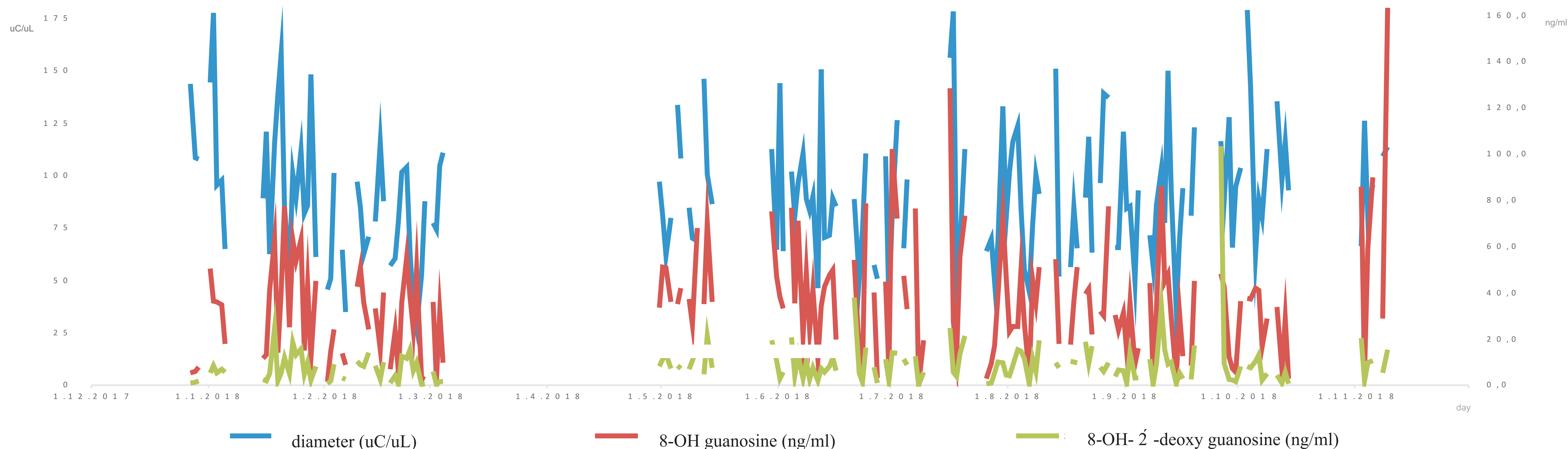


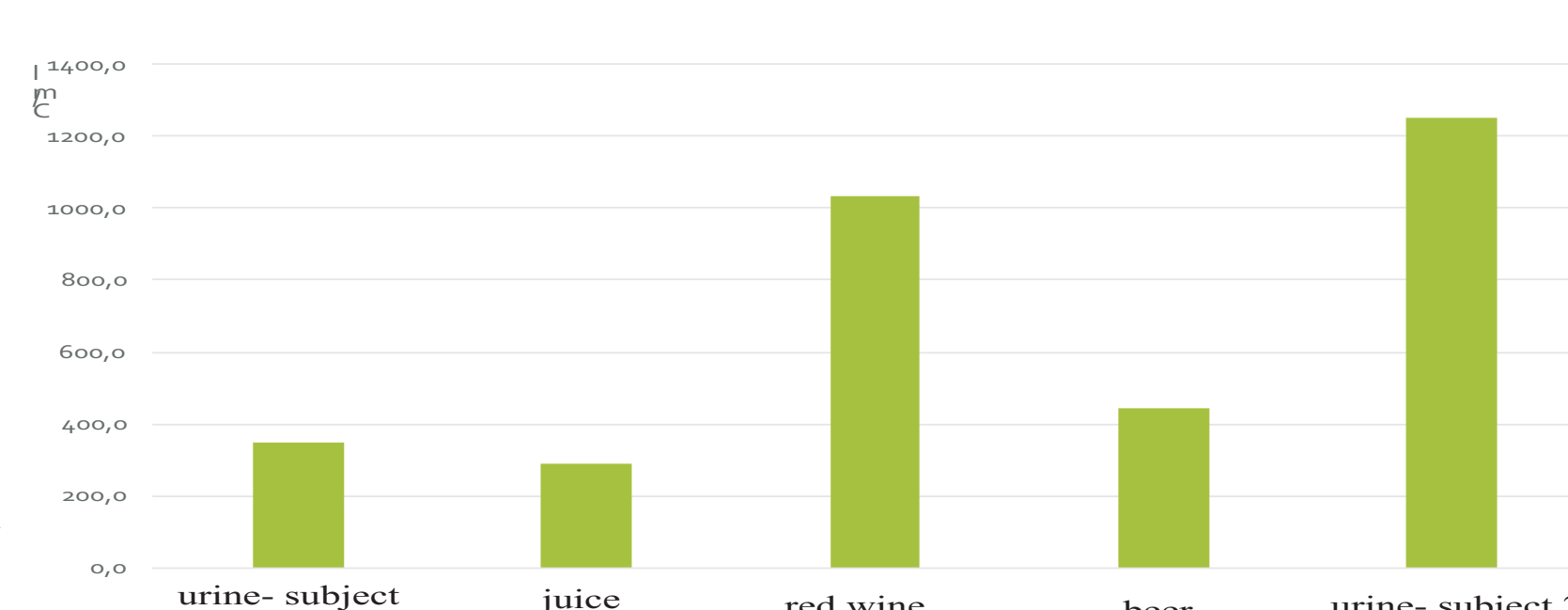
Fig.2: Chromatogram of monitored substances

FIA - COULARRAY CORRELATION



CONCLUSION

- UHPLC-QqQ method for monitoring of 8-OH guanosine and 8-OH-2'-deoxy guanosine was developed.
- Concentration levels of the analytes were determined in urine samples:
- 8-OH guanosine 50.45 ng/g creatinine (median)
- 8-OH-2-deoxy-guanosine 12.07 ng/g creatinine (median)
- Correlation between UHPLC-QqQ and FIA-COULARRAY method was found.
- Comparison with the samples of beer, wine and juice was made.



ACKNOWLEDGEMENT

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