A powerful and sensitive MEPS/UHPLC-PDA-based methodology for assessment of urinary biomarkers of oxidative DNA damage

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INTRODUCTION

The formation of reactive oxygen species within the cells causes damage of biomolecules and it plays an important role in carcinogenesis and aging. An important type of oxidative DNA damage is hydroxylation of DNA bases, leading to formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) and 5-hydroxymethyluracil (5-HMU). Measurements of these biomarkers in urine samples are challenging due to the low levels of the analytes and the complex matrix.

In this work a semi-automatic MEPS combined with UHPLC-PDA was developed in order to quantify urinary biomarkers of oxidative DNA damage, 8-OHdG and 5-HMU

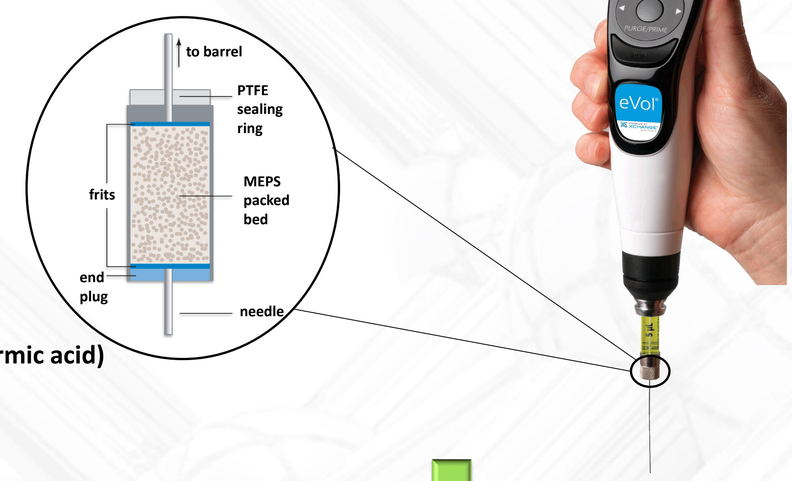
A previous optimization of the MEPS experimental influence parameters was carried out.

EXPERIMENTAL

Sampling

Urine sample diluted 1:10 and pH = 6

Extraction



MEPS procedure with the automated analytical syringe eVol®

Step 1: Conditioning

250 µL MeOH + 250 µL H_2O (0.01% formic acid)

Step 2: Sampling

5 x 50 µL urine sample

Step 3: Washing

50 µL H_2O (0.01% formic acid)

Step 4: Elution

3 x 30 µL H_2O (0.01% formic acid) MeOH (80:20, v/v)

Analysis

UHPLC-PDA conditions

Column: ACQUITY UPLC HSS T3

(100 mm 2.1 mm, 1.8 µm particle size)

Column temperature: 30 °C

Flow rate: 250 mL min^{-1}

UV detection wavelength: 215 nm; 246 nm; 285 nm

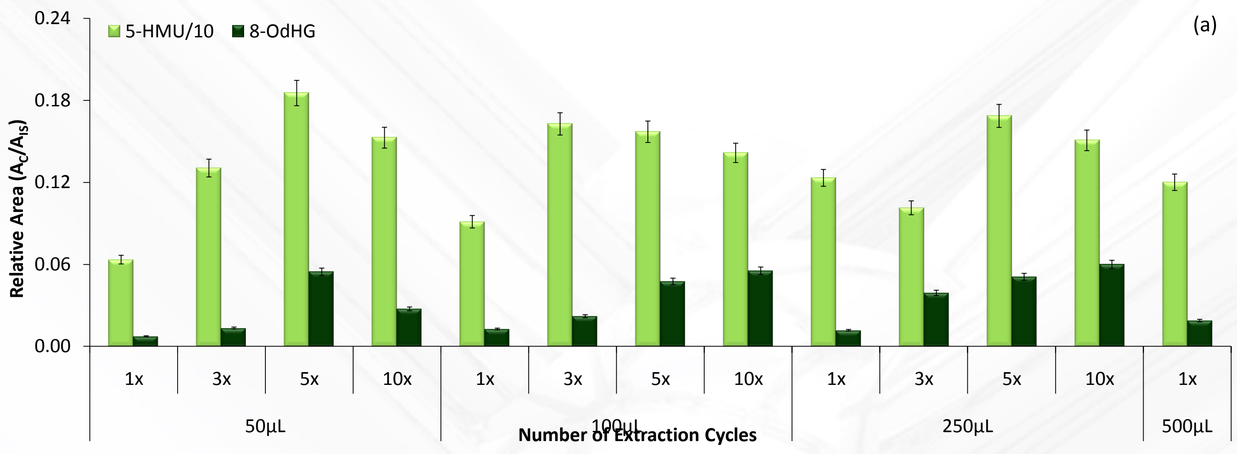
Injection volume: 2 µL

Mobile phase: 0.01% formic acid with 20% methanol

RESULTS

Optimization of MEPS influencing parameters

Figure 1. Effect of the main MEPS influencing extraction parameters: (a) comparison of the performance of five different MEPS sorbents; (b) influence of pH values. Error bars represent standard error of the mean (*n*=3 for each data point).



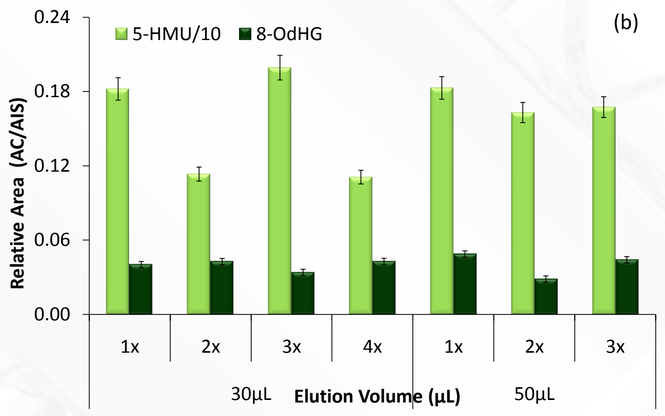


Figure 2. Effect of the main MEPS - influencing extraction parameters : (a) Influence of number of extraction cycles and volume of spiked urine sample; (b) Influence of elution volume in UPLC-PDA response. Error bars represent standard error of the mean (*n*=3 for each data point).

UHPLC-PDA profile of urinary biomarkers

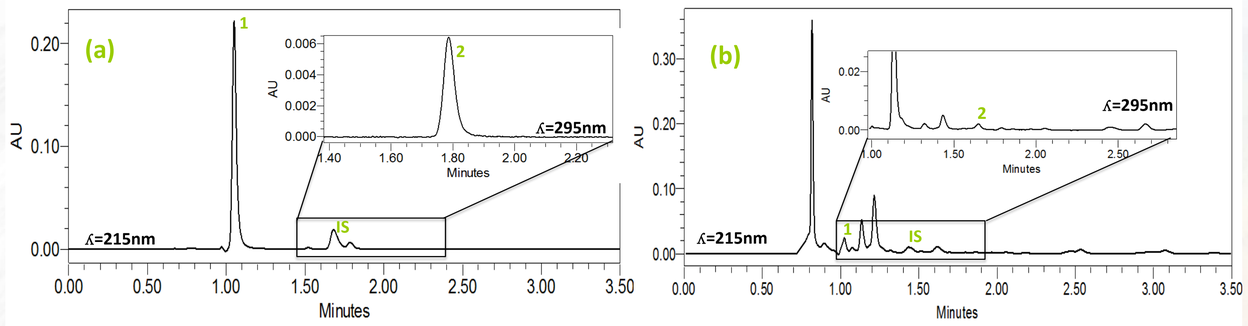


Figure 3. Representative chromatogram of standard mixture and human urine, of oxidative stress biomarkers: (a)- standart mixture; (b)- Urine Sample;(1)5-HMU; (2)- IS- Internal Standard (3)8-OHdG acquired (Aq) at different wavelenghts.

Analytical MEPS/UHPLC-PDA-based methodology performance

Table 1. Validation process data showing the concentration range inside which the linearity was tested, retention times (RT), and results of regression for total area *versus* concentration and analytical performance for the bioactive metabolites (biomarkers of oxidation DNA damage) on synthetic urine determined by the newly developed methodology (eVOL® $MEPSC_8$/UHPLC-PDA)

Peak nº.	RT (min)	Biomarker	λ_{max} [a] (nm)	Analytical Performance					
				Conc.Range (µg mL^{-1})	Regression equation	r^2 [b]	LOD [c] (µg mL^{-1})	LOQ [d] (µg mL^{-1})	% Matrix effect
1	1,05	5HMU	215	0.0005 – 0.01	1277.5x [e] + 0.8318	0.9906	0.00005	0.00023	80.11
2	1,60	IS	218	5	-	-	-	-	-
3	1,80	8OdHG	295	0.1 - 5	0.0724x + 0.0052	0.9946	0.04	0.13	82.21

[a] Maximum absorbance values obtained in PDA system detection
[b] Correlation coefficient, give an estimating how well the experimental points fit a straight line
[c] Limit of detection.
[d] Limit of quantification. Values obtained from ordinary least-squares regression data
[e] x= analyte concentration

CONCLUSIONS

- A Ultra fast (running time <3min), sensitive, reabible, time and resource, saving analytical method is reported for the successfully measurement of oxidative DNA biomarkers.
- The linearity of the method is demonstrated all along the range of concentration investigated, with correlation coefficient (R^2) ranging from 0.9906 to 0.9946 depending on analytes.
- The LODs and LOQs were in the range of 0.00005-0.04 µg.mL^{-1} and 0.00023-0.13 µg.mL^{-1}, respectively.
- The comparison between the biomarkers were found to be statistically different ($p<0.05$).
- The method provides a potential non-invasive screening for cancer diagnosis.

Acknowledgments

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