

A powerful and sensitive MEPS/UHPLC-PDA-based methodology DA MADEIRA for assessment of urinary biomarkers of oxidative stress in

oncologic pathologies





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- Oxidative stress has long been known to be involved in the pathophysiology of many human diseases, including to cancer. Cancer diseases are leading a few million deaths worldwide every year.
- The most common methods currently used to diagnose cancer can occasionally miss tumors because they depend on tumor size. In addition, they are costly and not amenable to widespread screening because they are not efficient in terms of time. In contrast, urine testing has long been recognized as a medical technique that allows diagnosis of disease by linking oxidative stress biomarkers (OxSB) in urine to medical conditions, including malondialdehyde (MDA), uric acid (UA), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 5-(hydroxymethyl)uracil (5-HMU). Creatinine (Cr) was used as normalizing factor.
- Recent improvements in instrumentation design have made ultra high pressure liquid chromatography combined with microextraction in packed syringe (MEPS/UHPLC-PDA) methodology an optimal choice for determination of OxSB, providing the advantages of higher specificity, sensitivity and resolution.
- The aim of this study was to compare OxSB levels between cancer patients and healthy individuals (control group) in order to explore the OxSB as potential biomarkers in cancer.
- Multivariate statistical methods were used to gain insight into the metabolomic differences between healthy and patients.

EXPERIMENTAL Sampling strategy Controls (n=15; age=44.1 ± 10.2) Breast Cancer (n=10, age= 42.0 ± 19.0) Lung Cancer (n=10, age= 49.0 ± 15.0) **Extraction procedure** Conditionir Sylvania Loading Sample (1:20) **Washing** Elution 100 H 0,1% 0.100 WeOH1 WEOH1 20000 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

RESULTS 0.30 A 0.20 1.70 0.10 **λ=295n λ=245nm λ=285nm λ=215nm** 0.00 0.50 2.50 0.00 1.00 1.50 2.00 3.00 3.50 Minutes

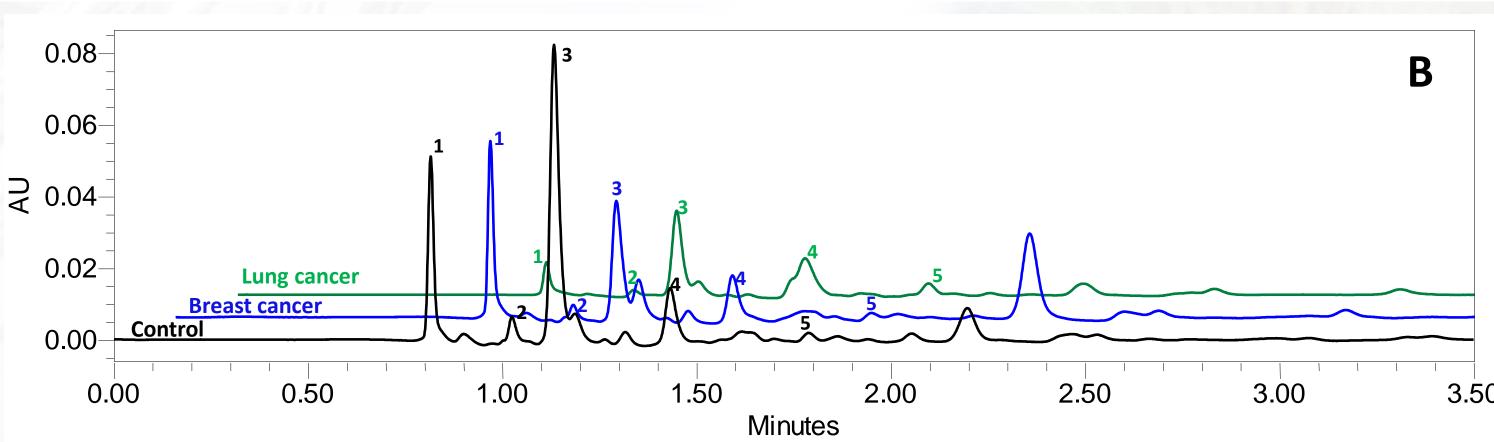


Figure 1. Representative chromatogram of standard mixture and human urine, of oxidative stress biomarkers: A- standart mixture; B- Urine Samples (aquisition at 245nm); 1-Cr; 2-5HMU; 3-UA; 4-MDA; 5-8OHdG

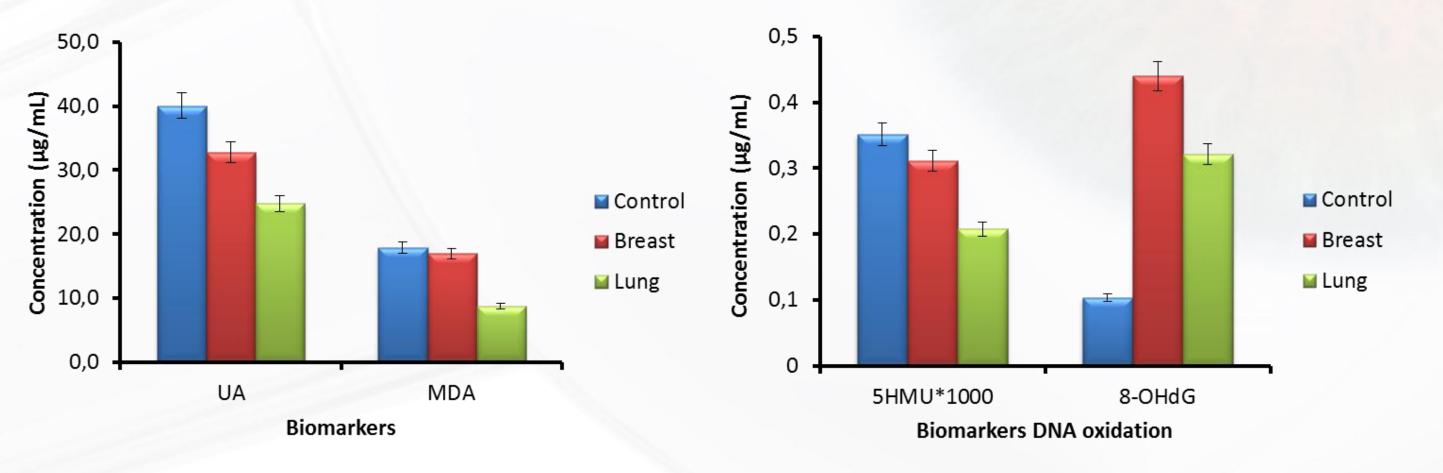


Figure 2. Total Concentration of oxidative stress biomarkers for control, breast and lung cancer patients.

CONCLUSIONS

- A powerful MEPS_{C8}/UHPLC-PDA methodolgy is reported for the sucessfully measurement of some oxidative biomarkers.
- The concentration of UA presents statistically significant differences between control and cancer patients. However, with MDA concentration the difference was detect just for lung cancer patients.
- According to biomarkers of DNA oxidation, it was found that 5-HMU decreases significantly in cancer patients whereas 8-OHdG increases specially for breast cancer patients.
- * 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.

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

Injection volume: 2 μL

Mobile phase: 0.01% formic acid with 20% methanol