Semi-automatic MEPS* combined with UPLC®

as a fast and simplified methodology to assess oxidative damage through quantification of 8-iso-prostaglandin F2α biomarker in urine

MEPS* - micro-extraction in packed syringe, UPLC® - Ultra high pressure liquid chromatography

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INTRODUCTION

- Every oxygen-based living organism in earth produces free radicals as a result of their physiological metabolism. When the level of these species become too high, excessive oxidative stress is generated and this can be very harmful to the cell (particularly to membrane lipids, DNA, proteins and sugars). It is nowadays widely accepted that this oxidative damage is a hallmark in several degenerative processes, including neurodegenerative and cardiovascular diseases and carcinogenesis.
- Isoprostanes (IsoPs) are a unique serie of prostaglandin-like compounds formed *in vivo* via a non-enzymatic mechanism that involves the free radical-initiated peroxidation of arachidonic acid (AA) [1]. They accumulate in tissue, circulate in plasma and are excreted in urine.
- As AA is one of the main lipid components of cell membranes, the measure of IsoPs levels in body fluids, namely plasma and urine, is a reliable approach to assess oxidative stress damage *in vivo*. As a result, IsoPs are the most frequently measured biomarker of oxidative damage.
- 8-iso-prostaglandin F2 α (8isoPGF2α) is the IsoP most frequently used to access oxidative damage.
- Quantification of this biomarkers usually involve laborious and not very reproducible ELISA methodologies or the more reliable but expensive GC-MS or LC-MS.
- Here we propose a fast and accurate methodology for quantification of 8isoPGF2α in urine using microextraction in packed syringe (MEPS) combined with ultra-high performance liquid chromatography (UPLC).

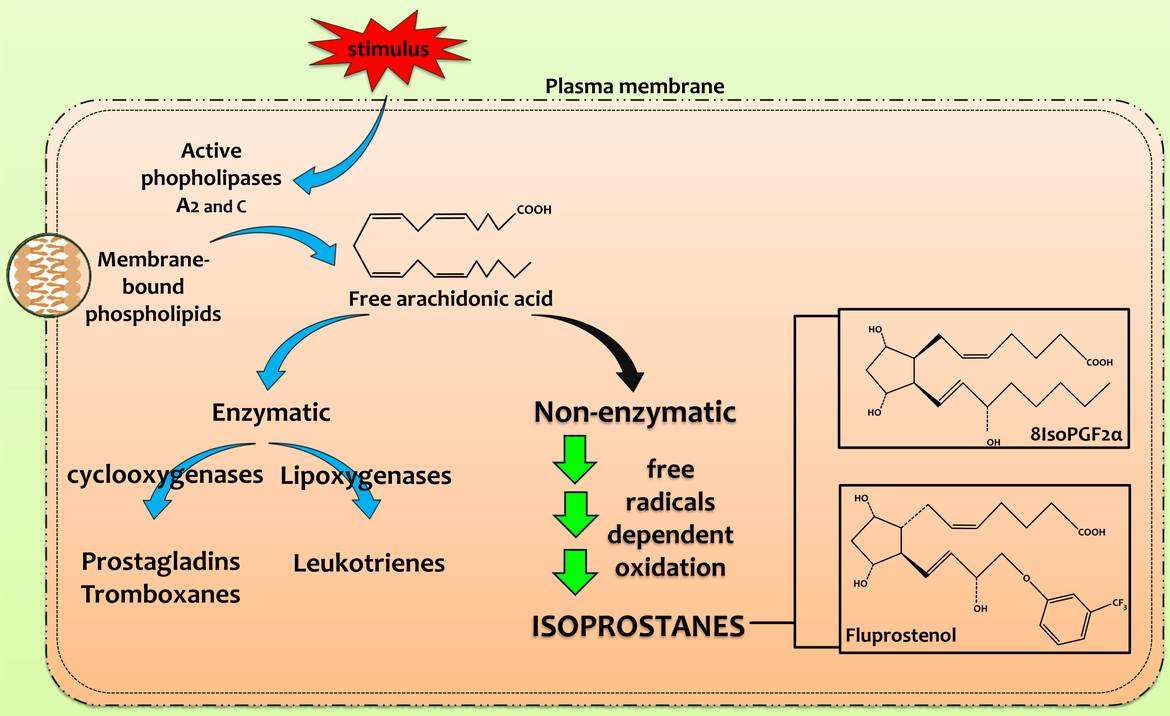


Figure 1: Outline of the free radical-induced (non-enzymatic) peroxidation of AA and consequent generation of IsoPs. 8isoPGF2α is the most relevant IsoP, being the most studied biomarker of oxidative damage to the cell. Fluprostenol is a sinthetic IsoP used as internal standard.

EXPERIMENTAL

Mighty MEPS™-UPLC® Procedure

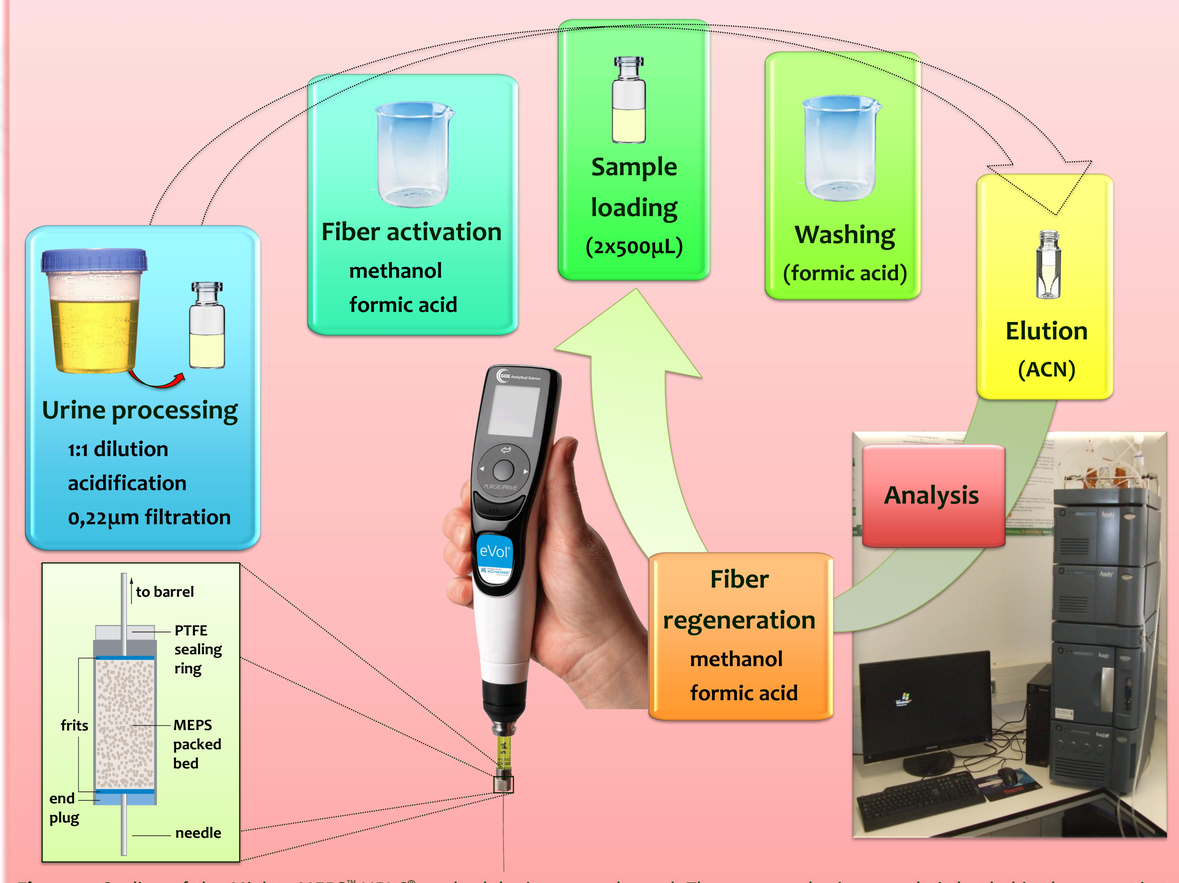
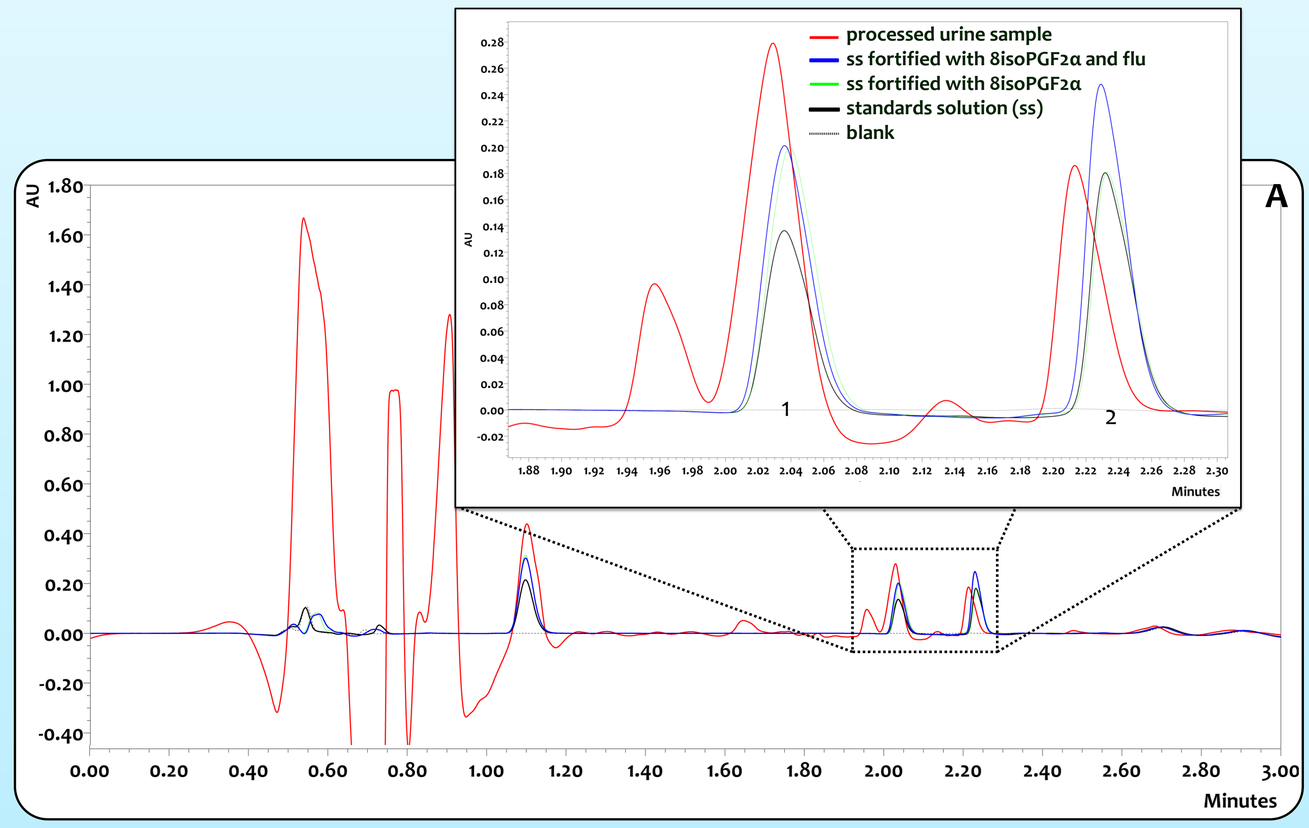
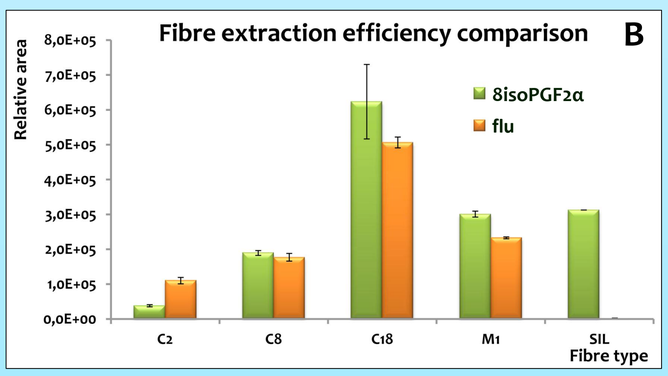


Figure 2: Outline of the Mighty MEPS™-UPLC® methodologic approach used. The processed urine sample is loaded in the pre-activated MEPS (micro-extraction by packed sorbent) C18X fiber coupled with the automated analytical syringe eVol®. Then the fiber is washed and the IsoPs eluted with acetonitrile (ACN, elution volumes <50µL). Finally, the elute is analysed in the Waters Acquity H-Class quaternary solvent manager UPLC® system (Waters, USA) equipped with an Acquity UPLC® HSS column and Waters Acquity PDA detector.

RESULTS





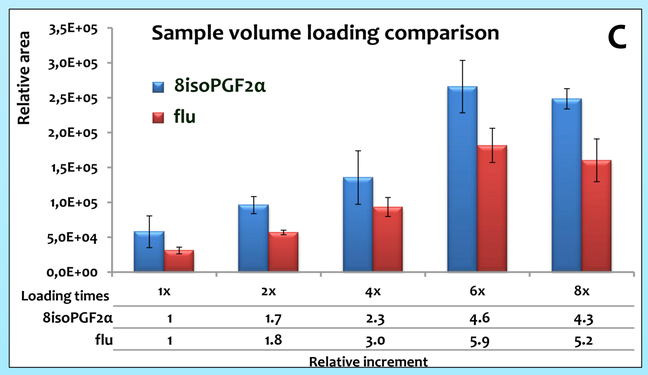


Figure 3: **A**) Representative UPLC® chromatograms of a standard solution (**ss**) containing the isosprostanes 8-iso-Prostaglandin α (**8isoPGF2α**, **1**) and fluprostenol (**flu**, **2**, internal standard) (**–––**); ss fortified with 8isoPGF2α (– – –); ss fortified with 8isoPGF2α and flu (– – –) and a processed urine sample previously fortified with the two (– – –); **B**) Comparison of the extraction efficiency between the five MEPS fibres commercially available: C2, C8, C18, M1 and SIL. C18 presents the best extraction efficiency for the two IsoPs analysed. **C**) Determination of the optimal sample volume for IsoPs extraction. As the maximum eVol syringe volume is 500µL (1x volume loading), several consecutive cycles of sample loadings are necessary. After two times cycle loading, the increment in the relative area for each IsoP is no longer proportional, which suggest that the fibre became saturated in the IsoPs analysed.

CONCLUSIONS

- **Here we described a new and optimized method to quantify IsoPs in urine in a simpler, faster and accurate way using microextraction in packed syringe (MEPS) prior to the ultra-performance liquid chromatography (UHPLC) analysis.**
- **The use of the automated analytical syringe eVol® coupled with a MEPS fibre (Mighty MEPS™ approach) allow full control of sample loading speed through the fibre, minimizing errors associated to repetitive hand manipulations.**
- **UPLC® analysis is another major advantage of the methodology, allowing the separation and quantification of 8isoPGF2α and the synthetic IsoP fluprostenol, here used as internal standard, in a 3 minutes ACN gradient run.**
- **Different parameters (urine volume and pH, stationary phase, wash solution and elution solvent and volume) were assayed.**
- **The optimized protocol involves two times loading of 500 µL of acidified and diluted urine through a C18 stationary phase in a MEPS syringe coupled to the analytical syringe eVol®. The stationary phase is then washed with formic acid and eluted with ACN. Finally, the extract is directly analysed in the UPLC® and the IsoPs are quantified by comparison with pure standards.**

Acknowledgments

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References

1. Morrow, J.D. *et al.* (1990) A series of prostaglandin F2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radicalcatalyzed mechanism. PNAS 87, 9383