Optimization of a new strategy coupling a semi-automatic micro-extraction by packed syringe and ultrahigh pressure liquid chromatography for determination of xanthohumol and isoxanthohumol in beers

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INTRODUCTION

The prenylflavonoid xanthohumol (XN) and its isomeric flavanone isoxanthohumol (IXN) has attracted considerable interest due its antiproliferative activity in human cancer cell lines, its antioxidant activity in inhibition low-density lipoproteins oxidation, antiangiogenesis and anti-inflammatory activity.

In this study a new strategy based on semi-automatic microextraction by packed sorbent (MEPS) combined with ultrahigh pressure liquid chromatography equipped with a photodiode array system detection (UHPLC-PDA), was optimized in order to determine the XN and IXN content in beers. Experimental parameters affecting the MEPS performance namely, the solvent, sorbent type, sample volume, number of extraction cycles, elution volume and pH, were tested and optimized.

EXPERIMENTAL

MEPS Analytical Procedure

Performance of six different MEPS sorbents (C2, C8, C18, C18 (eVol®) M1 and SIL) were tested.

Step 1: Conditioning

- 100 µL ACN
- 100 µL H_2O (0.1% formic acid)

Step 2: Sampling

- Multiple draw-eject sample cycles (1×, 5× and 10× with 50µL, 100µL, 250µL, 350µL and 500µL) were applied, in order to obtain the best extraction efficiency.

Step 3: Washing

- 100 µL H_2O (0.1% FA)

Step 4: Elution

- Different elution volumes and solvents were applied , in order to obtain the maximum response of UPLC-PDA.

UHPLC-PDA Conditions

Column: ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 µm particle size)
Column temperature: 40 ºC
Flow rate: 250 µL min^{-1}
UV detection wavelength: 289 nm
Injection volume: 2 µL
Gradient:

Time (min)	H_2O (0.1 % FA)	ACN
0	80	20
0,5	70	30
1	68	32
8	20	80
10	80	20

ACKNOWLEDGEMENTS

➢The authors acknowledge ECM (Empresa de Cervejas da Madeira) for the samples supply, Fundação para a Ciência e a Tecnologia (FCT) with funds from the Portuguese Government (Project PEst-OE/QUI/UI0674/2011) and MS Portuguese Network (Rede/1508/RNEM/2005).

➢A special acknowledge to **Pfizer Pharmaceutical Company** for the scholarship granted to Catarina Silva.

RESULTS

Optimization of MEPS influencing parameters

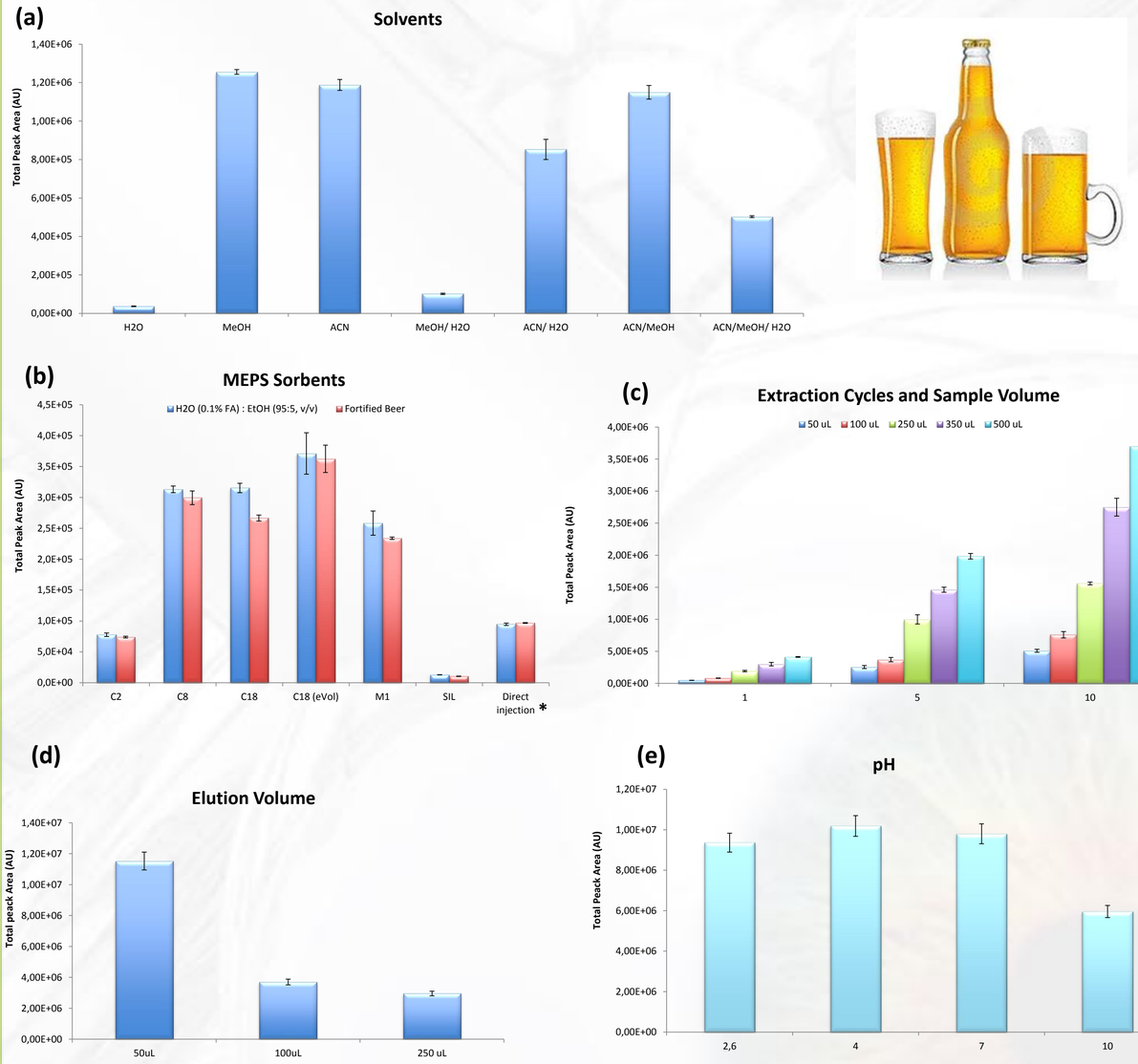

Fig.1- Effect of experimental parameters on XN and IXN extraction efficiency. **(a)** comparison of several partition solvents; **(b)** comparison of the performance of five different MEPS sorbents; **(c)** Influence of number of extraction cycles and volume; **(d)** Influence of elution volume in UPLC-PDA response; **(e)** influence of pH solution. Error bars represent standard error of the mean (*n*=3 for each data point).

(*)Direct injection- Standards in solvent.

MEPS Optimized Conditions	
Solvent	ACN
MEPS Sorbent	C18
Extraction Cycles	10
Sample Volume	500µL
Elution Volume	50µL
pH	4

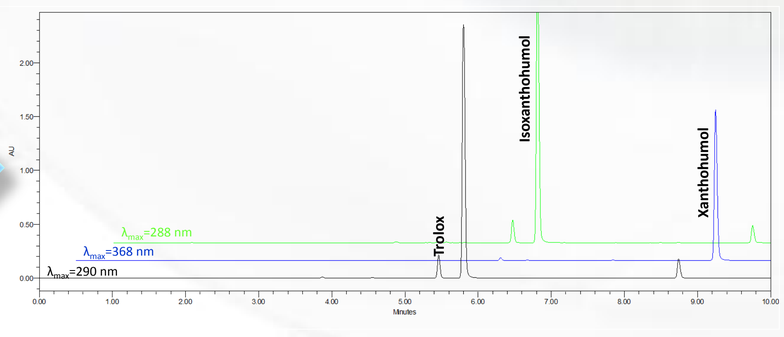

Fig.2- UHPLC chromatogram from the mixture of standards on their respective λ_{max}. Trolox (290 nm), Xanthohumol (368 nm) and Isoxanthohumol (288 nm).

CONCLUSIONS

➢ A novel, ultra-fast, sensitive and reproducible MEPSC8/UHPLC-PDA-based methodology, using a 100 mm analytical column (Acquity HSS T3) packed with 1.8 µm particle size, was developed, validated and successfully applied to the simultaneous determination of XN and IXN in beer samples.

➢ The extraction procedure is simpler, more efficient and low time-consuming, and moreover can be used for small sample volumes (50 µL) as well as large volumes (> 1000µL)

➢ The optimal conditions of MEPS extraction were obtained using C18 sorbent and small sample volumes (500 µL) in ten extraction cycle and in a short time period.

➢ The extraction efficiency is highest at beer pH (≈4).