

Micro-extraction by packed sorbent (MEPS) of andrographolide's derivatives in human plasma

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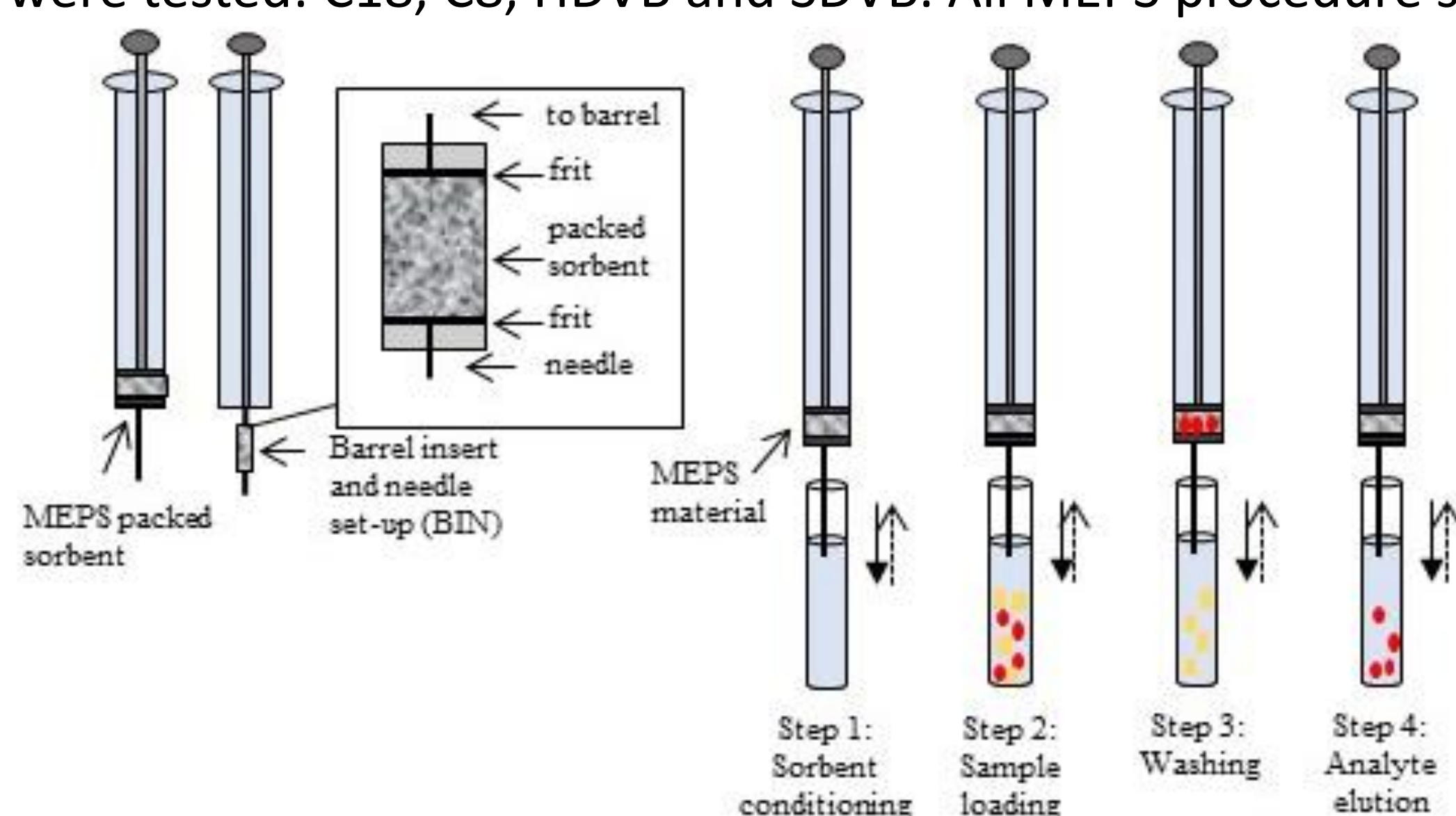
međunarodni znanstveno-stručni skup
19. Ružičkini dani
DANAS ZNANOST – SUTRA INDUSTRIJA
21. – 23. rujna 2022. | Vukovar, Hrvatska

INTRODUCTION

Inflammatory bowel disease is a general term that includes ulcerative colitis and Crohn's disease, disorders of unknown causes resulting in inflammation of the colon or small intestine. In addition to various medications, dietary supplements containing herbal extracts are used in the treatment of inflammation. One of them is andrographolide, an active compound from the plant *Andrographis paniculate* for the treatment of several inflammatory diseases [1, 2]. In this research, solid-phase microextraction by packed sorbent (MEPS) technique for sample preparation was examined with the aim of analysis of biological samples and determination of chemical components andrographolide (AND), neoandrographolide (NAND) and 14-deoxyandrographolide (DAND) in human plasma by high-performance liquid chromatography/ultraviolet spectroscopy (HPLC – UV).

METHODS AND MATERIALS

For plasma sample preparation and clean-up, solid-phase microextraction by packed sorbent (MEPS) was used. Various types of sorbents were tested: C18, C8, HDVB and SDVB. All MEPS procedure steps are showed in Fig. 1.



Final step

The analytes were eluted with 700 μL of methanol, evaporated to dryness at 60°C, re-dissolved with 100 μL of ultra-pure water and 25 μL injected into the HPLC-UV system.

Figure 1. MEPS procedure steps

RESULTS

SDVB sorbent showed to have the best extraction efficiency (extraction recoveries > 92%) and was chosen for the MEPS technique (Fig. 2). The selected chromatographic method was validated and result are shown in Table 1. Acceptable accuracy and precision of the method were indicated by the recovery of 91 – 100% and with Relative Standard Deviation (RSD) values lower than 7.6% for all analytes. The extraction recovery was found to be between 91.04 and 100.34% with RSD values not higher than 11.52%.

Table 1. Method calibration data

Analyte	Linearity range ($\mu\text{g/mL}$)	Slope (a)	Intercept (b)	r	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
AND	4 – 12	45.339	15.904	0.9989	0.043	0.145
NAND	4 – 12	39.255	7.130	1	0.097	0.322
DAND	4 – 12	44.837	-0.8066	0.9987	0.018	0.062

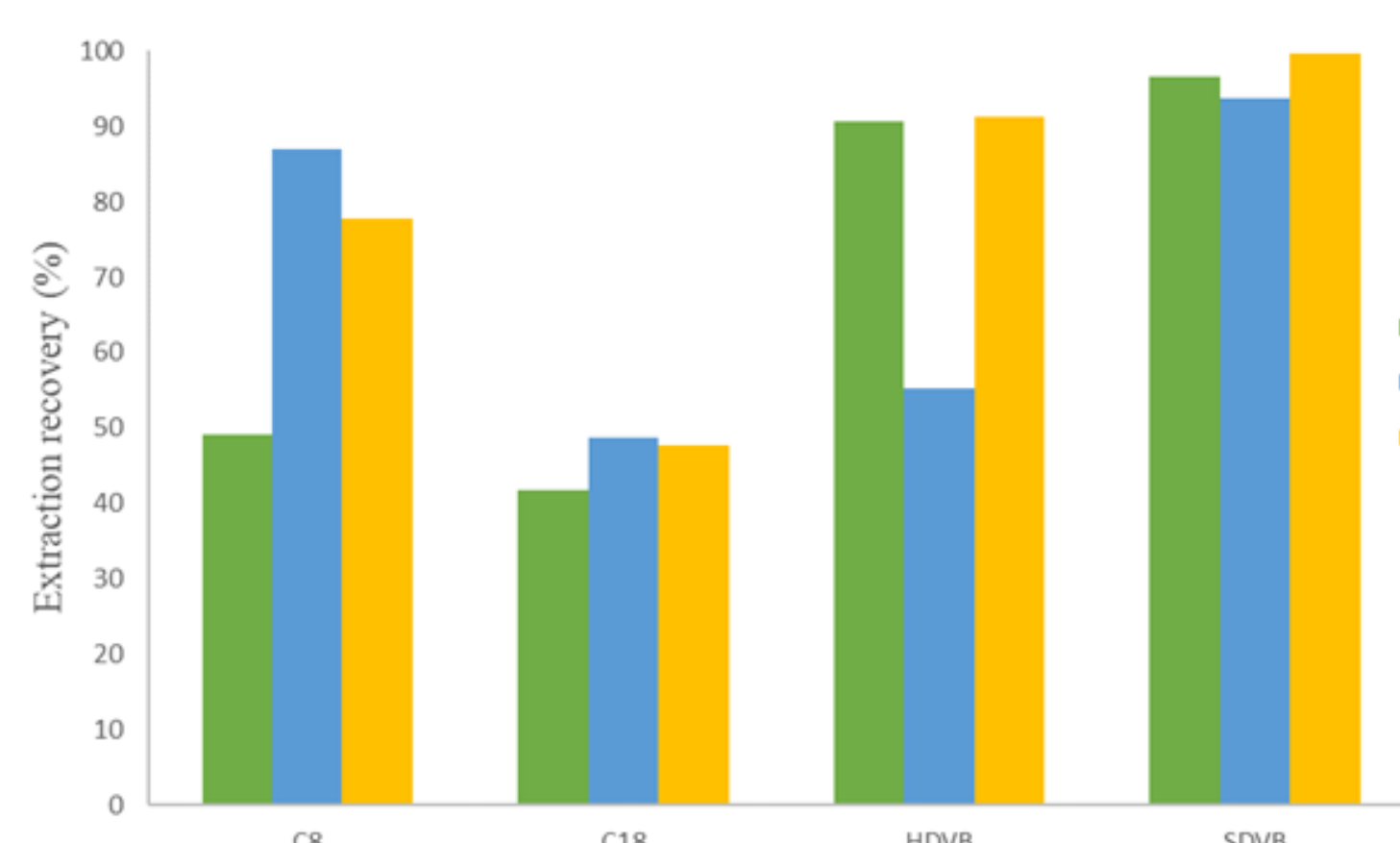


Figure 2. Extraction recoveries obtained by different types of MEPS sorbent

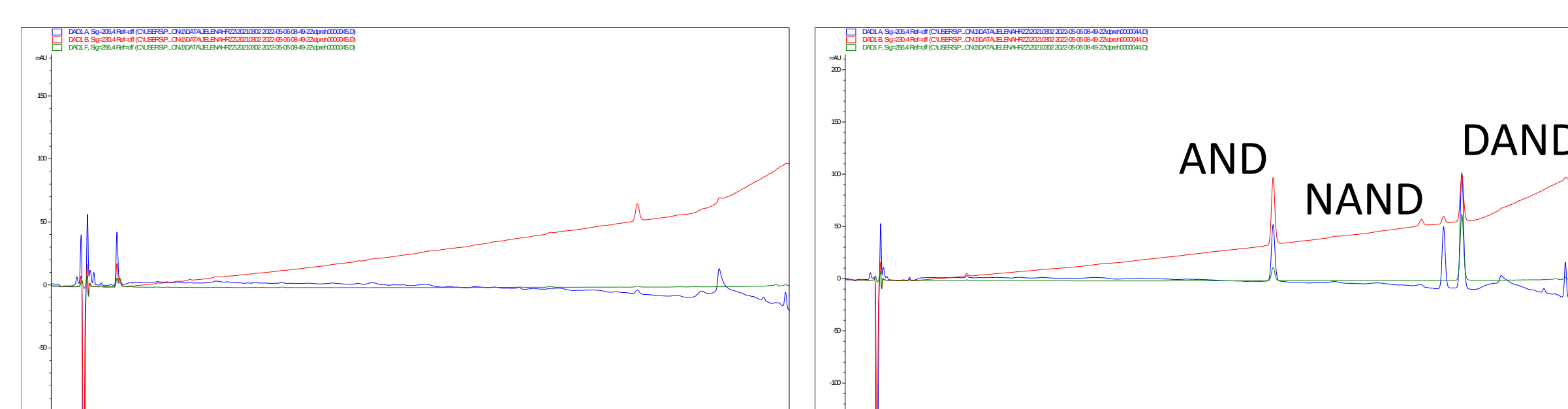


Figure 3. Representative chromatograms: a) Blank plasma sample, b) spiked plasma sample at ULOQ

CONCLUSION

Fast and easy MEPS pre-treatment procedures granted agreeable validation results and it represents a bioanalytical improvement (cheaper, greener and less time-consuming). The green MEPS/LC-UV method demonstrated high sensitivity, extraction recovery, precision, and accuracy, allowing andrographolide's derivatives to be quantified in human plasma.