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THERMALLY CONDENSED HUMIC ACIDS ONTO SILICA AS MIXED-MODE SORBENT FOR MULTICLASS EXTRACTION, CLEANUP AND PRE-CONCENTRATION OF STEROIDS IN HUMAN PLASMA

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In this work, a new, straightforward analytical method has been developed for multiclass determination of steroids (progestins, oestrogens, androgens and glucocorticoids) in human plasma. The key part of the procedure is the sample pre-treatment step, that is based on solid-phase extraction (SPE) using a newly proposed carbon-based sorbent able to retain the target compounds from plasma while excluding most of the matrix proteins, which represent serious interferences in the routinely used chromatographic analysis.
The sorbent is obtained by pyrolysis of commercial humic acids (10 wt%) onto micrometric silica [1]. This preparation yields a supported carbonaceous phase (HA-C@silica, carbon phase 1.9 wt%) characterized by an aromatic structure embedding polar oxygenated groups, thus a hydrophilic-lipophilic balanced sorbent able to give multi-type interaction with the solutes. Interestingly, HA-C@silica is able to exclude about 96% protein, similarly to the restricted access material based on modified carbon nanotubes (RACNTs), specifically designed for cleanup of protein samples [2].
The explorative part of the work was carried out on bovine serum albumin (BSA) solutions (phosphate buffer pH 7) to investigate the feasibility of extraction/cleanup of the analytes in a protein-rich matrix. For the preliminary tests, undertaken in 10 g/L BSA solutions using 50 mg sorbent, progesterone, 17β-estradiol, testosterone and cortisone were selected as representative steroids of the four classes. Results indicated full adsorption and elution (using 2 mL methanol), with recovery from 84 to 100% (RSD < 7%, *n*=3) depending on the analyte. The extraction was instead unsatisfactory on the RACNTs, which provided recovery in the range 58-91%, with a larger volume of eluting solvent, reasonably due to a very strong CNT-solute interaction. Control tests on bare silica (pyrolyzed with no humic acids) highlighted the major role of the silica-supported carbons in terms of retention.
The SPE conditions on HA-C@silica were then optimized by a chemometric approach (2^3 factorial design) considering sorbent amount, sample volume and protein concentration as the variables involved in the process. Recovery tests were done at 2 µg/mL spikes. Results clearly indicated a full adsorption working with 100 mg HA-C@silica and 2 mL sample, at a protein concentration of 10 g/L. After extraction, analytes were quantitatively eluted by 2 mL methanol, prior to LC-UV analysis. Good precision was observed, with RSD ≤ 12% (*n*=3). Subsequent trials at lower concentrations (0.2 µg/mL) evidenced that recovery was quantitative but for a complete desorption of progesterone the mixture methanol-acetonitrile (1:1) was required. To improve both cleanup and enrichment factor, several

washings were tested after extraction to remove the small fraction of retained protein, and smaller volumes of eluting solution were applied. It was found that washing with 2% v/v formic acid followed by 30% v/v MeOH (2 mL each) provided an improved cleanup (about 0.4% residual protein in the eluate, Bradford assay); at the same time, elution with 0.5 mL gave enrichment and reduced consumption of organic solvents. The SPE procedure was extended to 15 steroids and recoveries in the range 100-120% were observed. The optimized protocol was applied to human plasma spiked with 2-8 µg/mL before 1:8 dilution, with unchanged recovery. Basing on these findings, SPE experiments at lower concentrations (20-200 ng/mL) close to the steroids levels commonly detectable in human plasma are in progress using LC-MS/MS quantification (MRM mode).
The method avoids ultracentrifugation and protein precipitation, which often are required before extraction/cleanup, and basing on these results it is expected to be a useful tool for routine bio-medical analyses.

References

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