HPLC-FLUORESCENCE – BASED METHOD TO DETERMINATION OF UREMIC TOXIN IN BIOLOGICAL SAMPLES

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Introduction. Indoxyl (IS) and p-cresyl (pCS) sulfates are low molecular weight uremic toxins that bind to proteins, are constantly produced under physiological conditions but accumulate in plasma in chronic kidney disease (CKD), inducing an array of cellular responses.

Objective. To develop a high performance liquid chromatography (HPLC)-based platform to determine IS in cells and both IS and pCS in serum samples.

Method. We standardized two methods. For cultured cells, IS was separated with a C18, while for serum, IS and pCS were simultaneously analyzed with a C8 column, eluted with 50 mM ammonium formate and methanol. The toxins were detected with a fluorescence detector. Human mesangial cells incubated with IS and sera from CKD patients were used to test the methods.

Results. Incubation of cells for 1h with IS led to increased intracellular IS levels, compared with non-treated cells. The presence of calf fetal serum, as well as probenecid (IS transporter inhibitor) inhibited IS incorporation. All serum samples presented both IS and pCS, but CKD serum presented higher levels of both toxins, which increased with disease progression.

Conclusion. HPLC methods were developed to quantify IS uptake by mesangial cells and to quantify IS and pCS in serum samples. These methods will be useful to biochemically understand the role of intracellular IS and to investigate pharmacological or nutritional interventions in IS and pCS serum levels.

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Key words: chronic kidney disease, HPLC, uremic toxicity.