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Short communication

Sodium octanoate to reverse indoxyl sulfate and *p*-cresyl sulfate albumin binding in uremic and normal serum during sample preparation followed by fluorescence liquid chromatography

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ABSTRACT

Indoxyl sulfate and *p*-cresyl sulfate are protein-bound marker molecules in chronic kidney disease. Recent findings suggest that indoxyl sulfate and *p*-cresyl sulfate directly contribute to the uremic syndrome. A method for quantification of *p*-cresyl sulfate and indoxyl sulfate total serum concentrations was developed. We used sodium octanoate as competitor to replace non-covalent binding of *p*-cresyl sulfate and indoxyl sulfate to albumin. Total, within-run, between-run and between-day imprecision for indoxyl sulfate and *p*-cresyl sulfate were all below 6%. The limit of quantification was 3.2 μM for both analytes. Recovery, tested in hemodialysis patients, was 102% for indoxyl sulfate and 105% for *p*-cresyl sulfate. Deming regression demonstrated good agreement for indoxyl sulfate between this new method and an external HPLC method. Method comparison for *p*-cresyl sulfate of the new method with our in-house GC–MS method demonstrated good agreement, whereas method comparison with an external HPLC method revealed a small proportional bias. Sodium octanoate binding competition is a novel sample preparation that allows for direct quantification of indoxyl sulfate and *p*-cresyl sulfate.

1. Introduction

Indoxyl sulfate and *p*-cresyl sulfate are marker molecules in chronic kidney disease to study the behavior of protein-bound uremic retention solutes. Renal excretion is the predominant route of elimination and concentrations are increased in patients with chronic kidney disease (CKD). When excreted in urine, breakdown of indoxyl sulfate to indigo (blue) and indirubin (red) results in the purple urinary bag syndrome [1].

More recent findings suggest that indoxyl sulfate and *p*-cresyl sulfate directly contribute to the uremic syndrome [2]. Indoxyl sulfate might promote CKD progression [3]. Serum concentrations of *p*-cresol independently predict overall mortality [4] and cardiovascular disease in hemodialysis patients [5].

Reported total serum concentrations vary widely [6]. This is attributed in part to differences in deproteinization methods [7]. We previously demonstrated that combined heat and acid (H_2SO_4) deproteinization resulted in near-complete sulfate deconjugation of protein-bound uremic retention molecules. This allows for indirect measurement of *p*-cresyl sulfate after hydrolysis to *p*-cresol [8,9]. However, indirect quantification of indoxyl sulfate by combined heat and acid deproteinization proved unsuccessful. Indoxyl sulfate was not only hydrolysed to indoxyl, but further degraded as well. We therefore aimed at developing a method for direct measurement of total serum concentrations of *p*-cresyl sulfate and indoxyl sulfate, while avoiding heat and acid denaturing conditions during sample preparation.

Both indoxyl sulfate and *p*-cresyl sulfate are non-covalently bound to albumin at the Sudlow II binding site, which predominantly binds small aromatic molecules that are either neutral or bear a negative charge located peripherally on the molecule [10]. We used binding competition for the albumin Sudlow II binding site during sample preparation to measure total serum concentrations of indoxyl sulfate and *p*-cresyl sulfate.

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2. Experimental

2.1. Standards and reagents

1-Naphthalenesulfonic acid (internal standard, purity 70%) and trifluoroacetic acid (purity 99%) were obtained from Acros Organics (Geel, Belgium). Sodium octanoate (binding competitor, purity 99%), phosphate buffered saline and indoxyl sulfate were purchased from Sigma (St. Louis, MO, USA). Acetone, dichloromethane and acetonitrile (all HPLC grade) were from Fisher Scientific (Pittsburgh, PA, USA). We used Milli-Q water (Millipore, Billerica, MA, USA).

p-Cresyl sulfate was synthesized according to Feigenbaum and Neuberg [11]. Identity and purity (>99%) were confirmed by using nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS).

2.2. Sample preparation

For binding competition, 200 μl serum to which we added 20 μl 0.50 mM 1-naphthalenesulfonic acid (internal standard) was vortex-mixed with 250 μl 0.24 M sodium octanoate (binding competitor). After incubation at room temperature for 5 min, we added 2 ml cold acetone to precipitate proteins. Following vortex-mixing and centrifuging at 4 °C, 1860 × *g* for 20 min, the supernatant was transferred to 12 mm × 100 mm, GL 14 glass test tubes and 2 ml

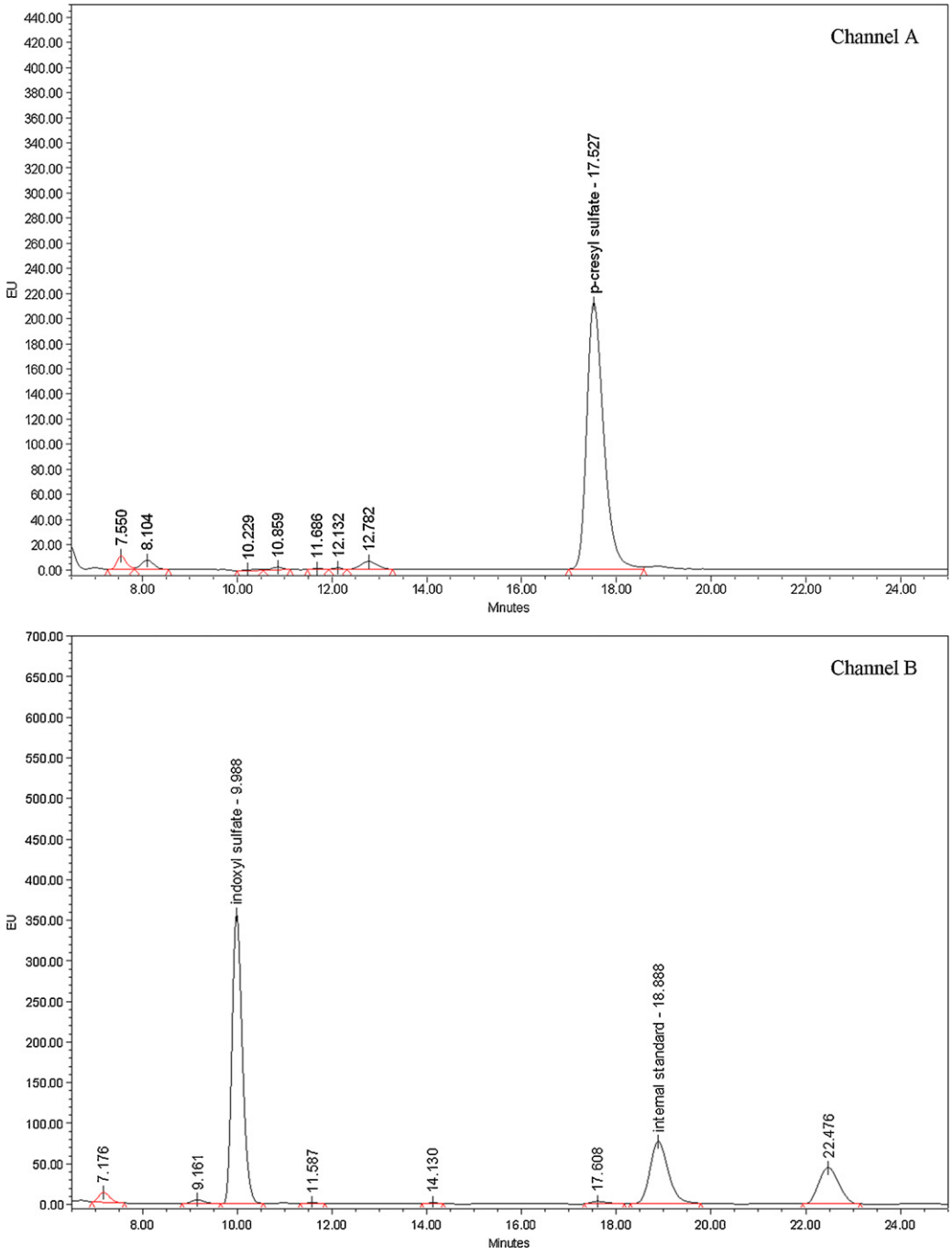

Fig. 1. Typical chromatogram in serum of a patient with chronic kidney disease demonstrating good separation of indoxyl sulfate, p-cresyl sulfate and 1-napthalenesulfonic acid (internal standard). Channel A: λ_{ex} 260 nm/λ_{em} 288 nm and channel B: λ_{ex} 280 nm/λ_{em} 390 nm.