

Potential for the speciation of Al in human serum using convective interaction media (CIM) fast monolithic chromatography with ICP-MS and cap nano LC ESI-MS detection

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An analytical procedure using anion-exchange separation support based on convective-interaction media (CIM) was developed for the speciation of Al in human serum. The separation of proteins was performed on a weak anion-exchange CIM diethylamine (DEAE) fast-monolithic disk. To prevent co-elution of low molecular mass (LMM) Al species with high molecular mass (HMM) Al compounds on CIM disk serum proteins were first separated from LMM-Al species by the use of size exclusion chromatography (SEC). For this purpose 1 mL of serum was injected onto SEC (Superdex 75 HR 10/30) column. Isocratic elution using 0.05 M TRIS-HCl + 0.03 M NaHCO₃ was applied and separation of proteins was followed by UV detection at 278 nm. It was experimentally proven that proteins were eluted in 5.5 mL peak that was collected into a polyethylene cup. A 0.1 mL of the sample aliquot was then injected onto the CIM DEAE disk. The separation of serum proteins was obtained in 10 min by applying linear gradient elution from 100% buffer A (0.05 M TRIS-HCl + 0.03 M NaHCO₃) to 100% buffer B (A + 1 M NH₄Cl) and followed by UV detection at 278 nm. Separated Al species were detected on-line by inductively coupled plasma mass spectrometry (ICP-MS). Well-resolved protein peaks were obtained. It was experimentally proven that $90 \pm 3\%$ of Al in spiked serum of renal patient was eluted under the transferrin peak. Transferrin was identified on the basis of the retention volume and also by the capillary nano liquid chromatography electrospray ionization mass spectrometry (cap nano LC ESI-MS).

The proposed speciation procedure removes LMM-Al species and enables reliable determination of the concentration and composition of Al bound to proteins by CIM DEAE-ICP-MS and cap nano LC ESI-MS when the concentration of Al in serum is higher than 5 ng mL^{-1} . In comparison to chromatographic columns CIM disks enable faster separation and simpler manipulation during cleaning procedure and coupling to ICP-MS.