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Multi-isotopic analysis of major cations in biological samples: method automation and application to chronic kidney disease

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Stable isotope ratios hold great potential as biomarkers of transmembrane ion flux, due to the distinct isotopic fractionations imparted during passive and active transport. One of the most promising systems to study with such an approach is the kidney, as renal reabsorption of major ions during urine formation has been shown to induce significant isotopic fractionation. Despite this potential, relatively little data exist to test the potential mechanisms of renal isotope fractionation and the modulation of this fractionation by various disease states. Part of the reason for limited study is the analytical difficulty of metal isotope measurements, which require tedious ion exchange chromatography and analytical effort for each individual element to be studied. To overcome this challenge, we developed an automated method for multi-element purification of major cations from biological sample matrices. Using a prepFAST MC (ESI) ion exchange chromatography system, and building on published protocols, we developed a method for sequential purification of Mg, K, Sr and Ca from synthetic and real biological sample matrices. We demonstrate the utility of this method with a pilot study of Ca, Mg and K isotope ratios in a population afflicted with chronic kidney disease of unknown etiology (CKDu). We observe significant enrichment of heavy Ca isotopes in CKDu patients compared to healthy individuals, whereas Mg and K did not show significant isotope fractionation despite consistent elemental enrichment. These patterns of elemental and isotopic enrichment can be used to refine assessments of renal physiology in the development of CKDu, highlighting the value of multi-isotopic datasets.