

A robust chromatographic method for single amino acid $\delta^{13}\text{C}$ measurement using LC-IRMS

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Isotopic analysis of carbon from amino acids has the potential to inform us about the original sources and perhaps the metabolic processes that make up the amino acids. This information can potentially be used to investigate dietary routing, metabolic pathways of proteins, and about turnover and growth of tissues made from protein. Furthermore, such information can be used to infer aspects of the biology, ecology and physiology of the individual that the tissue comprises and is applicable to a great many fields of science (e.g. physiology, metabolism, ecology, archaeology, forensics, palaeoenvironmental reconstruction and many more). Analysis of $\delta^{13}\text{C}$ of single amino acid fractions is technically difficult. Gas Chromatography-IRMS methods require derivatisation of the amino acids, involving the addition of carbon to the molecules, which increases the uncertainty (i.e. precision) in the $\delta^{13}\text{C}$ measurements. The Thermo-Scientific LC IsoLink interface allows for liquid chromatographic separation of amino acids prior to isotopic analysis. This system requires no derivatisation, however, complete baseline separation of the component amino acids is difficult as the technique does not allow for the use of organic solvents in the chromatographic separation. We present here a new LC-IRMS method using the Thermo-Scientific LC IsoLink capable of achieving baseline separation of 16 amino acids commonly found in protein hydrolysates. We will discuss the precision and accuracy of the method as well as the detection limits and required sample size. Furthermore, we will discuss its application to various protein types including: hair fingernail, bone collagen and milk protein.