

# High precision measurement of isotopic composition of amino acids by LC x GC/C/IRMS

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Nitrogen isotopic composition of amino acids has been widely applied to biochemical and geochemical studies in an attempt to trace nitrogen source and transformation processes. For accurate isotope analysis of individual amino acids, we validated a preparative method involving the isolation of underivatized amino acids by ion-pair chromatographic separation and confirmed the consistency of nitrogen isotope composition. Ion-pair reversed-phase liquid chromatography coupled with electrospray ionization mass spectrometry (LC/ESI-MS) and gas chromatography/ combustion coupled with isotope ratio mass spectrometry (GC/C/IRMS) were conducted for the purpose of separation of underivatized amino acids and nitrogen isotopic analysis, respectively. Firstly, we confirmed the resolution of proteinogenic and non-proteinogenic amino acids by the preparative ion-pair LC separation. Diagnostic product ions determined by mass spectrometry can support the rapid identification of individual amino acids in screening analyses. Secondly, we observed no dependency on nitrogen isotopic composition for the injection amount of underivatized amino acids and even for different chemical formula including neutral, acidic, sulfur-containing, heterocyclic, and aromatic species. The present method and strategy of LC coupled with GC/C/IRMS (i.e., LC x GC/C/IRMS) are useful for the high precision determination of the nitrogen isotopic composition of amino acids, in conjunction with an appropriate pre-treatment of chromatographic procedures.

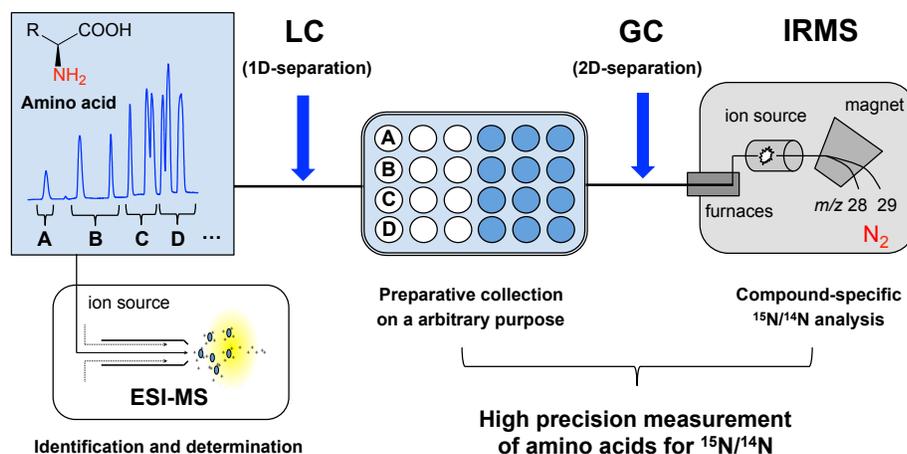


Figure 1: Scheme and developed system of liquid chromatography x gas chromatography/ combustion/ isotope ratio mass spectrometry (LC x GC/C/IRMS) for precise compound-specific isotope analysis (CSIA) and enantiomer-specific isotope analysis (ESIA) of amino acids.

## References

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- [2] Chikaraishi et al., 2010. *Earth, Life, and Isotopes* (Kyoto Univ Press), 367-386.
- [3] Takano et al., 2010. *Rapid Comm. Mass Spectrometry* **24**, 2317-2323.