A method for highly sensitive detection of D-amino acids

D-amino acids have recently attracted much attention in various research fields including medical, cliDical and food industry due to their important biological functions that differ from l-amino acid; thus, there has been an increasing need to develop rapid, robust, and highly sensitive methods for separation and quantification of both configurations of amino acids.

Most chiral amino acid separation techniques require complicated derivatization procedures in order to achieve the desirable chromatographic behavior and detectability. Here we introduce a highly sensitive analytical method for the enantioseparation of chiral amino acids without any derivatization process using liquid chromatography-quadrupole mass spectrometry (LC-QqQMS).

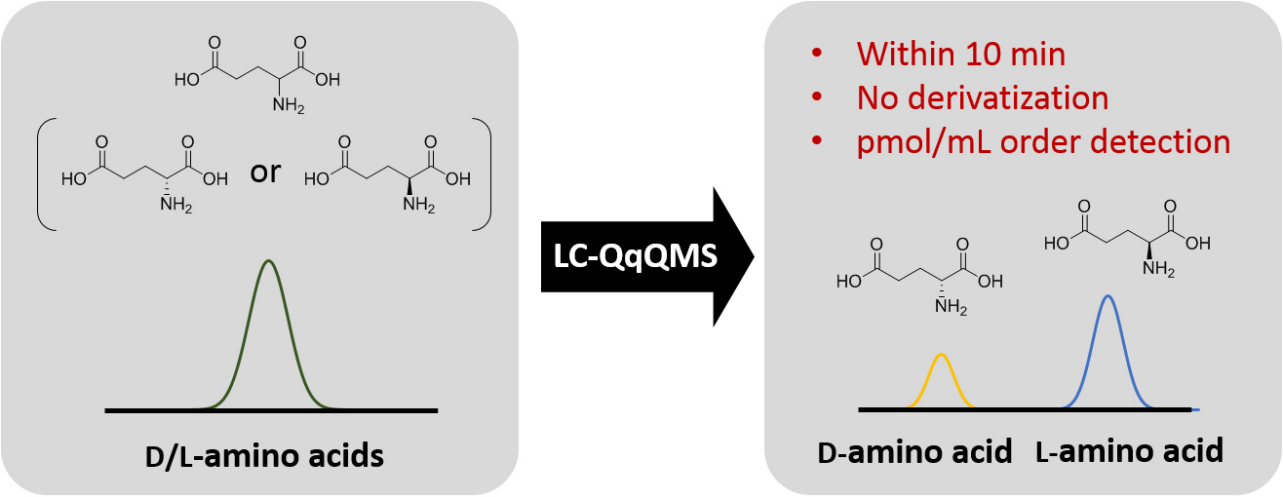

Fig. 1.

Previously, we developed an analytical method for simultaneous analysis of eighteen chiral proteinogenic amino acids using a combination of a chiral crown ether column and liquid chromatography-time of flight mass spectrometry (LC-TOFMS) (See: Technical revolution of D-amino acid profiling). This method enables the baseline enantioseparation of amino acids while maintaining excellent peak resolution within in a short time. Moreover, the method requires no derivatization steps and thus can avoid undesirable issues that may occur during derivatization. However, the method was constructed based on accurate mass measurement using a high-end TOFMS system, which hinders its versatility and usability. Compared to TOFMS, QqQMS is widely recognized as a powerful and universal analytical tool in terms of providing quantitative data. Multiple reaction monitoring (MRM) mode in QqQMS employs two stages of mass filtering that can remove impurity ions, which enables high sensitive and selective detection of trace target compounds.

The LC-QqQMS method allows the simultaneous analysis of 18 d-amino acids with high sensitivity (the limit of detection ranged from 5 pmol/mL to 500 pmol/mL) and repeatability (The RSD of all peak areas were £ 20%). Also, the linearity of all dilution curves in wide range was confirmed with R^2 values > 0.99, indicating good correlation of the data and that the system is capable of quantifying concentrations of chiral amino acids. Actually, this method can be applied to the quantification of minor d-amino acids in foods. Previously, we demonstrated that fourteen d-amino acids namely D-Ala, D-Ser, D-Val, D-Thr, D-*allo*-Ile, D-Leu, D-Asn, D-Glu, D-Met, D-His, D-Phe, D-Arg, D-Tyr, and D-Lys could be detected in vinegars.

Most recently, we have developed a method for comprehensive analysis of 115 chiral and non-chiral metabolites using the combination of two types of chiral columns (with binaphthyl-based crown ether and cinchona alkaloid-derived zwitterionic stationary phases). This method targets amines such as non-proteinogenic amino acid (*e.g*. GABA and ornithine), nucleic acid metabolite (*e.g*. adenine and guanine), and bioactive compound (*e.g*. dopa and serotonin) in addition to proteinogenic amino acids. We anticipate that these methods will enable advanced profiling to provide novel insights into the discovery of molecular markers utilized in various fields, especially for food analysis.

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