

Targeted LC-MS approach for indirect separation of chiral metabolites

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Abstract

Since the discovery of D-serine as an endogenous metabolite, chiral metabolomics has become more and more prevalent, exploring the role of chirality in development and progression of different diseases.

In biological systems, the most notable class of chiral metabolites is represented by amino acids. It is well known that in mammals the L- enantiomer of amino acids (AA) is naturally occurring. Nevertheless, D-AAs are present in very low concentrations (low nM to low μ M range) compared to L-AAs (low μ M to mM range), impeding their detection and quantification. Even though many analytical approaches have been developed, most of them are difficult to implement since they require special infrastructure (2D-LC-MS) or expensive chiral selectors.

The objective of our study was to develop a fast UHPLC-MS method capable to offer baseline enantiomeric resolution ($R_s > 2$) for all proteinogenic amino acids, using the indirect enantioseparation approach. The starting point were some several observations made in the past [1,2], where a dependance of chiral resolution on mobile phase pH was documented. Therefore, several qualitative variables were identified and included in the study, such as: chiral derivatization reagent (CDA), stationary phase, aqueous phase pH and the nature of organic modifier. Two CDAs were selected: (+)-1-(9-Fluorenyl)ethyl chloroformate ((+)-FLEC) and N-(4-Nitrophenoxycarbonyl)-L-phenylalanine 2-methoxyethyl ester ((S)-NIFE), together with five stationary phases (C18, Polar-C19, PS-C18, Phenyl and F5), two organic modifiers (acetonitrile and methanol) and a pH range between 2 and 8. After a comprehensive screening of these variables, it was observed that (S)-NIFE and acetonitrile have a positive impact on enantioseparation, as compared to (+)-FLEC and methanol, respectively. A further optimization using design of experiments was carried out for finding the best gradient and for fine tuning the pH of the aqueous phase.

In optimal conditions, baseline chiral separation of all proteinogenic D- and L-amino acids was achieved in less than 20 minutes.

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