Journal of Metabolic Syndrome

Tsyrlov I B., J Metabolic Synd 2021, 10:4

Short Article Open Access

Comparative enzyme kinetics analysis of the cDNA-expressed, microsomal and purified mammalian P450 monooxygenases family

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Received date: December 10, 2021**; Accepted date:** December 16, 2021**; Published date:** December 28, 2021

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Abstract

Since the descovery 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 µM range) compared to L-AAs (low µM 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 (Rs>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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