

Enantiomer separation of bioactive carboxylic acids derivatized with chiral reagent by LC-MS/MS

haruhito TSUTSUI

Analytical and Bio-Analytical Chemistry, Graduate School of Pharmaceutical Sciences

There are some differences of physicochemical properties between optically-active compounds. Especially in the field of medicines, attentions have gathered for the difference in the action of enantiomers in the body, because most of the physiologically active substance which controls the physiological function in the body has a chiral structure. Therefore, the technical advancement in enantioseparation is strongly required. The indirect determination method based upon diastereomer formation by using a chiral derivatization reagent in liquid chromatography is often used for the analysis of a couple of enantiomers. Target racemates are sensitively separated without the interference of foreign substances in the sample. However, most of chiral derivatization reagents are currently for fluorescent analysis, and the reagents for LC-MS/MS are very few. In this study, the efficiency of 8 commercially available chiral amines as the derivatization reagents for carboxylic acids was investigated in LC-MS/MS analysis for NSAIDs enantiomers.

DL-Ibuprofen (IBP), DL-Flubiprofen (FLP) and DL-Loxoprofen (LOP) were used as representative chiral NSAIDs. DL-IBP, DL-FLP and DL-LOP were reacted with chiral amine in the presence of triphenylphosphine and 2,2'-dipyridyl disulfide at room temperature for 90 min. The reaction solution was dried under a gentle nitrogen stream. The residue was redissolved in H₂O/CH₃CN and an aliquot was injected into the LC-MS/MS system. IBP, FPL and LOP were separated by derivatizing with (S)-(+)-1-(2-Pyrrolidinylmethyl)-pyrrolidine (PMP), (S)-(+)-DBD-APy (D-APy), (3R)-(+)-3-(Trifluoroacetamido)-pyrroli-dine (TFAP) and (R)-(-)-1-Aminoindan (AI) in LC-MS/MS system. The resulting diastereomers were efficiently separated by reversed phase chromatography and the R_s values were larger than 1.5. Furthermore, the sensitive detection was carried out by multiple reaction monitoring (MRM) in ESI-MS/MS.

In addition, we tried to the separation and detection of bioactive carboxylic acid enantiomers such as Lactic acid and 3-Hydroxybutyric acid. As the results, a pair of enantiomers of these carboxylic acids was clearly separated and sensitively detected by reversed-phase LC-MS/MS using PMP, D-APy and AI. The ability of other chiral amines to the bioactive carboxylic acids is now under investigation in our laboratory and will be reported elsewhere. Thus, these reagents seem to be useful for the enantioseparation and detection of many kinds of chiral carboxylic acid.