CHIRAL SEPARATIONS ON HPLC DERIVATIZED POLYSACCHARIDE CSPs:
TEMPERATURE, MOBILE PHASE AND CHIRAL RECOGNITION MECHANISM
STUDIES

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Direct chiral separations of the non-steroidal drugs of 2-methylarylpropionic acids (profens) on the chiral stationary phases (CSPs) of amyllose tris(3,5-dimethylphenyl-carbamate), Chiralpak AD, and cellulose tris(3,5-dimethylphenylcarbamate), Chiralcel OD, were investigated. Chiralpak AD and Chiralcel OD are CSPs coated on silica gel and have the same type of constituents. However, they have different higher order structures arising from their different arrangements of the glucose units, i.e., the former has an α-(1,4)-D-glucose linkage and the latter has a β-(1,4)-D-glucose linkage. The orders of optimum enantioselectivity of racemic acids were reversed on the two CSPs which demonstrated that the enantioseparating abilities of these CSPs are complementary. This phenomenon also confirmed that the chiral recognition abilities of both CSPs were dependent on their higher order structures.

Mechanisms for retention and chiral recognition for the separation of racemic 2-methylarylpropionic acids on Chiralpak AD and Chiralcel OD were explored. In depth studies of the dependence of retention and enantioselectivity on temperature and mobile phase compositions were made. The thermodynamic parameters, the differences in free energy, enthalpy, and entropy of association between enantiomers and the CSP were evaluated.

The results indicated that the retention of racemic acids on both CSPs is mainly dependent on the hydrogen bonding interaction between the acid proton of the carboxyl
moiety of the analyte and the carbonyl oxygen of the carbamate moiety of the CSP. The chiral recognition mechanism for Chiralpak AD involves: (1) the formation of transient diastereomeric analyte-CSP complexes through hydrogen bonding interactions between the carboxyl and the carbamate moieties of the acid and CSP, respectively; (2) stabilization of these complexes by insertion of the aromatic portion of the analytes into the chiral cavities of the CSP, as well as π-π, dipole-dipole, and additional hydrogen bonding interactions between analyte and CSP; and (3) chiral discrimination between enantiomer analytes arising from the additional hydrogen bond between analyte and CSP.

For Chiralcel OD, the chiral recognition mechanisms involve: (1) the formation of transient diastereomeric analyte-CSP complexes through hydrogen bonding interactions between the carboxyl and the carbamate moieties of the acid and CSP, respectively; (2) stabilization of these complexes by insertion of the aromatic portion of the analytes into the chiral cavities of the CSP, as well as π-π and dipole-dipole interactions between analyte and CSP; and (3) chiral discrimination due to: (a) the difference in the steric fit of enantiomers into the chiral cavity of the CSP (entropy controlled); and (b) dipole-dipole or π-π interactions between enantiomer analytes and CSP (enthalpy controlled).

Chromatographic and quantitative thermodynamic data showed that there are at least two different chiral recognition mechanisms for Chiralcel OD. One mechanism was characterized by negative values for the enthalpy and entropy differences of the association between enantiomers and CSP that classifies the enantioseparation to be enthalpy controlled. This behavior was exhibited by racemic 2-methylarylpropionic acids with fused rings that were favorably separated at low temperatures. The other mechanism was associated with positive values for the enthalpy and entropy differences of the association between enantiomers and CSP, and the enantioseparation is said to be entropy controlled. The analytes with “free” phenyl moieties favored high temperatures for their enantioseparations.

Both studies on the effects of temperature and mobile phase composition also indicated that the higher order structures of CSPs influence their chiral recognition abilities.
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