Improved Chiral Separations for Enantiopure D- & L-Amino Acids

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Several D-amino acids have been found to be important in brain neurochemistry and changes in the levels of these amino acids coincide with development of different diseases. For example, the D-form of Serine is involved in modulation of the NMDA receptor and has been implicated in a broad spectrum of disorders including schizophrenia, ischemia, and epilepsy.

D-amino acids have historically been considered unnatural since naturally produced proteins are composed exclusively of L-amino acids. While bacterial cell walls were known to contain D-amino acids, their presence in humans and higher organisms has been reported more recently.

Crown-ether chiral stationary phases (CSP) have been found to be especially well suited for separation of D- and L-amino acid enantiomers. Regis Technologies' crown-ether CSP ChiroSil® is a trifunctionally bonded (18-crown-6)-2,3,11,12-tetracarboxylic CSP available in both (+) and (-) conformations which thereby enables inversion of the elution order of the D- and L- isomers.

In this application note, we present separation of the D- and L-isomers of two neurophysiologically relevant amino acids Serine and Glutamic Acid by high pressure liquid chromatography (HPLC) using the CSP.

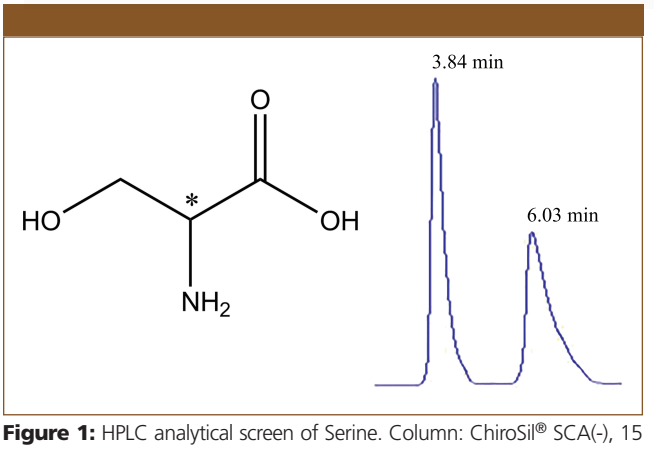

Figure 1: HPLC analytical screen of Serine. Column: ChiroSil® SCA(-), 15 cm × 4.6 mm, 5 µm. Mobile Phase: 84% MeOH/16%H_2O, 5 mM $HClO_4$. k'_1: 1.37 α: 1.99

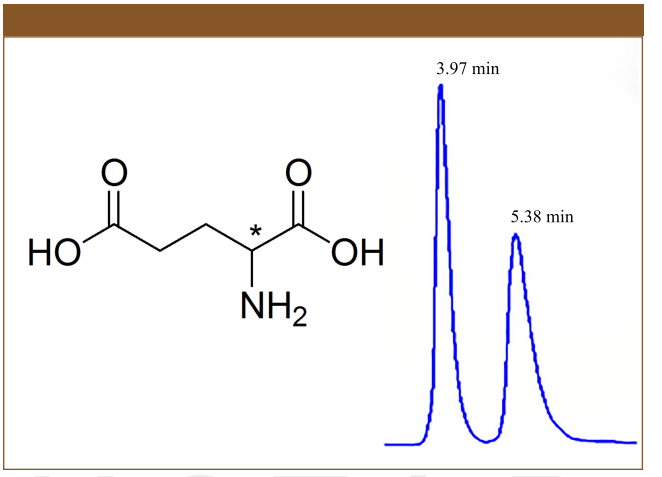

Figure 2: HPLC analytical screen of Glutamic Acid. Column: ChiroSil® SCA(-), 15 cm × 4.6 mm, 5 µm. Mobile Phase: 84% MeOH/16%H_2O, 5 mM $HClO_4$. k'_1: 1.45 α: 1.60

Experimental Conditions

Compounds were separated on ChiroSil® SCA(-) chiral columns using standard dimensions, 15 cm × 4.6 mm i.d. packed with 5 micron particles.

Conclusions

Baseline enantiomeric resolution of Serine and Glutamic Acid amino acids was achieved in less than 10 min using the crown-ether CSP method described here. The ChiroSil® CSP is a beneficial column in analyzing amino acids in the neurochemistry field.