Chiral HPLC Analysis of Underivatized Amino Acid Enantiomers

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In this brief article, we report a single, simple LC-MS-compatible mobile phase system that resolves the enantiomers of the common underivatized amino acids on an Astec CHIROBIOTIC® T column. Some experiments to provide insight into the variables that control retention and selectivity will also be touched upon.

Although L-amino acids dominate in nature, D-amino acids have been found in almost all species of bacteria, plants, and animals. Their presence has implications in physiology, nutrition, pharmacology, and toxicology that have spawned development of chromatographic methods to resolve in order to identify and quantify amino acid enantiomers (1,2). Resolving the enantiomers on polysaccharide-based chiral stationary phases (CSPs) is a challenge because native (underivatized) amino acids are zwitterionic and poorly soluble in non-polar solvents. Derivatization prior to separation can be used to improve solubility, or to create diastereomers that are resolvable by achiral HPLC (3). Derivatization however, adds an additional step and potential impurities. Direct analysis is preferred, and possible on macrocyclic glycopeptide-based CSPs.

Unlike polysaccharide-based CSPs, macrocyclic glycopeptides possess ionic groups (4) and are compatible with both organic and aqueous mobile phases. This makes them ideal CSPs for separating enantiomers of polar and ionic compounds, like amino acids. One such CSP that is particularly successful for resolving the enantiomers of underivatized amino acids is Astec CHIROBIOTIC T, which employs the macrocyclic glycopeptide teicoplanin as the chiral selector (5,6). The goal of this study was to develop a single mobile phase system that would resolve the majority of common amino acids on Astec CHIROBIOTIC T.

Approach

The retention of four representative amino acids, DL-arginine (positively charged), DL-aspartic acid (negatively charged), DL-threonine (polar, uncharged), and DL-tyrosine (hydrophobic) versus percentage of methanol in a water:methanol:formic acid mobile phase was measured and plotted **(Figure 1)**. The mobile phase composition that gave the best overall enantioselectivity was applied to the remaining amino acids.

Results

A simple mobile phase comprising water:methanol:formic acid (30:70:0.02) gave baseline resolution of most of the twenty chiral amino acids enantiomers on the Astec CHIROBIOTIC T column **(Table 1)**. The small amount of formic acid was necessary to produce elution of the charged acidic and basic amino acids. Enantiomers of histidine, cysteine, and proline were not resolved under these conditions, but could be resolved on the same column under slightly different conditions (see Table 1 footnote). Representative chromatograms of DL-tryptophan and DL-methionine appear in **Figures 2 and 3**, respectively.

Table 1. Screen of Underivatized Amino Acids Under Optimized Mobile Phase Conditions

column: Astec CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase: water:methanol:formic acid (30:70:0.02)
flow rate: 1.0 mL/min.
temp.: 25 °C

Amino Acid*	Rt1 (min.)	Rt2 (min.)	Selectivity	Resolution
DL-Arginine	7.367	9.623	1.31	3.78
DL-Histidine	9.132	9.737	1.07	0.67
DL-Lysine	7.128	9.464	1.33	4.12
DL-Aspartic Acid	4.477	5.165	1.15	2.68
DL-Glutamic Acid	4.340	5.321	1.23	4.59
DL-Serine	4.441	4.896	1.10	2.05
DL-Threonine	4.234	4.619	1.09	2.09
DL-Asparagine	5.267	6.835	1.30	4.64
DL-Glutamine	4.934	6.033	1.22	4.51
DL-Alanine	4.744	6.156	1.30	5.54
DL-Isoleucine	4.349	5.662	1.30	5.83
DL-Leucine	4.421	5.938	1.34	6.39
DL-Methionine	4.811	6.674	1.39	6.56
DL-Phenylalanine	4.994	6.170	1.24	6.17
DL-Tryptophan	5.095	6.275	1.23	3.90
DL-Tyrosine	4.578	5.594	1.88	4.25
DL-Valine	4.472	5.385	1.20	3.93

* Optimized conditions for histidine, cysteine, and proline on Astec CHIROBIOTIC T:
DL-Histidine: 160 mM sodium phosphate:ethanol, pH 4.5 (40:60)
DL-Cysteine: water:acetonitrile (30:70)
DL-Proline: water:acetonitrile (95:5)

For all amino acids tested, enantioselectivity increased with organic modifier concentration. Retention versus organic modifier concentration exhibited a U-shaped profile **(Figure 1)**. This observation has been well documented in small and large molecule achiral separations, and has also been reported for chiral compounds in methanol and acetonitrile on teicoplanin-based CSPs. It is likely due to the combined effects of analyte solubility and conformational changes in the CSP as a function of organic modifier content (7). The effect has also been reported on cyclodextrin (CD)-based CSPs, but only in acetonitrile. It is thought to be related to the accessibility of the CD cavity by the acetonitrile molecule (8).

It is interesting to note that the D enantiomer is always more strongly retained than the corresponding L enantiomer on macrocyclic glycopeptide CSPs. This is no coincidence since these molecules exert their antibiotic activity by interacting with terminal D-alanyl-D-alanine residues in bacterial cell membrane peptides (9).

Figure 1. Effect of Organic Modifier Concentration on Amino Acid Retention

column: Astec CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase: methanol:water:formic acid (20:70:0.02)
flow rate: 1.0 mL/min.
temp.: 25 °C
det.: UV at 205 nm
injection: 10 µL
sample: 0.2 mg/mL in 50:50, water:ethanol

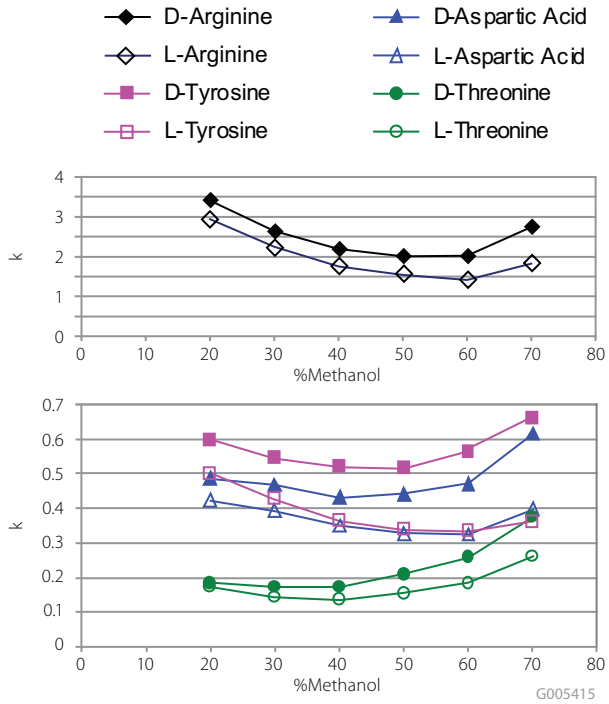


Figure 2. Representative Chromatogram of DL-Tryptophan on Astec CHIROBIOTIC T

Conditions same as Figure 1 except:
mobile phase: water:methanol:formic acid (30:70:0.02)

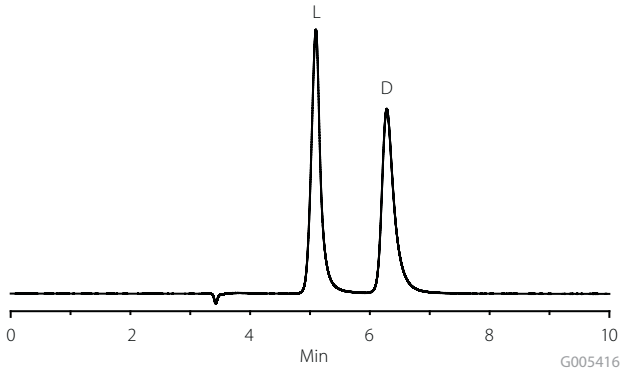
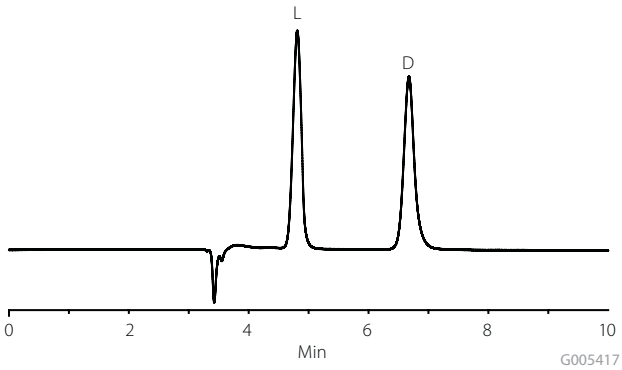


Figure 3. Representative Chromatogram of DL-Methionine on Astec CHIROBIOTIC T

Conditions same as Figure 1 except:
mobile phase: water:methanol:formic acid (30:70:0.02)



Conclusion

Because Astec CHIROBIOTIC T columns possess ionic functional groups and operate in mobile phase systems that favor polar analyte solubility, they are uniquely able to separate underivatized D- and L-amino acids. A simple LC-MS-compatible mobile phase system was found that resolved most amino acid pairs, and provides a foundation for future studies in this area.

References

1. Schieber, A.; Brückner, H.; Rupp-Classen, M.; Specht, W.; Nowitzki-Grimm, S.; Classen, H.-G. J. Chromatogr. B, 1997, 691(1), 1-12.
2. Péter, A.; Árki, A.; Vékes, E.; Tourwé, D., Lázár. L.; Fülöp, F.; Armstrong, D. W. J. Chromatogr. A, 2004, 1031(1-2), 159-170, 171-178.
3. Ilisz, I.; Berkecz, R., Péter, A. J. Pharm. Biomed. Anal. 2008, 47, 1-15.
4. Xiao, T. L.; Tesarova, E.; Anderson, J.L.; Egger, M.; Armstrong, D. W. J. Sep. Sci. 2006, 29(3), 429-445.Berthod, A.; Liu, Y.; Bagwill, C.; Armstrong, D. W. J. Chromatogr. A, 1996, 731(1), 123-137.
5. Berthod, A.; Liu, Y.; Bagwill, C.; Armstrong, D. W. J. Chromatogr. A, 1996, 731(1), 123-137.
6. Péter, A.; Töröka, G., Armstrong, D. W. J. Chromatogr. A, 1998, 793, 283-296.
7. Armstrong, D. W.; Liu, Y.; Ekborg-Ott, K H. Chirality, 1995, 7, 474-497.
8. Seeman, J.; Secor, H.; Armstrong, D. W.; Timmons, K.; Ward, T. Anal. Chem. 1988, 60(19), 2120-2127.
9. Wade, D.; Boman, A.; Wahlin, B.; Drain, C.; Andreui, D.; Boman, H.; Merrifield, R. Proc. Natl. Acad. Sci. USA, 1990, 87, 4761-4765.

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