A Simple Method for Resolution of 22 Amino Acids in LC

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Introduction

Amino acids are always of interest due to their availability in so many samples in biochemistry. They have many functions in metabolism critical to life and are seen as the building blocks of proteins. Linear chains can be formed in many sequences to produce a variety of proteins.
Key in nutrition, nutritional supplements, fertilizers, food technology and present in the chemical industry as additives in biodegradable plastics, drugs and chiral catalysts. Many industries therefore rely on accurate results when trying to measure amino acids in the products or by-products.

Amino acids are relatively simple molecules containing an amine group a carboxylic acid group and a side chain that alters. Due to the variability of the side chain and the potential for one or more of the groups to be charged amino acids can prove troublesome to retain and resolve from each other in LC or LC-MS. They can incorporate varying degrees of hydrophobic and hydrophilic nature, whose wide range can make it difficult to analyse them all in one run.

In this poster we show a simple reversed phase separation of all of the amino acids commonly referenced, using a polar-endcapped stationary phase and a aqueous:organic gradient. Good resolution of the compounds provides better qualitative results along with higher sensitivity and quantification levels. We discuss why this particular method is simple and yet comprehensive.

HPLC Analysis

Analysis of the amino acids is made challenging by the diversity of the various analytes involved. A variety of hydrophobicity and functionality are present basic, acidic and neutral. The amine and carboxylic functional groups present allow the amino acid to have amphiprotic or zwitterionic properties, either the carboxylic acid or the amino group being charged. This makes it difficult to choose the correct pH to aid retain and resolve the diversity present.

Conditions

Column: 5um Fortis™ H2o 150x2.1mm

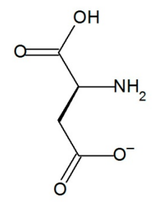
Buffer A: 2.72g NaOAc in 1000ml Water + 1.8ml TEA (pH=7.3) + 3ml THF

Buffer B: 2.72g NaOAc in 200ml Water + 400ml MeOH + 400ml ACN

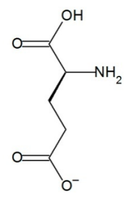
Gradient :

0.00min	flow - 0.45ml/min	%B - 2%
1.00min	%B - 2%	
17.00min	%B - 60%	
18.00min	%B - 100%	
18.10min	flow - 0.8ml/min	
23.90min	flow - 0.8ml/min	
24.00min	flow - 0.45ml/min	%B - 100%
25.00min	flow - 0.45ml/min	%B - 2%
30.00min	Equilibrate	

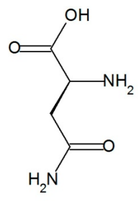
1. L-Aspartic acid



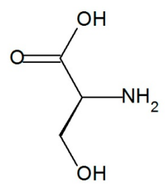
2. L-Glutamic acid



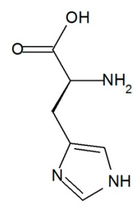
3. Asparagine



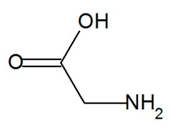
4. L-Serine



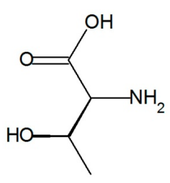
5. L-Histidine



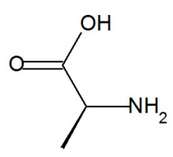
6. Glycine



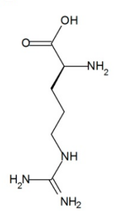
7. L-Threonine



8. L-Alanine

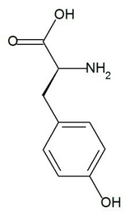


9. L-Arginine

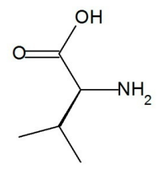


10. L-Gly-Tyr

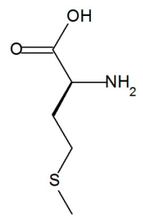
11. L-Tyrosin



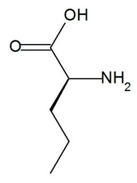
12. L-Valine



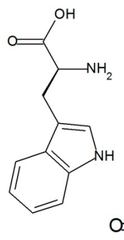
13. L-Methionine



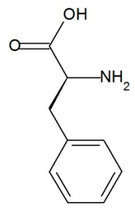
14. L-Norvalin



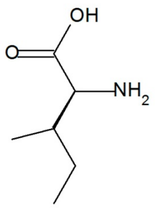
15. L-Tryptophan



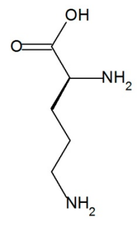
16. L-Phenylalanine



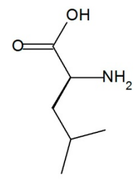
17. L-Isoleucine



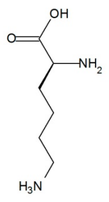
18. L-Ornithine



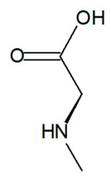
19. L-Leucine



20. L-Lysine



21. Sarcosin



22. L-Proline

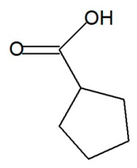
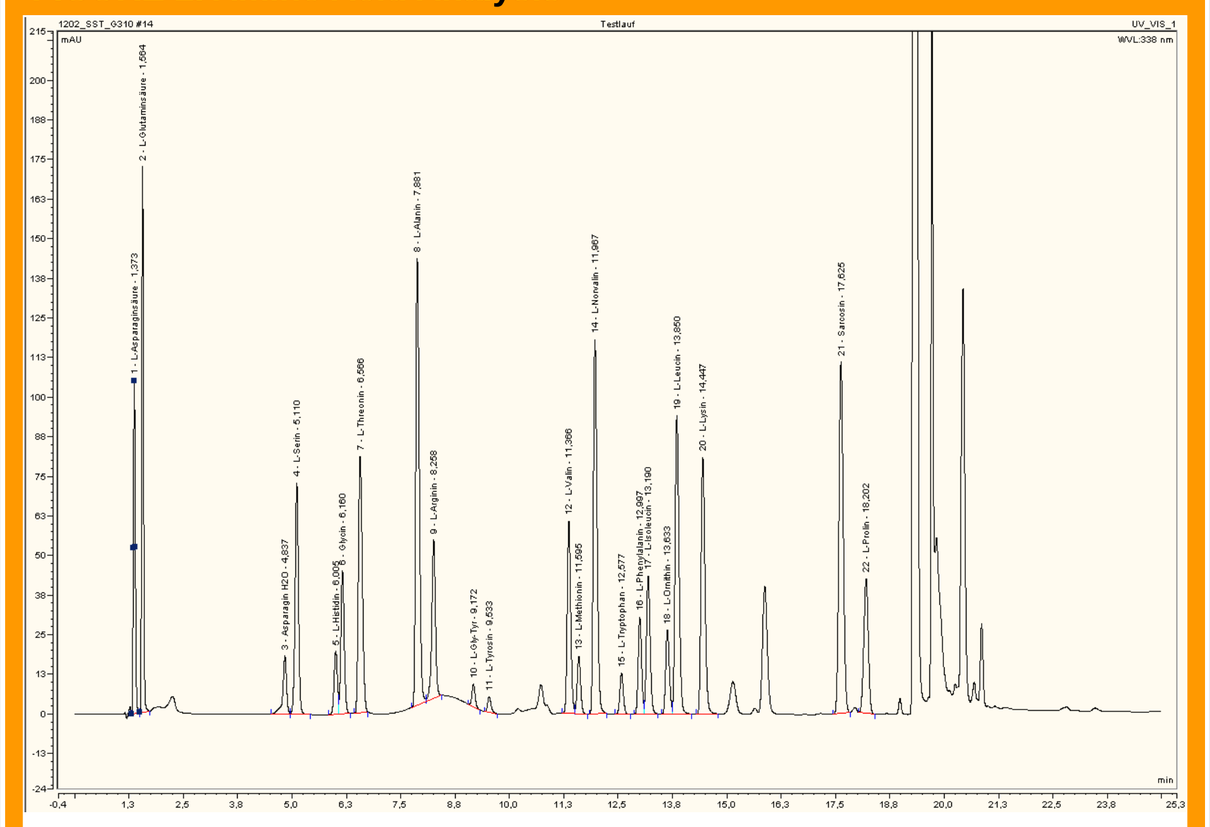


FIGURE 2. Amino Acid Analysis



Considerations

Analysis of the amino acids is made challenging by the diversity of the various structures and functionalities involved. Many methods incorporate complex mobile phase systems or ion-pair reagents meaning that compatibility with detection systems such as MS is compromised.
In the method shown in this poster a simple mobile phase system is utilised with a stationary phase possessing polar functionality as well as hydrophobic nature. This stationary phase allows the resolution of the various species both hydrophobic and hydrophilic in the same chromatographic run. Simplicity is maintained which leads to both good sensitivity and resolution.

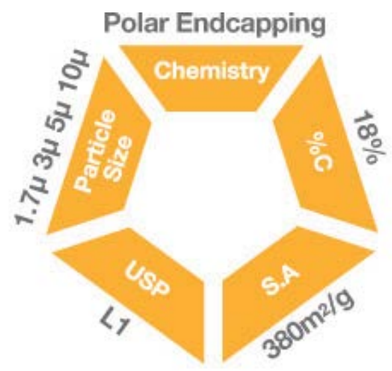
Advantages of polar endcapped

- Enhanced Resolution
- 100% Aqueous Compatible
- Fully Scaleable: UHPLC → Prep

Stationary Phase Characteristics

Fortis™ H2o

- Silica Template, Monofunctional C18 bonding
- Unique polar endcapped
- Sharp Peak Shapes
- Highly Selective



Conclusion

Amino acids are of interest in many industries, food, chemical and pharmaceutical, therefore their analytical determination is of great importance to many people. HPLC methods are generally challenged by the complexity of the mobile phase san ion-pair reagent or an additive that renders the method complicated and non-compatible with all detection methods, such as MS.

In the above method the mobile phase is simple, doesn't require any special additives and has an excellent result in terms of peak shape, efficiency and resolution of all the analytes. By achieving full resolution precision and accuracy of results can be achieved leading to confidence that the method is robust and reproducible.

Other polar molecules have been shown to retain well on this stationary phase, please request a copy of our HPLC 2011 poster (A New UHPLC Column for Polar Analyte Retention) if you wish to read more.