



Amino Acid Analysis with Temperature and Eluant gradients

Pickering laboratories specializes in manufacturing of cation-exchange columns and eluants for amino acid analysis. No other technique has been shown to match the reproducibility and sensitivity of ion-exchange analysis with post-column Ninhydrin detection. Nor is there a chromatography technique that provides as much information; the 570/440 nm signal ratio for each amino acid is a constant and so provides information about purity.

Pinnacle PCX post-column derivatization system provides a unique opportunity to combine eluant gradient capabilities of modern HPLC instruments with column temperature gradients. As might be expected this capability helps reduce analysis time. Even more significant is the ability to resolve coelutions: consider such metabolic markers as allo-Isoleucine (MSUD) and Argininosuccinic acid (ASA). Under isothermal conditions these amino acids coelute with Cystathionine and Isoleucine respectively but are each resolved using a localized temperature gradient.

The ability to accomplish this derives from the multiple retention mechanisms of the gel-type resins employed in ion-exchange. That all the amino acids appear in the same chromatogram is testament to the dominance of ion-exchange. However, the exact position is influenced by an array of mechanisms including partitioning, adsorption, charge exclusion, etc. So even though two amino acids might coelute their proximity is incidental. And since retention processes are affected differently by changes in pH, salt concentration and temperature all the parameters have significant influence on selectivity.

The importance of temperature profiles with complex retention mechanisms

METHOD

Analytical conditions

COLUMN:	High-efficiency Lithium cation-exchange column, 4x100 mm, Catalog number 0354100A, Lithium cation-exchange guard column, 2x20 mm, Catalog number 0352020
FLOW RATE:	0.4 mL/min
MOBILE PHASE:	Li292, Li365, Li375, RG003

Post-column conditions

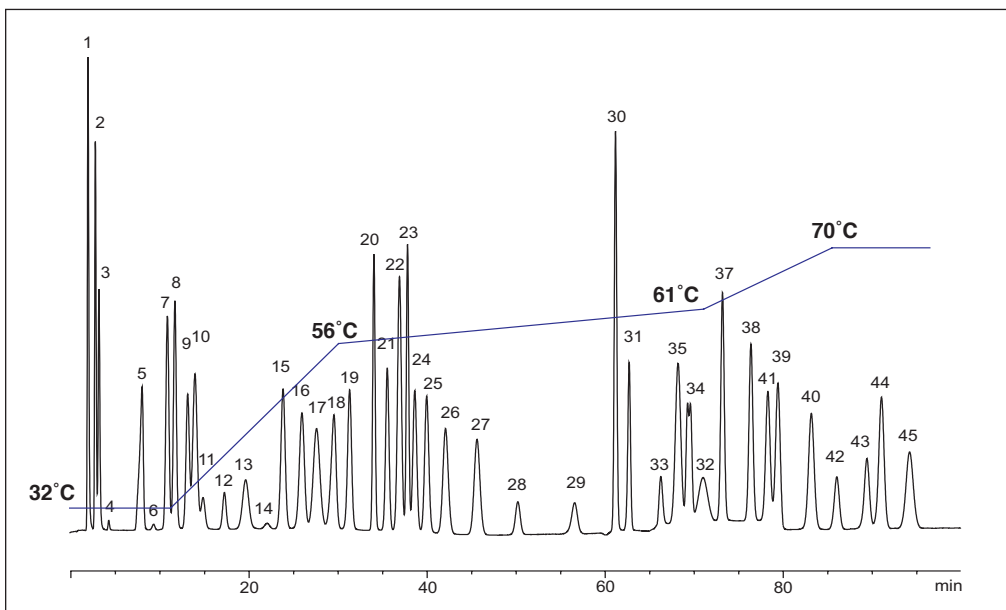
POST-COLUMN SYSTEM:	Pinnacle PCX
REACTOR VOLUMN:	0.5 mL
TEMPERATURE:	130 °C
REAGENT:	Trione Ninhydrin reagent
FLOW RATE:	0.3 mL/min
DETECTION:	UV/VIS 570 nm for primary amino acids 440 nm for secondary amino acids

NOTE

We recommend using Glucosaminic acid as internal standard with this method. Norleucine and α -Amino β -guanidinopropionic acid may not be suitable since they coelute with Tyrosine and 3-Methylhistidine respectively.

continued on back

Amino Acids calibration standard



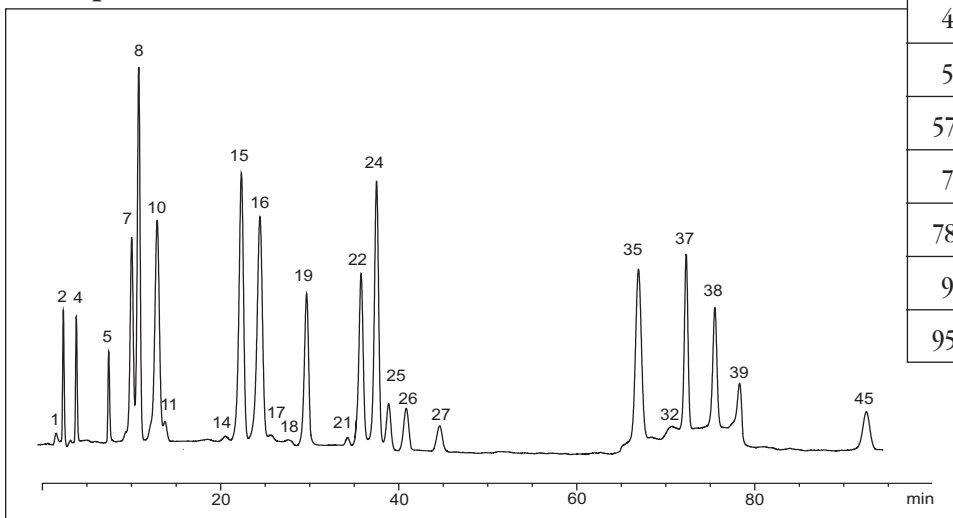
Column oven program

Time [min]	Temp [°C]
0	32
13	32
30	56
67	61
80	70
90	70
95	32

HPLC program

Time [min]	Li292 [%]	Li365 [%]	Li375 [%]	RG003 [%]
0	100	0	0	0
20	100	0	0	0
40	0	100	0	0
57	0	100	0	0
57.1	0	0	100	0
78	0	0	100	0
78.1	0	0	80	20
95	0	0	80	20
95.1	100	0	0	0

MSUD patient serum



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|------------------------|---|--|-----------------------|
| 1. Phosphoserine | 13. α -Amino adipic acid | 25. Leucine | 37. Ornithine |
| 2. Taurine | 14. Proline | 26. Tyrosine | 38. Lysine |
| 3. Phosphoethanolamine | 15. Glycine | 27. Phenylalanine | 39. Histidine |
| 4. Urea | 16. Alanine | 28. β -Alanine | 40. 3-Methylhistidine |
| 5. Aspartic acid | 17. Citrulline | 29. β -Amino- <i>i</i> -butyric acid | 41. 1-Methylhistidine |
| 6. Hydroxyproline | 18. α -Amino- <i>n</i> -butyric acid | 30. Homocystine | 42. Anserine |
| 7. Threonine | 19. Valine | 31. γ -Aminobutyric acid | 43. Carnosine |
| 8. Serine | 20. Cystine | 32. Tryptophan | 44. Homocarnosine |
| 9. Asparagine | 21. Methionine | 33. Ethanolamine | 45. Arginine |
| 10. Glutamic acid | 22. Allo-isoleucine | 34. Hydroxylysines | |
| 11. Glutamine | 23. Cystathionine | 35. Ammonia | |
| 12. Sarcosine | 24. Isoleucine | 36. Creatinine | |