Thermo Scientific Dionex AminoPac Column for Analysis of Amino Acids

Proteins and peptides are large macromolecules consisting of covalently bonded amino acids. Proteins commonly exist as folded structures, while peptides are shorter linear polymers consisting of only a few amino acids. Amino acid analysis refers to the methodology used to determine the individual amino acids in a protein or peptide, which may be part of a pharmaceutical preparation.

- Amino acid content determination can be used to establish the primary structure of a protein or peptide. It is necessary to hydrolyze the protein of interest, and the choice of hydrolysis procedures is key to accurate analysis as some sensitive amino acids may be destroyed during the hydrolysis.
- After hydrolysis, the hydrolyzing reagents are removed (typically by evaporation) and the hydrosylate is reconstituted in water or other compatible solvent. Most free amino acids are poor chromophores, so they cannot be directly detected at low concentrations using ultraviolet (UV), visible (vis), or fluorescence detection. Therefore, the hydrolysate must be derivatized with a chromophoric or fluorometric reagent before detection. This derivatization can be performed either before chromatography (precolumn derivatization) or after chromatography (postcolumn derivatization).
- Both pre- and postcolumn derivatization methods are costly in both reagents and labor. These methods create risk of toxic chemical exposure to personnel, and require hazardous waste removal. Electrochemical detection is a good alternative, that doesn't require derivatization after hydrolysis.
- Thermo Scientific™ Dionex™ Amino Pac™ PA10 columns separate free amino acids without the need for derivatization.

Column	Formats	Use For
Dionex AminoPac PA10	2 × 250 mm	A hydrophobic, polymeric, pellicular, anion-exchange resin stable over the range of pH 0–14. The unique pH stability allows the use of eluents that are conducive to anodic oxidation of amino acids at gold electrodes. This column is recommended for use with the Thermo Scientific™ Dionex™ AAA-Direct™ Amino Acid Analysis system, allowing direct detection of primary and secondary amino acids by IPAD, with no need for pre- or postcolumn derivatization.

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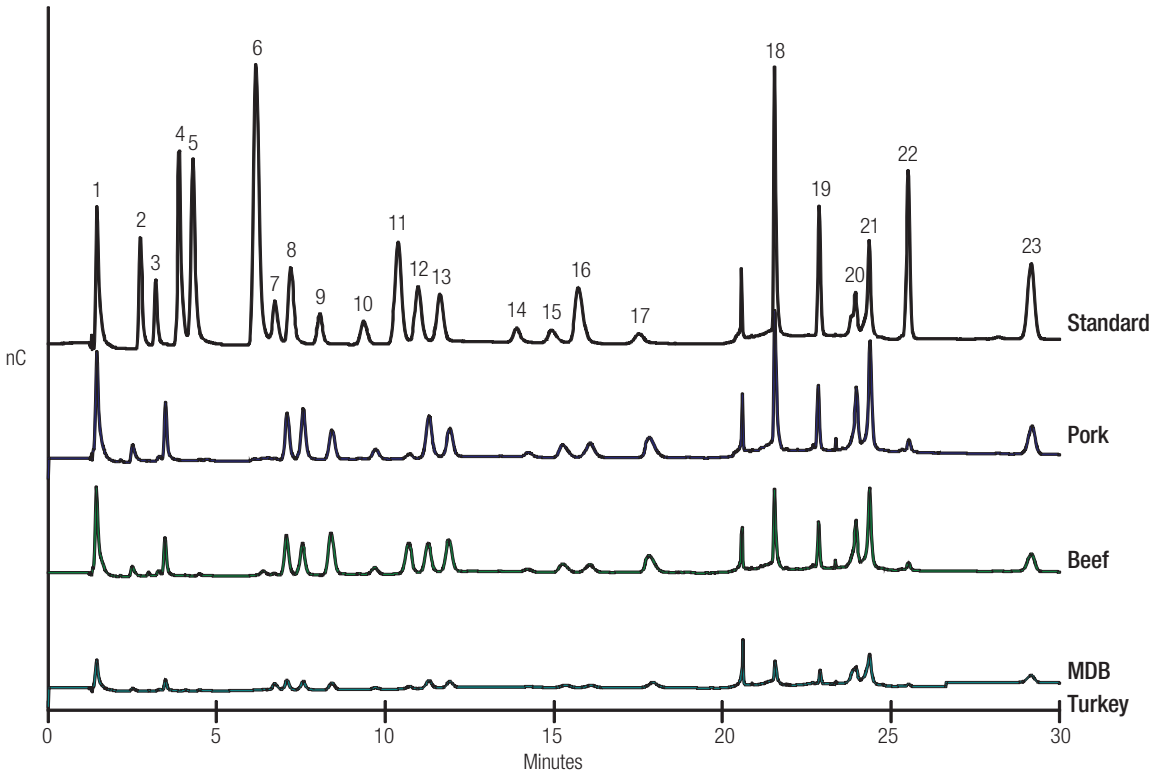
Figures 1 and 2 show examples of common amino acid separations in a variety of samples using a Dionex AminoPac PA10 column.

Figure 1. Analysis of meat hydrolysates.



Sample preparation:	Hydrolyze 0.1 g of meat in 5.0 mL of 4.0 M Methane Sulfonic Acid (MSA) for 16 hours at 100 °C Dilute 5x with water. In the next dilution step, dilute 500 fold with 8.0 µM norleucine azide diluent.
Injection Volume:	25 µL
Sample Concentration:	8.0 µM, all amino acids in "standard"
Column:	Dionex AminoPac PA10 analytical and guard columns
Column Temperature:	30 °C
Expected System Operating Backpressure:	< 3,000 psi
Eluent:	E1: Deionized water E2: 250 mM NaOH E3: 1 M Sodium acetate
Eluent Flow Rate:	0.25 mL/min
ED Waveform:	*AAA*-Direct Waveform
Gradient Conditions:	See table below

Time (min)	%E1	%E2	%E3	Curve	Comments
Init	84	16	0		Autosampler fills the sample loop
0.0	84	16	0		Valve from load to inject
2.0	84	16	0		Begin hydroxide gradient
12.1	68	32	0	8	
16.0	68	32	0		Begin acetate gradient
24.0	36	24	40	8	
40.0	36	24	40		
40.1	20	80	0	5	Column wash with hydroxide
42.1	20	80	0		
42.2	84	16	0	5	Equilibrate to starting conditions
65.0	84	16	0		

Peaks:

1. Arginine
2. Hydroxylysine
3. Lysine
4. Galactosamine
5. Glucosamine
6. Glucose
7. Alanine
8. Threonine
9. Glycine
10. Valine
11. Hydroxyproline
12. Serine
13. Proline
14. Isoleucine
15. Leucine
16. Methionine
17. Norleucine
18. Histidine
19. Phenylalanine
20. Glutamate
21. Aspartate
22. Cystine
23. Tyrosine

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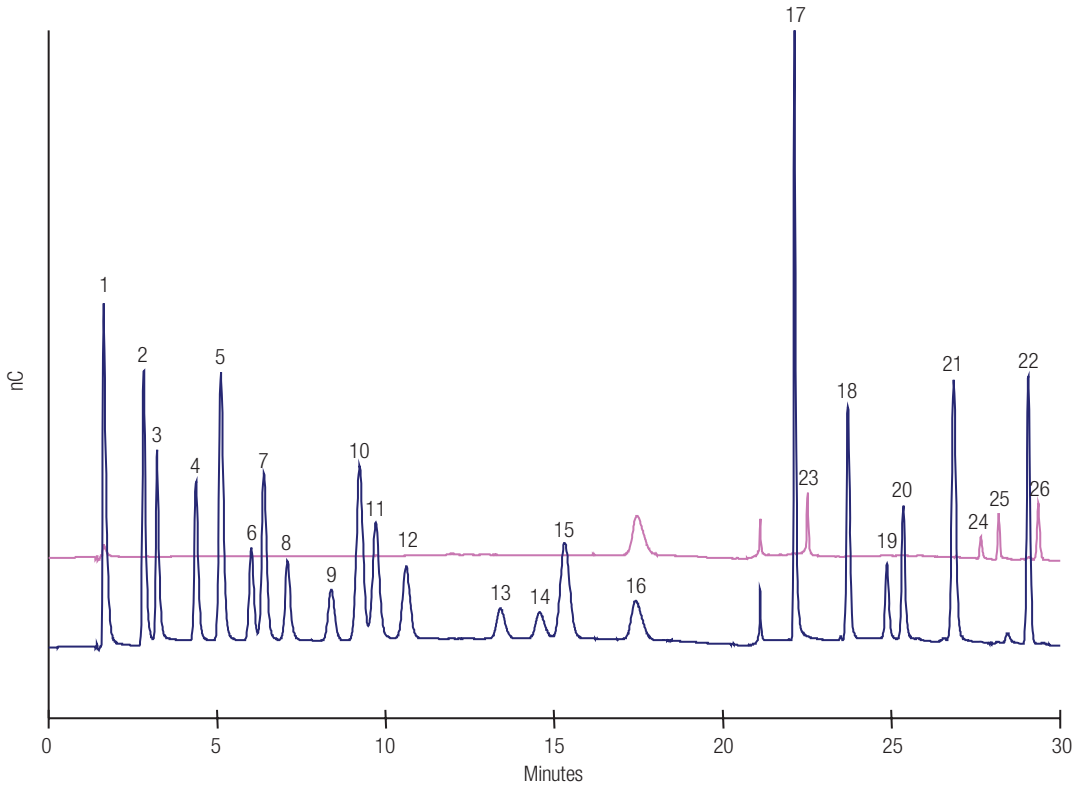
Figure 2. Analysis of phospho-amino acids.



Injection Volume: 25 µL
Standard: 8.0 µM, all amino acids in "standard"
Column: Dionex AminoPac PA10 analytical and guard columns
Column Temperature: 30 °C
Expected System Operating Backpressure: < 3,000 psi
Eluent: E1: 18.2 megohm-cm water
E2: 250 mM NaOH
E3: 1 M Sodium acetate
Eluent Flow Rate: 0.25 mL/min
ED Waveform: *AAA*-Direct Waveform
Gradient Conditions: See table below

Time (min)	%E1	%E2	%E3	Curve	Comments
Init	76	24	0		Autosampler fills the sample loop
0.0	76	24	0		Valve from load to inject
2.0	76	24	0		Begin hydroxide gradient, valve back to Load
8.0	64	36	0	8	
11.0	64	36	0		Begin acetate gradient
18.0	40	20	40	8	
21.0	44	16	40	5	
23.0	14	16	70	8	
42.0	14	80	0		
42.1	20	80	0	5	Column wash with hydroxide
44.1	20	80	0		
42.2	76	24	0	5	Equilibrate to starting conditions
75.0	76	24	0		

Peaks:

1. Arginine
2. Hydroxylysine
3. Lysine
4. Glutamine
5. Asparagine
6. Alanine
7. Threonine
8. Glycine
9. Valine
10. Hydroxyproline
11. Serine
12. Proline
13. Isoleucine
14. Leucine
15. Methionine
16. Norleucine
17. Histidine
18. Aspartate
19. Glutamate
20. Phenylalanine
21. Cystine
22. Tyrosine
23. *p*-Arginine
24. *p*-Serine
25. *p*-Threonine
26. *p*-Tyrosine

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