

Commission Regulation (EC) No 152-2009 published in Official Journal of European Union laid down the methods of sampling and analysis for the official control of feed. The Regulation describes methods of analysis to control the composition of feed materials and compound feed products. Establishing the Amino Acids profile is an important way to control quality and nutritional value of feeds. This regulation specifies HPLC with post-column derivatization with Ninhydrin reagent as the method of analysis for total and free amino acids. Pickering Laboratories developed the analytical method to comply with all the chromatographic requirements of Commission Regulation (EC) No 152-2009. The same method is used to analyze oxidized and unoxidized feed samples.

## Method

### Analytical Conditions

**Column:** High-efficiency Sodium cation-exchange column, 4.0 x 150 mm, Catalog Number 1154150T

**Guard:** Cation-exchange GARD™, Catalog Number 1700-3102

**Flow Rate:** 0.4 mL/min

**Mobile Phase:** Na270, Na740, RG011. See method in Table 1

**Injection Volume:** 10 µL

### Post-Column Conditions

**Post-Column system:** Onyx PCX or Pinnacle PCX

**Reactor Volume:** 0.5 mL

**Reagent:** Trione®

**Column Temperature:** See Method in Table 2

**Reactor Temperature:** 130 °C

**Flow Rate:** 0.25 mL/min

**Detection:** UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids  
Calibration

**Table 1. HPLC Program**

Time, Min	Na270, %	Na740, %	Rg011, %
0	100	0	0
16	100	0	0
40	54	46	0
45	0	100	0
66	0	100	0
66.1	0	0	100
70	0	0	100
70.1	100	0	0
80	100	0	0

**Table 2. Column Oven Program**

Time	Temp, °C
0	55
32	55
33	65
41	65
42	55

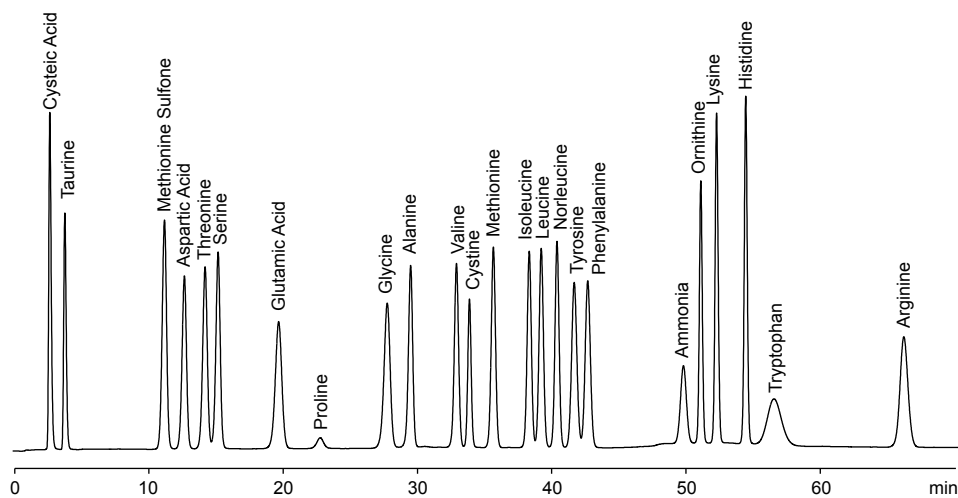


Fig 1. Chromatogram of a standard solution of amino acids

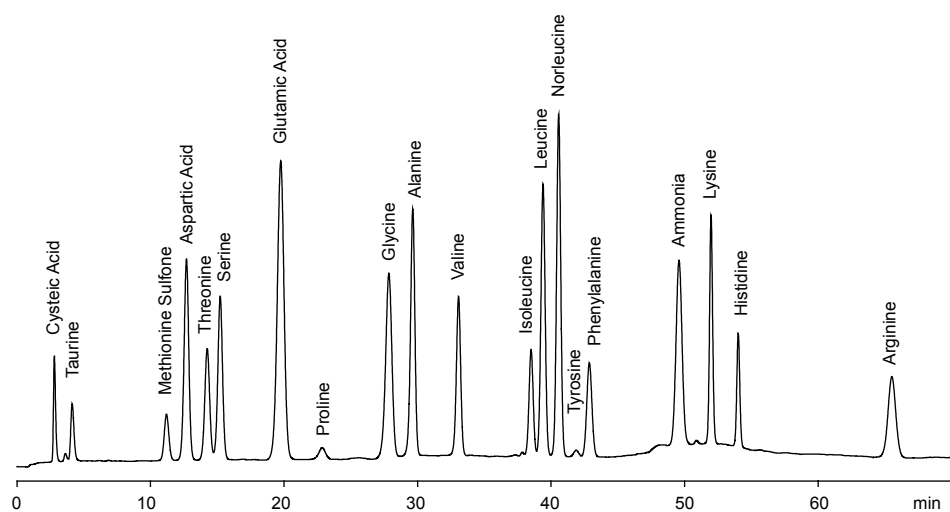


Fig 2. Chromatogram of an oxidized feed sample

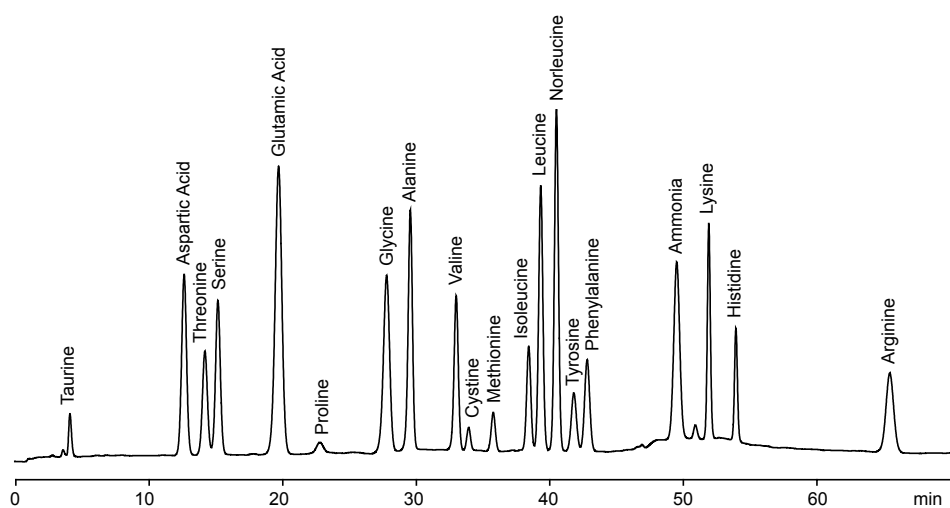


Fig 3. Chromatogram of a non-oxidized feed sample