



Sulfur Amino Acids in Foods and Feed Ingredients

Ion-exchange Chromatographic Method

One of the most important analyses performed on animal feeds is to determine the amino acid profile of the hydrolyzed feed. The retail price of the feed is generally dependent on specific amino acid concentrations: L-Methionine (Met) in the case of chicken feed and L-Lysine (Lys) for cattle feed.

Although the amino acid analysis of regular hydrolysate samples is straightforward, the hydrolysis presents problems. Met is partially oxidized (non-reproducibly) to Met-sulfoxide and Met-sulfone; L-Cysteine (Cys) is partially oxidized to L-Cystine and Cysteic acid. This inconsistency makes accurate quantitation difficult. In order to determine the sulfur amino acids accurately, the sample is first oxidized with performic acid to quantitatively convert Met and Cys to Met-sulfone and Cysteic acid, respectively.

These oxidized hydrolysate samples present a chromatography challenge: using the classical Na⁺ conditions, Met-sulfone and L-Aspartic acid (Asp) are not resolved. "Adjust chromatographic conditions as required to obtain >90% resolution for methionine sulfone peak1" is a tall order in AOAC Method 985.28. Adjusting the pH of buffers in 1-2 liter quantity is just not practical. Poor resolution, poor reproducibility, and amine contamination are typical problems.

Pickering Laboratories presents here a new set of buffers to work with our standard Na⁺ ion-exchange column and post-column system to improve quantitation of oxidized-sulfur amino acids in food and feed ingredients. The use of a special Na buffer, Na270, produces a near-baseline resolution (99+%) of Met-sulfone and Asp.

NOTE:

The liquid chromatograph (HPLC) should have a ternary pumping system to effect the gradient required for this method. In addition, ensure that the autosampler injection valve is fitted with a Tefzel[®] or PEEK rotor seal for compatibility with the high-pH column regenerant.

HPLC Ion-exchange Method for Oxidized Feed Hydrolysate

COLUMN: Na⁺ ion-exchange column,
4mm x 150mm (Cat. No. 1174150)
GUARD COLUMN: Na⁺ ion-exchange guard, 3mm x 20mm
(Cat. No. 1193020)
ELUENT FLOW RATE: 0.4 mL/min
COLUMN TEMPERATURE: 55° C
INJECTION VOLUME: 10µL

Step	Time [min]	Interval	%Na270	%Na740	%RG011	Comment
Equil.			100	0	0	0.4 mL/min.
0	0	0	100	0	0	inject up to 50µl
1	0 - 16	16	100	0	0	isocratic
2	16-48	32	0	100	0	linear gradient
3	48-58	10	0	100	0	isocratic
4	58-58.1	0.1	0	0	100	step change
5	58.1-60	1.9	0	0	100	regeneration
6	60-60.1	0.1	100	0	0	step change
7	60.1-75	14.9	100	0	0	re-equilibration

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Post-column Conditions

REAGENT: TRIONE[®] Ninhydrin
REAGENT FLOW RATE: 0.3 mL/min
REACTOR: 130° C, 0.5mL
DETECTION: Visible @ 570nm

Typical Retention Time Repeatability and Precision

Analyte	Retention Time Min. (Average)	Retention Time Repeatability (%RSD)	Area Precision (%RSD)
Cysteic acid	2.846	0.381	0.876
Met-sulfone	14.556	0.163	1.366
Aspartic acid	15.981	0.149	1.400
Threonine	17.493	0.142	1.648
Serine	18.666	0.131	1.370
Glutamic acid	24.577	0.125	1.644
Proline	26.499	0.100	1.450
Glycine	29.670	0.048	1.631
Alanine	30.243	0.052	1.880
Valine	31.816	0.022	1.520
Isoleucine	34.679	0.025	1.498
Leucine	35.312	0.041	1.638
Phenylalanine	38.733	0.047	1.536
Ammonia	46.477	0.009	1.594
Lysine	47.819	0.017	1.551
Histidine	52.406	0.020	1.789
Arginine	62.125	0.067	1.613