AccQ•Tag SOLUTIONS

Analyzing Feed Hydrolysate Samples Using the AccQ•Tag™ Method

A number of published studies provide substantial evidence that the AccQ•Tag method for amino acid analysis provides superb compositional analysis of hydrolyzed peptides and proteins. These samples are usually highly purified with a minimal amount of non-amino acid constituents. However, many samples are more complex in sample matrix. The high concentration of non-protein components creates the potential for significant interference in the analysis procedure. Does the AccQ•Tag method provide the same high quality results for these samples? This application note describes the analysis of animal feeds, a typical complex hydrolysate sample (1,2).

Complex Feed Samples

Animal feeds pose several significant challenges for accurate amino acid analysis. Although there are rarely limitations to the amount of sample available for analysis, the presence of high concentrations of complex carbohydrates, as well as fats (lipids), vitamins and inorganic salts can interfere with the analysis method. In addition, the samples are not completely soluble in any solvent and the entire contents must be sampled as a solid. It is necessary therefore, to hydrolyze the sample on a large scale relative to protein and peptide samples, to provide a representative sample for analysis.

Sample Preparation Method

Outlined below are the basic procedures for sample preparation, derivatization and chromatographic analysis for a feed hydrolysate.

Hydrolysis and Dilution

1. Weigh approximately 100mg of sample into a 16 x 25mm test tube and add 5ml of 6N HCl. Thoroughly flush the tube with nitrogen or argon. Cap the tube and place it in an oven at 112°C for 22 hours.
2. Add the internal standard (10ml of 2.5mM α-aminobutyric acid) to the cooled hydrolysate mixture, transfer to a 250ml volumetric flask, and fill to the meniscus with MilliQ® water. Filter approximately 0.5ml of the solution with a 0.45µm acid-compatible filter.

Derivatization

1. Mix 10µl of filtrate with 70µl of AccQ•Fluor™ derivatization buffer, and add 20µl of AccQ•Fluor reagent to derivatize.
2. Transfer the sample to a limited volume insert in an autosampler vial and heat the sample at 55°C for 10 minutes in a heating block.

Analysis

HPLC System: 626 LC System with heater
717plus Autosampler with heater/cooler
474 Scanning Fluorescence Detector
Millennium® Chromatography Manager

Column: AccQ•Tag column equipped with a Nova-Pak® C_{18} Sentry™ Guard column

Column Temperature: 37°C

Sample Volume: 5 or 10µl

Eluents: A = AccQ•Tag Eluent A
B = Acetonitrile
C = Water

Table 1

Time	Flow Rate	%A	%B	%C	Curve
Initial	1.0	100	0	0	-
0.5	1.0	99	1	0	11
18	1.0	95	5	0	6
19	1.0	91	9	0	6
28	1.0	83	17	0	6
35	1.0	0	60	40	11
38	1.0	100	0	0	11

The gradient above is modified from the standard protocol, published in the AccQ•Tag operations manual, to accommodate the use of the Sentry Guard column.

Note that the run time is increased from 45 to 47 minutes.

In addition to the standard HPLC system, Waters has documented the utility of several options for system configuration. Contact your Waters sales or technical service representative for details.

1. Detectors: UV detection at 248 or 254nm with the 996 PDA, 486 TUV, 490 or 441 detectors.
2. Solvent Delivery Systems: 616, 600, LCM1 or two 510 pumps.

Waters

Sample Solution

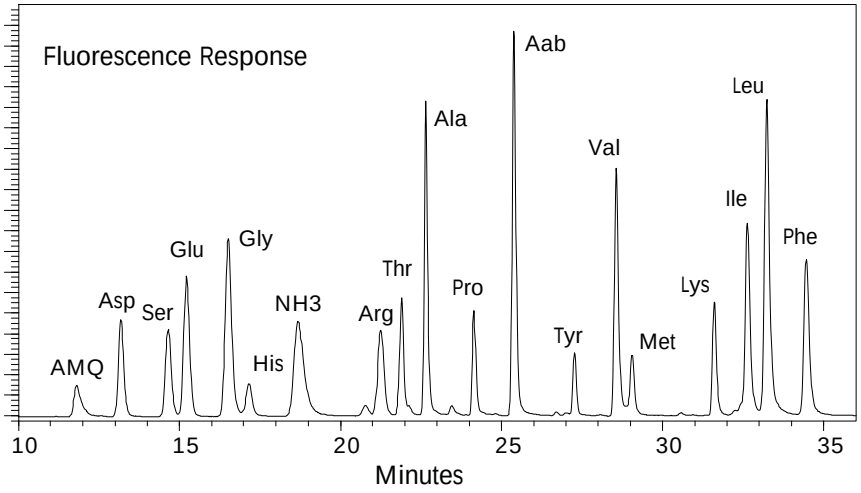

Figure 1 High Resolution Analysis of 17 Amino Acids in a Complex Peanut Meal Sample

Automate and Simplify Data Analysis

Using the Millennium Chromatography Manager data system significantly simplifies data reduction. The table below illustrates how custom fields and tailored processing methods can be used to automatically calculate and report individual amino acid concentrations and total protein in the sample.

Millennium Result Table Information					
SampleName:	CPeanut_Meal	Vial	40	Injection	1
Processing Method:	Fluor_Feed				
Protein mg per gram:	521.83				

Table 2 Partial Sample Results from an Analyzed Sample

Peak Results

AA	Ret Time (min)	Area (UV*Sec)	Height (uV)	Pmol	mg per g
Asp	13.19	1119610	94199	170.14	48.04
Ser	14.66	1076851	85357	126.15	26.95
Glu	15.23	1740836	137265	239.70	75.93
Gly	16.52	2447464	173319	308.64	43.20
His	17.17	452435	31730	37.26	12.54

This application note illustrates one example of how effectively Waters AccQ•Tag Method can be used in complex sample analysis.

References
1. S. A. Cohen and K. M. De Antonis, J. Chromatography, 661 (1994) 25-34.
2. H. J. Liu, B. Y. Chang, H. W. Yan, F. H. Yu, and X. X. Liu, JAOAC, 78 (1995) 736-744.

Waters

Waters Corporation
34 Maple Street
Milford, MA 01757
(508) 478-2000
FAX (508) 872-1990
http://www.waters.com/