ANALYTICAL SOLUTIONS FOR THE ANALYSIS OF BOUND AND FREE AMINO ACIDS IN FOODS AND FEEDS

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INTRODUCTION

The analysis of amino acids in foods and feeds is one of the most useful characterizations of these sample types. Quantitation of the amino acids released by hydrolysis is one important measure of nutritional value. A free amino acid profile can identify the origin of a food product, and in that way, detect adulteration. Free amino acids are also metabolic indicators that can be used to monitor and optimize processes such as fermentation.

The Waters® UPLC® Amino Acid Analysis Solution is a total system solution that can be used in all of these applications. It combines the well-established AccQ-Tag™ pre-column derivatization with the increased resolution and performance of the ACQUITY UPLC® system to assure accurate and precise qualitative and quantitative results.

The present study focuses on the nutritional analysis of foods and feeds. To confirm the accuracy of the determination, the proportions of amino acids in a pure protein were measured so that the experimental results can be compared to a known true result. The chromatographic method is then evaluated for the typical amino acids encountered in feed analysis. Because of the importance of sulfur-containing amino acids, a sample of chicken feed was analyzed with and without performic acid oxidation. Finally, the robustness of the method was examined in a collaborative study with four different feed types. Precision was assessed for each step in the analysis over a series of five days. The results of these experiments demonstrate the suitability of this analytical solution for assessing the amino acid nutritional value of feed samples.

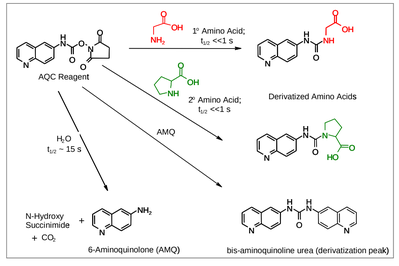

Figure 1. In the AccQ•Tag derivatization, the AQC reagent reacts quickly with unprotonated primary and secondary amino acids in a largely acqueous environment to form stable derivatives that are readily detected with UV. The excess reagent reacts with water on a slower time scale to form byproducts that are easily separated from the amino acids in the analysis.

METHODS

Sample Preparation and Derivatization
Hydrolyzed Bovine Serum Albumin (BSA) was supplied at an estimated concentration of 1.0 mg/mL. Hydrolyzed BSA was diluted 1:10 with 0.1 M HCl prior to derivatization.

Pelletized chicken feed was ground to a fine powder and hydrolyzed with and without performic acid oxidation, according to AOAC Official Method 994.12. Hydrolyzed samples were diluted 1:10 with 0.1 M HCl prior to derivatization.

Swine diet, poultry diet, whole soybean, and soybean meal samples were acid-hydrolyzed in an independent laboratory as part of a collaborative study. The samples were supplied at an estimated concentration of 1.0 mg/mL in 0.1 M HCl and sealed under argon in ampoules. The standard was NIST 2389 Amino Acids in 0.1 mol/L HCl Reference Material. The feed samples were diluted 1:16 and the with 0.1 M HCl prior to derivatization.

The standard derivatization protocol was modified to include neutralization of excess acid with 0.1 M NaOH. The samples were derivatized in batches according to Figure 2, and are stable at room temperature for one week when tightly capped.

Pre-column derivatization and analysis conditions are described in detail in the Waters UPLC Amino Acid Analysis Solution System Guide (P/N 71500129702).

Chromatographic Conditions

LC System:	Waters ACQUITY UPLC System
Column:	AccQ·Tag Ultra, 2.1 x 100 mm, 1.7 µm
Column Temp:	55 ˚C
Sample Temp:	20 ˚C
Flow Rate:	700 µL/min.
Mobile Phase A:	1:20 Dilution of AccQ·Tag Ultra Eluent A
Mobile Phase B:	AccQ·Tag Ultra Eluent B
Weak Needle Wash:	95:5 Water:Acetonitirile
Strong Needle Wash:	5:95 Water:Acetonitirile
Gradient:	AccQ·Tag Ultra Hydrolysate Method (see UPLC Amino Acid Analysis Solution Guide)
Total run time:	9.5 min
Injection volume:	1 µL, Partial Loop with Needle Overfill
Detection:	UV (TUV), 260nm

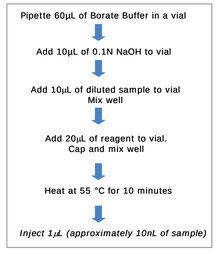

Figure 2. Steps in derivatization for UPLC Amino Acid Analysis

RESULTS

Method confirmation with a protein hydrolysate

The accuracy of an amino acid analysis method must be confirmed with the results obtained with a sample of known composition. An acid hydrolysate of a pure protein can be used as such a reference material. The expected composition is known from the sequence of the protein. The proportions of amino acids measured with the method should match this composition. The consistency of accurate compositional measurement is also a measure of the robustness of the method. The hydrolysate of BSA was used for this test.

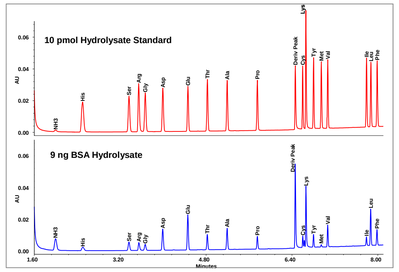

Figure 3. Comparison of the chromatographic separations of an amino acid standard with hydrolyzed BSA. The retention times are the same in the sample and standard for reliable peak identification. There are no significant extraneous peaks in the hydrolyzed sample.

Amino Acid	Expected # Residues	Observed # Residues
His	17	15.36 ± 0.19
Ser	28	26.00 ± 0.08
Arg	23	22.37 ± 0.08
Gly	16	17.68 ± 0.20
Asp	54	55.47 ± 0.21
Glu	79	80.68 ± 0.20
Thr	33	31.92 ± 0.06
Ala	47	47.51 ± 0.15
Pro	28	28.35 ± 0.14
Lys	59	57.78 ± 0.38
Tyr	20	20.19 ± 0.08
Met	4	4.16 ± 0.15
Val	36	35.67 ± 0.16
Ile	14	13.15 ± 0.15
Leu	61	63.13 ± 0.19
Phe	27	26.57 ± 0.13

Table 1. Comparison of observed amino acid composition with expected values from the sequence of BSA. The reported composition is the mean of five days of analysis, with five replicate derivatizations, each injected in triplicate, for a total of 75 independent analyses. The measured values match well to the known proportions, with the average deviation from expected less than 4%. The average variability of the compositional determination is less than 1% RSD.

Analysis of hydrolyzed feed samples

Feed samples present a more complicated analytical problem than pure proteins. In addition to the obvious matrix affects, the analysis must provide accurate measurement of the sulfur-containing amino acids that are often growth-limiting. Because cystine and methionine are partially and variably destroyed during the acid hydrolysis step, they must be protected. Performic acid oxidation is the most reliable solution, but this chemical treatment destroys other amino acids. Each sample must, therefore, be analyzed with and without oxidation. The chromatographic method must accommodate the oxidation products, cysteic acid and methionine sulfone. The complete quantitative analysis combines the results from the unoxidized and oxidized samples.

A sample of pelletized chicken feed was treated in both ways. The chromatograms were also examined for extraneous and unidentified components.

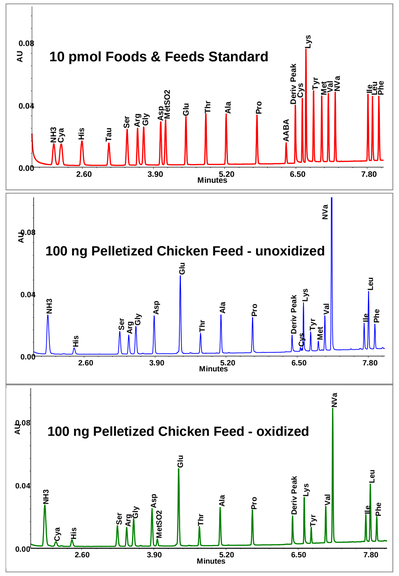

Figure 4. Standard and sample chromatograms for feed analysis. The standard chromatogram permits analysis of cysteic acid, methionine sulfone, taurine, as well as the internal standards alpha amino butyric acid and norvaline. There are no extraneous, interfering, or unidentified peaks in either of the chicken feed hydrolysates.

Stability of the analysis of feed samples

The amino acid analysis method must give reliable results for a range of sample types that could compromise the derivatizations or the chromatographic analysis. The same result must be obtained for all repeated tests.

Four types of hydrolyzed animal feed were provided as part of a collaborative study. The samples were analyzed on each of five days. On each day, each hydrolysate was derivatized five separate times, and each derivatizations was injected three times. Two different columns and five different bottles of eluent were used for the study.

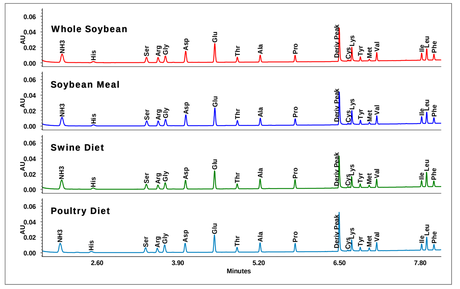

Figure 4. Chromatographic analysis of four animal feed hydrolysates. The different matrices have no effect on retention time and show no interfering peaks.

Amino Acids	Whole Soybean	Soybean Meal	Swine Diet	Poultry Diet
His	1.593 ± 0.027	1.867 ± 0.030	1.966 ± 0.028	1.948 ± 0.030
Ser	3.352 ± 0.016	3.650 ± 0.019	3.508 ± 0.022	3.608 ± 0.019
Arg	5.285 ± 0.025	5.822 ± 0.030	4.693 ± 0.027	5.178 ± 0.026
Gly	2.595 ± 0.016	2.976 ± 0.016	2.814 ± 0.018	3.522 ± 0.017
Asp	8.092 ± 0.037	9.061 ± 0.052	7.097 ± 0.043	7.629 ± 0.039
Glu	13.219 ± 0.058	14.489 ± 0.076	14.145 ± 0.087	14.114 ± 0.063
Thr	2.504 ± 0.013	2.918 ± 0.016	2.732 ± 0.017	2.819 ± 0.014
Ala	2.647 ± 0.013	3.172 ± 0.019	4.057 ± 0.027	4.065 ± 0.018
Pro	3.423 ± 0.022	3.862 ± 0.026	5.111 ± 0.034	5.037 ± 0.029
Cys	0.233 ± 0.004	0.269 ± 0.004	0.235 ± 0.003	0.241 ± 0.003
Lys	4.125 ± 0.027	4.674 ± 0.033	3.784 ± 0.028	3.955 ± 0.026
Tyr	2.525 ± 0.010	2.900 ± 0.016	2.485 ± 0.014	2.583 ± 0.013
Met	0.862 ± 0.020	1.082 ± 0.015	1.331 ± 0.017	1.823 ± 0.012
Val	3.152 ± 0.016	3.663 ± 0.019	3.540 ± 0.023	3.628 ± 0.019
Ile	3.014 ± 0.014	3.446 ± 0.019	2.946 ± 0.018	3.015 ± 0.015
Leu	5.307 ± 0.025	6.122 ± 0.033	7.472 ± 0.044	7.012 ± 0.034
Phe	3.459 ± 0.016	3.919 ± 0.022	3.773 ± 0.024	3.753 ± 0.020

Table 2. Quantitative analysis of the four animal feed types. The results are expressed as weight percents. The precision is expressed as absolute standard deviations. The results represent 75 independent determinations. The compositions of the samples are very similar but not identical. Lysine is more abundant in the soybean samples than in the mixed feeds. In contrast, the ratio of Leucine to Isoleucine is higher in the mixed feeds.
The variability across all amino acids, in all 75 determinations, for all four samples is well under 1% RSD.

CONCLUSIONS

- **UPLC Amino Acid Analysis Solution can be used for the determination of the nutritional content of amino acids in animal feeds**
- **The AccQ•Tag Ultra reagent reliably derivatizes primary and secondary amino acids in largely aqueous solutions that accommodate complex feed samples**
- **The analysis consistently gives the correct proportions of amino acids**
- **The chromatographic method is suitable for the analysis of oxidized feed samples to ensure accurate quantitative measurement of sulfur amino acids**
- **A range of complex feed samples can be analyzed consistently in replicate trials over multiple days**

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