1. SCOPE AND PURPOSE:

1.1. This method measures amino acids (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine) which are not bound in proteins (free amino acids excluding hydroxy acids) in food and feed ingredients, foods and feeds, premixes, feces, and fermentation products.

1.2. Upon request, monosodium glutamate (MSG) can be determined by converting glutamic acid to monosodium glutamate.

1.3. There is no assurance that matrices other than those listed can be tested using this method.

2. PRINCIPLE:

2.1. Free amino acids are extracted from the sample with dilute hydrochloric acid at room temperature. Internal standard is added and the extract is treated with 10% sulfosalicylic acid to precipitate any soluble proteins. The sample is partially neutralized, diluted with 0.1N HCl, and filtered. The amino acids are derivatized using Waters AccQ-Tag Ultra Derivatization Kit (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in acetonitrile) and separated by reverse-phase chromatography with UV detection. The concentration of each amino acid is quantitated against a series of reference standard solutions of amino acids which also contain internal standard.

2.2. Using a 250 mg sample, the lowest confidence level for each amino acid is 0.05g/100g sample.

2.3. Known Interferences:

2.3.1. Amino sugars may co-elute with some of the amino acids.

2.3.2. Dipeptides, not removed by treatment with sulfosalicylic acid, may interfere by co-eluting with some of the amino acids.

3. PRECISION:

Records of method precision based on Method Validation and/or known control summaries are located in the QA Master file for this test method. Assay precision may vary with test matrix and analyte level. Terms used to describe method precision are defined in NPSOP3000, *Validation of Quantitative Chemical Tests*.

4. REFERENCES:

4.1. Degussa, The Analytical Control of Supplemented DL-Methionine in Formulated Feeds, 1C-ATAV, 1.1982.

4.2. Fahnenstich, R., Tanner, H.: Determination of Supplemented Amino Acids in Feedstuffs and Formulated Feeds, Landwirtsch, Forsch., 27 (1), p. 46-50, 1974. (In German)

4.3. Official Methods of Analysis of the AOAC International, Method 2018.06.

4.4. Nestlé LI-00.594, Total Amino Acids By AccQ-TAG & UHPLC-UV.

4.5. Jaudzems, G. and Fuerer, C., 2022. Determination of Total Amino Acids in Infant Formulas, Adult Nutritionals, Dairy, and Cereal Matrixes by UHPLC–UV: Interlaboratory Validation Study, Final Action 2018.06. Journal of AOAC International, 105(6), pp.1625-1639.