

| | | |
|---------------------------|---|--------------------|
| EFFECTIVE DATE | N P Analytical Laboratories | Document ID |
| REVISION: 02/27/26 | STANDARD OPERATING PROCEDURE SUMMARY | TYHV |
| REPLACES: 12/06/24 | Tryptophan – Alkaline Hydrolysis | PAGE 1 OF 1 |

1. SCOPE:

- 1.1. This procedure measures tryptophan in foods, feeds, ingredients, and physiological samples.
- 1.2. There is no assurance that matrices other than those listed can be assayed using this method.

2. PURPOSE:

- 2.1. A portion of sample is treated with sodium hydroxide solution and hydrolyzed by heating at 110°C for 20 hours. The samples are cooled, opened, neutralized, and buffered to about pH 4.2. Hydrolysates are analyzed on a high performance liquid chromatograph (HPLC) using UV detection and quantitated from standards of known concentration.
 - 2.1.1. For the oven hydrolysis at 110°C for 20 hours, oxidation of the amino acids must be prevented as they are hydrolyzed.
- 2.2. Using a 300 mg sample, the lowest confidence level of this method is 0.05g/100g.
- 2.3. Known Interferences: If the defatting procedure fails to remove sufficient fat from a high fat sample (samples containing greater than 10% fat), low recovery of tryptophan may result.

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. Official Methods of Analysis of the AOAC International, Method 988.15
- 4.2. *Agri. Biol. Chem.*, 1984, Vol. 48, pp 2961-2969, “Determination of the Tryptophan Content of Feed and Feedstuffs by Ion Exchange Liquid Chromatography”, H Sato, T Seino, T Kobayashi, A Murai, and Y Yugari.
- 4.3. *Analyst*, 1981, Vol. 106, pp 968-973, “Determination of Tryptophan in Feeds and Feed Ingredients by High-performance Liquid Chromatography”, A.D Jones, H.S Hitchcock, and G.H. Jones.