1. SCOPE AND PURPOSE:

This method measures riboflavin (Vitamin B2) and thiamine (Vitamin B1), as thiamine hydrochloride, in foods, feeds, and ingredients by HPLC with fluorescence detector.

2. PRINCIPLE:

2.1. Thiamine and riboflavin are extracted by enzymatic treatment with alpha-amylase followed by acid hydrolysis. An aliquot is analyzed by HPLC fluorescence detection for riboflavin. A second aliquot is oxidized with potassium ferricyanide to form thiochrome and is analyzed by HPLC fluorescence detection for thiamine. Quantitation of both thiamine and riboflavin are calculated from a set of standards of known concentrations.

2.2. Using a 5g sample, the lowest level of confidence is 1ppm for both thiamine and riboflavin.

2.3. Known interferences:

2.3.1. Vitamin B1 analysis include samples with high concentrations of cocoa which contain interfering substances that inhibit the thiochrome reaction. These samples are not applicable for this method.

2.3.2. Photodegradation can occur if extracts are not properly protected with UV light filters or amber glassware/labware. Riboflavin is highly susceptible to photodegradation.

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. LI-00.689, *Determination of Vitamin B1(Thiamine) by HPLC-FLD or UPLC-FLD*

4.2. LI-00.688, *Determination of Vitamin B2(Riboflavin by HPLC-FLD or UPLC-FLD*