

EFFECTIVE DATE	N P Analytical Laboratories	METHOD CODE
REVISED: 02/20/26	LABORATORY TEST METHOD SUMMARY	CHOE
REPLACES: 07/07/24	Choline, Enzymatic Method	PAGE 1 OF 1
KEY WORDS: Choline, Choline Chloride, Choline Bitartrate, Choline Citrate, Choline Dihydrogen Citrate		

1. **SCOPE AND PURPOSE:**

This method measures choline in dry and intermediate moisture animal feeds and nutritional beverages using an enzymatic procedure. There is no assurance that matrices other than those listed can be assayed using this method.

2. **PRINCIPLE:**

- 2.1. Samples are acid digested at 70°C, releasing bound choline. Following pH adjustment, residual choline phospholipids are cleaved with phospholipase D. Free choline is then reacted with choline oxidase, liberating hydrogen peroxide. In the presence of peroxide, phenol is oxidized and quinoneimine chromophore is formed from 4-aminoantipyrine. Both the enzymatic and color-forming reactions take place concurrently. Absorbance is measured at 505 nm and choline content is quantitated from a multi-point standard curve using a set of standard solutions of known concentrations.
- 2.2. The lowest confidence level is 250 ppm using a 5 gram sample
- 2.3. For this procedure, choline is defined and reported as choline chloride.
- 2.4. Known Interferences – High levels of Vitamin C may interfere with the measurement of choline in the sample.

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 999.14, First Action 1999.
- 4.2. LI-00.694, "Enzymatic Determination of Choline"
- 4.3. Determination of Choline in Milk and Infant Formulas by Enzymatic Analysis: Collaborative Study, JAOACI, 83 (1), 131-138 (2000)