

EFFECTIVE DATE	N P Analytical Laboratories	METHOD CODE
REVISED: 04/10/26	LABORATORY TEST METHOD SUMMARY	VDMS
REPLACES: 12/26/25	Vitamin D, LCMS	PAGE 1 OF 1
KEY WORDS: Vitamin D, Vitamin D₂, Vitamin D₃, cholecalciferol, ergocalciferol		

1. SCOPE AND PURPOSE:

- 1.1. This method measures Vitamins D₃ and D₂ in foods, feeds, ingredients, and vitamin premixes.
- 1.2. There is no assurance that matrices other than these can be assayed using this method.

2. PRINCIPLE:

- 2.1. Samples are treated with ethanolic potassium hydroxide in the presence of the antioxidant pyrogallol. Fats and oils in the samples are saponified at room temperature to avoid the formation of previtamins D₃ and D₂. Following saponification, vitamins D₃ and D₂ are partitioned into hexane, taken to dryness, dissolved in hexane and derivatized.
- 2.2. Vitamins D₃ and D₂ are quantitated using liquid chromatography with tandem mass spectrometry and isotopically labeled internal standards. The small peak eluting before Vitamins D₂ and D₃ are epimer peaks and are not used for quantitation.
- 2.3. Using a 5 g sample, the lowest confidence level of this method is 0.02 IU/g vitamin D₃ or 0.1 IU/g vitamin D₂ activity per gram of sample.
- 2.4. Known Interferences: High levels of non-hydrolyzable oil, such as mineral oil, can interfere with extraction.

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. J AOAC Int., 1982, Vol. 6 (No. 3), pp 624 - 631, "High Performance Liquid Chromatographic Determination of Vitamin D in Fortified Milks, Margarine, and Infant Formulas", JN Thompson, G Hatina, WB Maxwell, and S Duval.
- 4.2. LI-03.701-3 Fat Soluble Vitamins in Premixes by HPLC
- 4.3. LI-75.215-01 Vitamin D by UHPLC-MS/MS