

EFFECTIVE DATE	N P Analytical Laboratories	METHOD CODE
REVISED: 01/09/26	LABORATORY TEST METHOD SUMMARY	B7LC
REPLACES: 02/12/25	Biotin by HPLC with post-column derivatization	PAGE 1 OF 1
KEY WORDS: B7, Biotin, Bound		

1. SCOPE AND PURPOSE:

This method measures bound forms of biotin in plant and animal tissues, foods, feeds, and ingredients. This method for the quantitative determination of biotin by HPLC with post-column derivatization and fluorescence detection. There is no assurance that matrices other than those listed can be assayed using this method.

2. PRINCIPLE:

- 2.1. Biotin in the bound form is extracted through acid hydrolysis. Extracts are further diluted with water. Biotin is determined by using HPLC, post-column derivatization, gradient mobile phase, with fluorescence detection at Excitation wavelength: 490 nm Emission wavelength: 520 nm.
- 2.2. Using a 10g sample, the lowest confidence level of this method is 0.025ppm.
- 2.3. There are no known interferences for this method.

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. "BIOTIN BY HPLC-FLD", Nestle method LI-00.674-1
- 4.2. *Methods of Vitamin Assay*, pp 43, 50-58 and 541-549, **1985**, Augustin, Jorg, et al
- 4.3. JAOAC, Vol. 49, No. 4, pp. 882-883, **1966**, *Extraction of Added Biotin From Animal Feed Premixes*, Scheiner, J.
- 4.4. J. Agric. Food. Chem, Vol. 23, No. 6, pp 1157-1162, **1975**, *Biotin Content of Feedstuffs*, Scheiner, J., and De Ritter, E.