

EFFECTIVE DATE	N P A n a l y t i c a l L a b o r a t o r i e s	METHOD CODE
REVISED: 09/26/25	LABORATORY TEST METHOD SUMMARY	B5LCMS
REPLACES: 10/23/22	Pantothenic Acid	PAGE 1 OF 1
KEY WORDS: calcium pantothenate, pantothenic acid, vitamin B5		

1. SCOPE AND PURPOSE:

The B5LCMS method measures total (bound and free) pantothenic acid (vitamin B₅) in plant and animal tissues, ingredients, foods, and feeds by UHPLC-MS/MS. There is no assurance that matrices other than those listed can be assayed using this method.

2. PRINCIPLE

- 2.1. Enzymatic extraction of total pantothenic acid.
- 2.2. Analysis by UHPLC-MS/MS.
- 2.3. Quantification using peak area ratio “analyte/IS”
- 2.4. Using a 2 g sample, the lowest confidence level of this method is 1.0 ppm total pantothenic acid .
- 2.5. Known Interferences:
 - 2.5.1. If the source of pantothenic acid activity is a combination of bound and free form, the high pH during the autoclave step prior to extraction for bound pantothenic acid activity may destroy a percentage of free pantothenic acid activity, producing a low estimate of total pantothenic acid activity.
- 2.6. There is no assurance that matrices other than those listed can be assayed using 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. Official Methods of Analysis of the AOAC Methods 945.74 and 992.07, AOAC, Arlington, VA. Modified.
- 4.2. Augustin, Jorg et. al., **1985**. *Methods of Vitamin Assay*, pp. 43, 50, 51-58 and 407-413, John Wiley & Sons, Inc., New York, NY.
- 4.3. Brennecke, Dorothy M. **1980**. *Evaluation of Enzymatic Extraction of Pantothenic Acid from Plant and Animal Tissues*, MR-X 3012, Central Research, Microbiology Department, Ralston Purina Company, St. Louis, Missouri.
- 4.4. Freed, M., **1966**. *Pantothenic Acid, Methods of Vitamin Assay*, pp. 197-207, Interscience Publishers, New York, London, Sydney.
- 4.5. Neilands, J. B. and Strong, F. M., **1948**. *The Enzymatic Liberation of Pantothenic Acid*, Arch. of Biochem., Vol. 19, pp. 287-291.
- 4.6. Rhody, D., Raltech Scientific Services Analytical Method of Analysis MP-PANNMA, Madison, Wisconsin, 6/15/79.
- 4.7. ISO-20639:2015 Pantothenic acid (Vit B₅) by UHPLC-MS/MS