Guide to Method Format

(Method shown is incomplete to allow space for description.)

Locator number identifies a method by chapter, subchapter, and sequence within the subchapter for ease of location. 50 = Chapter 50; 2 = subchapter 2; 10 = 10th method found in chapter 50, subchapter 2. The locator number is not a permanent number and is included only for convenient accessibility.

Applicability statement specifies utility and limitations on scope of method and other pertinent information.

Cautionary statement specifies precautions and possible hazards in carrying out method, including safety information on equipment, techniques and practices, and safe handling of chemicals, acids, alkalies, microorganisms, and solvents.

Calculation symbols are defined and show correct units.

Method may include **Chemical Abstracts Service (CAS) registry numbers**, unique identifiers used to search a number of data-retrieval systems.

50.2.10

AOAC Official Method 2016.05

Analysis of Vitamin D_2 and Vitamin D_3 in Fortified Milk Powders, Infant Formulas, and Adult/Pediatric Nutritional Formulas

Liquid Chromatography–Tandem Mass Spectrometry

First Action 2016

Final Action 2017

AOAC–ISO Method*

(Applicable to the determination of total vitamin D_2 and vitamin D_3 in fortified milk powders, infant formulas, and adult/pediatric nutritional formulas.)

Caution: Refer to the Material Safety Data Sheets for all chemicals prior to use. Use all appropriate personal protective equipment and follow good laboratory practices.

A. Principle

Samples are saponified at high temperature, and then lipid-soluble components are extracted into isooctane. A portion of the isooctane layer is transferred and washed, and an aliquot of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) is added to derivatize vitamin D to form a high-molecular-mass, easily ionizable adduct.

B. Apparatus

(a) *Ultra-high-performance LC (UHPLC) system*.—Nexera (Shimadzu, Kyoto, Japan) or equivalent LC system, consisting of a dual pump system, sample injector unit, degasser unit, and column oven.

C. Reagents

(a) *Vitamin D_2 (ergocalciferol)*.—CAS No. 50-14-6, purity: ≥99%.

D. Reagent Preparation

(a) *PTAD solution (10 mg/mL)*.—To a 5 mL volumetric flask, add 50 mg PTAD, then add 4 mL acetone, and dissolve; dilute to volume with acetone. Expiry: 1 day.

E. Standard Preparation

Vitamin D is sensitive to light. Perform all steps under UV-shielded lighting. If vitamin D_3 is exclusively required for analysis, then standards pertaining to vitamin D_2 need not be used and vice versa.

J. Calculations

(a) *Concentration of SIL vitamin D_2 in $SILD_2SS$.*—

$$SILD_2SS_{D2concn} = \frac{SILD_2SS_{abs(\lambda max)}}{E^{1\%}_{1\,cm}} \times 10000$$

where $SILD_2SS_{D2concn}$ = concentration of *d6*-vitamin D_2 in the stock standard (μg/mL); $SILD_2SS_{abs(\lambda\,max)}$ = UV absorbance of the stock standard at 265 nm (cm^{-1}); $E^{1\%}_{1\,cm}$ = extinction coefficient for vitamin D_2 in ethanol (461 dL/g·cm); and 10 000 = concentration conversion factor (g/dL to μg/mL).

References: *J. AOAC Int*. **99**, 1321(2016)
DOI: 10.5740/jaoacint.16-0160 (First Action)

J. AOAC Int. **101**, 256(2018)
DOI: 10.5740/jaoacint.17-0149 (Final Action)

J. AOAC Int. **95**, 292(2012) (AOAC SMPR 2011.004)
DOI: 10.5740/jaoac.int.11-0440

CAS-50-14-6 (ergocalciferol)

Revised: January 2019

Method number is a permanent codification that identifies a method by year and sequence of adoption. 2016 = First Action 2016; .05 = 5th method adopted in 2016.

Title typically includes analyte and matrix, technique, and official status (First Action, Final Action, Revised First Action, Repealed). May include adoption by other organizations.

Methods are divided into several **descriptive sections**, including specifications for necessary laboratory apparatus and reagent preparations. *See also Definition of Terms and Explanatory Notes*.

References direct the user to published validation studies (single- and/or multilaboratory), subsequent revisions, and relevant SMPRs®. Other informative references may be included.

Revision date at the end of method signifies that the method received minor editorial revisions (that do not affect method procedures).