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**NATIONAL STANDARD OF THE  
PEOPLE'S REPUBLIC OF CHINA**

**GB/T 17818-2025**

Replacing GB/T 17818-2010

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**Determination of vitamin D3 in feeds - High-performance liquid  
chromatography**

饲料中维生素D3的测定 高效液相色谱法

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## Table of Contents

Foreword

1 Scope

2 Normative references

3 Terms and definitions

4 Method 1.Saponification Extraction

4.1 Principle

4.2 Reagents or materials

### Foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for standardization work Part 1: Structure and drafting rules for standardization documents" Drafting.

This document replaces GB/T 17818-2010 "Determination of vitamin D3 in feed by high performance liquid chromatography".

Compared with 2010, in addition to structural adjustments and editorial changes, the main technical changes are as follows.

- a) The scope of application has been changed, the concentrate supplement has been added, and the quantitative limit of the "first method saponification extraction method" has been changed (see Chapter 1, 2010 Chapter 1 of the 2001 edition);
- b) The quantification limit of the "second method direct extraction method" has been changed (see Chapter 1, Chapter 1 of the 2010 edition);
- c) Added the "first method saponification extraction method" vitamin D3 standard series solution (see 4.2.21);
- d) The sample preparation method for compound feed, concentrate supplement and concentrated feed in the "First Method of Saponification Extraction" has been changed to Pass through a 1mm sieve (see 4.4, 3.5 of the 2010 edition);
- e) Changed the extractant of "First Method Saponification Extraction Method" to "petroleum ether" (boiling range 30°C~60°C) (see 4.5.1.2.1, 2010 Edition) 3.6.1.2);
- f) Added off-line solid phase extraction method (see 4.5.1.2.2) and online solid phase extraction method (see 4.5.1.2.3);
- g) Added liquid chromatography reference conditions (see 4.5.2.2, 4.5.2.3);

- h) Added qualitative detection methods and added multi-point calibration for quantitative detection (see 4.5.2.5, 4.5.2.6);
- i) Deleted the HPLC purification column purification (see 3.6.1.4 of the 2010 edition);
- j) Deleted the HPLC purification conditions (see 3.6.2.1 of the 2010 edition);
- k) Normal phase chromatography was deleted (see 3.6.2.2.1 of the 2010 edition);
- l) Added precision requirements for vitamin D3 content in the range of 100 IU/kg to 1000 IU/kg (see 4.7, 2010 edition) 3.6.2.5);
- m) The "Second Method Direct Extraction Method" has been changed. The water bath temperature should not exceed 65°C (see 5.5.1, 4.6.1 of the 2010 edition);
- n) Added qualitative detection method (see 5.5.2.3).

Please note that some of the contents of this document may involve patents. The issuing organization of this document does not assume the responsibility for identifying patents.

This document was proposed and coordinated by the National Feed Industry Standardization Technical Committee (SAC/TC76).

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The previous versions of this document and the documents it replaces are as follows.

- First published in 1999 as GB/T 17818-1999, first revised in 2010;
- This is the second revision.

Determination of Vitamin D3 in Feed High performance liquid chromatography

## 1 Scope

This document describes a method for the determination of vitamin D3 in feeds by HPLC.

The "first method of saponification extraction" in this document is applicable to vitamins in compound feeds, concentrate supplements, concentrated feeds, and compound premixed feeds.

Determination of vitamin D3. "The second method direct extraction method" is suitable for the determination of vitamin D3 in vitamin premix feed.

The first limit of measurement in this document is 100 IU/kg, and the second limit of measurement is  $2.00 \times 10^5$  IU/kg.

## 2 Normative references

GB/T 6682

GB/T 20195

## 3 Terms and definitions

There are no terms or definitions that require definition in this document.

## 4 Method 1. Saponification Extraction

Note. The glass surface of the separatory funnel piston is not oiled, the handling process is carried out under light protection; the extraction process is carried out in a fume hood.

### 4.1 Principle

The sample was saponified with potassium hydroxide ethanol solution, purified and concentrated by liquid-liquid extraction or solid phase extraction, and separated by two-dimensional chromatography system.

The compound premixed feed can also be directly separated by reverse phase chromatography column, detected by UV detector and quantified by external standard method.

### 4.2 Reagents or materials

Unless otherwise specified, only analytical grade reagents were used.

4.2.1 Water. GB/T 6682, Grade 1.

4.2.2 Anhydrous ethanol. chromatographic grade. 4.2.3 Anhydrous ethanol.

4.2.4 Petroleum ether (boiling range  $30^{\circ}\text{C} \sim 60^{\circ}\text{C}$ ).

4.2.5 Methanol. chromatographic grade.

4.2.6 Acetonitrile. chromatographic grade.

4.2.7 Formic acid. chromatographically pure.

4.2.8 Isopropanol. chromatographic grade.