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

GB/T 47386-2026

**Determination of brassinolide in fertilizers - High performance
liquid chromatography**

肥料中芸苔素内酯的测定 高效液相色谱法

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1. Structure and Drafting Rules of Standardization Documents". Drafting. Please note that some content in this document may involve patents. The issuing organization of this document assumes no responsibility for identifying patents. This document was proposed by the China Petroleum and Chemical Industry Federation. This document is under the jurisdiction of the National Technical Committee on Standardization of Fertilizers and Soil Conditioners (SAC/TC105). This document was drafted by: Shandong Provincial Institute of Product Quality Inspection and Testing, Shanghai Chemical Industry Research Institute Testing Co., Ltd., and the Institute of Eco-Environmental Sciences, Chinese Academy of Sciences. Research Center, Shandong Pengbo Biotechnology Co., Ltd., Shikefeng Chemical Co., Ltd., Guizhou Provincial Institute of Product Quality Inspection and Testing, Yunnan Provincial Chemical Industry Research Institute Industrial Product Quality Supervision and Inspection Station, Heilongjiang Provincial Institute of Quality Supervision and Inspection, Woda Agricultural Technology Co., Ltd., Shandong Provincial Fertilizer and Coal Chemical Industry Association, Guangdong Academy of Sciences Institute of Ecology, Environment and Soil, Sichuan Runer Technology Co., Ltd., Shanghai Chemical Industry Research Institute Co., Ltd. company. The main drafters of this document are. Zhang Juan, Sun Shengmin, Fan Xiukai, Duan Guilan, Zhu Hairong, Wang Xinhui, Zhang Xiaoying, Han

Chao, Shao Qingsong, and Chen Xuehang. Liu Shuang, Gui Suping, Xie Xiaomei, Meng Fancheng, Wu Fan, Sun Wendan, Zhang Shujuan, Yan Kun, Yin Yue, Liu Chuanping, Wang Jun. Determination of brassinolide in fertilizers High performance liquid chromatography Warning

---Personnel using this document should have practical experience working in a formal laboratory. This document does not address all possible safety issues. Users are responsible for taking appropriate safety and health measures and ensuring compliance with relevant national regulations.

1.Scope This document describes high-performance liquid chromatography-ultraviolet (HPLC-UV) and high-performance liquid chromatography-electrospray ionization (HPLC-ESI) methods for detecting brassinolide in fertilizers. This document applies to the determination of 24-epibrassinolide, 28-homobrassinolide, and 28-epihomobrassinolide in fertilizers.

3.Terms and Definitions This document does not contain any terms or definitions that need to be defined.

4.Sample Preparation Solid samples. Crush the sample and pass it entirely through a 0.50mm metal sieve (for moist samples, it can pass through a 1.00mm metal sieve). Sift, mix well, and set aside. Liquid samples. Weigh directly after mixing.

1 Scope

GB/T 47386-2026 is the Chinese national standard covering brassinolide in a fertilizer - a plant hormone active at very low concentrations, added to some products and claimed in many, which makes an analytical method the only way to check the label. First edition, in force since 1 November 2026. It was issued on 30 April 2026 and takes effect on 1 November 2026, as a first edition. The document is under the responsibility of the China Petroleum and Chemical Industry Federation. This page is published from the official record of the 2026 edition; the clause text of a standard this recent is not yet in circulation, and the figures, limits and tables it contains are those of the document itself, delivered in full with the English translation.

5.1.1 Principle 24-epibrassinolide, 28-homobrassinolide, and 28-epiborabrinolide were extracted from the sample using methanol as the solvent, and then treated with phenylboronic acid. The methanol solution was derivatized at room temperature for 30 min, and the reaction was detected by liquid chromatography-ultraviolet detection (or diode array detection) under the selected operating conditions. The determination was performed using a device (e.g., a measuring instrument), with retention time for qualitative analysis and external standard method for quantitative analysis.

2 High Performance Liquid Chromatography-Electro-Fogging Detection

5.2.1 Principle 24-epibrassinolide, 28-homobrassinolide, and 28-epibrassinolide lactone were extracted from the sample using methanol as the solvent. In selected... Under operating conditions, the determination was performed using a liquid chromatograph with an electrospray detector, with retention time used for qualitative analysis and external standard method for quantitative analysis.

5.1.2 Reagents or materials

5.1.2.1 Unless otherwise specified, only reagents confirmed to be of analytical grade and Grade I water as specified in GB/T 6682 shall be used in the analysis.

5.1.2.2 Methanol. chromatographic grade.

5.1.2.3 Acetonitrile. chromatographic grade.

5.1.2.4 Benzylboronic acid. purity $\geq 98.0\%$.

5.1.2.5 Phenylboronic acid methanol solution (

1.0 g/L). Accurately weigh

1.0 g of phenylboronic acid into a 1 L volumetric flask, dissolve it in methanol and dilute to the mark. 5.1.2.6 24-Epibrassinolide Standard (CAS No.. 78821-43-9). Purity $\geq 95.0\%$.

5.1.2.7 28-Homobrassinolide Standard (CAS No.. 82373-95-3). Purity $\geq 96.0\%$. 5.1.2.8 28-Epoxybrassinolide Standard (CAS No.. 80483-89-2). Purity $\geq 95.0\%$.

5.1.2.9 Mixed Standard Stock Solution (1000 $\mu\text{g/mL}$). Accurately weigh 24-epibrassinolide, 28-homobrassinolide, and 28-epibrassinolide... Dissolve

0.01 g (accurate to 0.0001

g) of each lactone standard in a 10 mL volumetric flask, dilute to the mark with methanol, shake well, and let stand. Store at -18°C away from light. Shelf life is 30 days.

5.1.2.10 Mixed Standard Solution (100 $\mu\text{g/mL}$). Transfer 1 mL of 1000 $\mu\text{g/mL}$ mixed standard stock solution (5.1.2.9) into a 10 mL... In a volumetric flask, dilute to the mark with methanol and shake well. Prepare fresh before use.

5.1.3 Instruments and Equipment

5.1.3.1 Commonly used laboratory instruments and equipment.

5.1.3.2 High performance liquid chromatograph, equipped with an ultraviolet detector or a diode array detector.

5.1.3.3 Electronic balance, accuracy 0.0001g.

5.1.3.4 Ultrasonic cleaner.

5.1.3.5 Vortex mixer.

5.1.3.6 Organic phase microporous filter membrane, pore size 0.45 μ m, nylon microporous filter membrane or equivalent.

5.1.4 Test Procedure

5.1.4.1 Preparation of test solution Weigh 0.5g to 2.0g (accurate to 0.0001g) of the sample into a 50mL plastic centrifuge tube, and accurately add 10mL of phenylboronic acid methanol solution. Add 10 mL of liquid to 10 mL of methanol, vortex for 1 min, sonicate at room temperature for 10 min, allow to stand for 30 min, transfer to a 25 mL volumetric flask, and dilute with methanol. Volume up to the mark, shake well, take 1 mL of supernatant and filter through a 0.45 μ m organic phase microporous membrane, ready for analysis.

5.1.4.2 Measurement 5.1.4.2.1 Chromatographic Reference Conditions The chromatographic reference conditions are as follows:

a) Chromatographic column. 100mm x 4.6mm (inner diameter), stainless steel column, packed with 2.7 μ m C18 material. Or a chromatographic column with equivalent performance. Spectral column.

b) Mobile phase. V(acetonitrile).V(water) = 85.15.

0.8 mL/min.

d) Column temperature. 30°C.

e) Detection wavelength. 222nm.

f) Injection volume. 5 μ L. 5.1.4.2.2 Plotting the Standard Curve Transfer

0.25 mL,

0.50 mL,

5.00 mL of the mixed standard solution (5.1.2.10) into 25 mL volumetric flasks, respectively. Add 10 mL of phenylboronic acid methanol solution to the solution, dilute to volume with methanol, shake well, and allow to stand for 30 min to prepare a solution with a mass concentration of 1.0 μ g/mL. Standard working solutions of 2.0 μ g/mL, 5.0 μ g/mL, 10.0 μ g/mL and 20.0 μ g/mL were analyzed under the chromatographic conditions described in 5.1.4.2.1. A standard working curve was plotted with the peak area of the chromatographic solution as the ordinate and the corresponding concentration of the standard working solution as the abscissa. (Brassinolide standard) A typical chromatogram of the quasi-working solution obtained by high performance liquid chromatography-ultraviolet detection is shown in Figure A.1 of Appendix A. 5.1.4.2.3 Measurement Take the sample