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## Determination of flavonol glycosides in epimedium extract as a feed additive - High performance liquid chromatography

饲料添加剂淫羊藿提取物中黄酮醇苷的测定 高效液相色谱法

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# Table of Contents





## 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. 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). This document was drafted by: Beijing Institute of Animal Husbandry and Veterinary Medicine, Chinese Academy of Agricultural Sciences, Institute of Medicinal Plants, Chinese Academy of Medical Sciences, Tianjin Institute of Feder Technology Co., Ltd., Hunan Agricultural University, Beijing Ailu Biotechnology Co., Ltd., and Feed Research Institute of Chinese Academy of Agricultural Sciences. The main drafters of this document are. Zhang Junmin, Zhang Huiyan, Zhao Qingyu, Tang Chaohua, Qin Yuchang, Guo Baolin, Deng Xuejuan, Zeng Jianguo, Yao Langqun, Zhang, Jingjing, Liu, Xiubin, Guo, Xiaoqing, and Gu, Xu. Feed Additive Epimedium Extract Determination of flavonol glycosides by HPLC

## 1 Scope

China's national method for determining flavonol glycosides in epimedium extract used as a feed additive, by high performance liquid chromatography. Epimedium is a traditional medicinal plant whose extracts have moved into animal nutrition as a phytogenic additive, used for immune and reproductive effects in place of the antibiotic growth promoters that China has progressively banned. The active constituents are the flavonol glycosides - icariin principally - and the extract is bought and dosed on their content, which is what makes the measurement commercially central. It is also where adulteration happens: the marker compound can be added synthetically to an otherwise weak extract, and an extract sold on total flavonoid content can meet that figure with compounds from a cheaper plant. Chromatographic separation of the individual glycosides is what distinguishes a genuine

extract from a made-up one.

This document describes a HPLC method for the determination of flavonol glycosides in the feed additive Epimedium brevicornum extract. This document applies to the five flavonol glycosides (icariin, epimedin A, epimedin B, epimedin C) in the feed additive epimedin extract. and Baohuoside I). In this document, the limits of quantification for icariin, epimedin A, epimedin B, epimedin C and baucariside I are all

0.2 g/kg.

## 2 Normative references

The contents of the following documents constitute the essential clauses of this document through normative references in this document. For referenced documents without a date, only the version corresponding to that date applies to this document; for referenced documents without a date, the latest version (including all amendments) applies to This document.

GB/T 6682 Specifications and test methods for water used in analytical laboratories

GB/T 20195 Preparation of animal feed samples

## 3 Terms and definitions

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

## 4 Principle

The flavonol glycosides in the samples were extracted with methanol solution and determined by high performance liquid chromatography using the external standard method.

## 5 Reagents or materials

Unless otherwise specified, only analytical grade reagents were used.

5.1 Water. GB/T 6682, Grade 1.

5.2 Methanol. chromatographic grade.

5.3 Acetonitrile. chromatographic grade. 5.4 70% methanol solution. Measure 700 mL of methanol (5.2) into a 1000 mL volumetric flask, add water to make up to volume, and mix well.

5.5 Standard stock solution (2 mg/mL). Accurately weigh icariin (CAS. 489-32-7, purity >= 96%), epimedin A (CAS. 110623-72-8, purity >=95%), epimedin B (CAS. 110623-73-9, purity >=95%), epimedin C (CAS. 110642-44-9, purity 20 mg each (accurate to

0.01 mg) of baohuoside I (CAS. 113558-15-9, purity >= 95%) were placed in 10 mL brown Dissolve in methanol (5.2) in a volumetric flask and make up to volume, mix well. Store below -18°C, valid for 3 months.

5.6 Mixed standard intermediate solution (200 mg/L). Accurately pipette 1 mL of each standard stock solution (5.5) into a 10 mL brown volumetric flask. Add 70% methanol solution (5.4) to the volume and mix well. Store at 2°C~10°C, valid for 3 months.

5.7 Mixed standard series solution. Accurately pipette an appropriate amount of mixed standard intermediate solution (5.6) and prepare it with 70% methanol solution (5.4) to a mass concentration of