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Determination of poplar tree gum in propolis-Reversed-phase high performance liquid chromatography method

蜂胶中杨树胶的检测方法 反相高高效液相色谱法

(English Translation)

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Foreword

This Standard is prepared according to GB/T 1.1-2009 "Directives for Standardization - Part 1. Rules For the Structure and Drafting of Standards".

This products uses patent 201010180675.0 (application number) .

This Standard was proposed by Zhejiang University.

This product shall be under the jurisdiction of China Federation of Supply and Marketing Cooperatives.

Drafting organizations of this Standard. Zhejiang University, and China Beer Products Association.

Main drafters of this Standard. Hu Fuliang, Zhang Cuiping, Zheng Huoqing, and Lv Zetian.

Introduction

Issuing organization of this Document draws attention to that - when declaring compliance with this Document, it involves the uses of patent application related to this Document (application number. 201010180657.0).

Issuing organization of this Document has no standpoint for authenticity, validity and scope of this patent application.

The patent applicant has assured the issuing organization of this Document that he is willing to negotiate with any applicant for the relevant license authorization under reasonable and non-discriminatory terms and conditions.

Statements of the patent applicant have been put on records at issuing organization of this Document. Relevant information can be obtained via following contact information.

Patent applicant. Zhejiang University.

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Please note that except above-mentioned patent application, certain content of this Document may also involve patent [Translator note. other patents]. The issuing organization of this Document does not undertake the responsibility for identification of these patents.

Determination of Poplar Tree Gum in Propolis -Reversed-phase High Performance Liquid Chromatography Method

1 Scope

This Standard specifies determination method of reversed-phase high performance liquid chromatography on whether propolis contains poplar tree gum.

This Standard applies to the determination of poplar tree gum in propolis raw materials and in propolis ethanol extract.

In this Standard, when injection volume is 5 μL, minimum detection limit of salicin is 1.3 μg/mL; lower detection limit of poplar tree gum in propolis raw material is about 5%; lower detection limit of ethanol in extract of propolis is about 15%.

2 Normative references

Following documents are indispensable for application of this Document. For the dated documents so quoted, only the dated versions apply to this document. For the undated documents so quoted, the latest versions (including all corrigendum) apply to this Document.

GB/T 6682-2008 Water for analytical laboratory use - Specification and test methods GB/T 24282-2009 Propolis

3 Terms and definitions

Following terms and definitions apply to this document.

Propolis

Glutinous substance formed by mixing of resin secretions, etc. collected by worker bees and its palate gland and wax gland secretions, etc.

Salicin content (X) in test sample is expressed in milligrams per gram (mg/g) and calculated according to Formula (1).

Where.

Cs - Concentration of salicin standard solution, in milligrams per milliliter (mg/mL);

V - Final constant volume of sample, in milliliters (mL);

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A - Corresponding peak height or peak area of sample solution;

m - Sample mass, in grams (g);

As - Corresponding peak height or peak area of salicin standard solution.

Take arithmetic average value of 2 independent measured values that meet repeatability requirements AS the determination result (mg/g). Maintain 2 significant figures. Relative deviation of parallel test must not exceed 2.0%.

8.5 Results determination

Salicin and CCP measured in this Standard are substances that are contained in Poplar tree gum but not in propolis. If salicin is undetectable in test sample or salicin detected concentration in ethanol extracted propolis is less than 0.25mg/g, or the salicin concentration detected in propolis raw material sample is less than 0.15mg/g or CCP is undetectable, then this sample is the sample in which the poplar gum tree is undetectable. If salicin concentration detected in ethanol extracted propolis is greater than 0.25mg/g or salicin concentration detected in propolis raw material sample is greater than 0.15mg/g, then, regardless of whether CCP is detectable, this sample is determined to be the sample in which poplar tree gum is detectable. If salicin is undetectable in test sample while CCP is detectable, then use liquid chromatography tandem mass spectrometry for further verification (detection conditions of liquid chromatogram and are shown in Annex C). If CCP is determined, then this sample is determined to be the sample in which the poplar tree gum is detectable.

Annex C

(Informative Annex)

CCP Determination Conditions Determined by Liquid Chromatography Tandem Mass Spectrometry C.1 Liquid chromatography C.1.1 Chromatographic column. Sepax HP-C18 (150 mmx4.6mm, 5 μm).

C.1.2 Mobile phase. Acetonitrile. 0.2% formic acid= 5.95.

C.1.3 Column temperature. 30°C.

C.1.4 Injection volume. 100μL.

C.1.4 Flow rate. 1 ml/min.

C.2 Mass spectrum conditions C.2.1 Scan mode. negative ion sweeping.

C.2.2 Drying gas flow rate. 12.0L/min.

C2.3 Nebulizer pressure. 344.7 kPa (50 psi).

C2.4 Drying temperature. 350 °C.

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C.2.5 Capillary voltage. positive ion 4 000 V; negative ion 3 500 V.

C2.6 Lysis voltage. 100 V.

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