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BSI Standards Publication

Animal feeding stuffs: Methods of sampling and analysis — Determination of theobromine in feed materials and compound feed, including cocoa derived ingredients, by liquid chromatography

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National foreword

This British Standard is the UK implementation of EN 17270:2019.

The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Compliance with a British Standard cannot confer immunity from legal obligations.

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Amendments/corrigenda issued since publication

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English Version

Animal feeding stuffs: Methods of sampling and analysis - Determination of theobromine in feed materials and compound feed, including cocoa derived ingredients, by liquid chromatography

Aliments des animaux : Méthodes d'échantillonnage et d'analyse - Détermination par chromatographie en phase liquide de la teneur en théobromine dans les matières premières destinées aux aliments des animaux et dans les aliments composés pour animaux, y compris les ingrédients issus du cacao

Futtermittel: Probenahme- und Untersuchungsverfahren - Bestimmung von Theobromin in Einzelfuttermitteln, vor allem aus Kakao gewonnen sowie in Mischfuttermitteln mittels Flüssigchromatographie

This European Standard was approved by CEN on 28 July 2019.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (EN 17270:2019) has been prepared by Technical Committee CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis", the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by April 2020, and conflicting national standards shall be withdrawn at the latest by April 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a standardization request given to CEN by the European Commission and the European Free Trade Association.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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Introduction

Theobromine is naturally present in the cacao tree and its seeds and consequently in cocoa product and by-products. Cocoa bean shells, cocoa bean meal, cocoa germs and discarded confectionery are used for feed purposes in Europe. Maximum levels of theobromine in feeding stuffs are controlled by EU regulations.

WARNING — the use of this protocol involves hazardous materials, operations and equipment. This protocol does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this protocol to establish appropriate health and safety practices and determine the compatibility with regulatory limitations prior to use.

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1 Scope

This document specifies a test method for the determination of theobromine in feed material or compound feed in the working range 27 mg/kg to 307 mg/kg using liquid chromatography coupled to a UV detector (HPLC-UV) or in the working range 49 mg/kg to 307 mg/kg using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

This method has been fully validated using complementary compound feed for adult dogs and complementary compound feed for horses.

This method is also considered applicable for determining theobromine in baking chocolate using either HPLC-UV or LC-MS/MS systems.

The working range can be extended provided the extended range is validated.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

4 Principle

A test portion of finely ground and homogeneous material is defatted with hexane, an internal standard added and the theobromine extracted into ammonium acetate buffer. The extract is cleaned with the addition of Carrez reagents, filtered and the extract analysed by liquid chromatography with UV detection. Alternatively, the theobromine content can be determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) providing it can be demonstrated that there is no interference from the sample matrix.

5 Reagents

Use only reagents of recognized analytical grade unless otherwise specified. Commercially available solutions with equivalent properties to those listed may be used. References to products or vendors are for information only and do not preclude the use of products of similar quality from alternative suppliers.

For reagents specific to the analysis of the extracts by HPLC-UV see Annex A. For reagents specific to the analysis of the extracts by LC-MS/MS see Annex B.

WARNING — Dispose of waste solvents according to applicable environmental rules and regulations.

5.1 Ammonium acetate, analytical reagent grade

5.2 Glacial acetic acid, 99,5 %

5.3 Acetic acid, 1 mol/l