SLOVENSKI STANDARD
oSIST prEN 18003:2023

01-december-2023

Pristnost živil - Določanje vsebnosti 16-O-metilkafestola v zeleni in praženi kavi - Metoda HPLC

Food Authenticity — Determination of 16-O-methylcafestol content of green and roasted coffee — HPLC-method

Lebensmittelauthentizität - Bestimmung des Gehaltes an 16-O-Methylcafestol in Roh- und Röstkaffee - HPLC-Verfahren

Authenticité des aliments - Détermination de la teneur en 16-O-méthylcafestol du café vert et torréfié - Méthode CLHP

Ta slovenski standard je istoveten z: **prEN 18003**

ICS:

67.140.20 Kava in kavni nadomestki Coffee and coffee substitutes

oSIST prEN 18003:2023 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 18003

August 2023

ICS

English Version

Food Authenticity - Determination of 16-O-methylcafestol content of green and roasted coffee - HPLC-method

Authenticité alimentaire du café et des produits á base de café ? Détermination de la teneur en 16-O-méthylcafestol dans le café vert et le café torréfié ? Méthode par HPLC

Lebensmittelauthentizität - Bestimmung des Gehaltes an 16-O-Methylcafestol in Roh- und Röstkaffee - HPLC-Verfahren

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Ref. No. prEN 18003:2023 E

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European foreword

This document (prEN 18003:2023) has been prepared by Technical Committee CEN/TC 460 “Food authenticity”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

Introduction

This document was developed in response to demand for an efficient and reliable test method allowing the confirmation of coffee authenticity both for commercial quality control and for official food control.

The coffee species with the greatest commercial importance are *Coffea arabica* and *Coffea canephora var. robusta*, commonly known as “arabica” and “robusta”. Within these species, arabica coffees have a significantly higher market value than robusta coffees. In unblended coffee the botanical origin of coffee can be determined unambiguously by analysing the concentration of 16-O-methylcafestol because this marker substance is present in arabica coffee at very low concentrations (typically < 20 mg/kg). In robusta coffees the concentration levels are significantly higher in the approximate range of 800 to 2 500 mg/kg.

NOTE These published values are quoted as an orientation only and not intended as strict threshold recommendations.

1 Scope

This document specifies a high-performance liquid chromatography (HPLC) method for determining the 16-O-methylcafestol content in green and roasted coffee.

The method described is suitable with a reasonable precision for a content of 40 mg to 1 600 mg of 16-O-methylcafestol per kg of green and roasted coffee respectively. The collaborative study has shown that concentrations also between 20 to 40 mg/kg can be successfully analysed depending on the laboratory equipment.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3310-1, *Test sieves — Technical requirements and testing — Part 1: Test sieves of metal wire cloth*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply

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

— ISO Online browsing platform: available at https://www.iso.org/obp/

— IEC Electropedia: available at https://www.electropedia.org/

3.1
16-*O*-methylcafestol content
concentration of 16-O-methylcafestol in green coffee or roasted coffee, expressed in mg per kg

4 Principle

An aliquot of the ground and well-mixed sample is hydrolysed using ethanolic potassium hydroxide solution. Then, repeated shaking with *tert*-butylmethylether (*t*BME) isolates the unhydrolysable portion. The combined ether-extract phases are reduced to dryness after being washed. The residue is taken up in HPLC mobile phase, and the 16-*O*-methylcafestol content is examined by HPLC.

The substance is identified by comparison of its retention time with that of the standard substance, and quantification is carried out by the external standard method based on peak areas.

5 Reagents

Unless otherwise stated, analytical grade reagents shall be used. The water used shall be of quality grade 1, as described in ISO 3696.

5.1 Acetonitrile, HPLC grade.

5.2 L(+)-Ascorbic acid, 99,5 %, suitable for analytical purposes.

5.3 Ethanol, HPLC grade.

5.4 Mobile phase, acetonitrile/aqua bidest or methanol/aqua bidest (see 8.4.5).

NOTE The mixing ratio may be adjusted depending on the analysis system used.

5.5 Potassium hydroxide pellets.

5.6 Ethanolic potassium hydroxide solution, prepared by dissolving 10 g of potassium hydroxide in 10 ml of aqua bidest and making up to 100 ml with ethanol; the solution shall always be freshly prepared and may be used for a maximum of two days.

5.7 Methanol, HPLC grade.

5.8 Sodium chloride.

5.9 Sodium chloride solution, prepared by dissolving 2 g of sodium chloride in a 100 ml volumetric flask filled with water and making up to the calibration mark.

5.10 tert-Butylmethylether (tBME), HPLC grade.

5.11 liquid nitrogen or dry ice (optional), for cooling.

5.12 Standard 16-O-methylcafestol solutions.

5.12.1 Standard substance

A 16-*O*-methylcafestol standard shall be used that has a minimum purity of 95 %.

5.12.2 Stock solution

Weigh about 10 mg of 16-*O*-methylcafestol into a 50 ml volumetric flask (approximately 200 µg/ml). Dissolve the substance in acetonitrile (5.1) using an ultrasonic bath, and then make up the flask to the mark with acetonitrile (5.1).

NOTE The stock solution will remain stable for at least 12 weeks if refrigerated.

5.12.3 Calibration solutions for high calibration range (160 mg/kg to 1 600 mg/kg)

The stock solution (5.11.2) is diluted with acetonitrile (5.1) to produce five different dilute solutions for creating the five-point calibration and calculating the 16-*O*-methylcafestol content, shown in Table 1.

NOTE The calibration solutions will remain stable for at least three weeks if refrigerated or stored at ambient temperature (20 °C) away from direct sunlight.

Table 1 — Standard solutions for high calibration range (160 mg/kg to 1 600 mg/kg)

Standard solution	$V_{16\text{-}OMC}$ ml	$C_{16\text{-}OMC}$ µg/ml	$W_{16\text{-}OMC}$ mg/kg
H1	2,0	80	1 600
H2	1,5	60	1 200
H3	1,0	40	800
H4	0,5	20	400
H5	0,2	8	160

Pipette the specified volumes into a 5 ml volumetric flask and fill up with acetonitrile (5.1).

5.12.4 Calibration solutions for low calibration range (16 mg/kg to 120 mg/kg)

First the stock solution (5.12.2) is diluted with acetonitrile (5.1) 1:10 to produce a standard solution with 20 µg/ml (10 ml). Thereof prepare five different dilute solutions for creating the five-point calibration and calculating the 16-*O*-methylcafestol content, shown in Table 2.

Table 2 — Standard solutions for low calibration range (16 mg kg to 120 mg/kg)

Standard solution	$V_{16\text{-}OMC}$ ml	$C_{16\text{-}OMC}$ µg/ml	$W_{16\text{-}OMC}$ mg/kg
L1	1,5	6,0	120
L2	1,2	4,8	96
L3	0,8	3,2	64
L4	0,4	1,6	32
L5	0,2	0,8	16

Pipette the specified volumes into a 5 ml volumetric flask and fill up with acetonitrile (5.1).

If the content of the coffee measurement solution exceeds the highest value of the calibration curve, the measurement solution shall be diluted 1:5 or 1:10. The linearity of the calibration curves shall be checked individually.

6 Apparatus

Standard laboratory equipment shall be used, as well as the devices listed in 6.1 to 6.20.

6.1 Coffee mill, cold resistant and therefore suitable for grinding green coffee beans.

NOTE For example, cryogenic mills have been found to be suitable for grinding green coffee beans.

6.2 Mill, suitable for grinding roasted coffee beans.

NOTE For example, standard mills have been found to be suitable for grinding roasted coffee beans.

6.3 Sieves, with wire screens of mesh sizes 0,63 mm and 0,25 mm, which meet the requirements of ISO 3310-1.

6.4 Glycerine bath or heating block.

6.5 Heating plate with integrated agitator.

6.6 Screw capped tubes[1] **and caps for screw capped tubes**[2] (for hydrolysis).

6.7 Magnetic rod, triangular, 16 mm x 10 mm.

6.8 Heating device for stripping solvents[3].

[1] DURAN culture tubes (Z620262), 30 ml volume (180 mm × 18 mm, height × diameter) made of borosilicate glass are an example for commercially available screw capped tubes. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

[2] PBT cap for DURAN (2924011), temperature-stable up to 180 °C, size GL 18 is an example for commercially available caps for screw capped tubes. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

6.9 Ultrasonic bath, room temperature.

6.10 Graduated cylinder, 10 ml.

6.11 Volumetric flask, different sizes (5 ml, 10 ml, 50 ml, 100 ml).

6.12 Pasteur pipettes.

6.13 Screw top vials with cap, 60 ml volume (height x diameter 140 mm x 28 mm).

6.14 Round bottom flask, 50 ml.

6.15 Vacuum rotary evaporator or similar laboratory equipment.

6.16 Membrane filter, regenerated cellulose, pore size of 0,2 µm or similar membrane.

6.17 HPLC Vials 2 ml, clear glass or brown glass.

6.18 High-performance liquid chromatograph with UV detector, measurements at 224 nm, with evaluation system, preferably with a column oven.

6.19 Analytical column, Reversed Phase C_{18}[4].

6.20 vessel, stainless-steel, 600 ml.

7 Sampling

It is assumed that the sample will be representative.

8 Test method

8.1 General

WARNING — The use of this document can involve hazardous materials, operations and equipment. It does not purport to address all of the safety or environmental problems associated with its use.

The coffee sample (ground powder) shall be thoroughly homogenized to ensure the extracted sub-sample to be representative. Whole beans shall be thoroughly mixed, and then 200 g (or approximately 1 000 beans equally) shall be ground using a mill according to 6.1 or 6.2.

[3] Reacti-Therm™ by PIERCE is an example for commercially available stripping equipment. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

[4] Chromolith HR (100 mm × 4,6 mm, length × diameter), Phenomenex Luna 3 µm C18 (150 mm × 4,6 mm), LiChrospher 100 RP 18 (250 mm × 5mm), and Zorbax Eclipse XDB-C18 (150 mm × 3 mm), among others, were used in the collaborative study and are examples for commercially available Reversed Phase C_{18} HPLC columns. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.