SLOVENSKI STANDARD
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Kava in proizvodi iz kave - Določevanje kofeina z uporabo tekočinske kromatografije visoke ločljivosti (HPLC) - Referenčna metoda

Coffee and coffee products -- Determination of the caffeine content using high performance liquid chromatography (HPLC) -- Reference method

Café et dérivés du café -- Détermination de la teneur en caféine par chromatographie liquide à haute performance (CLHP) -- Méthode de référence

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Coffee and coffee products — Determination of the caffeine content using high performance liquid chromatography (HPLC) — Reference method

Café et dérivés du café — Détermination de la teneur en caféine par chromatographie liquide à haute performance (CLHP) — Méthode de référence

Reference number
ISO 20481:2008(E)

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 20481 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 15, *Coffee*.

This corrected version of ISO 20481:2008 incorporates the following corrections:

a) from Clause 2, ISO 565, ISO 648, and ISO 1042 have been moved to the bibliography, and the numbering of the bibliographic references and their citations adjusted accordingly throughout;

b) in 8.3, paragraph 1, the second mention of "8.2.1" has been deleted, and "8.2.2" inserted;

c) in 9.1, "w_x" (2 occurrences) has been deleted, and "w_c" inserted;

d) in 9.1 and 9.2, "A_c" (5 occurrences) has been deleted, and "A_{st}" inserted;

e) in 9.1 and 9.2, "A_x" (5 occurrences) has been deleted, and "A_s" inserted;

f) in 9.1 and 9.2, "ρ_c" (5 occurrences) has been deleted, and "ρ_{st}" inserted;

g) in 9.2, "w'_x" (2 occurrences) has been deleted, and "w'_c" inserted;

h) in 9.2, a comma has been inserted after w'_c;

i) in Table A.1, row 7 (Standard deviation of repeatability, s_r), column 13 (Soluble coffee, Regular agglomerated), "0,30" has been deleted, and "0,030" inserted.

Coffee and coffee products — Determination of the caffeine content using high performance liquid chromatography (HPLC) — Reference method

1 Scope

This International Standard specifies a high performance liquid chromatography (HPLC) method for the determination of the caffeine content of: green coffee; roasted coffee; soluble coffee, regular and decaffeinated; and mixed instant coffee products (e. g. coffee/chicory mix or cappuccino-type coffee drink).

2 Normative references

The following referenced documents are indispensable for the application 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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 3726, *Instant coffee — Determination of loss in mass at 70 °C under reduced pressure*

ISO 6673, *Green coffee — Determination of loss in mass at 105 °C*

ISO 11817, *Roasted ground coffee — Determination of moisture content — Karl Fischer method (Reference method)*

3 Principle

Caffeine is extracted from samples with water at 90 °C in the presence of magnesium oxide. After filtration, the caffeine content of the extract is determined by HPLC on a RP-18 column using isocratic elution with UV detection at approximately 272 nm.

Wherever appropriate, the caffeine content may be given on dry basis which requires a moisture determination by a suitable standard method.

4 Reagents

Unless otherwise specified, use only reagents of recognized analytical grade, and only water conforming to the requirements of ISO 3696, grade 1.

4.1 Methanol, HPLC grade.

4.2 Magnesium oxide (MgO), heavy, high grade [1)].

1) Merck 105867 is an example of a suitable, commercially available product. This information is given for the convenience of users of this International Standard and does not constitute an endorsement of this product by ISO.

NOTE The use of MgO is important for the lifetime of the analytical column, especially for green coffee. The amount of MgO used depends on the equipment and on the type of coffee product. The precision data in Annex A were acquired using the procedure described in this International Standard.

The appearance of interfering peaks in the chromatogram may be due to improper adsorption. In these cases, subject the MgO used to examination.

4.3 Caffeine (1,3,7-trimethylxanthine; 1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione; methyltheobromine; $C_8H_{10}N_4O_2$), pure anhydrous.

4.4 Mobile phase, 24 % volume fraction methanol in water.

SAFETY PRECAUTIONS — Wear gloves, eye protection and dispense reagents in a fume cupboard (fume hood).

Transfer 240 ml methanol (4.1) to a 1 l one-mark volumetric flask (5.11). Make up to the mark with water, mix and filter through a 0,45 µm filter (5.2).

NOTE By changing the ratio of methanol to water, the retention time of caffeine can be adjusted to optimize the HPLC separation on the column used.

4.5 Caffeine standard solutions.

4.5.1 Stock solution, corresponding to 200 mg/l.

Weigh (0,200 ± 0,001) g anhydrous caffeine (4.3) into a 1 l one-mark volumetric flask (5.11). Add sufficient warm water to half fill the flask. Swirl to dissolve the caffeine, cool to room temperature, make up to the mark with water and mix.

The solution is stable at +4 °C for one month. Store in a refrigerator.

4.5.2 Dilute standard solution for regular coffee, corresponding to approximately 40 mg/l.

Pipette (5.12) 50 ml of the caffeine standard stock solution (4.5.1) to a 250 ml one-mark volumetric flask (5.11). Make up to the mark with water and mix. Prepare fresh dilute standard solution daily.

4.5.3 Dilute standard solution for decaffeinated coffee, corresponding to approximately 4 mg/l.

Pipette (5.12) 5 ml of the caffeine standard stock solution (4.5.1) to a 250 ml one-mark volumetric flask (5.11). Make up to the mark with water and mix. Prepare fresh dilute standard solution daily.

4.5.4 Calibration plot. The use of a three to five point calibration plot is optional. Recommended concentration range is 5 mg/l to 25 mg/l for regular coffee and 0,5 mg/l to 2,5 mg/l for decaffeinated samples.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Analytical balance, capable of weighing to an accuracy of ± 0,1 mg.

5.2 Membrane filter units of pore size 0,45 µm, for filtration of mobile phases and diluted sample extracts.

5.3 High performance liquid chromatograph, equipped to perform an isocratic elution, with a UV detector set at 272 nm (270 nm to 280 nm) or a filter detector (254 nm), and with a data collection/integration system. The use of a degasser is optional.

5.4 Chromatographic column for HPLC, of minimum length 125 mm, packed with C18 material, preferentially 5 µm spherical particles, with an efficiency of separation of at least 5 000 theoretical plates. The

theoretical number of plates, N_{th}, of the column may be calculated by determining peak shape after injection of the caffeine standard solution by Equation (1):

$$N_{th} = \left(\frac{t_r}{b}\right)^2 \times 5{,}54 \quad (1)$$

where

t_r is the retention time, in seconds, of the peak;

b is the peak width, in seconds, at half peak height.

5.5 Magnetic stirrer, with heater and mounted water bath.

5.6 Ultrasonic bath.

5.7 Microlitre syringe.

5.8 Coffee mill, suitable for grinding roasted coffee beans.

5.9 Grinder with cogged wheel, with cooling jacket, or **analytic grinder**, with blades and cooling jacket, or any other device suitable for grinding green coffee beans.

5.10 Sieve, of nominal size of openings 630 µm, complying with the requirements of ISO 565[2], R20 series.

5.11 One-mark volumetric flasks, of capacities 1 l and 250 ml, complying with the requirements of ISO 1042[4], class A.

5.12 Pipettes, of capacities 50 ml and 5 ml, complying with the requirements of ISO 648[3], class A.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. The sampling procedure shall be subject to agreement by the interested parties.

Store the test sample in such a way that deterioration and change in its composition are prevented.

In case of mixed beverage powders, a minimum of 50 g of the product is required (for portion packages: a minimum of five portions).

7 Preparation of test samples

7.1 Green coffee

Grind (5.9) green coffee beans so that more than 50 % mass fraction of the sample passes through the sieve (5.10). Then take the test sample from the well-mixed total ground coffee.

7.2 Roasted coffee

Mill (5.8) roasted coffee beans so that more than 50 % mass fraction passes through the sieve (5.10).

Use roasted ground coffee in commercial packages without further treatment except homogenization.