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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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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.

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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.