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Coffee — Determination of caffeine content — Method using high-performance liquid chromatography

*Café — Détermination de la teneur en caféine — Méthode par
chromatographie liquide à haute performance*



Foreword

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International Standard ISO 10095 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 15, *Coffee*.

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Coffee — Determination of caffeine content — Method using high-performance liquid chromatography

1 Scope

This International Standard specifies a method for the determination of caffeine content by high-performance liquid chromatography (HPLC) of regular and decaffeinated green and roasted coffee beans and of regular and decaffeinated coffee extract powder.

NOTE 1 The reference method for determination of the caffeine content, using ultraviolet absorption spectrometry, is given in ISO 4052:1983, *Coffee — Determination of caffeine content (Reference method)*.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 1447:1978, *Green coffee — Determination of moisture content (Routine method)*.

ISO 3726:1983, *Instant coffee — Determination of loss in mass at 70 °C under reduced pressure*.

ISO 6673:1983, *Green coffee — Determination of loss in mass at 105 °C*.

3 Principle

Extraction of the caffeine from a test portion with water at 90 °C in the presence of magnesium oxide. Filtration and then purification of an aliquot portion

on a silica microcolumn modified with phenyl groups.

Determination of the caffeine content by HPLC with ultraviolet detection.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

4.1 Methanol, HPLC grade.

4.2 Ammonia solution (0,3 mol/l)/methanol, 90 + 10 mixture by volume.

4.3 Elution solvent, for purification column, methanol/water/acetic acid, 75 + 25 + 1 mixture by volume.

4.4 Mobile phase, methanol/water, 30 + 70 mixture by volume.

Put 600 ml of methanol (4.1) into a 2 l one-mark volumetric flask and make up to the mark with water. Mix and then filter the mixture through a filter of 0,45 µm pore size (5.7).

NOTE 2 By adjusting the methanol concentration, the retention time of caffeine can be modified so as to optimize the HPLC separation.

4.5 Ethanol/water, 1 + 4 solution by volume.

4.6 Magnesium oxide¹⁾.

4.7 Caffeine, stock solution, corresponding to 0,5 g of caffeine per litre.

Weigh, to the nearest 0,1 mg, 125 mg of caffeine into

1) Merck 5867 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.