INTERNATIONAL STANDARD

ISO 10727

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Tea and instant tea in solid form — Determination of caffeine content — Method using high-performance liquid chromatography

Thé et thé soluble sous forme solide — Détermination de la teneur en caféine — Méthode par chromatographie liquide à haute performance

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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 10727 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 8, *Tea*.

This second edition cancels and replaces the first edition (ISO 10727:1995), which has been technically revised.

Annex A of this International Standard is for information only.

Tea and instant tea in solid form — Determination of caffeine content — Method using high-performance liquid chromatography

1 Scope

This International Standard specifies a method for the determination by high-performance liquid chromatography (HPLC) of the caffeine content of teas and instant teas. It is applicable to green tea, black tea and decaffeinated tea products.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 1572:1980, *Tea — Preparation of ground sample of known dry matter content*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 7513:1990, *Instant tea in solid form — Determination of moisture content (loss in mass at 103 °C)*

3 Principle

The caffeine from a test portion is extracted by reflux with water in the presence of magnesium oxide. After filtration, the caffeine content is quantified using high-performance liquid chromatography with ultraviolet detection.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

4.1 Water, in accordance with grade 1 of ISO 3696.

4.2 Methanol, HPLC grade.

4.3 Mobile phase, methanol/water mixture.

Add 600 ml of the methanol (4.2) to a 2 litre one-mark volumetric flask. Dilute to the mark with water and mix. Filter the mixture through a filter of 0,45 μm pore size (5.3).

By adjusting the methanol concentration, the retention time of the caffeine can be modified to optimize the HPLC separation. This can also be done by increasing the column temperature, but a temperature of 60 °C should not be exceeded.

4.4 Ethanol/water mixture, 1/4 (volume fraction).

4.5 Magnesium oxide (so-called “dense magnesium oxide”).

NOTE Light magnesium oxide may result in inaccurate caffeine content values.

4.6 Caffeine stock standard solution, corresponding to 500 μg/ml.

Weigh, to the nearest 0,001 g, 0,125 g of caffeine into a 250 ml one-mark volumetric flask. Add sufficient ethanol/water mixture (4.4) to half fill the flask. Swirl to dissolve the caffeine and then dilute to the mark with the ethanol/water mixture.

This solution will remain stable for up to 1 month if stored in a refrigerator.

4.7 Caffeine standard solutions.

Standard solutions A, B, C and D shall be prepared on the day of use.

4.7.1 Caffeine standard solution A, corresponding to 15 μg/ml.

Allow the caffeine stock standard solution (4.6) to warm up to room temperature. Using a pipette, transfer 3,0 ml of this solution to a 100 ml one-mark volumetric flask. Dilute to the mark with water and mix.

4.7.2 Caffeine standard solution B, corresponding to 10 μg/ml.

Follow the procedure given in 4.7.1 but take, using a pipette, 2,0 ml of the stock standard solution (4.6).

4.7.3 Caffeine standard solution C, corresponding to 5 μg/ml.

Follow the procedure given in 4.7.1 but take, using a pipette, 1,0 ml of the stock standard solution (4.6).

4.7.4 Caffeine standard solution D, corresponding to 2 μg/ml.

Using a pipette, transfer 20 ml of the standard solution B (4.7.2) to a 100 ml one-mark volumetric flask. Dilute to the mark with water and mix.

5 Apparatus

Usual laboratory apparatus, and, in particular, the following.

5.1 High-performance liquid chromatograph, equipped with an ultraviolet detector, allowing measurements to be made at a wavelength between 254 nm and 280 nm, and a data collection/integration system or chart recorder.

NOTE A wavelength close to 280 nm is preferred since the maximum UV absorption of caffeine is at 272 nm.

Where diurnal variations in temperature are wide, a means of ensuring a constant column temperature should be provided, for example a column oven or water jacket.

5.2 Chromatographic column for HPLC, a reversed-phase C18 type, preferably with spherical particles and having an efficiency of at least 5 000 theoretical plates[1)]. The theoretical plate number N of a column can be calculated as follows, from the shape of the peak obtained by injection of one of the caffeine standard solutions (4.7):

$$N = 5{,}54 \left(\frac{t}{W_{0,5}} \right)^2$$

1) Spherisorb 5 ODS, Spherisorb 10 ODS, Nucleosil 5 C18, Nucleosil 7 C18, Nucleosil 10 C18, Zorbax BP C18, Hypersil ODS, CP-Sper C18, Bondapak C18, Supelcosil L C18 and Partisphere C18 are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.