INTERNATIONAL STANDARD

ISO 10727

First edition
1995-01-15

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

Reference number
ISO 10727:1995(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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.

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

Annexes A and B of this International Standard are for information only.

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Printed in Switzerland

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 of the caffeine content by high-performance liquid chromatography (HPLC) of teas and instant teas with a caffeine content of greater than 0,3 % (*m/m*).

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 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

Extraction of the caffeine from a test portion with water at 90 °C in the presence of magnesium oxide. Filtration followed by determination of the caffeine content by high-performance liquid chromatography with ultraviolet detection.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and water in accordance with grade 1 of ISO 3696.

4.1 Methanol, HPLC grade.

4.2 Mobile phase, methanol/water mixture.

Add 600 ml of the methanol (4.1) to a 2 litre one-mark volumetric flask. Dilute to the mark with water, mix, and filter the mixture through a filter of 0,45 µm pore size (5.3).

NOTE 1 By adjusting the methanol concentration, the retention time of the caffeine can be modified so as 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.

4.3 Ethanol/water mixture, 1/4 (*V*/*V*).

4.4 Magnesium oxide (so-called "dense magnesium oxide").

4.5 Caffeine stock solution, corresponding to 0,500 g of caffeine per litre.

Weigh, to the nearest 0,001 g, 0,125 g of caffeine into a 250 ml one-mark volumetric flask made of amber glass. Add sufficient ethanol/water mixture (4.3) to half-fill the flask. Swirl to dissolve the caffeine and then dilute to the mark with the ethanol/water mixture.

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

4.6 Caffeine standard solutions

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

4.6.1 Caffeine standard solution A, corresponding to 0,010 g of caffeine per litre (to be used for decaffeinated products).

Allow the caffeine stock solution (4.5) to warm up to room temperature. Using a pipette, transfer 2,0 ml of the stock solution (4.5) to a 100 ml one-mark volumetric flask. Dilute to the mark with water and mix.

4.6.2 Caffeine standard solution B, corresponding to 0,050 g of caffeine per litre (to be used for regular products).

Follow the procedure given in 4.6.1 but take, using a pipette, 10,0 ml of the stock solution (4.5).

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 chart recorder.

NOTES

2 A wavelength close to 280 nm is preferred since the maximum absorption by caffeine is at 272 nm.

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

5.2 Chromatographic column for HPLC, type C_{18}, 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 the caffeine standard solution (4.6):

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

where

t is the retention time of the peak;

$W_{0,5}$ is the peak width at half peak height.

5.3 Filters, of 0,45 µm pore size.

5.4 Water bath, capable of being maintained at 90 °C ± 1 °C, with continuous shaking.

5.5 Bottle, of 500 ml capacity, fitted with a screw cap.

5.6 Analytical balance, capable of weighing to an accuracy of ± 0,001 g.

6 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in

- ISO 1839 for regular green tea and black tea;
- ISO 7516 for instant tea.

7 Preparation of test sample

Grind the sample of green tea or black tea in accordance with ISO 1572.

NOTE 4 Grinding is not required for instant tea samples.

8 Procedure

NOTE 5 If it is required to check whether the repeatability requirement is met, carry out two single determinations in accordance with 8.1 to 8.5 under repeatability conditions.

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1) Spherisorb 5 ODS, Spherisorb 10 ODS, Nucleosil 5 C_{18}, Nucleosil 7 C_{18}, Nucleosil 10 C_{18}, Zorbax BP C_{18}, Hypersil ODS, CP-Spher C_{18}, Bondapak C_{18}, Supelcosil L C_{18} and Partisphere C_{18} 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.

In this International Standard, the chromatographic conditions and the composition of the mobile phase (4.2) specified are suitable for a Partisphere C_{18} cartridge column of dimensions 110 mm × 4,6 mm, filled in a Whatman HPLC cartridge system. If other types of column are used, an alternative mobile phase and alternative chromatographic conditions may be necessary.

8.1 Determination of dry matter content

Calculate the dry matter content from the moisture content (loss in mass at 103 °C) determined on a portion of the test sample (clause 7) in accordance with

— ISO 1572 for green tea or black tea;

— ISO 7513 for instant tea.

8.2 Test portion

8.2.1 Green tea and black tea

Weigh, to the nearest 0,001 g, 1,0 g of the test sample (clause 7) into the bottle (5.5).

8.2.2 Instant tea

Weigh, to the nearest 0,001 g, 0,5 g of the test sample (clause 7) into the bottle (5.5).

8.3 Extraction of caffeine

8.3.1 Add, to the tea in the bottle, 4,5 g ± 0,5 g of the magnesium oxide (4.4) and 300 ml of water. Fit the screw cap and weigh, to the nearest 0,1 g, the bottle, cap and contents.

8.3.2 Mix the contents. Warm the bottle, cap and contents in the water bath (5.4) set at 90 °C, with continuous shaking, for 20 min.

Remove the bottle, cap and contents from the water bath, cool, dry and weigh to the nearest 0,1 g. The mass of the cooled bottle, cap and contents shall be equal to the mass determined in 8.3.1.

8.3.3 If the masses differ, carry out another extraction (8.3.1 and 8.3.2) using a further test portion.

8.3.4 Leave the bottle to stand to allow the contents to settle. Remove the cap and filter a portion of the contents through a filter (5.3).

8.4 Dilution (for regular green tea and black tea and for regular instant tea only)

Place 1,0 ml of the filtrate obtained in 8.3.4 in a 10 ml one-mark volumetric flask. Dilute to the mark with water and mix.

NOTE 6 For decaffeinated products, no dilution is necessary.

8.5 Determination

8.5.1 Adjustment of the apparatus

Set up the chromatograph (5.1) in accordance with the manufacturer's instructions and adjust it as follows:

— flowrate of the mobile phase (4.2): 0,5 ml/min to 1,5 ml/min depending upon the column used (see 5.2);

— temperature of the column (5.2): optimally 40 °C (but see note 1).

8.5.2 HPLC analysis

Once the flowrate of the mobile phase (4.2) and the temperature are stable, inject into the column 10 µl of the test solution obtained in 8.3.4 (and 8.4 in the case of regular leaf tea or regular instant tea) and then an equal volume of the appropriate caffeine standard solution (4.6.1 or 4.6.2).

9 Calculation

9.1 For regular green tea and black tea and for regular instant tea

The caffeine content, expressed as a percentage by mass, is given by the formula

$$\frac{A_x}{A_c} \times c_B \times \frac{10 \times 300}{1 \times m_0 \times 1\,000} \times \frac{100}{w} \times 100$$

where

A_x is the area of the caffeine peak obtained with the test solution;

A_c is the area of the caffeine peak obtained with the caffeine standard solution;

c_B is the concentration, in grams per litre, of the caffeine standard solution B;

m_0 is the mass, in grams, of the test portion;

w is the dry matter content of the test sample, determined in accordance with 8.1.

9.2 For decaffeinated green tea and black tea and for decaffeinated instant tea

The caffeine content, expressed as a percentage by mass, is given by the formula

$$\frac{A_x}{A_c} c_A \times \frac{300}{m_0 \times 1\,000} \times \frac{100}{w} \times 100$$

where

A_x, A_c, m_0 and w have the meanings given in 9.1;

c_A is the concentration, in grams per litre, of the caffeine standard solution A.

10 Precision

Details of the interlaboratory test to determine the precision of the method are summarized in annex A.

10.1 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should not be greater than the value given in table A.1.

10.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the value given in table A.1.

11 Test report

The test report shall specify

— the method in accordance with which sampling was carried out, if known,

— the method used,

— the test result(s) obtained, and

— if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

The test report shall include all information necessary for the complete identification of the sample.

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test, carried out in 1989 under the auspices of the International Organization for Standardization, gave the statistical results (evaluated in accordance with ISO 5725) shown in table A.1.

Table A.1 — Repeatability and reproducibility values

Sample	Caffeine content, %	Repeatability, r	Reproducibility, R
Black tea, regular	2,9	0,192	0,358
Green tea, regular	1,8	0,126	0,167
Instant tea, regular	6,5	0,167	0,381
Black tea, decaffeinated	0,3	0,028	0,043
Instant tea, decaffeinated	0,1	0,027	0,029

Annex B
(informative)

Bibliography

[1] ISO 1839:1980, *Tea — Sampling.*

[2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*

[3] ISO 7516:1984, *Instant tea in solid form — Sampling.*

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