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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

Reference number
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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):

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

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[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.

where

t is the retention time of the peak;

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

NOTE In this International Standard, the chromatographic conditions and the composition of the mobile phase (4.3) specified are suitable for a Partisphere C18 cartridge column of dimensions 110 mm × 4,6 mm, fitted in a Whatman HPLC cartridge system. If other types of column are used, an alternative mobile phase and alternative chromatographic conditions may be necessary.

5.3 Filter, membrane filter unit of 0,45 µm pore size.

5.4 Electric heating mantle, to accommodate a 1 litre round-bottom flask.

5.5 Flask, round bottom, of capacity 1 litre, with ground glass neck.

5.6 Anti-bumping granules.

5.7 Condenser, water-cooled with ground glass joint to fit a 1 litre round-bottom flask.

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

6 Sampling

It is important that the laboratory receive a sample that 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. Recommended sampling methods are 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.

Grinding is not required for instant tea samples.

8 Procedure

8.1 General

If it is required to check whether the repeatability requirement is met, carry out two single determinations in accordance with 8.2 to 8.6 under repeatability conditions.

8.2 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 in accordance with:

- ISO 1572 for green tea or black tea;
- ISO 7513 for instant tea.

8.3 Test portion

8.3.1 Green tea and black tea

Weigh, to the nearest 0,001 g, 1,0 g of the test sample (clause 7) into a round-bottom flask (5.5).

8.3.2 Instant tea

Weigh, to the nearest 0,001 g, 0,5 g of the test sample (clause 7) into a round-bottom flask (5.5).

8.4 Extraction of caffeine

8.4.1 Add, to the tea in the flask, 4,5 g ± 0,5 g of magnesium oxide (4.5), a few anti-bumping granules (5.6) and 300 ml of water. Weigh, to the nearest 0,1 g, the flask and contents.

8.4.2 Mix the contents. Attach the condenser (5.7) and place the flask in the electric heating mantle (5.4) and rapidly bring to boiling. Reduce the heat and gently simmer for 20 min with occasional swirling.

8.4.3 Remove the flask and contents from the heating mantle and cool to room temperature. Dry the outside of the flask and weigh to the nearest 0,1 g. The mass of the cooled flask and contents shall be equal to the mass determined in 8.4.1. If the masses differ, add sufficient water to increase the mass to that determined in 8.4.1 and then mix contents.

8.4.4 Leave the flask to stand to allow the contents to settle, then remove approximately 10 ml of the supernatant solution and pass it through the filter (5.3).

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

Using a pipette, transfer 1,0 ml of the filtrate obtained in 8.4.4 to a 10 ml one-mark volumetric flask. Dilute to the mark with water and mix.

For decaffeinated products, no dilution is necessary.

8.6 Determination

8.6.1 Adjustment of the apparatus

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

- flow rate of the mobile phase (4.3): 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 4.3).

8.6.2 HPLC analysis

Once the flow rate of the mobile phase (4.3) and the temperature are stable, inject onto the column 20 µl of each of the caffeine standard solutions A, B, C and D (4.7.1 to 4.7.4) followed by an equal volume of the test solution (obtained using the procedure in 8.4.4 for decaffeinated tea and the procedure in 8.5 for regular leaf tea and regular instant tea). Repeat injection of standard solutions at regular intervals (typically after six test solutions). Collect and record the data for the peaks of all standards and test samples.

9 Calculation

Identify and measure the peak areas or heights (area is preferable) for all standards and samples. Construct a linear calibration graph of caffeine standard concentration (micrograms per millilitre) against peak areas or heights and obtain the response factor (RF) automatically using a data collection/integration system, or manually from a selected point on the calibration graph:

$$\text{RF} = \frac{C_{\text{std}}}{A_{\text{std}}} \quad \text{or} \quad \text{RF} = \frac{C_{\text{std}}}{h_{\text{std}}}$$

where

RF is the response factor for caffeine;

C_{std} is the concentration of caffeine in the caffeine standard solution, in micrograms per millilitre;

A_{std} is the peak area for the caffeine standard solution;

h_{std} is the peak height for the caffeine standard solution.

The caffeine content, w, expressed as mass fraction in percent of the sample on a dry basis, is given by the formula

$$w = A_{\text{samp}} \times \text{RF} \times \frac{300d}{m} \times \frac{100}{w_{\text{DM}}} \times \frac{100}{1\,000\,000}$$

or

$$w = h_{\text{samp}} \times \text{RF} \times \frac{300d}{m} \times \frac{100}{w_{\text{DM}}} \times \frac{100}{1\,000\,000}$$

where

A_{samp} is the peak area for the test sample;

h_{samp} is the peak height for the test sample;

RF is the response factor for caffeine;

d is the dilution factor (10 for regular leaf teas and instant teas);

m is the mass, in grams, of the test sample;

w_{DM} is the dry matter content (mass fraction, in percent), determined in accordance with 8.2.

10 Precision

10.1 Interlaboratory test

Details of the interlaboratory test to determine the precision of the method are summarized in annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

10.2 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, will in not more than 5 % of cases be greater than the repeatability limit (r) values given in Table A.1.

10.3 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, will in not more than 5 % of cases be greater than the reproducibility limit (R) values given in Table A.1.

11 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;
- 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 result(s) obtained; and
- if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test, carried out in 1999 under the auspices of the International Organization for Standardization, gave the statistical results (evaluated in accordance with ISO 5725-2) shown in Table A.1.

Table A.1 — Precision data

Sample identification	**Sample**[a]							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Number of participating laboratories	11	11	11	11	11	11	11	11
Number of accepted test results	8	8	8	8	8	7	8	8
Mean caffeine content, % (mass fraction), dry matter	4,120	3,932	0,256	0,091	0,118	0,260	7,729	5,116
Repeatability standard deviation, s_r	0,102 9	0,118 2	0,009 6	0,005 4	0,007 1	0,010 4	0,296 4	0,113 2
Repeatability coefficient of variation, %	2,50	3,01	3,75	5,93	6,02	4,00	3,84	2,21
Repeatability limit, r $(2{,}8 \times s_r)$	0,288	0,331	0,027	0,015	0,020	0,029	0,830	0,317
Reproducibility standard deviation, s_R	0,165 0	0,156 4	0,012 1	0,007 9	0,007 1	0,014 0	0,355 7	0,236 1
Reproducibility coefficient of variation, %	4,01	3,98	4,74	8,68	6,02	5,37	4,60	4,61
Reproducibility limit, R $(2{,}8 \times s_R)$	0,462	0,438	0,034	0,022	0,020	0,039	0,996	0,661

[a] Sample A: Green leaf tea
Sample B: Black leaf tea (orthodox)
Sample C: Decaffeinated black leaf tea
Sample D: Decaffeinated black leaf tea
Sample E: Decaffeinated black leaf tea
Sample F: Decaffeinated instant black tea
Sample G: Instant black tea
Sample H: Instant black tea

Bibliography

[1] ISO 1839:1980, *Tea — Sampling*

[2] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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

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