BRITISH STANDARD

BS 5752 :
Part 12 : 1992
ISO 10095 :
1992

Methods of test for

Coffee and coffee products

Part 12. Coffee: determination of caffeine content (routine method by HPLC)

STANDARDS

Committees responsible for this British Standard

The preparation of this British Standard was entrusted by the Agriculture and Food Standards Policy Committee (AFC/-) to Technical Committee AFC/15, upon which the following bodies were represented:

Association of Public Analysts
British Soluble Coffee Manufacturers' Association
Coffee Trade Federation Ltd.
Department of Trade and Industry (Laboratory of the Government Chemist)
Food and Drink Federation
Institute of Trading Standards Administration
Ministry of Agriculture, Fisheries and Food
Natural Resources Institute

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Contents

National foreword

This Part of BS 5752 has been prepared under the direction of the Agriculture and Food Standards Policy Committee. It is identical with ISO 10095 : 1992 *Coffee — Determination of caffeine content — Method using high-performance liquid chromatography,* published by the International Organization for Standardization (ISO). This Part embodies an agreement, to which the UK was a party, reached in Subcommittee 15, Coffee, of Technical Committee 34, Agricultural food products, of the International Organization for Standardization (ISO).

Cross-references

International standard	Corresponding British Standard
	BS 5752 *Methods of test for coffee and coffee products*
ISO 1447 : 1978	Part 2 : 1984 *Green coffee: determination of moisture content (routine method)* (Identical)
ISO 3726 : 1983	Part 6 : 1983 *Instant coffee: determination of loss in mass at 70 °C under reduced pressure* (Identical)
ISO 4052 : 1983	Part 3 : 1983 *Coffee: determination of caffeine content (reference method)* (Identical)
ISO 4072 : 1982	BS 6379 *Sampling of coffee and coffee products* Part 1 : 1983 *Method of sampling green coffee in bags* (Identical)
ISO 5725 : 1986	BS 5497 *Precision of test methods* Part 1 : 1987 *Guide for the determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (Identical)
ISO 6670 : 1983	BS 6379 *Sampling of coffee and coffee products* Part 2 : 1984 *Methods of sampling instant coffee in cases with liners* (Identical)
ISO 6673 : 1983	BS 5752 *Methods of test for coffee and coffee products* Part 7 : 1984 *Green coffee: determination of loss in mass at 105 °C* (Identical)

Compliance with a British Standard does not of itself confer immunity from legal obligations.

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 oxide[1].

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

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[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.

a 250 ml volumetric flask made of amber glass. Add sufficient ethanol/water (4.5) to half-fill the flask. Dissolve the caffeine and then make up to the mark with the same ethanol/water mixture.

This solution can be stored for 1 month in a refrigerator.

4.8 Caffeine, standard solutions.

4.8.1 standard solution A, corresponding to 0,010 g of caffeine per litre, to be used for the decaffeinated product.

Allow the stock solution (4.7) to warm up to room temperature. Using a pipette (5.12), transfer 2 ml of this solution to a 100 ml one-mark volumetric flask. Make up to the mark with water and mix.

Prepare this solution on the day of use.

4.8.2 Standard solution B, corresponding to 0,05 g of caffeine per litre, to be used for regular products.

Allow the stock solution (4.7) to warm up to room temperature. Using a pipette (5.12), transfer 5 ml of this solution to a 50 ml one-mark volumetric flask. Make up 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 between 254 nm and 280 nm, and a chart recorder. A wavelength close to 280 nm is preferred since the maximum absorption by caffeine is at 272 nm.

5.2 Chromatographic column for HPLC, type C_{18}, preferably with spherical particles and having an efficiency of at least 5 000 theoretical plates.[2]

The theoretical plate number N of a column can be calculated as follows, from the shape of the peak obtained by injection of the pure caffeine standard solution (4.8):

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

where

t is the retention time of the peak;

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

5.3 Purification column, for reversed-phase chromatography, of 3 ml capacity, filled with silica modified with phenyl groups, the particles of which have an average size of 40 µm.[3]

5.4 Coffee mill, suitable for milling roasted coffee beans.

5.5 Grinder with cogged wheel, with a cooling jacket, or **analytical grinder**, with blades and a cooling jacket, or any other grinder suitable for grinding green coffee beans.

5.6 Sieve, of woven wire cloth, with an aperture size of 630 µm.

5.7 Filters, of 0,45 µm pore size.

5.8 Water-bath, capable of operating at 90 °C ± 1 °C with continuous stirring.

5.9 Analytical balance, capable of weighing to the nearest 0,000 1 g

5.10 Bottle, of 250 ml capacity, fitted with a screw cap.

5.11 One-mark volumetric flasks, of 10 ml, 50 ml, 100 ml, 250 ml and 2 l capacity.

5.12 Pipettes, of 2,0 ml, 5,0 ml and 10,0 ml capacity.

6 Sampling

Sampling of green coffee in bags should have been carried out in accordance with ISO 4072.

Sampling of instant coffee in cases with liners should have been carried out in accordance with ISO 6670.

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[2] 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 (4.4) specified are suitable for a Partisphere C_{18} Cartridge column of dimensions 110 mm x 4,6 mm, filled in a Whatman HPLC Cartridge system. If other types of column are used, an alternative mobile phase and chromatographic conditions may be necessary.

[3] The 3 ml Baker SPE column, modified with phenyl groups in reversed phase, 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.

7 Preparation of the test sample

If necessary, grind the sample using the apparatus specified in 5.4 or 5.5 , as appropriate, until it passes through the sieve (5.6).

8 Procedure

8.1 Determination of dry matter content

Calculate the dry matter content from the moisture content determined on a portion of the test sample (clause 7) in accordance with

- ISO 1447 or ISO 6673 for green coffee,
- ISO 3726 for instant coffee, or
- a corresponding future International Standard for other types of coffee and coffee-derived products.

8.2 Test portion

8.2.1 Regular and decaffeinated green or roasted coffee

Weigh, to the nearest 0,000 1 g, 1 g of the test sample (clause 7).

8.2.2 Regular and decaffeinated coffee extract powder

Weigh, to the nearest 0,000 1 g, 0,5 g of the test sample (clause 7).

8.3 Extraction of caffeine

8.3.1 Put the test portion (8.2.1 or 8.2.2) into the 250 ml bottle (5.10). Add 4 g ± 0,5 g of magnesium oxide (4.6) and 100 g of water. Weigh the flask and its contents to the nearest 0,1 g.

8.3.2 Stopper the bottle and mix the contents. Place the bottle plus contents in a water-bath (5.8) and heat to 90 °C, under continuous stirring, for 20 min.

Cool the bottle plus contents and weigh again to the nearest 0,1 g. The mass of the cooled bottle plus 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 another test portion.

8.3.4 Leave the solution to stand. Remove a portion of the solution and filter it through the filter (5.7).

8.4 Purification of the solution

Before any separation of the solution occurs, activate the purification column (5.3).

8.4.1 Preparation of the purification column

Set up the purification column as shown in figure 1.

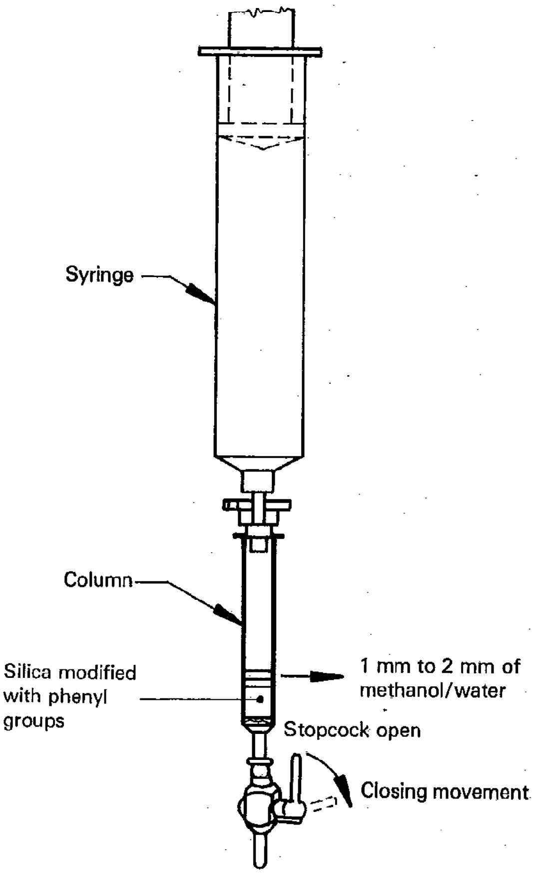

Figure 1 — Preparation of the purification column

Open the stopcock and rinse the column with 5 ml of the methanol (4.1), adjusting the stopcock to achieve a dropwise flow. When 1 mm to 2 mm of the methanol remains above the surface of the silica, close the stopcock. Add 5 ml of water, open the stopcock and close it again when 1 mm to 2 mm of water remains above the silica surface.

Do not allow the column to dry out, otherwise the preparation shall be repeated.

8.4.2 Absorption of caffeine

Using a pipette, introduce into the column either

a) 2,0 ml of the filtered solution obtained in 8.3.3 in the case of regular coffee or coffee extract, or

b) 10,0 ml of the filtered solution obtained in 8.3.4 in the case of decaffeinated coffee or decaffeinated coffee extract.

Adjust the stopcock to allow the solution to flow dropwise. Close the stopcock when the surface of the solution falls just below the surface of the silica.

8.4.3 Removal of unwanted compounds

Open the stopcock and add 2,5 ml of the ammonia solution/methanol mixture (4.2). Close the stopcock when the surface of this mixture falls just below the surface of the silica. Add a further 2,5 ml of the mixture (4.2) and allow it to flow completely through the column. Blow about 20 ml of air through the column in order to remove as much as possible of the mixture (4.2).

NOTE 3 The column may become dry at this stage of the procedure.

8.4.4 Elution of caffeine

Place a 10 ml one-mark volumetric flask (5.11) under the column. Open the stopcock and add 7,5 ml of the elution solvent (4.3), adjusting the stopcock to achieve a dropwise flow. Allow the elution solvent to flow completely into the volumetric flask. Make up to the mark with water and mix the contents.

NOTE 4 The purification column can be regenerated with methanol as indicated in 8.4.1. It may be used at most ten times for purification purposes.

8.5 HPLC analysis

8.5.1 Adjustment of the apparatus

Set up the chromatograph (5.1) and adjust it as follows:

- flow-rate of the mobile phase (4.4): 0,5 ml/min to 1,5 ml/min, depending on the column used (see 5.2)
- temperature of the column (5.2): 40 °C

NOTE 5 The peak separation can also be improved by raising the temperature of the column, but this should not exceed 60 °C.

8.5.2 Analysis

Once the flow-rate of the mobile phase (4.4) and the temperature are stable, inject into the column 10 µl of the test solution obtained in 8.4.4 and then an equal volume of the standard caffeine solution (4.8.1 or 4.8.2).

NOTE 6 In normal procedures Beer's law is obeyed for caffeine concentrations up to 0,025 0 g/l. This level is higher than the caffeine concentrations used in this test method. If, however, this should not be the case due to instrumentation deviations, a calibration curve for the extinction—caffeine concentration should be made.

9 Expression of results

The caffeine content of the sample, expressed in grams per 100 g of the dry matter content, is as follows.

9.1 For regular roasted or green coffee and instant coffee powder:

$$\frac{A_x}{A_c} \times c_1 \times \frac{10 \times 100}{2 \times m_0 \times 1\,000} \times \frac{100}{RS} \times 100$$

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 standard caffeine solution;

c_1 is the concentration of the standard caffeine solution (4.8.2), in grams per litre;

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

RS is the dry matter content, as a percentage by mass, of the sample (see 8.1).

9.2 For decaffeinated roasted or green coffee and decaffeinated coffee powder:

$$\frac{A_x}{A_c} \times c_2 \times \frac{10 \times 100}{10 \times m_0 \times 1\,000} \times \frac{100}{RS} \times 100$$

where

c_2 is the concentration of the standard caffeine solution (4.8.1), in grams per litre.

10 Precision

10.1 Results of inter-laboratory test

An inter-laboratory test was carried out at the international level in 1987 by ISO/TC 34/SC 15, *Coffee*, in which 18 laboratories participated. The repeatability and reproducibility values shown in table 1 were obtained. The sample plan and procedures followed in this inter-laboratory plan were in accordance with the split-level design as outlined in ISO 5725.

Table 1 — Repeatability and reproducibility values

Sample	Caffeine content g/100 g of coffee	Repeatibility g caffeine/100 g of coffee	Reproducibility g caffeine/100 g of coffee
Roasted coffee beans	≈ 2	0,07	0,34
	≈ 1	0,04	0,12
Decaffeinated roasted coffee beans	< 0,1	0,01	0,02
Instant coffee	≈ 4	0,09	0,36
Decaffeinated instant coffee	< 0,3	0,02	0,03

10.2 Repeatability

The absolute difference between two 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 exceed the value given in table 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, should not exceed the value given in table 1.

11 Test report

The test report shall specify the method used and the results 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 results.

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

Annex A
(informative)

Bibliography

[1] ISO 4072:1982, *Green coffee in bags — Sampling.*

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

[3] ISO 6670:1983, *Instant coffee in cases with liners — Sampling.*

List of references

See national foreword.

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