International Standard 4052

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Coffee — Determination of caffeine content (Reference method)

Cafés — Détermination de la teneur en caféine — Méthode de référence

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 4052 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in May 1982.

It has been approved by the member bodies of the following countries :

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Malaysia

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0 Introduction

The method described in this International Standard has been chosen from amongst several methods, a comparative study of which was carried out, because of its general applicability, its reproducibility, its specificity, its ease of application and its rapidity.

However, the method is particularly sensitive to variations in its application and it is therefore essential to follow the instructions in every detail.

1 Scope and field of application

This International Standard specifies the reference method for the determination of the caffeine content of coffee.

The method is applicable to green coffee, decaffeinated green coffee, roasted coffee, decaffeinated roasted coffee, extracts of coffee, both dried and liquid, and decaffeinated extracts, both dried and liquid.

The lower limit of detection is 0,02 % caffeine on the dry basis.

2 References

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

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

ISO 4072, *Green coffee in bags — Sampling.*

ISO 6670, *Instant coffee in cases with liners — Sampling.*[1)]

ISO 6673, *Green coffee — Determination of loss in mass at 105 °C.*[1)]

3 Principle

Extraction of the caffeine from a test portion, in an ammoniacal medium. Successive purification, with diethyl ether, on two chromatographic columns, the first in an alkaline medium, the second in an acid medium, followed by elution of the caffeine by chloroform.

Spectrometric measurement of the eluate at the wavelength of maximum absorbance (in the ultraviolet region).

4 Reagents

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity.

4.1 Sulphuric acid, 200 g/l solution [$c(H_2SO_4) \approx 2$ mol/l].

4.2 Sodium hydroxide, 80 g/l solution [$c(NaOH) \approx 2$ mol/l].

4.3 Diatomaceous earth

The product used shall ensure at least 98 % recovery of caffeine from the test portion.

NOTE — Celite 545 has been found to be suitable.

4.4 Ammonia, 70 g/l solution (1 volume of concentrated ammonia solution, $\varrho_{20} \approx 0{,}9$ g/ml, + 2 volumes of water).

4.5 Diethyl ether, pure, or repurified (see 7.5) by chromatography as follows, and saturated with water.

Pass 800 ml of diethyl ether through a column containing 100 g of basic aluminium oxide of activity grade 1. The diethyl ether, thus repurified, shall be kept in dark bottles until used.

(Alternatively, diethyl ether, recently distilled and free of peroxides, can be used instead of diethyl ether repurified by chromatography.)

4.6 Caffeine [1,3,7-trimethyl-2,6-dioxopurine ($C_8H_{10}N_4O_2$)], pure, anhydrous.

4.7 Chloroform, pure, or repurified (see 7.5) by chromatography as described in 4.5, and saturated with water.

1) At present at the stage of draft.

5 Apparatus

5.1 Chromatographic columns (see figure 1), 250 mm long, 21 mm in internal diameter (column I) and 17 mm in internal diameter (column II), with stopcocks preferably of PTFE.

5.2 Ultraviolet spectrometer, accurate to within 0,004 absorbance unit within the range used.

5.3 Silica cells, of optical path length 10 mm.

5.4 Usual laboratory equipment, including

5.4.1 Beakers, of capacity 100 ml.

5.4.2 Boiling water bath.

5.4.3 One-mark volumetric flasks, of capacities 50, 100 and 1 000 ml.

5.4.4 One-mark pipettes, of capacities 2 and 5 ml.

5.4.5 Analytical balance.

5.5 Coffee-mill, suitable for roasted coffee beans.

5.6 Toothed-disk mill, with cooling jacket, or **analytical mill,** with sparecutter and cooling jacket, or similar mill suitable for green coffee beans.

5.7 Sieve, of woven metal wire cloth, nominal aperture size 600 µm or 630 µm, complying with the requirements of ISO 3310/1.

6 Sampling

Sample in accordance with the method specified in the relevant International Standard.[1)]

7 Procedure

7.1 Preparation of test sample

If necessary, mill the sample, using the apparatus specified in 5.5 or 5.6, as appropriate, until it passes the sieve (5.7).

7.2 Determination of dry matter content

Calculate the dry matter content after determining the moisture content on part of the test sample (7.1) in accordance with the method specified in the relevant International Standard.[2)]

7.3 Test portion

7.3.1 Green coffee and roasted coffee

Weigh, to the nearest 0,1 mg, about 1 g of the test sample (7.1). Transfer it to a 100 ml beaker (5.4.1), add 5 ml of the ammonia solution (4.4) and warm for 2 min on the boiling water bath (5.4.2). Allow to cool, then transfer to a 100 ml volumetric flask (5.4.3), dilute to the mark with water and mix. Allow this turbid solution to settle, and then, using a pipette (5.4.4), transfer 5,0 ml of the solution into a 100 ml beaker (5.4.1), add 6 g of the diatomaceous earth (4.3) and mix carefully.

7.3.2 Dried coffee extract

Proceed in accordance with 7.3.1, but using a test portion of 0,5 g, and an aliquot portion of the turbid solution of 2 ml, and 3 g of the diatomaceous earth.

7.3.3 Liquid coffee extract

Proceed in accordance with 7.3.1, but using a test portion between 1 and 2,5 g corresponding to approximately 0,5 g of coffee solids, and an aliquot portion of the turbid solution of 2 ml, and 3 g of the diatomaceous earth.

7.3.4 Decaffeinated green coffee and decaffeinated roasted coffee

Weigh, to the nearest 0,1 mg, about 1 g of the test sample (7.1). Transfer it to a beaker (5.4.1), add 5 ml of the ammonia solution (4.4) and warm for 2 min on the boiling water bath (5.4.2). Add 6 g of diatomaceous earth (4.3) and mix carefully.

7.3.5 Dried decaffeinated coffee extract

Proceed in accordance with 7.3.4, but using a test portion of 0,5 g.

7.3.6 Liquid decaffeinated coffee extract

Proceed in accordance with 7.3.4, but using a test portion between 1 and 2,5 g corresponding to approximately 0,5 g of coffee solids, and 7 to 8 g of the diatomaceous earth.

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1) For the sampling of green coffee in bags, see ISO 4072; for the sampling of instant coffee in cases with liners, see ISO 6670. Methods for other types of coffee and coffee products have not yet been elaborated.

2) For the determination of the moisture content of green coffee, see ISO 1447, and, for the loss in mass at 105 °C of green coffee, see ISO 6673; for the determination of the loss in mass at 70 °C under reduced pressure of instant coffee, see ISO 3726. Methods for other types of coffee and coffee products have not yet been elaborated.

7.4 Determination

7.4.1 Filling of columns

7.4.1.1 Column I (alkaline column)

7.4.1.1.1 Layer A

Mix carefully, by kneading with a flexible spatula blade, 3 g of the diatomaceous earth (4.3) and 2 ml of the sodium hydroxide solution (4.2), until homogeneous (see the note). A slightly wet powder is obtained. Transfer this powder, in portions of approximately 2 g, into the 21 mm diameter chromatographic column (5.1), the lower part of which is packed with a wad of cotton wool or glass wool. Tamp down the mixture after each addition, without excessive force, using a glass rod one end of which is flattened to the diameter of the column, until a perfectly homogeneous and compact layer is obtained. A small wad of cotton wool or glass wool may be placed on the top of layer A.

NOTE — Column packing material may be prepared in bulk in advance and stored in closed containers. A mass of 5,16 g is required for each alkaline column.

7.4.1.1.2 Layer B

Transfer the mixture of the diatomaceous earth and the test portion (7.3) into the column on top of layer A. Dry the beaker twice with portions of about 1 g of the diatomaceous earth (4.3), transferring this into the column. Tamp down to obtain a homogeneous layer and place a wad of cotton wool or glass wool on the top of this layer B.

7.4.1.2 Column II (acid column)

Place in the 17 mm diameter chromatographic column (5.1), the lower part of which is packed with a wad of glass wool, 3 g of the diatomaceous earth (4.3) and 3 ml of the sulphuric acid solution (4.1), carefully mixed and packed into the column as described for layer A of column I in 7.4.1.1. Place a wad of glass wool on the top of this layer.

NOTE — Column packing material may be prepared in bulk in advance and stored in closed containers. A mass of 6,36 g is required for each acid column.

7.4.2 Chromatography

Mount the columns one above the other so that the effluent from column I can drip directly into column II. Pass 150 ml of diethyl ether (4.5) through the two columns. Adjust the stopcock of column II so that a quantity of supernatant liquid remains above the layer. Remove column I. Pass 50 ml of diethyl ether (4.5) through column II, using the initial portion to wash the tip of column I and passing this portion also into column II. Discard the effluent from column II.

NOTE — Used diethyl ether may be recovered by shaking it with iron(II) sulphate.

Pass a stream of air from the top to the lower part of column II (for example by using an inflated rubber blower), until no more diethyl ether drips from the column and the air flow from the stopcock carries only a faint smell of diethyl ether (see the warning below). Elute column II with 45 to 50 ml of chloroform (4.7). Collect the eluate in a 50 ml one-mark volumetric flask (5.4.3), dilute to the mark with the chloroform (4.7) and mix carefully.

The flow rate of the diethyl ether and the chloroform under conditions of natural flow should be between 1,5 and 3 ml/min. If this rate is exceeded, channelling should be suspected and the determination recommenced.

WARNING — The additions of diethyl ether and chloroform should be carried out in a well-ventilated fume-cupboard to prevent both the possibility of inhalation of solvent vapours and the possibility of an explosion.

7.4.3 Spectrometric measurement (see figure 2)

7.4.3.1 Measurement of the test solution

Avoiding error from evaporation of chloroform, measure the absorbance of the solution of caffeine in chloroform (7.4.2), using the silica cells (5.3), against chloroform (4.7) at the wavelength of maximum absorbance obtained on the spectrometer used (about 276 nm), and at wavelengths 30 nm above and below this wavelength in order to verify the purity of the caffeine obtained.

If the maximum absorbance exceeds the limit of accurate measurement of the instrument used, repeat the measurement on a diluted aliquot portion of the solution of caffeine in chloroform (7.4.2). In this case, take the dilution into account in the calculation; the appropriate factors of the formulae in 8.1.1, 8.1.2 and 8.1.3 will have to be adjusted accordingly. If the maximum absorbance measured is lower than 0,2, repeat the determination using a test portion of greater mass.

7.4.3.2 Preparation and measurement of reference solution

Prepare a reference solution of caffeine in the following manner.

Weigh, to the nearest 0,1 mg, 100 ± 20 mg of the pure anhydrous caffeine (4.6). Place in a 1 000 ml one-mark volumetric flask (5.4.3), dissolve in chloroform, and dilute to the mark. Using a pipette (5.4.4), transfer 5,0 ml of this solution into a 50 ml one-mark volumetric flask (5.4.3) and dilute to the mark with chloroform.

Measure the absorbance of this solution as described in 7.4.3.1. The corrected absorbance of the reference solution (see 8.1.1 and figure 2) should be in the region of 0,4.

7.4.4 Number of determinations

Carry out two determinations on separate test portions taken from the same test sample.

7.5 Blank test

Carry out a blank test on the reagents, using the procedure described, but omitting the test portion.

Before using repurified reagents (see 4.5 and 4.7), repeat the blank test to verify their purity.

8 Expression of results

8.1 Method of calculation and formulae

8.1.1 Green coffee and roasted coffee

The caffeine content of the sample, expressed in grams per 100 g of dry matter, is equal to

$$\frac{10^7 \times c \times A_1}{A_2 \times m \times P}$$

where

c is the concentration, in grams per millilitre, of caffeine in the reference solution (7.4.3.2);

A_1 is the corrected absorbance of the purified extract (7.4.2) obtained in 7.4.3.1, i.e.

$$(A_1)_\lambda - \frac{(A_1)_{\lambda\ -30\ nm} + (A_1)_{\lambda\ +30\ nm}}{2}$$

where the subscript λ refers to the wavelength of maximum absorbance (about 276 nm);

A_2 is the corrected absorbance of the reference solution of caffeine (7.4.3.2), i.e.

$$(A_2)_\lambda - \frac{(A_2)_{\lambda\ -30\ nm} + (A_2)_{\lambda\ +30\ nm}}{2}$$

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

P is the dry matter content, expressed as a percentage by mass, of the sample (see 7.2).

8.1.2 Dried coffee extract and liquid coffee extract

The caffeine content of the sample, expressed in grams per 100 g of dry matter, is equal to

$$\frac{25 \times 10^6 \times c \times A_1}{A_2 \times m \times P}$$

where the symbols have the same meaning as in 8.1.1.

8.1.3 Decaffeinated green coffee, decaffeinated roasted coffee, dried decaffeinated coffee extract and liquid decaffeinated coffee extract

The caffeine content of the sample, expressed in grams per 100 g of dry matter, is equal to

$$\frac{5 \times 10^5 \times c \times A_1}{A_2 \times m \times P}$$

where the symbols have the same meaning as in 8.1.1.

8.1.4 Result

Take as the result the arithmetic mean of the values obtained, provided that the requirement for repeatability (see 8.2) is satisfied.

8.2 Repeatability

The difference between the values obtained in the two determinations, carried out simultaneously or in rapid succession on the same sample in one laboratory by the same analyst, shall not exceed the value given in the table.

8.3 Reproducibility

The difference between the results of two determinations, carried out in two different laboratories on the same sample, shall not exceed the value given in the table.

9 Test report

The test report shall show the method used and the result obtained. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with any circumstances that may have influenced the result.

The test report shall give all details required for the complete identification of the sample.

Table — Repeatability and reproducibility

Sample	Amount of caffeine g/100 g coffee	Repeatability* g caffeine/ 100 g coffee	Reproducibility* g caffeine/ 100 g coffee
green coffee beans	about 2	0,12	0,38
	about 1	0,08	0,31
	decaffeinated < 0,1	< 0,01	0,01
roasted coffee beans	about 2	0,10	0,32
	about 1	0,04	0,19
	decaffeinated < 0,1	< 0,01	0,01
soluble coffee	about 4	0,17	0,39
	about 2	0,12	0,20
	decaffeinated < 0,3	0,01	0,01

* The values of repeatability and reproducibility are critical differences at the 95 % probability level.

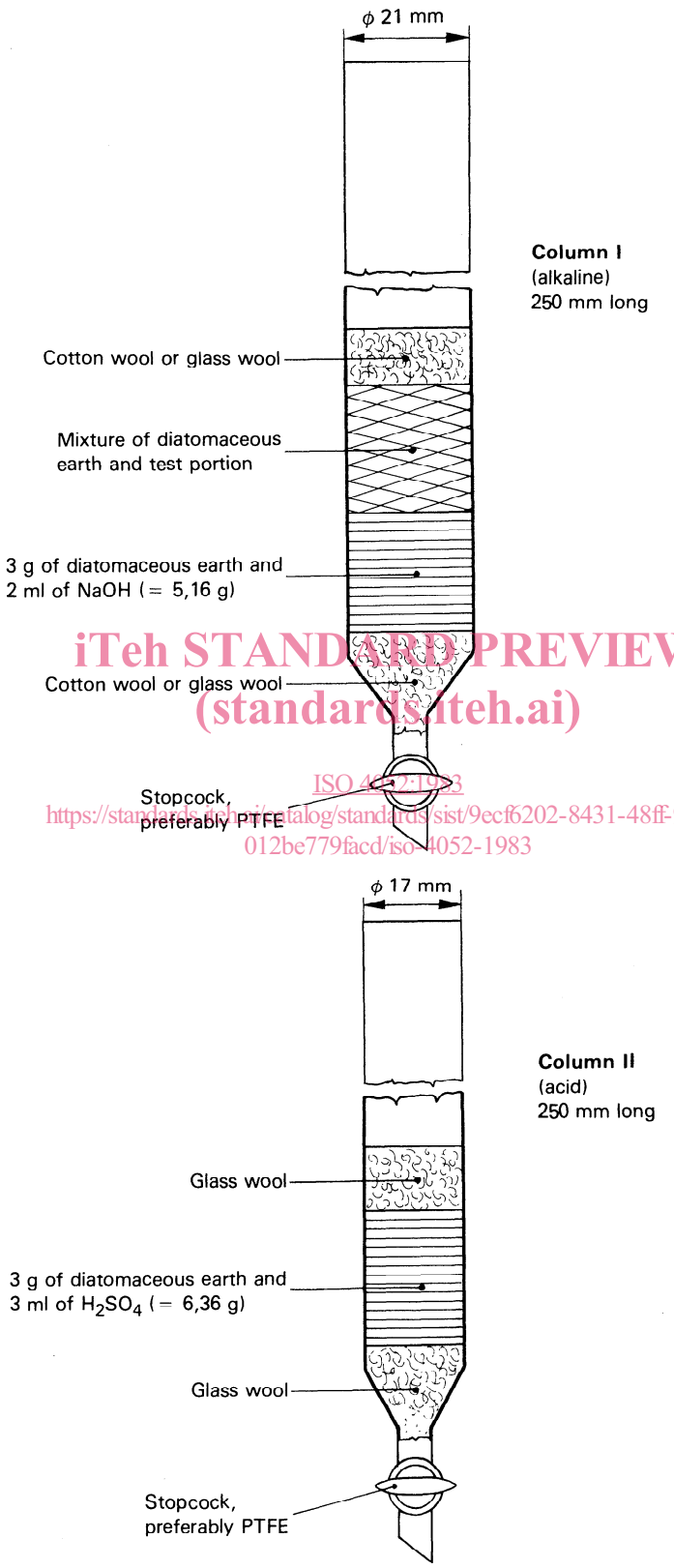

Figure 1 — Chromatographic columns

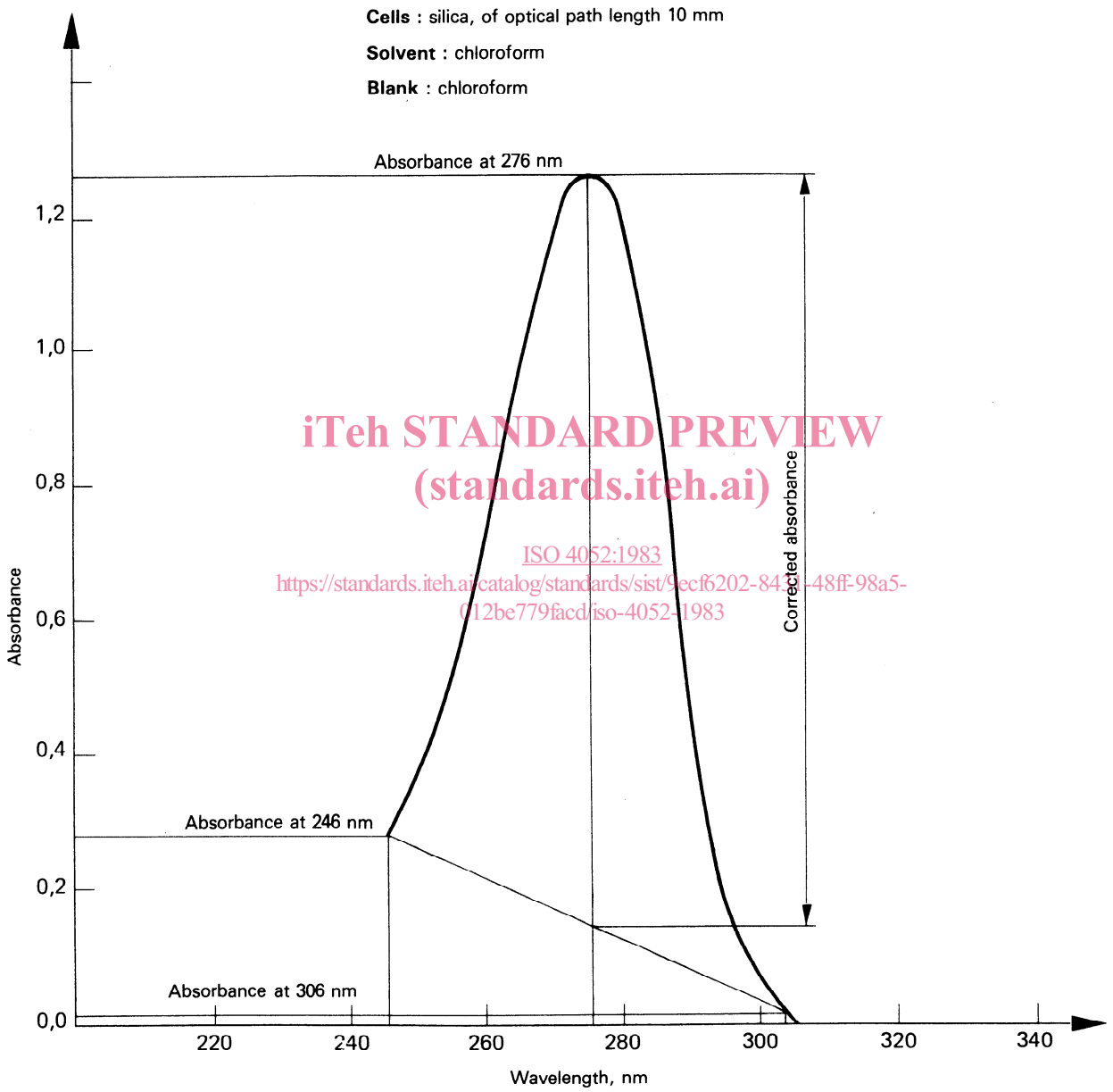

Figure 2 — Example of spectrometric measurement