COMMISSION

FIRST COMMISSION DIRECTIVE

of 13 November 1979

laying down Community methods of analysis for testing coffee extracts and chicory extracts

(79/1066/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 77/436/EEC of 27 June 1977 on the approximation of the laws of the Member States relating to coffee extracts and chicory extracts [1], and in particular Article 8 thereof,

Whereas under Article 8 of Directive 77/436/EEC, the composition and characteristics of coffee extracts and chicory extract are required to be checked according to Community methods of analysis;

Whereas a first series of methods for which studies are completed should now be adopted;

Whereas the measures provided for in this Directive are in accordance with the opinion of the Standing Committee on Foodstuffs,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Member States shall take all measures necessary to ensure that the analyses necessary for the verification of the criteria set out in Annex I are carried out in accordance with the methods described in Annex II.

Article 2

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive within 18 months of its notification. They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 13 November 1979.

For the Commission

Étienne DAVIGNON

Member of the Commission

[1] OJ No L 172, 12. 7. 1977, p. 20.

ANNEX I

SCOPE OF THE FIRST COMMUNITY METHODS OF ANALYSIS DIRECTIVE FOR COFFEE EXTRACTS AND CHICORY EXTRACTS

I. General provisions.

II. Determination of the caffeine content in decaffeinated coffee extracts using Annex II, method 1.

III. Determination of the dry matter content in dried coffee extract and dried chicory extract, soluble coffee and chicory, and instant coffee and chicory, using Annex II, method 2.

IV. Determination of the dry matter content in liquid coffee extract, liquid chicory extract, coffee extract paste and chicory extract paste using Annex II, method 3.

ANNEX II

METHODS OF ANALYSIS RELATING TO THE COMPOSITION OF COFFEE EXTRACTS AND CHICORY EXTRACTS

GENERAL PROVISIONS

1. PREPARATION OF THE ANALYSIS SAMPLE

1.1. General

The mass of the sample presented to the laboratory for analysis shall be at least 50 g.

1.2. Preparation of the sample for analysis in the laboratory

1.2.1. Mixing

The sample for analysis shall always be mixed thoroughly before any test portion is weighed out.

1.2.1.1. Samples in powder or paste form shall be removed from the container, any lumps broken down, the sample mixed in an appropriate manner and placed in a suitable container.

1.2.1.2. Samples in liquid form shall be mixed by stirring.

1.3. Containers

The sample shall always be kept in an airtight and moisture-tight container.

2. REAGENTS

2.1. Water

2.1.1. Wherever mention is made to water for solution, dilution or washing purposes, distilled water, or demineralized water of at least equivalent purity, shall be used.

2.1.2. Wherever reference is made to 'solution' or 'dilution' without further indication, 'solution in water' or 'dilution with water' is meant.

2.2. Chemicals

All chemicals used shall be of recognized analytical reagent quality except where otherwise specified.

3. EQUIPMENT

3.1. Lists of equipment

The lists of equipment contain only those items with a specialized use and items with a particular specification.

3.2. Analytical balance

Analytical balance means a balance capable of weighing to at least 0.1 mg.

4. EXPRESSION OF RESULTS

4.1. Results

The result stated in the analytical report is the mean value obtained from at least two determinations, the repeatability of which is satisfactory.

4.2. Calculation of percentage

Except where otherwise specified, the result shall be calculated as a percentage by mass of the

4.3. **Number of significant figures**

The result shall not contain more significant figures than are justified by the precision of the method of analysis used.

5. TEST REPORT

The test report shall identify the method of analysis used as well as the results obtained. In addition, it shall mention all details of procedure, not specified in the method of analysis, or which are optional, as well as any circumstances that may have influenced the results obtained. The test report shall give all the information necessary for the complete identification of the sample.

METHOD 1: DETERMINATION OF CAFFEINE CONTENT

1. SCOPE AND FIELD OF APPLICATION

This method determines the caffeine content of decaffeinated coffee extracts.

2. DEFINITION

Caffeine content: the content of caffeine as determined by the method specified.

3. PRINCIPLE

Caffeine is extracted from a test portion of the sample in an ammoniacal medium. It is then successively purified with diethyl ether on two chromatographic columns, the first in an alkaline medium and the second in an acid medium. The caffeine is then eluted from the column by chloroform and determined spectrophotometrically.

4. REAGENTS

4.1. Sulphuric acid, 2 M solution.

4.2. Sodium hydroxide, 2 M solution.

4.3. Celite 545 or equivalent.

4.4. Ammonia solution, approximately 4 M (prepare by adding 1 volume of concentrated ammonia solution, p_{20} ca. 0.9 g/ml to 2 volumes of water).

4.5. Diethyl ether, pure or repurified by chromatography on a column of basic aluminium oxide of activity grade 1.

Pass 800 ml of diethyl ether through a column filled with 100 g of aluminium oxide. The diethyl ether thus purified, shall be kept in dark bottles until used.

(Diethyl ether, recently distilled and free of peroxides, can be used instead of diethyl ether purified by chromatography.) Saturate the diethyl ether with water.

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

4.7. Chloroform, pure or repurified by chromatography according to the method specified in 4.5 and saturated with water.

5. APPARATUS

5.1. Chromatographic columns (see figure 1), approximately 250 mm long, 21 mm internal diameter (column I) and 17 mm internal diameter (column II), fitted with stopcocks.

5.2. Ultra-violet spectrophotometer: The spectrophotometer shall be accurate to within 0.004 absorbance unit within the range used.

5.3. Silica cells with 10 mm optical path length.

5.4. Usual laboratory equipment, including

5.4.1. Water bath, boiling.

5.4.2. One-mark volumetric flasks, 50 ml, 100 ml and 1 000 ml, complying with ISO 1042.

5.4.3. One-mark pipettes, 2 ml and 5 ml; complying with ISO 648.

5.4.4. Analytical balance.

6. PROCEDURE

6.1. Preparation of the test portion

Weigh, to the nearest 0.1 mg, about 0.5 g of dried coffee extract sample, between 0.5 and 0.7 g of coffee extract paste sample or between 0.8 and 3.2 g of liquid coffee extract sample. (These last two weights should be chosen to give test portions containing approximately 0.5 g of dried coffee extract.) Transfer sample to a 100 ml beaker add 5 ml of ammonia solution (4.4) and warm for two minutes on the boiling water bath (5.4.1). Add 6 g of Celite (4.3) and mix carefully.

6.2. Filling of the columns

6.2.1. *Column I* (alkaline column)

Layer A. Mix carefully, by kneading with a flexible spatula blade, 3 g of Celite (4.3) and 2 ml of the sodium hydroxide solution (4.2), until homogeneous (see note below). A slightly wet powder will be obtained. Transfer this powder, in small portions (of approximately 2 g), into a 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 internal 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 quantities in advance and stored in closed containers.

Layer B. Transfer the Celite-sample mixture (6.1) into the column upon layer A. Dry the sample beaker twice with portions of about 1 g of Celite (4.3) transferring this Celite into the column. Tamp down to obtain a homogeneous layer and place a wad of cotton wool or glass wool on the top of layer B.

6.2.2. *Column II* (acid column)

Place in a second column, the lower part of which is packed with a wad of cotton wool or glass wool, 3 g of Celite (4.3) and 3 ml of the sulphuric acid solution (4.1), carefully mixed as directed for layer A in 6.2.1 (see the note in that sub-clause). Place a wad of cotton wool or glass wool on the top of the layer to prevent damage to the top of the column packing.

6.3. 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. Keep open the stopcock in column I. Adjust the stopcock of column II so that a quantity of supernatant liquid remains above the column packing. 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 through column II with the stopcock open (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 note below). Elute column II with 45 to 50 ml of chloroform (4.7). Collect the eluate in a 50 ml volumetric flask (5.4.2), make up to volume with 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 through the packing should be suspected.

Note: This step 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.

6.4. **Spectrophotometric measurement** (see figure 2)

6.4.1. *Measurement of the test solution*

Avoiding error from chloroform evaporation, measure the absorbance of the solution of caffeine in chloroform (6.3) using silica cells (5.3), against chloroform (4.7) at 276 nm (absorption maximum). Measure also the absorbance at 246 nm (absorption minimum) and at 306 nm in order to verify the purity of the caffeine obtained.

If the absorbance at 276 nm exceeds 1.3, repeat the measurement on a diluted portion of the test solution. In this case, take into account the dilution factor; the appropriate factors of the formulae in 7.1 will have to be adjusted accordingly. If the absorbance measured at 276 nm is lower than 0.2, repeat the determination using a larger test portion.

6.4.2. *Preparation and measurement of a reference solution*

Prepare a reference solution of caffeine in the following manner:

Weigh, to the nearest 0.1 mg; 100 ± 20 mg of pure anhydrous caffeine (4.6). Place in a 1 000 ml volumetric flask (5.4.2), dissolve in chloroform, and make up to volume. With a volumetric pipette (5.4.3), take 5 ml of this solution and make up the volume to 50 ml with chloroform.

Measure the absorbance of this solution as described in 6.4.1. The corrected absorbance of the reference solution should be in the region of 0.4.

6.5. **Number of determinations**

Carry out at least two determinations on the same test sample.

6.6. **Blank test**

Carry out a blank test on the reagents following the procedure described above but omitting the test portion. Before using recovered reagents (see 4.5 and 4.7), repeat the blank test to verify their purity.

7. EXPRESSION OF RESULTS

7.1. **Formulae and method of calculation**

The caffeine content, in percent by mass of dry matter on the sample, is equal to:

$$\frac{5 \times 10^3 \times C \times A_1}{A_2 \times m \times p}$$

where:

C is the concentration of caffeine in the reference solution (6.4.2) in g/ml;

A_1 is the corrected absorbance of the purified extract (6.4.1) [i.e. absorbance at 276 nm – 0.5x (absorbance at 246 nm + absorbance at 306 nm)];

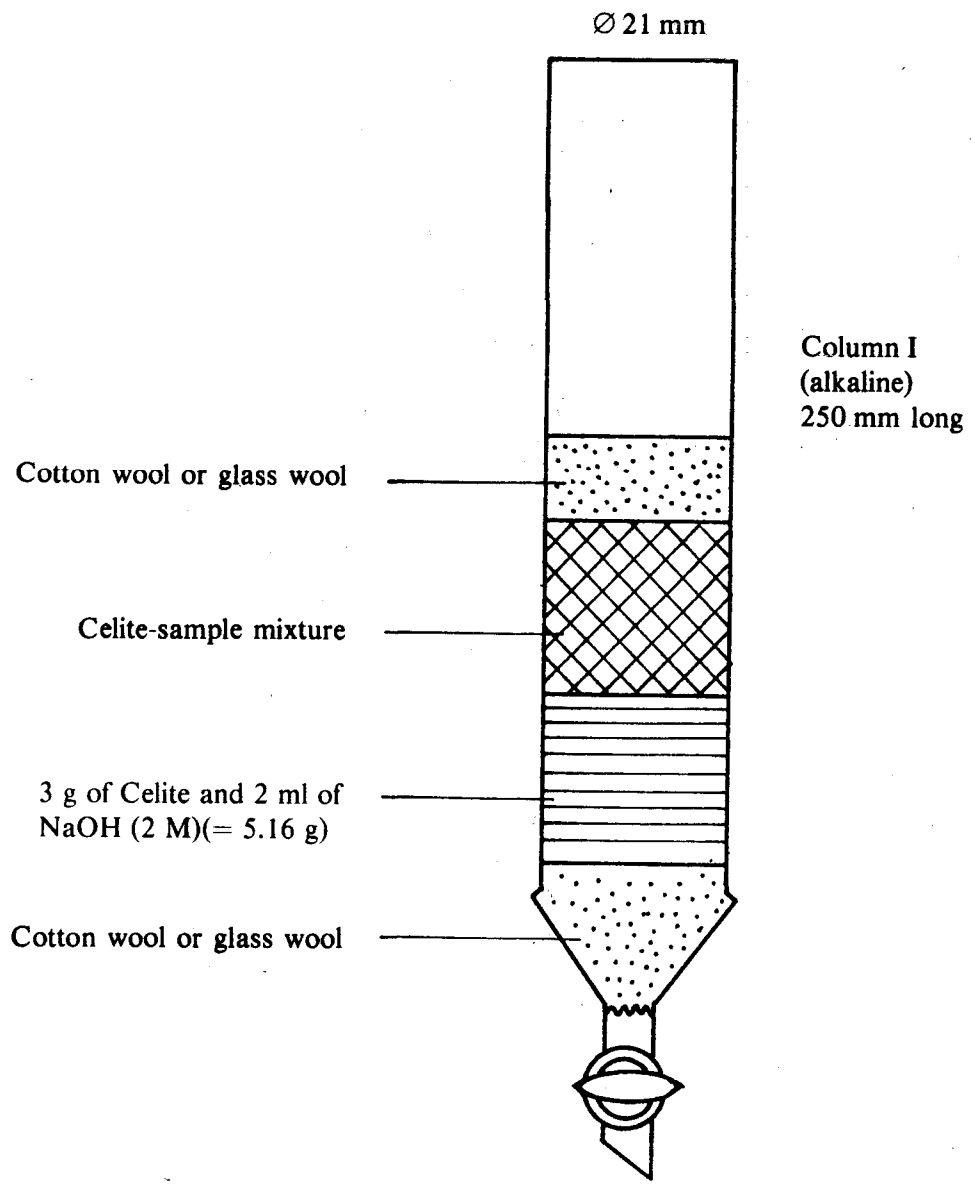
A_2 is the corrected absorbance of the caffeine reference solution (6.4.2) [i.e. absorbance at 276 nm – 0.5x (absorbance at 246 nm + absorbance at 306 nm)];

m is the mass in g of the test portion;

p is the dry matter content, expressed as a percentage by mass, of the sample, as determined by methods 2 or 3, Annex II.

7.2. **Repeatability**

The difference between the results of two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 0.01 g caffeine per 100 g sample on a dry matter basis.



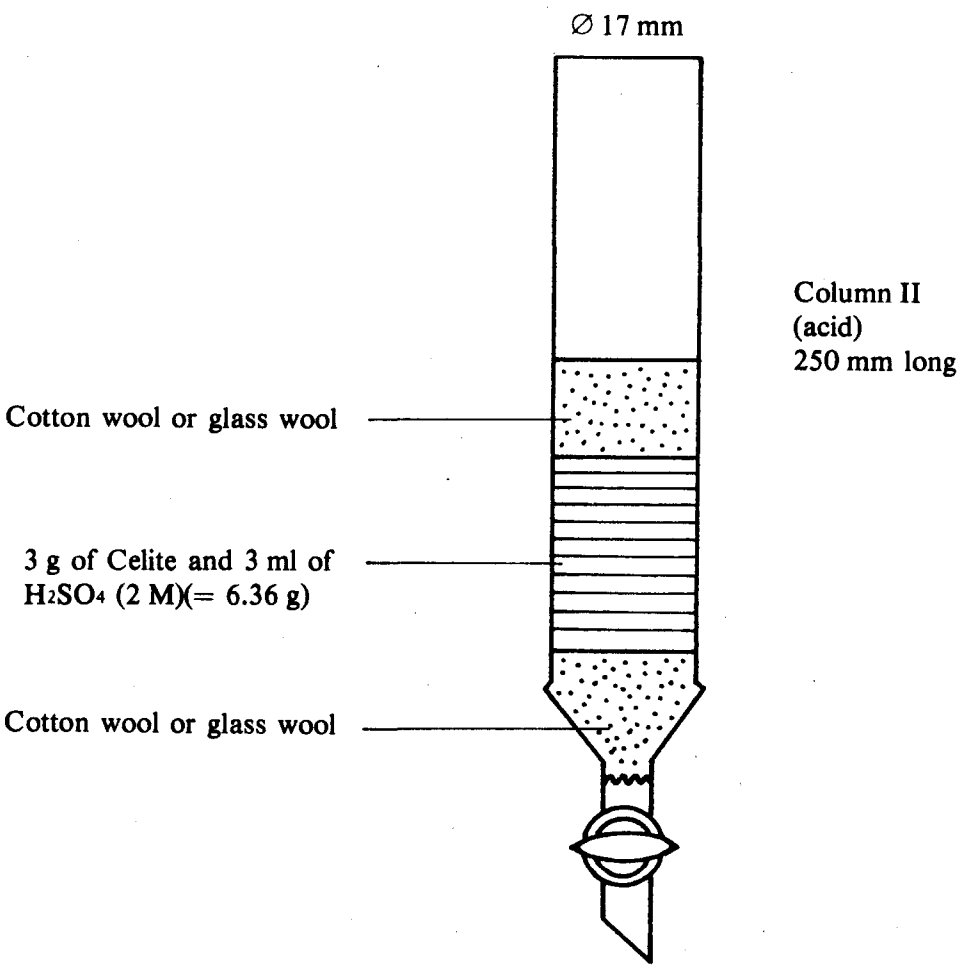


Figure 1

Chromatographic columns

Cells: silica with 10 mm optical path length
Solvent: chloroform
Blank: chloroform

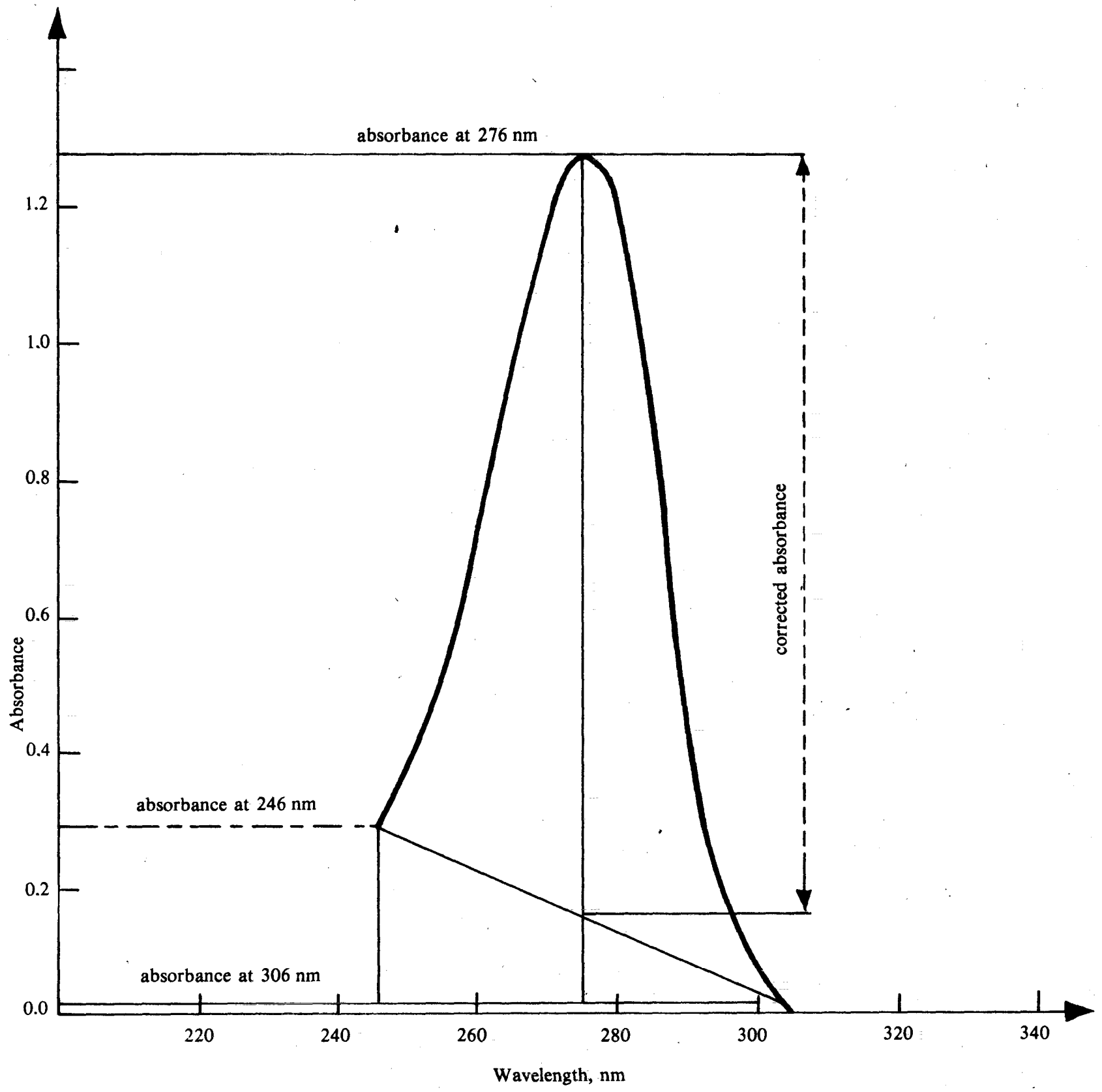


Figure 2

Spectrophotometric measurement

METHOD 2: DETERMINATION OF DRY MATTER CONTENT

1. SCOPE AND FIELD OF APPLICATION

This method determines the dry matter content of:

— dried coffee extract, soluble coffee, instant coffee,
— dried chicory extract, soluble chicory, instant chicory.

2. DEFINITION

Dry matter content: the content of dry matter as determined by the method specified.

3. PRINCIPLE

The residual mass of a test portion is determined after drying for 16 hours in a vacuum oven at a temperature of 70 °C and a pressure of 5.0 kPa and calculated as a percentage by mass of the sample.

4. APPARATUS

4.1. Weighing dishes, flat bottomed, resistant to attack by the sample and condition of the test, approximate dimensions 50 mm diameter by 30 mm height with closely fitting lids. Aluminium and stainless steel dishes are suitable.

4.2. Vacuum oven, electrically heated, temperature controlled by thermostat at 70 °C ± 1 °C throughout the volume of the oven equipped with a thermometer with a certificate of accuracy at 70 °C, indicating the temperature in the immediate vicinity of the shelf and a gauge indicating the internal pressure in kPa above zero pressure. This oven should have a uniform internal temperature distribution. The shelves shall be constructed and fitted so as to ensure good heat transfer to the dishes (4.1).

4.3. Drying oven, electrically heated, temperature controlled by thermostat at 102 °C ± 2 °C throughout the volume of the oven.

4.4. Vacuum pump capable of evacuating the vacuum oven (4.2) to an internal pressure of 5.0 kPa or less.

4.5. Air drying train, consisting of two glass washing bottles filled with glycerol to form a bubble train and two glass drying towers filled with freshly activated silica gel with a moisture content indicator. The bubble train and drying trains are connected in series with the vacuum oven (4.2) with the drying towers between the oven and the bubble train.

4.6. Desiccator, containing freshly activated silica gel (or an equivalent desiccant) with a moisture content indicator.

4.7. Analytical balance.

5. PROCEDURE

5.1. **Preparation of the dishes**

Place the clean dry empty dishes and lids (4.1) in a drying oven (4.3) set at 102 °C ± 2 °C for one hour. Covers should be placed beside dishes in order to expose all surfaces for drying.

Remove dishes and lids from the oven and place in a desiccator (4.6). Allow to cool and weigh dish and matching lid to the nearest 0.1 mg (M_0).

5.2. **Test portion**

Remove the lid from the prepared dish (5.1). As rapidly as possible place approximately 3 g of sample in dish and spread uniformly over the bottom of the dish. Cover dish with its matching lid and weigh with its contents to the nearest 0.1 mg (M_2). If more than one weighing is to be made place covered dishes in desiccator until all samples have been weighed and are ready to be placed in oven.

5.3. Place the dish and its lid separately in the vacuum oven (4.2).

5.4. Close the oven and reduce the pressure slowly (at least 2 to 2.5 minutes), to 5.0 ± 0.1 kPa.

5.5. Allow dry air to enter the oven slowly through the drying towers and bubble train (4.5) at about one bubble per second as observed in the liquid in the bubble train.

5.6. Dry in the vacuum oven at 70 °C ± 1 °C for 16 ± 0.5 hours maintaining the air stream.

5.7. At the end of the drying period allow the air to enter the oven slowly (two to three minutes) to prevent air turbulence that might cause some sample to be lost from dish.

Replace lid on matching dish and place covered dish in desiccator (4.6) and allow to cool to ambient temperature.

5.8. Weigh, to 0.1 mg, covered dish and contents (M_1).

6. EXPRESSION OF RESULTS

6.1. Formula and method of calculation

The content of dry matter calculated as a percentage by mass of the prepared sample, as given by:

$$\frac{M_1 - M_0}{M_2 - M_0} \times 100$$

where:

M_0 = mass of the dry prepared dish and lid;

M_1 = mass of the dish lid and test portion after drying;

M_2 = mass of the dish, lid and test portion before drying.

Take as the result the arithmetic mean of the results of two determinations provided that the evaluation concerning repeatability (6.2) is satisfied.

6.2. Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 0.06 g dry matter per 100 g of sample.

METHOD 3: DETERMINATION OF DRY MATTER CONTENT

1. SCOPE AND FIELD OF APPLICATION

This method determines the dry matter content of:

— liquid coffee extract,
— liquid chicory extract,
— coffee extract paste,
— chicory extract paste.

2. DEFINITION

Dry matter content: the content of dry matter as determined by the method specified.

3. PRINCIPLE

Test portions of the samples are mixed with sea sand and then dried for 16 hours in a vacuum oven at a temperature of 70 °C and a pressure of 5 kPa. The residual mass is calculated as a percentage by mass of the sample.

4. REAGENTS

Sea sand, washed in acid and then water till acid free and then ignited.

5. APPARATUS

5.1. Weighing dishes, flat bottomed, resistant to attack by the sample and condition of the test, approximately 80 mm in diameter and with closely fitting lids.

5.2. Glass rods of such a length that they lie wholly in the weighing dishes (5.1) for example 50 to 75 mm long.

5.3. Vacuum oven, electrically heated, temperature controlled by thermostat at 70 °C ± 1 °C throughout the volume of the oven, equipped with a thermometer with a certificate of accuracy at 70 °C, indicating the temperature in the immediate vicinity of the shelf and a gauge indicating the internal pressure in kPa above zero pressure.

This oven shall have a uniform internal temperature distribution. The shelves shall be constructed and fitted so as to ensure good heat transfer to the dishes (5.1).

5.4. Vacuum pump capable of evacuating the vacuum oven (5.3) to an internal pressure of 5.0 kPa or less.

5.5. Air drying train consisting of two glass washing bottles filled with glycerol to form a bubble train and two glass drying tubes filled with freshly activated silica gel with a moisture content indicator. The bubble train towers are connected in series with the vacuum oven (5.3) with the drying towers between the oven and the bubble train.

5.6. Desiccator containing freshly activated silica gel (or an equivalent desiccant) with a moisture content indicator.

5.7. Analytical balance.

5.8. Water bath boiling.

6. PROCEDURE

6.1. **Preparation of weighing dish**

Place 25 to 35 g of sea sand (4) in a weighing dish (5.1) together with a glass rod (5.2) and weigh. Place the dish with sea sand lid and rod in the vacuum oven (5.3).

The lid should be placed beside the dish in order to expose all surfaces for drying.

Remove the dish, with contents, and its lid from the oven and place in a desiccator (5.6).

Allow to cool and weigh, to the nearest 0.1 mg, dish, contents and matching lid.

Repeat till constant weight is obtained (M_0).

6.2. **Test portion**

Remove the lid from the prepared dish (6.1). Add (as rapidly as possible) a portion of the sample with a dry matter content which should correspond to 0.1 to 1 g.

Weigh, to 0.1 mg, dish, contents, including test portion, and lid (M_2).

6.3. Carefully mix the sea sand and the sample with the glass rod (5.2). If mixing is not carried out satisfactorily add a little water to aid the mixing. Re-heat on a water bath (5.8) stirring occasionally until a completely homogeneous sandy mixture is obtained. Should the mixture tend to become lumpy or crusty, stir or pound continuously so as to prevent any lumpiness.

6.4. Place the dish and its lid separately in the vacuum oven (5.3).

6.5. Close the oven and reduce the pressure slowly (at least 2 to 2.5 minutes) to 5.0 ± 0.1 kPa.

6.6. Allow dry air to enter the oven slowly through the drying towers and bubble train (5.5) at about one bubble per second as observed in the liquid of the bubble train.

6.7. Dry in the vacuum oven at 70 °C ± 1 °C for 16 ± 0.5 hours maintaining the air stream.

6.8. At the end of the drying period allow the air to enter the oven slowly (two to three minutes) to prevent air turbulence that might cause some sample to be lost from the dish.

Replace lid on matching dish and place covered dish in desiccator (5.6) and allow to cool to ambient temperature.

6.9. Weigh, to 0.1 mg, covered dish and contents (M_1).

7. EXPRESSION OF RESULTS

7.1. Formula and method of calculation

The content of dry matter, calculated as a percentage by mass of the prepared sample, is given by:

$$\frac{M_1 - M_0}{M_2 - M_0} \times 100$$

where:

M_0 = the mass of the prepared dish, and lid;

M_1 = the mass of the dish, lid and test portion after drying;

M_2 = the mass of the dish, lid and test portion before drying.

Take as a result the arithmetic mean of the results of two determinations provided that the evaluation concerning repeatability (7.2) is satisfied.

7.2. Repeatability

The difference between the results of two determinations when carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 0.06 g dry matter per 100 g of sample.