

Determination of substances characteristic of green and black tea —

Part 2: Content of catechins in green tea — Method using high-performance liquid chromatography

ICS 67.140.10

National foreword

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Summary of pages

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Determination of substances characteristic of green and black tea —

Part 2:

Content of catechins in green tea — Method using high-performance liquid chromatography

*Détermination des substances caractéristiques du thé vert et du thé
noir —*

*Partie 2: Dosage des catechins dans le thé vert — Méthode par
chromatographie en phase liquide à haute performance*



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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.

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Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 14502-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 8, *Tea*.

ISO 14502 consists of the following parts, under the general title *Determination of substances characteristic of green and black tea*:

- *Part 1: Content of total polyphenols in tea — Colorimetric method using Folin-Ciocalteu reagent*
- *Part 2: Content of catechins in green tea — Method using high-performance liquid chromatography*

Determination of substances characteristic of green and black tea —

Part 2: Content of catechins in green tea — Method using high-performance liquid chromatography

1 Scope

This part of ISO 14502 specifies a high-performance liquid chromatographic (HPLC) method for the determination of the total catechin content of tea from the summation of the individual catechins. It is applicable to both leaf and instant green tea, and with precision limitations to black tea (see Annex A).

Gallic acid and caffeine can also be determined by this method, as can theogallin and theaflavins.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 1572, *Tea — Preparation of ground sample of known dry matter content*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 7513, *Instant tea in solid form — Determination of moisture content (loss in mass at 103 °C)*

3 Principle

The total catechin content from a test portion of finely ground leaf tea is extracted with 70 % methanol at 70 °C. Instant teas are dissolved in hot water with 10 % (volume fraction) acetonitrile added to stabilize the extract. The individual catechins in the extract are determined by HPLC on a phenyl-bonded column using gradient elution with UV detection at 278 nm. External standards are used for quantitation. External catechin standards of defined purity and moisture content may be used directly. Alternatively, caffeine may be used as a standard in conjunction with individual catechin Relative Response Factors (RRFs) established by an ISO international interlaboratory test.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

4.1 Water, conforming to grade 1 of ISO 3696:1987.

4.2 Acetonitrile, HPLC grade.

4.3 Methanol.

4.4 Acetic acid, glacial HPLC grade.

4.5 Methanol/water extraction mixture, 70 % methanol (volume fraction).

Add 700 ml of the methanol (4.3) to a 1 litre one-mark volumetric flask. Dilute to the mark with water (4.1) and mix.

4.6 EDTA solution, 10 mg/ml.

Weigh $(1,00 \pm 0,01)$ g of EDTA (ethylenediaminetetraacetic acid disodium salt, dihydrate) into a 100 ml one-mark volumetric flask. Add sufficient warm water to half-fill the flask. Swirl to dissolve the EDTA, cool to room temperature, dilute to the mark with water and mix.

Prepare a fresh solution daily.

4.7 Ascorbic acid solution, 10 mg/ml.

Weigh $(1,00 \pm 0,01)$ g of L-ascorbic acid into a 100 ml one-mark volumetric flask. Dissolve in water, dilute to the mark and mix.

Prepare a fresh solution daily.

4.8 Stabilizing solution, 10 % (volume fraction) acetonitrile with 500 µg/ml of EDTA and ascorbic acid.

Using a pipette, transfer 25 ml of EDTA solution (4.6), 25 ml ascorbic acid solution (4.7) and 50 ml of acetonitrile (4.2) to a 500 ml one-mark volumetric flask. Dilute to the mark with water and mix.

Prepare a fresh solution daily.

4.9 HPLC mobile phases.

SAFETY PRECAUTIONS — Wear gloves, eye protection and dispense reagents in a fume cupboard.

4.9.1 Mobile phase A, 9 % (volume fraction) acetonitrile, 2 % (volume fraction) acetic acid with 20 µg/ml EDTA.

Transfer 180 ml of acetonitrile (4.2) and 40 ml of acetic acid (4.4) to a 2 litre one-mark volumetric flask. Add sufficient water to half-fill the flask and add 4,0 ml of EDTA solution (4.6). Dilute to the mark with water, mix and filter through a filter of 0,45 µm pore size (5.10).

4.9.2 Mobile phase B, 80 % (volume fraction) acetonitrile, 2 % (volume fraction) acetic acid with 20 µg/ml EDTA.

Transfer 800 ml of acetonitrile (4.2) and 20 ml of acetic acid (4.4) into a 1 litre one-mark volumetric flask. Add approximately 100 ml water and 2,0 ml of EDTA solution (4.6). Dilute to the mark with water, mix and filter through a filter of 0,45 µm pore size (5.10).

4.10 Stock standard solutions.

4.10.1 General.

If catechins of known purity are available, they may be used directly as external standards. In addition to the normally quoted HPLC purity, it is important that their moisture contents be also known, as high levels of water of crystallization will not be accounted for in the HPLC assessment. The purity and moisture content data on standards used in the interlaboratory test are given in Annex B. If comprehensive purity data are unavailable or cannot be determined, catechin materials should only be used as marker compounds to aid identification. In these circumstances, quantitation may be achieved using a caffeine external standard in conjunction with

consensus individual catechin RRF values (with respect to caffeine) obtained from interlaboratory tests (see 9.3 and Reference [3]).

4.10.2 Caffeine stock standard solution, corresponding to 2,00 mg/ml.

Weigh $(0,200 \pm 0,001)$ g of anhydrous caffeine into a 100 ml one-mark volumetric flask. Add sufficient warm water to half-fill the flask. Swirl to dissolve the caffeine then cool to room temperature. Dilute to the mark with water and mix.

4.10.3 Gallic acid stock standard solution, corresponding to approximately 1,00 mg/ml of anhydrous gallic acid.

Weigh $(0,110 \pm 0,001)$ g of gallic acid monohydrate (M.W. 188,14) into a 100 ml one-mark volumetric flask. Dissolve in water, dilute to the mark and mix.

Prepare fresh standard solution daily.

Gallic acid monohydrate is preferred over anhydrous, due to its greater solubility, reduced hygroscopic properties and availability of certified reagent grades, e.g. A.C.S., which is used to denote chemicals that meet specifications set by the American Chemical Society. If not known, the moisture content (loss in mass at 103 °C) should be determined on a portion of the standard material.

4.10.4 Preparation of individual catechin stock standard solutions

Accurately weigh the amounts of standards given in Table 1 into separate one-mark volumetric flasks. Dissolve in stabilizing solution (4.8), gently warming if necessary (max. 40 °C). Cool to room temperature, dilute to the mark with stabilizing solution and mix.

Table 1 — Catechin stock standard solutions

Standard component	Mass of standard g	Volume of stabilizing solution ml	Nominal concentration of stock standard mg/ml
(+)-Catechin	$0,020 \pm 0,001$	20	1,0
(-)-Epicatechin	$0,020 \pm 0,001$	20	1,0
(-)-Epigallocatechin	$0,040 \pm 0,001$	20	2,0
(-)-Epigallocatechin gallate	$0,040 \pm 0,001$	20	2,0
(-)-Epicatechin gallate	$0,040 \pm 0,001$	20	2,0

Where sufficient quantities (i.e. > 20 mg) are available, an analytical balance capable of weighing to an accuracy of at least 0,1 mg is required for the preparation of the individual stock standard solutions. For limited quantities (i.e. < 20 mg), an analytical balance capable of weighing to 0,01 mg is required.

4.11 Dilute standard solutions.

4.11.1 Dilute gallic acid standard solution, corresponding to approximately 200 µg/ml.

Using a pipette, transfer 20 ml of the gallic acid stock standard solution (4.10.3) to a 100 ml one-mark volumetric flask. Dilute to the mark with stabilizing solution (4.8) and mix.