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GB/T 8313-2018

Replacing GB/T 8313-2008

**Determination of total polyphenols and catechins
content in tea**

茶叶中茶多酚和儿茶素类含量的检测方法

(ISO 14502-1:2005, Determination of substances characteristic of green and black tea - Part 1: Content of total polyphenols in tea - Colorimetric method using Folin-Ciocalteu agent; ISO 14502-2:2005, Determination of substances characteristic of green and black tea - Part 2: Content of catechins in green tea - Method using high-performance liquid chromatography, NEQ)

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Determination of total polyphenols and catechins content in tea

1 Scope

This standard specifies a method for the determination of catechins in tea, by high-performance liquid chromatography (HPLC), AND the method for the determination of polyphenols in tea, by spectrophotometry.

This standard applies to the determination of catechins and polyphenols, in tea and tea products.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

GB/T 8302 Tea - Sampling (GB/T 8302-2013, ISO 1839:1980, NEQ)

GB/T 8303 Tea - Preparation of ground sample and determination of dry matter content (GB/T 8303-2013, ISO 1572:1980, MOD)

3 Detection of catechins in tea - HPLC method

3.1 Principle

The catechins in the ground tea sample are extracted by 70% methanol aqueous solution, on a 70 °C water bath. The catechins are determined by C₁₈ column, detection wavelength 278 nm, gradient elution, HPLC analysis; it is directly quantified by the external standard method of catechins standard materials, OR otherwise quantified by the relative correction factor (RRF_{Std}) of catechins and caffeine in the results of the ISO international ring test.

3.2 Instruments

3.2.1 Analytical balance: Accuracy 0.0001 g.

(EGCG) 2.00 mg/mL, epicatechin gallate (ECG) 2.00 mg/mL.

3.3.11 Standard working solution: Prepared by the stable solution (3.3.8).

Standard working solution's concentration: Gallic acid 5 µg/mL ~ 25 µg/mL, caffeine 50 µg/mL ~ 150 µg/mL, +C 50 µg/mL ~ 150 µg/mL, EC 50 µg/mL ~ 150 µg/mL, EGC 100 µg/mL ~ 300 µg/mL, EGCG 100 µg/mL ~ 400 µg/mL, ECG 50 µg/mL ~ 200 µg/mL.

3.3.12 0.45 µm organic phase filter membrane.

3.4 Operation method

3.4.1 Sampling

It is carried out according to the provisions of GB/T 8302.

3.4.2 Sample preparation

It is carried out according to the provisions of GB/T 8303.

3.4.3 Determination procedure

3.4.3.1 Determination of dry matter content

It is carried out according to the provisions of GB/T 8303.

3.4.3.2 Preparation of test solution

3.4.3.2.1 Mother liquor: Weigh 0.2 g (accurate to 0.0001 g) of uniformly ground sample (3.4.2), into a 10 mL centrifuge tube. Add 5 mL of 70% methanol aqueous solution (3.3.5), which was pre-heated at 70 °C. Use a glass rod to fully stir, to evenly moisten it. Immediately transfer it into a 70 °C water bath (3.2.2). Extract for 10 minutes (stir it once every 5 minutes). Cool to room temperature after extraction. Transfer it into a centrifuge at 3500 r/min. Centrifuge for 10 min. Transfer the supernatant to a 10 mL volumetric flask. Use 5 mL of 70% methanol aqueous solution, to extract the residue once again. Repeat the above operation. Combine the extracts. Make the volume reach to 10 mL. Shake well. Use a 0.45 µm membrane (3.3.12) to filter it. Prepare for use (the extract can be stored at 4 °C for up to 24 h).

3.4.3.2.2 Test solution: Use a pipette to transfer 2 mL of mother liquor (3.4.3.2.1), into a 10 mL volumetric flask. Use the stable solution (3.3.8) to make its volume reach to the mark. Shake well. Pass through a 0.45 µm membrane (3.3.12), to be tested.

3.4.3.3 Chromatographic conditions

The relative error of the two measured values of the total amount of catechins, in the same sample, shall be $\leq 10\%$. If the relative error of the measured value is within this range, take the arithmetic mean of the two measured values as the result; keep two decimal places.

4 Detection of polyphenols in tea

4.1 Principle

The polyphenols in the ground tea samples are extracted by 70% methanol aqueous solution, in a 70 °C water bath. The Folin phenol reagent oxidizes the -OH groups in the polyphenols and appears blue. The maximum absorption wavelength λ is 765 nm. Gallic acid is used as a calibration standard, to quantify the polyphenols.

4.2 Instruments

4.2.1 Analytical balance: Accuracy of 0.001 g.

4.2.2 Water bath: 70 °C $\pm$ 1 °C.

4.2.3 Centrifuge: Speed 3500 r/min.

4.2.4 Spectrophotometer.

4.3 Reagents

4.3.1 Methanol.

4.3.2 Sodium carbonate (Na_2CO_3).

4.3.3 70% methanol aqueous solution.

4.3.4 Folin-Ciocalteu: 1 mol/L.

4.3.5 10% Folin-Ciocalteu reagent (equipped before use): Transfer 20 mL of Folin-Ciocalteu reagent (4.3.4) into a 200 mL volumetric flask. Use water to make its volume reach to the mark. Shake well.

4.3.6 The 7.5% sodium carbonate (Na_2CO_3) solution: Weigh 37.50 g $\pm$ 0.01 g of sodium carbonate (Na_2CO_3) (4.3.2). Add an appropriate amount of water to dissolve it. Transfer it into a 500 mL volumetric flask. Make its volume reach to the mark. Shake well (it can be stored for 1 month, at room temperature).

4.3.7 Gallic acid standard stock solution (1000 $\mu\text{g/mL}$): Weigh 0.110 g $\pm$ 0.001 g of gallic acid (GA, relative molecular weight 188.14). Dissolve it in a 100 mL volumetric flask. Make its volume reach to the mark. Shake well (prepared

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