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**Tea — Determination of theaflavins  
in black tea — Method using high  
performance liquid chromatography**

*Thé — Détermination des théaflavines dans le thé noir — Méthode  
par chromatographie 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 8, *Tea*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

# Tea — Determination of theaflavins in black tea — Method using high performance liquid chromatography

## 1 Scope

This document specifies a high performance liquid chromatography (HPLC) or ultra-high performance liquid chromatography (UHPLC) method for the determination of content of the four major theaflavins of tea.

It is applicable to both leaf and instant black and oolong teas. The method is currently not validated for ready-to-drink (RTD) beverages.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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, *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 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

## 4 Principle

Extraction of the theaflavins from a test portion of finely ground leaf tea is achieved with 70 % methanol at 70 °C. Instant teas are dissolved in hot water with a volume fraction of 10 % acetonitrile added to stabilize the extract. The individual theaflavins in the extract are determined by HPLC on a reversed-phase column using isocratic elution with UV detection at 274 nm, optional at 375 nm (as an alternative detection wavelength not used in the method validation). External standards are used for quantitation. External theaflavin standards of defined purity and moisture content may be used directly. Alternatively, caffeine may be used as a standard in conjunction with individual theaflavins relative response factors (RRFs) established by an ISO international interlaboratory test (see [Table 3](#)).

## 5 Reagents

### 5.1 General

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

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