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**Determination of hydroxytyrosol  
and tyrosol content in extra virgin  
olive oils — Reverse phase high  
performance liquid chromatography  
(RP-HPLC)**

*Détermination de la teneur en hydroxytyrosol et tyrosol dans les  
huiles d'olive vierges extra — Chromatographie liquide à haute  
performance en phase inverse (CLHP-RP)*



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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 11, *Animal and vegetable fats and oils*.

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).

## Introduction

Biophenolic compounds of a secoiridoid nature and typical of extra virgin olive oil (*Olea europaea* L.), are derived from oleuropein and ligstroside and are correlated to different beneficial health effects for human beings other than particular sensorial characteristics. The biophenolic compounds contain, in an esterified form, two aromatic alcohols, namely hydroxytyrosol and tyrosol. The method given in this document is based on an extraction of the biophenolic fraction with a methanol/water solution and a subsequent hydrolysis reaction to produce free tyrosol and hydroxytyrosol<sup>[1][2]</sup>.



# Determination of hydroxytyrosol and tyrosol content in extra virgin olive oils — Reverse phase high performance liquid chromatography (RP-HPLC)

## 1 Scope

This document specifies a method for the quantitative determination of hydroxytyrosol and tyrosol content in extra virgin olive oils using reverse phase high performance liquid chromatography (RP-HPLC) with spectrophotometric detection. The method is also applicable to all other olive oils of a different commercial category.

## 2 Normative references

There are no normative references in this document.

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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/>

### 3.1

#### hydroxytyrosol and tyrosol

aromatic alcohols present in extra virgin olive oil typical of *Olea europaea* L. species as free or bound form

## 4 Principle

Hydroxytyrosol and tyrosol, present in free and esterified forms, are extracted from the oil with a methanol/water solution and then submitted to hydrolysis reaction with a 10 % of sulphuric acid ethanolic solution. The components are identified by means of HPLC and a spectrophotometric detector at 280 nm. The amount of free aromatic alcohols is calculated with the use of an external standard.

## 5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade.

**5.1 Ortho-phosphoric acid**, a volume fraction of 85 %.

**5.2 Methanol chromatographic grade.**

**5.3 Acetonitrile chromatographic grade.**

**5.4 Water chromatographic grade.**

**5.5 Ethanol**, a volume fraction of 96 %.