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**Infant formula and adult  
nutritionals — Determination of  
 $\beta$ -carotene, lycopene and lutein  
by reversed-phase ultra-high  
performance liquid chromatography  
(RP-UHPLC)**

*Formules infantiles et produits nutritionnels pour adultes —  
Détermination du bêta-carotène, du lycopène et de la lutéine par  
chromatographie liquide ultra haute performance à phase inversée*



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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*, in collaboration with AOAC INTERNATIONAL. It is being published by ISO and separately by AOAC INTERNATIONAL. The method described in this document is equivalent to the AOAC Official Method 2016.13: *Determination of Lutein,  $\beta$ -Carotene, and Lycopene in Infant Formula and Adult Nutritionals by Ultra-High-Performance Liquid Chromatography: Final Action ( $\beta$ -Carotene and Lycopene Only)*.

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

Lutein,  $\beta$ -carotene and lycopene are among the carotenoids present in human milk and are added to infant formula and adult nutritionals<sup>[1][2][3]</sup>. Lutein may play a role in vision and cognitive function, and  $\beta$ -carotene has provitamin A activity<sup>[4][5]</sup>. Accurate and precise measurements of these added ingredients are important for ensuring their presence in the allowable ranges.

This analytical method was originally presented to the Stakeholder Panel on Infant Formula and Adult Nutritionals through AOAC International, and a single-laboratory validation was previously published<sup>[6]</sup>. It was recommended as an AOAC Final Action method for  $\beta$ -carotene and lycopene after the collaborative study data was reviewed by the same panel<sup>[7]</sup>.



# Infant formula and adult nutritionals — Determination of $\beta$ -carotene, lycopene and lutein by reversed-phase ultra-high performance liquid chromatography (RP-UHPLC)

**WARNING** — The use of this method can involve hazardous materials, operations and equipment. This method does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices.

## 1 Scope

This document specifies a method for the quantitative determination of  $\beta$ -carotene and lycopene in infant formula and adult nutritionals in solid (i.e. powders) or liquid (i.e. ready-to-feed liquids and liquid concentrates) forms using reversed-phase ultra-high performance liquid chromatography (RP-UHPLC) and UV-visible detection. The application range runs from 1  $\mu\text{g}/100\text{ g}$  to 1 500  $\mu\text{g}/100\text{ g}$  for lycopene and from 1  $\mu\text{g}/100\text{ g}$  to 2 250  $\mu\text{g}/100\text{ g}$  for  $\beta$ -carotene. Based on the single-laboratory validation, the limit of detection (LOD) was 0,1  $\mu\text{g}/100\text{ g}$  and the limit of quantification (LOQ) was 0,3  $\mu\text{g}/100\text{ g}$  for each carotenoid.

The method does not apply to materials that contain measurable levels of  $\beta$ -apo-8'-carotenal. The reproducibility data meets the requirements given in References [8] and [10].

[Annex C](#) specifies the determination of lutein. The reproducibility data does not meet the requirements given in Reference [9].

## 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

#### **adult nutritional**

nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment, made from any combination of milk, soy, rice, whey, hydrolysed protein, starch and amino acids, with and without intact protein

### 3.2

#### **infant formula**

breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding

[SOURCE: Codex Standard 72-1981]