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**Fortified milk powders, infant  
formula and adult nutritionals —  
Determination of total biotin by  
liquid chromatography coupled with  
immunoaffinity column clean-up  
extraction**

*Poudres de lait fortifié, formules infantiles et produits nutritionnels  
pour adultes — Détermination de la teneur en biotine totale  
par chromatographie liquide après une purification sur colonne  
d'immunoaffinité*



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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 2016.02: *Determination of Total Biotin by Liquid Chromatography Coupled with Immunoaffinity Column Cleanup Extraction: Multilaboratory Testing, Final Action 2016.02*.

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).

# Fortified milk powders, infant formula and adult nutritionals — Determination of total biotin by liquid chromatography coupled with immunoaffinity column clean-up extraction

**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 and determine the applicability of regulatory limitations prior to use.

## 1 Scope

This document specifies a method for the quantitative determination of biotin and/or biocytin in fortified milk powders, infant formula and adult nutritionals in solid (i.e. powders) or liquid (i.e. ready-to-feed liquids and liquid concentrates) forms using liquid chromatography coupled with immunoaffinity column clean-up extraction.

Precision data from an interlaboratory study is given in [Annex B](#). A comparison between data obtained with the method in this document and EN 15607 is given in [Annex C](#).

## 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]

## 4 Principle

The sample is dispersed in sodium phosphate buffer and autoclaved at  $121\text{ °C} \pm 2\text{ °C}$  for 25 min. The sample is cooled to room temperature and then diluted to 100 ml in a volumetric flask. The extract is centrifuged and filtered using a glass microfibre filter. Clear filtrate is collected for clean-up and