



BSI Standards Publication

**Infant formula and adult nutritionals —
Determination of free and total choline and free
and total carnitine — Liquid chromatography
tandem mass spectrometry (HPLC-MS/MS)**

National foreword

This British Standard is the UK implementation of [ISO 21468:2020](#).

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Infant formula and adult nutritionals — Determination of free and total choline and free and total carnitine — Liquid chromatography tandem mass spectrometry (HPLC-MS/MS)

*Formules infantiles et produits nutritionnels pour adultes —
Détermination de la teneur en choline totale et la teneur en carnitine
par chromatographie en phase liquide et spectrométrie de masse en
tandem (CL-SM/SM)*



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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).

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).

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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.

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 2015.10: *Determination of Free and Total Choline and Free and Total Carnitine in Infant Formula and Adult/Pediatric Nutritional Formula by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)*.

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.

Infant formula and adult nutritionals — Determination of free and total choline and free and total carnitine — Liquid chromatography tandem mass spectrometry (HPLC-MS/MS)

1 Scope

This document specifies a method for the determination of total or free choline and carnitine in infant formula and adult nutritionals by liquid chromatography and tandem mass spectrometry (HPLC-MS/MS).

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

Samples are extracted in water for free carnitine and choline. For total carnitine and choline, samples are digested with nitric acid and microwave-assisted heating. Free and total extracts are both diluted with water, mixed with acetonitrile, and analysed using liquid chromatography (LC) with tandem mass spectrometric (MS/MS) detection.

5 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and high purity MS grade water or water of equivalent purity.

Non-specific binding can occur with these analytes when using glassware, so plasticware should be used at all times for standard/sample preparation. All laboratory plasticware should be single use where possible.

possible. Positive displacement pipets are also mandatory for pipetting to avoid contamination and for accuracy with organic solvents.

5.1 Water, purified, MS grade or equivalent purity.

5.2 Acetonitrile, MS grade.

5.3 Ammonium formate, MS grade or equivalent.

5.4 Formic acid, MS grade or equivalent.

5.5 Nitric acid, a mass fraction of 70 %, ACS grade or equivalent.

WARNING — All preparation steps with nitric acid should be performed within a fume hood. The necessary personal protective equipment should be used when handling.

5.6 Isopropanol, MS grade or equivalent.

5.7 Desiccant.

5.8 L-carnitine, inner salt, primary reference standard. Store in a desiccator.

5.9 Choline bitartrate, primary reference standard.

5.10 L-carnitine-d₃ HCL, primary reference standard.

5.11 Choline-1,1,2,2-d₄ chloride, primary reference standard.

6 Preparation of solutions

CAUTION — All mobile phase bottles should be rinsed thoroughly prior to use with purified water and isopropanol.

6.1 Mobile phase A

A mixture of 1 part per volume of 5 mmol/l ammonium formate in water and 1 part per volume of acetonitrile, with 0,2 % formic acid.

Weigh 0,32 g ± 0,01g of ammonium formate into a 1 l bottle ([7.16](#)) containing 500 ml of purified water. Add a stir bar and then mix on a stir plate until dissolved. Add 500 ml of acetonitrile and 2,00 µl of formic acid to the mobile phase container. Mix on a stir plate until thoroughly mixed, typically for about 2 min. The solution is stable for two weeks when stored at room temperature.

6.2 Mobile phase B

A mixture of 1 part per volume of 30 mmol/l of ammonium formate in water and 1 part per volume of acetonitrile, with 0,2 % formic acid.

Weigh 1,89 g ± 0,01g of ammonium formate into a 1 l mobile phase bottle containing 500 ml of purified water. Add a stir bar and then mix on a stir plate until dissolved. Add 500 ml of acetonitrile and 2,00 µl of formic acid to the mobile phase container. Mix on a stir plate until thoroughly mixed, typically for about 2 min. The solution is stable for two weeks when stored at room temperature.