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Infant formula and adult nutritionals — Simultaneous determination of total vitamins B_1, B_2, B_3 and B_6 — Enzymatic digestion and LC-MS/MS

Formules infantiles et produits nutritionnels pour adultes — Détermination simultanée de la teneur en vitamines B_1, B_2, B_3 et B_6 *— Digestion enzymatique et CL-SM/SM*

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Contents

Page

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).

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.

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 2015.14: *Simultaneous Determination of Total Vitamins B_1, B_2, B_3, and B_6 in Infant Formula and Related Nutritionals by Enzymatic Digestion and 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 — Simultaneous determination of total vitamins B_1, B_2, B_3 and B_6 — Enzymatic digestion and LC-MS/MS

1 Scope

This document specifies a method for the simultaneous quantitative determination of four water-soluble vitamins in infant formula and related nutritional products, including relevant forms of vitamins B_1, B_2, B_3 and B_6 by enzymatic digestion and UHPLC-MS/MS. This document is not intended to be used on products where vitamins have not been added.

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 prepared by enzymatic digestion with papain and α-amylase to hydrolyse protein and complex carbohydrate and acid phosphatase to free phosphorylated vitamin forms. Stable-isotope labelled internal standards are incorporated into the sample preparation to correct for variability in both the sample preparation and instrument response. A series of six mixed working standard solutions spanning two orders of magnitude in vitamin concentration are used to generate calibration curves based on the peak response ratio of the analyte to its stable-isotope labelled internal standard.

Prepared samples and working standard solutions are injected onto ultra-high pressure liquid chromatograph (UPLC) interfaced to a triple-quadrupole mass spectrometer (MS/MS) for analysis. The MS/MS is configured to monitor precursor-fragment ion pairs for each analyte and internal standard. This reaction forms the basis for method selectivity. Analytes are quantified by least squares regression using the response ratio of the analyte to its internal standard.