



BSI Standards Publication

**Infant formula — Determination of
 β -galactooligosaccharides — Ultra high performance
liquid chromatography (UHPLC) with fluorescence
detection after pre-column derivatization**

National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/5, Chemical analysis of milk and milk products.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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Infant formula — Determination of β -galactooligosaccharides — Ultra high performance liquid chromatography (UHPLC) with fluorescence detection after pre-column derivatization

*Préparation pour nourrissons — Détermination de la teneur
en β -galacto-oligosaccharides — Chromatographie liquide à
ultra-haute performance (CLUHP) couplée à une détection par
fluorescence après dérivation précolonne*

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Forewords

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC INTERNATIONAL. It is being published jointly by ISO and IDF and separately by AOAC INTERNATIONAL. The method described in this document is equivalent to the AOAC Official Method 2021.01.

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.

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IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

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The work was carried out by the IDF/ISO Action Team on C42 of the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader Mr S. Austin (CH).

Infant formula — Determination of β -galactooligosaccharides — Ultra high performance liquid chromatography (UHPLC) with fluorescence detection after pre-column derivatization

1 Scope

This document specifies a method for the determination of β -galactooligosaccharides (GOS) in infant formula (both powder and liquid) containing 0,2 g/100 g to 3,0 g/100 g of GOS in the product as prepared ready for consumption.

The method has been validated in a multi-laboratory study with reconstituted infant formula at levels of 0,236 g/100 g, 0,594 g/100 g, 0,616 g/100 g and 0,688 g/100 g and infant formula RTF at levels of 0,316 g/100 g and 0,858 g/100 g. During the single laboratory validation study^[1] spike-recovery experiments were performed up to 3 g/100 g in reconstituted infant formula powders (milk-based, partially hydrolysed milk-based and soy-based), and reconstituted adult nutritional powders.

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 terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

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

Powdered or concentrated samples are reconstituted in water and oligosaccharides present in samples are extracted at 70 °C. The ready-to-feed products (including reconstituted concentrates or powders) are diluted and two aliquots of the diluted sample are taken and both are treated with amyloglucosidase to hydrolyse any maltooligosaccharides present (Assay 1), one of the two aliquots is additionally treated with β -galactosidase (Assay 2) to hydrolyse all the GOS present. An internal standard (laminaritrise) is added to both aliquots and the oligosaccharides are fluorescently labelled with 2-aminobenzamide (2AB). Labelled extracts are diluted with acetonitrile prior to injection on an UHPLC-FLD system equipped with a hydrophilic interaction liquid chromatography (HILIC) analytical column. The analytes are separated using a gradient of aqueous ammonium formate in acetonitrile and detected with a fluorescence detector.

An external maltotriose calibration curve is prepared in the same way as the samples but without enzymatic treatment. Since it is the 2AB label that is detected, each oligosaccharide has an equivalent molar response. The maltotriose calibration curve can thus be used to determine the molar concentrations of the