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Infant formula — Determination of nucleotides by liquid chromatography

Formules infantiles — Détermination de la teneur en nucléotides par chromatographie liquide

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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Foreword

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The committee responsible for this document is 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 International Standard is equivalent to the AOAC Official Method 2011.20: *Nucleotides in infant formula*.

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Infant formula — Determination of nucleotides by liquid chromatography

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1 Scope

This International Standard specifies a method for the quantitative determination of 5′-mononucleotides in infant formula in solid (i.e. powders) or liquid (i.e. ready-to-feed liquids and liquid concentrates) forms using liquid chromatography.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
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]

3 Principle

The sample is dissolved in high-salt solution to inhibit protein and fat interactions. The 5′-mononucleotides — uridine 5′-monophosphate (UMP), inosine 5′-monophosphate (IMP), adenosine 5′-monophosphate (AMP), guanosine 5′-monophosphate (GMP), and cytidine 5′-monophosphate (CMP) — are separated from the sample matrix by strong-anion exchange solid-phase extraction (SPE), followed by chromatographic analysis using a C18 stationary phase with gradient elution, UV detection, and quantitation by an internal standard technique using thymidine 5′-monophosphate (TMP).[1]

4 Reagents and materials

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

4.1 Standards, ≥ 99 % pure (Sigma[1)] or equivalent). Nucleotide sodium salts or sodium salt hydrates may be substituted if free acid forms are not readily available.

4.1.1 TMP, thymidine 5′-monophosphate, CAS No. 365-07-1.

4.1.2 AMP, adenosine 5′-monophosphate, CAS No. 61-19-8.

4.1.3 CMP, cytidine 5′-monophosphate, CAS No. 63-37-6.

1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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