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**Milk-based infant formula powders —
Quantification of whey protein content
by sodium dodecyl sulfate-capillary
gel electrophoresis (SDS-CGE)**

*Formules infantiles en poudre à base de lait — Quantification de la
teneur en protéine de lactosérum par électrophorèse capillaire sur gel
contenant du dodécylsulfate de sodium (SDS-CGE)*

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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).

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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*, 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 AOAC 2016.15: *Quantification of Whey Protein Content in Milk-Based Infant Formula Powders by Sodium Dodecyl Sulfate-Capillary Gel Electrophoresis (SDS-CGE), First Action 2016, Final Action 2018*.

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.

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.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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The work was carried out by the IDF/ISO Action Team (C31) of the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader Mr E. Konings (CH).

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Milk-based infant formula powders — Quantification of whey protein content by sodium dodecyl sulfate-capillary gel electrophoresis (SDS-CGE)

1 Scope

This document specifies a method for the determination of the whey to casein protein ratio, ranging from 20:80 to 80:20 in cow milk-based infant formula powders.

This method does not apply to the analysis of infant formulas containing hydrolysed protein or proteins from other sources (e.g. plants or milk from other mammals).

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

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

In sodium dodecyl sulfate-capillary gel electrophoresis (SDS-CGE), proteins in infant formula samples are denatured by the anionic surfactant SDS and reduced by β -mercaptoethanol. The SDS-bonded, electrically charged proteins migrate in an electrical field filled with a separation gel and are detected by UV at 220 nm. Caseins and whey proteins are separated as two distinct, non-overlapping groups of peaks where the ratio can be established based on integrated areas without the need for a calibration curve. In the calculation of whey protein content, 1,4 was used as the mass-to-area correction factor for whey proteins versus caseins.

5 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.

Data from the multi-laboratory study was obtained with chemicals from the SDS-MW kit 390953 from Beckman Coulter/Sciex¹⁾. The SDS-MW gel separation buffer recipe described below (5.1) was tested in a separate study and found to be equivalent to the commercial SDS-MW gel separation buffer included in the kit. One laboratory used home-made reagents (5.2 to 5.5). All laboratories used the 10 kDa internal protein standard (5.6) and the SDS-MW size standard (5.7) from the kit. Use of equivalent standards requires prior verification.

5.1 SDS-MW gel separation buffer, tris(hydroxymethyl)aminomethane (Tris), boric acid, SDS, EDTA, glycerol and dextran 2000¹⁾.

Dissolve 0,726 6 g Tris base and 0,371 0 g boric acid in 9 ml water. Add 20 mg SDS, 1 g dextran 2000, 14,6 mg EDTA, and 1 ml glycerol. Mix thoroughly. Leave overnight to allow complete dextran solubilization.

NOTE Dextran 2000 has an average molecular weight of 2 000 000 Da.

5.2 SDS-MW sample buffer, 100 mmol/l Tris, pH 9,0, 1 % SDS.

Dissolve 109,4 mg of Tris base, 15,2 mg of Tris hydrochloride and 0,1 g of SDS in 10 ml water.

5.3 Acidic wash solution, hydrochloric acid, substance concentration $c = 0,1$ mol/l.

5.4 Basic wash solution, sodium hydroxide, $c = 0,1$ mol/l.

5.5 β -mercaptoethanol.

5.6 10 kDa internal protein standard, mass concentration $\rho = 5$ mg/ml.

5.7 SDS-MW size standard, 10 to 225 kDa, $\rho = 16$ mg/ml

5.8 Sample running pre-solution.

Mix the SDS-MW sample buffer (5.2) with the 10 kDa internal protein standard (5.6) using an 84:1 ratio. Prepare a sufficient volume based on the total number of samples to be analysed in the sample set (90 μ l of sample running pre-solution is needed per sample).

6 Apparatus

Usual laboratory glassware and equipment and, in particular, the following.

6.1 Capillary electrophoresis instrument, equipped with UV detector set at 220 nm and capable of maintaining the capillary and sample tray at $25 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$.

Suitable software should be available for peak integration.

6.2 Bare, fused-silica capillaries, of 50 μ m internal diameter \times 30 cm, with effective length of 20 cm.

6.3 Temperature controlled water bath or heating block, capable of maintaining a temperature of $95 \text{ }^\circ\text{C} \pm 5 \text{ }^\circ\text{C}$.

6.4 Micro centrifuge, with adaptors for 1,5 ml centrifuge vials.

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 either ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6.5 **Vortex.**

6.6 **Adjustable pipettes and tips**, volume of 20 µl, 100 µl and 1 000 µl.

6.7 **Safe-lock micro-centrifuge vials**, volume of 1,5 ml or 2,0 ml.

6.8 **Round-bottom centrifuge tubes**, volume of 10 ml.

6.9 **Analytical balance**, weighing to four decimal places.

7 Procedure

7.1 Sample preparation

7.1.1 Weigh 500 mg ± 20 mg of infant formula powder into a 10 ml centrifuge tube. A skim milk powder (SMP) sample is used as a reference to align the whey and casein regions in the infant formula samples. Prepare the SMP using a sample mass of 135 mg ± 5 mg.

7.1.2 Disperse the sample in 5 ml of water. Vortex each tube until the solution appears homogeneous. Each final solution contains between 10 mg/ml and 15 mg/ml of protein.

7.1.3 Pipette 10 µl of each sample solution into separate microcentrifuge vials.

7.1.4 Sequentially add 85 µl of the sample running pre-solution (5.8) and 5 µl of β-mercaptoethanol (5.5) to each micro-centrifuge vial. Mix well before heating the vials in a water bath or heating block at 95 °C ± 5 °C for 10 min. Cool down to room temperature. Centrifuge at room temperature for 1 min at about 5 000g (approximately 7 000 r/min in a table-top centrifuge).

7.1.5 Vortex before transferring each sample into corresponding injection vials.

7.2 CGE analysis

7.2.1 The separation and the quantification have proven to be satisfactory if the following experimental conditions are followed.

7.2.2 Load the reagents according to the instructions of the capillary electrophoresis instrument manufacturer (see Figure A.1 for an example).

7.2.3 Set up an optimized batch analysis separation method including a reagent blank (10 µl of water replacing the sample solution), a molecular weight size standard (5.7) and a SMP sample.

To prepare the reagent blank, replace the sample solution (see 7.1.3) by 10 µl water. Its function is to monitor any contamination in the buffer or reagents (there should be no peaks in the migration range of milk protein as defined from the 10 kDa internal standard protein). SMP works as a reference to verify the migration times for whey proteins and caseins.

7.2.4 For each separation cycle (45 min), precondition the capillary first with the basic wash solution (5.4) for 3 min, followed by the acidic wash solution (5.3) for 1 min and water for 1 min. Fill the capillary with the SDS-MW gel separation buffer (5.1) for 10 min. Set the separation run time to 30 min.

7.2.5 Introduce the samples electrokinetically by applying voltage at -5 kV for 20 s.