NQAC
Nestlé Quality Assurance Center
Dublin

Technical Datasheet

Analysis Name: Milk Powders and Similar Products
6 HMO in Infant Formula by UHPLC-FLD

Method Number: LI-08.014

Scope of Application: Quantitative determination of human milk oligosaccharides (HMOs) in milk-based infant formula and milk-based formula with partially hydrolysed protein (H.A.) by Ultra High Performance Liquid Chromatography with Fluorescence detection (UHPLC-FLD). The HMOs quantified in this method are: 2'-Fucosyllactose (2'FL), Difucosyllactose, 3'-Sialyllactose, Lacto-N-tetraose, Lacto-N-neotetraose, and 6'-Sialyllactose. The method may not be applicable to products containing galactooligosaccharides (GOS) such as BMOS, Vivinal-GOS, etc. Before analyzing such samples the laboratory should check if the HMOs are separated from the GOS. Quantification ranges of the 2'FL in the two matrices validated in-house are presented in Table 1 for IF powder and liquid formula.

Description: Oligosaccharides (OS) present in samples are extracted in water at 70 °C. An internal standard (IS) is added to the extracted OS and they are fluorescently labelled by reaction of anthranilamide (2AB) with the reducing end of the OS via formation of a Schiff base. The double bond is then reduced by reaction with sodium cyanoborohydride to give a stable OS 2AB derivative. Labelled extracts are diluted with acetonitrile (ACN) prior to injection on an UHPLC system equipped with HILIC analytical columns. Labelled OS are detected by a fluorimeter. Oligosaccharide concentrations are determined from a standard curve, plotting the relative response of the oligosaccharide authentic standard (OS) to the internal standard (Laminaritriose) against oligosaccharide concentration.

Sample Weight Required: 100 g

Analytical Platform: UHPLC-FLR

6625 Eiterman Rd, Dublin, OH 43017 **P** (614)526.5200 **E** nqacdublininfo@us.nestle.com

Analyte Reported	Alias	Unit of Measure	Limit of Quantification in powder	Reproducibility (%)
2'-Fucosyllactose	2'FL	mg/100 g	60	10
Difucosyllactose	DFL	mg/100 g	10	10
3'-Sialyllactose	3'SL	mg/100 g	10	10
Lacto-N-tetraose	LNT	mg/100 g	20	10
Lacto-N-neotetraose	LNnT	mg/100 g	10	10
6'-Sialyllactose	6'SL	mg/100 g	10	10