

Baby Milk Nucleotide Analysis

HPLC analysis of NMP's in baby milk powder

Aim: To determine the content of nucleotides monophosphate in baby milk powder, particularly CMP, UMP, GMP, IMP and AMP

Nucleotides are naturally present in milk (*i.e.* 2-5% of the non-protein nitrogen in Human milk). However, after studies had suggested that nucleotides have positive effects in infant nutrition, manufacturers have got used to adding nucleotides in baby food, for several years now and particularly in milk powder. Unsurprisingly, in a context where food additives are more and more subjected to regulation, nucleotide additions in baby milk powders do or are about to enter under the control of strict rules.

Nucleotides additives in baby food are generally monophosphate nucleotides. **NOVO CIB** proposes to analyze CMP, UMP, GMP, IMP and AMP contents in baby milk. Other nucleotides analysis are available.

Nucleosides & nucleotides separation and analysis: The extraction procedure of nucleotides has been optimized for milk powder analysis. It involves a specific extraction protocol from the powder matrix, a fixation-washing-elution step on a strong anion exchange (SAX, Supelco) cartridge and a filtration through a 0.45µm filter membrane (Roth).

Separation method is based on what is routinely performed for cell extract analysis (ref sheet # NA for further information), with slight adjustments of buffers pH and concentrations. Nucleotides are separated by ion-pairing HPLC (Agilent 1100) system with a Zorbax EclipsePlus C18 column and quantified using an Agilent ChemStation software

Linearity - Limit of Detection

Nucleotides monophosphate (NMP) standards are from Sigma-Aldrich, MPBiochemicals or Roche. 1mg/ml NMP stock solution is prepared in water and kept in the freezer (-20°C). Working standards are prepared daily at concentrations of 0.25, 0.5, 1.25, 2.5, 5, 10, 25 and 37.5 mg/100 g by dilution of stock solution with mobile phase A. The limit of detection is defined as the concentration where RSD (relative standard deviation) is <20%. A typical chromatogram of 5 nucleotide standards is shown in Figure 1. The related linearity and LOD results are shown in table 1.

Table 1. Linearity and LOD of NMP

Nucleotide	R ²	LOD (mg /100g)
CMP	0,9989	0,025
AMP	0,9990	0,025
IMP	0,9992	0,050
UMP	0,9987	0,025
GMP	0,9990	0,025

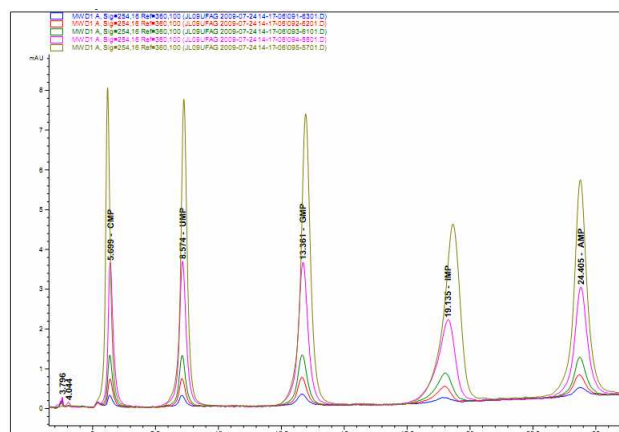


Fig. 1. Superposed spectra of 5 concentrations of standards (0.25, 0.5, 1.25, 2.5 and 5mg/100g).

Recovery and Reproducibility

The extraction of milk powder samples are done with and without spiked NMP (2.5mg of each NMP per 100g powder) for every provided sample.

Table 2. Recovery of 5 spiked NMP's in a baby milk powder (4 measurements)

	Mean	SD
CMP	77%	14%
UMP	94%	16%
GMP	89%	19%
IMP	84%	9%
AMP	97%	19%

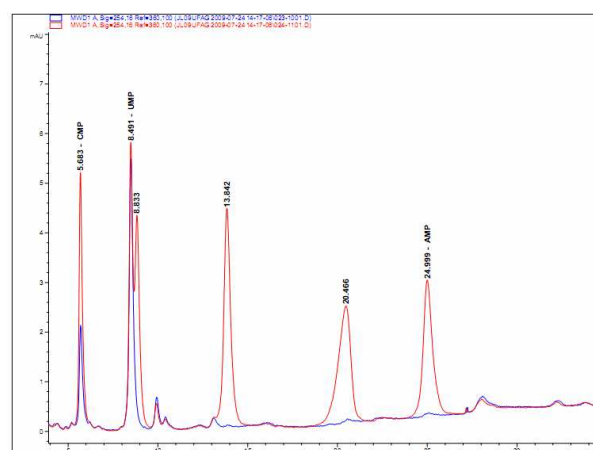


Fig. 2. Superposed chromatograms of milk powder without exogenous nucleotide (blue) and of a milk powder spiked with NMP at 2.5mg/100g (red).

- A. Singhal *et al.* (2008): Dietary nucleotides and fecal microbiota in formula-fed infants: a randomized controlled trial, *Am. J. Clin. Nutr.* 87(6), 1785–1792
J.P. Schaller *et al.* (2007): Ribonucleotides: conditionally essential nutrients shown to enhance immune function and reduce diarrheal in infants, *Semin. Fetal Neonatal. Med* 12(1), 35–44
+ *id.* Corrigendum 12(4), 326–328
A. Lerner and R. Shamir (2000): Nucleotides in infant nutrition: a must or an option? *IMAJ* 2(10), 172–174
O. Brunser *et al.* (1994): Effects of dietary nucleotide supplementation on diarrhoeal disease in infants, *Acta Paediatr.* 83(2), 188–191
J.D. Carver *et al.* (1991): Dietary nucleotide effects upon immune function in infants, *Pediatrics* 88(2), 359–363
C. DeLucchi *et al.* (1987): Effects of dietary nucleotides on the fatty acid composition of erythrocyte membrane lipids in term infants, *J. Pediatr. Gastroenterol. Nutr.* 6(4), 568–574
L.M. Janas and M.F. Picciano (1982): The nucleotide profile of human milk, *Pediatr. Res.* 16(8), 659–662