Anion Exchange Liquid Chromatography for the Determination of Nucleotides in Baby and/or Functional Foods

Pilar Viñas,† Natalia Campillo,‡ Ignacio López-García,† Sara Martínez-López,† Isabel Vasallo,‡ and Manuel Hernández-Córdoba*†

†Departamento de Química Analítica, Facultad de Química, Universidad de Murcia, E-30071 Murcia, Spain, and ‡Instituto de Nutrición Infantil (INUI), Grupo Hero España SA, Alcantarilla, Murcia, Spain

A sensitive, selective and solvent-free procedure is proposed for the rapid determination of monophosphate nucleotides (cytidine 5'-monophosphate, uridine 5'-monophosphate, adenosine 5'-monophosphate, and guanosine 5'-monophosphate) in baby foods. The method is based on the deproteinization of foods and direct analysis by anion exchange liquid chromatography (LC). Nucleotides were separated on an SAX anion exchange column with isocratic elution using 0.01 M dihydrogenphosphate buffer (pH 3.5) as mobile phase at a flow-rate of 1 mL min⁻¹ and detected by diode-array detection (DAD). The LC method rapidly separated the nucleotides (9 min) and was validated for linearity, detection and quantitation limits, selectivity, accuracy, and precision. The recoveries obtained for spiked samples were satisfactory for all the analytes. The proposed procedure allowed the only authorized nucleotides in infant formulas to be determined and was successfully applied to the analysis of different baby and/or functional food samples, including infant formulas, fermented milk, milk, and purees.

KEYWORDS: Anion exchange liquid chromatography; 5'-monophosphate nucleotides; baby foods; infant formulas; functional foods

INTRODUCTION

Childhood constitutes the life period when nutritional demands are at their highest. Baby foods combine a wide range of different matrices (1): nonfatty baby foods based on fruits and vegetables (fat content lower than 2%), fatty foods based on meat/egg/cheese, and cereal-based foods with different fat contents. Moreover, breast milk and infant formulas are also included. Research in the field of child nutrition has established the optimal requirements of proteins, carbohydrates, fats, vitamins, and minerals for each stage of child growth. More recent advances have allowed one to know the importance of other nutrients, such as fatty acids, lipids, and nucleotides, which take part in the processes of brain development, learning, language, and visual growth. Functional foods satisfy the basic nutritional needs and, moreover, provide health benefits; the range of functional products has strongly increased in recent years (2). Dairy functional foods and functional ingredients containing milk represent a growing market (3). Indeed, because of their health-giving properties, traditional dairy products (yoghurt, fermented milk, infant formula, or even milk) can themselves be considered functional foods, as well as containing different ingredients for the formulation of other functional foods.

Nucleotides are monomers which constitute the nucleic acids, DNA and RNA. Each nucleotide is formed by three units, a sugar with five carbon atoms (ribose for RNA or deoxyribose for DNA), a nitrogen base (purine or pyrimidine base), and one or more phosphate groups. The functions attributed to nucleotides are principally immune-, lipidic-, and digestive-related, to emulate breast feeding (4, 5). The addition of nucleotides to infant formulas began in Japan in 1965, and in Spain, the first European country to do so, in 1983 (6). In total, the maximum concentration authorized is 5 mg/100 kcal (0.26 mg g⁻¹), which is equivalent to the amount of free nucleotides in maternal milk (7). Permitted nucleotides are limited to five compounds: cytidine 5'-monophosphate (CMP), 2.5 mg/100 kcal (0.13 mg g⁻¹); uridine 5'-monophosphate (UMP), 1.75 mg/100 kcal (0.09 mg g⁻¹); adenosine 5'-monophosphate (AMP), 1.5 mg/100 kcal (0.08 mg g⁻¹); guanosine 5'-monophosphate (GMP), 0.5 mg/100 kcal (0.026 mg g⁻¹); and inosine 5'-monophosphate (IMP), 1 mg/100 kcal (0.05 mg g⁻¹). However, the latest recommendations in USA have increased the total maximum amount to 16 mg/100 kcal (0.83 mg g⁻¹) in infant formulas and 22 mg/100 kcal (1.13 mg g⁻¹) in infant formulas for premature babies (8). These values are based on the total amount of potentially available nucleotides (TPAN) in breast milk. Low weight newborn babies showed a higher growth rate in the first six months of life when fed with infant formula supplemented with nucleotides (9). There is no consensus on the concentration at which nutrients should be added to infant formula; however, it seems that the addition of nucleotides is safe and beneficial, especially for premature and low weight newborn babies, who have a low endogenous synthesis capacity. Consequently, supplementation levels need to be monitored.

Nucleotides, like many other biological compounds, are relatively nonvolatile and unstable, and LC has been the preferred