DEVELOPMENT AND VALIDATION OF A HILIC-UV METHOD FOR THE SIMULTANEOUS DETERMINATION OF NUCLEOTIDES IN INFANT FORMULA

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A method for the simultaneous determination of 5'-monophosphate nucleotides, adenosine 5'-monophosphate (AMP), uridine 5'-monophosphate (UMP), inosine 5'-monophosphate (IMP), cytidine 5'-monophosphate (CMP), guanosine 5'-monophosphate (GMP) in infant formulas by hydrophilic interaction liquid chromatography equipped with UV detector at 254 nm was developed. The complete chromatographic separation of five nucleotides was achieved isocratically through a polymeric zwitterionic column, ZIC-pHILIC with mobile phase consisted of acetonitrile and ammonium bicarbonate 100 mM (pH 8.8).

The preparation of the samples was conducted with centrifugal ultrafiltration tubes with molecular weight cut-off membrane. This preparation gave better results compared with two other procedures developed and based on the deproteinization of the sample using reagents of precipitation and specifically perchloric acid and trichloroacetic acid, respectively. This method was validated and satisfactory values of precision and trueness were obtained. The instrumental limits of detection and quantification of the standard solutions ranged from 0.092-0.15 mg/L and 0.28-0.45 mg/L, respectively. The limits of detection and quantification of the method were 3.1-7.3 mg/kg and 9.4-22 mg/kg, respectively. Recoveries ranged from 95.8 to 103.1. Thus, this method can be used for the determination of nucleotides in infant formulas.

KEYWORDS: nucleotides, infant formula, zic-p-hilic, centrifugal ultrafiltration