Development of Vitamin D Determination in Infant Formula by Column-Switching HPLC with UV Detector

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Abstract

This study was carried out to develop an analytical method for the determination of vitamin D in infant formula. Vitamin D was determined by column-switching high-performance liquid chromatography (HPLC) equipped with a reversed phase column and UV detector after saponification and extraction of the formula with an organic solvent. A pre-separation column (C8), focusing column (C18), analytical column (C18) and UV-Vis detector (254 nm) were used. The limits of detection (LOD) and the limits of quantification (LOQ) for vitamin D were estimated to be 1.51 µg/kg and 4.95 µg/kg, respectively. The linearity, recovery, precision and accuracy of the analytical method for vitamin D were evaluated through the application of a SRM (Standard Reference Material) 1846 (National Institute of Standard & Technology, USA). The linearity of this method was calculated with a value of the coefficient of determination (r2) ≥0.9999. The recovery of vitamin D was 85.20±3.00%. The intra-assay precision for vitamin D was between 1.68±0.03% and 5.75±0.33%, and the inter-assay precision for vitamin D ranged from 1.73±0.03% to 2.96±0.09%. The intra-assay accuracy for vitamin D was between 100.03±2.77% and 102.01±0.59%, and the inter-assay accuracy for vitamin D ranged from 99.00±1.53% to 102.01±3.04%. The proposed method is optimal for the separation and quantification of vitamin D from infant formula.

Key words: vitamin D, column-switching high-performance liquid chromatography, UV detection, infant formula

Introduction

Infant formula consists of various ingredients, including inorganic matter and vitamins to supply the nutritional requirements of infants and children (Codex, 2007). Infant formula is the only processed foodstuff that entirely satisfies the nutritional requirements of infants during the first months of life until the introduction of appropriate complementary feeding (The commission of the European communities, 2006). Among the nutritional ingredients in infant formula, the vitamin D is very low levels of fat-soluble vitamin (Heudi et al., 2004). Vitmain D is one of the most important essential bioregulators of the Ca2+ and phosphate metabolism in higher animals (Friedrich, 1988). Vitamin D and its hydroxyl metabolites play significant roles as hormonal regulators of calcium metabolism and are associated primarily with bone health (Bell et al., 1979). Vitamin D deficiency leads to rickets in children and osteomalacia in adults (Friedrich, 1988) and the quantification of vitamin D is widely used as a means of clinical testing for several pathophysiological states (e.g., parathyroid gland disorders, renal failure, vitamin-dependent rickets and sarcoidosis) (Donald and Gerhard, 1992). For this reason, the accurate determination of low levels of vitamin D in infant formula is crucial to safeguard the health of infants and children.

Traditionally, the existing procedures for vitamin D determination include colorimetric method (Gharbo and Gosser, 1974; Hassan, 1980), high-performance liquid chromatographic method with UV detection (AOAC, 2005; KFDA, 2009) and mass spectrometry (Heudi et al., 2004; Kamao et al., 2007; Soldin et al., 2009). Complex matrix sample, such as infant formula, requires a highly complicated sample preparation methods to remove interference for detection of low level vitamin D. The high-
performance liquid chromatographic (HPLC) method by KFDA (2009) and AOAC (2005) consists of two steps to remove interference in infant formula. Vitamin D fractions are collected from interfering substances on a cleanup column, and then an analytical procedure is carried out. However, this procedure is complicated and requires a long period of time; therefore, it is not suitable for handling a lot of samples. Recently, column-switching high performance liquid chromatographic method is used to eliminate the complicated sample cleanup step in sample preparation. Column-switching method is a rapid automated online extraction procedure and is successfully used to identify a wide range of analytes in complicated samples (Brunetto et al., 2004; Christians et al., 2000; Hartmann et al., 2001; Knebel et al., 2000). This study aims to develop vitamin D detection method in infant formula by column-switching high performance liquid chromatography with a rapid automated online extraction procedure without complicated sample cleanup step.

Materials and Methods

Reagents and materials

The experiments were performed using infant formula SRM (Standard Reference Material) 1849 (National Institute of Standard & Technology, USA), which contains 117.00±11.00 µg/kg of vitamin D. Sixteen kinds of commercial infant formula samples were collected from different markets in Daejeon, Korea. These samples were kept at room temperature. All reagents and solvents used were of HPLC or analytical reagent grade. The vitamin D standard was purchased from Sigma (USA). Water, methanol, absolute ethanol and hexane were obtained from E. Merck (Germany). Pyrogallol was supplied from Samchun Pure Chemical (Korea). Potassium hydroxide and sodium sulfate were purchased from Junsei Chemical (Japan). High-purity water was obtained through an Easy Pure system (Barnstead, USA).

Standard solutions

A stock solution (1000 mg/L) of the vitamin D standard was prepared in a 100 mL volumetric flask by dissolving 0.1 g of vitamin D in 100 mL of methanol. Working standards were prepared daily for analysis by diluting the vitamin D stock standard solution in a solution of methanol:H₂O (74.7:8.3:17, v/v) to the desired range of 10-100 µg/L.

Sample preparation

Sample preparation, except for the dissolving step in water, sample volume and final elute solution volume, was performed by the KFDA official method (KFDA, 2009). The sample preparation procedure was optimized by improving a previous extraction procedure with organic solvents. 1 g of infant formula (5 g liquid sample) was weighed accurately and transferred into a 250 mL brown round-bottom flask. After the addition of 3 mL of water, the flasks were shaken enough to dissolve the formula. 40 mL of 10% pyrogallol:ethanol (1:10, w/v) was added, and the flasks were shaken slowly. 10 mL of KOH:H₂O (9:1, w/v) was added, and the samples were refluxed for 30 min in a steam bath at 90°C. The samples were cooled rapidly under running H₂O. After the addition of 50 mL of hexane, the flasks were shaken vigorously three times for 10 min each.

The collected hexane extract liquid was transferred to brown separatory funnels with 100 mL of 1 N KOH. The funnels were shaken vigorously for 15 s. They were allowed to stand until both layers were clear, and then the aqueous layer was drained. 40 mL of 0.5 N KOH was added, and the mixture was shaken vigorously for 15 s. The funnels were allowed to stand until both layers were clear, and the aqueous layer was then drained. The remaining fraction was washed with 50 mL portions of H₂O until the last washing was neutral to phenolphthalein. The last few drops of H₂O were drained, and the remaining sample was dehydrated by sodium sulfate. The hexane layer was collected in a 250 mL brown round-bottom flask and evaporated to dryness under a vacuum by swirling in a water bath at ≤40°C. The residue was dissolved immediately in 5 mL of MeOH and filtrated by a PTFE filter. A 210 µL aliquot of this solution was then ready to be injected into the column-switching HPLC system.

Instruments

The experiments were performed using a liquid chromatographic Shiseido NANO SPACE SI-2 system (Shiseido, Japan) connected to a Shiseido UV-Vis detector and with SMC-21 software for instrument control and data collection and processing. Detection was carried out at 254 nm. Injections were made with a 100 µL loop. For column-switching purposes, a column-switching six-port valve (Shiseido, Japan) controlled by the SMC-21 software was used, along with an additional pump 3001 (Shiseido, Japan) to deliver the extraction mobile phase.