DETERMINATION OF VITAMIN D IN HUMAN BREAST MILK - METHODS

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Vitamin D is a hormone precursor with a steroidal structure that is found in animals, plants and yeast. This vitamin can be present in either of 2 different forms, vitamin D₂ (ergocalciferol) or vitamin D₃ (cholecalciferol). Breast milk contains 30% of the woman’s circulating concentration of vitamin D and only 1% of the circulating concentration of 25-OH-D (1). A lack of vitamin D in children can lead to soft, thin, and brittle bones, a disease known as rickets. Vitamin D deficiency has also been linked to osteoporosis, some cancers, heart disease and diabetes (2).

Due to the very low concentrations of vitamin D and its metabolites in human breast milk, its precise measurement in these samples is quite challenging and includes sample pre-treatment before analysis. Traditional sample preparation involves saponification, liquid-liquid extraction, solvent evaporation, manual solid phase extraction, and pre-concentration (3).

Techniques for the determination of vitamin D in milk can be categorized into immunological techniques (CPBA, ELISA, RIA) and non-immunological techniques (HPLC, LC-MS). LC is considered to be the primary method for the separation of vitamin D. Various types of detection have been used with LC, including MS², MS and UV. LC-MS/LC-MS² are the methods of choice in vitamin D analysis since this methodology is sensitive, accurate and provides high specificity and offers quantify multiple analytes in a single assay (4). The separation is usually performed on a reversed-phase analytical column packed with C18 particles. The best results are achieved by using isotope-labeled internal standards and MS detection (5).

Goal of this study is summarizing current chromatographic methods with sample preparation of vitamin D in human breast milk.

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