ABSTRACT

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Title of Doctoral Thesis: Using of separation methods for clinical research

This doctoral thesis deals with development and validation of a chromatographic determination of liposoluble vitamins and with optimization of biological materials preparation.

The first part of this work is focused on vitamins D, A and E assessment in serum. To separate studied analytes two different methods were developed successively. 1) Separation employing monolithic columns – two different monolithic columns Chromolith Performance RP-18e, 100×4.6 mm and SpeedRod RP-18e, 50×4.6 mm were connected in series. Vitamins $1,25(OH)_2D_3$, $25(OH)D_3$, retinol, tocol (internal standard), cholecalciferol, ergocalciferol and α-tocopherol were separated within 6.5 minutes using step gradient elution. 2) Separation exploiting a core-shell column – Ascentis Express RP-Amide 75×3.0 mm. To decrease the separation time a linear gradient was employed. The determination of $25(OH)D_2$, $25(OH)D_3$, tocol (internal standard) α-tocopherol took 8.5 minutes within separation of impurities. Developed solid phase (SPE) and liquid-liquid extraction (LLE) procedures were compared to select optimal method of sample preparation. A liquid-liquid extraction (LLE) was used as a preseparation step to release vitamins bound to carrier proteins. The addition of EDTA before deproteination and extraction agent acidification resulted in the optimized preseparation procedure. The sample preparation took less than 45 minutes providing recovery 98–109 %.

In the second part of this work a development of a human breast milk sample preparation is described. In this case, retinol and α-tocopherol were the analytes. The LLE was the method of choice preceded by deproteination and saponification. The whole procedure was validated according to FDA demands. The recovery at three concentration levels was 82–90 % and 92–109 % for retinol and α-tocopherol, respectively. The optimized method was applied on real samples and evaluated. A statistically significant (α=0,05) decrease in retinol concentration during lactation period was observed.