Abstract

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Diploma Thesis Title: Comparison of extraction procedures for HPLC determination of liposolubile vitamines in serum

In this thesis were compared extraction methods, solid phase extraction (SPE) and liquid liquid extraction (LLE) used for separation of human serum fat soluble vitamins: A (retinol), E (α-tocopherol), D_2 (ergocalcipherol), D_3 (cholecalcipherol), 25-(OH)D_3 (calcidiol). Vitamins were then determinated by high performace liquid chromatography (HPLC).

As a baseline method used the procedure that was developed and partially validated the thesis: Application of the SPE technology and monolithic columns in HPLC analysis of biologically active substances (Horčičková 2009). This procedure was further optimized in order to increase the extraction of target analytes 25(OH)D_3, D_2 and D_3 and shortening the preanalytical phase. Conditions were tested: sample volume, deproteination of serum (reagents, volume, temperature, time), the conditioning of SPE columns (water washing, the dosage), various SPE columns, elution process (agent, the dosage, use of vacuum), the effect of centrifugation on the purity of the extract, using the Eppendorf and glass tubes, sample evaporation conditions.

Based on the results of the optimization of the SPE extraction methods have been developed a new method using LLE extraction, which has also been optimized with regard to the environment deproteination and extraction, construction and extraction method of sample evaporation.

When comparing the two extraction methods were found using the LLE method, gave higher yield of extraction of target analytes D_2 (ergocalcipherol), D_3

(cholecalcipherol), 25-(OH)D_3 (calcidiol), also reduced the time required for sample preparation for HPLC analysis and reduced solvent consumption.

Keyworlds: solid phase extraction (SPE), liquid liquid extaction (LLE), vitamins: A (retinol), E (α-tocopherol), D_2 (ergocalcipherol), D_3 (cholecalcipherol), 25-(OH)D_3 (calcidiol), HPLC