

# Abstract

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Title of Doctoral Thesis: Development and validation of new HPLC methods for determination of biologically active compounds in clinical practise

The presented dissertation thesis is dealing with development and validation of HPLC methods for clinical research and clinical practice in Research laboratory of Department of Metabolic Care and Gerontology (GMC) in University Hospital in Hradec Kralove (UHHK). For this purpose modern technologies dealing with large sequences of biological material, speed separation, small volumes of organic solvent and sample were used.

New HPLC method for determination of 13-cis and all-trans retinoic acid, retinol, alpha- and gamma-tocopherol using internal calibration for patients suffers from acute promyelocytic leukemia treated with retinoic acid was developed and validated.

Solid phase extraction (SPE) and monoliths were selected for sample preparation and separation of the target compounds.

New method for determination of neopterin, creatinine, kynurenine and tryptophan in human serum using two monolithic column as stationary phase was developed and validated. This method was developed emphasis on large sequences of samples, which should be process. Biological samples were only diluted, deproteinized, centrifuged and filtrated. Micro titration plates with filters and special manifold which allow prepare 96 samples in one filtration step in very short time were used. The main advantage was combination of these technologies with special autosampler for micro titration plates. This sampler allows store samples in dark cooled place protected against evaporation. This method was partially validated for different biological fluids as amniotic fluid and exudates and will be using for determination of target analytes in patients of professor Melichar from Oncology department in University Hospital in Hradec Kralove and in University Hospital in Olomouc.

Some previously developed methods in Research laboratory GMC were modernized. Extraction procedure for determination of retinol and alpha-tocopherol was modernized.

Solid phase extraction was miniaturized; gamma-tocopherol and tocol (internal standard) were added. Consequently HPLC method was transported to HPLC system Prominence LC 20 (Shimadzu) and completely revalidated.

Kynurenine, the compound which helps reflect the stage of immune system activation was included as target analyte to method for determination of neopterin and creatinine in human urine which is in Research laboratory GMC very frequently using for large sequences of the samples. Advantages of hybrid Gemini-Twin technology were used for the separation. This column was very helpful in large sequences of the samples (1 column/1000 analysis of filtered urine) measurements. Method was revalidated.

All developed and modernized methods extend spectrum of methods in Research laboratory of Department of Metabolic Care and Gerontology in University Hospital in Hradec Kralove. In present time these methods are using in running clinical studies in various departments of UHHK and Oncology department in University Hospital in Olomouc.