

# ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Training Workplace      Department of Analytical Chemistry

Doctoral Degree Program      Bioanalytical Methods

**Candidate**      Mgr. Andrea Vernerová

Supervisor      Assoc. Prof. RNDr. Lenka Kujovská Krčmová, Ph.D.

Advisor      MUDr. Ondřej Sobotka, Ph.D.

**Title of Doctoral Thesis**      Application of modern analytical methods in clinical research

The dissertation thesis, composed as a compilation of five publications, is dealing with the development, optimization and validation of chromatographic methods and extraction procedures for the determination of early activation of the immune system biomarkers (neopterin, kynurenine, and tryptophan) in saliva and gingival crevicular fluid in oncological patients and patients with periodontal diseases. Furthermore, the potential of the use of Clark's sensor to determine the correct function of platelets by measuring mitochondrial respiration and its application in clinical research is under investigation. This work was based on cooperation with several clinical departments of the University Hospital Hradec Králové and Olomouc, and Oroboros Instruments (Innsbruck, Austria).

The theoretical part of the thesis is focused on detail description of the individual analytes, their clinical significance, used biological matrices and their preparation before the analysis. Methods of separation and detection, which were used in this work, are also discussed.

The experimental work is divided into two parts. The first part deals with development, optimization, and validation of HPLC methods and their subsequent use in applied research (Supplement 1–4). Two methods and review article are described in the chromatographic part of the work. Developed methods focused on determination of early biomarkers of inflammation in saliva and gingival crevicular fluid include a simple sample preparation suitable for large series of samples with an emphasis on non invasive sampling. The methods use connection with highly sensitive types of detection techniques such as mass spectrometry, fluorescence, and diode array detection. In the next section of this part biomedical article is discussed. Presented work deals with the determination of urinary neopterin in relation with oncological disease. I was involved in this study during my doctoral studies.

The second part describes the determination of mitochondrial platelet respiration in healthy blood donors using a polarographic oxygen sensor in combination with two different separation techniques for isolation of platelets (Supplement 5). The comprehensive methodological publication could be used for follow-up experimental works.