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Review of the Dissertation Thesis

RNDr. Anna Němečková: VOLTAMMETRIC AND AMPEROMETRIC DETERMINATION OF HOMOVANILLIC, VANILLYLMANDELIC, AND 5-HYDROXYINDOLE-3-ACETIC ACID

Search of inexpensive methods for monitoring of tumour biomarkers in biological fluids, moreover with a potentiality of miniaturization up to the point-of-care tests, is of wide biomedical and analytical interest. This dissertation thesis deals with the electrochemical detection of three selected acids from the biomarkers family. Therefore, the topic of this thesis should be recognized as very modern and important from several points of view analyses. Scientific value of the thesis is given also by 161 full literature references.

The main aim of presented dissertation thesis (as stated on the page 2) was the development of sensitive electrochemical methods for the determination of three important tumour biomarkers. The second aim was a simultaneous determination of three analytes in human urine. These aims are clearly stated and the results and the publications included into the thesis document the success achieved at their fulfilment.

The results obtained have been already published in peer-reviewed journals such as *Chemické Listy* 112 (2018), *Electroanalysis* 29 (2017) and 31 (2019), *Analytica Chimica Acta* 1087 (2019) and submitted to *Journal of Electroanalytical Chemistry* (2020) with indicated significant contributions by Anna Němečková Makrlíková. Thus the role of reviewer is somewhat simplified. Procedures with featured modern and effective working electrodes and arrangement of analytical experiment have been developed and utilized for particular applications including popular flow injection and HPLC techniques. The thesis demonstrates also wide conferences and projects activity of RNDr. Němečková. Advantages of the developed systems over others currently used methods I find in terms of sensitivity with sufficient precision, selectivity, short time of analysis, final operational simplicity and also costs.

Thus, the dissertation thesis represents surely a significant scientific and technical contribution to the knowledge in given fields possessing both new scientific information and the application protocols. From the point of view of further development, the author and whole research team work on the topic of high interest with respect to new and effective ways of bioanalytical diagnosis of the cancer biomarkers.

Questions and specific remarks:

On the page 14 the reference urinary concentrations are presented varying from 8.2 to 41.0 $\mu\text{mol}\cdot\text{L}^{-1}$ for HVA, from 11.6 to 28.7 $\mu\text{mol}\cdot\text{L}^{-1}$ for VMA, and 17.8 to 58.3 $\mu\text{mol}\cdot\text{L}^{-1}$ for 5-HIAA. What is the relation of the calibration ranges tested within the thesis and real concentration of these acids at patients with a tumor?

The LOD values are closely to the lower calibration limits tested, so we can evidently still assume a signal dependence on the concentration. How were the LOD value obtained?

What is/can be an influence of other urine components as potential interferences with anodic potentials in the range of three analytes tested? How can be such interferences recognized and what sample pre-treatment could be considered?

According to metrology, only the quantity (particle size, analyte concentration, etc.) can be subject of measurement. Together with true use of this term in the thesis, there are also laboratory expressions such as measuring of HVA, VMA, and 5-HIAA in biological fluids, measured solution, measured HVA, VMA, and 5-HIAA, and others.

Conclusions:

The questions and remark above are directed to discussion regarding presentation of the excellent results obtained. I can conclude that the dissertation thesis documents that RNDr. Anna Němečková is able to work scientifically and obtain new results of importance for practise. Therefore, I fully recommend RNDr. Anna Němečková's dissertation thesis as a basis for further process of obtaining the degree „philosophiae doctor“ (PhD.) according to corresponding rules.

Bratislava, 6 July, 2020

Ján Labuda