The evaluation of serotonin and 5-hydroxyindole-3-acetic acid levels in serum and urine samples from patients with neuroendocrine tumors

Natalia Miękus, Alina Plenis, Piotr Kowalski, Ilona Olędzka, Karolina Orlikowska and Tomasz Bączek
Medical University of Gdańsk, Poland

Introduction: Serotonin (5-HT) is a naturally occurring vasoactive substance found in the brain, enterochromatin cells and platelets. Elevated levels of 5-HT and its main metabolite 5-hydroxyindole acetic acid (5-HIAA) - are known to be associated with numerous life-threatening diseases as neurodegenerative disorders or cancer. The assessment of their concentrations in biological fluids and tissues could be helpful for early detection of on-going pathology. Nevertheless, it demands the development of reliable bioanalytical methods for their extraction and separation from biological samples.

Methods: Presented study was focused on establishment of the most accurate extraction and detection conditions for 5-HT and 5-HIAA from biological samples (serum and urine). The main aim was achieved by optimizing extraction of analytes. Various solid phase extraction (SPE) cartridges (C18, C8, HLB and SCN) as well as extraction buffers were tested. Optimal efficiency of extraction was achieved when simple C18 cartridges together with 20% of methanol and 80% of H_2O (pH 4) as eluent were applied.

Further optimization of separation conditions of analytes was carried out by use of either high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) with UV/VIS detection. Gained results confirmed that both separation techniques guarantee sensitive and precise determination of 5-HT and 5-HIAA levels in human body fluids in up to 15 min total analysis time.

Results and Discussion: The method has been validated for accuracy, precision, selectivity, linearity, recovery and stability. The limits of detection (LOD) and quantification (LOQ) were satisfactory for both analytes (LOD around 0.5 ng/mL and LOQ 0.25 ng/mL). Linearity was confirmed within a range of 0.5-50 ng/mL with the correlation coefficient greater than 0.9995 in both cases. Comparison of HPLC and CE showed good agreement between the two methods, while different wavelength was applied (200 nm for CE and 220 nm for HPLC).

Described methods were successfully applied for the quantification of 5-HT and 5-HIAA in real urine and serum samples derived from healthy controls (man and woman) as well as patients suffering from neuroendocrine tumors (NET). Data revealed significantly higher concentrations of both analytes in patients' samples. Therefore, 5-HT and 5-HIAA could be potentially used as biomarkers for detection of NET.

Presented experimental workflow for HPLC or CE of 5-HIAA and 5-HT detection and quantification could facilitate biomarkers' concentrations assessment from easy accessible patients' body fluids like urine or blood. That would have the great impact of early cancer detection what is especially important in rare, asymptomatic cancers like neuroendocrine tumors.

Biography

Natalia Miekus is a Ph.D. student at Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland (supervisor: Prof. Tomasz Bączek), Ph.D. thesis title: "Optimization of phosphoproteomic analytical methods for cancer biomarkers studies". She is a part of student research groups at The International Society for Pharmaceutical Engineering (ISPE) student research group at the Department of Applied Pharmacy, Medical University in Gdańsk, Gdańsk, Poland and International Organization: Engineering Pharmaceutical Innovation. She is a Member of Committee of the Pharmacy Faculty Council for the Plans and Program of Ph.D. studies for 2012-2016.

miekusn@gmail.com

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