amount of excretion moisturizes the eyelids and surrounding tissues on the 1st day after exposure, discharge from the eye is recorded during 5 days of observation (3 points)

The final classification assessment of the damaging effect of hexyl ester of 5-aminolevulinic acid by summing the intensity points of each of the symptoms of irritating effects on the mucous membranes of the eyes (8 points in total) allows us to classify, according to [1], this compound as class 3 — chemical compounds with pronounced irritative properties. The local inflammatory process caused by a single instillation of hexyl ester of 5-aminolevulinic acid is pathophysiologically characterized as serous blepharoconjunctivitis.

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ANALYSIS OF HUMAN CHORIONIC GONADOTROPIN USING HIGH-RESOLUTION TANDEM MASS-SPECTROMENRY

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Human chorionic gonadotropin (hCG) is tropic protein hormone secreted by the adenohypophysis. Hormone is included in the prohibited list in all sports. In this study, methodological approach of human chorionic gonadotropin specific peptides obtaining using «bottom-up» proteomics and its detection using liquid chromatography - high-resolution tandem mass spectrometry was developed.

Keywords: human chorionic gonadotropin, high performance liquid chromatography, tandem mass-spectrometry.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone with a molecular weight of about 36 kDa, consisting of two different alpha and beta subunits. Beta-subunit is specific for hCG, while alpha subunit is common for all gonadotropic hormones. The carbohydrate part, which is characterized by significant heterogeneity, accounts for about 30 % of the molecular weight of the protein. There are N- and O-linked carbohydrate chains.

Human chorionic gonadotropin is used by male athletes with the aim of enhancing the secretion of endogenous steroid hormones, while maintaining the testosterone/epitestosterone ratio have been described. hCG is included in prohibited list in all sports on competition and non-competition period (class S2 – peptide hormones, growth factors, related substances, and mimetics).

Due to the existence of several hCG isoforms, the heterogeneity revealed in the composition largely depends on the features of the analysis method used in the study. In the case of determining hCG in the urine, the situation is difficult, since the spectrum of isoforms is more complicated than in the case of blood serum.

In this study the methodological approach to obtain specific peptides of human chorionic gonadotropin using «bottom-up» proteomic approach and their analysis using liquid chromatography – high-resolution tandem mass spectrometry in human urine was developed.

Urine samples consisting hCG at a known concentrations were purified and concentrated using ultrafiltration. Hydrolysis of hCG was carried out using trypsin Proteomics Grade with preliminary protein alkylation. The peptides were separated by HPLC method on reversed-phase column and analyzed using high-resolution tandem mass-spectrometer LTQ Orbitrap Discovery. Mass-spectrometric detection was carried out using Full Scan, Auto MS/MS and Target MS/MS.

More than 90 % of the alpha and beta subunit peptides with varying degrees of protonation were identified. Protein detection limit was 1 ng/ml. List of characteristic peptides precursor and product ions, which will be used as indicators of hCG using as doping has been compiled. Figure 1 shows the mass spectrum of fragment ions of one of the specific hCG beta subunit peptides.

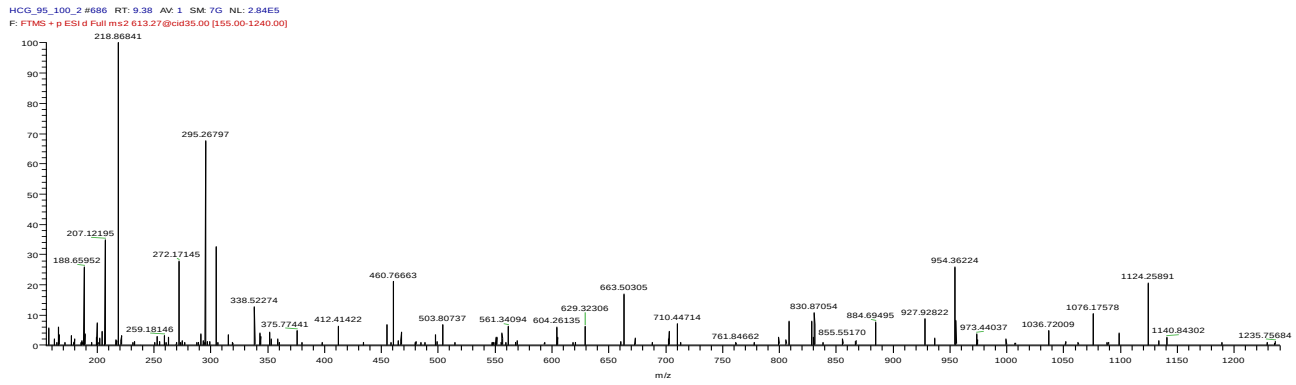

Fig.1. – Mass spectrum of fragment ions of hCG beta-subunit peptide DHPLTCDDPR, m/z 613.2626, protonation degree +2

Based on the data obtained, method for quantifying human chorionic gonadotropin using «bottom-up» proteomics based liquid chromatography – tandem mass-spectrometry in human urine for doping-control was developed.

THE FEATURES OF AP_4A IMPACT ON ADP-INDUCED PLATELET AGGREGATION IN PREGNANT WOMEN WITH PRE-ECLAMPSIA

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Pregnant women with pre-eclampsia have a significant increase in the degree of platelet aggregation in response to ADP, in comparison with a physiologically occurring pregnancy. In vitro experiments revealed that Ap4A inhibits ADP-induced platelet aggregation of pregnant women with pre-eclampsia.

Keywords: pre-eclampsia, platelets, ADP, Ap4A, aggregation.

Violations of the functional activity of platelets associated with their adhesion and aggregation lead to increase bleeding or increased thrombosis and development of circulatory pathology.

The addition of ADP to platelet-rich blood plasma in vitro leads to a change in the shape of the blood platelets and primary aggregation. By acting on P2-purinoreceptors, ADP activates phospholipase C, which leads to the formation of IP3, calcium mobilization from intracellular stores; inhibits adenylate cyclase, thereby reducing the level of intracellular cAMP, causing granule secretion and platelet aggregation. After primary aggregation, ADP activates phospholipase A2 and releases arachidonic acid from membrane phospholipids, which is converted to TxA2. TxA2 converts reversible aggregation into irreversible, also called the second wave of aggregation.

The experiment revealed that platelets of women with physiological pregnancy (n = 32) and pregnant women with pre-eclampsia (n = 32) reacted without showing any special differences in the responses to ADP in concentrations of $2{,}44\times10^{-5}$M and $2{,}44\times10^{-6}$M. With a further decrease of ADP concentration to $2{,}44\times10^{-7}$M, platelet aggregation was manifested exclusively in pregnant women with pre-eclampsia. Thus, the degree and rate of aggregation during physiological pregnancy was in the range of 0,8±0,69 % and 1,2 [0,35–2,2]%/min; whereas in case of pre-eclampsia – 5,46±1,72 % and 6,35 [3,7–10,7] %/min, respectively ($P < 0{,}05$). A slight increase in the concentration of ADP to $7{,}32\times10^{-7}$M cause platelet aggregation, both in women with physiological pregnancy and in pregnant women with pre-eclampsia. Satistical differences in the degree and rate of aggregation between norm and pathology (13,02±4,62 % and 13,65 [9,7–18,6] %/min; and 20,09±4,10 % and 21,4 [15,4–26,6]%/min, respectively; $P < 0{,}05$), made it possible to conduct further studies using antiplatelet agents on this model. There may be several reasons for increased platelet aggregation during pre-eclampsia: a decrease in the sensitivity of platelets to ADP and increased secretion of Ca^{2+} ions, ATP, and ADP from intracellular stores [1]; decreased intercellular levels of cAMP and cGMP.

Diadenosine-5',5'''-P1,P4-tetraphosphate (Ap4A) is a content of platelet dense granules [2], which is a molecule that is included in the processes of recovery, correction and protection of the body, both on the cellular and organism level. Inside the cell, Ap4A acts as a secondary messenger, initiates DNA repair, participates in the