

USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY IN THE ANALYSIS OF HUMAN CHORIONIC GONADOTROPIN

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Human chorionic gonadotropin (hCG) is secreted by cells of the adenohypophysis, placental syncytiotrophoblast, some normal non-placental tissues and tumors. Cases of the abuse of hCG in male athletes with the aim of enhancing the secretion of endogenous steroid hormones while maintaining the testosterone/epitestosterone ratio have been described.

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

Human chorionic gonadotropin is a glycoprotein hormone with a molecular weight of about 36 kDa, consisting of two different alpha and beta subunits. The carbohydrates, characterized by considerable heterogeneity, account for about 30% of the molecular weight of the protein. There are N- and O-linked carbohydrate chains.

Due to the heterogeneity of the protein, the presence of its isoforms and the content of impurities in the samples, the use of immunological methods of analysis to determine the hormone in biological fluids is complicated. The HPLC-mass spectrometry method described in this study helps to substantiate the positive results of tests on hCG. Tryptic hydrolysis allows to identify peptides that can act as unique markers for the qualitative and quantitative analysis of chorionic gonadotropin. This method would be more accurate and specific during doping control.

In this study the methodological approach of human chorionic gonadotropin detection by high performance liquid chromatography-mass spectrometry based on their prior tryptic hydrolysis ("bottom-up method") was developed. Hydrolysis of hCG was carried out using Proteomics Grade trypsin with preliminary protein alkylation. The peptides of the resulting hydrolyzate were separated by HPLC method on reversed-phase column and analyzed using high resolution mass spectrometer Agilent 6550 iFunnel Q-TOF. More than 90% of the alpha and beta subunits peptides with varying degrees of protonation were identified.

The developed approach was tested on urine samples with hCG in different concentrations. The samples were purified and concentrated using ultrafiltration. The role of the matrix in the process of enzymatic hydrolysis and subsequent instrumental analysis, as well as the need and influence of different types of protein denaturation (thermal, chemical and their combinations) on the yield of peptides and glycopeptides was studied.

During the analysis, glycopeptides of the beta subunit with N-glycosylation sites with different degree of heterogeneity of carbohydrate fragments were detected. Modeling of the oligosaccharide on the basis of MS and MS/MS analysis indicates the presence of a fucosylated core with a biantennary structure containing 2 residues of N-acetylglucosamine, galactose and N-acetylneuraminic acid.

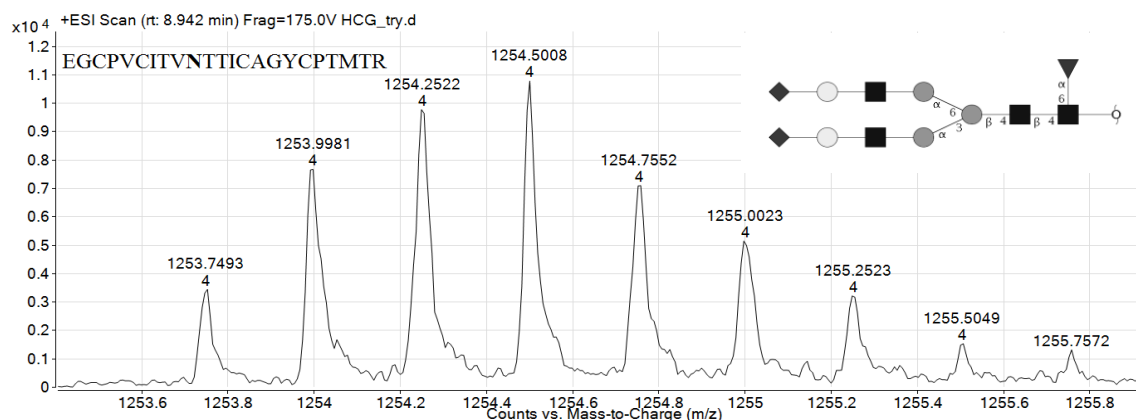


Fig. 1. Mass spectrum of one of the glycopeptide EGCPVCITVNTTICAGYCPTMTR isoforms with m/z 1253.7493 with a protonation degree of +4 and the structure of the oligosaccharide chain of the glycopeptide

Based on the data obtained, a list of characteristic peptides has been compiled which will be used to further develop a method for quantifying chorionic gonadotropin in human urine.

EPIDEMIOLOGICAL ASPECTS OF MORBIDITY OF THE POPULATION OF THE REPUBLIC OF BELARUS DISEASES OF THE CIRCULATORY SYSTEM

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The General and primary morbidity of diseases of the circulatory system in the Republic of Belarus among children and adults for 2006–2016 years was carried out. The data indicate that in the dynamics of the total incidence of diseases of the circulatory system in the adult population of Belarus, there is a marked trend in the increase in the incidence of CSD. Among children, there is a high tendency to reduce the overall incidence of diseases of the circulatory system in the Republic of Belarus.

Keywords: diseases of the circulatory system, morbidity, tendency.

Diseases of the circulatory system are the main cause of disability and death among the population. At this stage, diseases of the circulatory system in the Republic of Belarus are a threat in terms of the health and vitality of the nation. One of the leading tasks of modern health care is to establish and prevent the main causes of morbidity and mortality. First of all, this refers to diseases of the circulatory system, which in recent decades have firmly occupied one of the leading places in the structure of the morbidity of the population.

According to the results of medical and statistical studies submitted by WHO, there is still no tendency in the world to reduce mortality from circulatory system diseases.

Objective: to study the dynamics of the general and primary incidence of circulatory system diseases. The analysis was carried out according to official data of the Ministry of Health of the Republic of Belarus.

According to the results of the analysis of the incidence of the population of the Republic of Belarus from diseases of the circulatory system for the period from 2006 to 2016, it was revealed that circulatory system diseases occupy the first ranking places in the overall structure of morbidity. In 2006, they are in second place (19.9%), and in 2016 the structure has changed, the first ranked place was occupied by diseases of the circulatory system (23.3%).

In the dynamics of the primary incidence of circulatory system diseases, the adult population of the Republic of Belarus was not identified ($R^2 = 0.013$) upward / downward trend. In the dynamics of general morbidity, there is a pronounced ($R^2 = 0.854$) tendency for an increase in the incidence of circulatory system diseases from 2006 to 2016, which indicates that this type of disease is chronic.

When analyzing the dynamics of the overall incidence of diseases of the circulatory system of the child population of the Republic of Belarus, the tendency to decrease is rather high ($R^2 = 0.783$), and when analyzing the dynamics of primary incidence, there is a low tendency ($R^2 = 0.218$).

It was also revealed that the incidence rates of diseases of the circulatory system among the adult population are significantly higher than among the children's population. The incidence rates (total and primary) of the adult population are higher than the corresponding incidence rates of the child population by 14.8 times when comparing the overall incidence, and 4.46 times when comparing the primary incidence.