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ANALYSIS OF HUMAN CHORIONIC GONADOTROPIN USING BOTTOM-UP PROTEOMIC APPROACH

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Human chorionic gonadotropin (hCG) is mainly the product of placental syncytiotrophoblast cells. It can also be secreted by several normal non-placental tissues and trophoblastic or non-trophoblastic neoplasms. Human chorionic gonadotropin is included in the lists of illegal drugs in some sports. In this study the methodological approach to human chorionic gonadotropin detection by HPLC-mass spectrometry is developed.

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

Human chorionic gonadotropin has a molecular weight of 38 000 Da with 237 amino acids organized in two subunits, alpha and beta, each consisting of a single polypeptide chain. Seventy percent of its structure is represented by the protein chains and 30 % by carbohydrate chains. The carbohydrate chains covalently bound to the peptide chains are of two types: O-linked and N-linked oligosaccharides. Regarding endogenous forms of hCG, there are various ways to categorize and measure them, including total hCG, free β-subunit hCG, β-core fragment hCG, hyperglycosylated hCG, nicked hCG, alpha hCG, and pituitary hCG.

In this study the methodological approach to human chorionic gonadotropin detection by high resolution mass spectrometry based on their prior tryptic hydrolysis (“bottom-up method”) is developed. The peptides obtained from tryptic hydrolysis are separated by HPLC method on reversed-phase column and are analyzed using a high resolution mass spectrometer Agilent 6550 iFunnel Q-TOF. The designed approach allows detecting 7 hCG peptides (figure 1).

The 3 peptides of the alpha subunit and 4 peptides of the beta subunit at various degrees of protonation are detected. Analyzing the peptides obtained after the tryptic hydrolysis of hCG, it is found that two peptides derived from the cleavage of the β-subunit correspond to peptides that come out during hydrolysis of the beta-core fragment.

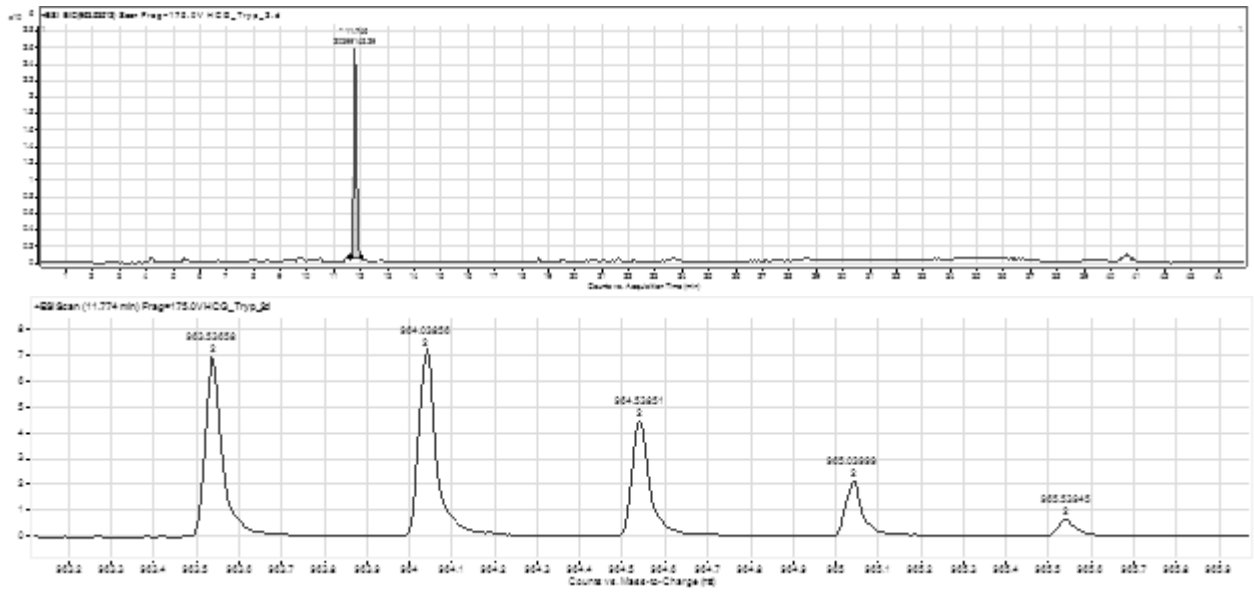

Figure 1 – Chromatogram and mass spectrum of peptide VLQGVLPALPQVVCNYR with m/z 963.5351 (+2)

Additionally, the mass spectrometric data analysis shows the presence of peptides with molecular masses that correspond to the glycopeptides of the chorionic gonadotropin. Due to the heterogeneity of the oligosaccharide fragments structure of this protein, the data obtained require additional processing and modeling.

Based on the data obtained, a list of peptides that can be used as specific for further studies has been compiled. The obtained data will be used to develop a method of quantifying chorionic gonadotropin in human urine.

CONTENT OF ALPHA-FETOPROTEIN IN PATIENTS WITH SYSTEMIC SCLERODERMA

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The analysis of the content of alpha-fetoprotein in patients with systemic scleroderma shows that serum alpha-fetoprotein levels could be used as a marker in the differential diagnosis of systemic scleroderma.

Keywords: alpha-fetoprotein, systemic scleroderma, embryonic markers.

Alpha-fetoprotein is a protein contained in mammalian embryonic serum, the level of which decreases to trace amounts in the blood of adults, but rises again in the case of hepatocellular carcinoma or a teratoblastoma of a testicle or ovary. In this regard, alpha-fetoprotein is widely used in the primary differential diagnosis of these tumors, as well as to evaluate the effectiveness of their treatment.

Normally, alpha-fetoprotein can be detected in the fetal serum as early as during the 4th week of pregnancy. Its concentration peaks between the 12th and 16th weeks and then gradually decreases until birth. In adults, alpha-fetoprotein is detected in normal hepatic tissue using an immunoblotting technique, as well as in follicular fluid. Since alpha-fetoprotein penetrates the placenta, it can be found in a fairly high concentration in the mother's serum, reaching a maximum between the 32nd and 36th weeks of pregnancy. This serves as an important indicator in monitoring the antenatal period. With the increase in pregnancy and in children in the early postpartum period, the level of alpha-fetoprotein decreases.

In man in the first year of life, the level of alpha-fetoprotein is subjected to strong fluctuations. A stable and regular increase in the level of alpha-fetoprotein in childhood is observed with tyrosinemia and ataxia – telangiectasia. Developmental delay or a liver structure disturbance in these conditions is responsible for maintaining a high level of alpha-fetoprotein.

An increase in the level of alpha-fetoprotein is observed in non-tumor liver diseases. It is temporary; sharp in acute viral hepatitis and less pronounced, undulate in cirrhosis of the liver.

An increased level of alpha-fetoprotein in the blood of pregnant women is a diagnostic sign of congenital pathology, mainly of neural tube defects; a low level is a marker of a high risk for Down's syndrome.