

## ANALYSIS REPORT № 06/06

1<sup>st</sup> of June 2017

**Sample name:** Gintropine – somatotrin, recombinant human growth hormone. Produced by: Europharm; Batch No: 201701002; Date of production: 2017.01.09; Exp. Date: 2018.12; 10 ME.

**Appearance of the sample:** lyophilized powder in vial of transparent glass (figure 1)



Figure 1 – The test sample

**Analysis technique:**

The determination of purity: by high-performance liquid chromatography (HPLC) with a diode-matrix detector (Agilent 1260 series, HPLC-DAD). The detection of  $\lambda=210$  nm.

Quality analysis: HPLC with a tandem quadrupole time-of-flight mass-detector (Agilent 6530 Accurate-Mass Q-TOF, HPLC-MS).

**Conditions of chromatographic analysis:** the column Hamilton PRP-3 300Å 150\*2,1 mm 10  $\mu$ m; mobile phase: A: 20 mM CH<sub>3</sub>COONH<sub>4</sub> in deionized water; B: 20 mM CH<sub>3</sub>COONH<sub>4</sub> / 25% H<sub>2</sub>O in acetonitrile; flow: 0,85 ml/min; thermostat temperature: 45 °C; injection volume: 10  $\mu$ l.

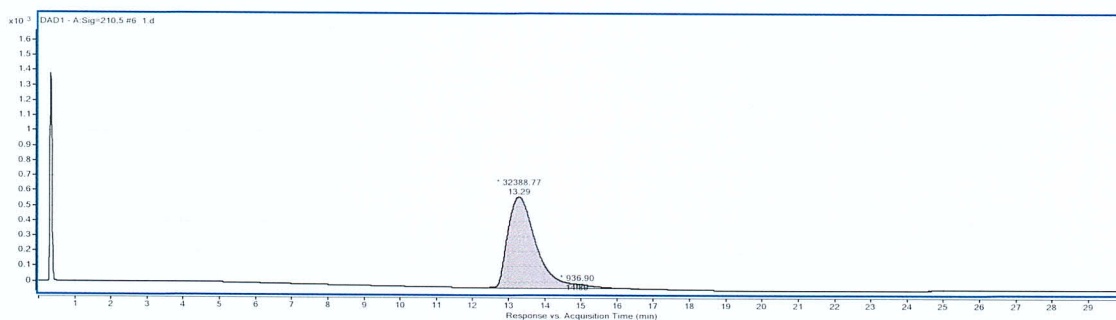
**Results of HPLC-DAD analysis:**

Figure 2 – The chromatogram of the test sample

The chromatogram of the sample solution is presented in the figure 2. Ultraviolet (UV) spectrum of the main peak of the test sample is presented in the figure 3.

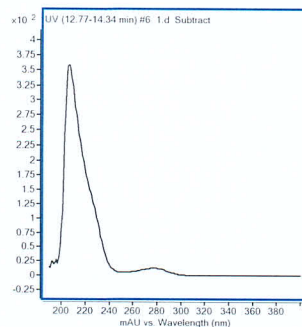


Figure 3 – UV-spectrum of the test sample  
(The range of wavelengths is 190 – 400 nm)

Relative component contents of the test sample are presented in the table 1. The content of the main component is 97,19%.

Table 1 – The results of HPLC-DAD integration of chromatogram.

№	Time; min	Area, conventional units	Relative content, %
1	13,29	32388,77	97,19
2	14,89	936,90	2,81
<b>Total</b>		33325,67	

**Results of HPLC-MS analysis:**

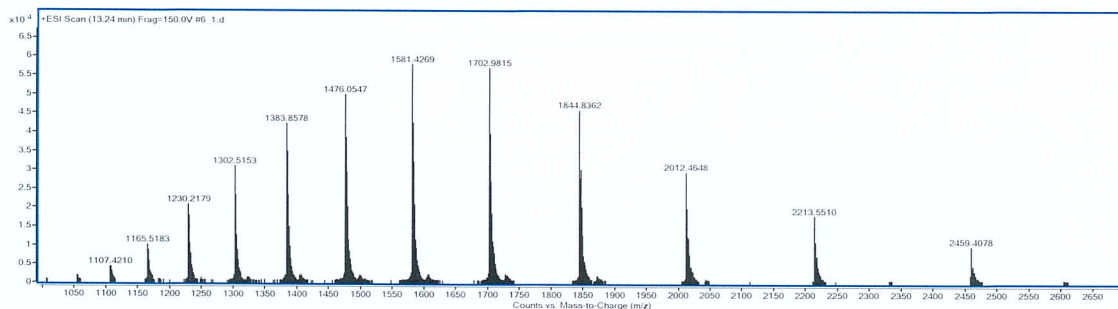


Figure 4 – Mass-spectrum of the test sample, RT= 13,29 min.

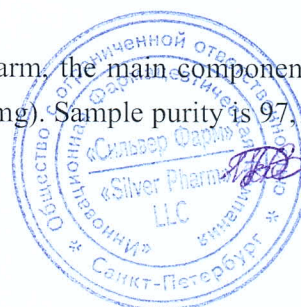
Mass spectra of protein molecules, obtained by the ionization electro-spray at atmospheric pressure are characterized by the formation of multicharged ions, exciting on different number of protons up to the maximum possible. Ions corresponding to one protein charge, form sets of isotope peaks in the cluster. In the sample of detected clusters 1844; 2012; 2213 m/z, where  $z = 12$ ; 11 and 10, which correspond to somatropin. According to the analysis of the molecular weight of the somatropin is 22125 daltons.

**Conclusion:**

In the sample «Gintropine» produced by Europharm, the main component is somatropin. The contents of HGH in the sample of 10,33 ME (3,44 mg). Sample purity is 97,19%.

«IPC «Silver Pharm» Ltd

Customer



Tsoy D.V.