

ANALYSIS REPORT № 01/061st of June 2017

Sample name: Norditropine® NordiLet® – somatropin, recombinant human growth hormone. Produced by: Novo Nordisk. Date of production: 05/2016; Use before 04/2018; Series: FC70864; 10 mg/1,5 ml.

Appearance of the sample: colorless transparent solution in a syringe pen for multiple injections (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 µm; mobile phase: A: 20 mM CH₃COONH₄ in deionized water; B: 20 mM CH₃COONH₄ / 25% H₂O in acetonitrile; flow: 0,85 ml/min; thermostat temperature: 45 °C; injection volume: 10 µl.

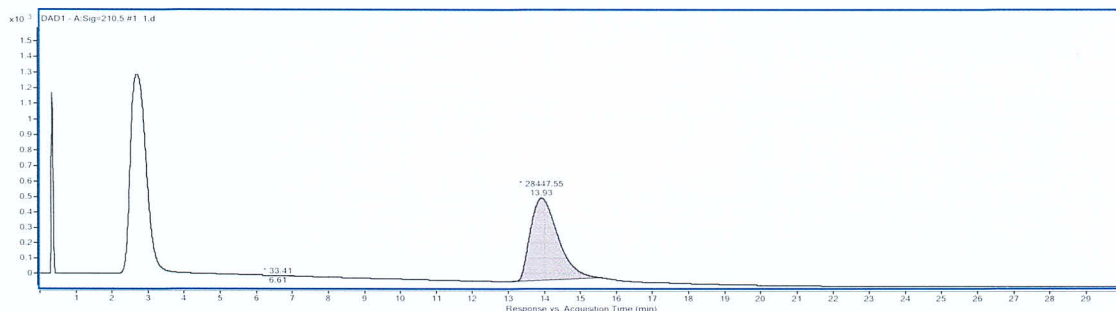
Results of HPLC-DAD analysis:

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.

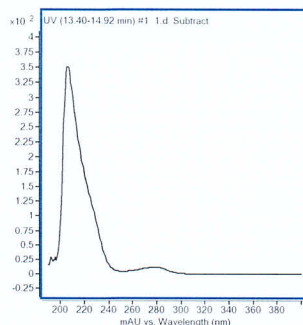


Figure 3 – UV-spectrum of the test sample
(The range of wavelengths is 190 – 400 nm)

Relative component contents of the test sample are presented in the table 1. The content of the main component is 99,88%.

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

№	Time, min	Площадь, усл. ед.	Relative content, %
1	6,61	33,41	0,12
2	13,93	28447,55	99,88
Total		28480,96	

Results of HPLC-MS analysis:

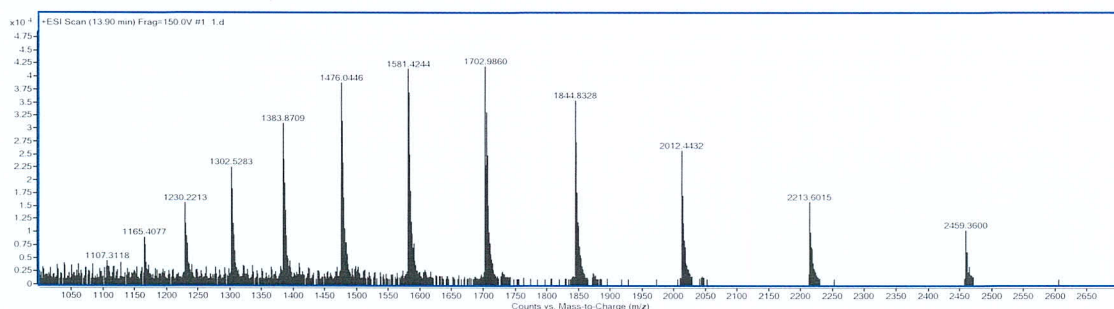


Figure 4 – Mass-spectrum of the test sample, RT= 13,93 min.

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.

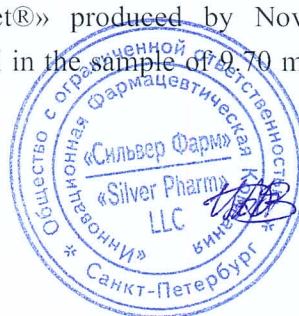
Conclusion:

In the sample «Norditropin® Nordilet®» produced by Novo Nordisk, the main component is somatropin. The contents of HGH in the sample of 9.70 mg/1.5 ml. The purity of the sample of 99.88 %.

Head of chemical analytical laboratory

«IPC «Silver Pharm» Ltd

Customer



Tsoy D.V.