

Drug analysis

REPORT

No. 17/11

November 19, 2014

Sample name: Unknown sample, perhaps TB-500 peptide drug, which is a peptide accelerating wound and injury healing, as well as promoting protective effect.

Sample appearance: white pressed lyophilized powder in a transparent glass vial with a black cap (figure 1).



Figure 1 – Sample

Dissolution: the drug was dissolved in deionized water to a concentration of 1 mg/ml. Its appearance upon dissolution: a transparent liquid without opalescence and foaming while pipetting.

Analysis technique:

Determination of purity: HPLC with a diode-matrix detector (Agilent 1100 series, HPLC-DAD).

Qualitative Analysis: HPLC with a tandem quadrupole time-of-flight mass-detector (Agilent 6530 Accurate-Mass Q-TOF, HPLC-MS) (figure 4).

Conditions of chromatographic analysis: the column: Merck LiChrospher WP 300 RP-18 250-4 mm; mobile phase: A: 100 % H₂O/0.1% TFA (v/v), B: 10 % H₂O/ 90% ACN/0.1% TFA (v/v/v); flow rate: 1 ml/min; thermostat temperature: 30°C; detection: UV 210 nm, injection volume - 20 µl.

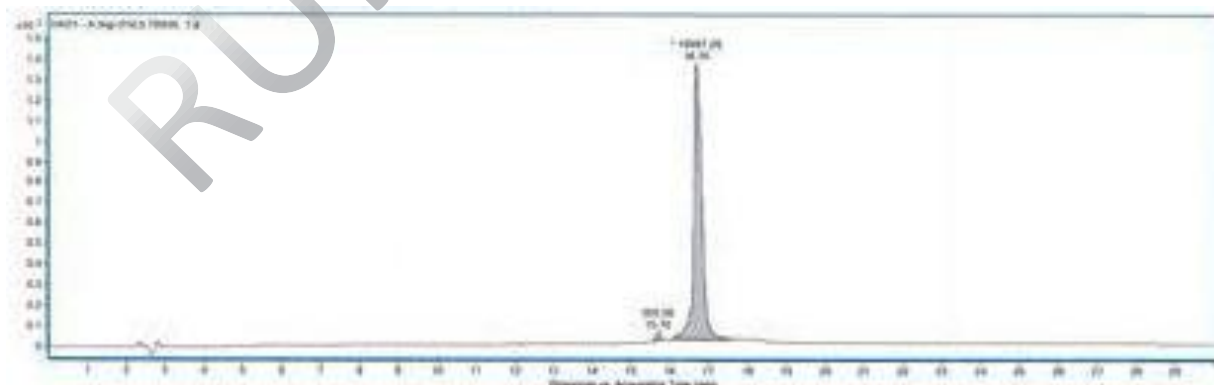
Results of HPLC-DAD analysis:

Figure 2 – Chromatogram of the sample

The peak value with a retention level of 16.70 min and UV absorption spectrum (figure 3) in the chromatogram (figure 2) correspond to the TB-500 drug.

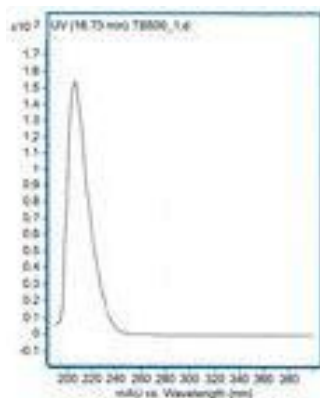


Figure 3 – UV spectrum of the sample
(wavelength range is 190-400 nm)

Relative abundance of components in the sample is presented in the table 1. The abundance of the TB-500 peptide in the sample is 98.49%.

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

No.	Time, min	Area, conv. units	Relative abundance, %
1	15.7	305.58	1.51
2	16.7	19997.29	98.49
Total		20302.87	

The results of HPLC-MS analysis:

Figure 4 – Mass-spectrum of the sample, RT = 16.7

The mass-spectrum (figure 4) shows the peak value of the analyzed drug with a retention time of 16.7 min, the peak with $m/z = 1241.87$ and with $m/z = 1655.17$; the isotopic pattern corresponds to the quadrivalent peptide ion $[TB-500 + 4H]^{4+}$ and trivalent peptide ion $[TB-500 + 3H]^{3+}$ respectively. According to the analysis report, the molecular mass of the peptide is 4963.5 Da.

Conclusion:

1) The main component of the unknown sample is TB-500 peptide. The sample purity is 98.49 %.

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