[redacted]

EXPERT RESEARCH PROTOCOL

from **09/09/2015**

Code:	15-08-20-1 (106q)
Customer:	[redacted]
Number of samples:	Quantitative analysis of the sample
Methods:	Agilent 1200, High-performance liquid chromatography (HPLC); Column: Zorbax SB-C18 150 mm×2.1 mm, 3 mkm; Detector – DAD, wavelength – 254 nm; Detector – MSD, ionization method APCI Positive, SCAN (100 - 500 m/z)
Number of samples:	1
Subject:	Clen powder (Clenbuterol)

1. The sample weighed 0.061 g is dissolved in 1 ml of solvent (MeOH)(A). The extraction is 30 min in an ultrasonic bath.

2. Sample is diluted 300 times

- B 10 µl A + 990 µl of the solvent;

- C 200 µl B+400 µl of the solvent.

3. 30 mg of the standard (Clenbuterol, C2248000, European Pharmacopoeia Reference Standards, Ph. Eur.) dissolved in 1 ml of the solvent (Outlet 1 – concentration - 30 mg/ml).

4. Standard (Outlet 1) diluted 100 times (10 µl Outlet 1+990 µl of the solvent). Received Solution is Outlet 2 – concentration - 0.3 mg / ml (300 mcg / ml).5. Standard (Outlet 2) diluted 6 times (200 µL Outlet 2+1000 µl of the solvent). The received Solution is Outlet 3 – concentration - 0.3 mg / ml (50 mcg / ml).

6. The Outlet 3 solution was used for making calibration dilutions (for injecting 1 µl of solution into the chromatograph):

T4 - Outlet 3 C = 50 mcg / ml;

T3 - 200 µl T4 + 200 µl of solvent C = 25 mcg/ml;

T2 - 200 µl T4 + 800 µl of solvent C = 10 mcg/ml;

T1 - 100 µl T4 + 900 µl of solvent C = 5 mcg/ml.

7. Research conditions:

Mobile phase: A - ACN-1% Formic acid (30%), B - H_20-1% Formic acid (70%).

The elution mode is an isocratic.

The flow rate through the column: 0.3 ml/min. Thermostat temperature is 30^0C.

8. Single quadrupole mass analyzer is used for identification of the chemical elements.The samples were ionized in the chemical ionization mode at atmospheric pressure (APCI) with fixation of positive ions (Fig. 1)

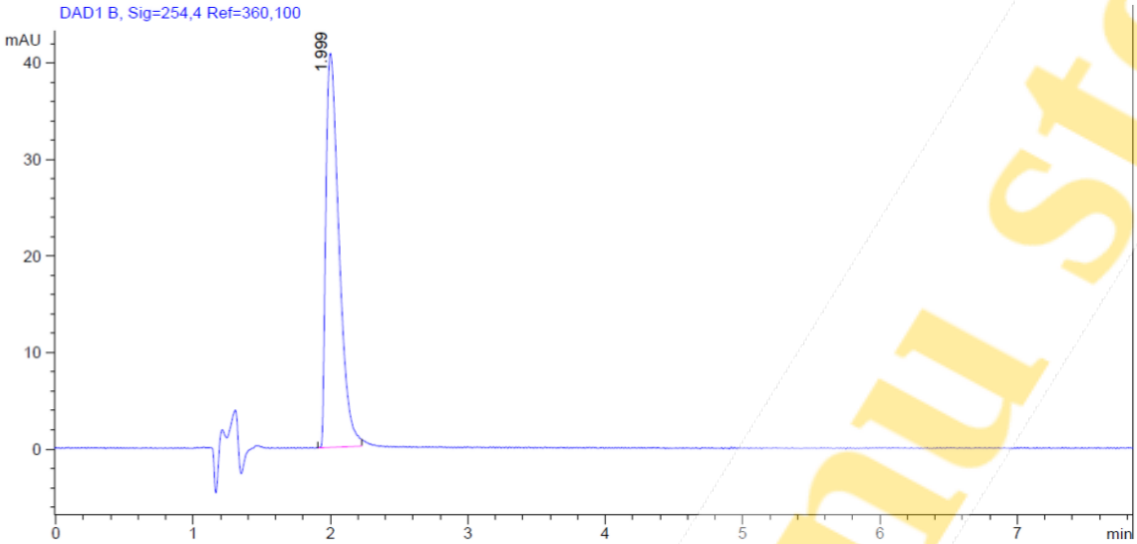

Fig.1. The component output chromatogram of the sample, detector DA

9. The analysis results of the received peaks are shown on Fig. 2.

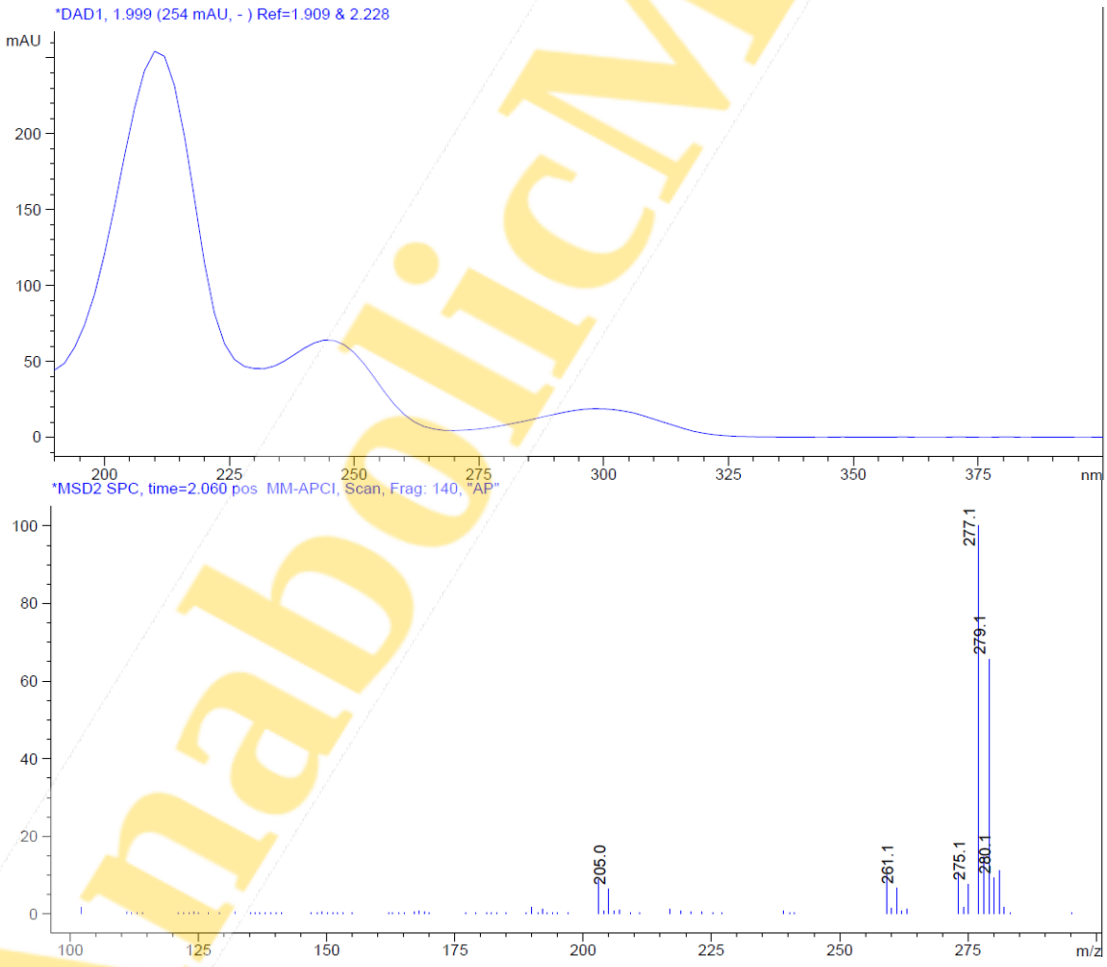

Fig.2. The analysis of the 1st peak

10. The received data of the analytical comparison results of MS and DA detectors with the calculated data on the test substance allow us to state that peak 1 refers to Clenbuterol.

11. The researched data of the standard samples for making a calibration chart is illustrated in the Tabl 1.

Tbl. 1. Peak values in accordance with the concentration of the test substance

Signal 1: DAD1 B, Sig=245,4 Ref=360,100

RetTime [min]	Sig	Lvl	Amount [mkg/ml]	Area	Amt/Area	Ref	Grp	Name
2.007	1	1	5.00000	17.96171	2.78370e-1			Clenbuterol
		2	10.00000	34.09997	2.93255e-1			
		3	25.00000	89.43427	2.79535e-1			
		4	50.00000	170.09660	2.93951e-1			

12. The calibration chart (Fig. 3) is made form received data of the Table 1.

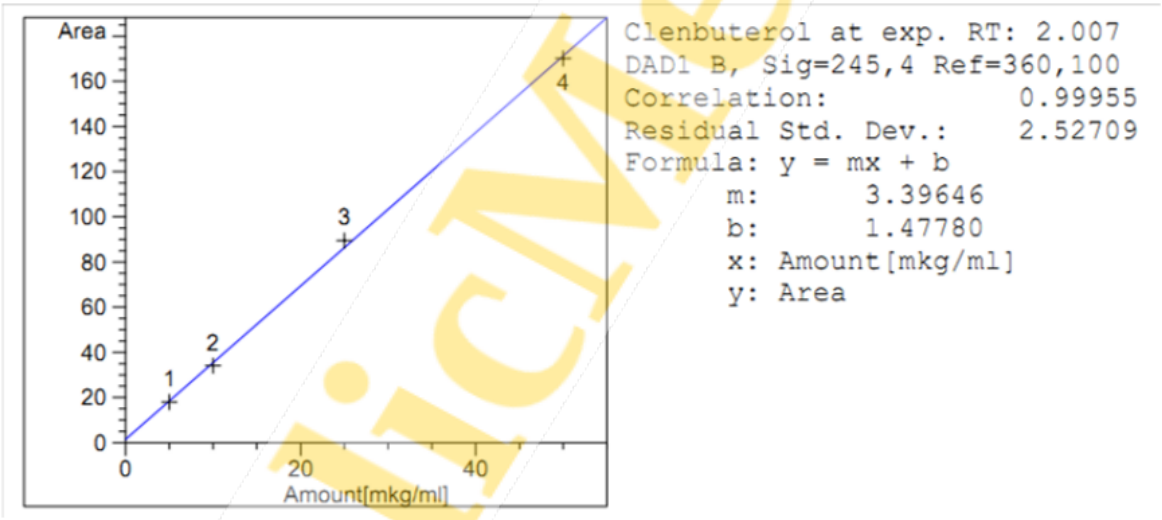

Fig.3. Calibration curve

13. The calculation of the concentration of the test sample from the HPLC data from the obtained calibration curve:

14.83 mcg/ml

14.72 mcg/ml

14.54 mcg/ml

14.97 mcg/ml14. The calculated average is 14.77 mcg/ml.

15. The test sample was diluted 300 times, so the value of the drug concentration

In the undiluted (original) sample will be equal to 14.77 mcg/ml x 300 = 4431 mcg/ml.

The sample of the preparation of 0.061 g is dissolved in 1 ml of solvent, therefore it will contain 4431 µg of preparation.

16. The amount of the sample in one gram is: 72639,34 mcg (72.64 mg).

Conclusion: Concentration of the test sample Clen powder (Clenbuterol) is 72.64 mg/g.

Remarks:

1. This conclusion was issued based on the results of examination of the provided samples. This conclusion does not guarantee the overall quality of the products.
2. This conclusion can not be used for advertising purposes. It can not serve as an evidence in a lawsuit or to be used for other purposes.