EXPERT RESEARCH PROTOCOL

from **09/09/2015**

Code:	15-08-20-1 (101q)
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 – 240 nm; Detector – MSD, ionization method APCI Positive, SCAN (100-500 m/z)
Number of samples:	1
Subject:	Dianabol (Methandrostenolone)

1. The sample weighed 0.122 g is dissolved in 1 ml of solvent (MeOH/Chloroform)(A). The extraction is 30 min in an ultrasonic bath.

2. Sample is diluted 60 times

- B 100 µL A + 900 µl of the solvent;
- C 100 µL B+500 µL of the solvent.

3. 100 mg of the standard (Methandrostenolone, analytical standard, 90151-100MG, European Pharmacopoeia Reference Standards, Ph. Eur.) dissolved in 1 ml of the solvent (Outlet 1-concentration-100 mg/ml).

4. Standard (Outlet 1) diluted 100 times (100 µL Outlet 1+900 µl of the solvent). The received Solution is Outlet 2 – concentration -1 mg/ml.

5. The Outlet 2 solution was used for making calibration dilutions (for injecting 1 µl of solution into the chromatograph):

T4 - 200 µl Outlet 2 + 200 µl of solvent C = 0.5 mg/ml;

T3 - 100 µl Outlet 2 + 300 µl of solvent C = 0.25 mg/ml;

T2 - 100 µl Outlet 2 + 700 µl of solvent C = 0.125 mg/ml;

T1 - 50 µl Outlet 2 + 750 µl of solvent C = 0.0625 mg/ml.

6. The research conditions:

Mobile phase: A - MeOH (65%), B - H 2 0 (45%). The elution mode is an isocratic.

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

7. 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)

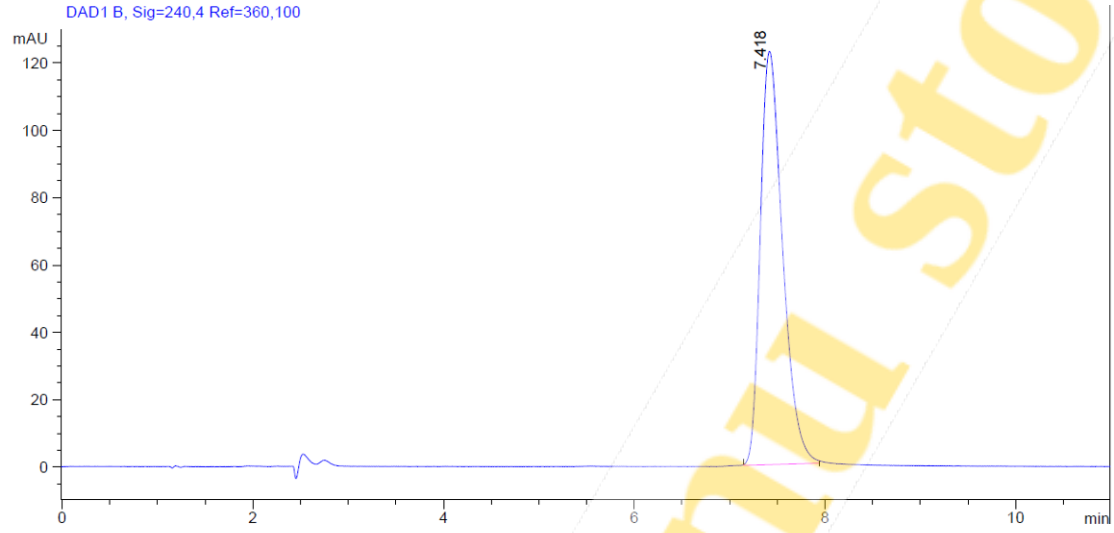


Fig.1. The component output chromatogram of the sample, detector DA

8. The analysis results of the received peaks illustrated on Fig. 2.

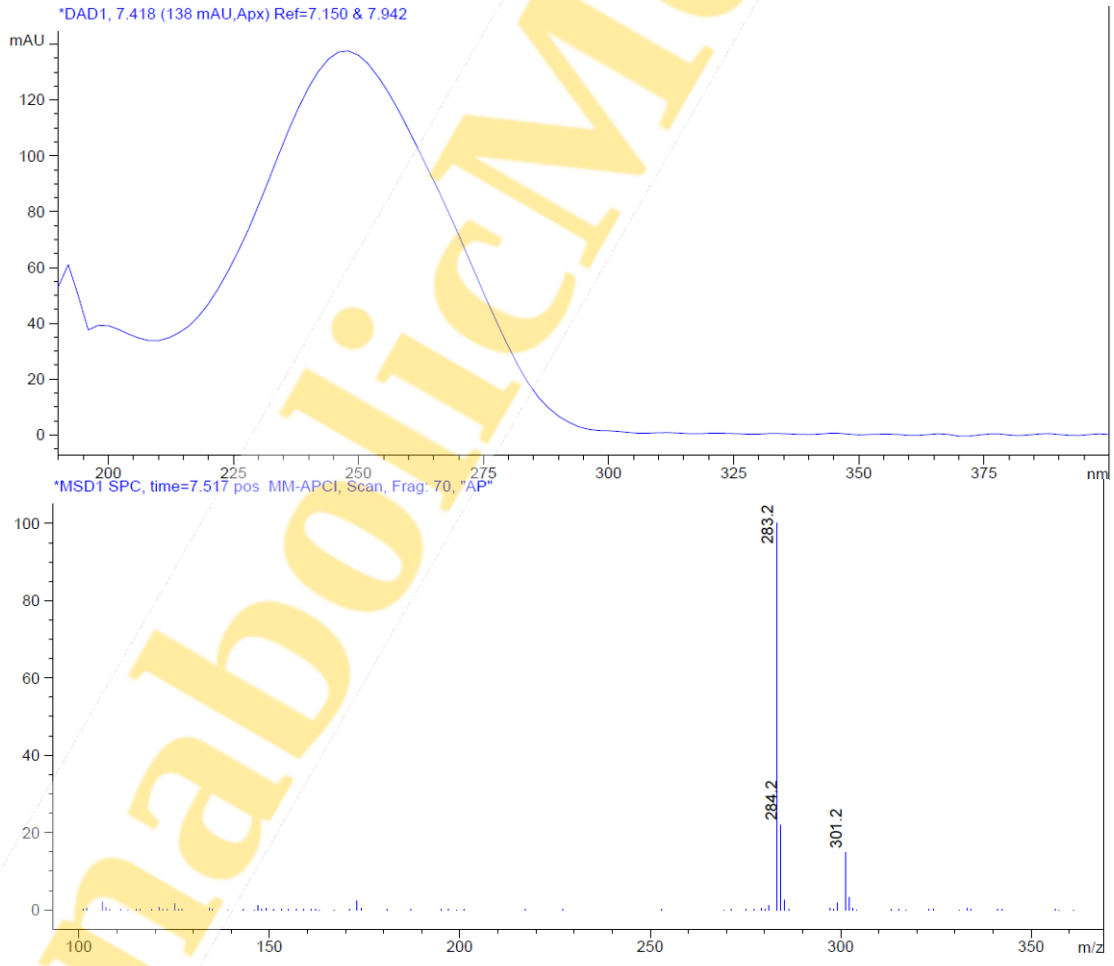


Fig.2. The analysis of the 1st peak

9. 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 Methandrostenolone..

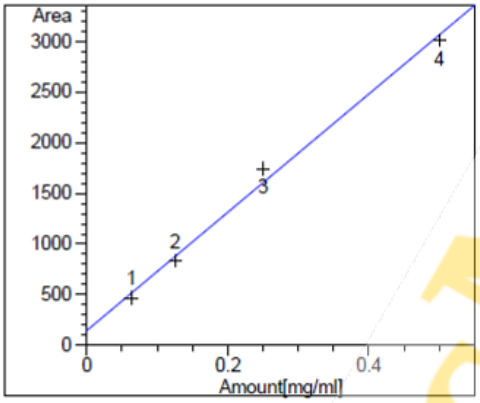
10. The researched data of the standard samples for making a calibration chart is illustrated in the Tabl 1.

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 [mg/ml]	Area	Amt/Area	Ref	Grp	Name
7.579	1	1	6.25000e-2	468.47192	1.33412e-4			Methandrostenolone
		2	1.25000e-1	836.27588	1.49472e-4			
		3	2.50000e-1	1743.35034	1.43402e-4			
		4	5.00000e-1	3012.96484	1.65949e-4			

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



Methandrostenolone at exp. RT: 7.579
DAD1 B, Sig=245,4 Ref=360,100
Correlation: 0.99674
Residual Std. Dev.: 111.94349
Formula: y = mx + b
m: 5837.44423
b: 147.11476
x: Amount [mg/ml]
y: Area

Fig.3. Calibration curve

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

0.168 mg/ml

0.167 mg/ml

0.167 mg/ml

13. The calculated average is 0.167 mg/ml.

14. The test sample was diluted 60 times, so the value of the drug concentration

In the undiluted (original) sample will be equal to 0.167 mg/ml x 60 = 10.02 mg/ml.

The sample of the preparation of 0.122 g is dissolved in 1 ml of solvent, therefore it will contain 10.02 mg of the preparation.

15. Weight of the pills: 0.122 g; 0.131 g; 0.126 g; 0.135 g; 0.131 g. Average is 0.129 g.

16. The amount of the sample in one pill is: 10.59 mg.

Conclusion: Concentration of the test sample Dianabol (Methandrostenolone) is 10.59 mg in a pill.

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.