

## EXPERT RESEARCH PROTOCOL

from **08/22/2015**

Code: 15-08-20-1 (100)  
Customer:   
Type of analysis: Qualitative analysis of the sample, determination of purity  
Methods: Agilent 1200, High-performance liquid chromatography (HPLC);  
Column: Zorbax SB-C18 150 mm×2.1 mm, 3 mkm;  
Detector – DAD, wavelength – 215, 240 nm;  
Detector – MSD, ionization method APCI Positive, ESI Positive, SCAN (100-500 m/z)  
Number of samples: 1  
Subject: Anavar (Oxandrolone)

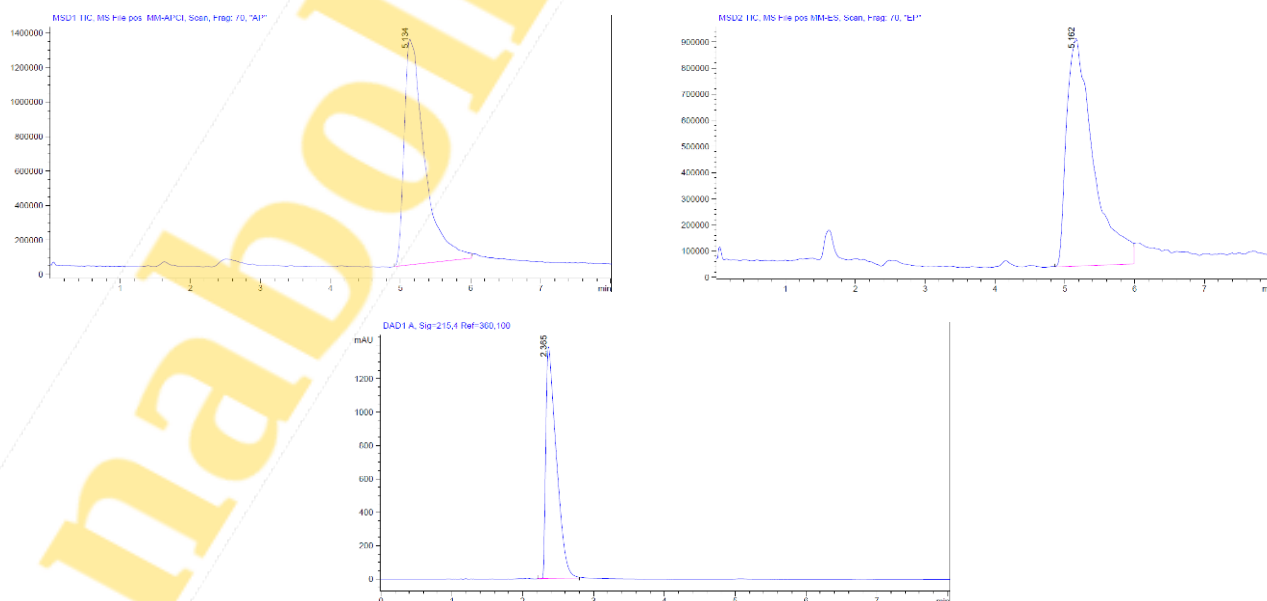
The solvent for the sample: Chloroform.

Mobile phase: A - MeOH (65%), B - H<sub>2</sub>O (45%).

The elution mode is isocratic.

The flow rate through the column: 0.3 ml/min. A thermostat: 300 °C.

Single quadrupole mass analyzer is used for identification the chemical elements. The samples were ionized at the electrostatic spraying (ESI) and atmospheric pressure with chemical ionization (APCI) mode with fixed positive and negative ions.



**Fig.1. The component output chromatogram of the sample, detector DA, MS in EP, MS in AP**

**Tbl. 1. The calculation results of the peak areas on Fig. 1**

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Area Percent Report  
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Sorted By : Signal  
Multiplier : 1.0000  
Dilution : 1.0000  
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	5.134	BB	0.2676	2.65592e7	1.30963e6	100.0000
Totals :				2.65592e7	1.30963e6	

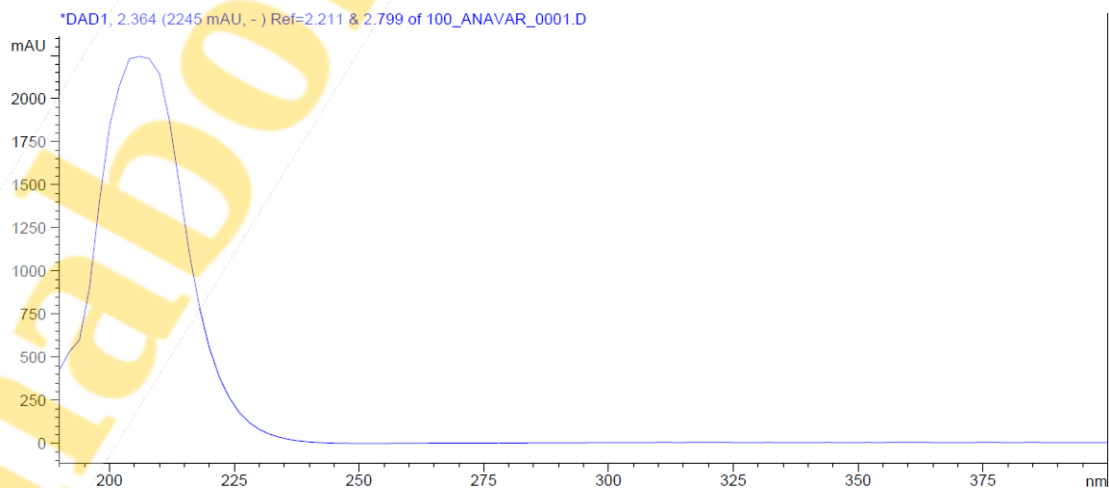
Signal 2: MSD2 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	5.162	VV	0.3800	2.33494e7	8.70826e5	100.0000
Totals :				2.33494e7	8.70826e5	

Signal 3: DAD1 A, Sig=215,4 Ref=360,100

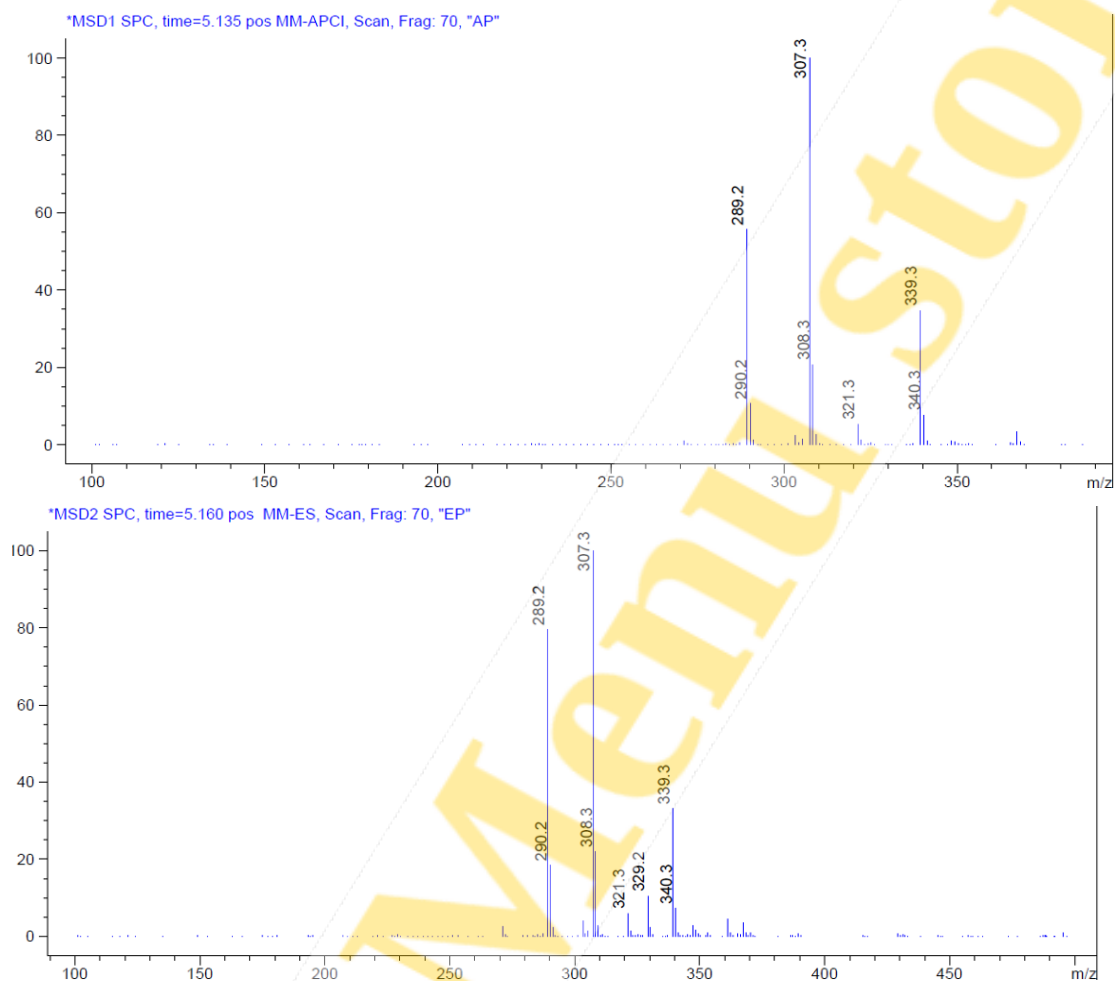
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.365	BB	0.1404	1.43101e4	1386.72034	100.0000
Totals :				1.43101e4	1386.72034	

The analysis results of the received peaks by detector DA are shown in Fig. 2



**Fig. 2. The analysis of the peak 1, DA detector**

The analysis results of the received peaks by detector MS are shown on Fig. 3



**Fig. 3. The analysis of the peak 1, MS detector (modes EP and AP)**

The received data of the analytical comparison results of MS detectors with the calculated data on the test substance allow us to state that peak 1 refers to Oxandrolone (mw 306.44).

The chromatographic purity of Oxandrolone by MS and DA detectors is 99%.

**Remarks:**

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