

EXPERT RESEARCH PROTOCOL

from 08/22/2015

Code: 15-08-20-1 (106)
Customer:
Number of samples: 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 – 225, 254 nm;
Detector – MSD, ionization method APCI Positive/Negative, SCAN (100 - 500 m/z)
Number of samples: 1
Subject: Clen powder (Clenbuterol)

The solvent for the sample: MeOH.

Mobile phase: A - ACN-1% Formic acid (30%), B - H₂O-1% Formic acid (70%).

The elution mode is isocratic.

The flow rate through the column: 0.3 ml/min. Thermostat: 30°C.

Single quadrupole mass analyzer is used for identification of 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.

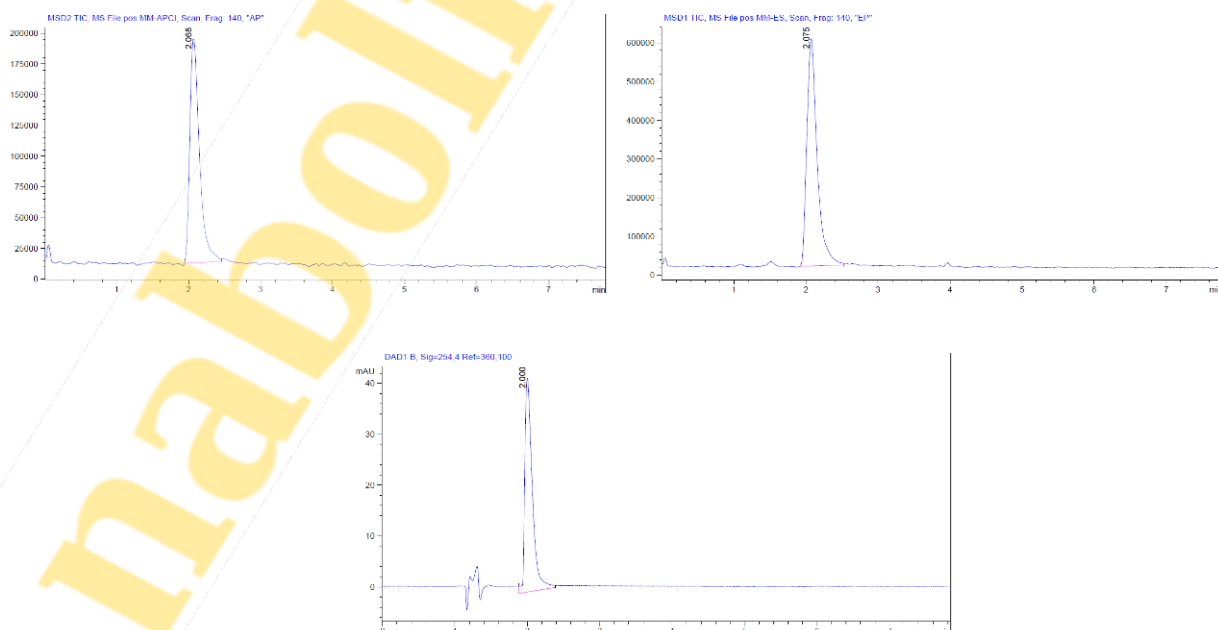


Fig.1. The component output chromatogram of the sample, detector DA, MS B AP, MS B EP

Tbl. 1. The calculation results of the peak areas on Fig. 1-3

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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: DAD1 B, Sig=254,4 Ref=360,100

| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|----------|---------------|------|-------------|--------------|--------------|----------|
| 1 | 2.000 | BB | 0.1086 | 301.01862 | 42.00079 | 100.0000 |
| Totals : | | | | 301.01862 | 42.00079 | |

Signal 2: MSD1 TIC, MS File

| Peak # | RetTime [min] | Type | Width [min] | Area | Height | Area % |
|----------|---------------|------|-------------|-----------|-----------|----------|
| 1 | 2.075 | BB | 0.1565 | 5.83861e6 | 5.91511e5 | 100.0000 |
| Totals : | | | | 5.83861e6 | 5.91511e5 | |

Signal 3: MSD2 TIC, MS File

| Peak # | RetTime [min] | Type | Width [min] | Area | Height | Area % |
|----------|---------------|------|-------------|-----------|-----------|----------|
| 1 | 2.068 | BB | 0.1388 | 1.70220e6 | 1.84308e5 | 100.0000 |
| Totals : | | | | 1.70220e6 | 1.84308e5 | |

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

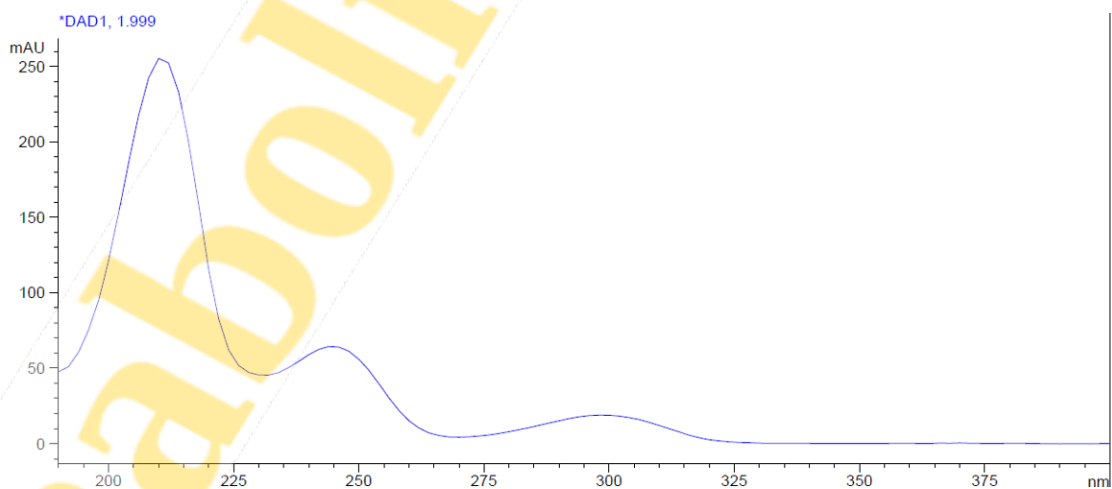


Fig. 2. The analysis of the peak 1, DA detector

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

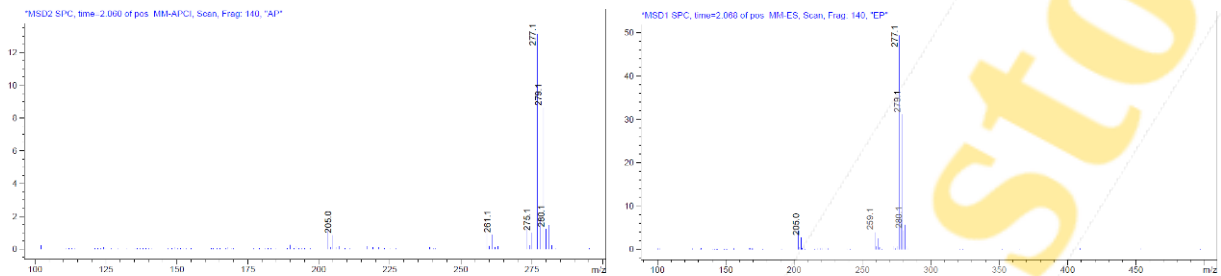


Fig. 3. The analysis of the peak 1, MS detector (modes AP and EP)

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 Clenbuterol (mw 277.1).

The chromatographic purity of Clenbuterol by MS and DA detectors is 98%.

Remarks:

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