# Evolution GC-MS/MS

## Transforming the best GC-MS system into something even better:


# Application Note Evo608

**Evolution-GC-MS/MS: Analysis of doping confirmation compounds**

This short application note summarizes the results of an Evolution MS/MS demo that was held in June 2006 in Idstein, Germany.

The customer supplied derivatized (TMS) urine samples to check the sensitivity and linearity for some commonly known substances in the field of doping analysis.

We adjusted the GC-MS parameters as close as possible to the values that are used in the customer´s lab.

Only the MS/MS-transitions were set up by Chromtech according to daughter scans of supplied standards. Transitions were found for all compounds and the collision energies were roughly optimized during one GCMS/MS run.

With the operating conditions described below, we checked for the linearity and sensitivity of the analytical system: GC 6890N/5973 upgraded to the Evolution Triple Quad system for MS/MS.

## Linearity

The linearity test was carried out with 19-norandrosterone in concentrations from 0.5 to 20 ng/mL on matrix. As internal standard 17α-methyltesto-sterone was used and one injection per calibration level was done. The peak identification and calibration was carried out automatically with MSD Chemstation software. Figure 1 shows the calibration curve together with the formula and correlation coefficient ($r^2$=0.998495, n=8).

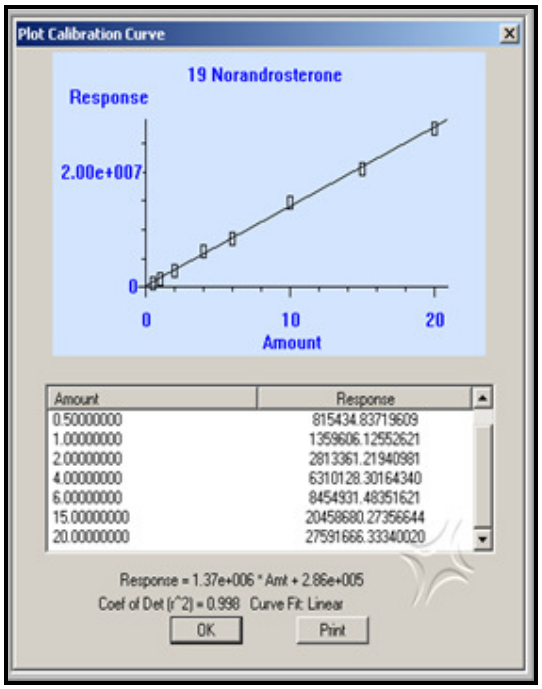


Figure 1: Linearity for 19-norandrosterone with a concentration range from 0.5 – 20ng/mL.

The customer assured us that the linearity of their ion trap instrument was less satisfactory than this. In addition, we checked the stability of the relative abundance of the two transitions that were used as target and qualifier in the data analysis method: The given ratio achieved was 97.3 with 2.7% relative standard deviation (n=8). Even at the lowest concentration (0.5 ng/mL) the ratio was excellent as shown in Figure 2.

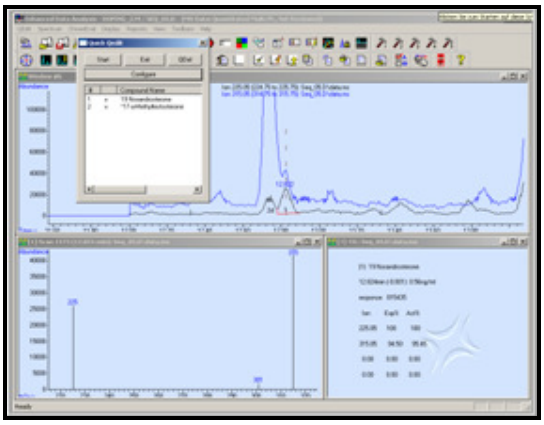

Figure 2: Lowest standard level (0.5 ng/mL) shown with the MSD Chemstation feature „Qedit Quant Result“: Note the good peak shape at retention time 12.822 min. Even at this low concentration the qualifier ratio of 95.7% was sufficient (94.7% required).

| *Compound* | *RT* | *MS/MS-Transitions ** | *SIM Trace*** |
|---|---|---|---|
| Clenbuterol | 7.65 | 335>300, -6<br>335>227, -6 | 300<br>227 |
| 19-Norandrosterone | 12.8 | 405>225, -6<br>405>315, -6 | 225<br>315 |
| Epimethendiol | 13.3 | 358>301, -10 | 301 |
| 19 Noretiocholanolone | 13.7 | 405>225, -6<br>405>315, -6 | 225<br>315 |
| Methyltestosterone – M1 | 15.5 | 435>345, -6<br>435>255, -6 | 345<br>255 |
| Methyltestosterone – M2 | 15.6 | 435>345, -6<br>435>255, -6 | 345<br>255 |
| 17-Epimethandienone | 16.0 | 444>206, -10<br>444>339, -10 | 206<br>301 |
| 17α-Methyltestosterone (ISTD) | 136 | 446>301, -6<br>446>356, -6 | 301<br>356 |

Table 1: Names, retention times, transitions and SIM traces of all analysed compounds.
*Format is Q1 [m/z] > Q3 [m/z], Collision energy [V] of Q2
**Automatically created SIM trace to use TripleQuad data in MSD Chemstation Data Analysis.

## Sensitivity

The following snapshots (Figures 3 and 4) were taken from a sample of urine spiked with 2 ng/mL of each compound. The sensitivity was as good as the customer hoped to see.

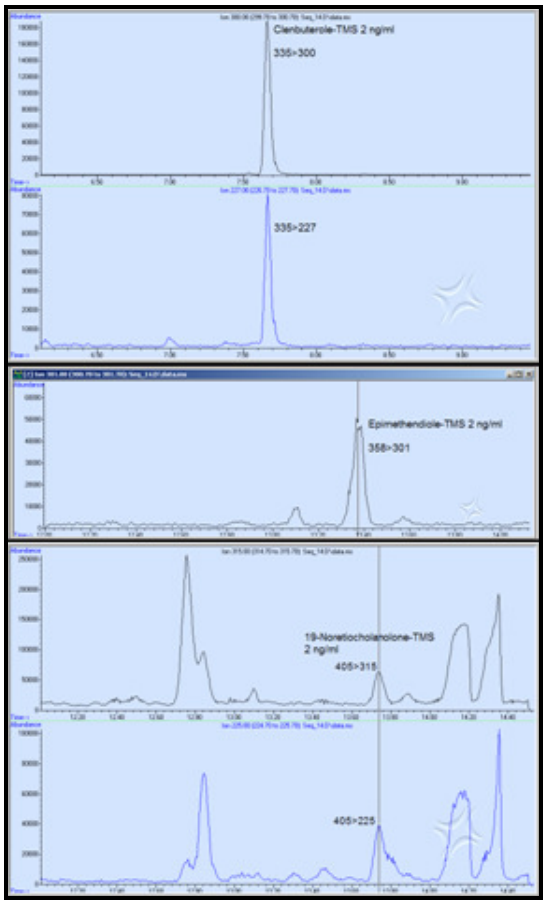


Figure 3: TIC of Clenbuterol, Epimethendiol and 10-noretiocholanone (all TMS-derivatives) each at 2 ng/mL. Despite the fact that the urine matrix is sometimes visible, a goof target and qualifier transition could be found in most cases.

In conclusion, the Evolution MS/MS proved to be a sensitive and linear Triple Quadrupole that is suitable for doping analysis with difficult matrices.

## Instrumentation

**Combi PAL Autosampler:**
Sample volume: 2 µL; Air volume: 700 nL; Preclean w/ Isooctane; Injection Speed 100µL/sec

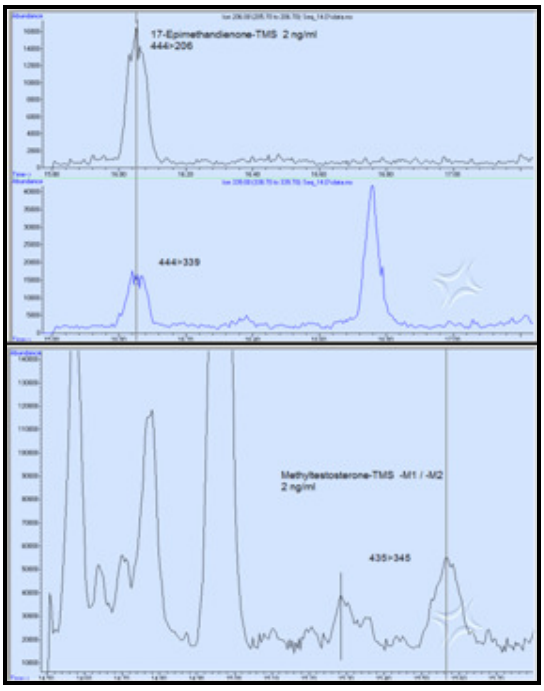


Figure 4: TIC of 17-Epimethandienone and methyltestosterone-M1 and -M2, both TMS-derivatives.

**GC Agilent 6890N:**
Injector: 260°C; temperature: 260°C; liner: 4mm; single tapered, empty; Mode: pulsed splitless: pulse pressure 2.40 bar; pulse time: 2.00 min; purge time: 1.00 min; purge flow: 30 mL/min
Oven program: 130°C (0 min)> 55°C/min> 185°C (0 min)> 5°C/min> 235°C> 5°C/min > 300 (5 min)
Column: VF-5MS 30m x 0.25mm x 0.25µm; 1.0 mL/min; constant flow, vacuum correction on.

**Evolution MS/MS:**
Transfer line temperature: 300°C; Ion source temperature: 230°C; quadrupole temperature 150°C; Ionization mode: EI; Detector voltage: 1400 V relative to autotune; Scan time: 200 ms; Resolution 1.0(Q1) / 1.0 (Q3).