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Performance Test of the new Mass Selektive Detector HP 5973 in Routine Dope Analysis of Anabolic Androgenic Steroids

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1. General

A HP5973 (MSD) was tested for about four weeks in the Institute of Biochemistry, German Sport University Cologne. This MSD was used as detector of a HP6890 (GC) equipped with a HP7673 (ALS).

The HP5973 was tested mainly under "routine conditions" for analysing anabolic androgenic steroids and their metabolites that means in SIM mode with EI ionisation.

A 17m HP Ultra1 (0.2 mm i.d., 0.11 µm film thickness) had been installed and 770 mainly silylated anabolic androgenic steroid samples were injected. Column installation could be performed easily and without any problems.

The head pressure was adjusted to 13 psi He (constant pressure). The transfer line was heated to 300° C and the quadrupole was heated to 100° C in all methods. In general the source was heated to 220° C and there was no evidence for peak broadening or signal suppression due to cold spots under these conditions especially in the analyses of silylated steroids and their metabolites.

2. Influence of the source temperature

The new HP5973 is equipped with a separate source heating so the influence of the source temperature on the sensitivity could be tested.

2µl of a standard solution containing 0.1 ppm 4ß-hydroxy-stanozolol, tris-TMS were analysed at different source temperatures.

GC conditions

Column:	HP Ultra1, 17m, 0.2mm i.d., 0.11µm film thickness
temperature program:	180°C, 20°C/min - 310°C, 3min
carrier:	13 helium
split:	10 ml He/min

MS conditions

SIM-Ions (dwell time: 20ms):	560 m/z (M^+) plus 6 additional ions
source temperature:	180°C - 280°C

result: Below 220°C source temperature a peak broadening for 4ß-hydroxy-stanozolol, tris-TMS (elution temperature: 308°C) can be detected. At temperatures higher than 220°C the noise increases and at temperatures higher than 240°C the signal decreases due to a more extensive fragmentation. A source temperature between 220°C and 240°C seems adequate for the GC/MS-analysis of this compound and for the silylated anabolic androgenic steroids and their metabolites (Table 1 and Figure 1-3)

source temp(°C)	Signal	noise (peak-peak)	S/N - ratio
180	1071	20	53.5
200	1560	21	74.3
220	2211	23	96.1
240	2790	32	87.2
260	2574	33	78.0
280	2652	34	78.0

Table 1: S/N ratios of the signal obtained from the m^+ (560 m/z) of 4ß-hydroxy-stanozolol, bis-TMS

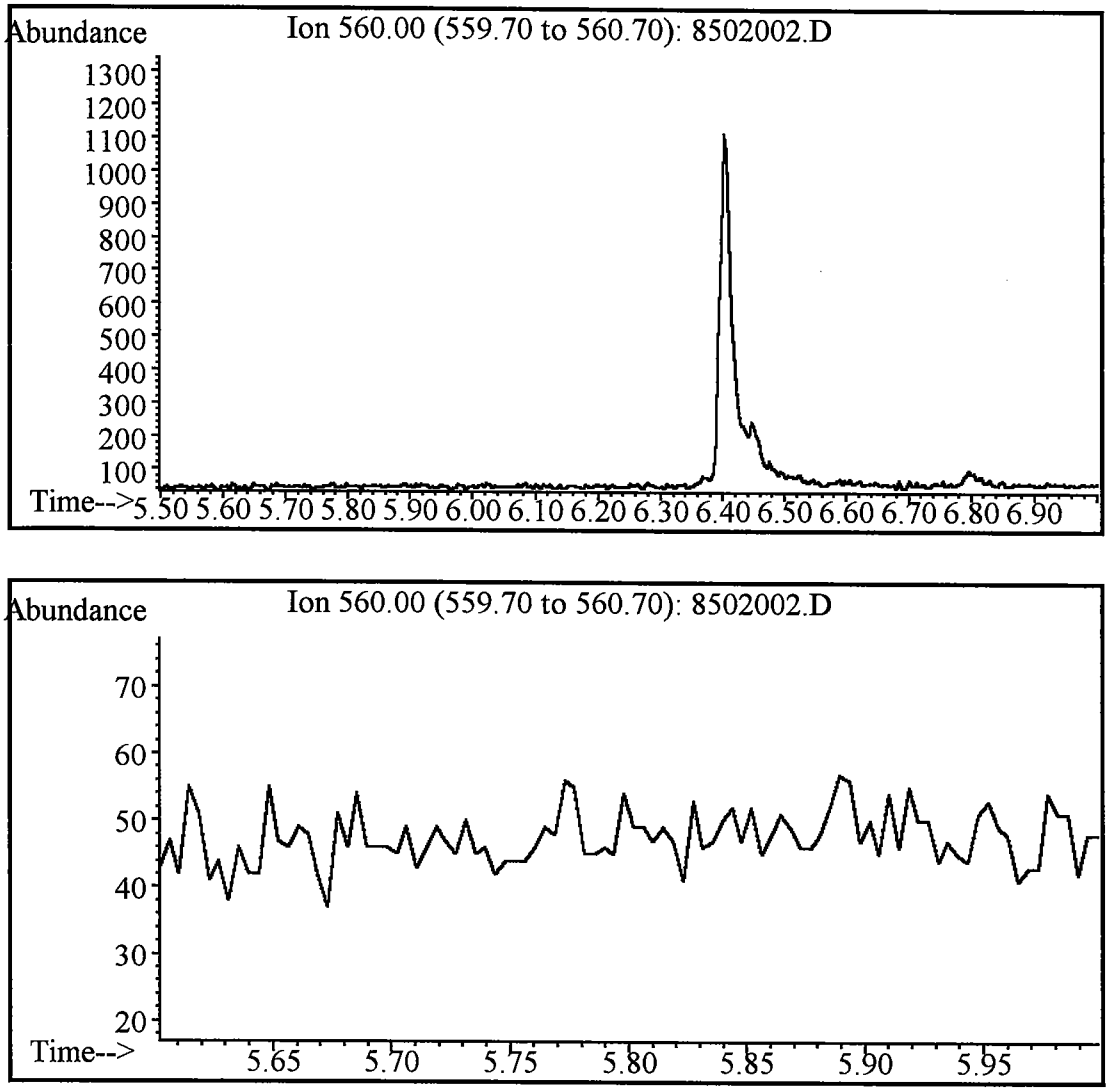

Figure 1: 4ß-Hydroxy-stanozolol,tris-TMS, ion m/z=560 (m^+)
Signal (Top) and noise (bottom) at 180°C source temperature

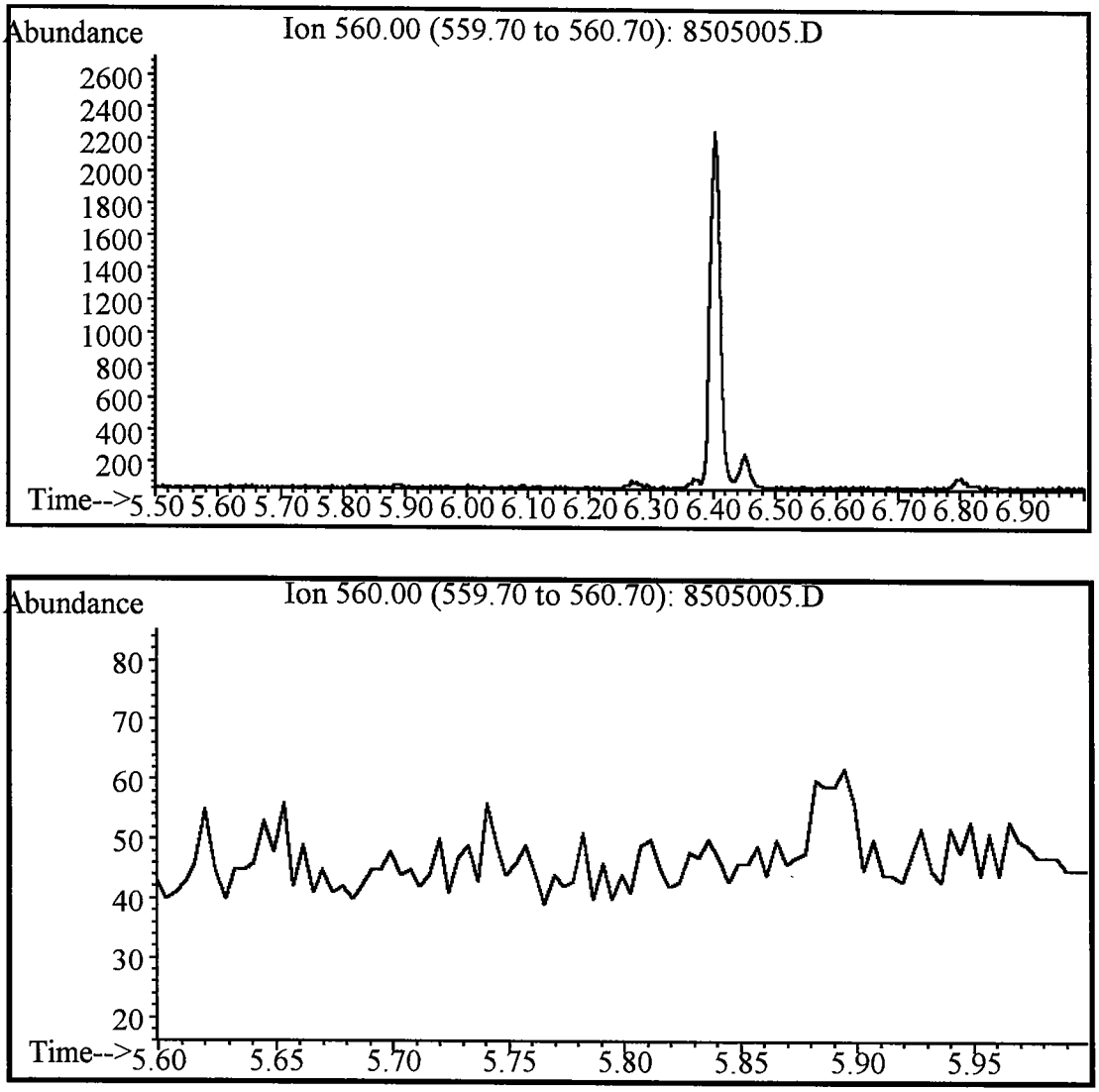

Figure 2: 4ß-Hydroxy-stanozolol,tris-TMS, ion m/z=560 (m^+)
Signal (Top) and noise (bottom) at 220°C source temperature

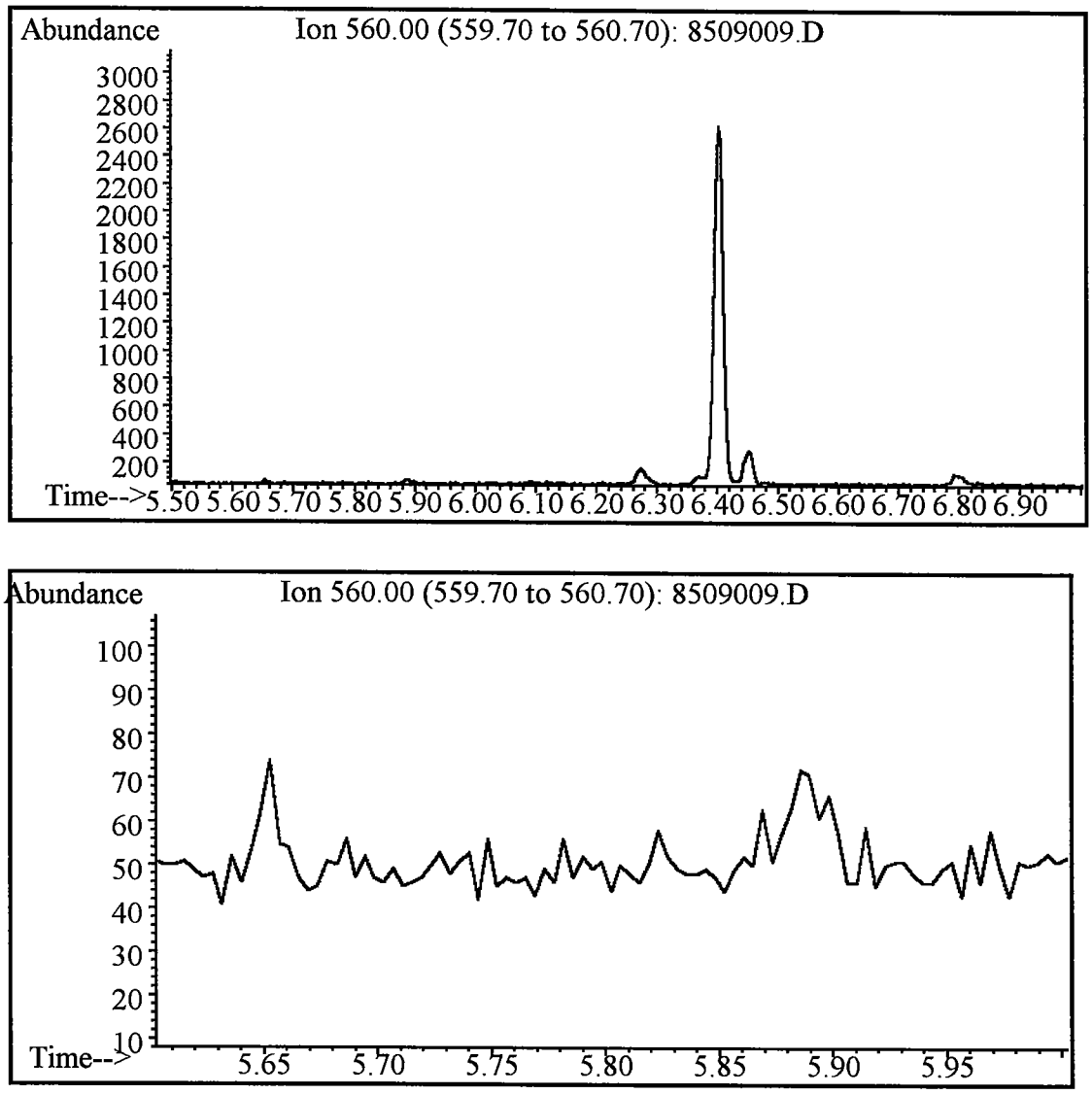

Figure 3: 4ß-Hydroxy-stanozolol,tris-TMS, ion m/z=560 (m^+)
Signal (Top) and noise (bottom) at 260°C source temperature

3. Sensitivity

The sensitivity (measured as signal-to-noise ratio) of the HP5973 was compared to the sensitivity of a HP5971 by the analyses of standards and biological samples loading the ion source with about 10pg in SIM mode under the same chromatographic conditions.

The new HP5973 is more sensitive by a factor of 5 to 10 in comparison to the HP5971. Figures 4 and 5 show norandrosterone, bis-TMS as example.

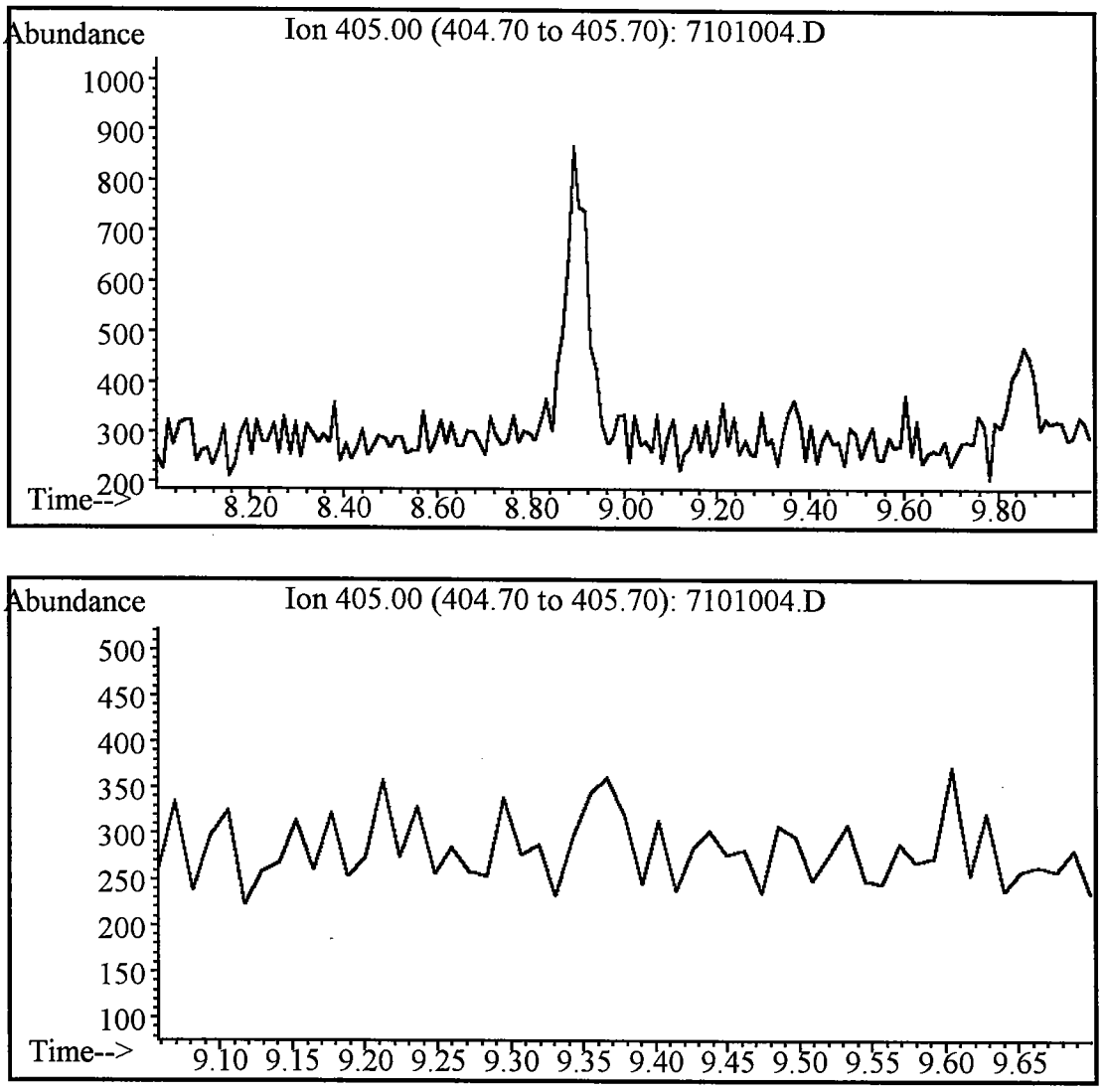

Figure 4: HP5971 - norandrosterone,bis-TMS, ion m/z=405 (m^+ - 15)
Signal (Top) and noise (bottom)
signal-to-noise ratio: 3.9

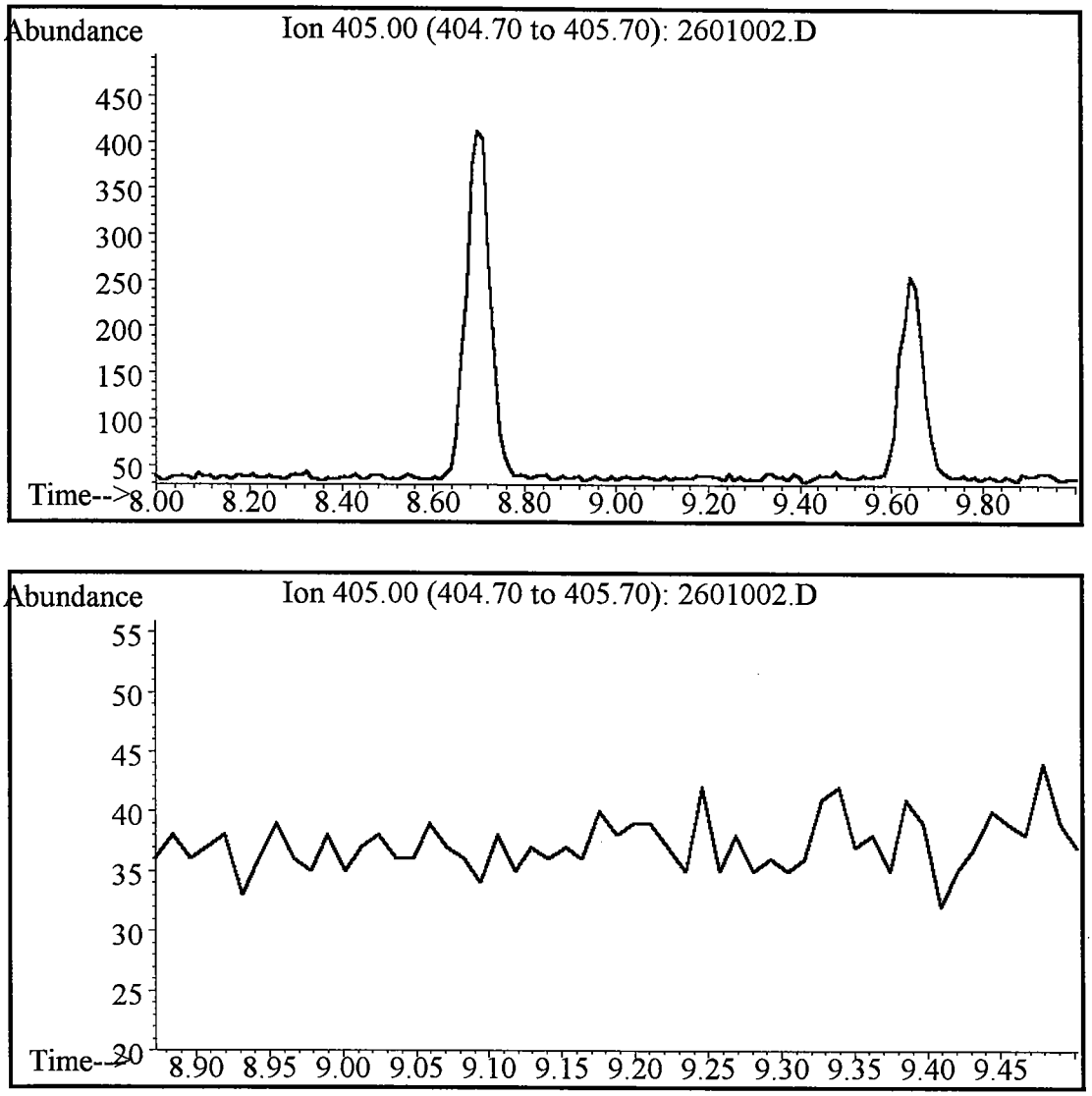

Figure 5: HP5973 - norandrosterone,bis-TMS, ion m/z=405 (m^+ - 15)
Signal (Top) and noise (bottom)
signal-to-noise ratio: 31.2

4. Stability

Several sequences of silylated anabolic steroids were analysed including quality controls. In table 2 the signal-to-noise ratios of norandrosterone, bis-TMS from those quality controls are listed over a period of 13 days where about 600 samples had been analysed. The sensitivity is very stable over this period and decreases slightly with the last analysis. The instrument has been tuned only once (after the first analysis) during this test period.

date of analyses	**time**	**Signal (height)**	**noise (peak-peak)**	**S/N**
24.10.96	16:45	375	12	31.2
29.10.96	07:13	351	13	27.0
29.10.96	15:29	436	13	33.5
30.10.96	10:03	421	15	28.1
30.10.96	12:39	549	20	27.5
31.10.96	20:49	513	17	30.2
01.11.96	14:13	507	16	31.7
02.11.96	06:41	497	19	26.2
06.11.96	21:43	292	15	19.5

table 2: signal-to-noise ratios of norandrosterone, bis-TMS (m/z=405 ,m^+ - 15) over a period of 13 days, about 600 samples analysed.

4. Conclusion

Operation, data evaluation and column exchange could be done easily and without any problems on this HP5973. The sensitivity for the tested silylated metabolites of anabolic androgenic steroids has been increased by a factor of 5 to 10 in comparison to the HP5971 used in our laboratory. This sensitivity has been stable over about 600 samples in a 13 days time period.