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Recent Advances in Doping Analysis (1). Sport und Buch Strauß, Köln 1994

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High-Resolution GC/MS

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INTRODUCTION

High-resolution GC/MS is a useful analytical technique, especially for long-term retrospective analyses in doping control, e.g., the detection of anabolic steroids long after their application has been discontinued. As a result of substantial improvements in instrument geometry and controlling electronics by mass spectrometer manufactures, high-resolution double-focusing mass spectrometers now can be operated for routine analysis in a single-ion detection mode, much like quadrupole mass spectrometers. In this contribution high-resolution single ion monitoring is described and a comparison is made between a quadrupole and a sector mass spectrometer.

Several terms are used to describe a method or analytical technique. With respect to mass spectrometry these include:

- **Resolution**: $R = m/\Delta m$, where Δm is the difference between two masses,
- **Sensitivity**: Change in signal response (measured ion current) with amount of analyte,
- **Detection Limit**: Minimum amount of analyte which can be detected,
- **Signal-to-Noise**: Signal of analyte divided by signal of noise (chemical and electronic).

It is important to note that the sensitivity is inversely proportional to the resolution. The mass resolution used in a particular analysis therefore depends on the sensitivity required and the level of chemical background noise. Normally a resolution between 3,000 and 5,000 is employed for GC/MS analyses.

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Resolution

A quadrupole mass filter operates at unit resolution: m/z 69 ± 0.5 amu
m/z 502 ± 0.5 amu

As such, an ion monitored at m/z 86, for example, could consist of any one of the following structures: $H_2CNHC(CH_3)_3^+$, $C_3H_8NCO^+$, $C_6H_{14}^{+\cdot}$, $C_5H_{10}O^{+\cdot}$, $C_2H_4NCS^+$.

A double-sector mass spectrometer operates at constant resolution:

at resolution 1000	m/z 69 ± 0.0345 amu
	m/z 502 ± 0.251 amu
at resolution 5000	m/z 69 ± 0.0069 amu
	m/z 502 ± 0.0502 amu

At high-resolution it is necessary to use the exact mass of the ion detected. By monitoring ion signals at high-resolution it is possible to suppress signals from ions with the same nominal mass, e.g., the ion $H_2CNHC(CH_3)_3^+$ can be measured using its exact mass m/z = 86.0970.

Exact mass calculation

Calculation of the exact mass is illustrated for testosterone $C_{19}H_{28}O_2$:

^{12}C = 12.00000 * 19
^{1}H = 1.00782 * 28
^{16}O = 15.99490 * 2
exact mass = 288.20893 Dalton

and for testosterone bis-TMS $C_{25}H_{44}O_2Si_2$:

^{28}Si = 27.97692
exact mass = 432.28798 Dalton.

Naturally occurring isotopes

Isotopic variants of carbon, silane, hydrogen and oxygen also exist:

^{1}H = 99.985% ^{2}H = 0.015%
^{12}C = 98.890% ^{13}C = 1.109%
^{28}Si = 92.230% ^{29}Si = 4.670% ^{30}Si = 3.099%

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For testosterone with 19 C atoms the ^{13}C isotopic peak at 289.21228 Dalton occurs with more than 20% abundance.

The isotopic peak of testosterone bis-TMS has contributions from both ^{13}C and ^{29}Si. The difference in the exact mass of these two isotopes is very small (Δm = 0.003790 Dalton).

^{13}C isotope = 433.29134 Dalton

^{29}Si isotope = 433.28755 Dalton

To resolve the ^{13}C and ^{29}Si isotopic peaks, $R = m/\Delta m$ = 433.29 / 0.003790 = 114,250.

EXPERIMENTAL

High-resolution GC/MS measurements are performed using a Finnigan MAT 95 mass spectrometer (Bremen, FRG), which is depicted in Figure 1. This is a reverse-geometry instrument, meaning that the magnetic sector is positioned after the ion source and before the electrostatic analyzer. The 1.7 Tesla magnet has a mass range of 3,500 Dalton at full acceleration voltage, 5 kV.

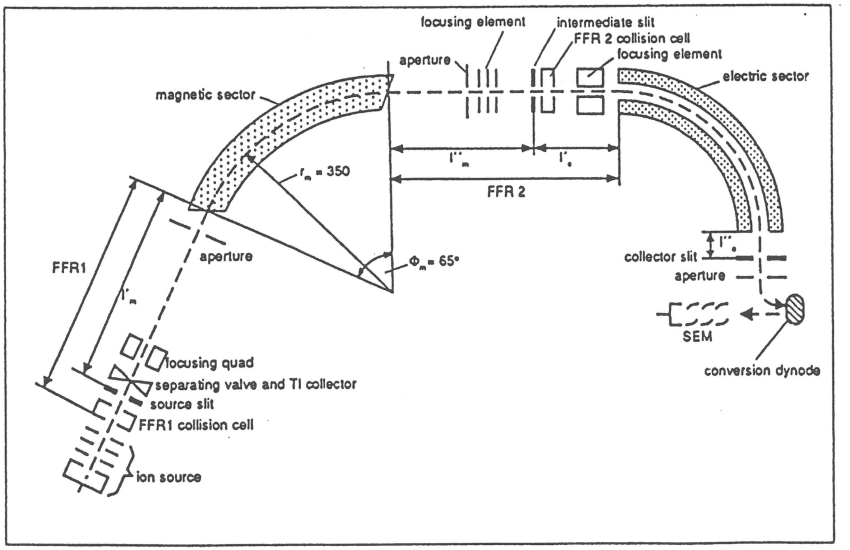

Figure 1: Schematic of the Finnigan MAT 95 mass spectrometer.

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Several ion focussing devices are incorporated into this instrument to insure that any ions formed in the ion source reach the detector with nearly 100% efficiency. The detector is well-separated from the ion source. This reduces background noise, especially noise generated from neutral species. In addition, the detector employs a conversion dynode which improves the detection of high mass ions and makes possible the detection of a single ion. The resolution is adjusted via the source slit and collector slit widths. The instrument has a resolving power of more than 60,000. For GC/MS analyses a resolution of 20,000 or more can be employed, e.g., for elemental composition determination, but normally a resolution of 3,000 is used.

To perform selected ion monitoring (SIM) analyses the MAT 95 mass spectrometer is operated in one of two ways: (i) the magnetic field is set to pass the ion mass of interest at full acceleration voltage, or (ii) the acceleration voltage and electric sector voltages are varied at constant magnetic field strength to pass the desired ion mass. Magnetic scanning is affected by hysteresis and therefore not desirable for rapid exact mass analysis over narrow GC peaks. The electric field, on the other hand, is easily varied at high speed under computer control and can be continuously calibrated during the analyses using a reference compound such as PFK or PFTBA.

At full acceleration voltage V_1, an ion of m/z m_1 passes through the magnet at a given magnet field strength, B (Gauss), as seen via the relation

$$m/z = 4.82 \times 10^{-5} B^2 r^2 / V,$$

where r (cm) is the radius of the ion path. If the acceleration voltage is decreased to V_2, at a constant magnetic field, a higher mass ion, m_2, passes through the magnet, where

$$m_2 = m_1(V_2/V_1).$$

In Figure 2 the parameters used in a high-resolution multiple ion analysis are shown. The ions registered are m/z 375.9807, 405.2645, 420.2880 and 463.9743. The first and last masses are those from the calibration compound, PFTBA, which is continuously leaked into the ion source. Masses 420.2880 and 405.2645 amu are the molecular ion and fragment ion masses of norandrosterone bis-TMS, respectively. In the analysis, the magnet is set to pass the ion m/z 375.9807 (the lock mass) at full acceleration voltage. To record the other ions,

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the acceleration voltage is precisely reduced by the mass ratio of the ion of interest, e.g., 375.9807/405.2645. The exact voltage is continuously recalculated using the lock mass and calibration mass (m/z 463.9743). This process is repeated with a cycletime of 0.5 seconds, which is adjusted to give an adequate number of sample points over a GC peak. Up to 31 ions can be monitored in a time window and 33 time windows can be included in an experiment.

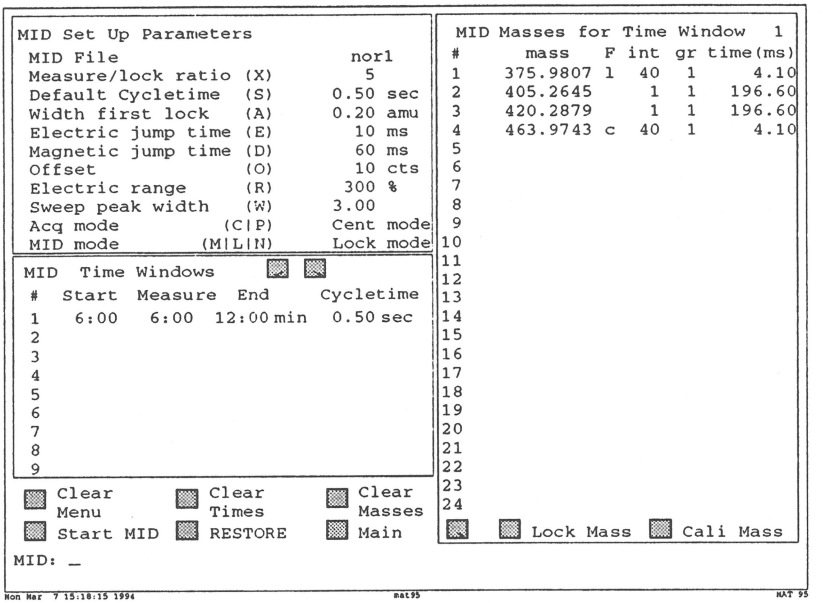

Figure 2: MID (multiple ion detection) parameters used on the Finnigan MAT 95 mass spectrometer for analysis of norandrosterone bis-TMS.

RESULTS AND DISCUSSION

The performance of the Finnigan MAT 95 in high-resolution single-ion monitoring mode is evaluated using a urine sample spiked with norandrosterone (2 ng/ml). 2 ml of urine are prepared for analysis and derivatized using 100 µl reagent (MSTFA/TMSI). 1 µl of sample is injected into the GC at a split ratio of 1:10, so that 4 pg of sample is eluted into the ion source. As a comparison the same test sample is also analyzed using a quadrupole instrument (Hewlett Packard 5971) operated in SIM mode under identical GC conditions.

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GC/MS ion chromatograms for norandrosterone bis-TMS obtained using the sector and quadrupole mass spectrometers are depicted in Figure 3. At a resolution of 3,500 and a multiplier voltage giving a 10^6 gain, the MAT 95 produces a much better defined signal than the quadrupole instrument, despite its lower resolution (unit mass). The sample, 4 pg, gives a molecular ion signal which is more than 70 times larger than the background noise. Using a simple extrapolation analysis and assuming a detection limit based on a signal-to-noise ratio of 3, it should easily be possible to detect as little as 85 pg/ml norandrosterone in a urine sample under these analysis conditions.

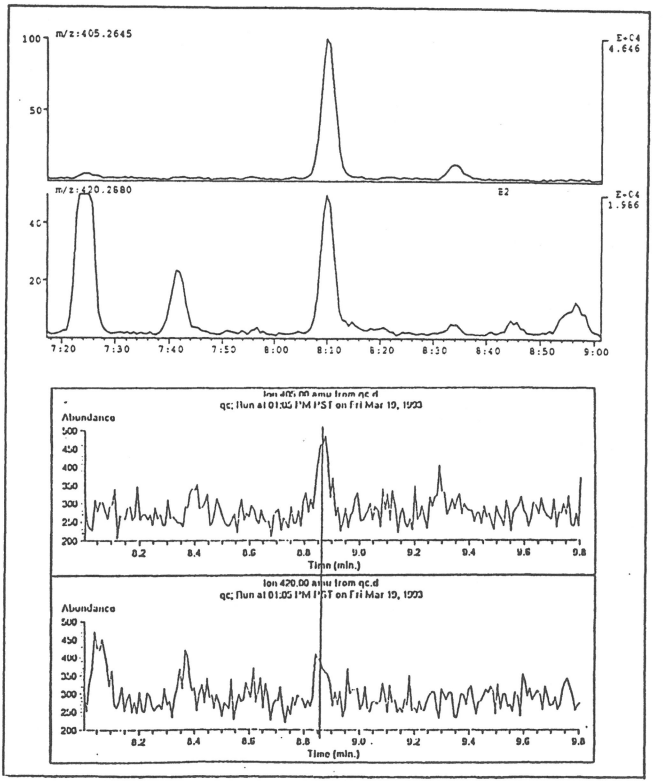

Figure 3: Selected ion monitoring GC/MS analysis of norandrosterone bis-TMS in urine (2 ng/ml or 7.2 nmole/l). Comparison of Finnigan MAT 95 (upper panel) and Hewlett Packard 5971 (lower panel) mass spectrometers for detection of 4 pg sample.

With the quadrupole mass spectrometer the molecular ion (m/z 420) signal cannot be distinguished from the background noise. Reduction of noise is one of the major advantages of high-resolution mass spectrometry. By suppressing all ion signals other than that of the exact ion mass chemical noise can be largely eliminated, although as the nominal mass of the ion increases so does the number of possible structures which have nearly the same exact mass, as was illustrated using the ^{13}C and ^{29}Si isotopes of testosterone bis-TMS in the Introduction. If sufficient sample clean-up steps are taken to remove chemical noise, it is possible to operate at lower mass resolution and hence the signal can be increased. It the above experiment with spiked urine is performed at 1,000 resolution the measured signal increases by 3.5 times. At this resolution, $\Delta m = \pm 0.21$ amu, which is comparable to the resolution of the quadrupole, $\Delta m = \pm 0.5$ amu, it is easily possible to analyze norandrosterone in urine at a concentration as low as 25 pg/ml.

CONCLUSION:

High-resolution GC/MS has many uses in doping analysis, most notably, the detection of trace amounts of sample in urine and other biological media. Another important use is the determination of elemental composition via exact mass measurements for the identification of unknown chemical substances. One new area of research in our laboratory which has been made possible with high-resolution GC/MS instrumentation is steroid profiling in blood. With detection limits in the low pg/ml range it is possible to determine steroid concentrations in plasma samples using straight-forward workup procedures. A further improvement in the detection limit can be made if additional sample clean-up steps are taken, e.g., the sample can be preconcentrated and purified by HPLC prior to GC/MS analysis. Another potential means of reducing chemical noise, which has not been explored, is high-resolution tandem mass spectrometry (MS/MS). Here one could envision an analysis procedure where two mass spectrometers are used, the first for sample clean-up at high mass resolution and the second for sample analysis at unit mass resolution.

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