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RECENT ADVANCES IN DOPING ANALYSIS (4)

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Basics of High Resolution Mass Sectrometry
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Basics of High Resolution Mass Spectrometry

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High resolution mass spectrometry coupled with gas chromatography (GC/HRMS) is a powerful analytical tool. At the 11th Cologne Workshop we described the use of GC/HRMS for the analysis of anabolic steroids and their metabolites and briefly compared a conventional quadrupole GC/MS system to the double focusing GC/HRMS instrument [1]. In this contribution, fundamentals of sector field mass spectrometry and the use of electric field scanning for selected ion monitoring are described. In the accompanying report we report on the screening of steroids by GC/HRMS.

A double-focusing mass spectrometer employs magnetic and electric sectors to separate and focus ions. The magnetic analyzer can either proceed the electric analyzer (BE geometry) or follow it (EB geometry). The magnetic analyzer (B) separates species with different mass-to-charge (*m/z*) ratios based on their different momenta. For the Finnigan MAT 95 (Bremen, Germany) BE geometry, double-focusing mass spectrometer, ions of different momenta are separated in the magnetic field following the equation

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

where B is the magnetic field strength in Gauss, r is the radius (35.0 cm), and V the acceleration voltage (5 kV). The electric sector (E) is an energy analyzer and only allows ions with a given kinetic energy to pass and reach the detector, thereby assuring mass and energy resolution of the charged species.

The resolution of the mass spectrometer is controlled by two slits, one positioned at the exit of the ion source and the other at the collector, just before the detector. Mass resolution can be used to separate species with identical nominal mass but different elemental composition. The

ELEMENTAL COMPOSITION AND MASS RESOLUTION

Element	Symbol	Atomic Mass	Exact Mass
Hydrogen	H	1	1.0078
Carbon	C	12	12.0000
Nitrogen	N	14	14.0031
Oxygen	O	16	15.9949
Fluorine	F	19	18.9984
Silicon	Si	28	27.9769

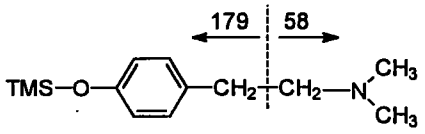
MASS RESOLUTION

Composition	Nominal Mass	Exact Mass
$C_3H_6O^+$	58	58.0419
$C_3H_8N^+$	58	58.0657

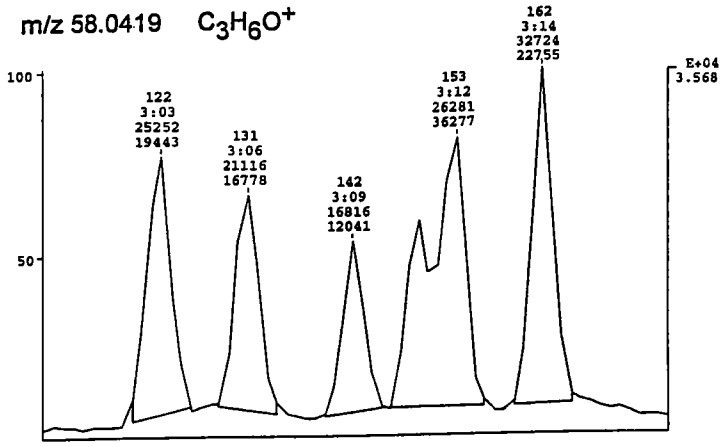
$$R = m/\Delta m$$

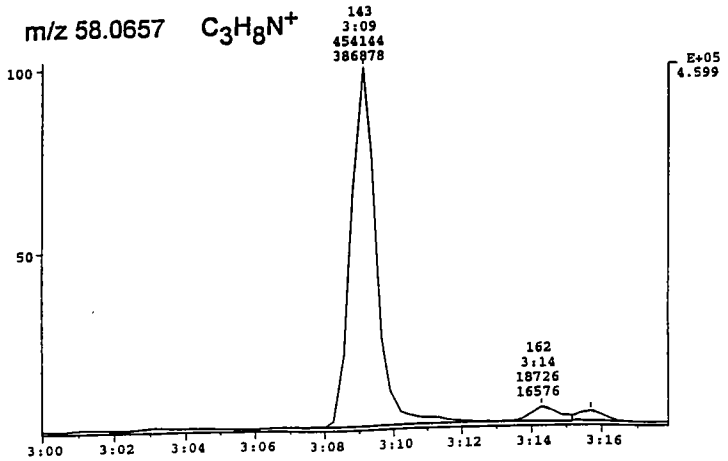
58.05 / (58.0657 - 28.0419) = **2439**

GC/HRMS DETERMINATION OF HORDENINE IN HORSE PLASMA



110 pg/ml plasma
Detected amount in MS 0.2 pg
MAT 95, R = 3000





resolution needed to separate two masses is defined as $R = m/\Delta m$, where Δm is the difference between two masses. The example in the table depicts the nominal mass and the exact masses of the elements which make up the ions $C_3H_6O^+$ and $C_3H_8N^+$ and the resolution needed to separate these two species differing in mass by only 0.024 amu. As illustrated in the accompanying figure, in a horse plasma sample spiked with 110 pg/mL hordenine it is possible to separate the nitrogen and oxygen containing ions and unambiguously determine the presence of hordenine. With a quadrupole mass analyzer it is not possible to separate the oxygen and nitrogen containing *m/z* 58 ions and the determination of hordenine is obscured.

The sector field mass spectrometer can be used to monitor selected ions by scanning either the magnet or the electric field. As magnetic scanning is affected by hystersis, it is not suitable for rapid exact-mass switching over the limited time of a GC peak. The electric field, on the other hand, is highly suitable for rapid, exact-mass monitoring since (i) it can easily be varied at high speed under computer control and (ii) the electric field is continuously calibrated during the analysis using ions with known masses from a reference marker, e.g., perfluorophenanthrene (Scientific Instrument Services, USA), which is leaked into the ion source. Electric field scanning is performed by setting the magnetic field at a given mass, e.g., an ion from the reference compound, and reducing the electric field accordingly to pass the desired higher mass ion of interest. At a reduced electric field (V_1), a higher mass ion (m_1) passes through the magnetic and electric sectors according to the equation

$$m_1 = m(V/V_1).$$

Unfortunately, as the electric field is reduced the ion transmission decreases. The loss in signal depends on the ion source condition (cleanliness) and tune parameters. With good tuning conditions there should be less than 30% loss in transmission when the electric field is reduced by a factor of 1.5 (33%). In some cases the loss is smaller (less than 10%), but it can be much larger. Due to this inherent limitation, the mass range which can be monitored is restricted. This is illustrated in the following figure. For the analysis of steroids, for example, ions in the mass range of 300 amu and higher are of interest and this is easily covered via electric field scanning. The figure shows that the range *m/z* 300 - 500 can be scanned by reducing the electric field by a factor of 1.67. Electric field scanning, however, is not suitable for monitoring ions over a wide mass range, e.g., *m/z* 143 to 560, as the intensity of the higher mass ion will be quite weak. The presence of these two ions must be determined via two separate analyses. Alternatively, one could

perform a magnetic field scan and record all the ions over this mass range. This would, however, result in a loss of sensitivity, and more importantly, a loss of mass specificity.

Multiple ion detection by electric field scanning is a multiple step process, see figure, and care must be taken to assure that the ion source is properly tuned and that the ion intensity varies appropriately with changing electric field strength. Thereafter, the resolution is adjusted and the electric field is calibrated using two masses from the reference compound (lock and calibration masses). When an analysis is started the magnet is first set slightly below the mass of the lock mass and the electric field is swept over a narrow range to find the peak maximum and the peak boundaries. During an analysis the peak maximum and the half-width mass boundaries are continuously sampled and displayed. Inspection of the peak shapes assures that the ion masses are properly monitored at the given resolution. The figure on the following page shows the multiple ion detection (MID) display generated by the mass spectrometer data system. The display shows on the left the lock mass (*m/z* 454.9728) sampled via appropriate setting of the electric field at three points, the mass at the peak maximum (*m/z* 454.9728) and the masses of the two points corresponding to the half-width mass boundaries, $m \pm m/4R$, *m/z* 454.9349 and 455.0107, respectively, at $R = 3{,}000$. The triangular shape of the lock mass (and calibration mass, shown on the right) intensity in the display indicates proper functioning of the mass spectrometer for electric field multiple ion detection. This in turn ensures that the accurate masses are monitored during the analysis.

In the multiple ion detection (MID) analysis, 31 ions can be acquired in a time group and as many as 33 time groups can be included in an analysis. The integration time for each ion is selected individually. Normally for GC analyses, the number of ions and their integration times are adjusted to obtain a scan cycletime of 0.5 s or less to ensure an adequate number of sample points over the GC peak. As an example, GC/HRMS analysis of norandrosterone bis-TMS (M^+ *m/z* 420.2879, M^+-15, 405.2645) extracted from urine (2 mL, 2 ng/mL) is shown. Included in the figure is a fragment ion (*m/z* 422.2308) from a metabolite of vitamin E. At 3,000 resolution there is no contribution from the vitamin E metabolite in the *m/z* 420.2879 and 405.2645 ion traces. This is in contrast to the result obtained using a quadrupole mass spectrometer where there is a 1% contribution of the *m/z* 422 ion intensity in the *m/z* 405 ion trace. The norandrosterone spiked

SELECTED ION MONITORING AND ELECTRIC FIELD SCAN

At acceleration voltage V, an ion of mass m passes through magnet

If acceleration voltage is reduced to V_1
and the magnetic field strength is kept constant
a higher mass ion m_1 will pass through

$$m_1 = m\,(V/V_1)$$

ESCAN ANALYSIS

Acceleration Voltage	ERATIO (V/V_1)	Mass Detected	Mass Detected	Mass Detected
100%	1.00	100	200	300
90%	1.11	111	222	333
80%	1.25	125	250	375
70%	1.43	143	286	428
60%	1.66	167	334	500
50%	2.00	200	400	600

MULTIPLE ION DETECTION

Multiple Steps Process

(1) Source is tuned, resolution adjusted and magnet set to a reference mass (fluorocarbon species)

(2) Electric field is calibrated using two known masses obtaining a slope (EDACG) an offset (EDACZ)

Instrument is now locked and in ESCAN mode
Magnetic (B) kept constant and masses selected by reducing Acceleration (V) and Energy Analyzer (E) voltages

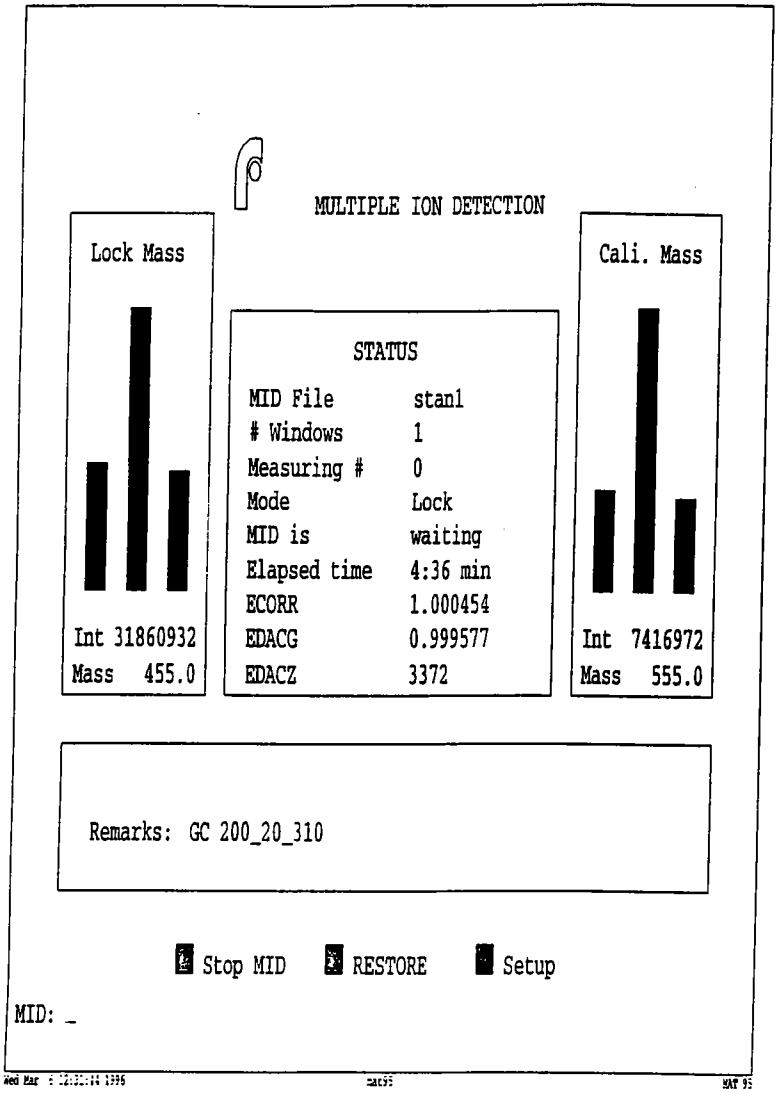
(3) Multiple Ion Detection

As analysis is started a sweep is made to find peak maximum

The resolution and mass are used to determine boundaries

Peak maximum and boundaries are sampled continuously during analysis and displayed

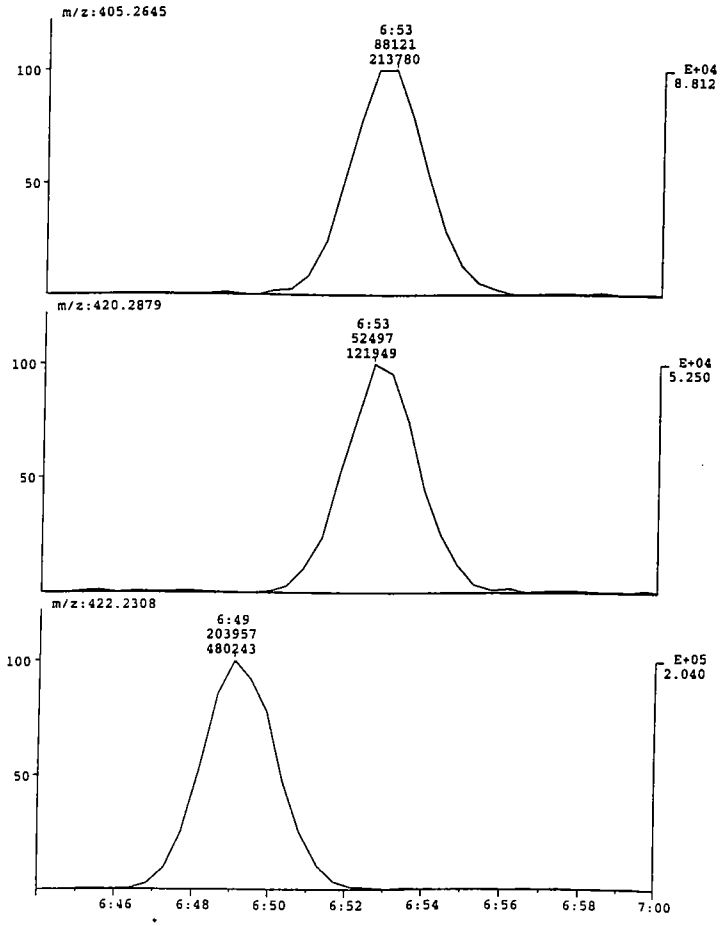
This process is repeated for each ion group, where in each group a new lock mass (magnet mass) can be selected



NORANDROSTERONE SPIKED URINE

Spiked at 2 ng/ml, 2 ml extracted, derivatized with 100 µl MSTFA/TMIS
2 µl injected with 1:20 split

4 pg on column
3000 resolution
10^6 gain



urine sample is used to monitor the instrument performance. The table on the following page shows that the MAT 95 instrument is very stable. In fact, the performance (based on the signal-to-noise ratio of *m/z* 405.2645 for 4 pg on-column of norandrosterone bis-TMS) has improved from the time of installation in 1993 to present.

To assess the limit of detection in the electric field scan mode at 3,000 resolution D_3-testosterone bis-TMS (M^+ *m/z* 435.3068) was measured at varying concentrations. The sample contains approximately 3% testosterone (M^+ *m/z* 432.2879). The lowest concentration standard, 5 pg/μL, was measured in split mode (0.5 pg on-column) and shows a well-defined signal for the deuterated component and no signal for testosterone. At the next higher concentration (1.5 pg on-column), there is a slight signal for testosterone (ca. 0.05 pg) which illustrates the detection limit. In the 5 pg on-column standard the signal for testosterone is clearly present and establishes the limit of detection at 0.15 pg on-column (or 1.5 pg/μL in solution). Another interesting feature of these data is the linearity of response of both deuterated testosterone and testosterone over the 30 fold (100 fold for testosterone) range of concentration.

The MAT 95 double focusing mass spectrometer is often termed as a high resolution mass spectrometer (HRMS). In this presentation we have shown that it is also an instrument with high sensitivity, high precision and high stability. With some practice it is possible to employ the instrument in routine steroid screening analysis in the same way as a conventional quadrupole mass spectrometer.

References

1. S. Horning and M. Donike, **High-Resolution GC/MS** in M. Donike (Editor), *Recent Advances In Doping Analysis - Proceedings of the 11th Cologne Workshop on Dope Analysis 1993,* Sport und Buch Strauß, Köln 1994, p. 155.

LONG TERM STABILITY

Control urine spiked with 2 ng/ml norandrosterone used to assess instrument sensitivity

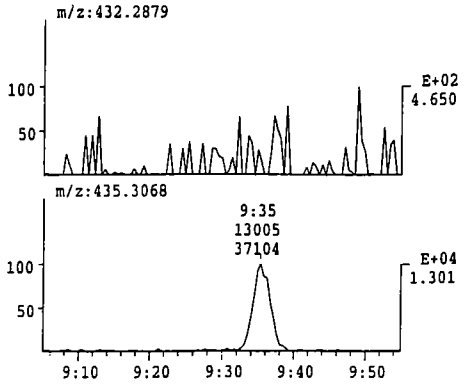
2 ml urine extracted, derivatized with 100 µl MSTFA/TMIS
2 µl injected with 1:20 split

Stability Measurements Performed at 3000 resolution and 10^6 gain			
m/z 405	Feb 1993	March 1994	March 1996
Signal	28506	45486	87282
Noise	761	942	2359
S/N (avg noise)	182	236	359

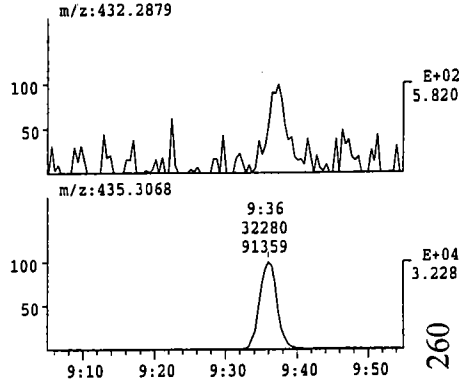
LIMIT OF DETECTION

0.5 ng/100 µl
2µl injected 1:20 split

0.5 pg on column

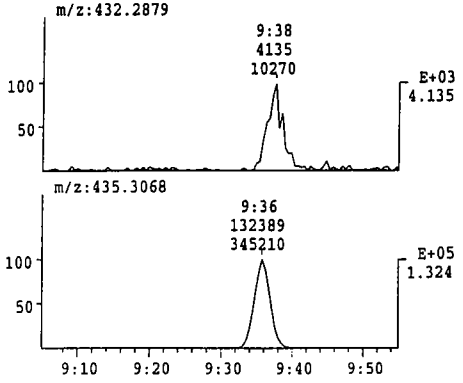


1.5 pg on column



260

5 pg on column
(432 ion 3% abundance
0.15 pg on column)



15 pg on column