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RECENT ADVANCES IN DOPING ANALYSIS (6)

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High Resolution Mass Spectrometry in the Antipodes
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High Resolution Mass Spectrometry in the Antipodes

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INTRODUCTION

We like other IOC laboratories were faced with the need to achieve lower detection levels for anabolic agents. There were two instrumental options available MS/MS and HRMS. In our hands bench-top MS/MS, whilst initially very promising, was not a useful routine procedure for large numbers of samples. However HRMS had demonstrated its reliability at the Atlanta games. It was decided to proceed with the purchase of a high resolution mass spectrometer in September 1996. This paper describes our experiences with the Finnigan MAT95S since its purchase and installation in December 1996.

DISCUSSION

Our first impression of the HRMS was its large size and weight. It exceeded the capacity of the existing laboratory air conditioning and meant that an additional air conditioner had to be installed. During its installation it became apparent that it was a much more complicated instrument than our bench-top MSDs as well as being very much more sensitive. The complexity was and is an ongoing concern due to the fact that the factory is in Germany which is a very long way from Australia. There are only two MAT95s in Australia and thus the local engineer whilst very competent can not be considered an expert in troubleshooting the MAT95. As the instrument was to become an integral part of our routine screening procedure it was vital that it be highly reliable and able to be easily maintained. Our laboratory can not afford the luxury of having a second instrument as a backup.

RESULTS AND DISCUSSION

The installation went smoothly in December 1996 and by the end of the month routine samples were being run successfully. Two factors assisted consisted considerably in this. The first was the Cologne software and the second was the laboratories prior experience in using both the Finnigan Incos software on a 4500 and the ICIS software on a TSQ700. Without these it would have taken considerably more time for the routine running of samples to be accomplished. Shortly after routine screening of conjugated steroids was begun a sample which was suspicious for stanozolol was detected in the MSD screen. The results obtained for this sample on LRMS (HP 5970), MS/MS (Finnigan GCQ), and HRMS (Finnigan MAT95S) are shown in Figure 1 below. It can be seen that whilst the detection of stanozolol requires the eye of faith in the LRMS screen it is clearly visible in the HRMS screen. The MS/MS data is better than the LRMS but is much inferior to the HRMS data.

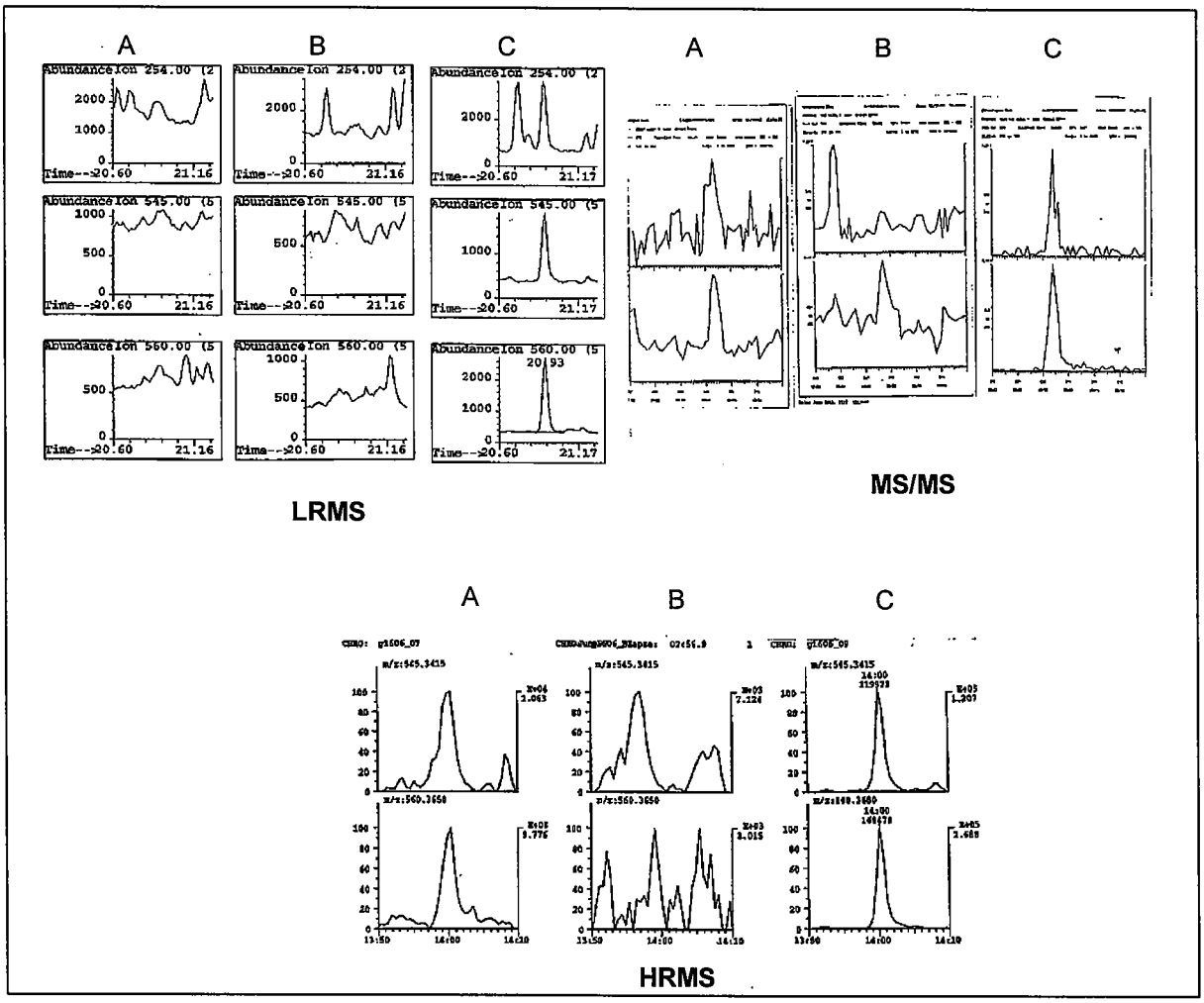


Figure 1. Ion traces for sample (A), blank (B), and standard (C) respectively for each of three MS techniques.

In order to evaluate the reproducibility and sensitivity of the MAT95S a series of standards at 1ng/mL, spikes at 0.25 ng/mL and blanks were injected 7 times. Samples were analysed under our standard conditions of 2uL injection at a 20 to 1 split, GC run of 18 minutes, MS resolution of 3500 with a gain of approximately 10^6. The compounds present were Nandrolone M1 and M2, 3'-Hydroxystanozolol, Epimetendiol (EMD, 17β-methyl-5β-androst-1-ene-3α-17α-diol), and Methyltestosterone M1 (5α) and M2 (5β). The samples were injected in the order standard, spike, blank so that there

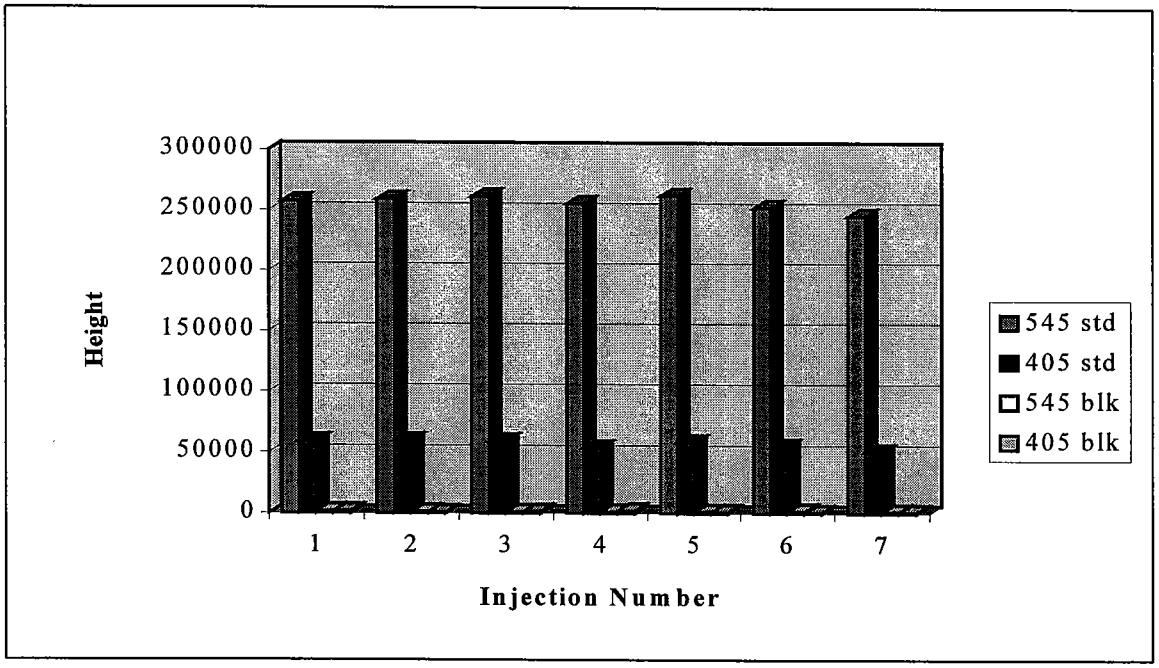


Figure 2. Repeatability for 1ng/mL Std of 3'OH-Stanozolol and Nandrolone M1

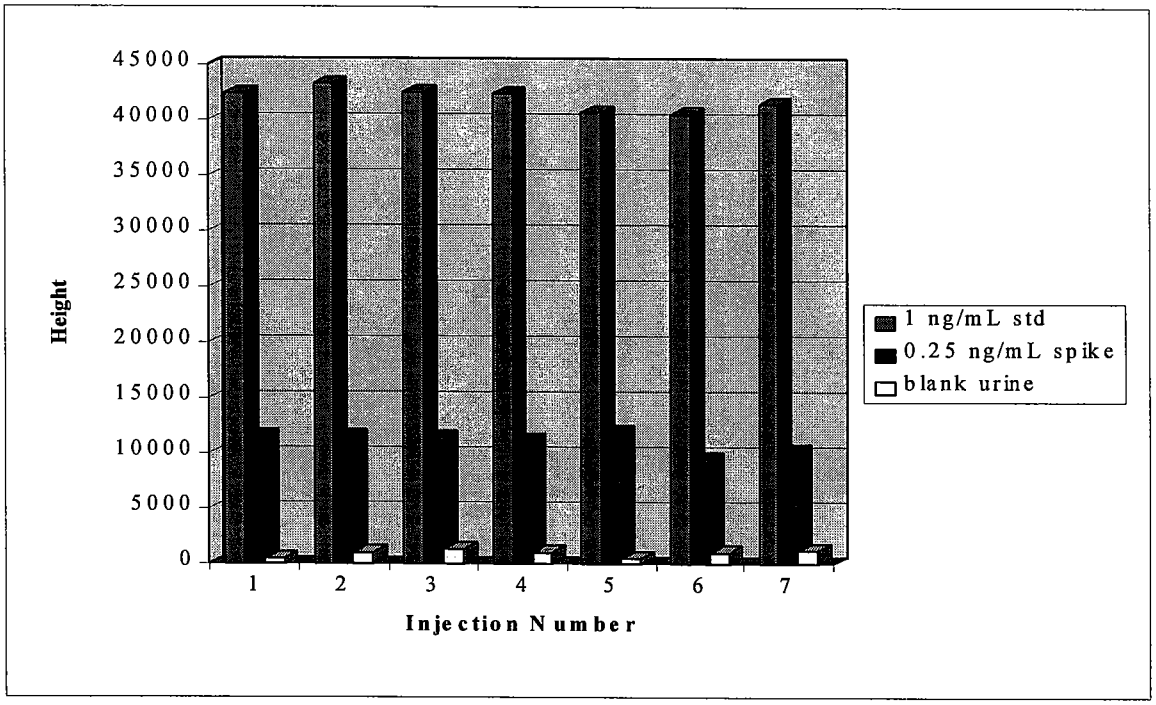


Figure 3. Variation in the 358 ion intensity of Epimetendiol

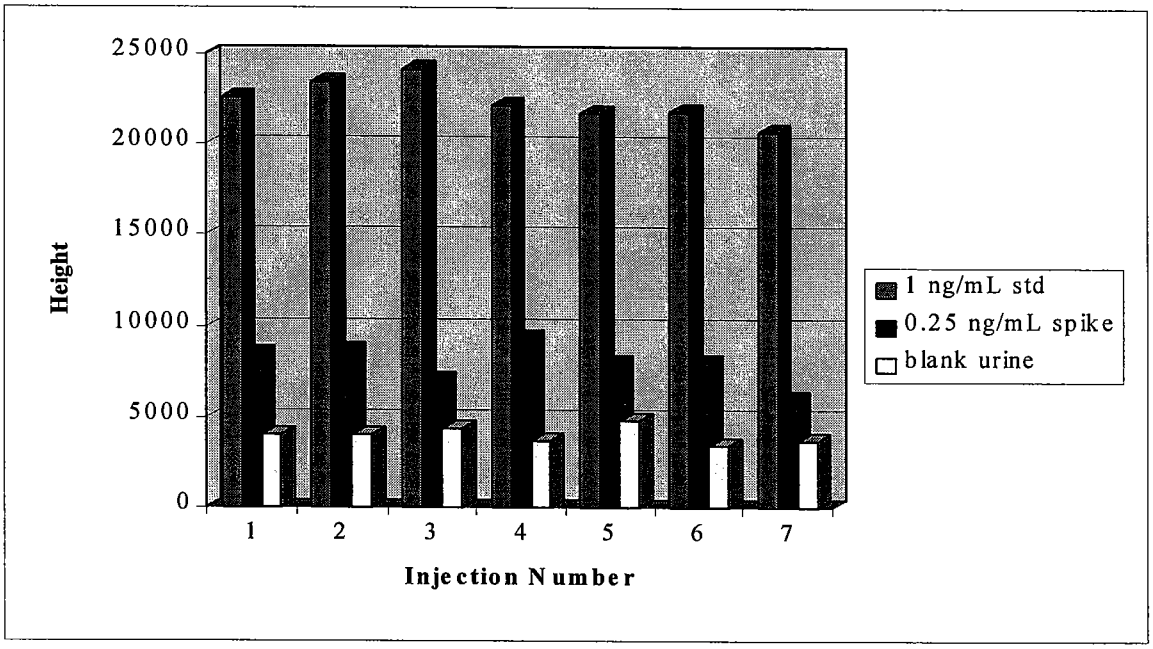
were actually 20 injections between the first and last injections of each sample type. Figures 2 and 3 show some of the results obtained for Stanozolol, Nandrolone and Epimetendiol. It can be seen that the reproducibility is good and the limit of detection will be significantly below 1 ng/mL for these compounds as the levels of the respective characteristic ions are low in the blank. The calculated LODs for Stanozolol, Nandrolone and Epimetendiol are shown in Table 1. The values have been calculated using a statistical method that is based on measuring the reproducibility of several spikes made at 3 to 5 times the estimated limit of detection.

Table 1. Results for 0.25 ng/mL spikes.

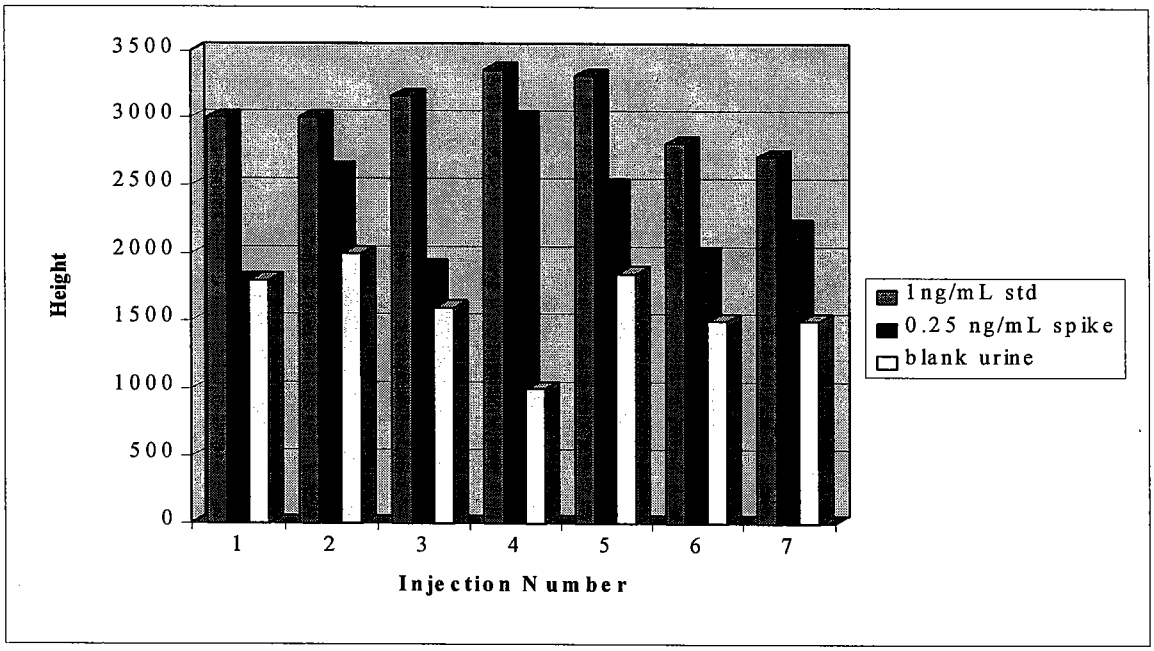
	Average Ht	% CV	Calc Concn	Calc LOD
Nandrolone M1				
Ion 405.2645	81700	6.35	0.32	0.05
Ion 420.2979	40854	4.69	0.35	0.04
3'OH Stanozolol				
Ion 545.3415	20035	11.07	0.35	0.09
Ion 560.365	14615	14.42	0.34	0.11
Epimetendiol				
Ion 358.2692	11079	7.95	0.26	0.06
Ion 448.3192	3047	6.67	0.36	0.05

For these three analytes spiking at 0.25 ng/mL demonstrates that the LOD is at or below 0.1 ng/mL. This is shown not only by the reproducibility of the measurements but also by the fact that the calculated values are close to the actual spiking levels. However for the Methyltestosterone metabolites the LOD is much higher due both to the lower ion abundances and to the higher background levels in the blanks. Because of the low ion abundances, the

limit of detection for the Methyltestosterone metabolites was found to be between 0.2 and 0.5 ng/mL



345 ion of Methyltestosterone M2



360 ion of Methyltestosterone M1

Figure 4. Ion abundances for Methyltestosterone metabolites M2 and M1

using repeat injections of the 1 ng/mL standard. Figure 4 shows the ion abundances of the 345 ion for M2 and for the 360 ion for M1 for the standard, spike and blank. In the case of the 360 ion it is apparent that the 1ng/mL standard response is only slightly above the blank and hence the 0.25 ng/mL spike is undetectable. The limit of detection of Methyltestosterone metabolites in a urine sample is likely to be above 2 ng/mL and will be very dependent on the urine background.

The Finnigan MAT 95S has shown itself to be more than capable of meeting the detection limits that we require. One effect of the increased sensitivity has been the routine detection of two coincident peaks of mass 405 and 420 in the Nandrolone M1 window. Our initial postulate was that this indicated the presence of low levels (<0.5 ng/mL) of Nandrolone in many samples. However further examination of these samples has shown that the ratio of the intensities of the 405 and 420 ions is incorrect (the 405 should be greater than the 420) and they lack the 315 ion which is also characteristic of Nandrolone M1. A typical ion trace and that of a Nandrolone standard is shown in Figure 5.

Since the initial observation there have been many samples with small levels of these coincident 405 and 420 peaks. Recently a cluster of samples was found with relatively high levels, which enabled a full scan spectrum to be run. The resulting spectrum is shown in Figure 6 and is clearly different from that of Nandrolone M1. The compound is still unidentified.

The reliability of the MAT 95S has been generally good over the last fifteen months with some notable exceptions. The water chiller that was supplied with the instrument developed an intermittent fault, which caused it to temporarily stop cooling in the middle of the night. Thus next morning the instrument would be shut down for no apparent reason as the cooler was functioning again. Eventually the failure was detected and the refrigeration unit was replaced. Later the cooling fan in the chiller failed. The other major problem resulted in a period of three weeks during which the instrument was out of operation. The instrument would not lock and hence would not run in monitored ion detection mode. The problem was eventually traced to a software bug that was fixed by a new software version and a patch.

There are two ongoing problems, which do not prevent the instrument operating, but which slow up routine operations. The first is the need to frequently reboot the instrument and the computer, either of which may lock up. The second is the short life of the electron multiplier.

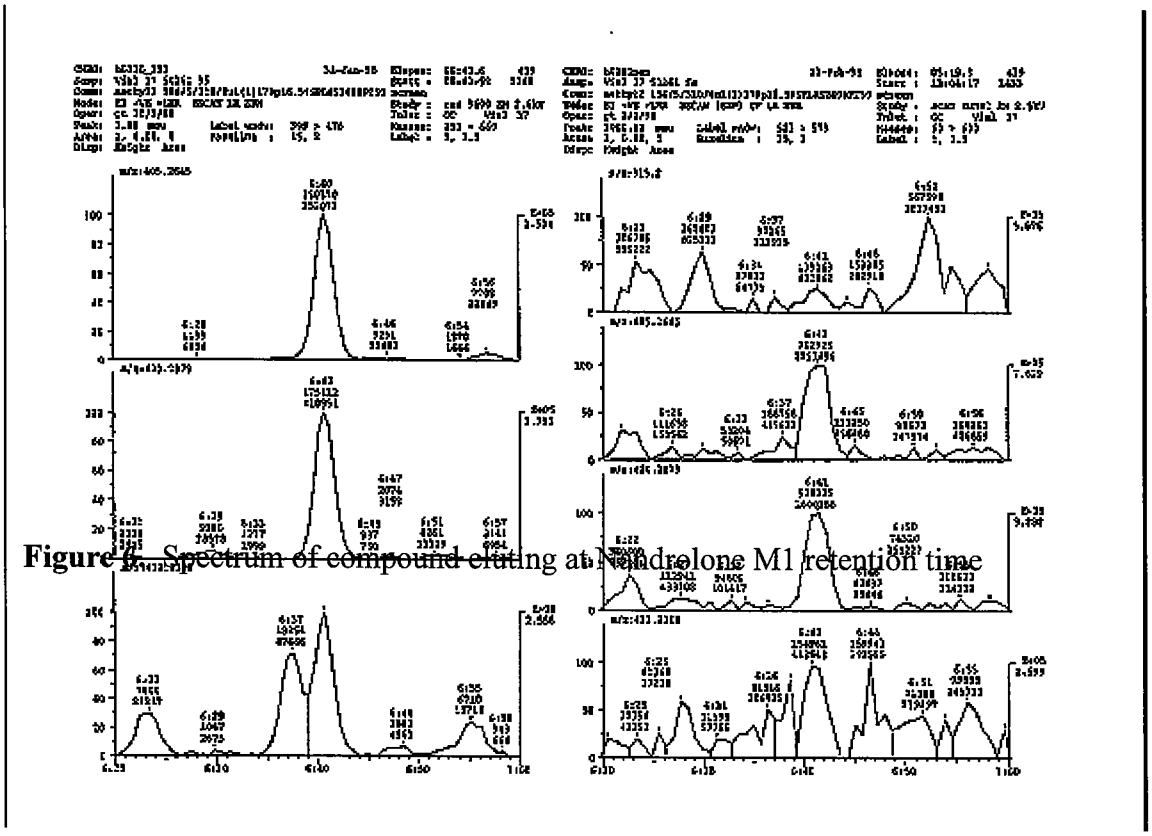


Figure 5. Ion chromatograms for Nandrolone M1 and suspect sample.

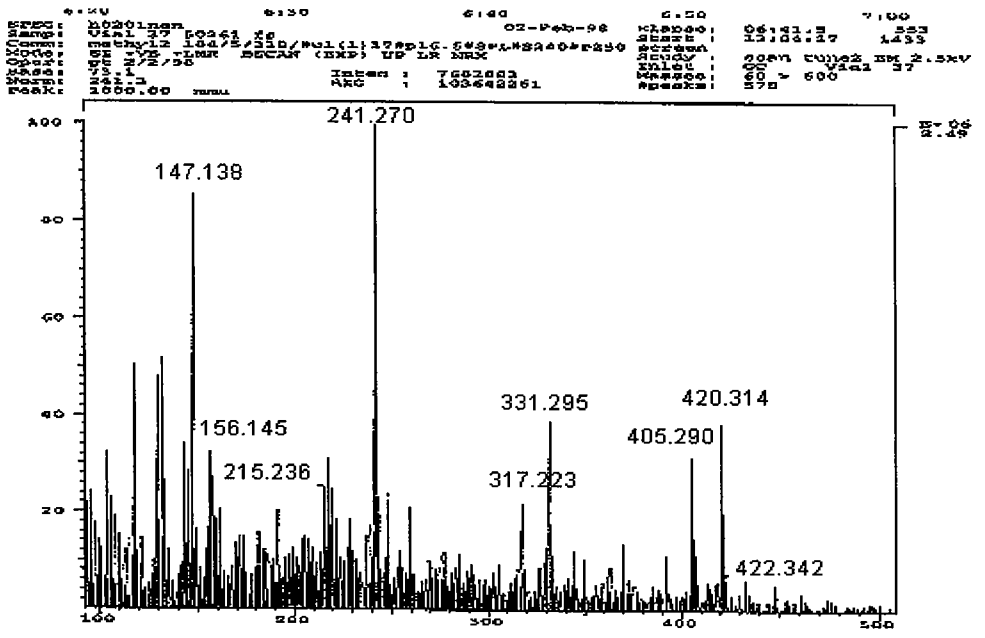


Figure 6. Spectrum of compound eluting at retention time of Nandrolone M1.

Figure 7 illustrates the increase in multiplier voltage with time needed to maintain a gain of 10^6. This short life is in complete contrast to that experienced by the Cologne laboratory.

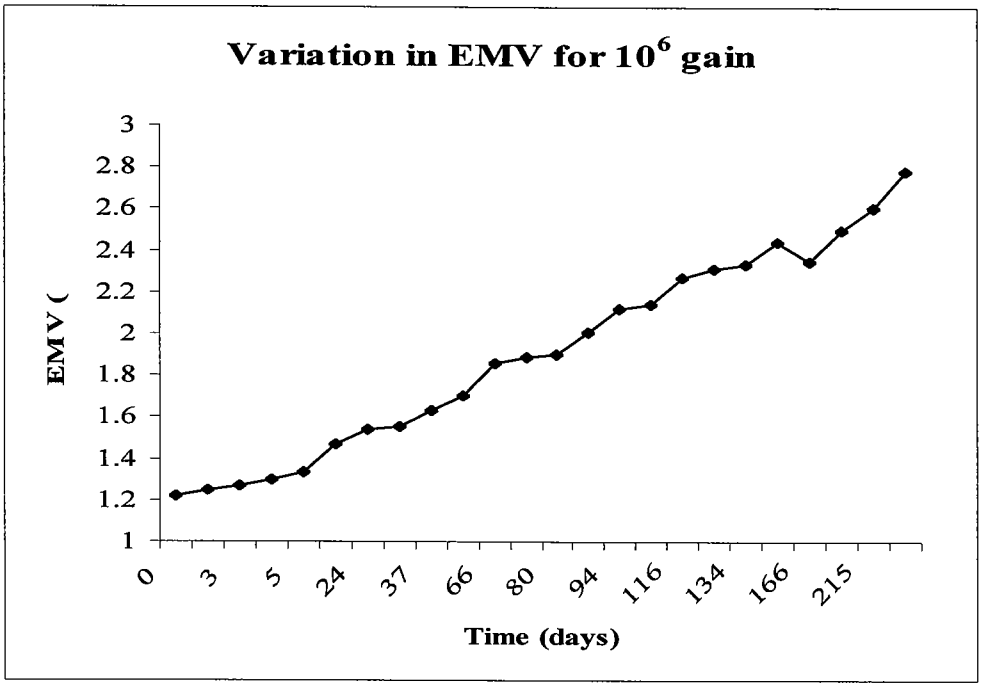


Figure 7. Rapid deterioration of electron multiplier.

CONCLUSIONS

The Finnigan MAT 95S has shown itself to be a reliable addition to our laboratory despite its ongoing quirks. The routine maintenance is somewhat difficult but needed infrequently. We change columns more often than we need to clean the ion volume. In summary much less routine maintenance is needed than with a typical bench-top MSD running steroids. Service for non-routine problems remains a problem.

The HRMS is used on every sample as part of our normal steroid screen. This results in some 8,000 to 9,000 injections per year. Both screening and confirmation methods have been validated and now form part of our ISO25 accreditation. More staff are being trained in the routine use of the MAT95S.