Reprint from

# RECENT ADVANCES IN DOPING ANALYSIS (7)

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Sport und Buch Strauß, Köln, 1999

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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (7). Sport und Buch Strauß, Köln, (1999) 249-254

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# A Single Screen for Steroids using HRMS

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## INTRODUCTION

In our laboratory all samples are analysed for steroids using both low resolution bench-top quadrupole gas chromatography mass spectrometry (LRMS) for all steroids of interest and then again by high resolution mass spectrometry (HRMS) for a selected range of anabolic agents at lower detection levels. The samples are first analysed by LRMS and then the vials are transferred to the HRMS for a second injection. This double handling causes time delays and so the possibility of developing a single HRMS run which would include all the compounds of interest in a single injection was investigated. A single analysis run for steroids would be particularly advantageous when short turn around times were required

## MATERIALS AND METHODS

The current steroid method has the prepared samples analysed on one of two HP 6890/5973 MSDs. The method used is similar to that adopted by many IOC laboratories using a 16.7m HP Ultra 1 column programmed at 3C/min from 183C to 233C and then at 20C/min to 280C and held for 5 minutes. The sample (2uL) was injected at a split ratio of 8 to 1. The MSD was operated in selected ion monitoring (SIM) mode using eleven ion groups containing from 5 to 27 ions. There are some 44 analytes in the screen which are predominantly steroids both natural and synthetic, but also includes other compounds such as salbutamol, morphine, and triamterene. The printed output for each sample covers ten pages. The original HRMS method is a variation of that developed by Horning and Schanzer [1]using a HP 5890(II) GC coupled to a Finnigan MAT95S HRMS. The column used is the same as in the MSD method

and the GC conditions are similar except that the initial ramp rate is at 5C/min. The sample (2uL) is injected at a split ratio of 20 to 1. The HRMS is operated in locked monitored ion detection (MID) mode and has five ion groups containing up to 20 ions. The printed output for each sample covers three pages.

## RESULTS AND DISCUSSION

There are a number of advantages that would flow from having a single GC/MS run that would detect all the analytes required. These include:

1. The considerable time saving both in analysing data and in MS run time. This would be especially valuable for high volume fast turn around samples.
2. The removal of the need to inject each vial twice. With two injections there is always the potential for the sample to dry out after the first injection.
3. Fewer MSDs would be required.

There are however a number potential problems that must be overcome for a single HRMS screen to be a feasible option. These include:

1. All historical data on steroid profiles has been done using MSDs and so there is the possibility that the new HRMS data may not be directly comparable.
2. The laboratory becomes dependent on only one instrument for all its steroid analyses and hence the reliability and service backup becomes critical.

Bearing the above in mind it is possible to set a number of criteria that a single HRMS method should meet for it to be useful.

1. It should reach or surpass the specified IOC detection levels for anabolic agents. The reproducibility should be comparable to that obtained with the existing HRMS method.
2. It should be capable of detecting all the compounds in the LRMS screen with detection levels at least as good and preferably better than those currently achieved.
3. Quantitative and semi-quantitative measurements made for natural occurring steroids and their metabolites should be the same with the new method, because there is

considerable historical data on which decisions can be based. If there are differences these must be consistent such that corrections can be made.

4. The total run time must be less than the existing LRMS screen as only one HRMS instrument is available.

With these objectives in mind an extended HRMS method was developed so that it could be evaluated. The GC run used was based around that used for the original HRMS method (subsequently referred to as methyl3) with a slightly lower starting temperature and hence slightly longer run time. The modifications to the MS conditions were restricted by the need to avoid having compounds eluting at or near the time of ion group switches and hence it was not possible to merely translate the MSD ion groups to the HRMS. With an MSD the switching time from one ion group to the next is only a few milliseconds, and whilst it is not desirable to have compounds eluting at the time of group switching they will still be detected. However with the HRMS a group switch requires a magnetic field jump and the settling time is a few hundred milliseconds. A compound eluting at this time would not be detected. A problem also arises regarding which ions are used to detect the analytes of interest. With an MSD any combination of ions can be used in an ion group. However with an HRMS electrostatic switching is used within the ion group and with the MAT95 it is not desirable to have the masses differ by more than a factor of 1.5. Thus it is not possible for a compound such as clenbuterol to scan for the ions 86 and 335 in the one run.

The extended HRMS method (subsequently referred to as conj) had a GC run starting at 178C, ramping at 5C/min to 238C, then at 20C/min to 310C, and holding 3 minutes. The total run time was 19.1 minutes. The HRMS was operated in locked MID mode with six groups of ions having from 15 to 27 ions in each group. It should be noted that some analytes are very close to group switches and correct retention times for internal standards are critical.

The new method "conj" was compared to the original HRMS method "methyl3" by injecting a multi-spiked urine extract using both methods several times with the aim of determining whether the new method detected all the analytes in the original method with comparable sensitivity and reproducibility. The relative sensitivities for nine analytes are shown in Figure 1. It can be seen that the two methods are comparable with no analyte having a sensitivity difference of more than 20 percent. To compare the reproducibility of the two methods the same multi-spiked urine was extracted ten times and analysed over a period of seven weeks. The results are shown in Figure 2 where it can be seen that the CVs for the nine analytes show

no particular trend and it can be concluded that the new HRMS method "conj" gives similar results to those obtained from the earlier HRMS method "methyl3". As expected all the new analytes, which were added to the new HRMS method from the MSD steroid screen were detected with at least comparable and generally superior sensitivity.

Having established that exogenous anabolic steroids could be adequately detected using HRMS alone it was necessary to determine whether the HRMS method was capable of giving comparable results for steroid profiling. To investigate this the T/E ratios for some 400 samples were measured over a period of several weeks using both MSD and HRMS methods. The results are shown in Figure 3 where it can be seen that the results by HRMS are some 7% higher than those measured by MSD. During this work it was observed that T/E standard solutions gave the same results by MSD and HRMS, but high T/E urines tended to give higher results by HRMS. Experiments have shown that these higher results are not related to the faster temperature ramp of the HRMS method as similar results are obtained when the identical GC run from the MSD method is used with the HRMS. The most likely explanation for the higher T/E values is a better measurement of the epi-testosterone peak with the HRMS. Although the T/E results are not identical for the two methods there were no cases when a positive result by MSD (T/E > 6) was missed by the HRMS method. Given the results obtained it is likely that the MSD method will still be needed to confirm T/E positives and for comparisons with historical data.

## CONCLUSIONS

It is possible to set up a single HRMS method that meets the requirements of our laboratory for steroid screening. The method is capable of detecting all the required analytes with a sensitivity comparable or superior to that currently achieved with the existing dual injection MSD and HRMS methods. The quantitation of endogenous steroids is adequate with the new method although when T/E ratios are to be measured for violations it may be necessary to continue to use an MSD method for direct comparisons with historical data. For our laboratory to rely on this method it will be necessary to maintain our MSDs as a backup in the event of instrument failure or else have access to a second HRMS.

REFERENCES

S. Horning and W. Schanzer, Steroid Screening using GC/HRMS in Recent Advances in Doping Analysis (4) – Proceedings of the 14th Cologne Workshop on Dope Analysis 1996, Sport and Buch Strauss, Koln 1997.

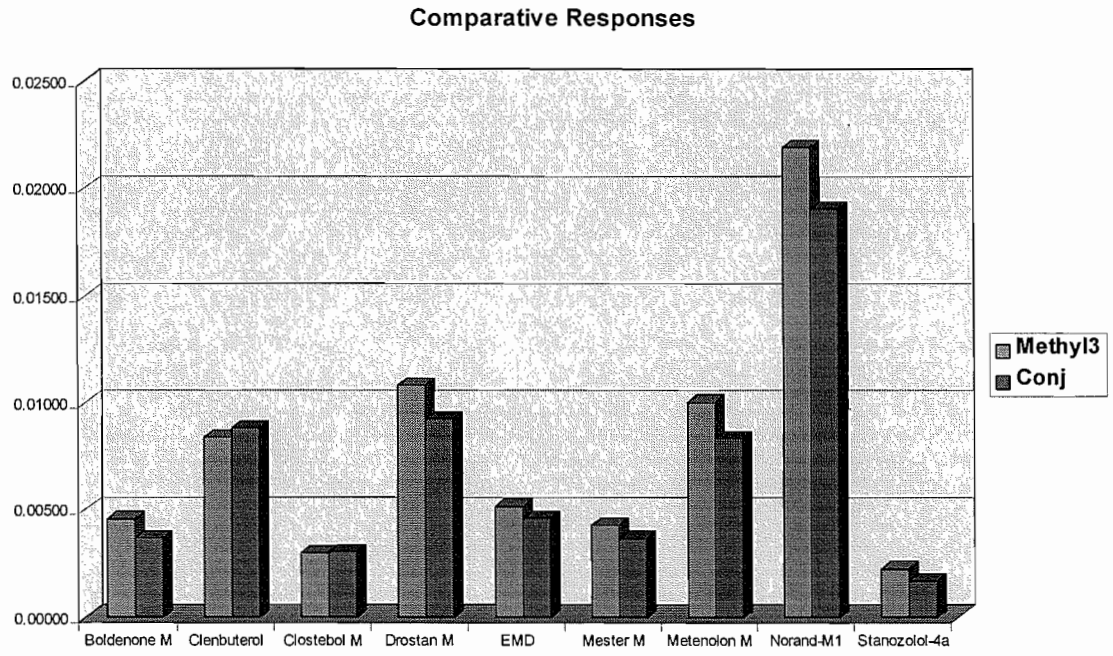


Figure 1. Comparative responses for a number of analytes using the old (methyl3) and new (conj) HRMS methods.

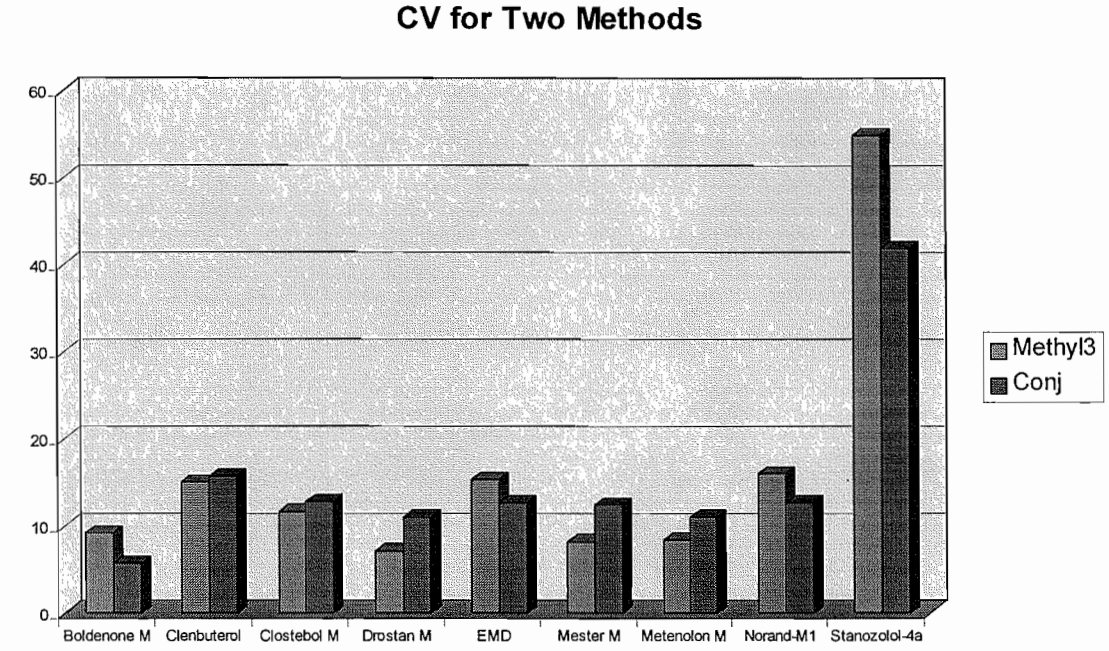


Figure 2. Comparison of the reproducibility of the old and new HRMS methods for a range of analytes.

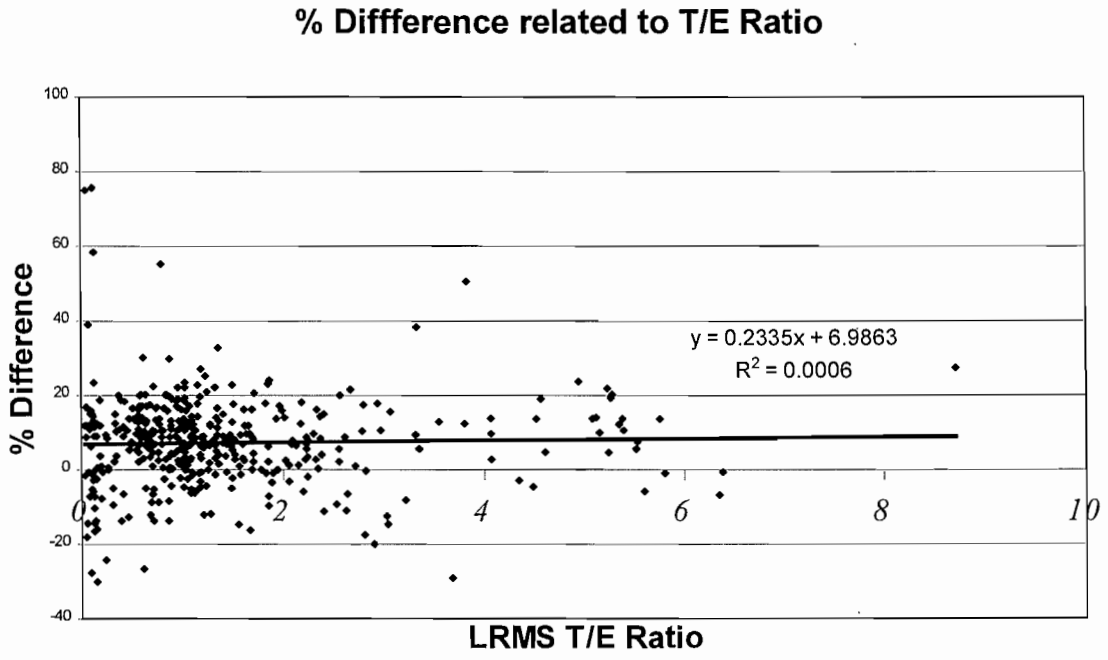


Figure 3. Comparison of T/E ratios as measured by MSD and HRMS.