focus on
Mass Spectrometry & Spectroscopy

The Identification of Illegal Anabolic Steroids in Customs Seizures by GCMS and High Resolution LCMS

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There have been numerous reports in the news over the last few years of new drugs of abuse becoming available on the UK market. These have included mephedrone, benzylpiperazine and the so-called synthetic cannabinoids found in 'Spice' type herbal high products. These have all led to revisions of the misuse of drugs act in an attempt to control their distribution and use.
A slightly older problem is the importation of anabolic steroids. In the year 2008/9 there were 802 seizures of anabolic steroids in the UK [1]. Of these, 259 were by the Borders Agency and the remainder by the Police forces. The misuse of drugs act now includes most known anabolic steroids and a generic wording to include most 'designer' anabolic steroids.

In contrast to previous decades where drugs of abuse were produced domestically or smuggled in to the country, many now arrive in bogusly labeled consignments destined for private addresses in the UK. The UK borders agency frequently intercepts shipments of significant size containing unknown white powders. These often do not contain the 'traditional' drugs of abuse that are readily identified by technology available at ports of entry to the UK. In such situations, seizures are sent for further analysis at laboratories, typically utilising GCMS, in an attempt to make an identification. This approach is often successful where the drug is amenable to GCMS analysis and a reference mass spectrum exists in a library or publication. There are occasions however where the drug is not suitable for analysis by GCMS or no reference spectrum is available. In such circumstances the use of high resolution LCMS can aid in the identification process.

In recent months, we have been forwarded samples of powders and oils seized by the UK borders agency that could not be identified by GCMS and mass spectral comparison.

Most have been from shipments of several kilograms of material. We have used LCMS consisting of a ThermoFisher Accela UPLC sytem interfaced to an LTQ Orbitrap to identify several anabolic steroids, all of which are controlled under the misuse of drugs act. We have also identified other steroids using GCMS and in-house databases.

The powders received for analysis were found to be anabolic steroids, all ofwhich are listed as Class C drugs under the Misuse of Drugs Act. The precise origin of these powders was unknown, but it is likely that they came from China. They were all destined for private addresses in the UK.

The use of high resolution LCMS proved to be very useful in the identification of these compounds and was certainly complementary to GCMS in that some compounds, for example stanozolol and trenbolone were best analysed by LCMS whilst others were detected only by GCMS. Where reference spectra did not exist, as with drostanolone propionate, the combination of both techniques led to identification based on the elemental composition derived from accurate mass measurement and the similarity of the GCMS derived EI mass spectrum to a reference spectrum of another compound.

The identification of customs seizures of this type can often be limited by databases and this is where the use of accurate mass can assist in giving additional information. As was shown with this work, accurate mass determination, followed by the assignment of probable elemental composition, often gives a good starting point in the identification process.

Experimental

Sample Preparation

Solutions of the materials were prepared in methanol.

For LCMS analysis, a sub aliquot of the solution was taken and diluted with water to produce a sample for injection with a concentration of 100µg/ml.

For GCMS analysis, 10µl of the initial solution was evaporated to dryness in a GC vial. To the residue, 200µl of derivatising solution (MSTFA:ethanethiol:ammonium iodide 1ml:6µl:3mg) was added. The vial was capped and heated for 2 hours at 80˚C before injecting on a GCMS system.

LCMS

The samples were analysed on a Thermo Fisher Accela UPLC system interfaced to a Thermo Fisher LTQ Orbitrap Discovery.

The chromatographic separation was performed using a Waters Symmetry C8 3.5µm x 2mm x 100mm column linked to a Phenomenex Security Guard C5 (4x2mm) pre-column.

The Orbitrap was calibrated for positive ion analysis using the standard Thermo Fisher Orbitrap calibration solutions and procedures. The instrument was operated in positive ion mode with a resolution setting of 30,000 with an internal lock mass of 113.03455 derived from uracil in the mobile phase. Data was acquired in full scan mode over a mass range of 100-650 Da.

10µl of sample was injected for analysis.

GCMS

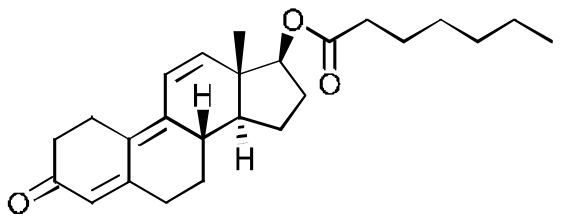
The samples were analysed on an Agilent 6890 GC interfaced to an Agilent 5973 MSD. The chromatographic separation was performed on a 25 meter x 0.25mm i.d. x 0.25µm film BPX5 capillary column from SGE.

The mass spectrometer was tuned and calibrated using perfluorotributylamine and data was acquired in full scan mode over a mass range of 50 to 700Da.

1µl of sample was injected on the system

Results

Trenbolone enanthate



Monoisotopic [M+H]+ = 383.2580

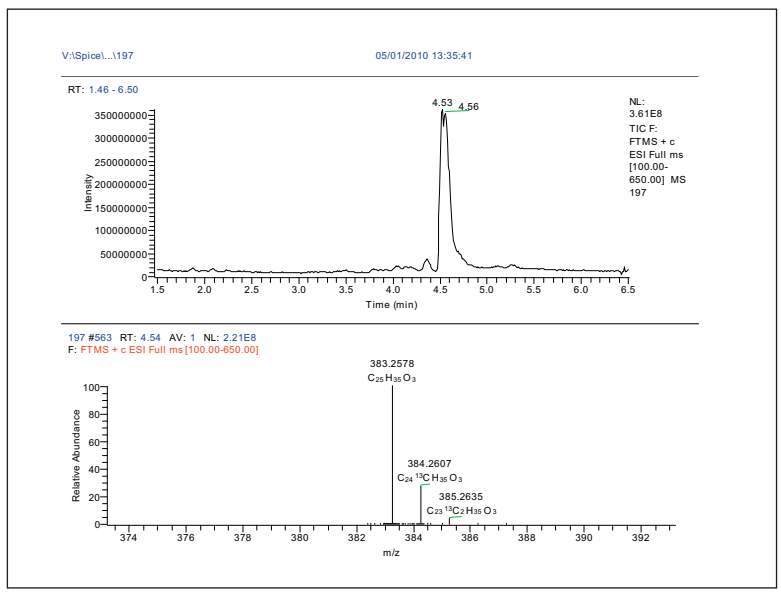


Figure 1. Accurate mass LCMS of trenbolone enanthate showing TIC and mass spectrum.

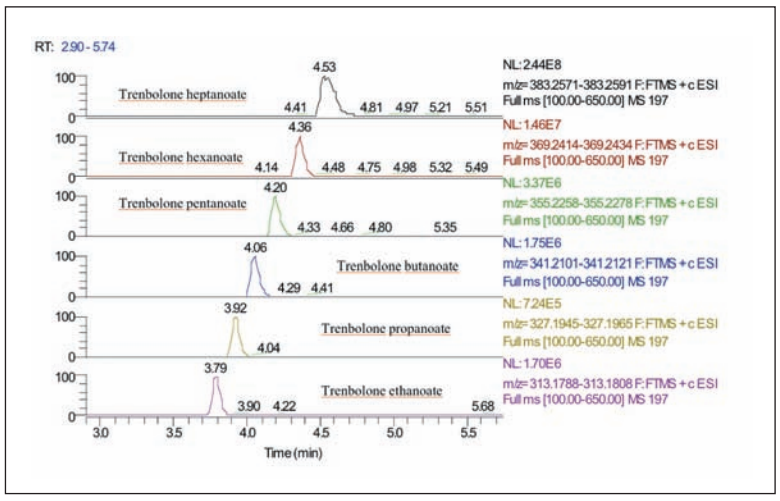


Figure 2. Accurate mass ion chromatograms of the trenbolone enanthate sample showing the range of additional trenbolone esters present as minor components.

LCMS

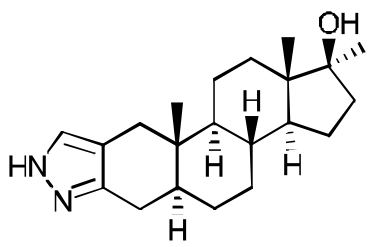
Analysis by accurate mass LCMS showed the presence of a large peak consisting of predominantly m/z 383.2579 (*Figure 1*). This was consistent, after having removed a proton, with an elemental composition of C25H34O3. A Google search of $C_{25}H_{34}O_3$ generated multiple hits for trenbolone enanthate. A repeat analysis acquiring MS^2 product ions of m/z 383 gave a spectrum similar to that of the MS^2 mass spectrum of trenbolone (MW 270). The 271.1689 product ion was the same as protonated trenbolone (271.1692). The m/z 253.1583 was also consistent with the MS^2 spectrum of trenbolone. This fragment is generated through the loss of H_2O from the trenbolone molecule

GCMS

Initial analysis by GCMS of underivatised material had failed to show any significant response although there were several small chromatographic peaks that gave poor matches for trenbolone against proprietary databases. Subsequent analysis as the enol TMS derivative yielded several related peaks. This is consistent with experiences with trenbolone as an enol TMS derivative in the author's laboratory.

Trenbolone enanthate is exclusive to the underground drug market and is not available via legitimate routes. Closer examination of the data revealed that all the esters from the ethanoate to the heptanoate (enanthate) were present in the sample (*Figure 2*). The other esters, even at lower levels as seen in this case, would not be present in a pharmaceutical product.

Stanozolol



Monoisotopic [M+H]+ = 329.2587

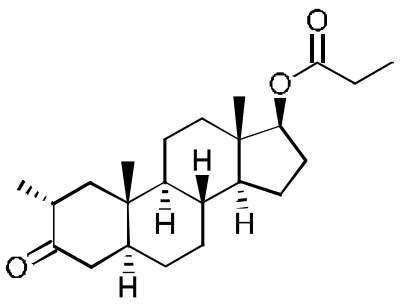
LCMS

Analysis by accurate mass LCMS gave a large chromatographic peak with a mass of m/z 329.2585. This was consistent, after having removed a proton, with an elemental composition of $C_{21}H_{32}N_2O$. A Google search of $C_{21}H_{32}N_2O$ generated multiple hits for stanozolol. The sample was reanalysed to acquire a MS^2 product ion spectrum of m/z 329. The resulting spectrum gave a very good match in our in house MSn library for stanozolol.

GCMS

Stanozolol is not suited to analysis by GCMS and as the identity of this sample had already been confirmed by LCMSMS, no GCMS analysis was performed.

Drostranolone propionate



Monoisotopic [M+H]+ 361.2737

LCMS

Initial analysis by accurate mass LCMS gave a peak predominantly of m/z 361.2736. In addition there were two other ions corresponding to $[M+NH4]^+$ (378.3002) and $[M+Na]^+$ (383.2553). This was consistent, after having removed a proton, with an elemental composition of $C_{23}H_{36}O_3$. Once again a Google search generated a hit for an anabolic steroid. This time it was for drostanolone propionate.

GCMS

Further analysis by GCMS gave a peak with a mass spectrum that was similar but not identical to mesterolone (*Figure 3*). Mesterolone is structurally very similar to drostanolone and the close spectral match to mesterolone would suggest that the

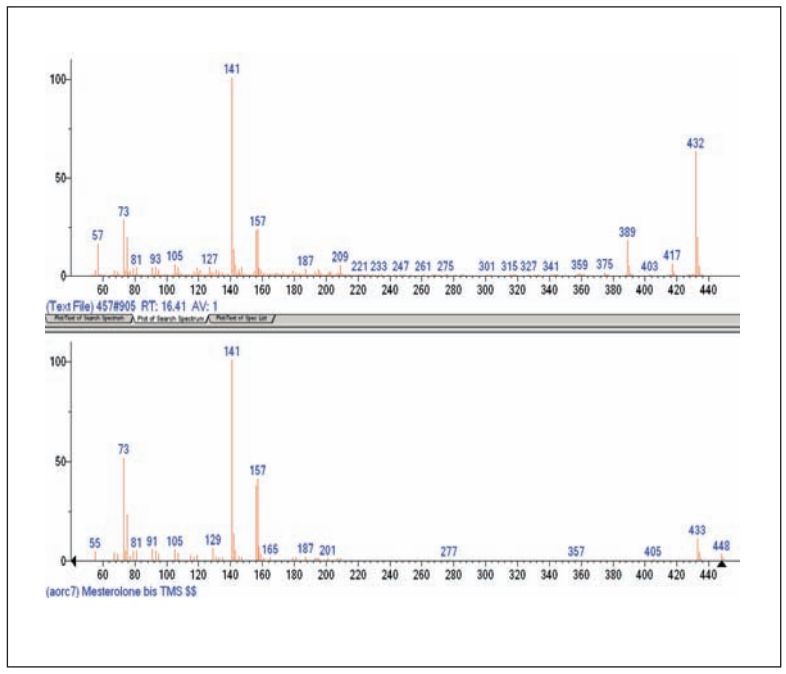
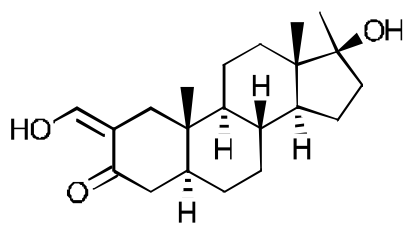


Figure 3. Mass spectrum from the peak at retention time 16.42 minutes giving a best hit for mesterolone bis TMS.

'unknown' steroid is structurally similar to drostanolone. The formation of the enol TMS derivative of drostanolone propionate would result in a compound with a mass spectrum showing a molecular ion of m/z 432. This was seen in the spectrum recorded for the largest peak in the TIC.

With a combination of the LCMS accurate mass data to confirm the elemental composition and the GCMS data to give an indication of structural similarity to mesterolone, we were confident in reporting drostanolone propionate in this sample.

Oxymetholone



LCMS

Not detected by LCMS. This is normal for fully saturated steroids.

GCMS

Analysis by GCMS showed a large peak at a retention time of 16.77 minutes (*Figure 4*). The mass spectrum from this peak was searched against a mass spectral library obtained from the Association of Official Racing Chemists (AORC) that has been compiled from entries received from member laboratories around the world. The spectrum matched with a library entry for oxymetholone.

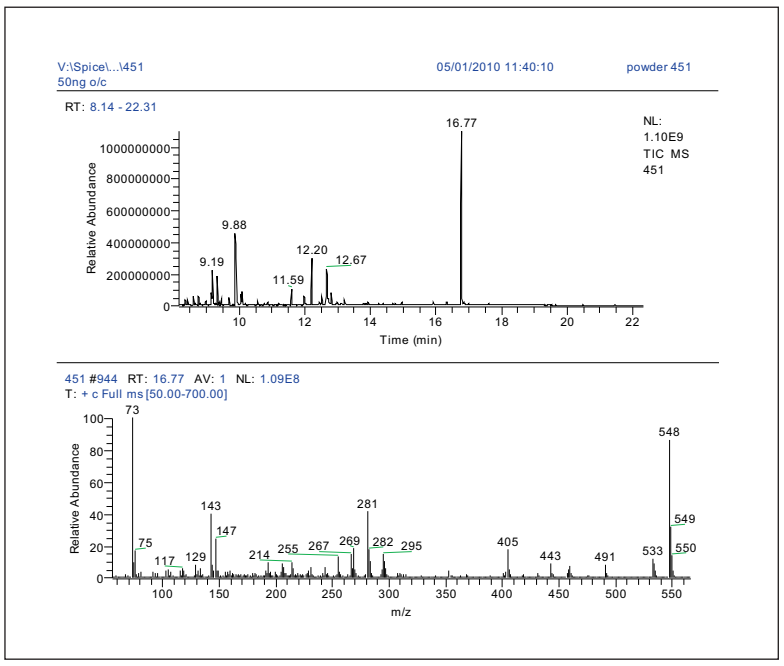


Figure 4. EI-GCMS of oxymetholone showing TIC and mass spectrum of tris TMS derivative

References

1. Seizures of Drugs in England and Wales, 2008/09: Home Office Report rds.homeoffice.gov.uk/rds/pdfs09/hosb1609.pdf

First published in MassMatters, the British Mass Spectrometry Society newsletter www.bmss.org.uk