Studies of Anabolic Steroids By ThermaBeam™ LC/MS

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Introduction

As a specific class of structurally complex molecules, steroid hormones have traditionally posed a special challenge to the analytical chemist. Previously, the mass spectral characterization of free steroids was dominated by gas chromatography/mass spectroscopy (GC/MS) with both electron (EI) and chemical impact (CI) ionization. Less volatile and thermally labile steroids and steroid conjugates required derivatization to more volatile and stable compounds prior to analysis. Successful characterization was then obtained using such techniques as DCI, FAB, FD, SIMS and thermospray (LC/MS)[1,2].

This prompted an investigation into the possibility of using the benchmark™ LC/MS equipped with a ThermaBeam™ particle beam interface in the analysis of anabolic steroids. The benchmark demonstrates the ability to provide data and analysis of each steroid compound. Our objectives for this study were (1) to demonstrate the generation of library searchable EI spectra with minimal pyrolysis fragments, 2) to alleviate the derivatization step necessary for GC/MS, and (3) to demonstrate useful quantitative data.

Experimental

All work described was performed on an EXTREL benchmark™ differentially pumped mass spectrometer. This instrument, equipped with an EI/CI switchable source, was interfaced with the liquid chromatography system by means of the ThermaBeam LC/MS interface. Data was acquired under full scan conditions from 100 to 500 amu at 250 amu/sec.

The chromatographic system consisted of a Waters Model 600 MS solvent delivery system, equipped with a Brownlee Labs SPHERI-5 RP-18 reverse phase column (4.6 mm i.d. x 100 mm). An isocratic elution program, using acetonitrile/water (90/10, v/v) as the solvent system, was run with a flow rate of 0.6 ml/min.

Testosterone (TEST), 17-methyltestosterone (METH-TEST), testosterone propionate (TEST-PRO) and testosterone cypionate (TEST-CYP) were chosen as the androgen compounds for analysis. A stock solution (1mg/1 ml = 1ug/1ul) containing all of these compounds was prepared by dissolving in a 50% acetonitrile/water solution. This stock solution was then further diluted to an optimal working solution of 100 ng/ul, which was utilized throughout the analysis. Appropriate sample injection volumes were made from this mixture.

Results and Discussion

A 250 ng sample of the working solution containing the four steroids resulted in the EI total ion current chromatogram (TIC), displayed in Figure 1A. This chromatogram shows four peaks representing each of the four steroids. Their elution order is as follows: 1.) testosterone (TEST), 2.) 17-methyltestosterone (METH-TEST), 3.) testosterone propionate (TEST-PRO) and 4.) testosterone cypionate (TEST-CYP). The extracted ion chromatogram at mass 124 for the same sample is shown in Figure 1B. This particular mass was chosen because it is common to all four compounds.

Using the TIC from the 250 ng sample, a mass spectrum was generated from the testosterone propionate (TEST-PRO) peak. Figure 2A shows the EI spectrum of TEST-PRO. The molecular ion is observed at mass 344. Other constituent fragments and isotope peaks are also shown. Figure 2B is a representative spectrum obtained from the positive ammonia CI ionization of TEST-PRO. The strong signal at 345 is M + H. The signal at 271 indicates the molecular ion without the propionic group.

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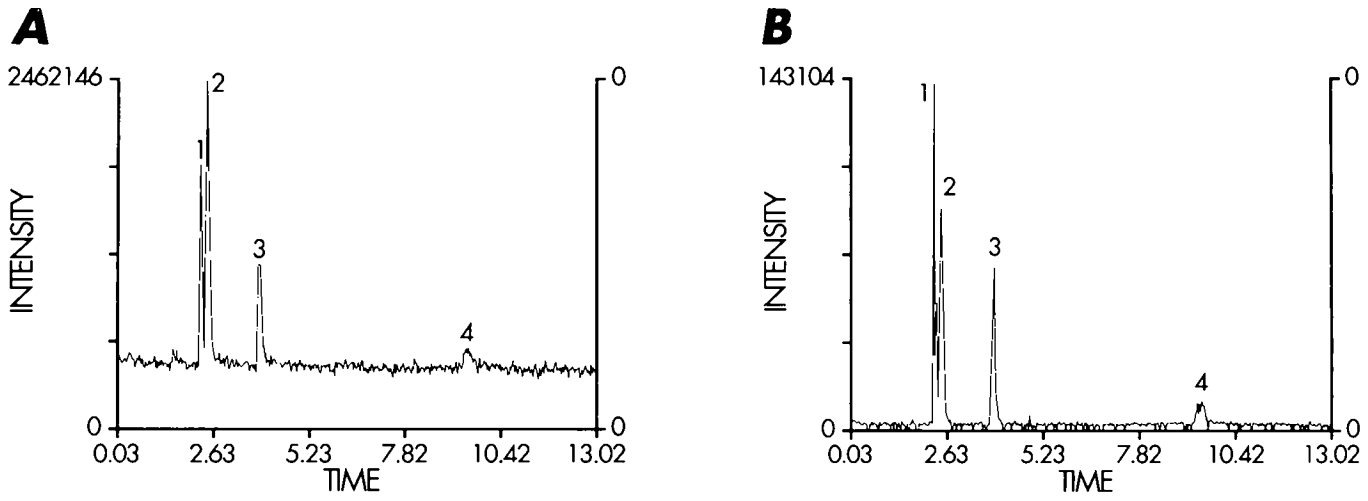

Figure 1 A) The EI total ion current chromatogram (TIC) for the four androgen compounds at 250 ng. The compounds eluted in the following order:

1.) testosterone (TEST),
2.) 17-methyltestosterone (METH-TEST)
3.) testosterone propionate (TEST-PRO) and
4.) testosterone cypionate (TEST-CYP), as indicated on the chromatogram.

B) The extracted EI ion chromatogram at mass 124.

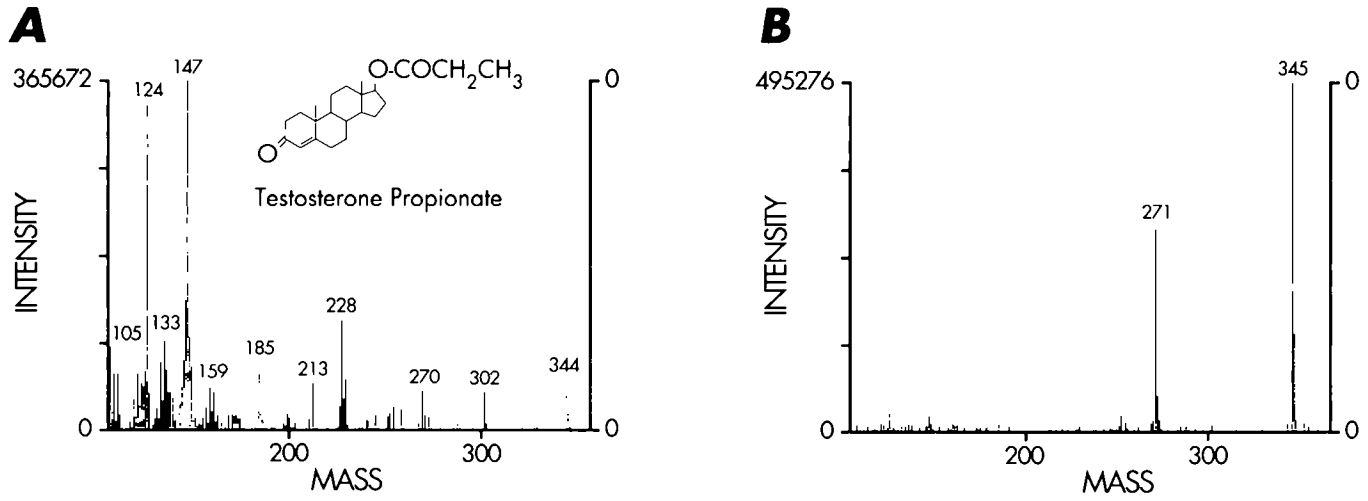

Figure 2 A) The EI mass spectrum of TEST-PRO showing the molecular ion at mass 344.
B) The positive ammonia ionization spectrum for TEST-PRO.

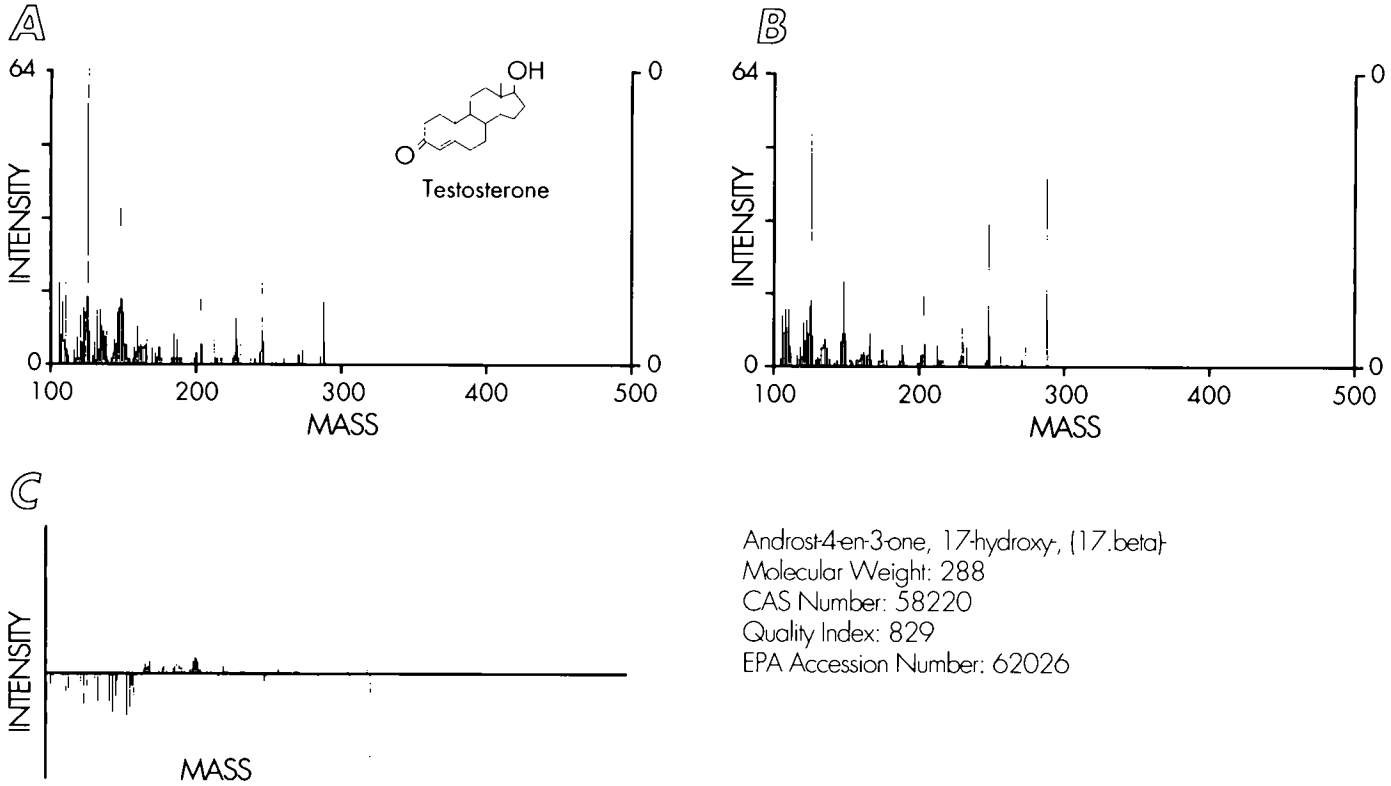

Figure 3 These figures show an example of EI library searchable spectra from the ThermaBeam™ interface:

A) The mass spectrum of TEST generated from data acquisition.

B) The top hit returned from a forward library search of the NIST library.

C) The comparison plot indicating the differences between the two spectra.

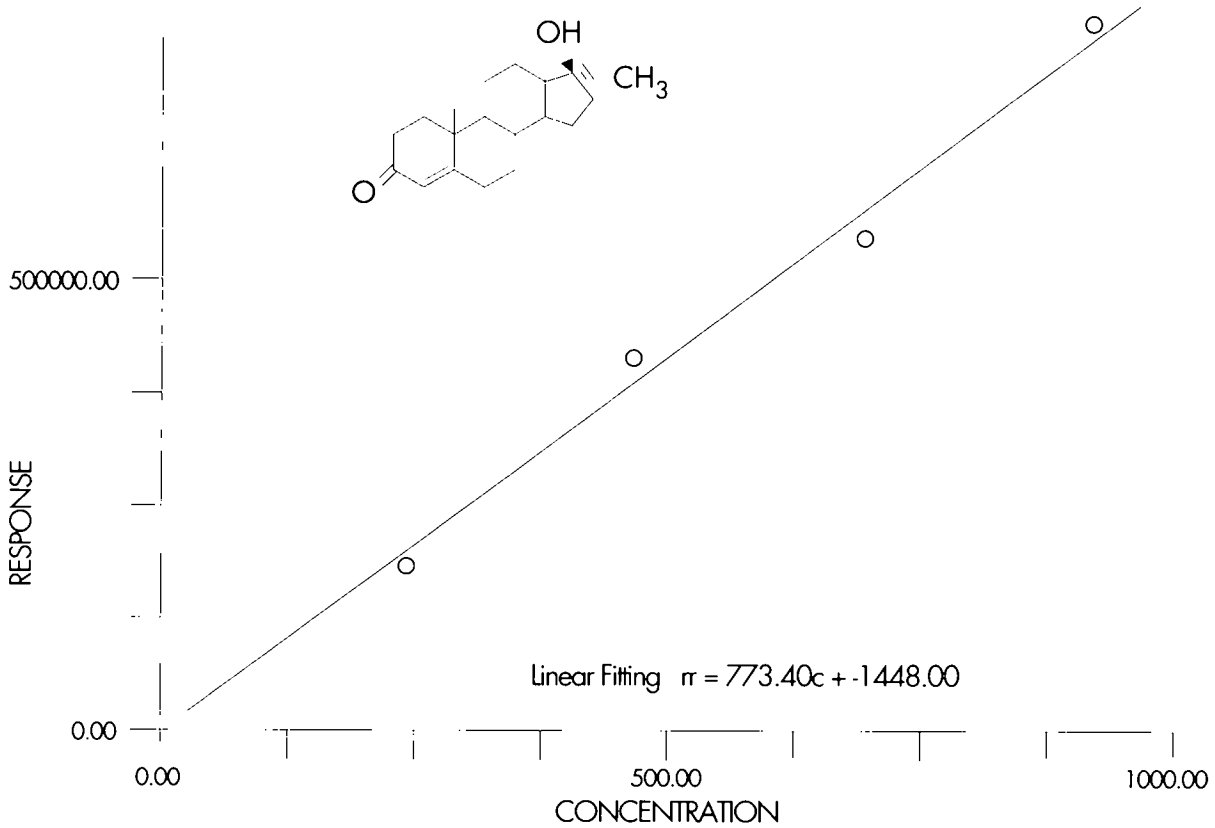

Figure 4 The linear plot calibration curve for METH-TEST.

The ability to generate EI library searchable spectra utilizing the ThermaBeam LC/MS interface is demonstrated in Figure 3. Testosterone (TEST) was chosen as an example to illustrate this capability. Figure 3A shows the mass spectrum of TEST. After the library search, a matching spectrum of TEST was isolated from the NIST library, Figure 3B. A comparison plot, indicating the differences between the two mass spectra, is shown in Figure 3C.

The capability to produce multiple point calibration curves utilizing the ThermaBeam LC/MS is demonstrated in Figure 4. Data generated at 250 ng, 500 ng, 750 ng and 1 ug was utilized for the calibration curves of each of the four steroids. The calibration plot for methyl testosterone (METH-TEST) is illustrated in Figure 4.

Conclusion

When acquired by EI and CI ionization techniques, all four androgen compounds generated interpretable spectra. The resulting EI spectra could then be library searched for further confirmation. All compounds exhibited response factors at the given injection volumes. These were then utilized in constructing linear calibration plots. Using the ThermaBeam LC/MS interface, decomposition during the chromatographic separation of the complicated spectra of fragment ions of less volatile steroids upon vaporization did not occur. As a result, the need to derivatize nonvolatile steroids to enhance the volatility was avoided.

Literature Cited

1. Lin,Y. Y., Smith, L. L. *Mass Spectrom.* 1984, Rev. 3, 319-355.
2. Gaskell, S. J., Rollins, K., Smith, R. W., Parker, C. E. *Biomed. Environ. Mass. Spectrom.* 1987, *14*, 717-722.

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