Determination of Estradiol in Blood by GC/MS/MS

GC/MS

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Introduction

Elimination of matrix interferences from biological fluids is a key step in improvement of analytical sensitivity for trace components which must be monitored in medical and pharmaceutical research. This applications note illustrates the selectivity and sensitivity advantages of an ion trap GC/MS/MS approach to enhance selectivity for the hormone estradiol (estrogen) in blood.

Experimental Design and Results

Sample Preparation. Estradiol standards were derivatized with BSTFA (with 1% TMCS as catalyst). Standards were prepared at levels of 10, 100, and 1000 pg/µL. Three blood samples were extracted and derivatized in a like manner.

MS/MS Method Development. Upon examination of the EI/MS mass spectrum of estradiol-di-TMS, it was determined that optimal sensitivity would be achieved through MS/MS isolation and collision-induced dissociation of the molecular ion at m/z 416. Because of its simplicity, the non-resonant mode of CID was chosen for the analysis. Two runs were performed with the Automated Method Development (AMD) feature of the MS/MS Toolkit for Windows to determine the optimal CID voltage for dissociation of the isolated parent ion at m/z 416 to characteristic product ions at m/z 285 and 326. The Ion Preparation Method (IPM) for the AMD process in Figure 1 shows the CID voltage increasing in 5V increments from 40-85V for Groups 0 through 9 of the method. When an analysis is performed with the AMD IPM file, the CID voltage is adjusted on successsive scans through the chromatogram which are also identified by Group number. Thus, one may quickly identify that 85 V is the optimal CID voltage for creating product ions of estradiol, as shown in Figure 2.

GC/MS Method.
8200 AutoSampler: Inject 1 µL sample @ 1µL/sec with 0.5 mL $MeCl_2$ solvent plug (5 µL sample for large volume injections). GC: 1078 Injector initial temperature 38°C for 0.2 min with split vent open. Close split vent, ramp to 275°C @ 150°C/min, hold. GC column 60°C for 2 min; 60-230°C @ 40°C/min; 5°C/min to 280°C.

MS: manifold temperature 250°C, filament emission 90 µA, multiplier 10^5 Gain + 200 V, background mass 95u, scan 100-420u @ 0.6 sec/scan, AGC Target 15,000.

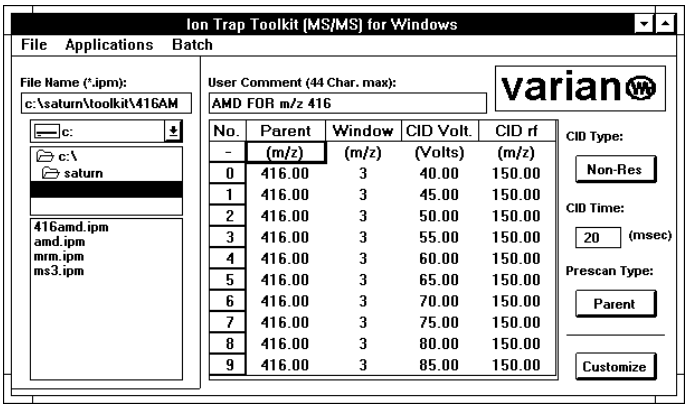

Figure 1. MS/MS Toolkit AMD method for Estradiol-di-TMS. Parent ion m/z 416; non-res CID voltage range from 40-85V.

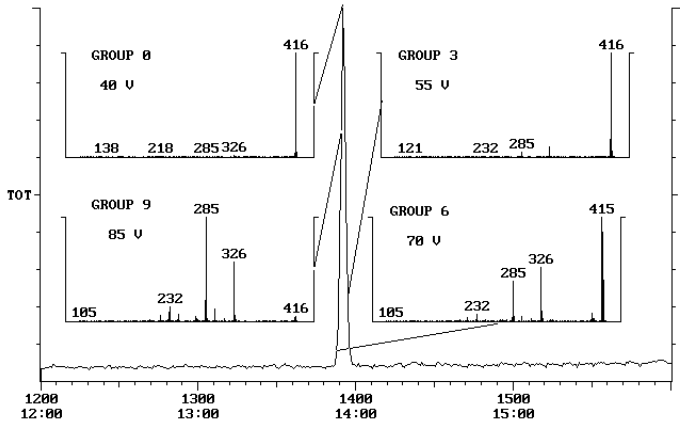

Figure 2. Automated Method Development for Estradiol-di-TMS. Optimal CID voltage is 85 V.

Calibration and Quantitation. An external standard calibration curve was prepared from derivatized standards at the 10, 100, and 1000 pg/µL level using the sum of product ions 285 + 326. The linearity is excellent (Figure 3). The RTICC and product ion mass chromatograms of a blood extract are shown in Figure 4. The MS/MS process eliminates interferences from the sample matrix; the estradiol peak is quantitated at 50 pg/µL.

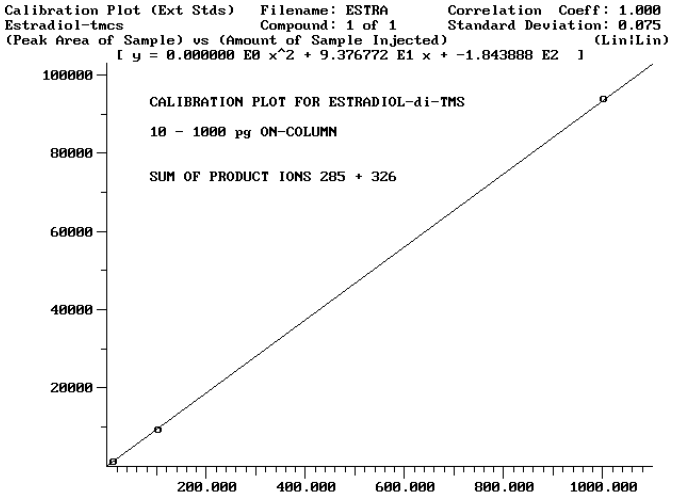

Figure 3. Calibration plot for estradiol-di-TMS for the concentration range 10 - 1000 pg/µL.

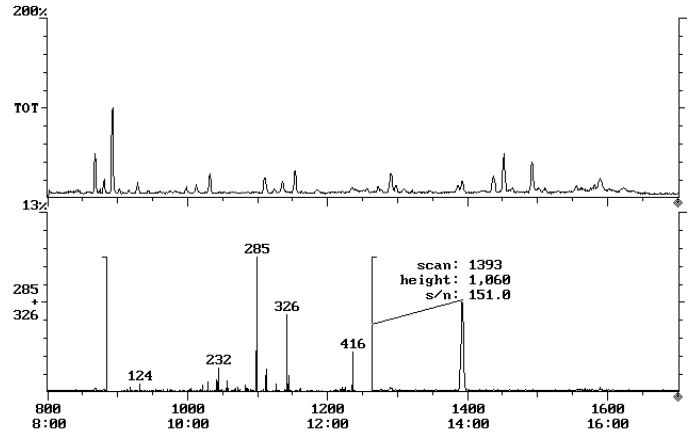

Figure 4. RTICC and Product Ion mass chromatograms of estradiol in a blood extract sample. Sample determined to contain 50 pg/µL estradiol-di-TMS.

Extension of sensitivity with solvent vent/splitless ramped injections. Using a novel injection approach with the 1078 injector on the 3400 CX gas chromatograph, it is now possible to perform large volume injections to extend sensitivity without compromising chromatographic separation. The principle of this process is simple. The sample is injected with the split vent open while the injector temperature is maintained at 5-20°C below the boiling point of the solvent; the solvent is vented to the split vent while analytes are maintained in the insert of the injector. The split vent is then closed as the injector temperature is ramped to drive analytes onto the GC column. Figure 5 illustrates the results obtained for a 5 µL injection of a 2 pg/µL estradiol standard. Using this approach, the GC/MS/MS method may be used to determine estradiol at < 1 pg/µL concentrations.

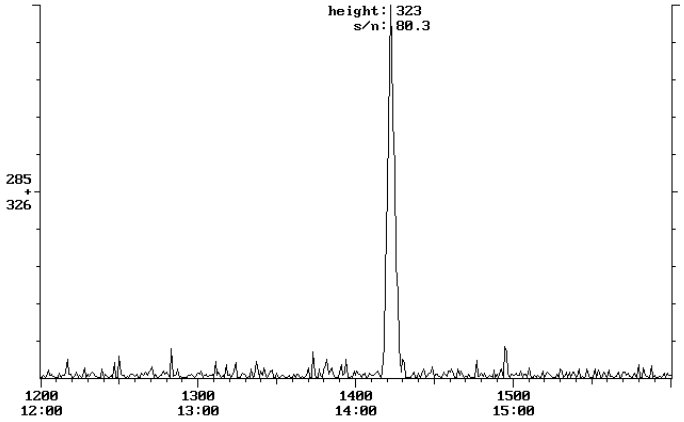

Figure 5. Product ion S/N >80 for a 5 µL injection of 2 pg/µL estradiol-di-TMS standard shows potential for determination of sub-pg/µL levels of estradiol using large volume injections with the 1078 injector.

Conclusions

GC/MS/MS has been demonstrated to be an excellent analytical approach for the determination of estradiol in blood extracts. Derivatization, MS/MS method development, calibration, and extract analysis were performed in less than one working day.