Quantification of and estradiol

Negative chemical ionization GC-MS

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Steroid hormones have long been known to enter the mammalian nervous system to influence its development and function. Commercially available assays for quantification of testosterone and estradiol in serum or in rat brain are based on radioimmunoassay. Because immunoassays can be subject to cross-reactivity with structurally related compounds, it is necessary to

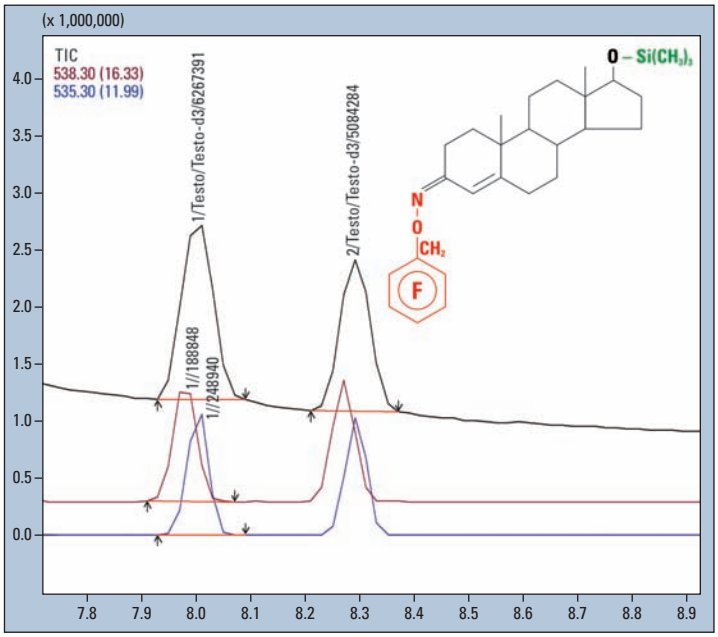

Figure 1a: Total ion current TIC of ECNCI mass spectrum of pentafluorobenzyloxime/ trimethylsilyl ether derivative of testosterone and testosterone-d3 showing the separation of the *syn* and *anti* forms

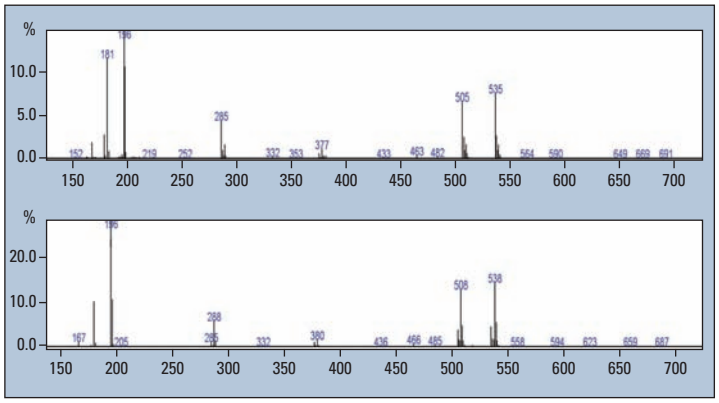

Figure 1b-1c: Full scans mass spectra and selected diagnostic ions at m/z 535 and 538

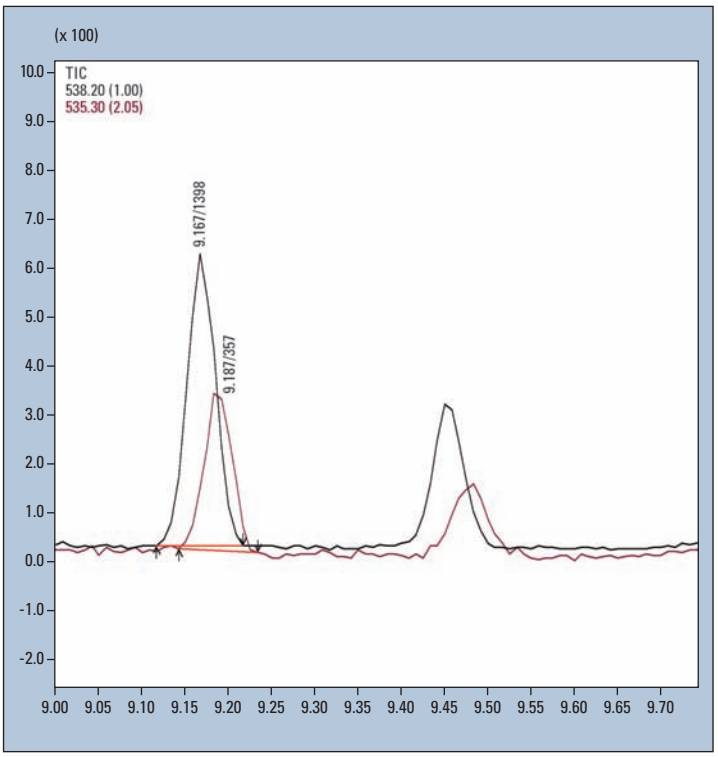

Figure 2: Selected-ion monitoring traces obtained from derivatized male rat brain structure extract. The m/z 535 ion channel is testosterone and the m/z 538 ion channel is the internal standard testosterone-d3. Endogenous testosterone concentration corresponds to 0.13 pg.

testosterone

have a reference method such as gas chromatography-mass spectrometry (GC-MS).

In this application a sensitive GC-MS procedure based on solid-phase extraction and electron capture negative chemical ionization (ECNCI) was developed.

Quantification is based on chromatographic peak areas of testosterone and estradiol relative to the internal standards trideuterated testosterone and estradiol.

Sample preparation for GC-MS

Internal standards 10 pg of testosterone-d3 and 100 pg of estradiol-d3 are added to 300 µL serum or 10-30 mg brain tissue. Partial purification is obtained by SPE C18 extraction. Extracted samples were evaporated and for testosterone, the pentafluorobenzyl-oxime derivative was formed by reacting 50 µL of PFBHA (Florox) in pyridine.

Reagents evaporated and trimethylsilyl derivatives were formed by adding 50 µL of BSTFA in ethyl acetate. Estradiol samples were derivatized with pentafluorobenzoyl chloride (PFBO) in 2.5 % triethylamine. Samples of 1 µL were injected into the GC.

When operated in the ECNCI mode, mass spectrometry is a very selective detection technique since only electron-capturing species give rise to an analytical signal.

Selected-ion monitoring at m/z 535 and 538 for testosterone and testosterone-d3 and ions at m/z 660 and 663 for estradiol and estradiol-d3 respectively were used for quantification.

Results

Chromatography and mass spectrometry

Typical for the ECNCI, the mass spectrum of pentafluorobenzyl-oxime/silyl ether derivative of testosterone shown in Figure 1 is fairly simple, consisting of a diagnostic ion at 535 m/z $(M-20)^-$, an ion cluster at 377 m/z $(M-178)^-$ and a base peak at 196.

Figure 2 shows selected-ion monitoring of the m/z ion channel obtained by ECNCI GC-MS from derivatized rat brain structure. The two peaks present for testosterone are the *syn* and *anti* isomers formed during the preparation of the oxime. Figure 3 shows selected-ion monitoring of the m/z ion channel 660 and 663 of estradiol and estradiol-d3.

Standard curve for testosterone

Figure 4 shows a standard curve (example for testosterone) obtained after addition of known quantities of testosterone to a plasma or brain rat pool sample from 0 to 32 pg. A plot of area ratio of 535 m/z relative to 538 m/z vs concentration of testosterone fits a straight line with $r^2 = 0.9997$.

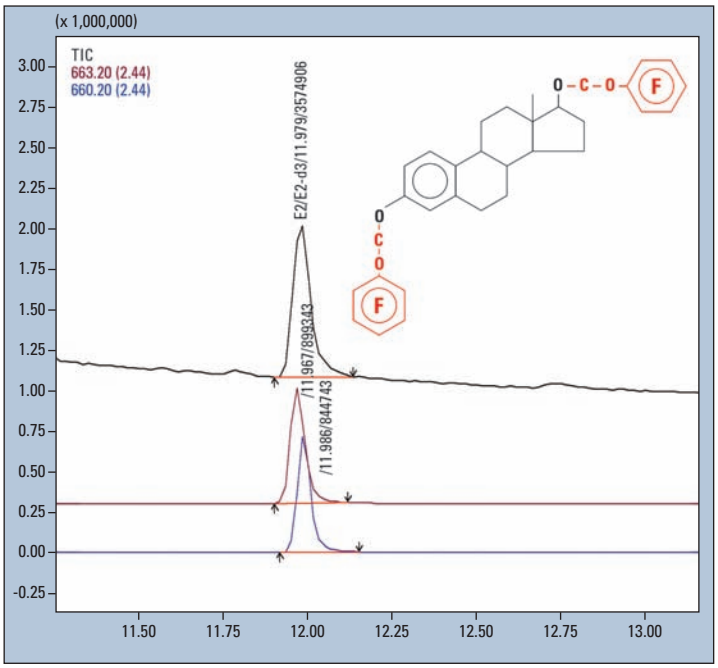

Figure 3a: Total ion current (TIC) of ECNCI mass spectrum of pentafluorobenzyl derivative of estradiol. The m/z 660 ion channel is estradiol and the m/z 663 ion channel is the internal standard estradiol-d3.

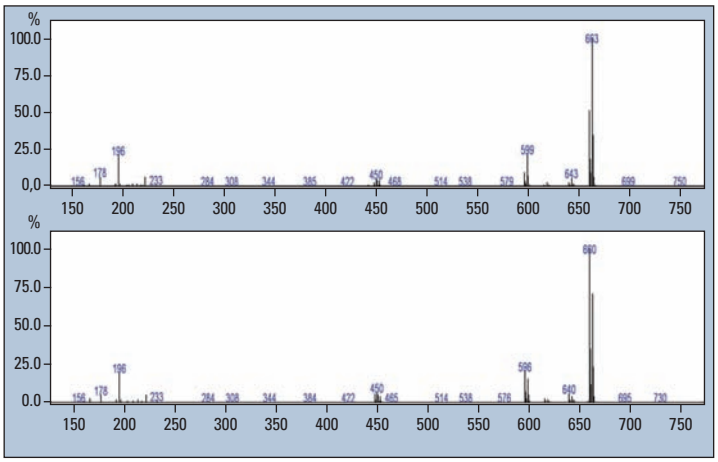

Figure 3b - 3c: Full scans mass spectra and selected diagnostic ions at m/z 660 and 663

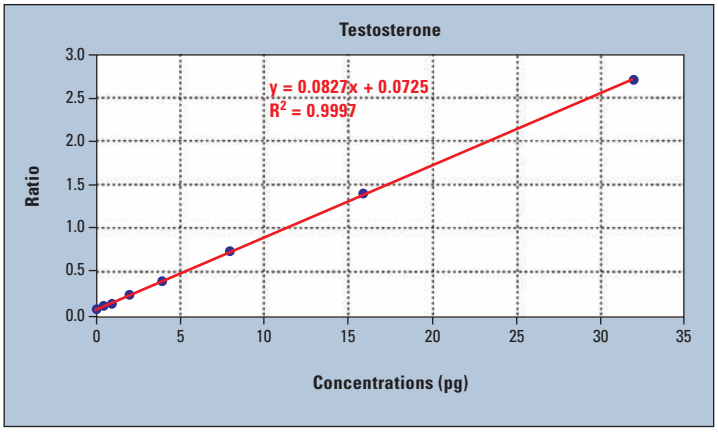

Figure 4: Standard curve obtained after addition of known quantities of testosterone from 0 to 32 pg in brain pool. Steroid amounts are plotted versus area ratios of their respective deuterium-labelled internal standards.