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Analysis of testosterone in serum using mass spectrometry

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Abstract

For either gas chromatography mass spectrometry (GC-MS) or liquid chromatography tandem mass spectrometry (LC-MS-MS) methods, the first step in the analysis is to add a deuterium-labeled internal standard such as testosterone-16,16,17-d(3). Testosterone in the sample is then isolated by liquid-liquid extraction and the extract is dried under a stream of nitrogen. For the GC-MS method we describe; the residue is transformed to the pentafluorobenzyl/trimethylsilyl derivative and is injected into the GC-MS, separated on a dimethypolysiloxane column, and ionized using electron capture negative chemical ionization (ECNCI). Quantification of testosterone in the samples is by selected ion monitoring, measuring peak ratios of testosterone relative to the deuterium-labeled internal standard. For the LC-MS-MS analysis of testosterone, the sample extract is reconstituted in mobile phase, injected on a C18 column, and quantified using multiple reaction monitoring of testosterone relative to the internal standard. There are no interferences from common steroids found in human serum. For both methods the run-to-run precision and accuracy is generally less than 6% and the methods are linear from 5 to 2000 ng/dL.

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