



Compounding Factors: The Analysis of Steroids

LC/MS/MS - A Reliable Instrument for the Detection of Anabolic Steroids in Urine Specimens

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The World Anti-Doping Agency (WADA) mandates an international standard for laboratories for the use of either GC/MS or LC/MS/MS to screen and confirm anabolic steroids in urine samples. Previous methods of steroid testing using urine samples were routinely conducted with the GC/MS. However, this procedure requires lengthy sample preparation and is time consuming. With the development of LC/MS/MS technology, laboratories have evolved toward using this technique for the detection of anabolic steroids because it's faster, more reliable, and provides acceptable reproducible results for the detection and quantitation of anabolic steroids. Additionally, this technique is highly sensitive as it detects the parent compound of the steroid and the breakdown compounds known as the metabolites.

Steroids can be taken by mouth, rubbed on skin as a cream or injected directly into the body with a needle. Eventually, the steroids enter the blood stream and muscle tissues to produce an anabolic effect.

The liver is the primary organ for the detoxification of the drugs. The steroid compounds are metabolized in the liver. The parent steroid compound and metabolites form glucuronic acid conjugates. These conjugates are more soluble in water making it easier for the kidneys to eliminate the toxins into the urine.

The steroid excreted in the urine includes the parent compound and its metabolites. The metabolism of the steroid is not completed with one pass through the liver. The parent compound continues to travel through the bloodstream and the liver until it is completely removed from the body. The metabolism and excretion process repeats itself as the body continues to remove toxins.

Depending on recent usage and the level of steroid ingested, determines the amount of the parent compound and its

metabolites contained in the specimen.

A gas chromatograph mass spectrometry analysis involves an extraction, derivatization and separation of the steroid prior to testing. These procedures are tedious and time consuming for laboratory personnel

One Source Toxicology Laboratory's analysis of steroids relies on the technology of Liquid Chromatography Mass Spectrometry (LC/MS/MS). The LC/MS/MS is a powerful instrument that detects a steroid's parent compound and its metabolites with the greatest sensitivity. The instrument is the most recent development in the analytical field. Processing samples with this instrument is simple and less time consuming. This technique allows the laboratory to identify the parent compound and its metabolites simultaneously.

The Steroids are initially separated on a column using Liquid Chromatography (LC) and are detected using *tandem* Mass Spectrometry (MS/MS). The identification of a steroid is based on the retention time that is specific for each steroid and a mass to charge (m/z) ratio of the individual steroid. One Source Toxicology Laboratory is currently using an LC/MS/MS to conduct its steroid analysis. Currently, the laboratory has the ability to screen and identify twenty steroid compounds. Research is underway to expand this panel to 45 steroid compounds with the corresponding metabolites. The detection limit for this instrument is parts per billion.

To date, the laboratory has conducted more than 4,000 steroid tests since it initiated its testing procedures in August 2006. One Source Toxicology Laboratory is recognized as the leader in steroid analysis in the southwest region of the United States.