K9 Development and Validation of a LC/MS Method for the Determination of Guanfacine in Urine

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After attending this presentation, attendees will become familiar with a validated liquid chromatography/mass spectrometry (LC/MS) method for detecting and quantifying guanfacine in urine specimens.

Guanfacine is a drug that was initially approved for the treatment of hypertension in adults, but has been recently approved (2007) for the treatment of attention deficit/hyperactivity disorder (ADHD) in adolescents. Due to the new therapeutic use, an increase in both availability and consumption of this drug required the development of an analytical method to detect the use or abuse of guanfacine. A validation of this LC/MS method will impact the forensic community by providing the field of toxicology with a rapid, robust analytical method that requires a small sample volume, and is also sensitive enough to detect drug use at a therapeutic dose.

The validation of a LC/MS method for the detection and quantification of guanfacine in urine is presented. Guanfacine was extracted from alkaline buffered urine using a liquid-liquid extraction scheme with ethyl acetate. Two hundred microliters of samples, controls, and calibrators were prepared with the addition of 10µL of protriptyline internal standard (2mg/L). Samples were buffered to a pH of 9.5 with 200µL saturated carbonate:bicarbonate solution. Five hundred microliters of ethyl acetate was added to the samples, followed by two minutes of rotation and five minutes of centrifugation at 3000rpm. The organic layer was transferred to a clean test tube, evaporated to dryness under a gentle stream of nitrogen, and reconstituted in 200µL of mobile phase. Guanfacine and protriptyline were separated and quantified on a reverse phase S-5 micron, 2.0 x 150mm column in a high performance liquid chromatography (HPLC) separations module coupled to a mass spectrometer (MS) with electrospray ionization operated in the positive ionization mode. The mobile phase consisted of 40% 10mM ammonium formate in methanol, and was delivered isocratically at a flow of 0.3 mL/min. Sample injection volume was 10µL. The MS was operated in selected ion resonance mode (SIR) using the following m/z ions: 246, 248, and 250 for guanfacine, and 264 and 265 for protriptyline. Under these conditions the retention time for guanfacine and protriptyline were 2.1 min and 3.6 min, respectively.

The analytical measurement range for guanfacine ranged from 5ng/mL to 2000ng/mL with a 5ng/mL limit of detection (LOD) and a 20ng/mL limit of quantitation (LOQ). The method was shown to be both precise and accurate. Precision for the assay was determined at concentrations of 40ng/mL, 100ng/mL, and 500ng/mL, (n=6), the %CV was <15% for all three concentrations. Percent recovery of guanfacine was also performed using the same concentrations and was shown to be 92%, 89%, and 93% respectively. Interference with other therapeutic drugs and drugs of abuse was assessed by analyzing two controls containing known concentrations of drugs in both categories. No interferences were noted. The method was used to analyze over 100 random post diagnostic specimens from children ranging in age from 4- to 18-years-old. Ten of the samples yielded positive results for the presence of guanfacine. Some of these samples were evaluated using a previously validated gas chromatography/mass spectrometry (GC/MS) method, and results were found to be comparable. Of the ten positive samples, seven were confirmed to be from patients who were prescribed guanfacine. No patient history was available for the remaining samples. This LC/MS method provides a rapid and reliable method for the routine determination and quantitation of guanfacine in urine specimens.

Guanfacine, LC/MS, Electrospray Ionization

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