



### K32 A Stability Study on Ritalinic Acid in Urine

Ayodele A. Collins, BS\*, Sam Houston State University, 2615 El Toro Dr, Apartment 127, Huntsville, TX; Joseph R. Monforte, PhD, Ameritox, 9930 West Highway 80, Midland, TX 79706; Ronald C. Backer, PhD, Universal Toxicology, Laboratories, LLC, 9930 West Highway 80, Midland, TX 79706; and Sarah Kerrigan, PhD, Box 2525 Sam Houston State University, Chemistry Forensic Science Building, 1003 Bowers Boulevard, Huntsville, TX 77341

The goal of this presentation is to evaluate the short-term stability of ritalinic acid in authentic urine specimens exposed to different storage conditions.

This presentation will impact the forensic science community by identifying adequate storage conditions for urine samples obtained from methylphenidate users; and contribute to proper interpretation of results from delayed analyses or reanalyses by reviewing indications from the assessment of ritalinic acid stability.

The stability of any drug and potential metabolites in biological samples must be considered when justifying the reliability of analytical results. Variation in drug concentrations in biological fluids is possible via thermal, chemical, enzymatic or matrix degradation. Stability studies can improve toxicological quality by identifying optimal storage conditions and time limits for analysis, after sample collection, and reanalysis. The examination of the short-term stability of ritalinic acid, assessed through quantitative results for eight positive ritalinic acid urine samples obtained from pain management patients prescribed methylphenidate, is presented.

Ritalinic acid is the primary metabolite of methylphenidate, a phenethylamine derivative employed in the treatment of attention-deficit hyperactivity disorder (ADHD), childhood hyperkinesis, depression and narcolepsy. Previous research has revealed that methylphenidate spontaneously hydrolyzes to ritalinic acid in vitro.<sup>[1]</sup> The conclusion from that scientific study recommends freezing or refrigeration conditions for specimen storage. Some commercial laboratories refuse analysis of specimens that have not been frozen. The methylphenidate degradation process is minimized at pH 2.9, or by addition of ethylenediaminetetraacetic acid (EDTA), and under "cool" storage.<sup>[1]</sup> Studies have confirmed the percentage of methylphenidate in urine to be minimal, less than 1% in a twenty four hour void.<sup>[2, 3]</sup> Consequently, urine methylphenidate concentrations are usually quite low and if conversion to ritalinic acid does occur, it is unlikely that the ritalinic acid content will be significantly increased. There have been no published studies on ritalinic acid stability to date.

The ritalinic acid concentration in three sets of urine aliquots from authentic cases (n=8), stored at room temperature, refrigerated or frozen was quantified by gas chromatography/mass spectrometry (GC/MS), once weekly, over a one-month period. The baseline concentration range of all specimens was 10,945-78,673 ng/mL. Loss of analyte appears to be concentration dependent. Deterioration is more rapid at higher ritalinic acid concentrations. There was a significant decrease in concentration of the target analyte over the twenty nine-day period; mean percentage loss of analyte was 32%, 36%, and 43% for highly concentrated ritalinic acid specimens (46,332-78,673 ng/mL) stored at room temperature, refrigerated and frozen, respectively. No statistically significant difference in the variation of ritalinic acid content among the samples stored under the three conditions was evident. At lower ritalinic acid content (10,945-18,594 ng/mL), change in concentration was insignificant. All statistical analysis was done at the 95 % confidence limit; P= 0.05. The results indicate that ritalinic acid is unstable in urine, particularly at high concentrations, and the concentration will decrease significantly upon storage at room temperature, refrigeration, or freezing.

**Stability, Ritalinic Acid, Storage Condition**