

Homogeneous Enzyme Immunoassay for Xylazine and Its Metabolites

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Background: Xylazine belongs to a class of compounds known as alpha-2 adrenergic agonists. It is a non-opiate sedative, analgesic, and muscle relaxant only authorized in the United States for veterinary use. However, xylazine has increasingly been identified in various illicit drug mixtures, associated with a rising incidence of drug overdose fatalities. It is most commonly found in combination with fentanyl known as, 'tranq' or 'tranq dope' in the USA, but has also been detected in mixtures containing cocaine, heroin, and a variety of other drugs.

The chronic use of xylazine can lead to severe side effects such as necrotic skin ulcerations, abscesses, and infections. Currently, there are no commercially available urine drug screening immunoassays for the detection of xylazine on automated chemistry analyzers. ARK Diagnostics has developed the first sensitive homogeneous enzyme immunoassay to detect xylazine in human urine at a cutoff concentration of 10 ng/mL.

Methods: The ARK™ Xylazine Assay is a liquid-stable homogenous enzyme immunoassay consisting of two reagents. The performance characteristics of this assay, including precision, spiked recovery, specificity, and method comparison to LC-MS/MS, were evaluated on the Beckman Coulter AU480 automated clinical analyzer.

Results: The ARK™ Xylazine Assay demonstrated acceptable precision, with $\leq 3.4\%$ CV in semi-quantitative mode and no overlap between cutoff (10 ng/mL) and $\pm 50\%$ control levels (5 ng/mL and 15 ng/mL) in a histogram overlap analysis. Spiked xylazine samples spanning the semi-quantitative assay range up to 500 ng/mL were recovered between 91.7% and 107.4% of the spiked levels. Two of the major metabolites, 3-hydroxyxylazine and 4-hydroxyxylazine, were detected as positive at concentrations of 10 ng/mL (100% cross-reactivity) and 25 ng/mL (40% cross-reactivity), respectively. Cross-reactivity to alpha-2 agonists, clonidine and romifidine, were determined to be $< 0.6\%$. In method comparison studies, the ARK™ Xylazine Assay accurately identified all 100 human urine samples confirmed negative by LC-MS/MS as negative, and all 32 samples with confirmed xylazine concentrations > 10 ng/mL were detected as positive.

Conclusion: The ARK™ Xylazine Assay enables a sensitive, rapid, and reliable measurement of xylazine and its metabolites in human urine, applicable to a wide range of clinical chemistry analyzers.

Keywords : Xylazine, Homogeneous Enzyme Immunoassay