New Emit® II Plus 6-Acetylmorphine Assay* on the V-Twin®/Viva-E® Analyzers

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Abstract

Background: 6-Acetylmorphine (6-AM) is a heroin metabolite and its presence in urine specifically confirms the illicit use of heroin. A new Emit® II Plus 6-AM Assay for human urine screening is currently being developed at the Siemens Healthcare Diagnostics laboratory. The assay has a cutoff of 10 ng/mL. The assay negligent provides quality and semi-quantitative results.

Methods: Precision was evaluated using the cutoff and +/- 25% controls according to CLSI EP5-A2. Recovery was studied by spiking 6-AM into human urine at levels that span the calibration range (0.05-30 ng/mL). 6-AM concentration was assayed using the cutoff and +/- 25% controls over a 2 day period. Urine specimens were assayed and the results compared to those from the GCMS. Cross-reactivity with structurally related drugs was assessed at different concentrations.

Results: The qualitative reproducibility (CV%) for the cutoff and +/- 25% controls ranged from 1.1 to 2.2% and the within run CV% ranged from 0.07 to 1.5%. The semi-quantitative reproducibility (CV%) ranged from 1.1 to 2.2% and the within run CV% ranged from 0.5 to 2.3%. The area under the curve for the 0-10 and 10-50 ng/mL ranges was 0.81 and 0.88 (0.0126). The results were stable over the analyzer for at least 30 days.

Conclusions: The new Emit® II Plus 6-AM Assay is suitable for competitive binding assay for urinalysis and can be performed both qualitatively and semi-quantitatively.

Introduction

6-Acetylmorphine (6-AM) is an active heroin metabolite and its presence in urine confirms the illicit use of heroin. It can be separated and identified using gas chromatography, which is currently considered a gold standard method. The Emit® II Plus 6-AM Assay is a homogeneous enzyme immunoassay. It is based on competition between drug (6-acetylmorphine) in the sample and drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding site. The enzyme conjugate activity decreases upon binding to the antibody. The enhanced immune conjugate converts the oxidized G6PDH to the active and inactive forms. The absorbance can be measured spectrophotometrically at 492 nm. The absorbance decreases upon binding to the antibody, allowing a 6-AM concentration in the sample to be measured in terms of inhibition.

Analytical Recovery / Linearity

Recovery was tested by spiked sample analysis on the Viva E® analyzer. 6-Acetylmorphine was spiked to various levels in urine and compared to nominal values. Linearity was tested by patient sample analysis of the Viva E® analyzer.

On-instrument Stability

On-board stability was determined quantitatively on the Viva E® analyzer. The 10 ng/mL cutoff and +/- 25% control commercial levels were assayed over 2 day period. The 6-AM Assay had at least 30 days of stability on the Viva E® analyzer.

Conclusions

The Emit® II Plus 6-AM Assay, currently under development, is a convenient method for detecting the heroin metabolite (6-acetylmorphine) in urine, should be a suitable screening method for urine screening for heroin, for both qualitative and semi-quantitative analysis. The results on precision, overlap, recovery, sensitivity, specificity, interfering substances and an instrumental stability were found to be excellent.