

## The Analysis of Fentanyl and Its Analogues in Human Urine by LC-MS/MS

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Synthetic opioid drugs, such as fentanyl and sufentanil, have very high analgesic potency. Abuse of these prescription opioids and their illicit analogue, acetyl fentanyl, is a growing public health problem. In this study, a simple dilute and shoot method was developed with an analysis time of less than 3.5 minutes for fentanyl, norfentanyl, acetyl fentanyl, and sufentanil in human urine by LC-MS/MS using the Raptor™ Biphenyl column.

Pooled human urine was fortified with the analytes. The urine sample was diluted 5-fold in a water/methanol solution with the addition of internal standards (fentanyl-d5, norfentanyl-d5, sufentanil-d5, acetyl fentanyl-<sup>13</sup>C<sub>6</sub>) prior to injection on the Raptor™ Biphenyl column (50x2.1mm, 5

0.1% formic acid in methanol (organic phase) and the chromatographic separation was achieved with a gradient elution of 30% - 80% organic phase in 2 minutes. The analysis was performed on a Waters ACQUITY UPLC® I-Class System coupled with a Waters Xevo TQ-S mass spectrometer using electrospray ionization in positive ion mode.

All four analytes were completely resolved on the Raptor™ Biphenyl column with a 2-minute gradient elution. No matrix interference was observed for quantitation. The calibration linearity ranged from 0.05 to 50 ng/mL for fentanyl, acetyl fentanyl, and sufentanil; and 0.25 to 50 ng/mL for norfentanyl with % deviation of less than 10.0% and the R<sup>2</sup> of ≥ 0.999. The LLOQ was 0.25 ng/mL for norfentanyl, and 0.05 ng/mL for fentanyl, acetyl fentanyl, and sufentanil in urine.

Three levels of QC samples were analyzed for accuracy and precision. Based on three independent experiments conducted on multiple days, the mean accuracy values ranged from 94 to 110% of the nominal concentrations for all compounds and the %RSD ranged from 0.2 to 9.2%.

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