Figure 1. Chromatograms of drug-free human serum spiked with analytes and internal standards. Chromatograms represent a 100 ng/mL drug mixture. The selected transition is indicated for each chromatogram and elution times are indicated above each peak.

Figure 2. Deming regression analysis of drug-free human serum spiked with morphine (A), morphine-6-β-glucuronide (B), morphine-3-β-glucuronide (C), and hydromorphone (D) comparing the described method (HPLC-MS/MS Vantage) to NMS Labs UPLC-MS/MS method. Slope, intercept, and Pearson's coefficient (R) are indicated for each analyte.

Figure S1. Chromatograms of drug-free human serum spiked with the 48-component interference mixture (does not contain hydromorphone-3-β-glucuronide) and extracted with IS spiked methanol. At retention times of interest, there are no interferences.

Figure S2. Stacked chromatogram plot of drug-free serum spiked with morphine-3-β-glucuronide, hydromorphone-3-β-glucuronide, and morphine-6-β-glucuronide at equimolar concentrations. The peak overlap of hydromorphone-3-β-glucuronide and morphine-6-β-glucuronide interferes with morphine-6-β-glucuronide integration on average 75%.