

Quantitation of Fentanyl, Norfentanyl, Buprenorphine, and Norbuprenorphine in Urine using WAX-XTR tips and LC-MS/MS

HIGHLIGHTS: Robust, linear, method with necessary sensitivity



INTRODUCTION

Fentanyl and buprenorphine are potent analgesics making them medicinally appealing and easy to abuse. Thus, they are two of the most common analytes seen in clinical and forensic laboratories. Due to their high potency and low dosages, urinary concentrations are much lower than other common opiates/opioids. This leaves most laboratories struggling to increase the sensitivity and speed of their methods. This method using INTip™ SPE (powered by Dispersive Pipette XTRaction technology) with weak anion exchange (WAX) sorbent promotes an easy, fast, and sensitive method to help laboratories increase efficiency of detecting these compounds and their metabolites.

800 µL DI water (wash) and 500 µL acetonitrile (ACN). XTR tips containing 10 mg of WAX in a 1 mL Hamilton format are then conditioned by aspirating 30% methanol (MeOH) solution from a solvent reservoir. Sample solutions are aspirated and dispensed five times in order to bind the analytes of interest to the sorbent. Two separate 400 µL aliquots of water wash are then aspirated and dispensed to remove salts and other common matrix interferences. Target compounds are eluted by aspirating and dispensing 500 µL of ACN three times. The elution solvent is then solvent evaporated and reconstituted in 100 µL of 20% methanol (aqueous). LC-MS/MS analysis was performed using a Sciex 6500+ triple stage quadrupole mass spectrometer coupled to an Agilent 1260 HPLC system. Chromatographic separation was performed on a Phenomenex biphenyl (2.6 µm; 50 x 3.0 mm) column with a 5 µL injection.

MATERIALS AND METHODS

Micro-plates containing urine (200 µL), 100 µL of acetate buffer, and 10 µL of ISTD are loaded onto the Hamilton Microlab NIMBUS96. Additional micro-plates are filled with

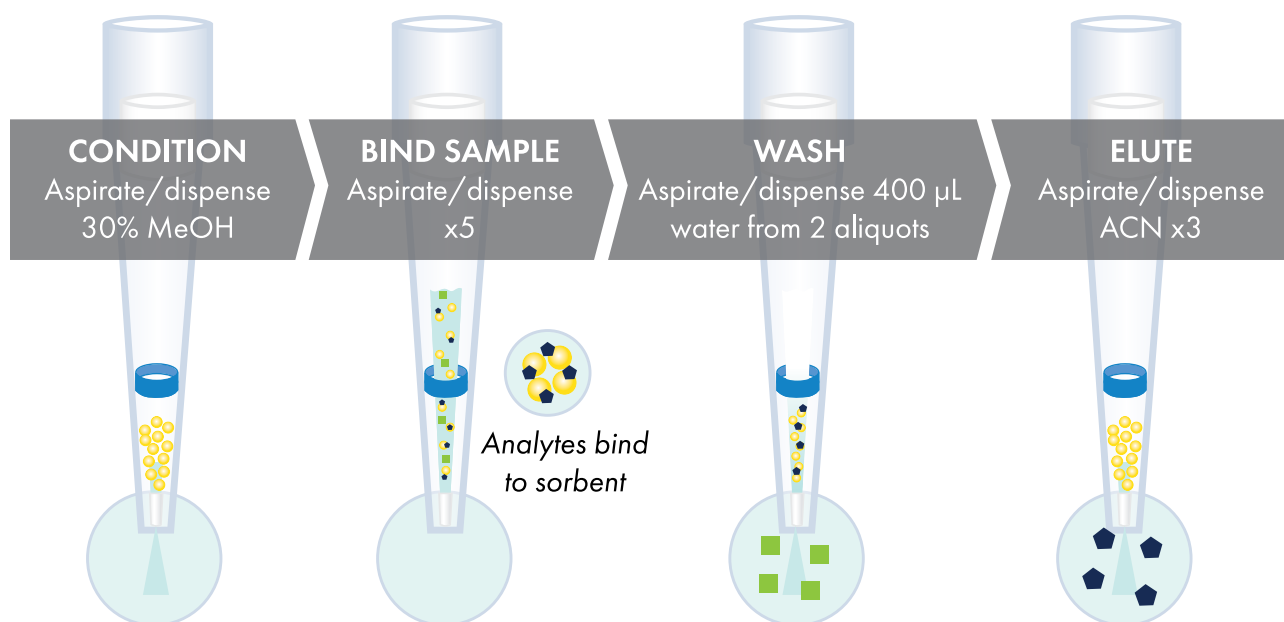


Figure 1. Schematic of INTip solid phase extraction (SPE) method

RESULTS

Analytical results are linear, accurate and precise. Correlation coefficients (R^2) were greater than 0.99 over the concentration range of 0.2-50 ng/mL for norfentanyl and fentanyl and 0.8-50 ng/ml for norbuprenorphine and buprenorphine, with all analytes exhibiting linearity over that range. Relative standard deviations (%RSDs) were calculated using 5 replicate extractions (0.2 ng/mL for norfentanyl and fentanyl and 0.8 ng/ml for norbuprenorphine and buprenorphine), and were under 4% for all four compounds. Limits of detection (LODs) were calculated as 3 times the standard deviation of the lowest non-zero calibrator. Limits of quantitation (LOQs) were chosen as 0.2 ng/ml for norfentanyl and fentanyl and 0.8 ng/mL for norbuprenorphine and buprenorphine.

Table 1. The yield, matrix effects, and Limit of Detection (LOD) are listed for Norfentanyl, Norbuprenorphine, Fentanyl, and Buprenorphine.

Compound	Recovery	Matrix Effects	LOD ng/mL
Norfentanyl	59%	-47%	0.02
Norbuprenorphine	97%	-65%	0.06
Fentanyl	107%	-70%	0.02
Buprenorphine	103%	-49%	0.07

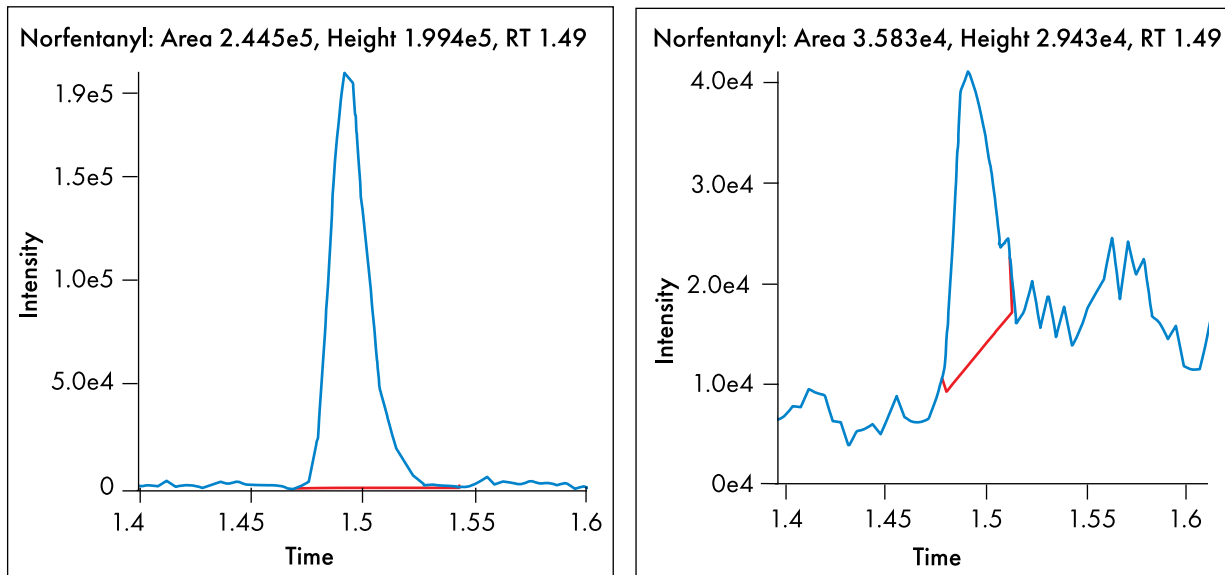


Figure 2. Norfentanyl quantities and qualifier chromatograms at LOQ of 0.2 ng/mL (233.2/84.1 left ; 233.2/55.1 right).

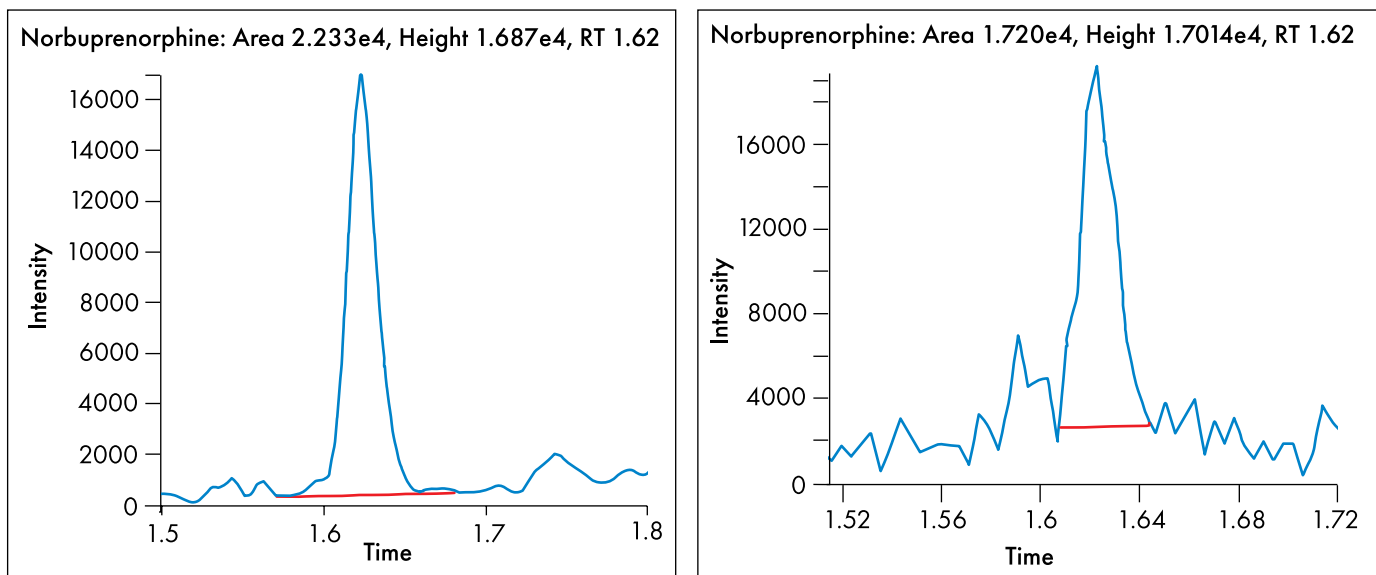


Figure 3. Norbuprenorphine quantities and qualifier chromatograms at LOQ of 0.8 ng/mL (414.2/152.1 left ; 414.2/115.1 right).

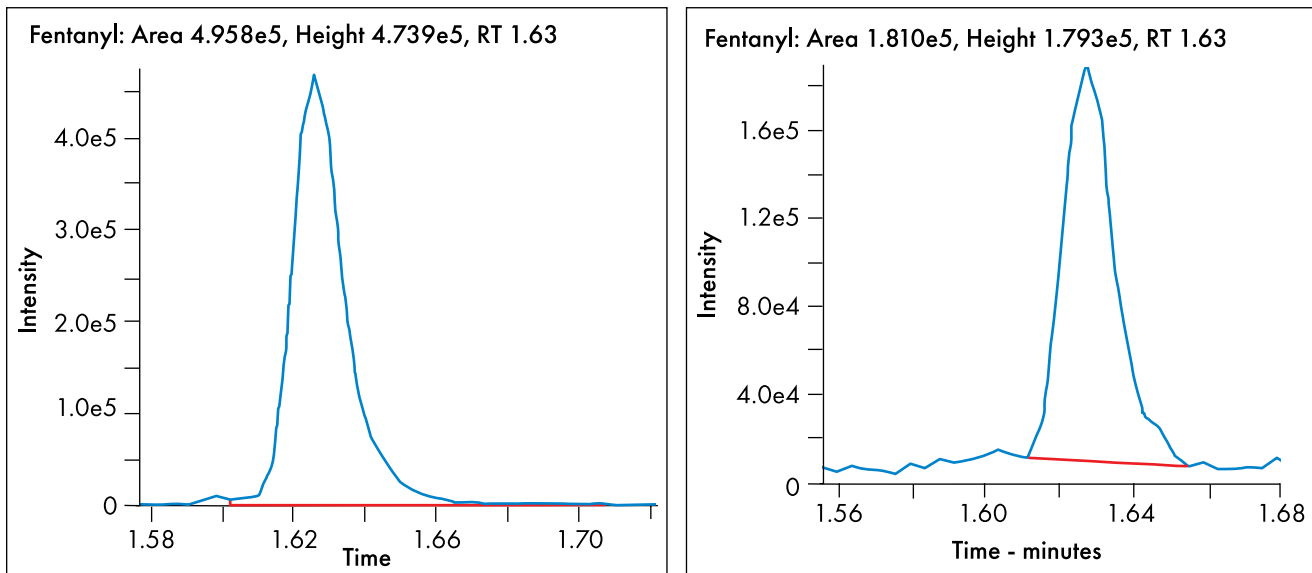


Figure 4. Fentanyl quantities and qualifier chromatograms at LOQ of 0.2 ng/mL (337.1/188.2 left ; 337.1/105.1 right).

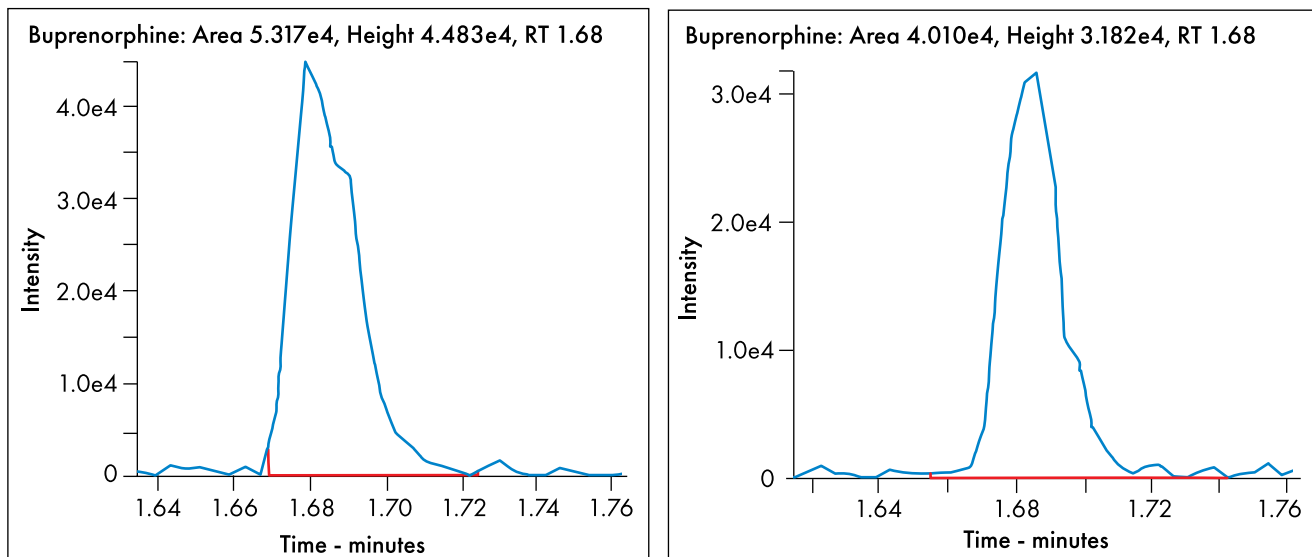


Figure 5. Buprenorphine quantities and qualifier chromatograms at LOQ of 0.8 ng/mL (468.3/396.2 left ; 468.3/414.3 right).

CONCLUSIONS

The WAX-XTR method described herein requires no complex solution preparation for wash or elution steps. This reduces solvent preparation time and streamlines sample preparation. This WAX-XTR method can process up to 96 samples in under 15 minutes allowing for a fast, automated, and high throughput workflow. The method is robust, linear, and provides the necessary sensitivity to meet the majority of laboratories' needs.

PRODUCT

To order the exact product in this application use this catalog number.

Catalog:	Description:
DPX170117	XTR tips: 10mg WAX (60 μ m) in 1 mL Hamilton

To customize this product choose sorbent amount and tip format from the available options listed on our website.