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

HIGHLIGHTS: 96 samples in < 15 minutes

SCX- XTR

INTRODUCTION

Fentanyl and buprenorphine are potent analgesics making them medicinally appealing and easy to abuse. Thus, they are two of the most common analytes monitored 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 DPX INTip technology with strong cation exchange (SCX) sorbent promotes an easy, fast, and sensitive method to help laboratories increase efficiency of detecting these compounds and their metabolites.

MATERIALS AND METHODS

Microplates containing urine (200 µL), 100 µL of acetate buffer, and 10 µL of ISTD are loaded on to the Hamilton Microlab NIMBUS96. Additional microplates are filled with 900 µL 0.1N HCl (wash #1), 600 µL of methanol (wash #2), and 300 µL 78:20:2 dichloromethane/isopropanol/ammonium hydroxide (DCM/IPA/NH_4OH) elution solvent. XTR tips containing 5 mg of SCX in a 1 mL Hamilton format are then conditioned by aspirating 30% methanol solution from a solvent reservoir. Sample solutions are aspirated and dispensed five times in order to bind the analytes of interest to the sorbent. Three separate 300 µL aliquots of wash solvent #1 are then aspirated and dispensed to remove salts and other common matrix interferences. Two separate 300 µL aliquots of wash solvent #2 are then aspirated and dispensed to remove common matrix interferences. Target compounds are eluted by aspirating and dispensing 300 µL of DCM/IPA/NH_4OH solution 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.

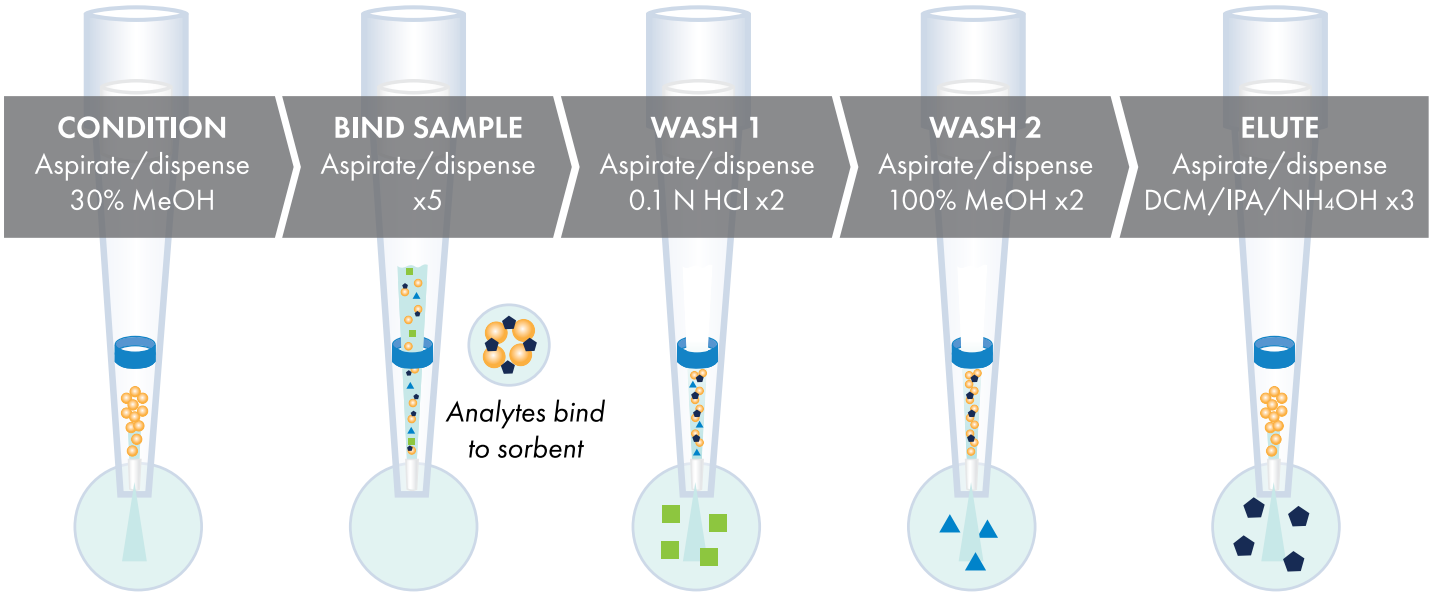
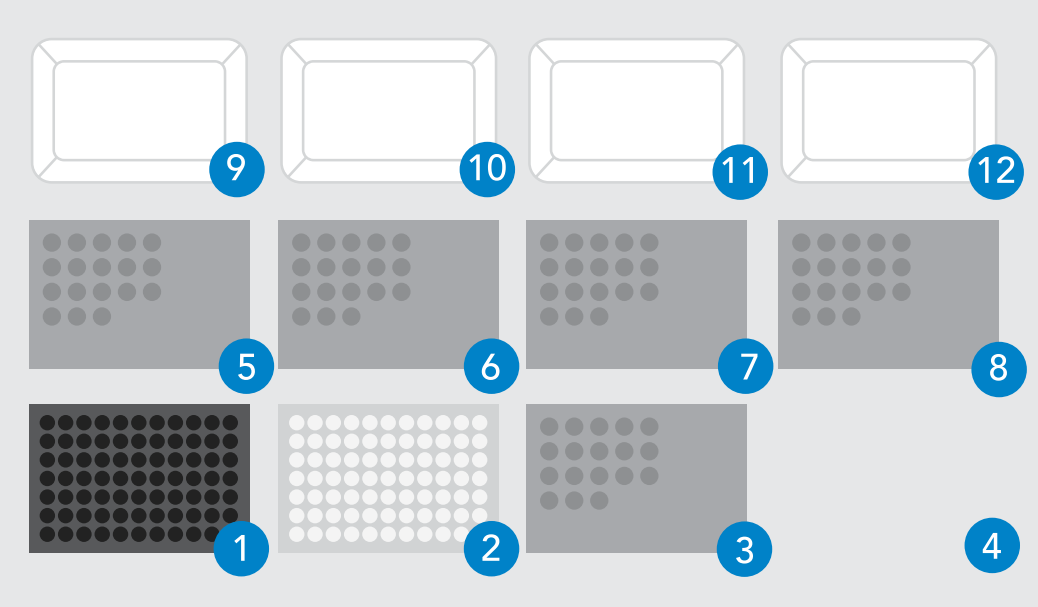


Figure 1. Schematic of INTip solid phase extraction (SPE) method



1. XTR Tips
2. Hamilton CO-RE 1 mL Tips
3. Condition
4. Empty Space
5. Sample
   200 µL urine, 100 µL acetate buffer, 10 µL ISTD
6. Wash 1 -*0.1 N HCl*
7. Wash 2 -*MEOH*
8. Elution -*DCM/IPA/NH_4OH*
9. 30% MEOH
10. 100% MEOH
11. 0.1N HCl
12. 78:20:2 DCM/IPA/NH_4OH

Figure 2. The NIMBUS96 deck configuration includes reservoirs, well plates and pipette tips to perform the fully automated DPX 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 (1.5 ng/mL) 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	Yield	Matrix Effects	LOD ng/mL
Norfentanyl	79%	-22%	0.022
Norbuprenorphine	89%	-38%	0.028
Fentanyl	88%	-57%	0.017
Buprenorphine	83%	-31%	0.28

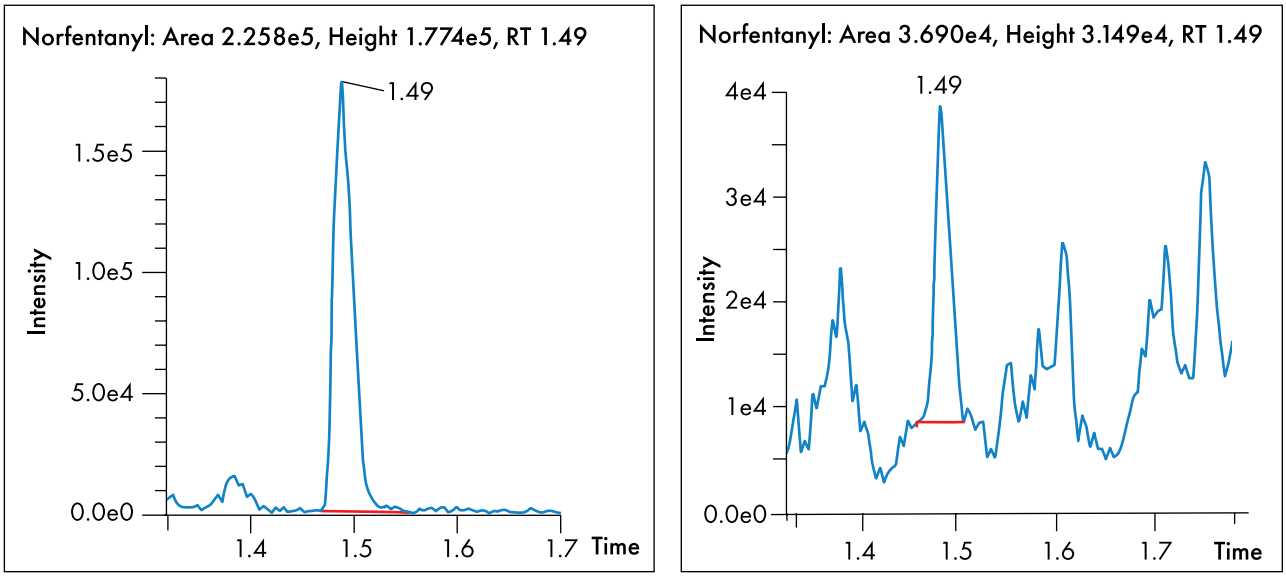


Figure 3. Norfentanyl quantities and qualifier chromatograms at LOQ of 0.2 ng/mL (233.2/84.1 left ; 233.2/55.1 right).

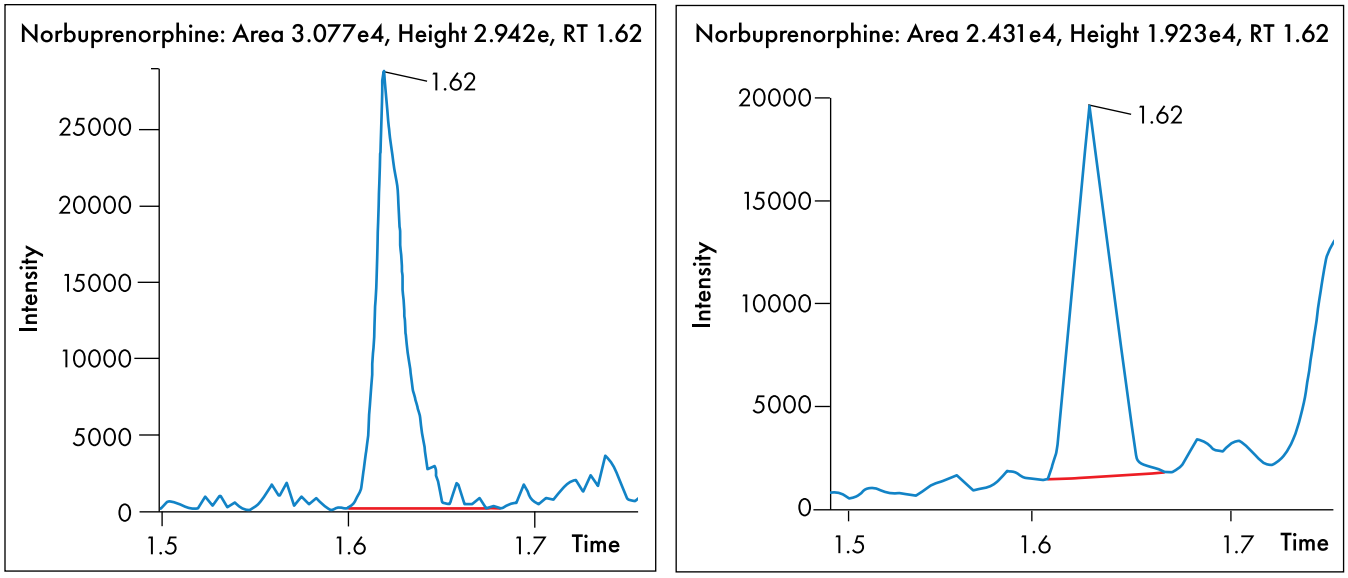


Figure 4. Norbuprenorphine quantities and qualifier chromatograms at LOQ of 0.8 ng/mL (414.2/152.1 left ; 414.2/115.1 right).

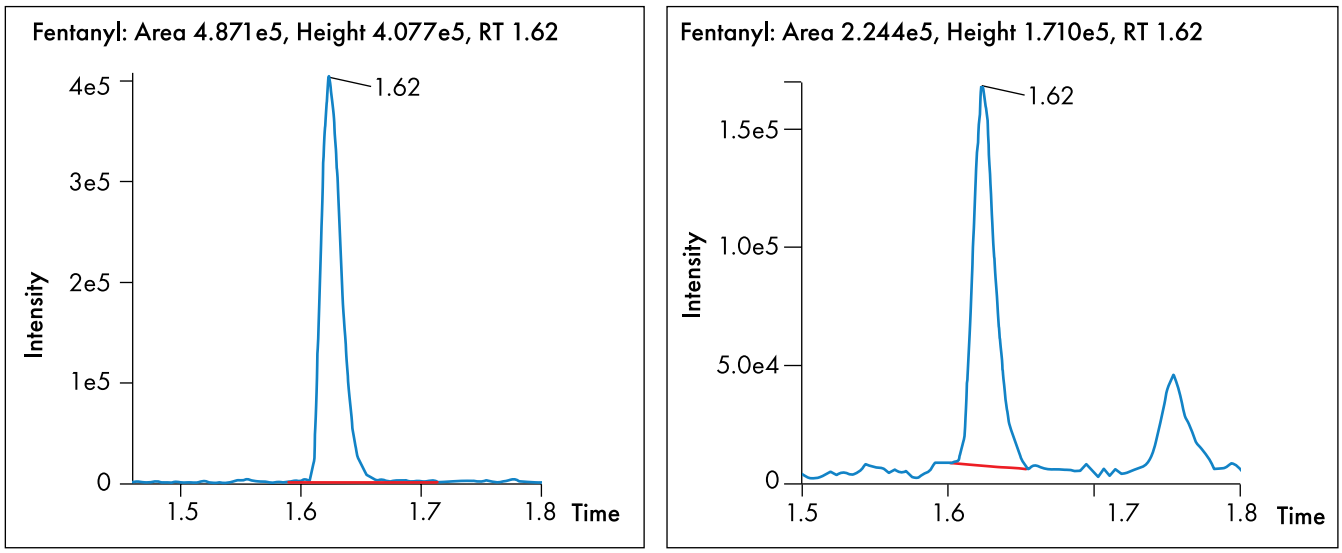


Figure 5. Fentanyl quantities and qualifier chromatograms at LOQ of 0.2 ng/mL (337.1/188.2 left ; 337.1/105.1 right).

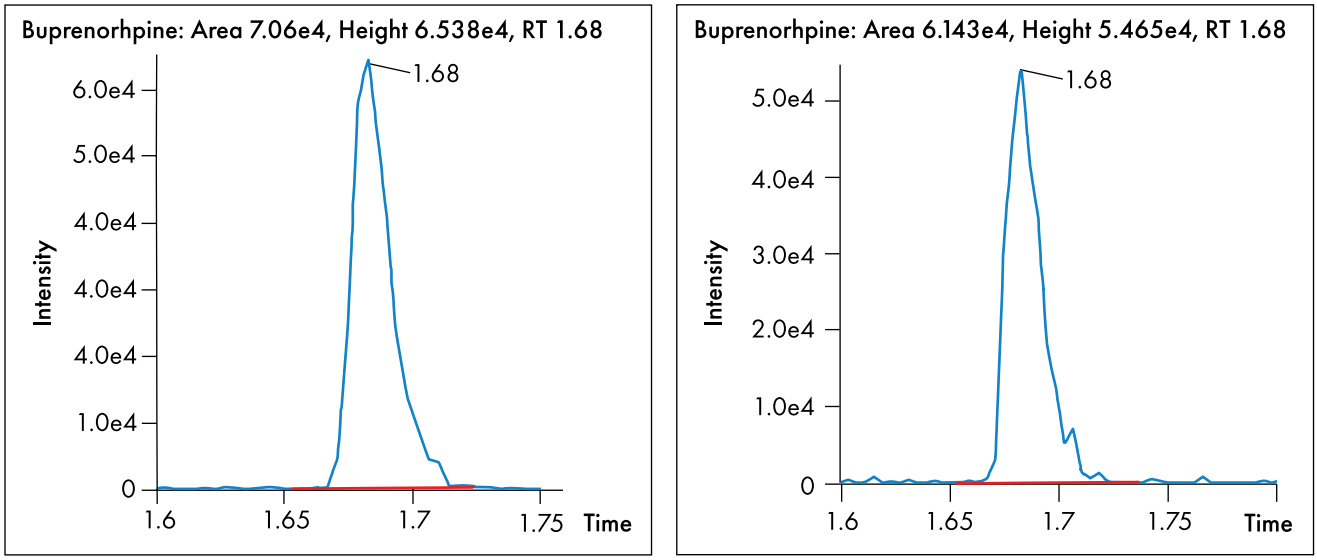


Figure 6. Buprenorphine quantities and qualifier chromatograms at LOQ of 0.8 ng/mL (468.3/396.2 left ; 468.3/414.3 right).

CONCLUSIONS

The SCX XTR method described herein requires more complex solvent preparation than the WAX method alternative, but it provides a greater reduction in matrix effects. When very high concentration factors are required, minimal matrix effects are pivotal in meeting the necessary cut-offs. This is often the case when laboratories do not have top-of-the-line instrumentation. This SCX 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:**
DPX170178	XTR tips: 5mg SCX (60 µm) in 1 mL Hamilton

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