

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

**HIGHLIGHTS:** 96 samples in < 15 minutes



### INTRODUCTION

Fentanyl and buprenorphine are potent analgesics making them medically 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  $\mu$ L), 100  $\mu$ L of acetate buffer, and 10  $\mu$ L of ISTD are loaded on to the Hamilton Microlab NIMBUS96. Additional microplates are filled with 900  $\mu$ L 0.1N HCl (wash #1), 600  $\mu$ L of methanol

(wash #2), and 300  $\mu$ L 78:20:2 dichloromethane/isopropanol/ammonium hydroxide (DCM/IPA/ $\text{NH}_4\text{OH}$ ) 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  $\mu$ L aliquots of wash solvent #1 are then aspirated and dispensed to remove salts and other common matrix interferences. Two separate 300  $\mu$ L aliquots of wash solvent #2 are then aspirated and dispensed to remove common matrix interferences. Target compounds are eluted by aspirating and dispensing 300  $\mu$ L of DCM/IPA/ $\text{NH}_4\text{OH}$  solution three times. The elution solvent is then solvent evaporated and reconstituted in 100  $\mu$ L 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  $\mu$ m; 50 x 3.0 mm) column with a 5  $\mu$ L injection.

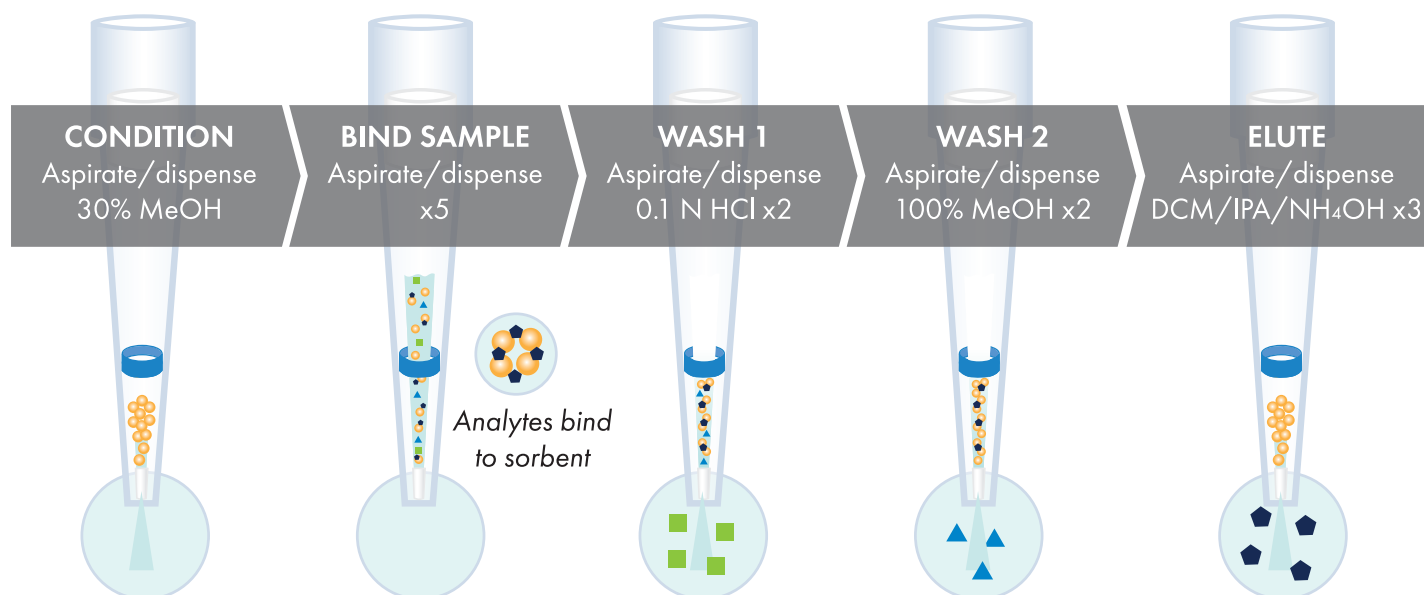


Figure 1. Schematic of INTip solid phase extraction (SPE) method

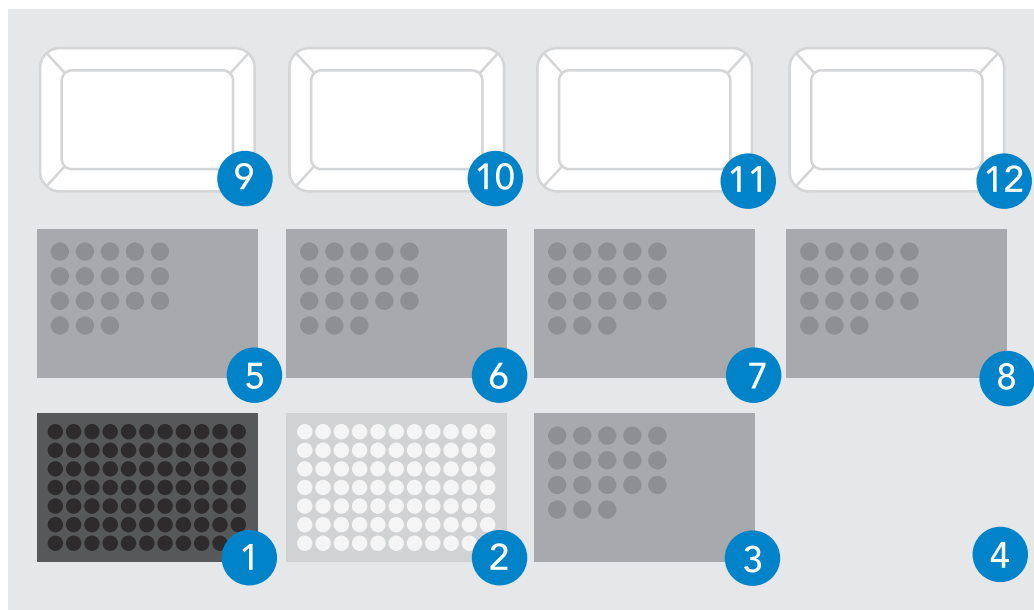


Figure 2. The NIMBUS96 deck configuration includes reservoirs, well plates and pipette tips to perform the fully automated DPX method.

## 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      |

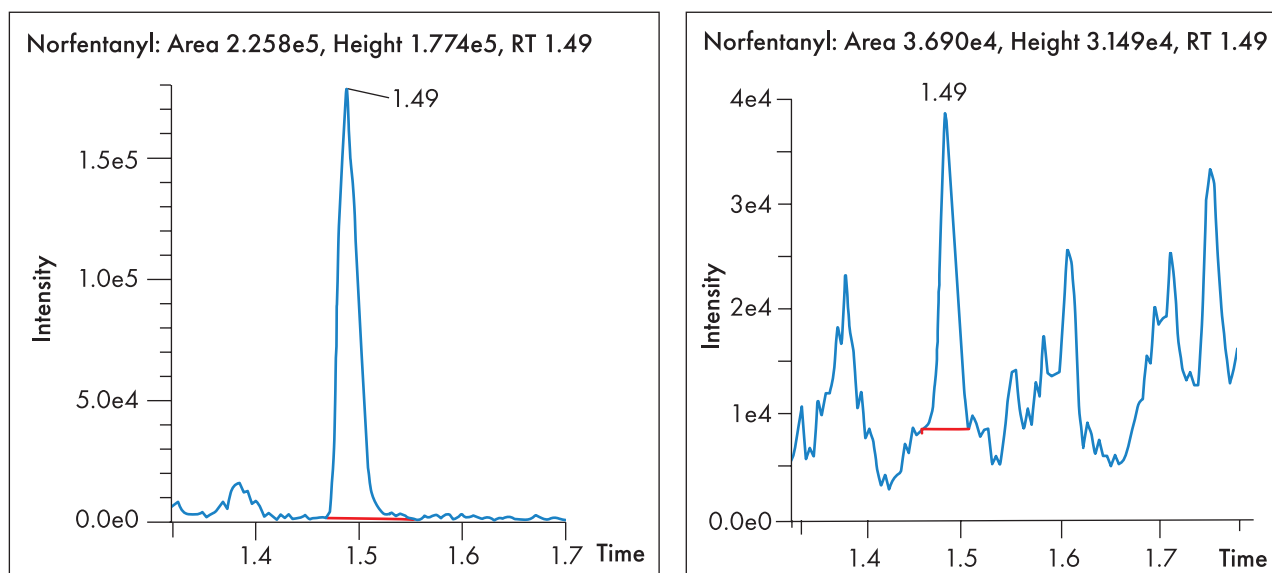


Figure 3. Norfentanyl quantifier and qualifier chromatograms at LOQ of 0.2 ng/mL (233.2/84.1 left ; 233.2/55.1 right).

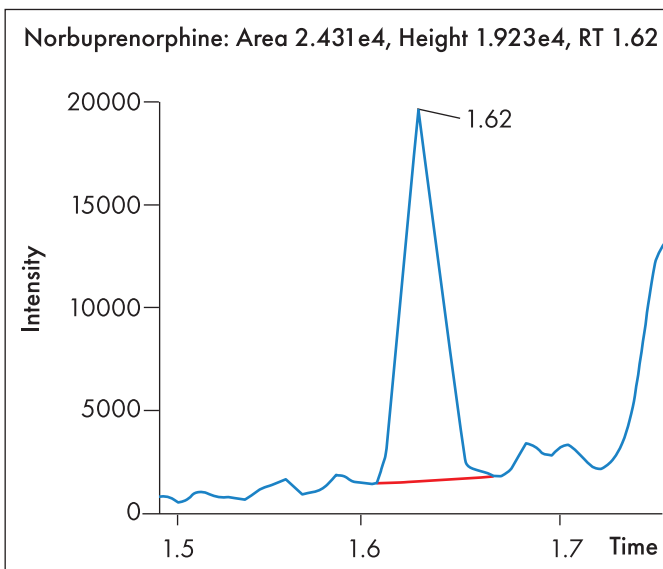
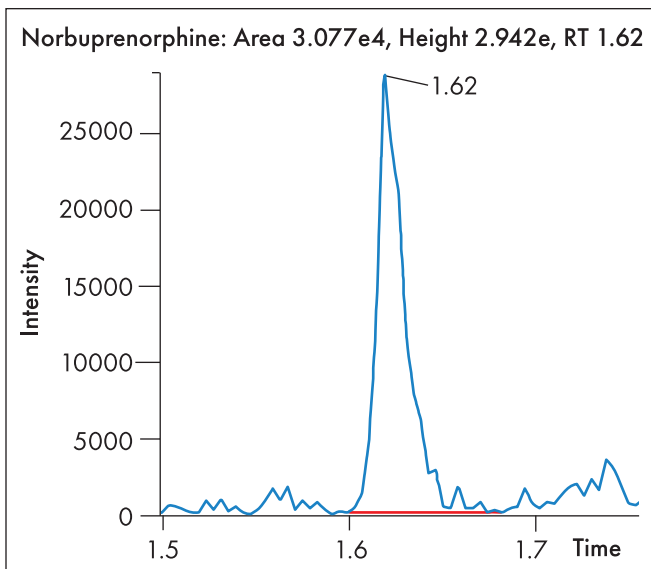


Figure 4. Norbuprenorphine quantifier and qualifier chromatograms at LOQ of 0.8 ng/mL (414.2/152.1 left ; 414.2/115.1 right).

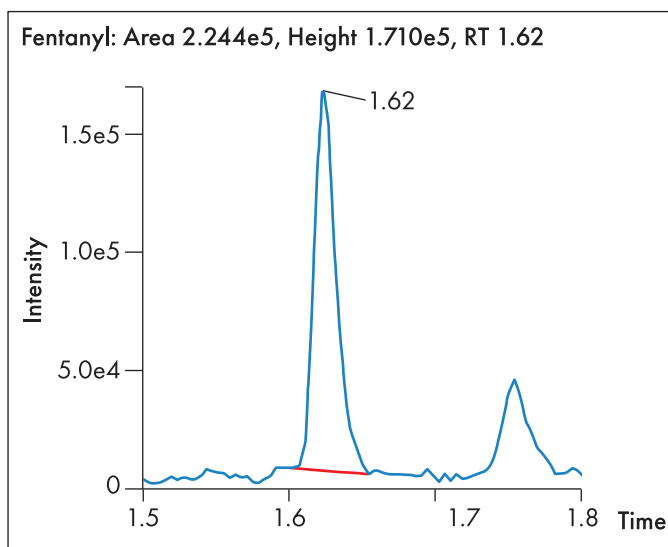
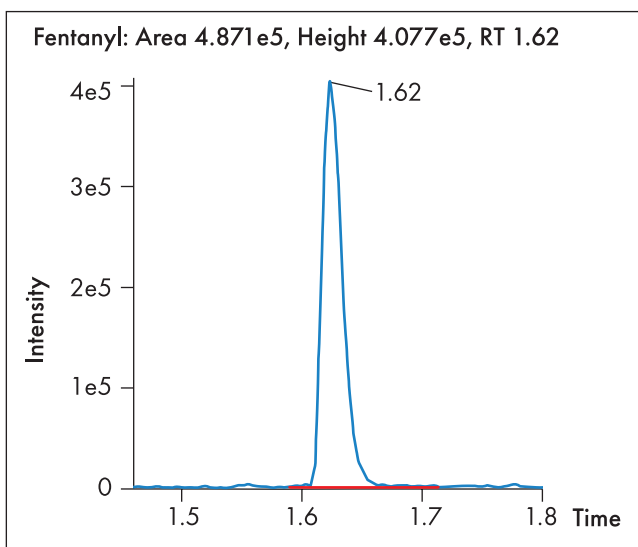


Figure 5. Fentanyl quantifier and qualifier chromatograms at LOQ of 0.2 ng/mL ( 337.1/188.2 left ; 337.1/105.1 right).

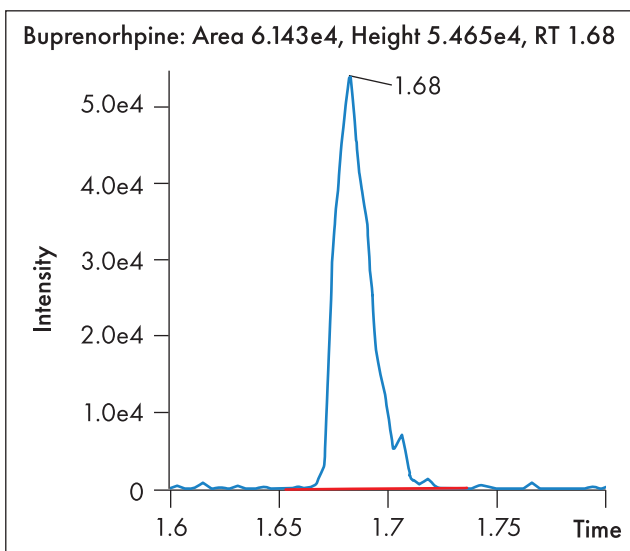
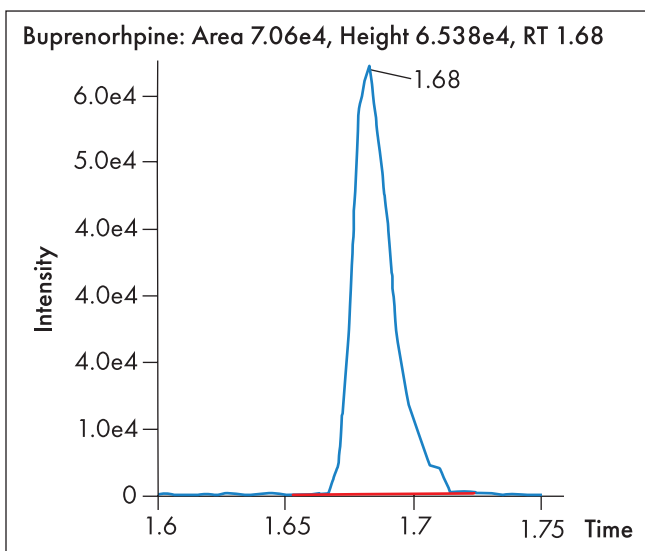


Figure 6. Buprenorphine quantifier and qualifier chromatograms at LOQ of 0.8 ng/mL (468.3/396.2 left ; 468.3/414.3 right).

## 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:**

DPX170178

**Description:**

XTR tips: 5mg SCX (60  $\mu$ m) in 1 mL Hamilton

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

## CUSTOM METHOD DEVELOPMENT

We'll help you validate with  
an INTip method.

✉ [info@dpxlabs.com](mailto:info@dpxlabs.com)  [dpxtechnologies.com](https://dpxtechnologies.com)

