Rapid, High Throughput Analysis of Pain Management Drugs in Urine with IONICS 3Q 220 Triple Quad Mass Spec

Quick Facts:

- High sensitivity method for quantitation of pain management drugs using IONICS 3Q 220 triple quadrupole mass spec
- Analysis of Fentanyl, Norfentanyl, Pentazocine and Meperidine in both water and diluted urine.
- R^2 of over 0.999 for all compounds.
- Quantitation as low as 250 ag/µL for Fentanyl and Norfentanyl with S/N of 6.

1. Introduction

Routine testing of urine or other biological specimens for pain medication can be used to monitor compliance and prevent misuse or abuse. Urine is the matrix of choice as it can be collected easily and in large volumes. However, urine composition is highly variable and dependent on factors such as diet, health and lifestyle. The variations within the urine matrix can adversely impact chromatographic separation and LC-MS/MS signal. The present study demonstrates that a simple "dilute and shoot" method coupled with the high sensitivity 3Q 220 triple quad mass spec system eliminates many of the complexities of sample preparation without compromising quantitation quality.

2. Method

Fentanyl, norfentanyl, pentazocine and meperidine standard stock solutions in liquid form were purchased from Cerilliant Inc. (Round Rock, Texas) and stored at -20°C. In order to test sensitivity, low levels of pure fentanyl and norfentanyl standards were prepared by diluting the high concentration stock with a 50/50 water/methanol in 0.1% formic acid solution. Drug free urine was purchased from UTAK (Valencia, CA). The urine was cleaned up by centrifuge for 15 minutes and the supernatant was filtered using a 0.2µm filter, then diluted by 0.1% formic acid 1000 times and used as the urine matrix. The calibration standards were prepared by making an addition of ten microliters of working solution to the diluted urine to obtain required concentration levels.

2.1 Mass Spectrometry Conditions

The LC-MS/MS analysis was performed using IONICS 3Q 220 triple quadrupole mass spectrometer. **Table 1** outlines the parameter settings used during this method.

Table 1: Settings used the 3Q 220 Instrument

ESI Voltage (V)	5000
HSID Temp (°C)	250
Nebulizer Gas Setting	450
Drying Gas Setting	200
Source Temp (°C)	325
Dwell Time (ms)	100
Pause Time (ms)	5

2.2 LC Conditions

This method utilized a Shimadzu UFLC system. Sample injections of 10µL were loaded onto an Imtakt Cadenza CD-C18HT column (50x2.0mm, 3µm) using the gradient (60% B isocratic for sensitivity test) as shown below in **Table 2** at a flow rate of 0.5mL/min. The composition of the two mobile phases was: Mobile phase A: 5% MeOH, 95% H_2O, 0.1% Formic Acid, 5mM NH_4OAc; Mobile phase B: 95% MeOH, 5% H_2O, 0.1% Formic Acid, 5mM NH_4OAc.

Table 2: Optimized MRM Parameters

Compound Name	Precursor	Fragment	CCL2	CE
Fentanyl	377.3	188.1	-60	32
Norfentanyl	233.3	84.1	-60	26
Pentazocine	286.3	69.1	-60	38
Meperidine	248.3	220.1	-60	28

Table 3: LC Cycle Time

Time (min)	Solvent B %
0.6	5
2.2	95
2.5	95
2.6	5
4.5	5

3. Results

3.1 Extracted Ion Chromatograms (EICs)

Figures 1 and 2 illustrate the results for duplicate injections of fentanyl and norfentanyl in water at blank, 250 ag/µL and 500 ag/µL concentrations.

Figure 1: Fentanyl: blank, 250 and 500 ag/µL in duplicate injections

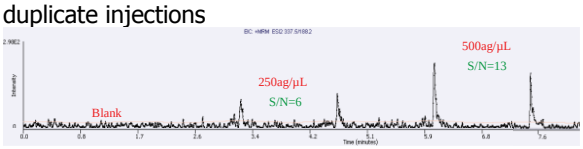
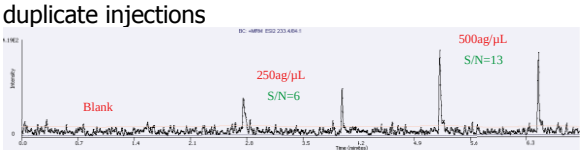


Figure 2: Norfentanyl: blank, 250 and 500 ag/µL in duplicate injections



Figures 3-6 show the results for fentanyl, norfentanyl, pentazocine, and meperdine in diluted urine.

Figure 3: Fentanyl (1 fg/µL, retention time = 2.46 min)

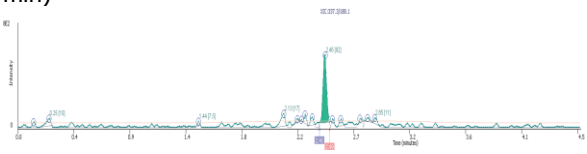


Figure 4: Norfentanyl (1 fg/µL, retention time = 2.24 min)

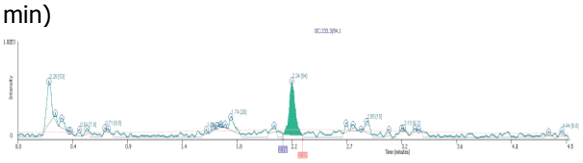


Figure 5: Pentazocine (4 fg/µL, retention time = 2.39 min)

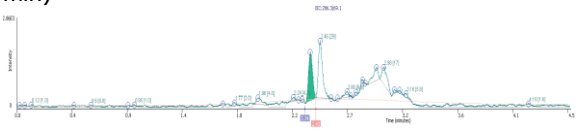
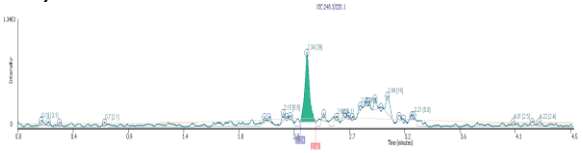
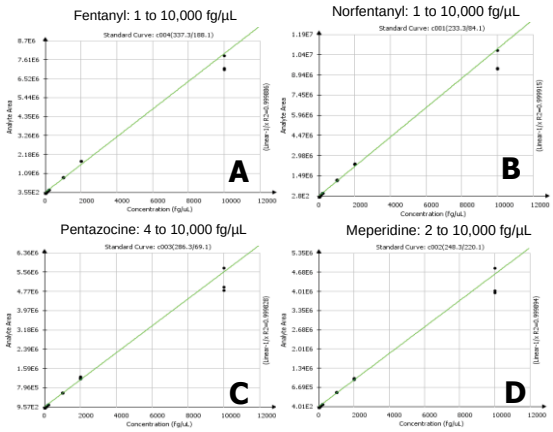


Figure 6: Meperidine (2 fg/µL, retention time = 2.34 min)



3.2 Linearity

Figure 7: Calibration Curves for **A)** Fentanyl, **B)** Norfentanyl, **C)** Pentazocine **D)** Meperidine



The calibration curves generated for fentanyl (377.3/188.1), norfentanyl (233.3/84.1), pentazocine (286.3/69.1) and meperidine(248.3/220.1) with triplet injections using 1/x weighting showed good linearity (R^2>0.999) for up to 4 orders of magnitude. The average accuracy and CV% at the LLOQ was 99.1% and 15.5% for Fentanyl, 94.5% and 13.0% for Fentanyl, 104.4% and 18.2% for pentazocine, 106.7% and 11.6% for meperidine, respectively. The matrix is minimized by diluting the urine (a thousand times in present study) and an excellent specificity is maintained through the LLOQs.

4. Conclusion

Detection limits in the attogram level were obtained for fentanyl and norfentanyl.

A fast, sensitive, and accurate LC-MS/MS method based on "dilute and shoot" methodology for fentanyl, norfentanyl, pentazocine and meperidine using IONICS 3Q 220 triple quadrupole mass spectrometer was developed and demonstrated.

The LLOQs achieved for the measured compounds in diluted urine were in the low femtogram per microliter levels utilizing a 10µL injection. Excellent linearity of four orders of magnitude was achieved with high levels of precision and accuracy for these compounds. This method requires little sample preparation and is well-suited for the routine quantitation of pain management drugs in urine.

5. Contact Information

To learn more about IONICS Mass Spectrometry, our products or services please visit our website or contact us directly.