APPLICATION NOTE

1599-9834

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Rapid, High Throughput Analysis of Compounds in Urine with PerkinElmer QSight® 220 Triple Quad Mass Spec

Introduction

Urine is the matrix of choice as it can be collected easily and in large volumes. 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 of PerkinElmer's QSight® 220 triple quadrupole mass spectrometer system, eliminates many of the complexities of sample preparation without compromising quantitation quality.

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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 the QSight 220 triple quadrupole mass spectrometer. Table 1 outlines the parameter settings used during this method.

Table 1: Settings used the QSight 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

Analysis was conducted using HPLC separation. 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.

Quick Facts:

- High sensitivity method for quantitation of pain management drugs using PerkinElmer QSight 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.

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 duplicateminjections 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

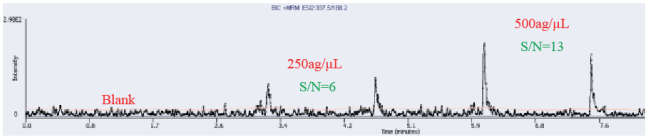
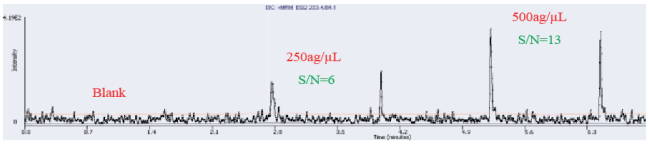


Figure 2: Norfentanyl: blank, 250 and 500 ag/µL in duplicate injections



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)

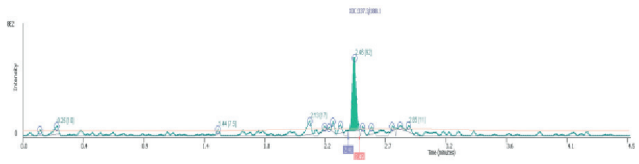


Figure 4: Norfentanyl (1 fg/µL, retention time = 2.24 min)

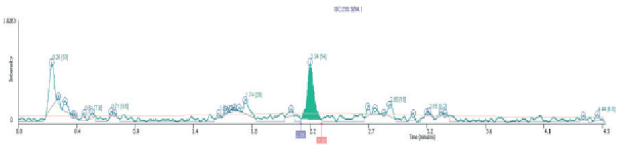


Figure 5: Pentazocine (4 fg/µL, retention time = 2.39 min)

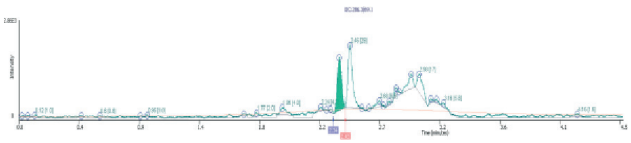
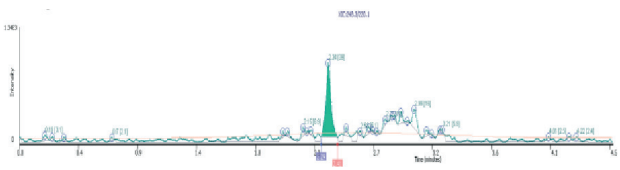
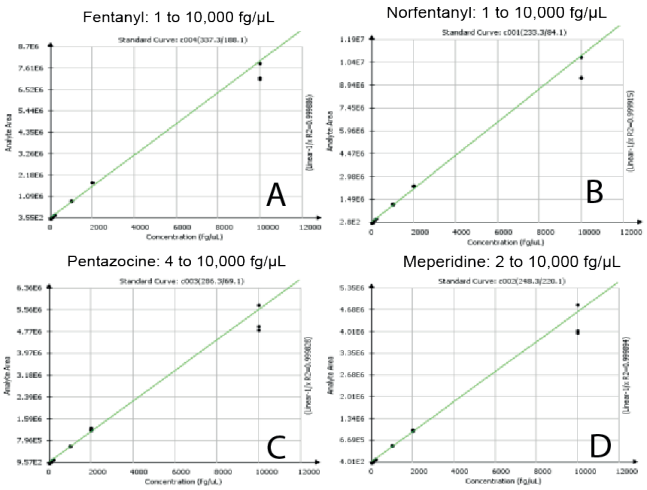


Figure 6: Meperidine (2 fg/µL, retention time = 2.34 min)



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 the QSight 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.

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