"Dilute and Shoot" Method for Determination of Synthetic Opioids in Urine using QSight triple quadrupole LC-MS/MS .

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1 Introduction

Internationally the opioid crisis has been gaining a lot of attention representing an ever-increasing threat to public health. Despite the levels in the US, also in Norway, increased prescription of potent opioids has triggered concern. In addition, there has been a considerable amount of seizing of illegal drugs recently by the Norwegian authorities.

It is therefore anticipated that the demand for rapid and robust diagnostics for synthetic opioids in body fluids will increase. Fast and efficient screening is important in order to be able to intervene in a potential overdose situation, but it is also important to measure correctly and quantitate from a legal point of view.

To address these challenges, we have thus developed robust and stable quantitative methods on the QSight LC-MS/MS system in order to support health providers and law enforcement to tackle the opioid crisis.

2 Experimental Conditions

Sample Preparation
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.
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.

Liquid Chromatography Conditions
Analysis was conducted using UHPLC separation with a 10µL injection on a C18 column (50x2.0mm, 3µm) at a flow rate of 0.5mL/min.

The composition of the two mobile phases was:
Mobile phase A: 5% MeOH, 95% H2O, 0.1% Formic Acid, 5mM NH4OAc;
Mobile phase B:95% MeOH, 5% H2O, 0.1% Formic Acid, 5mM NH4OAc.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.00	95	5
0.6	95	5
2.2	5	95
2.5	5	95
2.6	95	5
4.5	95	5

Table 1: LC Gradient Program

Mass Specrometry settings
The LC-MS/MS analysis was performed using the QSight® 220 triple quadrupole mass spectrometer (Figure 1). All instrument control, data acquisition and data processing were performed using Simplicity™ 3Q Software.

MS Conditions	
ESI Voltage	5000 V
Drying Gas setting	200
Nebulizer Gas	450
Source Temperature	325 °C
HSID Temperature	250 °C
Detection mode	Time managed MRM
Dwell Time	100
Pause Time	5 ms

Table 2: Settings used for the QSight 220 MS Instrument

Compound	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	284.3	220.1	-60	28

Table 3: Optimized MRM Parameters

Figure 1: PerkinElmer QSight ® LC-MS/MS system

3 Results

The LLOQs achieved for the measured compounds in diluted urine were in the low fg/ µL utilizing a 10 µL injection.

The matrix was minimized by diluting the urine (a thousand times in present study) and an excellent specificity is maintained throughout the LLOQs.

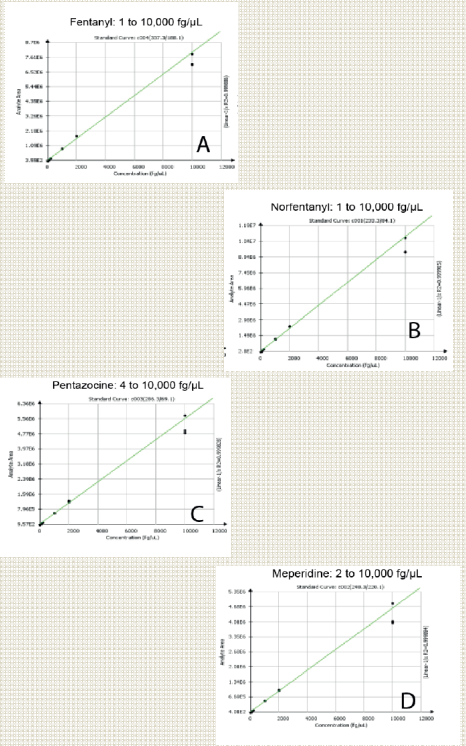

Figure 2: Calibration Curves for A) Fentanyl, B) Norfentanyl, C) Pentazocine D) Meperidine showing R2 > 0.999 and ranges low as 1 fg/ µL

Excellent linearity of four orders of magnitude was achieved with high levels of precision and accuracy for these compounds.
The calibration curves generated for fentanyl, norfentanyl, pentazocine and meperidine with triplet injections using 1/x weighting showed good linearity (R2>0.999).

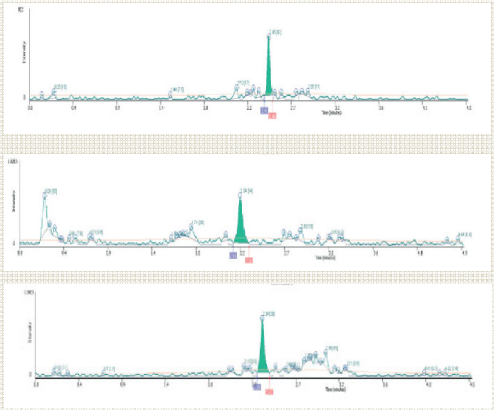

Figure 3: Shows chromatograms for the results of fentanyl and norfentanyl in diluted urine. On the top is fentanyl at 1 fg/µL and a retention time of 2.46 min). In the middle is norfentanyl at 1 fg/µL and a retention time of 2.24 min. On the bottom is meperidine 2 fg/µL a retention time of 2.34 min)

4 Conclusions

- Highly sensitivite method for quantitation of pain management drugs using the QSight® 220 LC/MS/MS
- Analysis of Fentanyl, Norfentanyl, Pentazocine and Meperidine in 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
- 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 was developed
- Excellent linearity of four orders of magnitude was achieved with high levels of precision and accuracy for these compounds.