Simple and Rapid Method Development for High Throughput Analysis of Pain Management Drugs in Urine by LC-MS/MS

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Introduction

LC-MS/MS has emerged as a reliable method to perform both screening and confirmation of the presence of drugs (of abuse or for pain management). Routine testing of urine or other biological specimens for prescribed pain medication is critical to monitor compliance and prevent misuse or abuse. Urine is used for analysis because it can be collected easily. However, patient urine composition is highly variable and dependent their diet, health and lifestyle. These variations in the urine matrix can have an adverse impact on the chromatographic separation and LC-MS/MS signal. The present study demonstrates that a simple dilute and shoot method coupled with a highly sensitive LCMS system is feasible to eliminate a typical sample clean-up step without compromising quantitation quality.

Method

Sample and Preparation: The Fentanyl, Norfentanyl, Pentazocine and Meperidine standard stock solutions in liquid form were purchased from Cerilliant Inc (Round Rock, Texas) and stored at -20°C. For sensitivity test, the low level of pure Fentanyl and Norfentanyl standards were prepared by diluting the high concentration stock with 50/50 water/methanol in 0.1% formic acid. The drug free urine was purchased from UTAK (Valencia, CA). The urine was cleaned up by centrifuge for 15 minutes and the supernatant was filtered by 0.2µm filter, then it is diluted by 0.1% formic acid 1000 times and used as 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.

LC-MS/MS Conditions: The LC-MS/MS was performed using IONICS' 3Q 200 Series,a triple quadrupole mass spectrometer (Bolton, ON Canada) with a Shimadzu UFLC system. Sample injections of 10µL were loaded on a Imtakt Cadenza CD-C18HT column (50x2.0mm, 3µm) using the gradient (60% B isocratic for sensitivity test) as shown below at a flow rate of 0.5mL/min. 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. The mass spec ion source settings are: Electrospray Voltage = 5000V, HSID=250°C, Nebulizer Gas=450, Drying Gas=200, Heating gas=350, Source temperature =325°C. And the optimized MRM parameters for all analytes are shown in the following table.

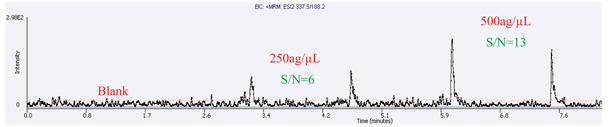
Time(min)	Solvent B%
0.6	5
2.2	95
2.5	95
2.6	5
4.5	5

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

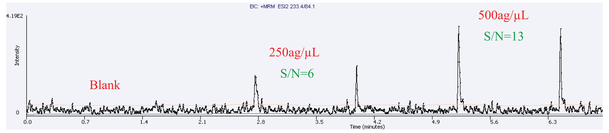
Results

LC-MRM Chromatograms of Fentanyl and Norfentanyl in Water

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

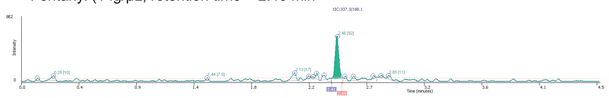


Norfentanyl: blank, 250 and 500ag/µL in duplicate injections

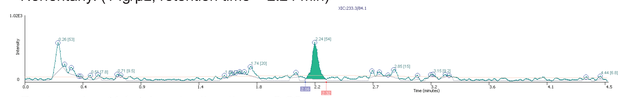


Extracted Ion Chromatograms of Four Mixed Compounds in Diluted Urine

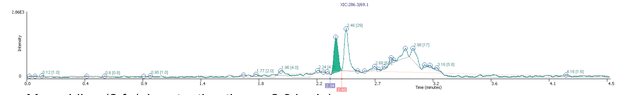
Fentanyl (1 fg/µL, retention time = 2.46 min



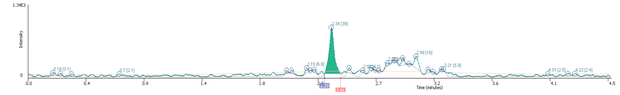
Norfentanyl (1 fg/µL, retention time = 2.24 min)



Pentazocine (4 fg/µL, retention time = 2.39 min

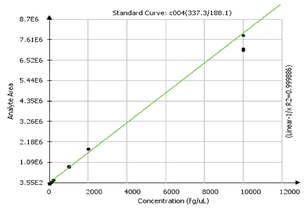


Meperidine (2 fg/µL, retention time = 2.34 min)

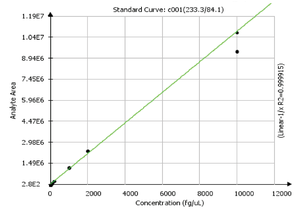


Calibration Curves

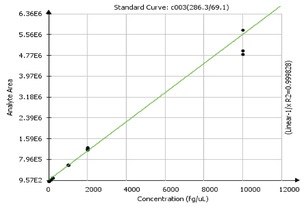
Fentanyl: 1 to 10,000 fg/µL



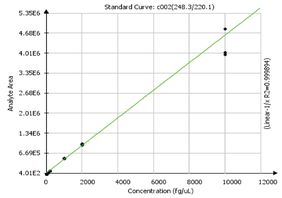
Norfentanyl: 1 to 10,000 fg/µL



Pentazocine: 4 to 10,000 fg/µL



Meperidine: 2 to 10,000 fg/µL



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 (R2 > 0.999) up to 4 orders of magnitude in concentrations ranging from low femtograms per microliter to 10,000 fg/µL. The average accuracy and CV% at LLOQ are 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.

Conclusion

A detection limit in the attogram level is found for Fentanyl and Norfentanyl with IONICS 3Q 200 series Molecular Analyzer triple quadrupole mass spectrometer.

A fast, sensitive, and accurate LC-MS/MS method based on "dilute and shoot" methodology for Fentanyl, Norfentanyl, Pentazocine and Meperidine using IONICS 3Q 200 series Molecular Analyzer was developed. The LLOQs achieved for these compounds in diluted urine were at low femtogram per microliter levels with 10µL injection. Excellent linearity over four orders of magnitude in concentration is also obtained with good precision and accuracy for these compounds . This method requires little sample preparation and is well-suited for routine drug of abuse quantitation.