# Development of a high-throughput LC-MS/MS assay for pain management panel from urine

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## INTRODUCTION

Opiates (vicodan, Percocet, oxycontin etc.) are some of the most frequently prescribed medications for chronic pain and prescriptions have increased more than four fold in the last decade.[1] Statistics show that patients requesting treatment for chronic non-cancer pain management are increasing at greater than 10% a year. Treating chronic pain is a complex and long term prospect. The widespread use of opiates and the potential for abuse, misuse, diversion and augmentation have increased the need and in some cases the requirement to screen patients on a routine basis. Urine drug testing is the method of choice and commonly used to inform the clinicians and provide more confidence to both the physician and the patient that the patient is following the prescribed medication regimen.[2] Pain panels continue to grow in complexity as more prescription and non-prescription compounds are added. There is a significant increase in the number and availability of drug analogues which has in turn, made the job of toxicological analysis ever more challenging. The demand for more accurate, faster, less expensive, and reliable methods for analyzing the presence and concentration of targeted and non targeted pain management drugs has increased.

The recent adoption of LC-MS/MS technology for pain management and therapeutic drug monitoring has been successful due to its high sensitivity, excellent selectivity, and low detection limits. In the work presented here, a LC-MS/MS method has been created for the analysis of a pain management panel comprising 12 analytes, on an IONICS 3Q 120 Triple Quadrupole Mass Spectrometer, combined with Biotage™ supported liquid extraction (SLE) platform for urine sample cleanup. This LC-MS/MS method provides a faster, more accurate and reproducible solution for the analysis of pain management drugs.

## METHOD

### Materials and sample preparation

Drug standards were purchased from Cerilliant Corporation. β-Glucuronidase was purchased from Sigma-Aldrich. Fresh urine was obtained from healthy male volunteer. Ammonium Acetate and Formic Acid were purchased from Sigma-Aldrich, and HPLC Grade solvents, Water and Methanol were purchased from Caledon Labs.

A urine matrix with internal standards was first spiked at 20 to1000 ng/mL for the drugs listed in Table 2. Hydrolyze of the matrix followed to convert the glucuronide metabolites to native form using β-Glucuronidase.[3,4] This was done by adding 950µL of ammonium acetate (100mM) pH=5 and 25µL β-Glucuronidase (5000 units) to 1mL urine sample. Samples were then incubated for 2 hours at 60°C. Aqueous ammonium hydroxide (2%) was then added to the incubated samples with 1:1 (v/v) ratio (the final pH is ~9). After the sample pretreatment step, 200µL of the pretreated solution was loaded onto a Biotage™ SLE plate for cleanup. With use of the Biotage™ pressure manifold to facilitate the flow of the sample into the SLE plate, the samples were finally eluted off the plates with ethyl acetate (1mL) and were dried using Biotage™ TurboVap vacuum workstation. The sample were then reconstituted in 200µL of the mobile phase (0.1% formic acid in water).

### Instruments

An IONICS 3Q 120 Mass Spectrometer system was used for all drug analysis. This instrument is equipped with heated coaxial flow ion source and hot “source-induced desolvation” interface, with a mult-iorthogonal channel and laminar flow sampling.

The urine spiked matrix samples were analyzed using Shimadzu Prominence LC system utilizing the gradient elution shown in Table 1 and the following conditions:

| | |
|---|---|
| Column: | Restek 5 µm, Ultra II Biphenyl Column, 50x2.1mm |
| Mobile Phase: | A (0.1% formic acid 100% H2O) |
| | B (0.1% formic acid in 100% MeOH) |
| Flow Rate: | 0.6 mL/min |
| Injection Volume: | 10 µL |
| Column Temperature: | 40 $^{0}$C |

| Time (min) | Solvent B % |
|---|---|
| 0 | 0 |
| 1 | 60 |
| 1.1 | 90 |
| 3 | 90 |
| 3.1 | 0 |
| 5 | 0 |

Table1: LC gradient, 5min Total Runtime

## RESULTS

### Separation of pain panel drugs

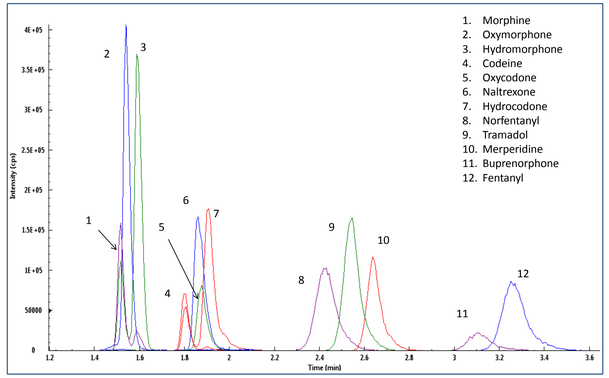



Fig.1. Overlaid MRM extracted ion chromatograms from 12 pain panel drugs in a 5-minute LC run.

### Quantitation Summary of pain panel drugs in spiked urine

Table 2. Summary of LLOQ, precision, and accuracy of pain panel drugs

| Analyte | Q1 | Q3 | LLOQ (ng/mL) | %CV | % accuracy | Linear range (ng/mL) |
|---|---|---|---|---|---|---|
| Morphine | 286.1 | 165 | 0.448 | <8 | 93-103 | 0.488-1000 |
| Oxymorphone | 302.1 | 227.1 | 0.122 | <4.3 | 90-110 | 0.122-500 |
| Hydromorphone | 286.1 | 185 | 0.122 | <8.7 | 92-109 | 0.122-500 |
| Codeine | 300.1 | 215.1 | 0.098 | <7.9 | 93-103 | 0.098-200 |
| Oxycodone | 316.1 | 241 | 0.048 | <7.9 | 90-101 | 0.048-100 |
| Naltrexone | 342.1 | 269.9 | 0.098 | <7.8 | 87-103 | 0.098-100 |
| Hydrocodone | 300.1 | 171 | 0.098 | <8.7 | 90-105 | 0.098-100 |
| Norfentanyl | 233.2 | 84.1 | 0.039 | <3.6 | 95-105 | 0.039-40 |
| Tramadol | 264.1 | 58.2 | 0.019 | <10 | 90-108 | 0.039-40 |
| Merperidine | 248.2 | 174.1 | 0.039 | <7 | 95-106 | 0.039-20 |
| Buprenorphone | 468.3 | 414.1 | 0.781 | <3.7 | 90-110 | 0.781-200 |
| Fentanyl | 337.1 | 188.2 | 0.039 | <5.8 | 90-110 | 0.039-20 |

### Calibration Curves

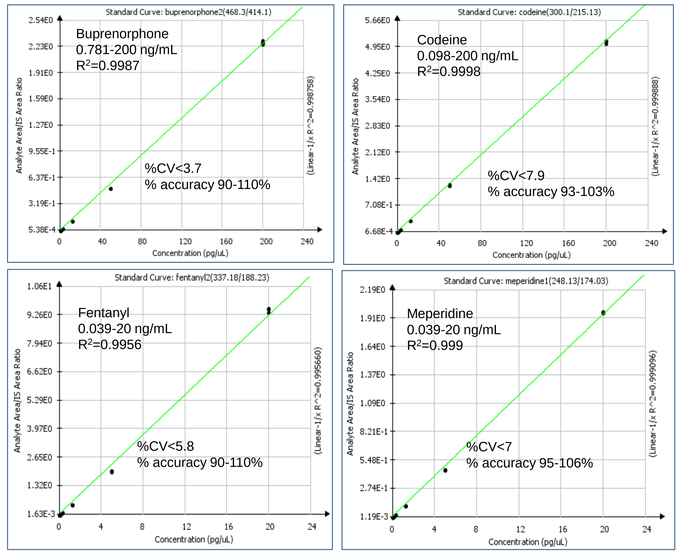



Fig.2. Representative calibration curves for pain panel drugs. Good linearity is obtained for all the analytes across the whole concentration range ($R^2$>0.99) with high accuracy, precision and reproducibility

## CONCLUSION

The results in this study show that in a 5-minute LC run, this LC-MS/MS method can effectively separate the 12 pain panel drugs. The quantitation results also indicate that this method is accurate, precise, and reproducible. The LLOQs for all the 12 drugs is in the range of 0.019 to 0.781 ng/mL, which is 3 to 4 orders lower than the typical screening cutoff concentration(300 ng/mL) and typical confirmation cutoff concentration (50 ng/mL) for drugs of abuse[2]. Therefore, the LC-MS/MS method outline above confirms that the IONICS 3Q 120 mass spectrometer is an effective combination for clinical pain management to monitor patient drug use and program adherence or for drugs of abuse or other workplace drug testing.

**References:**

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2. P. Christo, L. Machikanti, X. Ruan, et. al. Urine Drug Testing In Chronic Pain. Pain Physician, 2011; 14:124-143.
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