Opoids (codeine, Percocet, oxycodone 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] Statistic show that patients requesting treatment for chronic pain management are increasing at greater than 3.6% year over year with chronic pain and long term prospect. The widespread use of opiates and the potential for abuse, misuse, diversion and augmentation have increased and need in the recent requirement to screen patients on a routine basis. Techniques to change the method of choice and commonly used to inform the clinicians and provide more confidence to both the physician and the patient about the effectiveness of the prescribed medication regimen.[2] Pain panel continue to this expanding 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 panel comprising 12 analytes, on an IONICS 3Q 120 Triple Quadrupole Mass Spectrometer, combined with Biotaґre 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 Cerfiant Corporation, 3-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 Lab.

A urine matrix with internal standards was first spiked at 20 ng/1000 μL, for the drugs listed in Table 2. Hydrolysis of the matrix followed to convert the glucuronide metabolites to native form using 3-Glucuronidase(1.4). This was done by adding 100 μL of 100X ammnonium acetate (100mM HAc, pH 3.7) and 3-Glucuronidase 500 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 1/10(v/v) ratio (the final pH was 5.5). After sample precleanup step, 200 μL of the preservatives was loaded onto a Biotaґre SLE plate for cleanup. With use of the Biotaґre’s pressure manifold to facilitate the flow of the sample into the SPE plate, the samples were finally dried on the plate with ethyl acetate (1mL), and were dried using Biotaґre® TurboVac vacuum workstation. The sample were then reconstituted in 20μL of the mobile phase (0.1% formic acid in water).

Instrumentation

An IONICS 3Q 120 Mass Spectrometer system was used for all drug analysis. This instrument is equipped with heated capillary ion source and hot “source-induced desorption” interface, with a multi-orifical channel and tandem 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:

<table>
<thead>
<tr>
<th>Column</th>
<th>Resolved 5 μm, Ulta B 150mm Column, 5μLx1mm</th>
<th>A.O</th>
<th>0.1% formic acid in 100% H2O</th>
<th>B</th>
<th>0.1% formic acid in 100% MeOH</th>
<th>Flow Rate</th>
<th>0.6 mL-min</th>
<th>Injection Volume</th>
<th>10μL</th>
<th>Column Temperature</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time [min]</td>
<td>Sorbent % B</td>
<td>1</td>
<td>60</td>
<td>1.3</td>
<td>90</td>
<td>3.9</td>
<td>0</td>
<td>5.1</td>
<td>0</td>
<td>Table 1: LC gradient, 5min Total Runtime</td>
<td>5</td>
</tr>
</tbody>
</table>

**RESULTS**

Separation of pain panel drugs

- Morphine: 3.095-26.92 ng/mL, %CV<5.8
- Oxymorphone: 0.039-28.89 ng/mL, %CV<8
- Hydrocodone: 0.039-28.89 ng/mL, %CV<8
- Meperidine: 0.039-28.89 ng/mL, %CV<8
- Fentanyl: 0.039-28.89 ng/mL, %CV<8

- Morphine: 0.00-2.000 ng/mL, R<0.05
- Oxymorphone: 0.00-2.000 ng/mL, R<0.05
- Hydrocodone: 0.00-2.000 ng/mL, R<0.05
- Meperidine: 0.00-2.000 ng/mL, R<0.05
- Fentanyl: 0.00-2.000 ng/mL, R<0.05

**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 drugs is in the range of 0.015 to 3.781 ng/mL, which is 3 to 4 times 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 spectroscopy is an effective and precise lower than the clinical pain management patient drug use and program adherence or for drugs of abuse or other workplace drug testing.

**Acknowledgements**