A High-throughput Pain Management Panel in Urine Using IONICS 3Q 120 LC-MS/MS and an SLE Sample Prep Platform

Quick Facts:

- Method for high sensitivity quantitation of 12 pain panel drugs using IONICS 3Q 120 triple quadrupole mass spectrometer.
- LLOQ achieved ranged from 0.012-0.78 ng/mL with CV % ≤10.
- Calibration Curve R^2>0.99.

1. 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] 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. 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. 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 a 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.

2. Method

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 was then reconstituted in 200µL of the mobile phase (0.1% formic acid in water).

2.1 Mass Spectrometry Conditions

IONICS 3Q 120 equipped with with heated coaxial flow ion source and hot "source-induced desolvation" interface, with a mult-iorthogonal channel and laminar flow sampling.

The Q1 and Q2 mass filters were set to unit resolution. **Table 1** lists the transition for the 12 pain panel drugs.

Table 1. 12 pain panel drugs and MRM transitions

Analyte	MRM	Analyte	MRM
Morphine	286.1/186.0	Hydrocodone	300.1/171.0
Oxymorphone	302.1/227.1	Norfentanyl	233.2/84.1
Hydromorphone	286.1/185.0	Tramadol	264.1/58.2
Codeine	300.1/215.1	Merperidine	248.2/174.1
Oxycodone	316.1/241.0	Buprenorphone	468.3/414.1
Naltrexone	342.1/269.9	Fentanyl	337.1/188.2

2.2 LC Conditions

The separation was performed on a Shimadzu Prominence LC system which includes two pumps, autosampler, degasser, column oven. A 10 µL sample was loaded on a Restek Ultra II Biphenyl Column (50 x 2.0 mm, 5µ) at 40 °C. The flow rate used is 600 µL/min with a total LC cycle time of 5 min. Solvent A was composed of 0.1% formic acid in 100% H_2O. Solvent B was composed of 0.1% formic acid in 100% MeOH. A LC gradient time program was used as shown in following **Table 2**.

Table 2. LC Gradient Conditions for a total LC-MS/MS run time of 5 minutes

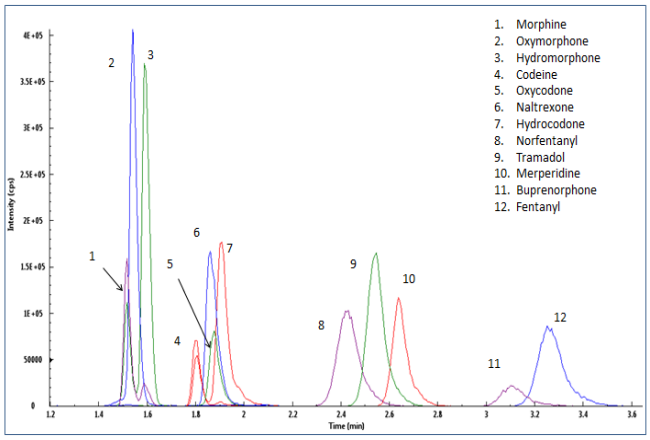
Time (min)	Solvent B composition (%)
0.0	0
1.0	60
1.1	90
3.0	90
3.1	0
5.0	0

3. Results

3.1 Extracted Ion Chromatogram (EIC)

A LC-MS/MS method was created to simultaneously monitor all 12 MRM transitions for the 12 pain panel drugs. **Figure 1** was shows an overlaid EIC for all 12 drugs.

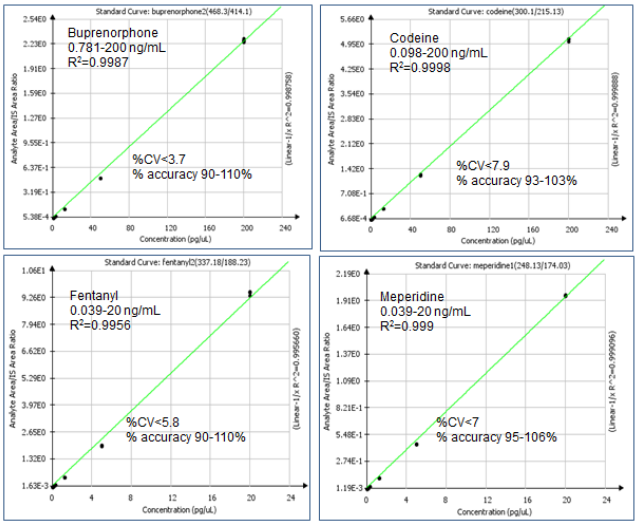
Figure 1. Overlaid MRM extracted ion chromatograms from 12 pain panel drugs in a 5-minute LC run



3.2 Linearity

Good linearity is obtained for all the analytes across the whole concentration range (given in **Table 2**) (R^2>0.99) with high accuracy, precision and reproducibility. All calibration curves use a linear regression of 1/x weighting. **Figure 2** shows 4 representative calibration curves for Buprenorphone, Codeine, Fentanyl and Meperidine.

Figure 2. Four representative calibration curves for pain panel drug analysis



3.3 Quantitation Results

All 12 of the pain panel drugs showed excellent CV and accuracy percentage across the concentration range shown in **Table 3.** All CV`s were ≤10%, and accuracy was between 87-110%.

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

Analyte	LLOQ (ng/mL)	%CV	% accuracy	Linear range (ng/mL)
Morphine	0.448	<8	93-103	0.488-1000
Oxymorphone	0.122	<4.3	90-110	0.122-500
Hydromorphone	0.122	<8.7	92-109	0.122-500
Codeine	0.098	<7.9	93-103	0.098-200
Oxycodone	0.048	<7.9	90-101	0.048-100
Naltrexone	0.098	<7.8	87-103	0.098-100
Hydrocodone	0.098	<8.7	90-105	0.098-100
Norfentanyl	0.039	<3.6	95-105	0.039-40
Tramadol	0.019	<10	90-108	0.039-40
Merperidine	0.039	<7	95-106	0.039-20
Buprenorphone	0.781	<3.7	90-110	0.781-200
Fentanyl	0.039	<5.8	90-110	0.039-20

4. 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 is an effective quantitative solution for clinical pain management monitoring of patient drug use and program adherence, or for the quantitation of drugs of abuse for workplace, legal or other testing.

5. Contact Information

To learn more about IONICS Mass Spectrometry, our products or services please visit our website or contact us directly.

References

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