Development of a High-Throughput LC-MS/MS Assay for a Pain Management Panel from Urine

Quick Facts:

- Fast LC-MS/MS method developed for 30 pain panel drugs covering opiates, sedatives, and stimulants using IONICS 3Q 120 mass spectrometer.
- Time-managed MRM with optimized dwell time allows more MRM transitions to be monitored, improving the reliability and accuracy of quantitation.
- Fast polarity switching (20 ms) enables simultaneous acquisition of both positive and negative analytes.
- LLOQs achieved range from 0.032 – 2 ng/mL for different drugs with CV ≤11%. Good linearity with R^2>0.99.

1. Introduction

The widespread use and the potential abuse of opiates, sedatives, and stimulant drugs have increased the need and in some cases the requirement to screen patients on a routine basis [1,2]. Pain panels have continued to grow in complexity as more prescription and non-prescription compounds are added. This has made the job of toxicological analysis even more challenging. To fulfill these requirements, a fast, reliable, and accurate LC-MS/MS method has been developed for the analysis of a pain panel comprised of 30 drugs, on an IONICS 3Q 120 triple quadrupole mass spectrometer. The fast scanning speed and shorter settle time (20 ms) for polarity switching on this system make it perfectly suited for the rapid analysis of a large number of drugs. Time-managed MRM in Molana™ software allows more MRM transitions to be monitored providing high quality data, and improving the reliability and accuracy of quantitation.

2. Method

Drug standards were purchased from Cerilliant Corporation. Fresh urine was obtained from a 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.

First, several vials each containing 50 µL urine matrix were prepared by adding 25 µL of mixed internal standard solution to each vial. Then the mixed drug standard solution was spiked into the urine matrix, and diluted with mobile phase A (100% H2O, 0.1% formic acid) to a total volume of 500 µL, making a series of calibrator solutions with concentrations over a range of 0.016 to 16 ng/mL. The final internal standard concentration was 10 ng/mL. The calibrator solutions were directly injected for analysis without further sample treatment.

2.1 Mass Spectrometry Conditions

IONICS 3Q 120 mass spectrometer was equipped with a heated coaxial flow ion source and a "Hot Source-Induced Desolvation" (HSID™) interface, which includes a multi-orthogonal channel and laminar flow sampling. The Q1 and Q2 mass filters were set to unit resolution.

The time-managed MRM in Molana™ software was used to optimize the dwell time for each MRM transition based on the retention times and the number of MRM transitions within given experiments. This allows more MRM transitions to be monitored without compromising data quality. **Table 1** lists the transitions for all 30 pain panel drugs (shown on the next page).

2.2 LC Conditions

The separation was performed on a Shimadzu Prominence LC system which includes two pumps, an autosampler, a degasser, and a column oven. A 10 µL sample was loaded onto a Restek Ultra II Biphenyl Column (50 x 2.1 mm, 5µm) kept at 40°C. The flow rate used was 600 µL/min with a total LC cycle time of 7.5 min. Solvent A was composed of 0.1% formic acid in 100% H2O. Solvent B was composed of 0.1% formic acid in 100% MeOH. A LC gradient time program was used as shown in **Table 2**.

Table 2. LC gradient conditions for a total LC-MS/MS run time of 7.5 minutes

Time (min)	Solvent B (%)
0.0	5
4.0	95
5.5	95
5.7	5
7.5	5

Table 1.: List of Pain Panel Drugs Analyzed:

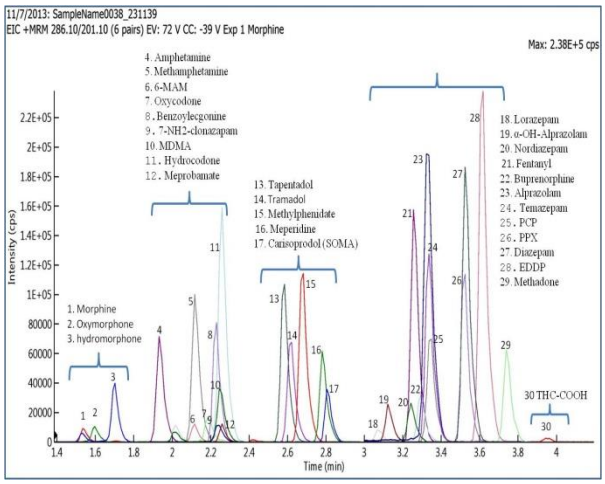
Category	Name	ESI polarity	Q1 (m/z)	Q3 (m/z)	IS	RT (min)
Opiates	Morphine	+	286.2	201.1	*Morphine-d3*	1.54
	Oxymorphone	+	302.2	227.1	*Oxymorphone-d3*	1.59
	Hydromorphone	+	286.2	185.1	*Hydromorphone-d3*	1.7
	Oxycodone	+	316.2	241.1	*Oxycodone-d3*	2.19
	6-MAM	+	328.2	211.1	*6-MAM-d3*	2.12
	Hydrocodone	+	300.2	199.1	*Hydrocodone-d3*	2.25
	Tramadol	+	264.2	58.1	*Tramadol-13C1d3*	2.61
	Tapentadol	+	222.2	107.1	*Tramadol-13C1d3*	2.58
	Meperidine	+	248.2	174.2	*Meperidine-d4*	2.78
	Fentanyl	+	337.2	188.1	*Fentanyl-d5*	3.26
	Buprenorphine	+	468.2	55.1	*Buprenorphine-d4*	3.3
	EDDP	+	278.2	234.1	*EDDP-d3*	3.61
	Methadone	+	310.2	105.1	*Methadone-d3*	3.74
	PPX	+	340.2	58.1	*PPX-d5*	3.52
Sedatives/Hypnotics	7-NH2-clonazapam	+	286.2	121.1	*7-NH2-clonazapam-d4*	2.24
	Lorazepam	+	321.2	229.1	*Lorazepam-d4*	3.08
	α-OH-Alprazolam	+	325.2	297.1	*α-OH-Alprazolam-d5*	3.13
	Nordiazepam	+	271.2	140.1	*Nordiazepam-d5*	3.24
	Alprazolam	+	309.2	281.1	*Alprazolam-d5*	3.33
	Temazepam	+	301.2	255.1	*Alprazolam-d5*	3.34
	Diazepam	+	285.2	193.1	*Diazepam-d5*	3.53
Stimulants	Amphetamine	+	136.2	91.1	*Amphetamine-d5*	1.93
	Methamphetamine	+	150.2	91.1	*Methamphetamine-d5*	2.12
	MDMA	+	194.2	163.1	*MDMA-d5*	2.25
	Benzoylecgonine	+	290.2	168.1	*Benzoylecgonine-d3*	2.23
	Methylphenidate	+	234.2	84.1	*Tramadol-13C1d3*	2.67
Abused/others	PCP	+	244.2	91.1	*PCP-d5*	3.34
	Meprobamate	+	219.2	158.1	*Hydrocodone-d3*	2.26
	Carisoprodol (SOMA)	+	261.2	176.1	*Meperidine-d4*	2.8
	THC-COOH	-	343.2	299.1	*THC-COOH-d3*	3.94

3. Results

3.1 Extracted Ion Chromatogram (EIC)

A LC-MS/MS method was created to simultaneously monitor all 57 MRM transitions for the 30 pain panel drugs with internal standard. **Figure 1** shows an overlaid MRM extracted ion chromatogram for all 31 drugs in a 7.5 minute LC run.

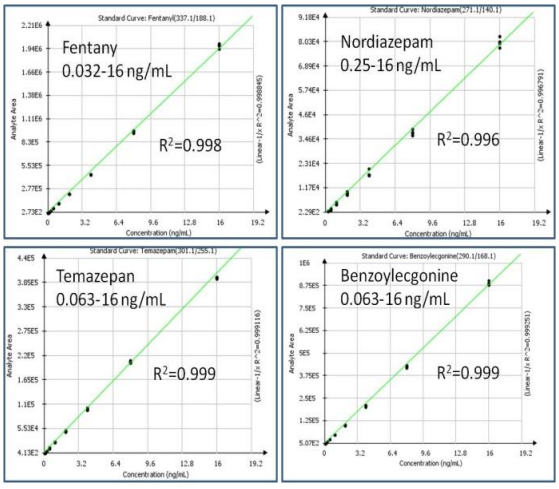
Figure 1. Example of an Overlay of Extracted Chromatograms of 30 Drugs in a 7.5 minute LC Run.



3.2 Linearity

Good linearity was obtained for all analytes across the whole concentration range (given in **Table 2**) (R^2>0.99) with high accuracy, precision and reproducibility. All calibration curves used a linear weighting regression of 1/x. **Figure 2** shows four representative calibration curves for fentanyl, nordiazepam, temzaepan, and benzoylecgonine.

Figure 2. Four Representative Calibration Curves for Pain Panel Drug Analysis



3.3 Quantitation Results

All 30 of the pain panel drugs showed excellent CV and accuracy percentages across the concentration range shown in **Table 3.** All CV's were ≤11%, and accuracy was between 84-114%.

Table 3. Summary of LLOQ, Precision, and Accuracy of Pain Panel Drugs

Analyte	Q1	Q2	LLOQ (ng/mL)	%CV	% accuracy
Morphine	286.1	201.1	0.5	<6.3	90-110
Oxymorphone	302.1	227.1	0.126	<11	85-112
Hydromorphone	286.1	185.1	0.126	<8.7	86-105
Oxycodone	316.1	241.1	0.126	<5.5	95-108
Hydrocodone	300.1	199.1	0.126	<6.3	94-109
Tramadol	264.1	58.1	0.063	<5.1	86-110
Merperidine	248.1	174.1	0.063	<6.5	91-110
Buprenorphone	468.1	55.1	0.25	<10.1	85-107
Fentanyl	337.1	188.1	0.032	<8.5	87-110
PCP	244.1	91.1	0.032	<10.5	84-108
6-MAM	328.1	211.1	0.5	<7	95-109
Amphetamine	136.1	91.1	0.032	<6.3	95-108
Methamphetamine	150.1	91.1	0.032	<9	92-109
MDMA	194.1	163.1	0.032	<3	96-110
Benzoylecgonine	290.1	168.1	0.063	<8	94-110
Meprobamate	219.1	158.1	0.5	<6.6	94-112
7NH2clonazapam	286.1	121.1	0.5	<5.7	97-110
Trapentadol	221.1	107.1	0.126	<7.2	93-114
Methylpenidate	234.1	84.1	0.126	<6.9	92-108
SOMA	261.1	176.1	0.063	<7	97-111
EDDP	278.1	234.1	0.063	<6.8	92-108
Diazepam	285.1	193.1	0.126	<6.5	92-112
Methadone	310.2	105.1	0.063	<7.1	89-109
PPX	340.1	58.1	0.063	<7	96-110
Temazepam	301.1	255.1	0.063	<10	97-111
Nordiazepam	271.1	140.1	0.25	<4	96-109
Lorazepam	321.1	229.1	1.0	<7.1	88-108
Alprazolam	309.1	281.1	2.0	<6.3	86-110
a-OH-Alprazolam	325.1	297.1	2.0	<7	97-112
THC-COOH	343.1	299.1	2.0	<5	93-108

4. Conclusion

The results of this study show that in a 7.5-minute LC run, this LC-MS/MS method can effectively separate the 30 pain panel drugs. The quantitation results also indicate that this method is accurate, precise, and reproducible. The LLOQs for all the 30 drugs are in the range of 0.032 to 2 ng/mL, which is 2 to 3 orders lower than the typical screening cutoff concentration (300 ng/mL), and also much lower than the typical confirmation cutoff concentration (50 ng/mL) for most of the drugs of abuse[1]. Therefore, the LC-MS/MS method outlined above confirms that the IONICS 3Q 120 mass spectrometer is an effective platform for monitoring patient drug use in pain management cases and detection and confirmation of drugs of abuse for program adherence or workplace testing.

5. Contact Information

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

References

1. Substance Abuse and Mental Health Services Administration, Results from the 2011 National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-44, HHS Publication No. (SMA) 12-4713. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2012.
2. P. Christo, L. Machikanti, X. Ruan, et. al. Urine Drug Testing In Chronic Pain. Pain Physician, 2011; 14:124-143.