APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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

Introduction

Opiates (Vicodin®, 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 has been 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, an LC/MS/MS method has been developed for the analysis of a pain management panel comprising 12 analytes, on a QSight™ 220 Triple Quadrupole Mass Spectrometer, combined with a Biotage™ Supported Liquid Extraction (SLE) platform for urine sample cleanup. The LC/MS/MS method described in this study provides a fast, accurate and reproducible solution for the analysis of pain management drugs.

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Experimental

Hardware/Software

The separation was performed on a PerkinElmer Altus® UPLC® system, which includes two pumps, autosampler, degasser and column oven. The UHPLC system was coupled to a PerkinElmer QSight 220 Mass Spectrometer with HSID™ (hot surface induced desolvation) interface, run in ESI mode. A PerkinElmer Brownlee™ Phenyl Hexyl Column (100 x 2.1 mm, 2.7 µm) was used for all analyses (PerkinElmer, Shelton, CT, USA). All instrument control, analysis and data processing was performed using the Simplicity 3Q™ software platform.

Method Parameters

The method parameters are shown in Table 1.

Table 1. LC Method Parameters.

Column	PerkinElmer Brownlee Phenyl Hexyl Column (100 x 2.1 mm, 2.7 µm) (Part# N9308485)
Supported Liquid Extraction (SLE):	Biotage SLE plate
Mobile Phase:	Solvent A: 0.1% formic acid in 100% water Solvent B: 0.1% formic acid in 100% MeOH
Analysis Time	5 min.
Flow Rate	0.6 µL/min.
Oven Temp	40 °C
Flow Rate	10 µ L

LC gradient conditions for a total LC/MS/MS run time of five minutes.

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

For MS/MS parameters, the Q1 and Q2 mass filters were set to unit resolution and Table 2 lists the transitions for the twelve pain panel drugs.

Table 2. 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

Solvents, Standards and Samples

Drug standards were purchased from Cerilliant® Corporation. β-Glucuronidase was purchased from Sigma-Aldrich®. Fresh urine was obtained from a healthy male volunteer. Ammonium acetate and formic acid were purchased from Sigma-Aldrich®, and UHPLC-grade solvents, water and methanol were purchased from Caledon Labs.

Urine was spiked with an internal standard (ISTD). Six aliquots of the ISTD-spiked urine were each spiked at different concentration levels (20-1000 ng/ml) of the drug standards listed in Table 2. Since drug metabolites are excreted as glucuronide conjugates, all urine samples were treated with the enzyme β-glucuronidase to convert any glucuronide conjugates of the drugs into the native form.[3,4] This was done by adding 950 µL of ammonium acetate (100 mM, pH 5.0) and 25 µL of β-glucuronidase (5000 units) to 1 mL of urine and incubating the samples for two hours at 60 °C. To stop the reaction, a 1:1 (v/v) ratio of aqueous ammonium hydroxide (2%) was then added to the incubated samples (final pH ~9). After the sample pre-treatment step, 200 µL of the pre-treated solution was loaded onto a Biotage SLE plate for clean-up. Using the Biotage pressure manifold to facilitate the flow of the sample into the SLE plate, the samples were finally eluted off the plates with 1-mL ethyl acetate and then dried using a Biotage TurboVap vacuum workstation. Following, the sample was reconstituted in 200 µL of mobile phase (0.1% formic acid in water), transferred to a vial and injected (10 µL).

Results

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

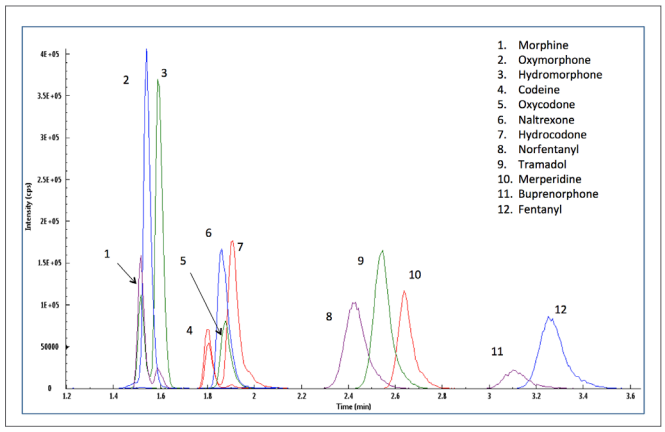

Figure 1. Overlaid MRM extracted ion chromatograms from 12 pain panel drugs in a 5-minute LC run.

Good linearity (R^2>0.99) was obtained for all 12 analytes across their respective concentration range, as shown in Table 2. All calibration curves used a linear regression with 1/x weighting. Figure 2 shows the four representative calibration curves for Buprenorphone, Codeine, Fentanyl and Meperidine.

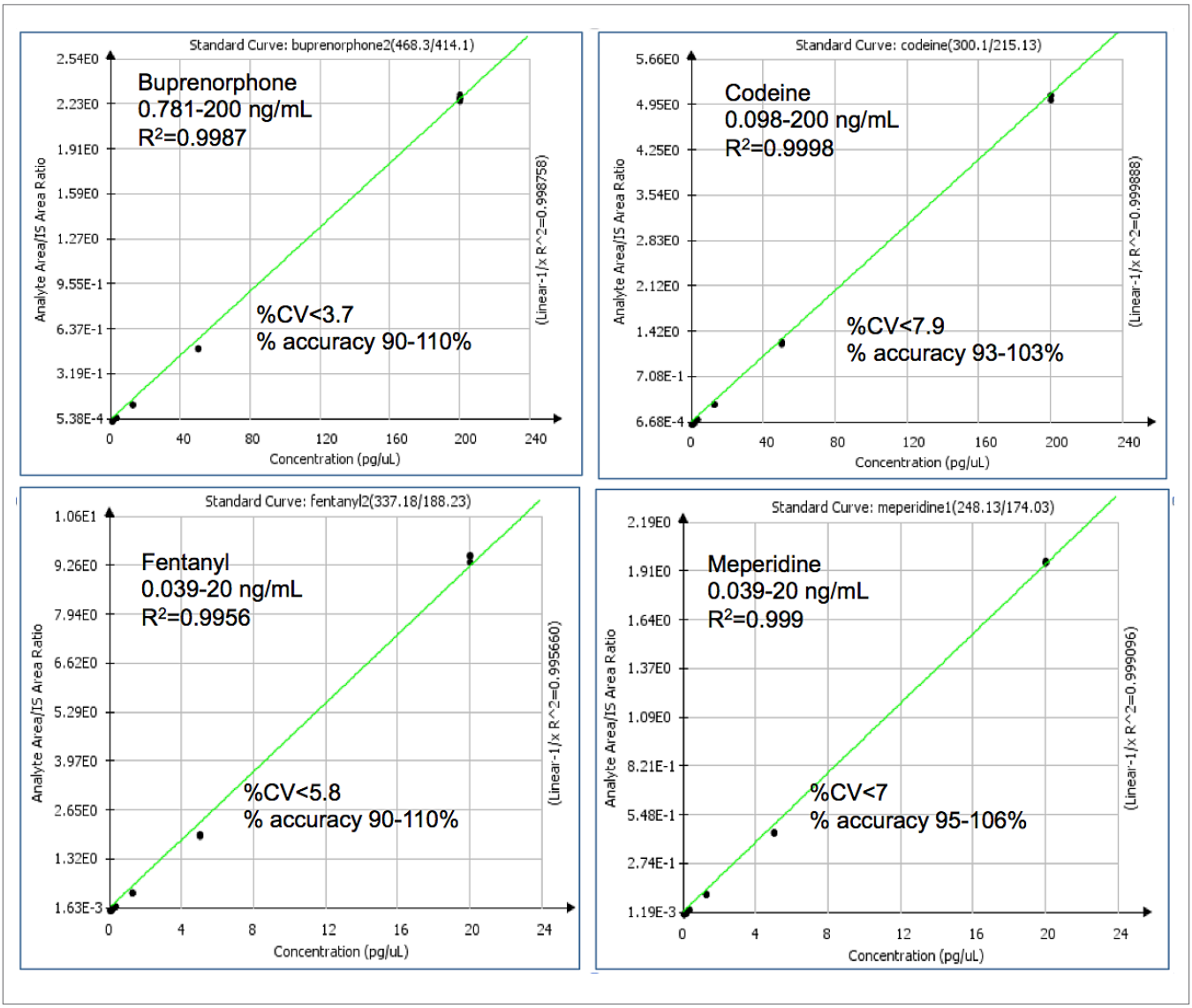

Figure 2. Four representative calibration curves for pain panel drug analysis.

Table 3. Summary of LOQ and linear range for all analytes..

Analyte	LOQ (ng/mL)	Linear Range (ng/mL)
Morphine	0.448	0.488-1000
Oxymorphone	0.122	0.122-500
Hydromorphone	0.122	0.122-500
Codeine	0.098	0.098-200
Oxycodone	0.048	0.048-100
Naltrexone	0.098	0.098-100
Hydrocodone	0.098	0.098-100
Norfentanyl	0.039	0.039-40
Tramadol	0.019	0.039-40
Merperidine	0.039	0.039-20
Buprenorphone	0.781	0.781-200
Fentanyl	0.039	0.039-20

Conclusion

We developed a 5-minute LC/MS/MS method that has shown to be effective in the separation and identification of 12 pain panel drugs. The LOQs for all 12 drugs were in the range of 0.019 to 0.781 ng/mL, which is three to four orders lower than the typical screening cutoff concentration (300 ng/mL) and the typical confirmation cutoff concentration (50 ng/mL) for drugs of abuse.[2] Based on the results, the Altus 30 UPLC® with the QSight 220 mass spectrometer was shown to be very effective for monitoring both patient drug use and program adherence for drugs of abuse.

References

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