

Rapid Analysis of Opiates from Low Volume Whole Blood Samples by LC-MS/MS Utilizing TurboFlow Methods

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Key Words

- Transcend TLX-1
- TurboFlow Technology
- TSQ Quantum Ultra
- Whole Blood
- Opiates

Introduction

The opiate morphine, and its derivatives, are medicines often used for pain-relief, cough-relief and as anti-diarrhoeals. For example, codeine and dihydrocodeine (morphine derivatives) are available in over-the-counter preparations in combination with paracetamol (acetaminophen) and are slowly metabolized to morphine and dihydromorphine respectively. However, the semi-synthetic opiate diacetylmorphine (heroin) is subject to wide abuse and has become such a major social problem that it is responsible for almost half of the drug-related deaths in the UK.¹

Heroin is deacetylated very rapidly (half-life ca. 3 mins in plasma) to its major active metabolite 6-monoacetylmorphine (6-MAM), which readily penetrates the blood-brain barrier to produce the desired euphoric effects.² 6-MAM also has a short plasma half-life of about 38 minutes (producing morphine), and thus, its detection in blood is very important to the forensic toxicologist in establishing the recent use of heroin.³ As a product of heroin metabolism, via 6-MAM, or from its own administration, morphine also undergoes further metabolism. The conjugation step produces inactive morphine-3-glucuronide (M3G) and the potently active morphine-6-glucuronide (M6G) along with other minor ones, including diglucuronides.

The forensic toxicologist is often asked to interpret results and possibly account for time of death in opiate (especially heroin) abuse cases. This task can be made easier if it is possible to identify and quantify the components such as 6-MAM, morphine, codeine, dihydrocodeine and the glucuronides in whole blood rather than urine. The volume of a human whole blood sample, however, may often only be available in the low microlitre range, thus presenting sample preparation and analysis sensitivity issues.

The analysis of free- and protein-bound opiate analytes in human whole blood by LC-MS/MS is routinely done after rigorous sample cleanup via solid phase extraction or liquid-liquid extraction in order to minimize ion suppression in the ionization source of the mass spectrometer. These

cleanup steps can be lengthy, laborious and expensive. Here we present a method to quantitatively analyze opiate compounds present in whole blood utilizing a simple, fast, low-volume extraction procedure followed by a Thermo Scientific TurboFlow method, an online extraction and chromatography coupled with selected reaction monitoring tandem mass spectrometry.

Goal

To replace laborious off line sample preparation with TurboFlow™ methodology and tandem mass spectrometry for the analysis of opiates in acetonitrile extracts from low volume whole blood samples.

Experimental

Sample Preparation

Horse blood was spiked with a mixture of opiates (codeine, morphine, 6-MAM, M3G, M6G and d6-codeine) at concentrations ranging from 1 ng/mL to 500 ng/mL. 150 µL spiked whole blood was mixed with 200 µL acetonitrile and vortexed. The resulting sample was then centrifuged for 10 min at 300 rpm. The supernatant was placed into a 96-well microtitre plate and 10 µL of the supernatant was used for the analysis.

TurboFlow Methodology

Thermo Scientific Transcend TLX-1 system	
Column:	Thermo Scientific TurboFlow Cyclone MAX 0.5 x 50 mm
Mobile phase A:	0.1% formic acid
Mobile phase B:	0.1% formic acid in acetonitrile
Mobile phase C:	10 mM ammonium bicarbonate pH 9
Mobile phase D:	10 mM ammonium acetate pH 6

Analytical LC

Column:	Thermo Scientific Hypersil GOLD aQ 50 x 2.1 mm, 1.9 µm
Mobile phase A:	0.1% formic acid
Mobile phase B:	0.1% formic acid in acetonitrile

The eluent gradients for both pumps are shown in Table 1.

Step	Start	Sec	TurboFlow Method								Analytical			
			Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	00:00	30	1.50	Step	-	-	100	-	====	out	0.30	Step	100	0
2	00:30	60	0.20	Step	100	-	-	-	T	in	0.10	Step	100	0
3	01:30	60	1.50	Step	-	-	-	100	====	in	0.30	Ramp	5	95
4	02:30	120	1.50	Step	99	1	-	-	====	in	0.30	Step	5	95
5	04:30	60	1.50	Step	-	-	100	-	====	out	0.30	Step	100	0

Table 1: Thermo Scientific Aria operating software gradient programs for the Transcend™ TLX-1 system with TurboFlow method and analytical LC method. Flow rate is reported as mL/min.

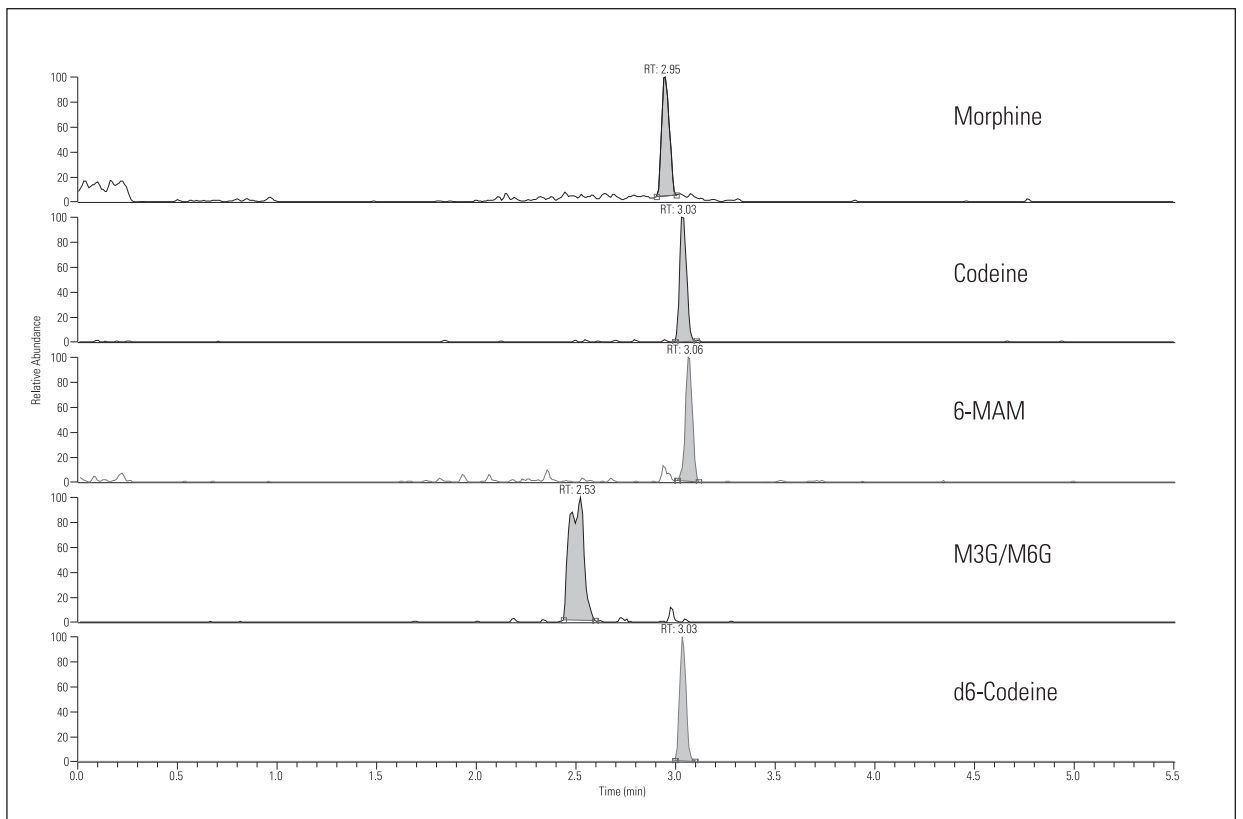


Figure 1: Extracted ion chromatogram for the lowest standard of each analyte

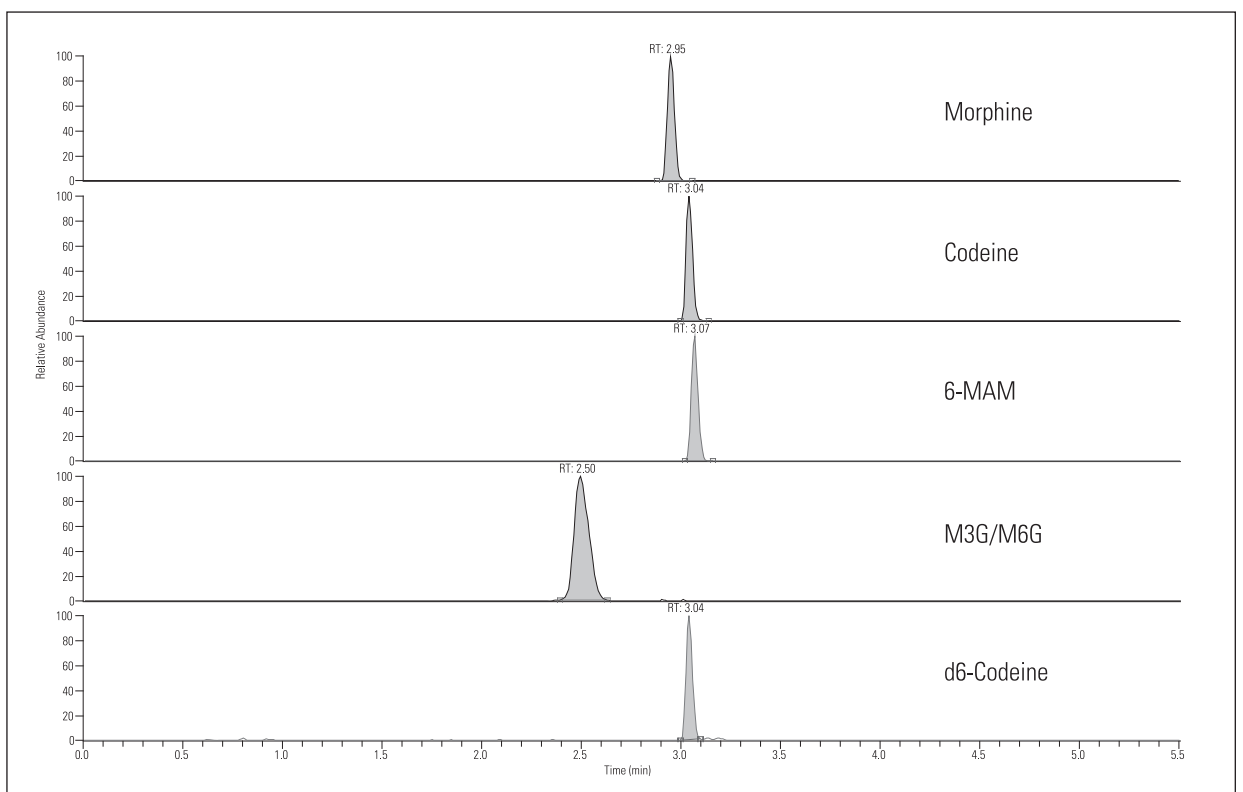


Figure 2: Extracted ion chromatogram for the highest standard of each analyte

Mass Spectrometry

Thermo Scientific TSQ Quantum Ultra

Ion Source & polarity: HESI, positive ion mode

Spray Voltage: 4750 V

Vaporizer Temperature: 450 °C

Sheath Gas: 50 units

Ion Sweep Gas: 5 units

Auxillary Gas: 60 units

Capillary Temperature: 200 °C

Collision Gas Pressure: 1.5 mTorr

The SRM transitions used for this experiment are presented in Table 2.

Analyte	Parent	Product	Scan Time	Collision Energy	Tube Lens
Morphine	286.13	165	5 ms	39	133
		201	5 ms	25	133
Codeine	300.14	165	5 ms	38	148
		215	5 ms	26	148
6-MAM	328.13	165	5 ms	38	145
		211	5 ms	25	145
M3G/M6G	462.16	286	5 ms	31	155

Table 2: SRM transitions monitored in the experiment

Results and Discussion

Prior to the analysis of spiked whole blood samples, opiate analytes were spiked into 100% acetonitrile and analyzed by the TurboFlow and LC-MS/MS method in order to demonstrate that the high organic content of the sample did not affect peak shape (peak splitting, etc.). The extracted, spiked whole blood samples were analyzed using the same TurboFlow method. Samples were run from low to high concentration with a solvent blank sample submitted after the highest concentration sample to calculate carryover. In all analyses, 10 μL of the extracted sample was injected and replicated to generate a calibration curve.

The extracted ion chromatograms of the lowest concentration sample and highest concentration sample are presented in Figures 1 and 2 respectively. The calibration curves for morphine, codeine and M3G/M6G covered 10–500 ng/mL (Figure 3, 4 and 6) and for the 6-MAM metabolite the curve covered 1–50 ng/mL (Figure 5). The isotopically labeled internal standard (d6-codeine) was spiked into each sample at 50 ng/mL. The concentration data for each analyte are provided as blood equivalents, i.e. the concentration in the blood before extraction. For example, 1 ng/mL blood equivalent was actual 0.43 ng/mL in the sample vial (150 μL diluted with 350 μL acetonitrile). Therefore, the equivalent on column amount of the lowest 6-MAM standard was 4.3 pg.

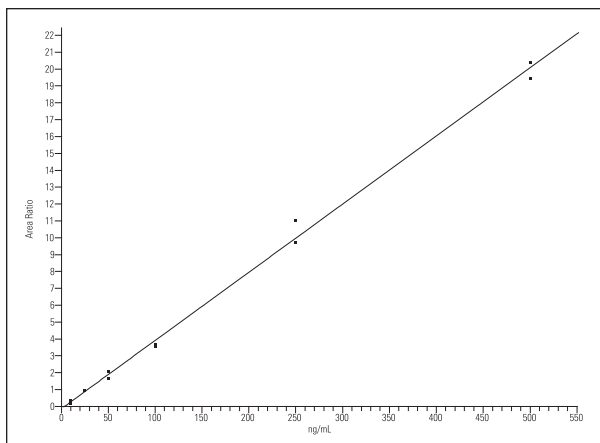


Figure 3: Calibration curve for the analyte morphine from 10–500 ng/mL

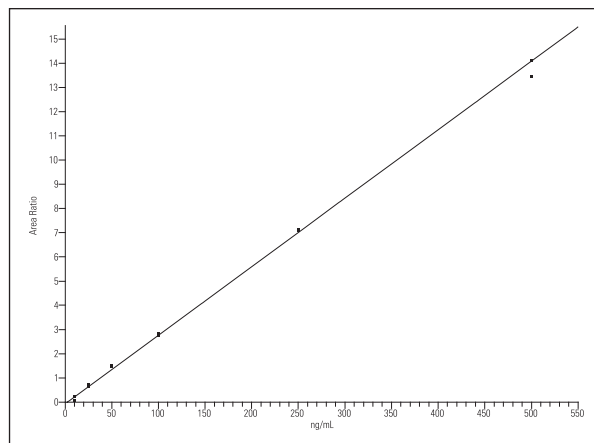


Figure 4: Calibration curve for the analyte codeine from 10–500 ng/mL

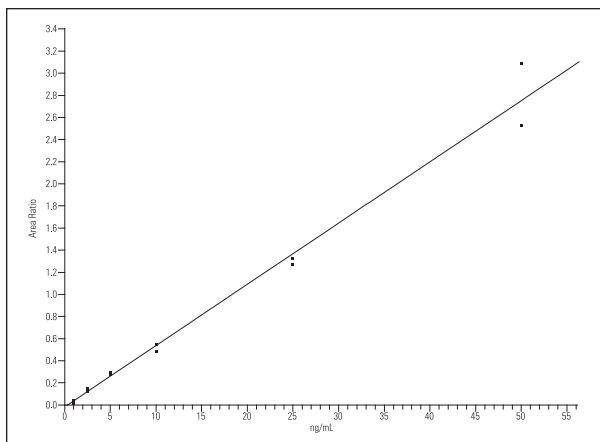


Figure 5: Calibration curve for the analyte 6-MAM from 1–50 ng/mL

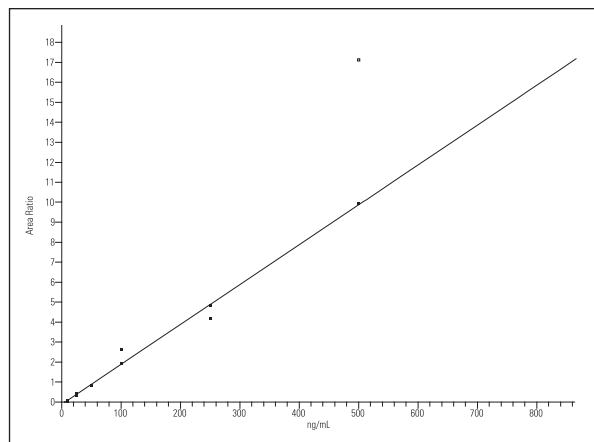


Figure 6: Calibration curve for the analyte M3G/M6G from 10–500 ng/mL

Conclusion

The use of a simple rapid acetonitrile work-up followed by a TurboFlow method (online extraction and chromatography) on the Thermo Scientific Transcend TLX-1 system with tandem MS/MS allowed the specific and sensitive analysis of various common opiates and their metabolites from a small volume of whole blood. Moreover, a limited portion of the acetonitrile extract volume was utilized in the analysis, thus, the method presents potential to scale down to a volume of blood achievable from a finger prick (5–10 µL). The calibration curves for all analytes analyzed were linear over the concentration range and carryover was calculated at less than 1% for all analytes. Since the method is ~ 4 minutes, 15 samples per hour may be completed, or indeed, doubled/quadrupled with the use of multiplexing. Significant time is saved in the absence of SPE sample preparation.

The method enables the forensic toxicologist to produce a full picture of the opiates and metabolites in blood to assist with the determination of time of injection (presence of 6-MAM) and the detection of M3G and M6G to determine prior use or accumulation following heavy use.

References and Acknowledgements

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