SAMHSA-Compliant LC/MS/MS Analysis of 6-Acetylmorphine in Urine with Agilent Bond Elut Plexa PCX and Agilent Poroshell 120

Application Note

Forensic Toxicology

Authors

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Abstract

New guidelines from the US Substance Abuse and Mental Health Services Administration (SAMHSA), effective October 2010, allowed LC/MS/MS methods to be used for confirmation of initial drug tests [1]. LC/MS/MS methods are often less complicated than previously employed GC/MS methods because they typically do not require a derivatization step. We present a method for analysis of 6-acetylmorphine that meets the most recent SAMHSA guidelines to demonstrate linearity, limit of detection (LOD), accuracy, and precision, as well as measurement of matrix effects, extraction recovery, and overall process efficiency. This is one of a suite of six simplified methods covering all classes of SAMHSA-regulated drugs and using premier Agilent products such as Agilent Bond Elut Plexa PCX mixed-mode polymeric SPE sorbent, Agilent Poroshell 120 EC-C18 2.7 µm superficially porous LC column, Agilent 1200 Infinity LC system, and Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source.

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Introduction

A metabolite, 6-Acetylmorphine, or 6-monoacetylmorphine (6-AM) is unique to heroin. Heroin (or diacetylmorphine) is an opioid drug synthesized from morphine. In the body, heroin is rapidly metabolized through deacetylation to 6-AM and then to morphine at a somewhat slower rate [2]. The updated SAMHSA confirmation cutoff concentration for 6-AM is 10 ng/mL, and a LOD at 10% of the cutoff would be 1 ng/mL.

The simple extraction method described here provides reproducible high recoveries of 6-AM due to the unique properties of Bond Elut Plexa. Unlike other polymeric sorbents, Plexa possesses an amide-free hydroxylated particle surface that excludes protein binding. This results in minimized ion suppression and maximum sensitivity. Fast flow and reproducible performance are due to the narrow particle size distribution with no fines to cause blockages.

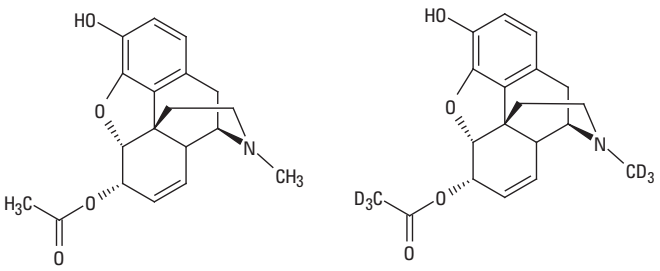
A Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm column was chosen due to its high capacity and excellent separation properties. With superficially porous 2.7 µm particles, Poroshell 120 provides similar efficiency to sub-2 µm UHPLC columns but with approximately 40% less back pressure, thereby allowing users of even 400 bar LC systems to increase resolution and to shorten both analysis and re-equilibration times by applying a higher flow rate.

With a low sample injection volume of 10 µL and no sample preconcentration, the presented method demonstrates excellent signal-to-noise ratios (> 190:1 at 1 ng/mL, 10% of the SAMHSA confirmation cutoff) due to the enhanced sensitivity of an Agilent 6460 Triple Quadrupole LC/MS system with the AJST electrospray source.

Previous methods from Agilent [3,4] used the Agilent 6410 Triple Quadrupole LC/MS system and other SPE/LC products and procedures.

Experimental

Analytes



6-Acetylmorphine
Log P 1.55
pK_{a1} 8.2

6-Acetylmorphine-D_6

Figure 1. 6-Acetylmorphine analytes and their structures.

Drug standards were purchased from Cerilliant Corporation as 1 mg/mL (6-acetylmorphine) and 100 µg/mL (6-acetylmorphine-D_6) solutions in acetonitrile.

Materials and instrumentation

SPE

- Bond Elut Plexa PCX cartridges 30 mg, 3 mL (p/n 12108303)
- Agilent vacuum manifold VacElut 20 (p/n 12234100)
- Agilent stopcock valves (p/n 12234520)
- Agilent 2-mL autosampler vials (p/n 5182-0716)
- Agilent screw caps for AS vials (p/n 5182-0717)

LC

- Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm column (p/n 699975-302)
- Agilent 1260 Infinity LC (G1379B microdegasser, 1312B binary pump in low delay volume configuration, G1367E autosampler, and G1330B thermostat)

MS

- Agilent 6460A Triple Quadrupole LC/MS system with AJST electrospray ionization source

Sample preparation

Pretreatment

Spike 1 mL of urine with ISTD at 20 ng/mL; use of 12 × 75 mm glass tubes is recommended. Add 1 mL of 2% formic acid, vortex; centrifuge if cloudy.

Extraction

1. Condition Bond Elut Plexa PCX column with 0.5 mL. methanol – soak, then let drip.
2. Load sample/supernatants.
3. Wash 1: 1 mL 2% formic acid.
4. Wash 2: 1 mL of methanol.
5. Dry 5–10 minutes under vacuum (10–15 in Hg).
6. Elute with 1 mL methanol: ammonium hydroxide (100:10), freshly prepared. Let eluate drip into collection vials, then apply low vacuum (2–3 in Hg).
7. Evaporate under stream of nitrogen to dryness.
8. Reconstitute in 1 mL initial mobile phase (10% methanol, 90% water, 0.1% formic acid).

LC/MS/MS

LC conditions

Mobile phase A	0.1% formic acid in water	
Mobile phase B	0.1% formic acid in methanol	
Flow rate	0.8 mL/min	
Gradient	Time (min)	% B
	0.0	10
	1.5	25
	2.0	60
	2.1	90
	5.0	90
	5.1	10
Stop time	5.2 min	
Post time	2 min	
Max pump pressure	400 bar	
Injection volume	10 µL	
Injection with needle wash		
Needle wash	Flush port 75:25 methanol:water for 10 s	
Disable overlapped injection		
No automatic delay volume reduction		

MS conditions

ES Source Parameters	
Ionization mode	Positive
Capillary voltage	2,800 V
Drying gas flow	13 L/min
Drying gas temperature	350 °C
Nebulizer gas	35 psi
Sheath gas flow	12 L/min
Sheath gas temperature	400 °C
Nozzle voltage	0 V
MS parameters	
Scan type	MRM
Pre-run script	SCP_MSDiverterValveToWaste() {MH_Acq_Scripts.exe}
Time segments	#1: 1.2 min - diverter valve to MS
Delta EMV (+)	400 V

Results and Discussion

At acidic pH, the tertiary amine of 6-acetylmorphine was protonated, and the analyte was efficiently retained on Bond Elute Plexa PCX polymeric sorbent by a combination of hydrophobic interaction and a strong cation exchange.

A 100% methanol wash eliminated most matrix interferences without 6-AM loss from the SPE column. A strong base was added to organic eluent to break ionic interaction between the analyte and strong cation exchange sorbent. 6-AM recovery was optimized with 10% NH_4OH added to methanol shortly before sample elution.

The Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm column provided fast separation of 6-AM in urine extract and good peak shape (Figure 2). The LC separation started with a low fraction of organic solvent (10%) to allow salts and other polar components of urine to elute at the beginning of the sample run. Each sample run started with diverting a first portion of flow (0 to 1.2 minutes) to waste to minimize source contamination. Data collection started at 1.2 minutes, immediately after the diverter valve switch. A flow rate of 0.8 mL/min allowed for short retention and re-equilibration times.

SAMHSA guidelines require one quantifier and at least one qualifier ion for both target compound and ISTD. A third transition for each target analyte (Table 1) was provided for additional confidence. Agilent MassHunter Quantitative software automatically calculated qualifier ion ratios, highlighting those out of acceptable range.

Table 1. MRM transitions.

Compound	Precursor	Product	Fragmentor	Collision energy
6-AM	328.2	165.1	140	40
6-AM	328.2	211.1	140	25
6-AM	328.2	193.1	140	25
6-AM-D_6	334.2	165.1	140	40
6-AM-D_6	334.2	211.1	140	25

Normal, rather than dynamic, MRM scan type can be used with this method, because dynamic MRM has no advantages for detection of a single compound.

A signal-to-noise ratio of > 190:1 for the 1 ng/mL peak (Figure 2, upper panel) illustrated a state-of-the-art performance of the Agilent 6460 Triple Quadrupole LC/MS capable of reliably detecting 6-AM at a small fraction (10%) of the SAMHSA cutoff concentration.

Figure 3 is an example calibration curve for extracted urine standards at five concentration levels of 6-acetylmorphine. Calibration standards were prepared by spiking negative urine at 1.0, 10, 50, 200, and 400 ng/mL. Deuterated internal standard 6-AM-D_6 was added at 20 ng/mL. The excellent linear fit with $R^2 > 0.999$ demonstrates linearity of the method across a broad dynamic range of concentrations, as required by SAMHSA guidelines.

Method evaluation

Method performance metrics in Table 2 were calculated according to the principles laid out in Matuszewski *et al* [5] and widely accepted as an industry standard approach for LC/MS/MS methods. Extraction procedure and LC/MS/MS measurement were performed for five replicates of negative urine spiked pre-extraction at the cutoff level, and five replicates of negative urine extract reconstituted in initial mobile phase and then fortified at 10 ng/mL with 6-AM (spiked post-SPE). The third measurement was of initial mobile phase (the reconstitution solvent) fortified to correspond to the cutoff concentration of 10 ng/mL in urine (spiked mobile phase).

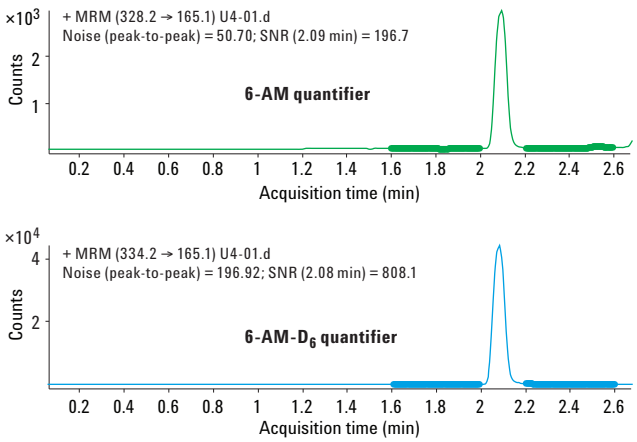


Figure 2. MRM extracted ion chromatograms for 6-AM (1 ng/mL) and 6-AM-D_6 (20 ng/mL) in urine extract. Agilent Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm column. Noise regions are shown in bold.

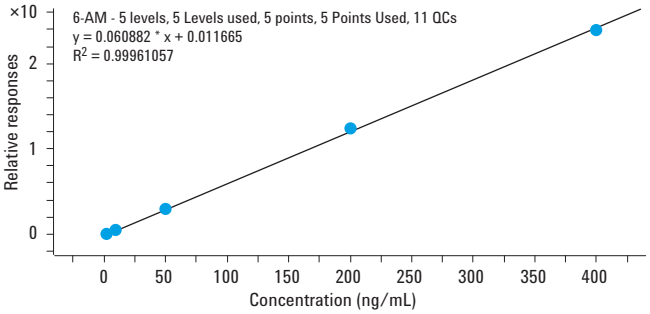


Figure 3. Example calibration curve for 6-AM in urine extract. Calibration range 1.0 to 400 ng/mL. Linear fit, $R^2 > 0.999$.

Table 2. Method performance for 6-Acetylmorphine, n = 5.

	%
Process efficiency*	83
Extraction recovery*	83
Matrix effect*	100
Accuracy**	106
Precision** (CV)	0.6

*determined at cutoff level **determined at 40% cutoff

Process efficiency (absolute recovery) is a ratio of a peak area of target analyte in urine sample spiked pre-SPE to its peak area in matrix-free spiked mobile phase. Extraction recovery is a ratio of a peak area of target analyte in urine extract spiked pre-SPE to its peak area in an extracted negative urine sample spiked post-SPE. Matrix effect is a ratio of a peak area of target analyte in urine spiked post-SPE to its peak area in spiked mobile phase. Accuracy is a ratio of a measured concentration calculated using the calibration curve to the expected concentration in a sample spiked with a known amount of target analyte. Precision or coefficient of variation (CV) is a measure of reproducibility and is calculated as a percent standard deviation over the mean of the five measurements.

Table 2 shows high extraction recovery for 6-acetylmorphine (83%) together with very good accuracy (106%) and precision (0.6 %). Matrix effect of 100% indicated no suppression or enhancement of a signal due to matrix interferences, thus confirming an exceptional cleanliness of Plexa-processed extracts.

Conclusions

The solid phase extraction procedure coupled with LC/MS/MS detection method described here is SAMHSA-compliant and provides accurate, precise, and reproducible results for forensic toxicology or other analytical environments with similar requirements for legally defensible data. The hardware setup is the same as in the other 2011 SAMHSA methods from Agilent. These methods are intended for all users of Agilent 1100 and 1200 Series LCs because the back pressure in the LC system does not exceed 400 bar. Source parameters can be easily modified to use this method with other models of the Agilent Triple Quadrupole LC/MS systems. Electronic copies of the LC/MS/MS acquisition and quantitation methods are available from Agilent Technologies.

References

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