

Nota applicativa

Opiates: Use or Abuse? Quantification of Opiates in Human Urine

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Abstract

In this application note, we describe a sensitive method for the simultaneous analysis of several opiates in urine. The method involves a simple SPE purification step prior to analysis using HPLC-MRM and may be used to identify cases of heroin abuse.

Introduction

Heroin is a highly addictive drug. It is processed from morphine, a naturally occurring substance extracted from the seedpod of the Asian opium poppy (Figure 1). Abuse of heroin is associated with serious health conditions, including fatal overdose, collapsed veins, and an increased risk of infectious diseases such as hepatitis, HIV/AIDS, and tuberculosis. Once inside the body it is rapidly metabolised to morphine (Figure 2), which is then excreted in the urine.



Figure 1. The Asian opium poppy, *Papaver somniferum*.

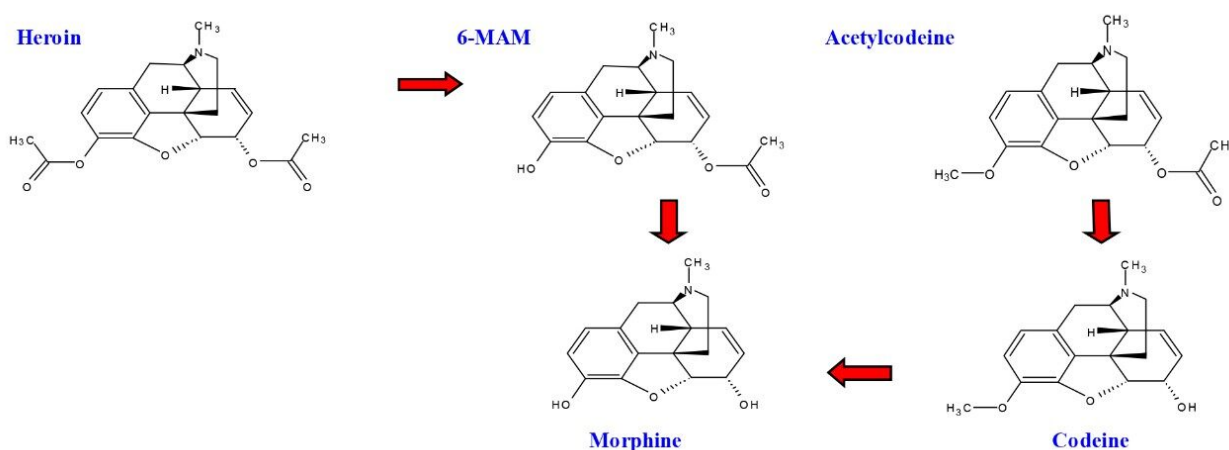


Figure 2. Metabolism of opiates.

The presence of morphine in urine cannot alone be used as a marker for illicit heroin abuse since morphine and codeine (which is also metabolised to morphine) can be found in prescriptive medicines and foods. For example, such medicines are valuable treatments for pain, coughs and diarrhoea. Ingestion of pastries containing poppy seeds has also been shown to lead to the presence of morphine and codeine in the urine (Hayes *et al.*, 1987). However, the intermediate metabolite of heroin, 6-monoacetylmorphine (6-MAM) can be used as a specific marker for heroin as it does not result from the metabolism of either morphine or codeine. In addition, acetylcodeine is a known impurity of illicit heroin synthesis and may be used to distinguish between the pharmacologically pure heroin that is used in heroin maintenance programs and illicit 'street' heroin.

We have developed a LC-MS/MS method that allows the simultaneous quantification of several opiates in urine. The method can also be used to establish whether morphine present in the urine has originated from illicit heroin use.

Experimental

Methodology

Sample Preparation

Urine samples were prepared for HPLC-MS/MS analysis by means of a simple, generic solid-phase

extraction (SPE) procedure. A Waters Oasis HLB Extraction Cartridge (1 cc/30 mg) was firstly conditioned with methanol (1 mL) followed by water (1 mL). Urine samples (spiked with deuterated internal standards) for SPE were diluted into water (300 µL urine into 700 µL water before applying to the pre-conditioned cartridge. The cartridge was washed with 5% methanol before elution of the sample using 1 mL 100% methanol. Ten microlitres (10 µL) of the eluant was analysed using HPLC in conjunction with multiple reaction monitoring (MRM).

A Quattro micro triple quadrupole mass spectrometer fitted with Z-Spray ion interface was used for all analyses. Ionization was achieved using electrospray in the positive ionisation mode (ES+). Details of the MRM conditions are given in Table 1.

HPLC analyses were performed using a Waters 2790 separations module. Chromatography was achieved using a Waters Nova-Pak CN HP Column (3.9 x 75 mm) eluted isocratically with 2 mM ammonium acetate:methanol (50:50) containing 0.1% formic acid at a flow rate of 0.3 mL/min. The column temperature was maintained at 30 °C. All aspects of system operation and data acquisition were controlled using MassLynx NT 3.5 Software with automated data processing using the QuanLynx program.

Compound	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)
Morphine	286	165	42	38
Morphine – d3	289	165	45	40
Codeine	300	165	45	40
Dihydrocodeine (DHC)	302	199	45	32
6-Monoacetyl Morphine (6-MAM)	328	165	50	38
6-Monoacetyl Morphine (6-MAM) – d6	334	165	45	38
6-acetylcodeine	342	165	50	38

Table 1. MRM transitions and conditions for the measurement of several opiates. The conditions for deuterated morphine and 6-MAM (d3 and d6 respectively) were also included for the purpose of internal standardisation.

Results and Discussion

A series of calibrators (0.5–250 ng/mL) were prepared by adding opiate standards to blank urine. Urine

samples (either calibrators or unknown samples) were then extracted using the SPE method described above prior to HPLC-MRM analysis.

Following HPLC-MRM analysis, the areas under the specific MRM chromatograms were integrated. Figure 3 shows the MRM chromatograms of various opiates obtained with a 10 μ L injection of the 5 ng/mL urine calibrator. The opiates were quantified by reference to the integrated area of the deuterated internal standards. Responses were linear for all compounds (Figure 4 shows a typical standard curve for 6-MAM in urine).

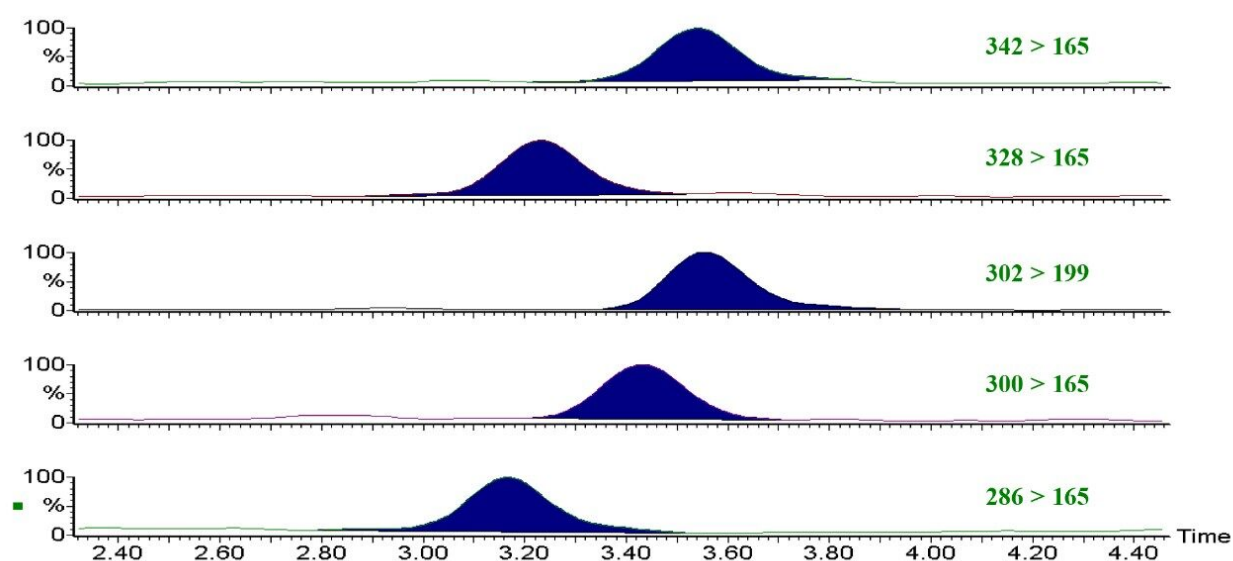


Figure 3. MRM chromatograms for: (top to bottom) acetylcodeine, 6-MAM, DHC, codeine, and morphine. Responses were obtained with a 10 μ L injection of the 5 ng/mL urine calibrator.

Compound name: 6-MAM
Coefficient of Determination: 0.999261
Calibration curve: $0.167559 * x + 0.0400415$
Response type: Internal Std (Ref 4), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

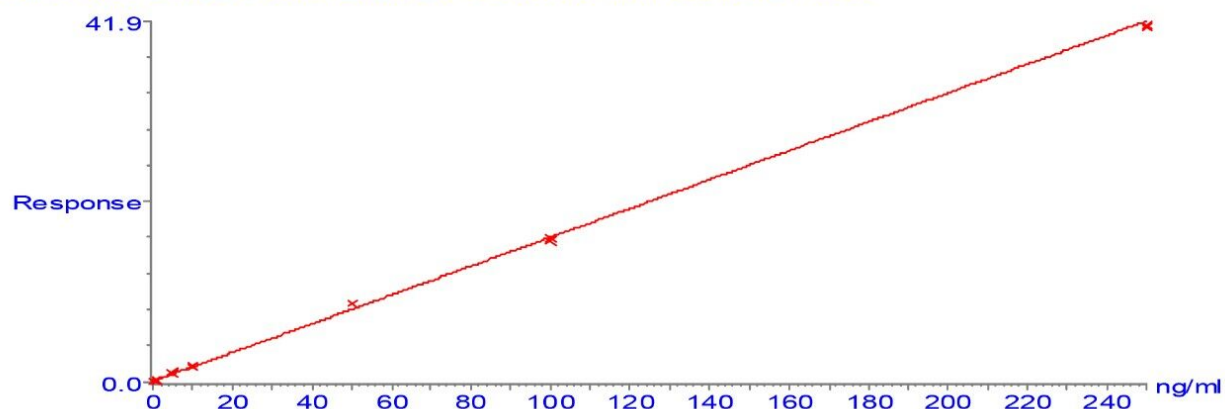


Figure 4. Standard curve for 6-MAM. Responses were calculated in reference to the integrated area of the deuterated internal standards.

Conclusion

We describe a sensitive method for the simultaneous analysis of several opiates in urine. The method involves a simple SPE purification step prior to analysis using HPLC-MRM and may be used to identify cases of heroin abuse.

References

1. Hayes LW, Krasselt WG and Mueggler PA. Concentrations of Morphine and Codeine in Serum and Urine After Ingestion of Poppyseeds. *Clinical. Chemistry*. 1987; 33: 806–808.

