

# Opiates: Use or Abuse?

## Quantification of Opiates in Human Urine.

Michelle Wood, Kevin Rush\*, Michael Morris and Allan Traynor\*

Clinical Applications Development Group, Micromass UK Limited, Manchester, UK

\* Medscreen Ltd. 1A Harbour Quay, 100 Prestons Road, London E14 9PH

### 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*.

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.

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

### HPLC-MS/MS

A Quattro *micro* triple quadrupole mass spectrometer fitted with Z-Spray ion interface was used for all analyses. Ionisation 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<sup>®</sup> CN HP column (3.9 x 75mm) eluted isocratically with 2mM ammonium acetate:methanol (50:50) containing 0.1% formic acid at a flow rate of 0.3mL/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<sup>™</sup> 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.

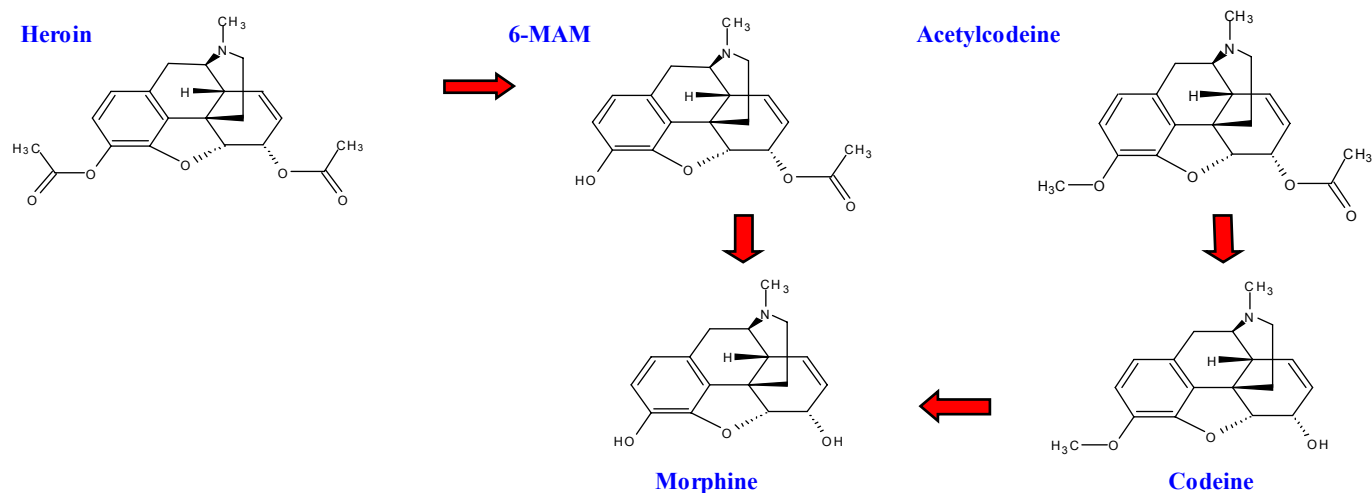


Figure 2. Metabolism of opiates

## Results

A series of calibrators (0.5-250ng/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 10µL injection of the 5ng/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).

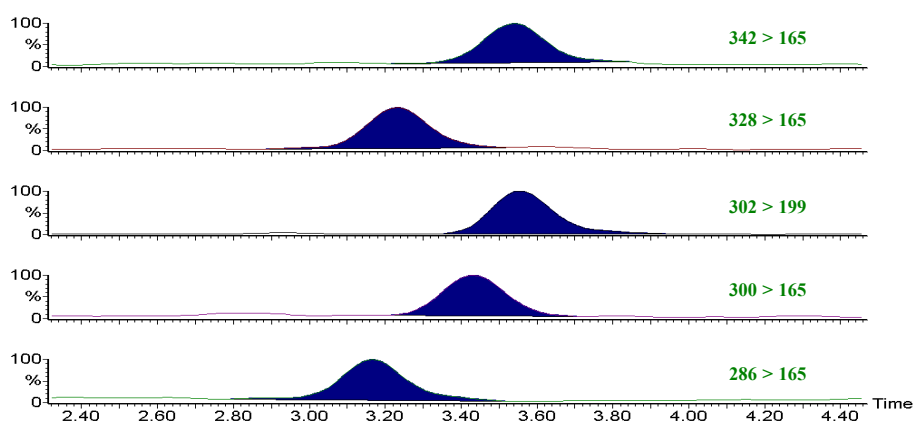


Figure 3. MRM chromatograms for: (top to bottom) acetylcodeine, 6-MAM, DHC, codeine and morphine. Responses were obtained with a 10µL injection of the 5ng/mL urine calibrator.

## Summary

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

Hayes LW, Krasselt WG and Mueggler PA. Concentrations of morphine and codeine in serum and urine after ingestion of poppy seeds. *Clinical Chemistry*. 1987; 33: 806-808.

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

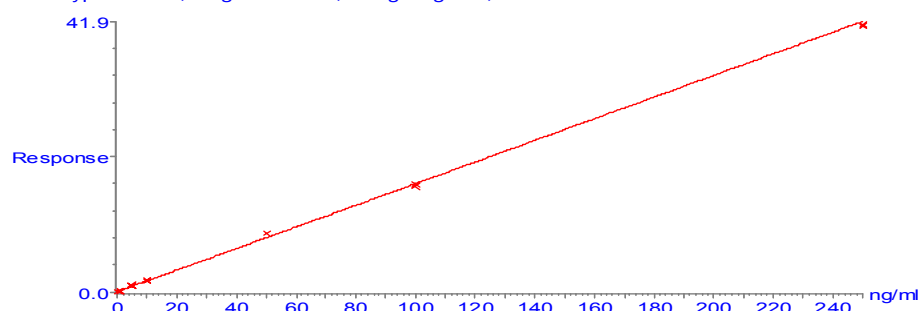


Figure 4. Standard curve for 6-MAM. Responses were calculated in reference to the integrated area of the deuterated internal standards.



USA Tel: 978 524-8200 Fax: 978 524-8210  
Europe Tel: +31 (0) 294-480484 Fax: +31 (0) 294-419052  
UK/International Tel: +44 (0) 161 946 2400 Fax: +44 (0) 161 946 2480  
<http://www.micromass.co.uk>