QUANTIFICATION OF MORPHINE, MORPHINE-3-GLUCURONIDE AND MORPHINE-6-GLUCURONIDE IN BIOLOGICAL SAMPLES BY LC/MS/MS

Michelle Wood1 and Michael Morris1.

1Waters Corporation, Manchester, UK.

INTRODUCTION

Morphine is a potent analgesic isolated from the opium poppy papaver somniferum and traditionally used for the treatment of moderate to severe pain. Analgesia results from the action of morphine at the opioid receptors of the spinal cord and brain (Figure 1), where it attenuates both the speed of the impulse and the perception of pain.

In human subjects, morphine is extensively metabolised (primarily by conjugation with glucuronic acid) to form morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Whilst, the principal metabolite i.e. M3G, has little or no analgesic effect, M6G has been shown to be highly effective and is believed likely to contribute significantly to the overall effectiveness of morphine1. Hence, quantification of both the parent drug and metabolites is desirable for pharmacokinetic studies.

Previously we have described a LC/MS/MS method that allows the quantification of morphine and several other opiates in urine2. Here we present a simple method that enables the quantification of morphine in plasma, whole blood and urine. Furthermore this procedure allows differentiation between two isobaric glucuronide metabolites.

METHODOLOGY

Sample preparation

Biological samples were prepared for LC/MS/MS analysis by means of a simple, solid-phase extraction (SPE) procedure. A Waters Oasis® HLB extraction Cartridge (1 cc/30 mg) was firstly conditioned with 1 mL volumes of each of the following: methanol, water and ammonium carbonate (10 mM, pH 8.8). Samples (100 μL, spiked with deuterated internal standards) were made up to a final volume of 1 mL with ammonium carbonate before applying to the pre-conditioned cartridge. The cartridge was then washed with 1 mL ammonium carbonate before elution of the sample using 100% methanol (0.5 mL). Eluents were dried using a Savant Speedvac Plus evaporator and then redissolved in 100 μL of mobile phase. Reconstituted samples were briefly vortex mixed before the analysis of 10 μL using LC in conjunction with multiple reaction monitoring (MRM).

LC/MS/MS

A Waters Quattro micro™ triple quadrupole mass spectrometer fitted with ZSpray™ 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.

Table 1: MRM transitions and conditions for the measurement of morphine and its metabolites. The deuterated analogues of morphine and morphine-3-glucuronide were also included for the purpose of internal standardisation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Cone Voltage (V)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>286</td>
<td>165</td>
<td>45</td>
<td>38</td>
</tr>
<tr>
<td>Morphine-d3</td>
<td>289</td>
<td>165</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>Morphine-M3G-glucuronide</td>
<td>462</td>
<td>286</td>
<td>45</td>
<td>28</td>
</tr>
<tr>
<td>Morphine-M3G-d3-glucuronide</td>
<td>465</td>
<td>289</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>Morphine-M6G-glucuronide</td>
<td>462</td>
<td>286</td>
<td>45</td>
<td>28</td>
</tr>
</tbody>
</table>
LC analyses were performed using a Waters 2795 separations module. Chromatography was achieved using a C18 column (3.9 x 150 mm) eluted isocratically with 0.1% formic acid:acetonitrile (97:3) at a flow rate of 0.3 mL/min. Column temperature was maintained at 30 °C. All aspects of system operation and data acquisition were controlled using MassLynx™ 4.0 software with automated data processing using the QuanLynx™ program.

RESULTS

A series of calibrators (0.5-500 μg/L) were prepared in duplicate by adding standards to blank plasma, whole blood or urine. Samples were then extracted using the SPE method described above prior to LC/MRM analysis.

Following LC/MRM analysis, the areas under the specific MRM chromatograms were integrated.

Figure 2 shows the extracted MRM chromatogram of morphine, M3G and M6G obtained with a 10 μL injection of the 5 μg/L plasma calibrator. Responses were linear (r > 0.999) over the range investigated for all 3 compounds and in each matrix (Figure 3 shows a typical standard curve for M3G in urine).

SUMMARY

We present a sensitive method for the quantification of morphine and its glucuronide metabolites. The method involves a simple SPE purification prior to analysis using LC/MRM and is suitable for plasma, whole blood or urine samples.

REFERENCES


*Medscreen Ltd., 1A Harbour Quay, 100 Prestons Rd, London.

Figure 2. MRM chromatogram for morphine (MOR), M3G and M6G. The above responses were obtained with a 10 μL injection of the 5 μg/L plasma calibrator. Due to the isobaric nature of M3G and M6G chromatographic resolution is required to enable identification.
The MassTrak™ systems are in vitro diagnostic devices compliant with EU directive 98/79/EC. The systems are manufactured in accordance with US and European regulations. Waters Corporation is ISO 13485:2003 certified and operates in accordance with international regulations, United States Food and Drug Administration Quality System Regulations and current Good Manufacturing Practices.

Figure 3. Standard curve for M3G in urine. Responses (duplicates) were calculated in reference to the integrated area of the deuterated internal standards. The inserted figure shows the response for the range 0-10 μg/L.