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Determination of morphine and morphine glucuronides in human plasma by 96-well plate solid-phase extraction and liquid chromatography–electrospray ionization mass spectrometry

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Abstract

There is considerable interest in quantifying morphine and its major metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Available assays use gas chromatography–mass spectrometry or high-performance liquid chromatography (HPLC) with single or tandem mass spectrometry, ultraviolet, electrochemical, or fluorimetric detection. Nevertheless, few methods provide adequate sensitivity for all analytes, in a single injection, with the desired rate of sample throughput. A rapid and sensitive method for quantification of morphine, M3G and M6G from human plasma using HPLC with electrospray ionization mass spectrometry was developed using a Waters Oasis MCX 96-well plate for extracting both lipophilic morphine and its hydrophilic glucuronides, C18 separation using an isocratic mobile phase (methanol, acetonitrile and formic acid), and selected ion monitoring. Recoveries of morphine, M3G and M6G, respectively, were 81, 90 and 82% at the low (2, 25 and 2 ng/ml), 80, 77 and 75% at the medium (10, 250 and 10 ng/ml), and 74, 62 and 72% at the high (100, 1000 and 100 ng/ml) quality control samples. The limit of quantitation was 0.5 ng/ml morphine and M6G, and 5 ng/ml M3G. Analytes were validated over a linear range of 0.5–200 ng/ml morphine and M6G, and 5–2000 ng/ml M3G. This assay represents an improvement over existing methods through solid phase extraction with increased sample throughput (96-well plates), use of small samples (0.5 ml), and sub-nanogram detection.

Keywords: Morphine; Morphine glucuronides

1. Introduction

Morphine is a mainstay in the treatment of acute and chronic pain. Glucuronidation is the main route of morphine metabolism, producing morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Simultaneous determination of morphine and metabolite concentrations has practical application in both pharmacokinetic studies and forensic assessment. Furthermore, it is now clear that M6G is pharmacologically active and a more potent analgesic than morphine itself, contributing to the analgesic and other pharmacologic effects of morphine under certain clinical conditions [1–3]. Since only 10%

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of a morphine dose is metabolized to M6G, assays with increased sensitivity for this metabolite are necessary.

A variety of techniques have been used to quantify morphine and its metabolites. Immunoassays offer adequate sensitivity for morphine but lack the specificity to distinguish opiates from their corresponding glucuronides [4]. Gas chromatography–negative chemical ionization mass spectrometry (GC–MS) provides the needed sensitivity and selectivity, but requires metabolite derivatization and thus additional time consuming sample manipulation [5,6]. Analysis of M3G and M6G without derivatization is possible with high-performance liquid chromatography (HPLC). Traditionally, methods for quantification of morphine and conjugated metabolites have used HPLC with ultraviolet, fluorescence or coulometric detection [7–11]. These provide adequate detection, however, sample run times may be long (>25 min). HPLC (to separate lipophilic morphine and hydrophilic metabolites) with mass spectrometry (usually tandem mass spectrometry, LC–MS–MS) has become the technique of choice for simultaneous analysis of low concentrations of morphine, M3G and M6G [12–15]. These methods have reported greater sensitivity compared to GC–MS [16–20]. While LC–MS–MS provides adequate sensitivity, tandem instruments are expensive and hence not universally available.

Recent improvements in extraction techniques have focused on solid phase extraction (SPE). SPE is faster, easier and has increased recovery compared with liquid–liquid extraction [12–15,17–21]. Although a variety of methods using SPE cartridges are available, inter-day variability due to matrix interferences has been problematic, presumably due to differences in sorbent packing and binding affinity [21,22]. A multi-cartridge SPE method was reported to have increased sensitivity and selectivity for morphine and its metabolites [21], but the time required for additional SPE extraction steps hindered sample throughput. Sample preparation and throughput are important factors in analytical methods selection. Extractions using 96-well plates rather than individual SPE cartridges decreases sample preparation time and increases throughput. There is a need for a simple and rapid SPE method using small sample volumes (<1 ml) with a low quantification limit (<1 ng/ml) for morphine and M6G. Presented in this paper is the development and validation of an SPE method for morphine, M3G, and M6G employing HPLC with single quadrupole electrospray ionization mass spectrometry.

2. Experimental

2.1. Materials

Morphine, morphine-3-β-D-glucuronide, d3-morphine, d3-morphine-3-β-D-glucuronide, and morphine-6-β-D-glucuronide were purchased from Cerilliant (Austin, TX) and d3-morphine-6-β-D-glucuronide from High Standard Corp (Westminster, CA). HPLC grade methanol and acetonitrile were from Fisher Scientific (Pittsburgh, PA), ammonium hydroxide from J.T. Baker (Phillipsburg, NJ) and formic acid from Aldrich (Milwaukee, WI). Oasis MCX cartridges (30 mg, 30 μm) and 96-well plates (30 mg, 30 μm) were obtained from Waters Corp (Milford, MA). All stock drug solutions, buffers, and HPLC mobile phase were prepared using Milli-Q grade water (Millipore, Bedford, MA). Outdated human plasma was pooled from several donors.

2.2. Sample preparation and extraction

Subject plasma, calibration or quality control samples (0.5 ml) were added to a polypropylene 96-well (1 ml) plate. Internal standard mix (0.5 ml, consisting of 10 ng/ml d3-morphine, 10 ng/ml d3-M6G, and 50 ng/ml d3-M3G in 0.1 N HCl, prepared daily from a concentrated stock) was added, and the samples vortexed for 2 min.

SPE was performed using Oasis MCX 96-well plates, a vacuum manifold and a vacuum source. The plate was conditioned with 1 ml methanol then 1 ml 0.1 N HCl. Samples were then loaded into the plate at 0.5 ml/min and washed with 1 ml 0.1 N HCl. The plates were thoroughly dried by vacuum (10–15 mm Hg) for 2 min. Analytes were eluted by gravity with 1 ml 5% ammonium hydroxide in methanol into a 96-well plate. Samples were evaporated to dryness under nitrogen in a TurboVap (Zymark, Hopkinton, MA) at 55 °C, reconstituted with 50 μl mobile phase, transferred to glass inserts, centrifuged (Eppendorf, Westbury, NY) at 12,000 rpm for 10 min, and inserts placed into autosampler vials.

2.3. LC–MS analysis

The LC–MS was an Agilent (Palo Alto, CA) 1100 series system, with a binary solvent pump, autosampler (15 μl injections), Atlantis dC_{18} (150 mm × 2.1 mm, 5 μm) column (Waters Corp, Milford, MA), and Phenomonex C_8 (4 mm × 2 mm) guard cartridge (Torrance, CA). The isocratic HPLC mobile phase was premixed acetonitrile, methanol and 10 mM formate (pH 3) in water (2.5:2.5:95%) delivered at 0.2 ml/min. Under these conditions, retention times were 4.1 min for M3G and d3-M3G, 6.3 min for morphine and d3-morphine, and 6.8 min for M6G and d3-M6G (Fig. 1). A ballistic gradient was run every 16 samples to regenerate the column and flush any accumulated buildup. The ballistic gradient was acetonitrile, methanol and 10 mM formate (pH 3) in water (2.5:2.5:95%), increased to 85% acetonitrile over 4 min, maintained at 85% acetonitrile for 4 min, then returned to the premixed mobile phase and equilibrated for 7 min.

The mass spectrometer was operated in positive electrospray ionization mode. Parameters were: nitrogen drying gas at 10 l/min and 325 °C; nebulizer pressure 138 kPa; capillary voltage 4500 V; and fragmentor 70 V. All analytes were monitored in the same ion group with *m/z* 286.2 and 289.2 for morphine and d3-morphine, *m/z* 462.2 for M3G and M6G, and *m/z* 465.2 for d3-M3G and d3-M6G.

2.4. Calibration standards and quality control samples

Dilutions of stock solutions (morphine, M3G, M6G, and the deuterated internal standards) were prepared in water and stored at −20 °C. Calibration curves were obtained by analyzing drug-free plasma fortified with 0.5, 1, 2, 5, 7.5, 10, 25, 50, 100, 200 ng/ml morphine and M6G, and 2, 5, 25, 50, 100, 200, 500, 750, 1000, 2000 ng/ml M3G. Quality control (QC) samples in plasma (2, 10, 100 ng/ml morphine and M6G; 25, 250, 1000 ng/ml M3G) were prepared from separate dilutions of stocks than those used for calibration curves. Calibration and QC samples were aliquotted and stored at −20 °C until extracted. Calibration and QC samples were analyzed daily with the analytical samples. Standard curves were constructed using weighted (1/*y*) linear regression. The acceptance standard for the calibration curves was a regression coefficient (r^2) >0.95 and back-calculated values of calibrations standards that deviated less than 15% from nominal and less than 20% at the limit of quantification.

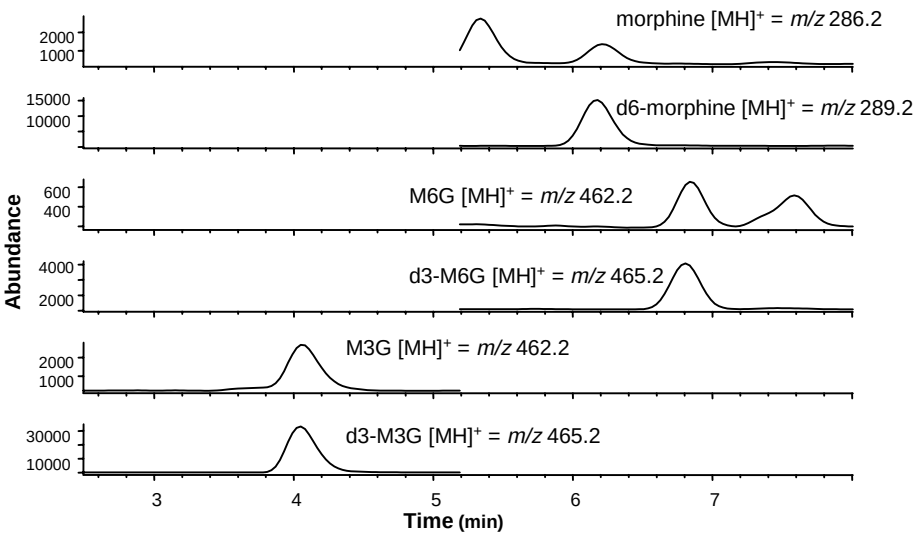

Fig. 1. Representative chromatogram of the lowest concentration plasma calibration standard (0.5 ml, 0.5 ng/ml morphine, 0.5 ng/ml M6G, 2.0 ng/ml M3G) extracted using 96-well SPE as described in Section 2.5. Internal standard concentrations were 10 ng/ml d3-morphine, 10 ng/ml d3-M6G, and 50 ng/ml d3-M6G.

2.5. Method validation

Accuracy and precision (coefficient of variation, CV(%)) were evaluated at three concentrations using QC samples for each analyte (2, 10 and 100 ng/ml for morphine and M6G, and 25, 250 and 1000 ng/ml for M3G). The assay was considered acceptable if the variation and deviation were <20% at the low QC (including diluted samples) and <15% for medium and high QC samples for intra- and inter-day runs.

Recovery was calculated by comparing the peak area of analyte, added to and extracted from plasma, with that of an unextracted sample. Stock solutions of low, medium, high, and internal standards were added into blank plasma, extracted, and compared to the same solutions placed directly into auto-sampler inserts. The assay was considered acceptable if recovery exceeded 60% for all concentrations.

Specificity testing evaluated potential interference from other sample components, since this method was designed for a clinical study involving morphine and quinidine coadministration. Two sets of plasma were prepared at medium (10 ng/ml morphine and M6G, and 250 ng/ml M3G) and high (100 ng/ml morphine and M6G, and 1000 ng/ml M3G) concentrations, to which was added 6 μg/ml quinidine, based on the maximum plasma quinidine concentration anticipated [23]. Also tested was potential interference from hemolyzed red blood cells. Red blood cells (25 μl) were added to the sample prior to each extraction.

The limit of detection (LOD) was defined as a signal to noise ratio of 2:1. The limit of quantification (LOQ) was the lowest concentration on the standard curve with an acceptable level of variation (<20%) and a signal to noise ratio >10:1.

Stability of morphine, M3G, and M6G was assessed in several ways. Unextracted QC plasma samples were subjected to three freeze/thaw cycles (thawed, left at room temperature for 3 hr then refrozen) on consecutive days. This sample set was compared to untreated QC samples extracted and run in the same session. Reconstituted extracted samples were subjected to two conditions: 48 h at 4 °C and 24 h at room temperature. Robustness (capacity of the assay to remain unaffected by deliberate changes) was determined by comparing results obtained from QC samples prepared by different analysts, extracted from cartridges and plates, different HPLC columns, and samples run on different analytical instruments.

Statistical difference was determined by Student's *t*-test. Significance was assigned at $P < 0.05$.

2.6. Method application

The method was applied to samples obtained from a clinical investigation of morphine disposition, which was approved by the University of Washington Institutional Review Board and performed after obtaining written informed consent. The subject received 30 mg oral morphine and venous blood samples were obtained for 8 h. Plasma was stored at −20 °C prior to analysis.

3. Results and discussion

3.1. Extraction procedure

An extraction process was needed to rapidly extract large numbers of clinical samples while providing high sensitivity. Sample volume was an issue since samples contained only 1.0–2.5 ml. Previous methods [12,13,17,19,21], with the exception of Shou et al. [20], used at least 1 ml. Using these volumes, insufficient sample for might have been available if re-extraction was needed.

Initially, Oasis HLB (3 ml, 30 μm) SPE cartridges were used with 1 ml plasma. Unidentified endogenous interferences persisted after extraction, which proved problematic at low analyte concentrations. When smaller samples were used to reduce interferent amounts, sensitivity was substantially and unacceptably reduced. Therefore, other sorbents were evaluated. Unlike other C_{18} sorbents, Oasis MCX can go to dryness during extraction, allowing consistent recovery without sorbent collapse as found problematic by Shou et al. [20]. Using Oasis MCX cartridges, only 0.5 ml plasma, and the generic quick start SPE method (condition with methanol then water, load sample, wash with 0.1 N HCl and methanol, elute with 5% ammonium hydroxide in methanol), a significant reduction in interference was achieved. However, morphine recovery (<35%) and hence sensitivity was low. MCX cartridges contain a mixed-mode polymeric sorbent with reversed-phase and cation

Table 1
Analyte recovery using Oasis MCX 96-well plates

	Recovery (%)		
	Low QC	Medium QC	High QC
Morphine (ng/ml)	2	10	100
Mean (CV (%))	81 ± 10 (12)	81 ± 5 (5.6)	74 ± 1 (1.8)
M3G (ng/ml)	25	250	1000
Mean (CV (%))	90 ± 13 (14)	77 ± 5 (6.1)	62 ± 3 (3.7)
M6G (ng/ml)	2	10	100
Mean (CV (%))	82 ± 4 (4.3)	75 ± 9 (12)	72 ± 5 (6.7)

Results are shown as the mean ± S.D. (CV (%)), $n = 6$.

exchange functionality which allows binding of both lipophilic morphine and hydrophilic metabolites. The methanol wash step in the generic procedure removes interferences retained by hydrophobic interactions, however a significant amount of morphine eluted in the methanol wash. Washing with as little as 10% methanol still reduced morphine recovery by 50%. Other organic solvents were evaluated at various concentrations, but also diminished recovery. Deleting the organic wash step improved morphine recovery to 80% at low concentrations (Table 1), comparable to or better than previously published methods. Endogenous interferences that remained were separated successfully by HPLC. To improve assay efficiency, the extraction was applied to 96-well plates, which increased throughput four-fold. No significant differences in recovery, precision or accuracy were found between extractions using 1 ml cartridges and 96-well plates.

3.2. Chromatography

Separation was initially evaluated with an Alltech Inertsil ODS-3 (150 mm × 2.1 mm, 5 μm) column. An endogenous compound, not removed by MCX SPE, co-eluted and interfered with morphine. Preliminary results indicated that a low (<10%) percentage of organic solvent in the eluent would be needed to separate morphine and the interferent. Due, however, to the minimal analyte retention under these conditions, small variations in organic composition resulted in significant changes in separation and retention times. In addition, use of low organic solvent concentrations (<10%) for long periods was not recommended by the column manufacturer. Thus, the assay was switched to an Atlantis dC_{18} column, which can accommodate very low organic solvent compositions to achieve optimal separation. Decreasing the organic fraction to 5% (2.5% methanol and 2.5% acetonitrile) at 0.2 ml/min separated the morphine interferent and provided baseline separations while allowing run times <8 min (Fig. 1). Shorter run times could separate the three analytes, however, 8 min runs were needed to achieve baseline separation from the morphine interferent. Due to the removal of the organic solvent SPE wash step and the low percentage of organic solvent in the mobile phase, interference buildup was noted after approximately 25 samples. The column was therefore flushed with a ballistic gradient every 16 samples. No carryover was evident for any of the analytes. A chromatogram of blank plasma is shown in Fig. 2.

3.3. Validation

Mean recoveries of morphine, M3G and M6G were 81, 90 and 82% at the low QC (2, 25 and 2 ng/ml), 81, 77 and 75% at the medium QC (10, 250 and 10 ng/ml), and 74, 62 and 72% at the high QC (100, 1000 and 100 ng/ml), respectively (Table 1). Lower recovery at high M3G concentrations was obviated by using a deuterated internal standard.

Precision and accuracy data for intra- and inter-day QC samples are summarized in Table 2. The coefficient of variation (CV) for both inter and intra-day determinations was <8% at all concentrations. Dilutions (10-fold) of medium and high QC samples were within 15% of expected concentrations with a CV <5% at the high QC concentration for all analytes (Table 3).

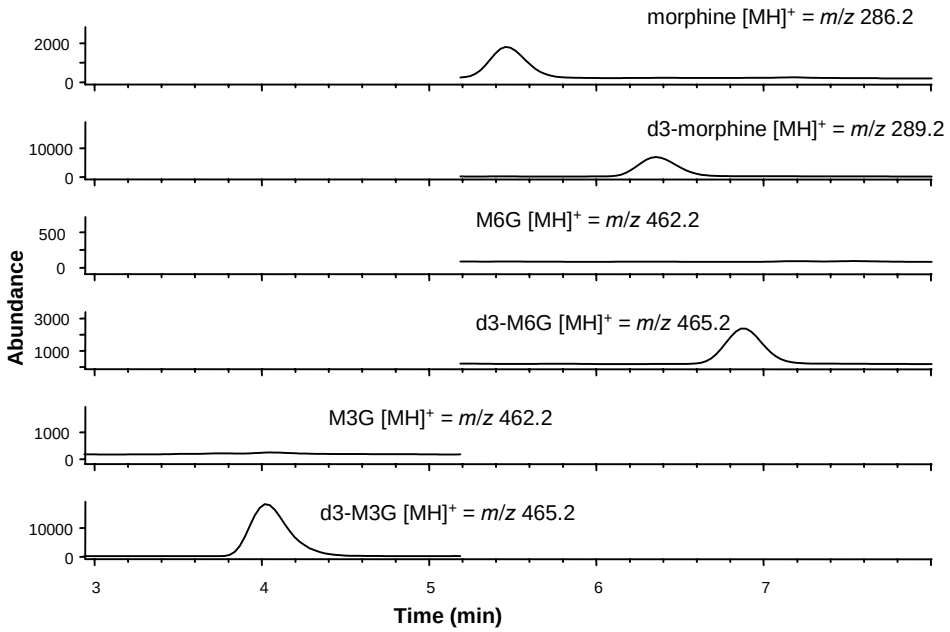

Fig. 2. Representative chromatogram of blank plasma with internal standards extracted using 96-well SPE as described in Section 2.5.

Inter-day comparison of calibration standards is provided in Table 4. The assay was linear up to the highest concentration (200 ng/ml morphine and M6G, and 2000 ng/ml M3G). The inter-day CV was <9% for all analytes. The accuracy for all calibration standards was within 5, 15, and 9% of the expected values for morphine, M3G, and M6G, respectively. Linearity of all calibration curves was excellent ($r^2 > 0.99$).

The LOQ was 0.5 ng/ml morphine and M6G and 5 ng/ml M3G. Although M3G peaks were detected with a signal to noise ratio >10:1 at concentrations <5 ng/ml, more than 10% of the blank plasma samples

Table 2
Accuracy and precision (CV (%)) of intra- and inter-day QC samples

	Intra-day ($n = 5$)			Inter-day ($n = 6$)		
	Low QC	Medium QC	High QC	Low QC	Medium QC	High QC
Morphine (ng/ml)	2	10	100	2	10	100
Mean ± S.D.	1.93 ± 0.06	9.36 ± 0.06	94.0 ± 1.1	1.86 ± 0.07	9.72 ± 0.45	96.8 ± 1.7
CV (%)	3.3	0.6	1.1	3.6	4.6	1.7
Accuracy (%)	97	94	94	93	97	97
M3G (ng/ml)	25	250	1000	25	250	1000
Mean ± S.D.	29.6 ± 0.1	272 ± 1	974 ± 6	29.3 ± 0.5	271 ± 11	996 ± 6
CV (%)	0.44	0.29	0.58	1.6	4.0	0.59
Accuracy (%)	118	109	97	117	109	100
M6G (ng/ml)	2	10	100	2	10	100
Mean ± S.D.	2.22 ± 0.14	11.1 ± 0.4	103 ± 2	2.31 ± 0.17	10.9 ± 0.3	99.0 ± 5.6
CV (%)	6.1	4.0	2.1	7.2	2.5	5.6
Accuracy (%)	111	111	103	116	109	99

Table 3
Dilution (10-fold) evaluations

	Intra-day ($n = 5$)		Inter-day ($n = 3$)	
	Medium QC	High QC	Medium QC	High QC
Morphine (ng/ml)	1	10	1	10
Mean ± S.D.	0.95 ± 0.04	8.84 ± 0.1	0.93 ± 0.07	9.59 ± 0.45
CV (%)	3.7	1.5	7.0	4.7
Accuracy (%)	95	88	93	96
M3G (ng/ml)	25	100	25	100
Mean ± S.D.	27.7 ± 0.3	103 ± 1	26.9 ± 1.5	111 ± 4
CV (%)	1.2	0.57	5.5	3.3
Accuracy (%)	111	103	107	111
M6G (ng/ml)	1	10	1	10
Mean ± S.D.	1.03 ± 0.19	10.2 ± 0.2	1.15 ± 0.05	10.4 ± 0.5
CV (%)	18	2.1	4.0	4.9
Accuracy (%)	103	102	115	104

contained interferences whose areas exceeded 25% of the LOQ. The LOD was 0.25, 0.25 and 1 ng/ml for morphine, M6G, and M3G, respectively.

Stability was assessed by comparing newly extracted calibration curves and QC samples with those that were extracted, reconstituted in mobile phase, and stored at room temperature for 24 h or 4 °C for 48 h (Table 5). There were no significant differences in mean values between the sample sets. QC plasma samples ($n = 5$) underwent three freeze/thaw cycles and were then extracted and analyzed, with no effects on compound stability (Table 5). No interference with any of the analytes by quinidine or red blood cell hemolysis was observed (data not shown).

Several factors were tested to ascertain robustness. There were no significant differences between results obtained from QC samples prepared, extracted, and quantified by a different analyst; extraction with different SPE formats (1 ml cartridges versus 96-well plates), analysis on different HPLC columns (Oasis Atlantis versus Alltech Inertsil), or using two different Agilent 1100 MSD instruments. Under the above robustness conditions, the CV between sets was less than 12% with all QC samples tested.

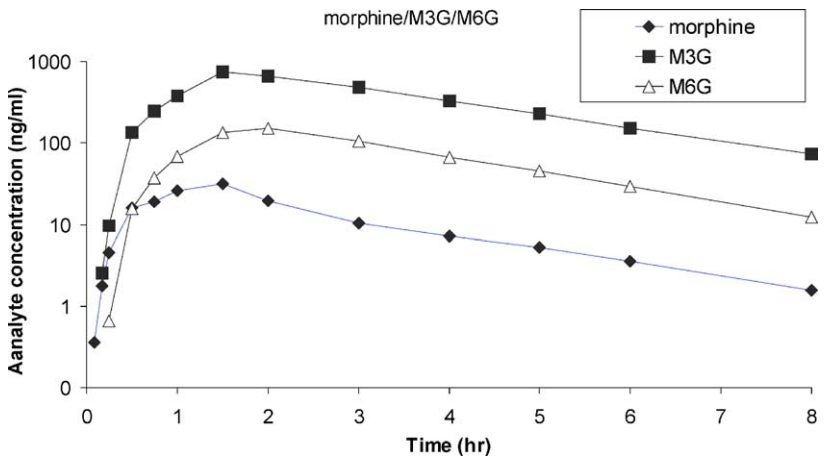

Fig. 3. Plasma morphine and glucuronide metabolite concentrations, analyzed with the 96-well SPE LC–MS assay, from a clinical trial subject dosed with 30 mg oral morphine.

Table 4
Accuracy, precision (CV (%)), and linearity of inter-day calibration standards ($n = 3$)

											Slope	r^2	y-intercept
Calibration standards (ng/ml)													
Morphine	0.5	1	2	5	7.5	10	25	50	100	200			
Mean ± S.D.	0.51 ± 0.01	0.97 ± 0.06	2.07 ± 0.06	5.01 ± 0.11	7.52 ± 0.28	1.00 ± 0.6	26.0 ± 0.5	50.8 ± 1.2	98.9 ± 2.1	191 ± 2	0.116 ± 0.01	0.997 ± 0.0	0.007 ± 0.01
CV (%)	2.8	6.1	2.8	2.3	3.7	5.7	1.9	2.4	2.2	1.3	5.54	0.1	
Accuracy (%)	101	97	103	100	100	100	104	102	99	95			
M3G	2	5	25	50	100	250	500	750	1000	2000			
Mean ± S.D.	1.95 ± 0.01	5.09 ± 0.02	27.7 ± 0.1	54.1 ± 0.1	108 ± 1	261 ± 1	512 ± 2	738 ± 6	959 ± 1	1690 ± 18	0.018 ± 0.0	0.993 ± 0.0	0.010 ± 0.0
CV (%)	0.4	0.4	0.3	0.1	1	0.3	0.4	0.8	0.1	1.1	2.73	0.09	
Accuracy (%)	98	102	111	108	108	105	102	98	96	85			
M6G	0.5	1	2	5	7.5	10	25	50	100	200			
Mean ± S.D.	0.50 ± 0.03	0.99 ± 0.09	2.03 ± 0.09	5.08 ± 0.07	7.92 ± 0.37	10.1 ± 0.3	26.1 ± 1.3	51.4 ± 1.7	99.3 ± 1.4	182 ± 7	0.112 ± 0.0	0.996 ± 0.0	0.01 ± 0.0
CV (%)	5.7	8.9	4.4	1.3	4.6	2.6	5.1	3.2	1.4	3.7	1.01	0.33	
Accuracy (%)	100	99	101	102	106	102	104	103	99	91			

Table 5
Stability evaluation

	Freeze/thaw[a] ($n = 5$)			48 h at 4 °C ($n = 5$)			24 h at 21 °C ($n = 5$)	
Morphine (ng/ml)	10	100	2	10	100	2	10	100
Mean ± S.D.	9.17 ± 0.11	90.7 ± 1.2	1.92 ± 0.08	9.60 ± 0.15	93 ± 2	1.96 ± 0.13	9.55 ± 0.20	94.0 ± 2.9
CV (%)	1.2	1.3	4.0	1.5	1.7	6.7	2.1	3.1
Accuracy (%)	92	91	96	96	93	98	95	94
M3G (ng/ml)	250	1000	25	250	1000	25	250	1000
Mean ± S.D.	271 ± 2	960 ± 6	29.2 ± 0.2	277 ± 1	983 ± 5	28.7 ± 0.9	271 ± 8	976 ± 20
CV (%)	0.6	0.6	0.8	0.5	0.5	3.1	2.8	2.0
Accuracy (%)	108	96	117	111	98	115	108	98
M6G (ng/ml)	10	100	2	10	100	2	10	100
Mean ± S.D.	10.9 ± 0.2	100 ± 2	2.32 ± 0.09	11.0 ± 0.4	99.1 ± 2.7	2.35 ± 0.33	10.9 ± 0.4	97.6 ± 1.5
CV (%)	2.1	1.6	4.1	3.8	2.7	14	3.3	1.6
Accuracy (%)	109	100	116	110	99	117	109	98

[a] Samples underwent a series of three freeze/thaw cycles from −20 °C to room temperature.

Fig. 3 presents preliminary data from a clinical trial subject dosed with 30 mg of oral morphine and plasma samples analyzed with the method validated in this paper.

4. Conclusion

The present assay compares favorably to and has some advantages over existing methods. In published methods, recoveries range from 48% for morphine [20] to >94% for all analytes [15], with large variability between different extraction methods. Using 96-well MCX plates, recovery was 75–90% at low and intermediate QC concentrations. Sensitivity was excellent, with an LOQ of 0.5, 0.5 and 5 ng/ml morphine, M6G and M3G. This was better than the 0.8, 5 and 2 ng/ml using larger, 1 ml samples and LC–MS [12]. It was also comparable to LC–MS–MS techniques. For example, the lowest LOQ using LC–MS–MS (and 1 ml plasma) was 0.5, 0.25 and 0.5 ng/ml for morphine, M6G and M3G, respectively [18]. Throughput was substantially improved over single cartridge methods. Total LC–MS run time for 70 samples was <12 h. High throughput 96-well plate extraction combined with LC–MS analysis enabled one analyst to evaluate >70 samples per day.

In summary, a LC–MS method for single-quadrupole mass spectrometry for the quantification of morphine and morphine glucuronides in human plasma was designed and validated. The assay is sensitive, precise, accurate, robust, and permits a high degree of throughput for a manual assay. The assay was well suited for pharmacokinetic studies of morphine, M3G and M6G.

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