Journal of Chromatography B, 980 (2015) 65–71

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Capillary electrophoresis-mass spectrometry determination of morphine and its isobaric glucuronide metabolites

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ARTICLE INFO

Article history:
Received 14 September 2014
Received in revised form
20 November 2014
Accepted 29 November 2014
Available online 19 December 2014

Keywords:
Morphine
Glucuronide metabolites
M3G
M6G
Capillary electrophoresis
Quadrupole time of flight mass spectrometry

ABSTRACT

The determination of morphine and its isobaric metabolites morphine-3-beta-D-glucuronide (M3G) and morphine-6-beta-D-glucuronide (M6G) is useful for therapeutic drug monitoring and forensic identification of drug use. In particular, capillary electrophoresis with mass spectrometry (CE-MS) represents an attractive tool for opioid analysis. Whereas volatile background electrolytes in CE often improve electrospray ionization for coupled MS detection, such electrolytes may reduce CE separation efficiency and resolution. To better understand the effects of background electrolyte (BGE) composition on separation efficiency and detection sensitivity, this work compares and contrasts method development for both volatile (ammonium formate and acetate) and nonvolatile (ammonium phosphate and borate) buffers. Peak efficiencies and migration times for morphine and morphine metabolites were optimal with a 25 mM ammonium borate buffer (pH = 9.5) although greater sensitivities were achieved in the ammonium formate buffer. Optimized CE methods allowed for the resolution of the isobaric morphine metabolites prior to high mass accuracy, electrospray ionization quadrupole time-of-flight (ESI-QTOF) MS detection applicable to the analysis of urine samples in under seven minutes. Urine sample preparation required only a 10-fold dilution with BGE prior to analysis. Limits of detection (LOD) in normal human urine were found to be 1.0 μg/mL for morphine and 2.5 μg/mL for each of M3G and M6G by CE-ESI-QTOF-MS. These LODs were comparable to those for CE-UV analysis of opioid standards in buffer, whereas CE-ESI-QTOF-MS analysis of opioid standards in buffer yielded LODs an order of magnitude lower. Patient urine samples ($N = 12$) were analyzed by this new CE-ESI-QTOF-MS method and no significant difference in total morphine content relative to prior liquid chromatography-mass spectrometry (LC-MS) results was found as per a paired-t test at the 99% confidence level. Whereas the LC-MS method applied to these samples determined only total morphine content, this new CE-ESI-QTOF-MS method allowed for species differentiation in addition to total morphine determination. By this method, it was found that M3G and M6G metabolites were present in a 5:1 concentration ratio, on average, in patient samples. Therefore, the CE-ESI-QTOF-MS method not only allows for total morphine concentration determination comparable to established LC-MS methods, but also allows for differentiation between morphine and its trace glucuronides, yielding additional biochemical information about drug metabolism.

1. Introduction

Morphine is an opioid prescribed for therapeutic pain management, although it is also used illicitly. Morphine is predominately metabolized to morphine-3-beta-D-glucuronide (M3G) and morphine-6-beta-D-glucuronide (M6G) via phase II metabolism, which involves conjugation with uridine diphosphoglucuronic acid [1,2]. Structures are shown in Fig. 1. The isolation and characterization of drug metabolites is essential to understanding the pharmacological effects induced by the parent drug for individual patients. For example, the main metabolite of morphine, M3G, has no opioid action whereas M6G, which is produced in smaller amounts, is 200 times more potent than morphine and also possesses analgesic properties [3,4]. Therefore, the separation and structural characterization of metabolites is very important to the process of drug analysis and monitoring [2].

Drug metabolites are often studied by high performance liquid chromatography (HPLC); however, since phase II metabolites

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http://dx.doi.org/10.1016/j.jchromb.2014.11.035
1570-0232/

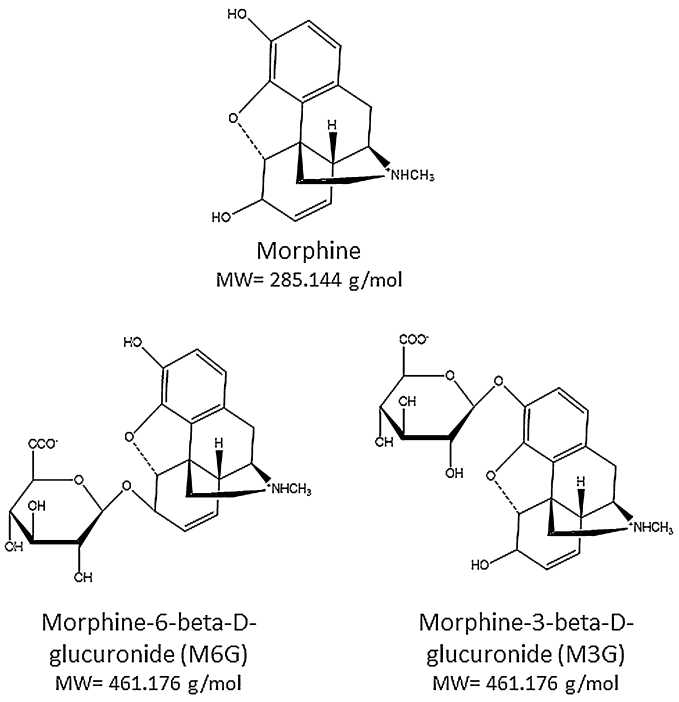

Fig. 1. Molecular structures of morphine and its isobaric metabolites morphine-3-beta-D-glucuronide (M3G) and morphine-6-beta-D-glucuronide (M6G).

are generally acidic, thermally unstable, and very polar, they are difficult to analyze. Capillary electrophoresis (CE) is becoming an increasingly attractive separation technique for these metabolites due to its ability to separate charged compounds with high efficiency, small sample size, fast analysis times, and low solvent consumption [4,5]. The most widely used detector for CE is UV–Vis absorbance; however, this detection method provides limited sensitivity due to the short optical path length provided by the capillary itself [6]. Although CE with laser-induced fluorescence detection (CE-LIF) offers greater sensitivity than CE-UV, it often necessitates additional sample work-up in order to render the analytes fluorescent. The coupling of CE with mass spectrometry (MS) provides improved sensitivity (without the need for sample derivatization), as required to detect often low levels of drug metabolites. Furthermore, CE-MS can determine both the exact mass of analytes and structural information including the possibility to identify and determine co-migrating species in overlapping peaks through the use of specific MS-MS transitions [7,8]. Therefore, even though M3G and M6G are isobars of one another, complete resolution and quantification should be achievable using CE-ESI-QTOF-MS.

The coupling of CE to MS is most commonly done through the use of electrospray ionization (ESI). ESI is useful in this capacity because: (i) it allows for the detection of species of high molecular mass with multiple charges; (ii) it facilitates the direct transfer of analyte molecules from the separation capillary to the mass spectrometer with minimal additional make-up flow; and (iii) it provides both stable spray current and high ionization efficiency [5,9,10]. When CE is coupled to MS, an additional requirement is for the interface to provide electrical grounding of the CE high voltage employed for separation without compromising analyte ionization or ESI spray stability. Since CE provides fast and efficient separations that often yield very small peak widths, the mass spectrometer must be able to produce a sufficient number of data points across the peak width. A time-of-flight (TOF) mass analyzer can be used to achieve this due to its high data acquisition rate, providing spectra on the order of milliseconds. Time-of-flight (TOF) offers numerous other advantages, including higher mass accuracy, larger upper mass-to-charge limit, lower detection limits, faster scan rates, and relatively lower cost compared to other high mass accuracy mass analyzers [8].

Typically, the background electrolyte (BGE) employed for CE separations coupled to ESI-QTOF-MS detection is volatile and of relatively low ionic strength [11]. Some of the most widely used BGEs for CE-MS are acetic acid, formic acid, and their ammonium salts. Unfortunately, CE separation efficiency and resolution may not be optimal when using volatile buffers, which can result in peak broadening during the electrophoretic separation process [12]. While the use of nonvolatile buffers may improve CE separation, it may adversely affect MS detection because of signal suppression due to salt deposits on the electrospray needle and/or decreased response due to ion-pairing with the analyte [7,9].

Whereas Oliveira et al. [13] recently described an HPLC method for the determination of morphine, M3G and M6G in a single 35-min run necessitating a multi-step sample extraction and purification procedure, we sought to develop a CE-based method to provide optimal resolution and quantitation of these analytes in lesser time and with lesser sample preparation, without sacrificing sensitivity. As such, we first undertook a study of the effect of BGE volatility on CE separation efficiency with UV–Vis absorbance detection of analytes. Prior work by Gottardo et al. [12] explored the use of nonvolatile buffers for the analysis of drugs, finding that ammonium phosphate buffer provided good separation of MDA, MDMA, methadone, morphine, codeine, 6-MAM, and cocaine by CE compared to the more volatile ammonium formate separation buffer. In our present work, optimized separation conditions for morphine, M3G, and M6G were translated to a CE-ESI-QTOF-MS system, with no significant reduction in detection sensitivity for urine samples despite the choice of a nonvolatile buffer to favor separation efficiency. To our knowledge, the simultaneous determination of morphine and its isobaric metabolites M3G and M6G by the CE-ESI-QTOF-MS method developed herein represents the first such report able to selectively quantify the levels of parent drug and both major metabolites in a single CE run. These results were validated by comparison to total morphine levels in patient urine samples determined by HPLC-MS.

2. Materials and methods

2.1. Reagents, solutions, standards, and samples

Standards of morphine, M3G, and M6G were purchased from Cerilliant (Round Rock, TX, USA). Each standard came prepared in methanol (1 mg/mL) and was diluted to 40 μg/mL with BGE prior to analysis for the CE-UV method optimization. Morphine-D_3 (Cerilliant) was prepared to 5 μg/mL in BGE and was used as an internal standard in CE-ESI-QTOF-MS studies.

Two volatile BGE solutions (ammonium formate and ammonium acetate) and two nonvolatile BGE solutions (ammonium phosphate and ammonium borate) were compared during CE-UV method optimization studies. Each BGE was prepared to 15, 25, 50, 75, or 100 mM by appropriate dilution (with Milli-Q distilled, deionized water (Millipore, Bedford, MA, USA)) of 1 M stock solutions of formic acid (Acros Organics, New Jersey, USA), boric acid (Sigma Aldrich, St. Louis, MA, USA), or acetic acid or phosphoric acid (Fisher Scientific, Fair Lawn, NJ, USA), with pH adjustment achieved by the addition of 1 M ammonium hydroxide (Fisher Scientific).

Certified drug-free normal human urine (UTAK Laboratories, Valencia, CA) was spiked with morphine-D_3 to prepare urine standards with a fixed concentration of 5 μg/mL morphine-D_3 upon 10-fold dilution with BGE. Authentic patient positive samples (provided in-kind from Ameritox, Ltd.) were treated likewise. Samples were analyzed immediately after preparation by CE-ESI-QTOF-MS.