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High-sensitivity analysis of buprenorphine, norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide in plasma and urine by liquid chromatography–mass spectrometry☆

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ABSTRACT

A new method using ultra-fast liquid chromatography and tandem mass spectrometry (UFLC–MS/MS) was developed for the simultaneous determination of buprenorphine and the metabolites norbuprenorphine, buprenorphine-3β-glucuronide, and norbuprenorphine-3β-glucuronide in plasma and urine. Sample handling, sample preparation and solid-phase extraction procedures were optimized for maximum analyte recovery. All four analytes of interest were quantified by positive ion electrospray ionization tandem mass spectrometry after solid-phase microextraction. The lower limits of quantification in plasma were 1 pg/mL for buprenorphine and buprenorphine glucuronide, and 10 pg/mL for norbuprenorphine and norbuprenorphine glucuronide. The lower limits of quantitation in urine were 10 pg/mL for buprenorphine, norbuprenorphine and their glucuronides. Overall extraction recoveries ranged from 68–100% in both matrices. Interassay precision and accuracy was within 10% for all four analytes in plasma and within 15% in urine. The method was applicable to pharmacokinetic studies of low-dose buprenorphine.

1. Introduction

Buprenorphine is an opioid used for decades in treating acute and chronic pain [1–3]. A transdermal formulation was recently approved for treating moderate-severe chronic pain. Buprenorphine sublingual tablets or films are also used for opioid addiction therapy [4], and for reducing addiction-related infectious diseases [5]. Clinical advantages of buprenorphine in addiction treatment include approval for use by private (non-specialized) practitioners, and a narrow dose range enabling rapid dose titration [5]. Reported advantages in pain therapy include a ceiling effect for respiratory depression but not analgesia at clinically relevant doses, fewer adverse events than other opioids, and the rarity of withdrawal symptoms [6–10].

Buprenorphine is extensively metabolized, primarily to norbuprenorphine, and both also undergo glucuronidation, to buprenorphine-3β-glucuronide and the secondary metabolite, norbuprenorphine-3β-glucuronide (Fig. 1) [11,12]. Plasma metabolite concentrations can approximate or exceed those of the parent drug, and relative norbuprenorphine, buprenorphine-3β-glucuronide, and norbuprenorphine-3β-glucuronide exposures, based on molar area under the plasma concentration vs time curves, are 200%, 100%, and 600% those of buprenorphine [13]. These buprenorphine metabolites are pharmacologically active in animals [14–17]. Metabolism therefore may potentially constitute a bioactivation pathway, although the role of these metabolites in mediating buprenorphine effects in humans is unknown. Additional pathways of buprenorphine metabolism have also been recognized [18]. Clinical disposition and metabolism of buprenorphine are subject to well-described drug interactions [19–21].

Methods for quantification of buprenorphine and one or more major metabolites in plasma and/or urine include immunoassay [22,23], gas chromatography with various detectors [24–28], HPLC with single quadrupole or tandem quadrupole mass spectrometry [13,29–35], and most recently UPLC methods with mass spectrometry [36–40]. Various methods of sample preparation have also been described, including protein precipitation [40], liquid/liquid extraction [39], and solid-phase extraction [37,41,42]. The most sensitive of the above assays generally have a lower limit of quantification of approximately 0.1 ng/mL for buprenorphine and 0.25 ng/mL for norbuprenorphine, and the glucuronide metabolites are often not analyzed.

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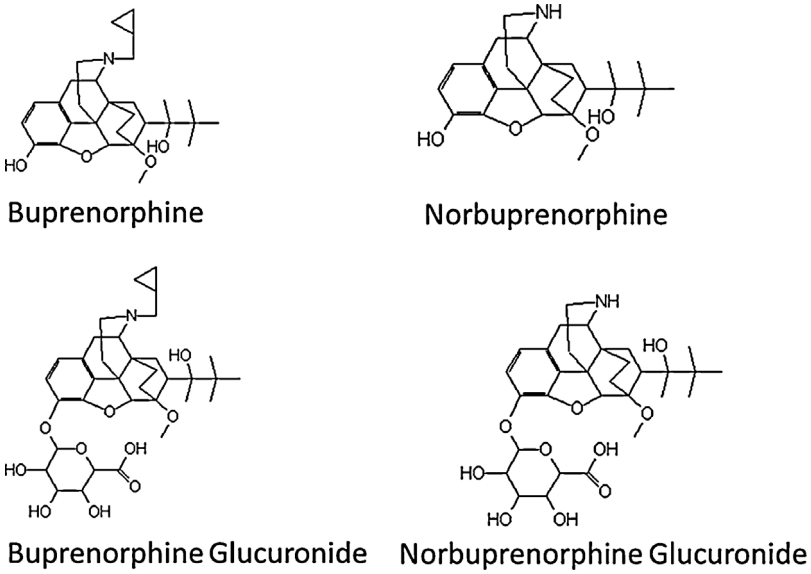

Fig. 1. Structures of buprenorphine and the metabolites norbuprenorphine, buprenorphine-3β-glucuronide and norbuprenorphine-3β-glucuronide.

Increasing interest in the clinical use of buprenorphine, recognition of metabolites formation and their potential clinical significance, use of lower buprenorphine doses for pain, and the desire to study buprenorphine drug interactions in healthy volunteers (at much lower doses than used in patients for prevention of opioid withdrawal) have accentuated interest in analytical methods with greater sensitivity for buprenorphine and metabolites in both plasma and urine. Buprenorphine is commonly administered sublingually at 4–32 mg/d for addiction maintenance therapy, with steady-state plasma buprenorphine concentrations averaging 5–10 ng/mL, and 2–16 mg intravenous buprenorphine results in peak buprenorphine and norbuprenorphine concentrations of 20–130 and 0.5–3.5 ng/mL respectively [43]. In contrast, lower doses (0.15–0.2 mg intravenously, 2 mg sublingually) must be used in non-dependent healthy volunteers [44]. Assuming linear pharmacokinetics, 0.2 mg/kg intravenously would have an expected peak plasma concentration (C_{max}) of approximately 2 ng/mL, declining to approximately 0.005 ng/mL after 72 h, and a 2 mg sublingual dose gives an expected Cmax of 1.6 ng/mL [45]. With transdermal buprenorphine patches delivering 5, 10 and 20 μg/h maximum plasma buprenorphine concentrations were 176, 191 and 476 pg/mL, respectively [10]. These studies exemplify the need for assay sensitivity.

Therefore, the purpose of this investigation was to develop and validate an analytical method for the analysis of buprenorphine and the metabolites norbuprenorphine, buprenorphine-3β-glucuronide, and norbuprenorphine-3β-glucuronide, in plasma and urine, with greater sensitivity, particularly over the expected plasma concentration range of low pg/mL to low ng/mL. A target limit of quantification of 1 pg/mL was established.

2. Experimental

2.1. Reagents

Buprenorphine, buprenorphine-d4, norbuprenorphine, norbuprenorphine-d3, buprenorphine-3β-glucuronide (B3G) and norbuprenorphine-3β-glucuronide (N3G) were all purchased from Cerilliant Corporation (Round Rock, TX). Phosphoric acid, formic acid, glacial acetic acid, methanol and acetonitrile were all obtained from Sigma-Aldrich (St. Louis, MO). Ammonium hydroxide was from JT Baker (Center Valley, PA). Water was filtered by a Milli-Q water filtration system, Millipore (Billerica, MA). Outdated human plasma and human urine were obtained from the local university hospital. Oasis MCS 96 well microextraction plates for method development purposes were kindly donated by Waters (Milford, MA).

2.2. Instrumentation

LC–MS/MS analysis was performed on an ultra-fast liquid chromatography system from Shimadzu Scientific Instruments (Columbia, MD) consisting of a CMB-20A system controller, two LC-20ADXR pumps, a DGU-20A3 degasser, a SIL-20AC autosampler, and a CTO-20A column oven. An external Valco switching valve was installed between the chromatography system and the mass spectrometer. The chromatography system was coupled to an API 4000 QTrap LC–MS/MS linear ion trap triple quadrupole tandem mass spectrometer from Applied Biosystems/MDS Sciex (Foster City, CA).

2.3. Solutions

Methanol stock solutions of buprenorphine, norbuprenorphine, B3G and N3G (100 μg/mL) from Cerilliant Corporation were stored at −20 °C. Composite 1 μg/mL standard solutions containing buprenorphine, norbuprenorphine, B3G, and N3G were prepared in duplicate in methanol from the 100 μg/mL stock solutions (CS1 and CS2). CS1 was utilized to make the working standard solutions for the standard curve and CS2 was used to make the working standard solutions for the quality control samples. Composite standards (10 μg/mL) were prepared and used to make urine calibrators and quality control samples (UCS1 and UCS2) which were prepared in a similar manner to the plasma standards. Internal standard solutions were made by spiking 100 ug/mL methanol stocks of buprenorphine-d4 and norbuprenorphine-d3 into 5% phosphoric acid to a final concentration of 0.5 ng/mL of each compound. This solution was found to be stable for several months stored at 4 °C. The solid-phase extraction (SPE) elution solution was acetonitrile, methanol and 30% ammonium hydroxide (12:8:1).

2.4. Standard and quality control preparation

Working plasma standards were prepared in methanol at the following concentrations of buprenorphine, norbuprenorphine,