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Simultaneous analysis of codeine and its active metabolites in human plasma using liquid chromatography–tandem mass spectrometry: Application to a pharmacokinetic study after oral administration of codeine

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ABSTRACT

A rapid and sensitive bioassay based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developed and validated for the simultaneous determination of codeine and its active metabolites, including morphine, morphine 3β-glucuronide (M3G) and morphine 6β-glucuronide (M6G), in human plasma. Sample preparation of plasma after the addition of naloxone as internal standard (IS) involved solid-phase extraction (SPE) on C18 cartridges. Reversed-phase chromatography using a gradient elution with methanol and 0.04% formic acid solution (pH 3.5) was used for separation in a run time of 5 min. The analytes were detected in the positive ion mode using multiple reaction monitoring (MRM) of the transitions at m/z 300.4 → 215.2 for codeine, 286.2 → 152.0 for morphine, and 462.2 → 286.2 for M3G and M6G. The method has the following performance characteristics: a reliable response range of 0.05–80 ng/ml for codeine, M3G and M6G and a response range of 0.05–5.0 ng/ml for morphine with correlation coefficients (r) of >0.997 for all analytes. The lower limit of quantitation (LLOQ) for all four analytes was 0.05 ng/ml. The intra- and inter-day precision and accuracy of the quality control samples at low, medium and high concentration levels showed <12% relative standard deviation (RSD) and −6.9 to 8.1% relative error (RE) for all the analytes. The method was successfully applied to a pharmacokinetic study of codeine in healthy Mongolian Chinese volunteers after a 30 mg oral dose.

1. Introduction

Codeine, as shown in Fig. 1, is a powerful and effective analgesic acting on μ-opiate receptors, and has been widely used in the treatment of cough and various types of pain [1]. Codeine is mainly metabolized by the liver. About 10% of the dose is O-demethylated to morphine by the polymorphic cytochrome P450 isoenzyme 2D6 (CYP2D6). Morphine is further glucuronidated to form M3G and M6G (structures in Fig. 1) [2]. M6G has substantially greater analgesic effects when compared to morphine. Human experiments have demonstrated that codeine is a prodrug, and its analgesic, antitussive and antidiarrheal effects are strongly contributed to morphine and M6G [3–5]. Although M3G has no analgesic activity, it is thought to contribute to the neuroexcitatory effects attributed to morphine [6]. Due to the polymorphism of CYP2D6, the O-demethylation of codeine varies among different populations [7]. Poor metabolizers (PM) lack CYP2D6 activity and have extremely low plasma concentrations of morphine and morphine glucuronides leading to diminished pain relief and even tolerance. However, ultra-rapid metabolizers (UM) have enhanced CYP2D6 activity and may suffer from severe opioid side effects after the intake of codeine because of the high exposure to morphine and morphine glucuronides [8,9]. Therefore, development of a sensitive method for the quantitation of codeine and its active metabolites, including morphine, M3G and M6G, in plasma samples is needed to better understand the pharmacokinetic and pharmacodynamic properties of codeine in different populations.

Various analytical methods have been reported for the simultaneous determination of codeine, morphine and their respective glucuronides in biological samples, including methods based on gas chromatography-mass spectrometry (GC–MS) [10–12], liquid chromatography–mass spectrometry (LC–MS) [13] and liquid

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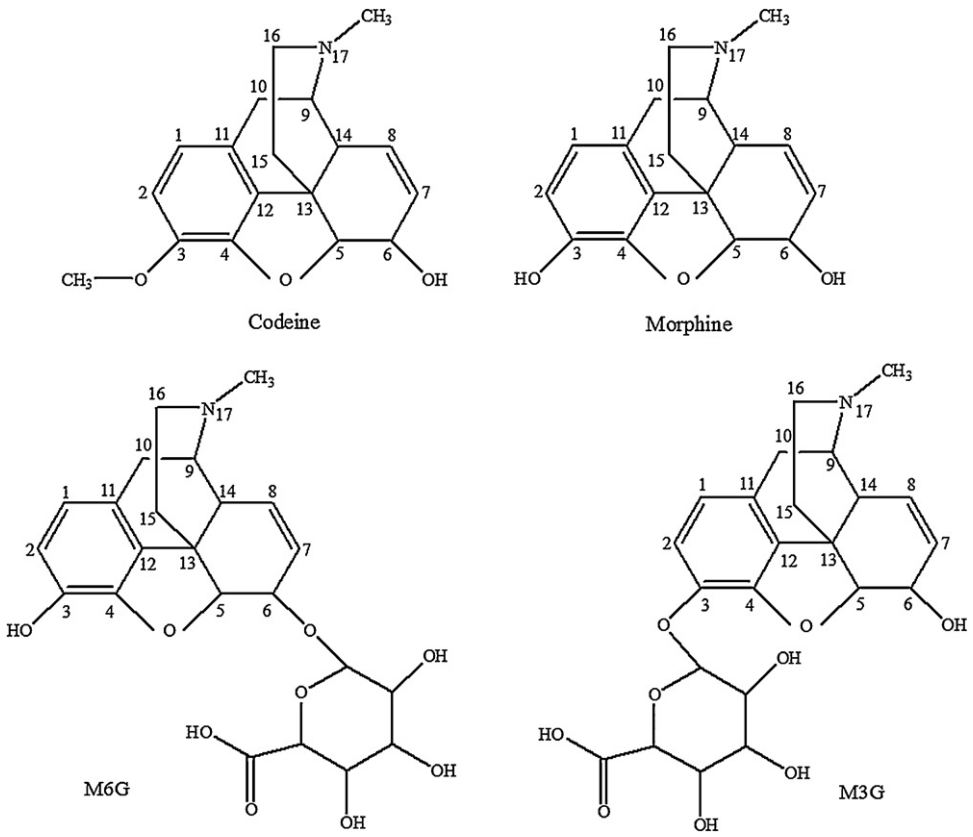

Fig. 1. Chemical structures of codeine, morphine, M3G and M6G.

chromatography–tandem mass spectrometry (LC–MS/MS) [14–17]. The GC–MS method has some disadvantages that limit its use in routine sample analysis, including complicated and time-consuming sample preparation, incomplete derivatization, derivatization side reactions, inadequate recovery during hydrolysis and extended run times. Although several LC–MS or LC–MS/MS methods have been developed for the simultaneous determination of codeine and other compounds, there are only a limited number of reports on the simultaneous analysis of codeine, morphine, M3G and M6G in human plasma. Bogusz et al. [13] reported the simultaneous determination of six analytes including codeine, morphine, M3G and M6G in serum using an LC–MS method with atmospheric pressure chemical ionization (APCI) and single ion monitoring (SIM) that gave LLOQs of 1 ng/ml for morphine and 5 ng/ml for codeine, M3G and M6G. However, besides poor sensitivity, this assay also suffered from a long chromatographic run time (17 min) and lower specificity of the SIM mode. Later, Dienes-Nagy et al. [14] published an LC–MS/MS assay with electrospray ionization (ESI) for the quantitation of morphine and its 3- and 6-glucuronides, codeine and other compounds in human blood (analysis time 15 min) with LLOQs of 0.5 ng/ml for morphine, 1 ng/ml for codeine and M6G but only 5 ng/ml for M3G. Very recently, Taylor and Elliott [17] reported a method capable of measuring morphine, M3G, M6G and other opioids including codeine in plasma, whole blood and postmortem blood using a hybrid quadrupole linear ion-trap mass spectrometer with an ESI source. In this assay, the run time was shortened to 11 min but the assay suffered from poor specificity and low sensitivity with limits of detection of 1.5 ng/ml for all analytes. In addition, this and other methods were mostly developed for use in forensic toxicology studies or illicit drug monitoring, and the large sample volumes (≥1 ml) and long run times limit their application in pharmacokinetic studies.

In this paper, we report a rapid and sensitive LC–MS/MS method for the simultaneous determination of codeine, morphine, M3G and M6G in human plasma which achieves an LLOQ of 0.05 ng/ml for all analytes in a short run time (5 min) using a small sample volume (0.35 ml). The assay is at least ten times more sensitive than any previously published method and has been applied to a pharmacokinetic study of codeine in healthy Mongolian Chinese volunteers given a low single oral dose of 30 mg of codeine. This is the first report of a pharmacokinetic study of codeine in a Mongolian Chinese population.

2. Experimental

2.1. Chemicals and reagents

Codeine, morphine, M3G, M6G and naloxone (internal stand, IS) were purchased from Cerilliant Corporation (Round Rock, TX, USA). HPLC-grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were analytical grade and used without further purification. Doubly distilled water was used throughout the study. Blank human plasma (drug-free) was obtained from the Changchun Blood Donor Service (Changchun, China).

2.2. Preparation of calibration standards and quality control samples

Stock solutions of codeine and morphine at 1 mg/ml in methanol and M3G and M6G at 100 μg/ml in methanol: water (50:50, v/v) were obtained directly from the manufacturer. Similarly, stock solution of IS (1 mg/ml) in methanol was obtained directly from the manufacturer. Intermediate standard solutions at the desired concentration for the preparation of the calibration curve and