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ORIGINAL ARTICLE

Rapid analysis of urinary opiates using fast gas chromatography–mass spectrometry and hydrogen as a carrier gas

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Abstract A sensitive and specific fast gas chromatography–mass spectrometry (FGC–MS) analytical method using hydrogen as a carrier gas is developed for the rapid simultaneous determination of morphine, codeine, hydrocodone and hydromorphone in human urine. Urine samples were spiked with deuterated internal standards, morphine-d3, codeine-d3, hydrocodone-d3 and hydromorphone-d3, subjected to acid hydrolysis, treated with hydroxylamine to convert the keto-opiates to oximes and then extracted using a positive pressure manifold and silica based solid phase extraction columns. The extracts were derivatized using BSTFA with 1% TMCS.

Gas chromatographic–mass spectrometric analysis was performed in electron ionization mode by selective ion monitoring, using hydrogen as a carrier gas, a short narrow bore GC capillary column, and fast temperature program, allowing for a rapid analytical cycle to maximize the instrument time for high throughput laboratories. While maintaining specificity for these drugs, concentrations in human urine ranging from 50 to 5,000 ng/mL can be measured with intraday and interday imprecision, expressed as variation coefficients, of less than 2.3% for all analytes within a run time of less than 3.5 minutes.

1. Introduction

Codeine and morphine are naturally occurring alkaloids derived from the seedpod of the opium poppy while hydrocodone and hydromorphone are semi-synthetic opiate derivatives and heroin is a diacetyl derivative of morphine. The opiates are classified as narcotic analgesics with codeine, hydrocodone and hydromorphone additionally having antitussive properties. Heroin, a Schedule I Controlled Substance in the United States, is generally administered by intravenous or subcutaneous injection, or less frequently by smoking or nasal insufflation. Morphine, codeine, hydrocodone and hydromorphone are Schedule II Controlled Substances. Morphine can be administered by intravenous, intramuscular, or oral routes, while codeine, hydrocodone and hydromorphone are usually administered orally. Pharmacologic effects of the opiates, in addition to analgesia,

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include euphoria, sedation, pupillary constriction, respiratory depression, and constipation at therapeutic dosages. Overdose of morphine/heroin can cause coma[1] and death by cardiopulmonary collapse[2] and an overdose of codeine can cause unconsciousness and convulsions; death may result from respiratory failure.[3] Toxic effects of hydrocodone and hydromorphone include stupor, muscle flaccidity, respiratory depression, hypotension, cold and clammy skin and coma.[4]

Morphine is rapidly absorbed in the body. Plasma peak levels following an oral dose occur after 15–60 min, and following injection can occur after 15 minutes.[5] Extensively metabolized by the liver, only 2–12% is excreted as unchanged drug, while 60–80% is excreted as morphine-3-glucuronide. The elimination half-life of morphine ranges from 1 hour to 8 hours. Heroin is rapidly metabolized (plasma half-life is approximately 3 minutes), first to monoacetylmorphine and further to morphine. Morphine is the primary metabolite excreted in urine after heroin abuse. Approximately 7% is excreted as unchanged morphine and 50–60% as glucuronides. Additionally, the specific heroin metabolite, 6-monoacetylmorphine (6-MAM) may be detected in urine 4 h–8 h after the ingestion of heroin. Codeine is also rapidly absorbed and metabolized following an oral dose, principally to codeine-6-glucuronide, with 10%–15% metabolized to morphine and norcodeine; 5%–17% of the codeine dose is excreted as a free drug.

Hydrocodone is more toxic than codeine and metabolized in the liver with most metabolites being pharmacologically active. About 26% of a single dose is eliminated in the 72 h urine as unchanged drug. Hydromorphone is metabolized in the body to hydromorphone-3-glucuronide and hydromorphol. About 6% of an average dose is excreted as free and 30% as conjugated hydromorphone in the 24 h urine. Opiates may be detected in the urine for 24 h–72 h following ingestion.

The opiates/opioids are encountered frequently in forensic toxicology as they are heavily prescribed and abused. The recent upsurge in pain clinics throughout the United States and the dispensing of large quantities of oxycodone and hydrocodone further highlights the forensic importance of this drug class.[6–8] According to the Centers for Disease Control and Prevention (CDC), 100 people in the United States die from drug overdoses every day, and death rates as a result of drug overdoses have more than tripled since 1990. The CDC also reports that nearly three out of four prescription drug overdoses are caused by opiates.

Drug testing for opiates under the Mandatory Guidelines for Federal Workplace Drug Testing Programs[9] in the United States currently requires immunoassay screening and confirmation by gas chromatography–mass spectrometry (GC–MS) or the recently approved technique of liquid chromatography–mass spectrometry (LC–MS) for morphine and codeine. Substance Abuse and Mental Health Services Advisory (SAMHSA) is proposing the addition of hydrocodone and hydromorphone to this mandatory testing program. The immunoassays available for opiate testing have variable cross-reactivity to codeine, morphine, and other opiates.[10] Detection and quantitation of keto-opiates like hydrocodone, hydromorphone, oxycodone, and oxymorphone are desirable (1) because of their potential interference with the measurement of codeine and morphine and (2) because of their increasing potential for abuse.

Recent advances in instrumentation like liquid chromatography with tandem mass spectrometry (LC–MS–MS) have demonstrated simultaneous detection of naturally occurring opiates and their synthetic derivatives such as hydrocodone and hydromorphone in various matrices,[11–14] but this instrumentation is more expensive than traditional electron impact gas chromatography (EI-GC)–MS systems and may be cost-prohibitive in many toxicology laboratories.

Several GC–MS methods have been developed for the analysis of codeine, morphine, and/or other opiates. The extraction, derivatization, and detection details of many of these methods have been published in review articles.[15,16] Stability and characteristics of various derivatives used for opiate anal-ysis[17,18] as well as the hydrolysis efficiency of acid and enzymes have also been studied.[19]

Problems encountered in GC–MS methods for simultaneous analysis of morphine and codeine include interference from keto-opiates like hydrocodone, hydromorphone, oxyco-done and oxymorphone in the analysis of codeine and morphine, incomplete derivatization, instability of derivatives, poor chromatography, inefficient hydrolysis, especially in case of enzyme hydrolysis, and extended run times. Techniques to improve separation of these opiates include pretreatment with borohydride,[20] sequential derivatization,[21] and multiple ramp temperatures.[22] Several methods have been reported that utilize dual derivatization with hydroxylamine to form oxime derivatives of the keto-opiates followed with BSTFA treat-ment.[23–26] Others have used methoxyamine and propionic anhydride with pyridine as a catalyst[27] for derivatization or a three step derivatization with methoxyamino/propionyl/ TMS groups.[28]

The method presented here is a modification of previously published methods that utilized hydroxylamine, and was developed to address the high throughput laboratory needs for faster turnaround times that the previously reported GC–MS methods did not address. The procedure includes acid hydrolysis of urine samples followed by reaction with hydroxylamine, extraction on solid-phase columns, and derivatization with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Codeine, morphine, hydrocodone, and hydromorphone are separated using a short, narrow bore capillary column, fast temperature programing and hydrogen as a carrier gas, within 3.5 min, and without cross-interference. Quantitation was performed with deuterated internal standards in selected ion monitoring (SIM) mode.

2. Materials and methods

2.1. Reagents and consumables

Certified drug-free urine was obtained from UTAK Laboratories (cat# 88121-CDF). BSTFA with 1% TMCS was purchased from Pierce Chemical Company (Rockford, IL). Hydroxylamine hydrochloride was obtained from Sigma Chemical Company (cat# H-9876). Sodium phosphate, mono-basic, monohydrate and sodium phosphate, dibasic, anhy-drous (cat# 3818–01 and 38828–01) were purchased from J.T. Baker (Phillipsburg, NJ). Hydrochloric acid, acetic acid, ammonium hydroxide, methanol, dichloromethane, and isopropyl alcohol were obtained from Spectrum Chemicals (Gardena, CA). All solvents were of HPLC grade or better and all chemicals were of ACS grade. Solid phase extraction columns (Clean Screen) were obtained from United Chemical Technologies, Bristol, PA. Gas chromatographic capillary column (CP-SIL 5CB, cat# CP7684) was obtained from Agilent, Inc. (Lake Forest, CA).