

Simultaneous identification and quantitation of codeine, morphine, hydrocodone, and hydromorphone in urine as trimethylsilyl and oxime derivatives by gas chromatography–mass spectrometry

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Following enzymatic hydrolysis of urine, a gas chromatography–mass spectrometry method for the simultaneous determination of codeine, morphine, hydrocodone, and hydromorphone uses hydroxylamine to form oxime derivatives of the keto-opiates (i.e., hydrocodone, hydromorphone, oxycodone, and oxymorphone). These trimethylsilyl-derivatized forms no longer interfere with the detection and quantitation of codeine and morphine. Samples are extracted on solid-phase columns and quantitated by deuterated internal calibrations of each analyte with selected ion monitoring. Codeine, morphine, hydrocodone, and hydromorphone are completely separated, allowing simultaneous quantitation without interference and a chromatographic analysis time <9 min.

INDEXING TERMS: abused drugs • drug monitoring • opiates • forensic toxicology

Drug testing for opiates under the Mandatory Guidelines for Federal Workplace Drug Testing Programs requires immunoassay screening and confirmation by gas chromatography–mass spectrometry (GC/MS) for morphine and

codeine. The immunoassays available for opiate testing have variable cross-reactivity to codeine, morphine, and other opiates [1]. Detection and quantitation by GC/MS of opiates such as the keto-opiates hydrocodone, hydromorphone, oxycodone, and oxymorphone are desirable not only because of their potential interference with the measurement of codeine and morphine but also because of their potential for abuse. Several GC/MS methods have been developed for the analysis of codeine, morphine, or other opiates. The extraction, derivatization, and detection details of many GC/MS methods have been reviewed by Goldberger and Cone [2] and Wasels and Belleville [3]. Chen et al. [4] and Grinstead [5] studied the stability and characteristics of various derivatives used for opiate analysis.

Problems encountered with some methods include instability of derivatives, poor chromatography, unsuitable ions and abundances, incomplete derivatization, derivatization side reactions, inadequate recovery, loss during hydrolysis, extended run times, and interference or coelution by other opiates. The potential interference of other opiates, particularly the keto-opiates hydrocodone, hydromorphone, oxycodone, and oxymorphone, with the analysis of codeine and morphine is a major concern. Techniques to improve separation of these opiates include pretreatment with borohydride [6], sequential derivatization [7], and multiple ramp temperatures [8]. The method presented here incorporates the use of hydroxylamine after enzymatic hydrolysis to form oxime derivatives of the keto-opiates [9,10], whose derivatized forms do not interfere with codeine or morphine detection and quantitation. The procedure includes enzymatic hydrolysis of 2.0-mL urine samples followed by reaction with hydroxylamine, extraction on solid-phase columns, and derivat-

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ization with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA).⁵ Codeine, morphine, hydrocodone, and hydromorphone are separated without cross-interference and quantitated with deuterated internal standards and selected ion monitoring (SIM).

Materials and Methods

INSTRUMENT

A Hewlett-Packard (HP) (Palo Alto, CA) 5890 gas chromatograph with splitless injection and a 5970 mass selective detector were used. The capillary column used was a J&W Scientific (Folsom, CA) DB-1 [15 m × 0.32 mm (i.d.), 0.25- μ m-thick film]. Helium was the carrier gas at a flow rate of 0.7 mL/min and a linear velocity of 38 cm/s. The temperature program was: initial temperature, 150 °C for 1.5 min; ramp at 20 °C/min to 250 °C; injection temperature, 250 °C; and transfer line, 280 °C.

MATERIALS

Codeine, morphine, hydrocodone, and hydromorphone used to prepare calibrators and controls were obtained from Sigma Chemical Co. (St. Louis, MO) and Radian (Austin, TX). Deuterated codeine, morphine, hydrocodone, and hydromorphone (used as internal standards) and oxycodone, oxymorphone, and norcodeine (used for interference studies) were obtained from Radian. Hydroxylamine hydrochloride and *Helix pomatia* (type H-2) β -glucuronidase were obtained from Sigma Chemical Co. BSTFA with 10 mL/L trimethylchlorosilane (TMCS) was from Regis Chemical Co. (Morton Grove, IL). All other solvents and reagents were of reagent or HPLC-grade.

EXTRACTION AND DERIVATIZATION

A 2.0-mL volume of urine (calibrators, controls, samples) was combined with 100 μ L of a 10 mg/L internal standard solution (deuterated codeine, morphine, hydrocodone, and hydromorphone) and 100 μ L of 2.0 mol/L acetate buffer (pH 4.8) in an appropriately labeled 16 × 100 mm screw cap tube. All samples were vortex-mixed. Conjugates were hydrolyzed by the addition of 100 μ L of β -glucuronidase solution (99.2 U/L) to all calibrators, controls, and donor samples and incubation at 56 °C for 2 h. After hydrolysis, extraction derivatization of keto-opiates was performed by the addition of 100 μ L of aqueous hydroxylamine (100 g/L) to each tube, then heating for 15 min at 56 °C. All tubes were centrifuged, and the supernatant was placed on solid-phase bonded silica extraction columns [Varian (Palo Alto, CA) Bond ElutTM or United Chemical Technology (Bristol, PA) Clean ScreenTM], previously activated by the sequential addition and elution of 3 mL of both methanol and

deionized water. Columns were then washed by the sequential addition and elution of 3 mL of water, 2 mL of 0.1 mol/L acetate buffer (pH 4.0), and 3 mL of methanol and then dried under maximum vacuum for 5 min. The analytes were eluted by the addition of 3 mL of freshly prepared methylene chloride:isopropyl alcohol:ammonium hydroxide elution solvent (78:20:2 by vol) at a rate of ~1–2 mL/min. The eluants were dried under a stream of air at 56 °C. The extracted residue was reconstituted with 100 μ L of BSTFA with TMCS, 10 mL/L, and the tubes were capped and heated for 20 min at 56 °C. Hexane (100 μ L) was added to each tube and transferred to a correspondingly labeled autoinjector vial, and 1 μ L of this solution was injected in the GC/MS with a HP 6890 Automatic Liquid Sampler.

DATA ACQUISITION AND ANALYSIS

SIM was used, and the data system was a HP DOS ChemstationTM. The following ions were monitored (quantitative ions are indicated in parentheses) for the derivatized analytes: codeine, 234, 343, (371); d₃-codeine, 346, (374); morphine, 234, 401, (429); d₃-morphine, 417, (432); hydrocodone, 297, 371, (386); d₃-hydrocodone, 300, (389); hydromorphone, 429, 444, (355); and d₃-hydromorphone, 447, (358). Quantitation was based on a calibration curve consisting of calibrators (prepared by appropriate dilutions of a 1 g/L solution of codeine, morphine, hydrocodone, and hydromorphone in 2.0 mL of drug-free urine) extracted with each batch of samples. The concentration of the calibrators in the curve were 120, 300, 1000, and 2000 μ g/L.

Results and Discussion

CHROMATOGRAPHY

The total ion chromatographs of the trimethylsilyl (TMS) derivatives of codeine, morphine, and norcodeine accompanied by the TMS-oxime derivatives of hydrocodone and hydromorphone are shown in Fig. 1. The chromatographic peaks are gaussian-shaped and demonstrate near-baseline resolution. Under these analysis conditions all peaks are eluted within 9 min. This resolution was maintained on the same column for >3500 injections. The retention times and relative (to codeine) retention times of these and other compounds of interest are shown in Table 1. A single peak was obtained for each of the keto-opiates in scan mode, indicating completeness of oxime formation.

LINEARITY

The procedure exhibits linearity for all four analytes with an upper limit of linearity of 2000 μ g/L. As part of each analysis, a best-fit linear regression calibration curve consisting of the four calibrators and a forced y -intercept of zero is obtained. Correlation coefficients of 0.995 or greater for the calibration of each analyte have been obtained on a daily basis for a 9-month period.

⁵ Nonstandard abbreviations: SIM, selected ion monitoring; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; HP, Hewlett-Packard; TMCS, trimethylchlorosilane; TMS, trimethylsilyl; LOD, limit of detection; LOQ, limit of quantitation.

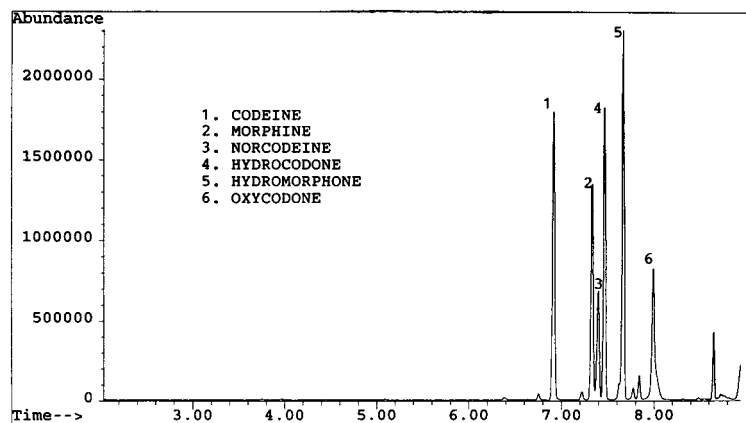


Fig. 1. Chromatograph of urine sample containing 1000 $\mu\text{g/L}$ codeine, morphine, norcodeine, hydrocodone, hydromorphone, and oxycodone processed through the procedure.

PRECISION

Between-run precision ($n = 47$) around the 300 $\mu\text{g/L}$ cutoff was determined by daily analysis of samples with target concentrations of 240 and 360 $\mu\text{g/L}$. CVs at these concentrations were: 5.4% and 6.0% for codeine, 8.4% and 6.3% for morphine, 4.2% and 6.0% for hydrocodone, and 4.0% and 6.6% for hydromorphone, respectively.

INTERFERENCE

Possible cross-interference with quantitation of codeine, morphine, hydrocodone, and hydromorphone was assessed by adding to urine samples containing each analyte at the cutoff concentration of 300 $\mu\text{g/L}$ another 10 000 $\mu\text{g/L}$ of each potential interferant. For example, urine samples with codeine and morphine target concentrations of 300 $\mu\text{g/L}$ were supplemented (in separate samples) with 10 000 $\mu\text{g/L}$ hydrocodone, hydromorphone, oxycodone, oxymorphone, and norcodeine. In all cases the quantitation of the analyte of interest was not affected by the presence of the most common potential interferants (i.e., codeine, morphine, hydrocodone, and hydromorphone concentrations were within 20% of the target concentration, ion ratios were within 20% of the calibrators, and there was no chromatographic interference). These results demonstrate that the presence of large

concentrations (10 000 $\mu\text{g/L}$) of hydrocodone, hydromorphone, oxycodone, oxymorphone, and norcodeine does not interfere with the analysis of codeine and morphine, and the presence of large concentrations of codeine, morphine, and norcodeine does not interfere with the analysis of hydrocodone and hydromorphone. In particular hydromorphone and norcodeine do not interfere with morphine quantitation, and hydrocodone does not interfere with codeine quantitation. These interferences are of concern because of common ions shared when the TMS derivatives alone are used for the analysis of these opiates.

DETECTION LIMITS (LOD/LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by serial dilution analysis as described below and not by calculations based on standard deviation ratios. Samples containing decreasing concentrations of the analytes of interest are assayed in triplicate. The LOQ is defined as the concentration at which two of the three specimens meet the criteria that all chromatographic variables (retention time, peak shape, qualifier ion ratios, etc.) for a positive sample are acceptable and the concentration obtained is within 20% of that expected. The LOD is the concentration at which two of the three specimens meet the criteria required for a positive determination but the quantitative result does not have to be within 20% of the expected concentration. The experimentally determined LOD and LOQ for each analyte were equal according to these definitions. The LODs and LOQs for codeine, morphine, hydrocodone, and hydromorphone are 50, 100, 75, and 75 $\mu\text{g/L}$, respectively. Because LOD and LOQ values may vary between laboratories, the National Laboratory Certification Program (Directive 025, May 1993, J.H. Autry) believes that all laboratories should be able to identify and quantitate drugs at concentrations between the cutoff and at least 40% of the cutoff. The LOQ and LOD concentrations obtained here are all below this minimum requirement (40% of the 300 $\mu\text{g/L}$ cutoff is 120 $\mu\text{g/L}$).

Table 1. Retention times of TMS and oxime derivatives of opiates.

	Retention time, min	Relative retention time
d_3 -Codeine	6.91	0.998
Codeine	6.92	1.000
d_3 -Morphine	7.32	1.058
Morphine	7.33	1.059
Norcodeine	7.40	1.069
d_3 -Hydrocodone	7.45	1.076
Hydrocodone	7.46	1.078
d_3 -Hydromorphone	7.65	1.105
Hydromorphone	7.66	1.107
Oxymorphone	7.91	1.145
Oxycodone	7.99	1.155

In conclusion, the above-described method, which allows the simultaneous quantitation of codeine, morphine, hydrocodone, and hydromorphone, demonstrates acceptable precision, linearity, sensitivity, and lack of cross-interference and interference from other opiates. It has been used in our laboratory for the analysis of >2500 samples in 9 months and has been found to be reliable as demonstrated by calibrator and control reproducibility and absence of interference.

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