Disposition of Hydrocodone in Hair

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Abstract

The use of prescription drugs, including synthetic opiates, is increasing in the U.S., with emergency room reports showing a dramatic rise in prescription opiate abuse. As part of an ongoing study, the hair of admitted opiate users was analyzed for hydrocodone and hydromorphone, as well as codeine, morphine, and 6-acetylmorphine in order to determine if there was any correlation between self-reported frequency of opiate intake and the concentration of drug detected in hair. The hairs were confirmed using gas chromatography–mass spectrometry following screening by enzyme linked immunosorbent assay (ELISA). Twenty-four hair specimens collected from volunteers showed the presence of hydrocodone (130–15,933 pg/mg); four of those also contained hydromorphone (59–504 pg/mg). The specimens were also analyzed for morphine, codeine, and 6-acetylmorphine. Hair specimens from five self-reported codeine users showed concentrations of hydrocodone between 592 and 15,933 pg/mg. In addition, codeine was present at concentrations of 575–20,543 pg/mg, but neither morphine nor hydromorphone were present in any of those hair specimens. Though the analysis of some opiates in hair has been previously published, this is the first study where the hydrocodone and hydromorphone concentrations have been measured following self-reported opiate intake.

Introduction

Prescription opioids such as Vicodin®, Lortab® (hydrocodone), and Dilaudid® (hydromorphone) are becoming increasingly abused in the U.S. Data from the 2003 Drug Abuse Warning Network, which collects information on drug-related episodes from hospital emergency departments in metropolitan areas, as well as from Medical Examiner offices, listed opiates or opioids as present in 20,830 cases of emergency room (ER) visits because of overmedication. Non-medical use of opioids accounted for 17% of ER visits. Various surveys indicate an increase in the use of prescription opiates, with hydrocodone being among the drugs showing the most pronounced trends of increasing abuse (1). In a recent study of prescription drug abuse in Miami, 36% of ecstasy users also reported hydrocodone use (2).

Procedures for the detection of these drugs in urine have been published (3,4) and there are several publications regarding the incorporation of opiates into hair (5,6), including studies employing the analysis of hair to prove heroin intake and/or distinguish heroin use from morphine or codeine ingestion (7,8). As far back as 1994, Nakahara et al. (9) described the importance of the efficient extraction of opiates from hair, comparing five extraction methods. Other procedures for the determination of drugs used in the treatment of heroin addiction, such as methadone (10), l-α-acetylmethadol (11), and buprenorphine (12), in hair have also been reported. However, we found only one publication specifically describing the detection of hydrocodone and hydromorphone, as well as oxymorphone and oxycodone simultaneously in human hair using gas chromatography–mass spectrometry (GC–MS) (13). The paper describes the methodology of analysis, but has no results from the analysis of samples where drug intake history was available.

Materials and Methods

Subjects

Our study enrolled subjects from the Drug and Alcohol Recovery Team (DART) in Fullerton, Orange County, Southern California and was approved under Immunalysis Institutional Review Board (IRB # 2004-05-001). Subjects were made aware of the purpose of the study and gave informed consent. Although information on drug use, including time of last use, frequency of use, ethnicity, age, gender, and hair color were recorded for each subject, names, addresses, or other identifying information were not collected on the interview sheet. Complete anonymity was established during the sample collection and laboratory testing procedures. Each subject provided a hair specimen taken from the vertex portion of the head at the time of interview. The subjects were asked if they took opiates, not specifically heroin, codeine, or other synthetic opiates. None of the subjects reported having a pre-
scription for opiates. Hair was transported to the testing facility and stored at room temperature.

**Experimental**

Hair screening kits were obtained from Immunalysis Corporation (Pomona, CA). The Opiates Direct ELISA Kit (Catalog # 207-0480) was used for screening the hair specimens and was used according to the manufacturer’s instructions. For confirmatory procedures, deuterated morphine-d₃, codeine-d₃, hydrocodone-d₃, hydromorphone-d₃, and 6-acetylmorphine-d₁, as internal standards as well as the corresponding unlabelled drug standards were obtained from Cerilliant (Round Rock, TX). Solid-phase mixed-mode cation exchange–hydrophobic phase extraction columns (CSDAU020, 200 mg) were obtained from United Chemical Technologies Inc. (Bristol, PA). The derivatizing agent, N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), was obtained from Pierce Chemical Co. (Rockford, IL). All solvents were high-performance liquid chromatography grade or better, and all chemicals were American Chemical Society grade.

**Screening assay**

The Immunalysis Opiates Direct ELISA kit is based upon the competitive binding of enzyme-labeled antigen and unlabeled antigen to antibody in proportion to their concentration in the reaction well. Hair was cut into small segments (3–5 mm), and an aliquot of 10 mg was weighed. The hair was washed briefly with methanol (2 mL /10 min), the solvent was decanted, and the hair was allowed to dry. To the hairs, 0.025M phosphate buffer (pH 2.7, 0.5 mL) was added; the tubes were capped and incubated at 60°C for 2 h. Phosphate buffer (0.5M, pH 9.0, 50 μL) was added to neutralize the acid environment, and the liquids were transferred to corresponding clean glass tubes. A standard curve consisting of a drug-free negative hair specimen, a drug-free hair specimen spiked at 100 pg/mg of morphine, and a drug free hair specimen spiked at 400 pg/mg of morphine, as well as a cut-off calibrator at 200 pg/mg morphine were included in duplicate in every batch. All specimens, calibrators, and controls were diluted 1:5 by adding 400 μL of phosphate buffer saline (PBS), with 0.1% bovine serum albumin (pH 7.0) to 100 μL of extract. An aliquot of the diluted hair extract (20 μL) was then added to the individual microplate well, and the screening assay was performed according to the manufacturer’s instructions. The assay performance was optimal when 20–60 pg of morphine were placed into the microplate well. A sample size of 20 μL of the diluted hair extract gave good separation at the screening cut-off concentration of 200 pg of morphine equivalents per milligram of hair. The assay showed broad spectrum crossreactivity with other opiates, including codeine (200%), hydrocodone (93%), 6-acetylmorphine (83%), hydromorphone (81%), oxycodone (21%), and oxy- morphine (20%).

**Efficiency of screening extraction**

The extraction efficiency of the screening assay was determined. Three hair specimens already confirmed for the presence of opiates were selected and analyzed according to the described procedure. After the first extract solution was removed, the hair was rinsed, dried, and re-extracted for 1 h (Extract 2). The solution was removed, and a third extraction was carried out (Extract 3). The extracts were screened as described or, if necessary, further diluted into the linear range of the screening assay.

**Confirmatory assay**

Specimens showing inhibition lower than that of the cut-off calibrator (200 pg/mg) were considered to be presumptively positive for opiates and were carried forward to confirmation using GC–MS, operating in electron impact mode.

A separate aliquot of hair was weighed out (10 mg). Low (100 pg/mg), medium (200 pg/mg), and high (500 pg/mg) controls were also prepared. The internal standard solution contained deuterated morphine-d₃, codeine-d₃, acetylmor­phone-d₁ (6-AM), hydromorphone-d₃, and hydrocodone-d₃ in acetone (200 ng/mL). The samples were incubated in acetone (1.5 mL) for 5 min at room temperature to remove gels, sprays, or extraneous cosmetic treatments on the hair, and then the acetone was discarded. Internal standard (50 mL) was added to each calibrator, control, or hair specimen at a concentration of 1000 pg/mg. Methanol (3 mL) was added, and the samples were allowed to sonicate (2 h/70°C). The methanol was decanted and evaporated to dryness. To the remaining hair, 0.1M hydrochloric acid (1.5 mL) was added, and the tubes were capped and incubated overnight at 55°C. The acid was decanted into the corresponding dried methanol tube, and 0.1M phosphate buffer (pH 6.0; 3 mL) was added. Solid-phase mixed mode extraction columns (CleanScreen CSDAU0133) were placed into a vacuum manifold. Each column was conditioned with ethyl acetate (2 mL), methanol (2 mL), and 0.1M phosphate buffer (pH 6.0; 2 mL). The samples were allowed to flow through the columns, then the columns were washed with deionized water (1 mL), 0.1M hydrochloric acid (1 mL), methanol (1 mL), and ethyl acetate (1 mL). The columns were allowed to dry under pressure (15 psi, 5 min). The drugs were finally eluted using freshly prepared ethyl acetate/2% ammonium hydroxide (98:2, 3 mL). The extracts were evaporated to dryness under nitrogen and reconstituted in ethyl acetate (25 μL) and BSTFA–1% TMCS (25 μL). The tubes were capped and heated at 70°C for 20 min. The extracts were transferred to autosampler vials for analysis by GC–MS. Controls and calibrators were included in each batch as described.

**Analytical procedure (GC–MS)**

For confirmation of the presence of opiates, an Agilent Technologies 6890 GC coupled to a 5975 mass selective detector operating in electron impact mode was used for analysis (GC–MS). The GC column was a DB-5 MS (0.25-mm i.d., 0.25-μm film thickness, 15-m length, J&W Scientific, an Agilent Company), and the injection temperature was 250°C. The injection mode was splitless. The oven was programmed from 100°C for 0.5 min, ramped at 40°C/min to 230°C, then ramped at 5°C/min to 250°C. The transfer line was held at 280°C, the MS source was 230°C, and the quadrupole at 150°C. The ions
monitored were as follows: deuterated codeine and hydrocodone \(m/z\) 374 (237); unlabelled codeine and hydrocodone \(m/z\) 371, (234, 196); deuterated morphine and hydromorphone \(m/z\) 432 (417); unlabelled morphine and hydromorphone \(m/z\) 429 (414, 401); deuterated 6-acetylmorphine \(m/z\) 402 (343); unlabelled 6-AM \(m/z\) 399 (340, 287). The qualifying ions are shown in parentheses. All the drugs were well separated by retention time. Specimens were considered positive for the opiate drugs if present above the limit of quantitation of the assay (50 pg/mg).

### Efficiency of confirmatory extraction

The extraction efficiency of the method was established. Six hair specimens containing high concentrations of opiates, relative to the 200 pg/mg screening cut-off, were selected and analyzed according to the extraction protocol. The remaining hair was dried and extracted again; the hair remaining from that was subjected to a third extraction procedure. The extracts were confirmed using the GC–MS procedure described.

### Results and Discussion

#### Method validation

**GC–MS.** The precision of the assay was determined by analyzing hair specimens containing codeine, morphine, and 6-acetylmorphine at concentrations of 50, 100, 200, 500, and 1000 pg/mg. The interday precision of the assay at 200 pg/mg for codeine, morphine, and 6-acetylmorphine was 2.20%, 2.97%, and 2.27%, respectively, \(n = 6\). The intraday precision was 2.37%, 2.78%, and 0.53% for codeine, morphine, and 6-AM, respectively, \(n = 5\). The limit of quantitation of the system was 50 pg/mg for all analytes; the correlation coefficient for all calibration curves was greater than \(r^2 = 0.99\), and the upper limit of linearity was 1000 pg/mg. Specimens exceeding the upper limit were either re-extracted using less sample volume or diluted so the analytical value lay within the calibration curve parameters.

**Extraction efficiency.** The extraction efficiency of both the screening procedure and the confirmatory extraction methods were determined. The screening method was efficient, with
an average extraction recovery of 79.2% of morphine equivalents in the first hour of incubation. After the second hour, the mean recovery was 91.7% with a coefficient of variation of 6.3% (n = 3). The confirmatory procedure was also extremely efficient with recoveries for all four opiates over 93% (n = 6). The results are shown in Tables I–III.

**Specimens**

Overall, 24 hair specimens tested positively for hydrocodone or hydromorphone.

*Hydrocodone.* Hydrocodone was present in all 24 synthetic opiate-positive samples. Eight of the subjects did not admit to opiate use of any kind. Five subjects reported specifically to the ingestion of codeine, and all provided hair samples that tested positively for hydrocodone and codeine (it should be noted that one of the subjects admitted hydrocodone intake in addition to codeine). Three individuals reported hydrocodone use in addition to heroin or codeine intake, and all were positive for hydrocodone in the hair.

The self-reported drug intake of the subjects is shown in Table IV along with the analytical results. Hydrocodone was detected in all hair samples following admitted codeine intake, but, notably, morphine was not detected in measurable concentrations.

*Hydromorphone.* Four samples contained hydromorphone above the limit of quantitation (Table III). Two were from

<table>
<thead>
<tr>
<th>Self-Reported Opiate Intake</th>
<th>Concentration Detected in Hair (pg/mg)</th>
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<tbody>
<tr>
<td>Drug</td>
<td>Codeine</td>
</tr>
<tr>
<td>Heroin 4 times/week</td>
<td>841</td>
</tr>
<tr>
<td>Heroin 2 times/week</td>
<td>285</td>
</tr>
<tr>
<td>Heroin 3 times/week</td>
<td>-</td>
</tr>
<tr>
<td>MTD 1 time/day</td>
<td>-</td>
</tr>
<tr>
<td>Heroin 1 time/month</td>
<td>-</td>
</tr>
<tr>
<td>Heroin 14 times/week</td>
<td>5743</td>
</tr>
<tr>
<td>Heroin 7 times/week</td>
<td>1958</td>
</tr>
<tr>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Opiates 3 times/week</td>
<td>192</td>
</tr>
<tr>
<td>Heroin 4 times/week</td>
<td>399</td>
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<tr>
<td>HCY 3000 mg/day</td>
<td>289</td>
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<tr>
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<td>-</td>
</tr>
<tr>
<td>Codeine 500 mg/day</td>
<td>6516</td>
</tr>
<tr>
<td>HCY 750 mg/day</td>
<td>-</td>
</tr>
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<td>-</td>
</tr>
<tr>
<td>Heroin 1 time/month</td>
<td>339</td>
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<td>Heroin 1 time/week</td>
<td>2197</td>
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<td>1763</td>
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<td>Heroin 1 time/month</td>
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</tr>
<tr>
<td>HCY 1 time/week</td>
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<td>NA</td>
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<td>NA</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
</tr>
<tr>
<td>Codeine 3000 mg/day</td>
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</tr>
<tr>
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<tr>
<td>Codeine 1000 mg/day</td>
<td>20,543</td>
</tr>
<tr>
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<td>-</td>
</tr>
<tr>
<td>Codeine 500 mg/day</td>
<td>20,543</td>
</tr>
</tbody>
</table>

* Abbreviations: 6-AM, 6-acetylmorphine; HYC, hydrocodone; HYM, hydromorphone; MTD, methadone; and NA, no admission of opiate intake.
subjects admitting frequent heroin use; two were from subjects not admitting any opiate intake. As for the two admitted heroin users, their hair specimens also contained codeine (1958 and 5743 pg/mg), hydrocodone (202 and 161 pg/mg), morphine (9160 and 15,206 pg/mg), and 6-acetylmorphine (9925 and 7623 pg/mg). The morphine levels in these two samples were the highest from any of the positive specimens.

There are several reports of extraction and analytical procedures for the determination of opiates in hair (14), but none focus on hydrocodone or hydromorphone following self-reported intake. Research groups have administered known amounts of codeine to subjects and subsequently measured the opiate content of hair. Morphine was not detected in hair following multiple oral doses of codeine (15,16). Scheidweiler et al. (17) reported codeine concentrations in hair from subjects taking low doses of codeine on alternate days and did not consider the detection of morphine in hair specimens following codeine administration to be significant because concentrations did not exceed 83 pg/mg in any subject (n = 10). None of these studies included hydrocodone in their analytical hair assay. However, in rat hair, codeine, morphine, and morphine glucuronide were incorporated in hair in a dose-related manner following codeine administration (18), and in one further publication regarding human subjects, the presence of morphine at a concentration of 400 pg/mg following low doses of codeine administration (3 x 60 mg per week) was reported (19). This was the only publication found that detected measurable morphine in hair following codeine intake, and the morphine was only present in one sample from one subject. Though our study is consistent with the majority of reported data, in that morphine is generally not detected above 20 pg/mg in hair specimens taken from admitted codeine users, we are the first to report the presence of hydrocodone in all the samples. Oyler et al. (20) reported the identification of hydrocodone in human urine following controlled codeine administration, and our results appear to support the observation that hydrocodone is a metabolite of codeine. However, it should be noted that the presence of hydrocodone could have been present both as a result of codeine metabolism and/or ingestion of hydrocodone itself.

There was no apparent correlation between the concentration of the opiates in hair and the amount of codeine reported by the subjects. The same was true following heroin use: there appeared to be no correlation with codeine, morphine, or 6-acetylmorphine levels in hair, although nine out of 10 hair specimens from these subjects were positive for 6-acetylmorphine (Figure 1). The only sample that did not show the presence of 6-acetylmorphine was from an individual admitting to heroin use only once a month. The only two samples from heroin users, which were positive for hydromorphone, had correspondingly high levels of morphine; in fact, they were the highest concentrations of morphine detected in this specimen pool. The ratio of hydromorphone to morphine in both cases was 0.03. This observation supports the possibility that hydromorphone is a minor metabolite of morphine, which has been reported (21).

Limitation of the study

The main limitation to our study is the reliance upon self-reported drug intake. When subjects take tablets (as in the case of hydrocodone and codeine) it is more likely that the amount ingested is known, compared with heroin use, where the potency and frequency of use may be suspect. Studies conducted in the 1980s concluded that individuals provide valid information on illegal drug use when the conditions of interview are appropriate. However, it has been shown that clients report heroin use while in treatment programs, but at the post-treatment follow up, heroin use is significantly under-reported (22). Colon et al. (23), based on hair analysis, reported a sensitivity of self-reports for heroin use of 78.6%. More recently, Fishbain et al. (24) noted that the self-reporting of cannabis and cocaine use in chronic pain patients was not well supported by urine toxicology, with sensitivity results indicating that a significant percentage (8.8%) of subjects who claimed to be drug-users were not actually taking the drug or were not taking it correctly. Apparently, the reliability of self-reported drug use depends largely on the situation of the subjects. In certain circumstances, where adverse results are not associated with admission, reliable self-reporting of opiate use has been shown (25).

![Figure 1. Opiate concentrations in hair (pg/mg) following self-reported heroin use.](image)

![Figure 2. Mean concentrations of opiates in grey, black, and brown hair.](image)
Correlation

There appeared to be no correlation between amount of drug ingested and the concentration of any drug or drug metabolite detected in hair. Such a correlation has been reported for codeine, when the codeine levels are normalized to the melanin (phaeomelanin and eumelanin) content of the hair. Unfortunately, the amount of hair needed to carry out the determination of total melanin, eumelanin, screening, and confirmation of each hair was impractical in our study. According to the method of Kronstrand et al. (26), 50 mg of hair was necessary for total melanin determination and 30 mg for eumelanin. Our study required 10 mg for screening and 20 mg for confirmation, requiring a total of 110 mg of hair per subject, assuming no repeats were necessary.

Because melanin is predominantly responsible for hair coloration, we assessed the average opiate concentration based on the hair color of subjects in the study (Figure 2). There was only one red-haired subject and only one blonde subject positive for morphine and 6-acetylmorphine; thus, these hair colors were not included in the average values reported. Overall, grey-haired subjects had the highest average concentration of codeine (5069 pg/mg) and hydrocodone, (6337 pg/mg; x = 54 years) in hair. The mean age of black-haired subjects was 48.8 years, and they showed the highest average concentration of morphine (6144 pg/mg) and 6-acetylmorphine (2931 pg/mg). However, the number of subjects in each hair color class was limited; therefore, no definitive conclusion can be drawn regarding hair color and opiate concentration. The age of the subjects ranged from 26 to 62 years, but only two were younger than 42 years.

Conclusions

In summary, hydrocodone can be readily detected in hair specimens. Following admitted codeine use, all hair samples contained hydrocodone, and none showed the presence of morphine, 6-acetylmorphine, or hydromorphone. In two of the cases, the hydrocodone concentration was significantly higher than the codeine. There appeared to be no correlation between self-reported opiate intake and concentration in hair.

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