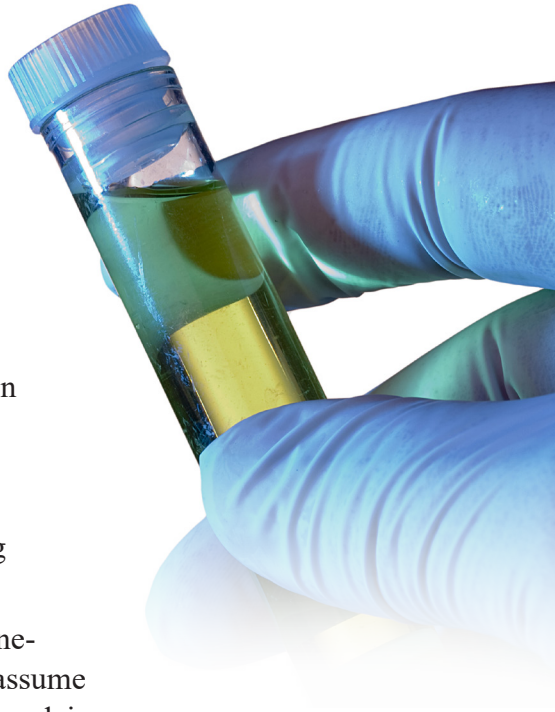
2018

Opioids Result Interpretation Issues

This is the fourth of a four-part Drug Testing Matters series on opioids.



Minor Metabolites and Process Impurities

Minor metabolites, such as hydromorphone in morphine samples and hydrocodone in codeine samples, can confound the interpretation of opioid positives. What was thought perhaps not metabolically possible is proving to be possible, although the mechanisms of biotransformation are not always clearly defined. Perhaps "never-say-never" is the new motto when it comes to predicting opioid drug metabolism within the limits of reasonable metabolic reactions.

In a morphine-positive specimen with hydromorphone or a codeine-positive specimen with hydrocodone, one should not automatically assume a donor is taking a combination of morphine and hydromorphone or codeine and hydrocodone, respectively. Typically, the relative percentages of the "minor" metabolites are low and can aid in the interpretation of results, but one should be cautious in drawing "black and white" conclusions from these results.

To further cloud test result interpretation, many investigators have postulated biotransformation products that may in fact be "process impurities"—contaminants introduced during the pharmaceutical manufacturing process. For instance, some investigators have suggested that codeine is a minor metabolite of morphine. However, evidence supports the existence of codeine as a process impurity during the commercial preparation of morphine, where codeine may be present in proportions up to 0.04%. The United States Pharmacopeia official monographs include impurity tables with specific acceptance criteria expressed as percentages of the synthesized product of interest. As an example, the impurity table for the synthesis of oxycodone hydrochloride allows for 0.15% oxymorphone, 0.15% noroxymorphone, 0.15% 10-hydroxyoxycodone, 0.25% 6-α oxycodol, 0.15% 7,8-dihydro-8β-14-dihydroxycodeinone, 0.15% hydrocodone, and 0.15% of an "individual unspecified impurity" (1). In this scenario, patients chronically taking oxycodone could potentially test positive for low levels of hydrocodone without taking hydrocodone.

Opioids Detection in Urine and Oral Fluid

The detection of morphine in urine and oral fluid can be explained by at least four different scenarios. Morphine can be present because of 1) morphine use; 2) codeine use, as morphine is a metabolite of codeine in CYP2D6-competant individuals; 3) heroin (diacetylmorphine) use, as morphine is a metabolite of 6-acetylmorphine; and 4) the ingestion of poppy seeds containing morphine (2). A fifth postulated scenario is that a very small percentage of morphine may be present as a process impurity from the manufacture of other semisynthetic opioids.

Codeine may be detected in urine and oral fluid after 1) codeine use or 2) the ingestion of poppy seeds containing codeine. A very small percentage may be present as a process impurity during the commercial preparation of morphine. A 2014 study investigated the metabolic profile of codeine (total urine codeine, morphine, hydrocodone, and hydromorphone) in a pain management population (3). The prevalence of codeine metabolized to morphine was found to be considerably higher than that of codeine metabolized to hydrocodone. As the total amount of codeine and its active metabolites increased, the fraction of codeine increased, and the fraction of active metabolites decreased. Based on physician-prescription data, the presence of CYP2D6 inhibitors, such as paroxetine, bupropion, fluoxetine, and methadone, significantly reduced the morphine fraction excreted by reducing or inhibiting the conversion of codeine to morphine.

In addition to codeine and morphine, most confirmatory assays for opioids in urine detect hydrocodone, oxycodone, hydromorphone, and oxymorphone. The presence of hydromorphone and oxymorphone in urine may be attributable to the prescribed use of these opioids. Alternatively, they may be present as the O-demethylated metabolites of hydrocodone or oxycodone, respectively. The addition of the N-demethylated metabolites norhydrocodone and noroxymorphone to an assay can facilitate identifying which compounds were ingested as the parent drug (4). These “nor” metabolites are not available as prescription drugs and are solely present in urine as direct metabolites of hydrocodone or oxycodone. All three types of compounds (i.e., the parent drug and its N-demethylated and O-demethylated metabolites) are typically found in urine after subjects ingest hydrocodone or oxycodone (4). However, because of the individual pharmacokinetic and pharmacogenetic differences within a population and the time and chronicity of dosing relative to specimen collection, not all metabolites or parent drugs may be present at the time of specimen collection.

A 2016 study followed the urinary excretion of both total and free hydromorphone after a single, 8-mg dose of an extended release formulation of hydromorphone (Exalgo ER) was administered (5). That study demonstrated the need to analyze hydromorphone glucuronide or develop an efficient hydrolysis method, as only 2% of the dose was detected as free hydromorphone.

Two 2014 studies investigated oxycodone disposition in pain patient populations. A 2014 study by Elder et al. investigated the disposition of oxycodone and metabolites in urine (6). The authors revised their data analysis (7) based on data provided by another research group (8). The oxycodone, oxymorphone, and noroxycodone mole fractions were consistent with the common finding of noroxycodone as the predominant analyte in urine after oxycodone administration. Moy et al. (11) compared oxycodone and metabolite excretion in urine and saliva, noting that parent oxycodone was predominant over noroxycodone in saliva (similar to plasma), while the opposite relationship was observed in urine. Much greater concentrations were found in saliva than would be expected in plasma.

The disposition of oxycodone in oral fluid and blood has been delineated after a single administration of 20 mg of OxyContin® (an extended release formulation), and oxycodone was found to be the primary substance detected in oral fluid and blood, followed by noroxycodone (15). The concentration of oxycodone in oral fluid was generally greater than twice that in blood.

Two studies in which patients were dosed with oxycodone found only oxycodone and noroxycodone in oral fluid; no oxymorphone was detected (12,13). In both studies, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays targeted all three analytes. In one of the studies, naive patients were dosed with oxycodone to achieve steady state (13), whereas the other selected cancer patients who had received at least 5 days of sustained-release oxycodone dosing (12). Heltsley et al. detected oxycodone, oxymorphone, and noroxycodone in various combinations in oral fluid obtained from a population of chronic pain patients (14). However, noroxycodone was detected in more patients (80%) than oxymorphone (43%), and oxycodone was present in 77% of the 2,445 oxycodone/oxymorphone screen positive oral fluid specimens. This finding is in contrast to the results obtained in urine by the same researchers; in that study, oxycodone, oxymorphone, and noroxycodone were detected at relatively equal prevalence rates: 38.4%, 34.6 %, and 36.2%, respectively (4). All three oral fluid studies showed that the oxycodone metabolite, noroxycodone, was present at a higher prevalence than oxymorphone in oral fluid. Furthermore, the retrospective study

(14), which included 2,445 oxycodone/oxymorphone-positive oral fluid specimens, did not differentiate between patients dosed with oxymorphone versus oxycodone. The differences in findings between the first two oral fluid controlled studies and the large retrospective study may reflect differences in the number of patients tested, the actual compound dosed (oxycodone versus oxymorphone), acute versus chronic dosing, or possible analytical differences in confirmation detection limits between laboratories

It is worth noting at this point that different methods of collecting oral fluid may affect the quantifiable levels of analytes in oral fluid specimens. As an example, the amount of oral fluid obtained in a so-called pad device, which is placed into a buffer/preservative post-oral fluid collection, may vary ±10%, influencing the amount of drug and/or drug metabolite measured relative to the true value. Additionally, drugs and drug metabolites obtained from a pad, regardless of whether the pad was placed in a buffer/preservative, may adsorb partially or completely on the collection pad, resulting in a lower recovery than anticipated. In contrast, the use of neat oral fluid eliminates questions concerning the actual amount of oral fluid collected. However, using unstabilized neat oral fluid presents its own set of sample handling and stability issues (16).

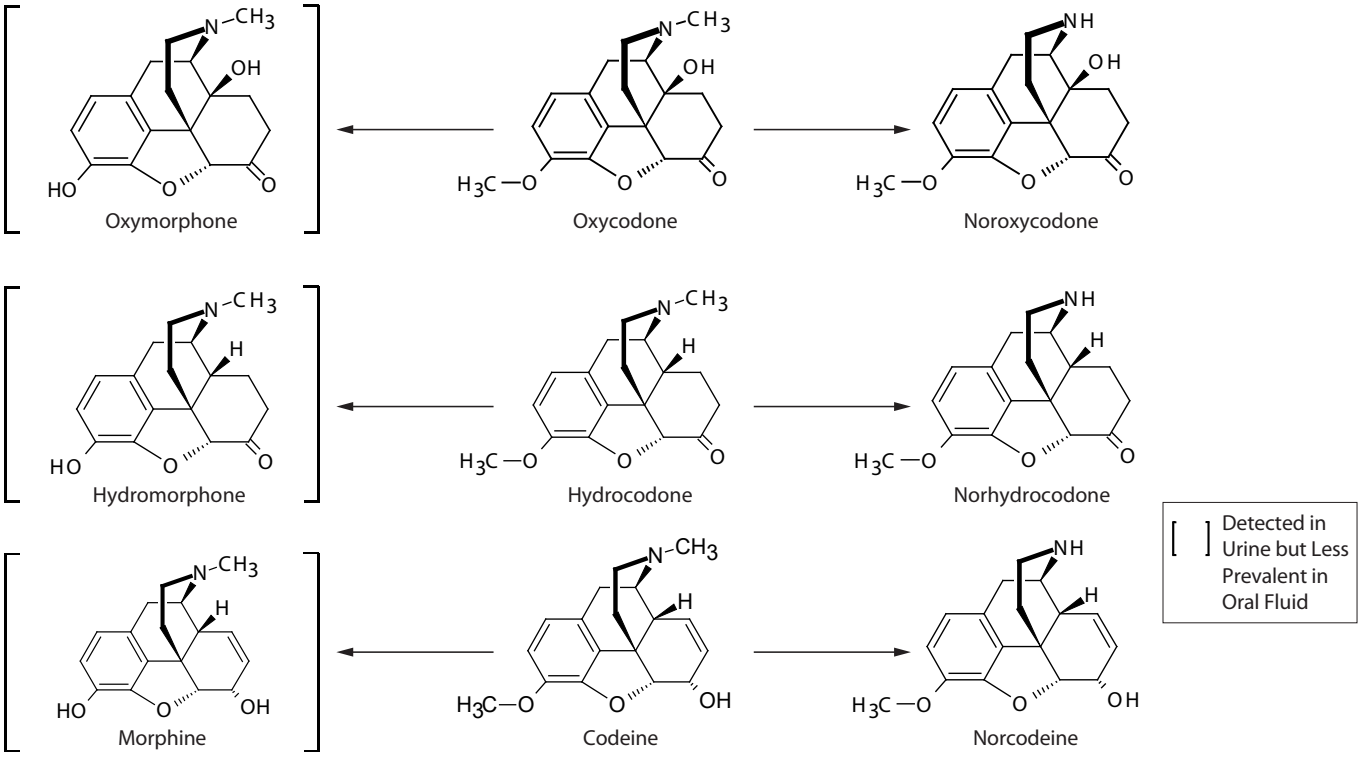
The disposition of hydrocodone in oral fluid and blood following a single, 12.1-mg dose of the free base hydrocodone was reported in 2015 (17), and the pharmacokinetic parameters for hydrocodone, norhydrocodone, and dihydrocodeine in blood and oral fluid were tabulated. Hydrocodone was the predominant analyte and norhydrocodone the predominant metabolite in both matrices studied. Hydrocodone and its metabolites were also studied by another group that compared oral fluid results to those in urine (18). In that study, the metabolic ratio of norhydrocodone to hydrocodone was found to be much lower in oral fluid than in urine.

In a retrospective study of chronic pain patients, hydrocodone, hydromorphone, norhydrocodone, and dihydrocodeine were all detected in various combinations in oral fluid specimens confirmed positive for a hydrocodone/hydromorphone-related compound (14). This study was not a controlled-dose study and, therefore, did not rule out patients dosed directly with hydromorphone. The prevalence rates of hydrocodone, norhydrocodone, hydromorphone, and dihydrocodeine were 51%, 73%, 55%, and 48%, respectively, in 561 hydrocodone/hydromorphone-related oral fluid positives. When hydromorphone-only findings were removed (i.e., to potentially rule out hydromorphone dosing), hydromorphone was detected in only 119 (34%) of the 561 oral fluid hydrocodone-related positive specimens, compared to 412 (73% prevalence) for norhydrocodone.

It would be logical to expect little to no morphine in the oral fluid of subjects dosed acutely with codeine and to find only codeine and norcodeine—or, at least, higher prevalence rates of these analytes—present at detectable levels. One hypothesis explaining why O-demethylated metabolites are detected with less prevalence in oral fluid than in urine relates to the limited ability of these more polar metabolites to cross from the bloodstream to the oral fluid compartments. At least five known factors affect the movement of substances from plasma into formed saliva, which becomes oral fluid: 1) molecular mass and size, with the diffusion

coefficient being inversely proportional to the molecular radius; 2) lipophilicity, with lipophilic substances diffusing more easily than lipophobic substances; 3) ionization, with nonionized or weakly basic substances diffusing more easily than acidic substances; 4) salivary pH, with the pH of the formed saliva being lower than the pH of blood, favoring ion-trapping of basic substances in the formed saliva; and 5) plasma protein binding, with the free or unbound substance being available to pass across membranes from plasma into formed saliva, whereas protein-bound drug cannot (16).

The following metabolic pathways are presented to clarify the postulated prevalence differences in metabolic profiles detected in oral fluid and urine:



DePriest et al. compiled a comprehensive review of opioids and their metabolism (19).

Workplace Drug Testing

The recent inclusion of the semisynthetic opioids hydrocodone, hydromorphone, oxycodone, and oxymorphone as analytes in federally regulated urine testing (20,21) and the potential expansion of federal workplace drug testing to include oral fluid as a specimen matrix (22) will certainly present new challenges both analytically and in the interpretation of toxicology results. Likewise, the possible future inclusion of hair as a matrix in federally regulated testing will further complicate interpretation.

Summary

Numerous factors can affect the presence and absence of opioids in urine or oral fluid and the interpretation of test results. A summary list is presented below:

1. The matrix (matrices) on which testing was performed (e.g., urine, neat oral fluid, absorbent pad-type collector for oral fluid, hair)
2. Opioid(s) prescribed and its form (e.g., oral, parenteral, suppository)
3. The dose of the opioid(s) and whether dosing was acute or chronic
4. The time and date of the last dose before specimen collection
5. Disease states that may affect metabolism (e.g., hepatitis) or the matrix itself (e.g., Sjögren's syndrome causes dry mouth)
6. The use of non-targeted drugs that may affect metabolic enzyme activity, especially CYP2D6 inhibitors (e.g., paroxetine) or CYP3A4 inhibitors (e.g., clarithromycin).

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