Clinical Biochemistry 112 (2023) 6–10

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

A preliminary study of hydrocodone and hydromorphone to oxycodone ratios for distinguishing impurities from independent opioid use

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ARTICLE INFO

Keywords:
Oxycodone
Oxymorphone
Hydrocodone
Hydromorphone
Urine drug testing
Impurities

ABSTRACT

Background: Urine drug testing (UDT) monitors prescription compliance and/or drug abuse. However, interpretation of UDT results obtained by liquid chromatography-tandem mass spectrometry (LC-MS-MS) can be complicated by the presence of drug impurities that are detected by highly sensitive methods. Hydrocodone is a drug impurity that can be found as high as 1% in oxycodone pills.

Objectives: We evaluated the frequency and concentration of hydrocodone and its metabolite, hydromorphone, in patients taking oxycodone to check if the ratio of hydrocodone or hydromorphone to oxycodone could distinguish between oxycodone only use from those consuming additional opiates.

Design & methods: We correlated LC-MS/MS results with medication records of 319 patients with positive oxycodone results over 7 months (4/2021-11/2021).

Results: Fifteen of 319 patients with positive oxycodone results were taking oxycodone only. For these 15 patients, the mean ratio of hydrocodone to oxycodone was 0.57% (range 0.05%-3.35%), and the mean ratio of hydromorphone to oxycodone was 0.81% (range 0.18–3.51%).

Conclusions: Hydrocodone and/or hydromorphone are detectable in patients taking only oxycodone and can likely be identified as an impurity if their calculated ratio to oxycodone is <1 %. Further validation of the ratios in a larger sample size is recommended.

1. Introduction

A key part of pain management, especially for patients with chronic pain, is urine drug testing (UDT) to monitor compliance with prescribed therapy and detect the use of nonprescribed or illicit substances. A positive UDT for a nonprescribed or illicit drug can have severe consequences for patients, including the physician's refusal to refill their opioid prescription or expulsion from their pain management program. Consequently, inappropriate result interpretation may lead to inappropriate actions that can leave many patients without access to life-altering pain medications. Unfortunately, the availability of different testing modalities (immunoassays vs mass spectrometry), variable genetic polymorphisms and certain co-medications affecting drug metabolism, and pharmaceutical drug impurities renders UDT result interpretation rather complicated [1–3].

Immunoassays are commonly used in urine drug screening for selected drug classes (like opiates, benzodiazepines, and amphetamines). The problem is that immunoassays often lack the specificity for a particular drug and sometimes even lack the sensitivity to detect low concentrations of these drugs. Therefore, all positive drug screen tests and unexpected negative results by immunoassays should be considered presumptive until confirmed using a more specific and sensitive methodology, such as mass spectrometry [4–6]. However, the interpretation of UDT results obtained by liquid chromatography-tandem mass spectrometry (LC-MS/MS) can be complicated due to drug impurities detected by high analytical sensitivity methods. For example, the detection of trace amounts of codeine by LC-MS/MS in the urine of patients taking morphine may indicate nonprescribed codeine use, except for the fact that morphine itself is pharmaceutically synthesized from codeine, and there is <0.5 % codeine impurity in the morphine pill itself [1,7]. Similarly, patients taking oxycodone can have trace levels of hydrocodone present in their urine [8], owing to the <1 % of

Abbreviations: UDT, urine drug testing; LC-MS/MS, liquid chromatography-tandem mass spectrometry; DAU, drugs of abuse; ESI, electrospray ionization; MRM, multiple reaction monitoring mode; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

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https://doi.org/10.1016/j.clinbiochem.2022.12.009
Received 14 October 2022; Received in revised form 9 December 2022; Accepted 14 December 2022
Available online 16 December 2022

hydrocodone present in the pharmaceutical preparation of oxycodone [9]. The exact concentrations of hydrocodone impurities are uncharacterized and vary between brands and batches.

Recently, our group encountered patients taking oxycodone with measurable levels of hydromorphone (a hydrocodone metabolite) with or without the presence of hydrocodone, complicating the interpretation of these urine drug results. Hydromorphone is a prescribed oral pain medication under the brand names, Dilaudid® and Exalgo® and is also a metabolite of morphine, but there are no reports on trace levels of it in patients taking oxycodone. It is essential to distinguish between hydromorphone from the metabolism of residual hydrocodone in an oxycodone prescription and hydromorphone from other sources that may suggest non-compliance.

We conducted a retrospective study to evaluate the frequency and concentration of hydrocodone and hydromorphone in patients only prescribed one opioid, oxycodone, and to evaluate if the ratio of hydrocodone and hydromorphone to oxycodone may be useful to separate those taking oxycodone only from those taking oxycodone and other opiates.

2. Materials and methods

2.1. Sample selection

This study was approved by Yale University's institutional review board (IRB). Two criteria were implemented to consider patients as taking only oxycodone:

1. Urine LC-MS/MS results should be negative for all opioids other than oxycodone, oxymorphone, hydrocodone, and hydromorphone.
2. Patients should have no newly prescribed or ongoing opioid medication other than oxycodone in the last seven days before sample collection.

We reviewed the LC-MS/MS results and medication records of 319 patients who had a confirmatory positive oxycodone test by LC-MS/MS over seven months (4/2021-11/2021). An illustration of the process we followed to screen patients is summarized in Fig. 1.

Opioids are detectable in urine samples for 1 to 3 days [9]. However, since we are tracking lower detectable concentrations and detection time depends on the dose, frequency of use, and individual metabolism, we decided to use 1-week as a cut-off. To ensure we captured all ongoing prescribed opioid medications, we also reviewed patients' medical history up to three months from the time of sample collection. Per the Centers for Disease Control and Prevention guidelines, "Clinicians should evaluate benefits and harms of continued therapy with patients every-three months or more frequently" [5]. Table 1 summarizes the LC-MS/MS results and list of active opioid medication for the 34 patients who tested positive for oxycodone and had detectable levels of hydrocodone and/or hydromorphone. Noteworthy exceptions: The patient of sample 20 in Table 1 has been prescribed hydromorphone, but it was included in the "oxycodone only" analysis group when calculating ratio of hydrocodone-to-oxycodone (Fig. 2), since hydrocodone is not a hydromorphone metabolite. However, the hydromorphone-to-oxycodone calculation for sample 20 was plotted on the "Oxycodone + other opioids". Sample 30 in Table 1 was positive for hydromorphone with a concentration higher than that of oxycodone, and Sample 31 in Table 1 was positive for cocaine metabolite. Both samples have evidence of polysubstance use and thus were added to the oxycodone and other opioids group.

2.2. Statistics analysis

All ratios were calculated using excel sheet. Discrimination power between oxycodone only and oxycodone and other opioids was done by Mann-Whitney test using GraphPad Prism (version 9.2).

2.3. Mass spectrometry

Briefly, positive urine samples by immunoassay screening were hydrolyzed using beta glucuronidase enzyme followed by the addition of ice-cold methanol. Samples were then vortexed, and spun, and the supernatant was transferred into a 96-well collection plate to run on the mass spectrometer. The LC-MS/MS system used was a Waters Acquity I Class UPLC coupled to the Waters XEVO TQS tandem mass spectrometer equipped with an electrospray ionization (ESI) probe operating at multiple reaction monitoring mode (MRM) positive ion mode. Two MRM transitions are monitored per analyte, a quantifier MRM channel

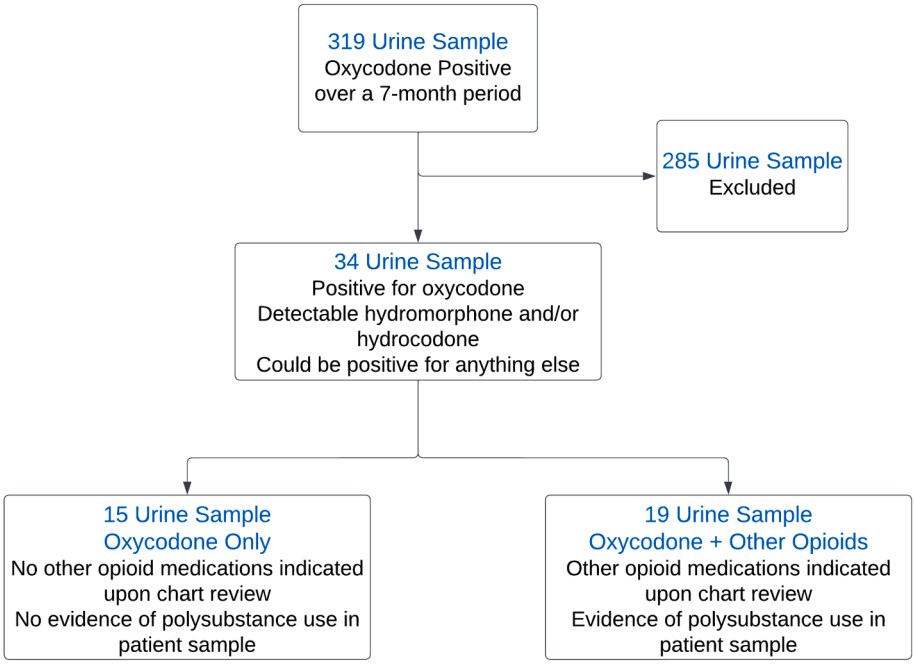

Fig. 1. Flowchart for selection and inclusion criteria for oxycodone positive urine samples in the study. All results are based on LC-MS/MS analyses.

Table 1
Oxycodone, hydrocodone and hydromorphone concentrations and ratios in 34 patients who met the inclusion criteria.

#	OC (ng/mL)	HC (ng/mL)	HM (ng/mL)	HC/OC	HM/OC	HC/OC (%)	HM/OC (%)	Active Opioid Medication
1	4802	ND	14	NA	0.003	NA	0.29	OC Only
2	4726	11	10	0.002	0.002	0.23	0.20	OC Only
3	10189	5	ND	0.001	NA	0.05	NA	OC Only
4	5940	6	ND	0.001	NA	0.10	NA	OC Only
5	8949	27	ND	0.003	NA	0.30	NA	OC Only
6	2776	9	ND	0.003	NA	0.34	NA	OC Only
7	9207	63	ND	0.007	NA	0.69	NA	OC Only
8	3574	11	ND	0.003	NA	0.32	NA	OC Only
9	9340	8	ND	0.001	NA	0.09	NA	OC Only
10	5693	ND	10	NA	0.002	NA	0.18	OC Only
11	4695	ND	17	NA	0.004	NA	0.37	OC Only
12	3549	ND	10	NA	0.003	NA	0.29	OC Only
13	584	ND	21	NA	0.035	NA	3.51	OC Only
14	4197	140	ND	0.03	NA	3.35	NA	OC Only
15	12367	375	ND	0.03	NA	3.03	NA	OC Only
16	1536	ND	2795	NA	1.819	NA	182	OC, HM
17	69	ND	4534	NA	66	NA	6585	OC, HM
18	575	ND	391	NA	0.68	NA	68.1	OC, HM
19	53	ND	1552	NA	29	NA	2938	OC, HM
20	9624	21	14648	0.002	1.5	0.2	152	OC, HM
21	761	ND	1914	NA	2.5	NA	252	OC, HM
22	3046	ND	2026	NA	0.67	NA	66.5	OC, HM inj
23	395	ND	6228	NA	15.8	NA	1578	OC, HM inj
24	1221	ND	608	NA	0.50	NA	49.8	OC, HM inj
25	1517	ND	911	NA	0.60	NA	60	OC, HM inj
26	1834	ND	290	NA	0.16	NA	15.8	OC, HM inj
27	259	ND	44	NA	0.17	NA	16.9	OC, HM inj
28	1021	ND	29	NA	0.03	NA	2.86	OC, HM inj
29	461	ND	60	NA	0.13	NA	13.01	OC, M inj
30	237	56	545	0.24	2.3	23.6	230	OC, PU
31	72	405	27	5.6	0.37	564	37.4	FENT, PU
32	381	1983	872	5.2	2.29	521	229	OC, HC-ACET
33	2215	305	37	0.14	0.02	13.8	1.68	OC, HC-ACET
34	61	317	370	5.2	6.1	518	606	OC, HC-ACET

AMR is 2500 and values above were not confirmed on dilution and are under recovered.
HC: Hydrocodone, OC: Oxycodone, HM: Hydromorphone, M: Morphine, ACET: acetaminophen, FENT: Fentanyl, inj: injection, PU: polysubstance use.
ND: none detectable (below limit of detection).

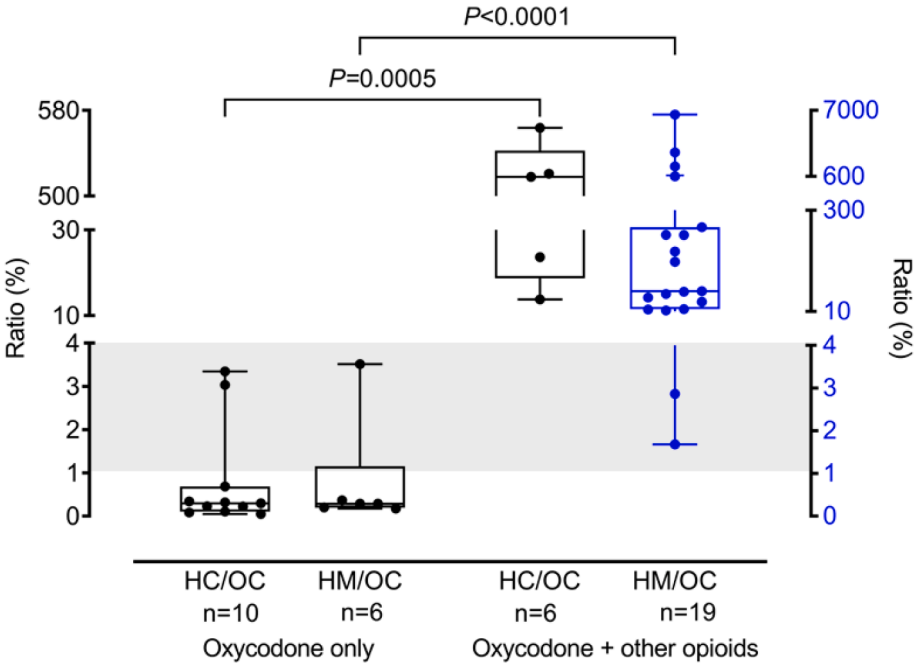

Fig. 2. Hydrocodone and Hydromorphone ratios percentage to Oxycodone in urine samples identified as on oxycodone only and in urine samples in patients on oxycodone and other opioids. HC: Hydrocodone, OC: Oxycodone, HM: Hydromorphone.

and a qualifier MRM channel, and a multipoint internal standard calibration, using isotopically labeled internal standards, was used to generate a calibration curve. The ion-ratios between the quantifier ion and qualifier ion in addition to the retention time window are used to confirm the identity of analytes. The concentrations of morphine, oxymorphone, hydromorphone, codeine, naloxone, oxycodone, hydrocodone, 6-monoacetylmorphine, benzoylecgonine, normeperidine, meperidine, phencyclidine, methadone metabolite (EDDP) and methadone in a sample were determined from each compound's calibration curve. Cutoff values for all analytes are at ≥50 ng/mL except for 6-monoacetylmorphine with a cutoff value of ≥10 ng/mL. Commercial quality control (QC) materials from UTAK Laboratories, Inc (Valencia, CA, USA) with target concentrations were used. Two QC levels with morphine-glucuronide and codeine-glucuronide are used to assess hydrolysis efficiency, which ranged 91–93 % for both.

3. Results

Out of 319 urine samples with positive oxycodone results, 19 had evidence of polysubstance or an indication of opioid medication use in the last seven days recorded in their medical chart. Also, after review, 15 urine samples had no evidence of polysubstance or opioid medication use (other than oxycodone) indicated in their medical chart. The remaining 285 samples did not have any detectable hydrocodone or hydromorphone by LC-MS/MS, and were therefore excluded from the study (Fig. 1). For samples collected from patients only on oxycodone medication, the mean percent ratio of hydrocodone to oxycodone was 0.79 % (range 0.05 %–3.35 %), and the mean percent ratio of hydromorphone to oxycodone was 0.81 % (range 0.18 %–3.51 %) (Table 1 and Fig. 2). On the other hand, samples from patients who were taking oxycodone and other opioids showed mean percent ratios of 327.98 % (range 13.77 %–563.6 %) for hydrocodone to oxycodone and 688.6 % (range 1.68 %–6585.1 %) for hydromorphone to oxycodone (Table 1 and Fig. 2). The difference between the ratio of hydrocodone to oxycodone between oxycodone only and oxycodone and other opioids was significant with a P value of 0.0005 and that of hydromorphone to oxycodone was $P < 0.0001$.

4. Discussion

Pain management can be very challenging; therefore, laboratory testing provides an objective assessment of drug exposure and adherence to treatment. Mass spectrometry is a sensitive and specific tool for the qualitative and quantitative analysis of drugs, overcoming the interference and poor sensitivity issues that plagued immunoassays. However, the exceptionally high sensitivity of mass spectrometry is a double-edged sword, because we can now detect the presence of impurities in pharmaceutical preparations of certain drugs, which makes interpreting the mass spectrometry-based results much more complicated.

Previous attempts to look at detectable impurities in patients prescribed oxycodone by West R. et al. focused on the absolute concentrations of hydrocodone in patients taking oxycodone and concluded that hydrocodone concentrations > 500 ng/mL with oxycodone < 100,000 ng/mL or hydrocodone > 1,500 ng/mL with oxycodone > 100,000 ng/mL is indicative of nonprescribed hydrocodone intake [8]. Concentration-based cutoffs for decision-making in urine can be problematic because of variable hydration status, genetic polymorphisms affecting metabolism, and variable drug doses and frequency of administration, all of which can affect drug and impurity concentrations in urine.

In this work, for the first time, we demonstrated that trace levels of hydromorphone, a hydrocodone metabolite, can be present in patients' urine who have only been prescribed oxycodone. We also identified that detectable hydrocodone or hydromorphone in patients taking only oxycodone can be identified as an impurity if their calculated ratio to oxycodone is <1 %. So, we offer an alternate approach that is concentration independent. Based on our preliminary findings, the ratio of hydrocodone and hydromorphone to oxycodone may offer more practical help to laboratorians interpreting positive oxycodone UDT results.

Our data showed that trace amounts of hydrocodone and/or hydromorphone are possible in patients taking only oxycodone. For hydrocodone, those values ranged from 5 to 375 ng/mL, while they were much lower for the metabolite hydromorphone, ranging from 10 to 21 ng/mL. Therefore, it is not surprising that laboratories often encounter positive hydrocodone results in patients taking oxycodone, but hydromorphone is unlikely to be positive unless laboratories use much lower cutoffs (below 20 ng/mL for positivity). Either way, the source of hydrocodone and hydromorphone can be confirmed by calculating their ratio to oxycodone. Ratios < 1 % strongly suggest that the source of hydrocodone or hydromorphone is the impurity from the pharmaceutical preparation of oxycodone.

Interestingly, 14 out of 15 samples with prescribed oxycodone only were positive for either hydrocodone or hydromorphone but not both, with hydromorphone concentrations typically much lower. This observation agrees with the literature published; hydromorphone is detected in urine in much lower concentrations than hydrocodone, but it is detectable for more extended hours after each dose [10].

This study is limited by the small sample number (15 oxycodone only group vs 19 oxycodone plus other opiates group) dictated by the manual nature of retrieving the mass spectrometry and opioid medication data. However, given how consistent the ratios were among patients taking oxycodone only, its alignment with the suggested purity reported by the manufacturer (<1%) and the novelty in reporting trace levels of hydromorphone, we believe these small findings are significant to report and hope this paper will influence a follow-up study with a higher number of samples. Other important limitations include the fact that each patient is taking a different dose of the medication with different release forms, and we do not have information about the last dose and the frequency of doses taken before giving the sample. However, that did not seem to affect the discriminatory power of the ratio calculation. In addition, some of the concentrations reported are outside the tested analytical measurement range (AMR). Validated AMR for oxycodone, hydromorphone and hydrocodone are from 5 to 2500 ng/mL. Nevertheless, the majority of samples (13 out of 16) with oxycodone and other opioids with ratios > 1 % were within the AMR. Only in the oxycodone-only samples with ratios < 1 %, where the oxycodone values were above the AMR. In testing concentrations above the AMR, up to 14,000 ng/mL (the highest oxycodone concentration detected), there was an under-recovery up to 50 %. However, if we were to dilute and rerun to measure more accurate oxycodone values, the ratios would have been even lower, offering more discriminatory power. We identified three samples with ratios between 1 and 4 % where we could not confirm other opioid use (Fig. 2 gray area). It is essential to be careful in interpreting such results, as it can be due to an oxycodone impurity contributed by individual differences in metabolism or other opioid use. Considering the under-recovery reported with oxycodone above AMR, the actual oxycodone concentration could be high enough to bring the ratio of hydromorphone or hydrocodone to oxycodone much closer to 1 than it is currently. Samples with oxycodone above AMR with detectable hydromorphone or hydrocodone should be diluted and rerun to get an accurate concentration to calculate ratios.

5. Conclusion

We have reported for the first time the detection of trace levels of hydromorphone as a metabolite of an impurity (hydrocodone) from prescribed oxycodone. Our work will help laboratorians and clinicians interpret impurities related to oxycodone testing, raising the awareness of the possibility of detecting not only hydrocodone but also its metabolite, hydromorphone, in urine samples from patients taking oxycodone only. Calculating ratios of hydrocodone and hydromorphone

to oxycodone may confirm that the detectable hydrocodone or hydromorphone results from the impurity from the pharmaceutical preparation of oxycodone (if < 1 %), independent of their concentrations in urine. Considering the low sample number used in drawing this conclusion, we recommend that laboratorians use this data cautiously, until a larger study confirms our findings.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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