ORIGINAL ARTICLE

Urinary metabolic ratio of pain management and substance abuse treatment drugs: Reference intervals

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ABSTRACT

We present data that show that quantitative urine drug concentrations obtained from individuals monitored for drug compliance as part of their participation in chronic opioid or substance abuse treatment can be used to quantify drug metabolism. We quantitatively monitor 18 drugs and their Phase 1 metabolite. These drugs were dextromethorphan, morphine, oxycodone, hydrocodone, quetiapine, tapentadol, tramadol, buprenorphine, clonazepam, fentanyl, imipramine, ketamine, carisoprodol, alprazolam, methadone, and amitriptyline. By using the ratio of metabolite/parent drug (prescribed medication), the expected or reference values for 18 drugs were obtained. Ratio values outside of this reference range could be considered to be caused by genetic metabolizing variants, drug–drug interactions, age, or deception. Alerting providers of the variance in metabolism from the expected norm might reduce overdosing or underdosing patients.

INTRODUCTION

A significant source of interindividual variability in the drug response is drug metabolism, where differences in the ability to metabolize drugs result in varying levels of systemic exposure.[1] Several factors such as genetics, age, sex, disease states, lifestyle, environmental factors, and concomitant medications can contribute to this variability by altering plasma concentrations of drugs. Genetic variations in the major drug metabolizing enzymes, cytochrome (CYP) 450, can produce CYP enzymes with reduced/total absence or enhanced functionality and alter drug metabolism. The concurrent use of medications that inhibit or induce the CYP enzymes, or substrates of the CYP enzymes, results in drug–drug interactions (DDIs) that also alter drug metabolism. These medications include antidepressants and antipsychotics, for example, fluoxetine, paroxetine, sertraline, citalopram, duloxetine, venlafaxine, and bupropion.[2] Compliance, dosage, route of administration, timing and frequency of administration, renal and liver functions are other factors known to impact variability and drug response.

The purpose of this paper is to help providers better understand the metabolism of opioids and some drugs used to treat pain or substance abuse disorder. Providers often do not consider genetic differences of patient metabolism. Patients given these treatment medications often use marijuana and are also given medications for depression and smoking cessation. All these can affect drug metabolism with possible negative consequences for the patient. These include the potential of overdose, or ineffective treatment. Metabolic studies have traditionally used blood specimens to perform pharmacokinetic analysis of drug metabolism. We argue and present data that quantitative urine drug metabolism can also be used to better understand metabolism in real-world situations. To do this, we present urine test drug data on 18 drugs and their Phase 1 metabolic products (Table 1).

Urine drug testing is performed on patients on chronic pain medications and those undergoing substance abuse rehabilitation. The reasons for these tests are to monitor medication compliance, detect unexpected drug use, and detect illicit drug use. Our laboratory has performed over 4 million drug tests

Table 1. Table of metabolite–parent drug pairs and associated CYP metabolizing enzymes

Metabolite–parent drug pair	CYP metabolizing enzyme
Dextrorphan/ dextromethorphan	CYP2D6[6,7]
Oxymorphone/oxycodone	CYP2D6[8]
Hydromorphone/ hydrocodone	CYP2D6[9]
O-Desmethyltramadol/ tramadol	CYP2D6, CYP3A4[10]
Hydromorphone/morphine	??CYP2D6[11,12]
Norbuprenorphine/ buprenorphine	CYP3A4/5[13]
Norfentanyl/fentanyl	CYP3A4/5[14]
Noroxycodone/oxycodone	CYP3A4/5[8]
Norhydrocodone/ hydrocodone	CYP3A4/5[15]
7-Aminoclonazepam/ clonazepam	CYP3A4/5[16]
α-Hydroxyalprazolam/ alprazolam	CYP3A4/5, CYP2C9[17]
Norquetiapine/quetiapine	CYP3A4/5, CYP2D6[18,19]
Meprobamate/carisoprodol	CYP2C19[20]
N-Desmethyltapentadol/ tapentadol	CYP2C9, CYP2C19, CYP2D6[21]
Norketamine/ketamine	CYP3A4, CYP2B6, CYP2C9[22]
EDDP/methadone	CYP3A4, CYP2B6, CYP2D6, CYP2C19[23]
Desipramine/imipramine	CYP2C19, CYP1A2, CYP3A4, CYP2D6[24,25]
Nortriptyline/amitriptyline	CYP2C19, CYP2D6, CYP2C9, CYP3A4, CYP2C8[26,27]

CYP: cytochrome; EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

since the year 2016. Many of these urine drug tests quantitatively measure the administered drug and its metabolite. Both the administered drug and its corresponding metabolite appearing in urine reflect the final processing of the medication and offer a way of monitoring its metabolism. Analysis of these data offers an opportunity to study the metabolism of these drugs. In this study, we term the parent drug as the taken medication, and the metabolite as the medication processed by any Phase 1 CYP450 pathway. The metabolic ratio (MR) of metabolite/parent drug, therefore, reflects the ability of the CYP450 pathway to process the drug. Some data proving the hypothesis that drug metabolism can be studied using urine specimens include studies showing that MRs follow predicted gene frequencies for fast and slow metabolizers in a test population.[3,4] A second proof is that known DDIs such as those due to serotonin norepinephrine reuptake inhibitors inhibit the metabolism of those drugs metabolized by the CYP2D6 pathway.[5] That is, in the presence of these drugs, the MR is markedly decreased in urine specimens. A third proof is that from a pharmacokinetic analysis, the MR follows the predicted relationship between first order and zero order as a function of increasing dosage. That is, as the dosage increases, the CYP450 system is saturated and proportionately more drug is not processed by that system. A fourth proof is that the MR decreases with age, that is, the metabolic pathways processing ability decreases with age. Our method of examining MRs using drug excretion data is unusual and differs from the usual methods of giving a known dose and monitoring blood levels of the test drug and one or more of its metabolites. However, as shown in other studies, the urinary metabolites of drugs such as dextromethorphan can be used to show effects on drug metabolism.[6] Studies on the metabolism of oxycodone to oxymorphone using a combination of pharmacogenomics and MRs follow the expected correlation of gene allele prevalence and MR.[3,4]

This work is divided into several studies to explore the use of the MR of metabolite to parent drug excreted into urine. The analyses used in these studies were estimates of drug metabolism. In the first study, we define reference intervals (RIs) for each of the MRs, the results of which are presented in this paper. In the other studies, we reclassify the metabolic ratio cutoff based on CYP metabolism, analyze the effect of DDIs and age on MR, explore how MRs allow pharmacokinetic differentiation of zero and first order metabolism, and use MRs to estimate attempts at deception of the drug test process. These studies will be presented separately in subsequent papers. Table 1 presents the CYP450 metabolizing enzymes associated with the drug pair.

The pairing of the CYP enzymes(s) with the drug pair was determined using studies that depict the main CYP enzyme(s) responsible for metabolism.

In this analysis, we chose to define a RI for each metabolite/parent drug. A RI is defined in this study as the range of MRs expected from a healthy population.[28-30] We defined our healthy population as the specimens without interfering drugs present. In this study, interfering drugs include fluoxetine, paroxetine, bupropion, citalopram, sertraline, venlafaxine, duloxetine, risperidone, trazodone, haloperidol, aripiprazole, cyclobenzaprine, amphetamine, and tetrahydrocannabinol (THC). A lower MR implies less drug processing, while a higher MR implies more drug processing compared to that defined by the RI. The final metabolism is determined by factors such as the patient's pharmacogenetic makeup and DDIs. As part of our urine drug testing for pain practices and substance abuse clinics, we test for 18 metabolite/parent drug pairs (Table 1). Data from 2 million specimens collected between January 2020 and September 2024 were used in the analysis. The RI was estimated using the 95 percent interval limits. This means using the 2.5th percentile and 97.5th percentile of the data for each metabolite-parent pair to estimate the lower and upper endpoints. These endpoints were back-transformed to the original, arithmetic units through the anti-log. For ease of comparison, we used the median concentration in ng/mL as the comparative metric. Graphs show log-transformed MRs: log transformation produces a more tractable basis for presentation and analysis.[28] The data tables include both logarithmic and arithmetic calculations. The arithmetic calculations better expedites understanding of the variance.

METHODS

Populations

This was a retrospective study of around 2.2 million data derived from patient specimens submitted for urine drug testing by pain physician clinics and rehabilitation facilities located in 50 United States states and the District of Columbia.[31] Quantitative urine drug testing of these specimens, collected between January 2, 2020, and September 30, 2024, was performed at Precision Diagnostics using a highly sensitive clinically validated liquid chromatography tandem mass spectrometry (LC–MS/MS) method capable of detecting low concentrations of the analytes.[32] No exclusion criteria were applied in the selection of the 581,614 patients included in this study. None of the data was from retested samples as repeats were excluded. Data were deidentified by assigning unique patient identification numbers and specimen numbers to patients and specimens, respectively. All specimens that also contained a potential interference drug were excluded. Interfering drugs include fluoxetine, paroxetine, bupropion, citalopram, sertraline, venlafaxine, duloxetine, risperidone, trazodone, haloperidol, aripiprazole, cyclobenzaprine, amphetamine, and THC. Figure 1 has a diagram of the selection and filtering of the patient population for the generation of the RIs for MR. For oxycodone, hydrocodone, and morphine, we extracted a second population set that

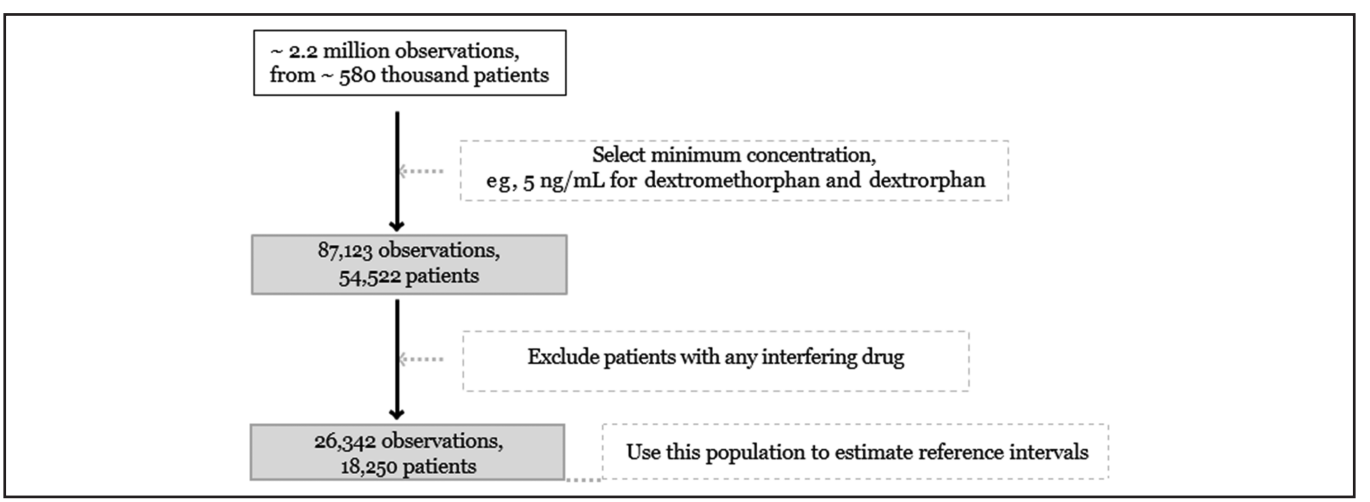


Figure 1. Diagram of the selection and filtering of the patient population for the generation of reference intervals for MR.

included only samples from patients with prescribed parent drug while excluding those with prescribed oxymorphone or hydromorphone.

Analytical method

Urinary concentrations of the different parent drugs and metabolites were analyzed using LC–MS/MS.[32] Briefly, a Shimadzu 20-XR series binary pump systems, well-plate autosampler, and thermostatted column oven paired with a Sciex 6500/6500+ mass spectrometer were used for the analysis of all drugs. Chromatographic separation was achieved using a methanol–formic acid–water gradient on a 50 × 4.6 mm, 2.6 µm Kinetex phenyl-hexyl column (Phenomenex, Torrance, California) kept at 40°C. Samples were prepared by the "dilute and shoot" method and hydrolyzed with β-glucuronidase prior to analysis. Thus, calculated concentrations represent the sum total of both free and conjugated forms. Results were analyzed using Indigo Bio Automation Ascent software (Indianapolis, Indiana), using a 4-point calibration curve with linear fit and $1/x^2$ weighting. Calibrators were deemed acceptable if they were within 20 percent of expected concentrations and with R^2 value greater than 0.98. The interassay coefficient of variation (CV) of all analytes at the lower limit of quantitation (cutoff values) was evaluated to be within 20 percent (most were within 10 percent CV). Table 2 lists the minimum cutoff values for detection of the parent drug and metabolites.

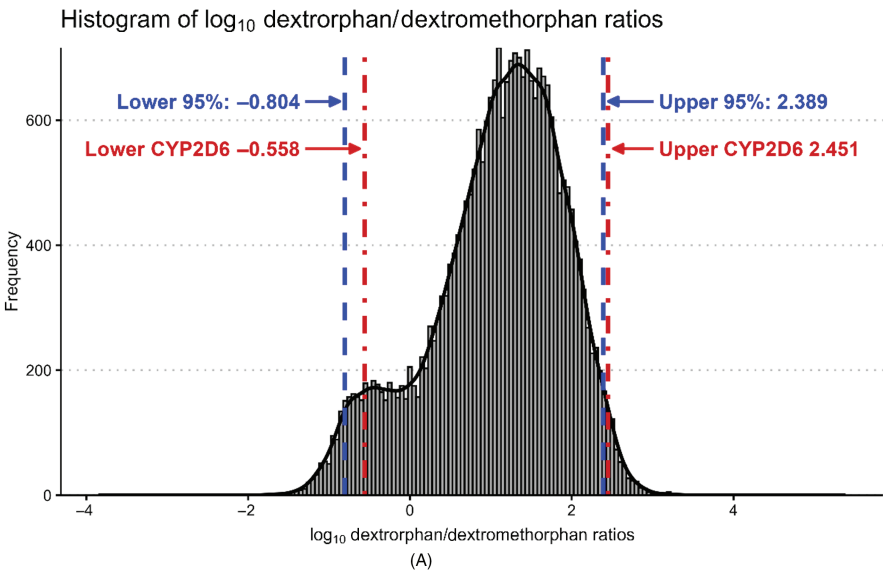
Data and statistical analysis

The urinary concentrations of the analytes (ng/mL), uncorrected for creatinine, were used to calculate MRs, defined as concentration of metabolite divided by concentration of parent drug. Our

Table 2. Minimum cutoff values for detection of the parent drug and metabolite

Parent drug	Cutoff (ng/mL)	Metabolite	Cutoff (ng/mL)
Alprazolam	5	α-Hydroxyalprazolam	5
Amitriptyline	10	Nortriptyline	10
Buprenorphine	5	Norbuprenorphine	5
Carisoprodol	10	Meprobamate	100
Clonazepam	5	7-Aminoclonazepam	5
Dextromethorphan	5	Dextrorphan	5
Fentanyl	1	Norfentanyl	2
Hydrocodone	5	Hydromorphone	5
		Norhydrocodone	10
Imipramine	5	Desipramine	5
Ketamine	2	Norketamine	2
Methadone	50	EDDP	100
Morphine	50	Hydromorphone	5
Oxycodone	10	Oxymorphone	10
		Noroxycodone	25
Quetiapine	5	Norquetiapine	25
Tapentadol	2	N-Desmethyltapentadol	25
Tramadol	25	O-Desmethyltramadol	10

EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.



(A)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.804	2.389	0.157	245.140
2D6	−0.558	2.451	0.277	282.480

(B)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
15.371	41.878	11.669	18,250	26,342

(C)

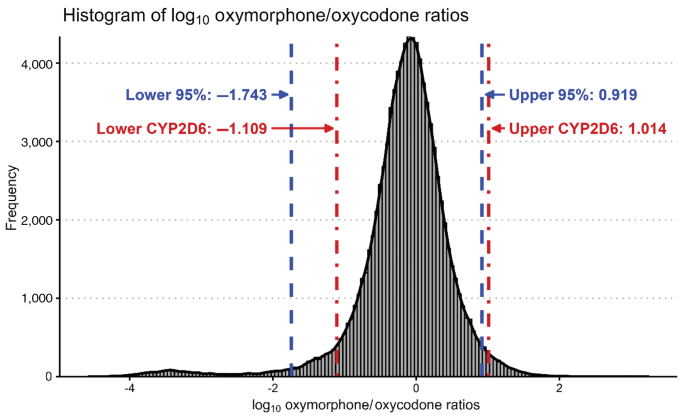
Figure 2. Reference interval histogram and interval limits for dextrorphan/dextromethorphan ratios. (A) Reference interval histogram for dextrorphan/dextromethorphan log-ratios. (B) Table of interval limits for dextrorphan/dextromethorphan ratios. (C) Median, mean, and N for 95 percent interval dextrorphan/dextromethorphan ratios.

aim in generating RIs was to appropriately capture our population's metabolism variance. RIs formed by using all observations or by using a single observation from each patient were found to be consistent across these two approaches. Thus, we formed RIs using all available criteria-matching data. Standard upper- and lower-limit fences were constructed for outlier identification. Only oxycodone and tramadol exhibited outliers; less than 5 such outliers were excluded.

Figures 2-19 illustrate log-MR histograms and interval tables for each metabolite-drug pair. Histograms depict log-MR values since the log transformation yields Gaussian-shaped distributions better suited for analysis.[28] Tables indicate log-MRs and respective normalized (non-log) MRs. RIs use standard 95 percent intervals derived from the 2.5th and 97.5th percentiles. These standard RIs do not account for the differences in metabolism due to genetic factors. Thus, we added percentiles to the tables tied to the expected genomic variance. These genomic variance values are those published by Zhu et al.[4] Data cleaning, extraction, and analysis were performed in the R studio using R.[33,34] Graphs were generated using base R as well as the ggplot package.

RESULTS AND DISCUSSION

The histograms and tabulated data from the 18 drug pairs provide clinicians who interpret urinary excretion data with a range of expected values (Figures 2-19). In the 18 histograms, the y-axis is the number of specimens, and the x-axis is the log-transformed MR. Percentiles are denoted by



(a)

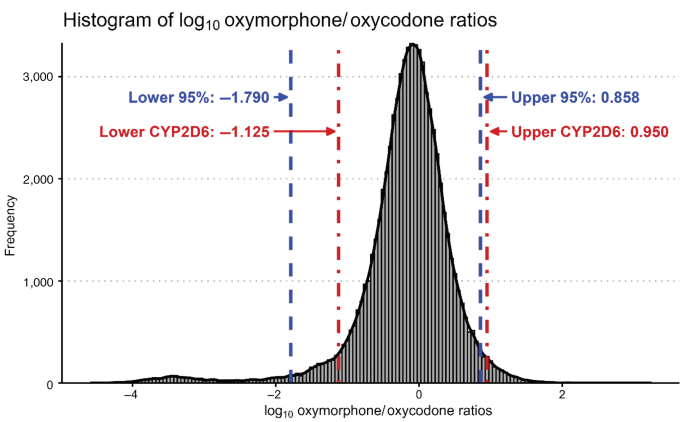
Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−1.743	0.919	0.018	8.307
2D6	−1.11	1.014	0.078	10.324

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.784	1.669	0.681	41024	93878

(c)

(A)



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−1.791	0.858	0.016	7.216
2D6	−1.126	0.950	0.075	8.907

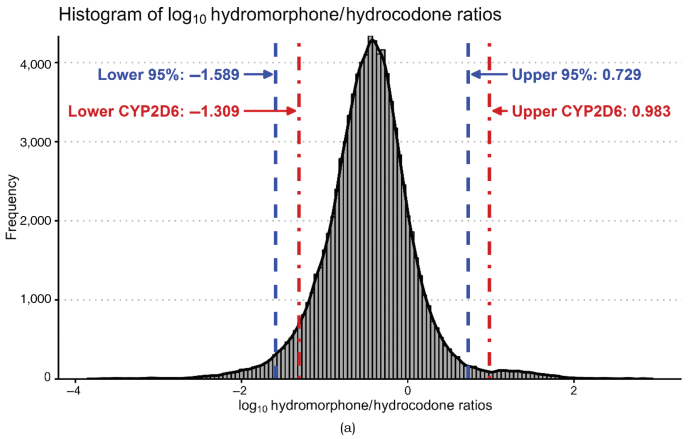
(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.769	1.461	0.657	32,833	71,363

(c)

(B)

Figure 3. (A) Reference interval histogram and interval limits for oxymorphone/oxycodone ratios (nonmedications). (a) Reference interval histogram for oxymorphone/oxycodone log-ratios. (b) Table of interval limits for oxymorphone/oxycodone ratios. (c) Median, mean, and N for 95 percent interval oxymorphone/oxycodone ratios. (B) Reference interval histogram and interval limits for oxymorphone/oxycodone ratios (medications). (a) Reference interval histogram for oxymorphone/oxycodone log-ratios. (b) Table of interval limits for oxymorphone/oxycodone ratios. (c) Median, mean, and N for 95 percent interval oxymorphone/oxycodone ratios. Limits estimated using samples from patients prescribed the oxycodone while excluding those prescribed oxymorphone.



(a)

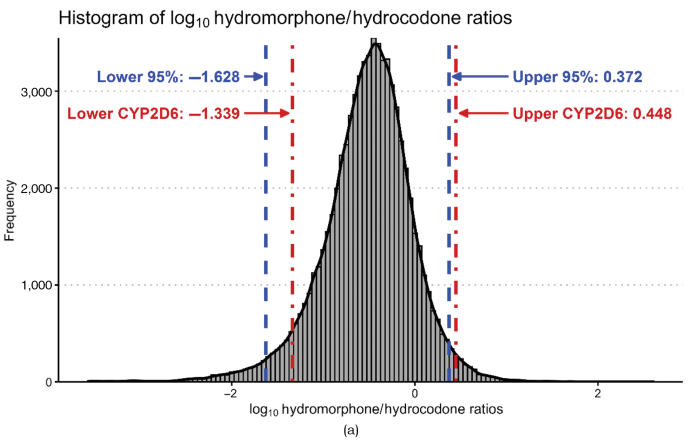
Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−1.589	0.729	0.026	5.353
2D6	−1.309	0.983	0.049	9.610

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.352	4.383	0.349	44,415	90,952

(c)

(A)



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−1.628	0.372	0.024	2.354
2D6	−1.339	0.448	0.046	2.806

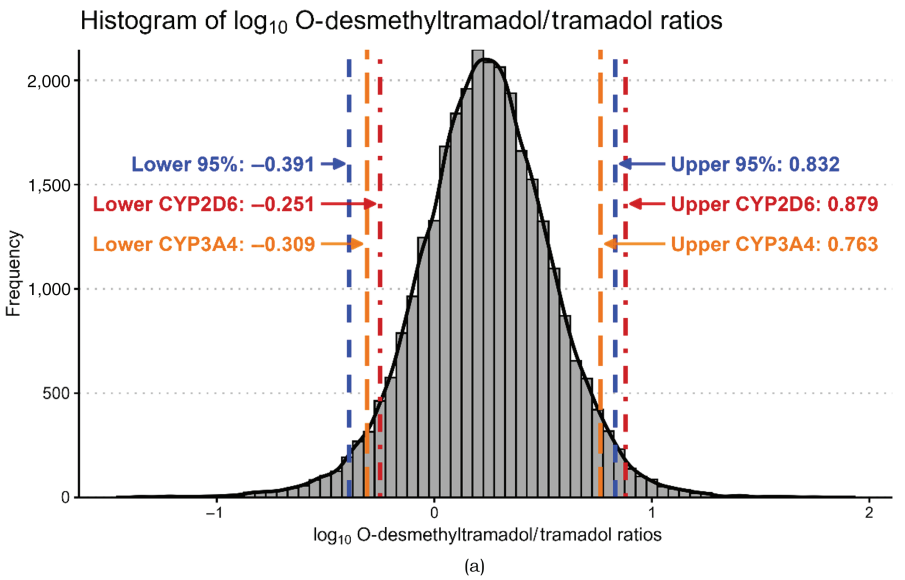
(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.329	0.588	0.301	33,747	71,509

(c)

(B)

Figure 4. (A) Reference interval histogram and interval limits for hydromorphone/hydrocodone ratios (nonmedications). (a) Reference interval histogram for hydromorphone/hydrocodone log-ratios. (b) Table of interval limits for hydromorphone/hydrocodone ratios. (c) Median, mean, and N for 95 percent interval hydromorphone/hydrocodone ratios. (B) Reference interval histogram and interval limits for hydromorphone/hydrocodone ratios (medications). (a) Reference interval histogram for hydromorphone/hydrocodone log-ratios. (b) Table of interval limits for hydromorphone/hydrocodone ratios. (c) Median, mean, and N for 95 percent interval hydromorphone/hydrocodone ratios. Limits estimated using samples from patients prescribed the hydrocodone while excluding those prescribed hydromorphone.



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.391	0.832	0.406	6.787
2D6	−0.251	0.879	0.562	7.562
3A4	−0.309	0.763	0.491	5.796

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
1.722	2.218	1.704	17,376	29,705

(c)

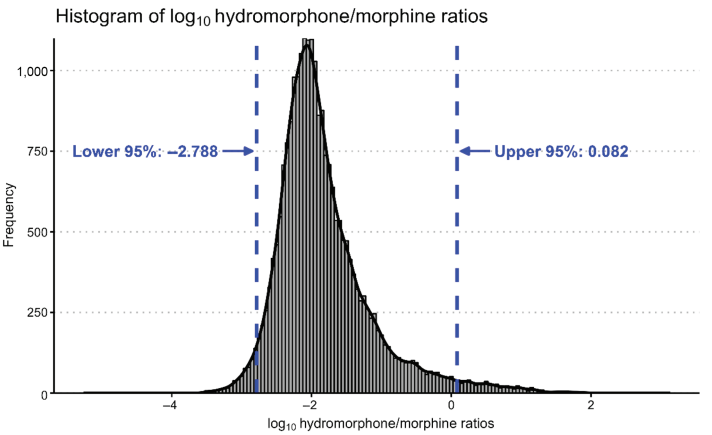
Figure 5. Reference interval histogram and interval limits for O-desmethyltramadol/tramadol ratios. (a) Reference interval histogram for O-desmethyltramadol/tramadol log-ratios. (b) Table of interval limits for O-desmethyltramadol/tramadol ratios. (c) Median, mean, and N for 95 percent interval O-desmethyltramadol/tramadol ratios.

vertical lines. These depict the 2.5th and 97.5th percentiles (blue/dashed), as well as the percentiles estimated from the pharmacogenomic literature (red/dotted-dashed, orange/long dashed, purple/dotted, and green/two-dash line). The accompanying table contains the respective values of the 95 percent RI (low limit and high limit), median, mean (arithmetic and geometric), and number of patients and observations (specimens). We also added the cutoff estimates derived from the literature on the pharmacogenomic frequency estimates of CYP450 enzymes. Results for the genomic variance are discussed in a separate paper. Calculated cutoff values outside the reference range are estimates to identify patients with unusual metabolism. Values outside the reference range encountered in practice can be due to genetic variance or DDIs that alter drug metabolism.

Figures 2-6 denote those drugs metabolized by the CYP2D6 pathway. Figures 7-13 denote those drugs metabolized by the CYP3A4/5 pathway. The remaining drugs are processed through other pathways. Histograms of the log-MRs for these drug pairs exhibited a normal distribution. The MR mean values for most of the 18 drug pairs aligned with previous results. An interesting observation is the significant variability in the RIs (95 percent interval) of the 18 drug pairs. Table 3 summarizes the MR results from previous studies.

Dextromethorphan → dextrorphan

Dextromethorphan is a widely used antitussive drug as over-the-counter treatment for cough and cold.[35] It is primarily metabolized by O-demethylation through CYP2D6 to form dextrorphan.[36,37]



(a)

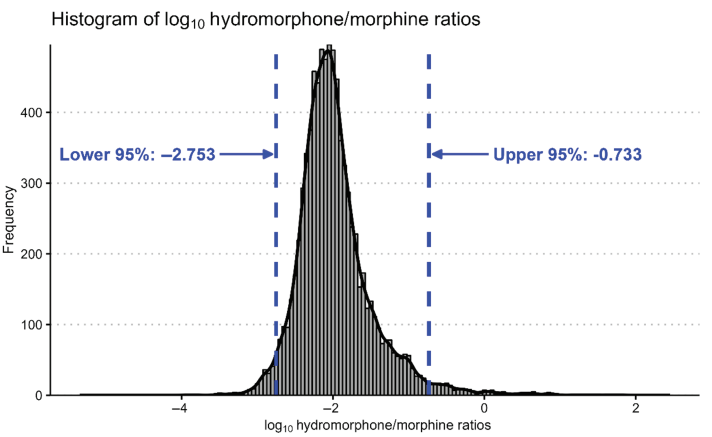
Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−2.788	0.082	0.0016	1.209
2D6	−2.621	0.315	0.0024	2.064

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.011	0.234	0.015	12,231	23,644

(c)

(A)



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−2.753	−0.733	0.0018	0.185
2D6	−2.604	−0.547	0.0025	0.284

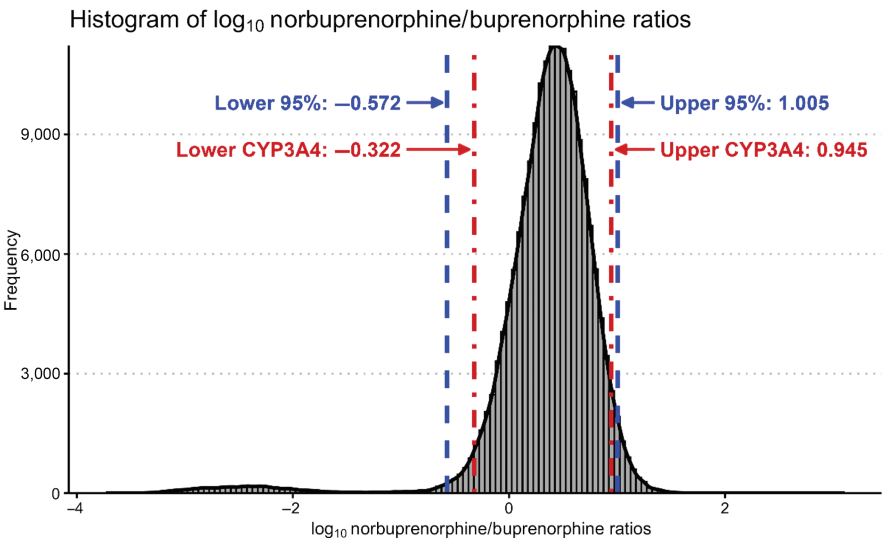
(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.009	0.063	0.011	4,205	8,418

(c)

(B)

Figure 6. (A) Reference interval histogram and interval limits for hydromorphone/morphine ratios (nonmedications). (a) Reference interval histogram for hydromorphone/morphine log-ratios. (b) Table of interval limits for hydromorphone/morphine ratios. (c) Median, mean, and N for 95 percent interval hydromorphone/morphine ratios. (B) Reference interval histogram and interval limits for hydromorphone/morphine ratios (medications). (a) Reference interval histogram for hydromorphone/morphine log-ratios. (b) Table of interval limits for hydromorphone/morphine ratios. (c) Median, mean, and N for 95 percent interval hydromorphone/morphine ratios. Limits estimated using samples from patients prescribed morphine while excluding those prescribed hydromorphone.



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.572	1.005	0.268	10.125
3A4	−0.322	0.945	0.477	8.804

(b)

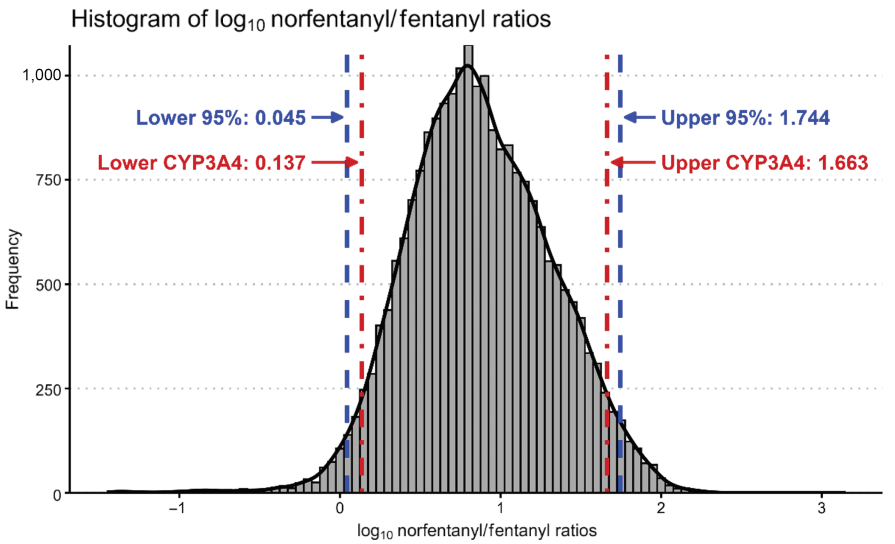
Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
2.556	3.24	2.206	37,793	18,4537

(c)

Figure 7. Reference interval histogram and interval limits for norbuprenorphine/buprenorphine ratios. (a) Reference interval histogram for norbuprenorphine/buprenorphine log-ratios. (b) Table of interval limits for norbuprenorphine/buprenorphine ratios. (c) Median, mean, and N for 95 percent interval norbuprenorphine/buprenorphine ratios.

The histogram of the log [MR] of dextrorphan/dextromethorphan showed a distinct bimodal distribution not seen in the other drug pairs (Figure 2). CYP2D6 is highly polymorphic with more than 100 variant alleles identified.[38,39] As a result, CYP2D6 activity differs significantly within a population, resulting in distinct phenotypes classified according to metabolizing status. These include poor metabolizer (PM), intermediate metabolizer, normal/extensive metabolizer (EM), and ultrafast metabolizer (UM). The bimodal distribution in the log [MR] of dextrorphan/dextromethorphan indicates that two distinct populations are involved in the metabolism of dextromethorphan, eg, EM (major peak) and PM (minor peak). The latter is characterized by low amounts of dextrorphan with increased amounts of the unmetabolized dextromethorphan. These results are consistent with previous studies, which demonstrated a bimodal distribution in the urinary log [MR] of dextromethorphan/dextrorphan that was attributed to distinct phenotypes of extensive and PMs.[36,40,41]

Our study determined a MR mean of 41.88 for dextrorphan/dextromethorphan (Figure 2), which agrees with the mean of 45.45 ± 31.25 obtained by Spina et al.[42] It is also comparable to the MR mean obtained from other studies.[36,41,43,44] We estimated the 95 percent RI for the MR of dextrorphan/dextromethorphan at 0.157-245.14, which showed a wide variability consistent with the findings of others (reflected in their large standard deviation [SD] values) (Table 3).[36,41-43] The influence of the CYP2D6 polymorphism on dextromethorphan metabolism, which determines the formation of dextrorphan and the amount excreted in urine, contributes significantly to the variability in MR. Other factors such as dose, sampling time after dose and differences in absorption, distribution, and excretion can also contribute to the variability.



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	0.045	1.744	1.109	55.488
3A4	0.137	1.663	1.372	46.049

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
7.050	12.459	7.336	11,861	22,085

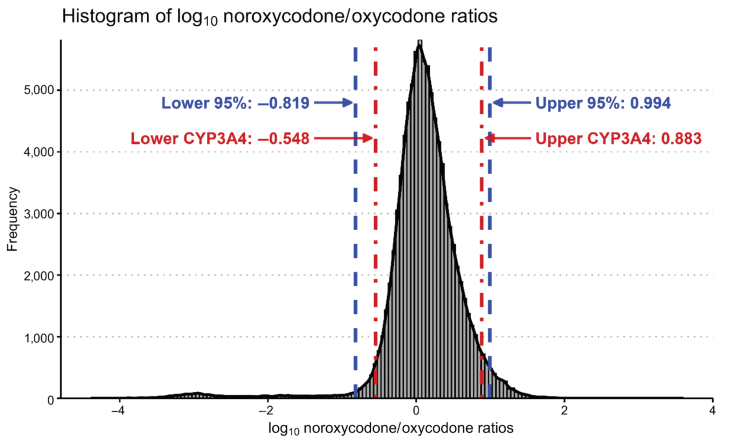
(c)

Figure 8. Reference interval histogram and interval limits for norfentanyl/fentanyl ratios. (a) Reference interval histogram for norfentanyl/fentanyl log-ratios. (b) Table of interval limits for norfentanyl/fentanyl ratios. (c) Median, mean, and N for 95 percent interval norfentanyl/fentanyl ratios.

Oxycodone → oxymorphone and noroxycodone

Oxycodone, a potent opioid analgesic, is metabolized by N-demethylation to noroxycodone (major metabolite) through CYP3A4/5, and O-demethylation to oxymorphone (minor metabolite) by CYP2D6.[8] Our study found a mean of 1.67 and a geometric mean (GM) of 0.68 for the MR of oxymorphone/oxycodone (Figure 3A). Our results were slightly higher than the mean of 1.27 and GM of 0.41 obtained by Yee et al.[45] but were within the mean of 1.38 ± 1.24 obtained by Cone et al.[46] Both of their samples (Yee et al.[45] and Cone et al.[46]) were from patients prescribed oxycodone or from patients who reported the use of oxycodone but not oxymorphone. Our study, on the hand, included all samples from patients positive for oxycodone and oxymorphone (lower limit of quantitation (LLOQ) ≥ 10 ng/mL), regardless of the reported use of oxycodone and oxymorphone (n = 93,878 samples/41,024 patients). Oxymorphone is an opioid analgesic that is also commonly prescribed for pain. Yee et al.[45] observed a great overlap in the distribution of urinary oxymorphone concentrations excreted due to oxycodone metabolism and those from oxymorphone use alone.

To exclude oxymorphone use, we extracted a second set of population to include only samples from patients with known oxycodone prescription, while excluding samples from patients prescribed oxymorphone (n = 71,363 samples/32,833 patients), and re-evaluated the MR of oxymorphone/oxycodone. This resulted in a mean of 1.46 and a GM of 0.66 for the oxymorphone/oxycodone MR (Figure 3B). These re-evaluated values were only slightly lower than our initial estimate. Our median of 0.78 (initial estimate) and 0.77 (re-evaluated estimate) agree very well with the



(a)

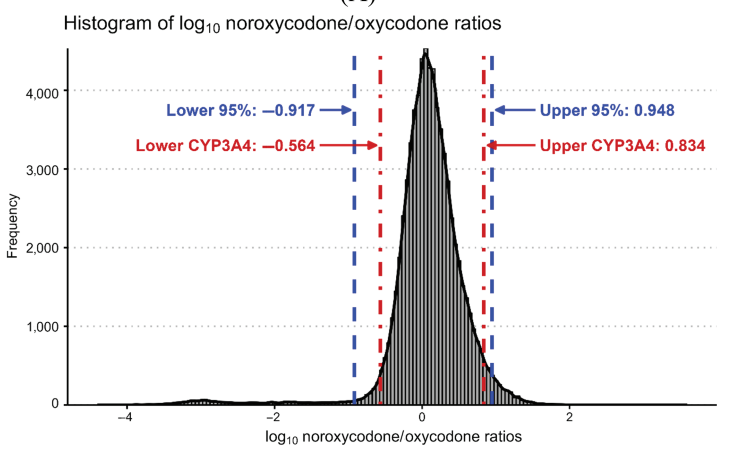
Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.819	0.994	0.152	9.874
3A4	−0.548	0.883	0.283	7.643

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
1.277	2.174	1.251	41,198	94,405

(c)

(A)



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.917	0.948	0.121	8.864
3A4	−0.564	0.834	0.273	6.83

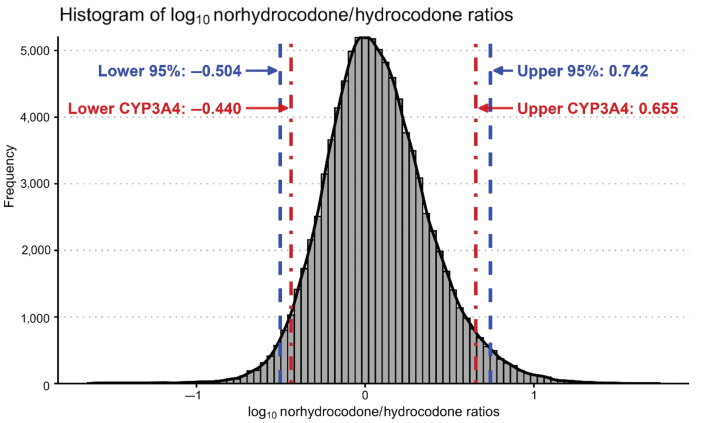
(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
1.245	2.034	1.198	33,001	71,845

(c)

(B)

Figure 9. (A) Reference interval histogram and interval limits for noroxycodone/oxycodone ratios (nonmedications). (a) Reference interval histogram for noroxycodone/oxycodone log-ratios. (b) Table of interval limits for noroxycodone/oxycodone ratios. (c) Median, mean, and N for 95 percent interval noroxycodone/oxycodone ratios. (B) Reference interval histogram and interval limits for noroxycodone/oxycodone ratios (medications). (a) Reference interval histogram for noroxycodone/oxycodone log-ratios. (b) Table of interval limits for noroxycodone/oxycodone ratios. (c) Median, mean, and N for 95 percent interval noroxycodone/oxycodone ratios. Limits estimated using samples from patients prescribed oxycodone while excluding those prescribed oxymorphone.



(a)

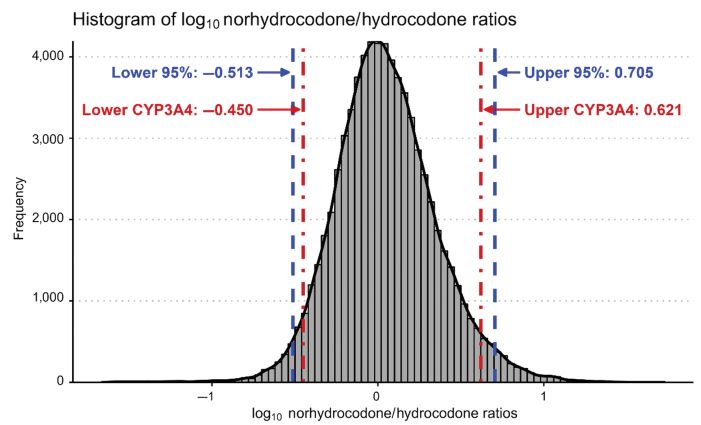
Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.504	0.742	0.313	5.518
3A4	−0.44	0.655	0.363	4.523

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
1.121	1.555	1.161	45,705	93,567

(c)

(A)



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.513	0.705	0.307	5.069
3A4	−0.45	0.621	0.355	4.175

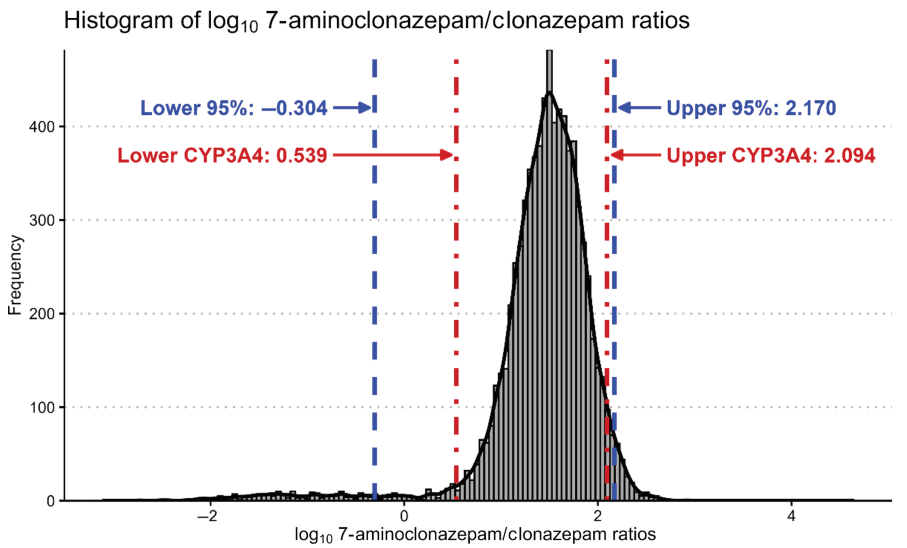
(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
1.076	1.469	1.109	34,230	72,838

(c)

(B)

Figure 10. (A) Reference interval histogram and interval limits for norhydrocodone/hydrocodone ratios (nonmedications). (a) Reference interval histogram for norhydrocodone/hydrocodone log-ratios. (b) Table of interval limits for norhydrocodone/hydrocodone ratios. (c) Median, mean, and N for 95 percent interval norhydrocodone/hydrocodone ratios. (B) Reference interval histogram and interval limits for norhydrocodone/hydrocodone ratios (medications). (a) Reference interval histogram for norhydrocodone/hydrocodone log-ratios. (b) Table of interval limits for norhydrocodone/hydrocodone ratios. (c) Median, mean, and N for 95 percent interval norhydrocodone/hydrocodone ratios. Limits estimated using samples from patients prescribed hydrocodone while excluding those prescribed hydromorphone.



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.304	2.170	0.496	147.770
3A4	0.539	2.094	3.458	124.200

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
31.512	42.500	26.794	3,582	7,721

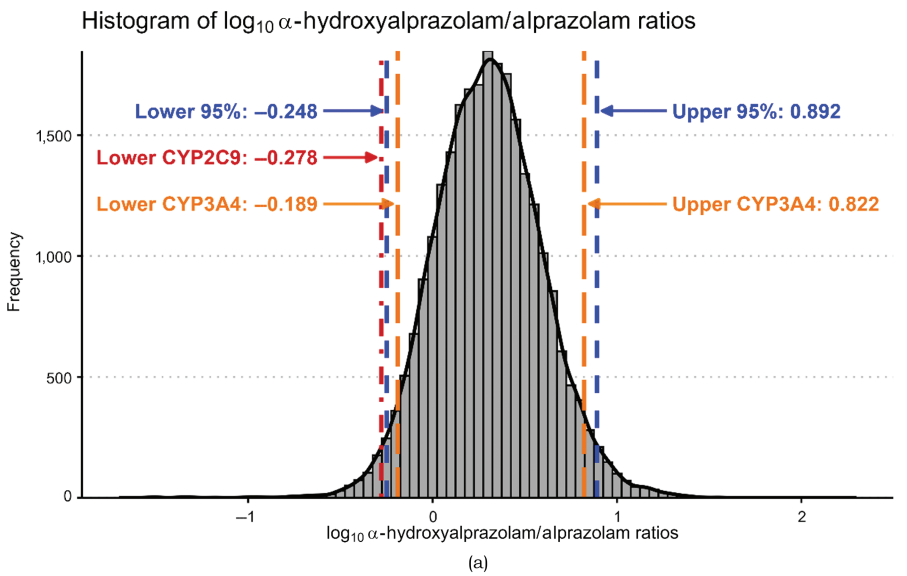
(c)

Figure 11. Reference interval histogram and interval limits for 7-aminoclonazepam/clonazepam ratios. (a) Reference interval histogram for 7-aminoclonazepam/clonazepam log-ratios. (b) Table of interval limits for 7-aminoclonazepam/clonazepam ratios. (c) Median, mean, and N for 95 percent interval 7-aminoclonazepam/clonazepam ratios.

median of 0.76 obtained by Zhu et al.[4] Our study estimated range for oxymorphone/oxycodone MR at 0.018-8.307 (initial estimate) and 0.0162-7.216 (re-evaluated estimate), which has a wider range than the 0.058-2.38 RI obtained by Bevins et al.[3] We used the 2.5th percentile and the 97.5th percentile, while Bevins et al.[3] used the 10th percentile and the 95th percentile to define the lower and upper limits, respectively, which may explain the difference in the RI seen.

The histogram of the log [MR] of oxymorphone/oxycodone showed a small secondary peak in the low MR region, which could be attributed to a second population of CYP2D6 PMs (Figure 3). This was consistent with the findings of Bevin et al.[3] and Zhu et al.[4] Patients practicing deception, who spike their urine with oxycodone resulting in high oxycodone with little oxymorphone, could also be part of this secondary population of low MR. It could also simply indicate samples from patients at the beginning or end of their dose interval.

Regarding noroxycodone/oxycodone MR, our study estimated a mean of 2.17 (Figure 10A), which agrees well with the mean of 2.34 ± 1.4 obtained by Cone et al.[46] Our GM of 1.25 was slightly less than the GM of 1.74 obtained by Moy et al.[47] Our median of 1.28 was also slightly less than the median of 1.70 obtained by Zhu et al.[4] but was within their interquartile range (1.06-2.93). Our study estimated the 95 percent RI for noroxycodone/oxycodone MR at 0.152-9.874, which has a wider range than the 0.424-3.7 RI obtained by Bevins et al.[3] We used the 2.5th percentile and the 97.5th percentile, while Bevins et al.[3] used the 5th percentile and the 95th percentile, to define the lower and upper limits, respectively, which may explain the difference in the RI seen.



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	–0.248	0.892	0.565	7.797
2C9	–0.278		0.527	
3A4	–0.189	0.822	0.647	6.633

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
1.99	2.538	2.001	13,494	25,898

(c)

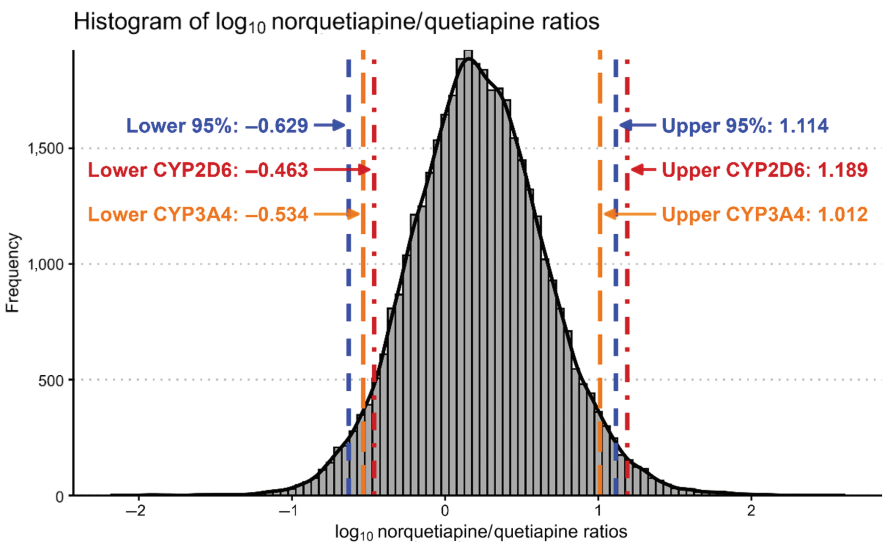
Figure 12. Reference interval histogram and interval limits for α-hydroxyalprazolam/alprazolam ratios. (a) Reference interval histogram for α-hydroxyalprazolam/alprazolam log-ratios. (b) Table of interval limits for α-hydroxyalprazolam/alprazolam ratios. (c) Median, mean, and N for 95 percent interval α-hydroxyalprazolam/alprazolam ratios.

We re-evaluated MR to include only samples from patients prescribed oxycodone and excluding those prescribed oxymorphone. This resulted only in a small change in the MR values (Figure 10B), indicating that oxymorphone use does not significantly affect noroxycodone/oxycodone MR.

Elder et al.[48] reported their observations on the distribution of urinary oxycodone and metabolites as mole fractions and found that noroxycodone had the highest mean mole fraction followed by oxycodone and oxymorphone (0.54 vs 0.44 vs 0.31, respectively). We did a similar calculation (not-creatinine corrected) and found a similar pattern: The mean mole fraction of noroxycodone was highest, followed by oxycodone and then oxymorphone (0.41 vs 0.33 vs 0.26, respectively). Our results agree with previous studies showing that urinary noroxycodone concentrations are higher compared to oxymorphone, supporting the observation that noroxycodone is the major metabolite of oxycodone.[46,49]

Hydrocodone → hydromorphone and norhydrocodone

Hydrocodone is a semisynthetic opioid used to manage pain. Hydrocodone metabolism occurs by O-demethylation to hydromorphone through CYP2D6 and N-demethylation to norhydrocodone by CYP3A4.[15,50] Our study initially estimated a mean of 4.383 for the MR of hydromorphone/hydrocodone (Figure 4A), which was much higher than the mean of 0.223 observed by Valtier and Bebarta.[51] Our GM of 0.349 was also higher than the GM of 0.161 obtained by Barakat et al.,[52] although they observed wide variability in their



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.629	1.114	0.235	12.996
2D6	−0.463	1.189	0.344	15.463
3A4	−0.534	1.012	0.292	10.284

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
1.632	2.913	1.665	13.497	39.660

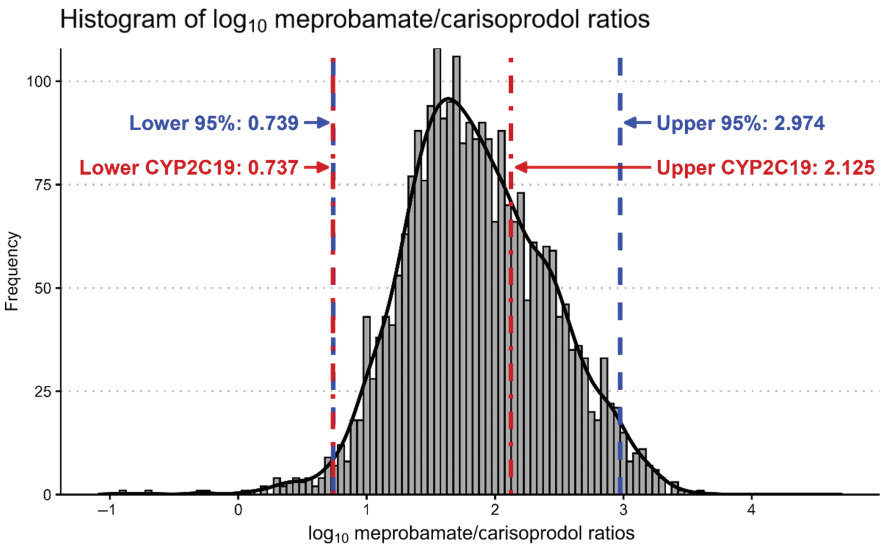
(c)

Figure 13. Reference interval histogram and interval limits for norquetiapine/quetiapine ratios. (a) Reference interval histogram for norquetiapine/quetiapine log-ratios. (b) Table of interval limits for norquetiapine/quetiapine ratios. (c) Median, mean, and N for 95 percent interval norquetiapine/quetiapine ratios.

hydromorphone/hydrocodone MR with a standard deviation of ±3.34. The results of previous studies were obtained from patients with reported use of hydrocodone but not hydromorphone.[51,52] Hydromorphone is an opioid that is also used to treat pain. Our initial MR evaluation of hydromorphone/hydrocodone was performed using samples from patients positive for hydrocodone and hydromorphone (LLOQ ≥ 5 ng/mL), regardless of the reported use of hydrocodone and hydromorphone (first population: n = 90,952 samples/44,415 patients).

To exclude hydromorphone use, we extracted a second population set to include only samples from patients with reported use of hydrocodone while excluding samples from patients with reported use of hydromorphone (second population: n = 71,509 sample/33,747 patients), and re-evaluated hydromorphone/hydrocodone MR. This resulted in a much lower mean of 0.588 (Figure 4B), which was closer in value to the mean of 0.223 from the previous study. Our results indicated that urinary concentrations of hydromorphone from hydromorphone use may have skewed the MR to a higher mean value in the initial population. The initial estimated 95 percent RI of 0.026-5.353 (first population) was reduced to 0.024-2.354 in the second set of population.

For the norhydrocodone/hydrocodone MR, our study estimated a mean of 1.555 (Figure 9A), which agrees very well with the mean of 1.47 (0.82-2.49) obtained by Valtier and Bebarta.[51] We estimated the 95 percent RI at 0.313-5.518. Barakat et al.,[53] who reported the distribution of urinary hydrocodone and metabolites in pain patients as mole fraction, found norhydrocodone with the highest mean mole fraction, followed by hydrocodone and hydromorphone (0.49 vs 0.39 vs 0.11, respectively). Our results



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	0.739	2.974	5.483	942.04
2C19	0.737	2.125	5.456	133.44

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
64.056	163.03	66.835	1,497	2,627

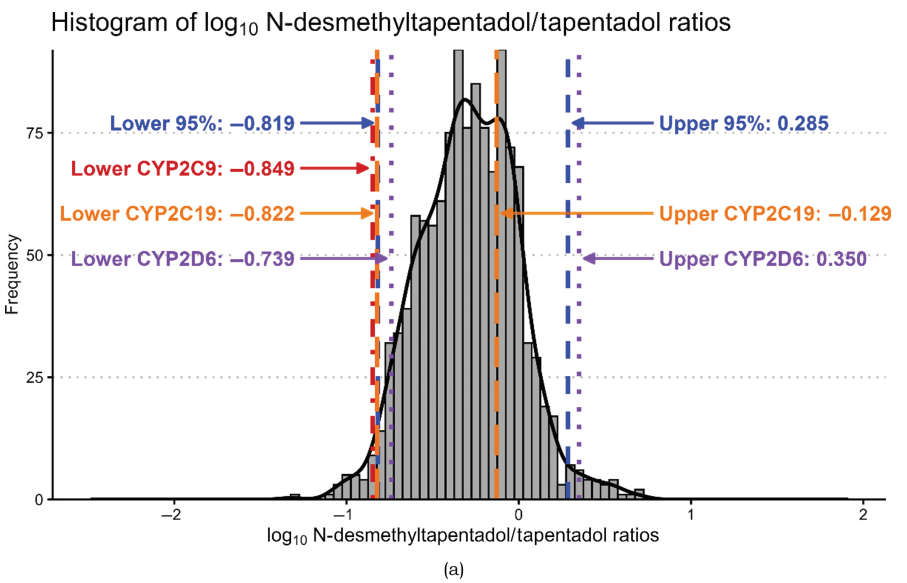
(c)

Figure 14. Reference interval histogram and interval limits for meprobamate/carisoprodol ratios. (a) Reference interval histogram for meprobamate/carisoprodol log-ratios. (b) Table of interval limits for meprobamate/carisoprodol ratios. (c) Median, mean, and N for 95 percent interval meprobamate/carisoprodol ratios.

on mean mole fractions followed the same pattern: The mean mole fraction of norhydrocodone was highest, followed by hydrocodone and then hydromorphone (0.45 vs 0.39 vs 0.16, respectively). Our results agree with previous studies describing higher urinary norhydrocodone concentrations compared to hydromorphone, supporting the observation that norhydrocodone is the major metabolite of hydrocodone.[49,54] We re-evaluated MR to include only samples from patients prescribed hydrocodone and excluding those prescribed hydromorphone. This resulted only in a small change in the MR values (Figure 9B), indicating that hydromorphone use does not significantly affect norhydrocodone/hydrocodone MR.

Tramadol → O-desmethyltramadol

Tramadol, an opioid analgesic used to treat pain, is metabolized by O-demethylation through CYP2D6 to form the active metabolite O-desmethyltramadol.[10,55,56] Although metabolized by the CYP2D6 pathway, the log [MR] distribution of O-desmethyltramadol/tramadol did not show an apparent secondary population of PMs (Figure 5). In particular, its histogram showed a unimodal distribution that was consistent with previous studies.[56-58] The absence of a clear bimodal distribution in the log [MR] of O-desmethyltramadol/tramadol can be attributed to a large overlap in the MR distributions between the CYP2D6 phenotypes. This, in turn, is probably due to the involvement of non-CYP2D6 pathways in tramadol metabolism. CYP2D6-mediated metabolism of tramadol to O-desmethyltramadol contributes only 30 percent to the overall body clearance of tramadol.[59] In contrast, CYP2D6-mediated metabolism of dextromethorphan to dextrorphan is responsible for 97 percent of the oral clearance of dextromethorphan.[60,61] In addition



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.819	0.285	0.152	1.929
2C9	−0.849		0.142	
2C19	−0.822	−0.129	0.151	0.743
2D6	−0.739	0.350	0.182	2.240

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.516	0.638	0.511	620	1,215

(c)

Figure 15. Reference interval histogram and interval limits for N-desmethyltapentadol/tapentadol ratios. (a) Reference interval histogram for N-desmethyltapentadol/tapentadol log-ratios. (b) Table of interval limits for N-desmethyltapentadol/tapentadol ratios. (c) Median, mean, and N for 95 percent interval N-desmethyltapentadol/tapentadol ratios.

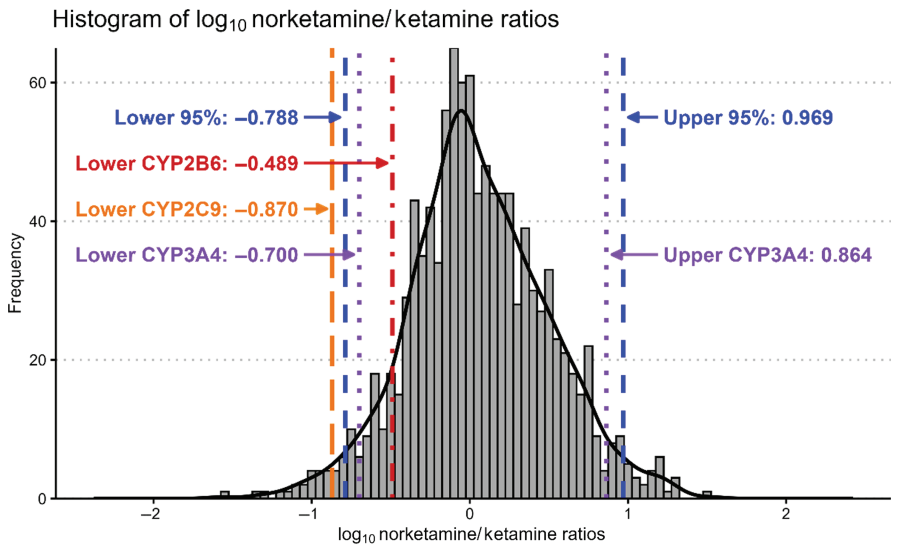
to CYP2D6-mediated O-demethylation, tramadol is also N-demethylated to N-desmethyltramadol by CYP3A4 and CYP2B6.[10] These tramadol metabolites are further metabolized by Phase II enzymes.[62-64] The inherent variability in each of these pathways adds to the variability in the urinary concentrations of tramadol and its metabolites, which may explain the overlap in the MR distribution.

Our study determined a MR mean of 2.218 for O-desmethyltramadol/tramadol (Figure 5), which was almost twice the MR mean (1.23 ± 0.322) obtained by Paar et al.[56] Pedersen et al.[57] obtained a much lower MR mean of 0.588, with MR values ranging from 0.122 to 2.5. Our mean of 2.22 was within these ranges. We estimated 95 percent RI at 0.406-7.787.

Morphine → hydromorphone

The pathway that converts morphine to hydromorphone is not fully understood. One hypothesis is that morphinone reductase mediates the conversion of morphine to an intermediate, morphinone, which is subsequently converted to hydromorphone by morphine reductase.[11,65] Another possibility is that morphine is converted to hydromorphone through a minor metabolic pathway similar to the formation of hydrocodone from codeine.[66]

In this study, we initially estimated the MR mean of hydromorphone/morphine at 0.234 with a 95 percent RI of 0.0016-1.209 (Figure 6A), which was much higher than previous observations (Table 3).[11,12,67-69] Our GM at 0.015 was also higher than the GM of



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−0.788	0.969	0.163	9.307
2B6	−0.489		0.324	
2C9	−0.870		0.135	
3A4	−0.700	0.864	0.200	7.315

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
1.053	1.943	1.144	819	1,077

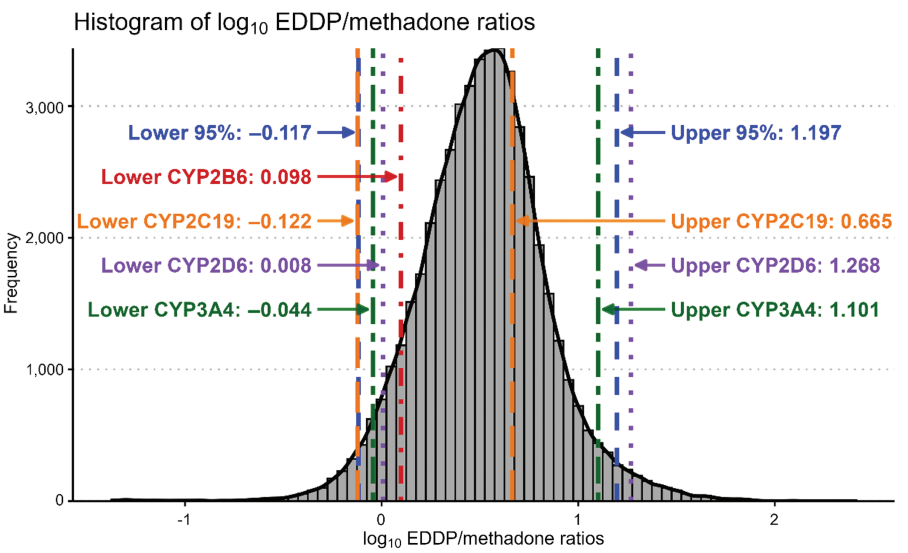
(c)

Figure 16. Reference interval histogram and interval limits for norketamine/ketamine ratios. (a) Reference interval histogram for norketamine/ketamine log-ratios. (b) Table of interval limits for norketamine/ketamine ratios. (c) Median, mean, and N for 95 percent interval norketamine/ketamine ratios.

0.008 found by Hughes et al.[69] but was within their 95 percent CI (0.001-0.04). Cone et al.[11] noted that a patient prescribed daily doses of hydromorphone, who consistently excreted hydromorphone at 3,400-10,000 ng/mL, had a hydromorphone/morphine MR ranging from 1.06 to 1.97. Our initial estimated upper limit of 1.209 (95 percent RI: 0.0016-1.209) indicated that we may have included samples from patients with hydromorphone use (first population: n = 23,644 samples/12,231 patients). We may also have included samples from patients with heroin use (metabolizes to morphine). To exclude patients with hydromorphone or heroin use, we extracted a second set of populations to include only patients prescribed morphine while excluding samples from patients with known hydromorphone prescriptions (second population: n = 8,418 samples/4,205 patients), and re-evaluated the hydromorphone/morphine MR. This resulted in a much lower MR mean of 0.063 (Figure 6B), which is closer in value to the MR mean from previous results. We estimated the 95 percent RI in the second population at 0.0018-0.185, which was well within the expected range.

Buprenorphine → norbuprenorphine

Buprenorphine is mainly metabolized by N-dealkylation to norbuprenorphine by CYP3A4, and to some extent by CYP2C8.[70] Both buprenorphine and norbuprenorphine undergo extensive conjugation. Because hydrolysis was performed prior to analysis, our assay measured total concentrations of the compounds. Our study found a mean of 3.234 for norbuprenorphine/buprenorphine MR



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	–0.117	1.197	0.763	15.752
2B6	0.098		1.255	
2C19	–0.122	0.665	0.754	4.62
2D6	0.008	1.268	1.018	18.517
3A4	–0.044	1.101	0.904	12.606

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
3.311	4.433	3.276	10,554	49,698

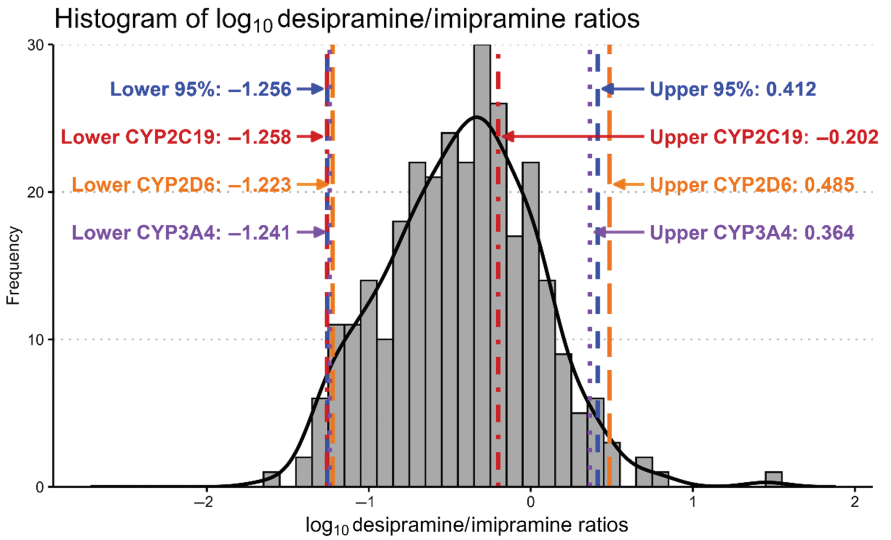
(c)

Figure 17. Reference interval histogram and interval limits for 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)/methadone ratios. (a) Reference interval histogram for EDDP/methadone log-ratios. (b) Table of interval limits for EDDP/methadone ratios. (c) Median, mean, and N for 95 percent interval EDDP/methadone ratios.

and the 95 percent RI (0.268-10.125) (Figure 7), which were within the MR values obtained from previous studies (Table 3).[71-76] CYP3A4 is subject to genetic variations, which accounts for the variability in norbuprenorphine/buprenorphine MR. The ratio of norbuprenorphine to buprenorphine is also influenced by the time interval between sampling and dose intake. Norbuprenorphine/buprenorphine MRs were found to be higher in samples collected with at least 24-hour time interval after dose intake (mean = 6.1; min-max values = 1.0-10) than in those with a shorter than 24-hour interval (mean = 1.5; min-max values = 0.5-2.6).[78] The 95 percent RI (0.272-10.125) in our study is well within these ranges. Despite the genetic variations in CYP3A4, the log [MR] of norbuprenorphine/buprenorphine did not show an apparent bimodal distribution. However, histogram does show a small population with very low MR, whose cutoff can be visually estimated at –log 1.75, giving an antilog value of 0.018. Previous studies have indicated that a norbuprenorphine/buprenorphine MR of 0.02 is the threshold at which a urine sample is considered tampered.[73,77,78] This small population of low MR values (≤0.018) probably represents those practicing deception.

Fentanyl → norfentanyl

Fentanyl is metabolized by oxidative N-dealkylation to norfentanyl, mediated mainly



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−1.256	0.412	0.055	2.584
2C19	−1.258	−0.202	0.055	0.628
2D6	−1.223	0.485	0.06	3.054
3A4	−1.241	0.364	0.057	2.313

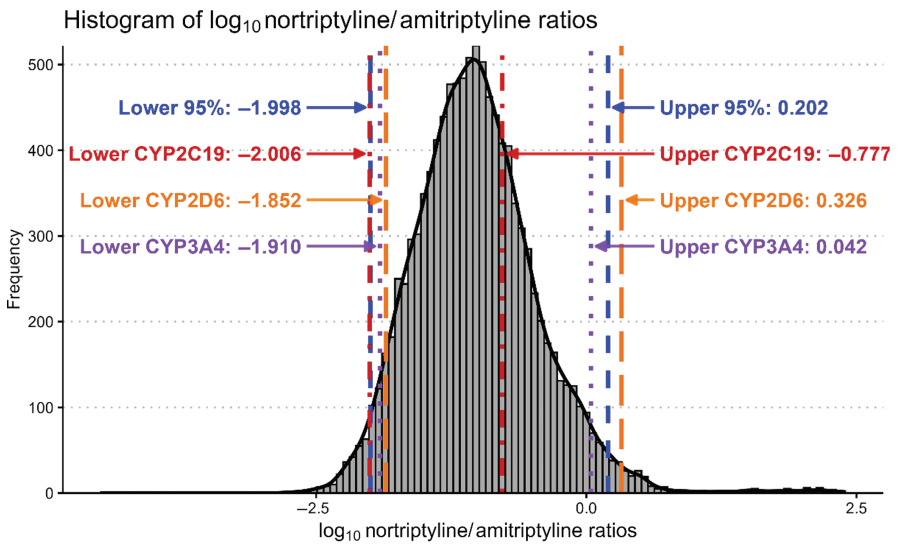
(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.382	0.710	0.374	147	298

(c)

Figure 18. Reference interval histogram and interval limits for desipramine/imipramine ratios. (a) Reference interval histogram for desipramine/imipramine log-ratios. (b) Table of interval limits for desipramine/imipramine ratios. (c) Median, mean, and N for 95 percent interval desipramine/imipramine ratios.

by CYP3A4, with a minor contribution from CYP3A5.[14,79-81] Due to extensive first-pass metabolism that limits the bioavailability of the oral formulation, fentanyl is often given as a transdermal patch to treat chronic pain. Previous studies on urinary excretion of fentanyl and norfentanyl have focused on patients administered fentanyl by transdermal patch (Table 3). Our study population, on the other hand, includes all patients positive for fentanyl, regardless of the route of administration. Our study determined a GM of 7.336 for norfentanyl/fentanyl MR and observed wide variability in MR (95 percent RI: 1.109-55.488) (Figure 8). Our results agree with the GM of 6.2 ± 2.4 obtained previously by Cole et al.,[82] even though their samples were from patients on a fentanyl patch. Cole et al.[82] observed significant interindividual variability (95 percent CI: 1-37), as well as significant intraindividual variability (95 percent CI: 2-20) in norfentanyl/fentanyl MR. Cummings et al.,[83] who conducted their study in a larger population (n = 69,316), also with patients on a fentanyl transdermal patch, presented their urinary results as mean fentanyl (19-139) or maximum fentanyl (120-494) and mean norfentanyl (149-680) or maximum norfentanyl (2,499-2,512) concentrations, depending on dosage. Their study supports the results presented by Poklis and Backler[84] in a smaller population [max fentanyl (167-350) and max norfentanyl (860-1,670)]. Our study determined a mean fentanyl of 276 and a mean norfentanyl of 1,643, which are within the maximum concentrations observed by Cummings et al.[83] and Poklis and



(a)

Interval name	Low limit (log)	High limit (log)	Low limit (normalized)	High limit (normalized)
95 percent interval	−1.998	0.202	0.010	1.593
2C19	−2.006	−0.777	0.010	0.167
2D6	−1.852	0.326	0.014	2.118
3A4	−1.91	0.042	0.012	1.101

(b)

Median	Mean (arith.)	Mean (geom.)	No. patients	No. obs.
0.09	1.204	0.098	5,500	10,911

(c)

Figure 19. Reference interval histogram and interval limits for nortriptyline/amitriptyline ratios. (a) Reference interval histogram for nortriptyline/amitriptyline log-ratios. (b) Table of interval limits for nortriptyline/amitriptyline ratios. (c) Median, mean, and N for 95 percent interval nortriptyline/amitriptyline ratios.

Backler.[84] Variable metabolism due to genetic polymorphism in CYP3A4/5 and variable dermal absorption can explain the large variability in the MR of norfentanyl/fentanyl.

Clonazepam → 7-aminoclonazepam

In this study, we evaluated the MR of two benzodiazepines: clonazepam and alprazolam. Clonazepam is extensively metabolized by nitroreduction to 7-aminoclonazepam (7-AC) through CYP3A4, which is further acetylated by N-acetyl transferase (NAT2).[85-87] The metabolites also undergo hydroxylation and subsequent glucuronidation.[88] Only about 0.5 percent of the parent drug (clonazepam) is excreted unchanged in the urine. 7-AC, which is excreted mainly in the urine, can have prolonged elimination (2-3 weeks), especially in individuals who have repeatedly ingested clonazepam.[89] This study determined a mean of 42.50 for the MR of 7-AC/clonazepam (Figure 11), which was slightly higher than the MR of 31.56 from a previous single case study.[89] We obtained GM of 26.79 and 95 percent RI at 0.496-147.767. Genetic variations in CYP3A4 and NAT2 (both polymorphic), resulting in differential clonazepam-metabolizing capacity, can account for the high variability in the MR ranges.[90,91] Nongenetic factors such as hormones, disease state, age, and medication can also alter CYP3A4 and NAT2 activities, thus influencing clonazepam metabolism.[16] The histogram of the log [MR] of 7-AC/clonazepam shows peak tailing of small population with very low MR, characterized by unusually high clonazepam and low 7-AC concentrations. This could

Table 3. Summary of MR means and ranges from previous studies

Metabolite/parent drug	n (samples)	MR, mean ± SD [range type]	References
α-Hydroxyalprazolam/ alprazolam	26,469	96 (22-878) [median alprazolam conc. (95 percent CI)]	96
	25,667	209 (32-1,955) [median α-hydroxyalprazolam conc. (95 percent CI)]	
		209/96 = 2.18 [median MR]	
Nortriptyline/amitriptyline		N/A	
Norbuprenorphine/ buprenorphine	33	2.27 ± 1.86	71
	148	2.86 ± 2.45	74
	371	1.11 (± 0.56) to 3.33 (± 2.5)	76
	300	2.94 (1.01-12.5)	72
	166	4.52 ± 3.97 (0.07-23.0)	73
	9	1.5 (0.5-2.6) [<24-hours after dose intake]	75
	13	6.1 (1.0-10.0) [≥24-hours after dose intake]	
Meprobamate/carisoprodol	4,982	70.8 ± 3.64 [GM ± SD]; 77.1 [median]	106
	12,203	74 (72.1-76) [GM]	107
7-Aminoclonazepam/ clonazepam	1	561.7/17.8 = 31.56	134
Dextrorphan/dextromethorphan	268	76.9 (± 35.71) [men] to 114.9 (± 90.9) [women]	41
	99	61.72 ± 57.14	43
	246	45.45 ± 31.25	42
	74	58.5 ± 35.71	36
	4,301	71.4	44
Norfentanyl/fentanyl	492	4.22 (3-5.4)	84
	69,316	6.41 (4.89-7.84) to 11.86 (6.49-20.95)	83
	368	6.2 ± 2.4 [GM ± SD]; 1-37 [95 percent CI]	82
Hydromorphone/hydrocodone	125	0.223 (0.088-0.426)	51
	25,200	0.161 ± 3.34 [GM ± SD]	52
	21,177	0.39 (0.38-0.39) [mean mole fraction hydrocodone]	53
		0.11 (0.11-0.13) [mean mole fraction hydromorphone]	
Norhydrocodone/hydrocodone	125	1.47 (0.82-2.49)	51
	21,177	0.39 (0.38-0.39) [mean mole fraction hydrocodone]	53
		0.49 (0.48-0.49) [mean mole fraction norhydrocodone]	
Desipramine/imipramine	201-397	1.38 (men) to 1.51 (women) [GM]	133

Table 3. Summary of MR means and ranges from previous studies (continued)

Metabolite/parent drug	n (samples)	MR, mean ± SD [range type]	References
Norketamine/ketamine	31	1.35 (range: 0.392-7.69)	120
	9	1.52 (range: 0.328-3.506)	121
	6	Range: 0.28-2.04	123
	31	1.38 (range: 0.327-3.03)	122
EDDP/methadone	8	1.731 (0.36-2.92)	135
	5	1.96-5.24	136
	21	3.2 (1.3-12.7)	137
	8,083	1.75 ± 2.38 [GM ± SD]; 0.44-13.7 [95 percent RI]	128
	11	1.6 (0.45-5.4) [median (range)]	138
	64	0.85 ± 1.45 (0.275-7.143)	130
Hydromorphone/morphine	13	0.015-0.024	11
	34	0.008 (0.002-0.022)	67
	21	0.024 ± 0.017	12
	73	0.002-0.12	68
	4,650	0.008 (0.001-0.04) [GM]	69
Oxymorphone/oxycodone	8,752	1.27; 0.41 [GM]	45
	87	1.38 ± 1.24	46
	24,731	0.76 (0.39-1.49) [median (IQR)]	4
	536	0.058 (0.03-0.079) to 2.38 (1.96-3.03) [lower to upper limit]	3
	106,229	0.44 [mean mole fraction oxycodone]	48
		0.31 [mean mole fraction oxymorphone]	
Noroxycodone/oxycodone	87	2.34 ± 1.4	46
	27,974	1.74 [GM]	47
	28,701	1.70 (1.06-2.93) [median (IQR)]	4
	1,216	0.424 (0.355-0.467) to 3.7 (3.22-4.54) [lower to upper limit]	3
	53,394	0.44 [mean mole fraction oxycodone]	48
		0.54 [mean mole fraction noroxycodone]	
Norquetiapine/quetiapine	8	1.43 ± 0.909 (min-max: 0.294-10.0) [postmortem]	103
N-desmethyltapentadol/ tapentadol	8	0.273 ± 0.061	116
O-desmethyltramadol/tramadol	104	1.23 ± 0.322 [24-hours urinary MR]	56
	139	0.588 (0.122-2.5) [8-hours urinary MR]	57

GM: geometric mean; IQR: inner quartile range; SD: standard deviation; CI: confidence interval; NA: no references found; MR: metabolite/parent drug; RI: reference intervals; EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

indicate PMs, the beginning of a dose, or patients attempting deception by spiking clonazepam into the urine samples.

Alprazolam → α-hydroxyalprazolam

Alprazolam is metabolized mainly by hydroxylation to 4-hydroxyalprazolam (the major metabolite) and α-hydroxyalprazolam (a minor but pharmacologically more active metabolite), mediated by the CYP3A4/5 enzymes.[17,92-94] The metabolites are further conjugated before excretion. About 10 percent of the parent drug (alprazolam) is excreted unchanged in the urine.[95] This study determined a mean of 2.538 for the MR of α-hydroxyalprazolam/alprazolam (Figure 12). The GM was determined to be 2.001 and 95 percent RI at 0.565-7.797. Our study may be among the first to evaluate the urinary MR of α-hydroxyalprazolam/alprazolam. Previous studies measured the urinary concentrations of alprazolam and α-hydroxyalprazolam, but none evaluated the MR of α-hydroxyalprazolam to alprazolam. In particular, Feng et al.[96] determined median concentration values for urinary alprazolam (n = 26,469) and α-hydroxyalprazolam (n = 25,667) to be 96 ng/mL Range: 22-878) and 209 ng/mL (Range: 32-1,955), respectively. For comparison, we also determined the median concentrations for alprazolam and α-hydroxyalprazolam (n = 25,718) and found that these values were 69.90 ng/mL (Range: 6.63-742.48) and 138.75 ng/mL (Range: 10.83-1,718.60), respectively. Our median values were slightly lower than those of Feng et al.,[96] most likely due to our lower cutoff (LLOQ = 5 ng/mL vs 20 ng/mL). We can use these median values to calculate the MR of α-hydroxyalprazolam/alprazolam, resulting in a median MR of 1.98 (our study) and 2.18 (Feng et al.[96]). These median MR values agree well with each other.

Quetiapine → norquetiapine

Quetiapine is an atypical antipsychotic drug approved for the treatment of schizophrenia and bipolar disorders, as well as an adjunct treatment for major depressive disorders.[97-99] Quetiapine undergoes extensive metabolism through sulfoxidation, dealkylation, oxidation, and hydroxylation, resulting in the formation of desalkyl, hydroxy-desalkyl, sulfoxide, and carboxylic acid metabolites.[18] CYP3A4/5 is the main enzyme that mediates sulfoxidation, dealkylation, and partially hydroxylation, while CYP2D6 catalyzes hydroxylation.[18,100] Norquetiapine (N-desalkylquetiapine) is the main active metabolite formed. Genetic polymorphism in CYP3A4/5 and CYP2D6, as well as the presence of cosubstrates, can markedly influence quetiapine metabolism.[100-102]

In this study, we determined a mean of 2.91 for the MR of norquetiapine/quetiapine, GM of 1.67, with the 95 percent RI at 0.235-13.00 (Figure 13). Except for a study on the urinary MR of norquetiapine/quetiapine in postmortem samples,[103] there appear to be no other published data on the urinary distribution of quetiapine and norquetiapine. In the postmortem urine samples, the mean MR of norquetiapine/quetiapine was determined to be 1.43 ± 0.909 (min-max: 0.294-10.0). The potential redistribution of analytes complicates the interpretation of drug levels in postmortem samples. However, in the case of quetiapine, Saar et al.[104] noted minimal changes in quetiapine concentrations during a postmortem investigation (7 days), indicating that the risk of redistribution is low. Norquetiapine is also less affected by postmortem redistribution. Hence, the norquetiapine/quetiapine MR in the postmortem urine samples may reflect the urinary distribution of quetiapine and norquetiapine in real life. The MR mean of our study was slightly higher, but the MR ranges were similar: 2.913 (95 percent RI: 0.235-13.00) (our study) vs 1.43 ± 0.909 (min-max: 0.294-10.0).[103]

Carisoprodol → meprobamate

Carisoprodol is mainly metabolized to meprobamate by the highly polymorphic CYP2C19.[20,105] This study estimated a GM of 66.84 for the MR of meprobamate/carisoprodol (Figure 14), which compares well with the GM of 70.8 (± 3.64) to 74, obtained from previous studies[106,107] (Table 3). Our median of 64.06 was slightly lower than the median of 77.1 previously observed. We observed a wide variability in the meprobamate/carisoprodol MR (95 percent RI: 5.48-942.62) that can be attributed to the CYP2C19 polymorphism. Approximately 35 alleles of CYP2C19 have been identified,[108-110] including those associated with the PMs and UM phenotypes. Heterozygous individuals with 1 copy of the CYP2C19*1 allele are often classified as normal extensive metabolizers.

However, a study observed heterozygous individuals (CYP2C19*1) with a decreased metabolizing capacity for carisoprodol, suggesting a unique intermediate metabolizing phenotype with specific regard to carisoprodol metabolism.[111] These studies support the significant contribution of genetics to the variability in the MR of meprobamate to carisoprodol. Carisoprodol is extensively metabolized and excretes only a small amount (1 percent of the dose) of unchanged carisoprodol.[112] Carisoprodol also has a shorter half-life compared to meprobamate (2.0 ± 0.5 vs 9.6 ± 1.5 hours, respectively).[113] This suggests that carisoprodol is present in lower amounts in the urine, while meprobamate accumulates over a period of time due to its longer half-life and extensive formation from carisoprodol. Previous studies used the LLOQ of 100 ng/mL as the cutoff point to detect carisoprodol and meprobamate.[106,107] The higher cutoff point may have caused previous investigators to miss samples with lower carisoprodol concentrations. On the other hand, our study used the LLOQ of 5 ng/mL for carisoprodol (meprobamate at the LLOQ of 100 ng/mL). The lower LLOQ allowed us to detect samples with low levels of carisoprodol, resulting in a high MR of meprobamate to carisoprodol in these samples. The presence of these samples with low carisoprodol concentration and high MR may have skewed the histogram of log[MR] of meprobamate/carisoprodol slightly to the left. This may also explain the high upper limit of 942.62 (95 percent RI) in our study. Ultrarapid metabolizers can also contribute to this region of high MR.

Tapentadol → N-desmethyltapentadol

Tapentadol undergoes extensive metabolism through Phase II conjugation (70 percent). The Phase I oxidative process, in which tapentadol is N-demethylated to N-desmethyltapentadol by CYP2C9, CYP2C19, and CYP2B6, and hydroxylated by CYP2D6, occurs to a lesser extent (15 percent).[114,115] Because hydrolysis was performed prior to analysis, our assay measured total concentrations of the analytes. This study estimated a MR mean of 0.638 for desmethyltapentadol/tapentadol (Figure 15), which was approximately twice the mean of 0.273 ± 0.061 obtained previously by Bourland et al.[116] (Table 3). The previous result was obtained from a small sample size of 8, while our study consisted of 1,215 samples (620 patients). The relatively larger sample size in our study provided MR values from a wider range of the population and may explain the difference in results. This study also determined the GM at 0.511 and 95 percent RI at 0.152-1.929.

Ketamine → norketamine

Ketamine is used clinically for its anesthetic, sedative, and analgesic properties.[117,118] Ketamine is metabolized by N-demethylation to norketamine, mediated mainly by CYP3A4, with minor contributions from CYP2B6 and CYP2C9.[25,119] This study determined a mean of 1.94 for the MR of norketamine/ketamine (Figure 16), which was minimally higher than the MR mean of 1.35-1.52 from previous studies[120-122] (Table 3). The 95 percent RI estimated at 0.163-9.307 was higher compared to the ranges (0.28-2.04, 0.328-3.506, and 0.327-3.03) obtained previously[121-123] but was close to the range of 0.392-7.69 obtained by Moore et al.[120] The subtle differences in the MR values could have been due to the small sample size of previous studies, the dose, and the sampling time after the dose.

Methadone → 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)

Methadone is metabolized mainly by N-demethylation to EDDP by CYP3A4 and CYP2B6,[112] with minor participation by CYP2D6 and CYP2C19.[124-127] Except for the study by Leimanis et al.,[128] previous results on urinary EDDP/methadone MR were determined mainly from patients on methadone maintenance therapy. Our study population, similar to Leimanis et al.,[128] includes chronic pain patients prescribed opioid analgesics. We determined the MR mean (4.43) and median (3.31) for EDDP/methadone (Figure 17). These values were slightly higher than the mean/median from previous studies, but within their range of MR values (Table 3). Our study also determined the GM (3.28) and 95 percent RI (0.763-15.752) that were within the ranges (GM = 1.75 ± 2.38; 95 percent RI: 0.44-13.7) observed by Leimanis et al.[128] Genetic variants in the CYP450 enzymes, sampling time after dose, frequency of dosing,[129] inconsistency in prescribed medications, and urinary pH[130] can contribute to variability in EDDP/methadone MR.

Table 4. Summary of the median, lower and upper limits (95 percent RI) of MR reference interval. Drug pairs labeled with "medications" indicate limits estimated using samples from patients prescribed the parent drug while excluding those prescribed oxymorphone or hydromorphone

Drug pair	Median MR	95 percent RI	
		Lower limit	Upper limit
Dextrorphan/dextromethorphan	15.371	0.157	245.14
Oxymorphone/oxycodone	0.784	0.018	8.307
Oxymorphone/oxycodone (medications)	0.769	0.016	7.216
Hydromorphone/hydrocodone	0.352	0.026	5.353
Hydromorphone/hydrocodone (medications)	0.329	0.024	2.354
O-Desmethyltramadol/tramadol	1.722	0.406	6.787
Hydromorphone/morphine	0.011	0.0016	1.209
Hydromorphone/morphine (medications)	0.009	0.0018	0.185
Norbuprenorphine/buprenorphine	2.556	0.268	10.125
Norfentanyl/fentanyl	7.050	1.109	55.488
Noroxycodone/oxycodone	1.277	0.152	9.874
Noroxycodone/oxycodone (medications)	1.245	0.121	8.864
Norhydrocodone/hydrocodone	1.121	0.313	5.518
Norhydrocodone/hydrocodone (medications)	1.076	0.307	5.069
7-Aminoclonazepam/clonazepam	31.512	0.496	147.77
α-Hydroxyalprazolam/alprazolam	1.990	0.565	7.797
Norquetiapine/quetiapine	1.632	0.235	12.996
Meprobamate/carisoprodol	64.056	5.483	942.04
N-Desmethyltapentadol/tapentadol	0.516	0.152	1.929
Norketamine/ketamine	1.053	0.163	9.307
EDDP/methadone	3.311	0.763	15.752
Desipramine/imipramine	0.382	0.055	2.584
Nortriptyline/amitriptyline	0.090	0.010	1.593

MR: metabolite/parent drug; CI: confidence interval; RI: reference intervals; EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

Tricyclic antidepressants (TCAs): Imipramine → desipramine; amitriptyline → nortriptyline

In this study, we determined the MR of two commonly used TCA, imipramine and amitriptyline. Both analytes undergo similar metabolic pathways, specifically N-demethylation forming desipramine from imipramine and nortriptyline from amitriptyline. N-demethylation is mediated mainly by CYP2C19, with minor contributions from CYP1A2 and CYP3A4.[24,131,132] Both metabolites (desipramine and nortriptyline) are further metabolized by hydroxylation to form hydroxyl metabolites, mediated mainly by CYP2D6. Genetic variations in

CYP2C19 and CYP2D6 can markedly influence the metabolism of imipramine and amitriptyline.

Only a small number of patients in our study population tested positive for imipramine and desipramine (n = 298 samples/147 patients). From this small population, we determined a GM of 0.374 for the desipramine/imipramine MR (Figure 18), which was much less than the GM of 1.38 (men) and 1.51 (women) obtained by Ramey et al.[133] The arithmetic mean was 0.710, with 95 percent RI at 0.055 to 2.584. We found that urine desipramine concentrations ([GM] = 179.00 ng/mL) were lower than imipramine concentrations ([GM] = 480.12 ng/mL). This was in contrast to a previous study, in which Ramey et al.[133] observed urine desipramine concentrations to be slightly higher than imipramine concentrations. Ramey et al.[133] reported concentrations of imipramine and desipramine as mg analyte per gram creatinine, while no creatinine-correction was applied in our study. This may explain the differences in the results. What remained consistent was the variability observed in the urinary excretion of imipramine and desipramine.

For nortriptyline/amitriptyline MR, our study determined a mean of 1.204 and 95 percent RI of 0.01-1.593 (Figure 19). GM was determined to be 0.098. Urinary nortriptyline concentrations ([GM] = 99.84 ng/mL) were much lower than amitriptyline concentrations ([GM] = 1,028.6 ng/mL). As with imipramine and desipramine, we also observed a wide variability in urinary excretion of amitriptyline and nortriptyline. Our study may be among the first to evaluate the urinary MR of nortriptyline/amitriptyline, as we could not find other studies for comparison.

LIMITATIONS

This study is retrospective, and no data were collected with regard to dose, the frequency of dosing (whether single dose or multiple doses administered over time), and the time interval between collection and last dose. Samples were not differentiated with respect to age, gender, and ethnicity. Liver and kidney function and disease states were also unknown. All these factors will affect metabolism, which together with genetic polymorphism, may have contributed to the variability in the MRs. Despite these limitations, the MR values (95 percent RI) obtained in this study can be used to represent the patient population suffering from pain, as well as those undergoing substance abuse rehabilitation.

CONCLUSION

This study provides the 95 percent RI of the MR for 18 metabolite–parent drug pairs. Table 4 summarizes the lower and upper limits, as well as the median MR. The MR mean values for most of the 18 drug pairs compared well with previous studies, confirming previously reported MRs. Although these are estimates of drug metabolism, knowing the expected limits of the ratio of metabolite/parent drug is helpful. Calculated MR outside the reference range can be used to identify patients with unusual metabolism. The latter can be due to genetic variances, DDIs, age, or deception. Alerting providers of the variance in metabolism from the expected norm might reduce overdosing or underdosing patients.

For oxycodone, hydrocodone, and morphine, we included MR limits from two sets of populations. The first population is where we included all samples from patients positive for the parent drug and metabolites. The second population (indicated by medications in Table 4) is where we included only samples from patients prescribed for the parent drug while excluding those with prescribed oxymorphone or hydromorphone. We noticed that excluding oxymorphone use did not significantly change the lower/upper limits of oxymorphone/oxycodone and noroxycodone/oxycodone MR. However, excluding hydromorphone use significantly lowered the upper limit of hydromorphone/hydrocodone and hydromorphone/morphine MR, but not norhydrocodone/hydrocodone MR.

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