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Normalizing Oral Fluid Hydrocodone Data Using Calculated Blood Volume

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Abstract

Oral fluid testing to assist in the assessment of treatment adherence for chronic pain patients is attractive for a number of reasons. However, efforts focused on interpreting patient results have been modest when compared to urine drug testing. This work details a retrospective approach developed to transform and normalize oral fluid testing results to provide a historical picture of patient values in this important test fluid. Using this approach, a model was developed using data from 6,800 independent patients who were both prescribed hydrocodone and tested positive (with limitations: reporting cutoff < X < upper limit of quantitation) by liquid chromatography–mass spectrometry. Patient demographic data were used to calculate the relevant parameters (e.g., calculated blood volume (CBV)) used in the transformation and normalization of the oral fluid data. The crucial normalizing factor in oral fluids was found to be the CBV which parallels the use of creatinine to normalize drug concentration levels in urine and is consistent with the view that oral fluid samples reflect plasma concentrations of the respective drugs. The resulting near Gaussian distribution is dose independent and as such should be of value to physicians in quickly assessing whether their patient is consistent with this historical population in the broad terms of this model. While this comparison alone is not definitive for adherence with a treatment regimen, together with patient interviews, prescription history and other clinical criteria, it can add an idea of expected patient values from oral fluid testing.

Introduction

Hydrocodone is the most prescribed opioid in the USA. In 2011 alone it was the opioid responsible for the second highest number of emergency department (ED) visits (82,480) (1). Hydrocodone is also among the most abused and diverted opioids in the USA (2–4). It is relatively inexpensive when compared to drugs in the same class which fosters its popularity (5, 6). Given the propensity for abuse of hydrocodone containing medications and the high incidence of ED visits associated with abuse (1), monitoring patients' usage while being prescribed a hydrocodone pain regimen is an important component of their care.

Patients on opioid therapy regimens are typically screened periodically to monitor compliance with the prescribed therapy because of known dependency risks (7). Oral fluid testing is becoming more prevalent in this area because of the ease with which samples can be collected without the need for specialized professionals or dedicated facilities as reported in a number of earlier reports (8–14). However, aside from qualitative information about drugs of interest, oral fluid data is not easily correlated with either corresponding blood (15, 16) or urine (12, 13) test results nor are quantitative data informative to the physician other than to indicate whether the patient is "positive or negative". Efforts by Cone *et al.* reported hydrocodone detection in oral fluid as a strong indicator of hydrocodone presence in blood; however, the ratio of oral fluid and blood concentrations of hydrocodone is not directly correlated and vary considerably between patients (16). Normalized "curves" for a series of drugs have been published for urine drug samples (17) such that a physician can quickly compare the patient's results with normalized data from a patient population to help determine the likelihood that the patient is consistent with their prescribed medication plan. There

Table I. Reported ranges and median values for hydrocodone in oral fluids compared to our hydrocodone model (comparison of hydrocodone oral fluid ranges)

Study	Range (ng/mL)	Median (ng/mL)	Mean (ng/mL)	*N*
Heltsley *et al.* (11)	1.4–494	22.6	84.7	40
Heltsley *et al.* (9)	1.0–33,438	67.8	178.4	1,843
Cao *et al.* (12)	1.6–6,902	122	N/A	600
Our hydrocodone model	10–4,000	126	239	3,944

are reports of some issues with pushing these models too far in terms of demonstrating constancy with a given dose of hydrocodone—e.g., works by Nofziger and Bertino (18). Nevertheless, some physicians do find a quick, visual comparison of their patient's test result with a similar population of patients to be useful in their monitoring. Still, normalized urine derived curves have no utility in other fluids, and normalized oral fluid curves are to date unavailable to clinicians.

Several authors have published reports of oral fluid data including ranges and median values for hydrocodone. As shown in Table I, these data vary greatly. Certainly, the time post dosing of sample collection is important in these values. Perhaps more imperative are the lower and upper limits of quantitation used in these studies. For example, the lower limit of quantitation (LOQ) in work by Heltsley *et al.* (11) was 1.0 ng/mL while the upper limit of quantitation (ULOQ) was 500 ng/mL. This may have limited the observed values artificially as suggested by the additional references. Further, the median values are consistently lower than the mean for these data sets. The observation that the median does not coincide with the middle of the data range indicates these data do not fit a Gaussian distribution without some transformation as utilized by Cao *et al.* (12).

This work details statistical methods developed for retrospectively transforming and normalizing oral fluid hydrocodone drug results to be consistent with a Gaussian distribution. The results provide a look at historical data for hydrocodone that is both independent of daily dose and normalized to a constant blood volume. Unlike urine, where creatinine is accepted as a patient specific normalization factor, a patient specific normalization factor for oral fluid data has not been reported. Data herein demonstrate the utility of using a patient specific parameter, calculated blood volume (CBV), as that unique normalization factor for oral fluid data. A historical Gaussian distribution was developed using data from 6,800 patients who were both prescribed hydrocodone and tested positive for hydrocodone in oral fluids. A "positive" test for hydrocodone referred to in the body of this text applies to patients who tested within the limits of quantification of the analytical method. For model development purposes, patients with test values >ULOQ (~1.5% of total population) were excluded. The use of CBV is critical to the normalization of the mathematically transformed data. The utility of the methods detailed in this work is expected to extend to other opioids and other drug classes in oral fluids.

Methods and materials

Patient oral fluid specimens were submitted for quantitative liquid chromatography–mass spectrometry (LC–MS-MS) hydrocodone confirmation (Enders and McIntire (19)) and the resulting data were used to develop the statistical distributions. The LOQ for this method is 10 ng/mL while the ULOQ is 4,000 ng/mL. The data analysis and model development was conducted using R version 3.1: a language and environment for statistical computing (20). Data smoothing was completed using a kernel density process. The kernel density estimation is a well-accepted mathematical tool that "smooths" continuous data (e.g., Histograms) such that mathematical curve fitting and modeling can be accomplished. While the variables used to construct the kernel density estimation plot can be subjective, the result for a continuous data set retains the mean value and closely reflects the variance of the original data set itself. The kernel density estimation plot is simply used to "clean up" the display for inspection (21).

To ensure all the information required to adequately develop the model was available, only patients who had demographic information (gender, weight and height), a prescribed daily hydrocodone dosage, and positive oral fluid hydrocodone concentrations were included in the model. The resulting population size (N) used to simulate the hydrocodone models consisted of 3,944 independent individual patient results of which 60% were females and 40% were males. The average age of patients included in the model was 57 years old with an average lean body weight of 54 kg. The average daily dosage of hydrocodone taken by patients included in this model was 36 mg and their median and average oral fluid hydrocodone concentrations were 126 ng/mL and 239 ng/mL, respectively.

The developed method utilized a transformation and normalization of hydrocodone concentration in oral fluid of patients. The term normalization refers to the concentration of hydrocodone that has been modified to correct for one or more parameters associated with the patient. Part of the normalization process requires adjusting the concentration of hydrocodone and other parameters associated with the patient so that they share a common scale—caution was taken to ensure that all units were consistent.

The raw hydrocodone drug concentration measured in oral fluid of the patient is transformed and normalized as a function of patient height, weight, gender, prescribed drug dosage and calculated patient parameters including: body mass index (BMI), lean body weight, body surface area and CBV as described in Equation (1):

$$\mathrm{NORM}_{\mathrm{CONC}} = \frac{\ln\left(\frac{H_{\mathrm{CONC}} * \mathrm{LBW} * \mathrm{BSA}}{\mathrm{Dose}}\right)}{\mathrm{CBV}}, \tag{1}$$

where ln is the natural log, H_{CONC} is the concentration of hydrocodone in kg/L; LBW is the lean body weight of the patient in kg; BSA is the body surface area of the patient in meters squared; Dose is the patient prescribed daily drug dosage in kg; and CBV is the calculated blood volume in liters. This value is then transformed into its corresponding value on the standard normal distribution using Equation (2):

$$H_{\mathrm{STD}} = \frac{\mathrm{NORM}_{\mathrm{CONC}} - \mu_A}{\sigma_A} = Z_{\mathrm{SCORE}}, \tag{2}$$

where H_{STD} is the standardized normal value—referred to as the *z*-score for simplicity—and μ_A and σ_A are the mean and the standard

Table II. The association of the bmi chart and a modified version Gilcher's Rule of Five utilized in the development of the hydrocodone model

BMI index chart (26)		Modified Gilcher's Rule of Five (25)		
		Average blood volume (mL/kg of body weight)		
BMI (kg/m^2)	Category	Classification	Male	Female
<18.5	Underweight	Thin	65	60
18.5–24.9	Normal	Normal	70	65
≥25	Overweight-obese	Obese	60	55

deviation (SD) of the population used to construct the model described in Equation (1). The values of μ_A and σ_A for this model are—0.169 and 0.243, respectively; the resulting mean and standard deviation of the standardized normal distribution, H_{STD}, are "0" and "1", respectively. Equation (2) essentially centers the Gaussian distribution at 0 on the X-axis where X is a function of H_{CONC} as given in Equation (1) and H_{STD} is moved on the X-axis to yield a mean of "0" and a standard deviation of "1" unit.

Specific parameters were all utilized in some modified or direct form to mathematically transform and normalize the available oral fluid hydrocodone data points.

The LBW parameter accounts for the sum of everything in the human body with the exception of fat including but not limited to bones, muscles and organs. The LBW is calculated using the James Formula described in Equation (3) (22, 23):

$$\text{LBW (kg)} = \text{fact}_a * \text{weight (kg)} - \text{fact}_b * \left(\frac{\text{weight (kg)}}{100 * \text{height (m)}}\right)^2, \quad (3)$$

where fact_a equals 1.1 for Men and 1.07 for Women and fact_b equals 128 for Men and 148 for women, respectively.

The BSA parameter is the calculated surface area of the human body or the patient in this specific case. This accounts for patient BSA which is considered a better indicator of metabolic mass than the raw weight of the patient. The BSA is calculated using the Mosteller Method as shown in Equation (4) (24):

$$\text{BSA (m}^2) = \sqrt{\left(\frac{\text{height (cm)} * \text{weight (kg)}}{3{,}600}\right)}. \quad (4)$$

The CBV parameter accounts for the volume of blood (both red blood cells and plasma) in the circulatory system of a patient. The CBV of each patient is estimated using Equation (5):

$$\text{CBV (L)} = \text{weight (kg)} * \text{AVG\_BV (L/kg)} \quad (5)$$

where AVG_BV is the estimated average blood volume in L/kg of each patient which is determined using a modified version of Gilcher's Rule of Five (25) and the BMI chart classification of weight categories. The BMI parameter is used as an assessment of body fatness and to place patients into weight categories. The BMI is calculated using Equation (6) (26):

$$\text{BMI (kg/m}^2) = \frac{\text{weight (kg)}}{\text{height (m)}^2} \quad (6)$$

Gilcher's Rule of Five used as the primary method of estimating the AVG_BV in Equation (5) classifies male, female and infant patients into four categories (Obese, Thin, Normal and Muscular) and determines an average blood volume for those patients. In the modified version developed and utilized in this model, infants are excluded and patient muscularity is not considered. Patient calculated BMI is used to categorize patients in a way that parallels Gilcher's Rule of Five as shown in Table II. Moreover, for the purpose of this study, BMI indexes in the underweight, normal and overweight/obese categories are paralleled with Gilcher's classification as thin, normal and obese, respectively. Based on this assessment, patients' genders were taking into account and the AVG_BV was assigned according to Table II.

The use of CBV is critical to the normalization of the mathematically transformed data. Unlike urine, wherein creatinine concentration is commonly used to establish the level of "hydration" of the patient and further to normalize data to that level of hydration, creatinine is not expressed in oral fluid. However, given that a concentration of a drug and/or its plasma resident metabolites observed in oral fluid is representative of the concentration in blood or plasma, the CBV seems obvious to normalize all the patients to the same blood volume resulting in a "normalized" historical Gaussian distribution.

Results and discussion

In the broadest sense, this model provides a method of determining whether a patient's hydrocodone oral fluid test result is consistent with a historical population of hydrocodone positive patients. The method requires determining the concentration of hydrocodone in the oral fluid of the patient and prescribed daily dose of hydrocodone associated with the patient; determining the weight, height and gender associated with the patient; subsequently estimating patient LBW, BMI, BSA and CBV. This information is used in Equations (1) and (2) to determine the normalized hydrocodone concentration determined from an oral fluid sample from a patient and comparing that mathematically transformed and normalized drug concentration to the historical Gaussian distribution prepared from a body of known test patients who were both prescribed the drug of interest and tested positive for the drug and/or metabolite in oral fluids.

Figure 1 shows a histogram of the hydrocodone oral fluid concentrations observed from the collected oral fluid test results used to generate the mathematically transformed and normalized historical Gaussian distribution for hydrocodone in oral fluids. These data look like an exponential decay making it difficult to use standard criteria such as mean and standard deviation to adequately characterize these data. This is why the results in Table I appear to have off-centered median and mean values as hydrocodone concentrations are not normally distributed. Cao *et al.* (12) did transform

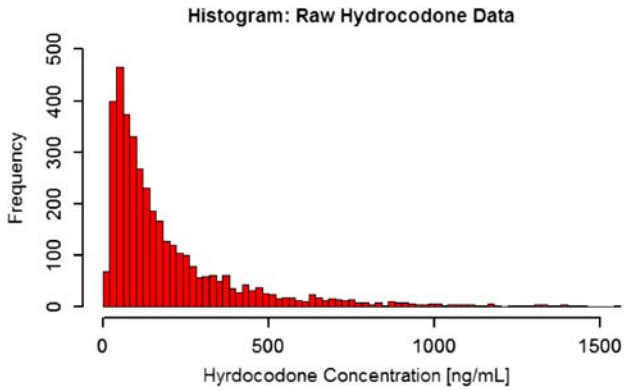

Figure 1. Histogram of the hydrocodone oral fluid concentrations observed from a body of collected oral fluid test results used to generate the mathematically transformed and normalized standard curve for hydrocodone in oral fluids.

their data into the log (base 10) space to generate means and standard deviations which they then reverse transformed to generate the same values in the original linear space. However, their transformation did not take any patient specific information into account.

When these data are transformed and normalized using CBV, the histogram takes on the appearance shown in Figure 2 (top). These transformed and normalized data are closer to a Gaussian distribution where characteristics of mean and standard deviation have meaning. Figure 2 (bottom) shows the kernel density estimation plot derived from the transformed, normalized and standardized raw hydrocodone data overlaid with the standard normal distribution. The transformed hydrocodone drug concentration data normalized using CBV and standardized using the population mean and standard deviation as detailed in Equations (1) and (2) are consistent with a Gaussian distribution (a normally distributed symmetric bell curved function).

A true Gaussian population distribution has 68% of the data within ±1 SD, 95% of the data within ±2 SDs and the other 5% greater than ±2 SDs. However, without a clinically determined compliance correlation with these data, any discussion of adherence to dose paradigms is filled with uncertainty (i.e., greater than ±2 SDs) (20). Notably, this distribution does not reflect a given dose but rather indicates that the patient is consistent with a historical population having both prescriptions and positive drug test results.

This model is based on steady-state drug concentrations. Patients who are taking hydrocodone "as needed" may not be consistent with this historical Gaussian distribution. Arbitrarily, patient results that fall outside ±2 SDs are less consistent with the larger body of the historical distribution suggesting perhaps they are not in a "steady-state" or may have some condition not considered by the model hence causing them to be outside the 95% range of the model. For example, CYP2D6 is known to affect hydrocodone metabolism (27, 28). For those patients falling outside of −2 SDs from the mean of the historical standard distribution, it may be that they are ultra-rapid metabolizers and have cleared the drug from their blood volume (e.g., a CYP2D6 genetic issue), or are taking their drug less frequently than prescribed for any number of reasons such as expense, improved efficacy (less dose required) or in the worst case, they may be diverting their drug to a different use (e.g., for someone else, or for resale). On the other side, if their score falls beyond +2 SDs from the mean of the standard distribution, it is possible that they are poor metabolizers (e.g., a different type of CYP2D6 genetic issue) leading to a build-up of drug in their blood (27, 28) or

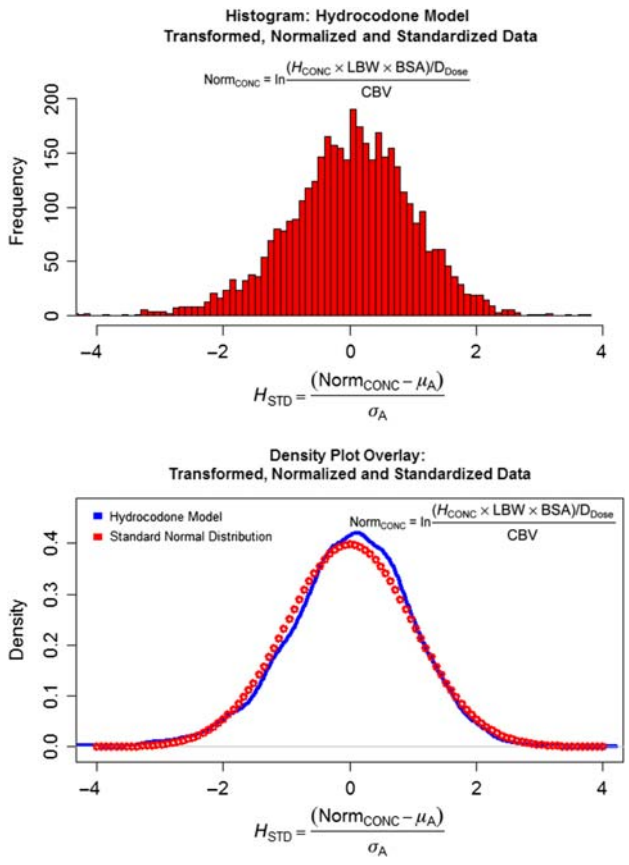

Figure 2. Histogram of the transformed, normalized and standardized raw hydrocodone oral fluids data (top) and the kernel density estimation plot derived from the transformed, normalized and standardized raw hydrocodone data overlaid with the least squares minimized best fit Gaussian distribution curve (bottom).

are taking larger amounts of drug than prescribed due to inefficacy. In any event, the results of a comparison to the standard distribution provide an additional piece of information to the benefit of the patient.

Hydrocodone oral fluid concentration results, demographic information (gender, weight, height and age), and the prescribed dosage of hydrocodone for 55 randomly selected patients—not included in the patient population used to design the model—were used to assess the validity and robustness of the model. The summary data is presented in Table III and a detailed list of patient parameters is shown in Table S1 included in the Supplementary material section. The scores for hydrocodone oral fluid concentrations for all patients were calculated using Equations (1) and (2) following the calculation of LBW, BSA, BMI, AVG_BV and CBV according to Equation (3) through Equation (6). For the data transformed according to Equations (1) and (2), 69% of the patients fall within ±1 SD, 93% fall within ±2 SDs and 7% fall outside the ±2 SD range. If we examine the data presented in Table III closely, it is evident that this sample population has a similar distribution to what is expected from a randomly selected population with sample size (n) —where $n \geq 32$ data points (patients). Furthermore, a detailed step-by-step example for how the z-scores were calculated is included in the Supplementary material section. Table III also shows typical patient parameters of patients used in the model data set versus patients used in the model validity assessment. The z-scores and

Table III. Comparison of demographic and hydrocodone medication information for the population data set used to develop the model and the data set used to assess model validity. (Summary of hydrocodone model and validity testing data set)

Total population	Model data set	Validity testing data set
	$N = 3{,}944$ subjects	$N = 55$ subjects
Data collection period	16 months	1 month
Gender distribution	F: 60% M: 40%	F: 65% M: 35%
Patient population averages* with standard deviations		
Age	57 ± 14 years	62 ± 15 years
Height	1.69 ± 0.11 m	1.69 ± 0.11 m
Weight	89 ± 25 kg	88 ± 23 kg
Hydrocodone dosage	36 ± 18 mg	21 ± 15 mg
Oral fluid hydrocodone concentration		
Mean	239 ± 345 ng/mL	116 ± 142 ng/mL
Median	126 ng/mL	79 ng/mL
Lean body weight (LBW)	54 ± 12 kg	54 ± 11 kg
Body surface area (BSA)	2 ± 0.31 m^2	2 ± 0.29 m^2
Calculated blood volume (CBV)	5.3 ± 1.4 L	5.4 ± 1.3 L

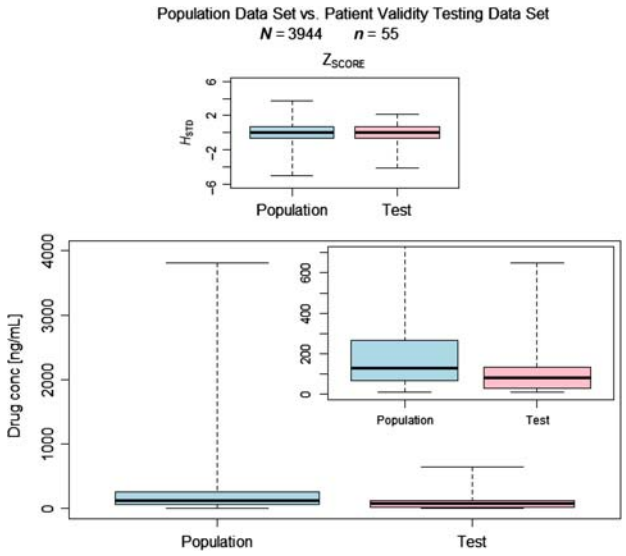

Figure 3. Box plot showing a comparison of the *s* score and hydrocodone concentrations for the population used to create the hydrocodone model and the patient set used to assess the validity of the model. The inset shows a magnified version of the hydrocodone concentrations box plots which excludes the upper range of the population set.

drug concentrations of the model and patient validity data sets are compared graphically in the boxplots shown in Figure 3.

Different variations of Equation (1) not detailed in this publication were examined in our studies. Equation (1), however, was found to be the most robust and preferred model used to determine whether the patients fall within the population of patients normally distributed around the standardized population mean. For example, a simple logarithmic transformation of the drug data as reported by Cao *et al.* (12) results in less correspondence to a true Gaussian distribution. However, it may be mathematically simpler for physicians to access such a model using a simple $\log_e$ transformation found on any smart phone or calculator.

This approach includes several assumptions and has several limitations. If the hydrocodone concentration is measured as greater than the 4,000 ng/mL ULOQ or negative—zero or below the 10 ng/mL LOQ of the LC–MS-MS method used for a patient prescribed hydrocodone—Equations (1) and (2) cannot be utilized and said patient will be deemed as "cannot be assessed". The concentration of hydrocodone in oral fluid of the patients is assumed to be a steady state concentration or level. The term "steady state" refers to an equilibrium hydrocodone concentration obtained after at least five days and on or before seven days (29, 30) and should remain considerably constant if the dose and the frequency of administrations remain substantially constant.

As opposed to conventional (i.e., urine) standard curves where carefully controlled, relatively small data sets (i.e., prospective clinical trials), are used to construct "normal" curves for comparison to current drug testing results, the present method uses data obtained for the hydrocodone drug concentration in oral fluid and the accompanying demographics and dose data to construct a mathematically transformed and normalized historical distribution of oral fluid testing results regardless of dose, time of sample donation, time of dosing and concurrent medications (if any). Thus, the samples used for this mathematically transformed and normalized standard distribution may include samples from patients that are fast or slow metabolizers, patients with impaired kidney or liver function, patients using drugs with overlapping metabolites on the same day and/or patients taking medication on an inconsistent schedule. However, this process does exclude samples without a quantifiable value for the drug concentration in question (i.e., >ULOQ or <LOQ), and samples that might have been positive for the drug of interest but obtained from patients that were not prescribed that drug, etc. Furthermore, patients with missing demographic information will not be normalized using this model and would be reported as "cannot be assessed".

Conclusion

Transforming and normalizing a historical oral fluid hydrocodone data set results in a near Gaussian distribution that can be used to assess patient consistency with this historical population. The key normalization factor is CBV derived from the relationship of oral fluid to plasma. While lean body weight, BMI and body surface area, calculated using patient specific parameters such as weight and height are important features of the transformation of these data, the CBV stands out as the primary normalization factor.

Supplementary material

Supplementary material is available at *Journal of Analytical Toxicology* online.

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