Urine drug testing concentration ranges for select benzodiazepines

Abstract
Benzodiazepines have been important drugs for the treatment of anxiety and pain since their introduction in the 1950s. Urine drug testing (UDT) is often used to help establish whether the patient is adherent to their prescribed benzodiazepine or is misusing the drug. As such, it is important for physicians to recognize drug levels that are outside expected ranges. However, to date there has been little research into “normal” urine drug ranges for patients prescribed benzodiazepines. This work is designed to present the UDT benzodiazepine results from a large patient population from 6 months of testing, specifically, alprazolam, clonazepam, diazepam, lorazepam, temazepam, and oxazepam. In an effort to make these results useful to the clinician, the raw data are emphasized rather than any mathematical transformations. The ranges from this population are presented as a tool to reduce subjectivity in determining if a patient is likely adherent to their medication or needs additional counselling.

Keywords: benzodiazepine, alprazolam, LC/MSMS, patient results

Introduction
Benzodiazepines were first synthesized in the 1950s and successfully marketed in the 1960s12 leading to a rapid rise in prescriptions of these compounds for the treatment of anxiety, insomnia, and seizures, among other conditions.1 They were seen as safer than, but just as effective as, barbiturates and other older drugs used as sedatives and hypnotics. However, by the 1980s it became clear that they were also saddled with similar liabilities for abuse and dependence as the older medications they were designed to replace.3,4 Despite this risk, as well as the availability of other safer medications with anxiolytic properties, the number of prescriptions written for benzodiazepines has not significantly dropped.3,4

Urine Drug Testing (UDT) is often used to help assess a patient’s adherence to their prescribed medication.1 Benzodiazepines and their metabolites are excreted in the urine as glucuronide conjugates and as such require hydrolysis for analysis.6–8 Benzodiazepine testing can be amenable to rapid analytical techniques.9,11 However, testing is most often accomplished by liquid chromatography-mass spectrometry mass spectrometry (LC-MS/MS)12–14 with limits of quantitation (LOQ) of 20ng/mL or lower.11

When UDT results are returned to the prescribing physician, they can often only confirm that the patient is positive or negative for the drug and/or metabolite(s) tested. However, there is relevance of UDT quantitative values in determining adherence.12–14 Several authors have attempted to draw more information out of UDT than just a positive or negative result, such as clinically relevant ranges. Pesce et al.15–19 demonstrated the relationship between drugs and metabolites in urine in a number of papers focused on metabolic ratios. The derived ratios demonstrated normalization and transformation have been demonstrated for a number of additional drugs and/or metabolites.23,24

While data modelling has been successful for other drugs and/or metabolite(s), a large number of data points is required (i.e., >4,000). To date, Xanax® or alprazolam, has been the only benzodiazepine to provide enough data points to use traditional modelling.23–24 Therefore, raw UDT concentrations were investigated with the goal to determine if a clinical UDT range of “normal patients” could be determined for benzodiazepines. This range would be analogous to existing blood ranges and assist a physician in quickly estimating whether their patient is consistent with a “normal population” and likely adherent with their prescription. To the best of the author’s knowledge, this is the first time the raw UDT data has been reported for use as a quick reference without the need for any patient demographic input to determine apparent adherence.

Materials and methods
The benzodiazepines analysis used in this report is part of a larger method for testing a wide variety of drug classes including opiates, opioids, amphetamines, and others. Details of the full method and validation can be found in an earlier report by Enders et al.25 Alprazolam, α-hydroxyalprazolam, lorazepam, nordiazepam, oxazepam, temazepam, and nordiazepam-D5 standards were purchased from Cerilliant Corporation (Round Rock, TX) as 1mg/mL stock solutions. An enzyme solution was prepared by diluting IMCSzyme® β-glucuronidase solution (IMCS, Irvine, SC) to 10,000units/mL in 0.02M sodium phosphate buffer, pH 7.5. Normal, drug-free urine was purchased from UTAK (Valencia, CA). Samples (30µL) were diluted 6x with 120µL of enzyme solution and 30µL of 1,000ng/mL nordiazepam-D5 internal standard. After dilution, samples were incubated at 60°C for 60 minutes for hydrolysis and then extracted using a solid-phase extraction method. Ultimately, samples were diluted 10x in 300µl of 10% methanol:90% water prior to injection and LC-MS/MS analysis. A morphine-3β-D-glucuronide
Figure 2 (a) Histogram of the normalized, transformed and standardized raw alprazolam data. (b) Kernel density estimation plot derived from the normalized, transformed and standardized raw alprazolam data overlaid with the least squares minimized best fit Gaussian distribution curve.

Alprazolam was investigated to determine if data modelling could be successful for benzodiazepines as shown in Figure 3. The data analysis and model development for alprazolam were conducted using R Project version 3.3.26 Data smoothing was conducted by kernel density estimation to smooth continuous data (e.g., histograms).27 Model development is detailed in earlier reports22,23 and results in equation 1.

Figure 3 (a) Alprazolam concentration ranges with corresponding dose levels. (b) α-hydroxylprazolam concentration ranges with corresponding dose levels. (c) 7-aminoclonazepam concentration ranges with corresponding dose levels. (d) Lorazepam concentration ranges with corresponding dose levels. (e) Nordiazepam concentration ranges with corresponding dose levels.

Urine drug testing concentration ranges for select benzodiazepines

Lorazepam

<table>
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<tr>
<th>Dosage</th>
<th>4.5mg</th>
<th>15mg</th>
<th>20mg</th>
<th>&gt;20mg</th>
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<tr>
<td>Sample size</td>
<td>76</td>
<td>230</td>
<td>2570</td>
<td>4mg</td>
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Nordiazepam

<table>
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<th>Dosage</th>
<th>0.25mg</th>
<th>0.5mg</th>
<th>&gt;0.5mg</th>
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</thead>
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<tr>
<td>Sample size</td>
<td>536</td>
<td>2468</td>
<td>4943</td>
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</table>

Mathematical normalization and transformation model is preferred, as dose is included in the calculations (Equation 2).

The impact of dose on the range and median values are as predicted as shown in Figure 2 for alprazolam, alpha-hydroxyalprazolam, 7-aminoclonazepam, lorazepam, and nordiazepam. We did not generate dosage dependent “box and whisker” plots for oxazepam and temazepam as the sample sizes were too small. While the median values of alprazolam increase with dose (Figure 3A), the range increases almost exponentially. Some drug ranges showing unexpected lower or higher ranges than the trend might be due to the small sample size (Table 2) for that dose.

Discussion

Alprazolam (Xanax®) and its metabolite, α-hydroxyalprazolam exhibit median values of 96ng/mL and 209mg/mL, respectively. When compared with most other benzodiazepine UDT levels, these levels are lower except those of the primary metabolite of clonazepam, 7-aminoclonazepam, with a median value of 189ng/mL. Oxazepam and Temazepam demonstrate the highest levels and are consistent with the fact that they are dosed at higher levels. To better understand the UDT range for different benzodiazepine doses, alprazolam data was divided into different daily doses and plotted in Figure 2A. The overlapping ranges make it difficult to use a UDT result to discretely determine dose adherence, and is why the normalization and transformation model is preferred, as dose is included in the calculations (Equation 2).

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Table 2 Sample size for drugs at various doses

<table>
<thead>
<tr>
<th>Alprazolam</th>
<th>Alpha-alprazolam</th>
<th>7-aminoclonazepam</th>
<th>Lorazepam</th>
<th>Nordiazepam</th>
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<tr>
<td>Dosage</td>
<td>Sample size</td>
<td>Dosage</td>
<td>Sample size</td>
<td>Dosage</td>
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<tr>
<td>&gt;10mg</td>
<td>99</td>
<td>&gt;10mg</td>
<td>94</td>
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Part of the focus for this paper is to aid physicians in determining patient adherence. To be successful, alprazolam outliers should be readily identified from a comparison with Figure 3A. The ability to differentiate adherence from abuse from this “box and whisker” (Figure 3A) representation of alprazolam data is probably restricted to very low doses where a UDT result above the range presented for that low dose would suggest abuse or possible genetic/metabolic variations. Making decisions from population based data displays is difficult for those patients who fall above, but near the upper limit of the “normal range” and should be made in conjunction with other clinical observations of the individual patient.

The verification of urine drug screen results by LC-MS/MS for routine monitoring of patients prescribed benzodiazepines is not currently standard of care in most mental health clinics or primary care practices (where the majority of benzodiazepines are prescribed). These medications carry significant abuse-potential and the risk of lethal overdose. The ability to quickly compare UDT results without further mathematical manipulation to results from a large test population may help physicians determine patient adherence from their UDT data. While various normalizations and transformations have been reported, they all require additional mathematical manipulations often using demographic data that may or may not be available. Thus, direct comparison of individual patient data with raw data ranges (albeit filtered for inconsistent results) may be the easiest and most impactful way to help assess patient adherence.

Conclusion

This paper and the data contained within provide an important step forward in a clinicians’ ability to monitor the safety and effectiveness of treatment for anxiety/panic disorders with benzodiazepines. Given the recent rise in deaths attributable to multi-drug overdoses, many of which include benzodiazepines, it is imperative that prescribers are well equipped to determine how patients are using these medications. Well-defined reference ranges are integral in this endeavor.

Acknowledgments

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Conflicts of interest

The authors do not have any conflicts of interest with this work.

References


