

Commentary

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# Immunoassay Testing of Benzodiazepines in Urine Fails to Protect Patients

Amadeo J Pesce\* and Keith Tran

Precision Diagnostics LLC, San Diego, CA, USA

**\*Corresponding author:** Amadeo J Pesce, Professor, Precision Diagnostics LLC, 4215, Sorrento Valley Boulevard, San Diego, CA 92121, United States.

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## Abstract

**Objective:** Current immunoassays are yielding misleading benzodiazepine test results. Many current immunoassays use a 200ng/mL cutoff to define a positive test result.

**Experimental:** Using an LC-MS/MS method we observed that the median observed value for the benzodiazepine metabolites 7-aminoclonazepam and alprazolam were below this concentration. The fraction of patients positive for both an opiate and a benzodiazepine were greater than twenty percent.

**Conclusion:** The current immunoassays fail to detect concomitant drug use potential hazardous drug use in patients on pain medications.

**Keywords:** Benzodiazepines, Opiates, Immunoassays, LC-MS/MS

## Introduction

Benzodiazepines are one of the most commonly prescribed class of drugs in the world for the treatment of insomnia and anxiety [1]; they are also used as anticonvulsants [1]. When used as prescribed, benzodiazepines are relatively safe, but have abuse potential and enhance CNS and respiratory depression when used concomitantly with opioids [2]. Therefore, compliance monitoring using urine drug testing for these drugs is employed. There are problems with the immunoassays used for this type of testing. There are two reasons for this 1. the assay cutoff of 200ng/mL is set too high and 2. the assays do not cross react well with the excreted benzo-glucuronide metabolized drug. Several benzodiazepines are excreted as the glucuronide metabolite [3,4]. The current cutoff for a positive benzodiazepine assay is commonly set at 200ng/mL. However there has been a large change in the amount of drug prescribed. Early benzodiazepines were prescribed in dosages of

10 or more milligrams while some of the more recently approved drugs are given in dosages of 1mg or less (Table 1).

The poor cross reactivity with some of the benzodiazepine glucuronide metabolites is well known. To avoid this problem assay package inserts only list the cross reactivities with the parent drug and not the glucuronide metabolite. For example, the Thermo Scientific assay does not list the cross reactivity of any glucuronide [7]. In contrast the Roche Cobas assay lists the cross reactivity of the Temazepam glucuronide at 0.7% and lorazepam glucuronide at 1% [8]. In addition to the poor cross reactivity. The excretion of these drugs is often below the 200ng/mL cutoff for a positive test result. We list our median observed concentration for several benzodiazepines obtained from our pain management and rehabilitation facility clients performed by our LC-MS/MS method in the following table [9,10] (Table 2).

**Table 1:** Benzodiazepine equivalence Table [5,6].

Benzodiazepines	Half-life (hrs) [active metabolite]	Approximately Equivalent Oral dosages (mg)
Chlordiazepoxide (Librium) (1960)	5-30 [36-200]	25
Diazepam Valium (1963)	20-100	10
Alprazolam (Xanax, Xanor, Tafil) (1981)	6-12	0.5
Temazepam (Restoril, Normison, Euhypnos) (1981)	8-22	20
Clonazepam (Klonopin, Rivotril) (1997)	18-50	0.5

**Table 2:** Median benzodiazepine concentration values (after hydrolysis) in urine from LC-MS/MS data.

Benzodiazepine	Median ng/mL	Number Observed
7 aminoclonazepam	160	70,995
Clonazepam	11	22,081
ahydroxyalprazolam	149	75,394
alprazolam	88	70,113
Diazepam	10	9,226
Nordiazepam	97	51,880
Temazepam	304	56,199
Oxazepam	224	83,143
Lorazepam	339	37,154

## Results

The data shows that the median concentration of the excreted 7-aminoclonazepam and alphahydroxyalprazolam are below the 200ng cutoff implying that these observations would be deemed negative by immunoassay.

This poor reactivity has been known for about 13+ years.

However, these immunoassays are popular because they have FDA approval and are classified as moderate complexity and can be use in physician offices. There are some studies showing improved detection by hydrolyzing the urine with glucuronidase improves assay sensitivity [11]. However, this modification makes the assay a lab developed test requiring a more complex and costly validation.

Even though the CDC has recommended not prescribing an opiate and a benzodiazepine together, we have observed a significant fraction of patients on opioids also positive for a benzodiazepine.

We examined the co positive findings for hydrocodone, oxycodone, and morphine with the following benzodiazepine drugs or their metabolites. The time frame was Jan 1, 2020 to Jan 24, 2024. More than twenty percent of patients on man opiate also were positive for a benzodiazepine. We also observed that 25% of benzodiazepine use in individuals using fentanyl. Further patients on medication assisted substance abuse therapy were also observed to use benzodiazepines. This data reinforces the need to appropriately monitor patients on opioid therapy. Current immunoassay tests are not appropriate.

## Conclusion

We and others have shown the poor sensitivity of these immune-assays and have used definitive testing to overcome these poor-quality results. Newer immunoassays using glucuronidase and lower cutoffs are required to effectively monitor benzodiazepines (Table 3).

**Table 3:** Observed Frequency of benzodiazepine and opiates in urine specimens.

Opiate	Benzodiazepine	Cutoff	Number positive
<b>Hydrocodone 276,330 positive 5ng/mL cutoff</b>	Alphahydroxyalprazolam	5 ng/mL	21,345
	7-aminoclonazepam	5 ng/mL	13,328
	lorazepam	10 ng/mL	7,450
	oxazepam	10 ng/mL	16,701
	Co-positive percentage		21%
<b>Oxycodone 266,250 positive 10ng/mL cutoff</b>	Alphahydroxyalprazolam	5 ng/mL	21,035
	7-aminoclonazepam	5 ng/mL	12,700
	lorazepam	10 ng/mL	7,004
	oxazepam	10 ng/mL	17,006
	Co-positive percentage		22%

<b>Morphine 114,471 positive 50ng/mL cutoff</b>	Alphahydroxyalprazolam	5 ng/mL	9,651
	7-aminoclonazepam	5 ng/mL	7,416
	lorazepam	10 ng/mL	3,642
	oxazepam	10 ng/mL	8,258
	Co-positive percentage		25%
<b>Fentanyl 111,869 Positive 1ng/mL cutoff</b>	Alphahydroxyalprazolam	5 ng/mL	8,449
	7-aminoclonazepam	5 ng/mL	8,176
	lorazepam	10 ng/mL	3,712
	oxazepam	10 ng/mL	7,264
	Co-positive percentage		25%
<b>Buprenorphine 571, 622 Positive 5ng/mL cutoff</b>	Alphahydroxyalprazolam	5 ng/mL	29,534
	7-aminoclonazepam	5 ng/mL	35,791
	lorazepam	10 ng/mL	10,466
	oxazepam	10 ng/mL	25,018
	Co-positive percentage		18%
<b>Methadone 143,651 Positive 50ng/mL cutoff</b>	Alphahydroxyalprazolam	5 ng/mL	8,144
	7-aminoclonazepam	5 ng/mL	10,702
	lorazepam	10 ng/mL	2,918
	oxazepam	10 ng/mL	6,226
	Co-positive percentage		20%

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## Conflict of Interest

There are no conflicts of interest.

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