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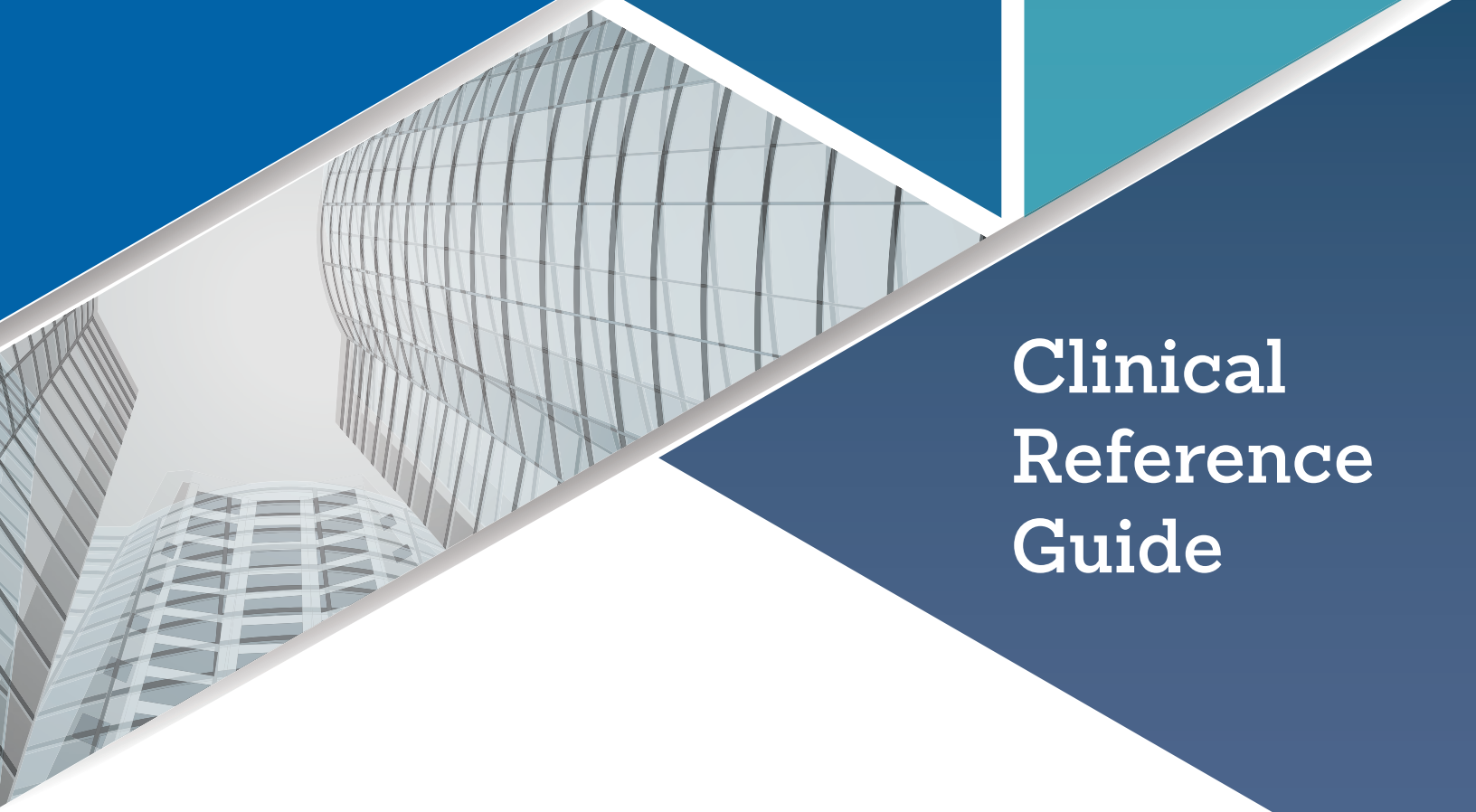

Clinical Reference Guide

Testing for Benzodiazepines

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Some benzodiazepines share the same metabolites; therefore, distinguishing which drug was ingested is not always possible. Benzodiazepines such as alprazolam, lorazepam, and clonazepam may not be detected by common benzodiazepine immunoassays.

A. Prescription Benzodiazepines

Many benzodiazepines, like opioids, are metabolized in the liver by cytochrome P450 (CYP450) enzymes and phase II conjugation. Parent drugs are not always detectable in urine; rather, the presence of metabolites may indicate that a benzodiazepine was ingested. Several prescription benzodiazepines undergo metabolism to common shared metabolites (see Figure 13.1). In these cases, distinguishing which compound was ingested may be impossible. Aegis reports the presence of these metabolites (nordiazepam, oxazepam, and temazepam) collectively as "Benzodiazepine Metabolites." The benzodiazepines which may contribute to this result are listed in Table 13.1.

Figure 13.1: Benzodiazepine Metabolism[1-2]

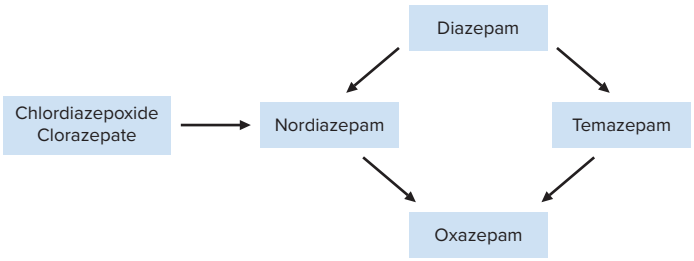

Table 13.1: *Benzodiazepines Which Are Reported as "Benzodiazepine Metabolites"*

DRUG	MARKERS
Chlordiazepoxide (Librax®, Librium®) Clorazepate (Gen-xene®, Tranxene®)	Nordiazepam Oxazepam
Diazepam (Valium®)	Nordiazepam Temazepam Oxazepam
Oxazepam	Oxazepam
Temazepam (Restoril®)	Temazepam Oxazepam

Conversely, there are some benzodiazepines which are metabolized to unique metabolites. In these cases, the identity of the parent compound may be easily identified (see Table 13.2). Many of these drug-specific markers, including alpha-hydroxyalprazolam, 7-aminoclonazepam, 2-hydroxyethylflurazepam, and lorazepam glucuronide do not react well to benzodiazepine immunoassays. This is concerning, because alprazolam and clonazepam are commonly abused and are included in the top 25 most frequently identified drugs through law enforcement seizures as reported by the National Forensic Laboratory Information System (NFLIS).[3] Relying on immunoassay results and failure to test patient specimens with definitive techniques such as gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/tandem mass spectrometry (LC/MS/MS) can result in false negatives for up to 53% of patients for the benzodiazepine class.[4-8]

Table 13.2: *Unique Markers of Benzodiazepine Metabolism[1]*

PARENT DRUG	METABOLITE
Alprazolam (Xanax®)	α-hydroxyalprazolam
Clonazepam (Klonopin®)	7-aminoclonazepam
Flurazepam	2-hydroxyethylflurazepam
Lorazepam (Ativan®)	Lorazepam glucuronide

B. Designer Benzodiazepines

Benzodiazepines were first studied in the 1950s by scientists at Roche. Throughout the 1960s and 1970s, other scientists studied the class, and several other compounds were developed and marketed. The research of benzodiazepines eventually yielded more than 3,000 different compounds, although most were never marketed.[9]

More recently, clandestine laboratories have studied the work of these early scientists and are producing "designer benzodiazepines," which are analogs that possess similarities to pharmaceutical benzodiazepines, but are intended to avoid regulation or detection. The first designer benzodiazepines developed were phenazepam and etizolam, which

were originally marketed by pharmaceutical companies in some countries as non-controlled substances. As countries began to regulate and schedule phenazepam and etizolam as controlled substances, clandestine laboratories began producing and distributing diclazepam, flubromazepam and pyrazolam as "research chemicals," labeled not for human consumption. Recently, clonazolam, deschloroetizolam, flubromazolam, nifoxipam, and meclonazepam have been marketed on various research chemical websites.[10]

In some cases, these research chemicals may metabolize to, or share a common metabolite with, a commercially available drug in the U.S. and lead to unexpected results with definitive testing. Due to their structural similarities, these research chemicals may cross-react in immunoassay testing and, unless they share common metabolites with prescription benzodiazepines, may not be detected by mass spectrometry. The prevalence of designer benzodiazepine abuse has not been extensively studied to date; therefore, the impact on presumptive positive immunoassay results is unknown at this time. When considering causes of a false-positive immunoassay result, the use of designer benzodiazepines and potential for cross-reactivity may be worthy of consideration in select circumstances.

REFERENCES:

1. Baselt RC. *Disposition of toxic drugs and chemicals in man*. 11th ed. Seal Beach, CA: Biomedical Publications; 2017.
2. Mandrioli R, Mercolini L, Raggi MA. Benzodiazepine metabolism: an analytical perspective. *Curr Drug Metab*. 2008;9(8):827-44.
3. U.S. Drug Enforcement Administration, Diversion Control Division. (2018). *National Forensic Laboratory Information System: NFLIS-Drug 2017 Annual Report*. Springfield, VA: U.S. Drug Enforcement Administration.
4. Pesce A, Rosenthal M, West R, et al. An evaluation of the diagnostic accuracy of liquid chromatography-tandem mass spectrometry versus immunoassay drug testing in pain patients. *Pain Physician*. 2010;13(3):273-81.
5. Mikel C, Pesce AJ, Rosenthal M, West C. Therapeutic monitoring of benzodiazepines in the management of pain: current limitations of point of care immunoassays suggest testing by mass spectrometry to assure accuracy and improve patient safety. *Clin Chim Acta*. 2012;413(15-16):1199- 202.
6. Dixon RB, Floyd D, Dasgupta A. Limitations of EMIT benzodiazepine immunoassay for monitoring compliance of patients with benzodiazepine therapy even after hydrolyzing glucuronide metabolites in urine to increase cross-reactivity: comparison of immunoassay results with LCMS/MS values. *Ther Drug Monit*. 2015;37(1):137-9.
7. Darragh A, Snyder ML, Ptolemy AS, Melanson S. KIMS, CEDIA, and HSCEDIA immunoassays are inadequately sensitive for the detection of benzodiazepines in urine from patients treated for chronic pain. *Pain Physician*. 2014;17(4):359-66.
8. Kirsh KL, Heit HA, Huskey A, Strickland J, Egan K, Passik SD. Trends in drug use from urine drug testing of addiction treatment clients. *J Opioid Manage*. 2015;11(1):61-8.
9. Sternbach LH. The benzodiazepine story. J Med Chem. 1979;22(1):1-7.
10. Moosmann B, Link LA, Auwärter V. Designer benzodiazepines: a new challenge. *World Psychiatry*. 2015;14(2):248.