

# Detection of Benzodiazepines in Oral Fluid by Homogeneous Enzyme Immunoassay

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## Abstract

Benzodiazepines (after marijuana) are the most widely detected drugs in DUID cases. Oral fluid has become a useful biological testing matrix to detect recent drug usage, mainly due to observed collection and difficulty of adulteration of specimens. As roadside screening in some jurisdictions is moving to allow oral fluid, new assays are necessary for high volume screening. The sample volumes with oral fluid are limited and it typically contains lower concentrations of drugs. Also, the low saliva:plasma ratio (<1) for benzodiazepines, requires that new oral fluid assays be very sensitive and cross-reactive to the lower dosage drugs such as alprazolam, lorazepam, clonazepam, etc.

## Oral Fluid Collection and Drug stability

Using the Quantisal™ oral fluid collection device, 1 mL of neat oral fluid is collected. The collection pad is immersed in a tube containing saliva extraction buffer (3 mL), capped and sent to a testing laboratory. To date, oral fluid analysis for benzodiazepines has been limited to an ELISA platform due to the lack of a homogeneous immunoassay (HEIA) and the need for a pretreatment step to reduce the viscosity problems associated with neat oral fluid. This collection method greatly simplifies the oral fluid testing.

Benzodiazepines are relatively stable in the saliva extraction buffer. Based on GC-MS analysis, when various benzodiazepines were spiked into saliva extraction buffer and stored in the Quantisal™ device at room temperature for 5 days, the recovery was 71.3 % for oxazepam, 92.1% for diazepam, 86.7% for alprazolam, 83.4% for lorazepam, 88.1% for clonazepam and 84.8% for temazepam.

## Methods

This homogenous immunoassay uses a benzodiazepine antibody and is based on the competition of oxazepam labeled glucose-6-phosphate dehydrogenase (G6PDH) and the free drug in the oral fluid sample for a fixed amount of antibody binding sites. In the absence of free drug in the sample, the antibody binds the drug enzyme conjugate and enzyme activity is inhibited. This creates a dose response relationship between drug concentration in the oral fluid and enzyme activity. The enzyme activity is then measured spectrophotometrically at 340 nm by the conversion of NAD to NADH.

## Assay Performance

### Precision:

Inter-assay (5 days): 1-6 (S.D.); <1 (C.V.%)  
 Intra-assay (n=5): shown in table below

Oxazepam Conc (ng/mL)	Mean $\Delta$ abs	S.D.	C.V.%
0	591	4.81	0.82
10	611	2.96	0.48
20	629	3.27	0.52
30	642	3.22	0.5
40	653	4.22	0.65

## Cross-reactivity

Compound	Conc (ng/mL)	Oxazepam equivalents (ng/mL)	% Cross-reactivity
Oxazepam	20	20	100
Alprazolam	10	25	250
Hydroxy Alprazolam	25	30	120
Clonazepam	50	35	70
7-aminoclonazepam	50	20	40
Diazepam	25	30	120
Estazolam	25	30	120
Flurazepam	10	10	100
Lorazepam	50	45	90
Nitrazepam	50	35	70
Temazepam	50	100	200
Triazolam	50	37.5	75
Flunitrazepam	25	15	60

Disclosure: Immunalysis Corporation manufactures and distributes the immunoassay described in this presentation

Non-related drugs and other interfering substances spiked in oral fluid, at a concentration of 10,000 ng/mL, showed no cross-reactivity and no false positive results in the assay.

## Accuracy

HEIA	LC-MS	
	+	-
+	22	1
-	0	19

Sensitivity: 100%

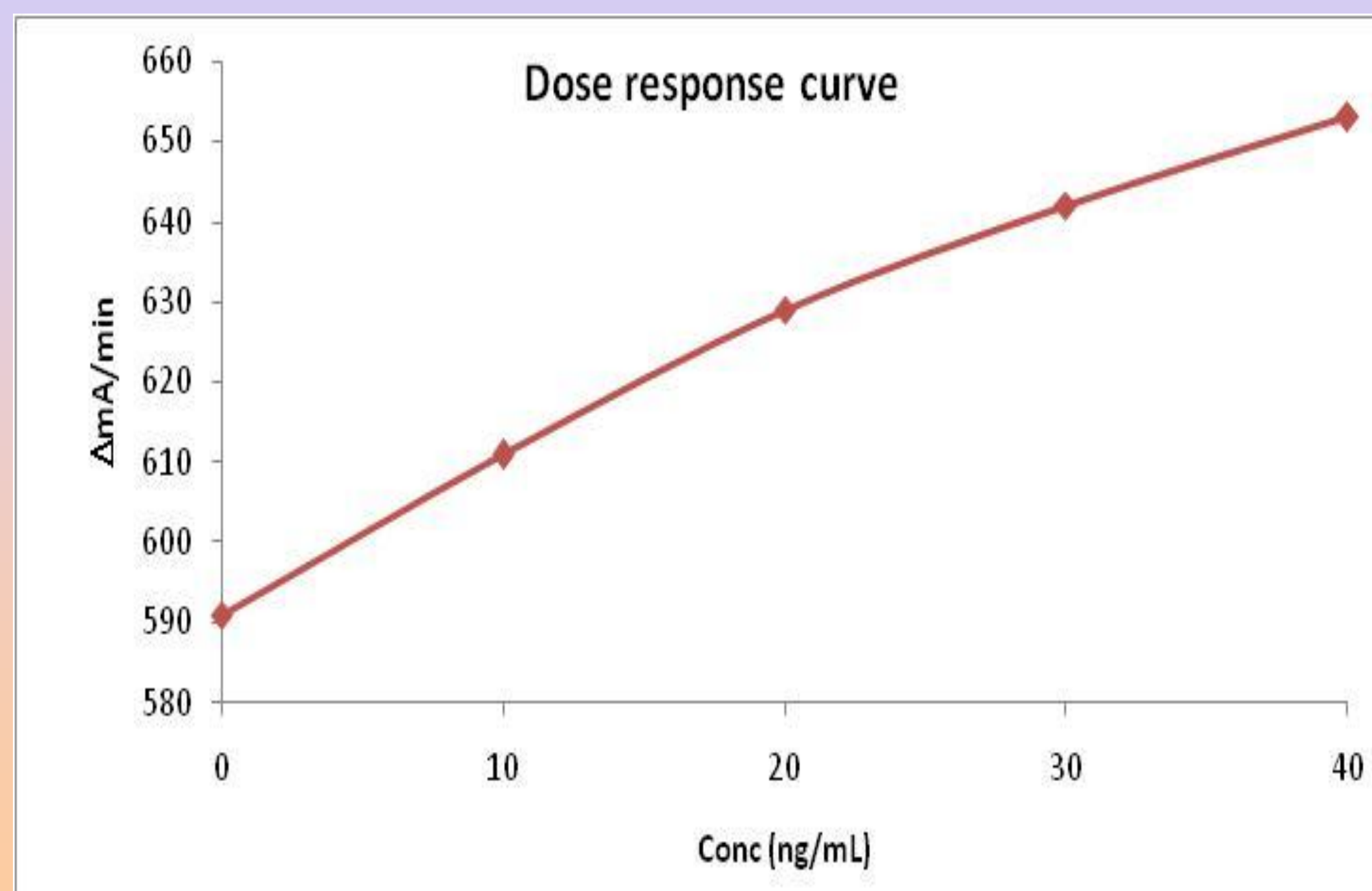
Specificity: 95%

Accuracy: 97.6%

The one sample which tested positive by HEIA was screened at 18 ng/mL by GC-MS.

## Assay Profile

Cutoff: 20 ng/mL; Detection limit: 1 ng/mL



## Summary

The homogeneous immunoassay is sensitive, specific and precise for the detection of a range of benzodiazepines in oral fluid, which are of significance in the toxicology field. The assay format is compatible with commercially available chemistry analyzers.

## References

Saliva testing for drugs of abuse: Saliva as a diagnostic fluid, Cone, E.J. *Annals NY Acad. Sci.* **1993**, 649, 91-127.

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