

# Rapid Analysis of Benzodiazepines and “Z-drugs” in Five Different Biological Matrices using Dispersive Pipette XTRaction

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**HIGHLIGHTS:** Reduce transfer steps, reduce solvent volumes, reduce conditioning and wash steps



WAX - XTR

## INTRODUCTION

Benzodiazepines and sedatives are some of the most commonly abused drugs in the nation and are often found in cases involving driving under the influence of drugs, sexual assaults and overdoses.

Unfortunately, most existing methods used to identify benzodiazepines and sedatives across matrices are labor intensive and time-consuming. A simple cleanup method that can be applied across multiple biological matrices would be an ideal solution to increase the throughput capabilities of forensic laboratories.

Unlike traditional methods for SPE, Dispersive Pipette XTRaction technology for clean-up focuses on removing matrix interferences, while keeping the benzodiazepines in the sample solution. XTR tips containing loose weak anion exchange (WAX) sorbent remove phospholipids and small non-amphoteric acids that can cause ion suppression. By simply aspirating and dispensing the sample solution with WAX XTR tips, matrix interferences are removed with minimal loss of the drug. This reduces the time required for extraction by minimizing several conditioning and washing steps, reducing the volume of solvent required for elution and eliminating analyst error from multiple transfer steps. The initial addition of acetonitrile to the sample solutions keeps the benzodiazepines and sedative hypnotics from binding to the sorbent. (1)

We reported an LC-MS/MS analysis of benzodiazepines using Dispersive Pipette XTRaction technology for cleanup. WAX- XTR tips can provide sample preparation across five matrices for a simple and fast INTip™ solution.

## MATERIALS AND METHODS



All sample preparation was performed on a 5 mL Pneumatic Extractor with 5 mL XTR tips that contained 60 mg of weak anion exchange (WAX) sorbent from DPX Technologies, Columbia, SC.

Figure 1. DPX 5 mL Pneumatic Extractor is a syringe device that operates under gas to create positive and negative pressure to aspirate and dispense solutions in and out of 5 mL tips

Table 1. Sample Preparation

<b>Pretreatment</b>	<ul style="list-style-type: none"> <li>250 <math>\mu</math>L sample of the following matrices: urine, blood, liver tissue, brain tissue and stomach content</li> <li>50 <math>\mu</math>L internal standards</li> <li>750 <math>\mu</math>L acetonitrile</li> <li>Centrifuge</li> </ul>
<b>Sample</b>	Aspirate/dispense supernatant solution 3 times - 60 seconds using WAX-XTR tips
<b>Prep for injection</b>	Transfer 50 $\mu$ L and add 80 $\mu$ L of the LC mobile phase for injection

## RESULTS AND DISCUSSION

The following studies were performed for all drugs in the method following the SWGTOX Guidelines: limits of detection, limits of quantitation, limits of linearity, accuracy, precision, dilution integrity carry-over, selectivity, absolute recovery, ion suppression/enhancement and stability.

Different regression models were examined and the quadratic model weighted  $1/x$  with no forcing through zero for all drugs was determined to be the most appropriate model.

Drug	Concentration (ng/mL)	Aqueous	Blood	Liver	Brain	Stomach Contents	Urine
Zopiclone	40	88.07	73.63	74.88	74.40	76.78	87.66
Zolpidem	40	85.64	75.47	75.08	76.06	77.34	88.19
Chlordiazepoxide	400	83.01	63.35	63.27	63.15	66.45	74.45
Midazolam	40	80.08	69.43	70.02	70.99	71.54	81.59
Flurazepam	8	87.61	69.60	76.31	75.80	80.69	83.59
Zaleplon	16	91.07	78.78	77.45	78.92	83.67	90.37
Estazolam	40	86.34	70.41	75.60	75.52	75.44	87.38
Oxazepam	400	73.87	60.84	63.98	64.40	63.04	72.95
Lorazepam	40	77.80	64.28	62.85	66.39	66.85	76.25
Clonazepam	40	91.50	76.98	76.02	81.86	79.57	93.11
Alprazolam	40	91.02	79.41	79.78	80.32	77.87	93.12
Nordiazepam	400	65.23	56.00	55.34	55.15	54.30	58.98
Triazolam	4	76.95	71.87	68.99	72.94	68.06	83.19
Flunitrazepam	8	72.17	61.80	59.87	60.58	59.83	67.19
Temazepam	400	79.59	67.59	68.66	69.18	68.76	79.37
Phenazepam	8	76.55	66.59	64.92	68.41	65.46	71.11
Diazepam	400	73.40	62.80	63.18	63.61	61.77	66.75
Doxylamine		60.63	68.48	74.12	71.05	78.86	71.31
Diphenhydramine		72.63	69.56	76.30	72.20	81.61	73.04
Zaleplon		77.07	73.58	75.58	74.76	78.22	79.63
Hydroxyzine		74.53	71.01	74.12	73.38	75.78	71.56
Suvorexant		57.74	57.35	64.78	64.04	51.70	59.46

## CONCLUSION

A rapid quantitative extraction and LC-MS/MS method has been developed for benzodiazepines and z-drugs in five different matrices. The method can extract 48 standards and samples in 1 hour and has been validated at Orange County Crime Lab, reducing their extraction and LC-MS/MS times in half.

## REFERENCES

1. Mata, Danielle C. (2015) Ultra Performance Liquid Chromatography with Tandem Mass Spectrometry for the Quantitation of Seventeen Sedative Hypnotics in Six Common Toxicological Matrices. *Journal of Analytical Toxicology*, 40, 58-63

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