



ANALYSIS OF BENZODIAZEPINES AND "Z-DRUGS" IN 5 MATRICES USING dSPE TIPS

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 extraction technology for clean-up focuses on removing matrix interferences, while keeping the benzodiazepines in the sample solution. dSPE 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 dSPE 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 extraction technology for clean-up. dSPE tips with WAX sorbent can provide sample preparation across five matrices for a simple and fast INTip solution.

WORKFLOW

Platform	DPX Pneumatic Extractor
Pretreatment	<ul style="list-style-type: none">• 250 μL sample of the following matrices: urine, blood, liver tissue, brain tissue and stomach content• 50 μL internal standards• 750 μL acetonitrile• Centrifuge
Dispersive Pipette Extraction	5 mL Universal dSPE Tips with 60 mg WAX
Condition	None
Sample	Aspirate and dispense supernatant solution 3 times - 60 seconds
Prep for injection	Transfer 50 μ L and add 80 μ 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.

Table 1. Average extraction recoveries using dispersive pipette extraction with dSPE WAX tips for cleanup across various matrices at calibrator 5.

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 Country Crime Lab, reducing their extraction and LC-MS/MS times in half.

REFERENCES

1. Mata, Dani 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