Extraction of Z-Drug/Metabolite Panel From Urine Using Clean Screen® XCEL I SPE and LC-MS/MS

UCT Part Numbers:
Clean Screen® CSXCE106: 130 mg/6mL
SLDA100ID21-3UM: Selectra® DA HPLC Column 100 x 2.1, 3µm
SLDAGDC21-3UM: Selectra® DA Guard Column 10 x 2.0, 3µm
SLDGRDHLDR-Guard Cartridge Holder

April 2015

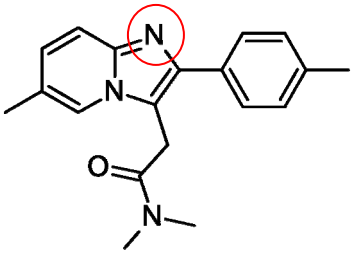
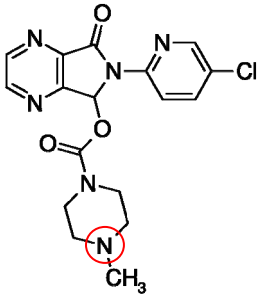
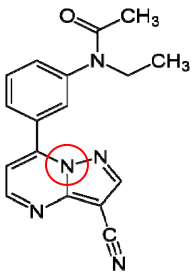
SUMMARY:

Z-drugs or nonbenzodiazepines are a class of psychoactive drugs whose pharmacological actions are similar to those of the benzodiazepines and have shown efficacy in treating sleep disorders. Although they are chemically different, they have similar benefits, risks and side effects as benzodiazepines. Z-drugs bind to GABA receptor complexes which are close to or actually coupled with benzodiazepine receptors. Noted to be more selective, these compounds bind mainly to the a1 GABA receptor subtype which mediates hypnotic effects. Benzodiazepines worsen sleep architecture whereas Z-drugs have little to no effect on sleep architecture, making them more preferable.

The three primary groups of Z-drugs are:

- **Zolpiclone** (Lunesta, Imovane, Zimovane, Imrest)
- **Zolpidem** (Ambien, Ambien CR, Stilnoct, Intermezzo, Stilnox)
- **Zaleplon** (Sonata, Starnoc)

Zolpiclone is partially metabolized in the liver to form an inactive N-demethylated derivative and an active N-oxide metabolite. These metabolites account for about 30% of the initial dose in urine while 7-10% of the drug is excreted unchanged. Less than 1% of both Zolpidem and Zaleplon get excreted in urine unchanged. Due to these low concentrations, sample clean up using SPE is normally required to eliminate undesirable matrix as well as to concentrate the sample.

Zolpidem	**Zopiclone**	**Zaleplon**
pKa=5.65	**pKa=6.89**	**pKa=0.3**
		

Clean Screen® XCEL I columns were chosen because of their excellent capability to extract basic compounds while eliminating the need for time consuming column conditioning. This method reduces the amount of time needed to extract a panel of samples and minimizes solvent use for sample cleanup. To ensure the functional groups on the various compounds were fully ionized, the urine samples were adjusted to a pH of 4 prior to passing them through the column. At this pH samples were effectively retained on the column. A wash step was performed which removed unwanted matrix and interferences without losing any of the analytes in question. The Z-drugs were eluted using a highly basic solvent combination of $MeCl_2$: IPA: NH_4OH (pH 11-12).

The majority of the compounds included in the evaluated panel are true bases with ionizable functional groups. However, Zaleplon is structurally more similar to a benzodiazepine. Because this drug only becomes charged in extremely acidic pH conditions, it functions more as a neutral compound. Retained primarily via hydrophobic interactions, the amount of organic in the wash needed to be optimized to not compromise the overall recovery of Zaleplon but still provide sufficient cleanup for the remainder of the analytes.

PROCEDURE:

1. **Sample Preparation**
   a. To 1 mL of urine sample, add 3 mL of 0.1% Formic Acid Solution.
   b. Vortex Sample.

2. **Apply Sample to Clean Screen® XCEL I column**
   a. Load sample directly to column without any preconditioning.
   b. Pull sample through at a rate of 1-2 mL/ minute.
   c. Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. **Wash**
   a. 1 x 3 mL 75:25 100mM Acetic Acid:MeOH
   b. 1 x 3 mL Hexane
   c. Dry column thoroughly under full vacuum or positive pressure for 5-10 minutes.

4. **Elution**
   a. 1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2)
   b. Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. **Dry Eluate**
   a. Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. **Reconstitute**
   a. Reconstitute sample in 100 µL of mobile phase.

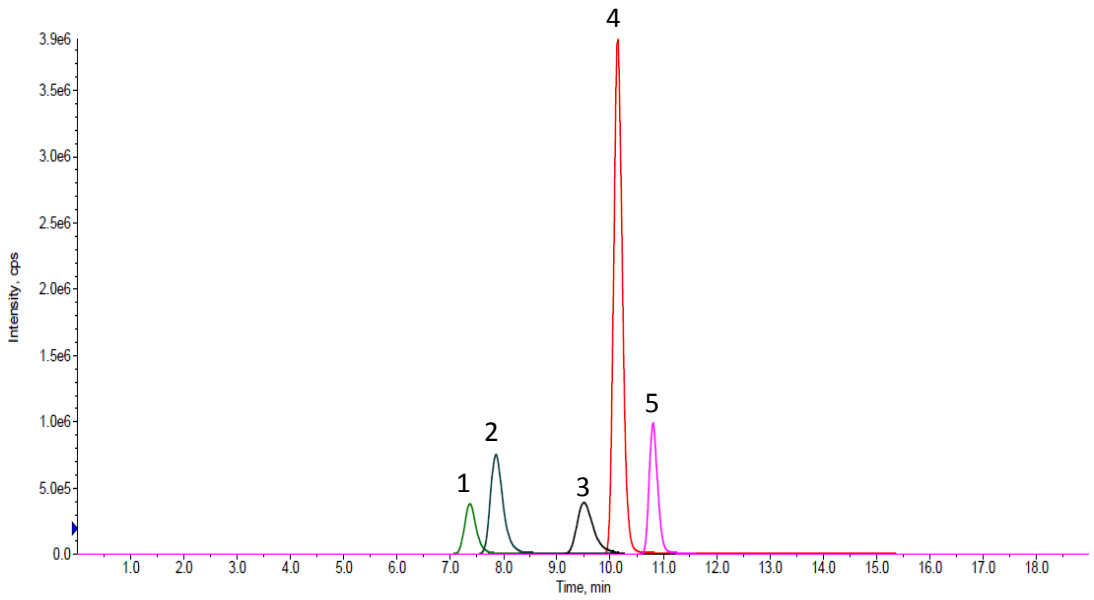
INSTRUMENTAL:

HPLC Conditions	
Instrumentation	Agilent 1200 Series Binary Pump SL
HPLC column	Selectra® DA, 100 × 2.1 mm, 3 µm
Guard column	Selectra® DA, 10 × 2.0 mm, 3 µm
Guard column holder	p/n: SLDGRDHLDR
Column temp.	50°C
Autosampler temp.	10°C
Injection volume	10 µL
Mobile phase A	0.1% formic acid in H_2O
Mobile phase B	0.1% formic acid in ACN
Flow rate	300 µL/min

LC gradient		
Time (min)	**A (%)**	**B (%)**
0	95	5
0.5	80	20
6	80	20
7.5	0	100
12.0	0	100
13.2	95	5
18	95	5

MS Conditions	
Instrumentation	API 4000 QTRAP MS/MS
Ionization mode	ESI^+
Spray voltage	4200 V
Vaporizer temperature	650°C
Sheath gas pressure	40 arbitrary units
Auxiliary gas pressure	5 arbitrary units
Collision gas pressure	Medium

CHROMATOGRAM:



Analyte		MRM Transitions		RRT (min)
		Q1	Q3	
1	N-Desmethylzolpiclone	375.033	244.900	7.36
2	Zopiclone	388.988	244.900	7.86
3	Zopiclone-N-oxide	404.966	143.000	9.50
4	Zolpidem	308.289	234.900	10.12
5	Zaleplon	305.963	264.000	10.80

RESULTS:

Compound	50 ng/mL (n=5)		300 ng/mL (n=5)	
	Extraction Recovery	Matrix Effect	Extraction Recovery	Matrix Effect
N-Desmethyl Zopiclone	88%	8%	89%	5%
Zopiclone	90%	44%	91%	23%
Zopiclone-N-Oxide	71%	46%	75%	37%
Zolpidem	80%	26%	93%	26%
Zaleplon	90%	13%	102%	20%

**Insignificant loss of Zaleplon was observed using 3mL 75:25 100mM Acetic Acid:MeOH wash in this study. To correct for any residual recovery or matrix issues, the use of deuterated internal standards is strongly recommended.*

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255 • www.unitedchem.com • Email: methods@unitedchem.com