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Toxicological Confirmation of Intoxications Involving the Designer Benzodiazepine Clonazolam

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Disclaimer

- The Sciex TripleTOF[®] 5600+ instrumentation included in this presentation is part of a collaborative agreement with Sciex Corporation.

Benzodiazepines

- First benzodiazepine was chlordiazepoxide
 - Developed by Leo Sternbach in 1955
- Replaced barbiturates due to lack of respiratory depression
- Used for treatment of sleeping disorders, anxiety and seizures
- Current most prescribed benzodiazepines
 - Alprazolam, Diazepam, Clonazepam, Lorazepam

Misuse of Benzodiazepines

- Commonly used to ease withdrawal symptoms associated with other drugs of abuse
- Sexual assault
 - Flunitrazepam (Rohypnol)
- Contraindicated with alcohol, barbituates, opiates, tricyclic antidepressants

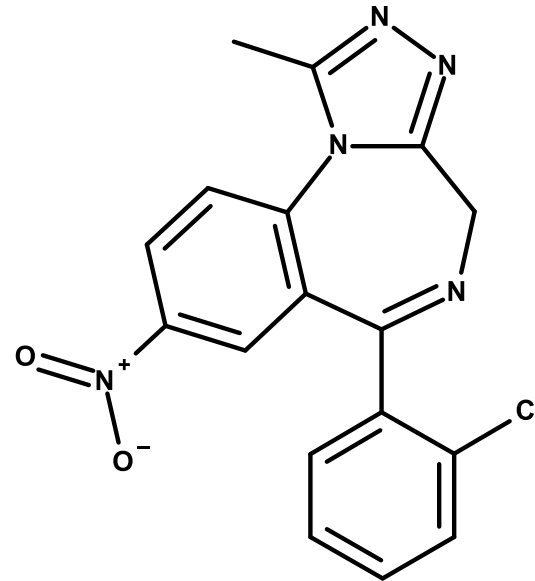


Emergence of “Designer Benzodiazepines”

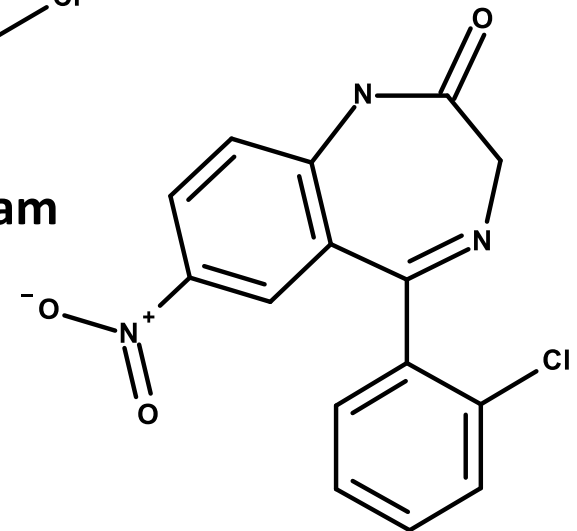
- 2007-EMCDDA reported phenazepam as a novel psychoactive substance
- 2012-Diclazepam, flubromazepam, and pyrazolam appeared in online markets
- 2014-EMCDDA recognizes these substances in a separate class, generally referred to as “designer benzodiazepines”
- 2015-Five additional compounds emerged
 - Clonazolam, deschloroetizolam, flubromazolam, nifoxipam and meclonazepam

Clonazolam

- First reported in 1971 by Hester et al.
 - The Upjohn Company
- Is a triazolo-analog of clonazepam
- Due to high potency, can cause sedation and amnesia at doses as low as 0.5 mg



Clonazolam



Clonazepam

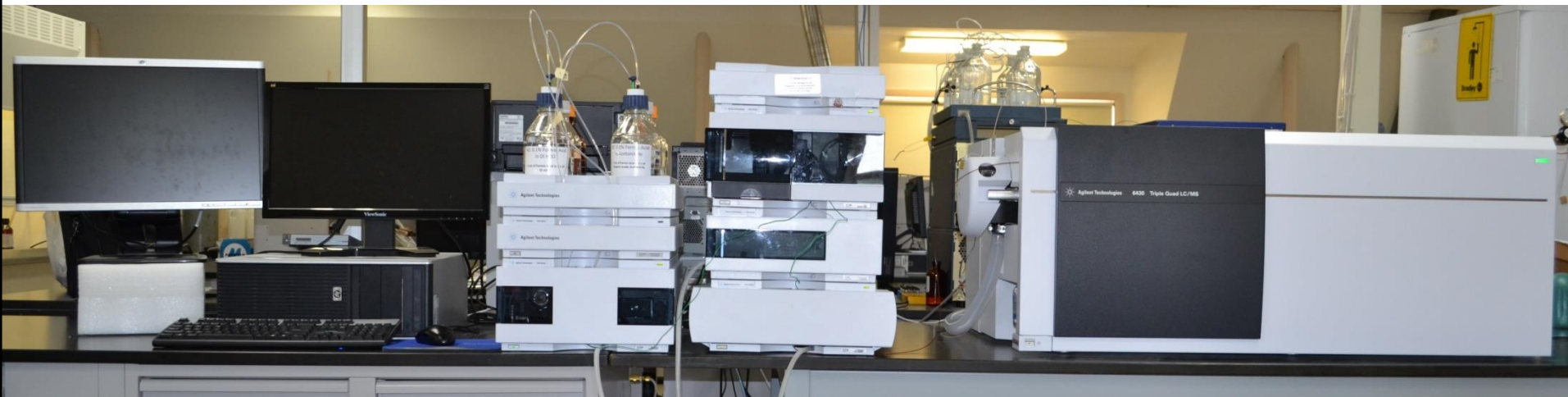
METHODS & INSTRUMENTATION

Extraction

1. Aliquot 0.2 mL urine sample
2. Add 100 uL internal standard (IS)
 - IS: 100 ng/mL (Clonazepam D4)
3. Vortex samples for 30 seconds
4. Add 0.2 mL of 0.1 M bicarbonate buffer (pH=9)
5. Add 2 mL of Methyl-tert-butyl ether
6. Cap, rotate and centrifuge samples at 3750 RPM for 5 minutes
7. Transfer supernatant using a freeze/pour acetone bath method
8. Dry down samples at 35°C for 35 minutes
9. Reconstitute samples in 200 uL 60A:40B mobile phase

Instrumentation

- Agilent 1100 Series Liquid Chromatograph coupled with a Agilent 6430 Tandem Mass Spectrometer (LC-MS/MS)



LC Parameters

- Agilent 1100 Series Conditions:

- Mobile phase A: 0.1% formic acid in water
- Mobile phase B: 0.1% formic acid in methanol
- Column: Restek UltraAqueous C18 (2.1x100mm, 3 μ m)
- Flow rate: 0.45 mL/min
- Column Temperature: 50°C
- Injection Volume: 10 μ L

Time (min)	%A	%B
Initial	60	40
3	5	95
6	5	95
6.5	60	40
9	60	40

Method Validation

Method Parameters	
Limit of Detection/Quantitation	5 ng/mL
Calibration Range	5-500 ng/mL
Carryover Limit	500 ng/mL
Commonly Encountered Interferences	None
Recovery	81%

Method Bias and Precision									
	Bias (%)			Within-Run (%CV)			Between Run (%CV)		
	LLOQ	Low	High	LLOQ	Low	High	LLOQ	Low	High
Clonazepam	12.6	4.4	4.9	13.5	6.1	6.6	3.6	6.1	6.2

Stability Data

Stability					
Matrix Stability					
		30 Day	7 Day	2 Day	1 Day
Low Control	Room Temperature	Stable	Stable	Stable	Stable
	Refrigerated	Stable	Stable	Stable	Stable
	Frozen	Stable	Stable	Stable	Stable
High Control	Room Temperature	Stable	Stable	Stable	Stable
	Refrigerated	Stable	Stable	Stable	Stable
	Frozen	Stable	Stable	Stable	Stable

CASE HISTORIES AND RESULTS

Case 1, Patient 1

A 20 year old male with a history of anxiety and depression was admitted to the emergency room after being found slumped over in his car. Upon arriving at the hospital he was drowsy, but became alert and informed medical personal he ingested three 0.7 mg “Pinzor” tablets.

- **Vital signs upon admission**
 - Initially stable and then became hypotensive and bradycardic
- **Results of hospital urine drug screen**
 - Benzodiazepines

Case 1, Patient 2

An 18 year old male with no prior medical history was admitted to the emergency room after being found slumped over in a car. He was alert upon arrival and admitted to taking two 0.7 mg “Pinzor” tablets prior to being awakened by emergency medical services.

- **Vital signs upon admission**
 - Tachycardia and Hypertension
- **Results of hospital urine drug screen**
 - Benzodiazepines, THC and Opiates

Clonazepam



- Received 10 yellow tablets
 - Front: Inscribed with a letter “Z” going through a letter “P”
 - Back: Score line, inscribed with a “.5” on the bottom

Pinzor Pellets

Clonazepam Pellets



PINZOR
Pure Clonazepam Pellets

www.shymolecule.com

Product Code: Pinzor Research Pellets

Availability: **In Stock, Ships within 24 hours**

\$90.00

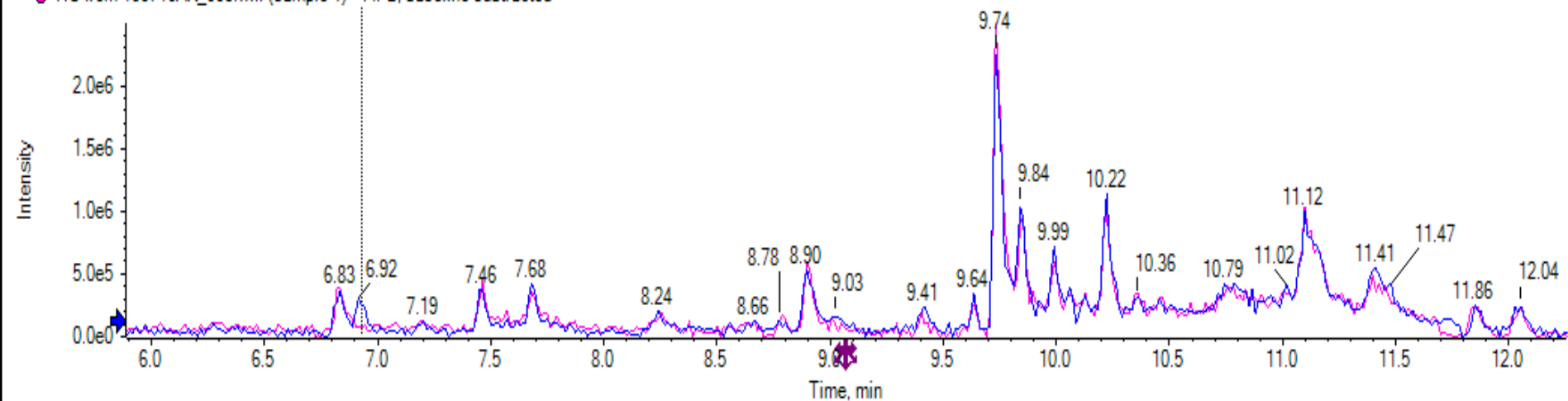
▼ Pinzor Research Pellets

90 Pellets

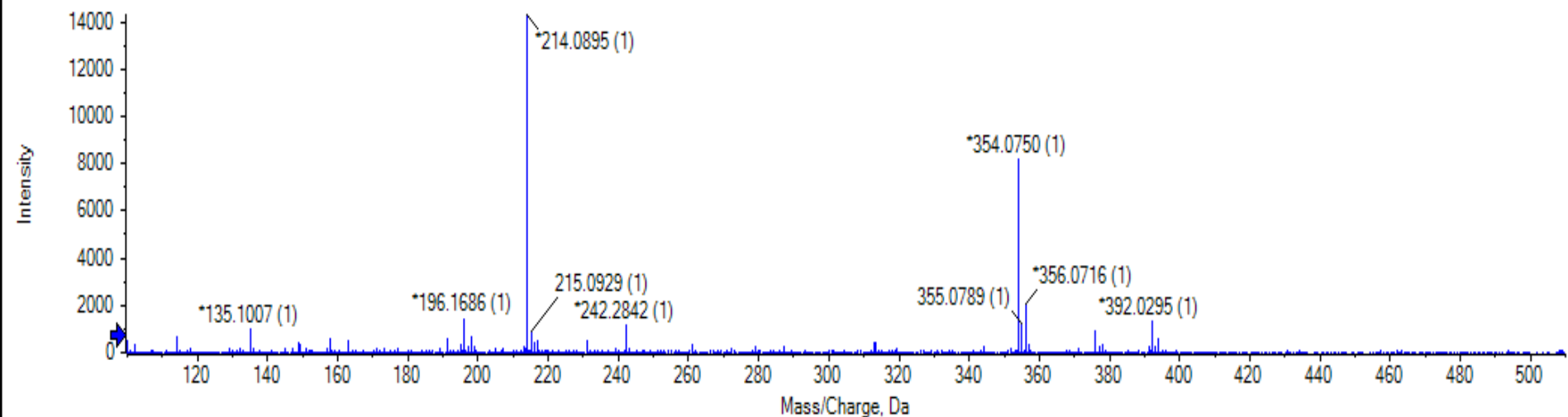


*This product is NOT evaluated by the FDA, is not meant to be consumed in any way, is not a drug, and **sold for research purposes only!**

● TIC from 100716AK_009.wiff (sample 1) - Tablet, baseline subtracted
● TIC from 100716AK_008.wiff (sample 1) - MPB, baseline subtracted



Spectrum from 100716AK_009.wiff (sample 1) - Tablet, Experiment 1, +TOF MS (100 - 510) from 6.930 min



Tablet Purity

	Tablet 1	Tablet 2	Tablet 3
Weight of Tablet (mg)	273.9	271.6	242.3
Quant Result (mcg/mL)	0.38	0.43	0.40
Purity	0.38%	0.44%	0.40%
Amount of Clonazolam in Tablet (mg)	1.0	1.2	1.0

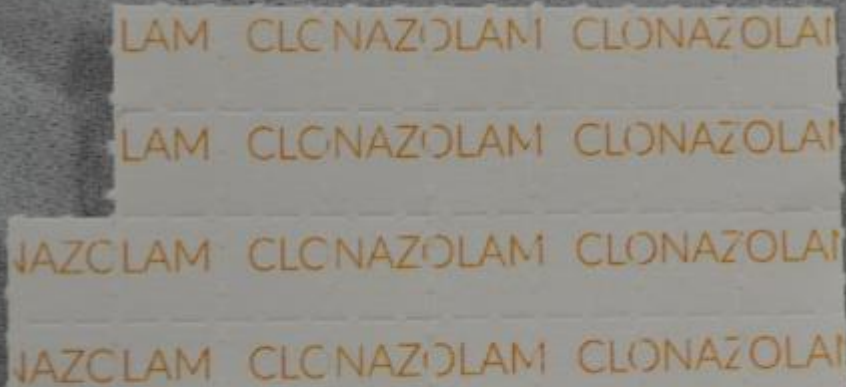
Patient 1 and 2 Results

	Patient 1	Patient 2
Vital Signs at Hospital	Bradycardic and Hypotension	Tachycardia and Hypertension
Hospital Screen	Benzodiazepines	Benzodiazepines, Opiates and THC
QTOF Screen	Cotinine, Clonazepam	Cotinine, Caffeine, Diphenhydramine, Morphine, Clonazepam
Tablets Taken	3	2
Urine Concentration (ng/mL)	60	34

Case 2

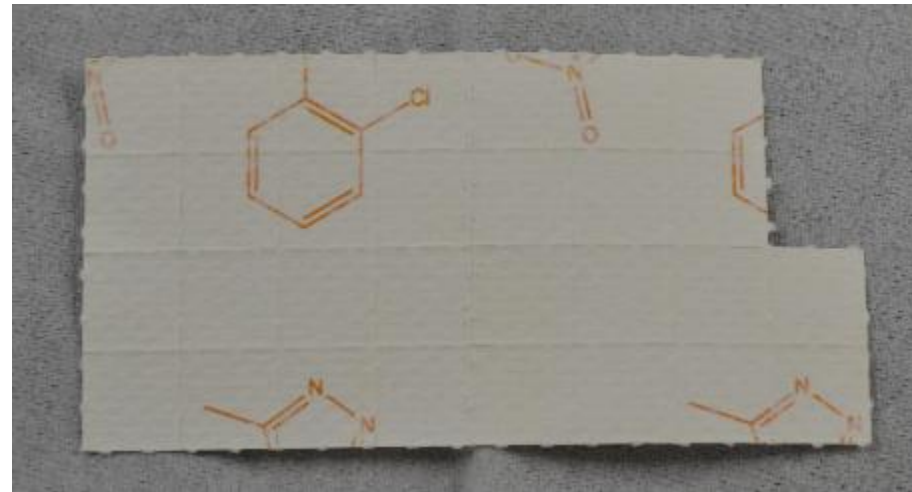
1. An 19 year old male was presented to the emergency department with urinary retention following several hours of nausea, vomiting, headache and tremulousness
2. He was displaying slurred speech and somnolence
3. 24 hours into his hospital stay he discloses taking several capsules of powdered tianeptine and since admission, had been continuing to self-administer blotter tabs labeled “Clonazepam”

Blotter Tab



LAM CLONAZOLAM CLONAZOLAM
LAM CLONAZOLAM CLONAZOLAM
CLONAZOLAM CLONAZOLAM CLONAZOLAM
CLONAZOLAM CLONAZOLAM CLONAZOLAM

Front



Back

- Front labeled with repeating “CLONAZOLAM”
- Back is labeled with partial Clonazepam structure
- Each individual tab is 1 cm x 1 cm (30 present)

<http://nootropicsusa.com/>
50 count CLONAZOLAM .5mg blotter

★★★★★ (13 customer reviews)

~~\$65.00~~ **\$55.00**

Clonazepam (or Clonitrazepam)

These 0.5mg Clonazepam labeled blotter.

Clonazepam IUPAC name: 6-(2-chlorophenyl)-1-methyl-8-nitro-4H-s-triazolo(4,3-a)-(1,4)-benzodiazepine

Clonazepam Mol. mass: 353.07 g/mol

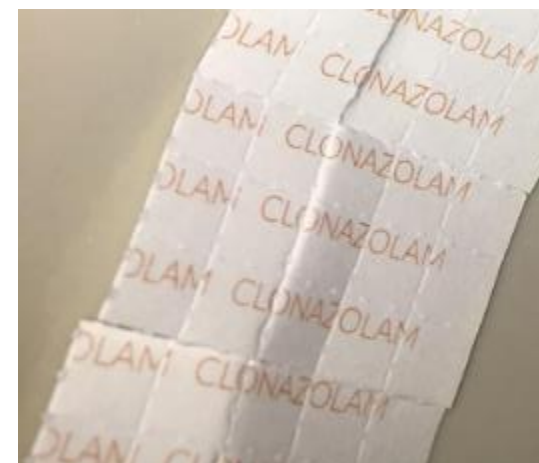
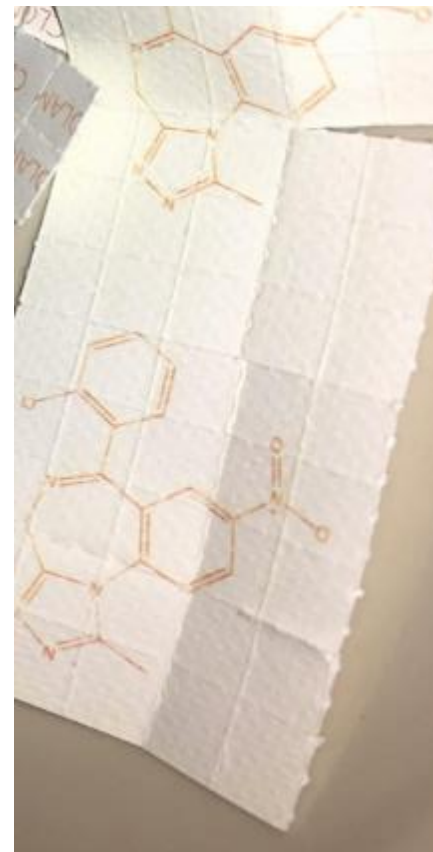
Disclaimer: For research purposes only. This product is NOT for human consumption.

Only 4 left in stock

1

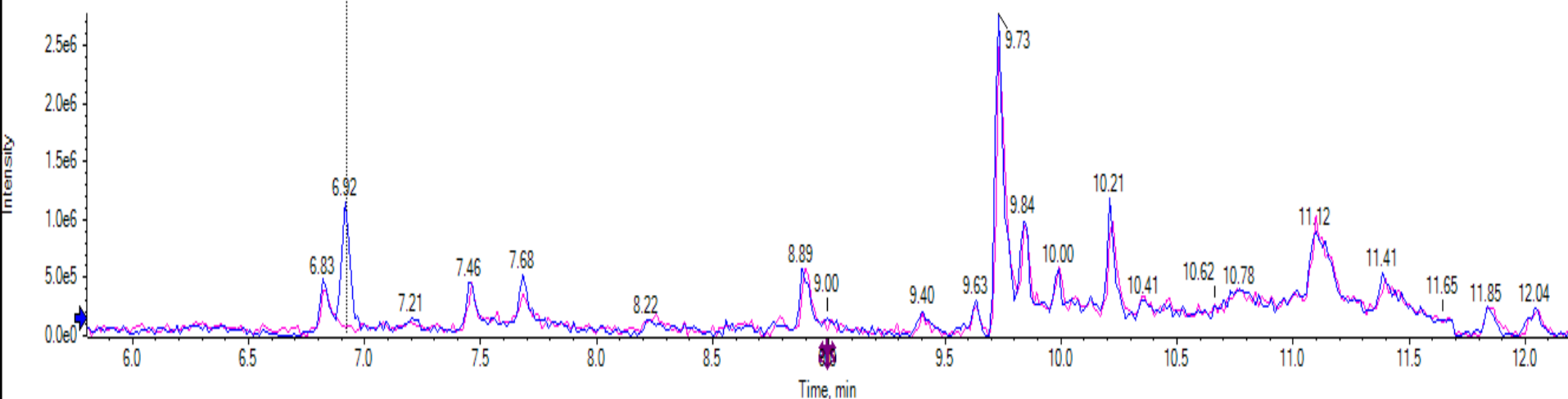
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SKU: clon50 Category: [Nootropic Supplement](#)

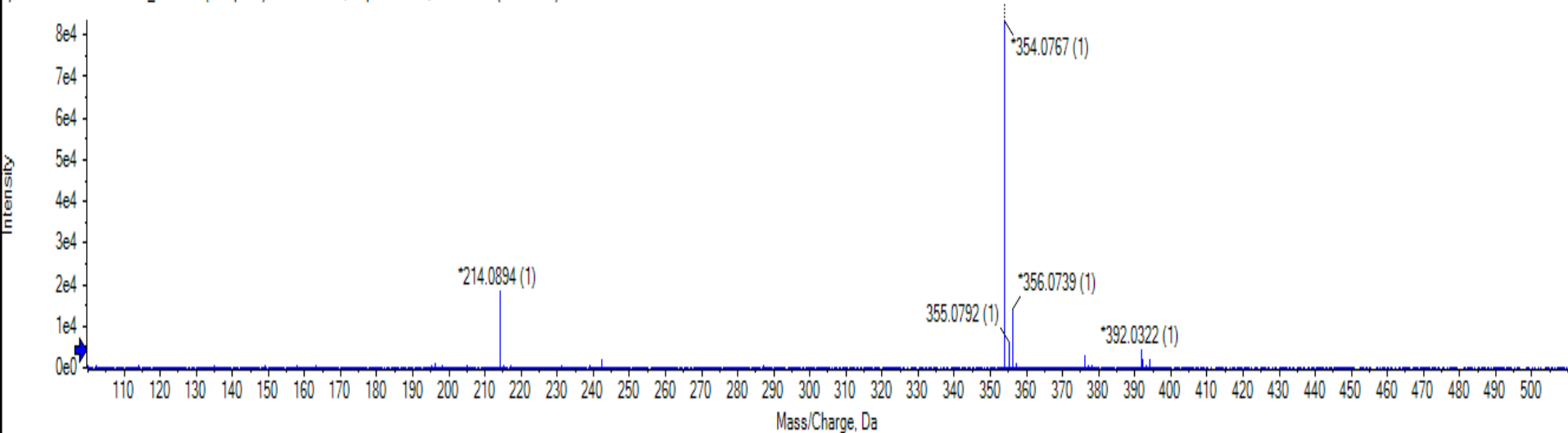


● TIC from 100716AK_010.wiff (sample 1) - Blotter Tab, baseline subtracted

● TIC from 100716AK_008.wiff (sample 1) - MPB, baseline subtracted



Spectrum from 100716AK_010.wiff (sample 1) - Blotter Tab, Experiment 1, +TOF MS (100 - 510) from 6.916 min



Blotter Tab

	Tab 1	Tab 2	Tab 3
Weight of Blotter Tab (mg)	16.3	16.3	16.3
Quant Result (mcg/mL)	0.109	0.076	0.085
Amount of Clonazepam on Blotter Tab (mg)	0.54	0.38	0.42
Average	0.44 mg		

Discussion

- Hospital drug screen positive for benzodiazepines
 - Cross reactivity
 - In absence of traditional benzodiazepines check for designers
- Reported urine clonazepam concentrations produced a ranged from 7.3-23 ng/mL (n=8)
 - Bergstrand et al. 2016
 - Patient 1: 60 ng/mL
 - Patient 2: 34 ng/mL

Acknowledgments

- SOFT
- Emily Salib
 - Department of Medicine: Highland Hospital
Rochester, NY

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Questions?

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