



DEVELOPMENT OF A HIGH RESOLUTION MASS SPECTROMETRY METHOD FOR DESIGNER DRUGS

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Disclosures



- Western Slope Laboratory, LLC-salary
- Thermo Fisher Scientific-research support
- Phenomenex-research support
- U-Toledo College of Pharmacy and Pharmaceutical Sciences-board member

Outline



- Why we investigated this problem
- What compounds were involved
- The methodology
- Results and Conclusions

Designer drugs

Why we investigated this problem

Bath salts could be more addictive than meth, study finds

Published July 11, 2013 / FoxNews.com

Military Members Getting High on Bath Salts?



Meet two Marines who are fighting their own battles away from the frontlines.

07/09/2013

Bath Salts and Other Synthetic Drugs Making "Millions" for Terrorists Drug in bath salts caused teen's overdose death, Dr. G says

June 27, 2013

By JACK CLOHERTY and SALLY ZHANG via [WORLD NEWS](#)

17-year-old Krystopher Sansone died of drug overdose in February

Published On: Apr 09 2013 05:34:01 PM EDT | Updated On: Apr 05 2013 07:22:11 PM EDT

'Spice' Drug, AKA 'K2' Or 'Potpourri,' Is Legal & Popular Among Teens In Southern California (VIDEO)

Posted: 05/10/2013 3:46 pm EDT | Updated: 05/12/2013 1:56 pm EDT

Bath salts blamed for Vt man's death

Posted: Aug 01, 2012 5:50 AM EDT

Updated: Aug 03, 2012 5:00 PM EDT

By Molly Smith - [bio](#) | [email](#)

Taft mother blames son's suicide on drug spice

By Carol Ferguson, Eyewitness News | Published: Jul 8, 2013 at 9:58 PM PDT (2013-07-9T4:58:38Z) | Last Updated: Jul 9, 2013 at 12:24 PM PDT (2013-07-9T19:24:11Z)

Spice/K2 at root of recent Oakland County deaths

Published: Tuesday, May 29, 2012

By CAROL HOPKINS
carol.hopkins@oakpress.com Twitter: [@waterfordreport](#)

'Spice' drug linked to sudden kidney failure in area teenagers

By STEVEN DuBOIS, Associated Press

Saturday, October 6, 2012

Legal designer drugs like bath salts proliferating, confounding authorities: U.N. report

Synthetic or plant-based drugs classified as new psychoactive substances (NPS) include bath salts, spice and herbal incense. They are legal, and many believe them to be safe and recreational, but they pose serious health risks, United Nations officials warned.

AFP RELAXNEWS

THURSDAY, JUNE 27, 2013, 11:06 AM

The War against Designer Drugs



- Headlines are riddles with stories about their dangers and abuse
- Emergency departments and law enforcement are prepared
- Parents and other family stakeholders are afraid that this evil drug will take over their children
- Taken together, you have a society that is primed to take on the designer drug problem

Is the science ready?



- Most laboratories rely on a screen-confirm mechanism for testing
- Designer drugs change so frequently and do not cross react well with the generation I designer drug immunoassays
 - e.g. JWH-018 vs AKB48
- Requires “confirmation” testing each time or MS-based screening which is expensive

The labels



- ❑ Synthetic cannabinoids
- ❑ Synthetic cathinones
- ❑ Piperazines
- ❑ Phenethylamines
- ❑ Tryptamines
- ❑ And derivatives of the aforementioned

Just when you think you've found
it....

The culprits

Moving Targets...

- As legislation and enforcement change, so does the compounds
 - ▣ We are on 4th generation with “spice/k2” and “bath salts”
 - ▣ Getting certified reference materials and reference standards is difficult
 - ▣ Validating methodologies is unending with the addition or changing of compounds



Solution? MS Screening



- With MS Screening with a HR-MS instrument both targeted and untargeted analysis can be accomplished
- Includes targeted analysis of 63 compounds as well as allow for the presumptive positive testing for new, emerging compounds prior to reference materials being available

Targeted Compounds

- Amphetamine, methamphetamine, phentermine, MDMA, MDA, and MDEA
- Alprazolam, α -hydroxyalprazolam, diazepam, flunitrazepam, nordiazepam, oxazepam, temazepam, and 7-aminoclonazepam
- PCP, TFMPP, mCPP, and BZP
- Cocaine and BZE
- methylone, α -PVP, pentedrone, methylhexanamine, 4-MEC, ethylone, pentylone, butylone, MDPV, and mephedrone
- MAM2201, RCS-8, RCS-4, JWH398, JWH018, AM2201, HU210, JWH073, JWH200, AM694, AKB48, JZL184, JZL195, STS135, UR144, UR144 N-pentyl metabolite, URB447, URB597, URB602, URB754, URB937, XLR11, JWH018 pentanoic acid metabolite, JWH073 butanoic acid metabolite, AM2201 N-hydroxypentyl metabolite, JWH018 Ω and Ω -1 pentyl metabolites, JWH019 6-hydroxyhexyl metabolite, JWH022, JWH081 N-hydroxypentyl metabolite, JWH122 N-hydroxypentyl metabolite, and JWH210 N-carboxyl metabolite



methodology

Turboflow-Exactive HRMS

Sample Prep



- 50 total samples were submitted for “bath salt” testing: 48 urine and 2 saliva
- Urine prep
 - ▣ Enzymatic hydrolysis
 - ▣ Dilute & Shoot
 - ▣ Online sample extraction
- Saliva prep
 - ▣ LLE
 - ▣ Online sample extraction

Technology, In brief



- MCX turboflow column
- Biphenyl analytical column
- Methanol, Water Mobile Phases with Ammonium Formate and Ammonium Acetate
- 7 min method

Scope of the Method Validation

Parameter	Specification
Analytes	Parent and known metabolites (63)
Concentration range	100pg/mL – 1000ng/mL
Calibration curve	Linear, preferred
Suppression/Enhancement	< $\pm 25\%$
Imprecision	< $\pm 20\%$, < $\pm 10\%$ preferred
Inaccuracy (low=250pg/mL; med=50ng/mL; high=650ng/mL)	< $\pm 20\%$, < $\pm 10\%$ preferred
LLOD	At least 100pg/mL
LLOQ	At LLOD
Multiplexing-requires the validation of each pump	
Comparative results to the current 118 compound LC/MS/MS methodology (for confirmed positives)	

Turbulent Flow Online Sample Clean-up

The parameters of the clean-up and LC methodology for the designer drug method

Step Control
Variables
Method Info
Pressure Profile

Step Number: **10**

Length: 30 s

Start: 05:27

Comment: Empty

FlowRate	%A	0.0
1.200	%B	100.0
	%C	0.0
	%D	0.0
Step		
FlowRate	%A	0.0
0.800	%B	5.0
	%C	95.0
	%D	0.0
Step		

Total Method Duration 06:27

Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B	%C	%D
00:00	40	1.30	Step	100.0	-	-	-	=====	out	0.80	Step	90.0	10.0	-	-
00:40	2	0.70	Step	-	-	100.0	-	=====	out	0.70	Step	90.0	10.0	-	-
00:42	60	0.50	Step	-	100.0	-	-	T	in	0.70	Step	98.0	2.0	-	-
01:42	45	1.10	Step	-	-	-	100.0	=====	in	0.70	Ramp	80.0	20.0	-	-
02:27	45	1.10	Step	-	-	-	100.0	=====	in	0.70	Ramp	2.0	98.0	-	-
03:12	30	1.10	Step	-	-	-	100.0	=====	in	0.70	Step	2.0	98.0	-	-
03:42	45	1.10	Step	-	-	100.0	-	=====	in	0.70	Ramp	98.0	2.0	-	-
04:27	30	1.10	Step	-	100.0	-	-	=====	in	0.70	Step	98.0	2.0	-	-
04:57	30	1.10	Step	40.0	10.0	-	50.0	=====	in	0.70	Step	-	-	-	100.0
05:27	30	1.20	Step	-	100.0	-	-	=====	in	0.80	Step	-	5.0	95.0	-



results

What we found

Summary of Results



- All 50 samples were positive for at least one compound
- 49/50 samples were positive for cotinine
- 24/50 samples were positive for acetaminophen
- 17/50 samples were positive for opiates
- 15/50 samples were positive for benzodiazepines
- 13/50 samples were positive for amphetamine or methamphetamine (9 were positive for both)

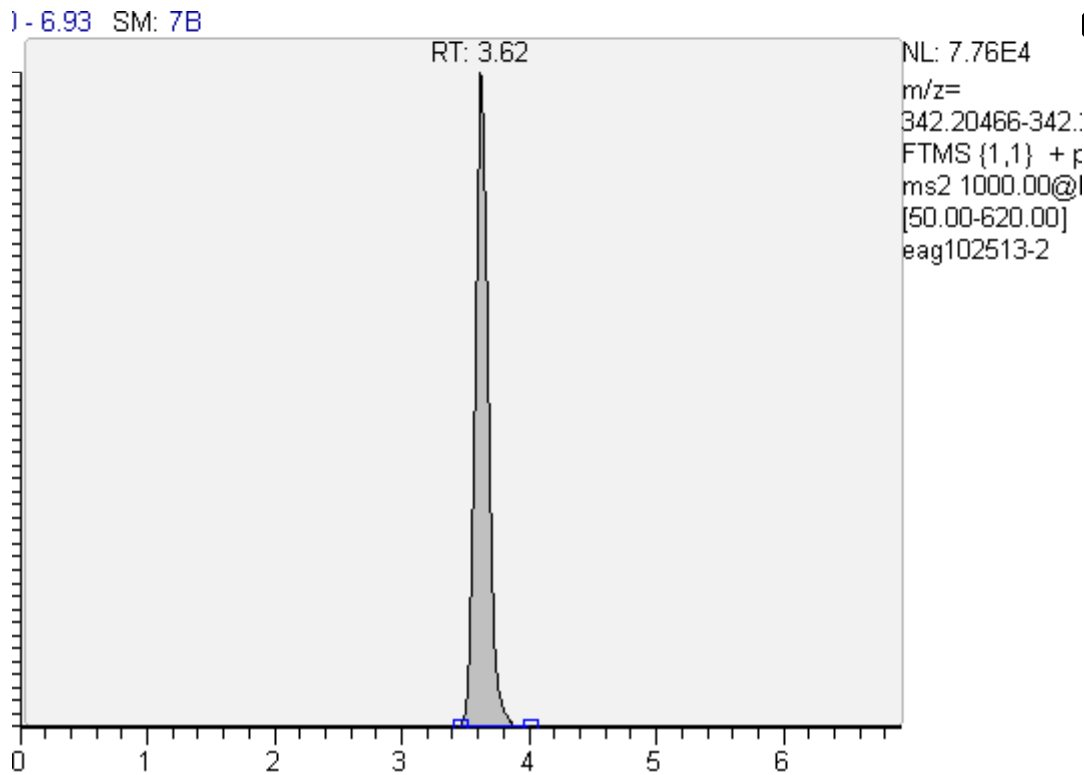
Summary of Results, cont.

- Methadone, Gabapentin, Pregabalin, Buprenorphine, Zolpidem, Duloxetine, Fluoxetine, Paroxetine, Amitriptyline, Citalopram, Cyclobenzaprine, Ritalinic Acid, Venlafaxine, Tramadol, Tapentadol, Meprobamate, and Naproxen were also positive in some of the samples
- There were also positives for UR-144 (2 samples), DMAA (4 samples), α -PVP (5 samples), and ethylone (1 sample)

Comparison of positives

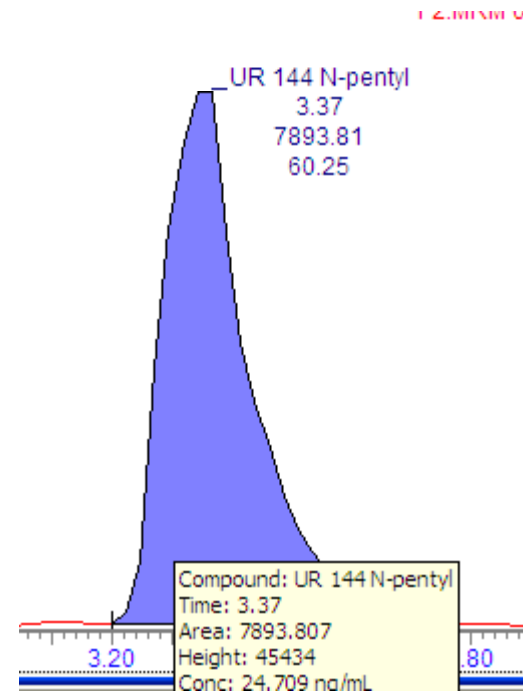
HR-MS Methodology

□ UR-144 metabolite



LC-MS-MS

□ UR-144 N-pentanoic acid

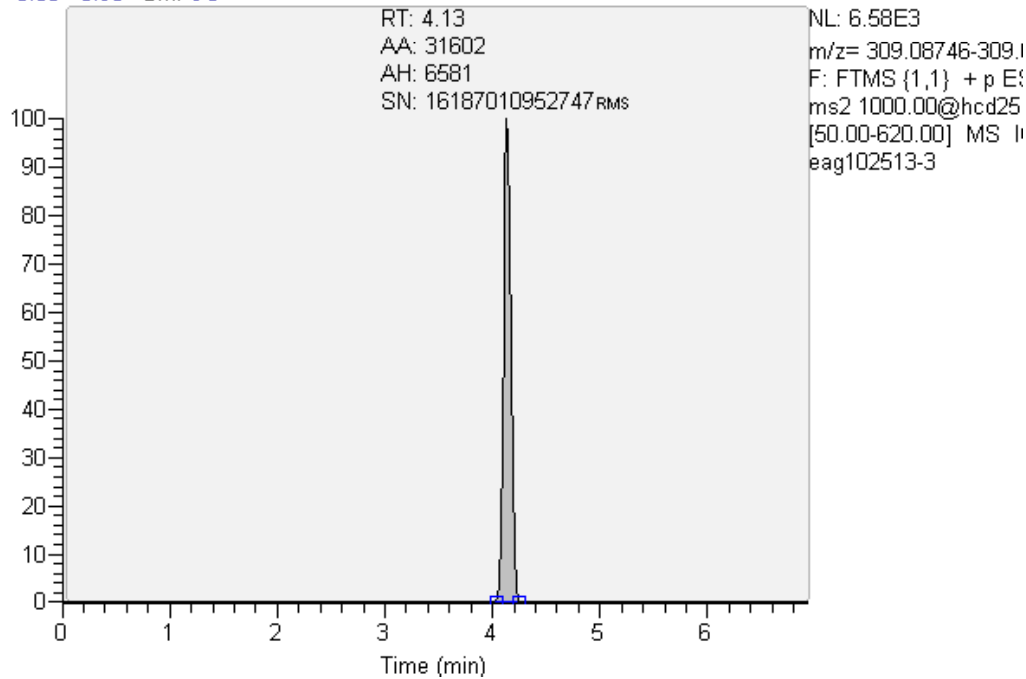


Comparison of positives

HR-MS

□ Alprazolam

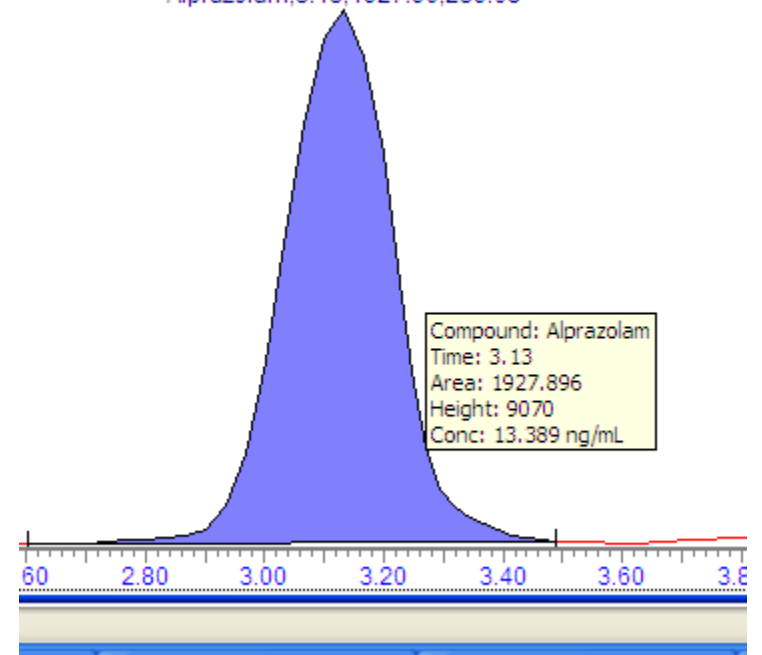
0.00 - 6.93 SM: 9G



LC-MS-MS

□ Alprazolam

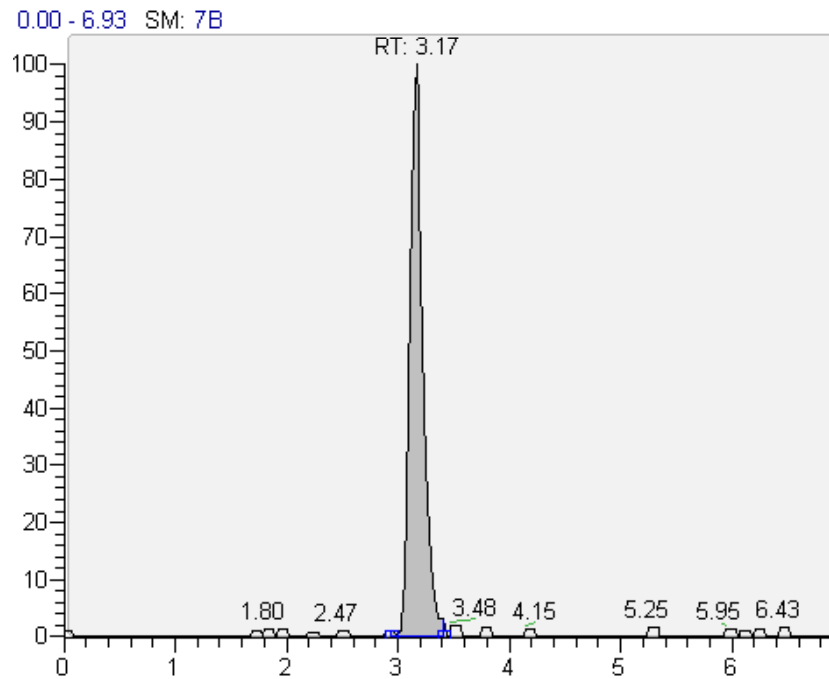
Alprazolam;3.13;1927.90;239.68



Comparison of positives

HR-MS

□ Ethylone



NL: 1.79E4

m/z=

222.11135-222.11357

FTMS (1,1) +p ESI I

ms2 1000.00@hcd25

[50.00-620.00] MS

eag102513-2

LC-MS-MS

□ Ethylone

INE;3.53;1173736.38;4692827

Conclusions

- ❑ Confirmations for “bath salts” seems to contain many compounds while only 20% contained a commonly characterized “bath salt” with α -PVP and DMAA being the most common
- ❑ Opiates and benzodiazepines were the most common non-OTC compound
- ❑ Smoking is common amongst persons submitted for “bath salt” testing. Only one person did not smoke and most had concentration ranges in the non-passive smoking range (Max 12619ng/mL)
- ❑ Unless packaging is found to suggest a specific class, a designer drug panel might be the best testing option.
- ❑ Further directions include adding more targeted compounds once certified reference materials are available. e.g. NBOMe

Thanks



- Co-Authors, especially Kevin Ebert
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Questions?

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