WESTERN SLOPE LABORATORY

Development of an LC/MS/MS method for 30 synthetic cannabinoids and metabolites

Erica A. Guice, Matt Pentis, Brad Holden, and Mounira Ozoor

Western Slope Laboratory, LLC

A professional society dedicated to the application of science and law

American Academy of Forensic Sciences

Disclosures

- All were employed (or interns) at Western Slope Laboratory during the course of the research
- Thermo Fisher Scientific provided the analytical instrumentation
  - Exactive MS with a TLX-2

The Problem

- Testing for synthetic cannabinoids is mostly reactive:
  - Limited analytical methods available
  - Certified standards can be difficult to obtain
  - Laboratories who use screen-confirm methodology being limited by available screens
  - Ban of the compounds leading to ever-changing target list

Great laws lead to reactive labs

With great legislature like Michigan Law (Senate Bill 1082; MCL 333.7212): “(e) Synthetic cannabinoids. As used in this subdivision, "synthetic cannabinoids" includes any material, compound, mixture, or preparation that is not otherwise listed as a controlled substance in this schedule or in schedules II through V, is not approved by the federal food and drug administration as a drug, and contains any quantity of the following substances, their salts, isomers (whether optical, positional, or geometric), homologues (analogs), and salts of isomers and homologues (analogs), unless specifically excepted, whenever the existence of these salts, isomers, homologues (analogs), and salts of isomers and homologues (analogs) is possible within the specific chemical designation”

The ten clauses...

- Any compound containing a 3-(1-naphthoyl)indole structure, also known as napthoylindoles
- Any compound containing a 1h-indol-3-yl-(1-naphthyl)methane structure, also known as napthylmethylindoles
- Any compound containing a 3-(1-naphthoyl)pyrrolestructure, also known as naphtoylpyrroles
- Any compound containing a naphthylideneindene structure
- Any compound containing a 3-phenylacetylindole structure, also known as phenacetylindoles
- Any compound containing a 2-(3- hydroxycyclohexyl)phenol structure, also known as cyclohexylphenols
- Any compound containing a 3-(benzoyl)indole structure, also known as benzoylindoles
- Any compound containing a 11-hydroxy-δ8-tetrahydrocannabinol structure, also known as dibenzopyrans
- Any compound containing a 3-(l-adamantoyl)indole structure, also known as adamantoylindoles
- Any other synthetic chemical compound that is a cannabinoid receptor agonist and mimics the pharmacological effect of naturally occurring cannabinoids that is not listed in schedules ii through v and is not approved by the federal food and drug administration as a drug

The Solution?

- Develop a method that can do targeted as well as untargeted detection
  - Requires the use of an instrument with exact mass capabilities to do full scan
  - Requires an instrument that can also provided fragmentation information
  - Have standards for many compounds

Scope of the Method Validation

Parameter	Specification
Analytes	Parent and known metabolites (29)
Concentration range	100pg/mL – 1000ng/mL
Calibration curve	Linear, preferred
Suppression/Enhancement	< ±25%
Imprecision	< ±20%, <±10% preferred
Inaccuracy (low=250pg/mL; med=50ng/mL; high=650ng/mL)	< ±20%, <±10% preferred
LLOD	At least 100pg/mL
LLOQ	At LLOD
Multiplexing-requires the validation of each pump	
Comparative results to the current 8 compound LC/MS/MS methodology (for confirmed positives)	

Analytes

JWH-018	JWH-073
HU-210	AM-2201
AM-694	JWH-210
JWH-200	MAM-2201
JWH-019	JWH-398
JWH-122	JWH-081
JWH-250	STS-135
XLR-11	

Method Details

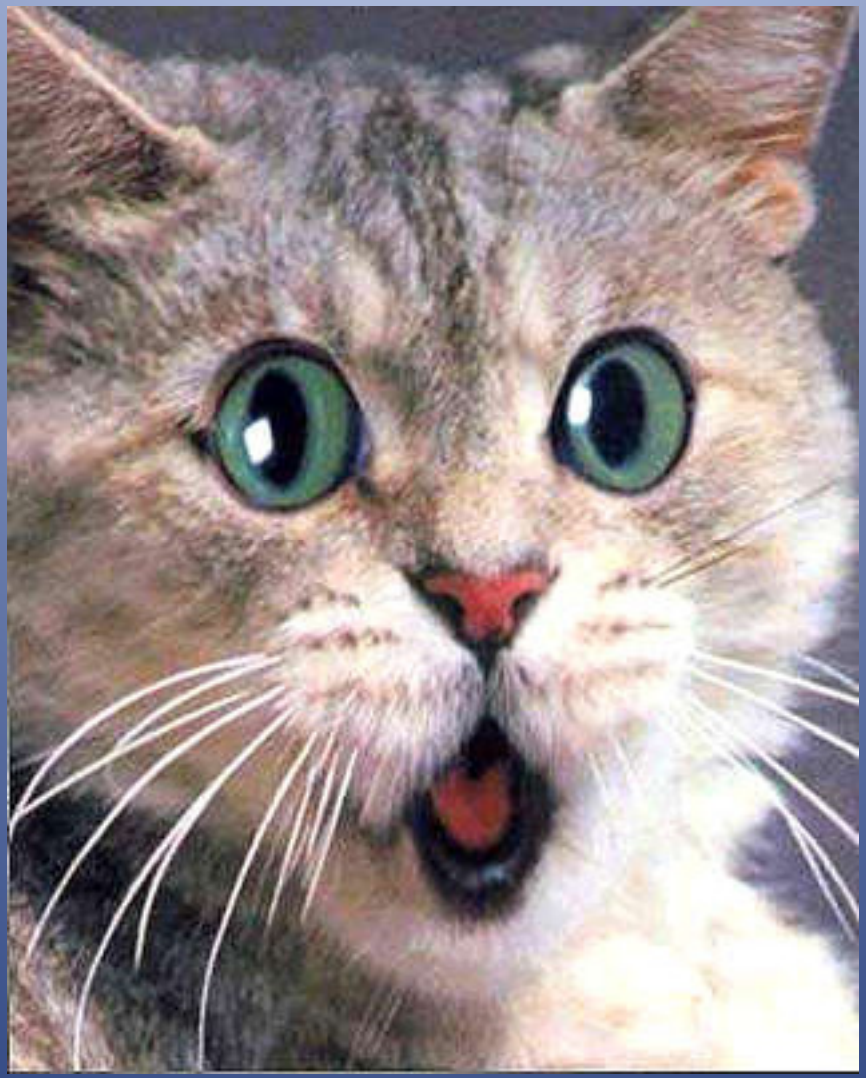
- Uses turbulent flow online sample extraction
  - Cyclone P column
- Uses liquid chromatography, full scan, CID
  - It is a 12 minute method; gradient 5-95%B in 5mins (mobile phases are water and methanol with ammonium formate and ammonium acetate modifiers; Biphenyl Restek analytical column
  - Collision energy of 40eV (100-600 m/z; 50-500m/z)

Results, stage one

- Able to produce a method within specification limits previously mentioned for 18 compounds
- Patient samples which tested positive by the previous 8 compound method had comparable results

Method interruptus…

- Or the problem continues….
  - Samples were being sent to laboratory screening positive by immunoassay, but negative by LC/MS/MS
  - Samples were tested on commercially available kit by kit manufacturer which provided cross-reactivity to several more compounds but unknown cross-reactivity to "3rd generation" compounds were also strongly positive even for samples both LC/MS/MS and other IMA were negative



What's a Mass Spectrometrist to Do?

- First, I read the package insert and did a gap analysis
- Second, I investigated information from other reference laboratories, government agencies, and research institutions
- Lastly, I consulted the experts….

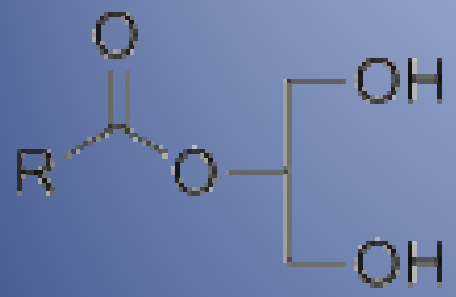
When all else fails, erowid

- Persons who seek to use synthetic cannabinoids are having to be more resourceful:
- “Being an amateur pharmacologist, I consulted the literature. My current theory about these effects comes from a recent paper from Nichols, in which he proposes that traditional psychedelic drugs acting on the 5HT-2A receptors cause a release of 2-AG, an endocannabinoid. If Prozac causes activation of the 5HT-2A receptor without triggering the release of 2-AG, then it's possible that smoking pot would activate the same receptors 2-AG binds to, namely CB1/2 (I think), causing a psychedelic experience to take place. I'm hoping this is the case, as I've heard anecdotal reports that tripping from pot is a sign that one may be latently schizophrenic? So I'm hoping for the best, and worrying that this may be a warning of nasty mental issues to come. “

Amateur Pharmacology….

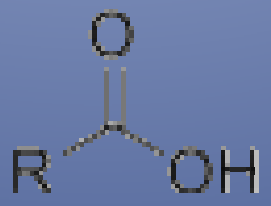
- Further quotes from Parker et al about the benefits of marijuana when used in conjunction with a FAAH regulator
  - "desired effects are obtained…"
  - "…effects last longer"
- Never mind that the paper is researching using this combination to reduce nausea and vomiting in cancer patients


HUMBLE

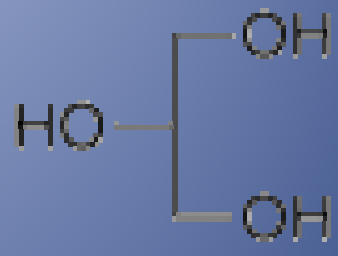


MAG

MGLL



FFA



Glycerol

Fatty Acid Amid Hydrolase and Monoacylglycerol Lipase

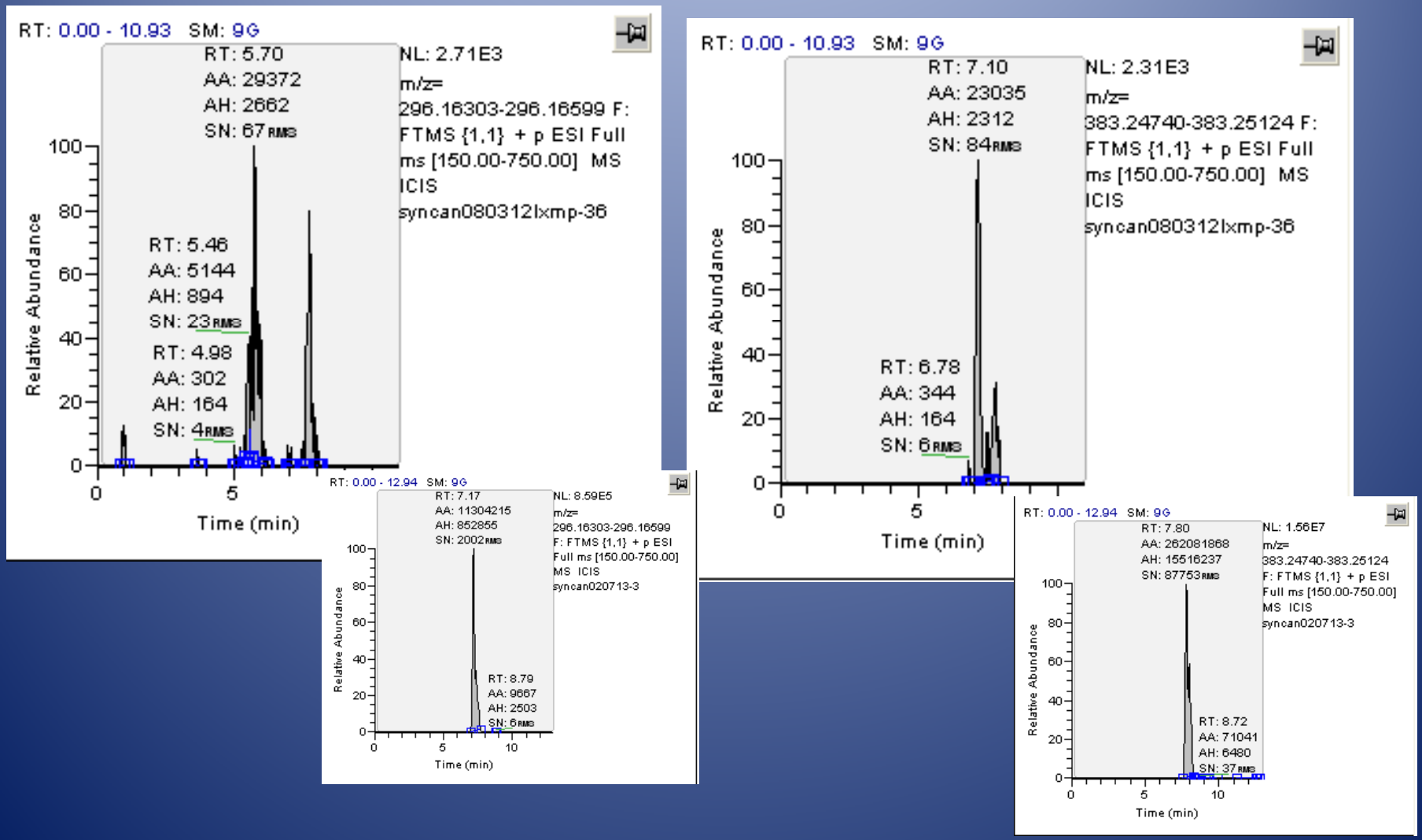
- FAAH and MAGL regulators attenuate the effects of 2-arachidonolyglycerol (2-AG), which is the endogenous CB1 receptor agonist
- So, these compounds can make the desirable effects feel greater and last longer

$\therefore$ Taking a synthetic cannabinoid with a FAAH or MAGL regulator can be dangerous, but deemed desirable to the user

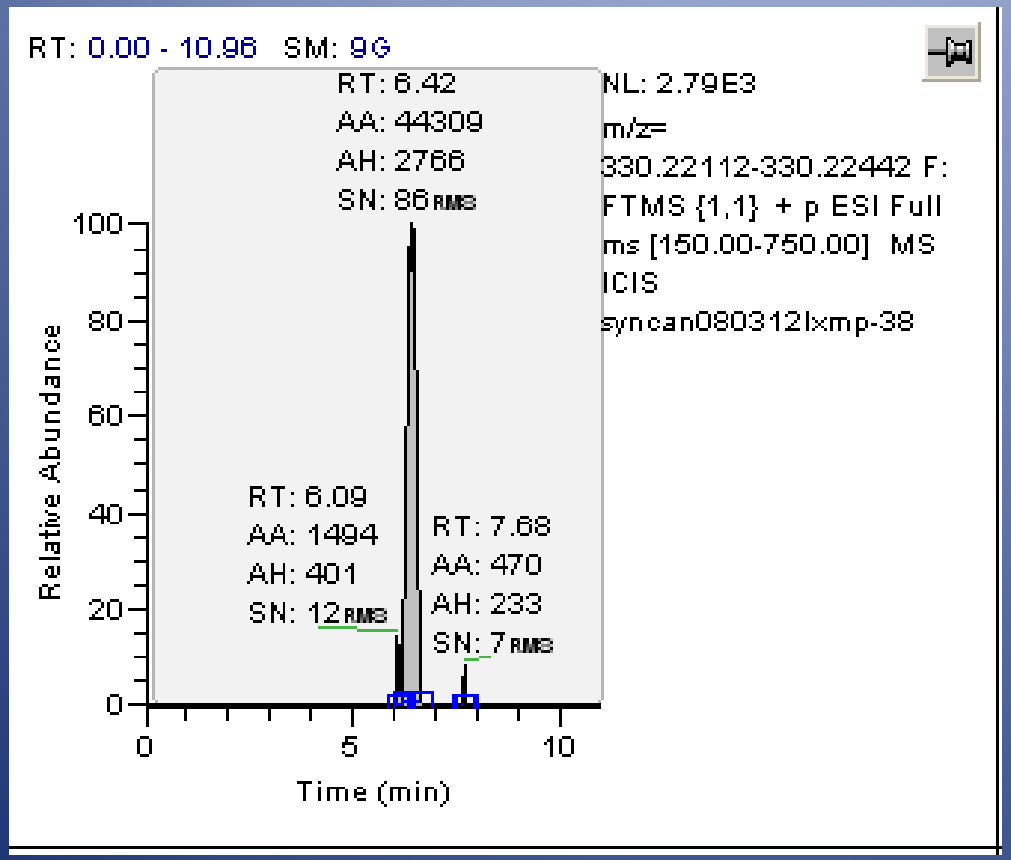
So we added…

- 5 FAAH regulators and 2 MAGL regulators to the method…
- And we got positives…

Both a drug and modulator



XLR11 in patient sample



Future Plans

- Collect samples from known spice users
- Continue to test confirms with expanded panel
- Continue to learn from the experts

Acknowledgements

- Thermo Fisher
  - Jeff Zonderman
  - Susan DiPietro
  - Jeff Fischer
- Cayman Chemical
  - Greg Endres
  - Dan Tew
  - Paul Kennedy
  - Andrew Pavlik, Toni Myles

Thank you for your attention
and the Scientific Committee for
accepting our work

References

- www.erowid.com
- Wiki MAGL
- Westlawnext.com (MCL 333.7212)
- Google image “surprised”
- Google image “humble pie”
- Parrish JC, **Nichols** DE. J Neurochem. 2006 Nov;99(4):1164-75.
- Parker LA, Rock EM, Limebeer CL.Br J Pharmacol. 2011 Aug;163(7):1411-22.

Questions