Innovation Applied

Analysis of Synthetic Cannabinoids (Spice) in Urine Utilizing HRAM

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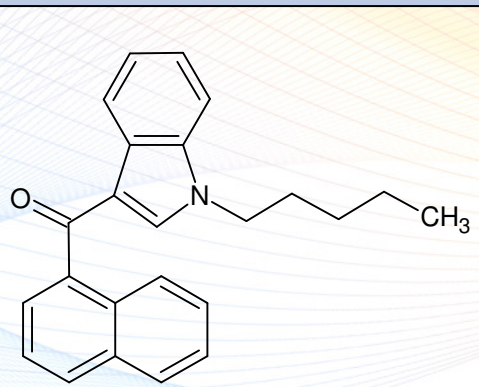
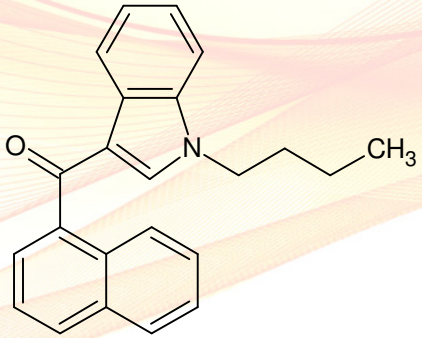
History of Synthetic Cannabnoids

- Originally made for research in 1980s.
- Around 2008, they have found their way into the designer drug markets as a “legal high.”
- Sprayed onto herbal products as “incense” and go by the names Spice and K2.
- DEA has recently regulated five as Schedule I compounds.
- JWH-018, JWH-073, JWH-200, CP-47,497, and CP-47,497-C8 homolog (cannabicyclohexanol)

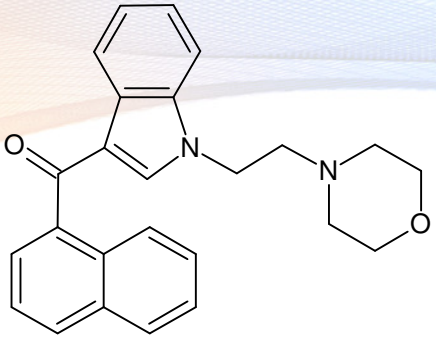
Newly Regulated Synthetic Cannabinoids



JWH-018

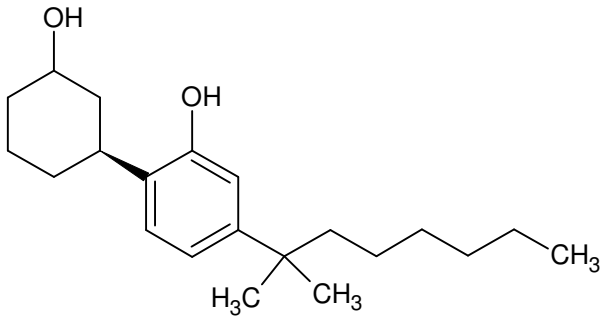


JWH-073

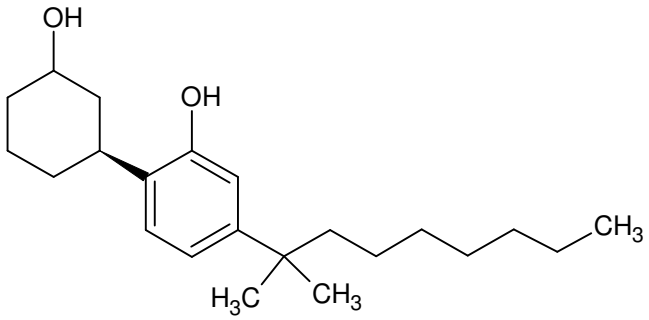


JWH-200

Sobolevsky et al./Forensic Science International 200 (2010) 141-147



CP-47,497



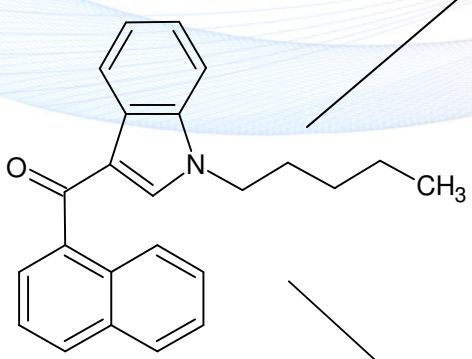
CP-47,497 C8 homolog

Analytical Approaches – What to look for?

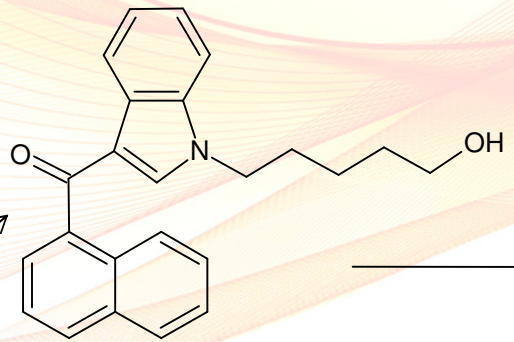
- Human liver microsomal incubations…
- …used as a starting point to screen human urine.
- Metabolism screening software

- What has been found (so far)…

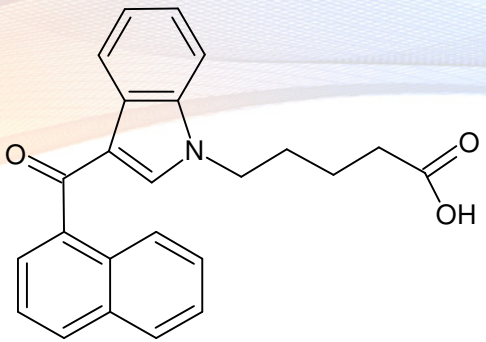
Structures and Metabolism JWH-018



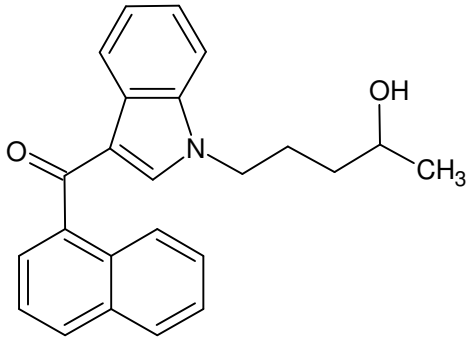
JWH-018
m/z+ = 342.1852



JWH-018-N-(5-hydroxypentyl)
m/z+ = 358.1802



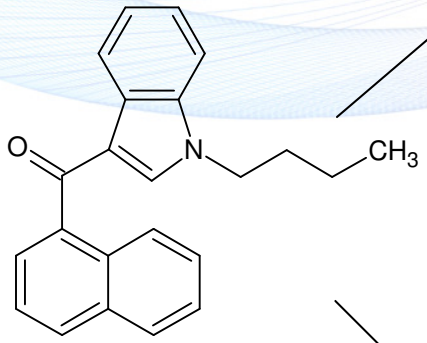
JWH-018-N-pentanoic acid
m/z+ = 372.1594



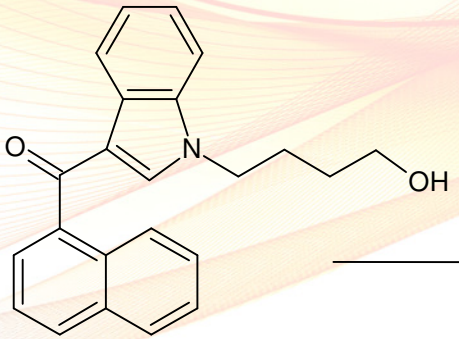
JWH-018-N-(4-hydroxypentyl)
m/z+ = 358.1802

- No parent seen in urine.
- Corresponding glucuronides are also seen in urine.

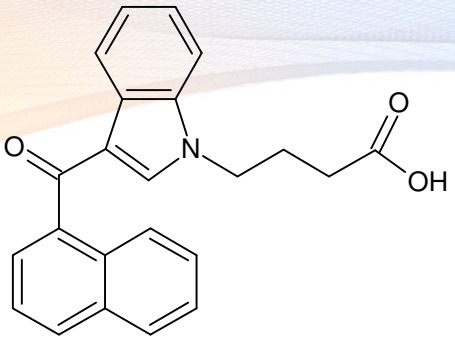
Structures and Metabolism JWH-073



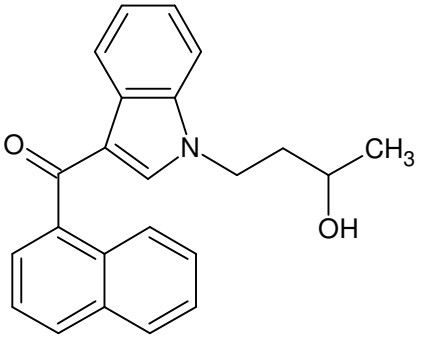
JWH-073
m/z+ = 328.1700



JWH-073-N-(4-hydroxybutyl)
m/z+ = 344.1645



JWH-073-N-butanoic acid
m/z+ = 358.1438



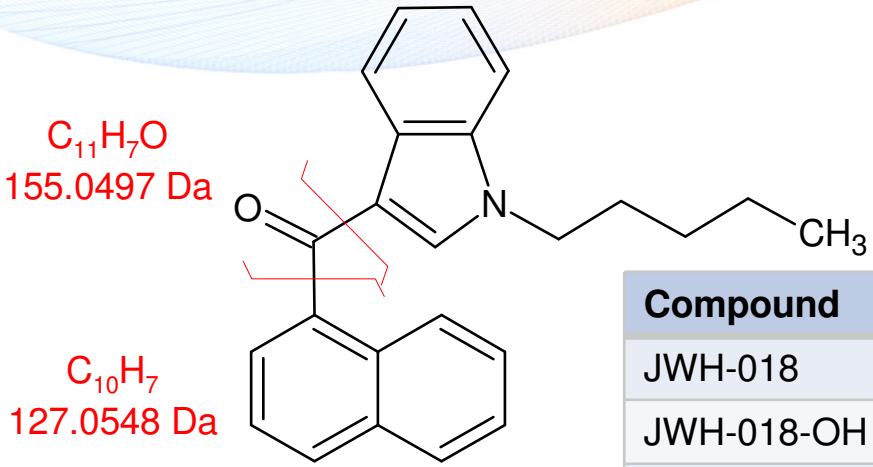
JWH-073-N-(3-hydroxybutyl)
m/z+ = 344.1645

- No parent seen in urine.
- Corresponding glucuronides are also seen in urine

LC- MS Analytical Approaches

Analytical Challenges in JWH analysis

- Compounds have same fragments
- Two key metabolites have identical SRM's.
- Must be separated chromatography.
- The same metabolites have different exact mass (Δ by 100 ppm)
- Additional chromatographic challenge of Ω and (Ω-1) alkyl-OHs.



Compound	Formula	Exact m/z+	SRM-1	SRM-2
JWH-018	C24 H23 N O	342.1852	127.04	155.04
JWH-018-OH	C24 H23 N O2	358.1802	127.04	155.04
JWH-018-COOH	C24 H21 N O3	372.1594	127.04	155.04
JWH-073	C23 H21 N O	328.1700	127.04	155.04
JWH-073-OH	C23 H21 N O2	344.1645	127.04	155.04
JWH-073-COOH	C23 H19 N O3	358.1438	127.04	155.04

Method Development/Validation

- Separation of JWH-018-OH and JWH-073-COOH
- Separation of Ω and (Ω-1) -OHs
- Check for glucuronides and hydrolysis conditions.
- Check for matrix effects.
- Precision/accuracy/LOQ/carryover/linearity (the usual suspects).

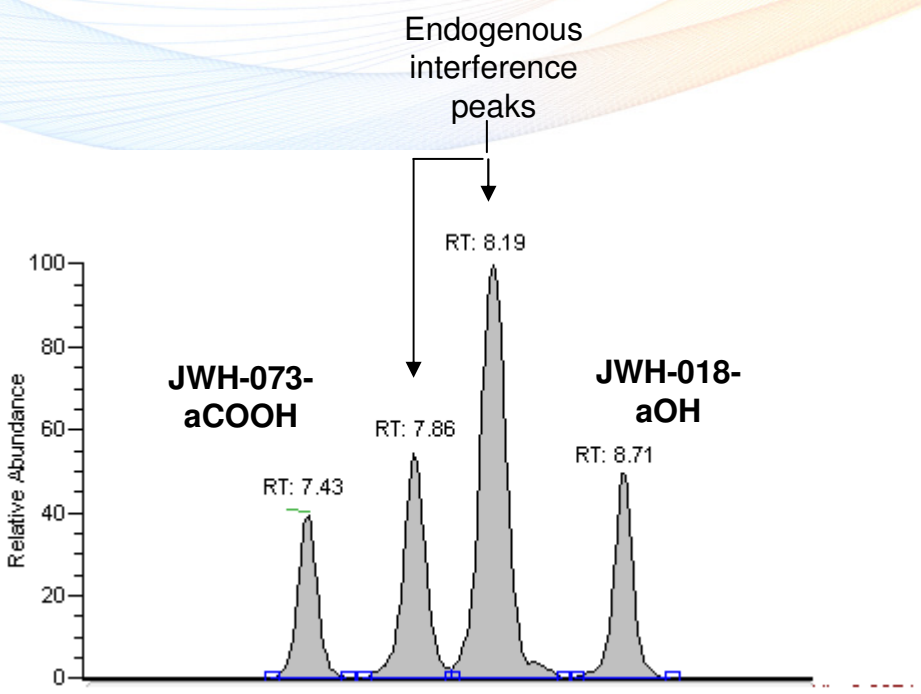
Sample Processing Method

- Aliquot of urine
- Add IS and β-glucuronidase enzyme
- Incubate, quench, centrifuge, dilute supernatant and inject

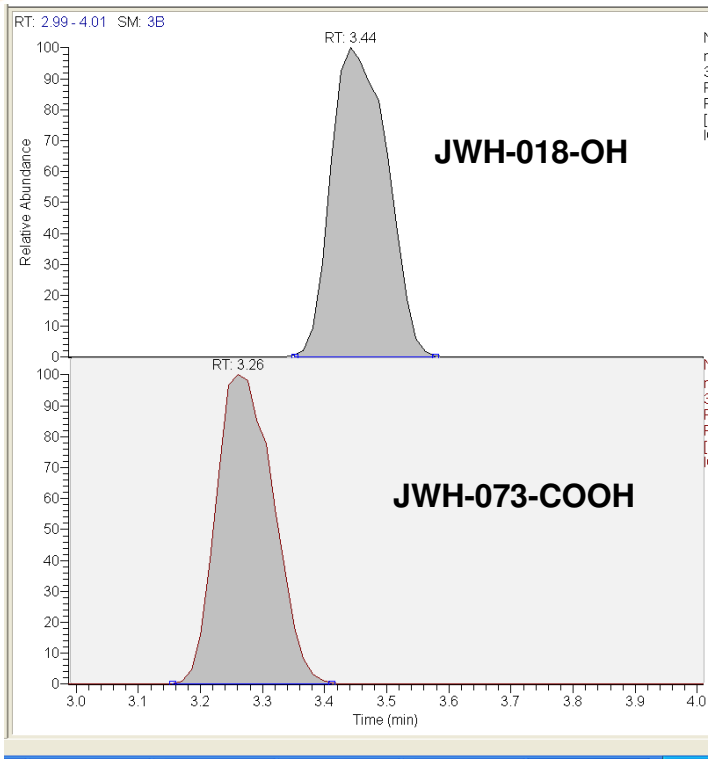
Separation of 018-OH and 073-COOH

HRAM analysis time 3X faster than SRM

Triple Quadrupole



HRAM



Exactive Data Acquisition Method

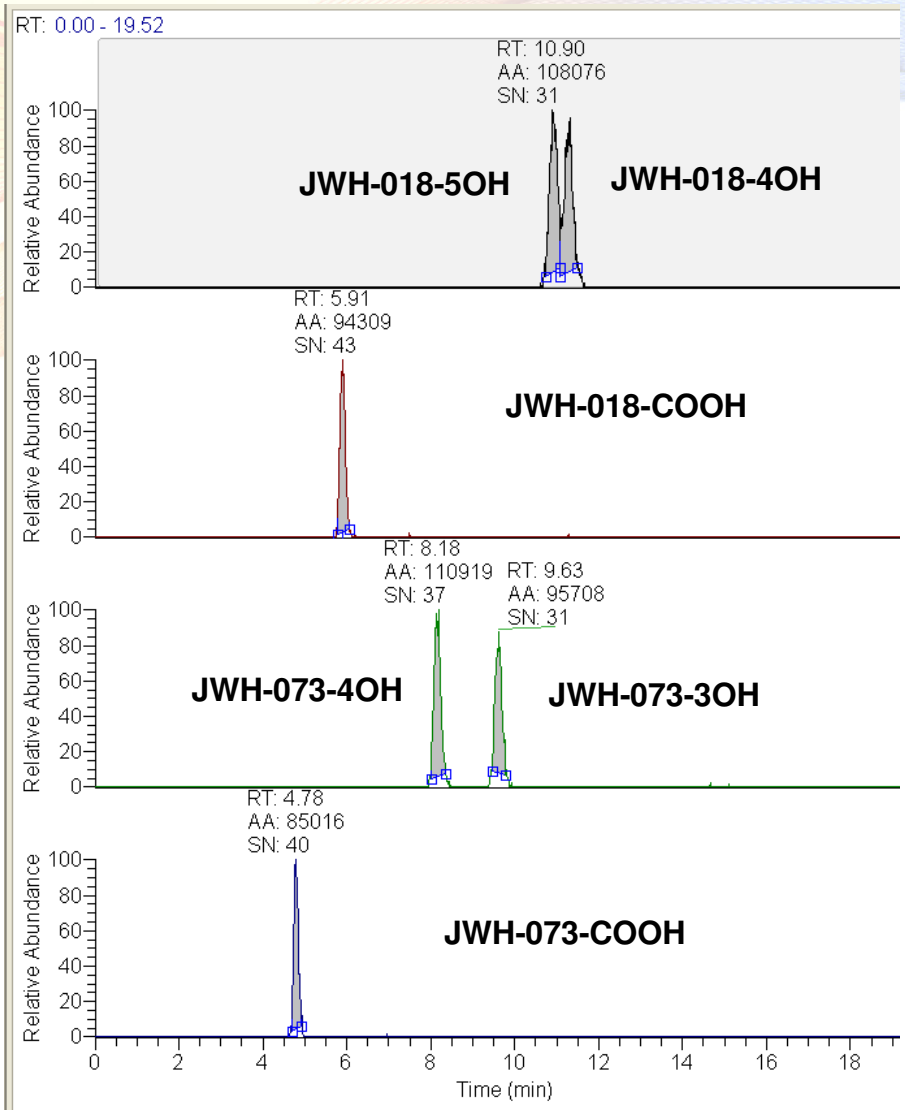
- HESI Source
- 2 Scan Events
  - Event 1
    - Full Scan
    - Mass Range m/z+ 300-2000
    - Resolution 50,000
  - Event 2
    - HCD Fragmentation
    - Collision Energy = 30
    - Mass Range m/z+ 50-1000
    - Resolution 25,000

Best separation of Ω and (Ω-1) hydroxies

- JWH-073-OH separate easily.
- JWH-018-OH are difficult to separate.

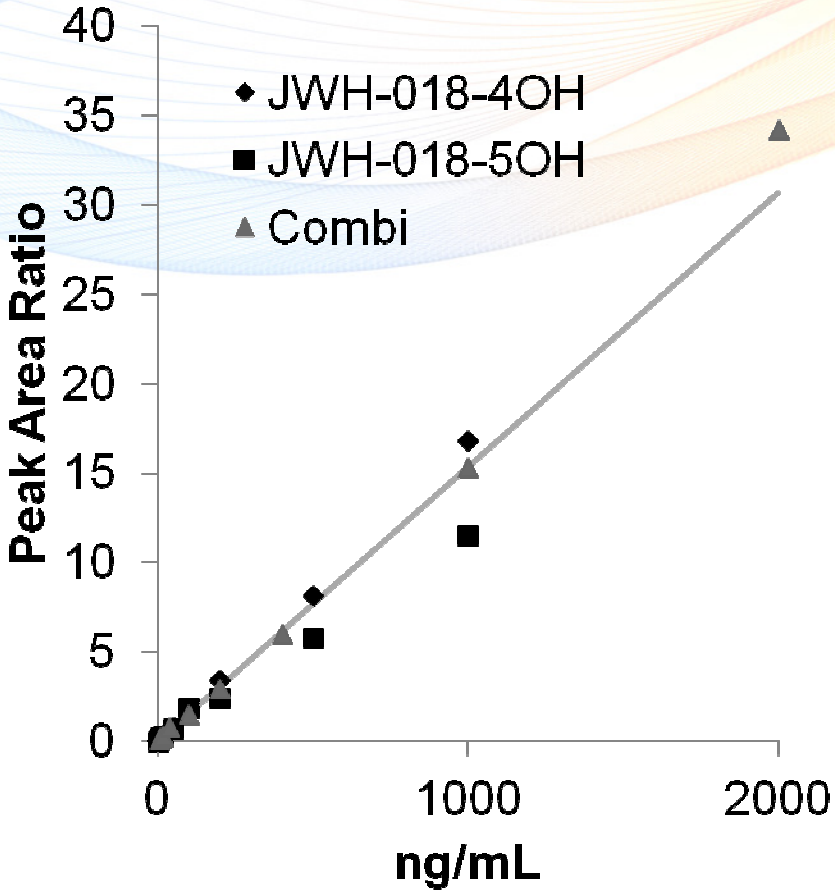
Column:
Hypersil GOLD aQ, 1.9 µm particle, 100x2.1 mm

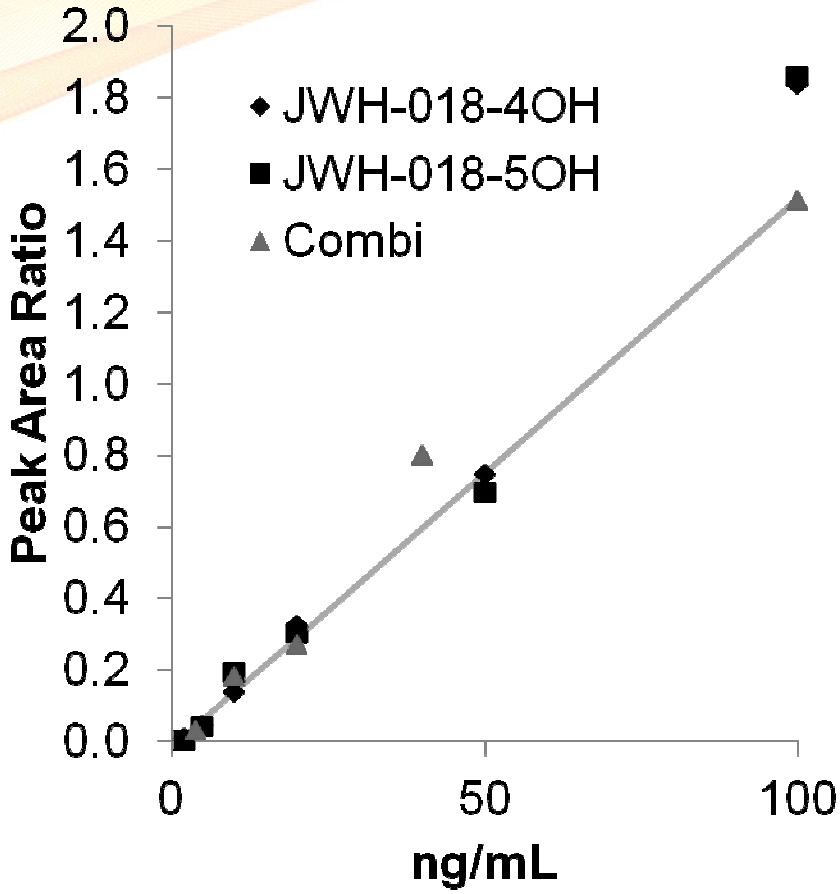
LC Conditions:
5 mM NH_4COOH
Isocratic at 55% methanol



Equivalence of JWH-018-OHs

Individual OHs back calculated to within 30% on combination curve





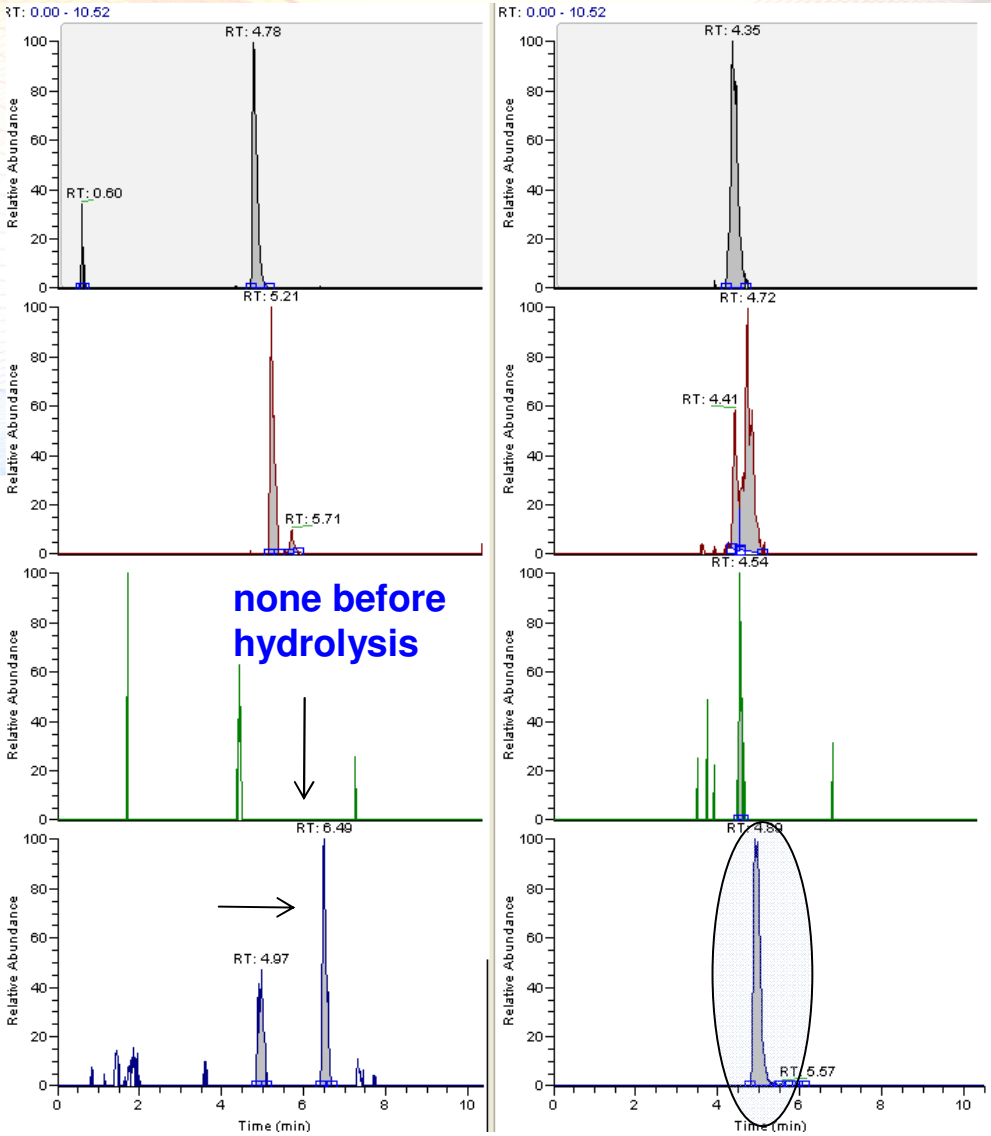
Do we have glucuronides?

JWH-073-COOH

JWH-018-COOH

JWH-073-OH

JWH-018-OH



none before hydrolysis

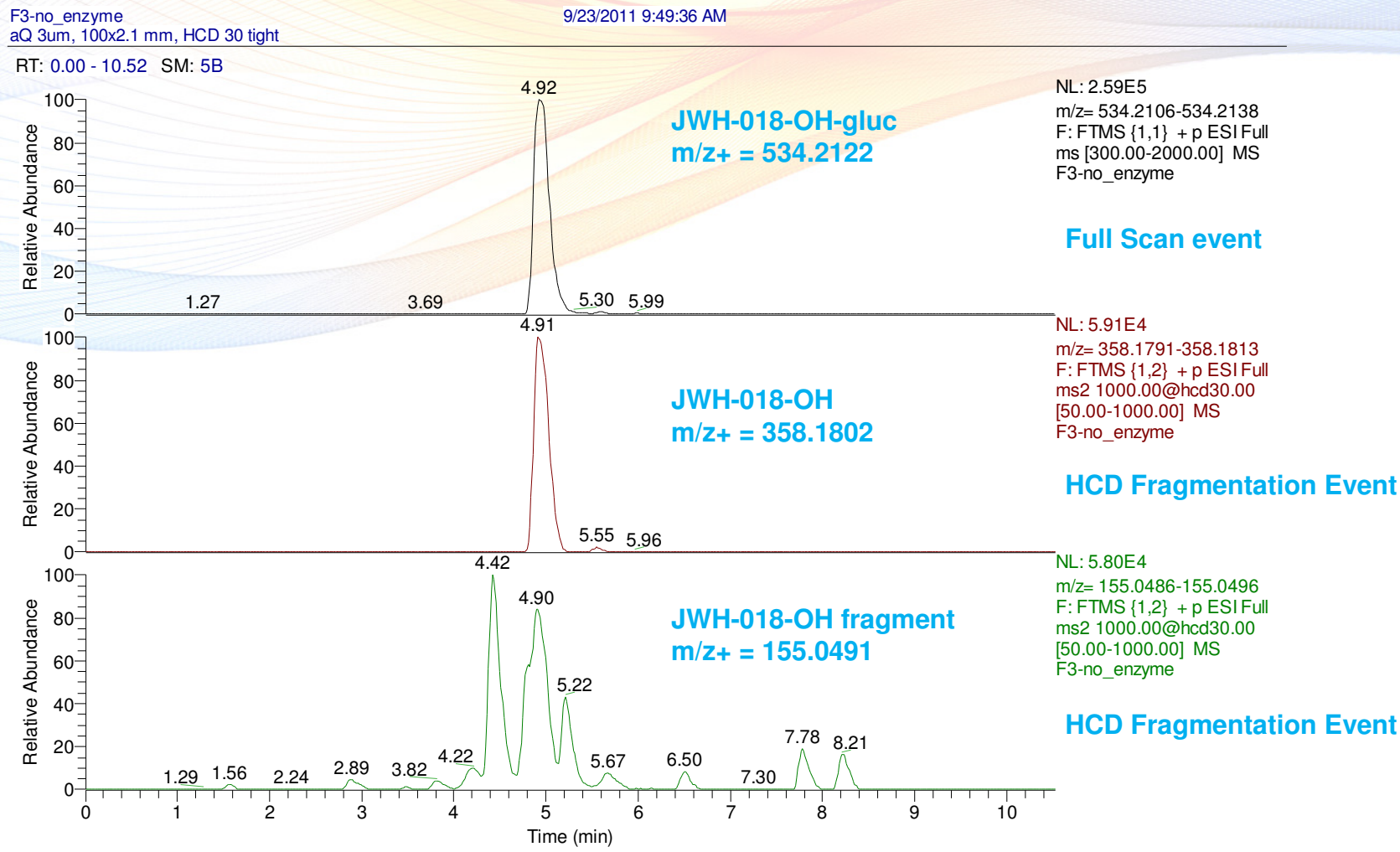
JWH-073-COOH-gluc

JWH-018-COOH-gluc

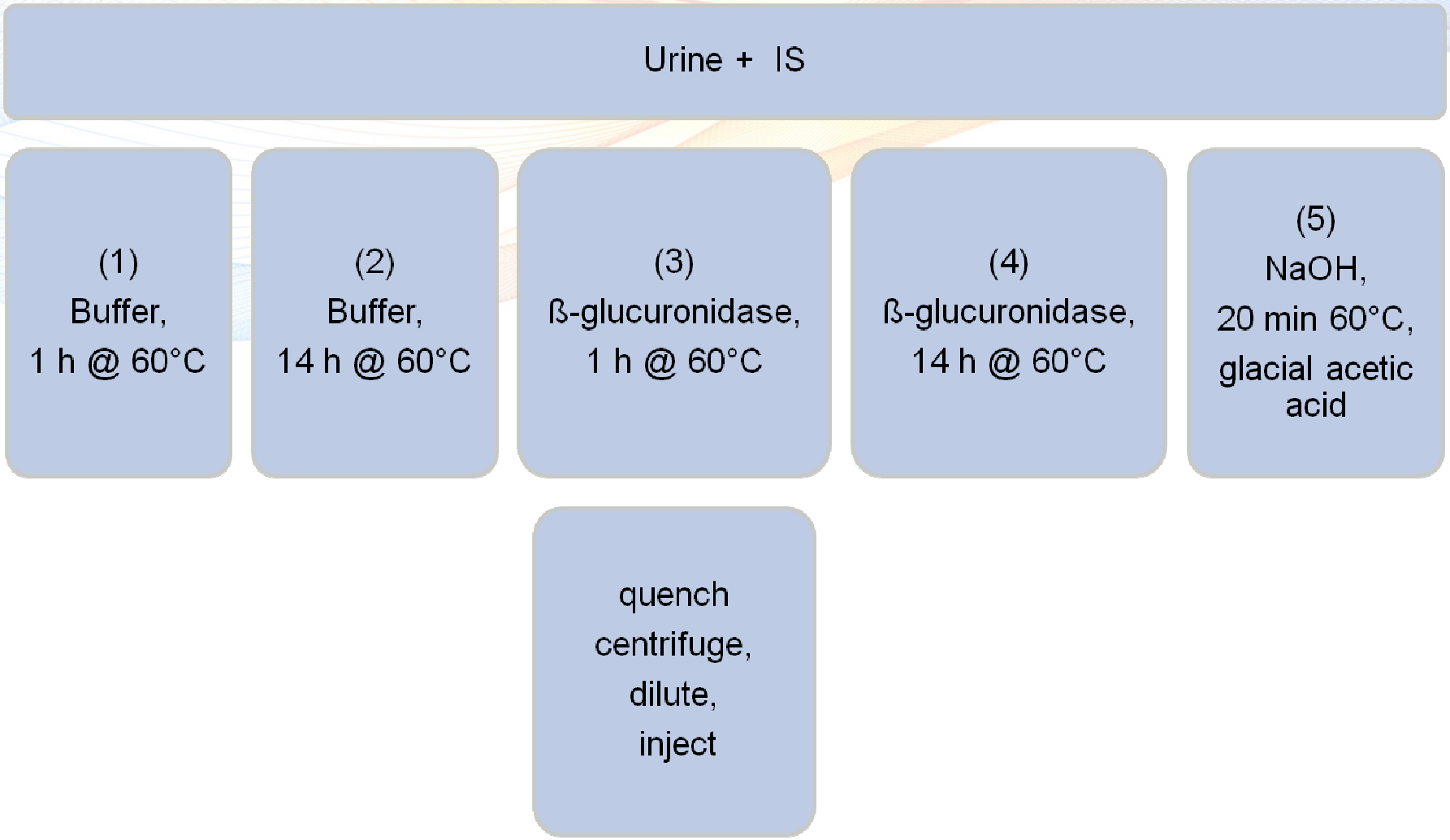
JWH-073-OH-gluc

JWH-018-OH-gluc

Confirmation of JWH-018-OH-glucuronide

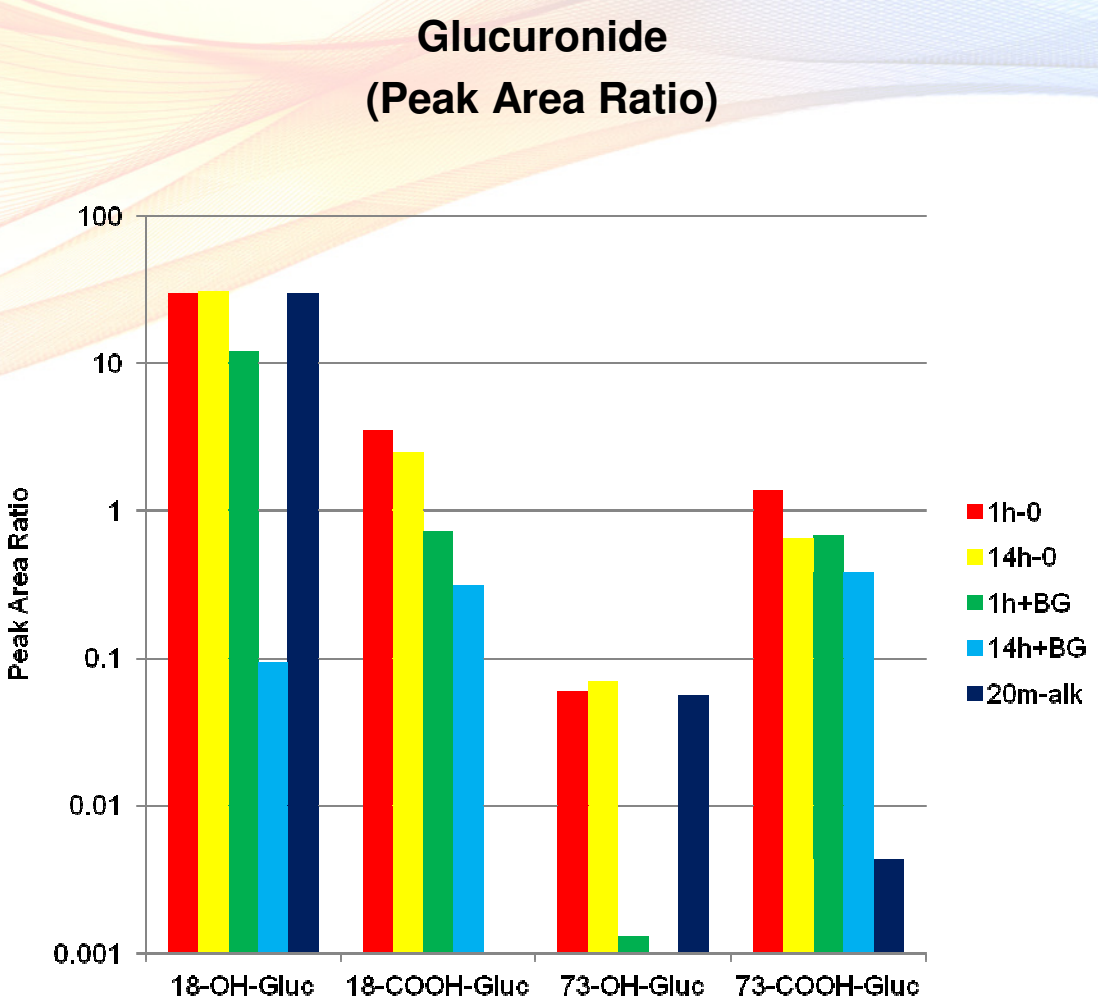


Testing Hydrolysis Methods



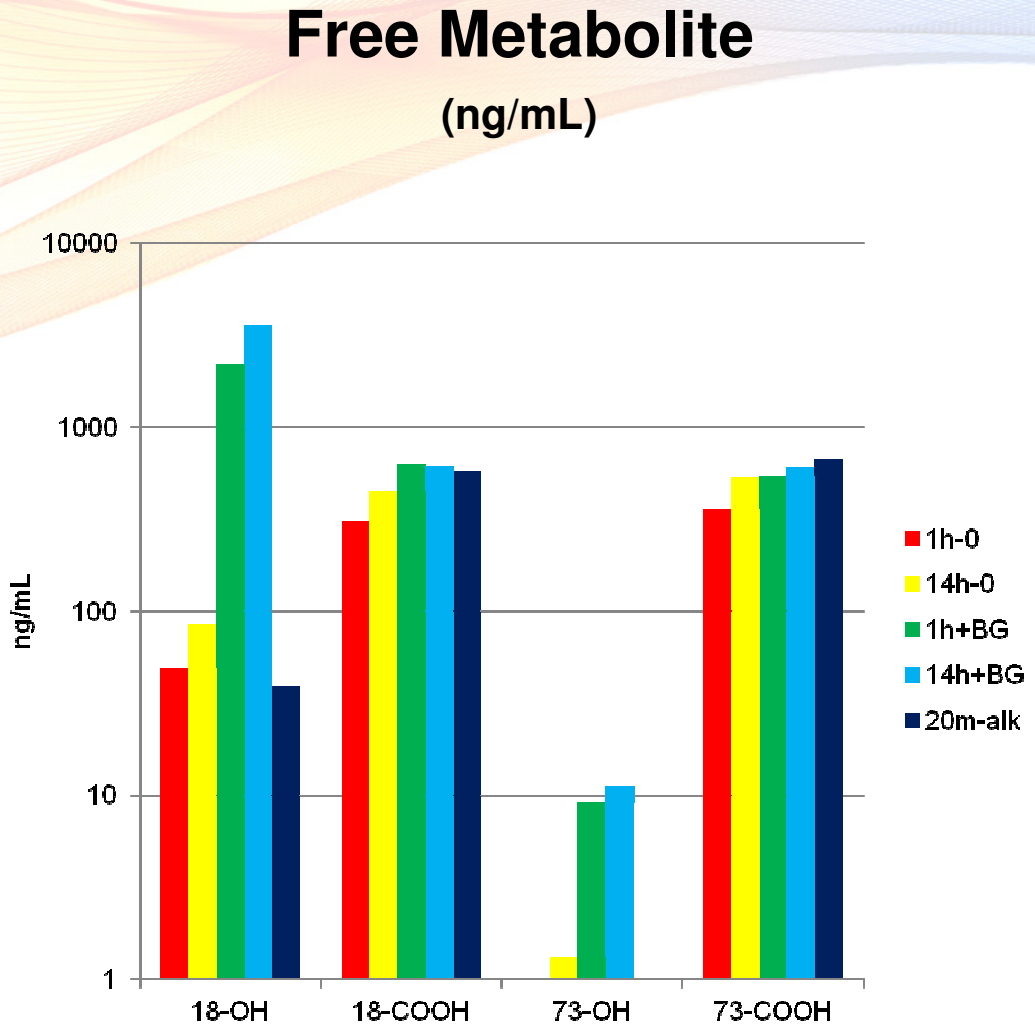
Differing hydrolysis susceptibility

	Enzyme	Basic
-OH-gluc	++	—
-COOH-gluc	+	+++



Appearance of Free Metabolite

	Enzyme	Basic
-OH	++	—
-COOH	++	++



IS Peak Area Compared to Standard Curve

No matrix effects

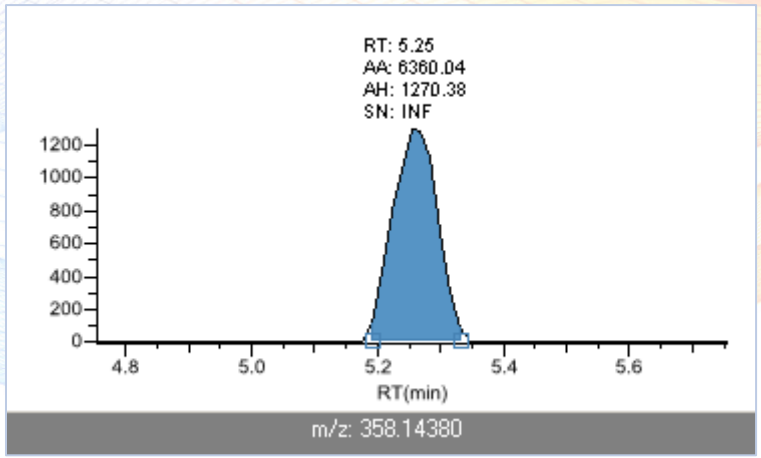
Sample	JWH-073-COOH-d5	JWH-018-COOH-d4	JWH-073-3OH-d5	JWH-018-4OH-d4
F1	104%	106%	108%	95.2%
F2	114%	116%	108%	112%
F3	92.5%	87.8%	99.2%	105%
F4	101%	108%	100%	108%
F5	102%	103%	102%	98.1%
F6	109%	103%	105%	95.9%
F7	93.2%	100%	97.8%	92.9%
F8	104%	116%	108%	101%

Method Validation Results

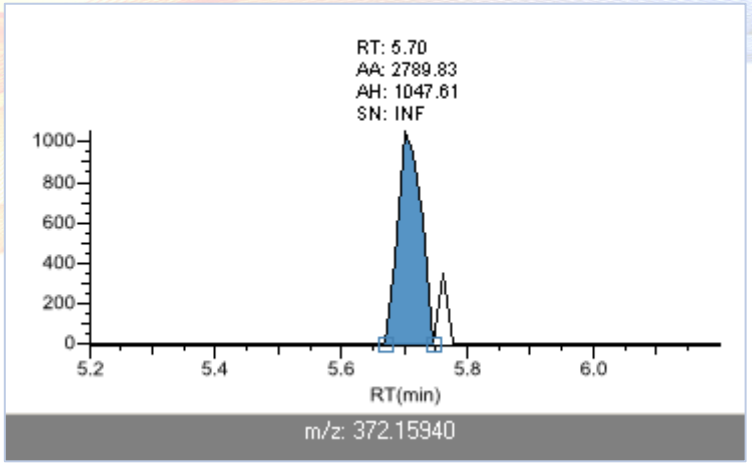
- LQC Inter-Assay accuracy and precision within 20%
- HQC Inter-Assay accuracy and precision both within 10%
- LOQ 2-5 ng/mL for all compounds.
- Linear to 1000 ng/mL, 1/x weighting
- No carryover seen in blank injected after high QC sample

Chromatograms at 5 ng/mL

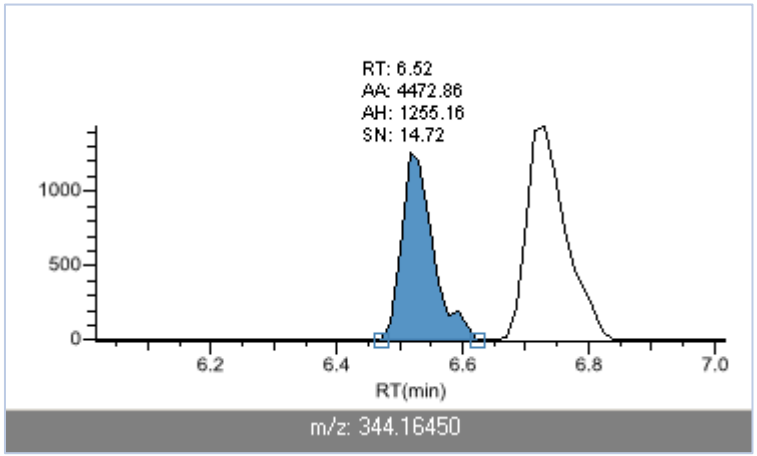
JWH-073-COOH



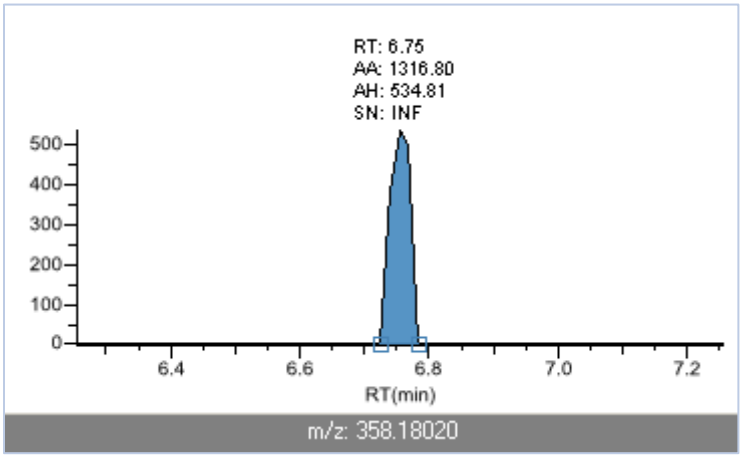
JWH-018-COOH



JWH-073-OH



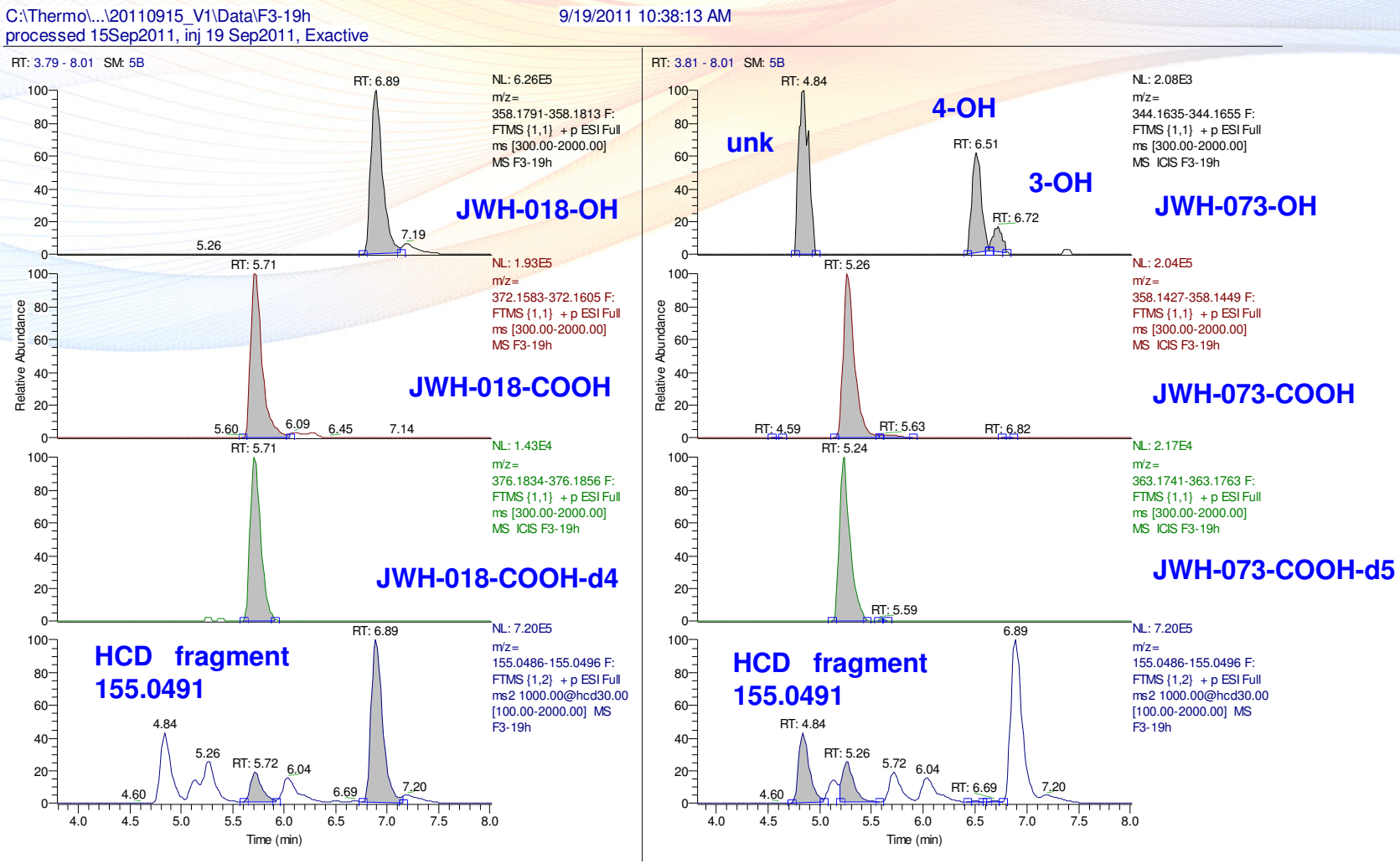
JWH-018-OH



Results of self confessed consumption samples

ng/mL	JWH-073-COOH	JWH-018-COOH	JWH-073-3OH	JWH-073-4OH	JWH-018-OH
F1	2.26	2.59	ND	ND	10.2
F2	22.9	19.4	ND	ND	63.6
F3	724	552	2.67	6.78	3370
F4	ND	ND	ND	ND	10.8
F5	ND	ND	ND	ND	ND
F6	ND	ND	ND	ND	ND
F7	ND	ND	ND	ND	ND
F8	2.25	3.93	ND	ND	4.04

Sample F3 showing HCD fragment confirmation



Retrospective Analysis

Putative identification of other metabolites

JWH-073-COOH

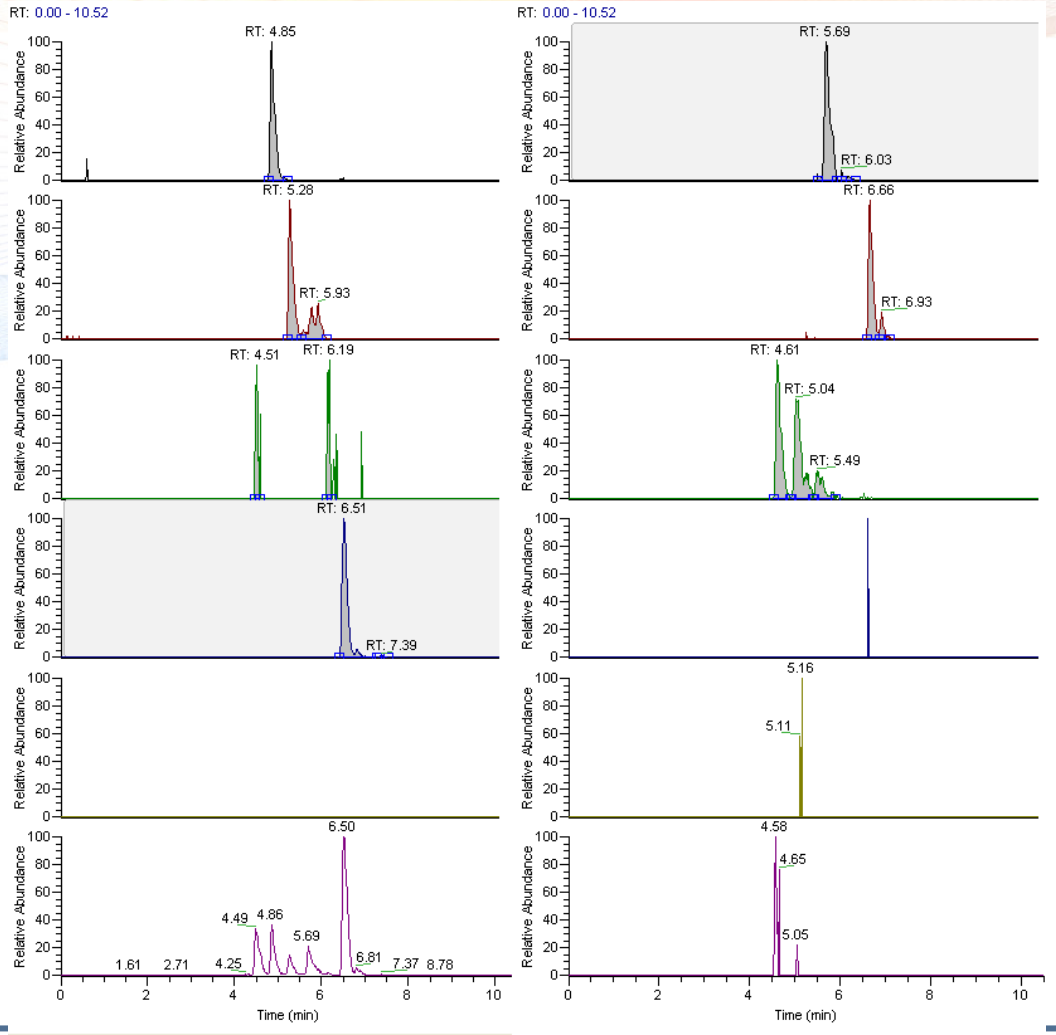
JWH-018-COOH

JWH-073-OH

JWH-018-OH

JWH-des-alkyl

HCD 155.0491



JWH-018-di-OH

JWH-018-di-OH+2H

JWH-018-tri-OH+2H

JWH-073-di-OH

JWH-073-di-OH+2H

JWH-073-tri-OH+2H

Summary

- Newly scheduled synthetic cannabinoids need to be analyzed.
- Look for Ω- and (Ω-1)-alkyl-OH and alkyl-COOH in urine
- Analytical challenges due to identical fragments, isobaric nominal masses and isomeric metabolites.
  - Chromatography must be used to separate isomers
  - Equivalent detector response for W /(W-1) isomers
- Faster analysis by HRAM when Ω and (Ω-1) don't need to be separated
- -OH and –COOH respond differently to hydrolysis conditions
- Good precision, accuracy, linearity, carryover and no matrix effects

Acknowledgements

- Marta Kozak, Thermo Fisher Scientific
- Kent Johnson, Pacific Hospital of Long Beach (Fortes Laboratories)
- Robert Hara, Fortes Laboratories
- Cerilliant