In Vitro Metabolism Studies of Synthetic Cannabinoids

New Drugs 2014
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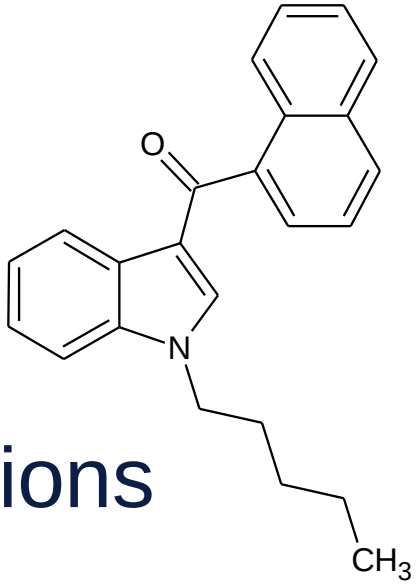
12 Synthetic Cannabinoid Classes

- Adamantoylindole
- Benzoylindoles
- Cyclohexylphenols
- Classical dibenzopyrans
- Indazole based
- Naphthoylindoles & WIN 55,212-2
- Naphthoylpyrroles
- Naphthylmethylindoles
- Naphthylmethylindenes
- Phenylacetylindoles
- Quinolinyl ester (indole based)
- Tetramethylcyclopropyl ketone indoles
- New synthetic cannabinoids constantly emerging

Analytical Challenges



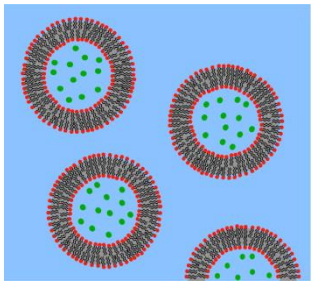
- Vast array of target analytes
- High potency means low concentrations
- Similar chemical structures makes chromatographic separation difficult
- Common metabolites for different targets
- Changing target analyte availability requires constant method addition & validation
- Lack of information about human urinary metabolites of new cannabimimetics

Metabolite Identification

- To determine best metabolites to identify synthetic cannabinoid intake
- To identify potential active metabolites
- To identify potential toxic metabolites
- Supply single known parent drug for hepatocyte metabolism
- Produce phase 1 & 2 metabolites
- Accurate mass provides elemental composition of molecular ion & fragments

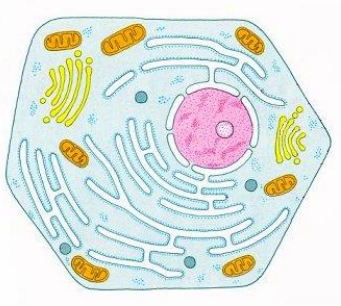
Identifying Human Metabolites with Human Liver Microsomes & Hepatic Cell Cultures & High-Resolution Mass Spectrometry

HLM Preparation $T_{1/2}$ determination



1

Incubation with human hepatocytes

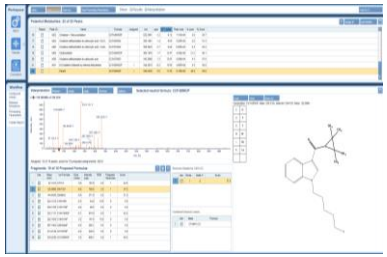


2

Analysis by LC-HRMS

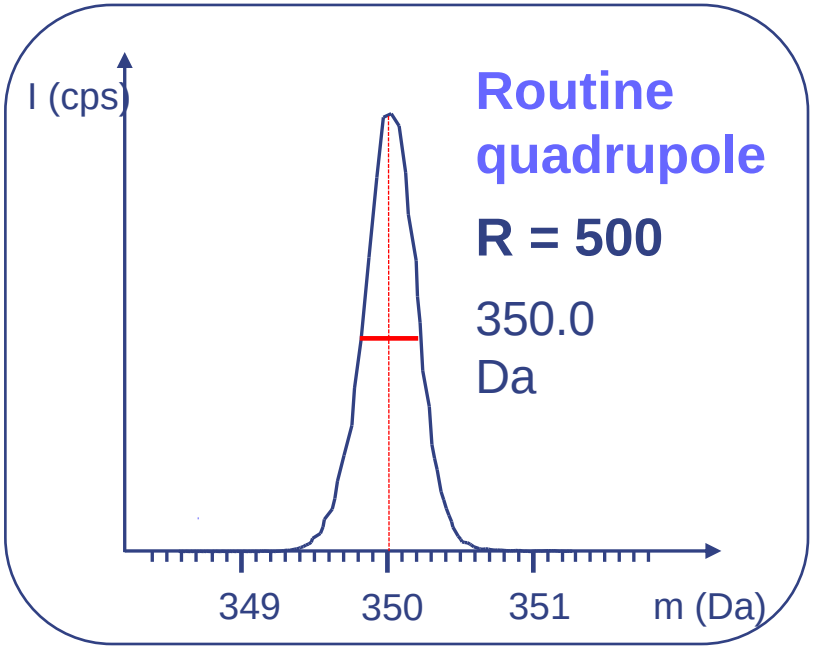
3

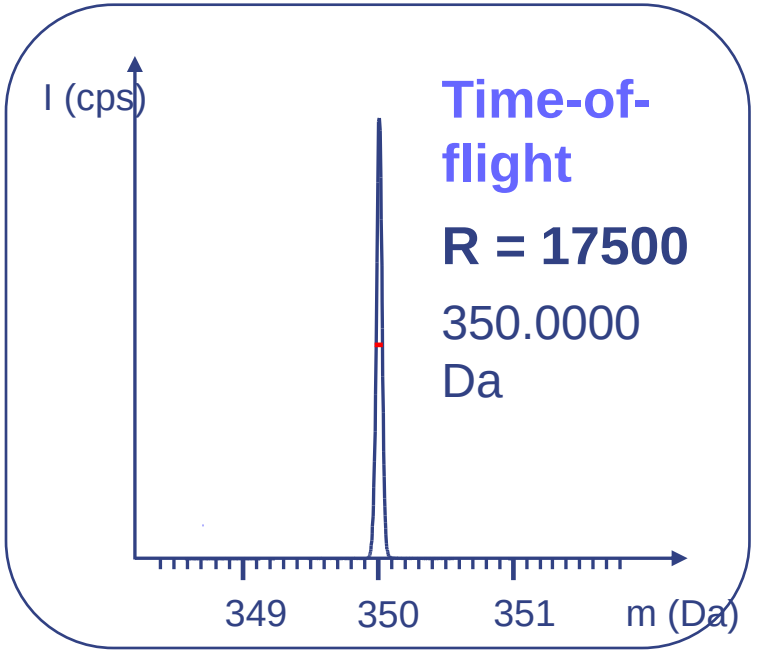
Software-assisted data analysis



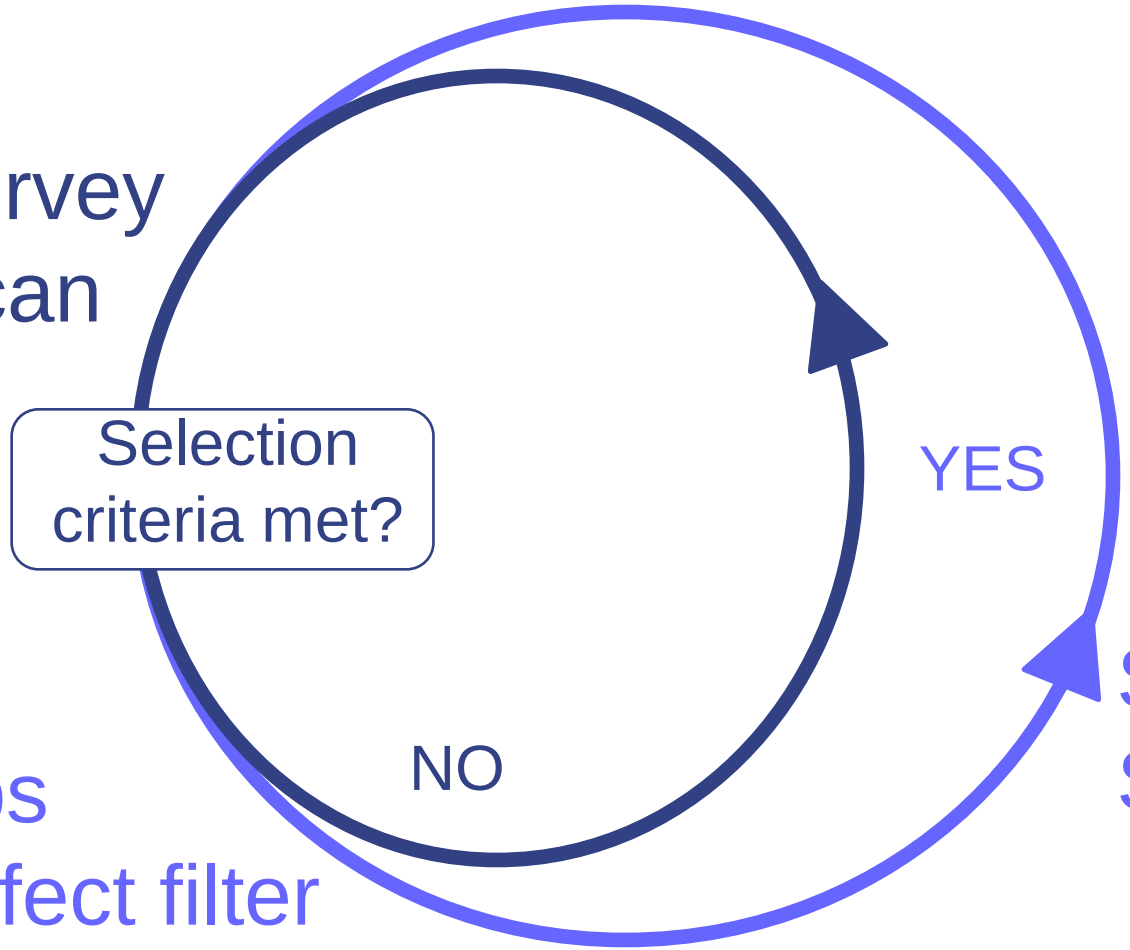
Advantages Of High Resolution MS

- Enhanced specificity
- Simplified sample preparation
- Elemental composition: intact [MH]+ & fragments
- Retrospective analysis is possible





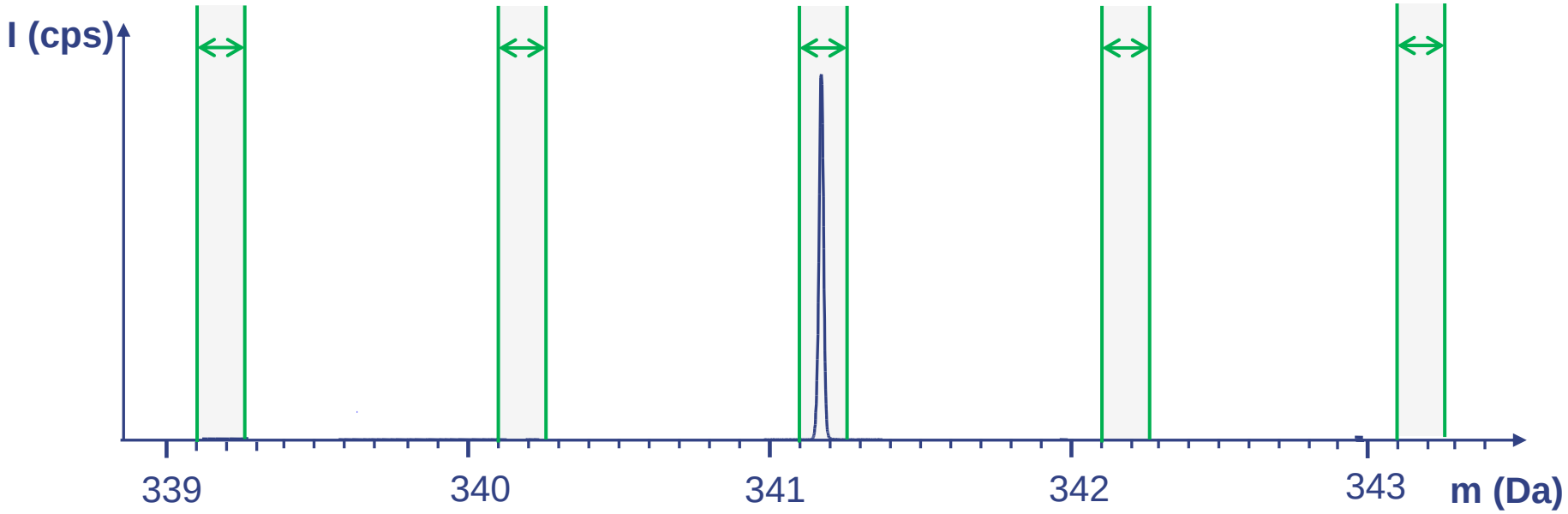
Information-Dependent Acquisition



Intensity
> 500 cps
Mass defect filter
(range ± 50 mDa)
Dynamic Background Subtraction

Mass Defect Filtering

- Exact mass of JWH-018 $[MH]^+$ 341.1779 Da
  → mass defect 177.9 mDa
- Mass defect filter: filters substances that fall within a specific window of mass defect



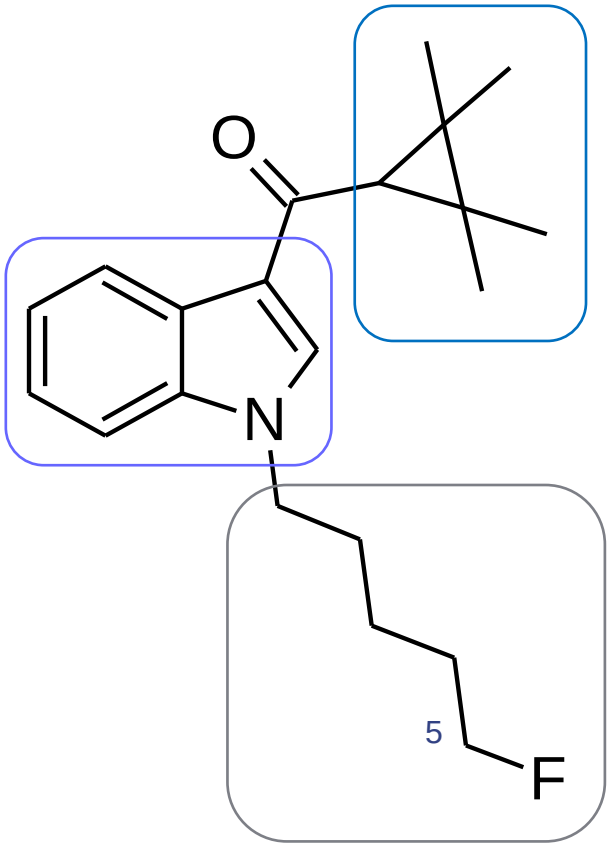
Typical Biotransformations Only Minimally Change Mass Defect

+/- 50 mDa window around the parent drug

	Nominal mass	Mass defect
Oxidation	+ 16 Da	- 5 mDa
Hydration	+ 18 Da	- 16 mDa
Demethylation	- 14 Da	- 16mDa
Reduction	2 Da	+ 16 mDa
Glucuronidation	+ 176 Da	+ 32 mDa
Sulfation	+ 80 Da	- 43 mDa

XLR-11

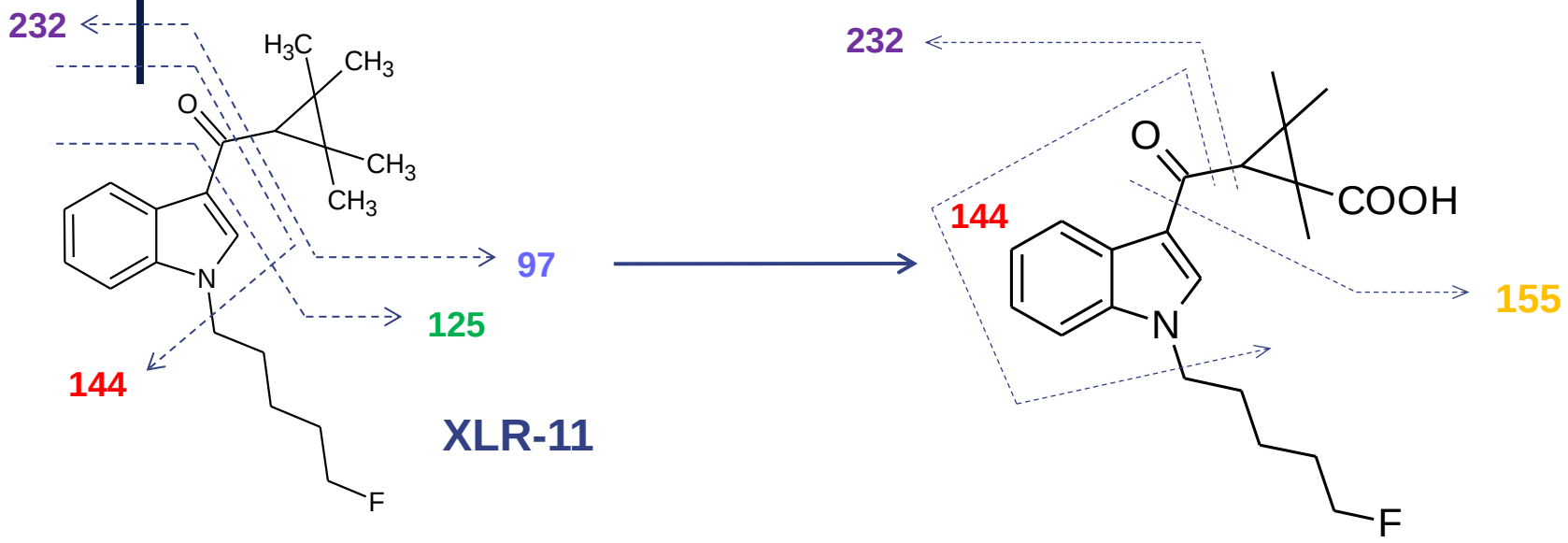
Indole

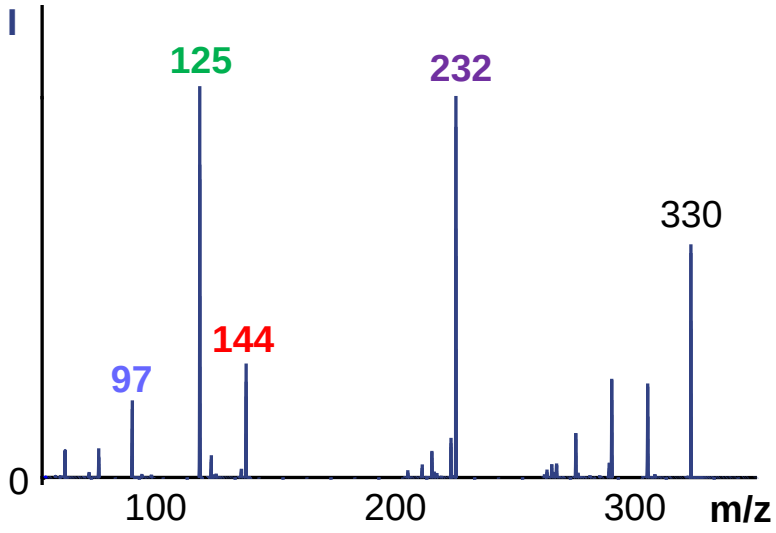


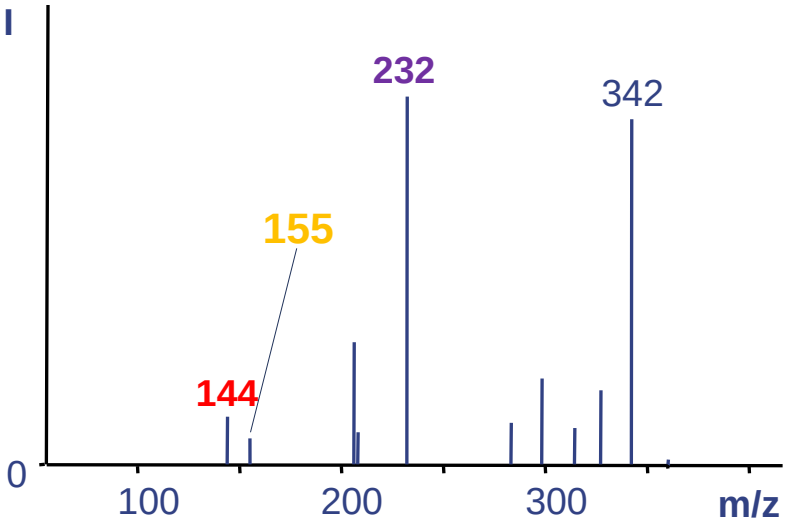
Tetramethylcyclopropyl structure

5-Fluoropentyl chain

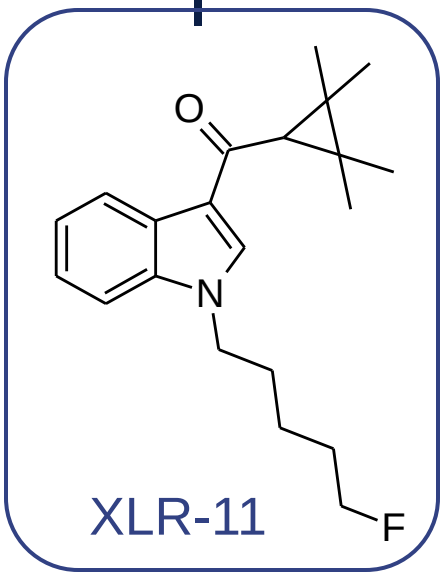
XLR-11-2’-carboxylic acid





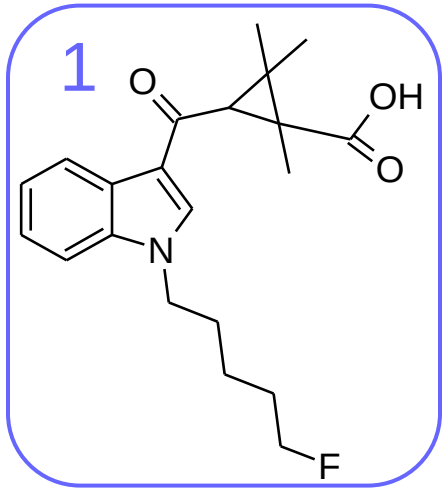


Metabolic XLR-11 Profile

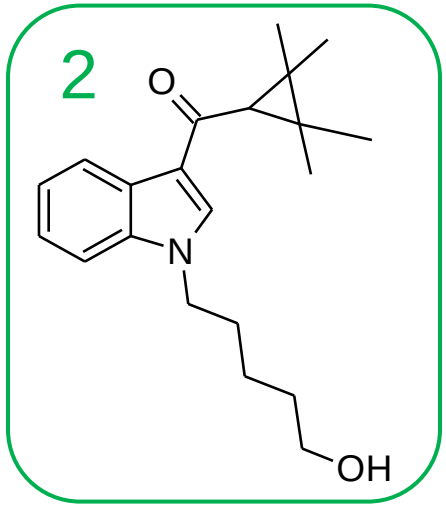


XLR-11

& more than 25 other metabolites

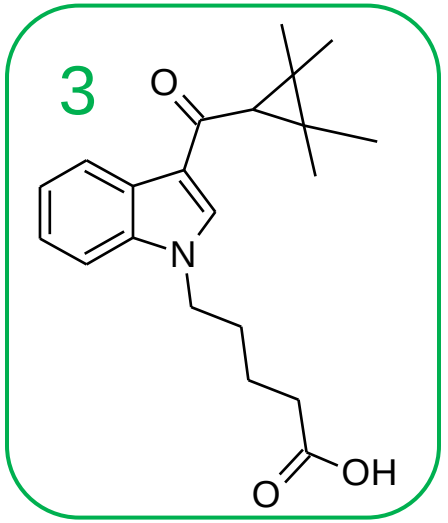


Carboxy-XLR-11 (specific)



UR-144 5-OH-pentyl

Commercially available



UR-144 pentanoic acid

Wohlfarth et al. *Clinical Chemistry* (2013)

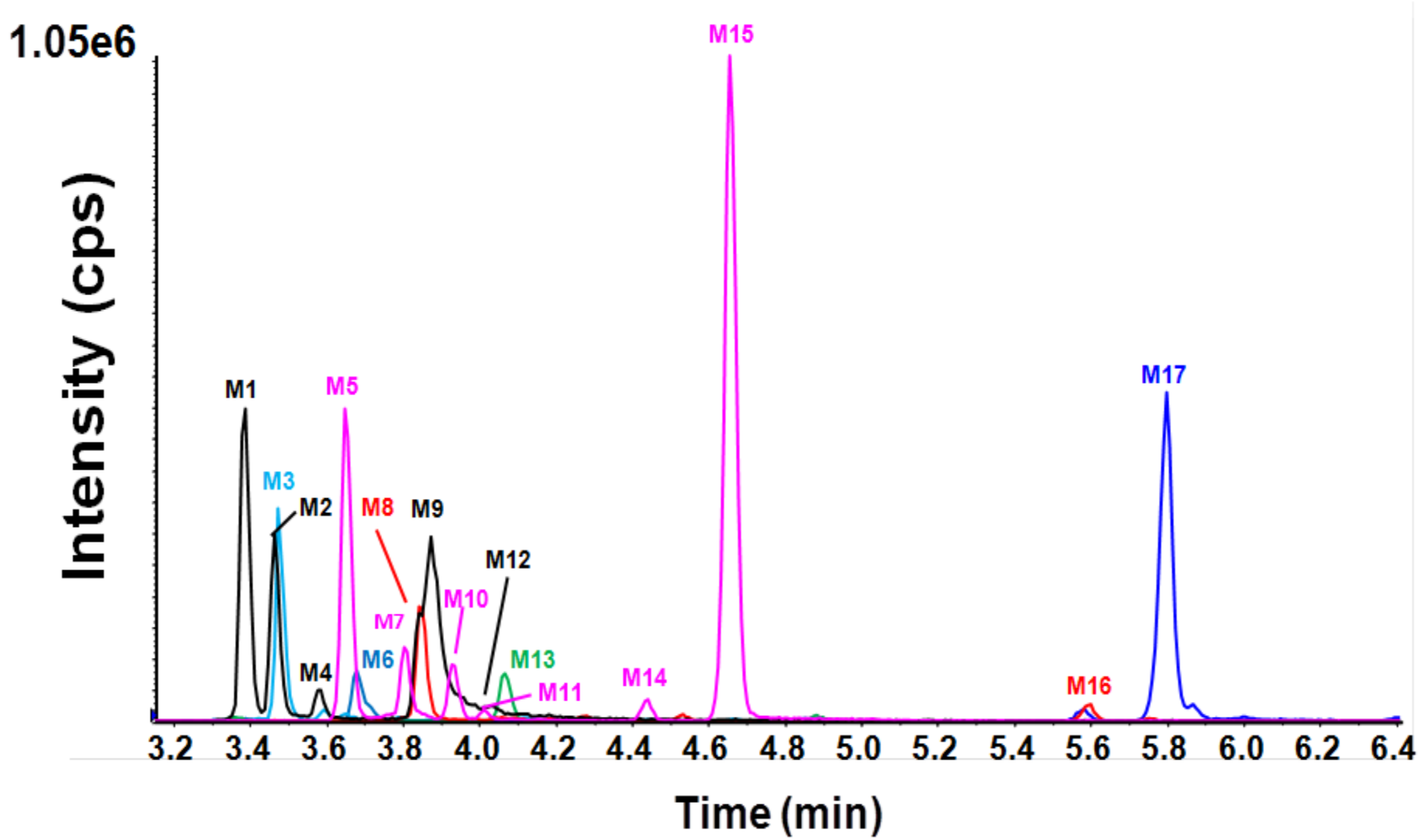
XLR-11 Metabolism

- Biotransformations: hydroxylation, carboxylation, oxidative defluorination, hemiketal & hemiacetal formation, internal dehydration & glucuronidation
- Defluorination leads to UR-144 metabolites
- As expected, more phase 2 metabolites after 3 h incubation
- Main modifications are terminal position on pentyl chain & tetramethylcyclopropyl ring

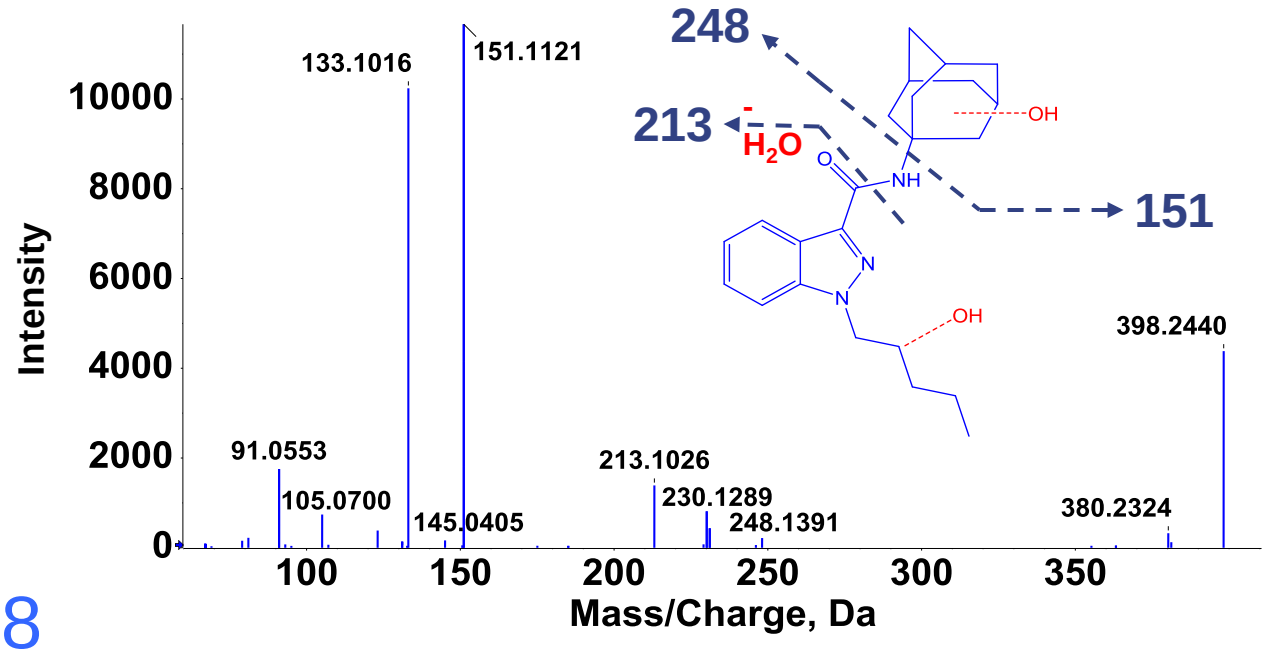
AKB-48 Metabolism

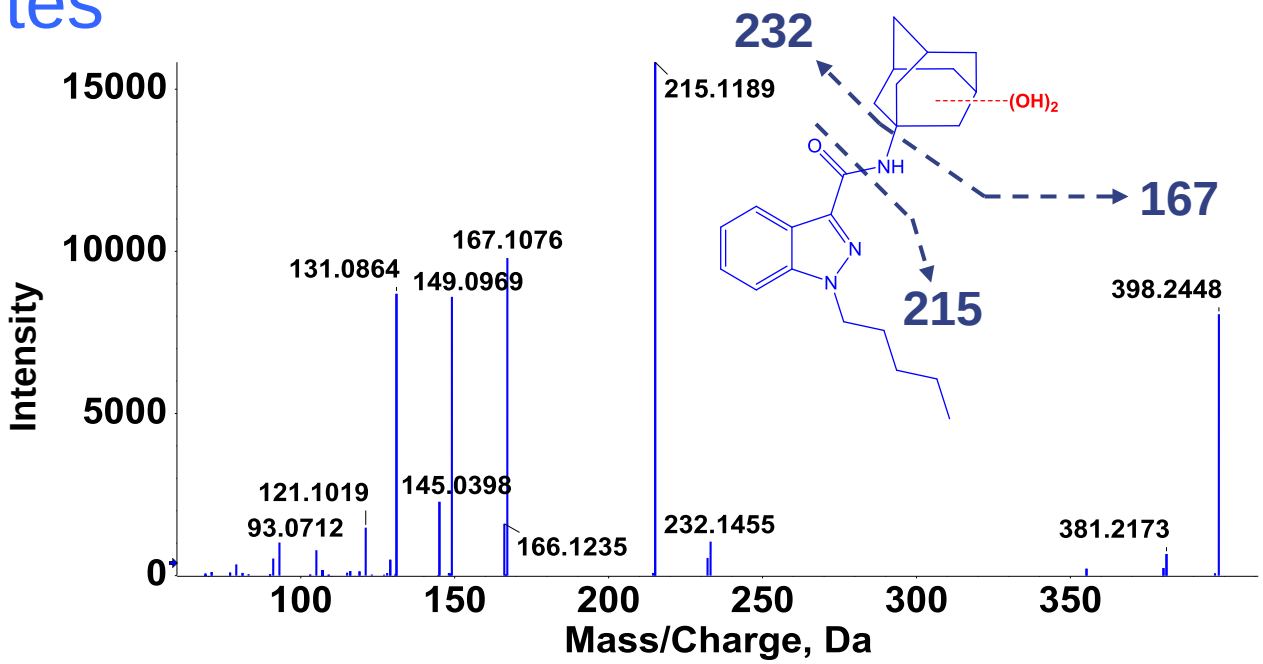
- APINACA, N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide)
- Novel synthetic cannabinoid found in herbal smoking blends in Japan in 2012
- Strong binding affinity (2x at CB_1 than CB_2)
- DEA Schedule I drug May 2013
- Goal is to profile metabolites of AKB-48 for detection in forensic & clinical laboratories

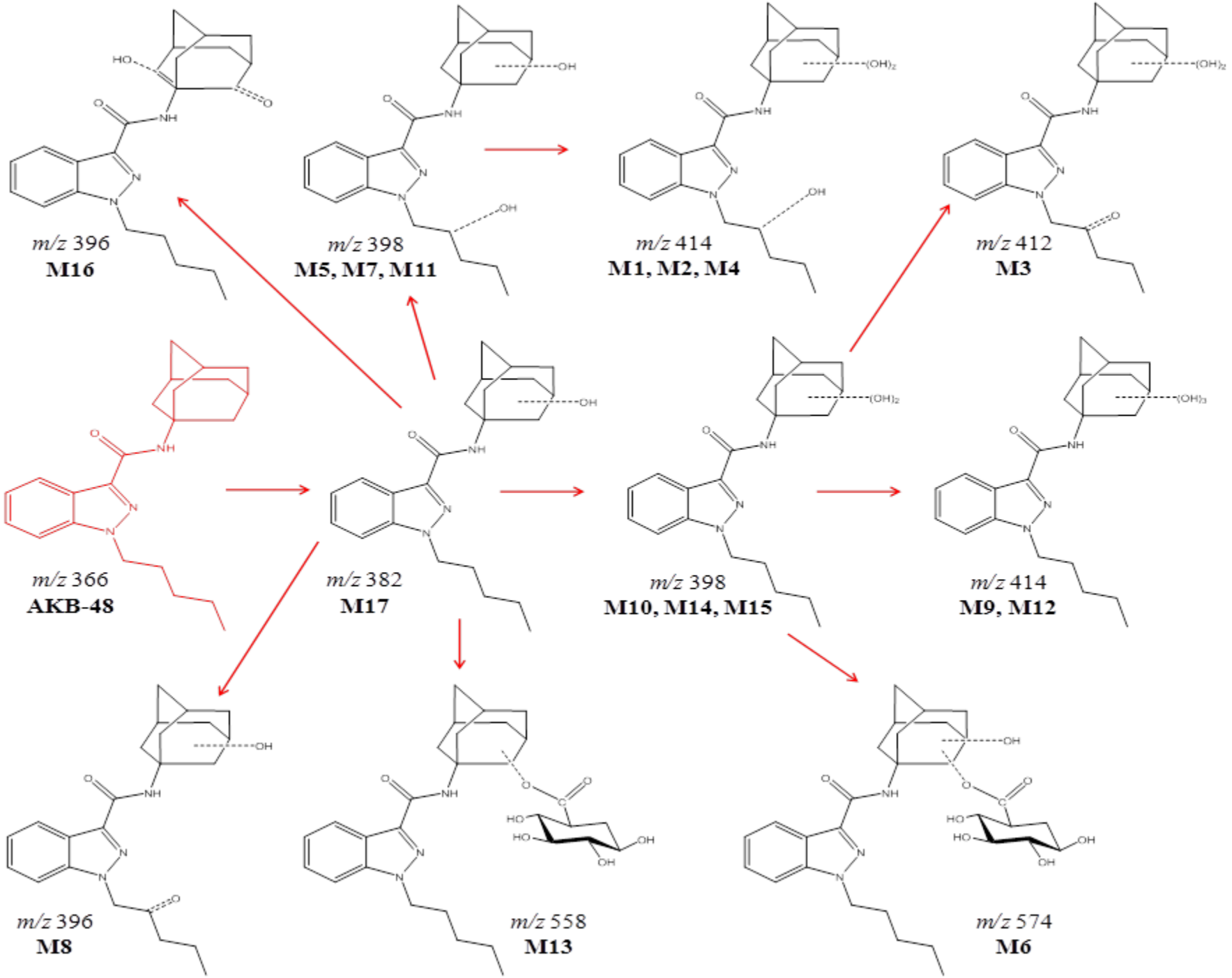
AKB-48 Metabolism



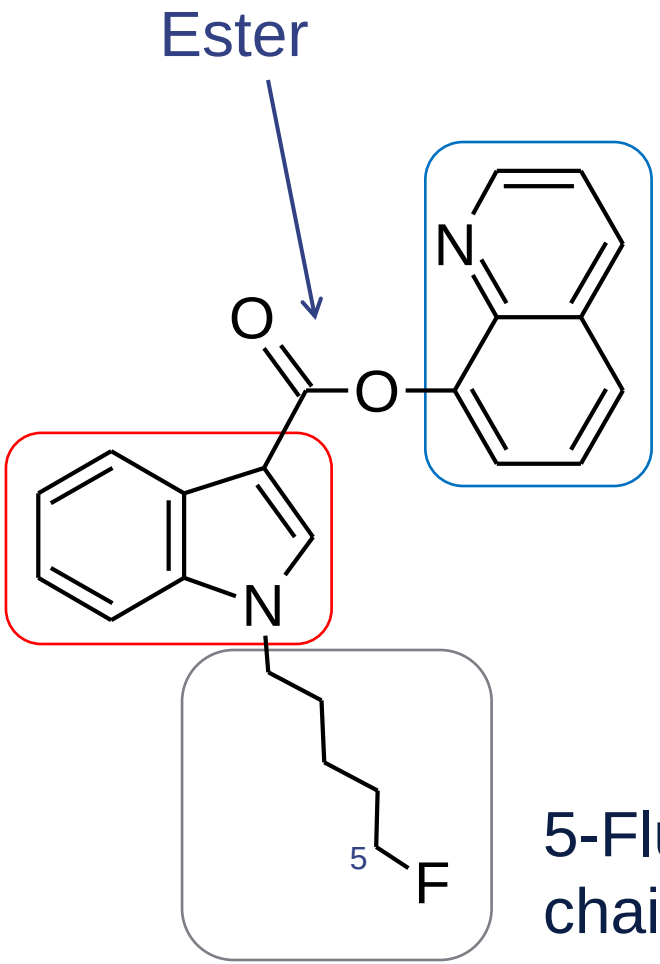
AKB-48 Metabolites





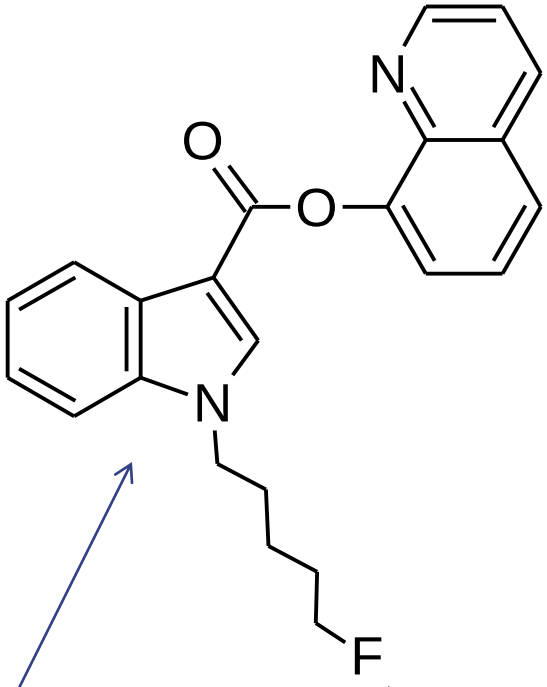

m/z 396
M16
m/z 398
M5, M7, M11
m/z 414
M1, M2, M4
m/z 412
M3
m/z 366
AKB-48
m/z 382
M17
m/z 398
M10, M14, M15
m/z 414
M9, M12
m/z 396
M8
m/z 558
M13
m/z 574
M6

5F-PB-22

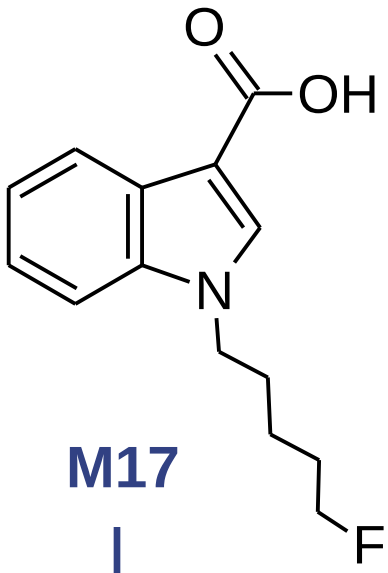


1st Metabolism Ester Hydrolysis

Epoxide formation + hydrolysis



Ester hydrolysis



M17

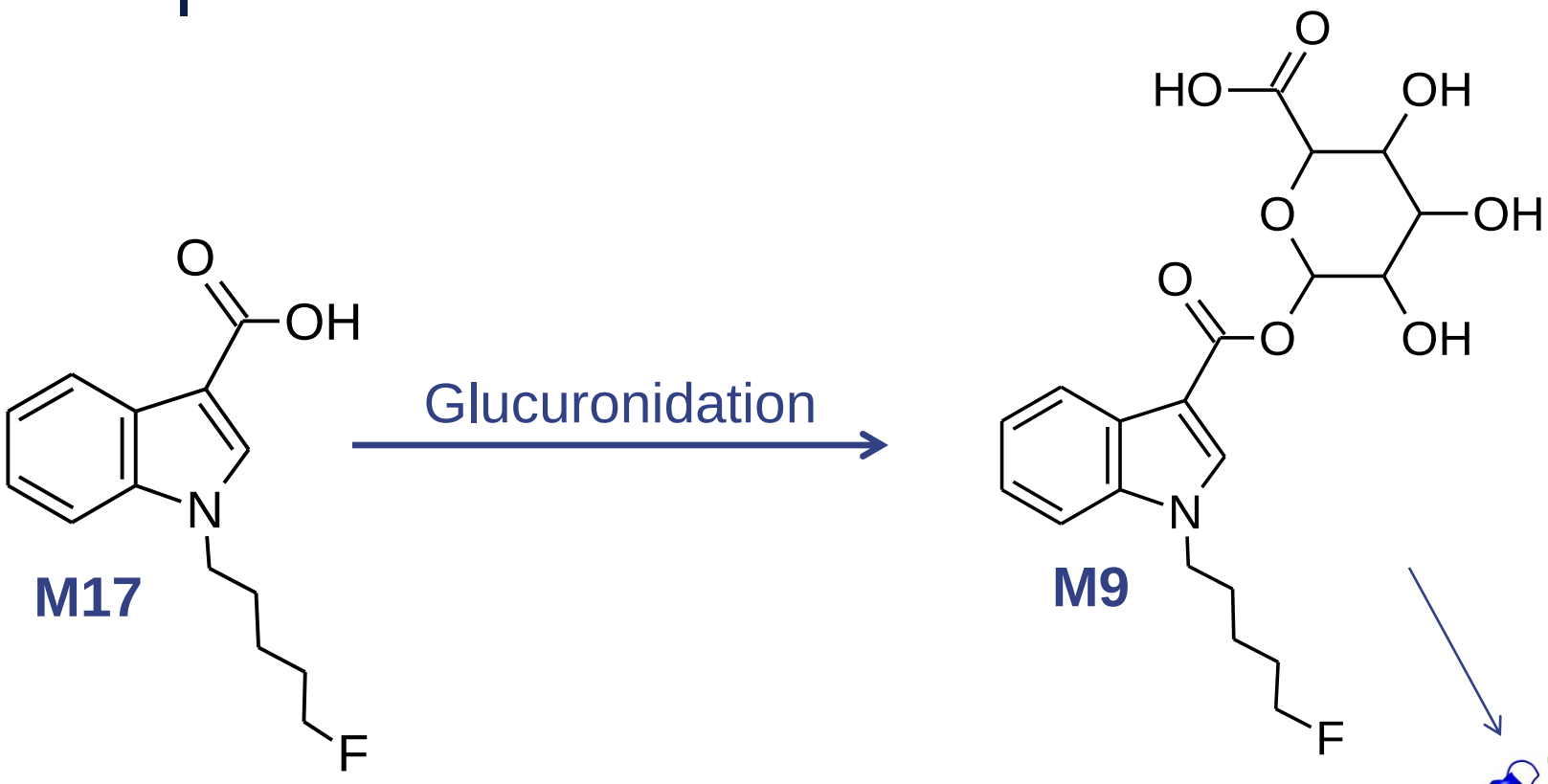
Oxidation
(1x, 2x, 3x)

Oxidative defluorination

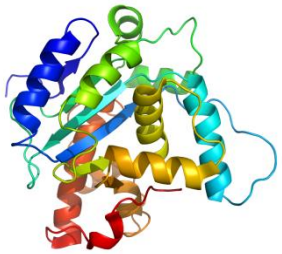
10 other metabolites

Phase II: Glucuronidation

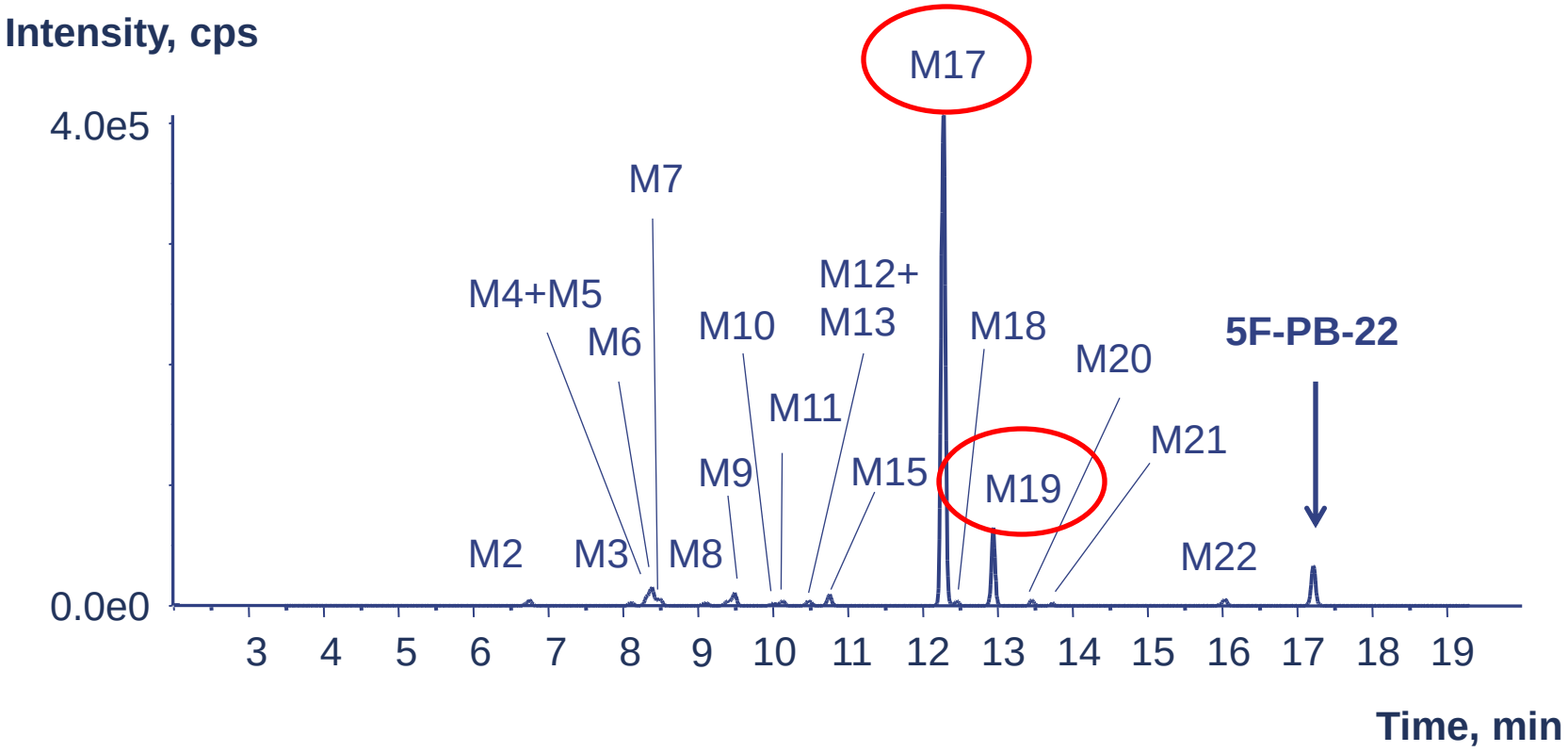
Acyl Glucuronides Reactive & Potentially Toxic



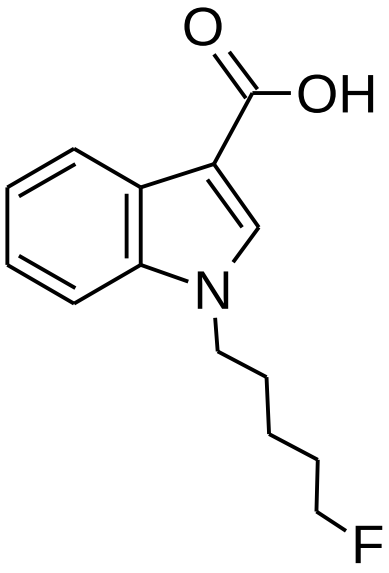
Acylation of amino & thiol groups in proteins



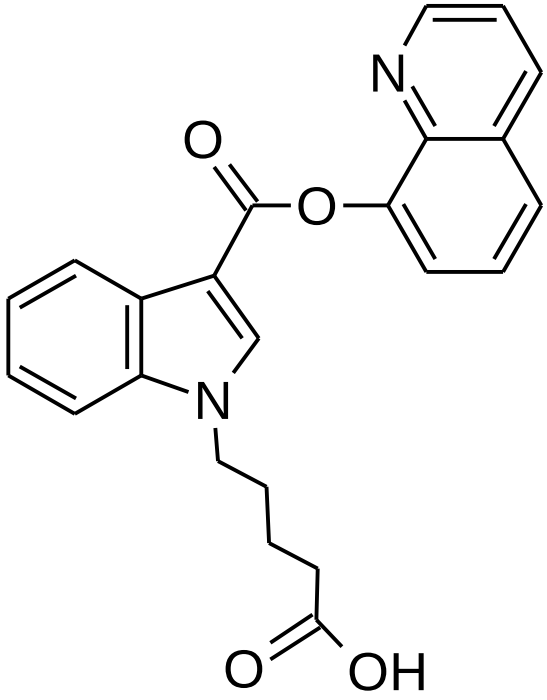
5F-PB-22 Metabolites



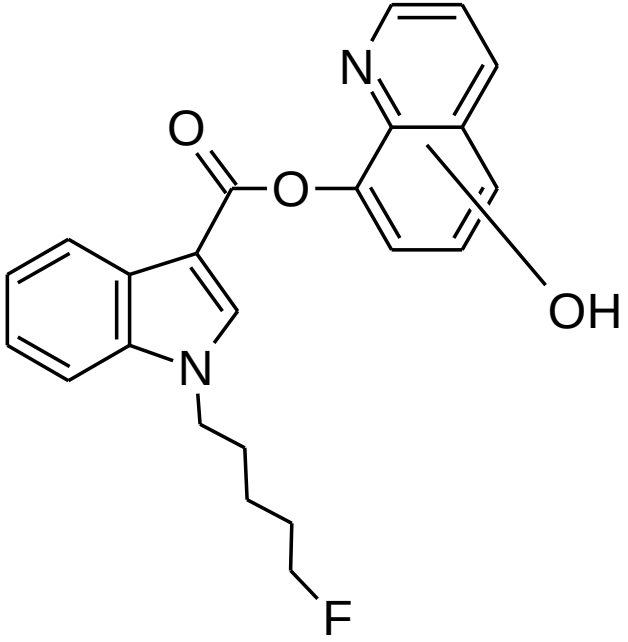
5F-PB-22 Recommended Targets



M17

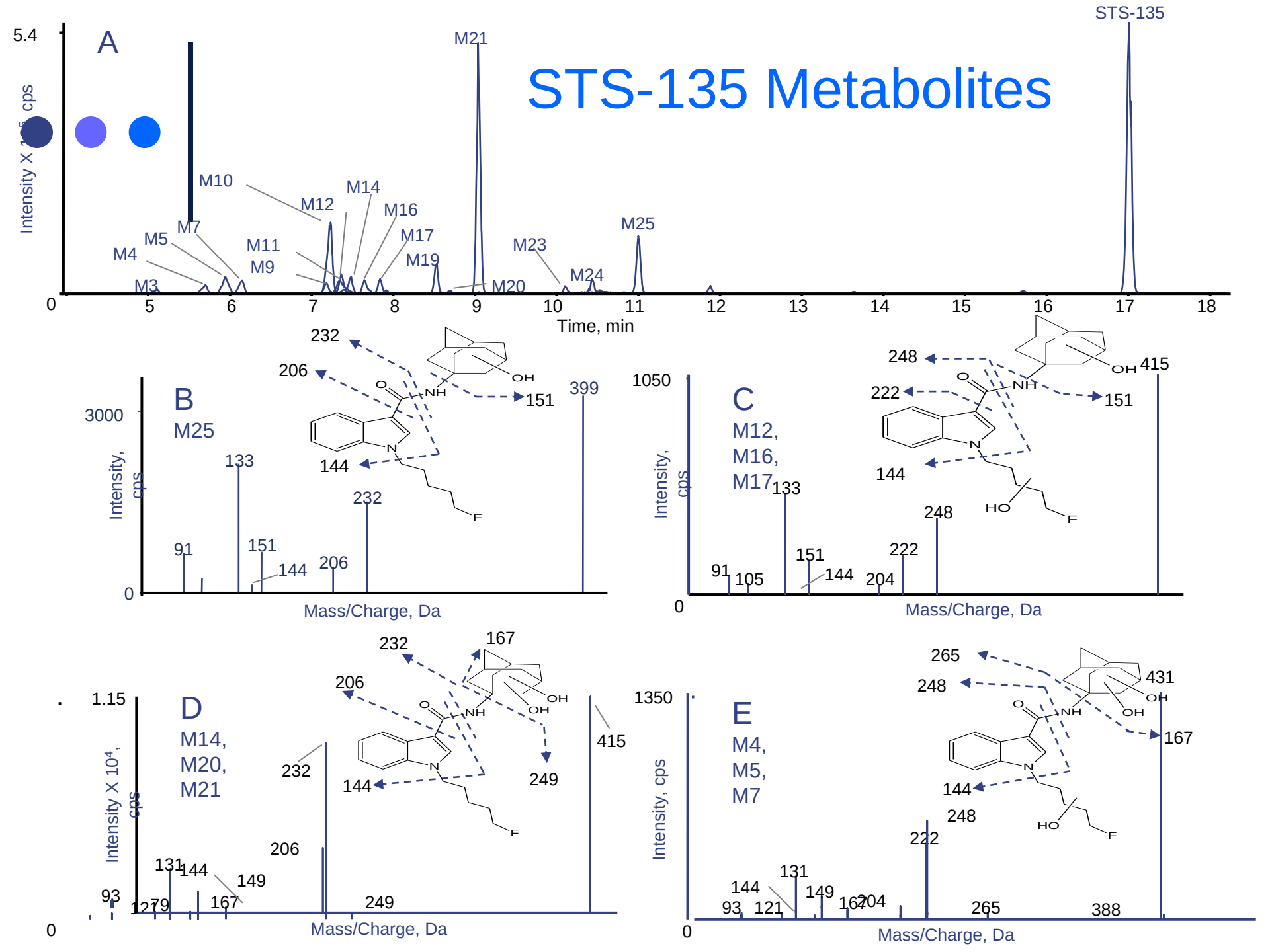


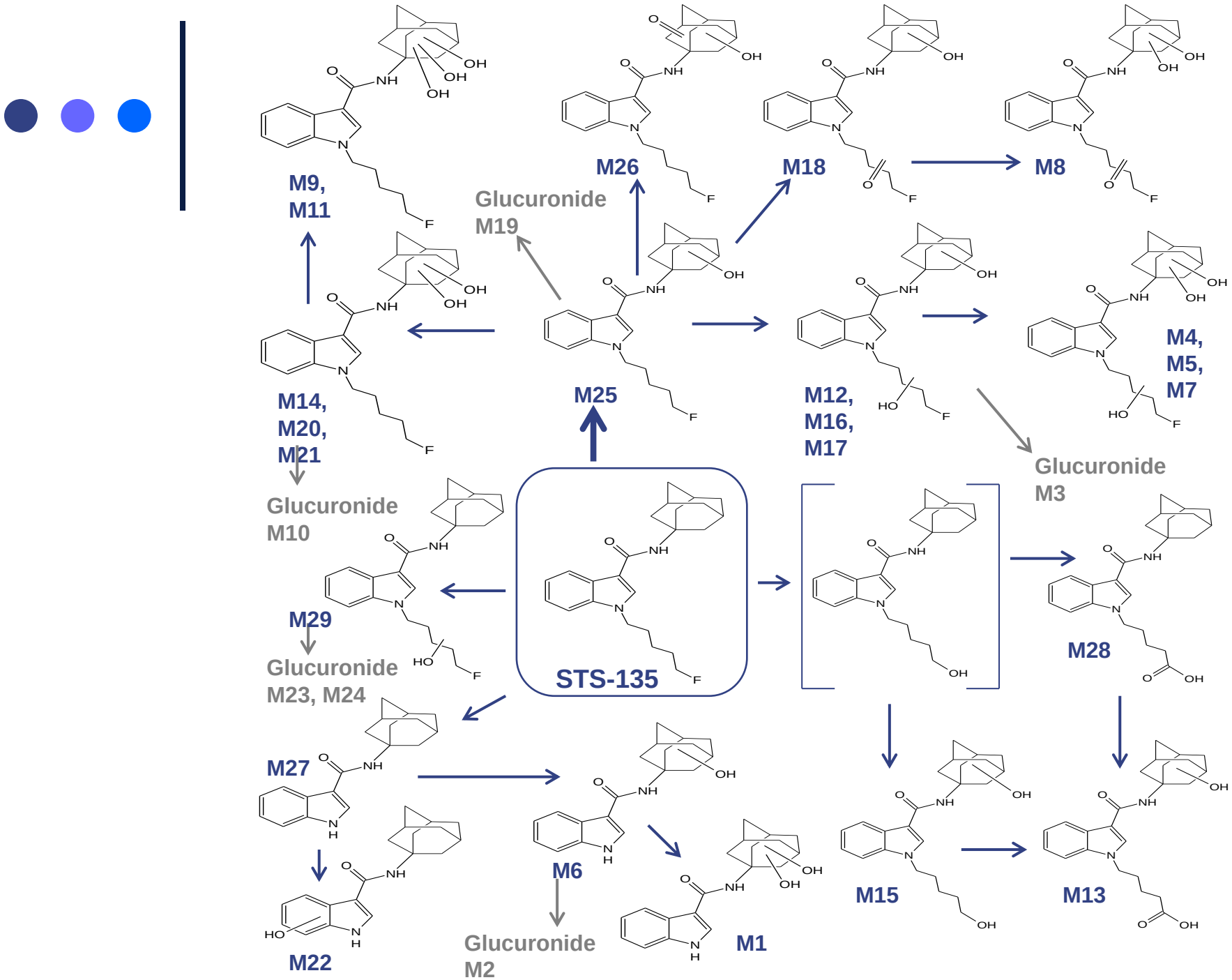
M19



M22

STS-135 Metabolites




M9,
M11
M26
Glucuronide
M19
M18
M8
M14,
M20,
M21
M25
M12,
M16,
M17
M4,
M5,
M7
Glucuronide
M10
Glucuronide
M3
M29
STS-135
M28
Glucuronide
M23, M24
M27
M6
M15
M13
M22
Glucuronide
M2
M1

Determining Primary Human Urinary Metabolite Targets of Synthetic Cannabinoids

- Only interested in synthetic cannabinoids where nothing is known about human metabolism
- To date published targets for XLR-11, AKB-48, RCS-4, RCS-8, PB-22, 5F-PB-22, STS-135
- Ultimate goal is to conduct a controlled administration study of synthetic cannabinoid to document human pharmacology
- Compare others based on CB-1 & CB-2 cannabinoid receptor binding

Synthetic Cannabinoids

- Prosecutors & judges indicate need for human pharmacology data
- Designer drugs are the new face of drug abuse
- Present real challenges for clinicians, treatment providers, emergency departments, police, poison control centers, laboratories, legislators, prosecutors, workplace & anti-doping programs
- Important public health & safety issue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Thank you for your attention!