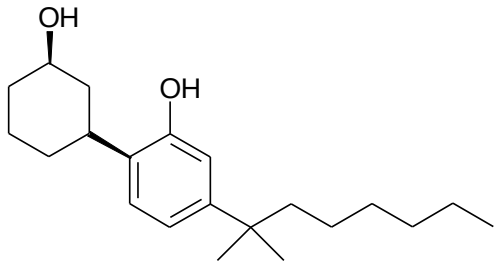
COLOR TESTS AND ANALYTICAL DIFFICULTIES WITH EMERGING DRUGS OF ABUSE

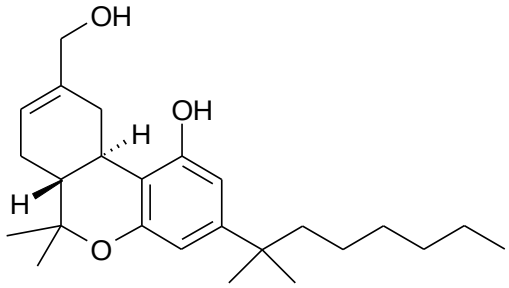
Jeremiah Morris

Johnson County Sheriff's Office
Criminalistics Laboratory

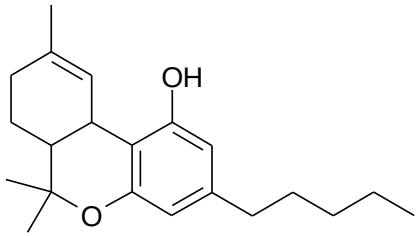
Synthetic cannabinoids



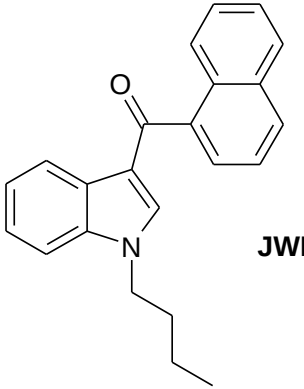
CP-47,497



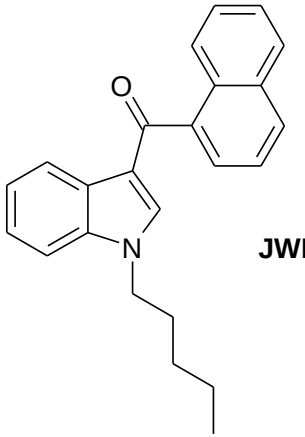
HU-210



Tetrahydrocannabinol



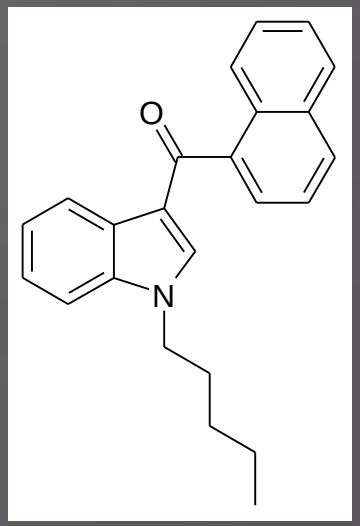
JWH-073



JWH-018

Presumptive tests - failures

- Nothing suitable published so far
- No color with Duquenois-Levine
- Structural interferences
  - para-Dimethylaminobenzaldehyde reagent (Ehrlich's)
  - Test with glutaconic aldehyde
- Vegetation interferences
  - Fast Blue B and 2B reagents
  - Sulfuric based color tests
  - UV fluorescence of indole nucleus
  - Color test for aromatic carbonyls



Presumptive test – success!

- Most cannabinoids react with Liebermann's

Reference	Cannabinoid Chemical Class	Source	Color
JWH-307	Naphthoylpyrrole	Reference Collection	Dark Yellow
AB-001	Adamantoyl indole	Reference Collection	Dark Yellow
CB-13	Dinaphthlyene methanone	Reference Collection	Dark Green
JTE-907	1,2-Dihydroquinoline-3-carboxamide	Reference Collection	Black (Bubbling)
UR-144	Tetramethylcyclopropanoylindole	Reference Collection	Dark Red
URB597	FAAH inhibitor	Reference Collection	Yellow-Brown
URB602	FAAH inhibitor	Reference Collection	Dark Brown
URB754	FAAH inhibitor	Reference Collection	Light Brown
AM-1248	Adamantoyl indole	Reference Collection	Dark Yellow
AB-034	Tetramethylcyclopropanoylindole	Reference Collection	Red-Orange>Dark Red
A-796, 260	Tetramethylcyclopropanoylindole	Reference Collection	Red-Orange>Dark Red
A-834-735	Tetramethylcyclopropanoylindole	Reference Collection	Red-Orange>Dark Red
FUR-144	Tetramethylcyclopropanoylindole	Reference Collection	Dark Red
AKB48	Adamantyl amidoindazole	Reference Collection	No color change
JWH-073	Naphthoylindole	Cayman Chemical	Yellow-Brown
JWH-018	Naphthoylindole	Cayman Chemical	Yellow-Brown
JWH-200	Naphthoylindole	Cayman Chemical	Dark Yellow-Brown
AM-2201	Naphthoylindole	Cayman Chemical	Yellow-Brown
JWH-203	Phenylacetylindole	Cayman Chemical	Yellow-Orange
RCS-4-C4 homolog	Benzoylindole	Cayman Chemical	Brown
AM694	Benzoylindole	Cayman Chemical	Dark-Yellow
MAM2201	Naphthoylindole	Cayman Chemical	Green-Brown
AM2233	Benzoylindole	Cayman Chemical	Yellow
STS-135	Adamanty lamidoindole	Reference Collection	Brown

Extraction procedure

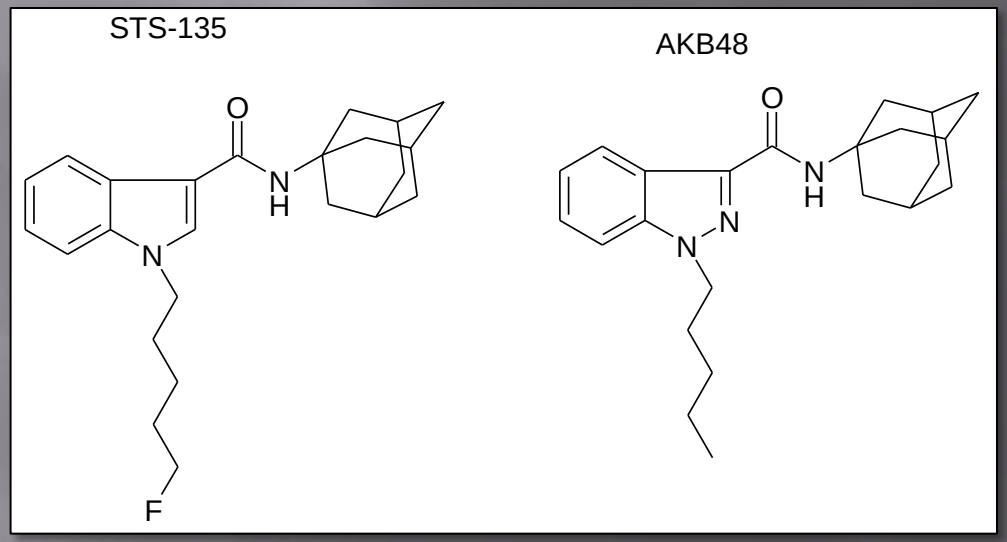
- A small amount of vegetative sample was added to a clear test tube followed by enough methylene chloride-acetonitrile solution to fully immerse the sample. The tube was then shaken quickly and the liquid was immediately pipetted off of the sample and into another clear test tube. Several drops of Liebermann's reagent were then added to the liquid and mixed thoroughly. Samples containing synthetic cannabinoids formed a yellow, yellow-orange, orange, to orange-red color. A negative result was indicated by no color change or a white color. A blank was also prepared for side-by-side comparisons of the blank and the samples.

Results of commercial products

Product Name	Cannabinoids Present	Color
Green Buddha	AM-2201, JWH-122, AM-2233	Yellow-Orange
Zombie Matter	Fluoro UR-144	Yellow-Orange
Space Monkey	AM-2201, JWH-122	Yellow
K4	Fluoro UR-144	Orange
Juicy Chong	AM-2201	Light Yellow
Cherry Cheech	AM-2201	Light Yellow
Hindu Magic	AM-2201	Light Yellow
Ultra Cloud 10	Fluoro-amphetamine, AM-2201	Yellow-Green
Happy Hour	None	White
Wicked X	None	Clear
Canna Boost	No Cannabinoids, Yohimbine	Light Orange
Bang!	JWH-250, JWH-081	Yellow-Orange
Devilz Lettuce	None	White-Yellow
Mary Jane Private	UR-144, JWH-122	Orange
Mary Jane Watermelon	UR-144	Orange
Impact Cotton Candy	AM-2201	Yellow
Impact Blueberry	UR-144	Orange
Mind Wave Blueberry	UR-144	Orange
Marijuana	None	Dark Brown
Salvia	None	Light Brown
Mary Jane Kratom	None	Brown (Bubbling)
White Rabbit KratomMaeng Da	None	Blue>Brown (Bubbling)

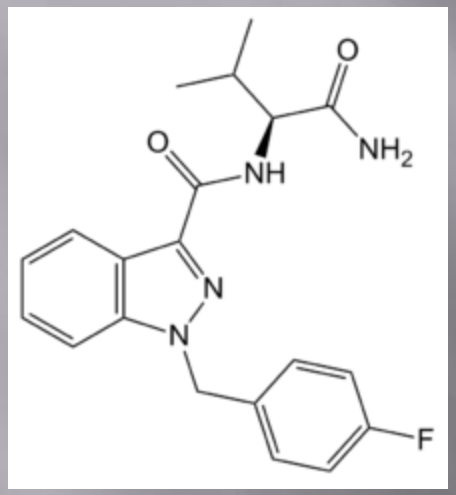
Other comments about presumptive testing

- AKB48 (an adamantyl indazole carboxy amide) does not produce a color with Liebermann's

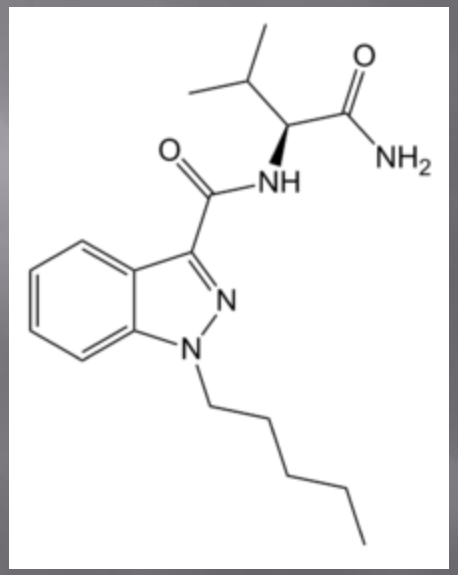


- Other positive reactions reported with Meyer's and other general alkaloid reagents

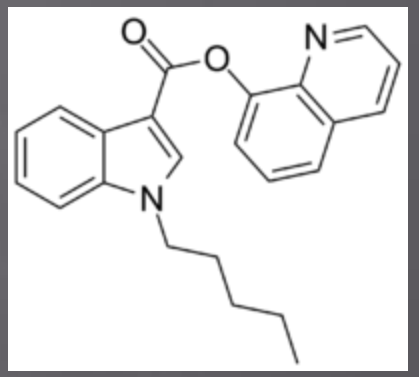
What about these cannabinoids?



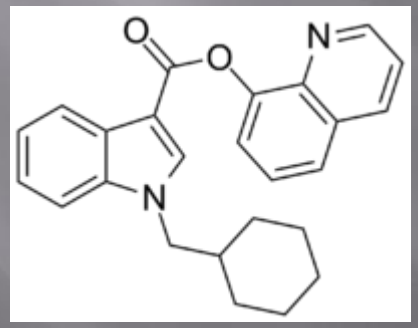
AB-FUBINACA



AB-PINACA



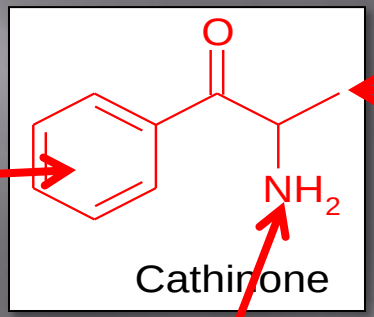
PB-22



BB-22

Substituted cathinones

Unsubstituted
3- or 4-methyl
3- or 4-halo (F, Cl, Br, or I)
3- or 4-ethyl
3- or 4-hydroxy
3- or 4-methoxy
3,4-methylenedioxy
3,4-dimethyl
3,4-dihalo (F, Cl, Br, or I)
Replace phenyl with naphthyl



Propyl
Butyl
Pentyl
Hexyl

Unsubstituted
N-methyl
N-ethyl
N,N-dimethyl
Pyrrolidine
Phthalamido
N-benzyl

Grand total of 672 possible combinations

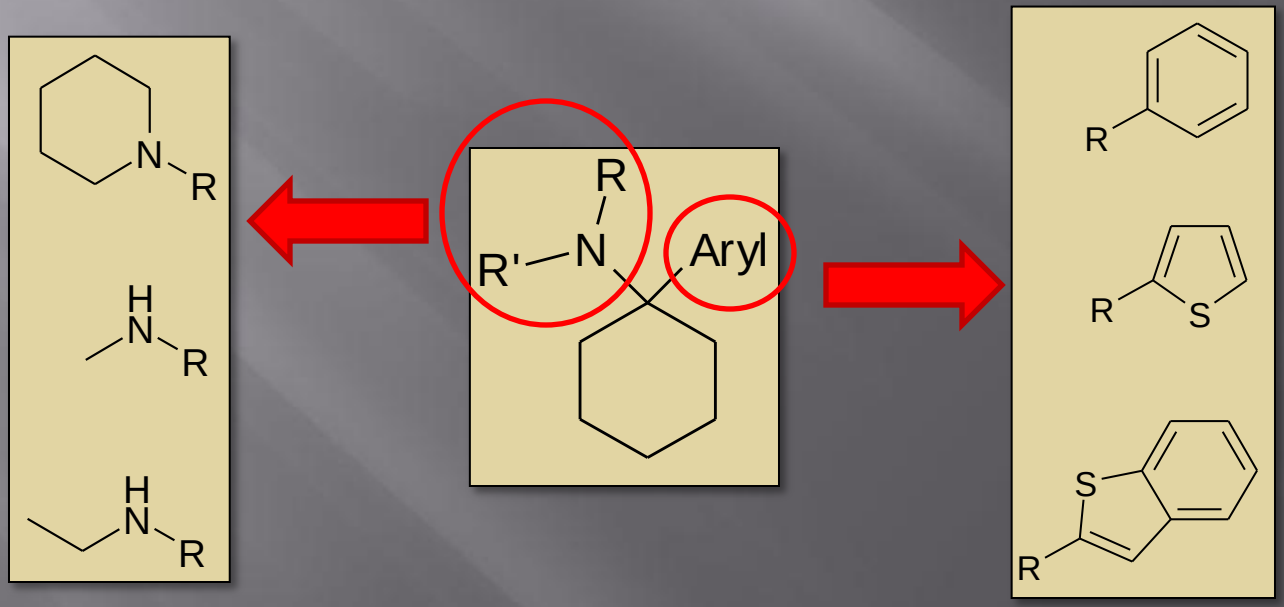
Presumptive tests

Compound	Marquis	Liebermann's	Mecke	Froedhe	CoSCN (n)	Na nitro	Modified CoSCN
4-Me-PPP	---	Orange	---	---	Blue	Slow blue	---
Brephedrone	---	Yellow	---	---	---	Blue	Purple
4-MEC	---	Orange	---	---	Blue	Blue	Purple
Pentedrone	---	Yellow	---	---	Blue	Blue	Light purple/blue
4-methyl buphedrone	---	Yellow	---	---	Blue	Blue	Blue
Buphedrone	---	Yellow	---	---	---	Blue	---
Butylone	Yellow	Yellow→ brown	Yellow→ orange	Yellow→ green	Blue	Blue	Purple
3, 4-DMMC	Green particles (?)	Orange	---	Light brown	Blue	Blue	---
Naphyrone	Green	Brown	Brown-orange	Orange	---	---	---
Benzedrone	---	Orange	---	---	---	---	---

Note – all produce either no color or just blue specks with *acidified* CoSCN

Arylcyclohexylamines

- Comprise the most common class of dissociatives
  - Complex pharmacology
  - CNS appears dose dependent and spans entire range

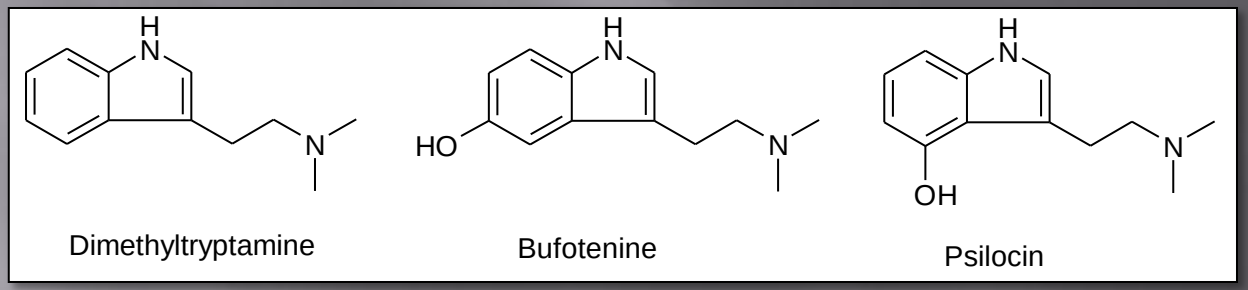


Presumptive tests

Compound	Marquis	Liebermann's	Mecke	Froedhe	CoSCN (n)	Na nitro	Modified CoSCN
4-MeO-PCP	Slow red	Brown	Yellow→ green→ red	Light yellow	Blue	---	---
Methoxetamine	Slow pink	Orange-brown	Yellow→ green→ red	Yellow-green	Blue	---	---
Ethketamine	---	Pale yellow	---	---	Blue	---	Lavender ppt
3-HO-PCE	Brown	Dark brown	Brown	Black	Weak blue	---	---

Tryptamines

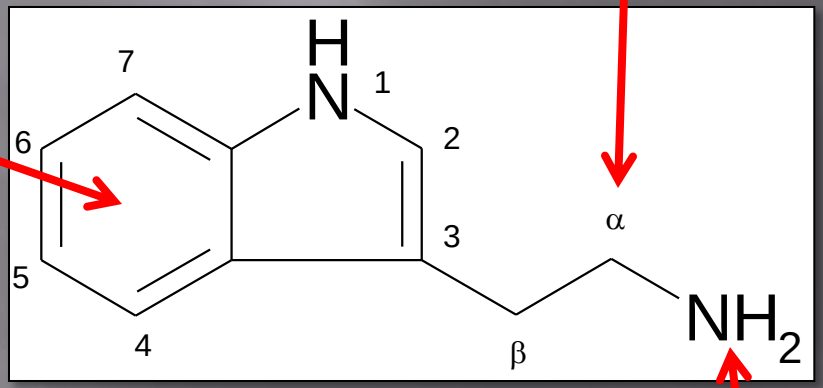
- Class of highly potent hallucinogens
  - Present in a diverse group of botanical materials
  - All contain substituted indole compound



Variations on a tryptamine theme

Methyl or ethyl with mono-N alkyl substitution

Hydroxyl, methoxy, acetoxy, halo



Alkyl or dialkyl substitution (methyl, ethyl, propyl, isopropyl, allyl

Presumptive tests

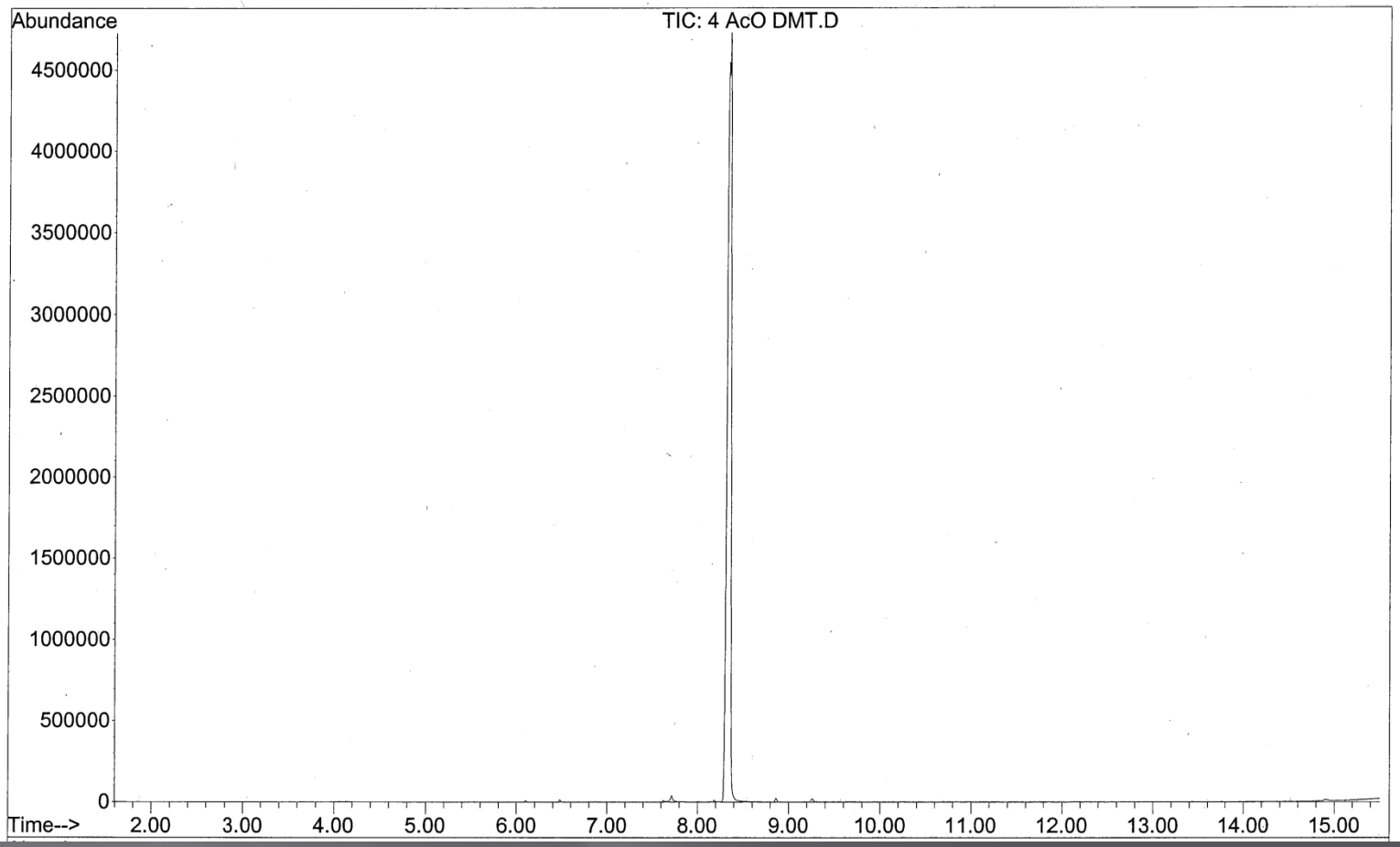
Compound	Marquis	Liebermann's	Mecke	Froedhe	CoSCN (n)	Na nitro	PDMB
5-MeO-DALT	Olive→ black	Dark brown/black	Olive→ brown	Yellow	---	---	Purple
4-methyl-aET	Light brown	Brown	Brown	---	---	---	Purple
4-AcO-DALT	Yellow→ brown	Black	Black	Yellow→ green	Blue	---	Purple
4-HO-MET	Yellow→ brown	Black	Black	Yellow→ green	Blue	---	Purple
4-HO-MIPT	Yellow→ brown	Black	Black	Yellow→ green	Blue	---	Purple
4-AcO-DET	Yellow→ brown	Black	Black	Yellow→ green	Blue	---	Purple

Issues with acetoxy compounds

- Vendors beginning to sell acetoxy tryptamines
  - 4-AcO-DMT (acetylated psilocin)
  - 5-AcO-DALT
- A number of reports about 4-AcO-DMT being unstable and converting into psilocin
  - As solid (slightly over a few months)
  - In solution (within a day)
  - During acid-base extractions
- This is a concern because psilocin is controlled while 4-AcO-DMT is not.

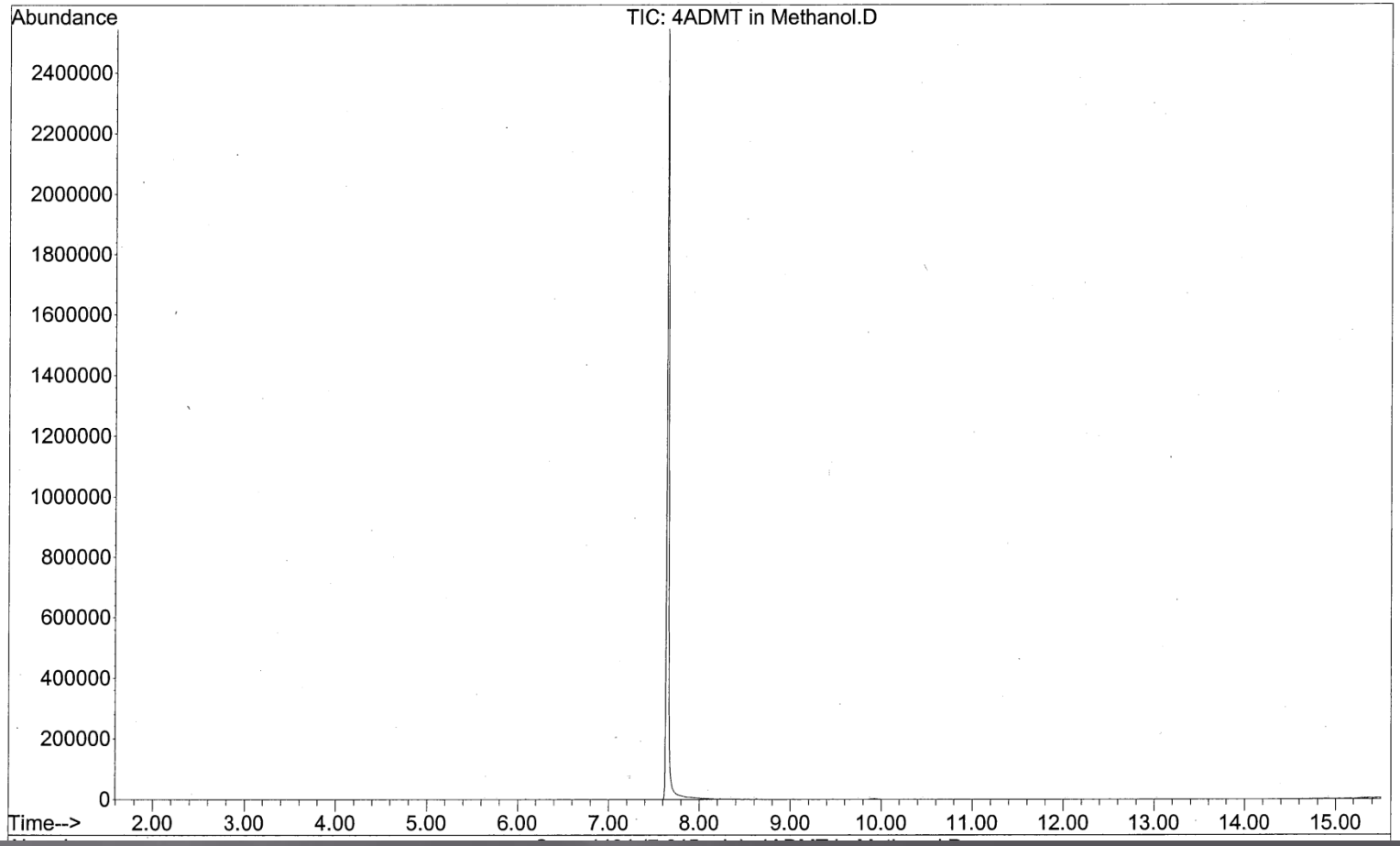
Initial analysis

```
File         :C:\MSDChem\1\DATA\MFRC screen data\4 AcO DMT.D
Operator     :
Acquired     : 21 May 2012  14:21     using AcqMethod TEMPRO.M
Instrument   :    HP1
Sample Name: 4-acetoxy-dimethyltryptamine
Misc Info    : 4-AcO-DMT, lot# MFRC-2012-A44
Vial Number: 13
```



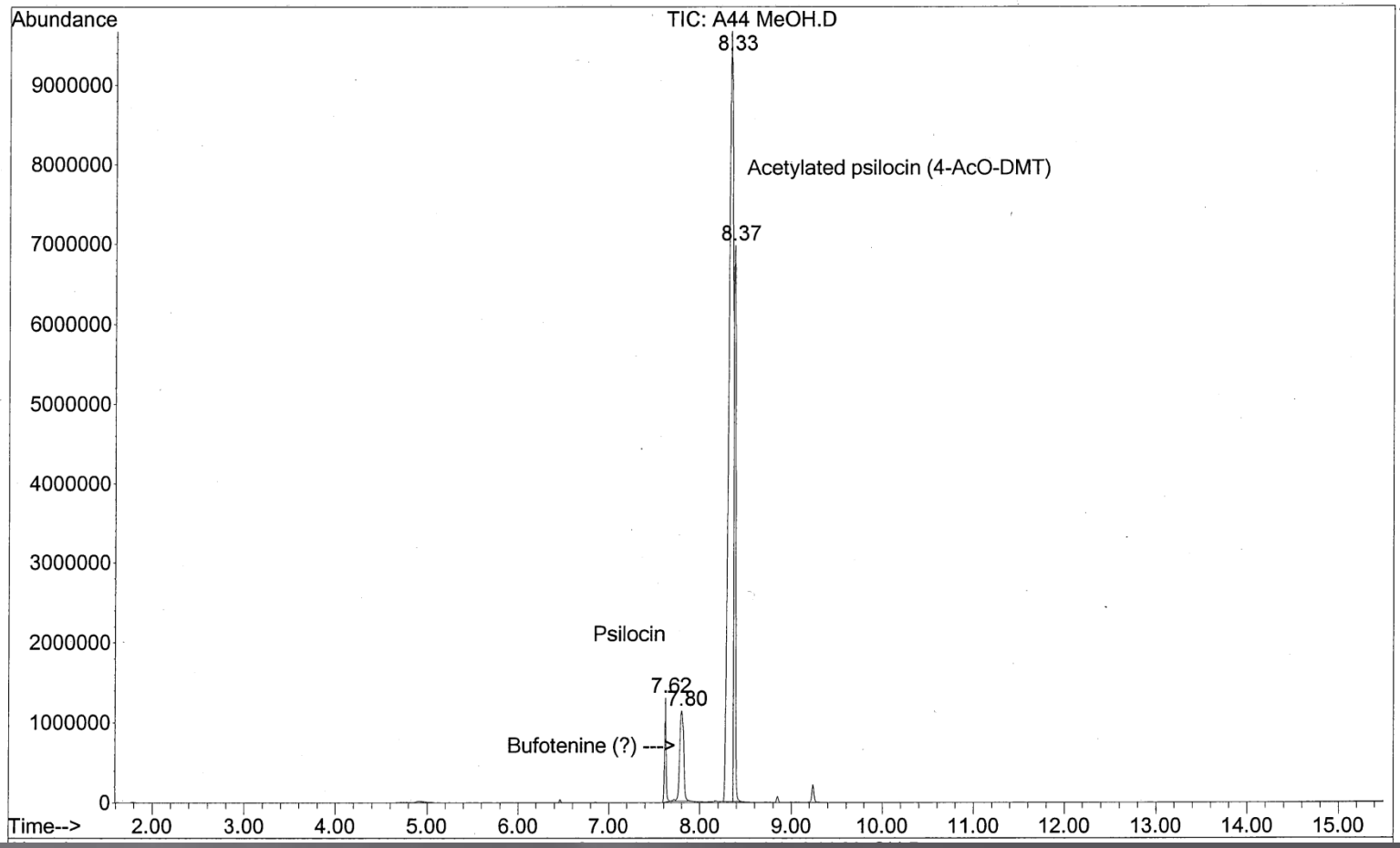
One month later

```
File         :C:\MSDChem\1\DATA\MFRC OFFICIAL DATA\4ADMT in Methanol.D
Operator     :
Acquired     : 16 Jun 2012  00:21      using AcqMethod TEMPRO.M
Instrument   :     HP1
Sample Name: 4-Aco-DMT A44
Misc Info    : Lot# MFRC-2012-A44, in basic Methanol
Vial Number: 43
```



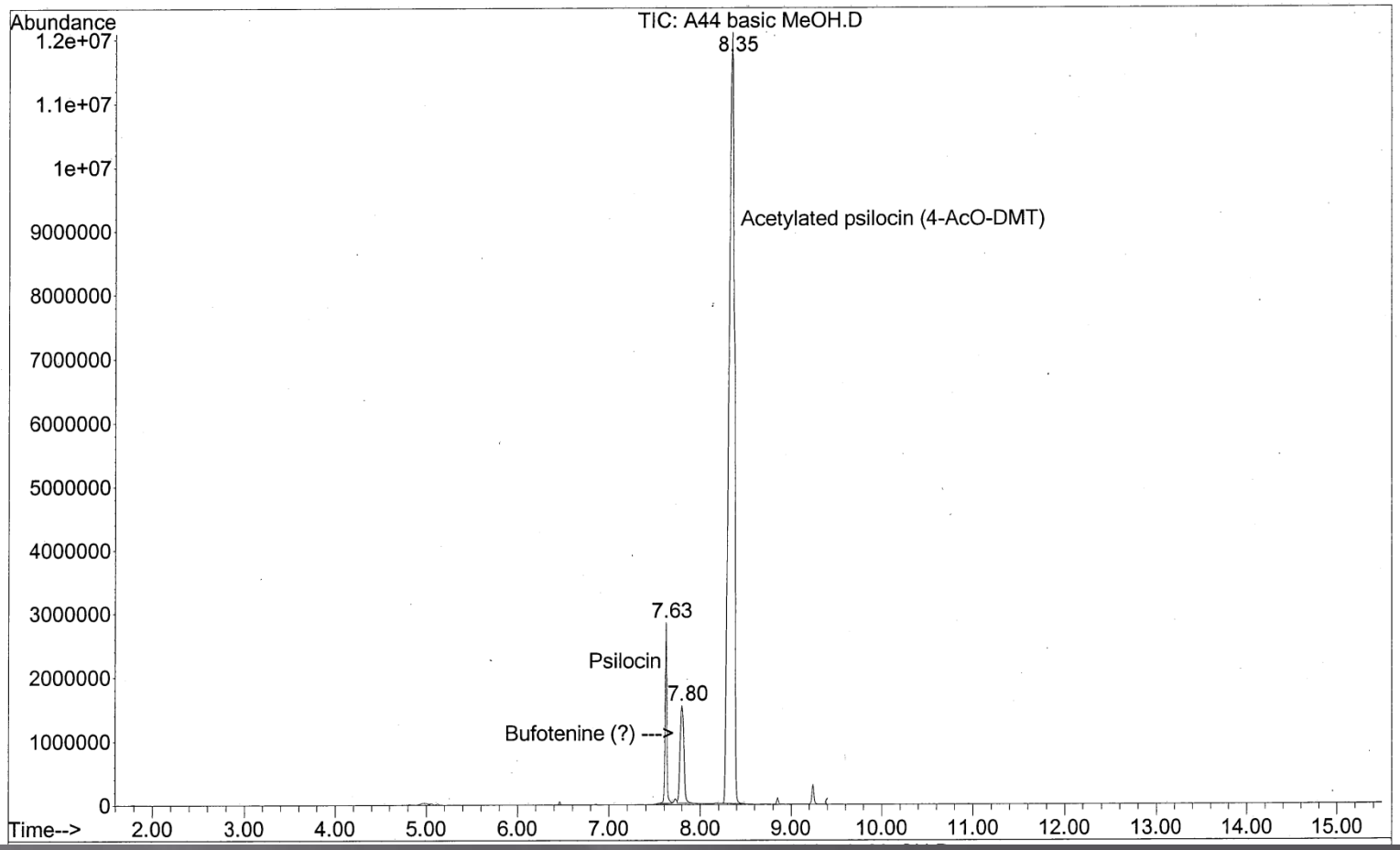
Two months later

```
File       :C:\MSDChem\1\DATA\080712\A44 MeOH.D
Operator   :
Acquired   :  7 Aug 2012  16:21     using AcqMethod TEMPRO.M
Instrument :    HP1
Sample Name: A44
Misc Info  :
Vial Number: 22
```



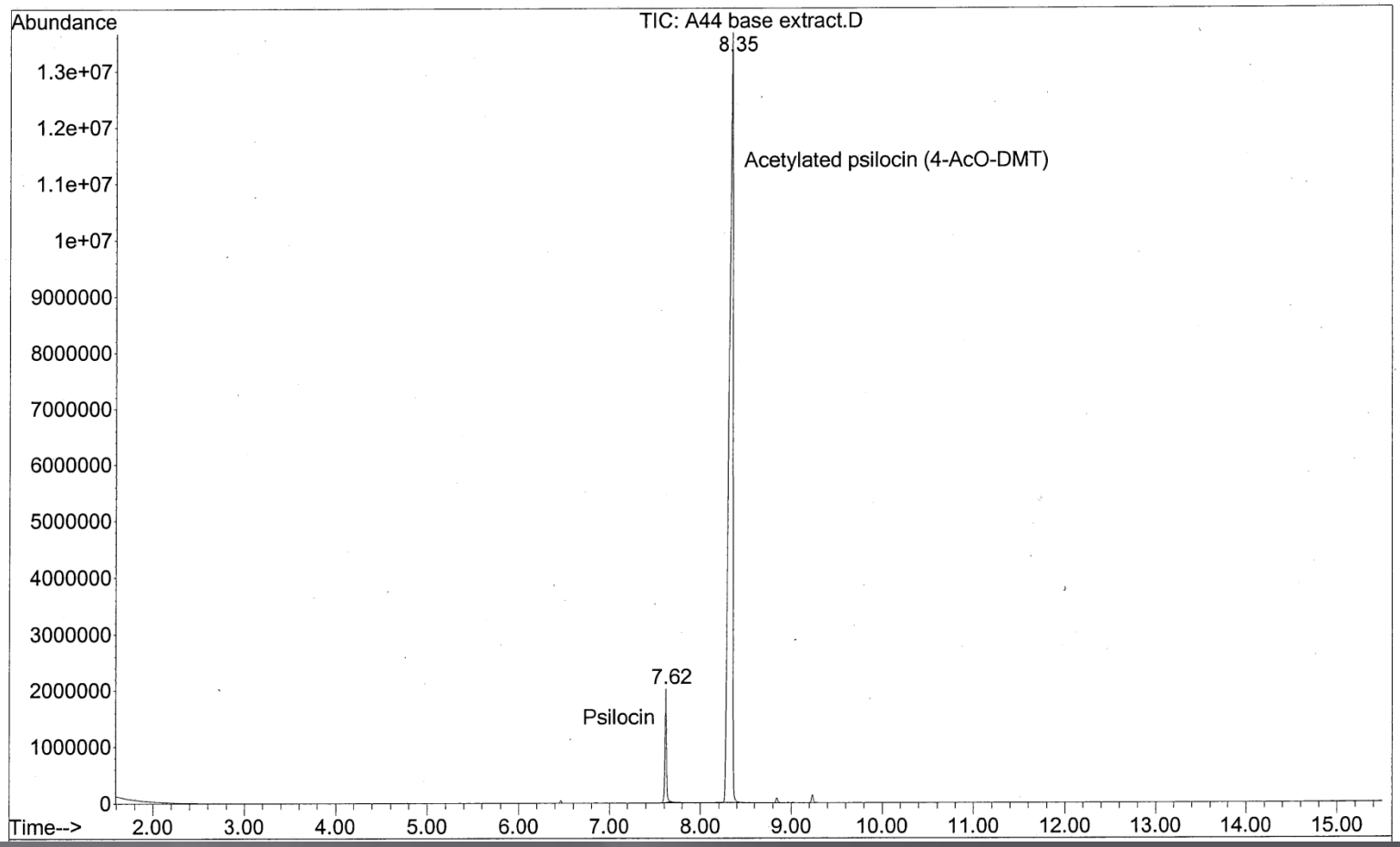
Basic methanol

```
File        :C:\MSDChem\1\DATA\080712\A44 basic MeOH.D
Operator    :
Acquired    :  7 Aug 2012  16:41     using AcqMethod TEMPRO.M
Instrument  :    HP1
Sample Name: A44
Misc Info   :
Vial Number: 23
```



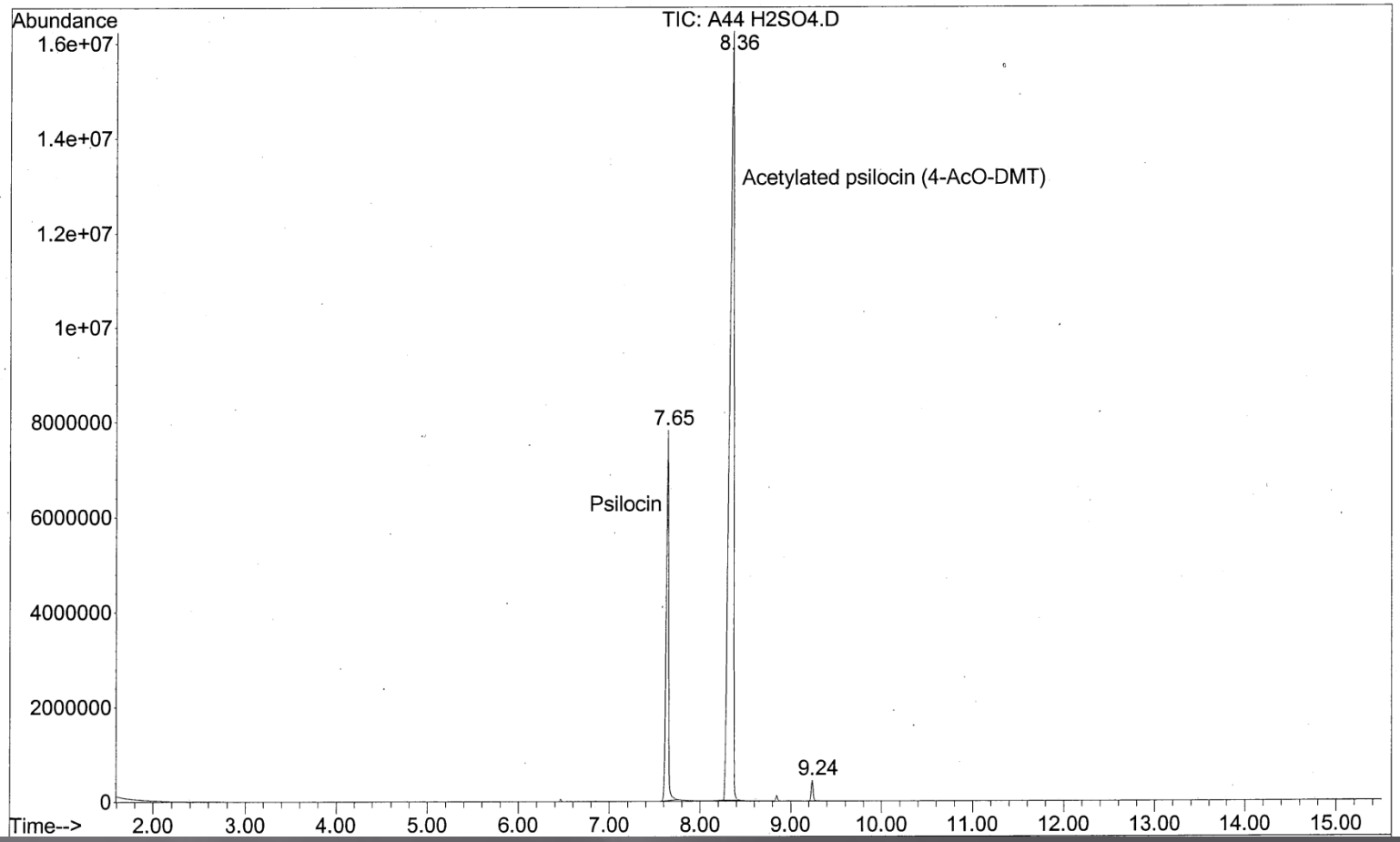
Basic extract

```
File       :C:\MSDChem\1\DATA\080712\A44 base extract.D
Operator   :
Acquired   :  7 Aug 2012  17:00     using AcqMethod TEMPRO.M
Instrument :    HP1
Sample Name: A44
Misc Info  :
Vial Number: 24
```



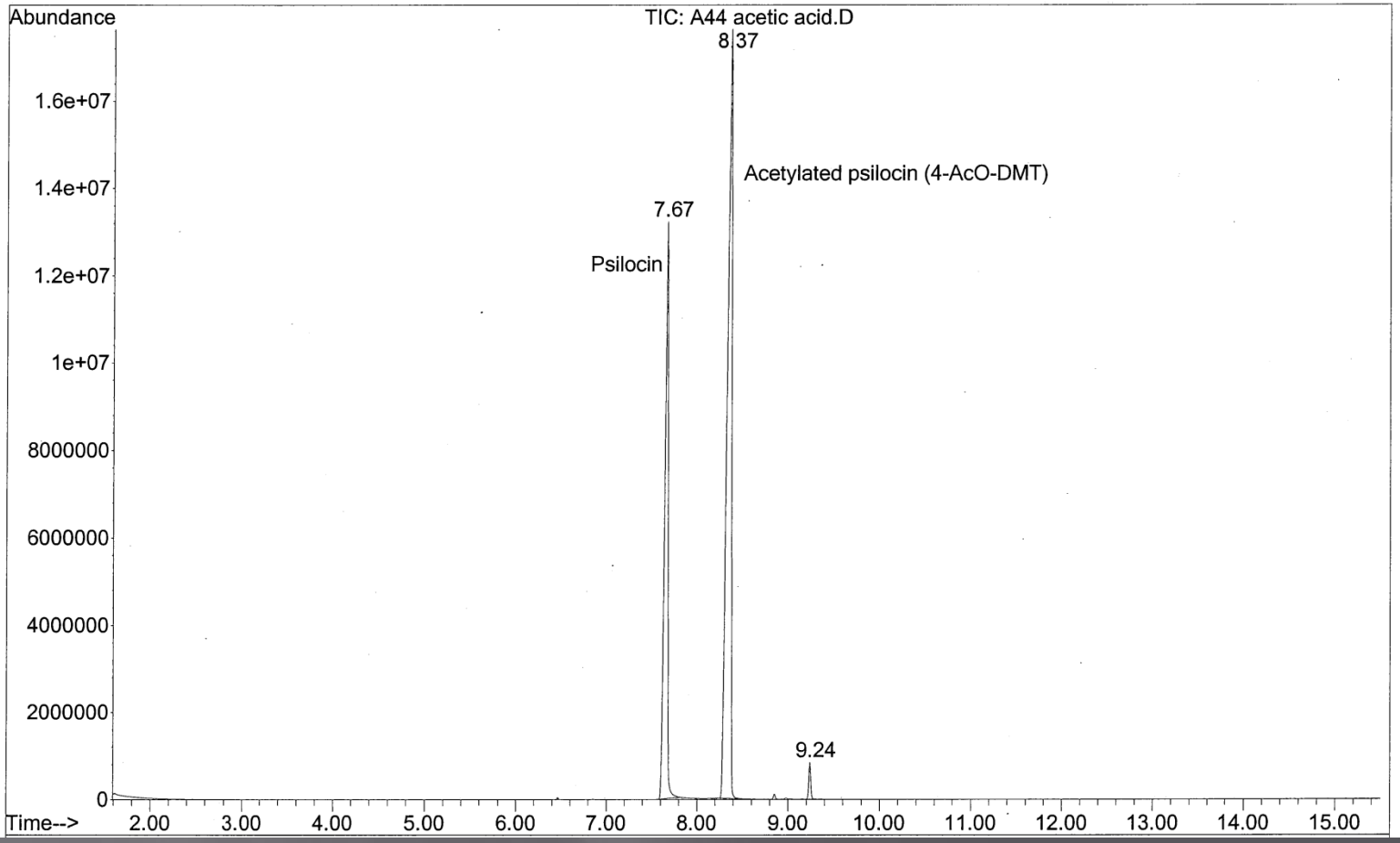
Acid/base extract (H_2SO_4)

```
File        :C:\MSDChem\1\DATA\080712\A44 H2SO4.D
Operator    :
Acquired    :  7 Aug 2012  17:20     using AcqMethod TEMPRO.M
Instrument  :    HP1
Sample Name: A44
Misc Info   : 0.1 N H2SO4 then base extract
Vial Number: 25
```



Acid/base extract (acetic acid)

```
File         :C:\MSDChem\1\DATA\080712\A44 acetic acid.D
Operator     :
Acquired     :  7 Aug 2012  17:40     using AcqMethod TEMPRO.M
Instrument   :    HP1
Sample Name: A44
Misc Info    : 10% acetic acid then base extract
Vial Number: 26
```

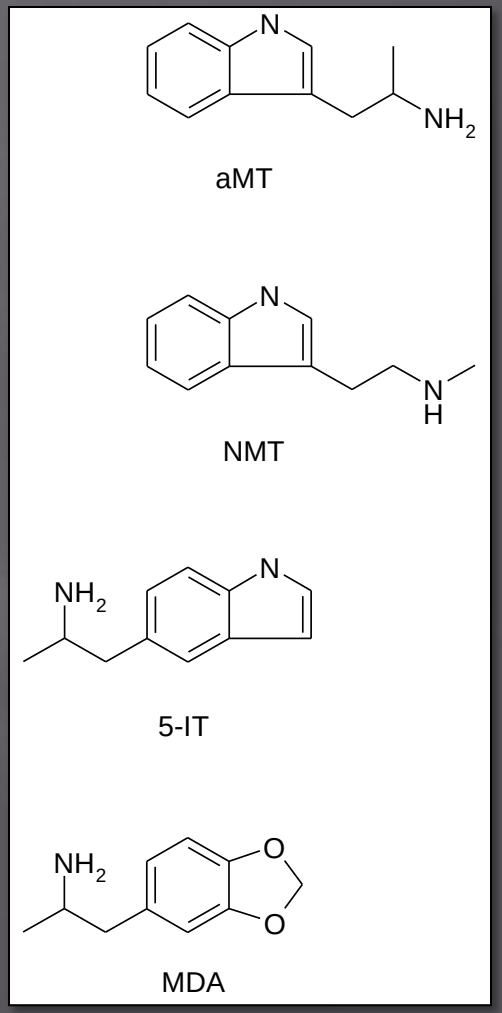


Recommendations

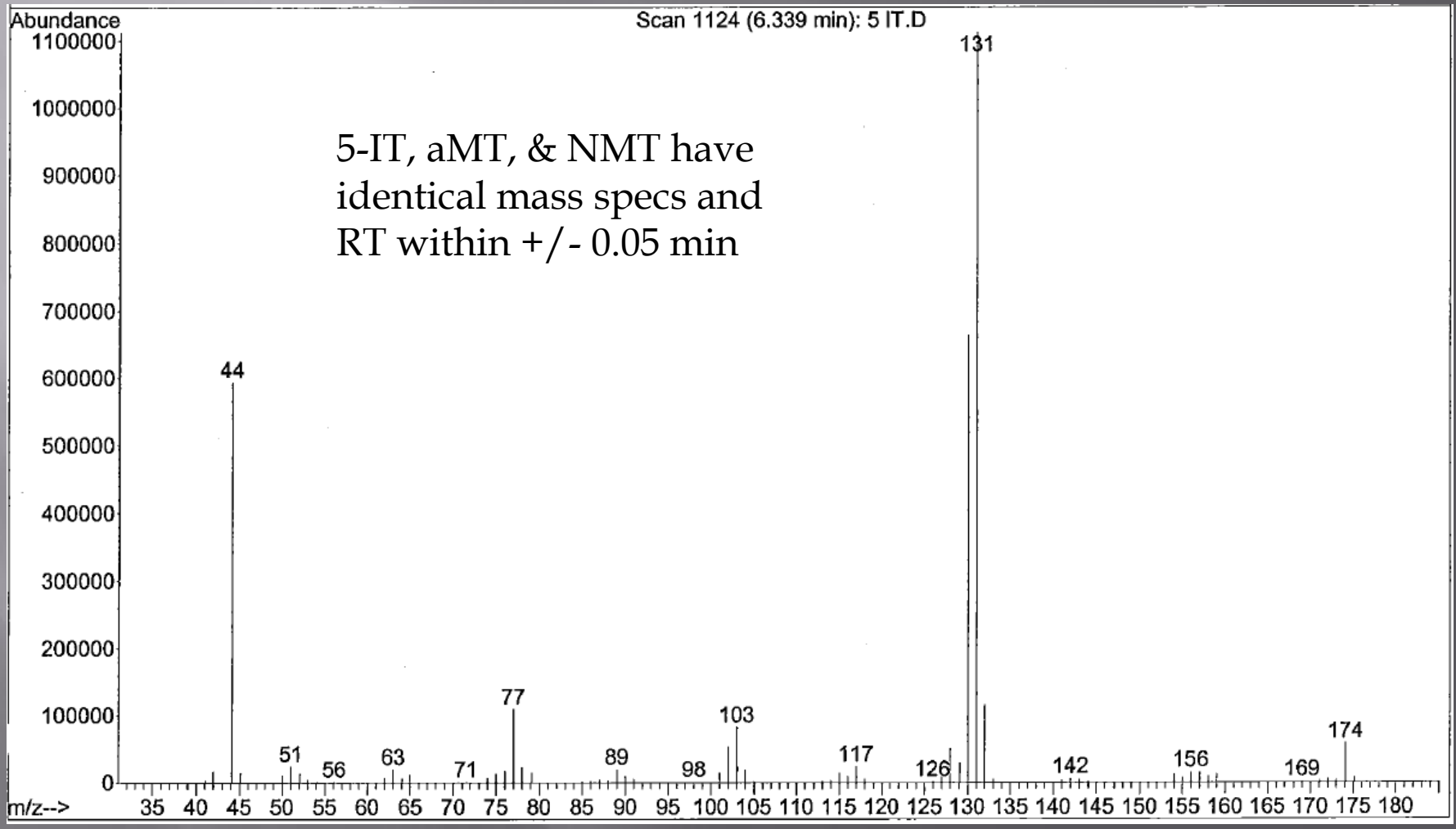
- Remember, these are just my thoughts!
- If your 4-AcO-DMT sample has low levels of psilocin you don't know if it was there originally (degradation over time)
- All psilocin/4-AcO-DMT mixtures should be re-analyzes using either methanol extract or basic methanol extract to exclude extraction degradation
- Be *extremely* cautious about reporting out psilocin in these cases

5-IT

- 5-(2-Aminopropyl)indole
  - 5-IT
  - Only recently hit markets in Europe
  - Positional isomer of *alpha*-methyltryptamine (aMT) and N-methyltryptamine (NMT)
  - 5-IT and aMT often sold by same vendors
- This is a problem

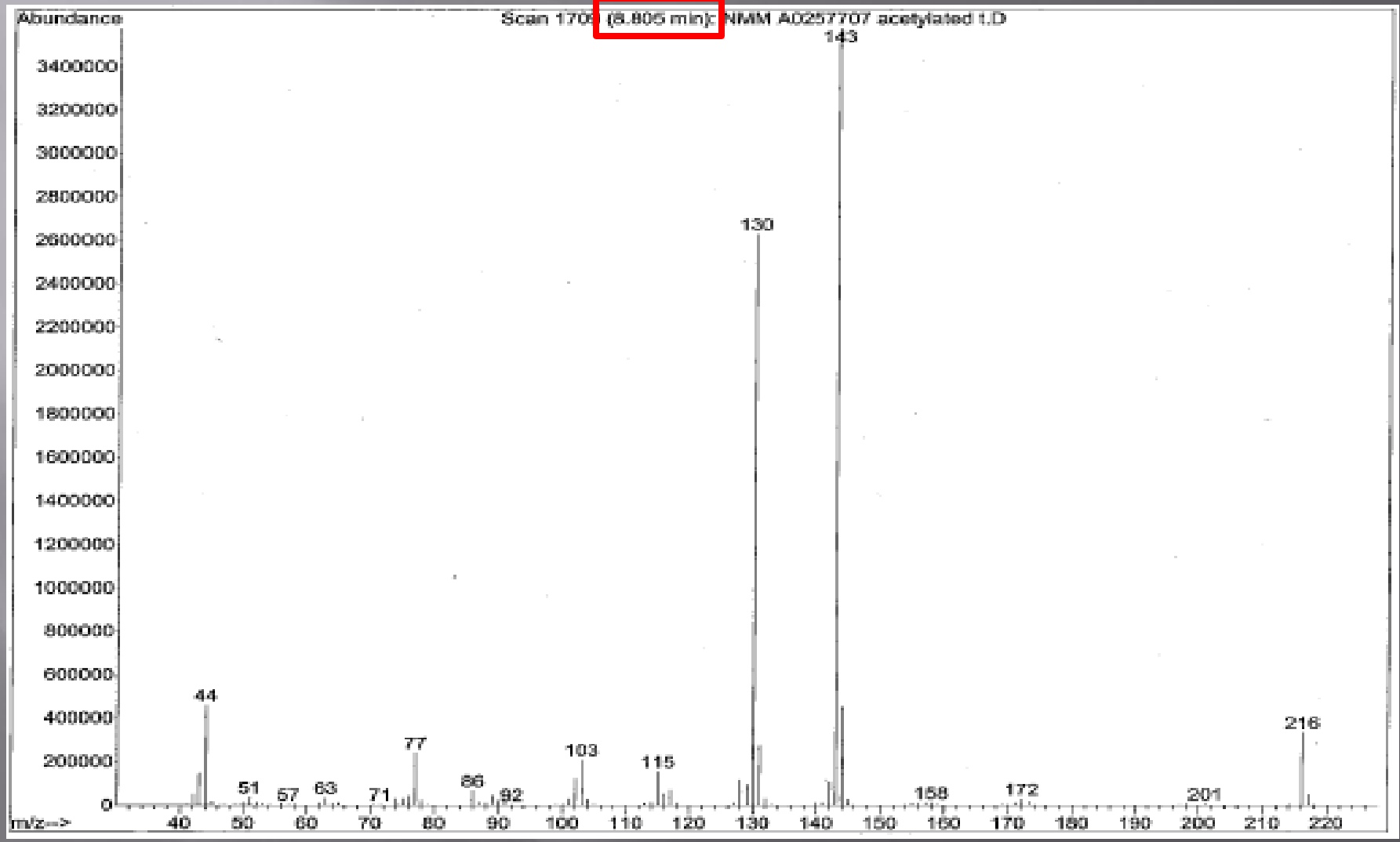


GC/MS results

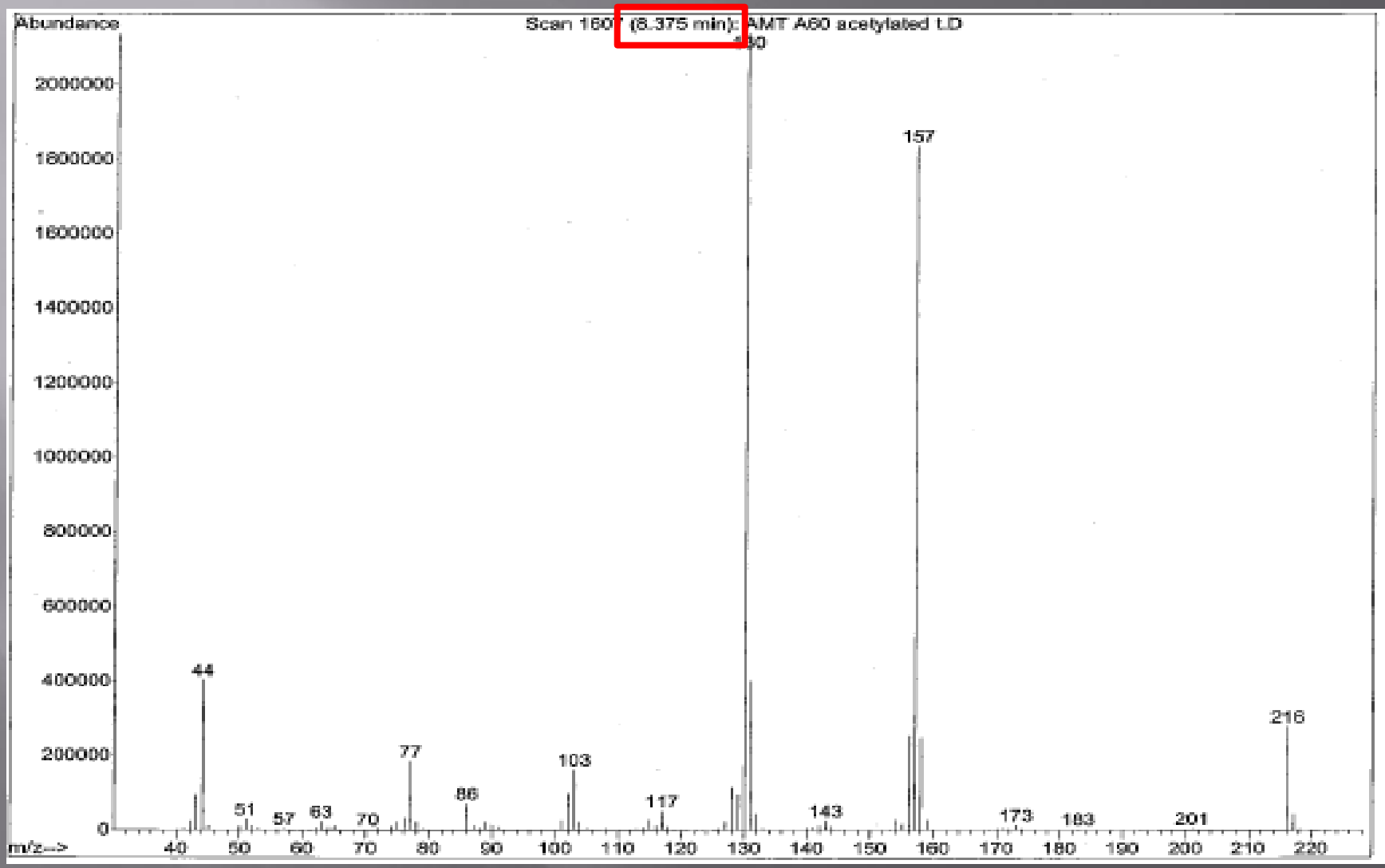


5-IT, aMT, & NMT have identical mass specs and RT within +/- 0.05 min

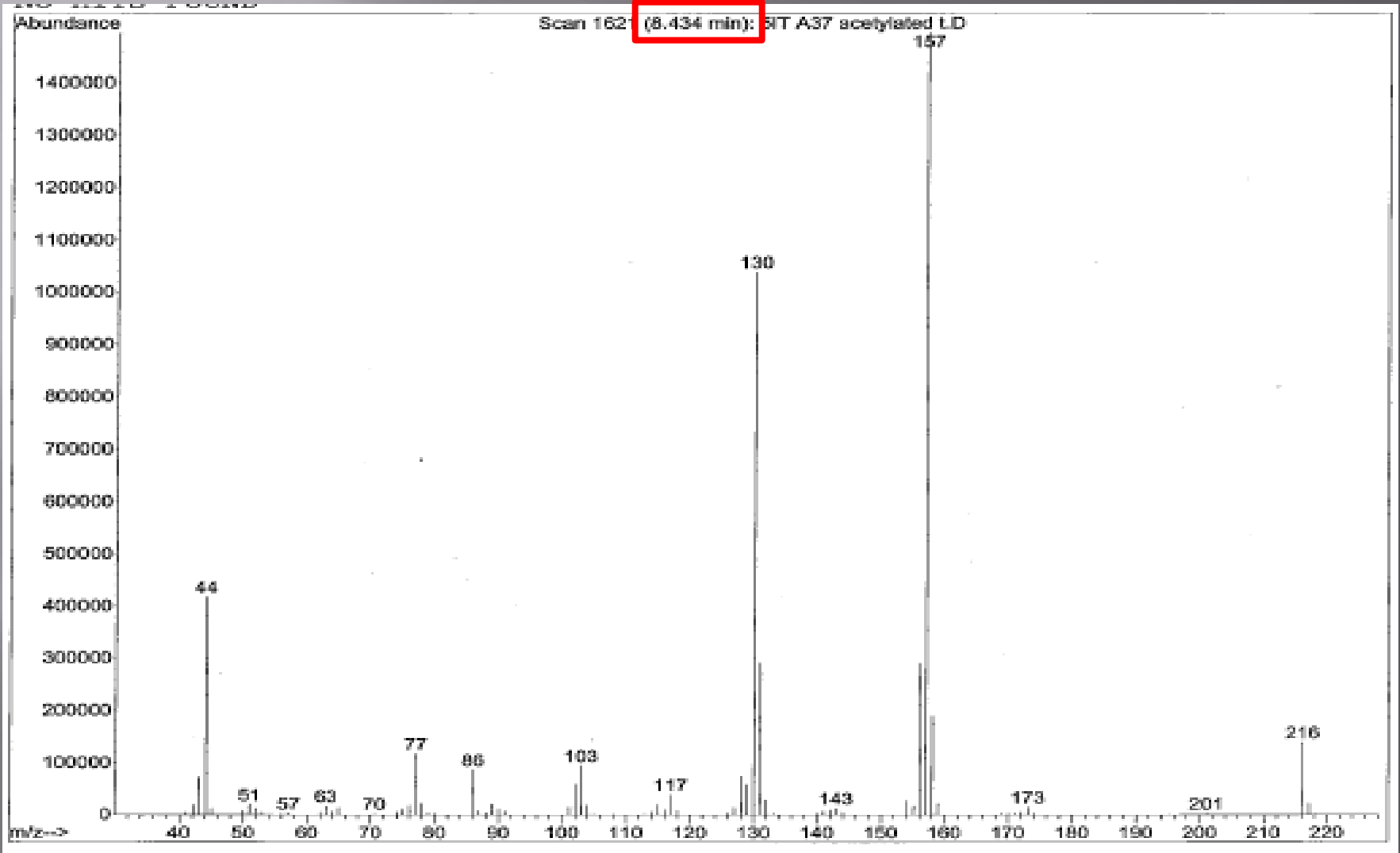
Acetylated NMT



Acetylated aMT



Acetylated 5-IT



Where we stand

- All three have identical MS and indistinguishable retention times as parent compounds
- NMT separates out from aMT and 5-IT when acetylated
- aMT and 5-IT have indistinguishable RTs but slightly different mass specs

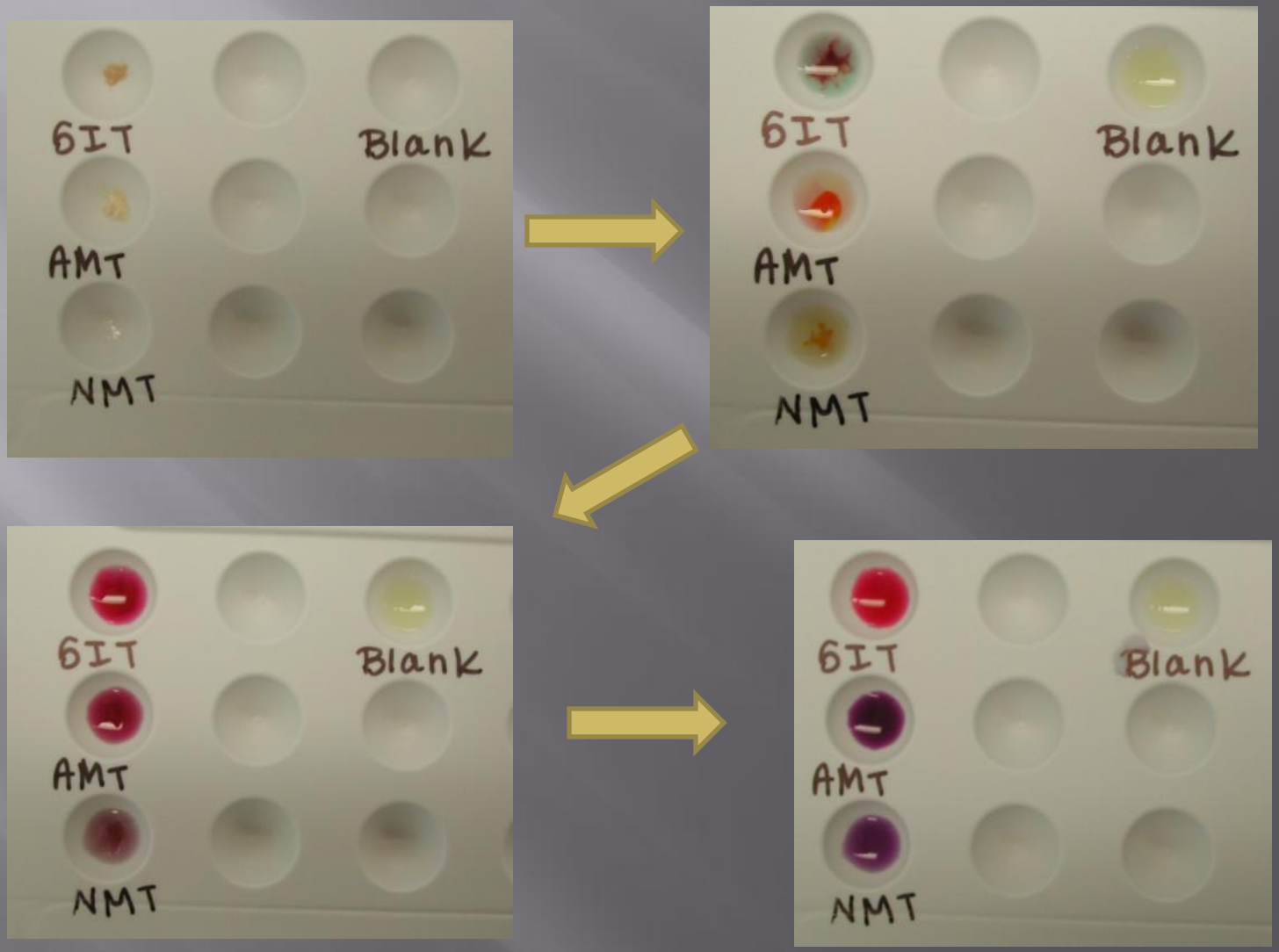
- Is this enough?

Other options

- FTIR
  - All three easily differentiated
  - Sample purity is critical
- TLC
  - Some Rf differences with *Clarke's* TA solvent (10 cm plate)
  - What about 20 cm plate?
- Color tests

Compound	Marquis	Liebermann	Mecke's	Froehde's	PDMB	Cinnamoyl
aMT	Yellow-brown	Black	Brown	Yellow	Purple	Red → purple
5-IT	Dark red	Dark brown	Brown	Red brown	Red	Red

p-Dimethylaminocinnamaldehyde reagent



What does this mean?

- NMT easily excluded with aceytlation
- 5-IT verses aMT? → Your call
  - If pure enough for IR, you're golden
  - Are aceytlated derivatives different enough?
  - Are color test differences enough?
- You must carefully consider what you can actually report out with possible aMT sample

Presumptive color tests (misc)

Compound	Marquis	Liebermann	Mecke's	Froedhe's	CoSCN	Na Nitro
5-APB	Black	Black	Black	Dark purple	---	---
6-APB	Purple	Dark purple	Purple	Purple	---	---
Camfetamine	Orange → red	Dark red	Yellow	Tan→ dark yellow	Blue	Blue
Methiopropamine	Dark brown	Dark brown	Black	Light brown	---	Blue
MDAI	Orange	Green→ black	Green	Green→ black	---	---
5-IAI	Brown	Dark brown	Brown	Orange	---	---
Allylescaline	Dark red	Brown-black	Yellow→ brown (fast)	Green→ black (fast)	---	Green particles
2C-T-2	---	Red	Orange→ red→ purple	Orange → purple	---	Green specks
2C-P	Yellow	Green	Green	Green	---	---
β-methoxy-2C-D	Purple	Green	Brown→ green	Red	---	---

Acknowledgements

- Special thanks to:
  - Malinda Spangler
  - Bethany Poyner
  - Jason Stenzel
  - Elizabeth Kiely

- This work was funded by subcontract SC-12-370 through Ames Laboratory and the Midwest Forensic Resource Center

Questions?