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Identification of Regioisomers via Gas Chromatography Coupled with Vapor-Phase Infrared Detection

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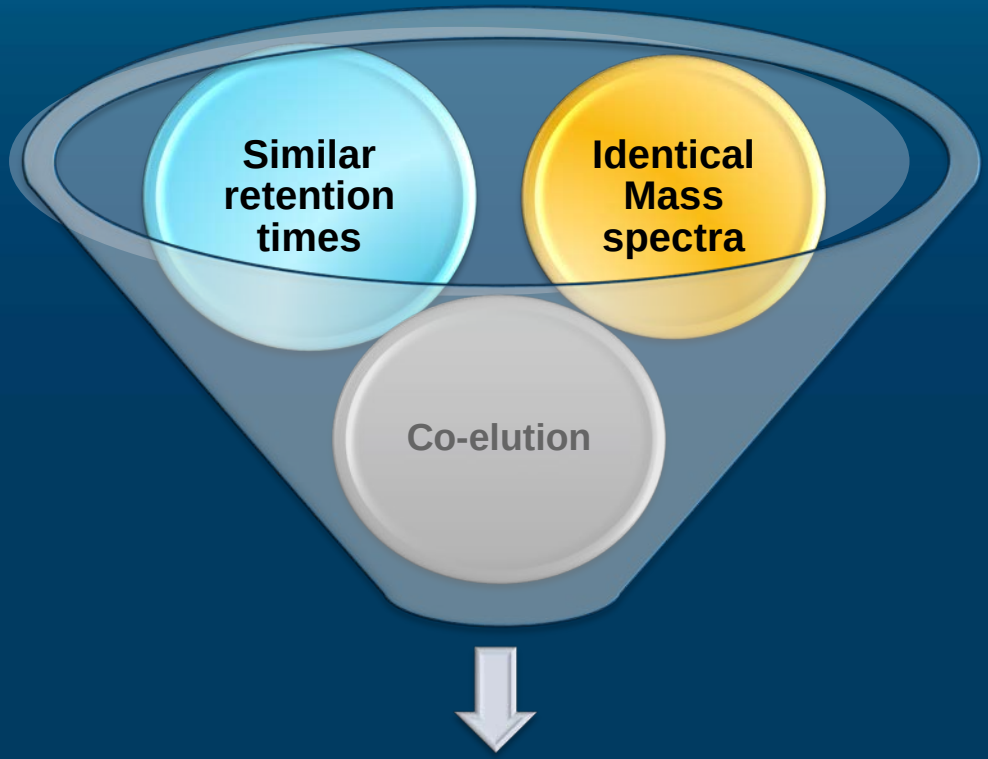
Disclosure

The content of this activity does not relate to any product of a commercial interest; therefore, there are no relevant financial relationships to disclose.

Regioisomer Identification Problem

- **Regioisomers present a problem in the Forensic Science community**

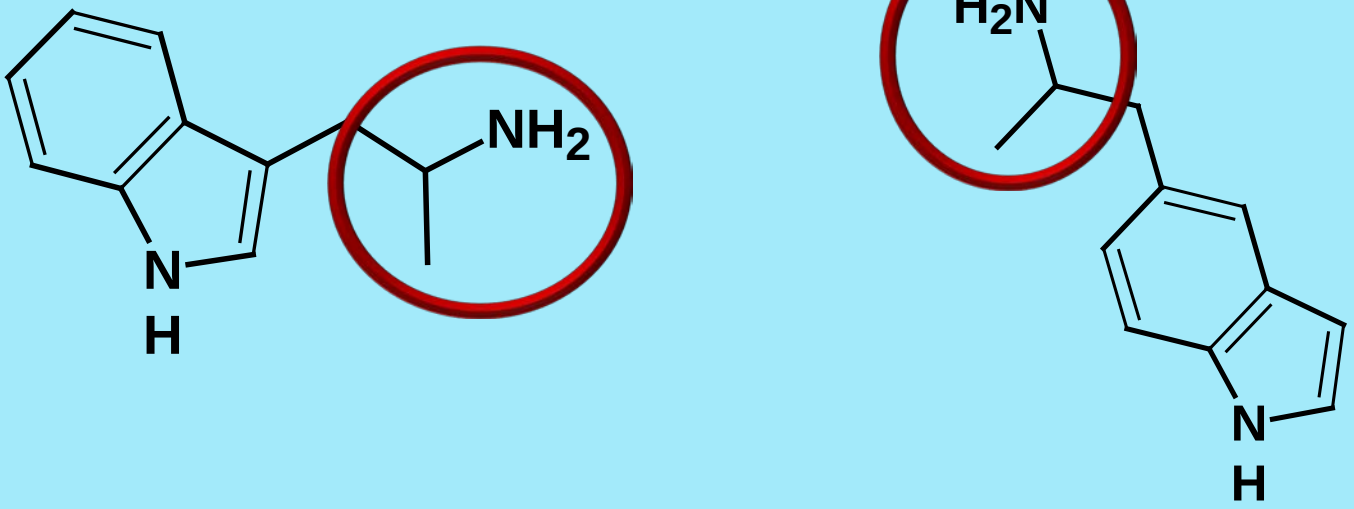


Issues observed in a Drug Chemistry Lab

AMT and 5-IT Issue at HCIFS

- **Elute at nearly identical retention times**
- **Display identical mass spectra**

AMT (α-Methyltryptamine)	5-IT (5-(2-Aminopropyl)indole)



AMT and 5-IT Issue at HCIFS

- **Cannot confidently differentiate between the two**
  - **Reported as AMT <Not confirmed> or 5-IT <Not confirmed>**

AMT vs. 5-IT

- First developed in the 1960s and highly uncommon until the 1990s
- Alternative to LSD
- AMT temporarily controlled Federally in April 2003
- Permanently in September 2004

- 5-IT was synthesized to replace AMT
- A positional isomer of AMT
- 5-IT is more potent
- Temporarily controlled Federally in June 2013

Summer Internship Project

Can we differentiate between 5-IT and AMT using GC-IRD?

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Huntsville, TX

GC-IRD Theory

- Combines separation power of GC with identification power of infrared spectrometry
- Uses a heater light pipe flow cell to keep the sample in a vapor state while interacting with the IR
- Allows molecules to freely rotate in a low energy environment
- Keeps the molecular geometry intact during analysis resulting in a unique & highly reproducible IR spectra
- Derivatization not required
- Confirmation method for identifying organic compounds due to the uniqueness of IR spectra

GC Conditions

- Carrier Gas: Hydrogen
- GC Column: DB-1MS
  - 12.0 m x 0.20 mm x 0.33 um
- Flow Rate: 2.0 mL/min
- Initial Temp: 100 °C
- Final Temp: 290 °C
- Initial Time: 30 sec
- Ramp Rate:
  - 40 °C /min to 200 °C
  - 30 °C /min to 290 °C
- Final Hold Time: 5.0 min
- Total Run Time: 11.0 min
- Split Ratio: 3:1
- Inj. Port Temp: 290 °C

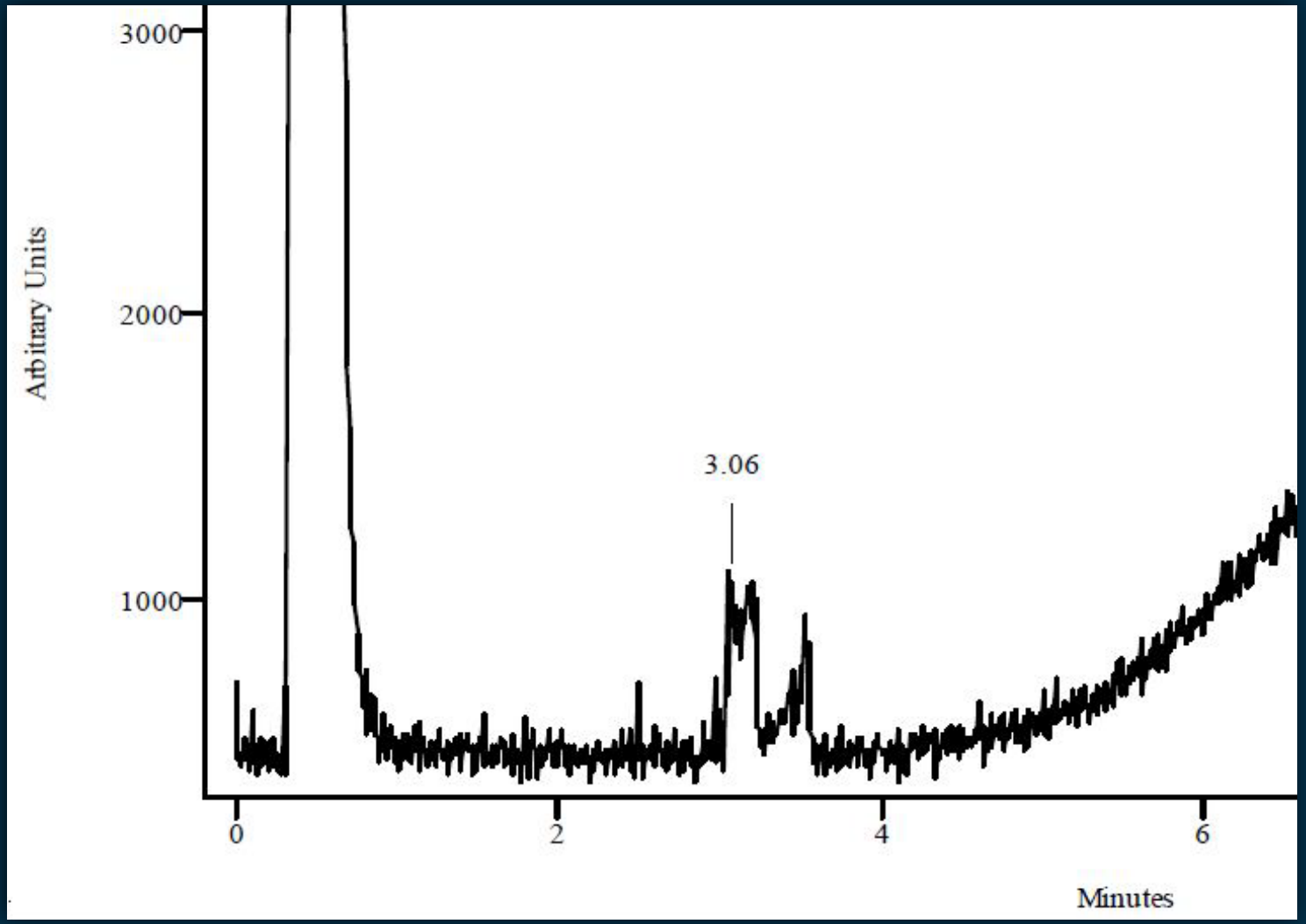
IRD3 Conditions

- **Flow Cell: 290 °C**
- **Transfer Line A: 290 °C**
- **Transfer Line B: 290 °C**
- **Scans per Second: 8**

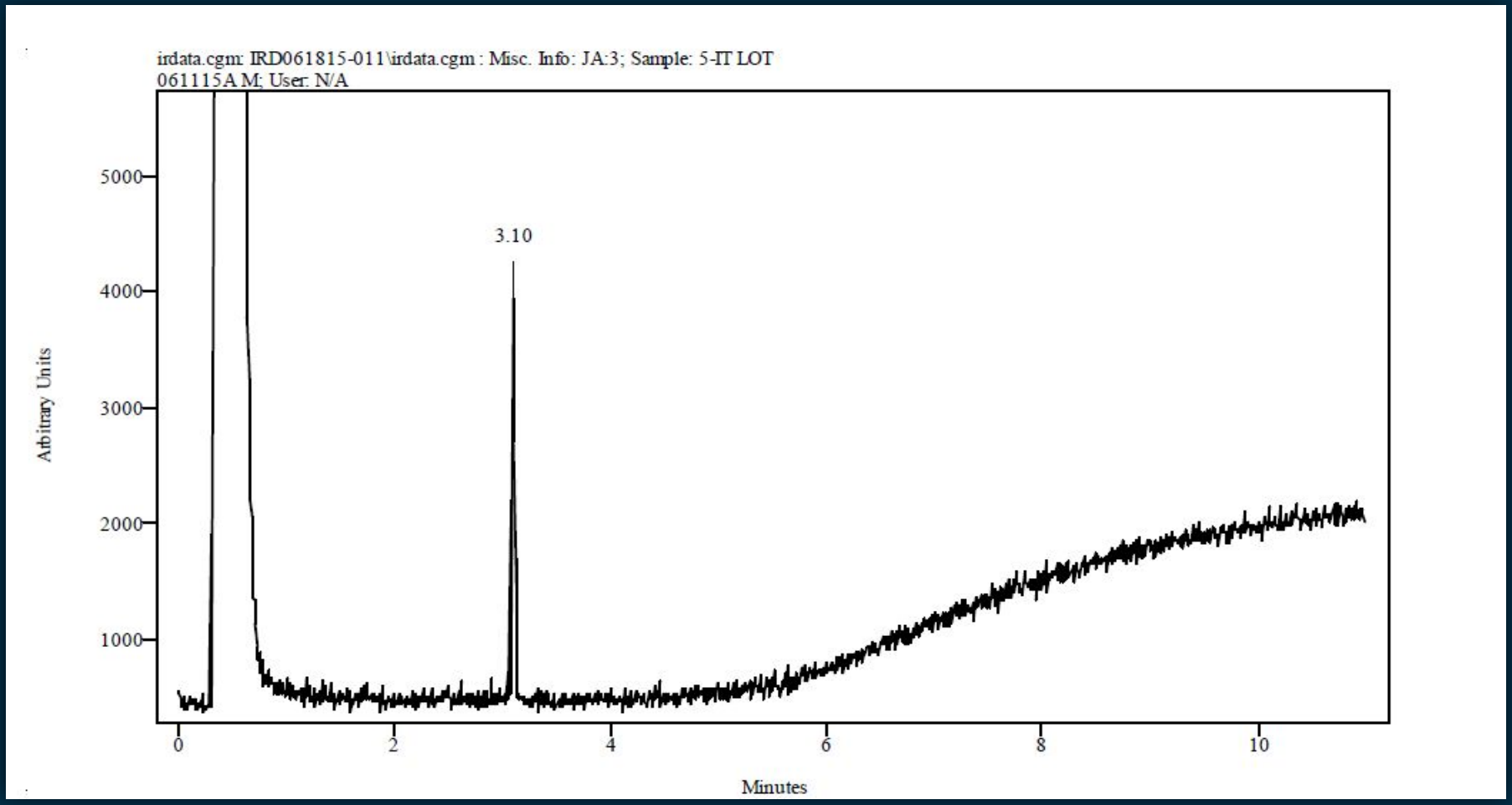
AMT and 5-IT Controls

- 2 mg constituted in 2 mL of methanol
- Storage Temp: 4 °C
- Previously analyzed via GC-FID and GC-MS
- Analyzed twice daily over five days via GC-IRD
- Comparison of chromatography and vapor phase IR spectra
  - Variation between days
  - Reproducibility of minute details

AMT Chromatography

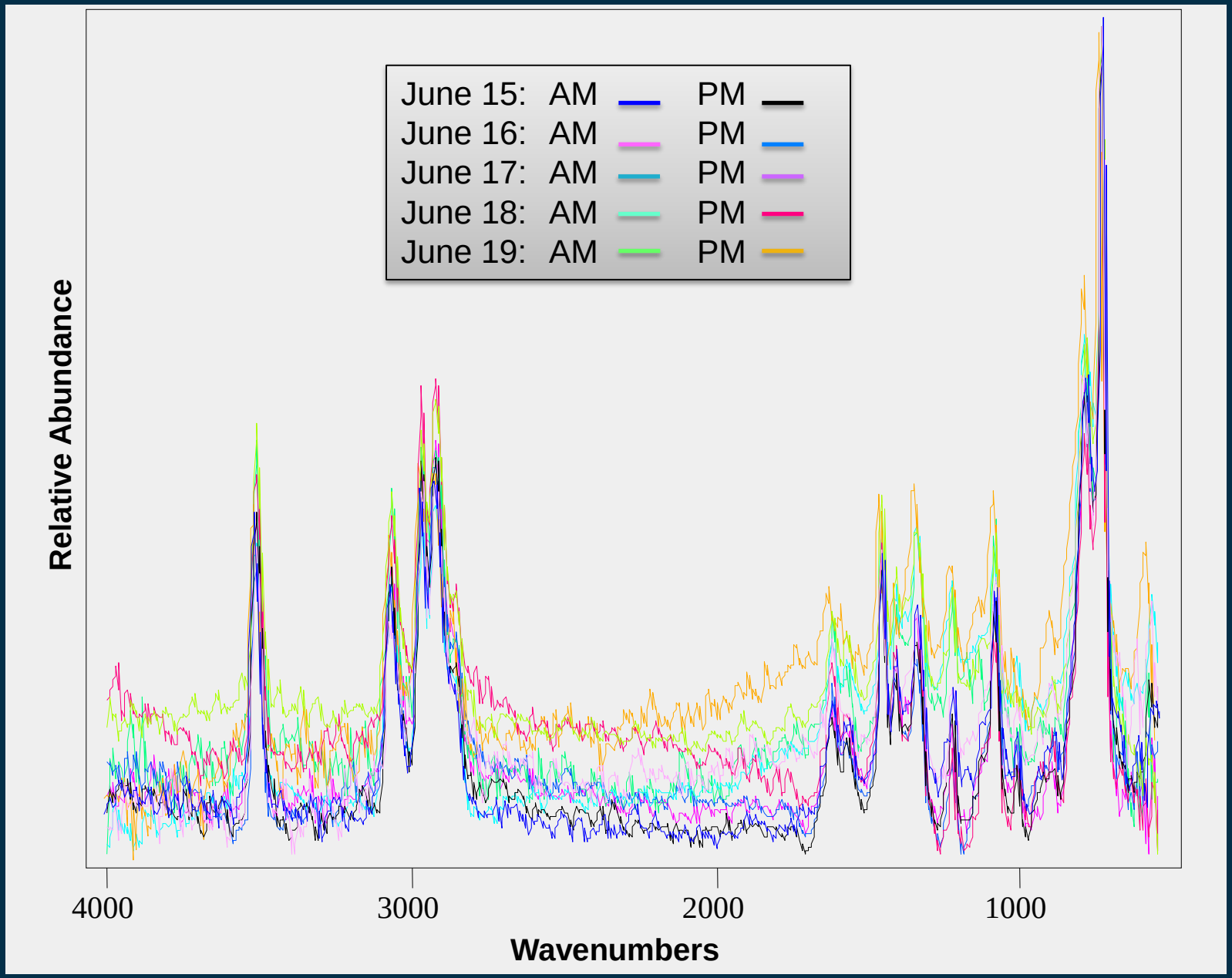


5-IT Chromatography

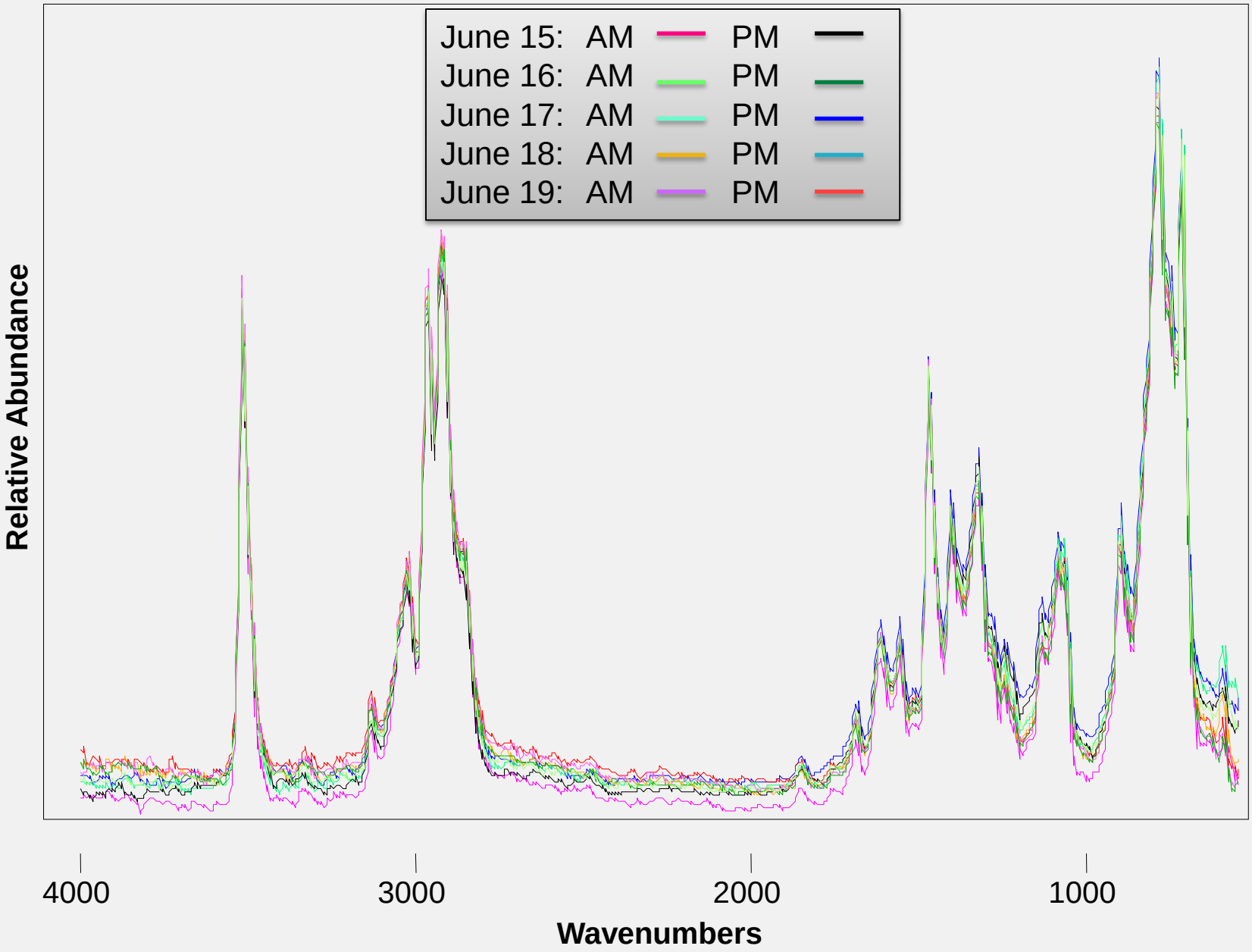


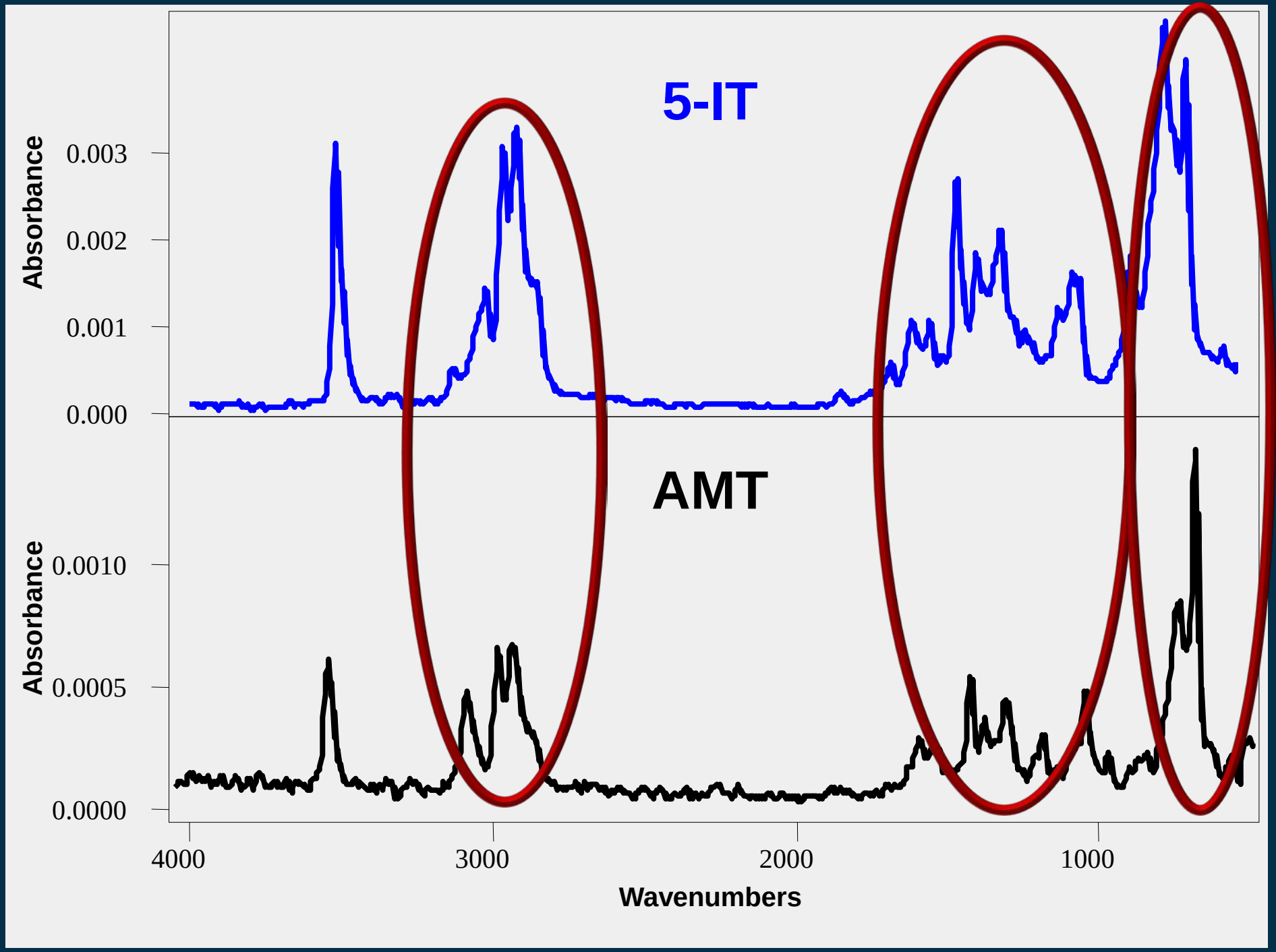
- **Peak chromatography reproducible**
  - **No significant factors observed**

AMT Overlay of IR Spectra (June 15, 2015 – June 19, 2015)



5-IT Overlay of IR Spectra (June 15, 2015 – June 19, 2015)




5-IT
AMT
Absorbance
0.003
0.002
0.001
0.000
Absorbance
0.0010
0.0005
0.0000
4000
3000
2000
1000
Wavenumbers

5-IT and AMT Conclusions

- **Differences observed in IR spectra were sharpness and general peak shape**
- **Despite AMT producing poor chromatography it can still be used to help ID which compound may be present.**
- **Not concerned about chromatography because additional information will be available**
  - **GC-FID**
  - **GC-MS**

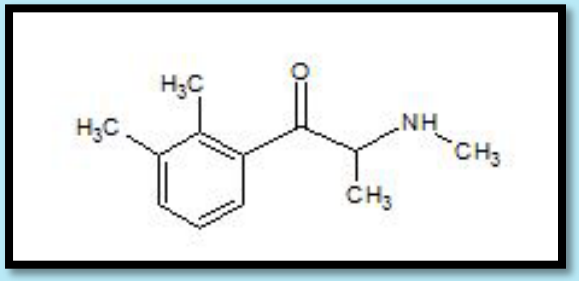
In the end, it was found you can differentiate between on 5-IT and AMT via GC-IRD.

Expansion of Project

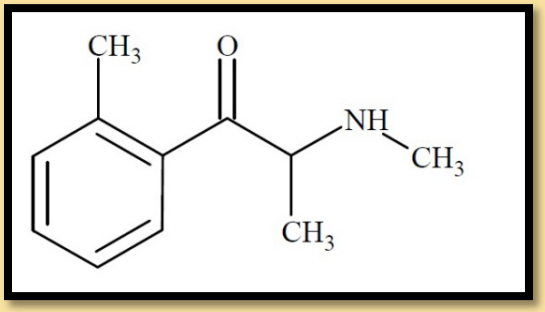
Continuation of a Prior Study of Cathinone Controls that Presented Potential Data Quality Issues

Cathinone Standards

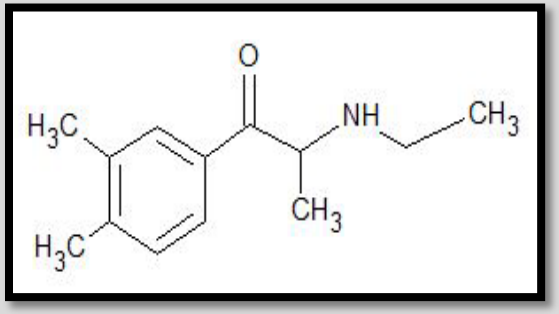
2,3-Dimethylmethcathinone (2,3-DMMC)



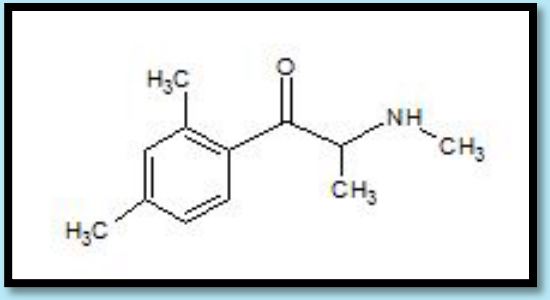
2-Methylmethcathinone (2-MMC)



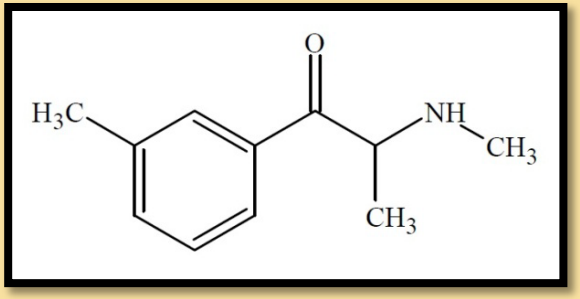
3,4-Dimethylethcathinone (3,4-DMEC)



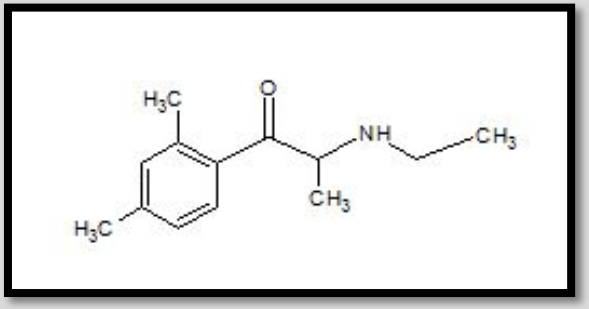
2,4-Dimethylmethcathinone (2,4-DMMC)



3-Methylmethcathinone (3-MMC)



2,4-Dimethylethcathinone (2,4-DMEC)



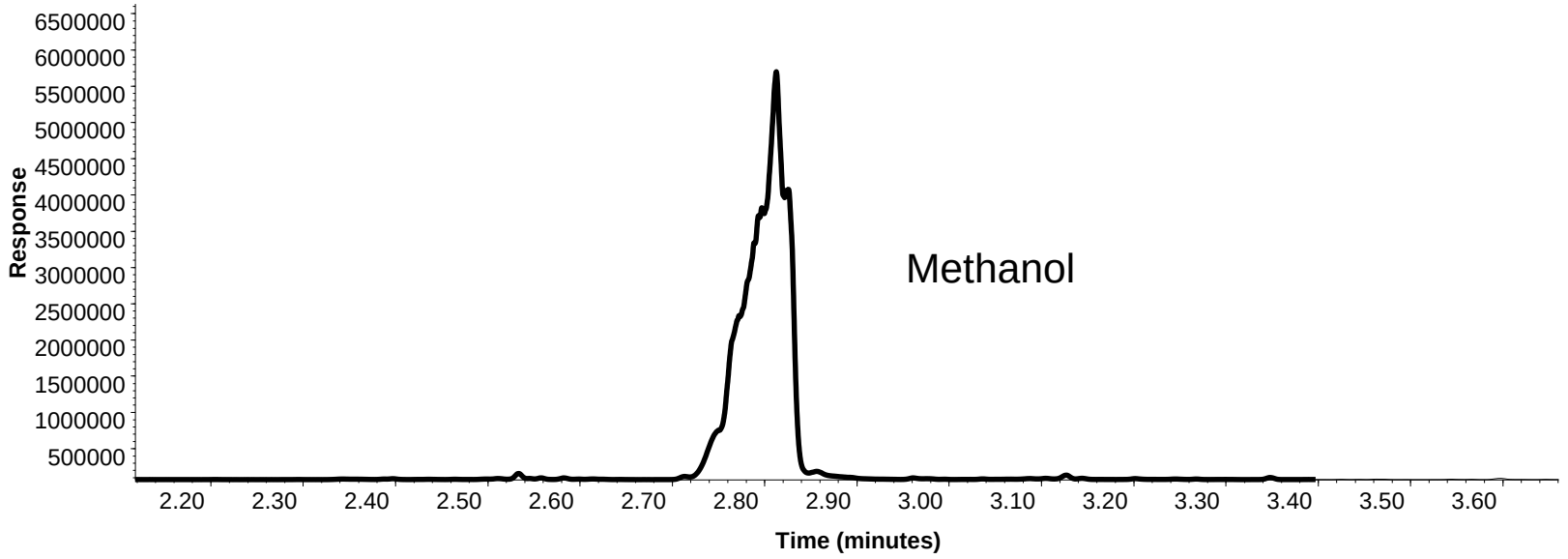
Cathinone Controls

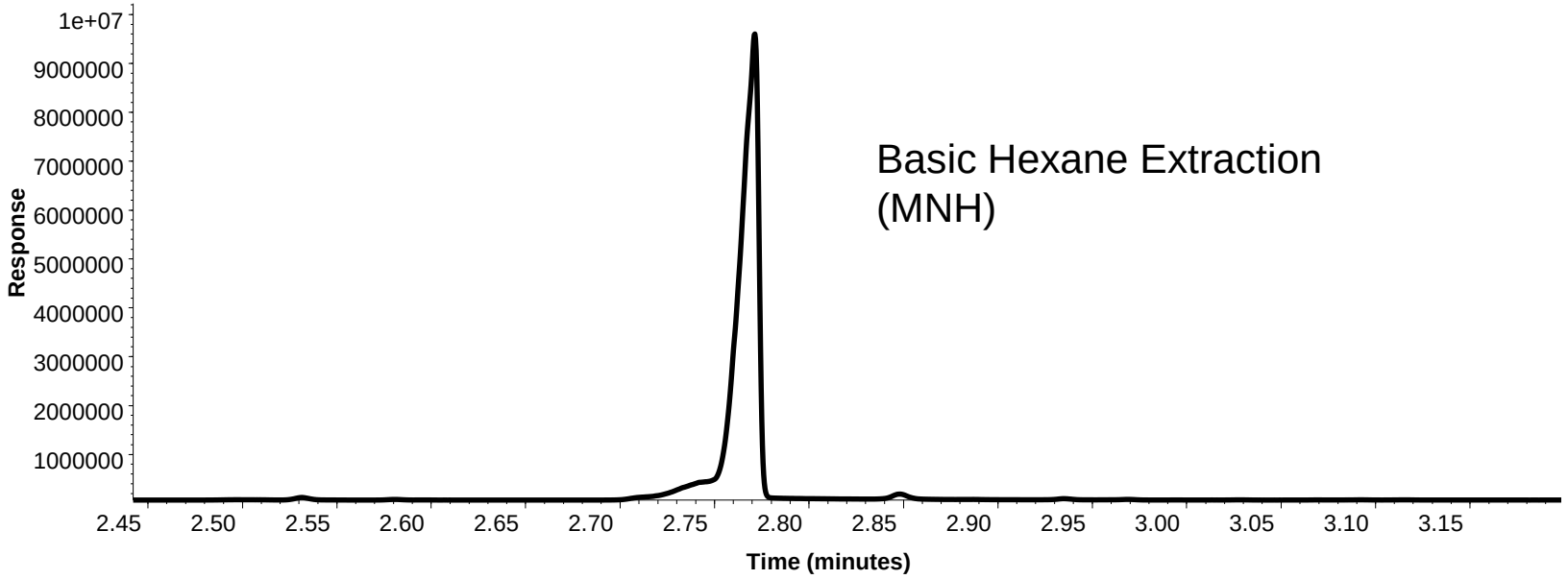
- **2,3-DMMC, 3-MMC, 2-MMC and 3,4-DMEC produced low reproducibility of IR spectra**
  - **Resulted in incorrect library matches**

- **GC-FID displayed poor chromatography**
  - **Basic (0.45 N sodium hydroxide) hexane extraction**
  - **Extraction improved chromatography**

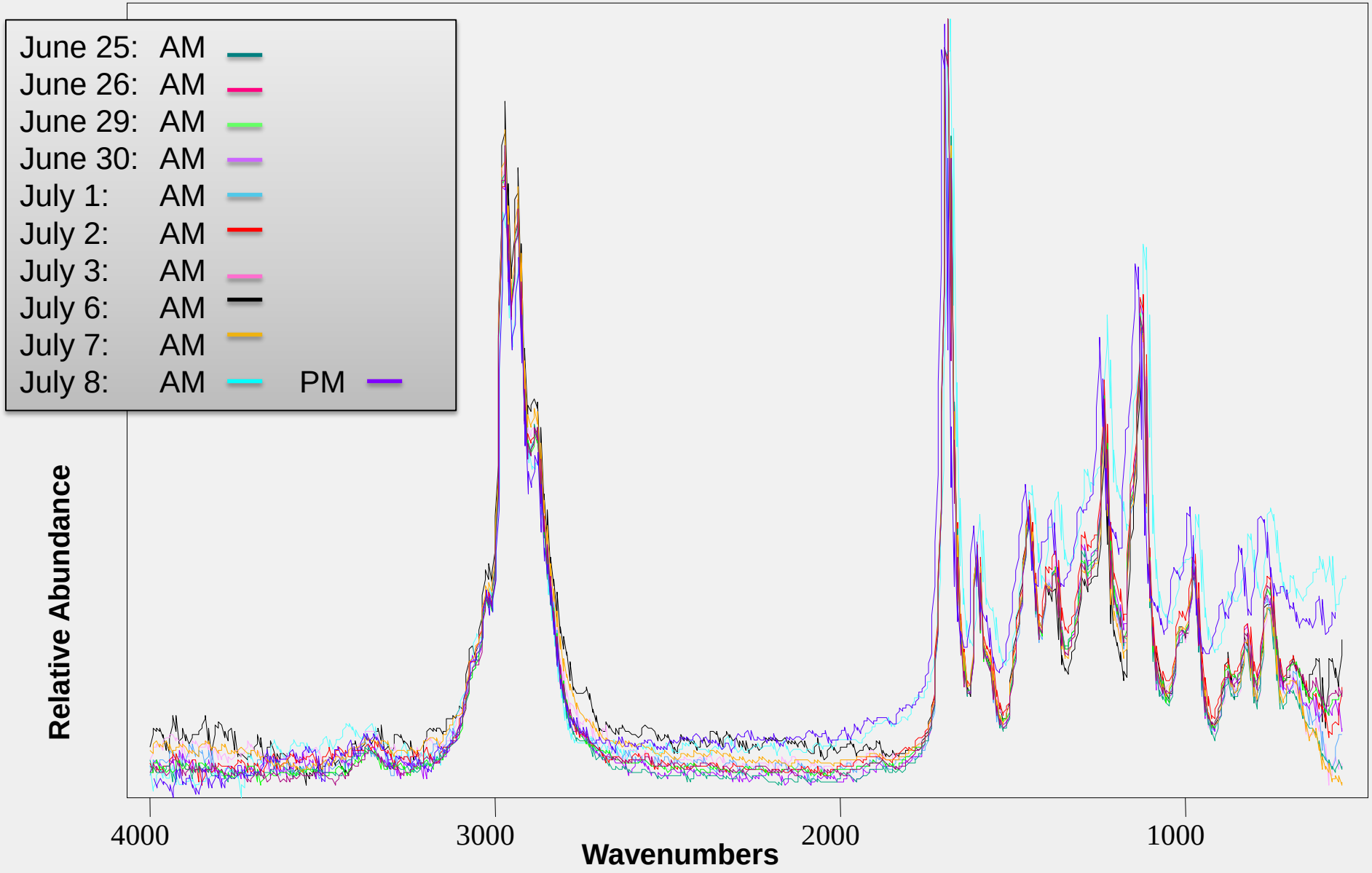
GC-FID Data

3,4-DMEC Methanol vs. MNH

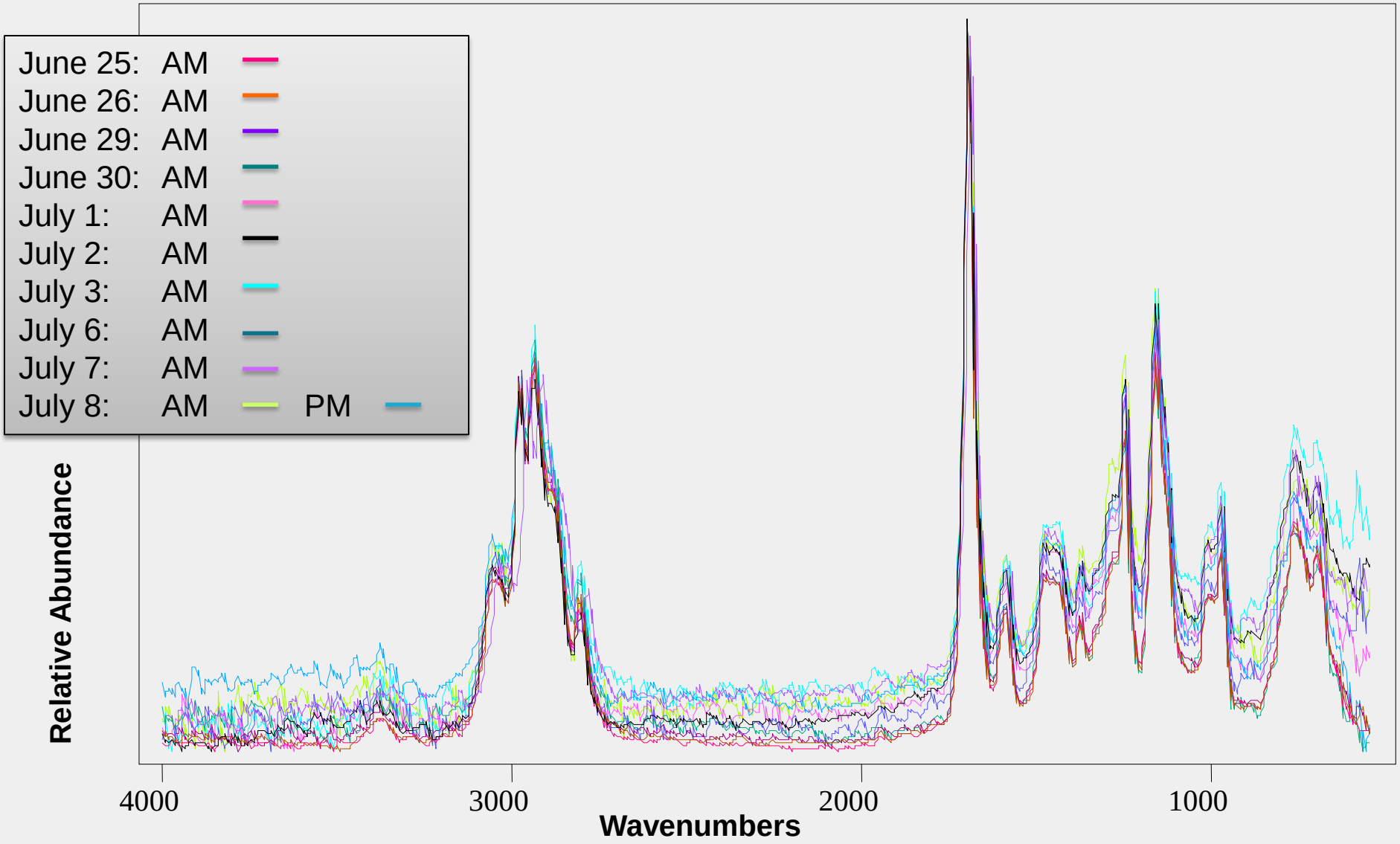




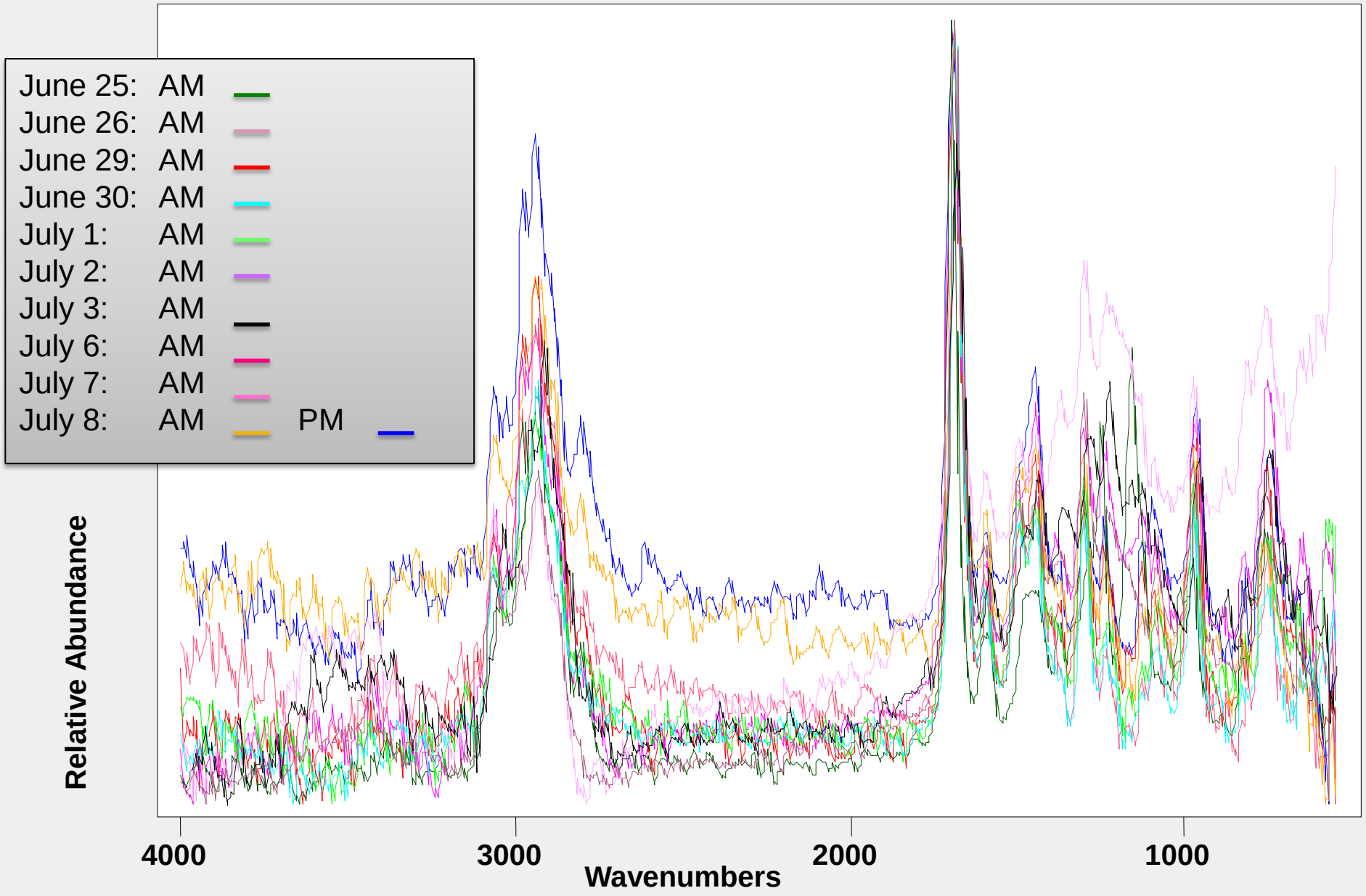
3,4-DMEC Overlay (June 25, 2015 to July 08, 2015)



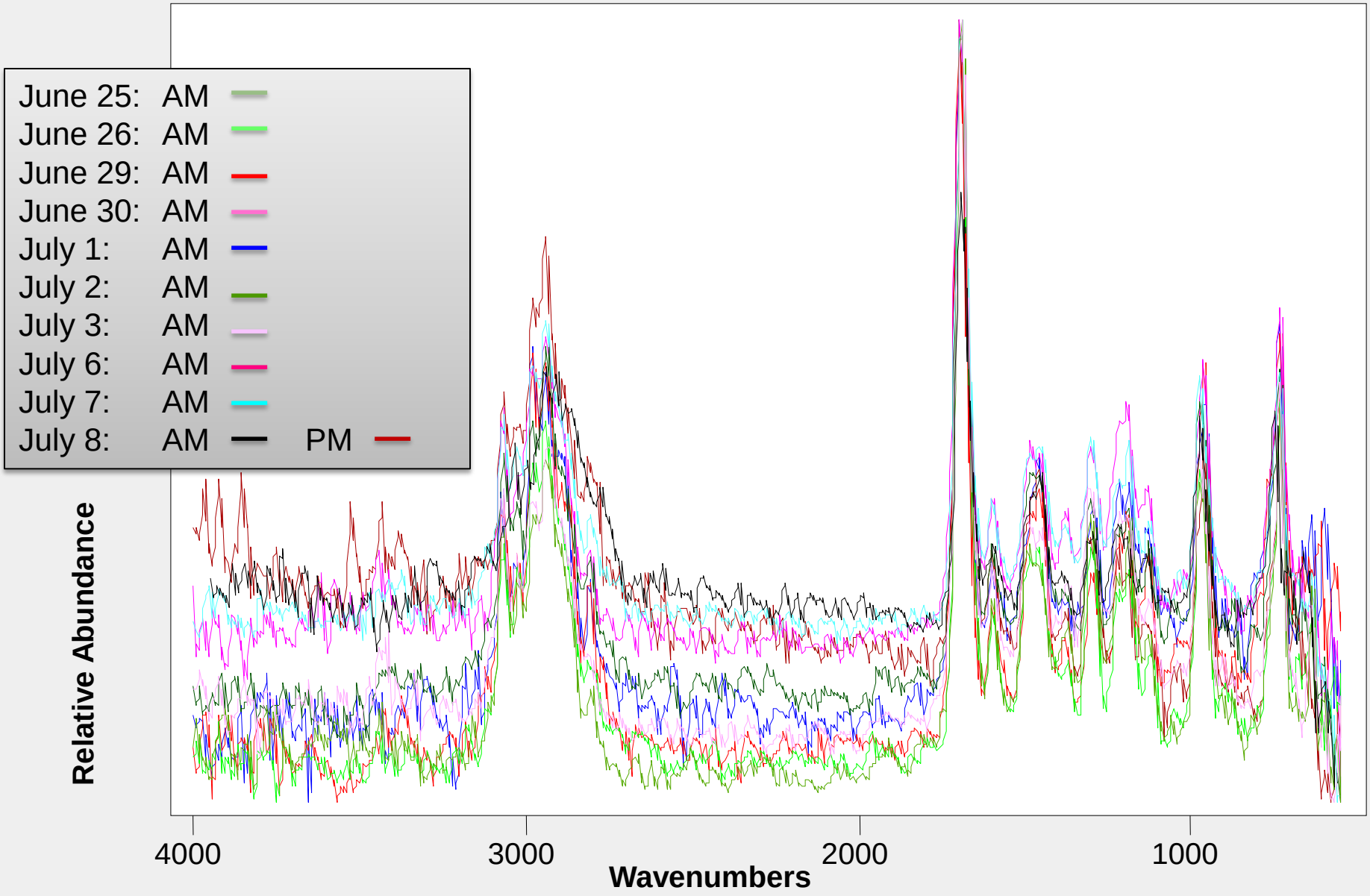
3-MMC Overlay (June 25, 2015 to July 08, 2015)



2,3-DMMC Overlay (June 25, 2015 to July 08, 2015)



2-MMC Overlay (June 25, 2015 to July 08, 2015)



Cathinone Conclusions

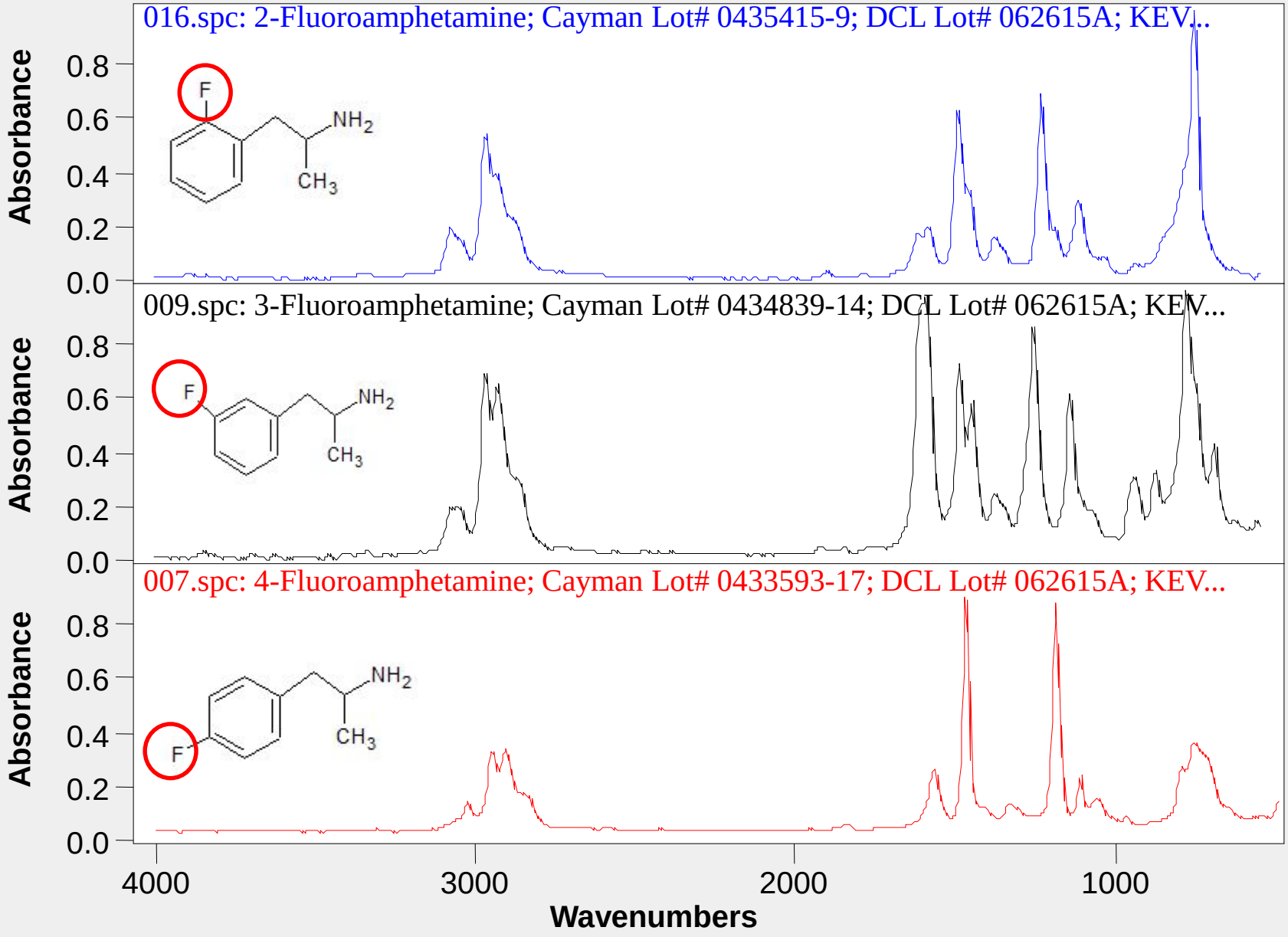
- **2-MMC and 2,3-DMMC presented degradation issues while 3-MMC and 3,4-DMEC presented no complications.**
  - **Cause of degradation of samples is not fully understood**
  - **Could be due to instability of compounds, method parameters and/or interaction of the sample with the reflective coating of the light pipe**

Limitations of the GC-IRD

- **Sensitivity**
  - **High LOD**
  - **Sensitive to internal vibrations**
- **May require concentrated samples**
  - **1 mg/mL samples were weak**
- **Chromatography issue on some compounds**
  - **Method parameters**
  - **Heated light pipe flow cell interaction**
- **Not all compounds are IR active**

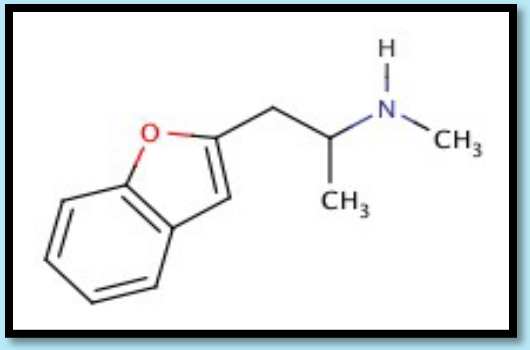
GC-IRD Used for Casework

Comparison of FA IR Spectra

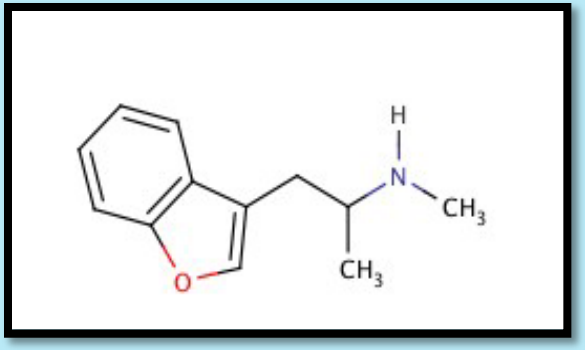


MAPB Series

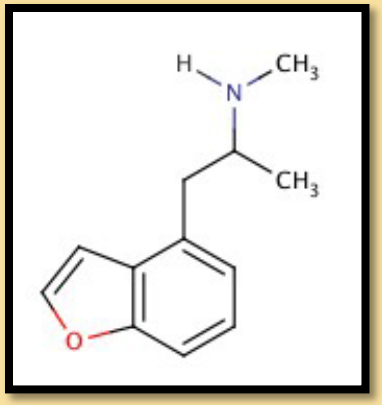
N-α-Dimethyl-2-Benzofuranethanamin (2-MAPB)



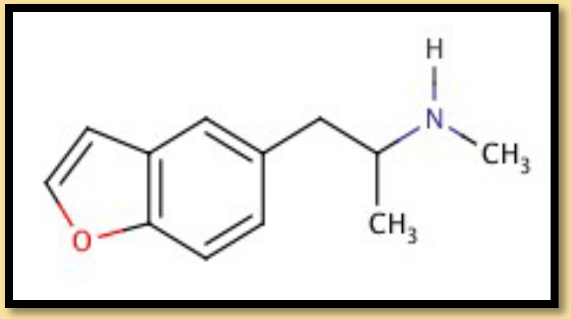
1-(benzofuran-3-yl)-N-methylpropan-2-amine (3-MAPB)



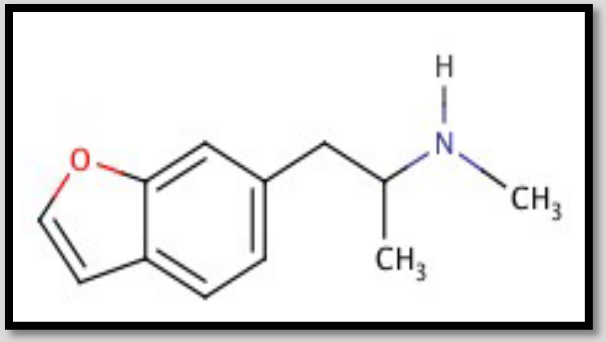
1-(benzofuran-4-yl)-N-methylpropan-2-amine (4-MAPB)



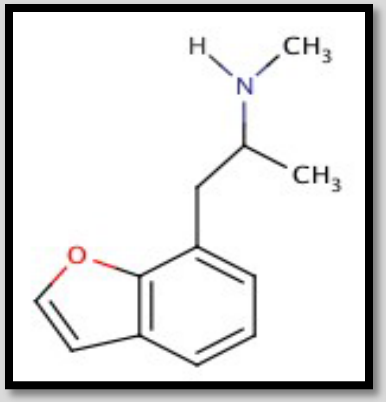
N-α-Dimethyl-5-Benzofuranethanamin (5-MAPB)

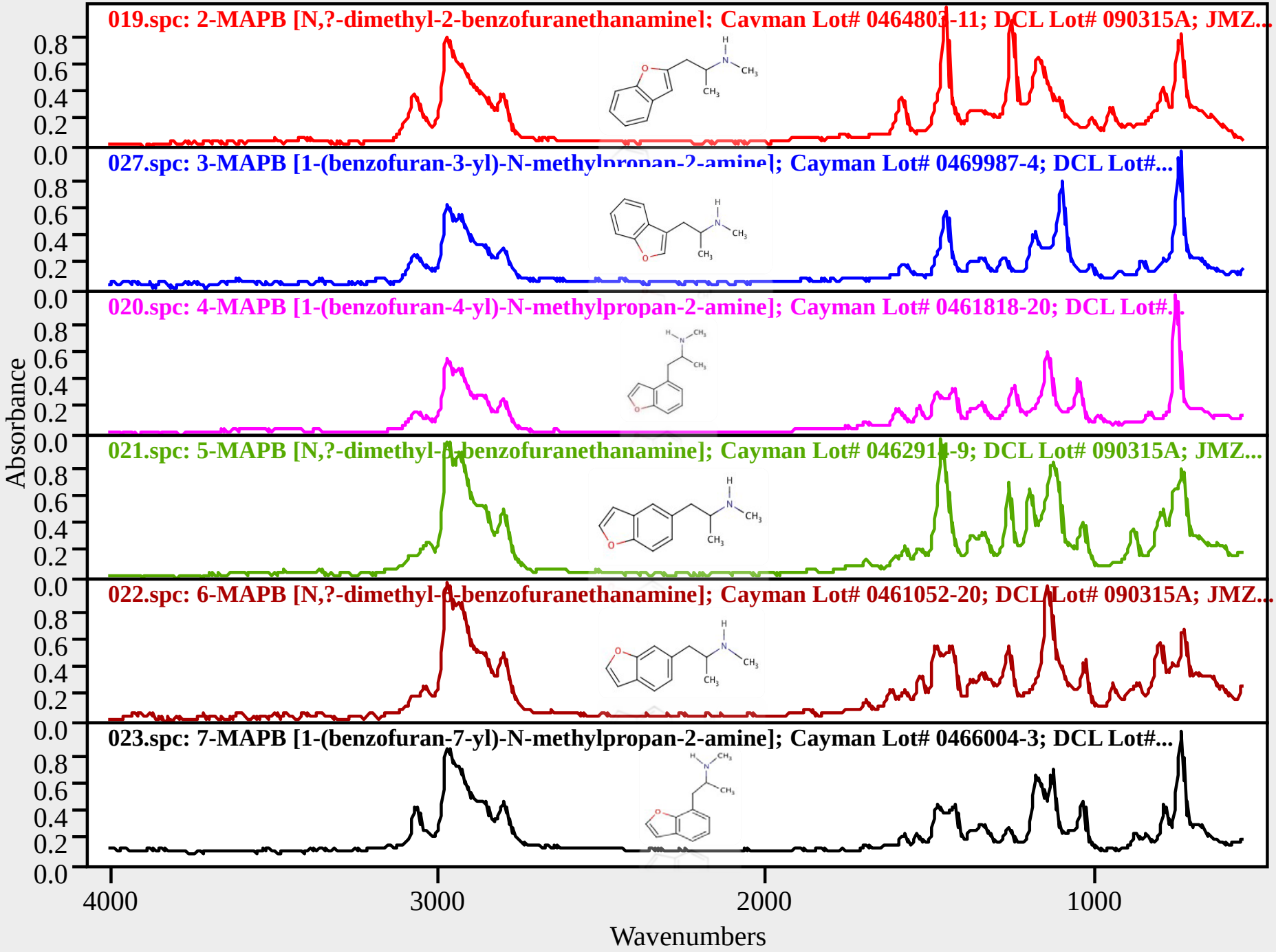


N-α-Dimethyl-6-Benzofuranethanamin (6-MAPB)



1-(benzofuran-7-yl)-N-methylpropan-2-amine (7-MAPB)




019.spc: 2-MAPB [N,?-dimethyl-2-benzofuranethanamine]; Cayman Lot# 0464803-11; DCL Lot# 090315A; JMZ...
027.spc: 3-MAPB [1-(benzofuran-3-yl)-N-methylpropan-2-amine]; Cayman Lot# 0469987-4; DCL Lot#...
020.spc: 4-MAPB [1-(benzofuran-4-yl)-N-methylpropan-2-amine]; Cayman Lot# 0461818-20; DCL Lot#...
021.spc: 5-MAPB [N,?-dimethyl-5-benzofuranethanamine]; Cayman Lot# 0462914-9; DCL Lot# 090315A; JMZ...
022.spc: 6-MAPB [N,?-dimethyl-6-benzofuranethanamine]; Cayman Lot# 0461052-20; DCL Lot# 090315A; JMZ...
023.spc: 7-MAPB [1-(benzofuran-7-yl)-N-methylpropan-2-amine]; Cayman Lot# 0466004-3; DCL Lot#...
Absorbance
0.0
0.2
0.4
0.6
0.8
4000
3000
2000
1000
Wavenumbers

Recommendations

- **Solubility of samples**
- **CAREFULLY review the GC-IRD data**
- **Run controls over a period of time to ensure stability and reproducibility of IR spectra**

Acknowledgments

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Kay McClain, B.S., Drug Chemistry Manager

Kyle Vircks, B.S., Forensic Chemist II Specialist

&

All Drug Chemistry Staff

Jasmine Drake, Ph.D., Assistant Professor (Advisor)

Thank you for this wonderful opportunity!

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Thank you!

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