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BILL LEE
Governor

DAVID B. RAUSCH
Director

MEMORANDUM

TO: Chad Johnson, Quality Assurance Manager
Brett Trotter, Forensic Chemistry Technical Leader / Nashville Supervisor

FROM: Hannah Peterson, Special Agent Forensic Scientist II

DATE: August 23, 2022

SUBJECT: Validation plan for the Extension of the Accepted Retention Time Standard Window

The experiments outlined here are designed to demonstrate the feasibility for extending the accepted retention-time standard window from 24 hours to 120 hours, or 5 days. Updating policy to reflect this could improve efficiency by shaving a minimum of 45 minutes off a single GC/MS run and add up to 15 hours of additional instrument run time each week in the Knoxville Regional Crime Laboratory alone. A number of crime laboratories already use this standard at minimum for their own casework as demonstrated by their policies. [1, 2.3.4,6,7]

Validation experiments will include the following:

- Working standard mixes of multiple drug standards
- Repeatability of RT values across instruments
- Known conditions that would change RT
- Historical Data showing the stability of RT

A. A wide range of drug compounds, including different drug classes and known different elution times, will be used. To save time and increase repetitions of standard runs, the drug standards are combined in a working standard mix. Two different mixes will be used for this experiment. They include:
P-MOM Standard Mix: Phentermine, Methamphetamine, Tramadol, 4-ANPP, Heroin, para-Fluorofentanyl, Fentanyl
Standard Mix: Pethidine, Cocaine, Hydrocodone
Additional information on the drug standard sources and lot numbers that were used to make these mixes can be found in Table 1 at the end of this proposal.

B. As with any scientific testing determining whether or not results are consistent between multiple testing of the same sample is important. The currently accepted standard for retention time standard matching is a 2% window. In other words, the retention time of the sample must be within 2% of the retention time of the standard to be considered a “match” and be used to support the conclusion of an identification of a substance[5]. For this experiment, showing that repeatability within a specific range, in this case 2%, over an extended period of time demonstrates the stability of the retention time values on a given instrument and the feasibility for extending the accepted retention time window. If the

repeated retention times fall outside the accepted 2% window, extending the accepted window would not be feasible.
It is important to demonstrate that the results of retention time values within 2% are independent of the instrument used; therefore, the experiment will be performed on two instruments. Two samples taken from the same standard mixes will be run in the same manner on two GC/MS instruments. To further demonstrate the independence of the instruments, two different models have been selected for this study. The two instruments are: 6890/5973N Agilent GC/MS-Tobias and 7890B/5977A Agilent GC/MS-Kreacher.
Due to the nature of GC/MS and the data it provides (both retention time and mass spectral data), additional confidence and confirmation is provided on the identity of drugs in samples or standard mixes.

C. Within GC/MS maintenance and care, there are circumstances that are known to alter the RT value for a known standard: changing the length of the column and changing the method parameters such as temperature programming. Changing the length of a column is a routine part of GC/MS maintenance. Any time injection port or interface maintenance is done, the column is clipped. This changes the overall length of the column. Changing method parameters will not be included in this study because that is a situation that is not generally a part of routine GC/MS maintenance or casework. As per normal operating policy, the liners will be checked weekly and changed if necessary. In addition, the liner will be changed at multiple points throughout the week on one instrument to demonstrate that the liner should not affect the RT value.

D. Historical data shows that over extended periods of time the retention time values for known standards do not vary beyond the accepted 2% window. The only exceptions are when underlying conditions are present. These exceptions included GC/MS column maintenance, instances of changes in instrument sensitivity due to a need for injection port or column maintenance, and when the working standard had become overly concentrated due to the age of the working standard. In these instances, once the condition described was addressed, the retention time values returned to well within the 2% window.

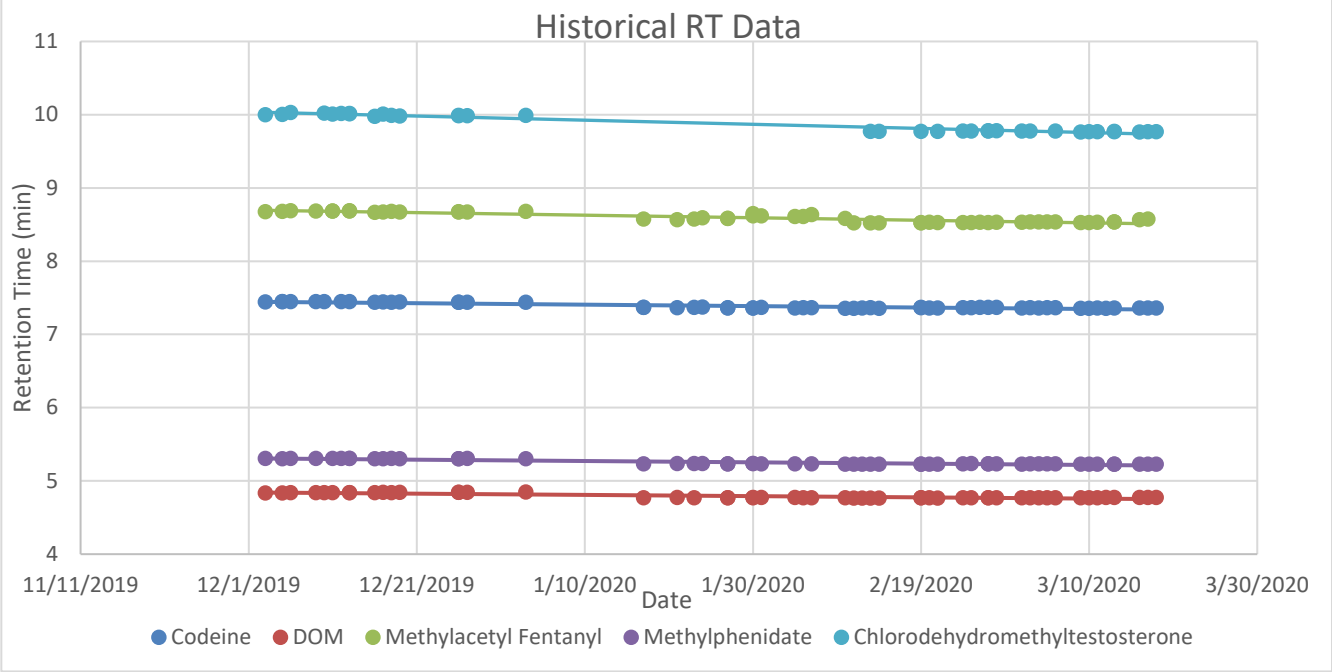

TABLE 1

Drug	Source	Lot #
4-ANPP	Cayman	0540831-18
Cocaine	Sigma	59H0640
Fentanyl	Cayman	0530926-33
p-Fluorofentanyl	Cayman	0541563-41
Heroin	Cayman	0559235-10
Hydrocodone	Sigma	047F0128
Methamphetamine	Sigma	071K1580
Pethidine	SB Penick	413-NMN-027
Phentermine	Sigma	074K1059
Tramadol	Cayman	0540757-30

References:

1-Arizona Department of Public Safety Scientific Analysis Bureau – Controlled Substances Analytical Protocol, Revision 5. *15.7.* September 28, 2020.

2-Houston Forensic Science Center – Seized Drugs Standard Operating Procedures. *7.4.6*. September 7, 2020.

3-Ohio Bureau of Criminal Investigation Crime Laboratory – Chemistry Methods Manual, Revision 22. *16.4.* March 01, 2021.

4-San Diego Police Department Crime Lab – Seized Drug Manual, latest revision. *11.4.2.2.* October 21, 2020.

5-Tennessee Bureau of Investigation Forensic Services Division – Forensic Chemistry Standard Operating Procedure Manual, Revision 6. *21.1.5, 21.1.7.* October 02, 2021.

6-Texas Department of Public Safety – Seized Drugs Manual, Revision 4. *5.3.B* August 8, 2022.

7-Washington State Patrol Crime Laboratory Division – Materials Analysis Technical Procedures, Revision 9. *9.6.3.* October 11, 2021.

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MEMORANDUM:

GC/MS Retention Time Extension Validation

This validation was performed at the Knoxville laboratory on Gas Chromatography / Mass Spectrometer instrumentation. However, it applies to those types of instruments across the state in the Nashville and Jackson laboratories as well. Methodology, Instrument manufactures, and models are consistent throughout the state. The training for this modified methodology will consist of reading the validation study and understanding which types of retention time analyses are impacted.

Sincerely,

Special Agent Forensic Scientist Supervisor / Technical Leader
Brett Trotter

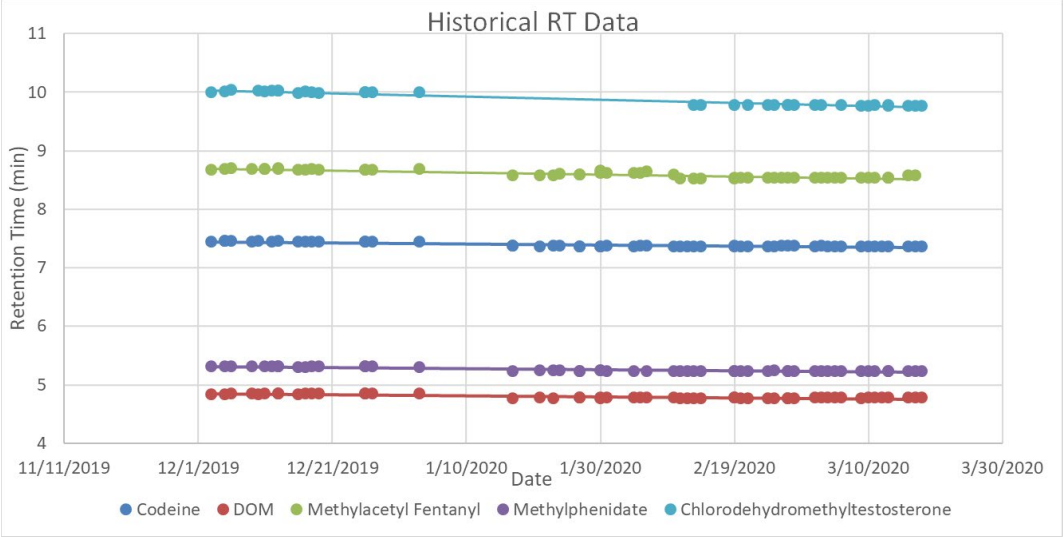
Retention Time Validation Study

Extension of Accepted Retention Time Window

Knoxville Lab, August 2022

1.Introduction

The purpose of this study was to investigate the validity of extending the length of a retention time (RT) standard window. Currently, the accepted retention time window is 24 hours, meaning that a sample RT can be compared to a standard RT only if the times of injection for the sample and standards are within 24 hours of one another. Reviews of historical data show that the RTs of standard data run over a larger timespan, such as a week (even upwards of a month), show little variance. (Figure 1) The aim of this study was to systematically demonstrate that this variance is not greater than the 2% window required by current policy for casework and thus demonstrating the case for extending the accepted RT standard window from 24 hours to 120 hours, or 5 days.



2. Methods and Materials

For approximately two weeks, on two GC/MS instruments (6890/5973N Agilent GC/MS-Tobias, 7890B/5977A Agilent GC/MS-Kreacher) known RT standard mixes were run twice daily, once in the morning and once in the afternoon/evening. The effects of specific GC/MS maintenance tasks were demonstrated by performing full injection port maintenance(replacing the gold seal/ferrule, clipping the column, and changing the liner) on one instrument involved in the study. Injection port maintenance was not performed on the other instrument as a control. As per normal operating policy, the injection port liner was checked weekly and changed if necessary. Additionally, the liner was changed at multiple points throughout the two week period on one instrument. Figure 2 provides a breakdown of the standard runs and maintenance tasks for this study.

	Monday	Tuesday	Wednesday	Thursday	Friday
Tobias-week 1	Liner changed		Liner changed	Liner changed	
	Run 1	Run 1	Run 1	Run 1	Run 1
	Run 2	Run 2	Run 2	Run 2	Run 2
Tobias-week 2	Injection port maintenance				Liner changed
	Run 1	Run 1	Run 1	Run 1	Run 1
	Run 2	Run 2	Run 2	Run 2	Run2
Kreacher-week 1	Liner changed				
	Run 1	Run 1	Run 1	Run 1	Run 1
	Run 2	Run 2	Run 2	Run 2	Run2
Kreacher-week 2	Liner changed				
	Run 1	Run 1	Run 1	Run 1	Run 1
	Run 2	Run 2	Run 2	Run 2	Run2

Figure 2

A variety of different drug standards were used in this study to demonstrate the consistency of retention times across a wide range of elution times. Table 1 lists the drug standards, suppliers, and lot numbers used for this study.

Drug	**Source**	**Lot #**
4-ANPP	Cayman	0540831-18
Cocaine	Sigma	59H0640
Fentanyl	Cayman	0530926-33
p-Fluorofentanyl	Cayman	0541563-41
Heroin	Cayman	0559235-10
Hydrocodone	Sigma	047F0128
Methamphetamine	Sigma	071K1580
Pethidine	SB Penick	413-NMN-027
Phentermine	Sigma	074K1059
Tramadol	Cayman	0540757-30

Table 1

3. Results and Conclusions

Of the 10 drug standards used for this study, all maintained consistent retention times within the accepted 2% window for the entirety of the two week period. Table 2 provides the average retention time values and their associated standard deviations and the average percent difference from the first run and associated standard deviations. Figures 3-12 show the RT vs the run number. The 20 runs were completed over a two week period. The graphs demonstrate the stability and reproducibility of the RTs over an extended period of time.

To demonstrate the effects of injection port maintenance and clipping the column on the RTs, injection port maintenance was performed on Instrument 1 (Tobias) at the beginning of week 2. The changes in the RTs, due to this maintenance, were small but distinguishable. This can be seen in the visual representations of the RTs in Figures 3b–12b in Appendix A. In Table 2, there are two sets of values for Instrument 1 (Tobias): one from before injection port maintenance and one after.

Table 2

Drug	Average Retention Time			Standard Deviation of Retention Time			Average % Difference from 1st Run			Standard Deviation of % Difference from 1st Run		
4-ANPP	7.329	7.305	8.946	0.006	0.006	0.014	0.061	0.063	0.164	0.046	0.087	0.149
Cocaine	6.598	6.575	6.506	0.007	0.007	0.008	0.092	0.064	0.090	0.053	0.088	0.086
p-Fluorofentanyl	7.954	7.927	9.844	0.006	0.007	0.020	0.052	0.054	0.217	0.057	0.089	0.198
Fentanyl	8.068	8.040	10.022	0.007	0.009	0.026	0.064	0.070	0.279	0.057	0.102	0.255
Heroin	7.795	7.771	9.609	0.007	0.008	0.023	0.067	0.066	0.262	0.063	0.099	0.233
Hydrocodone	7.283	7.259	8.903	0.005	0.005	0.006	0.045	0.054	0.055	0.041	0.050	0.057
Methamphetamine	2.613	2.589	3.601	0.003	0.003	0.004	0.088	0.155	0.110	0.044	0.122	0.115
Pethidine	5.109	5.086	6.506	0.006	0.005	0.005	0.104	0.051	0.085	0.054	0.078	0.077
Phentermine	2.491	2.467	3.433	0.002	0.003	0.004	0.080	0.158	0.089	0.046	0.112	0.096
Tramadol	5.806	5.783	7.270	0.004	0.004	0.006	0.064	0.066	0.093	0.033	0.076	0.086
	Tobias		Kreacher	Tobias		Kreacher	Tobias		Kreacher	Tobias		Kreacher
	Pre-Injection Port Maintenance	Post Injection Port Maintenance		Pre-Injection Port Maintenance	Post Injection Port Maintenance		Pre-Injection Port Maintenance	Post Injection Port Maintenance		Pre-Injection Port Maintenance	Post Injection Port Maintenance	

Injection port liners allow the instrument to assist in volatilization and filter out non-volatiles. Because of this, the liners can get dirty and must be changed periodically. Policy allows for the liner to be checked weekly to determine if a change is necessary. For this study, liners were changed within policy guidelines on Instrument 2 (Kreacher). Liners were changed on Instrument 1 (Tobias) at various times throughout the two weeks. Overall, there were no significant effects noticed on the retention times due to changing the liners.

One notable exception to the effects of liner changes on the retention times was noted from week one to week two on the instrument Kreacher. At the beginning of week two, the liner was changed which caused a significant increase in sensitivity on the instrument, to the point where the first standard run of week two was unusable due to the chromatography of the runs. To remedy this problem, an aliquot of the prepared standard was taken and diluted to be used for week 2 of the study. Once the prepared standards were appropriately diluted, the retention times were within the 2% window where they stayed consistently for the entirety of the week. It is important to note that the chromatography issues caused by the marked increase in sensitivity that prevented the standard runs from being used in the study would have also prevented the standard runs from being used in casework. Thus, while the liner change did change the retention times initially, functionally the times remained within the acceptable window once the standards were appropriately diluted.

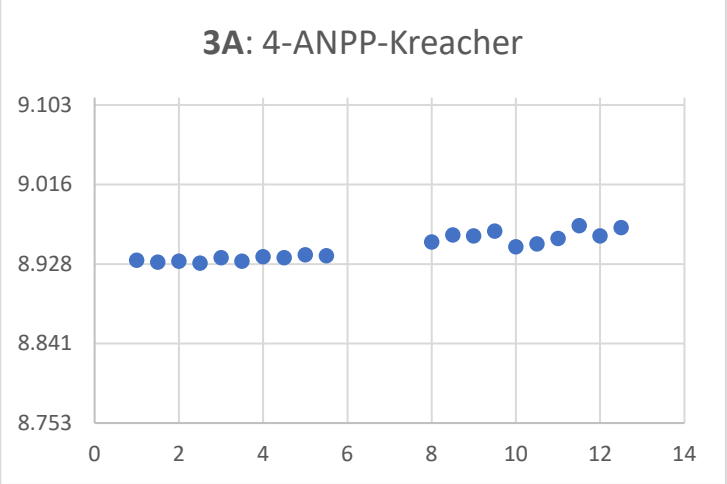
The results from this study demonstrate that the RT for working standards remains consistent within the same instrument conditions. None of the usable standard runs fell outside the accepted 2% window. This study demonstrates that the RTs were stable well outside of the proposed time frame for extension. Even the largest percent difference from the first run for both instruments (a 0.70 % difference in the fentanyl standard on Kreacher, and a 1.16% difference on Tobias -note this difference was between the first run and the first run after injection port maintenance) was well within the accepted two percent window further demonstrating the long term stability of RT values. Based on these results, it is recommended that the accepted RT standard window be extended to 120 hours, or 5 days.

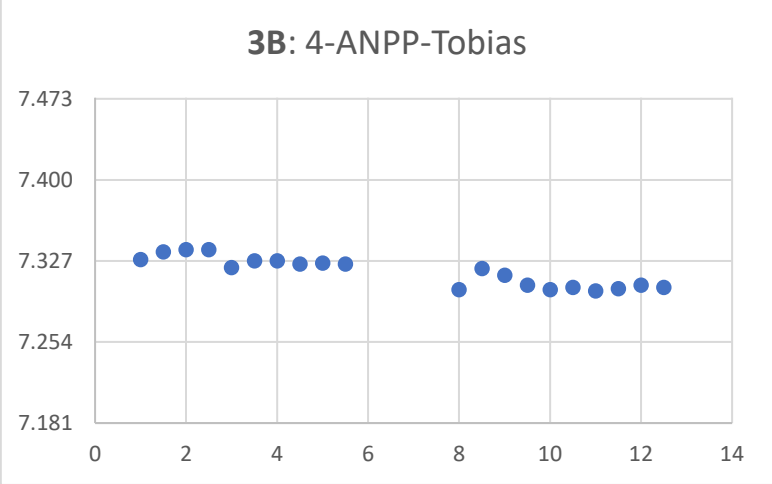
4.Training and Competency

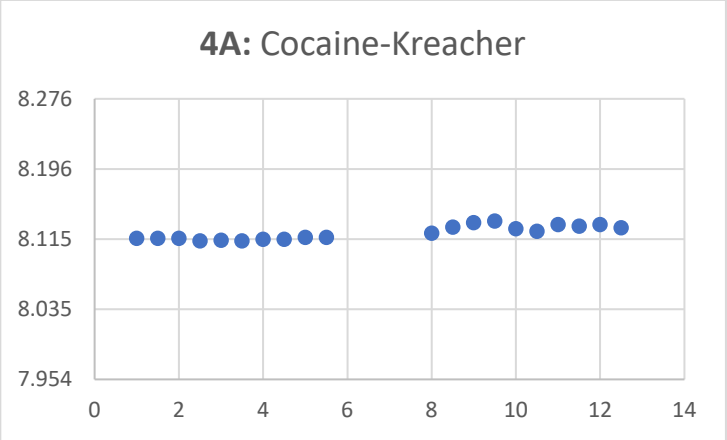
There are no significant changes to procedures that would warrant an initial demonstration of proficiency. All of the analysts have shown competency in casework involving GC/MS analysis. Reading the new policy extending the acceptable retention time window to 5 days (120 hours) would be sufficient training for using this new policy standard in casework.

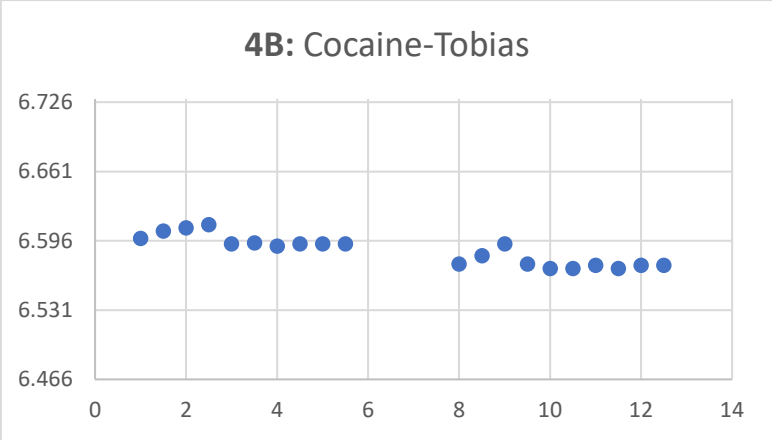
Appendix A

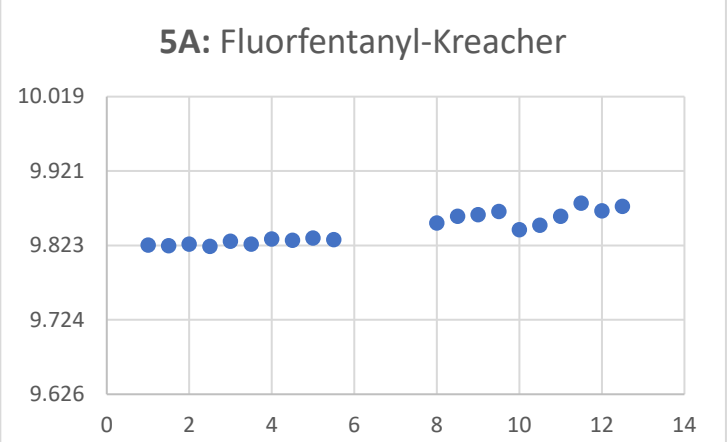
Figures 3-12 show the RT vs the run number from the two week period. The gaps in the data from day 6 and 7 are the weekend in the middle of the study. The y-axis for each graph represents the 2% window surrounding the initial run of each standard. Each major axis line represents one percent from the 1st run of the two week period.

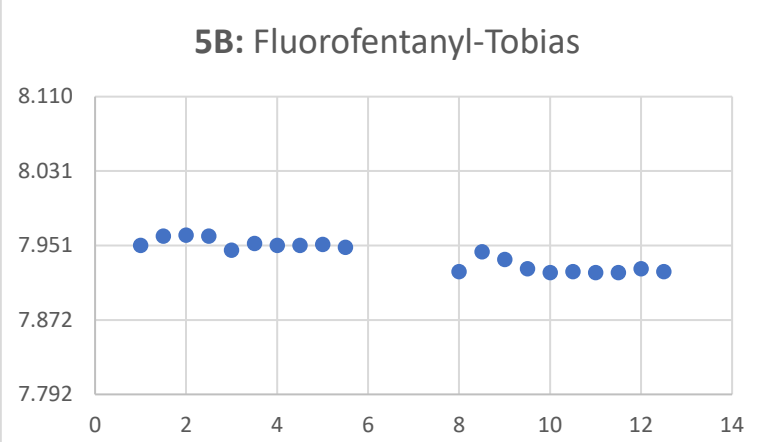


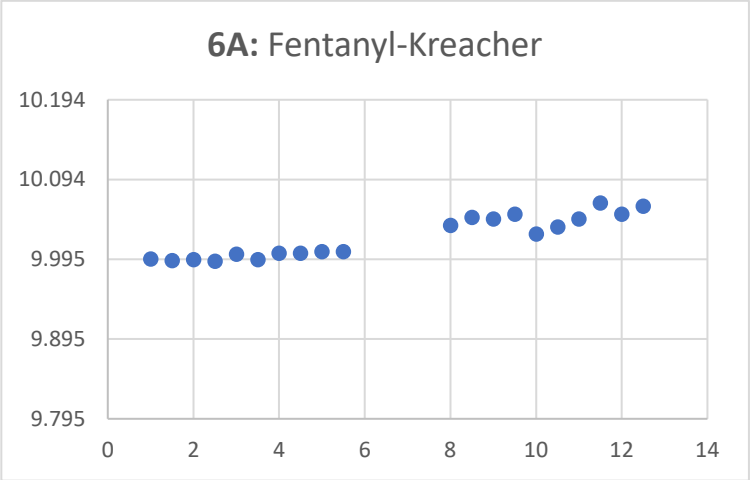


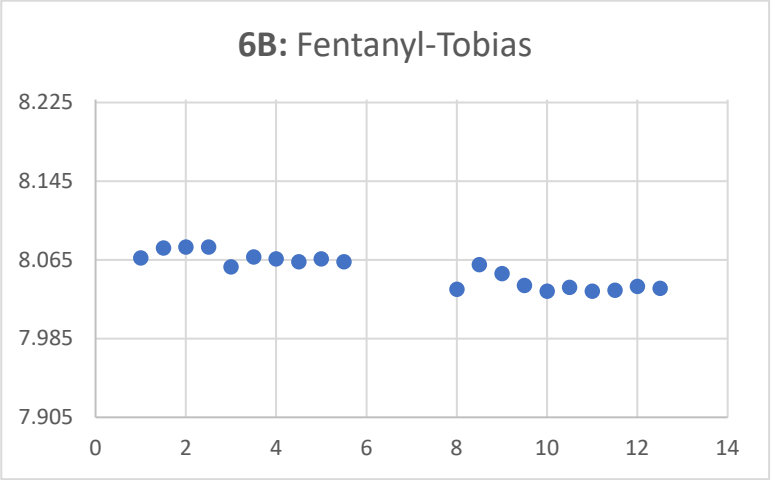


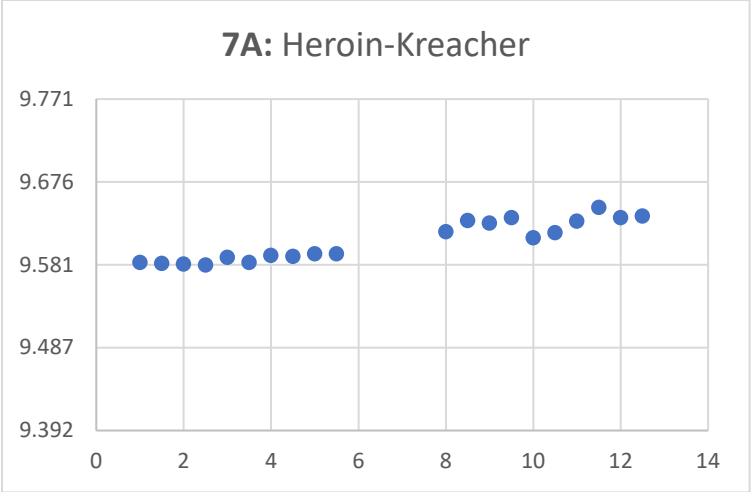


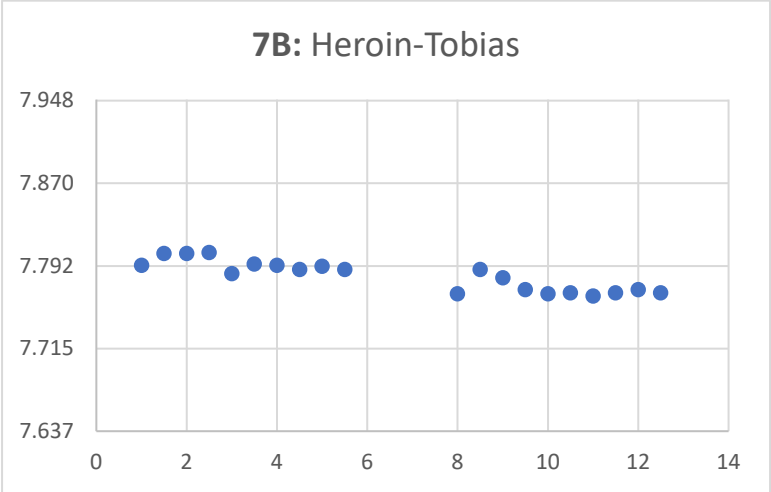


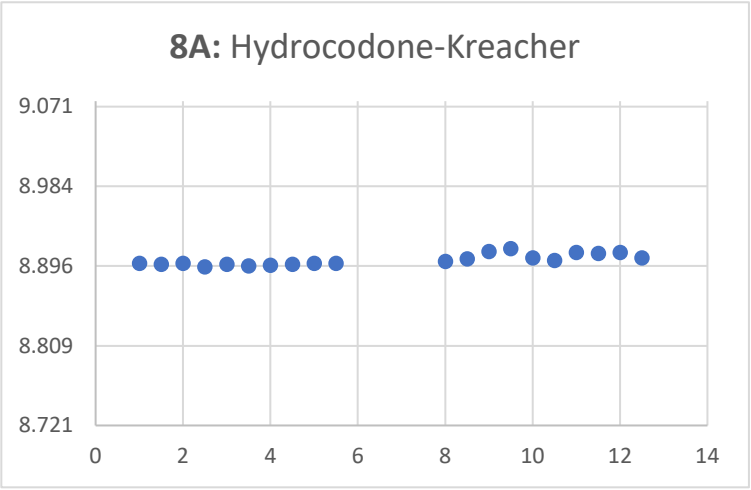


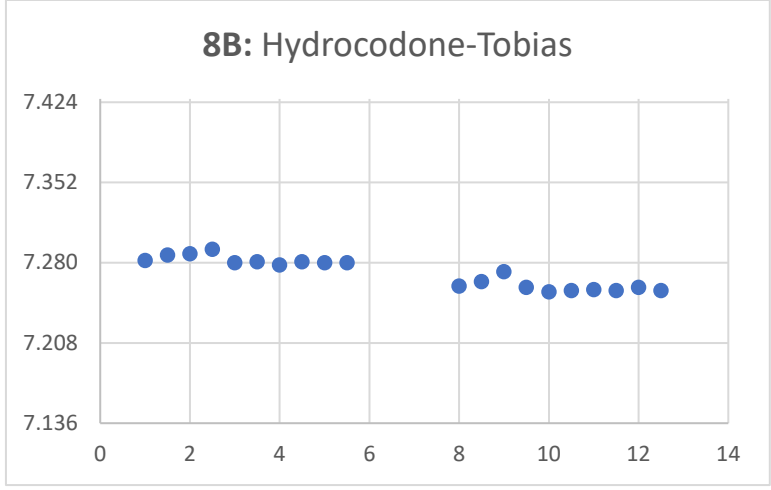

6A: Fentanyl-Kreacher
10.194
10.094
9.995
9.895
9.795
0 2 4 6 8 10 12 14

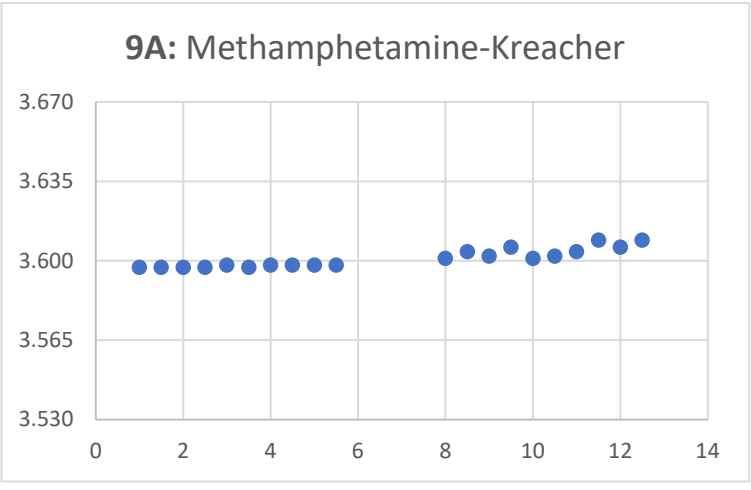

6B: Fentanyl-Tobias
8.225
8.145
8.065
7.985
7.905
0 2 4 6 8 10 12 14

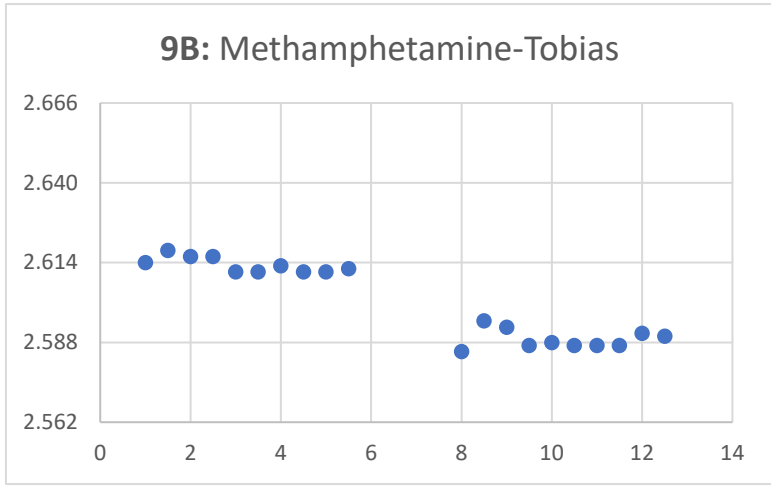

7A: Heroin-Kreacher
9.771
9.676
9.581
9.487
9.392
0 2 4 6 8 10 12 14

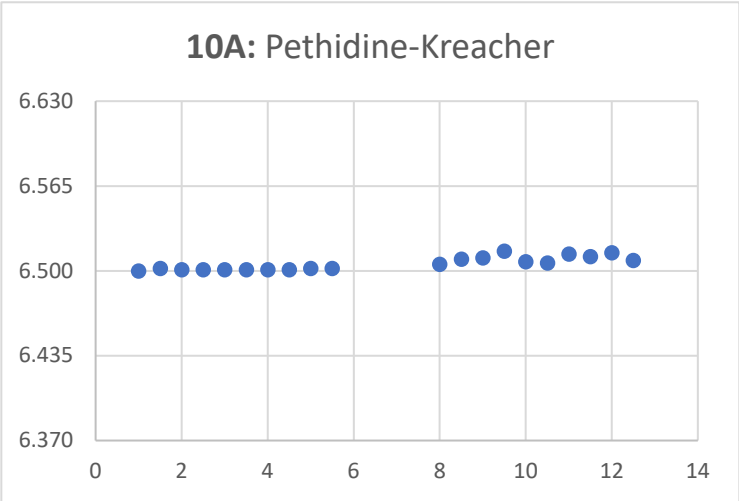

7B: Heroin-Tobias
7.948
7.870
7.792
7.715
7.637
0 2 4 6 8 10 12 14

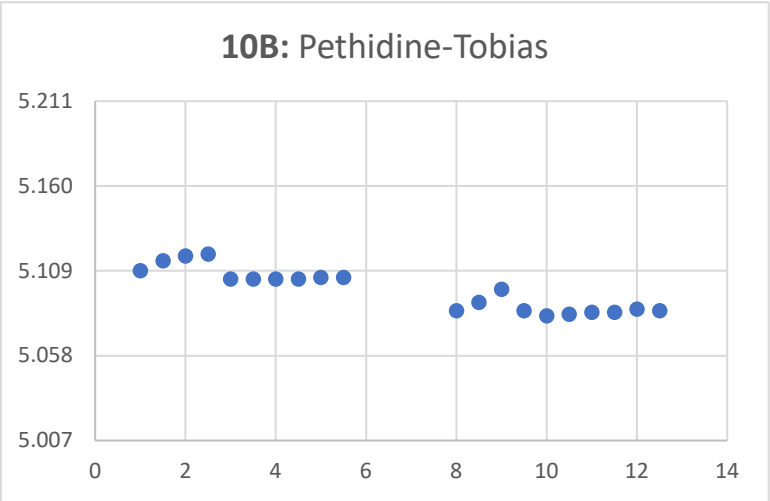

8A: Hydrocodone-Kreacher
9.071
8.984
8.896
8.809
8.721
0 2 4 6 8 10 12 14

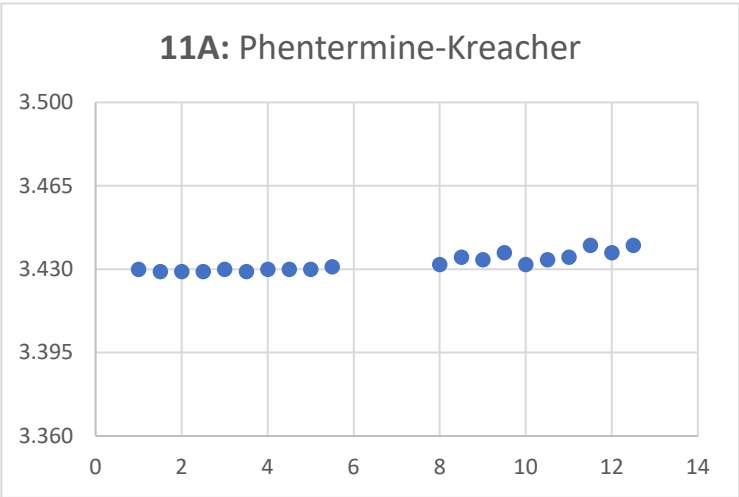

8B: Hydrocodone-Tobias
7.424
7.352
7.280
7.208
7.136
0 2 4 6 8 10 12 14

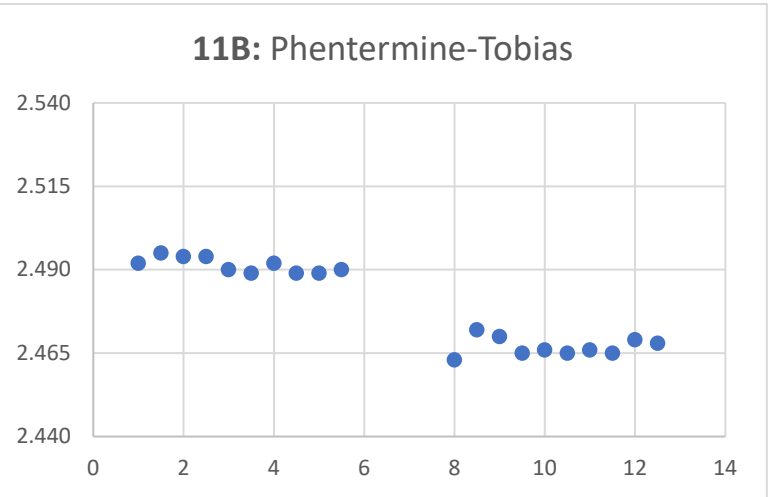

9A: Methamphetamine-Kreacher
3.670
3.635
3.600
3.565
3.530
0 2 4 6 8 10 12 14

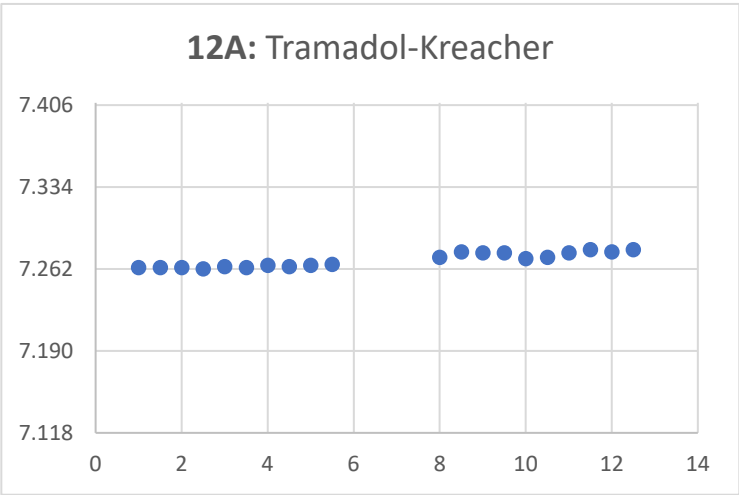

9B: Methamphetamine-Tobias
2.666
2.640
2.614
2.588
2.562
0 2 4 6 8 10 12 14

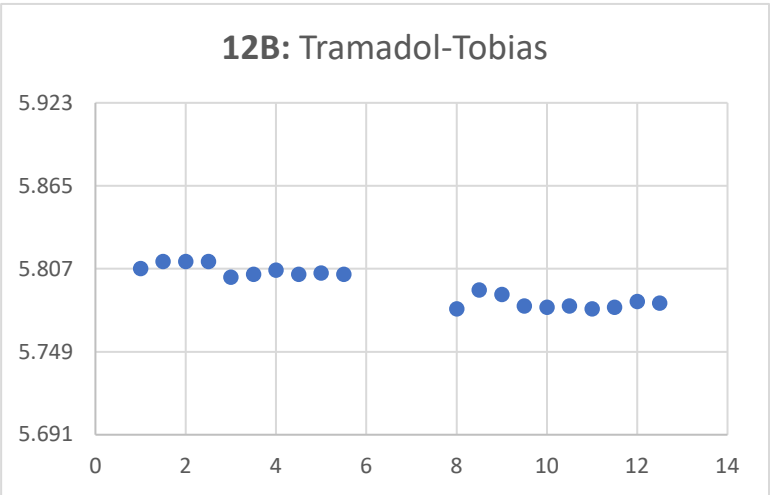












Appendix B

Appendix B contains the printed GC/MS data files for the study. For each instrument, the first run is printed out with the accompanying mass spectra for each standard. Each subsequent run is printed as an integrated TIC. The TIC for the unusable run from week 2 on Kreacher is also provided in appendix B.

Appendix C

Appendix C contains printouts of the excel spreadsheets used to create Table 2 and Figures 3-12. It also contains a printout of the retention time values as they were transferred from the GC/MS TICs.