Third Quarter Report to the Midwest Forensics Resource Center

1. *Fast Gas Chromatography Capabilities in Drug Identification*

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3. Third Quarterly Progress Report: July 1, 2011 – September 30, 2011

4. Project Description:

Fast gas chromatography with hydrogen mobile phase (Fast GC-H_2) enables significant increases in sample throughput, chromatographic resolution, and budget savings in drug identification involving GC separations. Peer-reviewed research in Fast GC and recent work by the PI with Fast GC-H_2 in ignitable liquid identification demonstrates significant improvements in each listed figure of merit. These benefits should translate to Scheduled compound analysis by Fast GC-H_2. This work will assess Fast GC-H_2 in drug identification, provide data for the use of H_2 in GC-MS, and create webinar modules and discussion networks for laboratory personnel to discuss the potential implementation of Fast GC-H_2.

5. Project Objectives:

The objectives of the project are three-fold:

a) assess the primary impact of Fast GC and hydrogen carrier gas usage in the area of drug identification as each aspect may provide a substantial time savings per analysis,

b) explore the limitations that Fast GC may place on MS signal collection;

c) create webinars for a more robust dissemination of meritorious results from Fast GC in drug analysis as well as the capabilities of hydrogen carrier gas in GC-MS instruments with one area of interest being safety issues.

6. Current Status/Results

a) Progress to date:

Instrumentation/Methodology:

This quarter included the Summer 2011 term in which the undergraduate student and PI worked on completing the analysis of a library of Scheduled compounds. These compounds were purchased from Cerilliant and Sigma Aldrich (Table 1). To evaluate specifically the effects of shorter retention times and narrow peaks associated with Fast GC, 1.0 mg/mL standards of lysergic acid (LSD) and cocaine were analyzed by

conventional GC-MS (Agilent 5890/5972; 20m DB-5 stationary phase) with helium mobile phase and hydrogen mobile phase. The minibore (0.18 mm ID) column and rapid heating cycles of Fast GC were not employed for this preliminary experiment given that the UWP GC-MS is not adapted; however, flow and oven parameters for this experiment were wildly exaggerated (no resolution required in a single component sample) to mimic to narrow peak widths of Fast GC. These preliminary experiments demonstrate no issues with the mass spectrometer and ion fragment pattern recognition with the narrower peaks resulting from faster elution.

Scheduled Compound	Supplier (1.0 mg/1.0 mL)
zolpidem	Cerilliant
delta9-THC	Cerilliant
methamphetamine	Cerilliant
phencyclidine	Cerilliant
amphetamine	Cerilliant
morphine	Cerilliant
LSD	Cerilliant
flunitrazepam	Cerilliant
ketamine	Cerilliant
buprenorphine	Sigma Aldrich
cocaine hydrochloride	Sigma Aldrich
3,4-methylenedioxymethamphetamine	Sigma Aldrich
alprazolam	Sigma Aldrich

Table 1. Scheduled Compound Standards Selected for Study

This experiment also provided further information related to hydrogen use as a mobile phase in GC and the mass spectrometer. First the theoretical flow maximum according to the Van Deemter equation permits much higher (> 2X) flow rates for hydrogen, and the data illustrate this as high theoretical plate counts were observed for flow rates between 1.50 and 2.50 mL/min with hydrogen. The Agilent 5972 did experience slight increases in ion gauge pressure pumping the hydrogen (5.3×10^{-5} torr) compared to helium (3.5×10^{-5} torr).

In addition, hydrogenation did not appear to be an issue in this experiment. If sufficient time remains and equipment is inventory following completion of the goals of this project, an additional experiment will be performed using steel and nickel lines to see if the theoretical (and antidotal) possibility of significant hydrogenation is possible. While this is primarily associated with purge and trap techniques and that is not prevalent in drug identification, purge and trap may be used in other units in crime laboratories (i.e. Trace) which use GC-MS.

Chromatographic results from this experiment provide the first data point related to hydrogen. Laboratories benefits from hydrogen usage include sharper peaks, accessible shorter retention times without conversion to fast GC through increased flow, and more secure market access and stable prices with hydrogen. This result is significant in that

some facilities may be affected by infrastructure or budget constraints that impede implementation of Fast GC yet these analysts could still access improvements and, in cases where appropriate, perhaps use the financial savings to justify later conversion to Fast GC.

Given that the data from the comparison of hydrogen and helium demonstrate the benefits of simply switching mobile phase to hydrogen, the next experiment was designed to assess the addition of Fast GC to the equation. In a comparison of Fast to Conventional GC two variables were singularly assessed while controlling for column length and stationary phase. These included use of the minibore (0.18 mm ID) column length and higher oven temperature ramping. From these experiments, a sharpening of peaks (narrower widths) was observed with aggressive GC oven ramps (> 30˚C/min) and resulted in reduced retention times across the standards (Figures 1a and 1b).

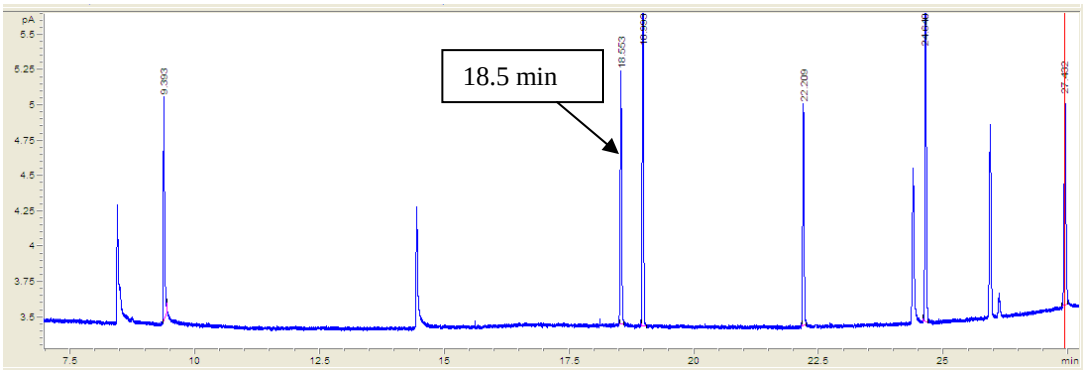

Figure 1a. Drug Mix Separated on 20 Meter, DB-5 GC Colum Using Hydrogen Carrier (Mobile) Phase; Oven Ramp 10ºC/min

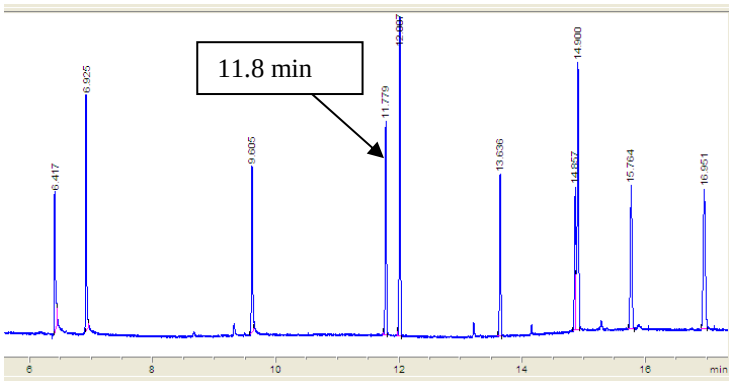

Figure 1b. Drug Mix Separated on 20 Meter, DB-5 GC Colum Using Hydrogen Carrier (Mobile) Phase; Oven Ramp 30ºC/min

Another concern entering this project was the sample overloading of the smaller minibore column (0.18 mm ID compared to 0.25 mm ID). All analysis to date has involved the use of 1.0 µL samples. From Fig 1b, the peaks appear generally symmetric even with the 1.0 µL sample. A comparison of symmetry values demonstrates no difference ($p < 0.05$) with Fast GC-H_2. As a matter of professional opinion and common practice in drug identification, analysts may create samples with concentrations well in excess of 1.0 mg/1.0 mL. This may provide a potential problem with column overloading. A final experiment is planned following the installation of the conversion kit at WSCL-M to assess this potential issue further.

Given the relatively low number of peaks in typical GC analysis of illicit drug samples, resolution concerns are generally lower than in other analytical fields with a multitude of chromatographic peaks to resolve such as ignitable liquid analysis. Figure 2. Examines peak width changes as the retention times decrease during the Fast GC-H_2 process. This experiment demonstrates that peak width general decreases in proportion to the shortened retention time which means that Fast GC-H_2 can be used very aggressively on samples with few peaks to exploit the chromatographic properties of hydrogen for incredible fast analysis times without loss of mass spectral features for identification. For example, the current WSCL-M method results in an 18 minute retention time for the strongly retained species alprazolam, but with Fast GC-H_2 this was easily cut to 11 minutes. Conversely, more complicated matrices or samples with multiple components can be analyzed in somewhat less aggressive flow and oven conditions to maximize the benefits of the hydrogen for improved resolution compared to helium. This provides an outstanding duel capability for the analyst.

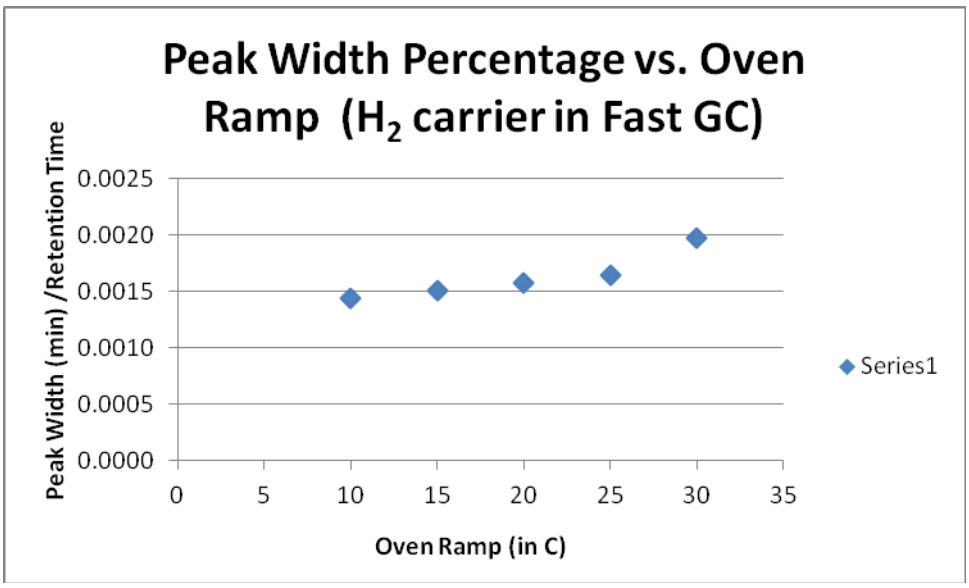

Figure 2. Analyte Peak Width /Retention Time as a Function of GC Oven Ramp

Outside of the experimental results, there have been two issues with equipment related to the grant. The first is the installation (upgrade) of the Fast GC at Wisconsin State Crime Laboratory-Milwaukee. Work is just now being completed to supply the necessary 208V line for the more robust Fast GC oven. There is also some clarification required on buck-boost on the 208V line as that is the "traditional" way of substituting 15A/ 208V service

for the true 240V. The second issue is an autosampler tower failure on the UWP Fast GC. While the initial problem appeared to be repaired in June, the tower lost all functionality. This slowed work somewhat until WSCL-M loaned a tower for troubleshooting. The problem has been addressed, but there is a request to use supply budget funds for this repair.

Administrative:
Complete tower failure on the UWP Fast GC was temporarily dealt with by a loan of a working tower from WSCL-M. The repair estimate is $800. Supply budget is running in the black for this stage of the grant, and I would like to use funds from here to affect this repair.

Work to date was presented in Lombard, IL on Sept 22, 2011 as part of the Drug ID forum for the annual Midwest Association of Forensic Scientists meeting. The work was also presented as a poster. There were three questions during the oral with at least six analysts engaging in further, later conversation as a result of these presentations. My estimation is that the drug identification community is very interested in the results, and two analysts were eager to know when the webinar would be made available.

Abstracts (poster and oral) for the American Academy of Forensic Sciences annual meeting in Atlanta GA (Feb 2012) have been informally accept (notice by personal email). We would like to request a one-quarter extension through Mar 31, 2012 for dissemination purposes

b) Work that lies ahead:

The experimental and analysis work is drawing to a close. Ms. Macans and I are preparing to review data within the next two weeks to determine if any further experiments are necessary before installation of the conversion kit at WSCL-M.

While some outline work on the webinar took place this past month, effort here needs to increase. Timelines may be affected by staff availability as well. Completion of the webinar certainly did not occur in this third quarter but is a second priority behind installation of the upgrade in Milwaukee.

c) Assessment of time and budget

At this point, the project work appears to be within the time allotted, but dissemination at the AAFS conference in Feb 2012 necessitates requesting a one-quarter extension. From a monetary standpoint, the project appears to be well within budget even with the repair of the UWP Fast GC autosampler tower.

7. Discussion of problems that have arisen:

The issues to arise this quarter have been mentioned in various sections of this report. There was an autosampler tower failure that delayed work slightly during the third quarter and necessitated a repair. There were also delays, somewhat expected though, in infrastructure adaptations in the WSCL-M facility. Delays also arose in the webinar as a result of scheduling.

These issues should not pose delays in completion; however, the dissemination to the American Academy of Forensic Sciences annual meeting will require an extension to March 31, 2012. This additional period will certainly cover any future minor delays in the webinar completion should they arise.