Final Report

1. Title of Project — *Fast Gas Chromatography Capabilities in Drug Identification*

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3. Report ID and Period — Final Report (January 1, 2011 – June 30, 2012)

4. Abstract

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 Description

While autosamplers have increased sample throughput in trace units across the nation's crime laboratories, many labs still find themselves desiring higher sample throughput, especially in the area of chromatography. From an instrumental perspective, the stable, rapid heating cycles of Fast GC coupled with narrower capillary columns and high phase ratio result in more theoretical plates per meter, and this increase enables fast separations with potentially superior resolution compared to conventional GC if hydrogen replaces helium as the carrier gas.

Switching to Fast GC from a conventional unit is a relatively simple, inexpensive proposition. For example, an Agilent 6890 GC adaptation to Fast GC involves upgrading the power source to either a 220 V or 240 V (preferred) service, installation of a high-heat oven shroud, power cord, and narrower capillary column. Using hydrogen as a carrier gas in Fast GC results in further increases in species resolution. The instrumentation upgrade may cost as little as $1,000-$2,000 depending on infrastructure in place.

This project is designed to assess the use of Fast GC and the switch from helium to hydrogen mobile phase in the area of illicit drug identification and provide the Wisconsin State Crime Laboratory-Milwaukee (WSCL-M) the opportunity to convert a conventional GC with helium mobile phase to Fast GC with hydrogen mobile phase (Fast GC-H_2). The goal of the project is to provide a realistic step-by-step assessment of this conversion and disseminate it to the drug analysis community through traditional means (peer-reviewed conference presentations and publication) as well as relatively novel formats such as an asynchronous webinar.

6. Project Objectives

The central objective of this project is providing a pathway to more efficient, cost effective separation in the identification of illicit drugs in the regional, state, and federal crime laboratories. To this point, the project has three specific objectives and four experiments to achieve these.

The first objective of this project is an assessment of the expected theoretical gain in resolution and sample throughput for a drug identification unit using a combination of Fast GC and hydrogen carrier gases. The second objective is an assessment of hydrogen use in GC and its effects on mass spectrometry data as some analysts express concern over loss of signal and a potential for hydrogenation with the conversion to H_2. The final objective is the improved dissemination that includes an asynchronous webinar in addition to traditional conference presentations and publication.

7. Procedures

Materials and Equipment

This project utilizes Wisconsin State Crime Laboratory – Milwaukee (WSCL-M) protocols for conventional GC parameters and sample preparation of <1 mg/mL sample solutions for those compounds not available to UW-Platteville through commercial purchase should the appropriate SUA and DEA licenses be delayed. The WSCL-M lab will select at least 30 Scheduled compounds for analysis and generate baseline data for those compounds using their current GC-MS protocols. Commercially available ASTM standards are purchased from suppliers such as Grace, Sigma Aldrich, and Cerilliant. Extraction solvents are purchased from Sigma Aldrich and Fischer Scientific.

The primary instrumentation for the project includes three GC's (one Fast GC-FID and two GC-MS units). The UWP Chemistry Program recently (2006) acquired an Agilent 6890 Fast GC-FID/ECD unit through the University of Wisconsin System *Laboratory Modernization* program. Standards and samples will be analyzed using this Fast GC, the UWP Agilent 5890-5972 GC-MS, and an Agilent 6890-6972 GC-MS at WSCL-M. Experiment 1 will compare the effects of hydrogen and helium as a carrier gases in the analyses of these compounds with respect to resolution. Experiment 2 seeks to quantify the maximum gain in efficiency available to drug and toxicology analysts using Fast GC-H_2 with no loss in current resolution. Experiment 3 will examine the issue of sample size and column loading on the smaller Fast GC column.

Research / Project Plan

The Project Plan calls for research experiments to provide answers to three fundamental questions and creates an improved dissemination to further increase the probability of implementation. While there are many facets of the proposed work that could be implemented in a crime laboratory, the most important question for directors and analysts appears to be, "how can I switch to hydrogen safely without losing effectiveness?" The second set of questions that we seek to answer is, "what is Fast GC?" and "how can it help me?" The third set of questions ask, "what will happen to sample sizes and column loading?" and "how fast can I go without compromising the resolution of my MS"

Experimental

Fast GC and hydrogen carrier gas usage can provide crime laboratory analysts with superior resolution in significantly less time in screening samples for compounds. If this hypothesis is accepted, crime laboratories would reap potentially large savings in terms of time, money, and/or space allocated to the instrumental phase of analysis. A library of at least thirty Scheduled compounds suitable for GC analysis will be prepared by using WSCL-M protocols and split for comparison. The first phase of Experiment 1 establishes a baseline for and compares each of the three instruments using the current mobile phase of helium and similar analysis parameters. From there, the second phase of the Experiment assesses simply the impact of hydrogen on both separation and MS aspects. For this phase, the UWP instruments switch to hydrogen carrier gases to assess the separation resolution (GC-FID) and MS effects (5890-5972).

Experiment 2 is designed to assess the impact of Fast GC using the more aggressive temperature profiles and smaller columns of Fast GC. This experiment involves the UWP Fast GC operating with hydrogen carrier gas and optimized Fast GC-H_2 conditions. Column overloading is somewhat of a concern with Fast GC. Therefore, Experiment 3 examines column loading to provide a more universal assessment of the technique. Controlled alterations in sample size and appropriate assessment of the chromatography are used in this assessment. The potential instrumental time savings will be calculated for Fast GC using retention times of common drug analytes. Experiment 4 compares the mass spectra generated in a variety of conditions involving Fast GC-H_2 and Conventional GC-He. The experimental outcome here is an assessment of mass spectral resolution as related to significantly shorter retention times and decreased scans.

8. Results/Discussion

Experiment 1 (Listed as Experiments 1 and 2 in the Proposal and Library Changes)

After consultation with crime laboratory drug analysts through the Midwest Association of Forensic Sciences annual meeting, the list of Scheduled compounds in the study is was focused to twenty (Table 1) compounds from the original thirty. The consensus opinion was that the twenty selected compounds were more than sufficient to span the gamut of functional groups and retention times commonly encountered and included common separation issues such as heroin and 6-MAM. The Conventional GC – He instrument (He@1.0 mL/min) in use in the WSCL-M and the newly installed and validated Fast GC-H_2 WSCL-M instrument (H_2@3.0 mL/min) were compared in Experiment 1 using the "long run" procedures of WSCL-M with these results also listed in Table 1.

Compound	He@1.0 mL/min	H_2@3.0 mL/min		Compound	He@1.0 mL/min	H_2@3.0 mL/min
Methamphetamine	4.117	2.335		BTCP	10.867	6.791
BZP	6.053	3.609		THC	10.889	6.860
TFMPP-basified	6.248	3.744		Oxycodone	11.047	6.919
TFMPP	6.250	3.774		6-MAM	11.039	6.925
MDMA-basified	6.300	3.797		Heroin	11.438	7.214
MDMA	6.302	3.900		Clonazepam	12.120	7.881
Ketamine	8.087	4.973		Alprazolam	12.518	7.961
MDPV	9.412	5.843		LSD	13.284	8.358
Cocaine	9.734	6.097		Buprenorphine	14.875	9.112
Diazapem	10.727	6.741		Testosterone	16.549	9.889

Table 1. Scheduled Compound Library for Experiments (Methanolic Standards)

Several key findings resulted from Experiment 1. The data in Table 1 illustrate a WSCL-M "long" run procedure which is generally utilized for samples in which the color are inconclusive or negative. Data from the WSCL-M and UWP Fast GC analyses of these compounds demonstrated that Fast GC-H_2 provided up to a 60% decrease in reproducible retention times. Other data from similar experiments using the UW-Platteville instrumentation demonstrate that peak shape improves with hydrogen use (Figures 1a and 1b). The Fast GC-H_2 analysis of the alprazolam standard exhibited a peak symmetry of 0.963 whereas the conventional GC-He analysis returned a peak symmetry of 0.889. In addition, methanolic standards of the compounds in Table 1 were used to create mixes to assess the changes in resolution because of the relative simplicity of drug matrices compared to ignitable liquids for instance. These drug mix data indicated that the use of hydrogen instead of helium resulted in resolution gains ranging from 30 to 60%.

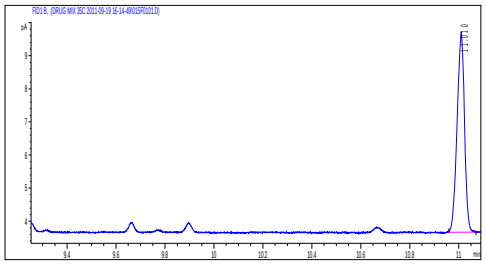

Fig 1a. Alprazolam by Fast GC with Hydrogen (Ret.Time = 11 min.)

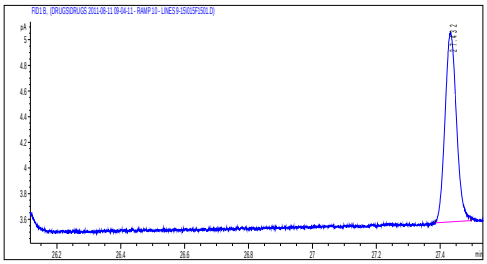

Fig 1b. Alprazolam by Conventional GC with Helium (Ret. Time = 27 min.)

This experiment provided useful information on the benefits of Fast GC-H_2 with respect to maximizing sample throughput on "short run" procedures used following positive color tests. Generally speaking, these were traditionally more aggressive profiles on the conventional GC and used for samples with strong presumptive indications (i.e. presumptive/color tests) of a straightforward sample and matrix. These procedures reflected the limitations of heating cycles with conventional GC in reproducing short retention times. The Fast GC however operated on the higher 208/220V service and provided significant increases in heating ramps (30ºC/min) with reproducible retention times. A cocaine "short run" comparison was selected for Figures 2a/2b.

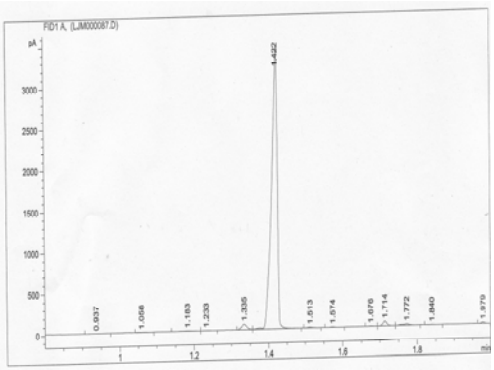

Fig 2a. Cocaine by Fast GC with Hydrogen (Ret.Time = 1.422 min.)

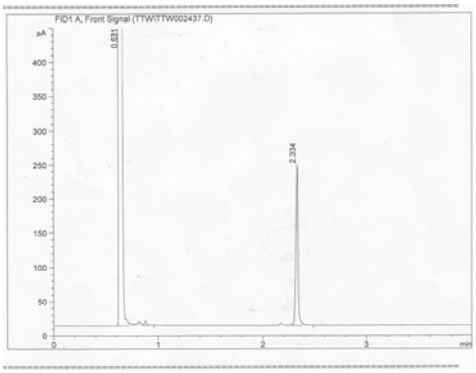

Fig 2b. Cocaine by Conventional GC with Helium (Ret. Time = 2.234 min.)

As Figures 2a and 2b illustrate Fast GC-H_2 has the capability to bring short-run analyses into the realm of tens of seconds with reproducible retention times. The question here becomes sample throughput in terms of bench time. Assuming that the sample preparation is the bulk of time spent on the analysis of any one sample, these reductions by Fast GC are unlikely to shorten the analysis of a sample. However, if the opportunity exists to load more samples per autosampler sequence, a gain may be realized. Alternatively, the shorter run times made possible by Fast GC could provide more time for more blanks and/or standards per tray of samples for improved quality assurance.

Experiment 2

In Fast GC applications with minibore (0.18 mm ID) columns, column overloading and resulting tailing occurs with traditional 1.0 µL injections. While manual injection provides a continuum of responses, the Agilent software controlled autosamplers used in this project are limited to 1.0 and 0.2 µL injection volumes. The more straightforward means of addressing sample on column is an increase in the split off amount. The primary finding here is that column overloading must be addressed when transitioning to minibore columns.

Experiment 3

In discussions surrounding this project proposal, a few colleagues expressed a variety of concerns regarding the use of hydrogen and mass spectrometers. Two colleagues expressed concerns regarding the response rate of the mass spectrometers, especially the standard quadrupole in service in most drug units. Additionally, there were passing questions regarding potential hydrogenation in mass spectra.

Experimental data from Fast GC-H_2 did not provide any reason for concern on either of these issues. While the faster elution of compounds with Fast GC-H_2 resulted in narrower peaks or short time intervals for sampling, there were ample ions collected with no observed difficulties in mass spectral output library matching. The effect of Fast GC oven ramp rates on peak width normalized to compound retention time was also measured and plotted in Figure 3. In Figure 3 the trend peak narrowing was not significant until 30 ºC/min ramp rates were reached, and as stated, the quadrupole (Agilent 5972 MSD) was quite capable of reliably detecting the compounds of interest even at ramps of 35 and 40 ºC/min.

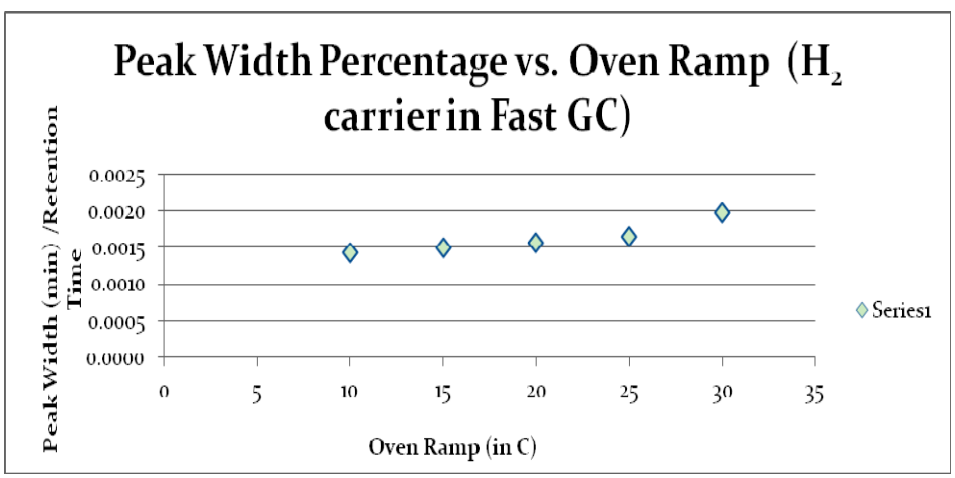

Figure 3. Peak Width Normalized to Retention Time as a Function of Ramp Rate

On the topic of hydrogenation, there was no noticeable effect with hydrogenation using a standard configuration of Agilent 5890 GC – 5972 MSD at the University of Wisconsin-Platteville facility. The data support literature discussions which do not suggest hydrogenation issues. There is some indication though that contact with steel (i.e. purge and trap device) may catalyze this process if present. Discussion of this project at the Midwest Forensics Resource Center annual meeting in Columbus, OH in May 2012 resulted in a related issue though. This discussion brought to light somewhat abnormal chromatographic tailing for a laboratory that had recently switched from helium to hydrogen. This tailing was present for several weeks and eventual reached acceptable QA-QC levels according to discussion with the laboratory's director and analyst. Subsequent discussion developed a potential explanation that a hydrogen generator was supplying the hydrogen in a fresh re-start situation which may have induced the chromatogram artifact.

In summarizing the results of this project, Fast GC-H_2 provides several opportunities for improvements in the identification of Scheduled compounds. In situations where color tests and preliminary screenings permit rapid analysis of straightforward matrices, the Fast GC capabilities mean that laboratory can shorten run times further and use the savings in instrument time in a manner that best suits their needs. For samples in which color tests are inconclusive or negative and longer screening GC runs are required, the Fast GC-H_2 combination can also reduce retention times by as much as 60% while actually increasing resolution which is useful in separating compounds with similar retention times.

Perhaps the most significant outcome of this project was the increased flow rates available with H_2. The switch to H_2 from He mobile phase (carrier gas) provides a significant cost savings as bottled (cylinder) hydrogen costs are approximately 60% the cost of helium currently, and the supply of hydrogen is more stable which eliminates concerns over suppliers refusing to guarantee annual helium contracts. The ability to access flow rates up to 3.0 mL/min provided some significant decreases in retention times without the need for Fast GC and the same improvements in resolution. This finding certainly points to the switch to the H_2 mobile phase as the more advantageous single factor in this project, but incorporating the Fast GC as well produces a more powerful tool. One note of concern involves hydrogen generators. At this point in time, discussions have indicated that there may be a chromatographic tailing effect present in analyses performed with hydrogen supplied by recently dormant generators. This should be examined more closely.

Ultimately each laboratory needs to assess the best manner to capitalize on the potential gains. Obviously the use of H_2 is going to save laboratories significant funds, and these savings likely could be increased through hydrogen generators. The Fast GC and shortened retention times must be assessed against the bench throughput to ascertain value; however one area of potential gain lies with "long" run screening, especially with the dire need for reliable color tests for the synthetic cannabinoids. In the case of the WSCL-M lab, the current Conventional GC – He long run is twenty minutes. With Fast GC-H_2 this long run could easily be reduced to 12 or possible 10 minutes which would help alleviate the increasing case load in this area.

It is important to note that hydrogen is a flammable gas with a wide range of explosive concentrations. This however should not be a deterrent to accessing the immense potential of its use as a mobile phase. Agilent, for one instance, has covered the topic of hydrogen safety (and its use with GC-MS) very well in documents; however, each laboratory needs to integrate thorough safety protocols into their SOP if adopting this technology.

9. Dissemination Discussion

This work was presented in the poster session[1] of the Midwest Association of Forensic Scientists 2011 annual meeting in Lombard, IL. An oral presentation[2] was also given in the (Drug) Chemistry section of this meeting. The work was also presented orally[3] in the Criminalistics Section of the American Academy of Forensic Sciences annual meeting in Atlanta, GA in February 2012 and as a presentation[4] to the annual meeting of the Midwest Forensics Resource Center in Columbus, OH in May 2012.

Project Objective #3 focused on enhanced dissemination through the creation of an asynchronous webinar. This was produced using software available through the University of Wisconsin-Platteville Media Services and has been housed on UWP server for asynchronous viewing at:

http://mediasite.uwplatt.edu/uwp/Play/d71f97a70a0c4157aa65633a1a1598ab1d

In addition, a discussion board specific to Fast GC and H_2 use in forensic applications has been created. The URL for this board is:

http://fastgc-hydrogen.proboards.com/index.cgi

The PI encourages any and all distribution of these links to colleagues in the affected fields.

Furthermore, the success of this project prompted the PI to work with the MAFS organization to organize a workshop for the Fall 2012 annual meeting which, fortuitously, happens to be in Milwaukee, WI which will tentatively provide an opportunity for workshop participants to tour the unit and see the implementation first-hand.

[1]R. Hansen, L. Macans, and C. Cornett (2011) *Fast Gas Chromatography Capabilities in Illicit Drug Identification*; Midwest Association of Forensic Scientists Annual Meeting; Lombard, IL.

[2]C. Cornett, R. Hansen, and L. Macans (2011) *Evaluation of Fast Gas Chromatography Coupled with Hydrogen Mobile Phases in Drug Identification;* Midwest Association of Forensic Scientists Annual Meeting; Lombard, IL.

[3]C. Cornett, R. Hansen, and L. Macans (2012) *Evaluation of Fast Gas Chromatography Coupled with Hydrogen Mobile Phases in Drug Identification;* American Academy of Forensic Sciences Annual Meeting; Atlanta, GA.

[4]C. Cornett, R. Hansen, and L. Macans (2012) *Evaluation of Fast Gas Chromatography Coupled with Hydrogen Mobile Phases in Drug Identification;* Midwest Forensics Resource Center Annual Meeting; Columbus OH.