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Feasibility Study of Waste Isolation and Characterization by Instrumental Methods

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Feasibility Study

of

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Instrumental Methods

Presented by

Janith A. Wiehe-Wazio

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ABSTRACT:

The original focus of this project was to develop an in house procedure for the recovery of commonly used science laboratory solvents consisting of Acetone, Ethyl (Ethanol) Alcohol, Hexane, Methanol and Hydrochloric Acid from everyday laboratory waste. However, the projects scope shifted a little further back to basics when it was discovered that Laboratory Waste Safety and Accounting practices were insufficient. It was assumed that identification of waste products was being recorded accurately. It became apparent with the very first waste bottle analysis that chemicals recorded on the standardized waste log sheets were not always what was in the waste bottles. Therefore the focus was changed to verify if the existing waste disposal procedures are properly being followed with the expectation that future ongoing experiments can focus on the original recovery aspect of the project.

The experimental procedures of this project have zeroed in the Non-Halogenated and Halogenated labeled Waste. These chemicals have been chosen for the case study for the following reasons. These groups of chemicals:

1) Are a standard in numerous labs across the chemistry disciples.
2) Generate the largest volumes of waste.
3) Analysis can be conducted using currently available instrumentation.
4) Theoretically will provide quality standards of recovery

The experimental procedures have been theorized and developed from various chemistry curriculums over the past 13 years from the perspective of the Lab Manager.

INTRODUCTION:

Governors State University CAS-Science Division is about to celebrate three years in the long awaited renovated F-Wing. One of the major goals of the relocation into state of the art university laboratories was to implement a whole new "thinking" process. This process was designed to focus from inside out on the concept of "sustainability" as stated in the Governors State Mission statement.[1]

Expanding on the concept of Green Chemistry sustainability includes the 21st Century version of the 3R's. Replace, Recover, Reuse. Replace focuses on the concept of whenever possible use of alternative, less hazardous chemicals should be introduced into science teaching and research laboratory procedures. Recover generally focuses on the concept of recycling of waste products and unused reagents which ties into the concept of Reuse.

The focus of this Research Project is on the Recover and Reuse concepts. This focus includes the development of in house experimental procedures for handling the ever increasing chemical and biological waste products generated by the Biology and Chemistry curriculum and research classes. This proposed project study has evolved from an environmental point of view to an economic perspective due to the high cost of waste disposal.

METHODOLOGY:

An examination of guidelines and procedures is in process encompassing the various academic and governmental agencies that have been working in the area of Green Chemistry since its inception. Preliminary literature research has basically encountered a trend by the Environmental Protection Agency, American Chemical Society, Industry and Academia to recommend the outsourcing of the removal of laboratory waste by specialized handlers. Therefore, a back to basics approach is part of the experimental design of this in house analysis and recovery of laboratory waste materials.

Basics begin with an evaluation of the categories of laboratory waste GSU Labs generate. The Biology Labs' generation of waste is generally non-hazardous elements of plants and animal products. The exception being the Microbiology and Cell Biology Lab curriculum that conduct extensive studies of biological materials that require autoclaving procedures before disposal. This has lead to narrowing the scope of discovery to the Chemistry Lab curriculum which has been increasingly generating lab waste that has been at times difficult to find proper safety storage procedures. Once the scope of laboratory waste was chosen, the next step was to procedurally review the categories of chemical materials that are routinely used in the Chemistry Labs.

METHODOLOGY:

Experimental Procedures:

The following is a category listing of common laboratory chemicals:[2]

Halogenated Waste (any organic chemical that contains F, Cl, Br, or I)
Chloroform (Cl)
Benzalkonium Chloride (Cl)
Bromophenol blue (Br)
Crystal violet (Cl)
Eosin (Br)
Methylene Chloride (Cl)
Methylene blue (Cl)
Safranin (Cl)

Non-Halogenated Waste (organic solvents that do not contain F, Cl, Br, or I)
Acetone
Acetonitrile
Bis / Tris solutions
Cyclohexane
DAPI—2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride
DMSO—Dimethyl sulfoxide
EDTA—Ethylene-diamine-tetraacetic acid
Ethyl Alcohol _24%
Ethyl Ether
Fluorescein
Hematoxylin
HEPES—4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
Hexanes
Hybridization buffer (sodium dodecyl sulfate / sodium phosphate dibasic buffer)
Isopropyl Acetate
Isopropyl Alcohol _24%
Methanol _24%
Oil Red O

METHODOLOGY:
Experimental Procedures:

The following is a category listing of common laboratory chemicals:[2 cont'd]

Non-Halogenated Waste (organic solvents that do not contain F, Cl, Br, or I) cond't

Petroleum Ether (mineral spirits)
Phenol
2-Propanol _24%
TEMED—1,2-Bis(dimethylamino)ethane
tert-Butanol
Tetrahydrofuran
Toluene
TAE-Tris Acetate EDTA buffer

Non-Halogenated Waste (organic solvents that do not contain F, Cl, Br, or I)
Tris base
Tris borate (TBE)
Xylene cyanol

Aqueous Waste – Predominantly Water-Based
Buffers (water-based)
Sulfuric Acid
Hydrochloric Acid
Acetic Acid
Nitric Acid
Phosphate buffered saline (PBS)
Sodium Hydroxide
Trace metals?
Any alcohol <24% (Ethanol, Isopropanol, Methanol, 2-Propanol, etc)
*** (Sewer disposal is allowed for alcohols containing <24%, if
Alcohol is the only hazardous constituent)*

METHODOLOGY:

Experimental Procedures:

The following is a category listing of common laboratory chemicals:[2] [cont'd]

Solid Waste

Silica Gel
Magnesium Sulfate
Sodium Bicarbonate
Sodium Sulfate
Calcium Chloride

Separate Waste Streams for each of these

Ethidium Bromide gels and contaminated filters (solutions may go through filtration)
Osmium-containing products (Osium Tetroxide, Osmium Dioxide)
Mercury-contaminated debris
Formaldehyde, Formalin, or Paraformaldehyde aqueous solutions
Chromium-containing solutions
Lead-containing solutions

METHODOLOGY:

Experimental Procedures:

In an effort to incorporate as many laboratory procedures and techniques as possible to experimentally determine which would be the most effective in a waste identification and recovery system, the methodology of this project will start with experiments based on the basic theory of qualitative and quantitative analysis. The following experimental procedures evolved as a template for the development of waste identification methods for chosen case study chemicals.

Safety Precautions
Follow Safety Data Sheets procedures as indicated for the chemical being recovered.
Use personal protection equipment at all times:
--Safety glasses
--Lab gloves
--Chemical apron
--No loose clothing, open shoes, shorts, etc.

Experimental Procedure I:
Identification of Laboratory Waste
❖ Assemble the apparatus as depicted in Figure 1 as follows in a chemical hood:
   ✓ Standard ring stand and clamps—in stock
   ✓ 500ml Erlenmeyer flask—clean Kimax 27060
      • Rinsed with RO water only. Do not want to add any cleaning reagent (ex. Acetone) since trying to obtain a quantitative waste sample.
      • Attached flask to ring stand via clamps.

METHODOLOGY:

Experimental Procedure I:

- ✓ Buchner Funnel and rubber stopper—new/clean sized to fit 500mL flask
    - • Insert Buchner Funnel into the Erlenmeyer Flask checking for tight fit for efficient filtration to occur.
- ✓ Qualitative Filter Paper—VWR #28297-868 #417 Paper 5.5cm Lot#G1823096

    Note: Filter paper may need to be review for fast vs slow filtration rate depending on type of waste solution being analyzed.
    - • Place one VWR filter paper into Buchner Funnel checking for perfect fit to avoid waste solution being drawn directly into flask around edges.
- ✓ TygonTubing—size to fit Erlenmeyer flask and water line.
    - • Attach tubing to flask and water line. Test for tight fit to ensure efficient filtration.
- ✓ Graduated cylinder new/clean Flinn PC#5 100mL
    - • To be utilized to measure and transfer waste solution from waste bottle for filtration.

- ✓ Review Waste Collection Sheets to determine which waste bottles would result in reproducible Quantitative and Qualitative results. Figure 2
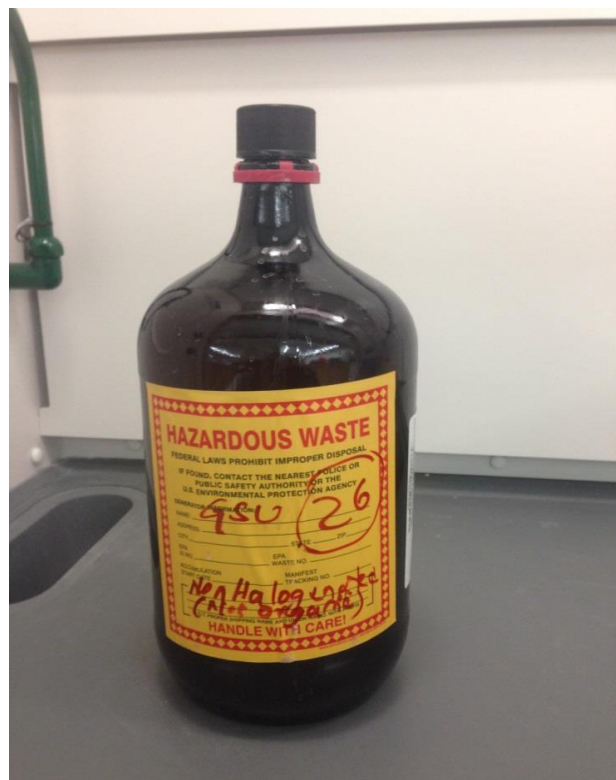
Figure 1:

Filtration Set-up

METHODOLOGY:

Experimental Procedure I:

Figure 2: Waste Bottle 26 and GSU Waste Accumulation Log Sheet

After establishing a Methodology and experimental (instrumentation) procedures, the waste bottle pictured was chosen for the initial analysis for the following reasons:

1) It was clearly labeled with a number (26) that matched a GSU Waste Accumulation Log.
2) It had a completed sheet GSU Waste Accumulation Log—chemical listed, dated, amounts, signed.
3) Chemicals listed should be identifiable.
4) The waste bottle and label were in good shape.
5) It didn't emit a "toxic" odor.

METHODOLOGY:

Experimental Procedure I:

1) Determine if waste bottles should be allowed to settle or agitated. Caution: review waste collection sheets to determine if chemicals contained in bottle can be safely agitated.
2) Place graduated cylinder in a plastic container/pan in chemical hood order to avoid spillage. Carefully pour contents of waste bottle into 50mL glass graduated cylinder thru plastic funnel. Choice of glass graduated cylinder allows for less chance of additional contamination to waste solution. Glass transfer funnel would be preferred choice; however in this experiment only plastic funnel available. Immediately clean up any spills.
3) Proceed with filtration. Let filtrate run for approximately 5 minutes. Prepare next waste filtration. Repeat this step 5 times to obtain 500mL of filtrated waste product/solution.
4) Once the allocated filtrations are completed continue the suction for approximately ½ hour in order to observe if any color changes occur in the filtrate due to air intake.
5) Record observations.

OBSERVATIONS:

Experimental Procedure I:

As soon as the solution from Waste Bottle 26 was transferred into the graduated cylinder and measured to the meniscus at the 100mL mark, the solution appeared to be the color of amber red. What could be causing this color? Possible the combination of the chemicals listed on the waste collection sheet. Tested to make sure filtration was working by adjusting the water flow. Continued adding waste into the Buchner funnel at a rate of about 25mL at time until the first 100mL completed filtration. Continued filtration for approximately 5 minutes before adding next 100mL of waste.

Prepared second 100mL returning the overage of waste to reach the meniscus mark to the waste bottle since it is already a waste bottle. Repeated filtration as before. Decided not to change filter paper since at this point no obvious solid waste had been collected. Continued with second and third filtration with no change in color of filtrate as it was collected.

Decided to cautiously agitate the waste bottle in order to mix the waste. At this point as the fourth filtration 100mL sample was added to the Buchner funnel at 25mL increments, the filtrate and filter paper stated to turn a bluish color. Checked for an odor to the sample filtrate by the whiffing technique. No odor was detected. As the filtration proceeded, a little foaming occurred in the flask that quickly disappeared.

Continued with preparation and addition of fifth and final filtration. At this point the color of the filtrate in the flask appeared to be a murky seaweed green. Continued the filtration for an additional ½ hour to check for any additional color change due to possible air intake. No significant color change was observed. At this point the water suction was turned off and the filtrated 500mL sample was allowed to settle as the separatory funnel experiment was set up.

QUESTIONS:

Experimental Procedure I:

Why did filtrate change colors between filtrations?
One possibility could be once the waste bottle was agitated, it mixed up various heavy aqueous materials that settled to the bottom of the waste bottle.

What caused the foaming in the filtrate during the fourth filtration?
Possible agitation of the waste bottle and mixing of waste chemicals resulted in a foaming action as the 100mL filtrate sample was added to the Buchner funnel. Another possibility could have been a surge in the water suction.

METHODOLOGY:

Experimental Procedure II:

- ✓ Separatory Funnel—new/clean Kimble 500mL from stock.
  - Rinsed with RO water only. To be utilized to verify separation of waste solutions into layers.
  - Attach Separatory funnel to ring stand.
  - Add 10mL to 20mL of Deionized water to Separatory to test stop cock closure.
- ✓ Erlenmeyer Flask 500mL—clean/dry
  - Set up as secondary collection. Figure 3

1) Carefully transfer filtrated 500mL sample from Erlenmeyer flask from Experiment I via plastic funnel to Separatory Funnel.
2) Let stand for approximately ½ hour in order to allow for possible layer separation.
3) If no visible separation, release the solution into the original 500mL Erlenmeyer flask from the filtration procedure. This is to avoid any additional contamination.
4) Pipette off 50mL using a glass pipette and place into a medium vial in order to check for any separation that may have been missed. Shake the vial in order to mix the sample. Let stand in order for any layer separation to take place.
5) Seal the 500mL Erlenmeyer flask containing the filtered 500mL sample with parafilm, label and place under chemical hood for further observations.
6) Clean and rinse all glassware with DI water. Place rinse water into a plastic container. Do not dispose down lab sink since may contain hazardous waste.
7) Label all samples, materials and waste with content information and dates.

METHODOLOGY:

Experimental Procedure II: cont'd

8) Clean up the surrounding work area. Dispose of all cleaning materials into a clearly label container for further treatment as waste. Treat as Non-Halogenated or Halogenated liquid/solid waste.

9) Store all glassware and other materials in a safe location. Preferable under a chemical hood for additional experiments on lab waste.

Figure 3: Separatory Funnel
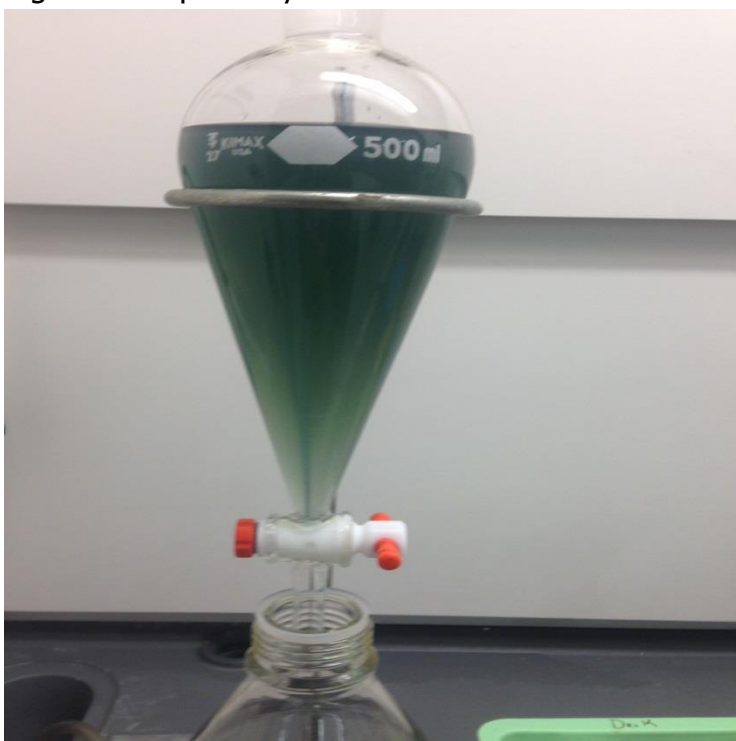
OBSERVATIONS:

Experimental Procedure II:

No obvious separation of layers was observed at start after all of the 500mL of filtered solution had been transferred to the Separatory Funnel. Expected to observe a water layer. Color of solution remains a seaweed green.

At 15min mark still no separation of layers and/or color change. Again at the 30min mark no separation of layers and/or color change. After an hour there was no apparent evaporation of possible water or any other solvents such as methanol and no layers.

Four days after the initial experiments, the 500mL filtered solution that was drained back into the 500mL Erlenmeyer flask for storage in the chemical hood needed to be moved to another location since a lab class required use of the chemical hood. At this time it was discovered that the seaweed murky solution had settled over the course of the four days. There now was a greenish/blue layer of approximately 100mL at the bottom of the 500mL Erlenmeyer flask and an almost clear solution for the remaining 400mL.
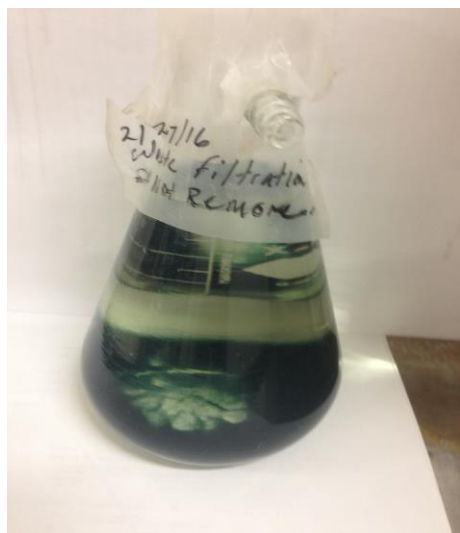
QUESTIONS:

Experimental Procedure II:

What caused the sample solution to separate into two layers and change color over the four days is stood in the chemical hood?
There is no obvious explanation as to why it took four days for the sample to separate into layers. Possible answers could be a solvent such as Acetone or Methanol evaporated off while under the chemical hood despite the fact the Erlenmeyer Flask was sealed with parafilm. Or did it just take time for the sample to naturally settle.

What could the now clear solution be since it settled as the top layer?
In order to answer this question, an approximately 10mL sample of the clear solution was decanted out by a glass pipette into a clean 50mL beaker. The solution pH was tested using pHydrion Controls pH papers. The solution tested positive at pH 6 which is close to the neutral pH 7 of water.

METHODOLOGY:

Experimental Procedure III:

Decanting of Sample Solution:
Development of this experimental step stems from the unexpected late settlement of the sample solution from Experiment II Separation by Separatory Funnel.

✓ Set up clean/dry 500mL Erlenmeyer Flask under a chemical hood.

1) Slowly decant solution from the settled sample flask into the clean/dry flask to avoid decanting any of the bottom settlement getting into the new flask.
2) Clearly label both the original sample solution flask and the new sample solution flask.
3) Seal both flasks with parafilm and place in a chemical hood to let settle for a few hours.

Observations:
The new sample unknown separated into two layers. A clear solution top layer and a blue/green bottom layer.   The bottom layers characteristics appeared to be that of Nickel. However, there should not be any metals in the sample waste bottle.   Waste Bottle #26 is labeled as "Non-Halogenated" waste.   Therefore, what chemical waste is actually in the bottle and/or what type of reaction is taking place that would create such a bottom layer?

METHODOLOGY:

Experimental Procedure III:

✓ Based on observations, the following additional experimental steps were developed:

1) Carefully draw out by glass pipette a few drops out of the blue/green solution and place onto the type of filter paper used for the original filtration. However, any available filter paper should work.
2) For comparison, repeat procedure one and place a few drops onto a watch glass.
3) Observe the filter paper and watch glass drops for any separation in the form of an inner and outer ringlet.

Observations:

Drops on filter paper indicated a mixture of water and known chemicals. The water evaporated off quickly leaving a bluish spot on filter paper.

The watch glass appeared to produce a phase within a phase with unknown chemicals trying to phase out.

METHODOLOGY:

Experimental Procedure III:

✓ The following procedures were added based on no clear formation of separate layers.

1) Fill a small GC/LC sample glass vial half full by glass pipette with B&J Dichloromethane solvent.
2) Fill the remaining half of the vial with the blue/green unknown solution by glass pipette.

Figure 4

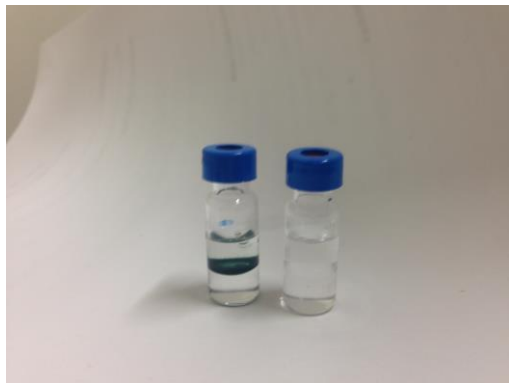3) For comparison blank repeat above procedure with ½ B&J Dichloromethane solvent and ½ water. (Blank on right)

Figure 5

4) Let both vial stand and observe if the two solutions will mix or separate into layers.

METHODOLOGY:

Experimental Procedure III:

Observations:
Figure 4: Sample in B&J Dichloromethane solvent separated into two layers
Figure 5: Blank ½ B&J Dichloromethane solvent and ½ water no visual separation observed.

✓ Based on these observations, the following additional experimental steps were developed:

5) Draw as much of the remaining clear solution out of the 500mL Erlenmeyer as possible using a clean 9" glass pipette. Careful not to draw off any of the sample settled at the bottom of the flask.
6) Place 10ml of the sample unto a petri dish and 10mL onto a medium sized watch glass. Place this set of dishes into a drying oven setting the temperature at a starting point of about 75°C. Increase as needed but do not let temperature exceed 100°C.
7) Repeat placing 10mL of the sample unto a petri dish and 10mL medium sized watch glass.   Place this set of dishes under a chemical hood to allow for air drying.
8) Check both sets of dishes at 5 minute intervals until any remaining water is evaporated off.

Observations:
The petri dish and watch glass with the sample solution dried quickly in the drying oven at a temperature of 80°C. The petri dish dried in 5 minutes and the watch glass dried in 15minutes.
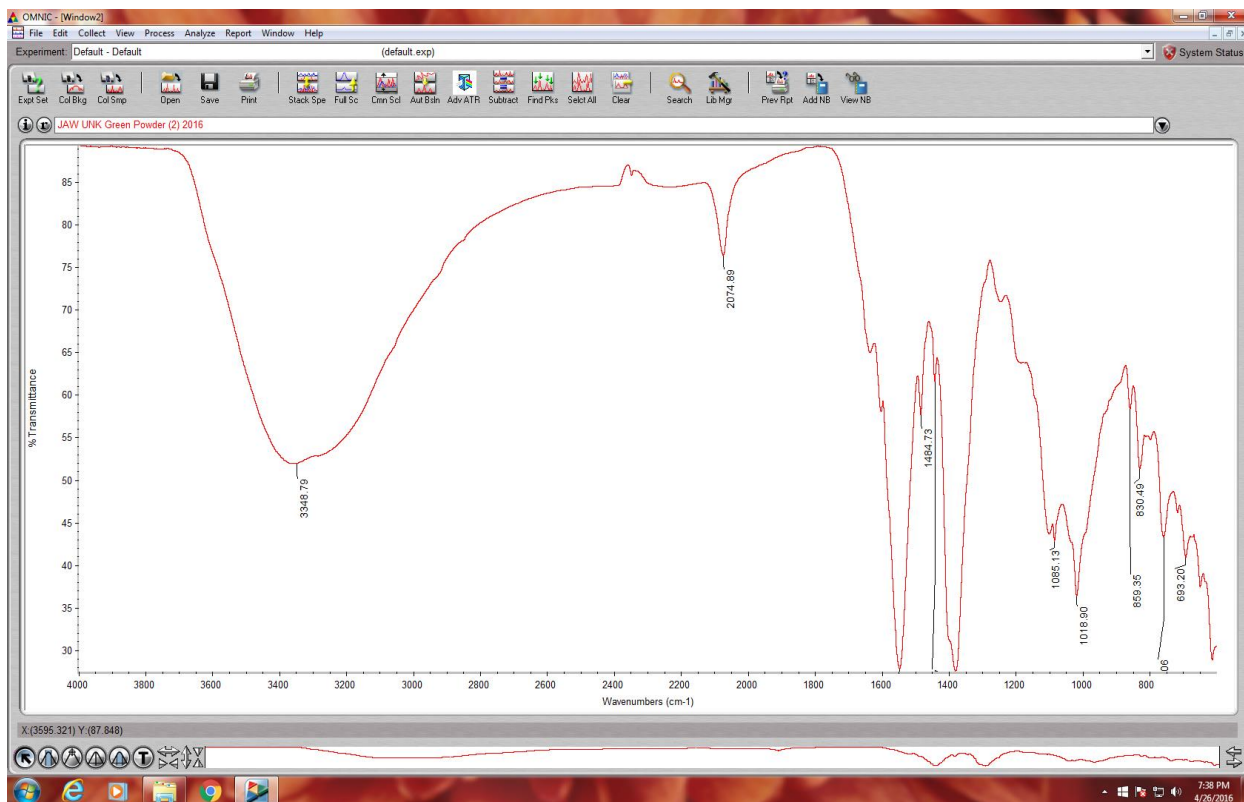
INSTRUMENTATION:

At this point in the waste study, the chemical analysis of the waste bottles was determined to consist of four of the types of instrumentation available in the GSU chemistry labs.

- Infrared Spectroscopy – typically used for analysis of pure compounds and limited mixtures.
- Gas Chromatography – for the separation of mixtures containing volatile and semi-volatile compounds.
- Gas Chromatography/Mass Spectrometry – for the separation of components of a mixture by (GC) and the identification of the analytes (compounds) by (MS) mass spectrometry based on their mass to charge ratio (M/Z).
- Liquid Chromatography/Mass Spectrometry – for the highly sensitive separation, general detection and potential identification of chemicals of particular masses in the presence of other chemicals such as complex mixtures.

INSTRUMENTATION:

The first Waste Bottle number 26 was now ready for instrumental analysis. Since solid samples were collected procedurally by filtration, separation and oven drying, the first instrument selected for a sample run was the FT-IT. The Nicolet iS5 FT-IR (Fourier Transform Infrared Spectroscopy) was chosen for initial identification since liquid and/or solid samples can be collected on this instrument. This technique obtained an infrared spectra of the filtered solid material utilizing the iD3 ATR (Attenuated Total Reflectance) accessory with the germanium crystal.

Infrared data for Waste Bottle 26 sample was collected in the region of Wavenumber region of 4000 cm^{-1} to 600 cm^{-1}

INSTRUMENTAL ANALYSIS:

Since a spectral match was not conclusive for this IR spectrum using the NIST libraries, spectral interpretation was necessary to try to characterize the unknown based on what information was listed on the waste bottle log sheet.

The OH stretch typically associated with alcohols is usually a broad and strong absorption near 3400 wavenumbers as shown. The over subtraction at about 2400 cm is due to Carbon Dioxide since the IR bench is not purged with nitrogen gas. But no alcohols were listed on the Waste Log for Waste Bottle 26.

The next peak at 2074 wavenumbers was not correlated to any specific functional group, however the next strong absorption bands seen at 1390 cm and 1550 cm can be attributed to an Aromatic C=C stretch or more likely due to a N-O stretch or bend for compounds containing the functional group of Nitro type compounds. Which was a possibility since the Library search did come up with a 30-39% match for various Nitro based compounds including Nitromethane, Nitrocyclohexane and 2-Nitropropane.

Once again none of those compounds were listed on the GSU Waste Accumulation Log. Therefore they could not be ruled out or confirmed.

Close to 1000 Wavenumbers the absorption bands (peaks) are indications of a strong C-O absorption. Again the Waste Log indicated Non-Halogenated (Non-Organic) waste which would rule out the carbon in the C-O absorption. Finally the range of peaks in the 600 cm to 850 cm region are characteristic of no known compounds.

Since this Infrared Data was generated from a solid sample that had been prepared based on a filtrated sample from waste bottle 26, the preparation procedures used may have not captured the true components of the waste. Traceable compounds may have been filtered out.

Based on the FT-IR analysis, focus was shifted to the analysis of samples using GC and GC/MSD.

INSTRUMENTATION:

Based on the FT-IR results for the filtrated solid sample, a liquid sample from Waste Bottle 26 was selected for analysis by Gas Chromatography. The Gas Chromatography Conditions consisted of the following:

- HP 7890 GC with FID detection
- HP-5 capillary column 0.32mm x 30 M x 0.25uM
- Nitrogen carrier gas
- Temperature Program
- 40°C to 250°C at a 10°C ramp rate
- Injection Port Temp 200 $^\circ$C   Split Ratio 50:1
- Detector temp 300 $^\circ$C
- 1uL manual injection

Waste Bottle 26 sample
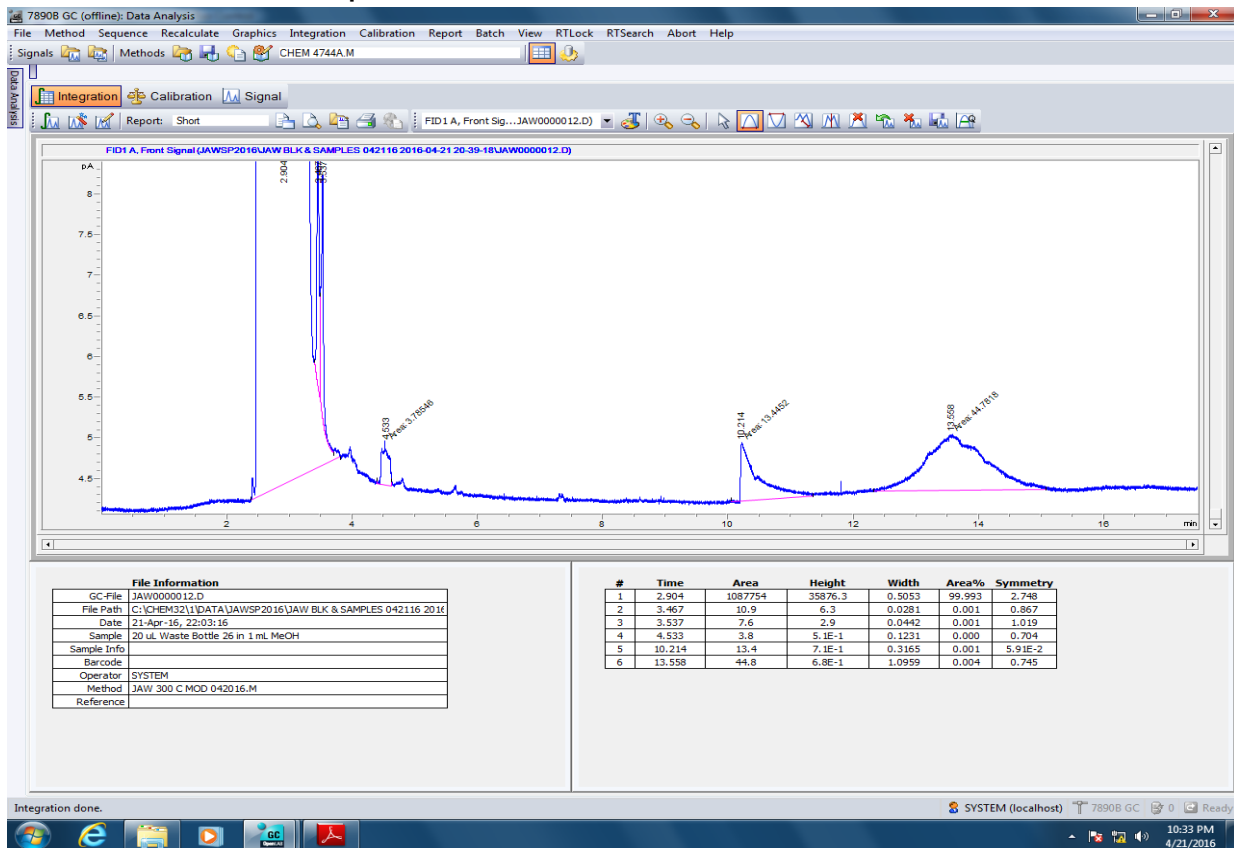
INSTRUMENTAL ANALYSIS:

This is a Gas Chromatogram for Waste Bottle 26 using the Agilent GC 7890B . This is the initial Chromatogram that was generated. It is obvious the first peak known as the solvent Peak is off scale. The GC Chromatogram display was enhanced or magnified to show the small analyte peaks. The area counts were very small in relation to the solvent peak and therefore should be considered as trace level components in the sample.

Based on the results of the IR Spectrum and in accordance with general GC practice a Methanol Blank was injected in order to assist in the further identification of Waste Bottle 26 sample. Therefore, compared to the sample chromatogram, the following chromatogram confirms that the first large peak is the MeOH 1mL solvent that was used to spike the 20uL Waste Bottle 26 sample.

Methanol Blank
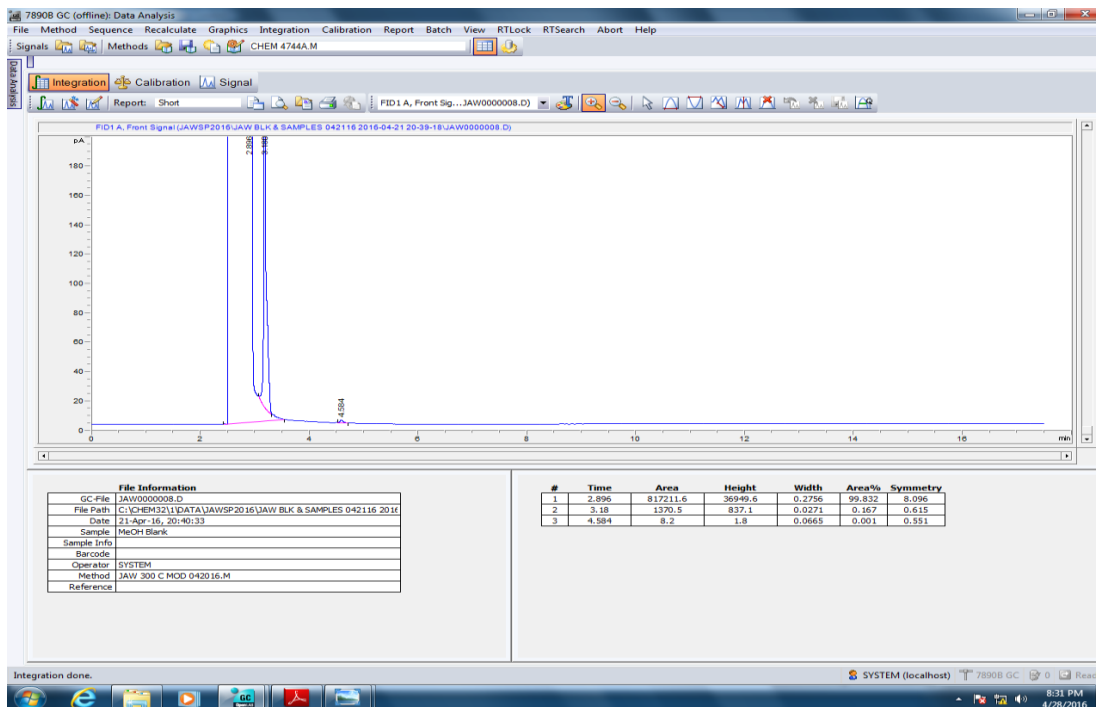
INSTRUMENTAL ANALYSIS:

Based on the FT-IR and GC results for the filtrated solid sample, a liquid sample from Waste Bottle 26 was selected for analysis by Gas Chromatography/Mass Selective Detector.

The Gas Chromatography Conditions consisted of the following:

- Agilent 7890 GC/5977 Quadrupole mass spectrometer

- Mass Hunter Software for data acquisition and analysis

- Temperature Program

- 40°C to 250°C at a 10°C ramp rate

- 1uL injection using autosampler

- Typical solvent cut 1.0min however, in last method 1.9 min to maximize peak height for analytes.

- Split Ratio 50:1

- Mass Range 15 to 1000 A.M.U. (atomic mass units)

INSTRUMENTAL ANALYSIS:

Waste bottle 26 sample
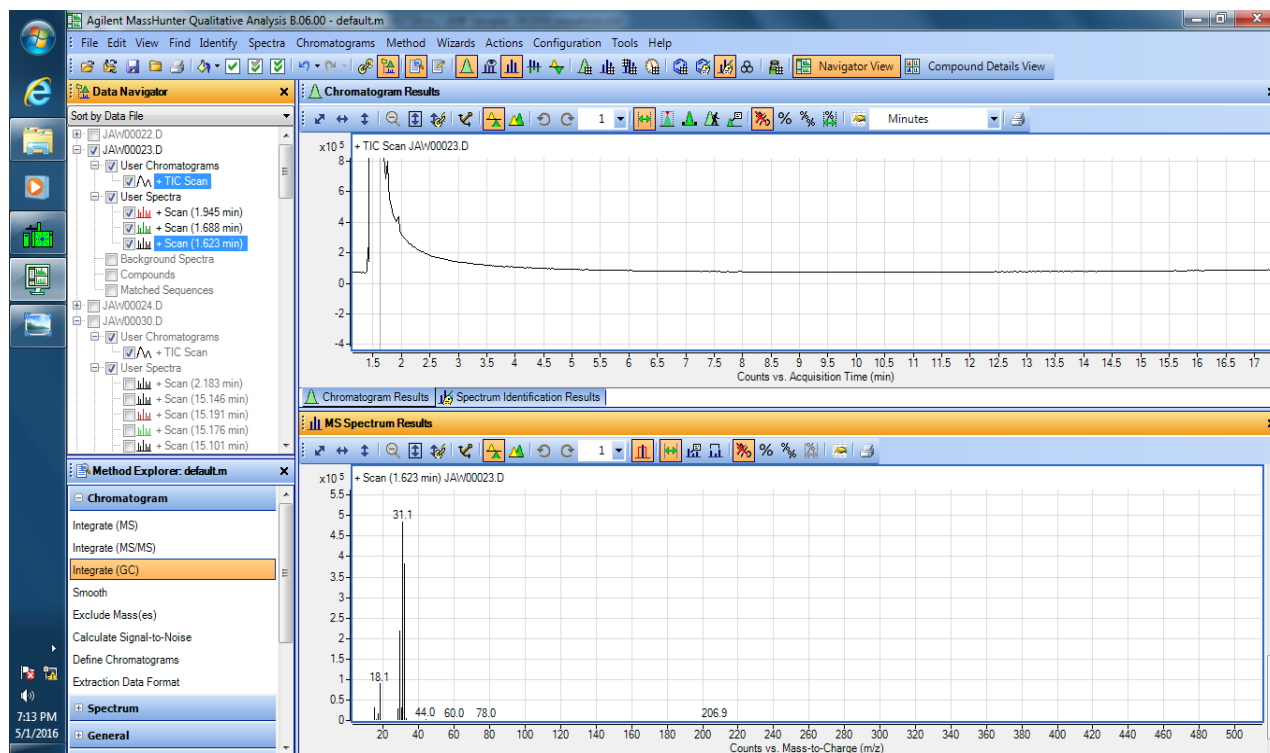
Top view is the Total Ion Chromatogram (TIC) or Reconstructed Ion chromatogram (RIC).

Bottom view is the Mass Spectrum generated by scanning over at a scan rate 1 scan/sec the corresponding chosen peak in the total ion chromatogram.

In this TIC, the first peak at retention time of 1.623 min indicates methanol is present in the solvent mixture. The mass spectrum generated by scanning over this peak shows the base fragment ion for methanol as 31.1 m/z and the NIST libraries search of this spectrum confirmed methanol by the (M-1) fragment and the actual Mol. Wt. of MeOH is 32.04.

INSTRUMENTAL ANALYSIS:

Waste Bottle 26: NIST Library Search
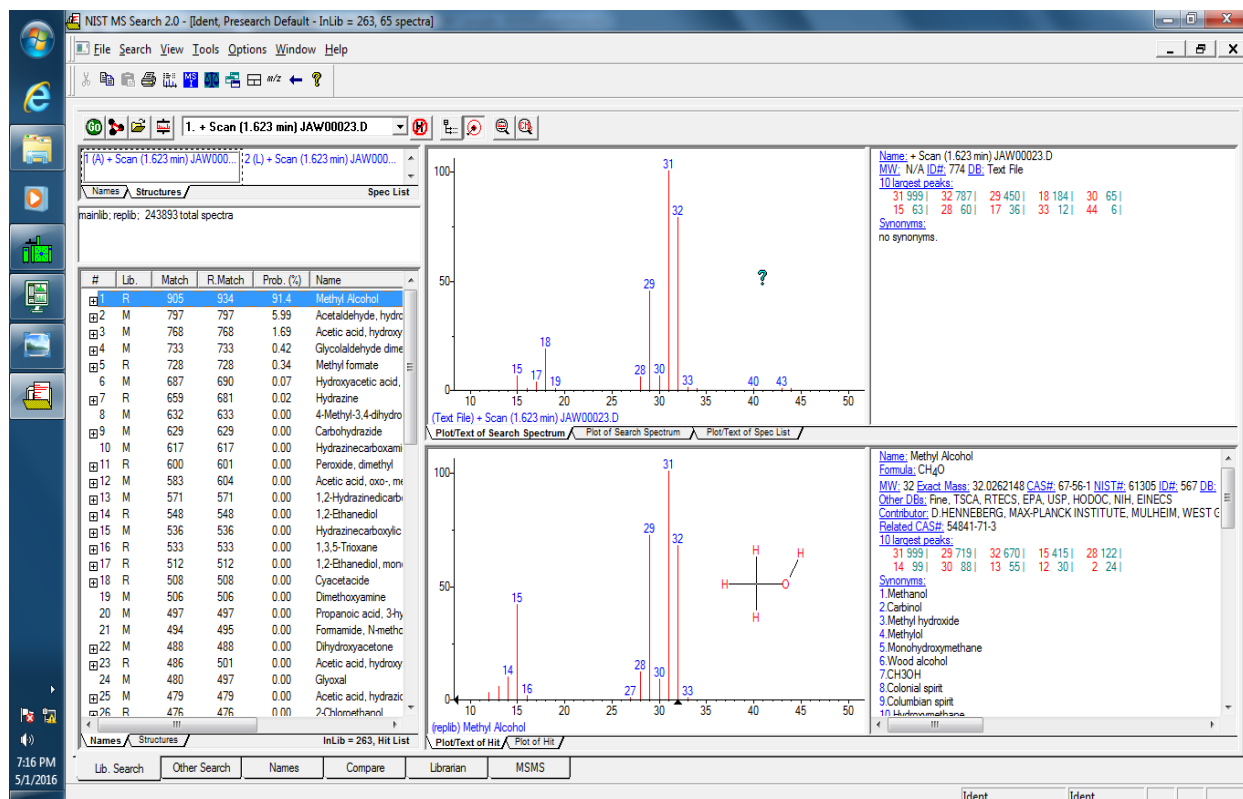
This is the NIST Library search that produced a 91.4% match for Methyl Alcohol also known as Methanol (MeOH).

Despite all the chemicals listed on the GSU Waste Accumulation Log. The only chemical detected in Waste Bottle 26 sample was Methanol. One reason could be the filtration and separation procedures could have removed the chemicals that were actually in the bottle as listed. It is also possible the waste log was not correct.

INSTRUMENTAL ANALYSIS:

HPLC Infinity 1260 Solvent Waste:

This Waste Bottle was discovered in the Instrumentation Lab F2206 on the floor under the lab bench full to the top connected by a waste hose to the Agilent Infinity 1260. The date 2-9-15, initials WS and Methanol, Acetonitrile, H20 was all that was written on the Hazardous Waste label. No other fields of information were filed in. No GSU Waste Accumulation Log was located. Agilent 1260 Waste was added to the label when removing the waste bottle and replacing it with a correctly labeled glass waste bottle.
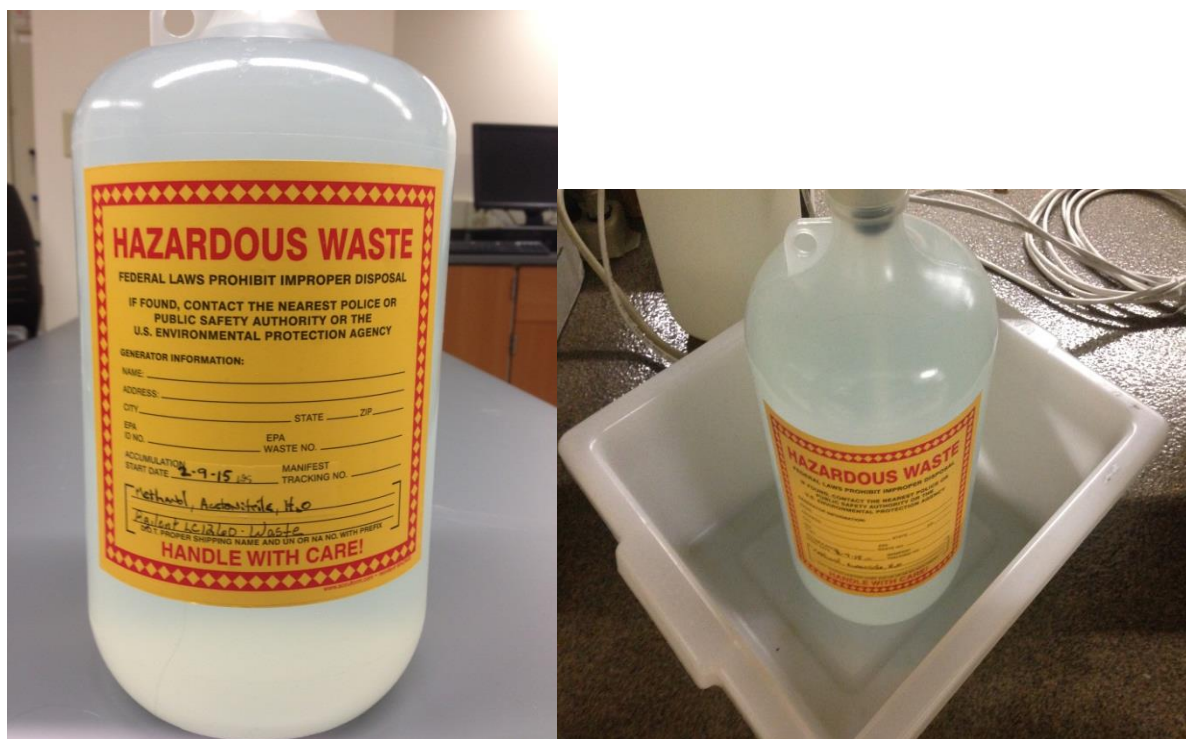
INSTRUMENTAL ANALYSIS:

Agilent LC1260 Waste Bottle:
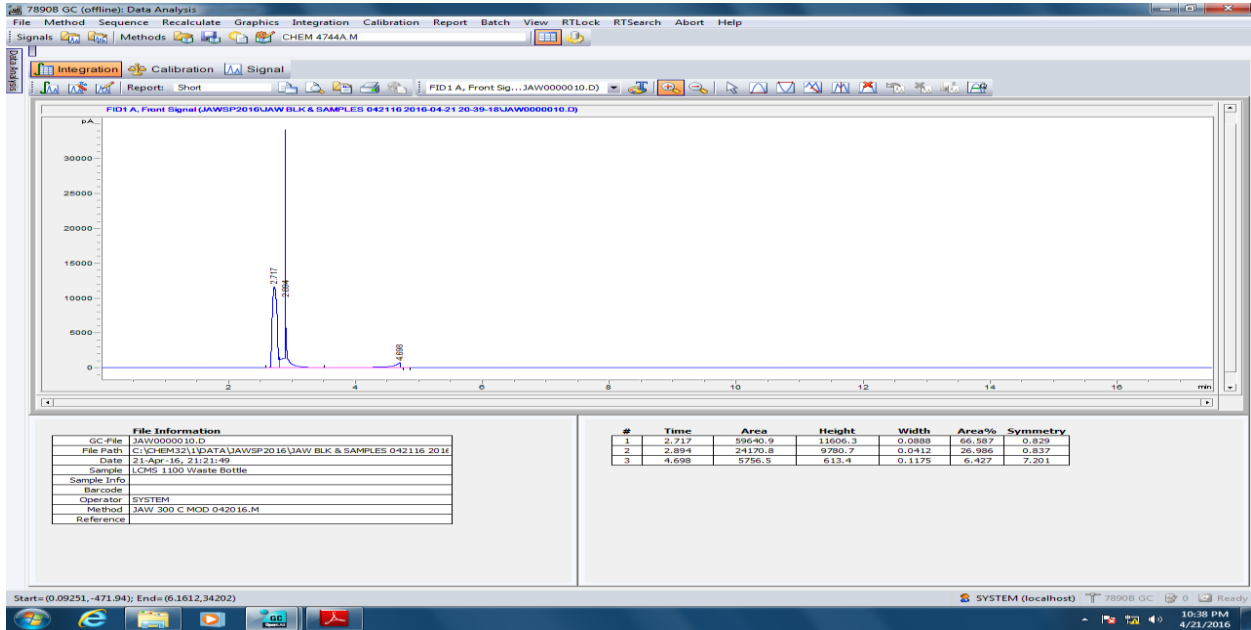
Methanol (MeOH) Blank
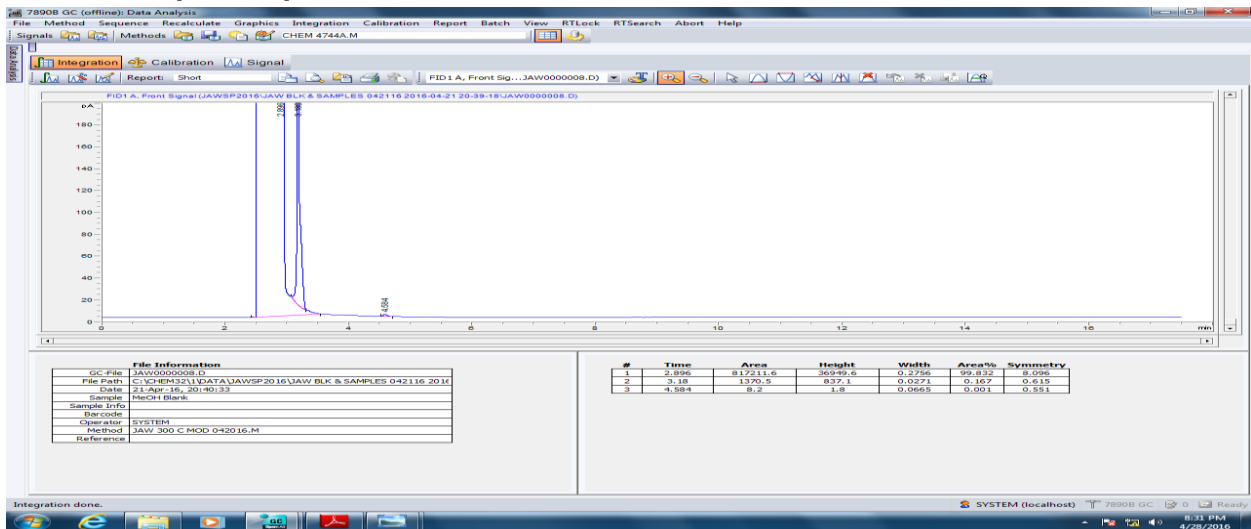
Upon examination of the MeOH Blank the LC1290 sample doesn't seem that out of place. The MeOH is coming out first as expected and this peak in larger than the LC1290 peak in the above chromatogram due to the concentration.

INSTRUMENTAL ANALYSIS:

Agilent LC1260 Waste Bottle:

The chromatogram obtained from the Agilent 7890 GC with FID (Flame Ionization Detection) for the LC 1260 Waste Bottle is shown and clearly reveals two peaks at Retention Times (RT) of 2.717 and 2.894 minutes respectively. The peak at 2.717 minutes represents the methanol solvent as recorded on the 1260 Waste Bottle. A 1uL (micro liter) standard of methanol was also injected into the GC to verify methanol at this retention time. However, the small peak on the shoulder of the solvent peak represents an impurity of unknown origin. The peak at 2.894 minutes represents the ACN (acetonitrile) solvent that was written on the label of the LC 1260 Waste Bottle. The retention time of this component was also confirmed by injecting 1ul standard of acetonitrile using the same GC conditions. The small peak at retention time 4.698 minutes was attributed to acetic acid. A standard of acetic acid in methanol was injected into the GC to confirm this retention time. The quantitative results from the GC report suggests that in area percent the methanol is the major solvent in the LC 1260 Waste Bottle at 66.59%. The acetonitrile component in the waste bottle is the other major solvent with an area percent of 26.99%. Combining the percentages of the solvent peaks equals 93.6%. The peak at retention time 4.698 minutes represents acetic acid and has an area percent of 6.43%. The total area percent representing the three components of the mixture in the chromatographic results is 100%.

However, it must be noted that the water component of the solvent mixture in the LC 1260 Waste Bottle could not be accounted for using quantitative results by GC since the FID detector is not sensitive to water molecules in the flame of the detector.

It can be noted that the GC/MSD reveals the water component in the solvent mixture as shown in the next chromatogram.

INSTRUMENTAL ANALYSIS:
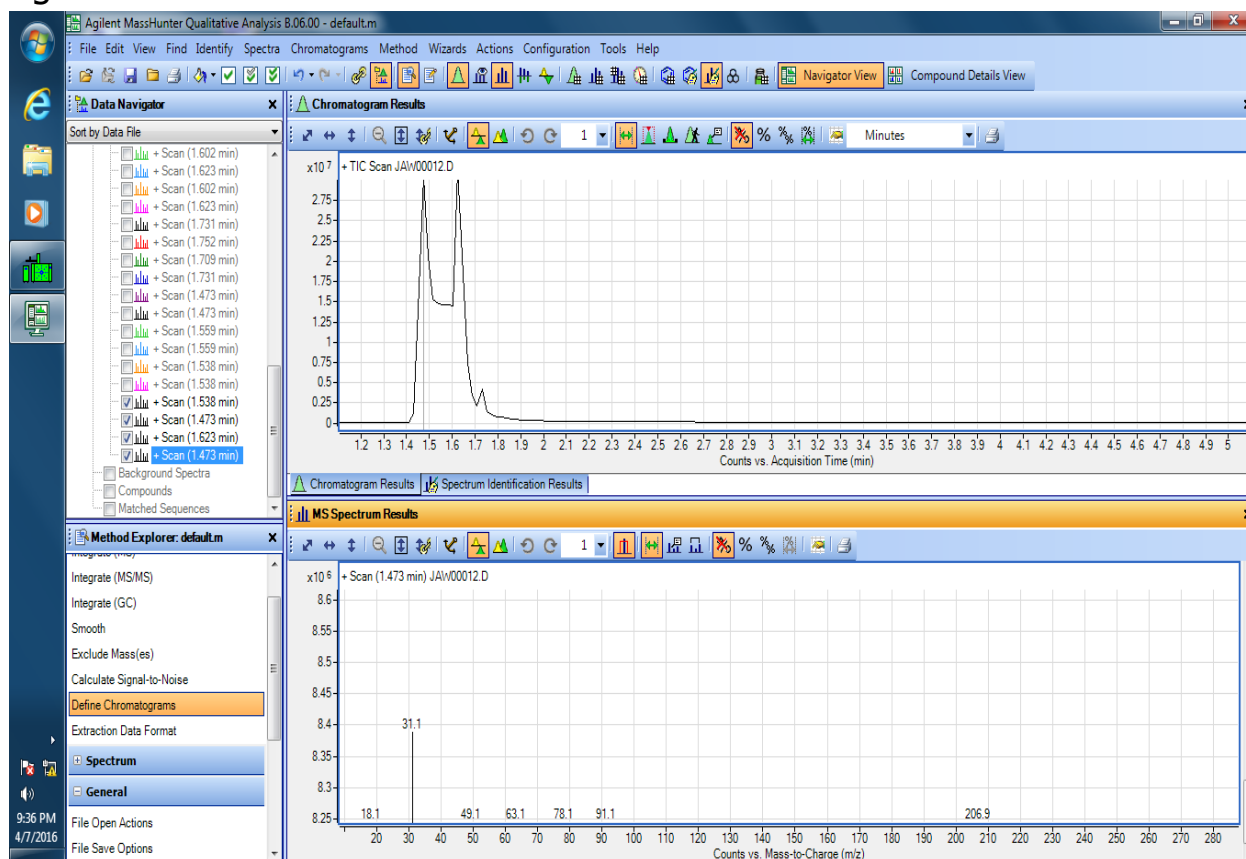
Agilent LC1260 Waste Bottle:

Top view is the Total Ion Chromatogram (TIC) or Reconstructed Ion chromatogram (RIC).

Bottom view is the Mass Spectrum generated by scanning over the corresponding or chosen peak at a scan rate of 1 scan/sec in the total ion chromatogram.

In this TIC, the first peak at retention time of 1.473 min indicates methanol is present in the solvent mixture. The mass spectrum generated by scanning over this peak shows the base fragment ion peak for methanol as 31.1 m/z and the NIST library search of this spectrum confirmed methanol by the (M-1) fragment and actual Mol. Wt. of MeOH is 32.04

INSTRUMENTAL ANALYSIS:

Agilent LC1260 Waste Bottle:
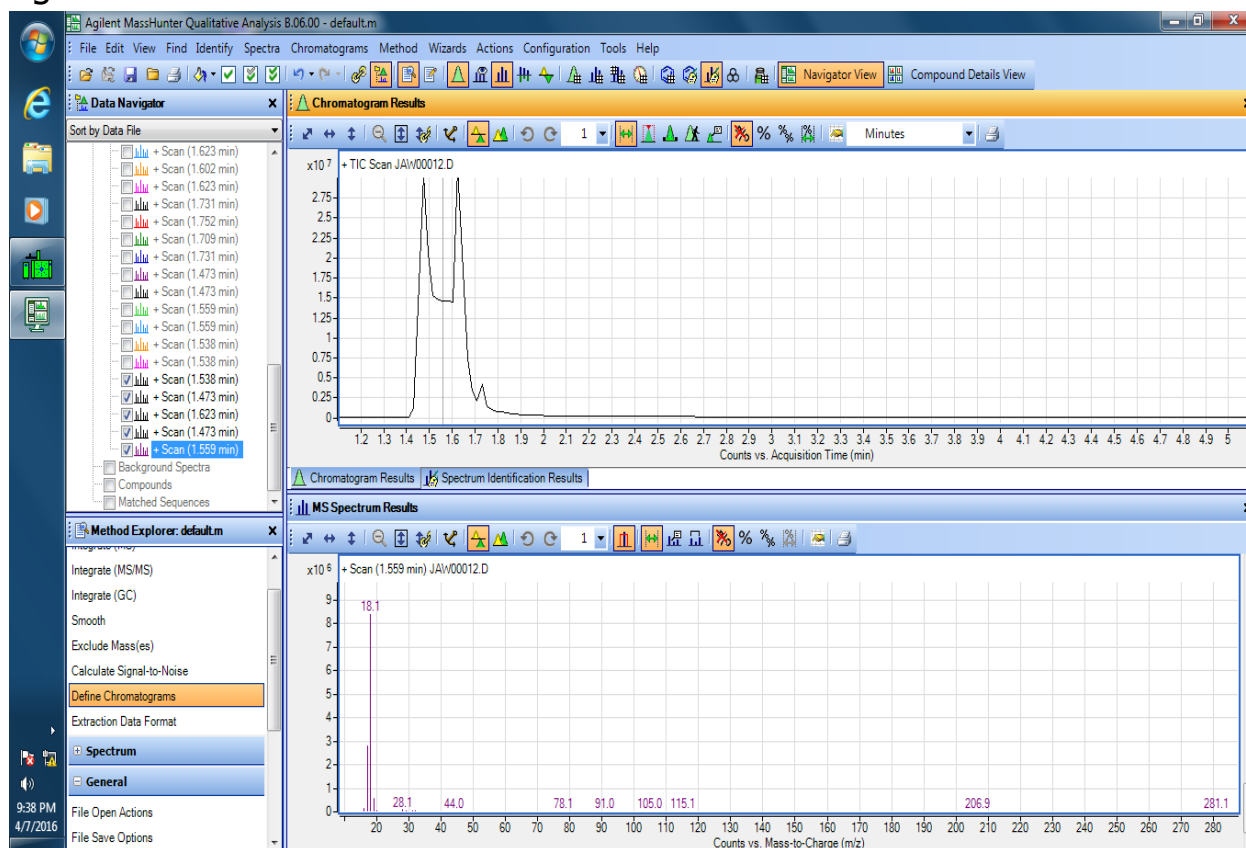
The middle region of the bi-modal peak in the TIC at retention time of 1.559 min indicates water is present in the solvent mixture. The mass spectrum generated by scanning over this region shows the base peak as 18.1 m/z that is also the molecular ion for water.

INSTRUMENTAL ANALYSIS:

Agilent LC1260 Waste Bottle:
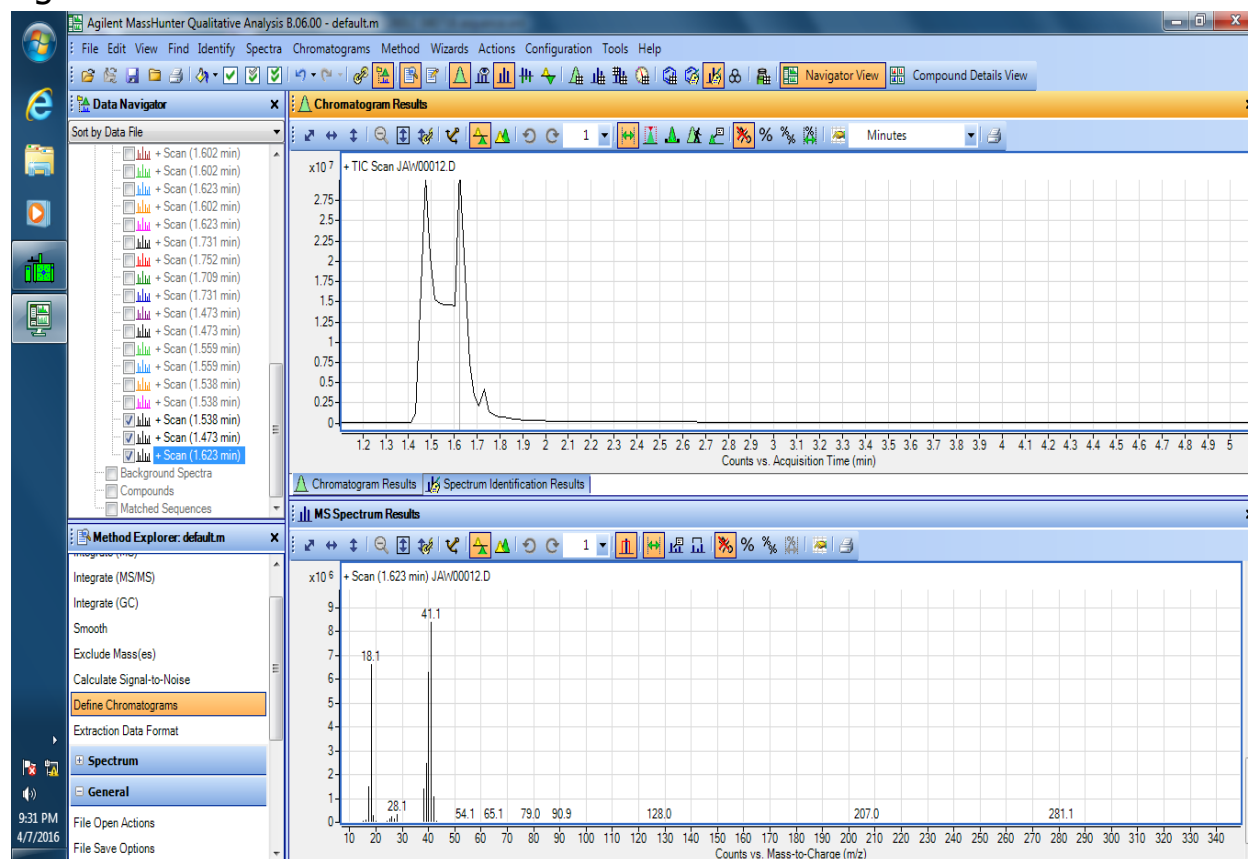
The TIC of the second or peak on the right produced two dominate fragment ions in the mass spectrum indicating water at 18.1 m/z and the molecular ion for Acetonitrile at 41.1 m/z The NIST library search confirmed the presence of acetonitrile in the solvent mixture at Mol. Wt. 41 The fragment ion for water at 18.1 m/z is due to the background interference from water in this mass spectrum. There is a resolution problem for the peaks that cannot be baseline resolved for methanol and acetonitrile due to the presence of water in the solvent mixture or there would be two distinct peaks.

This conclusive evidence confirms the fact that despite no official GSU Waste Accumulation Log for this Agilent LC 1260 Waste Bottle the label is correct. The mixture primarily consist of methanol, water, and acetonitrile.

INSTRUMENTAL ANALYSIS:

The next waste bottle to be examined turned out to be a total mystery starting with the label. The label was almost illegible. Upon close examination the following was determined:

- Dr. Fu Waste Bottle
- Halogenated Organic Solvent Waste
- #9 (circled)
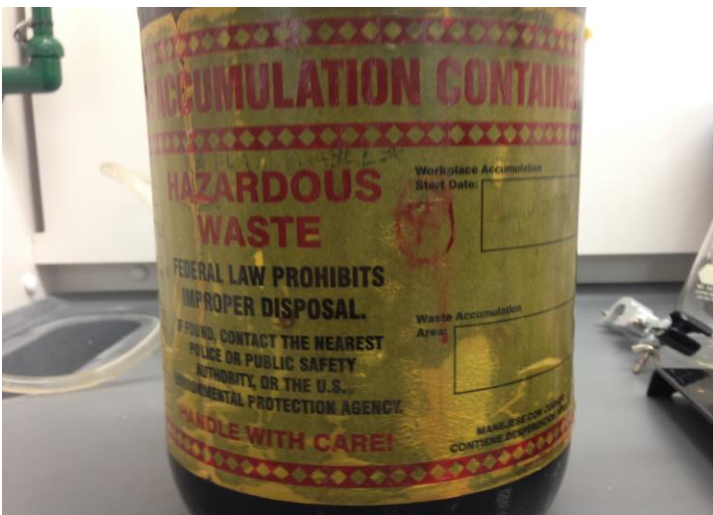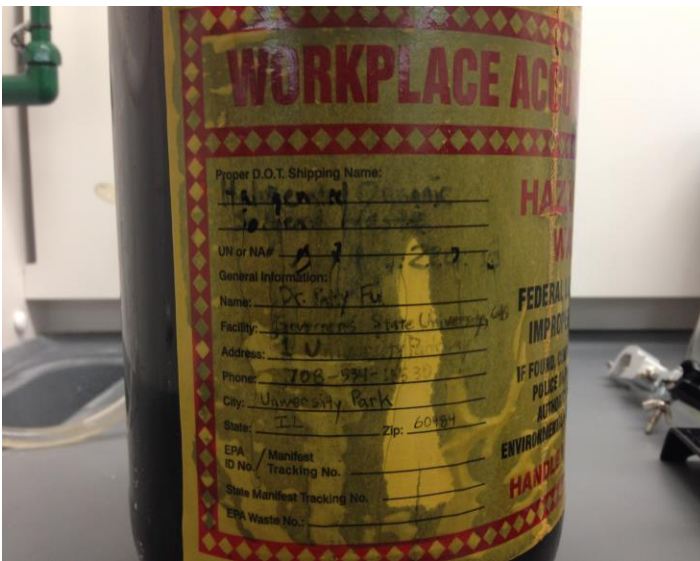
Experimental Procedure/Observations:

A search for a GSU Waste Accumulation Log sheet resulted in not finding one in any lab or among lab waste files. No clue what this bottle could actually contain or where it originated. Once again this waste bottle was filled to the top.

Filtration and separatory procedures were conducted as described in the Experimental Procedures outlined in the beginning of the study. The resulting solution appeared to be in two layers with an oily top layer and a green to yellow bottom layer with a couple red oily spots at the bottom of the Erlenmeyer flask.

Dr. Fu Waste Bottle sample
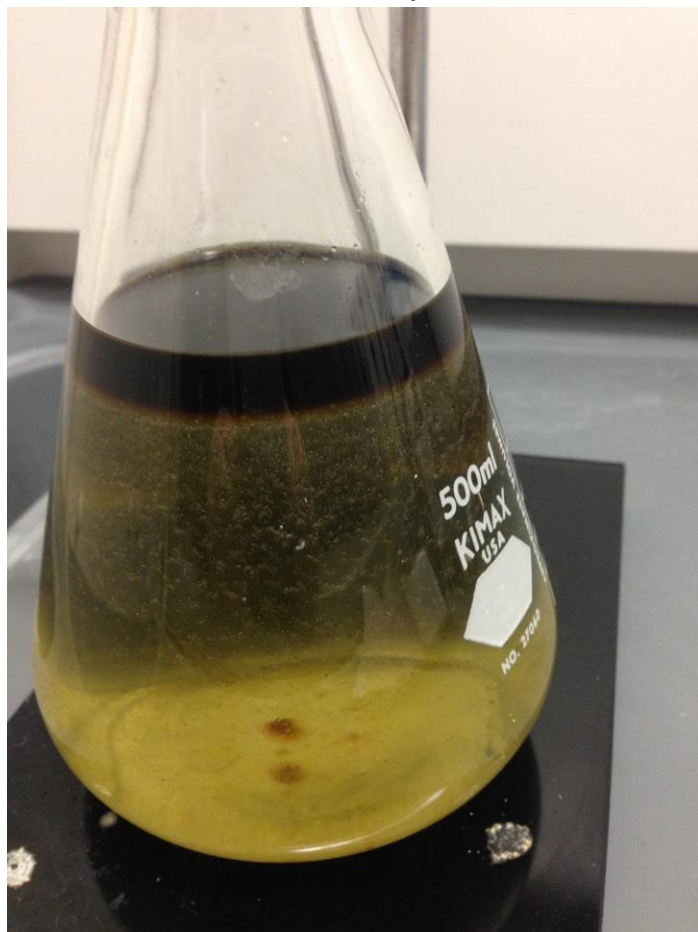
INSTRUMENTAL ANALYSIS:

This waste bottle presented a few challenges. Would it be possible to analyze? What instrumentation should be utilized? Which layer or both layers should be analyzed?

A decision was made to analyze both the top and bottom layers starting with the Agilent 7890 GC.

Analyzed peaks 1-15
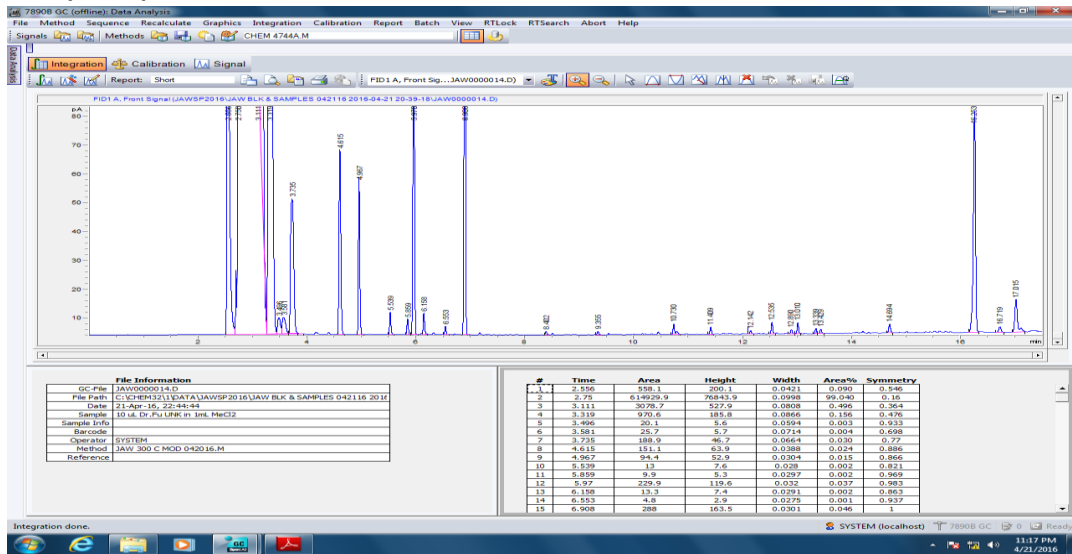
Analyzed peaks 16-29
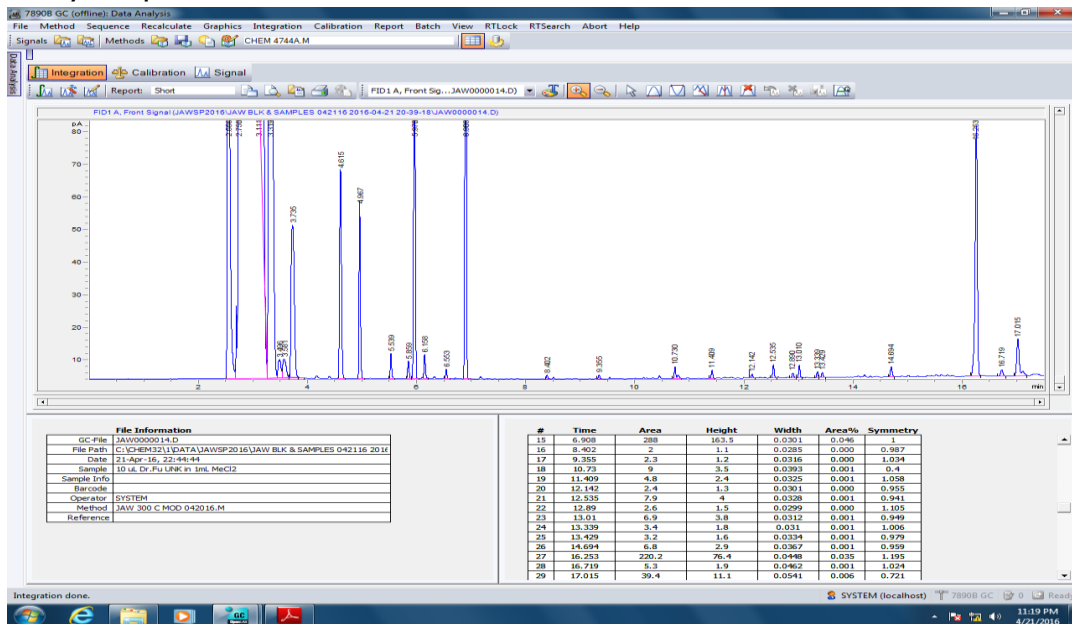
INSTRUMENTAL ANALYSIS:

Dr. Fu Waste Bottle:

There is so much activity in this 7890 GC Chromatogram that it takes two separate chromatographic displays to see all the possibilities.   The GC analysis revealed 29 peaks in the top layer from this waste bottle. One of the reasons for all the activity is samples were taken from the two separated layers. Based on these GC results the top and bottom layers were next analyzed in the 7890 GC/5977 MSD

In review, a few GC/MSD conditions and parameters were adjusted to optimize the method used for the analysis.   Based on previous data collected the split ratio was changed to 10:1 for certain samples. And the solvent cut was adjusted to 1.9 min vs the typical 1.0 min. This was done to minimize the solvent peak and consequently improve the peak heights for the analytes. The mass range was adjusted to 15 to 700 atomic mass units. The solvent used for the preparation of these samples was mehtylene chloride 84 mol.wt. which was detected 99% at retention time of 2.75 minutes.

INSTRUMENTAL ANALYSIS:

Dr. Fu Waste Bottle: Top Layer Analysis
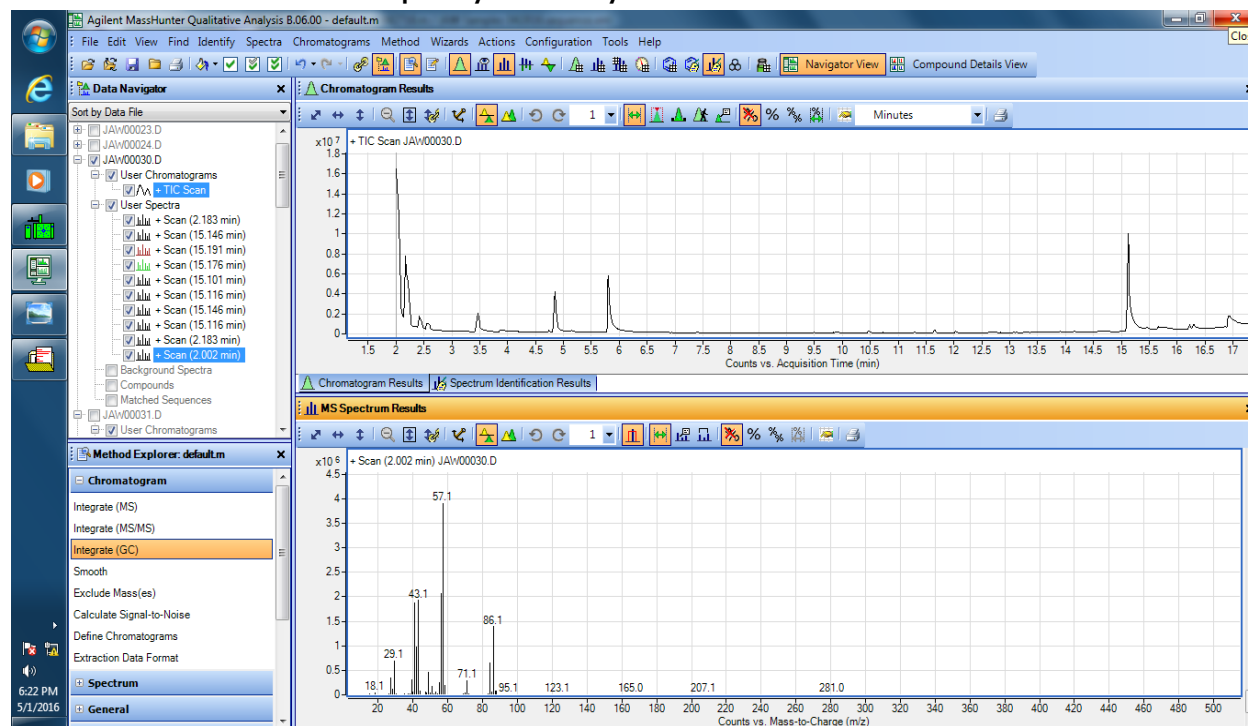
The Total Ion Chromatogram (TIC) using the GC/MSD indicates a minimal solvent peak at the 1.9 min solvent cut. The analyte peaks in the TIC are then normalized to the remaining solvent response based on the Ion counts. As shown in the Reconstructed Ion Chromatogram (RIC), there are an 4 to 5 analyte peaks, however the peaks heights (that are based on Ion counts) are smaller than the analyte peaks shown in the GC/FID results for the same sample.

In the GC/MSD top layer of the Dr. Fu Waste Bottle a retention time of 2.002 min represents the solvent hexane or possibly a combination of hexane and cyclohexane. The TIC scan of this peak clearly shows fragment ions at 86.1, 71.1, 57.1, 43.1, 29.1 in the mass spectrum. A search of the mass spectrum utilizing the NIST mass spectral library revealed a match for n-Hexane at a probability of 71.0%
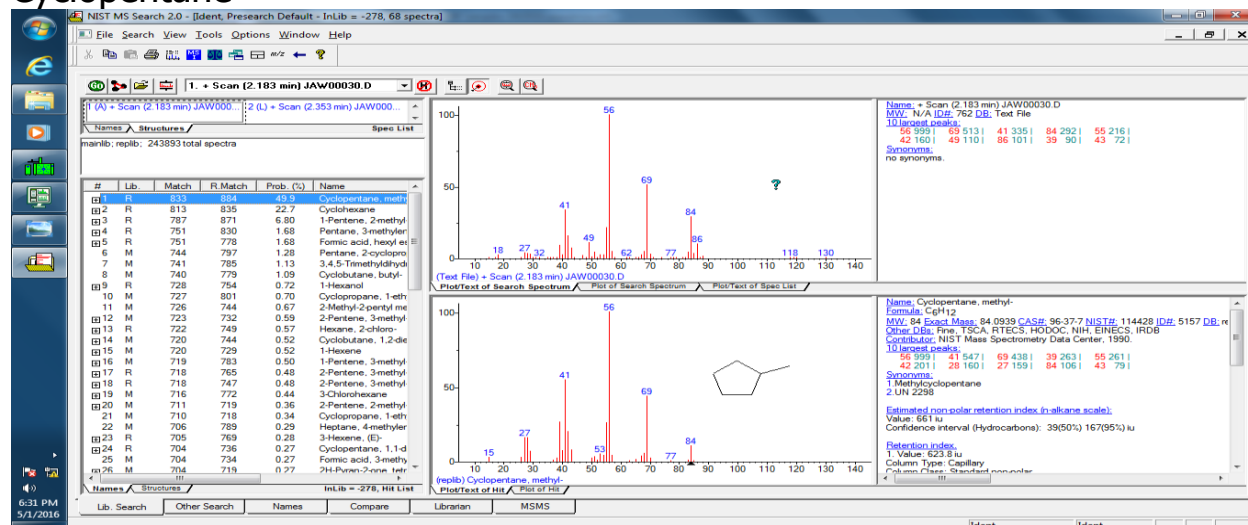
INSTRUMENTAL ANALYSIS:

Dr. Fu Waste Bottle: GC/MSD NIST Library Search Top Layer:

N-Hexane
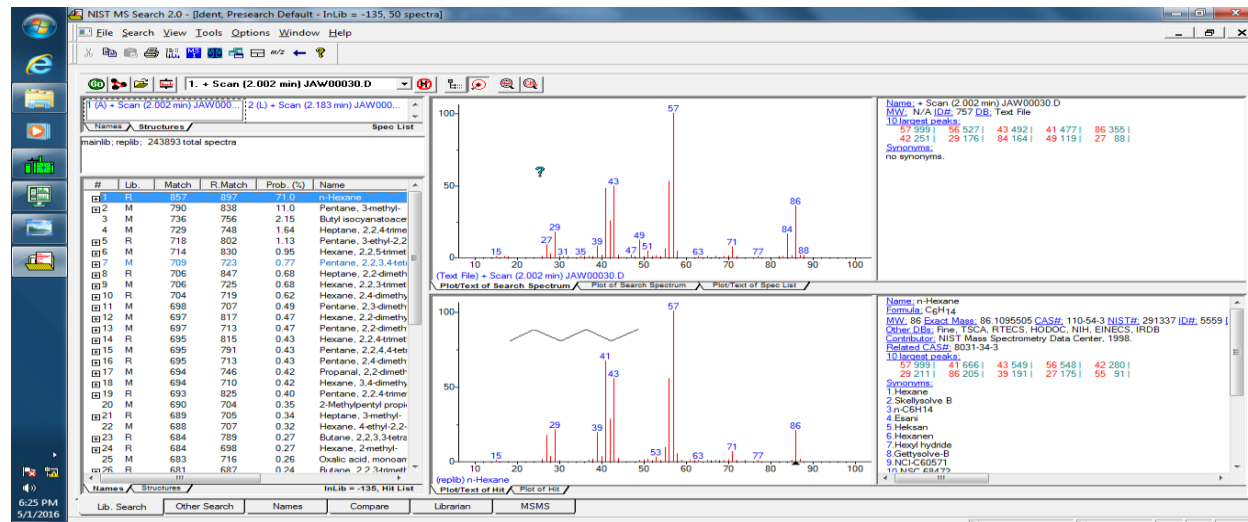
At retention time 2.002 min there is a 57.1 peak which is characteristic of an Aliphatic straight chain of hydrocarbons and based on the fragmented patterns. A search of the mass spectrum utilizing the NIST mass spectral library revealed a match for n-Hexane at a probability of 71.0%.
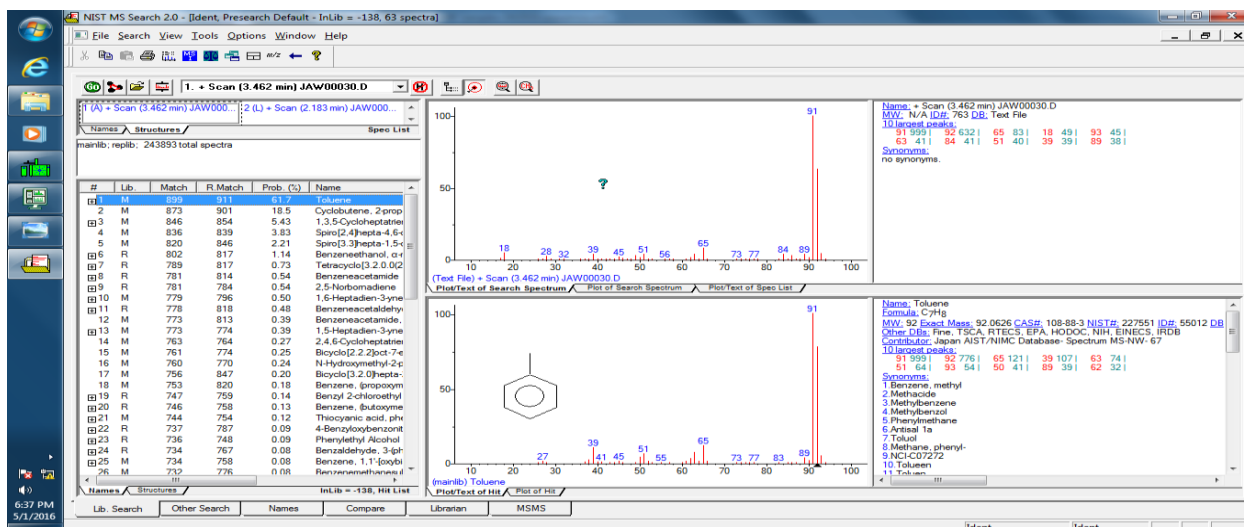
Cyclopentane

AT retention time 2.183 min there is a base peak at 56 and another match at peak 84 with a 49.9% match for cyclopentane and a 22.7% match for cyclohexane.
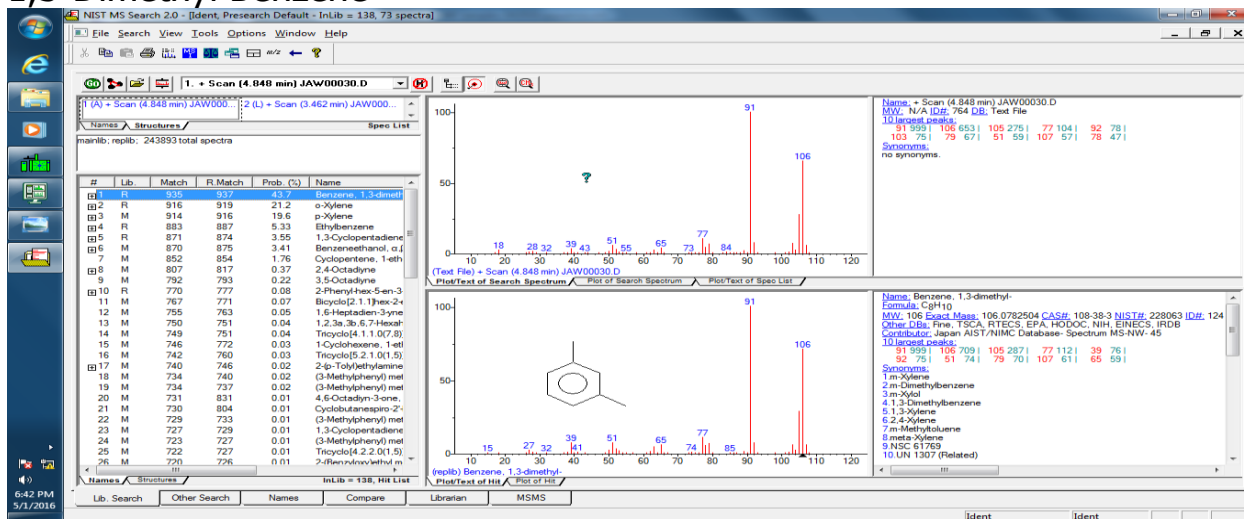
INSTRUMENTAL ANALYSIS:
Dr. Fu Waste Bottle: GC/MSD NIST Library Search
Top Layer:

Toluene

At retention time 3.462 min there is a molecular base peak at 91 with a M-1 and a 61.7% probability match.
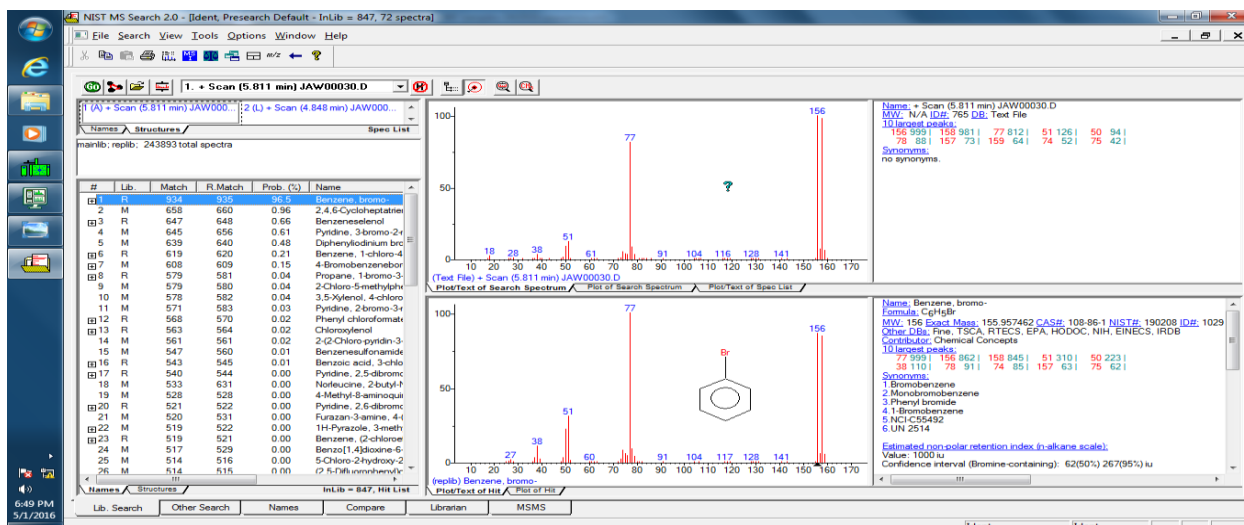
1,3-Dimethyl Benzene

At retention time 4.848 min there is a strong base peak at 91. A molecular ion peak at 106 results in a 43.7% probability match. This compound is also known as Meta-Xylene and the NIST search reveals the first three matches are for m,o,p-Xylene.
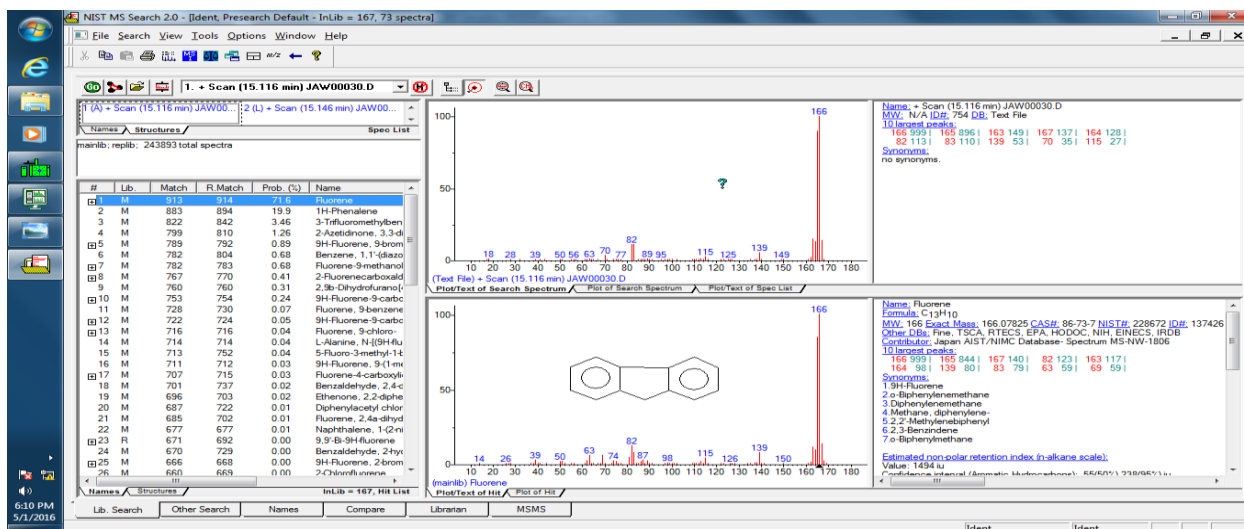
INSTRUMENTAL ANALYSIS:
Dr. Fu Waste Bottle: GC/MSD NIST Library Search
Top Layer:

Bromo Benzene

At retention time 5.811 min there is a base peak at 77. A molecular ion peak at 156 reveals an excellent 96.5% probability match.
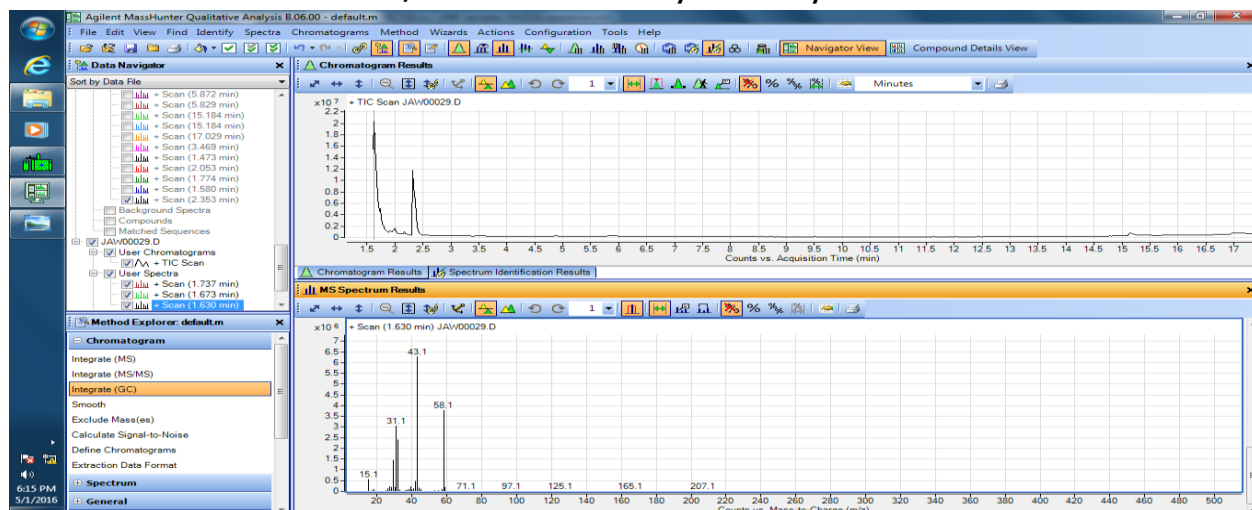

Fluorene

At retention time 15.116 min there is a molecular ion peak at 166 is due to the stability of the ring statue resulting in a strong 71.6% probability match.
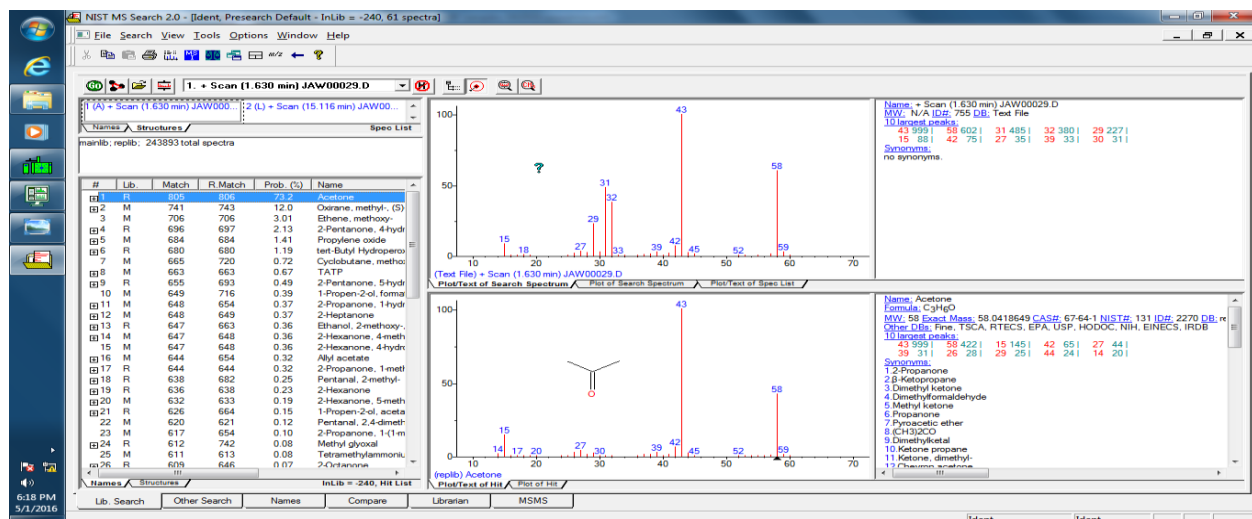
INSTRUMENTAL ANALYSIS:

Dr. Fu Waste Bottle: GC/MSD Bottom Layer Analysis

The retention time of 1.630 min shown in the TIC for the GC/MSD results for the lower layer from the Dr. Fu Waste Bottle represents the solvent acetone. The scan of this peak at edge of the solvent cut in the TIC clearly shows fragment ions at 58.1, 43.1, 31.1 in the mass spectrum. Much smaller fragment ions were also noted at 59 and 60 atomic mass units. A search of this mass spectrum utilizing the NIST mass spectral library revealed a match for acetone with a probability of 73.2%.

Acetone

NIST mass spectral library search

INSTRUMENTAL ANALYSIS:

Dr. Fu Waste Bottle: GC/MSD Bottom Layer Analysis
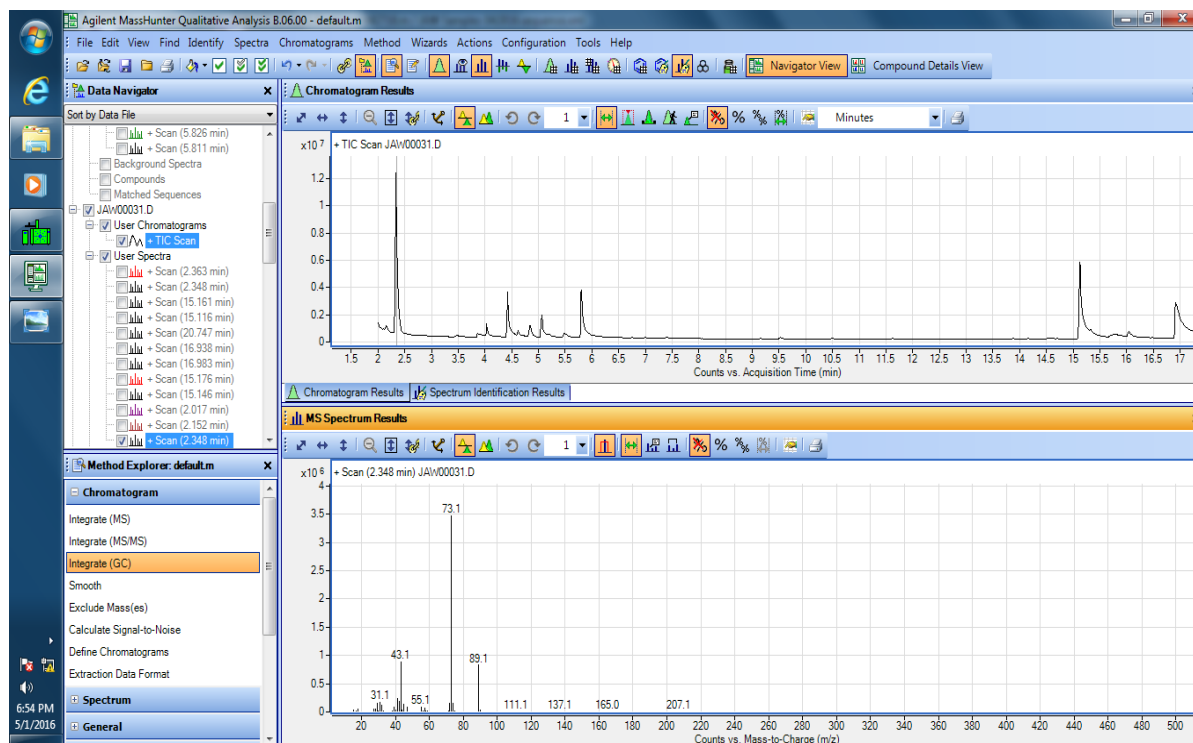
The data file shown represents a 1ul injection of the lower phase for the Dr. Fu Waste Bottle using the GC/MSD. It can be noted that the solvent cut used was 1.9 min as opposed to the previous solvent cut of 1.0 min, therefore the acetone solvent peak was vented away from the GC capillary column. Another change as previously mentioned involved a 10:1 split ratio instead of a 50:1 split ratio used for the previous analysis of this waste sample.

INSTRUMENTAL ANALYSIS:
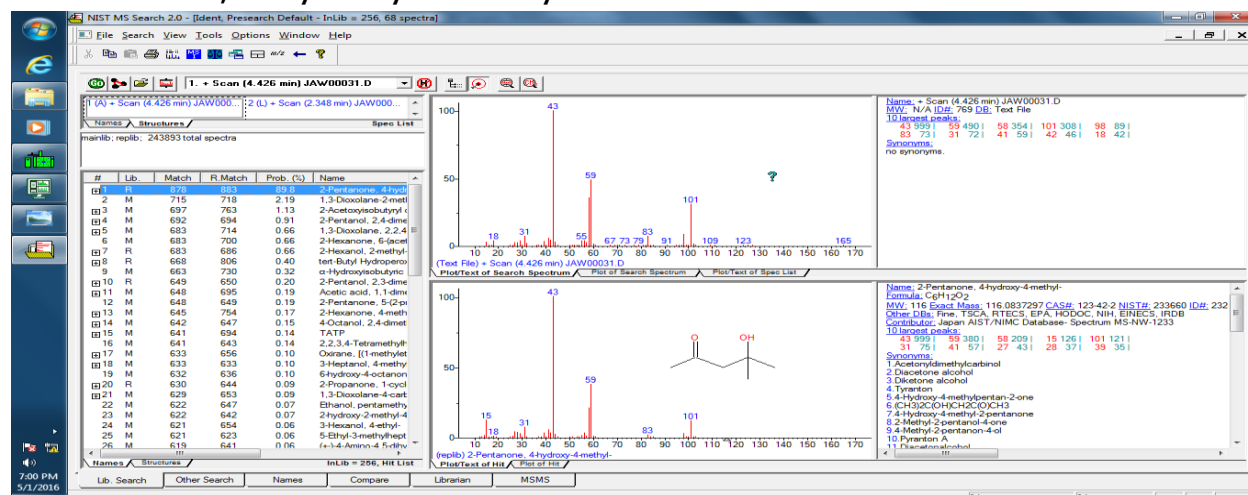
Dr. Fu Waste Bottle: GC/MSD NIST Library Search

Bottom Layer:

2,2-dimoxy Propane
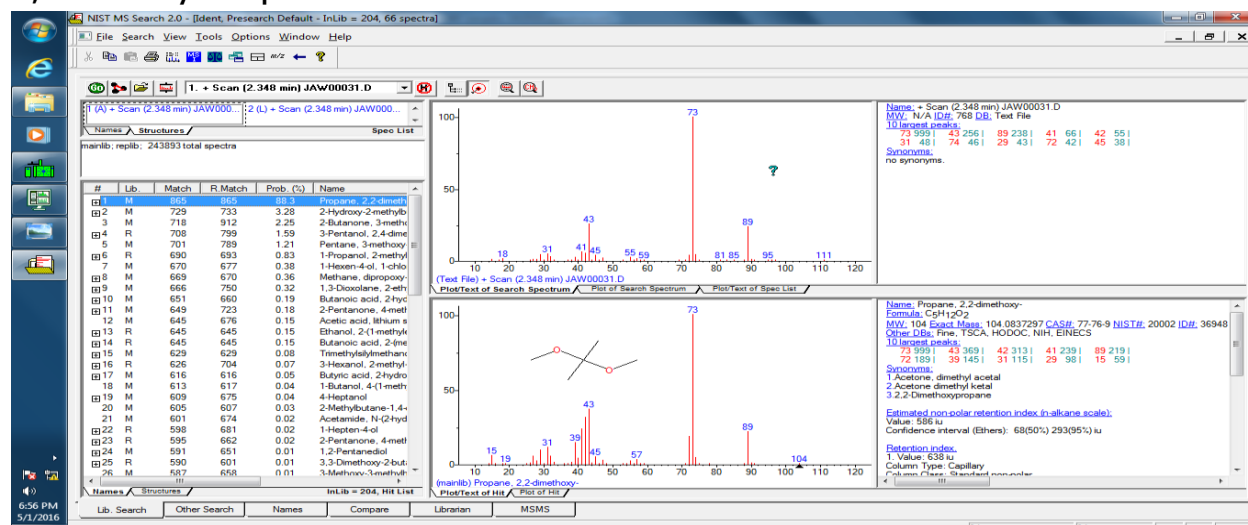
At retention time 2.348 min there is a strong base peak at 73. The molecular ion peak at 104 results in an excellent 88.3% probability match.

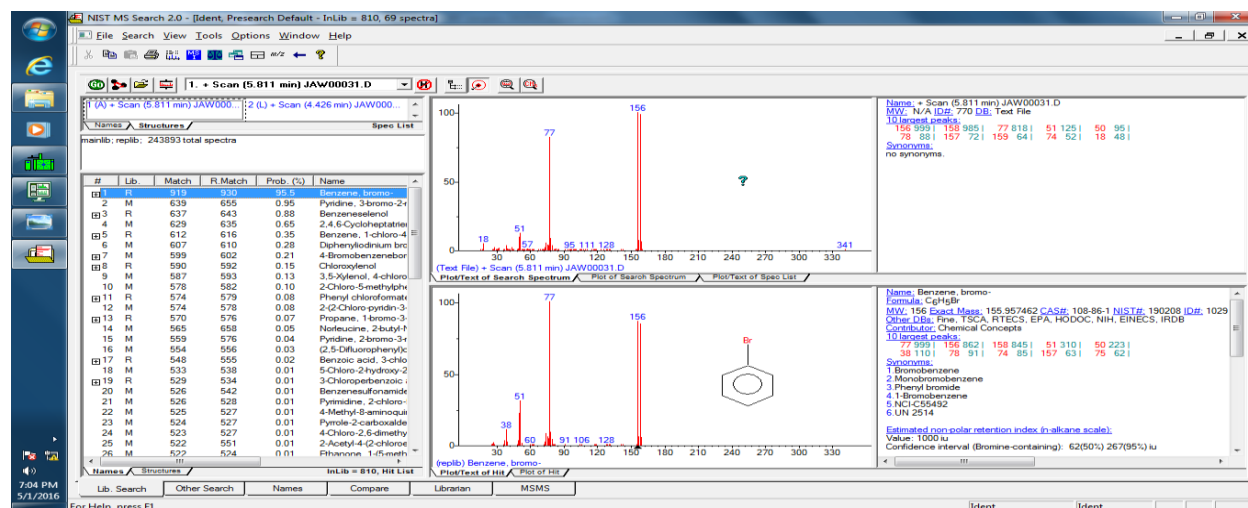2-Pentanone, 4-hydroxy-4-methyl

At retention time 4.426 min there is a strong base peak at 43 and a high 89.8% probability match

INSTRUMENTAL ANALYSIS:
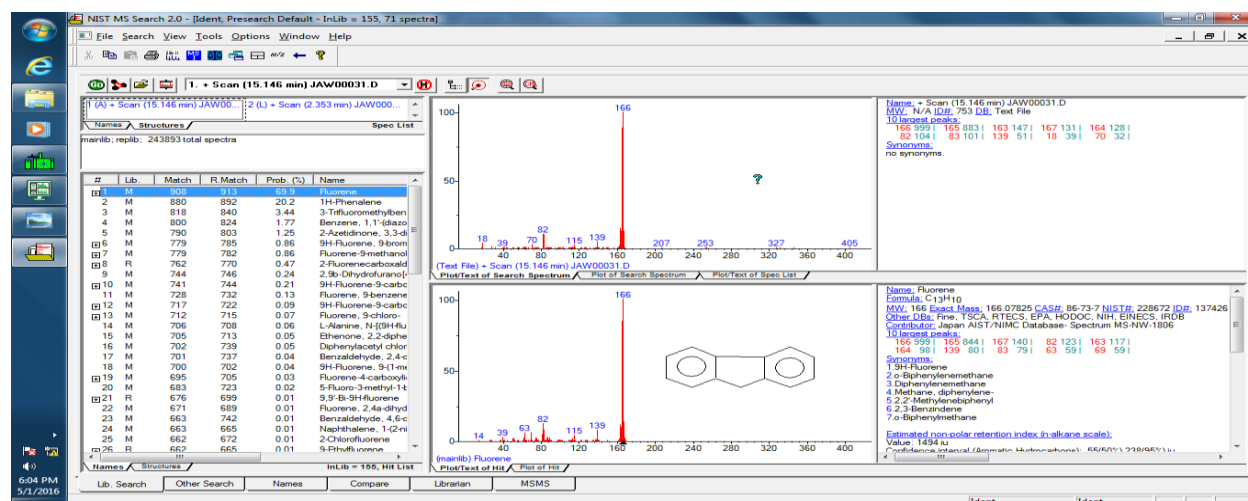Dr. Fu Waste Bottle: GC/MSD NIST Library Search
Bottom Layer:

Bromo-Benzene

At retention time 5.811 min there is a strong base peak at 77. The molecular peak at 156 reveals an astonishing 95.5% probability match.
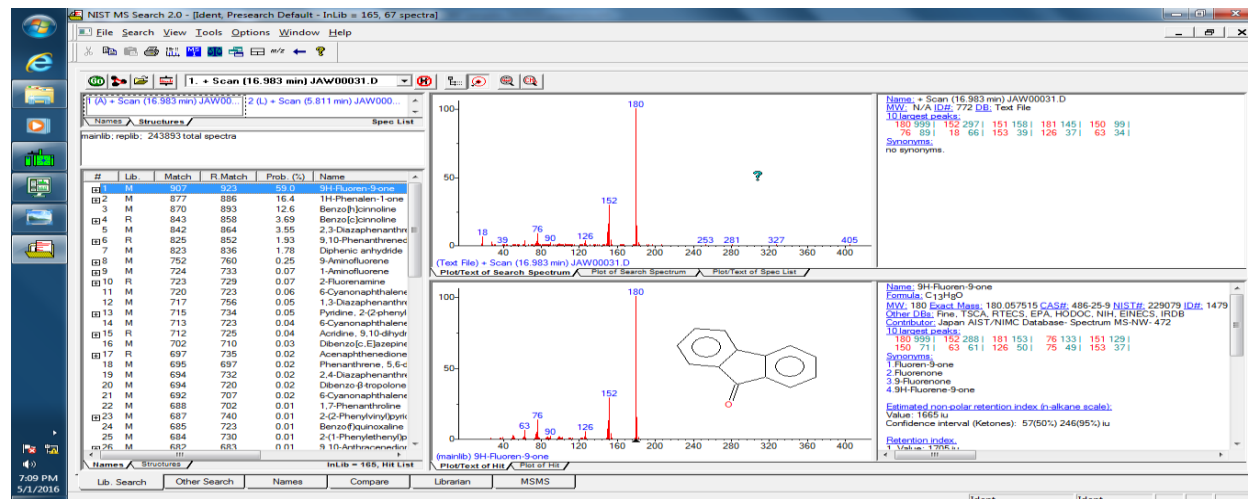

Fluorene

At retention time 15.146 there is a molecular ion peak at 166 due to the stability of the ring statue resulting in a good 69.9% probability match.

INSTRUMENTAL ANALYSIS:

Dr. Fu Waste Bottle: GC/MSD NIST Library Search

Bottom Layer:

9H-Fluoren-9-one

At retention time 16.983 there is a very strong base peak and molecular ion at 180 resulting in a 59.0% probability match.

It can be noted that the component fluorene was identified in the non-polar top layer (hexane based solvents) and also the bottom layer which was more polar due to the acetone solvent. Additionally, the chemical components of bromo-benzene were also found in both layers when analyzed by GC/MSD.

The methodology used for the GC and GC/MSD proved to be successful in characterizing the contaminants in the waste bottle labeled as Dr. Fu Hazardous Waste.

RESULTS:

Waste Bottle 26

Despite all the chemicals listed on the GSU Waste Accumulation Log. The only chemical detected in Waste Bottle 26 sample was Methanol.
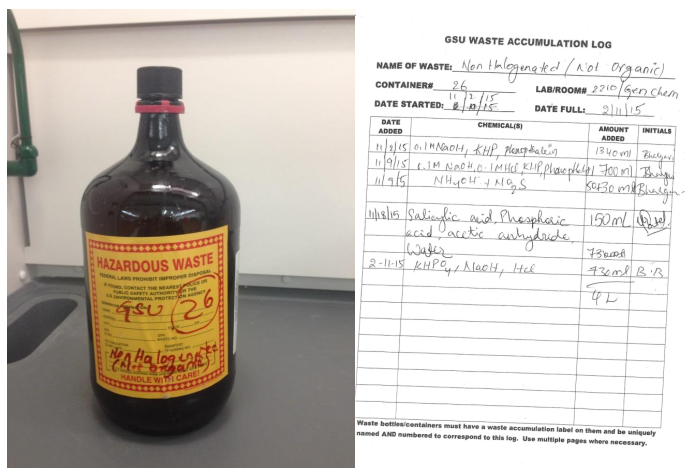
Agilent LC 1260 Waste Bottle

This conclusive evidence confirms the fact that despite no official GSU Waste Accumulation Log for this Agilent LC 1260 Waste Bottle the label is correct. The mixture primarily consists of methanol, water, and acetonitrile.
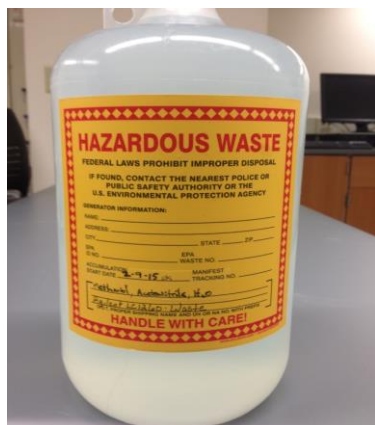
RESULTS:

Dr. Fu Waste Bottle

Analysis of the Dr. Fu Waste Bottle resulted in the identification of two separate layers containing the following chemicals:

Top Layer: NIST Library Match

N-Hexane	71%
Cyclopentane	22.7%
Toluene	61.7%
1,3-Dimethyl Benzene	43.7%
Bromo Benzene	96.5%
Fluorene	71.6%

Bottom Layer: NIST Library Match

Acetone	73.2%
2,2-dimoxy Propane	88.3%
2-Pentanone	89.8%
Bromo Benzene	95.5%
Fluorene	69.9%
0H-Fluoren-9-one	59.0%
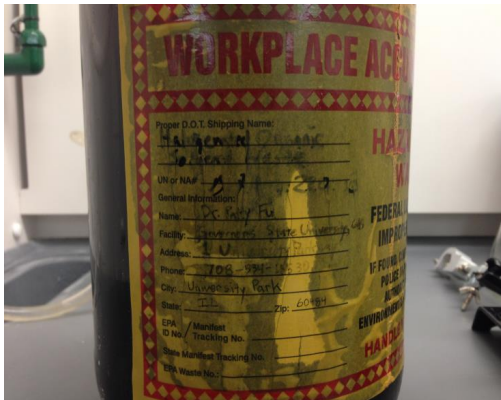
DISCUSSION:

The GC and GC/MSD instrumental analysis of Waste Bottle 26 resulted in the definitive identification of methanol. No other chemicals listed on the GSU Waste Accumulation Log were detected in the filtered samples tested. The results point to one reason for the limited results being the filtration and separation procedures outlined in the methodology could have removed chemicals that were actually in the bottle as listed. It is also possible the waste log was not correct.

Based on the results for the analysis of Waste Bottle 26 (and the revisions in sampling methodology), clearly resulted in a successful analysis and characterization of the Agilent LC 1260 Waste Bottle. NIST Library mass spectra confirmed the presence of the solvents methanol, acetonitrile, and water as listed on the bottle's Hazardous Waste Label.

The methodology and instrumental analysis of the mysterious Dr. Fu Waste Bottle did present a formidable challenge. However, the resulting analysis of 29 peaks by GC and the subsequent GC/MSD identification using the NIST library search for the 12 peaks detected in the two layers was encouraging. This evidence supported the development of the sampling techniques and the possible analysis by GC/MSD for chemicals contained in the waste bottles. The information presented can be used as a basis for future waste characterization and analysis.

CONCLUSION:

The purpose of this feasibility study of the chemical waste generated in the science teaching and research labs was to develop an efficient system of isolation and characterization. Proper waste segregation and disposal is possible based on carefully outlined procedures starting with complete waste accumulation log sheets. The most effective approach is detailed identification and recording of all starting chemicals and the resulting chemical compounds generated in all teaching and research labs. If necessary, waste characterization by GC and GC/MSD can be utilized to assist in the preparation of waste materials for disposal pick up.

The procedures developed in the methodology may not always be necessary or the best path to identification of unknown waste materials. Filtration and separation procedures may only be effective when there is solid materials present and/or the liquid waste appears to be in two or more layers. Aggressive filtration may result in the removal of the very chemical that needs to be characterized for proper disposal since the unknown compound may be inadvertently removed. The theoretical reasons for the filtration and separation of the waste is to avoid damage to instrumentation due to particulate matter. Additionally, decision making based on experience in the operation of the various instrumental methods is the first step in determining the proper characterization and treatment of waste samples.

There really is no end to this feasibility study. As the science teaching curriculum and research investigations evolve there will continue to be a need for constant review and revision of proper identification and disposal of resulting waste. This project has put GSU on the path to fulfilling the commitment to the modern day three R's of Reduce, Recover, Reuse.

FUTURE STUDY:

This feasibility study of waste isolation in the science labs at GSU is the beginning of what hopefully will develop into new and revised procedures for the collection, isolation, and the disposal of laboratory waste. There are a number of procedures already being implemented to improve the current waste system such as:

- New training procedures for faculty, staff and laboratory assistants in regards to the safe handling of waste and correct labeling.

- Review accountability procedures and effectiveness of the GSU Waste Accumulation Log sheets.

- If budget permits, provide industry approved waste disposal containers for each individual lab experiment.

- Develop procedures for general consolidation of waste in preparation for outside service removal.

- Review lab curriculum for "green chemistry" alternatives.

- Encourage faculty to develop labs in their area of expertise that deal with isolation and identification of lab waste.

- Develop an instrumentation workshop and eventually a semester course in the isolation and identification of lab waste.

- Encourage faculty and graduate students to continue with and improve upon this waste study as a research project within CAS.

- Develop an ongoing undergraduate and graduate level independent project whereby students review and contribute to the improvement of waste disposal for other areas within the university.

- Continue to always look for ways to Reduce, Recover, Reuse and Recycle.

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