

Analysis of Polycyclic Aromatic Hydrocarbons
In
Air and Precipitation Samples

Integrated Atmospheric Deposition Network Project

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I. INTRODUCTION

This document outlines the instrumental analysis and quantitation of polycyclic aromatic hydrocarbons (PAHs) measured in atmospheric (vapor and particle phases) and precipitation samples collected at six sites on the Great Lakes. The work is conducted as part of the Integrated Atmospheric Deposition Network (IADN) at the O'Neill School of Public and Environmental Affairs, Indiana University – Bloomington. This research is supported by the Great Lakes National Program Office of the U.S. Environmental Protection Agency.

II. SAMPLE PREPARATION

Vapor, particle and precipitation samples sent to the laboratory from the various field stations are processed by IADN laboratory personnel. The details of laboratory protocols and procedures is provided in the IADN Sample Preparation Standard Operation Protocol (SOP). A summary of those procedures is provided in Chart 1. Instrument analysis for polycyclic aromatic hydrocarbons (PAHs) is conducted on the first and second eluted fractions of all vapor, particle and precipitation samples.

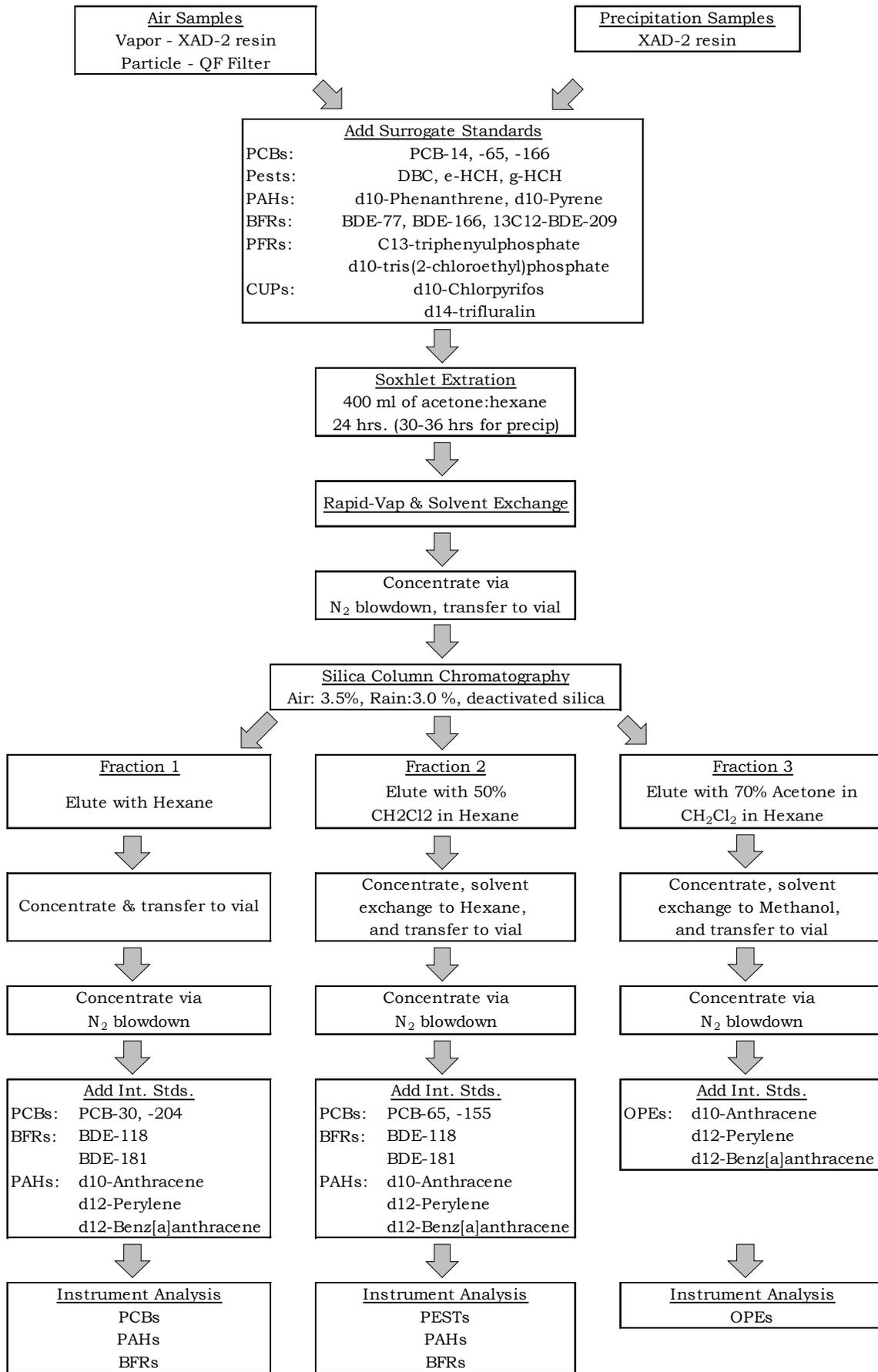


Figure 1. Flow chart summarizing IADN sample preparation

III. INSTRUMENT ANALYSIS

PAH analyses are performed on an Hewlett-Packard (HP) 6890 gas chromatograph coupled with an HP 5973 mass spectrometer (GC/MS) operated in electron ionization (EI) mode.

HP GC 6890 model # G1513A, serial # US00008627

HP 5973 MS model #G1099A, serial # US72020797

GC autosampler controller model #G1512A, serial # US72202110

Chromatographic resolution is achieved with a 30 m x 250 μm x 0.25 μm film thickness HP-5MS UI capillary column (J&W Scientific, Folsom, CA) with helium as the carrier gas. 1 μl injections are analyzed by GC/MS using selected ion monitoring (SIM). Monitoring ions are divided into 9 groups as outlined in Table 1.

Table 1. Selected ion monitoring window groups and ions (m/z).

Group	Ion (m/z)
1	152, 153, 154, 165, 166
2	178, 188
3	202, 212, 219, 234
4	228, 240
5	252, 264
6	276, 278
7	300

Instrument control parameters and method details are provided in Appendix I (page 26).

Quantitation is performed using Agilent's proprietary software package, Mass Hunter, using the method of internal standards. Relative response factors (RRFs) for each analyte are determined from the calibration standard's peak areas using equation 1:

$$RRF_{std} = \left(\frac{mass_a}{area_a} \right)_{std} \div \left(\frac{mass_{istd}}{area_{istd}} \right)_{std} \quad (1)$$

Where $mass_a$ is the analyte's known mass in the injected amount of the calibration standard, $area_a$ is the analyte's peak area, $mass_{istd}$ is the known mass of the appropriate internal standard, and $area_{istd}$ is that internal standard's peak area. The response factors for target compounds are calculated relative to the internal standards d₁₀-anthracene, d₁₂-benz(a)anthracene, and d₁₂-perylene as summarized in Table 2.

An analyte's mass in a sample ($mass_a$) is calculated from the RRF_{std} above and the internal standard response in the sample by the following equation:

$$(mass_a)_{sample} = (area_a)_{sample} \times RRF_{std} \times \left(\frac{mass_{istd}}{area_{istd}} \right)_{sample} \quad (2)$$

where $area_a$ is the analyte's peak area in the sample, $mass_{istd}$ is the mass of internal standard spiked into the sample, and $area_{istd}$ is the internal standard's peak area in the sample. The analyte concentrations are tabulated by Mass Hunter and transferred to an excel spreadsheet.

The list of PAHs analyzed for in this study is given in Table 2, along with quantitation and confirmation ions for each compound, internal standards used for calculating each target amount, and SIM group.

Table 2. IADN PAH analyte list with quantitation parameters.

Compound	Elute Order	Type	Internal Standard	Quant Ion	Confirmation Ion	Sim Group
Acenaphthylene	1	Target	d10-Anthracene	152	--	1
Acenaphthene	2	Target	d10-Anthracene	154	153	1
Fluorene	3	Target	d10-Anthracene	166	165	1
d10-Phenanthrene	4	Surrogate	d10-Anthracene	188	--	2
Phenanthrene	5	Target	d10-Anthracene	178	--	2
d10-Anthracene	6	Internal	--	188	--	2
Anthracene	7	Target	d10-Anthracene	178	--	2
Fluoranthene	8	Target	d10-Anthracene	202	--	3
d10-Pyrene	9	Surrogate	d10-Anthracene	212	--	3
Pyrene	10	Target	d10-Anthracene	202		3
Retene	11	Target	d10-Anthracene	219	234	3
d12-Benz(a)anthracene	12	Internal	--	240	--	4
Benz(a)anthracene	13	Target	d12-Benz(a)anthracene	228	--	4
Chrysene + Triphenylene	14	Target	d12-Benz(a)anthracene	228	--	4
Benzo(b)fluoranthene	15	Target	d12-Perylene	252	--	5
Benzo(k)fluoranthene	16	Target	d12-Perylene	252	--	5
Benzo(e)pyrene	17	Target	d12-Perylene	252	--	5
Benzo(a)pyrene	18	Target	d12-Perylene	252	--	5
d12-Perylene	19	Internal	--	264	--	5
Indeno(1,2,3-cd)pyrene	20	Target	d12-Perylene	276	--	6
Dibenz(a,h)anthracene	21	Target	d12-Perylene	278	--	6
Benzo(g,h,i)perylene	22	Target	d12-Perylene	276	--	6
Coronene	23	Target	d12-Perylene	300	--	7

IV. ROUTINE GC/MS MAINTENANCE

1. Gas Tanks

Check the gas tanks (helium and methane). They should not go dry. When either tank is at 500 PSI the tank should be replaced. While changing the tank lower the temperature of the GC oven to 40°C and turn the gas saver off for about 15 minutes to get rid of air or oxygen that was drawn in. Check for leaks using an electronic leak detector. Do not use liquid leak check solution as it may be drawn into the instrument and may cause damage to the column.

2. Liner

The liner needs to be replaced when the response and resolution begins to lower. Turn down the injection port temperature to 30°C to prevent burn hazard. Wear clean, lint-free gloves to prevent

contamination of parts with dirt or skin oils. Remove tower. Remove the inlet sealing apparatus with specialized tool so that the liner can be replaced. Replace with new liner and o-ring using tweezers. Re-install the inlet sealing apparatus with specialized tool. Do not over tighten. Replace tower. Reset inlet temperature to normal conditions. Turn off the gas flow saver so that the gas flows continuously for 5 minutes to purge the lines, then turn the gas saver back on. Recommendation: replace frequently (every 80-130 injections), and simultaneously with the septum.

3. Septum

The septum needs to be replaced when necessary. Symptoms such as shifting retention times, loss of response, loss of column head pressure, and/or increased signal-to-noise indicate that the septum needs to be replaced. Turn down the injection port temperature to 30°C to prevent burn hazard. Wear clean, lint-free gloves to prevent contamination of parts with dirt or skin oils. Remove tower. Remove septum retaining nut. Remove old septum using tweezers. Replace with new septum. Finger tighten septum retaining nut. Watch the gas pressure of the injection port until it becomes stable, then tighten the nut about ½ turn. Put tower back. Reset inlet temperature to normal conditions. If the pressure does not remain stable, tighten retaining nut further. Recommendation: replace frequently (every 80-130 injections), and simultaneously with the inlet liner.

4. Gold Seal

The gold seal should be replaced when necessary. Turn down the inlet temperature to 30°C and GC oven to 30°C to prevent burn hazard. Wear clean, lint-free gloves to prevent contamination of parts with dirt or skin oils. Remove inlet end of GC column. Remove insulation cover by loosening screws holding it in place. Remove the reducing nut with wrench. Replace gold seal located in reducing nut. After a new gold seal is in place, re-installed the reducing nut and insulation cover. Re-install the column into injector port. Return oven and inlet temperatures to normal. Turn off the gas flow saver so that the gas flows continuously for 5 minutes to purge the lines, then turn the gas saver back on.

5. Pump Oil

Pump oil for the MS's roughing pump should be replaced every 6 months. The used oil should be placed in a closed container labeled as hazardous waste and when full picked up by the IU Health and Safety Department for proper disposal.

6. Column

The column will need to be replaced or clipped if the peaks have bad shapes, show tailing, have low response, or has an early elution time. Procedures for replacing and clipping columns are provided below.

A. INSTALLING A NEW COLUMN

- a. Turn down the temperatures for GC, injection port, and the auxiliary (interface) on the front keypad of the GC
- b. In the Chemstation software that operates the GC/MS, under **View** select **Tune and Vacuum Control** from the drop down list
- c. In the new screen, under **Vacuum**, select **Vent**
- d. When prompted (in approximately 40 minutes) turn off the MS
- e. Take off the cover of the instrument after the MS is off. *BE SURE TO WEAR LINT-FREE GLOVES TO DO ANY WORK ON THE MS DETECTOR*
- f. Open the vent valve to break the vacuum – it will be audible. Once the instrument has vented close the vent valve (be sure to not tighten too much)
- g. Unplug the cables (side board control cable and the source power cable) that are connected to the vacuum manifold
- h. Open the door of the vacuum manifold

In the GC oven

- i. Hang the column in the center of the GC oven to insure the column is uniformly headed during operation and remove rubber seals from either end of column
- j. Loosen one end of the column (free ~1 foot) so that it will be easy to work with
- k. Place a septum, a new inlet column nut, and a graphite/vespel ferrule on the inlet end of the column (the ferrule should “point” towards the point of injection)
- l. Remove ~2 cm from the end with ceramic cutter. Verify that the cut end is straight using a magnifying glass. *If the cut end of the column is jagged or at an angle it will negatively impact peak shapes and overall chromatogram quality.*
- m. Slide the septum to place the nut and ferrule so that only 4 to 6 mm of the column is showing beyond the upper point of the ferrule.
- n. Holding the column at a point just below the septum, carefully insert the injector end of the column into the lower end of the reducing nut below the injection point. Tighten the column nut to secure the column in place
- o. Start carrier gas flow.
- p. Loosen the other end of the column. Place this loose end in a small beaker of hexane to verify proper gas flow through the column – look for small bubbles
- q. After confirming gas flow, allow the gas to flow through the column for 15 without heating the GC oven. This allows the column to bleed off and make for a better baseline following installation.

- r. Condition the column by ramping oven temperature starting at 30°C and increasing by 5°C per minute to 300°C, and holding it at that temp for 60 minutes
- s. Following column conditioning, return GC oven temperature to 30°C
- t. Loosen the MS end of the column (free ~1 foot) so that it will be easy to work with
- u. Place a septum, a new MS column nut, and a graphite/vespel ferrule on the MS end of the column (the ferrule should “point” towards the point of injection)
- v. Remove ~2 cm from the end with ceramic cutter. Verify that the cut end is straight using a magnifying glass. *If the cut end of the column is jagged or at an angle it will negatively impact peak shapes and overall chromatogram quality.*
- w. Slide the column into the GC/MS interface transfer line until the tip of the column becomes visible in the MS vacuum manifold. With the goal of having 1-2 mm of column projecting beyond the transfer line into the MS vacuum manifold, adjust the septum/nut/ferrule to an appropriate position along the length of the column. Tighten the column nut to secure the position of the column and seal off the MS.
- x. Close the vacuum manifold and make sure the vent valve is closed
- y. Re-attach the side board control cable and source power cable to the door of the MS vacuum manifold
- z. Firmly press the door of the MS vacuum manifold shut while turning the power of the MS back on. You should hear the roughing pump kick back on and the vacuum created should hold the door shut.
- aa. In Chemstation, in **Tune and Vacuum Control**, prompt the MS to **Pump Down** (generally, this will not be necessary, due to the fact that pump down is initiated when the MS is turned back on in the step before). Click **OK**.
- bb. Reinstall the MSD and vent valve covers. Make sure the temperatures increase toward set points, and that the turbo pump speed advances to 100%.
- cc. Prepare for autotuning by allowing the instrument to reach thermal equilibrium by letting it run for 4 hours before initiating autotune
- dd. Auto tune (detailed later)
- ee. Check for air/water
 - i. In the **Tune and Vacuum Control** window, go to **Parameters, Manual Tune, More Parameters**, and then **Tune Parameters**. Make sure the parameters show Mass 1 = 69.0, Mass 2 = 32.0, and Mass 3 = 18.0. Click OK. The air and water should be 1! And under for the instrument to be free from air and water.
- ff. Correct SIM windows if necessary

B. CLIPPING THE COLUMN

- a. Reduce GC oven and injection port temperatures to 30°C using keypad on the front of the GC
- b. With a ceramic cutter, cut the inlet end of the column just below the inlet column nut
- c. Remove the old inlet column nut/ferrule/column by unscrewing it from the reducing nut
- d. With a ceramic cutter, remove a portion of the column from the inlet end. If the column is contaminated, at least 30 cm of column length should be removed. If tailing trends are the issue, shorter lengths are acceptable to remove.
- e. Place a septum, a new inlet column nut, and a graphite/vespel ferrule on the inlet end of the column (the ferrule should “point” towards the point of injection)
- f. Remove ~2 cm from the end with ceramic cutter. Verify that the cut end is straight using a magnifying glass. *If the cut end of the column is jagged or at an angle it will negatively impact peak shapes and overall chromatogram quality.*
- g. Slide the septum to place the nut and ferrule so that only 4 to 6 mm of the column is showing beyond the upper point of the ferrule.
- h. Holding the column at a point just below the septum, carefully insert the injector end of the column into the lower end of the reducing nut below the injection point. Tighten the column nut to secure the column in place
- i. Return GC temperatures to normal operating conditions using keypad
- j. Turn off the gas flow saver so that the gas flows continuously for 5 minutes to purge the lines, then turn the gas saver back on
- k. Check for air/water
- l. Correct SIM windows if necessary

7. Ion Source Cleaning

The frequency of cleaning the ion source depends on the number and type of samples being run through the instrument. Check column response and peak shape to determine whether the ion source needs cleaning. It is most convenient to review and overlay chromatograms of calibration standards within and between runs to see instrument conditions are changing. Removing contaminants restores the electrostatic properties of the ion source lensing system. *WHEN HANDLING THE ION SOURCE BE SURE TO USE LINT-FREE GLOVES.*

A. Materials Needed

- a. Alumina abrasive powder
- b. Aluminum foil

- c. Cotton Swabs
- d. Beaker
- e. Gloves – nylon lint-free & nitrile
- f. Acetone
- g. Methanol
- h. Methanol squirt bottle
- i. Ultrasonic bath
- j. Ion Source dis-assembly tool kit
- k. Methylene Chloride

B. Removing the Ion Source

- a. In the Chemstation software that operates the GC/MS, under **View** select **Tune and Vacuum Control** from the drop down list
- b. In the new screen, under **Vacuum**, select **Vent**
- c. When prompted (in approximately 40 minutes) turn off the MS
- d. Take off the cover of the instrument after the MS is off. *BE SURE TO WEAR LINT-FREE GLOVES TO DO ANY WORK ON THE MS DETECTOR*
- e. Open the vent valve to break the vacuum – it will be audible. Once the instrument has vented close the vent valve (be sure to not tighten too much)
- f. Unplug the cables (side board control cable and the source power cable) that are connected to the vacuum manifold
- g. Open the door of the vacuum manifold
- h. Disconnect the 7 colored wires from the ion source. Disconnect the 4 wires for the ion source heater and temperature sensor from the circuit board. Remove the 2 thumb screws from the ion source body holding it in place within the MSD, and place them loosely inside the MSD.
- i. Place ion source on clean piece of aluminum foil. Close vacuum manifold door.



Figure 2. EI ion source in open vacuum manifold

C. Ion Source Disassembly

- a. Remove screws holding the 2 filaments in place. Set screws & filament pieces aside.
- b. Remove Hex nut attached to repeller projecting through EI source heater assembly. Set aside nut, washers, and insulator.
- c. Remove screws holding EI source heater assembly to source body. Set screws aside.
- d. With screws removed, the EI source heater assembly can be detached and set aside
- e. With EI source heater assembly removed the repeller insulator can be set aside, and repeller removed to be cleaned
- f. Remove small set screw from the side of the EI source body. Set aside. Don't lose.
- g. Slide out EI lens insulator/entrance lens/ion focus lens. Disassemble. Set entrance lens and ion focus lens with items to be cleaned. Set insulator aside.
- h. Remove draw out cylinder and draw out place from EI source body. Add all three to items to be cleaned.
- i. Remove transfer line socket from source body. Add to items to be cleaned.
- j. With ion source completely disassembled, wrap the pieces that will not be cleaned in a piece of clean aluminum foil until ready to reassemble ion source.

D. Ion Source Cleaning

- a. Use nitrile gloves while cleaning the ion source

- b. 7 pieces that should be cleaned (shown in image order below L-R)
 - i. Repeller
 - ii. Draw out plate
 - iii. Source body
 - iv. Ion focus lens
 - v. Entrance lens assembly
 - vi. Draw out cylinder
 - vii. Transfer line socket



Figure 3. EI ion source parts to clean

- c. Place a small amount of alumina powder in a beaker and add reagent grade methanol to make a slurry mixture. Use this slurry mixture and a cotton swab to abrasively clean the parts listed above. Clean all surfaces that come into contact with the sample or ion beam
- d. Rinse away all abrasive residues with reagent grade methanol. Make sure all residues are rinsed away before the ultrasonic cleaning. If the methanol becomes cloudy or contains visible particles, rinse again.
- e. Place the parts in a clean beaker. Making sure to cover all parts with the solvent and loosely covering the beaker with aluminum foil, ultrasonically clean the parts for 15 minutes in each of the following reagent grade solvents in the order listed below
 - i. DCM
 - ii. Acetone
 - iii. Methanol
- f. Dry the cleaned parts by letting them air dry in aluminum foil for 30 minutes. Make sure that the parts are covered by foil.

E. Reassembling the Ion Source

- a. Be sure to wear lint-free gloves when handling components of the ion source
- b. Insert draw out plate, then draw out cylinder into the source body.
- c. Insert entrance lens & ion focus lens into lens insulator. Slide entire assembly into source body. Secure in place with small set screw.
- d. Screw transfer line socket back in place into the side of the source body
- e. Assemble repeller/heater with insulators and hex nut in place
- f. Align source body with repeller/source heater assembly. Secure all together using the screws that pass through heater assembly into source body.

- g. Secure 2 filaments in position with screws. *New filaments may be needed. This would be the time to replace old ones with a new ones.*

F. Re-install the Ion Source

- a. Secure the reassembled EI ion source within the MSD using the two thumb screws
- b. Reattach all wires
- c. Close the vacuum manifold and make sure the vent valve is closed
- d. Re-attach the side board control cable and source power cable to the door of the MS vacuum manifold
- e. Firmly press the door of the MS vacuum manifold shut while turning the power of the MS back on. You should hear the roughing pump kick back on and the vacuum created should hold the door shut.
- f. In **Chemstation**, in **Tune and Vacuum Control**, prompt the MS to **Pump Down** (generally, this will not be necessary, due to the fact that pump down is initiated when the MS is turned back on in the step before). Click **OK**.
- g. Reinstall the MSD and vent valve covers. Make sure the temperatures increase toward set points, and that the turbo pump speed advances to 100%.
- h. Prepare for autotuning by allowing the instrument to reach thermal equilibrium by letting it run for 4 hours before initiating autotune
- i. Auto tune (detailed later)
- j. Check for air/water
- k. Correct SIM windows if necessary

8. MS Autotuning

- a. Autotune the instrument with perfluorotributylamine (PFTBA) calibration gas after the instrument has equilibrated for at least 4 hours.
- b. In the Chemstation software that operates the GC/MS, under **View** select **Tune and Vacuum Control** from the drop down list
- c. In the new window, under **Tune** select **Autotune**
- d. After the software has completed its Autotuning procedure, select **File** then **Save Tune Values**. Save using the same file name (ATUNE.U)
- e. When the autotuning is complete, a report will be generated. This report should automatically print to the attached printer. File this and all other autotune reports.
- f. Under **View** select **Instrument Control** to return to the primary Chemstation screen

Table III. Suggested maintenance check list (may vary according to instrument usage).

TASK	FREQUENCY			
	WEEKLY	6 MONTHS	YEARLY	AS NEEDED
Tune the MSD				x
Change injection port liners & septa	x			
Check foreline pump oil level	x			
Gas ballast the foreline pump	x			
Check the calibration vial		x		
Replace the foreline pump oil		x		
Replace traps and filters			x	
Clean the ion source				x
Change the carrier gas trap(s) and purifier				x
Replace worn out parts				x
Lubricate seals (where appropriate)				x
Replace column				x

V. ACQUISITION

1. The instrumentation for acquisition is provided in section I.
2. The method details are provided in Appendix I.
3. Software operation
 - a. Loading & altering a method
 - i. With Chemstation in the “Instrument Control” view, methods can be loaded under **File** select **Load Method**
 - ii. To alter MS parameters of the acquisition method -- under **Instrument** select **MS SIM/Scan Parameters**
 1. Here you will be able to adjust solvent delay, SIM windows, EM voltage, and select between SIM or SCAN modes
 - iii. To alter GC parameters of the acquisition method – under **Instrument** select **GC Edit Parameters**
 1. Here you will be able to adjust GC oven parameters, injector settings, and run length
 - iv. If changes have been made to a method, they can be saved under **File** select **Save Method** or **Save Method As** (if you don’t want to over-write the previous version)
 - b. Preparing a run sequence

- i. In the “**Instrument #1MSTop/Enhanced**” view, go to **Sequence** and select **Load** to get an existing sequence or create a new one by using “default.s”
- ii. Go to **Sequence** and select **Edit Sample Log Table** to edit sequence parameters
 1. Data Path: select Browse and specify your data directory. This is where your data files will be stored
 2. Method Path: select Browse and select from C:\msdchem\1\methods
 3. Fill out type (sample, blank, calibration), vial (placement in autosampler tray), sample (name of sample), and comments
 4. Method/keyword – select box – a “?” will appear on right side of box. Click on the “?” to select method to use
 5. Data File – select box – a “?” will appear on right side of box. Do not click on “?”. Instead, double click in the box and cursor will appear in box. Enter the name here and the computer will create the data file with that name. Be careful to ensure every sample has a unique data file name, otherwise the data will be overwritten.
 6. Click **OK**
- iii. The sequence table should include the following
 1. Hexane blanks
 2. Calibration standards
 3. QC samples (common reference standards)
 4. Your samples

c. Saving sequence

- i. Under **Sequence** select **Save** which will prompt you to specify sequence name and file location.

d. Running sequence

- i. Under **Sequence** select **Load and Run Sequence** which will open a new window.
- ii. Select “Full Method”
- iii. Click on the option for not overwriting existing data
- iv. Enter your initials in “Operator Name” box
- v. Do not change Data File Directory
- vi. Click **Run Sequence**, instead of just clicking “OK”
- vii. The run may be stopped at any time by clicking the red “**STOP**” button in the **Instrument Control Panel**
- viii. To monitor the run, click on the “total ion” thumbnail window in the “instrument control panel” to enlarge the view. Click the “instrument #1 Data Analysis” desktop icon. From the pull down menus select **File** then **Take Snapshot**

4. Example Chromatograms from acquisition (Figures 4 & 5)

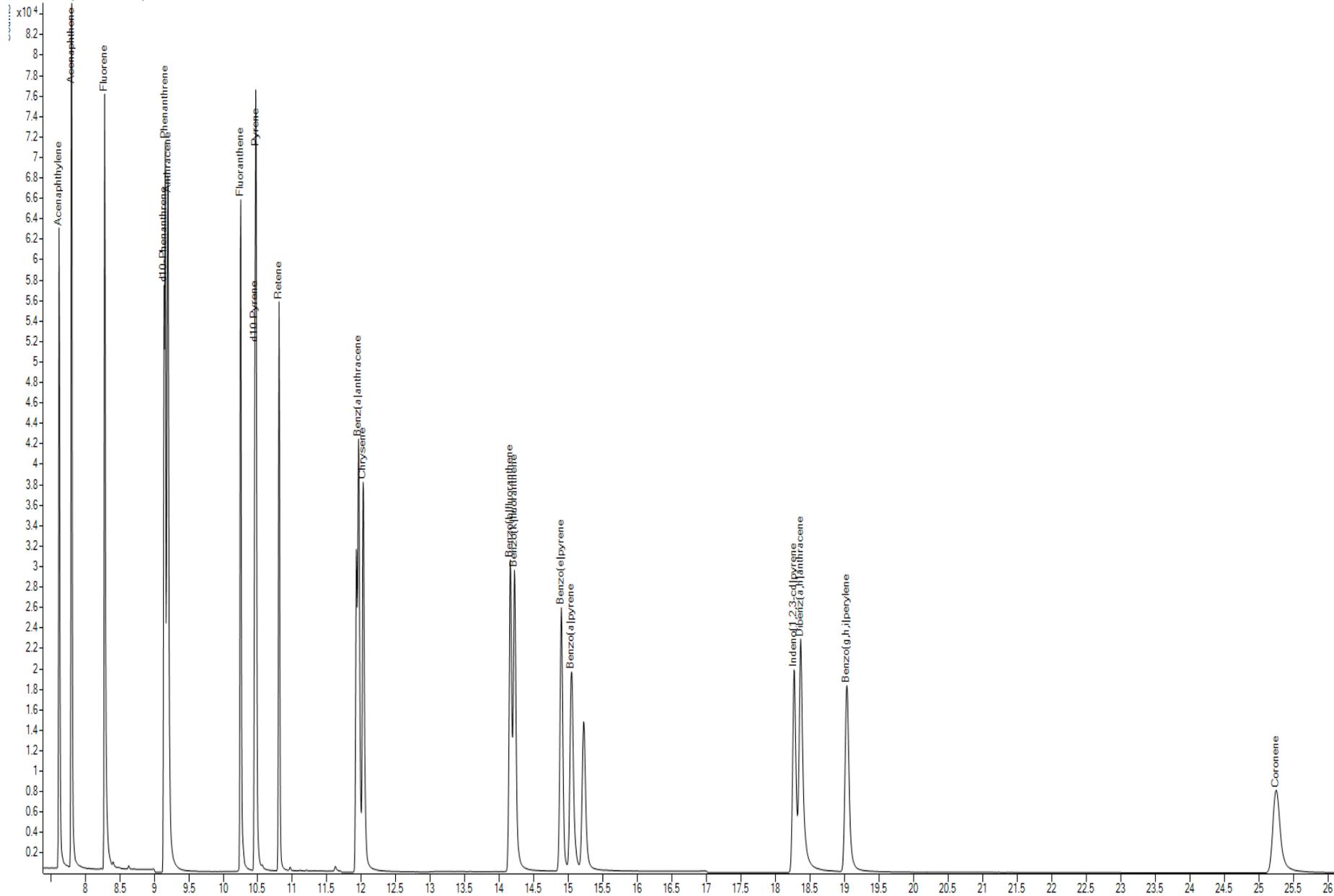


Figure 4. Example of a PAH calibration standard chromatogram.

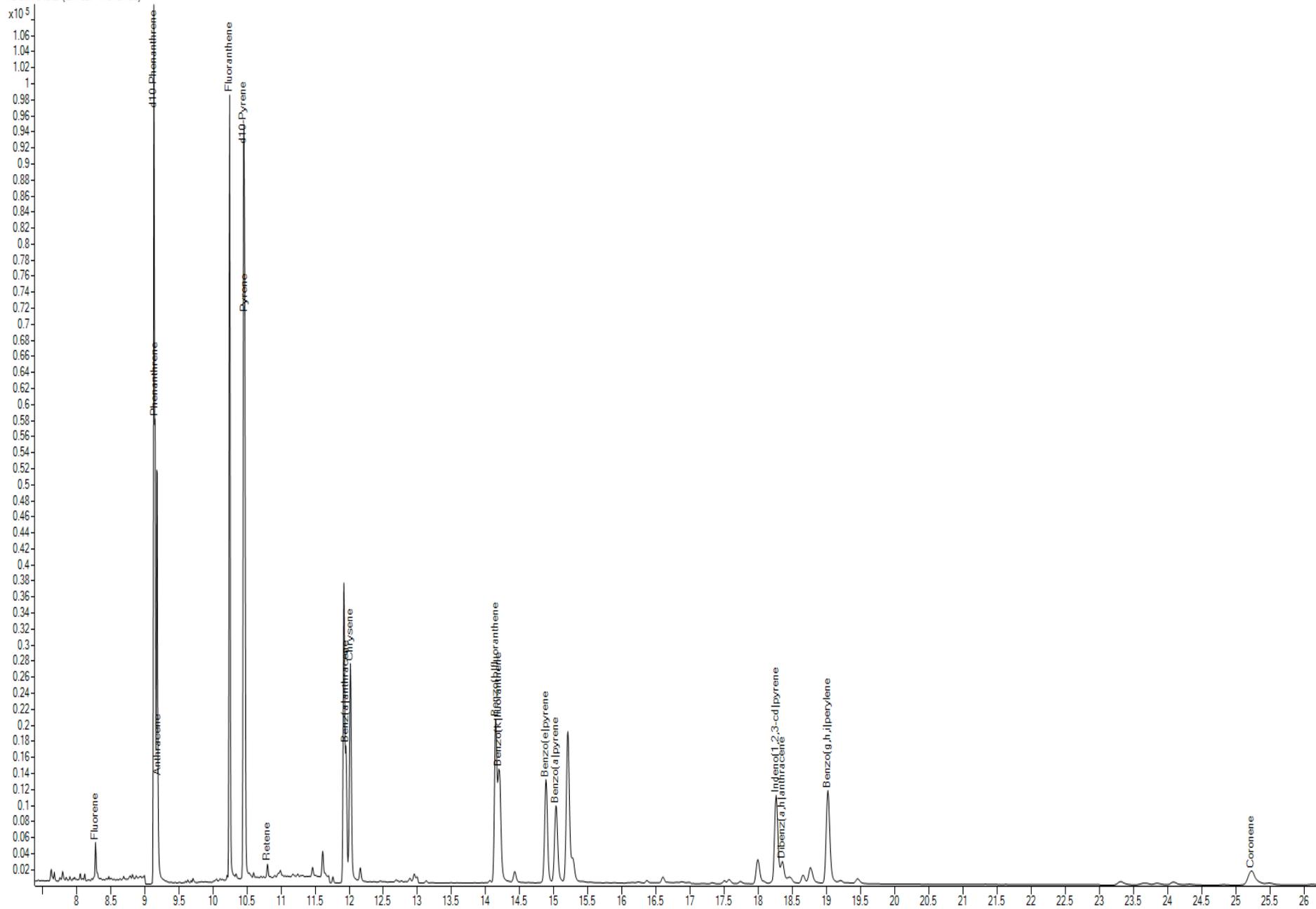


Figure 5. Example of a PAH sample chromatogram.

VI. QUANTITATION

Mass Hunter software is used to quantitate the chromatograms produced from acquisition.

The data files generated in acquisition will need to be copied to the computer where quantitation will occur.

1. Mass Hunter Setup

For quantitation and data review/reporting purposes it is important to include certain sample and compound information.

Making changes to Mass Hunter sample table

- a. Sample table information can be added or removed by selecting **View**, then **Add/Remove Columns...**
- b. In the newly opened “Columns” window, verify that “Sample” is selected in the drop-down list at the top left.
- c. Available fields will appear in the left-hand box, while actively used fields appear in the right hand box. Fields can be moved back and forth using the *add* or *remove* buttons when a field is selected. The order of displayed fields can be adjusted in the right hand box.
- d. Sample fields/information to include
 - i. Quantitation Message Summary
 - ii. Outlier Summary
 - iii. Comment – *this will often include the batch ID*
 - iv. Name – *this will be the sample ID*
 - v. Data File
 - vi. Type
 - vii. Vial
 - viii. Acq. Date-Time
 - ix. Acq. Operator
 - x. Acq. Method File
 - xi. Level
 - xii. Sample Group
- e. Target Compound results can be adjusted by selecting “Compound Results” in the drop-down list at the top left of the “Columns” window
- f. Available fields will appear in the left-hand box, while actively used fields appear in the right hand box.
- g. Compound result fields/information to include
 - i. Final Conc.
 - ii. Resp.
 - iii. RT
- h. Internal standard results can be adjusted by selecting “ISTD Compound Results” in the drop-down list at the top left of the “Columns” window
- i. Available fields will appear in the left-hand box, while actively used fields appear in the right hand box.

- j. ISTD compound results fields/information to include
 - i. Resp.

2. Quantitating Data

- a. Open the Mass Hunter Quantitative Analysis program
- b. Under **File** select **New Batch**. This will open a new window that will allow you to navigate through your computer's folder/file directory.
- c. Navigate to the folder that contains your data files. Provide a name for the batch in the space for *File Name*. Click **Create**
- d. A new window will appear prompting you to add samples to the batch. All data files within the same folder as the batch file that you just created should appear. Highlight in blue the files that are to be quantitated. Click **OK**
- e. At this time, the data files may need to be translated. The program should do this automatically and the progression of the translation should be visible onscreen.
- f. Once translation is complete, the samples will be loaded to the Quantitative Analysis sample table.
- g. Under **File** select **Save Batch**
- h. At this time, make sure all calibration standards in the sequence table have "Cal" under the column *Type*
- i. At this time, make sure all calibration standards in the sequence table have "1" under the column *Level* (this is not the case if your calibration uses multiple levels – 1,2,3... would be appropriate in such a case)
- j. Set up sample groups for your quantitation.
 - i. This allows you to group samples within the sample table to calibration standards in the table.
 - ii. There is a column called "sample group"
 - iii. By entering in values of 1,2,3... in this column the analyst defines the grouping
 - iv. Each group must have one calibration standard, and should not have more than that.
 - v. Each sample (row) in the table must have a sample group listed
- k. Select a calibration standard by clicking on the far left box the row corresponding to a calibration standard. A black arrow should appear and the row become highlighted in dark green.

- l. Under **Method** select **Edit**, then select **Open Method From Existing File**
- m. Navigate to a quantitation method that you saved earlier in the window that pops up. Click **Open**
- n. Update the retention times
 - i. Select *Retention Time Setup* in the left hand method task list.
 - ii. The TIC is shown below compound list
 - iii. Update retention times either by manually entering in time in RT column based on TIC (this is generally done when the peak retention times have changed significantly from previous quant method usage), or by clicking **Update** and selecting **Update Retention Times**. Select all compounds. Click **OK**
- o. Update the qualifier ion ratios
 - i. Under **Update** select **Update qualifier ratios**
 - ii. Select all compounds. Click **OK**
- p. Validate your method by selecting Validate in the left-hand method task list. Resolve any errors that might arise
- q. Click **Save As** in the left-hand method task list. Navigate to the folder containing your data files and provide a name for you quantitation method.
- r. Click Exit in the left-hand method task list. A new window will open asking if you would "...like to apply this method to the batch?". Make sure that *Analyze* is selected below the yes/no options, then click **Yes**.
- s. Under **File** select **Save Batch**.
- t. To review and make adjustment of your integration peaks for the chromatograms – under **View** select **Compounds-at-a-glance**. This will open a new window with all compounds and all sample laid out in a grid of windows
- u. The grid of windows can be changed by selecting the **Panes: # x #** drop-down list from the upper ribbon
- v. The order of compounds and samples can be changed by clicking **Layout** then selecting **Setup Layout** and making adjustments within the newly opened window
- w. Allow the software to enable user peak adjustment by clicking on the icon of a green peak with two small black boxes on either side from the upper ribbon. This will enable a variety of other icons in the upper ribbon for manual integration
- x. Things to consider when quantitating...

- i. Look at the peak shape. The peak shape in a sample should be similar to what is in the calibration standard. If the peaks are tailing, fronting, flat tops, split tops, or of any other irregular shape, refer to the GC manual for chromatographic problems and how to resolve them. In some cases the sample will need to be rerun and/or recleaned. If these actions don't help then the compound needs to be deleted with a corresponding note in the data entry spreadsheet
- ii. Look at the ion abundances. The most abundant ion in the TIC should be the quantitation ion. Additionally, the abundance of the qualifying ion(s) relative to the quantifying ion in samples should match what is observed in the calibration standard

There are exceptions to this rule in the case of co-eluting peaks. Deuterated PAH congeners and their non-deuterated counterparts (anthracene and d10-anthracene, pyrene and d10-pyrene, phenanthrene and d10-phenanthrene, benz(a)anthracene and d12-benz(a)anthracene) will co-elute, and as a result when inspecting the spectra for one of these peaks, the quantitation ion of interest may not be the most abundant, but rather the quantitation ion of the co-eluting counterpart. This is most likely to occur at low concentrations of the non-deuterated compound. Therefore, in the case of these compounds the peaks should not be deleted even if the most abundant ion is different from the quantitation ion. See Appendix III for additional information on these spectra.

- iii. Look at the response of the peak from the base of the peak and the top of the peak. The overall area (response) of the peak should be at least 3x the baseline. As an example: the base of the peak is at 100, and the overall response of the peak is 800 – the area is greater than 3x the peak base value, so it is acceptable.
- iv. The bottom boundary of the quantitation area should reflect the baseline trend around the target peak. Meaning, all peaks should have a straight quantitation baseline in cases of a uniformly even baseline, or be at a complimentary angle if the baseline is increasing or decreasing.
- v. Look at the retention times. The retention time of specific peaks in samples should be very similar to the retention time of those same peaks in the calibration standard. Peak shifting does happen and when it does it does not necessarily happen uniformly throughout a single chromatogram. The analyst can use internal standard and surrogate standard peaks as markers to assess the degree and direction of retention time shifting that is taking place in the chromatogram. This can be tricky. The more you do it the easier it will become. If you see large peak shifting, then the sample may be too concentrated – dilute and rerun to confirm peaks. Use your judgement and experience when identifying and/or deleting peaks.
- y. When you have finished deleting/reviewing/manually adjusting peaks close the **compounds-at-a-glance** window. In the upper ribbon select **Analyze Batch**. This will requantitate all calculated values using the adjustments that have been made to samples and/or calibration standards

z. Under **File** select **Save Batch**.

aa. Export data for entry in IADN raw entry spreadsheet

i. Under File select **Export**, then **Export Table**

ii. Select file type as either CSV or Excel, and select location for file to be generated (ideally in the same folder as the data files)

bb. Generate Reports

i. Under **Report** select **Generate**

ii. Select report folder (ideally the in the same location as the data files)

iii. Select report method (specified by user)

iv. You can choose to report only specific samples or specific compounds

v. Click **OK**

vi. Save the resulting report PDF document on the network drive for permanent reference

cc. Entering data into raw entry spreadsheet

i. Copy data from CSV or excel file that was exported earlier into sorting spreadsheet provided by program data manager

ii. Run Sorting Macro

iii. Each tab of spreadsheet represents data for a specific field location

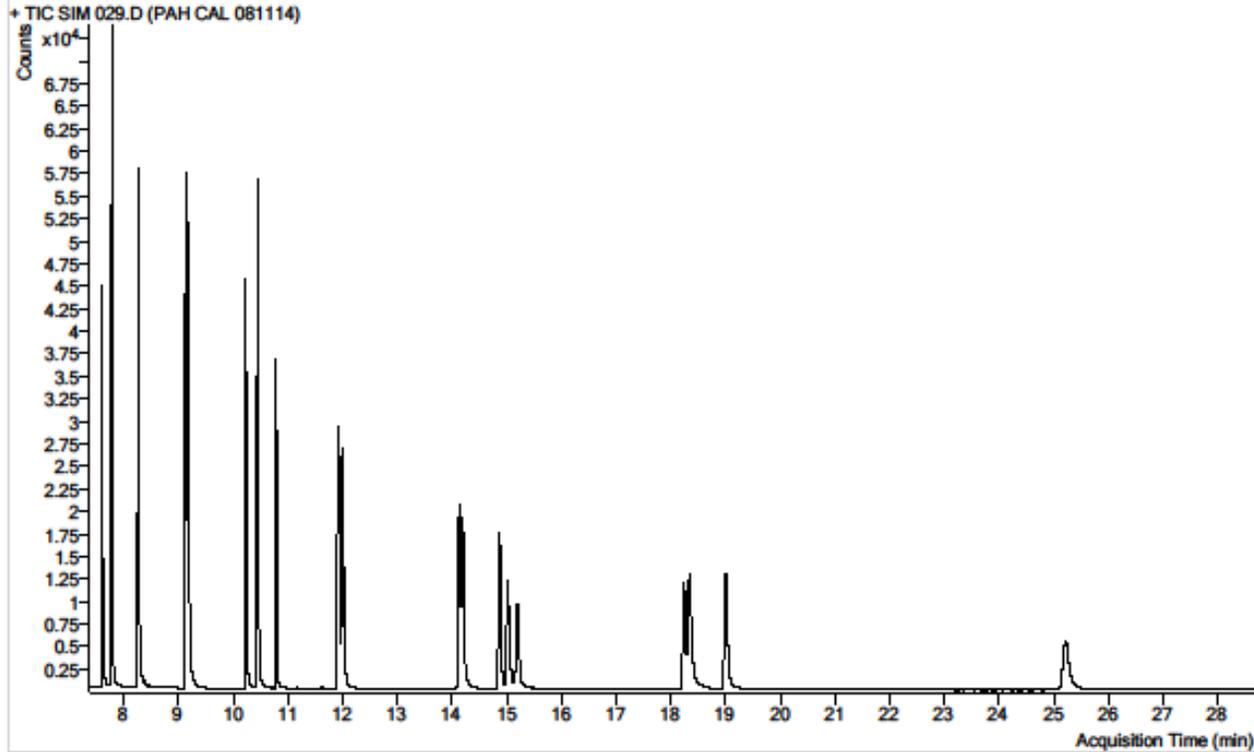
iv. Copy data directly from sorted tabs into the IADN raw data entry spreadsheet

3. Example quantitation reports (Figures 6 & 7)

Quantitative Analysis Report

Batch Path	G:\Labs\HitesR\Daniel\PAH Stuff\IADN PAH\2017\180529_JL17F_JL217F_JL17P\QuantResults\180529.batch.bin	Acquisition Time Stamp	5/30/2018 8:51:01 AM
Quantitation Time Stamp	6/4/2018 2:57:24 PM	Acquisition Method	PAH_2017(DCL)VERSION2.M
Quantitation Method	PAH_Quant.m	Acquisition Operator	DCL
Analyst Name	DCL	Instrument Name	Instrument #1
Quant Software Version	B.09.00		
Sample Name	PAH CAL 081114	Comment	200 NG/ML
Sample Type	Cal	Data File	029.D
Vial	22	Vol.	0

Sample Chromatogram



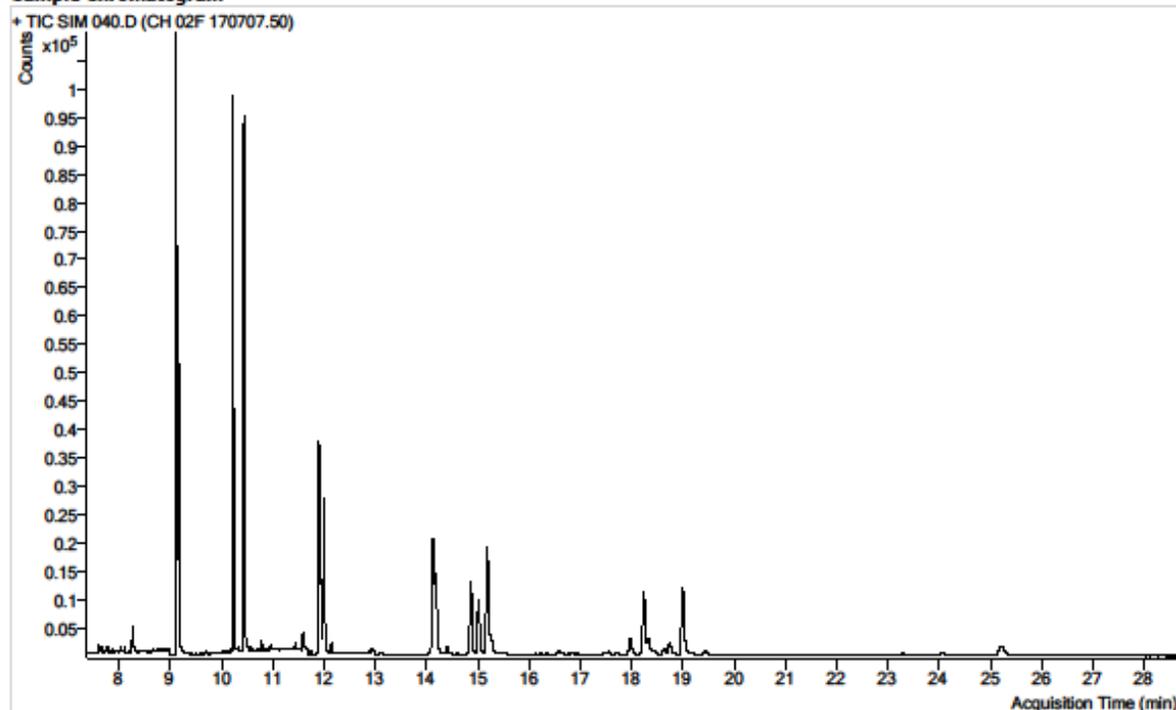
Name	RT	Target Ion	Target Response	Final Conc.	Units
Fluorene	8.280	166.0	42758	200.0000	ng
d10-Phenanthrene	9.143	188.0	47454	200.0000	ng
Phenanthrene	9.155	178.0	56765	200.0000	ng
Anthracene	9.197	178.0	61629	200.0000	ng
Fluoranthene	10.247	202.0	61991	200.0000	ng
d10-Pyrene	10.457	212.0	42553	200.0000	ng
Pyrene	10.470	202.0	64573	200.0000	ng
Retene	10.808	219.0	26763	200.0000	ng
Benz(a)anthracene	11.957	228.0	48234	200.0000	ng
Chrysene	12.024	228.0	59800	200.0000	ng
Benzo(b)fluoranthene	14.150	252.0	51793	200.0000	ng
Benzo(k)fluoranthene	14.217	252.0	60410	200.0000	ng
Benzo(e)pyrene	14.891	252.0	53130	200.0000	ng
Benzo(a)pyrene	15.036	252.0	46002	200.0000	ng
Indeno(1,2,3-cd)pyrene	18.264	276.0	41886	200.0000	ng
Dibenz(a,h)anthracene	18.361	278.0	45715	200.0000	ng
Benzo(g,h,i)perylene	19.022	276.0	53180	200.0000	ng
Coronene	25.231	300.0	46151	200.0000	ng

Figure 6. Example of quantitation report for PAH calibration standard.

Quantitative Analysis Report

Batch Path	G:\Lab\HitesR\Daniel\PAH Sturm\ADN PAH\2017\180529_JL17F_JL17F\QuantResults\180529.batch.blm		
Quantitation Time Stamp	6/4/2018 2:57:24 PM	Acquisition Time Stamp	5/30/2018 3:10:17 PM
Quantitation Method	PAH_Quant.m	Acquisition Method	PAH_2017(DCL)VERSION2.M
Analyst Name	DCL	Acquisition Operator	DCL
Quant Software Version	B.09.00	Instrument Name	Instrument #1
Sample Name	CH 02F 170707.50	Comment	JL17F
Sample Type	Sample	Data File	040.D
Vial	31	Vol.	0

Sample Chromatogram



Name	RT	Target Ion	Target Response	Final Conc.	Units
Fluorene	8.279	166.0	2721	9.0885	ng
d10-Phenanthrene	9.135	188.0	109030	320.2856	ng
Phenanthrene	9.154	178.0	50860	123.3141	ng
Anthracene	9.196	178.0	5811	14.0441	ng
Fluoranthene	10.246	202.0	111816	244.7345	ng
d10-Pyrene	10.450	212.0	103025	322.4969	ng
Pyrene	10.469	202.0	75142	155.6094	ng
Retene	10.801	219.0	1068	5.3507	ng
Benz(a)anthracene	11.951	228.0	24797	64.7330	ng
Chrysene	12.017	228.0	54430	123.2292	ng
Benzo(b)fluoranthene	14.149	252.0	52433	115.4170	ng
Benzo(k)fluoranthene	14.192	252.0	45789	88.7120	ng
Benzo(e)pyrene	14.884	252.0	38183	83.0108	ng
Benzo(a)pyrene	15.029	252.0	31809	78.4448	ng
Indeno(1,2,3-cd)pyrene	18.259	276.0	37112	100.5780	ng
Dibenz(a,h)anthracene	18.350	278.0	6730	16.5528	ng
Benzo(g,h,i)perylene	19.018	276.0	43265	95.0338	ng
Coronene	25.226	300.0	14726	39.8106	ng

Figure 7. Example of quantitation report for sample.

VII. STANDARDS and SPIKING

1. PAH Calibration Standard

Table 4. Contents of PAH Calibration Standard.

Compound	Amount (ng/ml)
Acenaphthylene	200
Acenaphthene	200
Fluorene	200
d10-Phenanthrene	200
Phenanthrene	200
d10-Anthracene	200
Anthracene	200
Fluoranthene	200
d10-Pyrene	200
Pyrene	200
Retene	200
d12-Benz(a)anthracene	200
Benz(a)anthracene	200
Chrysene + Triphenylene	200
Benzo(b)fluoranthene	200
Benzo(k)fluoranthene	200
Benzo(e)pyrene	200
Benzo(a)pyrene	200
d12-Perylene	200
Indeno(1,2,3-cd)pyrene	200
Dibenz(a,h)anthracene	200
Benzo(g,h,i)perylene	200
Coronene	200

2. Additional Standards

For additional information on stock standard and the preparation of various PAH standard mixtures, see IADN Standards Preparation SOP.

3. Internal Standard Spiking

Spiking of sample vials following nitrogen blowdown with internal standards (50 µl) results in amounts summarized in Table 5.

Table 5. PAH internal standard amounts in IADN samples.

Compound	Amount (ng/ml)
d10-Anthracene	200
d12-Benz(a)anthracene	200
d12-Perylene	200

4. Surrogate Standards.

When extraction are being set up, surrogate recovery standards are added to each sample by spiking with the surrogate recovery working mixture. The target recovery amounts for PAHs are noted in Table 6.

Table 6. PAH surrogate recovery standard amounts.

Compound	Amount (ng/ml)
d10-Phenanthrene	400
d10-Pyrene	400

Appendix I. Instrument Control Parameters for PAH method

INSTRUMENT CONTROL PARAMETERS

=====

6890 GC METHOD

=====

OVEN

Initial temp: 70 'C (On) Maximum temp: 350 'C
Initial time: 3.00 min Equilibration time: 0.50 min

Ramps:

#	Rate	Final temp	Final time
1	30.00	280	6.00
2	30.00	300	12.00
3	0.0(Off)		

Post temp: 0 'C
Post time: 0.00 min
Run time: 28.67 min

FRONT INLET (SPLIT/SPLITLESS) BACK INLET (UNKNOWN)

Mode: Pulsed Splitless
Initial temp: 285 'C (On)
Pressure: 14.50 psi (On)
Pulse pressure: 25.0 psi
Pulse time: 2.00 min
Purge flow: 500.0 mL/min
Purge time: 2.00 min
Total flow: 504.0 mL/min
Gas saver: On
Saver flow: 20.0 mL/min
Saver time: 2.00 min
Gas type: Helium

COLUMN 1	COLUMN 2
Capillary Column	(not installed)
Model Number: Agilent 19091s-433u	
hp-5ms ui, 30mx250umx.25um	

Max temperature: 325 'C
Nominal length: 30.0 m
Nominal diameter: 250.00 um
Nominal film thickness: 0.25 um
Mode: constant pressure
Pressure: 14.50 psi
Nominal initial flow: 1.5 mL/min
Average velocity: 46 cm/sec
Inlet: Front Inlet
Outlet: MSD
Outlet pressure: vacuum

FRONT DETECTOR () BACK DETECTOR ()

SIGNAL 1	SIGNAL 2
Data rate: 20 Hz	Data rate: 20 Hz
Type: test plot	Type: test plot
Save Data: Off	Save Data: Off
Zero: 0.0 (Off)	Zero: 0.0 (Off)
Range: 0	Range: 0
Fast Peaks: Off	Fast Peaks: Off
Attenuation: 0	Attenuation: 0

COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)

THERMAL AUX 2
Use: MSD Transfer Line Heater
Description: MSD
Initial temp: 285 'C (On)
Initial time: 0.00 min
Rate Final temp Final time
1 0.0(Off)

POST RUN
Post Time: 0.00 min

TIME TABLE

Time Specifier Parameter & Setpoint

7673 Injector

Front Injector:

Sample Washes	0
Sample Pumps	3
Injection Volume	1.0 microliters
Syringe Size	10.0 microliters
Nanoliter Adapter	Off
PostInj Solvent A Washes	2
PostInj Solvent B Washes	2
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0.00 minutes
PostInjection Dwell	0.00 minutes
Sampling Depth	-2.0 mm

Back Injector:

No parameters specified

Column 1 Inventory Number : USD632046H

Column 2 Inventory Number :

MS ACQUISITION PARAMETERS

General Information

Tune File : ATUNE.U

Acquisition Mode : SIM

MS Information

--

Solvent Delay : 7.30 min

EM Absolute : False

EM Offset : 0

Resulting EM Voltage : 1082.4

[Sim Parameters]

GROUP 1

Group ID : 1

Resolution : Low

Plot 1 Ion : 152.0

Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(152.0, 70) (153.0, 70) (154.0, 70)
(165.0, 70) (166.0, 70)

GROUP 2

Group ID : 2

Resolution : Low

Group Start Time : 9.00

Plot 1 Ion : 188.0

Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell)
(178.0, 165) (188.0, 165)

GROUP 3

Group ID : 3

Resolution : Low

Group Start Time : 10.00

Plot 1 Ion : 202.0

Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(202.0, 80) (212.0, 80) (219.0, 80)
(234.0, 80)

GROUP 4

Group ID : 4

Resolution : Low

Group Start Time : 11.70

Plot 1 Ion : 228.0

Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell)
(228.0, 165) (240.0, 165)

GROUP 5

Group ID : 5
Resolution : Low
Group Start Time : 13.00
Plot 1 Ion : 252.0
Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell)
(252.0, 165) (264.0, 165)

GROUP 6

Group ID : 6
Resolution : Low
Group Start Time : 17.00
Plot 1 Ion : 276.0
Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell)
(276.0, 165) (278.0, 165)

GROUP 7

Group ID : 7
Resolution : Low
Group Start Time : 23.00
Plot 1 Ion : 300.0
Ions/Dwell In Group (Mass, Dwell)
(300.0, 350)

[MSZones]

MS Quad : 150 C maximum 200 C
MS Source : 230 C maximum 250 C

END OF MS ACQUISITION PARAMETERS

TUNE PARAMETERS

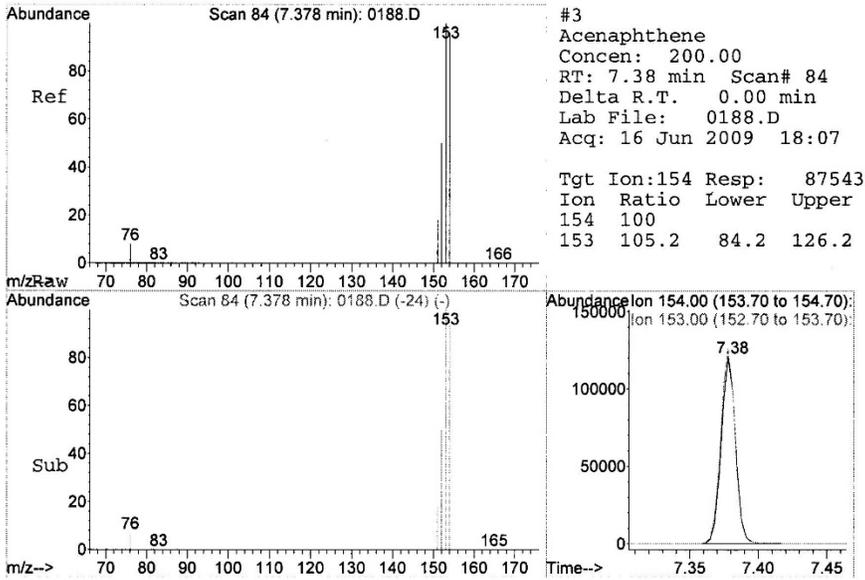
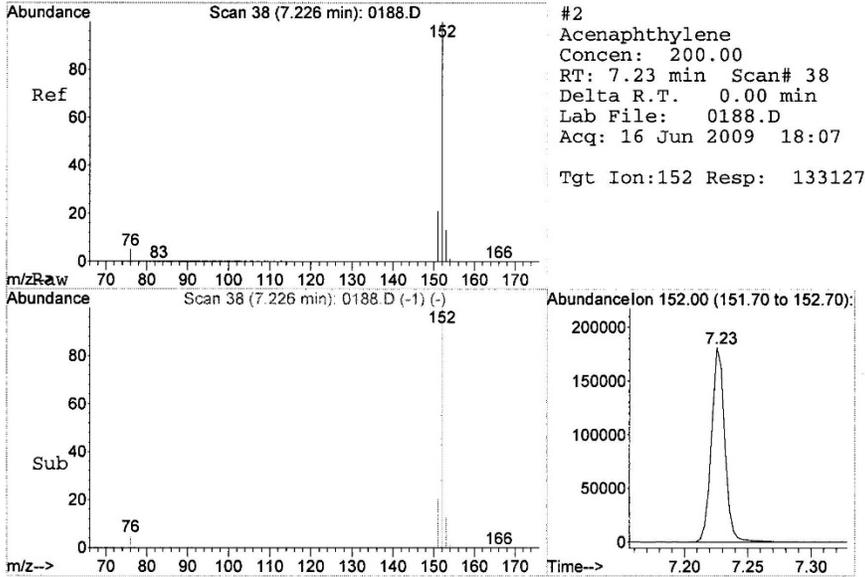
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ENERGY : 69.922
REPELLER : 31.128
IONFOCUS : 90.157
ENTRANCE_LE : 11.500

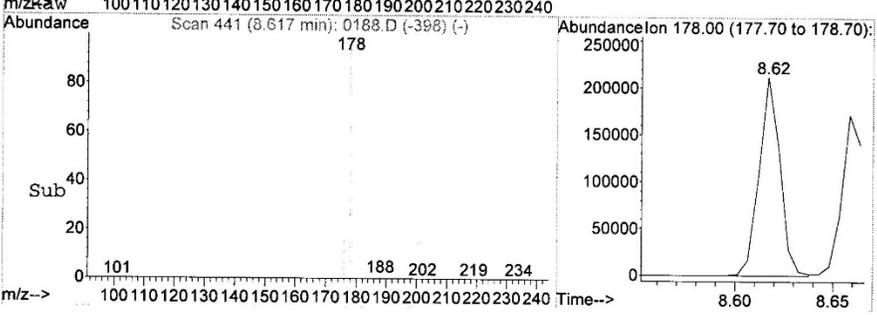
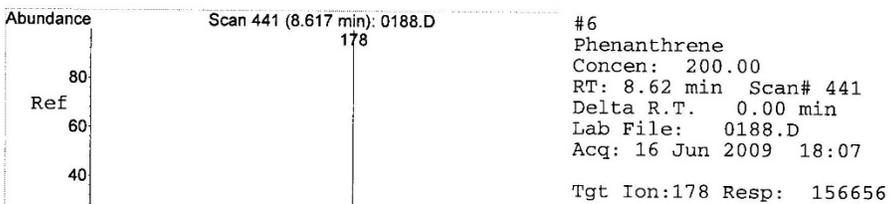
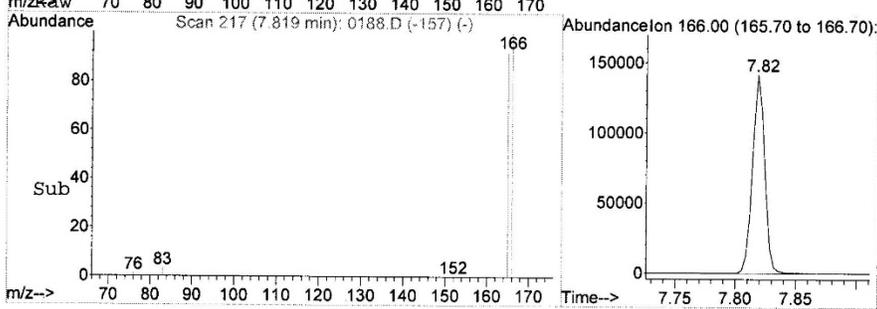
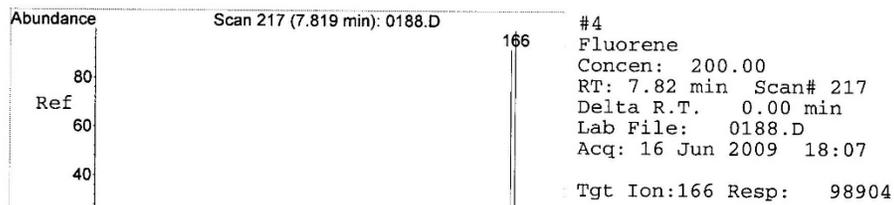
EMVOLTS : 1082.353
AMUGAIN : 1917.000
AMUOFFSET : 121.000
FILAMENT : 1.000
DCPOLARITY : 1.000
ENTLENSOFFS : 20.329
MASSGAIN : -230.361
MASSOFFSET : -12.096

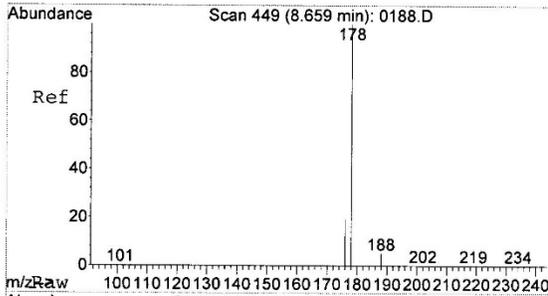
END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

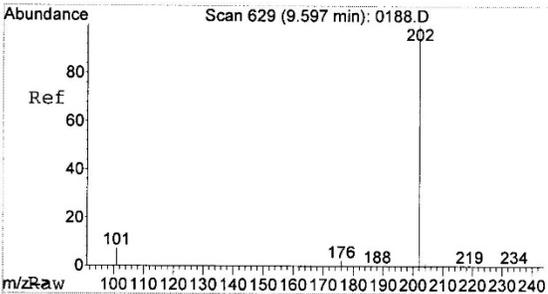
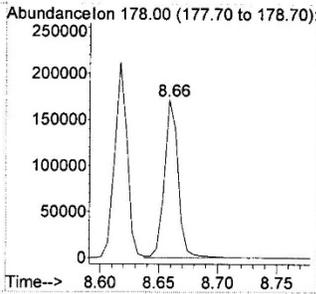
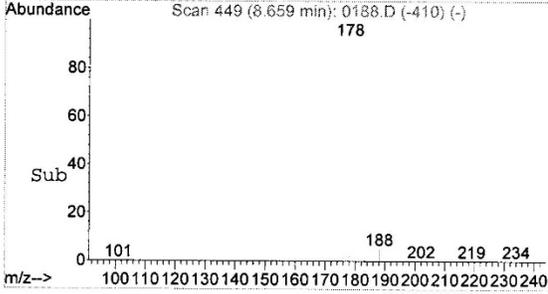
Appendix II. EI spectra for PAH target compounds



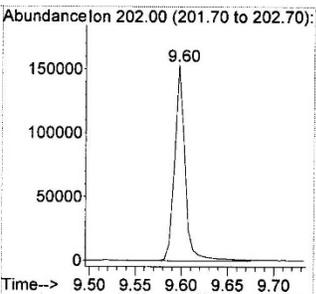
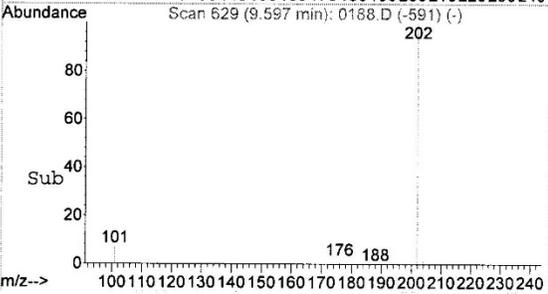


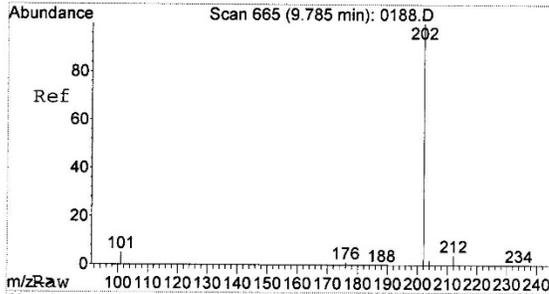


#7
 Anthracene
 Concen: 200.00
 RT: 8.66 min Scan# 449
 Delta R.T. 0.00 min
 Lab File: 0188.D
 Acq: 16 Jun 2009 18:07
 Tgt Ion:178 Resp: 137969

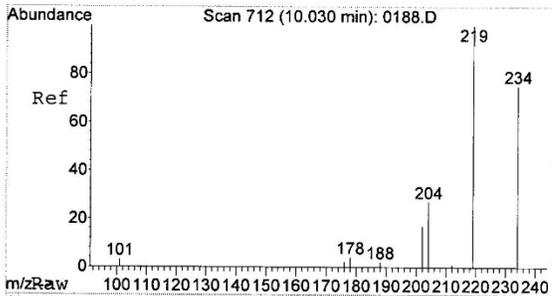
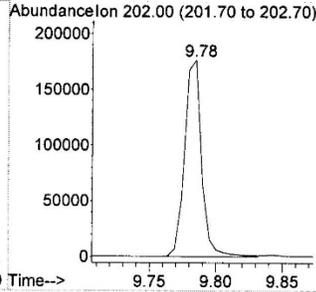
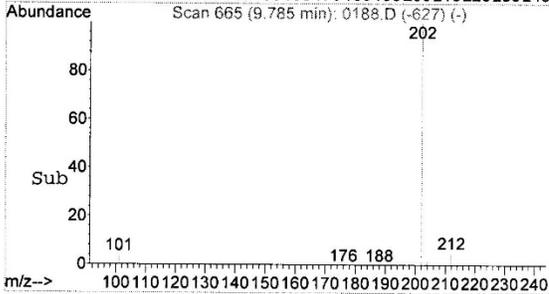


#8
 Fluoranthene
 Concen: 200.00
 RT: 9.60 min Scan# 629
 Delta R.T. 0.00 min
 Lab File: 0188.D
 Acq: 16 Jun 2009 18:07
 Tgt Ion:202 Resp: 121164

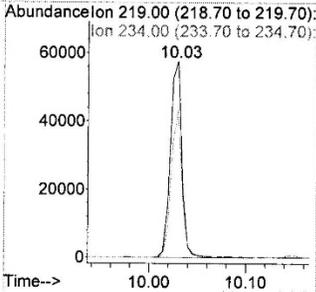
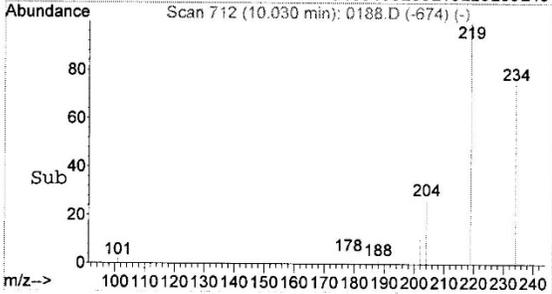


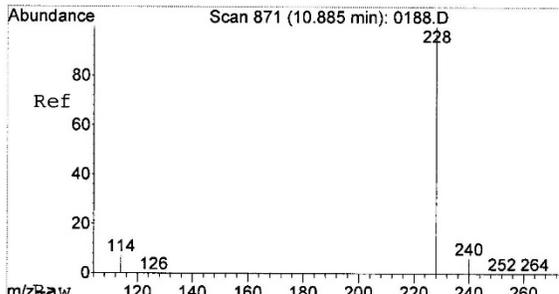


#9
 Pyrene
 Concen: 200.00
 RT: 9.78 min Scan# 665
 Delta R.T. 0.00 min
 Lab File: 0188.D
 Acq: 16 Jun 2009 18:07
 Tgt Ion:202 Resp: 155744

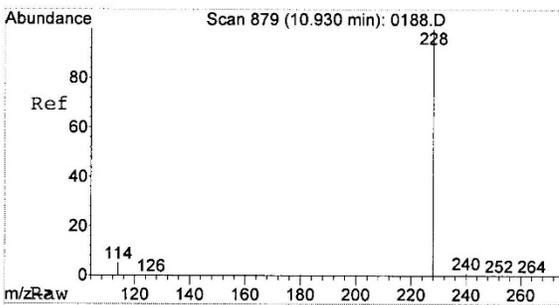
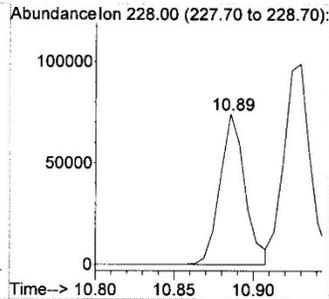
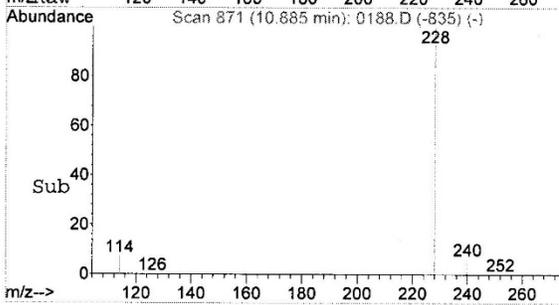


#11
 Retene
 Concen: 200.00
 RT: 10.03 min Scan# 712
 Delta R.T. 0.00 min
 Lab File: 0188.D
 Acq: 16 Jun 2009 18:07
 Tgt Ion:219 Resp: 47691
 Ion Ratio Lower Upper
 219 100
 234 70.9 35.4 106.3

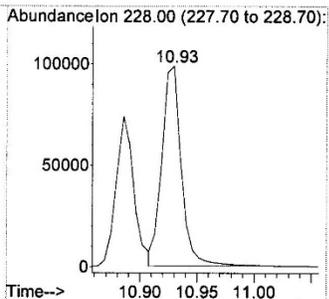
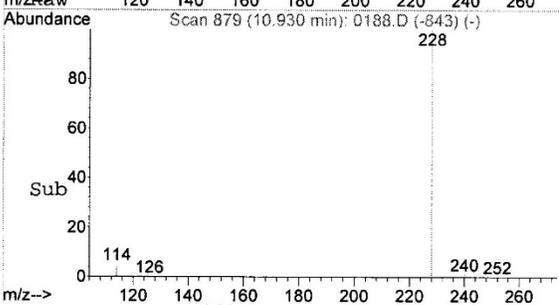


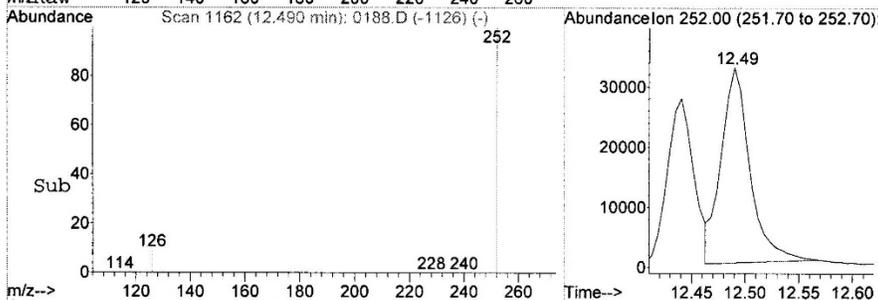
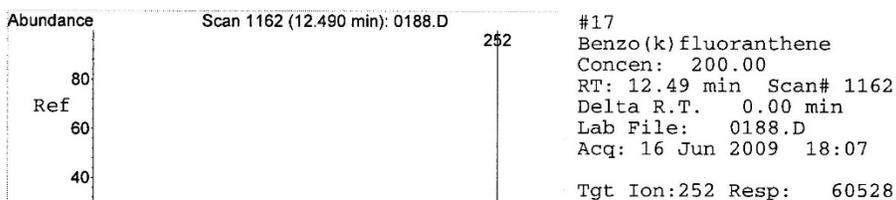
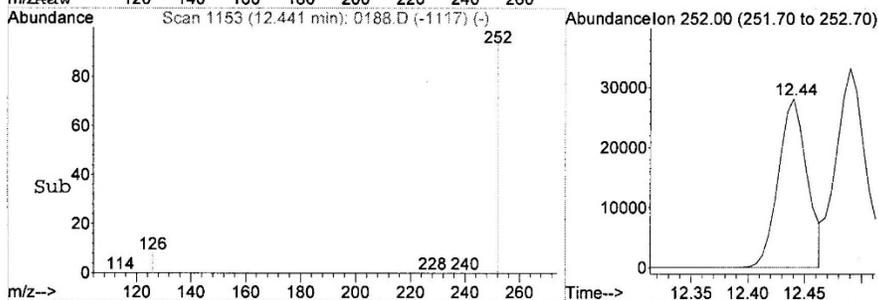
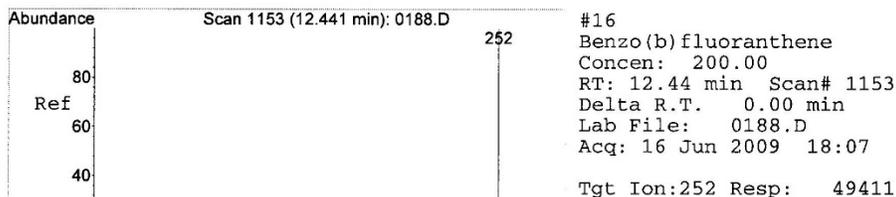


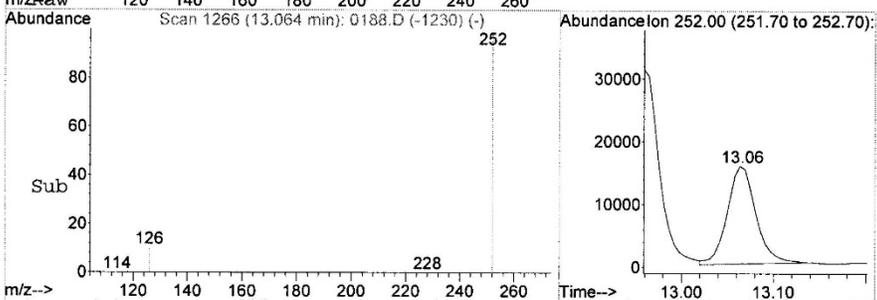
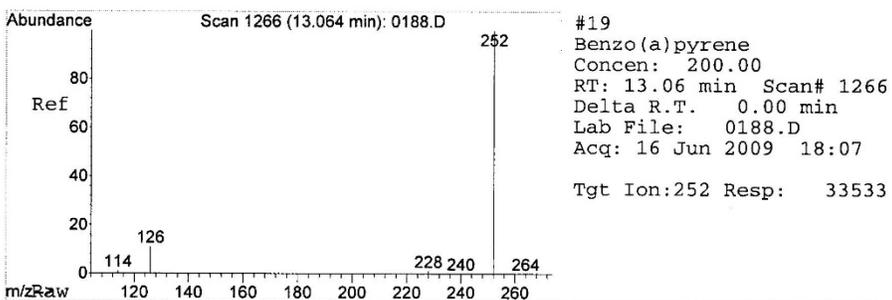
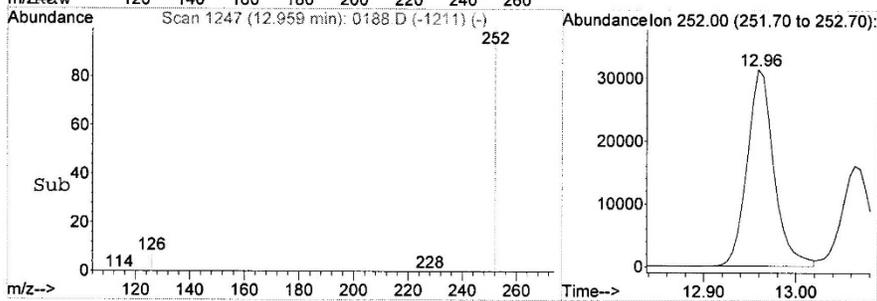
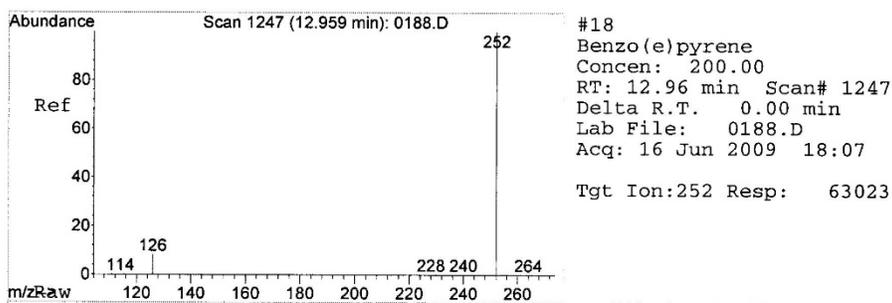
#13
Benz (a) anthracene
Concen: 200.00
RT: 10.89 min Scan# 871
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07
Tgt Ion:228 Resp: 81139

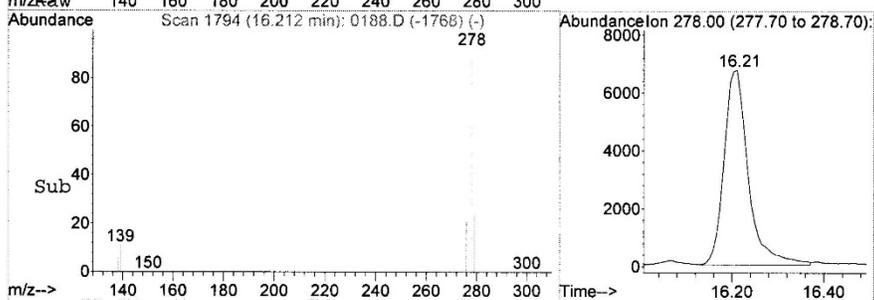
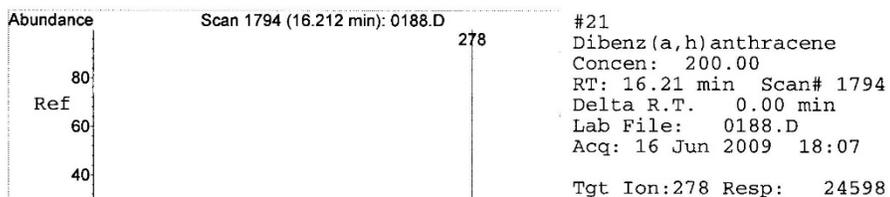
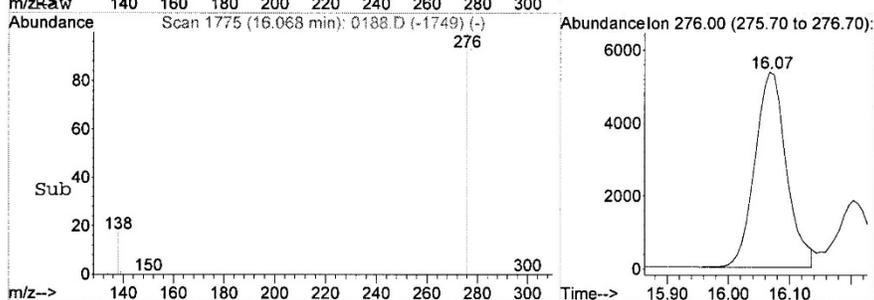
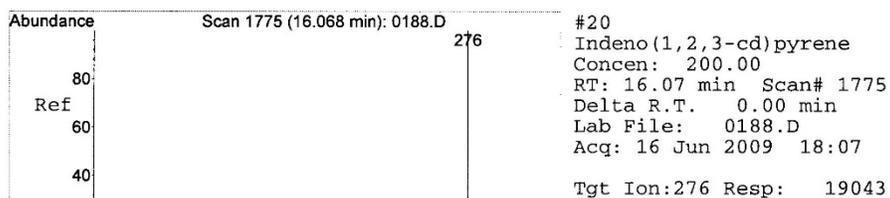


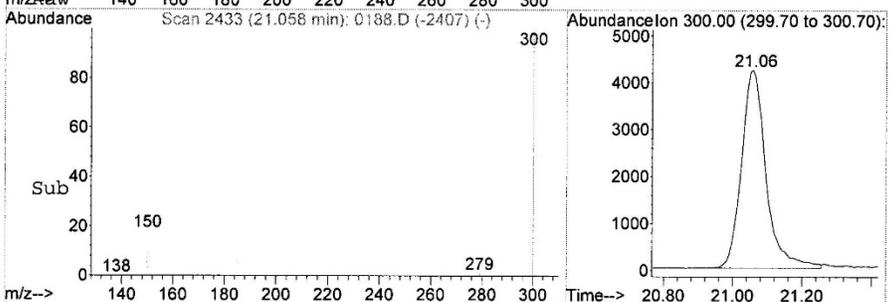
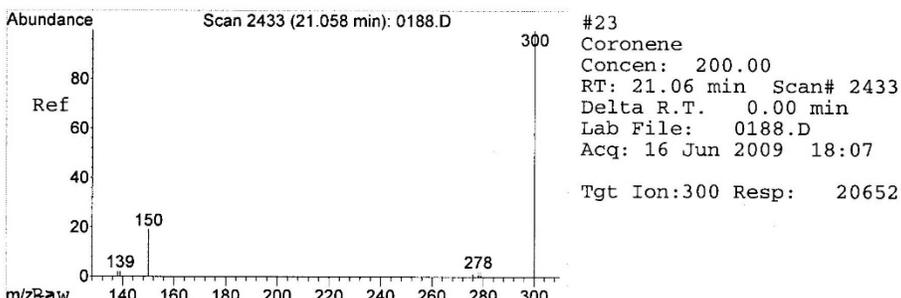
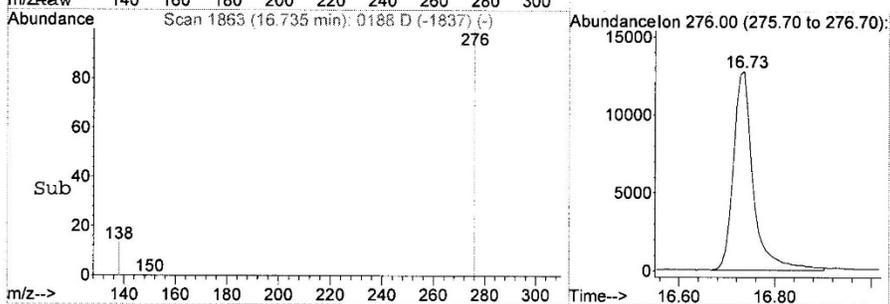
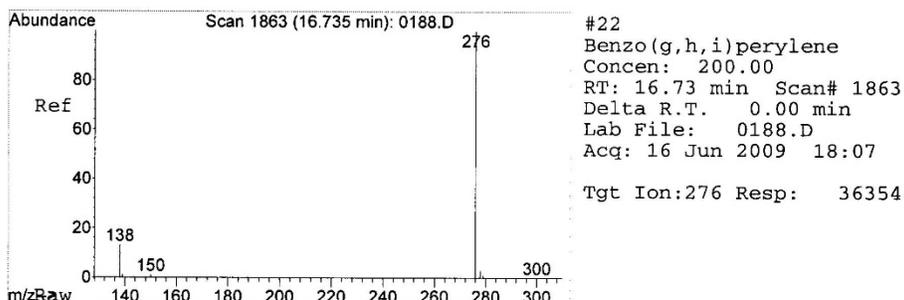
#14
Chrysene
Concen: 200.00
RT: 10.93 min Scan# 879
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07
Tgt Ion:228 Resp: 116990





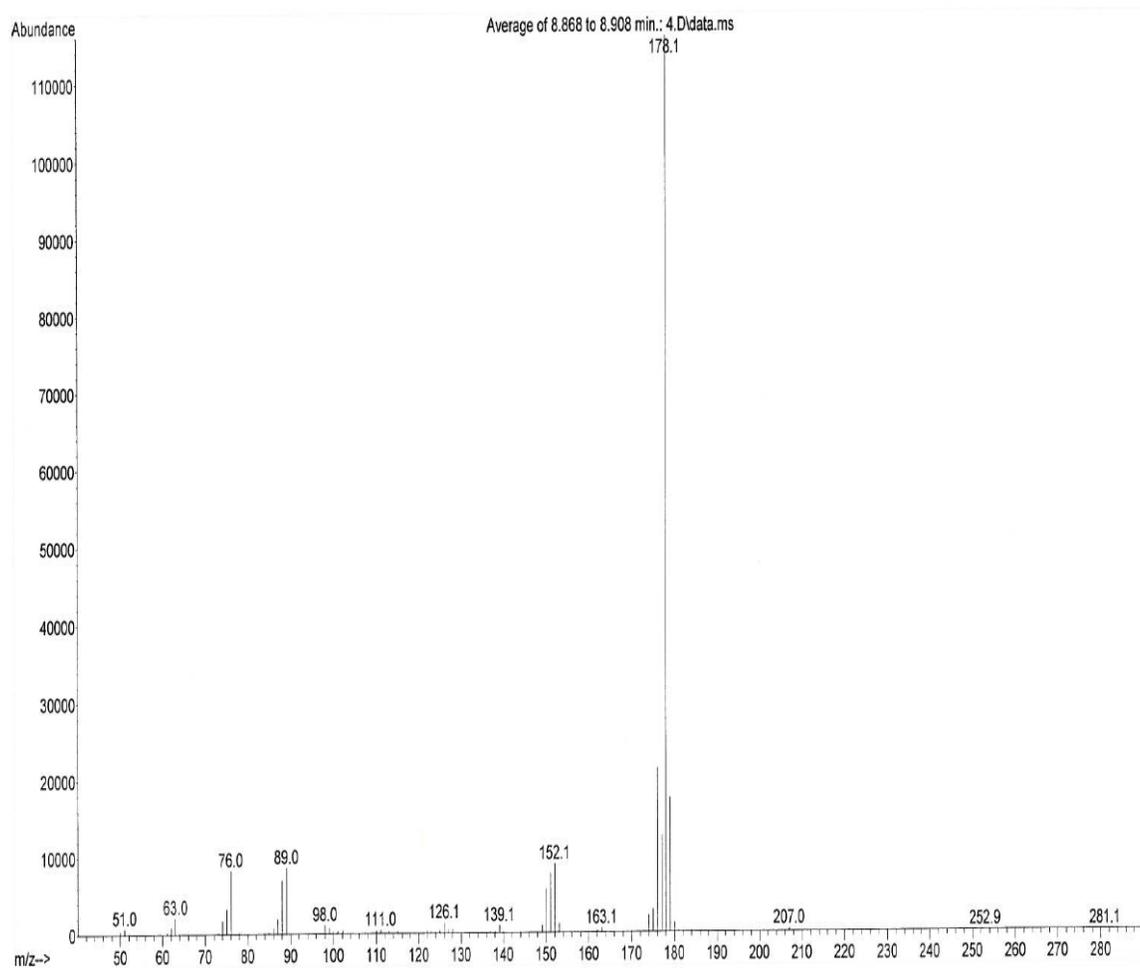




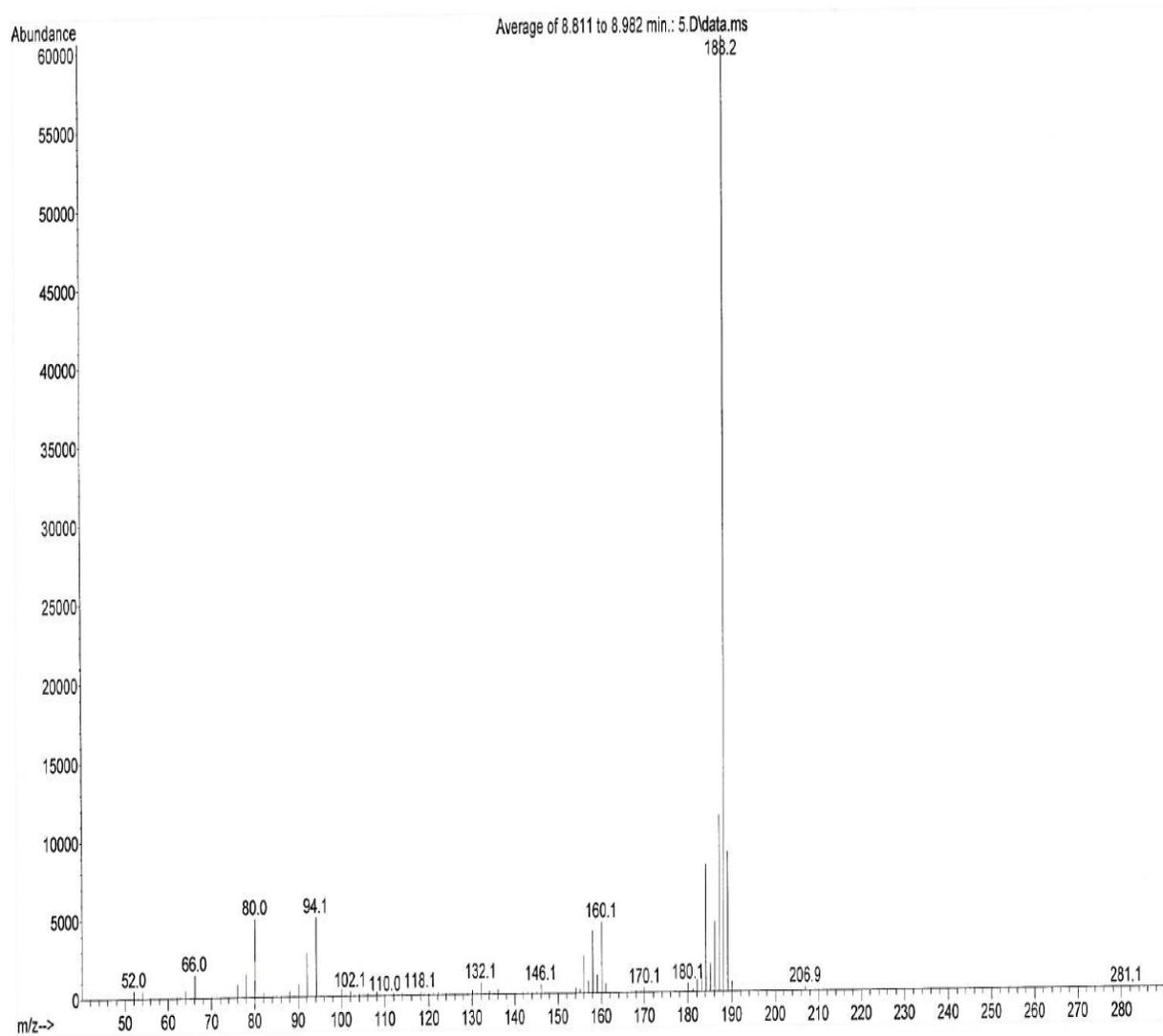


Appendix III. Highlighting deuterated & non-deuterated spectra of co-eluting compounds.

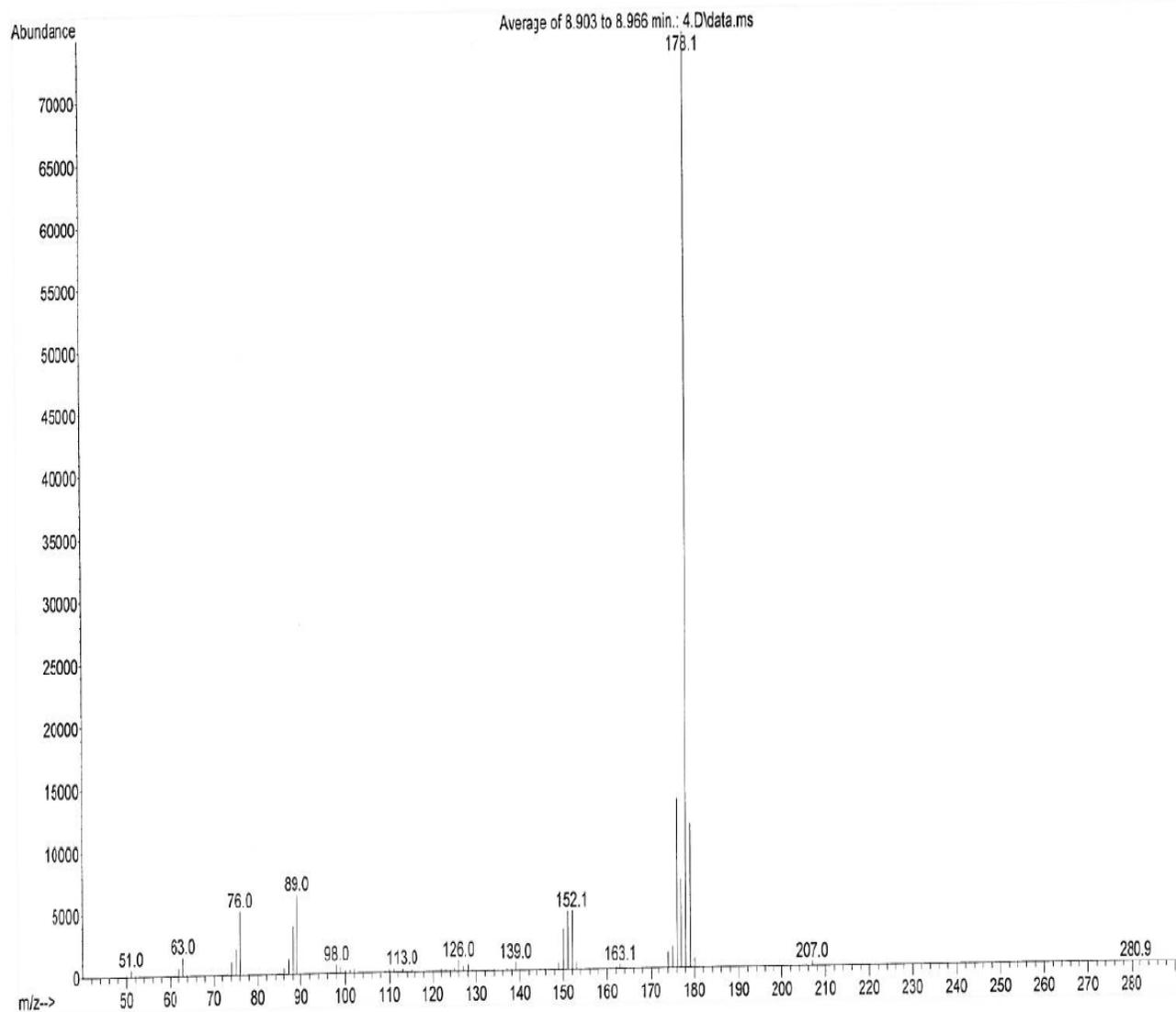
I. Phenanthrene



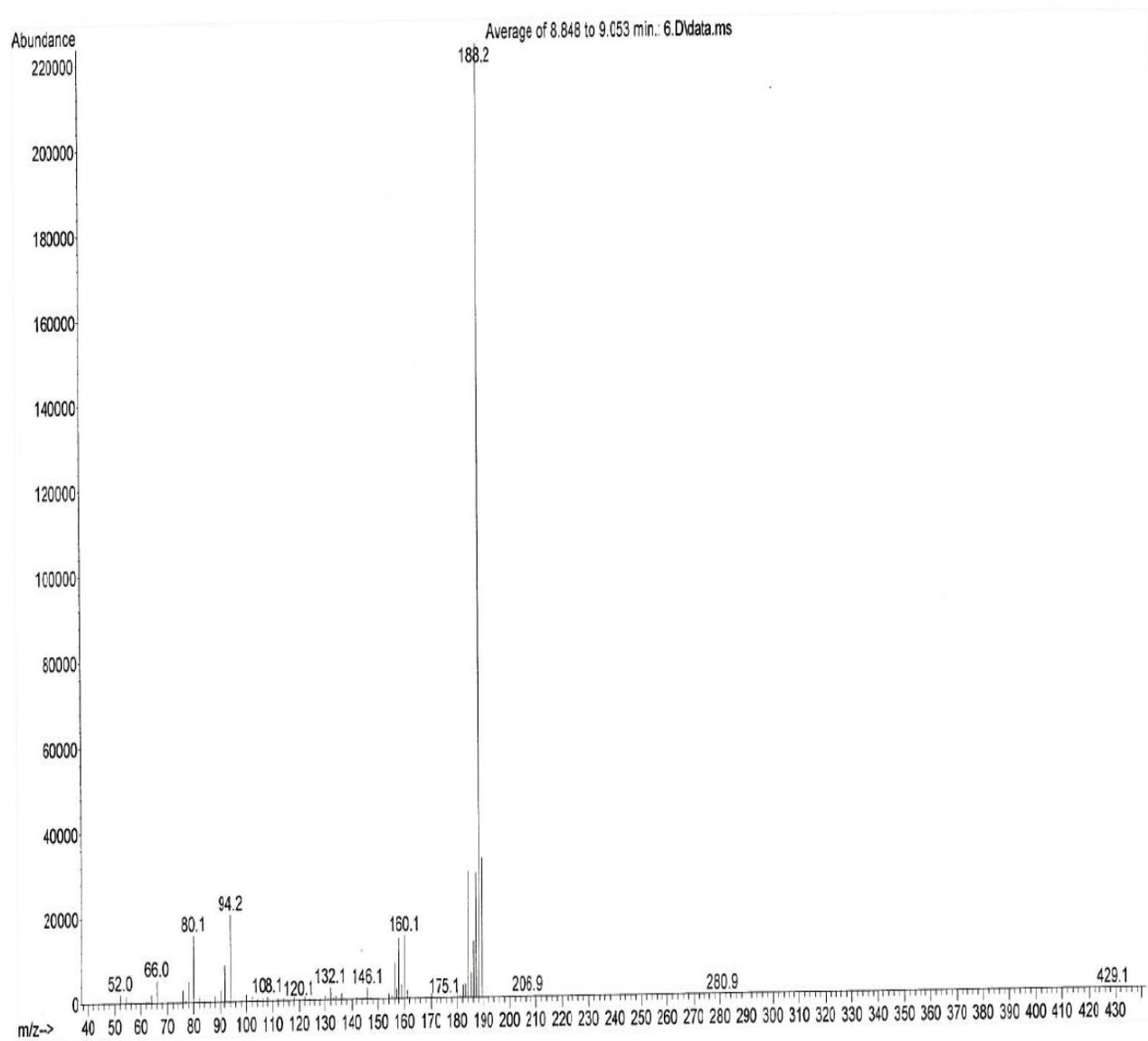
II. d₁₀-Phenanthrene



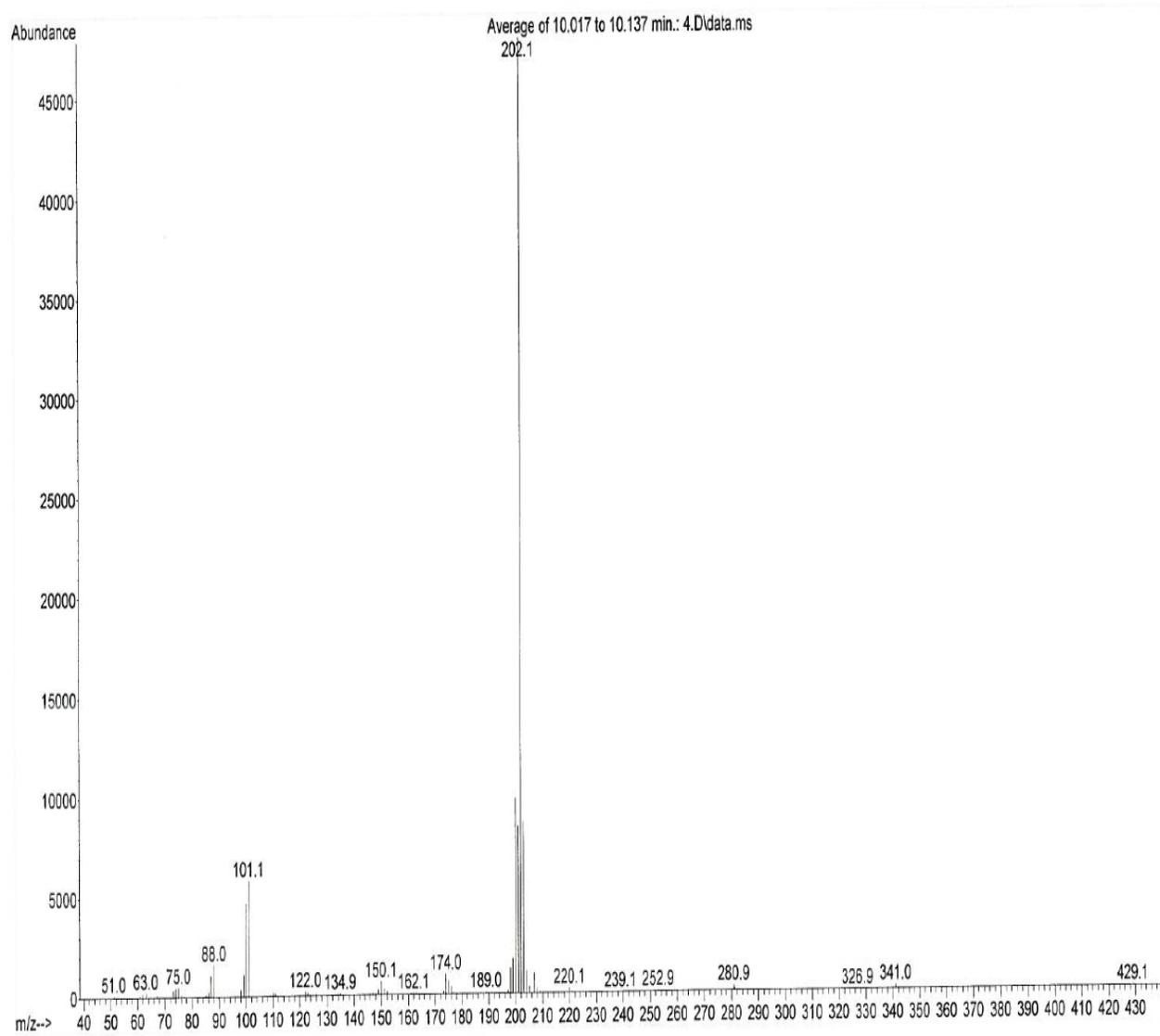
III. Anthracene



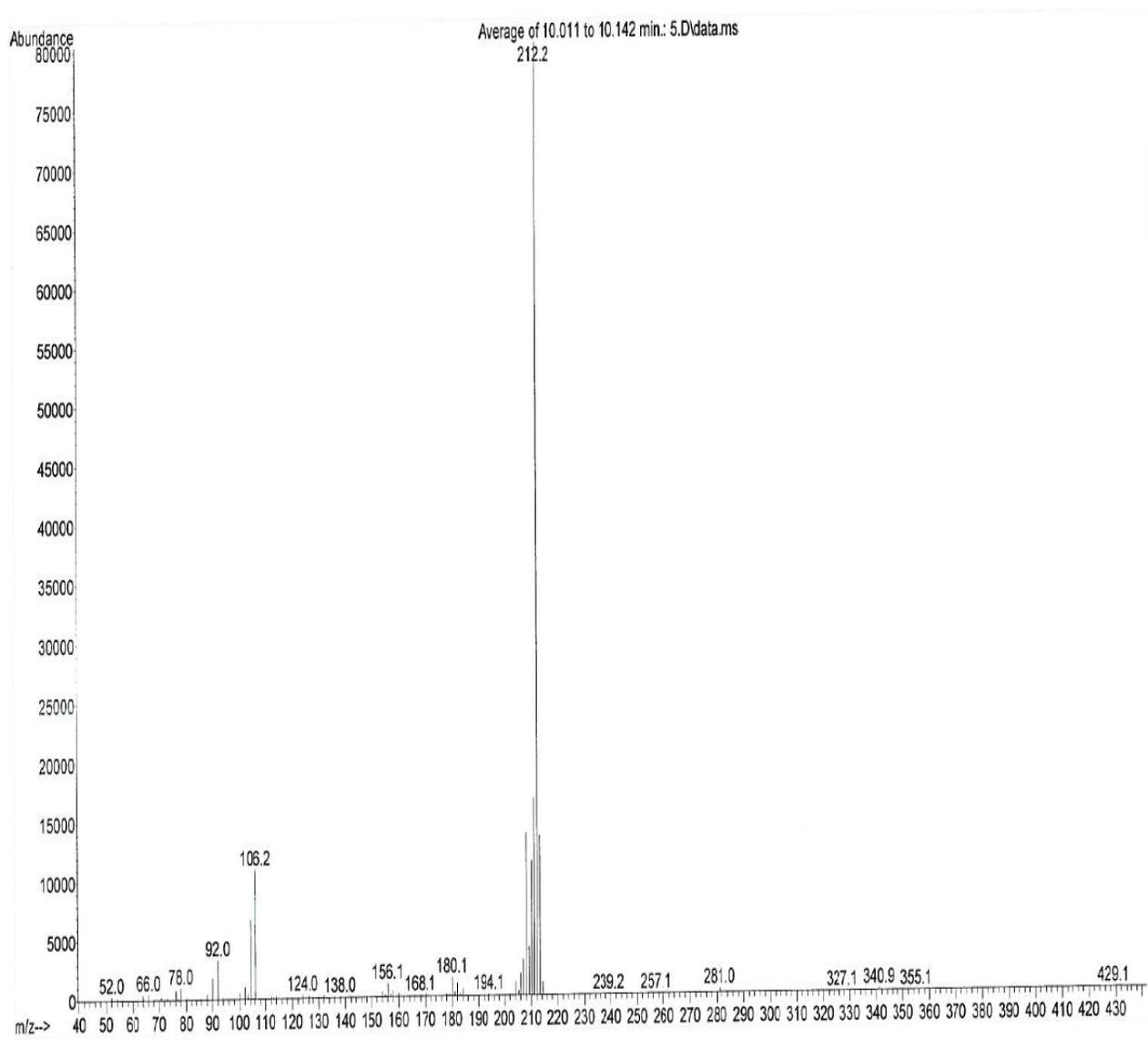
IV. d₁₀-Anthracene



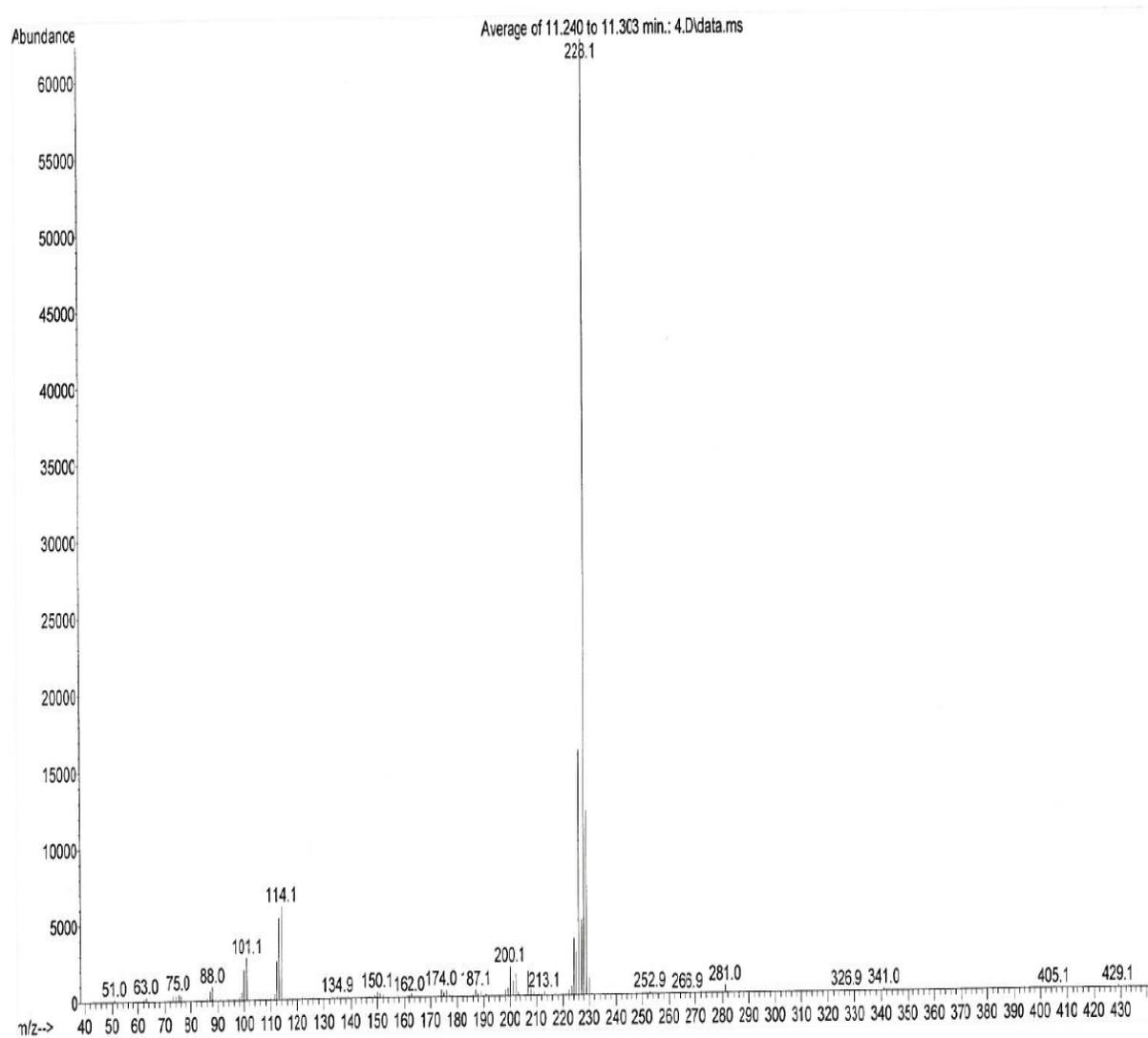
V. Pyrene



VI. d₁₀-Pyrene



VII. Benz(a)anthracene



VIII. d₁₂-Benz(a)anthracene

