

**Analysis of Polybrominated Diphenyl Ethers (PBDEs)  
and Other Flame Retardants  
In  
Air and Precipitation Samples**

Integrated Atmospheric Deposition Network Project

Prepared by

██████████ & ██████████

O'Neill School of Public and Environmental Affairs  
Indiana University  
Bloomington, IN 47405

Version 7.0 – January 2024

Table of Contents:

<b>Chapters</b>	<b>Page</b>
I. Introduction .....	2
II. Sample Preparation .....	2
III. Instrument Analysis .....	4
IV. Routine GC/MS Maintenance .....	6
V. Acquisition .....	15
VI. Quantitation .....	19
VII. Standards & Spiking .....	26

<b>Tables</b>	
Table 1 .....	4
Table 2 .....	5
Table 3 .....	14
Table 4 .....	26
Table 5 .....	27
Table 6 .....	27

<b>Figures</b>	
Figure 1 .....	3
Figure 2 .....	12
Figure 3 .....	17
Figure 4 .....	18
Figure 5 .....	24
Figure 6 .....	25

<b>Appendixes</b>	
Appendix I .....	28
Appendix II .....	35

## **I. INTRODUCTION**

This document outlines the instrumental analysis and quantitation of polybrominated diphenyl ethers (PBDEs) and other brominated flame retardants (BFRs) 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 Operating Protocol (SOP). A summary of those procedures is provided in Chart 1. Instrument analysis for BFRs is conducted on the first and second eluted fractions of all vapor, particle and precipitation samples.

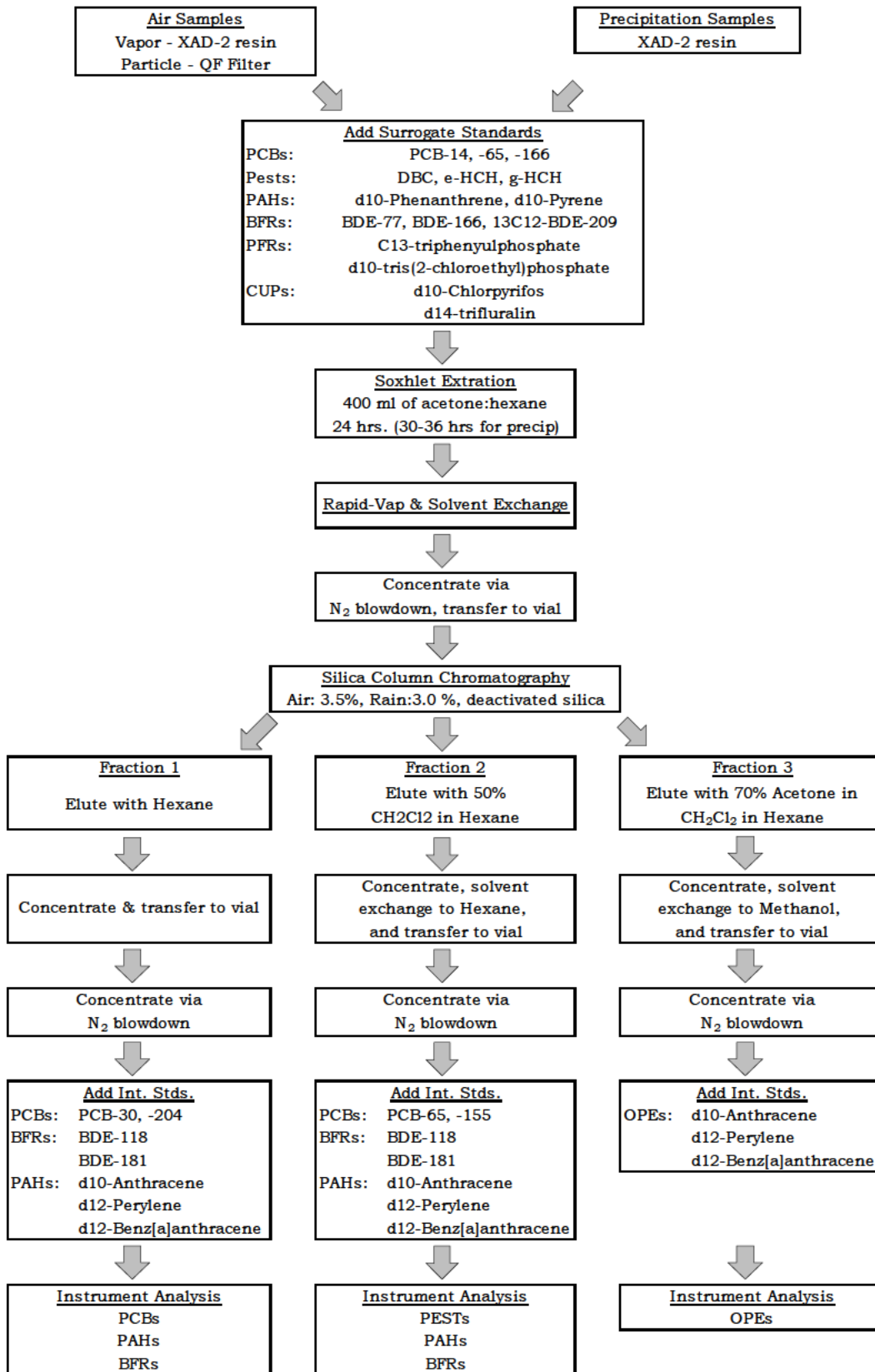


Figure 1. Flow chart summarizing IADN sample preparation.

### III. INSTRUMENT ANALYSIS

BFR analyses are performed on an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer (GC/MS) operated in electron capture negative ionization (ENCI) mode.

Agilent 7890A GC, serial # CN10271122

Agilent 5975C MS, serial # US10264402

Agilent GC Autosampler Controller, Model G4513A, serial # CN10240017

Chromatographic resolution is achieved with a Restek Rtx-1614 15 m, 0.25 mm ID, 0.1  $\mu\text{m}$  fused silica capillary column with helium as the carrier gas. Injections are analyzed by GC/MS using selected ion monitoring (SIM). Monitoring ions are divided into 5 groups as outlined in Table 1.

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

Group 1	Group 2	Group 3	Group 4	Group 5
79.0	79.0	70.0	486.8	79.0
81.0	81.0	81.0	488.8	81.0
159.8	406.7	486.8	494.6	
160.8	408.7	488.8	496.6	
357.0	461.7			
471.7	463.7			
	651.8			
	653.8			
	719.5			

Instrument control parameters and method details are provided in Appendix I (page 27)

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 I:

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

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 BDE-118 and BDE-181 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:

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

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 PBDE and BFRs analyzed in this study are listed 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 BFR analyte list with quantitation parameters.**

Compound	Elute Order	Type	Internal Standard	Quant Ion	Confirmation Ion I	Confirmation Ion II	Confirmation Ion III	SIM Group
BDE-15	1	Target	BDE-118	81	79			1
pTBX	2	Target	BDE-118	81	79			1
PBBZ	3	Target	BDE-118	81	79	471.7		1
BDE-17	4	Target	BDE-118	81	79	160.8		1
BDE-28	5	Target	BDE-118	81	79			1
PBEB	6	Target	BDE-118	81	79			1
HBB	7	Target	BDE-118	81	79	471.7		1
BDE-49	8	Target	BDE-118	81	79	160.8		1
BDE-47	9	Target	BDE-118	81	79	160.8		1
BDE-66	10	Target	BDE-118	81	79	160.8		1
BDE-77	11	Surrogate	BDE-118	81	79			1
BDE-100	12	Target	BDE-118	81	79			1
BDE-99	13	Target	BDE-118	81	79			1
EHTBB	14	Target	BDE-118	357	359	79	81	1
BDE-118	15	Internal		81	79			1
BDE-85	16	Target	BDE-118	81	79			1
BDE-154+BB-153	17	Target	BDE-118	81	79			1
BDE-153	18	Target	BDE-118	81	79			1
BDE-139	19	Target	BDE-118	81	79			1
BDE-140	20	Target	BDE-118	81	79			1
A-HBCD	21	Target	BDE-118	159.8	81	79		1
BDE-166	22	Surrogate	BDE-118	81	79			1
BDE-183	23	Target	BDE-118	81	79			1
TBE	24	Target	BDE-181	81	79			1
BDE-181	25	Internal		81	79			1
BEHTBP	26	Target	BDE-181	463.7	461.7	81	79	2
syn-DP	27	Target	BDE-181	653.8	651.8			2
BDE-201	28	Target	BDE-181	408.7	406.7			2
anti-DP	29	Target	BDE-181	653.8	651.8			2
BDE-197	30	Target	BDE-181	408.7	406.7			2
BDE-203	31	Target	BDE-181	81	79			2
BDE-208	32	Target	BDE-181	486.8	488.8	81	79	3
BDE-207	33	Target	BDE-181	486.8	488.8	81	79	3
BDE-206	34	Target	BDE-181	81	79	486.8	488.8	3
BDE-209	35	Target	BDE-181	486.8	488.8			4
<sup>13</sup> C <sub>12</sub> -BDE-209	36	Surrogate	BDE-181	494.6	496.6			4
DBDPE	37	Target	BDE-181	81	79			5

## IV. ROUTINE GC/MS MAINTENANCE

### 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.

### 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.

*Note on liners: The 7890A GC is equipped with two injection ports for two different types of injections. Split/splitless injections (1.0 – 2.0 µL) are carried out on the back injection port and require a double taper gooseneck liner. The multi-mode inlet (for large volume injections) is located on the front injection port, and requires a helical liner so that there is no “line of site” from top to bottom of liner.*

### 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.

### 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.

*Note: Gold seals are required for the rear injector port for split/splitless injections, but not for the front multi-mode inlet.*

## 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.

## 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.

### 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 Mass Hunter 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 **MS Vacuum Control...**
- d. In the next pop-up window, select **Vent**
- e. When prompted (in approximately 40 minutes) turn off the MS
- f. 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*
- g. 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)
- h. Unplug the cables (side board control cable and the source power cable) that are connected to the vacuum manifold
- i. Open the door of the vacuum manifold

### In the GC oven

- j. Hang the column in the center of the GC oven to insure the column is uniformly heated during operation and remove rubber seals from either end of column
- k. Loosen one end of the column (free ~1 foot) so that it will be easy to work with
- l. 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)
- m. Remove ~1 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.*
- n. Adjust the septum and column so that...



- i. Split/splitless inlet: 4 to 6 mm of the column is showing beyond the upper point of the ferrule.
  - ii. Multi-mode inlet: 12-14 mm of the column is showing beyond the upper point of the ferrule.
- o. Holding the column at a point just below the septum...
  - i. Split/splitless inlet: ... 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
  - ii. Multi-mode inlet: ... carefully inset the injector end of the column into the lower end of the MMI. Two small wrenches will likely be required to secure the column inlet nut to the MMI receiving nut. The MMI receiving nut may “wobble” a bit – it is designed to do this because it must deal with dramatic and rapid temperature changes of the MMI.
- p. Start carrier gas flow.
- q. 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
- r. 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.
- s. 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
- t. Following column conditioning, return GC oven temperature to 30°C
- u. Loosen the MS end of the column (free ~1 foot) so that it will be easy to work with
- v. 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)
- w. Remove ~1 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.*
- x. 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.
- y. Close the vacuum manifold and make sure the vent valve is closed
- z. Re-attach the side board control cable and source power cable to the door of the MS vacuum manifold
- aa. 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.
- bb. In **Mass Hunter**, 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). The software will prompt you to set MS temperatures specified in the loaded autotune file. Click **OK**.
- cc. Reinstall the MSD and vent valve covers. Make sure the temperatures increase toward set points, and that the turbo pump speed advances to 100%.
  - dd. Prepare for autotuning by loading the PCI tune file. Once loaded, allow that instrument to reach thermal equilibrium by letting it run for 4 hours before initiating autotune
  - ee. Auto tune (detailed later)
  - ff. Check for air/water
  - gg. Correct SIM windows if necessary

#### CLIPPING THE COLUMN

- hh. Reduce GC oven and injection port temperatures to 30°C using keypad on the front of the GC
- ii. With a ceramic cutter, cut the inlet end of the column just below the inlet column nut
- jj. Remove the old inlet column nut/ferrule/column by unscrewing it from the lower portion of the inlet assembly
- kk. 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, shorted lengths are acceptable to remove.
- ll. 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)
- mm. Adjust the septum and column so that...
  - i. Split/splitless inlet: 4 to 6 mm of the column is showing beyond the upper point of the ferrule.
  - ii. Multi-mode inlet: 12-14 mm of the column is showing beyond the upper point of the ferrule.
- nn. Holding the column at a point just below the septum...
  - i. Split/splitless inlet: ... 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
  - ii. Multi-mode inlet: ... carefully inset the injector end of the column into the lower end of the MMI. Two small wrenches will likely be required to secure the column inlet nut two the MMI receiving nut. The MMI receiving nut may “wobble” a bit – it is designed to do this because it must deal with dramatic and rapid temperature changes of the MMI.
- oo. 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
- pp. Return GC temperatures to normal operating conditions using keypad

- qq. 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
- rr. Check for air/water
- ss. Correct SIM windows if necessary

### **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

#### **B. Removing the Ion Source**

- a. In the Mass Hunter 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 **MS Vacuum Control...**
- c. In the next pop-up window, 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

- i. 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.
- j. Place ion source on clean piece of aluminum foil. Close vacuum manifold door.

### C. Ion Source Disassembly

- a. Remove screws holding filament & filament dummy in place. Set screws & filament pieces aside.
- b. With screws removed, the CI source heater assembly can be detached and set aside
- c. With CI source heater assembly removed the repeller insulator can be set aside, and repeller removed to be cleaned
- d. Remove small set screw from the side of the CI source body. Set aside. Don't lose.
- e. Slide out CI lens insulator/entrance lens/ion focus lens. Disassemble. Set entrance lens and ion focus lens with items to be cleaned. Set insulator aside.
- f. Remove draw out cylinder and draw out plate from CI source body. Add all three to items to be cleaned.
- g. 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. 6 pieces that should be cleaned (As shown L-R)
  - i. Repeller
  - ii. Draw out plate
  - iii. Source body
  - iv. Ion focus lens
  - v. Entrance lens assembly
  - vi. Draw out cylinder



**Figure 2. CI 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. Methanol
    - 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. Align source body with repeller, repeller insulator, source heater assembly, and filament components. Secure all together using the screws that pass through filament components. *A new filament may be needed. This would be the time to replace the old one with a new one.*
- F. Re-install the Ion Source
- a. Secure the reassembled CI ion source within the MSD using the two thumb screws
  - b. Reattach all wires
  - c. Verify interface tip seal is in place on end of transfer line
  - d. Close the vacuum manifold and make sure the vent valve is closed
  - e. Re-attach the side board control cable and source power cable to the door of the MS vacuum manifold
  - f. 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.
  - g. In **Mass Hunter**, 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). The software will prompt you to set MS temperatures specified in the loaded autotune file. Click **OK**.
  - h. Reinstall the MSD and vent valve covers. Make sure the temperatures increase toward set points, and that the turbo pump speed advances to 100%.
  - i. Prepare for autotuning by loading the PCI tune file. Once loaded, allow that instrument to reach thermal equilibrium by letting it run for 4 hours before initiating autotune

- j. Auto tune (detailed later)
- k. Check for air/water
- l. Correct SIM windows if necessary

## MS Autotuning

- a. In the Mass Hunter software that operates the GC/MS, under **View** select **Tune and Vacuum Control** from the drop down list
- b. In the new interface, under **File** select **Load Tune Parameters**, load PCI tune file **pcich4.u**
- c. You will be prompted to set temperatures for the source & quad (source: 250°C, quad: 150°C). Click **Apply**, then **OK**
- d. Allow instrument to equilibrate for 4 hrs.
- e. Under **Setup**, select **Methane Flow Setup**. This will take you through various steps of the pre-tune
- f. Once the pre-tune is complete, under **Tune** select **CI Autotune**. This will take you through the various steps of the autotune
- g. 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.
- h. Under **File** select **Save Tune Parameters**, select pcich4.u. Click **OK**
- i. Under **File** select **Load Tune Parameters**, load NCI tune file **ncich4.u**
- j. You will be prompted to set temperatures for the source & quad (source: 200°C, quad: 140°C). Click **Apply**, then **OK**
- k. Allow instrument to equilibrate for 4 hrs.
- l. Under **Tune** select **CI Autotune**. This will take you through the various steps of the autotune.
- m. 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.
- n. Under **File** select **Save Tune Parameters**, select ncich4.u. Click **OK**
- o. Under **View** select **Instrument Control** to return to the primary Mass Hunter 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. In Mass Hunter, acquisition methods can be loaded under **File** select **Load Method**
    - ii. To alter MS parameters of the acquisition method -- under **Instrument** select **MS Edit 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 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 Mass Hunter, to access the sequence table under **Sequence** select **Edit Sequence**. This will open sequence table window
    - ii. In the newly opened table you will be able to construct the sample run.
    - iii. To increase the number of rows for this table select *New Samples* in the upper left corner and specify the desired number of rows

iv. Columns specify

1. Sequence order
2. Sample name
3. Vial location
4. Operator's comments
5. Method file path
6. Method file name – this is the acquisition method described earlier
7. Acquired data path – this is where your data for the run will be stored
8. Date file ID – this has to be unique for every sample in the run
9. Sample type
  - a. Sample
  - b. Blank
  - c. Calibration
  - d. QC
10. Calibration level
11. Dilution

v. The sequence table should include the following

1. Hexane blanks
2. Calibration standards
3. QC samples (common reference standards)
4. Your samples

vi. The sequence table can have NO empty rows – it will give you an error

c. Saving sequence

- i. Once desired adjustments to the sample table are made, click the X in the upper right hand corner to return view to primary Instrument Control window.
- ii. Under **Sequence** select **Save Sequence As** which will prompt you to specify sequence name and file location.
- iii. If the sequence was saved earlier and new corrections or adjustments were made, **Save Sequence** can be selected to save the updates instead of having a completely new sequence file.

d. Running sequence

- i. Under **Sequence** select **Run Sequence** which will open a new window.
- ii. In the lower left hand corner of this new window click **Run**



e. Tips & Tricks

- i. Rather than creating a new sequence table from scratch every time, load an old sequence and simply update the sample IDs, acquired data path, vial IDs, etc.
- ii. If there is a piece of data the needs to be repeated in many rows of a single column, by *right clicking* and selecting **fill down** it will copy that same info down throughout the column.
- iii. If there is a piece of data that needs to be incrementally changed in many rows of a single column, by *right clicking* and selecting **fill down and increment** it will increment values down the column. This is most useful in the *data file* and *vial location* columns.

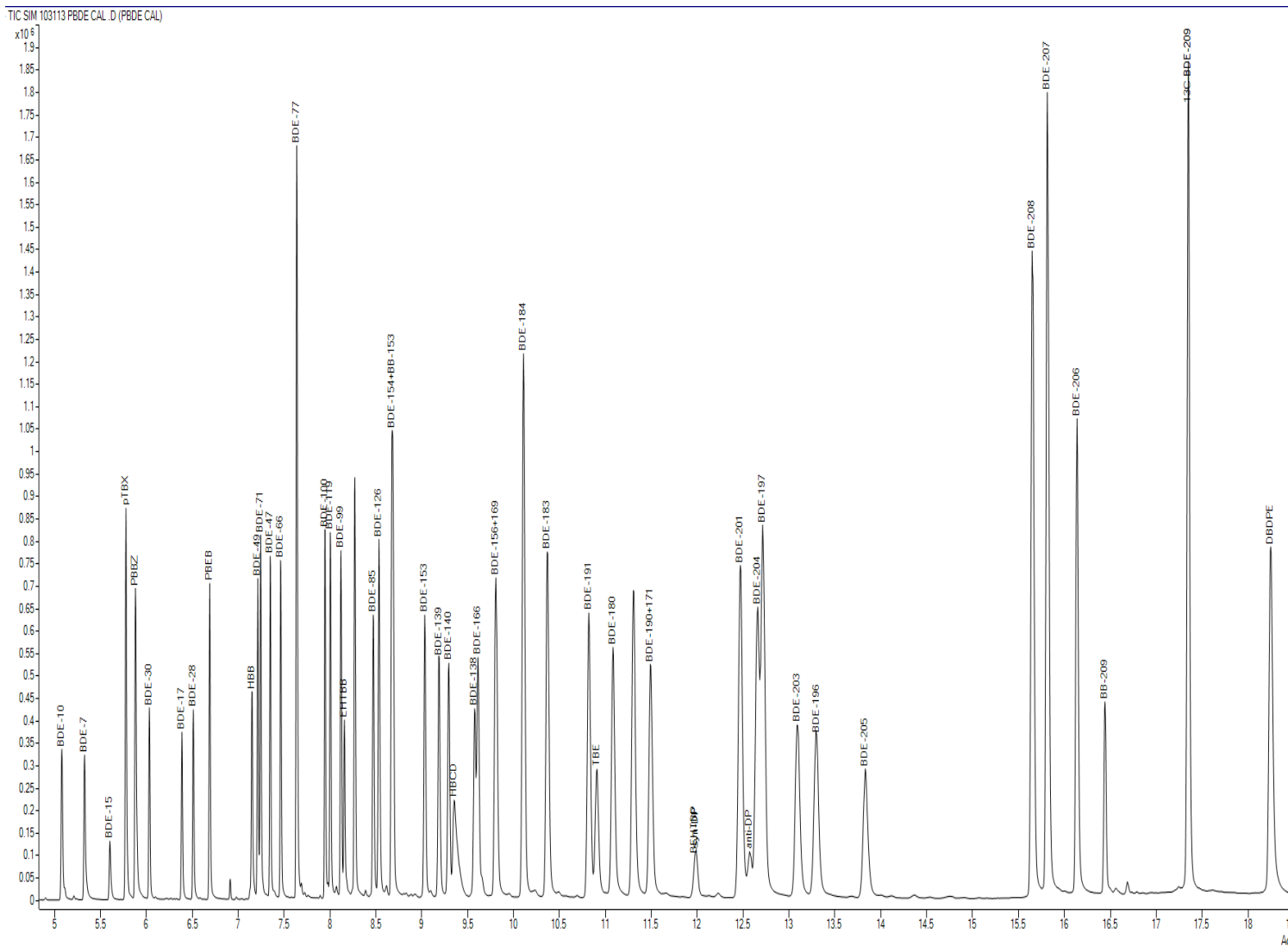


Figure 3. Example of a BFR calibration standard chromatogram.

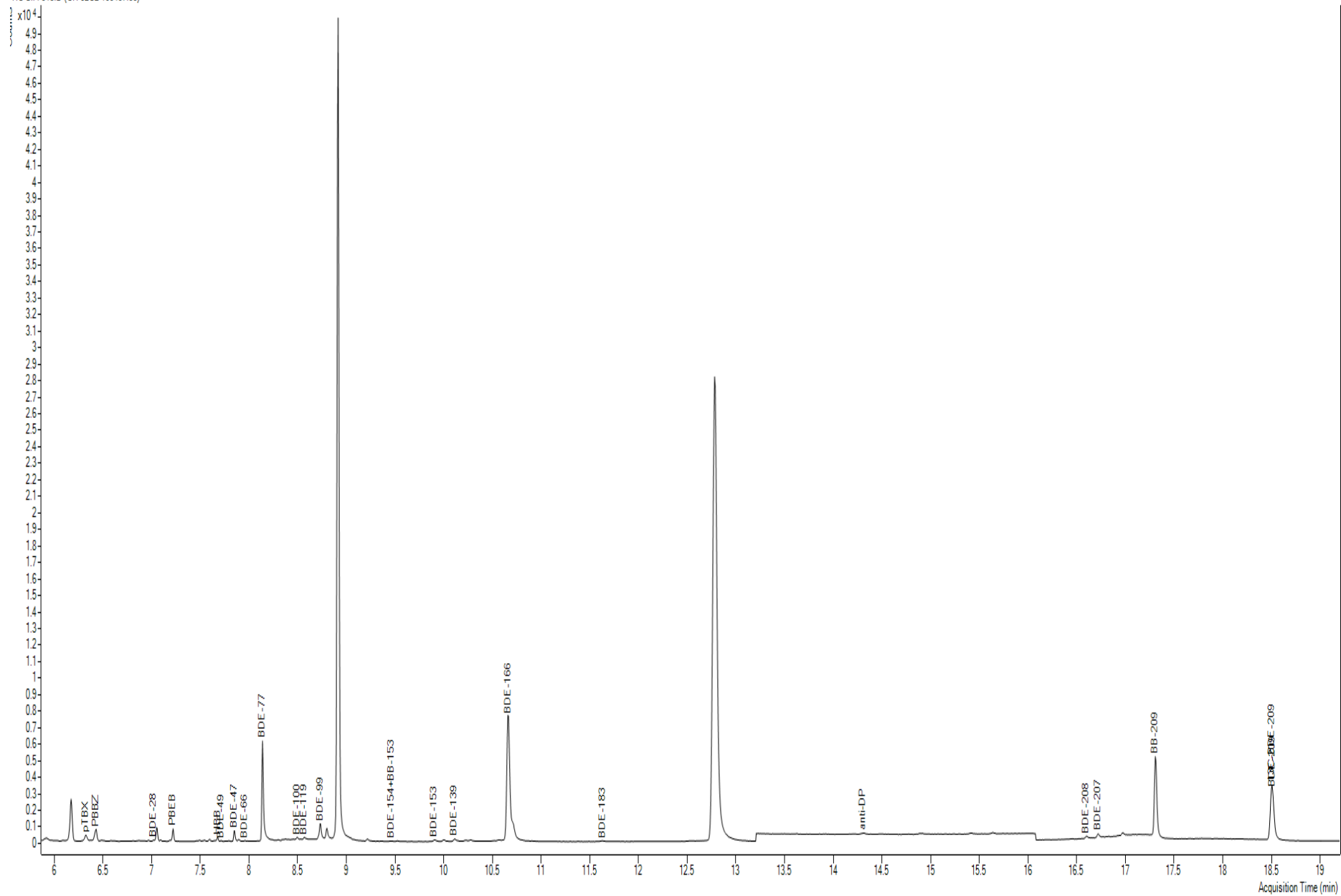


Figure 4. Example of a sample chromatogram.

## 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 be 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, page 70 for chromatographic problems and how to resolve them. In some cases the sample will need to be rerun and/or recleaned. If these actions down help then the compound needs to be deleted with a corresponding not 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. For example, BDE-209 & <sup>13</sup>C<sub>12</sub>-BDE-209 will elute simultaneously, so when inspecting the ion spectra for one of these compounds, the quant/qual ions used for the other peak may be at a higher abundance than those for the present target compound. This scenario is most likely to occur at low concentrations of the parent compound. The peaks for BEHTBP & syn-DP overlap, so this scenario also pertains to those two compounds.*

- 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

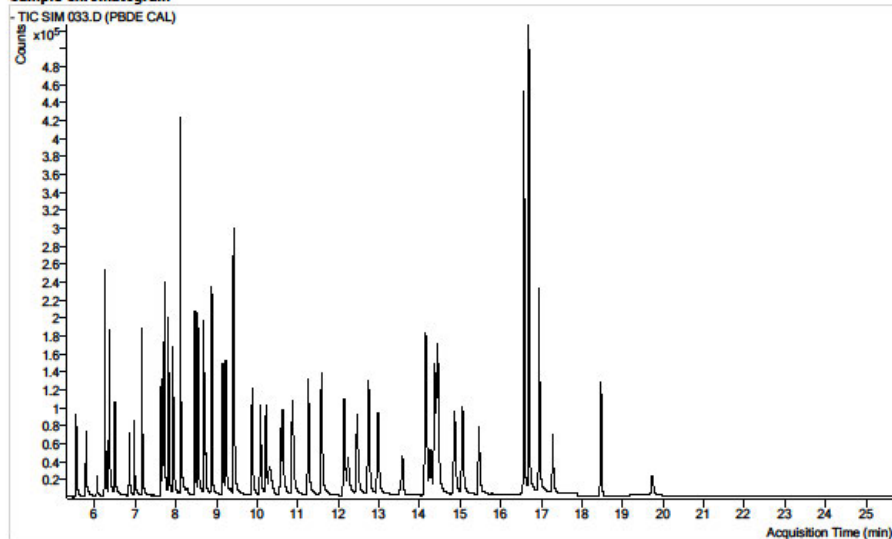
cc. Entering data into raw entry spreadsheet

- 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

### Quantitative Analysis Report

Batch Path	G:\Labs\Hites\RDaniel\PBDE Stuff\ADN PBDEs\2016\180320_MY16\QuantResults\180320.batch.bin		
Quantitation Time Stamp	4/6/2018 3:40:56 PM	Acquisition Time Stamp	3/23/2018 7:01:00 AM
Quantitation Method	Quant_BFRs.m	Acquisition Method	DCL_IADN_BFRs_1ul
Analyst Name	DCL	Acquisition Operator	DCL
Quant Software Version	B.09.00	Instrument Name	CN10271122
Sample Name	PBDE CAL	Comment	
Sample Type	Cal	Data File	033.D
Vial	12	Vol.	1

#### Sample Chromatogram



Name	RT	Target Ion	Target Response	Final Conc.	Units
BDE-10	5.559	81.0	73399	2.0000	ng/ml
BDE-7	5.805	81.0	52186	2.0000	ng/ml
BDE-15	6.074	81.0	15177	2.0000	ng/ml
pTBX	6.267	81.0	171650	2.5000	ng/ml
PBBZ	6.372	81.0	143769	2.5000	ng/ml
BDE-30	6.512	81.0	78660	2.0000	ng/ml
BDE-17	6.869	81.0	49838	1.9200	ng/ml
BDE-28	6.986	81.0	52540	2.0000	ng/ml
PBBB	7.185	81.0	114763	2.0000	ng/ml
HBB	7.653	81.0	79756	2.0000	ng/ml
BDE-49	7.699	81.0	100013	4.0000	ng/ml
BDE-71	7.734	81.0	147765	4.0000	ng/ml
BDE-47	7.834	81.0	125531	4.0000	ng/ml
BDE-66	7.945	81.0	110145	4.0000	ng/ml
BDE-77	8.126	81.0	252420	7.0000	ng/ml
BDE-100	8.484	81.0	129746	4.0000	ng/ml
BDE-119	8.555	81.0	141388	4.0000	ng/ml
BDE-99	8.703	81.0	127065	4.0000	ng/ml
EHTBB	8.741	357.0	5892	5.0000	ng/ml
BDE-85	9.167	81.0	112900	4.0000	ng/ml

### Quantitative Analysis Report

Name	RT	Target Ion	Target Response	Final Conc.	Units
BDE-126	9.243	81.0	145852	4.0000	ng/ml
BDE-154+BB-153	9.442	81.0	331104	8.0000	ng/ml
BDE-153	9.898	81.0	126113	4.0000	ng/ml
BDE-139	10.102	81.0	112699	4.0000	ng/ml
BDE-140	10.237	81.0	111425	4.0000	ng/ml
HBCD	10.325	81.0	87952	10.0000	ng/ml
BDE-138	10.600	81.0	76926	4.0000	ng/ml
BDE-166	10.646	81.0	110137	5.0000	ng/ml
BDE-156+169	10.892	81.0	190287	8.0000	ng/ml
BDE-184	11.278	81.0	165261	8.0000	ng/ml
BDE-183	11.605	81.0	193795	8.0000	ng/ml
BDE-191	12.167	81.0	158414	8.0000	ng/ml
TBE	12.255	81.0	85023	4.0000	ng/ml
BDE-180	12.483	81.0	159954	8.0000	ng/ml
BDE-190+171	12.997	81.0	158703	8.0000	ng/ml
BEHTBP	13.524	81.0	2754	5.0000	ng/ml
syn-DP	13.598	653.8	63213	5.0000	ng/ml
BDE-201	14.172	719.5	52741	8.0000	ng/ml
anti-DP	14.288	651.8	68717	5.0000	ng/ml
BDE-204	14.388	486.8	123554	8.0000	ng/ml
BDE-197	14.462	408.9	211404	8.0000	ng/ml
BDE-203	14.886	561.7	20852	8.0000	ng/ml
BDE-196	15.086	561.7	17494	8.0000	ng/ml
BDE-205	15.484	81.0	96950	8.0000	ng/ml
BDE-208	16.591	486.8	237064	20.0000	ng/ml
BDE-207	16.707	486.8	236890	20.0000	ng/ml
BDE-206	16.965	486.8	39386	20.0000	ng/ml
BDE-209	18.491	486.8	123950	20.0000	ng/ml
13C-BDE-209	18.485	494.6	25120	4.0000	ng/ml
DBDPE	19.750	81.0	41366	40.0000	ng/ml

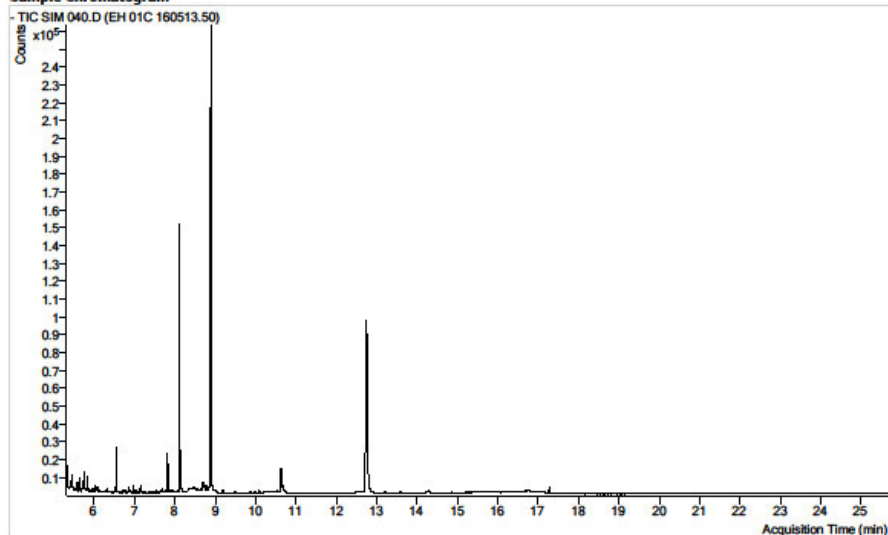
Figure 5. Example of quantitation report for BFR calibration standard.



### Quantitative Analysis Report

Batch Path	G:\Labs\HilleR\Danie\PBDE Stum\ADN PBDEs\2016\180320_MY16C\QuantResults\180320_batch.bin		
Quantitation Time Stamp	4/6/2018 3:40:56 PM	Acquisition Time Stamp	3/23/2018 10:28:07 AM
Quantitation Method	Quant_BFRs.m	Acquisition Method	DCL_ADN_BFRs
Analyst Name	DCL	Acquisition Operator	DCL
Quant Software Version	B.09.00	Instrument Name	CN10271122
Sample Name	EH 01C 160513.50	Comment	MY16C
Sample Type	Sample	Data File	040.D
Vial	84	Vol.	2

#### Sample Chromatogram



Name	RT	Target Ion	Target Response	Final Conc.	Units
BDE-10	5.582	81.0	0	0.0000	ng/ml
BDE-7	5.770	81.0	0	0.0000	ng/ml
BDE-15	6.097	81.0	0	0.0000	ng/ml
pTBX	6.278	81.0	418	0.0060	ng/ml
PBBZ	6.372	81.0	84	0.0014	ng/ml
BDE-30	6.565	81.0	0	0.0000	ng/ml
BDE-17	6.869	81.0	1526	0.0579	ng/ml
BDE-28	6.992	81.0	2337	0.0876	ng/ml
PBEB	7.185	81.0	1874	0.0321	ng/ml
HBB	7.664	81.0	174	0.0043	ng/ml
BDE-49	7.699	81.0	1021	0.0402	ng/ml
BDE-71	7.729	81.0	301	0.0080	ng/ml
BDE-47	7.834	81.0	10880	0.3413	ng/ml
BDE-66	7.951	81.0	702	0.0251	ng/ml
BDE-77	8.132	81.0	82127	2.2421	ng/ml
BDE-100	8.485	81.0	388	0.0118	ng/ml
BDE-119	8.908	81.0	0	0.0000	ng/ml
BDE-99	8.709	81.0	3501	0.1085	ng/ml
EHTBB	8.741	357.0	186	0.1550	ng/ml
BDE-85	9.173	81.0	62	0.0022	ng/ml

### Quantitative Analysis Report

Name	RT	Target Ion	Target Response	Final Conc.	Units
BDE-126	8.908	81.0	0	0.0000	ng/ml
BDE-154+BB-153	9.448	81.0	68	0.0016	ng/ml
BDE-153	9.898	81.0	436	0.0136	ng/ml
BDE-139	10.103	81.0	1632	0.0570	ng/ml
BDE-140	10.272	81.0	0	0.0000	ng/ml
HBCD	10.325	81.0	1016	0.1138	ng/ml
BDE-138	10.652	81.0	0	0.0000	ng/ml
BDE-166	10.652	81.0	14635	0.6541	ng/ml
BDE-156+169	10.699	81.0	0	0.0000	ng/ml
BDE-184	11.009	81.0	0	0.0000	ng/ml
BDE-183	11.617	81.0	250	0.0101	ng/ml
BDE-191	12.541	81.0	0	0.0000	ng/ml
TBE	12.565	81.0	0	0.0000	ng/ml
BDE-180	12.763	81.0	0	0.0000	ng/ml
BDE-190+171	12.763	81.0	0	0.0000	ng/ml
BEHTBP	13.190	81.0	0	0.0000	ng/ml
syn-DP	13.590	653.8	196	0.0228	ng/ml
BDE-201		719.5		ND	ng/ml
anti-DP	14.296	651.8	1492	0.1599	ng/ml
BDE-204		486.8		ND	ng/ml
BDE-197		408.9		ND	ng/ml
BDE-203	14.895	561.7	0	0.0000	ng/ml
BDE-196	14.895	561.7	0	0.0000	ng/ml
BDE-205	15.401	81.0	0	0.0000	ng/ml
BDE-208	16.448	486.8	0	0.0000	ng/ml
BDE-207		486.8		ND	ng/ml
BDE-206	17.040	486.8	0	0.0000	ng/ml
BDE-209	18.515	486.8	0	0.0000	ng/ml
13C-BDE-209		494.6		ND	ng/ml
DBDPE	19.495	81.0	0	0.0000	ng/ml

Figure 7. Example of quantitation report for sample.

## VII. STANDARDS and SPIKING

### BFR Calibration Standard

Prepared by combining PBDE Internal Standard stock, PBDE Surrogate Standard stock, Non-PBDE stock, and BFR-PAR

**Table 4. Contents of BFR Calibration Standard.**

<b>Elute Order</b>	<b>Compound</b>	<b>Amount (ng/ml)</b>	<b>Elute Order</b>	<b>Compound</b>	<b>Amount (ng/ml)</b>
1	BDE-10	20.0	27	HBCD	100.0
2	BDE-7	20.0	28	BDE-138	40.0
3	BDE-15	20.0	29	BDE-166	50.0
4	pTBX	25.0	30	BDE-156+169	80.0
5	PBBZ	25.0	31	BDE-184	80.0
6	BDE-30	20.0	32	BDE-183	80.0
7	BDE-17	19.2	33	BDE-191	80.0
8	BDE-28	20.0	34	BTBPE	40.0
9	PBEB	20.0	35	BDE-180	80.0
10	HBB	20.0	36	BDE-181	100.0
11	BDE-49	40.0	37	BDE-190+171	80.0
12	BDE-71	40.0	38	BEHTBP	50.0
13	BDE-47	40.0	39	syn-DP	50.0
14	BDE-66	40.0	40	BDE-201	80.0
15	BDE-77	70.0	41	anti-DP	50.0
16	BDE-100	40.0	42	BDE-204	80.0
17	BDE-119	40.0	43	BDE-197	80.0
18	BDE-99	40.0	44	BDE-203	80.0
19	EHTBB	50.0	45	BDE-196	80.0
20	BDE-118	50.0	46	BDE-205	80.0
21	BDE-85	40.0	47	BDE-208	200.0
22	BDE-126	40.0	48	BDE-207	200.0
23	BDE-154+BB153	80.0	49	BDE-206	200.0
24	BDE-153	40.0	50	BDE-209	200.0
25	BDE-139	40.0	51	13C12-BDE-209	40.0
26	BDE-140	40.0	52	DBDPE	400.0

### Additional Standards

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

### Internal Standard Spiking

Spiking of sample vials following nitrogen blow down with internal standards (50 µl spikes for vapor and particle phases, and 200 µl spike for precipitation) results in amounts summarized in Table 5.

**Table 5. Internal standard amounts in IADN samples**

<u>Vapor &amp; Particle Phase</u>		<u>Precipitation Phase</u>	
Compound	Amount (ng/ml)	Compound	Amount (ng/ml)
BDE-118	5.00	BDE-118	20.00
BB-209	10.00	BB-209	40.00
BDE-181	10.00	BDE-181	40.00

Surrogate Standards.

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

**Table 6. BFR surrogate recovery standard amounts**

Compound	Amount (ng/ml)
BDE-77	3.00
BDE-166	5.00
13C12-BDE-209	4.00

**Appendix I. Instrument Control Parameters for BFR split/splitless acquisition method.**

INSTRUMENT CONTROL PARAMETERS: CN10271122

-----  
Mon Jul 29 19:33:15 2019

Control Information

-----  
Sample Inlet: GC  
Injection Source: GC ALS  
Injection Location: Rear  
Mass Spectrometer: Enabled

No Sample Prep method has been assigned to this method.

GC

GC Summary

Run Time 16.983 min  
Post Run Time 0 min

Oven

Equilibration Time 0.25 min  
Max Temperature 350 °C  
Maximum Temperature Override Disabled  
Slow Fan Disabled  
Temperature  
Setpoint On  
(Initial) 100 °C  
Hold Time 2 min  
Post Run 0 °C  
Program

#1 Rate	24 °C/min
#1 Value	250 °C
#1 Hold Time	0 min
#2 Rate	3 °C/min
#2 Value	260 °C
#2 Hold Time	0 min
#3 Rate	25 °C/min
#3 Value	320 °C
#3 Hold Time	3 min

#### ALS

ALS Errors	Pause for user interaction
Back Injector	
Syringe Size	10 µL
Injection Volume	2 µL
Solvent A Washes (PreInj)	3
Solvent A Washes (PostInj)	3
Solvent A Volume	8 µL
Solvent B Washes (PreInj)	3
Solvent B Washes (PostInj)	3
Solvent B Volume	8 µL
Sample Washes	0
Sample Wash Volume	8 µL
Sample Pumps	6
Dwell Time (PreInj)	0 min
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	300 µL/min
Solvent Wash Dispense Speed	6000 µL/min
Sample Wash Draw Speed	300 µL/min
Sample Wash Dispense Speed	6000 µL/min
Injection Dispense Speed	6000 µL/min
Viscosity Delay	2 sec

Sample Depth	1 mm
Injection Type	Standard
L1 Airgap	0 µL
Solvent Wash Mode	A-A6,B-B4

Sample Overlap Mode	Sample overlap is not enabled
------------------------	-------------------------------

Front MM Inlet He

\*\*\*Excluded from Affecting GC's Readiness State\*\*\*

Mode	Pulsed Splitless
Pressure	Off
Total Flow	Off
Septum Purge Flow	Off
Injection Pulse Pressure	25 psi Until 0.5 min
Purge Flow to Split Vent	100 mL/min at 1 min
Cryo	Off
Cryo Type	N2
Temperature Setpoint (Initial)	Off 285 °C

Back SS Inlet He

Mode	Pulsed Splitless
Heater	On 300 °C
Pressure	On 7.0918 psi
Total Flow	On 54 mL/min
Septum Purge Flow	On 2.5 mL/min
Septum Purge Flow Mode	Switched
Gas Saver	On 20 After 6 min mL/min
Injection Pulse Pressure	25 psi Until 0.5 min
Purge Flow to Split Vent	50 mL/min at 1 min

Thermal Aux 2 (MSD Transfer Line)

Temperature

Setpoint On  
(Initial) 320 °C  
Post Run 0 °C

Column

Column Outlet Pressure 0 psi  
Column #1  
Column Information Restek Rtx-1614: 1281.66361  
Restek  
Temperature Range -60 °C—360 °C (360 °C)  
Dimensions 15 m x 250 µm x 0.1 µm

In Back SS Inlet He

Out MSD

(Initial) 100 °C

Pressure 7.0918 psi

Flow 1.5 mL/min

Average Velocity 64.594 cm/sec

Holdup Time 0.38703 min

Flow

Setpoint On

(Initial) 1.5 mL/min

Post Run 0.57353 mL/min

Valve 1

Name ?

Type Gas Sampling Valve

GSV Loop Volume 1 mL

Load Time 0.5 min

Inject Time 0.5 min

PCM C

PCM C He

\*\*\*Excluded from Affecting GC's Readiness State\*\*\*

Pressure

Setpoint	Off
(Initial)	10 psi
Post Run	0 psi

Aux PCM C He

\*\*\*Excluded from Affecting GC's Readiness State\*\*\*

Pressure

Setpoint	Off
(Initial)	10 psi
Post Run	0 psi

Signals

Signal #1: Test Plot

Description	Test Plot
Details	
Save	Off
Data Rate	50 Hz
Dual Injection Assignment	Front Sample

Signal #2: Test Plot

Description	Test Plot
Details	
Save	Off
Data Rate	50 Hz
Dual Injection Assignment	Front Sample

Signal #3: Test Plot



Description Test Plot  
Details  
Save Off  
Data Rate 50 Hz  
Dual Injection Assignment Back Sample

Signal #4: Test Plot

Description Test Plot  
Details  
Save Off  
Data Rate 50 Hz  
Dual Injection Assignment Back Sample

MS Information

-- -----

JetClean: No Cleaning

General Information

-----

Acquisition Mode: SIM  
Solvent Delay (minutes): 4.9  
Tune file: D:\MassHunter\GCMS\2\5975\ncich4.u  
EM Setting mode Gain: 6.000000

Number of SIM Groups: 5  
Run Time (if MS only): 650 minutes

[SIM Parameters]

Group 1 Group ID: 1  
Resolution: 1

Group Start Time: 4.9

Number of Ions: 7

Ions

Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)  
( 79.00,80 ) ( 81.00,80 ) ( 159.80,20 )  
( 160.80,20 ) ( 357.00,80 ) ( 359.00,80 )  
( 471.70,20 )

Group 2 Group ID: 2

Resolution : 0

Group Start Time : 11.846

Number of Ions : 11

Ions

Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)  
( 79.00,40 ) ( 81.00,40 ) ( 406.70,40 )  
( 408.70,40 ) ( 461.70,40 ) ( 463.70,40 )  
( 486.80,40 ) ( 488.80,40 ) ( 651.80,40 )  
( 653.80,40 ) ( 719.50,40 )

Group 3 Group ID: 3

Resolution : 0

Group Start Time : 13.37

Number of Ions : 2

Ions

Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)  
( 486.80,200 ) ( 488.80,200 )

Group 4 Group ID: 4

Resolution : 0

Group Start Time : 14.5

Number of Ions : 4

Ions

Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)  
( 486.80,100 ) ( 488.80,100 ) ( 494.60,100 )  
( 496.60,100 )

Group 5            Group ID:    5  
Resolution        :            0  
Group Start Time    :            15.55  
Number of Ions     :            2  
Ions  
Dwell In Group     : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)  
                      ( 79.00,200 ) ( 81.00,200 )

[MSZones]

MS Source         : 200 C maximum 300 C  
MS Quad           : 140 C maximum 200 C

Timed Events

-----

Number Events= 0

END OF MS ACQUISITION PARAMETERS

TUNE PARAMETERS for SN: US10264402

-----

Trace Ion Detection is OFF.

49.443 : EMISSION  
148.953 : ENERGY  
4.006 : REPELLER  
144.251 : IONFOCUS  
5.000 : ENTRANCE\_LENS  
1858.824 : EMVOLTS  
          2258.824 : Actual EMV  
          6.07 : GAIN FACTOR  
2178.000 : AMUGAIN  
126.375 : AMUOFFSET

1.000 : FILAMENT  
0.000 : DCPOLARITY  
28.361 : ENTLENSOFFSET  
-503.000 : MASSGAIN  
-35.000 : MASSOFFSET  
CI Flow Rate: 40  
CI A/B Gas : 1

END OF TUNE PARAMETERS

-----

END OF INSTRUMENT CONTROL PARAMETERS

-----

**Appendix II. Instrument Control Parameters for BFR multi-mode inlet large volume acquisition method.**

INSTRUMENT CONTROL PARAMETERS: CN10271122

-----

Sat Aug 15 20:50:03 2020

Control Information

-----

Sample Inlet: GC  
Injection Source: GC ALS  
Injection Location: Front  
Mass Spectrometer: Enabled

No Sample Prep method has been assigned to this method.

## GC

### GC Summary

Run Time 17.433 min

Post Run Time 0 min

### Oven

Equilibration Time 0.25 min

Max Temperature 350 °C

Max Temperature Override Disabled

Slow Fan Disabled

### Temperature

Setpoint On

(Initial) 40 °C

Hold Time 0.3 min

Post Run 0 °C

### Program

#1 Rate 120 °C/min

#1 Value 100 °C

#1 Hold Time 2.3 min

#2 Rate 21 °C/min

#2 Value 254 °C

#2 Hold Time 0 min

#3 Rate 3 °C/min

#3 Value 260 °C

#3 Hold Time 0 min

#4 Rate 30 °C/min

#4 Value 320 °C

#4 Hold Time 3 min

## ALS

ALS Errors                      Pause for user interaction

### Front Injector

Syringe Size	50 µL
Injection Volume	10 µL
Solvent A Washes (PreInj)	2
Solvent A Washes (PostInj)	2
Solvent A Volume	40 µL
Solvent B Washes (PreInj)	2
Solvent B Washes (PostInj)	2
Solvent B Volume	40 µL
Sample Washes	0
Sample Wash Volume	40 µL
Sample Pumps	3
Dwell Time (PreInj)	0 min
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	1500 µL/min
Solvent Wash Dispense Speed	30000 µL/min
Sample Wash Draw Speed	1500 µL/min
Sample Wash Dispense Speed	30000 µL/min
Injection Dispense Speed	100 µL/min
Viscosity Delay	0 sec
Sample Depth	1 mm
Injection Type	Standard
L1 Airgap	0 µL
Solvent Wash Mode	A-A6,B-B4

### Sample Overlap

Mode                              Sample overlap is not enabled

### Front MM Inlet He

Mode                              Solvent Vent

Pressure	On 14 psi
Total Flow	On 66.493 mL/min
Septum Purge Flow	On 3 mL/min
Gas Saver	On 20 After 4 min mL/min
Purge Flow to Split Vent	60 mL/min at 2.6 min
Vent Flow	100 mL/min per min
Vent Pressure	2.5 psi Until 0.1 min
Cryo	Off
Cryo Type	N2
Temperature	
Setpoint	On
(Initial)	46 °C
Hold Time	0.1 min
Program	
#1 Rate	700 °C/min
#1 Value	310 °C
#1 Hold Time	15 min
#2 Rate	700 °C/min
#2 Value	320 °C
#2 Hold Time	3 min

#### Back SS Inlet He

\*\*\*Excluded from Affecting GC's Readiness State\*\*\*

Mode	Pulsed Splitless
Heater	Off
Pressure	Off
Total Flow	Off
Septum Purge Flow	Off
Septum Purge Flow Mode	Switched
Injection Pulse Pressure	25 psi Until 0.5 min
Purge Flow to Split Vent	50 mL/min at 1 min

Thermal Aux 2 (MSD Transfer Line)

Temperature

Setpoint On  
(Initial) 300 °C  
Post Run 0 °C

Column

Column Outlet Pressure 0 psi  
Column #1  
Column Information Restek Rtx-1614: 1281.66361

Restek

Temperature Range -60 °C—360 °C (360 °C)

Dimensions 15 m x 250 µm x 0.1 µm

In Front MM Inlet He

Out MSD

(Initial) 40 °C

Pressure 14 psi

Flow 3.4931 mL/min

Average Velocity 95.845 cm/sec

Holdup Time 0.26084 min

Flow

Setpoint On

(Initial) 3.4931 mL/min

Post Run 1.6478 mL/min

Valve 1

Name ?

Type Gas Sampling Valve

GSV Loop Volume 1 mL

Load Time 0.5 min

Inject Time 0.5 min



PCM C

PCM C He

\*\*\*Excluded from Affecting GC's Readiness State\*\*\*

Pressure

Setpoint	Off
(Initial)	10 psi
Post Run	0 psi

Aux PCM C He

\*\*\*Excluded from Affecting GC's Readiness State\*\*\*

Pressure

Setpoint	Off
(Initial)	10 psi
Post Run	0 psi

Signals

Signal #1:	Test Plot
Description	Test Plot
Details	
Save	Off
Data Rate	50 Hz
Dual Injection Assignment	Front Sample

Signal #2: Test Plot

Description	Test Plot
Details	
Save	Off
Data Rate	50 Hz
Dual Injection Assignment	Front Sample

Signal #3: Test Plot

Description	Test Plot
-------------	-----------

Details

Save Off  
Data Rate 50 Hz  
Dual Injection Assignment Back Sample

Signal #4: Test Plot

Description Test Plot

Details

Save Off  
Data Rate 50 Hz  
Dual Injection Assignment Back Sample

MS Information

-- -----

JetClean: No Cleaning

General Information

-----

Acquisition Mode : SIM  
Solvent Delay (minutes) : 6.25  
Tune file : D:\MassHunter\GCMS\2\5975\ncich4.u  
EM Setting mode Gain : 12.000000

Number of SIM Groups : 5  
Run Time (if MS only) : 650 minutes

[SIM Parameters]

Group 1 Group ID 1  
Resolution : 0  
Group Start Time : 6.25  
Number of Ions : 7

Ions

Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)

( 79.00,60 ) ( 81.00,120 ) ( 159.80,90 )

( 160.80,20 ) ( 357.00,90 ) ( 359.00,20 )

( 471.70,20 )

Group 2 Group ID 3

Resolution : 0

Group Start Time : 12.8

Number of Ions : 9

Ions

Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)

( 79.00,20 ) ( 81.00,72 ) ( 406.70,20 )

( 408.70,72 ) ( 461.70,20 ) ( 463.70,72 )

( 651.80,20 ) ( 653.80,72 ) ( 719.50,20 )

Group 3 Group ID 4

Resolution : 0

Group Start Time : 14.05

Number of Ions : 4

Ions

Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)

( 79.00,20 ) ( 81.00,20 ) ( 486.80,200 )

( 488.80,80 )

Group 4 Group ID 5

Resolution : 0

Group Start Time : 15.15

Number of Ions : 4

Ions

Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)

( 486.80,125 ) ( 488.80,50 ) ( 494.60,125 )

( 496.60,50 )

Group 5 Group ID 6

Resolution : 0  
Group Start Time : 15.95  
Number of Ions : 2  
Ions  
Dwell In Group : ( Mass, Dwell) ( Mass, Dwell) ( Mass, Dwell)  
( 79.00,200 ) ( 81.00,200 )

[MSZones]

MS Source : 200 C maximum 300 C  
MS Quad : 140 C maximum 200 C

Timed Events

-----

Number Events= 0

END OF MS ACQUISTION PARAMETERS

TUNE PARAMETERS for SN: US10264402

-----

Trace Ion Detection is OFF.

49.443 : EMISSION  
125.276 : ENERGY  
4.006 : REPELLER  
153.741 : IONFOCUS  
0.000 : ENTRANCE\_LENS  
1529.412 : EMVOLTS  
2070.588 : Actual EMV  
12.10 : GAIN FACTOR  
2185.000 : AMUGAIN  
127.000 : AMUOFFSET  
1.000 : FILAMENT

0.000 : DCPOLARITY

30.118 : ENLENDOFFSET

-503.000 : MASSGAIN

-35.000 : MASSOFFSET

CI Flow Rate: 40

CI A/B Gas : 1

END OF TUNE PARAMETERS

-----

END OF INSTRUMENT CONTROL PARAMETERS

-----