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Chemical Instrumentation Facility

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LCMS 2020 Training Manual

Kamel Harrata (November 2014)

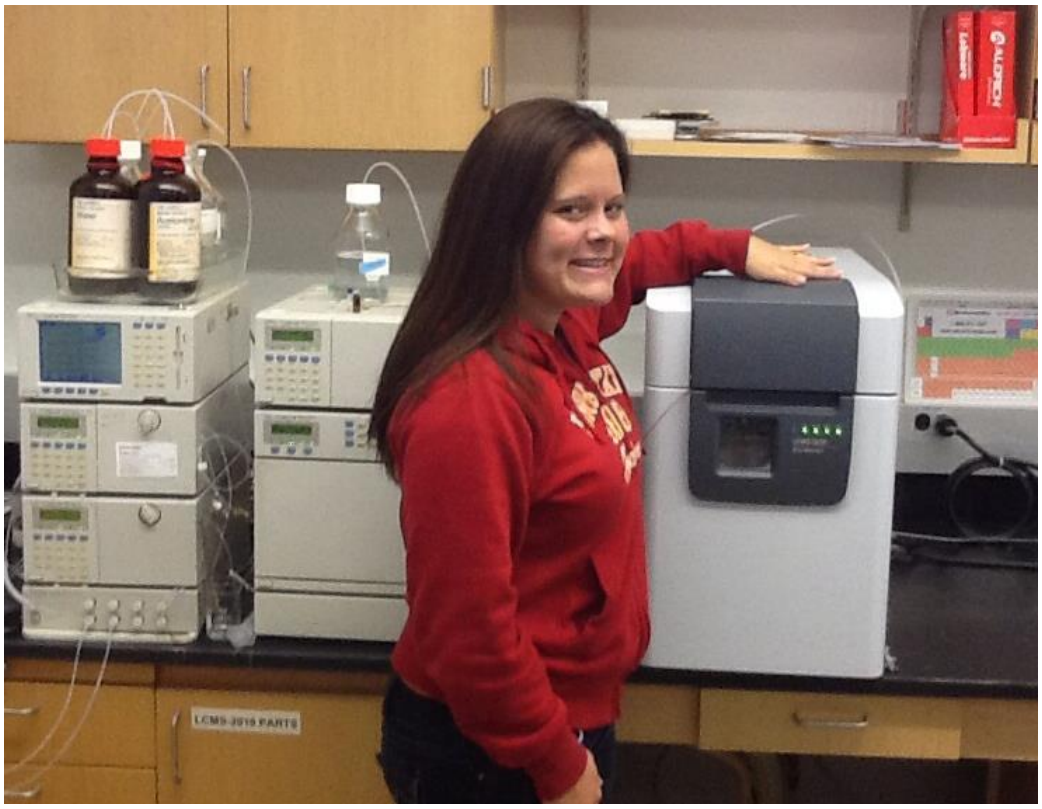

Photo: Toshia Zessin (Graduate Student)

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Important Notes:

This training manual is property of Chemical Instrumentation Facility of Iowa State University. A proper training, usually one-on-one, is provided along with this manual. For those students who are not well versed in Chromatography and Mass Spectrometry, a 1 hour course/session is usually provided by mass spec personnel prior to actual training.

Before starting analysis!!!!!

Samples must be completely dissolved prior to Injection. Please filter and/or centrifuge your sample. Next to the instrument there is a Vortex, Micro centrifuge, and 1,5 ml Micro centrifuge polypropylene tubes. For Flow Injection (FI) ESI work, sample concentrations of less than 100 ppm (100ng/ul) are usually enough.

Vortex Micro Centrifuge Tubes and vials

Take a look at the pressure reading from the Acquisition software (When IG is on).

The pressure value should be close to 6 10^{-4} Pa.

Vortex

Micro Centrifuge

Tubes and vials

Files structures:

Each user has data files folder located in his or her directory:

C:\LabSolutions\Data\username\filename

Username is the iastate email id, e.g. kamel

Filename is always: xyz-month-day-nm e.g. akh-8-20-01

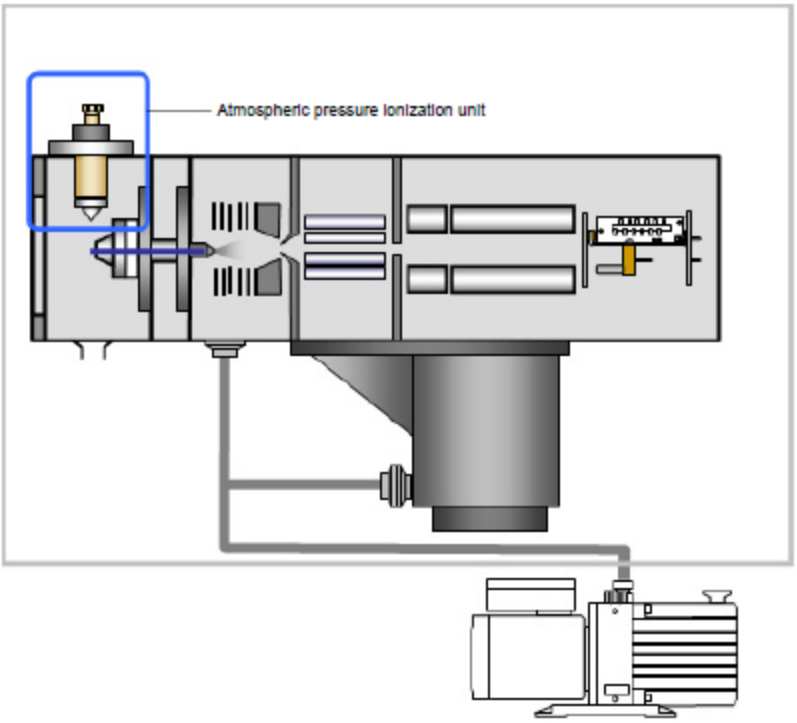
xyz is your initials

Method files are located in C:\Labsolutions\Methods

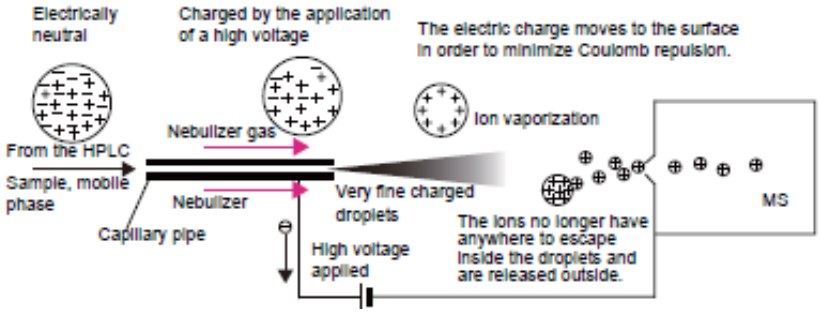
Tune files are located in C:\LabSolutions\Tune Files

LCMS 2020 Configuration:

LCMS 2020 is a single quadrupole mass spectrometer (mass range up to 2000) that is equipped with ESI source and ESI/APCI combination source called DUIS. This is the same as ESI with the addition of corona discharge. The DUIS probe offers the ability to collect data in either ESI or APCI modes, or both in the same acquisition method. The sensitivity for either mode is slightly less than would be attainable with a dedicated probe for that specific type of ionization, but it offers the flexibility to be able to run both modes without changing probes. The selection of the acquisition mode is in the method setup.The system will always be configured for LCMS using a XDB C18 column from Agilent. The column dimensions are 4.6*50 mm, 1.8 um. If other column is needed, please consult with lab personnel.



ESI (Electrospray Ionization)



The formation of positive or negative ions (depending on the sign of the applied electrical field) occurs in high yield. In the positive ion mode protonated and/or alkali adduct analyte molecules are generally observed in the mass spectra. In the negative ion mode operation peaks corresponding to deprotonated analyte molecules are observed. ESI is described as a very "soft" ionization technique where the surrounding bath gas has a moderating effect on the internal and translational energies of desorbed ions.

Advantages of ESI:

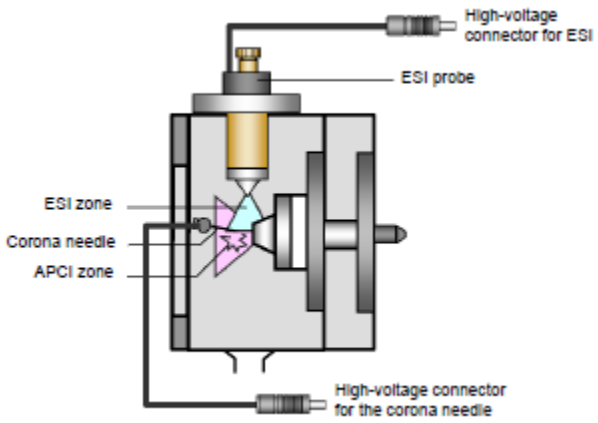
- Soft ionization process so intact molecular ions are observed

- ESI allows production of multiply charged ions. This results in the ability of analyzing very high molecular weight species using the most available mass analyzers (e.g. quadrupoles).

- ESI is an atmospheric pressure process. This makes it easy to use and easy to interface with HPLC and CE separation techniques.

DUIS (dual ion source)

A high voltage is applied to the corona discharge needle located just underneath the sprayer.



The DUIS ion source adds a corona discharge needle with a voltage of few kV. This latter induces ion formation from low polarity compounds that may not ionize with ESI.

Tuning and Calibration:

The mass spectrometer is tuned for sensitivity, resolution, and mass calibration.

The system can be tuned automatically or manually. This step is performed only when deemed necessary by lab personnel. Autotune is always performed without corona needle voltage, and with heater block of 400 deg. Tune results copies are stored in the computer and a binder.

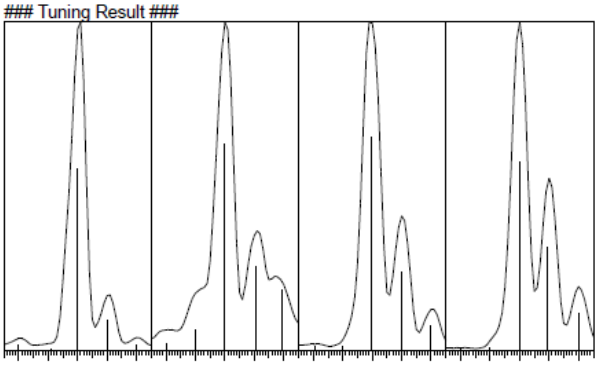
The system is tuned either in positive or negative ion modes or both simultaneously. Last tuning file is usually used in data acquisition unless otherwise specified. The following is a typical autotune results.

LCMS Tuning

Sample Information
Sample Type: PEG+PPG+Raffinose
Auto Tuning Mode: Both

Tuning Condition
Only Positive Tuning: Off
Resolution Adjustment: On
FWHM of Spectrum(Low): 0.60
FWHM of Spectrum(High): 0.60
Sensitivity Adjustment: On
Mass Calibration: On

Tuning Result

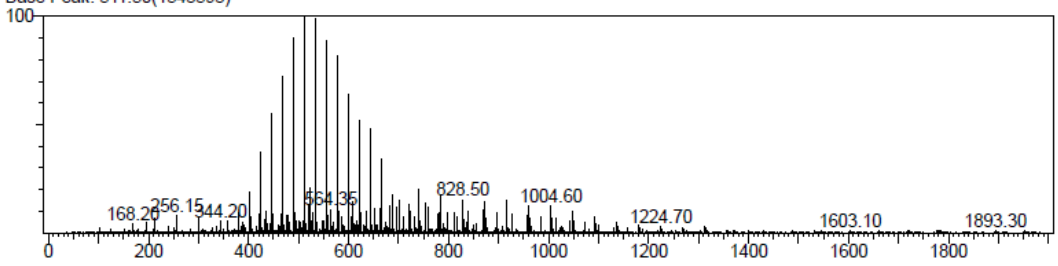


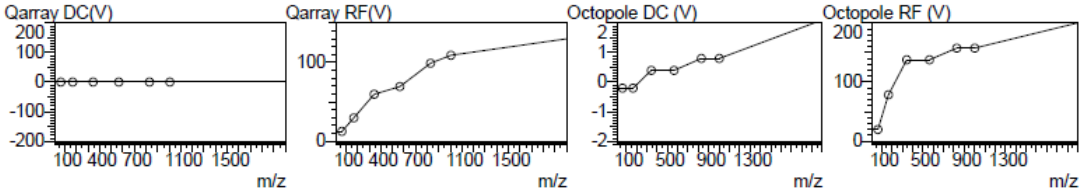
m/z: 256.15	m/z: 564.35	m/z: 828.50	m/z: 1004.60
Mag.: 52.11	Mag.: 31.66	Mag.: 20.86	Mag.: 27.59
Int.: 111644	Int.: 208688	Int.: 327845	Int.: 219239
FWHM: 0.59	FWHM: 0.67	FWHM: 0.69	FWHM: 0.62

Tuning Result
Model: LCMS-2020
Interface: DUIS
Polarity: Pos
Tuning Mode: Auto
Tuning Date: 2014 Aug 14 11:26:44
Nebulizing Gas Flow: 1.50 L/min
Drying Gas Flow: 15.00 L/min
Interface Bias: +4.50 kV
Interface Current: 0.5 uA

Heat Block Temp.: 400 C
Entrance Lens: -20.0 V
RF Gain: 4910
RF Offset: 5090
Mainrod Bias: -5.0 V
Conversion Dynode: -10.0 kV
Detector: -1.10 kV
PG: 9.9e+001 Pa
IG: 6.1e-004 Pa
DL Temp.: 250 C

Scan: 10.00 - 2000.00 Scan Speed: 29
Base Peak: 511.50(1543593)





	65.05	168.10	344.20	564.35	828.50	1004.60
DL	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
Qarray DC	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
Qarray RF	+12.5	+29.9	+59.6	+69.5	+99.2	+109.1
Octopole RF	+19.9	+78.9	+137.9	+137.9	+157.6	+157.6
Octopole DC	-0.2	-0.2	+0.4	+0.4	+0.8	+0.8
Entrance Lens	-25.1	-10.1	-5.2	-5.2	-5.2	-5.2

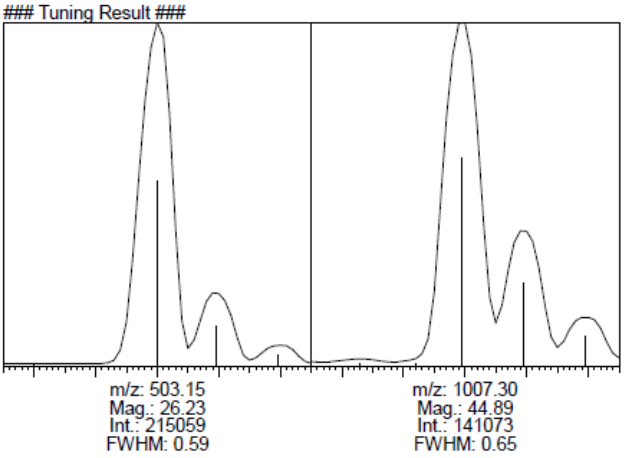
	107.15	1071.50
Prerod Bias	-5.0	-50.0

Sample Information
Sample Type: PEG+PPG+Raffinose
Auto Tuning Mode: Both

Tuning Condition

Only Positive Tuning:	Off
Resolution Adjustment:	On
FWHM of Spectrum(Low):	0.60
FWHM of Spectrum(High):	0.60
Sensitivity Adjustment:	On
Mass Calibration:	On

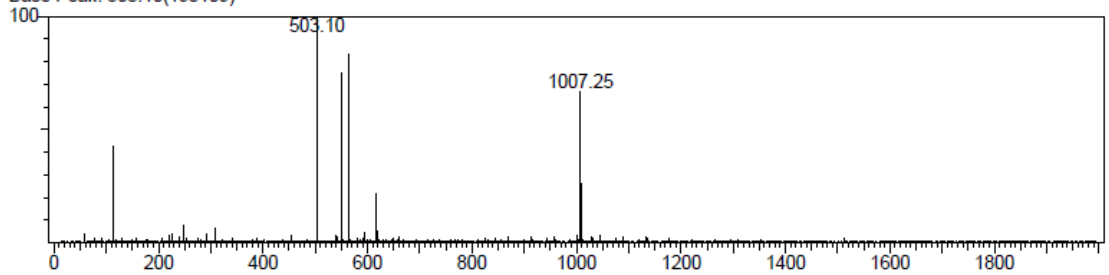
Tuning Result

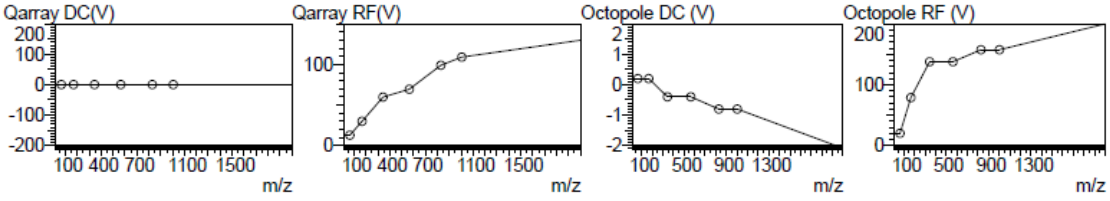


Tuning Result

Model:	LCMS-2020
Interface:	DUIS
Polarity:	Neg
Tuning Mode:	Auto
Tuning Date:	2014 Aug 14 11:34:45
Nebulizing Gas Flow:	1.50 L/min
Drying Gas Flow:	15.00 L/min
Interface Bias:	-3.50 kV
Interface Current:	0.5 uA
Heat Block Temp.:	400 C
Entrance Lens:	+20.0 V
RF Gain:	4948
RF Offset:	5123
Mainrod Bias:	+5.0 V
Conversion Dynode:	+10.0 kV
Detector:	-1.00 kV
PG:	9.8e+001 Pa
IG:	6.1e-004 Pa
DL Temp.:	250 C

Scan: 10.00 - 2000.00 Scan Speed: 29
Base Peak: 503.10(193109)





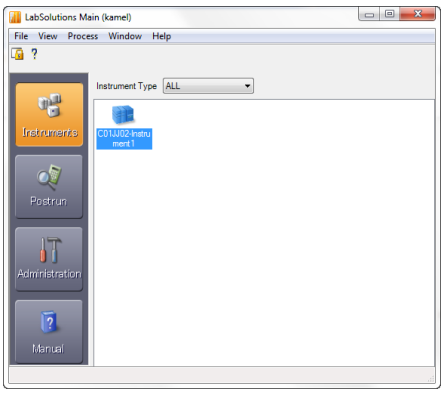
	65.05	168.10	344.20	564.35	828.50	1004.60
DL	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
Qarray DC	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
Qarray RF	+12.5	+29.9	+59.6	+69.5	+99.2	+109.1
Octopole RF	+19.9	+78.9	+137.9	+137.9	+157.6	+157.6
Octopole DC	+0.2	+0.2	-0.4	-0.4	-0.8	-0.8
Entrance Lens	+25.1	+10.1	+5.2	+5.2	+5.2	+5.2

	107.15	1071.50
Prerod Bias	+5.0	+50.0

Data Acquisition: (Step-by-Step)

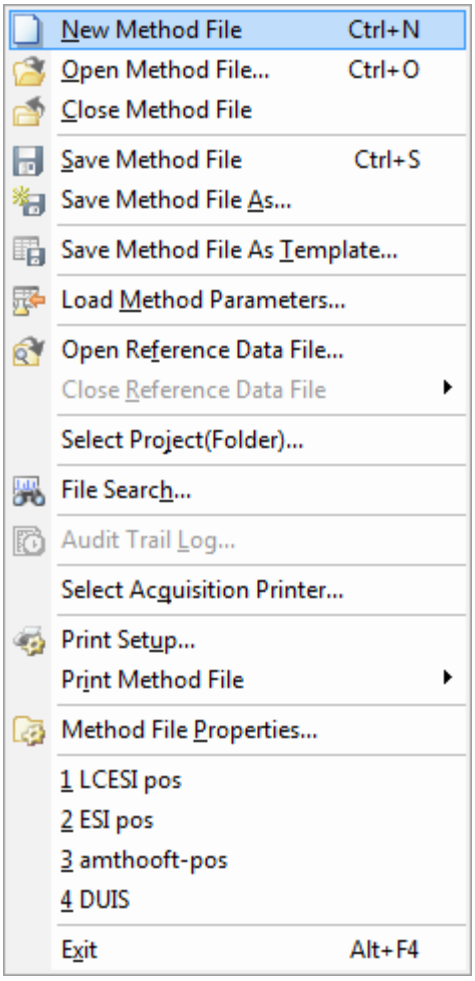
The acquisition software uses Realtime browser while data processing uses Postrun analysis.

Real time Analysis browser can be accessed from LabSolution Main program:



 Realtime Analysis

1- From Realtime Analysis: File > Open Method File… (Your method will be set and explained during training)



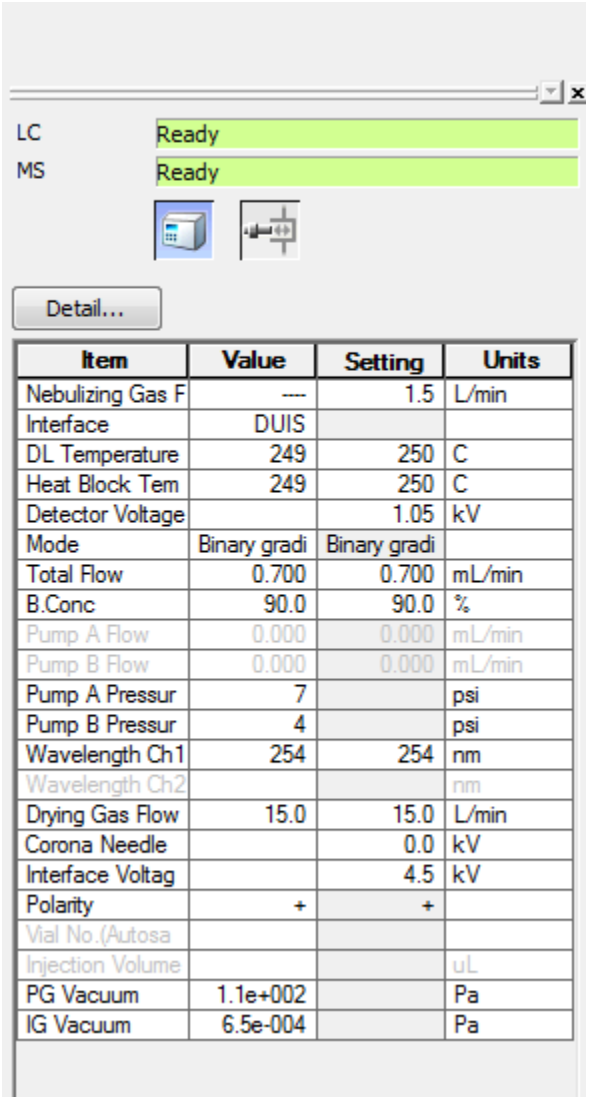
2- Make sure there is enough solvent in the solvent reservoir. Bottle A is always aqueous and bottle B is for organic solvent.

3- Turn on heaters DL and HEAT. They should always be left ON. Turn IG on (should be ON too.
4- Turn on NEBU, MS, and LC pumps.

Wait for IG Vacuum to be about 6 10^{-4} Pa. This will allow LC and MS to be in ready state.

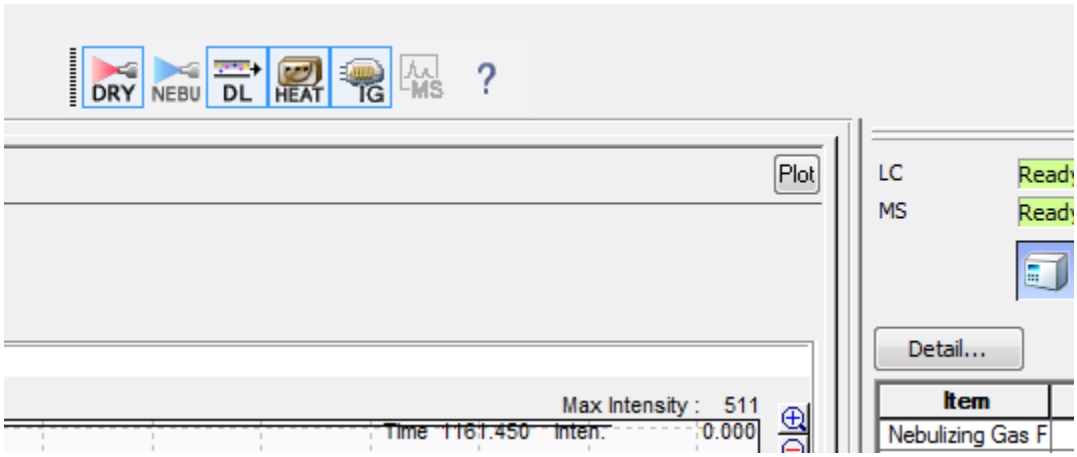
Start LC pump by clicking on  from 

If LC column is installed, increase Total Flow from setting slowly so that the back pressure do not go up abruptly.



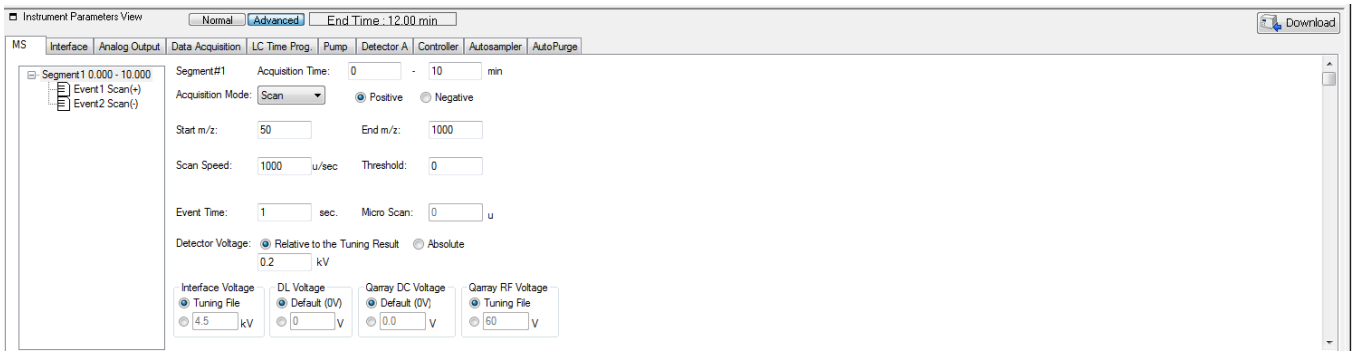
Item	Value	Setting	Units
Nebulizing Gas F	----	1.5	L/min
Interface	DUIS		
DL Temperature	249	250	C
Heat Block Tem	249	250	C
Detector Voltage		1.05	kV
Mode	Binary gradi	Binary gradi	
Total Flow	0.700	0.700	mL/min
B.Conc	90.0	90.0	%
Pump A Flow	0.000	0.000	mL/min
Pump B Flow	0.000	0.000	mL/min
Pump A Pressur	7		psi
Pump B Pressur	4		psi
Wavelength Ch1	254	254	nm
Wavelength Ch2			nm
Drying Gas Flow	15.0	15.0	L/min
Corona Needle		0.0	kV
Interface Voltag		4.5	kV
Polarity	+	+	
Vial No.(Autosa			
Injection Volume			uL
PG Vacuum	1.1e+002		Pa
IG Vacuum	6.5e-004		Pa

5- Check the system for ion formation: Click Plot located in the upper right corner



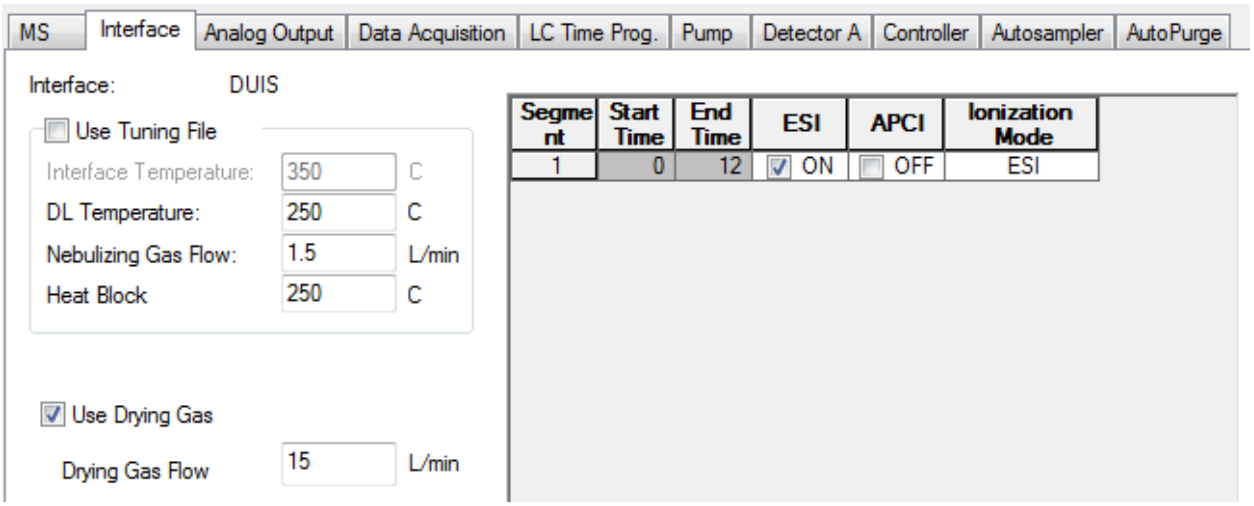
This allows observation of UV and MS data as it occurs. If background ions are observed, the plot can be stopped and data acquisition can proceed. Stop the plot.

Instrument or method parameters:

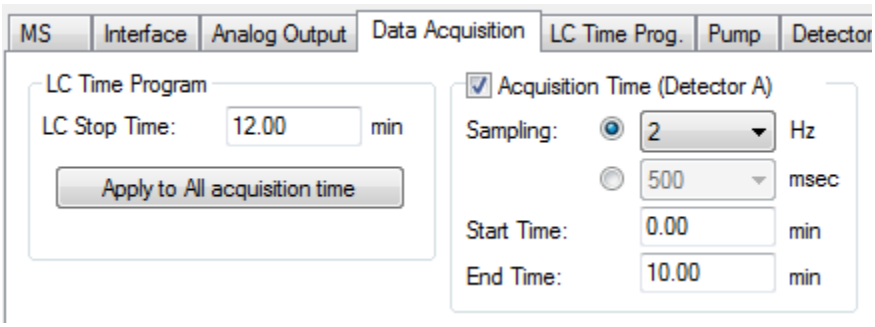


The system can be scanned in positive and negative ion modes simultaneously. This can be accomplished by setting events. Each event has its own parameters.

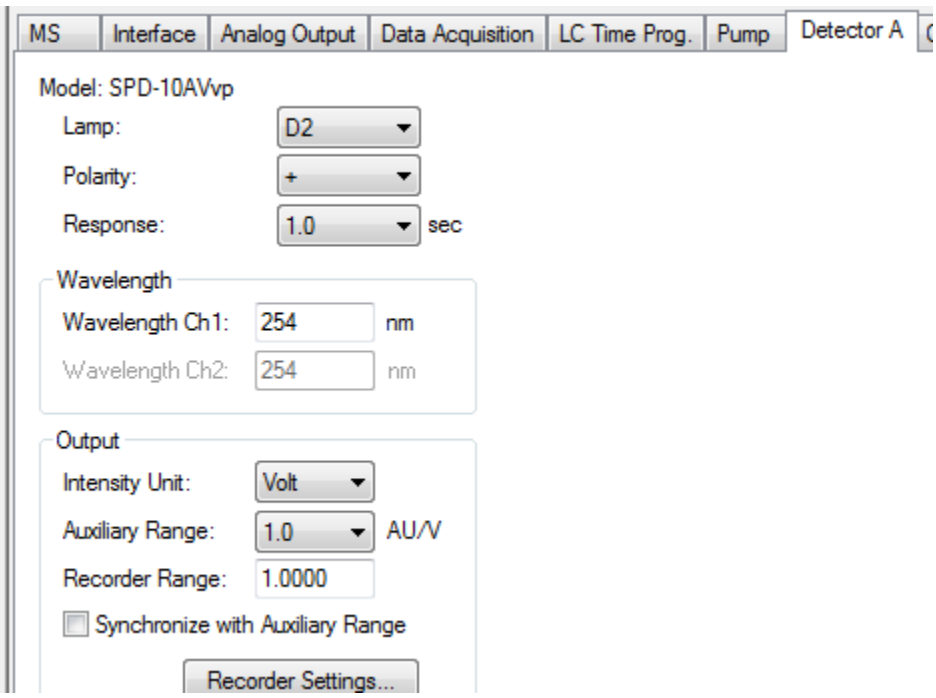
In Interface tab, the system can be set for ESI or DUIS.



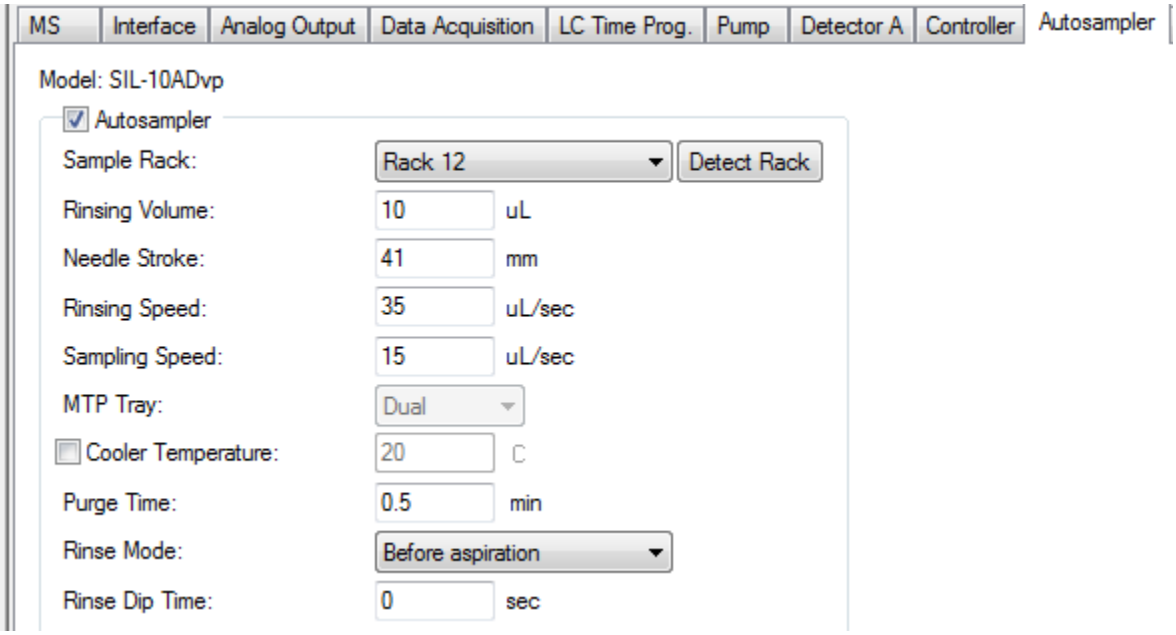
Data Acquisition Tab allows setting experiment duration in both MS and UV.



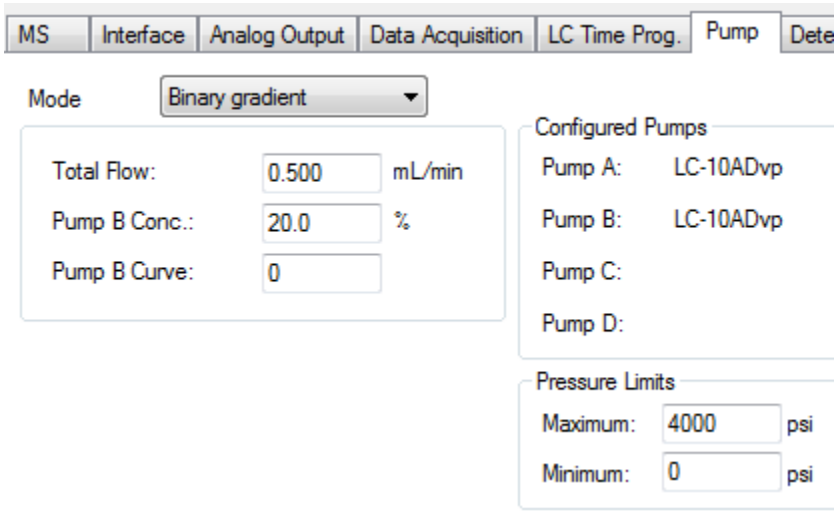
Detector Tab allows setting Wavelength value.



Autosampler tab allows setting Rack type and all other parameters.



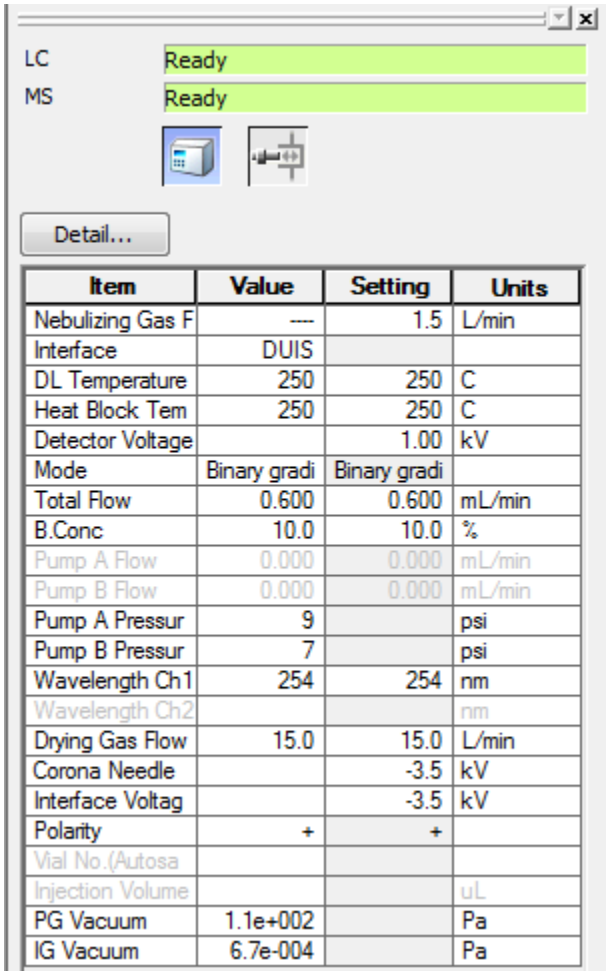
Pump Tab allows setting type flow, Flow rate, and Pressure limits.



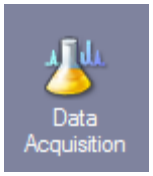
LC Time Prog. allows setting the binary LC gradient.

	Time	Module	Command	Value
1	0.10	Pumps	Pump B Conc.	20
2	10.00	Pumps	Pump B Conc.	90
3	12.00	Controller	Stop	
4	0.00			

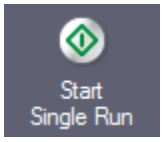
If any changes are made, click on Download and save method. Make sure that all the starting conditions are visible in the Detail section of the display.

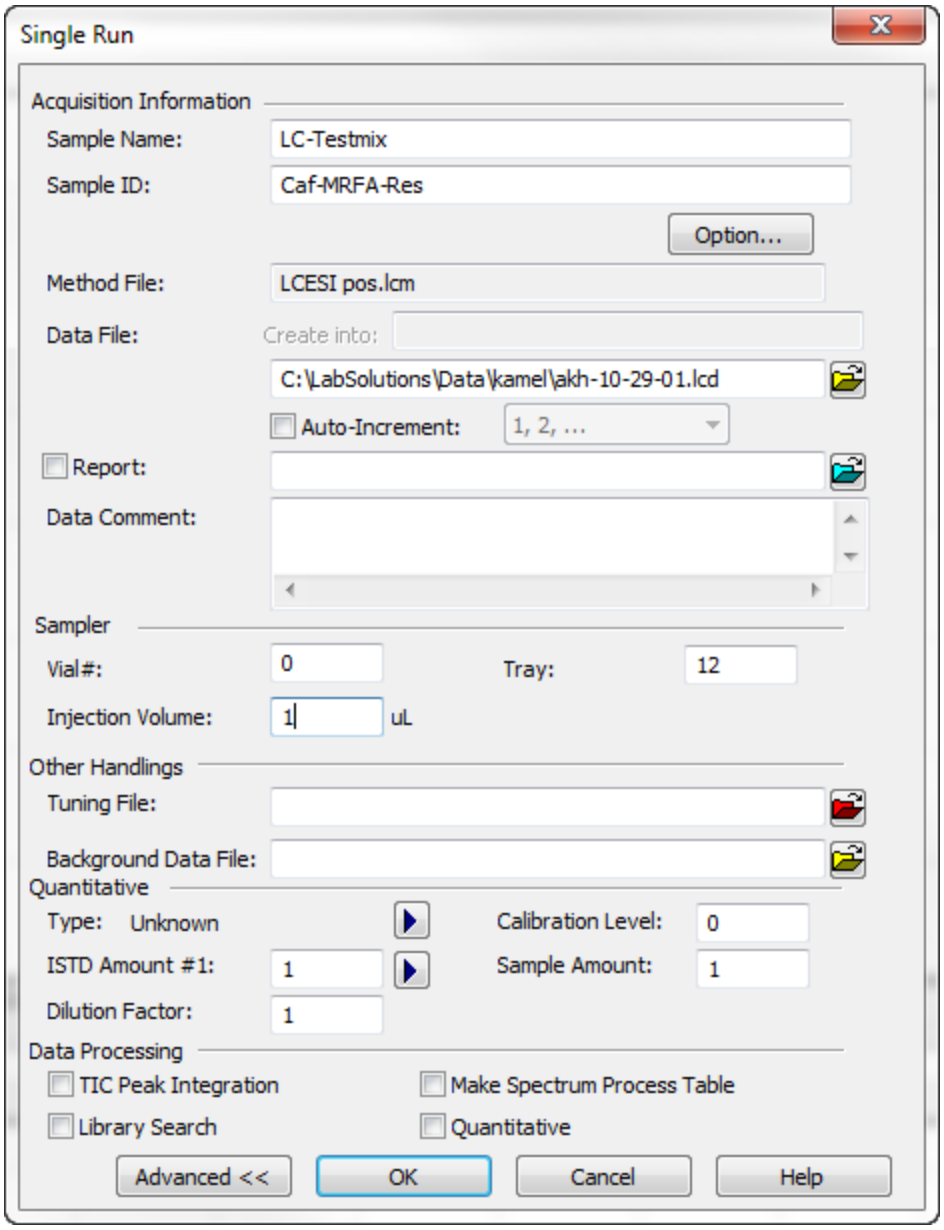


Item	Value	Setting	Units
Nebulizing Gas F	----	1.5	L/min
Interface	DUIS		
DL Temperature	250	250	C
Heat Block Tem	250	250	C
Detector Voltage		1.00	kV
Mode	Binary gradi	Binary gradi	
Total Flow	0.600	0.600	mL/min
B.Conc	10.0	10.0	%
Pump A Flow	0.000	0.000	mL/min
Pump B Flow	0.000	0.000	mL/min
Pump A Pressur	9		psi
Pump B Pressur	7		psi
Wavelength Ch1	254	254	nm
Wavelength Ch2			nm
Drying Gas Flow	15.0	15.0	L/min
Corona Needle		-3.5	kV
Interface Voltag		-3.5	kV
Polarity	+	+	
Vial No.(Autosa			
Injection Volume			uL
PG Vacuum	1.1e+002		Pa
IG Vacuum	6.7e-004		Pa

6- From Main click on Data Acquisition 

to start data acquisition

7- Click Start Run 



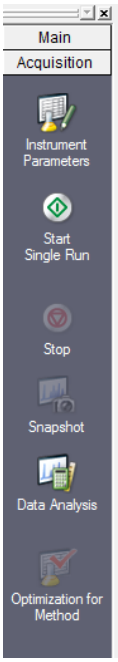
Fill in sample name, sample ID, Method file, and set you file name. Filenames can be incremented if Auto-Increment is checked. Fill in Vial# and Tray number. The Auto sampler Tray id is 12 unless changed with other tray. Fill in injection volume. This is the only place where injection volume is selected.

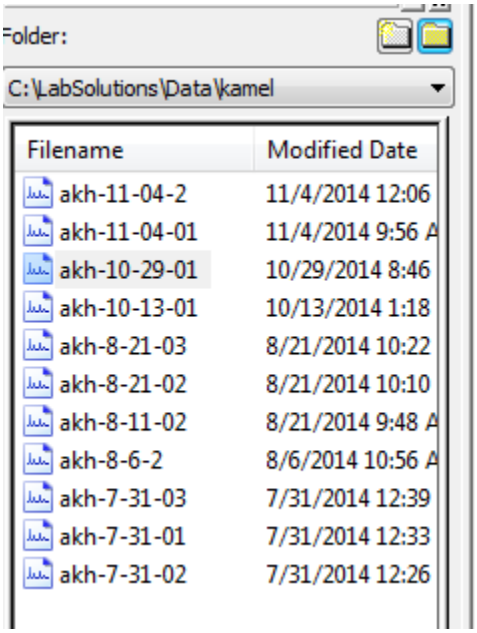
8- Click OK to start acquisition.

Data can be viewed processed and printed from Postrun Analysis browser.

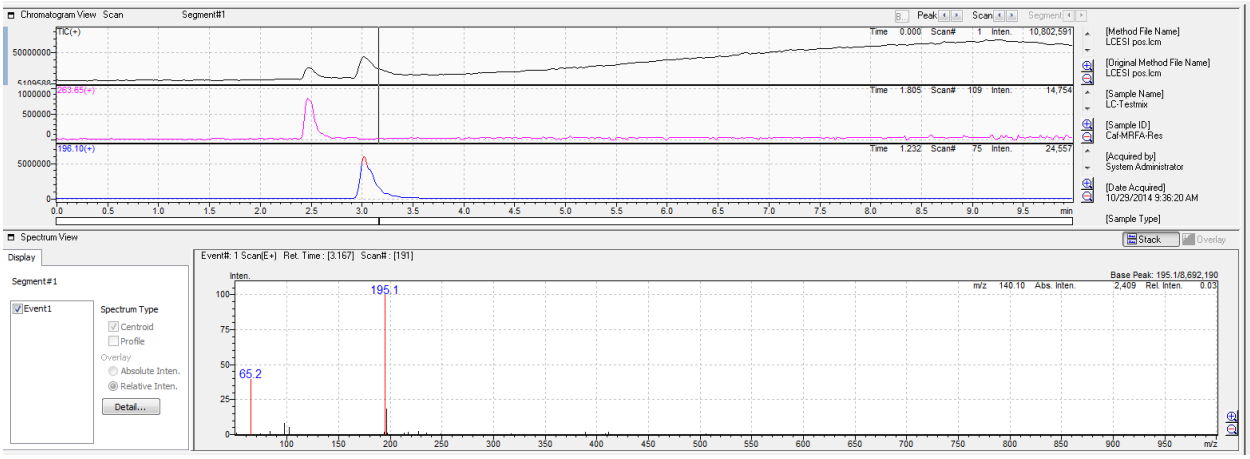
Postrun Analysis: (Data Processing)

From the **Main** menu of Realtime Analysis software, select **Data Analysis**.





Open your data folder and select the data file for processing.

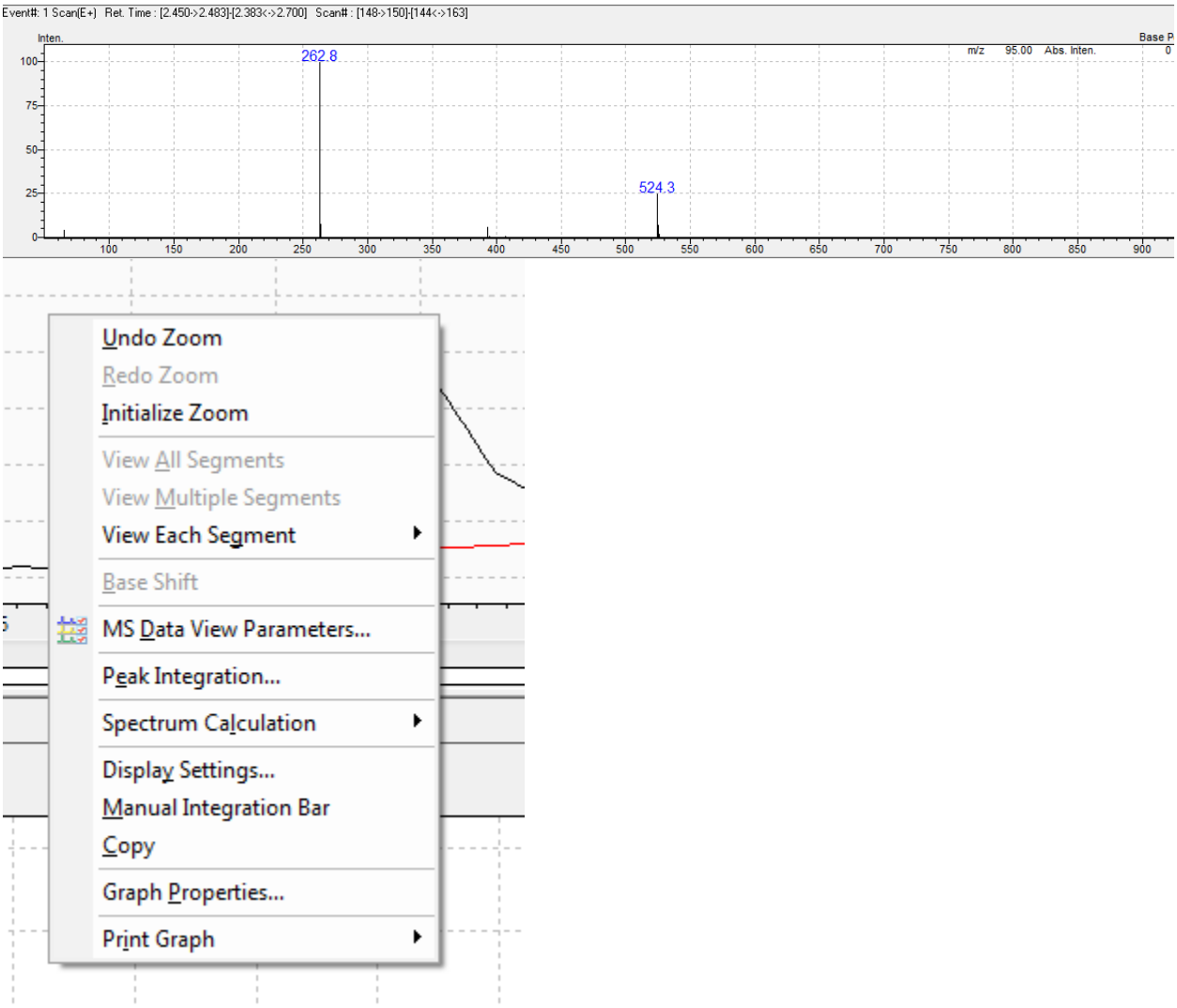


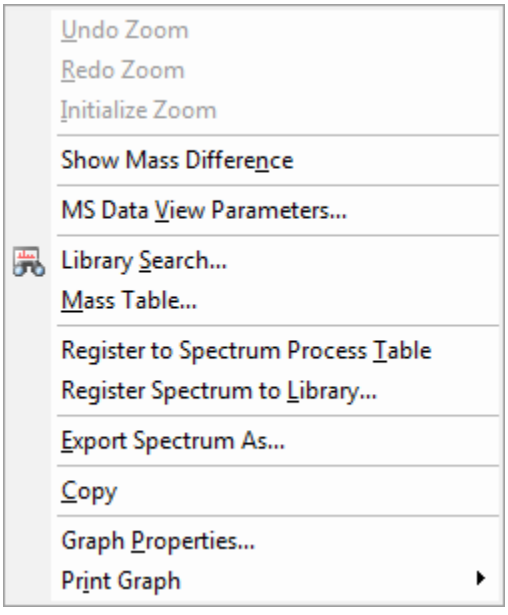
Average mass spectrum and background subtraction:

Click on the (+) button (for averaging)

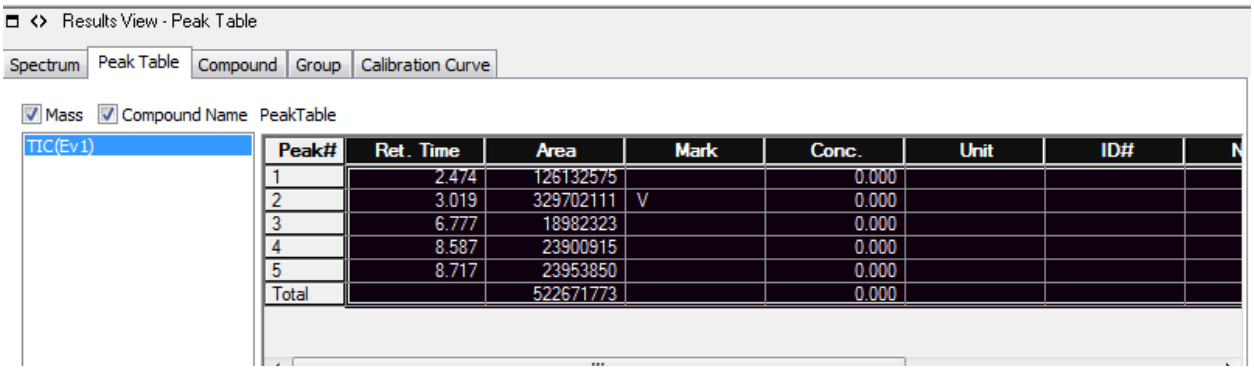
Click and drag on the desired TIC peak

Click the (-) button and drag to select the background or scans to be subtracted

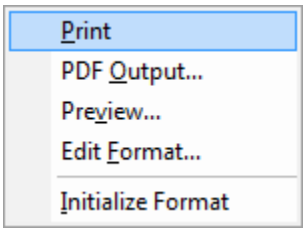

Event#: 1 Scan(E+) Ret. Time : [2.450->2.483]-[2.383<->2.700] Scan# : [148->150]-[144<->163]
Inten.
Base P
m/z 95.00 Abs. Inten. 0
262.8
524.3
Undo Zoom
Redo Zoom
Initialize Zoom
View All Segments
View Multiple Segments
View Each Segment
Base Shift
MS Data View Parameters...
Peak Integration...
Spectrum Calculation
Display Settings...
Manual Integration Bar
Copy
Graph Properties...
Print Graph


Undo Zoom
Redo Zoom
Initialize Zoom
Show Mass Difference
MS Data View Parameters...
Library Search...
Mass Table...
Register to Spectrum Process Table
Register Spectrum to Library...
Export Spectrum As...
Copy
Graph Properties...
Print Graph

Right clicking on the chromatogram (TIC or UV) will open up Peak table view showing all the areas for selected peaks.



Peak#	Ret. Time	Area	Mark	Conc.	Unit	ID#
1	2.474	126132575		0.000		
2	3.019	329702111	V	0.000		
3	6.777	18982323		0.000		
4	8.587	23900915		0.000		
5	8.717	23953850		0.000		
Total		522671773		0.000		



When you finish all experiment:

Uncheck: MS, NEB, and LC pump Flow

Solvents considerations

1. Solvent Bottles

The LC reservoir bottles are by defaults A (H2O/MeOH/Formic acid 90/10/.1) and B (MeOH/H2O/Formic acid 90/10/.1). You can use your own solvents in your own bottles, or bottles that have been assigned to you. You may never use solvents prepared for someone else, or solvent containers that are not yours. We have a supply of 500 mL and 1000 mL bottles especially designed for use with the LCMS. These can be loaned to you for the duration of your project. The content and the owners name must be CLEARLY visible on all containers in our lab. Unlabeled containers will be discarded without hesitation.

2. Solvent Filtering

Pure organic solvents do not need to be filtered prior to use. If any modifiers have been added to the organic phase, then the mixture should be filtered. The aqueous phase, regardless of the presence or absence of modifiers, should be filtered immediately prior to use. Bacterial growth, especially in aqueous solutions, can lead to clogs in the HPLC mixer, the high-pressure stainless

steel tubing, or the capillary transfer tubing, as well as high background and extraneous mass peaks in the mass spectrum. As part of your training, Dr. Harrata will show you how to use the filtering apparatus located in our wet lab.

3. Additives considerations

1- Acids

a- Do not use inorganic acids (may cause source corrosion).

b- Two possible choices are:

- 0.1 % Formic acid
- 0.1 % Acetic acid with 0,02 % TFA

c- Best results arise from acids stored in glass

d- Excellent results have been found with JT Baker acids

2- Surface Active Agents

Detergents and other surface active agents may suppress ionization.