

LCMS 2020 Training Manual

Kamel Harrata (November 2014)

Location: 1238 Hach Hall
Contact: Kamel Harrata, 1236 Hach Hall

Safety

All researchers working in 1238 Hach Hall must complete the EH&S courses: “*Fire Safety and Extinguisher Training*”, and “*Lab Safety: Compressed Gas Cylinders*”. When preparing samples in 1238A, please wear all appropriate personal protective equipment. Aprons, safety glasses, and rubber gloves are available.

Properly dispose of waste solvents and glass pipettes in the containers provided in 1238A. All of the data processing computers and many of the data acquisition computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.

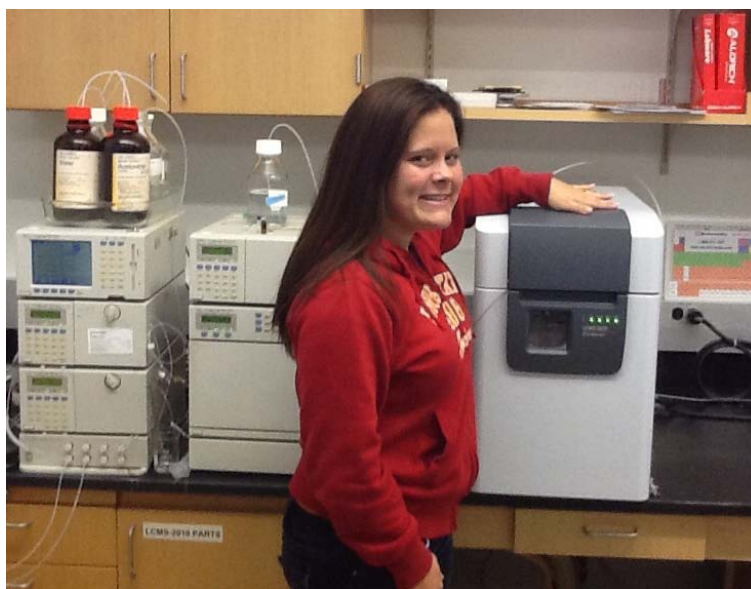


Photo: Toshia Zessin (Graduate Student)

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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).

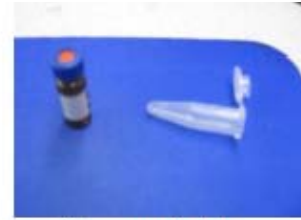
The pressure value should be close to $6 \cdot 10^{-4}$ Pa.



Vortex



Micro Centrifuge



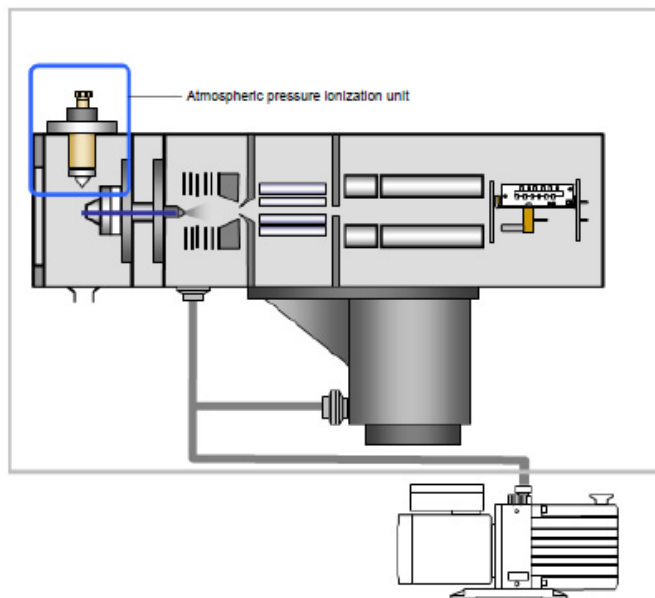
Tubes and vials

Files structures:

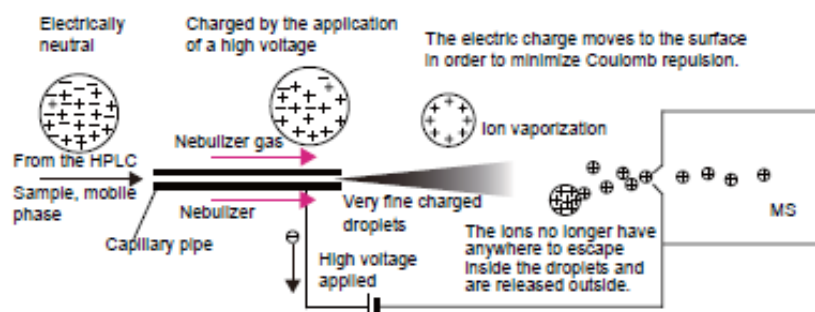
- Each user has data files folder located in his or her directory:
- C:\LabSolutions\Data\username\filename
- Username is the iastate email id, e.g. kamel
- Filename is always: xyz-month-day-nm e.g. akh-8-20-01
- xyz is your initials
- Method files are located in C:\Labsolutions\Methods
- Tune files 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. It is important to indicate that there is no true APCI source with our system. 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)



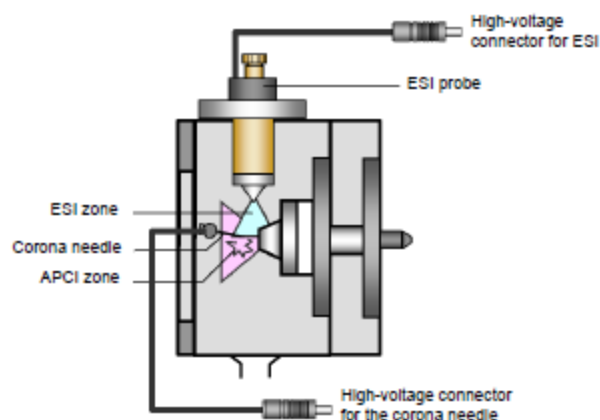
The formation of positive or negative ions (depending on the sign of the applied electrical field) occurs in high yield. In the positive ion mode protonated and/or alkali adduct analyte molecules are generally observed in the mass spectra. In the negative ion mode operation peaks corresponding to deprotonated 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.

8/14/2014 11:35:39 AM Page 1 / 2

LCMS Tuning

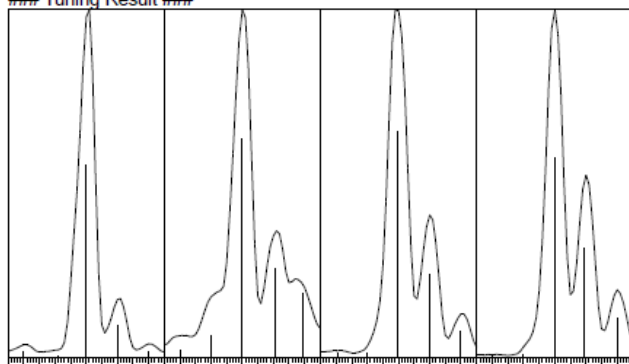
Sample Information

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

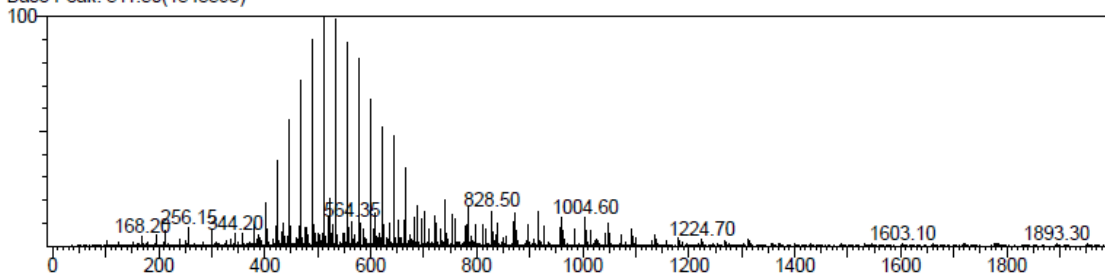


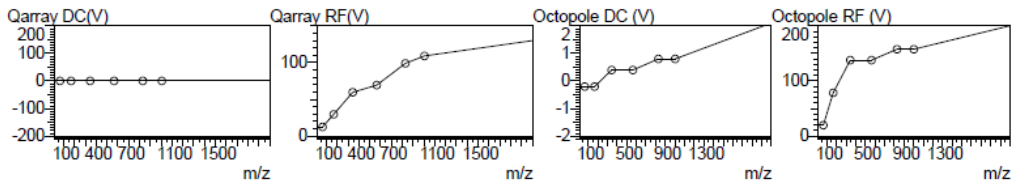
m/z	Mag.	Int.	FWHM
256.15	52.11	111644	0.59
564.35	31.66	208688	0.67
828.50	20.86	327845	0.69
1004.60	27.59	219239	0.62

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

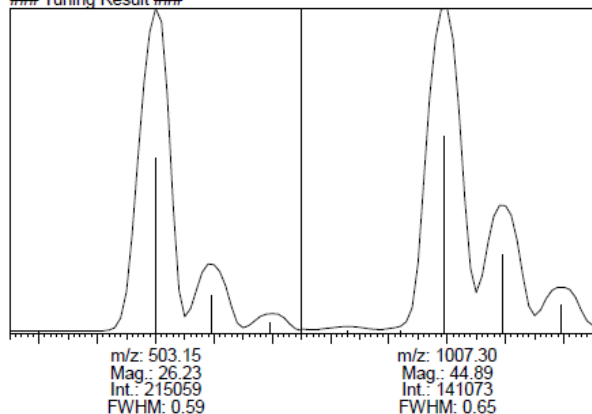
Sample Information

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

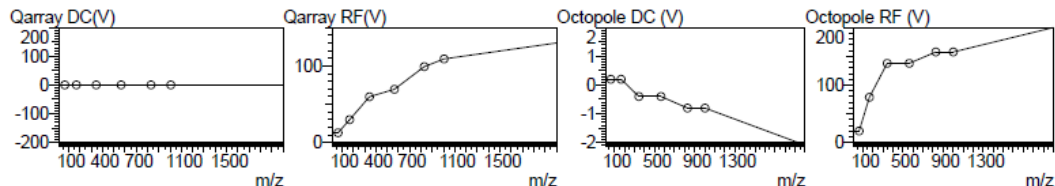
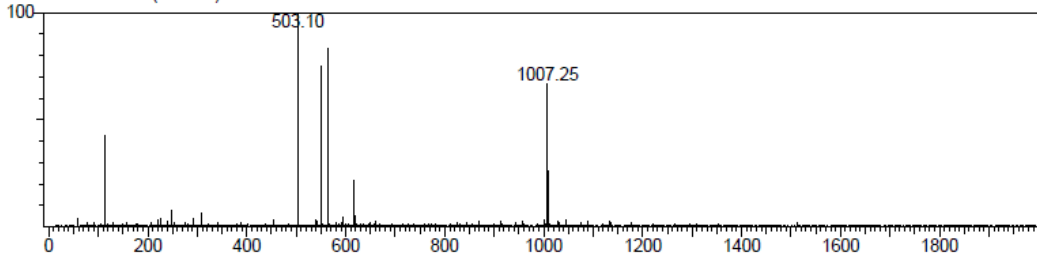
Tuning Result



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)



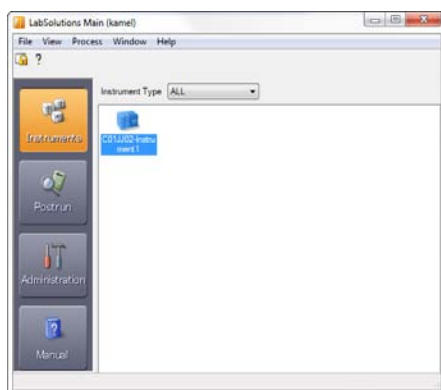
	65.05	168.10	344.20	564.35	828.50	1004.60
DL	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
Qarray DC	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
Qarray RF	+12.5	+29.9	+59.6	+69.5	+99.2	+109.1
Octopole RF	+19.9	+78.9	+137.9	+137.9	+157.6	+157.6
Octopole DC	+0.2	+0.2	-0.4	-0.4	-0.8	-0.8
Entrance Lens	+25.1	+10.1	+5.2	+5.2	+5.2	+5.2

	107.15	1071.50
Prerod Bias	+5.0	+50.0

Data Acquisition: (Step-by-Step)

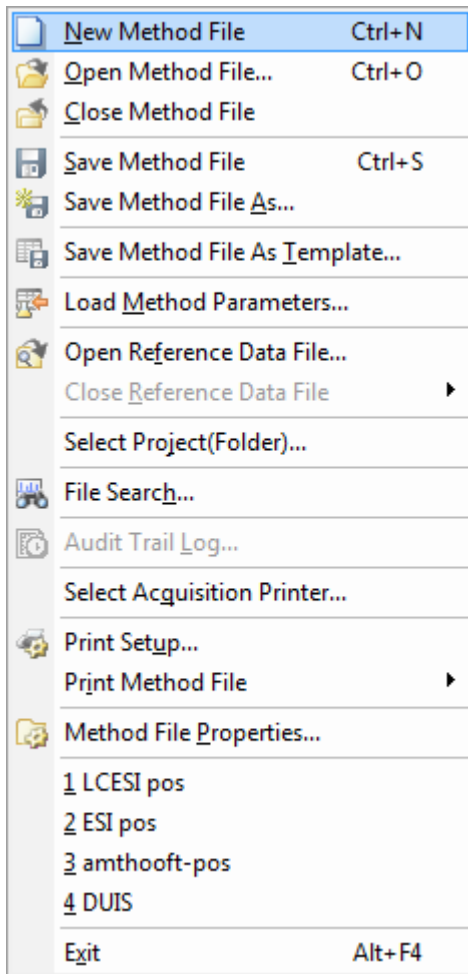
The acquisition software uses Realtime browser while data processing uses Postrun analysis.

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 $6 \cdot 10^{-4}$ 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.

LC Ready
MS Ready

Detail...

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

DRY NEBU DL HEAT IG MS ?

Plot

LC Ready
MS Ready

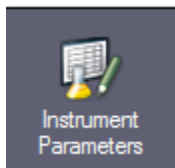
Detail...

Item
Nebulizing Gas F

Max Intensity : 511
Time 1161.450 Inten: 0.000

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:

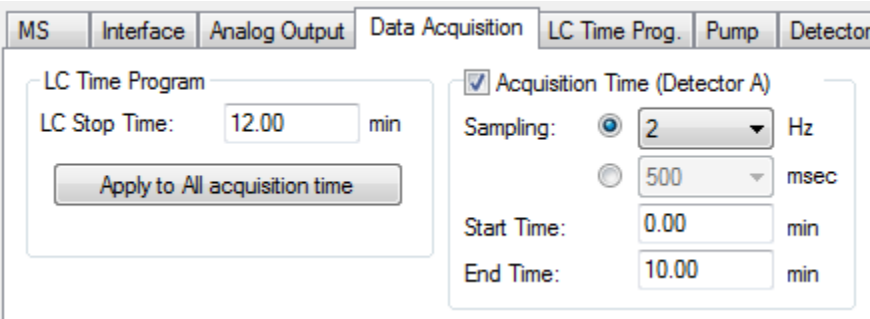


The system can be scanned in positive and negative ion modes simultaneously. This can be accomplished by setting events. Each event has its own parameters.

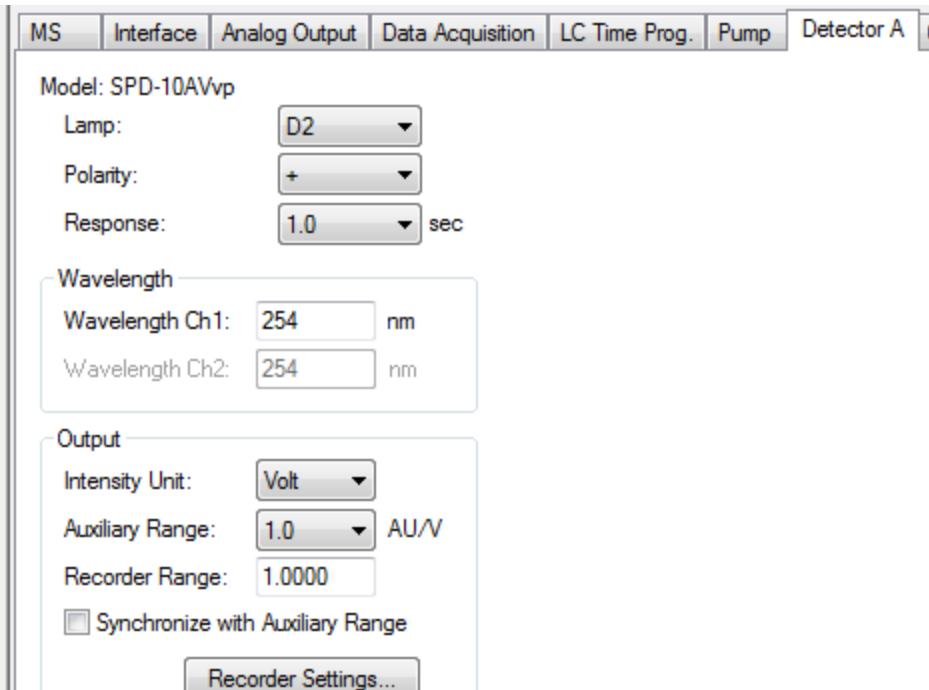
In Interface tab, the system can be set for ESI or DUIS.

Segment	Start Time	End Time	ESI	APCI	Ionization Mode
1	0	12	<input checked="" type="checkbox"/> ON	<input type="checkbox"/> OFF	ESI

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.

MS	Interface	Analog Output	Data Acquisition	LC Time Prog.	Pump	Detector A	Controller	Autosampler
----	-----------	---------------	------------------	---------------	------	------------	------------	-------------

Model: SIL-10ADvp

Autosampler

Sample Rack: Rack 12 Detect Rack

Rinsing Volume: 10 uL

Needle Stroke: 41 mm

Rinsing Speed: 35 uL/sec

Sampling Speed: 15 uL/sec

MTP Tray: Dual

Cooler Temperature: 20 C

Purge Time: 0.5 min

Rinse Mode: Before aspiration

Rinse Dip Time: 0 sec

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

MS	Interface	Analog Output	Data Acquisition	LC Time Prog.	Pump	Detector A	Controller
----	-----------	---------------	------------------	---------------	------	------------	------------

Mode: Binary gradient

Total Flow: 0.500 mL/min

Pump B Conc.: 20.0 %

Pump B Curve: 0

Configured Pumps

Pump A: LC-10ADvp

Pump B: LC-10ADvp

Pump C:

Pump D:

Pressure Limits

Maximum: 4000 psi

Minimum: 0 psi



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.


LC Ready

MS Ready

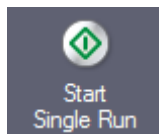
 

Detail...

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



Single Run

Acquisition Information

Sample Name: LC-Testmix

Sample ID: Caf-MRFA-Res

Option...

Method File: LCESI pos.lcm

Data File: Create into:

C:\LabSolutions\Data\kamel\akh-10-29-01.lcd

Auto-Increment: 1, 2, ...

Report:

Data Comment:

Sampler

Vial#: 0 Tray: 12

Injection Volume: 1 uL

Other Handlings

Tuning File:

Background Data File:

Quantitative

Type: Unknown Calibration Level: 0

ISTD Amount #1: 1 Sample Amount: 1

Dilution Factor: 1

Data Processing

TIC Peak Integration Make Spectrum Process Table

Library Search Quantitative

Advanced << OK Cancel Help

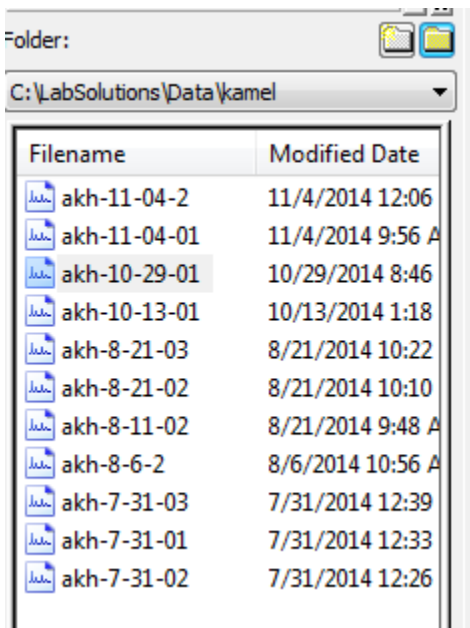
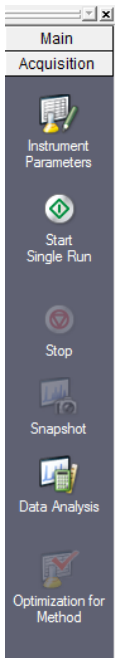
Fill in sample name, sample ID, Method file, and set you file name. Filenames can be incremented if Auto-Increment is checked. Fill in Vial# and Tray number. The Auto sampler Tray id is 12 unless changed with other tray. Fill in injection volume. This is the only place where injection volume is selected.

8- Click OK to start acquisition.

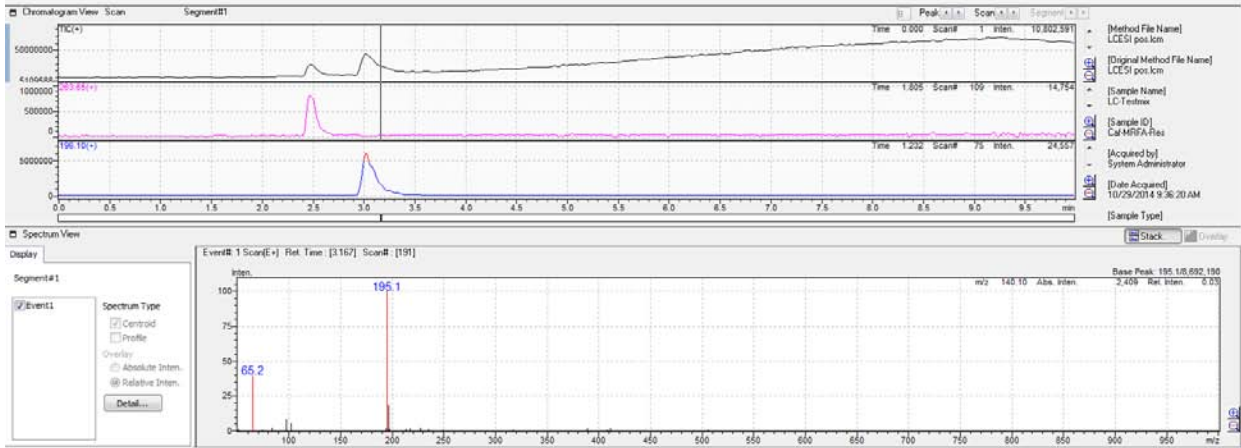
Data can be viewed processed and printed from Postrun Analysis browser.

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.



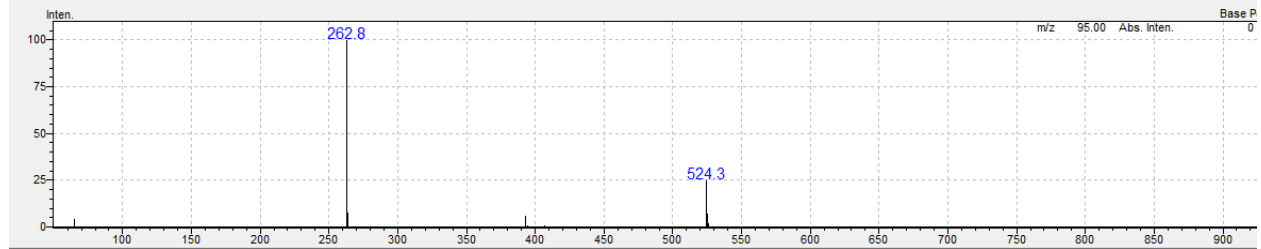
Average mass spectrum and background subtraction:

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]

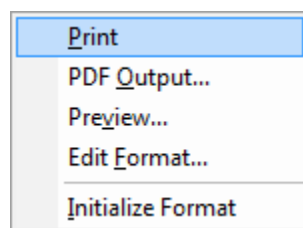


- Undo Zoom
- Redo Zoom
- Initialize Zoom
- View All Segments
- View Multiple Segments
- View Each Segment ▶
- Base Shift
- MS Data View Parameters...
- Peak Integration...
- Spectrum Calculation ▶
- Display Settings...
- Manual Integration Bar
- Copy
- Graph Properties...
- Print Graph ▶

- 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#	N
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 consideration

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.