

# IOWA STATE UNIVERSITY

Chemical Instrumentation Facility

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## QTOF Training Manual

(11/1/2011 K.H.)

**Location: 1238 Hach Hall**

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### 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.



## Table of Contents

Important Notes

p.3

Overview of QTOF Hardware	p.5
Overview of LC System	p.5
Electrospray Ionization Principle (ESI)	p.6
Atmospheric Pressure Chemical Ionization Principle (APCI)	p.7
Tuning and Calibration	p.8
<b>Data Acquisition</b>	p.10

- Acquisition software overview
- Starting and verifying LC and QTOF functionality
- Checking resolution and background ions
- Setting up LC pumps flow and LC gradient, UV, Column,
- Autosampler, and Acquisition parameters
- Setting up worklist

<b>Data processing</b>	p.13
------------------------	------

- Opening layout
- Opening file
- Chromatogram, Spectrum, Navigator
- Averaging scans and subtracting background
- Peak list and integration
- Formula calculator
- Mass calculator
- Bioconfirm software
- Spectrum Deconvolution

## **Appendix**

Additive and buffer summary	p.17
-----------------------------	------

Common button actions in the chromatogram and spectrum view	p.17
Solvent Bottles	p.18
Solvent Filtering	p.19
Samples Rules	p.19

**Important Notes:**

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 (100 ng/ul) are usually enough.



and vials

Vortex

Micro Centrifuge

Tubes

**Actuals**

Parameter	Value
Q-TOF: Not Ready Text Ld	
Q-TOF: TOF Vac	2.04E-07 Torr
Q-TOF: Quad Vac	4.51E-05 Torr
Q-TOF: Rough Vac	2.10E+00 Torr

Take a look at the pressure readings from the Acquisition software.

If any value differs greatly from those, please alert the mass spec personnel.

Make sure that the desired solvents are in the right place:

Solvent A1 is always Water (90%)/Methanol (10%)/.1% Formic Acid

Solvent B1 is always Methanol(90%)/Water(10%)/.1% Formic Acid

Solvents A2 and B2 are reserved to any other solvents. We prefer to keep B2 with Acetonitrile (90%)/Water(10%).

The instrument is always in standby mode when not used. If all solvent lines and connections are in place, open your Method File and turn on the system. Background ions are always present when the system is in the ON state. IF NO BACKGROUND ION IS PRESENT, IMMEDIATELY ALERT PERSONNEL!!!!

ESI response is concentration and sample dependant. Minimum sample concentration is strongly recommended. This will result in better data and minimal memory effects. 10 ppm (0.01 mg/ml) is a good start.

When experiments are finished, take your sample out from the tray if you want it back, otherwise it will be thrown away.

Experiments offered:

- Flow injection nominal mass measurement.
- Flow injection accurate mass measurement, better than 3 ppm.
- ESI and APCI MS/MS are performed with special request.
- House Columns include: XDB C18, Eclipse C18, Extend C18.

!!!When you finish, make sure to return the system to Standby!!!!

## **QTOF Overview:**

**m/z is proportional to flight time squared. Heavy ions will spend longer time to reach the detector.**

$$T = eV = \frac{mv^2}{2}$$

The kinetic energy of ions leaving the source.

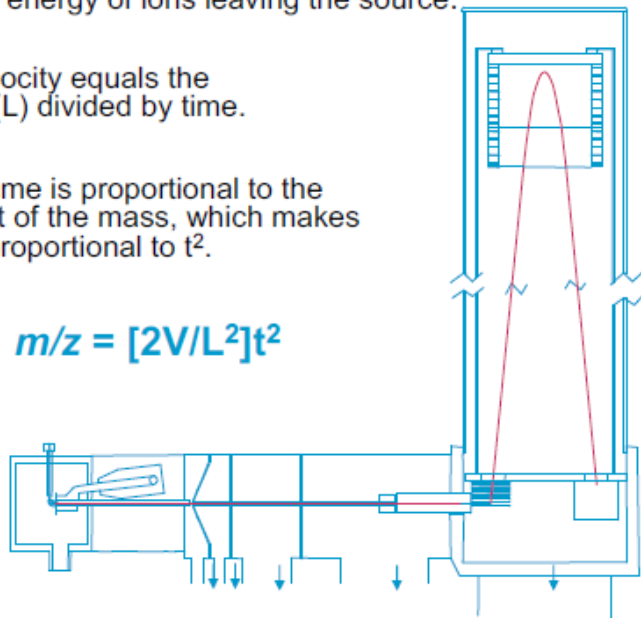
$$v = \frac{L}{t}$$

The ion velocity equals the flight path (L) divided by time.

$$t = L \sqrt{\frac{m}{e \cdot 2V}}$$

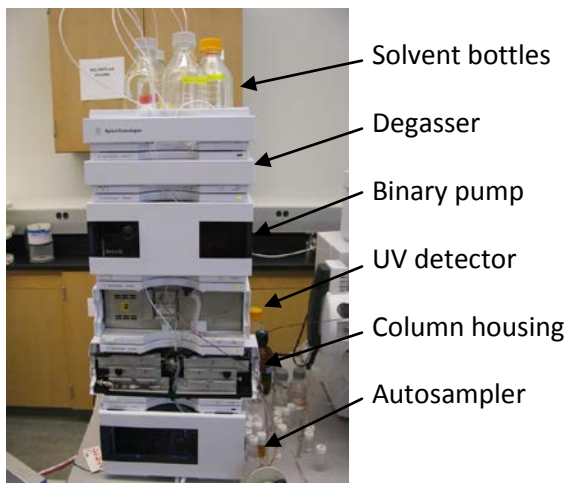
The flight time is proportional to the square root of the mass, which makes the mass proportional to  $t^2$ .

$$m/z = [2V/L^2]t^2$$



The Agilent QTOF 6540 is equipped with exchangeable ESI/APCI ion sources. The ESI source is the JetStream source. The instrument is capable of MS and MS/MS and it is using MassHunter software for data acquisition and processing. LC pumps can handle up to 8000 psi. Mass Accuracy of better than 3ppm is routinely achieved with a resolution (FWHM) of 40,000 for mass 1000. The system is capable of rapid switching between positive and negative ion modes.

## LC System Overview:

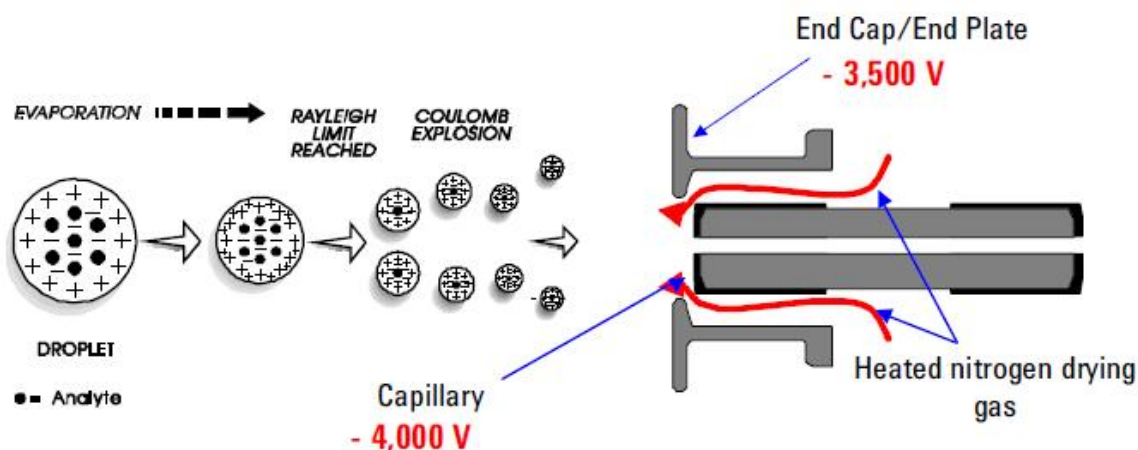


The LC system comprises: A Binary pump, Autosampler, Columns compartment, UV detector.

Typical applications are high throughput methods with fast gradients on high resolution 2.1 mm columns. The pump is capable of delivering flow in the range of 0.1 - 5 mL/min against up to 600 bar (8000 PSI) back pressures.

A switching valve located between injector and detector can be set to either column path or bypass path mode. We have set Column 1 position to column path and column 2 to bypass position. If you observe any solvent leak and/or high back pressure, please alert the mass spec lab personnel.

## Electrospray Ionization principle:



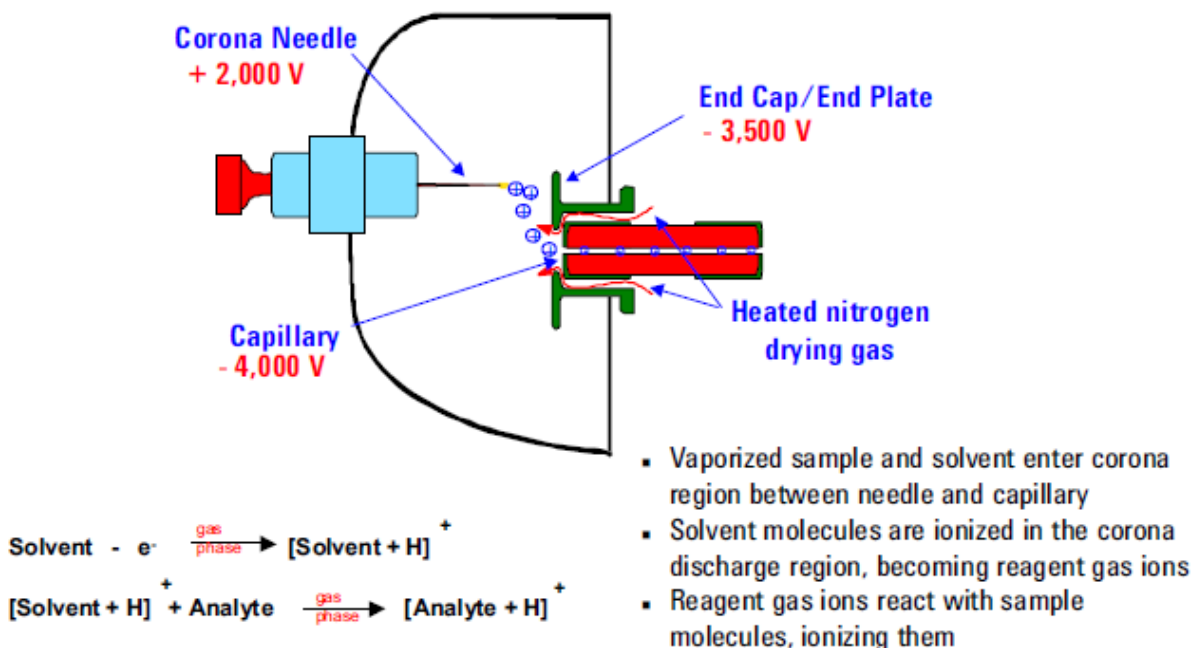
The formation of positive or negative ions (depending on the sign of the applied electrical field) occurs in high yield. In the positive ion mode protonated and/or alkali adduct analyte molecules are generally observed in the mass spectra. In the negative ion mode operation peaks corresponding to deprotonated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

## Atmospheric Pressure Chemical Ionization (APCI)



APCI uses a corona discharge technique to produce ions. This ionization technique is used mostly for low molecular samples that are more or less polar. It is an excellent LC/MS interface that it's easy to use and can accommodate high flow rates. Like ESI, APCI can be used in positive ion mode or negative ion mode.

## Tuning and Calibration

The mass spectrometer mass accuracy is always within a one decimal place. For accurate mass measurement (4 decimal places), the mass spectrometer should be calibrated and calibration masses are always present during acquisition. It is possible to run the experiments without prior calibration. In this case, external calibration can be achieved using known accurate masses that happen to be in the mass spectral results.

Typically the instrument is set to High Resolution mode with standard mass range (3200 m/z)



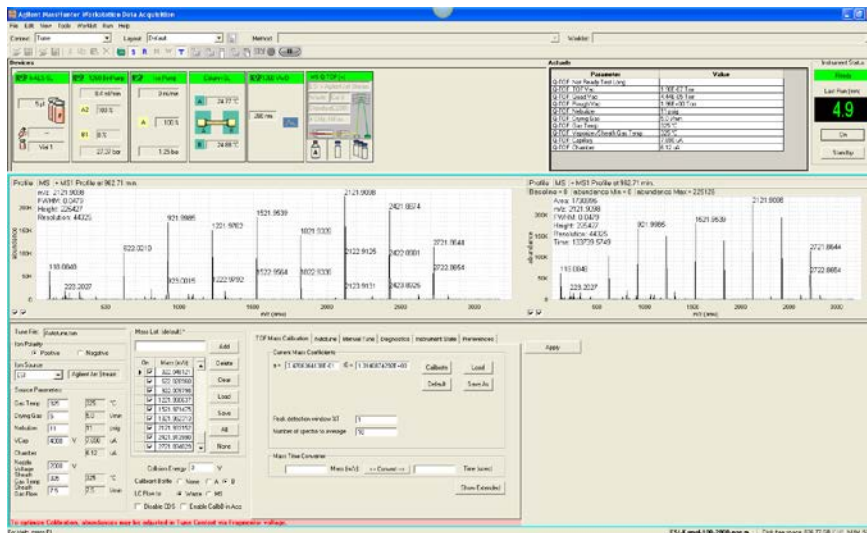
If there is a need for Extended Mass Range, please inform the mass spec personnel. The instrument is capable of measuring masses up to 10,000 dalton when operated under 1 GHz conditions.

The instrument is tuned only by the mass spec personnel.

## Calibration:

### ESI - L Calibration Ions (formerly ESI-TOF) Empirical Formulas for High Resolution MS

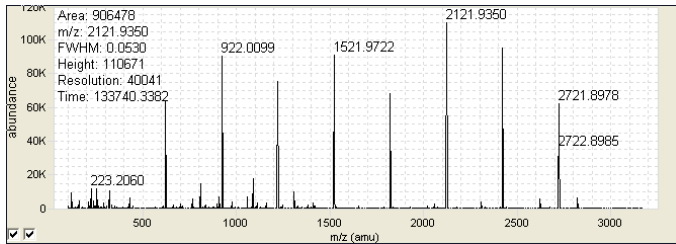
Positive Ion (m/z)	Empirical Formula	Negative Ion (m/z)	Empirical Formula
118.086255	C5.H12.O2.N	112.985587	C2.O2.F3
322.048121	C6.H19.O6.N3.P3	301.998139	C6.H.O.N3.F9
622.028960	C12.H19.O6.N3.P3.F12	601.978977	C12.H.O.N3.F21
922.009798	C18.H19.O6.N3.P3.F24	1033.988109	C20.H18.O8.N3.P3.F27
1221.990637	C24.H19.O6.N3.P3.F36	1333.968947	C26.H18.O8.N3.P3.F39
1521.971475	C30.H19.O6.N3.P3.F48	1633.949786	C32.H18.O8.N3.P3.F51
1821.952313	C36.H19.O6.N3.P3.F60	1933.930624	C38.H18.O8.N3.P3.F63
2121.933152	C42.H19.O6.N3.P3.F72	2233.911463	C44.H18.O8.N3.P3.F75
2421.913990	C48.H19.O6.N3.P3.F84	2533.892301	C50.H18.O8.N3.P3.F87
2721.894829	C54.H19.O6.N3.P3.F96	2833.873139	C56.H18.O8.N3.P3.F99



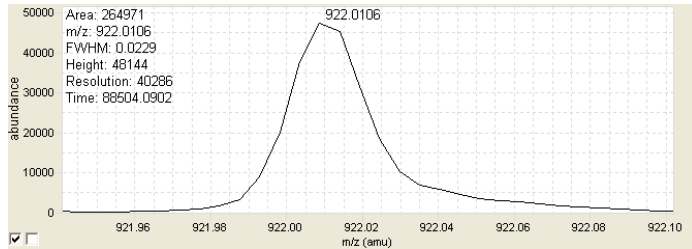
Put the system in the ON state: In the **Context** pull down, select **Tune**.

Select calibrant **bottle B** for the calibrant mixture to flow

into the ion source. The flow stream from LC should go to waste. Select polarity. Make sure all the calibrant masses are selected.



Visually inspect the MS profile. All calibrant ions should be observed.



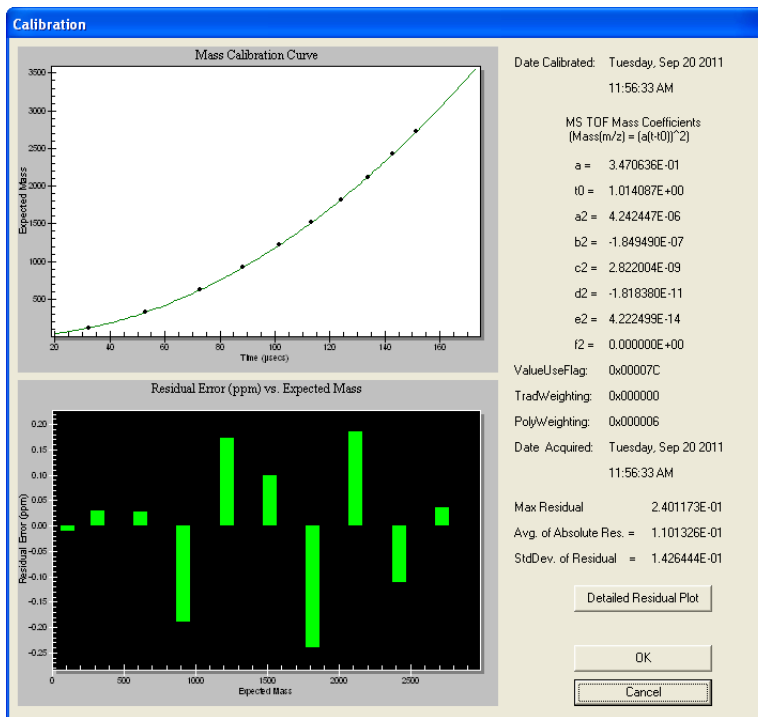
Verify that the resolution of mass 922.0097 (Positive ion mode) mass 1033.9881 (negative ion mode) is about 40,000 (FWHM).

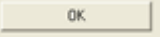
TOF Mass Calibration software interface showing the following settings:

- Current Mass Coefficients:
  - a = 3.4706364138E-01
  - t0 = 1.0140874292E+00
- Buttons: Calibrate, Load, Default, Save As
- Peak detection window %T: 1
- Number of spectra to average: 10
- Mass Time Converter:
  - Mass (m/z): [ ]
  - Time (usec): [ ]
  - Convert button: <-- Convert -->
- Show Extended button

From the TOF Mass Calibration menu click Calibrate.

The result should show mass accuracies less than 3 ppm for all masses.



Click  to accept the calibration

Calibrant Bottle  None  A  B  
 LC Flow to  Waste  MS

Reset calibrant Bottle to None and LC flow to MS.

Return to Acquisition mode and proceed to acquiring data. The calibrant A (Lock masses or reference masses should be allowed in order to acquire data in high accuracy mode).

## **Data Acquisition:**

Open your Method File and your Worklist. Visually inspect all devices and vacuum status. Place your sample in the autosampler plate.

Samples can be placed in positions P1A1, P1A2 etc... It is always wise to inject blank (solvent only) first. This will constitute the instrument background that eventually can be subtracted.

Note that all MS files are located in the **D:\MassHunter\Data\kamel2** directory.

Data file naming is always QTOF1100001, QTOF1100002 etc... where the 11 implies the year (2011 in this case).

For a single sample run you can use the following procedure or you can skip and use the worklist.

*Sample information and experiment parameters field*


Fill in Sample name or ID, Injection volume **h-ALS-SL**; Set Pump conditions **1260 BinPump**; Column bypass state **Column-SL**; Set UV conditions **1260 VWD**; Set MS Q-TOF conditions **MS Q-TOF**.

All these fields are already correctly set in your Method File except Sample Name.

Set Stop time. The stop time is usually set to coincide with the time of the total gradient LC time.

From **1260 BinPump** and **Timetable** edit the LC gradient

	Time	B%	Flow	Max. Press.
1	0.00	0.0	0.800	400
2	5.00	100.0	0.800	400
3	5.10	0.0	0.800	400
4	11.00	0.0	0.800	400

For a single sample run click 

The sample is injected, and data can be viewed in the Profile Display window and/or in the processing software.

Acquisition can be stopped manually 


Data can be processed in MassHunter Qual program. (See data processing)

Acquiring data using Worklists:

Open Worklist: Ctrl+W and select your worklist. You can Edit and save your worklist. A user can create only one worklist. The naming of worklist should be "USERNAME.wkl".

Edit worklist: **Sample name, Sample Position, Method File, Data File.**

	✓	Sample Name	Sample Position	Method	Data File	Sample Type
1	✓	meOH	P1-A1	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100539.d		Sample
2	✓	Se-0	P1-A2	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100540.d		QC
3	✓	Se-1	P1-A3	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100541.d		Sample
4	✓	Se-2	P1-A4	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100542.d		Sample
5	✓	Se-3	P1-A5	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100543.d		Sample
6	✓	L2-0	P1-B1	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100544.d		Sample
7	✓	L2-1	P1-B2	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100545.d		Sample
8	✓	L2-2	P1-B3	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100546.d		Sample
9	✓	L2-3	P1-B4	ESI-Kamel-100-1000-Grad1-Rich-Kemin-p D:\MassHunter\data\Kamel1\QTOF1100547.d		QC

Highlight the targeted files and click start experiment 

Acquisition can be stopped manually  or automatically when LC gradient is finished.

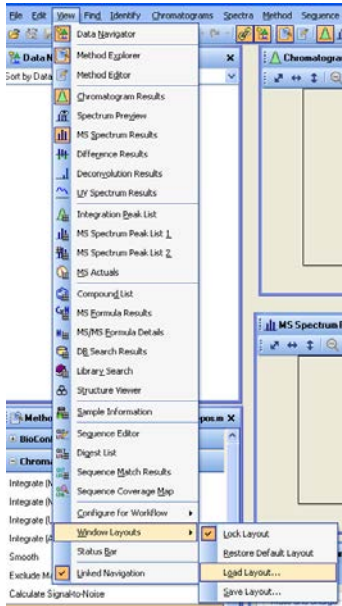
After each run, enough time should be given to the starting LC conditions in order to calibrate the column. Rule of thumb the volume necessary to calibrate the column is 10 to 20 times the volume of the column.

## Data Processing:



Data processing is accomplished using the MassHunter Qualitative Analysis software

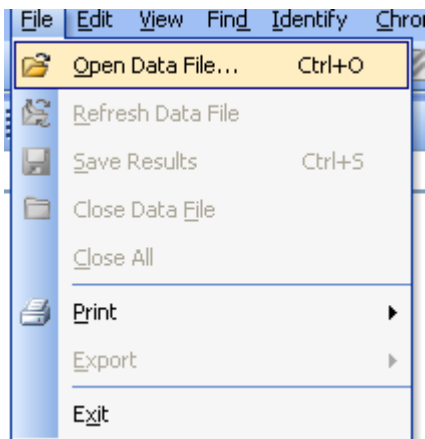
The following are the essential steps to taken to obtain Chromatograph and mass spectra:



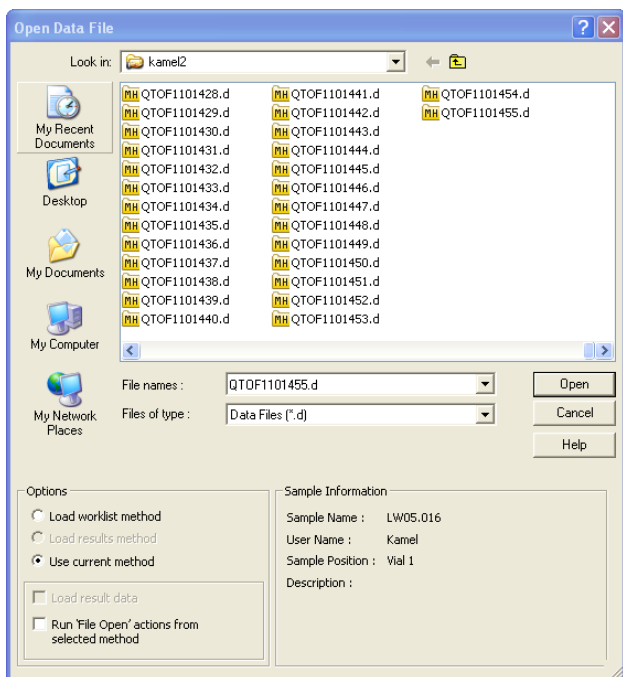
Load you preferred layout

Your layout will be set during the actual training and will probably be “Yourname.xml”

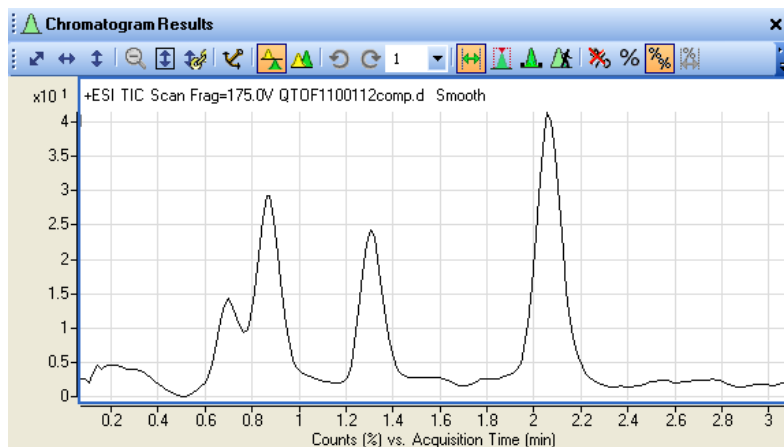
For the purpose of this training I am using “kamel-routine.xml” layout that comprises: Data Navigator, Method Explorer, Chromatogram Results, MS Spectrum Results, Formula Calculator, and Mass Calculator.






Open Data File

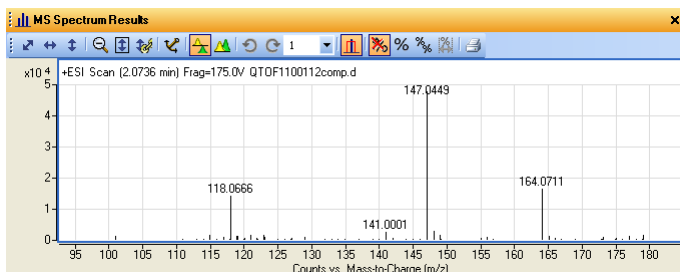


Double Click with left mouse button on the target data file



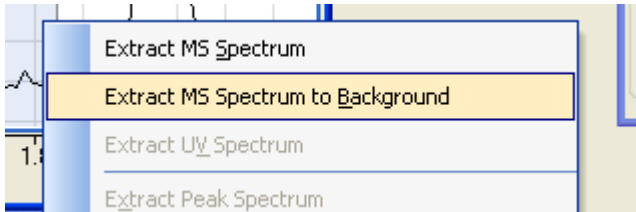
-  Display all data
-  Normalize display
-  Separate LC traces

Right mouse button hold and drag can be used to zoom on a specific region of the Chromatogram.

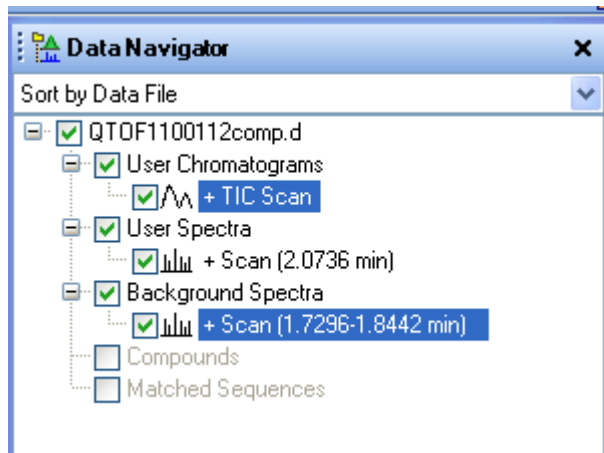


Left mouse button double Click will display the mass spectrum of that scan.

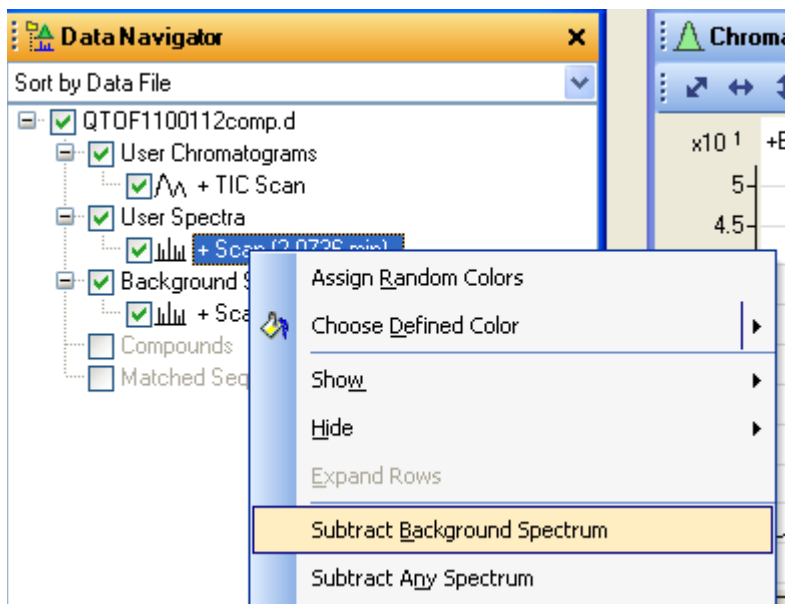
Left button and drag, then double click inside the shaded area to average scans.



Left mouse button and drag and release followed by Right click in the shaded area to specify the background spectrum.

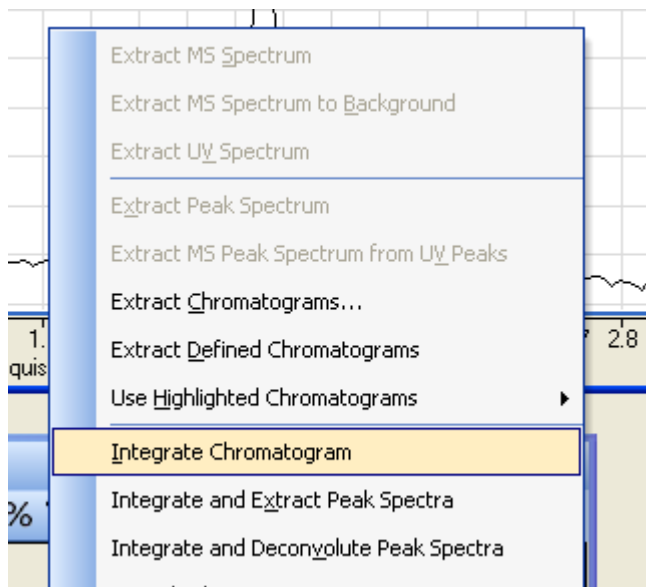


Background spectrum will be display in the Navigator window.



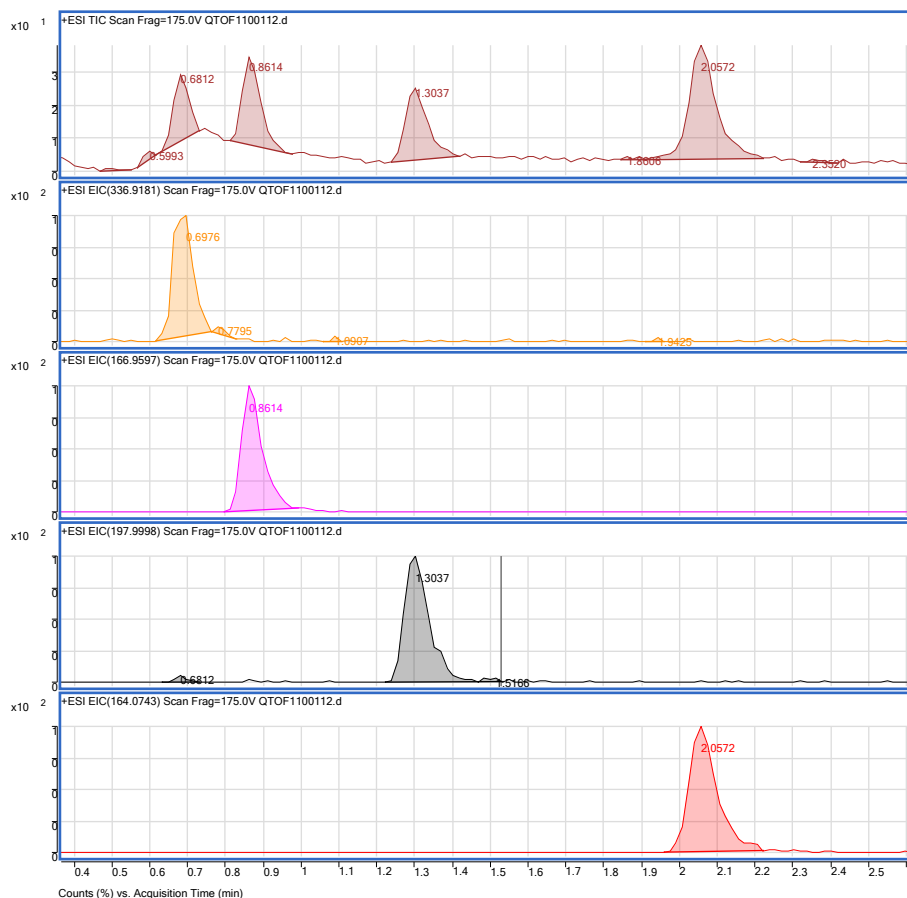
Right click on the averaged spectrum and select Subtract Background Spectrum



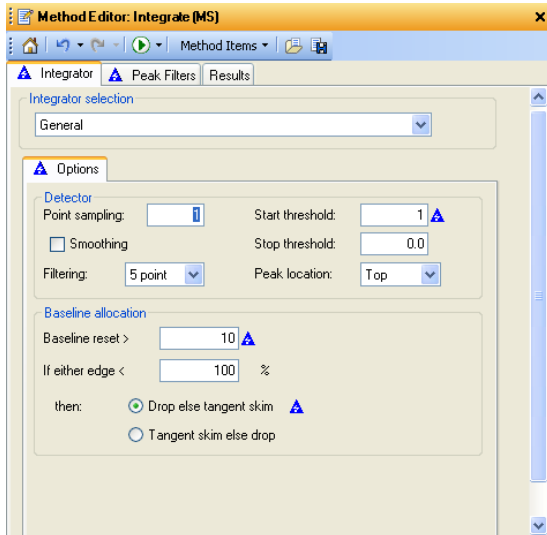


Right click in Chromatogram and select Peak Integration to integrate peaks.

Also double click on any mass peak (in spectrum) to display selectively that mass in the Chromatogram (EIC)



Colors can be set by Right clicking and “Choose Define Color”



From Method Editor-Chromatogram-Integrate (MS), Peak Area and Heights can be manipulated.



Left mouse button for printing the window

## Appendix

### Common button actions in the chromatogram and spectrum view:



Reset display



Reset display without normalizing



Normalize display



Toggles previous display



Autoscale Y-axis during zoom



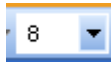
Set Anchor display



Separate displays



Overlaid displays



Set number of displays



Range select



Peak select



Manual integration



Walk chromatogram



Print chromatogram display

## **Additive and buffer summary:**

For positive ion mode ESI use proton donor, e.g. Acetic Acid or Formic acid.

For negative ion mode ESI, use proton acceptor, e.g. TEA

Trifluoroacetic acid (<0.1 % v/v) is often used to assist the chromatographic separation.

Ammonium Acetate and Ammonium Formate are often used as buffer systems.

## **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.

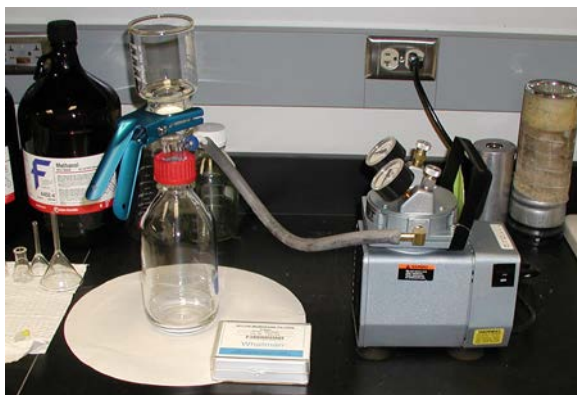
## Solvents:

### 1. Solvent Bottles

You must 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. Unlabelled 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.



## Samples Rules:

### 1. Preparation

If possible, samples should be prepared in your own lab. In emergency situations you may use the equipment in our wet lab, including the balance, centrifuge, acid-washed

glassware, and sample filtration cartridges. We do have a supply of nanopure water available. This can be used for final rinse or sample dilutions. If you use any of our glassware, *do not* return it to the glassware rack or shelf. Put it in the sink and we will eventually clean and acid wash it. At no time should phosphate detergents be brought into or used in our lab.

## **2. Sample Filtration**

All samples should be filtered through a 0.45 micron filter just prior to analysis. No exceptions. Ignoring this rule will be cause for suspension of privileges. We have a limited supply of these filters for use in cases of emergency. You should order and use your own.

## **3. LC Columns and Syringes**

You must provide your own LC column, as appropriate for your project. You must provide your own syringes (blunt-tip; especially designed for LC use). You WILL be charged for damage to rotor seals if you use sharp-tip syringes.

