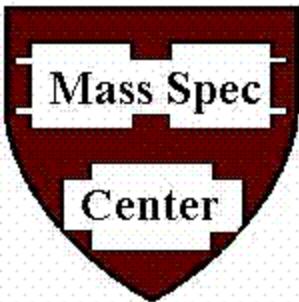


Open Access Training

Agilent LC/MS TOF

Mass Spec Center

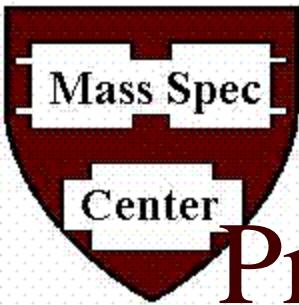


Open Access Setup: You are responsible for the following:

- Get a RC account to get access to SPINAL-make sure your lab admin has a RC account (also follow link from our website)

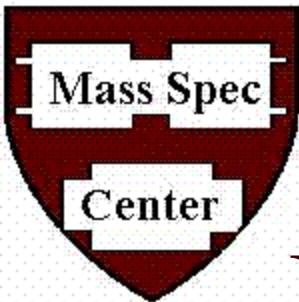
<http://rc.fas.harvard.edu/account-access-request-forms/division-of-scienceresearch-computing-domain-account/>

- Inform our staff (Sunia) when you have RC account, so you can be added to the SPINAL user group
- Once you are added to the user group, you must login and send request to your lab admin for 33 digit billing code. Please do it for both slotbased and hourly reservation
- Once this is approved by your lab admin-you are ready to use₂, open access



Procedure for Using the Instrument

- Please note there is no use of the instrument allowed without logging into SPINAL and making a “reservation”. If you are running one sample, you make a single slot based reservation. The time on the reservation for slot based reservations does not matter. The samples are queued up and run. However making the “reservation” is essential for payment.



Instrumentation & Software

Instrumentation

Agilent 1200 Series LC system

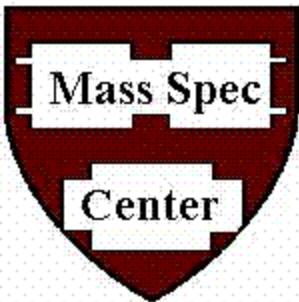
6210 TOF MS with ESI source

Software

MassHunter

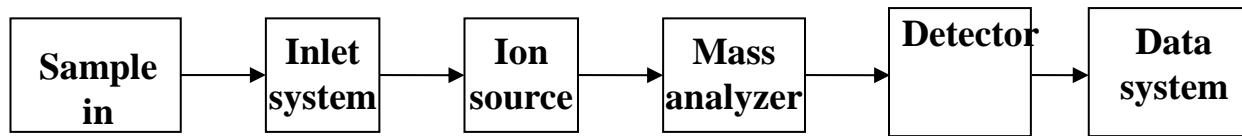
EasyAccess





Introduction to Mass Spectrometer and LC/TOF

Mass spectrometer
Schematic



Inlet systems:

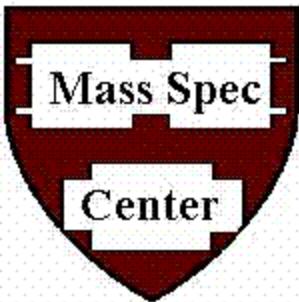
- HPLC
- GC

Ion source:

- Electrospray (ESI)
- MALDI
- Electron ionization (EI)
- APCI
- APPI

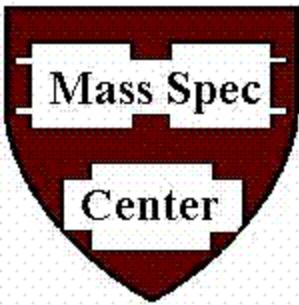
Mass analyzer:

- Quadrupole
- Time-of-flight
- Ion trap
- Magnetic sector
- FTMS

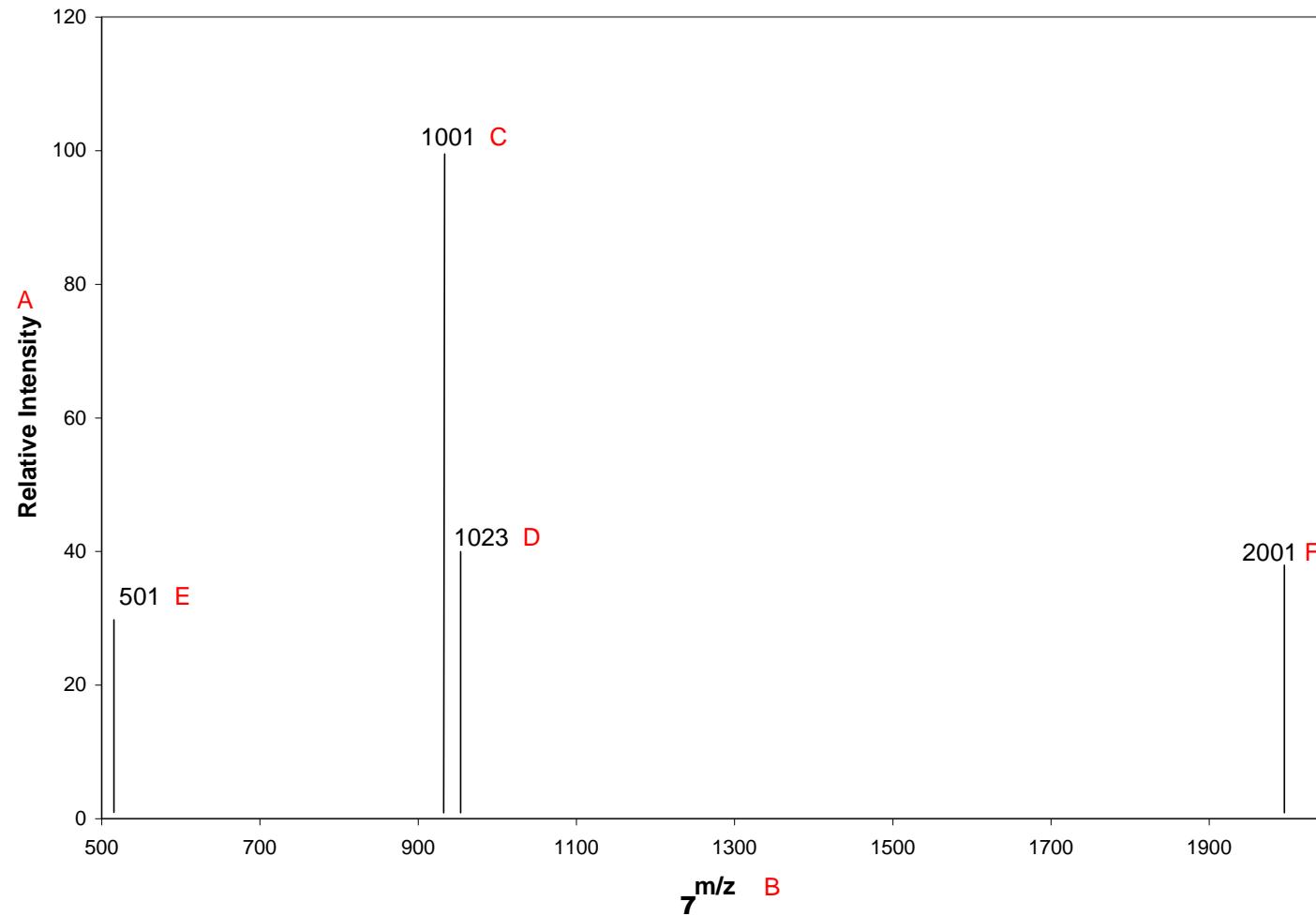


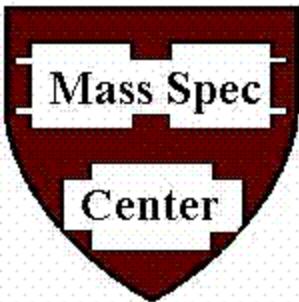
Introduction to TOF analyzer

- The basic concept of TOF Mass analyzer (Time-of-flight) is that the ions are separated based on the time it takes for the ion to drift down the flight tube to the detector in a field free region. Lighter ions have higher velocities than heavier ions and reach the detector first.



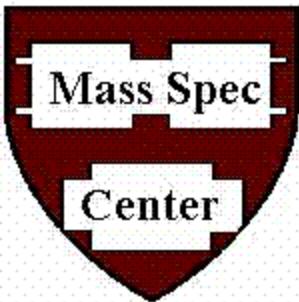
Interpreting Mass spectra





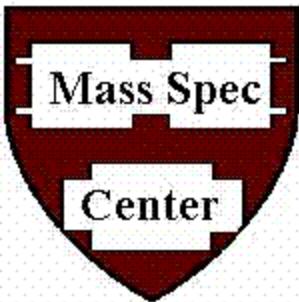
Interpreting Mass spectra

- The Y axis is labeled as relative intensity. This is the intensity relative to the tallest peak in the spectrum with the tallest peak set to 100%.
- m/z, mass divided by charge
- base peak: the tallest peak in the spectrum
- In LC-TOF, ions usually have multiply charge
- Sodium adduct: 1000+23
- Double charge: $(1000+2)/2$
- Dimer: 2000+1, when the concentration of the sample is too high
- At higher resolution peaks may be isotopically resolved and appear as multiple peaks separated by a 1 Da mass difference



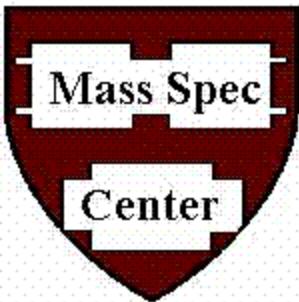
Important sample preparation

- Samples must be completely dissolved
- Keep sample concentrations below 0.01 mg/mL to avoid severe system contamination. If you can see the sample (by color), it's too much!
- Filter samples after dissolution to remove solids
- Centrifugation is a good alternative if filtration is a problem.
- For moisture sensitive compounds, use an aprotic solvent CH₂Cl₂, Acetonitrile, THF can be used. Please note there is water in the elution buffer in the HPLC. You may want to have these samples run under special solvent conditions by our staff.



OpenAccess Overview

- Walk-up MS instrument to users 24/7
- ESI source – suitable for polar, acidic and basic molecules < 3200 m/z
- Capable of carrying out low and high resolution (4 decimals) analysis



OpenAccess Overview

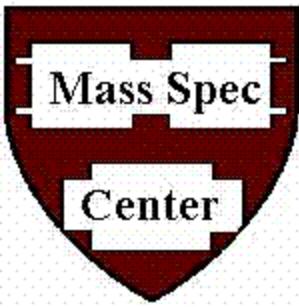
FIA-MS

(flow injection analysis-
direct injection/no
separation)

- Direct flow injection
- Suppression if complex mixture or detergents/salts present
- Confirmation of molecular formula
- Fast, run-time ~ 3 mins

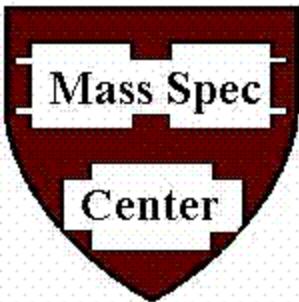
LC-MS

- Running through column (C18, 30mm x 2.1mm, 3.5µm)
- Separation of components
- Additional info from UV data
- Run time ~ 12 mins



OpenAccess Methods

| Method | Description |
|---------------------|---|
| OA_FIA_POS | Direct flow, positive ion mode , high accuracy, formula required |
| OA_FIA_NEG | Direct flow, negative ion mode , high accuracy, formula required |
| OA FIA POS NOM | Direct flow, positive ion mode , nominal mass (not high accuracy-nominal mass) |
| OA FIA NEG NOM | Direct flow, negative ion mode , nominal mass (not high accuracy-nominal mass) |
| OA_LCMS_POS | General LCMS method ~15 minutes, positive ion mode |
| OA_LCMS_POS_Formula | General LCMS method ~15 minutes, positive ion mode With formula requirement |
| OA_LCMS_NEG | LCMS, negative ion mode |
| | General LCMS method ~15 minutes, negative ion mode With formula requirement |

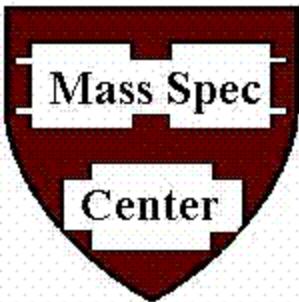


Methods in Open Access

| MethodID | MethodName | MethodInfo | Time |
|----------|-----------------------------------|---|-------|
| 1 | D:\MassHunter\FIA_Neg_50_3000 | wide range FIA | 2.00 |
| 2 | D:\MassHunter\LC_Neg_LargeMW | large MW LC-MS method | 16.00 |
| 3 | D:\MassHunter\LCMS_LargeMW_pos_RC | Reference correction | 20.00 |
| 4 | D:\MassHunter\OA_FIA_NEG_NOM | Nominal mass measurement | 2.00 |
| 5 | D:\MassHunter\OA_FIA_POS_NOM | OA FIA POS NOM | 3.00 |
| 6 | D:\MassHunter\OA_FIA_NEG | Negative ion mode flow injection | 2.00 |
| 7 | D:\MassHunter\OA_FIA_POS | FIA positive ion mode | 2.00 |
| 8 | D:\MassHunter\OA_LCMS_NEG_FORMULA | negative ion LCMS method | 16.00 |
| 9 | D:\MassHunter\OA_LCMS_NEG_SMALL | LCMS method for small molecules | 20.00 |
| 10 | D:\MassHunter\OA_LCMS_POS_FORMULA | formula confirmation for positive ions | 20.00 |
| 11 | D:\MassHunter\OA_LCMS_POS_SMALL | LCMS method for small molecules | 20.00 |
| 12 | D:\MassHunter\OA_POS_LARGEMW | high accuracy method with large molecules | 2.00 |
| 13 | D:\MassHunter\OA_PROTEIN_LCMS | LC-MS method for proteins | 20.00 |
| 14 | D:\MassHunter\PROTEIN_FIA | FIA method for proteins | 2.00 |

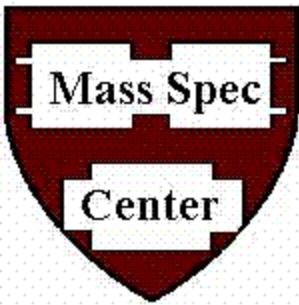
A complete list of current methods. Please ask if you want a customized method and we will be happy to work with you to optimize this

The LCMS methods with “formula” are accurate mass methods which will search for the specified formulas and print a report if these are found



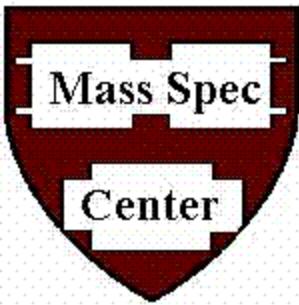
Policy

-
- SPINAL slot based submission necessary for each sample or by the hour
 - Suitable solvents are Methanol, Methylene Chloride, Acetonitrile or Water.
 - Concentration is sample dependent but a good start point is **0.001** mg/mL for FIA and **0.01** mg/mL for LC samples.
 - Use 2 mL vials with insert (available in stockroom). Fill up to at least 1/3 of the insert.
 - Take your sample out from the tray if you want it back, otherwise it will be thrown away.
 - Please show courtesy to your peers if there is a line.



Good Practice

-
- Before login, please check the standard spectrum to make sure the instrument is in good condition for the day. Facility staff will perform calibration daily.
 - You do not have to, but to avoid possible carryover from previous sample, run a blank (such as methanol). There is no charge on blank run.
 - Don't overload the machine with too concentrated sample, a response comparable to our daily standard is good enough. Concentration > 0.5 mg/ml may produce dimers and carryovers. $> 10^6$ for Pos ion and $> 10^5$ for Neg ion is an indication of too much material.
 - If there is a problem, please notify us ASAP so we can help.

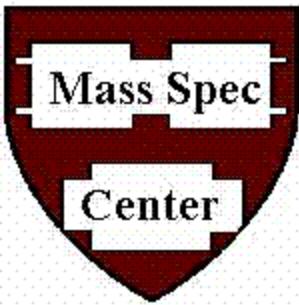


Data Analysis

- Open Access is set up to process and email you a report automatically.
- The automatic data processing is specific to the type of analysis carried out. For the methods that do not require formula, major peaks are reported. For methods where formula is required, report generation is done after a script uses the accurate mass of the predicted ions to search for the target formula and confirms with isotope pattern match
- Masshunter Qual Browser (green icon) can be used to analyze the raw data. There is a computer across from the ESI-TOF labeled SPINAL login computer that has this software. If you like, you can transfer your raw .d data files on a flash drive and use this computer to look at the raw data.

MS Interpretation Hints:

- Signal Intensities - distinguish real signals from noise.
- MS and UV Peaks - confirm elution on LCMS with redundant signals.
- Isotope Pattern - determine a valid ion, spike or saturated signal.
- Ion Adducts - salt or solvent adducts add mass to parent ion.
- Dimer Adducts - concentrated solution form cluster ions ($2M+H^+$).



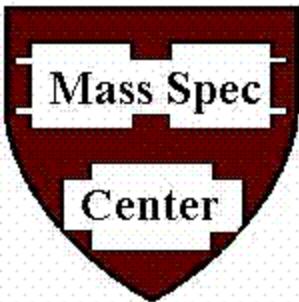
Adduct Ions

□ ESI Positive

- M + 18 (NH₄)
- M + 23 (Na)
- M + 39 (K)

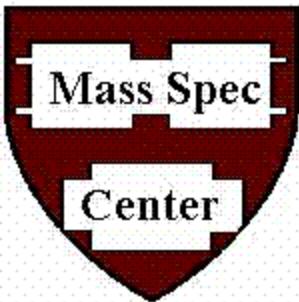
□ ESI Negative

- M + 45 (formic acid)
- M + 35 (Cl)



Data Delivery

- PDF email data
- Raw data (files are saved for a limited time)



Save as PDF File

- Find compounds by formula function in MassHunter Qual Browser helps you look for target formulas, compare to predicted isotope pattern, and get ppm relative error for target compounds.
- Print compound report function will allow you to generate a .pdf report for “compounds” found.