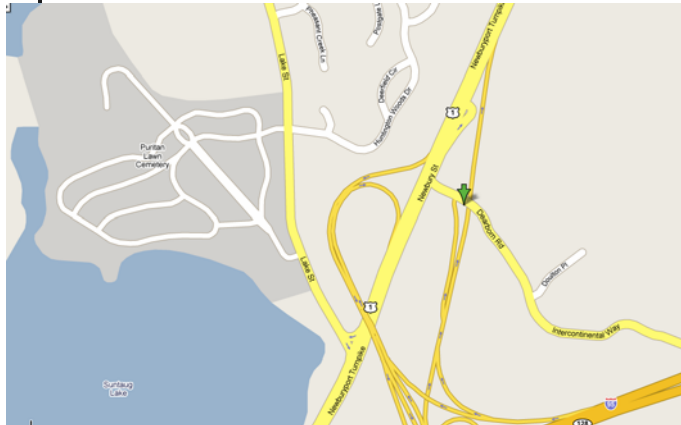


AccuTOF™ LC Training Course

JEOL USA, Inc.
11 Dearborn Road
Peabody, MA 01960

Area map



Restaurants:
Wendy's
Bennigan's
Bertucci's

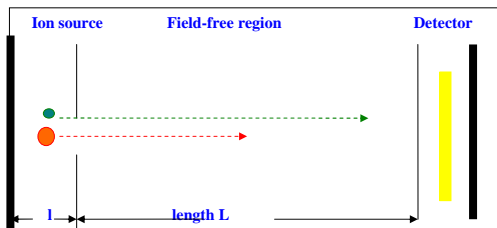
AccuTOF™ LC Training Course

- **Monday**
 - Basic principle and history of TOF MS
 - Instrument configuration and connections
 - MassCenter™
 - HPLC control
 - Tuning procedure
 - Single data acquisition
- **Tuesday**
 - DART basic principle
 - DART operation
 - Mass calibration
 - MS acquisition method editor
 - Multiple function switching
 - Automatic data acquisition
- **Wednesday**
 - Data processing
 - Exact mass measurement
 - Post-acquisition calibration
 - Data Manager
 - Basic maintenance and troubleshooting
- **Thursday**
 - DART applications
 - Discussion



Instructor: Zhanpin Wu
Robert Cody
Chip Detmer

The basic principle of TOF MS



- All ions leave the ion source at the same time with the same kinetic energy
- The larger the ion, the slower its velocity and thus the longer time it takes to traverse through the field-free region. Ions with different m/z values arrive at the detector in different time.



The basic principle of TOF MS

■ Basic equation

The kinetic energy which the ions obtain after passing the accelerating region is shown below.

$$qeV_a = 1/2 mv^2 \quad \rightarrow \quad v = \sqrt{\frac{2qeV_a}{m}}$$

e : unit charge (coulombs) q : charge number of ion V_a : accelerating voltage (volts)
 m : mass (kilograms) v : velocity (m/s)

The flight time t of an ion having mass m is as shown below when the effective flight distance is L .

$$t = \frac{L}{v} = \frac{L}{\sqrt{2qe\frac{V_a}{m}}}$$

Flight time is inversely proportional to the square root of the mass/charge ratio. As the flight time depends on mass, you can obtain a mass spectrum as a graph with mass numbers on the X-axis and ion intensities on the Y-axis.

■ Definition of mass resolution for TOF MS

$$R = m/\Delta m = t/2\Delta t$$

Δt is usually the full-width at half-maximum (height) of the peak (FWHM).



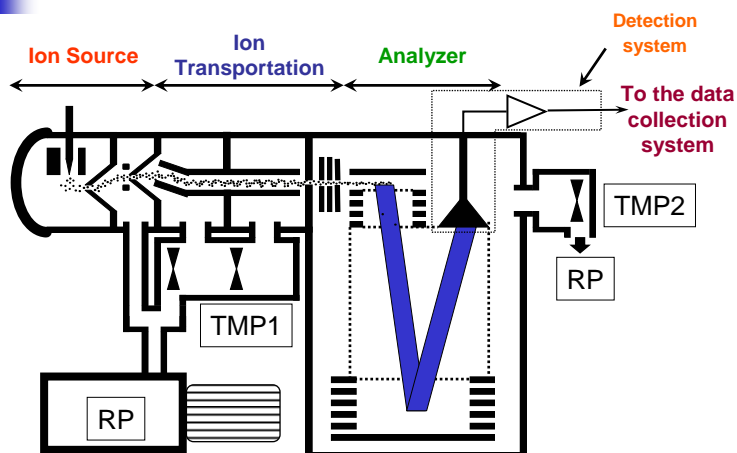
The history of TOF MS

- 1946, Stephens *et al*/proposed the idea of TOF MS
- 1948, Cameron and Eggers announced the first TOF mass spectra, $R \sim 5$.
- 1955, Wiley and McLaren developed a technique to focus the spread of ionization position and initial ion energy, achieving a mass $R > 300$, the commercial systems distributed by Bendix.
- 1970s, reflectron TOF MS developed, $R \sim$ a few thousand.
- 1980s, Dawson and Guilhaus, and Dodonov developed orthogonal acceleration, allowed an efficient combination of TOF MS and continuous ion source.

Introduction of AccuTOF™ LC system



The AccuTOF™ LC diagram





Specifications

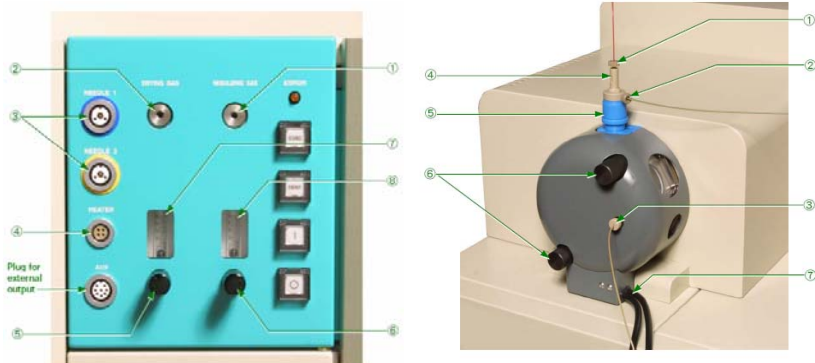
- Resolution: $R \geq 6,000$ (FWHM) at m/z 609.28
- Mass range: 7 – 10,000
- Sensitivity: ESI positive-ion mode, $s/n \geq 10$ with 10 pg reserpine
- Mass accuracy: 2 mmu or 5 ppm
- Acquisition rate: 10 spectra/s
- Dynamic range: 10^4



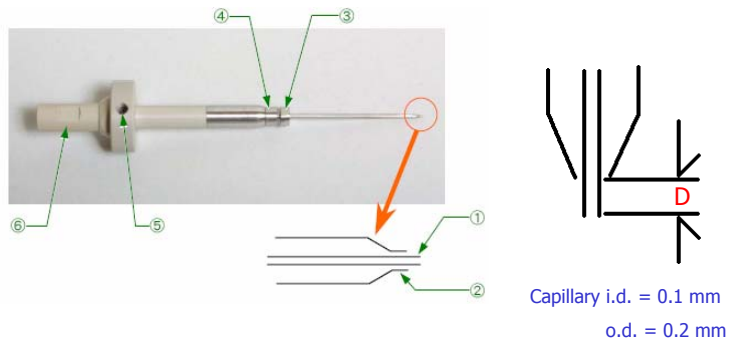
Optional attachments

- ESI (standard)
- DART
- APCI
- microESI
- nanoESI
- DualESI
- CoronaESI
- AP-MALDI

Front panel and ESI source

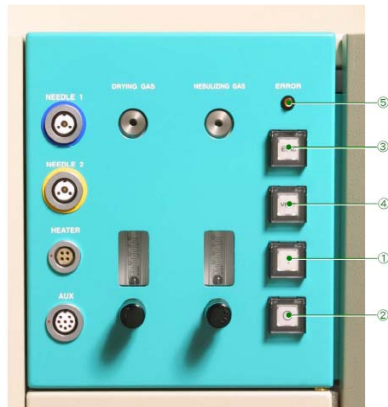


ESI spray needle assembly



Inner needle protrudes 0.5 mm for the flow rate of 200 $\mu\text{L}/\text{min}$, 2 - 3 mm for the flow rate of 10 $\mu\text{L}/\text{min}$.

System startup and shutdown



Check N₂ pressure
through the side
panel

Check the oil level

Final vacuum
pressure in the
analyzer should be 1
or 2×10^{-5} Pa or less

Condition MCP


MassCenter™ 1.3.x

- Basic features in MassCenter™ Main
 - HPLC control
 - MS control
 - Chromatogram and spectrum viewers
 - MS watch viewer
 - Isotopic simulator
- Force termination of MassCenter™



Create new projects & analysis lists

- Create a new project
- Switch to other projects
- Create a new analysis list
- Open an analysis list



Agilent 1100/1200 HPLC control

- Add or delete Agilent 1100/1200 HPLC modules
- Choose autosampler and detector
- Reset communication
- Display status
- Send and receive settings
- Set parameters for each module

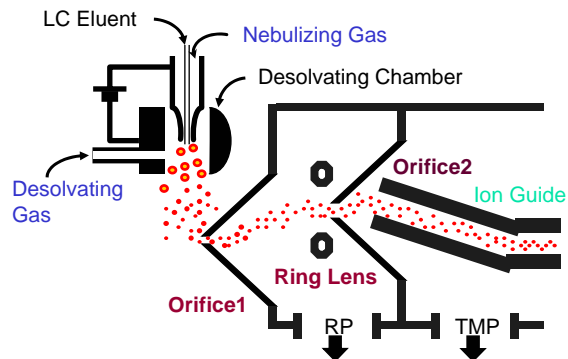
MS Tune Manager

- MCP conditioning
- FastFlight noise coefficient
- Instrument modes

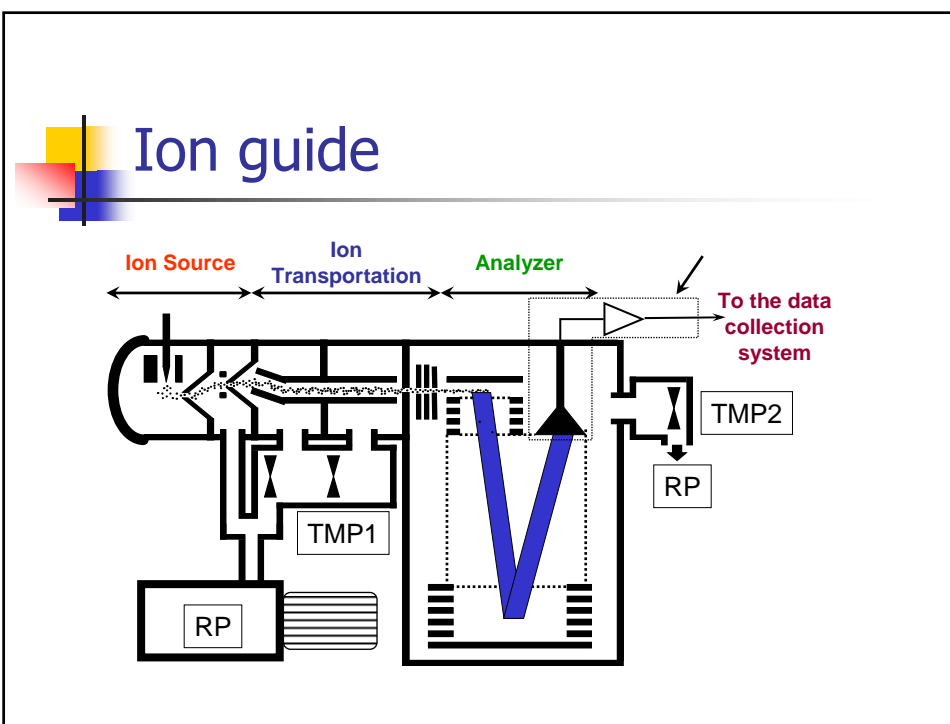
Status	High Voltage			Temperature
	MCP	Acceleration	Ion Source	
Evacuation Ready	OFF	OFF	OFF	OFF
Warm Up	ON	OFF	OFF	OFF
Standby	ON	ON	OFF	ON
Operate	ON	ON	ON	ON

- Ionization modes
- MS spectrum monitor

MS Peak Tuning (ESI)



The actual voltage values read in MS Watch Viewer are:
 Ring Lens voltage = Ion Guide bias Voltage + Orifice 1 + Orifice 2 + Ring Lens Setting Voltages.
 Orifice 1 voltage = Ion Guide bias Voltage + Orifice 2 + Orifice 1 Setting Voltages.
 Orifice 2 voltage = Ion Guide bias Voltage + Orifice 2 Setting Voltage.



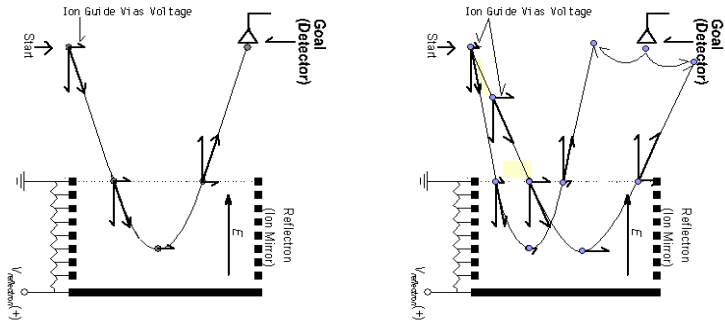
Ion guide peaks voltage

Peaks Voltage	Lowest m/z
500	50
1000	100
1500	150
2000	200
2500	250

An approximation for easy recall; a value of 600 - 1500V useful for small-molecule analysis (up to m/z 1000).

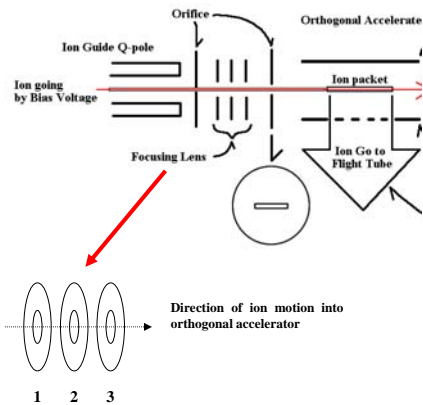
The quadrupole ion guide resonating frequency is set with the configuration tool and the value is stored in the registry. The correct value is set for each instrument and is listed in the parameter list in the final test document. If the software is reinstalled, this value may have to be set again.

Ion guide bias voltage



The ion guide bias voltage is theoretically 27 V, although it varies from system to system. Do not change it since it is optimized at the factory or at the time of installation.

Focus Lens

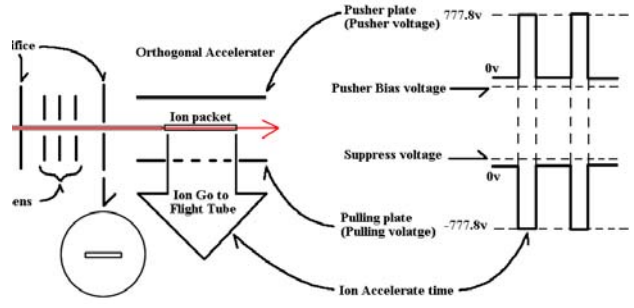


The first lens is segmented into quadrants to steer the beam up and down and left and right.

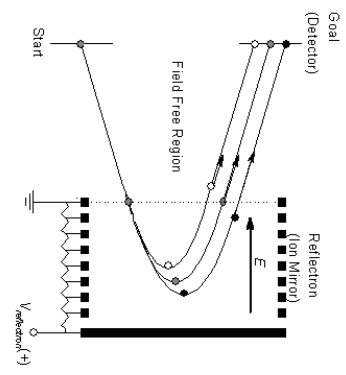
The second lens is an einzel focusing lens.

The third lens is grounded.

Orthogonal Accelerator



Reflectron



- Doubling the flight distance
- Focusing the kinetic energy spread



Detector

- AccuTOF uses 2 micro channel plates (MCP) as detector
- Detector conditioning
- Set detector voltage to 0 to save its lifetime when instrument is not in use



ESI tuning example I

- Sample: reserpine ($[M+H]^+$ at m/z 609.2812, 100 ng/mL in methanol)
- Flow rate: 100 - 200 $\mu\text{L}/\text{min}$
- Initial settings:
 - Needle: 2000V
 - Orifice1: 65V
 - Orifice2: 5V
 - Ring lens: 10V
 - Peaks voltage: 2500V
 - Drying gas: 2.5 -3 unit
 - Nebulizing gas: 0.5 - 1 unit
 - Desolvating T: 250 $^{\circ}\text{C}$
 - Orifice1 T: 80 $^{\circ}\text{C}$



ESI tuning example II

- Sample: acrylamide $[M+H]^+$ at m/z 72.04494, 1.0 $\mu\text{g/mL}$ in water
- Flow rate: 100 $\mu\text{L/min}$
- Initial settings
 - Needle voltage: 2000 V
 - Orifice 1 voltage: 30 V
 - Orifice 2 voltage: 5 V
 - Ring lens voltage: 10 V
 - Ion guide peaks voltage: 600 V



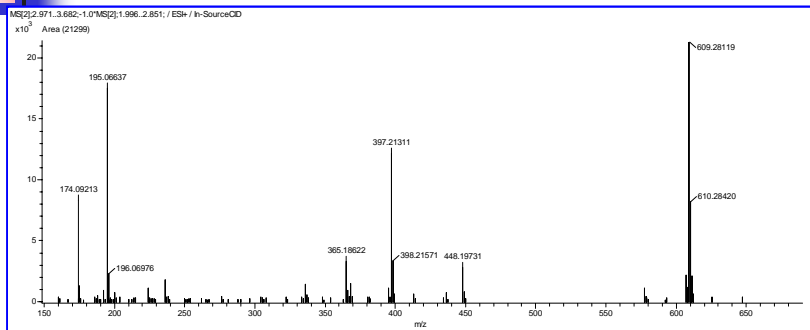
ESI tuning example III

- Sample: insulin in methanol/1% formic acid (50/50)
- Flow rate: 100 $\mu\text{L/min}$
- Initial settings
 - Needle voltage: 2000 V
 - Orifice 1 voltage: 30 V
 - Orifice 2 voltage: 5 V
 - Ring lens voltage: 10 V
 - Ion guide peaks voltage: 2500 V

In-souce CID

- Increase orifice 1 voltage
- Decrease peaks voltage
- Adjust ring lens voltage slightly

Reserpine in-source CID spectrum



<i>m/z</i>	composition	<i>m/z</i>	composition
174.0921	C ₁₁ H ₁₂ NO	195.0664	C ₁₀ H ₁₁ O ₄
365.1856	C ₂₂ H ₂₅ N ₂ O ₃	397.2136	C ₂₃ H ₂₉ N ₂ O ₄
448.1969	C ₂₃ H ₃₀ NO ₈	609.2812	C ₃₃ H ₄₁ N ₂ O ₉

ESI conditions
 Needle Voltage[V]:2000
 Orifice1 Voltage[V]:110
 Orifice2 Voltage[V]:5
 Ring Lens Voltage[V]:10
 Ion Guide Peaks Voltage[V]:1500
 Detector Voltage[V]:2600



Single data acquisition

- Acquisition with current settings
- Acquisition with new settings
- Acquisition with MS acquisition method
- Sweeping orifice 1 voltage and ion guide peaks voltage



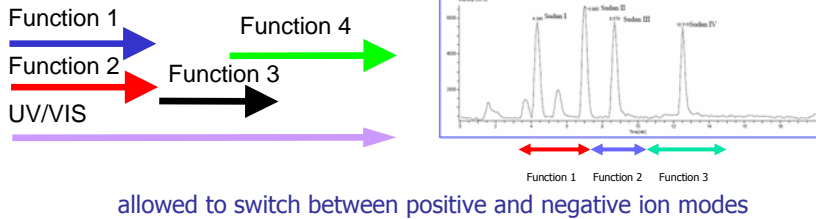
Mass Calibration

- Common calibration standards
 - PEG
 - PPG
 - NaTFA
 - CsI
 - Ultramark
 - ...
- When a calibration is needed?
 - Low resolution
 - Poor mass accuracy
 - Parameters changed in TOF mass analyzer (new tuning settings)
- Mass Calibration file is shared by all projects
- Calibration procedure
 - Automatic peak assignment
 - Manual peak assignment
 - Register new calibration
 - Reference Editor



Mass acquisition method

- Mass acquisition method editor
 - Multiple functions switching
 - Alternatively switching between different MS tuning settings
- not allowed to switch between positive and negative ion modes
- Setting up time widows for switching different MS tune settings



An example of function - switching for HPLC separation

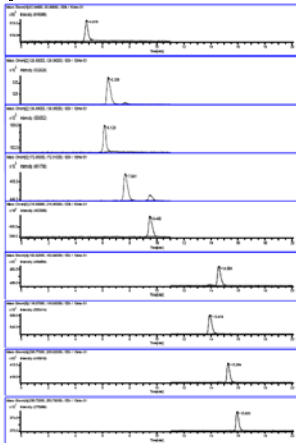


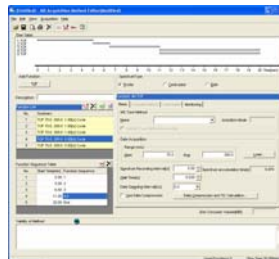
Fig 1. Mass Chromatograms of HAAs.

Table 2. MS Conditions

MS Functions	Needle Voltage (V)	Orifice1 Voltage (V)	Orifice2 Voltage (V)	Ringers Voltage (V)	Ion Guide Voltage (V)	Desolv. Temp (T1 °C)	Orifice1 T1 (°C)	Desolv. Temp (T2 °C)	Nebulizing gas (L/min)
Function1	-30	-3	-3	400					
Function2	-30	-4	-4	1025					
Function3	-2000	-30	-4	-4	1225	250	100	2.0	5.0
Function4	-40	-5	-4	1350					
Function5	-40	-3	-2	180					

Table 3 Selected ions for mass chromatograms and "function-switching"

MS	BEKA	BEKA	BEKA	BEKA	BEKA	BEKA	BEKA	BEKA	BEKA
BEKA	92.9743	125.9254	136.9236	172.9826	216.9323				
BEKA						162.8536	116.8066	206.8034	250.753
Function	1	2	2	3	3	1	5	4	4
RT	0.956	0.965	0.9579	0.9573	0.9584	0.9581	0.9581	0.9584	0.9585





Voltage sweeping

- Sweep orifice 1 voltage
- Sweep ion guide peaks voltage



Automatic Sample Acquisition

- Before running samples automatically, you need:
 - A calibration file
 - An HPLC method file
 - An MS tune settings file
 - An MS acquisition method file
 - An analysis list
- Automatic shutdown of the system after acquisition



Data Processing

- Generate mass spectra from chromatographic peaks
- Convert profile spectrum to centroided spectrum
- Baseline subtraction
- Create mass chromatograms (mass chromatogram process list)
- Peak integration, S/N and mass resolution display
- MS Peak detection and list
- Mass spectrum processing list
- UV trace and spectra
- Peak labeling
- Automatic data processing



Exact mass measurement and elemental composition estimation

- Internal standard ("lock mass")
 - Mix with sample and inject together
 - Inject right before or after sample injection
 - Deliver through a tee by syringe pump
 - Post-column injection through a manual or automatic injector when running HPLC
 - Dual ESI source
- Operating procedure
 - Exact mass measurement
 - Elemental composition estimation



Post-acquisition calibration

- Recalibrate existing data but keep the original calibration
- Recalibrate existing data and save the new calibration into the data



Data Manager

- Copy, move or delete data
- Convert profile data into centroided data
- Convert MassCenter data into netCDF format
- Search project or acquisition data
- Data properties



Basic maintenance

- Ion source cleaning

- API interface cleaning

- Rotary pump oil and filter change

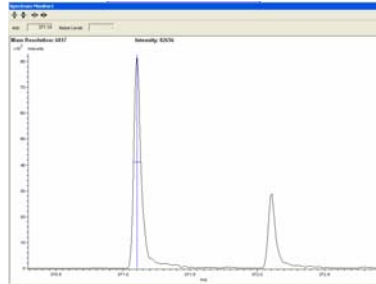


Troubleshooting

- The instrument can not be set to Operate mode
 - Condition MCP
- No signal after the instrument is set to Operate mode
 - Check if the flange is closed
 - Check if the nebulizing gas is on
 - Check if the mobile phase is on
 - Check if the ion guide peaks voltage and reflectron voltage are on (correct tuning parameters)
 - Check the detector voltage
 - Check if orifice 1 is clogged
 - Check if the mass range is correct
 - Check if the mass calibration file is registered
 - Check RF ion guide status
 - Check if DART is on and the discharge needle voltage is on
 - Check the DART electrode potentials E1 and E2 should both be positive for positive-ion mode, negative for negative-ion mode
- The HPLC pressure is too high
 - Check if the spray needle is clogged
 - Wash the HPLC column
 - Replace the mobile phase filter
 - Check the HPLC pumps

Troubleshooting

- The instrument can not be vented or evacuated
 - Check N₂ pressure
- Difficult to get a good mass calibration
 - Check peak shape
 - Make sure ions are not saturated
 - Make sure the peak assignment is correct
- Mass accuracy is poor even after a fresh calibration
 - Check the correct peaks are assigned for the calibration
 - Check the mass resolution, if poor retune the instrument $R \geq 6,000$ at m/z 609.28
 - Signal intensity is too low
 - The m/z values of internal reference and the sample molecules are too far away
 - The background ions, internal reference ions and sample ions have overlapped isotopic distribution
- Mass number m/z on MS monitor is not stable
 - General, you need to wait approximately 2 hours to obtain stable m/z value after switching the instrument mode from Evacuation Ready to Operate.



Troubleshooting

- Error message when creating mass chromatograms
 - This might happen if using some versions of MassCenter™.
 - Set the running time in MS Acquisition Method longer than the actual stopping time
 - Do not use mass chromatograms for real time monitoring
 - Upgrade MassCenter™.