



HRAM iQuan 2016: Thermo Scientific™ Q Exactive™ Mass Spectrometer Series Calibration & Maintenance

Tatjana Talamantes and Roberto Gamez
North America Omics Markets Field Support, Center of Excellence

Tuning and Calibration

- Tune Software Interface (v.2.6)
- Preparation
- Tuning for Calibration Solvents
- Calibration Procedure

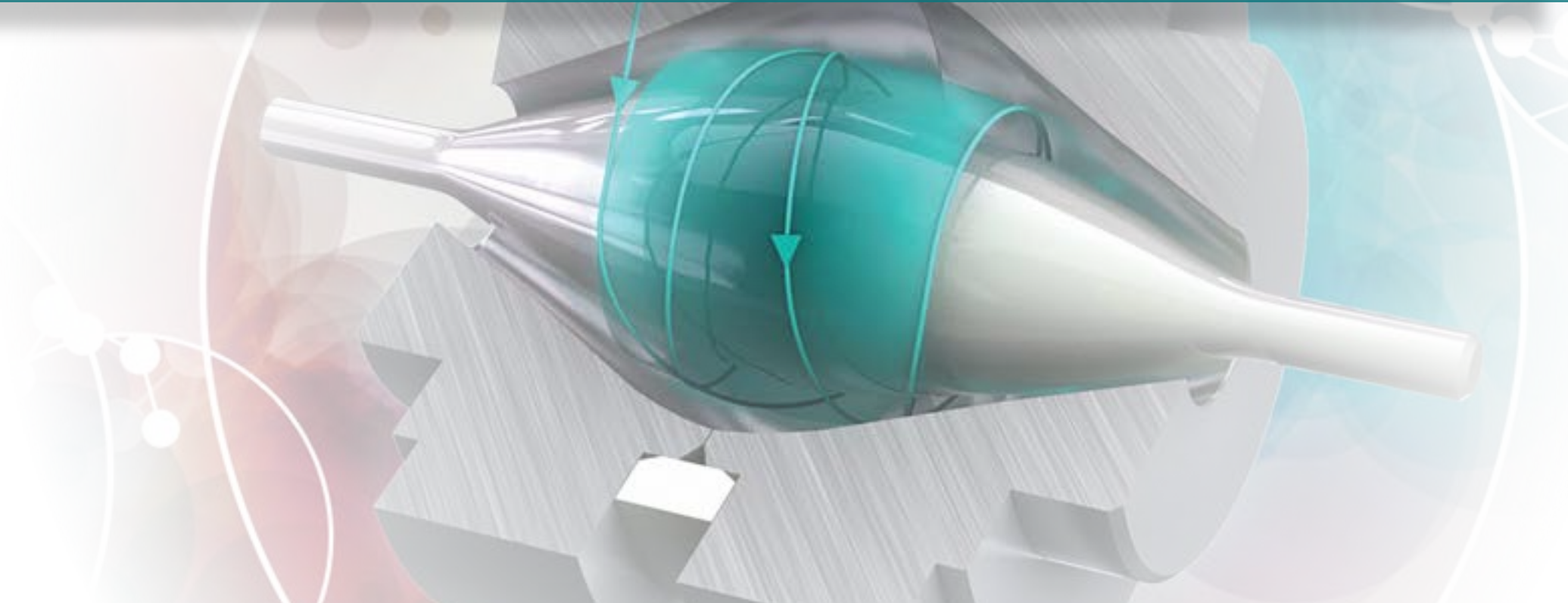
Maintenance

- Considerations
- Schedules
- How-to's



Thermo Scientific™ Q Exactive™ Mass Spectrometer Calibration & Maintenance

Preparation, Tuning, Calibration



Q Exactive MS Series Tune Interface (Tune v2.6): Overview

Menu Bar

Toolbar

User Role

Tasks Panel

Display Panel

The screenshot displays the Thermo Q Exactive MS Series Tune Interface (Tune v2.6) with the following components:

- Menu Bar:** File, Windows, Reports, Help.
- Toolbar:** Contains icons for file operations, instrument control, and data analysis.
- User Role:** Role: Advanced.
- Instrument Control Panel:**
 - Mass Traces:** Shows the current mass trace.
 - Calibrate:** Includes a Calibrate button and a progress bar (0.0 %).
 - Calmix Calibration:** A list of calibration items with their status and timestamps.

Item	Status	Timestamp
Base Calibration	OK	2016-05-11 14:03
Electronics		
Quadrupole RF Frequency	2016-05-11 12:27	
Flatapole RF	2016-05-11 12:27	
HCD RF	2016-05-11 12:28	
Basics - Positive Ion	OK	2016-05-11 14:03
Ion transfer (pos)		
Quadrupole Basic (pos)		
Quad Iso Mass/Res Cal (rough)	2016-05-11 14:03	
Quad RF freq. fine adjustment	2016-05-11 14:03	
eFT Parameter (pos)	OK	2016-05-11 14:03
Analyzer Accuracy (pos)	OK	2016-05-11 14:04
Basics - Negative Ion	OK	2016-05-12 09:46
Ion transfer (neg)		
Quadrupole Basic (neg)		
eFT Parameter (neg)	OK	2016-05-12 09:46
Analyzer Accuracy (neg)	OK	2016-05-12 09:47
Isolation Mass and Res. (pos)	OK	2016-05-11 14:36
Iso Mass/Res. Cal. (wide iso)	2016-05-11 14:25	
Iso Mass/Res. Cal. (narrow)	OK	2016-05-11 14:36
Isolation Mass and Res. (neg)	OK	2016-05-12 10:04
Iso Mass/Res. Cal. (neg)(wide iso)	2016-05-12 09:54	
Iso Mass/Res. Cal. (neg)(narrow)	OK	2016-05-12 10:04
MS Mass Calibration (pos)	OK	(due in 7 days)
MS Mass Calibration (neg)	Overdue	(2016-05-12 10:17)
 - Customized Calibration:** A section for customized calibration settings.
 - Evaluate:** A button to evaluate the calibration.
 - Vacuum / Bakeout:** A button to manage the vacuum system.
- Spectrum Panel:** Displays the mass spectrum with relative abundance (%) on the y-axis and m/z on the x-axis. Key peaks are labeled with their m/z values: 195.088, 262.636, 360.238, 524.265, 1121.997, 1221.991, 1321.984, 1421.977, 1521.972, 1721.960, 1621.967.
- Instrument Status Panel:** Shows the status of various instrument components.

Component	Status
Instrument	
Current Scan	
Total Ion Current	2035.07 E6 ions/sec
TIC Variation	4 %
Inject time	0.64 ms
AGC Target reached	100 %
AGC Prescan Mode	-1
Scan Rate	13.3 scans/sec
Lock masses	
Control	
System	
Analysis Graph	
Performance	Ok
Electronics	
ESI Ion Source	
Peripheral Devices	
Vacuum System	
- Analysis Graphs Panel:** Displays the mass accuracy graph with m/z deviation (ppm) on the y-axis and Scan on the x-axis. The graph shows the mass accuracy for various m/z values over 20 scans.

Q Exactive MS Series Tune Interface (Tune v2.6): Display Panel Options

The screenshot displays the Thermo Q Exactive MS Series Tune Interface (Tune v2.6) software. The interface is divided into several panels:

- Instrument Control:** Contains scan parameters and HESI source settings.
- Spectrum:** Displays a mass spectrum plot with relative abundance (%) on the y-axis and m/z on the x-axis. The plot shows several peaks, with the base peak at m/z 195.088. Other labeled peaks include 262.636, 360.238, 524.265, 1022.003, 1221.990, 1321.985, 1421.978, 1521.971, 1621.967, and 1721.960.
- Instrument Status:** Provides a summary of the instrument's current state, including scan parameters and system health.
- Messages:** A log of system messages and events.

Instrument Control Panel:

Parameter	Value
Scan type	Full MS
Scan range	150.0 to 2,000.0 m/z
Fragmentation	None
Resolution	17,500
Polarity	Positive
Microscans	1
Lock masses	Off
AGC target	1e6
Maximum inject time	50

HESI source Panel:

Parameter	Value	Actual
Sheath gas flow rate	5	0
Aux gas flow rate	1	0
Sweep gas flow rate	0	0
Spray voltage (kV)	3.50	0.00
Spray current (μA)		0.00
Capillary temp. (°C)	300	300
S-lens RF level	50.0	
Aux gas heater temp (°C)	0	41

Instrument Status Panel:

Parameter	Value
Total Ion Current	2001.20 E6 ions/sec
TIC Variation	2 %
Inject time	0.65 ms
AGC Target reached	100 %
AGC Prescan Mode	-1
Scan Rate	13.3 scans/sec
Lock masses	
Control	
System	
Analysis Graph	
Performance	Ok
Electronics	
ESI Ion Source	
Peripheral Devices	
Vacuum System	
Status	Ok - HV enabled
Fore Vacuum	1.48e+00 mbar
High Vacuum	Turned off
Ultra High Vacuum	1.76e-10 mbar
Source TMP Speed	100 %
UHV TMP Speed	100 %

Messages Panel:

Priority	Source	Time	Description
✓		14:53:52.82	Starting procedure Calibrate()
✓		14:53:52.84	== Running Calibration... ==
✓		14:53:52.84	'Spectral Mass Calibration (pos)' running...
✓		14:54:08.28	'Spectral Mass Calibration (pos)' Ok: (rms = 0.25/0.27 ppm)
✓		14:54:08.51	Calibration successful. Saving calibration results.
✓		14:54:09.60	Document C:\Xcalibur\system\Exactive\instrument\reports\901B0E665870_Calibration_2016-06-06_14-53-52.pdf generated.
✓		14:54:10.37	Document C:\Xcalibur\system\Exactive\instrument\reports\901B0E665870_Spectral Mass Calibration (pos)_2016-06-06_14-53-52.pdf generated.
✓		15:00:56.33	Set user role to Standard.
✓		15:00:56.37	Set user role to Standard.
✓		15:01:41.46	Set user role to Advanced.
✓		15:01:41.53	Set user role to Advanced.

Instrument Messages:

- Displayed here and saved in log files
- Alerts the user if the system noticed a problem

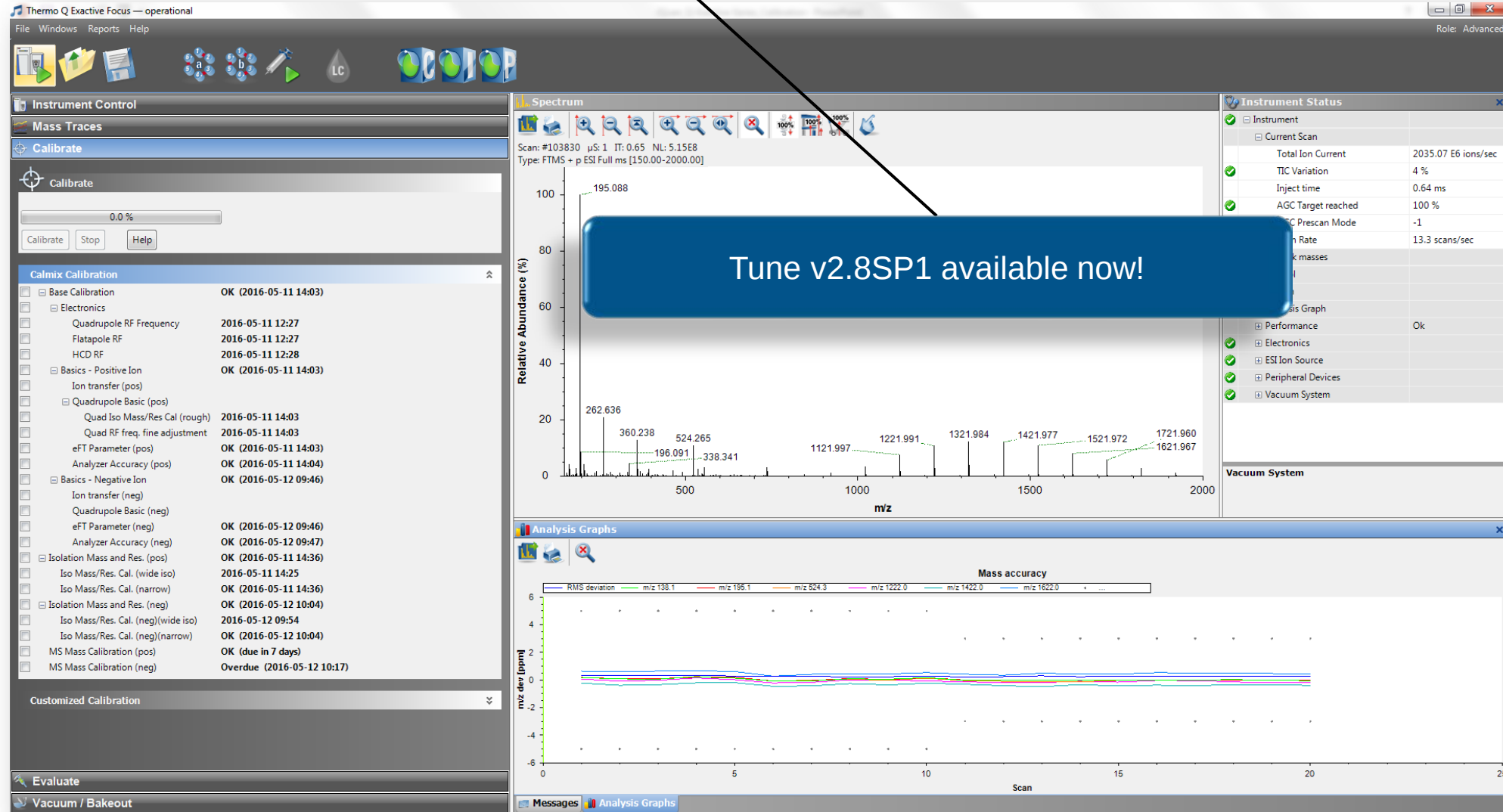
Q Exactive MS Series Tune Interface (Tune v2.6): Display Panel Options

The screenshot displays the Thermo Q Exactive Focus software interface. The left sidebar contains the 'Instrument Control' panel with 'Mass Traces' and 'Calibrate' options. The 'Calibrate' panel shows a 'Calmix Calibration' list with various parameters and their status (OK or Overdue). The main area is divided into three sections: 'Spectrum' (top right), 'Analysis Graphs' (bottom right), and 'Instrument Status' (far right). The 'Spectrum' plot shows relative abundance versus m/z with several peaks labeled. The 'Analysis Graphs' plot shows mass accuracy (m/z deviation) versus scan number. A blue callout box with the title 'Analysis Graphs' is overlaid on the spectrum plot, containing the following text:

- When instrument plots something, the graphs will display here
- Graphs can be copied to the clipboard
- Graphs are not automatically saved

A red box highlights the 'Analysis Graphs' icon in the bottom left corner of the interface.

Q Exactive MS Series Tune Interface (Tune v2.6): Display Panel Options



Instrument Control Software Update

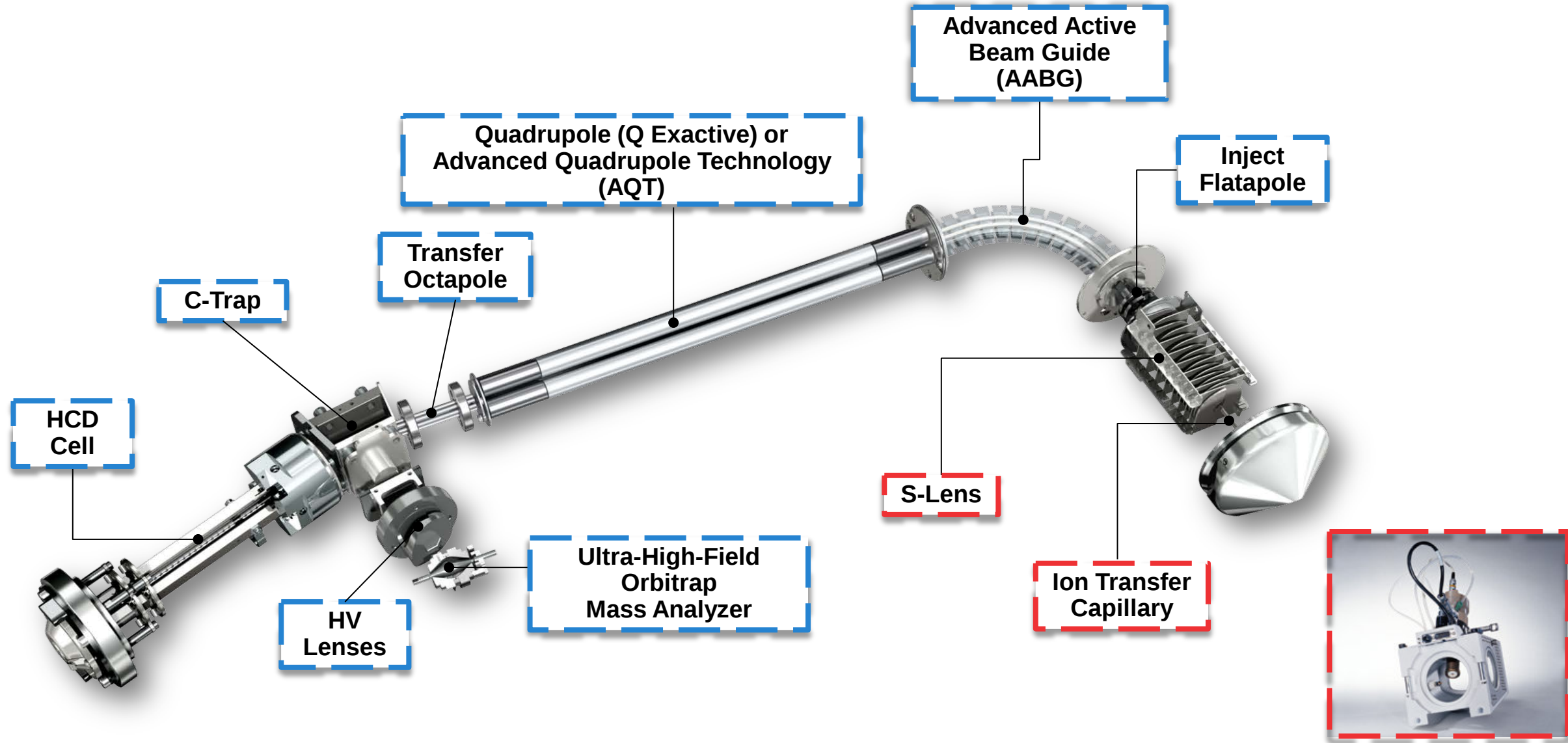
- Q Exactive MS Series 2.8 SP1 is released for Windows 7 32-bit and 64-bit
- Recommended to upgrade all instruments running Q Exactive MS Series Tune 2.7 or older to this release
- **BRE0008597** Exactive MS Series 2.8SP1 / Xcalibur 3.1 (for Windows 7 32-bit)
- **BRE0008596** Exactive MS Series 2.8SP1 / Xcalibur 4.0 (for Windows 7 64-bit).
- Full list of improvements and defect fixes can be found in release notes included with the software
- Main changes with the release of 2.8 SP1 are:
 - Modified bakeout procedure to improve lifetime of turbo pumps
 - Defect fix for some calibration procedures (Isolation Transmission Endurance Test, normal mode and HMR mode eFT)
 - TIC variation readback in v 2.7 did not generate reliable output



Calibration is Qualitative, Tuning is Quantitative

- Calibration ... *Mass spectrometer optimization*
 - Calibration is independent of source type
 - Exactive MS series calibrations must be performed using the ESI or HESI-II probe
 - Successful calibrations are saved automatically
 - Only one calibration file exists
- Tuning ... *Compound optimization*
 - Efficiently generating ions from the source into the mass spectrometer
 - Tune files must be manually saved after the tuning process
 - Compound/Application/Method dependent
 - Multiple tune files can be saved to a method

Q Exactive MS Series: What is **Tuned** and What is **Calibrated**?



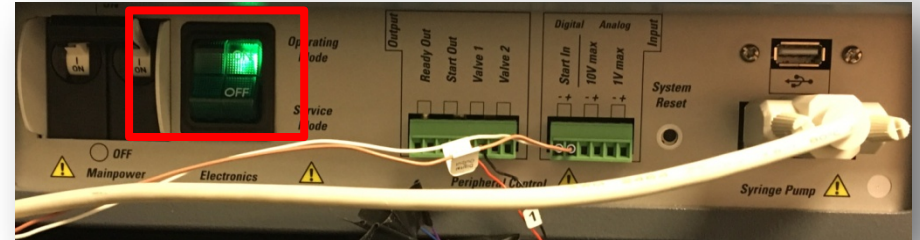
Calibration: Preparation

- Calibrations can be performed immediately if the instrument switched from “STANDBY” to “ON” mode
- If the instrument switched from “OFF” to “ON” mode, scan in FTMS mode for 80 minutes prior to calibration
 - Does not require spray voltage or liquid flow
- Instrument reset, instrument shut down, bakeout and leaving the source open for more than 60 min, will place the instrument’s electronics in “OFF” mode
 - Make sure your instrument has warmed up (scanned) for 80 minutes if the electronics have been off
- Use the correct calibration solutions for your instrument (store in the dark at room temp.)
 - Pierce™ LTQ Velos ESI Positive Ion Calibration Solution (*Catalog no. 88323*)
 - Pierce™ Negative Ion Calibration Solution (*Catalog no. 88324*)



Q Exactive MS Series Calibration – Standard User Role

- Electronics service switch is in the Operating Mode position



Q Exactive MS Series Calibration – Standard User Role

- Electronics service switch is in the Operating Mode position
- Check that the gas pressure is within the operational limit

Nitrogen: 800 ± 30 kPa (8 ± 0.3 bar, 116 ± 4 psi)

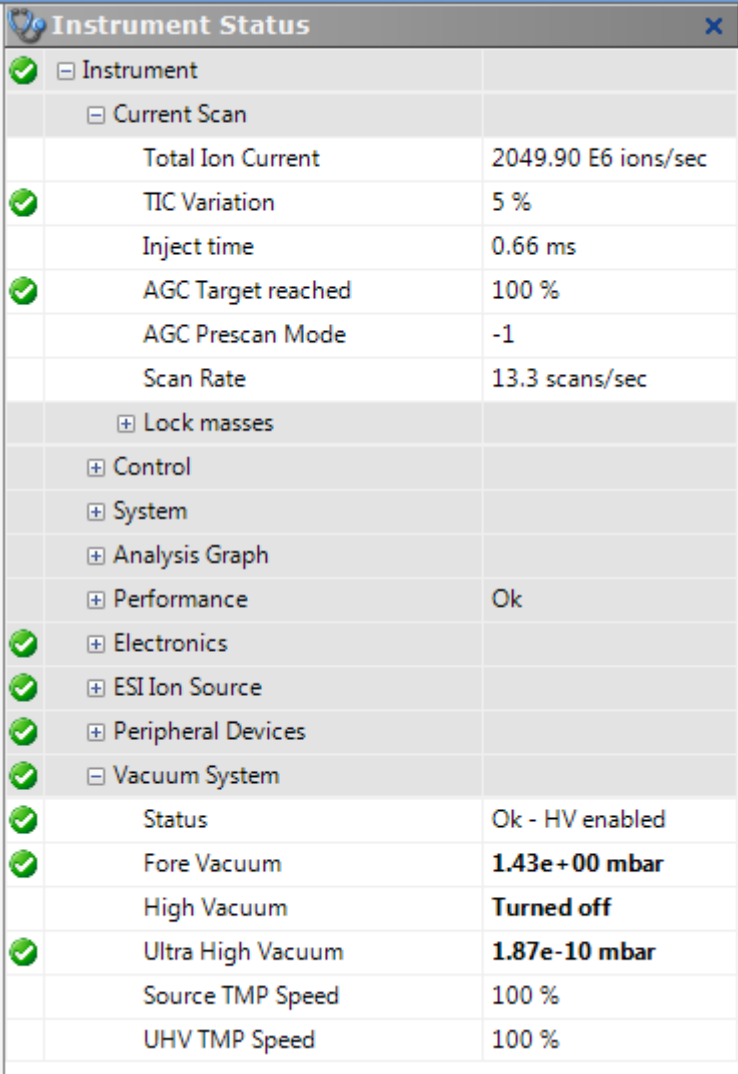


Q Exactive MS Series Calibration – Standard User Role

- Electronics service switch is in the Operating Mode position
- Check that the gas pressure is within the operational limit

Nitrogen: 800 ± 30 kPa (8 ± 0.3 bar, 116 ± 4 psi)

- Check that the vacuum levels are sufficient for operating the instrument



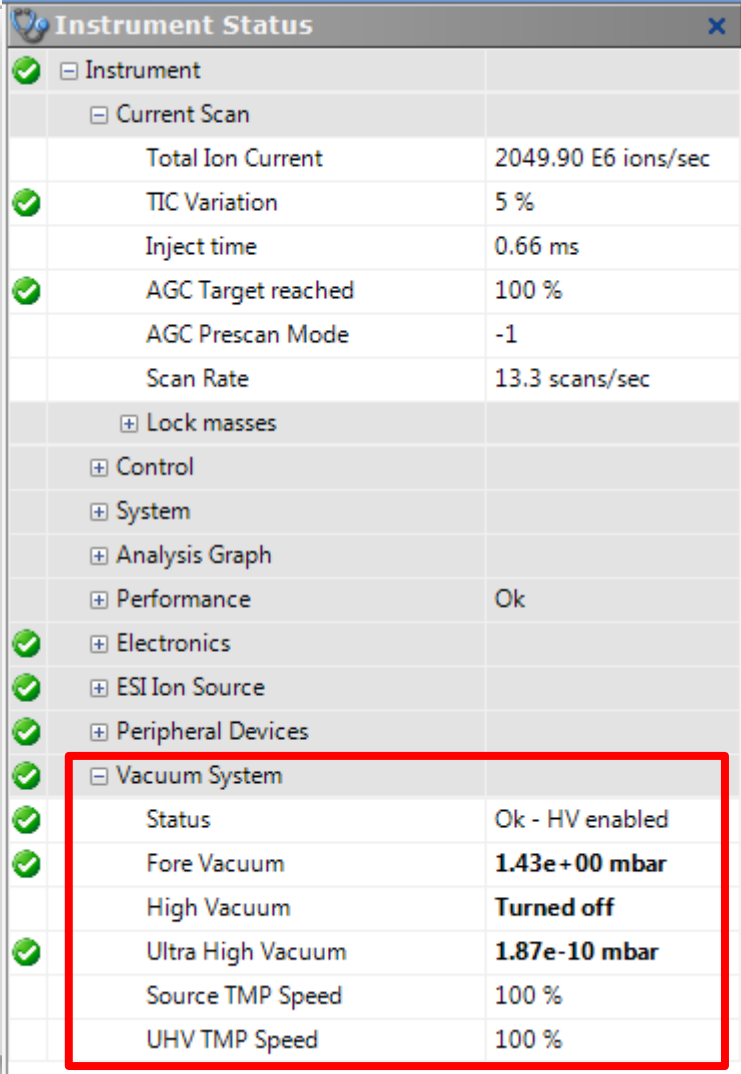
Instrument Status		
✓	Instrument	
	Current Scan	
	Total Ion Current	2049.90 E6 ions/sec
✓	TIC Variation	5 %
	Inject time	0.66 ms
✓	AGC Target reached	100 %
	AGC Prescan Mode	-1
	Scan Rate	13.3 scans/sec
	Lock masses	
	Control	
	System	
	Analysis Graph	
	Performance	Ok
✓	Electronics	
✓	ESI Ion Source	
✓	Peripheral Devices	
✓	Vacuum System	
✓	Status	Ok - HV enabled
✓	Fore Vacuum	1.43e+00 mbar
	High Vacuum	Turned off
✓	Ultra High Vacuum	1.87e-10 mbar
	Source TMP Speed	100 %
	UHV TMP Speed	100 %

Q Exactive MS Series Calibration – Standard User Role

- Electronics service switch is in the Operating Mode position
- Check that the gas pressure is within the operational limit

Nitrogen: 800 ± 30 kPa (8 ± 0.3 bar, 116 ± 4 psi)

- Check that the vacuum levels are sufficient for operating the instrument
 - Fore Vacuum < 2 mbar
 - High Vacuum “Turned off”
 - Ultra High < $5\text{E}-10$ mbar
 - Source and UHV TMP Speed 100%



Instrument Status	
Instrument	
Current Scan	
Total Ion Current	2049.90 E6 ions/sec
TIC Variation	5 %
Inject time	0.66 ms
AGC Target reached	100 %
AGC Prescan Mode	-1
Scan Rate	13.3 scans/sec
Lock masses	
Control	
System	
Analysis Graph	
Performance	Ok
Electronics	
ESI Ion Source	
Peripheral Devices	
Vacuum System	
Status	Ok - HV enabled
Fore Vacuum	1.43e+00 mbar
High Vacuum	Turned off
Ultra High Vacuum	1.87e-10 mbar
Source TMP Speed	100 %
UHV TMP Speed	100 %

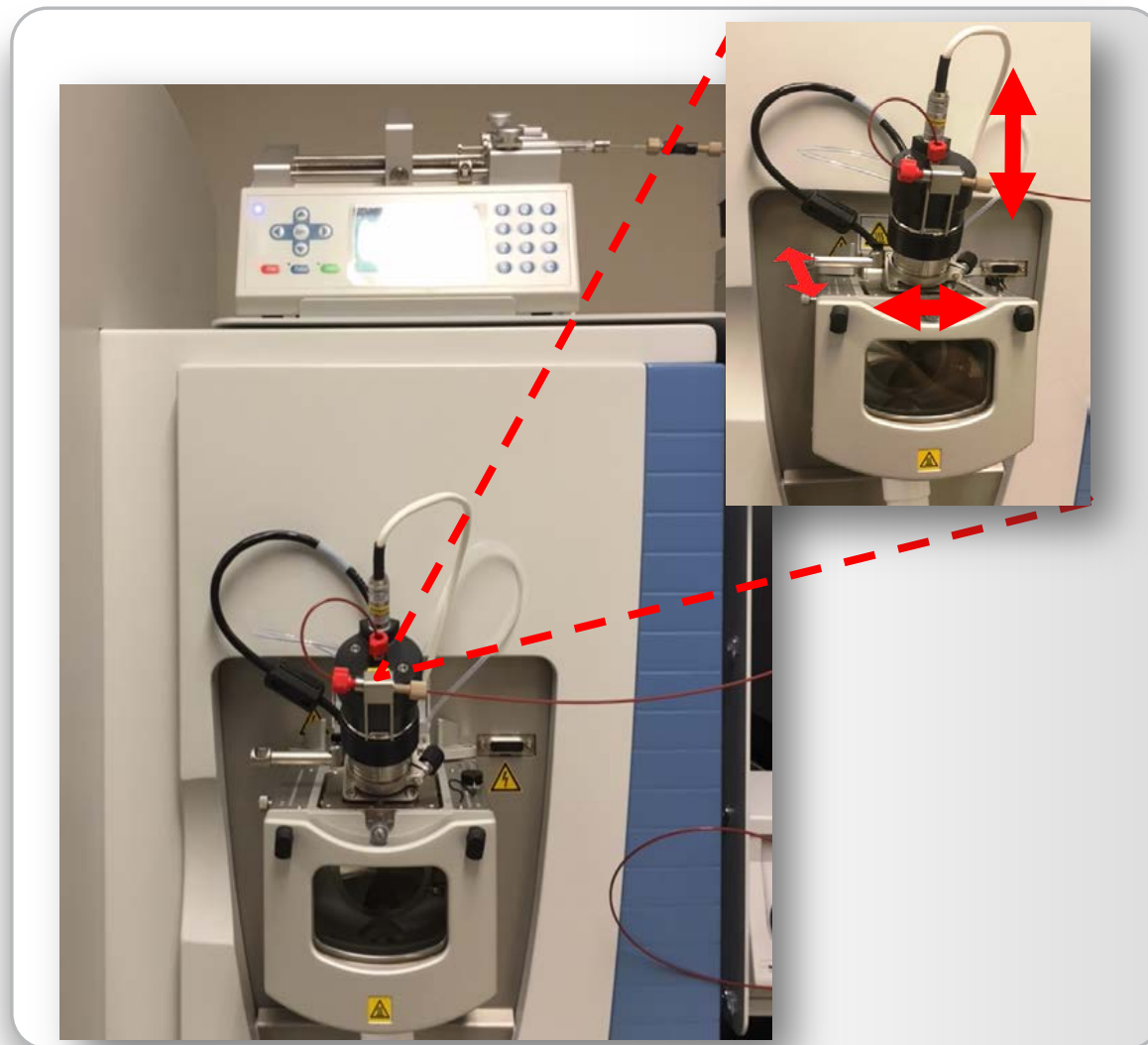
Q Exactive MS Series Calibration – Standard User Role

- An infusion line should be installed between the syringe pump and the grounding union that is held by the grounding bar of the Ion Max API source
- Clean 500 μ L Hamilton (Thermo) syringe
- Pierce LTQ Velos ESI Positive/Negative Ion Calibration Solution
- Tubing, PEEK™ (0.005-in ID $\times$ 1/16-in. OD (red))
- Fitting, fingertight, Upchurch Scientific (natural) (used with (red) PEEK™ tubing)



Q Exactive MS Series Calibration – Standard User Role

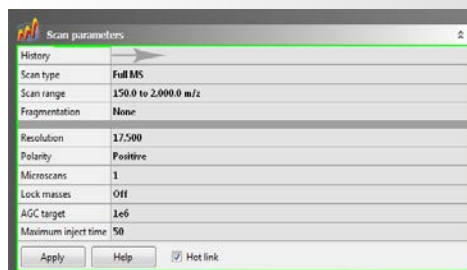
- An infusion line should be installed between the syringe pump and the grounding union that is held by the grounding bar of the Ion Max API source
- Clean 500 μ L Hamilton (Thermo) syringe
- Pierce LTQ Velos ESI Positive/Negative Ion Calibration Solution
- Tubing, PEEK™ (0.005-in ID $\times$ 1/16-in. OD (red))
- Fitting, fingertight, Upchurch Scientific (natural) (used with (red) PEEK™ tubing)
- Ion Max API source with HESI II probe are installed on the mass spectrometer and proper position of the HESI II probe is established



Establishing a Tune: Conditions for Calibration Solutions

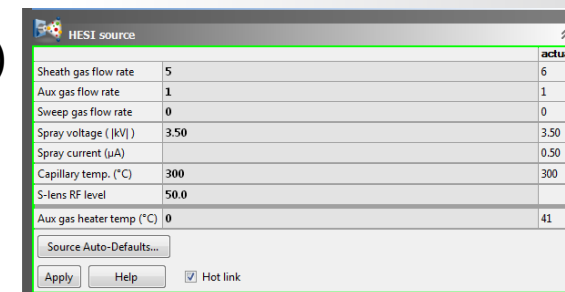
- Remember to create a tune under the conditions used for calibration

- Fill syringe with calmix solution
 - Use 500 μ L syringe
 - Start with positive calmix first
- Set flow rate to 5 μ L/min
- Set up scan parameters appropriately
 - Mass range 150-2000 m/z
 - AGC target 1e6
 - 17,500 resolution



- Optimize source conditions for best signal stability

- Source conditions include: gas flows, voltages, temperatures, source probe adjustments
 - TIC Variation <10% RSD
 - NL signal (min 1e8)
 - IT < 2 ms



- Save Tune File (pos/neg)

Typical Source Settings for Calibration Solutions

- Example for HESI-II probe and syringe pump infusion
- Syringe Pump Flowrate: 5 $\mu\text{L}/\text{min}$

		actual
Sheath gas flow rate	5	6
Aux gas flow rate	1	1
Sweep gas flow rate	0	0
Spray voltage (kV)	3.50	3.50
Spray current (μA)		0.50
Capillary temp. ($^{\circ}\text{C}$)	300	300
S-lens RF level	50.0	
Aux gas heater temp ($^{\circ}\text{C}$)	0	41

Source Auto-Defaults...

Apply Help ☒ Hot link

Sheath gas flow is typically set low, Aux gas flow is either not required or set lower than Sheath

Spray voltage for positive and negative mode is between 3 and 4.5 kV

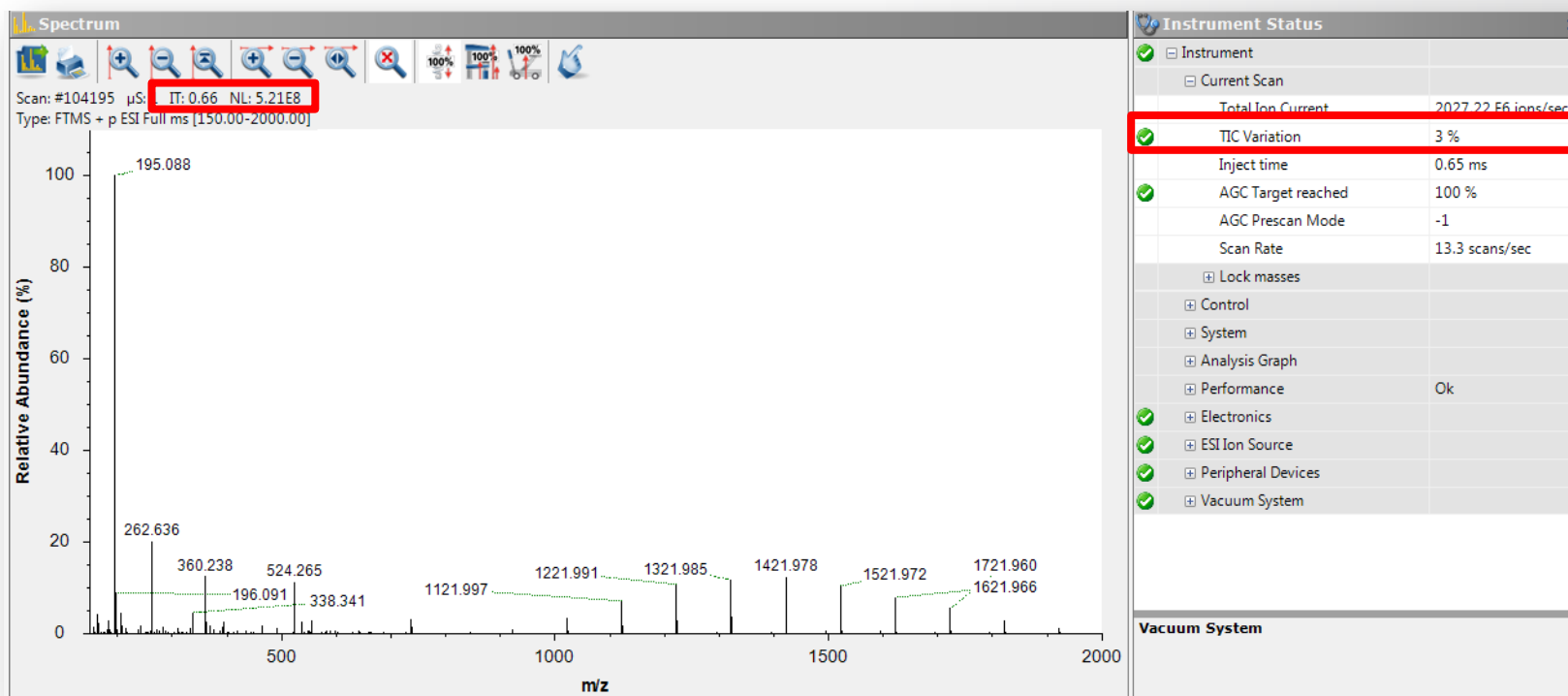
Capillary temp (set at 320 $^{\circ}\text{C}$ for calibration)

S-lens level may have to be adjusted manually (typical range is 50-60)

HESI-II Probe Heater temp is NOT used and can be set to 0 $^{\circ}\text{C}$

Establishing a Tune: Requirements

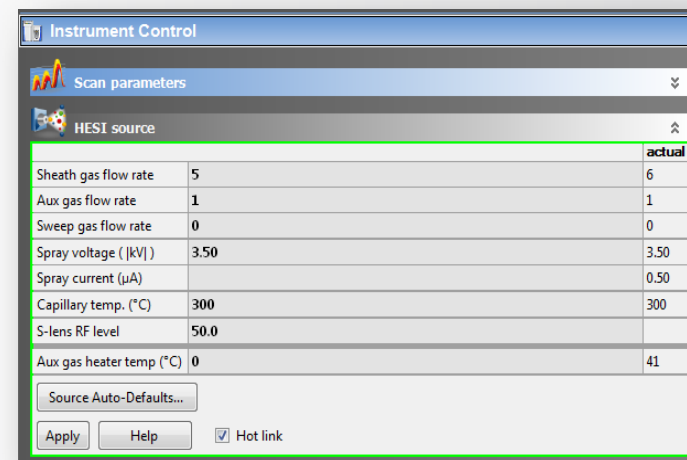
- Optimize source settings for calmix solution using 5 $\mu\text{L}/\text{min}$ flow (see *next slides*)
- Check spray stability: monitor the **TIC Variation** (RSD < 10%)
- Monitor **injection time (IT)**: < 2 ms and establish strong **signal (NL)** ($\geq 1\text{e}8$)



Establishing a Tune: What to Know About the Tune File

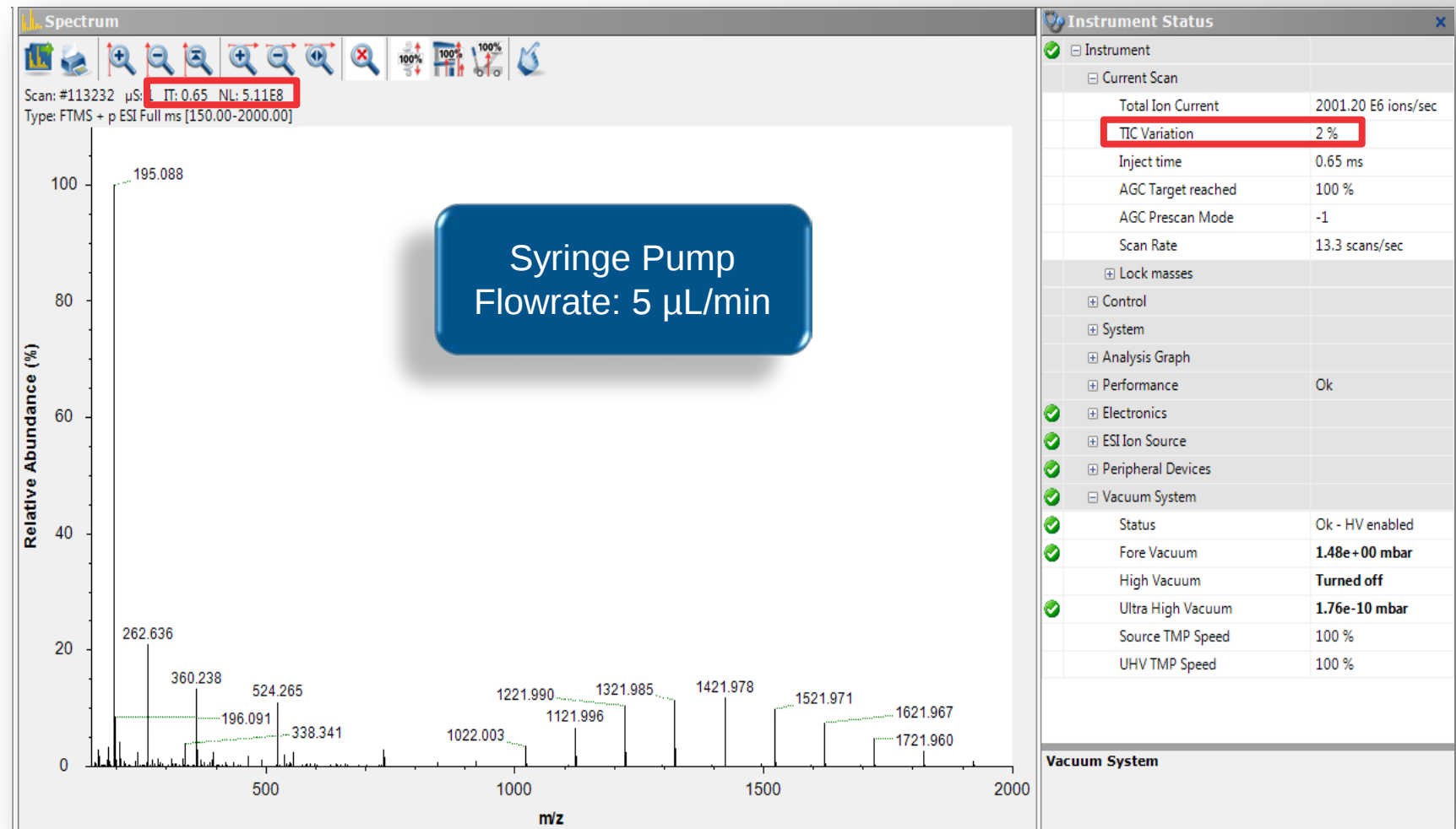
- Unlike calibration files, results of tuning procedure are **not** saved automatically
- Tune files only save the source parameters (e.g. gases, voltages, temps)
- The source parameters for both positive and negative polarity modes can be saved in the same tune file
- To save optimized parameters, select **File > Save Tune** to overwrite the previous Tune file **or File > Save Tune as** to capture the conditions in a new file

Next order of operation ... calibration!



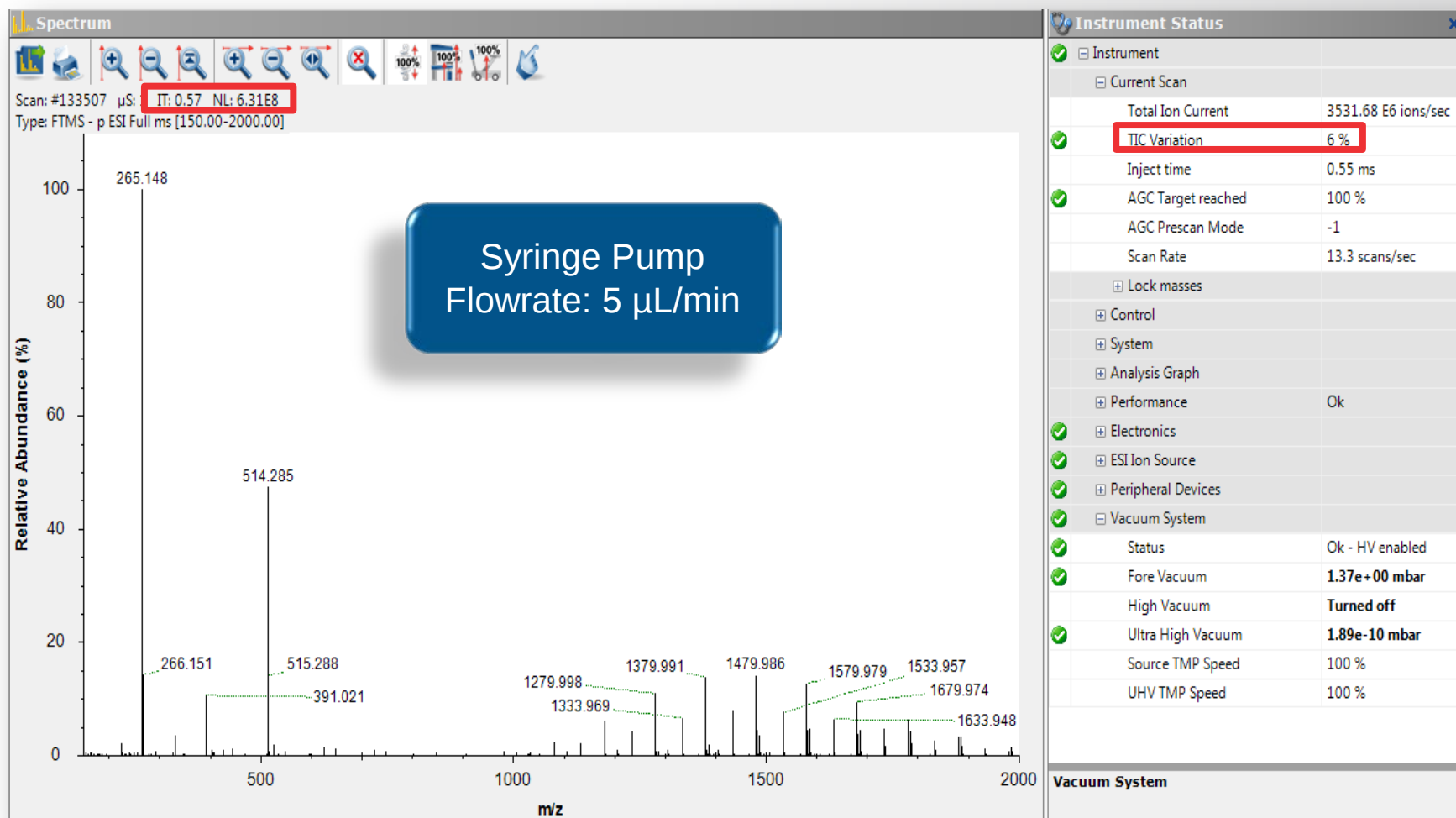
Q Exactive MS Series: A Good Positive Calibration Spectrum

- TIC Variation < 10%
- Intensity > 1e8
- IT < 2 ms



Q Exactive MS Series: A Good Negative Calibration Spectrum

- TIC Variation < 10%
- Intensity > 1e8
- IT < 2 ms

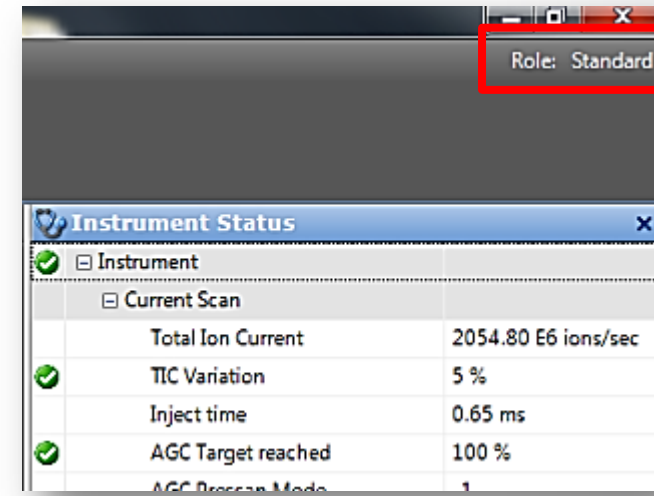


Calibration: Tips for Success

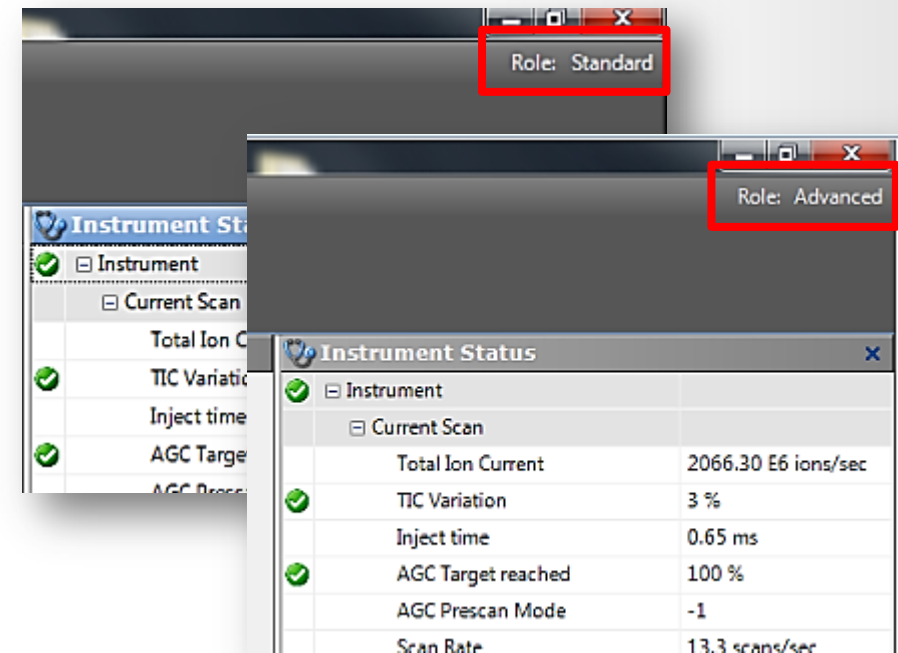
- Make sure calibration solutions are fresh
- Ensure infused calibration solution contains all calibrant masses (pos/neg)
- Maintain stable spray
 - Focus on the injection time (**IT, < 2 ms**) and normalized level (**NL, minimum 1e8**) in the Tune Page scan header
 - In the instrument status pane, monitor the TIC Variation, **RSD < 10%**
- All successful calibration procedures are updated and saved automatically in the “master.cal” file



Q Exactive MS Series Calibration – Standard User Role

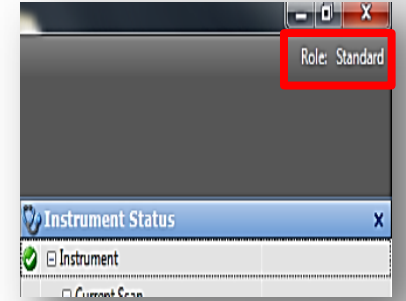


Q Exactive MS Series Calibration – Standard User Role



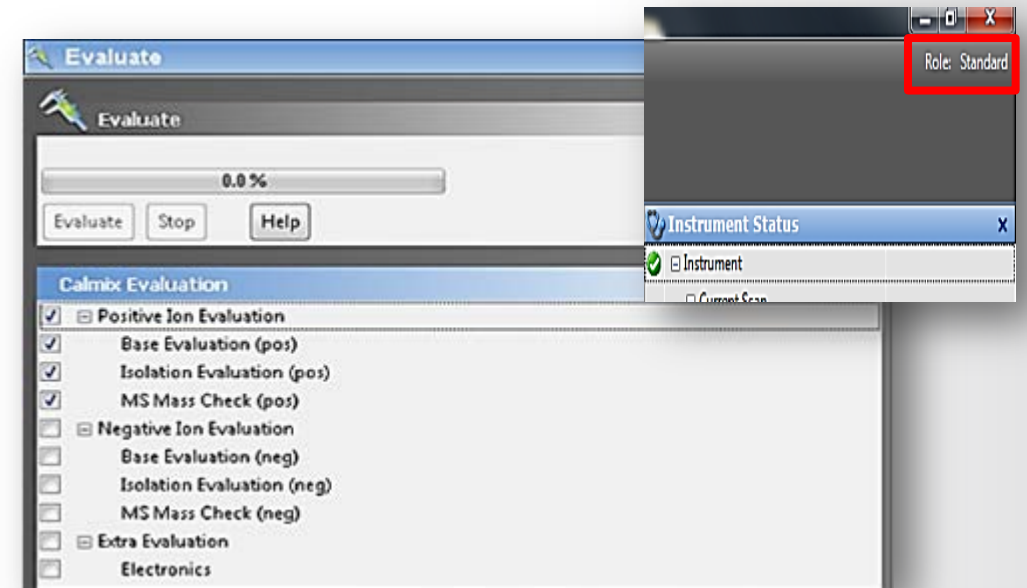
Q Exactive MS Series Calibration – Standard User Role

- NOTE: Always **check/evaluate**, if a procedure fails then calibrate it



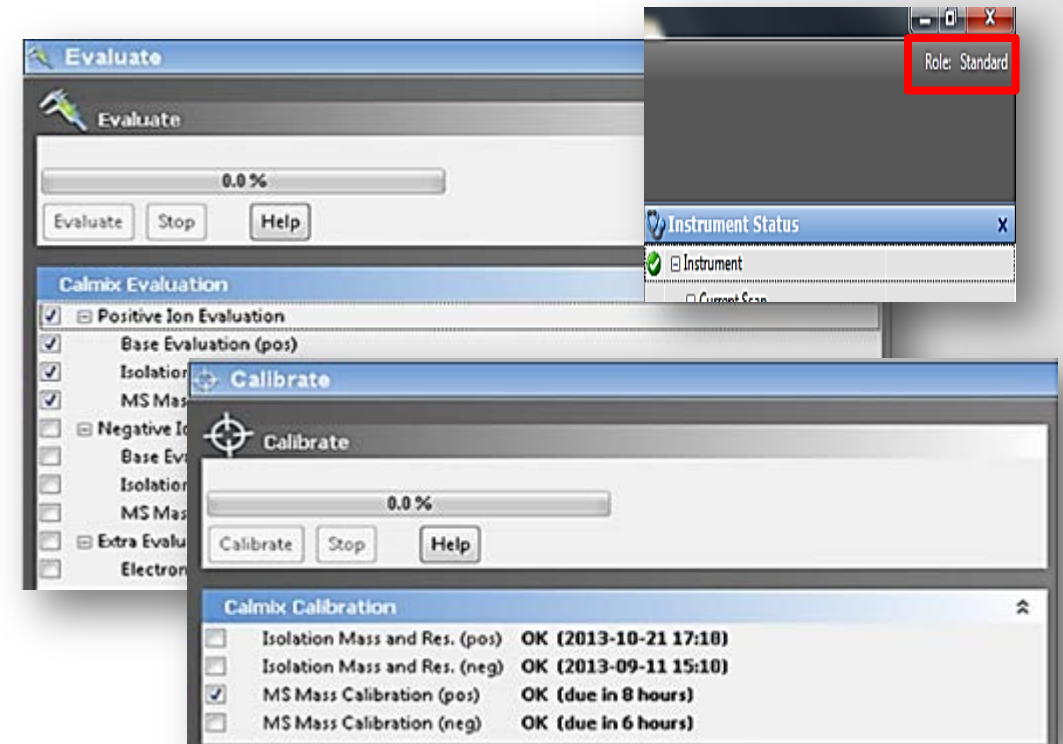
Q Exactive MS Series Calibration – Standard User Role

- NOTE: Always **check/evaluate**, if a procedure fails then calibrate it
- **Positive Ion Evaluation:** evaluate every month; if it fails, calibrate Isolation Mass and Resolution



Q Exactive MS Series Calibration – Standard User Role

- NOTE: Always **check/evaluate**, if a procedure fails then calibrate it
- **Positive Ion Evaluation:** evaluate every month; if it fails, calibrate Isolation Mass and Resolution
- **MS Mass Calibration** (positive/negative): evaluate daily/weekly; if it fails, calibrate
- Calibrate unused polarity modes (e.g. negative mode) at least every 3 months



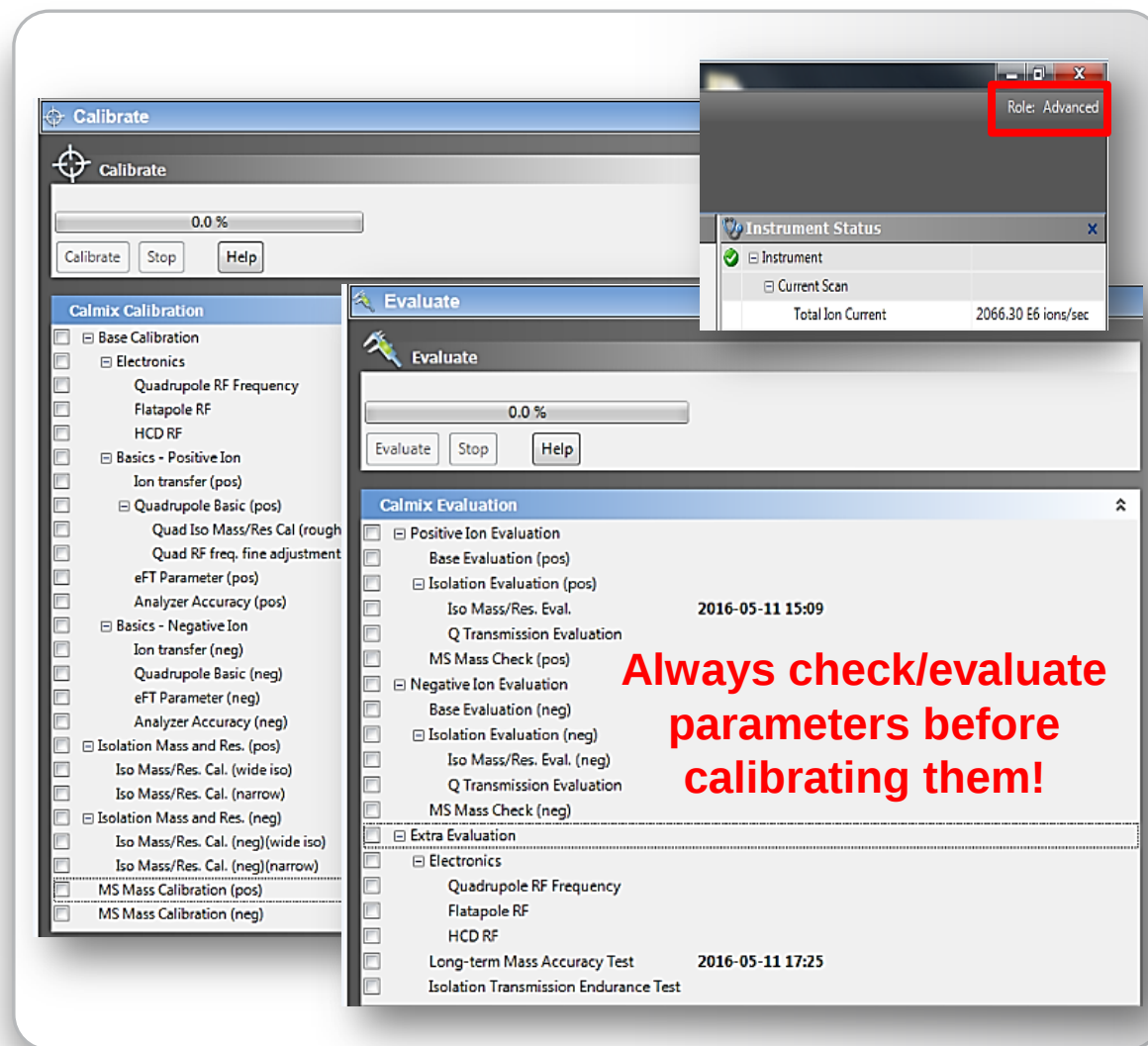
Q Exactive MS Series Calibration – Advanced User Role

- **Electronics:**

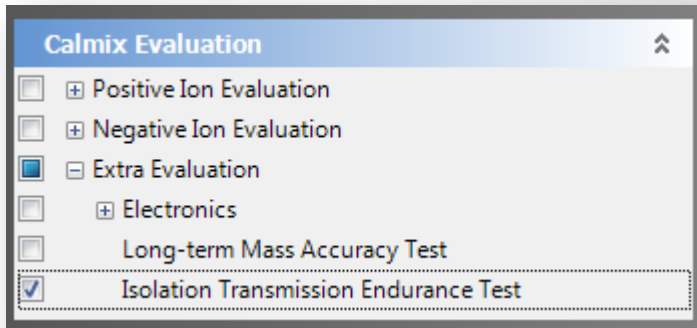
- Calibrated in factory, calibrate only when hardware is replaced

- **Basic Positive or Negative:**

- **Ion transfer:** calibrated in the factory
- **Quadrupole Basic:** evaluate monthly, calibrate only when Isolation Mass and Resolution calibration fails
- **eFT:** evaluate monthly, calibrate if it fails. Always perform a MS Mass Calibration after eFT calibration
- **Analyzer Accuracy:** evaluate monthly, if it fails calibrate (C-trap Charge Detection)
- **Isolation Mass and Resolution:** evaluate monthly, if it fails calibrate. If calibration fails, check quadrupole Basic calibration
- **MS Mass Calibration (positive/negative):** evaluate daily/weekly; if it fails, calibrate



Extra Evaluation – Calmix Evaluation (Introduced in ICSW v2.5)



Dialog with Thermo Q Exactive Plus



Instrument procedure running.

- Evaluating instrument performance ...
- Evaluate
- Extra Evaluation
- Isolation Transmission Endurance Test
- Phase FS : 7 of 56 min (equilibrating - syringe pump is stopped and will re-start automatically)

Please wait ...

Cancel

Dialog with Thermo Q Exactive Plus



Evaluation done.

- Done: Isolation Transmission Endurance Test: Transmission score at 0.98

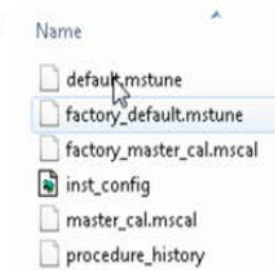
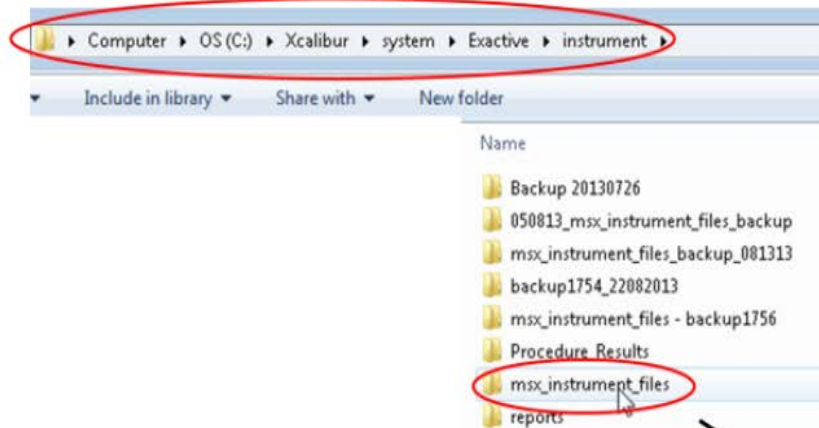
OK

Electronics evaluations are NOT recommended on a regular basis

These evaluations should be performed upon request with guidance from tech support

The evaluation **REQUIRES** a stable spray of calmix (Intensity > 1e8; TIC Variation < 10%; IT < 2 ms)
Calmix infusion starts automatically

Calibration: Regular Backup of the Calibration File



Backup the 'msx_instrument_files' folder **quarterly**

Calibration: Reports (Tune v2.6)

The screenshot displays the Thermo Q Exactive Focus software interface, version 2.6. The main window is titled "Thermo Q Exactive Focus — operational". The "Reports" menu is highlighted, showing options for "Calibration", "Spectral Mass Calibration (neg)", "Spectral Mass Calibration (pos)", and "All Reports". The "Calibration" option is further expanded, showing "Latest..." and "List..." buttons.

The "Scan parameters" section on the left shows the following settings:

- Scan type: Full MS
- Scan range: 150.0 to 2,000.0 m/z
- Fragmentation: None
- Resolution: 17,500
- Polarity: Positive
- Microscans: 1
- Lock masses: Off
- AGC target: 1e6
- Maximum inject time: 50

The "HESI source" section shows the following settings:

- Sheath gas flow rate: 5
- Aux gas flow rate: 1
- Sweep gas flow rate: 0
- Spray voltage (kV): 3.50
- Spray current (μA): 0.40
- Capillary temp. (°C): 300
- S-lens RF level: 50.0
- Aux gas heater temp (°C): 0

The "Acquisition" section shows the following settings:

- Acquisition state: ready
- Progress: 0.00 min
- File in use: unknown
- Destination file: C:\Xcalibur\Data\Bus_NoLC2
- Method file: by time

The "Mass Traces" section shows the following settings:

- Calibrate
- Evaluate
- Vacuum / Bakeout

The "Spectrum" section displays a mass spectrum plot with the following data points:

m/z	Relative Abundance (%)
195.088	100
262.636	20
360.238	10
524.265	10
196.091	5
338.341	5
1221.991	10
1322.989	5
1321.985	10
1421.978	10
1521.972	10
1621.968	10
1721.960	5

The "Instrument Status" section on the right shows the following status:

- Instrument: OK
- Current Scan: OK
- Total Ion Current: 1995.84 E6 ions/sec
- TIC Variation: 3 %
- Inject time: 0.66 ms
- AGC Target reached: 100 %
- AGC Prescan Mode: -1
- Scan Rate: 13.1 scans/sec
- Lock masses: OK
- Control: OK
- System: OK
- Analysis Graph: OK
- Performance: OK
- Electronics: OK
- ESI Ion Source: OK
- Peripheral Devices: OK
- Vacuum System: OK
- Status: OK - HV enabled
- Fore Vacuum: 1.43e+00 mbar
- High Vacuum: Turned off
- Ultra High Vacuum: 1.87e-10 mbar
- Source TMP Speed: 100 %
- UHV TMP Speed: 100 %

The "Analysis Graphs" section at the bottom shows a plot of mass accuracy over time, with the y-axis labeled "Mass accuracy" and the x-axis labeled "Scan". The plot shows a stable line at approximately 0.00 mbar.

Calibration: Reports (Tune v2.6)

The screenshot displays the Thermo Q Exactive Focus software interface. The 'Reports' menu is open, showing options for Calibration, Spectral Mass Calibration (neg), Spectral Mass Calibration (pos), and All Reports. The 'Calibration' option is selected, and the 'Latest...' button is highlighted. The main window shows the 'Calibration Report' for the file '90180665870_Calibration_2016-06-06_14-53-52.pdf'. The report includes the following sections:

Calibration Report

Date of calibration: 2016-06-06 14:53:52
Windows user name: FDKW762(Thermo)
Q Exactive Focus
Instrument identification: Exactive Series slot #150 (90180665870)

Type of calibration: Spectral Mass Calibration (pos)

Scan settings:
scan range: 150.0 - 2000.0
resolution: 17500
AGC target: 1000000
micro scans: 1

Check ok: Ion signal (Ion s/n 2683, TIC 1.59e+09)

Mass accuracy without lock mass

Scan	138.06619	195.08765	524.26496	1221.99064	1421.97786	1621.96509
1	138.06621	195.08769	524.26505	1221.99070	1421.97754	1621.96612
2	138.06620	195.08767	524.26500	1221.99054	1421.97751	1621.96606
3	138.06620	195.08767	524.26499	1221.99060	1421.97738	1621.96612
4	138.06622	195.08789	524.26510	1221.99081	1421.97759	1621.96616
5	138.06621	195.08769	524.26503	1221.99065	1421.97758	1621.96608
6	138.06618	195.08764	524.26492	1221.99037	1421.97721	1621.96558
7	138.06618	195.08764	524.26495	1221.99042	1421.97725	1621.96574
8	138.06620	195.08767	524.26488	1221.99064	1421.97748	1621.96578
9	138.06620	195.08766	524.26486	1221.99063	1421.97735	1621.96570
20	138.06621	195.08768	524.26500	1221.99055	1421.97753	1621.96595
mean value	138.06620	195.08767	524.26500	1221.99059	1421.97742	1621.96593
std. dev. (ppm)	0.093	0.097	0.101	0.106	0.096	0.131

Mass accuracy with lock mass 195.08765

Scan	138.06619	195.08765	524.26496	1221.99064	1421.97786	1621.96509
11	138.06619	195.08765	524.26499	1221.99045	1421.97742	1621.96576
12	138.06619	195.08765	524.26491	1221.99042	1421.97727	1621.96564
13	138.06619	195.08765	524.26491	1221.99039	1421.97716	1621.96588
14	138.06619	195.08765	524.26489	1221.99052	1421.97738	1621.96578
15	138.06619	195.08765	524.26494	1221.99044	1421.97723	1621.96577
16	138.06619	195.08765	524.26495	1221.99059	1421.97732	1621.96594
17	138.06619	195.08765	524.26494	1221.99050	1421.97726	1621.96592
18	138.06619	195.08765	524.26495	1221.99046	1421.97739	1621.96585

Mass accuracy

Mass Calibration Trend/History

Procedure result: passed (rms = 0.25/0.27 ppm)

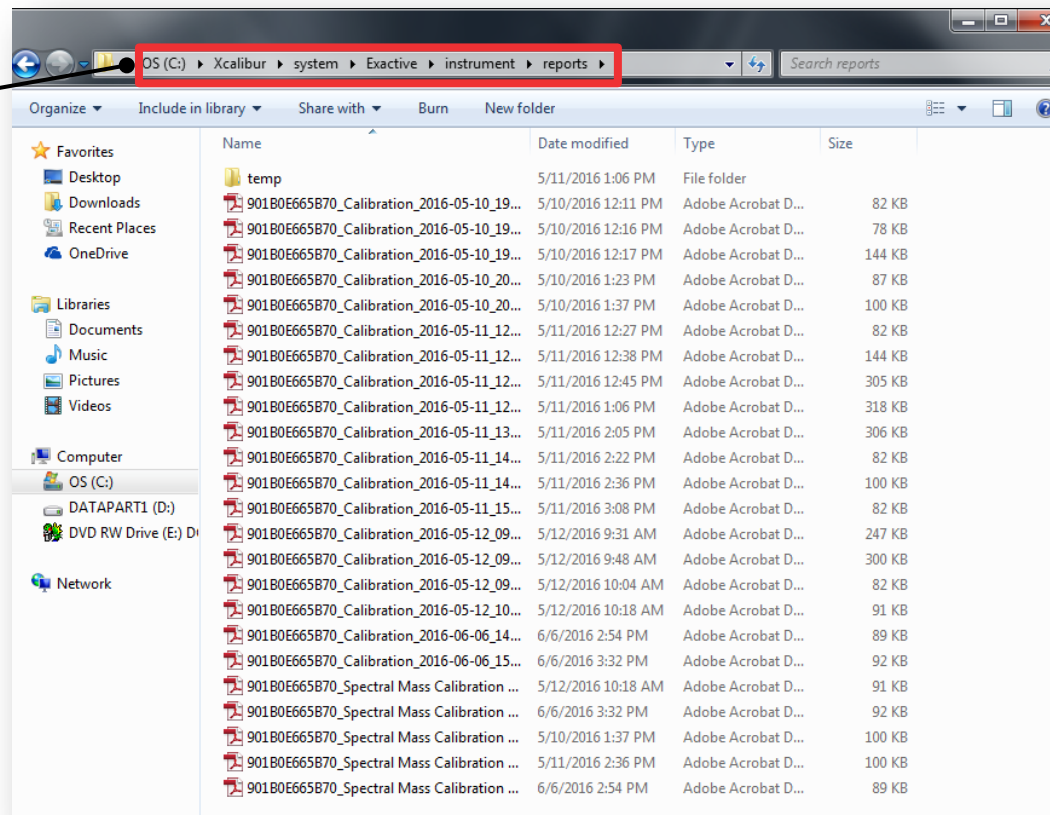
Calibration Reagent: _____
Lot Number: _____
Expiration Date: _____

Name of Instrument Operator: _____
Signature: _____ Date: _____

Calibration: Reports (Tune v2.6)

Report Files

- Saved in data system's local
C:\Xcalibur\system\Exactive\instrument
\reports

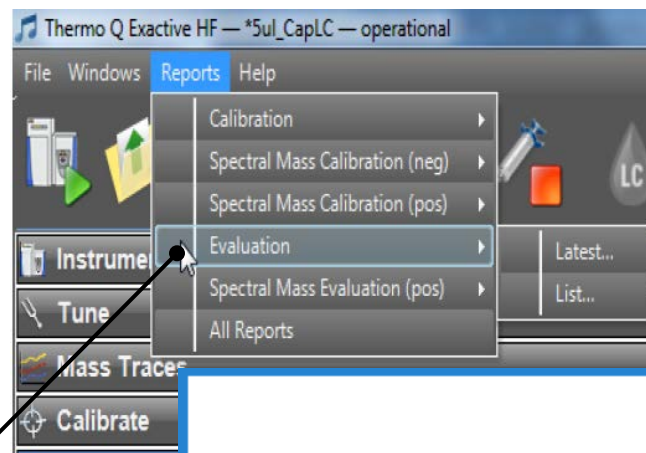


Calibration: Reports (Tune v2.6)

Tune v 2.8 SP1

- Evaluation reports are now available
- Structure of drop-down menu under reports sorted and cleaned up to enable quick finding of reports

NEW



Evaluation Report

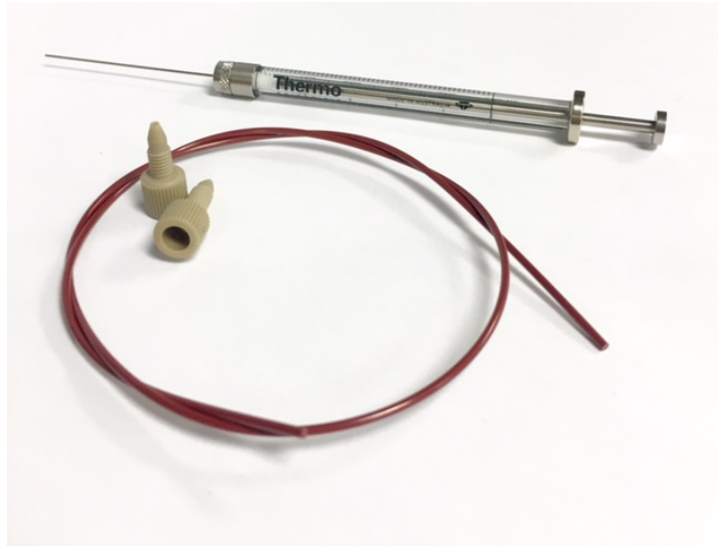
Thermo
SCIENTIFIC

Date of evaluation: 2016-08-24 11:01:50
Windows user name: WINDOWS-3FU3DB0\Thermo(Thermo)
Q Exactive HF: 2.8-268801/2.8.0.2688
Instrument identification: Exactive Series slot #1 (90180E49DA3D)

Type of evaluation: C-Trap Charge Detection Scaling

Scan settings:
scan range: 150.0 - 2000.0
resolution: 60000
AGC target : 1000000.0
micro scans : 1

- To avoid persistent contamination with Ultramark or Caffeine in the Ion Max API source



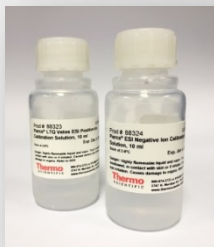
Reminder:

- Use dedicated syringe and infusion line for calibration
- Use dedicated ion transfer capillary for calibration
- After calibrations, flush lines with LC/MS grade methanol

Tuning and Calibration: Take Home Message

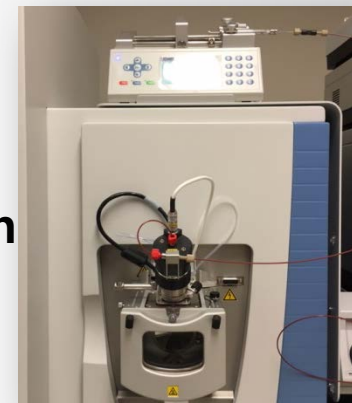
- **Key Considerations:**

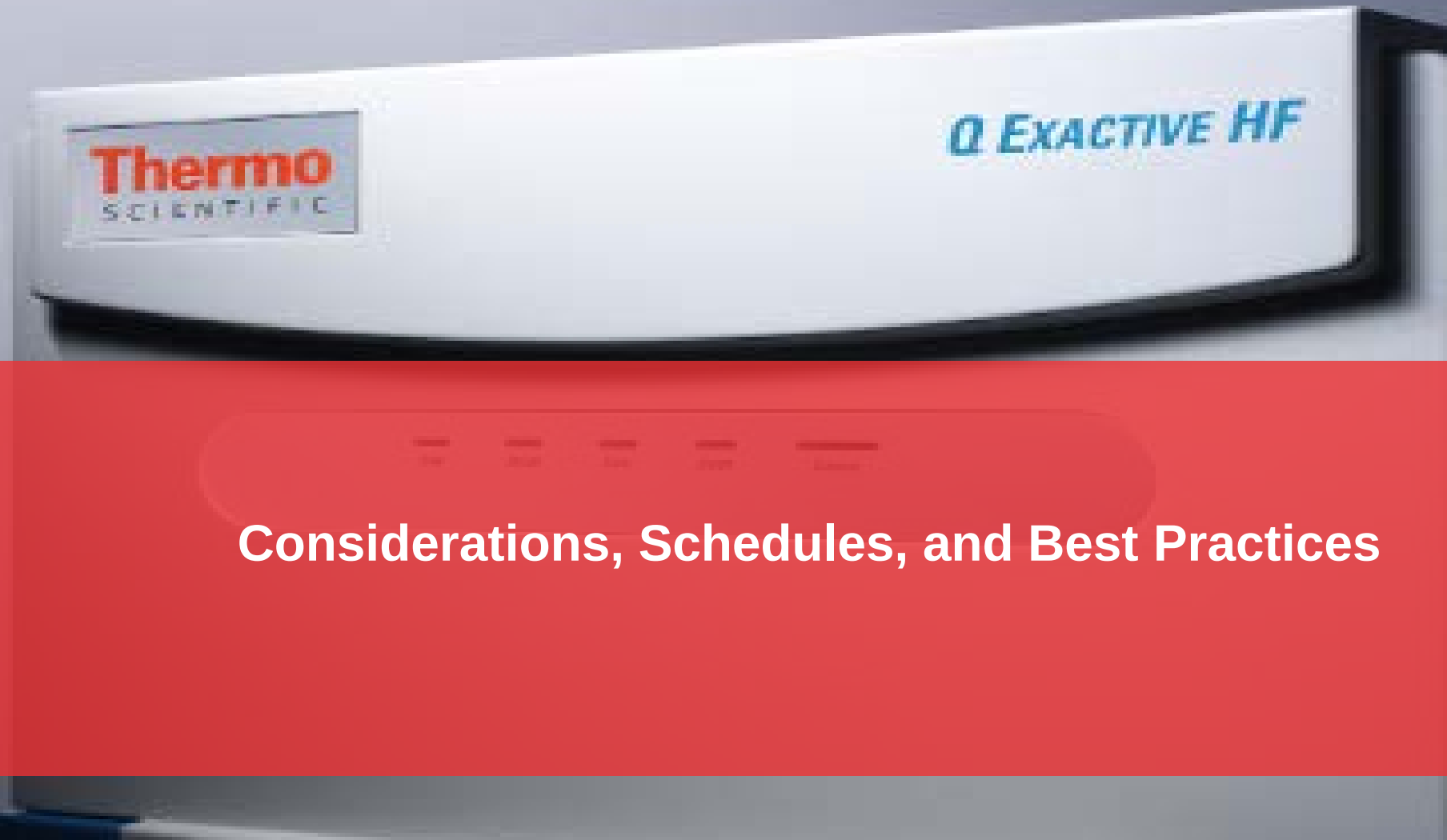
- Successful evaluation/calibration must be performed under stable spray conditions using HESI-II probe
 - Ensure <10% TIC Variation for >100scans located in instrument status panel via tune page
- Use fresh calmix for all calibrations
 - Pierce LTQ Velos ESI Positive Ion Calibration Solution
 - *Catalog no. 88323*
 - Pierce Negative Ion Calibration Solution
 - *Catalog no. 88324*



- **Calibration Schedule:**

- Weekly
 - Perform **MS Mass Calibration**
- Monthly
 - Run **Ion Transfer** calibration
 - Run **Base Evaluation**, if failed run **Analyzer Accuracy** calibration and **eFT** calibration
 - Run **MS Mass Calibration**
 - Run **Isolation Evaluation**, if failed, calibrate **Wide Isolation** and then **Narrow Isolation**
- Following vent/clean → perform monthly procedure





Considerations, Schedules, and Best Practices



Maintenance Considerations

- Always wear clean, talc-free and lint free gloves when handling instrument components
- Always place the components on a clean, lint-free surface, such as Aluminum foil
- Never overtighten a screw or use excessive force
- Ensure tools are clean; dirty tools can contaminate your system
- Never insert a test probe into the sockets of female cable connectors on Printed Circuit Boards (PCBs)

Recommended Maintenance Schedule

• Daily

- Check the vacuum gauge pressures and make sure the vacuum system is operational. Typical values are:
 - Fore Vacuum: <2 mbar
 - High Vacuum: Turned off
 - Ultra High Vacuum : < 5e-10 mbar
 - Source and UHV TMP Speed: 100%
- If bottled nitrogen gas is being used, check nitrogen gas pressure. Recommended value for the Q Exactive MS series is 800 ± 30 kPa (8 ± 0.3 bar, 116 ± 4 psi)
- Make sure the solvent waste bottle for drain tube is empty
- Flush (clean) sample transfer line
- Perform MS Mass Calibration (*optional*)

Recommended Maintenance Schedule

- **Weekly**

- Check the forepump oil level and check for condensation in the pump

NOTE: An increase of the pump oil level is a sign of vapor condensation in the pump

- Fill the forepump with oil as needed
- Ballast the forepump to drain oil back into the pump (~30 min)
- Clean the ion transfer capillary
- Perform MS Mass Calibration

- **Monthly**

- Evaluate Q Exactive MS series calibrations and recalibrate only if the evaluation(s) fails

Recommended Maintenance Schedule

- **Quarterly**

- Back up the data folder and master calibration file
- Check relevant websites for available software updates
 - Thermo Fisher Scientific BRIMS portal for Omics software - <https://portal.thermo-brims.com/>
 - Thermo Fisher Scientific Customer Download Site - <https://thermo.flexnetoperations.com/control/thmo/login>

- **Yearly**

- Schedule Preventative Maintenance (PM)
- Leak Check Gas Lines
- Defragment computer hard drive
- Replace the forepump oil

Removing the Sweep Cone



CAUTION: During operation of the mass spectrometer, sweep cone might reach temperatures of up to 450° C.

Let the spray cone cool down for at least 60 minutes before removing.

Remove the ion sweep cone by grasping its outer ridges and pulling the cone straight off of the API cone seal.

Clean the ion sweep cone by wiping the inside and outside a lint-free tissue soaked in LC/MS grade methanol.

Remove and inspect the O-ring that is seated in the spray cone under the entrance end of the ion transfer capillary and clean with LC/MS grade methanol or replace if necessary.

Removing the Sweep Cone



CAUTION: During operation of the mass spectrometer, sweep cone might reach temperatures of up to 450° C.

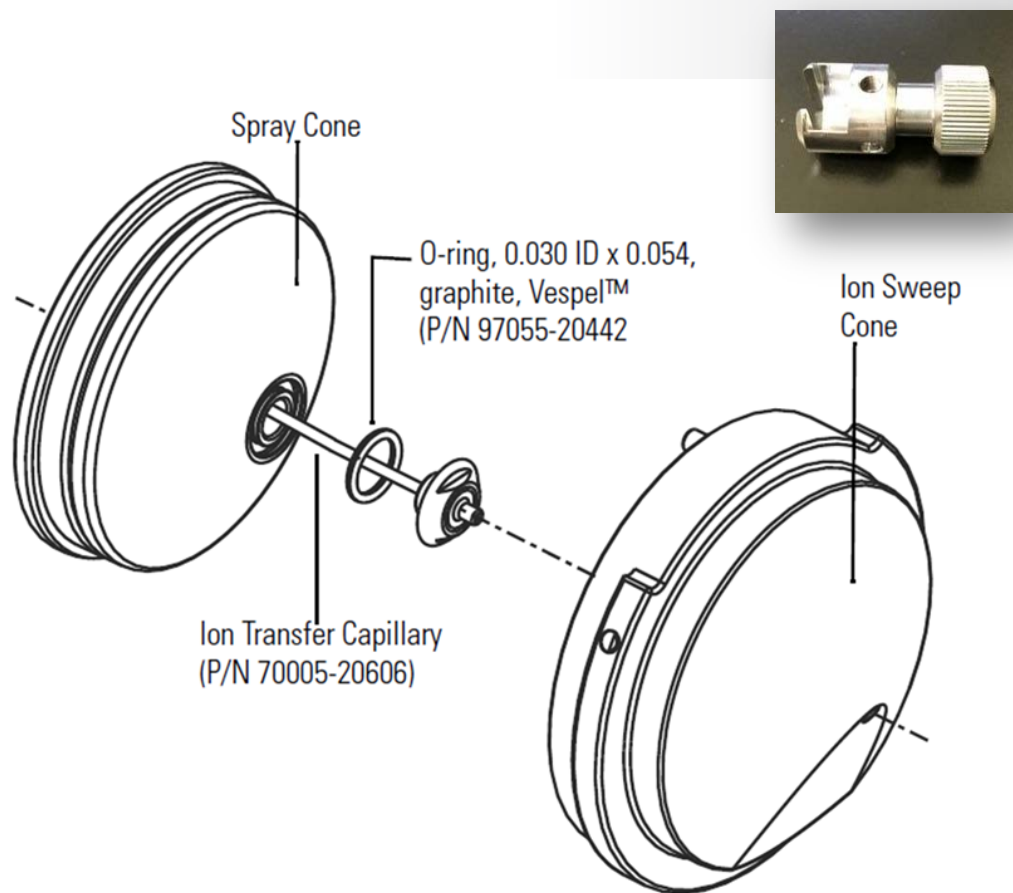
Let the spray cone cool down for at least 60 minutes before removing.

Remove the ion sweep cone by grasping its outer ridges and pulling the cone straight off of the API cone seal.

Clean the ion sweep cone by wiping the inside and outside a lint-free tissue soaked in LC/MS grade methanol.

Remove and inspect the O-ring that is seated in the spray cone under the entrance end of the ion transfer capillary and clean with LC/MS grade methanol or replace if necessary.

Removing Ion Transfer Capillary



Turn off flow from LC.

Turn off Capillary temperature and allow Ion Transfer capillary to cool (temperature needs to be below 200°C).

Remove source housing from the front of the mass spectrometer.

Remove the Ion Sweep Cone.

Remove Ion Transfer Capillary by turning it counterclockwise with the custom removal tool.

Removing the Ion Transfer Capillary

- Click here to see [video](#)

Cleaning Ion Transfer Capillary



Cleaning Ion Transfer Capillary



Cleaning Ion Transfer Capillary



Sonicate the Ion Transfer Capillary for 30 minutes in 50:50 solution of methanol/water containing 20% formic acid.

Rinse the Ion Transfer Capillary thoroughly with LC/MS grade water.

Sonicate the Ion Transfer Capillary in LC/MS grade water for 15 minutes.

Rinse the Ion Transfer Capillary with LC/MS grade methanol.

Sonicate the Ion Transfer Capillary in LC/MS grade methanol for 15 minutes.

Blow the Ion Transfer Capillary dry with a stream of nitrogen gas.

In Case of Extreme Contamination



Add a cleaning step that uses Liquinox™ to the standard procedure as follows:

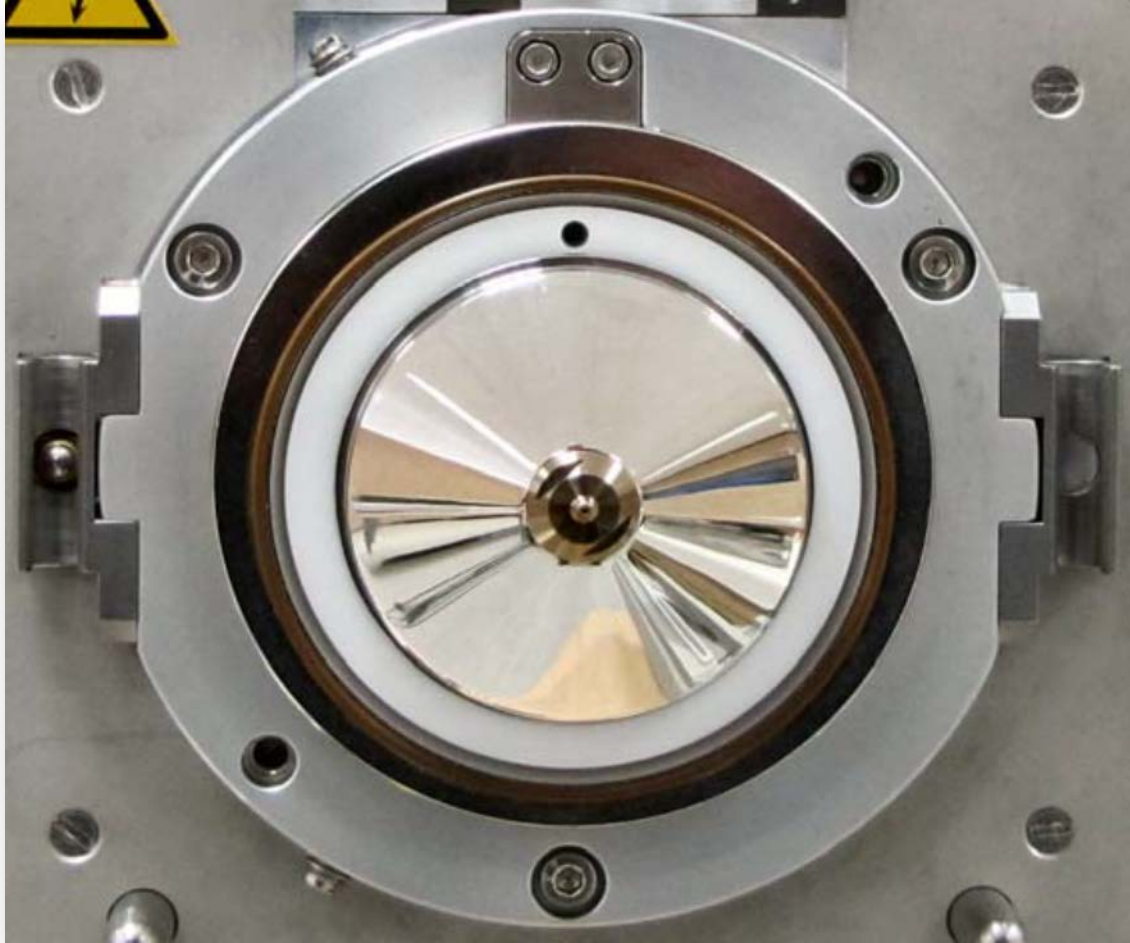
Sonicate the Ion Transfer Capillary overnight in a 10% Liquinox™ solution in LC/MS grade water.

Rinse the Ion Transfer Capillary, forcing a strong stream of LC/MS grade water through the orifice for 2 minutes.

Sonicate the Ion Transfer Capillary for 30 minutes in LC/MS grade water.

Continue with standard procedure
(*see previous slide*).

Reinstalling Ion Transfer Capillary



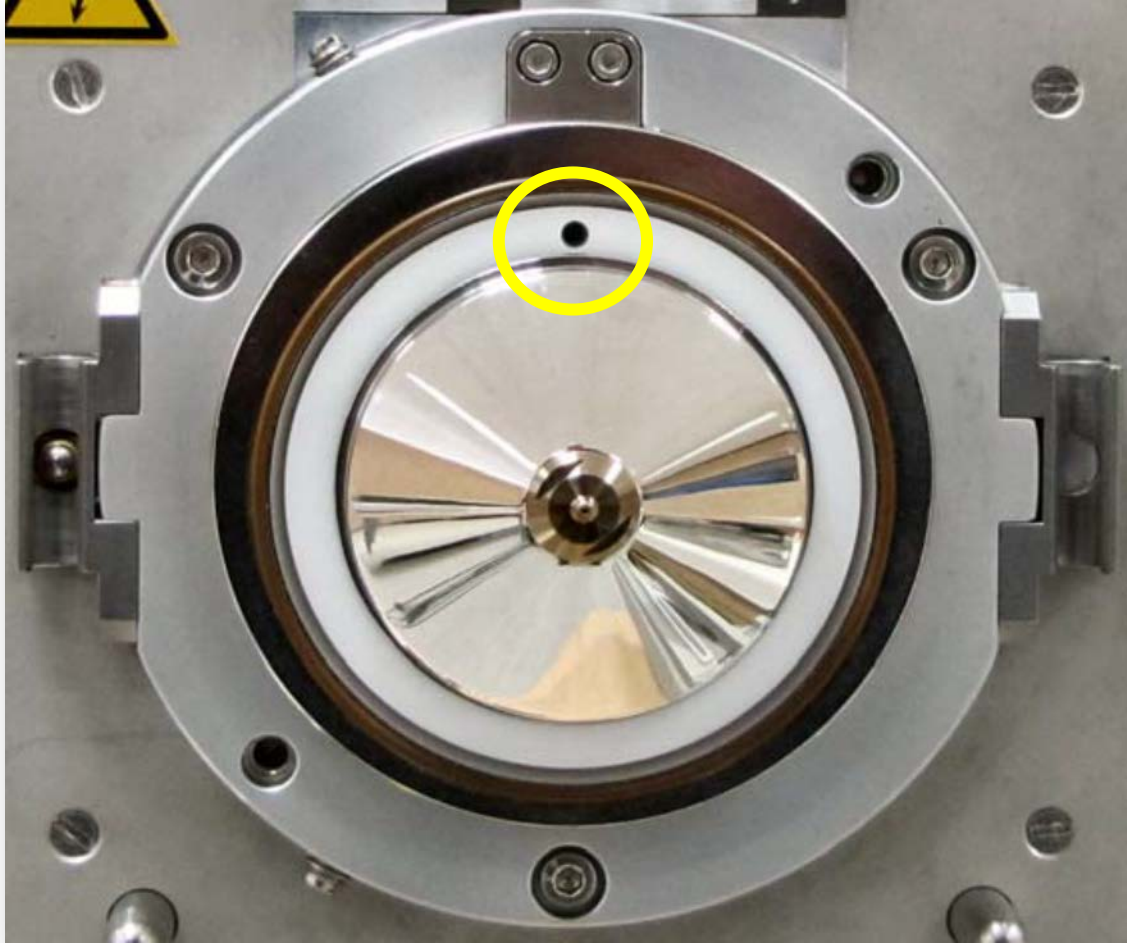
NOTICE: Use caution when reinstalling the Ion Transfer Capillary.

Ensure that everything is properly aligned to prevent stripping the threads on the Ion Transfer Capillary.

Insert the Ion Transfer Capillary into the heater block and rotate the Ion Transfer Capillary as you insert it.

After it is inserted, turn the Ion Transfer Capillary clockwise until it is finger tight.

Reinstalling Ion Transfer Capillary



NOTICE: Use caution when reinstalling the Ion Transfer Capillary.

Ensure that everything is properly aligned to prevent stripping the threads on the Ion Transfer Capillary.

Insert the Ion Transfer Capillary into the heater block and rotate the Ion Transfer Capillary as you insert it.

After it is inserted, turn the Ion Transfer Capillary clockwise until it is finger tight.

Align the gas inlet on the Ion Sweep Cone with the sweep gas supply port on the ion source mount. Firmly press the Ion Sweep Cone onto the ion source mount.

Instrument Shutdown Procedure



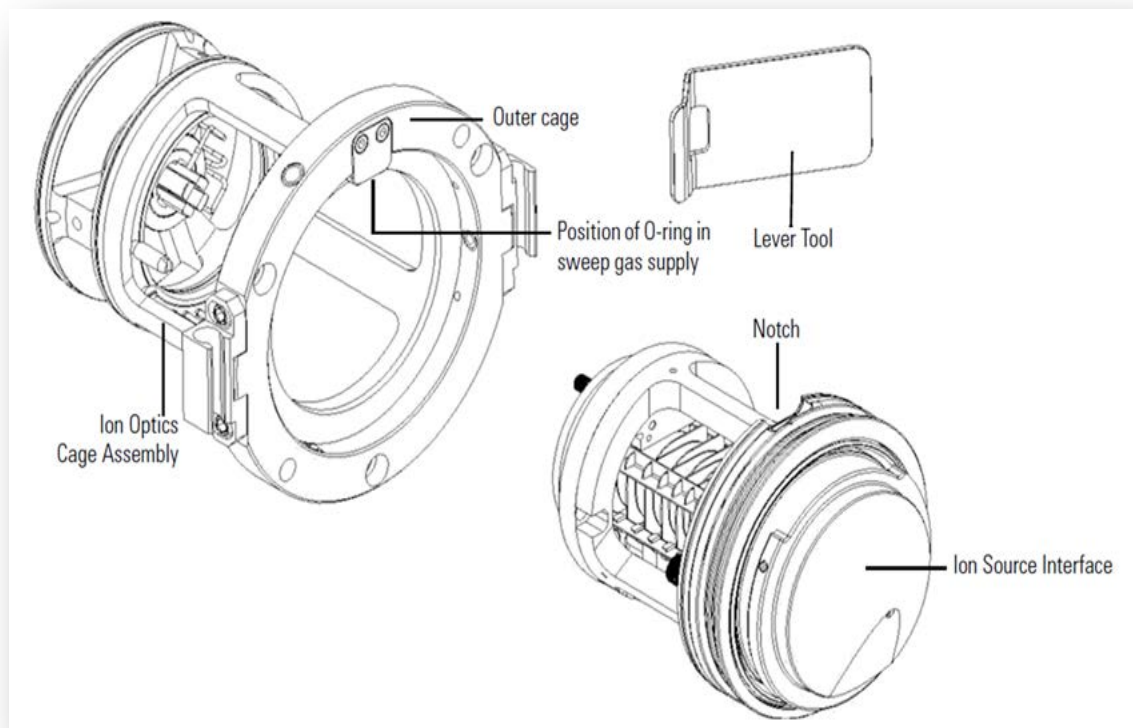
In the Tune software window, click the On/Standby button to put the instrument in the Off condition.

Place the electronics service switch (located on the power panel) in the Service Mode position.

Put the main power circuit breaker switch of the mass spectrometer in the Off position.

NOTICE: An instrument that is shut down still consumes nitrogen because the vent valve is connected to the nitrogen supply of the laboratory. Keeping on the nitrogen flow prevents humidity from contaminating the vacuum system of the mass spectrometer.

Removing the Ion Source Interface

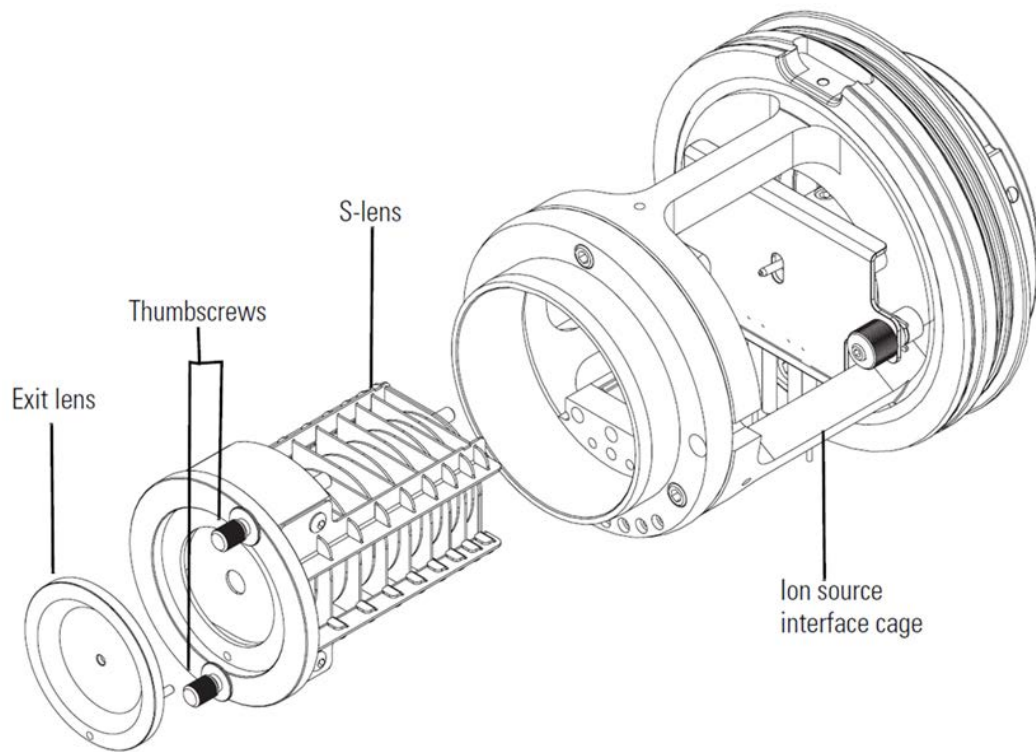


- **CAUTION:** The ion source interface can become hot enough to cause severe burns when the mass spectrometer is in operation. Do not touch the ion source interface immediately after removing the ion source housing. Allow the ion source interface to cool to room temperature before removing it from the mass spectrometer.
- Use the tool to lever out the interface assembly.
- Grasp the ridges on either side of the ion source interface and firmly pull the assembly straight out.

Removing the Ion Source Interface

- Click here to see [video](#)

Removing S-Lens



Prepare a clean work surface by covering the area with lint-free paper or Aluminum foil.

Put on a new pair of lint- and powder-free gloves.

Loosen and extend the two thumbscrews that secure the S-lens to the ion source interface cage and the exit lens to the S-lens.

Remove the exit lens from the S-lens and place it on a clean, lint-free surface.

Grasp the two thumbscrews and carefully pull the S-lens straight out of the ion source interface cage and place it on a clean, lint-free surface.

Cleaning the S-Lens



DO NOT DISASSEMBLE THE S-LENS!

NOTICE: Do not clean the exit lens or S-lens with abrasives, acidic or caustic substances, or detergents not stated.

Clean the S-lens by sonicating the unit for 10–15 minutes in a 50:50 solution of LCMS-grade methanol and water or a 1% solution of Liquinox in LCMS-grade water.

Rinse the components thoroughly with LCMS-grade water, then rinse the exit lens and S-lens with fresh LCMS-grade methanol.

Blow dry the exit lens and S-lens with oil-free nitrogen gas.

Cleaning the S-Lens



Ensure that all solvent has evaporated from the components before reassembly.

Inspect the components under magnification for any lint or particulates.

NOTICE: Inspect the orifices to confirm that no lint or particulates are present in the bore of the orifices.

Use tweezers or a similar tool to remove the lint or particulate.

Removing and Cleaning the S-Lens

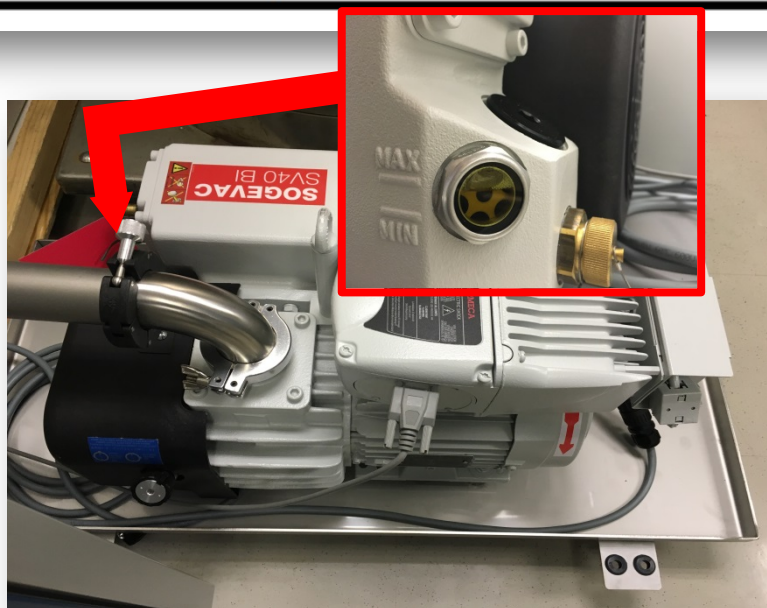
- Click here to see [video](#)

Reinstalling Ion Source Interface

- Click here to see [video](#)

Forepump Maintenance

Maintenance job	Frequency
Check oil level	Daily
Check oil condition	Depends on process
Check gas ballast valve	Monthly
Change oil	Every 8000 h (~ one year) of operation
Replace exhaust filter	If oil mist appears at exhaust or annually
Check anti-suckback valve	Annually
Clean fan cover	Annually



Oil level should be close to the MAX marks. If below the MIN mark, add the required amount.

Make certain the oil is not turbid.

Open the ballast valve and operate the pump for 30 minutes.

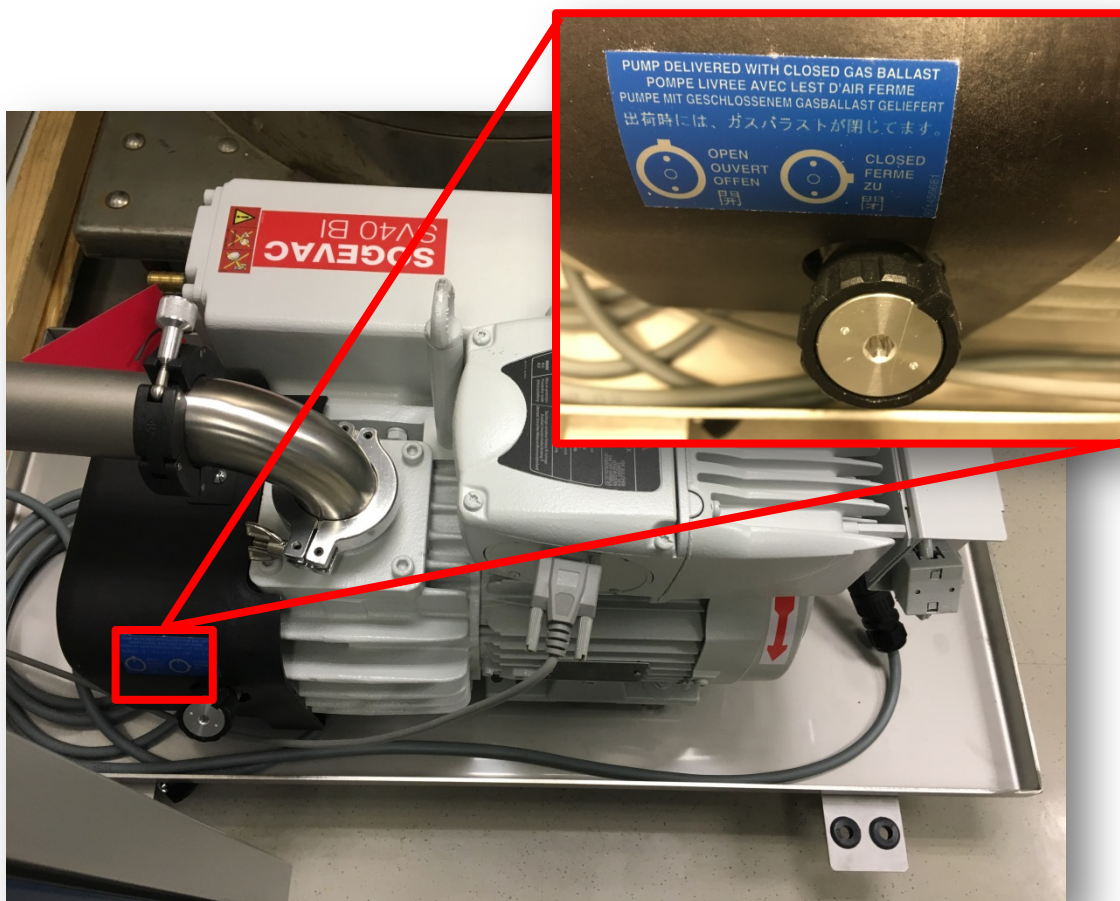
Oil change must be done with a switched off and still warm pump.

Pump switched off, open the exhaust hood, take out the filter and replace it.

If dirty, clean with an appropriate solvent.

Remove the filter and clean with stream of air.

Purging the Oil of the Forepump



Turn off the flow of liquid from the LC (or other sample introduction device to the API source).

Put the MS into Standby mode.

Open the gas ballast valve on the forepump.

Allow the pump to run for 30 minutes with the gas ballast valve open.

After 30 minutes, close the gas ballast valve.

Instrument Startup Procedure After a Shutdown



Switch on the computer and wait until the operating system is completely loaded.

Start the Tune software. NOTICE: data system must be running before start up of instrument, otherwise the instrument will not operate.

Turn on nitrogen flow at the tank, if it is off. NOTICE: Make sure the main power circuit breaker switch is in the off position (O) and the electronics service switch is in the Service Mode position.

Place the main power circuit breaker switch in the On (I) position to start up the forepump and turbomolecular pumps (TMPs); all LEDs on the front panel will remain off.

Instrument Startup Procedure After a Shutdown



Within a short time, a significant pressure decrease must be observed. The quality of the vacuum can be estimated by the rotation speed of the TMPs.

Allow the mass spectrometer to pump down for 5 minutes.

Place electronics service switch into Operating Mode position and continue to monitor the vacuum readings in the Tune software.

Proceed to the bake out procedure to bake out the system before data acquisition occurs.

Baking Out the System



In the Q Exactive MS Series Tune software window, click the On/Standby button to put the instrument in Off condition (see *image*).

Baking Out the System



Vacuum / Bakeout



Vacuum / Bakeout

Fore vacuum (mbar) 1.46E+00 ●
UHV (mbar) 1.77E-10 ●

Bakeout time (h) 12.0

Enter standby after Bakeout ☒

0.0 %

Bake out

Stop

Help

In the Q Exactive MS Series Tune software window, click the On/Standby button to put the instrument in Off condition (see *image*).

In the Tasks panel of the Q Exactive MS Series Tune software window, click to display the Vacuum / Bakeout.

Baking Out the System



Vacuum / Bakeout

Vacuum / Bakeout

Fore vacuum (mbar) 1.46E+00 ●

UHV (mbar) 1.77E-10 ●

Bakeout time (h) 12.0

Enter standby after Bakeout ☒

0.0 %

Bake out Stop Help

In the Q Exactive MS Series Tune software window, click the On/Standby button to put the instrument in Off condition (see *image*).

In the Tasks panel of the Q Exactive MS Series Tune software window, click to display the Vacuum / Bakeout.

Enter the baking duration (in hours) into the spin box. The available range is 4 to 30 hours (v 2.7 SP1), 12 hours is recommended as the maximum.

Baking Out the System



Vacuum / Bakeout

Vacuum / Bakeout

Fore vacuum (mbar) 1.46E+00 ●

UHV (mbar) 1.77E-10 ●

Bakeout time (h)

Enter standby after Bakeout ☒

0.0 %

Bake out Stop Help

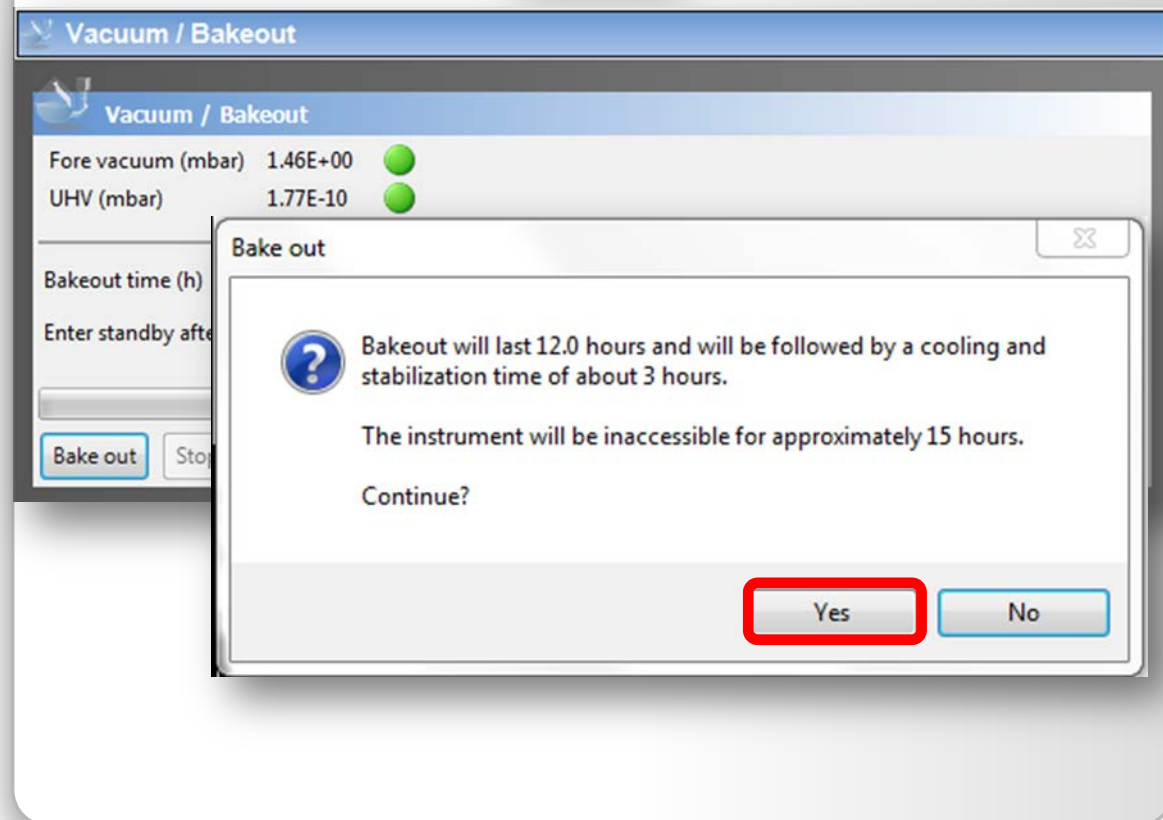
In the Q Exactive MS Series Tune software window, click the On/Standby button to put the instrument in Off condition (see *image*).

In the Tasks panel of the Q Exactive MS Series Tune software window, click to display the Vacuum / Bakeout.

Enter the baking duration (in hours) into the spin box. The available range is 4 to 30 hours (v 2.7 SP1), 12 hours is recommended as the maximum.

Click Bakeout.

Baking Out the System



In the Q Exactive MS Series Tune software window, click the On/Standby button to put the instrument in Off condition (see *image*).

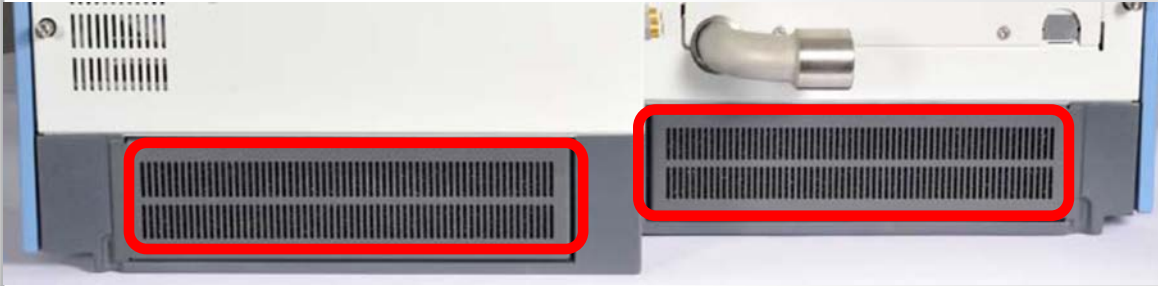
In the Tasks panel of the Q Exactive MS Series Tune software window, click to display the Vacuum / Bakeout.

Enter the baking duration (in hours) into the spin box. The available range is 4 to 30 hours (v 2.7 SP1), 12 hours is recommended as the maximum.

Click Bakeout.

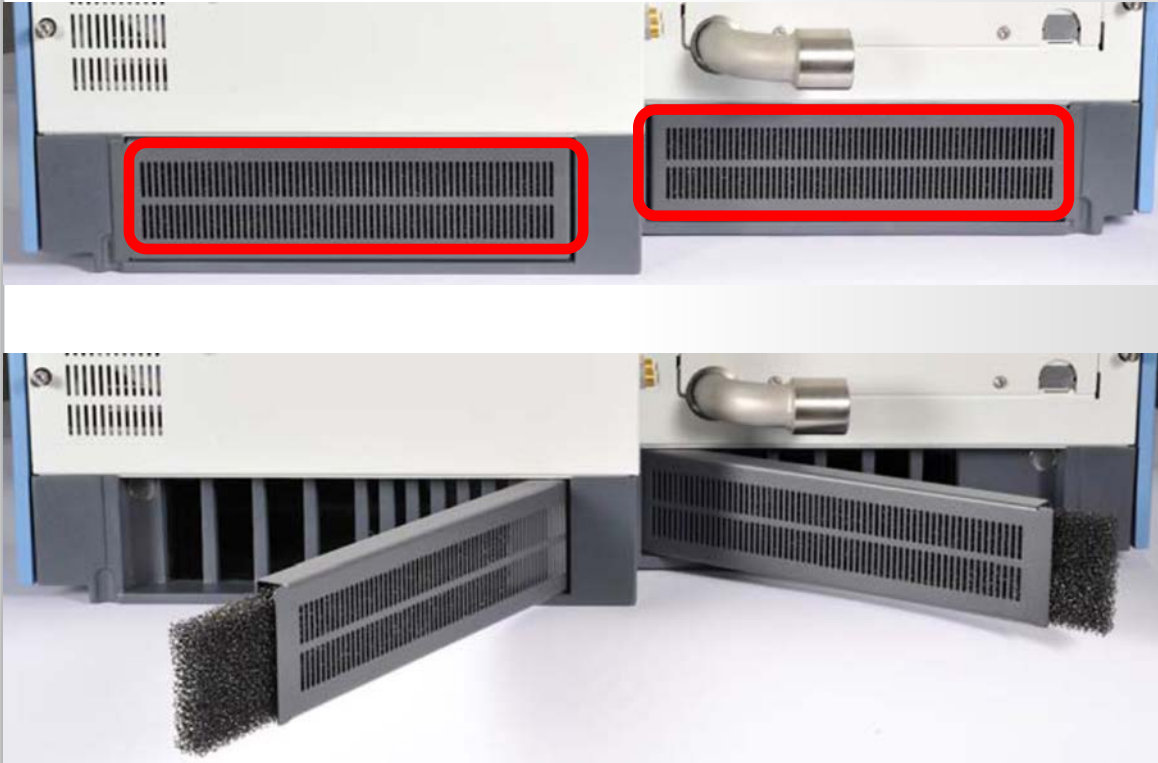
A dialog box shows the duration of the baking procedure. Click Yes to confirm the message.

Fan Filters Maintenance



Each fan filter bracket is mounted on hinges. Insert a finger into the recess in the instrument frame and pull at the fan filter bracket to open it.

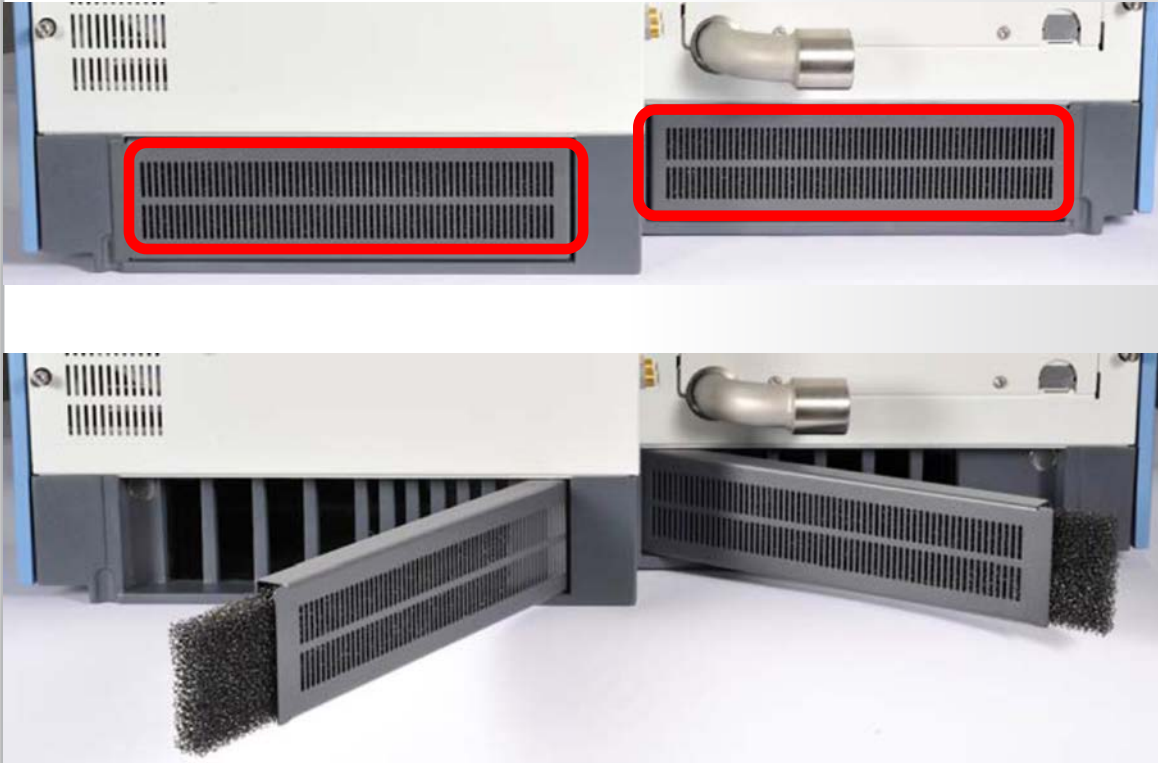
Fan Filters Maintenance



Each fan filter bracket is mounted on hinges. Insert a finger into the recess in the instrument frame and pull at the fan filter bracket to open it.

Remove each fan filter from the rear of the mass spectrometer by pulling it out of the filter bracket.

Fan Filters Maintenance



Each fan filter bracket is mounted on hinges. Insert a finger into the recess in the instrument frame and pull at the fan filter bracket to open it.

Remove each fan filter from the rear of the mass spectrometer by pulling it out of the filter bracket.

Wash the fan filters in a solution of soap and water and rinse the fan filters with tap water.

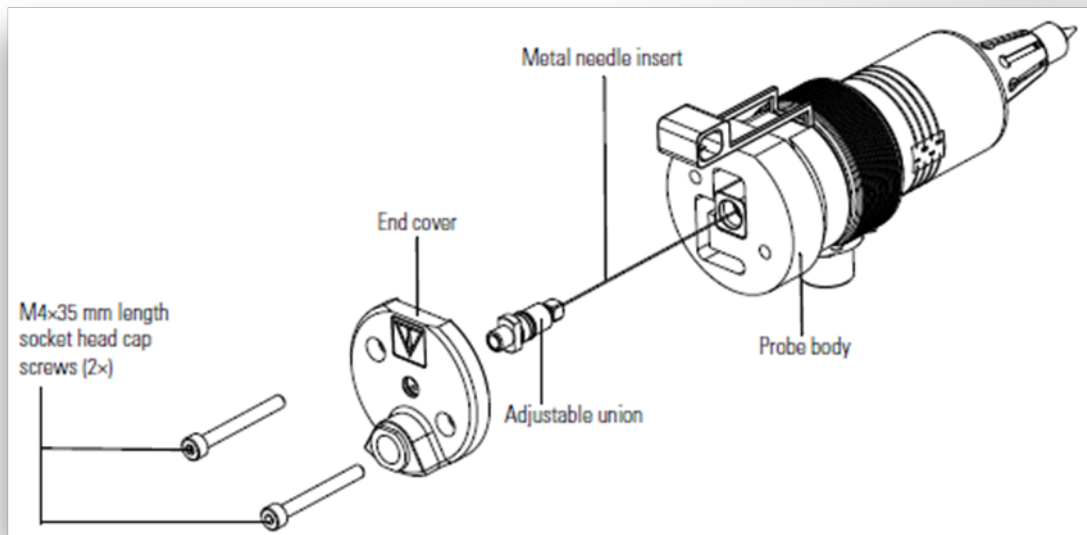
Squeeze the water from the fan filters and allow them to air dry, then reinstall the fan filters in the fan filter brackets.

Replacing Metal Needle Insert

Remove the HESI-II probe from the Ion Max API source.

Unscrew the finger tight fitting from the sample inlet port.

Replacing Metal Needle Insert

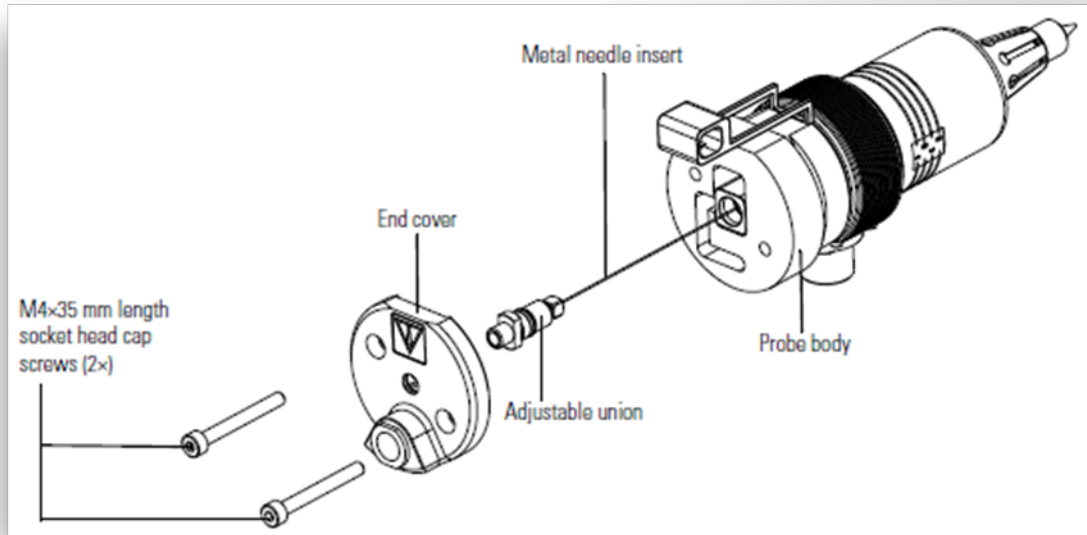


Remove the HESI-II probe from the Ion Max API source.

Unscrew the finger tight fitting from the sample inlet port.

Remove the metal needle insert from the probe using a 3 mm (7/64 in.) hex wrench or ball driver, by removing the two M4x35 mm length, socket head cap screws.

Replacing Metal Needle Insert



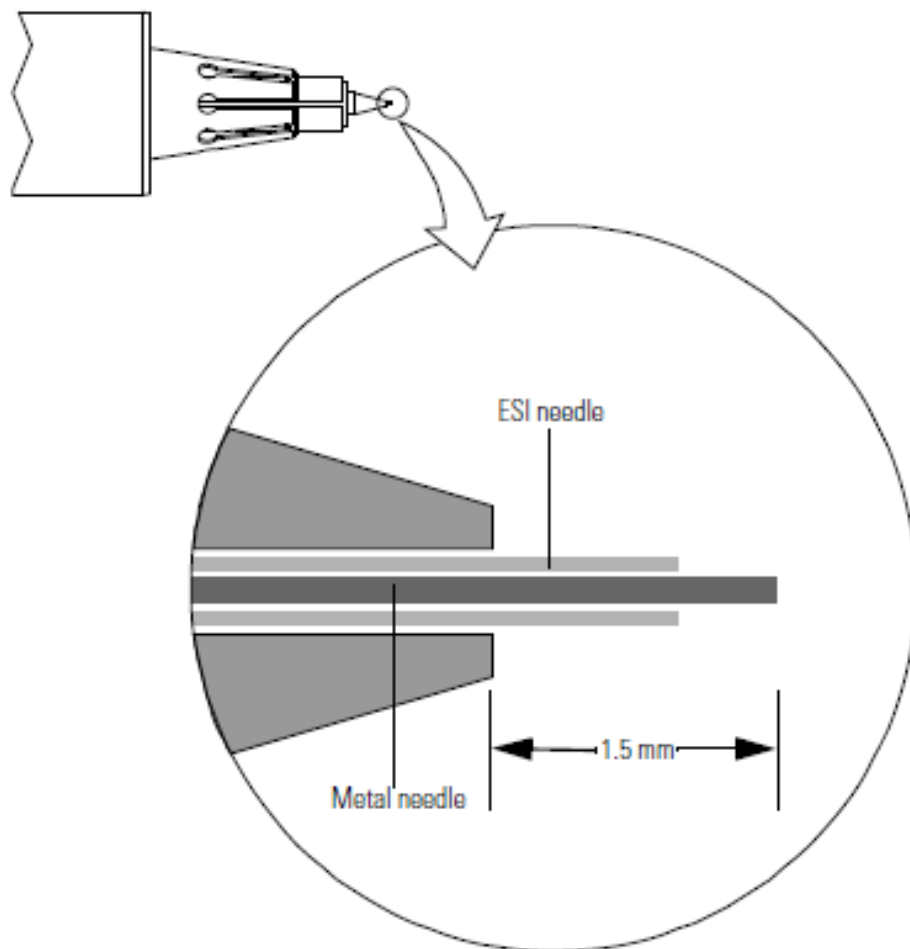
Remove the HESI-II probe from the Ion Max API source.

Unscrew the finger tight fitting from the sample inlet port.

Remove the metal needle insert from the probe using a 3 mm (7/64 in.) hex wrench or ball driver, by removing the two M4x35 mm length, socket head cap screws.

Pull off the end cover of the probe and unscrew the metal needle insert, and then pull it out of the probe body.

Replacing Metal Needle Insert



Remove the HESI-II probe from the Ion Max API source.

Unscrew the finger tight fitting from the sample inlet port.

Remove the metal needle insert from the probe using a 3 mm (7/64 in.) hex wrench or ball driver, by removing the two M4×35 mm length, socket head cap screws.

Pull off the end cover of the probe and unscrew the metal needle insert, and then pull it out of the probe body.

Insert a new metal needle insert into the probe body and hand tighten the adjustable union fitting until the tip of the needle insert protrudes from the probe nozzle by 1.5 mm.

Maintenance: Take Home Message

- **Key Considerations:**

- Practice good lab habits
 - Work bench surfaces should be clean and lint-free
 - Wear clean, talc-free and lint free gloves when handling instrument components
 - Ensure tools are clean; dirty tools can contaminate your system

- **Maintenance Schedule**

- Weekly
 - Check vacuum and gas tank pressures
 - Ballast forepump
 - Clean ion transfer capillary
 - Perform MS Mass Calibration
- Monthly
 - Evaluate Q Exactive MS series calibrations, only calibrate procedures that fail
- Quarterly
 - Back up data and master calibration file
 - Check for software updates (*see next slide*)
- Yearly
 - Schedule Preventative Maintenance (PM)
 - Replace forepump oil

Useful Reminders

- Technical Support-North America

- Priority issues:
 - Call 800-532-4752, Select option 2
- Non-urgent issues:
 - Webform:
<http://www.unitylabservices.com/contact.php>
 - Email:
US.Techsupport.Analyze@ThermoFisher.com
 - Enter serial number in subject line
 - Provide brief Description of Issue

- Technical Support-Europe

- Email:
EU.techsupport.CMS@thermofisher.com

Websites

- planetorbitrap.com
- <https://portal.thermo-brims.com>
- www.mytracefinder.com
- <https://appslab.thermofisher.com>
- Thermo Fisher Scientific Customer Download Site:
<https://thermo.flexnetoperations.com/control/thmo/login>

Thank You for Your Attention!

HRAM iQuan 2016

Acknowledgments

Content Support

- Eric Stover
- Gabriela Grigorean
- James Hogya

CoE Field Support

- Detlef Schumann
- Katie Southwick
- Roberto Gamez
- Tatjana Talamantes

Content Support

- Andreas Boegehold
- Bob Stathers
- Nicole Zehethofer