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TECHNICAL NOTE 73050

Quick and easy tuning and troubleshooting of ISQ EC and ISQ EM mass spectrometers

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Goal

Demonstrates the procedures for autotune and diagnostic tune and how to troubleshoot the Thermo Scientific™ ISQ™ EC and ISQ EM single quadrupole mass spectrometer.

Introduction

The Thermo Scientific™ ISQ™ EC and ISQ EM mass spectrometers are robust detectors that allow both novice and advanced users to master LC-MS analysis. With prior LC-MS systems, when deviations from expected results were noted, such as missing target ions or decreases in signal intensity, the operator would start troubleshooting by changing the solvents, preparing fresh samples, cleaning the column, etc. If a solution was not found after trying these common troubleshooting steps, the operator may have reluctantly tried to determine whether there was any issue with the mass detector and whether it was detecting ions accurately. When there is no simple way to check this, a common way is to run a system suitability test with a known standard and check whether the desired mass is seen in the mass detector with expected intensity.

Failure resolution often requires tuning the mass detector in order to correct the detection of the ions. Tuning is also recommended when the instrument is relocated. Tuning a mass detector can be laborious for a variety of reasons, including changing connections to the LC system, preparation of the calibrant, or creation of new methods or sequences. However, the ISQ EC and ISQ EM mass detectors have a built-in calibration delivery system to allow tuning with just a few mouse clicks and without any changes to the fluidic connections. Now, any user can simply perform auto-tuning and return the system to operation with minimal down time.

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In addition, acquisition of high quality data depends on regular verification of system performance, e.g. mass accuracy and resolution. Preparing standards and performing injections to perform this check, including changing columns if necessary, is troublesome and time-consuming. On the ISQ EC and ISQ EM, the automated calibrant delivery system and availability of real time scans with the calibrant can easily be used to check whether the mass detector is working correctly.

In regulated environments, it is often required to perform tuning of mass detectors at regular intervals and document the results. The autotune procedure available in the ISQ EC and ISQ EM records all performed steps in the audit trail and generates a tune report for review. Thus, tuning becomes fully compliant, meeting GxP and 21 CFR Part 11 regulations.

In some cases, tuning of the mass detector might not solve the issue. Typically, the next step is to change the connecting tubing and spray needle, clean the source, or even clean the front optics in the mass detector. A common issue with mass detectors is not knowing which parts need to be cleaned or maintained. To assist with this, the ISQ EC and ISQ EM mass spectrometers come with algorithms for diagnostic-only tuning. They perform an automated series of experiments utilizing the calibrant to check the performance of the mass spectrometer. The tests can often identify exactly which portion of the instrument requires attention. This can save the time and expense of swapping parts that are not faulty.

This technical note explains how to perform the autotune and the diagnostic-only tune and how instrument users can take advantage of these tests to minimize instrument downtime and get reliable analyses.

Equipment

- Thermo Scientific™ Vanquish™ UHPLC system, Thermo Scientific™ UltiMate™ 3000 UHPLC system or any other LC pump
- Thermo Scientific ISQ EC or ISQ EM Mass Detector
- Restriction capillary (included in ship kit). Thermo Scientific™ nanoViper™ 50 µm x 950 mm fused silica capillary (PN 6041.5125)

Chromatography Data System

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software version 7.2.6 and later can be used for both autotuning and diagnostic-only tuning.

It is recommended to use the latest Chromeleon release whenever possible.

Note: If you are using Chromeleon 7.2.9 or later, tune reports will be available as pdf files for you to view from tune window.

Reagents and standards

Chemicals	
Fisher Scientific Water Optima™ LC/MS grade	W6-212
Calibrant solution – 250 mL	1R120590-6204

Instrument set up

Default LC and MS parameters for positive and negative mode auto tune, and diagnostic-only tune. MS parameters are automatically set at the start of the tuning procedure. The LC flow rate is verified if a Thermo Scientific pump is used and associated with the MS in the instrument configuration.

Table 1. Source settings for tuning.

	Positive mode	Negative mode
Liquid flow rate	0.050 mL/min	0.050 mL/min
Vaporizer temperature	Off, set to 0 °C	Off, set to 0 °C
Ion transfer tube temperature	350 °C	350 °C
Sheath gas	30 psig	42 psig
Aux gas	2.0 psig	6.7 psig
Sweep gas	0 psig	0 psig
Source voltage	3000 V	-2500 V

Note: You have the option to use either 100% water or any other solvent composition to push the calibrant from its loop towards the mass spectrometer.

Experimental details

Autotuning

The ISQ EC and EM mass detectors feature an autotuning procedure. The procedure relies on a sophisticated algorithm, but it is straightforward for an operator to perform with a few mouse clicks. The mass detectors are equipped with an automated calibrant delivery system and therefore, the user does not need to perform any kind of sample preparation or plumbing modifications before tuning. Before starting the tuning, a quick check of the spray stability and whether the calibrant ions are detectable is advisable.

This can be done using real time scan. In the ePanel of the ISQ EC and ISQ EM (Figure 1), you can directly access real time scan and tuning pages. When you click on the “real time scan” button, a new window will appear, and the operator can modify instrument settings and see the changes in real time. In addition, you can check the spray stability and flush the internal PEEK capillary lines with the calibrant by checking the “use calibrant” check box. It is important to perform any analysis using the calibrant delivery system with the flow rate set to 0.050 mL/min. In order to make sure that the correct flow rate is set prior to tuning, the system checks for the correct flow rate if the pump is associated with the mass detector in the instrument configuration. Operators may also use the real time scan in order to check whether mass accuracy and sensitivity is still good, or if tuning should be performed.

Note: The ISQ EM can be configured with dual sources (HESI and APCI) but tuning only needs to be performed with HESI positive and negative modes. The tune results in the HESI mode will be used for APCI mode as well. Diagnostic-only tuning is available for both HESI and APCI modes.

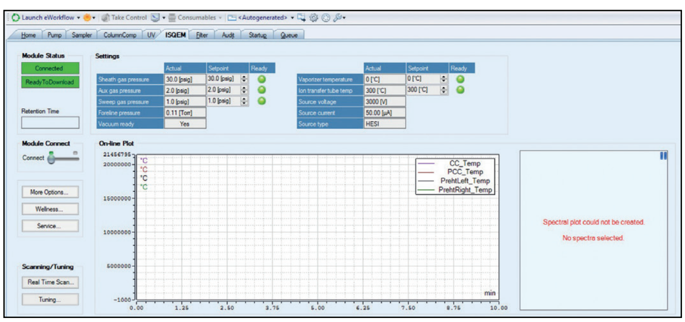

Figure 1. ISQ EC/EM ePanel.

Note: The calibrant solution (250 mL) consists of poly-tyrosine (1 mg of Tyrosine, 3 mg of Tyrosine - 3, 6 mg of Tyrosine - 6) in 49.9% methanol, 49.9% water with 0.2% formic acid.

Table 2. The expected masses with the corresponding ionization mode.

	Expected *m/z*	
	Positive mode ([M+H]$^+$)	Negative mode ([M-H]$^-$)
Methanol adduct (2 CH_3OH + H)	65.1	
Tyrosine (Tyr)	182.1	180.1
Tyrosine - 3 (Tyr-Tyr-Tyr)	508.2	506.2
Tyrosine - 6 (Tyr-Tyr-Tyr-Tyr-Tyr-Tyr)	997.4	995.4

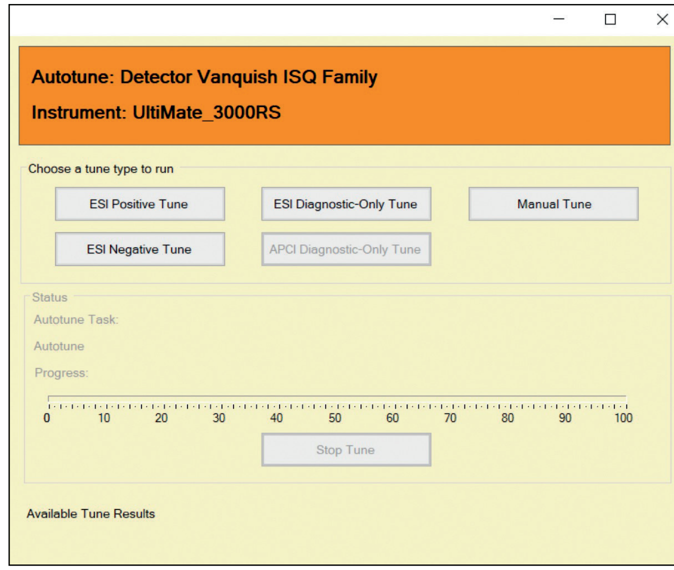

Figure 2. Auto-tuning window.

In order to check whether the mass detector is still performing within specifications, the operator can use the real time scan option available in the ISQ EC/EM ePanel (Figure 1). First, the positive mode should be tested. After opening the “real time scan” window by clicking on the respective button the operator should enter the source parameters listed in Table 1 and any additional acquisition parameters as depicted in Figure 3. Data acquisition can started by clicking the play button at the top right corner. After a couple of minutes, you will see a spectrum as seen in Figure 3. If the correct masses for the calibrant ions are observed, and spray stability is less than 5%, then mass detector calibration is valid for positive mode. After stopping the current scan, set the “ion polarity” to “negative” and then click “play” button. The spectrum will be displayed after couple of minutes. If the correct masses are shown for the calibrant ions, then the calibration is still valid for negative mode.

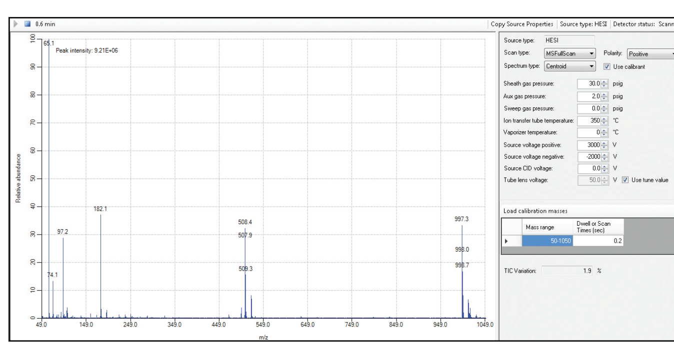

Figure 3. Real time scanning window with positive mode settings and spectrum.

Occasionally, the calibrant ions do not appear in the mass spectrum, or the mass/charge ratio is different than expected. Common causes for undetected calibrant ions include insufficient calibrant solution, a leak in the system, no nitrogen gas flowing to the calibrant system, or the LC pump is not pumping solvent to push the calibrant. If these causes are ruled out, the mass spectrometer may need tuning in order to correct the mass detection.

To start the autotune, close the real time scan window, then click on the tuning button on the ePanel. The instrument then performs the positive autotune, which can take up to an hour to complete. When the tune completes successfully, a tune report will be generated, and the tune file will be updated. If the tuning fails, a tune report will be generated stating that it failed but the tune settings will not be updated. The negative mode tuning can then be started (negative tuning is typically faster, taking approximately half an hour).

IMPORTANT: The positive autotune runs the detector gain tune. Even if the system is primarily being used for negative analysis, the positive tune should be run when the system is calibrated to ensure that the detector has the correct gain settings. If the system is used only for positive mode acquisitions, negative tuning is not required to be performed.

Diagnostic tune

The ISQ EC and ISQ EM mass detectors come with a built-in diagnostic tool to assist in identifying issues in the instrument. The diagnostic-only tune can be performed in the same way as autotune. Diagnostic-only tuning will neither change any values in the current tune file nor create any new tune files. Rather, it checks the performance of the mass detector using the current tune settings. The ESI diagnostic-only tune can be started by clicking "tuning" on the ISQ EC and ISQ EM ePanels, and then selecting "ESI Diagnostic-Only Tune" (Figures 1 and 2). The setup of the instrument is similar to the autotune and the flow rate again needs to be 0.050 mL/min. The diagnostic-only tune will then check all components in the ion beam path and generate a report at completion identifying any issues.

Both auto tune and diagnostic-only tune can be performed from a sequence, allowing, for example, the operator to perform tuning at the start of a sequence. When the diagnostic-only tune is performed from a sequence, it allows the selection of individual diagnostic algorithms.

Results and discussion

Operators can open saved tune reports as a pdf file, but tune settings are only updated if tuning passes.

If both positive and negative tuning passes, then the operator can commence regular analyses. In the case of failed tuning, the operator needs to examine the audit trail to identify where the tuning failed. One potential area of failure is ESI spray stability, with following possible reasons:

- LC pump is not stable
- The nitrogen gas flow is unstable
- The electrospray capillary is not properly adjusted in the electrospray probe
- There are liquid leaks
- The electrospray capillary is damaged

Many LC pumps produce a smooth pressure trace only with higher back pressures, which can be difficult to reach at these low flow rates. At the flow rate required for the tuning (0.050 mL/min), some LC pumps do not have enough backpressure and will not produce a stable flow, resulting in an unstable electrospray. If the LC pump being used has this problem, then a restriction capillary can be used in order to add enough back pressure so that the pump delivers a stable liquid flow. When the recommended restriction capillary (nanoViper 50 µm x 950 mm fused silica capillary - PN 6041.5125) is used with water as the mobile phase, a back pressure of 50 – 100 bar can be expected.

Another factor affecting the spray stability is the protrusion of the ESI capillary from the electrospray probe; this can be checked by using the marking on the front of the source housing (Figure 4). If the spray intensity is unstable, the capillary can be adjusted in or out in quarter turn increments to help stabilize the beam. It is recommended to have the total ion count (TIC) variation to be less than or equal to 4% for the tests. Checking the spray stability can be done in real time scan (as mentioned above).

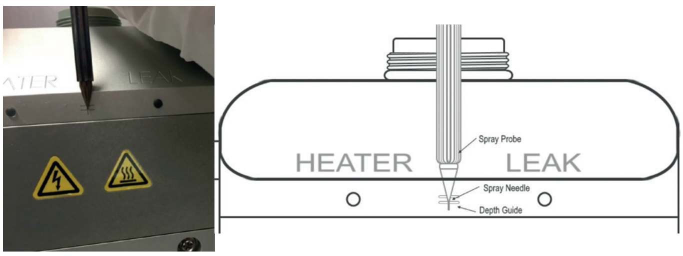

Figure 4. ESI capillary depth adjustment markings on the source housing.

Note: When the APCI mode is used in the diagnostic-only tune, no adjustment is needed for the APCI capillary as it is a cut-to-length silica capillary.

In the diagnostic-only tune, the components in the ion path (Figure 5) and source are checked individually.

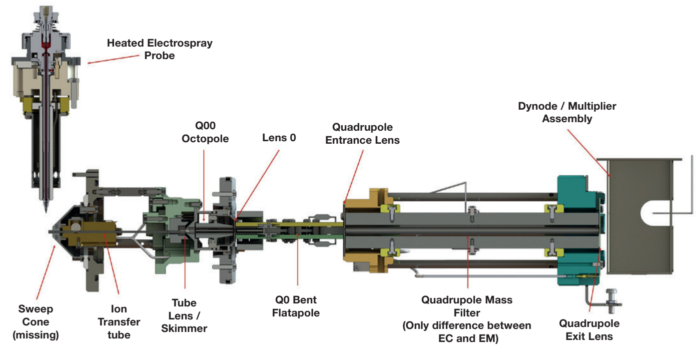

Figure 5. Schematic showing the components of the ion flow path.

Table 3. Summary of tests performed. A short description of the respective tests is given, along with a list of root causes for test failure.

Test	Description	If failed, modules that could be faulty
Heater check	Checks the temperatures of the ion transfer tube and vaporizer after heating them and ensures that they reach the required setpoints	Ion transfer tube heater assembly, **vaporizer heater, vaporizer board**, pressure control board, lens driver board, main cable harness, power supply module
Ion transfer tube check	Checks the Foreline pressure and compares it to expected values based on ion transfer tube temperature	**Clogged ion transfer tube, leaky seal under ion transfer tube**, leak in the hose between turbo pump and Foreline pump, power supply module
Lens check	Ramps the lenses and ensures that the readback matches the setpoint across their ranges	**Lens driver board**, bridge board, power supply module, **lenses shorted to one another**
Lens charging test	Uses ion beam to attempt to discharge charged up lenses to find dirty	**Dirty lenses, unstable/missing ion beam**, faulty boards on system, power supply module, problems with dynode/multiplier
Lens continuity check	Attempts to block the ion beam with each lens, looking for lenses that are disconnected from the lens driver board	**Lens not plugged in, faulty lens drive board**, power supply module, **unstable/missing ion beam**, problems with the dynode/multiplier
Power supply check	Looks at the readbacks for the power supplies and ensures that they are within an acceptable range of voltages for each supply	**Faulty power supply,** electrical short somewhere in the system or on a board
Q0 frequency check	Ramps the Q0 RF amplitude to ensure that it can reach its max value	**Q0 assembly, RF board**, RF feedthrough, power supply module
Q1 frequency check	Ramps the Q1 RF amplitude to ensure that it can reach its max value	**Q1 assembly, RF board**, RF feedthrough power supply module
Q1 RF DC check	Evaluates the Q1 RF and Q1 resolving DC to look for problems	**Q1 assembly, RF board, DC rod driver**, RF feedthrough, power supply module
Detector check	Looks for noise on the various components of the detector	**Dynode, electron multiplier, electrometer, electrometer cable**, multiplier feedthrough dynode feedthrough, electrometer anode feedthrough, dynode/ multiplier power supply, power supply module

Depending on the report of the diagnostic-only tune results, the operator can narrow-down the problem to one of a few modules or boards in the mass detector and can swap those modules. While some repairs, such as cleaning a clogged ion transfer tube or swapping it with new one, can easily be done by the operator, many modules or parts are not user accessible and may require Thermo Fisher Scientific service engineers to perform the repair.

IMPORTANT: If the system has no calibrant ion beam in real time scan, it will fail some of the diagnostic tests that use the ion beam. This includes the charging test and the continuity check. Ensure that there is a calibrant beam present before running diagnostic tests.

Best Practices in ISQ EC and EM tuning

In order to get the best results of both autotuning and the diagnostic-only tune, the following should be checked:

- Install the HESI source if you have an ISQ EM with APCI source option
- Make sure there is sufficient calibrant solution and it is not expired
- There is a stable spray (TIC variation 4% or lower). If the TIC variation is greater than this, check for liquid leaks in liquid flow path (including the rear seal of the pump) and stable nitrogen gas flow
- By adjusting the needle protrusion spray stability can be optimized. If it does not help replace the HESI needle with a new one.
- All calibrant ions are detected in real time scan.
- If the instrument is being powered up for the first time or after being vented for a long period, wait about 40 minutes from "vacuum ready" before starting the tuning.
- Perform autotune only when required for compliance, after maintenance, after moving the mass detector to a new location, if you see mass drift, or missing ions.
- Iterative tuning should not be performed as a system suitability test (SST) as it results in detector aging. In order to verify performance defined samples could be injected and compared vs. previous results.

Conclusion

This technical note demonstrates how to perform the autotune and diagnostic-only tune and the advantages these features bring the Thermo Scientific ISQ EC and ISQ EM single quadrupole mass detectors. By using these automated routines, operators can satisfy the compliance requirements to have tune reports recorded, and all users can correct mass detection if any drift has occurred. By using the diagnostic-only tuning users are able to diagnose if they have an issue and quickly identify any faulty part or module, saving money and time.

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