Quick Start Guide

ExD AQ-25x Option

1. Introduction

This document serves as a quick reference for end-users operating the ExD AQ-25x Option for Agilent 6500 LC/Q-TOFs. Contents include an overview of ExDControl, guidelines for routine setup, and best practices. See the *ExD AQ-25x Option User Guide* for more information, including important safety precautions.

2. Getting started

The ExD-equipped Q-TOF is controlled via two standalone software applications: MassHunter Acquisition and ExDControl. The basic setup of both applications is described here, but see Agilent documentation for more details on MassHunter Acquisition use. This guide is accurate for software versions MassHunter Acquisition 11 and ExDControl 3.6.

2.1 Introduction to ExD Control 3.6

ExDControl software controls the ExD cell and is used alongside MassHunter to operate the ExD-equipped Q-TOF. ExDControl controls voltages to the ExD cell lenses as well as the current applied to the electron-emitting filament. ExDControl autotunes allow the user to establish appropriate voltages without manual tuning. “MS1” and “MS2” as used in ExDControl refer to Total Ion and Isolation modes, respectively, in MassHunter Acquisition. The ExD cell can be tuned to transmit ions with or without performing ECD.

The Profile Tab

A “profile” is a set of voltages for each of the lens elements in the ExD cell. Profiles for MS1 and MS2 are chosen in the main ExDControl window under the Profile tab (Figure 1). The ExD cell is situated in the middle of the ion optics rail, therefore setpoints for instrument optics must be taken into account when making a suitable ExD profile. Separate profiles must be established for MS1 and for MS2. ExDControl switches automatically from the MS1 profile to the MS2 profile when the quadrupole state changes from Total Ion to Isolation, and vice versa.

Having the filament ON is a requirement for ExD experiments and is advantageous for transmission only or CID experiments, because it tends to result in greater transmission. A current of 2.4A is a reasonable starting current for most filaments. Starting from this setpoint, the filament current can be manually optimized further for transmission or ECD. Changes to filament current should be done in small increments such as +/- 0.05A.

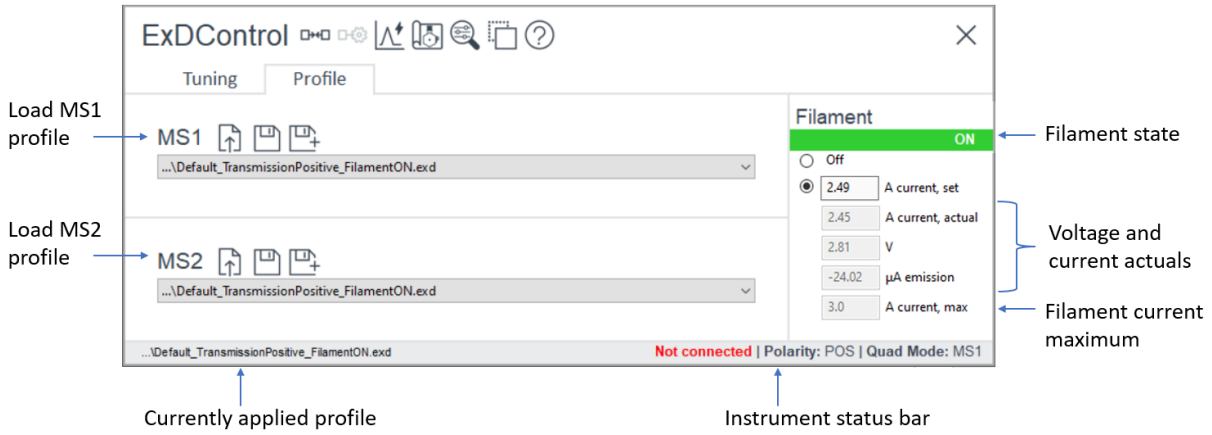

Figure 1 - ExDControl profiles and filament tab in ExDControl 3.6

Tuning Tab

Tuning is the process of adjusting voltages to optimize the abundance of certain *m/z* peaks. Tuning the ExD cell can be performed manually or by using an autotune method (Figure 2). Iteratively tuning the ExD cell and instrument is needed ensure the instrument and ExD cell voltages are compatible. See 2.2 Tuning the System for step-by-step instructions for tuning the ExD cell alongside MassHunter.

Loading a profile in ExDControl 3.6 loads a set of voltages that are applied to the lenses of the ExD cell. L1, L2, L4, L6, and L7 are electrostatic lenses. LM5 and LM3 are permanently polarized magnets with electric potentials applied to them. FB is the filament bias voltage applied to the electron-emitting filament. Default profiles are provided in the profiles directory as a starting point for the most common applications. These profiles can be optimized using one of the autotune methods. Mass lists for several common standards are provided in the drop-down menu. The Edit button opens an editor so that mass lists can be altered or entered.

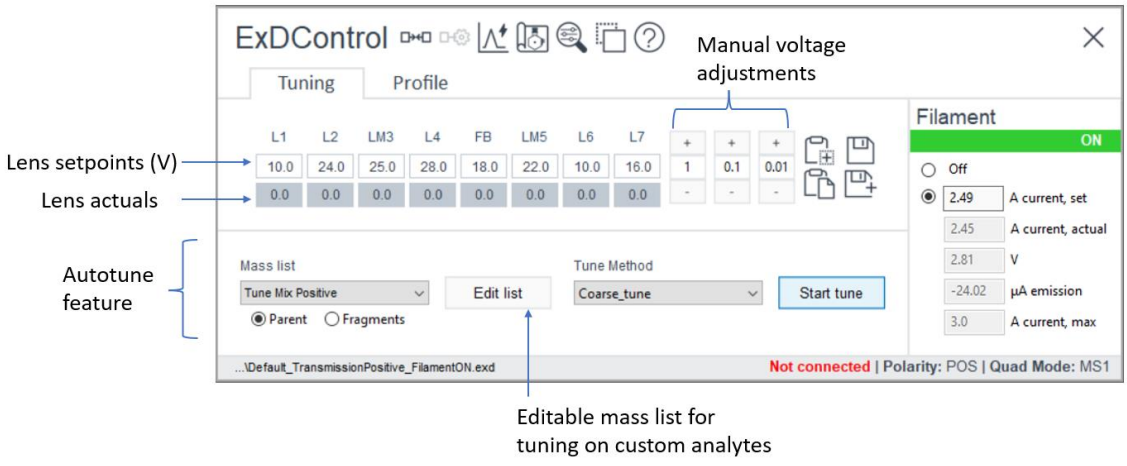

Figure 2 – Tune tab in ExDControl 3.6

2.2 Tuning the System

Iterative tuning of the ExD cell and MassHunter is necessary to optimize the system as a whole. The following procedures rely on ExD autotunes. For guidelines on manual tuning, see the User Guide. Once a profile is established, retuning should be performed whenever there is a noticeable decrease in signal intensity.

Tuning for Transmission (MS1)

The following guidance is for setting up the instrument for MS1 transmission in positive polarity. A separate profile is needed for MS2 transmission (see Tuning for Transmission (MS2)).

1. Set up MS1 transmission profile
    - 1.1. Set the initial MassHunter and ExD parameters given in Table 1 and Table 2.
    - 1.2. Set the filament current to 2.4A.
    - 1.3. Infuse Tunemix. Ensure MassHunter is in the Tune Context and Total Ion mode.
    - 1.4. In the ExDControl tune tab, select the Tunemix mass list and the Parent sub-list.
    - 1.5. Select the Refine_tune method, which searches a relatively narrow search space around the applied profile
    - 1.6. Select "Start tune" to begin an ExD refine tune on Tunemix
    - 1.7. Adjustments to the filament current may improve signal abundance. The filament can be raised in small (0.05A) steps as long as an increase in analyte signal is observed.
    - 1.8. After changing the filament current, another Refine tune may further improve signal.
2. Run a MassHunter tune
    - 2.1. After the ExD cell is tuned once, run a system tune in MassHunter with the "Tune using current parameters" box checked. Tune both TOF and quad. The slicer should be set to High Resolution.
3. Refine MS1 profile
    - 3.1. Ensure the instrument is in Total Ion mode.
    - 3.2. Select Tunemix from the mass list and run another ExD refine tune.

Table 1 – Adjusted MassHunter parameters

MH Parameter	Setpoint
Oct1DC	36 V
Lens1	34.5 V
QuadDC	33 V
PostFilterDC	32.5 V
HexDC	21 V
HexDelta	-6 V
Hex2DC	13.5 V
Hex2DV	-1 V
CCGP	24 psi

Table 2 – Default profiles for MS1 and MS2 transmission

	L1	L2	LM3	L4	FB	LM5	L6	L7
MS1	18	26.5	26.5	29	23.5	26	24	22
MS2	34	0	32	33	28	32	15	26

Tuning for Transmission (MS2)

The following guidance is for setting up the instrument to transmit ions after isolation in positive polarity. This method should be used anytime ions will be transmitted in MS2 mode without ECD fragmentation.

1. Establish MS1 transmission profile following the process described in Tuning for Transmission (MS1).
2. Set up MS2 transmission profile
    2.1. Set the initial ExD parameters for MS2 transmission given in Table 2.
    2.2. In MassHunter, isolate *m/z* 922.
    2.3. In the ExDControl tune tab, select the Tunemix mass list and the Parent sub-list.
    2.4. Select the Refine_tune method.
    2.5. Select "Start tune" to begin an ExD refine tune on *m/z* 922.

Tuning for ECD (MS2)

The following guidance is to set up the system for ECD experiments, using Substance P as an example.

1. Establish MS1 transmission profile following the process described in Tuning for Transmission (MS1).
2. Set up MS2 ECD profile
    2.1. Infuse Substance P. Ensure MassHunter is in the Tune Context.
    2.2. Set the initial ExD parameters for MS2 ECD given in Table 3.
    2.3. In MassHunter, isolate the parent ion at *m/z* 674.
    2.4. In the ExDControl tune tab, select the Substance P mass list and the Fragments sub-list.
    2.5. Select the Refine_tune method.
    2.6. Select "Start tune" to begin an ExD refine tune on Substance P ECD fragments.
    2.7. Adjustments to the filament current may improve ECD signal abundance. The filament can be raised in small (0.05A) steps as long as an increase in analyte signal is observed.
    2.8. After changing the filament current, another Refine tune may further improve signal.

Table 3 – Default voltage profiles for ECD.

L1	L2	LM3	L4	FB	LM5	L6	L7
35.0	5.0	38.0	40.0	33.8	36.4	20.0	26.0

3. Best Practices

3.1 Filament

- Allow the system to warm up for 15 minutes after turning the filament on. Allow the electrospray source to stabilize for a few minutes after beginning infusion.
- Avoid frequent large changes to filament current. Turning the filament on and off a few times per day is OK.
- Turn the filament off when the instrument isn't in use for more than a few hours, e.g. overnight.

3.2 Tuning

- Check that MS1 and MS2 transmission profiles are optimized before running a MassHunter tune, or else the MassHunter tune may fail or produce poor results.
- Re-tune the ExD cell after running a MassHunter System Tune or Transmission Tune, or whenever the main optics values change.
- In the drop-down menu of autotune methods, the 'Coarse' method ramps voltages over a fairly wide range centered on the profile active when the autotune is begun. A 'Fine' tune ramps selected voltages over a narrower range. An 'Extended' tune consists of a course tune followed by a refine tune.
- Keep good records of day-to-day system performance using standard analytes such as Tunemix and Substance P.
- After tuning on Tunemix and/or Substance P, it is recommended to re-tune on a standard that more closely resembles your analyte of interest in terms of mass, charge, and composition. For example, one profile may work well for most peptides but a different profile may be needed for proteins.
- Edit the autotune samples list to enable automatic tuning on custom analytes.

3.3 Method Setup

- Averaging spectra tends to improve data quality when ECD fragment ions are low abundance. Prioritize high-value precursors with a preferred list in auto MS2, or add duplicate targets to a targeted list to ensure multiple scans per precursor are collected.
- Singly charged precursors are not amenable to ECD, so exclude them from the precursor list.
- Collision energy should generally be set to zero for ECD experiments. ECD efficiency will deteriorate as collision energy is applied unless all the voltages in the ECD profile are increased by the number of volts of collision energy.

4. References

See the following references for additional details about setting up, operating, and troubleshooting the ExD AQ-25x Option. Refer to Agilent documentation for details on using Agilent products.

- ExD AQ-25x Option User Guide
- ExDControl Software User Guide
- Application Note: Top-down Disulfide Mapping, Revision R001, July 2021
- Application Note: Modification Localization, Revision R002, May 2022
- J. S. Beckman *et al.*, "Agilent/e-MSion - JASMS Industrial Special Issue Improved Protein and PTM Characterization with a Practical Electron-Based Fragmentation on Q-TOF Instruments," *J. Am. Soc. Mass Spectrom.*, p. jasms.0c00482, Apr. 2021, doi: 10.1021/jasms.0c00482.

Questions or need support? Contact e-MSion at **support.pdl-emsion@agilent.com**

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