# TopSpec - 829157

## WP6 - Modification of the Orbitrap mass spectrometer

## **Deliverable:** D6.1 **-** Modified Orbitrap Q Exactive HF X installed

**Task:** In parallel to Task 6.1 (Installation of a loaned Q Exactive instrument to Fasmatech to support Omnitrap development), a standard Orbitrap Q Exactive HF-X (or a similar high-end instrument) will be modified to improve its performance for desolvation and transmission of intact antibodies. Based on research using Q Exactive UHMR and standard HF-X instruments, there is clearly a reserve for optimizing the desolvation region of the atmosphere-to-vacuum interface that deserves a more detailed exploration. In parallel to this, a joint work with Spectroswiss and Fasmatech will be started on integration of instrument control software using application programming interface (API) to be provided by TF. This work includes also development of tuning and calibration procedures specific for antibody analysis in order to ensure best top-down performance, integration of data for all fragmentation methods and cross-section measurements.
After testing of all functional units, the resulting will be delivered and installed at KI and performance protocol will be completed for a test set of compounds.

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## Content:

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**Authors:** Alexander Makarov and Kyle Fort, Thermo Fisher

Deadline: 11/30/2022

# 1. Description of action

## Part 1. Initial assembly and testing of the final instrument.

Successful installation of a new Orbitrap Exploris 480 instrument in Fasmatech lab and its coupling to Omnitrap back-end was described in the Month 30 WP6 report and is illustrated in Appendix 1. This document contains also illustration of the modified time sequence of Orbitrap Exploris 480 MS needed for such integration.

In addition to and beyond the initial scope of this work package, multi-platform integration of Omnitrap with the older Exactive family of instrument was implemented for the most popular Q Exactive Plus and HF instruments. In process, unexpected residual ions were discovered to leak through the closed ion gate of Exactive instruments and additional research allowed to resolve this issue (**Appendix 1**).

Following this, an Q Exactive HF/Omnitrap instrument was successfully installed at Institute Pasteur partner as a part of WP1.

The extensive process of integrating Omnitrap and Exploris required development of two-way communication utilizing application-programming interface (API). **Appendix 2** contains more detail on this important software tool. On Orbitrap Exploris instruments, access to API is obtained via a click-through license agreement.

## Part 2. Installation and testing of the final instrument at partner KI.

Successful installation of the complete Orbitrap Exploris 480/Omnitrap/IMS instrument in Karolinska institute is covered by Installation Protocol of **Appendix 3**. This report contains also description of user interface and methods of operations of the system.

# 6. Performance

Deliverable D6.2 was completed as detailed in Appendices 1-3.

Appendix 1

# WP6: Modification of the Orbitrap Mass Spectrometer

## Appendix 1. Initial installation of Orbitrap™ Exploris™ instrument

Kyle Fort, Alexander Makarov

22-07-21

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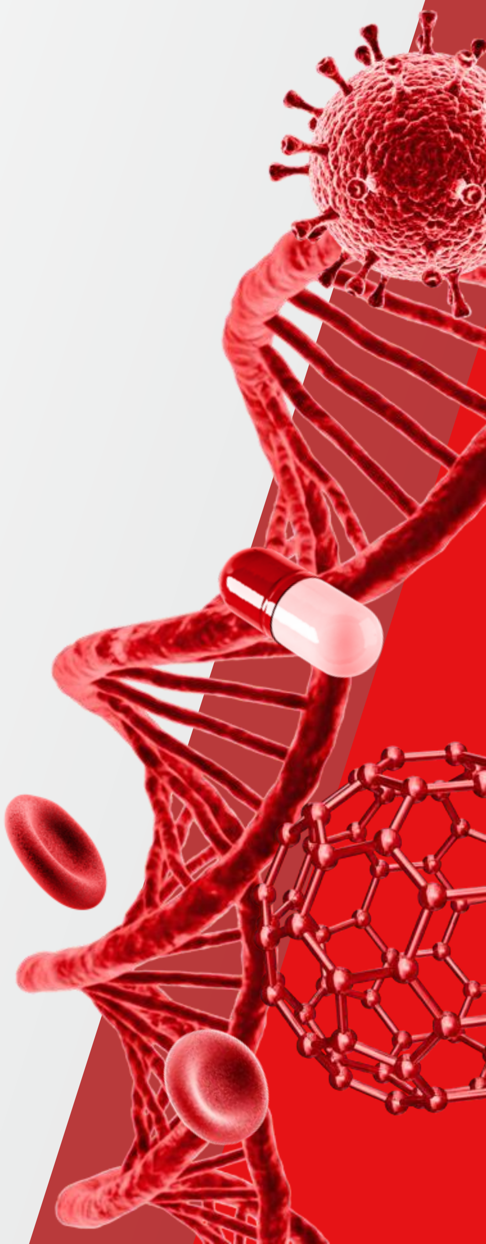
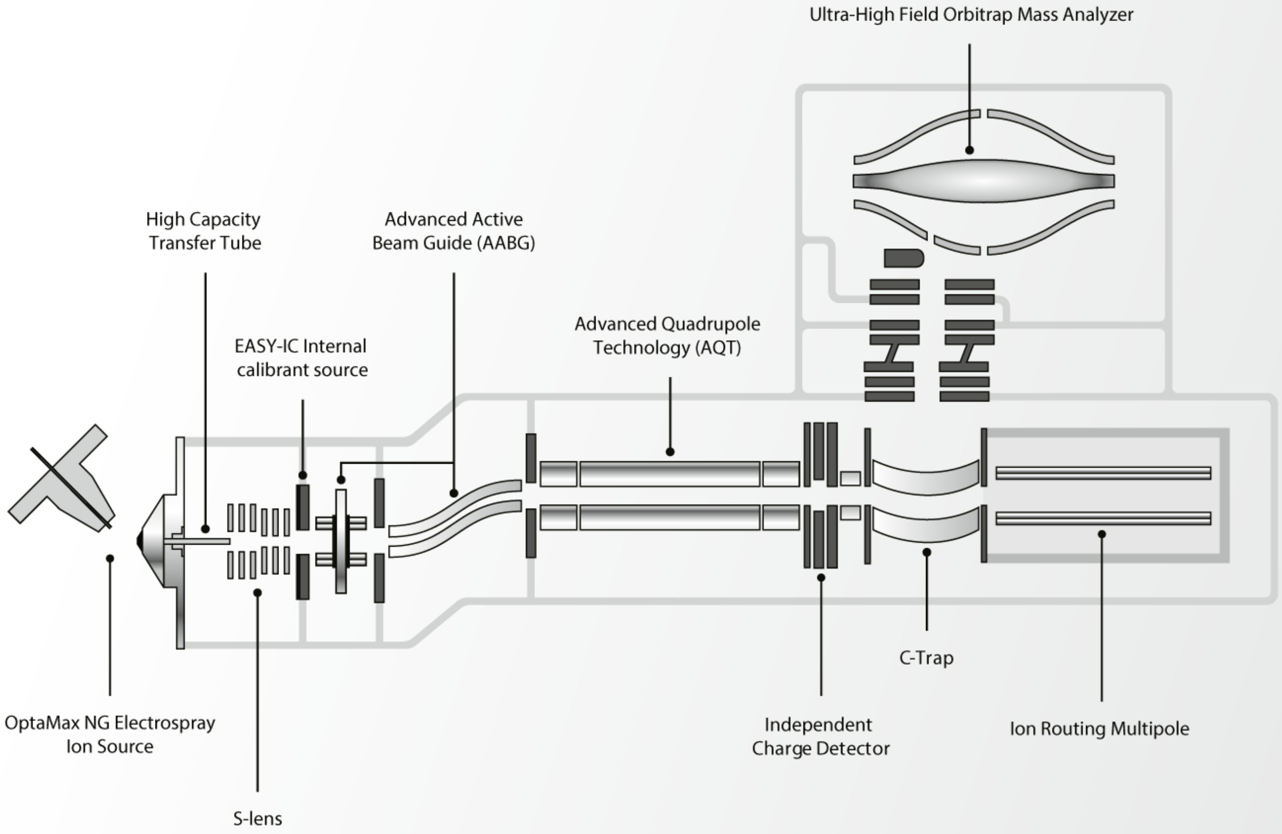
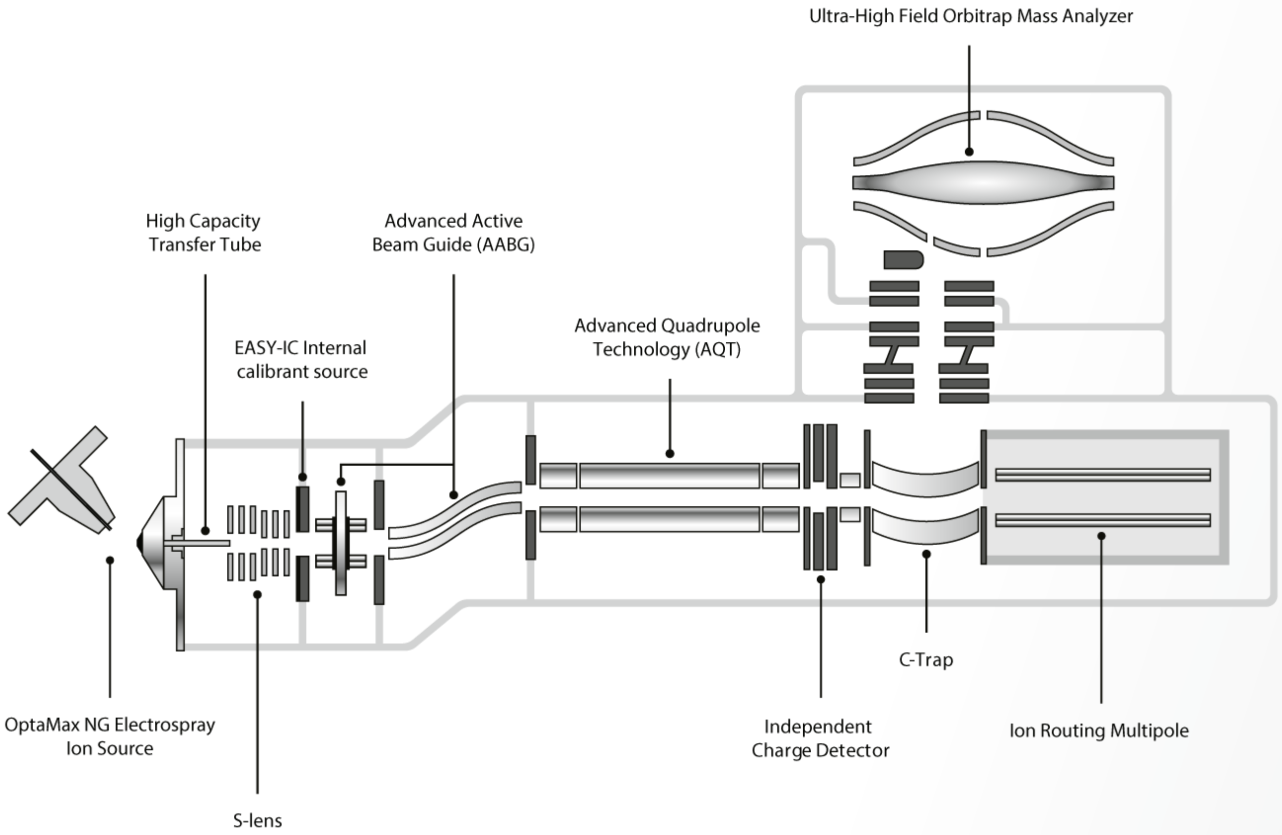


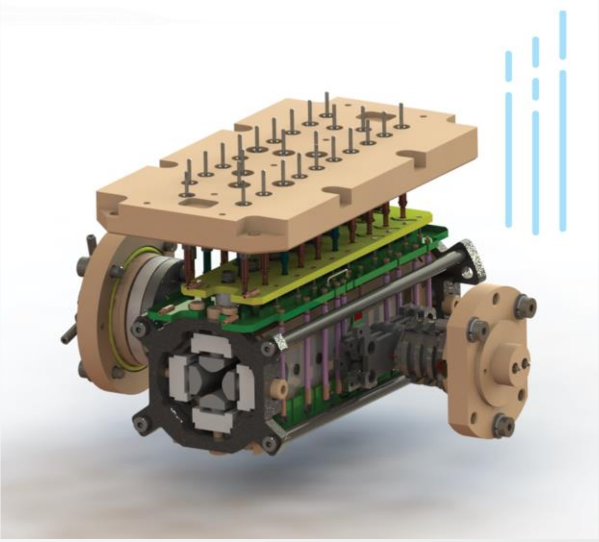
# Orbitrap Exploris 480 Mass Spectrometer



- Exploris 480 Mass Spectrometer was delivered to Fasmatech and was installed on December 14-18th, 2020
- Instrument offers increased robustness, higher resolution, and easier access for hardware modification
- The Exploris is compatible with Field Asymmetric-waveform Ion Mobility, enabling additional specificity

# Orbitrap Exploris 480 Mass Spectrometer: Modification with the Omnitrap



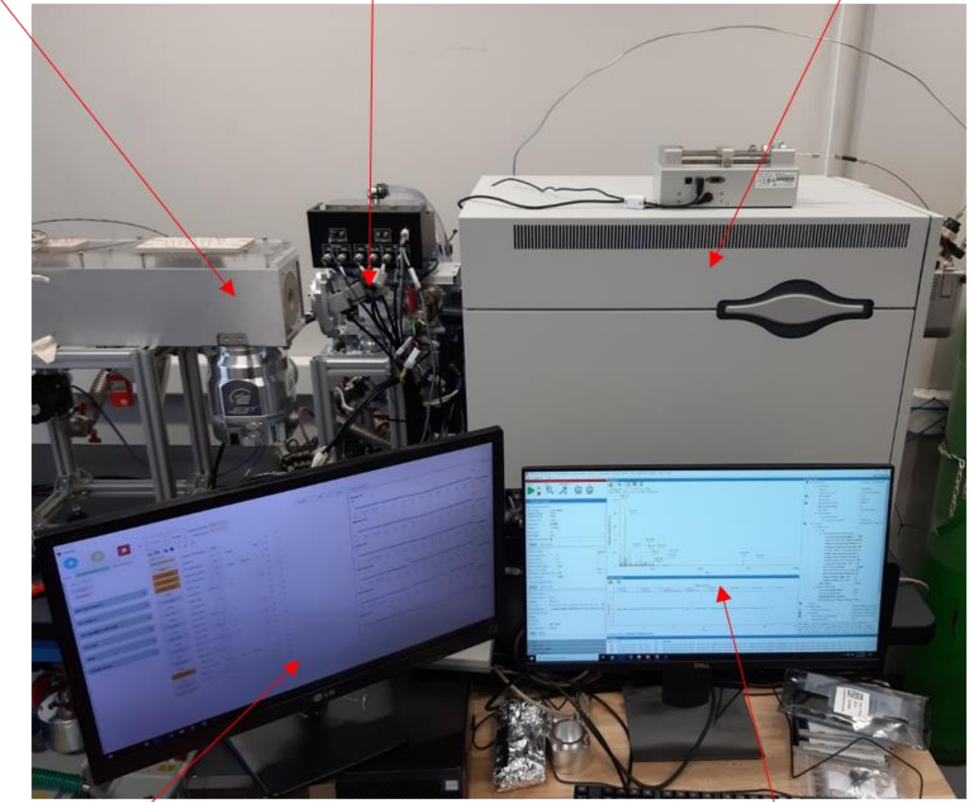


- Combination of the Omnitrap with the Exploris 480 MS will occur by installing the Omntrap on the back of the Ion Routing Multipole (IRM, formally termed HCD-cell)
- Ions will be transported from the IRM to the Omnitrap for fragmentation and the returned to the Exploris for mass analysis
- Customized hardware developed in collaboration between Thermo Fisher and Fasmatech to enable mechanical attachment
- Customized MS instrument control software to enable ion transport to and from the Omnitrap

Omnitrap Photo Source: http://erevna.minedu.gov.gr/index.php/en/news/2065-omnitrap-among-top-10-innovations-2018-the-scientist

# Omnitrap Installed on Exploris 480 MS

**Side View**



Ion Mobility Drift Tube (not yet installed)

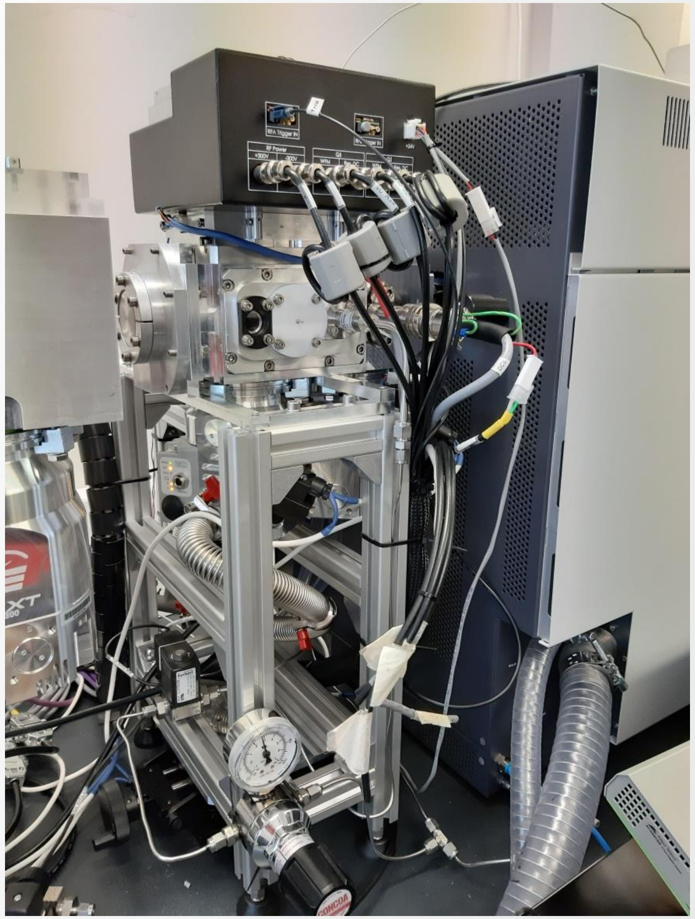
Omnitrap

Orbitrap Exploris 480 MS

Omnitrap Tune Software

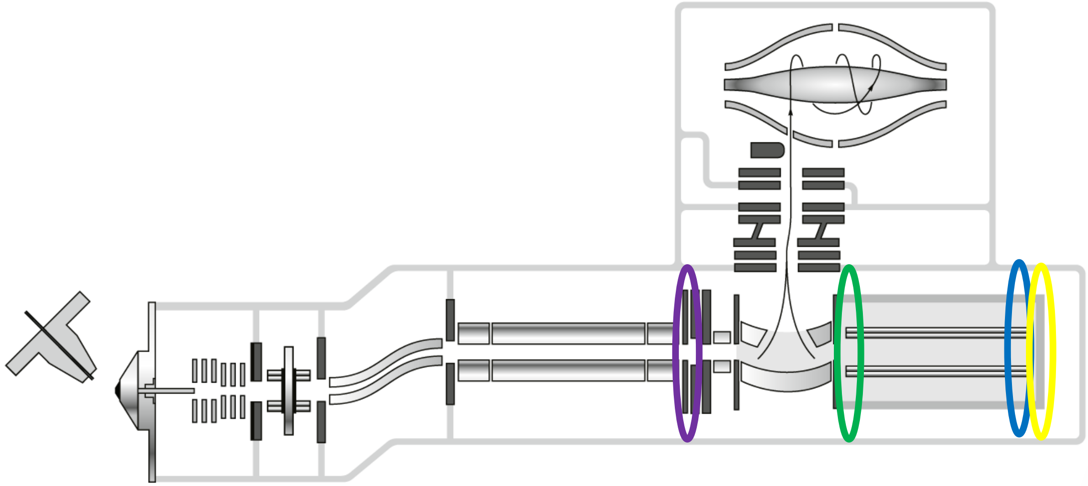
Orbitrap Exploris 480 MS Tune Software

**Rear View**



# Customized Instrument Control Software: External Instrument Mode

## Modified Operation of Ion Optics



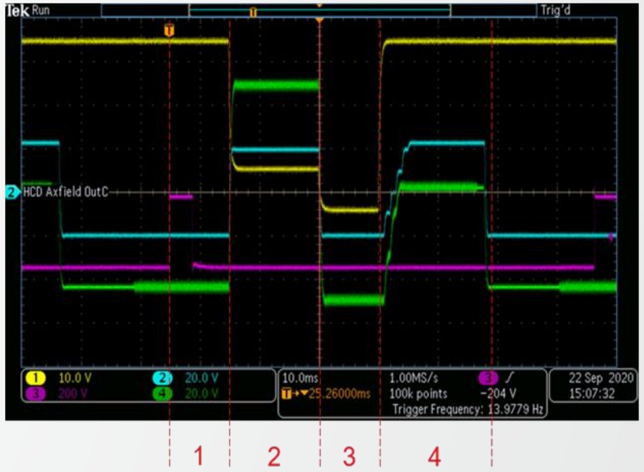
Voltages:

- 🟪 **Split Lens** (trigger, Purple)
- 🟩 **HCD-cell Front** (Green)
- 🟦 **HCD-cell Back** (Blue)
- 🟨 **HCD-cell Exit Lens** (Yellow)

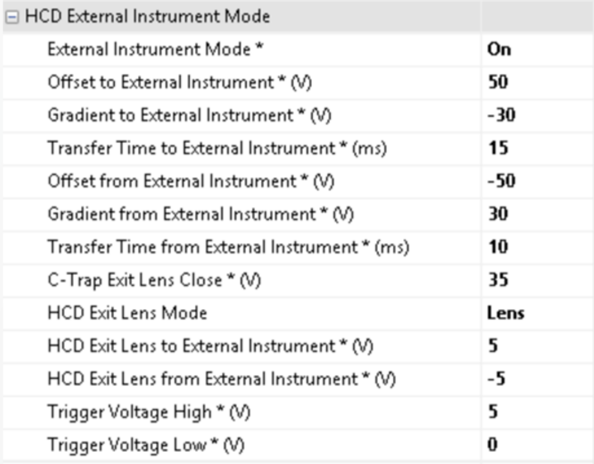
## Customized Voltage and Timing Control

Timing Steps:

| | |
|---|---|
| 1: Ion Injection – | Ions are sent to the HCD-cell for trapping or fragmentation |
| 2: Ions to Ext. Inst. - | Ions are sent to the external instrument through the **HCD-cell Exit Lens** |
| 3: Ions from Ext. Inst. - | Ions are sent from external instrument to the HCD-cell through the **HCD-cell Exit Lens** |
| 4: Ion Purge - | Ions present in the HCD-cell are removed and sent to the C-trap |



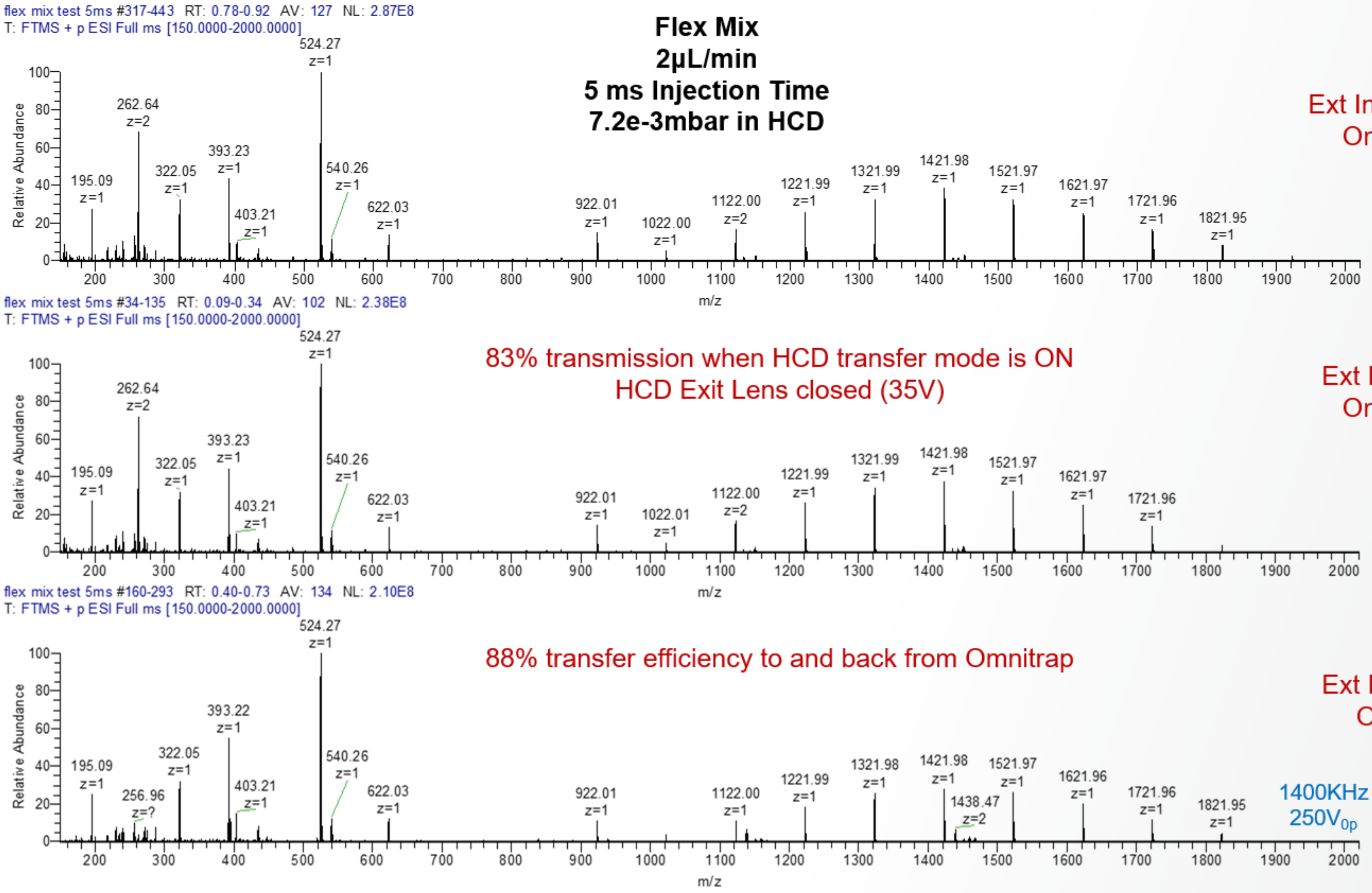
## User Interface Built-in to Instrument Control Software



| ⊟ HCD External Instrument Mode | |
|---|---|
| External Instrument Mode * | On |
| Offset to External Instrument * (V) | 50 |
| Gradient to External Instrument * (V) | -30 |
| Transfer Time to External Instrument * (ms) | 15 |
| Offset from External Instrument * (V) | -50 |
| Gradient from External Instrument * (V) | 30 |
| Transfer Time from External Instrument * (ms) | 10 |
| C-Trap Exit Lens Close * (V) | 35 |
| HCD Exit Lens Mode | Lens |
| HCD Exit Lens to External Instrument * (V) | 5 |
| HCD Exit Lens from External Instrument * (V) | -5 |
| Trigger Voltage High * (V) | 5 |
| Trigger Voltage Low * (V) | 0 |

Appendix 1

# Exploris 480/Omnitrap Combination: Initial Data



**Flex Mix**
**2µL/min**
**5 ms Injection Time**
**7.2e-3mbar in HCD**

Ext Instr OFF
Omni OFF

83% transmission when HCD transfer mode is ON
HCD Exit Lens closed (35V)

Ext Instr ON
Omni OFF

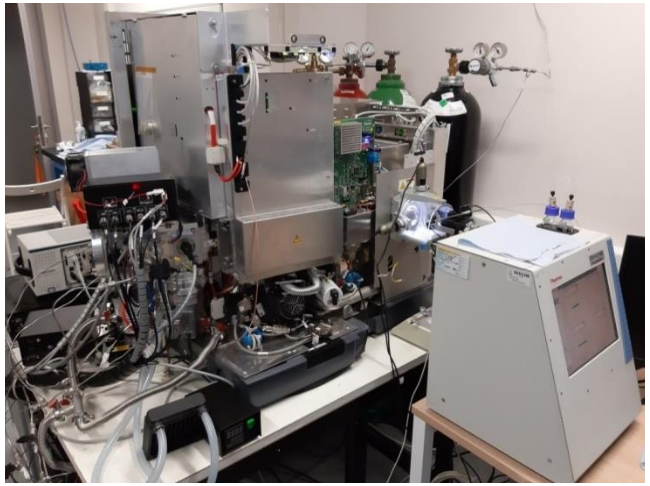
88% transfer efficiency to and back from Omnitrap

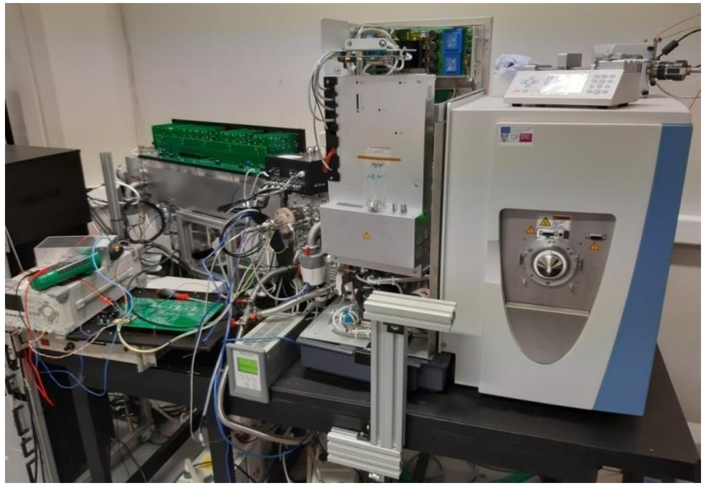
Ext Instr ON
Omni ON

1400KHz
$250V_{0p}$

- After modification and software implementation, initial data was collected using the MS calibration solution, Flexmix
- The hardware addition of the Omnitrap showed no noticeable effect on the operation of the MS
- When the External Instrument Mode is enabled, but ions are not transported to the Omnitrap, >80% ion transmission is preserved
- Upon transmitting ions to the Omnitrap with the External Instrument Mode, transmission efficiency is ~90%

# Beyond WP6: Multiplatform Integration

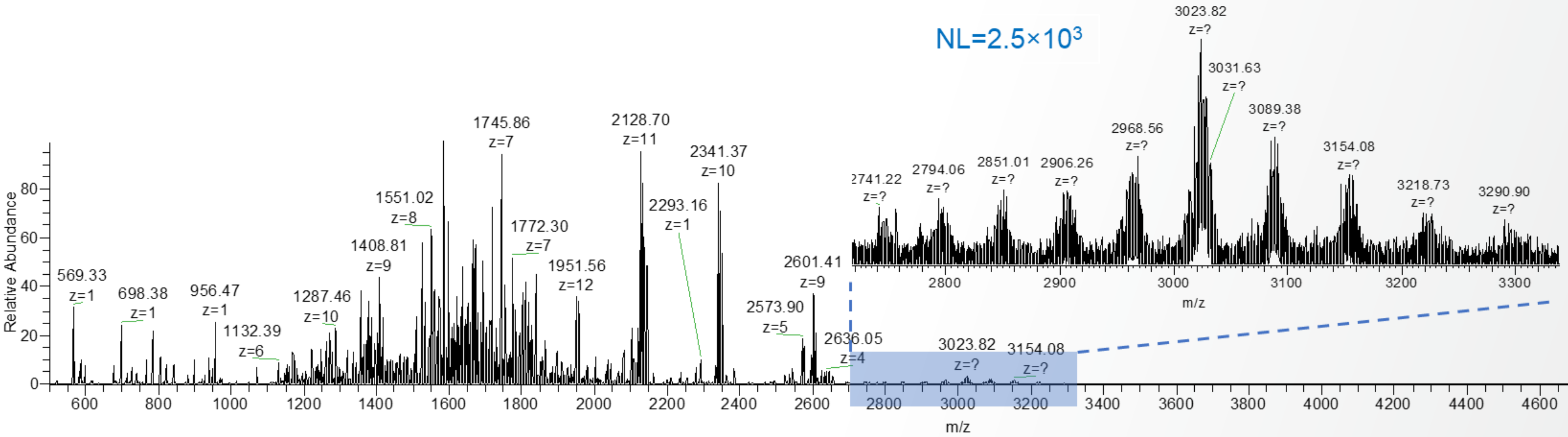




- The Omnitrap was enabled on two other Orbitrap Mass Spectrometer platforms prior to the coupling to the Exploris 480: Q Exactive Plus and Q Exactive HF MS
- These mass spectrometers acted as a test bed for optimizing operation of the Omnitrap operation and initial data collection
- Transfer of instrument development to the new Orbitrap Exploris platform improves analytical performance of the complete instrument and long-term commercial prospects for Omnitrap but required significant additional work on all aspects of integration

Appendix 1

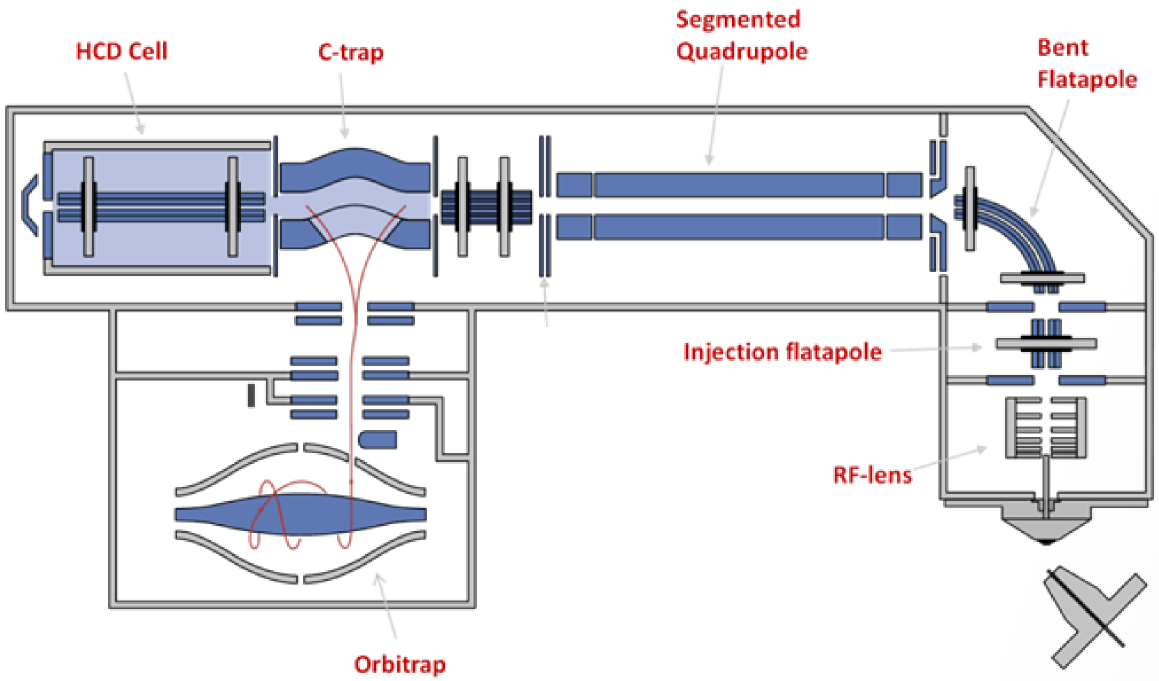
# Beyond WP6: Advanced Modification and Troubleshooting Required for MS3/MS4 Analysis



residual ions (intensity ~10³) were always present after the QMF isolation of the precursor, affecting the MSn spectra

- Initial experiments on Exactive-based Mass Spectrometers, modified with the Omnitrap, showed that very small amounts of the precursor remained even after “elimination” in the Omnitrap
- These residual precursor ions overlapped with higher m/z ions from the MS$^3$/MS$^4$ experiments, limiting sequence coverage
- In-depth troubleshooting and root-cause-analysis was needed in order to determine the source and find a solution to eliminate these ions

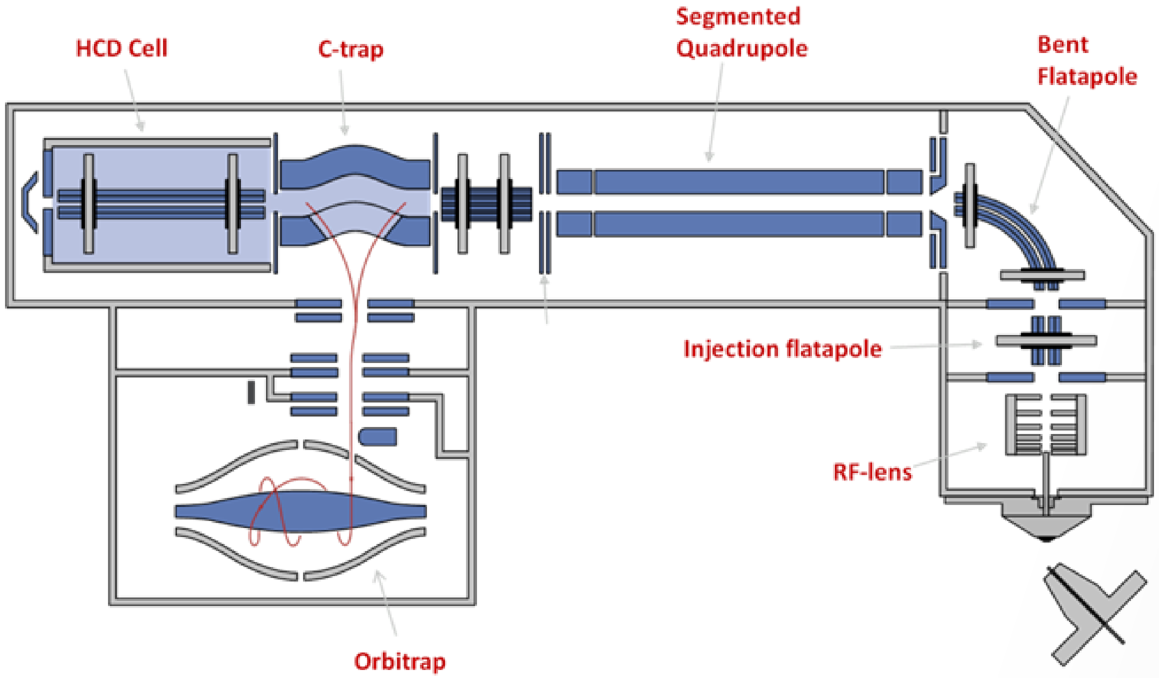
# Beyond WP6: Advanced Modification and Troubleshooting Required for MS3/MS4 Analysis



Q Exactive Plus/HF Ion Optics

- The ion optics were modified to install the Omnitrap at the end of the HCD-cell
- Where are the Residual Precursor ions coming from? Multiple possibilities:
  - Ions being trapped in the HCD Cell
  - Ions being trapped in the C-Trap
- Issue was difficult to replicate off-site and non-reproducible, adding to complexity

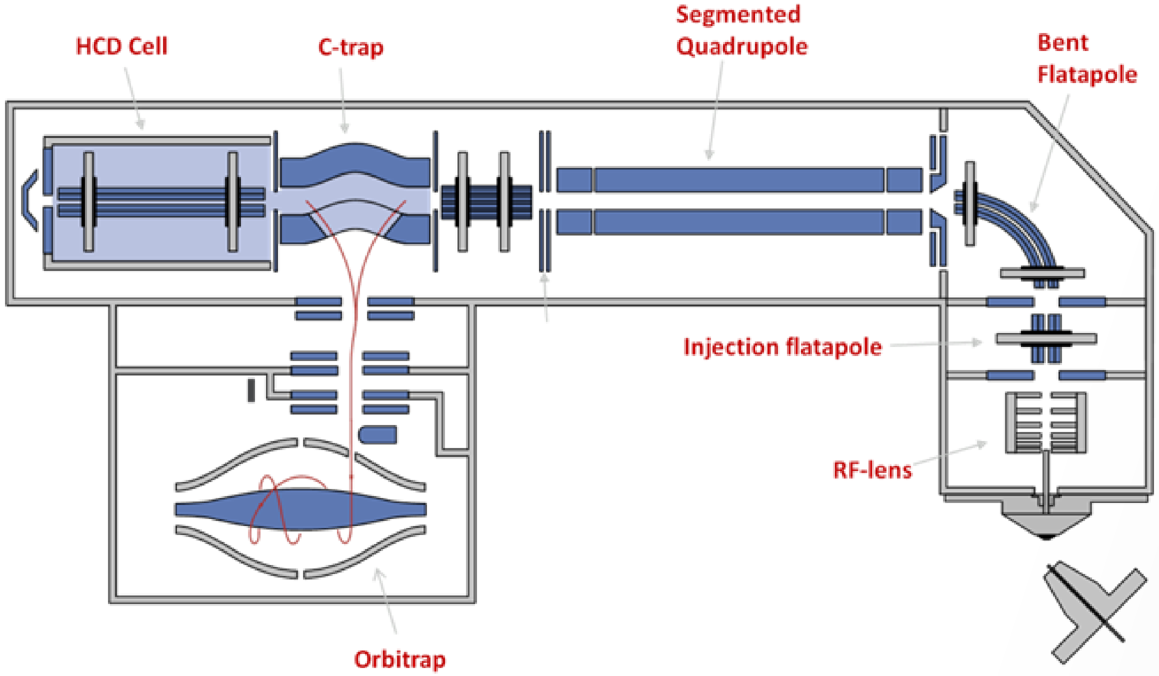
# Beyond WP6: Advanced Modification and Troubleshooting Required for MS3/MS4 Analysis



Q Exactive Plus/HF Ion Optics

- Causes, Multiple possibilities:
  - Ions being trapped in the HCD Cell
  - Ions being trapped in the C-Trap
- Potential Solutions:
  - Turning off HCD Cell RF when ions were in Omnitrap
  - Turning off C-trap RF when ions were in the Omitrap
  - Both
- Custom software was written for each iteration of potential solution; while each step showed improvement, neither nor both fully solved the issue

# Beyond WP6: Advanced Modification and Troubleshooting Required for MS3/MS4 Analysis

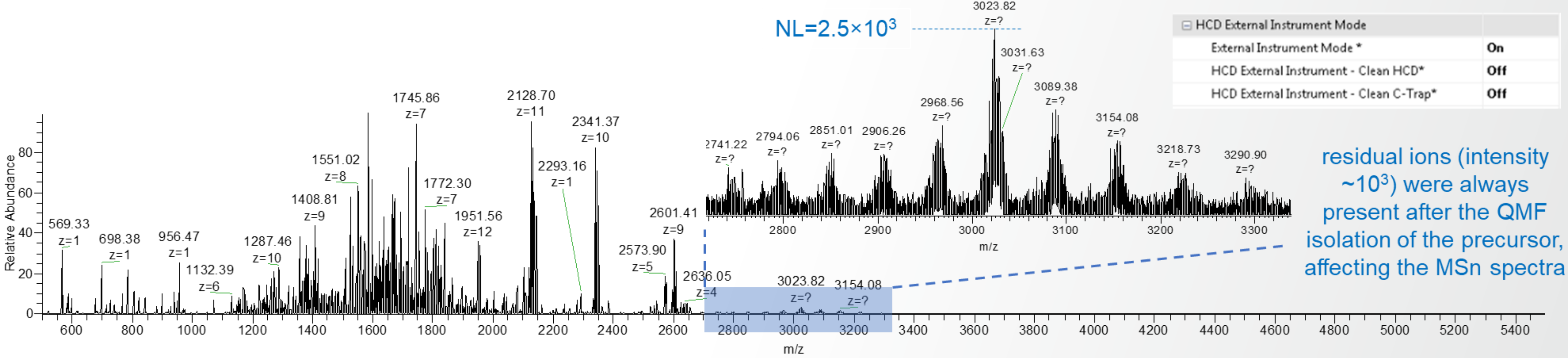


**Q Exactive Plus/HF Ion Optics**

- Causes, Multiple possibilities:
  - Ions being trapped in the HCD Cell
  - Ions being trapped in the C-Trap
  - Ions leaking from Quadrupole in spite of gating
- Potential Solutions:
  - Turning off HCD Cell RF when ions were in Omnitrap
  - Turning off C-trap RF when ions were in the Omitrap
  - Applying a high resolving DC potential to Quadrupole during “ion blocking”, i.e. when split gate was closed

Appendix 1

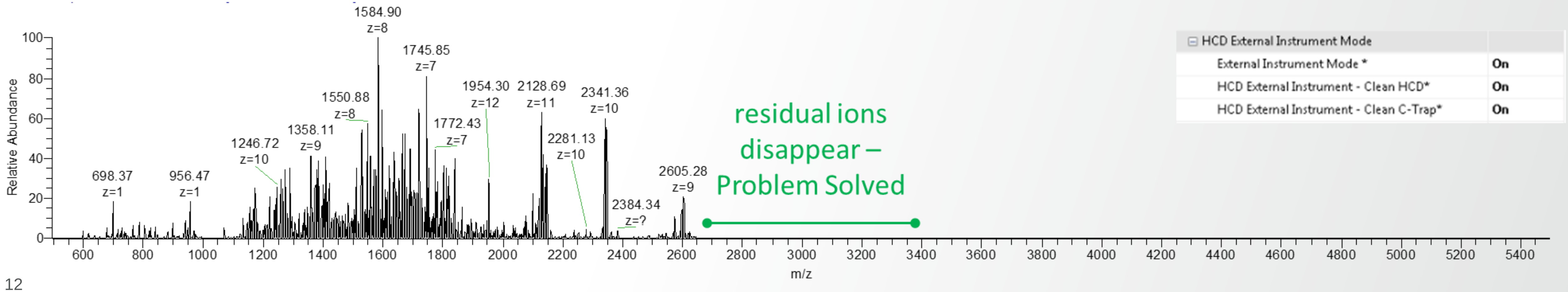
# Beyond WP6: Advanced Modification and Troubleshooting Required for MS3/MS4 Analysis



| ⊟ HCD External Instrument Mode | |
|---|---|
| External Instrument Mode * | On |
| HCD External Instrument - Clean HCD* | Off |
| HCD External Instrument - Clean C-Trap* | Off |

residual ions (intensity ~10$^3$) were always present after the QMF isolation of the precursor, affecting the MSn spectra

With both RF solutions and the high resolving DC solution

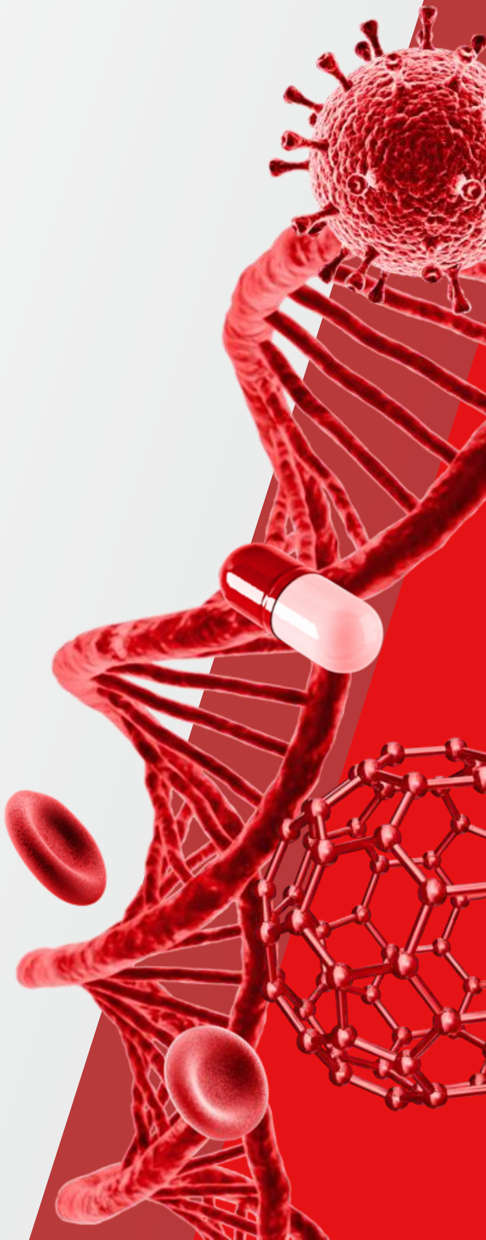


| ⊟ HCD External Instrument Mode | |
|---|---|
| External Instrument Mode * | On |
| HCD External Instrument - Clean HCD* | On |
| HCD External Instrument - Clean C-Trap* | On |

residual ions disappear – Problem Solved

# WP6: Modification of the Orbitrap Mass Spectrometer

# Appendix 2. Application Programming Interface for Orbitrap™ Exploris™ instrument



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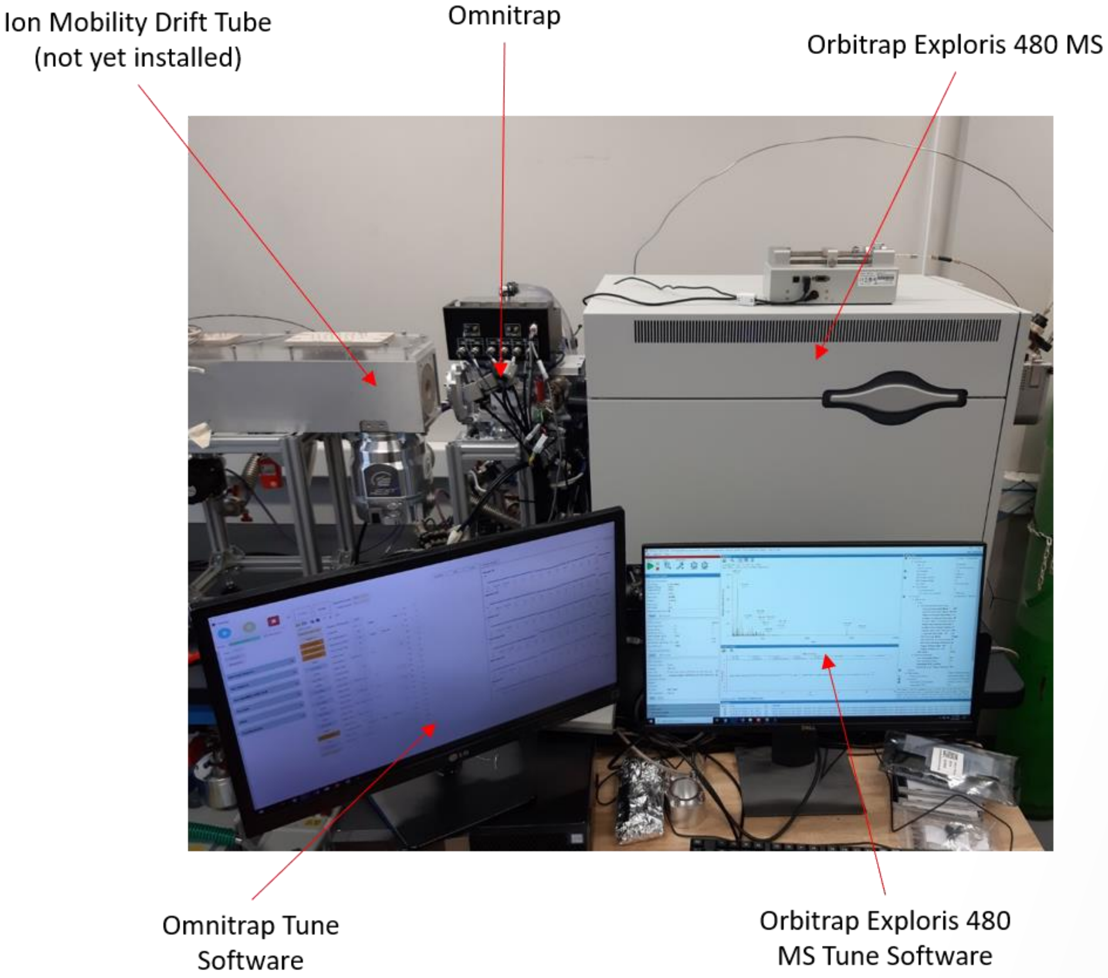
# Exploris 480/Omnitrap Combination: Developing High-Throughput Workflow via the API

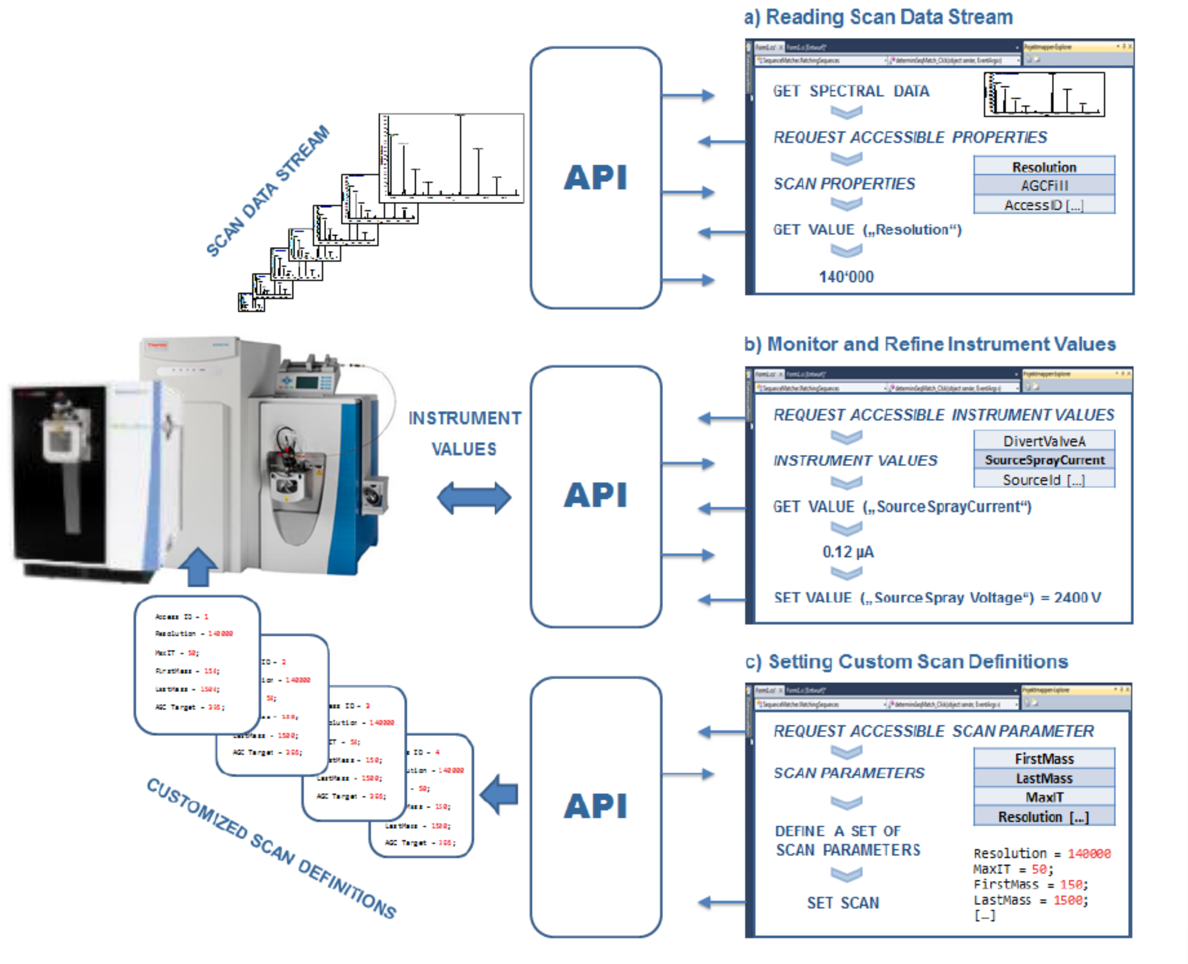
Appendix 2

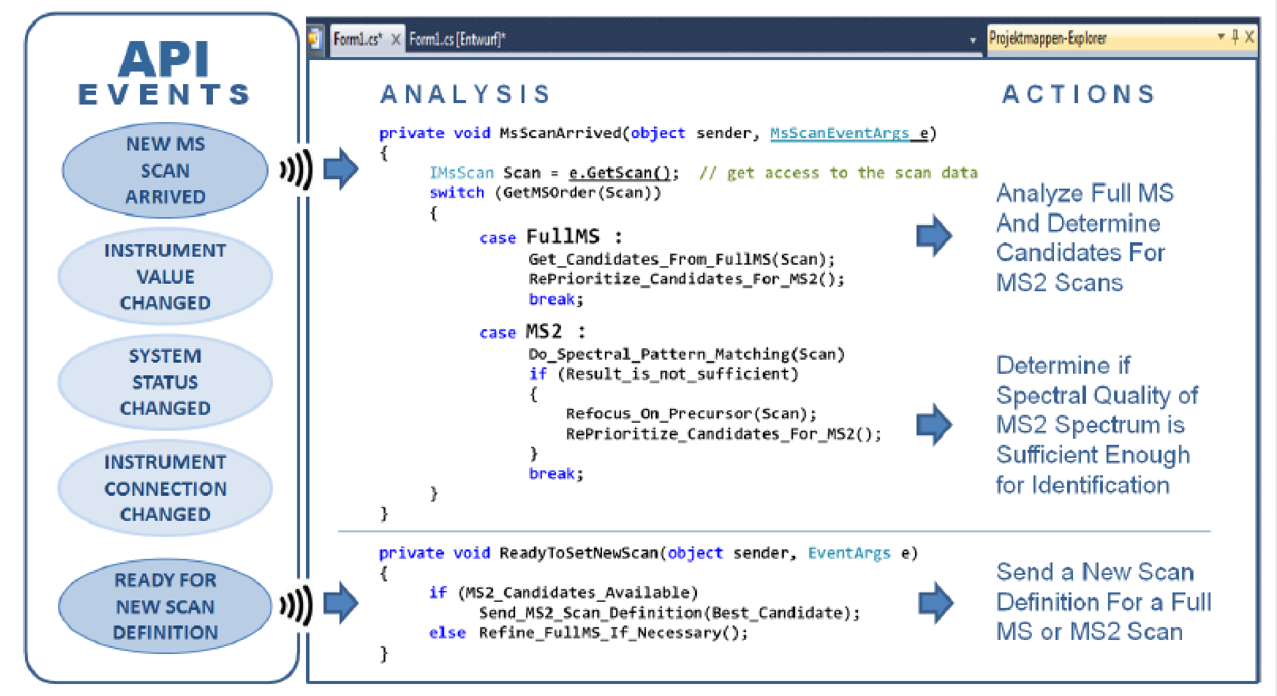


- For a standalone MS, DDA and targeted workflows are available; however, these are not directly compatible with the Omnitrap addition as there is no communication between the two instruments
- The API (**Application Programming Interface**) allows two-way communication
- Via the API, Fasmatech is able to get spectral information and send custom scans to the mass spectrometer, allowing high-throughput, e.g. DDA and targeted, workflows

# Exploris 480/Omnitrap Combination: Details

Appendix 2





```
private void MsScanArrived(object sender, MsScanEventArgs e)
{
    IMsScan Scan = e.GetScan();  // get access to the scan data
    switch (GetMSOrder(Scan))
    {
        case FullMS :
            Get_Candidates_From_FullMS(Scan);
            RePrioritize_Candidates_For_MS2();
            break;

        case MS2 :
            Do_Spectral_Pattern_Matching(Scan)
            if (Result_is_not_sufficient)
            {
                Refocus_On_Precursor(Scan);
                RePrioritize_Candidates_For_MS2();
            }
            break;
    }
}

private void ReadyToSetNewScan(object sender, EventArgs e)
{
    if (MS2_Candidates_Available)
        Send_MS2_Scan_Definition(Best_Candidate);
    else  Refine_FullMS_If_Necessary();
}
```

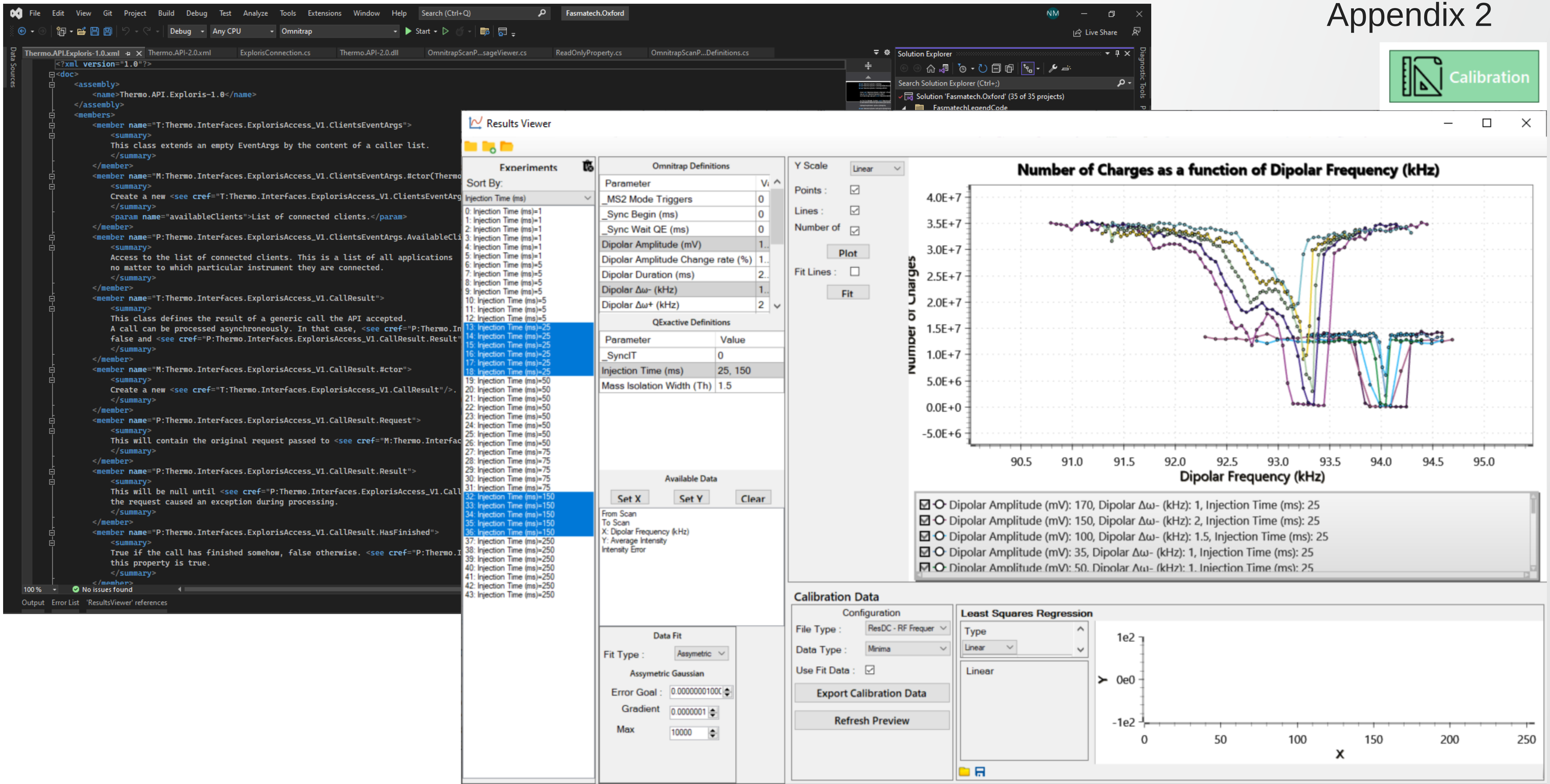
- API allows to automate essentially any operation available in Tune View of Exploris or Q Exactive MS
- API examples for the Exploris: https://github.com/thermofisherlsms/iapi/tree/master/examples/Exploris

Figures from: ASMS Poster, “Customized Real-Time Control of Benchtop Orbitrap MS” by Kuehn *et al.*

# Example of API usage for Omnitrap operation

Appendix 2

Calibration



```
<?xml version="1.0"?>
<doc>
    <assembly>
        <name>Thermo.API.Exploris-1.0</name>
    </assembly>
    <members>
        <member name="T:Thermo.Interfaces.ExplorisAccess_V1.ClientsEventArgs">
            <summary>
            This class extends an empty EventArgs by the content of a caller list.
            </summary>
        </member>
        <member name="M:Thermo.Interfaces.ExplorisAccess_V1.ClientsEventArgs.#ctor(Thermo
            <summary>
            Create a new <see cref="T:Thermo.Interfaces.ExplorisAccess_V1.ClientsEventArg
            </summary>
            <param name="availableClients">List of connected clients.</param>
        </member>
        <member name="P:Thermo.Interfaces.ExplorisAccess_V1.ClientsEventArgs.AvailableCli
            <summary>
            Access to the list of connected clients. This is a list of all applications
            no matter to which particular instrument they are connected.
            </summary>
        </member>
        <member name="T:Thermo.Interfaces.ExplorisAccess_V1.CallResult">
            <summary>
            This class defines the result of a generic call the API accepted.
            A call can be processed asynchroneously. In that case, <see cref="P:Thermo.In
            false and <see cref="P:Thermo.Interfaces.ExplorisAccess_V1.CallResult.Result"
            </summary>
        </member>
        <member name="M:Thermo.Interfaces.ExplorisAccess_V1.CallResult.#ctor">
            <summary>
            Create a new <see cref="T:Thermo.Interfaces.ExplorisAccess_V1.CallResult"/>.
            </summary>
        </member>
        <member name="P:Thermo.Interfaces.ExplorisAccess_V1.CallResult.Request">
            <summary>
            This will contain the original request passed to <see cref="M:Thermo.Interfac
            </summary>
        </member>
        <member name="P:Thermo.Interfaces.ExplorisAccess_V1.CallResult.Result">
            <summary>
            This will be null until <see cref="P:Thermo.Interfaces.ExplorisAccess_V1.Call
            the request caused an exception during processing.
            </summary>
        </member>
        <member name="P:Thermo.Interfaces.ExplorisAccess_V1.CallResult.HasFinished">
            <summary>
            True if the call has finished somehow, false otherwise. <see cref="P:Thermo.I
            this property is true.
            </summary>
        </member>
```

```
0: Injection Time (ms)=1
1: Injection Time (ms)=1
2: Injection Time (ms)=1
3: Injection Time (ms)=1
4: Injection Time (ms)=1
5: Injection Time (ms)=1
6: Injection Time (ms)=5
7: Injection Time (ms)=5
8: Injection Time (ms)=5
9: Injection Time (ms)=5
10: Injection Time (ms)=5
11: Injection Time (ms)=5
12: Injection Time (ms)=5
13: Injection Time (ms)=25
14: Injection Time (ms)=25
15: Injection Time (ms)=25
16: Injection Time (ms)=25
17: Injection Time (ms)=25
18: Injection Time (ms)=25
19: Injection Time (ms)=50
20: Injection Time (ms)=50
21: Injection Time (ms)=50
22: Injection Time (ms)=50
23: Injection Time (ms)=50
24: Injection Time (ms)=50
25: Injection Time (ms)=50
26: Injection Time (ms)=50
27: Injection Time (ms)=75
28: Injection Time (ms)=75
29: Injection Time (ms)=75
30: Injection Time (ms)=75
31: Injection Time (ms)=75
32: Injection Time (ms)=150
33: Injection Time (ms)=150
34: Injection Time (ms)=150
35: Injection Time (ms)=150
36: Injection Time (ms)=150
37: Injection Time (ms)=250
38: Injection Time (ms)=250
39: Injection Time (ms)=250
40: Injection Time (ms)=250
41: Injection Time (ms)=250
42: Injection Time (ms)=250
43: Injection Time (ms)=250
```

Omnitrap Definitions

| Parameter | V |
|---|---|
| _MS2 Mode Triggers | 0 |
| _Sync Begin (ms) | 0 |
| _Sync Wait QE (ms) | 0 |
| Dipolar Amplitude (mV) | 1. |
| Dipolar Amplitude Change rate (%) | 1. |
| Dipolar Duration (ms) | 2. |
| Dipolar Δω- (kHz) | 1. |
| Dipolar Δω+ (kHz) | 2 |

QExactive Definitions

| Parameter | Value |
|---|---|
| _SyncIT | 0 |
| Injection Time (ms) | 25, 150 |
| Mass Isolation Width (Th) | 1.5 |

From Scan
To Scan
X: Dipolar Frequency (kHz)
Y: Average Intensity
Intensity Error

Fit Type : Assymetric
Assymetric Gaussian
Error Goal : 0.0000000100(
Gradient 0.0000001
Max 10000

Y Scale Linear
Points : ☑
Lines : ☑
Number of ☑
Fit Lines : ☐

☒ ○ Dipolar Amplitude (mV): 170, Dipolar Δω- (kHz): 1, Injection Time (ms): 25
☒ ○ Dipolar Amplitude (mV): 150, Dipolar Δω- (kHz): 2, Injection Time (ms): 25
☒ ○ Dipolar Amplitude (mV): 100, Dipolar Δω- (kHz): 1.5, Injection Time (ms): 25
☒ ○ Dipolar Amplitude (mV): 35, Dipolar Δω- (kHz): 1, Injection Time (ms): 25
☒ ○ Dipolar Amplitude (mV): 50, Dipolar Δω- (kHz): 1, Injection Time (ms): 25

File Type : ResDC - RF Frequer
Data Type : Minima
Use Fit Data : ☑

Type
Linear

Linear

# WP6: Modification of the Orbitrap Mass Spectrometer

# Appendix 3. Installation test protocol of Omnitrap™ – Exploris™ 480 instrument

# The Omnitrap / Exploris 480 system

Appendix 3

- Omnitrap/IMS installed on an Exploris 480 MS at Fasmatech’s DemoLab, Athens, Greece.

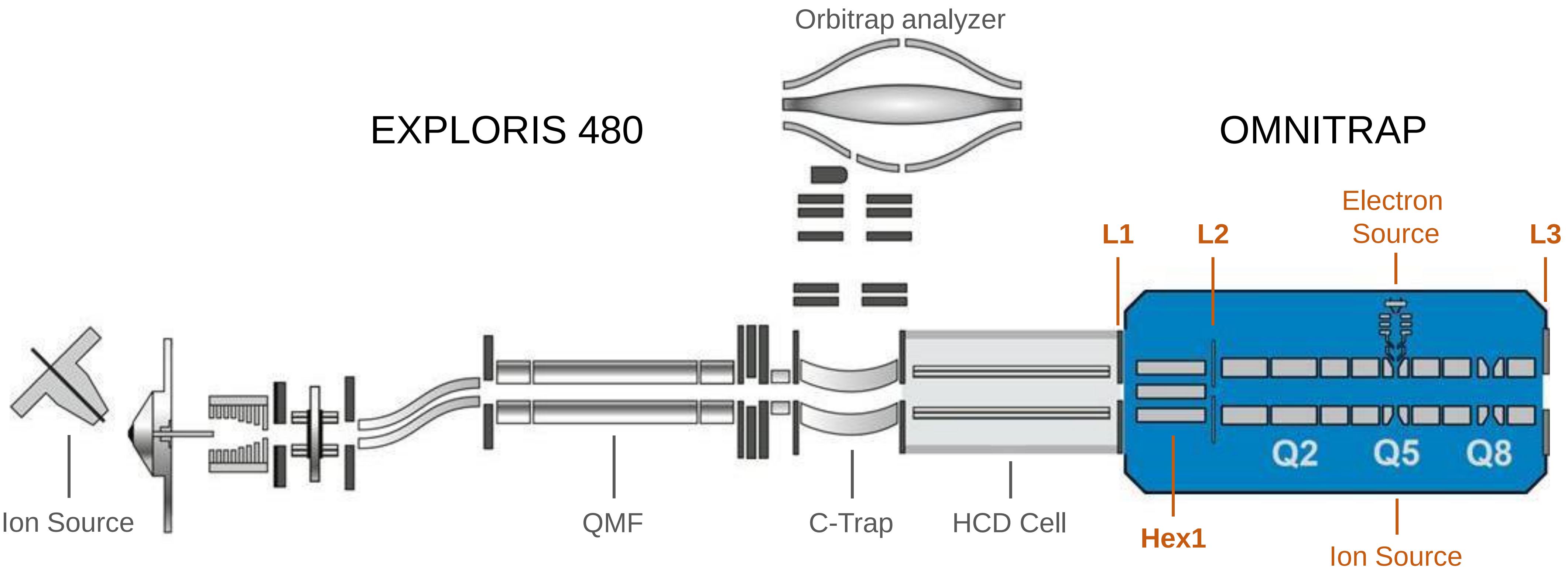
# The Omnitrap / Exploris 480 system

- Omnitrap/IMS/Exploris 480 system installed at Karolinska Institute, Stockholm (October 2022)

Appendix 3

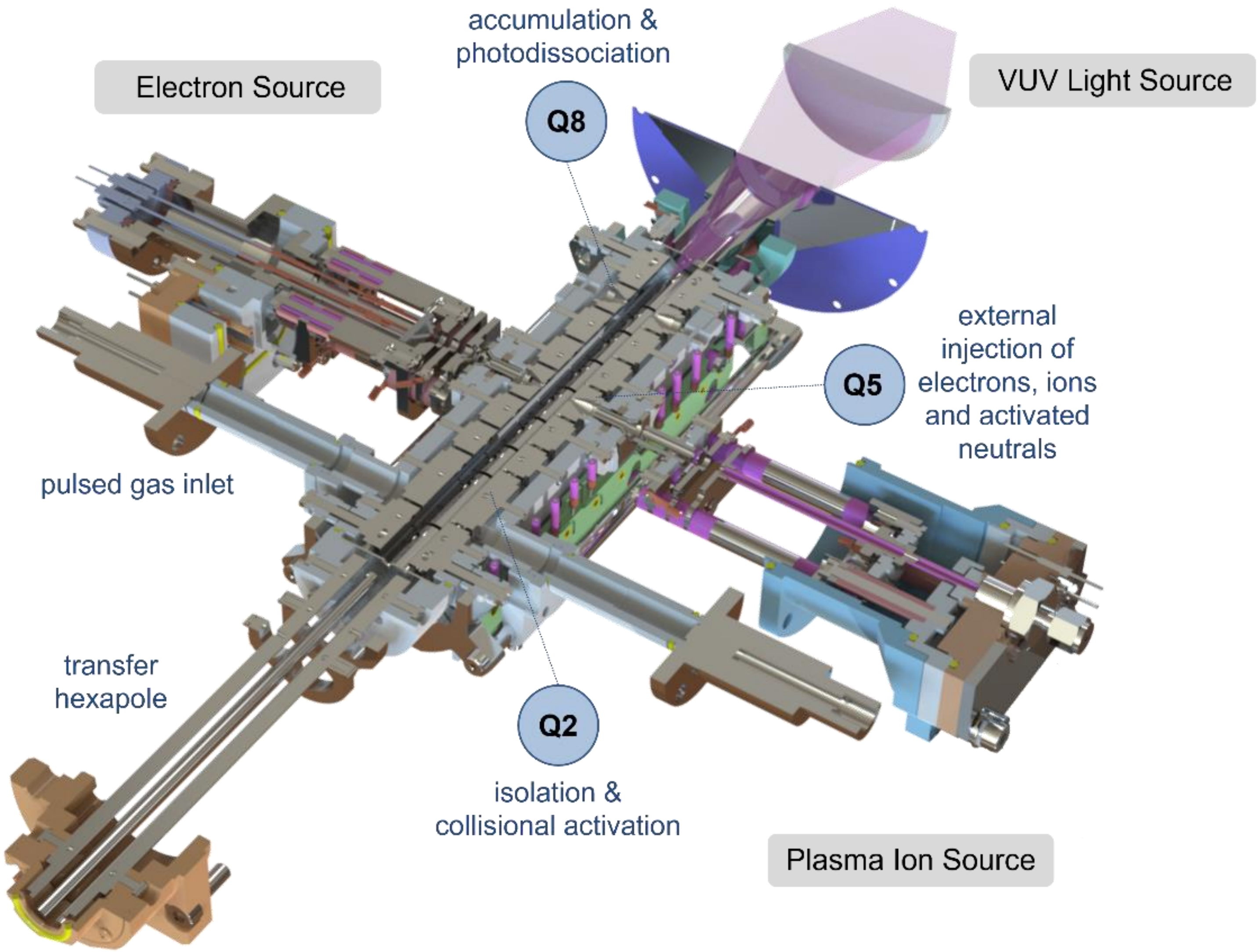
# The Omnitrap / Exploris 480 system

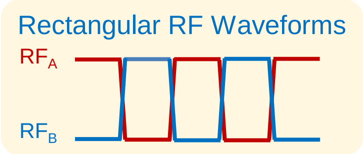
Appendix 3

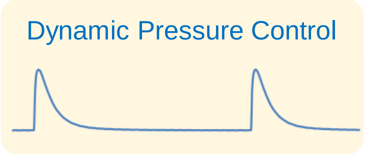


# The Omnitrap

Appendix 3




Rectangular RF Waveforms


Dynamic Pressure Control

- **Q2 segment**
  - slow heating CID
  - resolving DC isolation
  - sweep isolation
  - broadband or tailored excitation
- **Q5 segment**
  - electron ionization dissociation (EID)
  - electron capture dissociation (ECD)
  - hydrogen ion activated dissociation (HIAD)
  - slow heating CID
  - resolving DC isolation
  - sweep isolation
  - broadband or tailored excitation
- **Q7 segment**
  - UV photo-dissociation,
- **Q8 segment**
  - ion accumulation
  - UV photo-dissociation

# Gas lines installation

Appendix 3

Gas Lines Connections:

1/8' stainless steel tubing

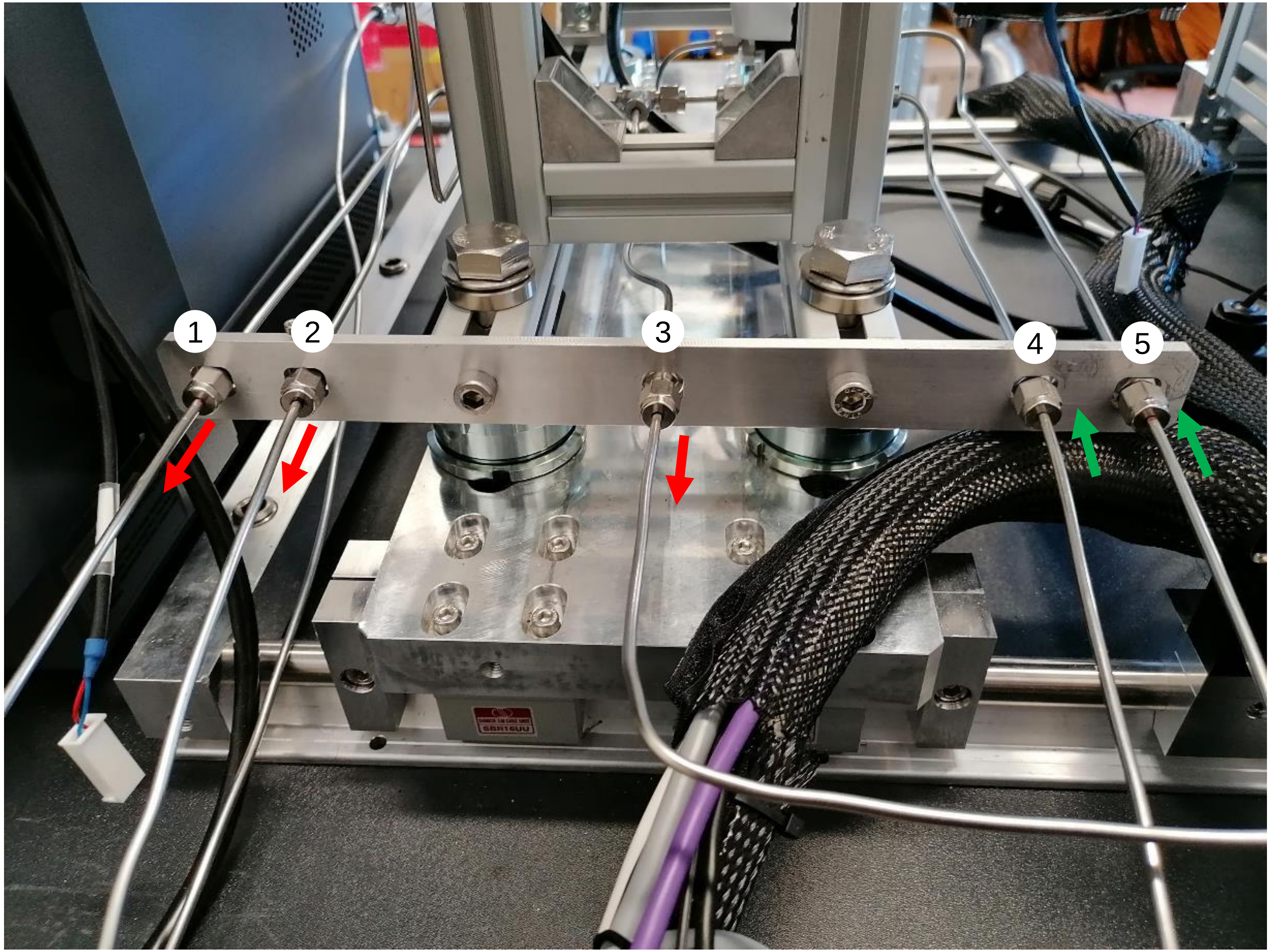
1. $H_2$ gas outlet (purge line)
   → ion source purge valve
2. $N_2$ gas outlet (purge line)
   → omnitrap purge valve
3. $N_2$ gas outlet to IMS drift cell
   → IMS needle valve
4. $N_2$ gas inlet (tank line)
   → omnitrap tank valve
5. $H_2$ gas inlet (tank line)
   → ion source tank valve

Gas & Regulator specs:

- Ultra-high purity gas supply (UHP > 99.999% pure) at 600 ±50 kPa [6.0 ±0.5 bar]
- Two-stage regulator connected to the gas tank is preferred – stainless steel tubing with double-ferrule compression fittings (1/8'' or 1/4'' fittings) must be used exclusively.

Backpressure settings:

- **$P(N_2)$ = 3 bar**
- **$P(H_2)$ = 5 bar**



# Omnitrap Software

# Omnitrap Software: Standard Mode

Appendix 3

Time: 88 ms State : Idle Loop Count : 0 Sequence File:

Mode : Omnitrap

Mode : Standard

**Initialization**

Number of Loops 65536
RF Freq (kHz) 1300
Min m/z 127.52
Max m/z 892.65

**Injection Q2**

Transfer Time (ms) 5
Trapping Time (ms) 10

**ResDC Isolation Q2**

Delay from Gas (ms) 1
RF Freq (kHz) 440.944
Mode ResDCtheoretical
Resolving DC (V) 53
Isolation Mass (m/z) 1422
Duration (ms) 6
RF Freq Return (kHz) 1309.77

**CID Q2**

RF Freq (kHz) 842.568
Mode CIDtheoretical
Secular Freq (kHz) 77.393
Amplitude (mV) 1
Duration (ms) 10
m/z 1071
q value 0.2

**Ejection Q2**

Ejection Lens (V) 6
Transfer Time (ms) 10

Normal Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 4 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Inject Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 5 | 5 | 4 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Confine Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 10 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Resolving DC Q2

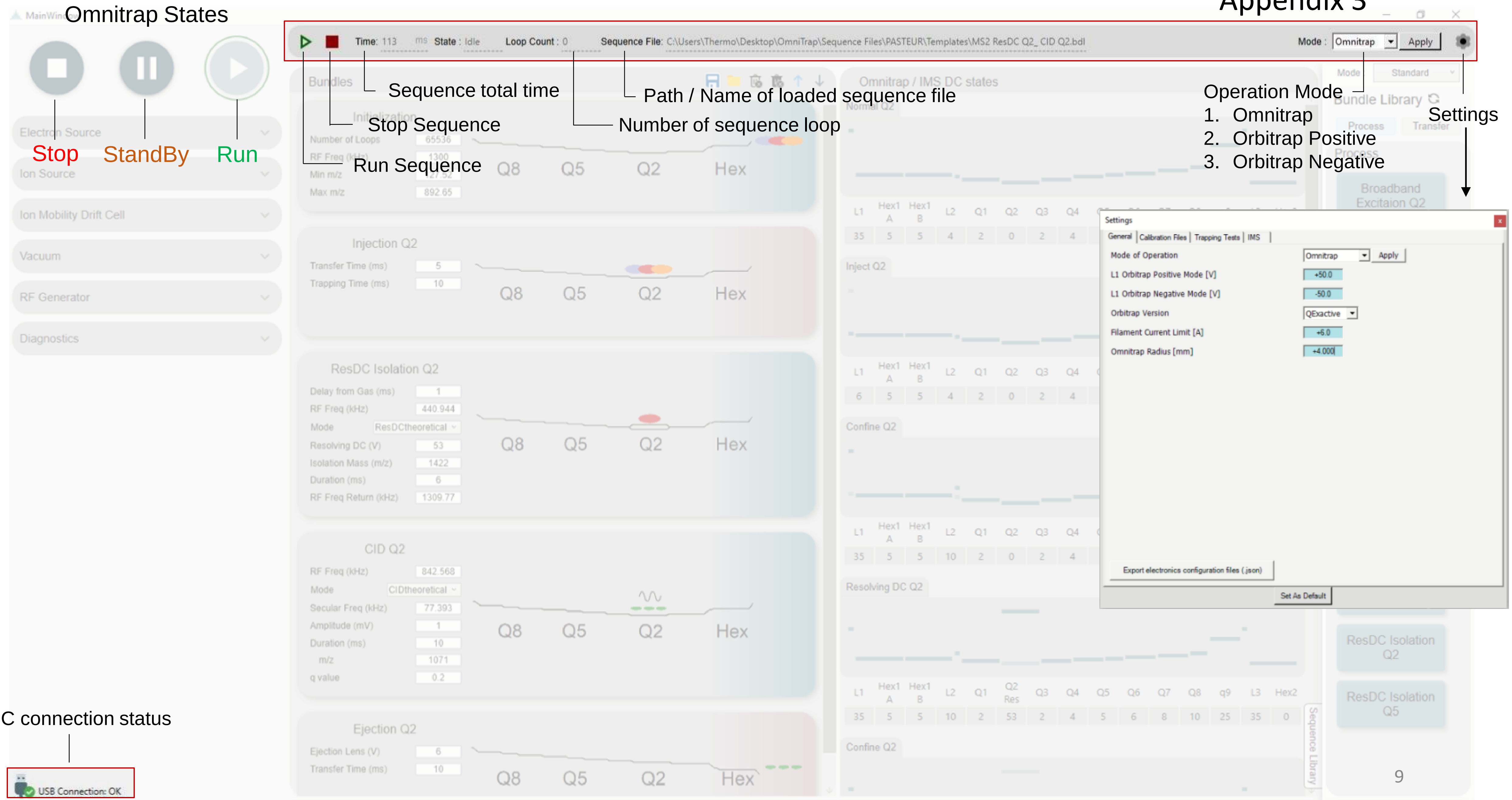
| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 Res | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 10 | 2 | 53 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Confine Q2

USB Connection: OK

# Omnitrap Software

## Appendix 3



Omnitrap States

Stop

StandBy

Run

Sequence total time

Stop Sequence

Run Sequence

Number of sequence loop

Path / Name of loaded sequence file

Operation Mode

1. Omnitrap
2. Orbitrap Positive
3. Orbitrap Negative

Settings

PC connection status

Time: 113 ms State : Idle Loop Count : 0 Sequence File: C:\Users\Thermo\Desktop\OmniTrap\Sequence Files\PASTEUR\Templates\MS2 ResDC Q2_ CID Q2.bdl Mode : Omnitrap Apply

Settings

General | Calibration Files | Trapping Tests | IMS

Mode of Operation: Omnitrap Apply

L1 Orbitrap Positive Mode [V]: +50.0

L1 Orbitrap Negative Mode [V]: -50.0

Orbitrap Version: QExactive

Filament Current Limit [A]: +6.0

Omnitrap Radius [mm]: +4.000

Export electronics configuration files (.json)

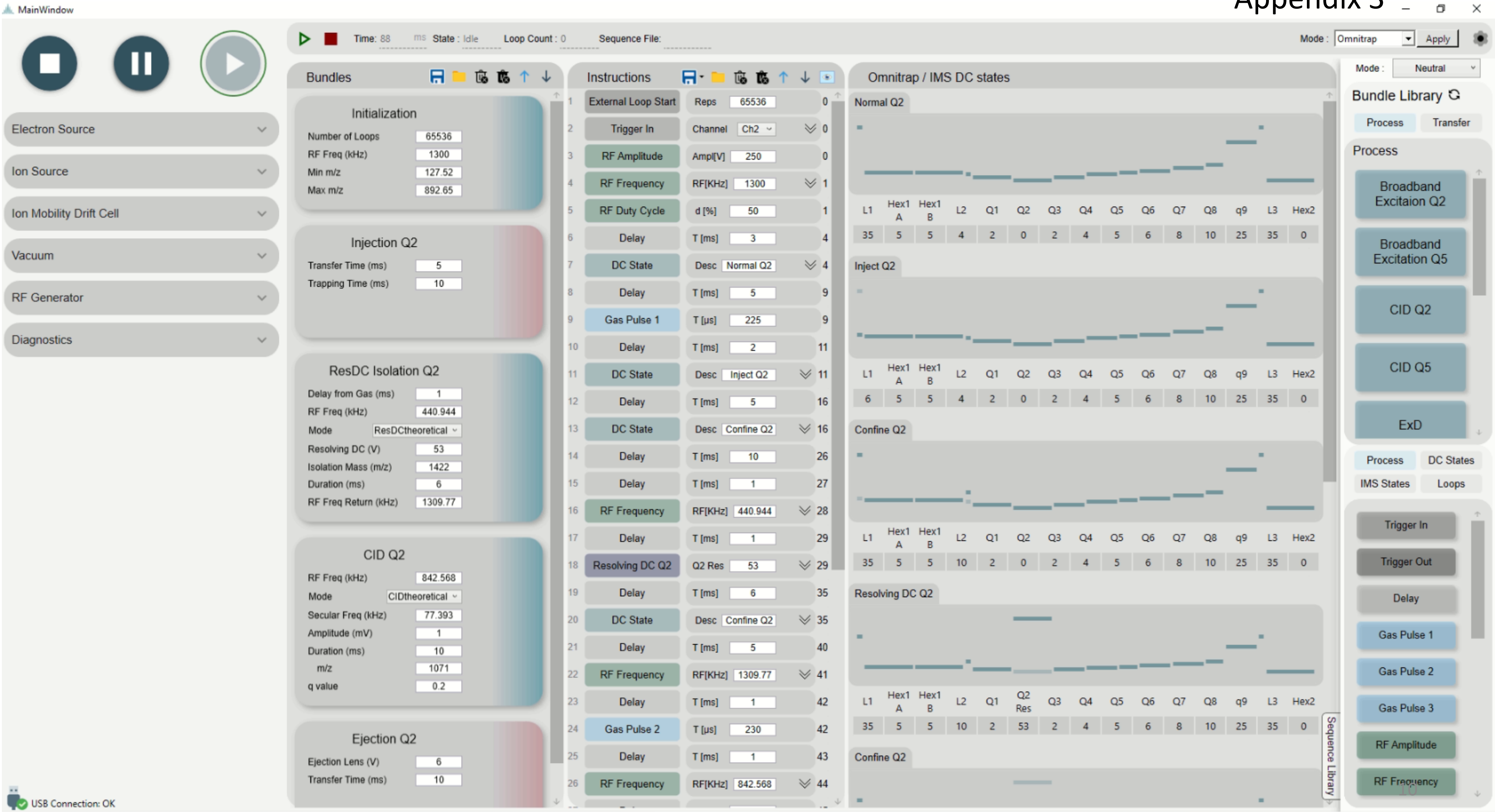
Set As Default

USB Connection: OK

# Omnitrap Software: Neutral Mode

FasmaTech science and technology

Appendix 3



Time: 88 ms State : Idle Loop Count : 0 Sequence File:

Mode : Omnitrap

Mode : Neutral

USB Connection: OK

**Initialization**

Number of Loops 65536
RF Freq (kHz) 1300
Min m/z 127.52
Max m/z 892.65

**Injection Q2**

Transfer Time (ms) 5
Trapping Time (ms) 10

**ResDC Isolation Q2**

Delay from Gas (ms) 1
RF Freq (kHz) 440.944
Mode ResDCtheoretical
Resolving DC (V) 53
Isolation Mass (m/z) 1422
Duration (ms) 6
RF Freq Return (kHz) 1309.77

**CID Q2**

RF Freq (kHz) 842.568
Mode CIDtheoretical
Secular Freq (kHz) 77.393
Amplitude (mV) 1
Duration (ms) 10
m/z 1071
q value 0.2

**Ejection Q2**

Ejection Lens (V) 6
Transfer Time (ms) 10

| | | | | |
|---|---|---|---|---|
| 1 | External Loop Start | Reps | 65536 | 0 |
| 2 | Trigger In | Channel | Ch2 | 0 |
| 3 | RF Amplitude | Ampl[V] | 250 | 0 |
| 4 | RF Frequency | RF[KHz] | 1300 | 1 |
| 5 | RF Duty Cycle | d [%] | 50 | 1 |
| 6 | Delay | T [ms] | 3 | 4 |
| 7 | DC State | Desc | Normal Q2 | 4 |
| 8 | Delay | T [ms] | 5 | 9 |
| 9 | Gas Pulse 1 | T [µs] | 225 | 9 |
| 10 | Delay | T [ms] | 2 | 11 |
| 11 | DC State | Desc | Inject Q2 | 11 |
| 12 | Delay | T [ms] | 5 | 16 |
| 13 | DC State | Desc | Confine Q2 | 16 |
| 14 | Delay | T [ms] | 10 | 26 |
| 15 | Delay | T [ms] | 1 | 27 |
| 16 | RF Frequency | RF[KHz] | 440.944 | 28 |
| 17 | Delay | T [ms] | 1 | 29 |
| 18 | Resolving DC Q2 | Q2 Res | 53 | 29 |
| 19 | Delay | T [ms] | 6 | 35 |
| 20 | DC State | Desc | Confine Q2 | 35 |
| 21 | Delay | T [ms] | 5 | 40 |
| 22 | RF Frequency | RF[KHz] | 1309.77 | 41 |
| 23 | Delay | T [ms] | 1 | 42 |
| 24 | Gas Pulse 2 | T [µs] | 230 | 42 |
| 25 | Delay | T [ms] | 1 | 43 |
| 26 | RF Frequency | RF[KHz] | 842.568 | 44 |

Normal Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 4 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Inject Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 5 | 5 | 4 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Confine Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 10 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

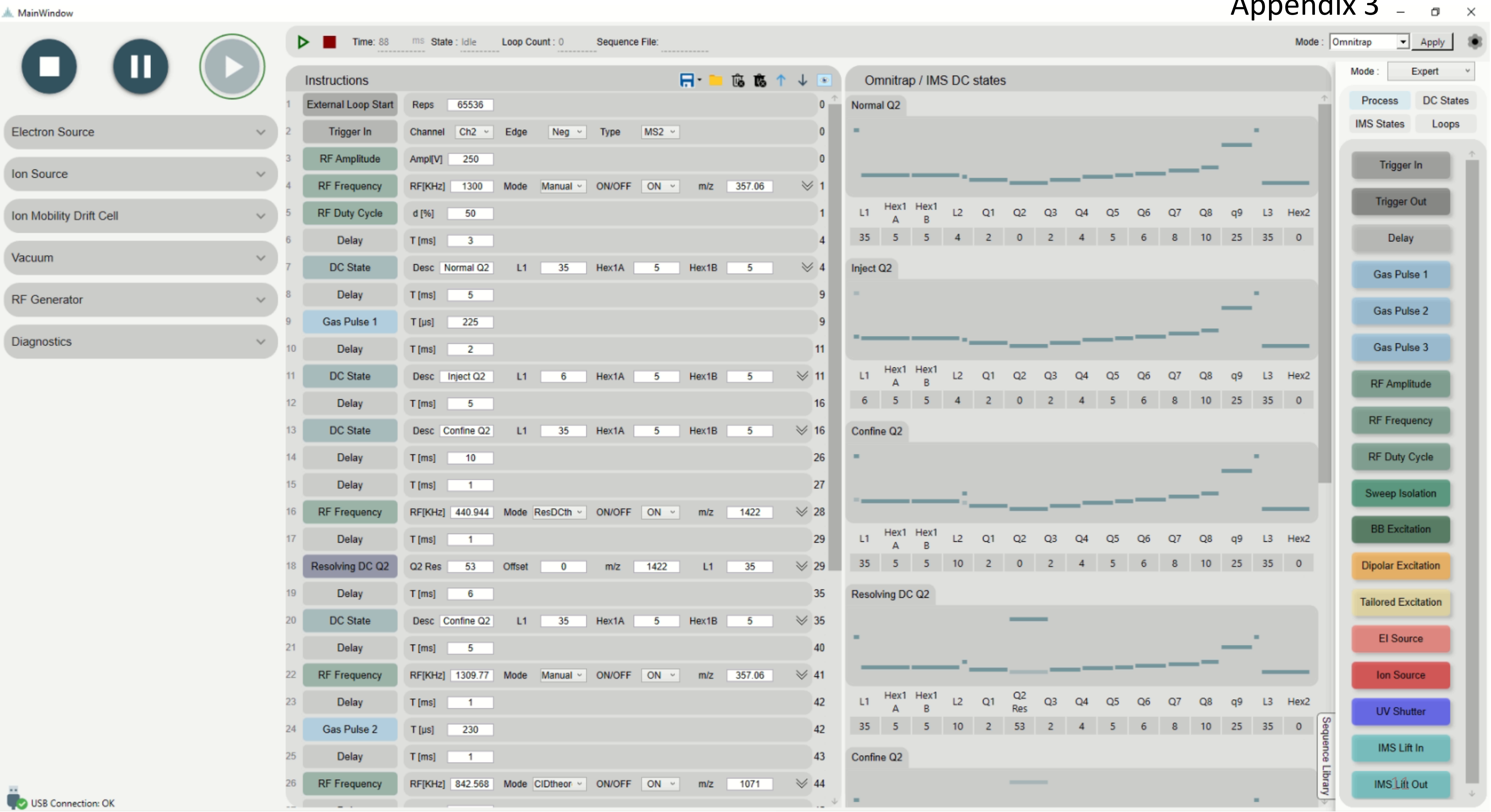
Resolving DC Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 Res | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 10 | 2 | 53 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Confine Q2

# Omnitrap Software: Expert Mode

Appendix 3



Time: 88 ms State : Idle Loop Count : 0 Sequence File:

Mode : Omnitrap

Mode : Expert

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | External Loop Start | Reps | 65536 | | | | | | | 0 |
| 2 | Trigger In | Channel | Ch2 | Edge | Neg | Type | MS2 | | | 0 |
| 3 | RF Amplitude | Ampl[V] | 250 | | | | | | | 0 |
| 4 | RF Frequency | RF[KHz] | 1300 | Mode | Manual | ON/OFF | ON | m/z | 357.06 | 1 |
| 5 | RF Duty Cycle | d [%] | 50 | | | | | | | 1 |
| 6 | Delay | T [ms] | 3 | | | | | | | 4 |
| 7 | DC State | Desc | Normal Q2 | L1 | 35 | Hex1A | 5 | Hex1B | 5 | 4 |
| 8 | Delay | T [ms] | 5 | | | | | | | 9 |
| 9 | Gas Pulse 1 | T [µs] | 225 | | | | | | | 9 |
| 10 | Delay | T [ms] | 2 | | | | | | | 11 |
| 11 | DC State | Desc | Inject Q2 | L1 | 6 | Hex1A | 5 | Hex1B | 5 | 11 |
| 12 | Delay | T [ms] | 5 | | | | | | | 16 |
| 13 | DC State | Desc | Confine Q2 | L1 | 35 | Hex1A | 5 | Hex1B | 5 | 16 |
| 14 | Delay | T [ms] | 10 | | | | | | | 26 |
| 15 | Delay | T [ms] | 1 | | | | | | | 27 |
| 16 | RF Frequency | RF[KHz] | 440.944 | Mode | ResDCth | ON/OFF | ON | m/z | 1422 | 28 |
| 17 | Delay | T [ms] | 1 | | | | | | | 29 |
| 18 | Resolving DC Q2 | Q2 Res | 53 | Offset | 0 | m/z | 1422 | L1 | 35 | 29 |
| 19 | Delay | T [ms] | 6 | | | | | | | 35 |
| 20 | DC State | Desc | Confine Q2 | L1 | 35 | Hex1A | 5 | Hex1B | 5 | 35 |
| 21 | Delay | T [ms] | 5 | | | | | | | 40 |
| 22 | RF Frequency | RF[KHz] | 1309.77 | Mode | Manual | ON/OFF | ON | m/z | 357.06 | 41 |
| 23 | Delay | T [ms] | 1 | | | | | | | 42 |
| 24 | Gas Pulse 2 | T [µs] | 230 | | | | | | | 42 |
| 25 | Delay | T [ms] | 1 | | | | | | | 43 |
| 26 | RF Frequency | RF[KHz] | 842.568 | Mode | CIDtheor | ON/OFF | ON | m/z | 1071 | 44 |

Normal Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 4 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Inject Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 5 | 5 | 4 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Confine Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 10 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

Resolving DC Q2

| L1 | Hex1 A | Hex1 B | L2 | Q1 | Q2 Res | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 5 | 10 | 2 | 53 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

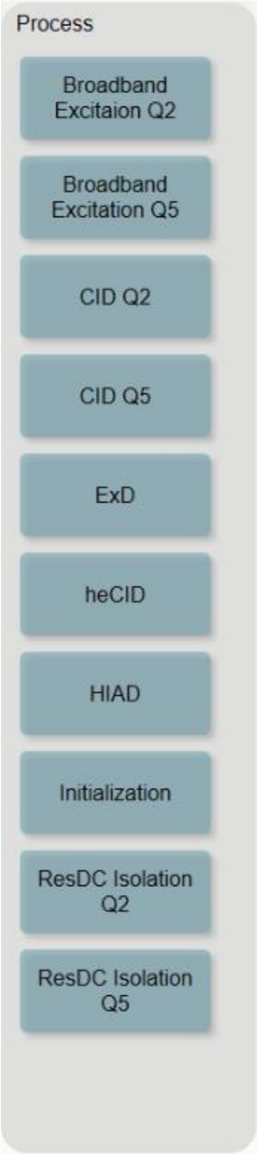
Confine Q2

# Omnitrap Software: Bundle & Instruction Libraries

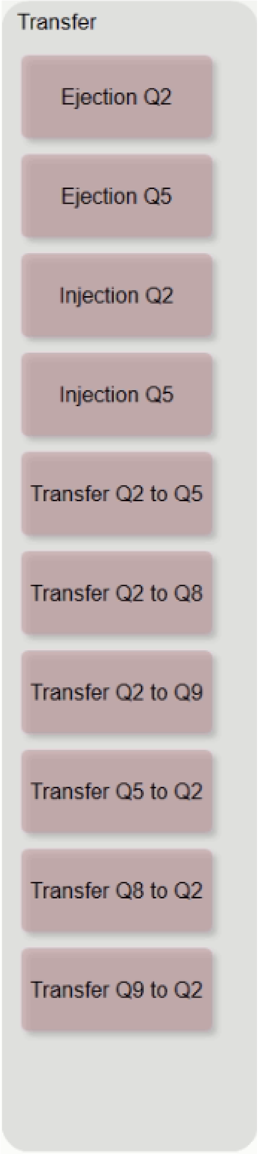
Appendix 3

## BUNDLES: STANDARD mode

Process



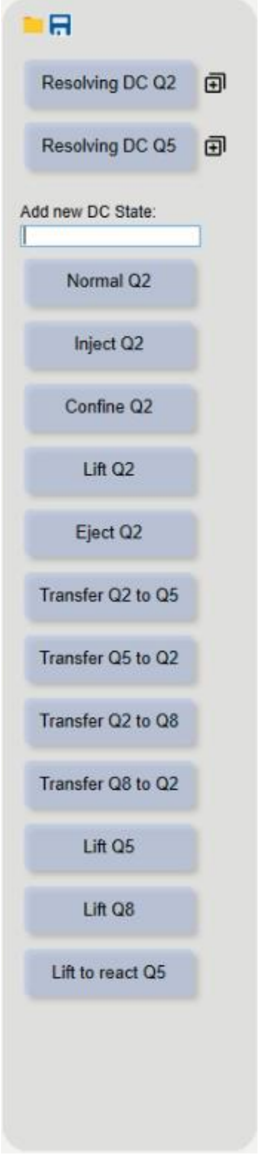
Transfer



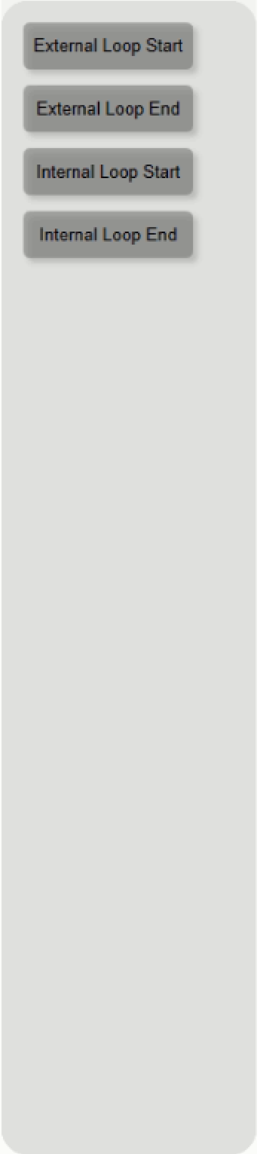
## INSTRUCTIONS: EXPERT mode

Process

DC states



Loops



IMS states

# List of Instructions

Appendix 3

| Instruction | Parameters | Values | Description |
|---|---|---|---|
| **Trigger In** | Channel | 1, 2, 1+2 | Set at 2 |
| | Edge | positive, negative | Set at negative |
| | Type | Normal, MS2 | Set at MS2 |
| **RF Frequency** | Frequency | 150kHz … 2500kHz | Set depending on the desired mass range. |
| | Mode | Manual, CIDTheoretical/Calibration, ResDCtheoretical/Calibration, SweepTheoretical/Calibration | |
| | State | ON/OFF | |
| | m/z | | |
| | q value | 0.01 – 0.7 | |
| | Min m/z , Max m/z | calculated | |
| **RF Amplitude** | Voltage amplitude | 0V … 400V | Always set at 250 V. |
| **RF Duty Cycle** | % duty cycle | 0 … 100 % | Always set at 50%. |
| **Delay** | Time | ms | |
| **Gas Pulse 1 ($N_2$)** | Time | µs | Set at 215 to 220 µs |
| **Gas Pulse 2 ($N_2$)** | Time | µs | Set at 220 to 225 µs |
| **Gas Pulse 3 ($H_2$)** | Time | µs | |
| **UV Shutter** | Time | ms | unavailable |

# List of Instructions

Appendix 3

| Instruction | Parameters | Values | Description |
|---|---|---|---|
| **Dipolar Excitation** | Quad segment | Q2, Q5 | |
| | Secular frequency ($\omega_{exc}$) | 1 … 300 kHz | |
| | Amplitude | 0 … 5000 mV | |
| | Duration T | ms | Use 10 ms or 20 ms |
| | q value | 0.01 – 0.7 | Use 0.1 , 0.15 or 0.5 depending on desired mass range |
| | m/z | | |
| | RF frequency | calculated | |
| **EI Source** | State | ON/OFF | |
| **Ion Source** | State | ON/OFF | unavailable |
| **Sweep Isolation** | Edit Waveform | | |
| | Quad. | Q2, Q5 | |
| | Gain | 0 … 5000 mV | |
| | m/z | | |
| | Q value | | |
| **DC States** | Lenses Voltages | -150…150 V | |
| | Q1-Q9 Voltages | -150…150 V | |
| | Hexapole Voltage | -150…150 V | |
| **Trigger Out** | Time | μs | Signal connected to the IMS picoscope |

# Omnitrap Bundles

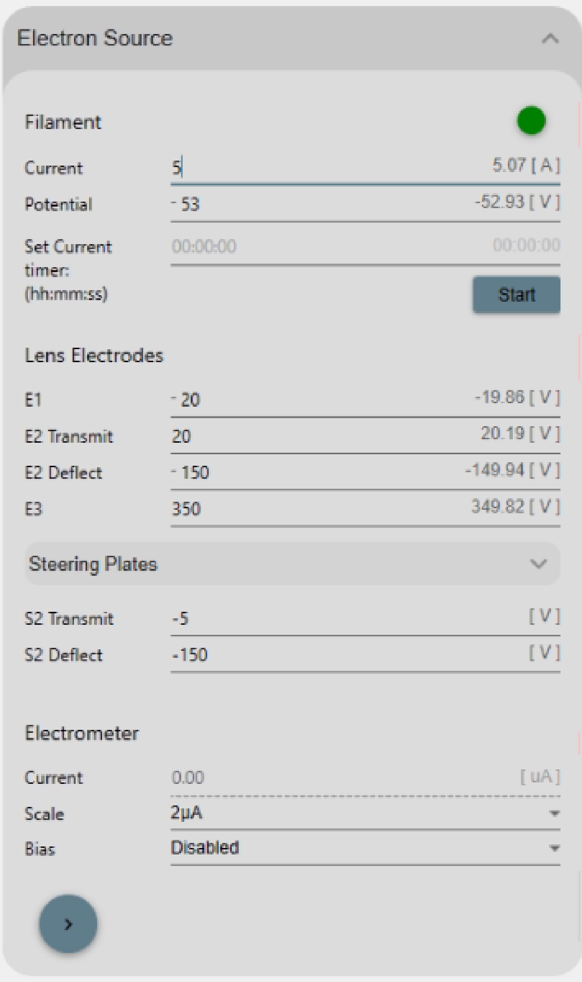
Indicative list of bundles:

Appendix 3

| **TRANSFER** | | **PROCESS** | |
|---|---|---|---|
| **External Transfer** | **Internal Transfer** | **Ion Activation** | **Ion Isolation** |
| Injection Q2 | Transfer Q2 to Q5 | Initialization | ResDC Isolation Q2 |
| Injection Q5 | Transfer Q2 to Q8 | CID Q2 | ResDC Isolation Q5 |
| Ejection Q2 | Transfer Q2 to Q9 | CID Q5 | Sweep Isolation Q2 |
| Ejection Q5 | Transfer Q5 to Q2 | Broadband Excitation Q2 | Sweep Isolation Q5 |
| … | Transfer Q8 to Q2 | Broadband Excitation Q5 | |
| | Transfer Q9 to Q2 | Tailored Excitation Q2 | |
| | … | Tailored Excitation Q5 | |
| | | UVPD | |

Note:
Every sequence must start with a "Initialization" bundle follows by an "Injection …" bundle, and finish with an "Ejection to …" bundle.

# Omnitrap Software: Modules



## Electron Source

**Filament** ●

| | | |
|---|---|---|
| Current | 5 | 5.07 [ A ] |
| Potential | -53 | -52.93 [ V ] |
| Set Current timer: (hh:mm:ss) | 00:00:00 | 00:00:00 |

**Lens Electrodes**

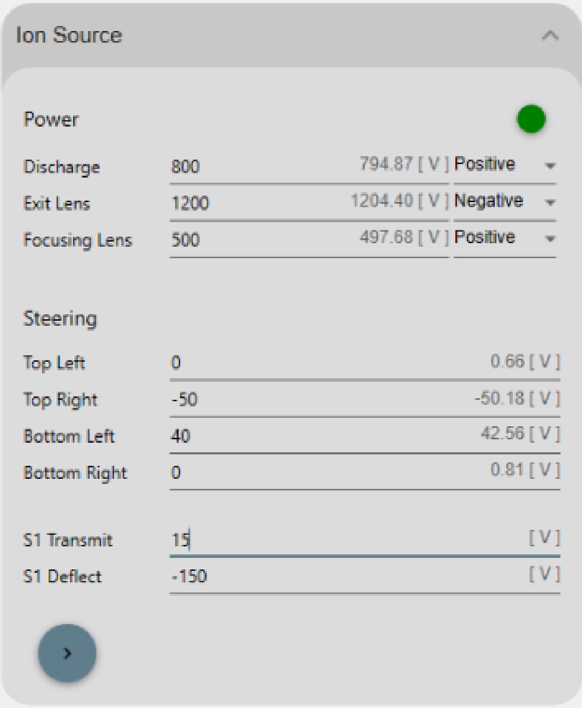
| | | |
|---|---|---|
| E1 | -20 | -19.86 [ V ] |
| E2 Transmit | 20 | 20.19 [ V ] |
| E2 Deflect | -150 | -149.94 [ V ] |
| E3 | 350 | 349.82 [ V ] |

**Steering Plates**

| | | |
|---|---|---|
| S2 Transmit | -5 | [ V ] |
| S2 Deflect | -150 | [ V ] |

**Electrometer**

| | | |
|---|---|---|
| Current | 0.00 | [ uA ] |
| Scale | 2µA | |
| Bias | Disabled | |



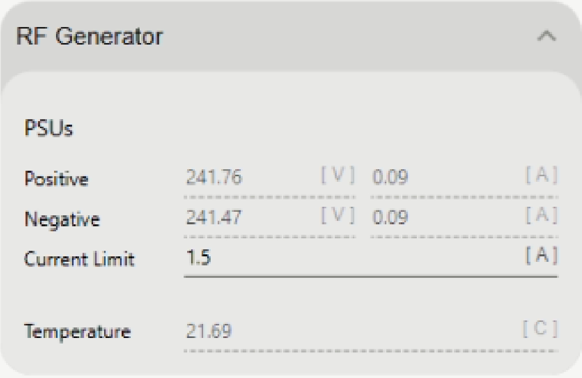
## Ion Source

**Power** ●

| | | | |
|---|---|---|---|
| Discharge | 800 | 794.87 [ V ] | Positive |
| Exit Lens | 1200 | 1204.40 [ V ] | Negative |
| Focusing Lens | 500 | 497.68 [ V ] | Positive |

**Steering**

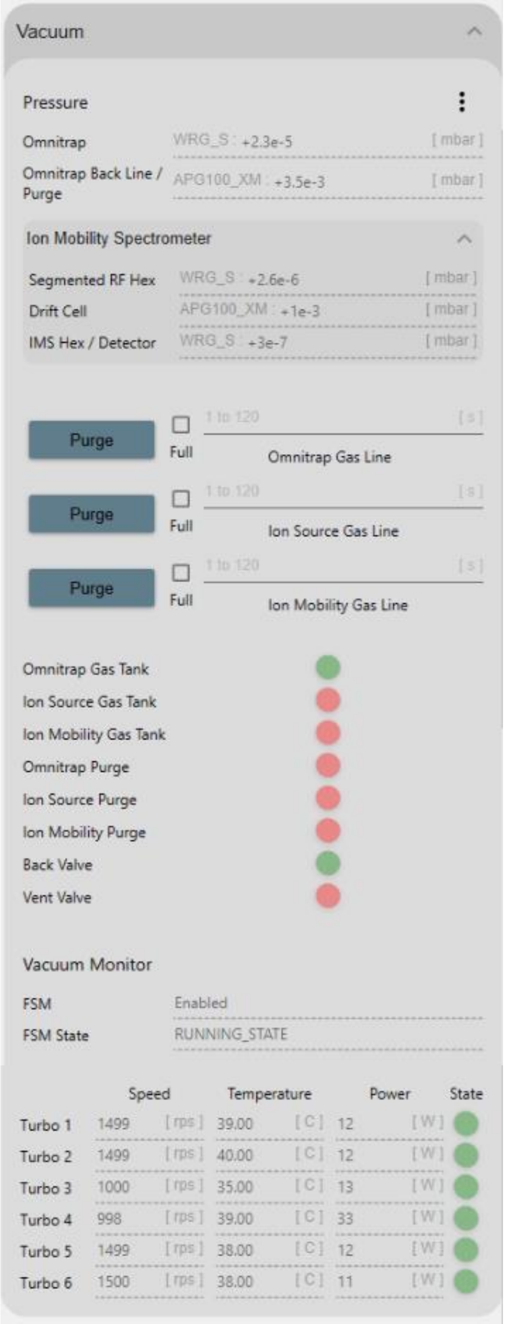
| | | |
|---|---|---|
| Top Left | 0 | 0.66 [ V ] |
| Top Right | -50 | -50.18 [ V ] |
| Bottom Left | 40 | 42.56 [ V ] |
| Bottom Right | 0 | 0.81 [ V ] |
| S1 Transmit | 15 | [ V ] |
| S1 Deflect | -150 | [ V ] |



## RF Generator

**PSUs**

| | | |
|---|---|---|
| Positive | 241.76 [ V ] | 0.09 [ A ] |
| Negative | 241.47 [ V ] | 0.09 [ A ] |
| Current Limit | 1.5 [ A ] | |
| Temperature | 21.69 [ C ] | |



## Vacuum

**Pressure**

| | | |
|---|---|---|
| Omnitrap | WRG_S : +2.3e-5 | [ mbar ] |
| Omnitrap Back Line / Purge | APG100_XM : +3.5e-3 | [ mbar ] |

**Ion Mobility Spectrometer**

| | | |
|---|---|---|
| Segmented RF Hex | WRG_S : +2.6e-6 | [ mbar ] |
| Drift Cell | APG100_XM : +1e-3 | [ mbar ] |
| IMS Hex / Detector | WRG_S : +3e-7 | [ mbar ] |

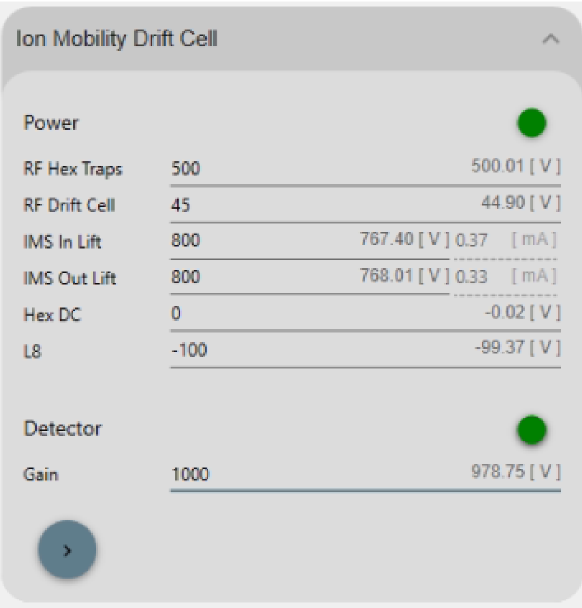
| | | | |
|---|---|---|---|
| Purge | ☐ Full | 1 to 120 [ s ] | Omnitrap Gas Line |
| Purge | ☐ Full | 1 to 120 [ s ] | Ion Source Gas Line |
| Purge | ☐ Full | 1 to 120 [ s ] | Ion Mobility Gas Line |

| | |
|---|---|
| Omnitrap Gas Tank | 🟢 |
| Ion Source Gas Tank | 🔴 |
| Ion Mobility Gas Tank | 🔴 |
| Omnitrap Purge | 🔴 |
| Ion Source Purge | 🔴 |
| Ion Mobility Purge | 🔴 |
| Back Valve | 🟢 |
| Vent Valve | 🔴 |

**Vacuum Monitor**

| | |
|---|---|
| FSM | Enabled |
| FSM State | RUNNING_STATE |

| | Speed | Temperature | Power | State |
|---|---|---|---|---|
| Turbo 1 | 1499 [ rps ] | 39.00 [ C ] | 12 [ W ] | 🟢 |
| Turbo 2 | 1499 [ rps ] | 40.00 [ C ] | 12 [ W ] | 🟢 |
| Turbo 3 | 1000 [ rps ] | 35.00 [ C ] | 13 [ W ] | 🟢 |
| Turbo 4 | 998 [ rps ] | 39.00 [ C ] | 33 [ W ] | 🟢 |
| Turbo 5 | 1499 [ rps ] | 38.00 [ C ] | 12 [ W ] | 🟢 |
| Turbo 6 | 1500 [ rps ] | 38.00 [ C ] | 11 [ W ] | 🟢 |



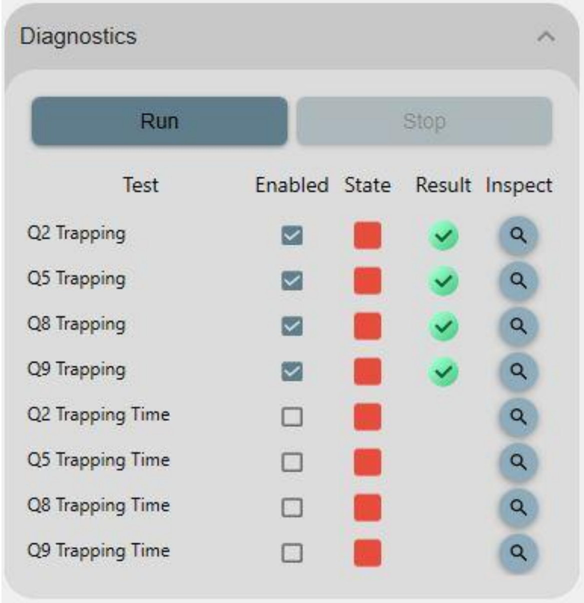
## Ion Mobility Drift Cell

**Power** ●

| | | |
|---|---|---|
| RF Hex Traps | 500 | 500.01 [ V ] |
| RF Drift Cell | 45 | 44.90 [ V ] |
| IMS In Lift | 800 | 767.40 [ V ] 0.37 [ mA ] |
| IMS Out Lift | 800 | 768.01 [ V ] 0.33 [ mA ] |
| Hex DC | 0 | -0.02 [ V ] |
| L8 | -100 | -99.37 [ V ] |

**Detector** ●

| | | |
|---|---|---|
| Gain | 1000 | 978.75 [ V ] |

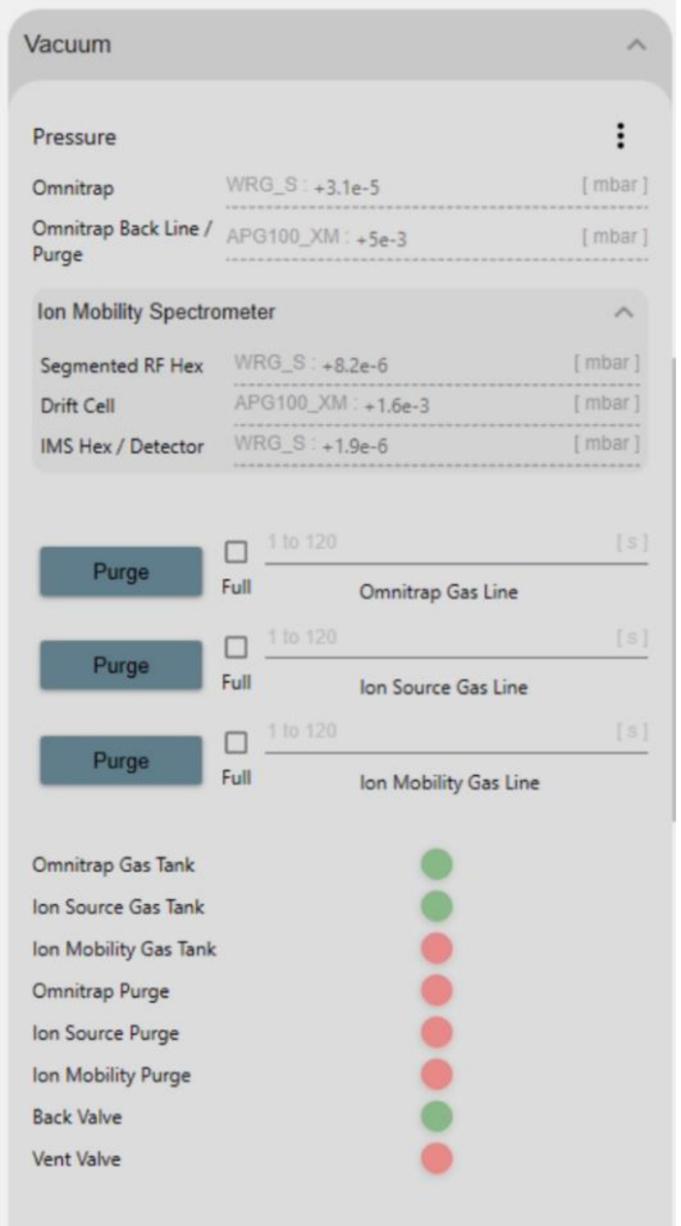


## Diagnostics

| Test | Enabled | State | Result | Inspect |
|---|---|---|---|---|
| Q2 Trapping | ☑ | 🟥 | ✓ | 🔍 |
| Q5 Trapping | ☑ | 🟥 | ✓ | 🔍 |
| Q8 Trapping | ☑ | 🟥 | ✓ | 🔍 |
| Q9 Trapping | ☑ | 🟥 | ✓ | 🔍 |
| Q2 Trapping Time | ☐ | 🟥 | | 🔍 |
| Q5 Trapping Time | ☐ | 🟥 | | 🔍 |
| Q8 Trapping Time | ☐ | 🟥 | | 🔍 |
| Q9 Trapping Time | ☐ | 🟥 | | 🔍 |

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Vacuum module – Pressure readings



Vacuum

Pressure

Omnitrap WRG_S : +3.1e-5 [ mbar ]

Omnitrap Back Line / Purge APG100_XM : +5e-3 [ mbar ]

Ion Mobility Spectrometer

Segmented RF Hex WRG_S : +8.2e-6 [ mbar ]

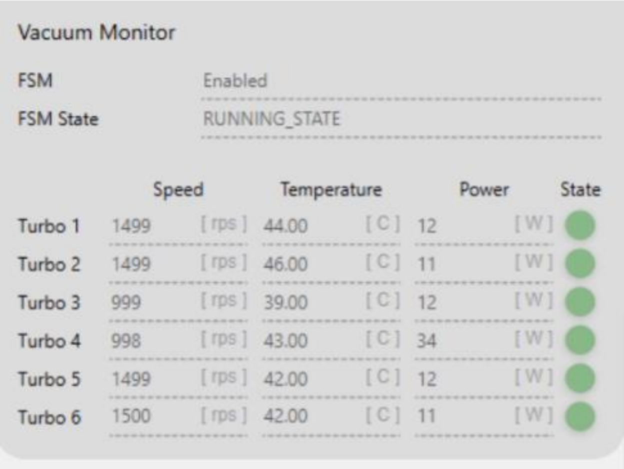
Drift Cell APG100_XM : +1.6e-3 [ mbar ]

IMS Hex / Detector WRG_S : +1.9e-6 [ mbar ]

| | |
|---|---|
| Omnitrap Gas Tank | 🟢 |
| Ion Source Gas Tank | 🟢 |
| Ion Mobility Gas Tank | 🔴 |
| Omnitrap Purge | 🔴 |
| Ion Source Purge | 🔴 |
| Ion Mobility Purge | 🔴 |
| Back Valve | 🟢 |
| Vent Valve | 🔴 |

- **Omnitrap pressure should be ~3e$^{-6}$ mbar** when Exploris is in StandBy or Run mode (N2 is injected into the HCD cell). Pressure should drop at **2 to 4e$^{-6}$ mbar** when Exploris is in Stop mode.

- **Back Line / Purge** pressure < 5e$^{-3}$ mbar
- **Segmented RF Hex** pressure < 1e$^{-5}$ mbar (with IMS needle valve closed)
- **Drift Cell** pressure ~ 1e$^{-3}$ mbar (with IMS needle valve closed)
- **IMS Hex/ Detector** pressure ~1e$^{-6}$ mbar (with IMS needle valve closed)



Vacuum Monitor

FSM Enabled

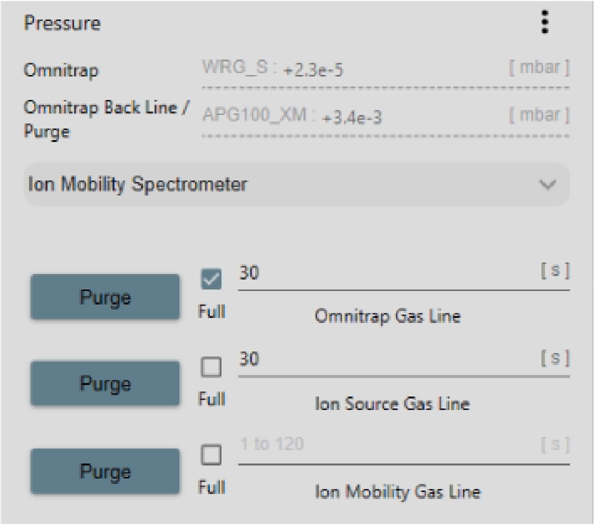
FSM State RUNNING_STATE

| | Speed | | Temperature | | Power | | State |
|---|---|---|---|---|---|---|---|
| Turbo 1 | 1499 | [ rps ] | 44.00 | [ C ] | 12 | [ W ] | 🟢 |
| Turbo 2 | 1499 | [ rps ] | 46.00 | [ C ] | 11 | [ W ] | 🟢 |
| Turbo 3 | 999 | [ rps ] | 39.00 | [ C ] | 12 | [ W ] | 🟢 |
| Turbo 4 | 998 | [ rps ] | 43.00 | [ C ] | 34 | [ W ] | 🟢 |
| Turbo 5 | 1499 | [ rps ] | 42.00 | [ C ] | 12 | [ W ] | 🟢 |
| Turbo 6 | 1500 | [ rps ] | 42.00 | [ C ] | 11 | [ W ] | 🟢 |

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Vacuum module: Purge Gas

Appendix 3



Pressure

Omnitrap WRG_S : +2.3e-5 [ mbar ]

Omnitrap Back Line / Purge APG100_XM : +3.4e-3 [ mbar ]

Ion Mobility Spectrometer

Purge ☑ Full 30 [ s ] Omnitrap Gas Line

Purge ☐ Full 30 [ s ] Ion Source Gas Line

Purge ☐ Full 1 to 120 [ s ] Ion Mobility Gas Line

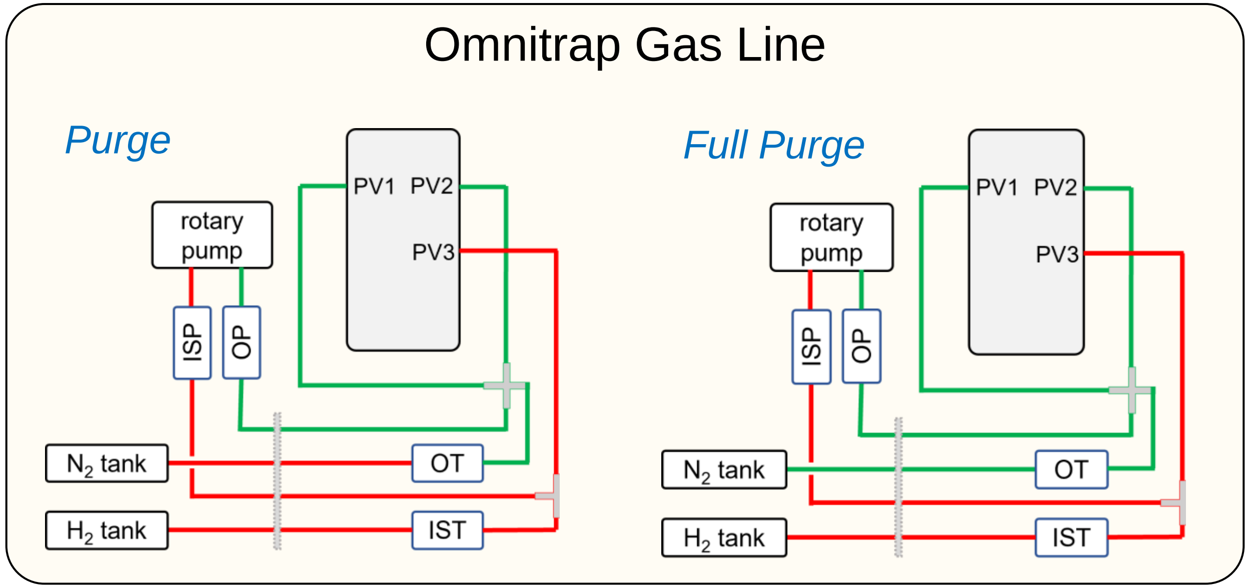
## Omnitrap Gas Line

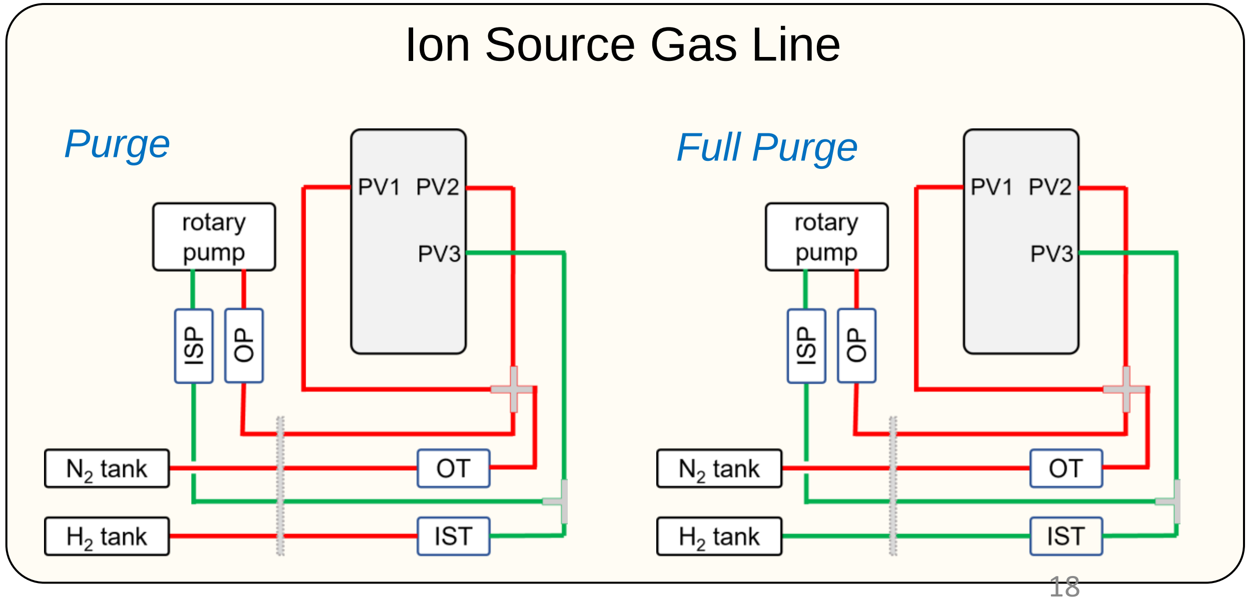
- *Purge*: The omnitrap purge (OP) valve is opened; omnitrap gas tank (OT) valve is closed, and the gas lines of pulse valves 1 and 2 ($N_2$) are purged.
- *Full Purge*: Both the omnitrap purge (OP) valve and omnitrap gas tank (OT) valve are opened and the gas lines of pulse valves 1 and 2 ($N_2$) are purged. The $N_2$ tank must be closed!

## Ion Source Gas Line

- *Purge*: The ion source purge (ISP) valve is opened; ion source gas tank (IST) valve is closed, and the gas line of pulse valve 3 ($H_2$) is purged.
- *Full Purge*: Both the ion source purge (ISP) valve and ion source gas tank (IST) valve are opened, and the gas line of pulse valve 3 ($H_2$) is purged. The $H_2$ tank must be closed!

➢ Monitor *Back Line* / *Purge* pressure and set time (s) accordingly until pressure drops at ~ $10^{-3}$ mbar.

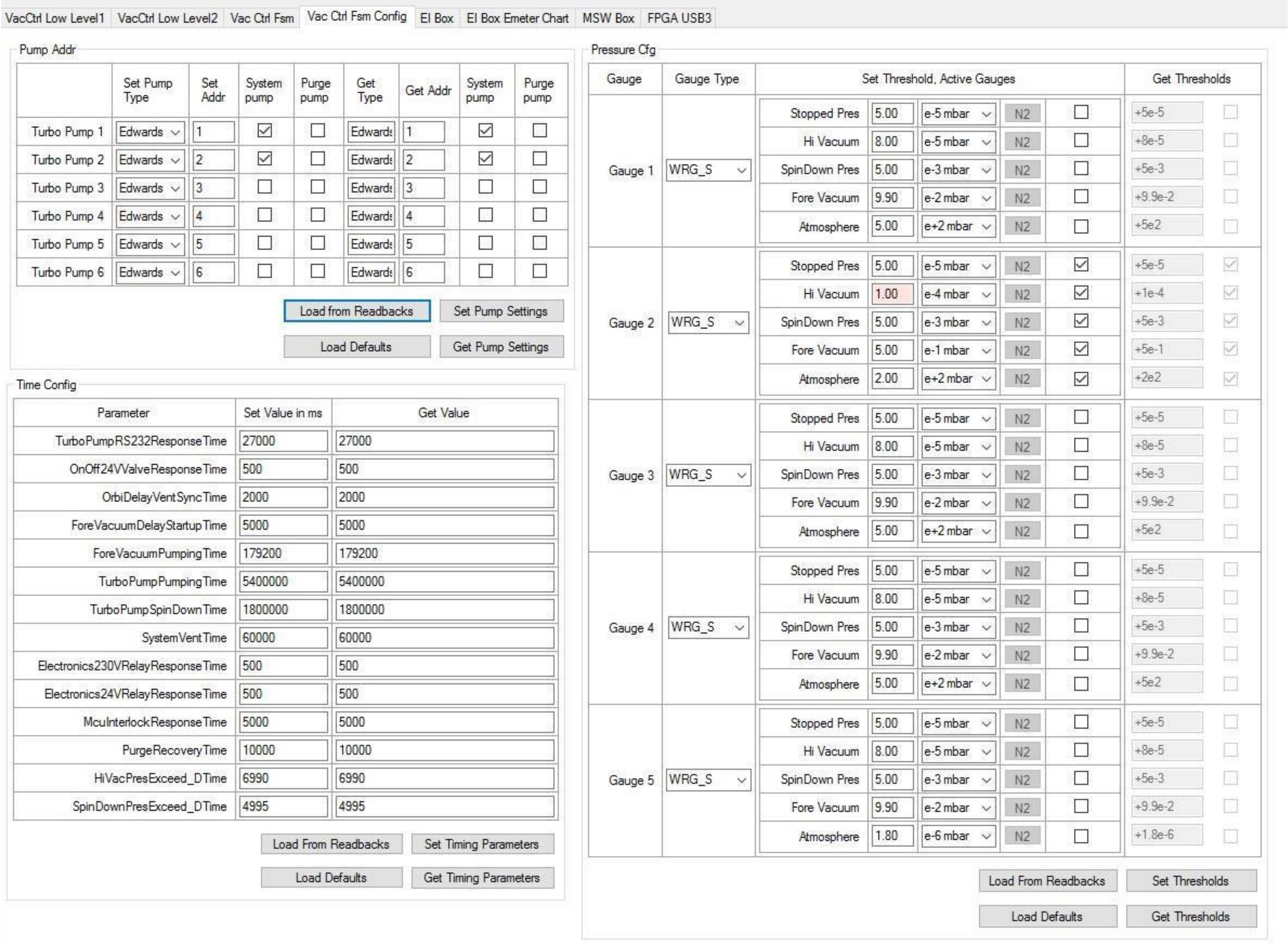




**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Vacuum control FSM settings

Appendix 3



Pump Addr

| | Set Pump Type | Set Addr | System pump | Purge pump | Get Type | Get Addr | System pump | Purge pump |
|---|---|---|---|---|---|---|---|---|
| Turbo Pump 1 | Edwards | 1 | ☑ | ☐ | Edwards | 1 | ☑ | ☐ |
| Turbo Pump 2 | Edwards | 2 | ☑ | ☐ | Edwards | 2 | ☑ | ☐ |
| Turbo Pump 3 | Edwards | 3 | ☐ | ☐ | Edwards | 3 | ☐ | ☐ |
| Turbo Pump 4 | Edwards | 4 | ☐ | ☐ | Edwards | 4 | ☐ | ☐ |
| Turbo Pump 5 | Edwards | 5 | ☐ | ☐ | Edwards | 5 | ☐ | ☐ |
| Turbo Pump 6 | Edwards | 6 | ☐ | ☐ | Edwards | 6 | ☐ | ☐ |

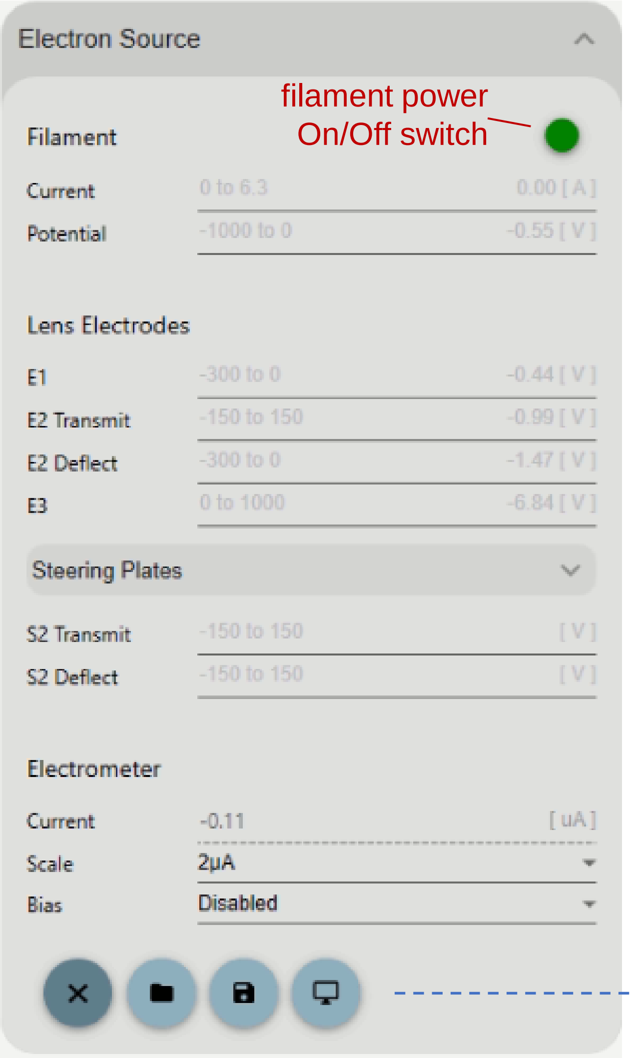
Time Config

| Parameter | Set Value in ms | Get Value |
|---|---|---|
| TurboPumpRS232ResponseTime | 27000 | 27000 |
| OnOff24VValveResponseTime | 500 | 500 |
| OrbiDelayVentSyncTime | 2000 | 2000 |
| ForeVacuumDelayStartupTime | 5000 | 5000 |
| ForeVacuumPumpingTime | 179200 | 179200 |
| TurboPumpPumpingTime | 5400000 | 5400000 |
| TurboPumpSpinDownTime | 1800000 | 1800000 |
| SystemVentTime | 60000 | 60000 |
| Electronics230VRelayResponseTime | 500 | 500 |
| Electronics24VRelayResponseTime | 500 | 500 |
| McuInterlockResponseTime | 5000 | 5000 |
| PurgeRecoveryTime | 10000 | 10000 |
| HiVacPresExceed_DTime | 6990 | 6990 |
| SpinDownPresExceed_DTime | 4995 | 4995 |

Pressure Cfg

| Gauge | Gauge Type | Set Threshold, Active Gauges | | | | | Get Thresholds | |
|---|---|---|---|---|---|---|---|---|
| Gauge 1 | WRG_S | Stopped Pres | 5.00 | e-5 mbar | N2 | ☐ | +5e-5 | ☐ |
| | | Hi Vacuum | 8.00 | e-5 mbar | N2 | ☐ | +8e-5 | ☐ |
| | | SpinDown Pres | 5.00 | e-3 mbar | N2 | ☐ | +5e-3 | ☐ |
| | | Fore Vacuum | 9.90 | e-2 mbar | N2 | ☐ | +9.9e-2 | ☐ |
| | | Atmosphere | 5.00 | e+2 mbar | N2 | ☐ | +5e2 | ☐ |
| Gauge 2 | WRG_S | Stopped Pres | 5.00 | e-5 mbar | N2 | ☑ | +5e-5 | ☑ |
| | | Hi Vacuum | 1.00 | e-4 mbar | N2 | ☑ | +1e-4 | ☑ |
| | | SpinDown Pres | 5.00 | e-3 mbar | N2 | ☑ | +5e-3 | ☑ |
| | | Fore Vacuum | 5.00 | e-1 mbar | N2 | ☑ | +5e-1 | ☑ |
| | | Atmosphere | 2.00 | e+2 mbar | N2 | ☑ | +2e2 | ☑ |
| Gauge 3 | WRG_S | Stopped Pres | 5.00 | e-5 mbar | N2 | ☐ | +5e-5 | ☐ |
| | | Hi Vacuum | 8.00 | e-5 mbar | N2 | ☐ | +8e-5 | ☐ |
| | | SpinDown Pres | 5.00 | e-3 mbar | N2 | ☐ | +5e-3 | ☐ |
| | | Fore Vacuum | 9.90 | e-2 mbar | N2 | ☐ | +9.9e-2 | ☐ |
| | | Atmosphere | 5.00 | e+2 mbar | N2 | ☐ | +5e2 | ☐ |
| Gauge 4 | WRG_S | Stopped Pres | 5.00 | e-5 mbar | N2 | ☐ | +5e-5 | ☐ |
| | | Hi Vacuum | 8.00 | e-5 mbar | N2 | ☐ | +8e-5 | ☐ |
| | | SpinDown Pres | 5.00 | e-3 mbar | N2 | ☐ | +5e-3 | ☐ |
| | | Fore Vacuum | 9.90 | e-2 mbar | N2 | ☐ | +9.9e-2 | ☐ |
| | | Atmosphere | 5.00 | e+2 mbar | N2 | ☐ | +5e2 | ☐ |
| Gauge 5 | WRG_S | Stopped Pres | 5.00 | e-5 mbar | N2 | ☐ | +5e-5 | ☐ |
| | | Hi Vacuum | 8.00 | e-5 mbar | N2 | ☐ | +8e-5 | ☐ |
| | | SpinDown Pres | 5.00 | e-3 mbar | N2 | ☐ | +5e-3 | ☐ |
| | | Fore Vacuum | 9.90 | e-2 mbar | N2 | ☐ | +9.9e-2 | ☐ |
| | | Atmosphere | 1.80 | e-6 mbar | N2 | ☐ | +1.8e-6 | ☐ |

# Electron Source module



Electron Source

filament power On/Off switch

Filament

| | | |
|---|---|---|
| Current | 0 to 6.3 | 0.00 [ A ] |
| Potential | -1000 to 0 | -0.55 [ V ] |

Lens Electrodes

| | | |
|---|---|---|
| E1 | -300 to 0 | -0.44 [ V ] |
| E2 Transmit | -150 to 150 | -0.99 [ V ] |
| E2 Deflect | -300 to 0 | -1.47 [ V ] |
| E3 | 0 to 1000 | -6.84 [ V ] |

Steering Plates

| | | |
|---|---|---|
| S2 Transmit | -150 to 150 | [ V ] |
| S2 Deflect | -150 to 150 | [ V ] |

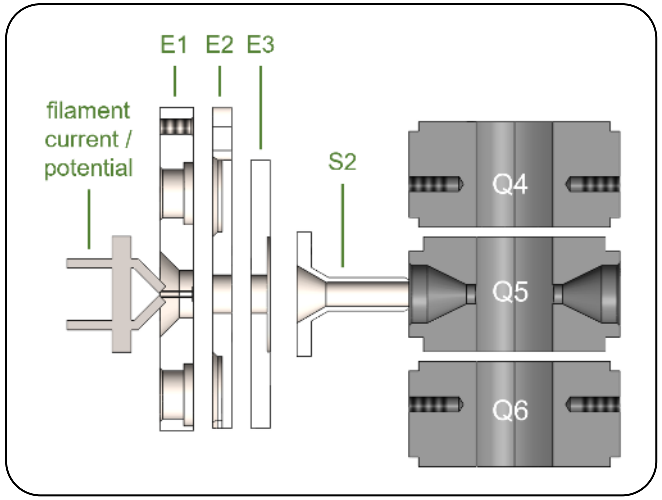
Electrometer

| | | |
|---|---|---|
| Current | -0.11 | [ uA ] |
| Scale | 2µA | |
| Bias | Disabled | |

- *Current* (A): Ignites the filament and controls operation temperature and emission current. Filament current limit is set at Settings → General tab
- *Filament Potential* (V): Defines the electron energy, along with Q5 DC voltage, where: $$E_{electron} = V_{Q5} - V_{filament}$$
- *Lens Electrodes E1*, *E2*, *E3* and *skimmer lens S2* are used to guide and focus the e$^-$ beam into the Q5 segment of the omnitrap.
- *Lens electrode E2* and *Skimmer S2* can take both transmit and deflect values.

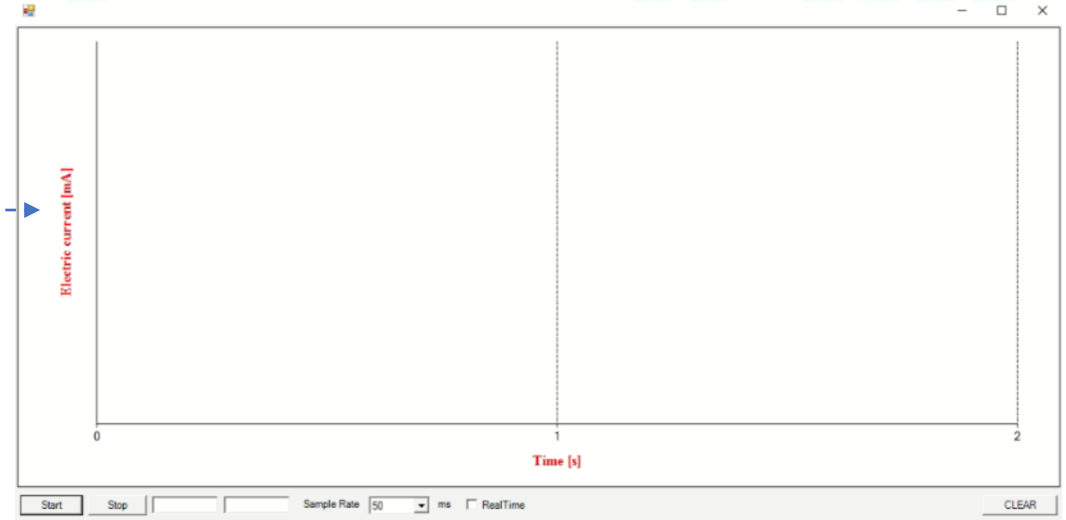
  - *Deflect Values* are applied when *EI Source* instruction state is set to OFF and used to deflect electrons in order not to enter the trap region.
  - *Transmit Values* are applied when *EI Source* instruction state is set to ON and allow electron irradiation of the ions in Q5.

- *Steering plates*: not available in this electron source version.



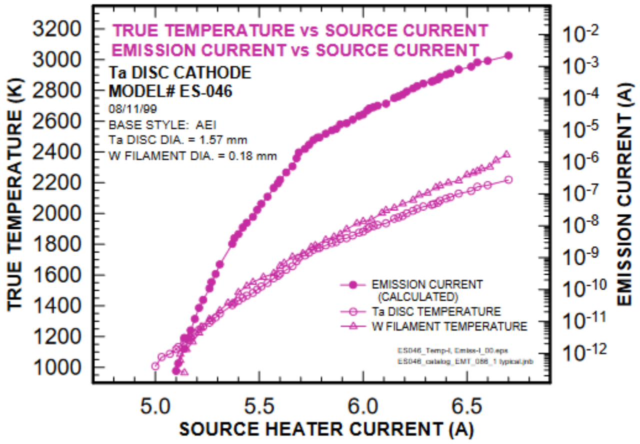
| | | | |
|---|---|---|---|
| DC State | Desc | Lift to react | Deflect |
| Delay | T [ms] | 5 | |
| EI Source | State | ON | Transmit |
| Delay | T [ms] | 50 | |
| EI Source | State | OFF | Deflect |
| Delay | T [ms] | 1 | |
| DC State | Desc | Confine Q5 | |
| Delay | T [ms] | 5 | |

An electrometer connected to skimmer lens S2 is used to monitor the emission current of the filament.



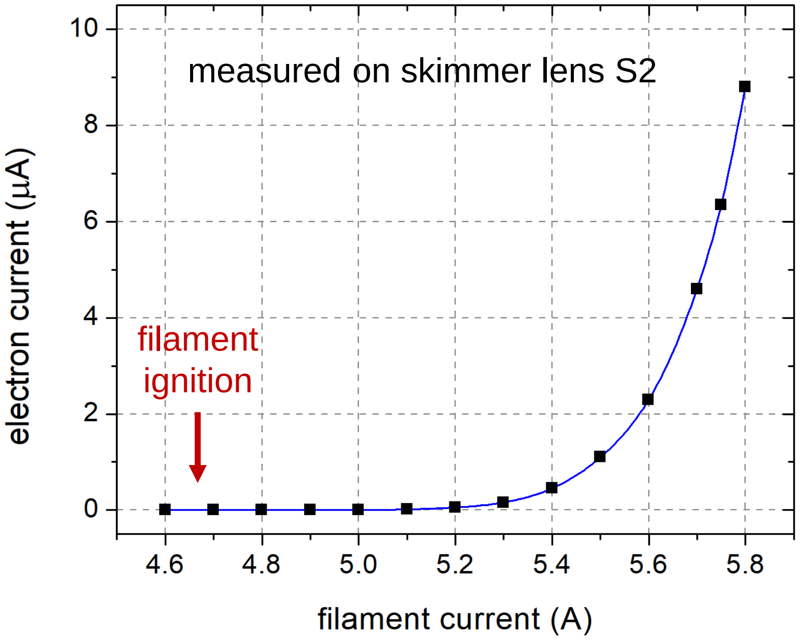
# Electron Source module: Filament Characterization Appendix 3

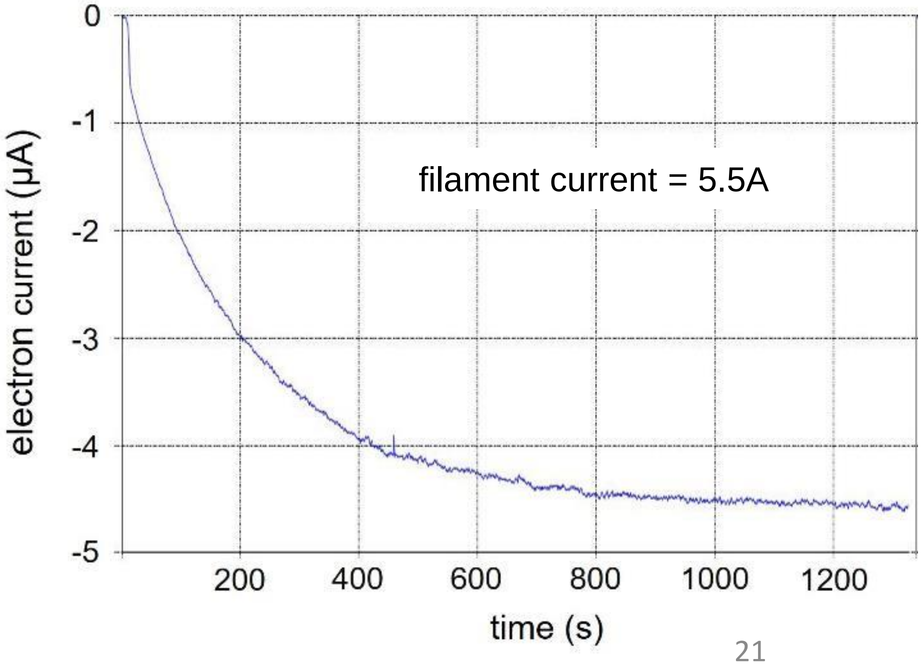
- Filament type: ES-046 Ta disc (from Kimball Physics)
- Turn-on: For the first time or if the filament has been exposed to air for a prolonged period, turn on filament power supply and gradually increase current to 5.5 amps to 5.7 amps while monitoring emission current and vacuum pressure. As the cathode heats up, small increases in vacuum pressure will most likely be noticed, due to out gassing. Maintain vacuum pressure at ~$10^{-5}$ torr or better. A filament that remains in vacuum or is exposed to air for a short time may be brought to the desired operating temperature almost instantly. Small changes in electron emission will occur during the first 20-30 minutes of operation, until thermal equilibrium is achieved.
- Turn-off: Heater current may be turned off slowly or instantly. Prior to venting, the filament and surrounding structure should be allowed to cool.
- Lifetime: Filament current should not exceed 6.3A to increase lifetime and avoid filament failure.



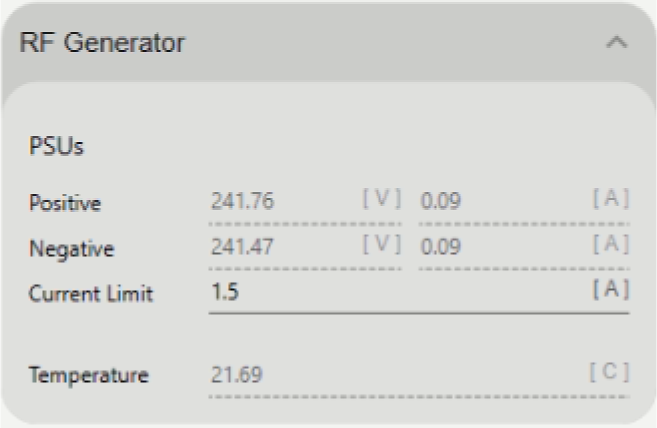
https://www.kimballphysics.com/downloadable/download/sample/sample_id/77/

Important note:
Always load the electron source ECD or EID settings before ignite the filament for warm up!





# RF Generator module



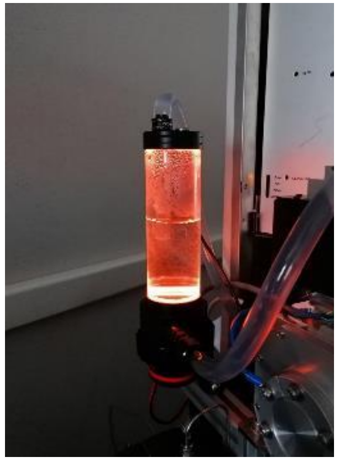
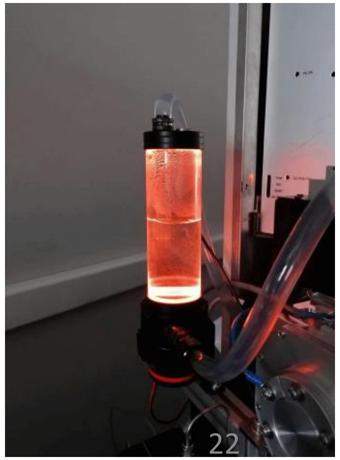
RF Generator

PSUs

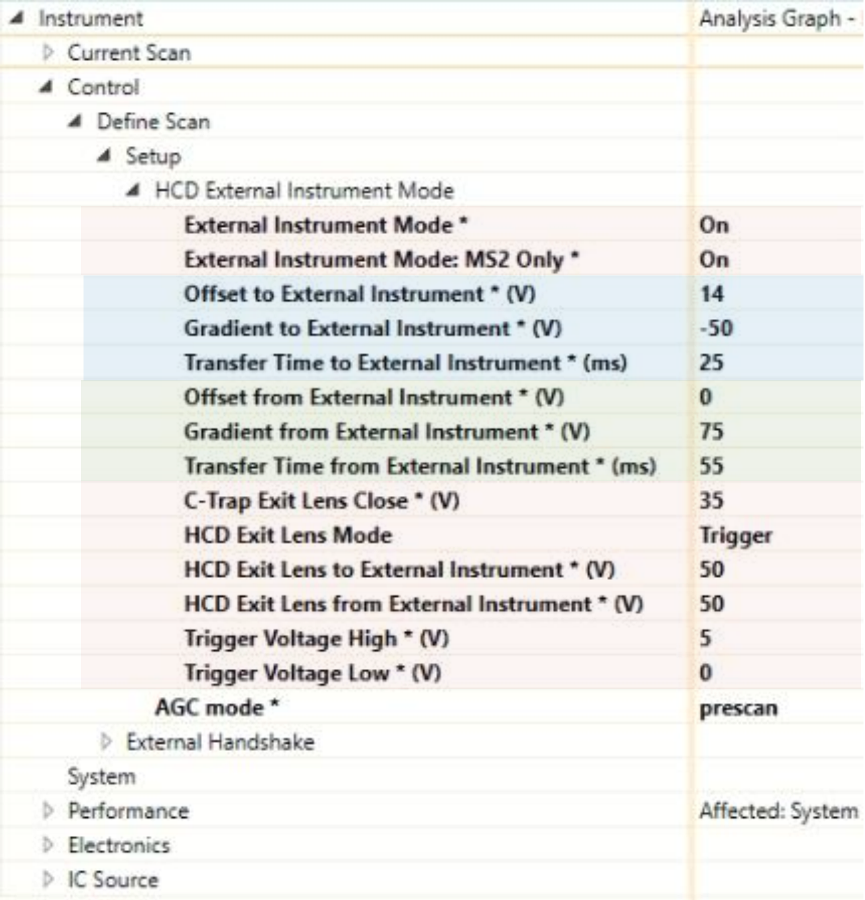
| | | | | |
|---|---|---|---|---|
| Positive | 241.76 | [V] | 0.09 | [A] |
| Negative | 241.47 | [V] | 0.09 | [A] |
| Current Limit | 1.5 | | | [A] |
| Temperature | 21.69 | | | [C] |

- *RF Amplitude* (V) (both positive and negative PSUs) is controlled by the "*RF Amplitude*" sequence instruction. Always set value at **250 V**.
- *Current Limit* (A) : default value set at 1.5A
- *Temperature* (°C) : The temperature of the RF Generator is continuously monitored and regulated by the water-cooling system.
- If temperature exceeds 42°C, a pop-up message appears to notify the user.
- If temperature exceeds 70°C, omnitrap automatically turns into "Stop" state, and all the electronics are switched off.

## LED COLOUR TEMPERATURE INDICATOR

| Temperature (°C) | 18 - 25 | 26 – 42 | 43 – 55 | 56 - 70 | 70 + |
|---|---|---|---|---|---|
| Water Tank Colour | blue | green | orange | red | blinking red |
| |  |  |  |  |  |

# Tune: External Instrument Mode (1/2)



| | |
|---|---|
| Instrument | Analysis Graph - |
| Current Scan | |
| Control | |
| Define Scan | |
| Setup | |
| HCD External Instrument Mode | |
| External Instrument Mode * | On |
| External Instrument Mode: MS2 Only * | On |
| Offset to External Instrument * (V) | 14 |
| Gradient to External Instrument * (V) | -50 |
| Transfer Time to External Instrument * (ms) | 25 |
| Offset from External Instrument * (V) | 0 |
| Gradient from External Instrument * (V) | 75 |
| Transfer Time from External Instrument * (ms) | 55 |
| C-Trap Exit Lens Close * (V) | 35 |
| HCD Exit Lens Mode | Trigger |
| HCD Exit Lens to External Instrument * (V) | 50 |
| HCD Exit Lens from External Instrument * (V) | 50 |
| Trigger Voltage High * (V) | 5 |
| Trigger Voltage Low * (V) | 0 |
| AGC mode * | prescan |
| External Handshake | |
| System | |
| Performance | Affected: System |
| Electronics | |
| IC Source | |

**External Instrument Mode** : This Mode is used for transferring ions out of the back of the HCD-cell to the Omnitrap, which is connected instead of the electrometer. Turning this feature "On" sends ions to the HCD-cell even during full scans with 3eV. In MS2 scans, the NCE or Direct eV setting defines the injection energy into the HCD-cell.

**External Instrument Mode: MS2 Only** : When External Instrume Mode: MS2 Only is "On", then this feature only applies to MS2 scans.

**Offset to External Instrument (V)** : Sets the voltage of the front the HCD-cell DC gradient when ions are transported to the Omnitrap.

**Gradient to External Instrument (V)** : Determines the gradient applied across the HCD-cell. This number, when added to the value of "Offset to External Instrument * (V)", is the voltage applied to the back of the HCD-cell DC gradient when ions are transported to the Omnitrap.

**Transfer Time to External Instrument (V)** : The time that the HCD-cell voltages are held to transport ions from the HCD-cell to the Omnitrap.

**Offset from External Instrument (V)** : Sets the voltage of the front the HCD-cell DC gradient when ions are returned to the HCD-cell from the Omnitrap.

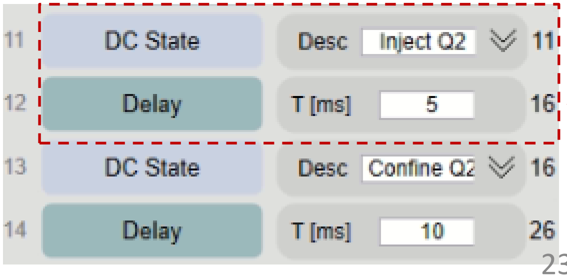
**Gradient from External Instrument (V)** : Determines the gradient applied across the HCD-cell. This number, when added to the value of "Offset from External Instrument * (V)", is the voltage applied to the back of the HCD-cell DC gradient when ions are returned to the HCD-cell from the Omnitrap.

**Transfer Time from External Instrument (V)** : The time that the HCD-cell voltages are held when ions are transported to the HCD-cell from the Omnitrap.

Omnitrap / Orbitrap Synchronization Rule:

***Transfer Time to Ext. Instrument ≥ Omnitrap Injection Q2 Time***

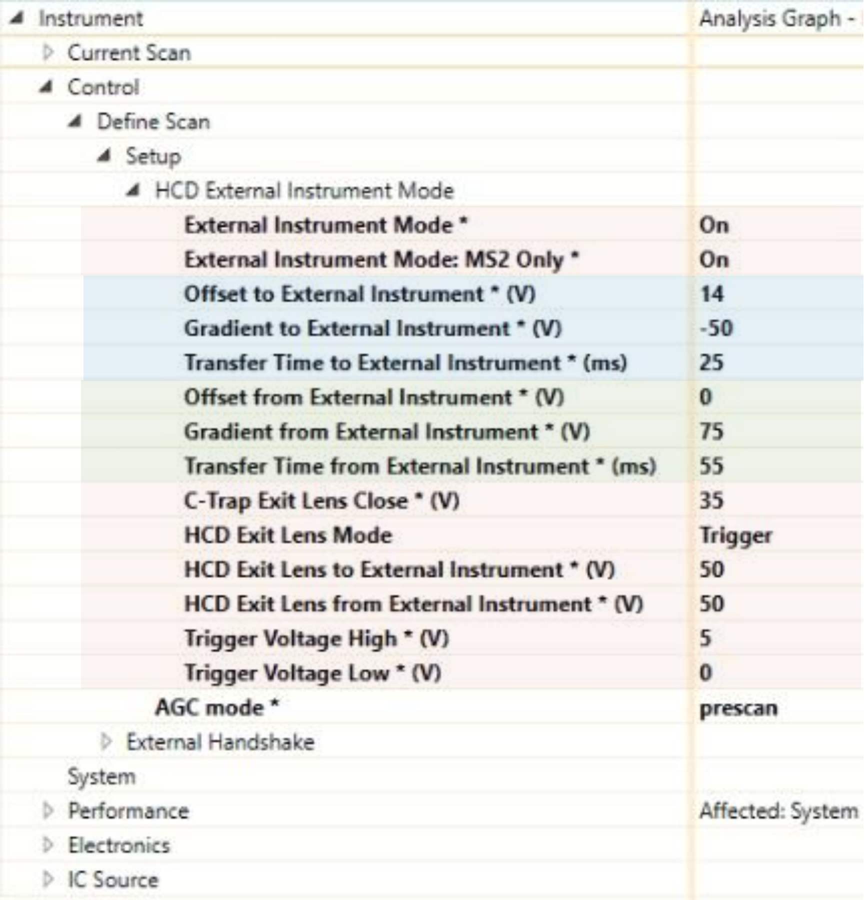
***Transfer Time to Ext. Instrument + Transfer Time from Ext. Instrument ≥ Omnitrap Total Sequence Time***



| | | | | |
|---|---|---|---|---|
| 11 | DC State | Desc | Inject Q2 | 11 |
| 12 | Delay | T [ms] | 5 | 16 |
| 13 | DC State | Desc | Confine Q2 | 16 |
| 14 | Delay | T [ms] | 10 | 26 |

Omnitrap Injection Q2 Time

# Tune: External Instrument Mode (2/2)



| | |
|---|---|
| Instrument | Analysis Graph - |
| Current Scan | |
| Control | |
| Define Scan | |
| Setup | |
| HCD External Instrument Mode | |
| External Instrument Mode * | On |
| External Instrument Mode: MS2 Only * | On |
| Offset to External Instrument * (V) | 14 |
| Gradient to External Instrument * (V) | -50 |
| Transfer Time to External Instrument * (ms) | 25 |
| Offset from External Instrument * (V) | 0 |
| Gradient from External Instrument * (V) | 75 |
| Transfer Time from External Instrument * (ms) | 55 |
| C-Trap Exit Lens Close * (V) | 35 |
| HCD Exit Lens Mode | Trigger |
| HCD Exit Lens to External Instrument * (V) | 50 |
| HCD Exit Lens from External Instrument * (V) | 50 |
| Trigger Voltage High * (V) | 5 |
| Trigger Voltage Low * (V) | 0 |
| AGC mode * | prescan |
| External Handshake | |
| System | |
| Performance | Affected: System |
| Electronics | |
| IC Source | |

C-Trap Exit Lens Close (V): Sets the voltage of the C-Trap Exit Lens during the transport of ions to and from the Omnitrap.

HCD Exit Lens Mode: Determines how the HCD Exit Lens behaves during HCD External Instrument Mode operation. In "Lens" mode, the "HCD Exit Lens to External Instrument * (V)" and "HCD Exit Lens from External Instrument * (V)" control the voltage. In "Trigger" mode, the "Trigger Voltage Low * (V)" and "Trigger Voltage High * (V)" control the voltage and create a 5 ms pulse when transfer to the Omnitrap starts.

HCD Exit Lens to Exteranl Instrument (V): The HCD Exit Lens voltage applied when ions are transported to the Omnitrap. For this value to be utilized, the "HCD Exit Lens Mode" must be set to "Lens".

HCD Exit Lens From External Instrument (V): The HCD Exit Lens voltage applied when ions are transported to the HCD-cell from the Omnitrap. For this value to be utilized, the "HCD Exit Lens Mode" must be set to "Lens".

Trigger Voltace High (V): Sets the voltage of the HCD Exit Lens for the first 5 ms of the "Transfer Time to External Instrument * (ms)". After 5 ms, the voltage is switched back to the value defined by "Trigger Voltage Low * (V)". For this value to be utilized, the "HCD Exit Lens Mode" must be set to "Trigger".

Trigger Voltace Low (V): Sets the voltage of the HCD Exit Lens after the first 5 ms of ions being transported to the Omnitrap. For this value to be utilized, the "HCD Exit Lens Mode" must be set to "Trigger".

**Off-line method for MS2 experiments (Large Molecules):**

1. Spray analyte.
2. Determine optimal injection time in Full MS mode with AGC Mode: “Prescan”.
3. Switch to MS2 mode and AGC Mode: "Fixed"; set injection time to the optimal determined in step 2.

For small molecules, you can run in prescan mode for either Full MS or MS2.

Appendix 3

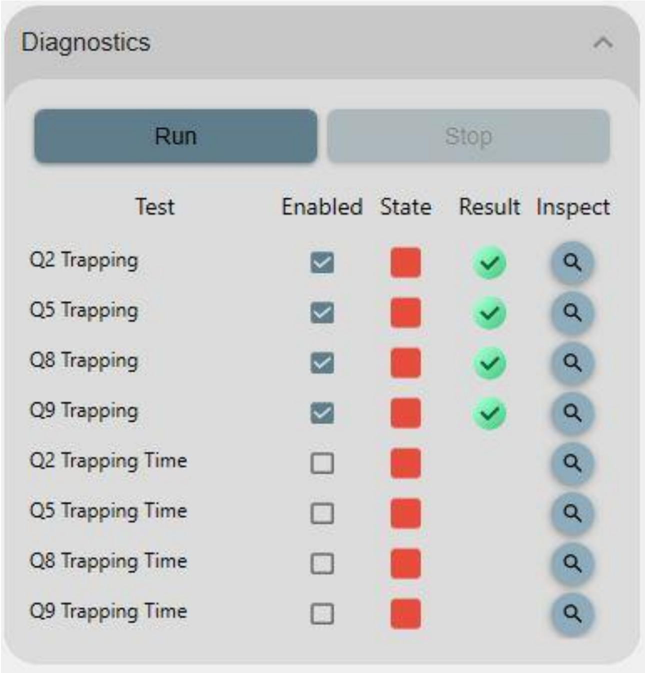
## Omnitrap Diagnostics

- Trapping tests Q2/Q5/Q8/Q9
- Trapping Time tests Q2/Q5/Q8/Q9

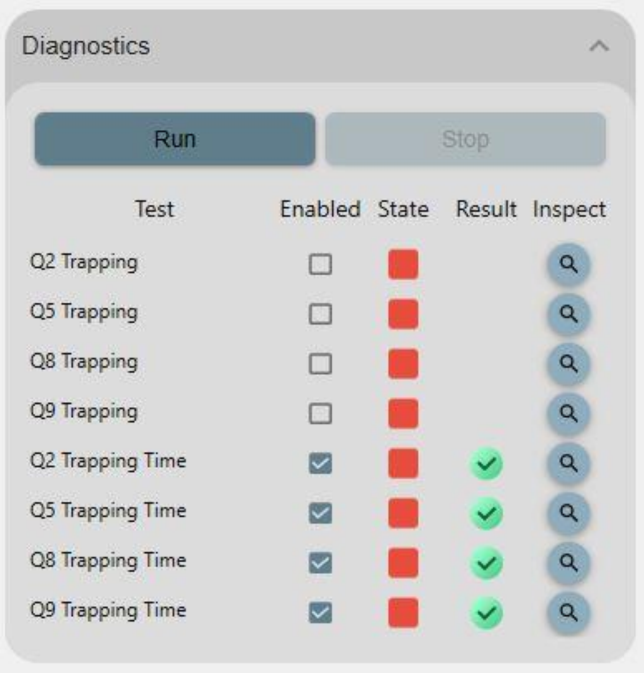
# Diagnostics modure: Trapping Tests

- Trapping tests are performed on segments Q2, Q5, Q8 and Q9 of the omnitrap in order to ensure that all injected ions are successfully transfer and trapped at all omnitrap segments.
- Additionally, trapping time tests are performed to check the trapping efficiency on different omnitrap segment during time.

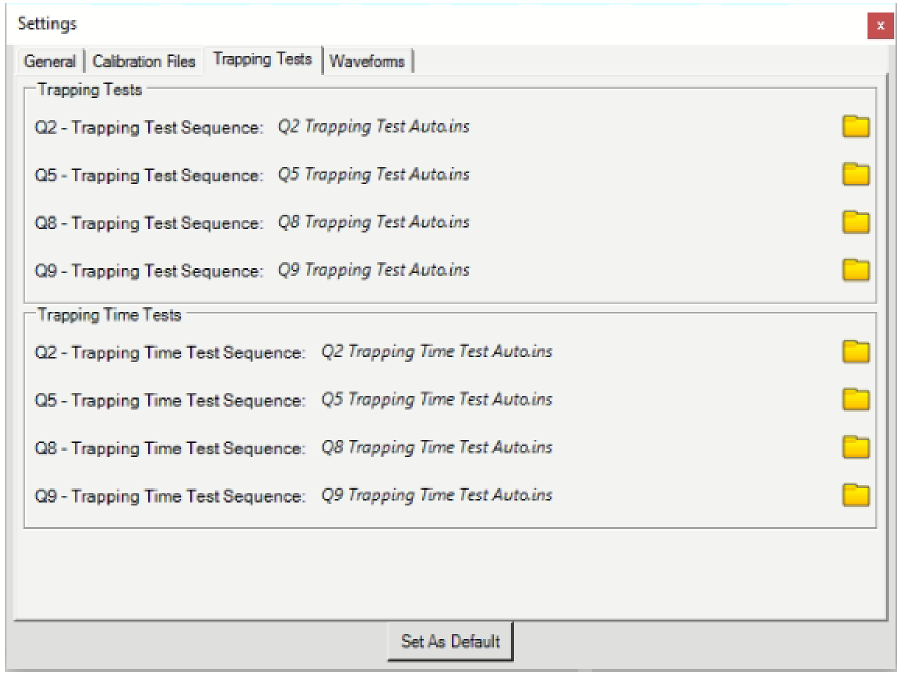
The sequence files that are assigned for every diagnostic test can be set on the settings menu/Trapping Tests tab.



| Test | Enabled | State | Result | Inspect |
|---|---|---|---|---|
| Q2 Trapping | ☑ | 🟥 | ✓ | 🔍 |
| Q5 Trapping | ☑ | 🟥 | ✓ | 🔍 |
| Q8 Trapping | ☑ | 🟥 | ✓ | 🔍 |
| Q9 Trapping | ☑ | 🟥 | ✓ | 🔍 |
| Q2 Trapping Time | ☐ | 🟥 |  | 🔍 |
| Q5 Trapping Time | ☐ | 🟥 |  | 🔍 |
| Q8 Trapping Time | ☐ | 🟥 |  | 🔍 |
| Q9 Trapping Time | ☐ | 🟥 |  | 🔍 |



| Test | Enabled | State | Result | Inspect |
|---|---|---|---|---|
| Q2 Trapping | ☐ | 🟥 |  | 🔍 |
| Q5 Trapping | ☐ | 🟥 |  | 🔍 |
| Q8 Trapping | ☐ | 🟥 |  | 🔍 |
| Q9 Trapping | ☐ | 🟥 |  | 🔍 |
| Q2 Trapping Time | ☑ | 🟥 | ✓ | 🔍 |
| Q5 Trapping Time | ☑ | 🟥 | ✓ | 🔍 |
| Q8 Trapping Time | ☑ | 🟥 | ✓ | 🔍 |
| Q9 Trapping Time | ☑ | 🟥 | ✓ | 🔍 |



Trapping Tests

Q2 - Trapping Test Sequence: *Q2 Trapping Test Auto.ins*
Q5 - Trapping Test Sequence: *Q5 Trapping Test Auto.ins*
Q8 - Trapping Test Sequence: *Q8 Trapping Test Auto.ins*
Q9 - Trapping Test Sequence: *Q9 Trapping Test Auto.ins*

Trapping Time Tests

Q2 - Trapping Time Test Sequence: *Q2 Trapping Time Test Auto.ins*
Q5 - Trapping Time Test Sequence: *Q5 Trapping Time Test Auto.ins*
Q8 - Trapping Time Test Sequence: *Q8 Trapping Time Test Auto.ins*
Q9 - Trapping Time Test Sequence: *Q9 Trapping Time Test Auto.ins*

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Q2 Trapping Test

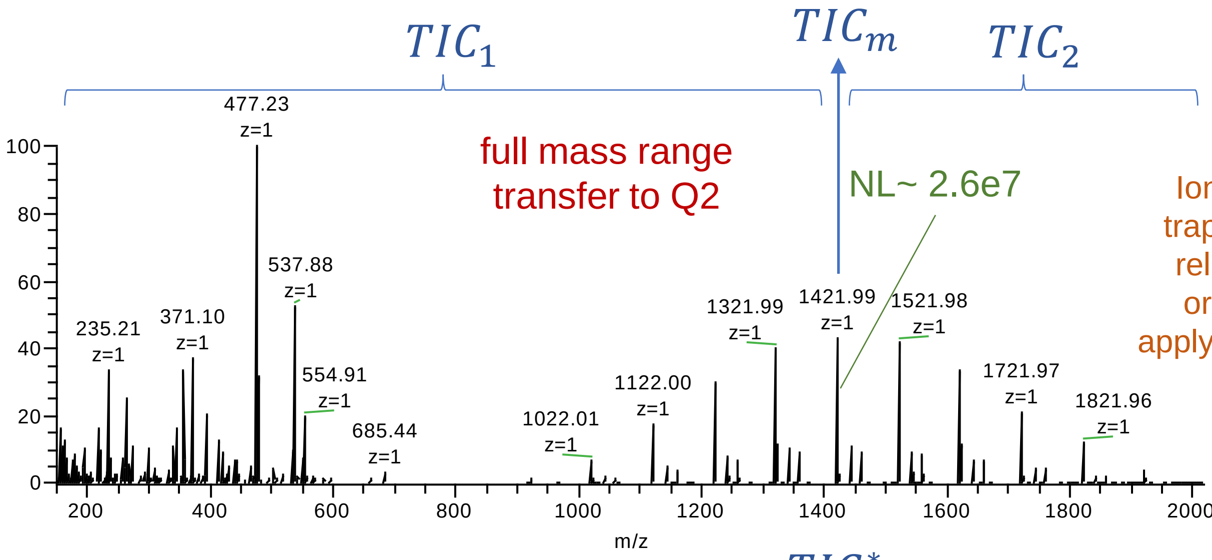
Appendix 3

- Ion trapping in segment Q2 is diagnosed by transferring and trapping ions to Q2, followed by a resolving DC signal applied to successfully isolate a single ion mass distribution, e.g., m/z=1422.
- Pass Criterion: The following conditions should be fulfilled:

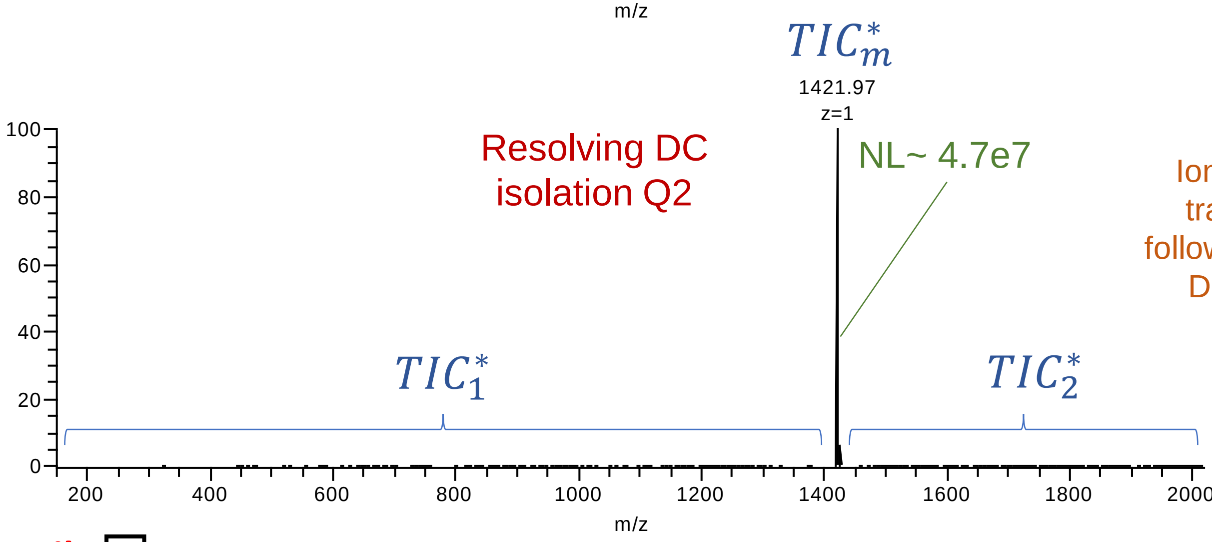
$$\frac{TIC_m^*}{TIC_m} > 0.9 \quad \& \quad \frac{(TIC_1^* + TIC_2^*)}{(TIC_1 + TIC_2)} < 0.01$$

*TIC*: total ion current

- Variable parameters of resolving DC isolation:
  1) RF frequency
  2) Resolving DC voltage (Q2)
- Values alternate between two sequence states:
  i) full mass range transfer to Q2
  ii) single ion mass isolation to Q2



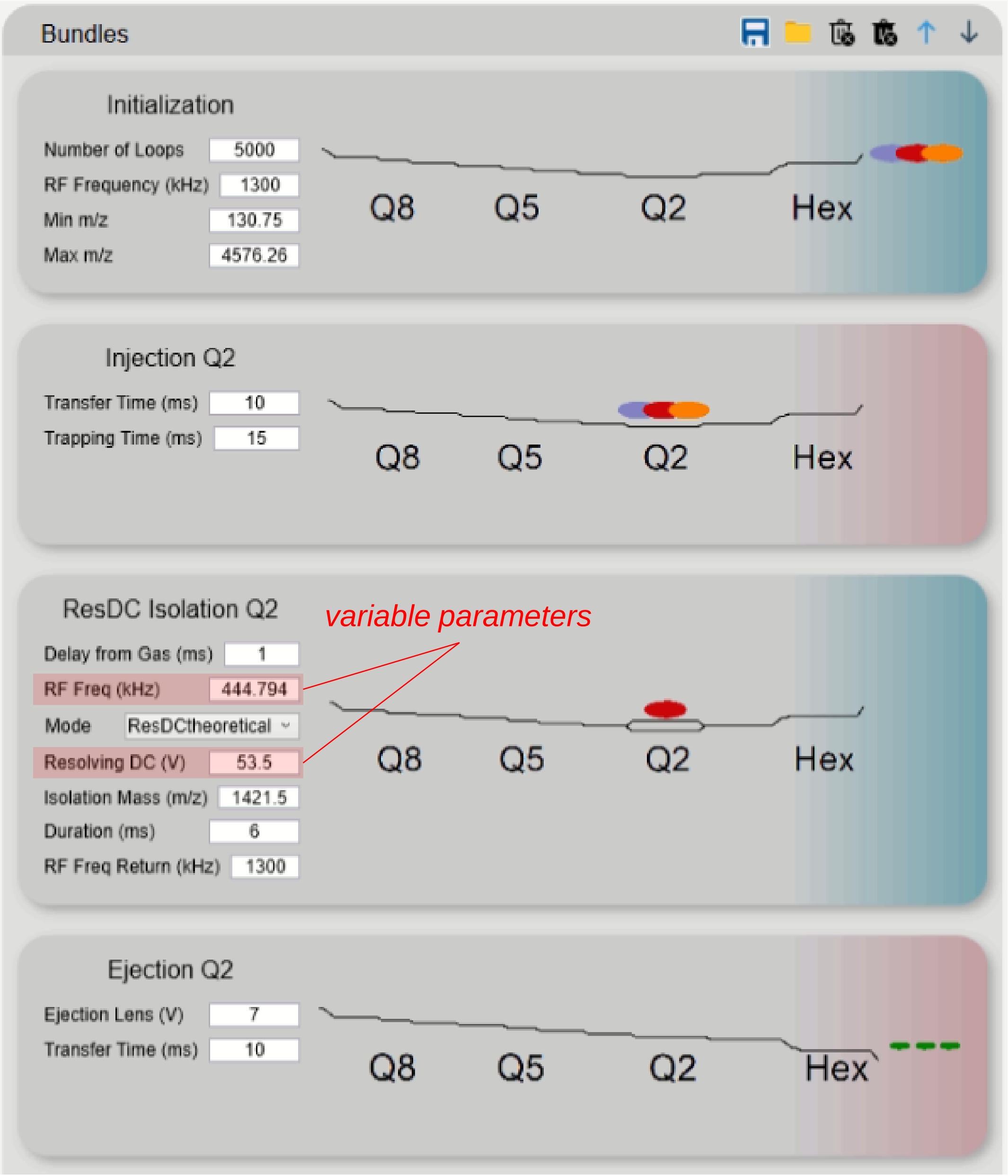
Ions transferred, trapped to Q2 and released back to orbitrap without applying resolving DC isolation.



Ions transferred, trapped to Q2 , followed by resolving DC isolation of m/z = 1422

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Q2 Trapping Test



Bundles

Initialization

Number of Loops 5000
RF Frequency (kHz) 1300
Min m/z 130.75
Max m/z 4576.26

Injection Q2

Transfer Time (ms) 10
Trapping Time (ms) 15

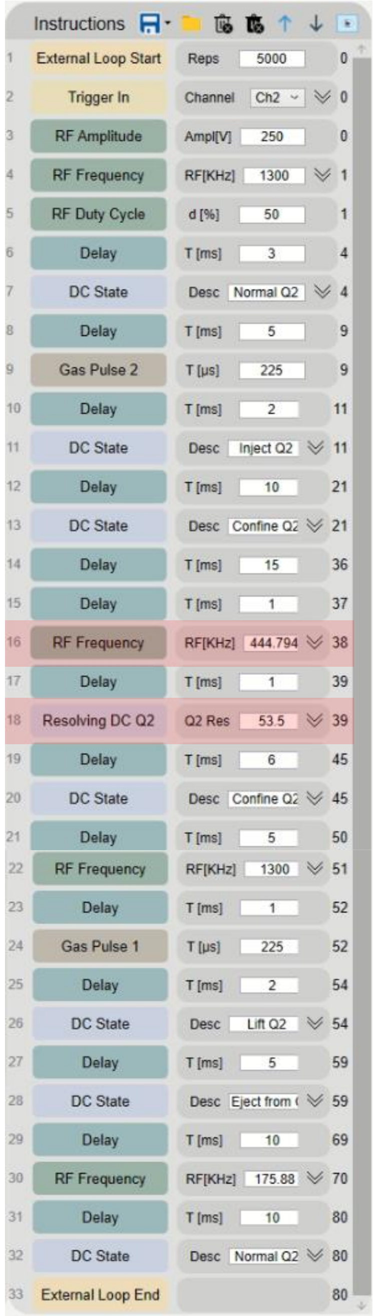
ResDC Isolation Q2

*variable parameters*

Delay from Gas (ms) 1
RF Freq (kHz) 444.794
Mode ResDCtheoretical
Resolving DC (V) 53.5
Isolation Mass (m/z) 1421.5
Duration (ms) 6
RF Freq Return (kHz) 1300

Ejection Q2

Ejection Lens (V) 7
Transfer Time (ms) 10



Instructions

| | | | | |
|---|---|---|---|---|
| 1 | External Loop Start | Reps | 5000 | 0 |
| 2 | Trigger In | Channel | Ch2 | 0 |
| 3 | RF Amplitude | Ampl[V] | 250 | 0 |
| 4 | RF Frequency | RF[KHz] | 1300 | 1 |
| 5 | RF Duty Cycle | d [%] | 50 | 1 |
| 6 | Delay | T [ms] | 3 | 4 |
| 7 | DC State | Desc | Normal Q2 | 4 |
| 8 | Delay | T [ms] | 5 | 9 |
| 9 | Gas Pulse 2 | T [µs] | 225 | 9 |
| 10 | Delay | T [ms] | 2 | 11 |
| 11 | DC State | Desc | Inject Q2 | 11 |
| 12 | Delay | T [ms] | 10 | 21 |
| 13 | DC State | Desc | Confine Q2 | 21 |
| 14 | Delay | T [ms] | 15 | 36 |
| 15 | Delay | T [ms] | 1 | 37 |
| 16 | RF Frequency | RF[KHz] | 444.794 | 38 |
| 17 | Delay | T [ms] | 1 | 39 |
| 18 | Resolving DC Q2 | Q2 Res | 53.5 | 39 |
| 19 | Delay | T [ms] | 6 | 45 |
| 20 | DC State | Desc | Confine Q2 | 45 |
| 21 | Delay | T [ms] | 5 | 50 |
| 22 | RF Frequency | RF[KHz] | 1300 | 51 |
| 23 | Delay | T [ms] | 1 | 52 |
| 24 | Gas Pulse 1 | T [µs] | 225 | 52 |
| 25 | Delay | T [ms] | 2 | 54 |
| 26 | DC State | Desc | Lift Q2 | 54 |
| 27 | Delay | T [ms] | 5 | 59 |
| 28 | DC State | Desc | Eject from ( | 59 |
| 29 | Delay | T [ms] | 10 | 69 |
| 30 | RF Frequency | RF[KHz] | 175.88 | 70 |
| 31 | Delay | T [ms] | 10 | 80 |
| 32 | DC State | Desc | Normal Q2 | 80 |
| 33 | External Loop End | | | 80 |

- Sequence file path/name:
  …*Diagnostics\Trapping Tests\Q2 Trapping Test Auto.ins*

- Variable parameters of resolving DC isolation:
  1) RF frequency
  2) Resolving DC voltage (Q2)

- Values alternate between two sequence states:
  i) full mass range tranfer to Q2
  ii) single ion mass isolation to Q2

| Sequence state | RF frequency | Resolving DC |
|---|---|---|
| full mass range transfer | $f_{initial}$ (=1300 kHz) | 0 V |
| single ion mass (m/z) isolation | $f_{m/z\ @\ q=0.55}$ * | 53.0 V |

* e.g., m/z = 1422 → f ≈ 444 kHz

# Q5 Trapping Test

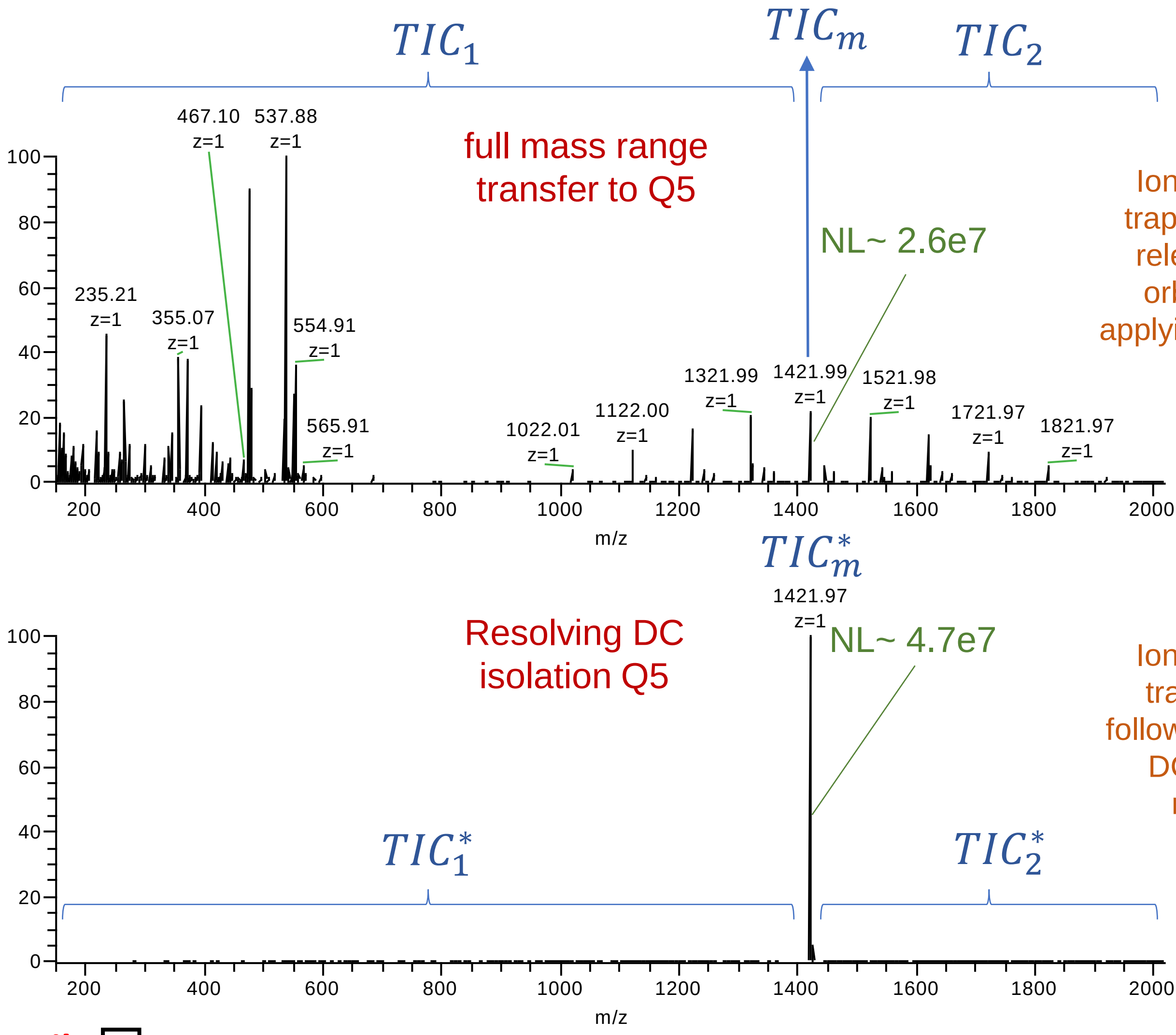
Appendix 3

- Ion trapping in segment Q5 is diagnosed by transferring and trapping ions to Q5, followed by a resolving DC signal applied to successfully isolate a single ion mass distribution, e.g., m/z=1422.
- <u>Pass Criterion</u>: The following conditions should be fulfilled:

$$\frac{TIC_m^*}{TIC_m} > 0.9 \quad \& \quad \frac{(TIC_1^* + TIC_2^*)}{(TIC_1 + TIC_2)} < 0.01$$

*TIC*: total ion current

- Variable parameters of resolving DC isolation:
  1) RF frequency
  2) Resolving DC voltage (Q5)
- Values alternate between two sequence states:
  i) full mass range transfer to Q5
  ii) single ion mass isolation to Q5

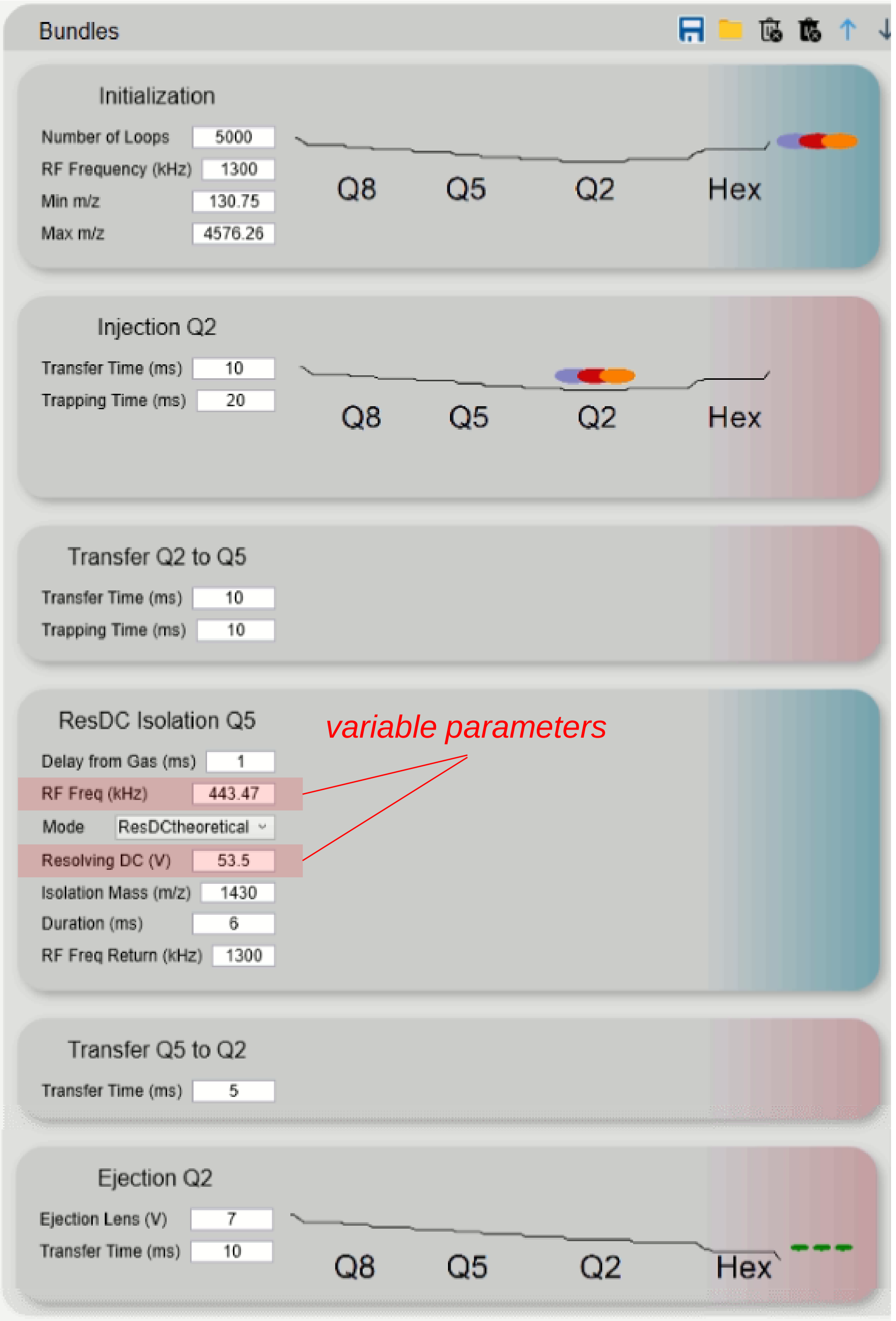


Ions transferred, trapped to Q5 and released back to orbitrap without applying resolving DC isolation.

Ions transferred, trapped to Q5 , followed by resolving DC isolation of m/z = 1422

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Q5 Trapping Test



**Initialization**
Number of Loops 5000
RF Frequency (kHz) 1300
Min m/z 130.75
Max m/z 4576.26

**Injection Q2**
Transfer Time (ms) 10
Trapping Time (ms) 20

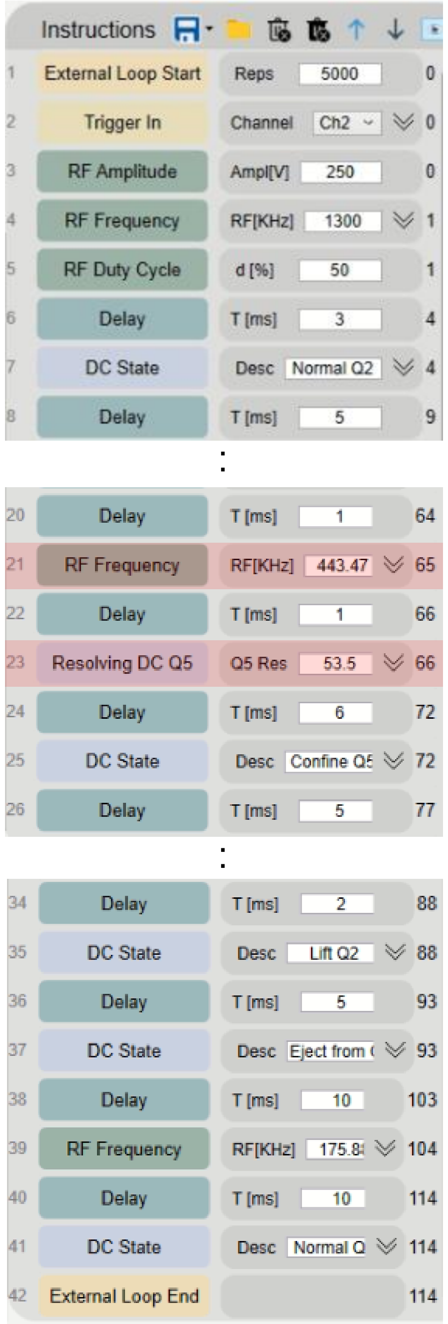
**Transfer Q2 to Q5**
Transfer Time (ms) 10
Trapping Time (ms) 10

**ResDC Isolation Q5**
Delay from Gas (ms) 1
RF Freq (kHz) 443.47
Mode ResDCtheoretical
Resolving DC (V) 53.5
Isolation Mass (m/z) 1430
Duration (ms) 6
RF Freq Return (kHz) 1300

*variable parameters*

**Transfer Q5 to Q2**
Transfer Time (ms) 5

**Ejection Q2**
Ejection Lens (V) 7
Transfer Time (ms) 10



| | | | | |
|---|---|---|---|---|
| 1 | External Loop Start | Reps | 5000 | 0 |
| 2 | Trigger In | Channel | Ch2 | 0 |
| 3 | RF Amplitude | Ampl[V] | 250 | 0 |
| 4 | RF Frequency | RF[KHz] | 1300 | 1 |
| 5 | RF Duty Cycle | d [%] | 50 | 1 |
| 6 | Delay | T [ms] | 3 | 4 |
| 7 | DC State | Desc | Normal Q2 | 4 |
| 8 | Delay | T [ms] | 5 | 9 |
| : | | | | |
| 20 | Delay | T [ms] | 1 | 64 |
| 21 | RF Frequency | RF[KHz] | 443.47 | 65 |
| 22 | Delay | T [ms] | 1 | 66 |
| 23 | Resolving DC Q5 | Q5 Res | 53.5 | 66 |
| 24 | Delay | T [ms] | 6 | 72 |
| 25 | DC State | Desc | Confine Q5 | 72 |
| 26 | Delay | T [ms] | 5 | 77 |
| : | | | | |
| 34 | Delay | T [ms] | 2 | 88 |
| 35 | DC State | Desc | Lift Q2 | 88 |
| 36 | Delay | T [ms] | 5 | 93 |
| 37 | DC State | Desc | Eject from | 93 |
| 38 | Delay | T [ms] | 10 | 103 |
| 39 | RF Frequency | RF[KHz] | 175.8 | 104 |
| 40 | Delay | T [ms] | 10 | 114 |
| 41 | DC State | Desc | Normal Q | 114 |
| 42 | External Loop End | | | 114 |

- Sequence file path/name:
  *…\Diagnostics\Trapping Tests\Q5 Trapping Test Auto.ins*

- Variable parameters of resolving DC isolation:
  1) RF frequency
  2) Resolving DC voltage (Q5)

- Values alternate between two sequence states:
  i) full mass range tranfer to Q5
  ii) single ion mass isolation to Q5

| Sequence state | RF frequency | Resolving DC |
|---|---|---|
| full mass range transfer | $f_{initial}$ (=1300 kHz) | 0 V |
| single ion mass (m/z) isolation | $f_{m/z\ @\ q=0.55}$ * | 52.8 V |

* e.g., m/z = 1422 → f ≈ 441 kHz

# Q8 Trapping Test

- Ion trapping in segment Q8 is diagnosed by transferring ions to Q8 and trapping them using segments Q6, Q7 and Q9. This causes total ion signal loss in Orbitrap detector. Releasing ions by “opening” segments Q6 and Q7 results in a full mass range spectrum.

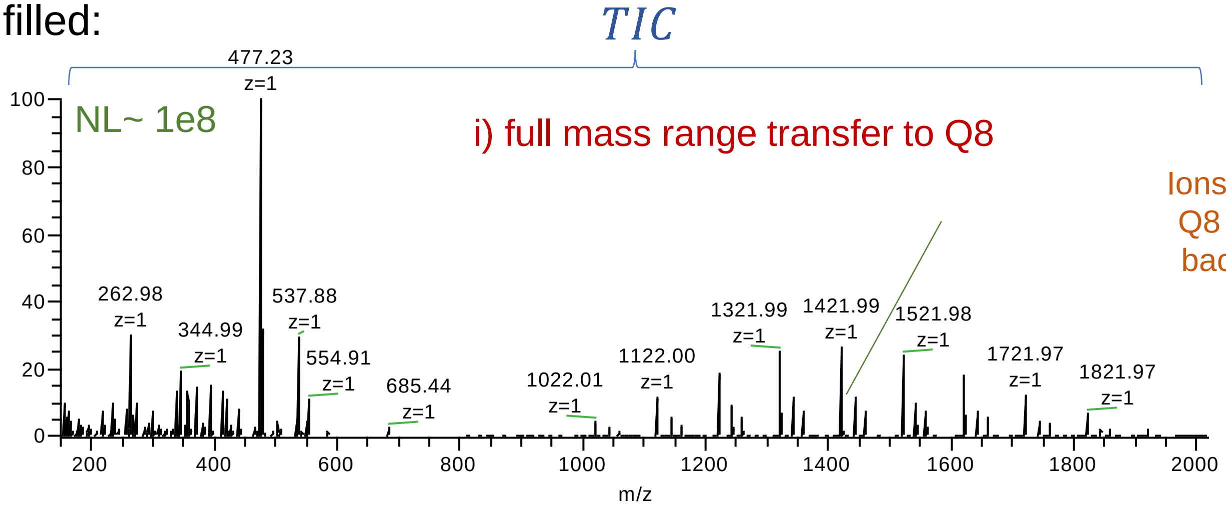
- Pass Criterion: The following condition should be fulfilled:

$$\frac{TIC^*}{TIC} < 0.001$$

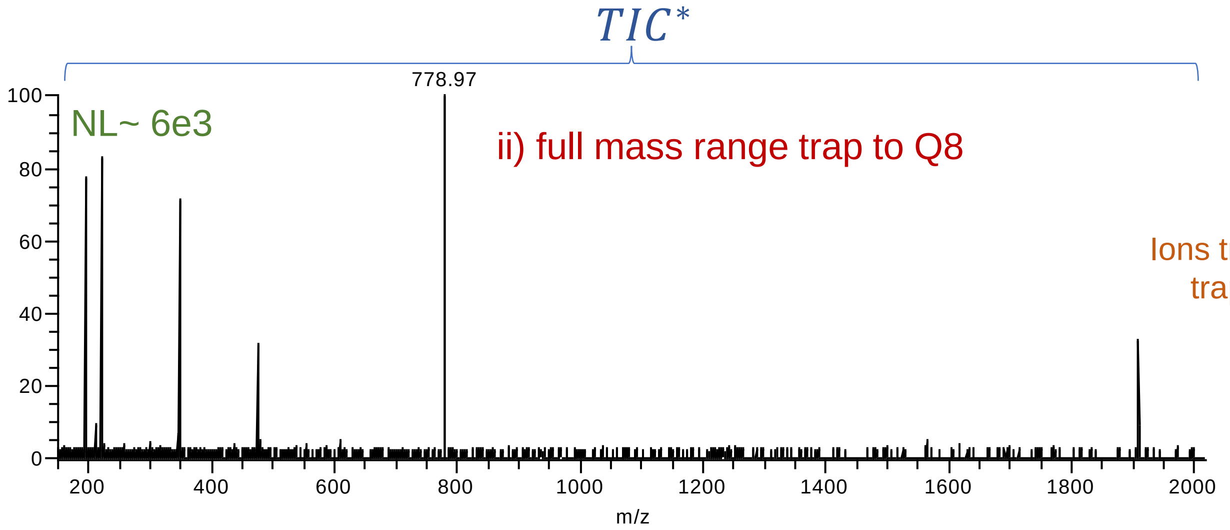
*TIC*: total ion current

- Variable parameters of resolving DC isolation:
  1) Q6 DC voltage
  2) Q7 DC voltage

- Values alternate between two sequence states:
  i) full mass range transfer to Q8
  ii) full mass range trap to Q8



Ions transferred to Q8 and released back to orbitrap.

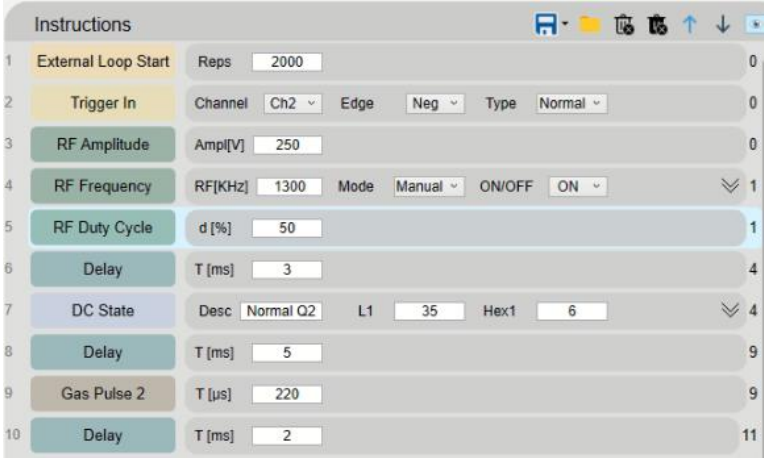


Ions transferred and trapped to Q8.

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Q8 Trapping Test

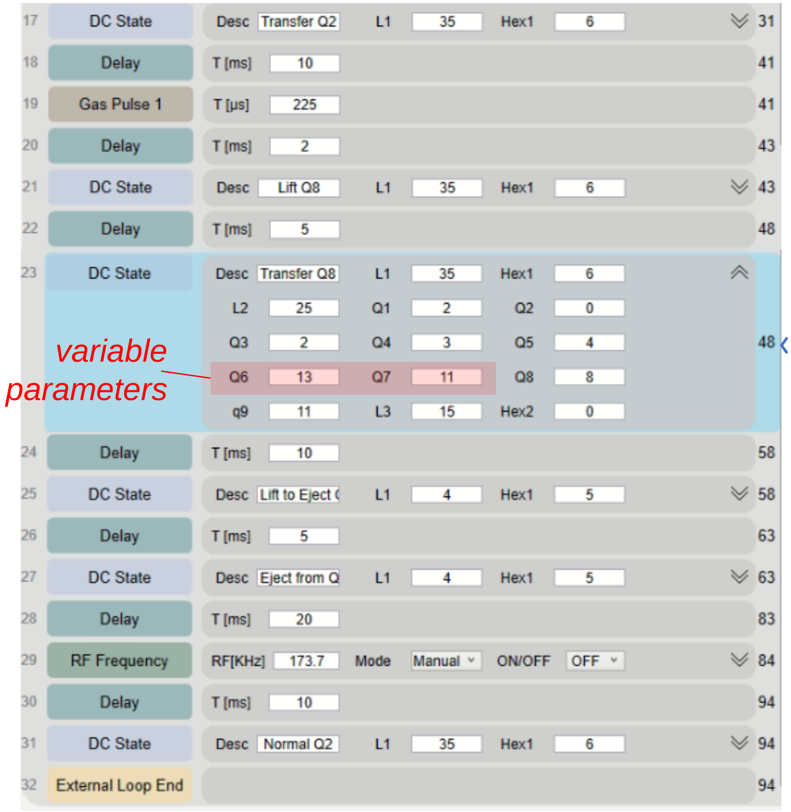
Appendix 3



Instructions

1 External Loop Start — Reps 2000 — 0
2 Trigger In — Channel Ch2, Edge Neg, Type Normal — 0
3 RF Amplitude — Ampl[V] 250 — 0
4 RF Frequency — RF[KHz] 1300, Mode Manual, ON/OFF ON — 1
5 RF Duty Cycle — d [%] 50 — 1
6 Delay — T [ms] 3 — 4
7 DC State — Desc Normal Q2, L1 35, Hex1 6 — 4
8 Delay — T [ms] 5 — 9
9 Gas Pulse 2 — T [µs] 220 — 9
10 Delay — T [ms] 2 — 11

:



17 DC State — Desc Transfer Q2, L1 35, Hex1 6 — 31
18 Delay — T [ms] 10 — 41
19 Gas Pulse 1 — T [µs] 225 — 41
20 Delay — T [ms] 2 — 43
21 DC State — Desc Lift Q8, L1 35, Hex1 6 — 43
22 Delay — T [ms] 5 — 48
23 DC State — Desc Transfer Q8, L1 35, Hex1 6, L2 25, Q1 2, Q2 0, Q3 2, Q4 3, Q5 4, Q6 13, Q7 11, Q8 8, q9 11, L3 15, Hex2 0 — 48
24 Delay — T [ms] 10 — 58
25 DC State — Desc Lift to Eject (, L1 4, Hex1 5 — 58
26 Delay — T [ms] 5 — 63
27 DC State — Desc Eject from Q, L1 4, Hex1 5 — 63
28 Delay — T [ms] 20 — 83
29 RF Frequency — RF[KHz] 173.7, Mode Manual, ON/OFF OFF — 84
30 Delay — T [ms] 10 — 94
31 DC State — Desc Normal Q2, L1 35, Hex1 6 — 94
32 External Loop End — 94

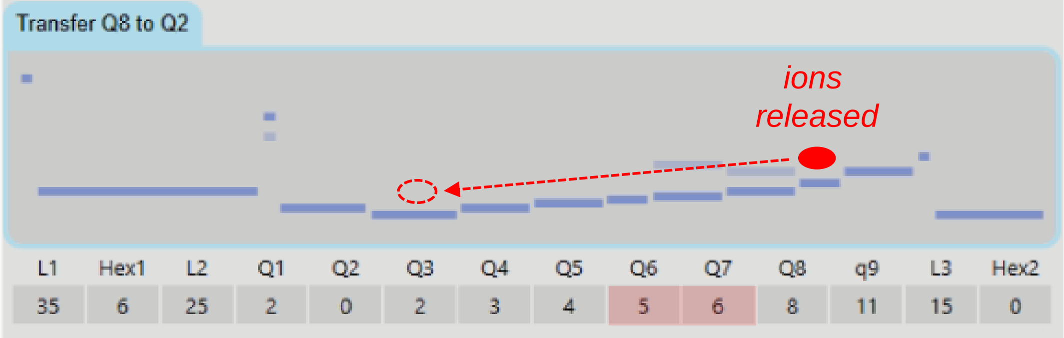
*variable parameters*

- Sequence file path/name:
  *…\Diagnostics\Trapping Tests\Q8 Trapping Test Auto.ins*

- Variable parameters of "Transfer Q8 to Q2" DC state:
  1) Q6 DC voltage
  2) Q7 DC voltage

- Values alternate between two sequence states:

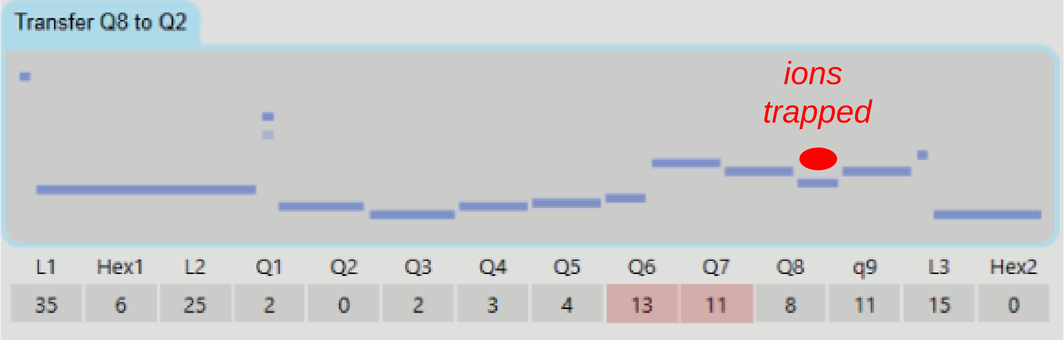
i) full mass range transfer to Q8



Transfer Q8 to Q2

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 25 | 2 | 0 | 2 | 3 | 4 | 5 | 6 | 8 | 11 | 15 | 0 |

ii) full mass range trap to Q8



Transfer Q8 to Q2

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 25 | 2 | 0 | 2 | 3 | 4 | 13 | 11 | 8 | 11 | 15 | 0 |

| sequence state | Q6 | Q7 |
|---|---|---|
| transfer | 5 V | 6 V |
| trap | 13V | 11 V |

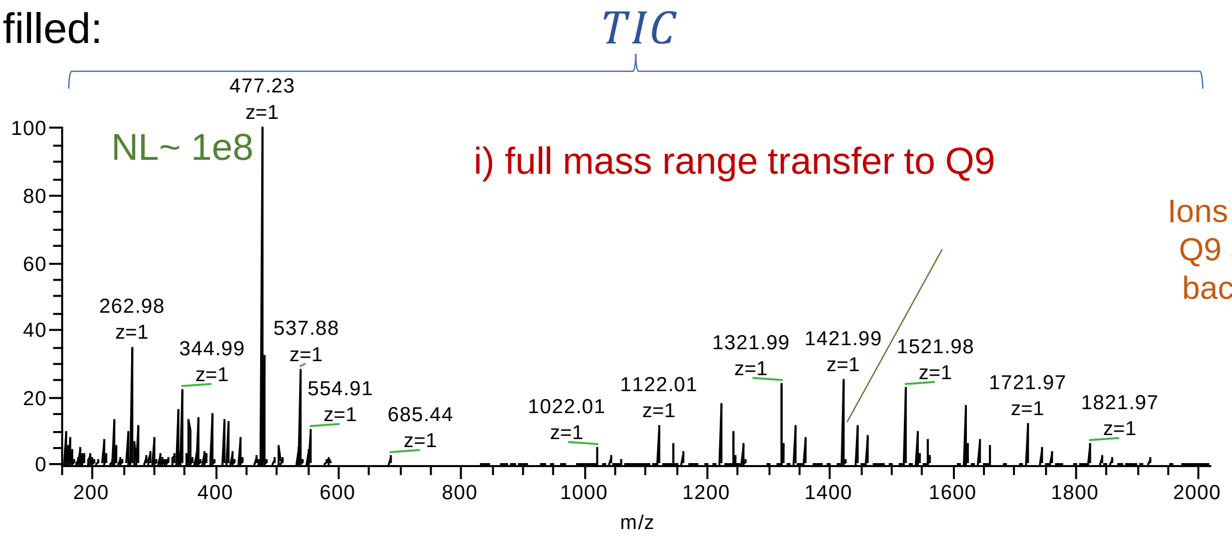
# Q9 Trapping Test

- Ion trapping in segment Q9 is diagnosed by transferring ions to Q9 and trapping them using segments Q7, Q8 and lens L3. This causes total ion signal loss in Orbitrap detector. Releasing ions by “opening” segments Q7 and Q8 results in a full mass range spectrum.
- Pass Criterion: The following condition should be fulfilled:

$$\frac{TIC^*}{TIC} < 0.001$$

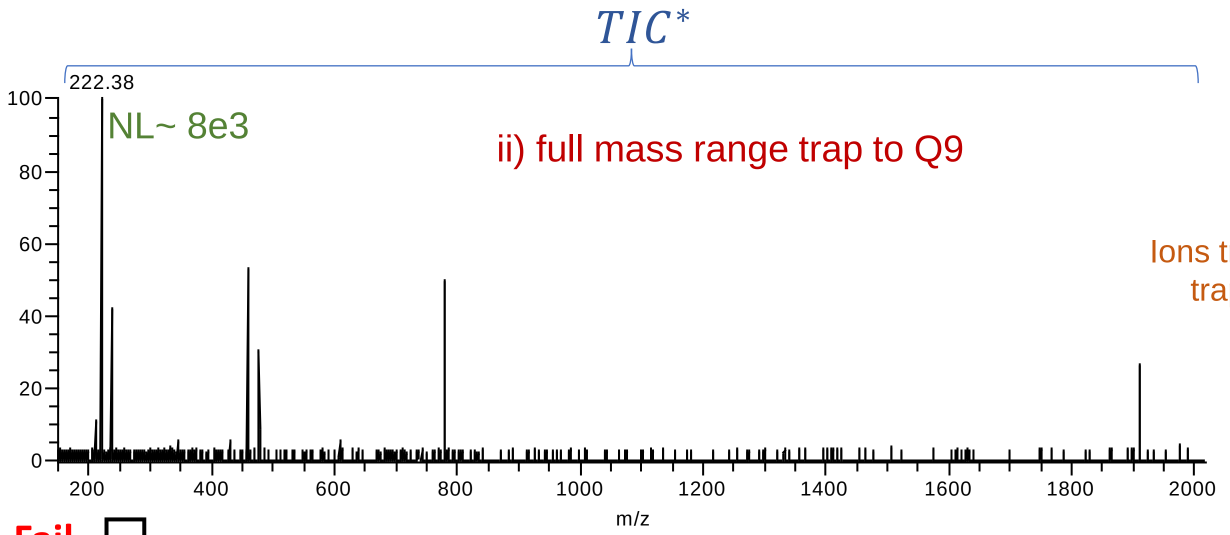
*TIC*: total ion current

- Variable parameters of resolving DC isolation:
  1) Q7 DC voltage
  2) Q8 DC voltage
- Values alternate between two sequence states:
  i) full mass range transfer to Q8
  ii) full mass range trap to Q8



i) full mass range transfer to Q9

Ions transferred to Q9 and released back to orbitrap.



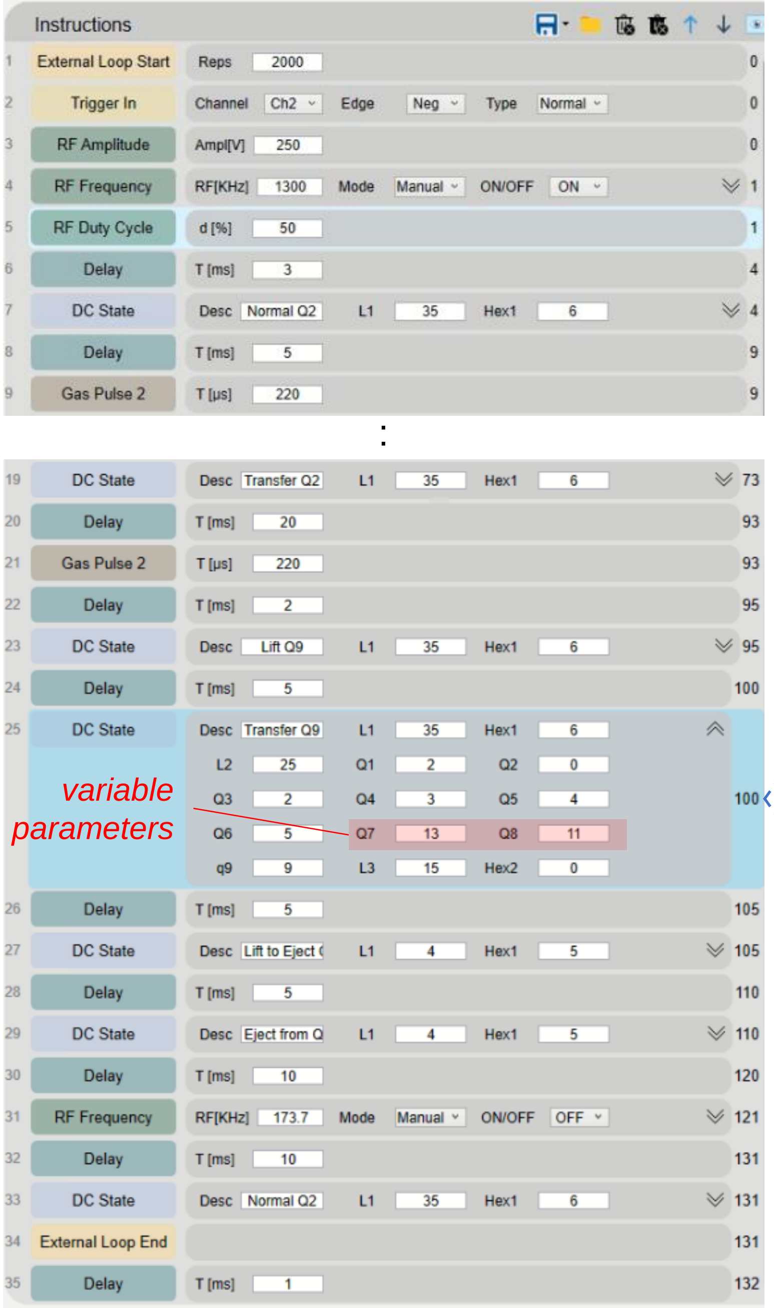
ii) full mass range trap to Q9

Ions transferred and trapped to Q9.

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Q9 Trapping Test

## Appendix 3

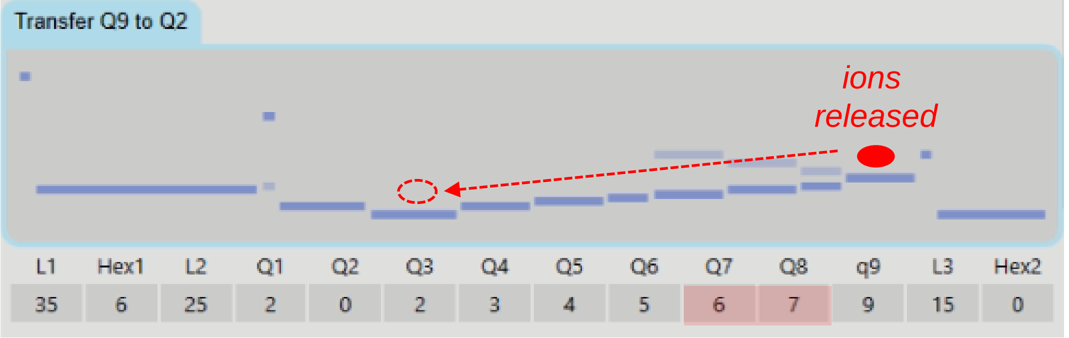


Instructions

|  |  |  |  |
|---|---|---|---|
| 1 | External Loop Start | Reps 2000 | 0 |
| 2 | Trigger In | Channel Ch2 Edge Neg Type Normal | 0 |
| 3 | RF Amplitude | Ampl[V] 250 | 0 |
| 4 | RF Frequency | RF[KHz] 1300 Mode Manual ON/OFF ON | 1 |
| 5 | RF Duty Cycle | d [%] 50 | 1 |
| 6 | Delay | T [ms] 3 | 4 |
| 7 | DC State | Desc Normal Q2 L1 35 Hex1 6 | 4 |
| 8 | Delay | T [ms] 5 | 9 |
| 9 | Gas Pulse 2 | T [µs] 220 | 9 |
| : |  |  |  |
| 19 | DC State | Desc Transfer Q2 L1 35 Hex1 6 | 73 |
| 20 | Delay | T [ms] 20 | 93 |
| 21 | Gas Pulse 2 | T [µs] 220 | 93 |
| 22 | Delay | T [ms] 2 | 95 |
| 23 | DC State | Desc Lift Q9 L1 35 Hex1 6 | 95 |
| 24 | Delay | T [ms] 5 | 100 |
| 25 | DC State | Desc Transfer Q9 L1 35 Hex1 6<br>L2 25 Q1 2 Q2 0<br>Q3 2 Q4 3 Q5 4<br>Q6 5 Q7 13 Q8 11<br>q9 9 L3 15 Hex2 0 | 100 |
| 26 | Delay | T [ms] 5 | 105 |
| 27 | DC State | Desc Lift to Eject ( L1 4 Hex1 5 | 105 |
| 28 | Delay | T [ms] 5 | 110 |
| 29 | DC State | Desc Eject from Q L1 4 Hex1 5 | 110 |
| 30 | Delay | T [ms] 10 | 120 |
| 31 | RF Frequency | RF[KHz] 173.7 Mode Manual ON/OFF OFF | 121 |
| 32 | Delay | T [ms] 10 | 131 |
| 33 | DC State | Desc Normal Q2 L1 35 Hex1 6 | 131 |
| 34 | External Loop End |  | 131 |
| 35 | Delay | T [ms] 1 | 132 |

- Sequence file path/name:
  *…\Diagnostics\Trapping Tests\Q9 Trapping Test.ins*
- Variable parameters of “Transfer Q9 to Q2” DC state:
  1) Q7 DC voltage
  2) Q8 DC voltage
- Values alternate between two sequence states:

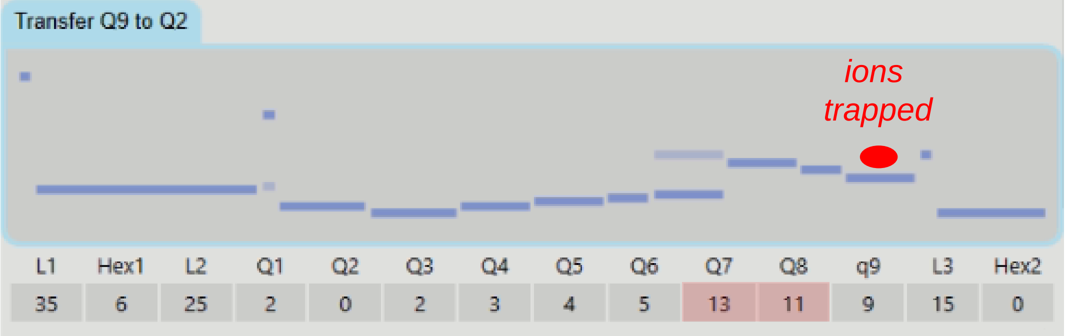
i) full mass range transfer to Q9



Transfer Q9 to Q2

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 25 | 2 | 0 | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 15 | 0 |

ii) full mass range trap to Q9



Transfer Q9 to Q2

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 25 | 2 | 0 | 2 | 3 | 4 | 5 | 13 | 11 | 9 | 15 | 0 |

| sequence state | Q7 | Q8 |
|---|---|---|
| transfer | 6 V | 7 V |
| trap | 13V | 11 V |

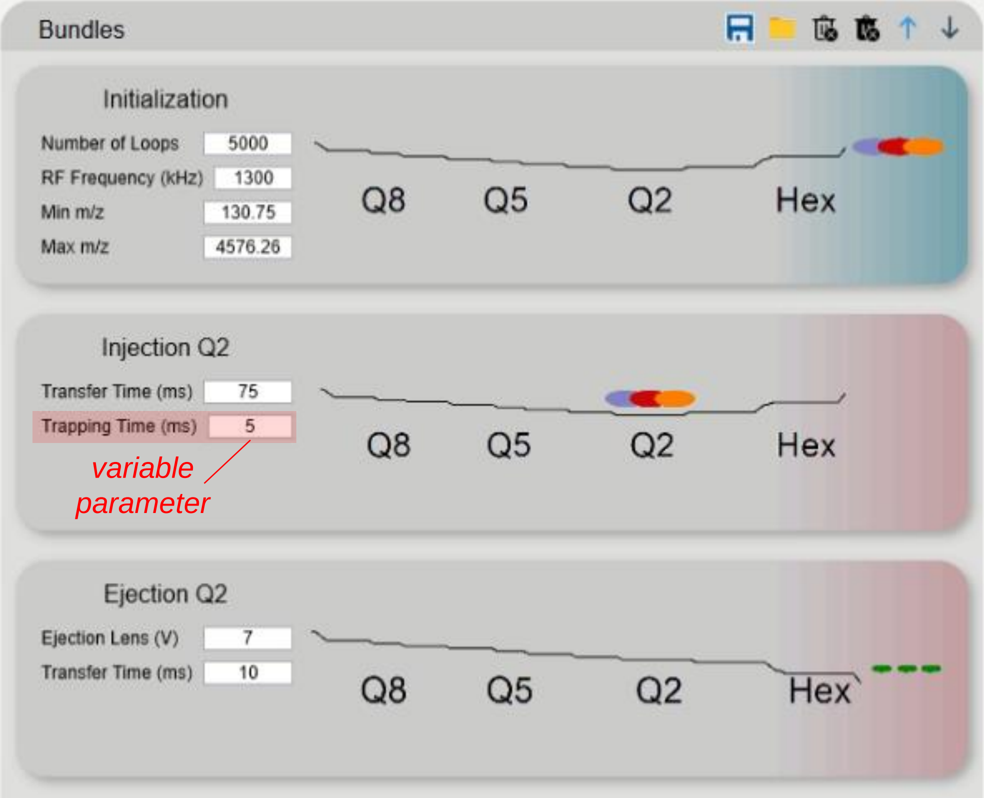
# Trapping Time test Q2

Appendix 3

- Sequence file path/name: …\Diagnostics\Trapping Time Tests\Q2 Trapping Time Test.ins
- Variable parameter: Injection Q2 bundle - Trapping Time *t* (ms)
- Scan values: 5, 10, 25, 50, 100, 250, 500 ms
- Pass Criterion:

$$\frac{TIC_{t=500ms}}{TIC_{t=5ms}} > 0.8$$

*TIC*: total ion current



Initialization

- Number of Loops: 5000
- RF Frequency (kHz): 1300
- Min m/z: 130.75
- Max m/z: 4576.26

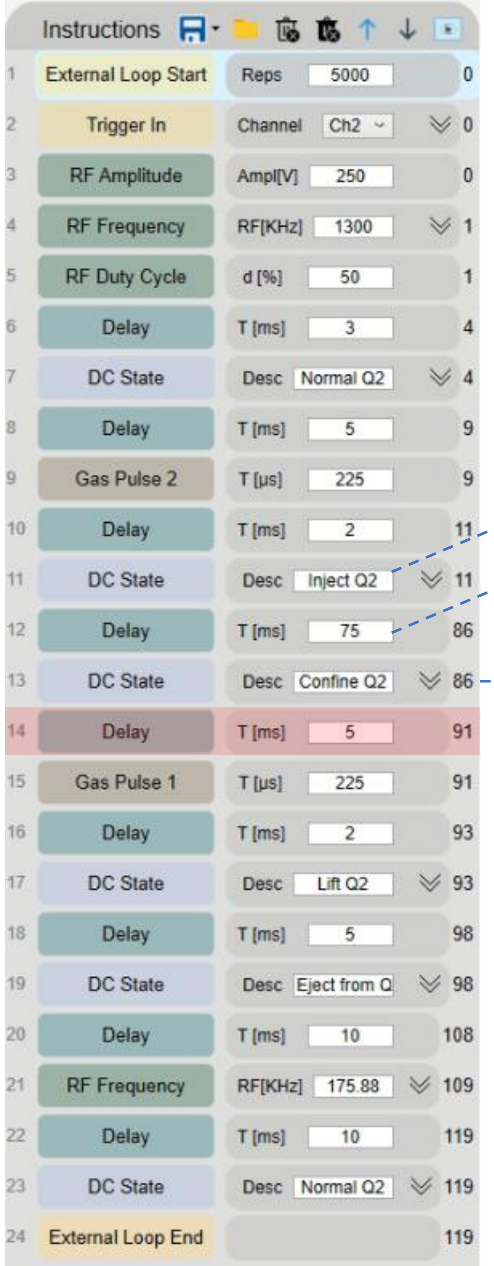
Injection Q2

- Transfer Time (ms): 75
- Trapping Time (ms): 5

*variable parameter*

Ejection Q2

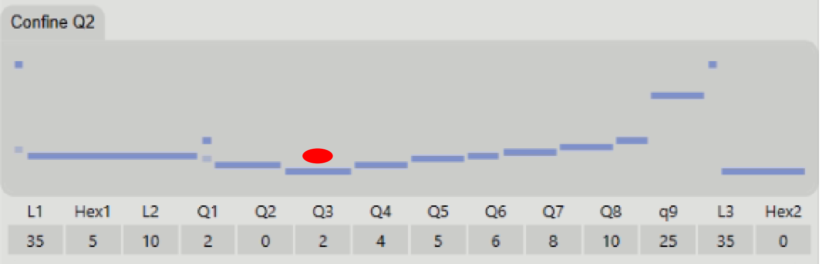
- Ejection Lens (V): 7
- Transfer Time (ms): 10



| | | | | |
|---|---|---|---|---|
| 1 | External Loop Start | Reps | 5000 | 0 |
| 2 | Trigger In | Channel | Ch2 | 0 |
| 3 | RF Amplitude | Ampl[V] | 250 | 0 |
| 4 | RF Frequency | RF[KHz] | 1300 | 1 |
| 5 | RF Duty Cycle | d [%] | 50 | 1 |
| 6 | Delay | T [ms] | 3 | 4 |
| 7 | DC State | Desc | Normal Q2 | 4 |
| 8 | Delay | T [ms] | 5 | 9 |
| 9 | Gas Pulse 2 | T [µs] | 225 | 9 |
| 10 | Delay | T [ms] | 2 | 11 |
| 11 | DC State | Desc | Inject Q2 | 11 |
| 12 | Delay | T [ms] | 75 | 86 |
| 13 | DC State | Desc | Confine Q2 | 86 |
| 14 | Delay | T [ms] | 5 | 91 |
| 15 | Gas Pulse 1 | T [µs] | 225 | 91 |
| 16 | Delay | T [ms] | 2 | 93 |
| 17 | DC State | Desc | Lift Q2 | 93 |
| 18 | Delay | T [ms] | 5 | 98 |
| 19 | DC State | Desc | Eject from Q | 98 |
| 20 | Delay | T [ms] | 10 | 108 |
| 21 | RF Frequency | RF[KHz] | 175.88 | 109 |
| 22 | Delay | T [ms] | 10 | 119 |
| 23 | DC State | Desc | Normal Q2 | 119 |
| 24 | External Loop End | | | 119 |

Ions are injected and trapped to Q2.

A 75 ms delay time is needed before trapping time starts, to ensure gas-free trapping evaluation.



Confine Q2

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 10 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

**Benchmark MS2 experiments**

- slow heating Collisional Induced Dissociation (CID)
- Electron Ionization Dissociation (EID)
- Electron Capture Dissociation (ECD)

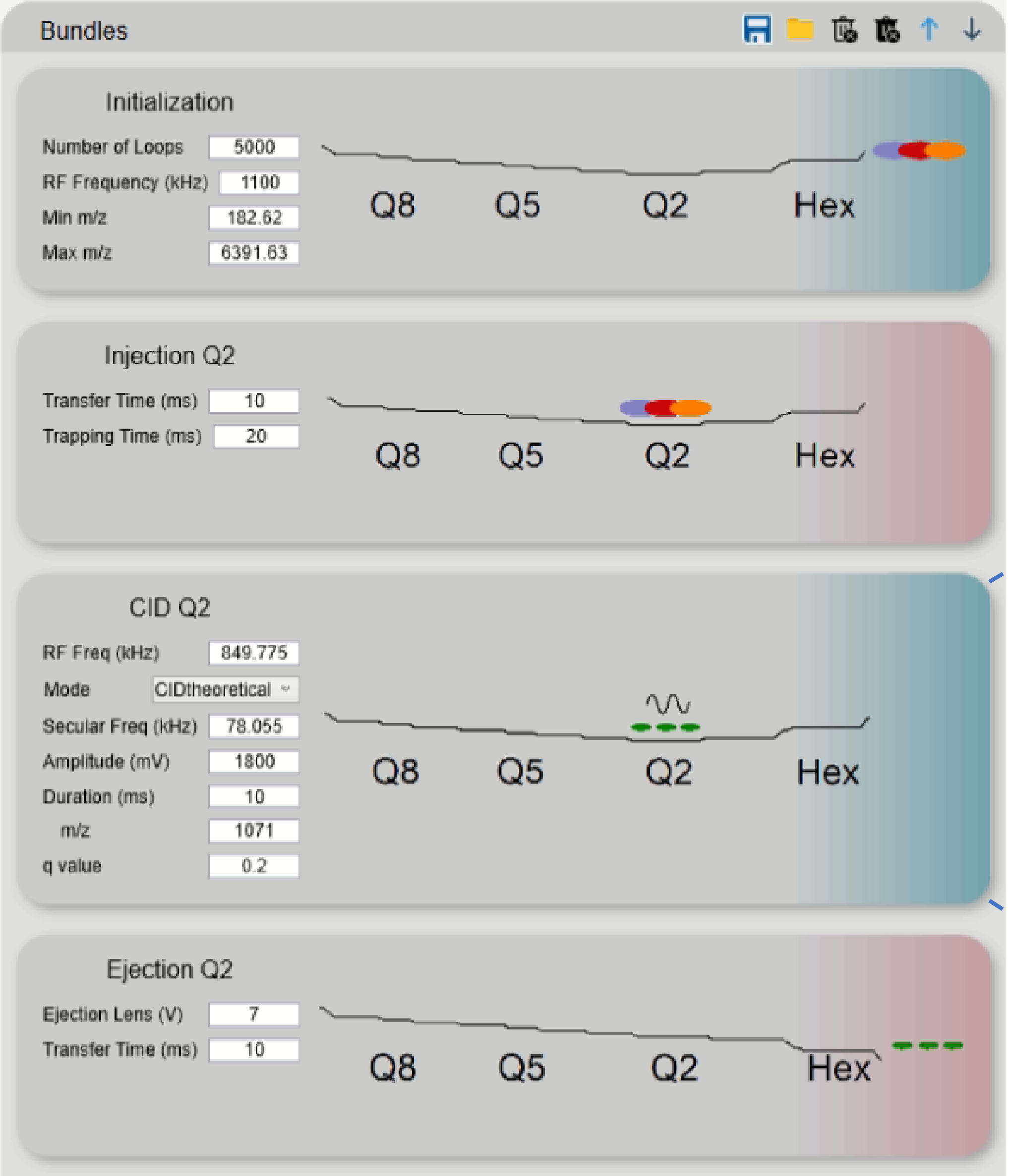
Appendix 3

# slow heating Collision Induced Dissociation

# (CID)

# Slow heating CID (Q2) : Omnitrap sequence

## Appendix 3



Bundles

Initialization

Number of Loops: 5000
RF Frequency (kHz): 1100
Min m/z: 182.62
Max m/z: 6391.63

Injection Q2

Transfer Time (ms): 10
Trapping Time (ms): 20

CID Q2

RF Freq (kHz): 849.775
Mode: CIDtheoretical
Secular Freq (kHz): 78.055
Amplitude (mV): 1800
Duration (ms): 10
m/z: 1071
q value: 0.2

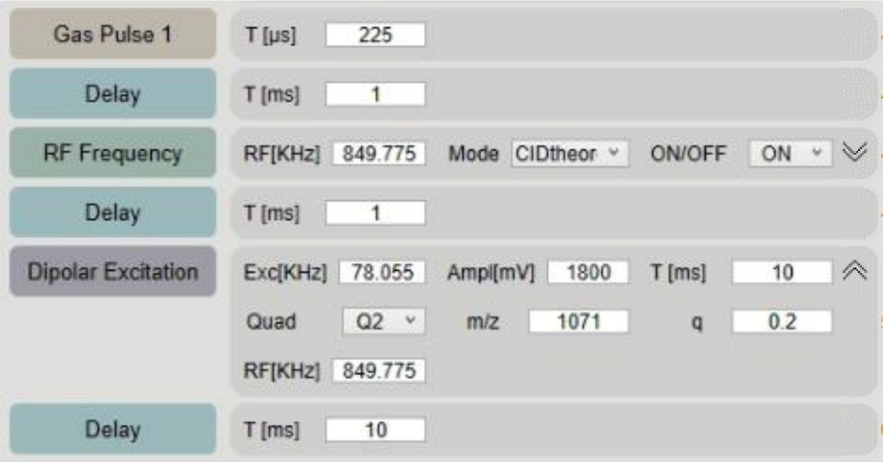
Ejection Q2

Ejection Lens (V): 7
Transfer Time (ms): 10

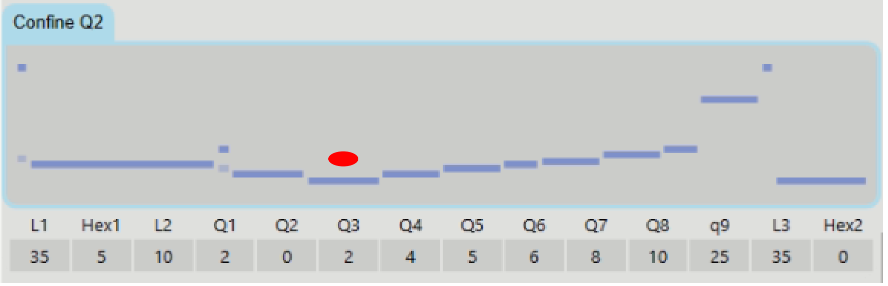
- Sequence file path/name:
  *…\OmniTrap\Sequence Files\Templates\MS2 CID Q2.bdl or .ins*

- CID parameters:
  *q value (RF frequency)*
  *excitation (secular) frequency*
  *excitation amplitude*
  *duration*

- Sample: ubiquitin (H2O:MeOH:AcOH, 49:50:1) , C=1μM



Gas Pulse 1 — T [μs]: 225
Delay — T [ms]: 1
RF Frequency — RF[KHz]: 849.775 Mode: CIDtheor ON/OFF: ON
Delay — T [ms]: 1
Dipolar Excitation — Exc[KHz]: 78.055 Ampl[mV]: 1800 T [ms]: 10
Quad: Q2 m/z: 1071 q: 0.2
RF[KHz]: 849.775
Delay — T [ms]: 10



Confine Q2

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 10 | 2 | 0 | 2 | 4 | 5 | 6 | 8 | 10 | 25 | 35 | 0 |

# Slow heating CID (Q5) : Omnitrap sequence

Appendix 3

**Bundles**

**Initialization**

Number of Loops: 5000
RF Frequency (kHz): 1300
Min m/z: 130.75
Max m/z: 4576.26

**Injection Q2**

Transfer Time (ms): 10
Trapping Time (ms): 10

**Transfer Q2 to Q5**

Transfer Time (ms): 5
Trapping Time (ms): 5

**CID Q5**

RF Freq (kHz): 849.775
Mode: CIDtheoretical
Secular Freq (kHz): 78.055
Amplitude (mV): 1700
Duration (ms): 10
Mass (m/z): 1071
q value: 0.2

**Transfer Q5 to Q2**

Transfer Time (ms): 5

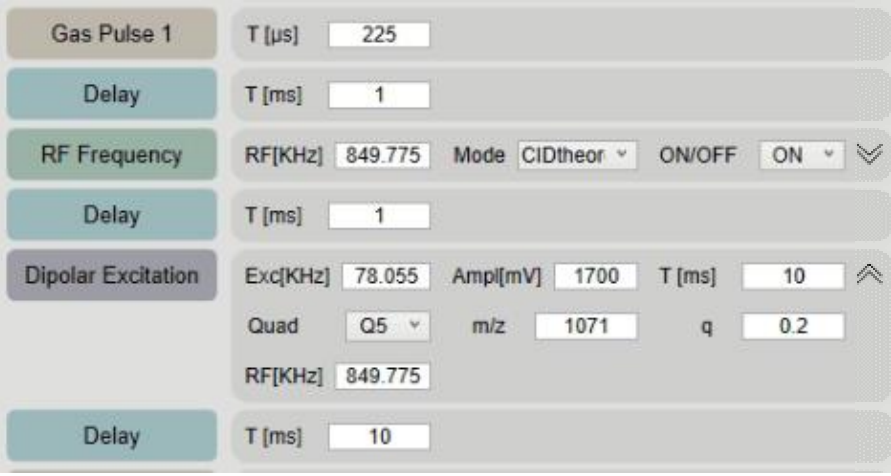
**Ejection Q2**

Ejection Lens (V): 7
Transfer Time (ms): 10

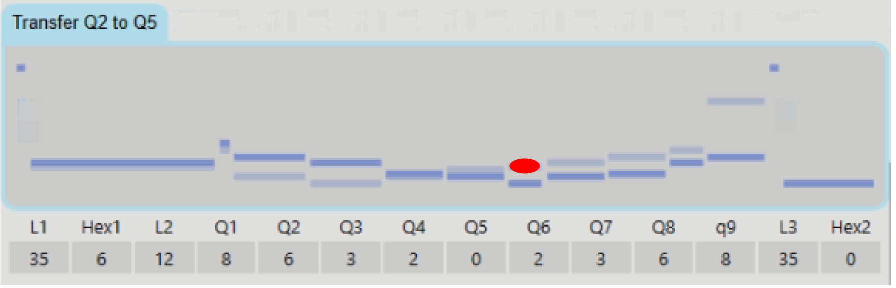
- Sequence file path/name:
  *…\OmniTrap\Sequence Files\Templates\MS2 CID Q5.bdl* or *.ins*

- CID parameters:
  *q value (RF frequency)*
  *excitation (secular) frequency*
  *excitation amplitude*
  *duration*

- Sample: ubiquitin (H2O:MeOH:AcOH, 49:50:1) , C=1μM



Gas Pulse 1: T [μs] 225
Delay: T [ms] 1
RF Frequency: RF[KHz] 849.775 Mode CIDtheor ON/OFF ON
Delay: T [ms] 1
Dipolar Excitation: Exc[KHz] 78.055 Ampl[mV] 1700 T [ms] 10
Quad Q5 m/z 1071 q 0.2
RF[KHz] 849.775
Delay: T [ms] 10

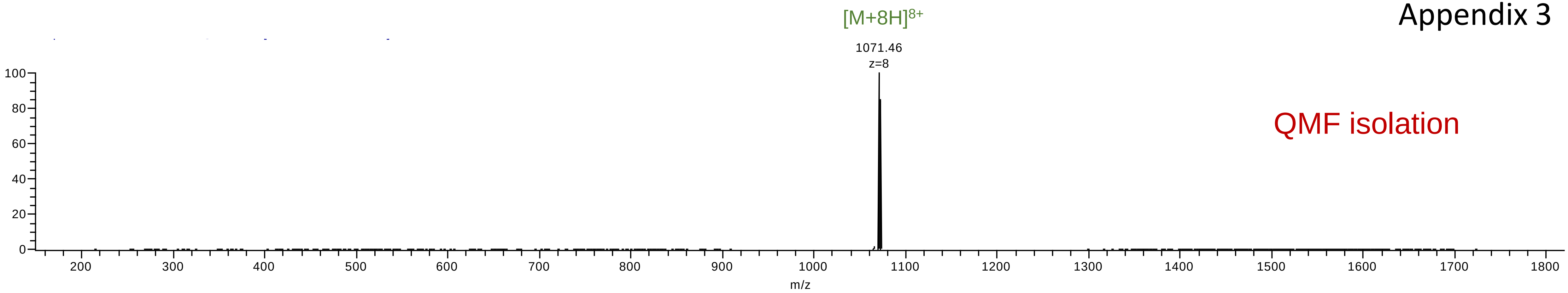


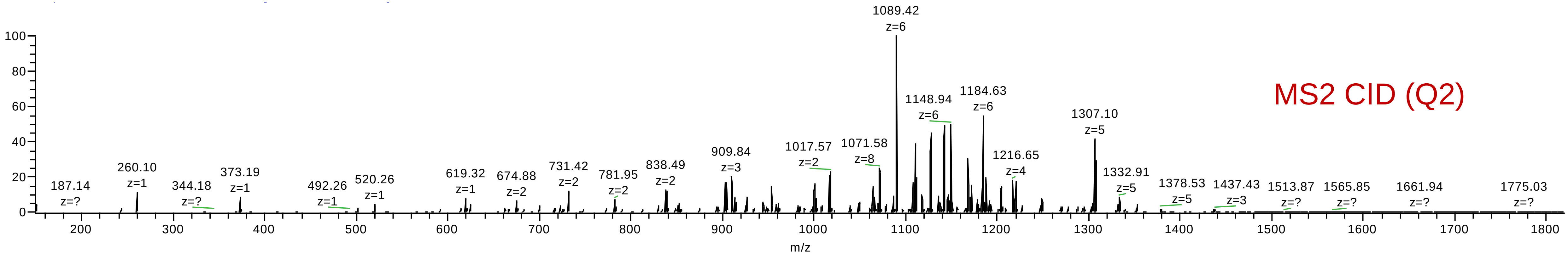
Transfer Q2 to Q5

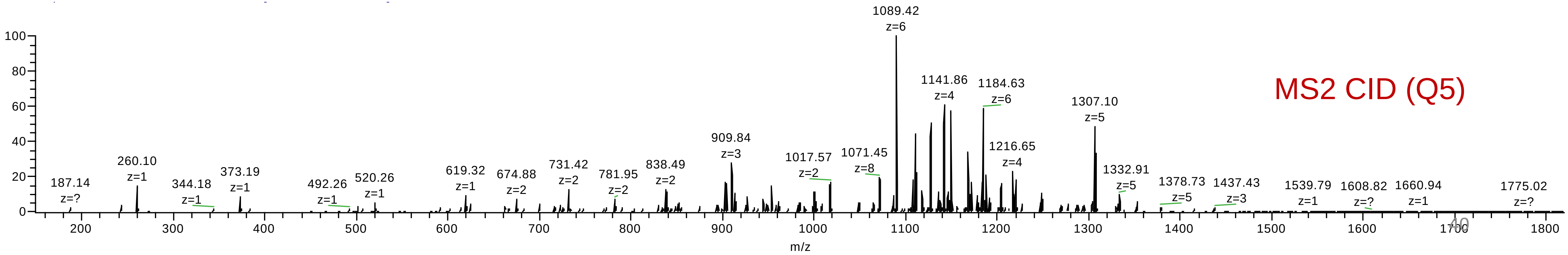
| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 12 | 8 | 6 | 3 | 2 | 0 | 2 | 3 | 6 | 8 | 35 | 0 |

# Slow heating CID (Q2 & Q5) – ubiquitin $[M+8H]^{8+}$

Appendix 3


QMF isolation

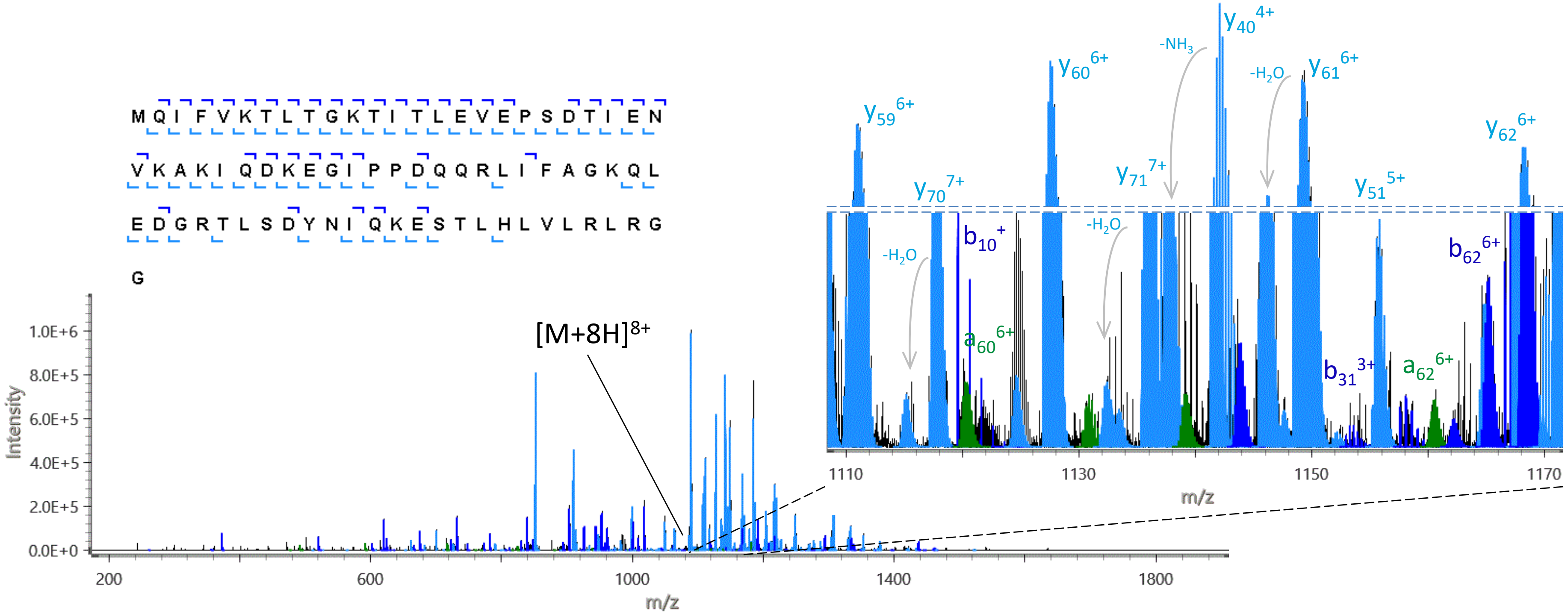

MS2 CID (Q2)


MS2 CID (Q5)

# Slow heating CID – ubiquitin $[M+8H]^{8+}$

Appendix 3

- Annotated slow heating CID mass spectrum of ubiquitin 8+ and corresponding sequence map.



<u>Pass Criterion:</u> >50% bonds cleaved

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

Appendix 3

# Electron Ionization Dissociation

# (EID)

# MS2 EID : omnitrap sequence / electron source settings

Bundles

Initialization

Number of Loops: 5000

RF Frequency (kHz): 1000

Min m/z: 220.97

Max m/z: 7733.88

Injection Q2

Transfer Time (ms): 10

Trapping Time (ms): 5

Transfer Q2 to Q5

Transfer Time (ms): 5

Trapping Time (ms): 5

ExD

Irradiation Time (ms): 50

Transfer Q5 to Q2

Transfer Time (ms): 5

Ejection Q2

Ejection Lens (V): 7

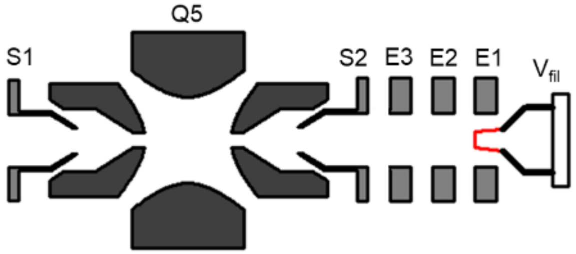
Transfer Time (ms): 10

## Appendix 3

- Sequence file path/name:
  *…\OmniTrap\Sequence Files\Templates\MS2 ExD.bdl* or *.ins*

- Electron Source settings:

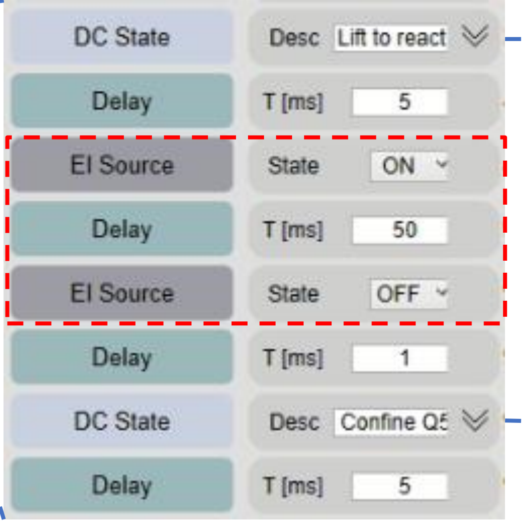
| | |
|---|---|
| *Current* | 5.6A |
| *Potential ($V_{filament}$)* | **-53V** |
| *E1* | -20V |
| *E2 Transmit* | 0V |
| *E2 Deflect* | -150V |
| *E3* | 450V |
| *S2 Transmit* | 5V |
| *S2 Deflect* | -150V |



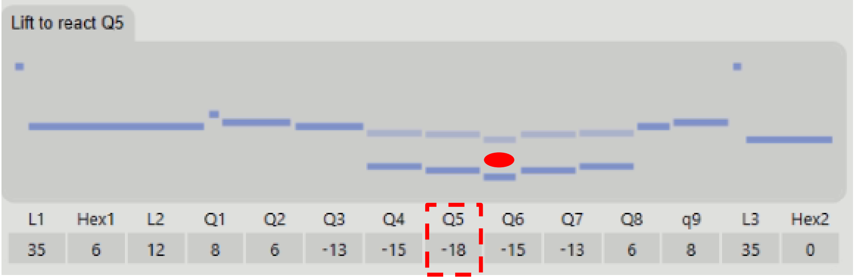
$$E_{electron} = V_{Q5} - V_{filament}$$

$$\approx 35eV$$

- Sample: ubiquitin (H2O:MeOH:AcOH, 49:50:1) , C=1μM

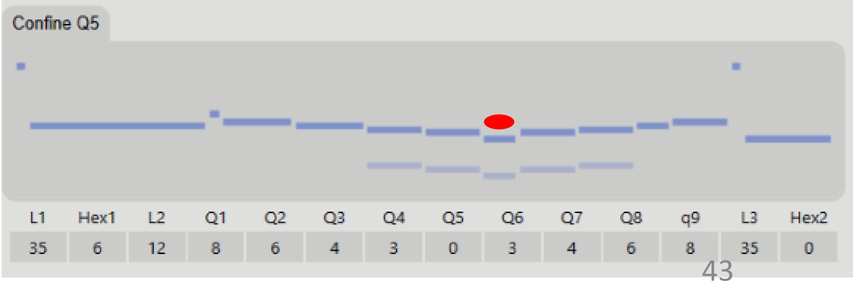


| | |
|---|---|
| DC State | Desc: Lift to react |
| Delay | T [ms]: 5 |
| EI Source | State: ON |
| Delay | T [ms]: 50 |
| EI Source | State: OFF |
| Delay | T [ms]: 1 |
| DC State | Desc: Confine Q5 |
| Delay | T [ms]: 5 |



Lift to react Q5

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 12 | 8 | 6 | -13 | -15 | -18 | -15 | -13 | 6 | 8 | 35 | 0 |

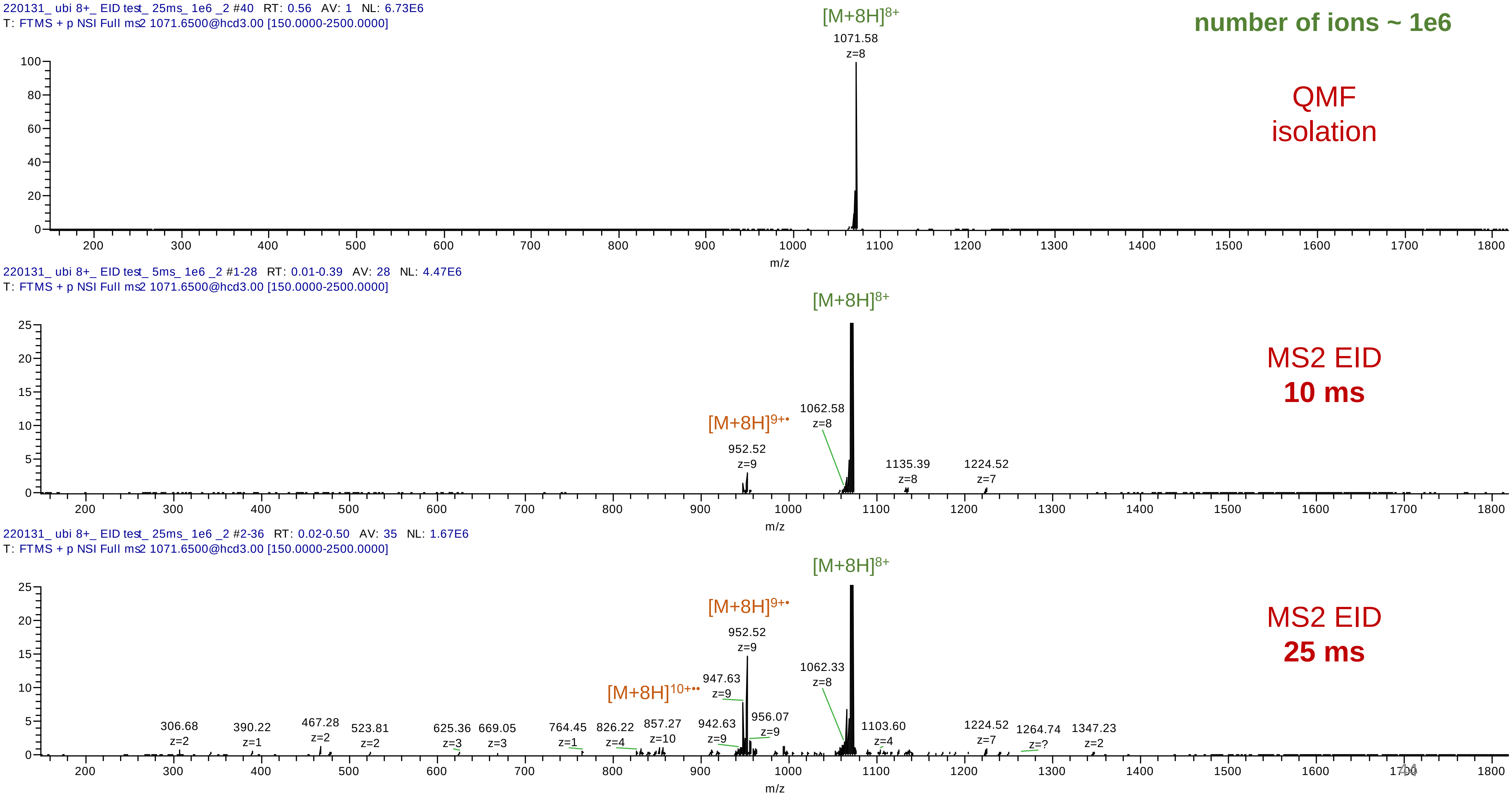


Confine Q5

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 12 | 8 | 6 | 4 | 3 | 0 | 3 | 4 | 6 | 8 | 35 | 0 |

# Electron Ionization Dissociation (EID) – ubiquitin $[M+8H]^{8+}$

Appendix 3



**number of ions ~ 1e6**

QMF
isolation

MS2 EID
**10 ms**

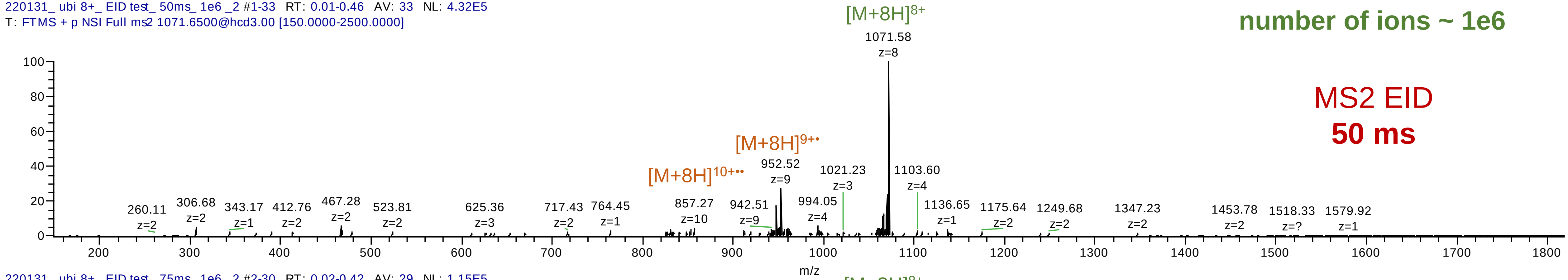
MS2 EID
**25 ms**

# Electron Ionization Dissociation (EID) – ubiquitin $[M+8H]^{8+}$

Appendix 3

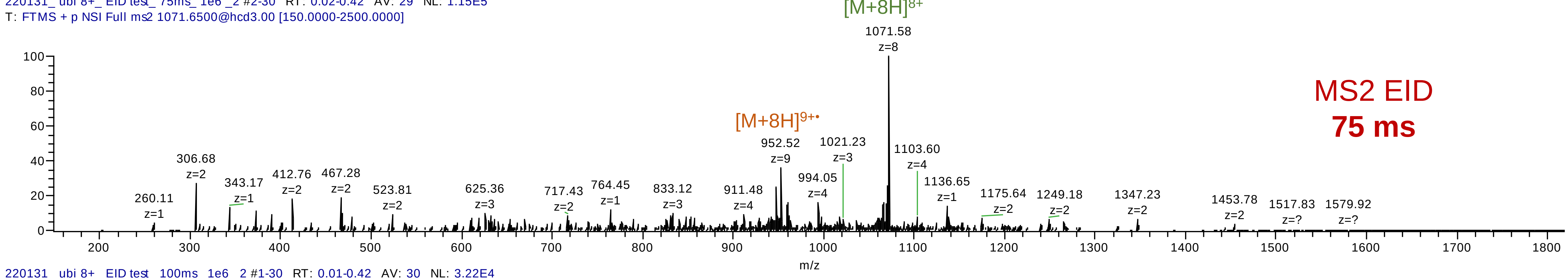
**number of ions ~ 1e6**

220131_ ubi 8+_ EID test_ 50ms_ 1e6 _2 #1-33 RT: 0.01-0.46 AV: 33 NL: 4.32E5
T: FTMS + p NSI Full ms2 1071.6500@hcd3.00 [150.0000-2500.0000]



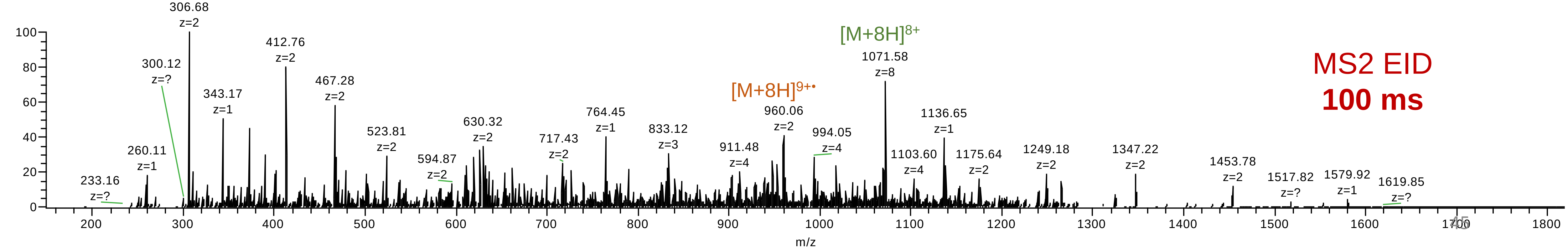
**MS2 EID**
**50 ms**

220131_ ubi 8+_ EID test_ 75ms_ 1e6 _2 #2-30 RT: 0.02-0.42 AV: 29 NL: 1.15E5
T: FTMS + p NSI Full ms2 1071.6500@hcd3.00 [150.0000-2500.0000]



**MS2 EID**
**75 ms**

220131_ ubi 8+_ EID test_ 100ms_ 1e6 _2 #1-30 RT: 0.01-0.42 AV: 30 NL: 3.22E4
T: FTMS + p NSI Full ms2 1071.6500@hcd3.00 [150.0000-2500.0000]

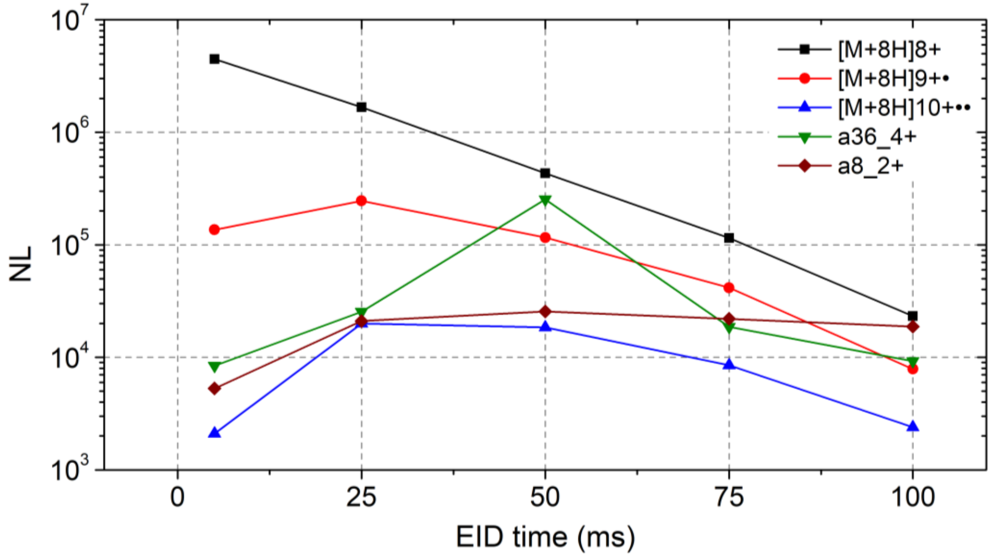


**MS2 EID**
**100 ms**

# Electron Ionization Dissociation (EID) – ubiquitin [M+8H]$^{8+}$

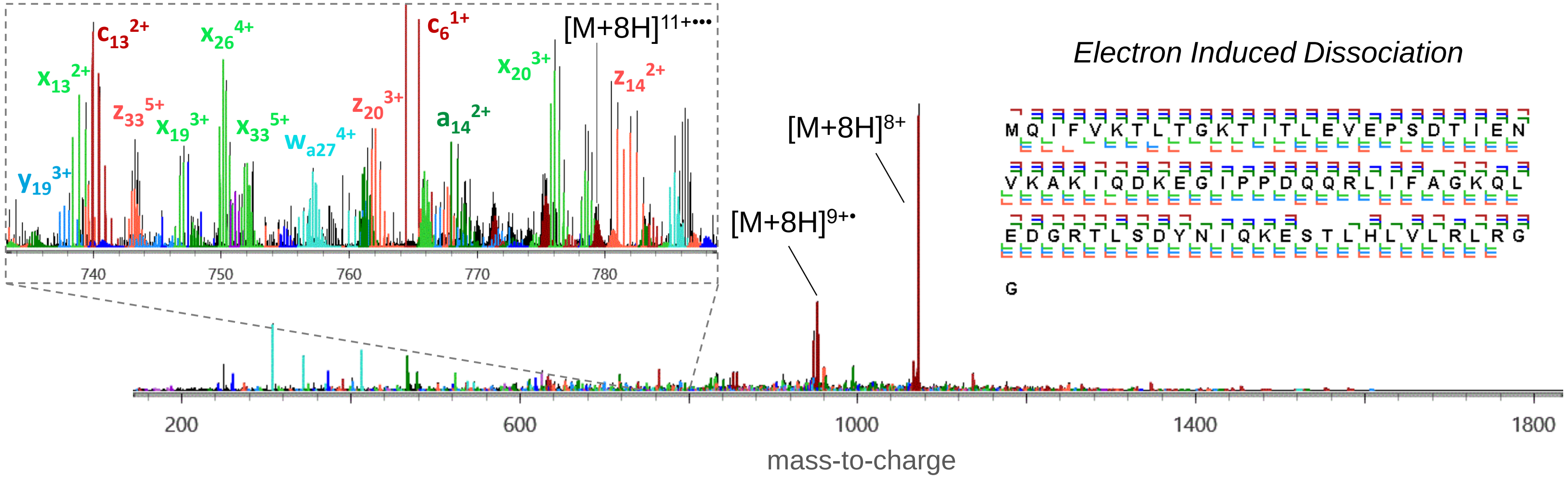
Appendix 3

- Precursor, ionized and selected fragment ions signal intensity (NL) as a function of electron irradiation time on MS2 EID experiments.



Pass Criterion: >97% bonds cleaved

- Annotated MS2 EID mass spectrum of ubiquitin [M+8H]$^{8+}$ ions and corresponding sequence map.



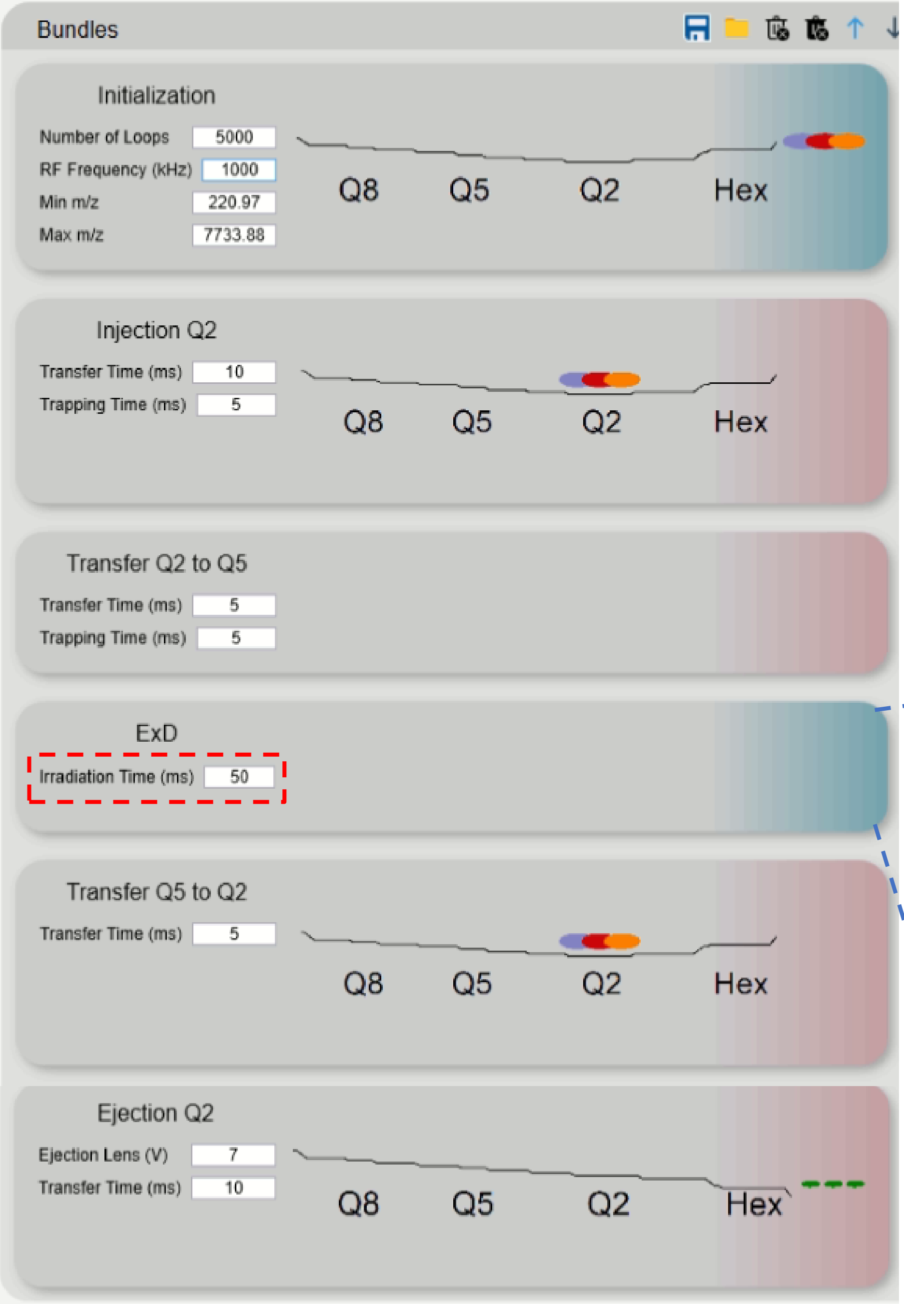
**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

Appendix 3

# Electron Capture Dissociation

# (ECD)

# MS2 ECD : omnitrap sequence / electron source settings



Bundles

**Initialization**

Number of Loops: 5000
RF Frequency (kHz): 1000
Min m/z: 220.97
Max m/z: 7733.88

**Injection Q2**

Transfer Time (ms): 10
Trapping Time (ms): 5

**Transfer Q2 to Q5**

Transfer Time (ms): 5
Trapping Time (ms): 5

**ExD**

Irradiation Time (ms): 50

**Transfer Q5 to Q2**

Transfer Time (ms): 5

**Ejection Q2**

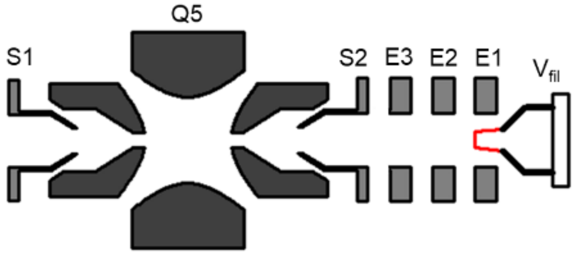
Ejection Lens (V): 7
Transfer Time (ms): 10

## Appendix 3

- Sequence file path/name:
  *…\OmniTrap\Sequence Files\Templates\MS2 ExD.bdl or .ins*

- Electron Source settings:

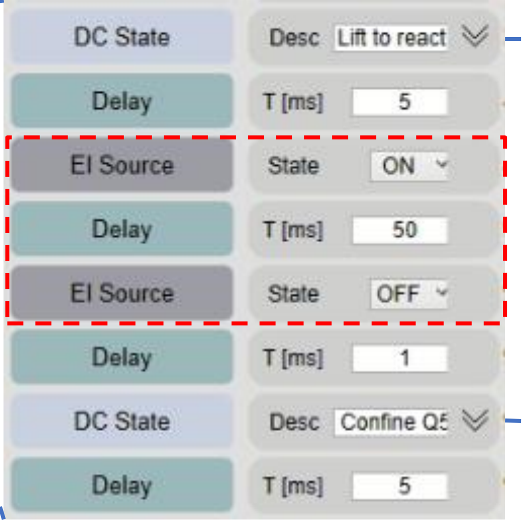
| | |
|---|---|
| *Current* | 5.7A |
| *Potential ($V_{filament}$)* | **-20V** |
| *E1* | *0V* |
| *E2 Transmit* | *20V* |
| *E2 Deflect* | *-150V* |
| *E3* | *400V* |
| *S2 Transmit* | 20V |
| *S2 Deflect* | -150V |



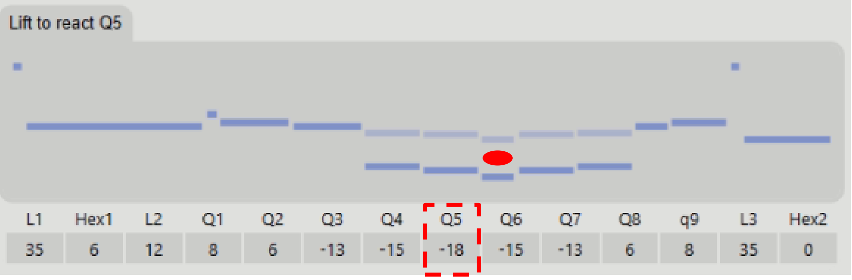
$$E_{electron} = V_{Q5} - V_{filament}$$

$$\approx \mathbf{0eV}$$

- Sample: ubiquitin ($H2O$:MeOH:AcOH, 49:50:1) , C=1μM

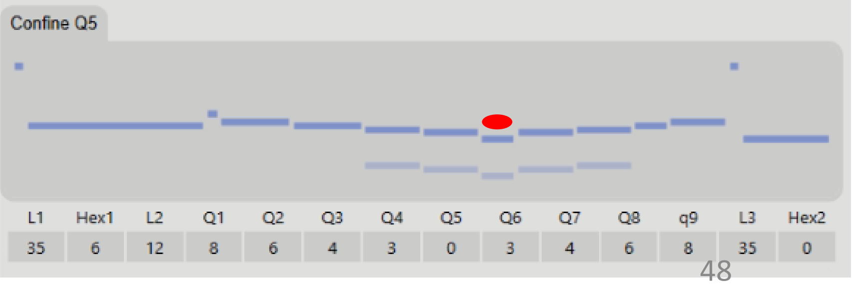


| | | |
|---|---|---|
| DC State | Desc | Lift to react |
| Delay | T [ms] | 5 |
| EI Source | State | ON |
| Delay | T [ms] | 50 |
| EI Source | State | OFF |
| Delay | T [ms] | 1 |
| DC State | Desc | Confine Q5 |
| Delay | T [ms] | 5 |



Lift to react Q5

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 12 | 8 | 6 | -13 | -15 | -18 | -15 | -13 | 6 | 8 | 35 | 0 |



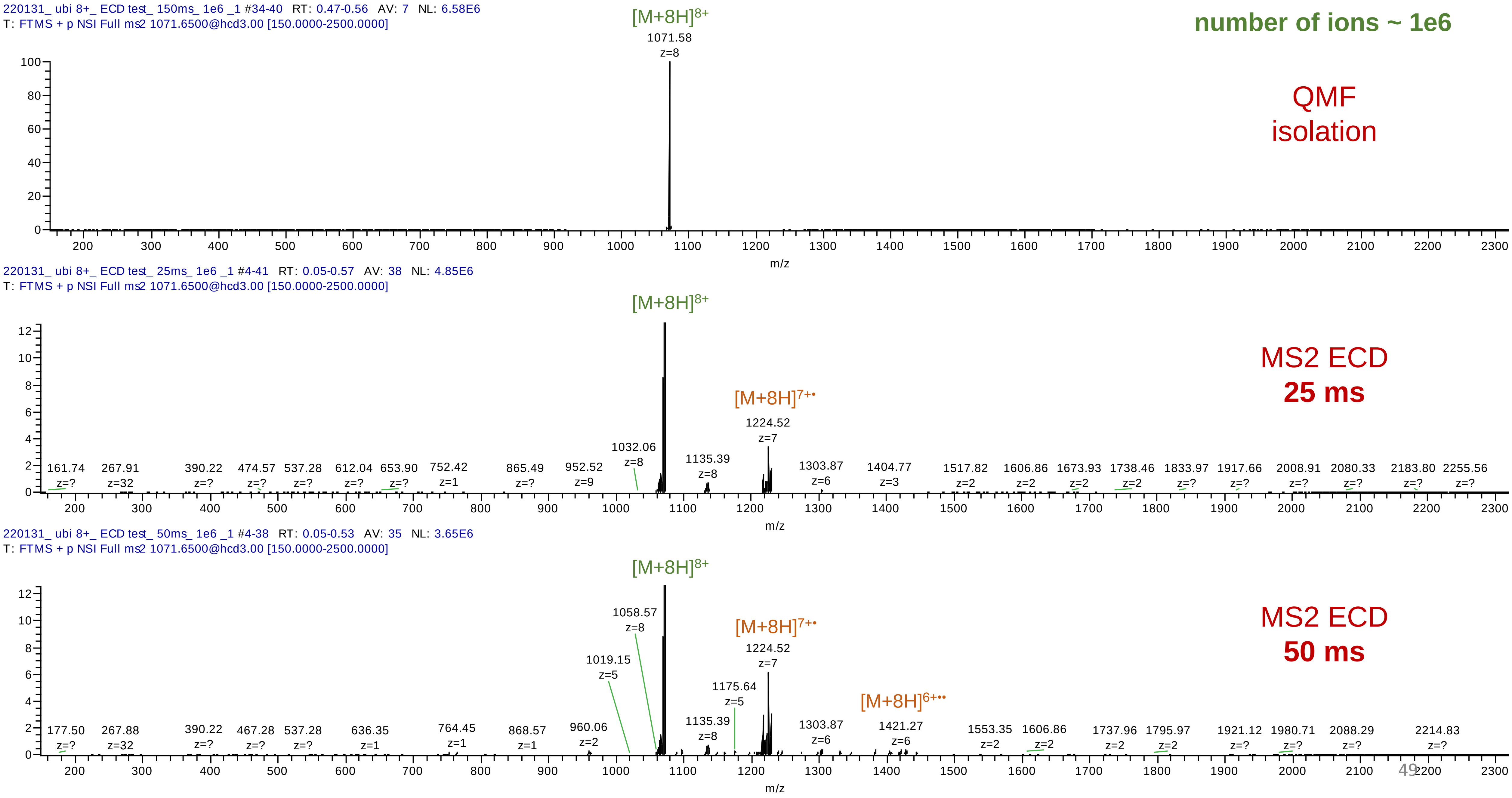
Confine Q5

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 12 | 8 | 6 | 4 | 3 | 0 | 3 | 4 | 6 | 8 | 35 | 0 |

# Electron Capture Dissociation (ECD) – ubiquitin $[M+8H]^{8+}$

Appendix 3

**number of ions ~ 1e6**

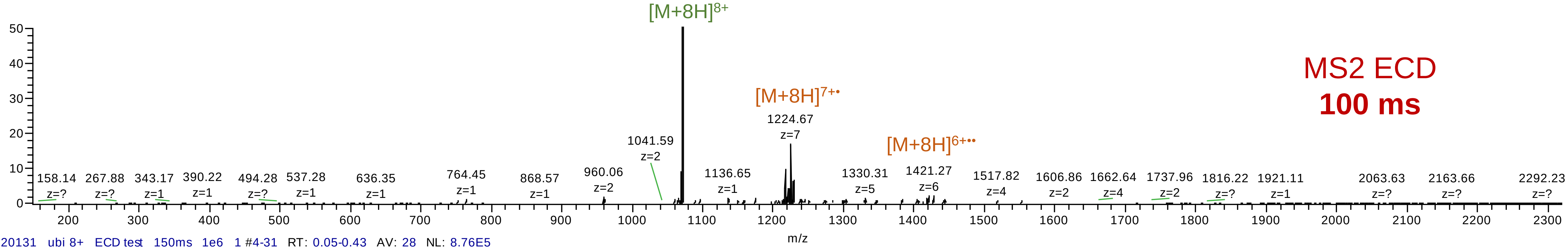


# Electron Capture Dissociation (ECD) – ubiquitin $[M+8H]^{8+}$

Appendix 3

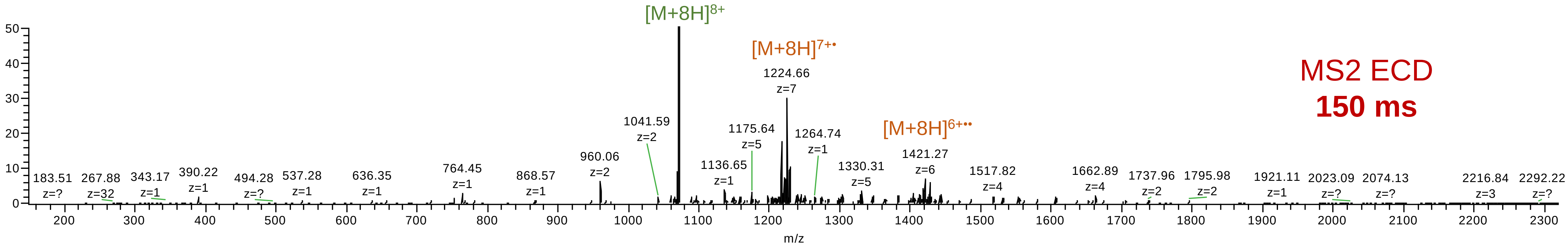
**number of ions ~ 1e6**

220131_ ubi 8+_ ECD test_ 100ms_ 1e6 _2 #2-35 RT: 0.02-0.49 AV: 34 NL: 1.86E6
T: FTMS + p NSI Full ms2 1071.6500@hcd3.00 [150.0000-2500.0000]



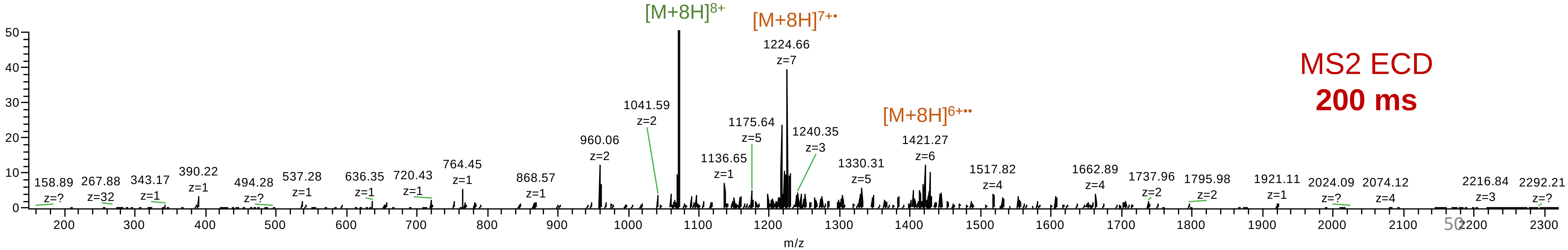
MS2 ECD
**100 ms**

220131_ ubi 8+_ ECD test_ 150ms_ 1e6 _1 #4-31 RT: 0.05-0.43 AV: 28 NL: 8.76E5
T: FTMS + p NSI Full ms2 1071.6500@hcd3.00 [150.0000-2500.0000]



MS2 ECD
**150 ms**

220131_ ubi 8+_ ECD test_ 200ms_ 1e6 _2 #2-30 RT: 0.02-0.42 AV: 29 NL: 5.47E5
T: FTMS + p NSI Full ms2 1071.6500@hcd3.00 [150.0000-2500.0000]

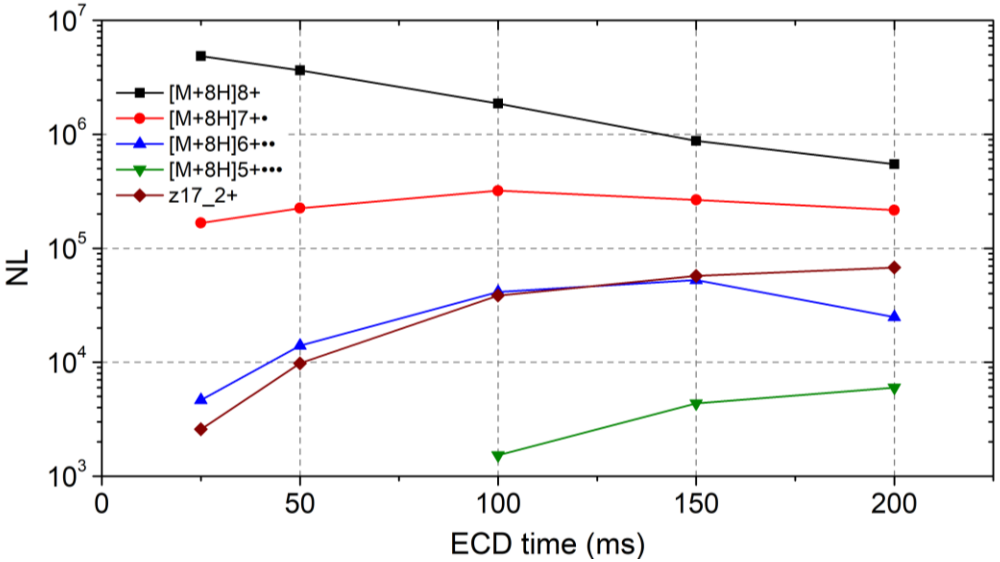


MS2 ECD
**200 ms**

# Electron Capture Dissociation (ECD) – ubiquitin [M+8H]$^{8+}$

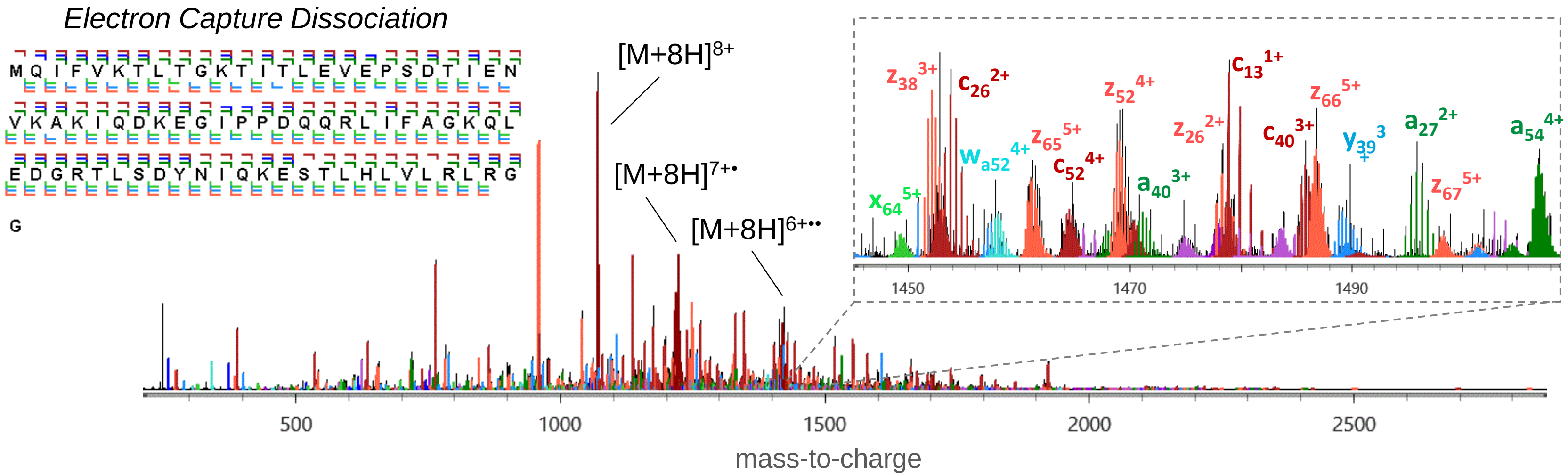
## Appendix 3

- Precursor, charged reduced and fragment ions signal intensity (NL) as a function of electron irradiation time on MS2 ECD experiments.



<u>Pass Criterion:</u> >97% bonds cleaved

- Annotated MS2 ECD mass spectrum of ubiquitin [M+8H]$^{8+}$ ions and corresponding sequence map.



**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

Appendix 3

# Accumulation Mode

# Accumulation mode in MS3 working flow: Omnitrap Sequence

Appendix 3

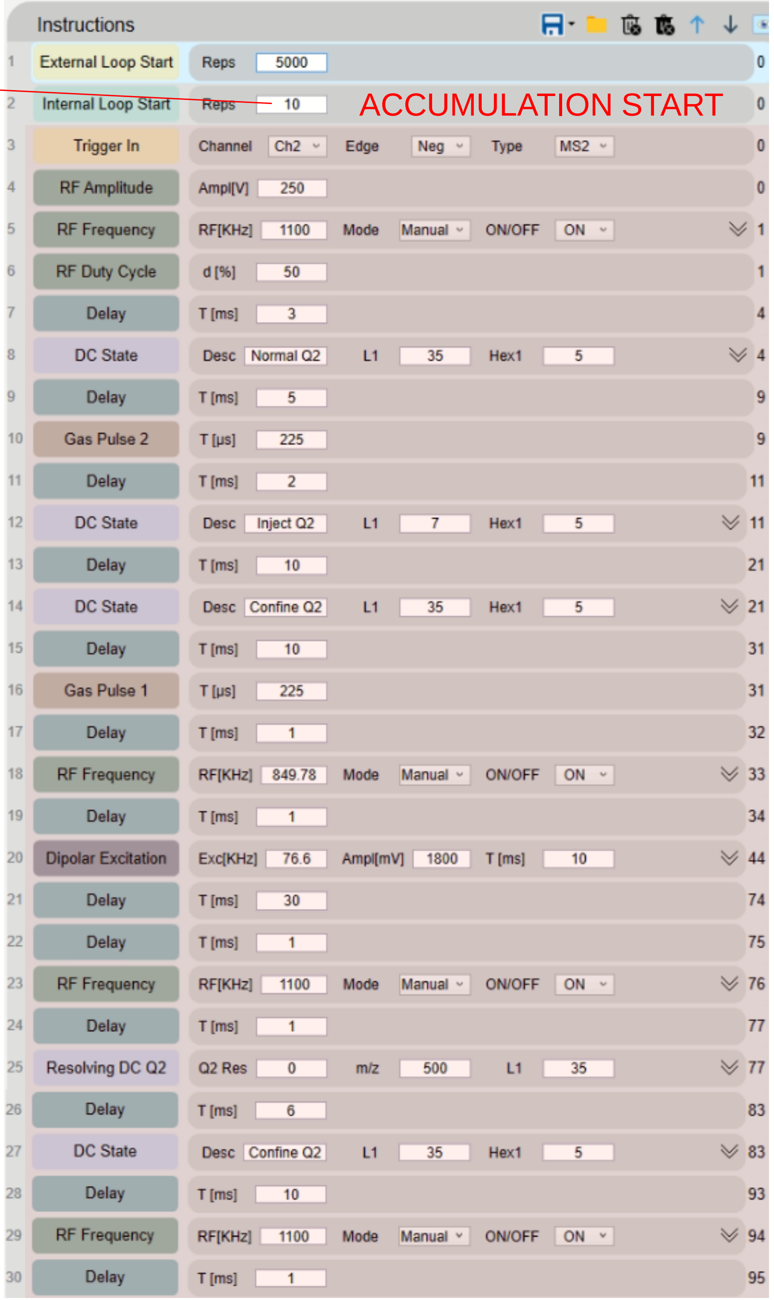
*number of accumulation loops (multiple injections to omnitrap)*

**Initialization**

**Injection to Q2**

**MS2 CID Q2**

**MS3 Resolving DC Isolation Q2**



Instructions

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | External Loop Start | Reps | 5000 | | | | | 0 |
| 2 | Internal Loop Start | Reps | 10 | ACCUMULATION START | | | | 0 |
| 3 | Trigger In | Channel | Ch2 | Edge | Neg | Type | MS2 | 0 |
| 4 | RF Amplitude | Ampl[V] | 250 | | | | | 0 |
| 5 | RF Frequency | RF[KHz] | 1100 | Mode | Manual | ON/OFF | ON | 1 |
| 6 | RF Duty Cycle | d [%] | 50 | | | | | 1 |
| 7 | Delay | T [ms] | 3 | | | | | 4 |
| 8 | DC State | Desc | Normal Q2 | L1 | 35 | Hex1 | 5 | 4 |
| 9 | Delay | T [ms] | 5 | | | | | 9 |
| 10 | Gas Pulse 2 | T [µs] | 225 | | | | | 9 |
| 11 | Delay | T [ms] | 2 | | | | | 11 |
| 12 | DC State | Desc | Inject Q2 | L1 | 7 | Hex1 | 5 | 11 |
| 13 | Delay | T [ms] | 10 | | | | | 21 |
| 14 | DC State | Desc | Confine Q2 | L1 | 35 | Hex1 | 5 | 21 |
| 15 | Delay | T [ms] | 10 | | | | | 31 |
| 16 | Gas Pulse 1 | T [µs] | 225 | | | | | 31 |
| 17 | Delay | T [ms] | 1 | | | | | 32 |
| 18 | RF Frequency | RF[KHz] | 849.78 | Mode | Manual | ON/OFF | ON | 33 |
| 19 | Delay | T [ms] | 1 | | | | | 34 |
| 20 | Dipolar Excitation | Exc[KHz] | 76.6 | Ampl[mV] | 1800 | T [ms] | 10 | 44 |
| 21 | Delay | T [ms] | 30 | | | | | 74 |
| 22 | Delay | T [ms] | 1 | | | | | 75 |
| 23 | RF Frequency | RF[KHz] | 1100 | Mode | Manual | ON/OFF | ON | 76 |
| 24 | Delay | T [ms] | 1 | | | | | 77 |
| 25 | Resolving DC Q2 | Q2 Res | 0 | m/z | 500 | L1 | 35 | 77 |
| 26 | Delay | T [ms] | 6 | | | | | 83 |
| 27 | DC State | Desc | Confine Q2 | L1 | 35 | Hex1 | 5 | 83 |
| 28 | Delay | T [ms] | 10 | | | | | 93 |
| 29 | RF Frequency | RF[KHz] | 1100 | Mode | Manual | ON/OFF | ON | 94 |
| 30 | Delay | T [ms] | 1 | | | | | 95 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 31 | DC State | Desc | Lift Q2 | L1 | 35 | Hex1 | 6 | 95 |
| 32 | Delay | T [ms] | 10 | | | | | 105 |
| 33 | Gas Pulse 2 | T [µs] | 220 | | | | | 105 |
| 34 | Delay | T [ms] | 2 | | | | | 107 |
| 35 | DC State | Desc | Transfer Q2 | L1 | 35 | Hex1 | 6 | 107 |
| 36 | Delay | T [ms] | 10 | | | | | 117 |
| 37 | Internal Loop End | ACCUMULATION END | | | | | | 1170 |
| 38 | Delay | T [ms] | 5 | | | | | 1175 |
| 39 | Gas Pulse 1 | T [µs] | 225 | | | | | 1175 |
| 40 | Delay | T [ms] | 1 | | | | | 1176 |
| 41 | DC State | Desc | Lift Q8 | L1 | 35 | Hex1 | 6 | 1176 |
| 42 | Delay | T [ms] | 5 | | | | | 1181 |
| 43 | DC State | Desc | Transfer Q8 | L1 | 35 | Hex1 | 6 | 1181 |
| 44 | Delay | T [ms] | 10 | | | | | 1191 |
| 45 | Gas Pulse 2 | T [µs] | 220 | | | | | 1191 |
| 46 | Delay | T [ms] | 2 | | | | | 1193 |
| 47 | DC State | Desc | Lift Q2 | L1 | 35 | Hex1 | 6 | 1193 |
| 48 | Delay | T [ms] | 5 | | | | | 1198 |
| 49 | DC State | Desc | Eject from Q | L1 | 4 | Hex1 | 5 | 1198 |
| 50 | Delay | T [ms] | 10 | | | | | 1208 |
| 51 | RF Frequency | RF[KHz] | 175.88 | Mode | Manual | ON/OFF | OFF | 1209 |
| 52 | Delay | T [ms] | 10 | | | | | 1219 |
| 53 | DC State | Desc | Normal Q2 | L1 | 35 | Hex1 | 5 | 1219 |
| 54 | External Loop End | | | | | | | 1219 |

**Transfer from Q2 to Q8**

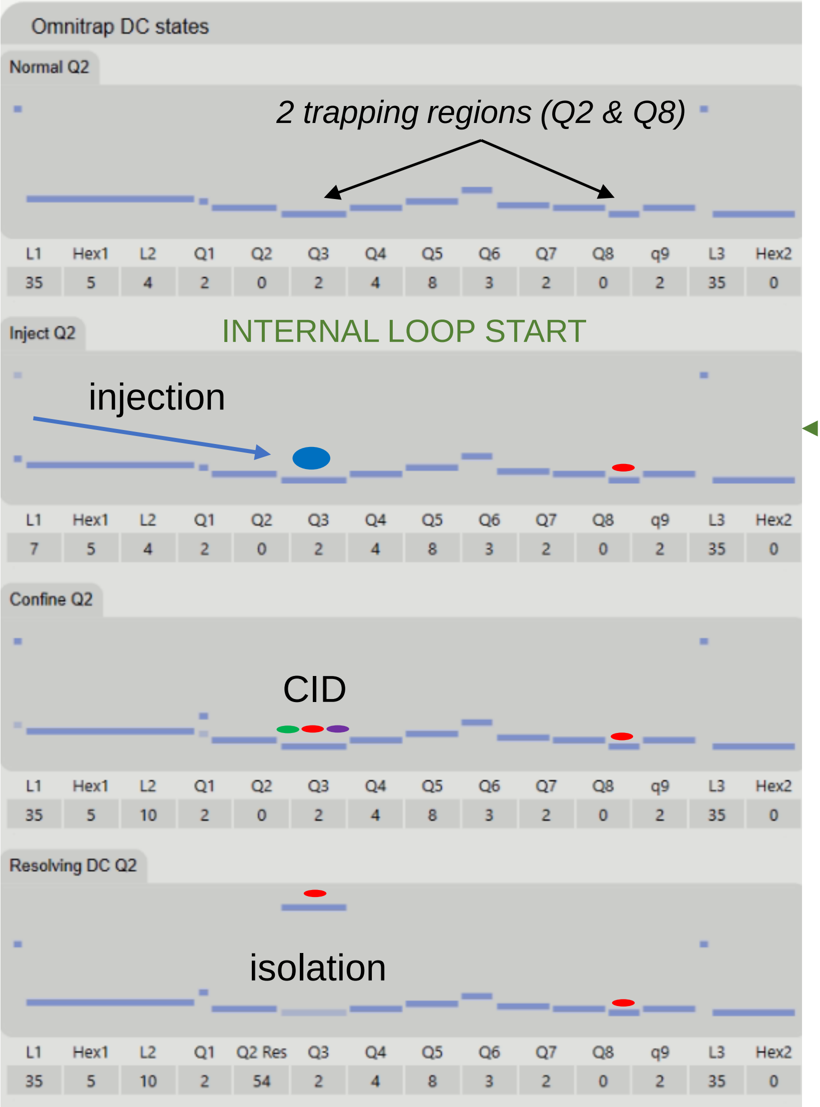
***Store to Q8 for ion accumulation***

**Transfer from Q8 to Q2**

**Ejection from Q2**

# Accumulation mode in MS3 working flow: Omnitrap DC states

Appendix 3



Omnitrap DC states

**Normal Q2**

*2 trapping regions (Q2 & Q8)*

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 4 | 2 | 0 | 2 | 4 | 8 | 3 | 2 | 0 | 2 | 35 | 0 |

**Inject Q2**

INTERNAL LOOP START

injection

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 5 | 4 | 2 | 0 | 2 | 4 | 8 | 3 | 2 | 0 | 2 | 35 | 0 |

**Confine Q2**

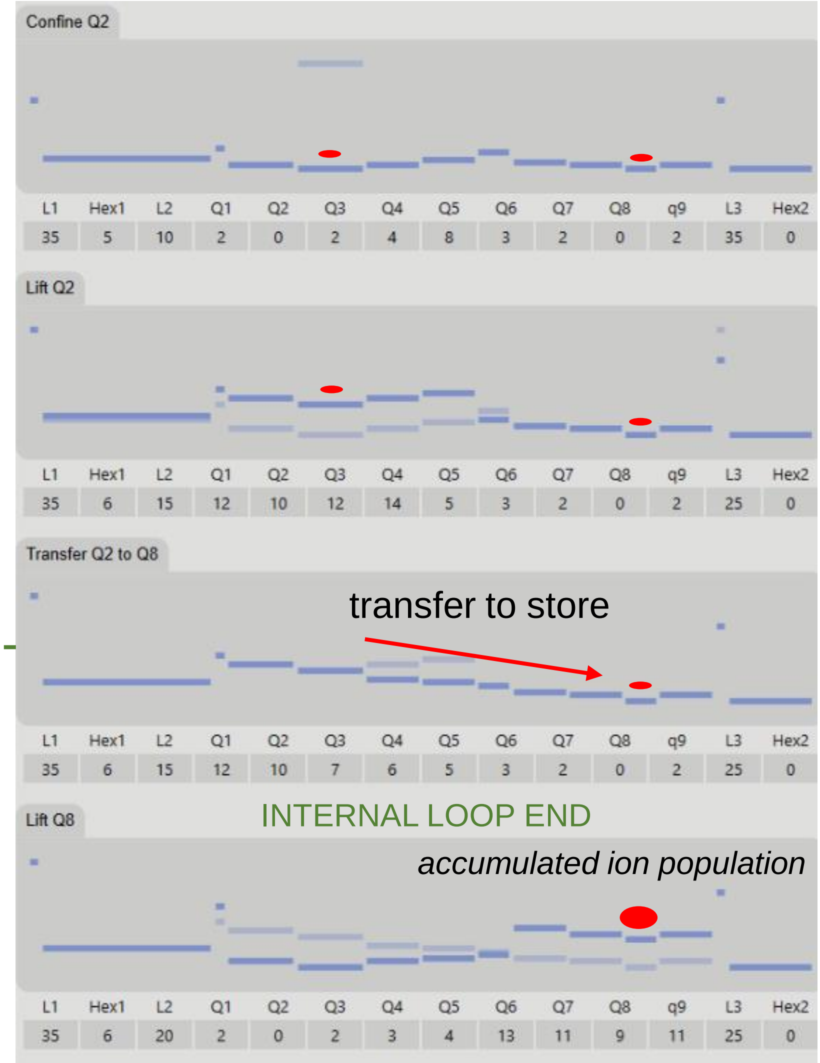
CID

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 10 | 2 | 0 | 2 | 4 | 8 | 3 | 2 | 0 | 2 | 35 | 0 |

**Resolving DC Q2**

isolation

| L1 | Hex1 | L2 | Q1 | Q2 Res | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 10 | 2 | 54 | 2 | 4 | 8 | 3 | 2 | 0 | 2 | 35 | 0 |



**Confine Q2**

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 10 | 2 | 0 | 2 | 4 | 8 | 3 | 2 | 0 | 2 | 35 | 0 |

**Lift Q2**

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 15 | 12 | 10 | 12 | 14 | 5 | 3 | 2 | 0 | 2 | 25 | 0 |

**Transfer Q2 to Q8**

transfer to store

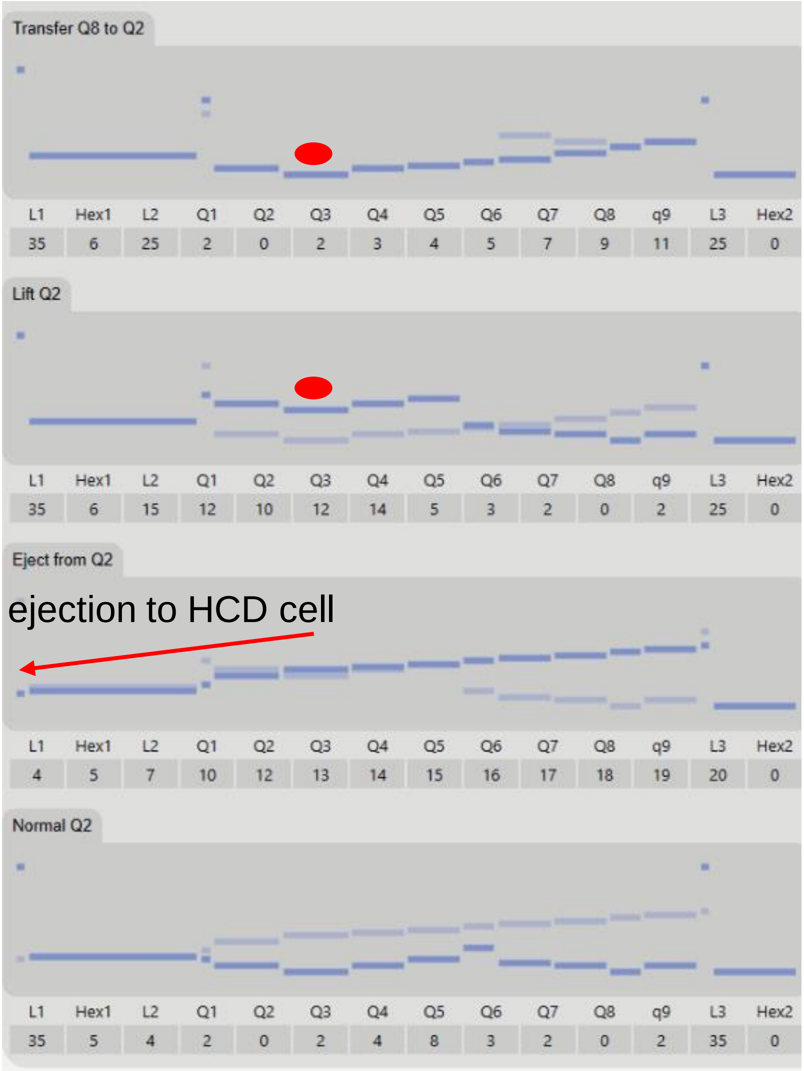
| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 15 | 12 | 10 | 7 | 6 | 5 | 3 | 2 | 0 | 2 | 25 | 0 |

**Lift Q8**

INTERNAL LOOP END

*accumulated ion population*

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 20 | 2 | 0 | 2 | 3 | 4 | 13 | 11 | 9 | 11 | 25 | 0 |



**Transfer Q8 to Q2**

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 25 | 2 | 0 | 2 | 3 | 4 | 5 | 7 | 9 | 11 | 25 | 0 |

**Lift Q2**

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 6 | 15 | 12 | 10 | 12 | 14 | 5 | 3 | 2 | 0 | 2 | 25 | 0 |

**Eject from Q2**

ejection to HCD cell

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 5 | 7 | 10 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 0 |

**Normal Q2**

| L1 | Hex1 | L2 | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | q9 | L3 | Hex2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 5 | 4 | 2 | 0 | 2 | 4 | 8 | 3 | 2 | 0 | 2 | 35 | 0 |

# Accumulation mode – Full Mass Range

Appendix 3

**Accumulation OFF**

**Accumulation ON**

× 2 loop

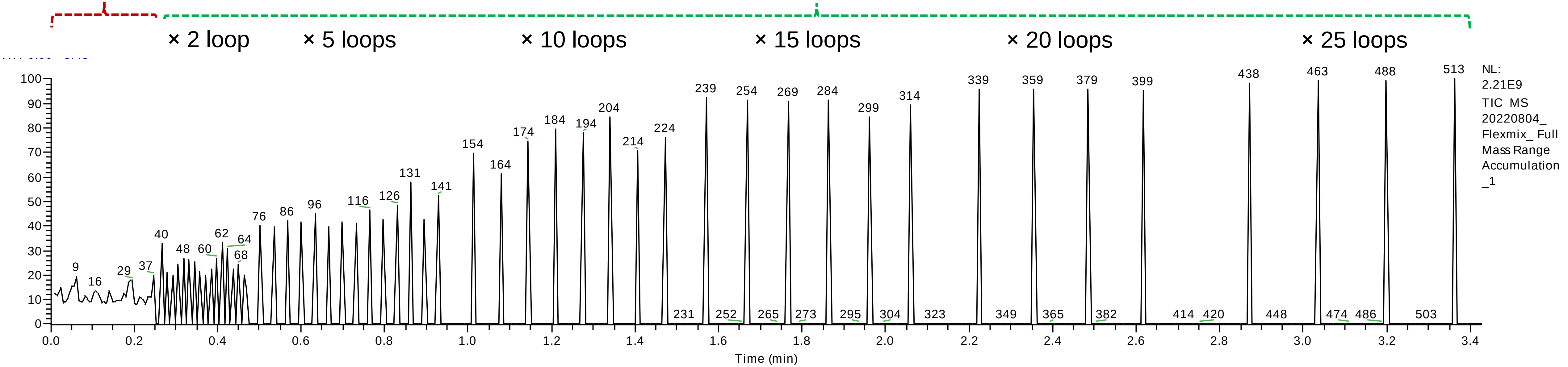
× 5 loops

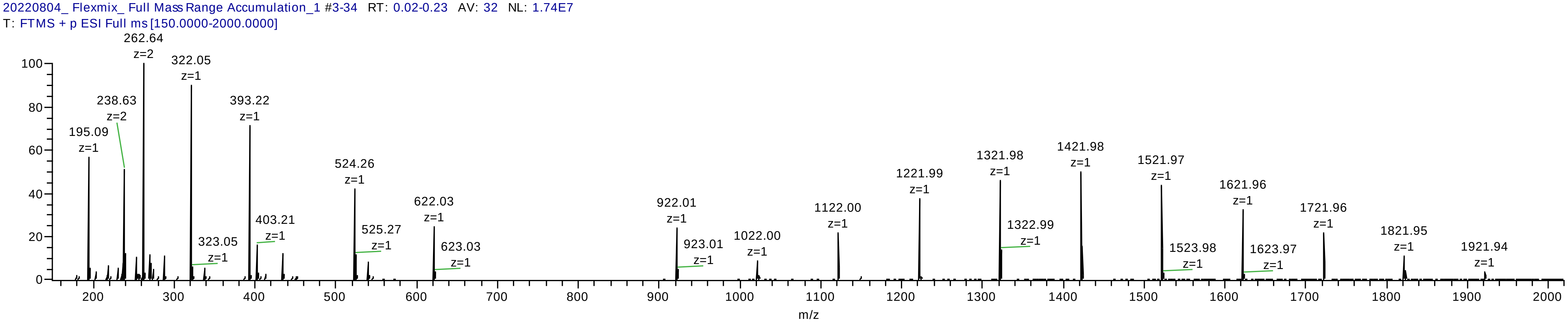
× 10 loops

× 15 loops

× 20 loops

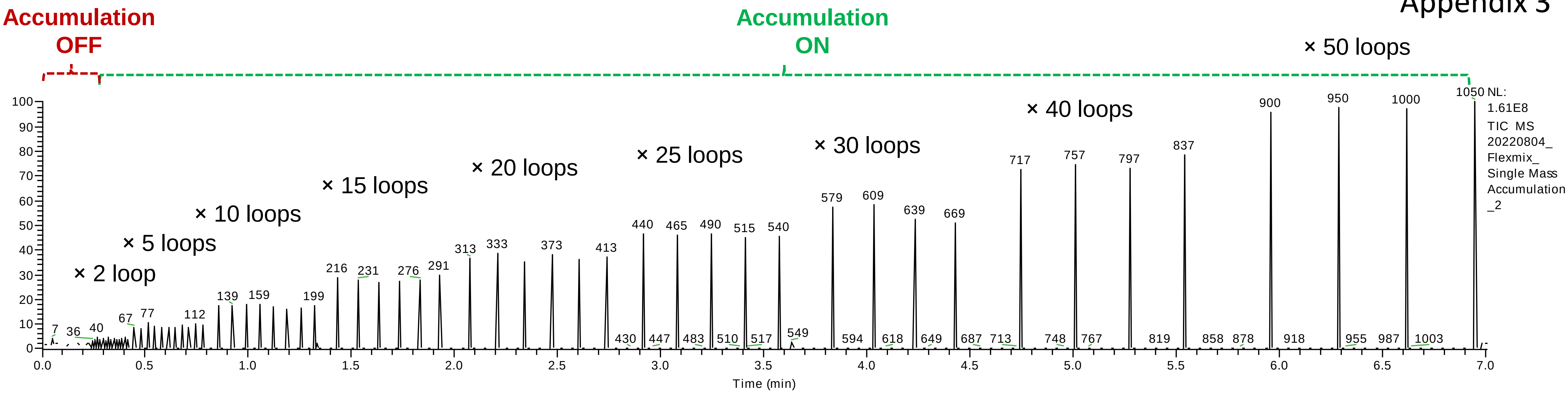
× 25 loops

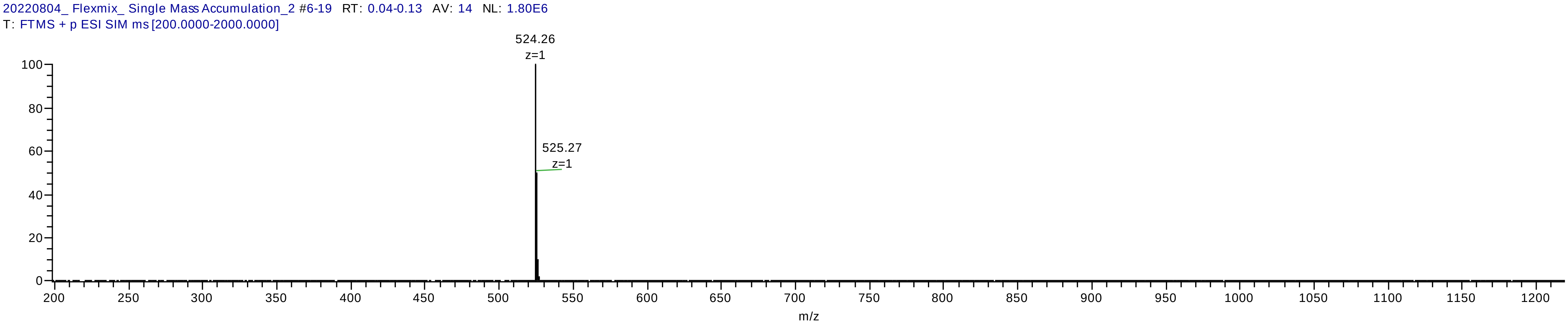




# Accumulation mode – Single Mass (m/z=524)

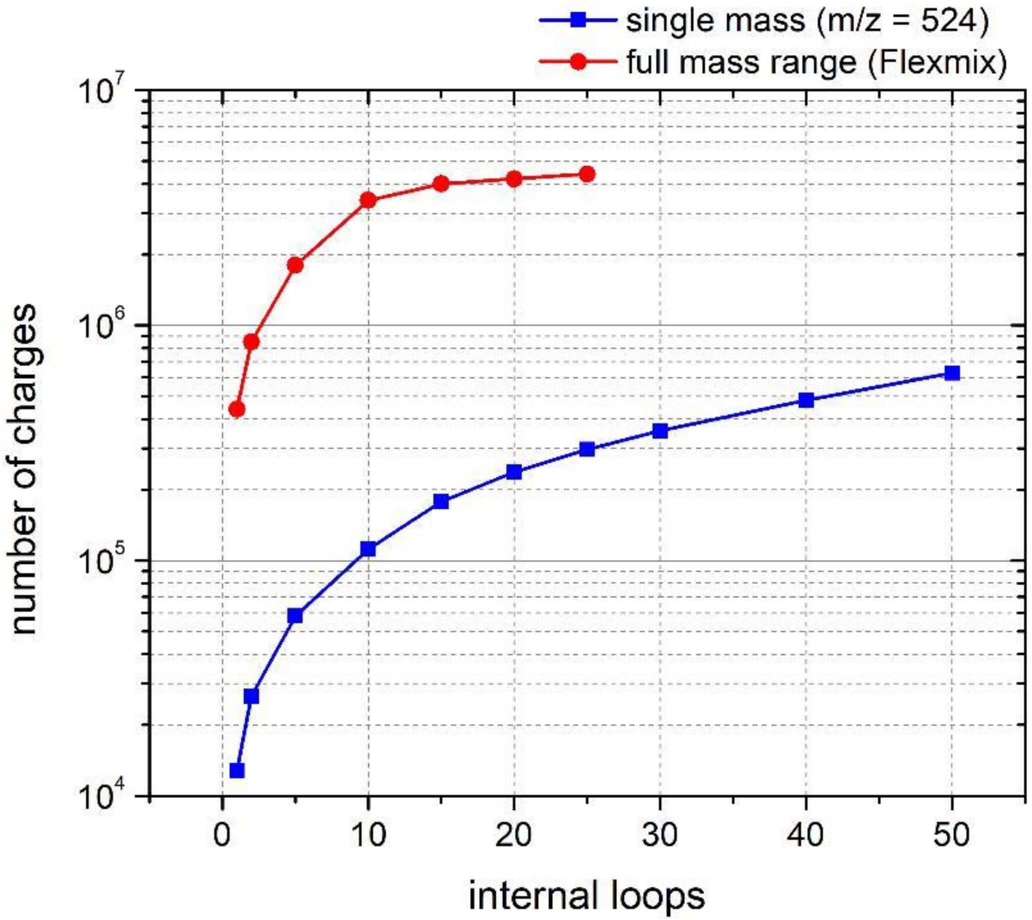
Appendix 3

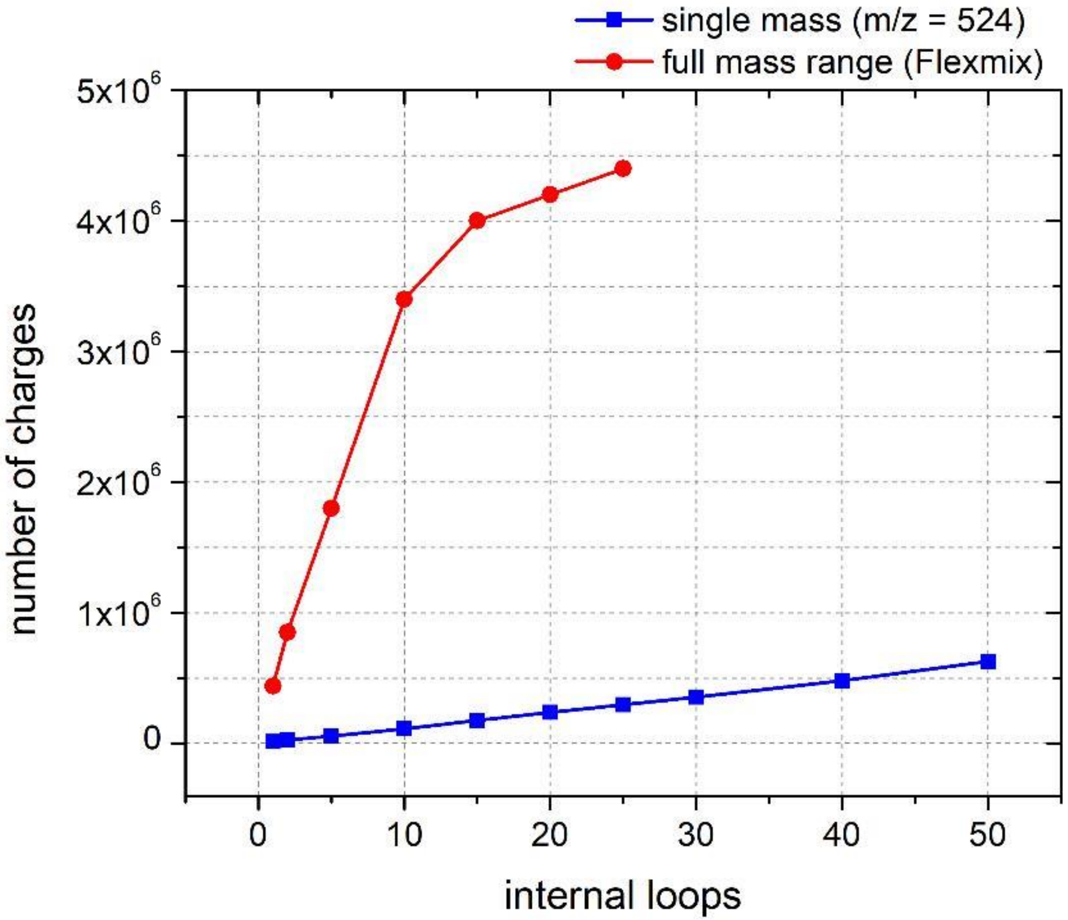




# Ion Accumulation Efficiency

Appendix 3





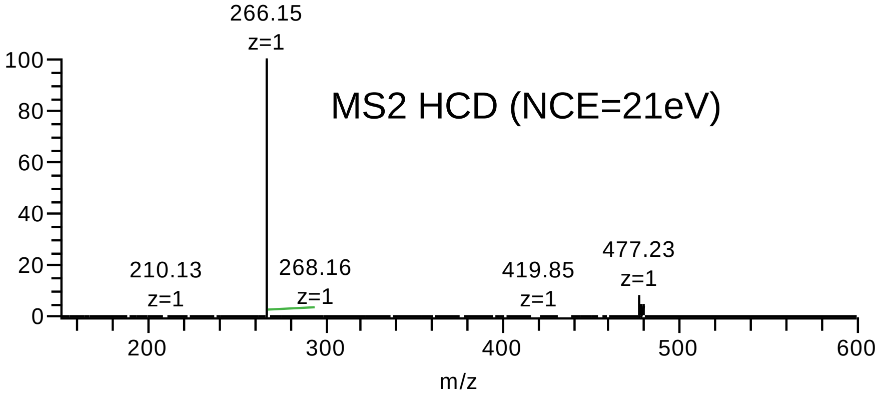
<u>Pass Criterion:</u> Gain >10-fold.

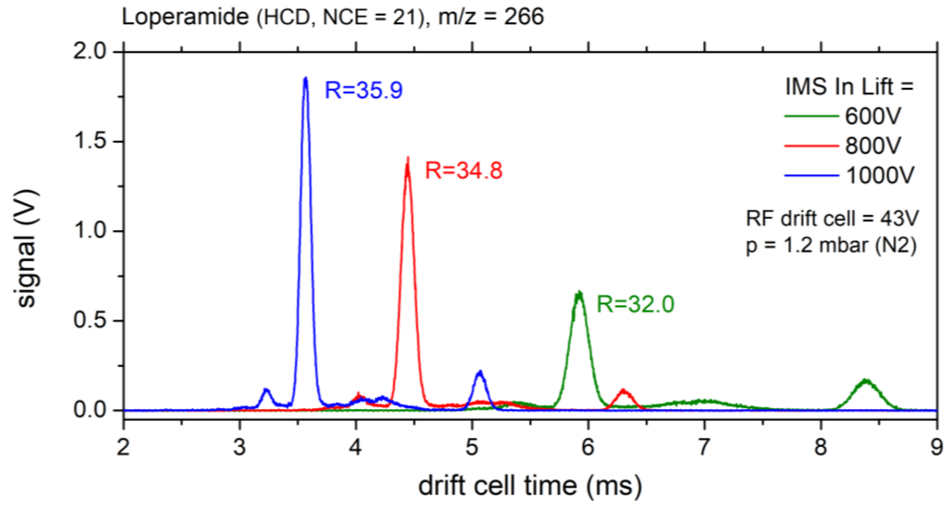
**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

# Ion Mobility Spectrometry experiments

# IMS experiments

## Loperamide:

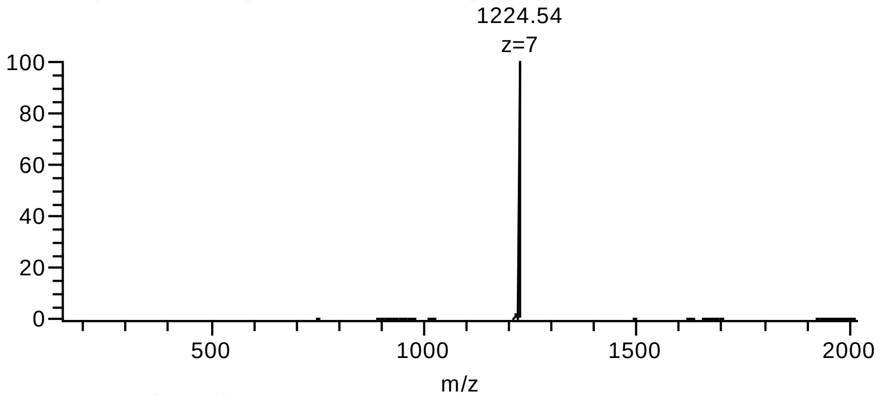




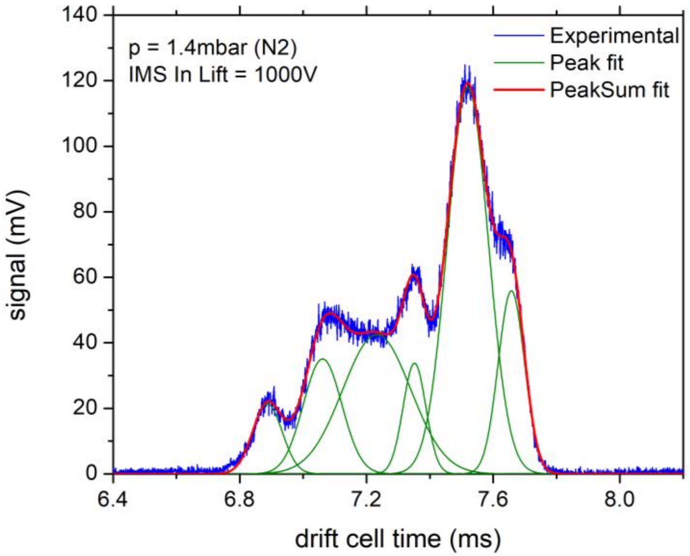
## Appendix 3

IMS resolution up to ~36 achieved for m/z = 266

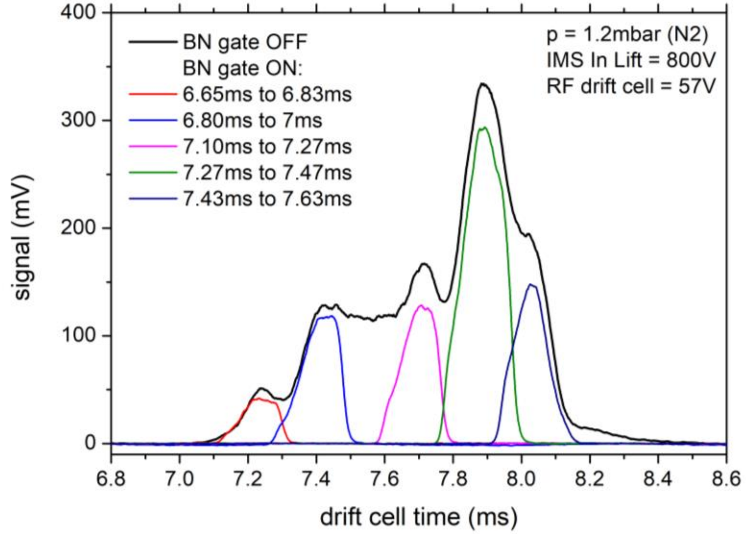
## Ubiquitin (z=7):



### Ion Mobility spectrum:



### BN gate selection experiment:



## Pass Criterion:

- IMS resolution on Loperamide >25
- At least 3 peaks resolved on Ub+7 IMS spectrum

**Pass** ☒ **Conditional Pass** ☐ **Fail** ☐

Appendix 3

# Conclusion

- Omnitrap/IMS/Exploris 480 system has successfully passed all acceptance criteria and was commissioned at Karolinska institute project partner