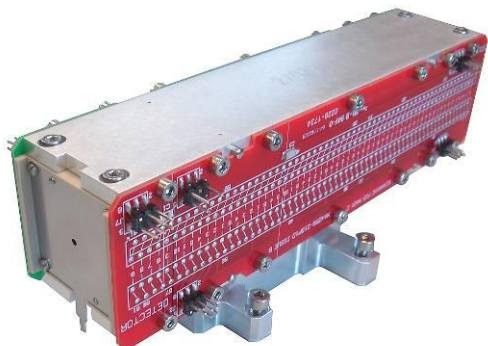


ScanWave Collision Cell Assembly

Part Number:	700004179	Where Used:	Xevo TQ-S / Xevo TQ-XS
---------------------	-----------	--------------------	------------------------

Functional description



- In standard MS modes the collision cell is operated as an ion guide and used to transfer ions from the first to the second quadrupole. When using MS1 mode, the collision gas is switched off, in MS2 mode it is switched on.
- In standard MS/MS modes, argon gas is bled into the collision cell. A DC offset (the collision energy) is then applied to all lenses before the collision cell. This creates a potential gradient that accelerates the ions. Collisions between the ions and the argon atoms in the collision cell result in fragmentation of the ions. The fragments then pass to the second quadrupole.
- In enhanced modes (ScanWave MS and DS) the ions are stored and then ejected from the collision cell according to their m/z . Release of the ions is controlled so that the masses of ions leaving the collision cell are synchronized with the scanning of the second quadrupole. This results in enhanced sensitivity. Collision gas is used in both MS and DS enhanced modes.

Location

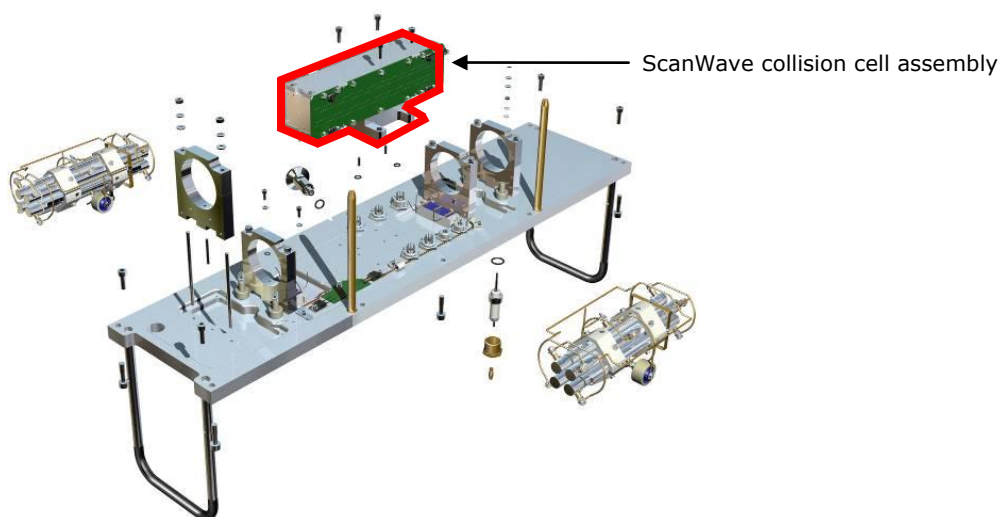
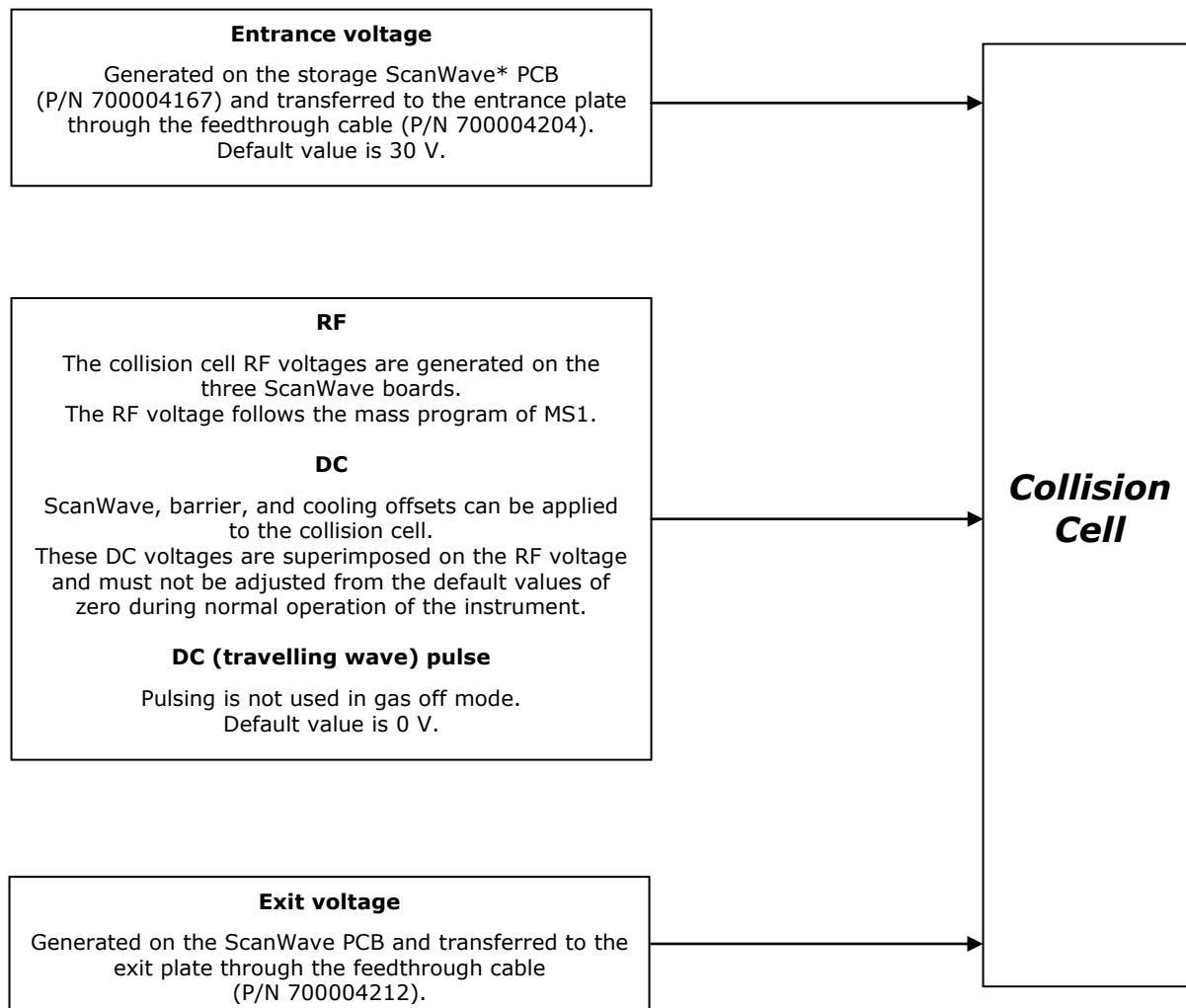


Figure 1 - Underside of the lid assembly (Xevo TQ-S shown)

Inputs/outputs

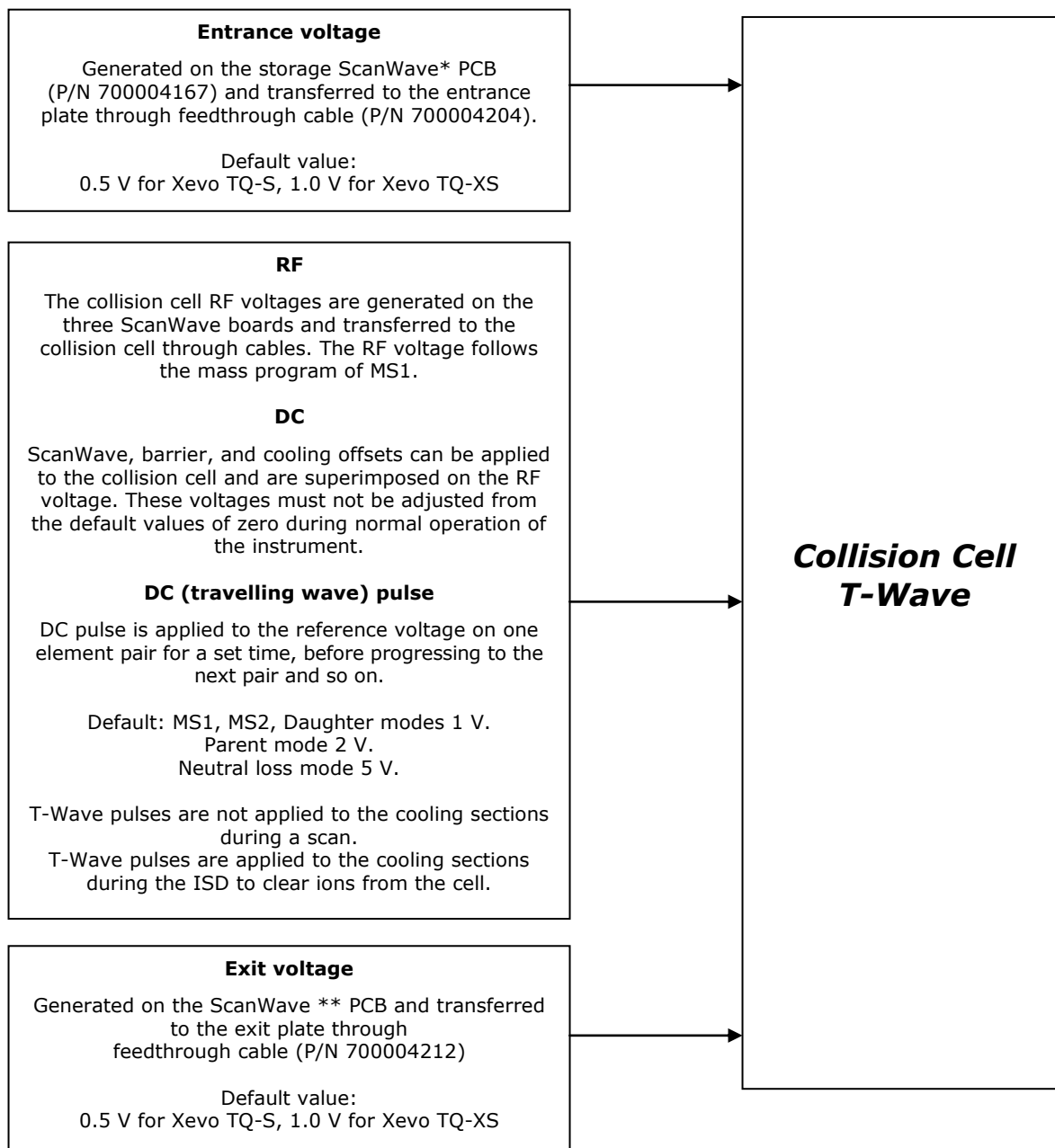
NOTE: *There are no outputs from the collision cell. All readbacks related to the collision cell are generated on the ScanWave PCBs.*

Collision gas off (MS1 mode)



NOTE: ** The storage ScanWave PCB is referred to as the trap ScanWave PCB in some drawings.*

Collision gas on (standard modes - MS, MS/MS)

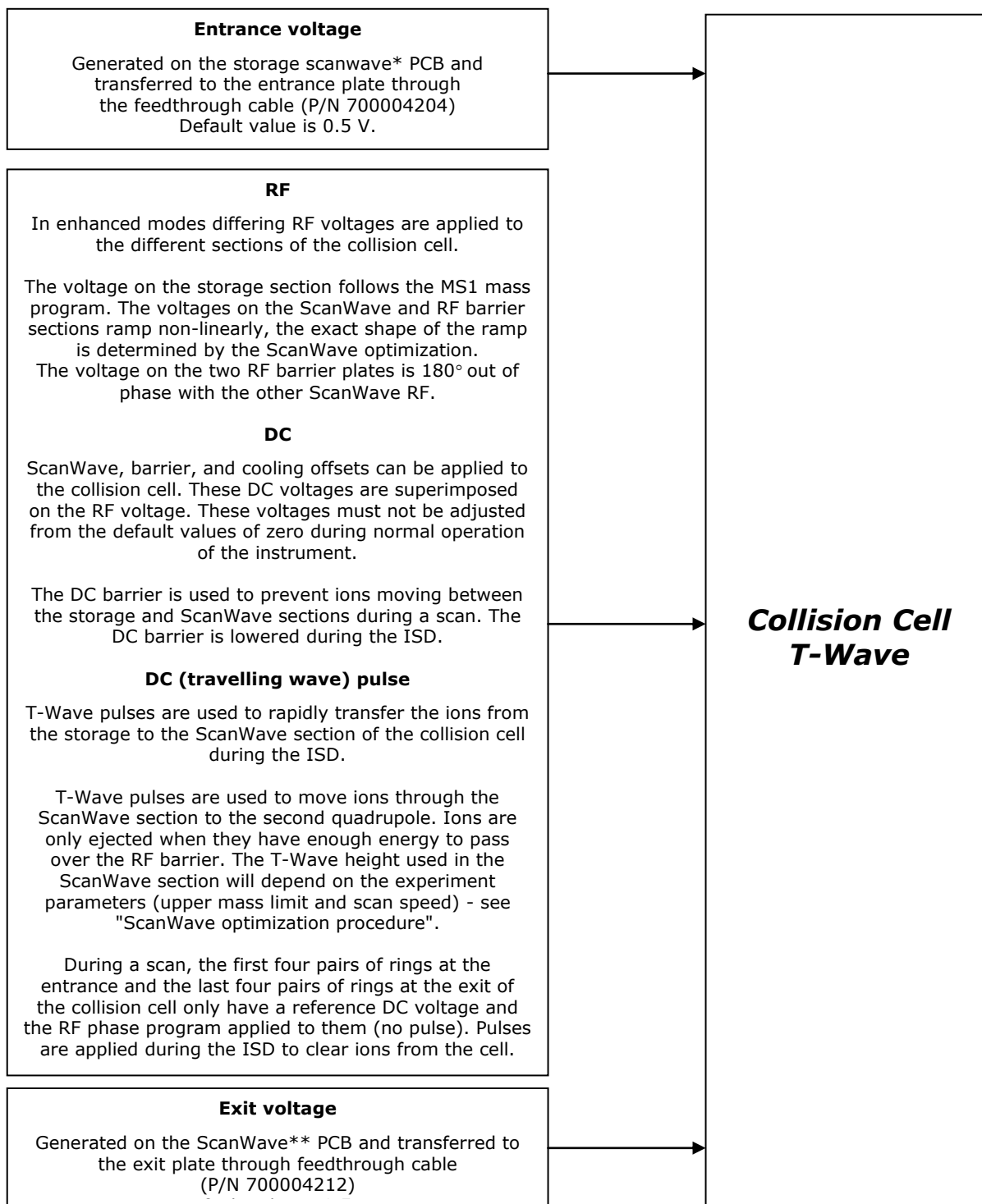


NOTE:

* The storage ScanWave PCB is also referred to as the trap ScanWave PCB in some drawings.

** The ScanWave PCB is also referred to as the analyzer PCB in some drawings.

Collision gas on (ScanWave enhanced modes - MS or DS)



NOTE:

* The storage ScanWave PCB is also referred to as the trap ScanWave PCB in some drawings.

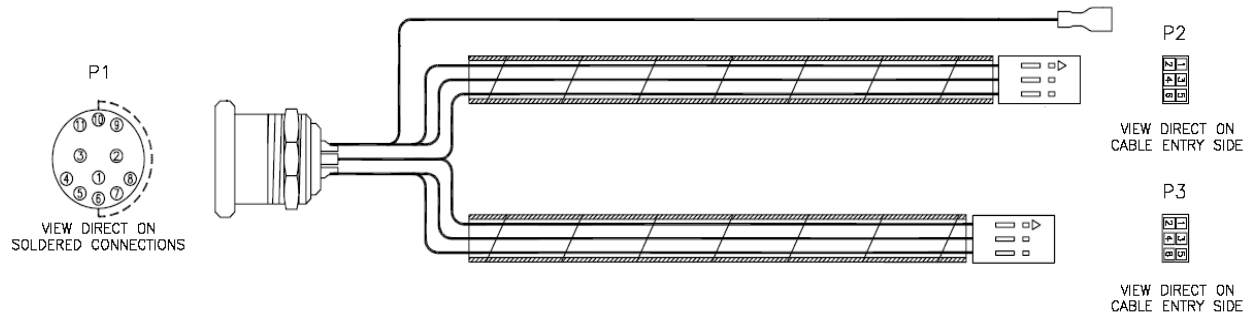
** The ScanWave PCB is also referred to as the analyzer PCB in some drawings.

Connections to the ScanWave PCBs

There are two color-coded PCBs (red and green) on the ScanWave collision cell assembly; each PCB has five connections. There are two further connections for the entrance and exit plates. Cable descriptions are given below.

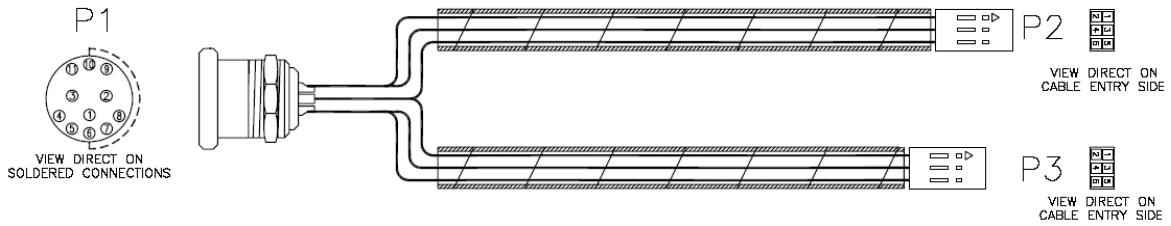
NOTE: In the tables below "POL PIN" denotes the position of the blanking pin used to polarize the connector so it can only fit on one connection point on the collision cell.

Storage section feedthrough (red) - 4265027BC1 (700004204)



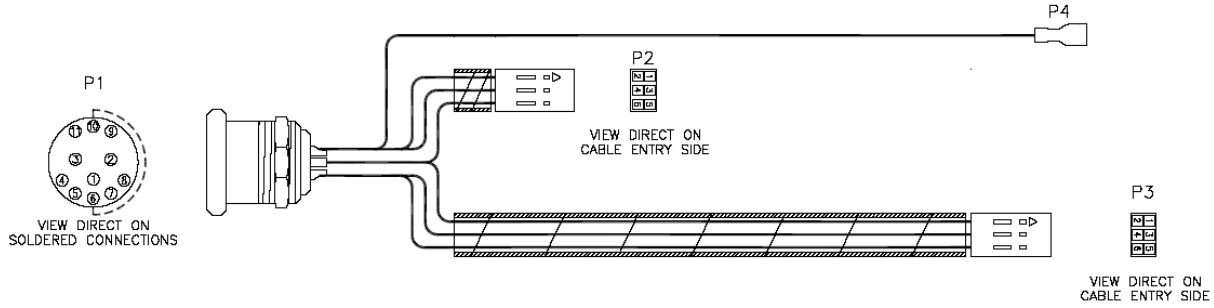
OUT 1 D-type connector from storage PCB pin no.	P1 11-way Fischer pin no.	P2 6-way Harwin pin no.	P3 6-way Harwin pin no.	P4 spade connector	Function
9	1	1	-	-	Storage OUT 1 (storage)
2	2	4	-	-	Storage OUT 2 (storage)
10	3	6	-	-	Storage OUT 3 (storage)
3	4	3	-	-	Storage OUT 4 (storage)
11	5	5	-	-	Storage OUT 5 (storage)
4	6	-	2	-	Storage OUT 6 (cooling)
12	7	-	4	-	Storage OUT 7 (cooling)
5	8	-	3	-	Storage OUT 8 (cooling)
13	9	-	5	-	Storage OUT 9 (cooling)
6	10	-	6	-	Storage OUT 10 (DC barrier)
8	11	-	-	*	ENTRANCE
-	-	2	1	-	POL PIN

Storage section feedthrough (green) - 4265023BC1 (700004211)

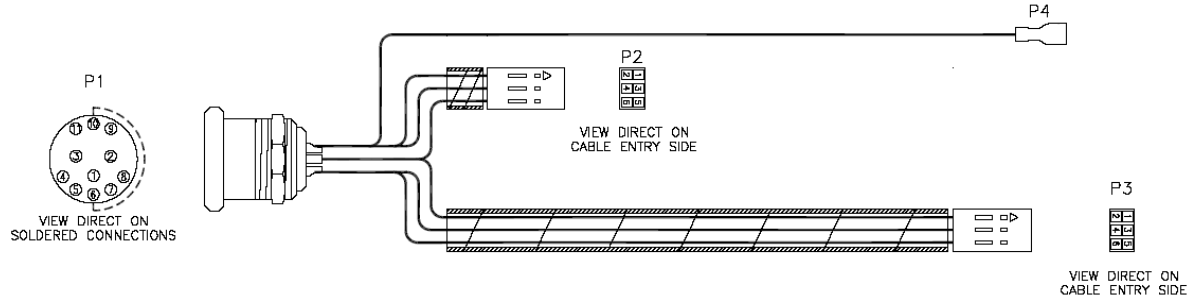


OUT 2 D-type connector from storage PCB pin no.	P1 11-way Fischer pin no.	P2 6-way Harwin pin no.	P3 6-way Harwin pin no.	Function
9	1	1	-	Storage OUT 1 (storage)
2	2	4	-	Storage OUT 2 (storage)
10	3	6	-	Storage OUT 3 (storage)
3	4	3	-	Storage OUT 4 (storage)
11	5	5	-	Storage OUT 5 (storage)
4	6	-	2	Storage OUT 6 (cooling)
12	7	-	4	Storage OUT 7 (cooling)
5	8	-	3	Storage OUT 8 (cooling)
13	9	-	5	Storage OUT 9 (cooling)
6	10	-	6	Storage OUT 10 (DC barrier)
-	-	2	1	POL PIN

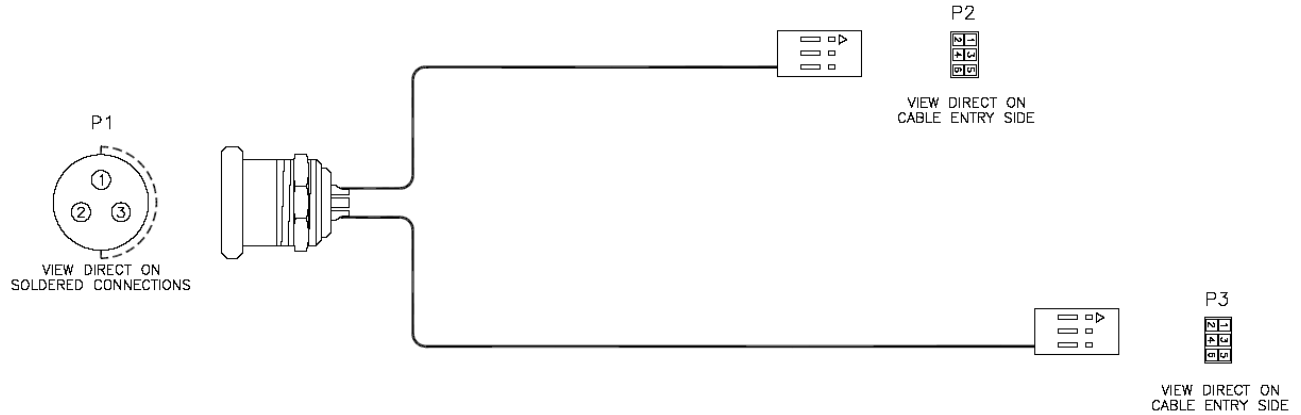
ScanWave section feedthrough (red) - 4265028BC1 (700004212)



OUT 1 D-type connection from ScanWave PCB pin no.	P1 11-way Fischer pin no.	P2 6-way Harwin pin no.	P3 6-way Harwin pin no.	P4 spade connector	Function
9	1	6	-	-	ScanWave OUT 1 (ScanWave)
2	2	1	-	-	ScanWave OUT 2 (ScanWave)
10	3	3	-	-	ScanWave OUT 3 (ScanWave)
3	4	2	-	-	ScanWave OUT 4 (ScanWave)
11	5	4	-	-	ScanWave OUT 5 (ScanWave)
4	6	-	4	-	ScanWave OUT 6 (cooling)
12	7	-	1	-	ScanWave OUT 7 (cooling)
5	8	-	3	-	ScanWave OUT 8 (cooling)
13	9	-	5	-	ScanWave OUT 9 (cooling)
6	10	-	2	-	ScanWave OUT 10 (ScanWave)
8	11	-	-	*	EXIT
-	-	5	6	-	POL PIN

ScanWave section feedthrough (green) - 4265029BC1 (700004214)

OUT 2 D-type connection from ScanWave PCB pin no.	P1 11-way Fischer pin no.	P2 6-way Harwin pin no.	P3 6-way Harwin pin no.	Function
9	1	6	-	ScanWave OUT 1 (ScanWave)
2	2	1	-	ScanWave OUT 2 (ScanWave)
10	3	3	-	ScanWave OUT 3 (ScanWave)
3	4	2	-	ScanWave OUT 4 (ScanWave)
11	5	4	-	ScanWave OUT 5 (ScanWave)
4	6	-	4	ScanWave OUT 6 (cooling)
12	7	-	1	ScanWave OUT 7 (cooling)
5	8	-	3	ScanWave OUT 8 (cooling)
13	9	-	5	ScanWave OUT 9 (cooling)
6	10	-	2	ScanWave OUT 10 (ScanWave)
-	-	5	6	POL PIN

RF barrier feedthrough - 4265030BC1 (700004210)

D-type connection from RF barrier PCB pin no.	P1 11-way Fischer pin no.	P2 6-way Harwin pin no.	P3 6-way Harwin pin no.	Function
13 (OUT 1)	1	5	-	BARRIER OUT 1 (RF Barrier)
13 (OUT 2)	2	-	5	BARRIER OUT 2 (RF Barrier)
-	-	1, 3, 4	1, 3, 4	POL PIN

Diagnostic procedures

Problems with the collision cell can cause poor sensitivity in MS/MS, ScanWave, and 'gas on' modes, and crosstalk in MRM experiments. Causes of these problems can include:

- Incorrect collision cell parameters (for example; entrance, exit, T-Wave, collision cell gas pressure)
- Incorrectly connected ScanWave cables
- Defective ScanWave PCBs, cables, or collision cell
- Dirty collision cell
- Bent or misaligned plates (entrance, exit plates, or ring pairs within the collision cell)

The following checks can be used to diagnose collision cell issues:

Table 1: Diagnostic tests

Test	Description
Dual scan function test	Use for diagnosing general problems with the collision cell and connections and as an initial check if gas cell performance is in doubt.
Crosstalk test	Use if the customer is complaining of cross talk in their MRM methods.
ScanWave PCB readbacks check	Use if dual scan function indicates a problem, see ScanWave PCB Technical Reference Sheet (P/N 715002671).
ScanWave PCB output check	Use if dual scan function fails, see ScanWave PCB Technical Reference Sheet (P/N 715002671).
Continuity check	Use if no faults can be found with the ScanWave PCB outputs (cables and connections).

NOTE: *The ScanWave enhancement check must be run if the collision cell has been removed from the instrument during diagnostics or maintenance. If the check fails, run the ScanWave optimization.*

MS/MS operation - dual scan collision cell diagnostic test

The dual scan test is used to assess the performance of the collision cell. Faults that can be diagnosed include bent plates, incorrectly placed entrance and exit lenses, wiring faults, and capacitance issues on the cables or the ScanWave PCBs.

Tune page scope parameters

The scan time is critical to the diagnostic side of this test. All of the information gathered is based on a scan time of 0.5 s and an Interscan time of 0.1 s.

NOTE: *Decreasing this time will make it more likely for a collision cell to falsely fail this test.*

1. Open an MS1 scan in window 1.

NOTE: *For Xevo TQ-S instruments, use Xevo TQ-S setup solution. For Xevo TQ-XS instruments, use Xevo TQ-XS setup solution.*

2. Infuse setup solution at 10 µL/min.
3. Tune only the source voltage parameters to optimize the 455.27 Da peak intensity.
4. Leave the temperatures on the source at **150 °C**, and the desolvation at **150 °C**.
5. Set the FWHH of the 455.27 Da to **0.5 Da**.
6. Record the exact position of the most intense part of the 455 Da peak. This will become the "set" mass.
7. Open a daughter scan window.
8. Turn on the collision gas and click the **MSMS** button to open the MS/MS default tuning parameters.
9. Set the entrance and exit value:
 - 0.5 V for Xevo TQ-S
 - 1.0 V for Xevo TQ-XS
10. Set the collision energy to **30 V**.
11. Set the collision cell gas flow rate to **0.15 mL/min** to achieve a pressure of **3.5E-3 mBar**.

NOTE: *Set = the exact mass, recorded earlier, of the MS1 455 Da peak.
Mass = 165 Da.*

12. There will now be a daughter peak. Record the exact position of the most intense part of the 165 Da peak. Change the mass of this window to this value.
13. Open a parent scan window.

NOTE: *Set = the exact mass, recorded earlier, of the 165 Da daughter peak.
Mass = the exact mass of the MS1 455 Da peak.*

14. Open a neutral loss scan window.

NOTE: *Set = the exact mass of the MS1 455 Da peak minus the exact mass, recorded earlier, of the 165 Da daughter peak. This will be 290** Da.
Mass = the exact mass of the MS1 455 Da peak*

15. Close the MS1 scan.
16. Set all three windows to the same "gain" value that exactly maximizes the daughter scan peak to just touch the top of its window.

T-Wave peak intensity

The software sets the travelling wave pulse voltage as follows:

- Daughter scan ScanWave tab/wave height = 1.0 V
- Parent scan Always 3 V
- Neutral loss Always 4 V

With these settings, the daughter peak will just fill the window. The parent peak may be a little lower and the neutral loss peak may be even less, see [Figure 2](#).

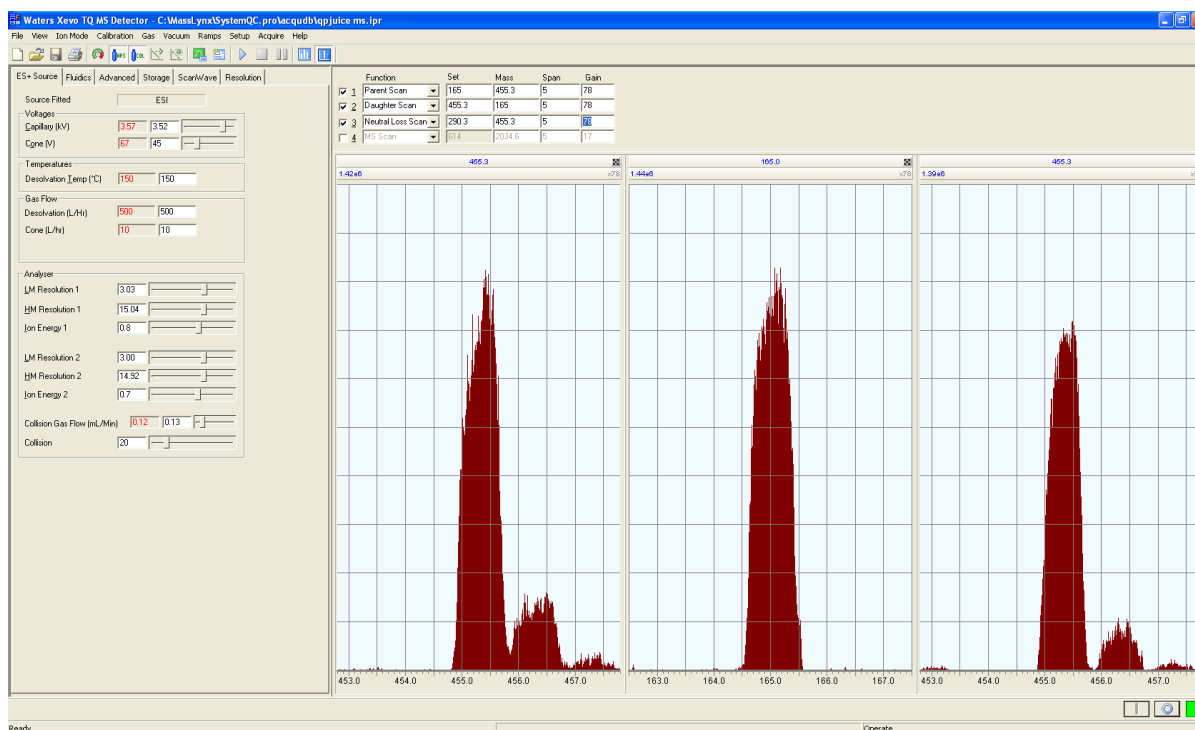


Figure 2 - Dual scan function test (example of good test)

Perform the following procedure to set the wave pulse voltage for all three peaks to 0.1 V:

1. Click **Start > Run** and type **telnet epc** then click **OK**.
2. Enter or copy/paste the following phrases:

NOTE: *Telnet is case sensitive and there are no spaces in the phrases.*

```
s_dParentScanTWaveHeight = 0.1      <enter>
s_dNeutralLossTWaveHeight = 0.1     <enter>
s_dParentScanTWaveVelocity = 300.0  <enter>
s_dNeutralLossTWaveVelocity = 300.0 <enter>
```

3. On the ScanWave Tune page tab, change the Wave Height to **0.1 V**.

NOTE: By reducing the traveling wave pulse voltage, it is possible to trap ions within the collision cell for the parent ion scan. However, this effect must be minimal at the slow scan speed of 0.5 s with an Interscan delay of 0.1 s.

Ion trapping is identifiable as a large extended shoulder on the low mass side of the parent ion (see Figure 3). The intensity of the daughter and parent ions also decreases. This happens after the T-Wave heights have been changed to 0.1 for parent, daughter, and neutral loss.

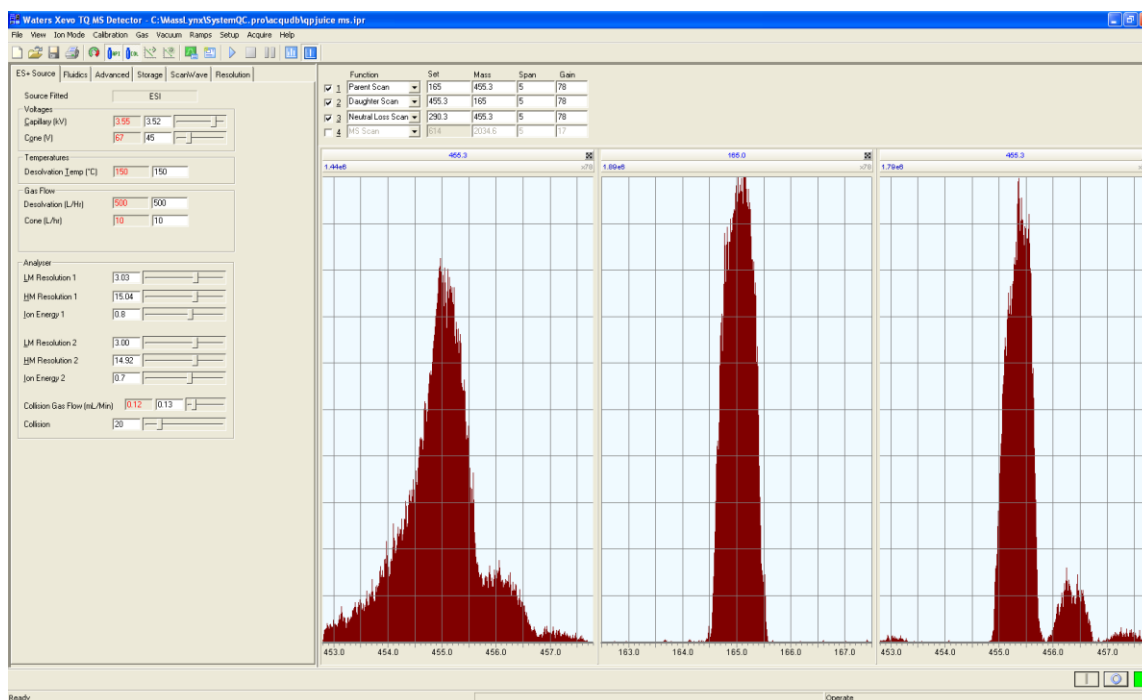


Figure 3 - Dual scan function test (example of bad test - ion trapping in parent mode)

NOTE: If any of the collision cell diagnostic checks fail, contact GSS before removing the collision cell.

Daughter ion

The daughter ion intensity should not degrade when using the lower pulse height. Perform the following procedure if degradation occurs:

1. Ensure that the collision cell vacuum level is between 3 to 4E-3 mBar, as too much gas may trap the ions.
2. Ensure that the drop in intensity is <10% with the entrance and exit lens voltages set to 0.5.

NOTE: If the drop in intensity is >10%, this could indicate ions are being trapped between the cooling region of the collision cell and the exit lens. This may be due to either bent plates or a poorly positioned exit lens; contact GSS.

Parent ion

With the pulse heights set at 0.1 V for all three windows and using the correct scope speeds, the parent ion must not achieve a shoulder greater than 10% of the peak height, nor a leading edge greater than 1.5 Da.

If the instrument fails this diagnosis test, perform the following procedure:

1. Ensure that the collision cell vacuum level is between 3 to 4E-3 mBar, as too much gas may trap the ions.
2. If the gas pressure is OK, but the parent ions are still being trapped then slowly increase the entrance and exit lens voltages from 0.5 V in 0.1 V increments.
3. If the parent ion intensity and peak shape is restored by an entrance and/or exit lens voltage of 1 V, then the amount of trapping is acceptable.
4. If more than 1 V is required, there may be a problem with the collision cell; contact GSS.

Peak heights

Once the daughter and parent ion peak shapes have been corrected, by either collision cell rebuild or entrance/exit voltages, the three peaks must be the same height within 10% and approximately the same peak width.

Resetting the pulse voltages to their correct values

WARNING: The pulse heights must be returned to the correct values. To guarantee this is done properly, check in the ScanWave Tune page tab that Wave Height = 1.0 V.

Perform the following procedure:

1. Click **Start > Run** and type **telnet epc** then click **OK**.
2. Enter or copy/paste the following phrases:

NOTE: *Telnet is case sensitive and there are no spaces in the phrases.*

```
s_dParentScanTWaveHeight = 3.0          <enter>
s_dNeutralLossTWaveHeight = 4.0          <enter>
s_dParentScanTWaveVelocity = 900.0        <enter>
s_dNeutralLossTWaveVelocity = 900.0       <enter>
```

3. Set the exit and entrance values back to their default setting.

Rapid MRM ScanWave cell diagnostic test

NOTE: *This tests the performance of the instrument when it is parked on a single MRM channel. As this instrument can use 1 ms dwell times, the beam has to be stable at the start of the acquisition so that sensitivity is not impaired. This test allows the user to see any variation in sensitivity at different dwell times. Any failures have been shown to be due to the cleanliness and build of the collision cell. To check the ScanWave cell for contamination, refer to the crosstalk test.*

Use default pulse heights and speeds for the test:

- Scan time 0.01 s
- Manual interscan / interchannel times 0.003 s
- Set the advanced Tune page settings to automatic

Procedure

1. Open a single MSMS mode daughter ion Tune page window using a set mass 455.27 Da, and looking at the 165.1 Da peak with a span of 3.
2. Set the advanced Tune page settings to automatic.

NOTE: *For Xevo TQ-S instruments, use Xevo TQ-S setup solution. For Xevo TQ-XS instruments, use Xevo TQ-XS setup solution.*

3. Infuse the setup solution with the following settings:
 - Infusion rate 10 $\mu\text{L}/\text{min}$
 - Collision gas flow rate 0.15 mL/min
 - Collision energy 30 V
 - Entrance and exit voltage (Xevo TQ-S) 0.5 V
 - Entrance and exit voltage (Xevo TQ-XS) 1.0 V
4. Check that the daughter peak (transition 455.27 > 165.1), is present and stable with a scope speed of 0.5 s scan, 0.1 s interscan delay.

NOTE: *If steps 1 to 4 achieve a stable beam on the Tune page, continue to step 5. If any drop outs are present investigate the probe and fluidics system for leaks.*

- In the sample list page, create an experiment called Scanwave_Trapping_Fast using one MRM function (Figure 4). Disable any method events associated with this experiment.

Function:1 MRM

Method

Ionization Mode: ES+ (dropdown)

Span: 0 (text box)

Use Tune Cone Voltage: ☒

Use Tune Collision Energy: ☐

Retention Window (Mins)

Start: 0 (text box)

End: 0.5 (text box)

Channels

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)
1	Verapamil	455.2700	165.1000	☐	0.001

Figure 4 - ScanWave_Trapping_Fast setup

- In the sample list page, create a second experiment called Scanwave_Trapping_Slow using one MRM function (Figure 5). Disable any method events associated with this experiment.

Function:1 MRM

Method

Ionization Mode: ES+ (dropdown)

Span: 0 (text box)

Use Tune Cone Voltage: ☒

Use Tune Collision Energy: ☐

Retention Window (Mins)

Start: 0 (text box)

End: 0.5 (text box)

Channels

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)
1	Verapamil	455.2700	165.1000	☐	0.1

Figure 5 - Scanwave_Trapping_Slow setup

- On the sample list add two rows called Scanwave_Trapping_Fast and Scanwave_Trapping_Slow containing the appropriate ms experiment files.

	File Name	File Text	MS File
1	Scanwave_Trapping_Fast	Dwell = 0.001s, ISD = 0.003s	ScanWave_Trapping_Fast
2	Scanwave_Trapping_Slow	Dwell = 0.1s, ISD = 0.003s	ScanWave_Trapping_Slow

Figure 6 - Sample list entries

- Check that there is more than half a syringe full of sample remaining in the fluidics system. Refill if necessary and infuse until the beam is stable again.
- Acquire both runs.
- When the acquisitions are complete open the chromatograms from both runs.

11. Smooth each mass chromatogram using the following parameters.

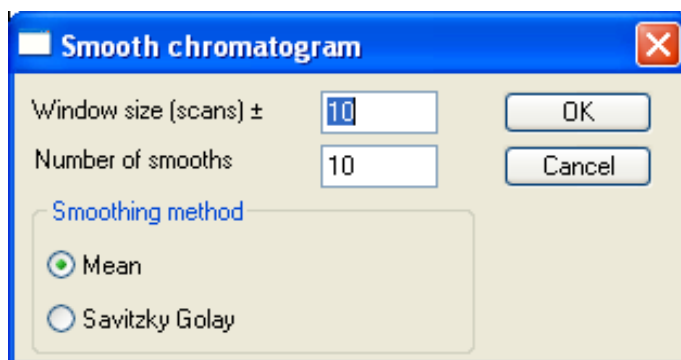


Figure 7 - Smoothing parameters

12. Calculate the percentage difference between the fast and slow MRM traces using the intensity of the smoothed acquisitions in the equation shown in Figure 8:

$$\%Diff = 100 \times \frac{\text{Intensity Scanwave_Trapping_Fast}}{\text{Intensity Scanwave_Trapping_Slow}}$$

Figure 8 - %Diff equation

13. Printout the chromatogram window displaying both smoothed extracted chromatograms and labelled with the calculated percentage.



Figure 9 - Chromatogram display

Specification

The intensity of the fast scan must be $\geq 80\%$ that of the slow scan. If the intensity is lower than 80%, this implies trapping within the ScanWave. This may be the result of poor cleanliness or bent plates in the ScanWave.

Fast crosstalk test

The crosstalk test checks for cleanliness and confirms that ions are not being trapped in the collision cell. Ion trapping is usually caused by the following:

- Incorrect collision cell parameters (for example; entrance, exit, T-Wave, collision cell gas pressure)
- Dirty collision cell
- Bent or misaligned plates

NOTE: For the crosstalk test, use Xevo TQ-S setup solution for Xevo TQ-S instruments, and use Xevo TQ-XS setup solution for Xevo TQ-XS instruments.

Before performing the test, ensure that the ICT is set to 30 and that the Spike Removal check box is selected.

LC conditions

1. Set the LC pump flow rate to 0.2 mL/min, using 70:30 acetonitrile/water as the mobile phase.
2. Ensure that there are no leaks from the ports and that the pump flow is stable.

Tuning

1. In combined flow mode, optimize the daughter ion fragmentation of verapamil using setup solution (455 to 165 Da).
2. Set the desolvation temperature to 600 °C to cope with the high LC flow rate.

MS acquisition

1. Using the exact* "set" mass for verapamil ($\sim 455.3 \pm 0.1$ Da) and the verapamil daughter ion ($\sim 165.3 \pm 0.1$ Da) set up an MRM acquisition to monitor the following transitions, ensuring the parameters in [Table 3](#) are used:

NOTE: * Recorded from the Tune page when tuning.

Table 2: Crosstalk MRM acquisition setup

Parent	Daughter	Dwell(s)
455.27*	165.3*	0.001
1000	165.3*	0.001
1500	165.3*	0.001

Table 3: Crosstalk MRM acquisition setup parameters

Parameter	Setting
Mode	ESI +ve
Span	0
ISD	0.003
ICD	0.003
Run time	60 minutes

Method

1. Start the acquisition and open the Chromatogram window.
2. Click **Display > Mass > Ch1** (then **CH2** and **CH3**) and monitor all three MRM channels.
3. Allow the solvent to run until the 455 to 165 chromatogram falls to a similar level to the 1000 to 165 channel.
4. Click the **Inject** button on the Fluidics tab.

NOTE: *If the baseline rises by more than 100%, wait until the noise falls back to the original level. Click **Refill** on the fluidics, then click the **Inject** button and check that the baseline remains steady.*

5. Make three injections of setup solution.

Specification

1. Ensure that the response to the injections in the 455 to 165 trace is between 1 and 2E7. If necessary, adjust the probe position to get the correct intensity.
2. Inspect the chromatograms for the three channels:
 - 1500 to 165 channel
 - 1000 to 165 channel
 - 455 to 165 channel
3. If there is a response in one or both of the dummy channels (1500/1000 to 165) then click **Edit > Integrated Peaks** and click **Add** on each of the peaks from all three channels in each chromatogram to integrate their areas.

NOTE: *The ratio of the average peak areas between the response for 1000 to 165 and 455 to 165 must be <0.01% (>5000:1).*

The ratio of the average peak areas between the response for 1500 to 165 and 455 to 165 must be less than 0.005% (>10000:1).

Diagnostic only

1. Reposition the probe away from the sample cone as far as necessary to achieve a signal intensity as close to 1.00E7 as possible, then repeat the experiment.
2. If the gas cell cleanliness test fails, repeat the test with the second channel set to 1000 to 900 Da (this is a completely dummy channel).
3. If the test still fails, contact GSS.

Checking the continuity of the ScanWave cables

Shorts and discontinuities within the collision cell or ScanWave cables and feedthroughs can be checked without venting the instrument.

1. Ensure the instrument is in Standby.
2. Disconnect the six D-type connectors from the ScanWave PCBs.
3. Using a DVM, ensure there is continuity between corresponding pins for example, between pin 1 on the Fischer connector and pin 6 on the Harwin connector, shown in the table on page 7.

NOTE: Use the DVM to either measure the resistance (Pass = $<0.5 \Omega$), or use the built-in continuity test function.

4. Using a DVM, ensure there is no continuity between other pins for example, between pin 1 on the Fischer connector and pins 1 to 5 on the Harwin connector, shown in the table on page 7.

NOTE: Use the DVM to either measure the resistance (Pass = $>1 M\Omega$), or use the built-in continuity test function.

Checking the continuity of the ScanWave connections

The analyzer lid must be removed to check the continuity of the collision cell connections and wiring.

WARNING: The ScanWave collision cell assembly could be contaminated with biohazardous and/or toxic materials; chemical-resistant, powder free latex or nitrile gloves must always be worn when handling the ScanWave assembly.

1. Vent the instrument.
2. Remove the analyzer lid with the ScanWave cables still connected to the lid.
3. Using a DVM, ensure there is continuity between the OUT1 D-type pins and corresponding outer solder points on the red ScanWave PCB (Figure 10).

NOTE: Use the DVM to either measure the resistance (Pass = $<0.5 \Omega$), or use the built-in continuity test function.

Table 4: Checking continuity on the ScanWave connections

Section	Action
ScanWave	Check continuity between the pins on the D-type connectors to the corresponding solder points. Figure 11 shows examples of where to check ScanWave outputs 1, 3, and 5.
Storage	Check continuity between the pins on the D-type connectors to the corresponding solder points.
RF barrier	Check continuity from output 1 (output 2 on the green PCB) to solder point 3, (Figure 11).

4. Using a DVM, ensure there is continuity between the OUT2 D-type pins and corresponding outer solder points on the green ScanWave PCB ([Figure 10](#)).

NOTE: *The entrance and exit outputs are not connected to the green PCB.*

There must only be continuity between the outer solder points and their corresponding pins on the D-type connector. Any continuity to non-corresponding pins or inner solder points indicates a short circuit.



Figure 10 - Checking for continuity from D-type connector to collision cell solder points

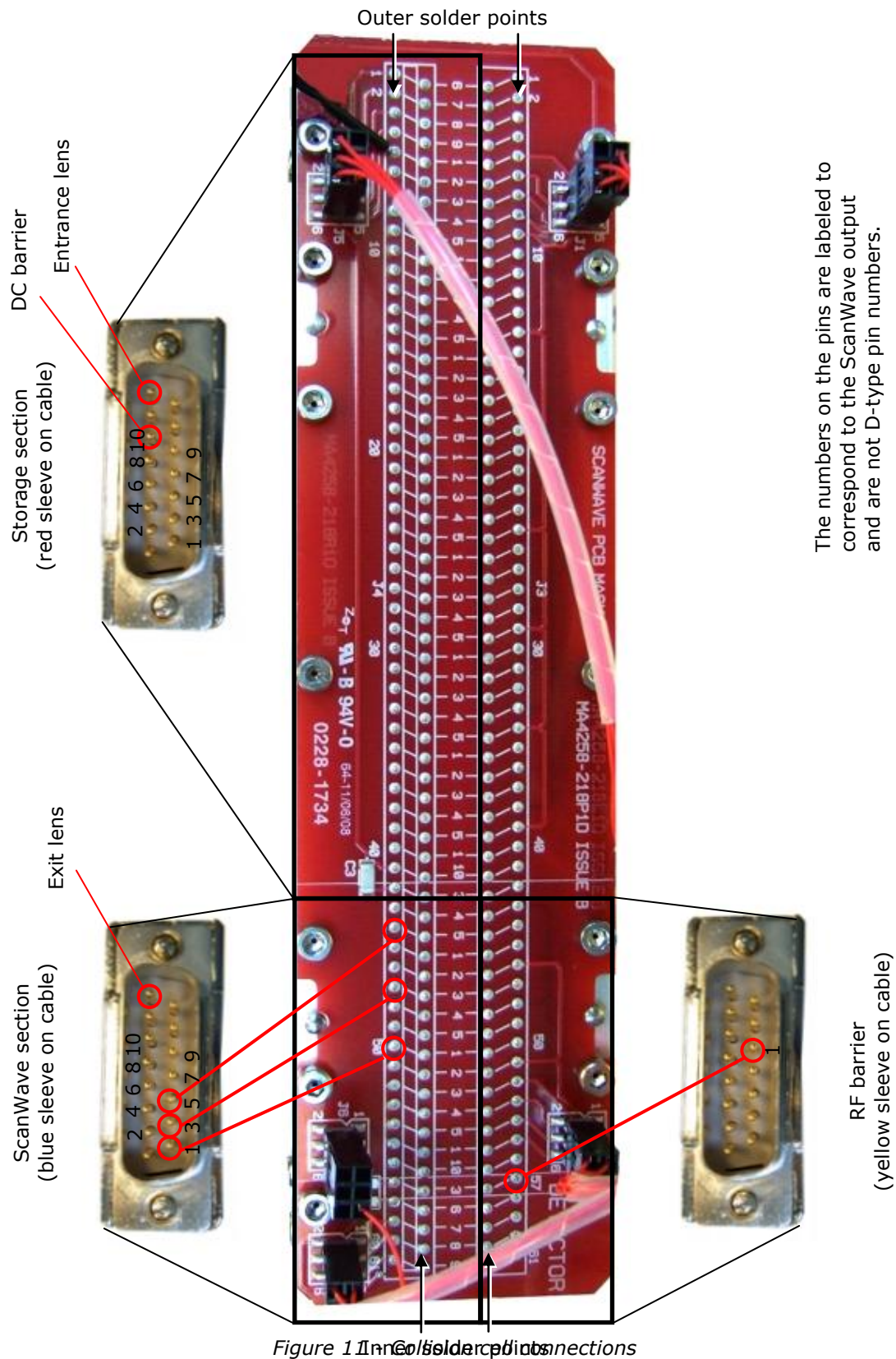


Figure 1 Inner solder point connections

Cleaning the ScanWave collision cell

WARNING: The ScanWave collision cell assembly could be contaminated with biohazardous and/or toxic materials; chemical-resistant, powder free latex or nitrile gloves must always be worn when handling the ScanWave assembly.

Removing the collision cell from the analyzer lid

1. Remove the analyzer lid from the instrument.
2. Disconnect the ten polarized Harwin connectors from each of the collision cell PCBs (five on each PCB).

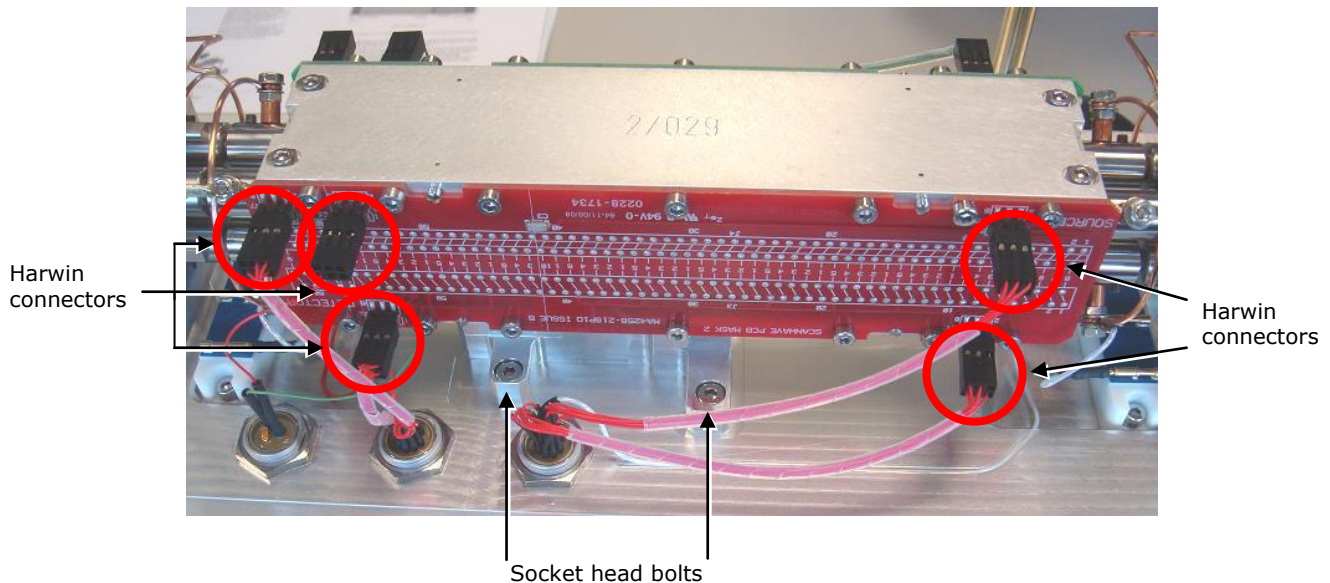


Figure 12 - Polarized Harwin connectors (red PCB)

3. Disconnect the entrance lens crimp connector.
4. Disconnect the exit lens crimp connector.
5. Remove the four socket head bolts that secure the collision cell to the analyzer lid.

CAUTION: Do not scratch the entrance and exit lenses or the MS2 pre and MS1 post-filters when removing the collision cell.

6. Remove the collision cell from the lid assembly and place on a clean flat surface.

Removing and cleaning the entrance and exit lenses

1. On the red PCB, loosen the two screws nearest the entrance lens.
2. Repeat step 1 on the green PCB.

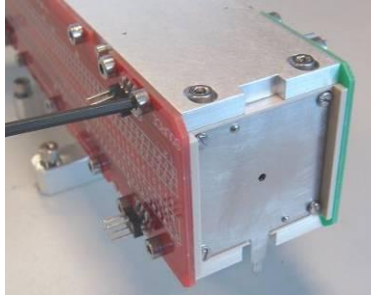


Figure 13 - Entrance/exit retaining screws

3. Record the position of the entrance lens relative to the collision cell, then remove the four screws that secure the entrance lens to the collision cell.

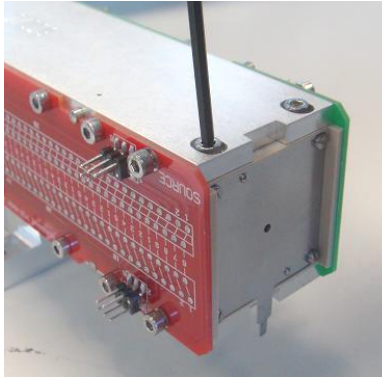


Figure 14 - Entrance lens retaining screws

4. Gently ease the entrance lens assembly out of the collision cell.
5. Repeat steps 1 to 3 at the opposite end of the collision cell to remove the exit lens assembly.
6. Remove the four screws that secure the entrance and exit lens plates within their PEEK housing and remove the plates.

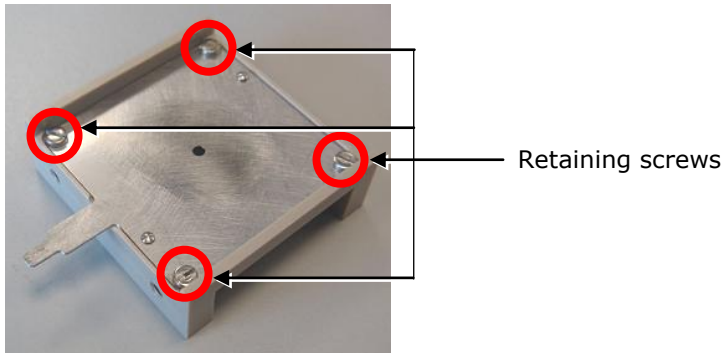


Figure 15 - Four retaining screws

7. Use a glass fiber pencil to clean around the entrance/exit apertures.
8. Immerse the entrance and exit plates in a glass vessel containing 1:1 methanol/water.

NOTE: *If the parts are obviously contaminated, use a mixture of 45:45:10 methanol/water/formic acid. Do not clean the collision cell with this mixture.*

WARNING: *Use extreme care when working with formic acid. Use a fume hood and appropriate protective equipment.*

9. Place the vessel in an ultrasonic bath for 30 minutes.
10. If formic acid was used in the cleaning solution, perform the following procedure:
 - a. Rinse the parts by immersing them in separate glass vessels containing water and placing the vessels in the ultrasonic bath for 20 minutes, to remove the formic acid.
 - b. Displace the water by immersing the parts in separate glass vessels containing methanol and placing the vessels in the ultrasonic bath for 10 minutes.
11. Carefully remove the parts from the vessels and blow-dry them using an inert, oil-free gas.
12. Wipe off any water spots with a lint-free cloth.
13. Fit the PEEK frames to the collision cell before attaching the metal entrance/exit plates so that the alignment of the PEEK can be easily checked.

CAUTION: *Misalignment of the entrance/exit plates will cause ion trapping within the collision cell, so it is important that the PEEK frames are fitted correctly. Ensure that the PEEK frame makes contact with and is parallel to the lens plate behind it. Take care not to use excessive force, as this will bend the lens plate and damage the collision cell.*

14. Reassemble the entrance and exit plates within their PEEK frames ensuring that the aperture orientation is correct.

NOTE: *Run the Dual Scan function and ScanWave enhancement check after reassembling the collision cell.*

Cleaning the collision cell T-Wave

1. Remove the seven screws from the green PCB that secure the aluminum lid of the collision cell.
2. Remove the seven screws from the red PCB.
3. Ease the aluminum lid away from the collision cell, revealing the rings inside.

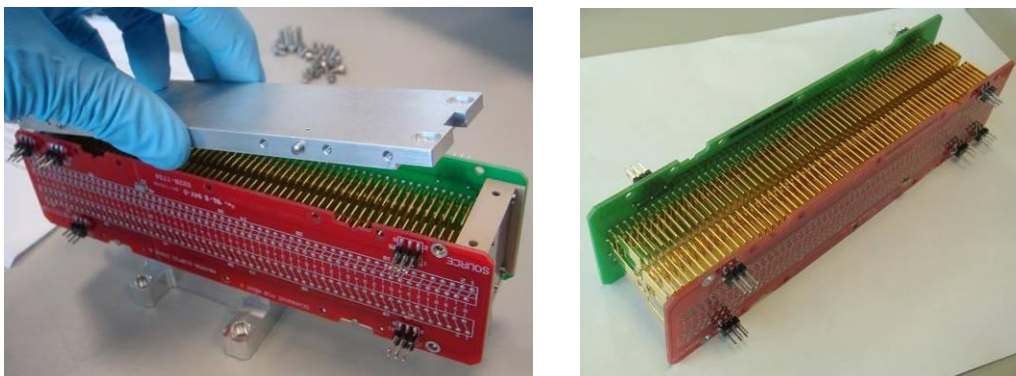


Figure 16 - Removing the collision cell lid and base

4. Remove the seven screws on the green PCB that secure the aluminum base of the collision cell.
5. Repeat step 4 for the red PCB.
6. Ease the aluminum base away from the collision cell.
7. Place the collision cell within the measuring cylinder, completely cover with methanol, and sonicate for 10 minutes.

CAUTION: Use IPA, MeOH, or MeCN solvents for cleaning. Do not use acetone, chlorinated solvents or any acids, as damage will occur.

8. Blow dry with nitrogen.

CAUTION: Ensure the collision cell is fully dry before reassembling.

9. Inspect the gaps between the ring electrodes and remove any particles or fibers as they may cause RF breakdown or charging to occur.
10. Inspect the gap between the ring electrodes at the entrance and exit.

CAUTION: When the entrance and exit lenses are fitted, care must be taken not to squash the end ring closer to the second ring as this may also create RF breakdown.

11. Rebuild the collision cell with its entrance and exit lenses, taking care not to press the end rings onto the next ring.
12. If available, use a Megger set to 500 V to check that breakdown does not occur between the two end collision cell plates.

Installing the collision cell onto the analyzer lid

1. Ensure that the argon gas connection O-rings are in place on the baseplate of the collision cell. Check that they are in good condition, clean, and dust free.

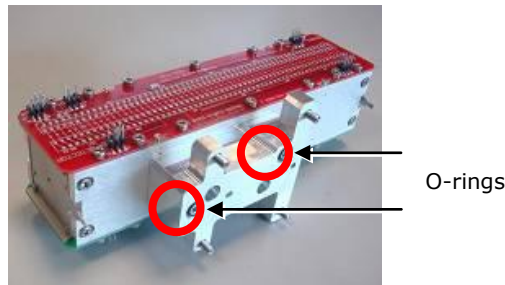


Figure 17 - Collision cell baseplate

2. Fit the collision cell brackets onto the two dowels on the analyzer lid and gently push down.

NOTE: When fitting the collision cell onto the analyzer lid, the writing must be upside down with the source writing towards the MS1 end and the analyzer writing towards MS2.

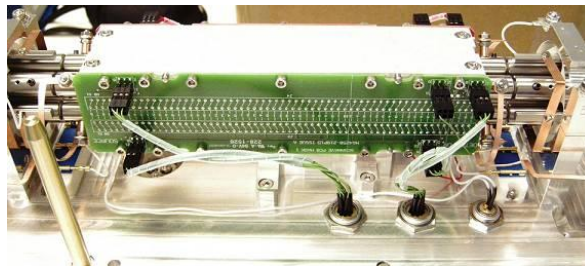


Figure 18 - Collision cell mountings

3. Screw the four M4 socket head bolts through the brackets into the analyzer lid to secure the collision cell.
4. Fit the Harwin connections (polarized to prevent incorrect fitting), entrance and exit crimp connections, and the MS1 and MS2 pre-filter connections, if they were disconnected.

NOTE: Push each pin within the Harwin connectors onto the collision cell with a small jeweler's screwdriver to ensure a good connection (Figure 19).

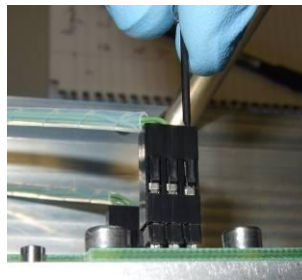


Figure 19 - Pushing in the pins

Installing the analyzer lid to the instrument and reconnecting the ScanWave cables

CAUTION: *Do not trap any of the cables near the source under the lid when installing the lid assembly to the vacuum housing.*

1. Blow the lid assembly with nitrogen to remove any dust particles and refit the assembly to the vacuum housing.
2. Secure the analyzer lid to the vacuum housing using the six M6 socket head screws.
3. Ensure the cables are connected correctly, see [Table 5](#), [Figure 20](#), and [Figure 21](#).

Table 5: Cable connections

Cable	Connection
Storage cable - D-types	Red sleeve on D-type to OUT 1 on the PCB (socket nearest the source) Black sleeve to OUT 2 (closest to detector)
Storage cable - Fischers	Red sleeve matched to red socket Black sleeve to unmarked socket
ScanWave cable - D-types	Blue sleeve on D-type to OUT 1 on the PCB (socket nearest the source) Black sleeve to OUT 2 (closest to detector)
ScanWave cable - Fischers	Blue sleeve matched to blue socket Back sleeve to unmarked socket
Barrier cable - D-types	Yellow sleeve on D-type to OUT 1 on the PCB (socket nearest the source) Black sleeve to OUT 2 (closest to detector)
Barrier cable - Fischer	Yellow sleeve matched to yellow socket

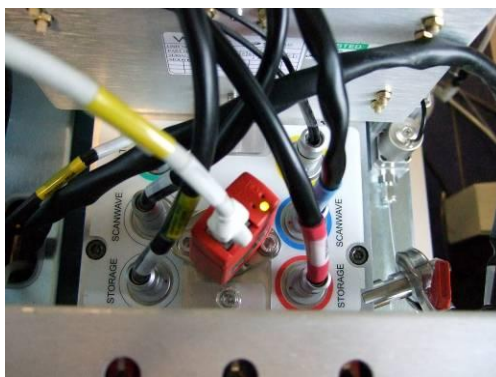


Figure 20 - Cable connections analyzer lid



Figure 21 - Cable connections ScanWave PCBs

Other relevant information

ScanWave collision cell overview

The ScanWave collision cell ([Figure 22](#)) consists of 61 lens pairs arranged into two main sections; a storage section and a ScanWave section. These two sections are separated by a DC barrier applied to one lens pair. RF and DC voltages for each section are provided by the storage and ScanWave PCBs respectively.

There is an RF barrier located within the ScanWave section. In ScanWave enhanced modes the voltages applied to the two plates forming the RF barrier are 180° out of phase with the voltages on the surrounding plates and are supplied by the RF barrier PCB.

Cooling regions at the front and rear of the cell are used to reduce the ion energy spread of the ions. Each cooling section is formed by four lens pairs; these sections do not have T-Wave pulses applied to them during a scan.

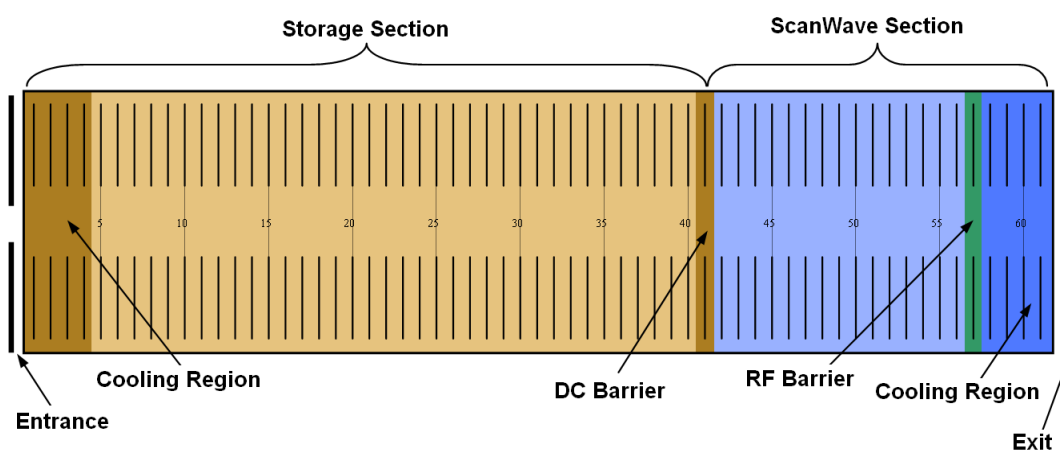


Figure 22 - Collision cell

The ScanWave collision cell can operate in either standard or ScanWave enhanced modes.

Standard mode

The collision cell operates in the same way as the Quattro Premier XE T-Wave collision cell. The storage, ScanWave, and RF barrier generator PCB outputs are synchronized. The RF barrier RF is applied in phase with the storage and ScanWave sections.

Enhanced modes (MS and MS/MS)

The ions are stored and then ejected from the collision cell in a mass dependent manner. This is achieved by ramping RF barrier voltage from high to low amplitude. High mass ions are released before low mass ions. The ramping of the barrier RF is adjusted so that the ejection of different masses is synchronized with the scanning of the second quadrupole, resulting in an improved sampling duty cycle and therefore enhanced sensitivity. The storage and ScanWave and RF barrier generator PCB outputs are each controlled independently. See [Figure 23](#) to [Figure 26](#) for the principles of ScanWave enhanced mode operation.

Principles of ScanWave enhanced mode operation

Ions accumulate in the Storage region. The DC barrier is high to prevent ions moving into the ScanWave region.

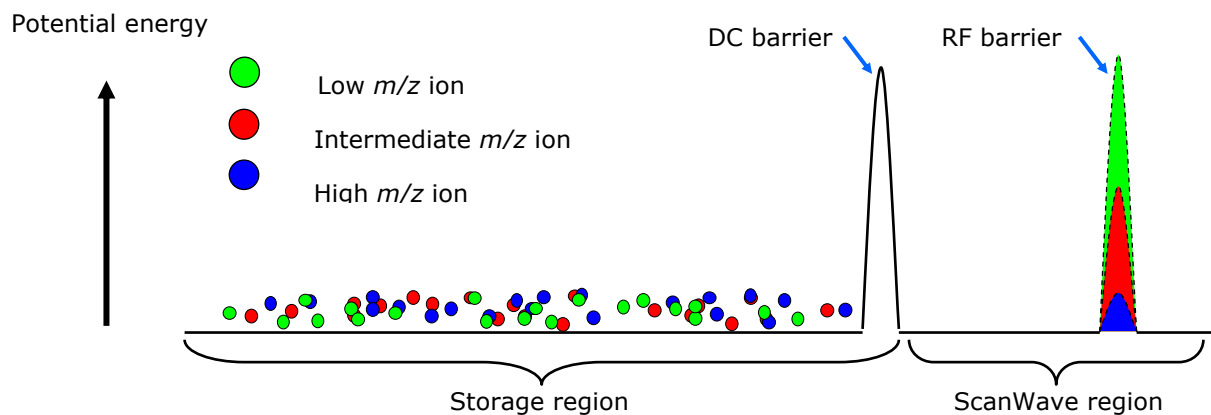


Figure 23 - High DC barrier

The DC barrier is lowered and a travelling wave applied to transfer ions to the ScanWave section. The RF barrier remains high so ions are not released from the ScanWave section.

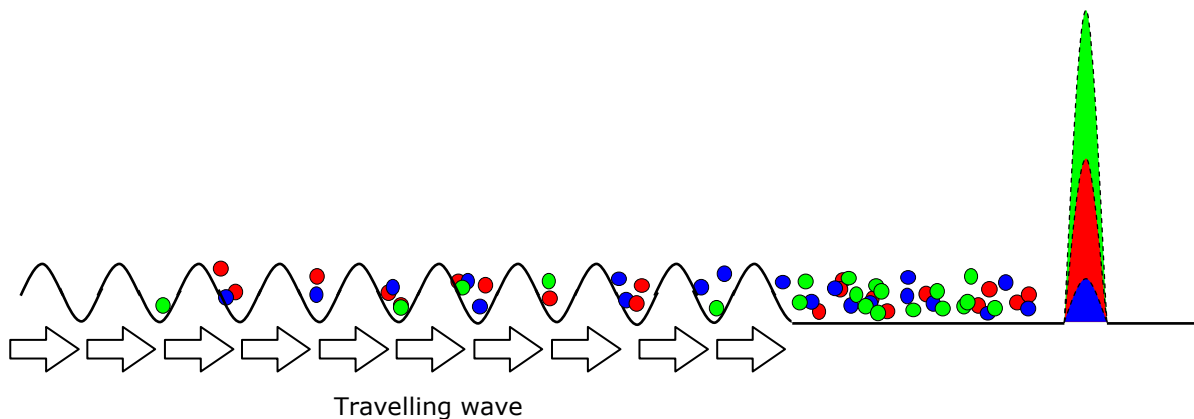


Figure 24 - Lowered DC barrier

The RF barrier voltage is lowered and high m/z is ejected from the ScanWave section. The storage section accumulates ions of all masses.

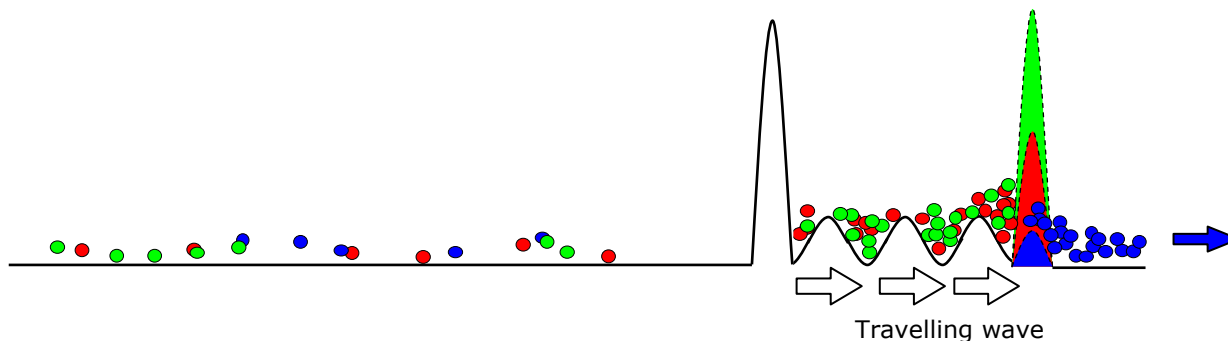


Figure 25 - Lowered RF barrier and high DC barrier

As the RF barrier voltage is lowered further medium m/z ions are ejected. The storage section continues to accumulate ions of all masses.

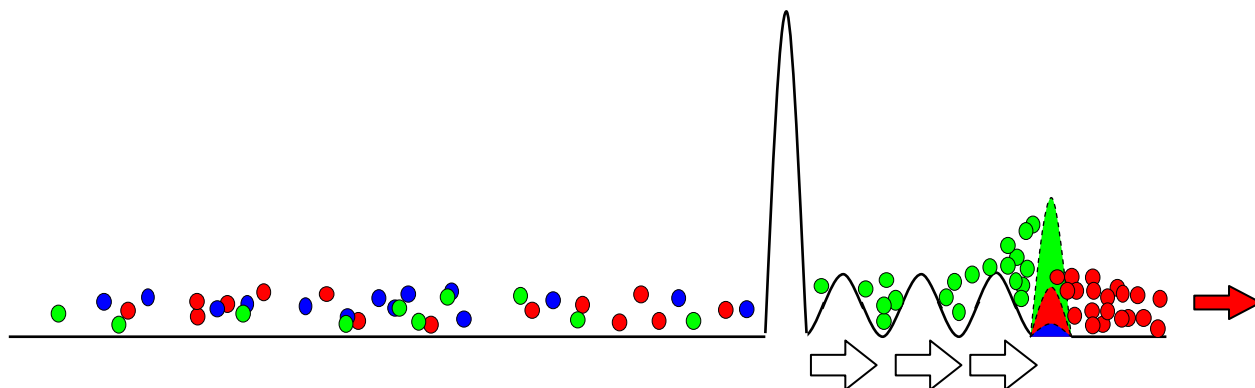


Figure 26 - Reduced RF barrier

Basic T-Wave collision cell principles

In non-ScanWave enhanced modes the Xevo collision cell works in a similar way to the Quattro Premier XE collision cell. Three types of voltage are applied to the plates within the collision cell:

- RF voltage
- DC offset(s)
- DC pulse voltage

RF voltage

The T-Wave optics consist of pairs of rings, each ring within a pair carries the opposite RF phase.

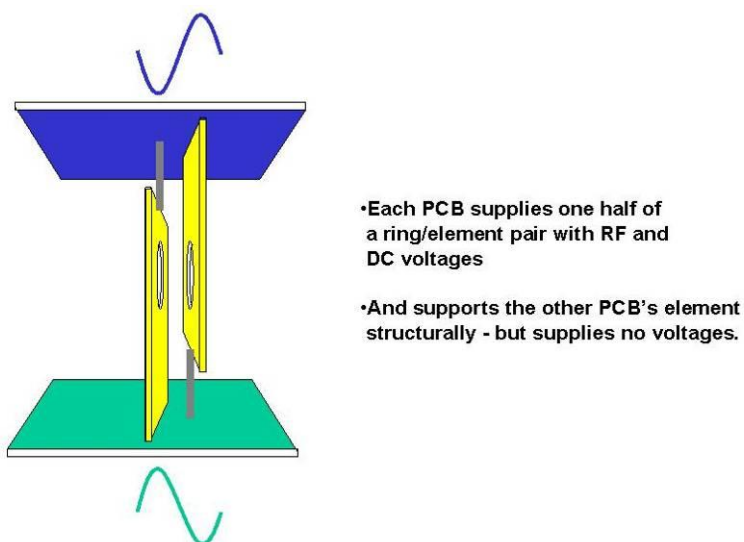


Figure 27 - Ring/element pairs

The peak-to-peak voltage of the RF applied to the rings, defines the mass range that passes through the ion guide.

- Low peak-to-peak voltages transfer low mass/charge ions more efficiently than high mass/charge ions
- High peak-to-peak voltages transfer high mass/charge ions more efficiently than low mass/charge ions

The RF voltage applied to the collision cell must therefore be ramped following the mass program of MS1.

The ScanWave RF generator boards supply RF at a frequency of 2.4 MHz.

DC offsets

The RF is superimposed onto three DC offset voltages applied to different sections of the collision cell. These DC offsets correspond to the Tune page parameters ScanWave, barrier and cooling (Figure 28).

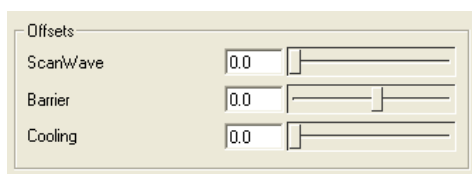


Figure 28 - Offset parameters

- The ScanWave DC offset is applied only to the storage section of the collision cell
- The barrier DC offset is applied only to the RF barrier plates
- The cooling DC offset is applied to the whole collision cell

CAUTION: *These voltages must only be changed from zero for diagnostic purposes and must always be returned to zero before leaving site.*

This is unlike previous tandem quadrupole instruments where the DC offset corresponds to the collision energy. The collision energy and collision cell offsets are applied to the lenses before the collision cell and not the collision cell itself.

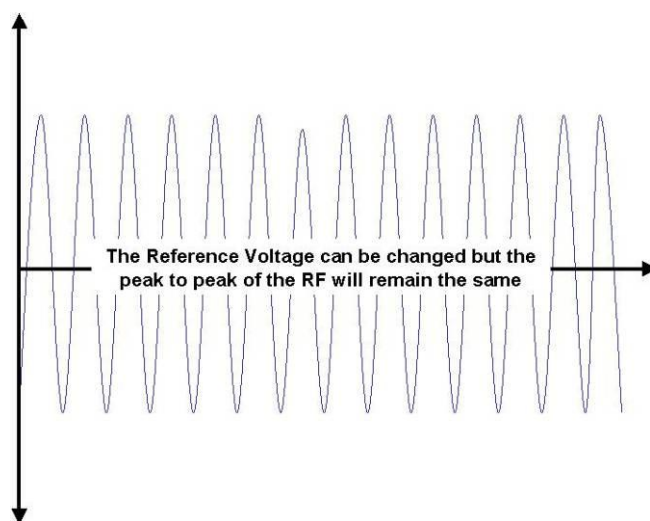


Figure 29 - Peak-to-peak of the RF

DC pulse

The traveling wave is a DC pulse that is applied to the DC offsets on one element pair for a set time, before progressing to the next pair, and so on.

NOTE: Default values in non-ScanWave mode are 300 m/s and 1 V; therefore the pulse width is 10 μ s. In ScanWave mode the storage velocity is 25 m/s and the ScanWave velocity is 140 m/s.

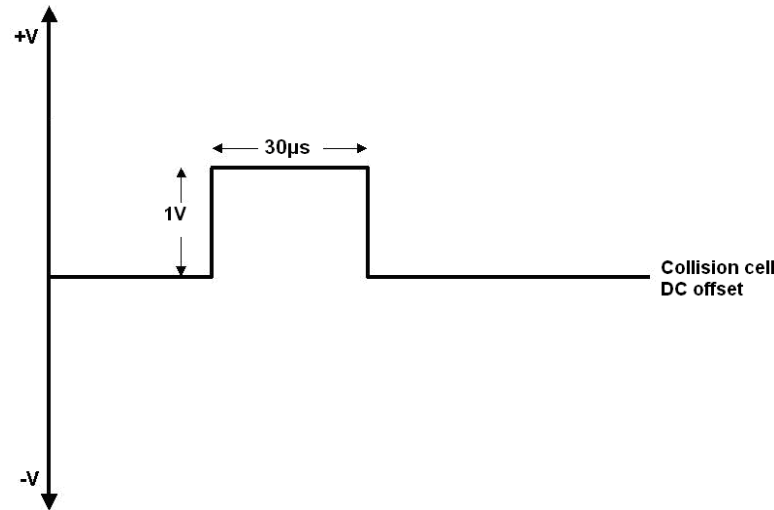


Figure 30 - DC pulse

NOTE: T-Wave pulses are only used when the collision gas is switched on.

The T-Wave pulse height is small compared to the peak-to-peak RF voltage, when superimposed on the RF it appears as shown in [Figure 31](#).

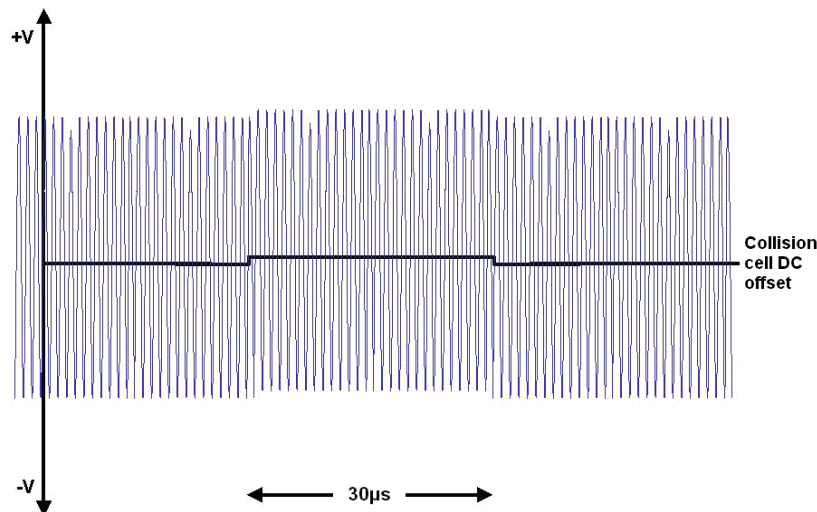


Figure 31 - RF voltage

A positively charged ion is more likely to sit in the potential energy well before or after a pulse, rather than at the crest of a pulse.

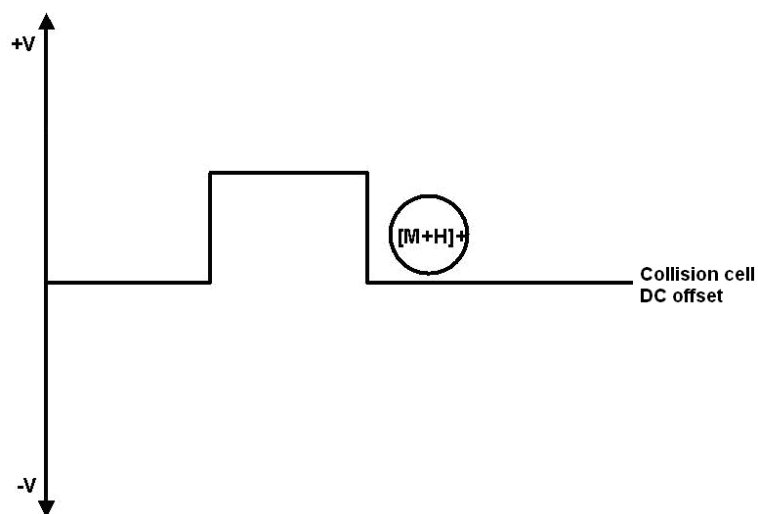


Figure 32 - Positive ions on the RF voltage

Switching the pulse on and off from one pair of rings to the next, results in a traveling wave that pushes the ions forward. A single RF/DC pulse program for every pair of rings is not necessary. The T-Wave generator produces five RF programs that have pulsed reference DC voltage.

The program applied to one ring pair is repeated every five ring pairs further into the ion guide. This confines or controls the ions transition through the ion guide.

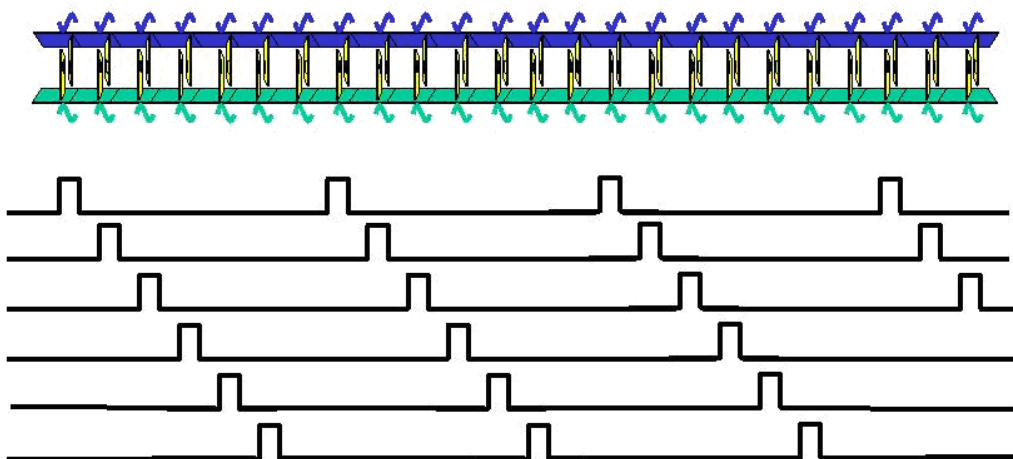


Figure 33 - RF/DC pulse programs

NOTE: There are five DC pulses which repeat within the cell

Tune page ScanWave parameters

A brief description of the ScanWave tuning parameters is given below.

CAUTION: *These settings must not be left accessible to customers.*

NOTE: *The default parameters must always be used during routine operation. The enhanced mode ScanWave parameters are optimized for an ISD of 20 ms. This value is not user selectable therefore the parameters do not require adjustment.*

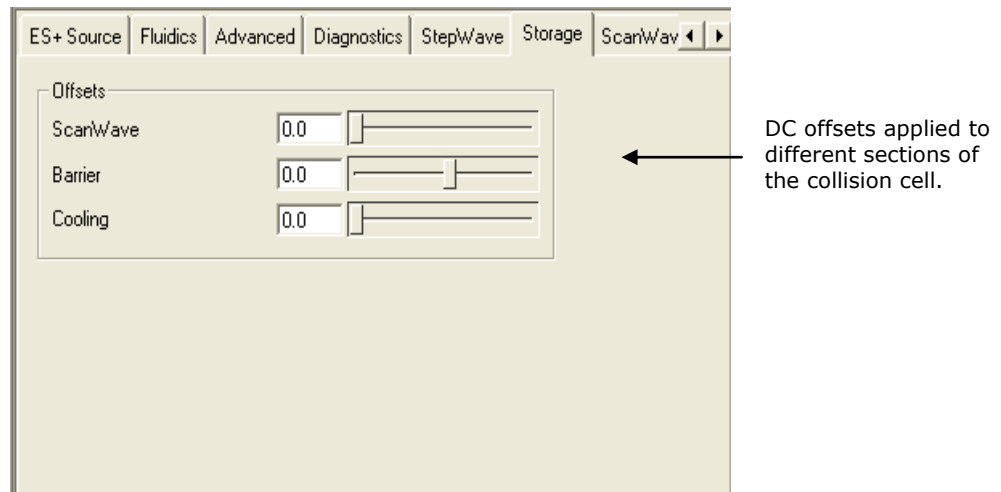


Figure 34 - Storage tab

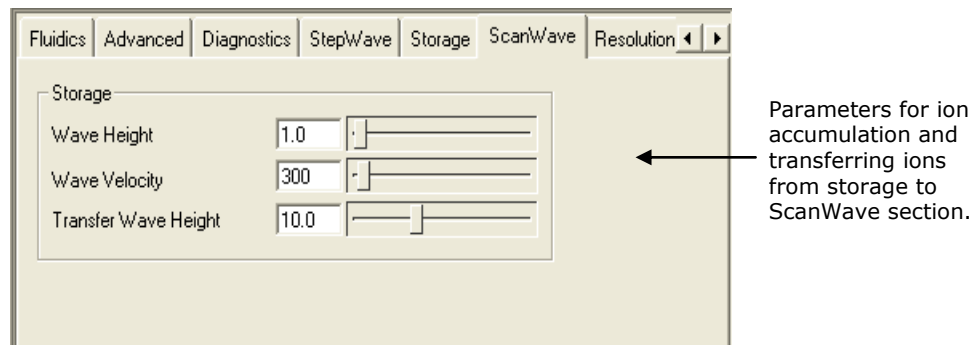


Figure 35 - ScanWave tab

ScanWave optimization

The ScanWave optimization procedure is slightly different for the Xevo TQ-S to Xevo TQ-XS, but the tuning, troubleshooting and end results are the same. The Xevo TQ-XS uses a different solution and a different reference file, refer to the procedures "[Positive Ion ScanWave optimization \(Xevo TQ-XS\)](#)", and "[Negative Ionization ScanWave Optimization \(Xevo TQ-XS\)](#)".

The ScanWave optimization procedure is run to synchronize the ejection of ions from the collision cell with the scanning of the second quadrupole. Each optimization is split into two sections; T-Wave height is optimized in the first part and the RF voltage in the second.

If the RF barrier is ramped in a linear fashion, ions are released from the collision cell in a non-linear manner. They will not be synchronized with the second quad which can only scan linearly with mass, therefore the RF barrier must be ramped non-linearly. The exact shape of this ramp is dependent on the mass range being scanned and the scan rate. The ScanWave Optimization wizard determines the correct ramp for various mass ranges and scan rates.

Different mass ranges, for example:

- 50 to 350 Da
- 200 to 2000 Da

Different scan rates, for example:

- 1000 amu/sec
- 5000 amu/sec

The mass ranges can be controlled by travelling wave height:

- A small T-Wave, for example 100 mV, will separate a large mass range.
- A large T-Wave, for example 1 V, will separate a smaller mass range (the larger m/z ions are swept straight over the barrier without being separated).
- The nonlinear ramp required to give linear separation for each TW height that is to be used must be calculated.

The nonlinear ramp is different depending upon scan rate:

- During a slow ramp, ions are ejected slightly earlier than during a long ramp.
- Ions see more T-Wave pulses and have more "chances" of being ejected past the barrier.
- The nonlinear ramp for each scan rate must be calculated.
- 10 T-Wave heights * 4 scan rates = 40 different "optimizations".

Each ScanWave optimization consists of two sections, T-Wave height optimization and RF voltage optimization.

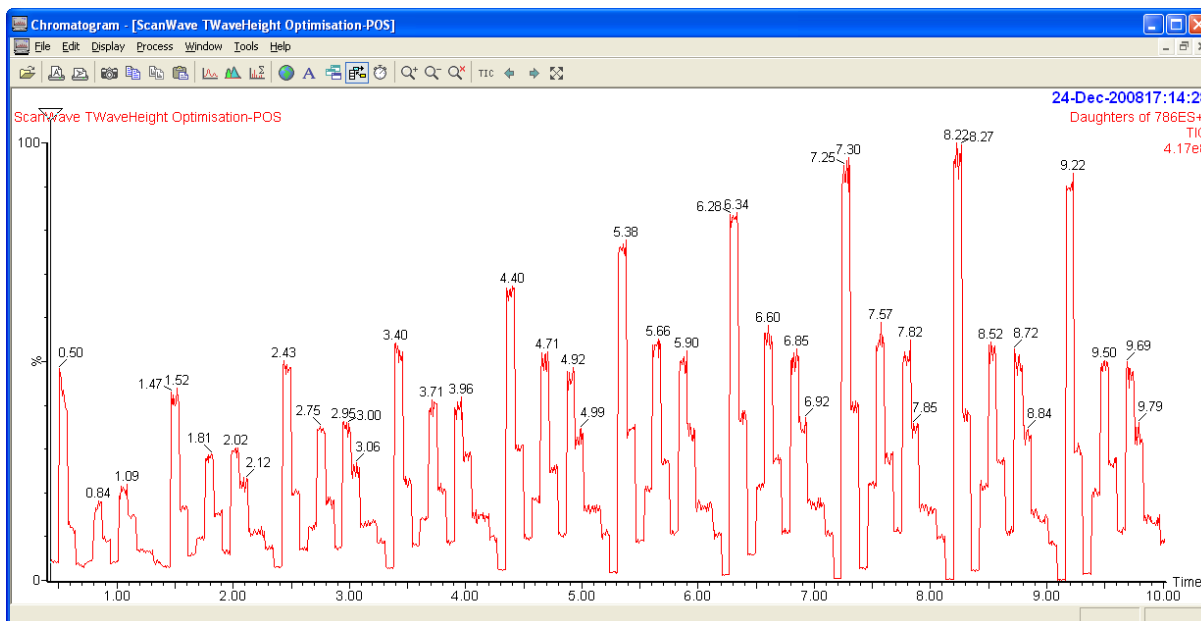


Figure 36 - Example of Positive ScanWave T-Wave height optimization chromatogram

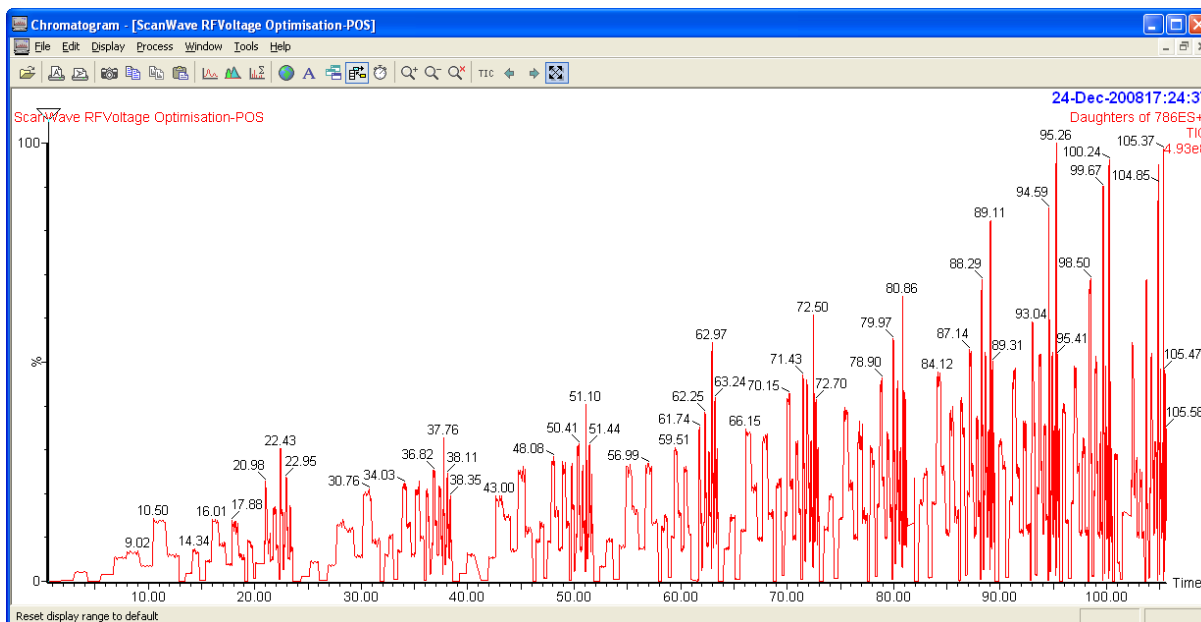
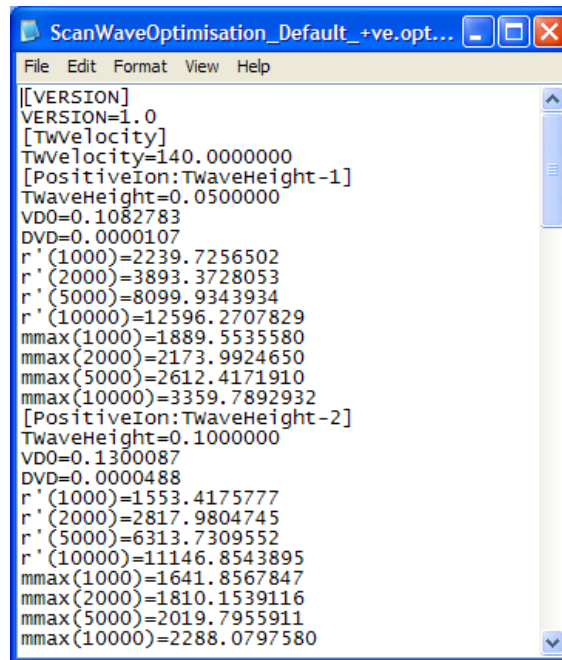


Figure 37 - RF voltage optimization

Optimization produces a look-up table in *.opt file format; the operator specifies where this is saved. The .opt file must contain results for ten different T-Wave heights; if any of these is missing, the optimization has failed and must be repeated.



```

[[[VERSION]
VERSION=1.0
[Twvelocity]
Twvelocity=140.0000000
[PositiveIon:TwaveHeight-1]
TwaveHeight=0.0500000
VD0=0.1082783
DVD=0.0000107
r'(1000)=2239.7256502
r'(2000)=3893.3728053
r'(5000)=8099.9343934
r'(10000)=12596.2707829
mmax(1000)=1889.5535580
mmax(2000)=2173.9924650
mmax(5000)=2612.4171910
mmax(10000)=3359.7892932
[PositiveIon:TwaveHeight-2]
TwaveHeight=0.1000000
VD0=0.1300087
DVD=0.0000488
r'(1000)=1553.4175777
r'(2000)=2817.9804745
r'(5000)=6313.7309552
r'(10000)=11146.8543895
mmax(1000)=1641.8567847
mmax(2000)=1810.1539116
mmax(5000)=2019.7955911
mmax(10000)=2288.0797580

```

Figure 38 - Optimization results (this example shows results for only two T-Wave heights)

Two separate .opt files must be created, one for positive ion and one for negative ion. The .opt files must then be loaded into the target registry by the engineer:

- For Xevo TQ-S instruments, refer to the [Xevo TQ-S Installation Manual](#) (P/N 715002461) for instructions on how to run the ScanWave optimization procedure and load the .opt files.
- For Xevo TQ-XS instruments, refer to "Positive Ion ScanWave optimization (Xevo TQ-XS)" for instructions on how to run the ScanWave optimization procedure and load the .opt files.

Optimizing the ScanWave collision cell parameters (Xevo TQ-S)

This section defines the procedure for optimizing the ScanWave collision cell parameters for Xevo TQ-S instruments.

The procedure described in the [Xevo TQ-S Installation Manual](#) (P/N 715002461) uses [Glu¹]-fibrinopeptide B as the sample, but other samples can also be used.

The following reference files are included in the ScanWave optimization wizard:

- Cyclodextrin_positive.ref
- Glufibrinopeptide_negative.ref
- Glufibrinopeptide_positive.ref
- PEG1002_positive.ref
- Reserpine_positive.ref
- SodiumIodide1476_negative.ref
- SodiumIodide1626_negative.ref

NOTE: *[Glu¹]-fibrinopeptide B can be used for both positive and negative ion modes. Cyclodextrin works well for positive but gives no signal in negative. Sodium iodide works well for negative but will foul the sample cone if run for a long period of time.*

If the ScanWave optimization procedure fails, perform the "Troubleshooting ScanWave" section.

CAUTION: *The automated ScanWave optimization may take substantial time to complete, but does not require user intervention once initiated. The procedure must be completed, but it can be performed at a convenient point during the installation.*

1. Install a bottle of 100 fmol/μL [Glu¹]-fibrinopeptide B in 50:50 acetonitrile/water + 0.1% formic acid on fluidics reservoir B.
2. Open the MS Tune page.
3. Click **View > System Diagnostics** to access the advanced MS Tune page tabs.
4. Click  to start the nitrogen flow, then click  to switch the instrument to Operate mode.
5. Click **Ion Mode > Electrospray +**.
6. Click  to select MS mode on the MS Tune page.
7. Click  to switch on the collision gas flow, adjust the collision gas flow rate so that the pressure in the collision cell is 3.0E-3 mbar.

8. Ensure the setting on the ES+ Source tab are as shown in [Table 6](#).

Table 6: Initial source conditions

Parameter	Setting	Comment
Capillary	3 kV	This value will be optimized later.
Cone Voltage	40 V	This value will be optimized later.
Source Offset	60 V	Fixed
Desolvation Temperature	200 °C	Fixed
Desolvation Gas Flow	500 L/h	Fixed
Cone Gas Flow	150 L/h	Fixed
Collision gas pressure	Tune	Gas cell pressure 3E-3 mbar

9. Click Fluidics > Reservoir B.
10. Click . The purge function automatically starts after reservoir A is selected.
11. Purge line B a second time.
12. Select Infusion as the flow state, and set infusion flow rate to 10 µL/min.
13. Ensure the isolation valve is open, then click .
14. Set the gain of the Tune window to 1 (mass 786.0, MS function).

NOTE: Keep the resolution sliders at 3 (LM) and 15 (HM) in both MS1 and MS2.

15. Adjust the capillary voltage so that the intensity of the peak at 786 is ~5 to 10% of the MS Tune window.

NOTE: The m/z 786 peak does not need to be fully resolved.

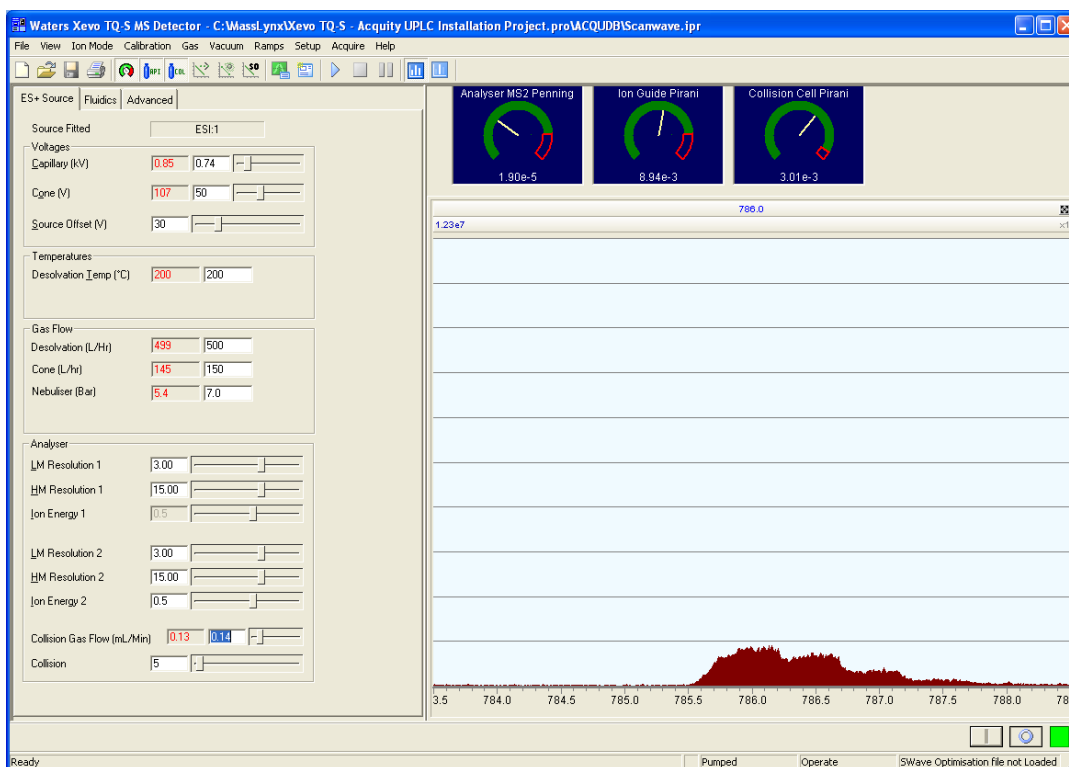


Figure 39 - Tune page optimized to run the positive ion scanwave optimization

16. Save the Tune page as Scanwave.ipr.

17. Click **Setup > ScanWave optimization > Run Optimization** to open the ScanWave Optimization Wizard.

18. Select **Positive Ion ScanWave Optimization**, then click **Next**.

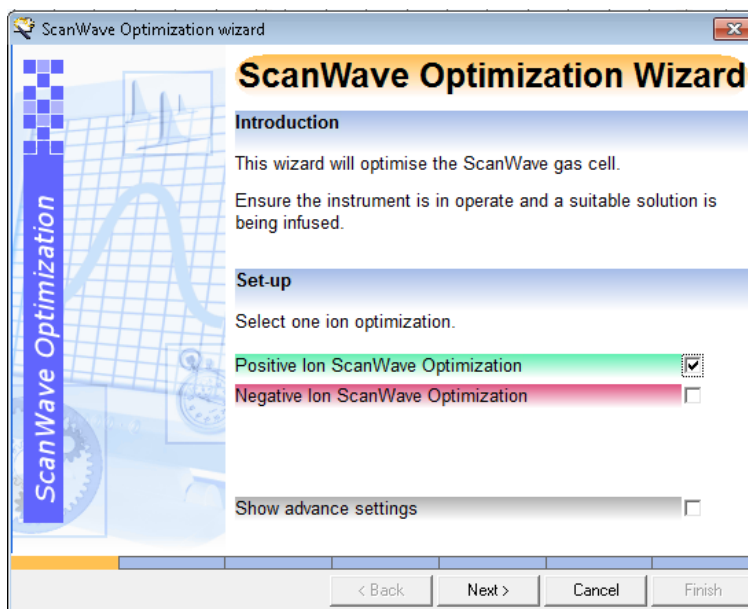


Figure 40 - Selecting Positive Ion ScanWave Optimization

19. Specify the values shown in Figure 41, then click **Next**.

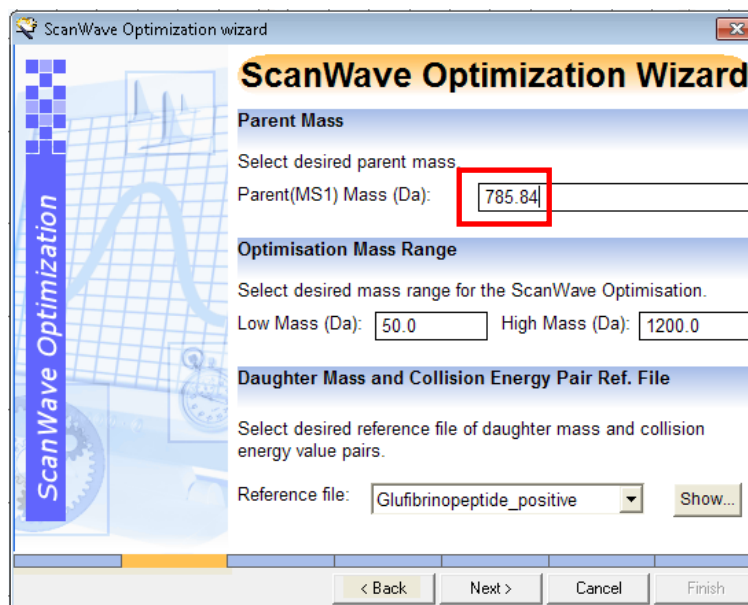


Figure 41 - Specifying the ScanWave optimization parameters

NOTE: Use the observed m/z value of the $[Glu^1]$ -fibrinopeptide B peak.

20. Ensure that the instrument is ready for the optimization procedure, then click **Next**.

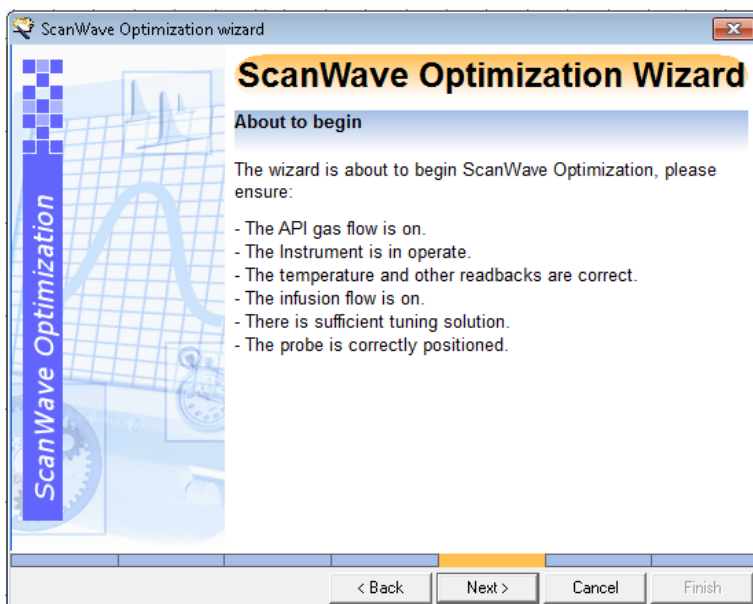


Figure 42 - Specifying the ScanWave scan parameters

NOTE: The ScanWave optimization will now proceed.

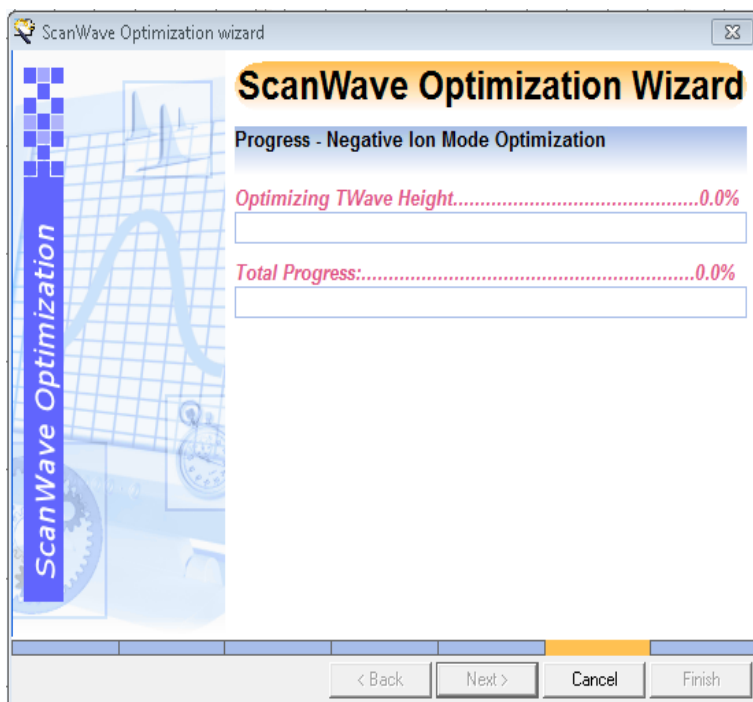


Figure 43 - ScanWave optimization status

21. When the wizard has completed, click **Use This**.

22. Save the optimization with the filename **ScanWave_POS_DDMMYY.opt** (where DDMMYY = date).

NOTE: The wizard saves the *.opt file to the C:\MassLynx\Default.pro\Acqudb folder by default.

23. On the MS Tune page, click **Setup > ScanWave optimization > Load Optimization** and browse to the ScanWave_POS_DDMMYY.opt file.
24. Click **Setup > ScanWave optimization > Optimization Enabled**, to ensure that optimization is enabled.
25. Repeat the procedure in Negative Ion mode using a fully tuned m/z 783.8 peak.
26. Save the optimization with the filename **ScanWave_NEG_DDMMYY.opt** (where DDMMYY = date).

NOTE: If necessary, adjust the infusion rate to 20 $\mu\text{L}/\text{min}$ to attain the required signal intensity.

Positive Ion ScanWave optimization (Xevo TQ-XS)

This section defines the procedure for optimizing the ScanWave collision cell parameters in Positive Ion mode for Xevo TQ-XS instruments. Perform the procedure using Xevo TQ-XS set up solution.

1. In the Tune page, click **View > Advanced > System**.
2. Using the integrated fluidics, infuse Xevo TQ-XS set up solution at 10 $\mu\text{L}/\text{min}$.

NOTE: A large amount of the solution will be used; fill a fluidics bottle with the solution.

3. Use MS1 to check the position of the peak at 2017.5 Da.
4. With the LM resolution at 3.0 and the HM resolution at 15, make a note of the position of the peak.
5. Increase the cone gas until the height of the precursor ion in a normal MS1 scan is $\leq 2.0 \times 10^7$.
6. Turn the argon gas supply on, and select **MSMS mode**.

7. Click **Setup > ScanWave Optimization > Run Optimization**.
8. Select Positive Ion mode and click **Next**.

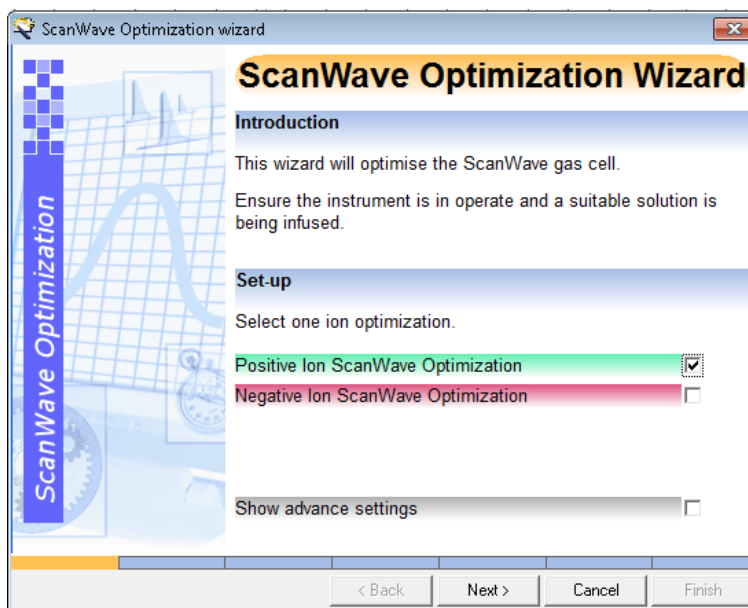


Figure 44 - Selecting Positive Ion ScanWave Optimization

CAUTION: Ensure that the Parent Mass is correctly set for Xevo TQ-XS set up solution based on the mass observed (positive = 2017.5).

9. Optimize from 50 to 1200 Da, and then select the **XEVO_TQXS_SETUP_POSITIVE** reference file.
10. Check that the instrument is ready to optimize, and then click **Next**.

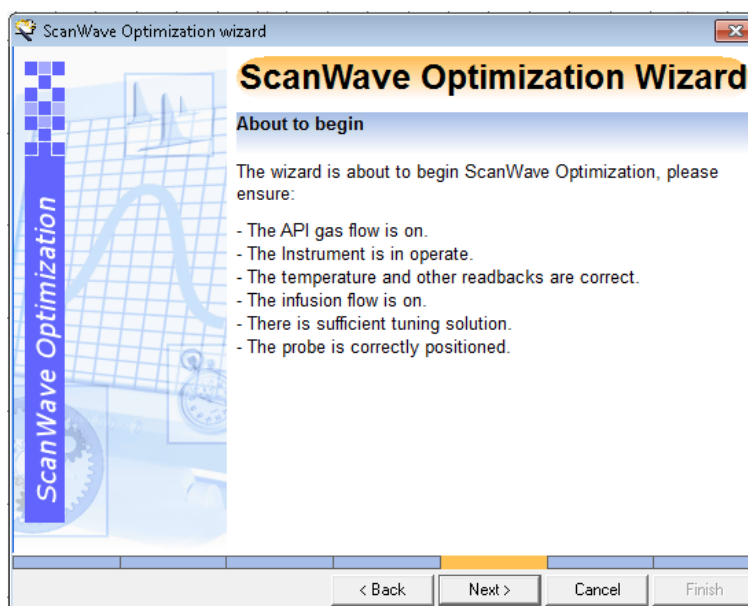


Figure 45 - Specifying the ScanWave scan parameters

CAUTION: *The instrument takes approximately one hour to optimize automatically. Ensure there is sufficient tuning solution.*

NOTE: *The fluidics will automatically pause the optimization and refill the syringe. Once the syringe is running again there is a 10 second wait before the acquisition is re-started.*

Once the optimization is completed, a final window opens.

11. Save the optimization as "WBA***_PositiveOptimization_160710", where "***" is the instrument serial number and the "160710" is the date in year, month, day format.
12. Click **Setup > ScanWave Optimization**, and confirm **Optimization Enabled** is selected.

Positive enhancement check at 5000 Da/s (Xevo TQ-XS)

1. Using the integrated fluidics, infuse Xevo TQ-XS set up solution at 10 $\mu\text{L}/\text{min}$.
2. Use MS1 to check the position of the peak at 2017.5 Da.
3. With the LM resolution at 3.0 and the HM resolution at 15, make a note of the position of the peak.
4. Increase the cone gas until the height of the precursor ion in a normal MS1 scan is $\leq 2.0\text{E}7$.
5. Turn the argon gas supply on, and select MSMS mode.
6. Check that the daughter entrance and exit values are set to the same figures as the enhanced daughter figures.
7. Create two experimental methods, one with a single daughter scan and the other with a single ScanWave DS, using the parameters in [Figure 46](#).

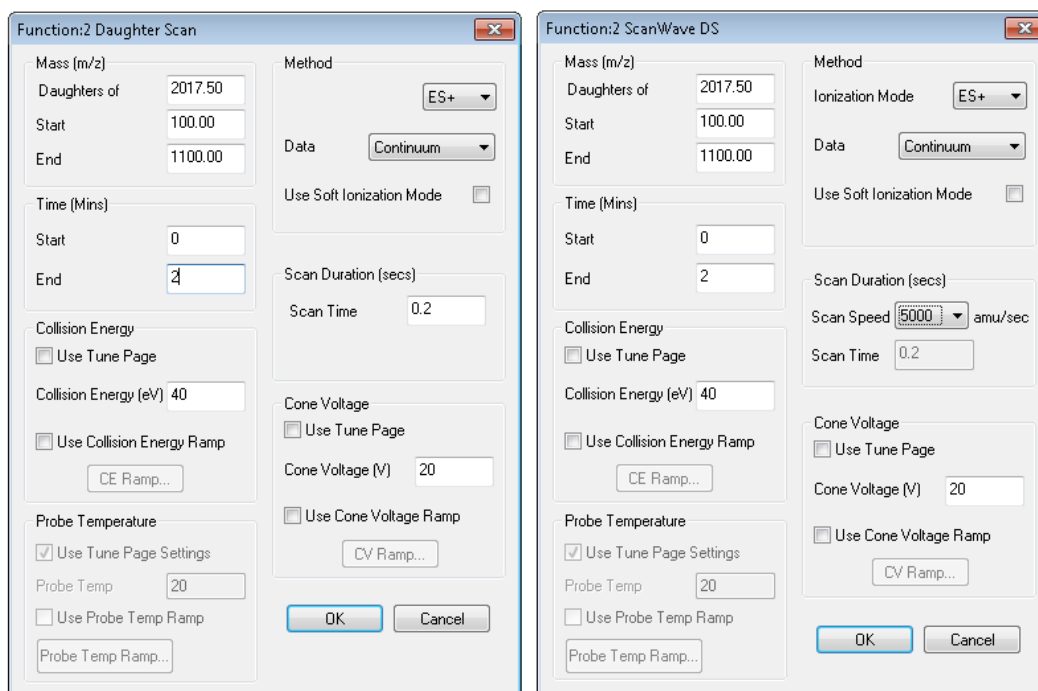


Figure 46 - Daughter scan parameters (left) and ScanWave DS parameters (right)

8. Create two runs on the sample list; one for the daughter scan experiment and one for the ScanWave DS experiment. Acquire both runs.

NOTE: *Ensure that the sample text indicates which experiment is being run.*

9. Open the resulting TIC 2 chromatograms on a single page and smooth both with the settings shown in [Figure 47](#).

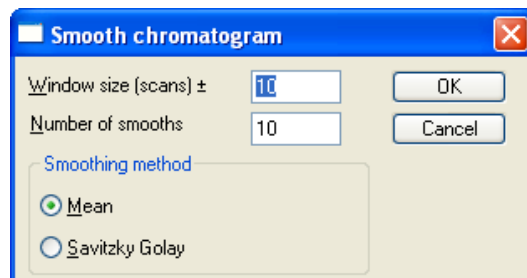


Figure 47 - Smoothing the chromatogram

10. Compare the smoothed chromatograms. Ensure that the improvement in TIC intensity is ≥ 9.1 .

Negative Ionization ScanWave Optimization (Xevo TQ-XS)

This section defines the procedure for optimizing the ScanWave collision cell parameters in Negative Ion mode for Xevo TQ-XS instruments. Perform the procedure using Xevo TQ-XS set up solution.

1. In the Tune page, click **View > Advanced > System**.
2. Using the integrated fluidics, infuse Xevo TQ-XS set up solution at 10 $\mu\text{L}/\text{min}$.

NOTE: *A large amount of the solution will be used; fill a fluidics bottle with the solution.*

3. Use MS1 to check the position of the peak at 1179.21 Da.
4. With the LM resolution at 3.0 and the HM resolution at 15, make a note of the position of the peak.
5. Increase the cone gas until the height of the precursor ion in a normal MS1 scan is $\leq 2.0 \times 10^7$.
6. Turn the argon gas supply on, and select **MSMS mode**.
7. Click **Setup > ScanWave Optimization**, and confirm **Run Optimization** is selected.

8. Select Negative Ion mode and click **Next**.

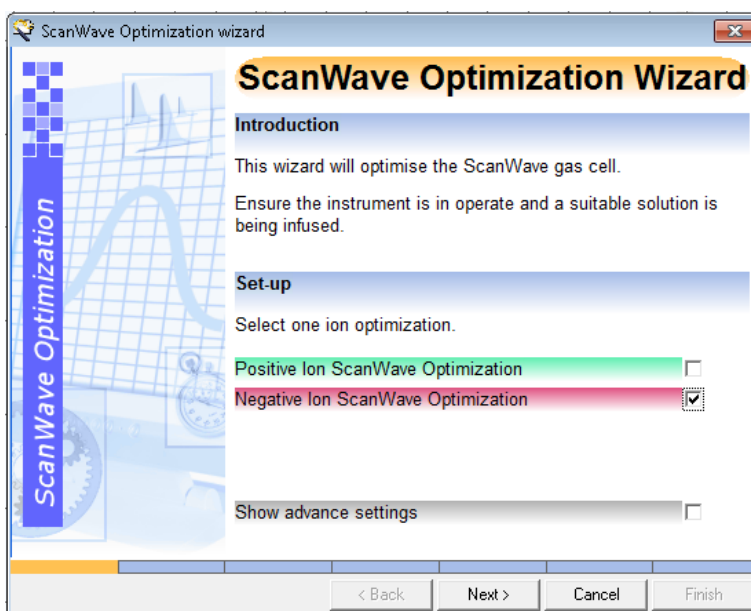


Figure 48 - Selecting Negative Ion ScanWave Optimization

CAUTION: Ensure that the Parent Mass is correctly set for Xevo TQ-XS set up solution based on the mass observed (negative = 1179.21).

9. Optimize from 50 to 1200 Da, and then select the **XEVO_TQXS_SETUP_NEGATIVE** reference file.
10. Check that the instrument is ready to optimize, and then click **Next**.

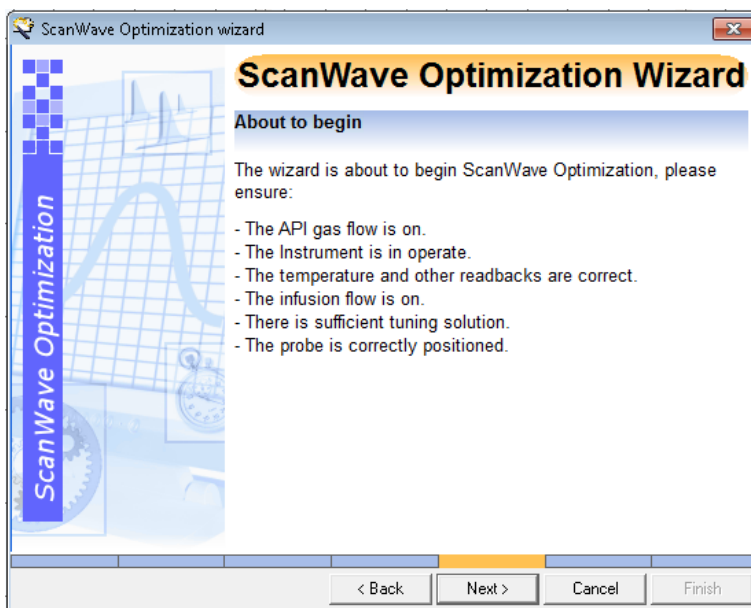


Figure 49 - Specifying the ScanWave scan parameters

CAUTION: *The instrument takes approximately one hour to optimize automatically. Ensure there is sufficient tuning solution.*

NOTE: *The fluidics will automatically pause the optimization and refill the syringe. Once the syringe is running again there is a 10 second wait before the acquisition is re-started.*

Once the optimization is completed, a final window opens.

11. Save the optimization as "WBA***_NegativeOptimization_160710", where "***" is the instrument serial number and the "160710" is the date in year, month, day format.
12. Load the saved calibration.
13. Click **Setup > ScanWave Optimization**, and confirm **Optimization Enabled** is selected.

Negative enhancement check at 5000 Da/s (Xevo TQ-XS)

1. Using the integrated fluidics, infuse Xevo TQ-XS set up solution at 10 $\mu\text{L}/\text{min}$.
2. Use MS1 to check the position of the peak at 1179.21 Da.
3. With the LM resolution at 3.0 and the HM resolution at 15, make a note of the position of the peak.
4. Increase the cone gas until the height of the precursor ion in a normal MS1 scan is $\leq 2.0\text{E}7$.
5. Turn the argon gas supply on, and select **MSMS mode**.
6. Check that the daughter entrance and exit values are set to the same figures as the enhanced daughter figures.
7. Using the parameters in [Figure 50](#) create two experimental methods, one with a single daughter scan and the other with a single ScanWave DS.

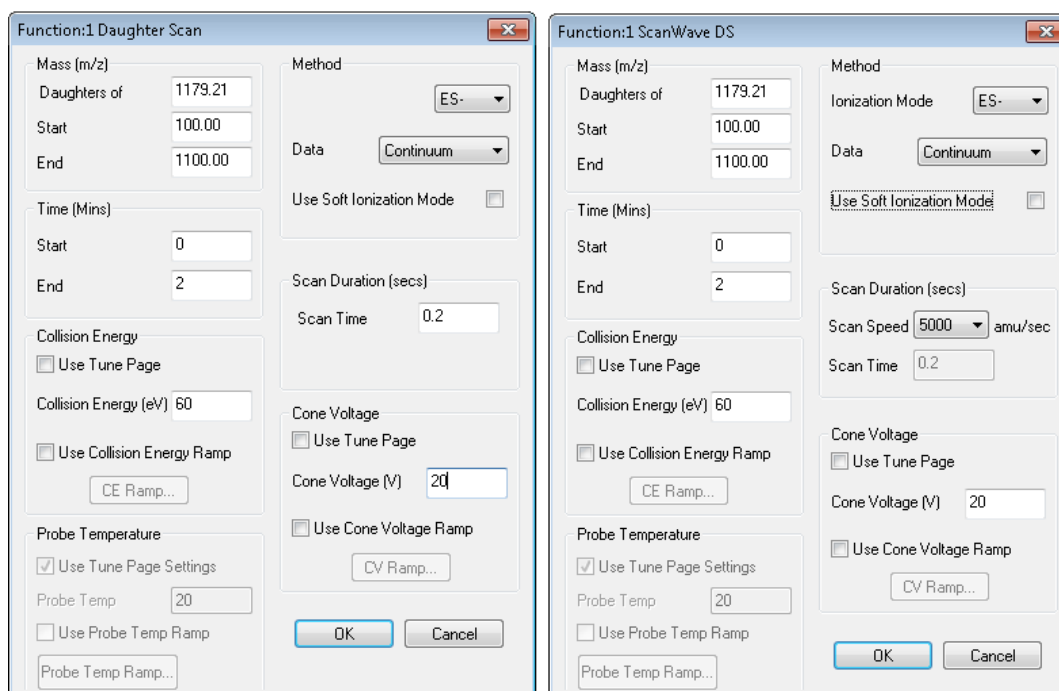


Figure 50 - Daughter scan parameters (left) and ScanWave DS parameters (right)

8. Create two runs on the sample list; one for the daughter scan experiment and one for the ScanWave DS experiment. Acquire both runs.

NOTE: Ensure that the sample text indicates which experiment is being run.

9. Open the resulting TIC 2 chromatograms on a single page and smooth both with the settings shown in [Figure 51](#).

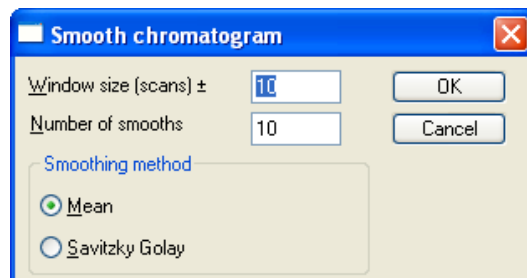


Figure 51 - Smoothing the chromatogram

10. Compare the smoothed chromatograms. Ensure that the improvement in TIC intensity is ≥ 3.4 .

Troubleshooting ScanWave verification test

1. Check the collision cell pressure.

NOTE: Enhanced ScanWave typically optimizes within $3.0E-3$ to $3.5E-3$ mbar. If the pressure is too high, the enhanced ScanWave function may fail.

The penning gauge pressure is normally within $1.8E-5$ to $2.0E-5$ mbar at this flow rate, or lower.

2. Check the intensity of the MS1 (no collision gas) is 786 Da (positive ion) and 784 Da (negative ion).
 - a. Set the gain value to **1**.
 - b. Tune the signal intensity to give a maximum of 10% of the window height (about **1.8E7**).
3. If the problem is caused during the negative ScanWave, perform the following steps:
 - a. Check that the infusion flow rate is 20 $\mu\text{L}/\text{min}$.
 - b. Tune until the enhanced TIC reads **>7E7** (when smoothed).

NOTE: The fault may be caused by the amount of sample entering the instrument, rather than the ScanWave function.

4. If automatic Advanced tuning is not being used for the differential aperture, entrance and exit lenses, ensure that the settings are the same for both daughter and enhanced daughter tuning.
5. If the ScanWave optimization procedure continues to fail, perform the "[Advanced troubleshooting](#)" section.

Advanced troubleshooting

1. If the specification has failed using the automatically set pulse height, navigate to C:/Masslynx/Scanwave/.
2. Load the default optimization file.
3. Run the verification using the factory optimization file.

NOTE: *If the factory optimization is not available, use the default optimization file.*

4. If the results are better when using the factory optimization, check the following:
 - a. Open the current project folder.
 - b. Navigate to the following file:
Data/Scanwave Optimization Logs/xxx/yyy/ScanWave RfVoltage Optimization.csv.

NOTE: *"xxx" represents the name of the dated folder which contains the latest positive or negative optimization calibration data." yyy" represents the term Positive Ion or Negative Ion.*

5. Examine the first table and ensure Input Data is fully populated for all ten T-Wave pulse heights.
6. If the table has not been populated with numerical data, repeat the optimization procedure.

NOTE: *This must be numerical data for the optimization to have completed fully.*

7. Examine the last table.

NOTE: *This table is the Final LU table and contains the pulse heights that the software uses for maximum masses at different scan speeds. It is populated with ten rows, one for each of the following pulse heights: 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.75, 1, 1.25 (all measurements in volts).*

8. If there are missing rows, repeat the optimization.
9. Look down the mmax(5000) column and locate the mass with the closest mass value, which is also greater than the maximum mass used within the specification (1100 Da).

NOTE: *The T-Wave height in this row is used by the software. It can be selected manually on the ScanWave tab of the Tune page for the specification to pass.*

Typically, the pulse height for the specification is 0.5 V.

10. If the software is selecting the incorrect optimal pulse height, physical conditions may have changed between running the optimization and verification tests; for example, any of the following:

- Argon flow rate
- Cleanliness of the collision cell
- Condition of the entrance and exit aperture
- Position and condition of the collision cell plates

CAUTION: *Do not open the analyzer at this stage.*

11. Delete all .opt files.

NOTE: *The location of these files is user definable.*

12. Note the engineer's resolution and mass position settings.

13. Load the factory target registry settings from the CD supplied with the instrument.

14. Re-enter the values recorded in step 12.

15. Delete the following files or folders:

- C:\MassLynx\VxWorks\TargetRegistryFiles
- C:\Program Files\Waters Instruments\Log\
- C:\Program Files\Waters Instruments\LogDatabase\
- C:\Program Files\Waters Instruments\Logs\

16. Perform the procedure in the "ScanWave optimization" section.

17. Examine the ScanWave Daughter spectra.

18. Inspect the combined spectra from the daughter scan and the enhanced daughter scan; the peaks of interest must be approximately 0.5 Da FWHH.

19. If the enhanced daughter scan peaks are reasonably intense but split, suspect an issue with either the software or hardware with respect to a sequence signal.

20. If the enhanced data is split, set the manual T-Wave pulse velocity setting to 142, and repeat the acquisition.

21. If the problem has resolved, re-activate automatic enhanced tuning; otherwise contact GSS.

Enhancement check

Details of the ScanWave enhancement check are given in the [Xevo TQ-S Installation Manual](#) (P/N 715002461) or the [Xevo TQ-XS Installation Manual](#) (P/N 715005249). This check must be run after making a new ScanWave optimization.