Waters
MALDI SYNAPT G2 HDMS
System Overview and Maintenance Guide

Revision B

Waters

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Customer comments

Waters' Technical Communications department invites you to tell us of any errors you encounter in this document or to suggest ideas for otherwise improving it. Please help us better understand what you expect from our documentation so that we can continuously improve its accuracy and usability.

We seriously consider every customer comment we receive. You can reach us at tech_comm@waters.com.

Contacting Waters

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Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, and radiological hazards. You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice, and consult your organization's safety representative for guidance.

When you develop methods, follow the "Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory," *American Journal of Medical Technology*, 44, 1, pages 30–37 (1978). This protocol addresses good operating procedures and the techniques necessary to validate system and method performance.

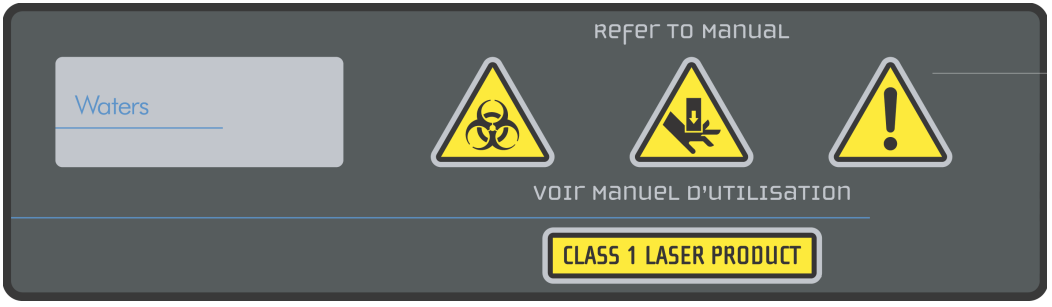
Considerations specific to the MALDI SYNAPT G2 HDMS system

Laser radiation hazard

Warning: To avoid personal exposure to potentially hazardous laser radiation, do not perform any procedure involving the instrument that is not set forth in this manual.

Do not operate any of the instrument's controls or adjustments if their purpose and use are not fully explained.

The MALDI SYNAPT™ G2 HDMS™ system uses a solid-state laser to produce a concentrated beam of invisible UV radiation. The instrument is a Class 1 laser product, as classified by EN 60825-1:2007 and indicated by a label affixed to the top of the instrument:



When you follow the operating procedures described in this manual, the laser beam remains contained within the instrument, and no risk of personal exposure to laser radiation ensues.

You must operate this instrument with all its exterior panels fitted. Removing any panel defeats the safety interlocks, creating the risk of personal exposure to a level of invisible radiation exceeding the Class 1 limit.

Only Waters service personnel qualified to service the instrument are authorized to open the safety cover that surrounds the laser. When this cover is open and the safety interlocks are defeated, the instrument becomes a Class 3B laser hazard, indicated by a warning label affixed to the safety cover:

Output specification of enclosed laser

Item	Specification A	Specification B
Wavelength	355 nm	355 nm
Average Power	20 mW @ 200 Hz	50 mW @ 1 kHz
Repetition Rate	Up to 200 Hz	1 kHz
Pulse Width	3 ns	2 ns
Pulse Energy	100 µJ @ 200 Hz	<50 µJ @ 1 kHz
Peak Power	34 kW	25 kW
Beam Divergence, Full Angle	<2 mrad	<2 mrad

Note: The laser fitted to your instrument will conform to one of the listed specifications only (A or B).

Hand crush hazard

Warning: To avoid hazards associated with the reciprocating or rotating parts in the source, keep clear of the regions marked with yellow and gray labels.

High voltage hazard

Warning:

- To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.
- To avoid nonlethal electric shock when the instrument is in Operate mode, avoid touching the areas marked with the high voltage warning symbol. To touch those areas, first put the instrument in Standby mode.

Hazards associated with removing an instrument from service

Warning: To avoid personal contamination with biohazardous or toxic materials, wear chemical-resistant gloves during all phases of instrument decontamination.

Warning: To avoid puncture injuries, handle syringes, fused silica lines, and borosilicate tips with care.

When you remove the instrument from use for repair or disposal, you must decontaminate all of its vacuum areas. These are the areas in which you can expect to encounter the highest levels of contamination:

- Source interior
- Waste tubing
- Exhaust system
- Rotary pump oil (where applicable)

The need to decontaminate other vacuum areas of the instrument depends on the kinds of samples the instrument analyzed and their levels of concentration. Do not dispose of the instrument or return it to Waters for repair until the authority responsible for approving its removal from the premises specifies the extent of decontamination required and the level of

residual contamination permissible. Management must also prescribe the method of decontamination to be used and the appropriate protection for personnel undertaking the decontamination process.

You must handle items such as syringes, fused silica lines, and borosilicate tips used to carry sample into the source area in accordance with laboratory procedures for contaminated vessels and sharps. To avoid contamination by carcinogenic, toxic, or biohazardous substances, you must wear chemical-resistant gloves when handling or disposing of used oil.

Safety advisories

Consult Appendix A for a comprehensive list of warning and caution advisories.

Operating this instrument

When operating this instrument, follow standard quality-control (QC) procedures and the guidelines presented in this section.

Applicable symbols

Symbol	Definition
	Manufacturer
	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
	Australia C-Tick EMC Compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Consult instructions for use

Audience and purpose

This guide is for operators of varying levels of experience. It is a comprehensive and detailed document that fully explains how to install and maintain the source component of a MALDI SYNAPT G2 HDMS system.

Intended use

Waters designed the MALDI SYNAPT G2 HDMS system to deliver authenticated, exact-mass measurement in both MS and MS/MS mode. The MALDI SYNAPT G2 HDMS is for research use only and is not for use in diagnostic procedures.

Calibrating

To calibrate the system, consult the calibration section of the operator's guide for the instrument you are calibrating. In cases where an overview and maintenance guide (not operator's guide) accompanies the instrument, consult the instrument's online Help system for calibration instructions.

Quality control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

ISM classification

ISM Classification: ISM Group 1 Class A

This classification has been assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements. Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment. Class A products are suitable for use in commercial, (that is, nonresidential) locations and can be directly connected to a low voltage, power-supply network.

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1 Preparing the MALDI SYNAPT G2 HDMS System for Operation

This chapter provides an overview of the system. It describes how to install the MALDI source, set up sample plates, and set up the camera for operation.

See the instrument's online Help for details on the system's operation.

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Waters MALDI SYNAPT G2 HDMS

The MALDI SYNAPT™ G2 HDMS™ system is a hybrid, quadrupole, ion mobility, orthogonal acceleration, time-of-flight (oa-TOF) mass spectrometer controlled by MassLynx™ software. The system combines exact-mass, high resolution mass spectrometry with high-efficiency ion-mobility-based measurements and separation (IMS).

See also: The *Waters SYNAPT G2 HDMS Operator's Overview and Maintenance Guide*.

The MALDI (matrix-assisted laser desorption ionization) source produces intact, gas-phase ions from large, nonvolatile and thermally-labile molecules such as proteins, synthetic polymers, and large inorganic compounds. Capable of high sample throughput, it also provides spatial data for MALDI imaging applications.

The MALDI interface switches between ESI mode and MALDI mode. After removing the ESI source, the MALDI source unit is transferred via a motorized stage to the source region and fixed in position.

MALDI SYNAPT G2 HDMS system

The image shows a MALDI SYNAPT G2 HDMS system with a LockSpray™ source fitted.

Installing the MALDI source

Caution: To avoid damage, do not move the MALDI source with the currently fitted source enclosure in place.

Before installing the MALDI source, you must first vent the instrument and then remove the currently fitted probe, source enclosure and ion block. Consult the *Waters SYNAPT G2 HDMS Operator's Overview and Maintenance Guide* for descriptions of these procedures.

Required materials

- Chemical-resistant, powder-free gloves
- Combined 2.5-mm Allen wrench and cone extraction tool
- 5-mm Allen wrench

To install the MALDI source

Warning: The source components can be contaminated with biohazardous and/or toxic materials. Always wear chemical-resistant, powder-free gloves while performing this procedure.

Warning: To avoid electric shock, prepare the instrument for work performed on its source before commencing this procedure (see page 2-4).

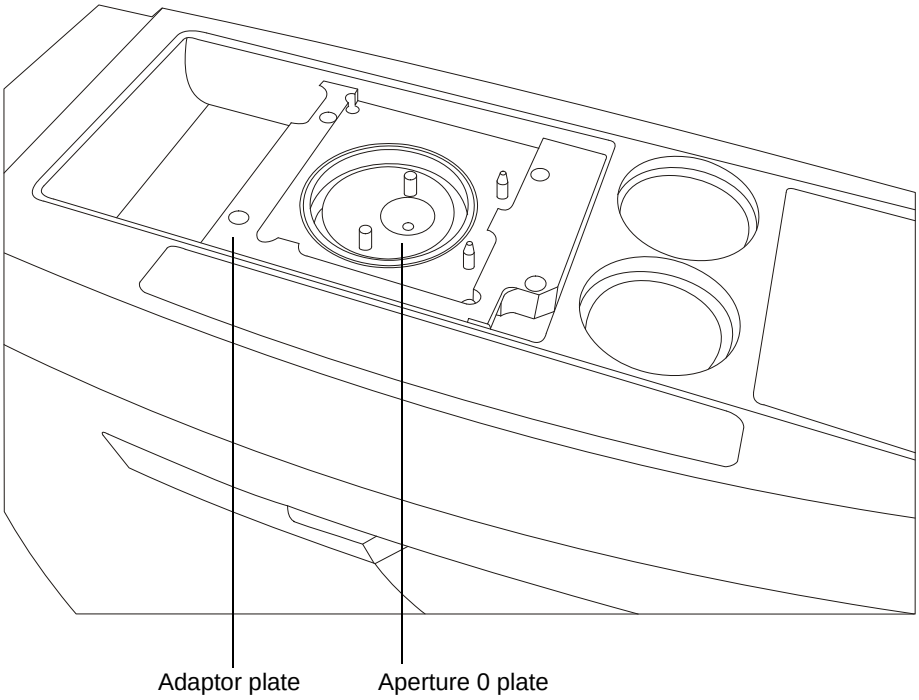
1. Vent the mass spectrometer (see the instrument's online Help).

   Warning: The probe and source can be hot. To avoid burn injuries, take great care while working with these components.

2. Remove the probe and source enclosure (see the *Waters SYNAPT G2 HDMS Operator's Overview and Maintenance Guide*).
3. Retrieve the combined 2.5-mm Allen wrench and cone extraction tool from its storage location on the source adaptor housing.
4. Remove the ion block assembly (see the *Waters SYNAPT G2 HDMS Operator's Overview and Maintenance Guide*).
5. Remove the top cover of the MALDI unit to expose the component repository.

Component repository



6. Locate the adaptor plate and aperture 0 plate, which are stored in the rectangular recess inside the component repository.

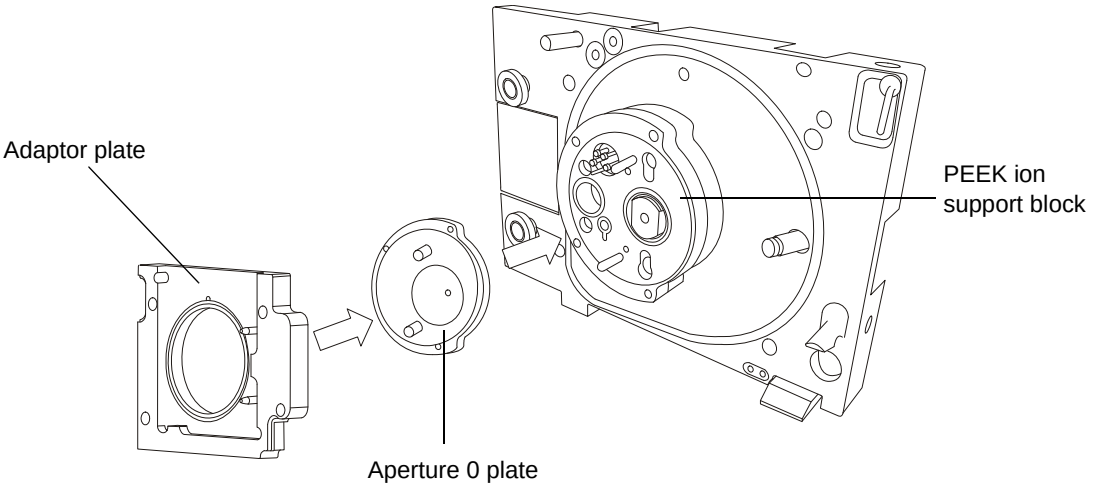
   Tip: You can store the ion block assembly (removed in step 4) in the circular recess under the adaptor plate, securing it using the captive screws on the assembly.

7. Fit the aperture 0 plate onto the two locating pillars, and then use the 2.5-mm Allen wrench to tighten the four captive screws until the plate fits tightly onto the PEEK ion support block.

   Caution: To avoid damage, do not overtighten the captive screws on the adaptor plate or aperture 0 plate.

8. Fit the adaptor plate onto the PEEK ion block support, and then use the 5-mm Allen wrench to tighten the four captive screws until the adaptor plate is square and tight.

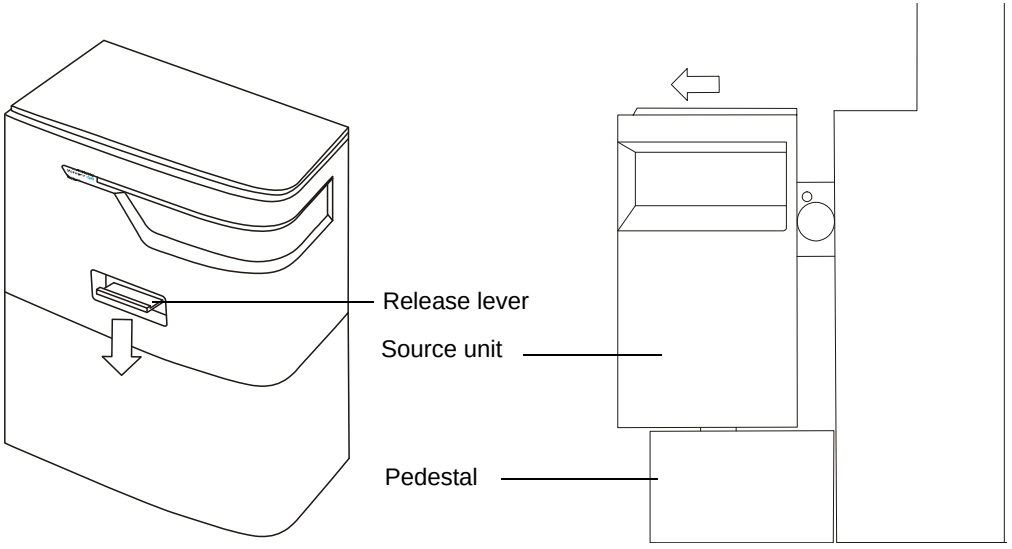
Fitting the adaptor and aperture 0 plates



9. Depress and hold the release lever while pulling the source unit gently back from the instrument, until the unit clicks into place.

Warning: To avoid crush injuries while moving the source unit, keep fingers away from all moving parts.

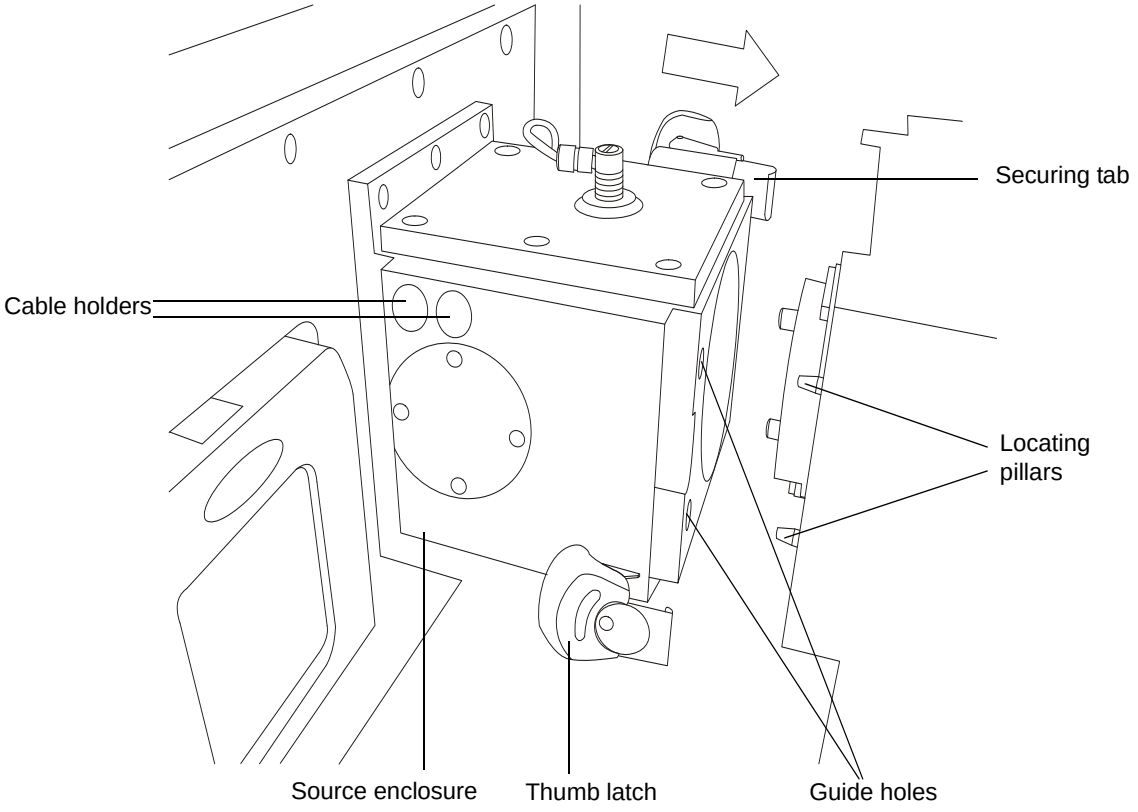
Moving the source unit



10. Press and hold the Raise button until the source unit fully extends above the pedestal.
11. Hold down the release lever while carefully pushing the source unit forward.

    Requirement: The two guide holes must fit over the locating pillars on the adapter plate.
12. Continue pushing the source unit until the source enclosure fits snugly against the adapter plate.

Attaching the source unit



13. Release the two securing tabs on either side of the source enclosure by pulling the thumb latches towards you and rotating them counterclockwise.

14. Hook the securing tabs onto the body of the aperture plate, rotate the thumb latches clockwise, and push them down to lock the source enclosure into place.
15. Remove the two color-coded cables from the cable holders and plug them into the corresponding sockets on the main instrument.

    Tip: The yellow cable carries the source activation signal, the blue cable carries the camera video signal.

To start the instrument

1. In the MassLynx Tune window, click Operate  .
2. In the Tune window, click Source > MALDI.

   Tip: If the MALDI source tab does not appear, the source activation cable (yellow) is incorrectly fitted. Refit the cable.

Troubleshooting vacuum errors

You cannot put the system in Operate mode unless an operating vacuum is established. The time the instrument requires to reach normal operating conditions depends on how long it has been vented.

Vacuum leaks prevent an instrument from achieving normal operating conditions. If you see a gap between the source and adapter plate and hear a hissing sound, the source is fitted incorrectly.

The following problems can cause a vacuum leak.

Possible cause	Corrective action
The source is not parallel with the instrument.	Vent the instrument and refit the source.
The aperture 0 plate is not tightly fitted to the ion block.	Vent the instrument and check the aperture 0 plate screws before refitting the source.
The O-rings have fallen out or are not seated correctly.	Vent the instrument and check the O-rings before refitting the source.
The MALDI source is not at the correct height.	Contact Waters®.

Removing the MALDI source

To remove the MALDI source

1. Unload the MALDI sample plate (see “To unload a sample plate” on page 1-12).
2. Click Vacuum > Vent and then OK.

   Tip: The instrument requires several minutes to vent completely.
3. When the instrument is vented, unplug the source activation (yellow) and camera (blue) cables, and return them to their holders.
4. Release the securing tabs using the thumb latches, and lock the tabs into their retracted positions (see the figure “Attaching the source unit” on page 1-7).
5. Hold down the release lever while carefully pulling back the source unit until the unit clicks into place.
6. Press and hold the Lower button until the source unit is fully lowered onto the pedestal.
7. Hold down the release lever while pushing the source unit forward until the unit clicks into place.
8. Remove the adaptor and aperture 0 plates, and store them in the component repository (see the figure “Fitting the adaptor and aperture 0 plates” on page 1-6).
9. Fit the source unit top cover.

The instrument is now ready for fitting other sources.

Setting up sample plates

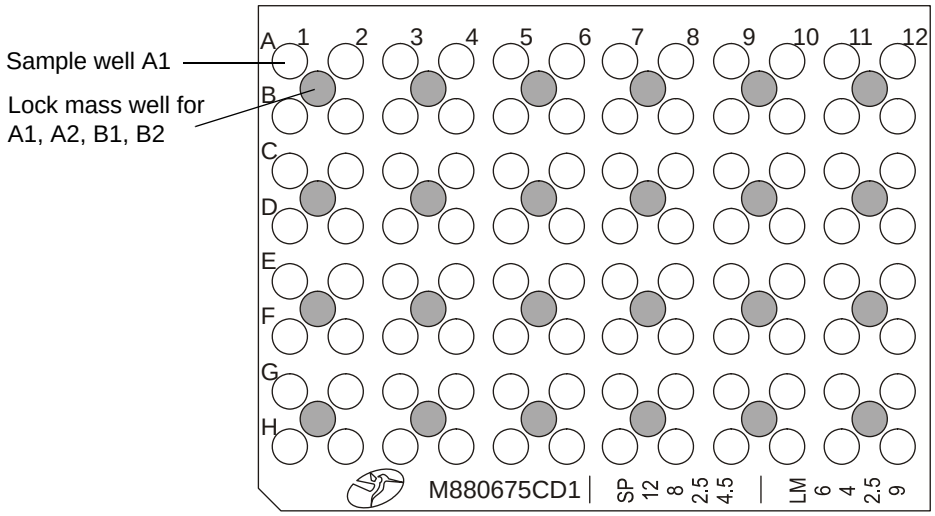
The system is compatible with all Waters sample plate formats. You can incorporate an optional, lock mass sample well for enhanced mass measurement accuracy. The system includes the following predefined sample plate formats:

- Standard 96 well
- 384 well
- BigSpot
- Imaging plate

You can select one of these predefined sample plate formats, or create your own user-defined sample plate format.

The system is also compatible with MALDI imaging plates and the imaging glass slide adaptor. For these options, use the standard 96-well plate format.

Standard 96 well sample plate



Defining a plate format

To select a predefined sample plate format

1. In the MassLynx Tune window, click Maldi > Sample Plate Definition.
2. In the Plate Type list of the Sample Plate Definition dialog box, select a plate format.
3. Click OK.

   Tips:

   - A sample log sheet, MALDI_SAMPLE_LOG.pdf, is included with your MassLynx installation, in the MassLynx folder.
   - With Adobe® Reader® installed on your PC, you can print log sheets and record sample details.

To create a user-defined sample plate format

1. In the MassLynx Tune window, click Maldi > Sample Plate Definition.
2. Click New.
3. Enter relevant parameters for the plate type you are using.
4. In the Index Type drop-down list, select Numerical or Alpha Numeric addressing of the sample wells.
5. To define wells for lock mass correction, select the Use Lock Mass Wells check box and specify the relevant parameter settings.
6. Click Save As, and save with an appropriate name.

   Tip: To view the definition of the newly created plate format and confirm its settings, click Open.

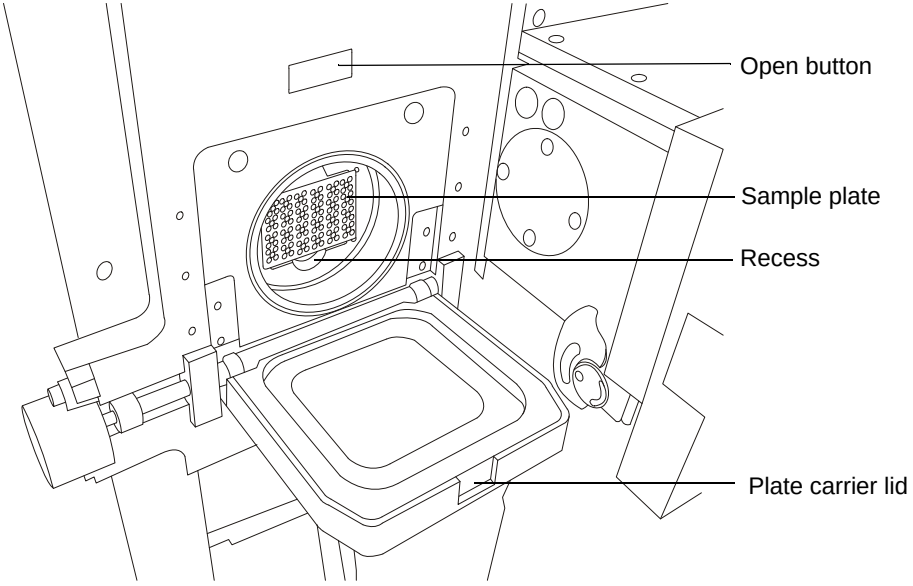
Loading and unloading sample plates

You can load and unload plates while the system is in Operate mode.

To load a sample plate

1. Press the plate carrier Open button to release the plate carrier lid.

Plate carrier



2. Hold the sample plate by the sides, orienting it so that the corner indentation faces the bottom left-hand side, and then place the plate into the recess within the plate carrier.
3. Close the plate carrier lid by lifting it manually until the magnetic clasps engage.
4. In the MALDI Source tab of the MassLynx Tune window, click Load  .

   Result: The system evacuates the plate carrier air lock and transports the sample plate into the source. This process requires approximately 3 minutes.
5. To align the laser beam with a sample well, in the MALDI Source tab's Sample text box, enter the sample well number.

   Tip: When you load a sample plate, the Unload button glows blue to indicate the unloading function is available. Neither button glows when the instrument is acquiring data.

To unload a sample plate

1. In the MassLynx Tune window, click Unload  .

   Caution: To avoid contamination, do not to touch the surface of the sample plate.
2. When the carrier stops moving, open the plate carrier lid, and then remove the sample plate.

Load/Unload button states

State	Meaning
Blue	Normal operation – Load/Unload
Red 	Recoverable error. The plate is out of position. Click the buttons to clear the error and reindex the plate. If it does not recover, contact Waters.
Both disabled	Indicates one of the following conditions: • Acquiring data • Vented error • Plate carrier error

Troubleshooting plate carrier errors

In the status bar of the MassLynx Tune window, the plate carrier control buttons display the status of the plate carrier (see the table above). In an error condition, the plate carrier generates additional information displayed in a message box in the Tune window.

These are the two types of plate carrier error:

- Position error
- Sample plate carrier error

In either case, you must clear the error before resuming normal operation.

Clearing a position error

The following problems can cause a position error:

- The sample plate carrier does not reach its requested position before an internal system timeout halts its movement.
- A requested move is beyond the limit of travel for the sample plate carrier (an inappropriate plate definition can cause this error).

In the case of a position error, the sample plate carrier control buttons change state to indicate the correct action to clear the error.

Clearing a sample plate carrier error

In the case of a sample plate carrier error, the control buttons are disabled, and an error message appears in the Plate Carrier Status Message dialog box.

If you connect the source activation (yellow) cable when a plate is already loaded, you receive a status message stating “Position Error - (Clear with Reindex)”, indicating that the sample plate control is restarting. To clear the error, remove and refit the source activation cable.

If the sample plate carrier error persists after the resetting sequence, contact Waters.

Using alternative sample plates

You can use MALDI sample plates from vendors other than Waters.

If the plates are of the same dimensions as a standard Waters plate, modify the sample plate definition (see "To create a user-defined sample plate format" on page 1-11).

If the plates are a different size from the standard Waters plate, you must remove the sample plate carrier insert, which is held in the carrier magnetically.

Glass microscope slides for MALDI imaging use a special holder supplied with the instrument. The holder fits into the sample plate carrier using the sample plate carrier insert.

When you use a sample plate of larger dimensions than a standard Waters plate, sometimes you cannot access all the rows and columns on the plate. If you select a sample well beyond the limit of movement, a plate carrier error occurs (see "Troubleshooting plate carrier errors" on page 1-13).

To remove the plate carrier insert

1. In the MassLynx Tune window, click Unload  .
2. Open the plate carrier lid, and remove the currently loaded sample plate (see "Loading and unloading sample plates" on page 1-11).
3. Remove the sample plate carrier insert.
4. Store the sample plate carrier insert in the source top cover, directly above the plate carrier lid.

   Tip: To revert to standard Waters plates, refit the carrier insert.

Setting up the MALDI camera

You can use the camera to display a real-time digital image of the sample target on the acquisition PC. Crosshairs associated with the camera image of the selected sample well indicate the firing point. When you fire the laser, a spot appears where the sample is consumed.

Controlling the camera

The camera operates in two image modes, Static and Live. In Static Image mode, click the image to move the crosshairs. In Live Image mode, you cannot move the crosshairs.

To operate the camera

1. To open the Camera Control window, in the MassLynx Tune window, click Camera  .
2. To toggle between live and static images, in the Camera Control window, click the status bar.
3. To move the camera position, click the status bar to toggle to Live Image mode, and then click and drag on the camera image.

Aligning the camera image with the laser position

When you fire the laser, the laser spot is not always perfectly aligned with the crosshairs. You can correct the misalignment from the Camera window.

To align the camera image with the laser position

1. In the MassLynx Camera Control window, click the status bar to change the camera status to Live Image mode.
2. Move the camera position to view a prepared sample well.
3. To fire the laser, in the Tune window, click Fire  .

   Rule: Do not move the laser until enough material is removed from the sample plate that you can see a laser spot.
4. Click the status bar to change the camera status to Static Image mode.
5. To move the camera crosshairs to the correct position, click on the laser spot in the camera image.
6. When the laser and crosshair positions align, click the status bar to toggle back to a Live Image mode.

   Result: The camera software stores the crosshair position.

Aligning the camera image and the sample well

After you align the camera crosshairs to the laser firing position, the center of the sample well does not always align with the camera crosshairs. You can align the sample well position relative to the camera image from the Tune window.

To realign the camera image with the sample well position

1. From the MassLynx Tune window, click Maldi > Source Settings > Password.
2. Type “access” and then click Apply.
3. In the Source Settings dialog box, click Sample Plate, and select Nudge Using Crosshairs.

   Result: The plate moves to sample well A1.
4. Adjust the sample plate crosshairs until the center of the sample well aligns with the camera crosshairs.
5. Click Mark Position  to store the current alignment.
6. Repeat steps 4 and 5 until the sample well center and the laser firing mark align satisfactorily.
7. Click Accept Position  to accept the current alignment.

   Result: The plate reindexes and moves to sample A1 using the latest alignment settings.

2 Maintenance Procedures

In addition to standard system maintenance, described in the *Waters SYNAPT G2 HDMS Operator's Overview and Maintenance Guide*, you must perform additional maintenance procedures for the MALDI source.

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Maintenance schedule

The following table lists the periodic maintenance schedules that ensure optimum instrument performance.

Maintenance schedule

Procedure	Frequency	For information...
Clean the hexapole.	When the sensitivity decreases to unacceptable levels.	See page 2-5.
Clean and replace the vacuum lock O-ring.	When the vacuum does not achieve <3E-6 mbar within a few minutes of loading a sample plate. When the vacuum does not reach <1E-6 mbar after pumping-down (evacuating) overnight.	See page 2-10.

Spare parts

Replace only the parts mentioned in this document. For spare parts details, see the Waters Quality Parts Locator on the Waters Web site's Services/Support page.

Safety and handling

Bear in mind the following safety considerations when performing maintenance procedures:

Warning: The instrument components can be contaminated with biologically hazardous materials. Always wear chemical-resistant, powder-free gloves while handling the components.

Warning: To prevent injury, always observe Good Laboratory Practices when handling solvents, changing tubing, or operating the instrument. Know the physical and chemical properties of the solvents used, see the Material Safety Data Sheets for the solvents in use.

Warning: To avoid electric shock,

- do not remove the instrument's panels. There are no user-serviceable items inside the instrument.
- ensure that the instrument is in Standby mode before commencing any maintenance.

Warning: The probe and source can be hot. To avoid burn injuries, take great care while working with these components.

Warning: To avoid puncture wounds, take great care while working with the source enclosure open if one or both of these conditions apply:

- An ESI probe is fitted (the probe tip is sharp).
- A corona pin is fitted (the pin tip is sharp).

See Appendix A for safety advisory information.

Preparing the instrument for work performed on its source

Warning: Follow the procedure below before working on the source (for example, when changing the probe, installing or removing the corona pin, operating the source isolation valve, and when maintaining the source).

To prepare the instrument

1. In the Instrument Console, click Stop Flow  to stop the LC flow, or if column flow is required, divert the LC flow to waste as follows:
   a. In the Instrument Console system tree, expand SYNAPT G2 Detector, Interactive Fluidics.
   b. Click Control  .
   c. Select Waste as the flow state.
2. In the Instrument Console, click Standby  .
3. Set the source temperature to 30 °C.
4. Wait 30 minutes to allow the desolvation gas flow to cool the probe and source.
5. In the Instrument Console, ensure that the API desolvation gas flow is stopped.

Cleaning the hexapole

The hexapole requires regular cleaning. In most cases, gently washing the hexapole with a wash bottle filled with propan-2-ol (IPA) suffices. In severe cases, you can place the whole assembly in a large beaker of propan-2-ol and sonicate it for 30 minutes.

Note: Cleaning the hexapole requires you to vent the instrument, following which you must condition the detector. See the The *Waters SYNAPT G2 HDMS Operator's Overview and Maintenance Guide*.

Required materials

- Chemical-resistant, powder-free gloves.
- 3-mm Allen wrench.
- Wash-bottle filled with propan-2-ol (IPA).
- Appropriately sized glass vessels in which to completely immerse components when cleaning. Use only glassware not previously cleaned with surfactants.
- Ultrasonic bath.
- Source of oil-free, inert gas (nitrogen or helium) for drying (air-drying optional).

To remove and clean the hexapole

Warning: The instrument components can be contaminated with biologically hazardous and toxic materials. Wear chemical-resistant powder-free gloves at all times while handling the components.

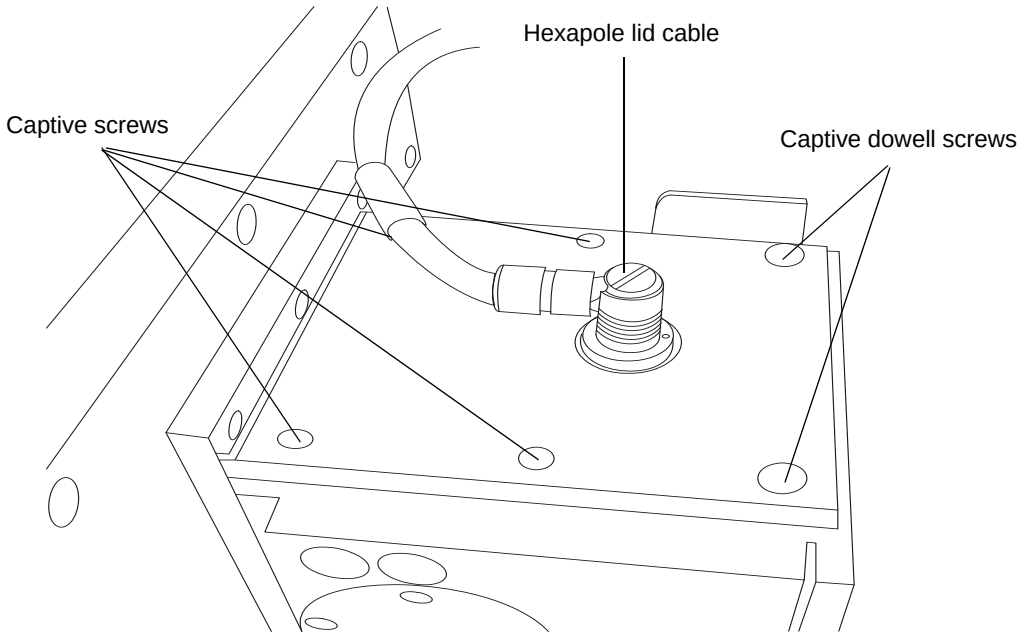
Warning: Ensure the instrument is in Standby mode and vented before removing the hexapole.

1. In the MassLynx Tune window, click Standby  , and ensure that the Operate LED glows red.
2. Click Vacuum > Vent and then click OK.

   Tip: The instrument takes several minutes to vent completely.

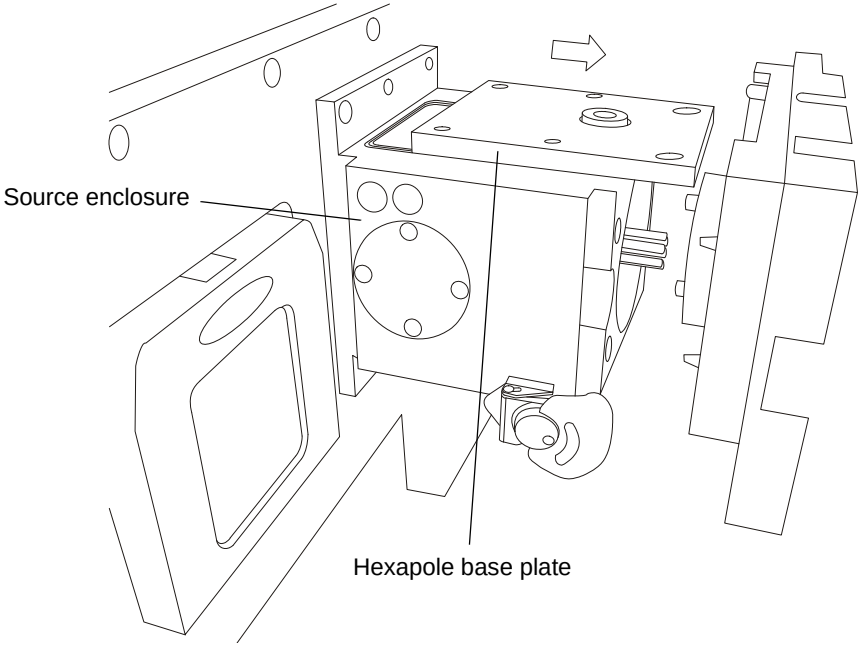
3. When the instrument is vented, unplug the source activation (yellow) and camera (blue) cables, and return them to their holders.
4. Release the securing tabs using the thumb latches, and lock the tabs into their retracted positions (see the figure “Attaching the source unit” on page 1-7).
5. Hold down the release lever while carefully pulling back the source unit, until the unit clicks into place.

Hexapole cable and screws

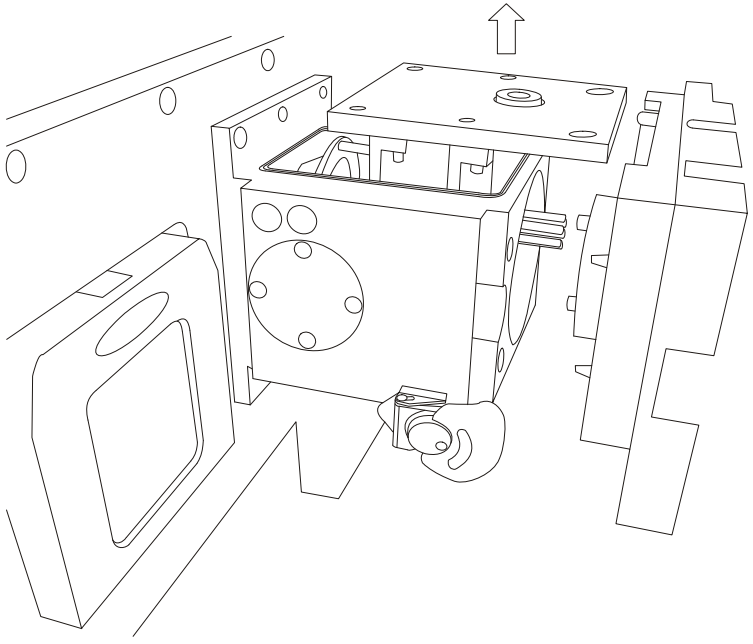


6. Disconnect the hexapole lid cable.
7. Use the 3-mm Allen wrench to undo the 4 captive screws and 2 captive dowell screws.
8. Slide the hexapole base plate forward, keeping it level with the source enclosure.
9. Holding the hexapole base plate firmly, lift the hexapole assembly partially free of the source housing.

Sliding the hexapole base plate forward

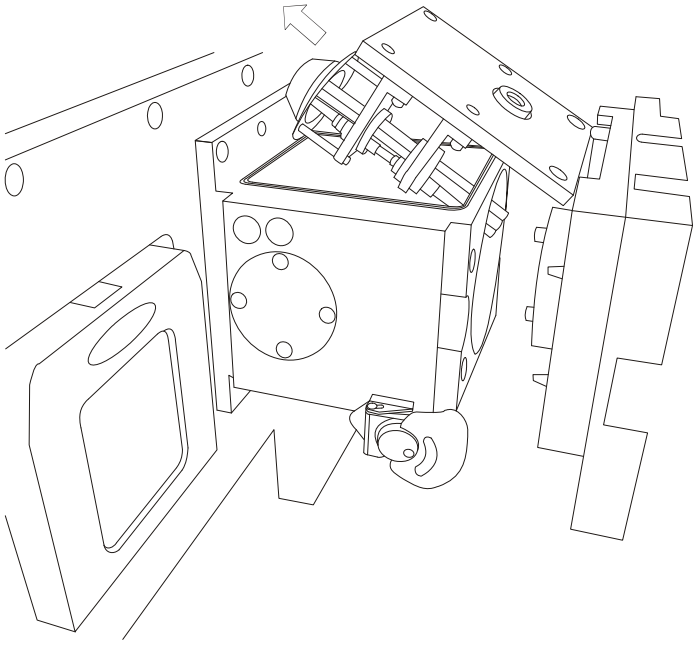


Lifting the hexapole assembly



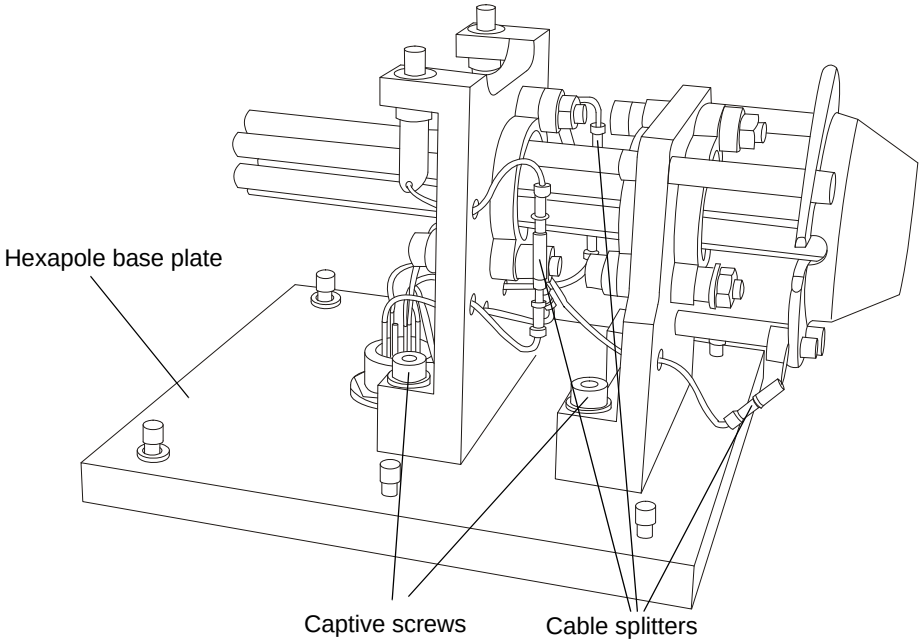
10. Tilt the hexapole assembly by approximately 45°, and then withdraw it carefully from the source housing.

Withdrawing the hexapole assembly



11. Remove the hexapole from the assembly by loosening the four captive screws, separating the three cable splitters and then detaching the hexapole from the base plate.

Hexapole assembly



12. Wash the hexapole using a wash bottle filled with propan-2-ol (IPA).

    Tip: If the contamination is severe, place the whole assembly in a large beaker of propan-2-ol and sonicate it for 30 minutes.

13. Blow dry the hexapole with inert, oil-free gas.
14. Refit the hexapole to the base plate, and tighten the four captive screws.
15. Reattach the three cables by snapping the cable splitters together.

    Requirement: The hexapole cables are color-coded to facilitate reattachment. Only connect cable ends of the same color together.

16. Insert the hexapole assembly into the source by following steps 8-11, in reverse order.
17. Refit the hexapole lid cable and then pump down (evacuate) the instrument.

    Requirement: Ensure that the hexapole lid cable is refitted before switching the instrument into Operate mode.

Cleaning and replacing the vacuum lock O-ring

If the sample plate carrier fails to pump the plate carrier lid within the three-minute system timeout period, a plate carrier error occurs (see "Troubleshooting plate carrier errors" on page 1-13).

The following conditions can cause a plate carrier error:

- The lid fails to seat correctly on the vacuum lock O-ring.
- The vacuum lock O-ring fails or is contaminated.

Required materials

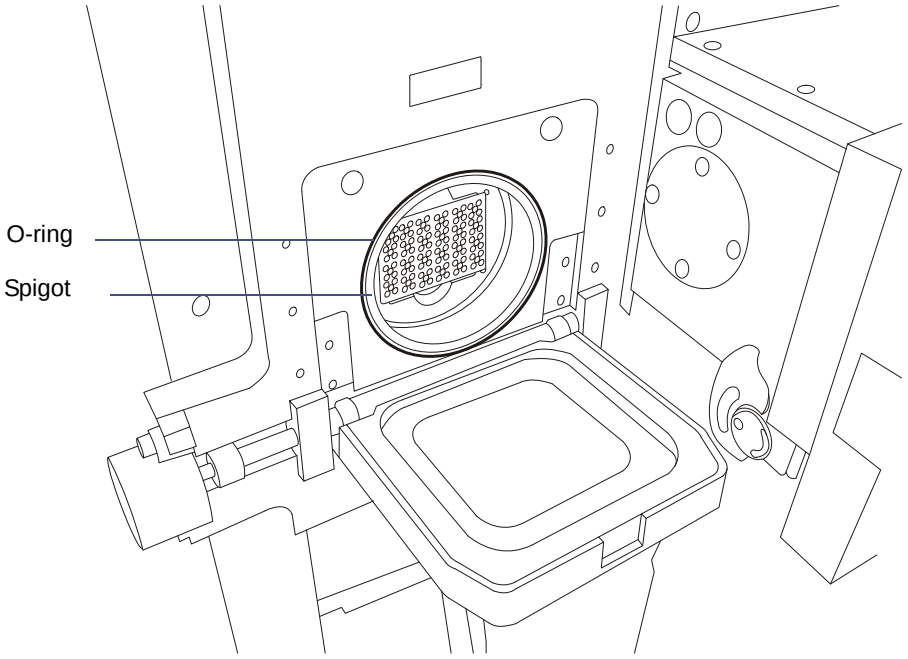
- Chemical-resistant, powder-free gloves
- Lint-free cloth
- Wash bottle containing methanol

To remove and clean the O-ring

Warning: The instrument components can be contaminated with biologically hazardous and toxic materials. Wear chemical-resistant powder-free gloves at all times while handling the components.

1. Unload the sample plate, and open the plate carrier lid. See "Loading and unloading sample plates" on page 1-11.
2. Lift the O-ring off the spigot.

Plate carrier O-ring



3. Clean the O-ring and its seating surface with a lint-free cloth and methanol.
4. Clean the inside surface of the lid (see step 3), and ensure that no fibers or other particles remain.
5. Examine the O-ring. If it is in good condition, refit it, seating it evenly around the spigot. If the O-ring is in poor condition, fit a new O-ring.

Warning: The seals can be contaminated with biohazardous and/or toxic materials. Ensure that they are correctly disposed of according to local environmental regulations.

6. Dispose of the O-ring in accordance with local environmental regulations.

3 Sample Preparation

Sample preparation is accepted as the most important step in mass analysis with MALDI mass spectrometers, and the impact of preparation quality on data quality cannot be over emphasized. This chapter gives sample and matrix details and protocols to act as a guideline. Waters recommends that you keep meticulous records of your procedures.

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Sample preparation considerations	3-3
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Safety

Warning: Many procedures in this chapter involve using flammable or caustic agents. There is danger of contamination by biological agents that constitute a threat to humans. Refer to Material Safety Data Sheets and take all necessary precautions.

Calibration standards

For mass analysis in positive ion mode, calibrate using a polyethylene glycol (PEG) mixture.

To prepare calibration standards for positive ion analysis

1. Prepare the following stock solutions:
   - 1 mg/mL of PEG 200, 400, 600, 1000, 2000, and 3000 in water.
   - 2 mg/mL of NaI in water.
   - 3 mg/mL alpha cyano in 50:50 water/acetonitrile.
2. Mix PEG oligomers with the NaI in the ratio 10:1 (v/v).
3. Mix the PEG mixture 1:1 with the alpha cyano matrix.
4. Spot 1 µL of the PEG matrix mixture onto the sample plate and allow it to air dry.

Washing stainless steel MALDI plates

To wash plates

1. Scrub the sample plate in 2% Decon 90 with a soft nylon brush to remove matrix deposits.

   Requirement: When analyzing synthetic polymers, use a suitable organic solvent such as dichloromethane.
2. Rinse the plate in distilled water.
3. Ultrasonically agitate the plate for 10 minutes in 1:1:1 dichloromethane/acetone/methanol or an alternative degreasing agent.

4. Ultrasonically agitate the plate for a further 10 minutes in methanol.
5. Dry the plate under a dry nitrogen stream.

Sample preparation considerations

Consider the following variables before preparing MALDI samples:

- Expected molecular mass range.
- Concentration or amount of sample supplied.
- Suitable solvent.
- Contaminants present, such as salts, buffers, or glycerol.

  Tips:

  – Higher purity samples yield better spectra with less interference.

  – You can decontaminate samples before or after preparing the sample spots.
- Sample type. For example, peptide, protein, protein digest, oligonucleotide, oligosaccharide, or synthetic polymer.
- Sample stability. For example, sensitivity to acid.

  Requirement: If the sample is acid-sensitive, omit TFA from the procedures outlined.
- Molecular structure or principal functional groups.

Matrixes

Matrixes and substrates

Matrixes and substrates

Matrix		Typical substrate
CHCA	α-cyano-4-hydroxy cinnamic acid	Peptides, polymers
Sinapinic acid	3,5-dimethoxy-4-hydroxycinnamic acid	Proteins, peptides, polymers
DHB	2,5-dihydroxybenzoic acid	Sugars, peptides, nucleotides, polymers
CMBT	5-chloro-2-mercaptobenzothiazole	Proteins, peptides
Dithranol	1,8-dihydroxy-9(10*H*)-anthracenone	Synthetic polymers
THAP	2,4,6-trihydroxyacetophenone	Oligonucleotides
HABA	2-(4-hydroxyphenylazo)-benzoic acid	Glycolipids, peptides, proteins
HPA	Hydroxypicolinic acid	Oligonucleotides, peptides, glycoproteins
IAA	β-indole acrylic acid	Polymethyl methacylates
	3-aminoquinoline	Sugars, peptides, nucleotides, polymers
	4-hydroxy-α-phenylcinnamic acid	Proteins, glycoproteins
	All trans-retinoic acid	Synthetic polymers

Matrix mixtures

Matrix mixtures and substrates

Matrix mixture	Substrate
S-DHB = 5-methoxysalicilic acid and 2,5-dihydroxybenzoic acid (mixed 1:10)	Peptides, proteins
α-cyano-4-hydroxy cinnamic acid and 2,5-dihydroxybenzoic acid (mixed 3:5)	Synthetic polymers
Anthranilic acid and nicotinic acid	Oligonucleotides

Preparing matrixes

The matrix:sample molar ratio must be >5000:1. Matrix solutions are light-sensitive, so prepare them fresh each day and keep them in a dark tube. A possible exception is hydroxypicolinic acid (HPA), which can improve sensitivity for oligonucleotides/DNA when the matrix solution has "aged" for up to two weeks before analysis, turning brown in color.

A washing stage or re-crystallization of the matrix before use can improve results, maybe due to a reduced contaminants, such as organic chemicals and metal ions.

Premix method

To mix the matrix

1. Mix 2 µL of sample with 2 µL of matrix solution.
2. Spot 1 µL of this mixture onto the MALDI sample plate.
3. Repeat step 1 and step 2 for the required samples and allow them to air dry.

Thin film technique

To improve signal strength, but with a reduction in sample longevity, reduce the matrix crystal size by seeding the sample spot with a thin film of matrix solution (dissolve the matrix in a volatile solvent such as acetone).

Tip: With this matrix preparation it can be beneficial to reduce the laser firing rate during the acquisition.

Use a low-volume pipette tip, such as a Gilson P2 pipette, for reproducible 1-µL spotting of the volatile matrix solution. Deposit the sample/matrix mixture directly onto the thin film and air dry.

CHCA (α-cyano-4-hydroxy cinnamic acid)

Use a concentration of 2 to 10 mg/mL in 1:1 aqueous (aq) 0.1% trifluoroacetic acid (TFA)/acetonitrile.

For thin film CHCA, use a concentration of 10 mg/mL in 495:495:10 ethanol/acetonitrile/0.1% TFA (aq). Dilute this solution 4:1 in 10 mg/mL nitrocellulose.

You can also dissolve CHCA in other organic solvents for nonaqueous sample preparations, such as synthetic polymers.

For peptide analysis at the low fmol level, to reduce the level of matrix clusters observed, use CHCA at a concentration of 3.6 mg/mL.

Sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid)

Use a concentration of 10 mg/mL in 4:6 acetonitrile/0.1% TFA (aq).

To improve the signal, mix sinapinic acid at a ratio of 3:1 matrix to the sample.

Thin film sinapinic acid

To obtain improved signal intensities with protein samples, use a thin film sinapinic acid matrix.

To prepare thin film sinapinic acid matrix

1. Prepare a thin film sinapinic acid matrix as described in "Sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid)" on page 3-6 and also at a concentration of 10 mg/mL in acetone.
2. Apply 1 µL of the thin film solution to the sample plate and allow it to air dry.
3. Mix the sample 1:1 with the standard sinapinic acid matrix preparation and apply 1 µL of this mixture over the thin film.

You can also dissolve sinapinic acid in organic solvents to prepare nonaqueous samples such as synthetic polymers.

DHB (2,5-dihydroxybenzoic acid)

Use a concentration of 10 mg/mL in 70:30 water/acetonitrile for general peptide analysis, or prepare a saturated solution in 20:80 water/acetonitrile for the analysis of oligosaccharides.

You can also dissolve DHB in organic solvents to prepare non-aqueous samples such as synthetic polymers. Use a concentration of 10 mg/mL in 1:1 methanol/chloroform.

S-DHB ("Super" DHB)

Add 1 mg of 5-methoxysalicilic acid to 9 mg of DHB, and dissolve the mixture in 9:1 0.1% TFA (aq)/acetonitrile.

CMBT (5-chloro-2-mercaptobenzothiazole)

Use a concentration of 10 mg/mL in 1:1:1 acetonitrile/methanol/aqueous 0.1% formic acid.

CMBT is not very soluble and must be ultrasonically agitated before use, to produce a saturated solution at the specified concentration.

Dithranol (1,8-dihydroxy-9(10*H*)-anthracenone)

Use a concentration of 20 mg/mL in tetrahydrofuran, dichloromethane, methanol, or hexafluoro-2-propanol (HFIP).

For synthetic polymer analysis use the matrix together with a trifluoroacetic acid salt such as sodium, potassium, or silver.

HABA (2-(4-hydroxyphenylazo)-benzoic acid)

Use a concentration of 3.5 mg/mL in methanol.

Use this matrix for peptide and protein analysis. It is also effective in the analysis of glycolipids.

HPA (hydroxypicolinic acid)

Use a concentration of 25 mg/mL in 25:75 water/acetonitrile.

Use this matrix together with an ammonium salt solution, or an ion exchange resin, to analyze oligonucleotides.

IAA (β-indole acrylic acid)

Use a concentration of 10 mg/mL in acetone.

Use this matrix to analyze acrylates.

THAP (2,4,6-trihydroxyacetophenone)

Use a concentration of 25 mg/mL in 1:1 water/acetonitrile.

Use this matrix together with an ammonium salt solution, or an ion exchange resin, to analyze oligonucleotides.

Anthranilic acid/nicotinic acid

Use a concentration of 27.9 mg of anthranilic acid and 12.3 mg of nicotinic acid in 500 µL acetonitrile/300 µL aqueous ammonium citrate (100 mM)/300 µL water.

4-hydroxy-α-phenylcinnamic acid

This matrix is used as an alternative to sinapinic acid. It is particularly useful for analyzing glycoproteins because it does not produce matrix adducts. However, the overall sensitivity is reduced compared to that of sinapinic acid.

3-aminoquinoline

Use a concentration of 10 mg/mL in 1:1 methanol/0.1% TFA (aq).

All *trans*-retinoic acids

Use a concentration of 10 mg/mL in THF.

These acids are especially useful for the analysis of high molecular weight polystyrenes.

Analysis of peptides and proteins

You can use MALDI to identify proteins from the unique masses of peptide fragments produced after specific digestion with protease enzymes. Trypsin is typically used to digest proteins that have been isolated electrophoretically from biological samples.

In most cases, peptides and proteins are water soluble and stable in the presence of 0.1% TFA. The presence of the acid prevents the analyte from becoming associated with the walls of the tube.

Prepare each sample at a concentration of between 10 and 500 fmol/µL (peptides) and 1 and 10 pmol/µL (proteins). For unknown sample concentrations, dilute over four orders of magnitude and load four separate spots on the sample plate. Dilution of the sample often enhances the higher molecular weight components, especially in a mixture.

Use the above concentrations as a starting point. For those samples that do not produce signals after trying various matrixes, try diluting the sample rather than concentrating the sample as a second step.

You can improve the data from salt or buffer contaminated samples by on-spot washing.

Analyze proteins above 12 kDa with a sinapinic acid matrix; for improved sensitivity, use the thin film technique. Sinapinic acid produces adducts (M + 208 Da) that correspond to the addition of sinapinic acid with the loss of water. Multiply-charged species and multimers are often observed, but the $[M+H]^+$ species typically predominates.

Preparing peptide and protein standards

Prepare and store standards at a concentration of 1 mg/mL in 0.1% TFA (aq) in a freezer. Dilute to obtain a working concentration of approximately 10 pmol/µL.

Example standards are shown in the following table.

Dilution factors for peptide and protein standards

Substance name	Average molecular mass (Da)	Dilution factor*
Leucine enkephalin	555.6	180
Bradykinin	1060.2	95
Angiotensin I	1296.5	77
Glu-fibrinogen	1570.6	64
Renin substrate	1759.0	57
ACTH (18-39 clip)	2465.7	40
Insulin (bovine)	5733.5	17
Cytochrome-C (horse heart)	12360	8
Myoglobin	16951	6
Trypsinogen	23980	4

*Dilution factor of 1 mg/mL solution to give 10 pmol/µL.

Store these solutions in a freezer and use as required, although solutions will deteriorate over time.

Preparing protein digests

Caution: To avoid risk of sample contamination from keratin, gloves that have been rinsed with water must be worn throughout the sample handling stages.

In-gel protein digests

To prepare in-gel protein digests

The following procedure is for manual in-gel digestion of proteins separated by 2D-polyacryamide gel electrophoresis (Coomassie blue stain).

1. Rinse the gel with distilled water and excise the bands of interest with a clean scalpel, cutting as close to the protein spot as possible. Chop the excised spot into pieces (1 × 1 mm).
2. If the gel pieces are still blue, rehydrate them in 100 to 150 µL 100 mM ammonium bicarbonate and heat to 37 °C. After 10 to 15 minutes add an equal volume of acetonitrile.
3. Vortex the solution for 30 seconds, then centrifuge, remove the supernatant, and shrink the gel with acetonitrile.
4. Dry the gel fragments in a vacuum centrifuge. If the Coomassie stain persists, repeat the destaining procedure.
5. Wash the gel fragments with 150 µL water for 5 minutes.
6. Centrifuge the gel fragments and remove the supernatant.
7. Add acetonitrile (3 to 4 × volume of gel pieces) to the gel fragments and wait for 10 to 15 minutes, until the gel fragments have shrunk and are white.
8. Dry the gel fragments in a vacuum centrifuge.
9. Swell the gel fragments in 1 mM 1,4-dithiothreitol (DTT) 100 mM ammonium bicarbonate, using the minimum volume to completely cover the gel and then incubate for 30 minutes at 56 °C to reduce the protein (this stage is recommended even if the proteins were reduced before electrophoresis).

10. Dehydrate the gel with acetonitrile, as above.
11. Centrifuge the gel, remove the supernatant, and then add 55 mM iodoacetamide dissolved in 100 mM ammonium bicarbonate, using the minimum volume to completely cover the gel.
12. Set aside the mixture for 30 minutes, at room temperature in the dark.
13. Centrifuge and remove the supernatant, wash with 15 µL 100 mM ammonium bicarbonate for 15 minutes, and then centrifuge and remove the supernatant.
14. Dehydrate the gel with acetonitrile.
15. Remove the supernatant and dry the gel fragments in a vacuum centrifuge.
16. Rehydrate the gel particles in a minimum volume of digest buffer [50 mM ammonium bicarbonate containing 12.5 ng/µL trypsin (w/v)] and incubate at 4 °C for 30 to 45 minutes. If the gel fragments absorb all the liquid in 15 to 20 minutes, add more buffer.
17. Incubate the gel at 37 °C for 16 hours.
18. To recover the peptides, add 10 µL of 50% acetonitrile/5% formic acid to the digest mixture and ultrasonically agitate the mixture for 10 minutes.
19. Perform 2 to 3 extractions of a suitable volume of the digest mixture, that is, double the volume required to immerse the gel pieces.
20. Transfer the supernatant after each wash using gel-loading pipette tips into Eppendorf™ micro test tubes.

    Note: The high acid concentration is used to minimize adsorptive sample loss.

    Tip: ZipTip™ extraction of samples into a lower volume can increase the concentration of the recovered peptides. Store the recovered peptides below -20 °C.

In-solution protein digests

The first procedure describes a method for the tryptic digestion of noncovalently bound proteins using Waters RapiGest™ SF. RapiGest SF is a reagent used to enhance in-solution enzymatic digests of proteins by solubilizing the proteins, making them more susceptible to enzymatic

proteolysis without inhibiting enzyme activity, thereby allowing the rapid digestion of proteins.

If the protein has covalent links (disulfide bonds), you must cleave and acetylate them.

To digest proteins without disulfide bonds

1. Prepare 50 mM ammonium bicarbonate solution (39.6 mg ammonium bicarbonate in 10 mL water).
2. Suspend 1 mg of RapiGest in 1 mL of 50 mM ammonium bicarbonate [resulting in a 0.1% (w/v) RapiGest solution].
3. Dissolve protein in 25 to 50 µL of 0.1% RapiGest solution and vortex the solution.
4. Equilibrate the sample at 37 °C for 2 minutes.
5. Add enzyme, at a ratio of 1:100 to 1:20 (enzyme/protein, w/w).
6. Incubate the sample at 37 °C for 20 to 60 minutes.
7. If the sample is particularly hydrophobic (for example, membrane proteins), boil the protein/RapiGest mixture at 100 °C for 5 minutes, and then cool the sample to room temperature before adding enzyme.

   Requirement: Once the sample is digested the you must remove the RapiGest from the sample before analysis by MALDI.

   To remove the RapiGest from the sample, add 2 µL neat TFA and incubate for 30 minutes at 37 °C, then centrifuge at 15,000 rpm for 15 minutes and remove supernatant.
8. Prepare a stock solution of 500 mM HCl.
9. Add 1:10 (v/v) HCl stock solution/sample (so that pH=2). The final concentration of HCl must be 30 to 50 mM.
10. Incubate the sample at 37 °C for 30 to 45 minutes.

    Tip: A slight cloudiness is normal.
11. Analyze the sample directly by MALDI, or first dilute to a suitable concentration.

To digest proteins with disulfide bonds

1. Prepare 50 mM ammonium bicarbonate solution (39.6 mg ammonium bicarbonate in 10 mL water).
2. Suspend 1 mg of RapiGest in 500 µL of 50 mM ammonium bicarbonate [resulting in 0.2% (w/v) RapiGest solution].
3. Dissolve protein in 25 to 50 µL of 0.2% RapiGest solution and vortex the solution.
4. Add DTT to the protein sample, to a final concentration of 5 mM.
5. Heat the sample to 60 °C for 30 minutes.
6. Cool the sample to room temperature.
7. Add iodoacetamide to the sample, to a final concentration of 15 mM, and then place the sample in the dark for 30 minutes.
8. Add enzyme, at a ratio of 1:100 to 1:20 (enzyme/protein, w/w).
9. Incubate the sample at 37 °C for 20 to 60 minutes.
10. If the sample is particularly hydrophobic (for example, membrane proteins), boil the protein/RapiGest mixture at 100 °C for 5 minutes, and then cool the sample to room temperature before adding enzyme.

    Requirement: Once the sample is digested, you must remove the RapiGest from the sample before analysis by MALDI.
11. Prepare a stock solution of 50 mM HCl.
12. Add 1:10 (v/v) HCl stock solution: sample (so that pH=2). The final concentration of HCl must be 30 to 5 mM.
13. Incubate the sample at 37 °C for 30 to 45 minutes.

    Tip: A slight cloudiness is normal.
14. Analyze the sample directly by MALDI, or first dilute to a suitable concentration.

Internal lock mass correction from trypsin autolysis peptides

The autolysis fragments of trypsin can be useful for internal lock mass correction of a protein digest spectrum. The masses of the observed autolysis fragments from porcine and bovine trypsin are shown in the following table.

Autolysis fragment monoisotopic masses from porcine and bovine trypsin

Porcine trypsin	Bovine trypsin
2211.1045	805.4168
	2163.0569

Analysis of other compounds

Phosphopeptides

MALDI analyses of phosphopeptides are compromised because of relative suppression by nonphosphopeptides, their relative instability (phosphopeptides readily lose H_3PO_4), and nonspecific binding to glassware. Your sample preparation protocol must therefore be meticulous.

To improve results, you can fractionate digests or mixtures to enrich fractions containing the phosphopeptide, using either HPLC or stepwise elution from ZipTips. To enrich phosphopeptide samples use immobilized metal ion affinity chromatography (IMAC).

Phosphopeptides tend to give an improved response with the instrument in negative ion mode relative to positive ion mode. Another strategy to identify phosphopeptides is differential MALDI mapping, whereby a sample is analyzed before, and after, treatment with a phosphatase enzyme giving mass shifts of 80 Da (exact mass 79.9663, HPO_3) for a phosphate removal.

Oligonucleotides

The matrix of choice is 25 mg/mL hydroxypicolinic acid in 75:25 acetonitrile/water prepared as outlined below.

The sample is dissolved in deionized water at 1 to 10 pmol/µL.

Analyze samples rapidly; do not leave them overnight because the sample/matrix will degrade.

Oligonucleotides readily form adducts with cations, leading to reduced resolution and sensitivity. Minimize these interactions. You can improve data quality by using HPLC/SPE purified samples.

Oligonucleotides are sensitive to enzymes present on the hands, so wear gloves when handling oligonucleotides. Only use deionized water to prepare both the sample and matrix, because other grades, such as HPLC-grade, contain metal ions.

HPA preparation with desalting

First desalt the oligonucleotides to remove cations that form adducts and result in peak broadening.

To prepare HPA

1. To desalt the sample, add strong cation exchange beads, which exchange the metal cation adduct for H^+.

   Recommendation: Use Dowex 50 W X8 beads, washed thoroughly and stored in deionized water.

2. Add a small number of beads to the matrix solution (25 mg/mL hydroxypicolinic acid in 75:25 acetonitrile/water), and ultrasonically agitate for at least 4 hours (or overnight).
3. Centrifuge the matrix and remove the supernatant (you can discard the beads).
4. Mix the matrix and sample 1:1 and spot directly onto the sample plate.

Oligonucleotide calibration

Use oligonucleotides of known masses.

Acquiring data

Use a higher laser energy setting than for peptides.

Oligosaccharides and sugars

The matrix of choice is a saturated solution DHB in 8:2 acetonitrile/water.

The required sample concentration is usually at pmol/µL concentration.

Sample preparation

Sugars readily form adducts with metal cations, which can result in a loss of resolution and mass accuracy. To reduce these effects, desalt the sample with ion exchange beads.

To prepare samples

1. Mix 2 µL of the matrix solution with 2 µL of sugar solution.
2. Spot 1 to 2 µL of the mixture onto the sample plate and air dry.
3. Add 0.5 µL of absolute ethanol to recrystallize the sample spot.

Oligosaccharide calibration

Use sugars of known masses.

Acquiring data

Use a higher laser energy setting than for peptides.

Glycoproteins

To resolve the glycoforms the sample must be rigorously desalted, because cations will adduct with the carbohydrate side-chains, resulting in peak broadening. You can apply the ion exchange desalting procedure detailed for oligonucleotides to glycoproteins, using 4-hydroxy-α-phenylcinnamic acid as the matrix.

Glycolipids

Matrix: HABA, 3.5 mg/mL in methanol.

Sample: 1 to 100 pmol/µL in methanol.

Sample preparation

The premix method of sample preparation is suitable for glycolipids.

To prepare samples

1. Mix 2 µL of the matrix solution with 2 µL of the sample solution.
2. Spot 1 µL of this mixture onto the sample well and air dry.

Glycolipids calibration

Peptide mixtures are suitable for calibrating the instrument for glycolipid sample analysis.

Analysis of synthetic polymers

MALDI can provide useful information on synthetic polymers, such as repeat unit mass and end group mass(es). You can also determine M_w and M_n values for monodisperse polymers that are in good agreement with other techniques such as GPC (gel permeation chromatography), viscometry, and light scattering.

It is well documented, however, that M_w and M_n values determined by MALDI for polydisperse polymers tend not to agree with values obtained using traditional methods. MALDI analysis tends to yield a polydispersity of approximately 1 and M_w and M_n skewed toward the low-mass end of the polymer distribution.

Several physical factors give rise to this effect, including more facile desorption/ionization of low-mass oligomers, 'dimerization' or multiple charging of long-chain polymers. The most common approach to overcoming this fundamental limitation of MALDI is fractionation using GPC before analysis.

Matrixes

Virtually all the common matrixes used for MALDI have been used to analyze synthetic polymers. A general starting point is to dissolve the matrix and sample in the same solvent, using as volatile a solvent as possible. Matrixes are typically employed at a concentration of 10 to 20 mg/mL.

Many synthetic polymers show good results by using methods containing the reagents 20 mg/mL dithranol in THF and 1 mg/mL lithium, sodium, potassium, or silver trifluoroacetate in THF.

To prepare samples

1. Prepare a sample concentration of 10 mg/mL in THF.
2. Mix 10 µL of sample and 10 µL of matrix.
3. Add 1 µL of salt (that is Na^+, K^+ or Ag^+).
4. Spot 1 µL of this mixture onto the sample plate.

   Tip: If there is no signal, vary the ratio of sample to matrix to salt. Diluting the sample can improve signal quality.

Common solvents suitable for dissolving synthetic polymers and matrixes

Matrix	Solvents
CHCA	Acetone, methanol, THF
Sinapinic acid	Acetone, methanol, THF
DHB	Acetonitrile, methanol, water
β-indole acrylic acid	Acetone
HABA	THF
All trans-retinoic acid	THF
Dithranol	THF, trichloromethane, hexafluoroisopropanol

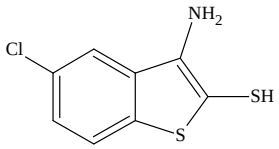
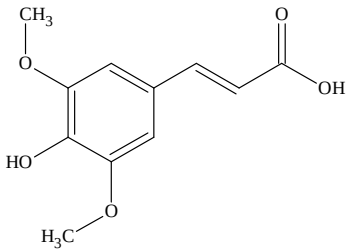
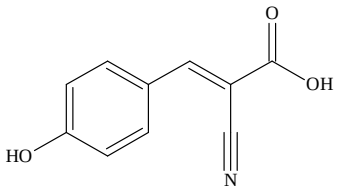
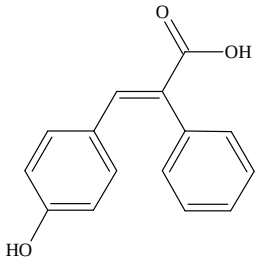
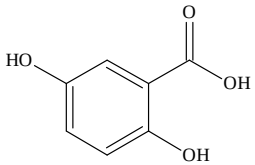
Polymer classification and suitable matrixes

Polymer	Suitable matrix
Acrylates	β-indole acrylic acid, dithranol
Unsaturated aromatic polyesters	Dithranol + silver trifluoroacetate
High molecular weight polystyrene	Retinoic acid + saturated ethnical silver nitrate
Resins	Dithranol
PEG	CHCA in acetone + sodium iodide

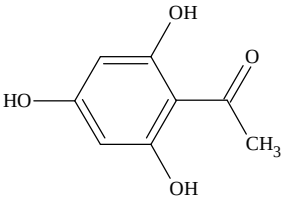
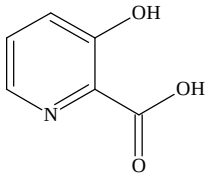
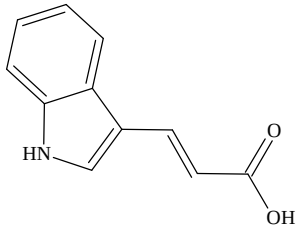
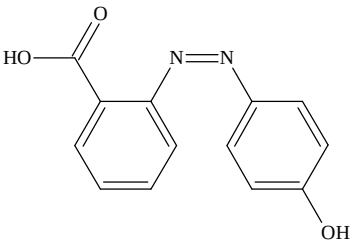
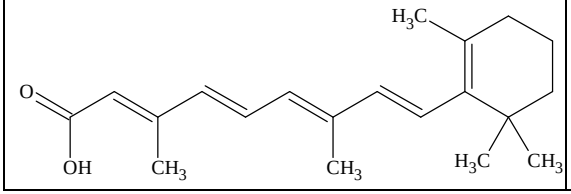
When running acrylates in β-indole acrylic acid, use acetone for the solvent of choice. When applying the mixture of sample and matrix to the sample well, drag the pipette tip across the surface during the drying process while applying more sample/matrix solution, until about 2 µL has been deposited.

Structures of MALDI matrixes

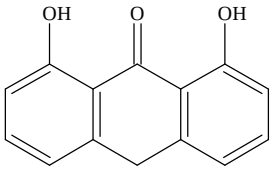
Structure and $[M+H]^+$ of common MALDI matrixes

Structure	Name	$[M+H]^+$
	CMBT (5-chloro-2-mercapto benzothiazole)	Average: 216.7322 Mono: 215.9708
	Sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid)	Average: 225.2212 Mono: 225.0763
	CHCA (α-cyano-4-hydroxycinnamic acid)	Average: 190.1784 Mono: 190.0504
	4-hydroxy-α-phenyl cinnamic acid	Average: 241.2619 Mono: 241.0865
	DHB (2,5-dihydroxybenzoic acid)	Average: 155.1301 Mono: 155.0344

Structure and $[M+H]^+$ of common MALDI matrixes (Continued)

Structure	Name	$[M+H]^+$
	THAP (2',4',6'-trihydroxy-benzoic acid)	Average: 169.1570 Mono: 169.0501
	HPA, Hydroxypicolinic acid (3-hydroxy-2-pyridinecarboxylic acid)	Average: 140.1185 Mono: 140.0347
	IAA (β-indole acrylic acid)	Average: 188.2059 Mono: 188.0711
	HABA (2,-(4-hydroxyphenyl-azo)-benzoic acid)	Average: 243.2420 Mono: 243.0769
	All trans-retinoic acid	Average: 301.4431 Mono: 301.2168

Structure and $[M+H]^+$ of common MALDI matrixes (Continued)

Structure	Name	$[M+H]^+$
	Dithranol (1,8-dihydroxy-9(10H)-anthracenone)	Average: 227.2395 Mono: 227.0708

A Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This appendix presents all the safety symbols and statements that apply to the entire line of Waters products.

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Topic	Page
Warning symbols	A-2
Caution symbol	A-5
Warnings that apply to all Waters instruments	A-6
Electrical and handling symbols	A-11

Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific risk and explains how to avoid it.

Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)

Warning: (Risk of burn injury from contacting hot surfaces.)

Warning: (Risk of electric shock.)

Warning: (Risk of fire.)

Warning: (Risk of sharp-point puncture injury.)

Warning: (Risk of hand crush injury.)

Warning: (Risk of exposure to ultraviolet radiation.)

Warning: (Risk of contacting corrosive substances.)

Warning: (Risk of exposure to a toxic substance.)

Warning: (Risk of personal exposure to laser radiation.)

Warning: (Risk of exposure to biological agents that can pose a serious health threat.)

Warning: (Risk of tipping.)

Warning: (Risk of explosion.)

Warning: (Risk of eye injury.)

Specific warnings

The following warnings can appear in the user manuals of particular instruments and on labels affixed to them or their component parts.

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.

Warning: Pressurized nonmetallic, or polymer, tubing can burst. Observe these precautions when working around such tubing:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, which significantly reduces the pressure at which the tubing can rupture.

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.

Warning: Where significant quantities of flammable solvents are involved, a continuous flow of nitrogen into the ion source is required to prevent possible ignition in that enclosed space.

Ensure that the nitrogen supply pressure never falls below 690 kPa (6.9 bar, 100 psi) during an analysis in which flammable solvents are used. Also ensure a gas-fail connection is connected to the LC system so that the LC solvent flow stops if the nitrogen supply fails.

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.

Warning: To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.

This warning applies to certain instruments when they are in Operate mode.

Warning: High voltages can be present at certain external surfaces of the mass spectrometer when the instrument is in Operate mode. To avoid non-lethal electric shock, make sure the instrument is in Standby mode before touching areas marked with this high voltage warning symbol.

Biohazard warning

This warning applies to Waters instruments that can be used to process material that might contain biohazards: substances that contain biological agents capable of producing harmful effects in humans.

Warning: Waters instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe Good Laboratory Practices, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Chemical hazard warning

This warning applies to Waters instruments that can process corrosive, toxic, flammable, or other types of hazardous material.

Warning: Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practices (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals.*

Caution symbol

The caution symbol signifies that an instrument's use or misuse can damage the instrument or compromise a sample's integrity. The following symbol and its associated statement are typical of the kind that alert you to the risk of damaging the instrument or sample.

Caution: To avoid damage, do not use abrasives or solvents to clean the instrument's case.

Warnings that apply to all Waters instruments

When operating this device, follow standard quality control procedures and the equipment guidelines in this section.

Attention: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Important: Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.

Achtung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Atencion: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

注意：未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

注意：未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。

주의**:** 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

注意：規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。

Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Attention: Manipulez les tubes en polymère sous pression avec precaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l'instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Vorsicht: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.

Attenzione: fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。

警告：当有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。

경고**:** 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告：圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があり、その場合、チューブは極めて低い圧力で破裂します。

Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Attention: L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.

Vorsicht: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwenddung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Attenzione: si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告：使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被消弱。

警告：使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用，那么该设备所提供的保护将被削弱。

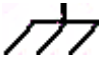
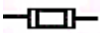
경고**:** 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告：ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

Electrical and handling symbols

Electrical symbols

These can appear in instrument user manuals and on the instrument's front or rear panels.

	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Protective conductor terminal
	Frame, or chassis, terminal
	Fuse
	Recycle symbol: Do not dispose in municipal waste.

Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!

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