# [USER'S GUIDANCE FOR TRANSFERRING LC/MS METHODS TO THE XEVO TQ-XS MASS SPECTROMETER]

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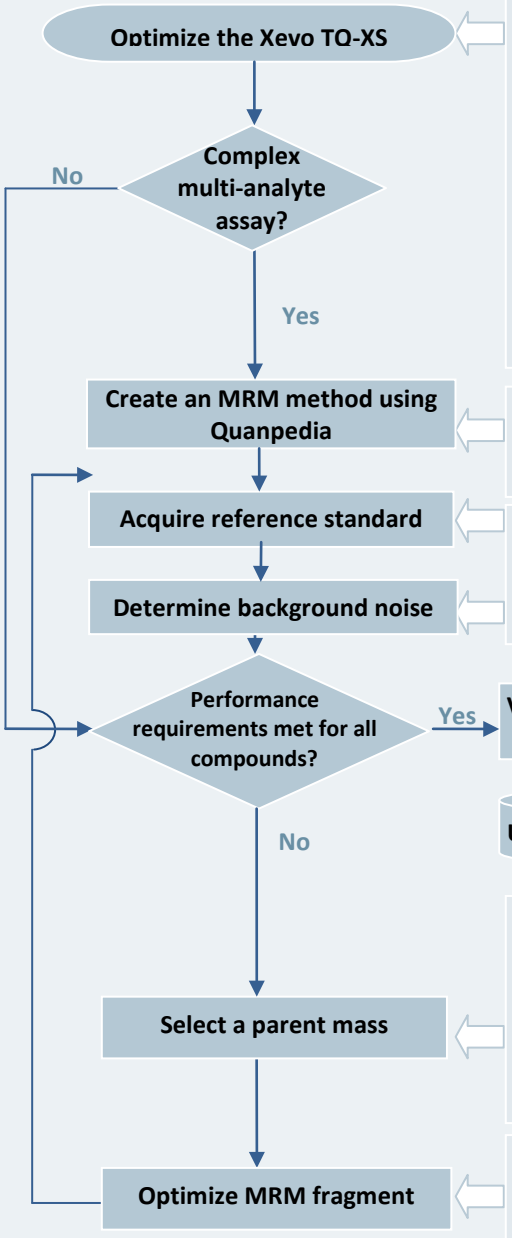
This document explains how to transfer multiple-reaction-monitoring (MRM) methods to the Xevo TQ-XS MS from a less-sensitive MS system. Owing to differences in the sample-cone orifice and StepWave technology between the Xevo TQ-XS MS and other systems, you must optimize your acquisition methods as shown here:

## WORKFLOW FOR MRM METHOD OPTIMIZATION

**Requirement:** Ensure that the calibration of the Xevo TQ-XS MS is valid.

For each workflow step, **>** indicates the path for selecting menu commands, for moving from one menu to another, or for performing a task.




**Console System Tree > Xevo TQ-XS > Manual Optimization**

1. From the existing MRM method, adopt the parameter settings specified for the source.
   **Note:** Ensure that the source and desolvation temperatures are stable.
2. Install the LC mobile phases and column specified in the existing MRM method.
3. Set the mobile phase composition and flow rate equivalent to those at the average retention time of the target compounds.
   **Note:** Ensure that the LC flow is via the instrument's onboard solvent-delivery system and that the LC pressures are stable.
4. Introduce a reference standard using the combined-infusion mode, and optimize the ESI probe position for maximum intensity on the target compound's parent mass.

**See the section** "Increase signal" and the online Help topic "Optimizing source parameters manually".

**MassLynx > TargetLynx > Quanpedia > Run Compounds**

**See the online Help topic** "Setting up acquisition methods for a set of compounds".

**See the online Help topic** "Acquiring data from the sample list".

Acquire the high calibration standard.

**See the online Help topic** "Acquiring data from the sample list".

**MassLynx > TargetLynx > Quanpedia**

**See the online Help topic** "Importing information into the Quanpedia database".

**MassLynx > Instrument tab > MS Tune**

1. Introduce a reference standard using the combined-infusion mode.
2. Confirm that the parent ion is the most abundant ion in the spectrum.
   **Note:** Otherwise, select the most abundant in-source fragment.
3. Optimize the ESI probe position for maximum intensity on the parent mass.

**See the online Help topic** "Acquiring data from the Tune window".

**MassLynx > Instrument tab > MS Tune**

1. Click MSMS mode.
2. Identify major fragments, and optimize the collision energy.

**See the online Help topic** "Optimizing source parameters manually ".



The Xevo TQ-XS Mass Spectrometer is for research use only. It is not for use in diagnostic procedures.

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## MAXIMIZE SENSITIVITY

# REDUCE BACKGROUND NOISE

- Use high-quality, ultra-pure solvents, buffers, and additives (LC/MS grade, prefiltered).
- Ensure that the LC system is clean (see the section "Clean the LC system").
- Consider alternative sample preparation techniques, such as Oasis PRiME HLB cleanup (see www.waters.com). Effective removal of matrix interferences during sample preparation offers these benefits:
    - Increases the signal-to-noise ratio by simplifying the chromatographic separation
    - Reduces variability in analytical results due to matrix inconsistencies
    - Higher, more consistent recovery
    - Minimizes matrix effects
    - Increases column lifetime
    - Concentrates the analyte of interest

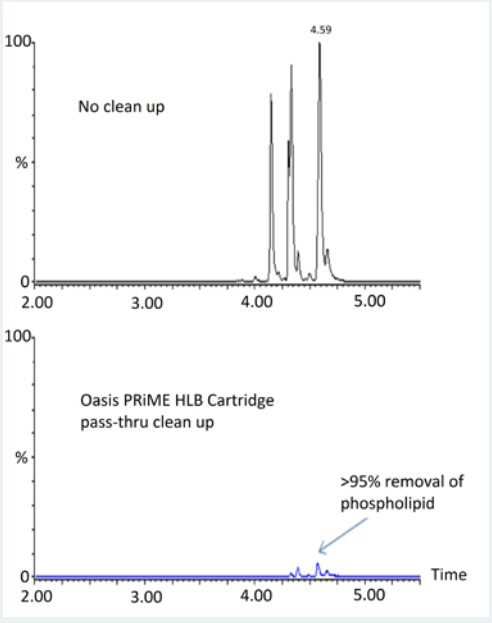
### To prepare tissue samples:

1. To 2.5 g of sample, add 10 mL of 0.2 % formic acid in 80:20 acetonitrile/water, and then vortex the solution.
2. Centrifuge at 12000 rpm for 5 min.
3. Pass 0.5 mL supernatant through the Oasis PRiME HLB cartridge at 1-2 psi.
4. Dilute the cleaned sample 1:3 with 10 mM acqueous ammonium formate buffer (pH 4.5).

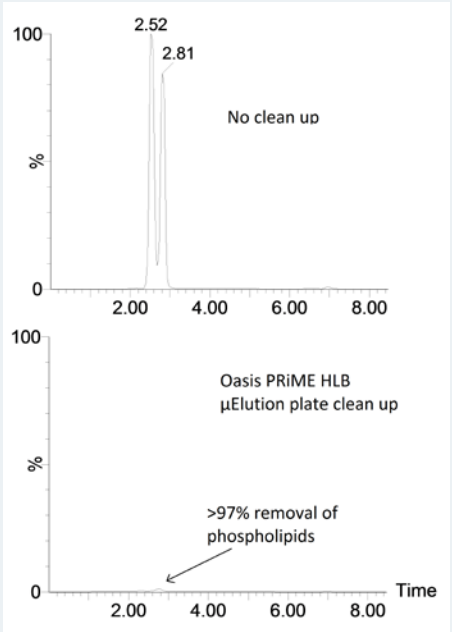
### To prepare plasma samples:

1. Precipitate 150 µL plasma with 300 µL 4:1 methanol/$ZnSO_4$.
2. Centrifuge at 3220 rpm for 10 min.
3. Dilute supernatant with 900 µL 4% $H_3PO_4$, and then load on to the Oasis PRiME HLB µElution plate.
4. Wash twice with 200 µL 25% methanol.
5. Elute twice with 25 µL 90:10 acetonitrile/methanol.
6. Dilute with 50 µL 25% methanol.

### Chromatograms showing the effective removal of >95% phospholipids from shrimp extract:




### Chromatograms showing the effective removal of >97% phospholipids from plasma:

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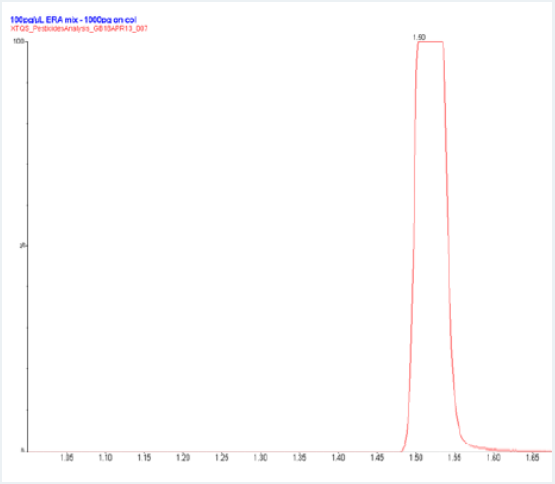
## MAXIMIZE SENSITIVITY
## INCREASE SIGNAL

- Always optimize ion-source parameter settings for the compounds that you intend to analyze.
- Always optimize at least two MRMs for each compound, and compare their signal intensities and signal-to-noise ratios.
- Follow the manual optimization procedure in the "Workflow" section, using the combined-infusion mode to deliver a standard solution with a mobile phase flow rate and composition equivalent to that at which the standard compound is expected to elute.
- To optimize the signal:

1. Position the ion probe close to the sampling cone, and move it progressively away (vertically and horizontally) until the signal intensity starts to decrease.
   **Note:** Choose one position for all compounds in an assay.
   **Tip:** For ionKey operation, to improve signal-to-noise ratios in front of the peak, position the stage away from the cone.
2. In ESI mode, optimize the capillary voltage (in APCI mode, optimize the corona current and voltage) for the maximum signal intensity (at typical UPLC flow, approximately 0.5kV).
   **Note:** Choose one setting for all compounds in an assay.
3. Optimize the ESI desolvation temperature (in APCI mode, optimize the probe temperature).
   **Note:** If the temperature is too low, ionization is inefficient. If the temperature is too high, the ion beam can become unstable, and performance for thermally-labile compounds degrades.
   Choose one setting for all compounds in an assay.
4. For each compound in the assay,
   a. Identify the optimal cone voltage.
   b. Determine whether soft or normal ionization mode is appropriate.
   c. Verify that the collision-energy values are optimal.

- Always verify that the highest calibration standards do not saturate the detector (as indicated by flat-topped chromatographic peaks with intensity 1. 38e9). Dilute samples, if necessary.

**Example chromatogram showing detector saturation:**



## INCREASE
## LC/MS SYSTEM ROBUSTNESS

### Sample dilution

Enhanced sensitivity allows you to use more dilute samples, which confer these benefits:

- Reduce matrix suppression effects
- Improve chromatographic peak shape
- Reduce contamination in the source

### LC flow diversion

To reduce contamination in the source, divert the flow from column to waste for the start and end portions of the chromatographic run.

**See the online Help topic** "Adding timed events to a method".



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## CLEAN THE LC SYSTEM

### Requirements:

- Ensure that the LC system is not connected to the mass spectrometer.
- Ensure that the ACQUITY columns are not connected.
- Install the injection loop and post-column tubing that you will use for the analysis.
- Use an LC method for which the duration is at least 3 min.
- Periodically flush the ESI probe with IPA.

| Solvents | ACQUITY Sample Manager position |
|---|---|
| Acid wash—1:1:1:1 Isopropanol:acetone:methanol:10% formic acid | 1:1 |
| Basic wash—10% ammonia | 1:2 |
| Isopropanol | 1:3 |

### To clean the LC system:

1. Prime the system with acid wash:
   - Flush all ACQUITY lines for 5 min.
   - Prime all syringes for at least 10 priming cycles.
   - Prime the seal wash for at least 5 min.
2. Prime the system with basic wash:
   - Flush all ACQUITY lines for 5 min.
   - Prime all syringes for at least 10 priming cycles.
   - Prime the seal wash for at least 5 min.
3. Prime the two primary solvent lines with acid wash for 3 min, allowing the basic wash to remain in the other lines.
4. Perform 25 injections (maximum volume) from vial 1:1 using method A.
5. Perform 25 injections (maximum volume) from vial 1:2 using method B.
6. Prime the system with basic wash:
   - Flush all ACQUITY lines for 5 min.
   - Prime all syringes for at least 10 priming cycles.
   - Prime the seal wash for at least 5 min.
7. From vial 1:3, perform 15 injections (maximum volume) using method A and 10 injections (maximum volume) using method B.

### Method A, flow rate 0.3 mL/min

| Time (min) | %A or A1 | %B or B1 |
|---|---|---|
| 0.00 | 95 | 5 |
| 0.50 | 95 | 5 |
| 2.00 | 5 | 95 |
| 2.50 | 5 | 95 |
| 3.00 | 95 | 5 |

### Method B, flow rate 0.3 mL/min

| Time (min) | %C or A2 | %D or B2 |
|---|---|---|
| 0.00 | 95 | 5 |
| 0.50 | 95 | 5 |
| 2.00 | 5 | 95 |
| 2.50 | 5 | 95 |
| 3.00 | 95 | 5 |

## ADDITIONAL INFORMATION

You can also consult the Waters Web site, www.waters.com.

| Resource | Part Number |
|---|---|
| Xevo TQ-XS online Help | Access via the instrument console |
| ACQUITY System online Help | Access via the instrument console |
| Quanpedia online Help | Access via the Waters MassLynx software |
| *Major Contaminants and Their Sources* | 715004193 |
| *Controlling Contamination in UPLC/MS and HPLC/MS Systems* | 715001307 |



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