

# Waters® ACQUITY TQD Empower 2154

## *Customer Familiarization Guide*



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## Introduction

The Customer Familiarization Guide contains five step-by-step procedures, but only three steps need to be performed during the installation, the extraction of chromatographic data from MS scans is optional.

|   |  |
|---|--|
| <b>Step 1</b>                             | Acquisition and IntelliStart with the ACQUITY TQD System   |
| <b>Step 2<br/>Quantitative processing</b> | SIR/MRM Processing with MS External Standards<br>or<br>SIR/MRM Processing with MS Internal Standards |
| <b>Step 3<br/>Qualitative processing</b>  | Full Scan Processing of MS Data  |
| <b>Optional</b>                           | Extraction of Chromatographic Data from MS Scans   |

If the system does not include an ACQUITY UPLC system, do not perform Step 1.

Once completed, the guide remains with the customer for their future reference and training.

## Software requirements

This training package requires the following software and files to be available:

- Empower version 2154 in Professional mode with Feature Release 4.0 or later
- For ACQUITY UPLC systems version 1.30 or later
- For MS systems version 1.32 or later
- Empower MS Familiarization project provided with this document

## Safety precautions

The customer must understand and follow all the necessary safety precautions described in the relevant Waters Operator's Guides provided with the system, prior to performing this procedure.

## Other operational assistance

The engineer must complete with the customer all the relevant operational and maintenance training described in the installation checklist, prior to performing this familiarization guide.

The engineer must demonstrate to the customer how to access the following information:

- Empower Online Help and electronic Operator's Guide
- Online ACQUITY and MS Console Help
- ACQUITY TQD Operator's Guide
- Waters Educational Services

# 1 Acquisition and IntelliStart with the ACQUITY TQD System

This procedure describes the basics of acquiring data using the ACQUITY TQD system with Empower. The procedure guides the operator through the following topics:

- Basic process of daily setup and operation
- Acquiring MS MRM data using IntelliStart
- Startup sequences for the ACQUITY system and the TQ Detector
- Instrument methods, method sets, and sample sets

The procedure consists of the following 16 sections:

|  |      |
|--|------|
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## Sample preparation for Familiarization Training

### Tools/materials required

- 100-mL graduated cylinder
- 1000-mL graduated cylinder
- 3 x 10-mL volumetric flasks
- 2 x 100-mL volumetric flasks
- 100- $\mu$ L adjustable pipette
- 1-mL adjustable pipette
- Column ACQUITY UPLC BEH C18 (2.1 mm x 50 mm, 1.7  $\mu$ m)
- System ACQUITY UPLC System with the TQ Detector
- MS Calibration Solution Naics2 (sodium iodide doped with cesium) (700002646-8)
- Sample ACQUITY UPLC MS Start-up Solutions Kit (700002741)

1. Prepare the mobile phase.

**Table 1: Mobile phase preparation**

| Solvent Lines      | Mobile Phase                               |
|--------------------|--|
| A1                 | 0.1% formic acid in 100% water             |
| B1                 | 0.1% formic acid in 100% acetonitrile      |
| A2/B2              | Methanol                                   |
| Seal Wash          | Methanol                                   |
| Strong Needle Wash | 90:10 methanol/water                       |
| Weak Needle Wash   | 50:50 methanol/water                       |
| Sample Diluent     | 10:90 acetonitrile/water +0.1% formic acid |

**NOTE:** This training procedure uses a series of sample dilutions of sulfadimethoxine.

## Diluent preparation

1. Measure 100 mL of MS-grade acetonitrile using the 100-mL graduated cylinder, and then transfer into a 1-L reservoir bottle.
2. Measure 900 mL of filtered MS-grade water using the 1000-mL graduated cylinder and transfer the water into the same 1-L reservoir bottle.
3. Add 1 mL of formic acid (Solution 3 from the solutions kit) into the same 1-L reservoir bottle and mix well.
4. Label the 1-L reservoir bottle as **Diluent - 10:90 acetonitrile:water plus 0.1% formic acid**.

## Sample tuning solution for developing an MS method

The sulfadimethoxine solution is provided during the start-up installation procedures and tests. This section describes how to make fresh solution from a stock solution of sulfadimethoxine 1 mg/mL in methanol.

### Solution A - 10 ng/µL sulfadimethoxine

1. Pipette 1 mL of 1 mg/mL sulfadimethoxine (Solution 1 from the solutions kit) into a 100-mL volumetric flask and dilute to 100 mL with diluent.
2. Stopper and invert the flask several times to mix fully.
3. Label the flask as **Solution A - 10 ng/µL sulfadimethoxine**.
4. Transfer the rest of Solution 1 to a scintillation vial, label as **1 mg/mL sulfadimethoxine** and store in a refrigerator at 4 °C.
5. Add 10 to 15 mL of **Solution A** into a tuning reservoir bottle and insert this into position B of the MS detector fluidics.
6. Transfer the remaining **Solution A** to an amber bottle and store in a refrigerator at 4 °C.

### Stock sulfadimethoxine 100 pg/µL

1. Pipette 1 mL of **Solution A** into a 100-mL volumetric flask and dilute to 100 mL with diluent.
2. Stopper and invert the flask several times to mix fully.
3. Label the flask as **Stock sulfadimethoxine 100 pg/µL**.

### Low Standard sulfadimethoxine 20 pg/µL

1. Pipette 2 mL of **Stock sulfadimethoxine 100 pg/µL** into a 10-mL volumetric flask and dilute to 10 mL with diluent.
2. Stopper and invert the flask several times to mix fully.
3. Label the flask as **Low Standard sulfadimethoxine 20 pg/µL**.
4. Place 1 mL of Low Standard sulfadimethoxine 20 pg/µL into a sample vial and insert this into position 1:A,1 of the ACQUITY UPLC sample manager.

**High Standard sulfadimethoxine 40 pg/µL**

1. Pipette 4 mL of **Stock sulfadimethoxine 100 pg/µL** into a 10-mL volumetric flask and dilute to 10 mL with diluent.
2. Stopper and invert the flask several times to mix fully.
3. Label the flask as **High Standard sulfadimethoxine 40 pg/µL**.
4. Place 1 mL of High Standard sulfadimethoxine 40 pg/µL into a sample vial and insert this into position 1:A,2 of the ACQUITY UPLC sample manager.

**Unknown Sample sulfadimethoxine 30 pg/µL**

1. Pipette 3 mL of **Stock sulfadimethoxine 100 pg/µL** into a 10-mL volumetric flask and dilute to 10 mL with diluent.
2. Stopper and invert the flask several times to mix fully.
3. Label the flask as **Unknown Sample sulfadimethoxine 30 pg/µL**.
4. Place 1 mL of Unknown Sample sulfadimethoxine 30 pg/µL into a sample vial and insert this into position 1:A,3 of the ACQUITY UPLC sample manager.

**NOTE:** This training exercise uses an injection of sulfadimethoxine at two standard concentrations and a pseudo-unknown. The final concentrations on the column for each of the samples are shown in Table 2.

**Table 2: Final concentrations on the column**

| Sulfadimethoxine Sample Concentration | Injection Volume | On Column |
|---------------------------------------|------------------|-----------|
| 20 pg/µL                              | 2 µL             | 40 pg     |
| 40 pg/µL                              | 2 µL             | 80 pg     |
| 30 pg/µL                              | 2 µL             | 60 pg     |

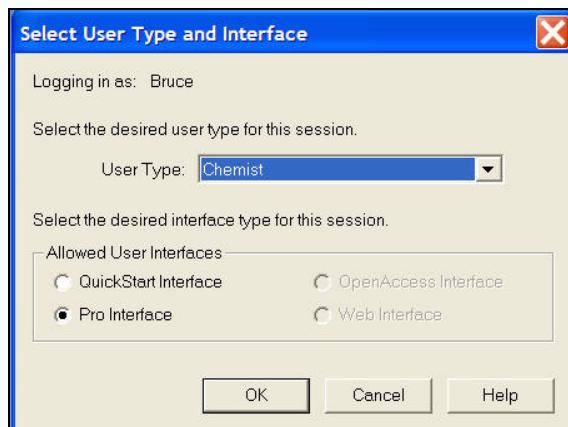
## Empower login and basic run sample setup

1. Double-click the **Empower** icon .
2. Type your user name as **System**, and password as **Manager** (or the user name and password assigned to you).



*Figure 1 - Empower Login window*

3. If the default interface is Pro mode, click **Advanced**.
4. Click the **User Type** drop-down list, and select your user type.
5. Select the **Pro Interface** check box, and then click **OK**.



*Figure 2 - Select User Type and Interface window*

6. Click the **Configure System** icon  to access the Empower Configuration Manager.
7. Right-click the **Projects folder**, and select **Restore Project(s)**.

8. Perform the following steps if loading the project for the first time:
  - a. Load the *Empower Familiarization CD* in the disk drive, and then click **Browse** to select the location of the "TQD Empower Familiarization" project.
  - b. Leave the Parent Project as the default, unless requested to put it in another location by your Empower system administrator.
  - c. Click **Next**, select the location of the parent project, and then click **OK**.
  - d. Leave the name of the project as displayed, and then click **Next** to restore the project.
  - e. Click **OK** after the project restores.
  - f. Click **File > Exit** to quit the Empower Configuration Manager.
9. Right-click the **Waters TQD Familiarization** project and select **Project Properties**.
10. Click the **General tab**, ensure that the **System Suitability** check box is enabled and then click **OK**.
11. Click the **Run Samples** icon  to start the sample acquisition of data.
12. Click **OK** and select the **Waters TQD Familiarization** project to acquire data and select the **ACQUITY TQD** system (or other name for the TQD MS system).

**NOTE:** *The TQD Familiarization already has the required methods and custom fields needed for IntelliStart including the LC/MS System Checks.*

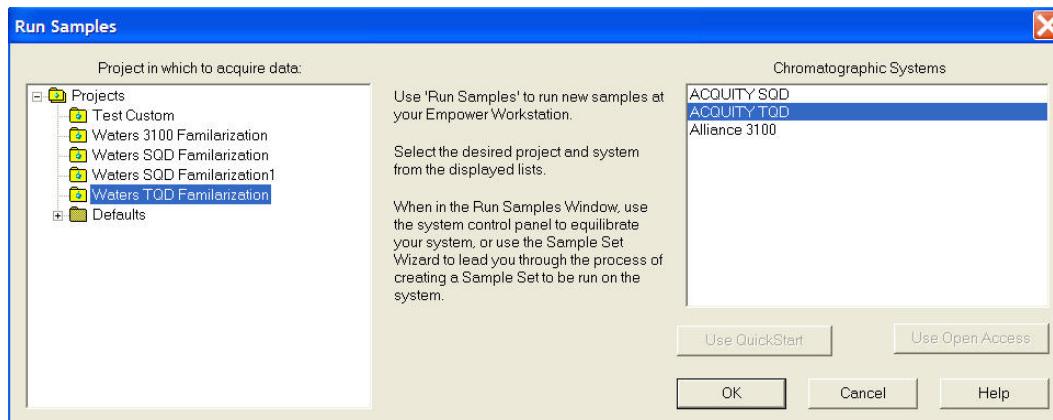


Figure 3 - Selecting the project and system

## Navigating the Run Samples window

This section describes the Run Samples window and basics of navigation and customization.

Run Samples is displayed with the last Table Display tab selected. The Run Samples window includes the following four tabs:

- Single                      Single injection mode
- Samples                      Sample set methods mode
- Sample Set                 Sample sets queue status
- Running                    Current sample set acquisition

A real-time plot of sample acquisition appears at the right-hand side with the control panels and system status at the bottom.

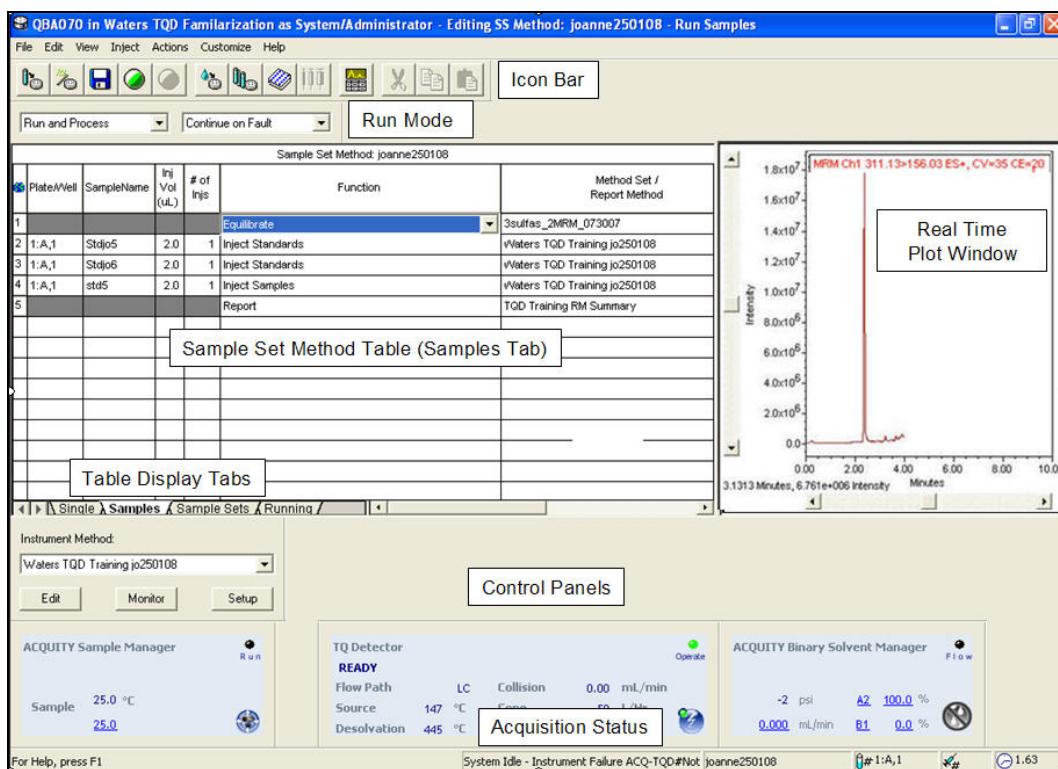


Figure 4 - Run Samples window

1. Drag and drop the ACQUITY control panels to the bottom of the Run Samples window.

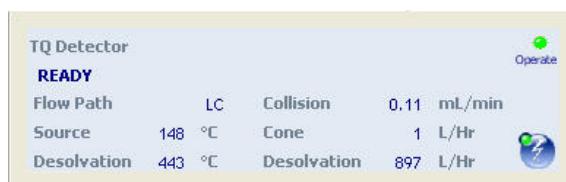


Figure 5 - Example ACQUITY control panel

2. Click **View > Toolbars** and deselect **Time Remaining** and **Solvent Required**.

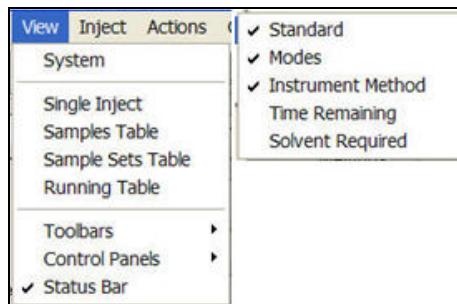


Figure 6 - Toolbar options

3. Click the **Samples** tab to display the sample set list.

**NOTE:** *The sample set list is used to create the samples set sequences, including sample names, vial-plate, injection volume, run times, method sets, MS tuning methods, and calibration methods.*

4. Right-click the **Globe** icon  at the top of the sample set list to customize the display in the sample set method editor of run samples.
5. Select the check boxes shown in Figure 7, and any other columns that do not need to be displayed, and then click **OK**.

**NOTE:** *Selected columns will be hidden from view.*

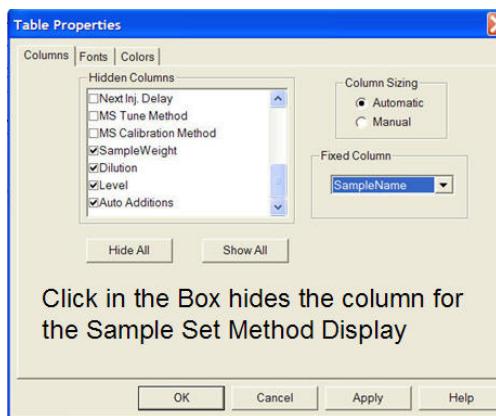


Figure 7 - Table Properties window

6. Click **File > Save Preferences**, to save the layout of the Run Samples window.

**NOTE:** *Preferences are saved by login user, project, and system. These preferences may be copied to other users and projects by the Empower system administrator.*

## Creating instrument methods and methods sets

To acquire data with an MS system you must create an instrument method and method set. This section describes how to create these methods.

**NOTE:** *This procedure creates all method conditions except for creating the TQD SIR/MMR acquisition conditions. We will be creating an assay for the analysis of sulfadimethoxine.*

1. Click **Edit > New Method Set**.
2. Click **Yes** to use the wizard.
3. Click the **Create New** icon to display the instrument method editor.
4. Click the **ACQUITY Binary Solvent Manager (BSM)** icon .
5. In the General tab, set the gradient conditions shown in Figure 8.
6. Click  to edit solvent A and B names.
7. Type the %B setting and the %A setting will be calculated automatically.

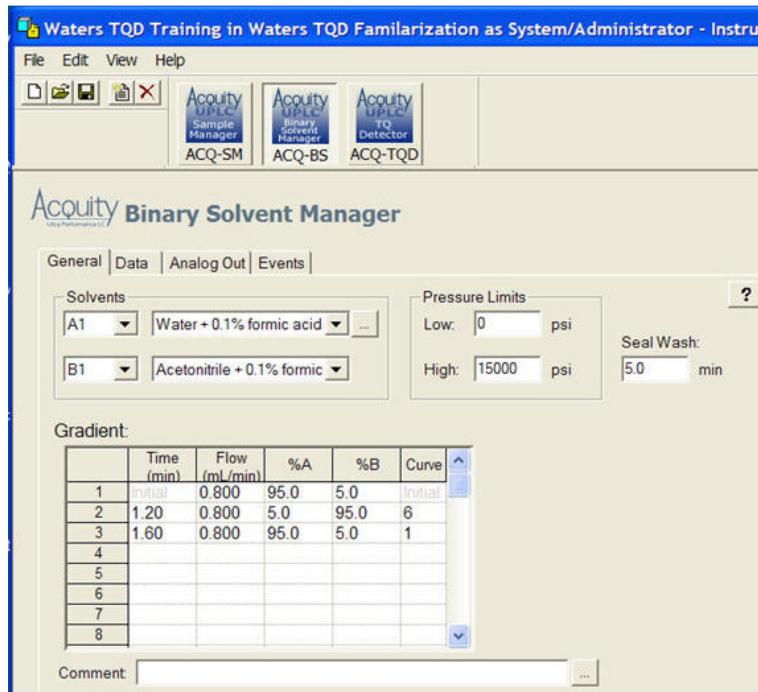


Figure 8 - Instrument Method Editor

**NOTE:** *The gradient curve changes solvent composition and flow rate over time. Curves are graded from 1 to 11, with curve one the most convex (the gradient changes immediately), and curve 11 the most concave (the gradient changes at the end).*

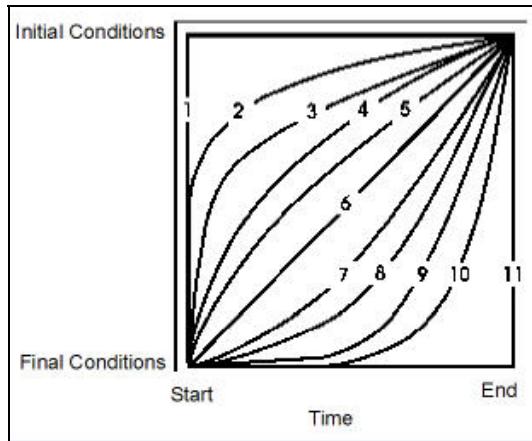


Figure 9 - Gradient curves

8. Click the **ACQUITY Sample Manager (ACQ-SM)** icon .
9. Click the **General** tab of the injector.
10. Set the acquisition conditions shown in Figure 10 for this analysis.
11. Select the **Partial Loop With Needle Overfill** check box (Table 3).

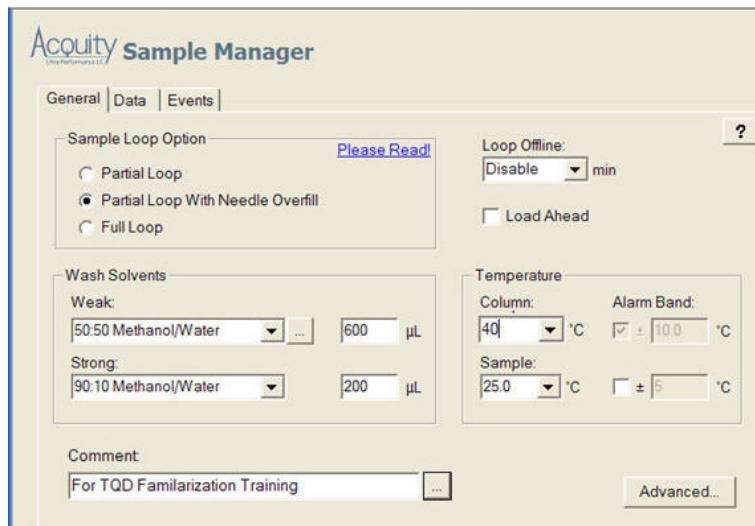


Figure 10 - Instrument Method Editor

**Table 3: Injection type selection and function**

| <b>Injection Type Selection</b>   | <b>Function</b>  |
|-----------------------------------|--|
| Partial Loop with Needle Overfill | Best accuracy and precision for partial loop injections        |
| Partial Loop (Pressure Assist)    | High throughput and limited sample volumes                     |
| Full Loop (Pressure Assist)       | Best accuracy and precision overall using 4 x injection volume |



12. Click **ACQ-TQD** and select the following parameters for this acquisition method:

- Ionization: ES
- Function: MRM, ES+, Start 0.00, Stop 1.20 (The precursor and product ions will be calculated using IntelliStart's Develop Method function)
- Leave all other settings at the default for this method

**NOTE:** *Leave the MRM at the default of 1000 to 166.95. The precursor and product ions will be calculated using IntelliStart's Develop Method function.*

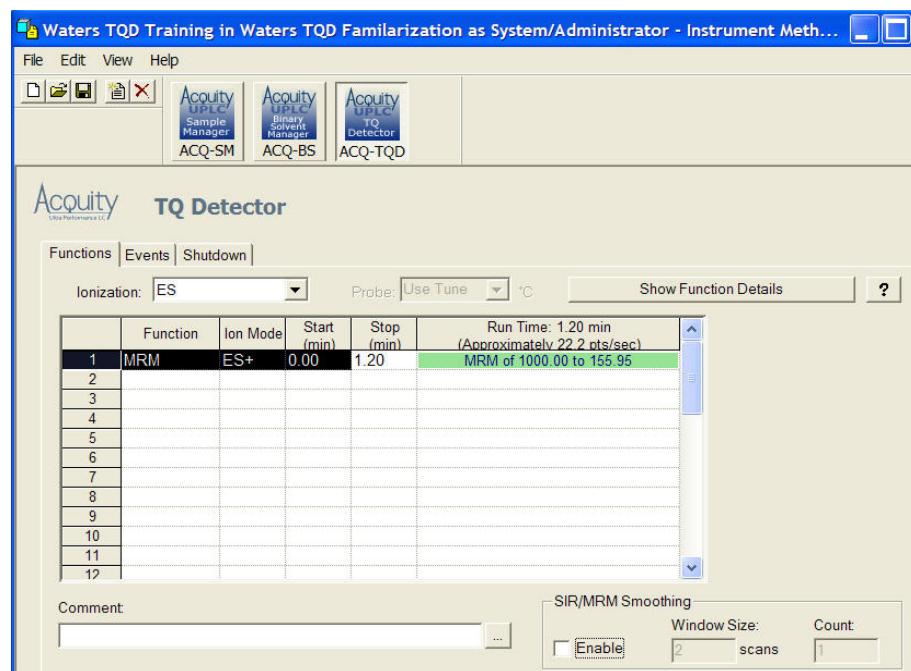


Figure 11 - Instrument Method Editor

13. TQD acquisition conditions:

- a. SIR and MRM ES+ methods can be developed using IntelliStart's Develop Method function.
- b. For ESI-, ESCI, and APCI MRM methods, use manual tune (APCI and ESCI require the corona discharge needle in place).
- c. For scanning experiments, select the first row of the table, click the drop-down list and select **MS2 Scan**.
- d. Click **Show Function Details** to set the MS scan range and the scan speed (Figure 12).

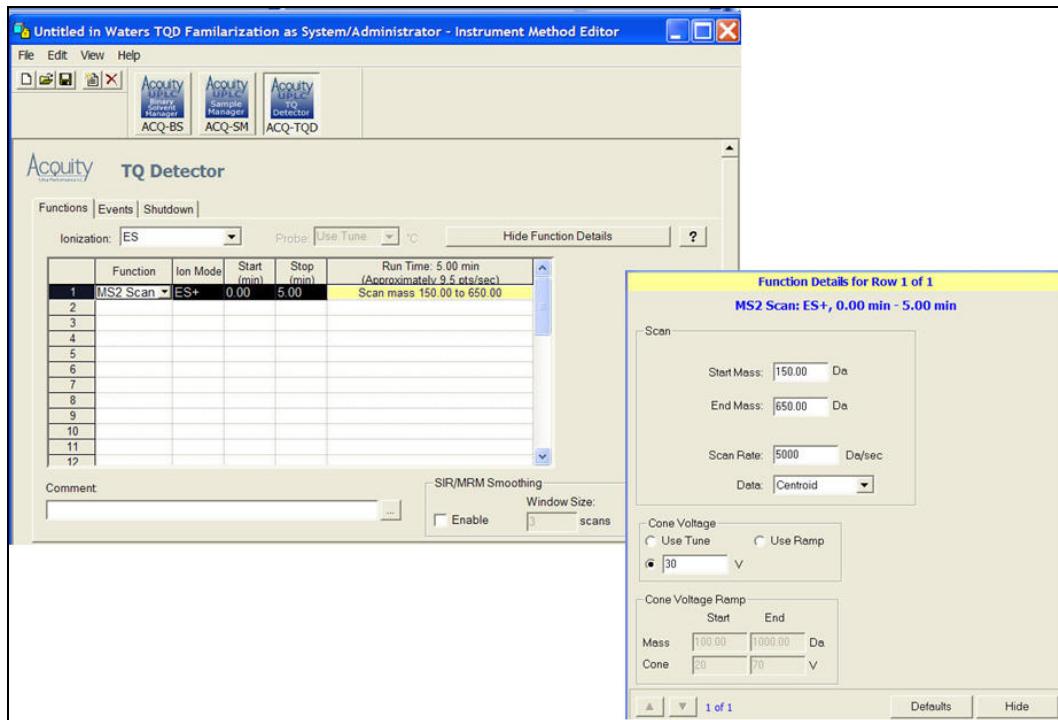


Figure 12 - Instrument Method Editor

14. For TUV/PDA detectors, click the **Detector** icon and enter a default setting of **20 points a second** for Sampling Rate, and set the Filter Time Constant to **Fast**.
15. Click **File > Save** and type the name of the instrument method as **TQD Training IM**.
16. Click **File > Exit**.
17. In the Methods wizard, click the **Instrument Method** drop-down list, select **TQD Training IM**, and then click **Next**.

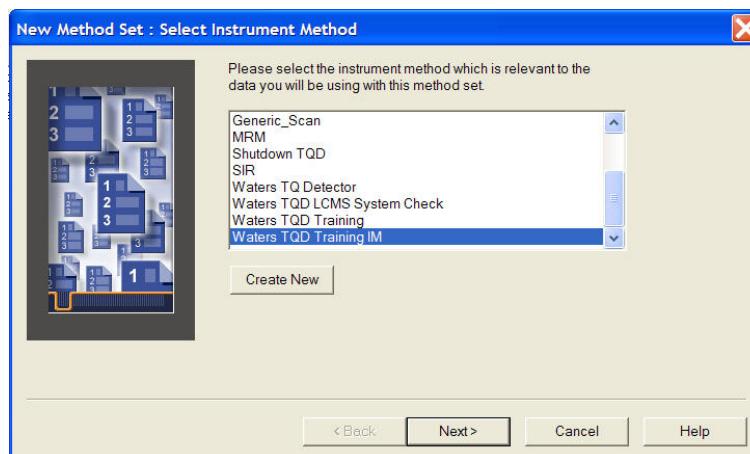


Figure 13 - Select Instrument Method window

18. Click the **Processing Method** drop-down list and select **TQD Training PM**.
19. Click the **Report Method** drop-down list and select **TQD Training RM**.
20. Leave the Export Method as **(No Exporting)** and then click **Next**.

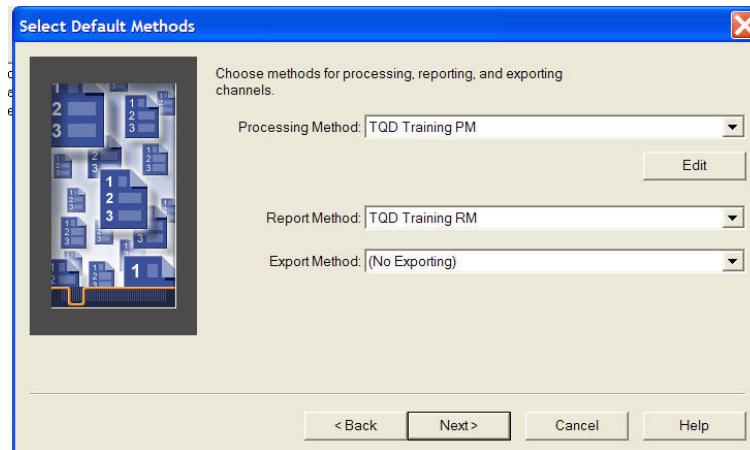


Figure 14 - Select Default Methods window

21. In the Name Method Set dialog box, type **TQD Training MS**.
22. Click **Finish**.
23. Click **File > Exit**.

## Creating TQD shutdown method and method set

The shutdown method set stops solvent flow, puts the TQ Detector into Standby, and turns off the nitrogen and collision gas.

1. Click **Edit > New Method Set**.
2. Click **Yes** to use the wizard.
3. Click the **Create New** icon to display the instrument method editor.

**CAUTION:** *It is important that the flow is off before shutting down the nitrogen flow to the TQ Detector.*

4. Click the **ACQUITY Binary Solvent Manager (BSM)** icon .
5. Click the **General** tab, and set a time of **zero** and a flow rate of **0.0**.
6. Leave all other settings at the default.
7. Leave the **ACQUITY Sample Manager (ACQ-SM)** settings at the default.

8. Click the  icon and leave the Function page at the default settings and then click the **Shutdown** tab.
9. Select the **Switch to Standby**, **API Gas Off**, and **Collision Gas Off** check boxes.

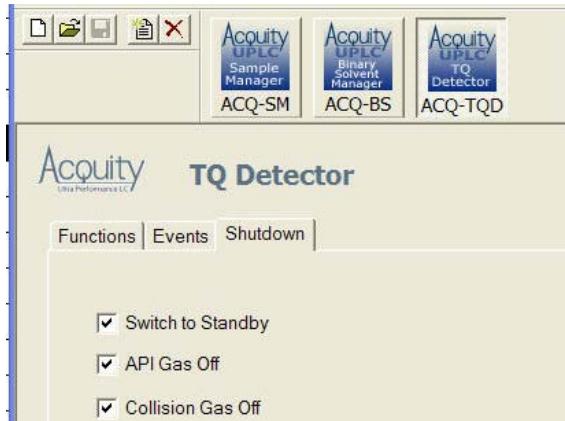


Figure 15 - Shutdown tab

10. If you have a UV detector, click the **Events** page of the UV detector and deselect the **Lamp** check box at time zero.
11. Click **File > Save** and type the name of the instrument method as **Shutdown TQD IM**.
12. Click **File > Exit**.
13. In the method set editor, click the **Instrument Method** drop-down list, select **Shutdown TQD IM**, and then click **Next**.

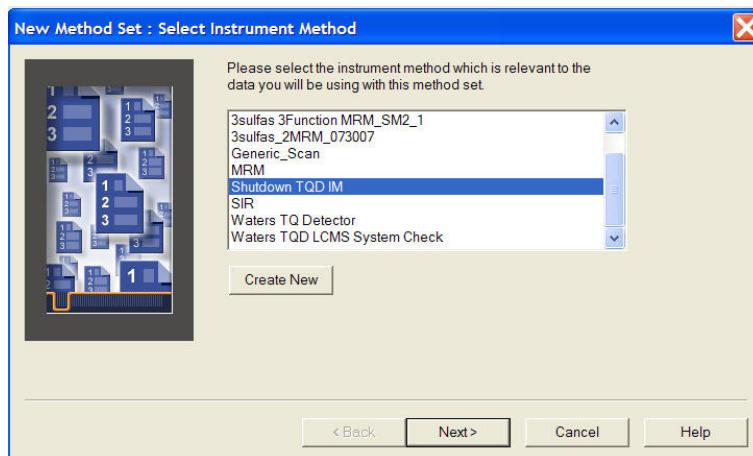


Figure 16 - Select Instrument Method window

14. Leave the Processing Method as **(No Processing)**, the Report Method as **(No Reporting)**, and the Export Method as **(No Exporting)**, and then click **Next**.

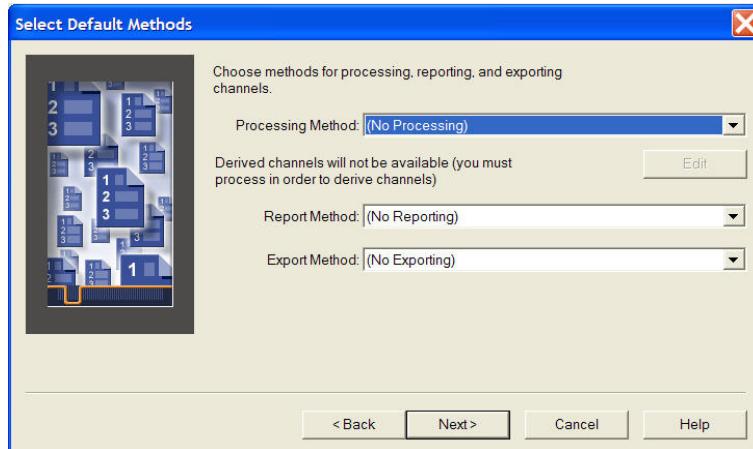


Figure 17 - Select Default Methods window

15. Click the **Name of the Method Set** dialog box and type **Shutdown TQD MS**.  
16. Click **Finish**.  
17. Click **File > Exit**.

## Routine TQ Detector and ACQUITY operation

This section describes routine TQ Detector and ACQUITY operation, including the control panel direct operations (starting the TQ Detector and priming the system).

**CAUTION:** *Ensure that the TQ Detector is switched on before applying solvent flow from the ACQUITY binary solvent manager.*

1. In the Run Samples field of the installation project, right-click the **TQD control panel** and select in sequence, **API Gas on** and then **Operate**, to turn on the mass spectrometer.



Figure 18 - Run Samples field

**CAUTION:** *Before putting any flow to the TQ Detector, wait to make sure the following settings are displayed in the control panel (source temperature may take a while if the TQ Detector was in Standby mode). If not, check the advanced Tune page settings in Appendix C.*

2. Ensure that the following settings are displayed before turning on solvent flow from the ACQUITY binary solvent manager:
  - API Gas on (nitrogen) is selected and turned on
  - Source Temperature: ~150 °C
  - Desolvation Temperature Range: ~250 to 450 °C
  - Desolvation Gas: ~500 to 1000 L/Hr
  - Cone Gas: Optional

**NOTE:** *The TQD control panel can also be used for quick access to commands (the instrument console and IntelliStart will be accessed later).*

3. Click **Refresh System (Sys Prep)** and select the solvent lines check box **Both A and B**, and then click **OK**.

**NOTE:** *This is for daily setup of the ACQUITY system. This primes your current selected solvent lines, the sample manager with one weak wash, and ignites the lamp UV detector (if a PDA or TUV detector was part of the system).*

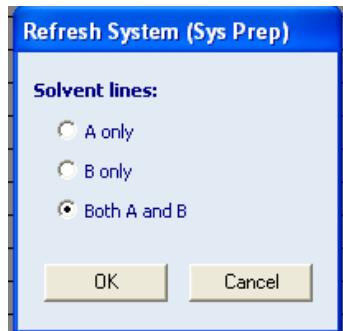


Figure 19 - Refresh System (Sys Prep) window

**NOTE:** *System Startup is used to set up the system, primes all solvent and needle wash lines, equilibrate flow and column temperatures, and optionally perform needle and sample loop characterization.*



Figure 20 - System Startup window

4. Turn on the ACQUITY flow rate and select a flow rate of **0.3 mL/min** at 50% A and 50% B.

**NOTE:** *This will be the flow rate used for combining LC flow during sample tuning.*

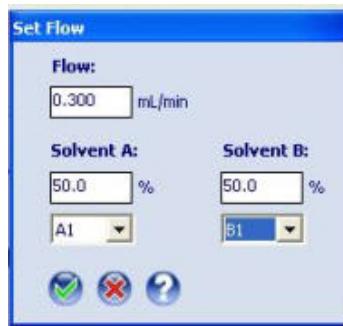


Figure 21 - Set Flow window

5. Right-click the **TQD control panel** and click **Launch Instrument Console** to access advanced TQD and ACQUITY controls and diagnostics.

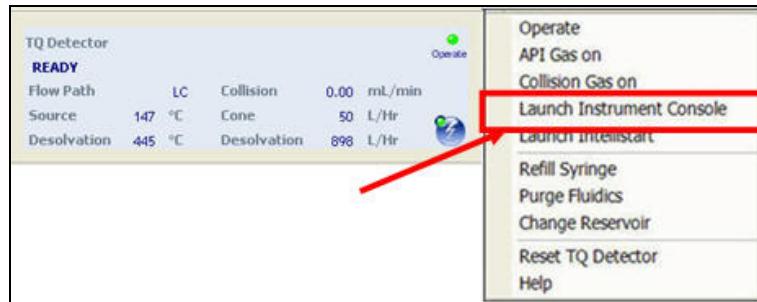


Figure 22 - Run Samples field

## The instrument console for the ACQUITY TQD

This section describes the process of MS calibration, sample tuning, and developing an MRM acquisition method.

1. In the Instrument Console field, select the **TQ Detector > MS Display**, to show the current readbacks of the mass spectrometer and aid diagnostic procedures.

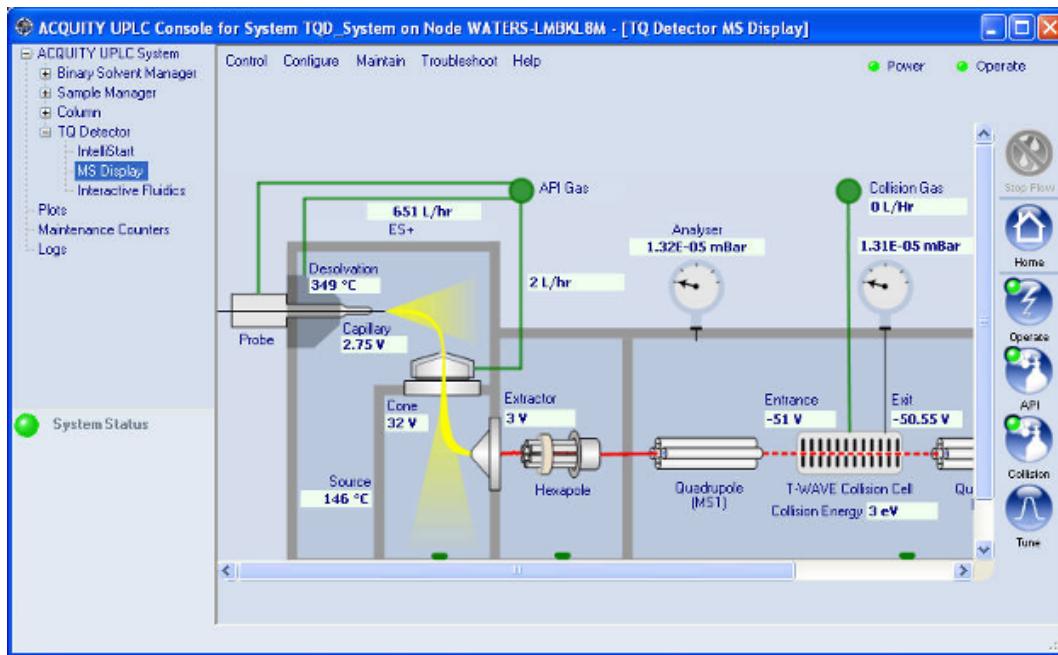


Figure 23 - TQ Detector MS Display window

2. Place a calibration solution of Naics2 into Reservoir A, and a sample tuning solution of 10 ng/ $\mu$ L of sulfadimethoxine into Reservoir B.
3. Click the **TQ Detector Interactive Fluidics and Interactive Control** icon .

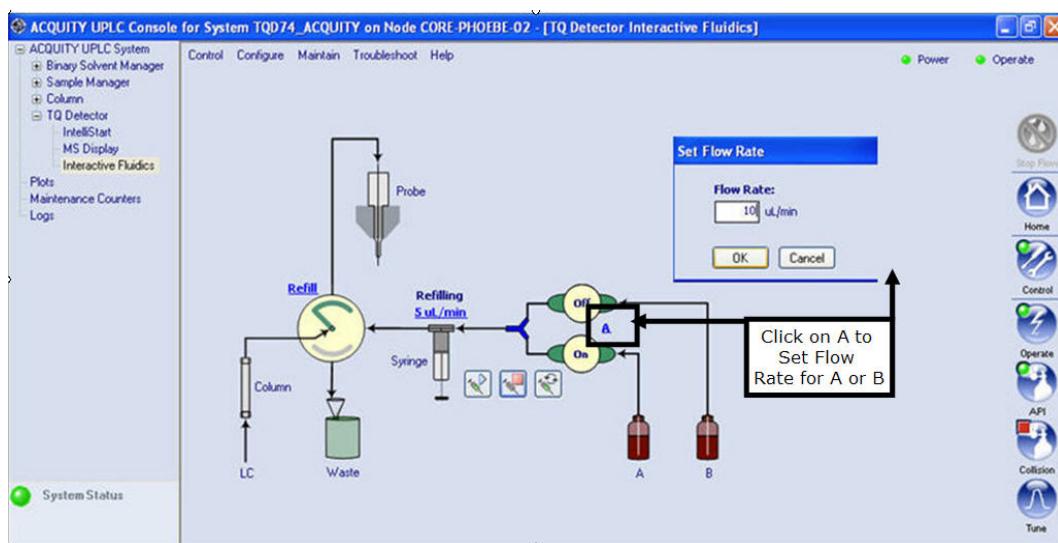


Figure 24 - TQ Detector Interactive Fluidics window

4. Click **A** and then click the **Purge** icon  to purge reservoir A.

**NOTE:** Clicking the Purge icon starts a sequence of three purges.

5. Click the **Refill Syringe** icon  after the purge, to ensure that the syringe is full before using the IntelliStart sequence.
6. If A is not displayed, click **B** and select **A** in the reservoir selection field.

## IntelliStart overview

### Instrument setup, sample tune, and develop methods

IntelliStart is designed to perform the following functions:

- Automatically optimize MS calibrant for signal optimization
- Calibrate MS for speed and mass resolution
- Optimize MS conditions for selected compounds at a selected LC flow rate
- Develop SIR and MRM instrument method conditions, including a printout of the optimized conditions

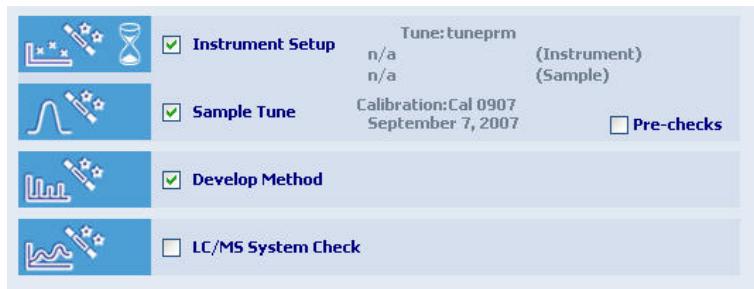


Figure 25 - IntelliStart window

### Instrument setup for mass calibration

Instrument setup locates a selected mass(es) signal of the calibration solution, and then calibrates the mass spectrometer for mass accuracy. Pre-entered mass resolutions settings for MS1 and MS2 determine proper scan speed and mass resolution parameters for the TQ Detector.

## Sample tune

Sample tune optimizes the mass spectrometer's signal intensity for a selected compound(s) selected mass. Mass optimization includes adjusting desolvation and gas temperatures for a selected LC flow rate condition.

Sample tune works by optimizing MS1 source parameters including cone voltage, capillary voltage, and ion energy. This is repeated for optimization of MS2 parameters. Sample tune can be used for one to four components.

IntelliStart uses a lookup table to adjust desolvation temperatures and gas flows for changes in LC flow rates.

**Table 4: Lookup table**

| Flow Rate (mL/min) | Source Temp (°C) | Desolvation Temp (°C) | Desolvation Gas Flow (µL/min) |
|--------------------|------------------|-----------------------|-------------------------------|
| 0.000 to 0.100     | 150              | 250                   | 500                           |
| 0.101 to 0.300     | 150              | 350                   | 600                           |
| 0.301 to 0.500     | 150              | 400                   | 800                           |
| >0.500             | 150              | 450                   | 900                           |

**NOTE:** *For compounds with molecular ions within ±1 Dalton of each other, it is recommended to do individual sample tunes, as IntelliStart will use the compound with the highest mass intensity to calculate the optimal settings.*

## Develop method

In conjunction with the sample tune, develop method optimizes SIR and/or MRM method conditions for a specific sample(s) and automatically prints out these conditions.

Develop method inserts these conditions into a pre-selected method set's instrument method.

Develop method can be used for up to four compounds, with the pre-cursor ion designated in the sample tune page and creates up to three transitions per component.

**NOTE:** *After using sample tune and the IntelliStart function, the LC/MS System Check is a separate function to test the TQ Detector as part of a system. This function is described in Appendix A.*

## IntelliStart operation

For this training section we will complete instrument setup, sample tune, and develop method. This exercise describes ESI+ operation.

**NOTE:** See Appendix B at the back for this document for a basic overview of other ionization modes.

For most daily operations, use sample tune and method. Instrument setup is advised to be used after system maintenance or at periodic time intervals.

1. In the ACQUITY UPLC console, click **Configure > IntelliStart configuration**.

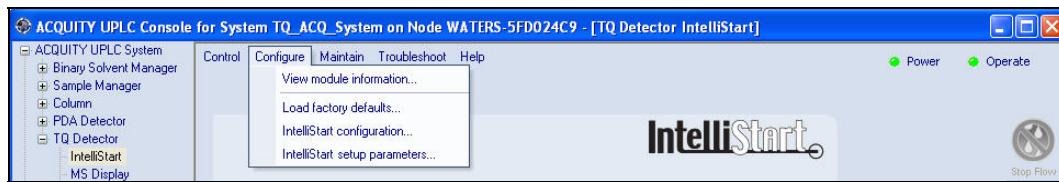


Figure 26 - ACQUITY UPLC console

**NOTE:** IntelliStart checks are used to check if the ACQUITY TQD system is working before operation. If a check is enabled, you will be flagged by a blinking light located on the TQ Detector, if there is a problem. It is recommended that all checks are investigated.

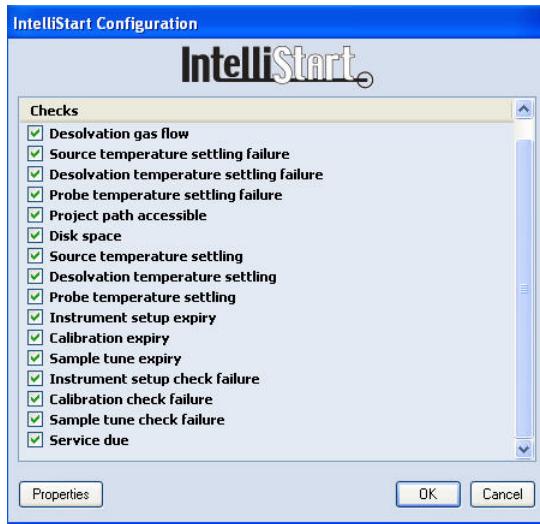


Figure 27 - IntelliStart Configuration window

2. In the UPLC console, click **TQ Detector > IntelliStart**.

3. Select the **Instrument Setup**, **Sample Tune**, and **Develop Method** check boxes.

**NOTE:** Pre-checks uses an existing sample tune to confirm a beam is present at the selected masses (ion intensity at that mass).

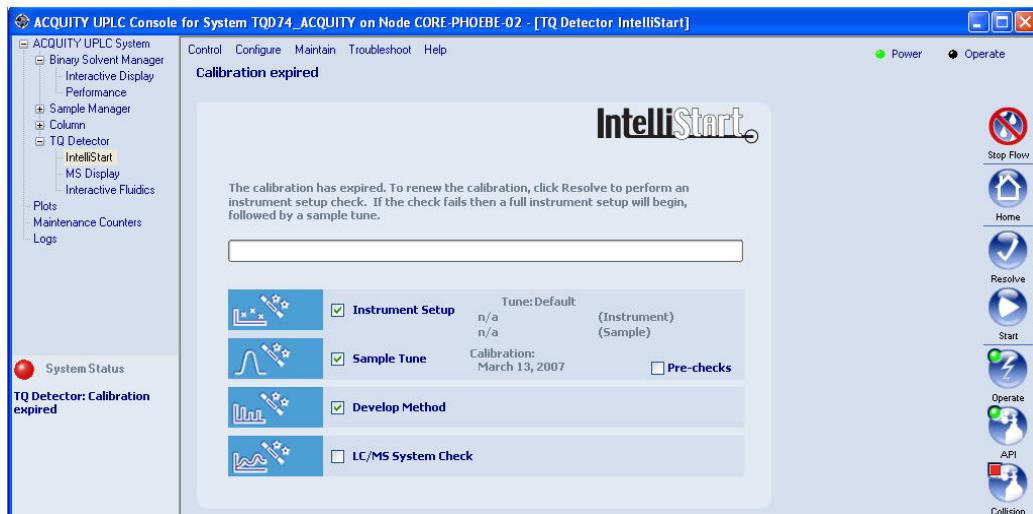


Figure 28 - ACQUITY UPLC console

4. Click the **Start IntelliStart Sequence** icon .
5. Click the **Instrument Setup** tab and set the instrument/calibrant tune masses, target resolution, calibration range, and flow path settings as shown in Figure 29.

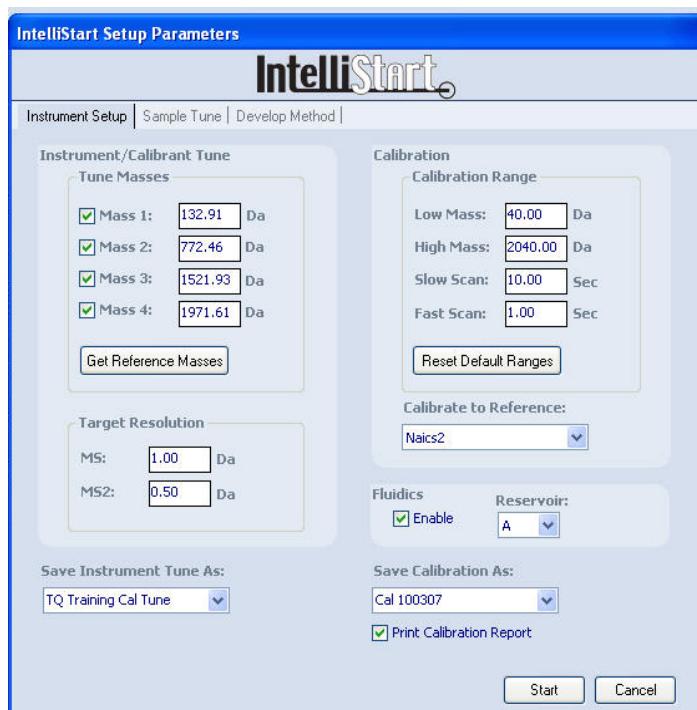


Figure 29 - Instrument Setup tab

6. Click the **Sample Tune** tab.
7. Refer to Table 5 and Table 6 for general recommendations for instrument setup and mass calibration.

**NOTE:** *Calibrants: For ESI it is recommended to use Naics2 (sodium cesium iodide) for calibration.*

**Table 5: Resolution settings**

| Calibrating for: | Scanning             | SIR/MRM                                    |
|------------------|----------------------|--|
| MS1              | 0.75 Da              | 0.7 to 1.0 Da                              |
| MS2              | 0.75 Da              | 0.7 to 1.0 Da                              |
| Notes:           | Use MS2 for Scanning | For Bio-Molecules use 0,75 for MS1 and MS2 |

**Table 6: Calibration range**

| Calibrating for: | Scanning | Default  |
|------------------|----------|----------|
| Low Mass         | 2 Da     | 2 Da     |
| High Mass        | 2040 Da  | 2040 Da  |
| Slow Scan        | 6 sec    | 10.0 sec |
| Fast Scan        | 0.2 sec  | 1.0 sec  |

**NOTE:** *The default settings for scanning are at 2000 amu/second. If you use the fast scanning capability of 10,000 amu/second, you must use MS2 for scanning (MS2 is closer to the detector which maintains resolution).*

8. Click the **Save Instrument Tune As** drop-down list and type **TQ Naics2 Tune**.
9. Click the **Save Calibration As** drop-down list and type **TQ Cal XXXX**, where XXXX is the date.
10. Select the **Print Calibration Report** check box.

11. Click the **Sample Tune** tab and set the following parameters:

- Tune Masses: Mass 1: 311.10 (sulfadimethoxine's m/z)
- Fluidics: Enabled
- Reservoir: B
- Flow Path: Combined
- Flow Rate: 0.8 mL

**NOTE:** The combined flow rate entry is set at 800  $\mu\text{L}/\text{min}$  to enable IntelliStart's lookup table for desolvation temperature, and gas flow settings for the LC analysis.

12. Click the **Save the Sample Tune Parameters As** drop-down list and type **Sulfadimethoxine Tune**.

**NOTE:** You can enter up to four separate masses using sample tune. Compound masses must be greater than  $\pm 1$  amu resolution apart or sample tune will optimize on the highest intensity compound of the components. For multiple compounds that have widely dissimilar optimizations, it is advised to use sample tune separately for each component.



Figure 30 - Sample Tune tab

13. Click the **Develop Method** tab and set the following parameters:

- Based On Method Set: Waters TQD Training
- Develop SIR Method: Deselected
- Develop MRM Method: Selected
- Stop Time: 1.60 min (the UPLC run time)
- Save As Method Set: TQD Training MS
- Optimization Range: Defaults
- Product Scan: Defaults
- Print AutoTune Report: Selected (Ensure that a printer is configured before using this function)

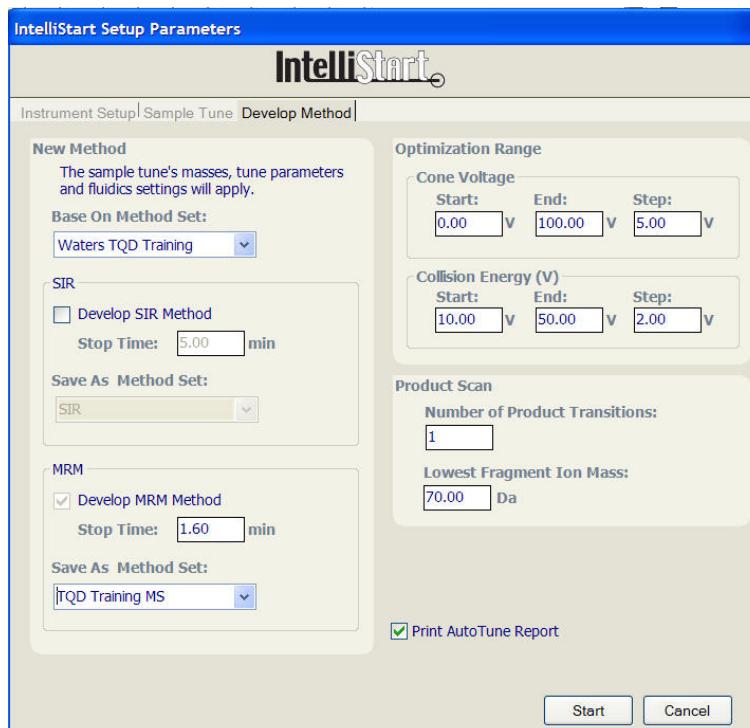


Figure 31 - Develop Method tab

14. Click **Start** and instrument setup with mass calibration, sample tune, and develop method will run sequentially.
15. Wait for 15 to 20 minutes for the IntelliStart sequence to complete.

**NOTE:** A printout of the MS calibration and sample tune reports is also produced for review and regulatory purposes.

## Reviewing IntelliStart's results and manual tuning

Once the tunes and calibration are completed, IntelliStart displays the method names and date, as well as a printout of the report.

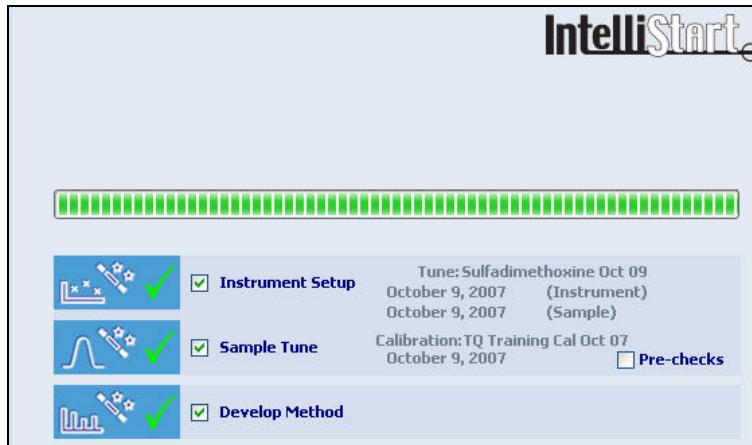


Figure 32 - IntelliStart window

The calibration method and tune method are stored in the Empower project where you acquired the data. This is stored in the Methods View tab. To acquire data in another Empower project using this calibration, you must copy the calibration and tuning method to the project you are acquiring from.

A second report is printed after the develop method is completed. This report shows the masses for MRM/SIR transitions selections and MRM/SIR optimizations.

1. Click the **Tune page** icon for manual tuning capabilities.
2. Change the cone gas flow from **0** to **50** to reduce solvent adducts and reduce background noise.

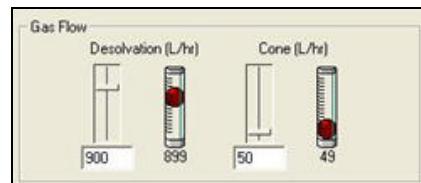


Figure 33 - Gas Flow field

3. Click **File > Save**, and then **File > Exit**.

## Edit the instrument method for MRM transition speed and smoothing

IntelliStart optimized the MRM for proper transitions. This section edits the instrument method for adjustments for proper acquisition data rates (dwell time) and smoothing selections.

1. Minimize the ACQUITY MS console and view the Run Samples window.
2. Click **Edit > Instrument Method**.
3. Select the **TQD Training IM MRM** instrument method from the list and click **Open**.
4. Click the **TQ Detector** icon.
5. IntelliStart has developed a method that has an optimized MRM transition. Further optimize the MRM transition for UPLC by clicking on the first row of the Functions table and clicking **Show Function Details**.
6. Change the dwell time from **0.1 seconds** to **0.040 seconds**.
7. Click **Hide**.
8. In the SIR/MRM Smoothing field, select the **Enable** check box, set a window size of **2 scans**, and a count of **1**. Leave all other settings as displayed.

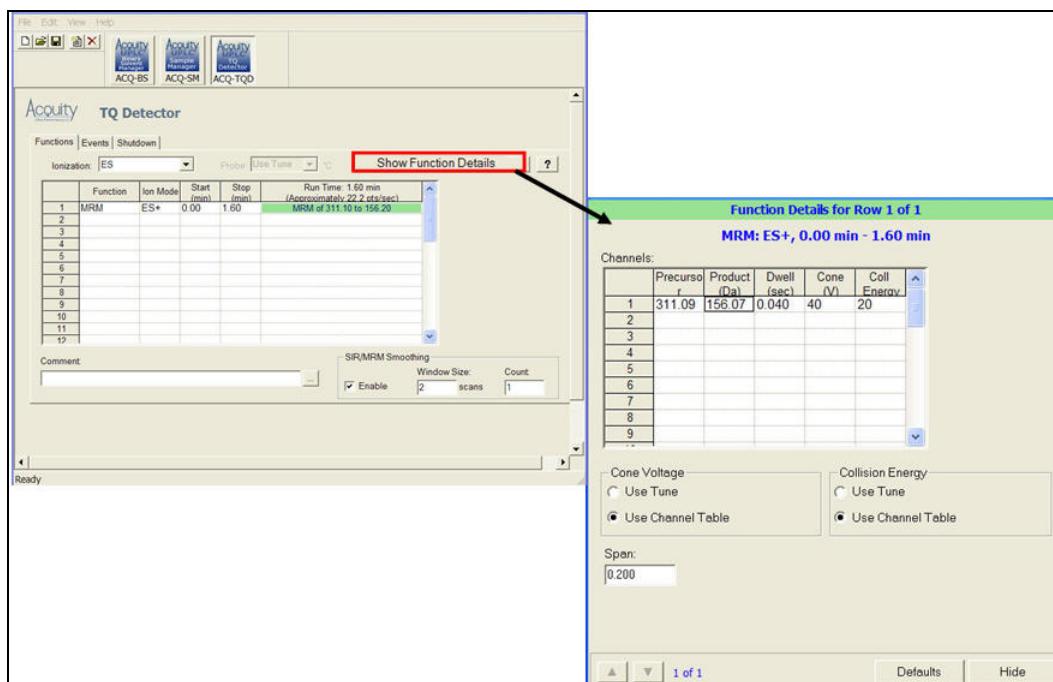


Figure 34 - Show Function Details

**NOTE:** IntelliStart's Develop Method uses a default dwell time of 0.1 second. For UPLC or multiple MRMs, change this to a faster dwell time to get enough data points across a chromatographic peak for good quantitation.

9. Click **File > Save**, and then **File > Exit**.

## Making a sample set

1. Click the **New Sample Set Wizard** icon .
2. Select **Create a Sample Set using this wizard** and then click **Next**.
3. Set the sample set type to **LC PDA/MS** and then click **Define Plates**.
4. Click in the Plate Type Name column and select **ANSI-48Vial2mLHolder**.
5. Click in the Plate Layout Position column and select **1**.
6. Click **Next**.

|   | Plate Type Name      | Plate Layout Positi |
|---|----------------------|---------------------|
| 1 | ANSI-48Vial2mLHolder | 1                   |

Figure 35 - Define Plates window

**NOTE:** All ACQUITY sample plates must be of the ANSI format.

7. Select the **At start of the sample set** check box.
8. In the Start loading vials in tray position box, select **1:A,1** (for well plate 1, position A1).
9. Click **Next**.

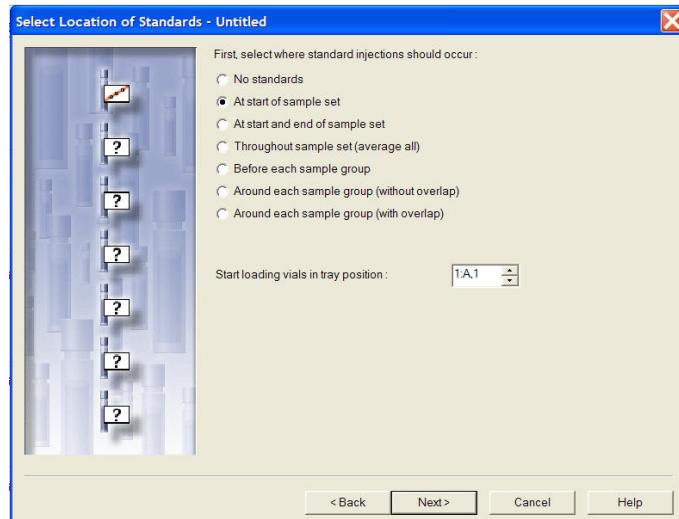


Figure 36 - Select Location of Standards window

10. Set the calibration options to **Each standard vial contain a different level standard**.
11. Click **Next**.

12. In the Standard Information field set the following parameters:

- Number of standard vials in each group: 2
- Number of injections per vial: 1
- Injection volume: 2
- Run time: 1.6
- Method Set: TQD Training MS

13. Click **Options**.

14. Set the next injection delay value to **0**.

15. Click **Next**.

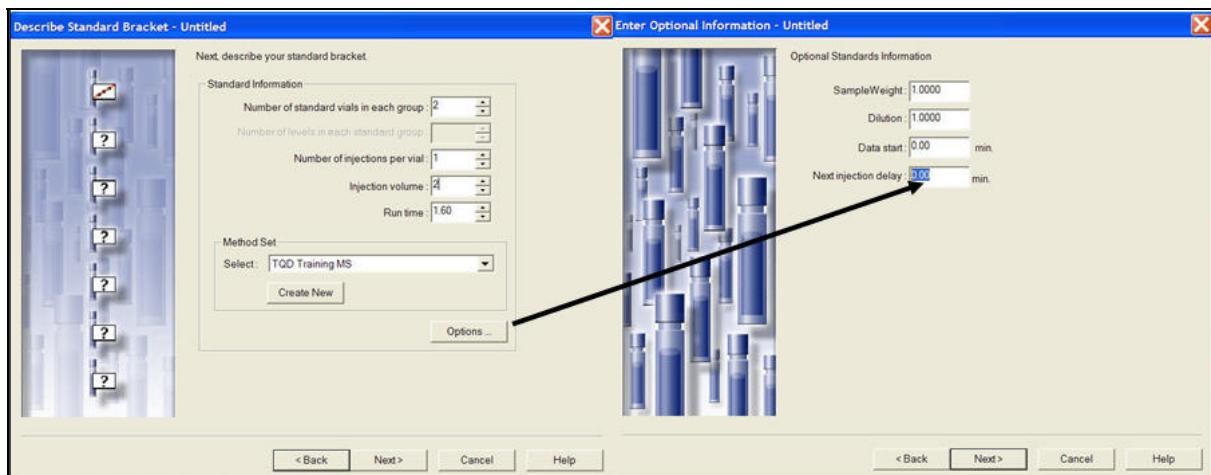


Figure 37 - *Describe Standard Bracket* window

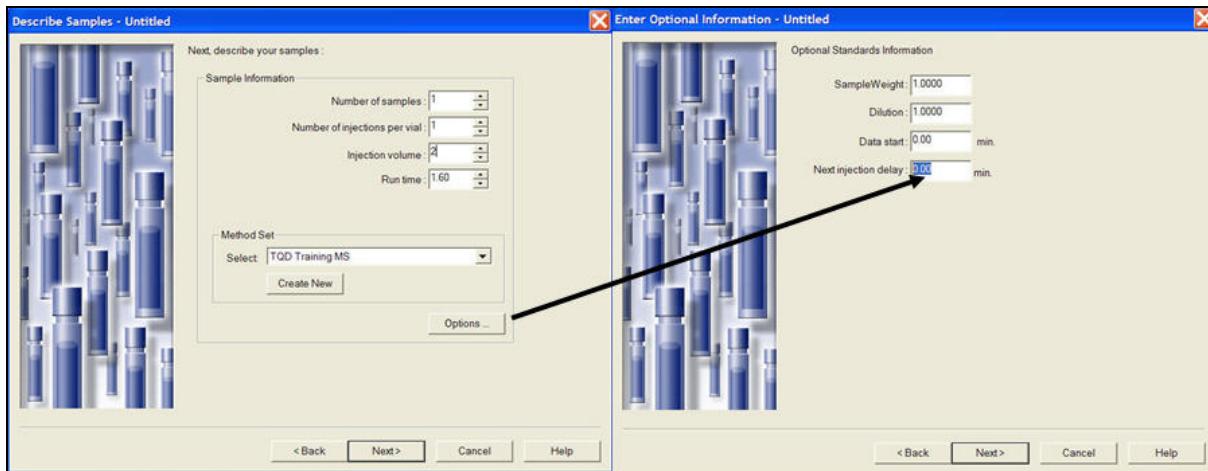
16. In the Sample Information field set the following parameters:

- Number of samples: 1
- Number of injections per vial: 1
- Injection volume: 2
- Run time: 1.6
- Method Set: TQD Training MS

17. Click **Options**.

18. Set the next injection delay value to **0**.

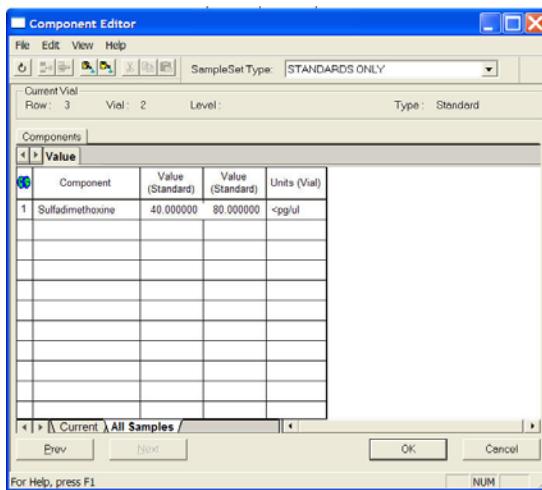
19. Click **Next**.



*Figure 38 - Describe Samples window*

20. In Identification, for the standard name, type **TQ Stan** and increment suffix use **1**.
  21. In the sample name, type **TQ Unk** and increment suffix use **a**.
  22. Click **Next**.
  23. Click **Run Mode to Run and Process**, and then click **Next**.
  24. Review the summary and then click **Finish**.
  25. In the Component Editor window, set the parameters shown in Figure 39 for component names and amounts and then click **OK**.

**NOTE:** Type < before the units in the last column.



*Figure 39 - Component Editor window*

26. Click **OK** and the sample set is created and displayed in Run Samples.
  27. In the Run Samples window, click the **Sample** tab.
  28. Highlight the first sample of the sample set below Clear Calibration and right-click and select **Insert Row**.

29. In the row added above the first sample, select the **Function** column, click the drop-down list and select **Condition Column**.

30. Set a time of **1.6** minutes.

**NOTE:** *The Condition column runs the gradient without a sample injection.*

31. In the Run Samples window, click the **Sample** tab

32. Highlight the last sample of the sample set, then right-click and select **Add Row**.

33. In the row added below the last sample, select the **Function** column, click the drop-down list, select **Report** and then select **TQ RM Report Summary**, to display the final sample set.

| Sample Set Method: Waters TQD Training |            |                    |              |           |                   |                            |                    |                           |
|--|------------|--------------------|--------------|-----------|-------------------|----------------------------|--------------------|---------------------------|
|  | Plate/Well | SampleName         | Inj Vol (uL) | # of Injs | Function          | Method Set / Report Method | Run Time (Minutes) | Next Inj. Delay (Minutes) |
| 1                                      |            |                    |              |           | Clear Calibration | TQD Training MS            |                    |                           |
| 2                                      |            |                    |              |           | Condition Column  | TQD Training MS            | 1.60               | 0.00                      |
| 3                                      | 2:A,1      | Sulfadimethoxine1  | 2.0          | 1         | Inject Standards  | TQD Training MS            | 1.60               | 0.00                      |
| 4                                      | 2:A,2      | Sulfadimethoxine2  | 2.0          | 1         | Inject Standards  | TQD Training MS            | 1.60               | 0.00                      |
| 5                                      | 2:A,3      | Sulfadimethoxine a | 2.0          | 1         | Inject Samples    | TQD Training MS            | 1.60               | 0.00                      |
| 6                                      |            |                    |              |           | Report            | TQD Training RM Summary    |                    |                           |

Figure 40 - Final Sample Set Method window

34. Click **File > Save Sample Set Method** and type the name as **TQD training Set**.

**NOTE:** *The Function column in the sample set table has some special functions in the drop-down list. The functions are described in the following table.*

**Table 7: Function column special features**

| Function                   | Description   |
|----------------------------|---|
| Equilibrate                | Runs at initial conditions without collecting data for a specified time using the method set.                         |
| Condition Column           | Runs the gradient without collecting data for a specified time.   |
| Purge Injector             | Purges the ACQUITY sample manager.  |
| Wet Prime                  | Primes the ACQUITY solvent manager with flow using the specified method set.  |
| Inject Immediate Standards | Collects standard data without making an injection. This is for collection from the MS console or Advanced Tune page. |
| Inject Immediate Samples   | Collects sample data without making an injection. This is for collection from the MS console or Advanced Tune page.   |
| Pause                      | Pauses the sample set during data collection.   |
| Sys Prep                   | Primes the ACQUITY sample manager, primes and wash the ACQUITY sample manager and equilibrates in one step.           |

35. In the sample set's samples table, scroll to the right and select **Sulfadimethoxine Tune** in the MS Tune Method column.

36. Select the **Naics2** calibration's from IntelliStart in the MS Calibration Method column.

| Plate/Well | SampleName         | Inj Vol (uL) | # of Inj | Function          | Method Set / Report Method | Run Time (Minutes) | Next Inj Delay (Minutes) | MS Tune Method          | MS Calibration Method  |
|------------|--------------------|--------------|----------|-------------------|----------------------------|--------------------|--------------------------|-------------------------|------------------------|
| 1          |                    |              |          | Clear Calibration | TQD Training MS            |                    |                          |                         |                        |
| 2          |                    |              |          | Condition Column  | TQD Training MS            | 1.60               | 0.00                     |                         |                        |
| 3 2:A,1    | Sulfadimethoxine1  | 2.0          | 1        | Inject Standards  | TQD Training MS            | 1.60               | 0.00                     | Sulfadimethoxine Oct 09 | TQ Training Cal Oct 07 |
| 4 2:A,2    | Sulfadimethoxine2  | 2.0          | 1        | Inject Standards  | TQD Training MS            | 1.60               | 0.00                     | Sulfadimethoxine Oct 09 | TQ Training Cal Oct 07 |
| 5 2:A,3    | Sulfadimethoxine a | 2.0          | 1        | Inject Samples    | TQD Training MS            | 1.60               | 0.00                     | Sulfadimethoxine Oct 09 | TQ Training Cal Oct 07 |
| 6          |                    |              |          | Report            | TQD Training RM Summary    |                    |                          |                         |                        |

Figure 41 - Complete Sample Set Method window

**NOTE:** If the MS Tune method and MS Calibration methods are not selected, Empower TQD will use the last tune and calibration that was acquired within the project.

37. Click **File > Save Sample Set Method** to use as a template sample set method for future analysis for the same type of samples.

38. Click  to acquire the sample set.

39. Set the following run sample set parameters:

- Sample set name Waters TQD Training (with your initials added)
- Run Mode Run and Report
- Suitability Mode Continue on Fault
- Printer Choose a printer
- Shutdown Method Shutdown TQD

40. Click **Run**.

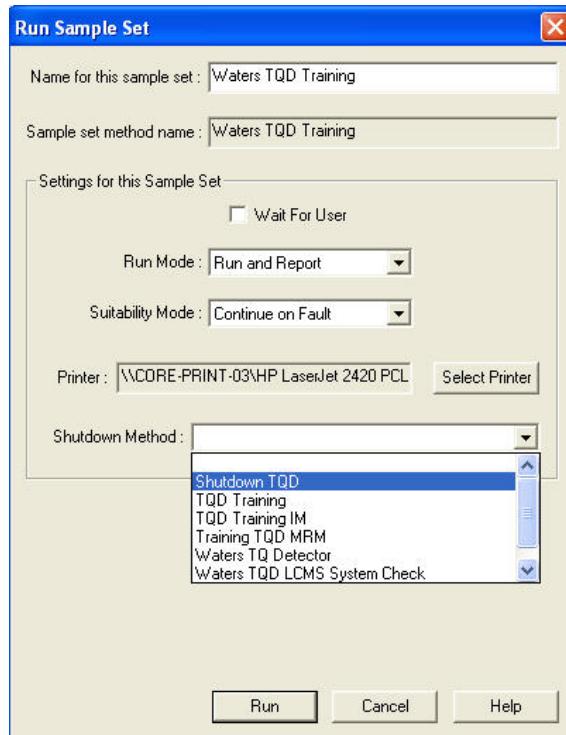


Figure 42 - Run Sample Set window

41. Wait for the three injections to occur and the Running tab to be displayed with the real time plot of data of the MRM data for the sulfadimethoxine analysis.

**NOTE:** After each injection a report of your result is printed, then after the final injection a summary report is printed.

42. After all acquisitions have occurred, click **File > Exit** to exit Run Samples.

## Example reports

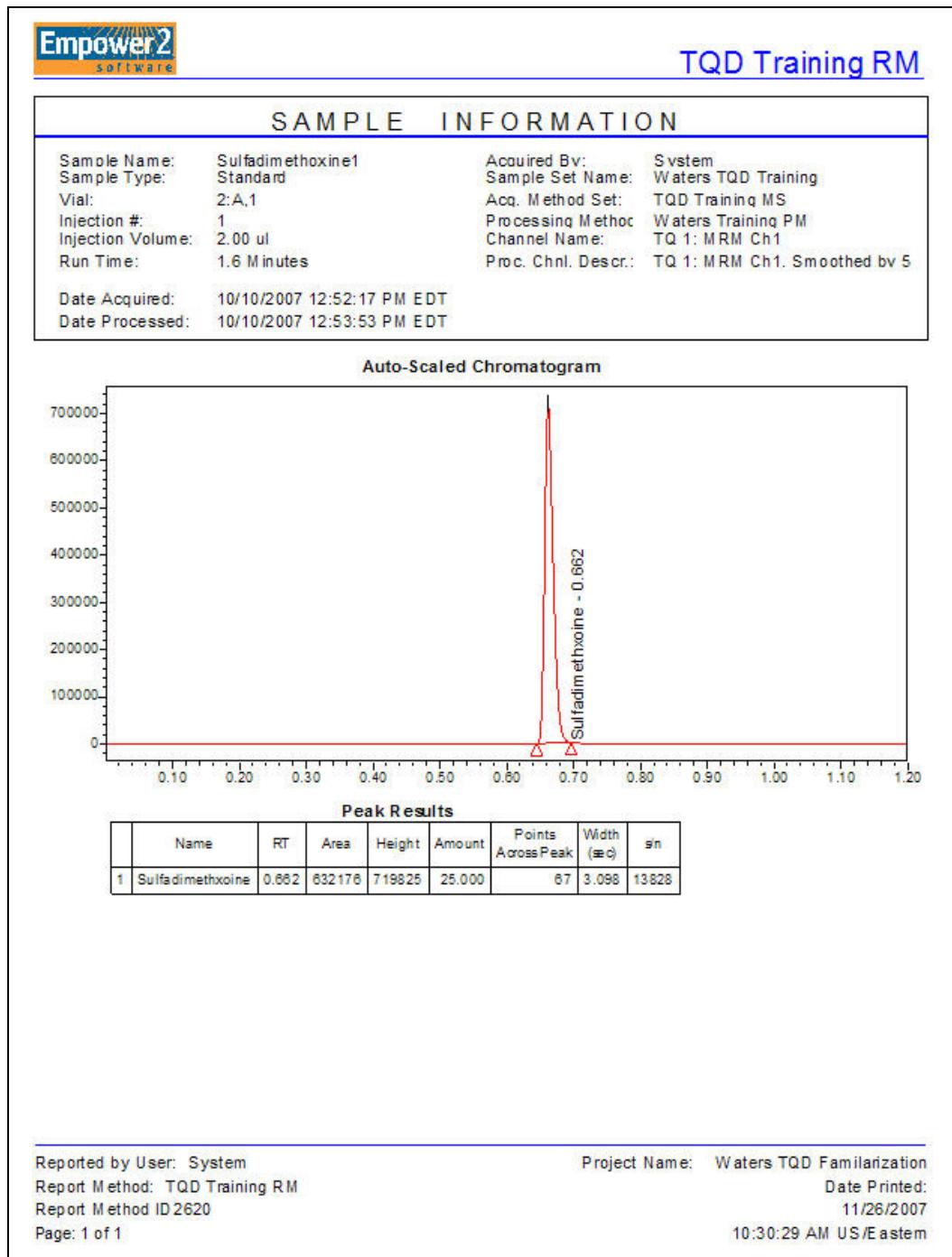


Figure 43 - Waters TQD individual report

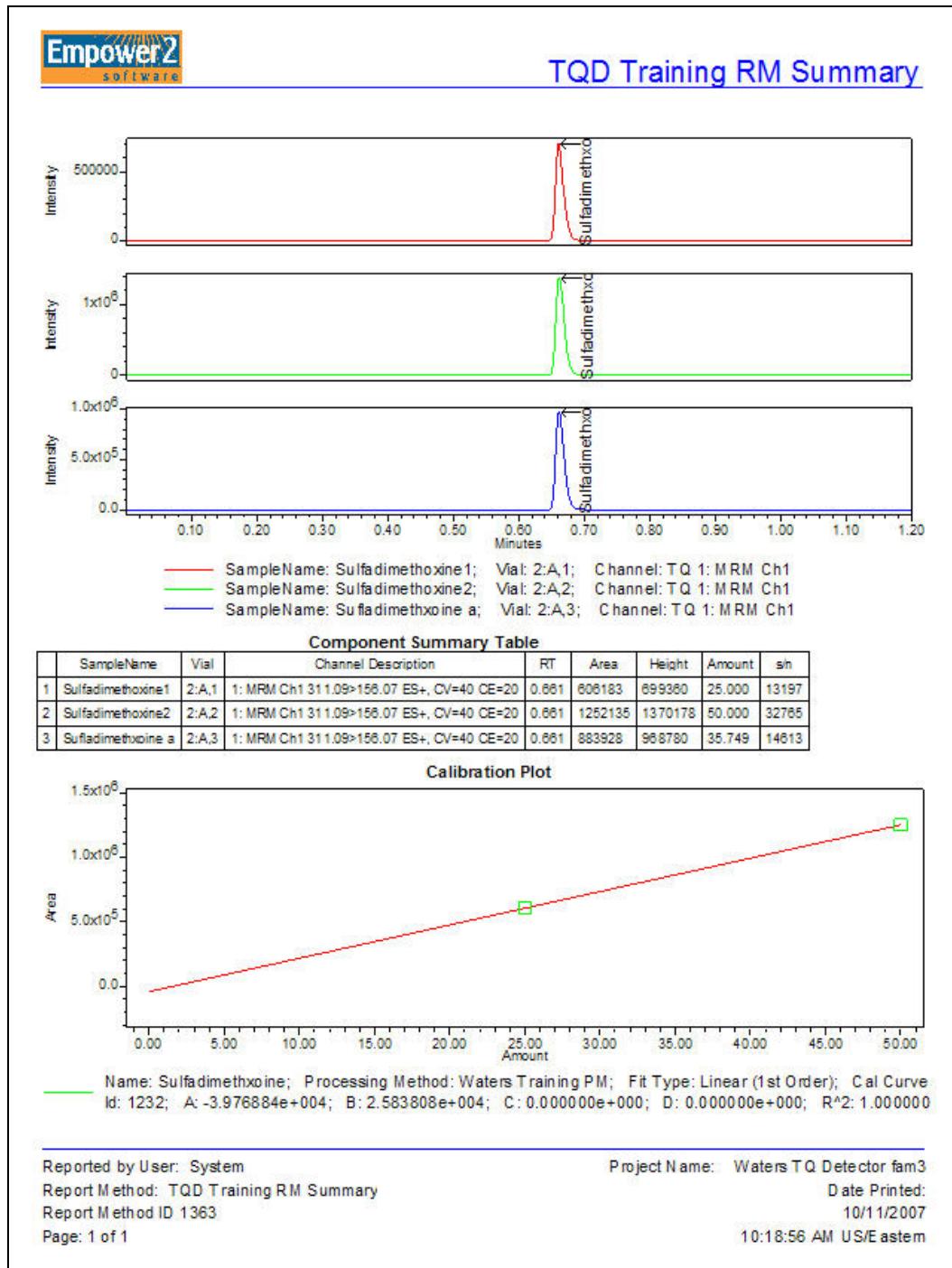


Figure 44 - Waters TQD summary report

## Addendum A: LC/MS system check

LC/MS system check uses a pre-defined instrument method, processing method, method set, and report method to check the ACQUITY TQD as a system.

This is done by injecting a single compound (sulfadimethoxine) six times at a pre-defined concentration. The resulting data is then processed and reported with checks of sensitivity (signal-to-noise ratio); response (peak area, peak height); and chromatographic performance (peak width, retention time).

System check can be run periodically to help confirm the performance of your ACQUITY TQD system.

**NOTE:** *The LC/MS system check has been pre-characterized with sulfadimethoxine.*

### Sample tuning solution for the LC/MS system check

The sulfadimethoxine solution is provided during the start-up installation procedures and tests. This section describes how to make fresh solution from a stock solution of sulfadimethoxine 1 mg/mL in methanol.

#### Solution A - 10 ng/µL sulfadimethoxine

1. Pipette 1 mL of Solution 1, from the solutions kit, into a 100-mL volumetric flask and dilute to 100 mL with diluent.
2. Stopper and invert the flask several times to mix fully.
3. Label the flask as **Solution A - 10 ng/µL sulfadimethoxine**.
4. Transfer the rest of Solution 1 to a scintillation vial, label as **1 mg/mL sulfadimethoxine** and store in a refrigerator at 4 °C.
5. Transfer the remaining **Solution A** to an amber bottle and store in a refrigerator at 4 °C.

#### Stock sulfadimethoxine 100 pg/µL

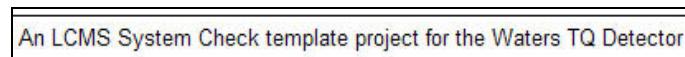
1. Pipette 1 mL of **Solution A** into a 100-mL volumetric flask and dilute to 100 mL with diluent.
2. Stopper and invert the flask several times to mix fully.
3. Label the flask as **Stock sulfadimethoxine 100 pg/µL**.

#### LC/MS system check sulfadimethoxine 20 pg/µL

1. Pipette 2 mL of **Stock sulfadimethoxine 100 pg/µL** into a 10-mL volumetric flask and dilute to 10 mL with diluent.
2. Stopper and invert the flask several times to mix fully.
3. Label the flask as **LC/MS system check sulfadimethoxine 20 pg/µL**.
4. Place 1 mL of **LC/MS system check sulfadimethoxine 20 pg/µL** into a sample vial and insert this into the default location for the LC/MS (position **2:A,7** of the ACQUITY UPLC sample manager).

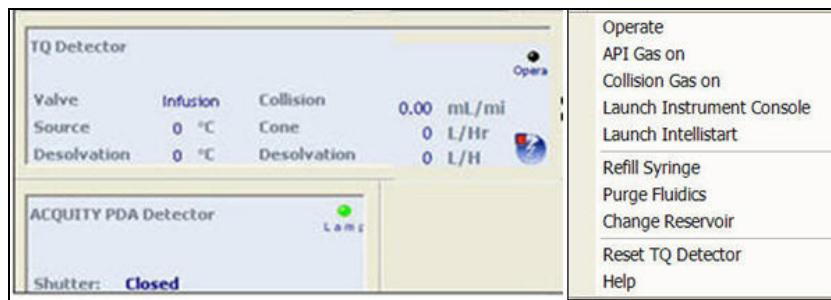
## Running the LC/MS system check

1. Before using the IntelliStart LC/MS system check, ensure that you select an MS project that has the LCMS system check custom fields and methods.
2. Click the **Configuration Manager** and then click the **Project** tab.
3. View the comments on the project and ensure that they are as displayed in Figure 45:



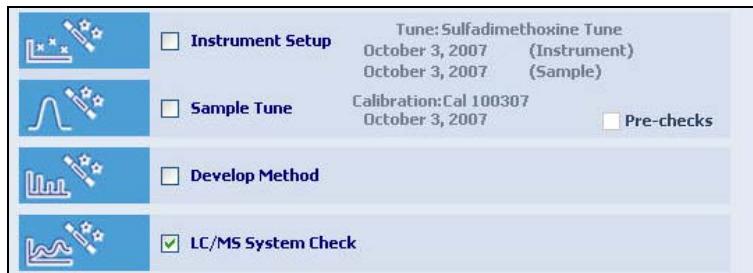
*Figure 45 - Project comments*

4. Open the **Waters TQD LC/MS System Check** and check the vial position before running the LC/MS system check.
5. In the Run Samples window, right-click the **TQD control panel** and click **Launch IntelliStart**.



*Figure 46 - Run Samples field*

6. In the IntelliStart window, select the **LC/MS System Check** check box.



*Figure 47 - IntelliStart window*

7. Click the **Start IntelliStart sequence** icon.
8. Click the **Sample Set Method** drop-down list, and select **Waters TQD LC/MS System Check**.
9. Click **Start**.

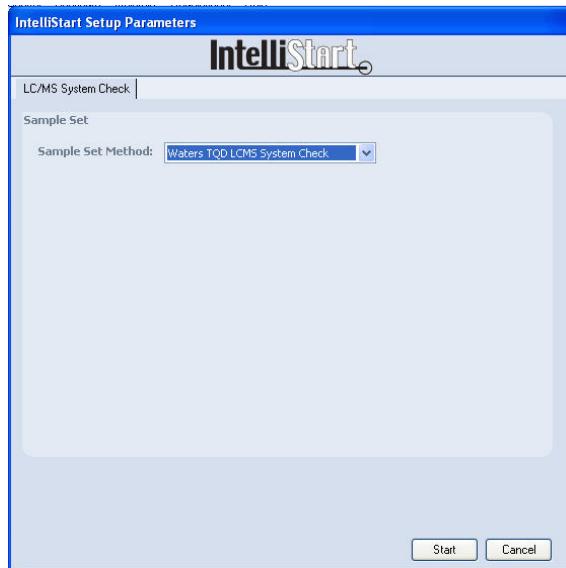


Figure 48 - IntelliStart Setup Parameters window

10. Ensure that the system automatically runs the sample set, as shown in Figure 49, and prints a processed result.

The screenshot shows the 'Sample Set Method' window. It has a toolbar at the top with various icons. Below that is a dropdown menu for 'Run Only' and 'Continue on Fault'. The main area displays a table for a 'Waters TQD LCMS System Check' method. The table columns are: Plate/Well, SampleName, Inj Vol (uL), # of Injs, Function, Method Set / Report Method, Run Time (Minutes), Next Inj. Delay (Minutes), and MS Method. The data in the table is as follows:

| Plate/Well | SampleName | Inj Vol (uL) | # of Injs | Function                | Method Set / Report Method   | Run Time (Minutes) | Next Inj. Delay (Minutes) | MS Method           |
|------------|------------|--------------|-----------|-------------------------|------------------------------|--------------------|---------------------------|---------------------|
| 2:A,2      | pre-run    | 5.0          | 2         | Inject Samples          | Waters TQD LCMS System Check | 5.00               | 0.00                      | Waters TQD LCMS Sys |
| 2:A,2      | run        | 5.0          | 6         | Inject Samples          | Waters TQD LCMS System Check | 5.00               | 0.00                      | Waters TQD LCMS Sys |
|            |            |              |           | Summarize Custom Fields |                              |                    |                           |                     |
|            |            |              |           | Report                  | Waters TQD LCMS System Check |                    |                           |                     |

Figure 49 - Sample Set Method window

11. Ensure that a report is displayed, with a pass or fail assigned to each of the test results (Figure 50 to Figure 52).

**NOTE:** If the LC/MS system check fails, click the resolve icon in the MS console of IntelliStart, check each component and re-run the LC/MS system check. If the LC/MS system check fails a second time, check your system settings and if necessary call technical services or your service engineer.

## Example LC/MC system check report

**Empower2** software

### LC/MS System Check

ACQUISITION INFORMATION

|  |  |
|--|--|
| System Name: T QD74 ACQUITY                      | Acquired By: System                              |
| Project Name: Waters TQD Familarization          | Build Version: Empower 2 Software Build 2154 SPs |
| Sample Set Method: Waters TQD LCMS System Check  | Sample Set Id: 1309 Result Set Id 1313           |
| Processing Method: Waters TQD LCMS System Check  | Sample Set Start Date: 10/10/2007 1:19:26 PM     |
| Report Method Name: Waters TQD LCMS System Check | Sample Set Name: Waters TQD LCMS System Check    |
| User Name: System                                |  |

This test has failed when any of the reported fields are seen in an underlined, italicized, red font.

All underlined, italicized, red values do not pass the set criteria

**Data and Results:**

|   | Name             | % RSD - Area | % RSD - Height |
|---|------------------|--------------|----------------|
| 1 | Sulfadimethoxine | 1.324        | 1.179          |

**Name: Sulfadimethoxine**

|   | Peak Name        | Inj # | Retention Time (min) | Area    | Height | Width @ 13.4% in seconds | S/N   |
|---|------------------|-------|----------------------|---------|--------|--------------------------|-------|
| 1 | Sulfadimethoxine | 1     | 1.578                | 1642019 | 611179 | 4.3                      | 18030 |
| 2 | Sulfadimethoxine | 2     | 1.576                | 1622014 | 596291 | 4.4                      | 20772 |
| 3 | Sulfadimethoxine | 3     | 1.576                | 1631804 | 593163 | 4.3                      | 18957 |
| 4 | Sulfadimethoxine | 4     | 1.573                | 1608106 | 594392 | 4.3                      | 16751 |
| 5 | Sulfadimethoxine | 5     | 1.574                | 1606831 | 597576 | 4.3                      | 17475 |
| 6 | Sulfadimethoxine | 6     | 1.567                | 1581660 | 591930 | 4.4                      | 19398 |

Printed 5:11:26 AM US/Eastern      10/16/2007      Page 1 of 3

Figure 50 - LC/MC system check (page 1 of 3)

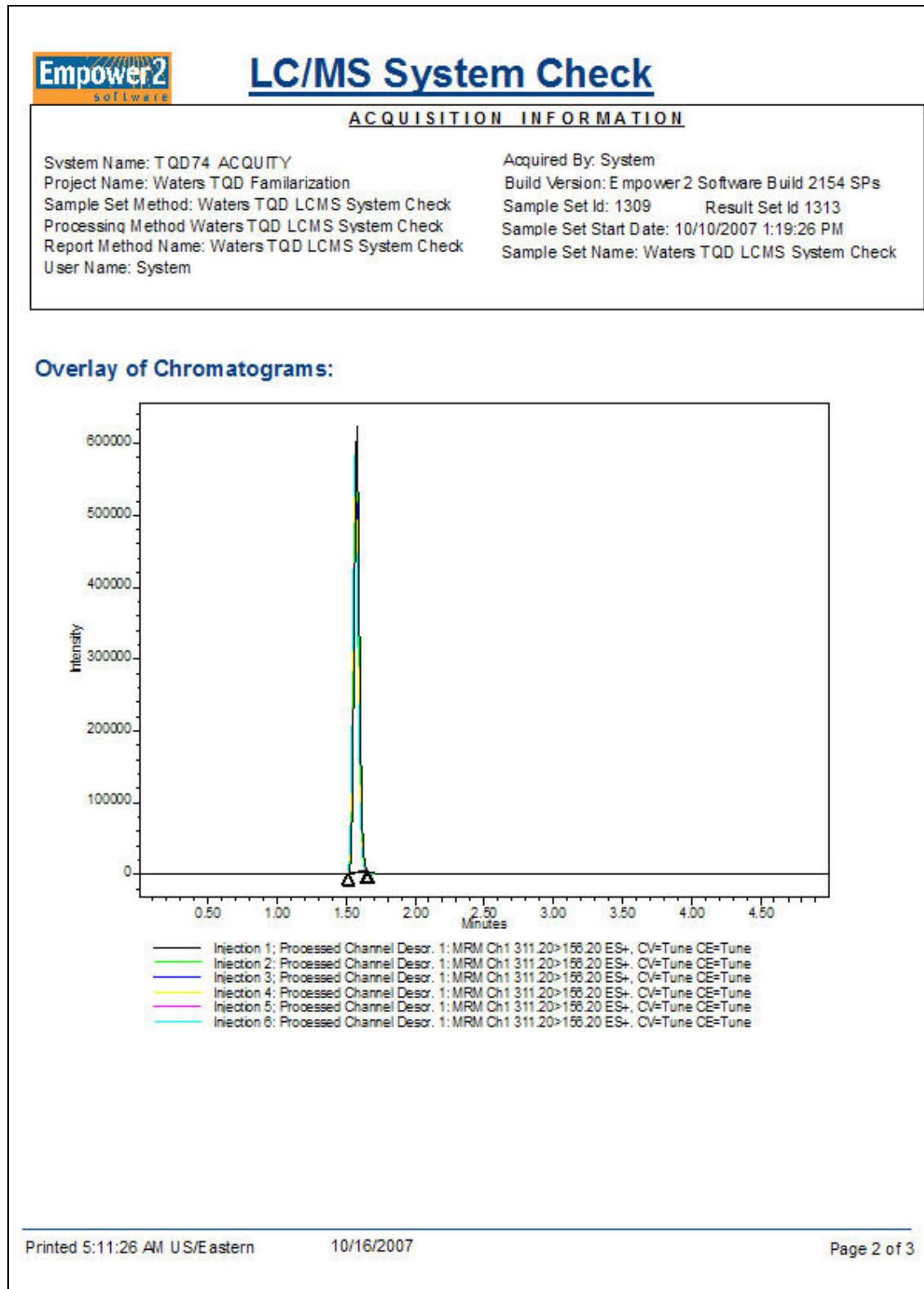


Figure 51 - LC/MC system check (page 2 of 3)

**Empower2** software

## LC/MS System Check

### ACQUISITION INFORMATION

|  |  |
|--|--|
| System Name: T QD74 ACQUITY                      | Acquired By: System                              |
| Project Name: Waters TQD Familiarization         | Build Version: Empower 2 Software Build 2154 SPs |
| Sample Set Method: Waters TQD LCMS System Check  | Sample Set Id: 1309 Result Set Id 1313           |
| Processing Method: Waters TQD LCMS System Check  | Sample Set Start Date: 10/10/2007 1:19:26 PM     |
| Report Method Name: Waters TQD LCMS System Check | Sample Set Name: Waters TQD LCMS System Check    |
| User Name: System                                |  |

**Pass/Fail Criteria:**

**Processing Method: Waters TQD LCMS System Check**

Type: MS      Stored: 9/27/2007 12:49:38 PM EDT

Suit Limits-'Sulfadimethoxine'

| Field Name          | Target | Error % | Lower Error Limit (LCL) | Upper Error Limit (UCL) | Warning % | Lower Warning Limit |
|---------------------|--------|---------|-------------------------|-------------------------|-----------|---------------------|
| 1 Area PercentRSD   |        |         |                         | 3.000                   |           |                     |
| 2 s/n               |        |         | 35.000                  |                         |           |                     |
| 3 Height PercentRSD |        |         |                         | 4.500                   |           |                     |
| 4 Wdth at 13p4 Sec  |        |         | 1.000                   | 10.000                  |           |                     |
| 5 SPSStdDevRT       |        |         |                         | 1.000                   |           |                     |

Suit Limits-'Sulfadimethoxine'

| Upper Warning Limit | Ignore Blank Values | Check Limits |
|---------------------|---------------------|--------------|
| 1                   | Nb                  | Always       |
| 2                   | Nb                  | Always       |
| 3                   | Nb                  | Always       |
| 4                   | Nb                  | Always       |
| 5                   | Nb                  | Always       |

Revision History  
This method contains 12 items in the revision history.

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Printed 5:11:26 AM US/Eastern      10/16/2007      Page 3 of 3

Figure 52 - LC/MC system check (page 3 of 3)

## Addendum B: General manual Tune page settings

MRM acquisitions using IntelliStart's operation has been optimized for ESI positive operation. If you are using the other ionization modes, you may need to manually tune. This section covers basic navigation of the Tune page including some initial settings for each of the MS ionization modes.

### Tune page

In the MS console, click the **Tune page** icon  for manual tuning capabilities (manual tune, manual calibrate) and to access advanced diagnostics.

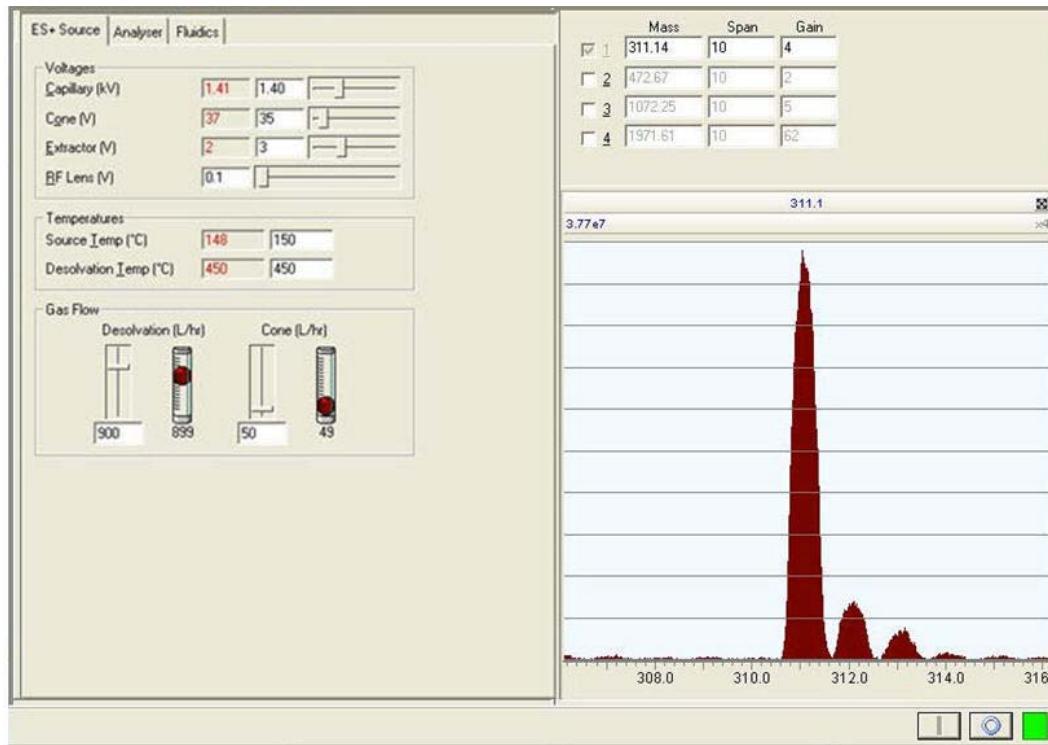


Figure 53 - Tune page

### Ion mode

Click the **Ion Mode** drop-down list to manually select ESI+, ESI-, APCI+, APCI-, ESCI+, or ESCI-.

**CAUTION:** *For APCI and ESCI, you must have the corona discharge pin installed in the TQ Detector source housing.*

## Source tab

1. In the Source tab, manually tune the cone, capillary, desolvation temperatures, and gas flows to optimize for LC flow rate and signal intensity while infusing.
2. In general, adjust the cone voltage and capillary to achieve proper mass signal intensity.
3. Set the gas flow and temperature settings as shown in Figure 54.

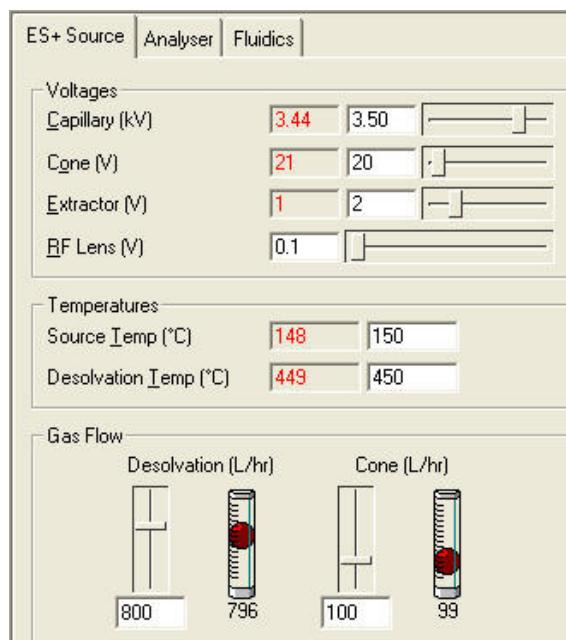


Figure 54 - Source tab

## Analyzer tab

1. In the Analyzer tab, manually change the mass resolution setup MRM signal optimization.
2. MRM signal optimization uses Entrance, Collision, and Exit for MRM transitions. Begin the collision energy settings for manual tuning between 3 to 40.

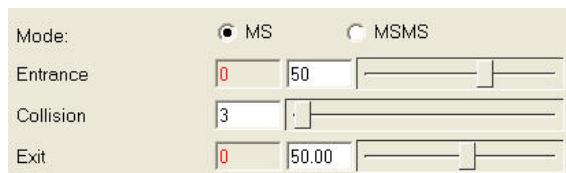


Figure 55 - Analyzer tab

3. In the Fluidics tab, manually infuse, purge, and refill the A and B reservoirs on the TQ Detector.

## Calibration

1. Manually calibrate the system (it is recommended to use IntelliStart to mass calibrate).

## Interscan setup

1. Click **Acquire > Interscan Setup** to set up the acquisition speed during time delays between one SIR and a second SIR. These must always be at the default settings.
2. Click **Setup > Interscan Setup** to ensure that the acquisition speeds are at the default settings as displayed in Figure 56.

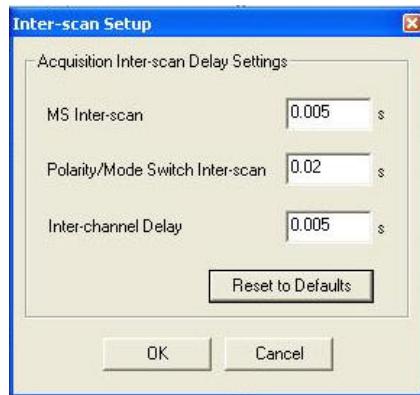


Figure 56 - Inter-scan Setup window

## Recommended start settings

Below are some initial start conditions for each of the ionization modes. These are not always optimal and you will need to optimize further.

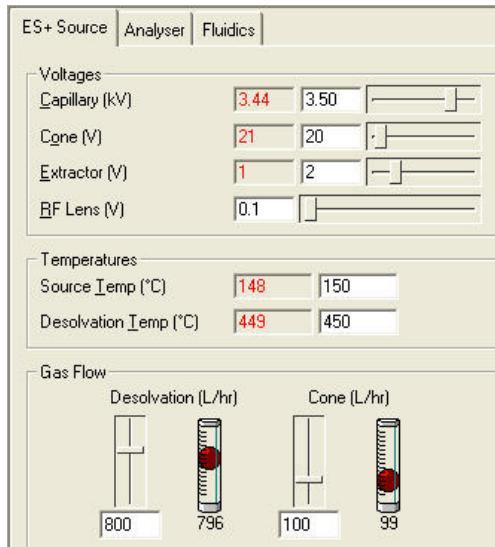


Figure 57 - General default settings for ESI+

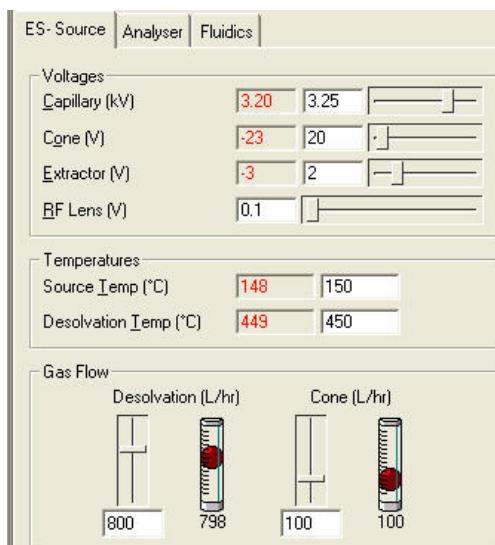


Figure 58 - General default settings for ESI-

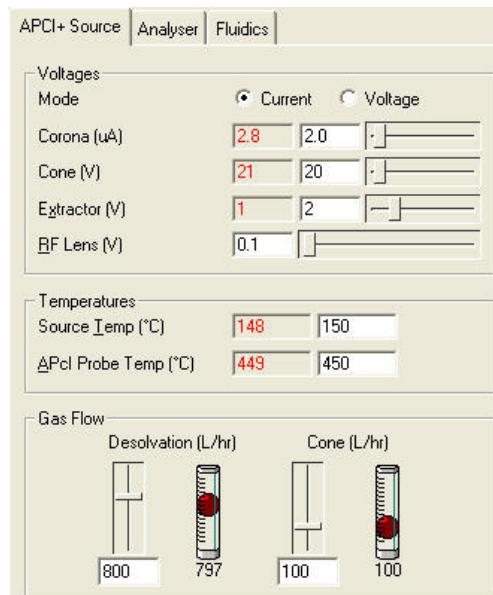


Figure 59 - General default settings for APCI+

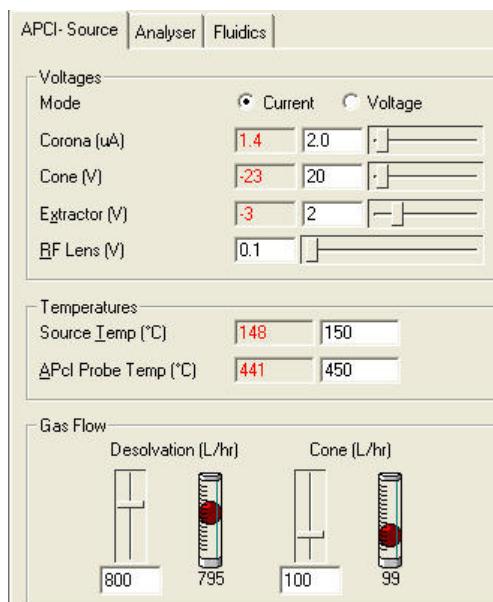


Figure 60 - General default settings for APCI-

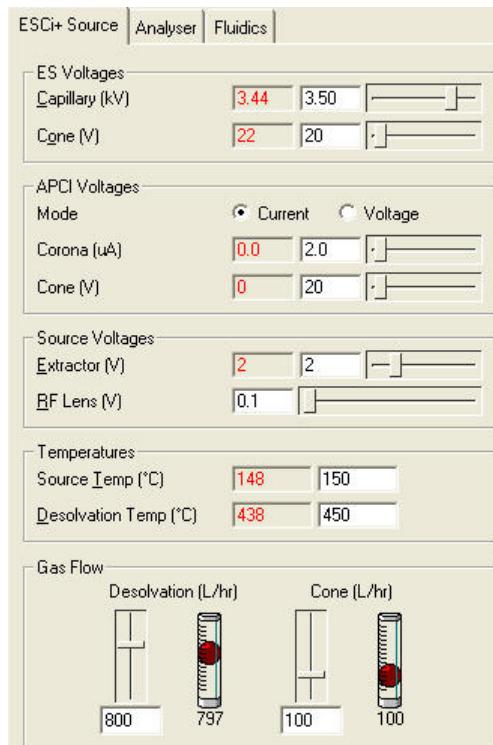


Figure 61 - General default settings for ESCI+

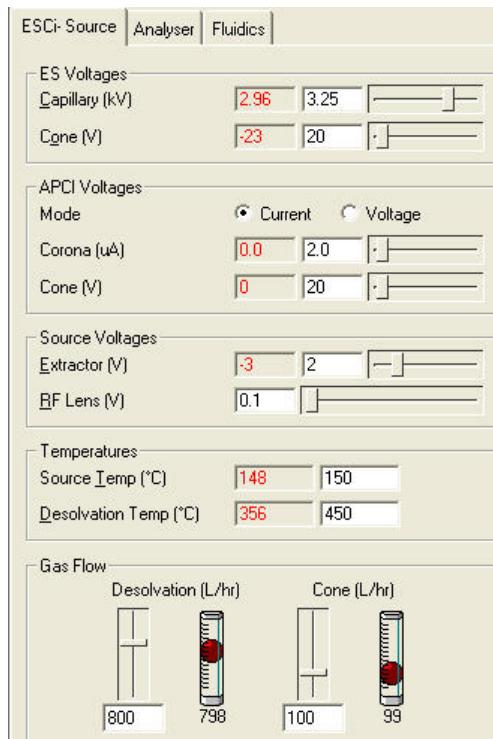


Figure 62 - General default settings for ESCI-

## Addendum C: Adjusting the TQ Detector gas and temperature settings from the Tune page

**CAUTION:** *Ensure that the TQ Detector is switched on before applying solvent flow from the ACQUITY binary solvent manager.*

1. In the Run Samples field of the installation project, right-click the **TQD control panel** and select in sequence, **API Gas on** and then **Operate**, to turn on the mass spectrometer.



Figure 63 - Run Samples field

**CAUTION:** *Before putting any flow to the TQ Detector, wait to make sure the following settings are displayed in the control panel (source temperature may take a while if the TQ Detector was in Standby mode).*

2. Ensure that the following settings are displayed before turning on solvent flow from the ACQUITY Binary Solvent Manager:
  - API Gas on (nitrogen) is selected and turned on
  - Source Temperature: ~150 °C
  - Desolvation Temperature Range: ~250 to 450 °C
  - Desolvation Gas: ~500 to 1000 L/Hr
  - Cone Gas: Optional
3. If the settings need to be changed, perform the following procedure to manually adjust the desolvation temperatures and gas flows.

## Manually adjusting the gas flows and desolvation temperatures

The TQ Detector's source temperature, desolvation temperature, and desolvation nitrogen gas must be set to the correct settings dependent upon flow rate.

IntelliStart uses a lookup table to adjust desolvation temperatures and gas flows for changes in LC flow rates.

1. In the Run Samples field of the installation project, right-click the **TQD control panel** and select in sequence, **API Gas on** and then **Operate**, to turn on the mass spectrometer.
2. Right-click the **TQD control panel** and click **Launch Instrument Console**.

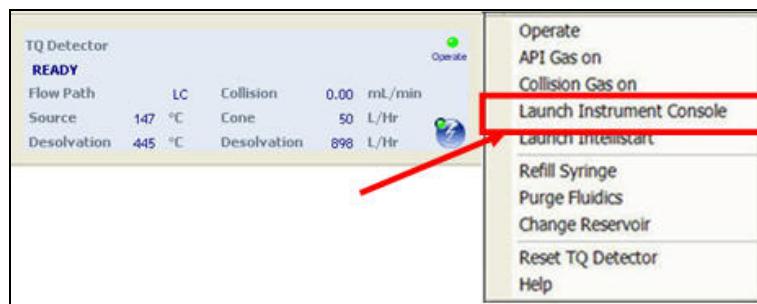


Figure 64 - Run Samples field

3. In the Instrument console, click the **Tune** icon .

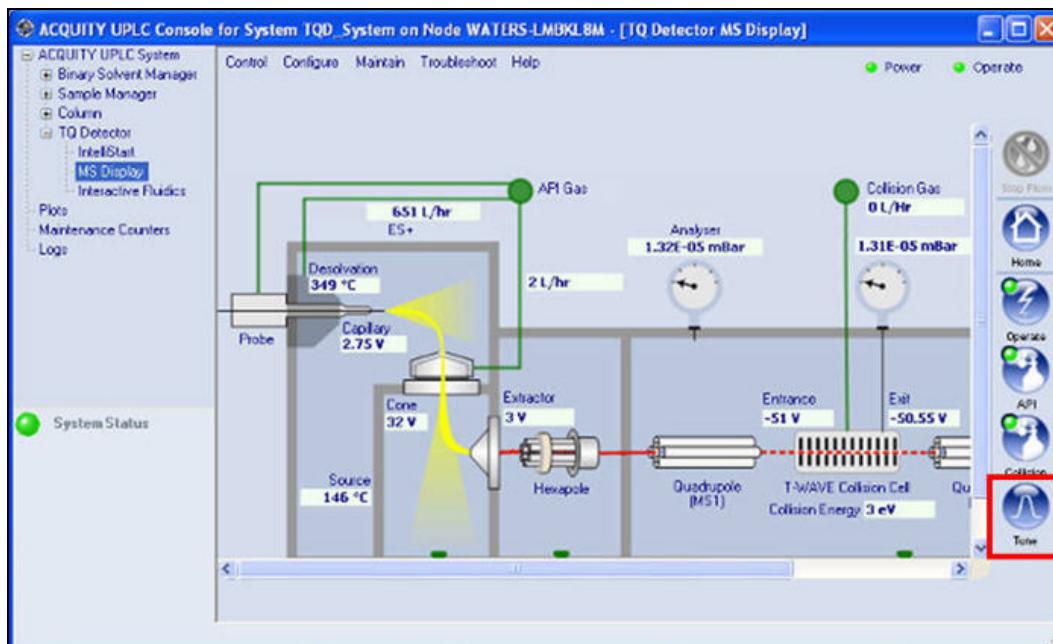


Figure 65 - Instrument console

4. In the Tune page, view the temperature and gas flow settings in the ES+ Source tab.

**NOTE:** The Source tab may be either ES-, ESCI+, ESCI-, APCI+, or APCI- depending on the Ion mode selected.

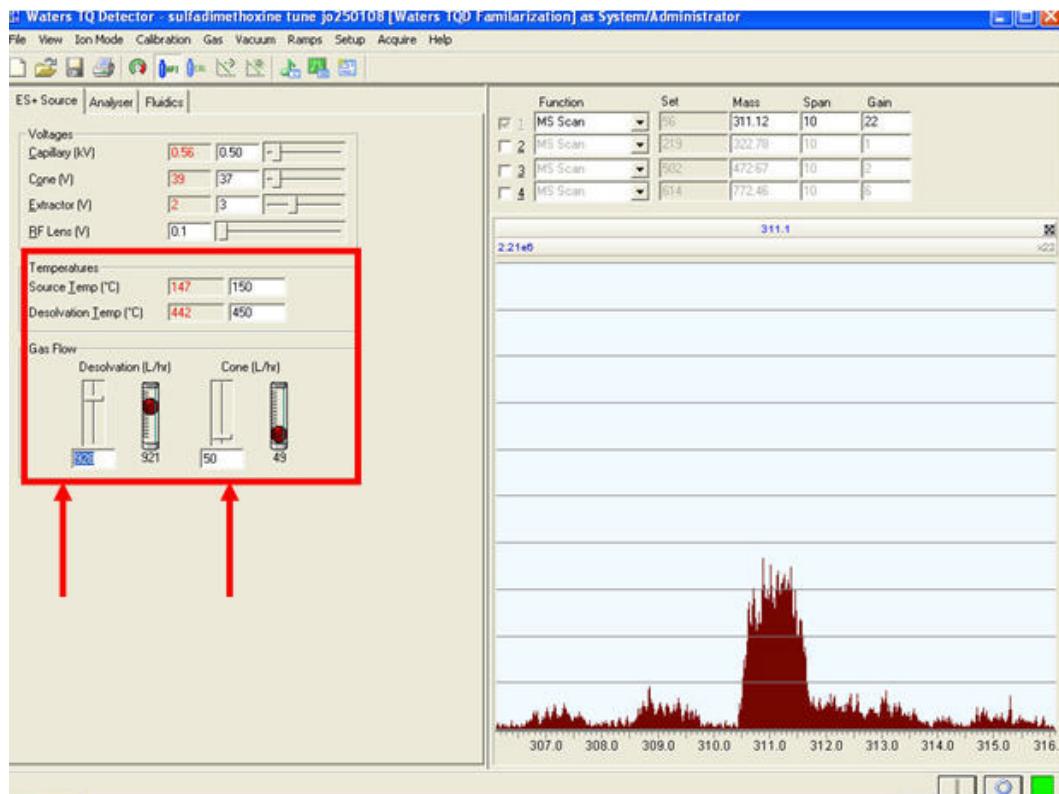


Figure 66 - Tune page settings

5. Adjust the source temperatures, desolvation temperatures, and desolvation nitrogen gas flow settings as shown in Table 8, by typing the correct flow and pressing **Enter**.

**Table 8: Gas flow and temperature settings**

| Flow Rate (mL/min) | Source Temp (°C) | Desolvation Temp (°C) | Desolvation Gas Flow (μL/min) |
|--------------------|------------------|-----------------------|-------------------------------|
| 0.000 to 0.100     | 150              | 250                   | 500                           |
| 0.101 to 0.300     | 150              | 350                   | 600                           |
| 0.301 to 0.500     | 150              | 400                   | 800                           |
| >0.500             | 150              | 450                   | 900                           |

6. Click **File > Save Tune**, and then **File > Exit**.  
 7. The gas flow setting is now set correctly.

**2**

## SIR/MRM Processing with MS External Standards

This generic procedure describes how to process MRM data for external standard quantitation using the ACQUITY TQD system with Empower. The same process can be used for SIR data from the ACQUITY SQD or Alliance 3100 systems. The procedure guides the operator through the following topics:

- Analyzing MRM data in Review
- Creating a basic processing method using the wizard with MRM external standards
- Batch processing the injections and reviewing the results
- Viewing basic individual and summary report methods

**NOTE:** *This data was acquired using a Waters TQ Detector; however, the technique for data processing for SIR external standards on other MS instruments, such as the SQ Detector or 3100 is the same as this procedure.*

The procedure consists of the following eight sections:

|   |      |
|---|------|
| Empower login and restoring the training project .....                  | 2-1  |
| Selecting the sample set and navigating the Review window .....         | 2-3  |
| Building a processing method for MS external standards.....             | 2-5  |
| Manually editing the processing method .....                            | 2-11 |
| Batch processing the 3 Sulfa MRM Ext sample set.....                    | 2-16 |
| Viewing the processed result sets and results in the Project view ..... | 2-17 |
| Viewing the processed data and calibration curves in Review.....        | 2-17 |
| Previewing individual results using Preview/Publisher .....             | 2-20 |

### Empower login and restoring the training project

1. Double-click the **Empower** icon .
2. Type your user name as **System**, and password as **Manager** (or the user name and password assigned to you).

3. Click **Advanced**.



Figure 67 - Empower Login window

4. Click the **User Type** drop-down list, and select **Administrator** (or the user type assigned to you).
5. Select the **Pro Interface** check box, and then click **OK**.

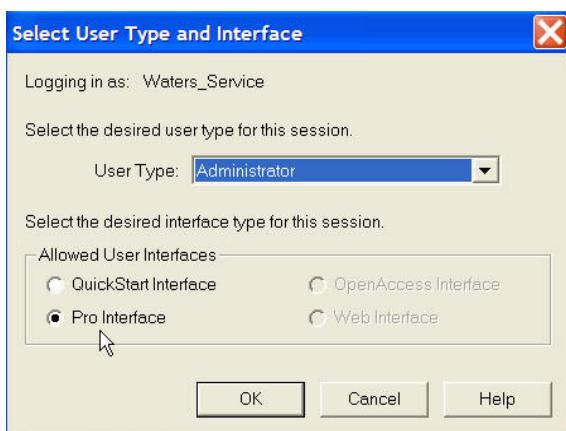


Figure 68 - Advanced dialog box

6. Click the **Configure System** icon  to access the Empower Configuration Manager.
7. Right-click the **Projects folder**, and select **Restore Project(s)**.
8. Perform the following steps if loading the project for the first time:
  - a. Load the *Empower Familiarization CD* in the disk drive, and then click **Browse** to select the location of the "TQD, SQD, or 3100 Empower Familiarization" project.
  - b. Leave the Parent Project as the default, unless requested to put it in another location by your Empower system administrator.
  - c. Click **Next**, select the location of the parent project, and then click **OK**.
  - d. Leave the name of the project as displayed, and then click **Next** to restore the project.
  - e. Click **OK** after the project restores.
  - f. Click **File > Exit** to quit the Empower Configuration Manager.

## Selecting the sample set and navigating the Review window

1. Click the **Browse Project** icon 
2. Select the appropriate **TQD, SQD ,3100 Empower Familiarization** project, and then click **OK**.

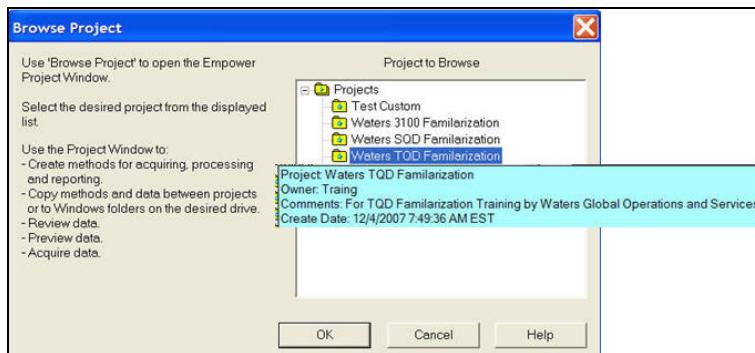


Figure 69 - Browse Project window

3. Click the **Sample Sets** tab.
4. Click the **Filter By** drop-down list, and select **Sample Set Information**.
5. View the additional information displayed about the sample set.
6. Highlight the **3 Sulfas MRM Ext** sample set, and then click the **Review** icon  or right-click and select **Review** to display the Review window.
7. Click **View > 3D Format Review window** to view the following information:
  - Complete sample set information on the left-hand side
  - Chromatogram in the center
  - Spectrum and spectrum information on the right-hand side

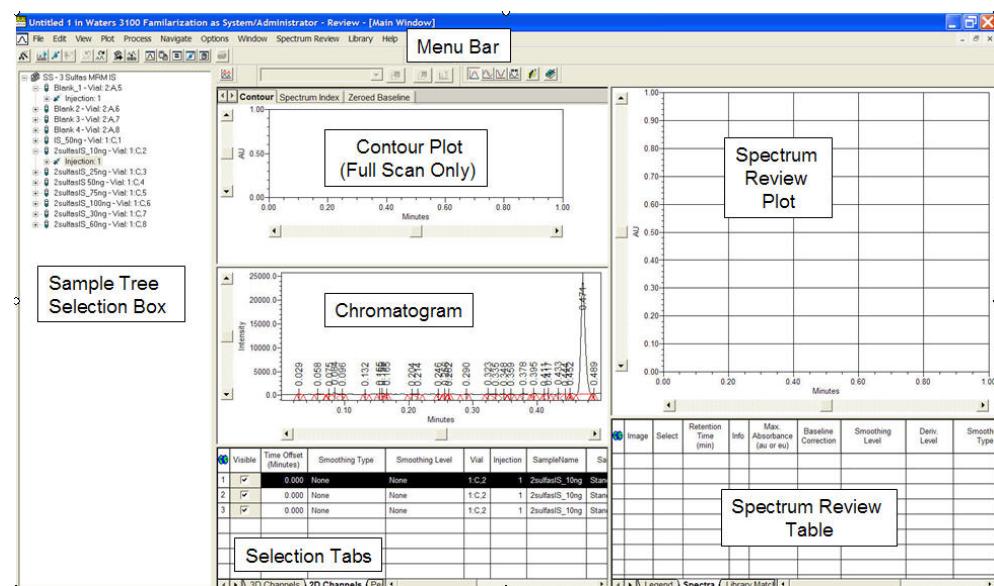
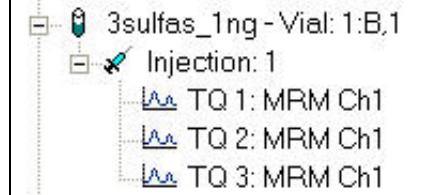


Figure 70 - Review window

8. Click **View > 2D Layout** to view the chromatogram only.
  9. In the Selection Tabs field, select the **2D Channels** tab to display the individual MRM channels of the selected sample.

**NOTE:** As a default the first collected sample's MRM Channel is displayed.

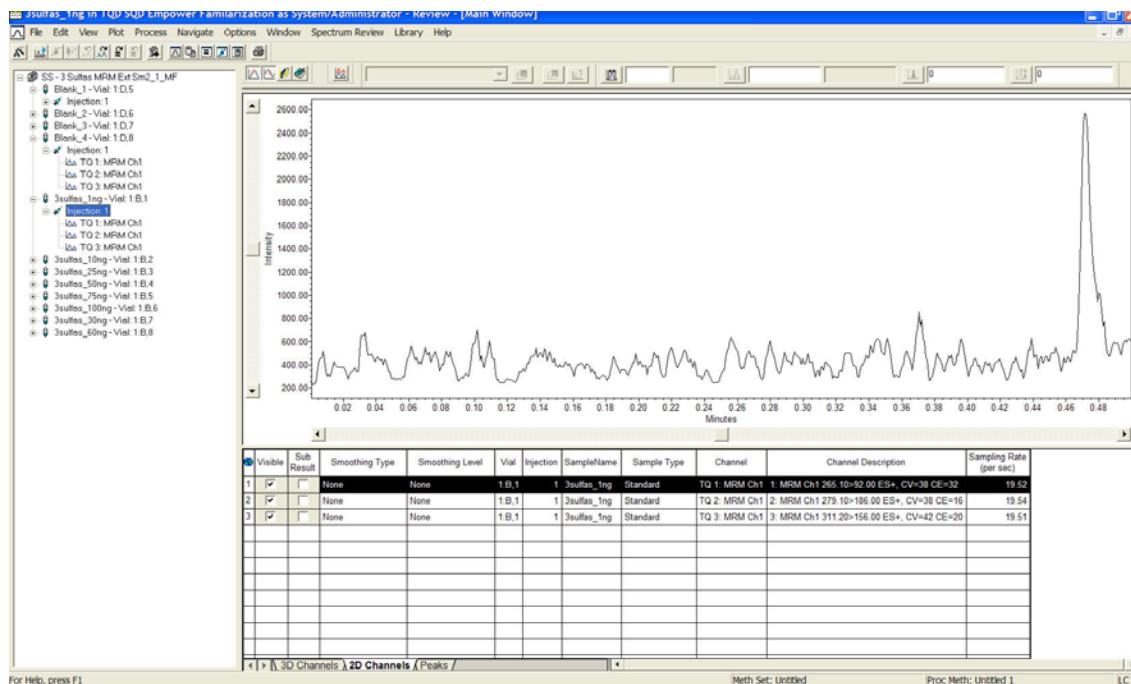
10. In the Sample Tree selection box, select the lowest concentration MRM injection standard **3sulfas\_1ng-Vial: 1:B,1**, and then click + to expand the information about the sample.
  11. Highlight **Injection: 1**, and then click + to display all the MRM data channels for the standard.



*Figure 71- MRM data channels*

**NOTE:** *Channels are also displayed in the 2D Channels tab below the chromatogram.*

The 3sulfas\_1ng standard is displayed with the TQD MRM Ch1 channel highlighted and the 2D chromatogram displayed (Figure 72). The chromatogram may be displayed integrated as well as additional channel information displayed in the table.



*Figure 72 - Displayed channels and chromatogram*

## Building a processing method for MS external standards

1. Click the **Processing Method Wizard** icon .
2. Select the **Create a New Processing Method** check box, and then click **OK**.
3. Click the **Processing type** drop-down list, and select **MS**.
4. Click the **Integration Algorithm** drop-down list, and select **ApexTrack**.
5. Select the **Use Processing Method Wizard** check box, and then click **OK**.

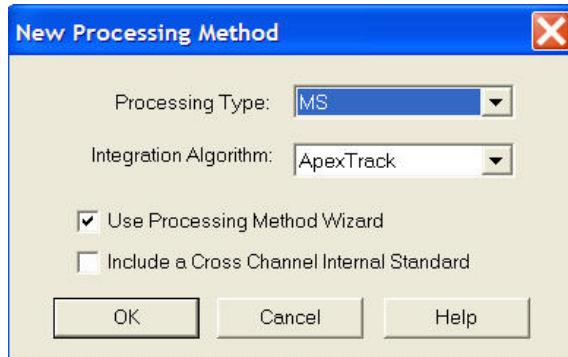


Figure 73 - New Processing Method window

6. For chromatographic smoothing of all MRM channels, click the **Smoothing Type** drop-down list and select **Mean**, and then click the **Smoothing Level** drop-down list and select **7**.

**NOTE:** *It is recommended for MS to have a smooth applied by the processing method. General recommendations are Mean, Smoothing Type, and a Smoothing Level of 5 to 15. Time offset is used to align different detector signals that are in series to each other (i.e. TQD and PDA).*

7. Ensure that the **Check here to have these empty parameters...** check box is unchecked, and then click **Next**.



Figure 74 - Time Offset and Smoothing window

8. Ensure that the **Start** and **End** boxes are empty, and then click **Next**.

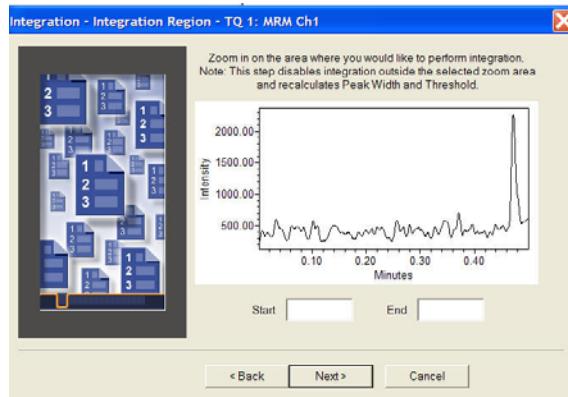


Figure 75 - Integration Region window

**NOTE:** *Start and End must not be used for SIR/MRM processing methods, as the data collection could be staggered for different SIR/MRM channels that are not displayed in the wizard.*

9. The chromatogram will be displayed with many peaks integrated. Ensure that the **Clear Peak Width and Threshold** settings are unchecked, and then click **Next**.

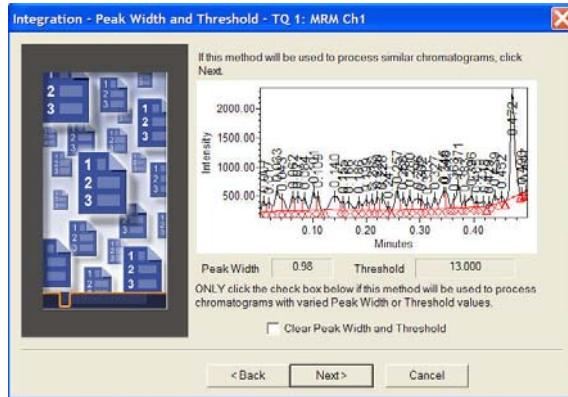


Figure 76 - Peak Width Threshold window

10. Click inside the peak at 0.472 minutes, and then select the **Minimum Height** check box.
11. Change the peak height values from 1703 to 800, and then click **Next**.

**NOTE:** *Default minimum height uses 90% of the peak height for peak rejection. In general it is recommended to set minimum height at ~10 to 50% of peak height.*

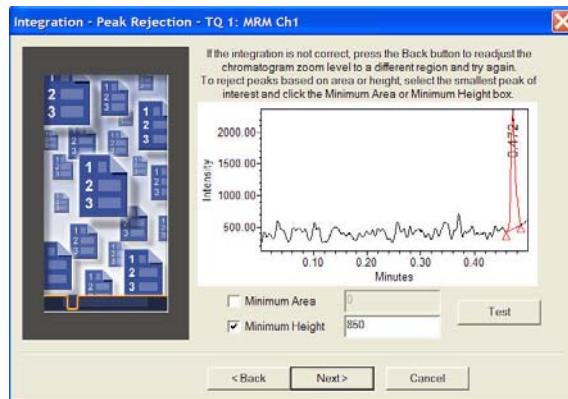


Figure 77 - Peak Rejection window

12. Click the drop-down lists, and select the following settings (Figure 78):
  - Select the method of quantitation **Area**
  - Component information **Amount**
  - Select the calibration curve fit type **Linear**
13. Click **Next**.

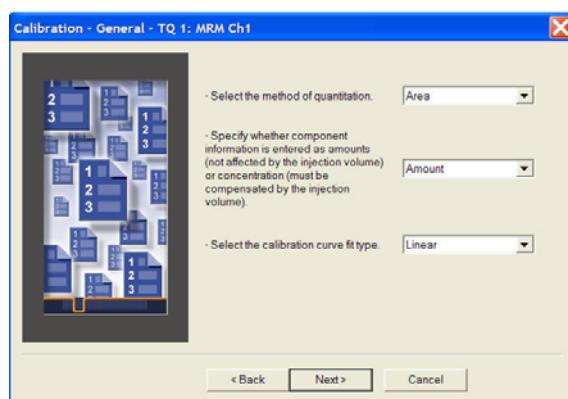


Figure 78 - Calibration window

14. Click **Yes** to use channel names to perform Cross Channel Internal Standard processing (Figure 79).

**NOTE:** *This is used to process the multiple MRM chromatograms as a single method.*

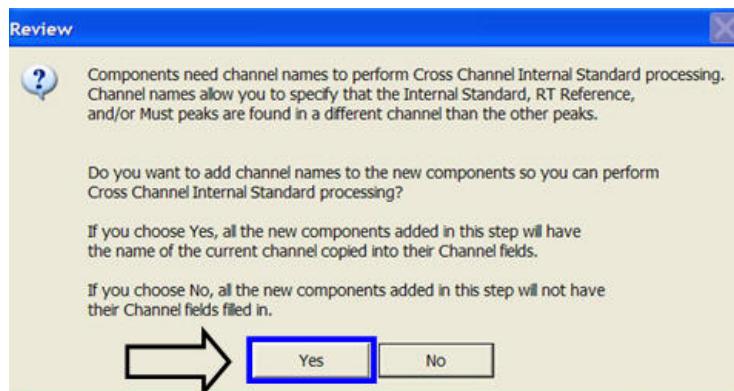


Figure 79 - Choosing channel names

15. In the Names and Retention Times window, click inside the Peak1 TQ 1: MRM Ch1, click the drop-down list, and then select **Sulfamerazine** (Figure 80).

**NOTE:** *The component names match the names entered in the standard Amounts from Run Samples. The final view for this dialog box should match the one displayed in Figure 80.*

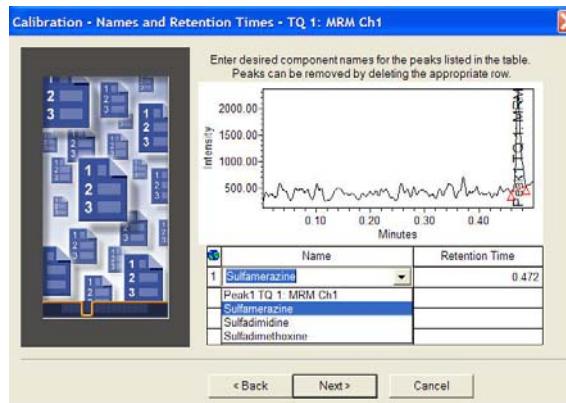


Figure 80 - Calibration window

16. Click **Next**.
17. Since multiple standard concentrations were acquired, skip the default amounts section, and then click **Next**.
18. Ensure that the **External Standard Calibration** check box is checked, and then click **Next**.

19. Ensure that the **MS Match Library** check box is unchecked, and then click **Next**.

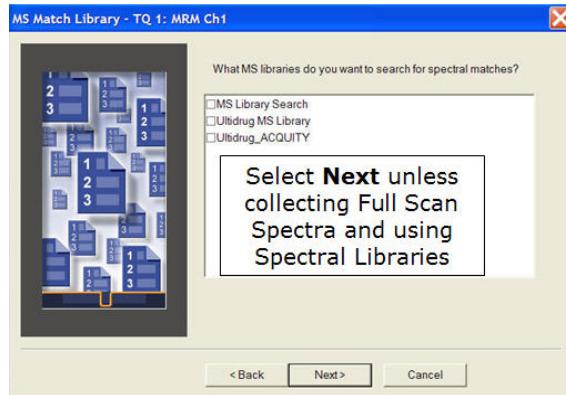


Figure 81 - MS Match Library window

**NOTE:** Libraries may be displayed from other scan sample sets. Since this is MRM data do not select a MS Library.

20. Type the processing method name as **3Sulfas MRM EX**, and then click **Finish**.

### Adding the second MRM chromatogram channel

1. In the 2D Channels Table field, highlight the **TQ2: MRM Ch1** row to add another MRM chromatogram channel to the processing method.
2. Select the channel as shown in Figure 82.

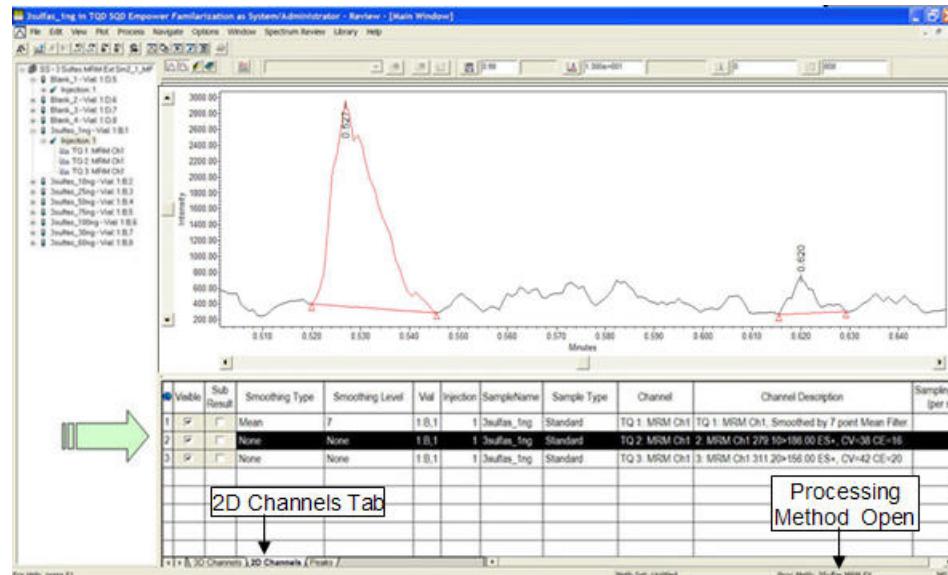


Figure 82 - 2D Channels Table tab

3. Click the **Processing Method Wizard** icon .
4. Select **Edit an Existing Processing Method**, and then click **OK**.

**NOTE:** *The processing method last opened will be displayed in the status bar at the bottom right-hand side of the Review window.*

5. Complete the wizard using the settings configured from the first edit of the processing method.
6. When prompted, ensure that **Yes** is selected to use channel names to perform Cross Channel Internal Standard processing.
7. In the Names and Retention Times window, click inside the Peak2 TQ 2: MRM Ch1, click the drop-down list, and then select **Sulfadimidine** (Figure 83).
8. Click **Next**.

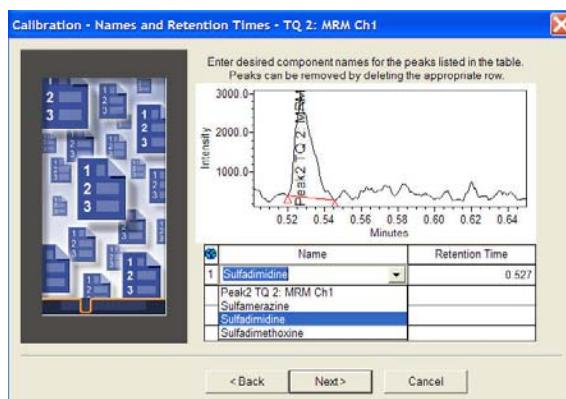


Figure 83 - Names and Retention Times window

9. Complete the Processing Method wizard setting the following parameters:
  - Calibration type **External**
  - MS Match Library **No**
10. Click **Finish**.

**NOTE:** *The second MRM chromatogram will be added to the processing method with the chromatogram displayed.*

### Adding the third MRM chromatogram channel

1. In the Chromatogram Table field, highlight the **TQ3: MRM Ch1** row to add another MRM chromatogram channel to the processing method.
2. Repeat the process of editing the processing method to add the third MRM chromatogram to the processing method.
3. Select the third component **Sulfadimethoxine**.

**NOTE:** *To add more MRMs to the processing method, repeat steps 1 to 3 or manually edit the processing method as described in the following section "Manually editing the processing method".*

## Manually editing the processing method

1. Click the **Processing Method** icon .
2. Click the **Components** tab, and view and edit the columns described in this section.
3. In the sulfamerazine row, click the **Channel** column to identify which MRM chromatogram is identified with the component.
4. Select the correct MRM from the drop-down list.

**NOTE:** *This may be used to identify MRM channels without using the processing wizard.*

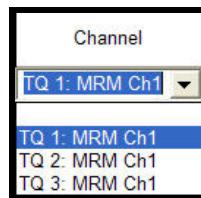


Figure 84 - Channel column drop-down list

5. In the sulfamerazine row, click the **Peak Match** column and then select **Greatest Area** from the drop-down list.
6. Use Ctrl-D to copy down.

**NOTE:** *This can be used when multiple peaks are within the same MRM window.*

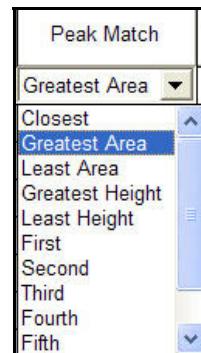


Figure 85 - Peak Match column drop-down list

7. In the sulfamerazine row, click the **Weighting** column and then select **1/X** from the drop-down list.
8. Use Ctrl-D to copy down.

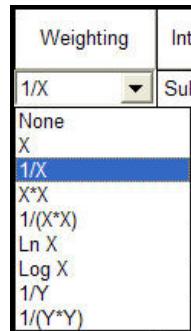


Figure 86 - Weighting column drop-down list

**NOTE:** MRM or SIR data from MS detectors frequently use a weighting value of  $1/X$  or  $1/(X^*X)$  to get a better fit at the lower end of the concentration curve.

9. Click the **Auto Peak Label** button to label the Peaks as 1, 2, 3.

| Name               | Peak Label | Retention Time (min) | RT Window (min) | Channel       | Peak Match    | Y Value | X Value | Fit    | Weighting | Internal Std | RT Reference | Rel RT Reference |
|--------------------|------------|----------------------|-----------------|---------------|---------------|---------|---------|--------|-----------|--------------|--------------|------------------|
| 1 Sulfamerazine    | 1          | 0.472                | 0.024           | TQ 1. MRM Ch1 | Greatest Area | Area    | Amount  | Linear | 1/X       |              |              |                  |
| 2 Sulfadimidine    | 2          | 0.527                | 0.026           | TQ 2. MRM Ch1 | Greatest Area | Area    | Amount  | Linear | 1/X       |              |              |                  |
| 3 Sulfadimethoxine | 3          | 0.716                | 0.036           | TQ 3. MRM Ch1 | Greatest Area | Area    | Amount  | Linear | 1/X       |              |              |                  |

Figure 87 - Example Processing Methods Component table

10. Click the **Noise and Drift** tab and select **Calculate Detector Noise and Drift**.
11. Set a Start Time of **0.050 min** and a Stop Time of **0.4 min**.

Figure 88 - Noise and Drift parameters

**NOTE:** Noise and drift is calculated for each data channel collected for the selected start and end time region. If a data channel is not collected during the time region, then a noise calculation is not calculated.

12. View the other processing tabs and variables as required, and then click **File > Save Processing Method** to save the method file.

## Overlay MRM chromatograms and save as a method set

1. Click the **Review Main Window** icon .
2. To view both the chromatogram and processing method, click **View > Processing Method Layout**.
3. Click the **Overlay** icon  to view all three MRM chromatograms together.
4. Ensure that the Visible column check box is selected on the MRM chromatograms you want displayed.

|   | Visible                             | Time Offset (Minutes) | Smoothing Type | Smoothing Level |
|---|-------------------------------------|-----------------------|----------------|-----------------|
| 1 | <input checked="" type="checkbox"/> | 0.000                 | Mean           | 7               |
| 2 | <input checked="" type="checkbox"/> | 0.000                 | Mean           | 7               |
| 3 | <input checked="" type="checkbox"/> | 0.000                 | Mean           | 7               |

Figure 89 - Visible column

5. Perform the following steps to display the component names for all the MRMs:
  - a. Right-click the chromatogram and select **Properties...**
  - b. Click the **Overlay** tab.
  - c. In the Chromatogram Annotation, select the **All Chroms in Overlay** check box.
  - d. Click **OK**.

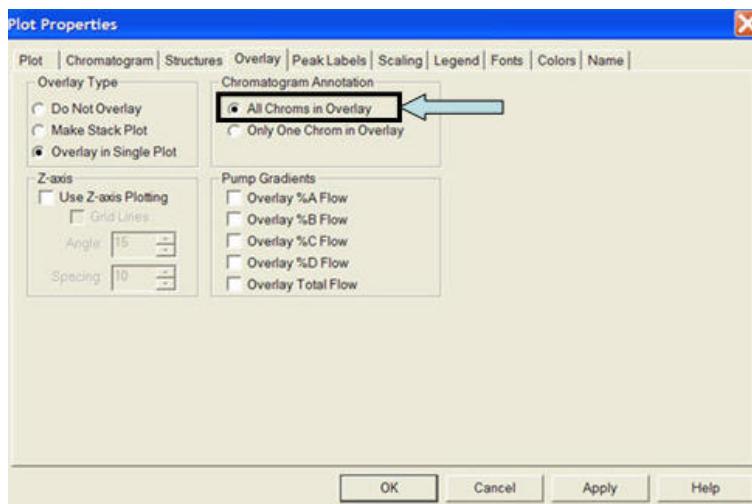


Figure 90 - Plot Properties overlay dialog box

6. Click **File > Save preferences** to save the default settings used in the Review window.

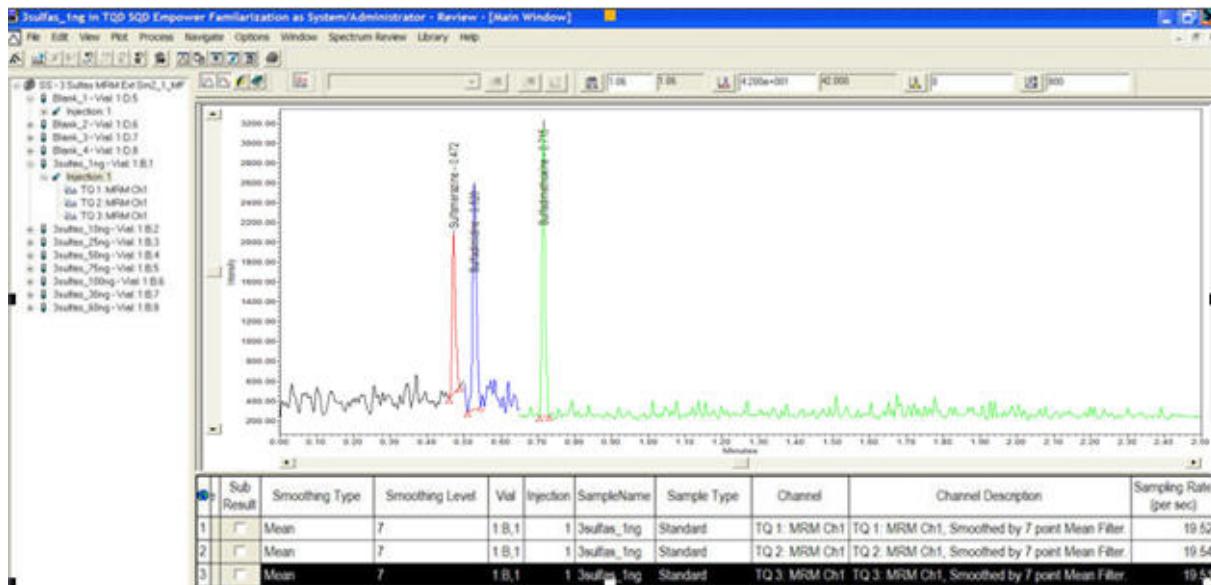


Figure 91 - Example Chromatogram overlay

7. Click the **Peaks** tab at the bottom of the Review window, and then view the result information.

| # | Name          | Retention Time (min) | Area ( $\mu\text{V}^*\text{sec}$ ) | % Area | Height ( $\mu\text{V}$ ) | Int Type | Amount | Units | Peak Type | Start Time (min) | End Time (min) | Baseline Start (min) | Baseline End (min) | Slope ( $\mu\text{V/sec}$ ) | Offset ( $\mu\text{V}$ ) | Points Across Peak | Width (sec) | ARTsec    |
|---|---------------|----------------------|------------------------------------|--------|--------------------------|----------|--------|-------|-----------|------------------|----------------|----------------------|--------------------|-----------------------------|--------------------------|--------------------|-------------|-----------|
| 1 | Sulfamerazine | 0.472                | 1024                               | 100.00 | 1618                     | BB       | 1.000  | ng/ml | Found     | 0.460            | 0.489          | 0.460                | 0.489              | 3.976273e+003               | -1.401148e+003           | 34                 | 1.742       | 28.332740 |

Figure 92 - Peaks Tab table

8. Right-click the **World** icon to set the table properties.

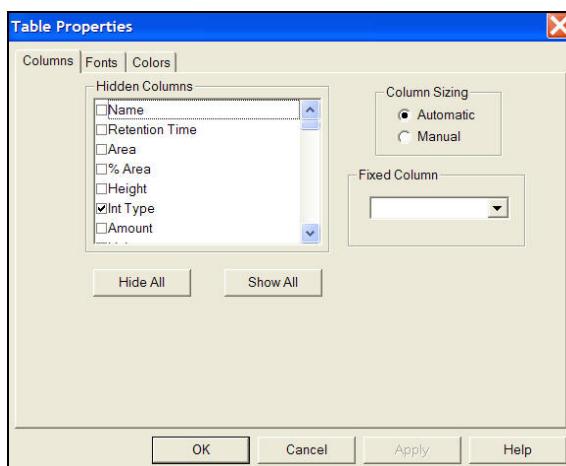


Figure 93 - Peaks Table properties

9. Click **Hide All**, scroll down to view all the column names and then uncheck the boxes as shown in Figure 94 to display only the desired columns.

10. Click **OK**.

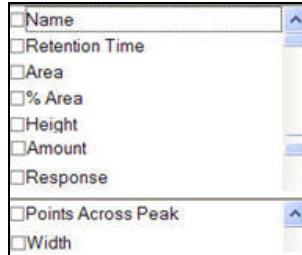


Figure 94 - Peaks Table Columns to be displayed

11. Select the check boxes for the columns that are required to be displayed, and then click **File > Save Preferences**.

|   | Name          | Retention Time (min) | Area ( $\mu\text{V}^{\text{sec}}$ ) | % Area | Height ( $\mu\text{V}$ ) | Amount | Response  | Points Across Peak | Width (sec) |
|---|---------------|----------------------|-------------------------------------|--------|--------------------------|--------|-----------|--------------------|-------------|
| 1 | Sulfamerazine | 0.472                | 12791                               | 100.00 | 22781                    | 10.000 | 12790.770 | 39                 | 1.998       |

Figure 95 - Peaks Table Columns to be displayed

12. Click the **Method Set** icon

13. Click the **Default Report Method** drop-down list and select **TQD Full Report**.

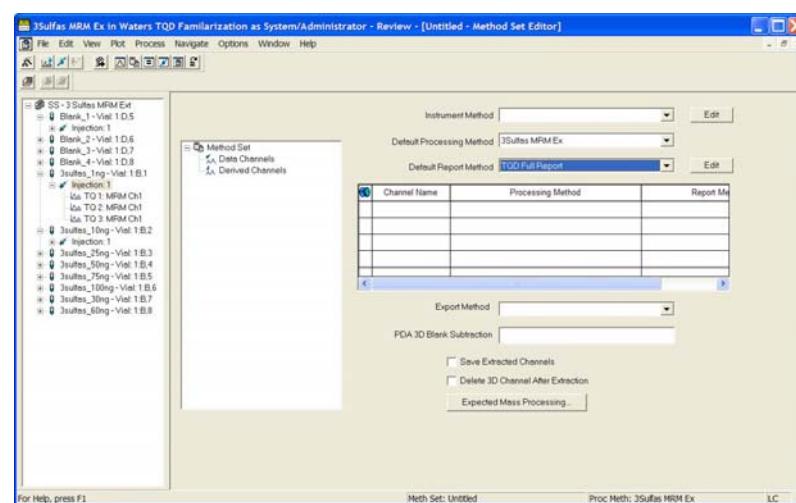


Figure 96 - Method set TQD Full Report method

**NOTE:** The method set window can be used to add additional processing and report functions to a method set for batch processing.

14. Click **File > Save As > Method Set**.

15. Save the method set as **3 Sulfas MRM Ex MS**.

16. Click the **Review Main Window** icon

17. Click **File > Exit** to quit the Review Main window.

## Batch processing the 3 Sulfas MRM Ext sample set

1. Click the **Sample Set** tab.
2. Click the **Filter By:** drop-down list and select **Sample set Information**.
3. Highlight the **3 Sulfas MRM Ext** sample set, and click the **Process** icon  or right-click and select **Process**.
4. In the Processing field, select the **Use specified method set** check box.
5. Click the drop-down list and select **3 Sulfas MRM Ex MS**.
6. Select the **Clear Calibration** check box.
7. Click the **How:** drop-down list and select **Calibrate and Quantitate**.
8. Leave the remaining settings as the default, and then click **OK** to process the data and create the results.

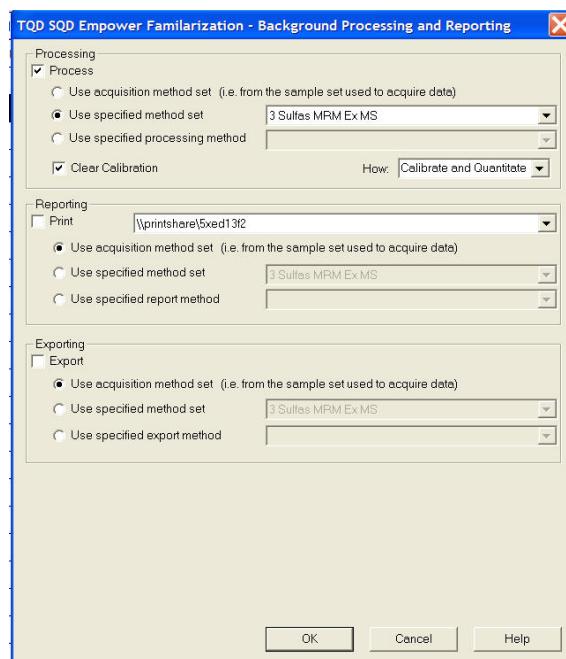


Figure 97 - Background Processing and Reporting window

**NOTE:** For SIR/MRM external standard data, it is best to process the data from the "Use acquisition method set" or "Use specified method set" check box, not the "Use specified processing method" check box. This is because some calculations use inter-channel information, which cannot be performed from the specified processing method selection.

## Viewing the processed result sets and results in the Project view

1. Click the **Result Sets** tab.
2. Click the **Filter By:** drop-down list and select **Processed Today** to show only today's results.
3. If the results are not displayed, click the **Update** button.
4. Highlight the **3 Sulfas MRM Ext** result set, right-click and select **View As Results** to show only the processed standard and unknown results from the processed sample set.
5. In the Results field, click the **Filter By:** drop-down list and select **3 Sulfas MRM Ext SM2\_1 Latest** to view only the unknown samples processed with this data set.
6. Highlight all six unknown MRMs by clicking on the **World** icon  to select all the results, and then click the **Review** icon .

## Viewing the processed data and calibration curves in Review

The Review window displays all three MRMs from the 3 Sulfas 30 ng sample and the sulfamerazine sample result peaks table displayed.

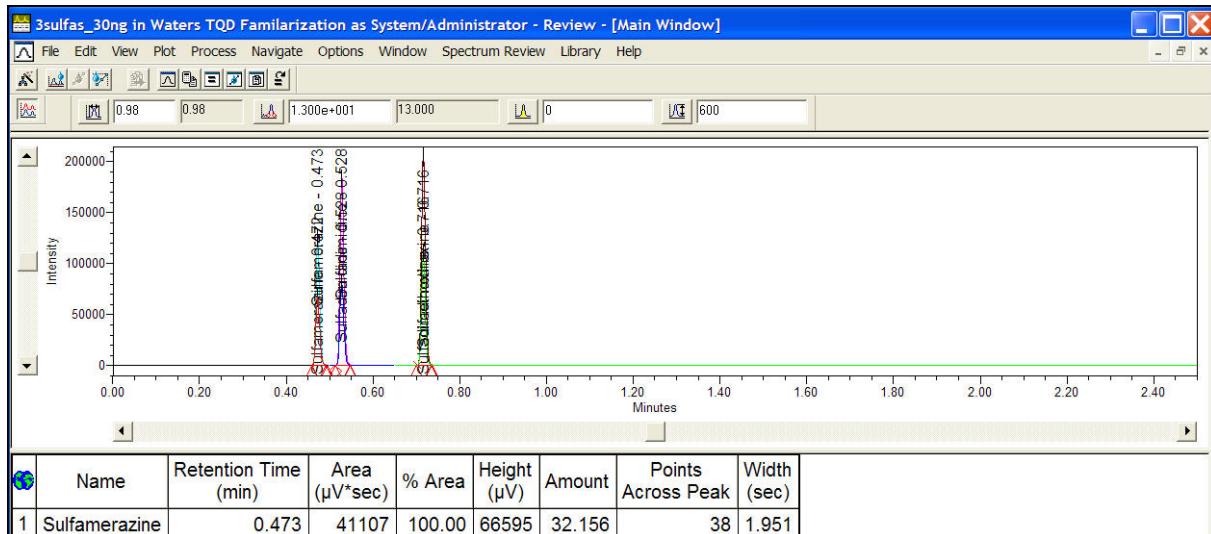


Figure 98 - Overlay of 3 Sulfas 30 ng MRMs

1. Click the **Overlay** icon  a second time to display only the current MRM viewed, "Sulfamerazine".
2. If the peaks table is not displayed, click the **Peaks** tab to display the results.

**NOTE:** *The description of the displayed sample is shown on the top of the Review Main window.*



Figure 99 - Sample description

3. Toggle between the **Previous Result** icon  or the **Next Result** icon  to view results MRM processed chromatogram and peak information.
4. Click the **Results** icon  located on the top right-hand side of the main icon display for a complete report of the details about the processed result.

**NOTE:** *All details of the result are displayed, including peaks information, chromatogram inforamtion, and calibration details.*

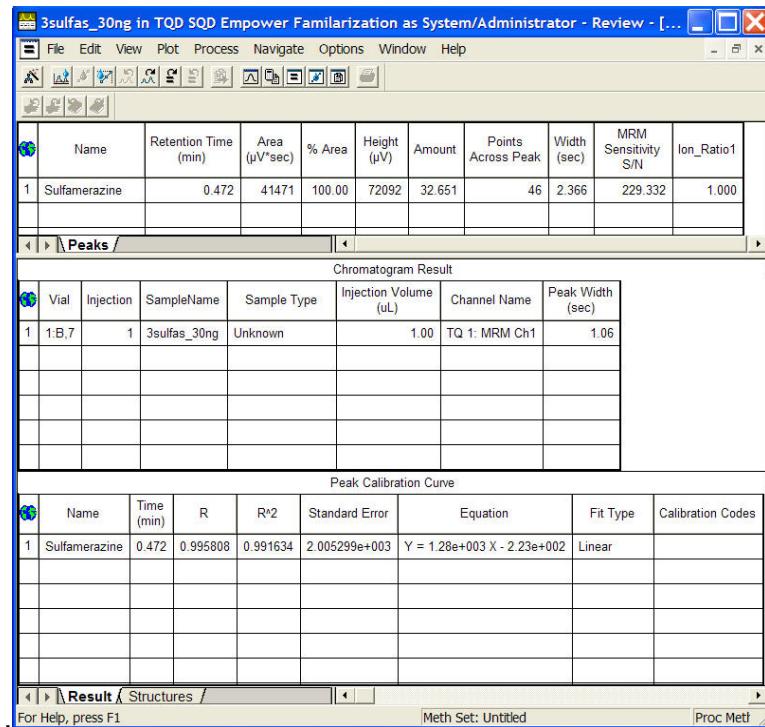


Figure 100 - Review window

5. Click the **Calibration Curve** icon  to view the first calibration curve.
6. Click the **Previous Result** icon  or the **Next Result** icon  to view the next components calibration curve.

**NOTE:** *The compound selected is displayed in the Compound selection box area of the Calibration Curve window.*

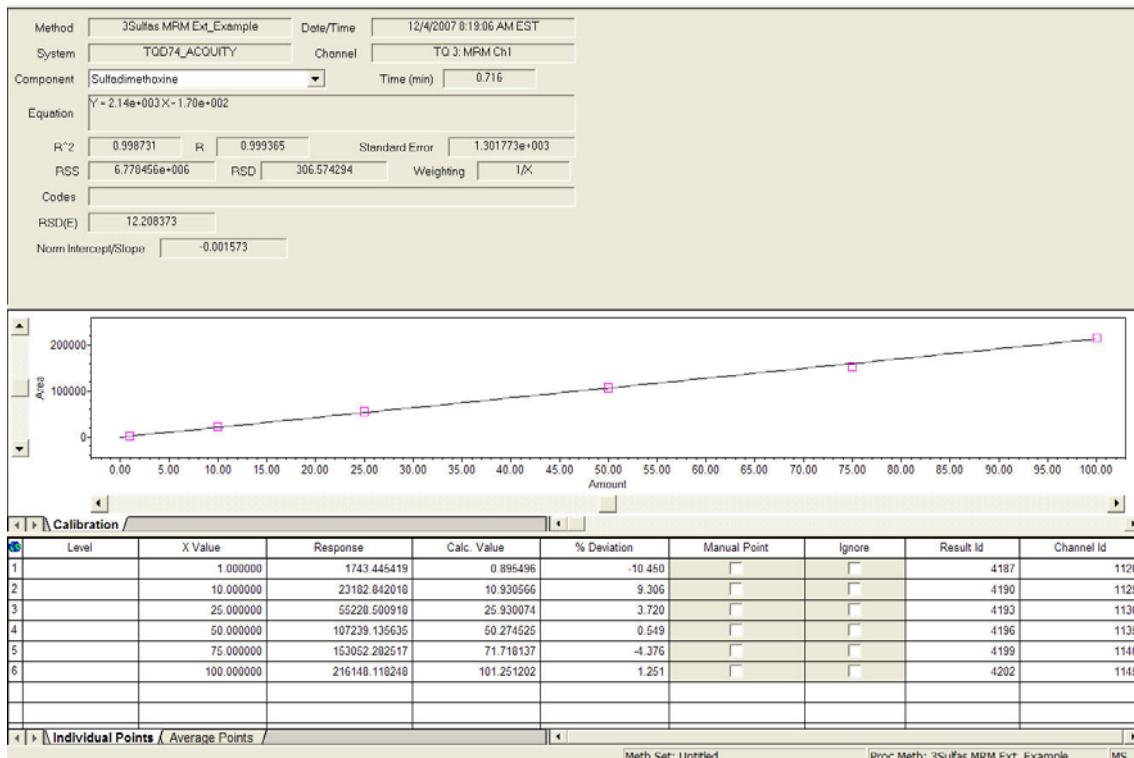


Figure 101 - Calibration Curve window

7. Click the **Review Main Window** icon , and then click **File > Exit from Review**.

## Previewing individual results using Preview/Publisher

The final section demonstrates how to review the processed data using reports through the Preview Publisher function. Reports have two modes, Individual and Summary.

### Individual unknown reports

1. Click the **Results** tab.
2. Click the **Filter By:** drop-down list, and select **3 Sulfas MRM Ext SM2\_1 Latest**.
3. Highlight all six unknown MRMs by clicking on the **World** icon  to select all the results, and then click the **Preview/Publisher** icon .
4. In the Preview window, Open Report Method dialog box; select the **Use the following Report Method** check box, select **TQD MRM Unknown Injections** from the drop-down list, and then click **OK**.

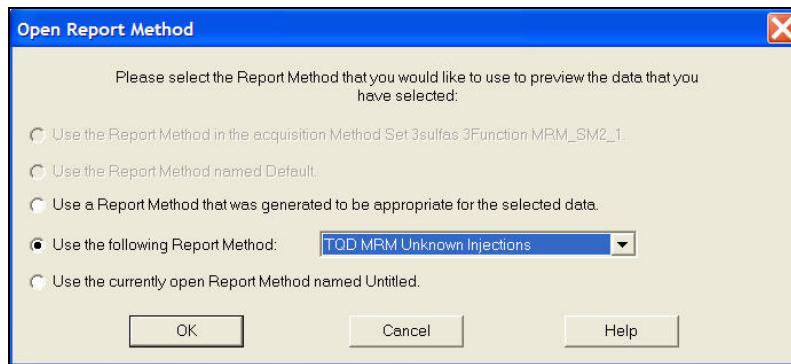


Figure 102 - Open Report Method dialog box

5. View the two reports for the two unknown samples, and then use the **Next Report** icon  or the **Previous Report** icon  to view the next sample.
6. Click the **Report** icon , and click **Save Report** to save the report as a PDF file.

**NOTE:** An example report is shown on page 2-22.

7. Click the **Open** icon , and then select the **TQD Full Report** to view the calibration curve plots and tables, and the MS calibration and tune conditions from the acquired data.
8. Click the **Next Page** icon  and the **Back Page** icon  to view all the pages of the report.
9. In the Report Publisher, click **Close > File Exit**.
10. After closing the Preview window, the Report Publisher is displayed for advanced editing of reports.
11. Click **File > Exit** to close the Report Publisher.

## Peaks summary report

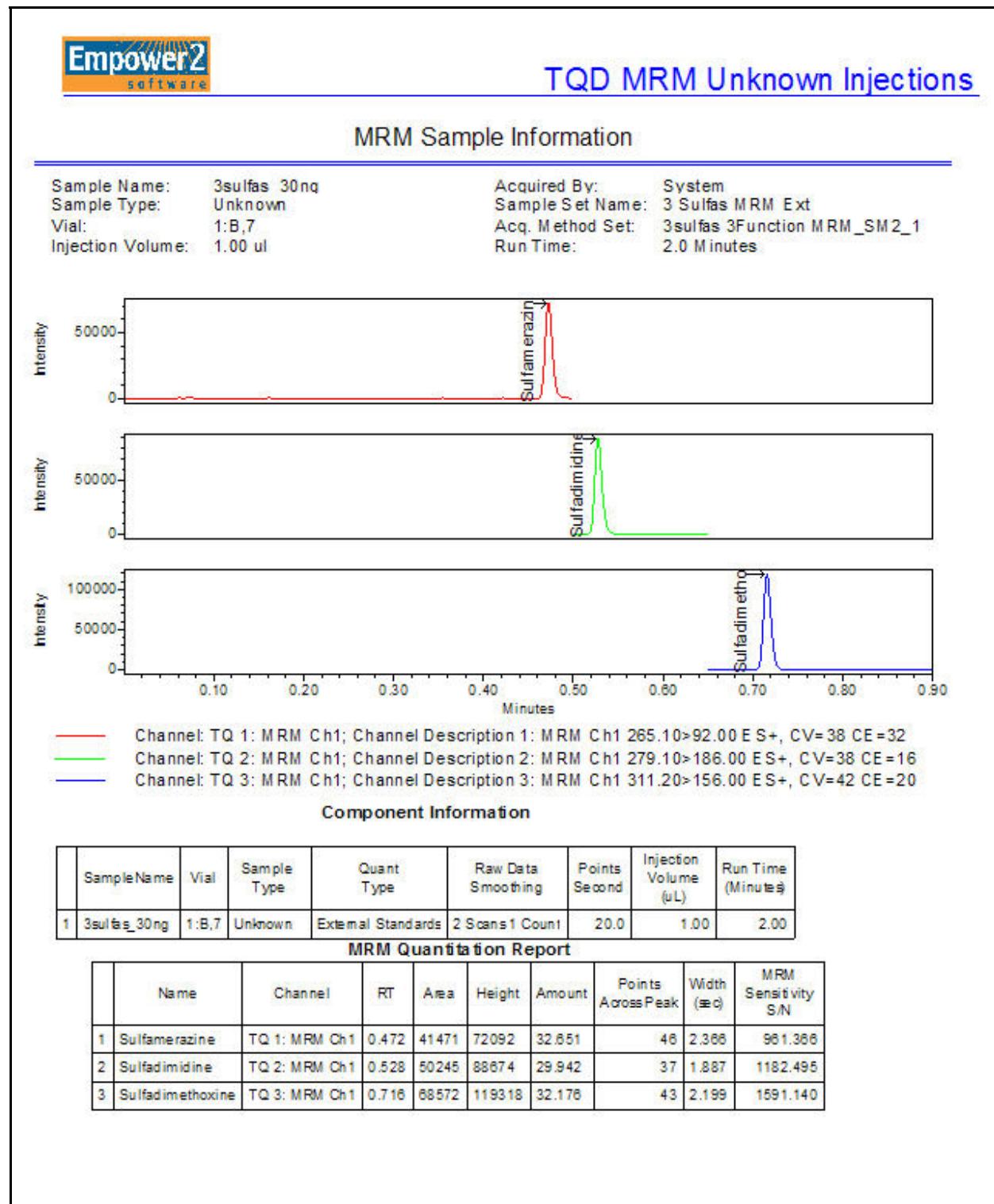
1. Click the **Result Sets** tab.
2. Highlight the **3 Sulfas MRM Ext** result set, and then right-click and select **Preview/Publisher**.
3. In the Preview window, select the **Use the following Report Method** and the **TQD MRM Peaks Summary** check boxes, and then click **OK**.
4. View the summary report, and then click the **Save Report** icon  to save the report as a PDF or the **Print** icon  to print the results.

**NOTE:** An example of the reports is shown on page 2-23.

5. In the Report Publisher, click **Close > File Exit**.
6. In the project view, click **File > Exit**.

**NOTE:** To learn more on quantitation, see the "SIR/MRM Processing with MS Internal Standards" section.

## Individual injection MRM External Standard report



## Examples of summary reports

| TQD MRM Peaks Summary   |               |             |       |   |                  |       |        |        |              |                     |  |
|-------------------------|---------------|-------------|-------|---|------------------|-------|--------|--------|--------------|---------------------|--|
| Name : Sulfadimethoxine |               |             |       |   |                  |       |        |        |              |                     |  |
|                         | Sample Name   | Sample Type | Vial  | Channel Description                       | Name             | RT    | Area   | Height | Amount ng/ml | MRM Sensitivity S/N |  |
| 1                       | 3aultas_1ng   | Standard    | 1:B.1 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 1743   | 2945   | 1.000        | 37.820              |  |
| 2                       | 3aultas_10ng  | Standard    | 1:B.2 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 23183  | 40048  | 10.000       | 537.521             |  |
| 3                       | 3aultas_25ng  | Standard    | 1:B.3 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 55229  | 94901  | 25.000       | 1129.470            |  |
| 4                       | 3aultas_50ng  | Standard    | 1:B.4 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 107239 | 186013 | 50.000       | 1963.843            |  |
| 5                       | 3aultas_75ng  | Standard    | 1:B.5 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 153052 | 257506 | 75.000       | 4029.176            |  |
| 6                       | 3aultas_100ng | Standard    | 1:B.6 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 216148 | 373322 | 100.000      | 4897.177            |  |
| 7                       | 3aultas_30ng  | Unknown     | 1:B.7 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 68572  | 119318 | 32.176       | 1591.140            |  |
| 8                       | 3aultas_60ng  | Unknown     | 1:B.8 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 131879 | 220334 | 61.808       | 2786.261            |  |
| Name : Sulfadimidine    |               |             |       |   |                  |       |        |        |              |                     |  |
|                         | Sample Name   | Sample Type | Vial  | Channel Description                       | Name             | RT    | Area   | Height | Amount ng/ml | MRM Sensitivity S/N |  |
| 1                       | 3aultas_1ng   | Standard    | 1:B.1 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 1667   | 2266   | 1.000        | 29.101              |  |
| 2                       | 3aultas_10ng  | Standard    | 1:B.2 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 16455  | 29387  | 10.000       | 394.438             |  |
| 3                       | 3aultas_25ng  | Standard    | 1:B.3 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 42091  | 74403  | 25.000       | 885.510             |  |
| 4                       | 3aultas_50ng  | Standard    | 1:B.4 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 84335  | 146572 | 50.000       | 1547.437            |  |
| 5                       | 3aultas_75ng  | Standard    | 1:B.5 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 118867 | 213594 | 75.000       | 3342.088            |  |
| 6                       | 3aultas_100ng | Standard    | 1:B.6 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 174707 | 297260 | 100.000      | 3899.418            |  |
| 7                       | 3aultas_30ng  | Unknown     | 1:B.7 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 50245  | 88674  | 29.942       | 1182.495            |  |
| 8                       | 3aultas_60ng  | Unknown     | 1:B.8 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 112268 | 200339 | 66.863       | 2533.415            |  |
| Name : Sulfamerazine    |               |             |       |   |                  |       |        |        |              |                     |  |
|                         | Sample Name   | Sample Type | Vial  | Channel Description                       | Name             | RT    | Area   | Height | Amount ng/ml | MRM Sensitivity S/N |  |
| 1                       | 3aultas_1ng   | Standard    | 1:B.1 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.472 | 1026   | 1792   | 1.000        | 23.017              |  |
| 2                       | 3aultas_10ng  | Standard    | 1:B.2 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.472 | 12791  | 22781  | 10.000       | 305.767             |  |
| 3                       | 3aultas_25ng  | Standard    | 1:B.3 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.472 | 31813  | 57191  | 25.000       | 680.665             |  |
| 4                       | 3aultas_50ng  | Standard    | 1:B.4 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.473 | 68038  | 120889 | 50.000       | 1276.292            |  |

| TQD MRM Peaks Summary |               |             |       |  |               |       |        |        |              |                     |  |
|-----------------------|---------------|-------------|-------|--|---------------|-------|--------|--------|--------------|---------------------|--|
| Name : Sulfamerazine  |               |             |       |  |               |       |        |        |              |                     |  |
|                       | Sample Name   | Sample Type | Vial  | Channel Description                      | Name          | RT    | Area   | Height | Amount ng/ml | MRM Sensitivity S/N |  |
| 5                     | 3aultas_75ng  | Standard    | 1:B.5 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32 | Sulfamerazine | 0.472 | 82868  | 143611 | 75.000       | 2247.071            |  |
| 6                     | 3aultas_100ng | Standard    | 1:B.6 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32 | Sulfamerazine | 0.472 | 135419 | 244570 | 100.000      | 3208.232            |  |
| 7                     | 3aultas_30ng  | Unknown     | 1:B.7 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32 | Sulfamerazine | 0.472 | 41471  | 72092  | 32.651       | 961.366             |  |
| 8                     | 3aultas_60ng  | Unknown     | 1:B.8 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32 | Sulfamerazine | 0.472 | 77106  | 135712 | 60.556       | 1716.162            |  |

## 3 SIR/MRM Processing with MS Internal Standards

This generic procedure describes how to process SIR or MRM data for internal standard quantitation using the ACQUITY SQD or TQD or Alliance 3100 systems with Empower. The procedure guides the operator through the following topics:

- Analyzing MRM data in Review
- Creating a basic processing method using the wizard with MRM internal standards
- Batch processing the injections and reviewing the results
- Viewing basic individual and summary report methods

**NOTE:** *This data was acquired using a Waters TQ Detector; however, the technique for data processing for SIR internal standards on other MS instruments, such as the SQ Detector or 3100 is the same as this procedure.*

The procedure consists of the following eight sections:

|   |      |
|---|------|
| Empower login and restoring the training project .....                  | 3-1  |
| Selecting the sample set and navigating the Review window .....         | 3-3  |
| Building a processing method for MS internal standards.....             | 3-5  |
| Manually editing the processing method .....                            | 3-14 |
| Batch processing the 3 Sulfa MRM IS sample set.....                     | 3-20 |
| Viewing the processed result sets and results in the Project view ..... | 3-21 |
| Viewing the processed data and calibration curves in Review.....        | 3-21 |
| Previewing individual results using Preview/Publisher .....             | 3-24 |

### Empower login and restoring the training project

1. Double-click the **Empower** icon 
2. Type your user name as **System**, and password as **Manager** (or the user name and password assigned to you).

3. Click **Advanced**.



Figure 103 - Empower Login window

4. Click the **User Type** drop-down list, and select **Administrator** (or the user type assigned to you).
5. Select the **Pro Interface** check box, and then click **OK**.

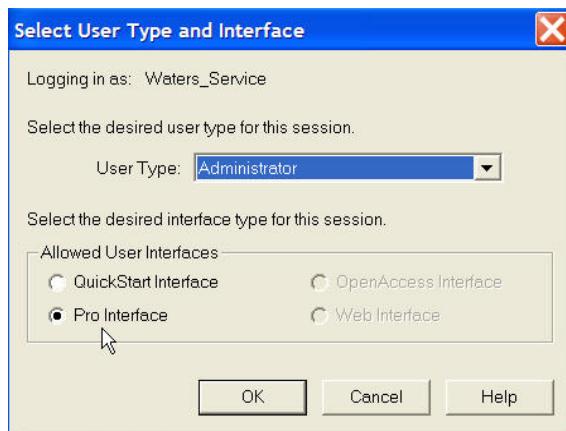


Figure 104 - Advanced dialog box

6. Click the **Configure System** icon  to access the Empower Configuration Manager.
7. Right-click the **Projects folder**, and select **Restore Project(s)**.
8. Perform the following steps if loading the project for the first time:
  - a. Load the *Empower Familiarization CD* in the disk drive, and then click **Browse** to select the location of the "TQD, SQD, or 3100 Empower Familiarization" project.
  - b. Leave the Parent Project as the default, unless requested to put it in another location by your Empower system administrator.
  - c. Click **Next**, select the location of the parent project, and then click **OK**.
  - d. Leave the name of the project as displayed, and then click **Next** to restore the project.
  - e. Click **OK** after the project restores.
  - f. Click **File > Exit** to quit the Empower Configuration Manager.

## Selecting the sample set and navigating the Review window

1. Click the **Browse Project** icon 
2. Select the appropriate **TQD, SQD ,3100 Empower Familiarization** project, and then click **OK**.

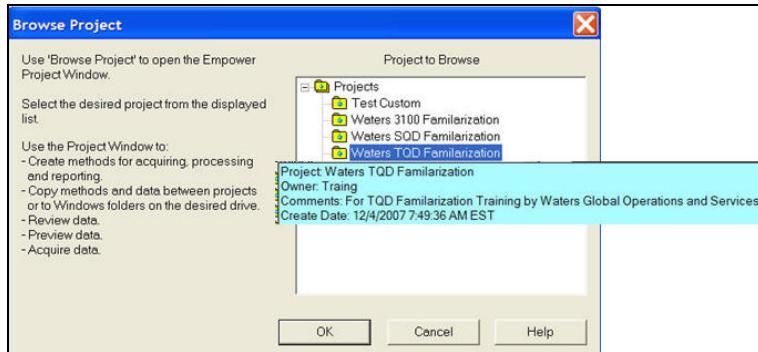


Figure 105 - Browse Project window

3. Click the **Sample Sets** tab.
4. Click the **Filter By** drop-down list, and then select **Sample Set Information**.
5. View the additional information displayed about the sample set.
6. Highlight the **3 Sulfas MRM IS** sample set, and then click the **Review** icon  or right-click and select **Review** to display the Review window.
7. Click **View > 3D Format Review window** to display the following information:
  - Complete sample set information on the left-hand side
  - Chromatogram in the center
  - Spectrum and spectrum information on the right-hand side

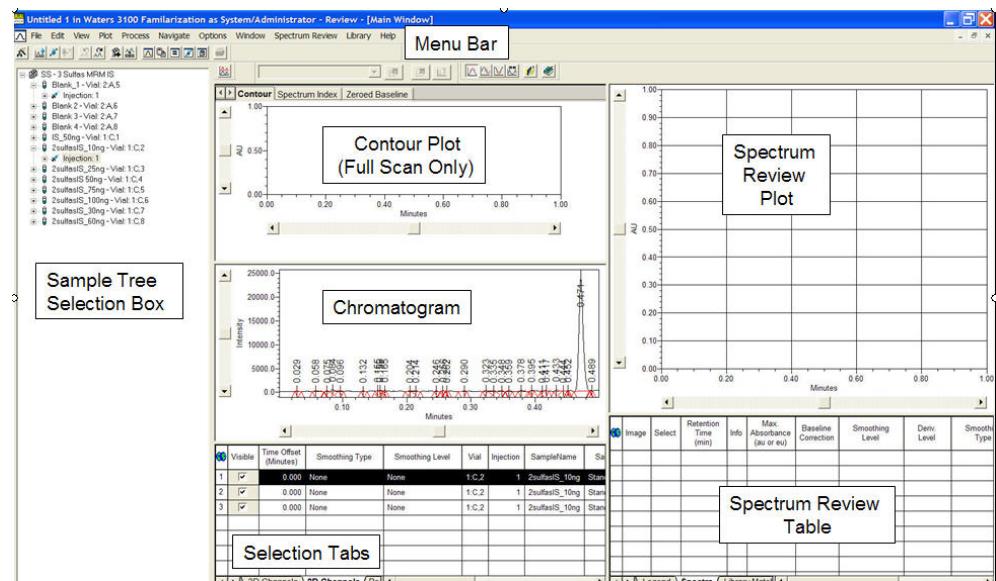


Figure 106 - Review window

8. Click **View > 2D Layout** to view the chromatogram only.
9. In the Selection Tabs field, click the **2D Channels** tab to display the individual MRM channels of the selected sample.

**NOTE:** As a default the first collected sample's MRM Channel is displayed.

10. In the Sample Tree selection box, select the lowest concentration MRM injection standard **2sulfasIS\_10ng-Vial: 1:C,2**, and then click **+** to expand the information about the sample.
11. Highlight **Injection: 1**, and then click **+** to display all the MRM data channels for the standard.

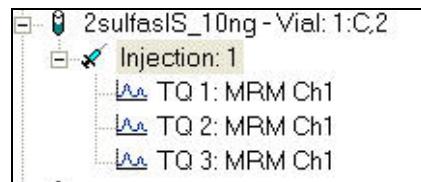


Figure 107 - MRM data channels

**NOTE:** The channels are also displayed in the 2D Channels tab below the chromatogram.

The 2sulfasIS\_10ng standard is displayed with the TQD MRM Ch1 channel highlighted and the 2D chromatogram displayed (Figure 108). The chromatogram may be displayed integrated as well as additional channel information displayed in the table.

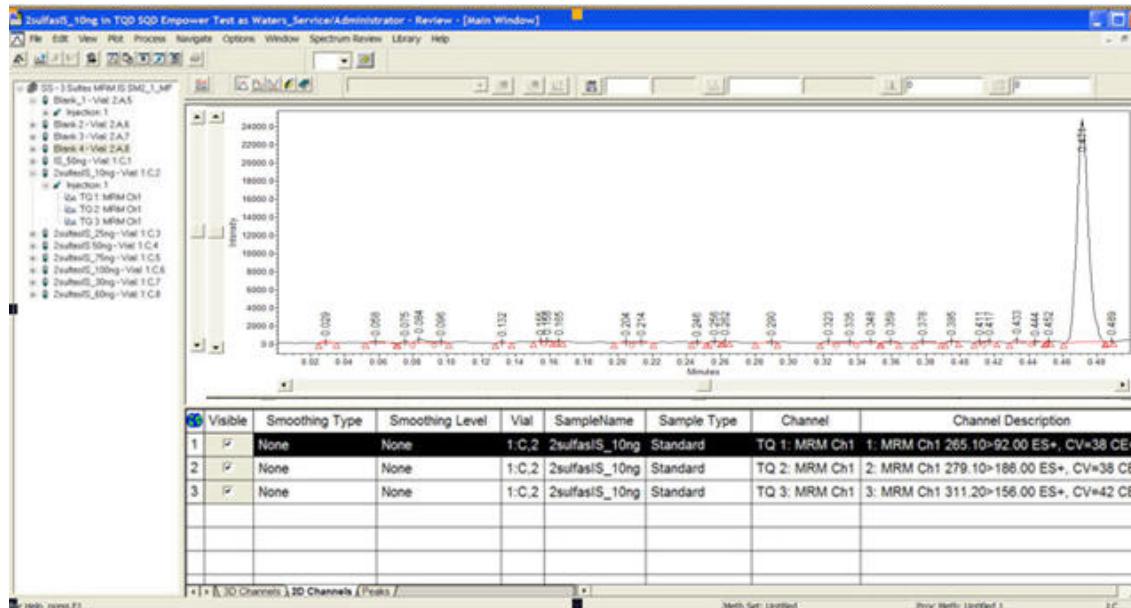


Figure 108 - Displayed channels and chromatogram

## Building a processing method for MS internal standards

1. Click the **Processing Method Wizard** icon .
2. Select the **Create a New Processing Method** check box, and then click **OK**.
3. Click the **Processing type** drop-down list, and select **MS**.
4. Click the **Integration Algorithm** drop-down list, and select **ApexTrack**.
5. Select the **Use Processing Method Wizard** check box.
6. Select the **Include a Cross Channel Internal Standard** check box, and then click **OK**.

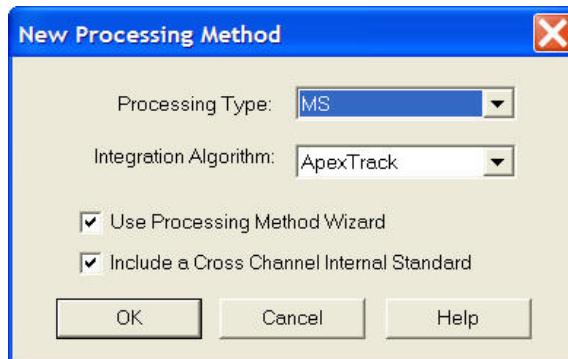


Figure 109 - New Processing Method window

7. Click the **Int Std Channel** drop-down list, and select the MRM channel to be used as the Internal Standard.
8. Select **TQ 2: MRM Ch1** (sulfadimidine) as the internal standard.
9. For chromatographic smoothing of all MRM channels, click the **Smoothing Type** drop-down list and select **Mean**, and then click the **Smoothing Level** drop-down list and select **7**.

**NOTE:** *It is recommended for MS to have a smooth applied by the processing method. General recommendations are Mean for the Smoothing Type, and a Smoothing Level of 5 to 15. Time offset is used to align different detector signals that are in series to each other (TQD and PDA).*

10. Ensure that the **Check here to have these empty parameters...** check box is unchecked, and then click **Next**.

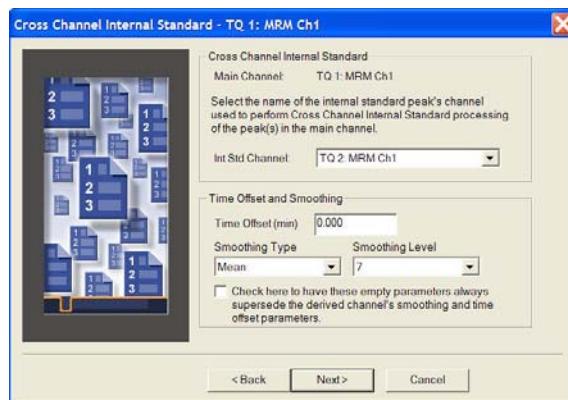


Figure 110 - Cross Channel Internal Standard, Time Offset and Smoothing window

11. Ensure that the **Start** and **End** boxes are empty, and then click **Next**.

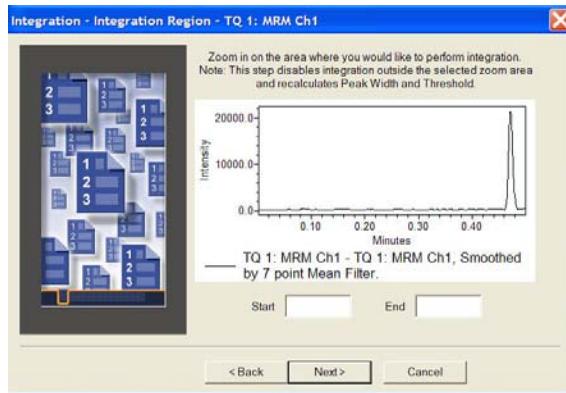


Figure 111 - Integration Region window

**NOTE:** *Start and End must not be used for SIR/MRM processing methods as the data collection could be staggered for different SIR/MRM channels that are not displayed in the wizard.*

12. The chromatogram will be displayed with many peaks integrated (Figure 112). Ensure that the **Clear Peak Width and Threshold** settings are unchecked, and then click **Next**.

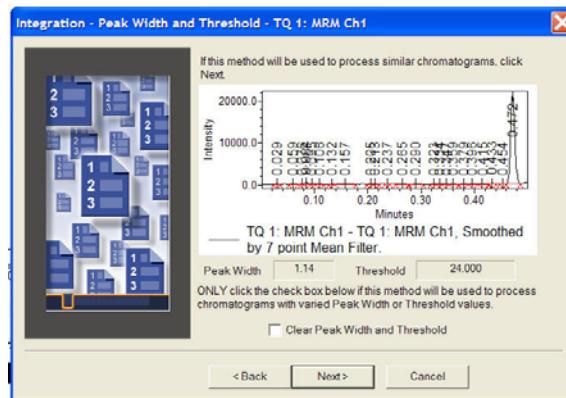


Figure 112 - Peak Width Threshold window

13. Click inside the peak at 0.472 minutes, and then select the **Minimum Height** check box.
14. Change the peak height values from 20024 to 2000, and then click **Next**.

**NOTE:** *Default minimum height uses 90% of the peak height for peak rejection. In general it is recommended to set minimum height at ~10 to 50% of peak height.*

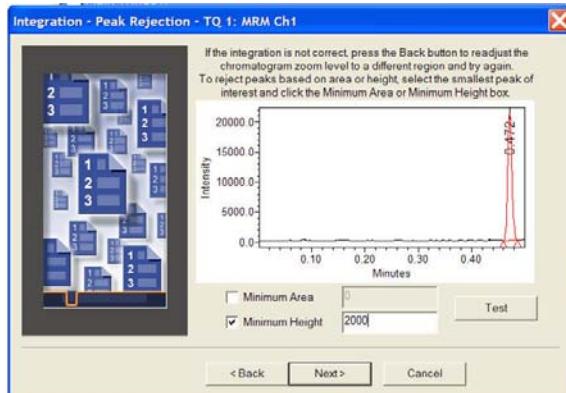


Figure 113 - Peak Rejection window

15. Click the drop-down lists, and then select the following settings (Figure 114):
 

|   |        |
|---|--------|
| • Select the method of quantitation     | Area   |
| • Component information                 | Amount |
| • Select the calibration curve fit type | Linear |
16. Click **Next**.

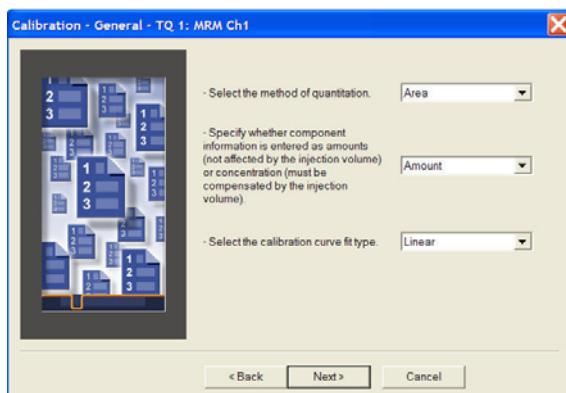


Figure 114 - Calibration window

17. The peak at 0.472 minutes is identified as Peak1 TQ 1: MRM Ch1. In the Names and Retention Times window, click inside the Peak1 TQ 1: MRM Ch1 box. Click the drop-down list, and then select **Sulfamerazine** for Peak1 TQ 1: MRM Ch1.

**NOTE:** *The component names match the names entered in the standard Amounts from Run Samples. The final view for this dialog box should match the one displayed in Figure 115.*

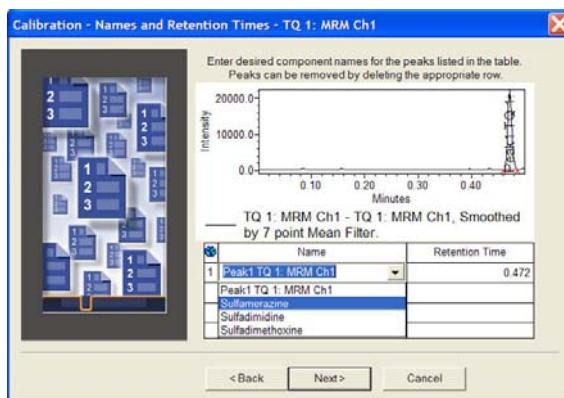


Figure 115 - Calibration Name and Retention Time window

18. Click **Next**.
19. Since multiple standard concentrations were acquired, skip the default amounts section, and then click **Next**.
20. Ensure that the **MS Match Library** check box is unchecked, and then click **Next**.

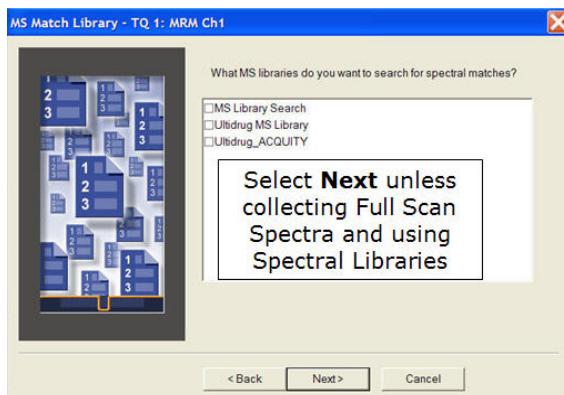


Figure 116 - MS Match Library window

21. Type the processing method name as **3Sulfas MRM IS**, and then click **Finish**.

## Adding the second MRM chromatogram channel

The TQ 2: MRM Ch1 (the channel that was identified as the internal standard in the wizard) is immediately displayed with the peak integrated.

1. Click **Next**.

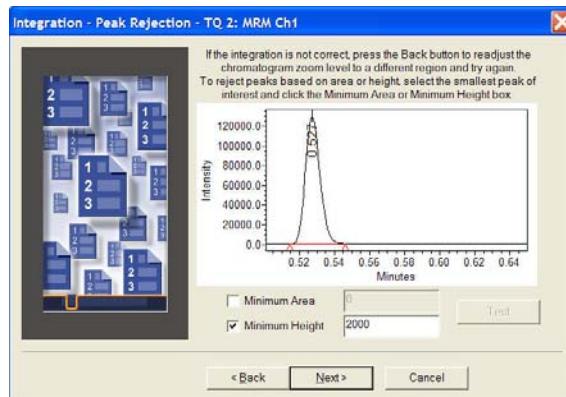


Figure 117 - Peak Rejection window

**NOTE:** If the peak was not properly integrated, you may have to adjust the minimum height or minimum area to a lower value.

2. In the Names and Retention Times window, click inside the Peak2 TQ 2: MRM Ch1, click the drop-down list, and then select **Sulfadimidine**.
3. Click **Next**.

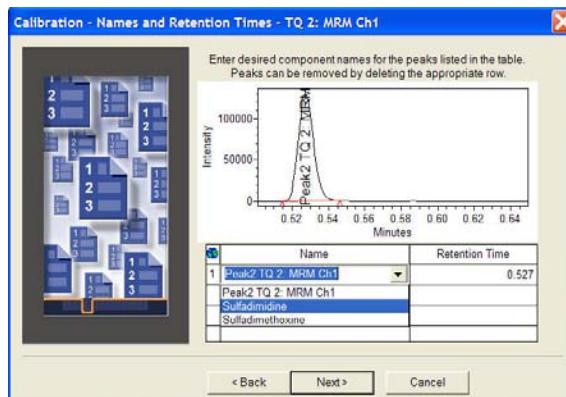


Figure 118 - Names and Retention Times window

4. Leave the Default Amounts as displayed, and then click **Next**.

**NOTE:** Default amounts are only used if you are using a single point calibration curve.

5. Confirm the information displayed for Internal Standards is correct, and then click **Next**.



Figure 119 - Internal Standards window

6. Click **Finish**.

**NOTE:** The Internal Standard MRM is saved to the processing method and the MRM Internal Standard Chromatogram is displayed in the Review window.

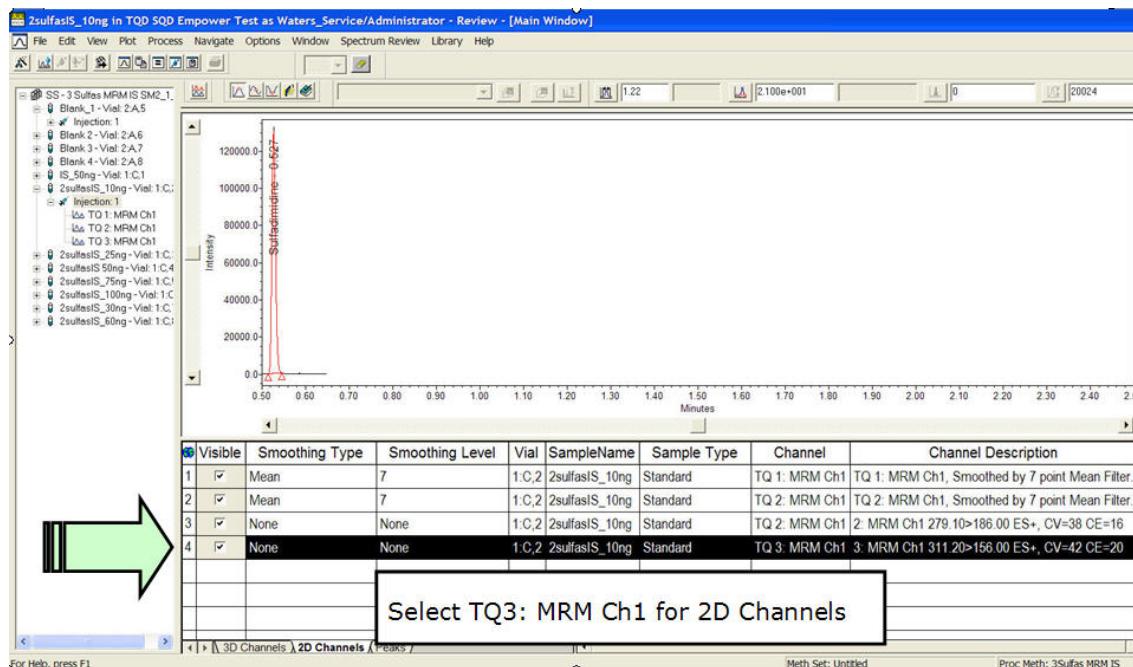
Both TQ2: MRM Ch1 Chromatograms are displayed in the 2D Channel view, one is displayed as smoothed data and the other is displayed unsmoothed. This is to show the internal standard's processed and unprocessed channels.

|   | Visible                             | Time Offset (Minutes) | Smoothing Type | Smoothing Level | Vial  | Injection | SampleName          | Sample Type | Injection Volume (uL) | Channel       | Channel Description                            |
|---|-------------------------------------|-----------------------|----------------|-----------------|-------|-----------|---------------------|-------------|-----------------------|---------------|--|
| 1 | <input checked="" type="checkbox"/> | 0.000                 | Mean           | 9               | 1.C.2 | 1         | 2sulfadimidine_10ng | Standard    | 1.00                  | TQ 1: MRM Ch1 | TQ 1: MRM Ch1, Smoothed by 9 point Mean Filter |
| 2 | <input checked="" type="checkbox"/> | 0.000                 | Mean           | 9               | 1.C.2 | 1         | 2sulfadimidine_10ng | Standard    | 1.00                  | TQ 2: MRM Ch1 | TQ 2: MRM Ch1, Smoothed by 9 point Mean Filter |
| 3 | <input checked="" type="checkbox"/> | 0.000                 | None           | None            | 1.C.2 | 1         | 2sulfadimidine_10ng | Standard    | 1.00                  | TQ 2: MRM Ch1 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16      |

Figure 120 - Internal Standards smoothed and unsmoothed channels

### **Adding the third MRM chromatogram channel**

1. In the 2D Channel table, highlight **TQ3: MRM Ch1** in the channel row to add another MRM chromatogram channel to the processing method.
  2. Select the channel as shown in Figure 121.



*Figure 121 - 2D Channels Table field*

3. Click the **Processing Method Wizard** icon .
  4. Select **Edit an Existing Processing Method**, and then click **OK** to display the integrated peak in the Processing wizard.
  5. Click **Next**.
  6. Leave the Peak Width and Threshold settings as displayed, and then click **Next**.
  7. Leave the Minimum Height and Minimum Area settings as displayed, and then click **Next**.
  8. Leave the Calibration General settings as displayed, and then click **Next**.
  9. When prompted, ensure that **Yes** is selected to use channel names to perform Cross Channel Internal Standard processing.

**NOTE:** Selecting Yes combines all the MRM chromatograms into one processing method.

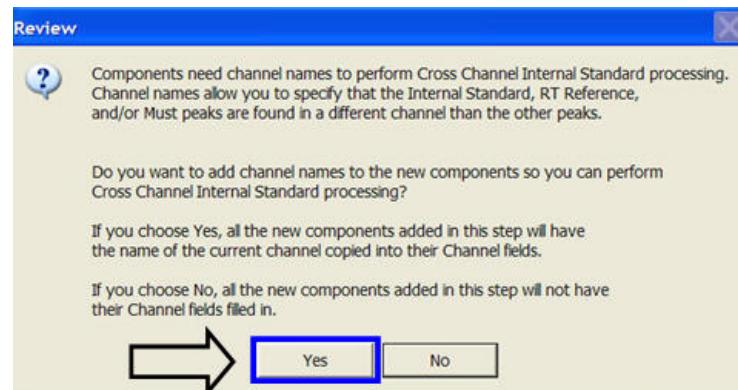


Figure 122 - Choosing channel names

10. In the Names and Retention Times window, click inside the Peak3 TQ 3: MRM Ch1, click the drop-down list, and then select **Sulfadimethoxine**.

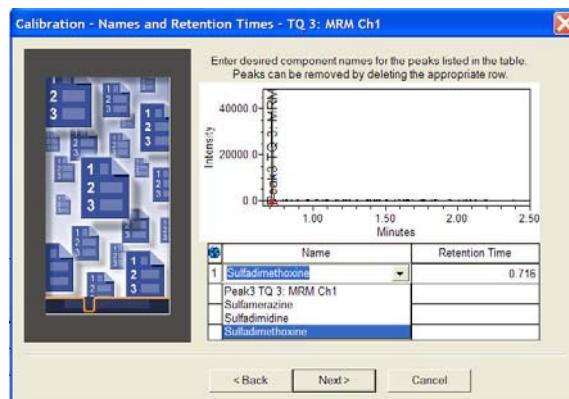


Figure 123 - Names and Retention Times window

11. Leave the Default Amounts as displayed, and then click **Next**.
12. Leave the component for an internal standard as Sulfadimidine, and then click **Next**.
13. Ensure that the **MS Match Library** check boxes are unchecked, and then click **Next**.
14. Click **Finish**.

**NOTE:** *The third MRM chromatogram will be added to the processing method with the chromatogram displayed.*

*To add more MRMs to the processing method, repeat steps 1 to 14 or manually edit the processing method as described in the section "Manually editing the processing method".*

*The processing method last opened is displayed in the status bar at the bottom of the Review window.*

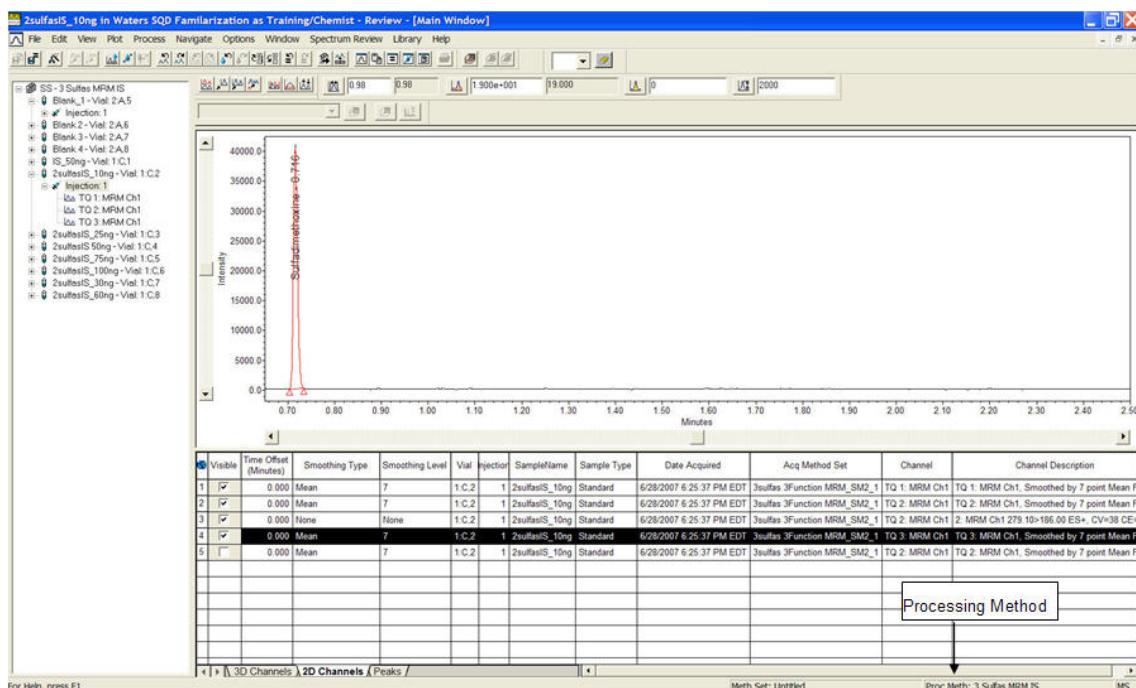


Figure 124 - Review window

## Manually editing the processing method

1. Click the **Processing Method** icon .
2. Click the **Components** tab, and view and edit the columns described in this section.
3. In the sulfamerazine row, click the **Channel** column to identify which MRM chromatogram is identified with the component.
4. Select the correct MRM from the drop-down list.

**NOTE:** *This may be used to identify MRM channels without using the processing wizard.*

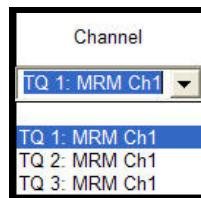


Figure 125 - Channel column drop-down list

5. In the sulfamerazine row, click the **Peak Match** column and then select **Greatest Area** from the drop-down list.
6. Use Ctrl-D to copy down.

**NOTE:** *This can be used when multiple peaks are within the same MRM window.*



Figure 126 - Peak Match column drop-down list

7. In the sulfamerazine row, click the **Weighting** column and then select **1/X** from the drop-down list.
8. Use Ctrl-D to copy down.

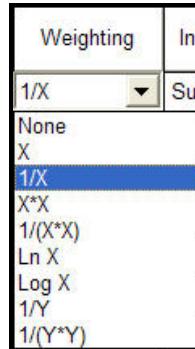


Figure 127 - Weighting column drop-down list

**NOTE:** MRM or SIR data from MS detectors frequently use a weighting value of **1/X** or **1/(X\*X)** to get a better fit at the lower end of the concentration curve.

9. Click the **Auto Peak Label** button to label the Peaks as 1, 2, 3.

|   | Name             | Peak Label | Retention Time (min) | RT Window (min) | Channel       | Peak Match    | Y Value | X Value | Fit    | Weighting | Internal Std | RT Reference | Rel RT Refer |
|---|------------------|------------|----------------------|-----------------|---------------|---------------|---------|---------|--------|-----------|--------------|--------------|--------------|
| 1 | Sulfamerazine    | 1          | 0.472                | 0.024           | TQ 1: MRM Ch1 | Greatest Area | Area    | Amount  | Linear | 1/X       |              |              |              |
| 2 | Sulfadimidine    | 2          | 0.527                | 0.026           | TQ 2: MRM Ch1 | Greatest Area | Area    | Amount  | Linear | 1/X       |              |              |              |
| 3 | Sulfadimethoxine | 3          | 0.716                | 0.036           | TQ 3: MRM Ch1 | Greatest Area | Area    | Amount  | Linear | 1/X       |              |              |              |

Figure 128 - Example Processing Methods Component table

10. Click the **Suitability** tab for signal-to-noise calculation.
11. Select **Calculate Suitability Results** and set a void volume time of **0.1 min**.
12. Click the **Noise Value for s/n** drop-down list and select **Detector Noise** to calculate noise using RMS (Root Mean Square Noise).

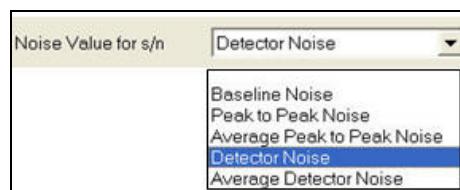


Figure 129 - Noise Value for s/n drop-down list

13. Click the **Noise and Drift** tab and select **Calculate Detector Noise and Drift**.
14. Set a Start Time of **0.050 min**, a Stop Time of **0.4 min**, and a Segment width of **10**.

**NOTE:** *Noise and Drift is calculated for each data channel collected for the selected Start and End Time region. If a data channel is not collected during the time region, then a noise calculation will not be calculated.*

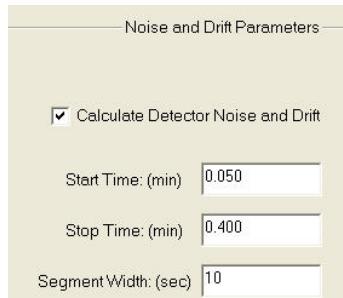


Figure 130 - Noise and Drift parameters

| Name               | Peak Label | Retention Time (min) | RT Window (min) | Channel       | Peak Match    | Y Value | X Value | Fit    | Weighting | Internal Std  | RT Reference |
|--------------------|------------|----------------------|-----------------|---------------|---------------|---------|---------|--------|-----------|---------------|--------------|
| 1 Sulfamerazine    | 1          | 0.472                | 0.047           | TQ 1: MRM Ch1 | Greatest Area | Area    | Amount  | Linear | 1/X       | Sulfadimidine |              |
| 2 Sulfadimidine    | 2          | 0.527                | 0.053           | TQ 2: MRM Ch1 | Greatest Area | Area    | Amount  | Linear | None      |               |              |
| 3 Sulfadimethoxine | 3          | 0.716                | 0.072           | TQ 3: MRM Ch1 | Greatest Area | Area    | Amount  | Linear | 1/X       | Sulfadimidine |              |

Figure 131 - Processing Methods Component table

15. View any other processing tabs and variables as desired and then click **File > Save Processing Method** to save the changes to the method.

## Overlay MRM chromatograms and save as a method set

1. Click the **Review Main Window** icon .
2. To view both the chromatogram and processing method, click **View > Processing Method Layout**.
3. Click the **Overlay** icon  to view all three MRM chromatograms together.
4. Ensure that the **Visible column** check box is selected on the MRM chromatograms you want displayed.

**NOTE:** *If the chromatograms are not displayed, ensure that the Visible Column check box in the Chromatogram table is checked for the MRM chromatograms to be displayed.*

5. Perform the following steps to display the component names for all the MRMs:
  - a. Right-click the chromatogram and select **Properties...**
  - b. Click the **Overlay** tab.
  - c. In the Chromatogram Annotation, select the **All Chroms in Overlay** check box.
  - d. Click **OK**.

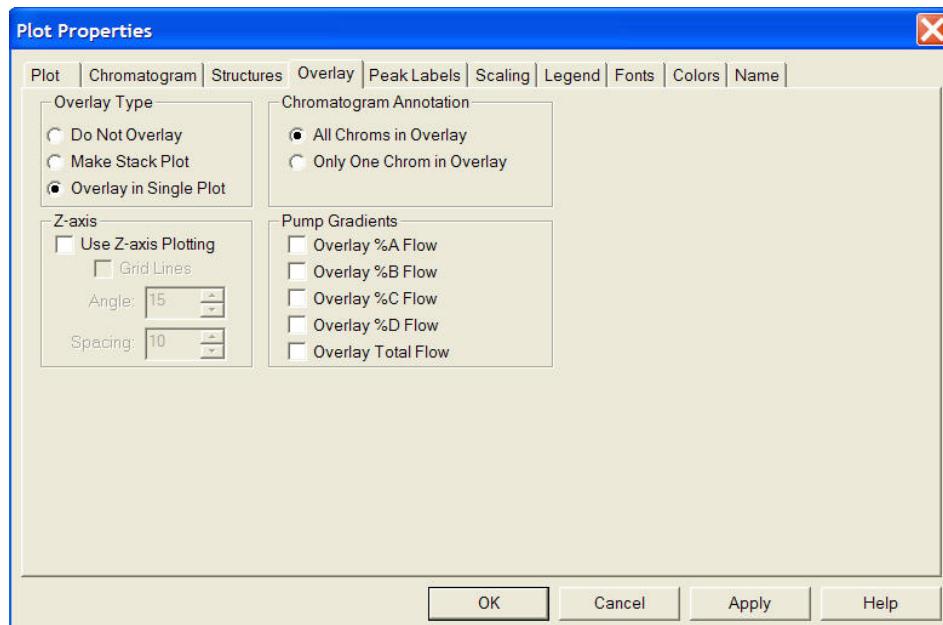


Figure 132 - Plot Properties Overlay tab

6. Click **File > Save preferences** to save the default settings used in the Review window.

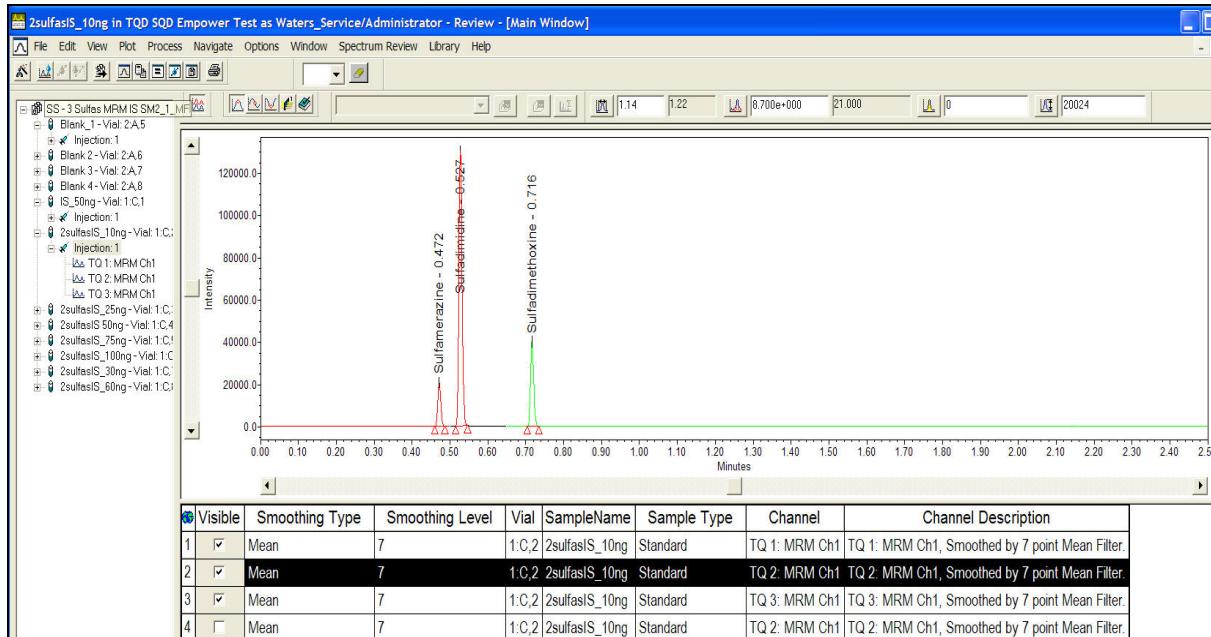


Figure 133 - Example Chromatogram overlay

7. Click the **Peaks** tab at the bottom of the Review window, to view the result information.

|   | Name             | Retention Time (min) | Area ( $\mu\text{V}^{\text{sec}}$ ) | % Area | Height ( $\mu\text{V}$ ) | Int Type | Amount | Units | Peak Type | Start Time (min) | End Time (min) | Baseline Start (min) | Baseline End (min) | Response   | Peak Level | Points Across Peak |
|---|------------------|----------------------|-------------------------------------|--------|--------------------------|----------|--------|-------|-----------|------------------|----------------|----------------------|--------------------|------------|------------|--------------------|
| 1 | Sulfadimethoxine | 0.716                | 23692                               | 100.00 | 40214                    | BB       | 10.000 | ng/ml | Found     | 0.704            | 0.734          | 0.704                | 0.734              | 1.569e+001 |            | 36                 |

Figure 134 - Peaks Tab table

8. Right-click the **World** icon to set the table properties.

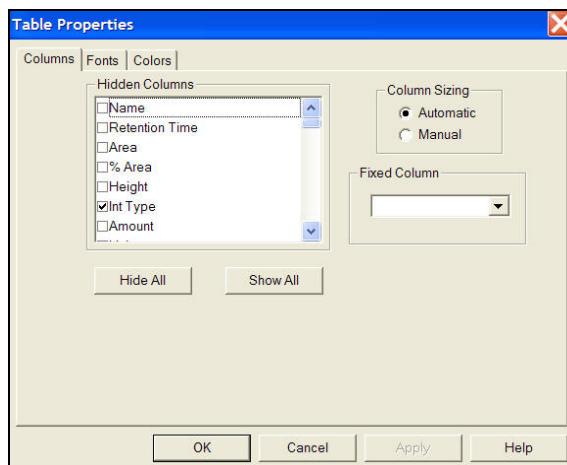


Figure 135 - Peaks Table properties

9. Click **Hide All**, scroll down to view all the column names and then uncheck the boxes as shown in Figure 136 to display only the desired columns.

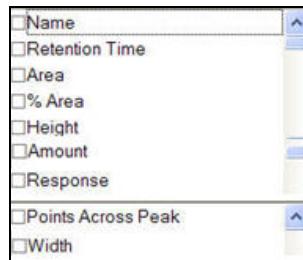


Figure 136 - Peak Table Columns to be displayed

10. Click **OK**.

11. Click **File > Save Preferences**.

12. Click the **Method Set** icon

located to the right side of the Main Icon bar to view the method set.

13. Click the **Default Report Method** drop-down list and select **TQD Full Report**.

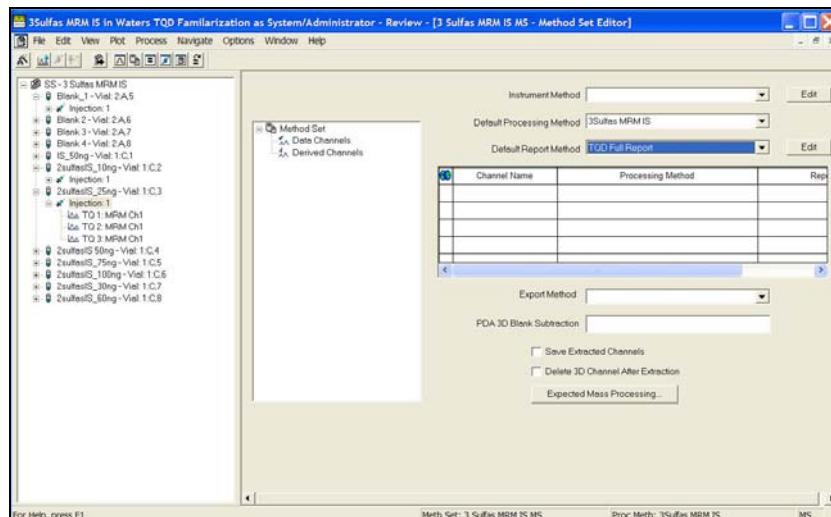


Figure 137 - Method set TQD Full Report method

**NOTE:** The Method Set window can be used to add additional processing and report functions to a method set process.

14. Click **File > Save As > Method Set**.

15. Save the method set as **3 Sulfa MRM IS MS**.

16. Click the **Review Main Window** icon

to return to the Review Main window.

17. Click **File > Exit** to quit the Review Main window.

## Batch processing the 3 Sulfas MRM IS sample set

1. Click the **Sample Set** tab.
2. Click the **Filter By:** drop-down list and select **Sample set Information**.
3. Highlight the **3 Sulfas MRM IS** sample set, and click the **Process** icon  or right-click and select **Process**.
4. In the Processing field, select the **Use specified method set** check box.
5. Click the drop-down list and select **3 Sulfas MRM IS MS**.
6. Select the **Clear Calibration** check box.
7. Click the **How:** drop-down list and select **Calibrate and Quantitate**.
8. Leave the remaining settings as the default, and then click **OK** to process the data and create the results.

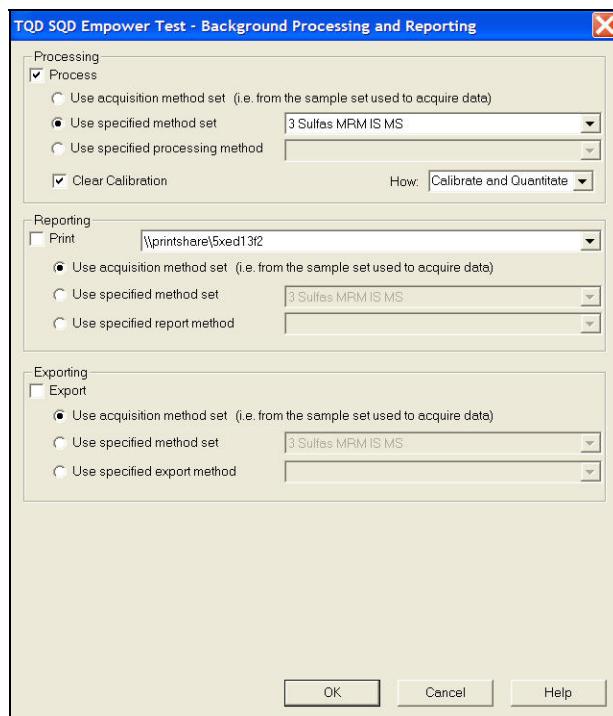


Figure 138 - Background Processing and Reporting window

**NOTE:** For SIR/MRM internal standard data, it is best to process the data from the "Use acquisition method set" or "Use specified method set" check box, not the "Use specified processing method" check box. This is because some calculations use inter-channel information, which cannot be performed from the specified processing method selection.

## Viewing the processed result sets and results in the Project view

1. Click the **Result Sets** tab.
2. Click the **Filter By:** drop-down list and select **Processed Today** to show only today's results.
3. If the results are not displayed, click the **Update** button.
4. Highlight the **3 Sulfas MRM IS** result set, right-click and select **View As > Results** to show only the processed standard and unknown results from the processed sample set.
5. In the Results field, click the **Filter By:** drop-down list and select **3 Sulfas MRM IS SM2\_1 Latest** to view only the unknown samples processed with this data set.
6. Highlight all six unknown MRMs by clicking on the **World** icon  to select all the results, and then click the **Review** icon .

## Viewing the processed data and calibration curves in Review

The Review window displays all three MRMs from the 2 Sulfas IS 30 ng sample and the sulfamerazine sample result peaks table displayed.

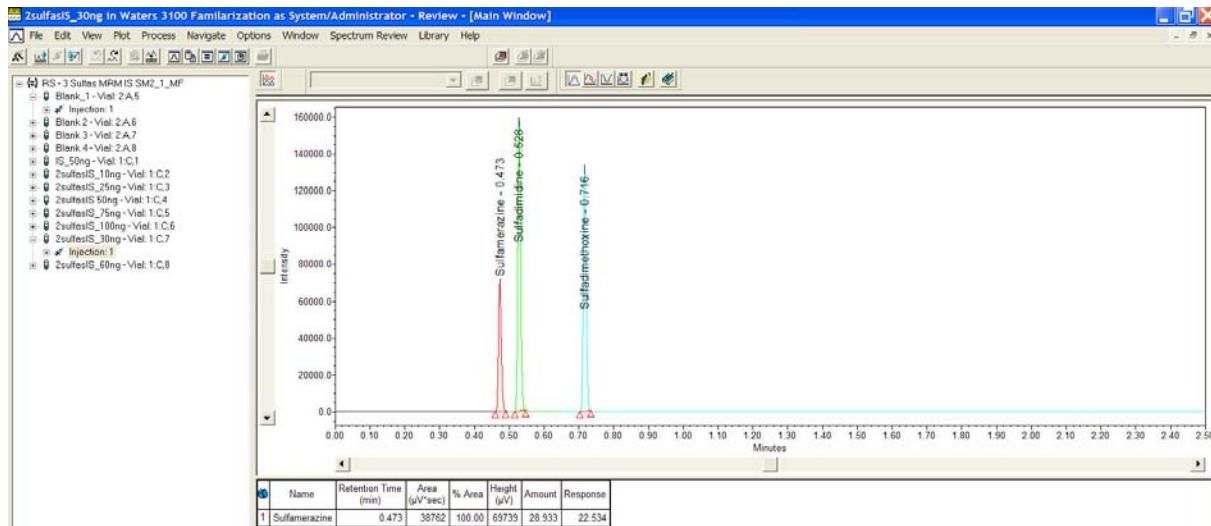


Figure 139 - Review window with three MRMs of 2 Sulfa IS 30 ng Overlaid

1. Click the **Overlay**  icon a second time to display only the current MRM viewed, "Sulfamerazine".
2. If the peaks table is not displayed, click the **Peaks** tab to display the results.

**NOTE:** *The description of the displayed sample is shown on the top of the Review Main window as shown in Figure 140.*



Figure 140 - Sample description

3. Toggle between the **Previous Result** icon  or the **Next Result** icon  to view results MRM processed chromatogram and peak information.
4. Click the **Previous Result**  or the **Next Result** icon  again until sulfadimethoxine is displayed.
5. Right-click inside the sulfadimethoxine peak and select the **Show Internal Standard** channel.

**NOTE:** *This displays the internal standard used for MRM Internal Standard quantitation. Use Return from Internal Standard View to display just the Quantitated MRM.*

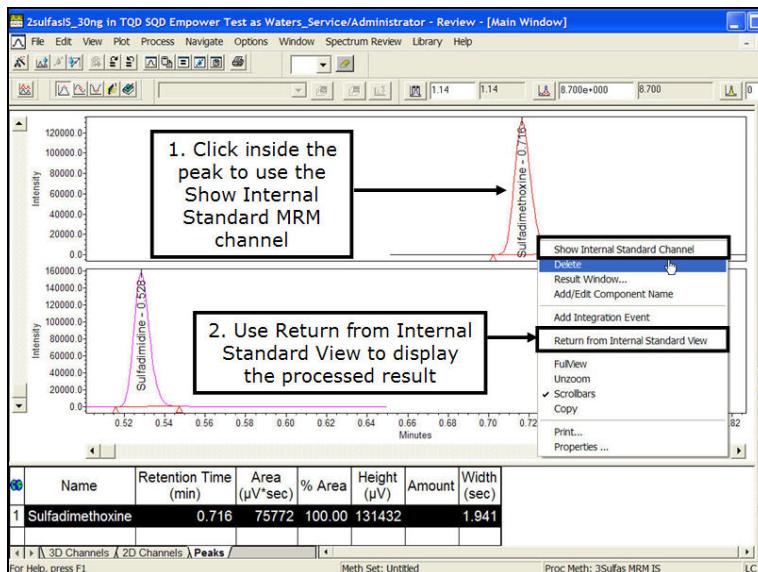


Figure 141 - Review window

6. Click the **Results Icon**  to display all details about the cross channel internal standard.
7. Click the **Sun Result Peaks** tab at the bottom of the Results window to display quantitative information about the internal standard.

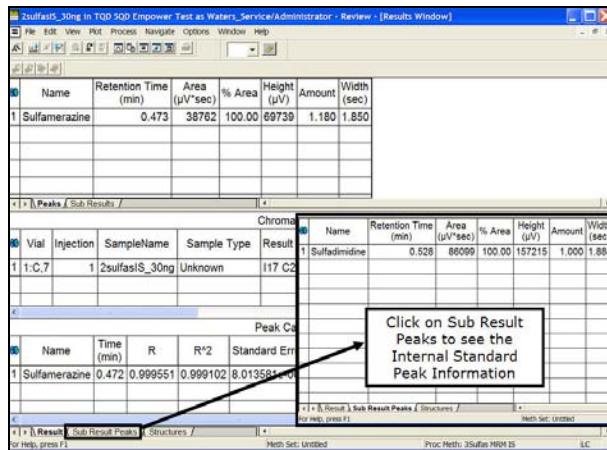


Figure 142 - Results window

8. Click the **Calibration Curve** icon  to view the calibration curve for sulfadimethoxine.

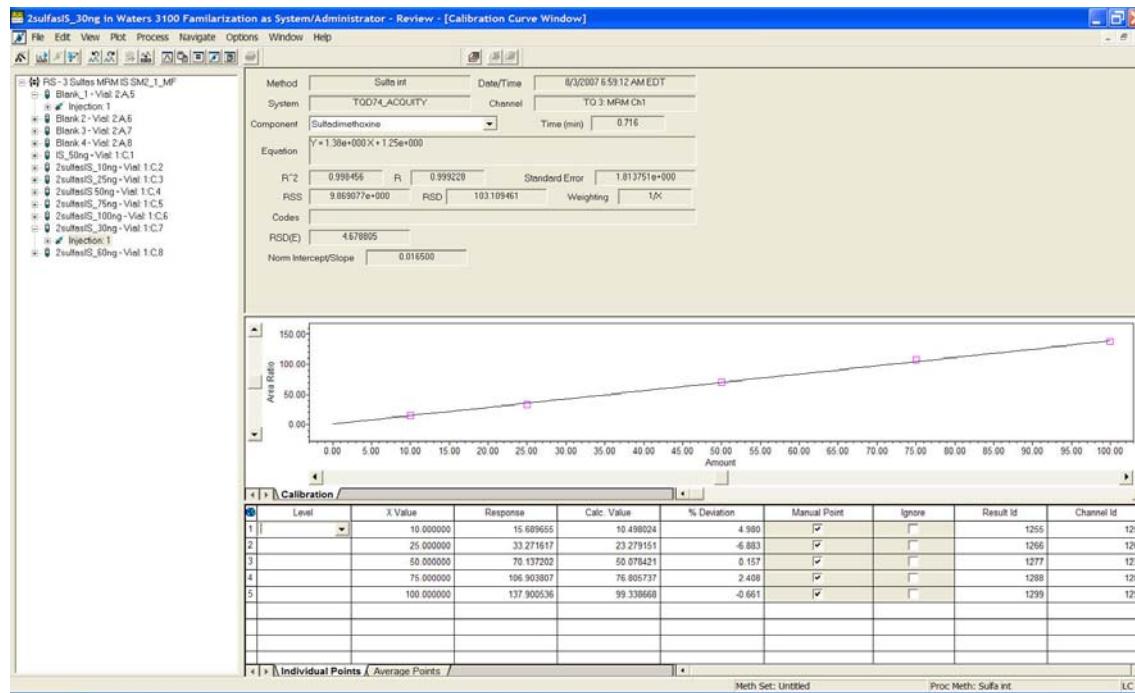


Figure 143 - Calibration Curve window

9. Click the **Previous Result** icon  or the **Next Result** icon  to scroll through the calibration curves.
10. Click the **Review Main Window** icon , and then click **File > Exit**.

## Previewing individual results using Preview/Publisher

The final section demonstrates how to review the processed data using reports through the Preview Publisher function. Reports have two modes, Individual and Summary.

### Individual unknown reports

1. Click the **Results** tab.
2. Click the **Filter By:** drop-down list, and select **3 Sulfa MRM Ext SM2\_1 Latest**.
3. Highlight all six unknown MRMs by clicking on the **World** icon  to select all the results, and then click the **Preview/Publisher** icon .
4. In the Preview window, Open Report Method dialog box; select the **Use the following Report Method** check box, select **TQD MRM Unknown Injections** from the drop-down list, and then click **OK**.

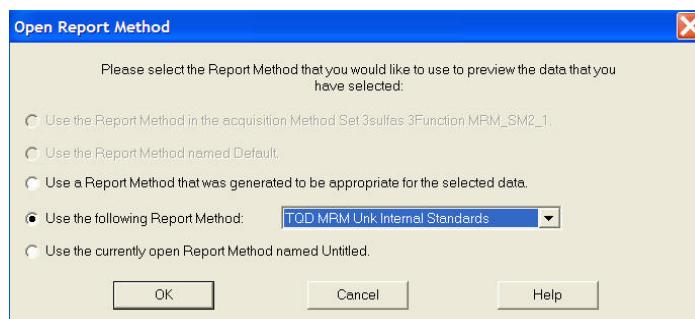


Figure 144 - Open Report Method dialog box

5. To view the reports for the two unknown samples, use the **Next Report** icon  or the **Previous Report** icon  to view the next sample.
6. Click the **Save Report** icon , to save the report as a PDF file or the **Print** icon  to print the results.

**NOTE:** An example report is shown on page 3-26.

7. Click the **Open** icon , and then select the **TQD Full Report** to view the calibration curve plots and tables, and the MS calibration and tune conditions used for the collection of the data.
8. Click the **Next Page** icon  and the **Back Page** icon  to view all the pages of the report.

**NOTE:** An example report is shown on pages 3-27 through 3-28.

9. In the Report Publisher, click **Close**.
10. After closing the Preview window, the Report Publisher is displayed for advanced editing of reports.

11. Click **File > Exit** to close the Report Publisher.

## Peaks summary report

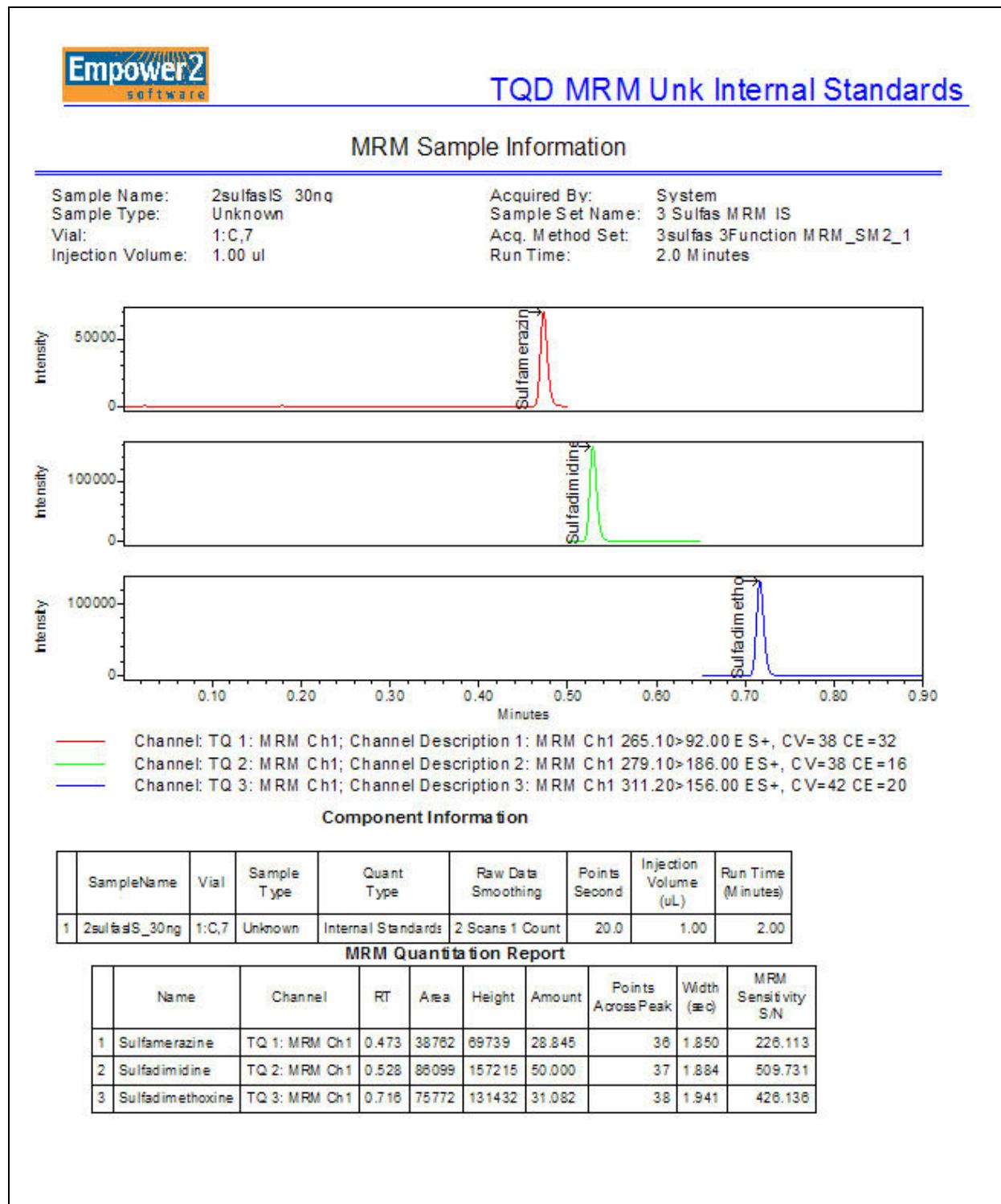
1. Click the **Result Sets** tab.
2. Highlight the **3 Sulfas MRM IS** result set, and then right-click and select **Preview/Publisher**.
3. In the Preview window, select the **Use the following Report Method** and the **TQD MRM Peaks Summary** check boxes, and then click **OK**.
4. View the summary report, and then click the **Save Report** icon  to save the report as a PDF file or the **Print** icon  to print the results.

**NOTE:** An example of the report is shown on page 3-29.

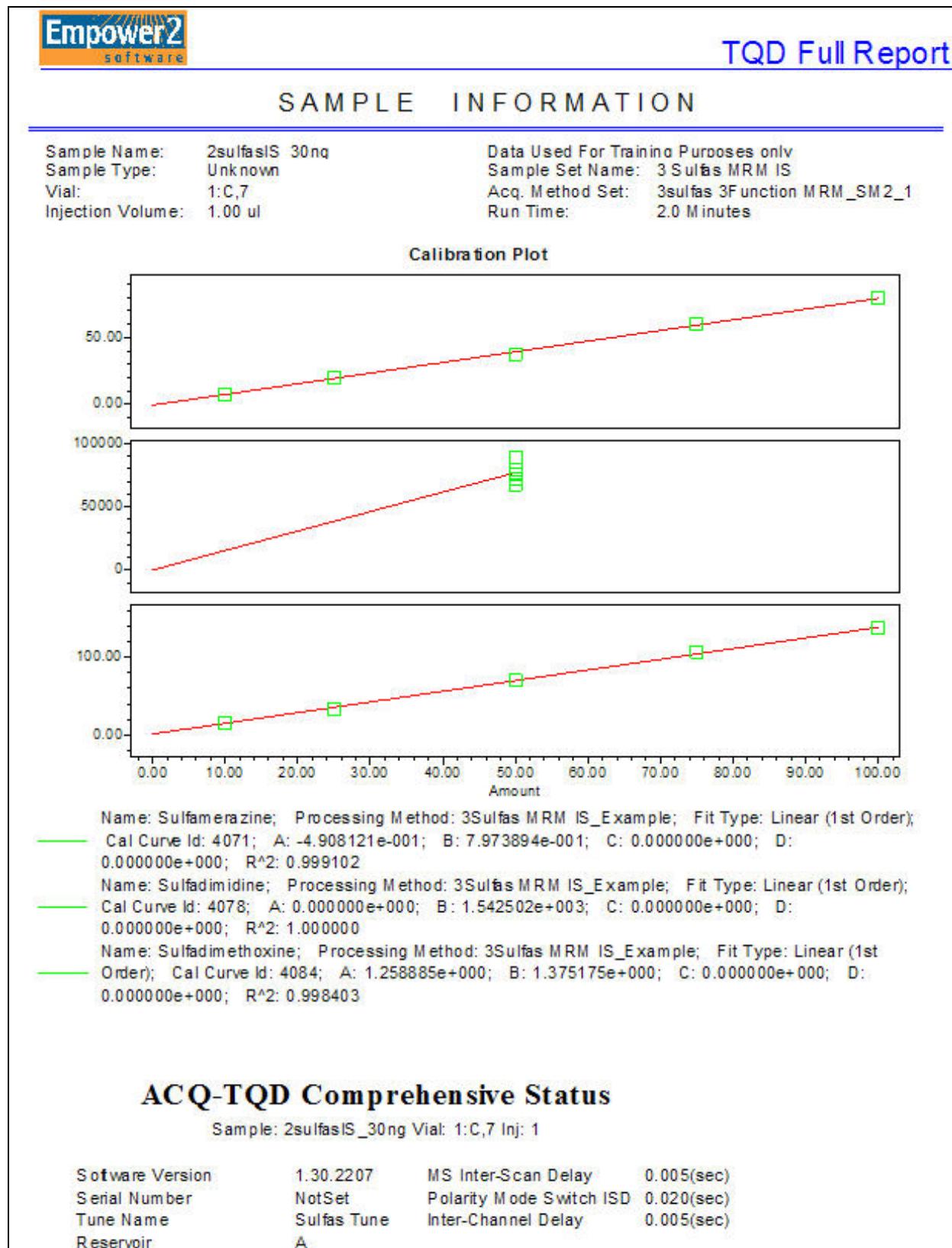
5. In the Report Publisher, click **Close > File Exit**.
6. In the project view, click **File > Exit**.

**NOTE:** To learn more on quantitation, see the "Empower 2 Software Data Acquisition and Processing Theory Guide" (p/n UG0000458) available in the Support Library at [www.waters.com](http://www.waters.com).

## Example of Individual unknown report



## Example of Full report



## SAMPLE INFORMATION

Sample Name: 2sulfasIS\_30ng  
 Sample Type: Unknown  
 Vial: 1:C,7  
 Injection Volume: 1.00 ul  
 Ion Energy 0.50(V)  
 Acquired By: System  
 Sample Set Name: 3 Sulfas MRM IS  
 Acq. Method Set: 3sulfas 3Function MRM\_SM 2\_1  
 Run Time: 2.0 Minutes

Quad2 Scanning Calibration

|                      |                     |
|----------------------|---------------------|
| Mass                 | 18.00 - 2040.00(Da) |
| High Mass Resolution | 15.37(Da)           |
| Low Mass Resolution  | 3.59(Da)            |
| Ion Energy           | 0.50(V)             |
| MS Inter-Scan Delay  | 0.005(sec)          |

Quad2 Scan Speed Compensation

|                      |                          |
|----------------------|--------------------------|
| Scan Rate            | 336.00 - 9863.00(Da/sec) |
| High Mass Resolution | 15.37(Da)                |
| Low Mass Resolution  | 3.59(Da)                 |
| Ion Energy           | 0.50(V)                  |
| MS Inter-Scan Delay  | 0.005(sec)               |

## ACQ-TQD Comprehensive Status

Sample: 2sulfasIS\_30ng Vial: 1:C,7 Inj: 1

|                          |             |                     |            |
|--------------------------|-------------|---------------------|------------|
| Software Version         | 1.30.2207   | Inter-Channel Delay | 0.005(sec) |
| Serial Number            | NotSet      |                     |            |
| Tune Name                | Sulfas Tune |                     |            |
| Reservoir                | A           |                     |            |
| MS Inter-Scan Delay      | 0.005(sec)  |                     |            |
| Polarity Mode Switch ISD | 0.020(sec)  |                     |            |

ES+ Tune Settings

|                            |              |
|----------------------------|--------------|
| Tune Cone Voltage          | 40(V)        |
| Capillary Voltage          | 3.50(kV)     |
| Extractor Voltage          | 3(V)         |
| RF Lens                    | 0.1(V)       |
| Source Temperature         | 150(C)       |
| Cone Gas Flow              | 0(L/hr)      |
| Desolvation Temperature    | 500(C)       |
| Desolvation Gas Flow       | 900(L/hr)    |
| LM1 Resolution             | 13.2(Da)     |
| HM1 Resolution             | 15.0(Da)     |
| Ion Energy 1               | 0.1(V)       |
| MS Mode Entrance           | 50.00(V)     |
| MS Mode Collision Energy   | 3.00(V)      |
| MS Mode Exit               | 50.00(V)     |
| LM2 Resolution             | 3.6(Da)      |
| HM2 Resolution             | 15.4(Da)     |
| Ion Energy 2               | 0.5(V)       |
| MSMS Mode Entrance         | 1.00(V)      |
| MSMS Mode Collision Energy | 20.00(V)     |
| MSMS Mode Exit             | 0.50(V)      |
| Collision Gas Flow         | 0.10(mL/min) |

## Example of Peaks Summary report

| TQD MRM Peaks Summary |                         |             |       |   |                  |       |        |        |              |                     |
|-----------------------|-------------------------|-------------|-------|---|------------------|-------|--------|--------|--------------|---------------------|
|                       | Name : Sulfadimethoxine |             |       |   |                  |       |        |        |              |                     |
|                       | Sample Name             | Sample Type | Vial  | Channel Description                       | Name             | RT    | Area   | Height | Amount ng/ml | MRM Sensitivity S/N |
| 1                     | 2aulfasS_10ng           | Standard    | 1:C.2 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.716 | 23692  | 40214  | 10.000       | 163.413             |
| 2                     | 2aulfasS_25ng           | Standard    | 1:C.3 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.716 | 52583  | 93157  | 25.000       | 334.531             |
| 3                     | 2aulfasS_50ng           | Standard    | 1:C.4 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.717 | 124982 | 221164 | 50.000       | 802.605             |
| 4                     | 2aulfasS_75ng           | Standard    | 1:C.5 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.716 | 144987 | 240244 | 75.000       | 837.533             |
| 5                     | 2aulfasS_100ng          | Standard    | 1:C.6 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.716 | 199253 | 336655 | 100.000      | 1430.417            |
| 6                     | 2aulfasS_30ng           | Unknown     | 1:C.7 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.716 | 75772  | 131432 | 31.082       | 426.136             |
| 7                     | 2aulfasS_60ng           | Unknown     | 1:C.8 | 3: MRM Ch1 311.20>156.00 ES+, CV=42 CE=20 | Sulfadimethoxine | 0.715 | 128768 | 217652 | 57.822       | 818.836             |
|                       | Name : Sulfadimidine    |             |       |   |                  |       |        |        |              |                     |
|                       | Sample Name             | Sample Type | Vial  | Channel Description                       | Name             | RT    | Area   | Height | Amount ng/ml | MRM Sensitivity S/N |
| 1                     | IS_50ng                 | Standard    | 1:C.1 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.527 | 79049  | 138082 | 50.000       | 544.526             |
| 2                     | 2aulfasS_10ng           | Standard    | 1:C.2 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.527 | 75809  | 129928 | 50.000       | 527.972             |
| 3                     | 2aulfasS_25ng           | Standard    | 1:C.3 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.527 | 79021  | 138268 | 50.000       | 496.528             |
| 4                     | 2aulfasS_50ng           | Standard    | 1:C.4 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 89094  | 159227 | 50.000       | 577.834             |
| 5                     | 2aulfasS_75ng           | Standard    | 1:C.5 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.527 | 67732  | 117345 | 50.000       | 409.085             |
| 6                     | 2aulfasS_100ng          | Standard    | 1:C.6 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 72346  | 130425 | 50.000       | 554.165             |
| 7                     | 2aulfasS_30ng           | Unknown     | 1:C.7 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 86099  | 157215 | 50.000       | 509.731             |
| 8                     | 2aulfasS_60ng           | Unknown     | 1:C.8 | 2: MRM Ch1 279.10>186.00 ES+, CV=38 CE=16 | Sulfadimidine    | 0.528 | 79709  | 142860 | 50.000       | 537.460             |
|                       | Name : Sulfamerazine    |             |       |   |                  |       |        |        |              |                     |
|                       | Sample Name             | Sample Type | Vial  | Channel Description                       | Name             | RT    | Area   | Height | Amount ng/ml | MRM Sensitivity S/N |
| 1                     | 2aulfasS_10ng           | Standard    | 1:C.2 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.472 | 11454  | 21078  | 10.000       | 85.653              |
| 2                     | 2aulfasS_25ng           | Standard    | 1:C.3 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.472 | 31027  | 55098  | 25.000       | 197.860             |
| 3                     | 2aulfasS_50ng           | Standard    | 1:C.4 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.473 | 67160  | 124585 | 50.000       | 452.118             |
| 4                     | 2aulfasS_75ng           | Standard    | 1:C.5 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.472 | 81193  | 143206 | 75.000       | 499.240             |
| 5                     | 2aulfasS_100ng          | Standard    | 1:C.6 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.472 | 115786 | 204543 | 100.000      | 869.083             |
| 6                     | 2aulfasS_30ng           | Unknown     | 1:C.7 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.473 | 38762  | 69739  | 28.845       | 226.113             |
| 7                     | 2aulfasS_60ng           | Unknown     | 1:C.8 | 1: MRM Ch1 265.10>92.00 ES+, CV=38 CE=32  | Sulfamerazine    | 0.473 | 70103  | 128691 | 55.763       | 472.865             |

# 4

## Full Scan Processing of MS Data

This generic procedure describes how to process and analyze full scan MS data using the ACQUITY SQD/TQD/3100 systems with Empower. The procedure guides the operator through the following topics:

- Extracting a chromatogram from full scan MS data
- Viewing and understanding how to display MS spectra
- Extracting MS data from PDA chromatograms
- Comparing spectra between different samples using a basic processing method
- Reporting for full scan MS data

The procedure consists of the following 12 sections:

|  |      |
|--|------|
| Empower login and restoring the training project .....   | 4-1  |
| Basic project navigation of MS/PDA data.....   | 4-3  |
| Basic navigation and customization of the MS/PDA 3D Review window.....                           | 4-4  |
| Basic PDA data chromatographic extraction.....   | 4-10 |
| Using PDA data to extract MS chromatograms.....  | 4-12 |
| Basic extraction of chromatographic data from MS scans .....                                     | 4-14 |
| Qualitative chromatographic processing for full scan MS data .....                               | 4-15 |
| Using MS spectra from MS chromatograms .....   | 4-18 |
| Summary of MS review process.....  | 4-21 |
| Batch processing for full scan MS data .....   | 4-21 |
| Viewing the processed results in review.....   | 4-22 |
| Viewing the processed results in preview/publisher for MS only and MS/PDA combined reports ..... | 4-25 |

### Empower login and restoring the training project

1. Double-click the **Empower** icon .
2. Type your user name as **System**, and password as **Manager** (or the user name and password assigned to you).

3. Click **Advanced**.



Figure 145 - Empower Login window

4. Click the **User Type** drop-down list, and select **Administrator** (or the user type assigned to you).
5. Select the **Pro Interface** check box, and then click **OK**.

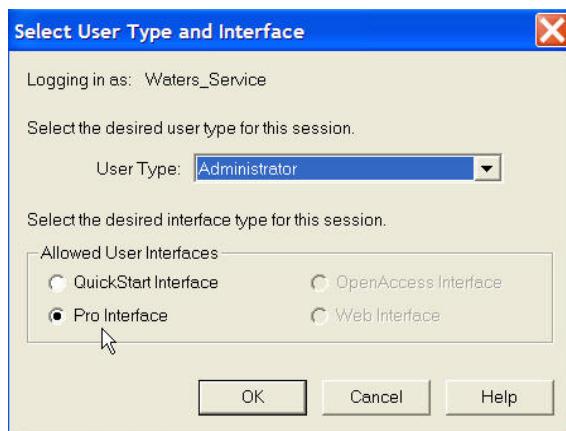


Figure 146 - Advanced dialog box

6. Click the **Configure System** icon  to access the Empower Configuration Manager.
7. Right-click the **Projects folder**, and select **Restore Project(s)**.
8. Perform the following steps if loading the project for the first time:
  - a. Load the *Empower Familiarization CD* in the disk drive, and then click **Browse** to select the location of the "TQD, SQD, or 3100 Empower Familiarization" project.
  - b. Leave the Parent Project as the default, unless requested to put it in another location by your Empower system administrator.
  - c. Click **Next**, select the location of the parent project, and then click **OK**.
  - d. Leave the name of the project as displayed, and then click **Next** to restore the project.
  - e. Click **OK** after the project restores.
  - f. Click **File > Exit** to quit the Empower Configuration Manager.

## Basic project navigation of MS/PDA data

This section covers basic project navigation for MS/PDA full scan data. Once the data has been acquired you can view MS/PDA data from three separate views (sample set, injections and channels).

1. Click the **Browse Project** icon 
2. Select the appropriate **TQD, SQD, 3100 Empower Familiarization** project, and then click **OK**.

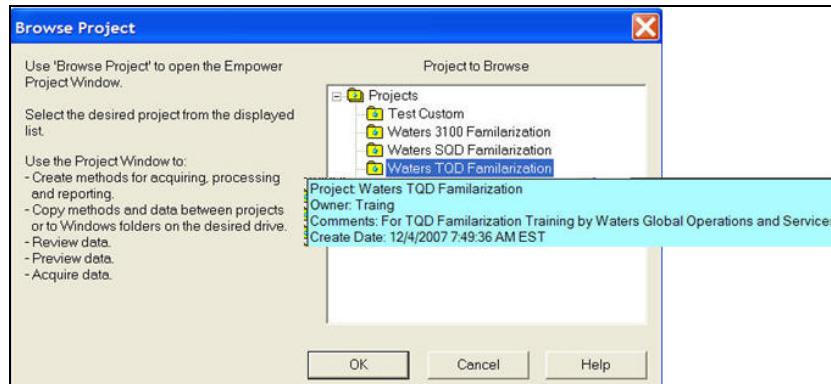


Figure 147 - Browse Project window

3. Click the **Sample Sets** tab.

**NOTE:** *Sample sets can be used to display data in 3D review as a group of compiled data.*

4. Click the **Filter By** drop-down list, and select **Sample Set Information**.
5. View the additional information displayed about the sample set.

**NOTE:** *The additional information displayed in this view filter was entered during sample acquisition with the use of custom sample set fields. For more information see the Empower on-line help.*

6. Click the **Training MS PDA Scanning** sample set, then right-click and select **View As > Injections**. This displays PDA and MS data that was collected for this analysis.

**NOTE:** *For MS and PDA scanning data, it is recommended to use either the sample set or injection views to review data. The Channel tab is only recommended for individual detector data.*

7. Highlight the **3 Sulfas** injection, and then click the **Review** icon  or right-click and select **Review** to display the Review window.

**NOTE:** *Use the Review window to analyze PDA/MS data for spectral and chromatographic analysis.*

## Basic navigation and customization of the MS/PDA 3D Review window

The 3D Review window displays the following information:

- Complete sample set information on the left-hand side
- Chromatogram in the center
- Spectrum and spectrum information on the right-hand side

**NOTE:** If the Contour Map and Spectra display are not viewed, click **View > 3D layout** from the Menu bar.

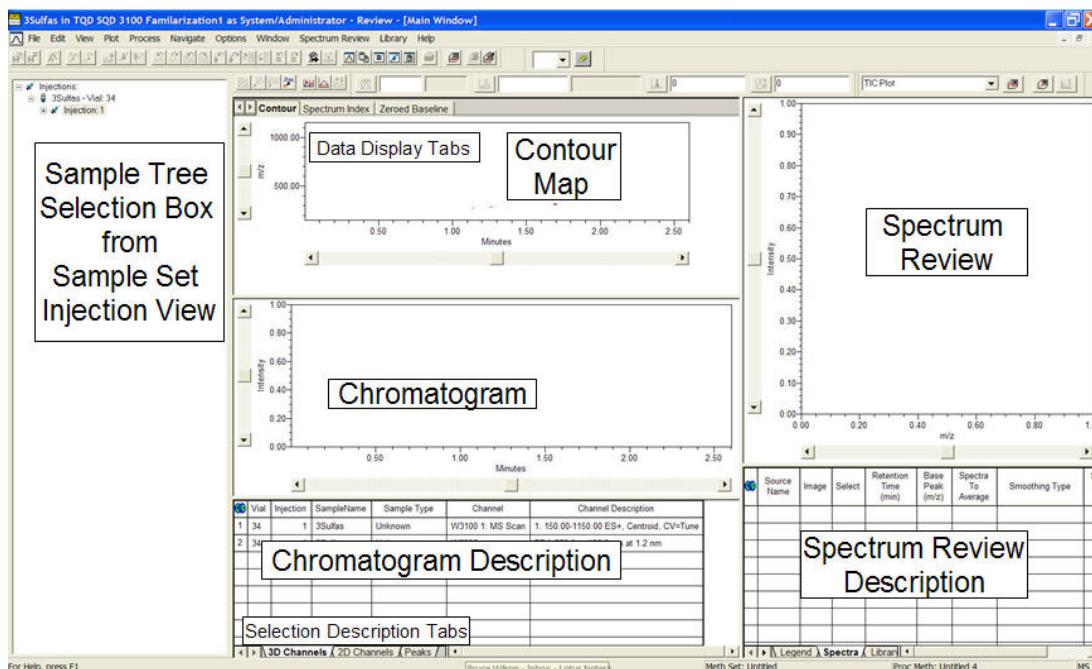


Figure 148 - 3D Review window

The following features of the Review window can be customized:

- Contour plot
- Toolbars
- Icons for display
- 3D Channels information table
- 3D MS plot

1. Click the **Main Window** icon , or select **Window > Main window** from the Menu bar to return to the Review Main window when customizations are complete.
2. Selecting **File > Save preferences** to save all display, toolbar, and icon display settings. This will save the review layout as selected for the next time the Review window is opened.

**NOTE:** Preferences can be saved by user and by project. Display preferences can be copied from one project to another by using the configuration managers Copy Preferences function.

## Customizing contour plot

1. Right-click in the **Contour Map** and click **Properties** to enhance MS contour data.

**NOTE:** The contour plot displays a topological map of MS intensity. PDA data shows greater details of a spectral contour map than MS data.

2. In the Levels field, set the Number to **13** and select the **Exponential Distribution** check box, to enhance the spectral definition of MS intensity of the full scan contour plot.
3. Click **OK**.

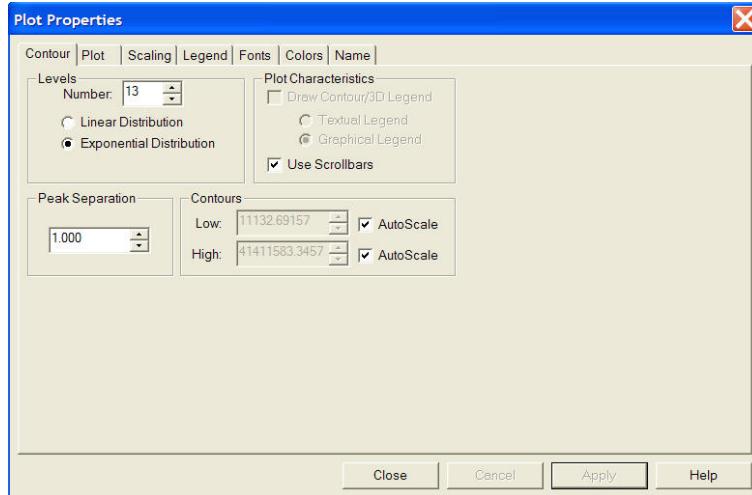


Figure 149 - Plot Properties window

## Customizing toolbars

PDA/MS Review has a selection of toolbars and icons that can be displayed or hidden.

1. Click **View > Toolbar**, and select the desired toolbars to be displayed.
2. For this exercise, deselect **Plot Annotations** to be removed from the toolbars displayed.

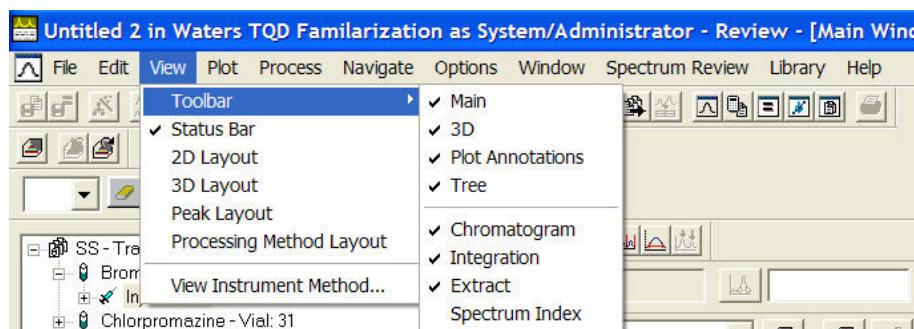


Figure 150 - Toolbars menu

## Customizing icons

Each toolbar has a selection of icons that can be displayed or hidden.

1. Right-click the **Wizard** icon (the icon may be greyed out) and select **Customize** to customize the Review window for basic MS/PDA analysis.

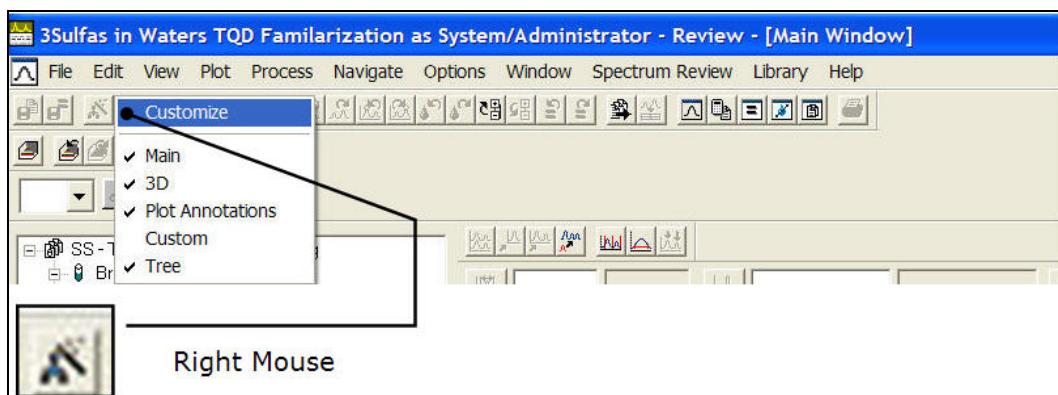


Figure 151 - Customizing icons

2. In the Customize toolbar dialog box, select icons from the current toolbar to hide. For this exercise we will hide the following icons:

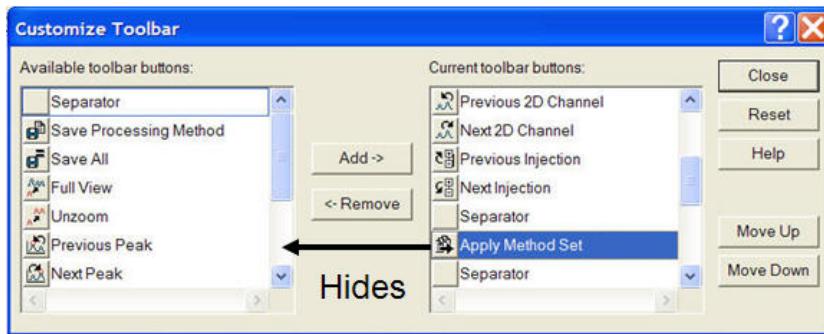
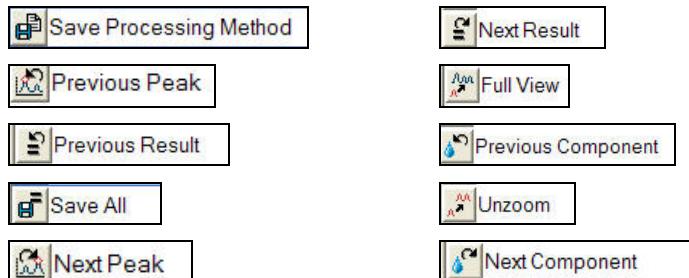


Figure 152 - Customize toolbar

3. Click **Close** to see the how the customized toolbar looks.

## Customizing the 3D Channels Information table

Each table tab can be customized to display selected columns.

1. Click the **3D Channels** tab to display the individual 3D scanning data channels from the selected sample.

|   | Vial | Injection | SampleName | Sample Type | Channel          | Channel Description                      |
|---|------|-----------|------------|-------------|------------------|--|
| 1 | 34   | 1         | 3Sulfas    | Unknown     | W3100 1: MS Scan | 1: 150.00-1150.00 ES+, Centroid, CV=Tune |
| 2 | 34   | 1         | 3Sulfas    | Unknown     | W2996            | PDA 220.0 to 400.0 nm at 1.2 nm          |

Figure 153 - 3D scanning data channels

2. Right-click the **World** icon  to set the table properties.

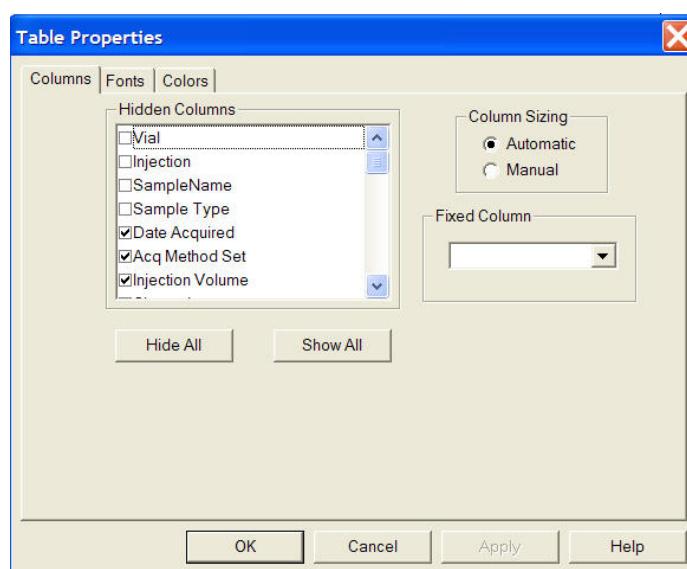


Figure 154 - Table Properties window

3. Click **Hide All**, then deselect the columns required to be displayed, see Figure 155.

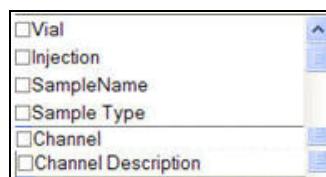


Figure 155 - Peak table columns to be displayed

## Customizing the 3D MS Plot display

The 3D view cube display can be used to determine what masses have intensity for full scan data and the isotope patterns for each peak (compound).

1. Highlight the first row of the 3D Channels tab to select the MS scan data for the sample.
2. Select the “**3Sulfas w3100 1 MS Scan**” data channel to display the contour map with MS full scan data.

|   | Vial | Injection | SampleName | Sample Type | Channel          | Channel Description                      |
|---|------|-----------|------------|-------------|------------------|--|
| 1 | 34   | 1         | 3Sulfas    | Standard    | W3100 1: MS Scan | 1: 150.00-1150.00 ES+, Centroid, CV=Tune |
| 2 | 34   | 1         | 3Sulfas    | Standard    | W2996            | PDA 220.0 to 400.0 nm at 1.2 nm          |

Figure 156 - 3D channels

3. Click the **3D** icon  to view the 3D plot.
4. Right-click inside the 3D plot area and select **Properties**.

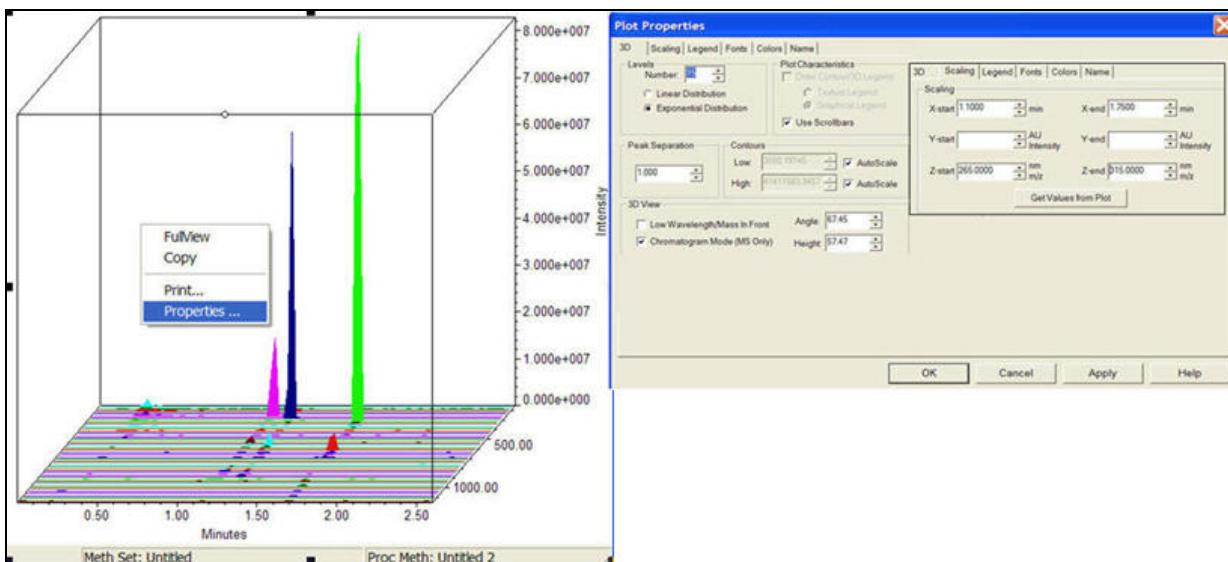


Figure 157 - 3D plot area

5. Set the following parameters in the Plot Properties window:

### 3D tab

- Levels Number 15
- Exponential Distribution Selected
- 3D View Angle 67.00, Height 57.00
- Chromatogram mode Selected

**Scaling tab**

- X-start 1.1
- X-end 1.75
- Z-start 265
- Z-end 315

6. Click **Apply** to display the 3D plot with the masses from the full scan data displayed for each chromatographic peak.

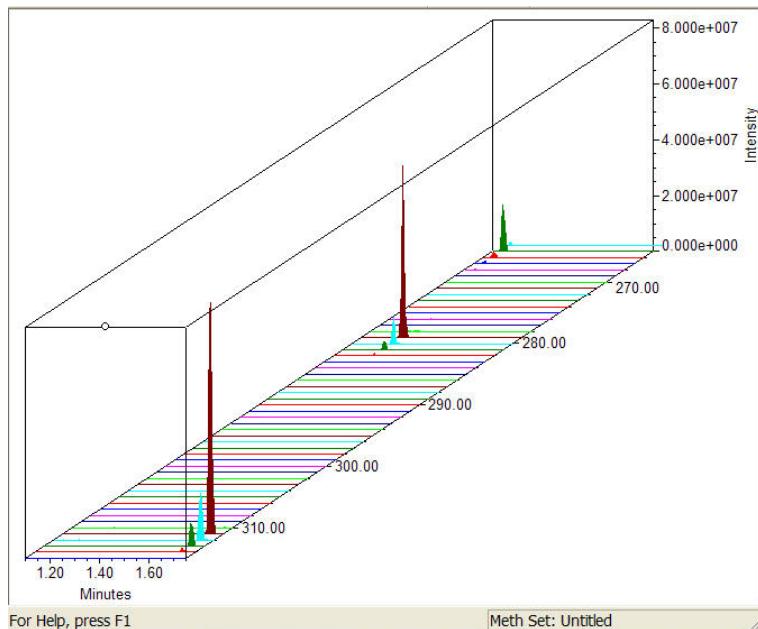


Figure 158 - 3D plot

7. Delete the X-start, X-end and Z-start, Z-end entries in the Scaling tab to reset the scaling to the default data range of the sample.
8. Click **OK** to exit the Plot Properties window.

## Basic PDA data chromatographic extraction

This section shows the basics of PDA chromatogram extraction from scanning data to show PDA spectral information. This will be the same technique for extracting chromatographic and spectral information for MS scanning data.

1. Select the **Main Window** icon  or select **Window > Main Window Switch** from the Menu bar to switch to the Review Main window.
2. Highlight the second row of the 3D channels tab to select the PDA scan data for the sample.

|   | Vial | Injection | SampleName | Sample Type | Channel          | Channel Description                      |
|---|------|-----------|------------|-------------|------------------|--|
| 1 | 34   | 1         | 3Sulfas    | Unknown     | W3100 1: MS Scan | 1: 150.00-1150.00 ES+, Centroid, CV=Tune |
| 2 | 34   | 1         | 3Sulfas    | Unknown     | W2996            | PDA 220.0 to 400.0 nm at 1.2 nm          |

Figure 159 - Selecting PDA scan data

3. Click the **Extract Choices** drop-down list and select **254 nm** wavelength.
4. Click **Enter** or use the **Extract Chromatogram** icon .
5. Manually integrate each of the three peaks by clicking and dragging across the baseline for each of the peaks at approximately **1.16 to 1.24**, **1.29 to 1.39**, and **1.71 to 1.79**.
6. Click **File > Open > Processing Method**.
7. Select “**PDA Extract 254 nm**” and then click **Open**.

**NOTE:** For training purposes we have pre-set a processing for the PDA data.

8. Click the **Integrate** icon  then the **Calibrate** icon  to identify the peaks.
9. Click the **Spectrum Index** tab to show the integrated peak.

**NOTE:** The corresponding spectra above the peak with the same spectra in the spectrum review are shown on the right-hand side. The Review window will be displayed as in Figure 160.

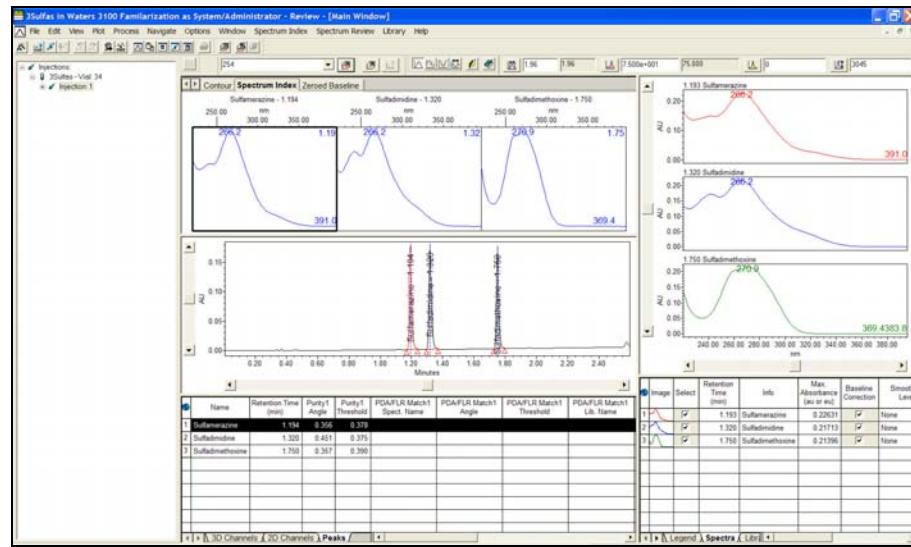


Figure 160 - Spectrum Index

10. Click **File > Save > Result** and then click **OK** to save the chromatogram result of the PDA extraction at 254 nm as the spectra for each component.

**NOTE:** We will use this PDA result combined with an MS result to get a combined PDA/MS report.

## Using PDA data to extract MS chromatograms

This section describes how to extract the MS spectra and chromatogram data from a PDA peak. This can be used to detect an unknown or impurity PDA peak, and to see the MS spectra of the same peak.

1. In the chromatogram view, right-click in the center of the chromatographic peak at retention time 1.19 minutes, and select **Extract MS Spectrum 1.190 min.**

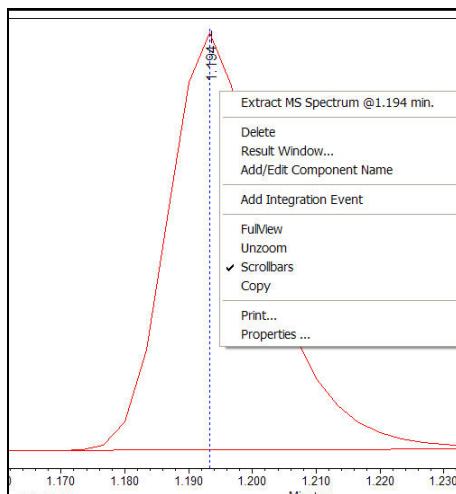


Figure 161 - Extracting a chromatogram

2. Set a time offset of **0.072**, and then click **OK** to display an extracted MS spectrum in the Spectrum Review field, see Figure 162.

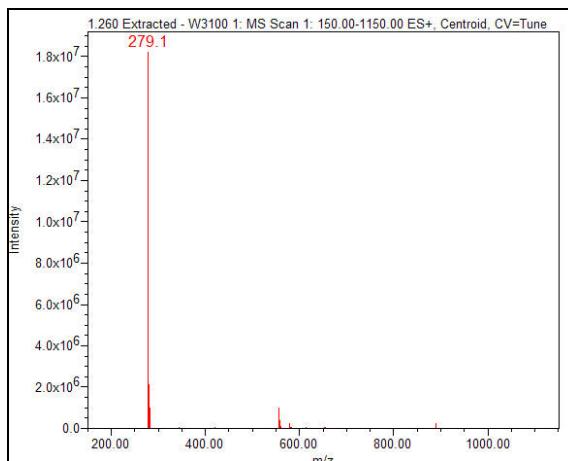


Figure 162 - Spectrum Review field

**NOTE:** The time offset is used to allow for correction of the tubing volume differences between the PDA and MS detector.

3. In the Menu bar of the Review window, click **Spectrum Review > Keep Spectra when Switching Channels**.

**NOTE:** This keeps the previously extracted MS spectra displayed as you extract data from other MS scans. If you switch back to PDA data then only the PDA spectra will be displayed and not the MS spectra.

4. Right-click the MS Spectra at m/z of 279.1 and select **Extract Chromatogram @279.12**, see Figure 163, to display the resulting MS chromatogram at a m/z of 279.12, see Figure 164.

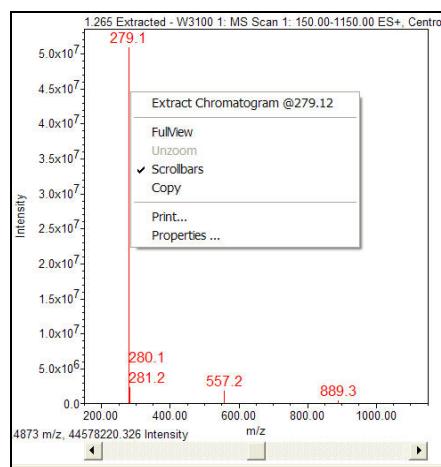


Figure 163 - Extracting chromatogram

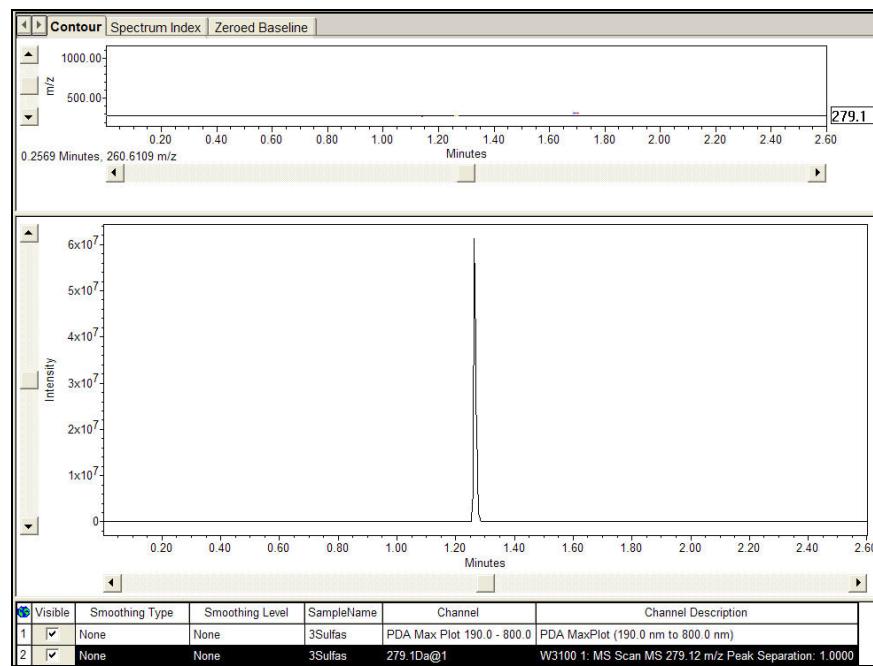


Figure 164 - Displayed chromatogram

**NOTE:** You can use the technique to extract MS data from PDA data but PDA data cannot be extracted from MS chromatograms/spectra.

## Basic extraction of chromatographic data from MS scans

This section describes how to extract chromatographic data from MS full scan data.

1. Click the **2D Channels** tab in the chromatogram description field at the bottom of the Review window to view any mass chromatographic extractions.
2. Click the **Extract Chromatogram** drop-down list and select **TIC Plot**.
3. Click the **Extract Chromatogram** icon  to display the Total Ion Chromatogram (TIC).

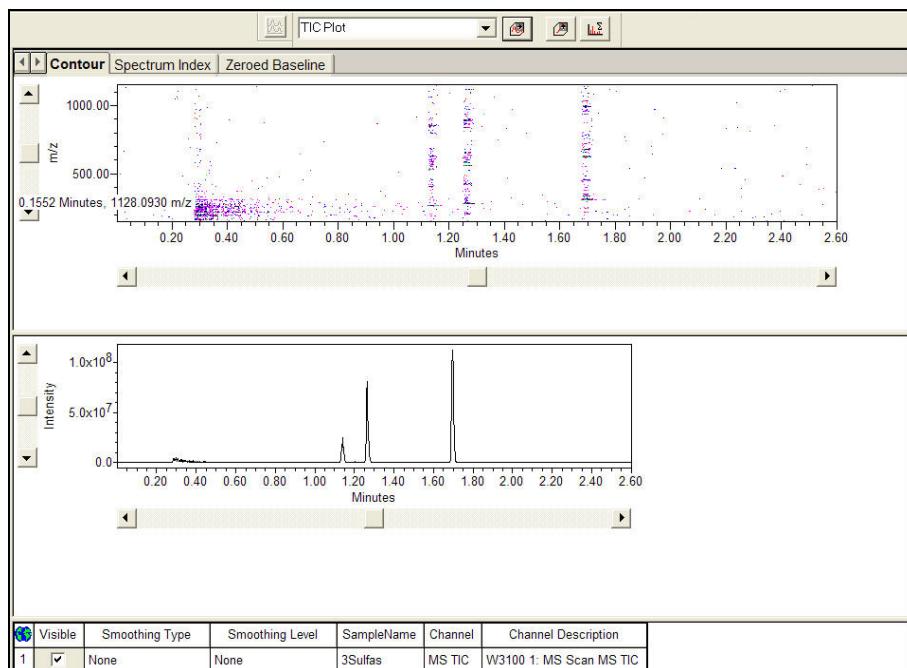


Figure 165 - Selecting a chromatogram

The direct extract chromatogram function can be used to extract specific masses, mass ranges, and addition/subtraction of mass ranges. See the following examples:

- TIC Plot Totals all the scanned masses together and plots the sum of these in the Chromatogram view
- 265.1 a specific mass
- 265.1+311.1 adding two or more masses together
- 265.1-311.1 subtracting two or more masses together
- 200.0:360.0 a range between 200 to 360 amu
- 150:200-200:360 subtract a range of masses from a range of masses

**NOTE:** *Each time you extract a mass, it will be displayed as a different chromatographic channel and added as a channel to the method set. If you extract two TIC chromatograms, you will get two different channels.*

## Qualitative chromatographic processing for full scan MS data

Qualitative identification of peaks can be completed by three methods:

- Manual identification from the Chromatogram window (as displayed below)
- The Processing Method wizard
- Manually using the Processing Methods Component field

**NOTE:** To see how to use the wizard or processing methods component field, see the procedure to Processing SIR/MRM External Standard Data with Empower.

1. In the 2D Channel display, set the smoothing type to **Mean** and smoothing level to **9** to display a smoothed chromatogram.

**NOTE:** MS chromatographic data is usually smoothed to eliminate the slight signal variations between data points.

2. Click the **Integrate** icon  or click **Process > Integrate**.
3. Click inside the peak at **1.141 minutes** and click the **Set Processing Method Minimum Height** icon .

**NOTE:** If you do not see the Set Processing Method Minimum Height icon, click **View > Toolbar > Integration** from the Menu bar to display the Integration toolbar.

4. Click the **Integrate** icon  again to see that only three peaks will be integrated, as well as each peaks apex spectrum displayed in the Spectrum Review field.

**NOTE:** You can manually integrate peaks by clicking and dragging the mouse across the baseline of each peak of interest.

5. Right-click inside each of the three peaks and select **Add/Edit Component Name** to identify components from the chromatogram view.
6. Enter the component name **sulfamerazine** for the first peak at 1.41 minutes; **sulfadimidine** for the 2nd peak at 1.26 minutes; and **sulfadimethoxine** for the 3rd component at 1.697 minutes; to automatically insert the name and retention time into the processing methods component table for each peak.

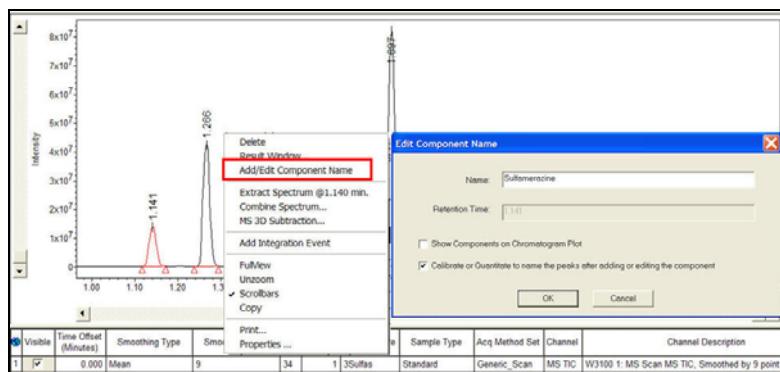


Figure 166 - Adding/editing component names

7. Click the **Integrate** icon  and then the **Calibrate** icon .

**NOTE:** The Quantitate icon  is used for samples labelled as unknowns.

8. Click the **Spectrum Index** tab and then the **Peaks** tab.

**NOTE:** The peaks table may look different from that displayed in Figure 167.

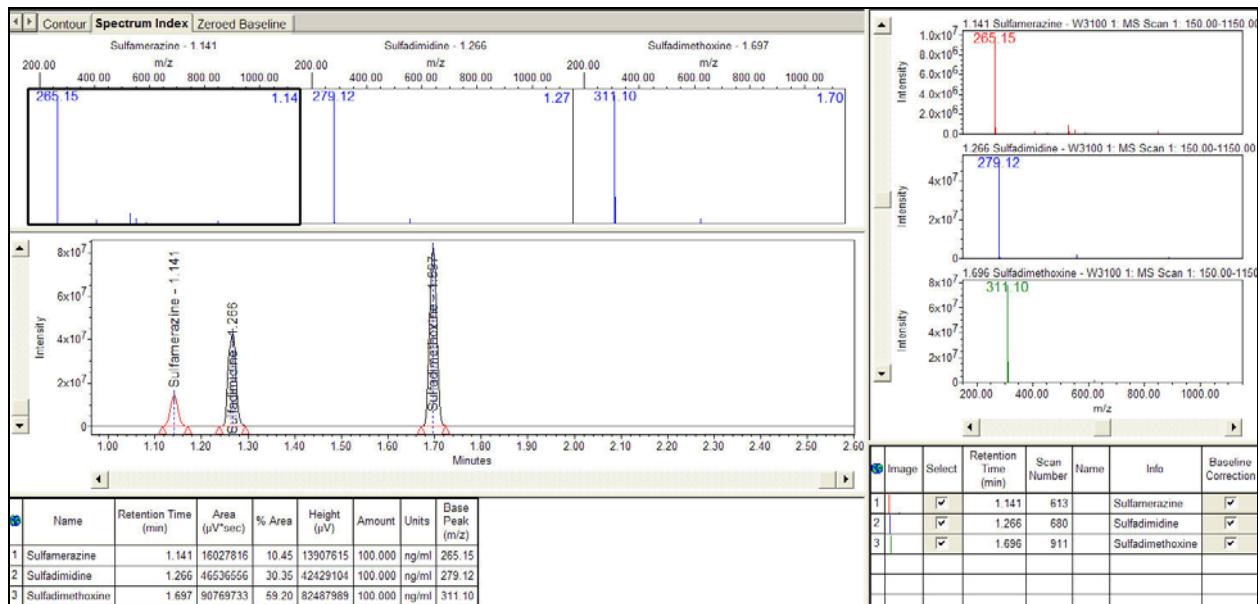


Figure 167 - Spectrum index

9. To change the peaks table, right-click the **World** icon  to set the Peak Table properties, then click **File > Save Preferences**.

10. Click **File > Save Processing method** and type **SulfaMix Qual** as the method name.

11. Click the **Method Set** icon  to view the chromatographic extractions and processing/report functions.

**NOTE:** The *Derived Channels* field is displayed with each extraction as well as the Channel table displaying the extractions and the processing/report method selections.

*Derived channels are added each time you extract an MS or PDA chromatogram. These may be saved for repetitive analysis of the same type of data.*

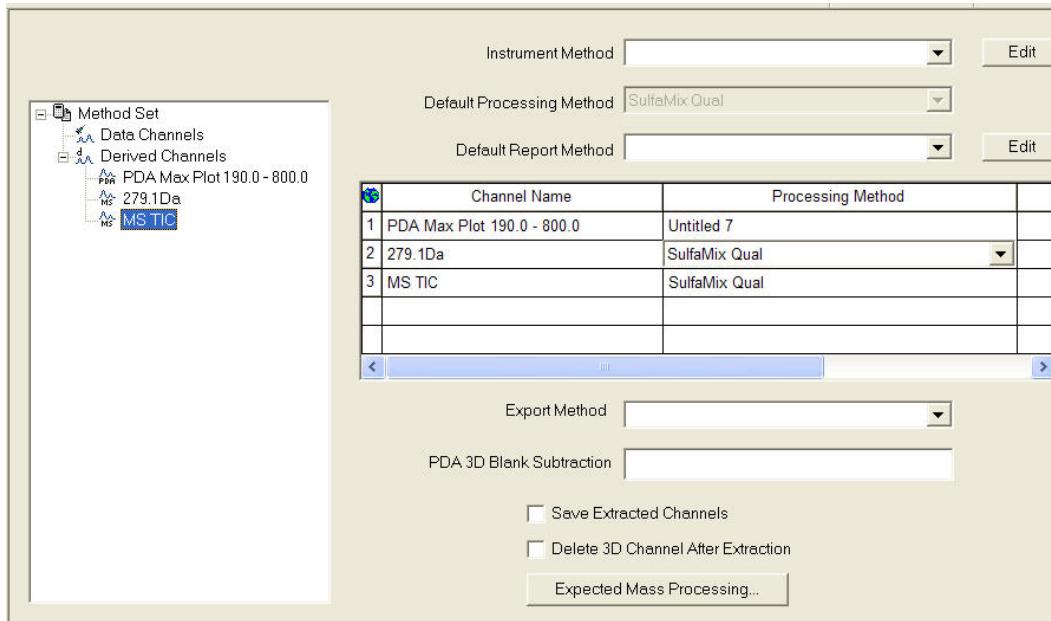


Figure 168 - Derived channels

12. Delete all derived channels, except for the MS TIC, from the Derived Channels field on the left-hand side of the window.

**NOTE:** *Deleting derived channels also removes these from the Channel table in the method set.*

13. In the Report Method field, select a pre-defined report method of **Basic MS Full Scan Report**.

14. Click **File > Save as > Method Set** and type **Sulfa Mix scan** as the method name, to save the extracted channels and avoid the extraction procedure being repeated.

15. Click the **Review Main Window** icon  to view the Chromatogram window.

16. Click the **Apply Method Set** icon  to see the extracted TIC chromatogram and the processing method applied to it.

17. Click **File > Save Results** to save the processed MS data as a result.

## Using MS spectra from MS chromatograms

This section describes the basics of the MS Spectrum Review window.

1. Box and zoom in on the peak at 1.69 minutes and then click the **Combine Spectrum** icon .
2. Type **1.7:1.8 minutes** as the average time to combine in the Average (min): box, or right-click and drag across the peak at half height.
3. Right-click in the Subtract (min) box before the peak start and after the peak end from **1.60 to 1.65**, and **1.75 to 1.80**.
4. Click **OK** to display a combined spectrum in the Review window.

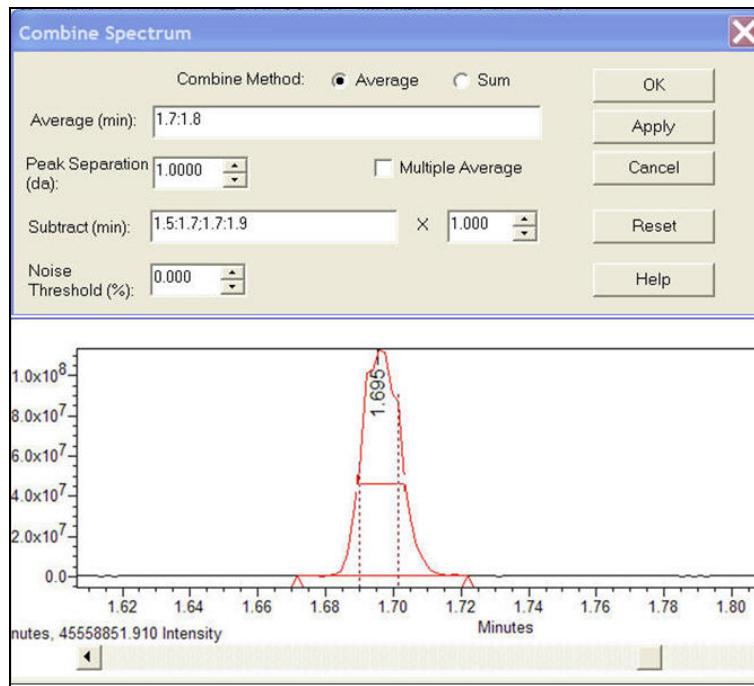


Figure 169 - Combined Spectrum window

**NOTE:** Combined spectrum can be used to subtract baseline noise and average noise spikes during data collection. This can be used when analyzing the spectra of very small analytes.

5. Right-click the **World** icon  in the Spectrum Review Table (on the right-hand side of the window) to set the columns to be displayed.
6. Click **Hide All**, then deselect the columns required to be displayed, see Figure 170.

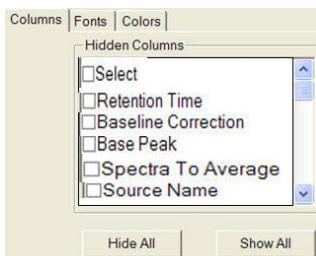


Figure 170 - Peak table columns to be displayed

**NOTE:** The information in the table includes the base peak. The base peak is the m/z with the highest peak intensity (usually the molecular ion of the compound).

|   | Select                              | Retention Time (min) | Baseline Correction                 | Base Peak (m/z) | Spectra To Average | Source Name   |
|---|-------------------------------------|----------------------|-------------------------------------|-----------------|--------------------|---|
| 1 | <input checked="" type="checkbox"/> |                      | <input checked="" type="checkbox"/> | 311.1           | 55                 | 3Sulfas - W3100 1: MS Scan 1: 150.00-1150.00 ES+, Centroid, CV=Tune |
| 2 | <input checked="" type="checkbox"/> | 1.141                | <input checked="" type="checkbox"/> | 265.2           | 1                  | 3Sulfas - W3100 1: MS Scan 1: 150.00-1150.00 ES+, Centroid, CV=Tune |
| 3 | <input checked="" type="checkbox"/> | 1.266                | <input checked="" type="checkbox"/> | 279.1           | 1                  | 3Sulfas - W3100 1: MS Scan 1: 150.00-1150.00 ES+, Centroid, CV=Tune |
| 4 | <input checked="" type="checkbox"/> | 1.696                | <input checked="" type="checkbox"/> | 311.1           | 1                  | 3Sulfas - W3100 1: MS Scan 1: 150.00-1150.00 ES+, Centroid, CV=Tune |

Figure 171 - Spectrum Review table

7. Right-click in the Spectrum Review Plot field, and click **Properties**.
8. Click the **Spectra** tab and set the following parameters:
  - MS Labels                    Multiple Masses
  - Intensity Label Level    2
  - Mass Precision            1
9. Click the **Legend** tab.
10. Click **MS Spectra > Source name** and then click **OK**.

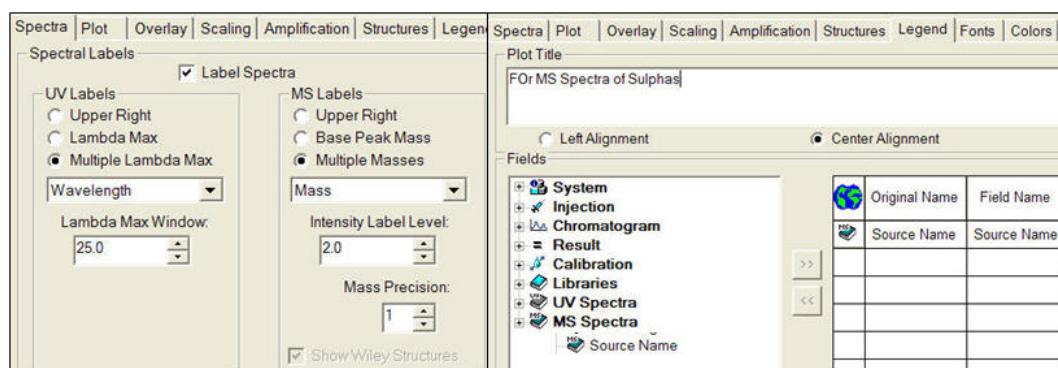


Figure 172 - Selecting MS spectra source name

**NOTE:** This shows more details of the mass intensities when displaying spectra information. These properties can be used for adding print information about the spectra directly from the Review window.

11. Click **File > Save Preferences** to display these fields in the spectrum review every time the Review window is opened.
12. Click inside the spectrum review plot, then click **File > Print Preview** to display the spectra with a description.

**NOTE:** This can be used to customize MS or PDA spectra for printouts.

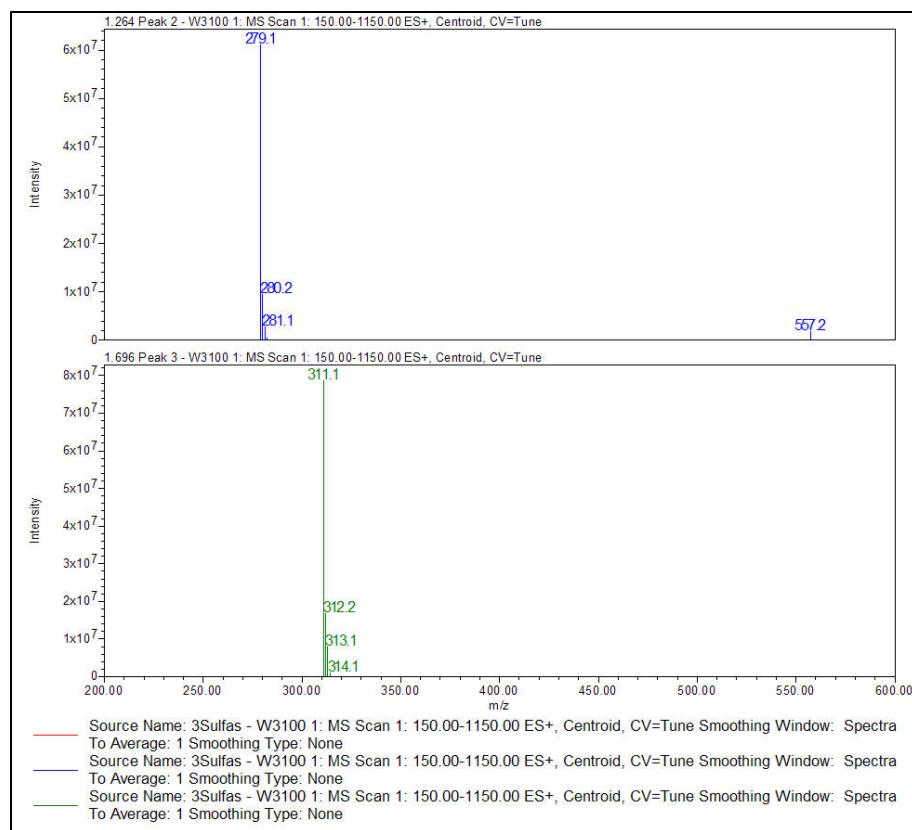


Figure 173 - Spectra print preview

13. In the Menu bar of the Review window, click **File > Exit**.

## Summary of MS review process

### Full scan qualitative data

The general process is to extract the MS data, integrate it, identify the peaks, and view the spectral information in either spectrum index or spectrum review. The chromatographic extractions may be saved in a processing method and method set for future analysis of similar samples.

### Full scan quantitative data

The general process is to extract the MS data, create a processing method, select a report method, and save the method set; then, exit review and batch process the sample set or injection data files. The processed results will show the processed data with area, heights, and amounts (if quantitation date was configured and set up).

### Batch processing for full scan MS data

Once you have set up a method set that includes extracted MS channels and a processing method, you can batch process a series of samples from a sample set to get extracted channels and processing for each of the injections.

1. In the Project window, click the **Injections** tab.
2. Click the **Filter By** drop-down list and select **3 Sulfas Injection**.
3. Select the **3Sulfas** sample injection then click the **Process** icon .
4. In the Process field, click **Use specified method set**.
5. Click the drop-down list and select **Sulpha Mix Scan**.
6. Click **Clear Calibration**.
7. Click **How:** and select **Calibrate and Quantitate**.
8. Leave all other settings set to default and click **OK** to process the data and create the results.

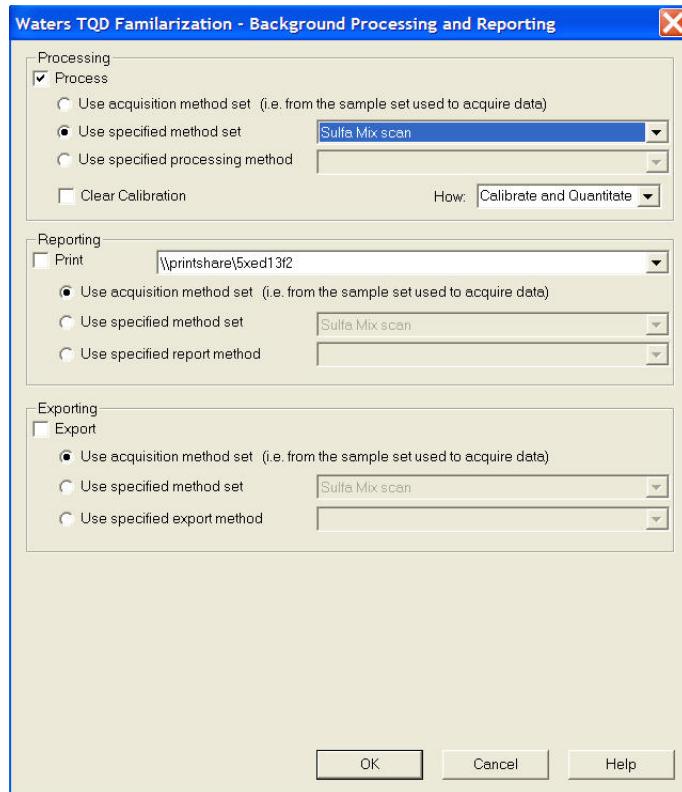


Figure 174 - Batch processing for full scan MS data

**NOTE:** For MS or PDA scanning data, process the data using "Use acquisition method set" or "Use specified method set".

## Viewing the processed results in review

Once the sample set has been processed we will review the processed results from the results set or results view in the Review window.

1. Click the **Results** tab.
2. Click the **Filter By** drop-down list and select **Sulfa Scan Results**.

| Sample Name | Vial | Sample Type | Channel          | Channel Description                      |
|-------------|------|-------------|------------------|--|
| 1 3Sulfas   | 34   | Standard    | W3100 1: MS Scan | 1: 150.00-1150.00 ES+, Centroid, CV=Tune |
| 2 3Sulfas   | 34   | Standard    | W2996            | PDA 220.0 to 400.0 nm at 1.2 nm          |

Figure 175 - Selecting results

3. Select both the **3Sulfas MS** and **PDA Results** and click the **Review** icon  to display the processed data in the Review window, see Figure 176.

**NOTE:** The Review window is displayed as it was last viewed. If the display is not as in Figure 176, click the **Review Main Window** icon, click **View > 3D Layout**, and then click the **Spectrum Index** tab.

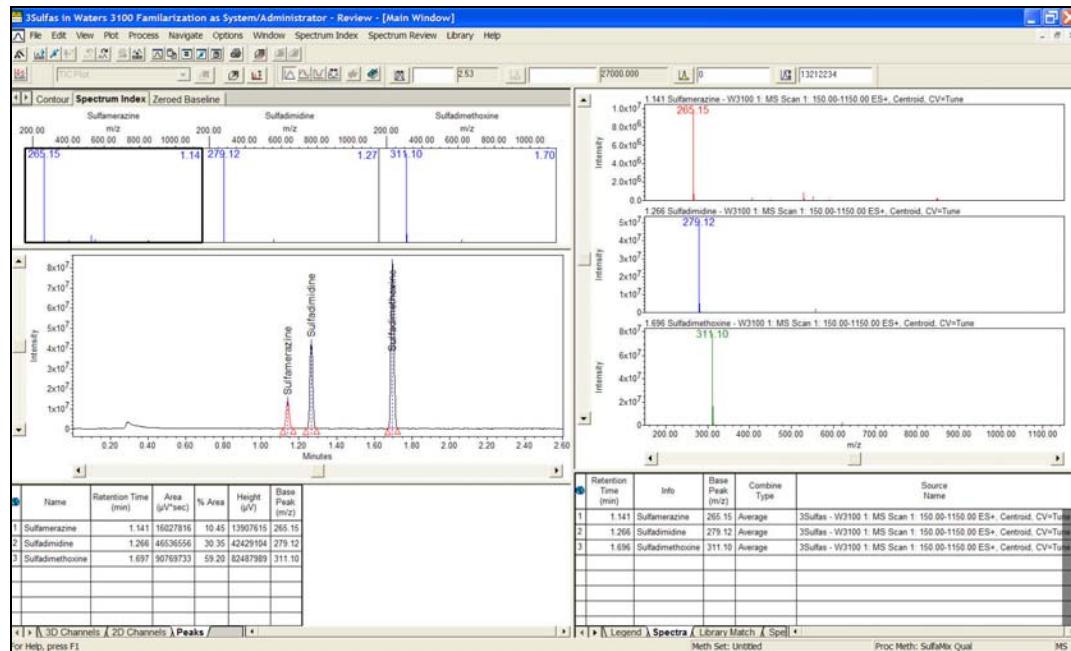


Figure 176 - Displayed results

- Right-click in the Chromatogram window and select **Properties**.
- In the Plot Properties window, click the **Overlay** tab and select the **Make Stack Plot** check box.
- Select the **All Choms in Overlay** check box, and then click **OK** to display both the MS and PDA chromatograms, see Figure 178.

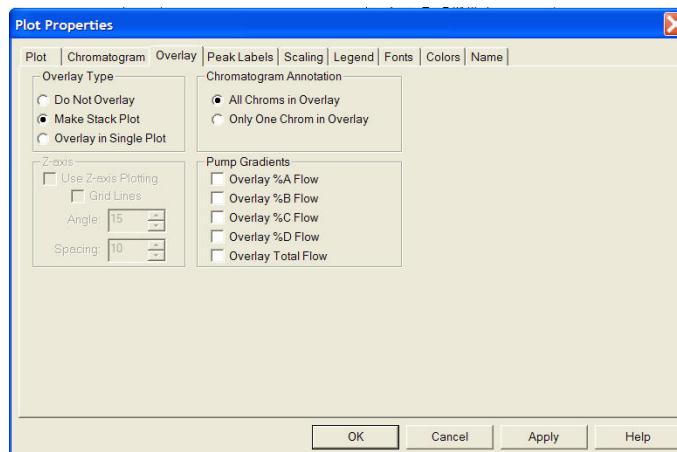


Figure 177 - Plot Properties window

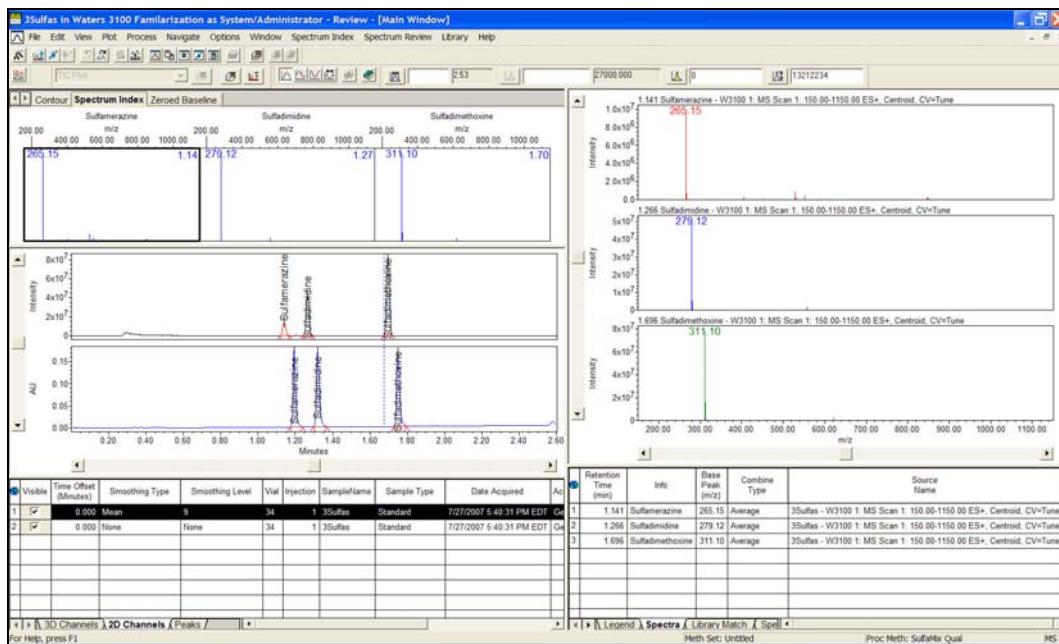


Figure 178 - Chromatograms in overlay

**NOTE:** The PDA data is offset from the MS data because this analysis used a split flow between the PDA and MS detectors.

- Click **File > Exit** to exit the Review window.

## Viewing the processed results in preview/publisher for MS only and MS/PDA combined reports

1. In the Project window results view select **3Sulfas**.
2. Click the **Preview** icon , or right-click and select **Preview-Publisher**.
3. In the Preview window select the **Use the following Report Method** check box.
4. Select **3 Sulfas Scanning Report** from the drop-down list and then click **OK**.

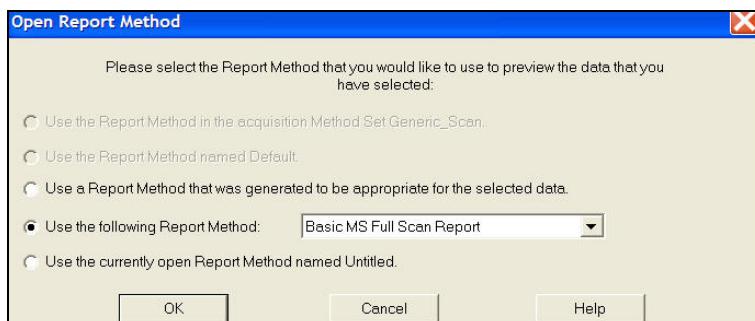


Figure 179 - Open Report Method window

**NOTE:** The Basic MS Full Scan report can be used as a template for basic individual reports of MS full scan data. It includes three sections: Chromatogram and Peak results table, Spectrum Index Plot, and MS Spectra of the apex of each integrated known peak. Figure 171 shows an example of this report.

5. Click the **Next Page** icon  and the **Back Page** icon  to view all the pages of the report.
6. Click the **Save PDF** icon  to save as a PDF, or the **Print Report** icon  to print the report.
7. Click the **Open Report** icon .
8. Select **Basic MS PDA Full Scan Report** from the drop-down list and then click **Open**.

**NOTE:** The Basic MS PDA Full Scan report can be used as a template for basic combined MS and PDA full scan data. It includes three sections: Overlaid Chromatograms of MS and PDA, a combined MS and PDA peak results table, and an MS and PDA Spectra of the apex of each integrated known peak.

9. Click **Close** to edit and customize the report as required.
10. To add the MS Tune and MS Calibration information to a report, select the **Comp Status Reports group** and click and drag it to the Report page.
11. Click the **Preview** icon  to see the amended report.
12. Click **File > Exit** to close the Report Publisher.

## Examples of reports

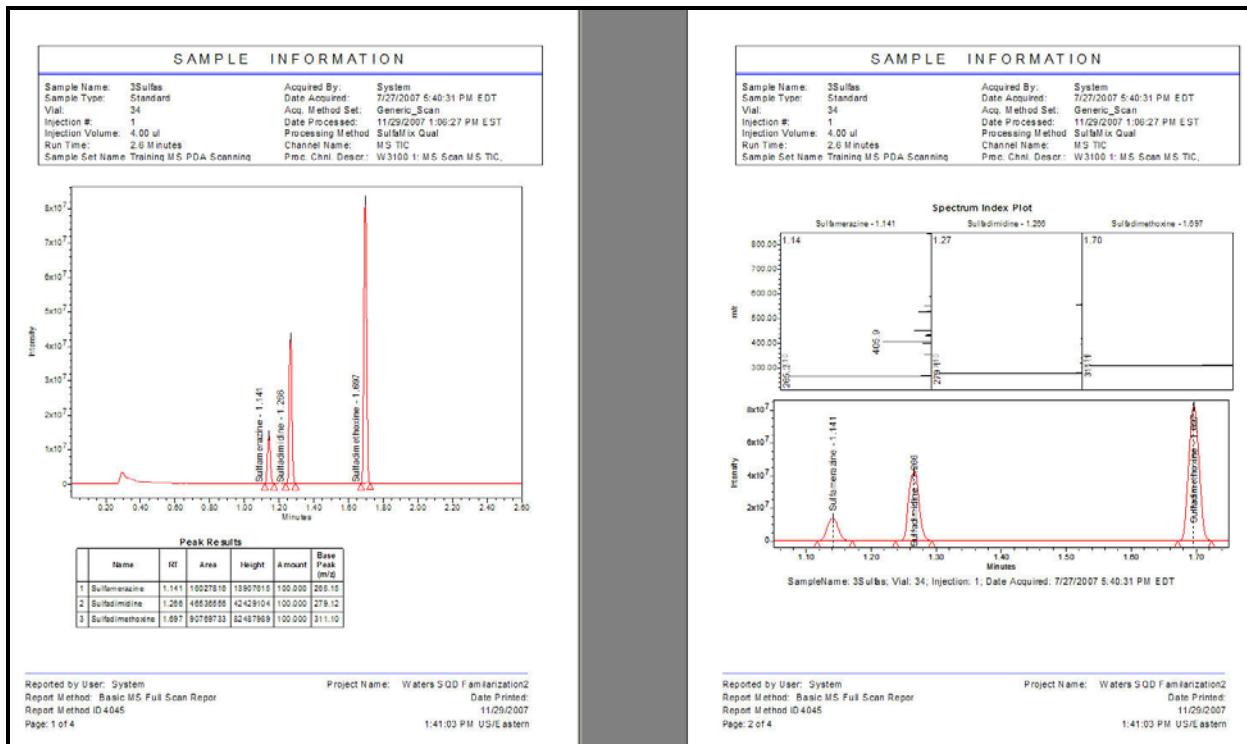


Figure 180 - Example Basic MS Full Scan Report

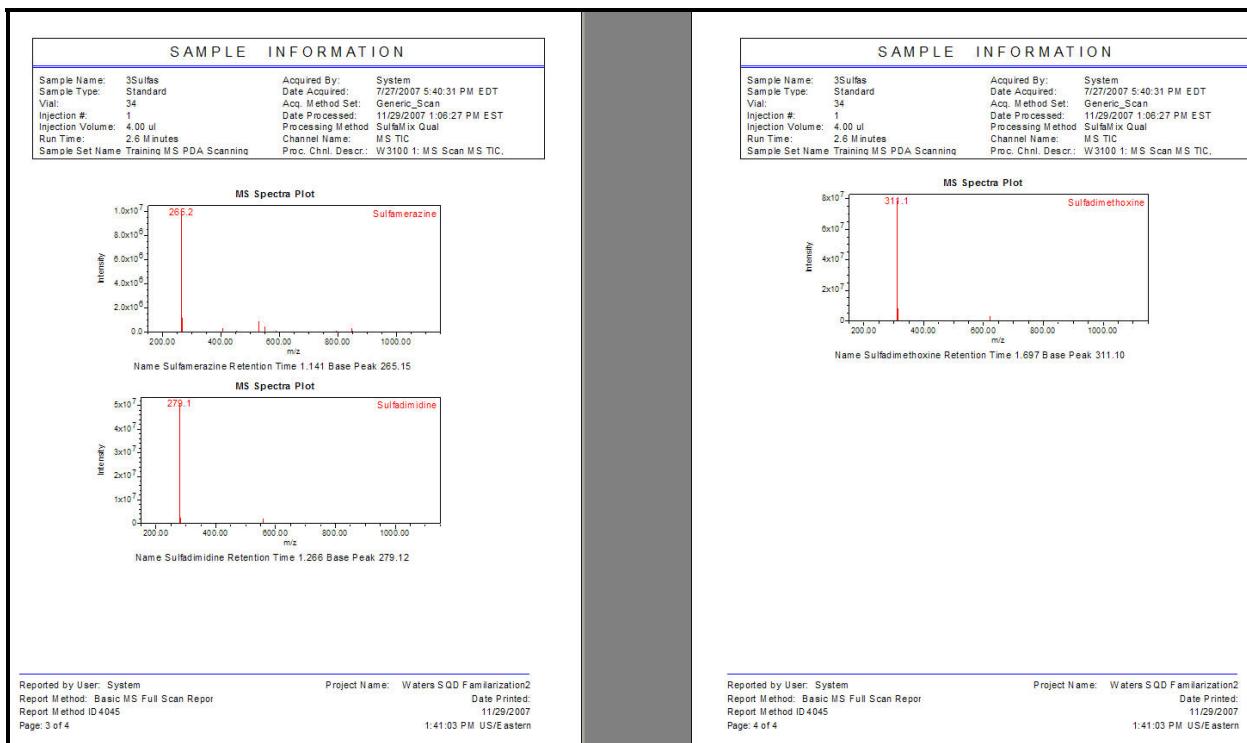


Figure 181- Example 2 of Basic MS Full Scan Report

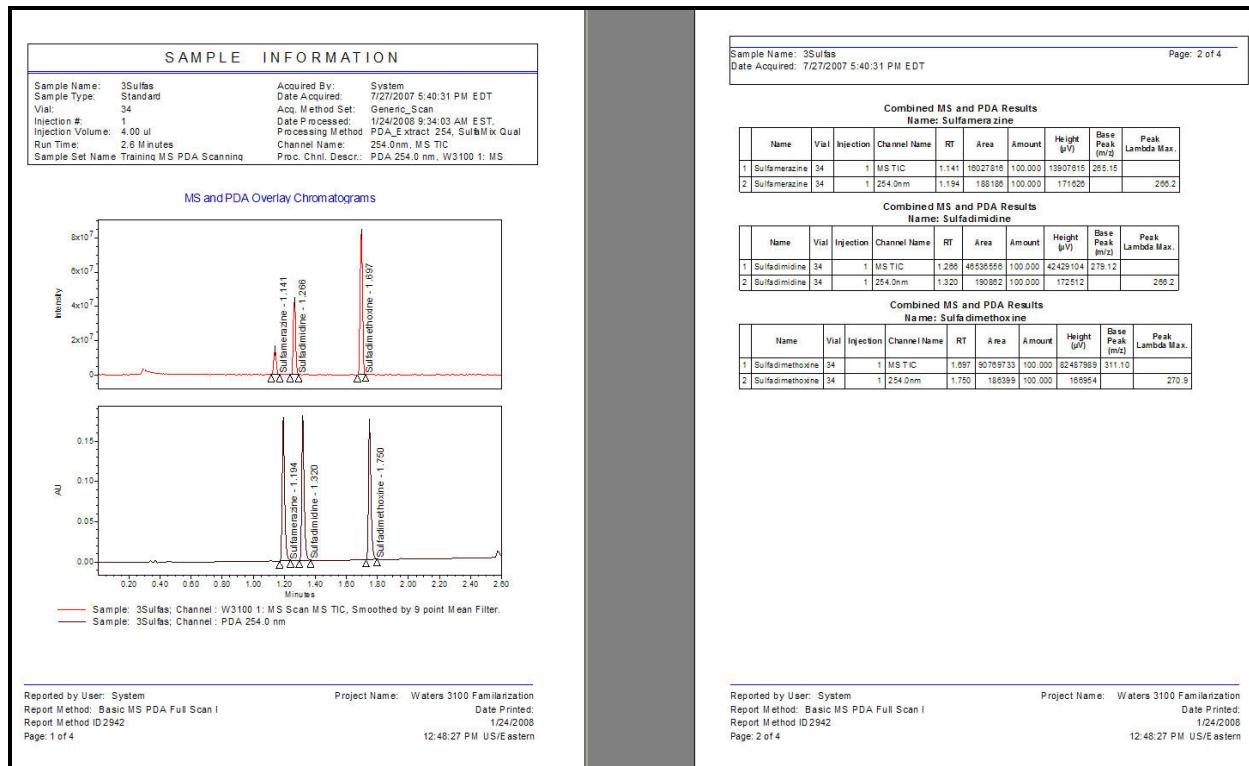


Figure 182 - Example MS/PDA Combined Full Scan Report

**5**

## Extraction of Chromatographic Data from MS Scans (optional)

This generic procedure describes some of the advanced MS scanning functions in Review. Before performing this section you must complete the "MS Basic Processing with Full Scan Data" section in this document.

The procedure consists of the following four sections:

- Selecting projects for review ..... 5-1
- Advanced chromatographic extraction of MS data from the Method Set dialog box.. 5-2
- MS spectra library functionality with MS chromatograms..... 5-6
- Component base expected mass MS processing ..... 5-10

Brompheniramine and chlorpromazine are used for the procedures in this section as they have the same nominal mono-isotopic mass.

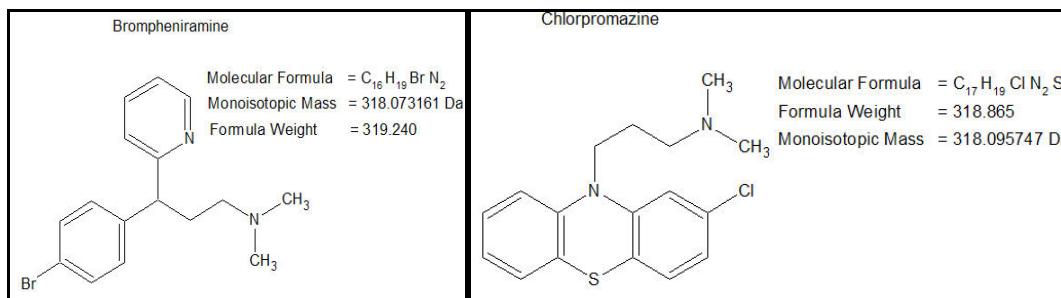


Figure 183 - Molecular structures of brompheniramine and chlorpromazine

### Selecting projects for review

1. Click the **Browse Project** icon .
2. Select the appropriate **TQD/SQD/3100 Empower Familiarization** project, and then click **OK**.
3. Click the **Sample Sets** tab.
4. Select **Training MS PDA Scanning**.
5. Right-click and select **View As > Channels** to display only the MS data that was collected for this analysis.
6. Select **brompheniramine** and **chlorpromazine**, and click the **Review** icon , or right-click and select **Review** to display MS data in the Review window.

|   |                 |    |   |          |                          |                  |  |
|---|-----------------|----|---|----------|--------------------------|------------------|--|
| 5 | Brompheniramine | 29 | 1 | Standard | 7/27/2007 4:56:10 PM EDT | W3100 1: MS Scan | 1: 150.00-1150.00 ES+, Centroid, CV=Tune |
| 6 | Chlorpromazine  | 31 | 1 | Standard | 7/27/2007 5:13:53 PM EDT | W3100 1: MS Scan | 1: 150.00-1150.00 ES+, Centroid, CV=Tune |

Figure 184 - Selecting channels

## Advanced chromatographic extraction of MS data from the Method Set dialog box

- Click the 3D Channels table and then select **chlorpromazine**.

|   | Vial | Injection | SampleName      | Sample Type | Channel          |
|---|------|-----------|-----------------|-------------|------------------|
| 1 | 31   | 1         | Chlorpromazine  | Standard    | W3100 1: MS Scan |
| 2 | 29   | 1         | Brompheniramine | Standard    | W3100 1: MS Scan |

Figure 185 - 3D Channels table

- In the Review window, click the **Method Set** icon  to access the advanced chromatographic extraction and processing functions.
- In the Method Set dialog box, right-click and select **New > TIC** to create a total ion chromatogram for the chlorpromazine sample.

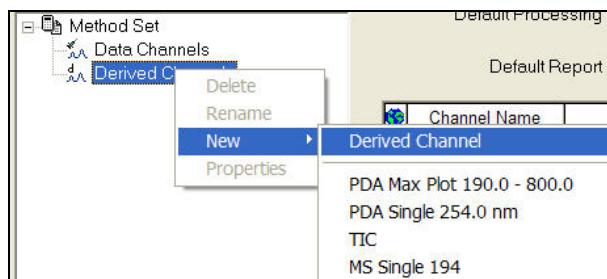


Figure 186 - Selecting a method set

- In the Method Set dialog box, right-click and select **New > Derived Channels**.
- Use the derived channels functions to create the following MS chromatograms:
  - Single mass channel: This is the same as using the Chromatographic Extraction bar.

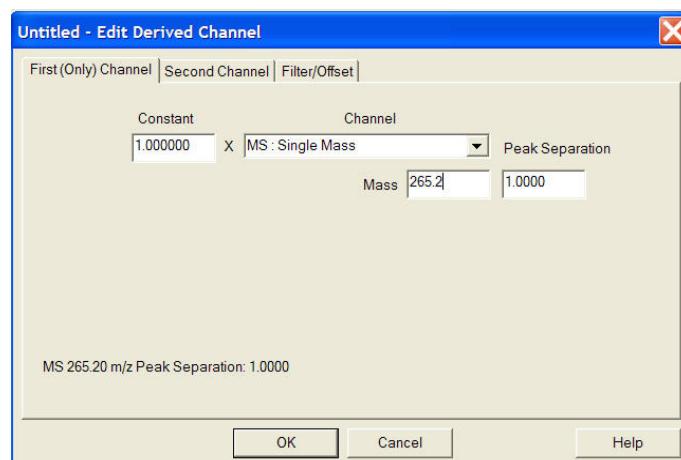


Figure 187 - Single mass channel

- b. Calculated mass chromatogram: Use this function to add two or more different masses together, subtract one mass from another, or multiply by one mass to another.

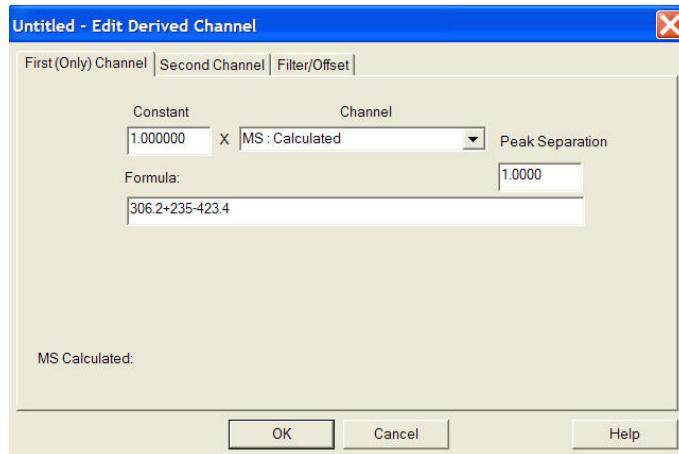


Figure 188 - Calculated mass chromatogram

- c. Timed mass chromatogram: This creates one chromatogram that contains a change of mass at a specific time. This can be used instead of extracting three different chromatograms for the three different masses that are the molecular ion for each component.

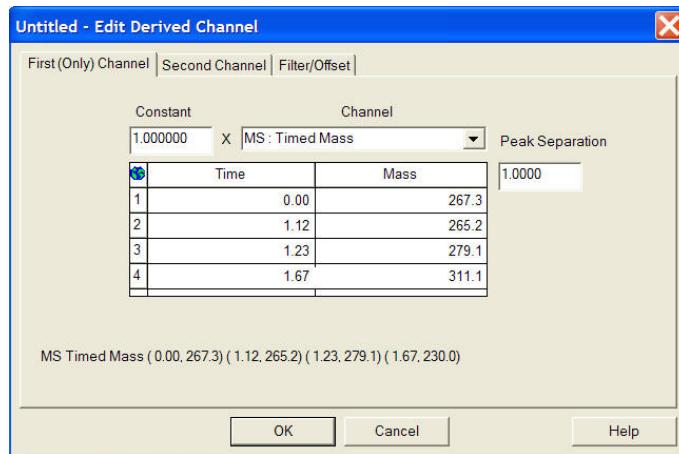


Figure 189 - Timed mass chromatogram

- d. Max plot: Plots each data point of the scanning data at its maximum mass intensity. Some programs call this a "Base Peak Intensity chromatogram".

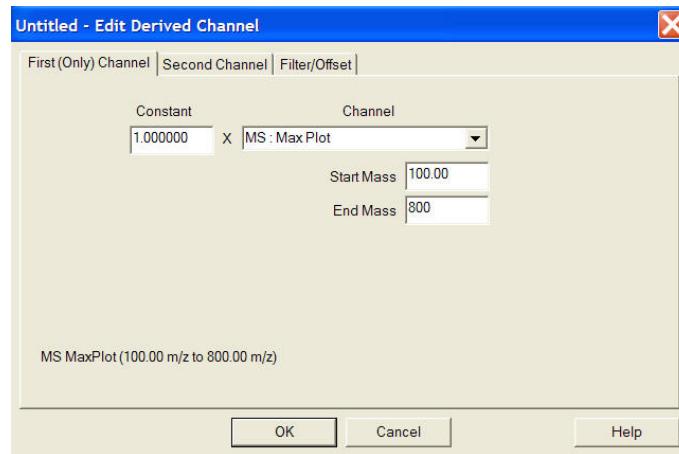


Figure 190 - Max plot

- e. Ratio plot of two masses: Used to get an ion ratio for full scan data. This can be used for confirmation of component identity as a specific compound will have a distinct fragmentation pattern.

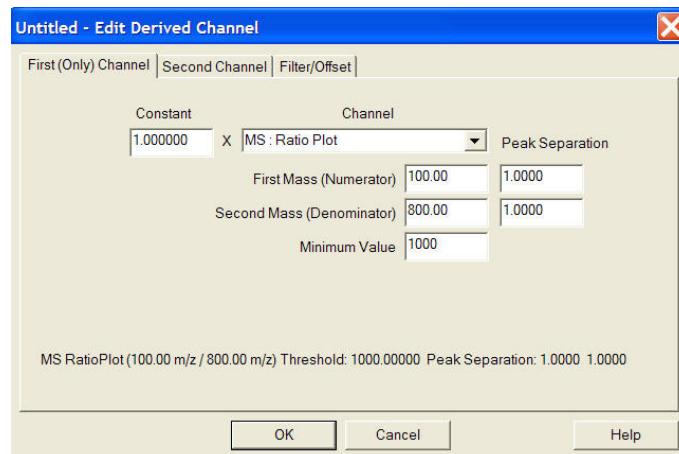


Figure 191 - Ratio plot of two masses

- f. Total plot of a range of masses: Used to create a TIC only at a specific mass range. This can be used to skip a mass range of the TIC that may have solvent interference.

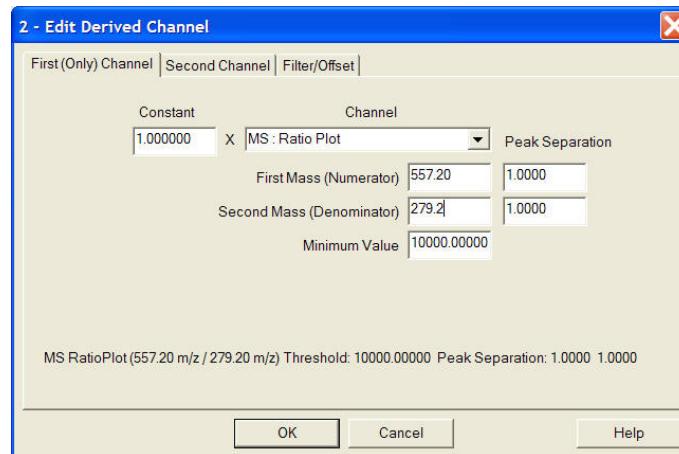


Figure 192 - Total plot of a range of masses

6. Right-click and selecting **Delete** to delete each of the derived channels except the TIC chromatograms.

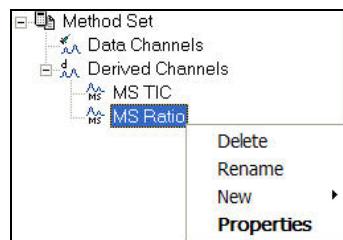


Figure 193 - Deleting the derived channels

7. Click the **Review Main Window** icon  to return to the chromatogram and spectra display.

## MS spectra library functionality with MS chromatograms

The MS Library matching function was designed for EI libraries (Electron Impact) and will not give proper numerical matches for API spectra. The library function can be used for visual comparison between samples, though the matching algorithm may give incorrect numbers or may not match. This section describes how to use library matches.

1. In the Menu bar of the Review Main window, click the **2D Channel** tab.
2. Set **Mean** as the smoothing type with a smoothing level of **9**.

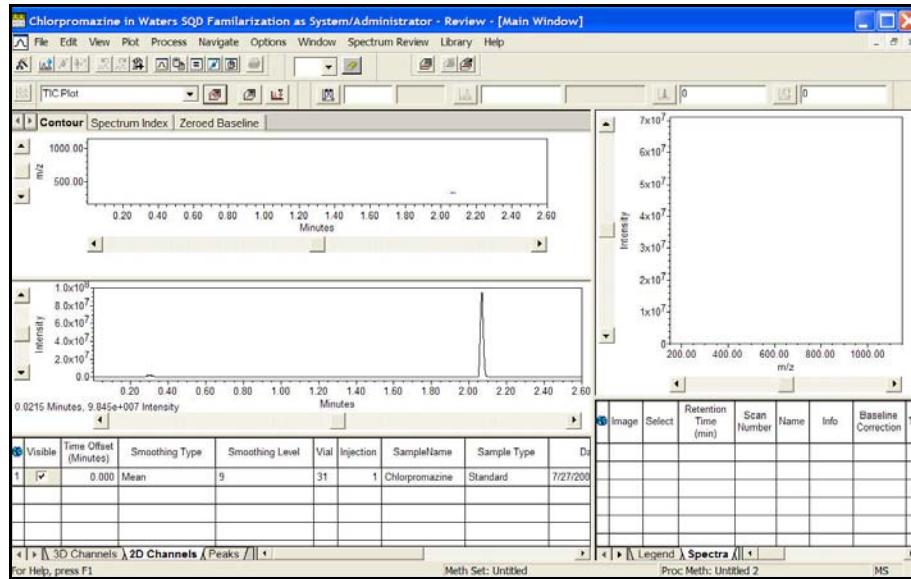


Figure 194 - Selecting a smoothing type

3. Manually integrate the peak at **2.069 minutes** by dragging and dropping the cursor across the peak. The spectra will be displayed in the Spectrum Review window.

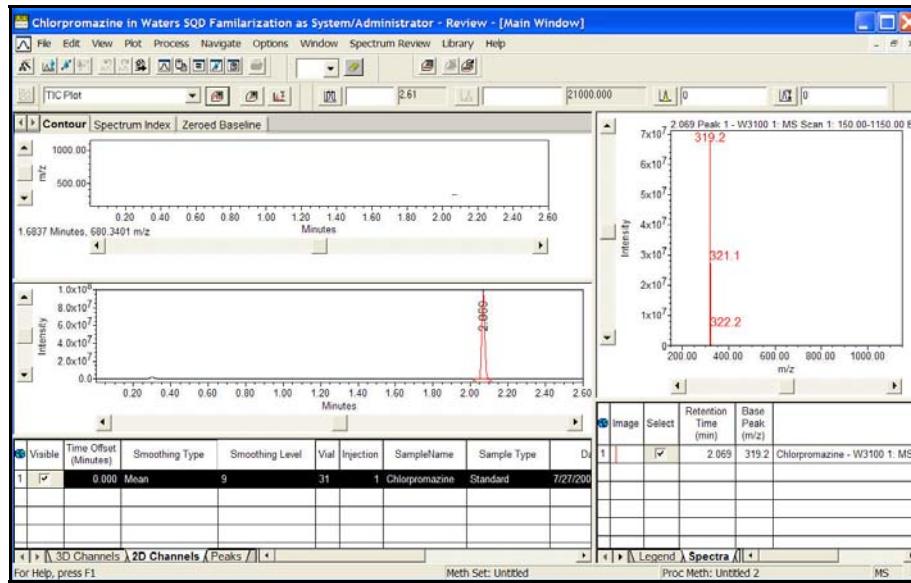


Figure 195 - Spectra displayed in the Spectrum Review window

4. Click **Library > New Library**.
5. Type **Chlorpromazine** as the name and then click **Create**.

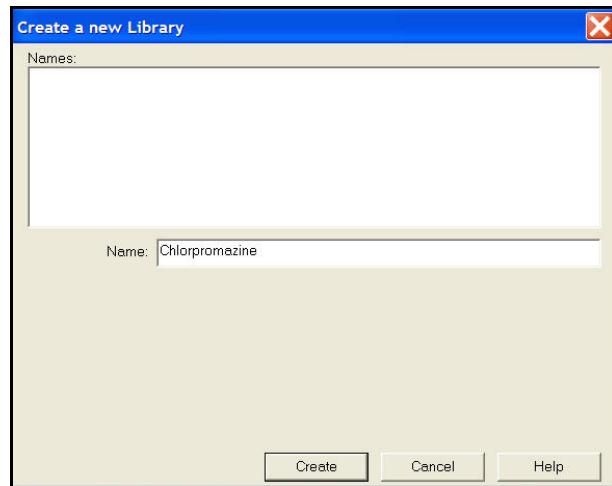


Figure 196 - Create a new Library window

6. Click **Library > Add to Library Chlorpromazine**.

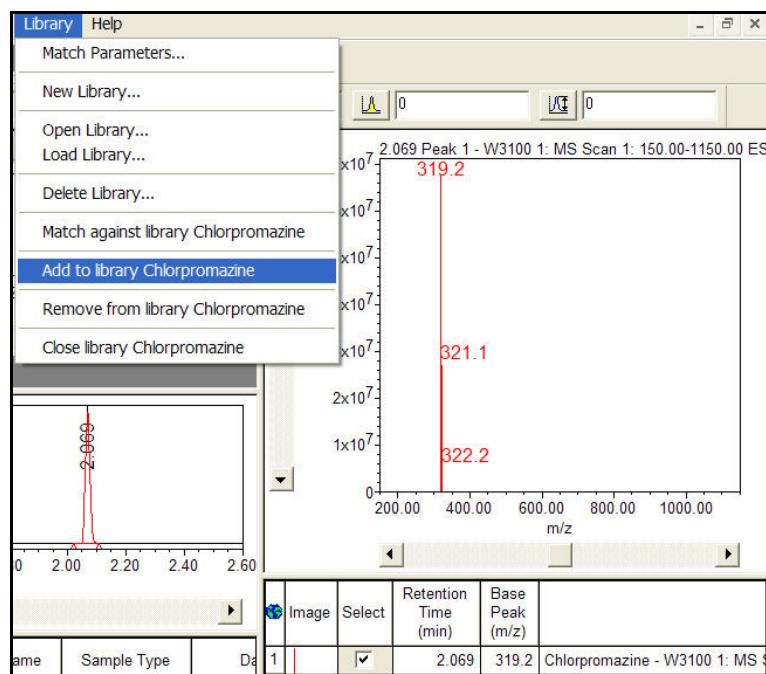


Figure 197 - Adding to library

7. Type **Chlorpromazine** as spectra one, and then click **OK** to add this spectra to the MS library.

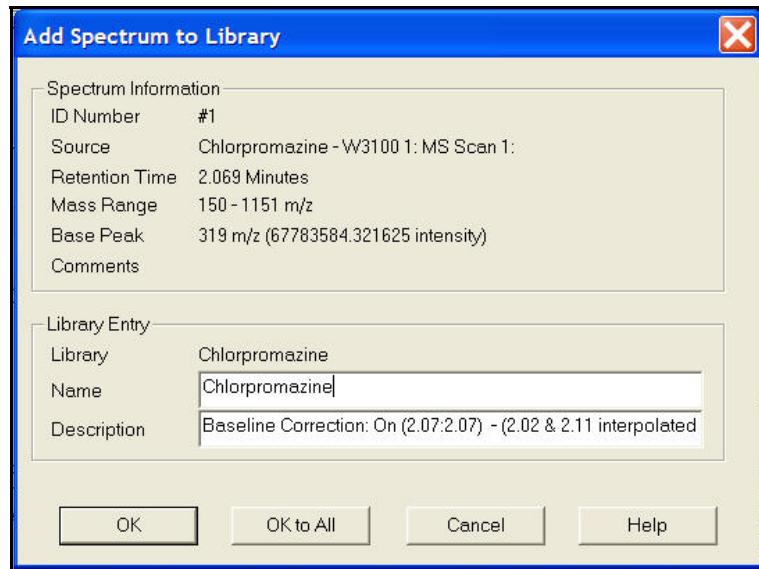


Figure 198 - Add Spectrum to Library window

**NOTE:** The Spectrum Review window is now displayed with two spectra, the original spectra and the spectra of chlorpromazine added to the library.

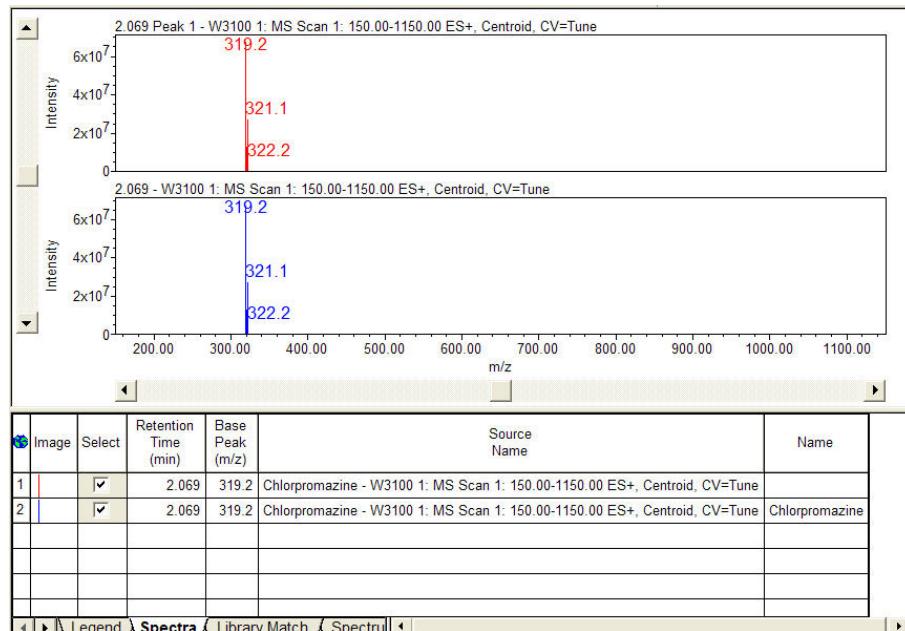


Figure 199 - Updated Spectrum Review window

8. Click **Chromatogram > 3D Channels** and select **Brompheniramine**, to search the library against a different sample.
9. Smooth the peak and manually integrate, and the brompheniramine spectra will be displayed below the chlorpromazine spectra.

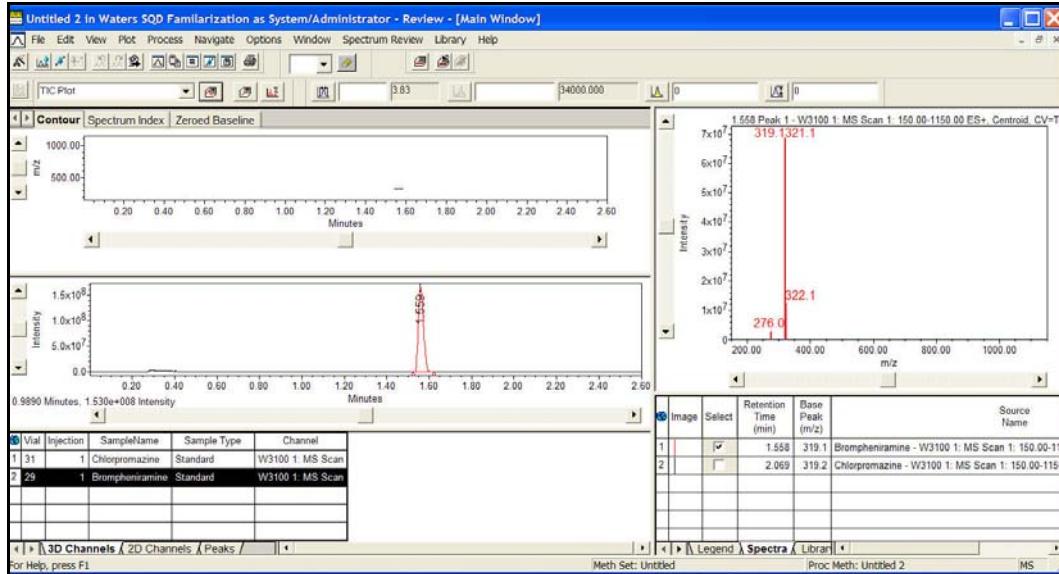


Figure 200 - Displaying multiple spectra

10. Click **Library > Load Library** to load spectra from libraries using a different sample.

**NOTE:** You can use the visual selection bar to visually compare two different MS spectra from different samples. Do not use the library match function.

11. In the Select column in the Spectrum Review table, select both **brompheniramine** and **chlorpromazine** to display both spectra in a stack plot.

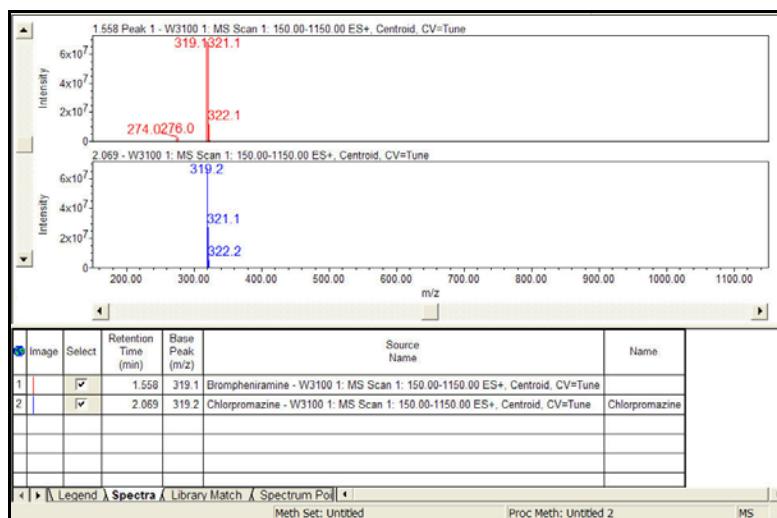


Figure 201 - Selecting to display both spectra

**NOTE:** This library can be added to the processing method, though the library function is more of a manual visual comparison process when following this procedure.

If you try to use the library matching routine by selecting File > Match against Library, you may get an error, see Figure 202.

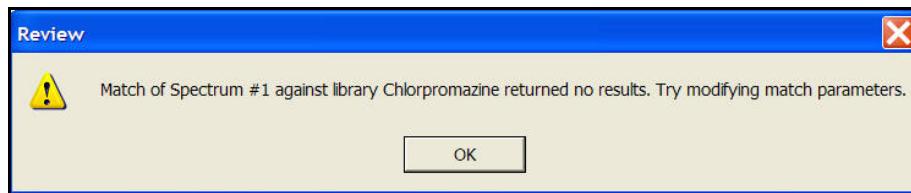


Figure 202 - Library matching error

## Component base expected mass MS processing

Expected mass processing is used to process a sample that should have a specific m/z (s).

For each component, you can set up to five expected masses for fragmentation confirmation of a component.

Expected mass processing uses the MS expected mass tab of the processing method and the component table. When Empower processes 3D scanning MS data, the apex spectrum is extracted for each chromatographic peak in the derived channel.

The software then scans this spectrum to determine if it contains a detectable peak at the specified mass.

- If Empower finds a peak for the target mass, it lists the exact mass and its intensity in the Peaks and Results tables.
- If Empower does not find a peak for the target mass, it marks the peak as faulted.

The following procedure describes how to use this functionality.

1. Click the **3D Channels** tab in the Review window.
2. Click the **Chlorpromazine** channel created in "MS spectra library functionality with MS chromatograms".

3. Click inside the peak at **2.069**, then click the **Peak Height** icon  to select the peak height limits for this sample.

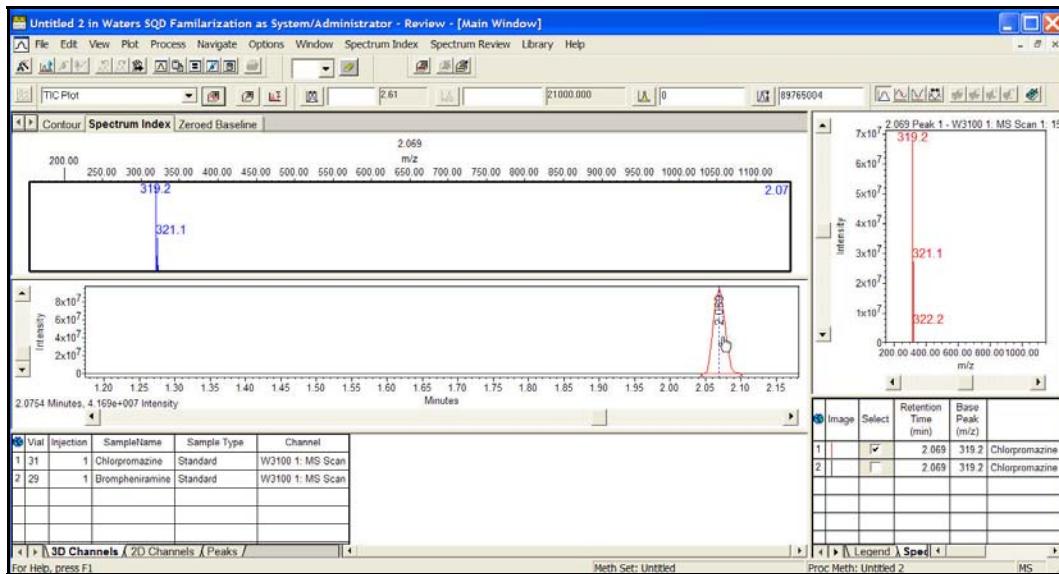


Figure 203 - Selecting peak height limits

4. Right-click inside the peak at **2.069** and select **Add/Edit Component name**.  
 5. Type **Chlorpromazine** as the component name and then click **OK**.

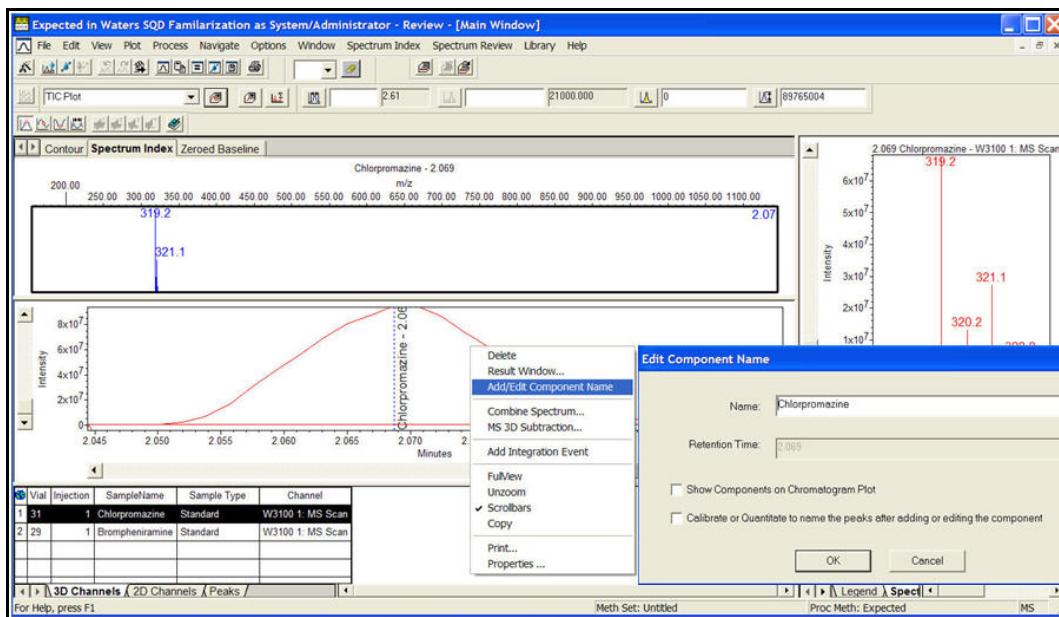


Figure 204 - Entering a component name

6. Click the **Processing Method** icon .
7. Click the **Component** tab.

8. Set the RT Window (min) column to **2.00**.

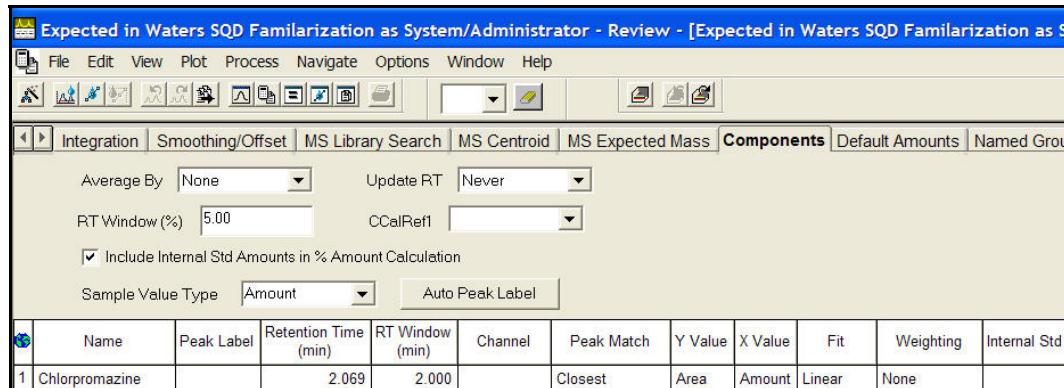


Figure 205 - Changing the RT window

9. Click the **MS Expected Mass** tab and select the **Component-based processing** check box.
10. Set the masses as displayed in Figure 206.

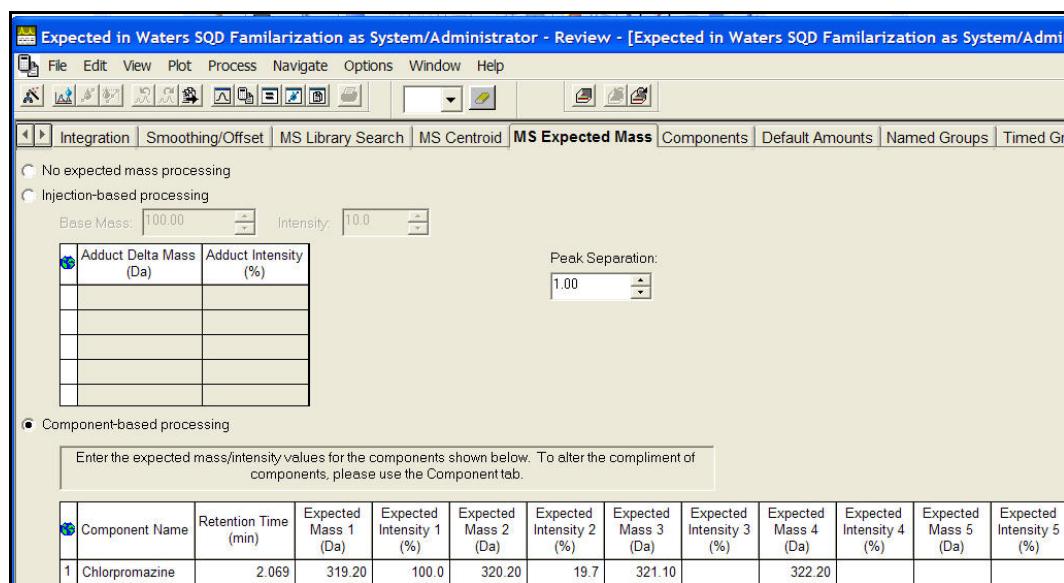


Figure 206 - Required masses

**NOTE:** Each injection for the specific component is searched for all the retention times, as well as the expected mass. This is then printed out in the Peaks table with the specific % of the base peak and specific % of each expected mass. Any change in tuning settings, cone voltage, mass calibration, or including cone voltage, can change the expected mass intensity values for a component.

11. Click the **MS Centroid** tab.
12. Set a peak separation of **0.5 Da** to search for an expected mass of 319.2 and a mass range of 318.7 to 319.7.



Figure 207 - Entering a peak separation

13. Click the **Review Main Window** icon  to switch to the Review Main window.
14. Click **File > Save** and type **Expected Mass PM** as the processing method name.
15. Click **File > Save Method Set** and type **Expected Mass MS** as the method set name.
16. Click the **Peaks** tab and right-click the **World** icon  to customize the table properties.
17. Click **Hide All**, then scroll to the bottom of the hidden columns and deselect all **Expected Mass** columns.
18. Click **OK**.

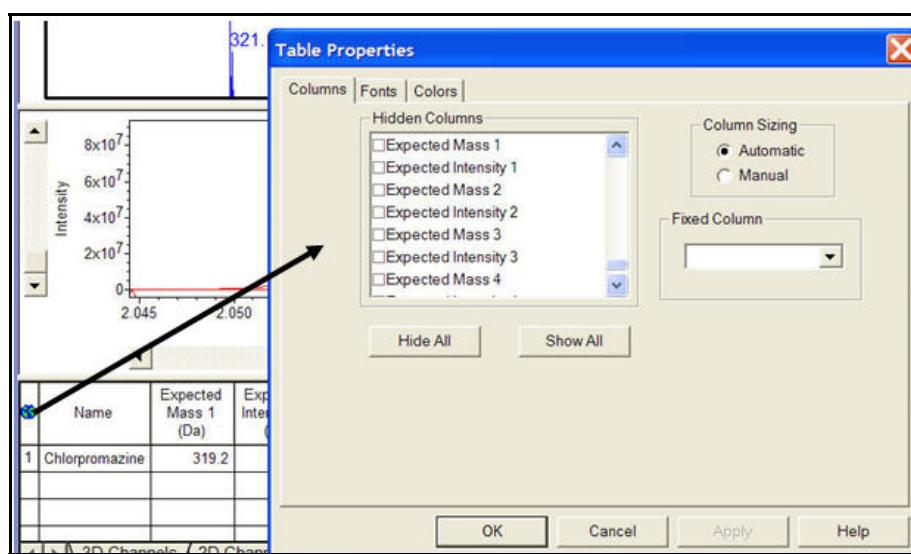


Figure 208 - Clearing Expected Mass columns

19. Click the **Integrate** icon and then the **Calibrate** icon.
20. View the Peak table to see the effects of expected mass processing for the chlorpromazine sample.

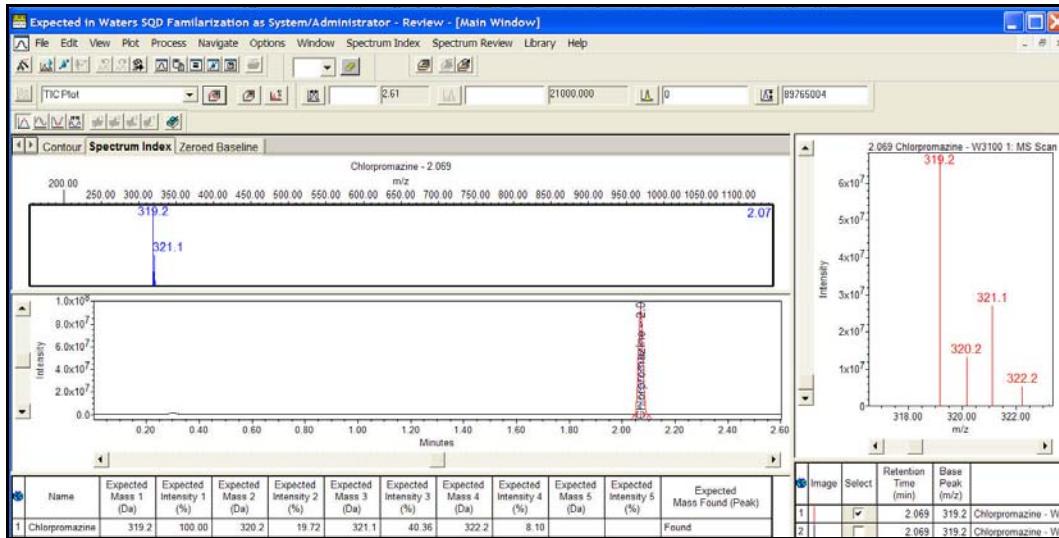


Figure 209 - Expected mass processing

21. Click the **3D Channels** tab.
22. Select **Brompheniramine** and then click the **Peaks** tab.

|   | Vial | Injection | SampleName      | Sample Type | Channel          |
|---|------|-----------|-----------------|-------------|------------------|
| 1 | 31   | 1         | Chlorpromazine  | Standard    | W3100 1: MS Scan |
| 2 | 29   | 1         | Brompheniramine | Standard    | W3100 1: MS Scan |

Figure 210 - Selecting a sample

23. View the Peak table to see the effects of expected mass processing for the brompheniramine sample.

**NOTE:** The peak was identified as chlorpromazine, though for Expected Mass 2 it was not there (results not matching will be highlighted in red), so it is probably not chlorpromazine.

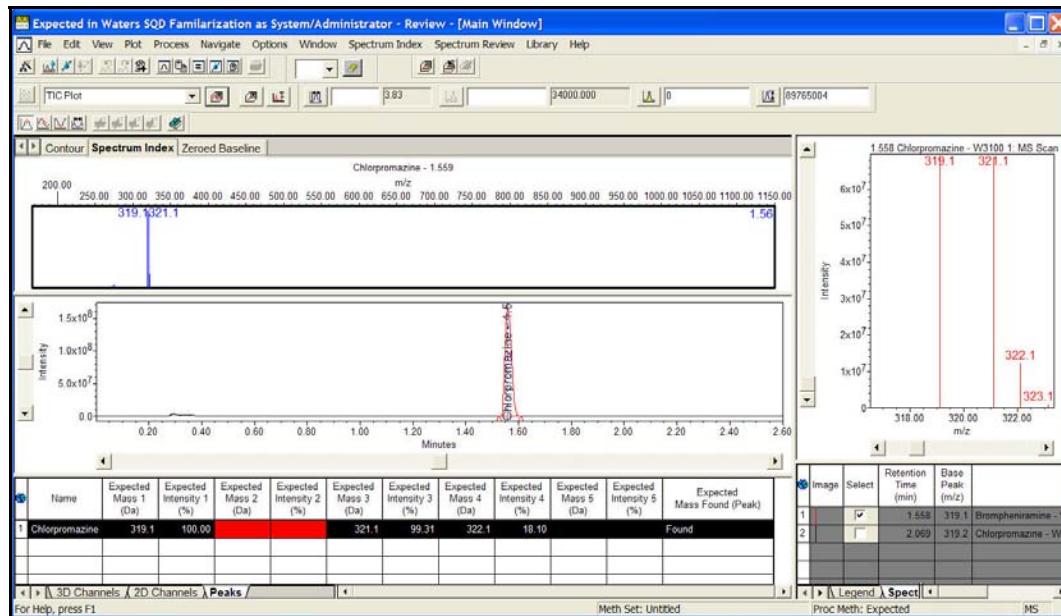


Figure 211 - Expected mass results

24. Use expected mass to differentiate samples that are similar in structure.
25. Click **File > Save All Results**.
26. Click **File > Exit** to close the Review window

## Familiarization Training Confirmation

I confirm on behalf of ..... that the familiarization  
Company Name

training has been satisfactorily completed on. ....  
System Serial Number

Signed: ..... Date: .....  
Customer

I certify that the familiarization training been successfully completed.

Signed: ..... Date: .....  
Waters Engineer