SOP 055 - Data Processing - LCQuan

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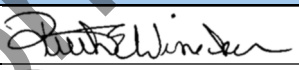
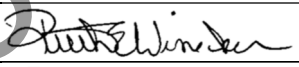
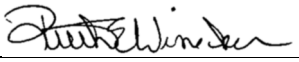
SOP Name: Data Processing - LCQuan		SOP #: 055
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	**Revision:** 5.2.4 – Revision to Ion Ratio update procedure	**Revision Date/Initials:** MSF – 11/13/15

Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/08/2015
Ruth E. Winecker, Ph.D.		06/27/2016
Ruth E. Winecker, Ph.D.		08/29/2017

1. **Principle**

    1.1. This method is designed to allow a user to process data and create reports from data acquired by a Thermo TSQ - LC/MS/MS

2. **Specimens**

    2.1. N/A

3. **Reagents and Materials**

    3.1. N/A

4. **Instrumentation and Equipment**

    4.1. Thermo TSQ LC/MS/MS

    4.2. LCQuan software

    4.3. Data reporting system (PC)

5. **Procedure**

    5.1. Data Processing

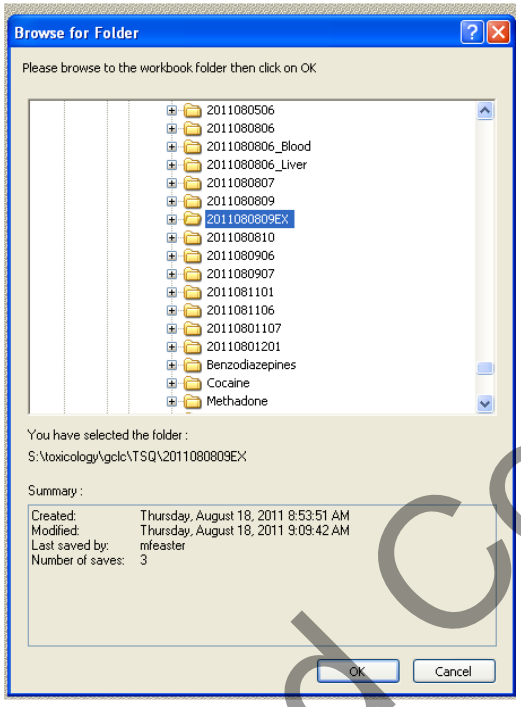
        5.1.1. On a networked PC with Thermo LCQuan software installed, open the LCQuan software by double-clicking the ICON.

            5.1.1.1. 

        5.1.2. Click on “Open an existing workbook” and click “Next” on the welcome screen.

        5.1.3. In the “Browse for Folder” dialog box, locate and click on the folder (named with Load#) to be processed: S:\toxicology\gclc\TSQ\”Load#”\. Click “OK”. (See below)

5.1.4.

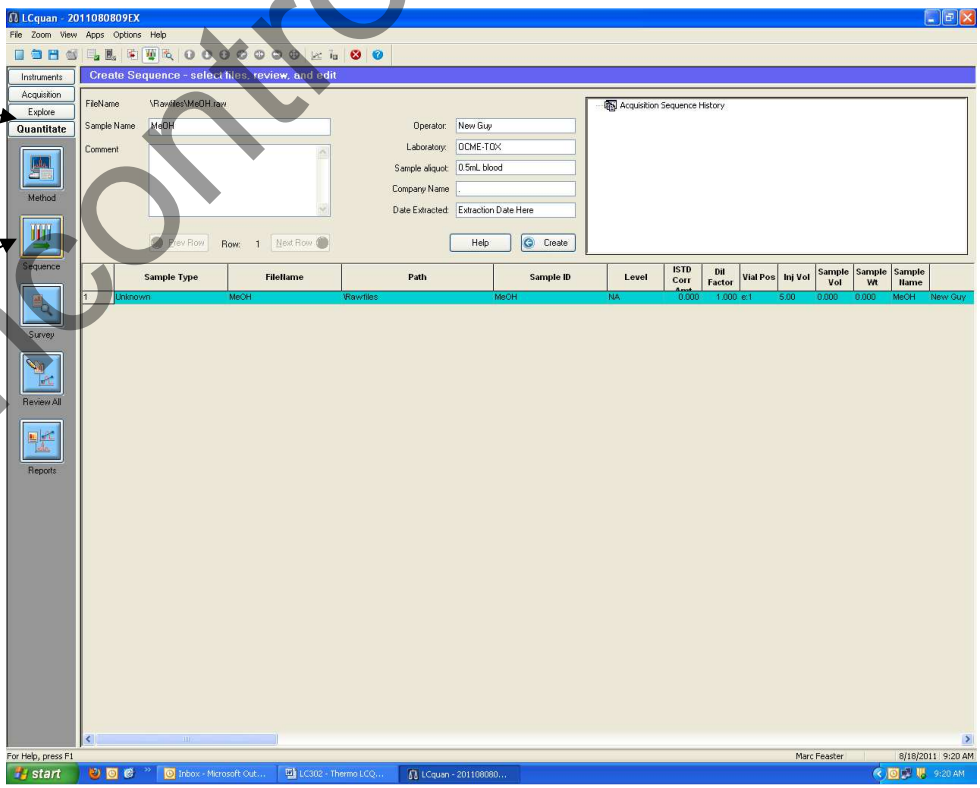


5.1.5. In the workbook screen, click the "Quantitate" tab (upper left), then the "Sequence" Button.

5.1.6.

1.

2.

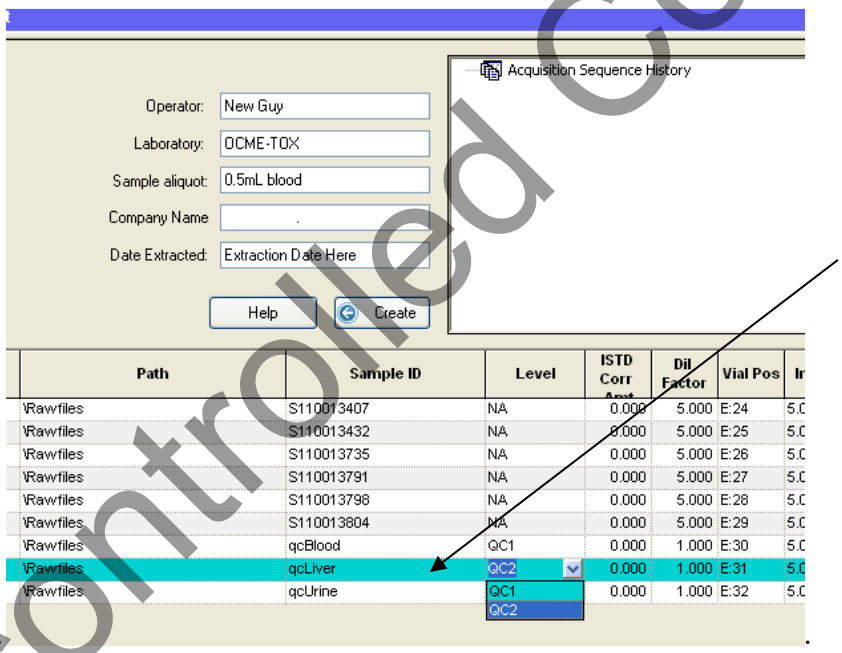


5.1.7.

5.1.8. To display the acquisition sequence, click “File” – “Import” – “Processing Sequence” – “Copy from Acquisition Sequence”. When prompted to overwrite levels, select “No”. The acquisition sequence should now be displayed.

5.1.9. If there are multiple QC levels for the assay, that were not assigned during sequence setup, now is the time to assign them. Click in the “Level” column(s) that correspond to the QC’s of interest. From the dropdown menu, select the appropriate QC level. (Dilution factors and any other typos need to be fixed now as well).

5.1.10.



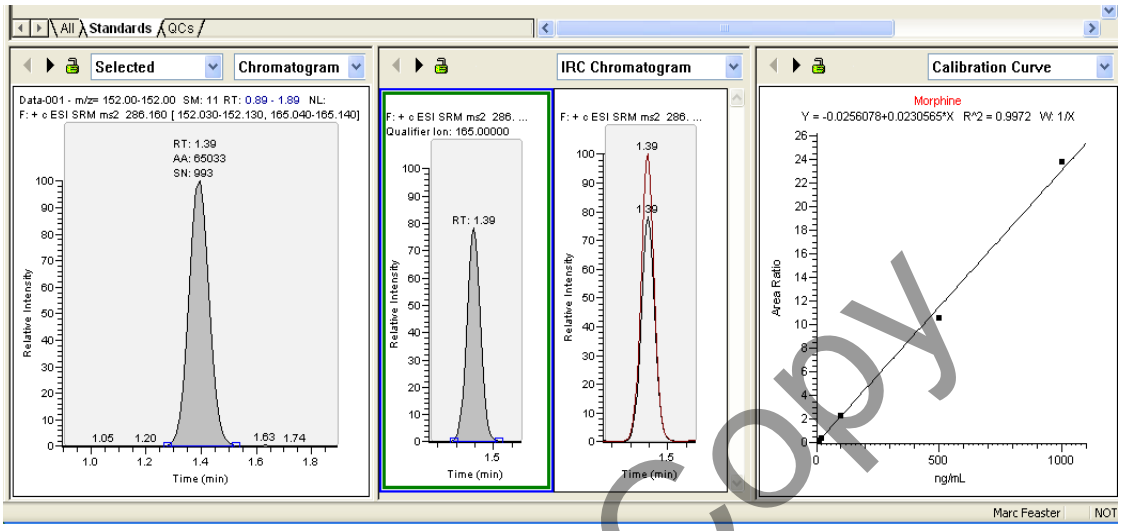
5.1.10.1. .

5.2. Update Ion Ratios & Retention Times

5.2.1. Click the “Survey” button on the left side of the screen.

5.2.2. If necessary, change the display windows (bottom) to the following configuration using the dropdown Menus:
Left window: “Selected” and “Chromatogram”
Center window: “IRC Chromatogram”
Right window: “Calibration Curve”
(See Below)

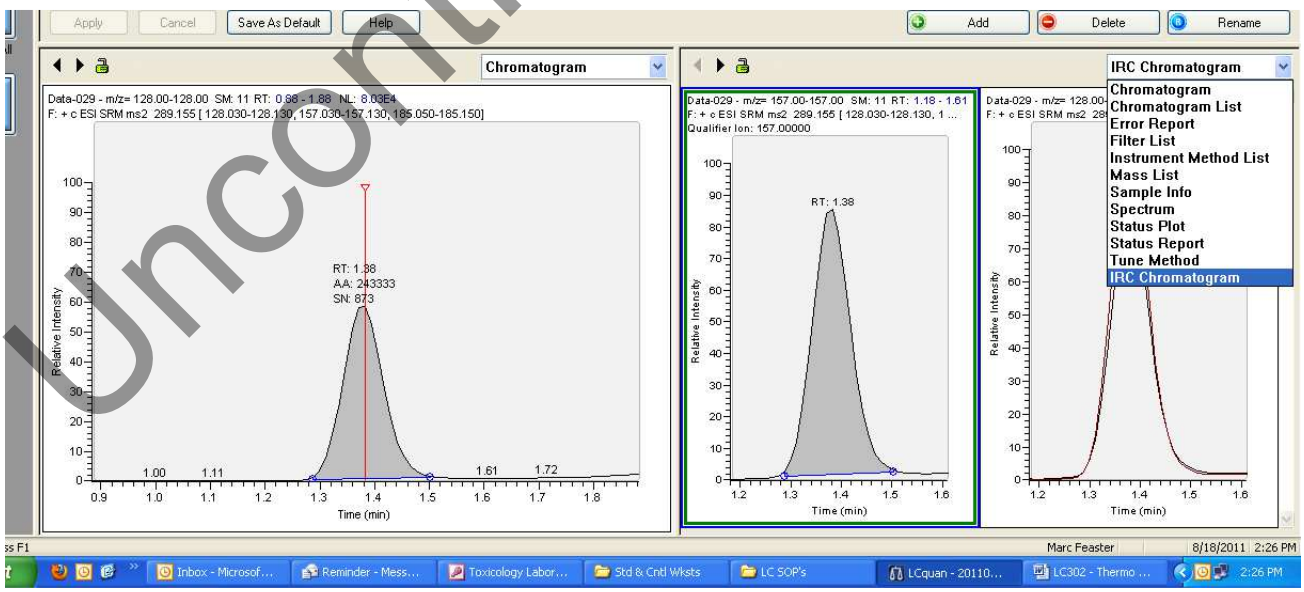
5.2.2.1.

5.2.3. 

5.2.4. Click the "Standards" tab (located directly above the above mentioned "Chromatogram" window). In the sequence table, click in the row in which the appropriate calibrator is displayed (Quality Manual section 2.13.4). The row should be highlighted green.

5.2.5. Click the "Method" button (Left). In the dropdown menu above the bottom-right window, select "IRC Chromatogram".

5.2.5.1.



5.2.6. Select each analyte in turn (upper right), and update the retention time for each.

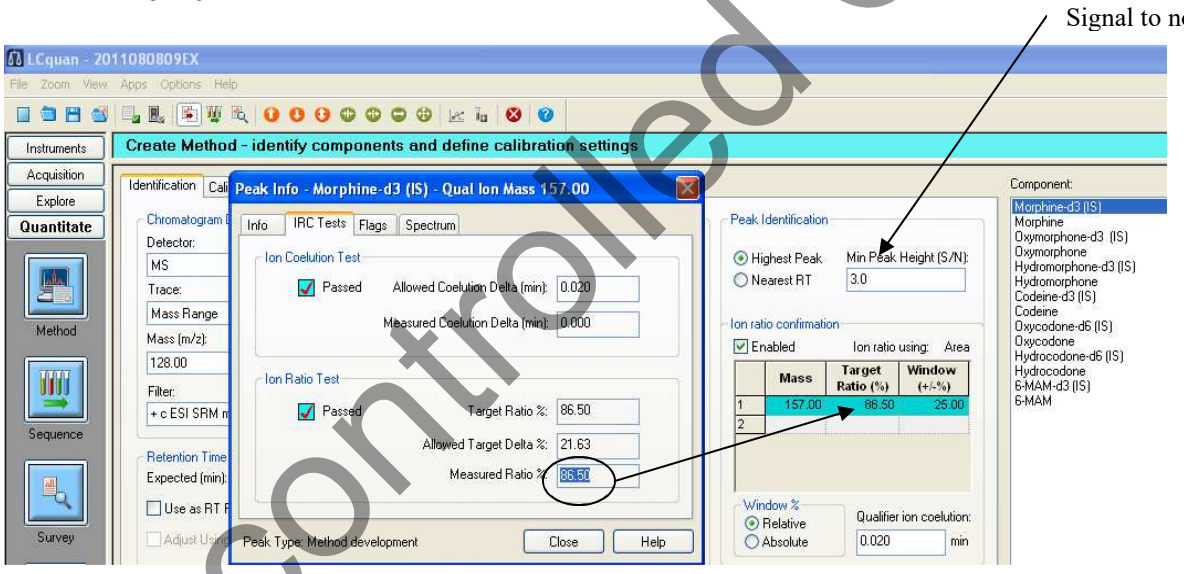
5.2.7. Right click on the "IRC Chromatogram" window and choose "Show Peak Info – Qual Ion Mass: xxx". The "Peak Info" dialog box should appear.

5.2.8. Drag the "Peak Info" dialog box into position so that it does not obscure the "Ion Ratio Confirmation" table displayed on the "Create Method" screen.

5.2.9. In the "Peak Info" box, select the "IRC Tests" tab. For each analyte in the "component list", copy/ paste the value listed in the "Measured Ratio %:" field into the "Target Ratio (%)" field of the "Ion Ratio Confirmation" table.

5.2.9.1. Note: The "Peak Info" dialog box can remain open as each component is selected from the list.

5.2.9.2.



5.2.10. When all analytes' ion ratios have been updated in the method, save the workbook. **Click the "Survey" button**.

5.3. Check Data Quality

5.3.1. With the "Standards" tab selected, check that the standard curves and percent difference of calculated calibration levels for each target analyte are within method parameters - R^2 (Calibration Curve Window) & (% Diff column of sequence table).

5.3.2. Switch to the "QC" Tab and check, for each target analyte, that the percent difference is within method parameters. Note: If there is a problem with the Calibration Curves or QC for an analyte, see a senior chemist for

solutions. Note: Steps 5.3.1 & 5.3.2 can also be viewed under the "Review All" button.

5.3.3. Click on the "Review All" button (left) and the "Blanks" tab. Check that each analyte is Not Found (NF). Note: If a target peak is integrated in a blank sample, or there is a substantial non-integrated peak, see a senior chemist for solutions.

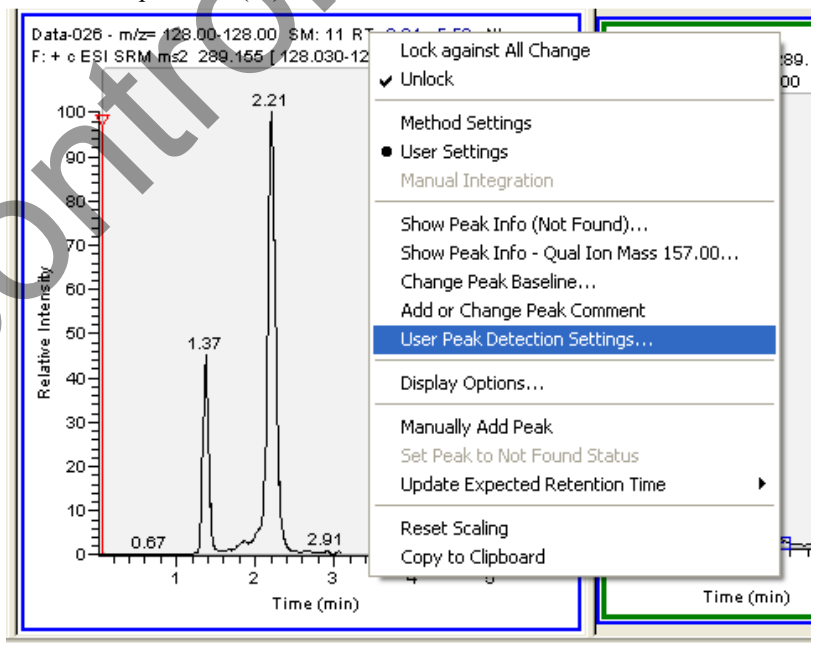
5.3.4. Select the "Unknowns" tab, click on each internal standard (upper right) in turn, checking the sequence table to be sure a peak was found in each sample. If an IS was not integrated, the corresponding row will be highlighted in red (See "Manual Peak Integration" section below).

5.3.5. Click on each target analyte (upper right) in turn. Compare results (Calculated Conc. Column) of duplicate samples. Note: See a senior chemist if results are inconsistent.

5.3.6. Manual Peak Integration – Occasionally, due to low recovery or matrix effects, internal standard and/or target analyte peak responses fall below integration thresholds.

5.3.6.1. Right click on the "Chromatogram Window" of the peak to be manually integrated and choose "User Peak Detection Settings…" from the dropdown menu (See Below).

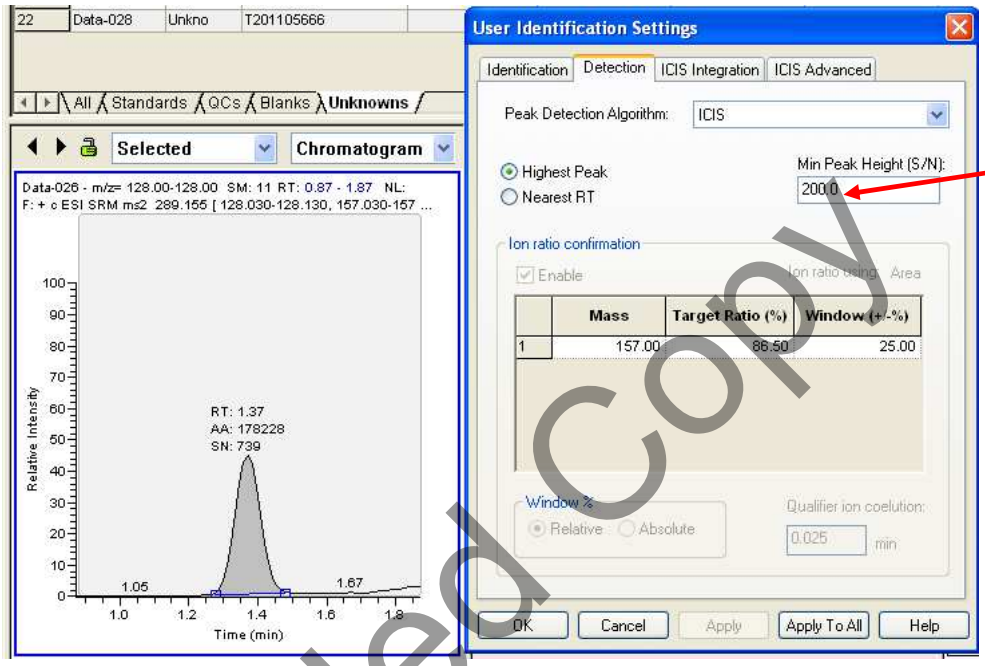
Morphine-d3 (IS)

5.3.6.2. 

5.3.6.3. In the "User Identification Settings" dialog box, choose the "Detection" tab. In the "Min Peak Hight (S/N)" field, enter a lower number than is currently displayed. Click "Apply". E.g. Change

from 500 to 200 or lower. (Some peaks cannot be integrated in this fashion).

5.3.6.4.



5.3.6.5. Click "OK" when complete. Right click on the "IRC Chromatogram" window. Choose "Show Peak Info – Qual Ion Mass: xxx…". Select "IRC Tests" tab and verify that both the "Ion Coelution Test" and "Ion Ratio Test" pass criteria. Close the Dialog Box. If integration criteria was not met, undo User Integration by right clicking the "Chromatogram Window" and selecting "Method Settings".

5.3.6.6. Note, if lowering the signal to noise setting is required to integrate Cal level 1 for an analyte, the setting should be changed in the method settings. (see 5.2.9.2)

5.3.6.7. For other tips on proper integration techniques, refer to *LCQuan and You* presentation[2].

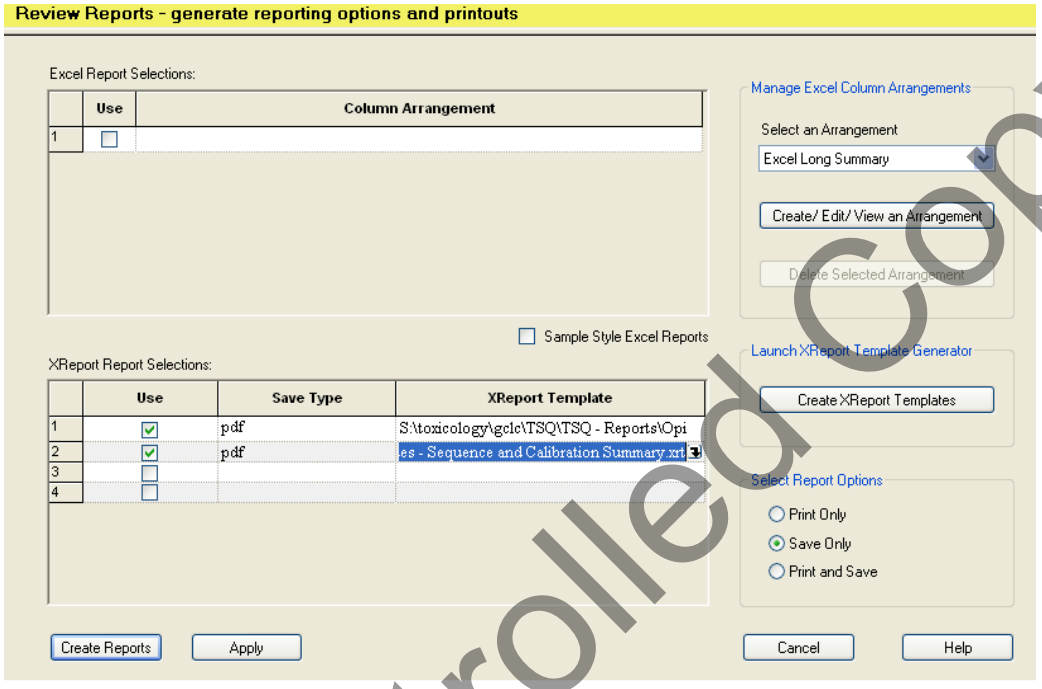
5.4. Create and Print Reports

5.4.1. Once all data has been reviewed and **Ion Ratios Re-Checked**, save the workbook and click the "Reports" button (Left).

5.4.2. In the "XReport Report Selections" table, select "PDF" from the dropdown menu in the first two rows of the "Save Type" column.

5.4.3. In the "XReport Report Selections" table, select the method specific "Sample Summary" and "Sequence and Calibration Summary" from the dropdown menu inRow1 and Row2 of the "XReport Templates" column respectively.

5.4.3.1. Report Template Path: S:\toxicology\gclc\TSQ\TSQ-reports\ (Screen Should Appear as Below)



5.4.4. Click "Create Reports". A dialog box will appear, click "Yes" to save the Workbook and create reports.

5.4.5. After several minutes, the above reports will be generated in PDF format and saved in the S:\toxicology\gclc\TSQ\"Load#"\Exports\ folder. Navigate to this folder in Windows Explorer, open, review, and print the reports.

5.4.5.1. Note: If any changes are made to the method and/or integration parameters after reports were generated, the above process is to be repeated and new set of reports generated.

5.5. Refer to the "Reporting" and "Preparation of Load" sections of the method SOP to complete the data package and report results.

6. References

6.1. Thermo Scientific. *Xcalibur LCQuan Quantitative Analysis User Guide*. Aug. 2007. XCALI-97166 Revision D. USA.

6.2. Feaster, Marc S. *LCQuan and You – Perfect Together*. Raleigh, NC: NC OCME Toxicology, August 8, 2012. PDF.