

Table of Contents

1. Principle ..... 3

2. Specimens ..... 3

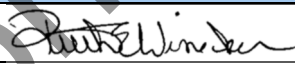
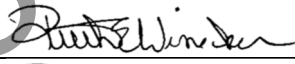
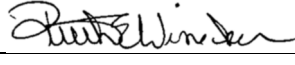
3. Reagents and Materials ..... 3

4. Instrumentation and Equipment..... 3

5. Procedure ..... 3

6. References..... 10

## SOP 053 - Sequence Setup – Thermo LCQuan

|                                                                                 |                                                                                     |                                |
|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------|
| <b>SOP Name:</b><br><b>LCQuan Sequence Setup</b>                                |                                                                                     | <b>SOP #:</b><br><b>053</b>    |
| North Carolina Office of the Chief<br>Medical Examiner<br>Toxicology Laboratory | <b>Revision:</b>                                                                    | <b>Revision Date/Initials:</b> |
|                                                                                 |                                                                                     |                                |
| <b>Approving Authority Name</b>                                                 | <b>Approving Authority Signature</b>                                                | <b>Approval Date</b>           |
| Ruth E. Winecker, Ph.D.                                                         |  | 04/08/2015                     |
| Ruth E. Winecker, Ph.D.                                                         |  | 06/10/2016                     |
| Ruth E. Winecker, Ph.D.                                                         |  | 08/29/2017                     |
|                                                                                 |                                                                                     |                                |
|                                                                                 |                                                                                     |                                |

## SOP 053 - Sequence Setup – Thermo LCQuan

### 1. Principle

- 1.1. This method is designed to allow the user to create a sequence and acquire LC/MS/MS data using Thermo LCQuan software.

### 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. Create Sequence

- 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. On the welcome screen, click on “Create a new workbook” and click “Next”.

- 5.1.3. “TSQ” should appear, in the “Study Name” field. In the “Workbook Name” field, enter the load number for which a sequence is to be created, click “Next” (see below).

## SOP 053 - Sequence Setup – Thermo LCQuan

**New Study or Workbook Wizard**

This wizard creates folders and imports data  
Create a new study and workbook and optionally import data

The Study Name and Workbook Name will be used to create a disk folder that contains all the workbook information in one place.

Study Name:  
TSQ Browse...

Workbook Name:  
Load Number Here Browse...

S:\toxicology\gcl\TSQ\Load Number Here\

☐ Change Study Root Folder

< Back Next > Cancel Help

5.1.4. Click “Next” in the following window leaving the box next to “import raw files” un-checked.

5.1.5. The following window should appear as below, click “Next”.

**New Study or Workbook Wizard**

This wizard creates folders and imports data  
Create a new study and workbook and optionally import data

Should prior data be imported to initialize the new workbook

☒ Import Instrument Method, Acquisition Sequence and Processing Parameters

☐ Import from existing workbook

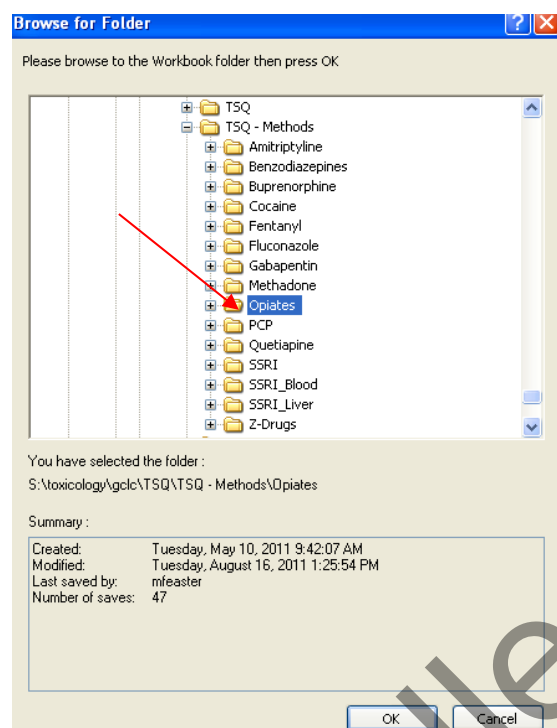
☐ Import legacy files

< Back Next > Cancel Help

5.1.6. In the following window, select “Browse” and choose the appropriate method folder corresponding to the assay being performed (Opiates used as example):

## SOP 053 - Sequence Setup – Thermo LCQuan

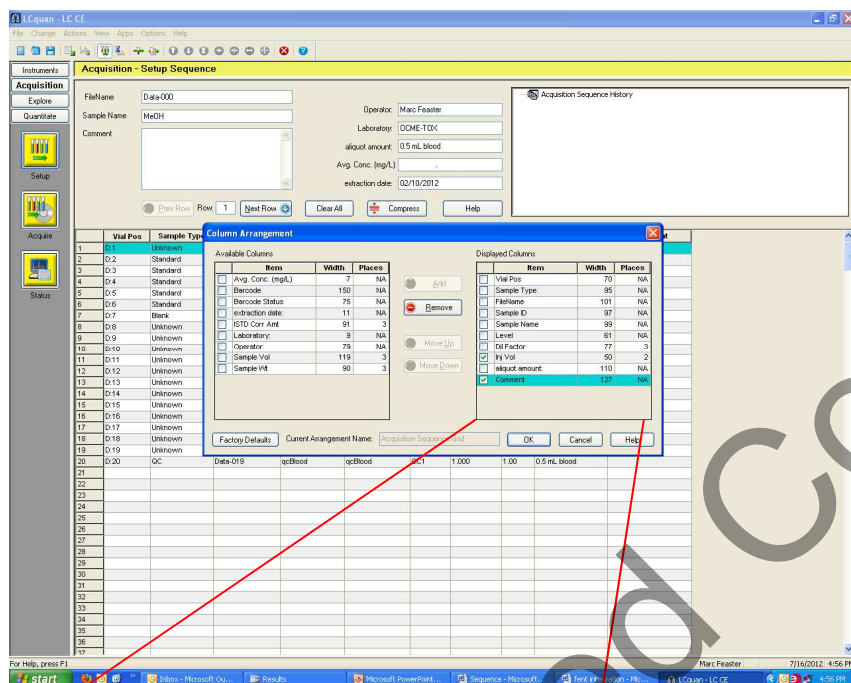
- 5.1.6.1. S:\toxicology\Instrument Methods\LC\Instrument Name). Click “OK”, then click “Next.”



- 5.1.7. The workbook template should now be open, click on the “Acquisition” then “Setup” tabs in the upper left side of the screen.
- 5.1.8. Right click anywhere in the sequence table and choose “Columns” from the dropdown menu. Click to check the boxes of columns to be removed and click “Remove” then “OK” (see below).
- 5.1.8.1. Columns to remain displayed (In the following order):
- 5.1.8.1.1. Vial Pos
  - 5.1.8.1.2. Sample Type
  - 5.1.8.1.3. FileName
  - 5.1.8.1.4. Sample ID
  - 5.1.8.1.5. Sample Name
  - 5.1.8.1.6. Level
  - 5.1.8.1.7. Dil Factor

## SOP 053 - Sequence Setup – Thermo LCQuan

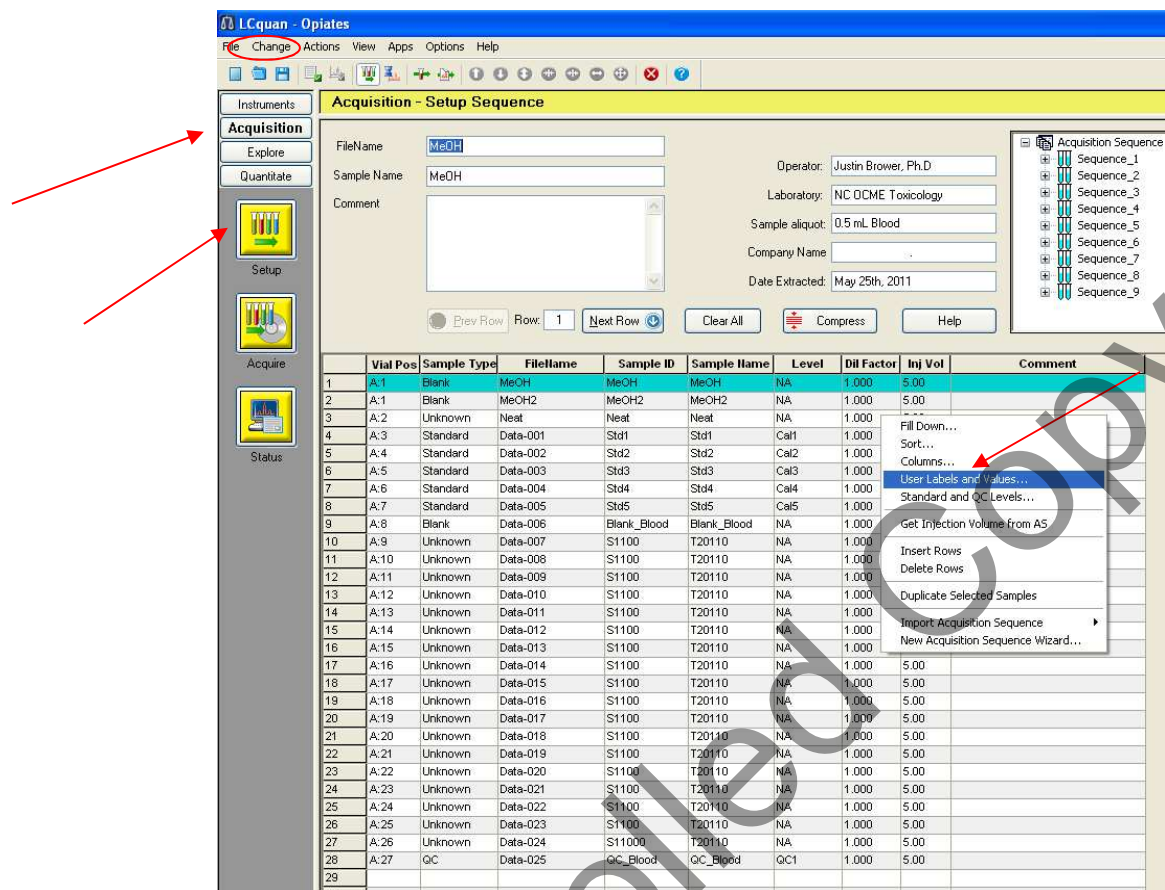
### 5.1.8.1.8. Aliquot amount



|                                     | Item            | Width | Places |
|-------------------------------------|-----------------|-------|--------|
| <input type="checkbox"/>            | Vial Pos        | 70    | NA     |
| <input type="checkbox"/>            | Sample Type     | 95    | NA     |
| <input type="checkbox"/>            | FileName        | 101   | NA     |
| <input type="checkbox"/>            | Sample ID       | 97    | NA     |
| <input type="checkbox"/>            | Sample Name     | 99    | NA     |
| <input type="checkbox"/>            | Level           | 61    | NA     |
| <input type="checkbox"/>            | Dil Factor      | 77    | 3      |
| <input checked="" type="checkbox"/> | Inj Vol         | 50    | 2      |
| <input type="checkbox"/>            | aliquot amount: | 110   | NA     |
| <input checked="" type="checkbox"/> | Comment         | 127   | NA     |

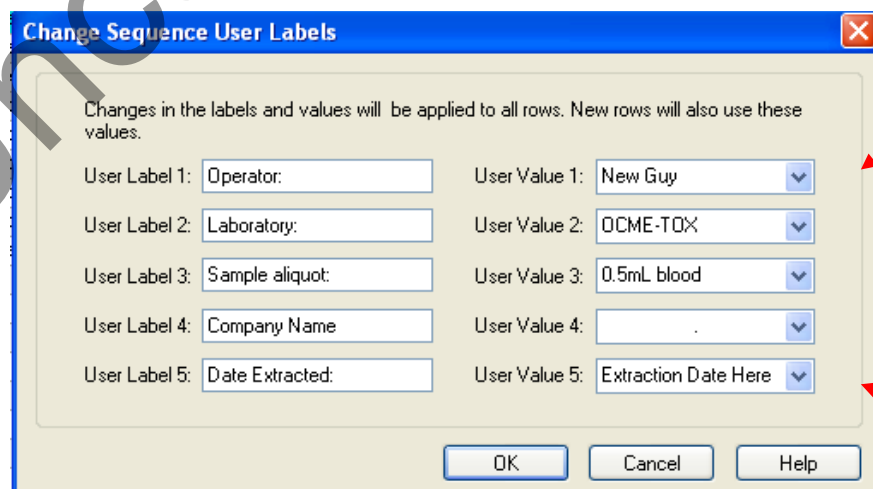
5.1.9. Right click anywhere in the sequence table and choose “User values and labels” from the dropdown menu. Alternatively, click “Change” - “User values and labels”. (see below)

## SOP 053 - Sequence Setup – Thermo LCQuan



5.1.10. In the “User Value 1” and “User Value 5” fields, enter operator name and extraction date respectively. Click “OK”.

5.1.11. In the “User Value 3” field enter default aliquot amount/SOP#/  
Instrument name. E.G. 0.5mL blood/LC208/TSQ01



## SOP 053 - Sequence Setup – Thermo LCQuan

5.1.12. Enter S#'s (Sample ID column) and T#'s (Sample Name Column) as appropriate. Enter dilution factors as needed (Dil Factor column) and enter aliquot amount for each row in the "Sample Aliquot" field at top center of screen. If needed, right click on the row numbers for the option to add or delete rows.

5.1.12.1. **Note: Enter "N/A" in "Sample Aliquot" field for MeOH blanks and neat.**

5.1.13. Shrink each column width to remove any extra spacing. This will allow for the sequence to print on one page.

|    | Vial Pos | Sample Type | Filename | Sample ID  | Sample Name | Level | Dil Factor | aliquot amount |
|----|----------|-------------|----------|------------|-------------|-------|------------|----------------|
| 1  | B:1      | Unknown     | Meoh     | meoh       | meoh        | NA    | 1.000      |                |
| 2  | B:2      | Standard    | Data-001 | Cal1       | Cal1        | Cal01 | 1.000      | 0.1 mL blood   |
| 3  | B:3      | Standard    | Data-002 | Cal2       | Cal2        | Cal02 | 1.000      | 0.1 mL blood   |
| 4  | B:4      | Standard    | Data-003 | Cal3       | Cal3        | Cal03 | 1.000      | 0.1 mL blood   |
| 5  | B:5      | Standard    | Data-004 | Cal4       | Cal4        | Cal04 | 1.000      | 0.1 mL blood   |
| 6  | B:6      | Standard    | Data-005 | Cal5       | Cal5        | Cal05 | 1.000      | 0.1 mL blood   |
| 7  | B:7      | Blank       | Data-006 | blankBlood | blankBlood  | NA    | 1.000      | 0.1 mL blood   |
| 8  | B:8      | Blank       | Data-007 | blankLiver | blankLiver  | NA    | 1.000      | 0.1 g liver    |
| 9  | B:9      | Unknown     | Data-008 | S120011637 | T201204482  | NA    | 1.000      | 0.1 mL blood   |
| 10 | B:10     | Unknown     | Data-009 | S120011639 | T201204482  | NA    | 4.000      | 0.1 g liver    |
| 11 | B:11     | Unknown     | Data-010 | S120011989 | T201204613  | NA    | 1.000      | 0.1 mL blood   |
| 12 | B:12     | Unknown     | Data-011 | S120012033 | T201201625  | NA    | 1.000      | 0.1 g blood    |
| 13 | B:13     | Unknown     | Data-012 | S120012513 | T201204819  | NA    | 1.000      | 0.1 mL blood   |
| 14 | B:14     | Unknown     | Data-013 | S120012515 | T201204819  | NA    | 4.000      | 0.1 g liver    |
| 15 | B:15     | Unknown     | Data-014 | S120012586 | T201204818  | NA    | 1.000      | 0.1 mL blood   |
| 16 | B:16     | Unknown     | Data-015 | S120012589 | T201204818  | NA    | 4.000      | 0.1 g liver    |
| 17 | B:17     | Unknown     | Data-016 | S120011637 | T201204482  | NA    | 1.000      | 0.1 mL blood   |
| 18 | B:18     | Unknown     | Data-017 | S120011639 | T201204482  | NA    | 4.000      | 0.1 g liver    |
| 19 | B:19     | Unknown     | Data-018 | S120011989 | T201204613  | NA    | 1.000      | 0.1 mL blood   |
| 20 | B:20     | Unknown     | Data-019 | S120012033 | T201201625  | NA    | 1.000      | 0.1 g blood    |
| 21 | B:21     | Unknown     | Data-020 | S120012513 | T201204819  | NA    | 1.000      | 0.1 mL blood   |
| 22 | B:22     | Unknown     | Data-021 | S120012515 | T201204819  | NA    | 4.000      | 0.1 g liver    |
| 23 | B:23     | Unknown     | Data-022 | S120012586 | T201204818  | NA    | 1.000      | 0.1 mL blood   |
| 24 | B:24     | Unknown     | Data-023 | S120012589 | T201204818  | NA    | 4.000      | 0.1 g liver    |
| 25 | B:25     | QC          | Data-024 | qcBlood    | QC_Blood    | QC01  | 1.000      | 0.1 mL blood   |
| 26 | B:26     | QC          | Data-025 | qcLiver    | QC_Liver    | QC02  | 1.000      | 0.1 g liver    |

5.1.14. If there are multiple QC levels for the assay, click in the "Level" column(s) that correspond to the QC's of interest. From the dropdown menu, select the appropriate QC level.

## SOP 053 - Sequence Setup – Thermo LCQuan

LCQuan - 2011080809EX

File Change Actions View Apps Options Help

Instruments

Acquisition

Explore

Quantitate

Setup

Acquire

Status

Acquisition - Setup Sequence

File Name: Data-031

Sample Name: qcUrine

Comment:

Operator: New Guy

Laboratory: OCME-TDXX

Sample aliquot: 0.5mL blood

Company Name:

Date Extracted: Extraction Date Here

Prev Row Row: 9 Next Row Clear All Compress Help

|    | Vial Pos | FileName | Sample Type | Sample ID  | Sample Name | Level | Dil Factor | Sample aliquot |
|----|----------|----------|-------------|------------|-------------|-------|------------|----------------|
| 1  | E:24     | Data-023 | Unknown     | S110013407 | T201105588  | NA    | 5.000      | 0.5mL blood    |
| 2  | E:25     | Data-024 | Unknown     | S110013432 | T201105594  | NA    | 5.000      | 0.5mL blood    |
| 3  | E:26     | Data-025 | Unknown     | S110013735 | T201105651  | NA    | 5.000      | 0.5mL blood    |
| 4  | E:27     | Data-026 | Unknown     | S110013791 | T201105664  | NA    | 5.000      | 0.5mL blood    |
| 5  | E:28     | Data-027 | Unknown     | S110013798 | T201105665  | NA    | 5.000      | 0.5mL blood    |
| 6  | E:29     | Data-028 | Unknown     | S110013804 | T201105666  | NA    | 5.000      | 0.5mL blood    |
| 7  | E:30     | Data-029 | QC          | qcBlood    | qcBlood     | QC1   | 1.000      | 0.5mL blood    |
| 8  | E:31     | Data-030 | QC          | qcLiver    | qcLiver     | QC2   | 1.000      | 0.5mL blood    |
| 9  | E:32     | Data-031 | QC          | qcUrine    | qcUrine     | QC2   | 1.000      | 0.5mL blood    |
| 10 |          |          |             |            |             | QC1   |            |                |
| 11 |          |          |             |            |             | QC2   |            |                |

- 5.1.15. Click the “Save” icon to save workbook. If the PC being used is not “attached” to the TSQ, close LCQuan and complete the following steps on the TSQ PC, otherwise skip to step 5.1.17.
- 5.1.16. Open LCQuan and choose “Open an Existing Workbook”. Choose the folder with the corresponding load number and click “OK”.
- 5.1.17. Determine which autosampler rack slot is to be used (A through E) and enter in the first row/Vial Pos column of the sequence table. (If the sequence starts with two MeOH blanks to be sampled from the same vial, enter “rack slot”:”vial position” in the first two rows. E.g. row1 - C:1; row2 - C:1.
- 5.1.18. To fill down the remaining vial locations, left click once and hold on the final sample location using slot1, and drag down to the last sequence entry. Right click on highlighted column and choose “Fill Down”. Click “OK” on the Fill Down dialog box.
- 5.1.19. Repeat step 5.1.17 for the “File Name” column starting with Data-001.
- 5.1.20. Save the workbook (Step 5.1.15).
- 5.1.21. Click “File” – “Print Sequence Info...” to print sequence.
- 5.1.22. Verify sequence vial locations with another analyst, obtaining their initials and date on the sequence sheet, and place the autosampler rack in the appropriate slot in the TSQ autosampler.

## SOP 053 - Sequence Setup – Thermo LCQuan

5.1.23. Check the fluid levels of the LC solvents, making sure there is enough to inject the entire load – refill if necessary.

5.1.24. Click the “Acquire” button on the left side of the screen. In the dialog box, verify that the appropriate number of rows is displayed in the “Run Rows” field in the upper right. Click “OK” to start acquiring data. It should appear as below.

5.1.24.1. **Note: For TSQ02 & TSQ03, do not check the boxes next to “Use Startup Method” and “Use Shutdown Method”.**

Run Sequence

Acquisition Options

User: tsq01 Run Rows: 1-41

Sequence Name: Sequence\_2

Comment:

Instrument Start Instrument

Surveyor MS Pump Plus

Surveyor AS Yes

TSQ Quantum

☒ Start When Ready Change Instruments...

Processing Actions

☐ Process after Acquisition

☐ Generate Selected Reports

Instrument Method

☒ Use Startup Method

☒ Use Shutdown Method

Programs

Pre-Acquisition: ...

Post-Acquisition: ...

Run Synchronously

☐ Pre-Acquisition ☐ Post-Acquisition

After Sequence Set System:

☐ On ☒ Standby ☐ Off

OK Cancel Help

5.1.24.1.1.

## 6. References

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