	Chemistry Department Bldg. 8 Research Group	SOP #	1
		Revision #	4
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Page Number	1 of 7	Last Reviewed Date	11/12/2018
SOP Owner	Tom Allston	Approval	

I. Purpose

To promote the effective use of the Q Trap 3200 – LC-MS/MS to collect scans. To educate students in the loop injection method for mass spectroscopy.

II. Scope

This SOP is intended for in-group use by trained and certified personnel in the Chemistry Department.

III. Prerequisites

The experimenter must be trained in proper instrument techniques before using this SOP.

IV. Responsibilities

The responsibility for this instrument lies with Tom Allston

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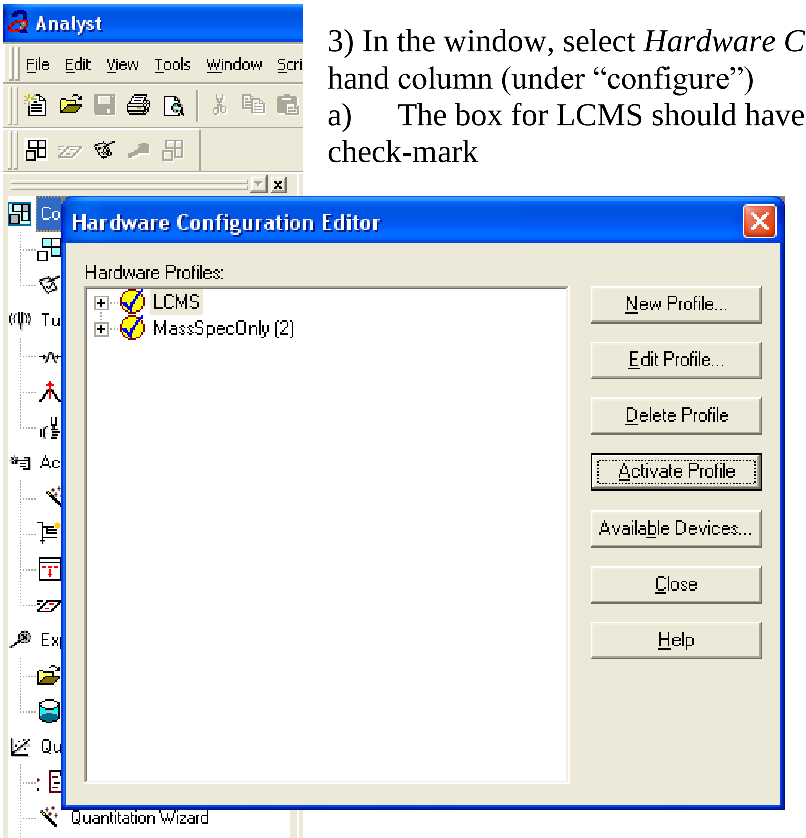
V. Procedures and information

1) Begin by powering on the computer and then the Shimadzu HPLC components. There are a total of four power buttons on the HPLC: UV-Vis, autosampler, both pumps, press the "A" or "Load" button on the Mass Spec which will set it for loop injection mode, *Mass Spec should already be on and should always be left on!*

2) After the computer has been powered up and logged into the appropriate user account, open the Analyst software using the icon found on the desktop.



3) In the window, select *Hardware Configuration* from the left-hand column (under "configure")

a) The box for LCMS should have a yellow circle with a check-mark

i) If it does: select it then press "Activate profile"

ii) If it does not: select it, deactivate, then reactivate

b) When activated, the check-mark in the box will be green and the MS should beep.

c) Close the configuration editor

4) Decide how many samples you will have, transfer 1.5 mL of each into the sample vials available in the stockroom (small glass vials with rubber seals around the lid).

a. Make sure that all of your samples have a maximum concentration of 500 ppb.

b. Make sure all of your samples are properly filtered.

5) Open the front panel on auto sampler (the panel to the right of the mouse)

6) Remove sample tray, insert samples into the tray and note the location numbers (out of the 70 sample locations), re-insert sample tray. Ensure that it is pushed back the entire way and that the lid is shut carefully as to not break the hinge of this cover.

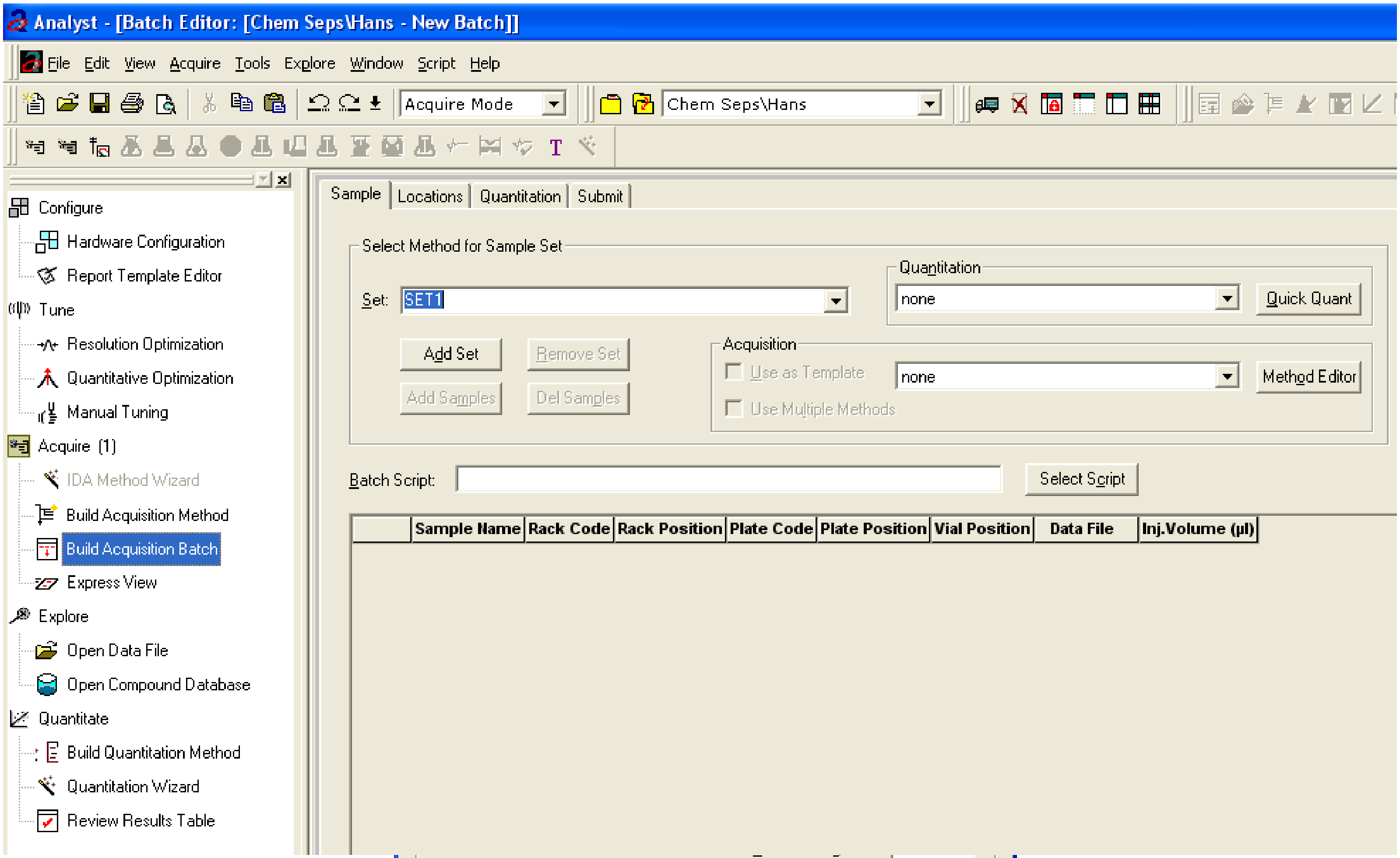
7) Select *Build acquisition Batch* in the left column.

a) This is where you will specify sample data and locations within the auto sampler

b) Under the sample tab, enter the name of your sample in the left-most white box. (says SET1 by default)

c) Click “Add set”

d) Click “Add samples”



Analyst - [Batch Editor: [Chem Seps\Hans - New Batch]]

Acquire Mode

Chem Seps\Hans

Set: SET1

Quantitation: none

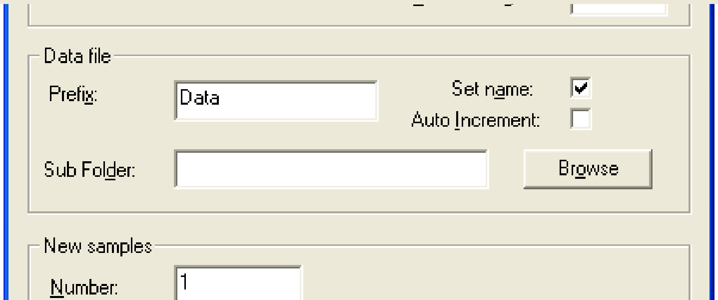
Acquisition: none

☐ Use as Template

☐ Use Multiple Methods

Batch Script:

	Sample Name	Rack Code	Rack Position	Plate Code	Plate Position	Vial Position	Data File	Inj.Volume (µl)



Data file

Prefix: Data

Set name: ☑

Auto Increment: ☐

Sub Folder:

New samples

Number: 1

RECOMMENDED FORMATTING:

Sample name Prefix: Replace the word sample with the name of the first sample in your list. If it is series it may be augmented by the number of digits in the digit box. Or use the word blank if your first sample is a blank vial and uncheck "sample number".

Sample Number: Change the number of digits to 1 or uncheck this field (and you will have no number after sample name).

Data file Prefix: specifies the first part of the name for your data set. It is recommended you start all file names with the date as date as **2014_01_13 (for example)** with the month and date and this will facilitate finding data in the future. Use the zero in 01 for example to retain proper filing order in month and date.

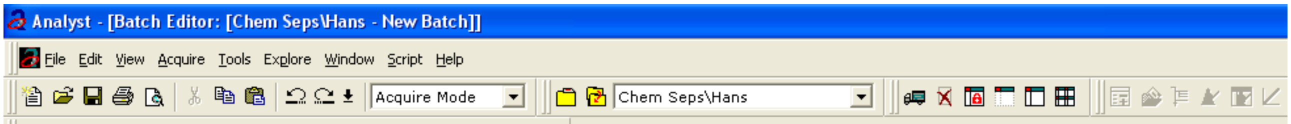
Sub Folder: Recommend to be left blank, subfolder can be used to further separate data but it is best to leave this blank.

Number: is where you put the <u>number of new samples</u> that you are testing

a) Under data file, prefix
b) If subfolder is left blank, the data file will be placed in the "data" folder within the analyst software folder tree

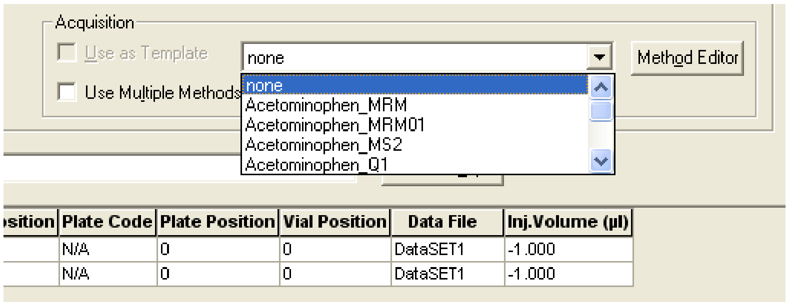
8) Under new samples, enter the number of samples you have inserted
a) A list of samples should be created in the sample tab. The only thing you should need to change is the vial position, which should be the number corresponding to the vial's place in the sample tray
    i) If you set it up this way, change the vial position of the first sample to 1.
    ii) To fill in the position for the rest of the samples, click the heading of the vial position column to highlight the whole column, right click the column and select Auto Increment

9) Now go to the acquisition box under the "Sample" tab and select from the drop down menu which of your acquisition methods you will be using.
    a. If you are going to be using multiple acquisition methods for different samples in the same batch check the "Use Multiple Methods" box and a new column will appear in your sample list with which you can



individually select which acquisition method the instrument will use for that sample. If none of the boxes are checked it will default to the method you selected in the acquisition box.

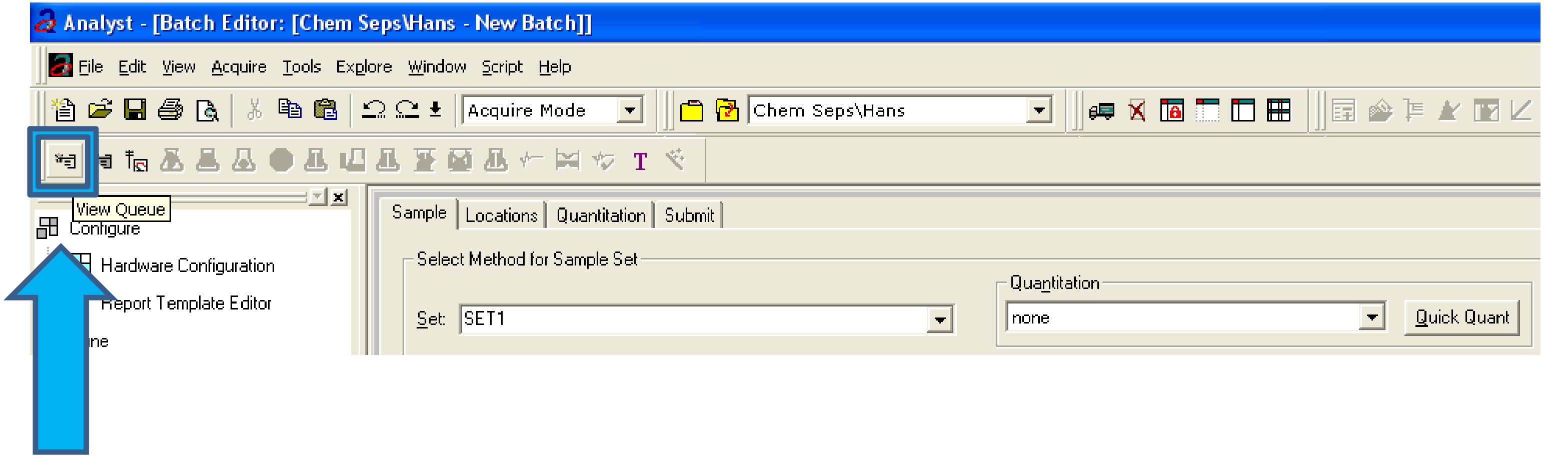
b. When you select a method (see blow up below) the injection volume will fill in. If you need to inject more or less of a sample than what the method you selected calls for you can change the amount of that particular sample injected using the "Inj. Volume (uL)" column in the sample list.



☐ Use as Template
☐ Use Multiple Methods

sition	Plate Code	Plate Position	Vial Position	Data File	Inj.Volume (µl)
	N/A	0	0	DataSET1	-1.000
	N/A	0	0	DataSET1	-1.000

10) Go to the "Submit" tab, review the information, then hit the submit button. This



Set: SET1

Quantitation: none

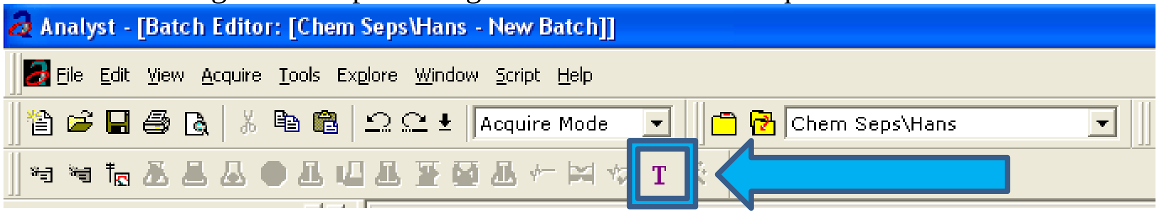
11) On the far left of the program window (beneath the file and new documents icons) there is a small, meaningless-looking icon with horizontal lines.

a) Click it, this opens the queue manager, which is a list of all jobs that the system is ready to do.

b) All of your samples should be listed as individual tasks within the queue.

c) Before you do anything, check that the four status symbols in the lower right of the window are green. (you will see these right above the time on the right of the computer screen)

    i) If they are yellow press the capital “T” button to reserve the instrument for tuning and then press it again to allow for data acquisition.

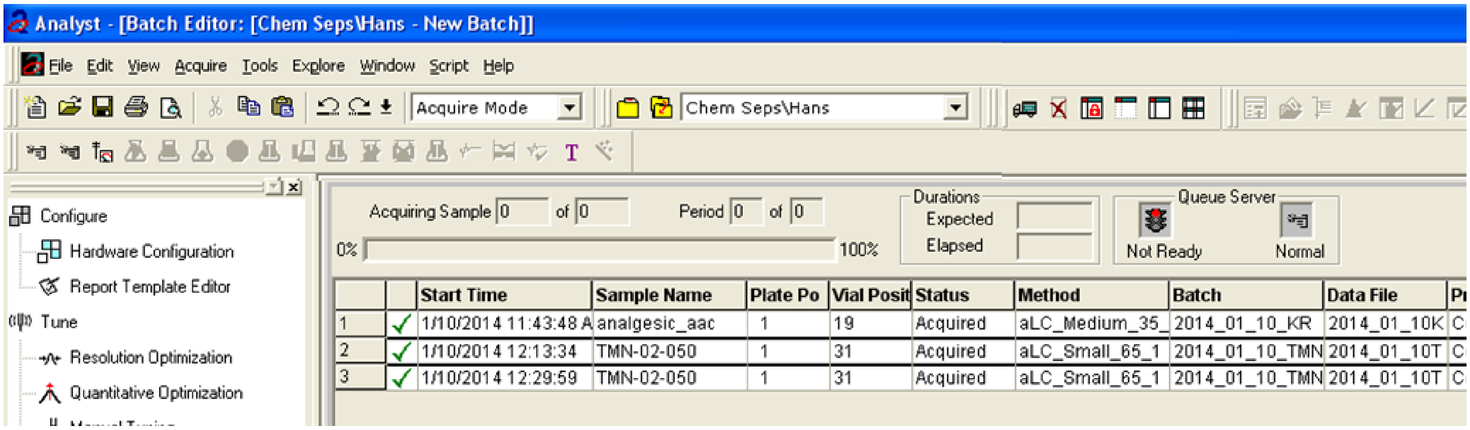


    ii) If one of the status symbols on the lower right of the window are red or display “Error” instead of “Idle” or “Ready” then you may have to restart the system or seek assistance.

d) With the queue manager window still open, select the *Acquire* button at the top of the program window, three over from “File” then choose “Start Sample” or click the first icon that looks like a flask (3 icons over from the “view queue”)

e) After the predetermined equilibration time, you should hear the autosampler start moving around inside the case.

12) Once your samples have started, you can watch their progress by selecting “open data file” under the explore tab and browsing to your data file (the one you specified in “build acquisition batch”).



Acquiring Sample 0 of 0 Period 0 of 0

0% 100%

Expected

Elapsed

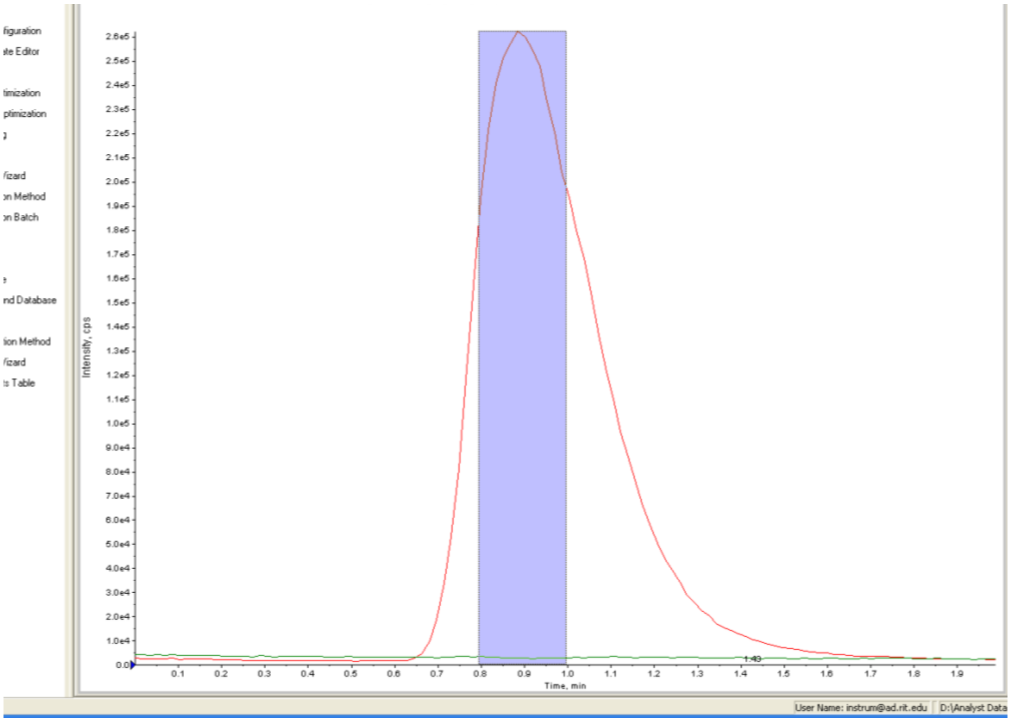
Not Ready

Normal

		Start Time	Sample Name	Plate Po	Vial Posit	Status	Method	Batch	Data File	Pr
1	✓	1/10/2014 11:43:48 A	analgesic_aac	1	19	Acquired	aLC_Medium_35_	2014_01_10_KR	2014_01_10K	C
2	✓	1/10/2014 12:13:34	TMN-02-050	1	31	Acquired	aLC_Small_65_1	2014_01_10_TMN	2014_01_10T	C
3	✓	1/10/2014 12:29:59	TMN-02-050	1	31	Acquired	aLC_Small_65_1	2014_01_10_TMN	2014_01_10T	C

    b. Click on the file you wish to view then select *ok*.

13) In order to view the MS data, highlight the area of the peak that appears by drop-and-dragging a box around the entire peak. Then you can either double click on this window or right click and select “Show Spectrum”, the MS data for the peak will show up in another window.



14) The spectrum can be saved as a pdf file by selecting file and then export, choosing a location to save to, and naming the file.

15) Once all the tasks in your queue have been completed, make sure to click the “stop queue” button in the queue manager (it is shaped like a stop sign seen on a road) and disconnect communications by selecting *Hardware Configuration* once again then select “deactivate” for the LCMS profile (the opposite of what we did in the initial setup). The UV-Vis should beep once the profile is successfully deactivated.

16) Before leaving the station:

    a. Turn off the four power buttons. **Leave the MS Portion on!**
    b. Remove all samples from the work space including from the autosampler. Any samples left behind will be disposed of!