

Standard Operating Procedure

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Revision History		
Version No.	Effective Date	Description

Approved By:

Date:

INSERT NAME AND TITLE HERE, Signature above

1 Purpose

The purpose of this document is to provide a step-by-step description of the how to setup a Sample Set and Calibration in Empower Pro software (v. 2005).

2 Impacted Individuals

3 Materials and Solutions Needed

- Autosampler vials

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- Autosampler vial inserts
- Analytical balance calibrated for weighing grams in the hundredths decimal place.
- General purpose mechanical pipettor, 1000uL (FisherSci, part no. FA10006MG)
- General purpose pipette tips, 1000uL (FisherSci, part no. 02-707-510)
-

4 Procedures

4.1 Preparation of Autosampler Vials

Note: Perform as much benchwork as possible in a sterile biological safety cabinet, while using individually wrapped and/or sterile glassware or consumable supplies.

- a. Aliquot 40mL of 1X PBS with 0.05% Tween 80 Solution into one sterile culture tube.

4.2 Creating a Calibration Batch

- a. Double-click Empower from Desktop.
- b. Select Run Samples.
- c. Load.
- d. Use the sample set wizard.
- e. Create a sample set method using this wizard.
- f. Select LC. Do not define plates or choose a dissolution type. Next.
- g. Select standard injections to occur at the start of sample set and update the vial position.
- h. Select Standard Level
 - i. Each standard vial contains a different level standard, if there are different concentrations of the same analyte of interest in separate vials.
 - ii. Different standard levels are created with different injection volumes using an autosampler, when standards are limited by

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volume and injections will be sampled from the same autosampler vial.

- iii. Next.
 - i. Update number of standard vials in each group, number of levels in each standard group, number of injections per level, injection volume, and run time as applicable. Select Method Set. Unless specifically instructed, do not to update Options: sample weight, dilution factors, data start and next injection delay. Next.
 - j. Update number of sample vials in each group, number of levels in each standard group, number of injections per level, injection volume, and run time as applicable. Select Method Set. Unless specifically instructed, do not to update Options: sample weight, dilution factors, data start and next injection delay. Next.
 - k. Optional: Identify prefix or suffix for SampleName of standards and samples. Next.
 - l. Select Run Only mode, Continue on Fault. Next.
 - m. Check the Display the Component Editor & Finish.
 - n. Minimize the Component Editor. It can also be opened using the Amounts icon in Run Samples window.
 - o. Within the Run Samples window, right-click Line 1 and Insert Row two times. If a sample or standard line is at the top or incorrect position, it can be moved to the correct line by a single-click and drag of the line number. Excess rows can be deleted in the same manner.
 - p. Update the Function of the first three lines to be: Purge Inj (10min), Purge Det (10min) and Equilibrate (30min).
 - q. Update Sample Name as desired. Update Level to reflect various concentrations (usually 100% is the method's injection volume, 50% & 200%).
 - r. Verify Vial, Inj Vol, # of Injs, Method Set, and Run Time are correct. Whole columns can be updated by right-clicking the Column Title, selecting Autofill and scrolling to Method Set, etc.
 - s. Walk over to the autosampler tray and verify it against vials in the Sample Set.

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- t. File, Save Sample Method As, following an example name format to include user, reason, and/or YYMMDD.
- u. Click Run Sample Set (green dot). Verify name. Run.
- v. Move to the Running tab to verify the batch is running or in queue.

4.3 Updating a Processing Method

- a. Open a similar processing method.
- b. Update Integration, Retention Times & Default Amounts parameters, if needed.
- c. File, Save As, file name to include column and date.

4.4 Processing the Calibration Sample Set Using Updated Processing Method

- a. Open Process Data.
- b. Select the desired Project and Data to Process: Sample Set. A new window opens. Select the same. Process.
- c. Background Processing and Reporting: Check Process,
 - i. If this is a fresh batch of standards previously calibrated on this system, select Use specified processing method and select the applicable from the drop-down list. Uncheck Clear Calibration & How: Calibrate Only. Uncheck Reporting and Exporting. Click OK. The window will minimize itself.
 - ii. If this is batch of standards which have analytes completely new to the system, **previously calibrated on this system, select Use specified processing method and select the applicable from the drop-down list. Uncheck Clear Calibration & How: Calibrate Only. Uncheck Reporting and Exporting. Click OK. The window will minimize itself.**
- d. From Review Data, open Result Sets tab. Select the Result Set Name from which you just processed, verifying date and time. Right-click, Review.
- e. Select the Calibration Curve icon. Verify Components have been imported correctly.

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- f. Select Review Main Window icon. Scroll through each injection using the Next Injection icon. When making changes to red integration line, select Process, Quantitate Standard to keep the change.
- g. File, Save, All. Exit or minimize the Review window.
- h. Within Results Set tab, right-click after selecting Result Set Name, Preview/Publisher, Report Method: LC Calibration Report, Save Report icon
- i. Also in Results Set tab, right-click after selecting Result Set Name, Preview/Publisher, Report Method: Multi-Sample Summary, Save Report icon
- j.

4.5 Reviewing, Processing & Printing Results from an Unknown Batch

- a. Double-click Empower from Desktop.
- b. Select Process Data.
- c. Select desired Project & Data to Process: Sample Sets.
- d. Highlight desired Sample Set Name and click Process.
- e. Within Background Processing and Reporting, check Process & select Use specified processing method and select the applicable method from the drop-down list & How: Quantitate Only. Uncheck Clear Calibration & Reporting and Exporting. Click OK. The window will minimize itself.
- f. Select Review Data from Empower Pro.
- g. Select desired Project & Data to Review: Sample Sets.
- h. Move to Result Sets tab, highlight desired Sample Set Name and click Review. A new Review window will open.
- i. Select the Calibration Curve icon. Verify all calibration information remains the same. Clicking Next Component icon will scroll faster than selecting from the drop-down menu.
- j. Select Review Main Window icon. Scroll through each injection using the Next Injection icon. When making changes to red integration line, select Process, Quantitate to keep the change.

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- k. File, Save, All. Exit or minimize the Review window.
- l. Within Results Set tab, right-click after selecting Result Set Name, Preview/Publisher, Report Method: Multi-Sample Summary, Save Report icon

5 References

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