CHEMICAL SOLUTIONS, Inc.

HPLC PERFORMANCE QUALIFICATION SYSTEM
QUICK START REFERENCE

Rev. 5.02

INTRODUCTION:

Welcome to the PQ Kit! These instructions are designed to help you to quickly familiarize yourself with the procedures needed to fully qualify your HPLC using the supplied NIST-traceable reference standards and the validated PQ test column. The total time to qualify your instrument should be about 1 hour for isocratic, with an additional hour if you have a quaternary gradient system. The supplied software will allow you to enter the data, and print out the results, along with a Certificate that can be signed and reviewed according to your normal SOPs. The most time consuming part of a first time qualification is writing the method programs – once that is done, they, along with the injection sequence, can be re-used in future Performance Qualifications on that instrument.

Sufficient volumes of solutions are supplied for several qualifications – the exact number depending upon the instrument and injector type. Mobile phase is stable for 60 days, and can be prepared in bulk if multiple instruments are to be qualified.

Here is an overview of the basic steps required for a Performance Qualification of your UV-Vis Detector HPLC:

Step 1	Perform any normally required Preventative Maintenance on the HPLC Typically, pump seals, check valves, rotor seals, lamps, etc. Or, confirm that the PM service was completed by the instrument vendor or service company.
Step 2	Qualify the HPLC for flow accuracy, column oven and refrigerated autosampler temperatures.
Step 3	Prepare the Mobile Phases
Step 4	Setup the HPLC Methods (re-use methods and sequences for subsequent qualifications)
Step 5	Perform the Wavelength Qualification with Holmiuim Oxide and Caffeine
Step 6	Prepare the Vials and Run the Injection Sequence
Step 7	Enter the Data into the software. All results are automatically calculated. Print and Review the Results
Step 8	Sign off on the printed Certificate, along with any reviewers. The HPLC is Qualified – ready for service!

DETAILED PROCEDURES:

INSPECT THE BOX CONTENTS –

There should be a total of 9 bottles containing the Wavelength Calibration Solution (WCS), Linearity Solutions (L1-L6) and the Gradient Visualization Solution (GVS), with a Certificate of Analysis (CoA).

A pre-tested, certified PQ column is provided (except in the replacement solution kit), along with its test Certificate.

A CD should be present, with the Excel-based Template program, along with electronic copies of the manuals and general background information. Instructions as to how to load and review the programs and instructions manuals will automatically come up on the screen when the CD is loaded. A demo copy of the template is provided, containing example qualification data. This should provide a sense of the type of data to be acquired, and typical results.

Support is available both from Chemical Solutions Inc. and MicroSolv Technologies, Inc.

For sales and technical support and questions, contact:

MicroSolv Technology Corporation
Telephone: 732-578-1777
website: www.mtc-usa.com

Contact us at with any questions.

Detailed discussion of the layout and interpretation of the various tests performed by this PQ Kit have been published in LC-GC Magazine. See:

> "Performance Qualification of HPLC Instrumentation in Regulated Laboratories",
> *LCGC North America, Volume 26 Number 5 May 2008*.

A reprint of that article is included on the CD disk, and should be referred to for more details on interpretation of the final results, and the assignment of Acceptance Criteria to the various test protocol results.

STEP 2 - PRE-QUALIFICATION PREPARATIONS -

Pre-qualification activities refer to the preventative maintenance and qualification activities normally performed on the instrument hardware prior to the actual performance qualification itself. Most laboratories will either have had a service provider already change pump seals, rotor seals, detector lamps, etc., or will have done these PM activities themselves, prior to starting the performance qualification. Remember to include any self-tests for the modules, such as internal wavelength qualification for diode array detectors, etc., prior to starting the formal HPLC qualification. We refer to these maintenance and modular component qualification test as "Pre-Qualification" for convenience.

For the flow and temperature qualification, these activities are assumed to be:

1. Pump maintenance and flow rate qualification
2. Temperature qualification of the column oven
3. Temperature qualification of a refrigerated autosampler (if present)

The accompanying PQ software provides two ways to accomplish this for any of the above tests

1. If the service provider of your instrument has already qualified the above items and you intend to reference that activity to satisfy your SOP requirements, simply select the box that says that this activity has already been performed, and enter the qualification date.

2. If you wish to perform these activities yourself, space is provided for entering the data either from your calibrated flow meter and thermometers, or to enter the volumetric flask sizes and timed collection data, for automatic calculation of the flow rates. There is space for entry of up to three flow rates and four column oven temperatures. You do not have to use all the spaces – simply leave the unused spaces blank, and the software will ignore the empty slots.

3. Flow Rate Qualification:

The easiest and fastest way to qualify flow rates is to use a calibrated liquid flowmeter. Simply enter that option into the cell, and record the values found. However, these flowmeters are expensive, and not every laboratory maintains a unit. If you have one, simply measure the flows and enter the values into the cells.

For manual flow rate qualification, a typical range for an analytical HPLC might be 0.5, 2.0 and 5.0 mL/min. Choose a qualification range that encompasses the intended use of the instrument.

To reduce errors, a collection time of at least one minute should be used. The flow rate is calculated as:

$$Flow = \frac{Volume(mL)}{Time(\text{min})}$$

Using a dry volumetric flask and calibrated digital timer, the following combinations of volumetric flasks and expected collection times are recommended, although others may be used as per your SOP:

Flow Rate:	Volumetric Flask Size:	Expected Time:
0.5 mL/min	5 mL	10.0 min
2.0 mL/min	5 mL	2.5 min
5.0 mL/min	10 mL	2.0 min

The software provides for time entry as minutes and seconds, as most timers use this format. It will automatically convert this to digital minutes and calculate the flow rate.

A flow accuracy specification of ±95% - 105% is recommended for manual qualifications, as it is difficult to achieve much tighter specifications given the uncertainties of the timing and collection procedures.

4. Column Oven Qualification:

   Temperature measurement for both the autosampler and column oven is most easily accomplished using a calibrated digital thermometer with a flexible wire thermocouple that can be inserted into the spaces and sealed. Most laboratories maintain such devices for this purpose.

   For the column oven, thread the thermocouple end into the column compartment, taking care the probe does not directly touch the heated metal surfaces. Allow the temperature to stabilize at each setting and record the temperature.

   The qualification range should encompass the intended use of the column oven.

   An acceptance criteria of ±5°C is recommended.

5. Refrigerated Autosampler Qualification:

   Insert the flexible thermocouple probe into a central vial well, and loosely seal the well with paper tissue or other means. Allow the temperature to equilibrate.

   Acceptance criteria:

   Most refrigerated autosamplers have relatively crude temperature control. The USP definition of refrigerated conditions is 2° - 8°C, with a target of 4°C. We recommend that the refrigerated autosampler be set the single temperature of 4°C, and that an acceptance criteria of 2° - 8°C be applied.

One final comment. Your company SOP's obviously take precedence over any of the above procedures and acceptance criteria. Maximum flexibility has been incorporated into the data entry, to accommodate the wide range of procedures used by various companies. On the other hand, it may be more efficient to follow the above recommendations, which have been field tested at many companies over many years of experience with the PQ kit, and to streamline and modify your internal SOP's to match the above recommendations. Obviously, your QA unit will have to make such final decisions.

STEP 3 – STARTING THE QUALIFICATION - MOBILE PHASE PREPARATION -

The approximate minimum volume of mobile phase for each HPLC to be Qualified is:

- 1L for full testing with a quaternary gradient
- 0.5L for isocratic only

Note that the mobile phase is stable for at least 60 days, and can be prepared in bulk for multiple qualifications.

Two equivalent mobile phases have been developed and qualified for testing with the PQ test column – 13% acetonitrile, or 30% methanol, both containing 1 mL/L (0.1% v/v) of glacial acetic acid. While both produce equivalent test data, the acetonitrile mobile phase will produce significantly lower pressure, and is preferred despite its higher cost. The lower percentage of acetonitrile means that only about 70 mL of the solvent are required for an isocratic qualification.

Either mobile phase may be adjusted to meet the system suitability retention time window of 1.0 – 1.5 minutes retention time for caffeine.

An equivalent reversed phase column may be used (C8, 5µm particle size, 120Ä, 4.6X75 mm), provided that system suitability can be achieved with only minor adjustment of the mobile phase.

The mobile phase is prepared by separately combining the following for every 1L:

130 mL of HPLC grade Acetonitrile or 300 mL HPLC methanol
870 mL of purified water or 700 mL for the methanol mobile phase
1 mL of glacial acetic acid
Mix and filter/degas using a membrane filter, or as per your current laboratory practice.

For a Gradient Qualification reserve about 500 mL of mp into a separate bottle (referred to as 'B*').
Add 3 mL of the ***Gradient Visualization Solution*** to this 500 mL, directly into the bottle.

Mix well.

(Note that the exact composition of the GVS solution in the B* mobile phase is not critical, with graduated cylinder accuracy being more than sufficient).

For a quaternary gradient qualification, put about 200 mL of unspiked mp into each of the reservoir bottles for C and D, or place the inlet lines for lines A, C and D into a single reservoir bottle with sufficient mobile phase.

Flush the HPLC with the new mobile phases.

If you have a binary or quaternary gradient pump for gradient qualification, flush the B* circuit with the GVS spiked mobile phase. Be sure to flush this line VERY THOROUGHLY! If the GVS-spiked mp is not of uniform composition, it will be misinterpreted as an error in the gradient delivery. If the gradient delivery steps look odd, repeat the test to ensure it was not due to incomplete flushing of the B* line. Alternatively, for overnight automated runs, simply perform the GRD method with 2 injections, using the first as a pre-conditioning injection, and the second run for the qualification results.

STEP 4 – WRITE THE METHODS –

The methods need only be written once for each instrument, and will be re-used for future qualifications.

Only a single method is required to perform an isocratic Performance Qualification (the PQ method below), with a second method for a gradient (GRD). However, it is usually more efficient to create an additional isocratic method (RTM), solely for use with the Resolution Test mixture injection, which needs a longer run time than the bulk of the caffeine-only injections. Also, if a diode array or scanning wavelength detector is being used to acquire the spectrum of caffeine, this can be accomplished most readily by adding spectral acquisition to this first method for the RTM and System Suitability injection, and not acquiring spectra for the bulk of the PQ injections.

The basic conditions for these three methods are listed below:

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PQ Method Summary:

Column: MicroSolv PQ Column, C8 5µm 75 X 4.6 mm

Parameter:	PQ:	RTM:	GRD:
Flow:	2 mL/min		
Injection Volume:	8 ΦL [Modify for injector volume linearity test and/or to extend detector linearity][b.]		
Wavelength:	273 nm		
Column Temperature:	Ambient [20EC-25EC]		
Run Time:	≤2 min	3 min	65 min
Other:	No scanning Set time constant as needed	Scan wavelengths 200-300 nm	
Gradient:	NA	NA	Time: %A %B %C %D 0 min 100% 0% 10 min 0% 100% 15 min 0% 100% 17 min 90% 10% 23 min 90% 10% 25 min 10% 90% 30 min 10% 90% 32 min 10% 90% 37 min 10% 90% 39 min 90% 10% 44 min 90% 10% 46 min 10% 90% 51 min 10% 90% 53 min 90% 10% 58 min 90% 10% 60 min 100% 0% 65 min 100% 0% (re-equilibrate)

[b.] If your data system permits, e.g. ChemStation, use the same method, but change the injection volume within the sequence for the injector volume linearity test. If this is not possible, copy and modify the same method, changing only the injection volume for each of the volumes to be tested, e.g., PQ5, PQ10, …PQ100.

SELECTING THE INJECTION VOLUME:

For a UV-Vis detector, except in volume or mass overload conditions, the absorbance linearity is determined by the ***HEIGHT*** of the injected peak (typically expressed as milli-absorbance units, or mAU. The PQ system, including the certified column, has been engineered to produce peak heights within the expected linear range of most detectors, based on a 10 mm pathlength for a typical analytical cell.

The peak height is determined the sample concentration, its molar absorptivity, the cell pathlength, the injection volume and the column efficiency. Since the sample concentration and type are controlled, along with the column efficiency, the only remaining variables are the cell pathlength and injection volume. The injection volume may be adjusted within the proven linear range of the autosampler (performed as part of the qualification) to set the absorbance range that will be observed for the set of linearity standards L1-L6.

The following Table outlines the approximate peak range of peak heights that should be found for the L1 - L6 linearity solutions, for various injection volumes, using a 10 mm or 6 mm pathlength flow cell.

Table of Approximate Expected Peak Heights for Various Injection Volumes and Flow Cell Pathlengths		
Injection Volume:	Cell Pathlength:	
	10 mm	6 mm
5 µL	0.8 – 800 mAU	0.5 – 500 mAU
8 µL	1.3 – 1300 mAU	0.8 – 800 mAU
10 µL	1.6 – 1600 mAU	1.0 – 960 mAU
15 µL	2.4 – 2400 mAU	1.4 – 1440 mAU
20 µL	3.2 – 3200 mAU	1.9 – 1900 mAU

Few detectors are linear at much above 1500 mAU, and many start to offer non-linear responses at 1000 mAU and above. You can decide what range of linearity to qualify for a given detector by selecting the appropriate injection volume.

For the typical 10 mm flow cell, an injection volume of 8µL will produce signals over the range of about 1 to 1300 mAU. The exact heights will depend on the specific instrument, its extra column dispersion and other factors. However, this is a typical range over which a modern detector would be qualified.

If you intend to use your detector at significantly higher absorbance values, you should inject larger volumes, e.g., 12µL, which calculates to produce peak heights on the order of 2000 mAU (the peak heights are assumed proportional to injection volume or cell pathlength). Note that this would be an extremely high absorbance value, and it would be unusual to expect an instrument to be fully linear to such an absorbance. However, you can perform this, and thus determine what the actual upper linear range of the detector is.

For the linearity test, the software requests the injection volume, and both peak heights and areas for the linearity solutions. The Dyanamic Linear Range of the detector is automatically calculated based on the peak heights, and the curvature from linearity.

You can input the maximum allowable error from linearity into the software. The ASTM test E1657-98 uses a value of 5% error to determine the upper limit of the Linear Dynamic Range. This implies that a single-point calibration standard at the upper limit, would have a ±5% error from another sample further down into the linear region.

The linearity of the AREA is also calculated separately, and the results reported. Typically, the area will be linear over a slightly greater range due to the integration of the signal over all absorbance values of a given peak.

One should note that the purpose of a routine *Performance Qualification* is not to determine the absolute limits of detector performance every time, which is more the function of an *Operational Qualification*. Thus, it is recommended that the peak region for a PQ encompass the normal range over which the detector is typically used. An 8 µL volume will typically be appropriate for modern instruments with a 10 mm flow cell. Other volumes can be used either for different flow cells, or if linearity needs to be demonstrated over a different range for trouble shooting purposes.

STEP 5 – PERFORM THE WAVELENGTH QUALIFICATION -

A full wavelength qualification requires obtaining spectra on two solutions – one of caffeine and the other of the holmium oxide solution. This will qualify the detector over the wavelength range of 205nm – 641nm (or 205nm – 361nm for the UV range only).

For a diode array detector, simply acquire the spectrum of caffeine during the RTM run, or any other injection of caffeine producing a reasonable signal height of 100 mAU or more.

For manual scanning detectors, where such spectra cannot be obtained automatically, and for holmium oxide, the spectra must be obtained by filling the flow cell with the appropriate solutions, as described below.

VWD or DAD (Scanning Detectors):

For scanning detectors (Variable Wavelength or Diode Array), the test solution is pulled through the flow cell using the spring-loaded syringe at the *detector outlet*, with the *detector inlet* (column outlet tubing) dipped into a small vial containing the desired solution. Start by pulling purified water into the flow cell. When pulling solutions through, small bubbles may be observed in the exit tubing, thus confirming flow. The vacuum connection on the spring-loaded syringe should be broken once the cell is flushed and before taking any spectra, so that a stable signal is obtained.

Write a short method (about 3 min total), with 0 flow and no injection. The method should acquire a spectrum within the first minute, while the Reference Solution is still in the cell. Then, it should acquire the WCS (Homium Oxide) or the L2 caffeine solution, once it has been pulled into the flowcell and the syringe vacuum broken.

Pull purified water through the flow cell, and break the syringe connection to release the vacuum. Start the method. After about 1 minute, switch the vial to the WCS or L2 solution, and pull it through the flowcell with the syringe for about 1 minute, establishing a strong steady signal, without any bubbles in the flowcell. Break the connection, and allow the signal to stabilize. Acquire the desired spectrum during this time. Note that this is a qualitative test to acquire spectra. The test is valid as long as a sufficiently strong signal is obtained that the software can process to produce a reliable spectrum.

Manual Variable Wavelength Detectors:

The wavelength qualification for a manual VWD is, in principle, the same as for a scanning instrument. However, for non-scanning VWD, it is necessary to manually step through discrete wavelengths before and after the spectral bands of interest, and interpolate the absorbance maxima by watching the absorbance values rise, plateau, then fall as the maximum is passed by. The PQ Template is designed such that you can enter these absorbance values into the cells. The program will interpolate and automatically calculate the spectral maximum for each absorbance band. Refer to the Holmium Oxide and Caffeine spectra shown in Figures 1 and 2. For some bands that are close together, such as the 278nm/287nm pair, you should be careful not to go too far away from the expected maxima, or else you might find a false maximum value.

Data Analysis - Primary Standard Holmium Oxide 241 nm - 641 nm:

The Wavelength Calibration Solution consists of Holmium Oxide (HoX) in 10% perchloric acid, at exactly the same concentration as the NIST SRM 2034. There are 14 absorbance bands over the range of 241nm to 641 nm, as shown in Figure 1 (also refer to the CoA, and to the full instructions). The Template allows you to select up to 5 HoX maxima for the qualification, in addition to the 2 bands of Caffeine. For a UV-only detector, there are 4 strong bands at 241nm, 278nm, 287nm and 361nm. To cover the Visible range, bands at 451nm, 537nm and 641nm are available.

For a DAD or scanning VWD, simply process the acquired HoX spectrum the data acquisition software, and enter these values into the template. The template program will regress and graph the results of the Found vs Theory values, and extrapolate the expected error at 200nm and 700nm, to show any trends in the monochrometer accuracy.

Data Analysis - Secondary Standard Caffeine 205 nm and 273 nm:

Acquire the spectrum of Caffeine over the range of about 200 nm to 300 nm. This is accomplished either manually, or by scanning the Caffeine peak in the RTM, or any other of the Caffeine injections. Use Mobile Phase as the Reference Solution. The UV spectrum of Caffeine is shown in Figure 2. Enter the data into the Excel^{tn} template. The data will be compared to the true absorbance maxima of 205nm and 273nm, and added to the regression line.

Figure 1: Spectrum of Holmium Oxide WCS Solution

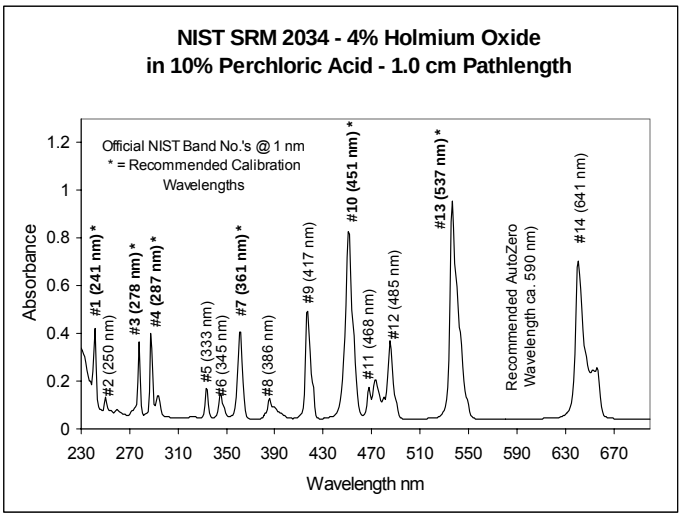
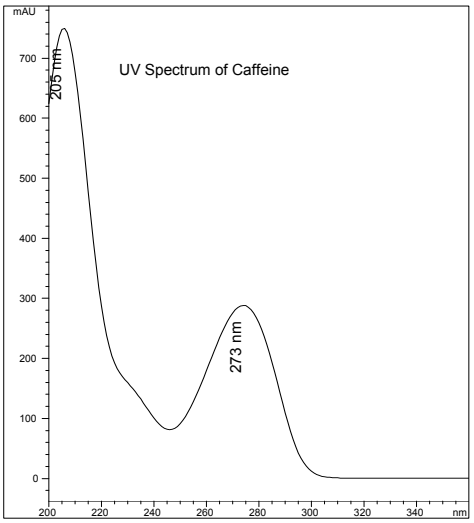


Figure 2: Spectrum of Caffeine in RTM



STEP 6 – PREPARE THE VIALS FOR THE REMAINING PQ TESTSAND RUN THE SEQUENCE -

If you are following the standard PQ sequence suggested below, you will need to fill the following numbers of vials, assuming a single injection from each vial.

Solution Vials Required[a.]									
Solution	Diluent (mp)	GVS Spiked MP B*	RTM	L1	L2	L3	L4	L5	L6
No. Vials (single injection per vial)	4	1	1	1	6	11	1	1	1
Using multiple injections per vial:	4	1	1	1	2	2	1	1	1

[a.] Assuming multiple injections per vial. If single injections per vial are planned, fill one vial per injection.

Write the *Injection Sequence and Run the Performance Qualification*

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Sequence PQ1:	**General HPLC Performance Qualification Example Injection Sequence**			
Line No. /Vial No.	**Sample Name**	**Method**	**Number of Injections**	**Comments:**
1	Mobile Phase Blank	RTM	1	System Suitability: Inject one or more Blanks until Clean, quiet baseline. Retention of Caffeine 1.0 – 1.5 min. Efficiency ≥ 2000 Rs of peaks before and after Caffeine ≥ 2.0 Use Blank for *Dynamic Noise* determination. *Noise Level* measured depends on the *Time Constant* used. Consult your detector/data system manual and set time constants at appropriate value. For DAD, acquire spectra if caffeine is used for 8 accuracy.
2	Resolution Test Mixture		1	
3	Linearity Solution L3	PQ	10	*Autosampler Precision* and *Pump Stability*.
4	Mobile Phase Blank		1	Ensures clean system prior to starting Linearity
5	Linearity Solution L1 (0.1%)	PQ	1	Begin *Detector Linearity* with 0.1% solution *System Sensitivity* will also be calculated from data. Conclude with the 100% level solution, L6
6	Linearity Solution L2 (1%)		1	
7	Linearity Solution L3 (20%)		1	
8	Linearity Solution L4 (50%)		1	
9	Linearity Solution L5 (75%)		1	
10	Linearity Solution L6 (100%)		1	
11	Mobile Phase Blank (for injector % Carryover)		3	*% Carryover* following the most concentrated solution. Note if a *wash vial* is used or not. The 1st injection is used for % carryover calculation The 2nd two injections are FYI, to show how long it takes to cleanse the needle prior to the next test.
12	Linearity Solution L2*	PQ (5ΦL)	1	Autosampler *Volume Linearity* at 5 volumes. * Injection volumes should be modified to suit autosampler or maximum loop volume. Area should remain within detector linear range (from above). For some data systems (e.g. Agilent ChemStation), the same method can be used, and the injection volume modified in the Sequence table.
13	Linearity Solution L2*	PQ (10ΦL)	1	
14	Linearity Solution L2*	PQ (25ΦL)	1	
15	Linearity Solution L2*	PQ (50ΦL)	1	

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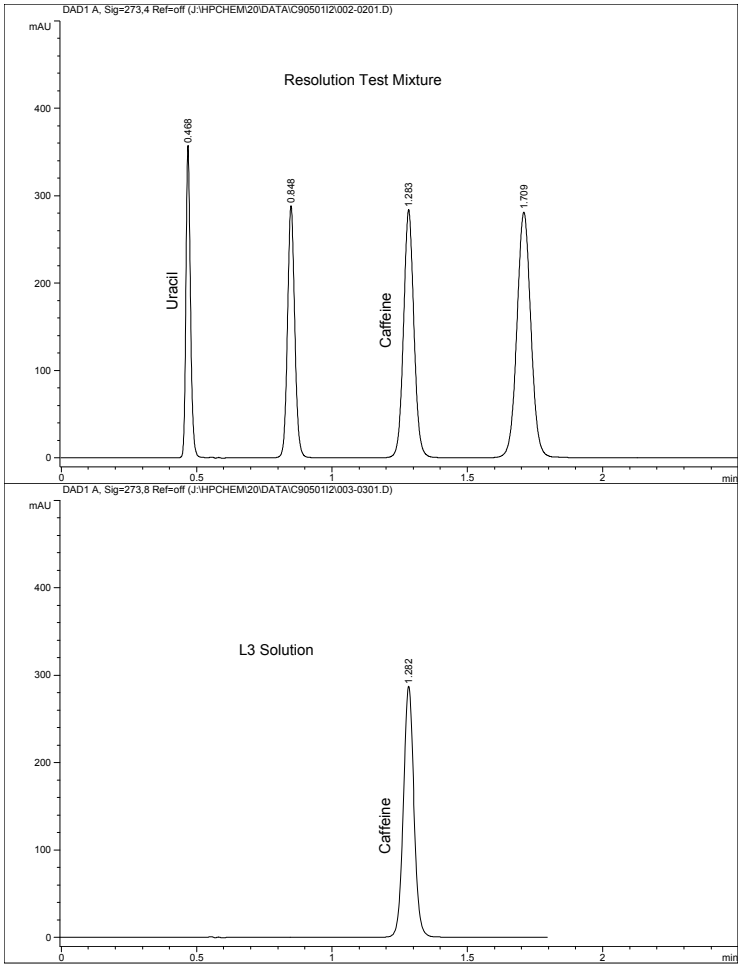
Sequence PQ1:	**General HPLC Performance Qualification Example Injection Sequence**			
Line No. /Vial No.	**Sample Name**	**Method**	**Number of Injections**	**Comments:**
16	Linearity Solution L2*	PQ (100ΦL)	1	
17	Mobile Phase Blank	PQ	1	Cleans system prior to start of Gradient Tests
18	GVS Spiked Mobile Phase B*	GRD	2*	Gradient *Dwell Volume* and *Accuracy* . *Use injection #2 for qualification. Or use a single injection if the system is adequately flushed.

Figure 3 shows a typical separation of the Resolution Test Mixture, used for System Suitability and for calculation of the Extra-Column dispersion. The total run time is 3 minutes, while the allowable retention time window for Caffeine (Peak #3) is 1.0 – 1.5 minutes. Subsequent injections of Caffeine only, using method PQ require only sufficient run time so that Caffeine can be eluted and integrated. For the separation in Figure 3, a run time of 1.5 min for method PQ is sufficient.

Figure 3: Typical separation of the Resolutioin Test Mixture and an L3 Precision Sample



Figures 4 and 5 show a typical gradient accuracy chromatogram for a quaternary pump. The gradient dwell volume is found by subtracting the retention time of the unretained peak Uracil (from the blank injection of the spiked mobile phase B*) from the apparent onset of the gradient. This Dwell Time, multiplied by the flow rate, gives the Dwell Volume. This value is automatically calculated by the software.

Figure 4: Gradient Accuracy for Quaternary Pump

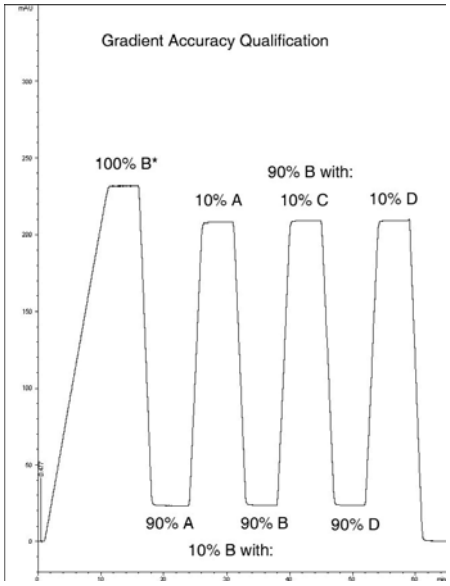
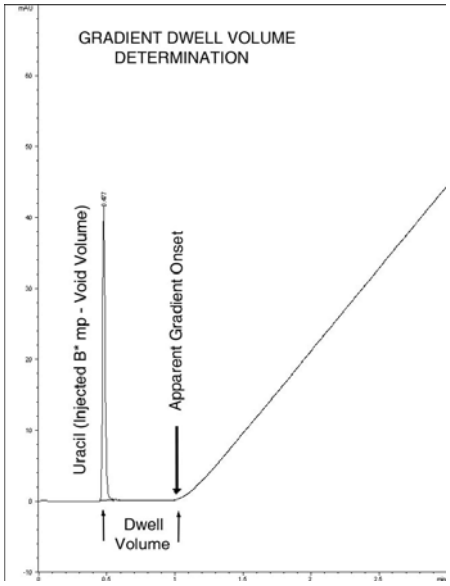


Figure 5: Gradient Dwell Volume



STEP 7 – ENTER THE DATA AND CALCULATE / REVIEW THE TEST RESULTS -

Ensuring that the data is integrated properly, enter the results into the Excel[tm] template. All data is entered into the Data Entry sheet. Be sure to enter the correct solution concentrations from the Certificate of Analysis provided with each kit. There are fields for the entry of instrument model and serial numbers, operator name, logbook pages, etc. Since every laboratory requires different documentation, these entry areas have been kept as flexible as possible. Modify and change the data entry labels and formats to conform to your own internal SOPs.

All cells are protected except those allowing data entry. Empty cells requiring data are red (for required data) or orange for optional data, and will turn to green once data is entered.

Not every test needs to be performed, and there may be times when only one or two tests are performed, as perhaps following repair of a module. Entry of a test date activates the corresponding data entry areas. If the test date field is blank, it is assumed that the test was not performed. If a date is entered, then data is expected, and a warning flag will become visible, requesting that either data be entered, or the date deleted.

Most of the data entry fields are self-explanatory, and many of the boxes contain optional drop down boxes to select units or other test conditions.

Once the data have been entered for all tests that were performed, click the “Show Results” button. In order for the macros to run properly, ***you must set the Security level of Excel to “Medium” or “Low”.*** You can consider the program as contained on the CD or downloaded from our website as a “trusted source”. Clicking the button will calculate and generate the test results in tabs on the spreadsheet – one tab per test. A Qualification Certificate will also be generated. Buttons are provided to print the Certificate alone, and/or the various test results sheets.

Don’t forget to SAVE THE TEMPLATE TO A NEW FILENAME!! Use SOPs at your laboratory to determine the spreadsheet name and file structures. Do this early in the PQ when first setting up, then save it early and often throughout the data entry process. The template is write-protected, so only the data entry cells on the first tab can be changed. The various graphs on the Results tabs will autoscale.

Failed tests will be highlighted in red. This first page gives you a compact single page summary of the entire instrument PQ results. It provides for easy review and sign off, and can be copied and pasted into the instrument logbook. The detailed test results are given in the remaining pages, where all the raw data for each test protocol is presented for reference.

Note that assigning the *Acceptance Criteria* is ultimately the responsibility of the laboratory. The program contains what Chemical Solutions feels are reasonable values, referencing the USP or ICH whenever possible, e.g., wavelength accuracy. However, for most tests, it is the responsibility of the laboratory to justify the Acceptance Criteria chosen. Your SOPs may call for tighter or looser specifications. This is a regulatory decision that must be made within your own company’s guidelines. It is also possible to use only the test solutions, column and general method conditions, and analyze the data without the Excel template, according to your own SOP requirements. The PQ Kit is designed to be flexible enough so that you can incorporate it into your SOPs to tailor it precisely to your needs.

STEP 8 – PRINT THE FINAL CERTIFICATE, CALCULATED RESULTS AND DATA

THE PERFORMANCE QUALIFICATION IS COMPLETED -

The HPLC is ready for service, with a comprehensive, NOST-Traceable Performance Qualification!

Close out the project by completing any signatures of the logbook, and application of stickers to the HPLC, etc., as required by your internal SOPs.