



## HPLC PERFORMANCE QUALIFICATION SYSTEM QUICK START REFERENCE

Rev. 5.01

### 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 2 hours 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 steps required for a Performance Qualification of your HPLC:

- |        |  |
|--------|--|
| Step 1 | Inspect the box contents                     |
| Step 2 | Perform any required PM Service              |
| Step 3 | Prepare the Mobile Phase                     |
| Step 4 | Setup the HPLC Methods                       |
| Step 5 | Perform the Wavelength Qualification         |
| Step 6 | Prepare the Vials \ Run the Sequence         |
| Step 7 | Enter the Data. Print and Review the Results |
| Step 8 | HPLC is Qualified – ready for service!       |



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### DETAILED PROCEDURES:

#### STEP 1 – 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 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.

#### STEP 2 - PRE-QUALIFICATION PREPARATIONS -

Perform any needed maintenance on your instrument. If you have a service contractor, or if you perform your own service, all routine items, such as pump seals, check valves, rotor seals, detector lamps, flow cell, etc. should be serviced prior to starting to the PQ testing. For DAD detectors, if there are any self-check procedures for internal wavelength calibrations, etc., that you wish to perform and document, do those now.

Note that some preparatory tests, such as flow rate qualification and column oven temperature, may have already been performed as part of the Preventative Maintenance/Service, and are technically not part of the PQ. If so, there is a section in the template for you to enter the data from those qualifications – you do not need to perform them again. The PQ tests assumes that the flow rate has been qualified, and is accurate. You may enter in an exact calibrated flow rate value if it is available.

#### STEP 3 - MOBILE PHASE PREPARATION -

Prepare for each HPLC to be Qualified:

- 2L for full testing with a quaternary gradient
- 1L for isocratic only

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

130 mL of HPLC grade Acetonitrile  
870 mL of purified water  
1 mL of glacial acetic acid  
Mix and filter/degas using a  $\leq 0.45 \mu\text{m}$  nylon or polycarbonate membrane filter.

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

For a quaternary gradient qualification, put about 250 mL of unspiked mp into reservoir bottles for C and D.

Fill the primary mobile phase A reservoir with about 1L of mp.

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, simply plan to run the GRD method with 2 injections, using the second run for the qualification results.

#### STEP 4 – WRITE THE METHODS –

Depending on your data system, 3 methods must be written. Their basic conditions 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 µL [Modify for injector volume linearity test and/or to extend detector linearity] <sup>b</sup>																																																																																												
Wavelength:	273 nm																																																																																												
Column Temperature:	Ambient [20EC-25EC]																																																																																												
Run Time:	≤2 min	3 min	65 min																																																																																										
Gradient:	NA	NA	<table border="1"> <thead> <tr> <th>Time:</th> <th>%A</th> <th>%B</th> <th>%C</th> <th>%D</th> </tr> </thead> <tbody> <tr><td>0 min</td><td>100%</td><td>0%</td><td></td><td></td></tr> <tr><td>10 min</td><td>0%</td><td>100%</td><td></td><td></td></tr> <tr><td>15 min</td><td>0%</td><td>100%</td><td></td><td></td></tr> <tr><td>17 min</td><td>90%</td><td>10%</td><td></td><td></td></tr> <tr><td>23 min</td><td>90%</td><td>10%</td><td></td><td></td></tr> <tr><td>25 min</td><td>10%</td><td>90%</td><td></td><td></td></tr> <tr><td>30 min</td><td>10%</td><td>90%</td><td></td><td></td></tr> <tr><td>32 min</td><td></td><td>10%</td><td>90%</td><td></td></tr> <tr><td>37 min</td><td></td><td>10%</td><td>90%</td><td></td></tr> <tr><td>39 min</td><td></td><td>90%</td><td>10%</td><td></td></tr> <tr><td>44 min</td><td></td><td>90%</td><td>10%</td><td></td></tr> <tr><td>46 min</td><td></td><td>10%</td><td></td><td>90%</td></tr> <tr><td>51 min</td><td></td><td>10%</td><td></td><td>90%</td></tr> <tr><td>53 min</td><td></td><td>90%</td><td></td><td>10%</td></tr> <tr><td>58 min</td><td></td><td>90%</td><td></td><td>10%</td></tr> <tr><td>60 min</td><td>100%</td><td>0%</td><td></td><td></td></tr> <tr><td>65 min</td><td>100%</td><td>0%</td><td></td><td></td></tr> </tbody> </table>	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%		
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<sup>b</sup> 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.

The injection volume of 8 µL should produce a maximum peak height of about 1500 mAU for solution L6. Use a larger injection volume to create a higher maximum absorbance value for the detector linearity, or reduce the volume if you desire a lower maximum signal.

### STEP 5 – PERFORM THE WAVELENGTH QUALIFICATION -

There are two techniques for obtaining spectra of the wavelength solutions – one for scanning detectors (either Variable Wavelength Detectors (VWD) or Diode Array (DAD)), the other for manually controlled instruments.

#### 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 with Reference Solution in 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, so that a stable signal is obtained.

Write a short method (about 5 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) once it has been pulled into the flowcell. For a DAD, it is easiest to simply acquire ALL SPECTRA throughout the runtime. For a VWD, it could acquire a single spectrum anytime after the WCS has been pulled into the flowcell, and the vacuum released so that a stable signal has been established.



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Pull the Reference Solution (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, 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 WCS spectrum during this time. Note that this is a qualitative test to acquire spectra. The test is valid as long as a strong signal is obtained that the software can process to produce a reliable spectrum.

The spectrum of Caffeine is most easily acquired by writing the Resolution Test Mixture method to acquire the spectrum of Peak #3 (Caffeine) when the RTM is run during System Suitability. If not, the caffeine spectrum of solution L2 can be manually acquired as described above.

### 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 sketch out 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 monochromator 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<sup>®</sup> template. The data will be compared to the true absorbance maxima of 205nm and 273nm, and added to the regression line.



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Figure 1: Spectrum of Holmium Oxide WCS Solution

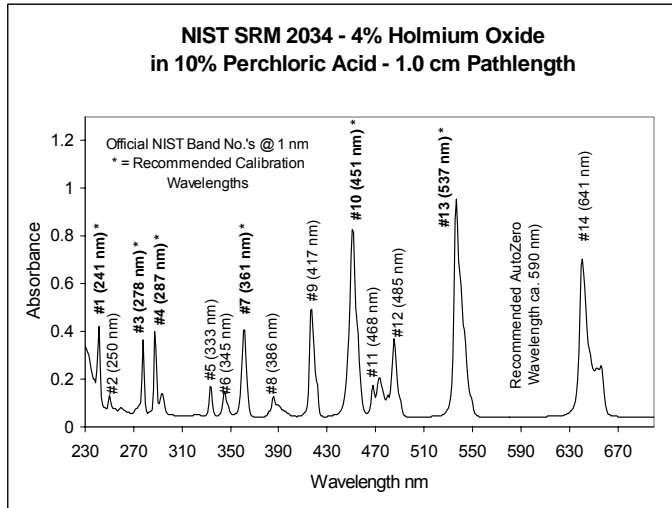
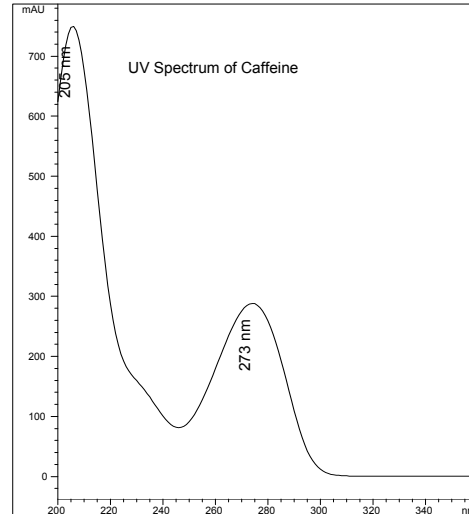


Figure 2: Spectrum of Caffeine in RTM



### STEP 6 – PREPARE THE VIALS AND RUN THE SEQUENCE -

If you are following the standard PQ sequence suggested below, you will need to fill the following numbers of vials:

Solution Vials Required <sup>a</sup>									
Solution	Diluent (mp)	GVS Spiked MP B*	RTM	L1	L2	L3	L4	L5	L6
No. Vials	4	1	1	1	6	1	1	1	1

<sup>a</sup> Assuming multiple injections per vial. If single injections per vial are planned, fill one vial per injection.

Write the *Injection Sequence* Run the Performance Qualification

Sequence PQ1: General HPLC Performance Qualification Example Injection Sequence				
Line No. /Vial No.	Sample Name	Method	# Inj	Comments:
1	Mobile Phase Blank	RTM	1	System Suitability: Inject Blanks until Clean, quiet baseline. Retention of Caffeine 1.0 – 1.5 min. Efficiency ≥ 3000 Rs of peaks before and after Caffeine ≥ 2.0  Use Blank for <i>Dynamic Noise</i> determination. <i>Noise Level</i> measured depends on the <i>Time Constant</i> used. Consult your detector/data system manual.  For DAD, acquire spectra if caffeine is used for 8 accuracy.
2	Resolution Test Mixture		1	



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Sequence PQ1: General HPLC Performance Qualification Example Injection Sequence				
Line No. /Vial No.	Sample Name	Method	# Inj	Comments:
3	Linearity Solution L3	PQ	10	<u>Autosampler Precision</u> and <u>Pump Stability</u> .
4	Mobile Phase Blank		1	Ensures clean system prior to starting Linearity
5	Linearity Solution L1 (0.1%)	PQ	3	Begin <u>Detector Linearity</u> with 0.1% solution <u>System Sensitivity</u> will also be calculated from data.  Triplicate injections at each level for precision  Conclude with 100% level solution, L6
6	Linearity Solution L2 (1%)		3	
7	Linearity Solution L3 (20%)		3	
8	Linearity Solution L4 (50%)		3	
9	Linearity Solution L5 (75%)		3	
10	Linearity Solution L6 (100%)		3	
11	Mobile Phase Blank (for injector % Carryover)		3	
12	Linearity Solution L2*	PQ (5 $\phi$ L)	3	Autosampler <u>Volume Linearity</u> and precision at each injection volume.  * Injection volumes may 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 (10 $\phi$ L)	3	
14	Linearity Solution L2*	PQ (25 $\phi$ L)	3	
15	Linearity Solution L2*	PQ (50 $\phi$ L)	3	
16	Linearity Solution L2*	PQ (100 $\phi$ L)	3	
17	Mobile Phase Blank	PQ	1	
18	GVS Spiked Mobile Phase B*	Grd	1	Gradient <u>Dwell Volume</u> and <u>Accuracy</u> .

Figure 3: Typical separation of the RTM



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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.

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.

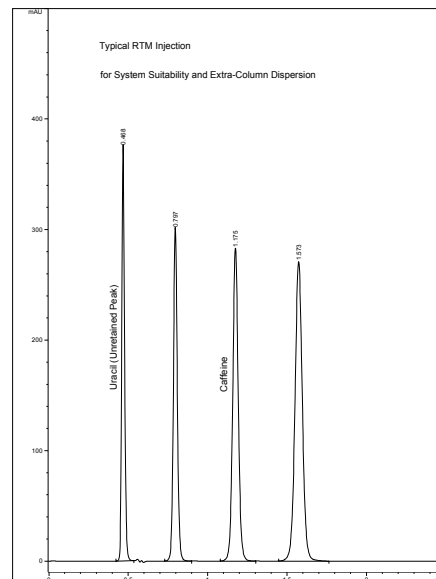


Figure 4: Gradient Accuracy for Quaternary Pump

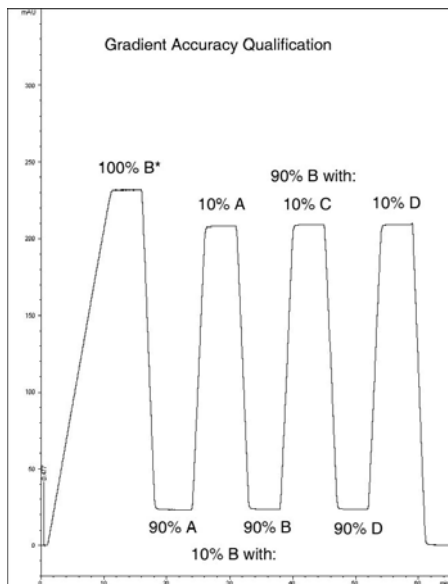
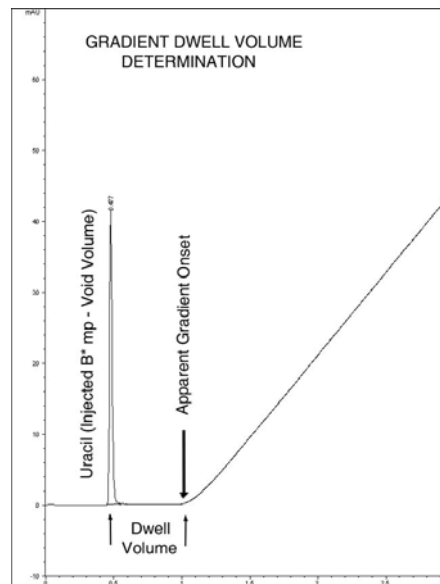


Figure 5: Gradient Dwell Volume





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

Enter the data into the Excel<sup>™</sup> template. All data is entered into the Data Entry tab. 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 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.

The data entry fields will generally follow the above example Injection Sequence. Data entry fields are light yellow, with numerous red warning labels indicating when data needs to be entered. Enter the Test Dates to indicate that test data was generated and will be filled in. 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 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 – PERFORMANCE QUALIFICATION 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.