CHEMICAL SOLUTIONS, Inc.

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HPLC PERFORMANCE QUALIFICATION SYSTEM
QUICK START REFERENCE

Rev. 5.05

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 and sequence – once that is done, they can be re-used in future Performance Qualifications on that instrument.

Sufficient volumes of solutions are supplied for multiple instrument qualifications. Mobile phase is stable for 60 days, and can be prepared in bulk and stored if multiple instruments are to be qualified.

The PQ Kit is a total Performance Qualification System comprised of the following components:

1. A set of certified, NIST-Traceable solutions that are analyzed just like normal samples in your laboratory, so that they test the entire HPLC *system* under realistic operating conditions

2. A pre-qualified, matched HPLC column, ensuring that all test data is consistent and comparable between different HPLCs.

3. Validated software, that automatically calculates and graphs the generated data, and prints a full PQ report, including a single page Certificate summarizing the results, that can be reviewed and approved for cGMP compliance. The software is Excel^{tm} –based, so it is intuitive and easy to use. The software is not licensed or restricted, so that it can support multiple qualifications on different instruments, thus making the kit economical and efficient in a large laboratory.

The entire PQ process is fully automated (except for flow, temperature and holmium oxide wavelength qualification). An analysis time of under two minutes per injection, with a total of less than 30 injections, produces a full qualification of an isocratic system in about an hour. Gradients are qualified for dwell volume and accuracy with an additional 60 minutes for a quaternary system, and less for a binary. Simply load the autosampler with the ready to use solutions, and run the sequence.

The results produce a comprehensive HPLC instrument qualification report, which includes the following major test protocols:

Pump/Column Oven	Autosampler	UV-Vis Detector	System Performance
Flow accuracy Flow stability Gradient Accuracy Gradient Dwell Volume Oven Temperature	Precision % Carryover Volume Linearity Temperature	Detector Linearity Linear Dynamic Range Noise Wavelength Accuracy (205-641 nm)	Extra-Column Dispersion Sensitivity

Some laboratories refer to such a comprehensive set of tests as an Operational Qualification (OQ), with much simpler tests comprising a PQ. The exact nomenclature is not important. The extensive results obtained ensure your HPLC is in good operating condition, and can reied upon to produce quality data, that can be defended against even the most stringent regulatory requirements.

A benefit of routine PQ testing is that results can be compared to previous qualifications on the same instrument, to quickly determine trends or instrument problems. Different HPLCs within the laboratory can be compared for differences in sensitivity, noise, precision, etc. Since the PQ kit represents a constant, it can be used to compare instruments in other laboratories around the world, to help troubleshoot method transfer issues that are instrument or laboratory related. It is also a convenient training tool to document the abilities of new laboratory personnel.

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

Step 1 Read this instruction guide and Inspect kit contents.

Step 2 Pre-Qualification Preparations
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 3 Qualify the pump for flow accuracy, and the column oven and refrigerated autosampler for temperatures, if not performed as part of the PM.

Step 4 Prepare the Mobile Phases

Step 5 Setup the HPLC Methods

(re-use methods and sequences for subsequent qualifications)

Step 6 Perform the Wavelength Qualification with Holmiuim Oxide and/or Caffeine

Step 7 Prepare the Vials and Run the Injection Sequence

Step 8 Enter the Data into the software. All results are automatically calculated.
Save, Print and Review the Results

Step 9 Sign off on the printed Certificate, along with any reviewers.
The HPLC is Qualified – ready for service!

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DETAILED PROCEDURES

STEP 1- INSPECT THE BOX CONTENTS –

1) There are a total of 9 bottles in the kit:
   Wavelength Calibration Solution (WCS)- holmium oxide solution
   Rs Test Mix
   Linearity Solutions (L1-L6)-caffeine ranging from 0.00035-0.35 mg/mL
   Gradient Visualization Solution (GVS)- uracil solution
2) Certificate of Analyses (CoA) for the above solutions.
3) A pre-tested, certified PQ column is provided (except in the replacement solution kit), along with its test Certificate.
4) A CD 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 instruction manuals will automatically come up on the screen when the CD is loaded.

   Two versions of the program are distributed on the CD, or are available for downloading. They are both identical, and either one can be used. The "demo" version contains typical qualification data, to provide a feel for what data is required for each test, and how the output looks. The empty program simply has all data deleted, but is otherwise identical. Use either program as your starting point. In any instance, the first step should be save a copy under a new filename.

Support is available both from Chemical Solutions Inc. and MicroSolv Technology Corporation

For sales, technical support and questions, contact:

MicroSolv Technology Corporation
Telephone: 732-380-8900
website: www.mtc-usa.com

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.

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STEP 2 - PRE-QUALIFICATION PREPARATIONS – Preventative Maintenance

Pre-qualification activities refer to the preventative maintenance (PM) and qualification activities normally performed on the instrument hardware prior to the actual performance qualification. 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. Remember to perform any self-tests for the modules, such as internal wavelength calibration for diode array detectors, etc., prior to starting the formal HPLC qualification. We refer to these maintenance and modular component qualification activities as “Pre-Qualification” for convenience.

STEP 3 - QUALIFY THE PUMP FLOW AND COLUMN COMPARTMENT/REFRIGERATED AUTOSAMPLER FOR TEMPERATURE

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

1. Pump 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 simply reference that activity to satisfy your SOP requirements, select the box provided in the software that says that this activity has already been performed, and enter the qualification date.

   Note that the entry of a valid date in the appropriate cell, acts as a switch, telling the software that it should expect data to be entered for that test protocol. ***If a test is not used, simply leave the date field blank to turn off the test.*** Various warnings will become visible if old data is left in cells if a test is not active.

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:

   There are two ways to qualify the flow rate. One is with a calibrated liquid flowmeter, and the other is with a timed collection into a volumetric flask or graduated cylinder. If you have a flowmeter, simply measure the flow rate and enter the values into the cells. Note that up to 3 flowrates can be entered. However, you can enter only one flow rate, or up to three total over an appropriate range for your instrument.

   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. You may also choose to qualify the pump at only a single flow. The software allows for maximum flexibility to suit your laboratory preferences. Simply leave the unused cells blank.

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

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

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Using a dry volumetric flask or graduated cylinder and calibrated digital timer, the following combinations of collection volumes and times are common. The qualification flow rates may be changed to suit your instrument or SOP's.

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 requires 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. The software also allows for any collection volume, and will automatically perform all calculations.

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. Digital liquid flowmeters are typically accurate to about 1.5%.

4. Column Oven Qualification:

   If the column oven and refrigerated autosampler has been qualified by the service provider, simply leave the section blank by not entering a date, or activate it by entering a date, along with the qualification results.

   If you are qualifying these components yourself, this 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. Rigid conventional thermometers can also be used, provided you can fit them into the cavity with draft shielding.

   For the column oven, thread the thermocouple end into the column compartment, taking care to replace any covers and sealing as necessary. Allow the temperature to stabilize at each setting and record the temperature.

   The qualification range should encompass the intended use of the column oven. The software provides for entry of up to 4 temperatures. As a default, we recommend the use of 20ºC (optional - only if capable of cooling), 30º, 40º and 50ºC. Special circumstances, such as the routine use of very high temperature methods, would obviously change these typical qualification points.

   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 a small piece of foil 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 to the single temperature of 4°C, and that an acceptance criteria of 2° - 8°C be applied.

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.

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STEP 4 – STARTING THE QUALIFICATION - MOBILE PHASE PREPARATION –

Prepare the mobile phases.

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

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

For a quaternary system, it is common to prep 2L of mobile phase, and to reserve 0.5 L of that for the gradient visualization. This will ensure an extra margin for good system flushing and for equilibration time.

The mobile phase is stable for at least 60 days when sealed to prevent evaporation, and can be prepared in bulk for multiple qualifications. It may be adjusted to meet the System Suitability requirement of a retention time of 1.0 – 1.5 minutes for the 3rd peak (caffeine) in the Resolution Test Mixture.

Two equivalent mobile phases have been developed and qualified for testing with the PQ test column – 14% 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 acetonitrile 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 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.

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

Acetonitrile:	**Methanol:**
140 mL acetonitrile	300 mL methanol
860 mL purified water	700 mL purified water
1 mL glacial acetic acid	1 mL glacial acetic acid
Gradient: 3 mL GVS to 500 mL of mp	Gradient: 3 mL GVS to 500 mL of mp

Mix and filter/degas using a membrane filter, or as per your current laboratory practice.

For a Gradient Qualification, reserve about 500 mL of mobile phase into a separate bottle (referred to as 'B*'). Add 3 mL of the ***Gradient Visualization Solution*** to this 500 mL (6 mL/L), directly into the bottle. The GVS is only semi-quantitative to define the gradient. A serological or electronic pipetor is sufficiently accurate for this purpose.

Purge lines A, C and D with the unspiked mobile phase (or only A for a binary system).

Purge the "B" line with the GVS-Spiked mobile phase.

Be sure to flush the "B" line VERY THOROUGHLY!

If the GVS-spiked mobile phase is not of uniform composition, it will be misinterpreted as an error in the gradient delivery accuracy. If the gradient delivery steps look odd, repeat the test to ensure it was not due to incomplete flushing of the B* line.

If you are not pressed for time, an easy protection against inadequate purging of the B mobile phase is to simply perform duplicate gradient runs. Specify the first as for conditioning only, and use the second injection for data analysis.

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STEP 5 – 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:

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][a]		
Wavelength:	273 nm		
Column Temperature:	Ambient [20°C-25°C]		
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)

a. 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.

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SELECTING THE INJECTION VOLUME FOR LINEAR DYNAMIC RANGE:

The test injection volume is a variable that can be adjusted to create a linear test range for the detector. For a UV-Vis detector, the absorbance linearity is generally determined by the ***HEIGHT*** of the injected peak (typically expressed as milli-absorbance units, or mAU, or µAU on Waters instruments. 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. Longer or shorter flowcells can be accommodated by changing the injection volume.

By selecting the injection volume, you are also choosing the range of heights produced, and thus can select the range of which you test the linear dynamic range of the detector. Until you are sure of what heights your detector produces, you might want to perform a test injection of the L6 solution, which will show you the greatest peak height and thus the upper limit of the range you will test. Adjust the injection volume to produce the desired maximum peak height. Enter the injection volume in the "HPLC Parameters" cell. This volume should be used for all of the test methods.

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 - Always confirm maximum height on a different detector with test injection of L6.		
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. For most routine PQ's, you would select conditions such that the upper absorbance is just within the linear range, or at least within the range of peak heights you use the instrument for. 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 usually 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. For reference, a 12µL injection would produce peak heights approaching 2,000 mAU, which is very high.

After testing, both peak heights and areas are entered in the appropriate cells. The Linearity and Dyanamic Linear Range are automatically calculated and graphed, including an array of statistics, to enable you to examine the behavior of the detector in detail.

You can input the maximum allowable deviation from linearity into the software. The ASTM test E1657-98 uses a value of 5% error to define 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. If this is excessive for your typical applications, you can input a lower acceptance value. An alternative value might be ±2%, similar to an acceptance criteria typically used for matched standard response factors.

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 typically not to re-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 8-10 µL volume will usually 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.

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STEP 6 – PERFORM THE WAVELENGTH QUALIFICATION –

Overview:

Confirmation of wavelength accuracy is required by the USP, EP and other regulatory agencies. The PQ kit offers two materials for wavelength qualification – holmium and caffeine.

Holmium oxide in perchloric acid (also referred to as holmium perchlorate) is an Internationally accepted primary standard covering the range of 241nm - 641nm. HPLC detectors are often used below 241nm, and caffeine, although being a secondary standard, is also widely used for qualification at its absorbance maxima of 205nm and 273nm. Caffeine has the advantage of being able to be chromatographed in a working HPLC, while the Holmium spectrum can only be obtained by manually filling the flow cell. However, holmium provides 14 sharp absorption bands, against only 2 much broader bands for caffeine.

A full wavelength qualification requires obtaining spectra on both solutions – one of caffeine (L2) and the other of the wavelength calibration solution (WCS). This will qualify the detector over the wavelength range of 205nm – 641nm (or 205nm – 361nm for the UV range only).

Some labs however, may decide to qualify their detectors only in the UV with caffeine. This is much easier to perform, and can be automated. The choice is yours, and should be decided with your Regulatory Affairs department.

Means of Obtaining Spectra:

There are basically three methods available to obtain spectra on HPLC UV-Vis detectors, in order of increasing complexity.

1. The easiest is with a Diode Array Detector (DAD) or some fast scanning instruments. Simply scan the peak of interest in the chromatogram during a run (caffeine), or upon filling the flow cell with solution in a static mode (holmium).

2. A more complex, but automated approach is to chromatograph a caffeine standard multiple times with different methods, where each method utilizes an incremental increase or decrease in wavelength (caffeine only). Usually 5 wavelengths are sufficient, such as 269, 271, 273, 275 and 277 nm, when looking for the 273 nm max of caffeine, with a +/- 3nm tolerance. Use the peak heights to find the wavelength of maximum absorbance. You can use more or fewer injections of greater or lesser changes in wavelength as necessary to define the maximum. This method, while tedious, has the advantage of being fully automated, but it can only be used for caffeine, and is not suitable for holmium.

3. If you have an older, manual variable wavelength detector, you can use either of the above methods. If you fill the flow cell with holmium or caffeine (don't forget to autozero the detector first with mobile phase in the cell), you can move the wavelength dial (or set it on the keypad of the detector – but don't autozero), and note the absorbance on the instrument readout. Conversely, you could chromatograph caffeine as in step 2 above, but stop to adjust the wavelength each time between injections.

   If you decide to fill the flow cell, a Flow Cell Filling kit is available with a spring loaded syringe, that will make this job easy and safe. The L2 solution should provide a good mid range absorbance, but if not select a linearity solution producing an absorbance of about 0.2 – 0.8 AU or so.

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Operational Details:

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 test 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 autozero within the first minute, while purified water is still in the cell. Then, it should acquire the WCS (Holmium 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 L2 or L3 solutions. 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.

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Figure 1: Spectrum of Holmium Oxide WCS Solution

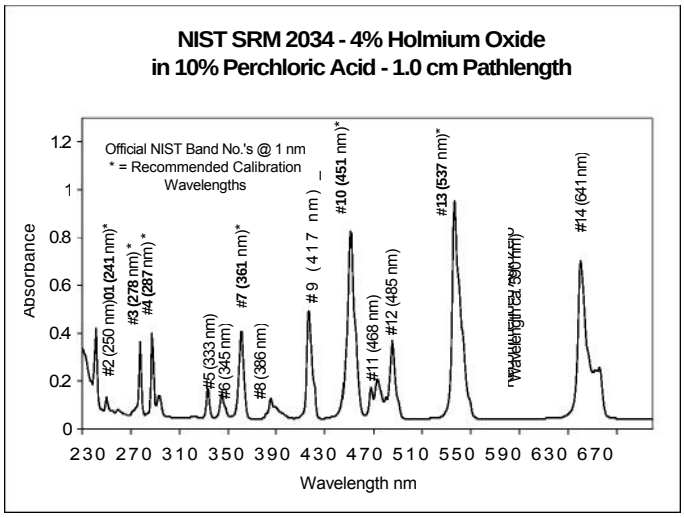
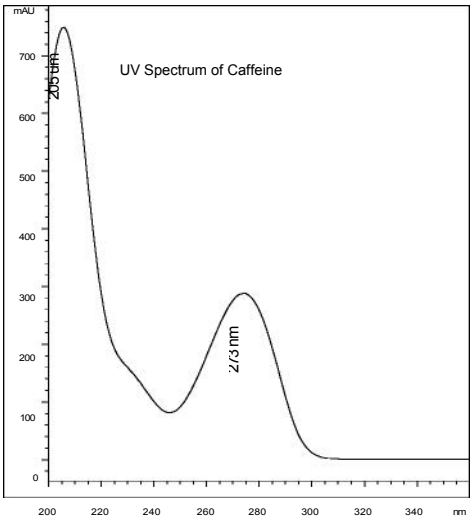


Figure 2: Spectrum of Caffeine in Rs Test Mix



STEP 7 – PREPARE THE VIALS FOR THE REMAINING PQ TESTS AND RUN THE SEQUENCE –

If you are following the standard PQ1 sequence, you will need to fill the following numbers of vials, depending on if you are using a single injection or multiple injections from each vial. Note this assumes you have a DAD. The wavelength qualification for holmium is a separate operation not included in this list.

Solution	Single injection/vial	Multiple Injections/vial
Diluent (MP)	4	4
GVS Spike MP B*	1	1
Rs Test Mix	1	1
L1, L4, L5, L6	1	1
L2	6	2
L3	11	2

System suitability is assessed on the injections of the blank and the Rs test mix and must meet the following criteria.

System Suitability	
Blank	Inject one or more Blanks until Clean, quiet baseline.
Rs Test Mix	Retention of Caffeine 1.0 – 1.5 min.
	Efficiency ≥ 2000
	Rs of peaks before and after Caffeine ≥ 2.0

You might want to perform a test injection after mobile phase preparation, to ensure that you have the correct retention time (ideally ~ 1.25 min) prior to committing to the full sequence. Adjust the mobile phase as necessary.

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Write the *Injection Sequence and Run the Performance Qualification*

Sequence PQ1: General HPLC Performance Qualification Example Injection Sequence				
Line No. /Vial No.	**Sample Name**	**Method**	**# Injections**	**Comments:**
1	Mobile Phase Blank	PQ	Min 1	Must meet System Suitability 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.
2	Rs Test Mix	RTM	1	Must meet System Suitability For DAD, acquire spectra.
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	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	
7	Linearity Solution L3		1	
8	Linearity Solution L4		1	
9	Linearity Solution L5		1	
10	Linearity Solution L6		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, remaining 2 injections clean autosampler prior to Linearity
12	Linearity Solution L2*	PQ (5µL*)	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 (10µL)	1	
14	Linearity Solution L2*	PQ (25µL)	1	
15	Linearity Solution L2*	PQ (50µL)	1	
16	Linearity Solution L2*	PQ (100µL)	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 #1 to condition, then #2 for qualification. Can use a single injection if the system is adequately flushed.

Note – It may help to peruse the "demo" version of the software in setting up your first qualification. That should give you a clear idea of exactly what data will be required from the sequence.

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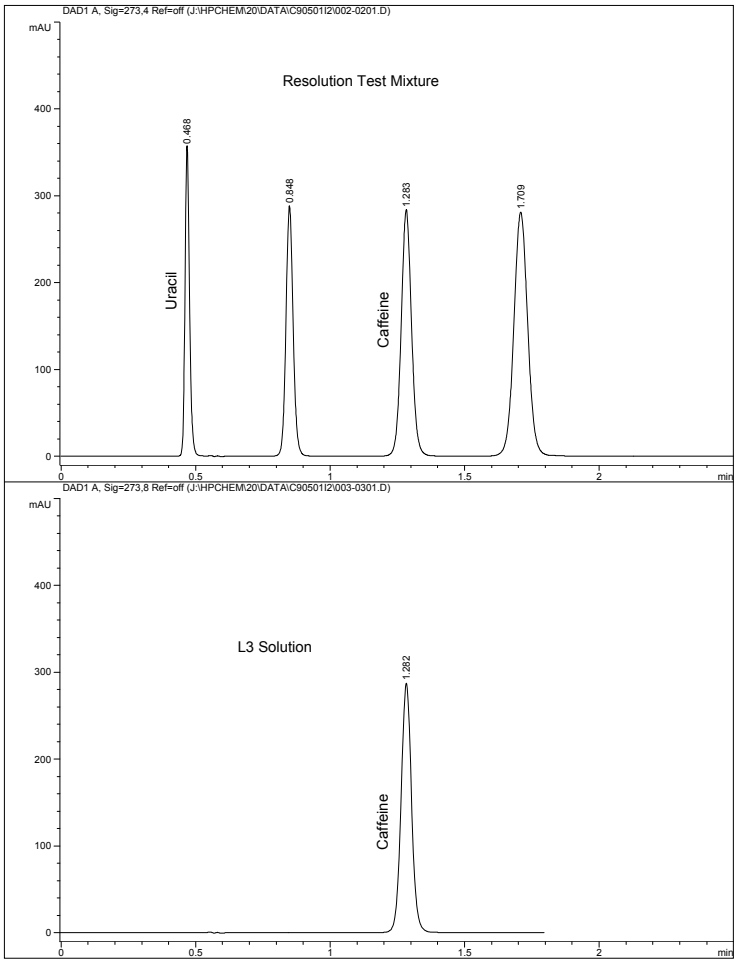
Figure 3 shows a typical separation of the Rs Test Mix, used for System Suitability and for calculation of the Extra-Column dispersion. The total run time is 3 minutes, while the allowable retention time window for Caffeine (Peak #3) is 1.0 – 1.5 minutes. Subsequent injections of Caffeine only, using 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 Rs Test Mix and an L3 Precision Sample



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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

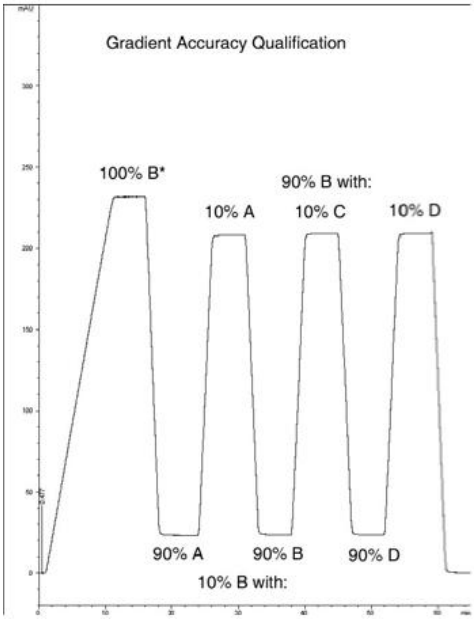
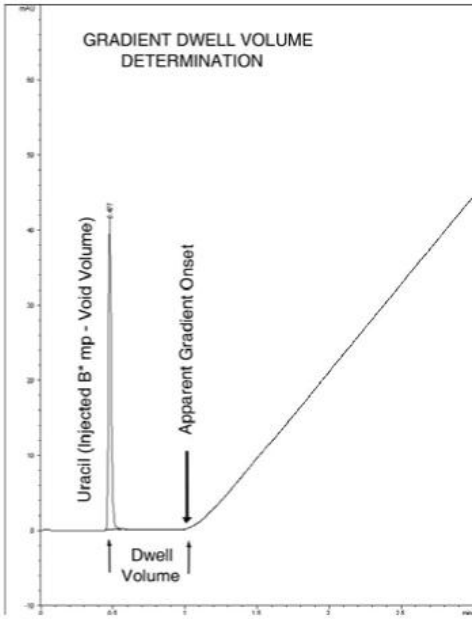


Figure 5: Gradient Dwell Volume – Expanded View



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STEP 8 – ENTER THE DATA AND CALCULATE / REVIEW THE TEST RESULTS –

Ensuring that the data is integrated properly, enter the results into the Excel™ 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.

The software has been tested and validated to run properly on both the older Excel ver. 97-2003, as well as the newest Office 2010. On either version, you must ***first enable the Analysis Tool Pak, and enable the Macros, by setting the security level to Low or Medium.*** Consult the Excel documentation to perform this. Basic instructions are also provided in the "Instructions" tab of the software. Most software problems are due to customers forgetting to perform these two Excel tasks.

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. 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.

The data analysis spreadsheet is preloaded with the values from the ***recommended acceptance criteria*** table. These may be modified to meet your internal company requirements.

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.

Refer to the reprint of the article "Performance Qualification of HPLC Instrumentation in Regulated Laboratories", *LCGC North America, Volume 26 Number 5 May 2008* which is included on the CD for a detailed discussion of data interpretation.

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.

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Recommended Acceptance Criteria			
Module	**PQ Test**	**Acceptance Criteria**	**Comment**
Pump	Flow rate accuracy	±5%	
	Flow stability	Drift NMT 1.0%	No outliers or indicators of unstable flow
	Gradient Dwell volume	Record value	Compare to previous values and to similar HPLCs
	Gradient Delivery Accuracy	±2%delivery error @ 10% and 90% delivery	Equivalent to ±0.2% absolute of A or B
Autosampler	Temperature	2-8°C	Only if refrigeration is used
	Precision	%RSD ≤1.0%	
	Injector Carryover	≤0.1%	1st injection following L6
	Volume linearity	R^2 ≥0.999	
Detector	Dynamic Short Term Noise	Record value	Compare to previous values and to similar HPLCs
	Linearity, peak area	R^2 ≥0.999	
	Dynamic linear range	±5%	As per ASTM
	Wavelength accuracy	±3nm	
Column Oven	Temperature	±5°C	
System performance	Extra-column instrument dispersion	Record value	Compare to previous values and to similar HPLCs

STEP 9 – PRINT THE FINAL CERTIFICATE, CALCULATED RESULTS AND DATA

THE PERFORMANCE QUALIFICATION IS COMPLETED –

The HPLC is ready for use, with a comprehensive, NIST-Traceable Performance Qualification!

Support is available both from Chemical Solutions Inc. and MicroSolv Technology Corporation

For sales, technical support and questions, contact:

MicroSolv Technology Corporation
Telephone: 732-380-8900
website: www.mtc-usa.com