CHEMICAL SOLUTIONS, Inc.

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HPLC PERFORMANCE QUALIFICATION SYSTEM FOR REFRACTIVE INDEX DETECTORS

QUICK START REFERENCE Rev. 5.01

INTRODUCTION:

Welcome to the PQ Kit for Refractive Index Detectors (RID). 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 hour, depending upon how many tests you choose to run.

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. You can also use your own column, which is a common C8, 5µm 4.6X75 mm.

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 and temperature qualification). 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 Oven Temperature	Precision % Carryover Volume Linearity Temperature	Detector Linearity Linear Dynamic Range Noise	Noise 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 relied 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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Note that this kit is specific for RI detectors. Due to the limitations of RID, some instrument parameters cannot be readily measured, such as gradient accuracy and dwell volumes. Chemical Solutions offers another, more comprehensive kit using caffeine for UV-Vis detectors. If your instrument has both an RID and a UV-Vis detector, it is recommended that the primary qualification be performed using our PQ kit for UV detectors (Cat. No. PQNC0101). We also have PQ kits available for FLD, ELSD and CAD detectors. All of our HPLC-PQ kits use the same mobile phase and column, so that your HPLC or UPLC can fully qualified using the HPLC kit. Then, only those additional tests relevant to the RID can be performed using these solutions – all within the same injection sequence.

Here is an overview of the basic steps required for a Performance Qualification of your FLD 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	Prepare the Vials and Run the Injection Sequence
Step 7	Enter the Data into the software. All results are automatically calculated. Save, Print and Review the Results
Step 8	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 7 bottles in the kit:
   Linearity Solutions (L1-L6)- caffeine ranging from 0.20-2.0 mg/mL
   Diluent Blank
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 Technologies, Inc.

For sales, technical support and questions, contact:

MicroSolv Technology Corporation
Telephone: 732-380-8900
email: customers@mtc-usa.com
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 control 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 capabilities or SOP requirements.

Flow Rate:	Volumetric Flask Size:	Expected Time:
0.5 mL/min	2 mL	4.0 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.

   Since column ovens vary widely in design, and are not high-accuracy devices, an acceptance criteria of ±5ºC 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. If your instrument is not capable of this temperature, enter a different temperature for the acceptance criteria.

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:

- 0.5L minimum for isocratic only

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 4-7 minutes for the salicylic acid peak.

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.

Either mobile phase may be adjusted to meet the system suitability retention time window.

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.

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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). Note that for the injection volume linearity test, most current instrument software packages (ChemStation, OpenLab, Empower, etc.) will allow you to specify the injection volume in the run sequence, using the same basic method. If not, then you will need to create a series of methods identical except for their specified injection volume.

PQ Method Summary: Column: MicroSolv PQ Column, C8 5µm 75 X 4.6 mm	
Parameter:	Recommended Values:
Flow:	1 mL/min
Injection Volume:	20 µL [Modify for injector volume linearity test and/or to extend detector linearity][a]
Column Temperature:	30°C
RID Cell Temperature:	35ºC
Run Time:	~4 minutes
Other:	Set time constant and attenuation as needed

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SELECTING THE INJECTION VOLUME FOR THE PERFORMANCE QUALIFICATION:

The test injection volume is a variable that can be adjusted to create a linear test range for the detector. Refractive Index detectors vary widely in their sensitivity. Within a given detector, most allow for various attenuation and time constant settings. Detector sensitivity can vary by 10X or more between different designs and models. Therefore, some specific settings, such as injection volume, may have to be modified to produce detectable peaks within the range of interest.

It is recommended that you perform several test injections under various attenuation settings, if your instrument is so designed, to find a combination of attenuation and Injection Volume suitable for the analysis of the caffeine solutions. Choose conditions that will produce a detectable signal for the L1 solution, yet allow the L6 peak to remain on scale. L1 to L6 represents a range of 10-fold. 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.

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. A 20 µL volume will usually be appropriate for modern instruments with a reasonably sensitive RID. 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 7 – PREPARE THE VIALS FOR THE 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 scanning instrument. More vials may be required if using multiple injections at different wavelengths.

Solution	Single injection/vial	Multiple Injections/vial
Diluent (MP)	4	4
L1, L4, L5, L6	1	1
L3 for Detector Linearity (1) and Injector Volume Linearity (6)	7	2
L4	11	2

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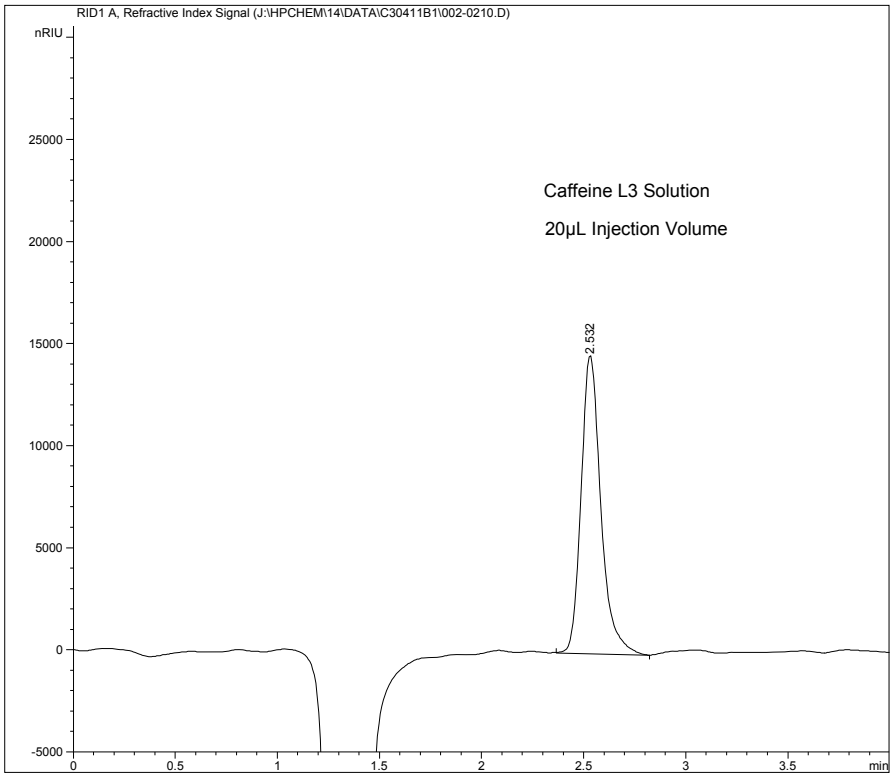
System suitability is assessed on the retention time of the first caffeine standard injection from Precision, and must meet the following criteria.

System Suitability	
Blank	Inject one or more Blanks until Clean, quiet baseline.
1st Injection for Precision	Retention of Caffeine 1.5 – 4 min.
	Efficiency ≥ 2000

You might want to perform a test injection after mobile phase preparation, to ensure that you have the correct retention time (ideally ~2.5 min) prior to committing to the full sequence. Adjust the mobile phase composition as necessary. Figure 1 shows a typical chromatogram of the L3 solution, injected at 20µL using a modern analytical RID.

Figure 1: Typical chromatogram of a 20µL injection of the L3 standard solution.



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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	RID	Minimum 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.
3	Linearity Solution L3 (or alternative)	RID	10	*Check Retention Time and N of first injection – adjust mp if necessary.* *Data used for Autosampler Precision* and *Pump Stability.*
4	Mobile Phase Blank		1	Ensures clean system prior to starting Linearity
5	Linearity Solution L1	RID	1	Begin *Detector Linearity* with 01% 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 ensure clean autosampler prior to Linearity
12	Linearity Solution L3*	RID (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 L3*	RID (10µL)	1	
14	Linearity Solution L3*	RID (25µL)	1	
15	Linearity Solution L3*	RID (50µL)	1	
16	Linearity Solution L3*	RID (100µL)	1	

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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STEP 8 – 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.

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%	Determine manually with volumetric and stopwatch, or use calibrated flowmeter. If Service Provider qualified flow, cite Service Date in cell.
	Flow stability	Drift NMT 1.0%	No outliers or indicators of unstable flow
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	Examine for curvature
	Dynamic linear range	±5%	As per ASTM
Column Oven	Temperature	±5°C	Measure using calibrated thermocouple, or cite Service Providers qualification date.

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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!

Extensive, unlimited support is available both from Chemical Solutions Inc. and MicroSolv Technologies, Inc.

Note that the software is provided with the kit at no charge, and is frequently updated. You can always obtain the latest revision software free of charge by contacting technical support, or by directly downloading from one of our websites.

For sales, technical support and questions, contact:

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