Title: System Suitability Requirements for SEC Chromatographic Methods Used in PA Laboratories

SOP Number: 22951

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1.0 Purpose

This procedure defines the system suitability requirements for SEC (size exclusion chromatography) methods used for Current Good Manufacturing Practices (cGMP) and Good Laboratory Practices (GLP) release and stability testing within Process Analytics/Quality Control (PA/QC) laboratories. The system suitability criteria are established from recommendations in the GOER (Center for Drug Evaluation Research) Guidance Document for the Validation of Chromatographic Methods, and from the United States Pharmacopeia (cUSP <621>).

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System Suitability Testing (SST) is used to verify that the complete analytical system (including column, instrument, and reagents) is suitable for the intended application on the day of analysis. Ideally, SST calculations are performed on standards before any samples are analyzed and an SST failure should stop the assay sequence immediately before any sample injections have been done. The analyst then diagnoses the system problem, makes adjustments or repairs, and performs the SST again. Analysis of actual samples should only commence after the system has passed all SST limits. With automated systems, it may not be possible to stop the system but any results are invalid. This does not trigger an Out-of-Specification (OOS) investigation because the SST results indicate the system is not suitable and the results are not valid. Most SEC HPLC SST failures will be due to an aging column.

2.0 Scope

This procedure applies to Process Analytics/Quality Control (PA/QC) personnel who perform chromatographic analyses, specifically to SEC methods.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

4.1 Validated high-pressure liquid chromatography (HPLC) system or ultra-pressure liquid chromatography (UPLC) system. This SOP is not specific and can be applied to any PA HPLC or UPLC system and to any SEC column.

4.2 The column(s) and mobile phases for the method of analysis (refer to the specific SOP or HPLC Technical Information Form for a complete materials and equipment list). Also see ***SOP 22720 - HPLC Technical Information Form***.

4.3 The System Suitability Standard (SSS) - Biorad Gel Filtration Standard, BDP PN 30223, or BDP-approved equivalent.

5.0 Procedure

5.1 **Initial System Suitability Assessment Prior to Analysis (Form 22951-01, Part I)** - Prior to any cGMP/GLP chromatographic analysis within PA/QC, the analyst must first determine whether or not the chromatographic system meets established system suitability criteria. This is determined by performing triplicate injections of the defined system suitability standard for the method of analysis. Each approved SSS will contain 2 or more compounds to be used for system suitability assessments. The compounds used for USP calculations, as well as the corresponding specifications, will be listed in the method-specific attachment for the particular analytical method. These are available in the form of a Microsoft Excel file (Form 22951-01) with multiple method-specific spreadsheets that contain the formulas needed to perform the calculations for system suitability assessment.

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NOTE: The resolution and selectivity of compounds in the SSS (shown as examples in the Excel spreadsheets) will vary with different running conditions (mobile phases, column dimensions, column chemistries, diluent formulation, etc.). The examples are meant to be general guidelines for identifying the compounds, through the typical elution order and pattern of the compounds.

5.2 Using the method-specific tab in the Excel spreadsheet (Form 22951-01), enter the results obtained from the raw data printouts from the initial system suitability assessment (refer to the instrument or method-specific SOP for instructions on data reporting). The results are automatically calculated as data is being entered. Mark each result as either "PASS" or "FAIL" according to the listed specification. In situations where the validated chromatographic system software performs the same USP calculations used for determination of system suitability, the analyst may enter this result in the "PASS/FAIL" column, followed by the word "PASS" or "FAIL" in parentheses, and line out and initial/ date the user input sections. Include a copy of the system suitability results with the raw data. If results for the initial system suitability assessment meet specifications, then the analyst can perform analysis on the product of interest. The initial system suitability criteria are valid for up to 24 hours for subsequent product analyses, after which, the initial system suitability assessment must be repeated.

5.3 The USP system suitability criteria used for the initial assessment are as follows:

5.3.1 **Capacity Factor (k') (Form 22951-01, Part I, 1)** - Capacity Factor is the time spent by the substance in the stationary phase divided by time spent by the substance in the mobile phase (i.e., the void volume), minus 1. This formula is expressed as $k' = (R_t / V_0) - 1.0$, where R_t is the retention time of the specified compound and V_0 is the void volume for the column, and will be defined as 2 minutes by default for all chromatographic methods (i.e., any compound eluting after 2 minutes following injection can be considered significant). In certain cases, such as reversed-phase methods using low flow rates and larger diameter columns, the void volume may be longer (i.e., the time it takes for mobile phase to pass through the column and to the detector is longer). This may necessitate utilization of a larger void volume. The larger void volume is usually detectable, typically by ultraviolet (UV) light, by the passing of buffer salts within the first few minutes following injection. In these cases, the void volume will have to be increased, resulting in an increase in the minimum allowable retention time of the component. Likewise, the void volume value may be less than 2 minutes for rapid LC methods, such as those using microbore column diameters, UPLC (ultra-high pressure liquid chromatography) columns, or monolith disk columns. **The specification for capacity factor for methods is k' > 2.0.**

5.3.2 **Theoretical Plate Count (N) (Form 22951-01, Part I, 2)** – Theoretical plate count is a measure of column efficiency and the formula is expressed as $N = 16 (t/W)^2$, where t is the retention time of the compound and W is the peak width at baseline. **The specification for theoretical plate count for methods is N > 7500.**

5.3.3 **Tailing Factor (T) (Form 22951-01, Part I, 3)** – Tailing Factor is a measure of peak symmetry, the value of which is near unity for perfectly symmetrical peaks and increases as peak tailing becomes more pronounced. The formula is expressed as $T = W_{0.05} / 2f$, where $W_{0.05}$ is the peak width at 5% total peak height and f is the peak width from start to apex at 5% peak height (i.e., the width of the leading half of the integrated peak). **The specification for tailing factor for methods is T ≤ 2.**

5.3.4 **Relative Retention Time (R_r) (Form 22951-01, Part I, 4)** - will follow a modified version of the USP method comparing the retention variation of each compound in the system suitability standard. This is performed by calculating the average retention time of each system suitability standard compound, from triplicate injections, as well as the standard deviation and relative standard deviation percent (%RSD) of each. These will be compared to an additional three injections (for six total) of the system suitability standard analyzed before, during, and after product analysis. **The specification for relative retention time for methods is ≤ 2% RSD.**

5.4 Manual peak integration (defined in the respective operational SOPs for various chromatographic systems) may be necessary to determine peak heights, retention times, peak start times, etc., of each standard compound in order to perform the USP system suitability calculations. In the case of calculating USP tailing factor, estimate where the 5% peak height starts by using the UV detector (or other method-specific detector) absorbance scale. Using an estimate is acceptable as long as peak integration is level across the peak (i.e., manually integrate the peak as a straight line from peak start to peak end).

5.5 **System Suitability of Complete Analysis (Form 22951-01, Part II)** – SST is also performed during and/or after the samples. The system suitability criteria used during product analysis are as follows:

5.5.1 **Modified USP Relative Retention Method (Form 22951-01, Part II, 1)** (see section 5.2.4). An additional three injections (for six total) of the system suitability standard will be performed at the start of the run sequence, during the run sequence, and at the end of the run sequence. During lengthy analyses of large quantities of in-process samples, the analyst may perform multiple single injections of the system suitability standard over the course of the run sequence in order to bracket the sample set for periodic determination of system suitability maintenance.

5.5.2 **Comparison of Initial Assessment Data and Complete Analysis Data (Form 22951-01, Part II, 2).** The average retention time of each compound in the system suitability standard from the initial assessment (injections 1 through 3) will be divided by the average retention time of each compound in the system suitability standard from the product analysis (injections 4 through 6). This is a method of measurement to show that system suitability has been maintained throughout the analysis. **The specification for methods is 0.98 – 1.02.**

5.5.3 T**he last section of the system suitability analysis sheet includes a Pass/Fail assessment.** There will be instances where observations made during analyses may not have an effect on chromatographic system suitability results, but will have an impact on the reliability of the data generated. This should be described in the Comments section. The analyst must explain or investigate such discrepancies and negate the passing system suitability results. These results must also be documented in the equipment logbook as per ***SOP 21531 - Equipment/Facility Logs***, and in any follow-up reports as appropriate for the corrective action.

5.6 As explained in section 5.4, the analyst must calculate the results and determine if system suitability has been met. In order for a valid test result to be reported, the system suitability results must pass specification, except under unique circumstances as described in this procedure.

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5.7 This procedure is designated for release testing and stability testing of cGMP and GLP products. There will be occasions where cGMP in-process samples may be submitted for analysis, the expedited results of which are needed to continue manufacturing. In these cases, performing system suitability testing is still necessary, but the analyst will be able to wait until the end of the run sequence to run the system suitability standard injections. A verbal result for stat in-process samples will be given to the requestor to allow for continued manufacturing, with the understanding that the results are not valid until it has been documented that system suitability was maintained throughout analysis. Scheduling analyses with production personnel will allow for performance of the initial assessment prior to sample submission and quick turnaround of results.

5.8 Save a copy of the Excel file on the BDP network at "H:\BDP_Public\PA\QC Files\System Suitability of Chromatographic Methods." Save the system suitability report on the BDP network at "SCIdata\BDP\PA\SEC\SEC-IEX-HPLC Reports" with the filename "QCXXXXXSEC system suitability" if a QC request is submitted. If no QC request form is submitted, use a six-digit date (yymmdd) followed by first initial/last name and a brief description of the analysis as a naming convention. For example, an SEC system suitability analysis performed by "John Doe" on September 2, 2016 for product X would be saved as "160902_jdoe_SEC System Suitability for Product X."

5.9 **When System Suitability Test Fails** – If possible, following an SST failure, the analyst should stop the assay sequence immediately, before any sample injections have been done. The analyst then diagnoses the problem, makes necessary adjustments or repairs, and performs the SST again. Analysis of actual samples should only commence after the system has passed all SST limits. Most SST failures are attributed to column aging, poor precision of the autosampler, pump problems, worn syringe, or mobile phase preparation.

6.0 Documentation

6.1 All instrument usage, maintenance, and service must be documented in an issued equipment notebook as per ***SOP 21531 - Equipment Logs.***

6.2 All solutions used for this procedure must be prepared and documented as per ***SOP 22702 - Solutions Used in Process Analytics.***

6.3 Include completed Form 22951-01 with the QC Test Request form or analysis report and create an electronic copy saved at the location described in section 5.8.

7.0 References and Related Documents

7.1 **SOP 21531** *Equipment Logs*

7.2 **SOP 22702** *Solutions Used in Process Analytics*

7.3 **SOP 22720** *HPLC Technical Information Form*

8.0 Attachments

8.1 Attachment 1 Form 22951-01, PA System Suitability Requirements for Size Exclusion Chromatography

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Attachment 1

NCI-Frederick
Form No.: 22951-01
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Process Analytics:

System Suitability Requirements for Chromatographic Methods Used in PA Laboratories
Size Exclusion Chromatography Methods

I. Initial System Suitability Assessment Prior to Analysis

Commercial Standard:
Biorad Gel Filtration Standard
Catalog Number 151-1901
BDP Part Number 30223

Lot #: _____

Expiration Date: _____

Column Description:

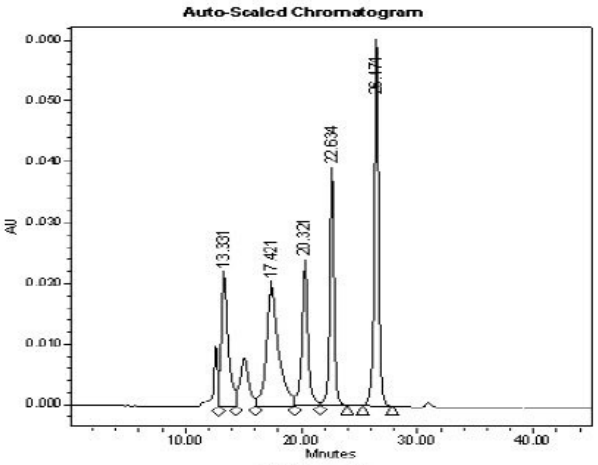
_____

BDP Part Number: _____

Lot #: _____

Expiration Date: _____

Example "Time Zero" Chromatograph:



Peak Results

	Peak Name	Ret Time (min)	Height (uV)	Area (uV*sec)	% Area	Int Type
1	Thyroglobulin	13.331	21186	906542	15.73	VV
2	Gamma globulin	17.421	19471	1442868	25.04	VV
3	Ovalbumin	20.321	23012	853978	14.82	VV
4	Myoglobin	22.634	38123	1006301	17.46	VB
5	Vitamin B-12	26.474	59341	1552493	26.94	BB

1) Capacity Factor (*k'*): $k' = (R_t / V_0) - 1.0$ *(where R_t = retention time of compound and V_0 = the void volume of 2 minutes or as stated)*

BioRad STD Injection Number	Compound	R_t (minutes)	V_0 (minutes)	*k'*	Specification	PASS / FAIL
Injection 1	Thyroglobulin		2.00	-1.0	> 2.0	
Injection 2	Thyroglobulin		2.00	-1.0	> 2.0	
Injection 3	Thyroglobulin		2.00	-1.0	> 2.0	

2) USP Theoretical Plate Count (*N*): $N = 16 (t/W)^2$ *(where t = retention time of compound and W = peak width at baseline)*

BioRad STD Injection Number	Compound	*t* (minutes)	*W* (minutes)	*N*	Specification	PASS / FAIL
Injection 1	Vitamin B-12			#DIV/0!	> 7500	
Injection 2	Vitamin B-12			#DIV/0!	> 7500	
Injection 3	Vitamin B-12			#DIV/0!	> 7500	

3) USP Tailing Factor (T): $T = W_{0.05} / 2f$ *(where $W_{0.05}$ = peak width at 5% height and f = peak width from start to apex at 5% height)*

BioRad STD Injection Number	Compound	$W_{0.05}$ (minutes)	*f* (minutes)	*N*	Specification	PASS / FAIL
Injection 1	Myoglobin			#DIV/0!	≤ 2	
Injection 2	Myoglobin			#DIV/0!	≤ 2	
Injection 3	Myoglobin			#DIV/0!	≤ 2	
Injection 1	Vitamin B-12			#DIV/0!	≤ 2	
Injection 2	Vitamin B-12			#DIV/0!	≤ 2	
Injection 3	Vitamin B-12			#DIV/0!	≤ 2	

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Attachment 1 (Continued)

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Process Analytics:
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Size Exclusion Chromatography Methods

4) Relative Retention Time: *% RSD of compound retention times during analysis*

BioRad STD Injection Number	Thyroglobulin Retention Time	Gamma globulin Retention Time	Ovalbumin Retention Time	Myoglobin Retention Time	Vitamin B-12 Retention Time
Injection 1					
Injection 2					
Injection 3					
Average:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Standard Deviation:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
%RSD:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Specification: ≤ 2% RSD					
PASS / FAIL:					

II. System Suitability of Complete Analysis

1) Relative Retention Time: *% RSD of compound retention times during analysis*

BioRad STD Injection Number	Thyroglobulin Retention Time	Gamma globulin Retention Time	Ovalbumin Retention Time	Myoglobin Retention Time	Vitamin B-12 Retention Time
Injection 4					
Injection 5					
Injection 6					
Average:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Standard Deviation:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
%RSD:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Specification: ≤ 2% RSD					
PASS / FAIL:					

2) Comparison of Initial Assessment Data and Complete Analysis Data:

BioRad STD Injection	Average Thyroglobulin Retention Time	Average Gamma globulin Retention Time	Average Ovalbumin Retention Time	Average Myoglobin Retention Time	Average Vitamin B-12 Retention Time
Initial Assessment (Inj. 1-3)	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Complete Analysis (Inj. 4-6)	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Comparison of Averages:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Specification: 0.98 - 1.02					
PASS / FAIL:					

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Attachment 1 (Continued)

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Process Analytics:
System Suitability Requirements for Chromatographic Methods Used in PA Laboratories
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4) **System Suitability Confirmation**

- ☐ Pass. Results meet the specifications. System is suitable for use.
- ☐ Fail. Results do not meet the specifications. System is not suitable for use.

Comments: _____

_____

_____

_____

_____

_____

Signature:_____ Date:_____

	Date:
Reviewed By:	Date:

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