Standard Operating Procedure

Title: Freeze/Thaw Stability Study by HPLC-SEC

SOP Number: 22501 **Revision Number: 03**

Supersedes: Revision 02 **Effective Date:** DEC 6 2015

Originator/Date: [redacted]

Approval/Date:

Approval/Date:

Table of Contents

1.0 Purpose

The purpose of this SOP is to study the effects of repeated freezing and thawing of products, by size exclusion chromatography following ***SOP 22935 - Size Exclusion Chromatograph of Proteins Using the Waters Millennium HPLC System*** in the event that reuse of the product in the lab or in the clinic is necessary.

2.0 Scope

Products manufactured at the Biopharmaceutical Development Program (BOP) may be analyzed for freeze/thaw stability by size exclusion chromatography (SOP 22935) to determine whether or not a particular product may be thawed and refrozen without the loss of product quality (i.e., increase in aggregation, dimerization, degradation, etc.). Some products are stored unfrozen and, therefore, are not subject to this procedure. The project team will decide whether this procedure applies to a particular product. This SOP applies to Process Analytics /Quality Control (PA/QC) personnel.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted]

3.0 Authority and Responsibility

3.1 The Director, Technical Operations, Process Analytics/Quality Control (PA/QC) has the authority to establish 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 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure

4.0 Materials and Equipment

4.1 Analytical HPLC system and peripherals (as described in ***SOP 22935 - Size Exclusion Chromatography of Proteins Using the Waters Millennium HPLC System***, Section 2.0, or equivalent).

4.2 Three vials/containers or 1%, whichever is smaller, of the product to be tested (container and volume requirements will be dependent upon the particular product, as determined by PA/QC personnel and/or the Project Scientist).

5.0 Procedure

5.1 If samples are already frozen, proceed to step 5.2, or store at appropriate temperature.

5.2 Just prior to analysis, completely thaw the product to be tested on the benchtop with gentle agitation, then store at 2-8°C until analysis is started. This mimics conditions in which a vial is placed on the bench top in the clinic to thaw. Alternatively, at the request of the project scientist, a vial can be placed at 2-8°C for a slow thaw in order to observe any potential adverse effects of gradual temperature change.

5.3 The first analysis (as per ***SOP 22935 - Size Exclusion Chromatography of Proteins Using the Waters Millennium HPLC System***) will be identified as “Initial Thaw.” This is the control sample that subsequent analyses will be compared to for determination of product stability.

5.4 Remove at least enough material to inject 25 - 50 µg onto the column.

5.5 Initiate testing within the same day initial thaw occurs.

5.6 Place the remainder of the material in the appropriate freezer (-20°C, -70°C , etc.) at least overnight, in an upright position.

5.7 Repeat steps 5.2 through 5.5 for a minimum of 3 freeze/thaw cycles (or as requested) using the same product containers. Be sure to inject the same volume/amount for repeat analyses.

6.0 Interpretation of Results

6.1 The purity specification for each analysis will be the same as the release specification for that product, as specified on the approved Certificate of Analysis.

6.2 Divide the product peak area in each analysis by the product peak area from the “Initial Thaw” analysis. This is the peak area percentage, and the specification for each analysis will be the same as the purity release specification for the product, as specified on the approved Certificate of Analysis.

6.3 Some products manufactured at the BDP are sensitive to both aggregation and degradation. Some observations of factors that attribute to this include:

6.3.1 Loss of peak height and/or area (i.e., loss of monomeric product, which could include an increase of dimer or multimer).

6.3.2 Loss of peak symmetry.

6.3.3 Presence of lower molecular weight peaks.

6.3.4 Increase in column back pressure (an indication of aggregation on the guard column or resin bed).

6.3.5 Increase in peak tailing (an indication of protein interaction with the resin; be aware that increased tailing can cause increased peak area).

7.0 Documentation

7.1 Document results on QC Test Request Form 22002-01, (if applicable) or in an issued laboratory notebook, and electronically on the designated computer.

7.2 A summary of the results of the study can be generated in a memorandum or spreadsheet format, or by completing Form 22501-01 (Attachment I), and attaching to the QC Test Request Form or laboratory notebook.

8.0 References and Related Documents

8.1 **SOP 22935** *Size Exclusion Chromatograph of Proteins Using the Waters Millennium HPLC System*

9.0 Attachments

9.1 **Attachment 1** Form 22501-01, Freeze/Thaw Stability for Products Manufactured at the BDP

Attachment 1

NCI-Frederick
Form No.: 22501-01
SOP No.: 22501
Revision 03: Sample ID#:_____

Biopharmaceutical Quality Control

Freeze / Thaw Stability Study by HPLC-SEC (SOP 22935)

Sample Name:_____ Lot#:_____

Requestor:_____ Study Start Date:_____

Number of vials/containers to be tested (3 or 1% of lot, whichever is less):_____

I. **Initial Thaw (Time Zero) Results:**

Vial Number	Retention Time **(minutes)**	**% Purity**	Peak Area	Comments
1				
2				
3				

II. Freeze / Thaw Cycle #1 Results:

Vial Number	**Retention Time (minutes)**	**% Purity**	**Peak Area**	**Peak Area % (peak area/initial thaw peak area x 100)**	**Comments**
1					
2					
3					

III. Freeze / Thaw Cycle #2 Results:

Vial Number	**Retention Time (minutes)**	**% Purity**	**Peak Area**	**Peak Area % (peak area/initial thaw peak area x 100)**	**Comments**
1					
2					
3					

IV. Freeze / Thaw Cycle #3 Results:

Vial Number	**Retention Time (minutes)**	**% Purity**	**Peak Area**	**Peak Area % (peak area/initial thaw peak area x 100)**	**Comments**
1					
2					
3					

(* additional freeze / thaw data can be recorded on a second form, if necessary)

Performed By/Date:_____ Reviewed By/Date:_____

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted]