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Evaluation of the Use of High-Pressure Liquid Chromatography Size-Exclusion Chromatography for the Detection of Aggregates in rhIL-15 Clinical Product

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I. Summary

The purpose of this report is to evaluate and discuss the data available to date evaluating the suitability of Size-Exclusion Chromatography High Pressure Liquid Chromatography (SEC-HPLC) for the detection of higher molecular weight degradation products in formulated intended for use in a Phase I clinical trial. Heat-stressed and freshly-thawed vials of were evaluated by both SEC-HPLC and by Analytical Ultracentrifugation (AUC). The results show that the level of aggregate detected by SEC is comparable to that detected by AUC.

II. Introduction

Reference is made to the , memorandum of meeting minutes regarding Pre-IND Application Under Chemistry, Manufacturing, and Controls, Section 2.2e the CMC reviewer made the following comment/request:

> ***The presence of aggregates in your product may contribute to the immunogenicity of the product and is considered an important product attribute. Please provide a release test specification for product aggregation in the drug substance and drug product testing such s SEC-HPLC and include evidence that aggregates are able to enter and transit the column matrix. As product development proceeds, the sensitivity of the SEC-HPLC method to detect aggregates should be validated with another orthogonal method such as ultracentrifugation.***

Data obtained during product development by the Biopharmaceutical Development Program, SAIC-Frederick, Inc., showed that exposure to elevated temperatures caused the appearance of aggregates detectable by SEC-HPLC. Although in common use for detection of aggregates, SEC is a solid phase matrix dependent method. Therefore, it can potentially exclude some aggregates from reaching the detector systems leading to an erroneously low estimate of the levels of aggregate in the test article. In order to “validate” the use of SEC for aggregate detection it has become a standard practice to compare SEC-HPLC to an orthogonal, non-matrix dependent analytical

method. Analysis of the sedimentation velocity of the species within a test article by analytical ultracentrifugation is a common approach.
The purpose of this study is to evaluate the reliability of SEC-HPLC for the quantitation of the level of aggregation in formulated by comparison with analytical ultracentrifugation.

III. Materials (Samples/Controls)

Recombinant human (concentration 0.45mg/ml) was used for this study. Reference Lot was used as a system suitability control for the HPLC -SEC analysis. For SEC-HPLC and Sedimentation Velocity Analytical Ultracentrifugation three vials were thawed at ambient temperature prior to analysis.

Earlier studies conducted during development showed that exposure to elevated temperatures can cause the generation of aggregates detected by SEC-HPLC. In contrast, up to five freeze/thaw cycles from -70°C did not result in the generation of detectable aggregates by SEC-HPLC. Three additional vials were heated to 65°C for one hour. Three heat-stressed vials were snap frozen and shipped on dry ice with three vials of the unstressed controls to the University of Connecticut Analytical Ultracentrifugation Facility for Analysis. The remaining heat-stressed material was returned to the BDP for SEC-HPLC analysis

IV. Procedures

SEC-HPLC was performed by the Quality Control unit of the Biopharmaceutical Development Program, SAIC-Frederick, Inc., following an approved procedure (SOP22935, available upon request). Sedimentation Velocity Analytical Ultracentrifugation was performed by the University of Connecticut Analytical Ultracentrifugation Facility. The method used is described in Attachment 1.

V. Results and Discussion

A comparison of the SEC-HPLC and Sedimentation Velocity Analytical Ultracentrifugation results are tabulated in Table 1. The original SEC-HPLC Quality Control Test Request (QCTR) report is available upon request. The University of Connecticut report is appended to this report as Attachment 1.

Recombinant human behaves as a homodimer in solution (data not shown). Comparison of unheated samples by both analytical methods indicates that the percentage of non aggregated material is comparable. The only exception is vial three of the unheated sample by Sedimentation Velocity Analysis. The reason for this one discrepancy in unclear. Following heating at 65°C for one hour, both SEC-HPLC and Sedimentation Velocity Analytical Ultracentrifugation detected comparable levels of aggregated material.

VI. Conclusions

Sedimentation Velocity Analytical Ultracentrifugation is a non solid matrix dependent method that is widely used to analyze the association of macromolecules in solution. It is often used as the “Gold Standard” method against which other methods are qualified. Although SEC-HPLC is an easy and widely used format for detection of protein aggregates it is possible that the percentage could be underestimated by rejection of the aggregates by the column matrix. Dilution of the sample upon application to the matrix may also underestimate the content of soluble, higher molecular weight species.

In this study we have performed a comparison of these two methods for evaluating the aggregate content of . The data indicate that the percentages are comparable to each other. Based on these data, the routine use of SEC-HPLC for monitoring the aggregate content of for release and stability testing is justified.

Table 1: Comparison of Tox Lot Sample Aggregate Content as Measured by SEC-HPLC and Sedimentation Velocity Analytical Ultracentrifugation

		Sedimentation Velocity (QC-037336)			[1]SEC-HPLC (QC-037568)	
		% Homodimer	% Monomer	% Aggregate	[2]% Main Peak	[2]% Aggregate
Unheated	Vial #1	95.8	3.3	0.9	99.75	0.24
	Vial #2	94.2	5.5	0.3	99.82	0.19
	Vial #3	95.2	2.4	2.4	99.79	0.22
	Average	**95.1**	**3.7**	**1.2**	**99.79**	**0.22**
Heated for 1 hr @ 65°C	Vial #4	86.5	4.9	8.6	91.72	8.28
	Vial #5	89.3	2.4	8.3	91.10	8.91
	Vial #6	91.5	2.0	6.5	91.87	8.13
	Average	**89.1**	**3.1**	**7.8**	**91.56**	**8.44**

[1] SEC-HPLC analysis was performed on samples after they were returned from University of Connecticut Analytical Ultracentrifugation Center
[2] Percentage reported for each vial is the average of 3 injections

Attachment 1: Sedimentation Velocity Analytical Ultracentrifugation Report from the University of Connecticut Analytical Ultracentrifugation Facility (QC-037336)