



MEMORANDUM

From Leslyn Aaron, OCBQ/DBSQC
Tao Pan, OCBQ/DBSQC
Grainne Tobin, OCBQ/DBSQC
Karen Smith, OCBQ/DBSQC
Kouassi Ayikoe, OCBQ/DBSQC
Ritu Agarwal, OCBQ/DBSQC
Mark Levi, OCBQ/DBSQC
Lokesh Bhattacharyya, OCBQ/DBSQC

To STN 125506/0

Through William M. McCormick, Director, OCBQ/DBSQC

Company Bio Products Laboratory Limited (BPL), Inc.

Product Coagulation Factor X (Human), High Purity Concentrate

Subject Review Memo for the Release Tests for the Drug Product and the Factor X Potency Assay for the (b) (4)

Recommendation: Approvable

Summary

A new BLA was submitted by Bio Products Laboratory Limited (BPL), Inc. for Coagulation Factor X (Human), STN: 125506, High Purity Concentrate for the treatment of hereditary factor X deficiency in July 2013. The application received a Complete Response (CR) letter due to significant CMC deficiencies, including unacceptable validation of the quality control lot-release test methods. The sponsor addressed the issues identified in the CR letter and resubmitted their application as Amendment 37. This memo constitutes review of the submissions in this amendment and their response to our subsequent IRs and includes review of the methods and their validations for the following quality control lot-release test.

1. Identity and Determination of Factor X Potency by Chromogenic Assay

2. Determination of Total Protein by (b) (4)
3. Determination of Factor II Potency by (b) (4) Assay
4. (b) (4) Moisture Determination
5. Determination of Factor IX Potency by (b) (4) Assay
6. Determination of Non-Activated Partial Thromboplastin Time (NaPTT)
7. Determination of Fibrinogen Clotting Time (FCT) at (b) (4)
8. Determination of (b) (4)
9. Determination of (b) (4)
10. Sucrose Determination by (b) (4)
11. Determination of Citrate by (b) (4)
12. Determination of Sodium Content by (b) (4)
13. Determination of (b) (4)

In this resubmission, the sponsor, Bio Products Laboratory Limited (BPL), Inc., provided an excellent method validation package for the tests listed above. We conclude that all methods can be approved as suitable for use in quality control lot-release testing.

Background of Submission

On July 10, 2013, Bio Products Laboratory Limited (BPL), Inc. submitted an original BLA for Coagulation Factor X (Human) product. The product contains a human coagulation factor X concentrate indicated for control and prevention of bleeding episodes as well as for peri-operative management in adults and children (aged 12 years and above) with a hereditary factor X deficiency. The product is formulated as a sterile, (b) (4), freeze-dried concentrate of coagulation factor X and is presented as two doses, 250 and 500 International Units (IU). Following reconstitution with sterile water for injection, the both formulations contain the same concentration of active ingredient and differ only in volumes at the point of use.

Having reviewed the initial BLA submission and responses to our information requests (IR) submitted by 31 January 2014, we concluded that only a few less critical methods used in the quality control lot-release testing could be considered sufficiently validated to be approved as suitable for use in quality control lot-release testing (Aaron et al., Review Memo for the Release Tests for the Drug Product and the Factor X Potency Assay for the (b) (4), dated 14 February 2015). Thus, the application was not approved and a Complete Response (CR) letter was issued. The sponsor addressed the issues identified in the CR letter and resubmitted their application in April 2015 as Amendment 37.

Submitted Information and Documents

Information submitted and reviewed includes:

- 125506/0 – 3.2.P.5.1 Specifications (Drug Product)
- 125506/0.37 – Response to FDA Request for Further Information (Q2)
 - 3.2.P.5.1 – Specifications of Drug Products
 - QCA/00179: (b) (4) Method for the Determination of Factor X, (b) (4)
 - 3.2.P.5.2.1.4 – Determination of moisture by the (b) (4) method
 - QCA/00089/Ver. 9: Preparation of In-house Standards and Control Samples for use within Biochemistry

- SOP QCA/00008 ver. 20: Non-Activated Partial Thromboplastin Time (NAPTT) Test
- SOP QCA/00011 Ver. 18: The Fibrinogen Clotting Time Test
- SOP QCA/00391 ver. 15: Revised Analytical Procedure for the determination of (b) (4)
- SOP QCA/00336/Version11: Determination of (b) (4)
- QAC/00452 ver. 04: (b) (4) Determination by (b) (4)
- LP/403/1/23/01: Validation for the Chromogenic Factor X assay with the Factor X Final Product
- LR/403/1/23/02: Validation Report for the Chromogenic Factor X assay with the Factor X Final Product
- 3.2.P.5.3.1.4 – Validation for (b) (4) Moisture Determination
- 3.2.P.5.3.3.2 – Validation of Method for Determination of Total Protein
- LR/403/1/24/01: Validation Report for the (b) (4) Factor II with Factor X Final Product
- LR 403/1/25/02: Validation Report for the Factor IX (b) (4) Assay with Factor X Final Product
- LR/403/1/21/01: Validation Report for the Non-Activated Partial Thromboplastin Time Test with Factor X
- LR/403/1/22/01: Validation Report for the Fibrinogen Clotting Time Test with Factor X
- 3.2.P.5.3.4.4 – Validation for the Procedure for Determination of Sucrose
- LR/403/1/06/03 – Revised Validation Reports of the Method used for the Determination of (b) (4)
- D2014-1721 – Validation of Procedure for Determination of (b) (4)
- LR40311602: Validation Report for the Sodium Determination in Factor X
- LR312401: Validation Report for the Evaluation of (b) (4) in Factor X using undiluted samples
- 3.2.P.5.4 –Batch Analysis
- 125506/41 – Response to FDA Request for Further Information, received 9 July 2015
 - SOP QAC/00391 Ver. 15: Determination of (b) (4)
- 125506/58 – Response to FDA Request for Further Information, received 28 September 2015

Review Narrative

1. Identity and Determination of Factor X Potency by Chromogenic Assay

The factor X assay is used for both the identity and potency of the (b) (4) the final container drug product. The specifications for the factor X activity in the final container product are proposed to be 80 – (b) (4) IU/mL and 200 – (b) (4) IU/vial for a 250 IU dose and 400 – (b) (4) IU/vial for 500 IU dose, after

reconstitution. The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. Please revise SOP QCA/00179 to clearly state the assay validity (acceptance) criteria for the standard.

Review of Response: An updated version of the SOP QCA/00179, version 09, was submitted, in which the assay validity criteria are detailed in section 11.1.19.

- b. Please describe clearly the details of the testing and calculation of potency in your SOP QCA/00089.

Review of Response: The updated version (version 19) of SOP QCA/00089 provides clear and detailed instructions for performing the factor X chromogenic assay method and calculating the factor X potency.

- c. You have not studied specificity of this assay citing that it is a (b) (4). procedure. However, evaluation of specificity is necessary to demonstrate that the method works for your product without interference from the product matrix. Please provide data to demonstrate specificity of this assay based on analysis of representative product samples and matrices.

Review of Response: Specificity for the method was assessed by (b) (4)

[Redacted]

The data submitted by the sponsor also show that different formulation buffer ingredients did not affect FX assay results. Sponsor’s response is acceptable.

- d. You have demonstrated accuracy of the method by testing one standard (3rd International Standard) against another standard ((b) (4)). Please provide results of accuracy of your method using (b) (4) the final container product for which this assay is intended. We suggest you evaluate accuracy using spike-recovery method in which unspiked samples at different concentrations and the same samples after spiking with known concentrations (IU/mL) of the standard are analyzed.

Review of Response: Accuracy of the method was demonstrated by (b) (4)

[Redacted]

[Redacted]

- e. You evaluated linearity only using the (b) (4)

[redacted] please evaluate linearity at different dilution of the product (dilution linearity) and show that the linear regression line of the standard and that of the product are parallel within the proposed assay range to validate that interpolation from the standard regression line is appropriate for the determination of the potency of the product.

Review of Response: Linearity was determined from the (b) (4)

[redacted]
The sponsor did not provide slopes or the slope ratio of the linear regression plots but provided plots for the standard and the final drug product samples. A visual examination of the plots shows that they are parallel.

- f. Please provide data to establish the range of the assay based on your results of repeatability, accuracy and linearity studies obtained using representative process intermediate and product samples over the intended range of the assay.

Review of Response: The range of the method was assessed between (b) (4)

- g. Please provide data to demonstrate appropriate robustness of the assay method using representative process intermediate and product samples for which this assay is intended. The data should demonstrate effect of small deliberate changes of critical method parameters, such as reagent concentration, incubation time, etc., as applicable.

Review of Response: Robustness of the of the Factor X assay method was evaluated by varying (b) (4)

[redacted] analyses of each set of parameters were assessed.

- i. (b) (4)

[redacted]

[redacted]

[redacted]

(b) (4)

Conclusion: Based on the review of submitted information, it is concluded that the method is adequately validated and is suitable for testing the potency of the factor X in the drug product manufactured by Bio Products Laboratory.

2. Determination of Total Protein by (b) (4)

Total protein was determined as a release test of the drug product. The proposed specification is (b) (4). The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. Please provide data of the linearity study using representative final container product samples and to demonstrate parallelism between the linear regression fits for the final container product samples and the standard protein used in the linearity study.

Review of Response: The sponsor validated the linearity of the assay by: (b) (4)

- b. Please provide data to establish the range of the assay based on your results of repeatability, accuracy and linearity studies obtained using representative product samples over the intended range of the assay.

Review of Response: The range of the assay has been re-evaluated with the data from repeatability, accuracy and linearity studies using representative drug product samples. The accuracy of the assay was demonstrated by (b) (4)

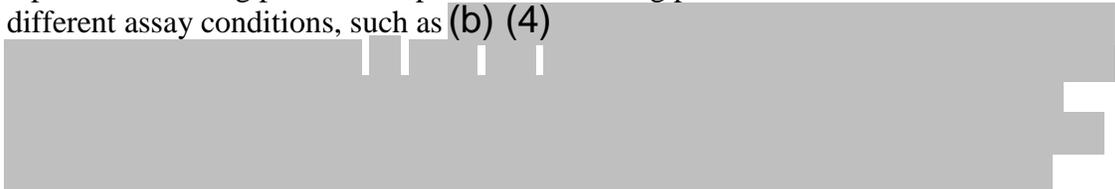
(b) (4)



- c. We don't agree with that your Internal Quality Control (IQC) sample is representative of the Factor X final container product and that any variation in the method will have similar effect on both final container product and IQC because we found that the composition of IQC is significantly different from that of the product, including the fact that the average protein concentration of IQC is (b) (4) whereas the specification limit for the Factor X product is (b) (4). Thus, the IQC sample will be (b) (4) during the assay compared to the product for this assay, which will lead to considerable (b) (4).

Please provide data for robustness studies performed with representative final container product samples.

Review of Response: The sponsor included additional result from robustness study using representative drug product sample. One lot of drug product was measured under different assay conditions, such as (b) (4).



With the newly added data, comment c) has been addressed adequately.

Conclusion: The CR comment was adequately addressed in the resubmission. Based on the information provided in original BLA, as well as the data provided in the resubmission, it can be concluded that this total protein by (b) (4) method has been validated adequately for its intended use.

3. Determination of Factor II by Chromogenic Assay

The assay measures indirectly the Factor II (FII) activity present in the final container product as a product-related impurity. The method adapted from (b) (4) is described in detail in the SOP entitled (b) (4) Method for the Determination of Factor II, document number QCA/00178/12/SOP. The proposed specification for the residual moisture in the drug product release is ≤ 1 IU/mL. The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. You have not studied specificity of this assay citing that the assay is performed as described in (b) (4). You need to perform specificity study to demonstrate that the method works for your product without interference from the product matrix. Please provide data to demonstrate specificity of this assay based on analysis of representative product samples.

Review of Response: Specificity was examined by (b) (4)



- b. You have demonstrated accuracy of the method by testing one standard (3rd International Standard) against another standard (b) (4). Please provide results of accuracy of your method using your product for which this assay is intended. We suggest you evaluate accuracy using spike-recovery method by analyzing unspiked samples at different concentrations and the same samples after spiking with known concentrations (IU/mL) of the standard in such a way that the total concentrations of factor II in the samples are between the Quantitation Limit of the assay and the proposed specification limit.

Review of Response: The accuracy of the method was assessed by (b) (4)



- c. Please provide data on the assessment of Quantitation Limit from analysis of representative samples of your product for which the assay is intended.

Review of Response: The quantitation limit of the assay was determined by measuring

(b) (4)
(b) (4)



- d. You evaluated linearity only using the standard. (b) (4) please evaluate linearity at different dilution of the product (dilution linearity) and show that the linear regression line of the standard and that of the factor II content are parallel within the proposed assay range to validate that interpolation from the standard line is appropriate for the determination of factor II content of the product.

Review of Response: Linearity was measured by (b) (4)



(b) (4)
Parallelism between the standard and drug product was not demonstrated. An IR was submitted (see below).

- e. Please reevaluate the range of the assay based on your results of repeatability, accuracy and linearity data obtained using representative product samples.

Review of Response: The range of the assay was determined from the precision, linearity and accuracy of samples tested at (b) (4)

- f. Please provide data to demonstrate appropriate robustness of the assay method using representative product samples for which this assay is intended. The data should demonstrate effect of small deliberate changes of critical method parameters, such as reagent concentration, incubation time, etc.

Review of Response: The robustness of the assay was assessed by (b) (4)

Additional Information Request and Review

- a. In your validation report (LR/403/1/24/02), you have presented linear regression analysis of your drug product measured against the standard. Please provide data to demonstrate parallelism between your drug product samples and standard. We suggest that you provide slopes and their ratio of the regression lines for the drug product samples and standard.

Review of Response: The sponsor provided linear regression analyses for (b) (4)

This is acceptable.

Conclusion: The validation report and additional information provided sufficient information to allow approval of this test method as part of this application.

4. Moisture Determination in Freeze-Dried Products by (b) (4) Method

The residual moisture of the final drug product, human coagulation factor X, was measured by (b) (4) method. The proposed specification for the residual moisture in the drug product is (b) (4). The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. You have conducted your method validation using (b) (4) standard but not the final container product for which the assay is intended to be used. Please provide you validation data using representative product samples over the intended range of the assay. The following characteristics should be addressed: specificity, accuracy (spike recovery), repeatability, intermediate precision (multiple analysts, multiple days), linearity, range, limit of quantitation and robustness of the assay. We suggest that you spike your sample with different known amount of (b) (4) and then assay both unspiked and spiked samples to calculate recovery.

Review of Response: In the resubmission, new data were provided to address the validation of the accuracy, precision, linearity, range, limit of quantitation and robustness of the assay.

To evaluate the accuracy of the assay, (b) (4)

. The accuracy of the assay was adequately validated using representative drug product samples.

The repeatability of the assay was assessed by measuring the (b) (4)

With the use of different lots of final drug product, the repeatability and intermediate precision were adequately validated.

The linearity of the assay was demonstrated by measuring the residual moisture of (b) (4)

. An IR was submitted to obtain further clarification.

In the resubmission, new data were provided on the evaluation of the range of the assay: three different lots of drug products were (b) (4)

However, the range was not clearly defined in this resubmission. The different (b) (4) spiking schemes used in accuracy (in $\mu\text{L}/\text{vial}$) and linearity/range (in $\mu\text{g}/\text{mL}$) make it difficult to interpret the accuracy and linearity data for the validation of range.

To determine the limit of quantitation. (b) (4)

(b) (4)

We agree with the assessment, however, both the calculated limit of quantitation and the preset acceptance criterion for the limit of quantitation should be expressed in the same term, either as (b) (4) with reference to dry weight of drug product or as $\mu\text{g/mL}$.

The robustness of the assay was evaluated for (b) (4)

(b) (4)

In this resubmission, assay characteristics such as accuracy, precision, linearity, range, and robustness of the (b) (4) method were evaluated with the use of samples representative of drug products. However, the range of the assay was not clearly defined; different (b) (4) spiking schemes used in different studies make it difficult to use the accuracy and linearity for the validation of the range; the range and limit of quantitation were defined in different terms, and they need to be consistent with each other, either as (b) (4) with reference to the dry weight of the drug product or as (b) (4)l. Please provide further clarification to address the above issues.

Information Requests and Review of the Responses to the IRs:

After reviewing the resubmission, additional IR were submitted in April 2015. The sponsor provided responses Amendment 41, received in July 2015. The responses are reviewed below.

- a. Please clearly define the range of the assay, either as (b) (4) with reference to the dry weight of drug product or as mg/ml for the (b) (4) content.

Review of Response: The sponsor explained that the range of the assay for Factor X was (b) (4) moisture, which was derived from the linearity studies (data were included in the response as Table 3.2.P.5.3.1.4-T5. Based on the data presented in the Amendment, the reviewer found the response is adequate.

- b. Please present the amount of (b) (4) spiked to drug product in a consistent manner between accuracy and linearity studies, so that the data from these studies can be interpreted to justify the range of the assay.

Review of Response: The accuracy and linearity data are recalculated and presented in Table 3.2.P.5.3.1.4-T1 and Table 3.2.P.5.3.1.4-T5 of the response in Amendment 41.

(b) (4)

(b) (4)

Based on the clarification provided in the Amendment, it can be concluded that the accuracy and linearity of the method has been validated over the assay range.

- c. Please express the limit of quantitation and the range in a consistent manner, either as (b) (4) with reference to the dry weight of drug product or as mg/ml.

Review of Response: The sponsor reported the limit of quantitation and the range data are in Table 3.2.P.5.3.1.4-T8 and Table 3.2.P.5.3.1.4-T7 of Amendment 41, with the residual (b) (4) now reported in a consistent manner as (b) (4) moisture and the (b) (4) spike reported as (b) (4). Thus, the sponsor has clarified that the QL of the method is (b) (4) which is adequate.

- d. Please demonstrate the range of the assay by summarizing and analyzing the data from accuracy, linearity and precision studies.

Review of Response: In the response, the sponsor provided the validation result of the method over a working concentration of (b) (4), which was presented in a consistent term as (b) (4). The results are summarized below.

Test	Result range, (b) (4)
Accuracy	(b) (4)
Linearity	(b) (4)
Precision (Repeatability)	(b) (4)
Assay Range, (b) (4)	(b) (4)

Based on the information, the review’s questions have been adequately addressed.

Conclusion: Based on the review of the data provided it is concluded that the description of the method is sufficient; appropriate validation characteristics have been selected; and the method has been validated for its intended use. Approval is recommended.

5. Determination of Factor IX ((b) (4) Assay)

Factor IX is present in the drug product as an impurity. The specification for the final container product is Not Greater Than (NGT) 1 IU/mL. The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. You performed specificity study using a factor IX product that contains (b) (4) of FIX concentration. However, your specification limit is NGT 1 IU/mL. Specificity should be evaluated at the expected concentration at which the analyte (factor IX) is present in the product. You assessed specificity at the factor IX concentration of (b) (4), which is significantly higher than the

(b) (4)

In that case, the test should have been validated as a limit test, which requires evaluation of specificity and detection limit. BPL demonstrated method specificity (see a., p. 12-13 of this memo) and quantitation limit (see response to Additional IR, p. 15 of this memo).

- e. Please reassess the range of the assay based on your results of repeatability, accuracy and linearity data obtained using representative product samples.

Review of Response: Range was determined from the precision, linearity and accuracy of (b) (4)

- f. Please provide data to demonstrate appropriate robustness of the assay method using representative product samples. The data should demonstrate effect of small deliberate changes of critical method parameters, such as reagent concentration, incubation time, etc.

Review of Response: BPL submitted data showing that robustness was determined (b) (4)

However, the study does not involve “deliberate changes to method parameters” as necessary to demonstrate robustness of the method.

- g. Please provide the SOPs QCA/00042 and QCA/00073. You referenced these two documents in your validation report but have not included them in your submission.

Review of Response: The data provided in the BLA (3.2.P.5.3.5.2.3) to demonstrate intermediate precision were obtained using (b) (4) and using the representative samples. The results from three batches of the drug product show overall RSD in the range (b) (4) which met the acceptance criteria, (b) (4)

Additional Information Request

Review of the submitted validation report led to an Information Request which was submitted to the sponsor on 10 June 2015. The response was received on 9 July 2015 as part of Amendment 41. The Information Request, response and review of responses are presented below.

- a. Please clarify what “deliberate changes to method parameters” were evaluated in your robustness studies.

Review of Response: BPL explained that the FIX method is run by a proprietary (b) (4)

(b) (4)

this is acceptable.

- b. Have you looked into sample stability during your robustness studies? For example, have you evaluated what effects (b) (4) may have on samples? Please provide the data.

Review of Response: BPL informed that sample stability study was not examined during robustness. It is not relevant to perform (b) (4) studies on the drug product since a lyophilized drug product sample is freshly prepared (reconstituted) each time the assay is run.

- c. In section 9.9.1 you stated that the (b) (4) generated standard line will not be used for the determination of linearity. Please explain what standard line is used for the determination of linearity. In addition, please provide data to show parallelism of concentration dependence of standard (regression line) you actually use and that of the drug product.

Review of Response: The (b) (4) generates (b) (4)

. As explained before, the test should have been validated as a limit test, which requires demonstration of specificity only (as per ICH Q2(R1)), which the sponsor did (section a.).

- d. You determined that the LOQ of your method is (b) (4). However, you have shown accuracy of the method at (b) (4). Please provide data either to demonstrate accuracy of your method at (b) (4) or revise your validation report to indicate that your LOQ is (b) (4).

Review of Response: BPL acknowledged that accuracy has not been measured at the previously defined limit of quantitation (b) (4). BPL conducted further evaluation, per FDA request, (b) (4)

. This is acceptable.

- e. We found a hand-written note in your validation report, which appear to state that you are unable to do linearity study because Factor IX concentration in your drug product is below the lowest concentration of your standard. We have found several hand-written pages in your validation report (pages 23, 26 and 27).
- i. Hand-written pages are not acceptable for review by CBER. Please submit typed information for the corresponding pages, identifying the page numbers in the validation report.

Review of Response: In response, BPL submitted typed transcripts as requested of the hand written comments from the validation protocol of the following pages: 23 (specificity), 26 – 27 (linearity).

- ii. Your reported LOQ to be (b) (4) and your proposed specification for the drug product is NGT 1 IU/mL. Therefore, we could not understand why you could not demonstrate linearity in the range (b) (4). If you change your LOQ to (b) (4) as request (d) above, please show linearity of the standard minimally in the range (b) (4) and parallelism between the regression lines of the standard and the drug product within that range.

Review of Response: BPL has redefined the LOQ as (b) (4) and showed linearity and accuracy data in the range (b) (4) and indicated that determining the linearity of samples against the standard line was not possible because the analyte concentration had been consistently below the lowest concentration of the standard. As discussed above, BPL made an incorrect attempt to validate the method as a quantitative test for impurity. It should have been validated as a limit test. BPL has presented sufficient data meeting the requirement for validating the test method as a limit test, as directed by the ICH Q2(R1) guideline.

Conclusion: The validation report and additional information provided sufficient information to allow approval of this test method as part of this application

6. Determination of Non-Activated Partial Thromboplastin Time (NAPTT)

The test is adapted from the test for '(b) (4)' described in (b) (4). The specification for the final container product is (b) (4) of the product sample. The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. In response to our IR, you responded that this is a qualitative method. Therefore, you will evaluate specificity only to validate these methods. We do not agree. You are assessing an impurity level that is related to safety of the product by measuring the clotting time. Thus, your reportable result is time. Therefore, the method is quantitative. You need to validate the method as a quantitative method with time as the measurand. Thus, in addition to specificity, please provide data on evaluation of other validation characteristics appropriate for a quantitative test for impurity in terms of the reportable result.

Review of Response: The sponsor submitted the validation report, LR/403/1/21/01, Validation of the Non Activated Partial Thromboplastin Time Test with Factor X. The validation followed ICH guidelines and examined the following: accuracy, precision, intermediate precision, specificity, detection limit at (b) (4) linearity, and robustness.

The accuracy of the method was measured by (b) (4)

which did not meet the

(b) (4)

Precision was determined by measuring the clotting time of (b) (4)

Specificity was determined by (b) (4)

. No data was provided for unspiked samples and the effect of formulation buffer alone was not examined. An IR was sent to address these issues (see 2.1 below).

The specifications of the NaPTT assay in 3.2.P.5.1 state the clotting time of the (b) (4) drug product must be (b) (4). Hence the detection limit of the assay was determined (b) (4)

Linearity was measured by (b) (4)

The robustness of the assay was examined by making small changes to the (b) (4)

(b) (4) . This suggests that the assay is robust under normal assay conditions only.

- b. Based on our analysis of the calibration (qualification) data for the control you submitted we found that the mean^{(b) (4)} SD values are (b) (4)

Please revise your SOP (QCA/00008) to include (b) (4) as the assay validity criteria.

Review of Response: The sponsor stated that the data for the control are continuously being reviewed using statistical software. Although the clotting times reported in the SOP were originally prepared in 2004, the clotting times currently being measured are within^{(b) (4)} SD of the original data and are appropriate to use. The specifications are^{(b) (4)}, hence it is appropriate for the acceptance criteria of the^{(b) (4)} dilution of the control to be (b) (4). Also, as detailed in (b) (4), the clotting time measured for the negative control must be within (b) (4). This is acceptable.

- c. You responded, “The operator will review the control chart and if the control result is (b) (4), the assay would be considered invalid, and the results would not be used.” We cannot agree. The assay validity criteria should be mentioned in the SOP and the assay should be considered invalid, if the results do not meet the criteria. Please revise your SOP to include assay validity criteria, as discussed above.

Review of Response: Assay validity criteria as described above are included in section 11.3 and 12.1. This is adequate.

- d. You mentioned that (b) (4) are necessary to ensure that there is no masking, due to either over dilution or matrix inhibition. In that case, results from both dilutions should be your reportable results. Please revise your SOP (QCA/00008) accordingly.

Review of Response: Section 12 of the SOP has been amended to include recording both the (b) (4) results. This is satisfactory.

- e. You indicated that (b) (4) step is not necessary for the factor X product. Please revise your SOP (QCA/00008) to include this clarification.

Review of Response: The sponsor clarified in the SOP that the (b) (4) step was not necessary for the FACTOR X drug product or (b) (4). This is adequate.

Additional Information Request

Review of the submitted validation report led to an Information Request which was submitted to the sponsor on 10 June 2015. The response was received on 9 July 2015 as part of Amendment 41. The Information Request, response and review of responses are presented below.

- a. In your validation report (LR/403/1/21/01), you state that Factor X samples both unspiked and spiked with the activated factors were measured in the assay to

demonstrate method specificity, but only the spiked data were submitted. Please provide the data for the unspiked samples. Also, please further show the specificity of your assay by demonstrating that there is no interference from the formulation buffer for the drug product.

Review of Response: The sponsor provided data demonstrating the clotting times of unspiked drug product in (b) (4) were (b) (4), the maximum time of the test. Since the purpose of the test is to measure activated factors in the drug product, and as nothing was detected in the drug product sample, the sponsor felt it was not necessary to measure (b) (4) alone as this would not contain any endogenous activated factors. This is acceptable.

Conclusion: The validation report and additional information provided sufficient information to allow approval of this test method as part of this application.

7. Determination of Fibrinogen Clotting Time (FCT)

The test measures the time required for thrombin (product-related impurity) in the test sample to clot a known concentration of fibrinogen. The specification for the final container product is (b) (4). The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. In response to our IR, you responded that this is a qualitative method. Therefore, you will evaluate specificity only to validate these methods. We do not agree. You are assessing the impurity level by measuring the clotting time. Thus, your reportable result is time. Therefore, the method is quantitative. You need to validate the method as a quantitative method. Thus, in addition to specificity, please provide data on evaluation of other applicable validation characteristics for a quantitative test for impurity in terms of the reportable result.

Review of Response: In response, the sponsor provided the validation report, LR/403/1/22/01, in which evaluation of accuracy, repeatability, intermediate precision, specificity, linearity, detection limit and robustness were reported.

Accuracy and specificity were evaluated by (b) (4)

. Comparable results in two different matrices show specificity and accuracy of the method.

The repeatability was assessed from (b) (4)

The results show adequate precision of the method.

The linearity of the assay was demonstrated by (b) (4)

The detection limit was determined as the (b) (4)

The robustness of the method was evaluated (b) (4)

demonstrating robustness of the method.

The results demonstrate adequate validation of the method. However, an IR was submitted requesting information on the amount of (b) (4) spiked in the repeatability and intermediate precision studies.

- b. Please revise your SOP QCA/00011/15: The Fibrinogen Clotting Time Test to include appropriate and justifiable the assay validity criteria and submit with your justification.

Review of Response: The sponsor informed that, based on the method validation results, Assay Validity Criteria were included in the SOP in terms of clotting times of (b) (4)

respectively. This is acceptable.

Additional Information Request

Review of the submitted validation report led to an Information Request which was submitted to the sponsor on 10 June 2015. The response was received on 9 July 2015 as part of Amendment 41. The Information Request, response and review of responses are presented below.

- a. In your validation report (LR/403/1/22/01), you reported repeatability and intermediate precision results using spiked samples. Please provide information on the concentration of (b) (4) spiked for repeatability and intermediate studies.

Review of Response: In response, the sponsor the sponsor informed that the repeatability and intermediate precision samples were (b) (4)

This response is satisfactory.

8. Determination of (b) (4)

(b) (4)

(b) (4)

10. Sucrose determination by (b) (4)

The sucrose content in the final container drug product was measured using an (b) (4) method with (b) (4). The specification for sucrose in the drug product was set to be (b) (4). The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. Please provide data, including your linear regression plots, to demonstrate parallelism between the linear regression fits for the final container product samples and the standard at different concentrations.

Review: Previously the linearity of the assay was demonstrated with the sucrose standard only. In the resubmission, the sponsor evaluated the linearity of the assay by (b) (4)

The related CR comment was adequately addressed and the linearity of the assay has been validated.

- b. Please provide data to establish the range of the assay based on your results of linearity, precision and accuracy evaluation using representative samples of final container product.

Review of Response: The range of the assay was evaluated with drug product (b) (4)

the related CR comment has been adequately addressed.

Conclusion: Based on the information provided in original BLA, as well as the data provided in this re-submission, it can be concluded that this sucrose by (b) (4) method has been validated for its intended use and approval is recommended.

11. Determination of Citrate by (b) (4)

Citrate is an excipient in the Factor X drug product. The proposed specification in the drug product is (b) (4) for lot release. The following issues were outstanding from the original submission and were addressed in response to the CR Letter as Amendment 37.

- a. The accuracy, repeatability and intermediate precision were studied at concentrations much lower than the target concentration of (b) (4). Please evaluate these validation characteristics over the actual assay range.

Review of Response: The sponsor presented revalidation data in the report LR/403/115/03.

Accuracy was determined by (b) (4)

Repeatability and Intermediate precision were determined (b) (4)

- b. The linearity of the method was evaluated in the range (b) (4) however the range of the method was determined to be (b) (4) based on the precision and accuracy results, which is different than the range in which linearity was studied. Please provide additional data for the linearity over the stated range of the assay or redefine your assay range that is supported by linearity, accuracy and precision results.

Review of Response: Linearity was studied using citrate standards in the range of (b) (4)

Conclusion: The method has been appropriately validated and is suitable for its intended use. However, it is recommended that the sponsor revise the assay range from (b) (4), since the sponsor has demonstrated accuracy in the range (b) (4) and linearity in the range (b) (4)

12. Sodium Content by (b) (4)

This assay directly measures the sodium level which is present in the final drug product as an excipient. The proposed specifications for the assay are (b) (4). The following issue was outstanding from the original submission and was addressed in response to the CR Letter as Amendment 37.

- e. Please provide data to show linearity and accuracy of sodium response using final container product. Also, show parallelism between the standard and sample regression lines to demonstrate assay linearity.

Review of Response:

(b) (4)

