



To STN # 125640/0

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Sponsor Instituto Grifols, S.A.

Subject: Primary Discipline Review Memo for Quality Control Lot-release Test Methods for Human plasma-derived Fibrin Sealant, VeraSeal Drug Product

Summary of Review and Conclusion

On November 3, 2016, a new BLA was submitted for human plasma-derived Fibrin Sealant, VeraSeal Drug Product (STN 125640) by Instituto Grifols, S.A. This document constitutes the primary review memo from DBSQ for the following analytical methods and their validations, as used for the quality control lot release of this product.

Fibronogen component

1. Determination of Fibrinogen (Clottable protein) by (b) (4) Method
2. Determination of Glutamic acid, Glycine, Arginine and Isoleucine by (b) (4)
3. Citrate Determination by (b) (4) Method
4. Determination of Chloride by (b) (4) method
5. Determination of Polysorbate 80 by (b) (4) method
6. Determination of Tri-n-Butyl Phosphate (TNBP) by (b) (4)
7. Sodium Determination by (b) (4)
8. Appearance of Frozen Product
9. Appearance of Solution after Thawing
10. pH

Thrombin component

11. Determination of Glycine by (b) (4)
12. (b) (4)
13. Determination of Chloride by (b) (4) method

14. Determination of Polysorbate 80 by (b) (4) method
15. Determination of Tri-n-Butyl Phosphate (TNBP) by (b) (4)
16. Sodium Determination by (b) (4)
17. Determination of Calcium by (b) (4)
18. Appearance of Frozen Product
19. Appearance of Solution after Thawing
20. pH

Review of the methods and their validations, led to three information requests (IR's). The first information request was submitted to the sponsor in two parts on 01 May 2017 and 05 May 2017. The second and third information requests were submitted on 10 July 2017 and 29 August 2017. The sponsor provided responses to the first IR on 10 May 2017, 25 May 2017 and 21 August 2017 as Amendments 27, 28 and 34 respectively. The response to the second IR was received on 5 August 2017 as Amendment 33. The response to the third IR is still pending at the time of writing this memo.

Conclusion

There are outstanding IR's for the following methods due to minor deficiencies in method validation:

Fibrinogen Component

1. Determination of Glutamic acid, Glycine, Arginine and Isoleucine by (b) (4)
2. Determination of Polysorbate 80 by (b) (4) method

Thrombin Component

3. Determination of Polysorbate 80 by (b) (4) method

The evaluation of sponsor's responses to assess adequacy of these methods will be reported in the addendum memo. All other methods have been described and validated adequately, and are suitable for lot-release testing of the product.

Background

Instituto Grifols, S.A. submitted a new BLA for their Veraseal drug product, which is a human plasma-derived Fibrin Sealant. Fibrin sealant is intended to be used as an adjunct to hemostasis for mild to moderate bleeding in adults (b) (4) undergoing surgery when control of bleeding by standard surgical techniques (such as suture, ligature, and cautery) is ineffective or impractical. Fibrin sealant is effective in heparinized patients. The plasma-derived Fibrin Sealant (FS) Grifols consists of two components, thrombin and fibrinogen. Both components are obtained from fraction 1 of human plasma and are extensively purified before assembly into a kit, which consists of two sterile syringes containing equal volumes of frozen solutions of human fibrinogen and thrombin, and a delivery device. The fibrin sealant Grifols is formulated for topical use, and is available in four presentations of 2 mL, 4 mL, 6 mL and 10 mL.

Submitted Information and Documents

This is an electronic submission. Information submitted and reviewed in support of this supplement included:

- 125640/0 — 1.2 Cover letter dated November 4, 2016
- 125640/0 — 2.3 Quality Overall Summary
- 125640/0 — 3.2.P.5.2. Analytical Procedures, Fibrinogen Component
 - Control Test Procedure: SOP IG_MA-000888: Determination of Fibrinogen by (b) (4) method
 - Control Test Procedure: SOP IG_MA-000358C_ING: Determination of Glutamic acid, Glycine, Arginine and Isoleucine by (b) (4)
 - Control Test Procedure: SOP IG_MA-000170B: Citrate Determination by (b) (4) method
 - Control Test Procedure: SOP IG_MA-000016A: Determination of chloride by (b) (4) method
 - Control Test Procedure: SOP IG_MA-000403C: Determination of polysorbate 80 by (b) (4) method
 - Control Test Procedure: SOP IG_MA-000281A: Determination TNBP by (b) (4)
 - Control Test Procedure: SOP IG_MA-000005A: Sodium Determination by (b) (4)
 - Control Test Procedures: SOP's IG_MA-000004A, IG_MA-000003A, IG_MA-000004B: pH, Appearance of Frozen product and Appearance of solution
- 125640/0 — 3.2.P.5.2. Analytical Procedures, Thrombin Component
 - Control Test Procedure: SOP IG_MA-000358A_ING: Determination of Glycine by (b) (4)
 - Control Test Procedure: SOP IG_MA-000456A: (b) (4) method
 - Control Test Procedure: SOP IG_MA-000016A: Determination of chloride by (b) (4) method
 - Control Test Procedure: SOP IG_MA-000403C: Determination of polysorbate 80 by (b) (4) method
 - Control Test Procedure: SOP IG_MA-000281A: Determination TNBP by (b) (4)
 - Control Test Procedure: SOP IG_MA-000005A: Sodium Determination by (b) (4)
 - Control Test Procedure: SOP IG_MA-000005A: Determination Calcium by (b) (4)
 - Control Test Procedures: SOP's IG_MA-000004A, IG_MA-000003A, IG_MA-000004B: pH, Appearance of Frozen product and Appearance of solution

- 125640/0 — 3.2.P.5.3. Validation of Analytical Procedures, Fibrinogen Component
 - Consolidated Validation Report, IG_IVMA-000408_ING: Determination of Fibrinogen by (b) (4) method
 - Consolidated Validation Report, IG_IVMA-FGDI358C_ING: Determination of Glutamic acid, Glycine, Arginine and Isoleucine by (b) (4) method
 - Consolidated Validation Report, IG_MA-000381_ING: Citrate Determination by (b) (4) method
 - Consolidated Validation Report, IG_IVMA-000367_ING: Determination of chloride by (b) (4) method
 - Consolidated Validation Report, IG_IVMA-FGDI403C_ING: Determination of polysorbate 80 by (b) (4) method
 - Consolidated Validation Report, IG_IVMA-000261_ING: Determination TNBP by (b) (4) method
 - Consolidated Validation Report, IG_MA-000373_ING: Sodium Determination by (b) (4) method
- 125640/0 — 3.2.P.5.3. Validation of Analytical Procedures, Thrombin Component
 - Consolidated Validation Report, IG_IVMA-THROM358A_ING: Determination of Glycine by (b) (4) method
 - Consolidated Validation Report, IG_IVMA-THROM456A_ING: (b) (4) method
 - Consolidated Validation Report, IG_IVMA-000374_ING: Determination of chloride by (b) (4) method
 - Consolidated Validation Report, IG_MA-000401_ING: Determination of polysorbate 80 by (b) (4) method
 - Consolidated Validation Report, IG_IVMA-000237_ING: Determination TNBP by (b) (4) method
 - Consolidated Validation Report, IG_IVMA-000415A_ING: Sodium Determination by (b) (4) method
 - Consolidated Validation Report: IG_IVMA-000062_ING: Determination Calcium by (b) (4) method
- 125640/27 — 1.11.1 Quality Information Amendment; Response to IR dated 01 May 2017; Received 10 May 2017
- 125640/28 — 1.11.1 Quality Information Amendment; Response to IR dated 05 May 2017; Received 25 May 2017
- 125640/33 — 1.11.1 Quality Information Amendment; Response to IR dated 10 July 2017; Received 5 August 2017
- 125640/34 — 1.11.1 Quality Information Amendment; Response to IR dated 05 May 2017; Received 21 August 2017

Review Narrative

Fibrinogen Component

1. Determination of Fibrinogen (Clottable protein) by (b) (4) Method

The specification for fibrinogen clottable protein is not less than (b) (4) and no more than (b) (4) of the stated content. In case of fibrinogen product, for a nominal value of 80 mg clottable protein/mL, the specification range is (b) (4).

Method

The Fibrinogen (clottable protein) content is determined using the (b) (4)

[Redacted]

Method Validation

(b) (4)

[Redacted]

[Redacted]

(b) (4)

(b) (4)

(b) (4)

(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 27 on 01 May 2017, is discussed below.

A. We have the following questions/comments regarding the method validation report for the determination of Fibrinogen (Clottable protein) by (b) (4) Method, Document IG_IVMA-000408_ING:

- i. You have indicated in Section 4.2 that Linearity was assessed using Fibrinogen in the range of (b) (4). This range is equivalent to a range of (b) (4), after considering the dilution specified in the test method SOP. Thus, your linearity data do not cover the lower specification limit of (b) (4) for the Fibrinogen component of your product. Please provide additional linearity data to cover the proposed specification range.

Review of response: The sponsor submitted additional linearity data and also precision data in the range of linearity study, as Amendment 27. Linearity was estimated from fibrinogen component of fibrin sealant sample containing (b) (4) of clottable fibrinogen protein. The sample was diluted (b) (4) times to obtain (b) (4) concentrations in the range of (b) (4) of fibrinogen. Three separate linearity runs were performed. The mean correlation coefficient (R) was (b) (4), and within the

acceptance criterion of R to be (b) (4). The average recovery as assessed from the three linearity runs was also within the acceptable range of (b) (4) at each concentration level. Intermediate precision was determined from the linearity runs, and repeatability was determined by testing the above (b) (4) dilutions of fibrinogen in (b) (4) within the same assay. The RSD's for repeatability were in the range of (b) (4). The RSD's for intermediate precision varied from (b) (4) at each concentration level. The overall RSD for intermediate precision, as calculated from the submitted data was also within the acceptable limit. The range of the assay is same as the linear range as evaluated from linearity/accuracy and precision data. The assay is acceptable for the quantitation of clottable fibrinogen protein in fibrinogen component of the drug product.

- ii. You have evaluated Accuracy using only the (b) (4) Standard but not your fibrinogen product. Please provide data using the Fibrinogen FDP to assess Accuracy over the proposed range of the assay. Alternatively, please provide data to support the suitability of the use of the standard in the study, e.g., recovery data from the Precision study in the proposed range.

Review of response: The sponsor explained that a spiking study with (b) (4) standard could not be performed over the range of the assay due to low fibrinogen content of the standard. Therefore, one concentration was evaluated by (b) (4)

Since, the sponsor was able to evaluate recovery at the highest concentration of the specification range, we do not agree that this assessment was not possible at other lower concentration levels. However, the sponsor has evaluated recovery in the additional linearity experiments, in the required assay range, therefore, no further IR is required.

- iii. Please provide robustness data for your method by evaluating the effect of variation of your method operating conditions parameters, including reagent concentrations.

Review of response: In response, the sponsor provided the robustness data as amendments 21 and 27. The first study was focused on variation of the following factors: (b) (4) equipment, human thrombin lots, (b) (4) calcium chloride preparations, and (b) (4) solutions. The effect of variation of each parameter was studied using the in-house qualified lot of fibrinogen drug product as a control for clottable protein. The results of control were within the acceptable range. In the second study, the effect of variation of concentration of calcium ((b) (4)), concentration of thrombin (IU/mL) and temperature of (b) (4) was evaluated. Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels. The influence of variation was evaluated in terms of (b) (4). Effect of none of the parameters studied was found to be statistically significant. Thus, the assay robustness is sufficiently demonstrated.

Conclusion: The method is has been adequately described and validated, and is acceptable as a lot-release test for the determination of clottable protein content in the fibrinogen component of fibrin sealant drug product.

2. Determination of Glutamic acid, Glycine, Arginine and Isoleucine by (b) (4)

Glutamic acid, Arginine and Isoleucine are excipients in fibrinogen component of fibrin sealant drug product. The specifications in fibrinogen are (b) (4) for glutamic acid, (b) (4) for arginine and (b) (4) for isoleucine. Glycine is a process derived impurity, and its specification is set at (b) (4) in the fibrinogen component of the drug product.

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

- A. For the (b) (4) procedure described in your SOP IG_MA-000358C_ING, please provide: (1) the composition of (b) (4); (2) the (b) (4); (3) (b) (4); and (4) composition of the control sample and the details of the qualification of the control.

Review of response: In response, the sponsor provided the details of the (b) (4) procedure used for the quantitation of amino acids. (b) (4)

The sample used as an assay control was a qualified lot of fibrinogen product, which was standardized against commercially available standards for glutamic acid, arginine, glycine and isoleucine.

- B. Regarding the Method validation report, document IG_IVMA- FGD1358C_ING:

- i. Please provide a representative (b) (4) of fibrinogen drug product, and (b) (4) of the four amino acids in fibrinogen product, and the (b) (4) of the corresponding amino acids in the standard solutions to establish both identity and specificity of your assay.

Review of response: In response to CBER IR, the sponsor provided additional specificity data wherein the fibrinogen product sample was analyzed in (b) (4). The (b) (4) of four amino acids was comparable to the (b) (4) observed in corresponding amino acid standard solutions. Thus, specificity of the assay was adequately demonstrated.

- ii. Please establish range of your assay for each amino acid based on the linearity, accuracy, and precision data obtained from the fibrinogen samples, and update your validation report accordingly.

Review of response: The sponsor submitted additional linearity data as Amendment 28. Linearity was estimated from (b) (4) concentrations of each amino acid, and by testing each concentration in three independent assays. The response was linear between (b) (4) for glutamic acid, (b) (4) for arginine, (b) (4) for isoleucine and (b) (4) for glycine. The correlation coefficient (R) was within the acceptance criterion of (b) (4) for three independent runs of all four amino acids. The recovery as assessed from the three linearity runs was also within the acceptable range of (b) (4) for all amino acids. The range of the assay is same as the linear range for each amino acid as evaluated from linearity/accuracy (IR response) and precision data (submitted as part of original submission). The assay is acceptable for the quantitation of glutamic acid, arginine and isoleucine. However, glycine is quantitated as an impurity in this assay, therefore, the sponsor needs to evaluate the linearity and accuracy at the specification limit of (b) (4). Another IR was submitted to the sponsor to address this issue.

iii. Please provide the robustness data by evaluating the effect of variation of critical method parameters.

Review of response: In response, the sponsor submitted the report IG_ITEC-002989_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of (b) (4) between the arginine and glycine (b) (4) for the lowest standard used in the preparation of calibration curve. The effects of above factors were statistically insignificant. The sponsor did not provide the results from drug product samples, however, they referred to compendia (USP and EP) guidance's for studying robustness of the method. Additionally, according to ICH (Q2) R1, for the evaluation of robustness of a (b) (4) method, a series of system suitability parameters should be established to ensure that the validity of the method is not susceptible to variations in analytical conditions. Based on the results obtained during the robustness study, and compendia guidance's, no further IR is required.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

A. With regard to the Method validation report, Document IG_IVMA-FGD1358C_ING:

i. Your response to Information Request dated May 25, 2017, and submitted

linearity data are acceptable for the quantitation of glutamic acid, arginine, and isoleucine. However, glycine is quantitated as an impurity in this assay. Please provide your results for linearity and accuracy evaluation for glycine in the interval between LOQ and the specification limit of (b) (4).

Review of response: The sponsor stated that the LOQ of the assay has been demonstrated earlier, and did not provide the complete response. The sponsor's linearity validation included three data points viz. (b) (4). The interval between the 2nd and 3rd data point was too large, and therefore, the sponsor was requested to submit additional data at the specification limit of (b) (4). Another IR was submitted to the sponsor to address this issue.

- ii. In your method validation for the glycine assay, the range of assay based on the linearity, accuracy, and precision results is (b) (4). Thus, the LOQ, which is the lowest concentration assessed with acceptable accuracy and precision, is (b) (4). However, you stated that the LOQ was (b) (4). Please provide data to show the accuracy and precision of the method at (b) (4) or correct your validation report to indicate (b) (4) as the LOQ.

Review of response: The sponsor explained that as per the validation report, the experimentally obtained value of LOQ is (b) (4). This value is the lowest concentration of the standard curve from the fibrinogen sample. However, we noted that the lowest point is (b) (4). Therefore, the sponsor has not corrected the LOQ result, as requested in the IR. A 3rd IR was submitted..

Third Information request: The following IR was submitted to the sponsor on 29 August 2017. The response to the third IR was not received at the time of writing this memo.

We have the following questions/comments regarding the Method validation report, Document IG_IVMA-FGD1358C_ING: In your (b) (4) assay for the quantitation of amino acids, glycine is measured as an impurity. Therefore, during the study of validation characteristics, it is critical to include the data point at the defined specification limit of (b) (4). As requested in our previous IR (sent on 10 July 2017), please provide the requested data to permit complete review of your assay.

Conclusion: The method is clearly described in the SOP. However, there are outstanding issues with the method validation as discussed in the third IR.

3. Citrate Determination by (b) (4) Method

Citrate is an excipient in fibrinogen component of fibrin sealant drug product. The specification in fibrinogen is (b) (4) for lot release.

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Regarding your testing instruction document IG_MA-000170B_ING, please provide the details of composition of the control sample used to assess the validity of citrate assay.

Review of response: In response, the sponsor stated that the in-house anti-thrombin secondary standard was used as an assay control. It is not clear how an anti-thrombin standard can be used as control for the citrate assay. The sponsor needs to clarify and provide the control qualification data. Therefore, another IR was generated for the sponsor.

B. Regarding the Method validation report, document IG_MA-000381_ING:

i. You have demonstrated linearity and accuracy of your assay using the data obtained from citrate standards only. Please provide data for the validation characteristics using representative fibrinogen drug product samples. Also, for linearity, please include an assessment of parallelism between the standard and sample regression lines of the plots of analyte concentration (or dilution) versus response.

Review of response: In response to CBER IR, the sponsor provided additional linearity and accuracy data obtained from the fibrinogen product sample. Linearity was assessed at (b) (4) concentration levels of citrate in the range of (b) (4). Three independent linearity runs were analyzed. A mean correlation coefficient (R) of (b) (4) was obtained, which met the pre-defined acceptance criteria for R to be (b) (4). The slope ratio of standard vs sample regression lines were close to (b) (4), and can be regarded as parallel. Accuracy was assessed in three separate assays at (b) (4) concentration levels of citrate in the range of (b) (4). The pre-set acceptance criteria for recovery was (b) (4), and the actual recovery varied from (b) (4). Thus, the range of the method was successfully demonstrated as (b) (4) of citrate.

ii. For your specificity study, please provide results obtained with fibrinogen drug product sample formulated with all other excipients, except citrate, and a comparison of the results obtained with the actual representative drug product formulation that contains citrate.

Review of response: In response, the sponsor explained that sodium citrate was used (b) (4) steps, and was present in (b) (4). Thus, it was not possible to obtain the sample without this excipient. Considering the specificity data and the additional linearity and accuracy data, submitted earlier by the sponsor, and the lack interference by excipients (other than citrate) and active component, no further IR is required.

iii. Please provide the results of the robustness evaluation for your assay.

Review of response: In response, the sponsor submitted the report IG_ITEC-002979_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4)

Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of results of citrate concentration in the control. The effect of above factors was statistically insignificant. The sponsor did not provide the results from drug product samples, however, the coupled (b) (4) for the estimation of citrate is specific and the validation results have clearly demonstrated that the matrix interference is negligible. Further, the control is run with every assay, and a significant effect due to the variation of analytical condition would also invalidate the control results. Therefore, the results are satisfactory to demonstrate method robustness.

Second Information request: Following the review of the first IR, another IR was submitted to the sponsor to provide the qualification details of the control sample used in the assay. This IR was sent on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

Review of response: In response the sponsor submitted the qualification report for the control sample that was currently in use. The sample was prepared from commercial available (b) (4). The citrate concentration in the control was established from the results obtained from (b) (4) laboratories using three different lots of (b) (4), followed by statistical analysis to define the control limits. The sponsor's qualification data is adequate.

The response to this IR has not been received yet.

Conclusion: Based on the review of submitted documents and response to our information requests, it is concluded that the method for the determination of citrate content in fibrinogen product is adequately validated, and can be used as a lot-release test.

4. Determination of Chloride by (b) (4) method

Chloride is an excipient in fibrinogen component of fibrin sealant drug product. The specification in fibrinogen is (b) (4) for lot release.

Method

(b) (4)

Method Validation

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Section 6.0 of your SOP (document IG_MA-000016A_ING) includes the assay validity criteria based on the control. Please provide the composition of the control sample.

Review of response: In response, the sponsor stated that the in-house anti-thrombin secondary standard was used as an assay control. It is not clear how an anti-thrombin standard can be used as control for the chloride assay. The sponsor needs to clarify and provide the control qualification data. Therefore, another IR was generated for the sponsor.

B. Please provide the results of the robustness evaluation for your assay.

Review of response: In response, the sponsor submitted the report IG_ITEC-003015_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4) . Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of results of chloride concentration in the control. The effects of the above factors were statistically insignificant. The sponsor did not provide the results from drug product samples, however, the method for the quantitation of chloride using (b) (4) is specific for chloride and the validation results have clearly demonstrated that the matrix interference is negligible. Further, the control is run with every assay, and any effect due to the variation of analytical condition would also invalidate the control results. Therefore, no further data is required.

Second Information request: The following IR was submitted to the sponsor 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

A. With regard to the Method validation report, Document IG_IVMA-000367_ING:

- i. You have demonstrated linearity and accuracy of your assay using the data obtained from chloride standards only. Therefore, you have not validated the assay adequately. Please provide linearity and accuracy data using representative fibrinogen samples. Also, for linearity please include an assessment of parallelism between the standard and sample regression lines.

Review of response: In response to CBER IR, the sponsor provided additional linearity and accuracy data obtained from the fibrinogen product sample. Linearity was assessed at (b) (4) concentration levels of chloride using the sodium chloride standard in the range of (b) (4) and using the fibrinogen product in the range of (b) (4). Three independent linearity runs were analyzed. A mean correlation coefficient (R) of (b) (4) was obtained for standard and fibrinogen samples. The pre-defined acceptance criterion for R was (b) (4). The slope ratio of standard vs sample regression lines were close to 1 for all three assays, indicating negligible interference from the matrix components. Accuracy was assessed from the three linearity runs. The results met the pre-set acceptance criteria of (b) (4) for recovery. Thus, the range of the method was successfully demonstrated as (b) (4) for the quantitation of chloride in fibrinogen samples.

- ii. Please provide data to show that the control sample used in each of the above assay is adequately qualified or standardized in your laboratory against an appropriate primary or secondary standard.

Review of response: In response the sponsor submitted the qualification report for the control sample that was currently in use. The sample was prepared from commercial available sodium chloride, (b) (4). The chloride concentration in the control was qualified against chloride volumetric standard from (b) (4). The control limits were established from the results obtained from (b) (4) laboratories using different lots of (b) (4), followed by statistical analysis. The sponsor's qualification data is adequate.

Conclusion: The method is has been adequately described and validated, and is acceptable as a lot-release test for the determination of chloride content in the fibrinogen component of fibrin sealant drug product.

5. Determination of Polysorbate 80 by (b) (4) method

Polysorbate 80 (Tween 80) is added during the fibrinogen manufacturing process to inactivate lipid-enveloped viruses and is substantially removed by the subsequent chromatography steps.

Thus, this is an assay for process-related impurity. The specification limit in fibrinogen component of fibrin sealant drug product is (b) (4).

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Regarding your testing instruction document IG_MA-000403C_ING: Please provide composition of the control sample mentioned in Section 4.2 of your test method SOP.

Review of response: In response, the sponsor provided the composition of the in-house secondary standard. To prepare this control, the sponsor diluted the commercial polysorbate 80 standard to a final concentration of (b) (4). However, the sponsor did not submit the qualification data, hence another IR was sent to the sponsor.

B. Regarding the Method validation report, document IG_IVMA- FGD1403C_ING:

- i. For your linearity studies, you have evaluated the results obtained using polysorbate 80 standard, but not the fibrinogen drug product sample. Please provide linearity data for your fibrinogen product over the proposed assay range, and demonstrate parallelism between the plots of analyte concentration (or dilution) versus response for the standard and your drug product.

Review of response: The sponsor provided additional linearity data wherein linearity was estimated from three independent runs of fibrinogen product samples, containing polysorbate 80 in the range of (b) (4). A mean correlation coefficient R of (b) (4) was obtained, which met the acceptance criteria of (b) (4). There was no significant difference in the slopes of standard and samples, and the ratio was close to 1 for all the three linearity runs. Thus, based on the submitted linearity, accuracy and precision data, the assay is suitable for quantitation of polysorbate 80 in the range of (b) (4).

- ii. You have concluded that the LOQ of the assay is (b) (4), based on polysorbate 80 standard curve. However, you have not provided accuracy, precision, and linearity data at LOQ. Please provide accuracy, precision, and linearity data from fibrinogen samples to support the LOQ of your assay.

Review of response: In response, the sponsor clarified that (b) (4) polysorbate 80/mL is the theoretical LOQ value. The experimental estimate is (b) (4), as evaluated from the linearity, accuracy and precision study. The sponsor's response is acceptable.

iii. Please provide the robustness data to show that your method is not susceptible to deliberate variations in analytical conditions.

Review of response: In response, the sponsor submitted the report IG_ITEC-002991_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of results of polysorbate 80 concentration in the control. The effects of above factors were statistically insignificant. The sponsor did not provide the results from drug product samples, however, as per the test method, the samples are prepared (b) (4) and the validation results have clearly demonstrated that the interference from other matrix components is negligible. Further, the control is run with every assay, and any effect due to the variation of analytical condition would also invalidate the control results. Therefore, no further data is required.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

A. With regard to the Method validation report, Document IG_IVMA-000367_ING:

i. Please provide data to show that the control sample used in each of the above assay is adequately qualified or standardized in your laboratory against an appropriate primary or secondary standard.

Review of response: In response the sponsor submitted the qualification report for the control sample that was currently in use. The sample was prepared by diluting commercially available polysorbate 80 secondary standard with (b) (4) to obtain a final concentration of (b) (4) of Tween 80 and (b) (4). The starting polysorbate 80 concentration of commercially available standard was accepted from its COA. The control limits were established from the results obtained from (b) (4) independent assays, followed by statistical analysis. The sponsor's qualification data is adequate.

Conclusion: Based on the review of submitted documents and response to our information requests, it is concluded that the method for the determination of polysorbate80 content in fibrinogen product is adequately validated, and can be used as a lot-release test.

6. Determination of Tri-n-Butyl Phosphate (TNBP) by (b) (4)

TNBP is added together with Polysorbate 80 (Tween 80) during the fibrinogen manufacturing process to inactivate lipid-enveloped viruses and is substantially removed by the subsequent chromatography steps. Thus, this is an assay for process-related impurity. The specification limit in fibrinogen component of fibrin sealant drug product is (b) (4) .

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 on 25 May 2017, is discussed below.

A. Regarding your method SOP IG_MA-000281A_ING, please provide composition of the sample used as an assay control.

Review of response: In response, the sponsor informed that the assay control is a (b) (4) sample that also contains (b) (4).

However, the sponsor did not submit the qualification data, hence another IR was sent to the sponsor.

B. Regarding the Method validation report, document IG_IVMA- 000261_ING:

i. For your specificity study, please provide (b) (4) of TNBP (b) (4) from the drug product and reference standard to establish (b) (4) and method specificity.

Review of response: In response to CBER IR, the sponsor provided additional specificity data wherein the fibrinogen product sample was analyzed in (b) (4). The (b) (4) of TNBP (b) (4) was approx. (b) (4), and was same as the (b) (4) of TNBP (b) (4) in the standard solution. Thus, identity of TNBP (b) (4) and method's specificity was sufficiently demonstrated.

ii. You have not provided accuracy data to support accuracy of your method at the upper specification limit of (b) (4). Please provide accuracy data at this concentration level.

Review of response: The sponsor did not provide the requested data. Another IR was submitted to the sponsor to address this deficiency.

- iii. You have calculated the LOQ of your assay as (b) (4) based on TNBP standard curve. However, you have not provided accuracy, precision, and linearity data at LOQ in your validation report. Please provide accuracy, precision, and linearity data from fibrinogen samples to support the LOQ of your assay.

Review of response: In response, the sponsor stated that (b) (4) is the theoretical LOQ value. The lowest reportable level at which linearity, accuracy and precision has been demonstrated is (b) (4).

- iv. Please provide robustness data for your method by evaluating the effect of variation of different (b) (4) parameters.

Review of response: In response, the sponsor submitted the report IG_ITEC-002086_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4)

(b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of resolution between the TNBP and (b) (4) for the standard at the lowest concentration of the calibration curve. The effect of variation of (b) (4) was statistically significant for some samples ((b) (4)), and therefore, non-significant intervals were determined for this factor, (b) (4) was obtained was strictly defined and incorporated in the experimental procedure.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

A. With regard to the Method validation reports for the determination of TNBP in fibrinogen product (Document IG_IVMA-000261_ING) and thrombin product (Document IG_IVMA-000237_ING):

- i. In response to Information Request dated May 05, 2017, regarding the robustness of the assay, you have submitted data wherein the effects of analytical parameters were evaluated by calculating the resolution between the TNBP and (b) (4), using calibration standards only. Please provide data for demonstrating robustness of the assay by evaluating the effect of variation of (b) (4) parameters on TNBP results from your fibrinogen and thrombin products.

Review of response: The sponsor stated that the system suitability results obtained for each (b) (4) condition studied are sufficient for evaluating the robustness of the method. The sponsor referred to compendia (USP and EP) guidance's for studying robustness in support of their statement. Based on the results obtained during the robustness study, and compendia guidance's, no further IR is required.

- ii. You have concluded that the LOQ of the assay is (b) (4) based on the analysis of the standard. However, you did not provide the data from fibrinogen and thrombin product samples in support of LOQ of this assay. Please provide linearity and accuracy data using the drug product to show that (b) (4) is the LOQ of your assay.

Review of response: In response to CBER IR, the sponsor submitted additional data wherein linearity was estimated from the precision results of fibrinogen sample (submitted as a part of original validation). For linearity, three independent intermediate precision runs were evaluated at TNBP concentrations ranging from (b) (4). A mean correlation coefficient (R) of (b) (4) was obtained, which met the pre-defined acceptance criteria for R to be (b) (4). For Accuracy, the sponsor referred to the original validation. None of these studies were conducted to cover the specification limit of (b) (4). Therefore, another IR was sent to the sponsor to submit the required linearity and accuracy data. Further, the sponsor's experimental LOD and LOQ value of (b) (4), as obtained in the additional validation studies was represented incorrectly as (b) (4), based on the lowest concentration evaluated using the TNBP standards.

- iii. Please provide data to show that the control sample used in each of the above assay is adequately qualified or standardized in your laboratory against an appropriate primary or secondary standard.

Review of response: In response the sponsor submitted the qualification report for the control that was currently in use. The sample was prepared from the in-process sample obtained during the manufacturing of another product by the company. The TNBP control was qualified against (b) (4) different lots of a secondary TNBP standard with the use of (b) (4) and three lots of (b) (4). The control limits were established from the results obtained from (b) (4) independent assays, followed by statistical analysis. The sponsor's qualification data is adequate.

Third Information request: The following IR was submitted to the sponsor on 29 August 2017. The response to the third IR was not received at the time of writing this memo.

We have the following question/comment regarding the Method validation reports for the determination of TNBP in fibrinogen product (Document IG_IVMA-000261_ING) and

thrombin product (Document IG_IVMA-000237_ING): In your method validation for the TNBP assay, the range of the assay as based on linearity, accuracy and precision results is (b) (4) for fibrinogen product and (b) (4) for thrombin product. Since TNBP is present as an impurity in your product, it is critical to have an assay range that includes the upper specification limit of (b) (4). Please provide linearity and accuracy from fibrinogen and thrombin samples to show that TNBP can be quantitated at the proposed upper specification limit of the assay.

The response to this IR has not been received yet.

Conclusion: The method is clearly described in the SOP. However, there are minor outstanding issues with the method validation as discussed in the third IR.

7. Sodium Determination by (b) (4)

The specification for sodium in fibrinogen is (b) (4) for lot release.

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Regarding your testing instruction document SOP IG_MA-000005A_ING: Please revise your SOP to include a detailed description or composition of the sodium secondary standard used as an assay control and submit for review.

Review of response: In response, the sponsor informed that the assay control is a qualified batch of albumin secondary standard. The standard mixture consists of ^{(b) (4)} albumin, (b) (4) of sodium and (b) (4). The sponsor did not submit the qualification data, hence another IR was sent to the sponsor.

B. Regarding the Method validation report, document IG_IVMA- 000373_ING:

- i. The linearity and accuracy of your assay was validated with the use of sodium standards only. Please provide data on linearity and accuracy using actual drug product samples at concentration levels covering the range of the assay.

Review of response: In response to CBER IR, the sponsor provided additional linearity and accuracy data obtained from the fibrinogen product sample. Linearity was estimated from ^{(b) (4)}

concentration levels of sodium in the range of (b) (4). Three independent linearity runs were performed. A mean correlation coefficient (R) of (b) (4) was obtained, which met the pre-defined acceptance criteria for R to be (b) (4). We evaluated the slope ratio of standard vs sample regression lines and found the value close to 1 for all three assays, indicating parallelism of the linear regression plots. Accuracy was assessed in three separate assays at (b) (4) concentration levels of sodium in the range of (b) (4). The pre-set acceptance criteria for recovery was (b) (4), and the actual recoveries varied from (b) (4). Thus, the range of the method was demonstrated as (b) (4) of sodium.

- ii. Please provide data obtained from fibrinogen drug product samples prepared without sodium-containing excipients (e.g., replacing sodium by (b) (4)) to substantiate your conclusion that the method is specific.

Review of response: In response, the sponsor explained that sodium is widely used throughout the purification process as sodium chloride, sodium citrate and monosodium glutamic acid. After taking into account, the purification process, it is not possible to obtain the drug product sample without sodium or by replacing sodium with (b) (4). Considering the specificity data submitted earlier by the sponsor, and the additional linearity and accuracy data, no further IR is required.

- iii. Please provide robustness data for your method by evaluating the effect of variation of different operating parameters of your assay.

Review of response: In response, the sponsor submitted the report IG_ITEC-002978_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of results of sodium concentration in the control. The effect of above factors was statistically insignificant. The sponsor did not provide the results from drug product samples, however, the method for the quantitation of sodium using (b) (4) is specific and the validation results have clearly demonstrated that the matrix interference is negligible. Further, the control is run with every assay, and any effect due to the variation of analytical condition would also invalidate the control results. Therefore, no further data is required.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

- i. Please provide data to show that the control sample used in each of the above assay is adequately qualified or standardized in your laboratory against an appropriate primary or secondary standard.

Review of response: In response the sponsor submitted the qualification report for the control. The control was prepared by diluting commercially available sodium chloride (b) (4) with (b) (4) albumin. The sodium concentration in the control was qualified against the (b) (4) calibration standard. The control limits were established from the results obtained from two laboratories, followed by statistical analysis. The sponsor's qualification data is adequate.

Conclusion: The method is has been adequately described and validated, and is acceptable as a lot-release test for the determination of sodium content in the fibrinogen component of fibrin sealant drug product.

8. Appearance of Frozen Product

The specification for appearance of frozen fibrinogen product is colorless or pale yellow and opaque solid.

Method

The frozen material is visually examined for color and transparency, as described in (b) (4). Visual inspection is appropriate to verify appearance of frozen product, and validation of this method is not necessary.

Conclusion

The assay is approvable as a release test for fibrinogen component of fibrin sealant drug product.

9. Appearance of Solution after Thawing

The specification for appearance of fibrinogen solution after thawing is colorless or pale yellow solution.

Method

The frozen fibrinogen sample is thawed at 37 °C. Appearance of the solution is examined visually for color in accordance with (b) (4). Visual inspection is appropriate to verify the appearance of solution after thawing, and validation of this method is not necessary.

Conclusion

The assay is approvable as a release test for fibrinogen component of fibrin sealant drug product.

10. pH

The pH specification for fibrinogen component of fibrin sealant drug product is 6.5-8.0.

Method

The pH of the (b) (4) is measured using a pH meter. Reference pH buffers ((b) (4)) are used to calibrate the pH meter. The method is compliant to the EP and USP test methods for pH determination. The method was not validated. However, measurement of pH is a widely used method and known to be dependent only on the hydrogen ion concentration, and is unaffected by other matrix components in aqueous solution. Hence, no validation should be necessary. The pH results of fibrinogen lots manufactured at the clinical and commercial scale during the scale-up of production process, and fibrinogen lots submitted for the stability studies were within the required specification limit.

Conclusion

This is a well-established method. Further information is not required. The assay is approvable as a release test for fibrinogen component of fibrin sealant drug product.

Thrombin Component

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

Glycine is an excipient in thrombin component of fibrin sealant drug product, and its specification is set at (b) (4).

Method

(b) (4)



(b) (4)

Method Validation

(b) (4)

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 on 25 May 2017, is discussed below.

A. For the (b) (4) procedure described in your SOP IG_MA-000358C_ING, please provide: (1) the composition of (b) (4); (2) the (b) (4); (3) (b) (4)

(b) (4) ; and (4) composition of the control sample and the details of the qualification of the control.

Review of response: In response, the sponsor provided the details of the (b) (4) procedure used for the quantitation of amino acids. (b) (4)

(b) (4) The sample used as an assay control was a qualified lot of product other than thrombin manufactured by the sponsor, and was standardized against commercially available standards for glutamic acid, arginine, glycine and isoleucine.

B. Regarding the Method validation report, document IG_IVMA- FGD1358A_ING:

- i. Please provide a representative (b) (4) of formulated thrombin product, and (b) (4) of glycine in thrombin product and glycine standard solution to establish (b) (4) and specificity of your assay.

Review of response: In response to CBER IR, the sponsor provided additional specificity data wherein the thrombin product sample was analyzed in (b) (4) . The (b) (4) of glycine was significantly different ((b) (4)) as compared to the (b) (4) observed in glycine standard solution ((b) (4)). Thus, another IR was submitted to the sponsor to explain this disparity.

- ii. Please establish range of your assay based on the linearity, accuracy, and precision data obtained from thrombin samples, and update your validation report accordingly.

Review of response: The sponsor submitted additional linearity data as Amendment 28. Linearity was estimated from (b) (4) concentrations of thrombin sample, and by testing each concentration in three independent assays. The response was linear between (b) (4) of glycine. The mean correlation coefficient (R) met the acceptance criterion of (b) (4) for three independent runs. Accuracy was assessed from the three assay runs in the range of (b) (4) of glycine, and the recovery was within the acceptable range of (b) (4) . Thus, the validated range of the assay is from (b) (4) , as evaluated from the linearity/accuracy data (submitted as amendment 28) and precision data (submitted as part of original submission). The assay is acceptable for the quantitation of glycine in thrombin product.

- iii. Please provide robustness data by evaluating the effect of deliberate variations of critical method parameters.

Review of response: In response, the sponsor submitted the report IG_ITEC-002115_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4)

(b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The evaluated variations did not affect resolution between the (b) (4) arginine (b) (4) at the lowest concentration of the standard used in the calibration curve. The effect of variation of initial (b) (4) was statistically significant, and therefore, non-significant intervals were determined for this factor, and the results were incorporated in the experimental procedure. The sponsor did not provide the results from drug product samples, however, they referred to compendia (USP and EP) guidance's for studying robustness of the method. Additionally, according to ICH (Q2) R1, for the evaluation of robustness of a (b) (4) method, a series of system suitability parameters should be established to ensure that the validity of the method is not susceptible to variations in analytical conditions. Based on the results obtained during the robustness study, and compendia guidance's, no further IR is required.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

A. With regard to the Method validation report, Document IG_IVMATHROMB358A_ING:

- i. The glycine (b) (4) obtained from the standard and product samples are (b) (4) (original submission) and (b) (4) (response to Information Request dated May 05, 2017), respectively. These two (b) (4) are significantly different. Please explain the discrepancy and provide justification for confirmation of glycine identity from your results.

Review of response: The sponsor explained that the difference in glycine (b) (4) observed is due to the fact that (b) (4) can be used for the analytical method IG_MA-000358A_ING and (b) (4) were used during the validation study. Each (b) (4) has a characteristic (b) (4). In the (b) (4) obtained from (b) (4), the glycine (b) (4), and in the (b) (4) obtained from (b) (4), the glycine (b) (4). In the Response to Information Request dated May 25, 2017, the additional (b) (4) for thrombin sample is obtained from (b) (4). In this case glycine (b) (4) and (b) (4). Thus, the sponsor's has adequately demonstrated the (b) (4) of glycine (b) (4) in thrombin samples.

Conclusion: The method is has been adequately described and validated, and is acceptable as a lot-release test for the determination of glycine content in the thrombin component of fibrin sealant drug product.

12. (b) (4) Determination by (b) (4) Method

(b) (4) is a process residual in thrombin component of fibrin sealant drug product. The specification in thrombin is (b) (4) for lot release.

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)



First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Regarding your testing instruction document IG_MA-000456A_ING, please provide the details of composition of the control sample used to assess the validity of (b) (4) assay.

Review of response: In response, the sponsor stated that the in-house anti-thrombin secondary standard was used as the assay control. It is not clear how an anti-thrombin standard can be used as control for the (b) (4) assay. The sponsor needs to clarify and provide the control qualification data. Therefore, another IR was generated for the sponsor.

B. Regarding the Method validation report, document IG_IVMA- THROMB456A_ING:

- i. You have demonstrated linearity of your assay using the data obtained from (b) (4) standards only. Please provide linearity data and plots of analyte concentration (or dilution) versus response using representative thrombin samples, and include an assessment of parallelism between the standard and sample regression lines.

Review of response: In response to CBER IR, the sponsor provided additional linearity data obtained from the thrombin product sample. Linearity was assessed at (b) (4) concentration levels of (b) (4) in the range of approx. (b) (4). Three independent linearity runs were analyzed. A mean correlation coefficient (R) of (b) (4) was obtained, which met the pre-defined acceptance criteria for R to be (b) (4). The slope ratio of standard vs sample regression lines were close to (b) (4) for all three assays, and which met the criteria of parallelism. Although not specified by the sponsor, the lowest amount of analyte ((b) (4)) in the sample which could be quantitatively determined with suitable precision and accuracy was (b) (4). Thus, based on the linearity (data submitted as amendment 27), accuracy and precision (data submitted in the original submission), the range of the method was successfully demonstrated as (b) (4). The experimental LOQ of the assay based from the submitted data from thrombin samples, is (b) (4).

- ii. For your specificity study, please provide results obtained from thrombin drug product sample formulated with all other excipients, except acetate.

Review of response: In response, the sponsor referred to the data submitted for the drug product (formulated product (b) (4)). The results of the thrombin sample as evaluated in the intermediate precision studies were below the quantifiable range of the calibration curve. Additionally, as per the linearity and accuracy studies, no significant interference from matrix was observed. Therefore, the specificity of the assay is acceptable, and no further IR is required.

- iii. You have not submitted the robustness data for your method. Please provide the results to permit a complete review of your assay.

Review of response: In response, the sponsor submitted the report IG_ITEC-002980_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4)

(b) (4) Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of results of (b) (4) concentration in the control. The effect of above factors was statistically insignificant. The sponsor did not provide the results from drug product samples, however, the (b) (4) for the estimation of (b) (4) is very specific and the validation results have clearly demonstrated that the matrix interference is negligible. Further, the control is run with every assay, and any effect due to the variation of analytical condition would also invalidate the control results. Therefore, no further data is required.

Second Information request: Following the review of the first IR, another IR was submitted to the sponsor to provide the qualification details of the control sample used in the assay. This IR was sent on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

Review of response: In response the sponsor submitted the qualification report for the control that was currently in use. Another product, (b) (4), manufactured by the sponsor was used as a control. The (b) (4) concentration in the control was established from the results obtained from (b) (4), followed by statistical analysis to define the control limits. The sponsor's qualification data is adequate.

Conclusion: Based on the review of submitted documents and response to our information requests, it is concluded that the method for the determination of (b) (4) content in thrombin product is adequately validated, and can be used as a lot-release test.

13. Determination of Chloride by (b) (4) method

Chloride is an excipient in thrombin component of fibrin sealant drug product. The specification in thrombin is (b) (4) for lot release.

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)

[Redacted]

[Redacted]

[Redacted]

[Redacted]

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Regarding your testing instruction document IG_MA-000016A_ING, Section 6.0 includes assay validity criteria based on the control. Please provide the composition of the control sample.

Review of response: In response, the sponsor stated that the in-house anti-thrombin secondary standard was used as an assay control. The standard solution contains (b) (4), sodium chloride and sodium citrate (b) (4). However, the sponsor did not provide the control qualification data. Therefore, another IR was generated for the sponsor.

B. Regarding the Method validation report, document IG_MA-000374_ING:

- i. Please provide data to show linearity and accuracy of chloride response in your thrombin drug product.

Review of response: In response to CBER IR, the sponsor provided additional linearity and accuracy data obtained from the thrombin product sample. Linearity was assessed at (b) (4) concentration levels of chloride using the sodium chloride standard in the range of (b) (4) and using the thrombin product in the range of (b) (4). Three independent linearity runs were analyzed. A mean correlation coefficient (R) of (b) (4) were obtained for the standard and thrombin samples respectively. The pre-defined acceptance criterion for R was (b) (4). The slope ratio of standard vs sample regression lines were close to 1 for all three assays, indicating parallelism and negligible interference from the matrix components. Accuracy was assessed in three separate assays at (b) (4) concentration levels of thrombin product in the range of (b) (4). The pre-set acceptance criteria for recovery was (b) (4), and the actual recovery varied from (b) (4). Thus, the range of the method was successfully demonstrated as (b) (4) for the quantitation of chloride in thrombin samples.

- ii. Please provide the data obtained from thrombin drug product formulated without chloride containing excipients to demonstrate method specificity.

Review of response: In response, the sponsor explained that chloride as sodium and calcium salts was used in several (b) (4) steps and in the final formulated product. Therefore, it was not possible to obtain the sample without this excipient. Considering the specificity data submitted earlier by the sponsor, and the additional linearity and accuracy data, the interference of excipients (other than chloride) and active component is negligible, therefore no further IR is required.

- iii. For your method robustness, please provide details of the parameters varied and their effect on chloride concentration

Review of response: In response, the sponsor submitted the report IG_ITEC-003015_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of results of chloride concentration in the control. The effect of above factors was statistically insignificant. The sponsor did not provide the results from drug product samples, however, the method for the quantitation of chloride using (b) (4) solution is very specific and the validation results have clearly demonstrated that the matrix interference is negligible. Further, the control is run with every assay, and any effect due to the variation of analytical condition would also invalidate the control results. Therefore, no further data is required.

Second Information request: Following the review of the first IR, another IR was submitted to the sponsor to provide the qualification details of the control sample used in the assay. This IR was sent on 10 July 2017. The response by Grifols received as Amendments 33 on 5 Aug 2017, is discussed below.

Review of response: In response the sponsor submitted the qualification report for the control sample that was currently in use. The sample was prepared from commercial available sodium chloride, (b) (4). The chloride concentration in the control was qualified against chloride volumetric standard from (b) (4). The control limits were established from the results obtained from (b) (4) laboratories using different lots of (b) (4) solution, followed by statistical analysis. The sponsor's qualification data is adequate.

Conclusion: The method is has been adequately described and validated, and is acceptable as a lot-release test for the determination of chloride content in the thrombin component of fibrin sealant drug product.

14. Determination of Polysorbate 80 by (b) (4) method

Polysorbate 80 (Tween 80) is added during the thrombin manufacturing process to inactivate lipid-enveloped viruses and is substantially removed by the subsequent chromatography steps. Thus, this is an assay for process-related impurity. The specification limit in thrombin component of fibrin sealant drug product is (b) (4).

Method

(b) (4)



Method Validation

(b) (4)



(b) (4) [Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

- A. Regarding your testing instruction document IG_MA-000403C_ING: Please provide composition of the control sample mentioned in Section 4.2 of your test method SOP.

Review of response: In response, the sponsor provided the composition of the in-house secondary standard. To prepare this control, the sponsor had diluted the commercial polysorbate 80 standard to a final concentration of (b) (4) in (b) (4) albumin. However, the sponsor did not submit the qualification data, hence another IR was sent to the sponsor.

B. Regarding the Method validation report, document IG_IVMA- FGD1403C_ING:

- i. For your linearity studies, you have evaluated the results obtained using polysorbate 80 standard, but not the thrombin drug product sample. Please provide linearity data for your thrombin product over the proposed assay range, and demonstrate parallelism between the plots of analyte concentration (or dilution) versus response for the standard and your drug product.

Review of response: The sponsor provided additional linearity data from three independent runs of thrombin product samples, containing polysorbate 80 in the range of (b) (4). A mean correlation coefficient R of (b) (4) was obtained, which met the acceptance criteria of (b) (4). There was no significant difference in the slopes of standard and samples, and the ratio was close to (b) (4) for all the three linearity runs. Thus, based on the submitted linearity, accuracy and precision data, the assay is suitable for quantitation of polysorbate 80 in the range of (b) (4).

- ii. You have concluded that the LOQ of the assay is (b) (4), based on polysorbate 80 standard curve. However, you have not provided accuracy, precision, and linearity data at LOQ. Please provide accuracy, precision, and linearity data from thrombin samples to support the LOQ of your assay.

Review of response: In response, the sponsor clarified that (b) (4) polysorbate 80/mL is the theoretical LOQ value. The experimental estimate is (b) (4), as evaluated from the linearity, accuracy and precision study. Considering that the sponsor has established a higher LOQ value, than the experimentally obtained result of (b) (4), as a more conservative estimate, the response is acceptable.

- iii. Please provide the robustness data to show that your method is not susceptible to deliberate variations in analytical conditions.

Review of response: In response, the sponsor submitted the report IG_ITEC-002991_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4)

(b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4) (b) (4)). The influence of variation was evaluated in terms of results of polysorbate 80 concentration in the control. The effect of above factors was statistically insignificant. The sponsor did not provide the results from drug product samples, however, as per the test method, the samples are prepared (b) (4) (b) (4) and the validation results have clearly demonstrated that the interference

from other matrix components or excipients is negligible. Further, the control is run with every assay, and any effect due to the variation of analytical condition would also invalidate the control results. Therefore, no further data is required.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

A. With regard to the Method validation report, Document IG_IVMA-000367_ING:

- i. Please provide data to show that the control sample used in each of the above assay is adequately qualified or standardized in your laboratory against an appropriate primary or secondary standard.

Review of response: In response the sponsor submitted the qualification report for the control sample that was currently in use. The sample was prepared by diluting commercially available polysorbate 80 secondary standard with albumin to obtain a final concentration of (b) (4) of Tween 80 and (b) (4) albumin. The starting polysorbate 80 concentration of commercially available standard was taken from its COA. . The control limits were established from the results obtained from (b) (4) independent assays, followed by statistical analysis. The sponsor's qualification data is adequate.

Conclusion: Based on the review of submitted documents and response to our information requests, it is concluded that the method for the determination of polysorbate 80 content in thrombin product is adequately validated, and can be used as a lot-release test.

15. Determination of Tri-n-Butyl Phosphate (TNBP) by (b) (4)

TNBP is added together with Polysorbate 80 (Tween 80) during the thrombin manufacturing process to inactivate lipid-enveloped viruses and is substantially removed by the subsequent chromatography steps. Thus, this is an assay for process-related impurity. The specification limit in thrombin component of fibrin sealant drug product is (b) (4) .

Method

(b) (4)



(b) (4)

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Method Validation

(b) (4)

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(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Regarding your method SOP IG_MA-000281A_ING, please provide composition of the sample used as an assay control.

Review of response: In response, the sponsor informed that the assay control is a qualified batch of (b) (4) sample that also contains (b) (4) at a concentration of (b) (4). However, the sponsor did not submit the qualification data, hence another IR was sent to the sponsor.

B. Regarding the Method validation report, document IG_IVMA- 000261_ING:

i. For your specificity study, please provide (b) (4) of TNBP (b) (4) from the drug product and reference standard to establish (b) (4) and method specificity.

Review of response: In response to CBER IR, the sponsor provided additional specificity data wherein the thrombin sample was analyzed in (b) (4). The (b) (4) of TNBP (b) (4) was approx. (b) (4), and was same as the (b) (4) of TNBP (b) (4) in the standard solution. Thus, identity of TNBP (b) (4) and method's specificity was sufficiently demonstrated.

ii. You have not provided accuracy data to support accuracy of your method at the upper specification limit of (b) (4). Please provide accuracy data at this concentration level.

Review of response: The sponsor did not provide the requested data. The sponsor had evaluated accuracy in the range of (b) (4), and this range does not include the upper specification limit of (b) (4). Another IR was submitted to the sponsor to address this deficiency.

iii. You have calculated the LOQ of your assay as (b) (4) based on TNBP standard curve. However, you have not provided accuracy, precision, and linearity data at LOQ in your validation report. Please provide accuracy, precision, and linearity data from thrombin samples to support the LOQ of your assay.

Review of response: In response, the sponsor stated that (b) (4) is the theoretical LOQ value. The lowest reportable level at which linearity, accuracy and precision has been demonstrated is (b) (4). Thus, the experimental LOQ value is (b) (4). However,

the sponsor did not include the data from thrombin samples to support this result. Another IR was submitted to the sponsor to submit the required data.

- iv. Please provide robustness data for your method by evaluating the effect of variation of different (b) (4) parameters.

Review of response: In response, the sponsor submitted the report IG_ITEC-002086_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4)

(b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of resolution between the TNBP (b) (4) for the lowest standard used in the preparation of calibration curve. The effect of variation of (b) (4) (b) (4) was statistically significant, and therefore, non-significant intervals were determined for this factor, and the information was incorporated in the experimental procedure. The sponsor did not provide the results from drug product samples, hence another IR was issued for the sponsor.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

A. With regard to the Method validation reports for the determination of TNBP in fibrinogen product (Document IG_IVMA-000261_ING) and thrombin product (Document IG_IVMA-000237_ING):

- i. In response to Information Request dated May 05, 2017, regarding the robustness of the assay, you have submitted data wherein the effects of analytical parameters were evaluated by calculating the resolution between the TNBP (b) (4) (b) (4), using calibration standards only. Please provide data for demonstrating robustness of the assay by evaluating the effect of variation of (b) (4) (b) (4) parameters on TNBP results from your fibrinogen and thrombin products.

Review of response: The sponsor stated that the system suitability results obtained for each (b) (4) condition studied are sufficient for evaluating the robustness of the method. The sponsor referred to compendia (USP and EP) guidances for studying robustness in support of their statement. Based on the results obtained during the robustness study, and compendia guidance's, no further IR is required.

- ii. You have concluded that the LOQ of the assay is (b) (4) (b) (4) based on the analysis of the standard. However, you did not provide the data from fibrinogen and thrombin product samples in support of LOQ of this assay. Please provide linearity and accuracy data using the drug product to show that (b) (4) (b) (4) is the LOQ of your assay.

Review of response: In response to CBER IR, the sponsor submitted additional data wherein linearity was estimated from the precision results of thrombin sample (submitted as a part of original validation). For linearity, three independent intermediate precision runs were evaluated at TNBP concentrations ranging from (b) (4). A mean correlation coefficient (R) of (b) (4) was obtained, which met the pre-defined acceptance criteria for R to be (b) (4). For Accuracy, the sponsor also referred to the original validation. None of these studies were conducted to cover the specification limit of (b) (4). Therefore, another IR was sent to the sponsor to submit the required linearity and accuracy data. Further, the sponsor's experimental LOD and LOQ value of (b) (4) as obtained in the additional validation studies was represented incorrectly as (b) (4) at the lowest concentration of the TNBP standards.

iii. Please provide data to show that the control sample used in each of the above assay is adequately qualified or standardized in your laboratory against an appropriate primary or secondary standard.

Review of response: In response the sponsor submitted the qualification report for the control sample that was currently in use. The sample was prepared from the in-process sample obtained during the manufacturing of another product by the company. The TNBP control was qualified against (b) (4) different lots of in house qualified secondary TNBP standard with the use of (b) (4) and three lots of (b) (4) used in the (b) (4). The control limits were established from the results obtained from (b) (4) independent assays, followed by statistical analysis. The sponsor's qualification data is adequate.

Third Information request: The following IR was submitted to the sponsor on 29 August 2017. The response to the third IR was not received at the time of writing this memo.

We have the following question/comment regarding the Method validation reports for the determination of TNBP in fibrinogen product (Document IG_IVMA-000261_ING) and thrombin product (Document IG_IVMA-000237_ING): In your method validation for the TNBP assay, the range of the assay as based on linearity, accuracy and precision results is (b) (4) for fibrinogen product and (b) (4) for thrombin product. Since TNBP is present as an impurity in your product, it is critical to have an assay range that includes the upper specification limit of (b) (4). Please provide linearity and accuracy from fibrinogen and thrombin samples to show that TNBP can be quantitated at the proposed upper specification limit of the assay.

The response to this IR has not been received yet.

Conclusion: The method is clearly described in the SOP. However, there are minor outstanding issues with the method validation as discussed in the third IR.

16. Sodium Determination by (b) (4)

The specification of sodium in thrombin is (b) (4) for lot release.

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Regarding your testing instruction document SOP IG_MA-000005A_ING: Please revise your SOP to include a detailed description or composition of the sodium secondary standard used as an assay control and submit for review.

Review of response: In response, the sponsor informed that the assay control is a qualified batch of albumin secondary standard. The standard mixture consists of (b) (4) albumin, (b) (4) of sodium and (b) (4). The sponsor did not submit the qualification data, hence another IR was sent to the sponsor.

B. Regarding the Method validation report, document IG_IVMA- 000415_ING:

i. The linearity and accuracy of your assay was validated with the use of sodium standards only. Please provide data on linearity and accuracy using actual drug product samples at concentration levels covering the range of the assay.

Review of response: In response to CBER IR, the sponsor provided additional linearity and accuracy data obtained from thrombin product samples. Linearity was estimated from (b) (4) concentration levels of sodium in the range of (b) (4). Three independent linearity runs were performed. A mean correlation coefficient (R) of (b) (4) was obtained, which met the pre-defined acceptance criteria for R to be (b) (4). We evaluated the slope ratio of standard vs sample regression lines and found the value close to be (b) (4) for all three replicates, indicating parallelism between the lines. The recoveries from the three linearity runs were also within the acceptable range of (b) (4) over the assay range.

Accuracy was also assessed in three separate assays at (b) (4) concentration levels of sodium in the range of (b) (4). The pre-set acceptance criteria for recovery was (b) (4), and the actual recovery varied from (b) (4).

Thus, the range of the method was demonstrated as (b) (4) of sodium.

ii. Please provide data obtained from thrombin drug product samples prepared without sodium-containing excipients (e.g., replacing sodium by (b) (4)) to substantiate your conclusion that the method is specific.

Review of response: In response, the sponsor explained that sodium is widely used throughout

the purification process as sodium chloride, sodium citrate and monosodium glutamic acid. After taking into account, the purification process, it is not possible to obtain the drug product sample without sodium or by replacing sodium with (b) (4). Considering the specificity data submitted earlier by the sponsor, and the additional linearity and accuracy data, the interference from matrix components is negligible, therefore no further IR is required.

iii. Please provide robustness data for your method by evaluating the effect of variation of different analytical parameters of your assay on sodium concentration.

Review of response: Review of response: In response, the sponsor submitted the report IG_ITEC-002978_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4). Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of results of sodium concentration in the secondary standard or control sample. The effect of above factors was statistically insignificant. The sponsor did not provide the results from drug product samples, however, the method for the quantitation of sodium using (b) (4) is specific and the validation results have demonstrated that the matrix interference is negligible. Further, the control is run with every assay, and any effect due to the variation of analytical condition would also invalidate the control results. Therefore, no further data is required.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

i. Please provide data to show that the control sample used in each of the above assay is adequately qualified or standardized in your laboratory against an appropriate primary or secondary standard.

Review of response: In response the sponsor submitted the qualification report for the control sample that was currently in use. The sample was prepared by diluting commercially available sodium chloride and (b) (4) with (b) (4) albumin. The sodium concentration in the control was qualified against (b) (4) calibration standard/s. The control limits were established from the results obtained from (b) (4) laboratories followed by statistical analysis. The sponsor's qualification data is adequate.

Conclusion: The method is has been adequately described and validated, and is acceptable as a lot-release test for the determination of sodium content in the thrombin component of fibrin sealant drug product.

17. Determination of Calcium by (b) (4)

Calcium (added in the form of calcium chloride) is an excipient in thrombin component of fibrin sealant drug product. The specification in thrombin is (b) (4) for lot release.

Method

(b) (4)



Method Validation

(b) (4)



(b) (4)

First Information request: The following IR was submitted to the sponsor on 5 May 2017. The response by Grifols received as Amendment 28 and 34 on 25 May 2017 and 21 August 2017 respectively, is discussed below.

A. Please revise your SOP (document IG_MA-000359A_ING) to include: (i) the composition of the assay control and (ii) procedure for (b) (4) and submit for review.

Review of response: (i) In response, the sponsor stated that the in-house calcium secondary standard was used as an assay control. The secondary standard was prepared from commercially available calcium chloride (b) (4) from (b) (4). However, the sponsor did not include the control qualification data. Therefore, another IR was generated for the sponsor.

(ii) The sponsor clarified that (b) (4) solution was prepared by (b) (4). The sample was diluted with (b) (4) before subjecting it for analysis. There was no additional (b) (4).

B. Regarding the Method validation report, document IG_IVMA- 000062_ING:

- i. The linearity and accuracy of your assay was validated with the use of calcium standards only. Please provide linearity and accuracy data using actual drug product samples at concentration levels covering the range of the assay.

Review of response: In response to CBER IR, the sponsor provided additional linearity data obtained from the thrombin product samples. Linearity was assessed at (b) (4) concentration levels of calcium in the range of approx. (b) (4). Three independent linearity runs were analyzed. A mean correlation coefficient (R) of (b) (4) was obtained, which met the pre-defined acceptance criteria for R to be (b) (4). The slope ratio of standard vs sample regression lines were close to 1 for all three assays, indicating parallelism between them. For accuracy, the sponsor clarified that in the original submission, this validation characteristic was evaluated in thrombin samples. Thus, based on the linearity (data submitted as amendment 27), accuracy and precision (data submitted in the original submission), the range of the method was demonstrated as (b) (4) for calcium. The specified range for calcium in this product is from (b) (4). As per the SOP, the sample is diluted (b) (4) fold before analysis. Therefore, the range required for validation is from (b) (4) after considering the dilution factor. The validated

range successfully covers the required limits, and the assay is appropriately validated for the quantitation of calcium in thrombin samples.

ii. To support your method specificity, please provide data obtained from thrombin drug product samples prepared without calcium-containing excipients.

Review of response: The sponsor submitted additional specificity data as Amendment 34. Method's specificity was performed in (b) (4) in three independent assays by analyzing the "(b) (4)" in-process sample, which was available just before calcium chloride addition step. The bulk in-process sample without calcium, was diluted (b) (4) with the (b) (4) as mentioned in the method SOP. In order to compare the responses, the blank of the test, the lowest calcium concentration (b) (4) standard, and a sample of thrombin product were tested in the same assay. Method's specificity was demonstrated since the responses obtained for the (b) (4) were of the same order as those obtained for the blank, and were (b) (4) folds lower than those obtained for the (b) (4) of calcium standard and for the thrombin component of FS. Considering the current submission, and results provided earlier (original submission) by the sponsor, the method's specificity is adequately demonstrated.

iii. Please provide robustness data for your method by evaluating the effect of variation of different analytical parameters of your assay on calcium concentration.

Review of response: In response, the sponsor submitted the report IG_ITEC-003006_ING which included the data obtained in the robustness study. This study was focused on variation of (b) (4) factors: (b) (4) Following a factorial design of experiments, (b) (4) independent assays were performed by varying each factor at (b) (4) levels ((b) (4)). The influence of variation was evaluated in terms of results of calcium concentration control sample. The effect of above factors was statistically insignificant. The sponsor did not provide the results from drug product samples, however, the validation results have clearly demonstrated that the matrix interference is negligible. Further, the control is run with every assay, and any effect due to the variation of analytical condition would also invalidate the control results. Therefore, no further data is required.

Second Information request: The following IR was submitted to the sponsor on 10 July 2017. The response by Grifols received as Amendments 33 on 5 August 2017, is discussed below.

i. Please provide data to show that the control sample used in each of the above assay is adequately qualified or standardized in your laboratory against an appropriate primary or secondary standard.

Review of response: In response the sponsor submitted the qualification report for the control that was currently in use. The control was prepared from commercially available (b) (4), arginine, albumin and calcium chloride from (b) (4). The calcium concentration in the control was qualified either against the standard calcium solution from (b) (4) at Instituto Grifols or against the standard solution of Calcium (b) (4) at the external Reference Laboratory. The control limits were established from (b) (4) independent test runs, followed by statistical analysis. The sponsor's qualification data is adequate.

Conclusion: The method is has been adequately described and validated, and is acceptable as a lot-release test for the determination of calcium content in the thrombin component of fibrin sealant drug product.

18. Appearance of Frozen Product

The specification for appearance of frozen thrombin product is colorless or pale yellow and opaque solid.

Method

The frozen material is visually examined for color and transparency, as described in (b) (4). Visual inspection is appropriate to verify appearance of frozen product, and validation of this method is not necessary.

Conclusion

The assay is approvable as a release test for thrombin component of fibrin sealant drug product.

19. Appearance of Solution after Thawing

The specification for appearance of thrombin solution after thawing is colorless or pale yellow solution.

Method

The frozen thrombin sample is thawed at 37 °C. The characteristic of the solution is examined visually for color against light in accordance with (b) (4). Visual inspection is appropriate to verify the appearance of solution after thawing, and validation of this method is not necessary.

Conclusion

The assay is approvable as a release test for thrombin component of fibrin sealant drug product.

20. pH

The pH specification for thrombin component of fibrin sealant drug product is 6.5-8.0.

Method

The pH of the (b) (4) is measured using a pH meter. Reference pH buffers ((b) (4)) are used to calibrate the pH meter. The method is compliant to the EP and USP test methods for pH determination. The method was not validated. However, measurement of pH is a widely used method and known to be dependent only on the hydrogen ion concentration, and is unaffected by other matrix components in aqueous solution. Hence, no validation should be necessary. The pH results of thrombin lots manufactured at the clinical and commercial scale during the scale-up of production process, and thrombin lots submitted for the stability studies were within the required specification limit.

Conclusion

This is a well-established method. Further information is not required. The assay is approvable as a release test for thrombin component of fibrin sealant drug product.