



MEMORANDUM

From Hsiaoling Wang, DBSQC/OCBQ
Ritu Agarwal, DBSQC/OCBQ
Lokesh Bhattacharyya, DBSQC/OCBQ

To STN 125555/0

Through Dr. William M. McCormick, Director OCBQ/DBSQC

Sponsor Octapharma

Product Antihemophilic Factor (Recombinant) – rAHF, B-domain deleted recombinant Factor VIII – BDDrVIII, plasma/albumin free (NUWIQ®), STN: 125555

Subject Final/Addendum Review Memo for the Method Validation for the Quality Control Release Tests for the Drug Product

Recommendation: Approval with PMC

Summary

A new BLA was submitted by Octapharma for Nuwiq, an Antihemophilic Factor (Recombinant) – rAHF, B-domain deleted recombinant Factor VIII (BDDrVIII) plasma/albumin free product for the prevention and control of bleeding episodes (also during and after surgery) in adults and children with Hemophilia A. This document constitutes the Final/Addendum review memo from LACBRP/DBSQC. The following analytical methods used for quality control lot release of the drug product, and validations of these methods were reviewed.

1. One-stage Factor VIII Clotting Assay
2. Identification by (b) (4)
3. (b) (4)
4. Total Protein by (b) (4)
5. Water by (b) (4)
6. Arginine by (b) (4)
7. Sucrose by (b) (4)
8. Poloxamer 188 Content by (b) (4)

9. Sodium Content by (b) (4)
10. Chloride by (b) (4)
11. Citrate by (b) (4)
12. Calcium by (b) (4)
13. Appearance (Lyo Cake) by Visual Inspection, Solubility of freeze-dried final products, and Visual inspection of solutions Solubility and Visual Inspection of solution
14. (b) (4)
15. (b) (4)

In the Primary Discipline Review memo (dated 22 January 2015) we concluded that, with the exception of the following test methods, the test methods listed above can be approved for quality control lot release test for NUWIQ.

- (b) (4)
- Sucrose by (b) (4), and
- Calcium by (b) (4)

Conclusion:

The three methods reviewed in this memo can be approved for lot-release testing of the drug product. However, there is an outstanding commitment from the sponsor to work with FDA/CBER (DBSQC) to implement and validate a new method for the (b) (4) which was provided to them by DBSQC.

Background

The B-domain deleted recombinant Antihemophilic Factor (factor VIII) (BDDrVIII) is a plasma/albumin free product that is supplied as lyophilized powder. Recombinant Antihemophilic Factor VIII (rAHF) is comprised of light and heavy chain complexes.

(b) (4) The lyophilized powder is formulated in single-dose vials containing 250 IU, 500 IU 1000 IU or 2000 IU recombinant factor VIII per vial, which is reconstituted as a single-dose with 2.5 mL of sterile water for injection before use from a pre-filled syringe supplied with the product.

Submitted Information and Documents

This is an electronic submission. Information submitted and reviewed includes:

- 125555.20 – 1.2 Response to FDA information request dated 16 January 2015, received on 06 February 2015
 - Doc. 138VAL168 FC 139/03: Validation of the method used for the Determination of Sucrose (revised version)
- 125555.23 – 1.2 Cover Letter
 - Outstanding response to FDA information request dated 15 January 2015, received on 27 February 2015

- Doc. 138VAL735 IP 137, FC 139/06: Updated validation report for (b) (4) of Human line recombinant human factor VIII (Human-cl rhFVIII) by (b) (4)
- (b) (4) method development for characterization of Human cell line recombinant human factor VIII (Human-cl rhFVIII), (b) (4)
- 125555.27 – 1.2 Cover Letter
 - Response to FDA information request dated 25 March 2015, received on 4 April 2015
 - SOP 130SOP735 ver. 07: (b) (4) of Human cell line recombinant human factor VIII (Human-cl rhFVIII) by (b) (4)
- 125555.30 – 1.2 Cover Letter
 - Response to FDA information request dated 9 April 2015, received on 15 April 2015
 - SOP 130SOP735 ver. 07: (b) (4) of Human cell line recombinant human factor VIII (Human-cl rhFVIII) by (b) (4)
- 125555.35 – 1.2 Cover Letter
 - Response to FDA information request dated 9 April 2015, received on 15 April 2015
 - SOP 130SOP735 ver. 08: (b) (4) of Human cell line recombinant human factor VIII (Human-cl rhFVIII) by (b) (4)
- 125555.37 – 1.2 Cover Letter
 - Response to FDA information request dated 22 April 2015 and 27 April 2015, received on 29 April 2015
 - SOP 130SOP735 ver. 09: (b) (4) of Human cell line recombinant human factor VIII (Human-cl rhFVIII) by (b) (4)
- 125555.45 (PMC Commitment) Response to FDA information request dated 8 May 2015.
- 125555.52 – Response to FDA information request dated 8 May 2015, received on 22 July 2015
 - SOP 130SOP735 ver. 10: (b) (4) of Nuwiq by (b) (4)
 - 138VAL735 IP 137 FC 139/07 Analytical method validation report: (b) (4) of Nuwiq by (b) (4)
- 125555.54 – Response to FDA information request dated 8 May 2015, received on 22 July 2015

Review Narrative

1. (b) (4)

The (b) (4) assay serves as an identification test for the protein Human-cl rhFVIII and as a quantitative method for relative percentages of Human-cl rhFVIII (b) (4) and impurities (b) (4) of the drug

product (DP). The method involves (b) (4)

The DBSQC laboratory required 4 days to complete analysis of 6 lots of the product (Testing Memo from Hsiaoling Wang and Alfred Del Grosso, dated 28 April 2015). In addition, even though the sponsor termed the method as an (b) (4) method, the (b) (4) used and the operating conditions are consistent with an (b) (4) assay.

The specification for the identity test for the DP is (b) (4)

each.

The method validation review, first IR and review of the responses were covered in the Primary Discipline Review memo (dated 22 January 2015).

Second Information Request

Based on the review of the initial submission, and the first subsequent IR on this assay, a second IR was sent to the sponsor on 20 January 2015. The responses to questions 1 and 2 were received as Amendment 23 and Amendment 20, respectively.

1. We disagree with your LOQ conclusions for (b) (4). The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. You have provided precision results at (b) (4) (proposed LOQ) but not the accuracy data at this level. For (b) (4) this is an impurity test. Therefore, LOQ is a critical validation characteristic. Please provide the accuracy data to demonstrate the accuracy at (b) (4)

Review of response: The sponsor provided data from analysis of samples that were fortified with (b) (4) by (b) (4) samples enriched in (b) (4) to the drug product samples containing (b) (4). The validation report (138VAL735 IP137 FC139/06) has been updated with this data showing linearity of the (b) (4) in the range (b) (4) and (b) (4) area%, respectively. The sponsor did not analyze the data however concluded that the data support (b) (4) as LOQ for (b) (4). Our analyses of the data submitted by the sponsor showed that the LOQ for the (b) (4), respectively. The proposed specifications for both (b) (4) in the DP are NMT (b) (4). Thus, LOQ values set by the sponsor as (b) (4) are above the true estimates of the LOQ and below the proposed specification limits, and are therefore acceptable.

2. Please provide the reference 18 cited in validation report page 34 "Study Report OC11-0289 (b) (4) method development for characterization of Human cell line

recombinant human factor VIII (Human-cl rhFVIII)', (b) (4)

(b) (4) You referred to this document for the results of the (b) (4) method you used (b) (4) but did not submit the document.

Review of response: The sponsor provided the document as part of amendment 20. The document provides details of the results obtained by an (b) (4) method, used as the (b) (4) method for the determination of (b) (4). The method was able to (b) (4)

(b) (4) method. In addition, the (b) (4) method was not validated and the critical issues such as (b) (4) recovery and method accuracy were not addressed in the report to judge the overall quality of data generated by the (b) (4) method.

The sponsor also indicated that due to the low precision of the (b) (4) method at low (b) (4) (at or below the specification limits) it was not possible to compare this method with the (b) (4) to evaluate accuracy at or below the proposed specification limits.

Third Information Request

A third IR was sent to the sponsor on March 25, 2015 after the first trial of testing run of the SOP in DBQSC Lab. The responses were received on 7 April 2015 as amendment 27.

1. According to the (b) (4) (b) (4). Please let us know your plans to address this issue.

Review of response: The sponsor indicated that they were aware of the fact that (b) (4) (b) (4). To address this issue, they had been (b) (4)

2. Our test results suggest that control sample is (b) (4) (b) (4). Thus, it is possible that a (b) (4) is required, as we previously suggested. At the same time, such procedure is not described in your SOP. Please clarify if such a step is necessary and if so, please revise the SOP accordingly and submit for review. In addition, please include RSD (%) of the (b) (4) in your SOP to ensure consistency of the results and (b) (4).

Review of response: The sponsor provided data, which indicate that the (b) (4) (b) (4) from (b) (4) batches require only (b) (4). The sponsor updated the SOP (SOP 130SOP735 version 07) to include (b) (4). The response is not satisfactory because it was obvious from the submitted data that (b) (4) was necessary.

We also noted in the revised SOP that the sponsor set the acceptance criterion for (b) (4) from (b) (4) to be (b) (4) which is very wide for a (b) (4) method.

3. You have demonstrated that the LOQ for both (b) (4). However, the results for (b) (4) are reported as (b) (4) for (b) (4) lots of the submitted samples. In addition, there are errors in the result provided for lots (b) (4)

Some of these appear to be typos. Please revise your results as appropriate and resubmit the data. We need specific results that are above your LOQ for your samples and controls. The results that are below LOQ should be expressed as less than (<).

Review of response: The sponsor provided reanalysis data from (b) (4) lots of the products, which they submitted for in-support testing. The results show that the (b) (4) are (b) (4) for all products and (b) (4) is present in (b) (4). The modified results meet the acceptance criteria from the current validation report. The response is satisfactory.

4. Please provide following information for samples (b) (4) and the control you submitted: 1) (b) (4) of all samples and the control, 2) (b) (4) of all samples and the control, 3) (b) (4) of all samples and the control.

Review of response: (b) (4) were provided. (b) (4) are set reasonable in (b) (4). There are discrepancies between the data provided in response to Question # 3 (above) and this question. It is not clear whether the results are from tests performed on different occasions. However, considering all results are within DP specifications, the response is acceptable.

Fourth Information Request

The fourth IR was sent to the sponsor on 9 April 2015 after reviewing the responses in amendment 27 (response to 3rd IR). The responses to Questions # 1-3 were received on 21 April 2015 (amendment 35) and Questions # 4-5 on 15 April 2015 (amendment 30).

1. The data you provided in response to our question #2 show that you have evaluated a limited number of (b) (4)

. You have not provided data to show that (b) (4) is sufficient. Please revise your SOP (130SOP735/07) (b) (4) you out-lined on page 6 of your Response to FDA Information Request Dated March 25, 2015 (Amendment 27) and submit for review.

Review of response: The sponsor revised the SOP to include (b) (4), as requested in the IR and submitted the revised SOP (130SOP735/08). The revised SOP is acceptable.

2. Your acceptance criteria of (b) (4) RSD (page 3 of the above mentioned document) is too large. Please reduce the acceptance criteria to no more than (b) (4) for the two Control sample (CTR) runs after the (b) (4) as requested above.

Review of response: The SOP was revised to change the acceptance criterion (b) (4) with respect to the (b) (4)

3. Were the data you reported on page 7 of the above mentioned document obtained after adequate (b) (4) ? If yes, please provide the batch number (b) (4) used. If not, you may be under-reporting the (b) (4). In that case, please reanalyze the samples of the same (b) (4) batches (b) (4) and submit the data to show that the results meet your proposed acceptance criteria for the (b) (4)

Review of response: The sponsor informed that the results previously reported for the (b) (4) lots that were submitted for in-support testing, were not obtained after (b) (4) (b) (4) or on a (b) (4). Therefore, (b) (4) were reanalyzed using a (b) (4) (as described in 130SOP735/08) and the results were provided. The (b) (4) for all of the (b) (4) when a (b) (4) however the results are still within the proposed specification limits. However, the difference between the results obtained (b) (4) is significantly smaller than those observed in the DBSQC laboratory. In addition, the (b) (4) obtained from the same samples in the DBSQC laboratory shows that the (b) (4), baseline (b) (4) are significantly affected, when a (b) (4) (memo from Hsiaoling Wang, 28 April 2015: Testing Memo for (b) (4) Analysis of Nuwiq® Drug Product).

4. The description in section 5.4.5 of your revised SOP (130SOP735/07) “an additional control (b) (4) FVIII sample for verification” is not consistent with the run sequence shown later in the same section. Please explain or revise as needed and submit for review.

Review of response: The sponsor agreed that it was a mistake on their part and revised the SOP to correct the error in ver. 08 of the document.

5. Please clarify whether both of the sequences in sections 5.1 (b) (4) and 5.4.5 (Sample Sequence) of your revised SOP (130SOP735/07) should be performed when (b) (4)

Review of response: The sponsor agreed that there is a lack of clarification and revised the SOP for clarification in ver. 08 of the document.

Fifth Information Request

The following IRs were sent to the sponsor on 22 April 2015 after reviewing the responses to the Fourth Information Request (Amendment 35). The response was received on 28 April 2015 as Amendment 37.

1. For Figures 2-4 you included in Amendment 35, you identified the start of the (b) (4). This is not acceptable.

Furthermore, it appears from Tables 5-7 that the results of batches (b) (4) would not meet your proposed specification limit for the (b) (4) if you (b) (4). Section 6.1 of your SOP for the (b) (4) assay (130SOP735/08) permits (b) (4). We have concern about this (b) (4) for the information we discussed above and also for the reason that such (b) (4) has the potential to introduce personal bias. Please revise your SOP to (b) (4) method using your (b) (4). Please recalculate the area percent for the batches (b) (4) using the method proposed above and submit the results.

Review of response: The sponsor informed that (b) (4). However, they agreed that (b) (4). Therefore the SOP has been updated (130SOP735/09) to describe in detail how to (b) (4). By application of this instruction a bias of the fragment result is excluded. The sponsor recalculated the (b) (4) lots of the product using this instruction. The results indicate that the (b) (4) would have failed to meet the proposed specification, if this corrected (b) (4) method was used.

2. Your (b) (4) in Amendment 35 show (b) (4). Please provide appropriate data to (b) (4). Unless your data show otherwise, this is (b) (4) and included in the calculation of the (b) (4). Please revise your SOP and resubmit, if necessary.

Review of response: The sponsor informed that this (b) (4) is treated as part of the (b) (4) and is included in the calculation (b) (4). This is acceptable.

3. a) You stated in Amendment 35, “The previous data were not obtained after (b) (4), therefore batches (b) (4) were reanalyzed with a (b) (4) and (b) (4) (as described in 130SOP735/08).” Please explain why the previous results for the above mentioned batches were not obtained using either a (b) (4).
b) Also, please clarify when the (b) (4) step was introduced in your SOP. We observe that the (b) (4) impacts the assay results; hence we consider this to be significant change. The critical question that we need addressed here is whether the method you validated included this (b) (4) step.
c) Please indicate if you used (b) (4) step during method validation and, if not, provide justification to indicate why your validation for this method (report # 138VAL735 IP 137, FC 139/03) will be acceptable to the agency and revalidation of the method is not necessary.

Review of response:

- a) The sponsor informed that the statement in Amendment 35 (quoted by FDA) is incorrect and misleading. These results were obtained after (b) (4) but with only (b) (4) as per the SOP # 130SOP735/07. This information explains the discrepancy of results obtained by DBSQC and the sponsor. DBSQC obtained significant difference in the percentage of (b) (4) between (b) (4) while the sponsor reported small difference in Amendment 35. The information also confirms that (b) (4) are necessary, as was determined by DBSQC and communicated to the sponsor.
- b) The sponsor informed that the (b) (4) was introduced in ver. 07 of the SOP (130SOP735/07) based on FDA's recommendation received April 9, 2015 after method validation. Thus, the method validation was conducted and all batch analysis results were obtained with (b) (4). The information suggests that the (b) (4) may have been under-reported during method validation and in all batch analysis data, including clinical samples.
- c) The sponsor provided data in this amendment show that there is (b) (4) difference in the (b) (4) without and with (b) (4). However, the results submitted in Amendment 27 shows as high as (b) (4) difference. Thus, the data provided by the sponsor are inconsistent, which the sponsor recognized and accepted the FDA request to revise the method to include (b) (4) and revised their SOP accordingly, which they did in ver. 09 of the SOP and revalidate the method post-approval as a PMC (see below).

PMC Request

As per the discussion during the Late-cycle Meeting on 05 March 2015 with Octapharma regarding the BLA submission for Nuwiq® (STN: 125555), FDA proposes that the sponsor commit to two Post Marketing Commitments (PMC), one short term and the other longer term, in relation to the (b) (4) assay for (b) (4) analysis of the recombinant Antihemophilic Factor (FVIII), as discussed below. Octapharma's commitment was received as Amendment 45.

Short-term Commitment

Octapharma will validate their current (b) (4) method, as described in their SOP # 130SOP735/09. Octapharma will reanalyze all retains from the clinical and validation lots of the product using the method after it is successfully validated. Octapharma will reevaluate their specifications for (b) (4) based on the results and submit to FDA for review. Octapharma will provide a plan and time-line for completing the proposed PMC for review and approval by FDA within one week of receiving the request.

Response from Octapharma

Octapharma will validate the current (b) (4) method, as described in SOP # 130SOP735/09 by July 15, 2015.

Octapharma will reanalyze all retains from the clinical and validation lots of the product using the method after it is successfully validated and Octapharma will reevaluate the specifications for (b) (4) based on the results and submit to FDA for review by August 17, 2015.

Long-term Commitment

Octapharma will receive via technology transfer from FDA an (b) (4) method, which involves (b) (4) analysis of Nuwiq® (STN: 125555). Octapharma will commit resources to work with FDA to evaluate the method within (b) (4) after receiving the procedure and come up with a plan and timeline to validate the method and reanalyze all retains from the clinical and validation lots of the product using the method after it is successfully validated and submit for approval by FDA. Octapharma will reevaluate the specifications for (b) (4) of Nuwiq® based on the results and submit new assay, validation, and product specifications to FDA for review as a Post Approval Supplement (PAS) Octapharma will not submit for a patent, publish or share the (b) (4) procedure they receive from FDA with any entity outside of Octapharma without written authorization from FDA. FDA retains the right to publish and share the method at its discretion.

Response from Octapharma

Octapharma commits to the long-term commitment. Regarding the long-term commitment: please let us know what the next steps will be in order to initiate the technology transfer from FDA to Octapharma for an (b) (4) method which involves (b) (4) analysis of Nuwiq® (STN: 125555).

Results from the Short Term Commitment by Octapharma and Review

Subsequent to the PMC agreement, Octapharma submitted a Major Amendment in a different area, as a result of which the Action Due Date (ADD) was extended by 3 months. During this extension, Octapharma fulfilled completion of the Short-term Commitment and submitted the new validation report on 22 July 2015 (Amendment 52) and results retains from the analysis of the clinical and validation lots on 31 July 2015. The new validation report and results retains from the analysis of the clinical and validation lots are reviewed below.

Method

The SOP for (b) (4) analysis was revised (SOP 130SOP735 ver. 10) to include the following:

- Changing the method title to (b) (4) of Nuwiq by (b) (4)
- Additional placebo run at the end of sequence for (b) (4) qualification
- Deleting (b) (4) requirement of (b) (4) from (b) (4). The new requirement is described as "The (b) (4)

These changes are logical and acceptable.

Method Validation

The following characteristics are evaluated in the validation report: specificity, linearity, repeatability and LOQ (for (b) (4))

Specificity was evaluated by identification of the (b) (4) of the test sample with that of the control. The (b) (4) of each sample must (b) (4) of the control sample as described in new SOP (version 10). The (b) (4) also demonstrated that the method is able to (b) (4)

However, the results show (b) (4). The (b) (4) results in an under-estimation of the (b) (4). This effect is maximum for the lowest formation dose (250 IU) in which the (b) (4) is underestimated by (b) (4). This difference is small. Furthermore, if the results are still within the specification limits, it does not pose a safety (over-estimation of impurities) or efficacy concern (under-estimation of active). The results also show (b) (4). To address this issue, the SOP is revised to include a (b) (4).

The linearity was evaluated to demonstrate that the (b) (4)

The linearities of the (b) (4) are evaluated by (b) (4) samples with samples, enriched in (b) (4). The samples (b) (4) were obtained with (b) (4). The correlation coefficient, r , between (b) (4) are between (b) (4) in the ranges (b) (4) meeting the acceptance criterion, (b) (4).

The accuracy of the method was evaluated by comparing the results of (b) (4) obtained by comparing the results from the (b) (4) method with those results obtained using an (b) (4) method, (b) (4). Samples from two different lots, both at 500 IU have been used to compare (b) (4) by inducing (b) (4). Such induction was necessary because the actual lots contain (b) (4) below LOQ (b) (4) by the assay method. The results from the determination of (b) (4) in samples containing (b) (4), each in three replicates, were evaluated by (b) (4). No statistical differences between the methods at 99% confidence interval (b) (4) were observed, indicating that the (b) (4) methods gave comparable results. The same statistical evaluation of results from samples containing (b) (4) also shows no statistical differences between the methods at 99% confidence interval (b) (4). tests were performed to ensure that (b) (4). The results from (b) (4).

(b) (4). The results demonstrate accuracy of the method for (b) (4).

Repeatability of the method determined from (b) (4) replicate measurements of a (b) (4)

Based on the results of the linearity study LOQ of the assay was set at (b) (4) for total protein concentration and (b) (4) for both (b) (4).

Conclusion

The method as described in ver. 10 of the SOP is adequate and validated adequately on revalidation.

2. Sucrose by (b) (4)

Sucrose is an excipient used in the Human-c1 rhFVIII drug product. The drug product specification for sucrose is (b) (4) for all drug product formulations.

Outstanding Information Request

After reviewing response from Octapharma to the first IR (received on 10 Oct 2014) (Amendment 7), a second IR was submitted to the sponsor on 16 Jan 2015. The response was received as Amendment 20 on 6 Feb 2015, which is reviewed below.

- a. In your accuracy determinations (Document 138VAL168 FC 139/02, section 6.4.2) it is stated that “on one occasion the sample was tested undiluted and diluted to (b) (4) (b) (4) respectively before analysis.” However, you diluted both solutions to about (b) (4). But, as per your SOP, the target nominal concentration of sucrose for analysis is approximately (b) (4). Thus, you have evaluated accuracy at one concentration only and at (b) (4) of the target nominal concentration. On the second occasion, accuracy was evaluated in the range of (b) (4) of sucrose in drug product, which does not cover the specified range (b) (4) of your assay. In our previous IR (dated 22 August 2014), we requested linearity and accuracy data of your product samples in the intended assay range. For the reasons explained above, the data that you have provided do not adequately address our IR. Please provide appropriate linearity and accuracy data to support your proposed assay range for sucrose.
- b. The results presented in tables on pages 41 and 42 appear to be incorrect. The mean measured sucrose concentration represented as (b) (4) (page 41) and nominal sucrose concentration and corresponding measured value represented as (b) (4) respectively (page 42) of the revised validation report. Please confirm the errors and provide the corrected table.

Review of response: In response, the sponsor provided additional method validation data in report 138VAL168 FC 139 /03. Linearity in sample matrix was reported in the range of (b) (4) µg sucrose/mL (corresponding to (b) (4) of sucrose at a working dilution of (b) (4). Linearity of sucrose standards was previously evaluated in the range of (b) (4). The regression coefficient R, for the plot of sucrose

concentration vs (b) (4) for the standard and NUWIQ drug product, respectively. The slope for the product was calculated by linear regression analysis as (b) (4) which compared well with the slope of (b) (4) for the standard (slope ratio (b) (4)), demonstrating parallelism between linear regression lines for the drug product and the standard.

Accuracy was determined from the data obtained in the linearity study. The recovery meets the acceptance criteria of (b) (4). Accuracy was also evaluated by fortification of the sample matrix with sucrose to concentrations from (b) (4) (final concentrations in the range of (b) (4)). The results show (b) (4) (without background correction), which met the acceptance criteria however the concentrations evaluated were above the proposed assay range. Since accuracy determined from the linearity data at concentrations that are within the proposed range, we conclude that accuracy has been demonstrated adequately.

Conclusion

The method and the validation report are clearly described and adequate for approval of this assay for lot release testing of the drug product.

3. Calcium by (b) (4)

This assay is performed using (b) (4). The proposed specification for the drug product is (b) (4) for all dosage formulations.

Outstanding Information Request

After reviewing response from Octapharma to the first IR (received on 10 Oct 2014) (Amendment 7), a second IR was submitted to the sponsor on 16 Jan 2015. The response was received as Amendment 20 on 6 Feb 2015, which is reviewed below.

- a. You have stated in your response to CBER IR (Received on 02 September 2014) regarding section 5.4 of document 138 VAL708FC139/01 that due to negligible interference from the product matrix, and that one point calibration is used as the standard solution (without product matrix), a blank solution containing only reagent should be the correct blank. We do not agree with your justification. Since you are using reagent only as the blank, it seems to us that there is a volume difference between the blank and the sample. Please provide additional explanation or include appropriate blank preparation in the test method and revise your SOP document.

Review of response: In response, the sponsor submitted comparative data of (b) (4) samples measured against the reagent blank alone or reagent blank with (b) (4) of WFI. Statistical evaluation of the test results of two series by difference t-test confirmed that there was no significant difference. Thus, the volume difference of (b) (4) between the blank and the sample did not influence the sample measurement at (b) (4). The sponsor's response is acceptable.

Conclusion

The method is described in sufficient details and is adequately validated for its intended use.