



## MEMORANDUM

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Office of Compliance and Biologics Quality (OCBQ)  
Center for Biologics Evaluation and Research (CBER)  
Food and Drug Administration (FDA)

**To:** Biologics License Application Submission Tracking Number # 125523/0

**Subject:** Addendum Review Memo of Analytical Procedures for Drug Product of Biologics  
License Application for Fibrocaps – (b) (4)

**Through:** Lokesh Bhattacharyya, Ph.D., Lab Chief, LACBRP/DBSQC/OCBQ/CBER  
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**Applicant:** ProFibrix

**Product:** Fribrocaps – Fibrin Sealant

**Biologics License Application (BLA) Submission Tracking Number (STN) #: 125523**

**Submission received by CBER:** January 31, 2014

**Review completed:** January 14, 2015

**Summary and Conclusion:**

This DBSQC reviewer found the method used to determine (b) (4) for the drug product (DP) of Fribrocaps is not suitable for the intended use because the sponsor could not demonstrate that the calibration model established on experimental spectra of actual samples could provide accurate and precise results for both (b) (4) trehalose. Thus this method could not adequately determine (b) (4) for DP at the proposed specification ((b) (4)) level.

The determination of (b) (4) assay was introduced as a method to compliment moisture content by (b) (4) method in Pre-IND stage in order to demonstrate that thrombin does not react with fibrinogen at residual moisture level of (b) (4). Subsequently, an (b) (4) method to assess premature activation of fibrinogen was introduced for the same purpose. The (b) (4) method show better sensitivity to the formation of (b) (4) fibrinogen in the DP. The review committee agreed that (b) (4) test for moisture combined with the (b) (4) can sufficiently monitor the integrity of the fibrinogen in the DP.

This assay of (b) (4) determination for DP was withdrawn by the sponsor in the amendment 22 dated on Dec. 17, 2014 after multiple communications between FDA reviewers and the sponsor through e-mails and conference-call.

**Background**

The deficiencies of this method were partially identified in the PDR memo from DBQSC (dated 09/05/2014). The major concerns at the time were 1) The determination of limit of qualification (LOQ) of the method, and 2) The unsatisfactory precision in the method transfer report between (b) (4)

A major deficiency of the method was confirmed after receiving sponsor's response dated Aug, 28, 2014 (Amendment 12). The calibration model used in the method was established on simulated spectra, which is one of the reasons resulting in a Major Amendment (125523/0.16) of this submission. After the teleconference with the FDA review committee on Dec. 08, 2014, the sponsor decided to withdraw this method from the submission.

## Information Request and Response Reviews

An IR was sent to the sponsor on Sep. 9, 2014 as follows:

- We found that (b) (4) out of (b) (4) spectra, which have (b) (4) calibration are digitally generated from the spectra of samples of (b) (4) and (b) (4) and were not obtained experimentally. This is unacceptable. The spectra used in calibration must be generated experimentally from actual samples. Please submit new (b) (4) data using actual samples.

- The procedure of calibration data generation described in your SOP needs to be modified to indicate use of actual samples rather than simulated ones for (b) (4) (b) (4). Similarly, your method validation is based on an unacceptable calibration model. Please revise the SOP and the validation report and submit both for FDA review.

A follow-up meeting was requested by the sponsor, which was held on September 19, 2014. During the meeting, the sponsor expressed that they would change this assay as a limit test instead of a quantitative test. FDA reviewers pointed out that only spectra from real samples should be used to establish the calibration model and both accuracy and precision data are required to determine limit of detection (LOD) and LOQ.

Additional information was communicated to the sponsor on Sep. 23, 2014 as follows:

In response to your inquiry, we would like to point out that we found the calibration model to be unacceptable because it was generated using theoretical data. The calibration model should be generated using experimental data from samples that have (b) (4) (the proposed specification limit) and the LOQ ((b) (4) whatever it is). You may use samples of higher (b) (4) if you want to but it is necessary to have data from samples at these (b) (4) levels. In addition, this should be done using no less than (b) (4) independent sample preparations at each (b) (4) level. In addition, the samples used to acquire LOQ data should be independent preparations from what were used in the generation of the calibration model. Using the same data for both is circular.

The responses were received on Oct. 24, 2014 in amendment 17.

Response 1.2 “Relationship between Moisture Content and (b) (4)”

The relationship between moisture content and (b) (4) was investigated. Results demonstrated that increasing moisture content correlated with higher (b) (4) on average, see Figure 1. However, this relation is most clear for moisture contents above the upper specification limit. For moisture content levels within the specification limit of (b) (4) (b) (4) below the LOQ of (b) (4) of the method is found. This prohibits numerical analysis of the relationship between moisture content levels within the specification range, as compared to the corresponding (b) (4) levels ((b) (4)). This limits the value of the (b) (4) method as a (b) (4) assay, and warrants its use as a limit test only.

(b) (4)

(b) (4)

Figure 1. Relationship between (b) (4) and Moisture content

#### Review of the response

The conclusion is invalid because the method used to determine (b) (4) was not a properly validated and the calibration model used simulated data.

#### Response 1.3 “Feasibility Assessment Experimental Calibration Model”

Feasibility of generating experimental calibration model was assessed in the two manners:

- 1) Assessment of experimental mixed samples against calibration line based on calculated spectra

The results demonstrate that the results for the actually (b) (4) trehalose samples at (b) (4) coincide with the calibration line based on calculated spectra at (b) (4) and actual (b) (4) spectra, therefore validating the use of the calculated calibration line.

- 2) Comparison of samples results calculated against a) an experimental calibration line and b) calculated calibration line

The brief summary of the results are listed in Table 1.

Table 1. Comparison of (b) (4) results of trehalose (b) (4) using two calibration lines

(b) (4)	Calculated calibration line	Experimental calibration line
(b) (4)	(b) (4)	(b) (4)
(b) (4) trehalose	(b) (4)	(b) (4)

\*ideal (b) (4) trehalose are (b) (4) respectively.

In conclusion, generating a calibration curve based on experimentally generated data is not considered feasible because of the high uncertainty.

#### Review of the response

The calculated calibration line, using spectra of (b) (4) samples plus (b) (4), is not acceptable as was discussed before. Thus all related results from this model are not valid.

The experimental calibration line with spectra of actual samples of (b) (4) could not provide required accuracy and precision for samples of (b) (4) trehalose. The results show unacceptable level of uncertainty. No accuracy and precision are evaluated for other samples. There is no data provided for method validation as an assay of limit test.

#### Conclusion

This method is not suitable for the intended use for the determination of (b) (4) in this DP.