Analytical Method Validation Memo - CINRYZE

Date:	December 27, 2007 **Time: 10:30 AM**
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Subject:	Final review memo for analytical method validation (Modules 2 & 3) in the Original BLA from Lev Pharmaceuticals, Inc. for C1 Esterase Inhibitor (Human), C1INH, [Cinryze™] for treatment of acute attacks of hereditary angioedema (HAE); STN 125267/0
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To the file:	STN 125267/0

Action recommended:

Based on my review of analytical methods validation, this part of the Original BLA STN 125267 for the manufacturing of C1 Esterase Inhibitor (Human) [Cinryze ™] is approvable with two recommendations (pp. 13 and 14) to be accepted by the firm.

SUMMARY

Submission date: 7-31-2007
CBER receipt date: 7-31-2007
DATS Log #: 422391
Type of submission: Original BLA, STN 125267/0.0
Sponsor: Lev Pharmaceuticals, Inc. (LPI)
Manufacturer: Sanquin Blood Supply Foundation (Sanquin)
Product: C1 Esterase Inhibitor (Human) nf, C1INH-nf
Proprietary name: Cinryze ™
Indications: treatment of acute HAE attacks

This memo is a review of analytical procedures validation as provided in Modules 2 and 3 (original submission STN 125267/0.0) for drug substance and drug product and in the firm's responses to CBER information requests (Amendments STN 125267/0.6 and 125267/0.7).

Background

The LPI's product Cinryze ™ is a preparation of C1INH derived from human plasma that is indicated for the treatment of acute attacks of HAE . C1INH has been used in Europe for over 30 years. However, currently in the US there is no licensed therapy to treat acute HAE.

C1INH is a multi-specific serine protease inhibitor (serpin) in the circulation. It is the major inhibitor of C1s and C1r proteinases that, together with C1q protein, regulate the C1 complement complex. C1INH also inhibits the contact system proteinases (kallikrein, XIa, and XIIa), thus regulating several pathways of inflammation, and the mannose-binding lectin-associated serpins (MASPs).

Genetic deficiencies of C1INH are of two types: functional deficiencies (protein is present, but not functional) and antigen deficiencies (protein is not present or more commonly present but at a low level); both may lead to life threatening HAE.

Finished product specifications

[(b)(4)]

VALIDATION OF ANALYTICAL PROCEDURES

Analytical procedures listed for Drug Product include:

7 EP methods, and
10 Sanquin procedures.

Compendial methods

The EP compendial methods listed in this submission are:

(b)(4)

The EP methods are compared with the USP procedures by the firm as follows:

M145	Sanquin method for ----(b)(4)---------- corresponds to the EP method --(b)(4)---, determination of -------(b)(4)----------, that is

comparable to the USP method<(b)(4)>.

M116/8.0	Sanquin method of ------(b)(4)-------- Content Measurement (--(b)(4)---- method).

C1INH activity assay

Sanquin method @0398/4.0 for C1INH activity assay is based on the inhibition of C1 esterase (C1s) by C1INH and measuring the ---(b)(4)-------- activity using ----------(b)(4)----------. This is a -(b)(4)--- assay that utilizes the reagents supplied by commercially available kit (----------(b)(4)----------). The -(b)(4)----- activity of C1s is determined from the ----(b)(4)---------- monitored at(b)(4) nm for --(b)(4)------. A no-serum sample, a normal serum sample and a product (C1INH or Cetor) are tested as controls. The assay is carried out at --(b)(4)----- °C. Validation report (@2347/2.0) includes the following validated parameters:

Accuracy (tested by determining the average C1INH activity in the serum samples of (b)(4) blood donors; test results: the average activity is --(b)(4)--, and SD is -(b)(4)-);

Reproducibility (test results,the average and the SD of (b)(4) independent assays for the C1INH concentrate (b)(4): average - (b)(4)--- , SD (b)(4) , and CV - (b)(4)- %; and the reproducibility of the results per analyst: for control sample <1562>: CV within acceptance criteria of (b)(4) %,and for product control sample <1569> NMT - (b)(4)- %);

Repeatability (acceptance criterion: CV of- (b)(4)-%;the result of - (b)(4)- series of dilutions: - (b)(4)- %);

Linearity (the measured activity of every independent dilution was plotted against the theoretical value; the theoretical activity per dilution was calculated from the average activity of - (b)(4)- assays (-- (b)(4)---); acceptance criteria: slope (b)(4) , intercept - (b)(4)- and - (b)(4) ;the results are within the target values);

Range (acceptance criterion: with which the results show a sufficient reproducibility, above; the results: the assay is linear between - (b)(4)- and - (b)(4)- ;

Sensitivity (calculated from the separate titration curves of the concentrate - (b)(4)- of the average activity of - (b)(4) ---------- extinction unit per unit of C1INH);

Determination limit (acceptance criterion: CV NMT (b)(4) % for the concentration range of the assay; the limit was determined by dilutions of concentrate - (b)(4)- ; the results revealed significant differences in the average values, but fulfilled the requirement of (b)(4) %); determination limit is --- (b)(4)----- ;

Robustness was evaluated by test results from (a) different batches from --(b)(4)-- kit using historical data (a total of - (b)(4)- observations) of the serum control sample

<1562>, (b) two brands of ----------(b)(4)---------- for concentrate --(b)(4)---- , and (c) three types of ----------(b)(4)---------- and temperature setting on the plate reader at (b)(4)---- and (b)(4)- °C. (a) There was no difference in results between two brands of --- (b)(4)---------- ; (b) the results from ---------- (b)(4) ------ are not identical (---(b)(4)----- provided a different average results); (c) no significant impact of the stated temperatures on the results;

Matrix effects (C1INH activity was determined for test mixtures of plasma and concentrate - (b)(4)- , followed by the regression analysis; the results lie within the target values (slope slope - (b)(4)-- , intercept - (b)(4)- and - (b)(4)-), thus, the degree of purity of the sample does not play a significant role.

Clinical assay validation

Antigenic C1INH assay

-(b)(4)------ assay for quantification of C1INH by using --------(b)(4)---------- was validated by --(b)(4)---------. The method is based on immunochemical reaction of C1INH with specific antibodies, which ----------(b)(4)----------. The -(b)(4) -- is proportional to the ----(b)(4)---------- of the - (b)(4)---- , and therefore, to C1INH concentration.

Precision (acceptance criterion: inter-assay CV NLT (b)(4) %; two levels of controls were the tested on (b)(4) different runs results: for mean of - (b)(4)- mg/L, CV =- (b)(4)- %; for mean of - (b)(4)- , CV =- (b)(4)- %; the data met the criterion);

Range (acceptance criterion: manufacturer's cutoff –- (b)(4)-- mg/L; the reference interval was represented by (b)(4) serum samples obtained from healthy donors; mean:-(b)(4)-- mg/L, SD (b)(4) ; the results: all- (b)(4)- donor's values were above the cutoff value of - (b)(4)- mg/L);

Comment : only the lower level of the assay (- (b)(4)- mg/L) is considered, but not an interval validated.

The firm provided Inter-laboratory comparisons for the determination of C1INH content in- (b)(4) -samples performed by --- (b)(4)---------- . All- (b)(4) -results from ---(b)(4) ------ were "- (b)(4)- mg/L" (reference range- (b)(4)-), the- (b)(4) -results varied from -- (b)(4) ---------- within the reference range of- (b)(4)- mg/L. Based on these results (Table 6, chapter 2.7.1, volume 1.1, Module 2) the sponsor concluded that "-(b)(4)- specimens tested by both labs showed 100% correlation".

Functional C1INH assay (- (b)(4)- **)**

The - (b)(4)- C1INH Enzyme Immunoassay assay for the quantification of functional inhibitor in human plasma (validated by -- (b)(4)-------- .) is a 4-step procedure:

(1) ----------;

(2) ----------(b)(4)----------;

(3) ----------;

(4) ----------.

The following parameters are reported in the validation:

Standard values (the standard curve was generated from absorbance data at- (b)(4) -nm for each standard sample as follows: S1 (C1INH target % function- (b)(4)- , calculated % function- (b)(4)-), S2 (-- (b)(4)--- , respectively), S3(- (b)(4) -and - (b)(4)- , respectively), S4 (-- (b)(4)--------- , respectively), S5 (-- (b)(4)---).

Precision (intra-and inter-assay CV)

Intra-assay CV (acceptance criterion: CV NLT- (b)(4)- %;the test results for- (b)(4)- determinations:for mean of- (b)(4) %,CV=- (b)(4) %; for mean of- (b)(4)- %, CV=- (b)(4)- %; the data met the acceptance criterion);

Inter-assay CV (acceptance criterion: not defined; the test results for (b)(4) determinations: for mean of- (b)(4)- %, CV = - (b)(4)- %; for mean of - (b)(4)- %, CV = - (b)(4)- %);

Linearity was evaluated using- (b)(4)- dilutions (---- (b)(4)----------) of the serum from- (b)(4) -donors; the results showed that this functional assay is non-linear across the entire dynamic range, but it is linear in the deficient range, i.e., -- (b)(4)-- %.

Comparison of the test results for- (b)(4) -clinical samples by --------- (b)(4) ---------- showed (Table 7) that- (b)(4)- results that were shown abnormal by the -- (b)(4) ----- were normal as determined by the --- (b)(4)----------.

Plasma C4 Concentrations

The assay for measurement of plasma C4 concentrations was performed by ----- (b)(4)---------- , a US Clinical Lab Improvement Amendments (CLIA)-certified lab, allows to measure complement C4 in serum by -- (b)(4) ---------- using the --- (b)(4) ---------- developed for use in clinical lab.

This is an FDA 510(k)- approved kit #-- (b)(4) --- (3/27/2000).

The turbidity of the sample after reaction with antibody specific to human complement C4 (read using the ---------- (b)(4) ---------- is proportional to the concentration of C4 in the sample.

The following parameterswere validated**:** Dynamic range (- (b)(4) --- mg/dL); Reference Interval (- (b)(4)- mg/dL); Analytical Sensitivity (- (b)(4)- mg/dL); Total Imprecision (at ---------- (b)(4)---------- ; Analytical Specificity (- (b)(4----- , and moderate to strong - (b)(4) - and - (b)(4) -- interfere with the assay); Stability(Ambient: (b)(4) days; Refrigerated: (b)(4) days; Frozen: - months); Carry-over (No carry-over was observed for measuring low concentration sample of known concentration after measuring high concentration sample).

LPI provides data on comparison of clinically defined samples by using -- (b)(4) --------- and --- (b)(4) ---------- was performed by analysis of (b)(4) specimens: --------- (b)(4)----------.

M289/1.0 Sanquin method for determination of sucrose

Determination of sucrose is performed by using (b)(4) . The following parameters were validated:

Linearity range (- (b)(4)- mmol/L as determined from- (b)(4) -runs and the integration of the- (b)(4) -at around- (b)(4)- min, using - (b)(4)- as an internal standard);

Repeatability (determined by measuring of the sucrose concentration of C1INH-nf and the reference solution (b)(4) times by the same analyst, within - (b)(4)- day, on the same sample by using the same equipment and materials; the results: for C1INH-nf: CV -- (b)(4) ---------- %; reference solution: CV - (b)(4)- %);

Reproducibility was determined by measuring of the sucrose concentration of C1INH-nf and the reference solution (b)(4) times by different analysts on- (b)(4) -different days; the results: for C1INH-nf: CV -- (b)(4) ---------- %; reference solution: CV - (b)(4)- %);

Accuracy (trueness) was determined by spiking the sample with known amount of sucrose; the added amount of sucrose was then plotted against the measured sucrose amount; the results: for batch 1 (- (b)(4)- runs),average recovery –- (b)(4)- %, - (b)(4)- ; for batch 2 (- (b)(4) -run), average recovery –- (b)(4)- %,

-(b)(4)- ; for batch 3 (- (b)(4) -run), average recovery –- (b)(4)- %,

-(b)(4)- ;

Limit of detection (determined as the lowest standard of the calibration curve, - (b)(4)- mmol/L);

Limit of quantification (- (b)(4)- mmol/L);

Specificity and selectivity: the assay is shown to be not specific for sucrose, but selective for it as shown by measuring sucrose with the internal standard (-- (b)(4)---), - (b)(4)- C1INH samples, and ---------- (b)(4)----------.

M303Determination of citrate by ---- (b)(4) ----------

The following parameters were validated:

Linearity range (- (b)(4)- mmol/L as determined from- (b)(4) -runs, - (b)(4)- for each of - (b)(4)- citrate concentrations; -- (b)(4)---);

Repeatability (determined by measuring of the citrate concentration of C1INH-nf and the reference solution- (b)(4)- times by the same analyst, within- (b)(4) -day, on the same sample by using the same equipment and materials; the results: for C1INH-nf: CV ---- (b)(4) -------- %; reference solution: CV - (b)(4)- %);

Reproducibility (determined by measuring of the citrate concentration of C1INH-nf and the reference solution- (b)(4)- times by different analysts on - (b)(4)- different days; the results: for C1INH-nf: CV -- (b)(4) ---------- %; reference solution: CV - (b)(4)- %);

Accuracy (trueness) was determined by spiking and recovery for- (b)(4) -batches;the results for batch- (b)(4)- average recovery -- (b)(4)---------- ; for batch ---- (b)(4)---------- , and for batch ----- (b)(4)---------- , respectively;

Limit of detection/Limit of quantification were determined as the lowest standard of the calibration curve,- (b)(4)-- mmol/L);

Specificity and selectivity : the assay is shown to be not specific for sucrose, but selective for it as shown by measuring sucrose with the internal standard (----- (b)(4) ------ C1INH samples, and ---------- (b)(4)----------);

Specificity and selectivity (investigated by measuring solutions with different components, as shown above for sucrose method, including the same amino acids and - (b)(4) ------ - used the internal standard); there was no components in C1INH-nf that show interference with the - (b)(4)- of the internal standard and citrate; therefore, the assay is selective for citrate.

Protein impurities

The presence of each one of the- (b)(4) -marker proteins (impurities) is being monitored to evaluate an effectiveness of removal of plasma protein impurities (according to the agreement with FDA). These chosen marker proteins are- (b)(4) -(Sanquin method @2562/3.0), - (b)(4) - (Sanquin method @2575/4.0),- (b)(4) (Sanquin method @2578/2.0), --- (b)(4) --- (Sanquin method @25738/2.0), and- (b)(4) -(Sanquin method @4693/1.0), that are validated (chapter 3.2.S.4.3).

M080/10.0Sanquin method for quantitative measurements of free amino acids

Sanquin M080/10.0 for quantitative determination of amino acid additives in the samples is based on the amino acid separation on ---- (b)(4) ---------- using buffers of

different ---- (b)(4) ---------- and - (b)(4)---------- , as well as variation in - (b)(4)- temperature (--- (b)(4) ---------- °C). The - (b)(4) ------- is mixed with -- (b)(4) ---- reagent followed by -- (b)(4) ------ determination at - (b)(4) ----- and - (b)(4)- . All - (b)(4) -- show a good - (b)(4) ----- of threonine (retention time- (b)(4)- min), alanine(- (b)(4)-), valine (- (b)(4)-).

Validation report (3.2.P.5.3 presents data for the reference solutions and (b)(4) batches to validate the following parameters:

Linearity (for threonine: - (b)(4) ----- mmol/l, alanine: - (b)(4) --- mmol/l, valine: -- (b)(4) -- mmol/l);

Repeatability (determined from- (b)(4)- measurements for each amino acid; CV (%) determined for reference solutions: threonine – (b)(4) , alanine –- (b)(4)- , valine –- (b)(4)- ; for amino acids in C1INH-nf: threonine – --- (b)(4)---------- ; alanine – ---- (b)(4)-- ---- ; valine – -- (b)(4)--------);

Reproducibility (determined from (b)(4) measurements for each amino acid; CV (%) determined for amino acids in C1INH-nf: threonine – -- (b)(4)--------- ; alanine – ---- (b)(4)---------- ; valine – ---- (b)(4)---------- ; for reference solutions: threonine – - (b)(4-) , alanine – - (b)(4)- , valine – - (b)(4)-);

Trueness (determined for three batches by spiking of C1INH-nf with amino acid; batch 1: threonine - average recovery - (b)(4)- %, - (b)(4)------ ; alanine - average recovery --- (b)(4)---------- ; valine - average recovery -- (b)(4)---------- ; batch 2: threonine - average recovery -- (b)(4)---------- ; alanine - average recovery -- (b)(4)------ ; valine - average recovery -- (b)(4)---------- ; batch 3: threonine - average recovery ---------- (b)(4)-- ; alanine - average recovery ---- (b)(4)---------- ; valine - average recovery -- (b)(4)----------);

Limit of detection and quantification (defined as the lowest standard of the linearity assay; the result: for threonine –- (b)(4)- -mmol/l, for alanine –- (b)(4)- -mmol/l, and for valine –- (b)(4)-- mmol/l);

Specificity and selectivity (investigated by measuring solutions with different components: - (b)(4)---------- , standard amino acids, internal standard ---------- (b)(4) ---------- with internal standard, and (b)(4) spiked with standard; the results showed no components that interfere with amino acids and internal standard).

During reviewing, two information requests were conveyed to the sponsor by RPM Nannette Cagungan, as described below.

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

Since --- (b)(4)---------- assay was not listed in the final product specifications, on September 25, 2007 the information request was faxed to the firm that contained a few

clinical questions and one CMC related question. The firm's response (A) to CBER recommendation (Q) was provided in STN 125267/0.6 on October 15, 2007 as follows.

Q. : **Please, indicate whether the -** (b)(4) --- **test currently being used is comparable to the USP method. You indicated that --** (b)(4) -- **testing is not required for release of the product. We recommend that -** (b)(4) -- **be included as a release specification. Please, comment.**

A. : -- (b)(4) --- testing will be included as a release specification. The specific method currently used is Sanquin Method M343, which is comparable to the USP method. The specific limit that will be set for release is -- (b)(4)---- .

This assay is indicated in 3.2.S.4.2 Item 39AL as the EP compendial method (EP chapter - (b)(4)-).

Regarding the limit of - (b)(4) ------ (that is set relatively high in comparison with that of other plasma protein products) its acceptance was evaluated as following. According to t he reviewer's calculations, one weekly dose (1000 U) of C1INH (- (b)(4)---) may result in total of- (b)(4)-of - (b)(4)--------. The worse case scenario (two weekly doses of 1000 U) may lead to a higher - (b)(4) - level, yet, not more than- (b)(4)-. This is lower than- (b)(4)-, the maximal limit of - (b)(4)- that is referred as ---- (b)(4) ----------.

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

On November 7, 2007 an additional information request was faxed to the sponsor. On November 23, 2007 the responses (A) to the CBER questions (Q) were amended (STN 125267/0.7) as follows:

Q. 14: In your response letter dated October 15, 2007, you indicated that -- (b)(4) --- **testing will now be included as a final release specification.**

a. Please submit updated lot release protocols.

b. Please provide justification for setting the specification limit to - (b)(4) --- **.**

A. 14:

14(a)

Please find attached the updated release protocol (FPSL370LEV1 2.0) which has now been revised with respect to adding the - (b)(4)- content as a release requirement.

14(b)

The rationale for setting an acceptance limit for (-b)(4) --- of- (b)(4) --- is based on the following formula as given by both the USP and the EP:

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

--(b)(4)---------------------------------- -- -- -- -------------- -- ------------------------------------- -- -------------------------.

Reviewer's comment : the response is adequate.

Q. 18:

Please provide rationale for not including a total protein specification in the final product specifications (Module 3. 2. P. 5. 1).

A. 18 :

In the finished product specifications the following items and their limits are defined: C1INH activity: (b)(4) ---U/ml and Specific activity: (b)(4) U/mg. These parameters are release parameters.

To calculate the specific activity the protein content of a sample has to be determined. The specific activity (U/mg) -----------------------(b)(4)-- -- ---.

Reviewer's comment: we recommend the total protein to be added to the final product specification to allow a verification of the product specific activity in the lot release protocols.

Q. 19:

Please provide the rationale for specifically selecting alanine, valine and threonine as amino acid (b)(4) **and provide justification for the amounts used in manufacturing.**

A. 19 :

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

---------- ---------- ---------- ----------.

---------- ----------.

---------- **----------(b)(4).**

----------(b)(4)----------.

Q. 20:

Please provide the justification for the broad ranges for sodium, sucrose, and amino acid additives in the finished product specifications (*e.g.*, for threonine: (b)(4) **mmol/L).**

A. 20 :

The ranges for sodium, sucrose and amino acids have been set during the early development of Cetor® , a C1INH product for the Dutch market. These components are added as ----------(b)(4)---------- ---------- ---------- ----------. ---------- ---------- ---------- ----------.
All data for the finished products comply with the specification. However, there are not enough data yet to redefine the ranges for the analysis of excipients in the finished product. Therefore, it is proposed to follow during the next six months the manufacturing of commercial batches and redefine tighter ranges as appropriate based upon the results.

Reviewer's comment : the tighter ranges for sodium, sucrose, and amino acid additives in the finished product specifications must be provided within next 6-8 months (to be submitted in a form of Changes Being Effective).

Q. 21:

Please clarify whether the two assays employed for the determination of functional CIINH ("Functional CIINH" in 2.7.1.4.2 and "Activity assay using -- (b)(4)

---- substrate" in 3.2.S.4.3) provide comparable results on quantification of functionally active ClINH in human plasma (serum and/or finished product).

A. 21 :

The assay used for determination of functional CIINH described in 2.7.1.4.2 is a US FDA licensed assay for clinical determination of functional C1INH in human plasma or serum. The assay using --- (b)(4) -----substrate described in 3.2.S.4.3 is an in-process test used by Sanquin to determine the functional ClINH in the in-process intermediates and final Cinryze product. Because the intended uses of the two assays are different, the two assays have not been compared to each other.

Reviewer's comment: the comparability of the test results for functional C1INH obtained by using -- (b)(4)----- assay and by functional --- (b)(4-------- has been recently compared by ----------(b)(4)----------. Quantitatively, the two assays showed good correspondence (92%). The firm's response is acceptable.

Q. 22 :

Regarding the comparison of ---- (b)(4) --------- results for determination of CIINH by ---------- (Module 2, volume l, 2.7, Table 6):

a. Please provide a valid reference range for the - (b)(4) ---- assay.

b. Please explain the basis for concluding that the results for - (b)(4)- are comparable to - (b)(4)----- .

A. 22:

22(a)
-- (b)(4) -- assayed serum samples from (b)(4) apparently healthy individuals. The range calculated from these results was -------- (b)(4)-- mg/dl (mean- (b)(4) -SD). The manufacturer's recommended reference range is-- (b)(4) ---mg/dl. Since the kit is FDA approved, and was tested on (b)(4) individuals, we adopted the manufacturer's reference range.

22(b)

The r^2 value for the two different methods run in two different laboratories was -- (b)(4)---. Please see the correlation graphs on page 8 of the validation document.

Reviewer's comment : the response is acceptable.

Q. 23:

Regarding the ------ (b)(4) ---------- analysis of CI-inhibitor:

a. Please indicate whether this method was validated.

b. Please define the - (b)(4) ---that are flanking the CI-inhibitor - (b)(4)- on the -- (b)(4)------ .

c. Do - (b)(4) - data correlate with native --- ---- (b)(4) --- data on the molecular integrity of the product? Please comment.

A. 23:

23(a)

The - (b)(4)- method M282 is performed according to the European Pharmacopoeia (- (b)(4)---). As this method is a EP method no additional validation was performed. For analysis of C1-inhibitor protein containing samples, method M916 was written based on M282. The running conditions of the (b)(4) described in M916 and M282 are similar.

At the start of the analysis of a series of protein containing samples, protein standards are analyzed to ensure a correct - (b)(4)-- performance. The- (b)(4)- is measured to ensure the quality of the ----- (b)(4)---------- . The - (b)(4) -is replaced when the - (b)(4)- is below -- (b)(4) ---- theoretical plates (using a ------ (b)(4)----------).

23(b)

- (b)(4) - analysis of a C1-inhibitor product results in a characteristic -- (b)(4) ----- consisting of a main C1-inhibitor protein -- (b)(4) -- additional- (b)(4)- . Several attempts have been taken to identify these additional - (b)(4)-- , but none of the experiments were conclusive.

A- (b)(4)- marker used before each- (b)(4)- run indicates that the first- (b)(4) -contains protein(s) of-- (b)(4)--- , the second- (b)(4)- contains protein(s) of - (b)(4)-- , whereas the third - (b)(4)- - contains protein(s) between -- (b)(4)---------- . The second - (b)(4)-- contains C1-inhibitor, which has been identified using the C1-inhibitor- (b)(4) - assay (@2587). The discrepancy in the molecular weight of C1-inhibitor compared to the - (b)(4)- marker is most likely caused by the - (b)(4)---------- of C1-inhibitor. Neither additional- (b)(4)-- results from the buffer components ----- (b)(4)---------- .

23(c)

Analysis of C1-inhibitor protein containing samples has always been performed using- (b)(4)- . We have not performed --- (b)(4) ---------- with Cl-inhibitor products and a correlation between - (b)(4)- data with ------- (b)(4) ---------- data on the molecular integrity of the product cannot be made. However, the integrity of the Cl-inhibitor protein

has been investigated using -- (b)(4) ---------- (Report 036-068 in 3.2.P.3.5. item 4). During the manufacturing process from plasma to Cl-inhibitor finished product, the -- (b)(4)--- -- point of the protein remained the same. In addition, the percentage in active C1-inhibitor has been determined in finished product. The percentage of inactive C1-inhibitor in finished product is- (b)(4)- %, which is similar to the percentage of inactive C1-inhibitor in plasma (Report 036-082 in 3.2.P.3.5 item 9). Both findings show that the integrity of C1-inhibitor protein is not affected by the manufacturing process.

Reviewer's comment: the response is adequate.

COMMENTS

From the standpoint of analytical methods validation, this part of the Original BLA STN 125267 for the manufacturing of C1 Esterase Inhibitor (Human) [Cinryze ™] is approvable with the above two recommendations (pp. 13 and 14) to be accepted by the firm.