

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

209210Orig1s000

PRODUCT QUALITY REVIEW(S)



**Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research**

Office of Biotechnology Products
Division of Biotechnology Review and Research III

Memorandum of Review

STN: NDA209210
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Serial: 0000 (2), 0022(24), and 0029(30)
Subject: review on the validation report of ELISA to detect anti-drug IgG/IgM/IgA antibodies

Submit Date: December 21, 2016
Review Date: August 9, 2107, September 14, 2017, and September 30, 2017

Primary Reviewer: Rong Wang, Ph.D., Product Quality Reviewer
CDER/OPQ/OBP/DBRR III

Secondary Reviewer: Ramesh Potla, Ph.D., Product Quality Team Leader
CDER/OPQ/OBP/DBRR III

Tertiary Reviewer: Susan Kirshner, Ph.D., Review Chief
CDER/OPQ/OBP/DBRR III

RPM: Richard Whitehead
Consult: Immunogenicity Consult

Sponsor: AstraZeneca Pharmaceuticals
Product: BYDUREON BCise®, Exenamide once-weekly suspension (EQWS)

Indication: Treatment of type 2 diabetes mellitus (T2DM)
Route of Admin: SC injection of 2mg once weekly

Clinical Division: Office of New Drugs, Division of Metabolism and Endocrinology Products

Wrap up meeting: September 14, 2017
PDUFA date: October 20, 2017

RECOMMENDATION:

The anti-exenatide antibodies (ADA) assay uses Enzyme-linked immunosorbent assays (ELISA) to screen for, confirm, and titer ADA, as well as detect cross-reactivity of the

ADA to GLP-1 and glucagon. The sensitivity of the ADA binding assay and the cross-reactivity assay is not adequately established. The Sponsor should validate ADA binding assay sensitivity and determine the low positive control concentration with a 1% false positive rate to ensure the assay performance. In addition, the Sponsor should develop new assays to directly assess anti-GLP-1 or glucagon antibodies for the cross-reactivity of the ADA to GLP-1 or glucagon. The Sponsor calculated the screening cut point using a parametric method on the log-normally distributed data with a false positive rate of 5% which is acceptable. The screening cut point for healthy subjects, calculated in the validation assay, is 0.066 lower than the screening cut point for T2DM patients. Therefore it is acceptable that the same cut point (0.154) for healthy subjects was used in all studies since the risk to have inaccurate results is very low. The titer-confirmatory assay is suitable with the confirmatory cut point to be (b) (4)% inhibition of the absorbance. For the ELISA screening assay, the Sponsor demonstrated the precision, specificity, selectivity, and robustness. Tests were performed to show positive controls sample stability.

Postmarketing commitments PMCs to the Sponsor:

The review team decided to request additional immunogenicity assay validation studies and clinical sample testing as post marketing commitments.

Three postmarketing commitments (PMCs) will be issued:

- (b) (4)
- (b) (4)
- Test samples from clinical trials BCB118 and BCB120 for the presence of anti-GLP-1 and anti-glucagon antibodies using (b) (4)
- (b) (4)
- Validate the sensitivity of the version of ELISA-0308 method used for the detection of anti-exenatide antibodies (ADA) in patient samples collected in NDA209210 clinical studies BCB118 and BCB120.

PMC Rationale:

1. Bydureon-BCise shares sequence homology with GLP-1 and glucagon. Therefore, theoretically antibodies to exenatide may also recognize and bind to GLP-1 or glucagon. In previous studies with Bydureon the Sponsor found that anti-drug antibodies did not cross-react with either GLP-1 or glucagon. The assay used in those studies only indirectly assessed for the presence of anti-GLP-1 and anti-glucagon antibodies by evaluating the ability of GLP-1 and glucagon to inhibit the anti-exenatide signal in the assay. Because many subjects in those studies had robust anti-exenatide antibody the presence of anti-GLP-1 and anti-glucagon antibodies many have been masked. GLP-1 and glucagon are endogenous human peptides. Therefore, it is important to understand whether anti-drug antibodies may interfere with the activity of those peptides. This may be performed as a post-marketing study because

currently the risk is theoretical as we are unaware of cases of GLP-1 deficiency associated with Bydureon use.

2. The assay sensitivity was not determined for the version of ELISA-0308 method used for the detection of anti-exenatide antibodies (ADA) in patient samples collected in NDA209210 clinical studies BCB118 and BCB120 per validation report REST090118R1. To fully understand assay performance the sponsor should determine the sensitivity of the assay.

REVIEW

I. Introduction

This application is for approval of BYDUREON BCise® (exenatide once-weekly suspension, EQWS) for the treatment of type 2 diabetes mellitus (T2DM). In this review I evaluate the assays used to test the ability of anti-exenatide antibody bind to EQWS, as well as the cross reaction activity of anti-exenatide antibody to other peptides.

BYDUREON BCise® contains the same active ingredient (i.e. exenatide) as the commercial products BYETTA® (exenatide) injection (NDA 021-773) and BYDUREON® (exenatide extended-release) for injectable suspension (NDA 022-220). The table below compares these three products.

	BYETTA®	BYDUREON®	BYDUREON BCise®
Approval date in the US	28 April 2005	27 January 2012	Under FDA review
Indication	improve glycemic control in adults with T2DM	improve glycemic control in adults with T2DM	improve glycemic control in adults with T2DM
Route of Admin	subcutaneously injection	subcutaneously injection	subcutaneously injection
Known as	exenatide twice daily	exenatide once weekly	exenatide once-weekly suspension
Formulation	no microsphere	extended-release aqueous suspension formulation contains biodegradable polymeric microspheres; provided as a powder to be combined with an aqueous vehicle to form a suspension for injection	extended-release non-aqueous suspension formulation, contains the same drug substance, drug load, and extended-release microspheres as BYDUREON, but with a non-aqueous medium-chain triglycerides (MCT) vehicle
Presentation	prefilled pen	single-dose tray or dual-chamber pen	autoinjector

Table prepared by the Reviewer.

The drug delivery technology for the EQWS formulation and BYDUREON formulation uses biodegradable polymeric microspheres. The microspheres consist of exenatide (b) (4)

and sucrose (b) (4) encapsulated within biodegradable poly (D,L-lactide-co-glycolide) (PLG) microparticles that entrap exenatide and provide extended release of the peptide over days to months. Once injected subcutaneously, exenatide is released gradually as the microspheres biodegrade (b) (4).

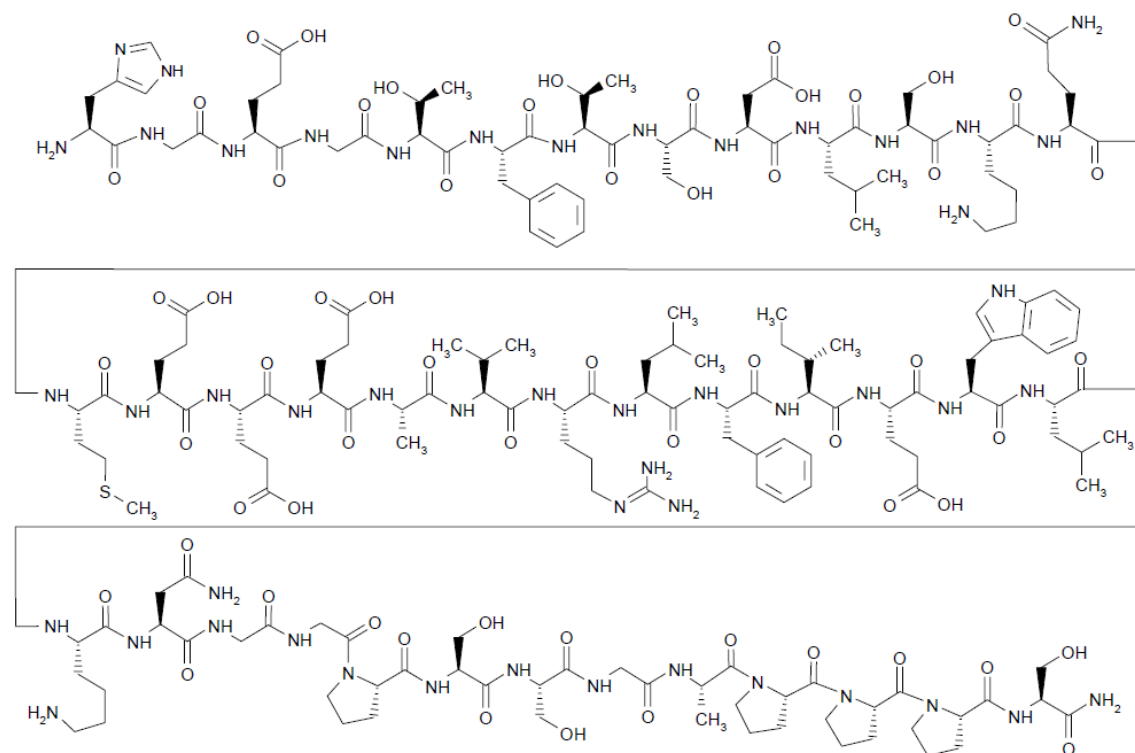
Exenatide

Exenatide (AC2993) is synthetic exendin-4 which is a hormone found in the saliva of the Gila monster. Figure 1 below (from NDA_021773) shows the structural formula of exenatide. The sequence of this 39 amino acid peptide is: H-His-Gly-Glu-Gly-Thr5-Phe-Thr-Ser-Asp-Leu10-Ser-Lys-Gln-Met-Glu15-Glu-Glu-Ala-Val-Arg20-Leu-Phe-Ile-Glu-Trp25-Leu-Lys-Asn-Gly-Gly30-Pro-Ser-Ser-Gly-Ala35-Pro-Pro-Pro-Ser-NH2.

Molecular formula is C184H282N50O60S with molecular weight of 4186.6 Daltons. (b) (4)



Figure 1: Exenatide Structure



Mode of Action

Exenatide shares a 53% amino acid homology to human glucagon-like peptide-1 (GLP-1) and is a GLP-1 receptor agonist. Exenatide binds to the human glucagon-like peptide-1 receptor (GLP-1R) and stimulates glucose-dependent insulin secretion from pancreatic beta cells and has additional antihyperglycemic functions. Exenatide has been shown to

reduce fasting and postprandial plasma glucose concentrations in nonclinical and clinical studies. In clinical studies, BYDUREON has shown sustained glycemic improvement and weight reduction in patients with T2DM.

Clinical Studies in this NDA

This submission is based on one Phase 2 study (BCB110) and two Phase 3 studies (BCB118 and BCB120). The trials included in this submission were carried out in 88 centers in the US under IND 107815. The table below lists the clinical studies of EQWS NDA.

Table 1 Overview of studies in the clinical development program for EQWS in patients with type 2 diabetes mellitus

Study code (Publication reference) CTD location Study period	Design	Primary and secondary efficacy variables	Background treatment	Dose/comparator	Duration of treatment	Number of randomized subjects
Phase 2						
BCB110 (Wysham et al 2016) 5.3.4.2 29-Apr-2009 to 08-Aug-2009	Two-cohort single and repeat dose PK, tolerability, and safety study	Primary: no primary efficacy variable Secondary: HbA1c, FPG, body weight	Cohort 2: Metformin, TZD, or a combination of metformin and a TZD	Cohort 1: EQWS 10 mg in healthy subjects	Single dose	EQWS 10 mg (30)
				Cohort 2: EQWS 2 mg/placebo in T2DM subjects	12 weeks	EQWS 2 mg (23) Placebo (12) (2:1 randomization ratio)
Phase 3						
BCB118 (NA) 5.3.5.1 28-Jan-2013 to 19-Aug-2014	Randomized open-label, parallel-group, comparator- controlled, 2-arm study	Primary: HbA1c Secondary: HbA1c <7%, FPG, body weight, 2-hour postprandial plasma glucose	Diet and exercise alone or with a stable regimen of metformin, SU, pioglitazone, or a combination of any 2 of these agents	Controlled treatment period: EQWS 2 mg /BYETTA	28 weeks (controlled/ randomized)	EQWS 2 mg (229) BYETTA (148) (3:2 randomization ratio)
				Extension period: EQWS 2 mg	+ 24 weeks (uncontrolled / extension)	EQWS 2 mg (375)
BCB120 (NA) 5.3.5.1 08-Feb-2013 to 04-Apr-2014	Randomized open-label, parallel-group, comparator and placebo-controlled 3-arm study	Primary: HbA1c Secondary: HbA1c <7%, FPG, body weight, 2-hour postprandial plasma glucose	Metformin	EQWS 2 mg/ sitagliptin 100 mg orally once daily /placebo orally	28 weeks	EQWS 2 mg (182) Sitagliptin (122) Placebo (61) (3:2:1 randomization ratio)

CTD common technical document; EQWS exenatide once weekly suspension; FPG fasting plasma glucose; HbA1c glycosylated hemoglobin; nNA not applicable; SU sulfonylurea; T2DM type 2 diabetes mellitus; TZD thiazolidinedione
Source: BCB110, BCB118, and BCB120 Clinical Study Reports

Results of Immunogenicity Studies

The analyses of anti-exenatide antibodies performed to support the three EQWS clinical studies (BCB110, BCB118, and BCB120) were conducted at (b) (4). The assay was originally developed and validated at (b) (4) where it was revalidated (see REST090118 and REST090118R1). In summary, the Sponsor evaluated the ADA and determined the titer for the ADA positive samples.

Overall, 74.0% of EQWS-treated subjects developed antibodies to exenatide during Phase 3 with 42.2% of EQWS-treated subjects developing low titer (< 625) and 31.8% developing high-titer (≥ 625) antibodies. The percentage of antibody positive subjects peaked at about 16 weeks of treatment followed with a slight reduction. The incidence of potentially immune-related AEs was 25.4% in antibody-positive subjects (29.6% and 22.3% in high- and low-titer subjects respectively) and 17.6% in antibody-negative subjects. 21.0% of antibody-positive subjects (27.2% and 16.3% in high- and low-titer subjects, respectively) and 15.7% of antibody negative subjects developed potentially immune response related injection site-related AEs.

II. Review of the Current Submission—" NDA209210 Method Validation Report: **REPORT #:** REST090118R1: Validation of an ELISA Method for the Detection of Anti-AC2993 Antibodies in Human Serum (b) (4),,

Enzyme-linked immunosorbent assays (ELISA) to screen for and confirm the anti-drug antibodies (ADA) in human serum were developed and validated by (b) (4). The method was validated using serum collected from normal humans.

During the review of this submission, a list of items need to be explained or clarified. The following IR was sent to the Sponsor on August 18, 2017:

We are currently reviewing NDA209210 Method Validation Report: **REPORT #:** REST090118R1: Validation of an ELISA Method for the Detection of Anti-AC2993 Antibodies in Human Serum (b) (4). To complete our review of your submission provide a response to the comments below:

1. FDA Guidances for Industry- Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products (2016) and Immunogenicity Assessment for Therapeutic Proteins (2014) recommend that confirmed anti-drug antibody (ADA) positive samples be tested for neutralizing activity using a neutralization assay. You did not provide any information on neutralizing antibody (NAb) assays or testing. Provide the neutralization assay validation report and results from the NDA 209210 clinical trials. It is important to characterize any neutralizing activity of the ADAs because the impact of ADAs on safety and efficacy may correlate with NAb activity rather than ADA incidence.
2. You provided results from the evaluation of human anti-exenatide antibodies for their cross-reactivity to glucagon like peptide -1 (GLP-1), glucogan, or sequence irrelevant peptides. However, these results were not from subjects who participated in the NDA 209210 clinical studies. Provide the validation report for

evaluating the cross-reactivity of anti-exenatide antibody to GLP-1, glucogan, and sequence irrelevant peptides, and the pertinent results from the NDA 209210 clinical trials. If different assays were used for different studies in NDA 209210 then also provide a table indicating which assays were used in each study.

3. FDA draft Guidance for Industry- Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products (2016) recommends that ADA assays be assessed for sensitivity, selectivity, prozone effect, robustness, and minimum required dilution. You did not provide adequate information on these assay parameters. Specifically:
 - a) You did not provide information on the sensitivity of your ADA screening assay. The sensitivity of the ADA screening assay is a key parameter and should be provided. The assay screening sensitivity is defined as the lowest concentration at which the antibody preparation consistently produces either a positive result or readout equal to the cut point determined for that particular assay. Provide the screening assay sensitivity.
 - b) You did not provide information on the selectivity of your ADA assay. Interference from the sample matrix may enhance or inhibit the activity of the therapeutic protein product, leading to incorrect results. Provide the assessment of assay selectivity, for example, evaluating factors present in the serum samples from diabetic patients that may potentially affect the ADA assay performance.
 - c) You did not provide information on whether there is any prozone effect for your ADA assay. High ADA concentrations may have a prozone effect by reducing the signal in the assay, leading to false negative results. Provide an assessment of the prozone effect for your ADA assay.
 - d) You did not provide information on the robustness of your ADA assay. Provide information showing that the method is robust, including ranges on critical method parameters, such as incubation times and temperatures.
 - e) You did not provide a description of how you determined the Minimum Required Dilution (MRD). Your established MRD for screening samples is 1/25. Provide a description on how the MRD is established.
4. A low positive control with an ADA concentration slightly above the assay sensitivity is used to ensure the assay sensitivity is consistent across assay runs and low positive samples are detected. You stated that the low positive control (LPC) was prepared by diluting the positive control 45.6 fold. However, the concentration of LPC was not provided. Therefore, the adequacy of the low positive control cannot be evaluated. Provide the concentration of the low positive control in mass units per mL neat serum. In addition, provide the high positive control (HPC) concentration.
5. Your description of screening cut point calculation did not include information on the data normality. You used a parametric method ($\text{mean} + 1.645 * \text{SD}$) to

- calculate cut point. Using a parametric method in cut point calculation requires that the dataset is normally distributed. In addition, you used the Grubbs' Outlier test for excluding outliers indicating that the data is assumed normally distributed. However, you did not state whether the data were normally distributed. Provide information showing the distribution of the data.
6. You used a floating cut point for the screening cut point, however there is no statistical analysis to support the use of a floating cut point. Provide information on the statistical approach you used for setting the floating cut point.
 7. You proposed the following formula to calculate the percent inhibition in your confirmatory assay: $[(\text{Mean signal of sample} - \text{Mean signal of treated sample}) / (\text{Mean signal of sample} - \text{Mean NC})] * 100$. You stated that the sample is confirmed positive if the percent inhibition $\geq$ (b)(4)% inhibition of signal upon treatment with the free drug. However, your approach for confirming the ADA as positive in your confirmatory assay is not in agreement with the FDA guidance, since it will lead to an increased risk of incorrect results. The guidance recommends using 1% false positive rate for confirmatory cut point calculation. You should calculate the confirmatory cut-point using a 1% false positive rate. Table 5A shows OD values of HPC and LPC samples are less than screening cut point (0.154) when treated with AC2993 at 10.0 µg/mL. However, you treated the samples with 100 µg/mL AC2993 in your confirmatory assay. Justify the use of 100 µg/mL AC2993 in your confirmatory assay.
 8. It is not clear from your submission whether the method used to test samples from clinical trial BCB120 is the same as the method used to test samples in trial BCB118. If the methods are different, state the ADA assay used for the detection, confirmation and titration of the anti-exenatide antibody in the clinical trial BCB120.
 9. It is unclear what cut points were used for assessing samples in the different clinical trials. Provide a justification if you used the screening cut point derived from the validation report in the NDA 209210 clinical trials immunogenicity assays. Provide a demonstration that the screening cut point derived from the normal human serum is similar to the one derived from the serum from target population handled the same way as clinical samples.
 10. Section 5.3.1.4 provides report REST090118R1, which is the validation report for version 6 of the screening, titering, and confirmatory assays. However, in section 5.3.5.1 MB001003a clinical study report on pages 31320 and 31322 you indicate that version 11 was used to test clinical samples and on page 31323 you provide version 11. Providing information in this way is inappropriate because it is difficult for FDA to locate all the information needed for the review. Assay

validation reports should be provided in the section 5.3.1.4. If different assays were used for different studies in NDA 209210 then also provide a table indicating which assays were used in each study.

The Sponsor provided a response to our IR on September 04, 2017 with five sets of documents:

1. Final Report for **REST080154R1**: “Results from the Evaluation of Anti-AC2993 Antibody Cross Reactivity to GLP-1 and Glucagon in Human Serum Specimens from Clinical Study LAR-105 using an Enzyme-Linked Immunosorbent Assay.”
2. Final Report for **REST080375R2**: “Validation Report: Enzyme-Linked Immunosorbent Assay (ELISA) to Detect Human Antibodies against Exenatide in Serum.”
3. Final Report for **REST080584R1**: “Results from the Partial Validation of an Anti-Exenatide (AC2993) Antibody Cross Reactivity ELISA using Tetramethylbenzidine (TMB).”
4. **CPD-Exenatide-JZ-001**: “Re-Analysis of Screening Cut Point Factor for Validation of an ELISA Method for the Detection of Anti-AC2993 Antibodies In Human Serum (REST090118R1).”
5. **CPD-Exenatide-JZ-002**: “Screening Cut Point Factor for an ELISA Method for the Detection of Anti-AC2993 Antibodies in Serum Type 2 Diabetes Mellitus Patients.”

The review below incorporates the Sponsor’s response to our IR.

note: QC is positive control; HQC is high positive control; LQC is low positive control. NQC is negative control.

1. Summary of validation results for anti-AC2993 antibody assay

The anti-exenatide antibody assay uses an ELISA method to determine the presence and titers of anti-exenatide antibodies in human sera. The summary of the validation results for the anti-AC2993 antibody assay are listed in the table below.

Table 5 Anti-exenatide antibody assay validation results at (b) (4)
(b) (4)

Assay parameter tested	Result
Minimum required dilution	1:25
Sensitivity	ND
Intra-assay precision (%CV)	7.2% to 7.3%
Inter-assay precision (%CV)	8.3% to 12.4%
Assay cut point and normalization factor (N-factor)	Assay plate cut point (OD) = mean plate NQC (OD) + N-factor N-factor = 0.104 (OD)
Specificity assessment (addition of 100 µg/mL exenatide)	>96.9% mean inhibition
Drug tolerance	ND
Stability	
Freeze/thaw	Stable through 8 cycles
Short-term ambient (15°C to 30°C)	Stable up to 21 hours
Short-term refrigerated (2°C to 8°C)	Stable up to 26 hours
Long-term frozen (-80°C to -60°C)	Stable for at least 1 year ^a

^a Based on Shankar et al 2008.CV Coefficient of variation; ND Not determined; NQC Negative control sera; OD Optical density.
Source: REST090118R1.

note:

- 1) The assay sensitivity was evaluated in a separate report and is discussed in Section II 4.5.
- 2) Drug tolerance was assessed as a part of assay specificity and is discussed in Section II 4.6.
- 3) The assay selectivity was evaluated in a separate report and is discussed in Section II 4.7.

2. Instruments and Reagents

The instruments include Flat Bottom, Nunc 96 well Maxisorp Microtiter® plates, Microplate reader (Molecular Devices SpectraMax), and Data Analysis Package (SoftMax® Pro). The reagents are listed in the table below.

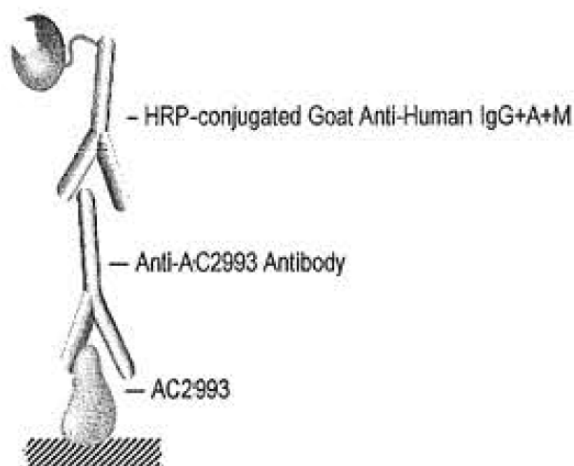
Controls and Others	Information
Coating Material	AC2993 coating material
PC (positive control)	human anti-exenatide sera pooled from ADA positive samples
LPC (low positive control), and HPC (high positive control)	LPC was diluted at 1/45.6, and HPC was diluted at 1/5.6 in normal human serum pool (Appendix 2)

immunoglobulin positive control	Human IgG whole molecule
Secondary antibodies	horseradish peroxidase conjugated Goat anti-Human Immunoglobulin
Matrix	normal human serum
NQC (negative control)	normal human serum pool

Table prepared by the Reviewer.

3. Method Summary

The quantitative ELISA method (method No. ELIZA-0308) is used to measure anti-drug antibodies (ADA) that bind with exenatide (AC2993). The signal intensity is proportional to the amount of human anti-drug IgG, IgM, and IgA in samples. The method work flow is listed below:



The screening and titer-confirmatory assays are presented below:

Screen Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	HQC	HQC	Aliquot 5	Aliquot 5	Aliquot 13	Aliquot 13	Aliquot 21	Aliquot 21	Aliquot 29	Aliquot 29	Aliquot 37	Aliquot 37
B	LQC	LQC	Aliquot 6	Aliquot 6	Aliquot 14	Aliquot 14	Aliquot 22	Aliquot 22	Aliquot 30	Aliquot 30	Aliquot 38	Aliquot 38
C	NQC	NQC	Aliquot 7	Aliquot 7	Aliquot 15	Aliquot 15	Aliquot 23	Aliquot 23	Aliquot 31	Aliquot 31	Aliquot 39	Aliquot 39
D	NQC	NQC	Aliquot 8	Aliquot 8	Aliquot 16	Aliquot 16	Aliquot 24	Aliquot 24	Aliquot 32	Aliquot 32	Aliquot 40	Aliquot 40
E	Aliquot 1	Aliquot 1	Aliquot 9	Aliquot 9	Aliquot 17	Aliquot 17	Aliquot 25	Aliquot 25	Aliquot 33	Aliquot 33	HQC	HQC
F	Aliquot 2	Aliquot 2	Aliquot 10	Aliquot 10	Aliquot 18	Aliquot 18	Aliquot 26	Aliquot 26	Aliquot 34	Aliquot 34	LQC	LQC
G	Aliquot 3	Aliquot 3	Aliquot 11	Aliquot 11	Aliquot 19	Aliquot 19	Aliquot 27	Aliquot 27	Aliquot 35	Aliquot 35	NQC	NQC
H	Aliquot 4	Aliquot 4	Aliquot 12	Aliquot 12	Aliquot 20	Aliquot 20	Aliquot 28	Aliquot 28	Aliquot 36	Aliquot 36	NQC	NQC

Titer-Confirmatory Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	HQC	HQC	Sample 1 1/5	Sample 1 1/5	Sample 2 1/125	Sample 2 1/125	Sample 4 Neat + AC2993	Sample 4 Neat + AC2993	Sample 5 1/5	Sample 5 1/5	Sample 6 1/125	Sample 6 1/125
B	LQC	LQC	Sample 1 1/25	Sample 1 1/25	Sample 2 1/625	Sample 2 1/625	Sample 4 Neat	Sample 4 Neat	Sample 5 1/25	Sample 5 1/25	Sample 6 1/625	Sample 6 1/625
C	NQC	NQC	Sample 1 1/125	Sample 1 1/125	Sample 3 Neat + AC2993	Sample 3 Neat + AC2993	Sample 4 1/5	Sample 4 1/5	Sample 5 1/125	Sample 5 1/125	HQC	HQC
D	NQC	NQC	Sample 1 1/625	Sample 1 1/625	Sample 3 Neat	Sample 3 Neat	Sample 4 1/25	Sample 4 1/25	Sample 5 1/625	Sample 5 1/625	LQC	LQC
E	HQC + AC2993	HQC + AC2993	Sample 2 Neat + AC2993	Sample 2 Neat + AC2993	Sample 3 1/5	Sample 3 1/5	Sample 4 1/125	Sample 4 1/125	Sample 6 Neat + AC2993	Sample 6 Neat + AC2993	NQC	NQC
F	LQC + AC2993	LQC + AC2993	Sample 2 Neat	Sample 2 Neat	Sample 3 1/25	Sample 3 1/25	Sample 4 1/625	Sample 4 1/625	Sample 6 Neat	Sample 6 Neat	NQC	NQC
G	Sample 1 Neat + AC2993	Sample 1 Neat + AC2993	Sample 2 1/5	Sample 2 1/5	Sample 3 1/125	Sample 3 1/125	Sample 5 Neat + AC2993	Sample 5 Neat + AC2993	Sample 6 1/5	Sample 6 1/5	HQC	HQC
H	Sample 1 Neat	Sample 1 Neat	Sample 2 1/25	Sample 2 1/25	Sample 3 1/625	Sample 3 1/625	Sample 5 Neat	Sample 5 Neat	Sample 6 1/25	Sample 6 1/25	LQC + AC2993	LQC + AC2993

Reviewer Comments: In screen and titer-confirmatory assays, LPC, and HPC were analyzed at least $n = 4$ in replicate, NC were analyzed at least $n = 8$ in replicate. In titer-confirmatory assay, test samples (TS) will be analyzed in 12 wells.

Overall, the ELISA assay appears suitable to detect the anti-drug antibody that binds with exenatide.

4. Individual validation parameters

4.1 Minimum require dilution (MRD)

The validation of MRD was provided in Report REST080375R2 at (b) (4).
(b) (4) The results recommended MRD at 1:20 or more. MRD of 1:25 was used in validation report REST080375R2 and in current method validation Report REST09118R1 at (b) (4) to ensure no matrix interference on the method.

Reviewer Comment: *Although the information was not sufficient, such as how many individual serum samples were calculated for MRD, the established MRD of 1:25 is acceptable. FDA Guidance on ADA assay development (2016) recommends an MRD < 1:100 and does not have specific recommendations on the number of samples that should be analyzed.*

4.2 Screening Assay -- Cut Point (CP) and Normalization Factor (NF)

Samples

72 normal human serum samples were tested in 3 runs performed on separate days by two analysts using a balanced design.

Reviewer Comment: *This is consistent with recommendations in FDA guidance and is acceptable.*

Outliers

The Grubb method was used for excluding outliers. A total of 56 individual normal serum samples were used to calculate the screening cut point.

Reviewer Comments: *Using the Grubbs test assumes the distribution of the dataset has a normal distribution. The data are not normally distributed unless log-transformed (discussed below). Therefore, the use of Grubb method on the data of original scale for exclusion of outliers may have the risk of bringing bias in screening cut point calculation (discussed below).*

Screening cut point

The screening cut-point was determined on the basis of the mean and standard deviation observed for the 56 individual normal serum samples.

In the original submission, for each plate,

Plate cut point = Mean normal serum signal + (1.645 * Stdev of mean normal serum OD)

N-factor = [Mean normal serum signal + (1.645 * Stdev of mean normal serum OD)] – Mean plate NC signal

note: 1.645 is the one-tail t-value at 95% confidence level

The results for the validation assay are listed below:

the screening cut point = 0.154 at 5% false positive rate, and
the screening cut point N-factor = 0.104.

Reviewer Comments: *The sponsor calculated floating cut points for the screening assay. There is no statistical analysis, such as comparison between means and among variances, to support the usage of a floating cut point. This is not optimal but acceptable since the floating cut point calculation is less complicated than dynamic cut point calculation, and more conservative than fixed cut point. Typically, we recommend the use of floating cut point over the fixed cut point or dynamic cut point.*

*Using a 5% false positive rate in cut point calculation is acceptable. The Sponsor used a parametric method (mean + SD * t-value) to calculate screening cut point which requires that the dataset is normally distributed. However, the data normality was not examined. We requested the data normality information in the information request.*

In the response to information request question #5, the Sponsor stated that the data is not normally distributed and the log-transformed data is normally distributed. The Sponsor re-calculated the screening cut point using a parametric method (mean + 1.645* SD) on the log-transformed data where the outliers were removed.

The recalculated results are listed below:

the recalculated screening cut point = 0.156 at 5% false positive rate, and
the recalculated screening cut point N-factor = 0.102, (the average of 0.095, 0.099, and 0.112 in three runs.)

The N-factor was calculated by subtracting the mean NC value from the recalculated cut point. In addition, the Sponsor provided a file presenting the recalculation of the screening cut point N-factor for the assay.

Reviewer's Comment: *I reviewed the file containing the screening cut point recalculation. It is suitable that the parametric method with a 5% false positive rate was used in the recalculation of the screening cut point on the log-normally distributed data. The calculation of the validated cut points is acceptable. However, the description of the recalculation of the cut point N-factor was not clear. Therefore, we sent out the second-round information request to the Sponsor for the clarification.*

The following second-round IR was sent to the Sponsor on September 20, 2017:

We are currently reviewing **CPD-Exenatide-JZ-001**: “Re-Analysis of Screening Cut Point Factor for Validation of an ELISA Method for the Detection of Anti-AC2993 Antibodies In Human Serum (REST090118R1).” Our understanding is that all calculations were performed on the log-transformed data, including the calculation of screening cut point and cut point N-factor. The values were transformed back to the original scale for reporting purpose after all of the calculation was finished. Please clarify whether our understanding is correct and present the calculation process for the plate specific cut point and N-factor.

The Sponsor provided a response to our IR on September 27, 2017 with one document: **CPO Exenatide JZ-003**: Statistical Analysis for “Exenatide once weekly suspension (EQWS) for Type 2 diabetes Response to NDA 209210 Information Request (20 September 2017).”

In Sponsor’s response, the sponsor confirms that the Reviewers’ understanding in cut point calculation is correct. The Sponsor stated that the data is not normally distributed and the log-transformed data is normally distributed. The Sponsor re-calculated the screening cut point using a parametric method on the log-transformed data where the outliers were removed. The recalculated values are listed below:
the recalculated screening cut point = 0.160 at 5% false positive rate, and
the recalculated cut point N-factor = 0.100 (average of 0.096, 0.092, and 0.112 in three runs.) The N-factor was calculated by subtracting the mean NC value from the recalculated cut point in the original scale.

Reviewer’s Comments:

The recalculated cut points and N-factors in the Sponsor’s response to two-rounds IR are very close (0.004 as cut point difference and 0.002 as N-factor difference.) The calculation of the validated cut points are acceptable. However, the Sponsor’s calculation of the cut point N-factor is inaccurate. The cut point N-factor calculation by subtracting the negative control value from the cut point in the original scale is inaccurate since the data are not normally distributed unless transformed to the log-scale. The cut point N-factor should be calculated by subtracting the NC value from the cut point in the log-transformed scale or by dividing the NC value from the cut point in the original scale.

Using the recommended method, the Reviewer calculated cut point N-factor = 2.73. The screening cut points are the same when the NC value is 0.058 (the average of NC values from three runs) using either the Sponsor’s calculation or the Reviewers’ calculation. The cut point is less using the Sponsor’s calculation if the NC value is larger than 0.058, vice versa. When the NC value is larger than 0.058, the N-factor calculated by the Sponsor is more conservative indicating higher false positive rates in the screening assay. It is not a concern since these false positive samples will be identified as ADA negative in the confirmatory assay. While when the NC value is less than 0.058, the N-factor calculated by the Sponsor is less conservative indicating higher false negative rates in the screening assay. The higher false negative rates does not negatively impact the ADA incidence in the clinical sample analysis since the same screening cut point 0.154 was used in all studies (see Appendix 1.)

Overall, the calculation of the screening cut point is acceptable.

4.3 Confirmatory Assay

The confirmatory assay was performed on potential positive samples identified in the screening assay. The samples were diluted 1/25 in plain assay buffer spiked with 100

µg/mL exenatide or without drug. The spiked and unspiked samples were tested in at least one run with n=2 replicates. A sample is confirmed positive if the sample meet the following criteria:

- 1) the untreated signal $\geq$ plate cut point;
- 2) $CV \leq \frac{(b)}{(4)}\%$; and
- 3) $\geq \frac{(b)}{(4)}\%$ reduction in signal is observed with addition of the drug.

Reviewer Comments:

The concentration of drug used to validate the confirmatory assay should be determined based on the data demonstrating inhibition in the presence of low levels PC. The method specificity data (see section II 4.6 below) demonstrate that 100 µg/mL of exenatide inhibit the detection of the low concentration PC. Based on these data, the 100 µg/mL of drug used for the confirmatory assay validation is acceptable.

$\frac{(b)}{(4)}\%$ inhibition of a sample signal used in confirmation cut point calculation equals to 0.1% false positive rate. The Guidance recommends using 1% false positive rate in the confirmatory cut point calculation. Because the main clinical impact of anti-exenatide antibodies is reduced efficacy with high titers the use of a 0.1% false positive rate still allows for sufficient understanding of the impact of ADA on safety and efficacy and is therefore acceptable.

4.4 Assay Precision

PC samples were analyzed by two analysts to have a total of six runs for each sample. The table below lists the assay validation results on precision.

Table 2
Within and Between Run Precision of Anti-AC2993 Antibody in Human Serum

Validation Sample	Assay Run	Analyst	Signal						Intra-assay (within-run) Statistics				Inter-assay (between-run) Statistics		
			1st Run	2nd Run	3rd Run	4th Run	5th Run	6th Run	n	mean	Stdev	%CV	Repeatability	Ancillary Statistics	Precision
High QC	Val 001	(b) (6)	1.948	1.823	1.780				3	1.850	0.1	4.7	ANOVA MS ₀ = 0.0	ANOVA MS ₀ = 0	mean = 1.809 s ₀ = Stdev = 0.1 %CV = 8.3
	Val 002		2.069	1.950	1.843				3	1.954	0.1	5.8	s ₀ = Stdev = 0.1	MS ₁ = 0	
	Val 003		1.866	1.765	1.469				3	1.700	0.2	12.1	%CV = 7.3	s ₀ = 0.1	
	Val 004		1.865	1.639	1.566				3	1.690	0.2	9.2		N _{obs} = 18	
	Val 007		1.747	1.791	1.809				3	1.782	0.0	1.8		N _{run} = 6	
	Val 008		2.018	1.799	1.814				3	1.877	0.1	6.5		N _{rep} = 3	
Low QC	Val 001		0.475	0.466	0.424				3	0.455	0.0	6.0	ANOVA MS ₀ = 0.0	ANOVA MS ₀ = 0	mean = 0.444 s ₀ = Stdev = 0.1 %CV = 12.4
	Val 002		0.522	0.530	0.479				3	0.510	0.0	5.4	s ₀ = Stdev = 0.0	MS ₁ = 0	
	Val 003	0.431	0.390	0.330				3	0.384	0.1	13.2	%CV = 7.2	s ₀ = 0.0		
	Val 004	0.429	0.381	0.371				3	0.394	0.0	7.9		N _{obs} = 18		
	Val 007	0.497	0.449	0.478				3	0.475	0.0	5.1		N _{run} = 6		
	Val 008	0.462	0.420	0.451				3	0.444	0.0	4.9		N _{rep} = 3		
NQC	Val 001	0.063	0.060	0.062	0.061	0.062	0.054	6	0.060	0.0	5.4	ANOVA MS ₀ = 0.0	ANOVA MS ₀ = 0	mean = 0.061 s ₀ = Stdev = 0.0 %CV = 23.8	
	Val 002	0.117	0.114	0.059	0.059	0.061	0.059	6	0.078	0.0	37.0	s ₀ = Stdev = 0.0	MS ₁ = 0		
	Val 003	0.059	0.054	0.059	0.056	0.048	0.042	6	0.053	0.0	12.7	%CV = 20.2	s ₀ = 0.0		
	Val 004	0.058	0.054	0.052	0.053	0.051	0.053	6	0.054	0.0	4.5		N _{obs} = 36		
	Val 007	0.060	0.063	0.057	0.062	0.057	0.060	6	0.060	0.0	4.2		N _{run} = 6		
	Val 008	0.063	0.060	0.060	0.058	0.059	0.062	6	0.060	0.0	3.1		N _{rep} = 6		

Reviewer Comment: Each PC has six runs performed by two analysts, which meets the guidance recommendation of at least six runs. For all six PC runs, each PC has three replicates which is not ideal but acceptable. NC has six runs and each NC run has six

replicates which is consistent with guidance. In addition, PCs (HPC and LPC) have inter-assay precision CV% < 13% and intra-assay CV% < 8%, meeting the acceptance criteria. NC has intra-assay CV% = 20.2% and inter-assay CV% = 23.8%. Therefore, the assay precision is acceptable.

4.5 Assay Sensitivity

In the response to information request question #3(a), the Sponsor stated that they did not determine the assay sensitivity in validation report REST090118R1. However, the assay sensitivity in units of concentration was determined in previous validations of the method using a mouse monoclonal anti-exenatide antibody, EXE4:2-8.4, as the assay positive control. The sensitivity was estimated as (b) (4) ng/mL at Amylin (Report REST080584R1) and (b) (4) ng/mL at (b) (4) Report REST080375R2 (b) (4)

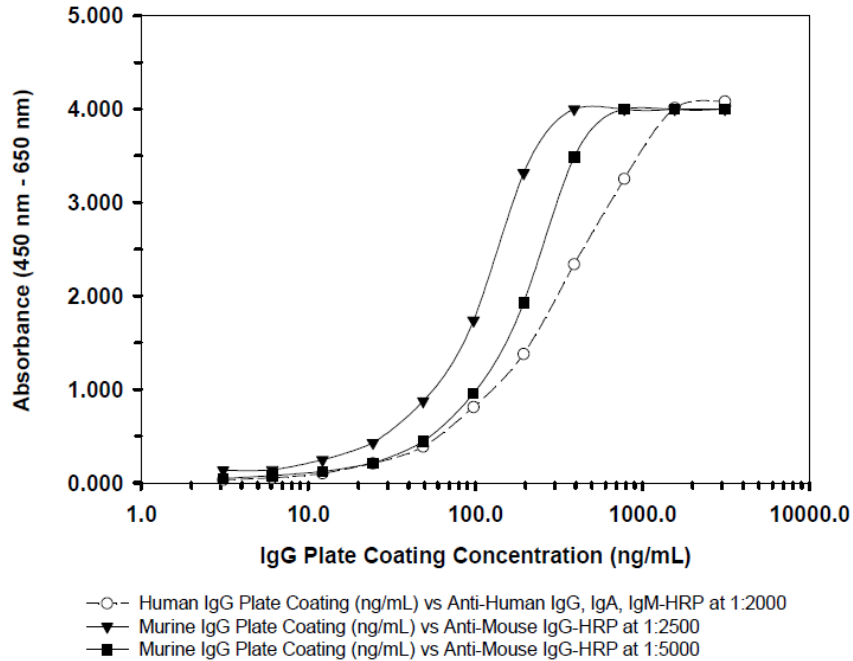
Reviewer Comment: *The assay in Report REST080584R1 was to detect whether anti-exenatide antibodies cross-react with GLP-1 or glucagon, which is discussed in Section III and Appendix 2.*

Below describes the assay sensitivity assay in Report REST080375R2 “Validation Report: Enzyme-Linked Immunosorbent Assay (ELISA) to Detect Human Antibodies against Exenatide in Serum.”

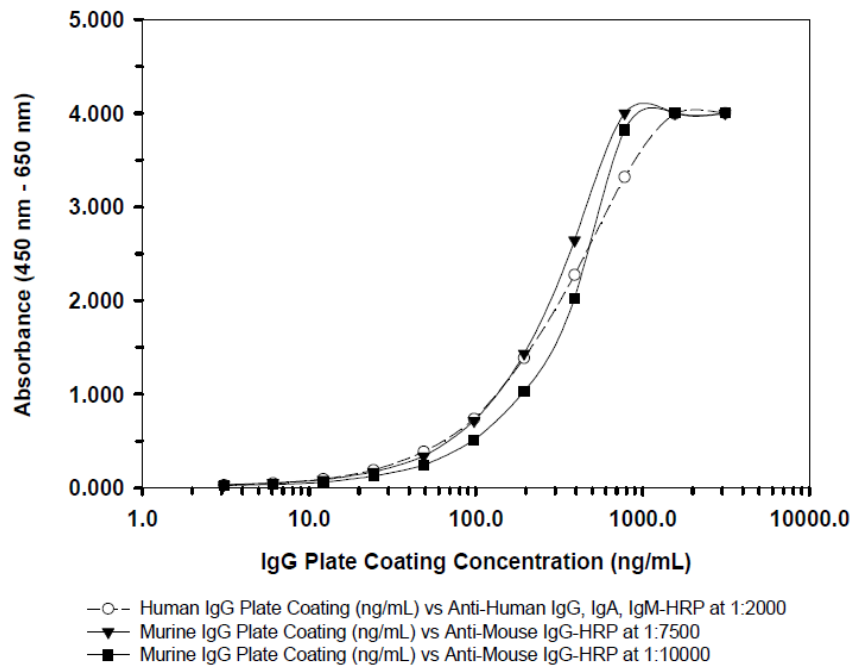
Monoclonal antibody EXE4:2-8 recognizes a C-terminal epitope on exenatide. Secondary anti-mouse IgG-HRP was diluted at 1:2,500, 1:5,000, 1:7,500, 1:10,000. The serial 1:2 dilutions of the purified mouse anti-exenatide antibody from 10.0 µg/mL to 0.019 µg/mL (covering 10 levels: 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039, 0.019 µg/mL) were tested n=2 in replicate and analyzed by one analyst over two days for a total of 4 runs. The figures below present the dose-response curves. The results show that 1:7,500 dilution of secondary antibody anti-mouse IgG-HRP is the closest to the 1:2,000 dilution of secondary anti-human IgG, IgA, IgM-HRP conjugate.

Attachment C: Sensitivity Part 1 Graphs

Assay A



Assay B



Reviewer Comment: Per FDA guidance it is preferable to use purified positive control antibodies for the assay sensitivity validation studies. The use of mouse monoclonal anti-

exenatide antibody, EXE4:2-8.4, meets the recommendations for assay sensitivity and is acceptable for that assay.

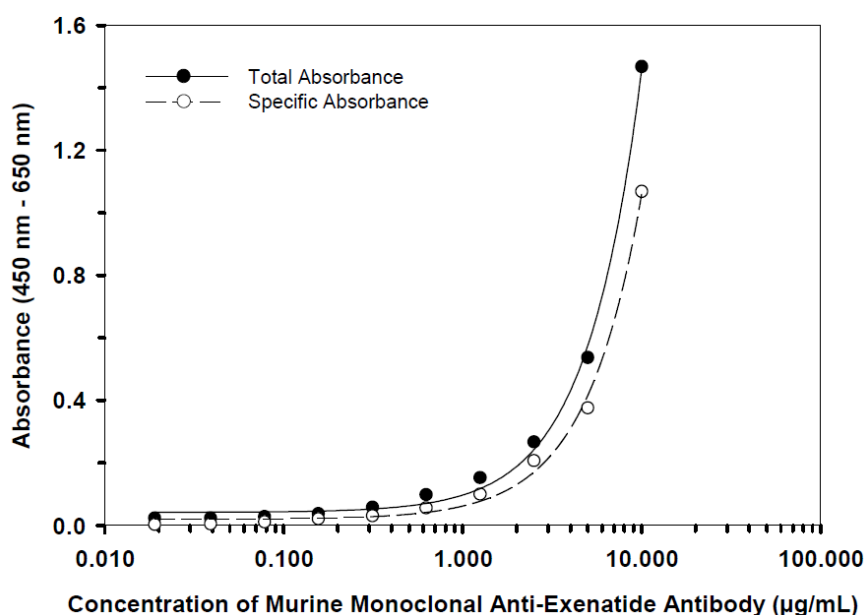
The assay sensitivity is determined as 625 ng/mL when the cut point is calculated using the formula below:

0.148 (fixed cut point) - 0.074 (negative control mean response) + 0.018 (NHS anti-mouse background, MoAb expt.) = 0.092 (MOAb cut point). The table and figure below list the results of the sensitivity assay.

Assay Date	Analyst	Assay Number	Absorbance (450 nm - 650 nm)										
			Concentration of Murine Monoclonal Anti-Exenatide Antibody (µg/mL)										
22-Feb-05	(b) (6)	PR50219	0.000	0.019	0.039	0.078	0.156	0.313	0.625	1.250	2.500	5.000	10.000
			0.018	0.019	0.020	0.025	0.035	0.053	0.085	0.155	0.255	0.510	1.178
			0.014	0.022	0.021	0.026	0.035	0.050	0.086	0.135	0.239	0.491	1.126
23-Feb-05		PR50220	0.022	0.029	0.025	^(b) NR	0.039	0.059	0.091	0.170	0.301	0.597	1.889
			0.017	0.022	0.026	0.034	0.041	0.069	0.132	0.150	0.272	0.549	1.678
Mean			0.018	0.023	0.023	0.028	0.038	0.058	0.099	0.153	0.267	0.537	1.468
n			4	4	4	3	4	4	4	4	4	4	4
Standard Deviation			0.003	0.004	0.003	0.005	0.003	0.008	0.022	0.014	0.027	0.047	0.375
Precision (%CV)			18.6	18.4	12.8	17.4	8.0	14.5	22.8	9.5	9.9	8.7	25.6

^(a)%CV of replicate responses is > 30%. Replicates 0.029 and 0.047.

Attachment E: Sensitivity Part 2 Graph



Reviewer Comments: The assay sensitivity is defined as the lowest concentration at which the antibody preparation consistently produces either a positive result or a readout equals to the cut point determined for that particular assay. The Sponsor evaluated the assay sensitivity using a different way from the guidance recommendations.

Using the Sponsor's data, the assay sensitivity estimated as the guidance recommends, is ^{(b) (4)} µg/mL (fixed cut point = ^{(b) (4)} Absorbance Units) indicating the assay sensitivity was inadequately established. This is not successfully resolved during our review cycle.

Therefore it is a PMC issue. The Sponsor should validate the screening ADA assay with adequate sensitivity. (b) (4)

4.6 Specificity

The Sponsor examined the assay specificity using a competitive binding assay. The assay is to test whether the binding of the positive samples to exenatide can be inhibited by the pretreatment of exenatide. The positive samples were spiked with anti-exenatide antibodies. The table below lists the specificity results.

Table 5A
Specificity; Inhibition of Anti-AC2993 Antibody Signal by AC2993 in Human Serum

(CB-0433-8200-497-Val-010)

HQC Treated with AC2993				HQC Treated with Human IgG			
Added Concentration of AC2993 (µg/mL)	Mean Signal	%CV	% Inhibition by AC2993	Added Concentration of Human IgG (µg/mL)	Mean Signal	%CV	% Inhibition by Human IgG
0*	1.565	8.3	0.0	0*	1.565	8.3	0.0
1.0	0.243	2.1	87.4	1.0	1.524	0.8	2.7
10.0	0.117	2.4	95.7	10.0	1.518	2.5	3.1
100.0	0.066	2.7	99.1	100.0	1.508	5.1	3.7
LQC Treated with AC2993				LQC Treated with Human IgG			
Added Concentration of AC2993 (µg/mL)	Mean Signal	%CV	% Inhibition by AC2993	Added Concentration of Human IgG (µg/mL)	Mean Signal	%CV	% Inhibition by Human IgG
0*	0.312	14.1	0.0	0*	0.312	14.1	0.0
1.0	0.077	1.3	90.4	1.0	0.353	2.9	-15.8
10.0	0.053	0.7	99.6	10.0	0.268	3.0	16.9
100.0	0.050	2.3	100.8	100.0	0.266	12.2	17.7

* Untreated QC values; used as baseline in this analysis

Reviewer Comment: The specificity was assessed using a competitive binding assay, which is consistent with the recommendation of guidance and is acceptable. The assay results show that anti-exenatide antibodies specifically bind to exenatide indicating the assay is specific.

4.7 Selectivity

In the response to information request question #3(b), the Sponsor stated that the validation of the assay selectivity was provided in Report REST080375R2 at (b) (4). The Reviewer found Section “Recovery” in Report REST080375R2 evaluated the assay selectivity. The matrix interference was evaluated in four normal human serum lots, each spiked with PCs or unspiked. Each sample was analyzed after a dilution of 1:25 to have a

total of six results with n=3 in replicate in two assays on two days by one analyst. The % Difference from Assay Buffer was calculated using the following formula:

$$\left(\frac{\text{Mean Pos. Ctrl. Response in NHS} - \text{Mean Neg. Ctrl. Response in NHS}}{\text{Mean Pos. Ctrl. Response in Assay Buffer} - \text{Mean Neg. Ctrl. Response in Assay Buffer}} \times 100 \right) - 100$$

In results, three of the four normal human serum lots have the % Difference from assay buffer ranged from 17.2% to 27.6% for the samples prepared at the low antibody spike level (1:100 dilution), and 11.3% to 17.7% for the samples prepared at the high antibody spike level (1:15 dilution). The %CV for all four lots of normal human serum ranged from 10.2% to 22.3% across the three levels.

Reviewer Comment: *The sponsor demonstrated that matrix does not have interference on the assay within the tested range indicating the assay is selective.*

4.8 Drug Tolerance

The interference by exenatide in the assay was assessed by testing two levels of PC (HPC and LPC) in plain assay buffer without exenatide and with three levels of exenatide (1.0, 10.0, and 100.0 µg/mL). The drug tolerance was analyzed n=2 in duplicate in one run with the assay results listed in the table in Section 4.6.

Reviewer Comment: *The drug tolerance assessment is not optimal but is acceptable. In the Phase 3 clinical trial BCB 118, the plasma exenatide concentration in low titer patients is ~ 180 pg/mL. Based on these data, the drug tolerance is acceptable.*

4.9 Titration Assay -- Titration Cut Point (TCP) and Prozone (Hook) Effect

Titration cut point

The Sponsor used the screening plate cut point as the titration cut point.

Reviewer Comment: *Using the screening cut point as the titration cut point is acceptable.*

Titration assay

The titer response assay was performed on five serial dilutions of HPC for at least two runs with n=2 replicates. Antibody titers are determined from the dilution series of ADA in the absence of exenatide and expressed as the reciprocal of the highest dilution of the sample serum that tests positive in the assay (ie, a dilution of 1:125 is expressed as an antibody titer of 125). The table below lists the titer response of anti-exenatide antibody in human serum.

Table 3
Titer Response for Anti-AC2993 Antibody in Human Serum

Dilution Factor	CB-433-8200-497-Val 009		CB-433-8200-497-Val 010	
	OD	%CV	OD	%CV
1	1.474	0.1	1.667	0.4
5	0.456	1.9	0.524	0.8
25	0.137	0.7	0.132	6.9
125	0.063	1.7	0.065	3.1
625	0.054	5.5	0.048	4.0
Plate Mean NQC Signal	0.048		0.050	
Plate Cut Point	0.138		0.140	
Individual Titer ¹	24.3		23.2	
Mean Titer	23.8			
% of Mean Titer ²	102.4		97.6	

¹ Titer = Dilution factor corresponding to the cut point.

² % of Mean Titer = (Individual Titer/Mean Titer) x 100

Reviewer Comment: The titration assay is suitable.

Prozone (Hook) Effect

The Sponsor did not evaluate the prozone effect of the assay. The figure below presents the data in the titration assay CB-433-8200-497-Val009 (as shown in the table3 above in section II 4.9).

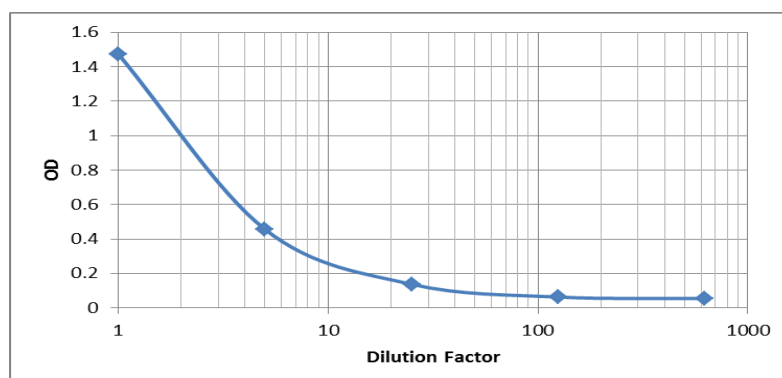


Figure prepared by the Reviewer.

Reviewer Comment: The sponsor included three points in the linear part of the curve. No hook effect was observed for the dilution factor of 1 for HPC.

4.10 Sample Stability

The long-term storing temperature for clinical samples is at -60°C to -80°C. The Sponsor assessed the HPC and LPC sample stability in n=3 replicates at the storage conditions listed below.

Stability	Storage	AR
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Bench top stability	stored at -60°C to -80°C for ≥ 12 hours, and thawed for ≥ 1 hour, then stored at ART (15°C-30 °C) for 21 hours	HQC: 95.0% LQC: 86.3%
Refrigerator stability	stored at -60°C to -80°C for ≥ 12 hours, then transferred to 2 to 8°C for 19 hours and 26 hours	HQC: 95.9% LQC: 103.9%
Freeze/thaw stability	stored at -60°C to -80°C for ≥ 12 hours, then thawed for ≥ 1 hour for up to 8 freeze/thaw cycles	HQC: 97.3-112.6% (4-8 F/T) LQC: 82.8-104.6% (4-8 F/T)
Long term stability	stored for one month at -60°C to -80°C, then thawed at ART for ≥ 1 hour	HQC: 103.0% LQC: 100.5%

Table prepared by the Reviewer.

Reviewer Comment: *HPC and LPC are stable at all tested conditions.*

4.11 Robustness

The sponsor provided information, such as variations in incubation time and rotation speed, to show assay robustness.

Reviewer Comment: *The assay is robust.*

5. Overall ADA Assay assessment:

The provided validation report on the ADA binding assay demonstrates:

- 1) The ELISA assay is suitable to detect the anti-drug antibody that binds with exenatide.
- 2) The assay method acceptance criteria are acceptable.
- 3) The assay is specific, selective, repeatable, and robust.
- 4) The HPC and LPC samples are stable.

Below lists the ADA validation assay deficiencies:

- 1) The assay sensitivity was not adequately established.
- 2) LPC concentration was not determined.

III. Review of the Current Submission— REPORT: REST080584R1: Results from the partial validation of an Anti-Exenatide (AC2993) Antibody Cross Reactivity ELISA Using Tetramethylbenzidine (TMB), and
REST080154R1: Results from the evaluation of Anti-AC2993 antibody cross reactivity to GLP-1 and Glucagon in human serum specimens from clinical study LAR-105 using an enzyme linked immunosorbent assay

1. Review background

In the response to information request question #2, the Sponsor stated that the anti-exenatide antibody cross-reactivity to GLP-1 or glucagon was not evaluated for the

clinical studies in NDA209210. The Sponsor provided evaluation report REST080154R1 which demonstrated that the anti-exenatide antibody does not have cross-reactivity to GLP-1 and glucagon. In the evaluation report the method TM-0163 was used to test the patient samples with higher antibody titers (≥ 625) in clinical study LAR-105 with Bydureon. REST080584R1 is the partial validation report of the method TM-0163 for testing the anti-exenatide antibody cross-reactivity to GLP-1 or glucagon.

2. Instruments and Reagents

The instruments include Immulon 2 HB Flatbottom Microtiter® plates and Molecular Devices VERSAmax plate reader. The reagents are listed in the table below.

Controls and Others	Information
Coating Material	exenatide coating material
PC (positive control)	EXE4:2-8.4 at 10.0 µg/mL in mouse NaEDTA plasma, and GLP1:3-3.1 at 5.0 µg/mL in mouse NaEDTA plasma
NC (negative control)	human K ₂ EDTA plasma, and human serum
Inhibitory peptides	exenatide (5 mg/mL), GLP-1 (2 mg/mL), glucagon (2 mg/mL), and AC3034 (Sequence Irrelevant Peptide) (5 mg/mL)
Primary antibody	anti-exenatide antibody
Secondary antibodies	horseradish peroxidase conjugated Goat anti-mouse Immunoglobulin

Table prepared by the Reviewer.

Monoclonal antibody EXE4:2-8 recognizes a C-terminal epitope on exenatide and does not cross react with GLP-1 or glucagon. Monoclonal antibody GLP1:3-3 recognizes an N-terminal epitope on both exenatide and GLP-1 and also cross reacts with glucagon. Therefore GLP1:3-3 antibody was used to confirm the ability of this assay to recognize antibodies that are cross-reactive to GLP-1 or glucagon.

3. Method Summary

Enzyme-linked immunosorbent assays (ELISA) (method No. TM-0163) to evaluate the cross reactivity of anti-exenatide antibodies to GLP-1 and glucagon in human serum were developed and validated by Amylin in San Diego, CA. The assay compares inhibition of the binding of anti-exenatide antibodies to exenatide coated on the plate in the presence of competitive peptides.

There were a total of twelve assays. Three analysts each perform three assays (two plates per assay) over three days. The assay was performed using PCs spiked into mouse NaEDTA plasma along with human K₂EDTA plasma and human serum. On day four, each analyst performed one five-plate assay comprised of one plate as mentioned above and four sensitivity plates included two PC antibody spiked into NCs and one set of PC and NC. The figures below present the coating, plate, and conjugate maps.

Example Coating Map

	1 - 2	3 - 4	5 - 6	7 - 8	9 - 10	11 - 12
A	AC2993	AC2993	AC2993	AC2993	AC2993	Human IgG (unlabeled)
B	AC2993	AC2993	AC2993	AC2993	AC2993	AC2993
C	AC2993	AC2993	AC2993	AC2993	AC2993	AC2993
D	AC2993	AC2993	AC2993	AC2993	AC2993	AC2993
E	AC2993	AC2993	AC2993	AC2993	AC2993	AC2993
F	AC2993	AC2993	AC2993	AC2993	AC2993	AC2993
G	AC2993	AC2993	AC2993	AC2993	AC2993	AC2993
H	AC2993	AC2993	AC2993	AC2993	AC2993	AC2993

Example Plate Map

	1 - 2	3 - 4	5 - 6	7 - 8	9 - 10	11 - 12
A	EXE4:2-8.4 in Blotto/Casein	EXE4:2-8.4 in Blotto/Casein w/ AC2993	EXE4:2-8.4 in Blotto/Casein w/ GLP-1	EXE4:2-8.4 in Blotto/Casein w/ Glucagon	EXE4:2-8.4 in Blotto/Casein w/ SIP*	Negative pool in Blotto/Casein
B	GLP1:3-3.1 in Blotto/Casein	GLP1:3-3.1 in Blotto/Casein w/ AC2993	GLP1:3-3.1 in Blotto/Casein w/ GLP-1	GLP1:3-3.1 in Blotto/Casein w/ Glucagon	GLP1:3-3.1 in Blotto/Casein w/ SIP	Blotto/Casein only
C	Negative pool in Blotto/Casein	Negative pool in Blotto/Casein w/ AC2993	Negative pool in Blotto/Casein w/ GLP-1	Negative pool in Blotto/Casein w/ Glucagon	Negative pool in Blotto/Casein w/ SIP	Blotto/Casein only
D	Sample 1 in Blotto/Casein	Sample 1 in Blotto/Casein w/ AC2993	Sample 1 in Blotto/Casein w/ GLP-1	Sample 1 in Blotto/Casein w/ Glucagon	Sample 1 in Blotto/Casein w/ SIP	
E	Sample 2 in Blotto/Casein	Sample 2 in Blotto/Casein w/ AC2993	Sample 2 in Blotto/Casein w/ GLP-1	Sample 2 in Blotto/Casein w/ Glucagon	Sample 2 in Blotto/Casein w/ SIP	
F	Sample 3 in Blotto/Casein	Sample 3 in Blotto/Casein w/ AC2993	Sample 3 in Blotto/Casein w/ GLP-1	Sample 3 in Blotto/Casein w/ Glucagon	Sample 3 in Blotto/Casein w/ SIP	
G	Sample 4 in Blotto/Casein	Sample 4 in Blotto/Casein w/ AC2993	Sample 4 in Blotto/Casein w/ GLP-1	Sample 4 in Blotto/Casein w/ Glucagon	Sample 4 in Blotto/Casein w/ SIP	
H	Sample 5 in Blotto/Casein	Sample 5 in Blotto/Casein w/ AC2993	Sample 5 in Blotto/Casein w/ GLP-1	Sample 5 in Blotto/Casein w/ Glucagon	Sample 5 in Blotto/Casein w/ SIP	

*SIP = Sequence Irrelevant Peptide

Example Conjugate Map

	1 - 2	3 - 4	5 - 6	7 - 8	9 - 10	11 - 12
A	Goat Anti-Mouse Ig-HRP	Goat Anti-Mouse Ig-HRP	Goat Anti-Mouse Ig-HRP	Goat Anti-Mouse Ig-HRP	Goat Anti-Mouse Ig-HRP	Goat Anti-Human Ig-HRP
B	Goat Anti-Mouse Ig-HRP	Goat Anti-Mouse Ig-HRP	Goat Anti-Mouse Ig-HRP	Goat Anti-Mouse Ig-HRP	Goat Anti-Mouse Ig-HRP	Goat Anti-Human Ig-HRP
C	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Mouse Ig-HRP
D	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	
E	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	
F	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	
G	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	
H	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	Goat Anti-Human Ig-HRP	

Reviewer Comments:

The Sponsor examined the cross-reactivity using the competitive binding method. Usually using competitive binding method to detect the cross-reactivity is acceptable. However,

this competitive binding method may not be adequately sensitive to test the ADA's cross-reactivity to endogenous GLP-1 or glucagon since many subjects in those studies had robust anti-exenatide antibodies the presence of anti-GLP-1 and anti-glucagon antibodies may have been masked leading to false negative results.

GLP-1 and glucagon are endogenous human peptides. Therefore, it is important to understand whether anti-drug antibodies may interfere with the activity of those peptides.

(b) (4)

In addition, the assay cut point was not determined. The Sponsor should determine the assay cut point to be used for the determination of positive and negative samples.

4. Acceptance Criteria

The sample is positive for each competitive peptide if it meets the following criteria:

- 1) sOD equals to or above (b) (4)
where $sOD = OD_{\text{specimen in buffer}} - OD_{\text{specimen in buffer + peptide}}$,
- 2) % inhibition > (b) (4)%,
where $\% \text{ Inhibition} = (sOD / OD_{\text{specimen in buffer}}) * 100$.

The criteria for positive and negative controls are provided in the evaluation report file and are not discussed in this review.

5. The individual validation parameters are reviewed in this section

5.1 Conjugate controls

The conjugate control anti-human Ig(H+L)-HRP with human IgG coat was tested in plate 1 and plate 2 in 9 assays and in plate 1 in the other 3 assays. The %CV are 1.420 and 1.6 for human K₂EDTA plasma pool and human serum pool for conjugate control run. The table below summarizes the conjugate control run with human K₂EDTA plasma pool.

Table 1: Results for Conjugate Controls Run With Human Serum and K₂EDTA Plasma Pool

Human IgG coat w/ Anti-Human Ig(H+L)-HRP PLASMA POOL							
Assay		Optical Density (OD) at 450/650 nm					
Date	Assay Number	Analyst	Plate 1 Mean OD	Plate 2 Mean OD	Overall Mean OD	Assay SD	Assay %CV
28-Aug-08	ExAbCr-110	(b) (6)	1.758	1.802	1.780	0.031	1.7
28-Aug-08	ExAbCr-111		1.876	1.862	1.869	0.010	0.5
28-Aug-08	ExAbCr-112		2.190	2.088	2.139	0.072	3.4
29-Aug-08	ExAbCr-113		1.789	1.782	1.786	0.005	0.3
29-Aug-08	ExAbCr-114		1.877	1.866	1.872	0.008	0.416
29-Aug-08	ExAbCr-115		1.929	2.033	1.981	0.074	3.712
02-Sep-08	ExAbCr-116		2.157	2.155	2.156	0.001	0.066
02-Sep-08	ExAbCr-117		2.071	2.018	2.045	0.037	1.833
02-Sep-08	ExAbCr-118		2.246	2.220	2.233	0.018	0.823
03-Sep-08	ExAbCr-119		2.251	NA	2.251	NA	NA
03-Sep-08	ExAbCr-120		2.044	NA	2.044	NA	NA
03-Sep-08	ExAbCr-121		2.406	NA	2.406	NA	NA
					Mean OD	SD	% CV
				MEAN	2.047	0.029	1.420
				STDEV	0.198		

NA= Not Applicable

Reviewer Comment: The assay is adequately precise for the conjugate control anti-human Ig(H+L)-HRP. All of the OD value > 1.70 indicates anti-human Ig(H+L)-HRP conjugate control specifically binds to human IgG.

5.2 Non-specific binding (NSB) controls

The conjugate controls goat anti-mouse Ig(H+L)-HRP and goat anti-human Ig(H+L)-HRP were tested in plate 1 and plate 2 in 9 assays and in plate 1 in the other 3 assays. The %CV are 11.5 and 8.7 for human K₂EDTA plasma pool and human serum pool. The table below summarizes NSB control run with human serum and K₂EDTA plasma pool.

Table 2: Results for NSB Controls Run With Human Serum and K₂EDTA Plasma Pools

GOAT ANTI-MOUSE Ig(H+L)-HRP							
Optical Density (OD) at 450/650 nm							
Assay	Assay		Plate 1	Plate 2	Assay		
Date	Number	Analyst	Mean OD	Mean OD	Mean OD	Assay SD	Assay %CV
28-Aug-08	ExAbCr-110	(b) (6)	0.010	0.008	0.009	0.001	15.7
28-Aug-08	ExAbCr-111		0.010	0.010	0.010	0.000	0.0
28-Aug-08	ExAbCr-112		0.008	0.006	0.007	0.001	20.2
29-Aug-08	ExAbCr-113		0.008	0.009	0.009	0.001	8.3
29-Aug-08	ExAbCr-114		0.010	0.009	0.010	0.001	7.4
29-Aug-08	ExAbCr-115		0.007	0.009	0.008	0.001	17.7
02-Sep-08	ExAbCr-116		0.009	0.010	0.010	0.001	7.4
02-Sep-08	ExAbCr-117		0.008	0.007	0.008	0.001	9.4
02-Sep-08	ExAbCr-118		0.007	0.009	0.008	0.001	17.7
03-Sep-08	ExAbCr-119		0.009	NA	0.009	NA	NA
03-Sep-08	ExAbCr-120		0.008	NA	0.008	NA	NA
03-Sep-08	ExAbCr-121		0.006	NA	0.006	NA	NA
					Mean OD	SD	% CV
				MEAN	0.008	0.001	11.5
				STDEV	0.001		

NA= Not Applicable

Reviewer Comment: The assay is adequately precise for nonspecific binding conjugate controls. All of the OD value ≤ 0.010 indicates goat anti-mouse Ig(H+L)-HRP and goat anti-human Ig(H+L)-HRP do not specifically bind to targets in human serum or human K₂EDTA plasma pool.

5.3 Negative controls

The negative controls human K₂EDTA plasma pool and human serum pool were tested in plate 1 and plate 2 in 9 assays and in plate 1, 3, and 5 in the other 3 assays. The table below summarizes negative control runs for human serum and K₂EDTA plasma pool.

Negative controls	Human K ₂ EDTA plasma pool		Human serum pool	
	Mean OD	%CV	Mean OD	%CV
w/o peptide	0.059	9.637	0.064	8.720
w/ exenatide	0.054	13.630	0.062	7.154
w/GLP-1	0.063	10.062	0.073	5.672
w/glucagon	0.061	6.454	0.071	5.723
w/AC3034	0.063	6.984	0.071	6.408

Table summarized by the Reviewer.

Reviewer Comment: The assay is adequately precise for negative controls. All of the OD value ≤ 0.010 indicates human K₂EDTA plasma pool and human serum pool do not specifically bind to potential inhibitory peptides.

5.4 Positive controls

The PCs were tested in both plates in nine assays and in all five plates in the other three assays. The table below summarizes PC runs.

Positive controls	Exe4:2-8.4 in mouse NaEDTA plasma				GLP1:3-3.1 in mouse NaEDTA plasma			
	Mean OD	%CV	sOD	% inhibition	Mean OD	%CV	sOD	% inhibition
w/o peptide	1.342	5.209	NA	NA	0.931	8.080	NA	NA
w/exenatide	0.016	5.116	1.326	98.804	0.016	6.711	0.915	98.083
w/GLP-1	1.197	17.871	0.145	11.987	0.019	5.631	0.912	97.736
w/glucagon	1.302	4.667	0.040	3.591	0.219	5.672	0.712	78.836
w/AC3034	1.263	7.128	0.079	6.745	0.962	8.433	-0.030	-3.668

Table summarized by the Reviewer.

Reviewer Comment: The assay is adequately precise for PCs. The inhibition indicates that

- 1) EXE4:2-8.4 is highly specific to exenatide;
- 2) GLP-1 and glucagon show much less inhibition of EXE4:2-8.4 compared with exenatide;
- 3) GLP1:3-3.1 has affinity for exenatide and GLP-1;
- 4) Glucagon shows cross reactivity with GLP1:3-3.1 but with less degree compared with exenatide and GLP-1;
- 5) AC3034 (SIP) shows no significant cross reactivity with EXE4:2-8.4 and GLP1:3-3.1.

5.5 Assay sensitivity

The acceptance criterion for PCs is calculated as one-sided 95/95 tolerance interval by (mean +/- k* stdev) for sOD or % inhibition. The table below lists the calculated sOD criterion for putative positive samples and % inhibition criterion for confirming positive samples.

Table 2: sOD putative positive and % inhibition confirmatory positive upper limits Sample is considered putative positive if it is greater than sOD upper limit and confirmed positive if additionally greater than % inhibition upper limit.

Antibody Spike	Matrix	sOD	% Inhibition
EXE4:2-8.4	Plasma	(b) (4)	(b) (4)
	Serum		
GLP1:3-3.1	Plasma		
	Serum		

A sample would have to be larger than these thresholds to be considered positive. The table below lists the estimated sensitivity using the criteria:

- 1) % inhibition criterion, or
- 2) sOD and % inhibition criteria.

Table 3: Sensitivity Estimates

Antibody Spike	Matrix	Sensitivity (ng/mL) of 3 assays % inhibition criterion only	Sensitivity (worst-case, maximum of 3 assays) % inhibition criterion only	Sensitivity (ng/mL) of 3 assays sOD & % inhibition criteria	Sensitivity (worst-case, maximum of 3 assays) sOD & % inhibition criteria
EXE4:2-8.4	Plasma	(b) (4)			
	Serum				
GLP1:3-3.1	Plasma				
	Serum				

Reviewer Comment: Using the criteria including both sOD and % inhibition, the sensitivity for the EXE antibody is (b) (4) ng/mL in both plasma and serum; and the sensitivity for the GLP-1 antibody is (b) (4) ng/mL in both plasma and serum.

6. Overall assessment on the anti-exenatide antibody cross-reactivity assay

The provided partial validation report on the cross-reactivity assay demonstrates:

- 1) The assay acceptance criteria are acceptable.
- 2) The assay is specific and repeatable.

Reviewer Comment: The competitive binding method is an inadequately sensitive method to detect the cross-activity of the anti-exenatide antibody to GLP-1 or glucagon. (b) (4)

(b) (4)

Appendix

1. Screening cut point in clinical studies BCB118 and BCB120

In the response to information request question #9, the Sponsor provided the screening cut point N-factor calculation using 488 samples collected from clinical studies BCB118 and BCB120. In summary, the SN (signal-to-negative-control) value was obtained by subtracting the negative control value from the sample's mean OD value for each sample. The data outliers (21 samples) were removed using Tukey's box plot method; then the cut point N-factor was calculated using nonparametric method since the data is not normally distributed. The N-factor is 0.097 for T2D patient samples in clinical studies BCB118 and BCB120. Comparing with the cut point factors of 0.096 and 0.104 calculated from healthy subjects and applied for studies BCB110, BCB118 and BCB120, the Sponsor justified that the cut points calculated from healthy subjects are suitable to be applied in the clinical studies and a T2DM-specific cut point is not required.

Reviewer Comment: *The statistical analysis for the cut point N factor calculation from T2DM patients is acceptable. However, the comparison between N-factors are not suitable for purpose. The Sponsor should compare the cut points not N-factors. We sent out the second round IR for the comparison between cut points in healthy people vs. T2DM patients.*

The following second-round IR was sent to the Sponsor on September 20, 2017:

You calculated the cut point N-factor from healthy subjects using a parametric method on log-transformed data. You also calculated the cut point N-factor to be 0.097 using patient samples collected from NDA 209210 clinical trials BCB118 and BCB120 using a non-parametric method on the original scale. To justify the appropriateness for the cut point calculated from healthy subjects to be used in the analysis of NDA209210 clinical trials, you compared N-factor calculated from healthy subjects *vs.* N-factor calculated from patients in clinical trials BCB118 and BCB120. We do not agree with your strategy of comparing N-factors because the relevant question is whether the cut-points are comparable between the populations. Compare the cut point calculated from healthy subjects with the cut point calculated from T2DM patient samples collected from NDA209210 clinical trials. Justify the usage of the cut point calculated from healthy subjects in the analysis of samples collected from clinical trials BCB118 and BCB120.

The Sponsor provided a response to our IR on September 27, 2017 with one document: **CPO Exenatide JZ-003:** Statistical Analysis for "Exenatide once weekly suspension (EQWS) for Type 2 diabetes Response to NDA 209210 Information Request (20 September 2017)."

In the response, the Sponsor stated that the cut point for T2DM patients (488 samples) was calculated in the following steps:

- 1) the outliers (18 samples) were removed using Tukey's box-plot method;

- 2) the data is normally distributed after log-transformation;
- 3) the cut point for T2DM patients was calculated using parametric method on the log-transformed data to have the cut point = 0.226.

The cut point for T2DM patients is ~ 0.066 higher than the cut point for healthy subjects (0.160). The Sponsor claimed the cut point difference was mainly caused by the fact that the NC value for BCB118/BCB120 clinical trial sample analysis in 2013 and 2014 is 0.062 higher than the one from the validation assays in 2009. The cut point difference is 0.004 after adjusting by the NC value difference. The same screening cut point (0.154, reported in validation Report REST090118R1) was used in all studies.

Reviewer Comments:

In the response to the first IR and the second IR, the Sponsor used different methods in the cut point and N-factor calculation although they removed the outliers using the same method—Tukey’s Box-plot method. In the response to the second IR, the cut point for T2DM patients was calculated using a parametric method with 5% false positive rate on the log-transformed data. The calculation of the cut point for T2DM patients in the response is acceptable.

Using the cut point for healthy subjects is more conservative and leads to higher false positive rates than using the cut point for T2DM patients. It is not a concern since higher false positive samples will be identified as ADA negative in the confirmatory assay. Therefore, applying the cut point for healthy subjects in the clinical trials BCB118/BCB120 sample analysis is acceptable.

2. HPC and LPC concentrations

The Sponsor did not provide the concentration for HPC and LPC in mass units per mL. We requested these concentration information in the information request.

Reviewer Comments:

In the response to the information request, the Sponsor estimated the LPC as ~ 3.8 µg/mL and HPC as ~14.2 µg/mL in Report REST080375R2 developed in (b) (4). In this report, positive controls were prepared by diluting human plasma containing anti-exenatide antibodies in human serum at 1:100 for LPC and 1:15 for HPC.

In Report 090118R1, the dilutions are 1/5.6 and 1/45.6 for HPC and LPC. Therefore the provided concentrations ~ 3.8 µg/mL for LPC and ~14.2 µg/mL for HPC are not the ones we asked for Report 090118R1. The Sponsor should provide the concentrations for HPC and LPC used in Report 090118R1 to demonstrate the properness of using LPC and ensure low positive samples are detected. If LPC is not close to the assay sensitivity, LPC should be determined by calculating the concentration of the ADA that is expected to fail the assay for ~1% of the time. The LPC concentration can be decided from this assay using (Mean PC concentration at PSCP + $t_{0.01,df} * SD$), with 1% possibility to fail the runs.

Since the assay sensitivity is inadequately established, we ask the Sponsor to fulfill a PMC for the validation of the assay sensitivity (b) (4)

3. Assay sensitivity in Report REST080584R1

Below describes the sensitivity assay in Report REST080584R1 developed in Amylin. The assay is to detect whether anti-exenatide antibody has the ability to cross-react with GLP-1 or glucagon. In the response to information request question #3(a), the Sponsor stated the sensitivity was estimated as (b) (4) ng/mL at Amylin (Report REST080584R1.) The tables below list the results of the serial dilution of positive control EXE4:2-8.4 spiked in human K₂EDTA plasma without peptide.

Table 7: Sensitivity for EXE4:2-8.4 Spiked in Human K₂EDTA Plasma

Summary of EXE4:2-8.4 Sensitivity In Human NaEDTA Plasma in The Presence of Various Peptides									
Inhibitory Peptide	EXE4:2-8.4 (µg/mL)	Optical Density (OD) at 450/650 nm			Overall Mean OD	SD	% CV	sOD	%Inhibition
		Analyst 1 Mean	Analyst 2 Mean	Analyst 3 Mean					
w/o peptide	1.0	0.239	0.206	0.176	0.207	0.032	15.2	NA	NA
	0.5	0.114	0.081	0.078	0.091	0.020	22.0	NA	NA
	0.25	0.053	0.045	0.040	0.046	0.007	14.3	NA	NA
	0.125	0.026	0.021	0.020	0.022	0.003	14.4	NA	NA
	0.063	0.014	0.014	0.012	0.013	0.001	8.7	NA	NA

Table 9: Sensitivity for EXE4:2-8.4 Spiked in Human Serum

Summary of EXE4:2-8.4 Sensitivity In Human Serum in The Presence of Various Peptides									
Inhibitory Peptide	EXE4:2-8.4 (µg/mL)	Optical Density (OD) at 450/650 nm			Overall Mean OD	SD	% CV	sOD	%Inhibition
		Analyst 1 Mean	Analyst 2 Mean	Analyst 3 Mean					
w/o peptide	1.0	0.418	0.291	0.280	0.330	0.077	23.3	NA	NA
	0.5	0.141	0.141	0.127	0.136	0.008	5.9	NA	NA
	0.25	0.065	0.062	0.063	0.063	0.002	2.4	NA	NA
	0.125	0.033	0.031	0.029	0.031	0.002	6.5	NA	NA
	0.063	0.020	0.019	0.018	0.019	0.001	5.3	NA	NA

Reviewer Comment: The assay sensitivity result of 125 ng/mL is not for the ADA binding assay that we requested in information request question #3(a), but the cross-reactivity assay. Therefore, this result is not considered for the sensitivity assay in ADA validation assays.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RONG WANG
10/10/2017

RAMESH B POTLA
10/10/2017

SUSAN L KIRSHNER
10/10/2017

OFFICE OF DEVICE EVALUATIONDIVISION OF ANESTHESIOLOGY, GENERAL HOSPITAL,
RESPIRATORY, INFECTION CONTROL, AND DENTAL DEVICES**GENERAL HOSPITAL DEVICES BRANCH
INTERCENTER CONSULT MEMORANDUM****Device Constituent Part Design Review: CDER NDA209210 - CDRH ICC1600904****Date:** September 15, 2017**To:** Richard Whitehead
CDER/OND/ODEII/DMEP**From:** Rong Guo
CDRH/ODE/DAGRID/GHDB**Through:** CAPT Alan Stevens, Branch Chief
CDRH/ODE/DAGRID/GHDB**Re:** NDA209210**Subject:** Device Constituent Part Design Review for ICC1600904, NDA209210 BYDUREON BCISE Exenatide suspension Autoinjector

Applicant	Astra Zeneca
Indication for Use	Adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus
Drug / Biologic Constituent	Drug/Device
Drug component	Exenatide injectable suspension
Device Constituent	Autoinjector

Recommendation: CDRH recommends approval based on review of the device constituent part of the combination product.

Digital Signature Concurrence Table	
Reviewer	Rong Guo -S 2017.09.15 14:15:53 -04'00'
Branch Chief	Alan M. Stevens -S Digitally signed by Alan M. Stevens -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=13001892 11, cn=Alan M. Stevens -S Date: 2017.09.21 11:33:40 -04'00'

Note: Unless otherwise stated, all figures and tables presented below were taken from this submission NDA209210.

Administrative

The CDRH/ODE reviewer performed a design review of submission materials intended to support the safety and functionality of the device constituent parts of the subject combination product. This evaluation covered the intended design and design control information for the subject device constituent part. Essential performance elements of the device under review include Dose accuracy, Needle extension, Activation force and Injection time, along with biological

evaluation and sterility of the device were covered in this review memo.

This review did not cover the following content:

- Review of primary container closure-drug product interaction, sterility, or toxicology
- Manufacturing of the drug product
- Manufacturing of the device constituent part of the combination product
- Review of human factor study

Documents Reviewed:

NDA 209210

- 3.2. R Medical Device –Autoinjector Sequence 0000 (SDN 2) December 21, 2016
- 1.11.1 Response to Questions –Quality, sequence 0024 (SDN25) September 5, 2017
- Response to Questions –Quality, received email on September 14, 2017

MAF (b) (4)

Cross-Referenced 510(k) # or DMF	Device	Letter of Authorization Included in NDA
MAF (b) (4)	(b) (4) Autoinjector	yes

Meetings attended:

Filing meeting	February 8, 2017
Mid-cycle meeting	May 24, 2017
Wrap up meeting	September 14, 2017

Purpose / Background

BYDUREON BCISE is an extended-release formulation of exenatide, administered as an injection once every 7 days (weekly). Exenatide is a glucagon-like peptide-1 (GLP-1) receptor agonist that exhibits many of the same glucoregulatory or glucose-lowering actions as GLP-1, a naturally-occurring incretin hormone. Exenatide has been shown to reduce fasting and postprandial plasma glucose concentrations in nonclinical and clinical studies through multiple mechanisms of action including increased synthesis and secretion of glucose-dependent insulin from pancreatic beta cells, decreased plasma glucose concentration in animal models and in humans, increased insulin sensitivity, suppression of inappropriately elevated glucagon in a glucose-dependent manner, increased pancreatic beta-cell mass, and slowing of nutrient absorption via a decrease in the rate of gastric emptying and reduced food intake.

AstraZeneca maintains two marketed formulations of exenatide as a treatment for type 2 diabetes mellitus (T2DM): BYETTA ([exenatide] injection; exenatide twice daily (BID)) and BYDUREON ([exenatide extended-release for injectable suspension]; exenatide once weekly (QW)). AstraZeneca is seeking approval for BYDUREON BCISE, a third exenatide formulation, exenatide injectable suspension, as an adjunct to diet and exercise to improve glycemic control in adults with T2DM. Unlike BYDUREON which utilizes an aqueous vehicle, the new product is suspended in a nonaqueous, medium-chain triglyceride (MCT) vehicle suitable for use with an autoinjector delivery platform, simplifying the process for dose preparation and self-injection by patients.

Device Description and Performance Requirements

Indications for Use	Adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus
Route of Administration	subcutaneous (SC) injection

The proposed combination product contains the drug product exenatide suspension and an autoinjector. The exenatide suspension is packaged in a 2-mL USP (b) (4) glass cartridge, which is pre-assembled into the autoinjector.

(b) (4)

Risk Analysis

Risk Analysis Attributes	Yes	No	N/A
Risk analysis conducted on the combination product	yes		
Hazards adequately identified	yes		
Mitigations are adequate to reduce risk to health	yes		
Version history demonstrates risk management throughout design / development activities			n/a

Summary of Risk Analysis

The Sponsor, AstraZeneca (AZ) is responsible for risk management activities for the autoinjector and has conducted risk analysis, evaluation, and acceptance activities for the final finished autoinjector. Under the direction of AZ, (b) (4) has performed separate risk management activities for the subassemblies. To ensure risks related to the drug product and intended use are properly accounted for within (b) (4) risk management activities, AZ and (b) (4) maintain close collaboration on risk documentation.

A hazard analysis was used to identify and evaluate the use of the autoinjector to identify the potential sources of patient / user harm or safety. Sources considered by the hazard analysis included energy, biological and environmental hazards as well as hazards related to the use of the product, functional failures, maintenance and aging. The identified risks were then evaluated using the Failure Mode and Effects Analysis (FMEA) tool. Risk assessments associated with user, design and process were carried out by a cross-functional team, reviewed and updated as part of the development process to assess effectiveness of mitigations and to consider potential new risks introduced by risk controls.

An example of the risk analysis provided in the submission, excerpted below, shows the risk analysis about autoinjector related risks including risk level, potential cause, potential failure mode, harm to the user and possible mitigation.

Table 7 **Examples of Risk Analysis**

Use Step	Potential Cause of Failure	Potential Failure Mode	Likely Effect of Failure	Harm to User	Current Controls	Occ	Det	Sev	Prob of Harm	Risk Level	Risk Acceptability	Action
Remove Device From Package	User drops device	Damaged needle	Device inoperable	Missed dose, delayed therapy, dissatisfaction, complaint	(b) (4)	3	5	5	2	1	Acceptable	None
					IFU contains instructions not to use device if any part appears broken or damaged							
Unlocking Device	User twists knob in wrong direction with excessive force	Device damaged	Device inoperable	Missed dose, dissatisfaction, delayed therapy	(b) (4) IFU contains arrow showing correct direction to turn, lock/unlock icons on device.	1	5	5	1	1	Acceptable	None
Dose Delivery	User attempts to inject through clothing	Needle blocked when passing through clothing	Needle blockage	Missed dose	IFU instructs to place device against skin and shows image of device against skin	2	5	5	2	1	Acceptable	None
					(b) (4)							

Reviewer Comment: *The proposed risk analysis is acceptable.*

Labeling

Container label:



Carton labeling:

(b) (4)



Instructions for Use

Once-weekly
BYDUREON® BCise™ 2 mg
(exenatide) extended-release injectable suspension
For subcutaneous use only
Single-dose Autoinjector once weekly



Read the Instructions for Use before you start using Bydureon BCise.
Before using Bydureon BCise, talk to your healthcare provider about how to use it the right way.

Before You Begin

The autoinjector:

- Is a single use, fixed dose autoinjector that automatically injects your medicine.
- Comes in the locked position before you use it. Do not unlock the autoinjector until you are ready to inject it.
- Needle is hidden. You do not see it before, during, or after using the autoinjector.
- **Do not** use the autoinjector if any parts look to be broken or damaged.
- Store flat in the refrigerator between 36°F to 46°F (2°C to 8°C).
- Never share your Bydureon BCise autoinjector with anyone else. You may give an infection to them or get an infection from them.
- Bydureon BCise should **not** be used by people who are blind or cannot see well, unless another person who is trained to use this device can help.
- Keep the autoinjector, and all medicines, out of the reach of children.

Before Use



Figure A

Supplies needed to give your injection:

- Bydureon BCise autoinjector
- Alcohol swab
- A clean, flat surface
- Sharps container (see “disposal” instructions at the end of these instructions)

STEP 1: Prepare for Injection

A. Let your autoinjector come to room temperature.

Remove 1 autoinjector from the refrigerator and rest it flat for 15 minutes.
Autoinjector can be kept at room temperature for up to 4 weeks.



Figure B

B. Check the expiration date (labeled EXP) printed on the autoinjector label.

Do not use the autoinjector past the expiration date.



Figure C

C. Wash your hands.

D. Choose your injection site.

In either your stomach, thigh, or back of the upper arm, see Figure D.
Each week you can use the same area of your body, but choose a different injection site in that area of your body.
Clean the area with an alcohol swab.

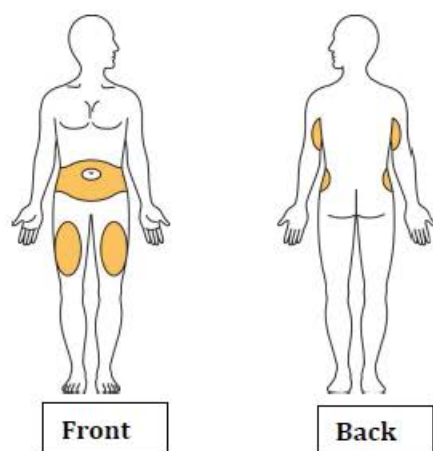


Figure D

STEP 2: Mix the medicine

A. Look in the window.

You may see white medicine along the sides, bottom or top. This means the medicine is not mixed evenly.

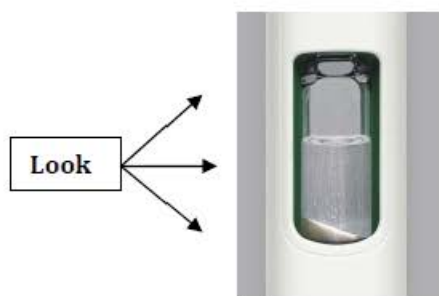


Figure E

B. Shake the autoinjector hard,

in an up-and-down motion, until the medicine is mixed evenly and you do not see any white medicine along the sides, bottom or top. Shake for at least 15 seconds.

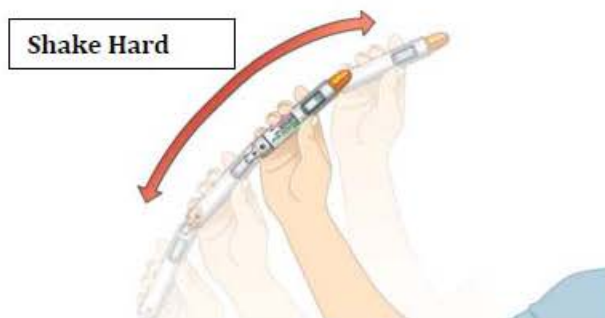


Figure F

C. Check the mix.

Hold the autoinjector up to the light and look through both sides and the bottom of the window. If not mixed well, repeat Step 2 and check again.

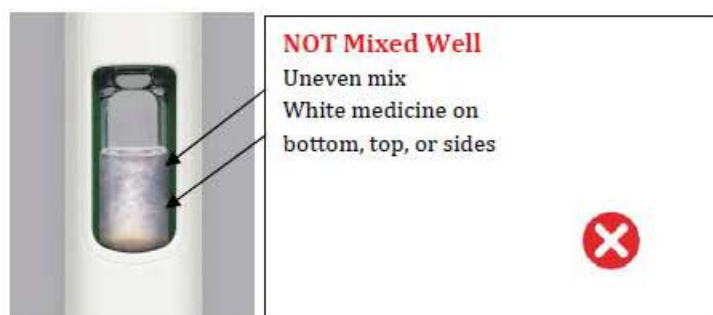


Figure G



Figure H



Do not go to the next step unless your medicine is mixed well. To get a full dose, the medicine must be mixed well and look cloudy.
If not mixed well, continue to shake hard.

STEP 3: Prepare the Autoinjector

Important: After the medicine is fully mixed, you must complete the preparation steps **right away**, and inject to get the full dose. Do not save it to use later.

Only unlock the autoinjector when you are ready to inject

A. Unlock the autoinjector.

Hold the autoinjector up straight with the orange cap toward the ceiling. Turn the knob from the Lock to the Unlock position until you hear a click.



Figure I



Figure J

B. While still holding the autoinjector straight up, firmly unscrew the orange cap.

- You may need to turn the cap a few times before it loosens (if you hear clicking you are turning in the wrong direction).
- Continue holding the autoinjector upright to prevent the medicine from accidentally leaking.

- A green shield will pop up after the cap is removed. The green shield hides the needle. It is normal to see a few drops of liquid inside the cap. **Do not** recap the autoinjector. Throw away the cap.

Hold upright & firmly unscrew

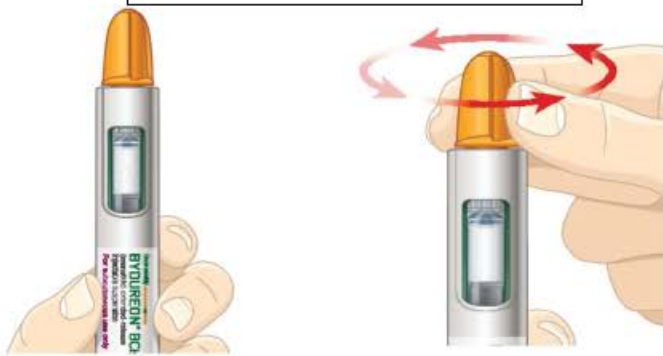


Figure K

Figure L

Removed Cap



Figure M

Green shield pops up

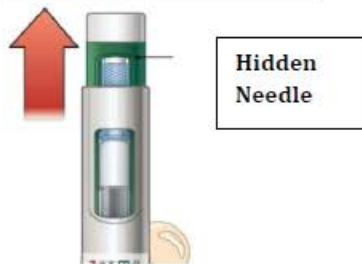


Figure N

STEP 4: Inject the Dose

A. Inject and hold:

- Push the autoinjector against your skin. You will hear a “click” when the injection begins.
- Keep holding the autoinjector against the skin for 15 seconds. This is to make sure you get the full dose.

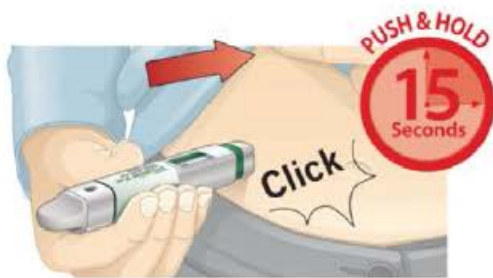


Figure O

B. Make sure you received your full dose.

After you receive your injection, you will see an orange rod in the window. After you lift the autoinjector from your skin, the green shield will move back up to lock over the needle. See the Common Questions & Answers for what to do if you do not see the orange rod after injection.



Figure P

STEP 4: Inject the Dose (continued)

C. Disposal.

Put your used autoinjector in a FDA-cleared sharps disposal container right away after use. Do not throw away (dispose of) loose needles and syringes into your household trash. If you do not have an FDA-cleared sharps disposal container, you may use a household container that is:

- Made of heavy-duty plastic
- Can be closed with a tight-fitting, puncture-resistant lid, that won't let sharps come out
- Upright and stable during use
- Leak-resistant, and
- Properly labeled to warn of hazardous waste inside the container



Figure Q

When your sharps disposal container is almost full, you will need to follow your community guidelines for the right way to dispose of your sharps disposal container. There may be state or local laws about how you should throw away used needles and syringes. For more information about safe sharps disposal, and for specific information about sharps disposal in the state that you live in, go to FDA's website at: <http://www.fda.gov/safesharpsdisposal>.

Do not dispose of your used sharps disposal container in your household trash unless your community guidelines permit this. Do not recycle your used sharps disposal container. See "Common Questions & Answers" for additional disposal information.

Please keep these instructions to use for your next dose.

Design Transfer Activities – Release Specifications

The following release specifications are included for the device constituent within eCTD Module 3.2.P.5:

(b) (4)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RICHARD E WHITEHEAD
09/21/2017



QUALITY REVIEW



Recommendation: **APPROVAL**

(including the Facility Review/Overall Manufacturing Inspection Recommendation)

NDA 209210

Review #1

Review Date (see last page)

Drug Name/Dosage Form	exenatide injectable suspension, extended release
Strength	2 mg
Route of Administration	SC injection
Rx/OTC Dispensed	Rx
Applicant	Astra Zeneca

SUBMISSION(S) REVIEWED NDA 209803	DOCUMENT DATE
0000	12/21/16
0008	3/24/17
0009	4/6/17
0014	7/7/17
0016	7/26/17
0026	9/12/17

Quality Review Team

DISCIPLINE	REVIEWER	DIVISION/OFFICE
Regulatory Business Process Manager	Anika Lalmansingh	Regulatory Business Process Management/OPRO
Application Technical Lead	Suong (Su) Tran	New Drug Products/ONDP
Drug Product	Christopher Galliford/Danae Christodoulou	New Drug Products/ONDP
Process	Delaram Moshkelani/Jane Chang	Process Assessment/OPF
Facility	Sherry Shen/Juandria Williams	Inspectional Assessment/OPF
Biopharmaceutics	Sandra Suarez/Haritha Mandula	Biopharmaceutics/ONDP
Microbiology	David Bateman/John Metcalfe	Microbiology Assessment/OPF

Quality Review Data Sheet

1. RELATED/SUPPORTING DOCUMENTS:

A. DMFs: Adequate (see Chapter II)

B. Other Documents: NDA 21773 BYETTA (exenatide injection) and NDA 22200 BYDUREON (exenatide for injectable suspension, extended release) by the same applicant

2. CONSULTS: CDRH Compliance (recommendation is included in the OPQ Facilities review; see separate CDRH review in DARRTS)

Executive Summary

I. Recommendation and Conclusion on Approvability

The final OPQ recommendation is for Approval, including the overall manufacturing inspection recommendation.

II. Summary of Quality Assessment

A. Product Overview

This is a 505(b)(1) NDA for exenatide but it is not a New Molecular Entity because the applicant has two approved NDAs for the same drug substance.

Reference is made to the applicant's approved NDA 21773 BYETTA for all CMC information on the drug substance.

The drug product is a suspension for subcutaneous injection, packaged in a single-dose pen injector. The formulation consists of drug microspheres in a non-aqueous, medium-chain triglycerides diluent.

- Reference is made to the applicant's approved NDA 22200 BYDUREON for CMC information on the drug microspheres. Both BYDUREON and the new product have the same exenatide microspheres.
- The differences between the new product and the approved BYDUREON are the dosage form, diluent, and delivery system: the new product is an injectable suspension in a non-aqueous diluent (medium chain triglycerides) to be marketed in pen injectors, and the approved BYDUREON is a powder requiring resuspending in an aqueous diluent (Na CMC, polysorbate 20, NaCl, buffer salts, and SWFI) marketed in a co-packaged vial/prefilled syringe presentation (drug powder in vials, diluent in prefilled syringes).

The pivotal Phase 3 studies were conducted with the commercial drug product formulation, manufactured at the commercial drug product manufacturing site at an acceptable pilot scale ((b) (4) commercial scale).

Proposed Indication(s)	[not finalized by GRMP goal; see CDTL's memo]
Duration of Treatment	[not finalized by GRMP goal; see CDTL's memo]
Maximum Daily Dose	[not finalized by GRMP goal; see CDTL's memo]
Alternative Methods of Administration	n/a

B. Quality Assessment Overview

Drug Substance

Exenatide is a 39-amino acid synthetic peptide amide with an empirical formula of $C_{184}H_{282}N_{50}O_{60}S$ and a molecular weight of 4186.6 Daltons. The amino acid sequence for exenatide is shown below.

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH₂

The drug substance is exenatide, a small (39-residue) synthetic peptide. Reference is made to the approved NDA 21773, from the same applicant, for all CMC information on the drug substance.

Drug Product

The drug product is an extended release injectable suspension, 2 mg of exenatide in 0.85 mL of the diluent medium chain triglycerides (MCT), packaged in a glass cartridge pre-assembled in a single-dose single-patient pen injector. To ensure the delivery of the minimum 0.85 mL volume, each cartridge has an overfill of (b) (4) mL, which is acceptable.

- Exenatide is formulated as microspheres with (b) (4) exenatide with the following excipients: (per 0.85 mL) 0.8 mg sucrose and (b) (4) mg 50:50 poly(D,L-lactide-co-glycolide). The microspheres are identical to those approved in the referenced NDA 22200.
- There is no novel excipient, and there is no human/animal-derived excipient. MCT is not a novel excipient because it has an NF monograph and it has been used in approved products. The compendial requirements include percentages of specific carbon-chain lengths. The subcutaneous chronic use proposed for this product is new for MCT (found acceptable by the Pharmacology Toxicology team).
- The pivotal Phase 3 studies were conducted with the commercial drug product formulation.

The drug product manufacturing process consists of

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

- The expiration dating period of the product is calculated based on the date of the suspension preparation (b) (4), which is different from the standard practice of calculating from the date of introduction of the drug substance into the

drug product manufacturing process. This deviation is acceptable from a risk-based perspective and based on the expiration dating period of the approved BYDUREON starting from the date of vial filling as well as on the supporting stability data.

Reference is made to the CDRH review of all information related to the device, including its components, assembly, biocompatibility, dose accuracy, and differences between the clinical device and commercial device.

The regulatory drug product specification is adequate based on the supporting release and stability data and ICH guidelines for this type of dosage form, including information on elemental impurities.

Reference is made to the CDRH review of all information related to the device, including dose accuracy.

Degradants – Even though the new product and BYDUREON have different dosage forms and different diluents, there is no difference in the degradants. The specification includes the same degradants that are approved for BYDUREON (b) (4), supported by qualification information (found acceptable by the Pharmacology Toxicology team). The limit on Total Impurities is the same (b) (4)% for both products.

(b) (4)

Primary container closure system: The primary packaging materials are a 2.0 mL USP (b) (4) clear glass cartridge with a (b) (4) plunger on one end and a (b) (4) aluminum cap on the other end. Information on extractables/leachables is included in the NDA and found acceptable by the Pharmacology Toxicology team.

Expiration Date & Storage Conditions: The shelf life of the drug product is 36-month at at 2-8 °C with an in-use storage for up to 4 weeks at room temperature, protected from light.

The long-term expiry is based on 36-month data at 5 °C and 6-month data at 25 C/60% RH for 3 batches of the complete combination products (cartridges assembled in pen injectors). All batches have the commercial formulation, packaged in the commercial cartridges, manufactured at the commercial site (b) (4) pilot scale (b) (4) commercial scale, and by a process representative of the commercial process. These 3 stability batches were also phase 3 clinical batches.

Comparability protocol.

- The proposal to submit a CBE30 supplement for the addition of a manufacturing line at the same commercial site (b) (4) is acceptable.

C. Special Product Quality Labeling Recommendation: not applicable

D. Life Cycle Knowledge Information/ Final Risk Assessment:

Drug product	none
Process	page 32 of Chapter V
Facilities	page 10 of Chapter IV
Biopharmaceutics	page 18 of Chapter VII
Microbiology	none

Application Technical Lead Signature:

I concur with the reviewers' recommendations.

Suong T. Tran
-S

Digitally signed by Suong T. Tran: 5
DN: cn=S, o=U.S. Government, ou=HHS,
ou=FDA, ou=People, cn=Suong T. Tran, 5
9 2242 1020000 100 1 1-200101829
Date: 2017.09.20 13:33:45 -0400

Suong (Su) Tran, Ph.D.
electronic signature also on the last page

BIOPHARMACEUTICS**BACKGROUND:****NDA: 209210 ORIG-1****Drug Product Name / Strength:** BYDUREON BCise (exenatide) extended-release injectable suspension**Route of Administration:** Subcutaneous injection**Applicant Name:** AstraZeneca

The Applicant is seeking approval of BYDUREON BCise® (exenatide) extended release injectable suspension) as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus (T2DM). BYDUREON BCise® contains the same active ingredient (i.e. exenatide) as the commercial products BYETTA (exenatide) injection (NDA 021-773) and BYDUREON (exenatide extended-release) for injectable suspension (NDA 022-200). The latter is approved by the FDA as an adjunct to diet and exercise to improve glycemic control in adults with T2DM to be administered once weekly.

The drug product under review, BYDUREON BCise® is also referred to as exenatide once-weekly suspension (EQWS). EQWS has the same exenatide drug load of (b) (4) encapsulated within biodegradable microspheres as in the BYDUREON formulation. The Applicant claims that the EQWS microspheres are identical to those used in commercially available BYDUREON (i.e., manufactured at the same site with the same manufacturing process). However, while BYDUREON utilizes an aqueous vehicle, the EQWS microspheres are suspended in a nonaqueous, oily medium-chain triglyceride (MCT) vehicle suitable for use with an autoinjector delivery platform. The EQWS formulation is supplied in an autoinjector device dispensing a 2 mg dose (single use).

This submission follows a 505 (b)(1) path and is based on one Phase 2 study (BCB110) and two Phase 3 studies (BCB118 and BCB120). Cross-references are also made to the BYETTA and BYDUREON marketing applications as appropriate. The Applicant intends to use Amylin LLC, Ohio for commercial and future clinical manufacturing of (b) (4) microspheres and assembly of the suspension filled cartridges into autoinjectors.

Summary of Biopharmaceutics Findings:

This 505b (b)(1) drug product (microspheres) containing the drug substance exenatide is to be administered subcutaneously (SC) for the treatment of T2DM. Exenatide has low solubility and has been classified as BCS Class II drug substance. BCS classification is leveraged in support of biowaiver for orally administered drug products. Therefore, BCS class for this drug product is

not relevant since it is to be administered SC. EQWS is to be marketed as an ER drug product. Evidence of extended release characteristics for EQWS is based on its *in vitro* and *in vivo* performance. Specifically, it was demonstrated that EQWS exhibits steady state plasma concentrations comparable to BYDUREON with lower fluctuations, and no evidence of dose dumping. EQWS comprises of identical microspheres as the approved extended release BYDUREON product. In addition, the Applicant claims that a consistent ER performance is assured (b) (4)

The drug product underwent some changes (e.g. inclusion of an autoinjector) through the phases of development. Data demonstrated that there is no change on the *in vitro* release among all batches tested in pivotal clinical trials. However, changing from syringe to autoinjector administration may have an impact on the *in vivo* performance of the drug product. This potential impact determination is under the purview of CDRH.

It should be noted that the proposed *in vitro* release method has been previously approved under NDA 22200 for the same type of microspheres based on its discriminating ability toward critical attributes. Briefly, data were submitted under NDA 22200 supporting the selection of temperature, volume of medium, osmotic pressure, pH, and agitation. As mentioned above, EQWS microspheres under the current NDA and under NDA 22200 (approved NDA) are similar except that EQWS microspheres are suspended in a non-aqueous vehicle, MCT. The analytical method for *in vitro* complete release for the exenatide suspension product from EQWS requires (b) (4)

Thus, the Applicant was requested during the review cycle to provide comparative data showing that the change in suspending vehicle does not affect the *in vitro* release. *In vitro* release profile comparisons with f2 test demonstrated that microspheres and exenatide suspension are equivalent. The following *in vitro* release method and acceptance criteria are deemed acceptable.

Apparatus	Water bath capable of maintaining a water temperature within $\pm 0.3^{\circ}\text{C}$ and 60 mL polypropylene bottles
Medium	300 mM TRIS buffer at pH 9.4 with 0.5% w/v poloxamer 188 and 4.0 mL per litre 5% sodium azide solution. To the above add 67g/1000mL carboxymethyl cellulose solution ^a . Ionic strength: 0.04M
Volume	50mL of medium added at start of test
Agitation	Samples are agitated manually. Agitation is performed before the start of the test and before and after each sampling point.
Temperature	37.0°C
Sampling times	1, 7, 14, 17, 21, 31, 42, 49 and 52 days
Acceptance criteria	Day 17: NMT (b) (4)% Day 31: (b) (4)% Day 52: NLT (b) (4)%

^a Also referred to as exenatide once weekly diluent

It is noted that manual agitation is implemented as part of the method which is an unusual procedure. The presence (manual horizontal agitation) and absence of agitation were evaluated. The Applicant showed, as part of NDA 22200 submission, that the presence of manual agitation does not have an effect on the in vitro release of the drug product. The lack of impact of manual agitation on the in vitro release is justifiable given that:

- The samples are agitated by horizontal circulation of sample bottles just before the sample is taken with the purpose of re-suspend/homogenize the samples.
- There are only three specification time points in a period of 52 days.
- The inter-sample variability in dissolution is relatively small.

Given the importance of microsphere PSD and its potential to impact in vitro release and in vivo performance, the Applicant was requested during the review cycle to revise their proposed PSD ranges (b) (4). Based on the Applicant's response, the inclusion of (b) (4) as part of drug product specification does not bring additional benefit to the control strategy. From Biopharmaceutics perspective, the proposed control strategy (including the in process controls attributes/parameters) are acceptable to assure product quality and performance; however, the specification proposed for PSD (i.e. (b) (4) microns) may need to be revised (e.g., (b) (4) microns) for the following reasons:

- The studies relating PSD to in vitro release evaluated ranges of particle size up to (b) (4) microns. The proposed (b) (4) microns was not evaluated;
- To ensure consistent performance as that seen for the pivotal phase 3 batches with a mean (b) (4) of about (b) (4) microns. This was communicated to the CMC reviewer via email on 09/06/17. Per the CMC reviewer, changing the PSD specification may not be warranted, since the same microspheres are approved under NDA 22200.

List of Submissions being reviewed (table):

SUBMISSION(S) DATE	SEQUENCE NO.
12/21/16	0000
3/24/17	0008
4/6/17	0009
7/7/17	0014

Highlight Key Outstanding Issues from Last Cycle: NONE

Concise Description of Outstanding Issues Remaining: NONE

*From Biopharmaceutics perspective, NDA 210209 for BYDUREON BCise (exenatide) extended-release injectable suspension is recommended for **Approval**.*

BCS Designation

Reviewer's Assessment:

Exenatide is a 39-amino acid peptide manufactured by chemical synthesis. The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. This information is leveraged in support of biowaiver for orally administered drug products. Therefore, BCS class for this drug product is not relevant since it is to be administered subcutaneously.

IVR Method and Acceptance Criteria

IVR Method

According to the Applicant, EQWS utilizes the same ER exenatide microspheres as the commercially available ER BYDUREON products. No change to the microsphere composition or manufacturing process has been made, and the fundamental physicochemical mechanisms governing extended release from exenatide microspheres remain the same. It is stated that the change from the aqueous vehicle in BYDUREON to a non-aqueous MCT vehicle in EQWS was not designed to alter the extended release profile, rather it was primarily undertaken to develop a formulation that does not require addition of the vehicle immediately before administration and is suitable for use with an autoinjector (this necessitates the use of a non-aqueous vehicle because the microspheres are sensitivity to water). The release mechanism of the exenatide QW microspheres (under NDA 22200) was characterized in vitro by incubating microspheres in release medium at 37°C and periodically sampling for drug release, polymer molecular weight, and particle morphology. The Applicant claims that this IVR method (Table 1), approved under NDA 22200 was used throughout the development of EQWS microspheres. Based on the biopharmaceutics reviews by Drs. Khairuzaman and Dorantes¹, the method was found acceptable with discriminating ability toward polymer molecular weight (Figure 1), one key quality attribute.

Data showing the effect of several parameters:

1. A sigmoidal drug release pattern is observed at 37°C and 45°C and simultaneous exponential decay in PLG molecular weight but the drug release at 25°C was linear with no apparent lag phase and a marginal drop in molecular weight.
2. At pH 9.4, the standard condition, the characteristic sigmoidal release pattern was observed. At pH 7.4, a similar sigmoidal pattern was observed, but with a longer lag phase ~35days and a more rapid primary release phase. At day 45 and 50 the cumulative release from both pH 7.4 and 9.4 buffers was comparable.

¹ Biopharmaceutics review for NDA 22200 entered in DARRT by Drs. Khairuzzamand and Dorantes on 09/22/11 and 10/19/11.

3. At low osmotic strength (24mOsm), drug release was continuous with no apparent lag. High osmotic pressure (~606mOsm) had little effect on drug release behavior compared to physiologic conditions (~306 mOsm). The decrease in molecular weight was comparable for all osmotic conditions.
4. Media agitation had no impact on drug release

Table 1. IVR method implement throughout drug product development

Apparatus	Water bath capable of maintaining a water temperature within $\pm 0.3^{\circ}\text{C}$ and 60 mL polypropylene bottles
Medium	300 mM TRIS buffer at pH 9.4 with 0.5% w/v poloxamer 188 and 4.0 mL per litre 5% sodium azide solution. To the above add 67g/1000mL carboxymethyl cellulose solution ^a . Ionic strength: 0.04M
Volume	50mL of medium added at start of test
Agitation	Samples are agitated manually. Agitation is performed before the start of the test and before and after each sampling point.
Temperature	37.0°C
Sampling times	1, 7, 14, 17, 21, 31, 42, 49 and 52 days

^a Also referred to as exenatide once weekly diluent

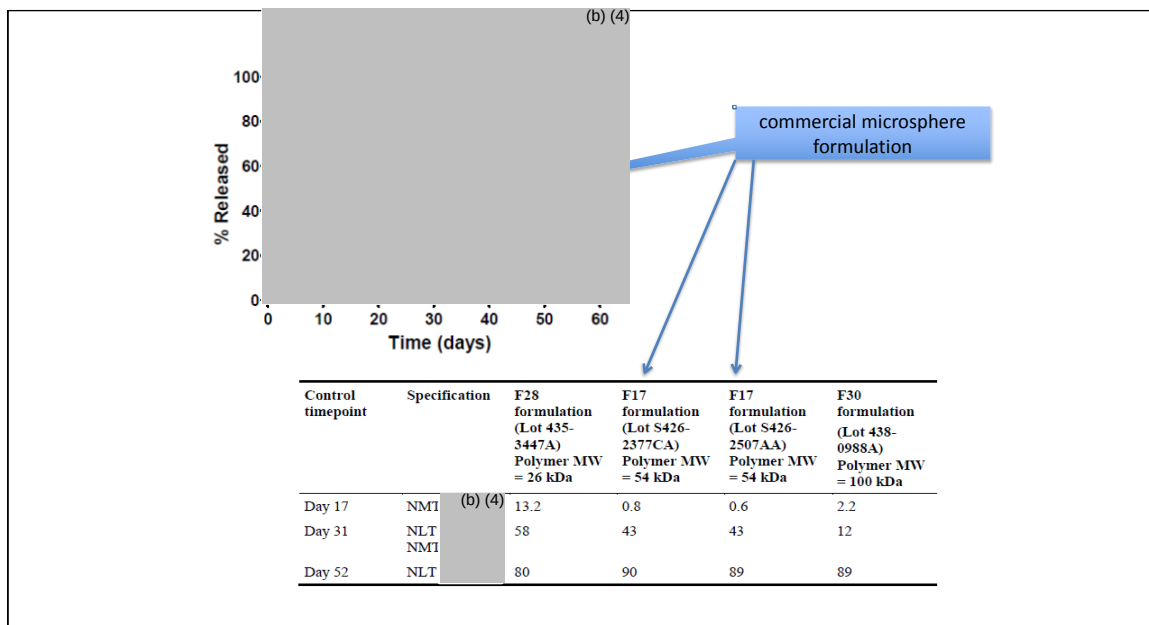


Figure 1. *In Vitro* Complete Release Profiles for Exenatide QW Formulations AC2993-F17, -F28, and -F30 at 37°C.

As mentioned above EQWS microspheres and under NDA 22200 (approved NDA) are similar except that EQWS microspheres are suspended in a non-aqueous vehicle, MCT. The analytical method for *in vitro* complete release for the exenatide suspension product from EQWS requires removal of the MCT, leaving dry BYDUREON microspheres. Thus, the Applicant was requested during the review cycle to provide comparative data showing that the change in suspending vehicle does not affect the *in vitro* release. On a submission dated 3/21/17, the Applicant stated that inclusion of MCT as a vehicle does not impact overall exenatide release, since this is controlled by the microsphere formulation and manufacturing process. In addition, data by means of an F2 test demonstrated that microspheres and exenatide suspension are equivalent (Figure 2, Table 3).

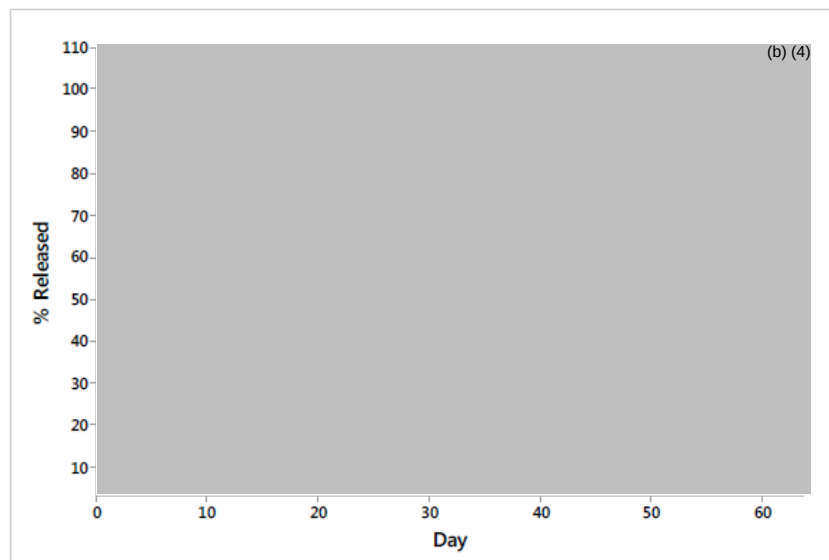


Fig. 2. In vitro release profile comparison of microspheres batch 10-011-007A (reference) and exenatide suspension batch AR-3680-057-52 (test).

Table 3. Results of F2 testing.

Microsphere batch	Exenatide suspension batch	F2
73888	AVOB01	(b) (4)
10-011-007A	AR-3680-057-52	

IVR Acceptance Criteria

The following acceptance criteria were originally proposed for the drug product under review. The data supporting these criteria are shown in Figure 8.

IVR Time Point	Acceptance Criteria
Day 17	NMT (b) (4)
Day 31	(b) (4)
Day 52	NLT (b) (4)

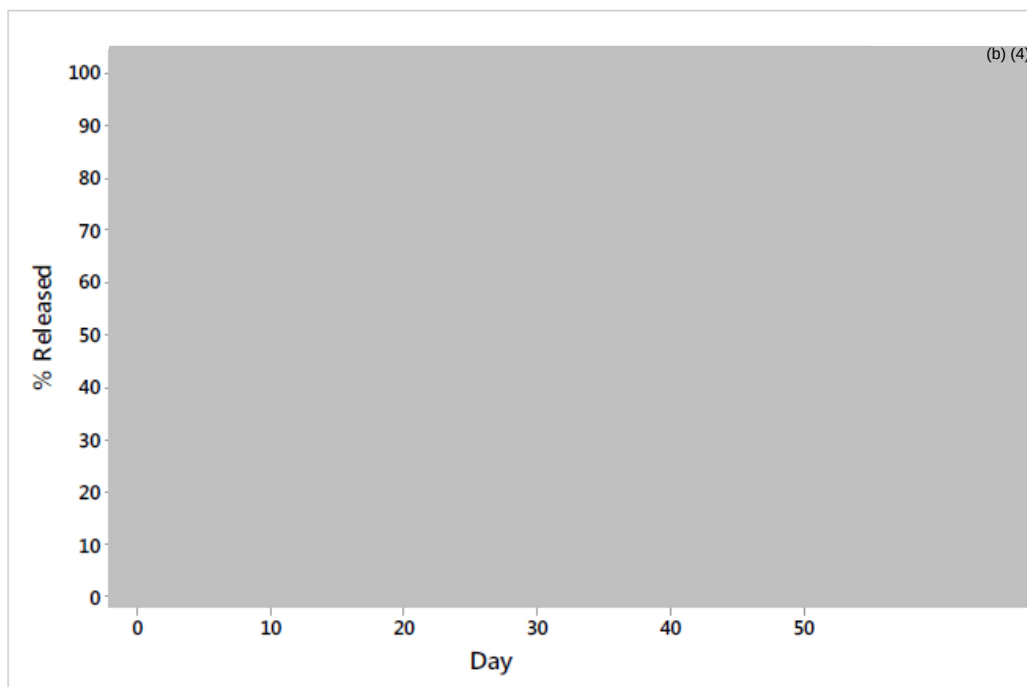


Figure 3. Typical IVR profiles for clinical pivotal batches.

Reviewer's comments

The time points proposed may not be sufficient to ensure consistent in vitro/in vivo performance throughout the life cycle of the drug product. Specifically, based on the results displayed in Figure 3, the proposed criteria/times point may not be able to reject for aberrant batches as higher variation on the profiles are seen upon stability testing. Therefore, during the review cycle, the Applicant was recommended to implement an additional time point at Day 41 of initiation of the test. On a response submitted Jul 7, 2017, the Applicant submitted an updated sheet of drug product specifications which reflect some of Agency's recommendations as follows:

IVR Time Point	Recommended Acceptance Criteria	Proposed (new) by Applicant
Day 17	NMT (b) (4)	NMT (b) (4)
Day 31	(b) (4)	(b) (4)
Day 41	(b) (4)	none
Day 52	NLT (b) (4)	NLT (b) (4)

As noted on the table above, AstraZeneca agreed (submission dated 07/07/17) to lower the range for the in vitro release at Day 17 to (b) (4)%. According to the Applicant, the extensive manufacturing history demonstrates that this specification can be consistently met at release and stability data confirming that there is little or no change on stability beyond that expected from analytical variability, as some batches had released above (b) (4)% at this time point (Figure 4). Therefore, their proposal is acceptable.

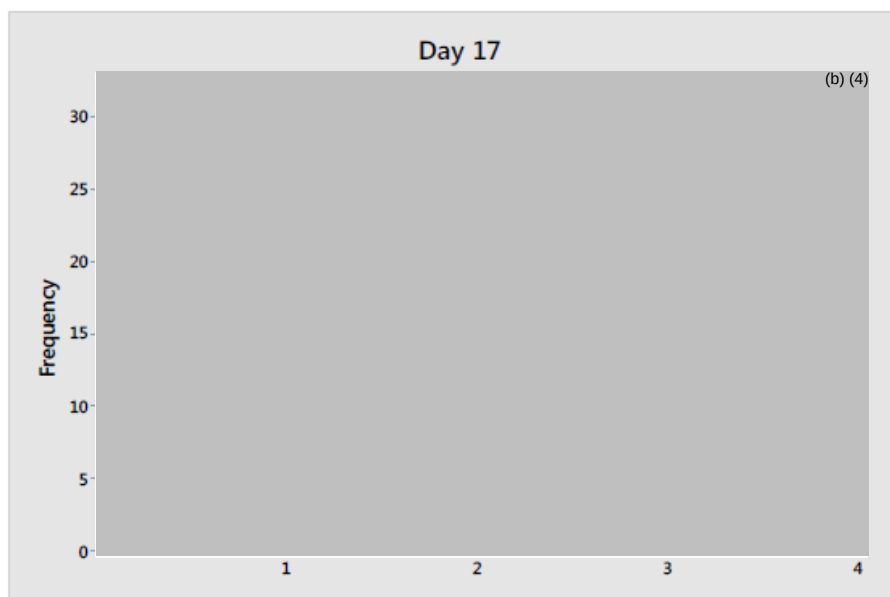


Figure 4. Histogram summary of *in vitro* complete release data for day 17 (n=100).

In relation to Day 41, the Applicant argues that an extra time point would not offer any additional control/assurance of quality. This claim is based on their manufacturing experience for BYDUREON microspheres between years 2015-2017 (Figure 5). The data show that the drug release rate is constant over this time-period at approximately (b) (4)% per day in such a way that if release at day 31 of between (b) (4)% and (b) (4)% is achieved, then it follows that the specification

of NLT ^{(b) (4)} % release at day 52 will also be met. Therefore, their proposal of not including an additional sampling time-point *is acceptable*.

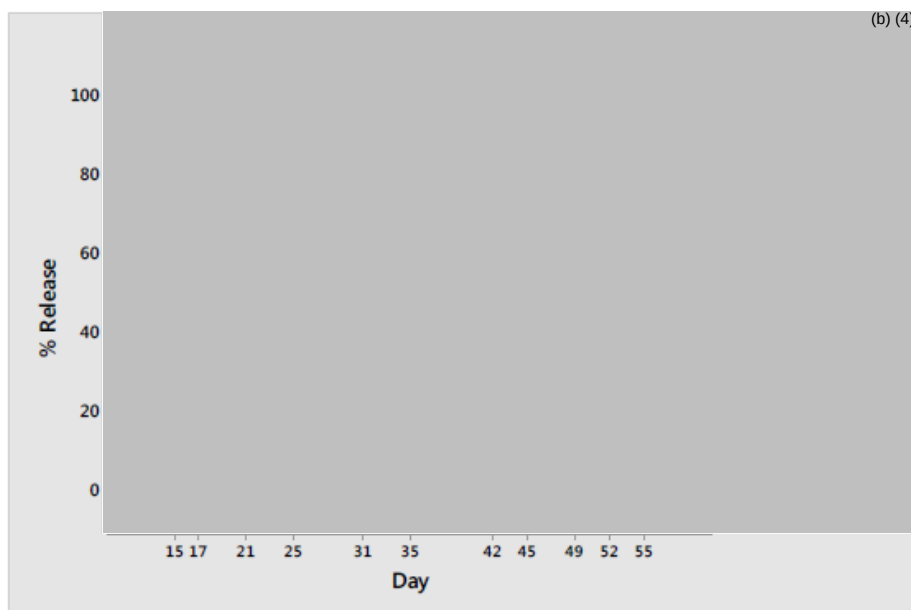


Figure 5. Demonstration of linear release between days 17 and 52 (n=100).

Reviewer's Assessment: ADEQUATE

The data provided demonstrated that the IVR method along with the recommended acceptance criteria are discriminating against critical quality attributes such as microsphere polymer weight and will ensure consistent in vitro and in vivo performance of the drug product throughout its life cycle.

Clinical relevance of dissolution method & acceptance criteria (e.g., IVIVR, IVIVC, In Silico Modeling, small scale in vivo)

Reviewer's Assessment: NA

There are no data relating variations on the critical quality attributes, in vitro release and in vivo performance (e.g. systemic exposure) to evaluate the clinically relevance of the in vitro release specifications (method and criteria). Based on the in vitro data provided, the method will be able to reject for batches with inadequate performance on key critical attributes. In addition, the CMC review team was recommended to tighten the ranges for the microspheres PSD (see section below) to comply with those observed for the clinical batches.

Application of Dissolution/IVIVC in QbD

The drug product quality target product profile, critical quality attributes and formulation development activities for exenatide suspension are summarized on the table below.

Product Profile	Requirements	Product critical quality attributes	Formulation development activities
Dosage form (type and administration related functionality)	Single use, fixed dose autoinjector device for subcutaneous injection, once weekly.	Description	Injection vehicle selection to produce a suspension, when mixed with BYDUREON® microspheres, to be filled in (b) (4) cartridge, enclosed within an autoinjector device.
Device	Should be suitable for use by the target user group, which includes the target patient group for self-administration.	Autoinjector device functionality	Evaluation performed during device development.
Injection volume	Less than or comparable to that of other products for similar indications. Must be clinically acceptable.	Delivered volume	Determination of injection volume, drug loading (core load) and suspension concentration. Injectability studies. Determination of target fill volume and overfill requirements to ensure drug product complies with specification for delivered volume.
Potency	2 mg exenatide per dose, assay within specification, complies with pharmacopoeial requirements for uniformity of dosage unit, USP<905>. Suspension can be injected readily through a 23 G needle.	Identity, assay, uniformity of content	Physicochemical characterisation of the formulation, including drug loading (core load), particle size distribution, suspension concentration, density, viscosity and settling rate to ensure drug product complies with specifications for assay and uniformity of content.

Product Profile	Requirements	Product critical quality attributes	Formulation development activities
Controlled release of exenatide	Delivers required plasma concentration-time profile for efficacy and tolerability.	Exenatide release	Physicochemical characterisation of the formulation, including drug loading (core load), polymer molecular weight, particle size distribution. Determination of initial and complete release profiles <i>in vitro</i> . Preclinical assessment of formulation performance.
Microbiological attributes	Complies with ICH and pharmacopoeial requirements for sterility, USP <71>, and endotoxins, USP <85>.	Sterility	Selection of formulation components and container closure. Sterilisation method selection and (b) (4) manufacturing process development. Demonstration of container closure integrity. Gamma irradiation of cannula; cycle development and validation performed as part of device development.
Product Profile	Requirements	Product critical quality attributes	Formulation development activities
Degradants and impurities	Complies with pharmacopoeial and ICH requirements.	Impurities	Determination of residual solvents to ensure drug product complies with ICH Q3C. Evaluation of excipient related impurities. Elemental impurities risk assessment to ensure drug product complies with ICH Q3D. Extractables and leachables evaluation to ensure any in the drug product are within permitted daily exposure limits. Stability evaluation to ensure impurities and degradation products meet ICH Q3B and Q6A requirements.
Pharmacopoeial compliance	Meets USP <788> requirements for subcutaneous injection dosage forms.	Particulate matter	Selection of appropriate raw materials, primary container and manufacturing processes to ensure USP requirements are met by the drug product.
Shelf life in relation to storage conditions, packaging configuration and handling.	Minimum shelf life of (b) (4) when stored at 2 to 8°C is required. In addition, the product must support an in-use period at room temperature (b) (4) for a period of (b) (4). (b) (4).	Stability	Selection of injection vehicle, based on development stability studies. Drug product stability evaluation in primary and in-use stability studies. Determination of drug product intermediates required to support the supply chain and associated stability.

The attributes affecting *in vitro* release and mitigation steps are as follows:

- 1) **Polymer molecular weight:** A specification clause is included on the raw material specification for PLG and on the (b) (4) microsphere (b) (4) specification. Appropriate limits have been set to control release rate of exenatide *in vivo*.

- 2) **Particle size distribution:** Given the importance of microsphere PSD and its potential impact on in vitro release, the Applicant was requested during the review cycle to provide the following data:

We acknowledge that particle size distribution (b) (4) is proposed (b) (4) for the (b) (4) (b) (4) exenatide microspheres with proposed limit of NMT (b) (4) μm . Note that the particle size distribution is a critical quality attribute (b) (4) (b) (4). Propose (b) (4) acceptance criteria for particle size distribution, (b) (4) (b) (4). In addition, provide supporting data to justify the proposed limits (e.g., data for all the pivotal phase 3 clinical batches).

On a submission dated 7/04/17, the Applicant stated that the addition of (b) (4) is of no additional benefit for the following reasons:

- It was acknowledged that although particle size distribution had been identified during development as a quality attribute with potential to impact the release of exenatide (b) (4), it has since been demonstrated experimentally that the particle size distribution of the microspheres has little or no impact on exenatide release (Figure 6).

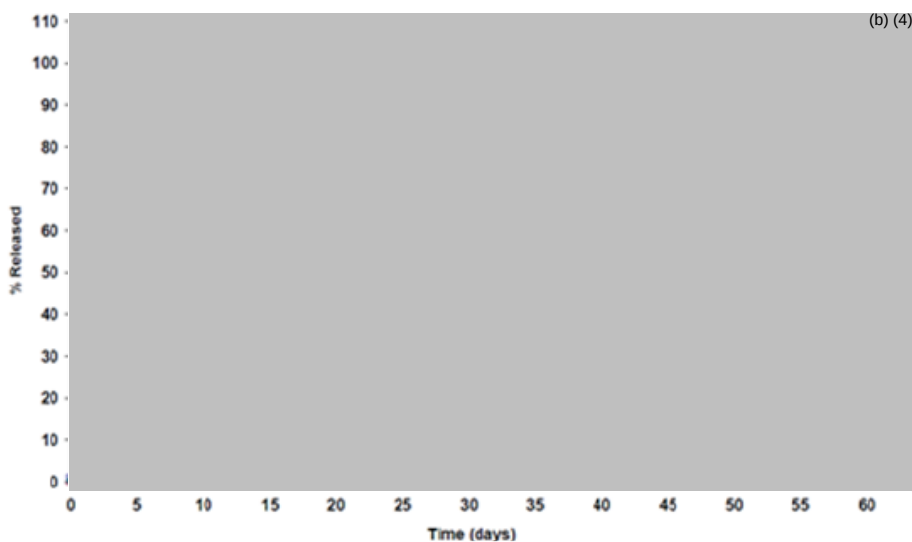


Figure 6. Effect of particle size on in vitro complete release of exenatide microspheres batch 07-017-112.

- It was stated that particle size is controlled by the (b) (4) (b) (4) manufacturing process and that the particle size range (b) (4), in the figure above showed a slight increase in initial release, is below the range of process experience and therefore can be considered worst case or of no practical significance.

The tabulated (b) (4) data for all pivotal phase 3 clinical and commercially representative batches of (b) (4) microspheres is shown in Table 5.

Table 5. Batch analyses for (b) (4) microspheres

Batch number	72815-02	72917-03	73079-02
Batch size	(b) (4)		
Batch number of drug substance	SF397-11AF1	P02948	P05318
Date of manufacture	Dec 2011	March 2012	June 2012
Site of manufacture	Amylin, Ohio, USA	Amylin, Ohio, USA	Amylin, Ohio, USA
Use of batch	Phase III studies Stability studies ^b	Phase III studies Stability studies ^b	Phase III studies Stability studies ^b
Immediate container closure	(b) (4)		
	microsphere container	microsphere container	microsphere container
Test	Acceptance criteria	Results	Results
Particle Size Distribution ^a			
(b) (4)			
a	Particle size distribution was previously tested at exenatide suspension.		
b	Exenatide suspension stability studies.		
NMT	Not More Than.		
NA	Not applicable		

3. **Drug Loading:** As stated previously, BYDUREON® microspheres are used in EQWS and core load (nominally (b) (4) % exenatide) and particle size are determined and controlled by the microsphere manufacturing process. According to the Applicant, (b) (4) (b) (4) microsphere concentration in each EQWS batch is adjusted to ensure 2 mg of exenatide can be delivered by the autoinjector device. Correct dose delivery is also dependent on particle size, including absence of agglomerates.

Reviewer's Assessment: ADEQUATE

From Biopharmaceutics perspective, the proposed control strategy (including the in process controls attributes/parameters) are acceptable to assure product quality and performance; however, the specification proposed for PSD (i.e. (b) (4) microns) may need to be revised (e.g., (b) (4) microns) for the following reasons:

- The studies relating PSD to in vitro release evaluated ranges of particle size up to (b) (4) microns. The proposed upper bound of (b) (4) microns was not evaluated;
- To ensure consistent performance as that seen for the pivotal phase 3 batches with a mean (b) (4) of about (b) (4) microns. This was communicated to the CMC reviewer via email on 09/06/17. Per the CMC reviewer, changing the PSD specification may not be warranted since the same microspheres are approved under NDA 22200.

MODIFIED RELEASE ORAL DRUG PRODUCTS –In-Vitro Alcohol Dose Dumping

Reviewer's Assessment: NA

This drug product is to be administered parenterally, and therefore, in vitro alcohol dose-dumping assessment is not applicable.

EXTENDED RELEASE DOSAGE FORMS –Extended Release Claim

During the review cycle, the Applicant was requested to provide evidence of extended release characteristics of their proposed product based on the following:

1. A bioavailability (BA) profile established for the drug product that rules out the occurrence of any dose dumping.
2. Data supporting that the drug product's steady-state performance is comparable (e.g., degree of fluctuation is similar or lower) to a currently marketed noncontrolled release or controlled-release drug product that contains the same active drug ingredient or therapeutic moiety and that was approved as an NDA.
3. Data supporting that the drug product's formulation provides consistent pharmacokinetic performance between individual dosage units.
4. Data supporting that the drug product has a less frequent dosing interval compared to a currently marketed non-controlled release drug product.

On a submission dated 04/05/17, the Applicant provided sufficient information (summarized) as follows to support that their proposed product has ER characteristics:

1. Lack of Dose-Dumping:

According to the Applicant, control of dose-dumping is ensured (b) (4) by adequate specifications of the *in vitro* complete release test.

(b) (4)

(b) (4)

(b) (4)

(b) (4). In addition, during the first 8 hours after injection of EQWS on Day 1, there was a minimal release of exenatide with a geometric mean

SE) maximum plasma concentration (C_{max}) of 34.9 (8.33) pg/mL, compared to a corresponding value of 124.4 (15.41) pg/mL on Day 1 for a 10 mg dose of the BYDUREON formulation, supporting no dose-dumping (Figure 6).

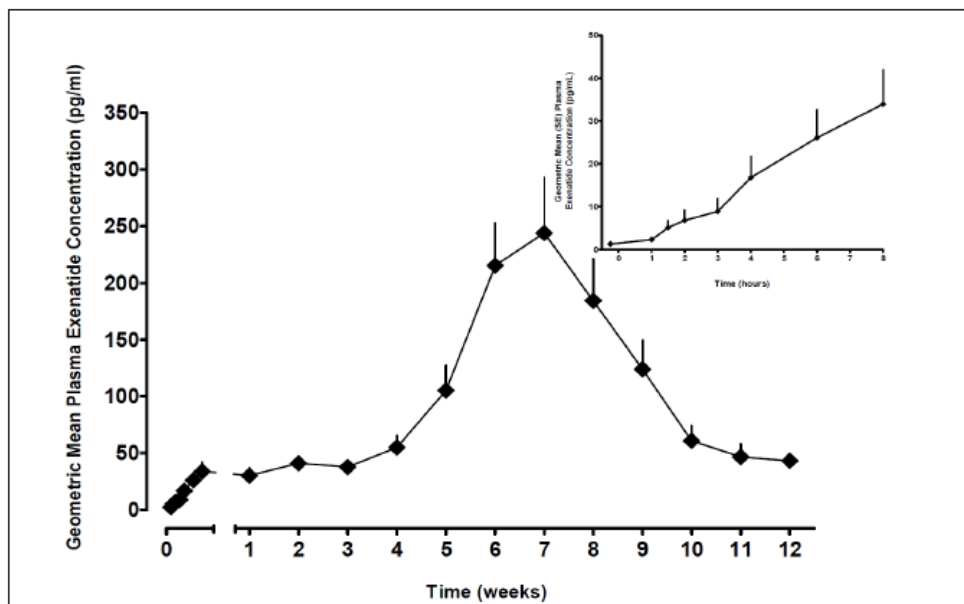


Figure 6. Geometric mean (+SE) exenatide plasma concentrations following a single 10 mg dose of EQWS from Day 1 to Week 12 (Study BCB110, Cohort 1 Pharmacokinetic-evaluable Population [N=30])

2. Drug Product's Steady-State Performance

Following once-weekly injection of 2 mg EQWS or BYDUREON, steady state concentrations ($C_{ss,ave}$) are comparable (Figure 7). In addition, during Week 15 to Week 16 of exenatide once-weekly dosing in study BCB118, the mean degree of fluctuation (calculated as $[C_{max} - \text{minimum plasma concentration}] / \text{average plasma concentration}$) was 41% compared to 230% and 78%, for BYETTA and BYDUREON, respectively supporting the ER claim.

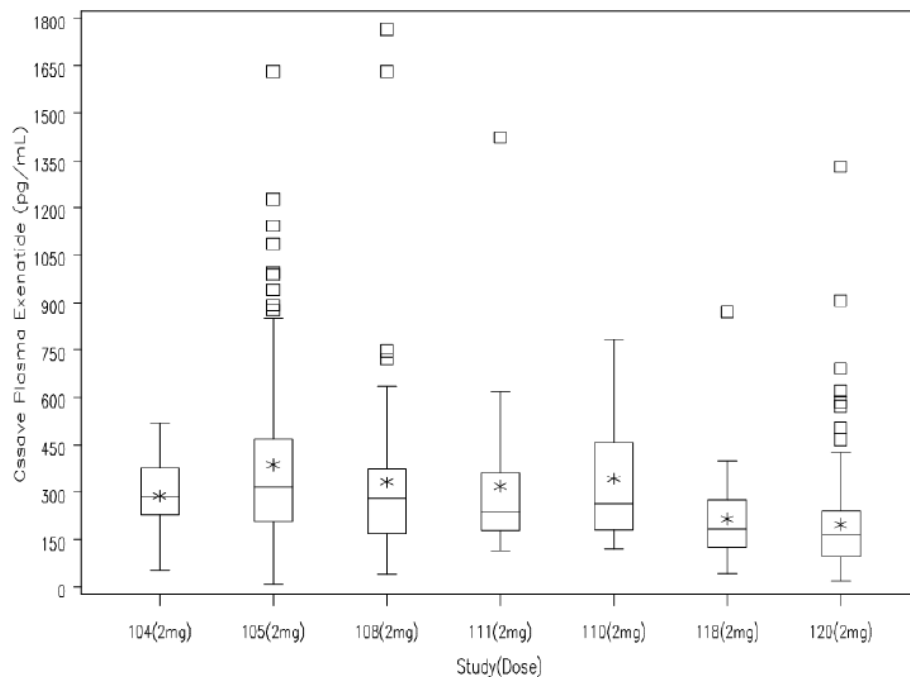


Figure 7. Boxplots of steady state exenatide plasma concentration for BYDUREON studies (2993LAR-104, 2993LAR-105, BCB108 and BCB111) versus EQWS studies (BCB110, BCB118 and BCB120).

3. Consistent PK Performance

The Applicant states that consistent extended release profile for EQWS and the marketed BYDUREON products is ensured through the following:

- Application of the control strategy for manufacture of exenatide extended release microspheres. Specifically, critical process parameters (b) (4) provide control (b) (4) and polymer molecular weight controls the complete release profile.
- The *in vitro* complete release test confirms that the expected release profile is consistently achieved.
- Application of two tests as part of the control strategy to ensure that a consistent dose is delivered to patients from individual autoinjector devices, specifically the Uniformity of Content test and the Delivered Volume test.
- EQWS exhibits comparable steady state plasma exenatide concentrations and a lower fluctuation ratio than the marketed ER product BYDUREON.

And lastly, EQWS has a less frequent dosing interval than the marketed immediate release exenatide product BYETTA, which is administered twice daily at doses of 5 µg or 10 µg.

Reviewer's Assessment: ADEQUATE

The in vitro release and in vivo PK data provided along with the characteristics of the dosing regimen, support the extended release designation claim for EQWS.

Bridging of Formulations Across Phases of Drug Product Development

According to the Applicant all batches of EQWS used in the clinical development program used commercial quality BYDUREON microspheres. Batch 0081 was dosed via a vial and syringe using a vial of exenatide microspheres with the MCT added extemporaneously. Batches AVLCO2, AVLI06 and AVMB02 were dosed via the exenatide suspension autoinjector. The Applicant claims that no change to the microsphere composition or manufacturing process has been made and therefore fundamental chemical mechanisms governing extended release from the exenatide microspheres remain the same throughout the EQWS clinical program. The changes during the clinical program reflect the mode of administration over the course of the EQWS development as follows:

1. During Phase II, study BCB110 batch 0081 was dosed through vial and syringe.
2. During Phase III, studies BCB118 and BCB120, batches AVLCO2, AVLI06 and AVMB02 were dosed via the autoinjector.

During the review cycle, the Applicant was requested to provide in vitro release profiles comparing all the batches used in pivotal clinical trials. On a submission dated 03/21/17, the Applicant submitted data demonstrating that the release across all batches is very similar (Figure 8).

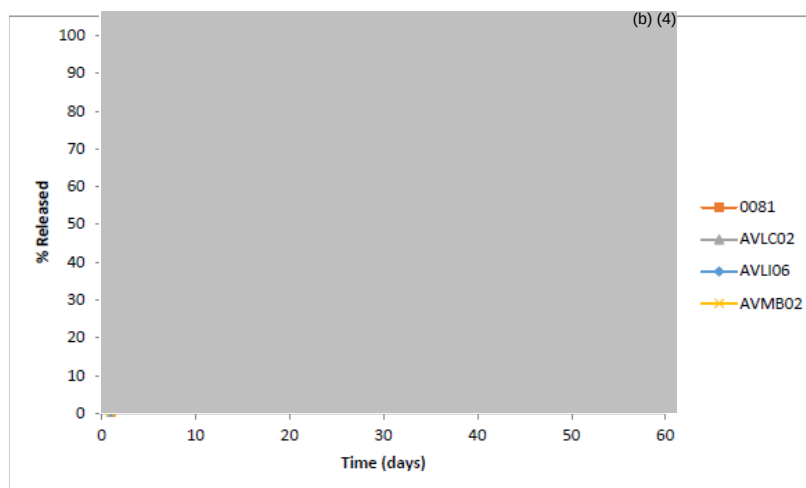


Figure 8. *In vitro* complete release profiles for all clinical batches used in BCB110, BCB118 and BCB120.

Reviewer's Assessment: ADEQUATE

The data demonstrated that there is no change in in vitro release among all batches tested in pivotal clinical trials. However, changing from syringe administration to autoinjector may have an impact on the in vivo performance of the drug product. This potential impact is being reviewed under the purview of CDRH.

Biowaiver Request**Reviewer's Assessment:**

No biowaiver requests were included as there were no major changes in formulation across phases of development.

Regional Information***Comparability Protocols*****Reviewer's Assessment: NA*****Post-Approval Commitments*****Reviewer's Assessment: NA*****Lifecycle Management Considerations***

The proposed control strategy is acceptable from biopharmaceutics perspective to assure product quality and performance and hence is adequate for lifecycle management of the product for changes within process/formulation ranges tested (see above comments on PSD for microspheres). However, during the lifecycle, if the changes are proposed beyond the ranges tested, depending on the criticality of the changes and its effect on drug product CQA and hence on product quality and performance, it would indicate a need of in vitro BE testing (e.g., SUPAC Level 3 process change).

List of Deficiencies:

NONE pending.

Primary Biopharmaceutics Reviewer Name and Date:

Sandra Suarez Sharp, Ph.D. (Branch 2DB\ONDP\OPQ), August 18, 2017

Secondary Reviewer Name and Date (and Secondary Summary, as needed):

Mandula Haritha, Ph.D., Acting Team Lead (Branch 2\DB\ONDP\OPQ), 9/11/2017

APPENDIX**Comments conveyed to the Applicant as part of the 74-day letter:**

1. *Your assertion that inclusion of MCT as a vehicle does not impact overall exenatide release is not supported. In addition, the in vitro release does not reflect the release of drug in vivo as plasma concentrations are measurable on the first hours after administration. On the contrary, no drug release is observed the first 2 weeks in vitro conditions. This indicates that your proposed method is not adequate since it is not sensitive to drug release (b) (4). Therefore, submit the dissolution method development report with data supporting the following:*
 - i) *Data demonstrating that the presence of MCT does not alter the release of the drug using a method that is discriminating (b) (4)*
 - ii) *Discriminating ability of the method for changes in Microsphere core load, polymer molecular weight and particle size distribution for your drug product suspended in MCT.*
2. *Submit In vitro release profile comparisons supporting the changes implemented to the clinical batch formulation.*
3. *Consider the development of an accelerated in vitro release method that is sensitive to both the initial and extended phases of the drug release for your microsphere formulation suspended in MCT.*
4. *Data supporting the ER designation claim. The following information should be submitted to support the extended release designation claim (refer also to CFR 320.25f):*
 - i) *The BA profile established for the drug product rules out the occurrence of any dose dumping;*
 - ii) *The drug product's steady-state performance is comparable (e.g., degree of fluctuation is similar or lower) to a currently marketed non-controlled release or controlled-release drug product that contains the same active drug ingredient or therapeutic moiety and that is subject to an approved full NDA;*
 - iii) *The drug product's formulation provides consistent pharmacokinetic performance between individual dosage units;*
 - iv) *The drug product has a less frequent dosing interval compared to a currently marketed non-controlled release drug product.*

Comments conveyed to the Applicant as part of the mid-cycle:

- 1) *It is not clear as to whether the exact in vitro release method (IVR) approved for BYDUREON is being implemented for your proposed drug product under NDA 209210. Submit detailed information on the apparatus, medium, pH, agitation speed, temperature, volume, osmotic strength, sampling times, sample handling procedure, sample filtration, etc., as well as in vitro release method validation report.*
- 2) *We acknowledge that particle size distribution (b) (4) is proposed (b) (4) for the (b) (4) (b) (4) exenatide microspheres with proposed limit of NMT (b) (4) μm. Note that the particle*

size distribution is a critical quality attribute as it impacts the release of exenatide (b) (4). Propose a (b) (4) acceptance criteria for particle size distribution, (b) (4). In addition, provide supporting data to justify the proposed limits (e.g., data for all the pivotal phase 3 clinical batches).

- 3) Provided we found the proposed IVR method acceptable, your proposed in vitro release acceptance criteria are insufficient to ensure accurate control of the in vitro release characteristics of your proposed drug product. We recommend the implementation of the following criteria and submission of the revised specifications table with the updated acceptance criteria:

Day 17: NMT (b) (4) %

Day 31: (b) (4) %

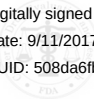
Day 41: (b) (4) %

Day 52: NLT (b) (4) %



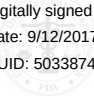
Haritha
Mandula

Digitally signed by Haritha Mandula
Date: 9/11/2017 05:29:56PM
GUID: 508da6fb000282df41459408f32a1ce0



Sandra
Suarez

Digitally signed by Sandra Suarez
Date: 9/12/2017 02:24:01PM
GUID: 5033874b000046a4a8b9c51d6f5bd8ba



MICROBIOLOGY**Product Background:**

NDA: 209210

Drug Product Name / Strength: Exenatide, 2 mg

Route of Administration: Subcutaneous injection

Applicant Name: AstraZeneca AB

Manufacturing Site:

Drug product: Amylin Ohio, LLC, 8814 Trade Port Drive, West Chester, OH 45071

Drug diluent:

(b) (4)

(b) (4)

Method of Sterilization:

(b) (4)

Review Recommendation: Recommended for Approval.

Review Summary: The single use autoinjector is comprised of an (b) (4) 2 mL cartridge containing the drug product and a (b) (4) needle assembly.

List Submissions being reviewed: December 21, 2016, July 7, 2017, July 26, 2017

Highlight Key Outstanding Issues from Last Cycle: N/A

Concise Description Outstanding Issues Remaining: None

Supporting/Related Documents:

DMF 10953 (type III)-10953mic34.doc, dated May 18, 2016 by M. Cruz-Fisher. Datwyler ISAFx washing process for rubber stoppers. (adequate)

NDA 22200 – N022200N000R1.doc, dated February 26, 2010 by R. Mello and N022200N000R2.doc, dated July 20, 2010 by R. Mello. Applicant cross references approved NDA for description of microsphere manufacturing and controls. (acceptable)

S Drug Substance

(1.4.4 Cross Reference)

The drug product is

(b) (4)

(b) (4). The applicant references the previously approved NDA 022-200 for all manufacturing, packaging, specifications and stability related to the (b) (4) microspheres. A cross reference letter dated November 23, 2016 by AstraZeneca is provided to NDA 022-200 for this information as allowed by 21 CFR 314.50(g)(1).

Reviewer's Assessment: Acceptable

The manufacturing at the specified location was reviewed and accepted in NDA022-200. A review of the container closure system, the manufacturing, sterility validation, specifications and stability program was conducted in microbiology reviews N022200N000R1.doc, dated February 26, 2010 by R. Mello and N022200N000R2.doc, dated July 20, 2010 by R. Mello. See these reviews for further information.

P.1 Description of the Composition of the Drug Product

- Description of drug product: A single use, fixed dose auto injector device with (b) (4) suspension of exenatide microspheres in medium chain triglycerides.
- Drug product composition:

Ingredient	Content per cartridge	Content per 0.85 mL dose
Exenatide	(b) (4)	2.0 mg
5050 DL (b) (4) Polymer		37.2 mg
Sucrose		0.8 mg
Medium chain triglycerides		774.4 mg

- Description of container closure system:

The applicant provided the following image of the autoinjector subassembly (pharmaceutical-development-process.pdf, page 10):

Figure 4 Exenatide suspension autoinjector subassemblies

(b) (4)

The applicant provided the following image of the needle assembly (pharmaceutical-development-microbiological.pdf, page 10):

Component	Description	Manufacturer
Cartridge	2 mL (b) (4) glass cartridge	(b) (4)
Plunger	10 mm (b) (4) plunger	
Seal	(b) (4)	
Autoinjector	(b) (4) need assembly	

Reviewer's Assessment: Acceptable

The applicant provided an adequate description of the drug product composition and the container closure system designed to maintain product sterility.

P.2.5 Microbiological Attributes

Container/Closure and Package Integrity

(3.2.P.8.3 Analytical procedure – container closure integrity by dye ingress)

Reviewer's Assessment: N/A

P.3 Manufacture

P.3.1 Manufacturers

Bulk Microspheres:

Amylin Ohio LLC

8814 Trade Port Drive, West Chester, Ohio, 45071

Drug product cartridges:

(b) (4)

Assembly of cartridges into autoinjectors and Release testing:

Amylin Ohio LLC

8814 Trade Port Drive, West Chester, Ohio, 45071

P. 3.3 Description of the Manufacturing Process and Process Controls

Overall Manufacturing Operation

17 Pages have been Withheld in Full as B4(CCI/TS)
Immediately Following this Page

P.5 Control of Drug Product

P. 5.1 Specification

(3.2.P.5.1 Specifications)

The product release specification includes the following microbiological tests:

Test	Test Method	Acceptance Criteria
Bacterial Endotoxins cartridge	USP<85>	NMT (b) (4) EU/mL
Bacterial Endotoxins device		NMT (b) (4) EU/device
Sterility	USP<71>	Must be sterile

Reviewer's Assessment: The applicant provided equivalent sterility and endotoxin test methods to USP methods.

P.5.2 Analytical Procedures

Reviewer's Assessment: See section P.5.1 and P.5.3

P.5.3 Validation of Analytical Procedures

Endotoxins

(3.2.P.5.3 Validation - Endotoxins)

Test Method: kinetic chromogenic method

Endotoxins Specification: (b) (4) EU/mL

Lysate sensitivity: (b) (4) EU/mL

Drug potency: 2 mg/0.85 mL

Calculated MVD: (b) (4)

Reviewer calculated MVD:

(b) (4)

Validation of the test shows no inhibition or enhancement using a dilution of 1:20000. However the applicant did not indicate the dilution for routine testing.

Maximum dose according to the package insert (nonannotated-draft-label.pdf, page 4): 2 mg per 0.85 mL once every 7 days.

Calculated endotoxin dose at the proposed endotoxins specification and maximum dose:

(b) (4)

On June 7, 2017 the following Microbiology Information Request was forwarded to the applicant by the CDER Project Manager. We acknowledge the endotoxins validation results provided for the cartridge. However the dilution which will be used for routine testing was not indicated

within the document. Provide the drug product dilution that will be used for routine endotoxins testing.

Applicant's Response (July 7, 2017): The applicant stated that the dilution of 1:20000 will be used for routine testing of the drug product.

Reviewer's Assessment: Acceptable

The applicant provided an acceptable routine testing dilution, which was validated and the endotoxin dose at the proposed endotoxins specification and maximum dose as calculated by this reviewer is within the USP <85> recommendation of 5 EU/kg/hr.

Sterility

(3.2.P.5.3 Validation - Sterility)

Test Method: Equivalent to USP<71>

Bacteriostasis/fungistasis testing was performed. The subject drug product was tested using *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Clostridium sporogenes* ATCC 11437, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis*. Forty samples were used with one rinse of 500 mL with 1% polysorbate.

The subject drug product did not inhibit recovery of the test organisms and the applicant indicates that growth was comparable to positive controls.

Reviewer's Assessment: Acceptable

The applicant provided an acceptable summary of the sterility test validation.

P.7 Container Closure

Summary table of the container closure system proposed

Reviewer's Assessment: See section P.1

P.8 Stability

P. 8.1 Stability Summary and Conclusion

(3.2.P.8.1 Stability Conclusion)

The Proposed expiry is 36 months when stored at 2-8 °C and protected from light.

Reviewer's Assessment: Acceptable

The applicant's proposed 36 month expiry is acceptable based on provided microbial data.

P. 8.2 Post-Approval Stability Protocol and Stability Commitment

(3.2.P.8.2 Post approval stability protocol and stability commitment)

The product stability specification includes the following microbiological tests:

Test	Test Method	Acceptance Criteria
Bacterial Endotoxins	USP<85>	(b) (4) EU/mL
Sterility	USP<71>	Sterile

The testing schedule in the post-approval protocol is as follows:

Stability storage conditions: 5 °C

Test	Time (Months)							
	0	3	6	9	12	24	36	48
Bacterial Endotoxins	X				X	X	X	X
Sterility	X				X	X	X	X

Post Approval Stability Commitment

The applicant commits to placing the first three commercial lots of the subject drug product into their stability program. Thereafter, (b) (4) one production lot will be added to the stability program.

Reviewer's Assessment: Acceptable

The applicant provided an acceptable stability program for microbial testing.

P.8.3 Stability Data

(3.2.P.8.3 Stability data)

The applicant provided bacterial endotoxin and sterility data up to 36 months at 5 °C and up to 6 months at 25 °C with 60% RH.

Reviewer's Assessment: Acceptable

The applicant provided acceptable microbiology stability data.

A Appendices

A.2 Adventitious Agents Safety Evaluation

Reviewer's Assessment: Not applicable.

A.2.1 Materials of Biological Origin

Reviewer's Assessment: Not applicable.

A.2.2 Testing at Appropriate Stages of Production

Reviewer's Assessment: Not applicable.

A.2.3. Viral Testing of Unprocessed Bulk

Reviewer's Assessment: Not applicable.

A. 2.4 Viral Clearance Studies

Reviewer's Assessment: Not applicable.

R Regional Information

Executed Batch Records

The batch records confirm that validated (b) (4) manufacturing processes were used for the manufacture of the exhibit batches.

Reviewer's Assessment: Acceptable

The applicant provided detailed batch records for batches AVLC02, AVLI06 and AVMB02, which used acceptable (b) (4) manufacturing processes.

Comparability Protocols

(3.2.P Comparability Protocol)

The applicant proposes to add a new manufacturing line (b) (4) (b) (4). The applicant also requests an increase in the maximum scale from (b) (4) units to approximately (b) (4) units. The applicant indicates (b) (4) will be of the same grade as current manufacturing line. (b) (4) (b) (4)

On June 7, 2017 the following Microbiology Information Request was forwarded to the applicant by the CDER Project Manager. We acknowledge the comparability protocol for the addition of a new manufacturing line along with an increase in batch size that is submitted within the application. However, the specific tests or studies and associated acceptance criteria that will be

performed to assess the proposed manufacturing changes were not described. Provide a list of the expected equipment that will be implemented for the new line, the specific studies that will be used to validate the equipment and the acceptance criteria that will be used. For additional information on comparability protocols please refer to:

<https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM496611.pdf>

Applicant's Response (July 7, 2017): The applicant indicated that the new line should be established and validated by middle of 2019. The applicant stated that there are no proposed changes to equipment type and the manufacturing equipment for the new line is identical in terms of materials, geometry and class currently validated

(b) (4)

(b) (4)

The applicant provided the following summary table for the planned (b) (4) work to be performed with the acceptance criteria (responses-quality-round4.pdf, page 41):

The applicant indicates that after successful technical batches and media fill simulation studies, the process validation will be performed with a minimum of 3 consecutive batches at the increased scale of manufacture. The applicant also indicates that after agreement with these details that they will be incorporated into the registered comparability protocol contained in Module 3.2.R.

On July 17, 2017 the following Microbiology Information Request was forwarded to the applicant by the CDER Project Manager. (b) (4)

(b) (4)

(b) (4)

Reviewer's Assessment: Acceptable

The applicant provided an adequate description

(b) (4)

(b) (4)

2. REVIEW OF COMMON TECHNICAL DOCUMENT – QUALITY (CTD-Q) MODULE 1

2.A. Package Insert

(1.14.1.3 Draft labeling text - nonannotated)

(b) (4)

Reviewer's Assessment: Acceptable

The applicant provided acceptable instructions which do not require additional microbiological studies.

Post-Approval Commitments: N/A

Lifecycle Management Considerations



QUALITY ASSESSMENT



N/A

List of Deficiencies: None.

Primary Microbiology Reviewer Name and Date:

David Bateman, Ph.D.

July 26, 2017

Secondary Reviewer Name and Date:

John W. Metcalfe, Ph.D.

I concur. July 27, 2017



Su (Suong)
Tran

Digitally signed by Su (Suong) Tran
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