



Food and Drug Administration  
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**MEMORANDUM**

Date: August 4, 2000

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DTP, OTRR

To: File

Through: John C. Hill, Ph.D., DTP, OTRR  
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Subject: STN 103979 (BLA 99-2865)  
Fabrazyme (recombinant human I-Galactosidase, CHO cells)  
for Treatment of Fabry's Disease, Genzyme,  
CMC-Product Review

**I. INTRODUCTION**

Fabrazyme contains a new active substance, agalsidase beta (recombinant human alpha galactosidase), used for the treatment of Fabry disease. Recombinant human alpha galactosidase (r-h alphaGAL), a purified recombinant form of the naturally occurring human glycoprotein, is isolated from cell culture supernatant following growth of a Chinese Hamster Ovary (CHO) cell line transfected with a recombinant expression vector containing the cDNA coding region for human alpha-galactosidase (hIGAL) and thereafter is purified using a series of chromatography steps.

alpha-Galactosidase is a lysosomal enzyme which catalyses the hydrolysis of the glycolipid, globotriaosylceramide (GL-3), to galactose and ceramide dihexoside. Further metabolism results in the formation of ceramide, an indispensable precursor for glycosphingolipids. Glycosphingolipids are present in all cell membranes where they act as structural components of cell membranes, and participate in a variety of immune recognition processes and in signaling mechanisms.

Fabry disease is a rare, X-linked, genetically inheritable disease. It is a progressive (lysosomal storage) disorder that is seriously debilitating and ultimately life-threatening. It is characterized by subnormal or absent enzymatic activity of alpha-Galactosidase and resultant accumulation of globotriaosylceramide (GL-3) and related glycolipids in the lysosomes of affected cells throughout the body. Progressive accumulation of GL-3 and related lipids leads to impaired tissue and organ function. The ultimate consequence of glycosphingolipid deposition in the vasculature and other tissues is end-organ failure, particularly in the kidney, but also heart and cerebrovascular system. In addition, involvement of the central, peripheral and autonomic nervous systems result in episodes of pain and impaired peripheral sensation. Vascular changes in the skin also result in angiokeratomas.



-----were also determined for the Process Qualification lots.

As detailed in the technical report (Appendix IIC-37), additional characterization of r-h alphaGAL Drug Substance PQ Lots: ----- revealed no significant differences among the three r-h alphaGAL Drug Substance PQ lots and the -----eference standard. The results obtained from this detailed biochemical analysis of the three PQ lots are summarized in Table 1 and the certificates of analysis are included in Table 3.



-beta-ceramide), thus the designation I-galactosidase. The activity assay measures the rate of alpha - galactosidase-catalyzed hydrolysis of a synthetic substrate, p-nitrophenyl-alpha-D-galactopyranoside, at

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of substrate per minute at -----under the assay conditions

B. Manufacturer(s)

1. Identification

Inoculum preparation, cell culture steps, and purification of r-hGAL is performed at Genzyme Corporation,-----

2. Floor Diagram

Appendix IIC-26 contains floorplans for manufacturing areas.

3. Other Products

4. Contamination Precautions

C. Method(s) of Manufacture

1. Raw Materials And Reagents

The raw materials qualification program is described. Compendial and non-compendial raw materials are identified through a combination of identity tests and review of certificates of analysis. Raw materials of animal origin, -----, are obtained from BSE-free countries and from suppliers that use controlled /monitored herds. Each lot of -----s qualified for use in manufacturing

2. Flow Charts

**Table 2: Manufacturing Process Flow Chart: Cell Culture to Drug Substance**

Production Step	Equipment and Materials Used
<p>-----</p>	<p>-----</p> <p>-----</p> <p>-----</p> <p>-----</p>
<p>-----</p>	<p>-----</p> <p>-----</p>
<p>-----</p> <p>-----</p> <p>-----</p> <p>-----</p>	<p>-----</p> <p>-----</p> <p>-----</p> <p>-----</p>
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<p>-----</p> <p>-----</p> <p>-----</p>	<p>-----</p> <p>-----</p> <p>-----</p> <p>-----</p> <p>-----</p>

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<p>-----</p>	<p>-----</p> <p>-----</p> <p>-----</p> <p>-----</p>

3. Detailed Description

a. Cell Substrate/Host Cell / Expression Vector System

(i). Recombinant DNA Products

(a). Host Cells

The host cell line, CHO-----  
 ----- was obtained originally from -----  
 -----This cell line has been maintained -----  
 at Genzyme. Briefly, CHO ----cells -----  
 -----  
 -----This cell line  
 was named-----  
 the isolation of -----

(b). Gene Construct

The gene encoding human alpha-galactosidase (halphaGAL) was isolated at Genzyme.  
 -----  
 -----  
 -----  
 -----  
 clone (----- contained a ----kb insert that represented the full-length h IGAL coding  
 region.

(c). Vector

The parent vector for -----is -----This vector is derived from constructs created at -----nd contains an ----- transcriptional unit. The vector containing full-length h IGAL coding region, ----- was used to create the h GAL expression vector. Briefly, -----

(d). Final Gene Construct

i. Transfection

-----growing -----cells were maintained in growth medium (-----  
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-----  
-----cells were plated onto -----mm dishes. T-----these cells were transfected with vector -----using minor modifications of the -----

ii. Drug Selection, Amplification.

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ii. Copy Number Analysis

Genomic DNA was isolated and treated with restriction enzymes. Samples of --ug of genomic DNA were digested ----- Copy number standards were plasmid -----DNA digested with restriction enzymes and subjected to electrophoresis in the presence of----- . All digests were subjected to electrophoresis on ----- gels in -----buffer, transferred to -----membrane, and hybridized to a -----probe, complementary to the h GAL coding region. The blot was -----and the expected size bands for the test samples ----- and digested-----were quantitated . The values obtained for -----were manually entered into a simple graphics program (-----and linear regression done to generate a standard curve representing DNA copy number .-----py number of each test sample was then calculated from the quantitated value of ----- relative to the standard curve.

Copy number values for the predicted -----basepair band were calculated to be:

- copies for the MCB cells;
- copies for the WCB cells;
- copies for the Bioreactor EOP cells.

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iii. Verification of the r-h alphaGAL coding sequence and regulatory sequences  
Sequence analysis of the alphaGAL expression cassette isolated from genomic DNA from the MCB cells, the WCB cells, and Bioreactor Day ---EOP cells was performed  
-----ndependent contractor ----- Briefly, total genomic DNA was isolated.  
-----overlapping PCR fragments that contained the -----romoter, the IGAL coding region, and the-----region were generated with standard techniques using total genomic DNA as the template and primers. Template and primers were added to the supplied -----  
----- The total reaction mix was placed in a ----- Thermocycler and run for ---cycles. The denaturing, annealing, and extensio-temperatures were -----C, respectively. The final reaction mix was separated on a ----- gel and the band of interest excised. The product was purified and then sequenced on a-----using the -----  
-----sequencing-----

The DNA sequences generated using DNA templates from the three cell sources are identical. There have been no detectable mutational events. These results confirm that the r-h IGAL coding and regulatory regions remained unchanged from the MCB through to the end of production.

(e). Cloning And Establishment Of The Recombinant Cell Lines.

The productivity was determined to be highest at cells amplified to -----  
These cells were cloned by -----Based on activity data,---clonal lines were chosen for further analysis. -----clones were analyzed for stability of r-h IGAL productivity in the absence of ----- These studies were performed for greater than---- generations to ensure suitable stability for production scale-up. Stability at production scale is assessed by continued monitoring of the production process. Based on the criterion of stable protein production in the absence of -----clone ----- was chosen as the candidate cell line for scale up and production optimization.

b. Cell Seed Lot System

The cell culture cell bank system for the production of r-h IGAL consists of the industry standard Master Cell Bank (MCB) and Manufacturers Working Cell Bank (MWCB or WCB) system. End of Production (EOP) Cells consisted of cells collected immediately following the end of the production phase of the cell culture process. Such EOP cells were collected and characterized from the 30L process (Harvest ---- corresponding to run day ---- and the 340L process (Harvest Day---- corresponding to run day ----- The latter represents the maximum number of days supported by these cell line characterization studies. The terms EOP and Post Production Cell Bank (PPCB) refer to cells collected immediately following the end of the cell culture production process.

(i). Master Cell Bank

The MCB was prepared from -----of the -----lonal cell line derived from a

development cell bank. A total of ----ampoules of the -----Master Seed, -----  
MCB (synonymous with -----MCB) were -----on -----at passage -----  
generation ----- Of these, a number have been used for initial release testing, cell line  
characterization, initial process development, and performance verification. As of February  
17, 2000, there were----ampoules remaining. The current use rate is l-----  
-----for the generation of new MWCB. At this rate, the current -----MCB should  
last for approximately -----. Ampoules of the -----  
----are maintained in dedicated storage in -----ocations, -----  
-----  
-----Access to these storage sites is restricted to authorized  
manufacturing personnel. The freezer banks are kept locked and the units are monitored  
regularly to ensure storage temperatures remain below -----C.

(ii). Working Cell Bank

The MWCB was prepared from -----of the -----Master Seed  
(Lot----- to support on-going production of r-h IGAL. The -----MWCB,  
Batch -----was-----on -----at passage ----generation -----and  
contained ----ampoules. Of these, a number have been used for initial release testing,  
cell line characterization, process development, production, and performance verification.  
As of February 17, 2000, there were ----mpoules remaining. The current use rate is  
approximately ----ampoules per yea---- manufacturing and on-going testing. At this rate,  
the -----MWCB (Batch-----should last for -----  
-----  
-----All ampoules of the -----MWCB (Batch ----- are  
maintained in dedicated storage in ----ocations: -----  
-----  
----- Access to these storage sites is restricted to authorized  
manufacturing personnel. The freezer banks are kept locked and the units are monitored  
regularly to ensure storage temperatures remain below -----.

(iii). End of Production Cells (EPC)

EOP cells were collected and characterized from the initial 30L process (designated Batch  
-----harvest day ---- corresponding to run day ----- and the 340L process (designated  
r-h Alpha-Gal-----2 Freeze, ----- harvest day ---- corresponding to run  
day ----- Currently, the latter represents the maximum number of days from which harvest  
is co-----ed in the r-h...GAL cell culture production process.

c. Cell Growth and Harvesting

The r-h alphaGAL Drug Substance is manufactured using a Chinese Hamster Ovary (CHO) cell  
culture process. The cell culture process takes place in a ----- bioreactor that is run at a 340L  
working volume in a-----mode. -----  
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C. Purification and Downstream Processing

1. Bioreactor Harvest Clarification

The purification process is initiated by transferring the bioreactor harvest from ----days of -----collection from one bioreactor into a -----processing tank. The harvest is conditioned by adding ----- of -----buffer/L of harvest. The harvest is then -----  
-----The----- fluid is pumped through a -----um filter onto the -----  
-----column as the load.

2.-----Column.

The-----column is a-----chromatography column that is operated in a -----mode. The r-h alphaGAL-----  
-----The -----fluid is loaded onto the -----  
column at a loading-----. The column is washed with a -----  
-----buffer and then with a -----  
-----

The r-h alphaGAL is eluted from the column with a -----  
buffer. The resulting eluate is collected into a steril-----  
appropriately. Samples are removed and tested for -----  
-----

3.-----Column

The-----column is an -----chromatography step in which r-h alphaGAL  
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After a rinse step of -----the column is pre-equilibrated with -----  
-----The column is equilibrated with a -----  
----- eluates are pooled to meet a loading capacity of -----g of r-halphaGAL/L of  
resin. This----- load is adjusted with -----to a pH of -----  
Samples of the-----load are taken fo-----  
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After applying the -----load, the column is washed with a -----  
buffer. The colum-----en washed with a -----  
buffer, which has been demonstrated to i-----  
-----buffer is performed to r-----

The r-halphaGAL is eluted from the column with a 1-----  
-----buffer. The eluate is filtered through a -----um filter into a sterile p-----

-----The eluate is tested for-----

4.-----Column

The-----column is effective in -----

After a rinse step of-----  
-----over the column. The column is then  
-----

-----umn equilibration is achieved with a -----  
-----buffer until the -----  
-----eluates are ----- loaded onto the column to achieve a loading level of  
-----g r-halpaGAL/L of resin. The column is washed with a -----  
-----buffer. The r-halpaGAL is eluted from the column with a -----  
-----buffer. The resulting eluate is filtered through a  
----- um filter into a sterile -----The eluate pool is tested for -----

5.-----Column

The -----column is a -----chromatography column purification step, which  
is effective in f-----

-----the column is equilibrated with a -----  
----- until the -----  
-----

The----- eluate is pH adjusted using -----  
pH of----- and loaded onto the column at a loading level of -----g r-h ----- GAL/L of resin.  
The column is washed with a-----buffer and  
then with a -----  
buffer.

The r-halpaGAL is eluted with a -----  
-----buffer. In order to minimize exposure of r-h IGAL to -----  
-----The pH of the elution is checked, and  
adjusted, if necessary, to pH -----using -----he  
eluate is filtered through a -----m filter into a sterile -----Samples of the  
pH-adjusted eluate are taken and tested for -----  
----- The eluate is stored at -----C for up to --months until further processing

6a. Concentration

The pH adjusted ---eluate is concentrated using a-----  
----- The concentration factor, and hence, the desired final volume is determined -----  
-----This

-----  
 -----

6b. Diafiltration and Dilution

The concentrated material is diafiltered into a -----uffer. -----  
 diafiltration volumes of buffer are passed through the system and the pH and conductivity of the  
 permeate are confirmed to be within ----pHunits an-----mS, respectively of the -----  
 The diafiltered pool is then further concentrated to --g/L to allow for the addition of two system  
 ----- The diafiltered pool is then diluted to a targeted final concentration of -----g/L  
 by adding the-----buffer, as  
 needed. This material is filtered through a -----um filter into -----  
 containers. Samples are taken and submitted for testing per the r-halphaGAL Drug Substance  
 specifications . Drug Substance is stored at -----C for up to -----until formulation .

2. Batch Records

a. Definition of a Batch

The first stage of production (cell culture) is run at a 340L working volume in a -----  
 bioreactor. Each run consists of -----phases and is denoted by a specific bioreactor run  
 number. The -----main phases after inoculation are -----  
 ----- During the harvesting phase, product is -----  
 for up to----days using ----- . Once sufficient harvest has been collected in a  
 -----L harvest vessel (every -----, it is transferred to the -----vessel  
 for processing. The first discrete batch consists of -----L of harvest, which has been  
 transferred to the microfiltration vessel for further processing.

b. Batch Record

**Table 3: Appendix Locations of Representative Batch Records**

Batch Record	Appendix
Alpha Galactosidase----- Culture to Seed -----iter Bioreactor	IIC-18
---- liter Bioreactor Production of rh IGAL CHO cells	IIC-19
340 liter Alpha-Galactosidase Harvest Clarification Operation Batch Record at -----	IIC-20
Alpha-Galactosidase 340 liter -----Column Operation Batch Record	IIC-21
Alpha-Galactosidase 340 liter -----Column Operation Batch Record	IIC-22

**Table 3: Appendix Locations of Representative Batch Records**

Batch Record	Appendix
Alpha-Galactosidase 340 liter-----Column Operation Batch Record	IIC-23
Alpha-Galactosidase 340 liter -----Column Operation Batch Record	IIC-24
Alpha-Galactosidase Concentration, Dialysis, and Formulation Production Record	IIC-25

D. Process Controls

1. In-Process Controls

Harvest Materials are tested for -----  
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Column Eluates are tested for -----  
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2. Process Validation

Validation of the r-h IGAL manufacturing process at Genzyme's -----acility was performed in accordance with current US and European regulations and guidelines. The facility utilized is an existing, approved and validated multi-product manufacturing site. New purification equipment was purchased and installed and some minor modifications were required for the existing, validated (340L working volume) -----L bioreactors (----- A traditional Installation Qualification (IQ), Operational Qualification (OQ), Process Qualification (PQ) validation philosophy was utilized by Genzyme. All new equipment was installation qualified, operationally qualified and, where applicable, performance qualified. Performance qualification and process validation was performed using-----testing approaches, when applicable, and sterilization validation was performed using an -----Process validation (cell culture process and the purification process) was performed subsequent to the successful completion of the equipment qualification.

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defines the preparation, testing and storage requirements to be met in establishing this primary reference standard. (Note: The remaining portion of Drug Substance Lot 1-----as used to formulate Drug Product Lot-----used in the Phase 3 Clinical Studies. Therefore, the Reference Standard -----has been used in patients.)

Approximately---liter (--mg/mL) of Drug Substance lot -----was aseptically dispensed into sterile microcentrifuge tubes -----uL /tube). -----vials were prepared, individually labeled and stored at ---- °C in a secured, continuously monitored and calibrated freezer. Vial labels included the reference standard designation, product name, aliquot size, date of manufacture and storage conditions.

## 2. Working Reference Standard (if used)

The working reference standard is defined as material to be used for calibration of the analytical system in a quantitative assay during routine Quality Control testing. The primary reference standard is to be used as the standard against which the working reference standard will be determined. A new lot of r-halphaGAL is obtained from manufacturing, dispensed into cryovials, and samples are analyzed by the full set of release tests and additional tests to be performed in the Research and Development laboratories.

## F. Specifications / Analytical Methods

### a. Specifications and Analytical Methods



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b. Certificates Of Analysis and Analytical Results



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G. Container/Closure System

Bulk drug substance is stored in ---Liter-----storage vessels. After formulation with mannitol, the formulated drug substance is stored in ---Liter -----storage vessels for shipment to the drug product manufacturing facility, Genzyme™s -----

H. Drug Substance Stability

The stability program for r-h alphaGAL was designed to profile its stability characteristics. The program follows the basic principles of the ICH document *Final Guideline on Stability Testing of Biotechnological/Biological Products*. Stability studies have been conducted on various process intermediates and drug substance to support the holding times allowed during the r-h GAL manufacturing process. Table 13 displays the relationship of the various lots used to support the stability specifications.

*Harvest Fluid*

The real time data collected in the stability studies indicate that the r-h IGAL harvest fluid is stable for up to----- when stored at ----- °C in -----ontainers with a -----Samples of-----batches of harvest fluid, t-----each from t-----bioreactor runs, were tested -----

-----*Column Eluate*

The real time data collected in the stability studies support a hold time of -----luates for up to-----when stored in -----at ----- °C. Samples of-----batches of -----column eluate were tested -----

-----*Column Eluate*

The real time data collected in the stability studies support a hold time of A----eluates for up to ----- when stored in -----at ----- °C. Samples of -----atches of -----column eluate were tested every -----for -----followed by every -----

-----*Column Eluate*

The real time data collected in the stability studies support a hold time of -----luates for up to----- when stored in -----at ----- °C. Samples of -----batches of -----eluate were tested every -----

----*Column Eluate*

The real time data collected in the stability studies support a hold time of ----eluate for up to -----when stored in -----at ----- °C. Samples of-----batches of ----column eluate were tested every -----

*Drug Substance*

Results for-----atches of Drug Substance meet stability specifications throughout the time period of the study. This stability data supports storage of Drug Substance for up to f--- weeks at ----- °C in -----containers. Samples of t-----ots of drug substance were tested -----or -----  
-----

*Formulated Drug Substance*

Results for-----batches of Formulated Drug Substance (Drug Substance to which -----has been added and then filtered) meet stability specifications throughout the time period of the study. This stability data supports storage of Formulated Drug Substance for up to -----months at ----- °C in -----containers. Samples of t-----lots of formulated drug substance were tested -----  
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III. DRUG PRODUCT

A. Composition

Fabrazyme TM (agalsidase beta) is a sterile lyophilized dosage form packaged in a container closure system consisting of a-----glass tubing vial, siliconized 20 mm gray butyl lyophilization stopper and a -----seal. Prior to lyophilization, each vial of Fabrazyme is filled with 7.4 mL of a buffered solution containing a nominal concentration of 5 mg/mL Formulated Drug Substance. A 0.4 mL overfill is included to provide a sufficient volume upon reconstitution for withdrawal of 35 mg (7 mL) reconstituted Fabrazyme TM . Each Fabrazyme 35 mg vial is intended for single use administration only, therefore a preservative is not present.

r-h alphaGAL is administered by I.V. infusion. This route of administration requires that the reconstituted solution be further diluted with 0.9% Sodium Chloride Injection, USP or equivalent diluent.

B. Specifications & Methods for Drug Product Ingredients

**Table 6: Composition of Drug Product - Fabrazyme**

Ingredient	Mass per vial	Mass per -----vials	Function	Reference to Standard
<b>Active Ingredient</b>				
r-h IGAL	37.0 mg	-----	Active Ingredient	-----Drug Substance -----ce standard
<b>Other Ingredients</b>				
Mannitol	-----	-----	Stabilizer/Bulking Agent	USP
Sodium phosphate, monobasic, monohydrate	-----	-----	Buffer	USP
Sodium phosphate, dibasic, heptahydrate	-----	-----	Buffer	USP
Nitrogen	--	--	Overlay	----
<i>Approximate Weight of Lyophilized Cake</i>				
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C. Manufacturer(s)

---- alphaGAL Drug Substance is formulated by the addition of mannitol to a final concentration of ----(w/w) at Genzyme's-----  
 -----ng vessel and shipped to Genzyme's  
 ----- The r-alphaGAL Formulated Drug Substance is aseptically filled in the fill/finish suite at -----The bulk is aseptically filled into -----vials at a target fill volume of 7.4 mL. Based on the Process Qualification run results, the filling process was validated for a bulk volume of -----  
 ----- which corresponds to a validated lot size of approximately -----ials.

D. Flowchart

**Table 7: Manufacturing Flow Chart: Formulated Drug Substance to Drug Product**

Production Step	Equipment and Material Used
Formulation	Mannitol -----storage vessel
Ship to -----	-----shipping vessel
Receipt at the -----	
Transfer to Storage	
Filtration into sterile vessel	-----container, filters,-----ubing
Filling and partial stoppering	-----Filling System  20 cc glass vials (Type I) Siliconized butyl stopper  ----- Transport System and Stoppering Mechanism
Lyophilization and final stoppering	Lyophilizer
Capping and Inspection	Capper  Plastic flip-off caps with aluminum crimps
Packaging and Labeling	Labeler, Cartoner, Embrosser, Imprinter

E. Methods of Manufacture And Packaging

The manufacturing process comprises four major steps:

1. r-h alphaGAL Formulation;

The r-h alphaGAL Drug Substance is formulated to-----mg r-h IGAL per mL by the addition of ----- Mannitol to achieve a final concentration ---- w/w and the resultant solution is the r-h alphaGAL Formulated Drug Substance. The Formulated Drug Substance is -----um filtered for storage at-----°C for up to-- months.

2. Pooling and shipping of the r-h alpha GAL Formulated Drug Substance;

-----lots of Formulated Drug Substance are pooled and filtered through a -----m filter for storage at ----- °C. The pooled bulk is then shipped to the filling operation. The r-h alphaGAL Formulated Drug Substance is released, tagged, and shipped from the ----- facility. The transfer vessel is packed for temperature-controlled

shipment using an approved and validated packaging procedure. This procedure specifies packaging and shipping configurations required for summer or winter shipment. Documented evidence was obtained to demonstrate that the bulk vessel maintains a liquid temperature between----- °C during worst case temperature/time conditions. The shipping configurations are validated for up to-----hours. The shipping vessel is received by the Genzyme ----- and is transferred to a controlled -----holding area where it is logged in and held until the date of sterile filtration and fill.

### 3. Filling, lyophilization and capping;

Prior to product transfer, the---L shipping vessel is weighed and a sample is taken from the outlet port for pre-filtration bioburden testing. A sterilized -----line is connected from the outlet of the ---L shipping vessel to the inlet of t-----um product filters, which are autoclaved as part of the filtrate vessel located in a curtained Class -----sterile storage area. The transfer tubing runs through a wall chase connecting room F-----  
The ----- shipping vessel is pressurized to -----psig using -----um filtered nitrogen. The outlet valve on the ---L shipping vessel is opened and the inlet valve on the filtrate vessel is opened initiating product flow through the sterilizing filters into the filtrate vessel. Displaced air from the filtrate vessel is vented through a ----um venting filter.

After transfer is complete, all line loss is collected. The ---L shipping vessel is weighed and the quantity of material transferred into the filtrate vessel is calculated. The calculated amount is compared to the claim on the tag and batch record.

The filtrate vessel is status tagged with the product lot number, part number, date, and the quantity transferred. The product filters on the filtrate vessel are post-use integrity tested by -----The maximum allowable time between initiating the filtration process and completing the filling process is -----hours.`

After the product filters have passed post-use integrity testing, the filtrate vessel is moved to a Class-----area and samples are drawn for bulk sterility testing. If the -----filler is used, the outlet tubing on the filtrate vessel is removed and the sample is taken at the o-----  
-----If the -----filler is used, the outlet tubing is l-----  
----- After sampling, the filtrate vessel is aseptically connected to either the -----filling manifold or the sterilized filling line of the -----pump.

The partially stoppered vials are placed in trays using an automated tray loader. The vials are then transferred from the trays directly onto the lyophilizer shelves that have been pre-cooled to ----- °C. Before sterile filtration and filling is initiated, a validated steam sterilization cycle is performed on the chamber, condenser and the filter assembly.

At the completion of the filling process the lyophilizer door is closed and the product is freeze dried using a validated cycle.

The final step of the freeze drying cycle is to -----  
-----The chamber is then vented to atmospheric equilibrium. Shelf temperature is set to ----- °C and the

stoppered product is removed from the unit and capped under HEPA filtered air

Filled vials are capped using the -----capper in room -----with -----caps under HEPA filtered air. Capped vials are stored under quarantine at 2-- °C in trays that are labeled with product lot number, part number, and date of manufacture. Vials may be labeled prior to completion of release testing.

Filled and capped vials are subjected to a ----% inspection process by trained and qualified manufacturing personnel.

#### 4. Labeling and packaging operations.

Fabrazyme - vials are labeled either manually or using a fully automated labeling machine.

For manual labeling, all labels are pre-imprinted with a lot number and expiration date using a validated label imprinter. QA assigns the expiration date for the lot and confirms that the imprinting device is printing both the proper expiration date and lot number before the labels are imprinted. After the labels have been imprinted, the information on each individual label is verified by a fill finish labeling operator prior to applying the label to the vial. The imprinted and verified labels are applied to the vials and each labeled vial is inspected by a fill finish labeling operator.

Automated labeling is initiated by feeding the unlabeled vials onto a rotary on-load table. The labeler is fitted with rolled stock vial labels and prints the expiration date and lot number onto each label. QA assigns the expiration date for the lot. -----

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-----Labeled vials exit the machine via an off-load rotary table and may be either removed at this point for future packaging or allowed to feed the automatic cartoner.

Fabrazyme - vials are packaged either manually or using a fully automated packaging machine.

For manual packaging, all cartons are pre-embossed with a lot number and expiration date using a validated carton embosser. QA assigns the expiration date for the lot and confirms that the embossing device is coding both the proper expiration date and lot number before the cartons are embossed. After the cartons have been embossed, a fill finish packaging operator verifies the information on each individual carton. Each embossed carton is filled with a labeled vial and a package insert. A fill finish packaging operator then inspects the assembled package.

Automated packaging is initiated by feeding the labeled vials to a rotary on-load table. QA assigns the expiration date for the lot and confirms that the packaging machine is embossing both the proper expiration date and lot number. The cartoner is supplied with cartons and package inserts and embosses the expiration date and lot number onto each carton. A validated bar code reader confirms that the carton and package insert are the correct revision. A labeled vial and a package insert are automatically inserted into an embossed carton and the completed package exits the machine.

F. Specifications & Test Methods For Drug Product

1. Sampling Procedures

2. Specifications & Methods

**Table 8: Drug Product Tests: Lyophilized**

Test/Assay	Method	Proposed Specification
-----	-----	-----
-----	-----	-----
-----	-----	-----
Residual Moisture	-----	-----

**Table 9: Drug Product Tests: After Reconstitution with 7.2 mL of WFI**

Test/Assay	Method	Proposed Specification
Activity	-----	-----
-----	-----	-----
-----	-----	-----
-----	-----	-----
Concentration	-----	-----
Identity	-----	-----
-----	-----	-----
Mannitol	-----	-----
-----	-----	-----
---	-----	-----
Phosphate	-----	-----
Purity	-----	-----
Purity	-----	-----
Sodium	-----	-----
Specific Activity	-----	-----
Sterility	-----	-----

\*One unit of r-h GAL is defined as that amount of activity which results in the hydrolysis of 1 micromole of substrate per minute at ---°C under the assay conditions.

Specifications for drug product were developed considering release data and ---month stability data for --- lots of drug product. Specifications are mean values -----standard deviations.

**Sponsor needs to include a structural identity test, such as a ----- already qualified and used in comparison study) or ----- (already qualified and used in comparison study), to distinguish IGalactosidase (the human protein) from IGalactosidase (the enzymatic activity).**

**G. Container/Closure System**

Drug Product container is a -----glass tubing, USP/EP, stoppered with a gray 20 mm butyl stopper ,USP/EP, and sealed with a 20 mm aluminum -----seal, with



*Drug Product - Lyophilized*

The ----- °C stability studies for r-h alphaGAL Phase 3 Drug Product lot numbers -----and----- are complete through the -----month time point. The ----- °C stability study for r-h alphaGAL Phase 3 Drug Product lot number -----s currently ongoing through ----months, with data available through the -----month time point. Preliminary statistical analysis of the data indicates that r-h alphaGAL Drug Product is stable for up to -----months when stored at 2 -8 °C.

Following actual storage, available stability data were analyzed and new proposed commercial stability specifications were established to reflect actual experience. The ----- °C stability studies for r-h alphaGAL process validation finished product lot numbers -----re currently ongoing, with data collected through the ----month time point on -----lots. Genzyme believes that the process validation lots will have the same profile as the clinical material, over the study period of -----months.

Based upon the data presented on Phase 3 and process validation lots, along with the accelerated data presented Genzyme requests an expiration date of twenty-four months for the product when stored at 2 -8°C.

The data in this section demonstrate that the Drug Product is stable when exposed to short-term temperature excursions outside the ----- °C storage conditions, up to --- °C with ---% RH.

Characteristics that were studied include activity, aggregation, appearance, endotoxi-- concentration, mannitol, moisture, oxygen headspace, particulates, pH, phosphate, ----- purity by -----and -----reconstitution time, sodium, specific activity, and sterility.

*Reconstituted Fabrazyme*

The----- °C and--- °C a-----% RH stability studies for lot -----ndicate that reconstituted Fabrazyme is stable for up to --- hours. This data supports the current package insert claim of 24 hours for product reconstituted with 7.2 mL of Sterile Water for Injection, USP/EP, when stored refrigerated or at room temperature.

Characteristics that were studied include -----  
-----  
-----

*Fabrazyme diluted for infusion*

The stability study for lot-----diluted in 0.9% sodium chloride injection, USP/EP, stored at ---- °C, and infused through a typical infusion apparatus indicates that diluted Fabrazyme is stable for up to ----hours in the infusion bag when stored at ----- °C, and then infused at room temperature over a six-hour period. This data supports the current package insert claim of 24 hours for product diluted with 0.9% sodium chloride injection, USP/EP, and stored at 2- 8°C.

## Stability Commitments

The stability studies for the commercial lots are ongoing at both ----- °C and ----- RH. The-----studies will be continued to -----months, and the accelerated studies to -----months. Genzyme will provide additional stability data as they become available. -----additional commercial lots will be evaluated in the reconstitution study, and in the infusion study in which diluted Drug Product is stored at ----- for -----hours prior to infusion.

Furthermore, -----lots will be evaluated in the infusion study in which diluted Drug Product is stored at-----% RH for --- hours prior to infusion. Data from these studies will be provided during the review process as they become available.

In addition to the ongoing studies----- Genzyme will commit to placing one additional Drug Product lot on long-term stability at ----- °C, reconstituted stability and diluted stability annually. Changes to the manufacturing process may require additional lots of Drug Product and/or process intermediates to be placed on stability. For significant process changes, Genzyme commits to evaluating the Drug Product in the long-term study at -----C, as well as the accelerated study, the reconstitution study, and the diluted product infusion studies per protocols.

## IV. INVESTIGATIONAL PRODUCT/FORMULATION

During clinical development, several manufacturing changes were made.

### 1. Scale-up Changes

For the Phase 1/2 clinical trials and acute toxicity studies, r-h alphaGAL was manufactured at a ---- and at a ----- bioreactor scale. For the Phase 3 clinical trial and repeat dose toxicity studies, a ----- bioreactor scale was implemented. The proposed commercial manufacturing process will be conducted at the 340L scale.

### 2. Cell Culture Process

In the 30L cell culture process, cells from the Manufacturers Working Cell Bank (MWCB) were expanded through----- bioreactor. The growth phase medium in the bioreactor consisted of -----plus d----- serum. The production phase medium was also -----Up to -----ays of production has been demonstrated for the 30L process.

For the 160L scale, cells from the MWCB were expanded in ----- rather than r-----to a 160L bioreactor. There was no change in the growth phase medium or the prod----- medium relative to the 30L bioreactor scale. The production process was run for a total of ----days for the 160L bioreactor scale.

The 340L bioreactor scale also utilizes ----- to expand cells from the MWCB to a 340L bioreactor. Again, the growth phase medium and production phase medium are the same as they were for the 30L and 160L scale.





The analyses indicate that the material produced at all three scales is biochemically equivalent.

Upon scaling the process from 30L to 340L there has been a reduction the amount of-----as determined by -----and ----- analysis. This change in-----has no effect on the specific activity of the preparations.

Material produced at the commercial (340L bioreactor) scale has a higher degree of purity as determined by the -----and the -----mpurity levels were equivalent at all three process scales.

The levels of----- were equivalent at the 160 and 340L bioreactor scales but both were higher than the levels observed at the 30L bioreactor scale. For both the-----and the -----teps, the -----does not bind to either column using the current loading conditions while the r-h alphaGAL binds tightly.



7. Finished Product Testing

Batch analysis data for the Drug Product produced at the three different scales demonstrates similar levels of potency, safety and purity across all three scales.

8. Batch History

**Table 11: Drug Product Batches**

Drug Product Lot	Bioreactor Scale	Manufacturing Facility	Use
-----	30 liter	-----	-----
-----	30 liter	-----	-----
-----	160 liter	-----	-----
-----	160 liter	-----	---
-----	340 liter	-----	-----
-----	340 liter	-----	-----
-----	340 liter	-----	-----
-----	340 liter	-----	-----
-----	340 liter	-----	-----
-----	30 liter	-----	-----
-----	340 liter	-----	-----
-----	340 liter	-----	-----
-----	340 liter	-----	-----

**Table 12: Correlation between Drug Substance and Drug Product Lots**

<b>Drug Substance Lot</b>	<b>Formulated Drug Substance Lot</b>	<b>Drug Product Lot</b>	<b>Bioreactor Scale</b>	<b>Use</b>
-----	-----		-----	-----
-----	-----		-----	---
-----	-----		30 liter	-----
-----	-----		30 liter	-----
-----	-----		30 liter	-----
-----	-----		30 liter	-----
-----	-----		30 liter	-----
----- -----			30 liter	----- -----
-----	-----		30 liter	---
-----	-----		30 liter	---
-----	-----	-----	30 liter	-----
-----	-----	-----	30 liter	-----
-----	-----	-----	160 liter	-----
-----	-----	-----	160 liter	---
-----	-----	-----	30 liter	-----

**Table 13: Correlation between Drug Substance and Drug Product Lots: Commercial Scale**

Drug Substance Lot	Formulated Drug Substance Lot	Pooled Formulated Drug Substance Lot	Drug Product Lot	Bioreactor Scale	Use
-----	-----		-----	-----	-----
	-----		-----	-----	-----
-----	-----	-----	-----	-----	-----
-----	-----				
-----	-----	-----	-----	-----	-----
-----	-----				
-----	-----	-----	-----	-----	-----
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-----	-----				

**V. ENVIRONMENTAL ASSESSMENT**

In accordance with the National Environmental Policy Act; Revision of Policies and Procedures, Final Rule published in the Federal Register (62 FR 145, 7/29/97) this Biologics License Application of Fabrazyme (r-h GAL) meets the criteria for categorical exclusion under 21 CFR Section 25.31(c). Section 25.31(c) provides for a categorical exclusion regarding an action on a BLA, for substances that occur naturally in the environment when the action does not alter significantly the concentration or distribution of the substance, its metabolites, or degradation products in the environment. In the case of the lyophilized r-h GAL drug product, the active drug substance is a recombinant version of a naturally occurring human enzyme, which would have the same metabolites or degradation products as the non-recombinant version. In addition, the concentration or distribution of the substance itself and therefore, its metabolites and degradation products, would be significantly less than one part per billion at the point of entry into the aquatic environment. The action, therefore, would not alter significantly the concentration in the environment.

## VI. METHOD VALIDATION

**Table 14: Assays, Procedures, and Validation Reports**

Test/Assay	Samples	SOP	Validation Protocol	Technical Report	Location
-----	----- ----- ----- ----- -----	QC-049-21	VP-025-47	98TRN091	Appendix IIC-40
-----	----- ----- ----- ----- -----	QC-049-51	VP-025-69	99TRN118	Appendix IIC-41
-----	----- ----- ----- -----	QC-025-07	NA	NA	Appendix IIC-42
-----	----- ----- ----- ----- -----	QC-040-09	VP-023-16	99TRN029 00TRN008	Appendix IIC-43
-----	----- ----- ----- -----	QC-045-16	QC-048-65	99TRN031 99TRN032	Appendix IIC-44
----- -----	----- ----- ----- -----	QC-049-33 QC-049-40	VP-025-56	99TRN012 99TRA005	Appendix IIC-45
----- -----	----- ----- ----- ----- -----	QC-049-22	VP-025-46	98TRN086	Appendix IIC-46
----- -----	----- ----- ----- -----	QC-039-77	NA	NA	Appendix IIC-47
----- -----	----- ----- ----- -----	QC-039-77	NA	NA	Appendix IIC-47
-----	----- ----- ----- ----- -----	QC-039-92	VP-025-66	99TRA032	Appendix IIC-48
-----	----- ----- ----- -----	QC-019-17	NA	NA	Appendix IIC-49
-----	----- ----- ----- -----	QC-021-04	USP	NA	Appendix IIC-50
----- -----	----- ----- ----- -----	QC-046-13	USP	NA	Appendix IIC-51
-----	----- ----- ----- -----	QC-049-72	VP-025-87	00TRN014	Appendix IIC-52
---	----- ----- ----- -----	QC-028-04	USP	NA	Appendix IIC-53
-----	----- ----- ----- -----	QC-049-04	VP-025-77	00TRN009	Appendix IIC-54









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**Residual Moisture**

The water content of the lyophilized final product is quantitated by an automated system known as----- . This methodology is analogous to that described in -----  
-----The assay is a coulometric, pyridine-free Karl Fisher titration of water present in the sample. This method is faster and more accurate than manual Karl Fisher titrations and measures total product water content.

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validation.

**Sodium**

Sodium is detected and quantitated by -----This is a highly sensitive and specific method.

**Specific Activity (U/mg)**

Calculation of r-h alphaGAL activity units per milligram of protein is performed by dividing the activity determined by QC-049-21 by the protein concentration determined by QC-049-22.

**Sterility**

The sterility of the Reconstituted Drug Product is evaluated by the -----  
-----

-----

-----is detected and quantitated by -----This is a highly sensitive and specific method.

The following information requests were sent to the sponsor in a letter from the Agency, dated October 13, 2000, and Amendment 007 was submitted in response on December 8, 2000.

These same information requests were reiterated as the first five requests in the Complete Review letter, dated December 22, 2000.

1. Please modify the Certificate of Analysis for bulk drug substance to include specifications for the following:

- a. -----
- b. -----
- c. -----
- d. -----content expressed as -----  
per mole of protein and moles of -----per mole  
of protein.
- e. -----content expressed as moles of -----  
-----
- f. -----
- g. -----

2. Please modify the Certificate of Analysis for drug product to include a -----density test in addition to the----- This identity test should be able to distinguish the human galactosidase protein from hamster enzyme.

3. Please include measures of -----stability in the stability protocol for drug product.

4. Please submit photostability data as part of the stress testing done on the product.

5. Please submit updated stability data for bulk drug substance and final drug product. Expiration dating will be based on real time data available prior to approval of the BLA.

**The sponsor responded to these requests on December 8, 2000 [BLA Reference No. 99-2865, Amendment 007].**

*Please modify the Certificate of Analysis for bulk drug substance to include specifications for f-----*

An assay has been developed and validated to assess the f----- content of r-huGAL in Fabrazyme bulk drug substance. This test has been employed for characterization of the Primary Reference Standard, -----as well as the demonstration of lot-to-lot consistency of the -----Process Qualification lots. This test will be added to the specifications and resulting Certificates of Analysis for bulk drug substance. The following interim specification is proposed: ----- Upon completion of analysis of at least -----lots of bulk drug substance, Genzyme will reassess further refinement of this specification.

*This response is satisfactory and adequate.*

Please modify the Certificate of Analysis for bulk drug substance to include specifications for  
-----

A ----- protocol, based on the -----  
-----has been utilized to assess the purity of r-háGAL in Fabrazyme bulk  
drug substance on -----as a complement to -----  
This test is perform-----omparison of test, reference and a co-mixture of  
test/reference materials. Upon completion of optimization and validation, a qualitative  
test for -----will be added to the specifications and  
resulting Certificates of Analysis for bulk drug substance. Since this is a qualitative test,  
a specification of -----s proposed at this time. The  
method and validation report will be submitted to CBER upon completion of the validation  
of this test.

*This response is satisfactory and adequate.*

Please modify the Certificate of Analysis for bulk drug substance to include specifications for -----  
-----

An-----test has been developed and validated to assess the  
microheterogeneity of r-huGAL in Fabrazyme bulk drug substance. This test has been  
employed for characterization of the Primary Reference Standard, ----- as well as the  
demonstration of lot-to-lot consistency of the -----Process Qualification lots. This test  
will be added to the specifications and resulting Certificates of Analysis for bulk drug  
substance. A specification of -----will be employed at this  
time. Upon completion of analysis of at least ----- lots of bulk drug substance,  
Genzyme will reassess further refinement of this specification.

*This response is satisfactory and adequate.*

Please modify the Certificate of Analysis for bulk drug substance to include specifications for -----  
content expressed as moles of -----and -----per mole protein.

An assay to determine the----- content of r-huGAL in Fabrazyme bulk drug  
substance has been developed and validated. This test has been employed for  
characterization of the Primary Reference Standard, -----as well as the demonstration  
of lot-to-lot consistency of the -----Process Qualification lots. This test will be added to  
the specifications and resulting Certificates of Analysis for bulk drug substance. The  
following specifications are proposed: -----  
-----

Upon completion of analysis of at least -----lots of bulk drug substance, Genzyme  
will consider further refinement of this specification.

*This response is satisfactory and adequate.*

Please modify the Certificate of Analysis for bulk drug substance to include specifications for m-----  
----- content expressed as moles of -----per mole of protein.

An assay to determine the -----content of r-huGAL in Fabrazyme bulk drug substance has been developed and validated. This test has been employed for characterization of the Primary Reference Standard, ----- as well as the demonstration of lot-to-lot consistency of the -----Process Qualification lots. This test will be added to the specifications and resulting-----ificates of Analysis for bulk drug substance. The following interim specification is proposed: -----  
r-háGAL monomer. Upon completion of analysis of at least t-----lots of bulk drug substance, Genzyme will consider further refinement of this specification.

For stability assessment of drug product, this assay will be revised to include a sample preparation step in order to -----  
----- After qualification of the sample preparation step, both assays will be added to stability protocols for new Drug Product lots. Revised methods will be provided to CBER upon completion of development and qualification.

*This response is satisfactory and adequate.*

Please modify the Certificate of Analysis for bulk drug substance to include specifications for  
-----

A test has been developed and validated to examine the -----  
----- from r-huGAL in Fabrazyme bulk drug substance. this test has been employed for characterization of the Primary Reference Standard, ----- as well as the demonstration of lot- to-lot consistency of the ----- Process Qualification lots. This test will be added to the specifications and resulting Certificates of Analysis for bulk drug substance. A specification of c-----  
----- will be employed at this time. Upon completion of analysis of at least ----- lots of bulk drug substance, Genzyme will consider further refinement of this specification.

*This response is satisfactory and adequate.*

CBER requested continued assessment of the -----mpurity in the drug substance.

Genzyme has developed and currently is completing the development and validation of a -----which is capable of detecting ----- and ----- in addition to other -----impurities which may be carried through the manufacturing process. -----f a specific test for -----Genzyme proposes to use the -----to measure the -----impurity content of the r-huGAL drug substance, which will also reflect a potential -----impurity content. The proposed commercial specification for first generation -----for r-huGAL drug substance is -----µg/mg. Upon completion of analysis of at least t-----lots of bulk drug substance using the -----, Genzyme will propose a revised specification based -----e ----- results. The n-----will then replace the first generation ---  
----- In the interim, all new r-háGAL drug substance lots will also be tested for -----in addition to the determination of -----mpurities using the ----- relative to the current propose-----ication.

*This response is satisfactory and adequate.*

*Please modify the Certificate of Analysis for drug product to include a -----identity test in addition to the -----assay. This identity test should be able to distinguish the human galactosidase from the hamster enzyme.*

A panel of ----- antibodies specific for recombinant human r-huGAL have recently been developed which do not cross react with the hamster enzyme. Genzyme proposes to develop a human galactosidase-specific-----using one of these reagents. Genzyme will provide the method and validation report to CBER upon completion of development and validation of this assay. After CBER review and approval of the method and validation report, this test will be added to the specifications and resulting Certificate of Analysis for drug product with a proposed specification of -----  
-----

*This response is satisfactory and adequate.*

*Please include a measure of o-----n the stability protocol for Drug Product*

For stability assessment of drug product, both assays will be revised to include a -----  
-----n order to remove any potentially -----  
----- After qualification of the -----both assays will be added to stability protocols for new Drug Product lots. Revised methods will be provided to CBER upon completion of development and qualification.

*This response is satisfactory and adequate.*

*Please submit photostability data as part of the stress testing performed on the product.*

Genzyme has not yet conducted a formal photostability study for Fabrazyme. A protocol has been drafted in accordance with ICH guideline Q1B, Photostability Testing of New Drug Substances and Products. Genzyme commits to conducting this photostability study on one lot of r-huGAL Drug Product during the year 2001. The data will be supplied to CBER upon completion of these studies.

*This response is satisfactory and adequate.*

*Please submit updated stability data for bulk drug substance and final drug product. Expiration dating will be based on real time data available prior to approval of the BLA.*

Genzyme provided additional stability data for the bulk drug substance, formulated drug substance, and final drug product. The update included data from the -----bulk drug substance and -----formulated drug substance. The upda-----ded data from the drug product real-time studies at -----°C, accelerated studies at 2-----C -----RH, reconstituted drug product studies at----- and-----RH, and diluted drug product studies at ----- and -----%R---

Genzyme provided stability data from -----lots each of -----bulk drug substance and-----formulated drug subst----- T-----rug substance was held for ----  
----- and the formulated drug substance for-----months, at -----C in -----

-----containers. Data from the -----lots of -----bulk drug substance support storage for up to -----when stored at -----C in ----- containers. Genzyme provided updated real-time and accelerated stability data on t----- lots of -----drug product. Studies conducted on r-háGAL Drug Product indicate that the product is stable for up to ----months real time at -----C with minimal degradation. The -----assay indicated a decrease of approximately ---- of initial -----over ----mo-----he -----assay indicated an increase of ----% over --- months.

*The updated stability data contributes to the real time data support of a ----month expiration date for the drug product and the nanofiltered drug product.*

**On March 11, 2002, additional responses were submitted to amend the BLA submission. These responses further substantiated information in the BLA and completed several commitments made on December 8, 2000 [BLA Reference No. 99-2865, Amendment 007].**

Attachment 10 updated two tables from the original submission listing the results of extended Quality Control testing of the Primary Reference Standard -----Table IIC-73) and the results of Initial Characterization of the Primary Reference Standard -----(Table IIC-74).

**Table IIC-73**  
**Extended QC Release Testing of Primary Reference Standard [REDACTED]**

Test/Assay	Number of Replicates	Proposed Commercial Specification	Result
[REDACTED]			

\* Results taken from Drug Substance lot [REDACTED]

Table IIC-74  
Initial Characterization Results for Primary Reference Standard [REDACTED]

Characterization Assay	Number of Replicates	Results
[REDACTED]		

Attachment 11 contains the Addendum to the Technical Report documenting a revision to the concentration determination in the reference -----the concentration of enzyme protein in -----was initially determined using-----as a Working Standard against the Interim Primary Reference Standard ----- The procedure was re-validated and the new values appear in Table IIC-73 and IIC-74.

In the response of December 8, 2000 , Genzyme proposed to use a new -----o measure the -----impurity content of the r-huGAL drug substance, which will also reflect a potential a-----y content. Attachments 12 through 15 report completion of this commitment.

Attachment 12 contains a copy of the new -----Assay; Attachment 13 is the ----- Assay Validation Protocol; Attachment 14 is the -----Assay Validation Technical Report; Attachment 15 is the Technical Report evaluating the specificity of the Protein Impurities Antisera used for the ----- Assay.

In the response of December 8, 2000, the sponsor agreed to develop and validate an assay to determine the -----content of r-huGAL in Fabrazyme bulk drug substance. Attachment 16 is the -----procedure

**On December 18,2002, Genzyme updated the CMC section of the Fabrazyme BLA STN 103979 (99-2865, Amendment 25).**

### **Specifications Update**

Genzyme completed the first annual product review that included lot release and stability data for Phase 3 clinical lots through recent production lots. The specification for Specific Activity was changed to represent the mean plus/minus three standard deviations for the ---lots evaluated. Several specifications have been added to the Certificates of Analysis, reflecting the regulatory requirements of the EMEA. The assays and data supporting these specifications had been previously qualified for use. Upon request, these assays were validated and introduced as release criteria for drug substance and drug product.

**Table 15: Drug Substance Specifications, First Anniversary - ---lots**

Test/Assay	Method	Current Specification	Proposed Specification
-----			
-----	----- -----	----- -----	-----
-----	-----	-----	-----
-----	----- -----	-----	-----
-----	-----	----- -----	-----
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**Table 16: Drug Product Specifications, First Anniversary -**

Test/Assay	Method	Current Specification	Proposed Specification
<b>Physical/Chemical Tests on Lyophilized Product</b>			
-----	-----	-----	-----
<b>Residual Moisture</b>	-----	-----	-----
-----	-----	-----	-----
-----	-----	-----	-----
<b>Identification and Quantification Tests</b>			
Activity	-----	-----	-----
Concentration	-----	-----	-----
Identity -----	-----	-----	-----
Mannitol	-----	-----	-----
Phosphate	-----	-----	-----
Sodium	-----	-----	-----
Specific Activity	-----	-----	-----
Identity	-----	-----	-----
Purity-----			
-----	-----	-----	-----
Purity	-----	-----	-----
Purity	-----	-----	-----
<b>Safety/Sterility Tests</b>			
-----	-----	-----	-----
-----	-----	-----	-----
Sterility	-----	-----	-----
-----			
-----	-----	-----	-----
-----	-----	-----	-----
---	-----	-----	-----

**Stability Update**

Genzyme submitted a stability update for Fabrazyme and requests a 24 month expiration date at time of licensure. This update reports the stability data for the-----process qualification lots (----- for ----- months, respectively. The drug product is stable for up to ---months real time at --- °C. The Activity assay indicated a decrease of approximately ---- initial activity over ----months for clinical lot -----For ----- initial specific activity of 7--U/mg, at ----months with ----U/mg, and at ----

months with---U/mg; for -----, the values range from -----U/mg over the same time span; for----- the values range from -----U/mg over the same time span. All values were within -----U/mg (previous specification) and -----U/mg (new specification). Similar trends are seen for the process qualification lots and will be monitored throughout the ----months of the study. The ----- assay indicated an increase of approximately ---- over the ----months. The ----- indicated -----of approximately ----% o---r the durati---of the study. Other assays indicated trends with minimal changes over the study durations.

Accelerated stability studies indicate that the product may be stored at ---\_ - °C with ---\_---- relative humidity for up to -----

*Based on the data currently available, Genzyme requests an expiration date of 24 months for Fabrazyme when stored at 2-8°C and a shipping configuration of up to --- °C.*

Genzyme provided updated stability data for one lot of drug product diluted for infusion in 0.9% sodium chloride injection, stored at 2-8°C for 72 hours, and then infused through a typical infusion apparatus. These data, along with data previously submitted, support a *hold time of 24 hours for product diluted with 0.9% sodium chloride injection, USP/EP, and stored at 2-8°C prior to infusion.*

Also, Genzyme reports the data for the 2000 Annual Lot of drug product at ---months, the 2001 Annual Lot of drug product at --months, and -----process qualification lots at ----months. -----of drug substance was requested by the EMEA and was implemented. -----process qualification lots as well as the-----were entered into the stability program.