CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125553Orig1s000

CHEMISTRY REVIEW(S)

BLA 125553

EP2006

SANDOZ, Inc.

PRODUCT QUALITY REVIEW AMENDMENT
February 6, 2015

Signature Block

Name and Title	Signature and Date
Gibbes Johnson, PhD Acting Director, Division of Biotechnology Review and Research IV	Gibbes R. Johnson -S Digitally signed by Gibbes R. Johnson -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000592128, cn=Gibbes R. Johnson -S Date: 2015.02.10 12:23:58 -05'00'
Maria-Teresa Gutierrez-Lugo, PhD Lead, Division of Biotechnology Review and Research III	Maria Teresa Gutierrez Lugo -S Digitally signed by Maria Teresa Gutierrez Lugo -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0011818863, cn=Maria Teresa Gutierrez Lugo -S Date: 2015.02.06 14:56:17 -05'00'

Updated sections: 3.2.S.6., 3.2.S.4.1, 3.2.S.4.5, 3.2.P.5.1, 3.2.P.5.6, and 3.2.S.5

This amendment summarizes review of the remaining responses to information requests listed below and corresponding BLA amendments:

Communication/Document	Date
Information Request 8	January 21, 2015
Information Request 10	February 3, 2015

Amendments:

Submission	Date Received	Review completed
Information Request 8	January 30, 2015	Y
Information Request 10	February 6, 2015	Y

1. You did not provide leachable and extractable data for the drug substance (DS) container closure system. To address this deficiency provide the following:

    a. Extractable and leachable data from the container closure system and leachables data from the EP2006 DS process using suitable methods. Analysis of extractables and leachables should include evaluation of organic non-volatile (e.g., HPLC-UV-MS), volatile (e.g., headspace GC-MS) and semivolatile (e.g., GC-MS) species, and metals (e.g., ICP-MS) (refer to Markovic, I. Evaluation of safety and quality impact of extractable and leachable substances in therapeutic biologic protein products: a risk-based perspective. Expert Opin. Drug Saf. (2007) 6(5)). The extractable and leachable assessment should include their chemical identification and quantification.

    b. Risk assessment of extractables and leachables identified in your proposed container closure system for EP2006 DS and leachables from the EP2006 DS process. You may consider the extractable data conducted by the manufacturers of the components of the container closure system and the materials used in the manufacture of EP2006 DS (b) (4) to conduct an initial risk assessment of potential extractables and leachables.

    Additional information regarding extractables and leachables should be provided per *FDA Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics (1999)*.

Sandoz provided data from extractable studies assessing volatile, semi-volatiles, non-volatiles, and metals from the container closure system. The conditions used are summarized in Tables 1-1 and 1-6 (the conditions for extracting metal were more stringent). The solvents used are listed in Table 1-2 (extraction of metals used the same solvents).

Table 1-1 Study conditions for evaluation of volatiles, semi-volatiles and organic non-volatiles substances in the EP2006 DS container closure system

Package component	Extraction Procedure
(b) (4)	Extraction days: 7 Extraction temperature: 30°C Position: Upright Last 8h: storage in downright position Manual shaking every 24h during 7 days of extraction

Table 1-6 Study conditions for evaluation of metals in the EP2006 DS container closure system

Package component	Extraction Procedure
(b) (4)	Extraction days: 14 Extraction temperature: 30°C Bottles: Cut into pieces HDPE screw: Cut into pieces Last 8h: Cap pieces were combined with bottle pieces and extraction continued

Table 1-2 Analytical methods and solvents used for the extractable study for EP2006 DS container closure system

Analytical method	Analysis of	Solvents used for the extractable study
HPLC-GC-MS DAD	Non-volatile substances	phosphate buffer, pH 2.5, phosphate buffer, pH 9.5, 2-Propanol (50%), EP2006 DS buffer at pH 4.4
Head space-GC-MS	Semi volatile substances	phosphate buffer, pH 2.5, phosphate buffer, pH 9.5, 2-Propanol (50%), EP2006 DS buffer at pH 4.4
GC-FID/MS	Volatile substances	phosphate buffer, pH 2.5, phosphate buffer, pH 9.5, EP2006 DS buffer at pH 4.4

Reviewer's comments: *The results from the studies showed that organic volatiles and non-volatile extractables and metals (15 different metals were investigated) from extracts using the solvents listed in Table 1-2 were <LOQ of the methods. LOQs for volatile, semi-volatiles and non-volatiles are* (b) (4) *respectively. LOQs for metals ranged from* (b) (4)

(b) (4)

(b) (4) *A toxicology assessment of these compounds indicated that possible exposure to the identified extractables is not associated with any reasonable risk to human health. The toxicological assessment report was provided. I reviewed the report and found that it supports Sandoz statement.*

Review of leachables and extractables data from the manufacturing process materials is described in the product quality review dated January 30, 2015.

2. Revise your release and stability specifications for drug substance (DS) and drug product (DP) to address the following:

    a. Establish objective and quantitative (when possible) acceptance criteria for identity methods (molecular size, hydrophobicity and isoelectric point). Acceptance criteria such as "correspond to reference" are not appropriate.

    Review incorporated in the CMC review dated January 30, 2015

b. Provide method validation and transfer reports (if applicable) for the peptide mapping method intended to be included as orthogonal identity tests in the DS release specifications.

Reviewer's comment: *I reviewed the peptide map validation and transfer reports provided in response to this IR. The method is validated for intended purpose: identity of EP2006 DS and DP. The method will be performed at* [redacted] (b) (4) *Briefly,*

The method was originally validated at [redacted] (b) (4) *in October, 2004. Method validation parameters included specificity, precision (repeatability and injection repeatability), range, and robustness. This approach covers additional parameters to those described in ICH Q2R1 for identity methods. EP2006 DS and EU-approved Neupogen samples as well as forced oxidized, deamidated and reduced/alkylated EP2006 samples were used in the method validation. The results show that the method is specific with respect to DS matrix components and with respect to the forced degraded samples referred above. Peptide maps of degraded samples have different profiles (e.g. additional peaks). The range of EP2006 protein of 0.1-0.75 mg/ml was determined to provide valid results (reproducible map). Precision was determined by means of retention time (RT) and peak area variability (RSD). For repeatability, the RSDs for RT and peak area were 0.02-0.25% and 0.7-3.7%, respectively. For injection repeatability, the RSDs for RT and peak area were 0.02-0.19% and 0.71-5.2%, respectively. These values were within the precision AC of <2% for RT and <10% for peak area and are acceptable.*

To transfer this method from [redacted] (b) (4) *to* [redacted] (b) (4) *specificity and stability of samples in the autosampler were evaluated at* [redacted] (b) (4)*. Specificity evaluation included EP2000, a product of similar molecular weight as EP2006* [redacted] (b) (4) [redacted] (b) (4)*. The results show that the method is able to distinguish these two products. The results of autosampler stability show that samples are stable at 2-10 °C for up to 48h.*

I noted that peptide map from the original validation is slightly different from the peptide map of the transfer exercise. However, the "transfer" exercise itself supports that the method is suitable for intended purpose. The data from the original validation further supports suitability because although the maps are different, they both provide the same information (i.e. identity of EP2006) given that the map cover the full sequence of the protein. The data also show that the peptide map method is reproducible with respect to RT and peak area. The evaluation of forced degradation EP2006 samples shows that this method is able to identify modifications of the protein when these are in a large proportion in the sample.

c. Process-related impurities such as E. coli host cell proteins and residual DNA are not expected to change during storage. Consider removing these tests from the stability specifications of EP2006 DS.

Review incorporated in the CMC review dated January 30, 2015

d. Your release and stability specification for extractable volume of EP2006 DP is "not less [redacted] (b) (4) (300 mcg/0.5 ml strength) and "not less than [redacted] (b) (4)" (480 mcg/0.8 ml strength). Revise your acceptance criterion for extractable volume to include two significant figures. In addition, specify the rounding procedures applied to extractable data.

Reviewer's comment: *Although the rounding rules described in response to the IR are acceptable, the proposed acceptance criterion of "not less than [redacted] (b) (4)" (300 mcg/0.5 ml) and "not less than [redacted] (b) (4)" (480 mcg/0.8 ml) for extractable volume is not appropriate. The reason is outlined in the IR was sent on February 3, 2015:*

Your proposed release and stability specification for extractable volume of EP2006 drug product (DP) is "not less than [redacted] (b) (4)" (300 mcg/0.5 mL strength) and "not less than [redacted] (b) (4)" (480 mcg/0.8 mL strength). The proposed acceptance criteria would result in [redacted] (b) (4) total amount of product for the 300 mcg/0.5 ml strength and [redacted] (b) (4) total amount of product for the 480 mcg/0.8 mL strength. The amount of product could be [redacted] (b) (4) if the protein concentration of the EP2006 DP is at the lower end of the specification. Revise your acceptance criteria to ensure that your drug product will deliver the stated amount of "not less than [redacted] (b) (4) (300 mcg/0.5 mL strength) and not less than [redacted] (b) (4) (480 mcg/0.8 mL strength).

In the response dated February 6, 2015, Sandoz changed the acceptance criteria to:

- "not less than [redacted] (b) (4)" (300 mcg/0.5 mL strength) and
- "not less than [redacted]" (480 mcg/0.8 mL strength)

The acceptance criteria are now acceptable.

e. Describe your control strategy for the levels of sub-visible particles [redacted] (b) (4) in the EP2006 DP.

Review incorporated in the CMC review dated January 30, 2015

f. You proposed to revise the acceptance criterion for pH of the EP2006 DP as [redacted] (b) (4) based on manufacturing experience of lots of EP2006 DP manufactured for the US market and for other markets. The data provided in Table 7-1 of the response to information request (question 1) dated January 14, 2015 indicate that your process is able produce EP2006 DP with pH in the range [redacted] (b) (4) Revise the upper limit of the acceptance criterion to better reflect manufacturing experience of the EP2006 DP for the US market.

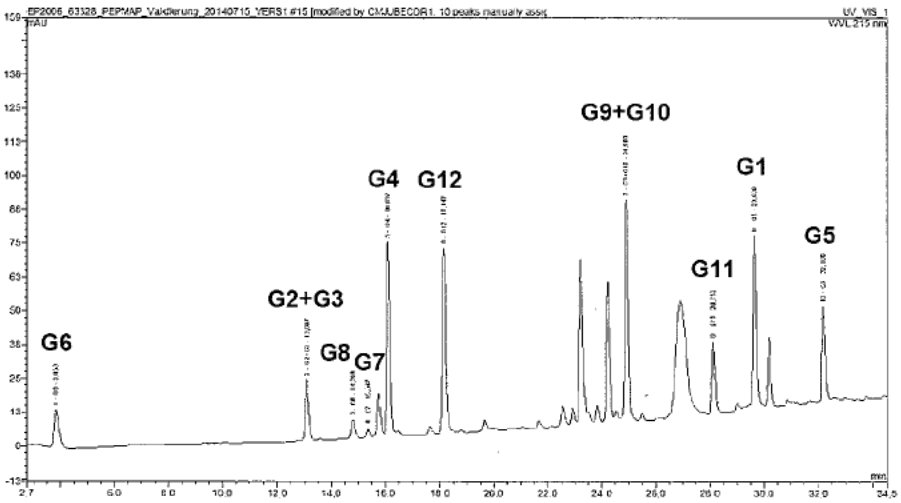
Review incorporated in the CMC review dated January 30, 2015

g. You proposed to introduce the relative retention time (0.8-0.9 min.) and relative peak heights (60-140%) of two EP2006 peptide peaks (G4, G12) as acceptance criterion for the peptide mapping method used as orthogonal identity test in the release specification of EP2006 DS. Your peptide map method has at least 12 well resolved peptide peaks. Additionally, based on the peptide map method data provided, it appears that your method is also quite reproducible. Revise your acceptance criterion for peptide mapping to include all major EP2006 peptide peaks to account for the complete sequence of the EP2006 protein.

Sandoz proposes that identity of EP2006 by peptide map be defined by visual detectability of 7 major peptide peaks G2+G3, G4, G12, G9+G10, G11, G1, and G5 (Figure 2-2). These peaks represent a 91% of the sequence of EP2006. Additionally, the criteria for relative retention and peak height of G4 and G12 will be maintained as system suitability test. All criteria will be defined in the method SOP and have to be fulfilled for the analysis result "corresponds to reference".

Reviewer's comment: *Sandoz proposal is acceptable because the current criteria of visual assessment of 7 major peaks are suitable for assessment of identity of EP2006 DS as the peptides cover 91% of the protein. Orthogonal methods such as potency and identity by RP-HPLC, IEF, and SEC further support evaluation of this QA. The proposed system suitability criteria are also acceptable.*

Figure 2-2 **Chromatogram of an EP2006.16REF Glu-C digest** Identified peptide peaks are annotated at the top of corresponding peaks.



3. You control the concentration of the excipients in the final EP2006 drug product (b) (4) [redacted] You should establish a more appropriate control strategy for the concentration of excipients in the final EP2006 DP. Establish a control strategy for the excipients of the final

EP2006 DP that includes measurements of the concentration of excipients as in-process controls and/or release testing.

Reviewer's comment: [redacted] (b) (4) *Osmolality was included in the EP2006 DP release specification in response to this IR.*

Sandoz proposes a specification [redacted] (b) (4) *to be implemented after method development. The implementation of an optimized control strategy* [redacted] (b) (4) *after approval is acceptable because there is currently a suitable control strategy for this excipient. The concentration* [redacted] (b) (4) *in EP2006 DP is being controlled by accurate* [redacted] (b) (4) (b) (4) (b) (4) (b) (4) *. The data provided in the submission from 26 EP2006 DP batches support* [redacted] (b) (4) *release and stability (≤0.1%). Thus the optimization of the control strategy as a PMC is acceptable.*

PMC: To enhance the control strategy [redacted] (b) (4) by establishing a release specification [redacted] (b) (4) in EP2006 drug product

4. The reference standards or materials section and the response to IR dated October 10, 2014 describe the procedures to declare the biological activity of EP2006 in-house primary and secondary reference materials. In the response to the above referred IR you state the following regarding the evaluation of the in vitro assay used to declare the potency of the EP2006 in-house reference materials:

   "The in-vitro assay is evaluated as follows: If the mean relative potency of the new EP2006 in house primary reference material is between [redacted] (b) (4) % of the used reference material, the new reference material will be assigned as having 100% potency, corresponding to 100% of the biological activity of the previous reference material (U/mg EP2006). If the mean relative potency of the new in-house primary reference material is outside this range, a correction factor may be introduced".

   The range of [redacted] (b) (4) proposed for declaration of 100% potency of your EP2006 in-house primary and secondary reference materials is too wide. Revise your proposed range to be more stringent [redacted] (b) (4). The variability of the biological activity data may be controlled by, for example, by increasing the number of replicates in the bioassay conducted to qualify the reference standard.

   Additionally, clarify whether the EP2006 in-house primary reference material will be calibrated against an international reference standard for GCSF and provide information about the procedures for declaration of potency of the EP2006 in house primary reference material.

Reviewer's comment: *For declaration of potency of the primary and secondary RSs, Sandoz proposes the following requirements:*

a. the determined potency value should be in the range of (b) (4) relative potency compared to the current in-house RS
b. the absolute 95% confidence interval of relative potency should include 100%
c. testing should be conducted on (b) (4) single determinations

The requirements are based on the following statistical considerations and mean relative potency of the last 4 RSs (Table 4-1):

CI= μ +- t(0,025;n-1)*SEM

Where SEM is standard error of the mean (i.e (b) (4)) and μ is ln(100%).

The means of the potencies are summarized in the table below.

Table 4-1 Mean relative potencies of the EP2006 in-house reference materials

In-house reference material	Mean relative potency against previous in-house reference [%] (b) (4)
E6WS12	(b) (4)
E6WS14	
EP2006.15REF	
EP2006.16REF	

The (b) (4)% is the precision expressed as relative standard deviation (RSD) between the means of 4 different RSs. This value is an estimator of the standard error of the means.

Reviewer's comment: *The proposed approach to declare potency of a RS is not appropriate. The reason is outlined in the following information request sent on February 3, 2015:*

Your justification for maintaining the (b) (4)% as criterion for assignment of equipotency of in house primary and secondary reference materials considering standard error of the last four reference materials is not appropriate because the variability is enhanced. You should establish acceptance criteria for assignment of equipotency from testing a single primary reference standard that has been calibrated using an international reference standard for GCSF. Revise your criterion for assignment of equipotency to be more stringent (b) (4) The variability of the biological activity data may be controlled, for example, by increasing the number of replicates in the bioassay conducted to qualify the reference standard

In the response, Sandoz agreed on tightening the AC for equipotency of a RS based on the intermediate precision of the method (b) (4). The criterion is now (b) (4) . Sandoz states that following:

"this limit (b) (4) will be valid for declaration of the in house primary and working reference materials of the newly established two tier reference material system. Additionally,

we can confirm that for the declaration of new two tier in house primary reference materials bioactivity will be:

- assigned using the previously valid in house primary reference material,
- and additionally calibrated against the international standard for G-CSF".

The criterion for establishing equipotency of RSs is now acceptable.

5. The method validation report for host cell proteins (P13/B03/04) entitled "Validation of the Sandwich ELISA to Determine the Concentration of Host Cell Proteins (HCP) in EP2006 test Items" states that "the reference item of this study was [redacted] (b) (4) [redacted] (b) (4) The IgG antibodies used were [redacted] (b) (4) [redacted] produced [redacted] (b) (4) with the described reference item". Provide information regarding the source of [redacted] (b) (4) [redacted] antibodies used in the HCP ELISA assay.

   Review incorporated in the CMC review dated January 30, 2015

6. Provide expansions of the ^{1}H-^{15}N HSQC spectra of EP2006, US-licensed Neupogen and EU-approved Neupogen (Figures 5-14 through 5-21, section 3.2.S.3.1). The expansions may be provided by quadrant (e.g. 4 quadrants per spectrum). In addition, please draw the cross-peaks in the overlaid spectra in different color and "transparent" so the cross-peaks of each product can be easily distinguished.

   Review incorporated in the CMC review dated January 30, 2015

In addition to the items listed above, the following IR was sent on February 3, 2015 regarding the acceptance criteria for purity by RP-HPLC:

Your proposed acceptance criteria for sum of impurities by RP-HPLC are [redacted] (b) (4) *for release and stability of EP2006 DP, respectively. Historical data of EP2006 DP provided in the submission show that sum of impurities of EP2006 DP are 0.9-2.4% at release and 3.4-5.3% at stability (36 months). These data include clinical EP2006 DP and process validation EP2006 DP batches. We are concerned that your current acceptance criterion for sum of impurities at release of* [redacted] (b) (4) *can led you to fail a stability specification for sum of impurities. Based on the stability data of EP2006 DP process validation batches, the sum of impurities can increase up to 2.7 % by the 24 month time point. This means that the sum of impurities of EP2006 DP lots released with a sum of impurities result of* [redacted] (b) (4) *will likely result in an out of specification. In addition, your analysis of US-licensed Neupogen by RP-HPLC indicates that the sum of impurities in the reference product is 3.5-5.9% for lots of different shelf life collected from the market.* [redacted] (b) (4) (b) (4)
Revise your acceptance criteria for sum of impurities determined by RP-HPLC taking into consideration your analysis of US-licensed Neupogen and your clinical and manufacturing experience with EP2006 DP

Reviewer's comment: *Sandoz justified the release AC* (b) (4) *total impurities by RP-HPLC for EP2006 DP based on the calculated degradation rate using stability data from 6 EP2006 DP validation batches. The rate of degradation was calculated as* (b) (4) *total impurities per year. Sandoz states that if a batch is released with* (b) (4) *total impurities, total impurities at the end of the shelf life of the batch will be* (b) (4) *which is within the proposed stability AC. However, Sandoz agreed on tightening the release AC to* (b) (4).

The stability AC was justified based on the estimation of total impurities at the end of shelf life of US-licensed Neupogen and EU-approved Neupogen (refer to the submission for raw data). According to Sandoz estimation, total impurities of US-licensed Neupogen at the estimated end of shelf life of 30 months is 7.9 % (95% prediction interval) or 6.4% if data of US-licensed Neupogen and EU-approved Neupogen data are used in the estimation. Sandoz states that the AC of (b) (4) *total impurities is conservative and is justified based on the US-licensed Neupogen data.*

Sandoz justification for the release and stability AC is acceptable and thus the AC for total impurities by RP-HPLC of EP2006 DP are (b) (4) *for release and stability, respectively.*

Recommendation: Approval

BLA 125553
Review 1
Review Date: February 6, 2015

Drug Name/Dosage Form	**Zarxio/injection**
Strength/Potency	**300 mcg/0.5 ml or 480 mcg/0.8 ml**
Route of Administration	**Subcutaneous or intravenous**
Rx/OTC Dispensed	**Rx**
Indications	**Zarxio is indicated to:** • **Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever** • **Reduce the time to neutrophil recovery and the duration of fever, following induction or consolidation chemotherapy treatment of patients with acute myeloid leukemia (AML)** • **Reduce the duration of neutropenia and neutropenia-related clinical sequelae, e.g., febrile neutropenia, in patients with nonmyeloid malignancies undergoing myeloablative chemotherapy followed by bone marrow transplantation** • **Mobilize autologous hematopoietic progenitor cells into the peripheral blood for collection by leukapheresis** • **Reduce the incidence and duration of sequelae of neutropenia (e.g., fever, infections, oropharyngeal ulcers) in symptomatic patients with congenital neutropenia, cyclic neutropenia, or idiopathic neutropenia**
Applicant/Sponsor	**Sandoz Inc.**

Submissions Reviewed

Submission	Date Received	Review Completed
Original Application	May 8, 2014	Y
Amendment 5	June 18, 2014	Y
Amendment 10	August 22, 2014	Y
Amendment 12	September 19, 2014	Y
Amendment 14	October 10, 2014	Y
Amendment 19	November 11, 2014	Y
Amendment 20	December 2, 2014	Y
Amendment 23	December 22, 2014	Y
Amendment 24	January 27, 2015	Y
Amendment 26	February 5, 2015	Y

Quality Review Team

DISCIPLINE	REVIEWER	BRANCH/DIVISION
Drug Substance	Maria-Teresa Gutierrez-Lugo	DBRR III
Drug Product	Maria-Teresa Gutierrez-Lugo	DBRR III
Analytical Similarity	Maria-Teresa Gutierrez-Lugo	DBRR III
Immunogenicity	Faruk Sheikh/Frederick Mills/Susan Kirshner	DBRR II/DBRRIV/DBRR III
Labeling	Jibril Abdus-Samad/ M-T Gutierrez-Lugo	OBP/DBRR III
Facility and Microbiology	Bo Chi/Steve Fong/ Patricia Hughes	DMA
Secondary Reviewer	Gibbes Johnson	DBRR IV
Tertiary Reviewer	Susan Kirshner	DBRR III

Multidisciplinary Review Team

DISCIPLINE	REVIEWER	OFFICE/DIVISION
RPM	Lara Akinsaya/Jessica Boehmer	OHOP/DHP
CDTL/Medical Officer	Albert Deisseroth	OHOP/DHP
Medical Officer	Donna Przerpiorka/A Deisseroth	OHOP/DHP
Pharm/Tox	Christopher Sheth/John Leighton	OHOP/DABT
Clinical Pharmacology	Sarah Schrieber/ Nam Atiqur Rahman	OCP/DCP V
Biostatistics	Kyung Lee/Lei Nie	OB/DBIV
CMC Statistics	Xiaoyu Dong/Yi Tsong	OB/DBVI

Signature Block

Name and Title	Signature and Date
Susan Kirshner, PhD. Review Chief, Division of Biotechnology Review and Research III	Susan L. Kirshner -S Digitally signed by Susan L. Kirshner -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300194629, cn=Susan L. Kirshner -S Date: 2015.02.10 15:37:55 -05'00'
Gibbes Johnson, PhD Acting Director, Division of Biotechnology Review and Research IV	Gibbes R. Johnson -S Digitally signed by Gibbes R. Johnson -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000592128, cn=Gibbes R Johnson -S Date: 2015.02.10 11:40:42 -05'00'

Quality Review Data Sheet

1. LEGAL BASIS FOR SUBMISSION: 351(k)

2. RELATED/SUPPORTING DOCUMENTS:

A. DMFs:

DMF #	TYPE	HOLDER	ITEM REFERENCED	CODE[1]	STATUS[2]	DATE REVIEW COMPLETED	COMMENTS
(b) (4)	III	(b) (4)		3	Adequate		Reviewed by CDRH
	III			2	Adequate		Memorandum of review for DMF (b) (4) completed by George Lunn, Ph.D., Review Chemist for material (b) (4) 3/10/2014
	III			1	Adequate		Reviewed by CDRH Review in DARRTS 8/30/2012. Adequate to support (b) (4)
(b) (4)	510(k)	(b) (4)		3	510 (k) reviewed by CDRH		Adequate

[1] Action codes for DMF Table: 1 – DMF Reviewed; Other codes indicate why the DMF was not reviewed, as follows: 2 – Reviewed previously and no revision since last review; 3 – Sufficient information in application; 4 – Authority to reference not granted; 5 – DMF not available; 6 – Other (explain under "Comments")

[2] Adequate, Adequate with Information Request, Deficient, or N/A (There is enough data in the application, therefore the DMF did not need to be reviewed)

B. Other Documents: none

3. CONSULTS:

DISCIPLINE/TOPIC	DATE REQUESTED	STATUS	RECOMMENDATION	REVIEWER
CDRH/ODE – Drug Product syringe and needle safety device	May 22, 2014	Completed	Adequate	Nicholas W. Werner/Ryan J. Mcgowan
CDRH/OC - Drug Product syringe	June 27, 2014	Under review		Rakhi Dalal/Francisco Vicenty
CDER/OSE/OMEPRM/DMEPA - Human factors	May 22, 2014	Completed	Adequate	Neil H Vora/Yelena L Maslov
CDER/OTS/OB/DBVI -Statistical Equivalence of bioactivity and protein content	August 27, 2014	Completed	Adequate	Xiaoyu Dong/Yi Tsong

Executive Summary

I. Recommendations

A. Recommendation and Conclusion on Approvability

a. Recommendation

The Office of Biotechnology Products, OPQ, CDER, recommends approval of BLA 125553 for Zarxio manufactured by Sandoz Inc., as a biosimilar to US-licensed Neupogen pending acceptable compliance checks. The analytical data submitted in this application are adequate to support the conclusion that Zarxio is highly similar to US-licensed Neupogen, and that the manufacture of Zarxio is well controlled and leads to a product that is pure and potent. It is recommended that Zarxio be approved for human use under conditions specified in the package insert.

b. Approval action letter language

- Manufacturing location:
  - Drug substance – Sandoz GmbH, Biochemiestrasse 10 A-6250 Kundl, Austria
  - Drug product –GP Grenzach Produktions GmbH, Emil-Barell-Strasse 7D-79639 Grenzach-Wyhlen, Germany
- Fill sized and dosage form – 300 mcg/0.5 mL and 480 mcg/0.8 mL in a pre-filled syringe, injection
- Dating period:
  - Drug product – 24 months at 5 ± 3ºC
  - Drug substance – (b) (4)
  - Stability option:
    We have approved the stability protocol in your license application for the purpose of extending the expiration dating period of your drug product under 21 CFR 601.12.
- Exempt from lot release
  - Yes
  - Rationale if exempted – Zarxio is a specified product as per 601.2a

c. Benefit/Risk Considerations

Zarxio is a biosimilar to US-licensed Neupogen and Sandoz requested all the indications for which the reference product (US-licensed Neupogen) is currently licensed. The indications are listed on page 1 of this review. These indications fall in the general categories of neutropenia and mobilization of hematopoietic stem cells.

Approval of Zarxio as a biosimilar is supported by analytical similarity studies. A total of 20 lots of Zarxio DP, 6 lots of EP2006 drug substance (DS), and 10-15 lots of US-licensed Neupogen were evaluated using the methods listed in Table A below. The results from these studies demonstrated that Zarxio is highly similar to US-licensed Neupogen notwithstanding minor differences in clinically inactive components. The analytical similarity studies did not raise residual uncertainties about the demonstration of highly similar between Zarxio and US-licensed

Neupogen. Therefore, based on the similarity data reviewed and from an analytical perspective, the benefit/risk profile of Zarxio is acceptable because Zarxio has been demonstrated to be highly similar to US-licensed Neupogen. From an analytical perspective, Zarxio would be expected to have a similar benefit/risk profile as US-licensed Neupogen.

Table A. Methods used to assess analytical similarity

Quality Attribute	Criticality[1]	Methods	Tier assignment[2]
Primary structure	Very High	• N-terminal sequencing • Peptide mapping with UV and MS detection • Protein molecular mass by ESI MS • Protein molecular mass MALDI-TOF MS • DNA sequencing of construct cassette • Peptide mapping coupled with MS/MS	2/3
Bioactivity	Very high	• Proliferation of murine myelogenous leukemia cells (NFS-60 cell line)	1
Receptor binding	Very high	• Surface Plasmon Resonance	2/3
Protein content	Very high	• RP-HPLC	1
Clarity	Very high	• Nephelometry	2
Sub Visible Particulates	High	• Micro flow imaging	3
Higher Order Structure	High	• Far and Near UV circular dichroism • ^{1}H nuclear magnetic resonance • ^{1}H-^{15}N heteronuclear single quantum coherence spectroscopy • LC-MS (disulfide bond)	2/3
High molecular weight variants/aggregates	High	• Size exclusion chromatography (validated using MALLS) • Reduced and non-reduced SDS-PAGE • 90º light scattering • Nile red steady-state fluorescence anisotropy	3
Oxidized variants	High	• RP-HPLC • LC/MS	2
Covalent dimers	High	• LC/MS	3

[1] Very high: criticality score of ≥140
High: criticality score of 90-104
Low: criticality score of 45-90
Very low: criticality score of ≤28

[2] Tier assignment for the purposes of analyzing the analytical data by statistical methods. The analytical data from quality attributes assigned to Tier-1 were analyzed by statistical equivalence testing. Attributes assigned to Tier-2 and Tier-3 were analyzed by comparison of individual data points to similarity acceptance criteria defined as mean ± 2 or 3 SD and by qualitative assessment of the data, respectively

Quality Attribute	Criticality	Methods	Tier assignment
Partially reduced variants	High	• LC/MS	3
Sequence variants: His→Gln Asp→Glu Thr→Asp	High	• RP-HPLC • LC/MS	3
Formyl-Met1	High	• RP-HPLC • LC/MS	3
Succinimide	High	• RP-HPLC • LC/MS	3
Phosphoglucunoylation and (b) (4)	High	• LC/MS	3
Acetylated variant	High	• LC/MS	3
N-terminal truncated variants	Low	• LC-MS/MS	2
Norleucine	Very low	• RP-HPLC • LC/MS	3
Deamidation	Very low	• RP-HPLC • LC/MS • IEF • CEX	3

The non-clinical and clinical program for Zarxio, including five PK/PD studies used a non US-licensed product (i.e. EU-approved Neupogen) as active comparator. Pair-wise analytical comparisons of Zarxio (up to 20 lots), US-licensed Neupogen (up to 10-15 lots) and EU-approved Neupogen (34-52 lots) supported a scientific bridge based on the relatively simple structure of the protein, lack of post-translation modifications, and the robustness of the pair-wise analytical characterization.

The results of the non-clinical studies did not identify discipline-specific uncertainties and supported similarity of the two products. The results from the clinical studies, including PK/PD support a demonstration of no clinically meaningful differences between Zarxio and US-licensed Neupogen. The totality of evidence support approval of Zarxio as a biosimilar to US-licensed Neupogen.

The manufacturing processes and control strategy of EP2006 DS and Zarxio drug product (DP) are well controlled and appropriate. A PMC to improve the control strategy of Zarxio drug product by development, validation, and implementation of an analytical method to assess (b) (4) concentration for release or in-process testing of Zarxio drug product has been agreed upon. Currently, the concentration of (b) (4) in Zarxio DP is controlled by (b) (4) (b) (4) The data provided in the submission support (b) (4) (b) (4) release and stability are below limit of quantitation (LOQ; ≤0.1%) of the analytical method.

Zarxio is administered subcutaneously and by intravenous infusion. For intravenous infusion, Zarxio is diluted to a concentration ranging between 5 mcg/ml to 15 mcg/ml in 5% glucose in presence of 2 mg/ml of human serum albumin (HSA). The in-use stability data provided in the submission and the analytical similarity of Zarxio to US-licensed Neupogen support the stability of Zarxio in 5% glucose. There is a PMC to confirm the stability of Zarxio drug product in 5% glucose at concentrations ranging from 5 mcg/ml to 15 mcg/ml Zarxio in the presence of 2 mg/ml HSA in glass bottles, PVC and polyolefin IV bags and polypropylene syringes. Testing will include potency and sub-visible particles.

B. Recommendation on Phase 4 (Post-Marketing) Commitments, Requirements, Agreements, and/or Risk Management Steps, if Approvable

1. To enhance the control strategy (b) (4) by development, validation and implementation of an analytical method to assess (b) (4) Zarxio drug product.

2. To confirm the stability of Zarxio drug product in 5% glucose at concentrations ranging from of 5 mcg/ml to 15 mcg/ml Zarxio in presence of 2 mg/ml human serum albumin in glass bottles, PVC and polyolefin IV bags and polypropylene syringes. Testing will include potency and sub-visible particles.

The data from these PMCs should be reported according to 21CFR601.12

II. Summary of Quality Assessments

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 1 below provides a summary of critical quality attributes identification and risk management. For the purposes of this table, critical quality attributes are limited to attributes intrinsic to the active pharmaceutical ingredient.

B. Drug Substance Quality Summary

Table 2 provides a summary of the identification, risk, and lifecycle knowledge management for drug substance CQAs that derive from the drug substance manufacturing process and general drug substance attributes, including process-related impurities.

Table 1: Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

CQA	Type	Risk	Introduction	Control Strategy[3]
Potency	Potency	Directly linked to efficacy (Very high criticality)	Intrinsic to the molecule	(b) (4) [redacted] RELEASE & STAB: Determination of potency and product-related impurities potentially influencing potency (by RP-HPLC and SEC)
Receptor binding	Potency	Directly linked to efficacy (Very high criticality)	Intrinsic to the molecule	(b) (4) [redacted]
Higher order structure	Potency	Directly linked to efficacy and MOA (high criticality)	Intrinsic to the molecule	(b) (4) [redacted]
Oxidized species	Product-related impurities	Efficacy Oxidized impurities exhibit low biological activity (high criticality)	(b) (4) [redacted]	(b) (4) [redacted]

[3] DC: Design Control, IPC: in-process control, STAB: Stability, PC: Process Control, PP: process parameter, RMC: raw materials control, CPP: critical process parameter

				(b) (4) RELEASE & STAB: Controlled by means of main peak and total impurities by RP-HPLC
Non-covalent HMW species	Product-related impurities	Efficacy and safety because HMW species can impact receptor binding and clearance (high criticality)	(b) (4)	(b) (4) RELEASE & STAB: Evaluated by SE-HPLC main-peak purity and levels of HMW impurities
Reduced species (partially and totally)	Product-related impurities	Efficacy. Unfolded EP2006 exhibits very low biological activity (high criticality)	(b) (4)	(b) (4) RELEASE and STAB: Evaluated by RP-HPLC by means of purity and sum of impurities

Covalent dimers	Potency and safety	Efficacy. Covalent dimers exhibit very low biological activity (high criticality) Covalent dimers have been present in up 0.3% in clinical studies without anti-therapeutic antibody formation	(b) (4)	(b) (4) RELEASE and STAB: by SEC by means of purity and sum of impurities
His-Gln Sequence variant	Product-related impurity	Immunogenicity due to altered sequence. (high criticality. CQA not detectable in EP2006 DS, worst-case assumption)		(b) (4) RELEASE: Controlled by RP-HPLC by means of main peak purity and sum of impurities. Quantitative depletion supported by MS on the level of DS.
fMet1 EP2006 species (N-formyl methionine)	Product-related impurity	Immunogenicity due to altered sequence. (high criticality. CQA not detectable in EP2006 DS, worst-case assumption)		(b) (4) RELEASE: Controlled by RP-HPLC by means of main peak purity and sum of impurities. Quantitative depletion supported by MS on the level of DS

(b) (4)

				(b) (4)
Phospho-glucunoylated species	Product-related impurity	Theoretical impact on efficacy (high criticality because QA not detectable in (EP2006 DS, worst case assumption)	(b) (4)	(b) (4) RELEASE: Controlled by RP-HPLC and SEC by means of main peak purity and sum of impurities (RP-HPLC).
Thr-Asp sequence variant	Product-related impurity	Immunogenicity efficacy (high criticality because QA not detectable in EP2006 DS, worst-case assumption)		(b) (4) (b) (4) RELEASE: Controlled by RP-HPLC by means of main peak purity and sum of impurities. Quantitative depletion supported by MS on the level of DS.
Acetylated species	Product-related impurity	Acetylation at free NH_2-groups can potentially inhibit target binding and reduce potency (high criticality because QA not detectable in EP2006 DS, worst case assumption)		(b) (4) RELEASE: Controlled by RP-HPLC by means of main peak purity and sum of impurities and by IEF. Quantitative depletion supported by MS on the level of DS.
Truncated species	Product-related	Efficacy and immunogenicity		(b) (4)

	impurity	(low criticality)		(b) (4)
Norleucine species	Product-related substances	Immunogenicity (very low criticality because no loss of bioactivity in samples containing up to 44% NLeu species. Low levels of NLeu species have been present in clinical studies.	(b) (4)	(b) (4) RELEASE: Controlled by RP-HPLC by means of main peak purity and sum of impurities.
Deamidated species	Product-related substances	Low risk (very low criticality. Deamidated EP2006 has no impact on bioactivity. Low levels of deamidation have been present in clinical studies)		(b) (4) RELEASE and STAB: Controlled by RP-HPLC by means of main peak purity and sum of impurities and also by IEF
Truncated species	Product-related substances	Low risk (low criticality)		(b) (4)

Table 2: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management

CQA	Source	Risk	Introduction	Control Strategy[4]
Host cell DNA	Host Cell (*E. coli*). Process-related impurity	Immunogenicity and safety	(b) (4)	(b) (4) RELEASE: Residual DNA is part of EP2006 DS release testing
Host cell proteins	Host Cell (*E. coli*). Process-related impurity	Immunogenicity and safety		(b) (4) RELEASE: residual HCPs are part of EP2006 DS release testing
(b) (4)				

[4] DC: Design Control, IPC: in-process control, STAB: Stability, PC: Process Control, PP: process parameter, RMC: raw materials control, PPQ: process performance qualification

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(b) (4)

Bacterial endotoxins	(b) (4)	Safety	(b) (4)	(b) (4) (b) (4) at release of DS
Leachables (b) (4)	Chemical	Safety	(b) (4)	PPQ: An extractables and leachables assessment is part of the process performance qualification. Supported by risk assessment.
Bioburden	(b) (4)	Safety	(b) (4)	(b) (4) RELEASE: Determination of bioburden is part of the release testing for EP2006 DS

(b) (4)

B. Drug Substance Quality Summary (cont.)

a. Names:

- Proprietary Name: Zarxio
- Trade Name: Zarxio
- Non-Proprietary/USAN: TBD
- CAS name: r-meth HuGCSF; recombinant methionyl human GCSF (CAS Registry number: 121181-53-1)
- Common name: EP2006
- INN Name: TBD
- Compendial Name: TBD
- OBP systematic name: RPROT P09919 (CSF3_HUMAN) Granulocyte colony-stimulating factor [EP2006]

b. Pharmacology category: Leucocyte growth factor

c. Description

EP2006 is a non-glycosylated recombinant methionyl human granulocyte colony stimulating factor (Met-GCSF) produced in *E. coli*. The primary sequence of EP2006 is identical to natural GCSF, except for an additional methionine residue at the N-terminus of EP2006. EP2006 has 175 amino acids and a molecular weight of 18798.9 g/mmol (native). EP2006 has a mostly alpha helical protein structure and two disulfide bridges between Cys37-Cys43 and between Cys65-Cys75. EP2006 is a proposed biosimilar to US-licensed Neupogen.

d. Mechanism of action

The biological activity of GCSF is initiated by binding of GCSF to the GCSF receptor on myeloid progenitor cells and mature neutrophils. This binding initiates transduction signals that lead to the proliferation and differentiation of neutrophil committed progenitor cells, increase of mature neutrophils in the blood (which is an relevant pharmacodynamic (PD) marker for neutropenia), and enhanced neutrophil function[1]. These functions are all relevant to the neutropenia indications.

The mechanism of action related to the mobilization of hematopoietic stem cells indication is not fully understood. However, reports in the literature strongly suggest that the GCSF receptor plays critical role in the mobilization of hematopoietic stem cells (HSC)[2]. In one model of GCSF-induced mobilization reported in the literature, mobilization of HSC is initiated by binding of GCSF to the GCSF receptor on monocytic cells in the bone marrow. This leads to changes in cells of the osteoblast lineage which results in the disruption of key interactions that regulate HSC. The absence of key interactions between cells of the osteoblast lineage and HSC result in mobilization of

[1] Panopoulus, A.D. and Watowich, S.S. (2008). Cytokine 42: 277-288

[2] Greenbaum AM and Link DC (2011). Leukemia, 25: 211-217

HSC into the blood stream. Hematopoietic stem cells are identified by the presence of the cluster differentiation protein 34 (CD 34) marker on their surface (which is a relevant PD marker for stem cell mobilization)[3].

e. Potency Assay

The biological activity of EP2006 is determined by measuring its ability to stimulate proliferation of NFS-60 cells compared. The NSF-60 cell line, which carries the GCSF receptor, was derived from mouse myelogenous leukemia cells. The assay is performed in 96-well plates. The number of viable cells is measured by quantification of intracellular ATP using a luciferase chemiluminescence system. The measured luminescence is proportional to the amount of ATP which is in turn proportional to the number of viable cells. The activity of EP2006 is reported relative to a calibrated in-house EP2006 RS using parallel line analysis.

f. Reference material(s)

Sandoz is currently implementing a two-tier reference standard system. A protocol for qualification and stability testing of in-house primary and working RSs was reviewed and found acceptable. The qualification of RSs includes calibration of potency using an international RS for GCSF. The current RS EP2006.16REF is acceptable because it was properly qualified and calibrated against WHO international standard for GCSF NIBSC 09/136.

g. Critical starting materials or intermediates

Master cell bank EP2006: (b) (4) (b) (4)

h. Manufacturing process summary

The EP2006 DS manufacturing consist of three processes: fermentation, isolation and purification. (b) (4) (b) (4) (b) (4) The EP2006 DS manufacturing process is well controlled.

i. Container closure

The container closure system for EP2006 DS consists of a (b) (4)

[3] Aiuti A, et al. (1997). J Exp Med, 185: 111–12

(b) (4). Compatibility of the container closure system by means of stability of EP2006 DS was demonstrated. Leachables from the container closure system were below reporting thresholds.

j. Dating period and storage conditions: (b) (4)

C. Drug Product Zarxio Quality Summary

Table 3 provides a summary of the identification, risk, and lifecycle knowledge management for drug product CQAs that derive from the drug product manufacturing process and general drug product attributes.

Table 3: Drug Product CQA Identification, Risk, and Lifecycle Knowledge Management

CQA (Type)	Risk	Introduction	Control Strategy[5]	Other
Protein concentration	Very high criticality Variable protein concentration causes variable dosage of the drug and may affect efficacy	(b) (4)	(b) (4) RELEASE & STAB: Protein concentration is part of DP release and shelf-life specification.	
Potency/target binding	Very high criticality, linked to efficacy	(b) (4)	(b) (4) RELEASE & STAB: Potency is part of the release and stability specifications of DP	

[5] DC: Design Control, IPC: in-process control, STAB: Stability, PC: Process Control, PP: process parameter, RMC: raw materials control, CPP: critical process parameter

Container closure integrity (CCI)	Very high criticality Failure in closure integrity may lead to contamination (absence of sterility) of DP or evaporation/leakage (impacting concentration or content).	Container design and control	(b) (4) RELEASE & STAB: Sterility (release) and CCI are part of release and shelf-life specifications. Gross CCI failure will be also detected by other methods such as protein concentration	
Clarity	Very high criticality. Clarity is impacted by the number of particles in a solution, which might be immunogenic, embolic, or cause a foreign body response	General	(b) (4) RELEASE & STAB: Clarity is part of DP release and shelf-life specification	
Color	Criticality: Very High. Variance in color is indicative for contamination or degradation	General	RELEASE / STAB: Color is part of DP release and end of shelf-life specification	
Sterility	Criticality: Very High. The use of a non-sterile injectable product exposes patients to the risk of microbial transmission	General	(b) (4)	

			(b) (4) [redacted] RELEASE & STAB: Sterility of DP is part of DP release and shelf-life specifications	
Endotoxins	Endotoxins can cause immunologic reactions	(b) (4) [redacted]	(b) (4) [redacted] RELEASE: Testing for endotoxin is part of drug product release and shelf-life specification	
Heavy metals	Criticality: Very High. Some inorganic impurities are known to impact protein folding and are toxic at low levels (safety). (b) (4) [redacted]	(b) (4) [redacted]		
Bioburden	Criticality: Very High. Bioburden might lead to endotoxin generation and/or overload	(b) (4) [redacted]	(b) (4) [redacted] [redacted]	

	(b) (4) with impact on endotoxin level in drug product and sterility and thus patient safety (potential microbial contamination after administration).		(b) (4)	
Particulate matter (SVP)	Criticality: High. SVP might be immunogenic	(b) (4)	(b) (4) RELEASE & STAB: Testing on SVP (particulate matter) is part of DP release and shelf-life specification	
Oxidized species	Criticality: High. (b) (4)	(b) (4)	(b) (4)	

			(b) (4) RELEASE & STAB: Oxidized species are detectable and measured by RP-HPLC. RP-HPLC is part of the DP release and shelf-life specification	
Foreign matter (visible particles)	Criticality: High. Particles might be immunogenic or precipitate a foreign body response	(b) (4)	(b) (4) RELEASE & STAB: Test for foreign matter (visible particles) is part of DP release and shelf-life specification	
pH	Criticality: High. Stability of DP is strongly dependent on the pH-range of DP.	General	(b) (4) RELEASE & STAB: pH is part of the DP release and shelf-life specification	
Sorbitol	Criticality: High. Sorbitol is a tonicity agent to obtain isotonic solutio (b) (4)	General	(b) (4) RELEASE: osmolality testing was added to the release specifications of DP	

Glutamic acid	Criticality: High. Glutamic acid serves as buffer (b) (4)	General	(b) (4) RELEASE & STAB: pH testing is part of the release and stability specifications	
Polysorbate 80	Criticality: High. Polysorbate 80 acts as surfactant (b) (4) (b) (4)	General	(b) (4)	(b) (4)
Higher order structure (HOS)	Altered secondary and tertiary structures can influence potency and may result in immunogenicity	Intrinsic to the molecule	(b) (4) (b) (4)	
HMW species and dimers	Criticality: High. HMWV and aggregates can contribute to immunogenic reactions	(b) (4)	(b) (4)	

			(b) (4) [redacted] RELEASE & STAB: HMW and aggregates testing are part of the release and stability specifications	
Viscosity	Criticality: Moderate. (b) (4) [redacted]	General	(b) (4) [redacted]	
[redacted]				
Osmolality	Criticality: Moderate. Variable osmolality may lead to altered composition of DP. Hypotonicity or hypertonicity can result in injection site or other safety issues.	General	(b) (4) [redacted] RELEASE: Osmolality was added as part of the DP specifications	
Extractable volume	Criticality: Moderate. Extractable volume	General	(b) (4) [redacted]	

	is essential for correct dosing		RELEASE & STAB: Extractable volume is part of release and shelf life specification for DP.	
Gliding force	Criticality: Moderate. Might have an impact on ease of administration	Container closure system	(b) (4) [redacted]	
Deamidated species	Criticality low. These are product-related substances	(b) (4) [redacted]	(b) (4) [redacted] RELEASE & STAB: Deamidated species by means of total impurities by RP-HPLC is part of release and shelf life specification for DP	
[redacted]			(b) (4)	
Leachables from process materials in DP manufacture and primary package	Low criticality	(b) (4) [redacted]	(b) (4) [redacted] Extractables/leachables studies performed on container closure systems for EP2006 DP solution show that EP2006 DP solution does not contain significant amounts of leachables after 12 months of storage at intended conditions.	

D. Drug Product Zarxio Quality Summary (Cont.)

a. Description and Strength:

- Zarxio (injection) is supplied as a single dose, pre-filled syringe
- Strengths: 300 mcg/0.5 ml and 480 mcg/0.8 ml

b. Summary of Product Design

The PFS has a needle safety device (NSD, Ultrasafe Passive Needle Guard (b) (4)). The NSD was cleared by CDRH under 510K ((b) (4)).

Zarxio was developed as a biosimilar to US-licensed Neupogen and thus to target the quality profile of the reference product.

c. List of Excipients

Glutamic acid (10 mM), Polysorbate 80 (0.004%), Sorbitol (b) (4) pH 4.4

d. Reference material(s)

The same reference material is used for DS and DP.

e. Manufacturing process summary

(b) (4)

f. Container closure

The container closure system consist of a 1 (b) (4), Type I glass syringe barrel with a hypodermic needle (27 Gauge ½ in). (b) (4). The needle shield is comprised by a rubber needle shield (b) (4) and a rigid needle shield (b) (4). The

syringe is closed with a rubber stopper [redacted] (b) (4) and plunger rod [redacted] (b) (4)). The PFS has a needle safety device (NSD, Ultrasafe Passive Needle Guard [redacted] (b) (4)) which was cleared by CDRH under 510K, submission [redacted] (b) (4)

g. Dating period and storage conditions: 24 months at 5 ± 3ºC
h. List of co-packaged components: not applicable

E. Novel Approaches/Precedents

- First biosimilar licensing application
- Application of statistics for evaluation of analytical similarity data

F. Any Special Product Quality Labeling Recommendations

- Protect from light
- Do not freeze
- If required, Zarxio may be diluted in 5% Dextrose Injection, USP. Zarxio diluted to concentrations ranging from 5 mcg/mL to 15 mcg/mL should be protected from adsorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2 mg/mL. When diluted in 5% Dextrose Injection, USP or 5% Dextrose plus Albumin (Human), Zarxio is compatible with glass bottles, polyvinyl chloride (PVC) and polyolefin intravenous bags, and polypropylene syringes.
- Dilution of Zarxio to a final concentration of less than 5 mcg/mL is not recommended at any time. Do not dilute with saline at any time; product may precipitate.

G. Establishment Information

OVERALL RECOMMENDATION:				
DRUG SUBSTANCE				
FUNCTION	SITE INFORMATION	DUNS/FEI NUMBER	INSPECTIONAL OBSERVATIONS	FINAL RECOMMENDATION
Drug substance manufacture	Sandoz GmbH Biochemiestrasse 10 A-6250 Kundl, Austria	FEI: 3002806523 DUNS: 300220969	**Observation 1: Production System** There are discrepancies between the current drug substance manufacturing process at Sandoz and the manufacturing process (b) (4) **Observation 2: Materials System** There is a lack of assurance of the quality of the (b) (4) a critical raw material used for (b) (4) drug substance manufacturing. Specifically, **Observation 3: Laboratory Control System** (b) (4) in-process samples and drug substance samples are not handled adequately. Specifically, as stated in SOP (b) (4), "Determination of the (b) (4) in-process samples and drug substance samples in BP", (b) (4) samples are allowed to be stored refrigerated and tested up to (b) (4) after sampling without supporting data for the sample handling process. **Observation 4: Facilities and Equipment System** The isolation and purification equipment (b) (4) clean hold time validation study was inadequate. Specifically, the equipment clean hold time validation (b) (4) was	Acceptable

			not sufficiently robust as it relied upon a single run (PQ46.764D). The equipment is used to manufacture (b) (4) drug substance (b) (4). (refer to 483-form for additional information on these observations)	
Stability testing	(b) (4)			Acceptable
E. coli host cell proteins, Residual DNA	(b) (4)			Under review
Potency assay	(b) (4)			Under review
Potency assay				Under review
DRUG PRODUCT				
FUNCTION	**SITE INFORMATION**	**DUNS/FEI NUMBER**	**INSPECTIONAL OBSERVATIONS**	**FINAL RECOMMENDATION**
Drug product manufacturing	GP Grenzach Produktions GmbH Emil-Barell-Strasse 7 D-79639 Grenzach-	FEI: 3006308309 DUNS:	**Observation1:** Procedures designed to prevent microbial contamination of drug product purporting to be sterile are not established and written. Specifically, during a tour of (b) (4) compounding operations in Building (b) (4) Room	Under review

	Wyhlen Germany	332090930	(b) (4) on 11/18/2014 […] **Observation 2:** The accuracy of test methods has not been established. Specifically, the gel clot endotoxin assay method used for IPC testing of the bulk drug product and drug product release testing has not been validated to be accurate in the presence of formulation components (polysorbate and glutamic acid) implicated in low endotoxin recovery. **Observation 3**: Control procedures are not established which monitor the output of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product. Specifically, there is no (b) (4) alert limit established for the (b) (4) bulk filgrastim drug product to initiate an investigation. The investigation into (b) (4) deviations could be relevant to the evaluation of compounding steps that pose a risk to microbiology quality. In addition, there is inadequate justification for the (b) (4) bulk drug product action limit of (b) (4) CFU/ (b) (4) mL. All batches manufactured in 2014 (b) (4) exhibited (b) (4) values of < (b) (4) CFU/ (b) (4) mL. **Observation 4:** Written records of investigations into unexplained discrepancies do not always include the conclusions and follow-up. Specifically, filgrastim lot numbers G700101, G700102 and G600001 were filled in (b) (4) 2014, 100% visually inspected in June/July 2014 with all defects removed, and released and shipped to (b) (4) After receipt, a 100% visual inspection was conducted by the (b) (4) customer that led to complaint #2014-029041 stating that 1.4% to 5% of the lots had deformed stopper lamellae. Currently, the root cause and product impact of the stopper defect is unclear, and corrective and preventative actions are incomplete. The same stoppers, (b) (4) stoppers from (b) (4), are proposed as a	

			component of the (b) (4) container closure system for BLA 125553/0. **Observation 5:** Time limits are not established when appropriate for the completion of each production phase to assure the quality of the drug product. Specifically, it was observed that clean hold times have not been established for product contact components within the (b) (4) filling line used for (b) (4) drug product fill **Observation 6:** Aseptic processing areas are deficient regarding the system for monitoring environmental conditions. For example, environmental monitoring procedures are not designed to give the most meaningful information regarding personnel practices and microbial quality. Specifically, on 11/21/2014 it was observed that an operator demonstrating the procedure for personnel monitoring only directly impressed her fingers on (b) (4) sampling plate rather than rolling her fingers to maximize sampling area. **Observation 7:** Procedures to prevent microbiological contamination of drug products purporting to be sterile do not include adequate validation of the sterilization process. Specifically, minimum loads are not assessed during (b) (4) requalification of the (b) (4) equipment sterilization (b) (4) (refer to 483-form for additional information on these observations)	
Stability testing optional release testing for IEF	(b) (4)			Acceptable
Potency assay				Under review

		(b) (4)		
Potency assay	(b) (4)			Under review

H. Lifecycle Knowledge Management

a. Drug Substance

i. Protocols approved

- Annual GMP stability protocol
- Protocol for concurrent validation of (b) (4) usage at scale
- Protocol for the qualification of new reference materials
- Protocol for the qualification of new master and working cell banks

ii. Outstanding review issues/residual risk: None

iii. Future inspection points to consider – None

b. Drug Product

i. Protocols approved:

- Annual GMP stability protocol
- Protocol for extension of DP shelf life

ii. Outstanding review issues/residual risk:

- Improve control strategy (b) (4)
- Confirm in use stability of DP in 5% glucose

iii. Future inspection points to consider: None

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

#	Checklist	Yes	No	N/A
Product Type				
1.	Recombinant Product	X		
2.	Naturally Derived Product		X	
3.	Botanical		X	
4.	Human Cell Substrate/Source Material		X	
5.	Non-Human Primate Cell Substrate/Source Material		X	
6.	Non- Primate Mammalian Cell Substrate/Source Material		X	
7.	Non-Mammalian Cell Substrate/Source Material	X		
8.	Transgenic Animal Sourced		X	
9.	Transgenic Plant Sourced		X	
10.	New Molecular Entity		X	
11.	PEPFAR Drug		X	
12.	PET Drug		X	
13.	Sterile Drug Product	X		
14.	Other_____		X	
Regulatory Considerations				
15.	Citizen Petition and/or Controlled Correspondence Linked to the Application (#_____)			
16.	Comparability Protocol(s)		X	
17.	End of Phase II/Pre-NDA Agreements tem)		X	
18.	SPOTS (Special Products On-line Tracking System		X	
19.	USAN Name Assigned	X		
20.	Other_____		X	
Quality Considerations				

21.	Drug Substance Overage			X	
22.	Design Space	Formulation		X	
23.		Process		X	
24.		Analytical Methods		X	
25.		Other		X	
26.	Other QbD Elements		X		
27.	Real Time Release Testing (RTRT)			X	
28.	Parametric Release in lieu of Sterility Testing			X	
29.	Alternative Microbiological Test Methods			X	
30.	Process Analytical Technology in Commercial Production			X	
31.	Non-compendial Analytical Procedures	Drug Product	X		
32.		Excipients		X	
33.		Drug Substance	X		
34.	Excipients	Human or Animal Origin		X	
35.		Novel		X	
36.	Nanomaterials			X	
37.	Genotoxic Impurities or Structural Alerts			X	
38.	Continuous Manufacturing			X	
39.	Use of Models for Release			X	
40.	Other _____			X	

Appears This Way On Original

BLA STN 125553

Product USAN name: TBD

EP2006

Sandoz

Maria-Teresa Gutierrez-Lugo, PhD
Gibbes Johnson, PhD
Susan Kirshner, PhD

OBP CMC Review Data Sheet

1. **BLA#:** STN 125553

2. **REVIEW DATE: January 30, 2015**

3. **PRIMARY REVIEW TEAM:**

Medical Officer:	Albert Deisseroth
Clinical Reviewer:	Donna Przerpiorka
Pharm/Tox:	Christopher Sheth
Product Quality Team:	Maria-Teresa Gutierrez-Lugo/Gibbes Johnson
Immunogenicity:	Faruk Sheikh/Frederick Mills/Susan Kirshner
BMT or Facilities:	Bo Chi/Steve Fong/Patricia Hughes
Clinical Pharmacology:	Sarah Schrieber
Statistics:	Kyung Lee/Lei Nie
	Xiaoyu Dong/Meiyu Shen
OBP Labeling:	Jibril Abdus-Samad
RPM:	Lara Akinsaya /Jessica Boehmer

4. **MAJOR GRMP DEADLINES**

Filing Meeting:	June 26, 2014
Mid-Cycle Meeting:	October 8, 2014 (DTP October 2, 2014)
Advisory Committee:	January 7, 2015
Wrap-Up Meeting:	February 23, 2015
Primary Review Due:	January 30, 2015
Secondary Review Due:	February 6, 2015
CDTL Memo Due:	February 15, 2015
PDUFA Action Date:	March 6, 2015

5. **COMMUNICATIONS WITH SPONSOR AND OND:**

Communication/Document	Date
Applicant Orientation Meeting	June 11, 2014
Information Request 1	June 9, 2014
Information Request 2	June 24, 2014
Information Request 3 (74-day letter)	July 22, 2014
Information Request 4	October 2, 2014
Information Request 5	October 31, 2014
Information Request 6	November 18, 2014
Information Request 7	December 27, 2014
Information Request 8	January 21, 2015
Information Request 9	January 27, 2015

See Appendix I

6. SUBMISSION(S) REVIEWED

Submission	Date Received	Review Completed (Yes/No)
Original Application	May 8, 2014	Y
Amendment 5	June 18, 2014	Y
Amendment 10	August 22, 2014	Y
Amendment 12	September 19, 2014	Y
Amendment 14	October 10, 2014	Y
Amendment 19	November 11, 2014	Y
Amendment 20	December 2, 2014	Y
Amendment 23	January 22, 2014	Y

7. DRUG PRODUCT NAME/CODE/TYPE:

a. Proprietary Name: Zarxio
b. Trade Name:
c. Non-Proprietary/USAN:
d. CAS name: R-methHuGCSF; Recombinant methionyl human GCSF (CAS Registry number: 121181-53-1)
e. Common name:
f. INN Name:
g. Compendial Name: TBD
h. OBP systematic name: RPROT P09919 (CSF3_HUMAN) Granulocyte colony-stimulating factor [EP2006]
i. Other Names: EP2006

8. **PHARMACOLOGICAL CATEGORY:** Leucocyte growth factor
9. **DOSAGE FORM:** Injection
10. **STRENGTH/POTENCY:** 300 µg/0.5 mL and 480 µg/0.8 mL (Cell proliferation potency assay)
11. **ROUTE OF ADMINISTRATION:** IV and SC
12. **REFERENCED MASTER FILES:**

DMF #	HOLDER	ITEM REFERENCED	Letter of Cross-Reference	COMMENTS (STATUS)
(b) (4)		(b) (4)	Yes	Sufficient information in application.
			Yes	Review in DARRTS 3/10/2014.

(b) (4)			(b) (4) is suitable for pharmaceutical purposes and adequate to support (b) (4) No further review is needed as sufficient information was provided in the BLA
(b) (4)		Yes	Review in DARRTS 8/30/2012. Adequate to support (b) (4)
(b) (4) 8	(b) (4)	n/a	Acceptable

13. INSPECTIONAL ACTIVITIES:

A PAI was conducted from September 8, 2014 through September 16, 2014. Information about the facility and FDA personnel involved in shown below:

Firm:	Sandoz GmbH
Location:	Biochemiestrasse 10 6250 Kundl, Austria
Phone:	0043 5338 200-0
FAX:	0043 5338 200-480
Mailing address:	Biochemiestrasse 10 6250 Kundl, Austria
Dates of inspection:	9/8/2014, 9/9/2014, 9/10/2014, 9/11/2014, 9/12/2014, 9/15/2014, 9/16/2014
Days in the facility:	7
FDA Participants:	Barbara J. Breithaupt, CSO, ORA/PR/SEA-DO Santos E. Camara, Microbiologist, ORA/PRLNW Bo Chi, CSO, BMAB/OC/CDER Maria T. Gutierrez Lugo, Chemist, DTP/OBP/CDER Michael R. Shanks, Biologist, BMAB/OC/CDER

A four-item 483 form was issue at the end of the inspection (Appendix II)

14. CONSULTS REQUESTED BY OBP:

DISCIPLINE/TOPIC	DATE REQUESTED	STATUS	RECOMMENDATION	REVIEWER
CDRH/ODE – Drug Product syringe and needle safety device	May 22, 2014			Nicholas W. Werner/Ryan J. Mcgowan
CDRH/OC - Drug Product syringe	June 27, 2014			Rakhi Dalal/Francisco Vicenty
CDER/OSE/OMEPRM/DMEPA - Human factors	May 22, 2014	Completed	Adequate	Neil H Vora/Yelena L

				Maslov
CDER/OTS/OB/DBVI -Statistical Equivalence of bioactivity and protein content	August 27, 2014	Completed	Adequate	Xiaoyu Dong/Meiyu Shen

15. QUALITY BY DESIGN ELEMENTS

The following was submitted in the identification of QbD elements (check all that apply):

X	Design Space
X	Design of Experiments
X	Formal Risk Assessment / Risk Management
	Multivariate Statistical Process Control
	Process Analytical Technology
	Expanded Change Protocol

Design space and design of experiments concepts were applied for characterization of the EP2006 DS manufacturing process. This is discussed in section 3.2.S.2.6

16. PRECEDENTS:

- First biosimilar application
- Application of statistics for evaluation of analytical similarity data

17. ADMINISTRATIVE

A. Signature Block

Name and Title	Signature and Date
Gibbes Johnson, PhD Acting Director, Division of Biotechnology Review and Research IV	Gibbes R. Johnson -S Digitally signed by Gibbes R. Johnson -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000592128, cn=Gibbes R. Johnson -S Date: 2015.01.30 13:41:37 -05'00'
Maria-Teresa Gutierrez-Lugo, PhD Lead, Division of Biotechnology Review and Research III	Maria Teresa Gutierrez Lugo -S Digitally signed by Maria Teresa Gutierrez Lugo -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0011818863, cn=Maria Teresa Gutierrez Lugo -S Date: 2015.01.30 13:34:45 -05'00'

B. CC Block

Recipient	Date
Clinical Division BLA RPM	
Division of Biotechnology Review and Research III File/BLA STN 125553	

SUMMARY OF QUALITY ASSESSMENTS

I. Primary Reviewer Summary Recommendation

I recommend approval of this 351(k) BLA application for EP2006 (Zarxio) as a biosimilar to US-licensed Neupogen

II. List Of Deficiencies To Be Communicated
Not applicable

III. List Of Post-Marketing Commitments/Requirement
Two post-marketing commitment are planned to improve the control strategy for EP2006 and to confirm the in-use stability of EP2006 in 5% glucose

IV. Review Of Common Technical Document-Quality Module 1
A. Environmental Assessment Or Claim Of Categorical Exclusion

A categorical exclusion is requested by Sandoz under 21 CFR Part 25.31(a). As stated in 21 CFR Part 25.31(a), if the action does not increase the use of the active moiety. Approval of this submission will not increase the overall use of the active moiety. Therefore we recommend approval.

V. Primary Container Labeling Review
Refer to review by Dr. Jibril Abdus-Samad

VI. Review Of Common Technical Document-Quality Module 3.2
See below

VII. Review of Immunogenicity Assays – Module 5.3.1.4
Refer to review by Dr. Faruk Sheikh, Dr. Frederick Mills and Dr. Susan Kirshner

218 Pages Have Been Withheld In Full As b4 (CCI/TS) Immediately Following This Page

3.R.3. ANALYTICAL SIMILARITY

The data and information discussed in this section was provided by Sandoz and corresponds to Sandoz analyses of the products under evaluation. Unless otherwise noted, all figures and tables referred in this section were excerpted from Sandoz 351(k) BLA submission.

The proposed biosimilar product, EP2006 was developed as a liquid, injection, filled in a pre-filled syringe in the same strengths (300 mcg/0.5 ml and 480 mcg/0.8 ml) approved for the reference product, US-licensed Neupogen. A comparison of the formulation of EP2006 and US-licensed Neupogen is provided below.

Ingredient/ Strength	EP2006		US-licensed Neupogen[35]	
	300 mcg/0.5 ml	480 mcg/0.8 ml	300 mcg/0.5 ml	480 mcg/0.8 ml
Recombinant mGCSF (mcg)	300	480	300	480
Acetate (mg)			0.295	0.472
Glutamic acid (mg)	0.736	1.178		
Sorbitol (mg)	25.0	40.0	25.0	40.0
Polysorbate 80 (%)	0.02	0.032	0.02	0.032
Sodium (mg)			0.0175	0.028
Sodium hydroxide (pH Adjustment)	q.s. for pH 4.4	q.s. for pH 4.4		
pH	4.4	4.4	4.0[36]	4.0
WFI (ml)	(b) (4)	q.s. ad 0.8	q.s. ad 0.5	q.s. ad 0.8

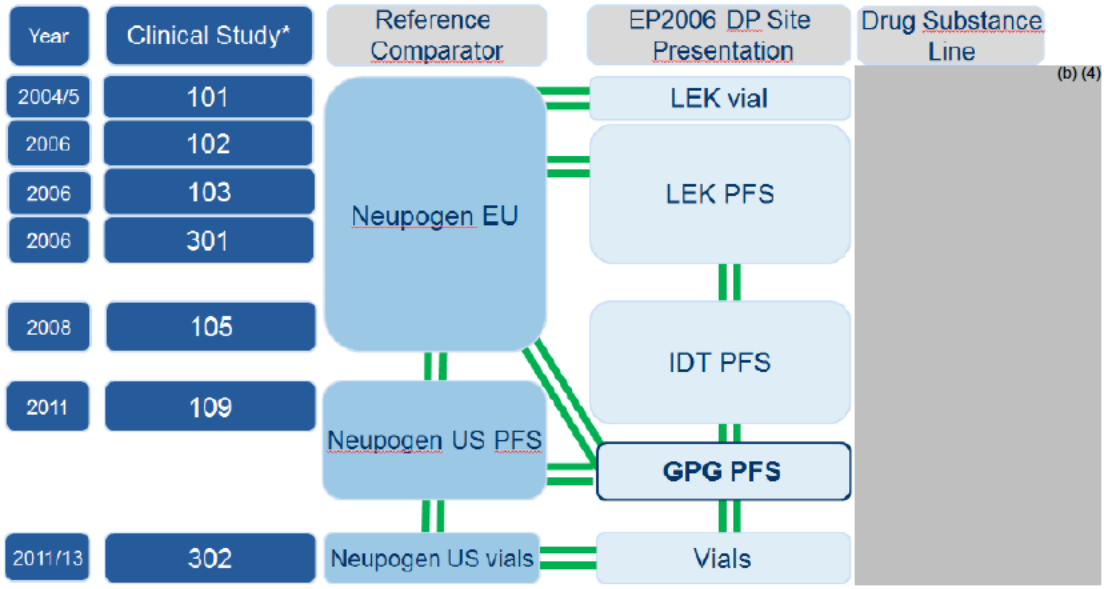
Note: *Table prepared by the CMC reviewer*

The clinical development program of EP2006 is supported by five PK/PD (EP06-101, EP06-102, EP06-103, EP06-105 and EP06-109) and two clinical (EP06-301 and EP06-302) studies (Figure 1-1). Studies EP06-101, EP06-102, EP06-103, EP06-105 and EP06-301 compare EP2006 with a non-US licensed comparator product (referred in Figure 1-1 as Neupogen EU. This product is referred through this submission as EU-approved Neupogen). Studies EP06-109 and EP06-302 compare EP2006 with US-licensed Neupogen (referred in Figure 1-1 as Neupogen US).

The non-clinical program of EP2006 consisted of five studies (EP06-001, EP06-002, EP06-003, EP06-004, and EP06-006. All of these studies used EU-approved Neupogen as active comparator.

[35] From US-licensed Neupogen labeling
[36] Based on Sandoz's analysis of the reference product, US-licensed Neupogen

Figure 1-1 Overview of EP2006 presentations throughout the history of development and link to clinics



Green lines: analytical link (e.g. comparability study)

* Clinical study codes: clinical phase 1: EP06-1NN; clinical phase 3: EP06-3NN; the timeline indicates the year(s) in which the clinical studies were conducted and is not applicable for the technical development

DP: drug product; PFS: pre-filled syringe

Drug substance manufacturing was done at Sandoz GmbH, Austria

The drug product presentation intended for commercialization in the US is highlighted in bold by a blue frame.

Sandoz proposes to leverage the data of the clinical and non-clinical studies conducted with EU-approved Neupogen as active comparator to support this application. Thus, an analytical bridge between EP2006, US-licensed Neupogen and EU-approved Neupogen needs to be established to justify the relevance of the data generated using EU-approved Neupogen as the comparator in the clinical and non-clinical studies referred above, intended to support a demonstration of biosimilarity to US-licensed Neupogen.

Sandoz GmbH, Kundl, Austria [redacted] (b) (4) is the proposed manufacturing site for commercial EP2006 DS. In 2004, the manufacturing process for EP2006 DS was validated at this site but in a different [redacted] (b) (4) line [redacted] (b) (4)). After this initial validation, the [redacted] (b) (4) process for the EP2006 DS was scale up, transferred to the commercial [redacted] (b) (4) line [redacted] (b) (4)) and re-validated in 2011 and in 2014.

GP Grenzach Produktions GmbH (GPG) is the proposed manufacturing site for commercial EP2006 DP. The clinical DP (PFS) was manufactured at LEK Pharmaceuticals d.d, Slovenia (LEK). The DP manufacturing process process was then transferred in 2006 to Impfstoffwerke Dessau-Tornau GmbH, Germany (IDT). This was also a clinical DP manufacturing site. The DP process was subsequently transferred to GPG in 2011. Validation of the EP2006 DP process at GPG was conducted in 2011.

Comparability between EP2006 DS manufactured at (b) (4) and comparability of EP2006 DP manufactured at LEK, IDT and GPG was established. Refer to section 3.2.S.2.6 (DS) and 3.2.P.2.6 (DP), respectively.

It is important to indicate that for this development program various analytical comparisons need to be made:

- The comparison between EP2006 and US-licensed Neupogen. This comparison is needed to demonstrate that EP2006 is "highly similar" to the reference product

- Pair-wise analytical comparisons between EP2006, US-licensed Neupogen and EU-approved Neupogen are used to support the analytical bridge

The similarity study results provided in the original application included DP lots used in clinical studies EP06-101, EP06-102, EP06-103, and EP06-301 and in non-clinical studies EP06-004 and EP06-006 as well as validation lots of the proposed commercial DP (referred as commercial process). The process used to manufacture the clinical lots is referred as clinical process. Detailed information on these lots is provided in Table 3-1 (Appendix XIX).

Over the course of the review of the analytical similarity data, the Agency requested Sandoz to include data from additional lots of the products under evaluation. A total of 20 lots of EP2006 DP, 6 lots of EP2006 DS, 10-15 lots of US-licensed Neupogen and 34-52 lots of EU-approved Neupogen were evaluated.

The US-licensed Neupogen and EU-approved Neupogen lots analyzed span approximately 5 and 10 years, respectively and correspond to lots across the shelf life of the products. Sandoz states that analyses were conducted 25-27 months before expiry of the EP2006 lots and 2-20 months before expiry of US-licensed Neupogen and EU-approved Neupogen lots.

Analytical similarity of EP2006, US-licensed Neupogen and EU-Neupogen was assessed using the methods listed in the Table A. The methods were validated or qualified at the time of testing and demonstrated to be fit for intended use.

Reviewer's comment: *methods used in the analytical similarity exercise that are also used for routine quality control were validated as per ICH Q2(R1). Validation of analytical procedures is discussed in section 3.2.S.4.3. Other methods, such as micro flow imaging, cation exchange chromatography, N-terminal sequencing, HPLC-ESI-MS, MALDI-TOF-MS, and CD were qualified according to Sandoz SOPs. The instruments/systems used have operational qualification (OQ) and performance qualification (PQ), system suitability testing (SST) procedures in place. Routine PQs procedure tests for system parameters are tracked in validated control charts with defined alert limits and acceptance criteria. Qualification of methods was reviewed on inspection and found acceptable.*

^{1}H NMR spectroscopy was performed in an academic environment according to (b) (4) *SOP NMR_001. No formal IQ/OQ/PQ of the instrument was performed. To ensure that the method is fit for the intended use, SST with acceptance criteria were conducted. In addition, the influence of the following parameters on the analysis results was tested: testing for local field homogeneity*

at the sample position; spiking of d4-TSP for reference for chemical shift calibration; shimming calibration and calibration of chemical shifts.

The qualification of the surface plasmon resonance method used in the receptor-binding studies consisted of an evaluation of precision, specificity and selectivity. In addition, experiments were performed to exclude that the mass transfer limitation influences the kinetic rate constants. The assays were performed on a qualified system using an appropriate SST. The RSD of the kinetic parameters was determined as follows: ≤16% for KD, ≤35% for kon and ≤40% for koff (refer to document entitled "EP2006 affinity to GCSFR determination by SPR- method characterization" located in section 3.2.R.

Table A. Methods used to assess analytical similarity

Quality Attribute	Criticality[37]	Methods	Tier assignment
Primary structure		• N-terminal sequencing • Peptide mapping with UV and MS detection • Protein molecular mass by ESI MS • Protein molecular mass MALDI-TOF MS • DNA sequencing of construct cassette • Peptide mapping coupled with MS/MS	
Bioactivity	Very high	• Proliferation of murine myelogenous leukemia cells (NFS-60 cell line)	1
Receptor binding	Very high	• Surface Plasmon Resonance	2/3
Protein content	Very high	• RP-HPLC	1
Clarity	Very high	• Nephelometry	2
SVP	High	• Micro flow imaging	3
Higher Order Structure	High	• Far and Near UV circular dichroism • ^{1}H nuclear magnetic resonance • ^{1}H-^{15}N heteronuclear single quantum coherence spectroscopy • LC-MS (disulfide bond)	2/3
High molecular weight variants/aggregates	High	• Size exclusion chromatography • Reduced and non-reduced SDS-PAGE • 90º light scattering • Nile red steady-state fluorescence anisotropy	3

[37] Very high: criticality score of ≥140
High: criticality score of 90-104
Low: criticality score of 45-90
Very low: criticality score of ≤28

Oxidized variants	High	• RP-HPLC • LC/MS	2
Covalent dimers	High	• LC/MS	3
Partially reduced variants	High	• LC/MS	3
Sequence variants: His→Gln Asp→Glu Thr→Asp	High	• RP-HPLC • LC/MS	3
Formyl-Met1	High	• RP-HPLC • LC/MS	3
Succinimide	High	• RP-HPLC • LC/MS	3
Phosphoglucunoylation	High	• LC/MS	3
Acetylated variant	High	• LC/MS	3
N-terminal truncated variants	Low	• LC-MS/MS	2
Norleucine	Very low	• RP-HPLC • LC/MS	3
Deamidation	Very low	• RP-HPLC • LC/MS • IEF • CEX	3

Note: Table prepared by the CMC reviewer based on Sandoz CQA assessment

Reviewer's comments: *Assessment of analytical similarity was made independently of Sandoz acceptance criteria (AC, not shown) because the acceptance criteria for some attributes were too wide and did not consider criticality of the quality attibute. In addition, during review of this application, the Agency requested additional similarity data and statistical analysis of selected quality attributes. The original AC did not consider the statistical analysis.*

The current recommendation for applying statistics in the evaluation of analytical similarity datais that attributes ranked higher in the CQA assessment be evaluated by a stringent statistical method such as equivalence testing (Tier-1). Attributes lower in the ranking may be evaluated by comparison to a similarity range defined as for example, mean ±2 SD (Tier-2) or by a qualitative comparison of the data (Tier-3). The similarity acceptance criteria should consider the criticality of the quality attribute, the method(s) used to evaluate the qualtiy attribute and other considerations such as amenability of the data to statistical evaluation. Refer to IR dated October 3, 2014.

I assigned the quality attributes evaluated in analytical similarity in the three-tier system based on Sandoz CQA assessment and the considerations mentioned above. Sandoz was asked to conduct this exersice (refer to IR dated October 3, 2014). However, based on the response to the IR, Sandoz interpreted the request differently and only focused on Tier-1 attributes. In addiiton, Sandoz did not interpret the concept of "equivalence testing" in the statistical context. Sandoz was not asked to propose attributes for Tier-2 and Tier-3 because the original proposal was similar to the Tier-2 and 3 concept.

Criticality of Quality Attributes

Criticality of quality attributes (QA) was evaluated using a risk ranking approach. This approach assesses the possible impact of the QA on efficacy, PK/PD, immunogenicity, safety, and stability (refer to section 3.2.P.2.2.3). Table A above lists the QA by criticality and tier assignment. The analytical similarity results will be discussed according to CQA raking.

Analytical similarity studies of EP2006, US-licensed and an EU-approved Neupogen

Reviewer's comments:

- *Sandoz provided statistical analysis of the analytical data to support analytical similarity. The Agency also conducted the statistical analysis to confirm Sandoz assessment. The data discussed below uses the Agency statistical analysis because Sandoz used statistical considerations that are not appropriate such as pooling data from US-licensed Neupogen and EU-approved Neupogen to establish analytical similarity criteria. This is not appropriate because EP2006 should be demonstrated to be "highly similar" to the reference product US-licensed Neupogen ("the single biological product, licensed under section 351(a) of the PHS Act").*

- *The data discussed below considers the Agency current recommendation on statistical analysis of analytical similarity data based on a tier system (refer to IR dated October 3, 2014). Tier 1 attributes are analyzed by statistical equivalence testing. Tier 2 attributes are analyzed by a quality range of mean ± XSD. Tier 3 is a qualitative assessment of the data. In some cases a single attribute may be analyzed by more than one approach (e.g. quality range and qualitative assessment)*

- *The statistical analysis for Tier-1 attributes (i.e. potency and protein content) was conducted by the Agency statistics reviewer, Dr. Dong according to the current recommendation (refer to statistics reviewer memo).*

- *I conducted the statistical analysis for Tier 2 attributes. The acceptance criterion (AC) for Tier-2 is as follows:*

  Analytical similarity is supported for the quality attribute if the 90 % test lot values fall within the quality range of mean ± 2SD or mean ± 3SD

- *The analytical results of EP2006 DP manufactured by the clinical and commercial processes were pooled because they are comparable (refer to section 3.2.P.2.6). Also, data from the two EP2006 strengths were combined because the only difference between the strengths is the volume of bulk DP filled in a syringe. The bulk EP2006 DP solution used to manufacture both EP2006 strengths (300 mcg/0.5 ml strength and 480 mcg/0.8 ml) has a concentration of 0.6 mg/ml of EP2006.*

Primary sequence

N-terminal Edman sequencing and peptide mapping were used to evaluate the primary sequence of EP2006, US-licensed Neupogen and EU-approved Neupogen. N-terminal sequencing provides information on the first N-terminal amino acids of the protein. Detailed information on the composition of the protein is obtained by peptide mapping coupled with mass spectrometry. In addition, protein molecular mass determination by LC-ESI-MS and MALDI-TOF was conducted to provide further support to the correctness of the primary sequence.

The number of lots analyzed in these assays is listed below:

Product	# lots			
	N-terminal sequencing	Peptide mapping	Protein molecular mass ESI	Protein molecular mass MALDI-TOF
EP2006 DP	6	11[38]	6	5
EP2006 DS	3	6		6
US-licensed Neupogen	4	4	4	4
EU-approved Neupogen	2	5	2	6

Note: Table prepared by the CMC reviewer

N-terminal sequencing

The results of N-terminal sequencing showed that the first 7 amino acids in the three products are: **MTPLGPA.**

Peptide mapping

Sandoz peptide map (Glu-C in phosphates buffer) consist in 12 peptide fragments (Figure 1-1) plus peptides resulting from unspecific cleavage (e.g. G11-1a and G-11-2a) or missed cleavage (e.g. G-8-10). The amino acid sequence of each peptide peak was based on the experimentally determined monoisotopic or average mass of the peptides and the theoretical mass calculated from the amino acid sequence of Met-GCSF (Table 1-7).

***Reviewer's comment**: The MS analysis shows that the peptide map provides 100% sequence coverage. I verified the correctness of the peptide map assignment, including peptides G4 and G5 (each of these peptides contain a disulfide bond) by digesting the met-GCSF in silico using the Protein Prospector tool (UCSF)[39] (refer to Appendix XX).*

[38] Monosiotopic and average masses were determined in evaluation 1 and 2, respectively. In addition, in evaluation 1, a more accurate instrument was used (LTQ Orbitrap). Therefore, for the purposes of computing a mean value, the data was analyzed separately.

[39] http://prospector.ucsf.edu/prospector/mshome htm

Figure 1-1 **Schematic representation of the amino acid structure (primary sequence) of Sandoz Filgrastim. Glu-C cleavage sites and peptides following Glu-C digest are indicated in red**

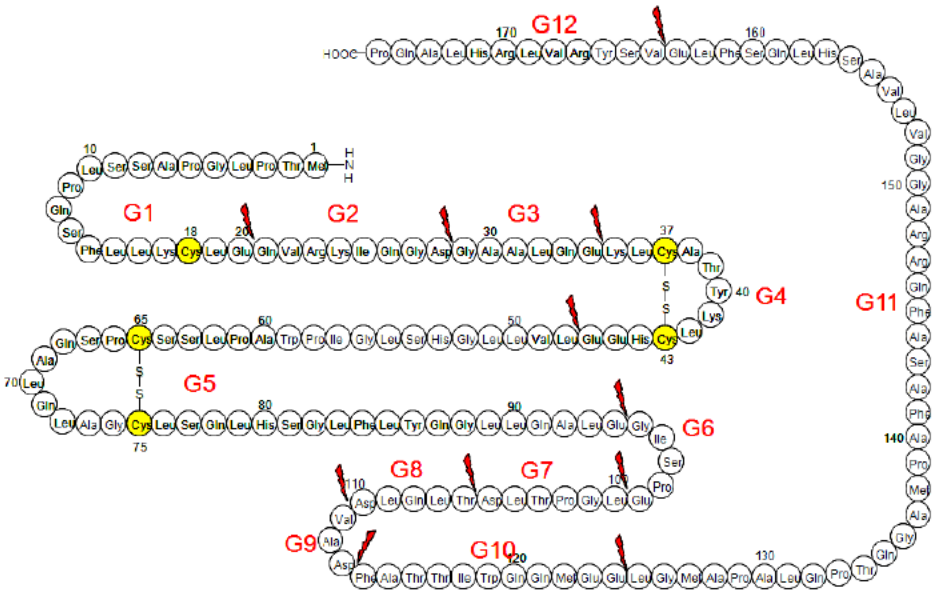


Table 1-7 **Expected fragments from a Glu-C-digest (phosphate buffer) of Filgrastim**

Label	Sequence position	Amino acid sequence
G1	Met(1)-Glu(20)	MTPLGPASSLPQSFLLKCLE
G2	Gln(21)-Asp(28)	QVRKIQGD
G3	Gly(29)-Glu(34)	GAALQE
G4 (S-S)	Lys(35)-Glu(47)	KLCATYKLCHPEE
		Intramolecular disulfide bridge Cys(37)-S-S-Cys(43)
G5 (S-S)	Leu(48)-Glu(94)	LVLLGHSLGIPWAPLSSCPSQALQLAGCLSQLHSGLFLYQG LLQALE
		Intramolecular disulfide bridge Cys(65)-S-S-Cys(75)
G6	Gly(95)-Glu((99)	GISPE
G7	Leu(100)-Asp(105)	LGPTLD
G8	Thr(106)-Asp(110)	TLQLD
G9	Val(111)-Asp(113)	VAD
G10	Phe(114)-Glu(124)	FATTIWQQMEE
G11	Leu(125)-Glu(163)	LGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLE
G12	Val(164)-Pro(175)	VSYRVLRHLAQP

Representative peptide maps of EP2006 in comparison to US-licensed Neupogen and an EU-approved Neupogen (lot #N1114AJ) are shown in Figures, 1-2, 1-3 and 1-4. The peptide map and individual peptide mass of EP2006 were compared to those of US-licensed Neupogen and EU-approved Neupogen in terms of the number of peaks, retention time, peak height and monosiotopic (or average) mass. Peptide MS data for all products tested are shown in Appendix XX.

Figure 1-2 **UV chromatograms of RP-HPLC of Glu-C digested Filgrastim from EP2006 drug product and Neupogen® 480 mcg/0.8 mL and 480 mcg/0.5 mL batches**

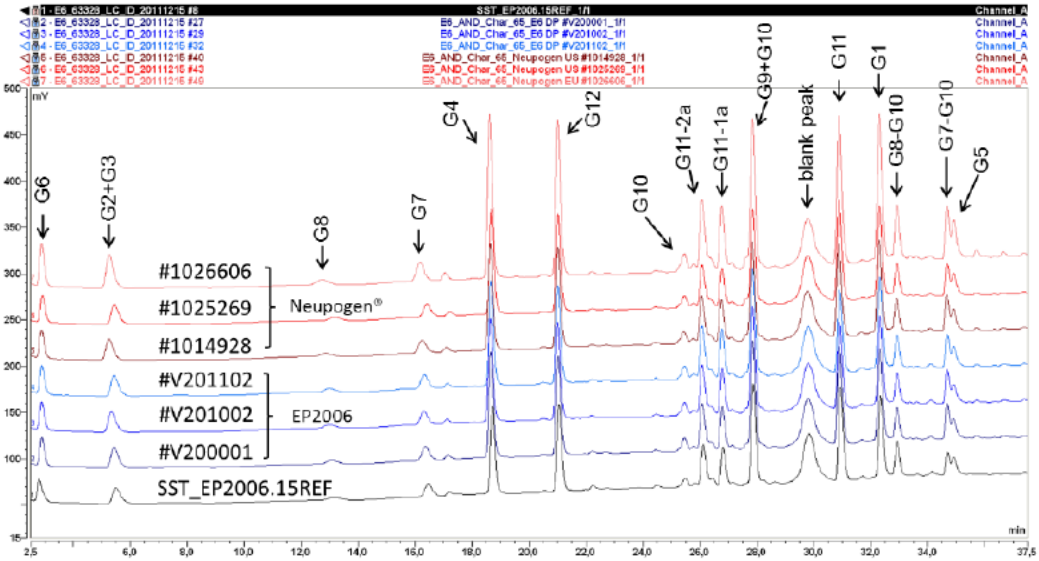


Figure 1-3 **RP-HPLC of a Glu-C digest peptide map from EP2006 drug product, #A03941609F**

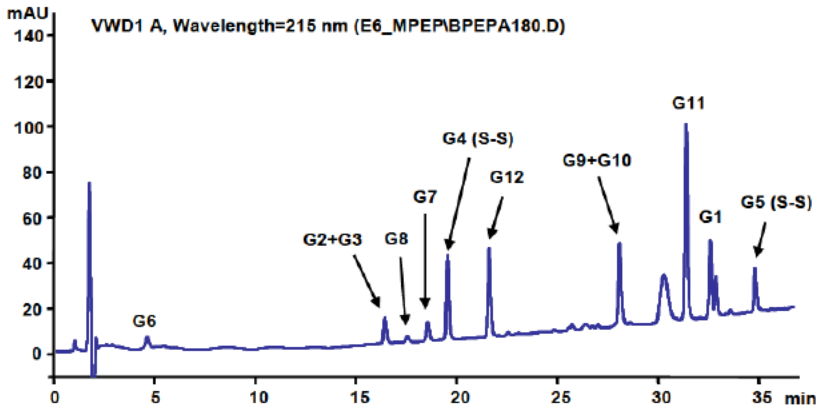
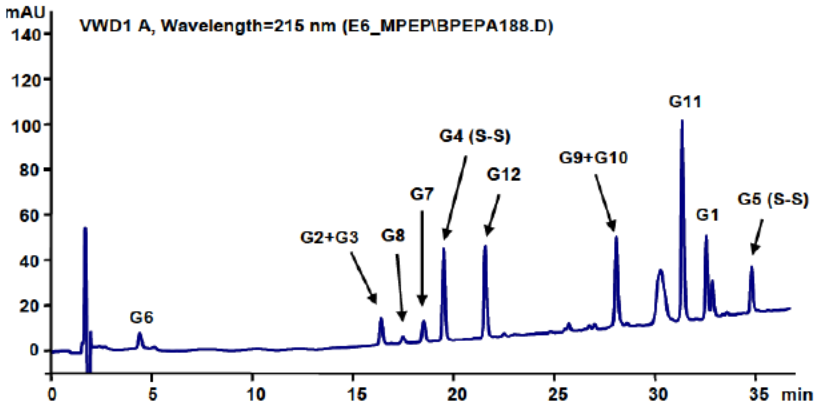


Figure 1-4 **RP-HPLC of a Glu-C digest peptide map from Neupogen, #N1114AJ**



Reviewer comments:

- *It appears that different peptide map methodologies were used in the comparison of EP2006 and US-licensed Neupogen (Figure 1-2) and in the comparison of EP2006 and EU-approved Neupogen (Figures 1-3 and 1-4) as the chromatography profile of Figure 1-2 is different from Figures 1-3 and 1-4. For example peptide G7-G10, corresponding to two missed cleavages is not observed Figures 1-3 and 1-4. This difference in methodology does not impact the evaluation of the primary sequence of the products because the amino acids of peptide G7-G10 are covered by other peptides (refer to Table 1-7), and the mass of the peptides determined by MS in both maps are correct and provide full sequence coverage.*

- *The mass analyzer (Thermo Scientific LTQ Orbitrap) used to determine the monoisotopic mass of the peptides provides results with high accuracy. The masses obtained are within ± 0.02 Da. Given the high accuracy of the results, the data were not analyzed statistically.*

Top down MS by LC-ESI-MS

Table 5-4 provides monoisotopic mass results for EP2006, US-licensed Neupogen and an EU-approved Neupogen.

Table 5-4 Summarized data of RP-HPLC-ESI-MS run (study 1)

Sample	RT [min]	Mass deconvoluted [Da]	Deviation to calculated mass [Da]
V200001, 480 mcg/0.8 mL, PFS, GPG	11.98	18786.7992	0.119
V201002, 480 mcg/0.8 mL, PFS, GPG	12.05	18786.8162	0.136
V201102, 480 mcg/0.8 mL, PFS, GPG	12.08	18786.8335	0.153
V201001, 300 mcg/0.5 mL, PFS, GPG	12.00	18786.8151	0.135
V201101, 300 mcg/0.5 mL, PFS, GPG	11.98	18786.8267	0.147
V200201, 300 mcg/0.5 mL, PFS, GPG	11.95	18786.8401	0.160
1014928, 480/0.8, PFS, N-US	12.00	18786.8315	0.151
1025269, 480/0.8, PFS, N-US	12.08	18786.8115	0.131
1020649, 300/0.5, PFS, N-US	12.05	18786.8634	0.183
1021957, 300/0.5, PFS, N-US	12.05	18786.8371	0.157
1026606, 480/0.5, PFS, N-EU	12.02	18786.8311	0.151
1025051, 300/0.5, PFS, N-EU	12.07	18786.8185	0.139

Product	Mean (Da)	SD	Similarity acceptance criteria (mean ±2 SD)
EP2006 DP	18786.8218	0.0147	
US-licensed Neupogen	18786.8359	0.0214	18786.7931-18786.8787
EU-approved Neupogen	18786.8248	0.0089	18786.8070-18786.8426

Note: Table prepared by the CMC reviewer

Reviewer's comments:

- *The molecular mass of each individual value (lot) of EP2006 is within the similarity acceptance criteria of mean ± 2 SD calculated from the US-licensed Neupogen data. With the exception of one data point (lot V200001, 16% of the population), the EP2006 lot values are within the AC calculated from the EU-approved Neupogen data. The EU-Neupogen data are within US-licensed Neupogen similarity acceptance criterion. The one data point outside*

of the AC is acceptable because the data is 0.0078 outside of the lower bound and the slight difference is within the instrument accuracy (1Da).

- *The molecular mass of the three products are also in agreement with the theoretical monoisotopic mass of Met-GCSF (oxidized form, 18786.68 Da[40]).*

Top down MS by MALDI-TOF

Table 5-5 provides average mass for EP2006, US-licensed Neupogen and an EU-approved Neupogen.

Table 5-5 Summarized results of MALDI-TOF-MS of tested samples

Samples	Average found mass	Standard deviation of 3 determinations	Deviation from theoretical mass (18798.9)
Similarity evaluation 1: Biosimilarity of EP2006 with reference product Neupogen			
V200001, 480 mcg/0.8 mL, PFS, GPG	18799.0	-	-0.1
V201002, 480 mcg/0.8 mL, PFS, GPG	18800.4	-	-1.5
V201102, 480 mcg/0.8 mL, PFS, GPG	18799.0	-	-0.2
V201001, 300 mcg/0.5 mL, PFS, GPG	18797.9	-	1.0
V201101, 300 mcg/0.5 mL, PFS, GPG	18798.0	-	0.8
V200201, 300 mcg/0.5 mL, PFS, GPG	18797.9	-	0.9
1014928, 480/0.8, PFS, N-US	18797.9	-	0.9
1025269, 480/0.8, PFS, N-US	18797.4	-	1.4
1020649, 300/0.5, PFS, N-US	18796.8	-	2.1
1021957, 300/0.5, PFS, N-US	18798.2	-	0.6
1026606, 480/0.5, PFS, N-EU	18798.3	-	0.6
1025051, 300/0.5, PFS, N-EU	18797.1	-	1.8
Similarity evaluation 2: Similarity of EP2006 with EU comparator product			
48200402, DS, Sandoz	18798.8	0.5	-0.1
48200403, DS, Sandoz	18797.4	0.4	-1.5
48200404, DS, Sandoz	18799.0	0.8	0.1
48200405, DS, Sandoz	18797.9	0.5	-1.0
48200406, DS, Sandoz	18798.5	1.1	-0.4
48200407, DS, Sandoz	18799.1	1.8	0.2
A03941609F, 960/1.0, vial, LEK	18798.5	0.9	-0.1
A00657409G, 300/0.5, PFS, LEK	18797.7	1.2	-1.2
A00675111G, 480/0.5, PFS, LEK	18797.7	0.2	-1.2
A00675011G, 300/0.5, PFS, LEK	18799.2	1.1	0.3
A00675211G, 480/0.5, PFS, LEK	18798.3	0.5	-0.6
N0875AA, 480/0.5, PFS, N-EU	18796.8	1.5	-2.1
N1144AE, 300/0.5, PFS, N-EU	18798.3	0.5	-0.6
N1113AG, 300/0.5, PFS, N-EU	18798.4	1.0	-0.1
N1114AJ, 480/0.5, PFS, N-EU	18797.5	0.4	-0.4

[40] http://web.expasy.org/peptide_mass/

Product	Mean	SD	Similarity acceptance criteria (mean ± 3SD) (Da)
EP2006 DP	18798.49 (includes DS and DP)	0.76	
US-licensed Neupogen	18797.58	0.61	18795.75-18799.41
EU-approved Neupogen	18797.73	0.69	18795.66-18799.8

Note: Table prepared by the CMC reviewer

Reviewer's comments:

- *With the exception of one data point (lot V201002, 6.3% of the population), each individual molecular mass value of EP2006 is within the similarity acceptance criterion of mean ± 3 SD of US-licensed Neupogen data. Same outcome is obtained from the comparisons of EP2006 and EU-approved Neupogen. All lots of EU-approved Neupogen fall in the similarity acceptance criterion calculated with US-licensed Neupogen data. The mean ± 3 SD acceptance criterion is acceptable because the expected accuracy of MALDI-TOF instruments is lower than that of ESI instruments (>1Da). Actual difference between the products with respect to mean values is less than 1Da. Given that ESI data is tight and met the similarity acceptance criteria, the MALDI-TOF data are supportive.*

- *The average molecular mass by MALDI-TOF is also in agreement with the theoretical average mass of Met-GCSF (oxidized form, 18798.82 Da[41]).*

Reviewer's assessment of the data supporting same primary sequence:

Sandoz analysis of the three products indicate that the first 7 amino acids of EP2006 (i.e. MTPLGPA) are the same 7 amino acids found in US-licensed Neupogen and EU-approved Neupogen. These 7 amino acids are identical to the amino acids deduced from the GCSF cDNA sequence with an additional N-terminal methionine (as expected for products expressed in E. coli) and to the sequence of Met-GCSF reported in the literature[42].

The peptide map data (e.g. Figures 1-2, 1-3 and 1-4) show that EP2006 has a similar map compared to US-licensed Neupogen and EU-approved Neupogen. No additional peptides or missing peptides between EP2006, US-licensed Neupogen and EU-approved Neupogen are detected. In addition, the experimentally determined peptide masses (Table 5-6, Appendix XX) of EP2006 are all consistent with US-licensed Neupogen and EU-approved Neupogen and with the theoretical masses deduced from the theoretical Met-GCSF sequence. MS analysis also confirms the presence of two disulfide bonds in peptides G4 and G5.

Correctness and similarity of the primary sequence of the three products is further supported by intact protein LC-ESI-MS and MALDI-TOF-MS. Judged by the individual data and mean values, the monoisotopic (LC-ESI-MS) and average (MALDI-TOF) mass of the three products is similar and in agreement with the theoretical mass of Met-GCSF.

[41] http://web.expasy.org/peptide_mass/

[42] Herman AC, et.al. (1996). Characterization, formulation, and stability of Neupogen (Filgrastim), a recombinant human granulocyte-colony stimulating factor. Pharm Biotechnol; 9, 303-328

In addition, the DNA sequence of the expression cassette (b) (4) *of the EP2006 producing plasmid encodes for human GCSF with an additional methionine at the N-terminus. Restriction analysis of the expression plasmid extracted from the producing strain cultured at and beyond the regular production period yields the expected pattern based on DNA sequence and restriction enzymes used (refer to characterization of cell bank section). In addition, the primary sequence of EP2006 deduced by amino acid analysis, peptide mapping using Glu-C, pepsin and chymotrypsin with MS and MS/MS analysis and N-terminal sequencing of Glu-C digested peptides was found to be identical to that of the Amgen's Filgrastim product, Neupogen[44] (refer to section 3.2.S.3.1).*

Taken together, these data provide strong evidence that the sequence of EP2006 is the same as the sequence of US-licensed Neupogen and the sequence of EU-approved Neupogen.

Biological activity

A cell proliferation assay using murine myelogenous leukemia cells (NFS-60 cell line) was used to evaluate the biological activity of the products. The results are reported in specific activity units (IU/mg) and in percentage relative to Sandoz reference standard (Table 5-3 and 2-8, Appendix XXI). Because biological activity data on limited number of reference product lots were submitted in the original application, additional data were requested (refer to IR dated October 3, 2014).

The mean values and standard deviation for specific activity expressed in U/mg and in percentage are shown below (Table 1 and Table 2, respectively). Figure 3 is a representation of the data in % bioactivity. In addition, to support analytical similarity and an analytical bridge between the three products, the bioactivity data (in percentage) were analyzed for statistical equivalence in a pair-wise fashion. Statistical equivalence was chosen for this attribute (Tier-1) because biological activity is a high risk attribute based on Sandoz CQA assessment. The three products were statistically equivalent with respect to mean values (Table 3. For details refer to CMC stats review).

Table 1. Biological activity expressed as specific activity (U/mg)

Product	# lots	Mean U/mg (x 10^8)	SD
EP2006 DP	11	1.02	0.040
EP2006 DS	6	1.10	0.063
US-Neupogen	4	1.10	0.082
EU-Neupogen	6	1.07	0.051

Note: Table prepared by the CMC reviewer

[43] http://www.uniprot.org/uniprot/P09919

[44] Herman AC, et.al. (1996). Characterization, formulation, and stability of Neupogen (Filgrastim), a recombinant human granulocyte-colony stimulating factor. Pharm Biotechnol; 9, 303-328

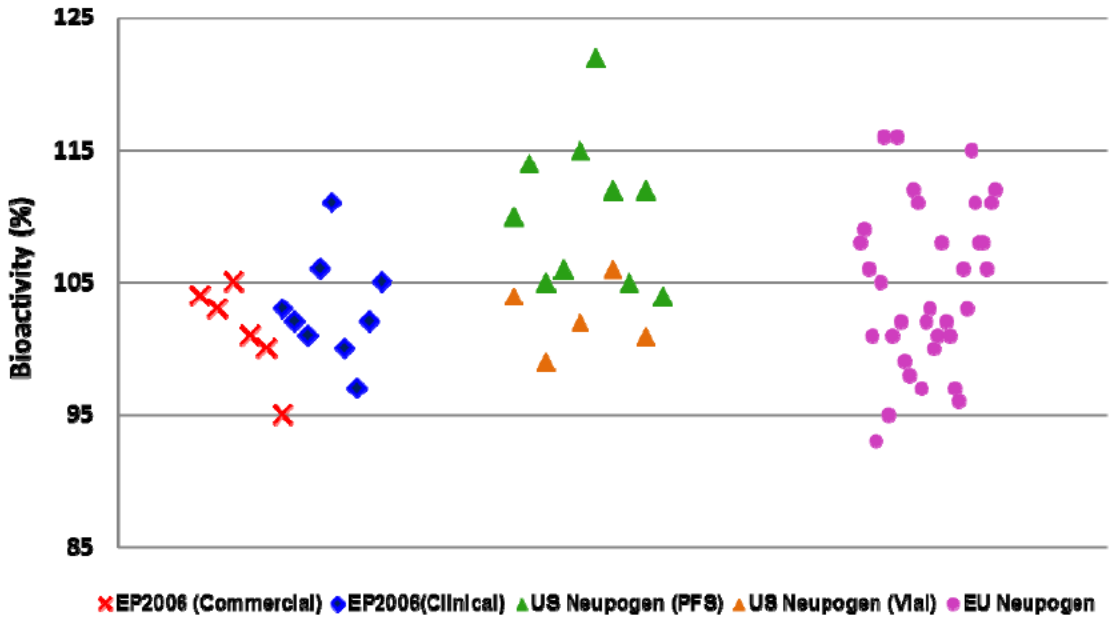
Table 2: Descriptive Statistics for Bioactivity (%)

Product	# batches	Min	Max	Mean	Standard Deviation	CV [a](%)
EP2006 (clinical and commercial, PFS)	15	95	111	102.3	3.81	3.72%
US-Neupogen (PFS and Vial)	15	99	122	107.8	6.21	5.76%
EU-Neupogen (PFS)	34	93	116	104.7	6.18	5.91%

[a] CV: coefficient of variability

Note: Table prepared by the CMC statistics reviewer

Figure 3. Biological activity of EP2006, US-licensed Neupogen and EU-approved Neupogen



Note: Figure prepared by the CMC statistics reviewer

Table 3. Equivalence testing of bioactivity data

Product*	# batches	Comparator Product	# batches	Statistical Equivalence?
EP2006 (clinical and commercial batches)	15	US-Neupogen (PFS and vials)	15	**Yes[a]**
EP2006 (clinical and commercial batches)	15	EU-Neupogen (PFS)	15	**Yes[b]**
EU-Neupogen	34	US-Neupogen (PFS and vials)	15	**Yes[c]**

* All PFS

[a] The 90% confidence interval (CI) of the mean difference between EP2006 and US-licensed Neupogen (-8.67%, -2.27%) is entirely within the equivalence acceptance criterion of (-9.32%, 9.32%)

[b] The 90% CI of the mean difference between EP2006 and EU-approved Neupogen (-5.47%, 0.54%) is entirely within the equivalence acceptance criterion of (-10.07%, 10.07%)

[c] The 90% CI of the mean difference between EU-approved Neupogen and US-licensed Neupogen (-6.34%, 0.10%) is entirely within the equivalence acceptance criterion of (-9.32%, 9.32%)

Note: Table prepared by the CMC statistics reviewer

Reviewer's assessment of data supporting similar biological activity:

The biological activity data expressed as specific activity (U/mg) provided in the original BLA (Table 5-3 in "Overview Biosimilarity Data" document located in 3.2.R) indicated that the mean specific activity of EP2006 DP appear to be slightly lower than that of US-licensed Neupogen (by ~7%) and EU-approved Neupogen (by ~2.4%). To address this potential difference, Sandoz was asked to provided data from additional lots of the three products and to conduct a statistical analysis (refer to IR dated October 3, 2014). In the statistical analysis, bioactivity data from lots of US-licensed Neupogen in both PFS and vials were included. The inclusion of both presentations is acceptable because both PFS and vials are used for the same indications and route of administration (ROA). Analytical comparison of both presentations is useful as they represent the profile that US-licensed Neupogen patients receive.

As shown in Table 3, the biological activity of the three products is statistically equivalent with respect to mean values. This assessment addresses the potential difference in biological activity between the products and support analytical similarity of EP2006 and US-licensed Neupogen and an analytical bridge between the three products.

Of note, the bioactivity data of EU-approved Neupogen spread wider and display higher variability (Figure 2 and Table 2) than that of US-licensed Neupogen. This is expected as EU-approved Neupogen data span ~10 years whereas US-licensed Neupogen data span ~5 years.

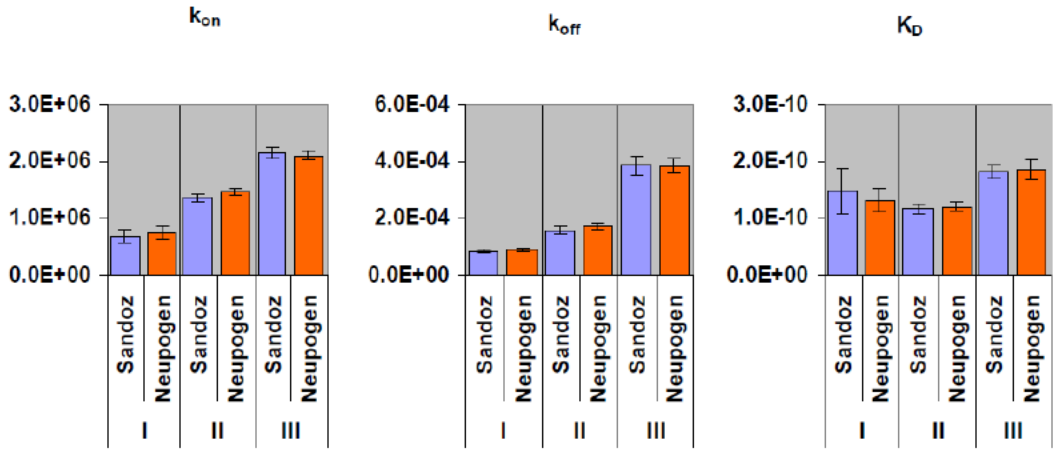
Receptor binding

Binding of the Met-GCSF to the GCSF receptor was investigated by SPR. Individual results of binding kinetic parameters for the three products are presented in (Table 2-14, Appendix XXI). Information on method performance provided by Sandoz indicate that the coefficients of variability for KD, Kon and Koff determinations are ≤16%, ≤35% and ≤40%, respectively. Sandoz states that the variability of the kinetic rate constants between individual repeats and in between experiments is most likely due to ageing of the sensor chip surface during storage.

Receptor binding is a high impact quality attribute and thus similarity of this quality attribute in the three products should be evaluated using a stringent approach. However, given that variability between experiments is high, pooling data from different experiments for a formal statistical analysis is not appropriate. Thus, review of the SPR data focuses on descriptive statistical analysis of side-by-side experiments, visual evaluation of binding curves and comparison of individual results to US-licensed Neupogen and EU-Neupogen ranges of mean ± 2SD (Tier 2/3 approach).

Figure 2-1 is an example of a side-by-side comparison of the kinetic parameters of EP2006 and EU-approved Neupogen. Differences in binding parameters between products in an experiment are within the confidence intervals. Student's t-test on these data revealed no difference at the significance level of 0.05 (data not shown). The binding parameters of the two products are similar.

Figure 2-1 Graphical comparison of k_{on}, k_{off} and K_D determined by surface plasmon resonance spectroscopy (mean and 95% confidence interval)



Reviewer's note: *The Roman numbers in the figure refer to experiment number, each having up to 21 replicates per sample*

Table 2-3 Comparison of results of surface plasmon resonance spectroscopy mean and 95% confidence interval

Exp.	Sample	k_{on} [* 10^6 l mol^{-1} s^{-1}]	k_{off} [* 10^{-4} s^{-1}]	K_D [* 10^{-10} mol l^{-1}]
1	Neupogen #N1144AE	0.76 ± 0.12	0.89 ± 0.08	1.30 ± 0.21
	Sandoz #000675011G	0.68 ± 0.12	0.82 ± 0.07	1.48 ± 0.40
2	Neupogen #N1144AE	1.46 ± 0.07	1.73 ± 0.12	1.20 ± 0.08
	Sandoz #000675011G	1.36 ± 0.07	1.57 ± 0.14	1.16 ± 0.09
3	Neupogen #N1144AE	2.09 ± 0.07	3.81 ± 0.27	1.84 ± 0.17
	Sandoz #000675011G	2.14 ± 0.10	3.85 ± 0.32	1.80 ± 0.12

To further support similarity of binding parameters, visual examination of binding curves and comparison of ranges was conducted. Representative sensograms are shown in Figure 2-7. Binding curves are similar. Descriptive statistics of binding parameters are summarized in Table C.

Figure 2-7 Sensograms of Neupogen® and EP2006 batches included in the final biosimilarity exercise

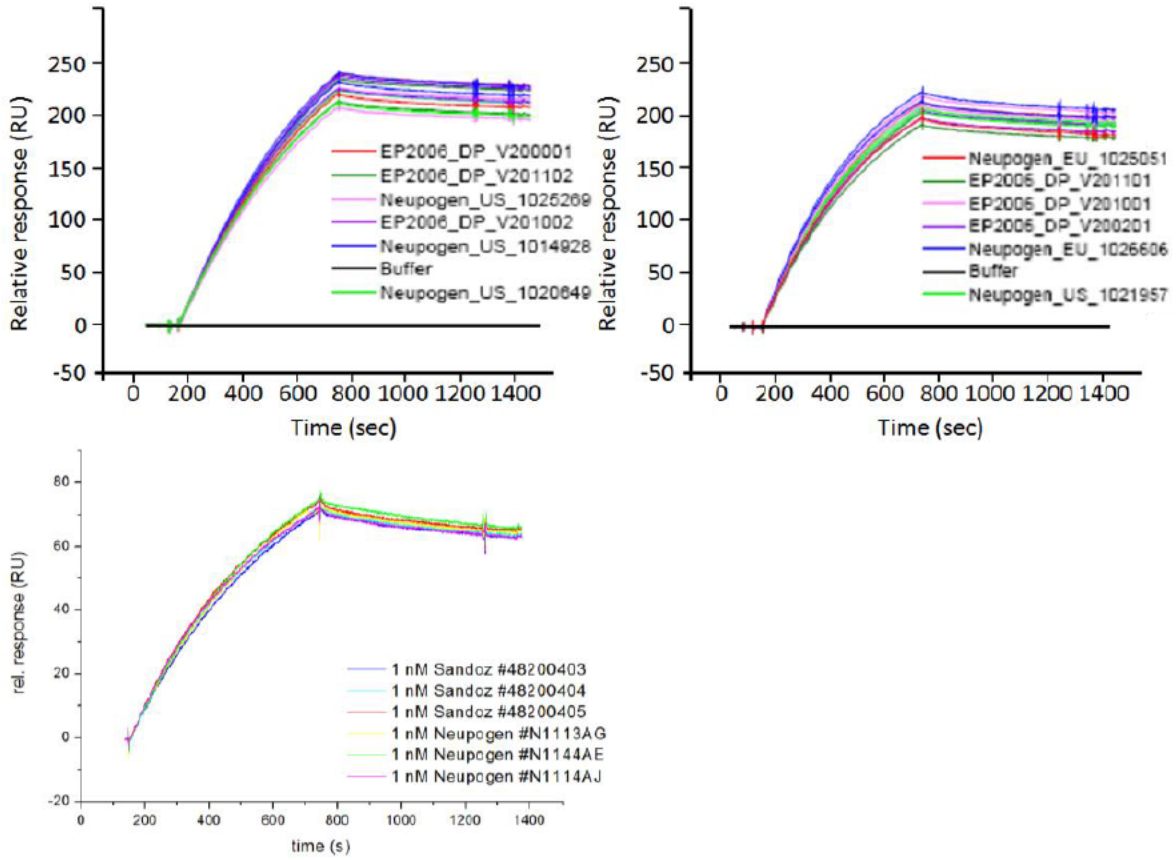


Table C. Descriptive statistics for binding parameters

Product	# lots	Mean Kon [a] (kM^{-1} s^{-1})	SD	Mean Koff [b] [45] (μ s^{-1})	SD	Mean K_D [c] [46] (pM)	SD
EP2006 DP	6	1.1	0.1	9.6	0.7	87.5	11.2
EP2006 DS*	3	1.8	0.3	10.7	0.6	56.6	6.6
US-licensed Neupogen	4	1.1	0.1	9.2	0.6	81.0	4.8
EU-approved Neupogen	5	1.4	0.2	10.6	1.2	77.5	14.2

* Data provided in response to information request dated October 3, 2014
[a] Association rate constant for formation of the complex ligand and receptor (l $mol^{-1}s^{-1}$)
[b] Dissociation rate constant (s^{-1})
[c] Equilibrium dissociation constant (mol
Note: Table prepared by the CMC reviewer

Table D. Similarity Acceptance Criteria

Range	Mean ± 2SD Kon (kM^{-1} s^{-1})	Mean ± 2SD Koff (μs^{-1})	Mean ± 2SD K_D (pM)
US-licensed Neupogen	0.9-1.3	8.0-10.4	71.4-90.6
EU-approved Neupogen	1.0-1.8	8.2-13.0	49.1-105.9

Note: Table prepared by the CMC reviewer

Additional biological activity and receptor binding data

Due to differences in bioavailability for the steady state administration between EP2006 and EU-approved Neupogen observed in study EP06-103, additional studies were conducted to investigate if differences in formulation between the products (EP2006: glutamate pH 4.4; EU-approved Neupogen: acetate, pH 4.0) could result in differences in receptor binding and biological activity. Bioactivity, receptor binding, HMW species, SVP, HOS and surface charge of the protein were evaluated in these studies.

Bioactivity individual results (4 replicates each) of EP2006 and EU-approved Neupogen lots used in the clinical and pre-clinical studies (Tables 2-1 and 2-1, pages 97-98 in document "Biosimilarity EU-comparator", located in 3.2.R) were used to calculate a 95% Confidence interval (CI_{90}) for each product:

EP2006, mean: 104.9%, CI_{90}: 101.03-108.74% (SD 9.3)
EU-approved Neupogen, mean: 107.2% CI_{90}: 104.71-109.65% (SD 4.6)

[45] Dissociation rate constant (s^{-1})
[46] Equilibrium dissociation constant (mol l^{-1})

***Reviewer comments**: the mean value for EP2006 is -2.3% lower than that of EU-approved Neupogen. However, statistical equivalence testing of more lots of EP2006 and EU-approved Neupogen show that the EP2006 lots manufactured by the clinical (LEK and IDT) and commercial (GPG) processes are statistically equivalent to EU-approved Neupogen (see previous section).*

Receptor binding parameters were evaluated for the EP2006 and EU-approved Neupogen lots used in study EP03-103. The products were evaluated in their original formulation (EP2006 in glutamates, pH 4.4 and Neupogen in acetates, pH 4.0) and in acetates (EP2006 was formulated in Neupogen formulation). The experiments were conducted at pH 7.4 and 6.0.

The results from the first study (products in their original formulation) shown in Figure 2-1 and Table 2-3 (see above) indicate that the two products have similar affinity (judged by KD). Although Kon and Koff are different between experiment I and III, both EP2006 and EU-approved Neupogen vary in similar proportion, suggesting that differences between experiment I and III are related to assay variability.

The results of the products in different buffer and at different experimental pH are shown in Table 2.6. The buffer composition of the EP2006 samples analyzed is listed in Table 2-5.

Table 2-6 Results (means) of comparison of EP2006 drug product and Neupogen by surface plasmon resonanc spectroscopy

pH	Sample	k_{on} [l mol^{-1} s^{-1}]	k_{off} [s^{-1}]	K_D [mol l^{-1}]
7.4	Neupogen #N1144AE	$1.85 * 10^6$	$1.09 * 10^{-4}$	$5.90 * 10^{-11}$
	Sandoz #000675011G	$1.80 * 10^6$	$1.07 * 10^{-4}$	$5.94 * 10^{-11}$
	Sandoz #0719ML96_1	$1.79 * 10^6$	$1.25 * 10^{-4}$	$7.04 * 10^{-11}$
	Sandoz #0719ML96_2	$1.81 * 10^6$	$1.05 * 10^{-4}$	$5.81 * 10^{-11}$
6.0	Neupogen #N1144AE	$1.78 * 10^6$	$1.95 * 10^{-4}$	$10.9 * 10^{-11}$
	Sandoz #000675011G	$1.72 * 10^6$	$1.88 * 10^{-4}$	$10.9 * 10^{-11}$
	Sandoz #0719ML96_1	$1.73 * 10^6$	$1.83 * 10^{-4}$	$10.6 * 10^{-11}$
	Sandoz #0719ML96_2	$1.70 * 10^6$	$1.87 * 10^{-4}$	$11.1 * 10^{-11}$

Table 2-5 Composition of EP2006 drug product samples

Sample	Composition
000675011G	10 mM Glutamic acid, 50 mg/mL sorbitol, 0.004% Tween 80, pH 4.4, 480 mcg/0.5 mL
0719ML96_1	10 mM Glutamic acid, 50 mg/mL sorbitol, 0.004% Tween 80, pH 4.4, 480 mcg/0.5 mL
0719ML96_2	10 mM Acetic acid, 50 mg/mL sorbitol, 0.004% Tween 80, pH 4.0, 480 mcg/0.5 mL

With the exception of EP2006 sample #0719ML96_1, tested at pH 7.4, EP2006 formulated in acetates or glutamates has similar kinetic parameters as EU-approved Neupogen when analyzed at pH 7.4 and 6.0. Note that the affinity (KD) of both products appears to be lower at pH 6.0 (increased Koff).

***Reviewer's comment:** I believe that although the buffer composition (formulation) appears to have a slight impact on binding kinetic parameters of EP2006 in vitro, the difference will not*

necessarily translate in differences in binding in vivo because the solvent conditions, including pH in the human body at the target cells overcome the DP buffer composition. However, the differences in formulation may impact absorption and distribution of the product.

The results for HMW variants, SVP, HOS and surface charge of the protein are discussed in the corresponding sections. In summary, the results of those methods showed that EP2006 contains less or equal amounts of HMW species and SVP.

Reviewer's assessment of data supporting similar receptor binding properties

The side-by-side binding results of EP2006 and EU-approved Neupogen (Figure 2-1 and Table 2-3) and the sensograms of the three products (Figure 2-7) support that the three products are similar. In general, the majority of individual binding parameter values of EP2006 DP and EU-approved Neupogen (Appendix XXI) are within the US-licensed Neupogen similarity acceptance criteria. A few data points, particularly KD values, are outside the similarity AC. However, the values are not all within the same order of magnitude. Taken together, the binding parameters of the three products are considered similar.

Reviewer's assessment of the data demonstrating same mechanism of action

As summarized in the drug substance section, the mechanism of action of GCSF related to the indication categories of neutropenia and mobilization HSC requires binding of GCSF to the GCSF receptor. The results summarized above show that binding of EP2006 and US-licensed Neupogen to the GCSF receptor is highly similar. The results of the cell-based bioassay, which also requires binding of the products to the GCSF receptor on NSF-60 cells to initiate signaling pathways that leads to their proliferation, are also highly similar. Based on these results, I conclude that EP2006 and US-licensed Neupogen have the same mechanism of action for each of the conditions of use for which licensure is sought. This is further supported by a demonstration that both products have the same primary structure, corresponding to Met-GCSF and have highly similar higher order structure (see below).

Protein content

The concentration of Met-GCSF (mg/ml) in EP2006, US-licensed Neupogen and EU-approved Neupogen was determined by RP-HPLC. This attribute is evaluated as part of analytical similarity and to determine if EP2006 has the same strength as US-licensed Neupogen. In this case, to determine whether EP2006 has the same strength as US-licensed Neupogen, EP2006 must have the same total content of the GCSF in mass in a PFS and same GCSF concentration (in mass/ml) as US-licensed Neupogen. Given the regulatory requirement of "same strength" this attribute was evaluated by comparison of the product's descriptive statistics and graphical representation data and by statistical equivalence testing.

In response to IR dated October 3, 2014, Sandoz provided protein concentration data (expressed as percentage of declared protein content) from analysis of the following drug product lots, along with results of a statistical analysis:

- 11 EP2006 clinical
- 6 EP2006 commercial (derived from four EP2006 bulk drug product lots)
- 12 US-licensed Neupogen
- 49 EU-approved Neupogen

The Agency also conducted a statistical analysis of these data to confirm the assessment provided by Sandoz. The Agency's statistical analysis of the submitted data using equivalence testing showed that the protein content of EP2006 (clinical and proposed commercial drug product, n=17 lots) was not statistically equivalent to US-licensed Neupogen. A combined analysis of clinical and proposed commercial drug product is appropriate because the clinical and commercial drug products are comparable. Given that the number of lots (n=11) of the EP2006 clinical drug product used in the PK and PD similarity and clinical studies comparing EP2006 with EU-approved Neupogen was sufficient to conduct equivalence testing, statistical equivalence testing of these data was conducted by the statistics reviewer. The result of this analysis showed that the protein content of the clinical EP2006 drug product (n=11 lots) was statistically equivalent to US-licensed Neupogen (a similar outcome was obtained when EP2006 was compared to EU-Neupogen). This indicated that the inclusion of the proposed commercial drug product was the reason for the failed statistical equivalence test result using the combined data. It is important to note statistical equivalence testing of the commercial EP2006 drug product alone is limited because of the limited number of lots (n=6).

Descriptive statistics results calculated by the Agency using the data submitted by Sandoz in response to IR dated October 3, 2014 (Table 4, Appendix XXII) indicated that the percentage of declared content of the EP2006 lots manufactured by the commercial process (mean 97.23%) was **2.92%** lower (mean differences) than that of US-licensed Neupogen (mean 100.15%).

On October 31, 2014, the Agency asked Sandoz to address the Agency's concern and provide data demonstrating that the EP2006 proposed commercial drug product has the same "strength"[47] as US-licensed Neupogen.

On November 25, 2014, Sandoz responded to the Agency request. In the response, Sandoz clarified that the actual number of EP2006 commercial drug product lots used in the statistical analysis referred to above is four lots instead of six lots. The six EP2006 commercial drug product lots initially considered were determined to be not independent because four of those six EP2006 commercial drug product lots were split-fill lots from two EP2006 bulk drug product lots, resulting in only four independent EP2006 commercial drug product lots. To have an adequate number of lots for the statistical analysis, Sandoz provided protein content data from three additional independent EP2006 drug product commercial lots, meaning that there were a total of seven independent EP2006 drug product commercial lots (i.e., seven EP2006 bulk drug product lots) and two additional EP2006 clinical drug product lots included in the analysis. A statistical analysis comparing EP2006 commercial and clinical drug product and the comparator products (US-licensed Neupogen and EU-approved Neupogen) was also provided. A total of 20 lots of EP2006 drug product (7 lots of commercial EP2006 drug product and 13 lots of clinical

[47] See section 351(k)(2)(A)(i)(IV) of the PHS Act

EP2006 drug product), 12 lots of US-licensed Neupogen, and 49 lots of EU-approved Neupogen were ultimately analyzed.

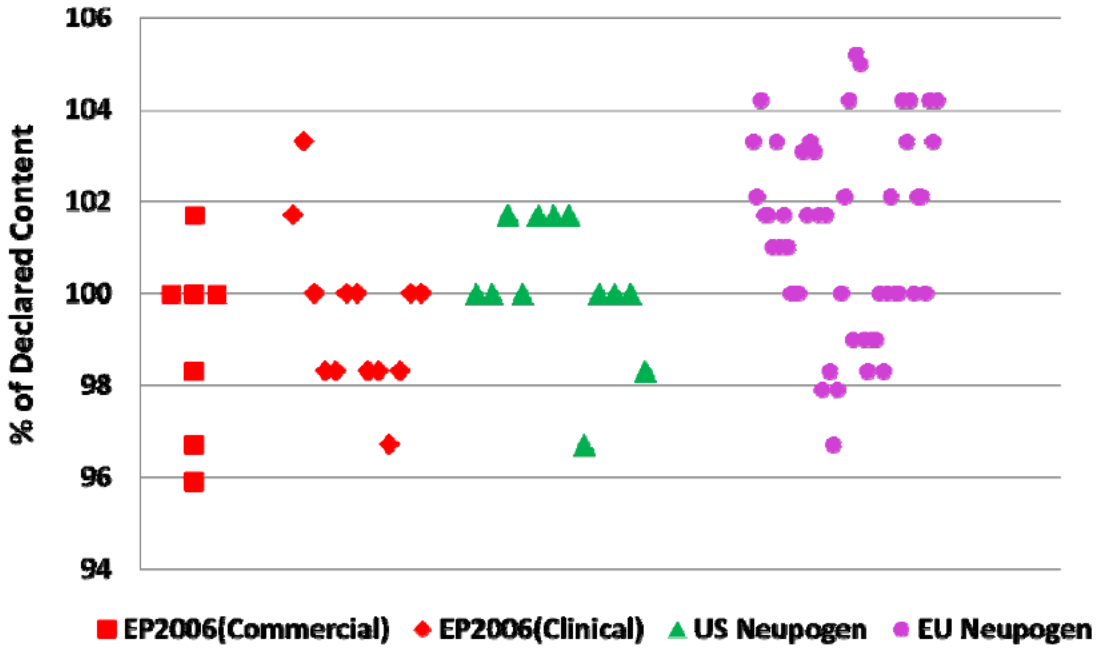
The Agency also conducted a statistical analysis of the submitted data (Table 2-2, Appendix XXII) to confirm the assessment provided by Sandoz. Table 1 and Figure 1 provide descriptive statistics and graphical representation of the percentage of declared content data. Table 2 summarizes the statistical equivalence testing results conducted by the Agency based on the data submitted by Sandoz.

Table 1: Descriptive Statistics for Protein Concentration Expressed as Percentage of Declared Content (%)

Product	# of lots	Min	Max	Mean	Standard Deviation	CV (%)
US-Neupogen	12	96.7	101.7	100.15	1.51	1.51%
EU-Neupogen	49	96.7	105.2	101.33	2.14	2.11%
Clinical EP2006	13	96.7	103.3	99.48	1.72	1.73%
Commercial EP2006	7	95.9	101.7	98.94	2.07	2.09%

Note: Table prepared by CMC statistics reviewer

Figure 1. **Content results of EP2006, US-licensed Neupogen and EU-approved Neupogen**



Note: Figure prepared by CMC statistics reviewer

Table 2. Statistical Equivalence Testing Results for Declared Content (%)

Product	# lots	Comparator product	# lots	Statistical Equivalence?
EP2006 (Clinical and commercial)	20	US-Neupogen	12	**Yes**[a]
EP2006 (Clinical and commercial)	20	EU-Neupogen	49	**Yes**[b]
EU-Neupogen	49	US-Neupogen	12	**Yes**[c]
EP2006 (Clinical)	13	US-Neupogen	12	**Yes**[d]
EP2006 (Clinical)	13	EU-Neupogen	49	**Yes**[e]
EP2006 (Commercial)	7	US-Neupogen	12	**No**[f]
EP2006 (Commercial)	7	EU-Neupogen	49	**No**[g]

Note: Table prepared by CMC statistics reviewer

[a] The 90% confidence interval (CI) of the mean difference between EP2006 clinical and commercial product and US-licensed Neupogen is (-1.87%, 0.15%), which is entirely within the equivalence acceptance criterion of (-2.26%, 2.26%)

[b] The 90% CI of the mean difference between EP2006 clinical and commercial product and EU-approved Neupogen is (-2.98%, -0.85%), which is entirely within the equivalence acceptance criterion of (-3.23%, 3.23%)

[c] The 90% CI of the mean difference between EU-approved Neupogen and US-licensed Neupogen is (0.27%, 2.09%), which is entirely within the equivalence acceptance criterion of (-2.26%, 2.26%)

[d] The 90% CI of the mean difference between EP2006 clinical lots and US-licensed Neupogen is (-1.78%, 0.43%), which is entirely within the equivalence acceptance criterion of (-2.26%, 2. 26%).

[e] The 90% CI of the mean difference between EP2006 clinical lots and EU-approved Neupogen is (-3.13%, -0.53%), which is entirely within the equivalence acceptance criterion of (-3.26%, 3.26%).

[f] The 85.2% CI of the mean difference between EP2006 commercial lots and US-licensed Neupogen is (-2.33%, 0.67%), which is not entirely within the equivalence acceptance criterion of (-2.28%, 2. 28%). A 85.2% CI is used to adjust for the limited number of lots.

[g] The 85.2% CI of the mean difference between EP2006 commercial lots and EU-approved Neupogen is (-3.71%, -0.20%), which is not entirely within the equivalence acceptance criterion of (-3.23%, 3.23%). A 85.2% CI is used to adjust the limited number of lots

The Agency's statistical analysis of the EP2006 protein content data (clinical and proposed commercial drug product) using equivalence testing now indicates that the percentage of declared content of EP2006 drug product (clinical and commercial, n=20 lots) is statistically equivalent to that of US-licensed Neupogen (n=12 lots) and EU-approved Neupogen (n= 49 lots) (Table 2).

Reviewer's assessment of the data supporting similar protein content and "same strength":

The results in Table 1 indicate that the mean value of the declared content (%) of EP2006 commercial drug product is ***1.21%*** *lower than that of US-licensed Neupogen. As noted in Table 2, the percentage of declared content of commercial EP2006 drug product lots alone (n=7) is not statistically equivalent to that of US-licensed Neupogen. The confidence interval (CI) for the*

mean difference was just -0.05% outside of the equivalence acceptance criteria. Similar equivalence testing results were obtained for the comparison of EP2006 commercial lots and EU-approved Neupogen (the CI for the mean difference was -0.48% outside of the equivalence acceptance criterion). The Agency interpreted the lack of statistical equivalence between commercial EP2006 drug product and US-licensed Neupogen to be the due to the limited number of EP2006 commercial lots (n=7) analyzed. However, as previously noted, a combined analysis of clinical and proposed commercial drug product is appropriate because the clinical and commercial drug products are comparable. These data support highly similar protein content between the three products under evaluation.

Although FDA had considered that the slightly lower protein content measurements of the proposed commercial EP2006 product was possibly due to a manufacturing issue (e.g., procedures to achieved the target protein content), the additional data (i.e. data from additional lots of EP2006 manufactured by the commercial process) provided by Sandoz indicate that the slightly lower content of the commercial product was due to the limited number of lots analyzed.

Based on FDA's review of the data provided, EP2006 (commercial and clinical) drug product was determined to have the same strength as the reference product, US-licensed Neupogen. The Agency's review of the Sandoz control strategy for protein content indicates that the strategy is adequate to ensure that the commercial drug product will have the same strength as the reference product, US-licensed Neupogen, throughout the shelf life of the EP2006 commercial drug product.

Clarity (Nephelometry)

Nephelometry measures the light scattering effect of suspended particles. It provides information about particle concentration and is therefore a measure of the clarity or turbidity of a solution. The results are provided in Appendix XXIII.

Similarity acceptance criteria

Product	**# lots**	**Mean (NTU)[48]**	**SD**	**Similarity acceptance criteria (range of mean ± 2SD)**
EP2006 DP	11	0.44	0.099	
EP2006 DS	6	0.56	0.175	
US-licensed Neupogen	3	0.47	0.081	0.31-0.63
EU-approved Neupogen	1	0.31		

Note: Table prepared by CMC reviewer

Reviewer's comment: *All lot values of EP2006 DP are within the acceptance criterion of mean ±2 SD calculated using the US-licensed Neupogen data. No acceptance criterion was defined using the EU-Neupogen data because there was only one lot. This one lot is within the US-licensed range. The turbidity of EP2006 DS is in general higher than that of EP2006 DP. This is*

[48] Nephelometric Turbidity Units

acceptable because the higher turbidity is likely due to higher concentration of GCSF (b) (4) *in EP2006 DS.*

Sub-visible particles by Micro flow imaging

Micro flow imaging (MFI) was used to determine the number of sub-visible particles (SVPs) in the range 2-5 µm, 5-10 µm and ≥ 25 µm. The results are provided in Appendix XXIII. In general, EP2006 DP (n= 6 lots) has lees particles of all evaluated sizes compared to US-licensed Neupogen (n=4 lots) and EU-approved Neupogen (n= 2 lots). Therefore no statistical analysis was conducted.

Reviewer's assessment on clarity and SVP

The results from nephelometry suggest that EP2006 contain less visible particles compared to US-licensed Neupogen and EU-approved Neupogen. Similarly, the MFI data (Appendix XXIII) show that EP2006 has fewer SVP in the ranges of 2-5 µm, 5-10 µm and ≥ 25 µm than US-licensed Neupogen and EU-approved Neupogen. Having less particles and SVP does not have a negative impact on drug product quality. EP2006 meets the current expectations for particles and SVPs for injectable products. Thus, the three products are determined to be similar with respect to these quality attributes.

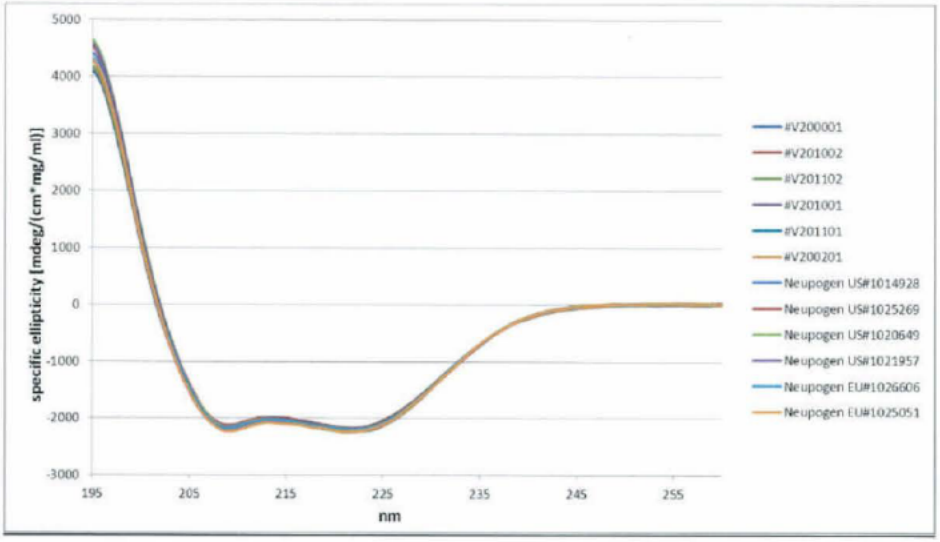
Higher order structure

Higher order structure (HOS) was evaluated by circular dichroism (far and near UV), ^{1}H NMR and ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) NMR spectroscopy. CD, 1D and 2D NMR spectra were analyzed visually whereas numerical CD data were analyzed by the mean ± 2SD approach (Tier 2/3).

<u>CD spectroscopy</u>

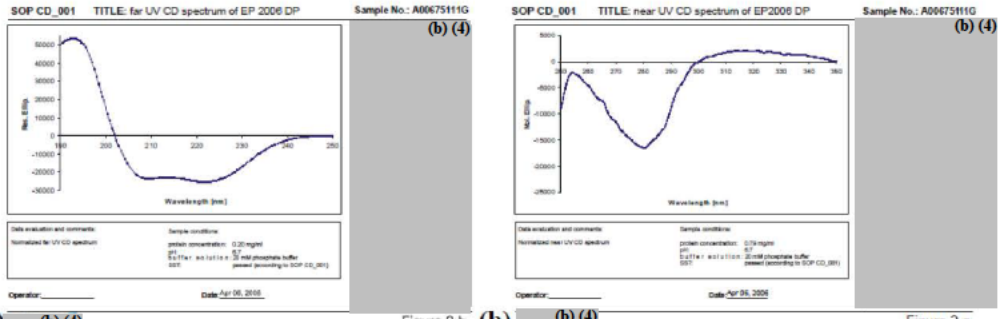
Far and near UV CD spectroscopy provides information about secondary (α-helix, β-sheet and random coil structures) and tertiary structure, respectively. Representative far and near UV CD spectra for EP2006, US-licensed Neupogen and EU-approved Neupogen are shown in Figures 5-9, 5-11 and 5-12.

Figure 5-9 Far UV CD spectra of EP2006 DP (GPG) and Neupogen® US and EU batches (study 1)



Overlay of CD spectra of EP2006 drug product PFS batches, manufactured at GPG (strength 300 mcg/0.5 mL: V201001, V201101, V200201; strength 480 mcg/0.8 mL: V200001, V201002, V201102), US licensed Neupogen® US PFS batches (strength 300 mcg/0.5 mL: 1020649, 1021957; strength 480 mcg/0.8 mL: 1014928, 1025269), and non-US licensed Neuogen® EU PFS batches (strength 300 mcg/0.5 mL: 1025051; strength 480 mcg/0.8 mL: 1026606).

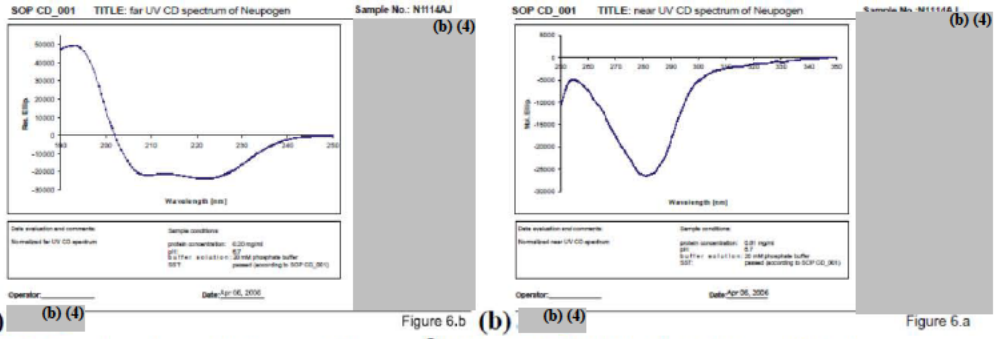
Figure 5-11 Far (a) and near (b) UV CD spectra of EP2006 DP (study 2)



(a) (b) (4) Figure 2.b (b) (b) (4) Figure 2.a

UV CD spectra of EP2006 drug product PFS batch A00675111G, 480 mcg/0.5 mL, manufactured at LEK.

Figure 5-12 Far (a) and near (b) UV CD spectra of Neupogen® EU (study 2)



a) (b) (4) Figure 6.b (b) (b) (4) Figure 6.a

UV CD spectra of non-US licensed Neuogen® EU batch N1114AJ, PFS, 480 mcg/0.5 mL

Spectrum transition point (specific ellipticity = 0) and ratio of specific ellipticity $(\theta_R)_{208}/(\theta_R)_{222}$ calculated from the far UV CD spectra are listed in Table 2-9 (Appendix XXIV). The table below provides similarity acceptance criteria calculated from these data.

Similarity acceptance criteria

Product	**# lots**	**$(q_R)_{208}/(q_R)_{222}$**			**Transition point (nm)**		
		mean	SD	Similarity AC (mean ± 2SD)	mean	SD	Similarity AC (mean ± 2SD)
EP2006 DP	6	201.7	0.16		0.957	0.014	
US-licensed Neupogen	10[49]	201.7	0.097	201.5-201.9	0.963	0.011	0.941-0.985
EU-approved Neupogen	2	201.6	0		0.976	0.002	

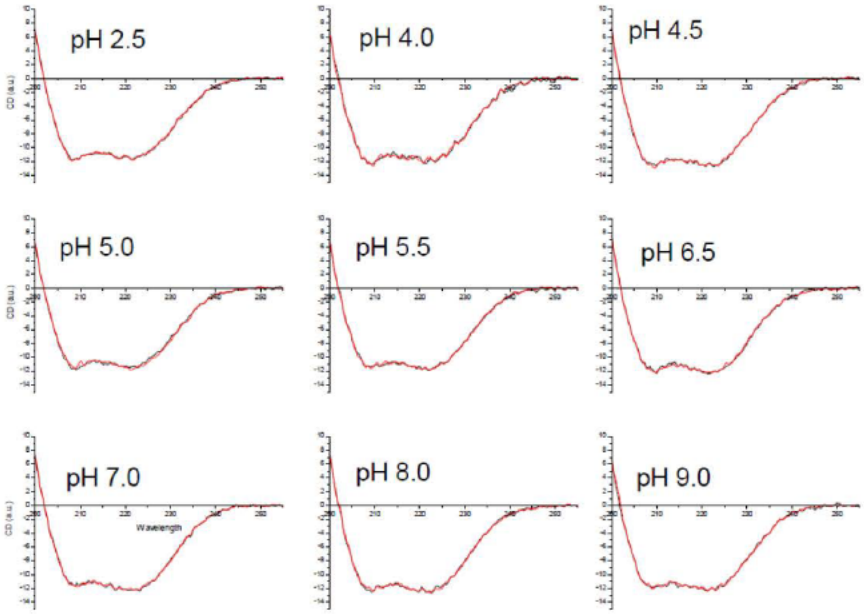
Note: Table prepared by CMC reviewer

Reviewer's comments: *all six lot values of EP2006 fall within the US-licensed Neupogen ranges of mean ± 2 SD calculated for specific ellipticity and transition point. No acceptance criteria were calculated for EU-Neupogen because only two lots were tested. However, as can be seen in Figure 5-9, the far UV CD spectra of US-licensed Neupogen, EU-approved Neupogen and EP2006 overlap. The near UV DC data were analyzed visually. Qualitatively, the near UV CD spectra are similar.*

In addition to the experiments described above, and in the context of evaluating if there are differences in HOS between EP2006 and EU-approved Neupogen that can explain the differences in the bioavailability for the steady state administration observed in study EP06-103, far and near UV CD at different pH were evaluated. No difference between the far and UV CD spectra of EP2006 and EU-Neupogen were identified (Figures 2-6 and 2-7).

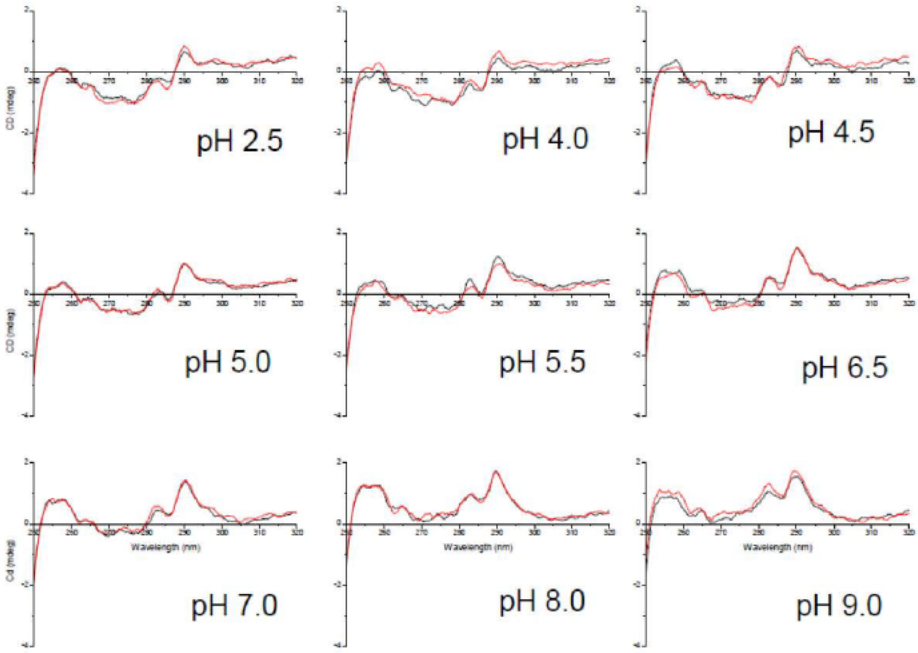
[49] Includes US-licensed Neupogen lots in vials (3 lots) and in PFS (7 lots). All other lot are in PFS

Figure 2-6 Far UV CD spectra of Neupogen and EP2006 drug product active ingredient at various pH values



Neupogen (red) and EP2006 drug product active ingredient (black). Spectra are normalized to same amplitude in order to highlight differences in spectra shape.

Figure 2-7 Near UV CD spectra of Neupogen (red) and EP2006 (black) active ingredient at various pH values

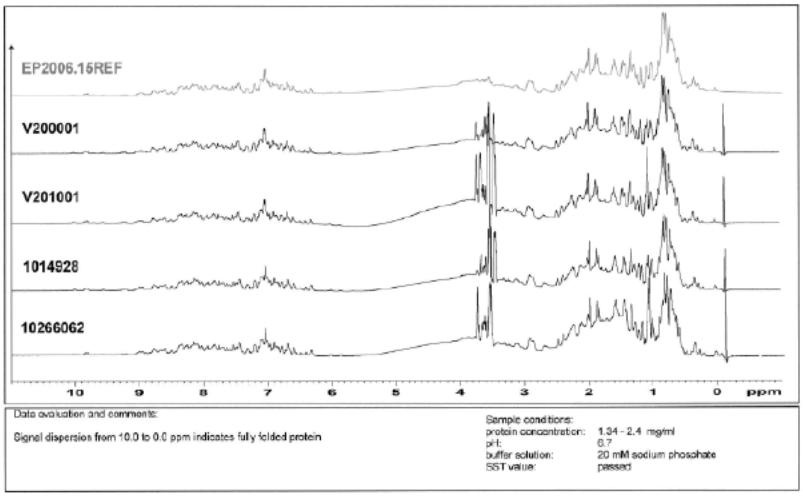


^{1}H NMR spectroscopy

^{1}H NMR spectroscopy provides information about the three dimensional structure of the protein. Presence of spread proton signals in the amide region (8.5 ± 3 ppm) is indicative of a protein in folded state. Overlapping of signals in the 8.5 ppm region is indicative of unfolded protein or a folded protein that requires different NMR conditions for its analysis. Three lots of EP2006 DP, one lot of EP2006 DS, one EP2006 RS, one lot of US-licensed Neupogen and two lots of EU-approved Neupogen were analyzed. Representative spectra of EP2006, US-licensed Neupogen and an EU-approved Neupogen are provided below. No significant difference in the spectra of EP2006 compared to US-licensed Neupogen and EU-approved Neupogen were detected.

Reviewer's comment: *the number of lots used for this assay is acceptable given that each experiment requires ~2 mg/ml protein (4-7 US-licensed Neupogen syringes).*

Figure 5-13 Superposition of full NMR spectra of selected batches (study 1)

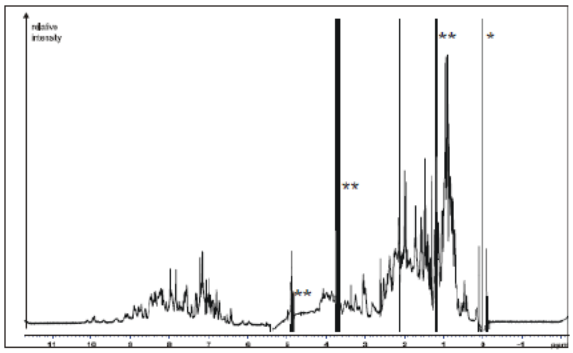


Superposition of full NMR spectra of selected batches (study 1): EP2006.15REF reference standard, EP2006 DP 480 mcg/0.8 mL and 300 mcg/0.5 mL from GPG (V200001, V201001), Neupogen® US 480 mcg/0.8 mL (1014928) and EU 480 mcg/0.5 mL (1026606) material are shown

Figure 5-15 1D-{^{1}H}-NMR spectrum of EP2006 DP 480 mcg/0.5 mL (A00675111G, LEK) (study 2)

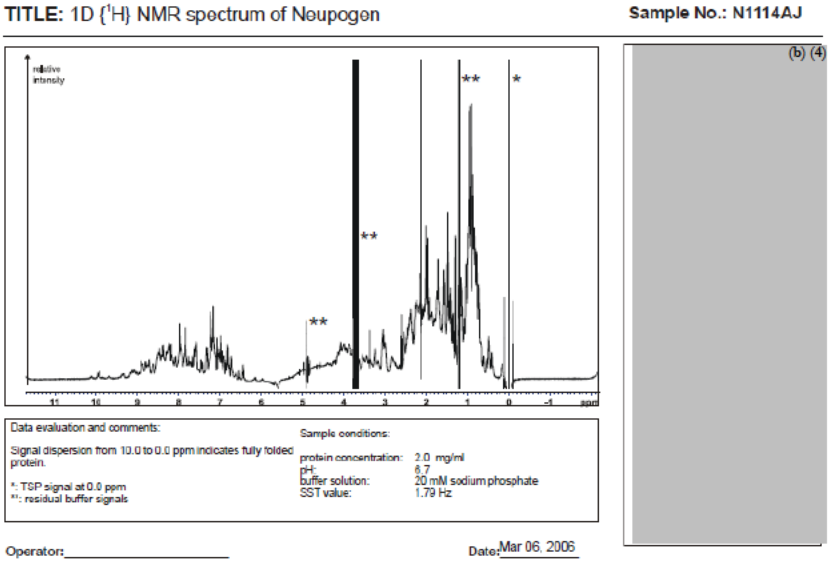
TITLE: 1D {^{1}H} NMR spectrum of EP2006 DP **Sample No.: A00675111G**

(b) (4)



Operator:_____ Date: Mar 06, 2006

Figure 5-16 1D-{^{1}H}-NMR spectrum of Neupogen® EU 480 mcg/0.5 mL (N1114AJ) (study 2)



Reviewer's note: *signals marked with asterisk correspond to buffer (phosphates) and polysorbate 80 signals. This is acceptable. NMR spectra usually show signals of the buffer and solvents used. Even a deuterated solvent has residual amounts of the corresponding non-deuterated solvent.*

^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) NMR

The signals in a ^{1}H-^{15}N HSQC spectrum correspond to N-H pairs (primarily amide groups). This technique provides better resolution than ^{1}H NMR and may be considered a structural fingerprint of the 3D structure of the protein. ^{1}H-^{15}N HSQC spectra of EP2006 DP (lots V200001, V201002 and V20112), US-licensed Neupogen and EU-approved Neupogen were acquired in both the Neupogen formulation (acetates, pH 4.0) and in the EP2006 formulation (glutamates pH 4.4.). The spectra provided in the submission correspond to the natural abundance isotopes (unlabeled proteins). Assignment of ^{1}H-^{15}N NMR signals has been reported previously[50]. Representative expansions of these spectra can be found in Appendix XXV.

[50] Zink, T. et al (1994). Structure ad Dynamics of the Human Granulocyte ColonyStimulating Factor Determined by NMR Spectroscopy. Loop mobility in a four-Helix-Bundle Protein. Biochemistry 33, 8453-8463 and http://www.bmrb.wisc.edu/data_library/summary/index.php?bmrbId=18291

Figure 5-15 **Overlay of EP2006 batch V201002 (cyan), Neupogen® US (orange) and Neupogen® EU (green) ^{1}H-^{15}N HSQC DP spectra in Neupogen® formulation.**

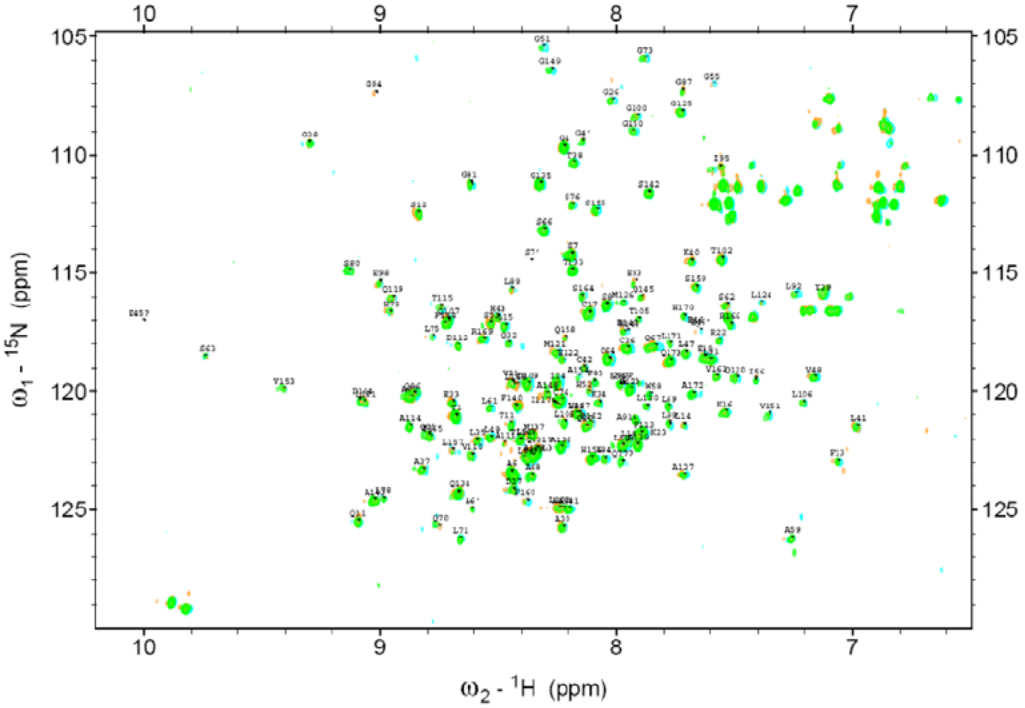
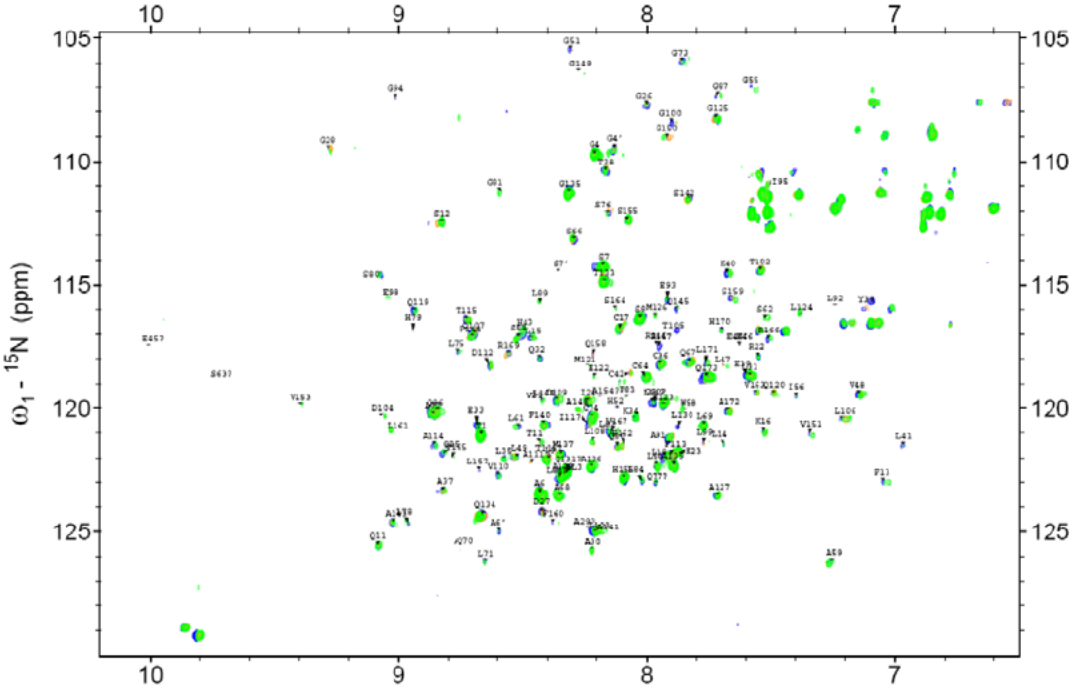


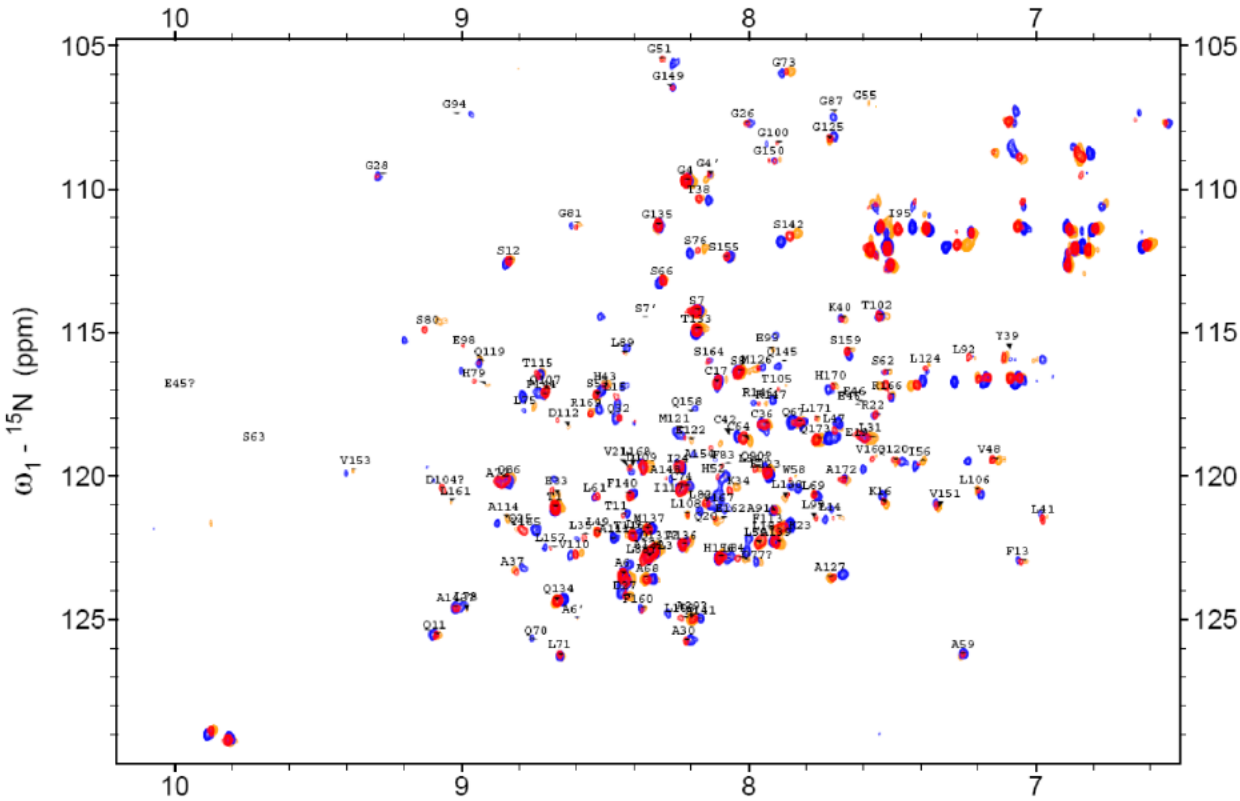
Figure 5-17 **Overlay of the EP2006 batch V200001 (blue), Neupogen® US (orange) and Neupogen® EU (green) 1H-15N HSQC DP spectra in the EP2006 DP formulation.**



Sandoz evaluated the sensitivity of the 2D NMR method by recording spectra at different pH (Figure 5-21). As expected, the chemical shifts of most of the N-H signals are different when the

pH of the formulation is changed. Also, satellite peaks corresponding to restricted dynamics of proline residues were observed for G4', A6', S7', and E98' residues. Representative spectra are provided below.

Figure 5-21 **Overlay of the EP2006 batch V201002 DP spectra in three different formulations – Sodium phosphate formulation pH 3.0 (blue), Neupogen® formulation, pH=4.0 (red) and EP2006 formulation, pH = 4.4 (orange).**



In addition to a qualitative comparison of the spectra, Sandoz conducted a statistical analysis of the data. The analysis considered chemical shifts of ^{1}H and ^{15}N signals, line width and signal to noise ratio. The evaluation met the predefined criteria of no significant peak shift between EP2006 and the comparator products in the two different formulations.

Reviewer's assessment of the data supporting similar HOS

HOS evaluated by far UV CD showed a folded protein with high content of α-helical secondary structure[51]. By far UV CD, the specific ellipticity and transition points of EP2006 are similar to those of US-licensed Neupogen and EU-approved Neupogen. The differences observed are within assay variability. Qualitative evaluation of the near UV CD spectra also shows similarity between the three products. This assessment is further supported by ^{1}H NMR and ^{1}H-^{15}N HSQC data. As show in Figures 5-13, 5-15, 5-16 (1D NMR) and 5-15 and 5-17 (2D NMR), the spectra of EP2006 are almost identical to the spectra of US-licensed Neupogen and EU-approved Neupogen.

[51] Tamada, T. *et. al.* (2006). PNAS, 103, **9**, 3135-3140.

An important piece of data provided by Sandoz was that the 2D NMR spectra of EP2006, US-Neupogen and EU-Neupogen were acquired in both the US-Neupogen and EP2006 formulations, reducing uncertainty about folding of the GCSF protein in these two different buffer compositions.

The use of sensitive methods such as 1D and 2D NMR, in addition to CD, provided strong evidence that the HOS of EP2006 is highly similar to that of the comparator products. Highly similar receptor binding and biological activity results also support a properly folded protein.

Product-related substances and impurities

A summary of product-related substances and impurities in the three products is presented in Table A below. Discussion of individual product-related substances and impurities follows this summary. Individual results for these species are presented in Appendix XXVI and their characterization is discussed in section 3.2.S.3.2.

Table A. Min-max levels of product-related substances and impurities

Species	EP2006 (%)	US-Neupogen (%)	EU-Neupogen (%)	Similar EP2006 vs US-Neupogen?	Similar EP2006 vs EU-Neupogen?	Similar US-Neupogen vs EU-Neupogen?
High molecular weight/aggregates (SEC)	≤ 1.0	≤ 1.0	≤ 1.0	Y	Y	Y
Oxidized variants by LC-MS[52] (sum 4 oxidized species at >22 months)	6.8-8.1	6.4 (one lot)	5.1-8.7	Y	Y	Y
Covalent dimers	0.0	0.0	0.0	Y	Y	Y
Partially reduced variants by RP-HPLC	0.0	0.0	0.0-0.3	Y	Y	Y
Formyl-methionine 1 By LC-MS	0.0	0.3	0.3-0.7	Y	Y	Y
His→Gln by LC-MS	0.0	0.0	0.0	Y	Y	Y
Asp→Glu by LC-MS	0.0	0.2-0.3	0.2-0.3	Y	Y	Y
Thr→Asp	0.0	0.0	0.0	Y	Y	Y

[52] Sites of oxidation are at the methionine residues 1, 122, 127 and 138

By LC-MS						
Succinimide By LC-MS	0.1-0.2	0.2	0.2-0.3	Y	Y	Y
N-terminal truncated (Sum of 8 variants)	1.4-2.1	1.4	0.9-1.5	Y	Y	Y
Norleucine variants[53] (Sum 4 variants)	0.2-0.7	0.3	0.0-0.5	Y	Y	Y
Deamidated variants	6.4-8.1	10.6	9.0-12.8	Y	Y	Y

Note: Table prepared by CMC reviewer

Reviewer's comment: *the assessment of stability indicating species (e.g. oxidized and deamidated species) was made by comparison of the levels of the species under evaluation in products of similar age.*

In general, the same type and levels of product-related species and impurities were identified in the three products. No impurity was found in EP2006 which was found absent in US-licensed Neupogen or Eu-approved Neupogen

High molecular weight variants/aggregates

High molecular weight (HMW) variants including oligomers, covalent dimers and aggregates were evaluated by size exclusion chromatography (SEC) and reduced and non-reduced SDS-PAGE (silver staining). Western blot was conducted for EP2006 DS (5 lots) and EU-approved Neupogen (1 lot).

In addition, orthogonal methods for aggregates were used to investigate whether there were differences in aggregates between the EP2006 and EU-approved Neupogen lots used in study EP06-103 that could be associated with the lower bioavailability of EP2006 compared to EU-Neupogen. The methods used include UV-Vis spectroscopy, 90° light scattering, and Nile red steady-state fluorescence anisotropy.

SEC

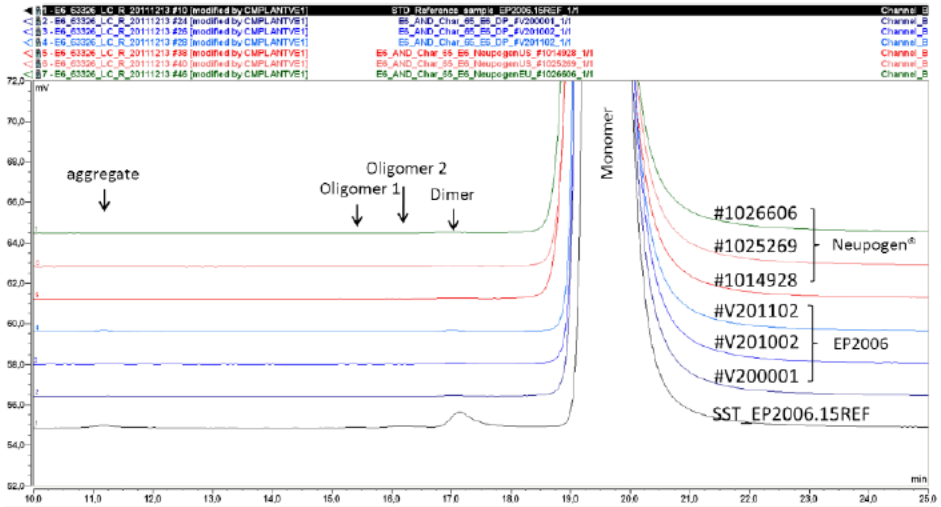
Tabular data for the lots analyzed are presented in Table 2-30, Appendix XXVI. The ability of the SEC method to detect monomeric, dimeric and HMW species was demonstrated by asymmetric filed flow fractionation and SEC-MALLS (Figure 4-24 show an aggregated sample WS07. Refer to sections 3.2.S.4.2 and 3.2.S.4.3).

A total of 10 lot of US-licensed Neupogen in PFS, 15 lots of US-licensed Neupogen in vials, 36 lots of EU-approved Neupogen in PFS and 17 lots of EP2006 in PFS were analyzed by SEC. All

[53] Methionine can be substituted nu Norleucine at positions 1, 122, 127 and 138

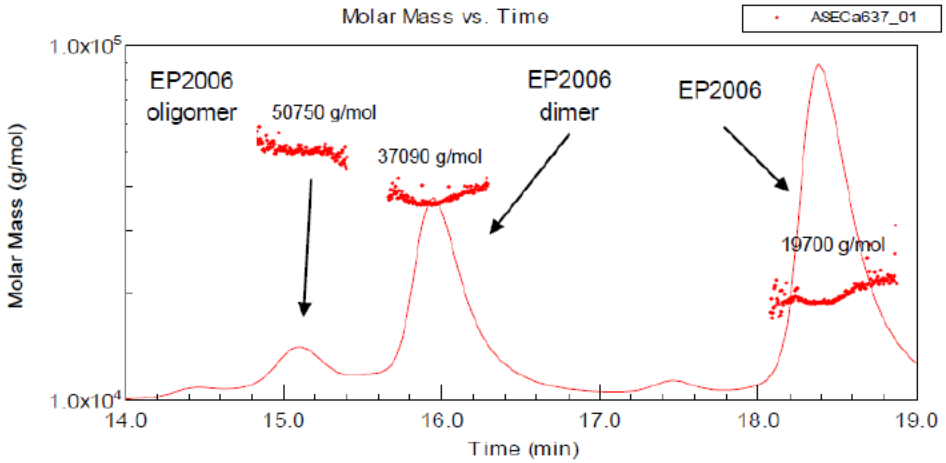
products had total of HMW species <0.1% (<LOQ). Representative chromatograms are presented below.

Figure 5-21 Superposition of SEC chromatograms of EP2006 DP, Neupogen EU and Neupogen US (study 1)



Superposition of SEC chromatograms of EP2006 DP 480 mcg/0.8 mL (GPG), Neupogen EU 480 mcg/0.5 mL (1026606) and Neupogen US 480 mcg/0.8 mL (1025269, 1014928)

Figure 4-24 SEC-MALLS analysis of EP2006 WS07

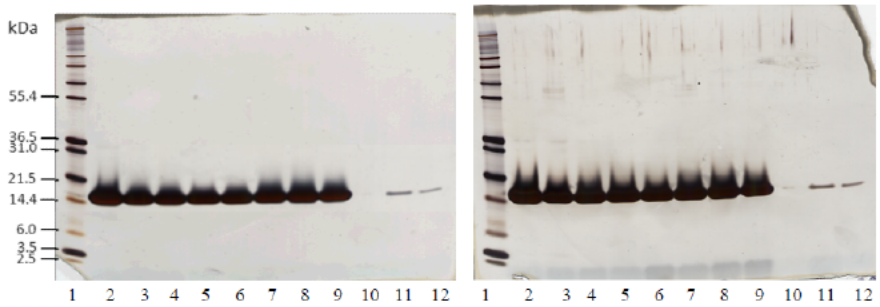


SDS-PAGE

Consistent with SEC, the SDS-PAGE gels for EP2006, US-licensed Neupogen and EU-approved Neupogen shown below indicate low levels of HMW species. No low molecular weight species are detected by SDS-PAGE. Western blot analysis of EP2006 DS and EU-Neupogen shows only one in each product band migrating at the same location (Figure 1-15).

Figure 5-33 SDS-PAGE gels with silver staining of LA-EP2006 DP (GPG) and Neupogen® US and EU (study 1)

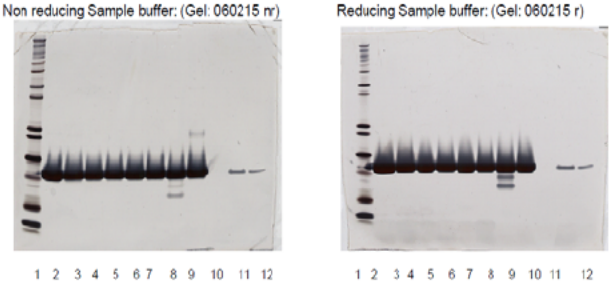
non reducing conditions 111220-A_n-red reducing conditions 111220-A_red



Lane	Sample	concentration [µg/ml]	amount applied [µg]
1	Novex Mark 12	/	/
2	EP2006.15 REF (Reference a)	600	9
3	EP2006 DP 480 mcg/0.8 mL (V200001)	300	4.5
4	Neupogen® US 480 mcg/0.8 mL (1014928)	300	4.5
5	Neupogen® EU 480 mcg/0.5 mL (1026606)	300	4.5
6	EP2006 DP 480 mcg/0.8 mL (V201002)	300	4.5
7	Neupogen® US 480 mcg/0.8 mL (1025269)	300	4.5
8	Neupogen® EU 300 mcg/0.5 mL (1025051)	300	4.5
9	EP2006 DP 480 mcg/0.8 mL (V201102)	300	4.5
10	sample buffer	/	/
Lane	**Sample**	**concentration [µg/ml]**	**amount applied [µg]**
11	EP2006.15 REF (Reference c)	2	0.02
12	EP2006.15 REF (Reference c)	2	0.01

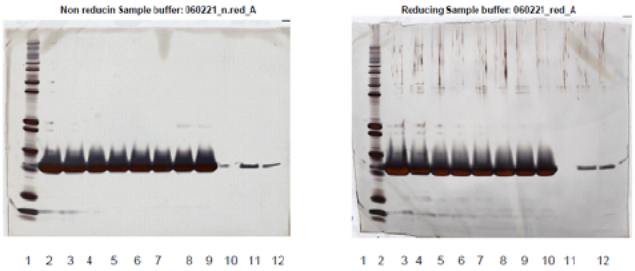
Figure 1-14 Silver stained SDS-PAGE gel of EP2006 drug product samples

Non reducing Sample buffer: (Gel: 060215 nr) Reducing Sample buffer: (Gel: 060215 r)



Lane	Sample	Concentration [mg/mL]	Amount protein applied [µg]
1	Novex Mark 12	/	/
2	Reference substance E6WS14	1.97 mg/mL	9
3	A03941609F	960 mcg/mL	4.5
4	A00657409G 300 mcg/0.5 mL	600 mcg/mL	4.5
5	A00675111G 480 mcg/0.5 mL	960 mcg/mL	4.5
6	A00675011G 300 mcg/0.5 mL	600 mcg/mL	4.5
7	A00675211G 480 mcg/0.5 mL	960 mcg/mL	4.5
8	Not relevant sample		
9	Not relevant sample		
10	Sample buffer	/	/
11	Reference substance E6WS14	1.97 mg/mL	0.02
12	Reference substance E6WS14	1.97 mg/mL	0.01

Figure 5-34 SDS-PAGE gels with silver staining of Neupogen® EU (study 2)

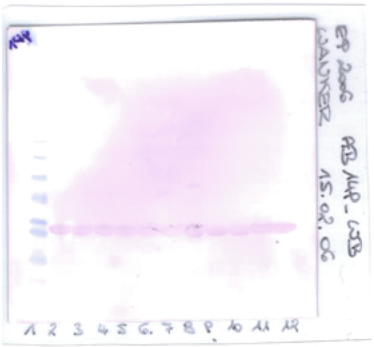


Lane	Sample	Concentration of sample	Mass applied [µg]
1	Novex Mark 12	/	/
2	Reference substance EP2006WS14	1.97 mg/mL	9
3	Neupogen® 480 mcg/0.5 mL (N0875AA)	960 µg/mL	4.5
4	Neupogen® 300 mcg/0.5 mL (N1144AE)	600 µg/mL	4.5
5	Neupogen® 300 mcg/0.5 mL (N1113AE)	600 µg/mL	4.5
6	Neupogen® 480 mcg/0.5 mL (N1114AJ)	960 µg/mL	4.5
7	Not relevant sample		
8	Not relevant sample		
9	Not relevant sample		
10	Sample buffer	/	/
11	Reference substance EP2006WS14	1.97 mg/mL	0.02
12	Reference substance EP2006WS14	1.97 mg/mL	0.01

Reviewer's note: *the 0.01 µg sample (lane 12) correspond to 0.22% of the sample loaded in the lane*

Reviewer's comment: *Note that the gels in Figures 1-14 and 5-34 include "non-relevant samples". The identity of the non-relevant samples was investigated on inspection. For these samples, lanes 8 and 9 correspond to RS21 and 030416S.*

Figure 1-15 SDS-PAGE gel with Western blot of EP2006 drug substance samples



Lane	Sample	Concentration [mg/mL]	Amount protein applied [µg]
1	See Blue marker		
2	RM/48200402/26	1.82	3.1
3	RM/48200403/29	1.98	3.1
4	RM/48200404/28	1.86	3.1
5	RM/48200405/27	1.73	3.1
6	RM/48200406/28	1.95	3.1
7	RM/48200407/27	1.82	3.1
8	Not relevant sample		
9	Not relevant sample		
10	Neupogen N0875AA	0.960	3.1
11	Gran 5X02U	0.250	3.1
12	Reference substance E6WS14	1.97	3.1

<u>HOS by UV-Vis spectroscopy, 90º light scattering, Nile red steady-state fluorescence anisotropy, zeta potential and hydrodymamic radius</u>

The results from UV-Vis spectroscopy, 90º light scattering, and Nile red steady-state fluorescence anisotropy studies indicated that EU-approved Neupogen has slightly higher levels of aggregates compared to EP2006. This assessment is based on:

1) Higher background absorption in the UV region of 320nm – 400 nm for EU-approved Neupogen compared to EP2006
2) Higher 90º light scattering for EU-approved Neupogen compared to EP2006
3) Higher anisotropy fluorescence observed for EU-approved Neupogen compared to EP2006

Representative data are presented in Figure 2-3 and 2-4, and 2-5, pages 104-106 in document "Biosimilarity EU-comparator", located in 3.2.R.

In addition, zeta potential and hydrodymamic radius of EP2006 formulated in acetates and glutamates were determined as a measure of the propensity of EP2006 to aggregate. Zeta potential is used to predict the relative stability of aqueous colloidal solutions. If the zeta potential is >30 mV or < -30 mV, protein molecules tend to repel each other, which increases their stability. As the zeta potential moves toward 0 mV (the isoelectric point), the possibility of aggregation increases, leading to reduced stability.

Zeta potential and hydrodynamic curves are shown below. As can be seen in the figures, the zeta potential of EP2006 (green line) is similar in both formulations (z potential is approximately 20 mV until pH 5.1). The isoelectric point (pI) of EP2006 is also similar in both formulations (pI 5.91 for glutamate and 5.89 for acetate).

In addition, Z-average; which provides a measure of the hydrodynamic radius, shows that in both formulations, EP2006 remains constant until pH 5.3 when the diameter of the particles increases rapidly.

The experiments show that both solutions exhibit similar zeta potential values over the evaluated range of pH 3.7-6.0 as an indicator of similar surface charge density of EP2006 which in turn indicate similar stability of EP2006 in in acetates and glutamates.

Figure 2-8 Results of zeta potential and z-average of EP2006 active ingredient formulated according to EP2006 drug product

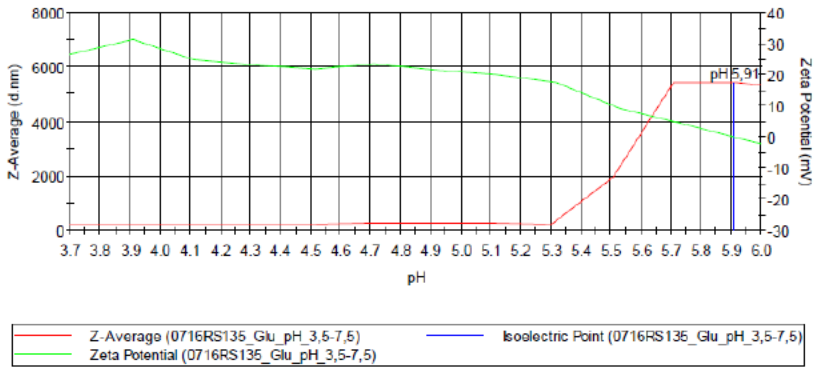
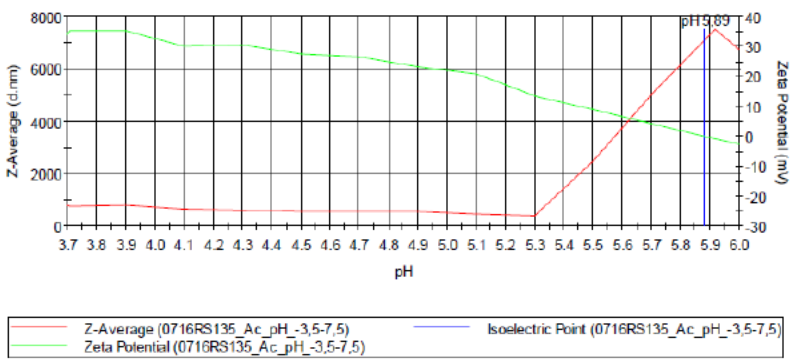


Figure 2-9 Results of zeta potential and z-average of EP2006 active ingredient formulated according to Neupogen



Reviewer's assessment of HMW species:

The total HMW species, including covalent dimers determined by SEC are similarly low (≤ 0.1%) in US-licensed Neupogen, EU-approved Neupogen, and EP2006. These data are in agreement with non-reducing and reducing SDS-PAGE analysis where no bands above 0.01 µg are observed (e.g. Figure 5-33). The ability of the SEC method to detect monomeric, dimeric and HMW species was demonstrated by asymmetric field flow fractionation and SEC-MALLS (refer to sections 3.2.S.4.2 and 3.2.S.4.3).

The low levels of HMW species detected in the three products by SEC and SDS-PAGE is in agreement with the results of the zeta potential and hydrodymamic radius experiments which

show that EP2006 in acetates and glutamates solutions exhibit similar zeta potential values over the pH range of 3.7-6.0. A similar zeta potential value is as an indicator of similar surface charge density of EP2006 which in turn indicate similar stability (i.e. propensity of EP2006 to aggregate) in acetates and glutamates.

In addition, no size variants are detected by Western blot. Western blotting show a single band for EP2006 DS and EU-approved Neupogen samples.

Taken together, the SEC, SDS-PAGE, the zeta potential and hydrodymamic radius, and Western blot data indicate that the levels of HMW species in EP2006 is similar (≤ 0.1% by SEC) to US-licensed Neupogen and EU-approved Neupogen.

Oxidized species

All four methionine residues (Met 1, Met 121, Met 122 and Met 138) in GCSF are susceptible to oxidation. Oxidized Met-GCSF species exhibit lower potency compared to native Met-GCSF[54]. The order of oxidation in EP2006 is Met 1> Met 138 > Met 127 > Met 122. Characterization of oxidized species is described in section 3.2.S.3.2.

Oxidized species were evaluated by RP-HPLC and LC-MS. Oxidized species at Met 122, Met 127, Met 138 and Met 127 +138 were monitored by RP-HPLC (Figure 1-21) whereas oxidized species at Met 1, Met 122, Met 127 and Met 138 were monitored by LC-MS. It is important to indicate that the quantitative results of RP-HPLC cannot be compared to the results from LC-MS because of different detection/quantification techniques (UV vs MS).

For analytical similarity purposes, oxidized species were evaluated by comparison of individual EP2006 values to an acceptance criterion defined as mean ±2SD (Tier-2) determined based on the data from the comparator products (US-licensed Neupogen or EU-approved Neupogen).

<u>RP-HPLC</u>

The oxidized species Met 122ox, Met 127ox, Met 138ox and Met 127ox +138ox were detected by RP-HPLC (Figure 1-21). Individual results for the three products are provided in Appendix XXVI. The data correspond to products stored at 2-8 ºC for 5 months for EP2006 (6 lots), 11-26 months for US-licensed Neupogen (4 lots) and 16-18 months for EU-approved Neupogen (5 lots). Similarity acceptance criteria and the results of the comparison of individual results of EP2006 (Appendix XXVI) against these criteria are summarized in Table A.

[54] Lu, H.S. et.al. (1999). Arch. Biochem. Byophis 362: 1-11

Figure 1-21 Overlay of RP-HPLC chromatograms of 480 mcg/0.5 mL and 480 mcg/0.8 mL batches (FLD)

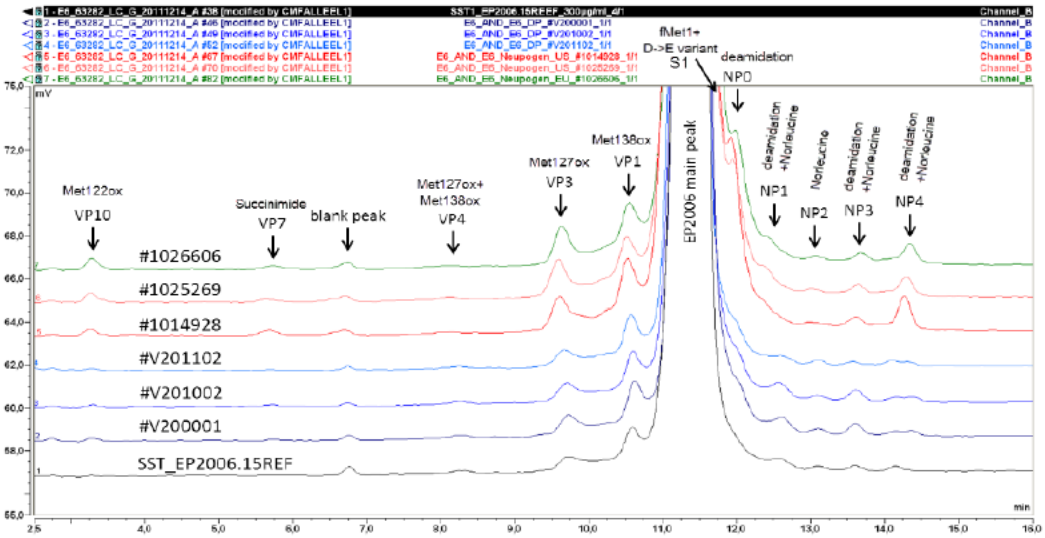


Table A. Similarity acceptance criteria (mean ± 2SD) for oxidized species determined by RP-HPLC

Species	Similarity Acceptance criteria (US-Neupogen)[55]	Similarity Acceptance criteria (EU-Neupogen)[56]	EP2006[57] vs US-Neupogen	EP2006 vs EU-Neupogen	EU-Neupogen vs US-Neupogen
%Met122ox (VP10)	≤0.1 No range was calculated because most values are ≤0.1	≤0.1 No range was calculated because most values are ≤0.1	Highly similar	Highly similar	Highly similar
%Met 127ox (VP3)	0.5 ± 0	0.31- 0.59	Highly similar	Highly similar	Highly similar
%Met 138ox (VP1)	0.66-0.94	0.8 ± 0	Highly similar	Highly similar	Highly similar
%Met127ox + 138ox (VP4)	≤0.1 No range was calculated because most values are ≤0.1	≤0.1 No range was calculated because most values are ≤0.1	Highly similar	Highly similar	Highly similar

Note: Table prepared by CMC reviewer

[55] The age of the reference product lots (n=4) was 11-26 months
[56] The age of the EU-Neupogen lots (n=4) was 16-18 months
[57] The age of EP2006 lots analyzed (n=6) was 5 months

In addition, sum of oxidized species (pre-peaks) data obtained from the RP-HPLC method was provided for the following products (Table 2-25, Appendix XXVI):

- EP2006 in PFS (17 lots)
- US-licensed Neupogen in PFS (12 lots)
- US-licensed Neupogen in vials (19 lots)
- EU-approved Neupogen in PFS (31 lots)

Min-max values of sum of oxidized species are shown in Table 2-29

Table 2-29 Oxidized variants: Comparison of min – max values

	EP2006 DP	Neupogen® US PFS	Neupogen® US PFS and vials	Neupogen® EU
Oxidized variant %	0.7 – 1.4	0.6 – 1.6	0.6 – 2.2	0.8 – 1.6

Reviewer's comment: *the levels of each of the four oxidized species and sum of oxidized species summarized in Tables A and 2-19 are similar between the three products.*

LC-MS

RP-HPLC coupled with MS was used to evaluate the following singly oxidized species: Met1ox, Met122ox, Met 127ox, and Met 138ox. The tested drug product samples did not contain sufficient amounts of variants oxidized at more than one methionine to enable their direct identification.

The RP-HPLC-MS data provided (Appendix XXVI) correspond to products stored at 2-8 ºC for 22 months (EP2006, 6 lots), 27 months for US-Neupogen (1 lot) and 16-29 months for EU-Neupogen (8 lots). Similarity acceptance criteria and the results of the comparison of individual results of EP2006 (Appendix XXVI) against these criteria are summarized in Table B.

Table B. Similarity acceptance criteria (mean ± 2SD) for oxidized species determined by LC-MS

Species	Similarity Acceptance criteria (US)[58]	Similarity Acceptance criteria (EU)[59]	EP2006[60] vs US-Neupogen	EP2006 vs EU-Neupogen	EU-Neupogen vs US-Neupogen
%Met1ox	1.9	0.58-3.18	The levels of this species are ~0.4-1.1% higher in EP2006. See discussion below	The levels of this species are ~0.1-0.6% higher in EP2006. See discussion below	Highly similar
%Met 122ox	0.3	0.21-0.51	Highly similar	Highly similar	Highly similar
%Met 127ox	2.0	1.42-2.54	Highly similar	Highly similar	The levels of this species are ~0.7% higher in EU-Neupogen. See discussion below
%Met 138ox	2.7	1.39-3.43	The levels of this species are ~0.4-0.6% higher in EP2006. See discussion below	Highly similar	The levels of this species are ~1.3% higher in EU-Neupogen. See discussion below

Note: Table prepared by CMC reviewer

Reviewer's assessment of oxidized species:

The RP-HPLC and LC/MS data along with characterization data of the 4 oxidized species indicate that EP2006, US-licensed Neupogen and EU-approved Neupogen have the same type of oxidized species. The levels of oxidized species evaluated by RP-HPLC (Table A) are similar in the three products.

Although LC-MS data do not necessarily reflect the actual levels of these species in the products because MS tend to enrich some ions and thus overestimate their amount. This technique can be used to compare levels. The LC/MS results show slight differences in the levels of the singly oxidized species Met1ox, Met 138ox and Met 127ox as discussed below.

EP2006 has ~0.4-1.1% more of Met1ox species and 0.4-0.6% more of Met138ox species compared to the levels of one lot of US-licensed Neupogen. The slightly Met1ox species (~0.4-

[58] The age of US-licensed Neupogen lots (n=1) was 27 months
[59] The age of the EU-approved Neupogen lots (n=8) was 19-29 months
[60] The age of EP2006 lots analyzed (n=6) was 22 months

1.1%, at 22 months) is unlikely to have an impact on potency based on the low levels and data reported in the literature that indicates that oxidation of Met-1 reduces potency by 20% [61]*. No biological data are reported for the singly oxidized 138ox species. However, oxidation of both Met-1 and Met-138 reduces potency by 80%. A slight difference in Met-138 oxidation of 0.4-0.6% is unlikely to have an impact on biological activity. This is confirmed by potency data monitored during stability under the recommended conditions of these same lots (commercial validation lots). During storage, the level of oxidized species are expected to increase over time, however, the potency of these lots remains stable for up to 24 months. Similar outcomes are obtained from the comparison of EP2006 and EU-Neupogen and between US-licensed Neupogen and EU-approved Neupogen.*

Overall, EP2006, US-licensed Neupogen and EU-approved Neupogen have the same type and levels of oxidized species.

Partially reduced species

Partially reduced species (b) (4) was identified in (b) (4) EP2006 (Figures 4-37 and 4-38, refer to characterization section). However, this variant is not present in EP2006 DS and DP. For the purposes of analytical similarity, this attribute was evaluated by comparison of min-max values (Tier-3). Refer to Table B below.

Sequence variants

Sequence variants were evaluated by top down LC-MS using a high accuracy Orbitrap system. Three types of sequence variants were identified in EP2006 and EU-approved Neupogen as follows (refer to section 3.2.S.3.2):

Table A. Levels of sequence variants

Sequence variant	(b) (4)	Present in EP2006 DS and DP?	Present in EU-approved Neupogen?	Comments
Asp → Glu		No	Yes	Met-GCSF has 4 Asp residues, D28, D105, D110 and D113. Substitution of D for E appears to occur in either of the last three Ds (Figures 4-32 and 4-33, refer to characterization section).
His → Gln		No	No	EP2006 has 5 His residues, H-44, H-53, H-80, H-157, and H-171. Two His → Gln sequence variants were identified. One variant elutes at RT of 31.94 min and has a Q for H substitution at either H-44 or H-53 (Figure 4-34). The second variant elutes at RT of 32.17 min and has a Q for H

[61] Lu, S.H. et al. (1999). Arch. Biochem Biophys. 362, 1-11.

	(b) (4)			substitution at H-157 (Figure 4-35, refer to characterization section).
Thr → Asp		No	No	The substitution of the EP2006 Thr → Asp sequence variant (TR 33.58 min) was located at position 134 (Figure 4-36, refer to characterization section).

Note: Table prepared by CMC reviewer

Evaluation of these species in the tree products by LC-MS was provided (Appendix XXVI). For the purposes of analytical similarity, this attribute is considered a Tier-3 attribute. The results are summarized in Tables B and C below.

Formylated Methionine-1

Formylated Met-1 was evaluated by LC-MS (Figure 4-31 in section 3.2.S.3.2). The fMet1 was identified in EU-approved Neupogen lot 1028082 and US-Neupogen but not in EP2006 DS, DP or (b) (4) Refer to analytical characterization for LC-MS data and Tables B and C below. For the purposes of analytical similarity, this attribute is considered a Tier-3 attribute.

Succinimide species

Formation of aspartate in EP2006 and US-licensed Neupogen was evaluated by LC-MS (ESI, Figure 4-30). Characterization of this variant is discussed in the characterization section. For the purposes of analytical similarity, this attribute was evaluated by comparison of min-max values (Tier-3).

Table B. Evaluation of succinimide, Norleucine, fMet1, D→E and partially reduced species by RP-HPLC

Product (Age) [62]	**# lots**	**Succinimide variant**	**Norleucine**	**fMet1+ D→E**	**Partially reduced**
EP2006 DS (18-30 months)	6		0.3-0.8	0.0	0.0
EP2006 DP (5 months)	6	0.0	0.4	0.0	0.0
US-licensed Neupogen (11-26 months)	4	<0.1	0.4	1.6-2.1	0.0
EU-approved Neupogen (16-18 months)	2-5	<0.1	0.0-0.4	0.0-1.7	0.0-0.3

Note: Table prepared by CMC reviewer

[62] Stored at 2-8 °C

Table C. Evaluation of succinimide, Norleucine, fMet1, D→E and partially reduced species by LC-MS

Product (Age) [63]	**# lots**	**D→E**	**Succinimide variant**	**fMet1**	**Covalent dimer**
EP2006 DP (22 months)	6	0.0	0.1-0.2	0.0	0.0
US-licensed Neupogen (27 months)	1	0.2	0.2	0.3	0.0
EU-approved Neupogen (16-29 months)	8	0.2-0.3	0.2-0.3	0.3-0.7	0.0

Note: Table prepared by CMC reviewer

(b) (4)

(b) (4)

Acetylated species

Acetylation in proteins expressed in E. coli can occur at the N-terminus or at lysine residues. No acetylated variants are identified in EP2006 DS or DP (b) (4) Given that the impurity is absent in the DP, there is no concern or impact in analytical similarity.

Phosphogluconoylated species

No phosphogluconoylated variants were identified in EP2006 DS or DP (b) (4) (b) (4) (b) (4) Given that the impurity is absent in the DP, there is no concern or impact in analytical similarity.

Reviewer's comment: *The levels of partially reduced, succinimide, formylated methionine-1,* (b) (4)*, acetylated, and phosphogluconoylated species and sequence variants is quite low in the three products. The results summarized in Tables A. B and C support that the type and levels of these species is highly similar in the three products.*

[63] Stored at 2-8 °C

N-terminal truncated variants

LC-MS/MS comparative analysis of EP2006, 16REF, EP2006 DP #201002 and EU-Neupogen 1036996 indicated that both products contain –M, –MT, –MTPL, and –MTPLG variants in different amounts (< 1.0% individual variants). EP2006 has also –MTPLGP whereas EU-approved Neupogen has –MTPLGPA (Figures 4-21 to 4-23). For the purposes of analytical similarity, this attribute was evaluated by comparison of individual values to an acceptance similarity criterion defined as mean ±2SD (Tier-2).

Figure 4-21 RP-HPLC-MS detection of truncated variants in EP2006 drug substance (EP2006.16REF)

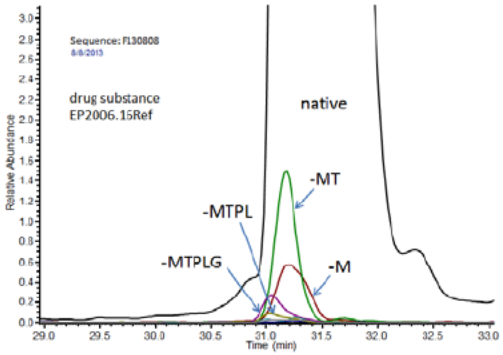


Figure 4-22 RP-HPLC-MS detection of truncated variants in EP2006 drug product #201002

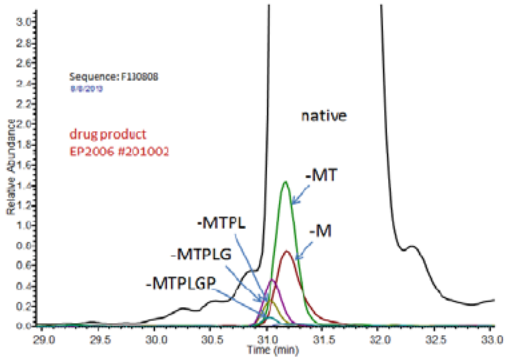
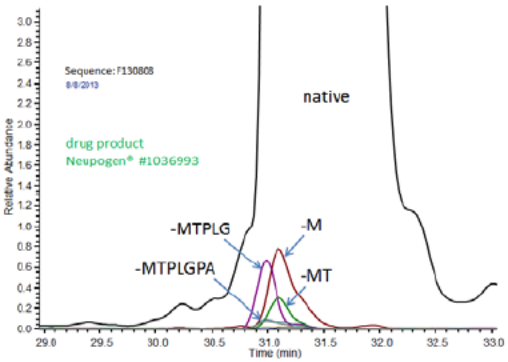


Figure 4-23 RP-HPLC-MS detection of truncated variants in Neupogen US #1036993



Semi-quantitative assessment of these species by LC-MS is summarized in Table D. Briefly, in EP2006 variants -M (≤0.7%) and –MT (≤0.9%) dominate the species of truncated variants in terms of abundance, followed by variants –MTPLG (≤0.3%) and –MPTL (≤0.1%).

In EU-approved Neupogen® variants -M (≤0.6%) and –MTPLG (≤0.5%) dominate, followed by variants -MT (≤0.2%) and –MTPLGPA (≤0.1%). A similar pattern is observed for US-Neupogen. Sandoz states that the "slight difference between EP2006 and Neupogen is considered not relevant in terms of safety and efficacy as the first 10 amino acids are neither part of the protein's structured region nor part of the receptor binding interface (Layton and Hall 2006)".

Table D. Similarity acceptance criteria and similarity assessment of the three products

Truncated Variant	EP2006 (10 lots, vial and PFS) % Min-max	US-Neupogen (7 lots, vials and PFS) Mean ± 2SD %	EU-Neupogen (8 lots) Mean ± 2SD %	EP2006 &US-Neupogen similar?	EP2006 & EU-Neupogen similar?	US-Neupogen & EU-Neupogen similar?
-M	0.3-0.7	0.25-0.7	0.5-0.7	Y	Y	Y
-MT	0.7-1.0	0.1-0.3	0.1-0.3	Y (See discussion in text)	Y (See discussion in text)	Y
-MTP	0.0	0.0	0.0	Y	Y	Y
-MTPL	0.1-0.2	0.0	0.0	Y	Y	Y
-MTPLG	0.1-0.4	0.2-0.6	0.3-0.5			
-MTPLGP	0.0-0.1	0.0	0.0	Y	Y	Y
-MTPLGPA	0.0-0.1	0.1	0.0-0.1 (min-max range)	Y	Y	Y
-MTPLGPAS	0.0	0.0	0.0-0.0	Y	Y	Y
Sum 8 variants	1.2-2.5%	1.0-1.4%	0.9-1.5%	Y (See discussion in text)	Y (See discussion in text)	Y (See discussion in text)

Note: Table prepared by CMC reviewer

In response to IR dated October 3, 2014. Sandoz provided additional data on sequence variants. These data correspond to the analysis of 31 lots of US-Neupogen (PFS and vials), 53 lots of EU-Neupogen (PFS) and 17 lots of EP2006. The data are in agreement with the analysis summarized above.

In addition, levels of total truncated species were evaluated by AEX. EP2006 DS and DP have slightly (0.6-0.8%) more truncated species than EU-approved Neupogen.

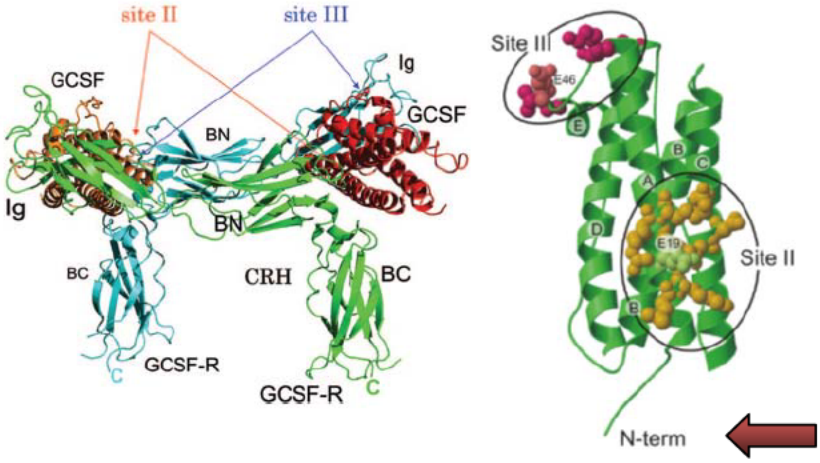
Table 4-13 Impurity levels by AEX; samples, EP2006 drug substance, EP2006 drug product and Neupogen

Product	Batch	Storage	Truncated variants	Deamidated variants
EP2006 drug substance	#RM/48200403	19 months at -20°C	1.0 %	0.1 %
	#RM/48200404	19 months at -20°C	1.0 %	0.2 %
	#RM/48200405	19 months at -20°C	1.1 %	0.2 %
EP2006 drug product	#A00657409G	5 months at 2-8°C	1.1 %	0.9 %
	#A00675111G	3 months at 2-8°C	1.1 %	0.8 %
	#A00675011G	3 months at 2-8°C	1.2 %	0.7 %
Reference product Neupogen®	#N1144AE (EU)	6 months at 2-8°C	0.4 %	2.4 %
	#N1114AJ (EU)	9 months at 2-8°C	0.4 %	2.1 %
	#N1113AG (EU)	9 months at 2-8°C	0.4 %	2.0 %

Reviewer's assessment of N-terminal truncated variants:

Truncated variants which lack MT are slightly more abundant in EP2006 by ~0.5-0.7%. Also, the total levels of truncated species by LC-MS and AEX is slightly higher in EP2006 (by ~0.7%) in EP2006 compared to US-licensed Neupogen and EU-approved Neupogen. I agree with Sandoz justification that this slight difference is unlikely to impact biological activity as the first 10 N-terminal amino acids are neither part of the protein structured region nor are part of the receptor binding epitopes as shown in the figure A below (red arrow)[64].

***Figure A**. Complex GCSF and the GCSF receptor and binding epitopes of GCSF.*



Nor-leucine species

NLeu species were characterized by top down MS and peptide mapping (refer to characterization section). Four NLeu variants were identified in EP2006 and US-licensed Neupogen: Met1Leu, Met122NLeu Met127NLeu and Met138NLeu. EP2006 contains up to 0.8% of NLeu variants. A quantitative comparison of NLeu variants in EP2006, US-licensed Neupogen and EU-approved

[64] Tamada, T. et. al. (2006). PNAS, 103, 3135-3140

Neupogen is presented below. For the purposes of analytical similarity, this attribute was evaluated by comparison of min-max values (Tier-3) because of their low abundance and low variability of their levels.

Table E. Semi-quantitative evaluation of NLeu species

Product (Age) [65]	**# lots**	**NLeu1**	**NLeu2**	**NLeu3**	**NLeu4**	**Sum of 4 variants**
EP2006 DP (22 months)	6	0.1-0.20	0.0-0.2	0.1-0.3	0.0	0.2-0.7
US-licensed Neupogen (27 months)	1	0.2	0	0.1	0.0	0.3
EU-approved Neupogen (16-29 months)	8	0.0-0.2	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.5
Similar to US-Neupogen		Y	Y, see justification below	Y, see justification below	Y	Y, see justification below
Similar to EU-approved Neupogen		Y	Y, see	Y, see justification below	Y	Y, see justification below

Note: Table prepared by CMC reviewer

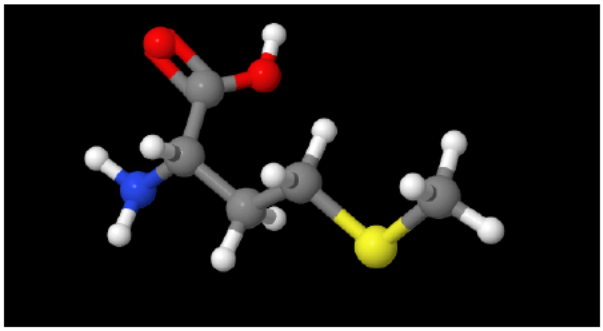
Sandoz provided an assessment of the potential impact of the presence of 0.8% NLeu variants in EP2006. The assessment considered data published in the literature, structural considerations of methionine in comparison to norleucine, immunogenicity results of five clinical studies and evaluation of the biological activity of a sample enriched with Nleu variants. A summary of Sandoz assessment is provided below:

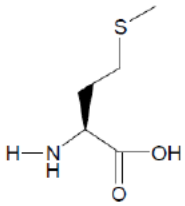
- N-Leu EP2006 variants are isosteric with EP2006 (Figure 5-1). Met-GCSF and the corresponding NLeu variants have the same number and configuration of valency electrons and equal specific volume with respect to space.

[65] Stored at 2-8 °C

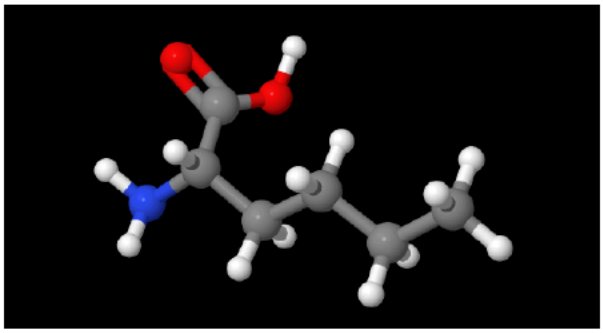
Figure 5-1 Chemical structures of methionine and norleucine

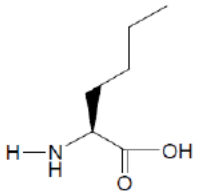
L-Methionine





Norleucine





- Scientific literature reports of recombinant proteins with NLeu substitution for methionine showing that this substitution has minimal or no impact on the function of the protein

- Met-GCSF effects on the immune system are dose-related and the NLeu variants are present at very low levels: less than 1%

- Met-GCSF is a "tolerogenic" cytokine and there is no evidence available which would indicate that this role could change for a isosteric variant

- Met-GCSF does not act on mast cells or cause the production of histamine or platelet activating factor in the investigated cells, and there is no scientific evidence which would indicate that this could change for an isosteric variant

- Non-clinical studies with EP2006 show no evidence of drug accumulation over prolonged continuous SC or IV infusion

- Immunogenicity data (Table 5-1) or DP containing NLeu variants up to 0.8% (Table and 5-2) show no increased immunogenicity.

Table 5-1 Summary of antibody assay results from EP2006 clinical studies

Study number	Number of patients randomized	Number of samples	Screening RIP cut-off Total binding [%] [1]	Number of samples with results [>cut-off in screening RIP]	Number of samples with results [>cut-off in confirmatory RIP[2]]	Number of positive samples in NAB assay
EP06-101	40	103	2.92%	3	0	N/A
EP06-102	26	75	0.90%	3	3	0
EP06-103	56	167	1.52%	7	0	N/A
EP06-105	24	72	0.68%	2	0	N/A
EP06-301	170	643	2.27%	14	0	N/A

[1] Study-specific cut-off: determined by measuring sera of volunteers or patients in the screening RIP assay before treatment with rhG-CSF.

[2] Positive if depletion rate > 20%;

Table 5-2 Batch content of norleucine in correlation to the number of subjects/patients exposed to EP2006, the number of applications, and the number of anti-EP2006 antibody positive samples in confirmatory RIP and NAB assays

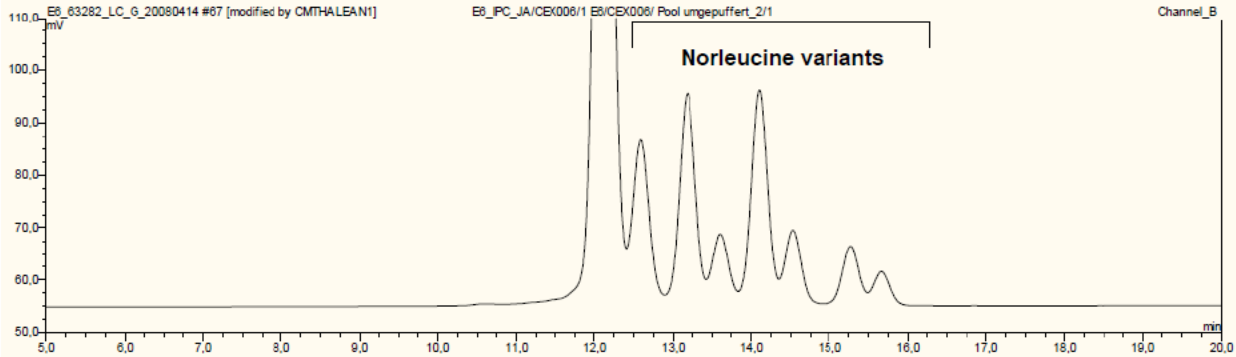
Batch	Strength	Date of manufacture	Related drug substance batch	Clinical use	Norleucine content	Number of subjects/ patients exposed to EP2006	Number of applications	Number of anti-EP2006 antibody positive samples considering both the (1)confirmatory RIP assay and the (2) NAB assay
#003941609F	480 mcg/ 0.5 mL	Sept. 2004	#48200402	EP06-101 Phase I	0.3%	36	252	0 (0%)
#000675011G	300 mcg/ 0.5 mL	Nov. 2005	#48200404	EP06-103 Phase I	0.5% (0.4%) **	28	196	0 (0%)
#000675111G	480 mcg/ 0.5 mL	Nov. 2005	#48200404	EP06-102 Phase I	0.5% (0.6%) **	24	24	0 (0%)
#000675211G	480 mcg/ 0.5 mL	Nov. 2005	#48200406	EP06-103 Phase I	0.3%	28	195	0 (0%)
#000657409G	300 mcg/ 0.5 mL	Sept. 2005	#48200405	EP06-301 Phase III	0.7% (0.8%) **	170*	5266*	0 (0%)
#000675011G	300 mcg/ 0.5 mL	Nov. 2005	#48200404		0.5% (0.4%) **			0 (0%)
#000675111G	480 mcg/ 0.5 mL	Nov. 2005	#48200404		0.5% (0.6%) **			0 (0%)
#000675211G	480 mcg/ 0.5 mL	Nov. 2005	#48200406		0.3%			0 (0%)

* Study EP06-301: Total number of patients and injections are presented.

** Results in brackets were determined during characterization, all other results during comparability. Slight variations observed are within the precision of the method for such small amounts of product related substances and impurities.

- A EP2006 DS sample containing 40% of NLeu variants deliberately produced (b) (4) had similar biological activity (0.9 x 10 U/mg) compared to EP2006 DS with < 1% NLeu variants (the proposed specification for biological activity for the DS is (b) (4) . Sandoz provided data to show that NLeu species are fully bioactive and thus are product-related substances.

Figure 5-3 RP-HPLC Chromatogram of EP2006 drug substance with increased levels of norleucine variants



Reviewer's assessment of NLeu species:

The slight differences in the levels of NLeu species (~0.8%) are not expected to have an impact on biological activity or immunogenicity of EP2006 because NLeu species are product-related substances, and the results from immunogenicity studies show that the immunogenicity rate of EP2006 and EU-Neupogen were similar. Also, the toxicology data of EP2006 support general safety of EP2006 containing up to 0.8% of NLeu variants. Based on these considerations and based on Sandoz justification described above, NLeu species are considered similar.

Deamidation

Deamidation was evaluated by isoelectric focusing (IEF, silver staining), cation exchange chromatography (CEX) and RP-HPLC. Characterization of these species is discussed in 3.2.S.3.2.

Number of lots analyzed by IEF, CEX

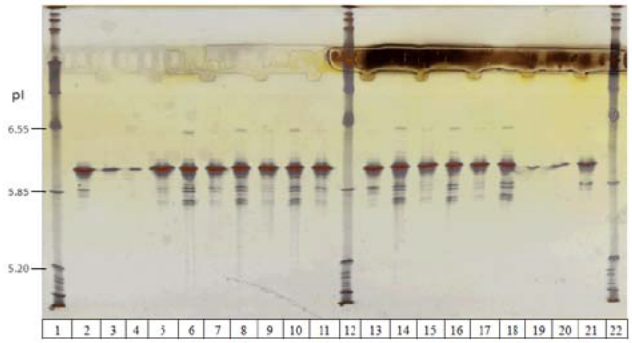
Product	# lots
EP2006 DP	11
EP2006 DS	6
US-licensed Neupogen	4
EU-approved Neupogen	6

Note: Table prepared by CMC reviewer

<u>IEF</u>

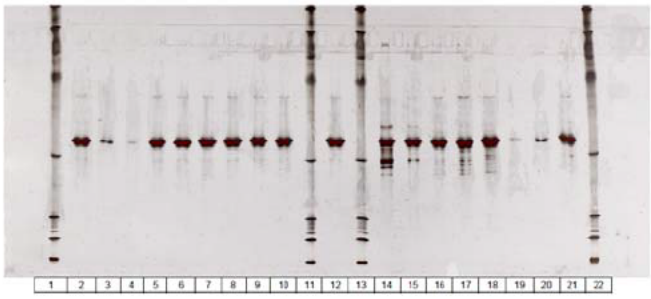
IEF results are reported as number of bands (i.e. charge variants) other than the main band observed as visible and at 2-5% and ≥5% (Appendix XXVI). Representative gels are presented below. For the purposes of analytical similarity, this attribute was evaluated by qualitative comparison of gels (Tier-3).

Figure 5-30 Analysis by isoelectric focusing (silver staining) of EP2006 DP (GPG) and Neupogen® US and EU samples (study 1)



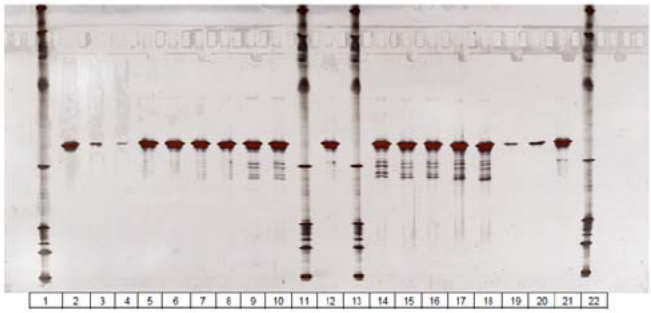
Lane	Sample	concentration [µg/ml]	amount applied [µg]
1	pI-Marker (dissolved in 200µl H_2O)	/	/
2	EP2006.15REF reference solution a	600	9
3	EP2006.15REF reference solution b (5%)	30	0.45
4	EP2006.15REF reference solution b (2%)	30	0.18
5	EP2006 DP 480 mcg/0.5 mL (V200001)	600	9
6	Neupogen® US (10149228)	600	9
7	EP2006 DP (V201002)	600	9
8	Neupogen® US (1025269)	600	9
9	EP2006 DP (V201102)	600	9
10	Neupogen® US (1020649)	600	9
11	EP2006 DP (V201001)	600	9
12	pI-Marker (dissolved in 200µl H_2O)	/	/
13	EP2006.15REF reference solution a	600	9
14	Neupogen® US (1021957)	600	9
15	EP2006 DP (V201101)	600	9
16	Neupogen® EU (1026606)	600	9
17	EP2006 DP (V200201)	600	9
18	Neupogen® EU (1025051)	600	9
19	EP2006.15REF reference solution b (2%)	30	0.18
20	EP2006.15REF reference solution b (5%)	30	0.45
21	EP2006.15REF reference solution a	600	9
22	pI-Marker (dissolved in 200µl H_2O)	/	/

Figure 5-31 Isoelectric focusing with silver staining of EP2006 DS, EP2006 DP (LEK) and Neupogen® EU (study 2)



Lane	Sample	concentration [µg/ml]	amount applied [µg]
1	pI-Marker	/	/
2	EP2006WS14 Reference solution a	1.97 mg/mL	9
3	EP20066WS14 Reference solution b (5% band)	1.97 mg/mL	0.45
4	EP20066WS14 Reference solution b (2% band)	1.97 mg/mL	0.18
5	EP2006 DS (RM/48200402/26)	1.82 mg/mL	9
6	EP2006 DS (RM/48200403/29)	1.98 mg/mL	9
7	EP2006 DS (RM/48200404/28)	1.86 mg/mL	9
8	EP2006 DS (RM/48200405/27)	1.73 mg/mL	9
9	EP2006 DS (RM/48200406/28)	1.95 mg/mL	9
10	EP2006 DS (RM/48200407/27)	1.82 mg/mL	9
11	pI-Marker	/	/
12	EP2006WS14 Reference solution a	1.97 mg/mL	9
13	pI-Marker	/	/
14	Not relevant sample		
15	Not relevant sample		
16	EP2006 DP 480 mcg/0.5 mL (A03941609F, LEK)	960 µg/mL	9
17	Neupogen® EU 480 mcg/0.5 mL (N0875AA)	960 µg/mL	9
19	EP2006WS14 Reference solution b (2% band)	1.97 mg/mL	0.18
20	EP2006WS14 Reference solution b (5% band)	1.97 mg/mL	0.45
21	EP2006WS14 Reference solution a	1.97 mg/mL	9
22	pI-Marker	/	/

Figure 5-32 Isoelectric focusing with silver staining of EP2006 DP (LEK) and Neupogen® EU (study 2)



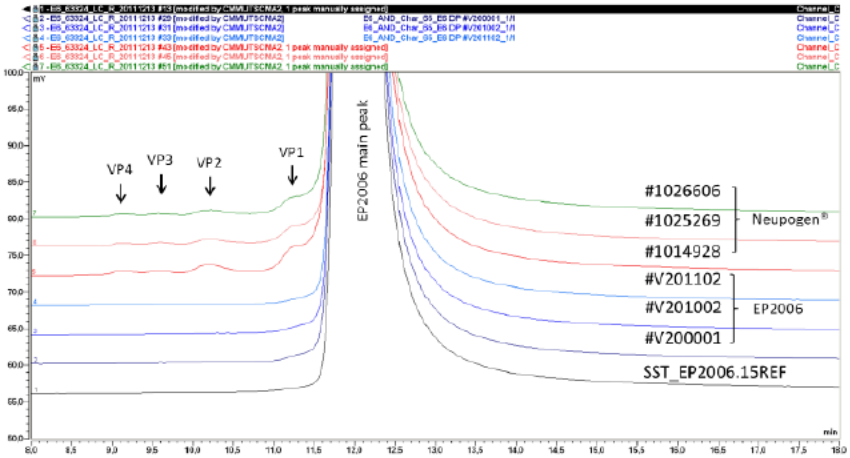
Lane	Sample	concentration [µg/ml]	amount applied [µg]
1	pI-Marker	/	/
2	EP2006WS14 Reference solution a	1.97 mg/mL	9
3	EP2006WS14 Reference solution b (5% band)	1.97 mg/mL	0.45
4	EP2006WS14 Reference solution b (2% band)	1.97 mg/mL	0.18
5	EP2006 DP 300 mcg/0.5 mL (A00657409G)	600 µg/mL	9
6	EP2006 DP 480 mcg/0.5 mL (A00675111G)	960 µg/mL	9
7	EP2006 DP 300 mcg/0.5 mL (A00675011G)	600 µg/mL	9
8	EP2006 DP 480 mcg/0.5 mL (A00675211G)	960 µg/mL	9
9	Not relevant sample		
10	Not relevant sample		
11	pI-Marker	/	/
12	E6WS14 Reference solution a	1.97 mg/mL	9
13	pI-Marker	/	/
14	Neupogen® EU 300 mcg/0.5 mL (N1144AE)	600 µg/mL	/
15	Neupogen® EU 300 mcg/0.5 mL (N1113AG)	600 µg/mL	9
16	Neupogen® EU 480 mcg/0.5 mL (N1114AJ)	960 µg/mL	9
19	E2006WS14 Reference solution b (2% band)	1.97 mg/mL	0.18
20	E2006WS14 Reference solution b (5% band)	1.97 mg/mL	0.45
21	E2006WS14 Reference solution a	1.97 mg/mL	9
22	pI-Marker	/	/

Reviewer's note: *the reference bands loaded at 0.45µg and 0.18 µg correspond to 2% and 5% of the loaded sample (9 µg), respectively. The intensity of the deamidation bands is compared to these standards to estimate the amount of the deamidated band. The identity of the "non-relevant samples" was investigated on inspection. In these gels, they correspond to MR/481004/166 and MR/48200105/25 (taken from copies of original signed documents).*

CEX

The deamidated species evaluated by IEF appear to co-elute as 4 peaks (VP1, VP2, VP3, and VP4) in the CEX chromatogram (Figure 5-25). In general more deamidated species are observed in US-licensed Neupogen and EU-approved Neupogen lots.

Figure 5-25 Superposition of CEX chromatograms of EP2006 DP, Neulasta® EU and Neulasta® US (study 1)



Superposition of CEX chromatograms of EP2006 DP 480 mcg/0.8 mL (GPG), Neulasta® EU 480 mcg/0.5 mL (1026606) and Neulasta® US 480 mcg/0.8 mL (1025269, 1014928) material taken from study 1

Reviewer's note*: the tittle of the Figure and the figure legend have a mistake. The reference product is not Neulasta.*

RP-HPLC and LC-MS

The deamidated species evaluated by IEF appear to co-elute as 4 peaks (NP0, NP1, NP3, and NP4) in the RP-HPLC and LC-MS methods. Quantitative evaluation of demidated species by these methods is summarized in Tables A and B below. In addition sum of deamidation data from the RP-HPLC method were provided for 12 lots of US-licensed Neupogen in PFS, 19 lots of US-licensed Neupogen in vials, 53 lot of EU-Neupogen and 17 lots of EP2006 DP. Min-max values are summarized in Table 2-35. Individual value results from these methods can be found in Appendix XXVI). For the purposes of analytical similarity, this attribute is evaluated by comparison of min-max ranges (Tier-3) because of their low criticality and because these species are product-related substances (refer to the characterization section of this document).

Figure 1-21 Overlay of RP-HPLC chromatograms of 480 mcg/0.5 mL and 480 mcg/0.8 mL batches (FLD)

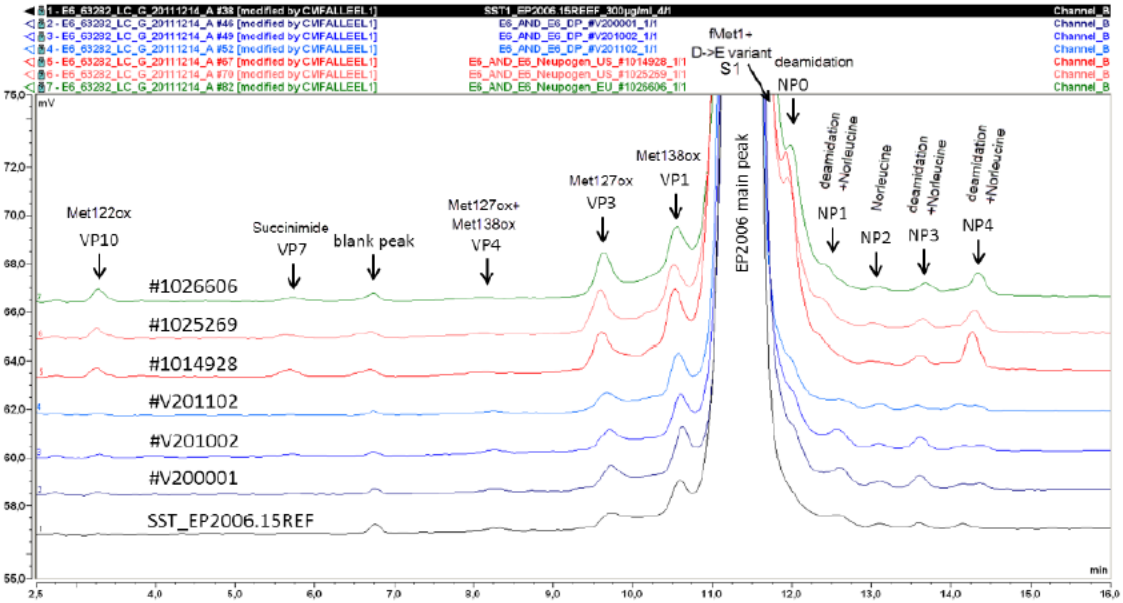


Table A. Min-max range of deamidated species by RP-HPLC

Product	# lots	NP0	NP1	NP3	NP4	Sum Deamidation
EP2006 DS (18-30 months)	6					0.9-1.5
EP2006 DP (5 months)	6	0.50	0.1-0.2	≤0.1-0.1	0.10	0.7-0.9
US-licensed Neupogen (11-26 months)	4	1.0-1.5	0.30	0.1-0.2	0.3-0.2	1.6-2.2
EU-approved Neupogen (16-18 months)	2-5	1.0-1.2	0.30	0.1-0.2	0.20	1.6-3.1

Reviewer's note: *NP1, NP3 and NP4 co-eluted with norleucine variants.*
Note: Table prepared by CMC reviewer

Table 2-35 Deamidated variants: Comparison of min – max values deamidation

	EP2006 DP	Neupogen® US PFS	Neupogen® US PFS and vials	Neupogen® EU
% deamidation	1.9	4.4	4.4	5.3

Table B. Min-max range of deamidated species by LC-MS

Product (Age) [66]	# lots	Deamid 1	Deamid 2	Deamid 3	Deamid 4	Sum deamidation
EP2006 DP (22 months)	6	5.0-6.0	0.5-0.8	0.2-0.5	0.7-0.8	6.4-8.1%
US-licensed Neupogen (27 months)	1	8.4	0.6	0.4	1.2	10.6%
EU-approved Neupogen (16-29 months)	8	6.9-9.7	0.7-0.9	0.3-0.5	1.1-1.7	9.0-12.8%

Note: Table prepared by CMC reviewer

Reviewer's assessment about deamidation

IEF and CEX and RP-HPLC and characterization data provided in section 3.2.S.3.2 show that EP2006 contain the same type of deamidated species as US-licensed Neupogen and EU-approved Neupogen.

The IEF profile of US-licensed Neupogen (Figure 5-30) show numerous acidic (deamidation) bands (pI < main band) and one basic band (pI ~6.55). A similar profile is observed for EU-approved Neupogen (Figure 5-31). EP2006 DP has a similar position and number of acidic and basic bands, but lower in amount compared to US-licensed Neupogen and EU-approved Neupogen. Similar results were observed by RP-HPLC (Table A). Comparison of lots of similar age by LC-MS (Table B) also indicates that EP2006 has lower levels of deamidated species. This is acceptable as deamidation species are degradation products of GCSF. Therefore, deamidation is considered similar in the three products.

Isoelectric point

The isoelectric point of EP2006 DS and DP and EU-approved Neupogen is shown in Table 1-20. For the purposes of analytical similarity, this attribute was considered a Tier-2 attribute with a similarity acceptance criterion of mean ± 2SD. The mean value for pI of EP2006DS, EP2006 DP and EU-approved Neupogen are 5.975 (SD 0.005), 6.03 (SD 0.03) and 6.00 (SD 0.025), respectively.

The acceptance criterion for EU-Neupogen is 5.95-6.05. All EP2006 DS and DP lots met the AC.

[66] Stored at 2-8 °C

Table 1-20 Isoelectric point determined for the EP2006 drug substance, EP2006 drug product and Neupogen samples by isoelectric focusing

Sample	Found pI	Δ to reference
Reference substance (n=4)	6.01	-
EP2006 drug substance #RM/48200402/26	5.97	0.04
EP2006 drug substance #RM/48200403/29	5.97	0.04
EP2006 drug substance #RM/48200404/28	5.97	0.04
EP2006 drug substance #RM/48200405/27	5.98	0.03
EP2006 drug substance #RM/48200406/28	5.98	0.03
EP2006 drug substance #RM/48200407/27	5.98	0.03
EP2006 drug product 480 mcg/0.5 mL #A03941609F	5.97	0.04
EP2006 drug product 300 mcg/0.5 mL #A00657409G	6.05	0.04
EP2006 drug product 480 mcg/0.5 mL #A00675111G	6.05	0.04
EP2006 drug product 300 mcg/0.5 mL #A00675011G	6.04	0.03
EP2006 drug product 480 mcg/0.5 mL #A00675211G	6.04	0.03
Neupogen 480 mcg/0.5 mL #N0875AA	5.97	0.04
Neupogen 300 mcg/0.5 mL #N1144AE	6.02	0.01
Neupogen 300 mcg/0.5 mL #N1113AG	6.02	0.01
Neupogen 480 mcg/0.5 mL #N1114AJ	6.02	0.01

Reviewers' comment: *Other product quality attributes such as appearance, pH, osmolality and extractable volume were evaluated in the context of the comparative stability studies (see below). Given that pH and osmolality are inherent to the formulation, and differences in formulation between a proposed biosimilar product and US-licensed Neupogen are permitted, similarity of these attributes is not considered.*

Comparative stability studies

Comparative stability data under the following conditions were provided: 5 ºC (up to 12 months), 25 ºC (up to 6 months) and 40 ºC (up to 1.5 months), mechanical stress, and photostability. The lots evaluated are listed in Appendix XXVII.

The quality attributes evaluated are those describe in the stability protocol for EP2006 and include: degree of coloration, clarity, pH, extractable volume, identity (by SEC, RP-HPLC and pI), purity (by SEC, RP-HPLC, and IEF), endotoxins, sterility, particulate matter, SVP, foreign matter, content and potency.

Storage at 5± 3 ºC (12 months study)

A summary of the review of the stability data under these conditions is provided below

Reviewer's comments: *By the 12 month time point, the three products showed no significant change in the parameters evaluated, except for the expected increase of impurities by RP-HPLC, EIF, and SDS-PAGE.*

By RP-HPLC, total impurities of EP2006 changed from ~1.1% to ~2.5% from release to 12 months storage. Total impurities by RP-HPLC for the reference product and EU-Neupogen were maintained to ~5% from the start of the study to 12 months of storage.

By IEF, EP2006 was relatively unchanged up to the 12 month time point. However, for the reference product and EU-Neupogen, the number of bands of 2-5% increased by the 12 month time point. By SDS-PAGE, an increase in the number of bands of ≥ 0.01 µg was observed for the three products. These differences are not considered significant because they are related to the age of the products. It is expected the comparator products to have more impurities because they are older (based on Sandoz estimation of the age of the products). Therefore, the three products are deemed similar with respect to degradation profiles under real time conditions.

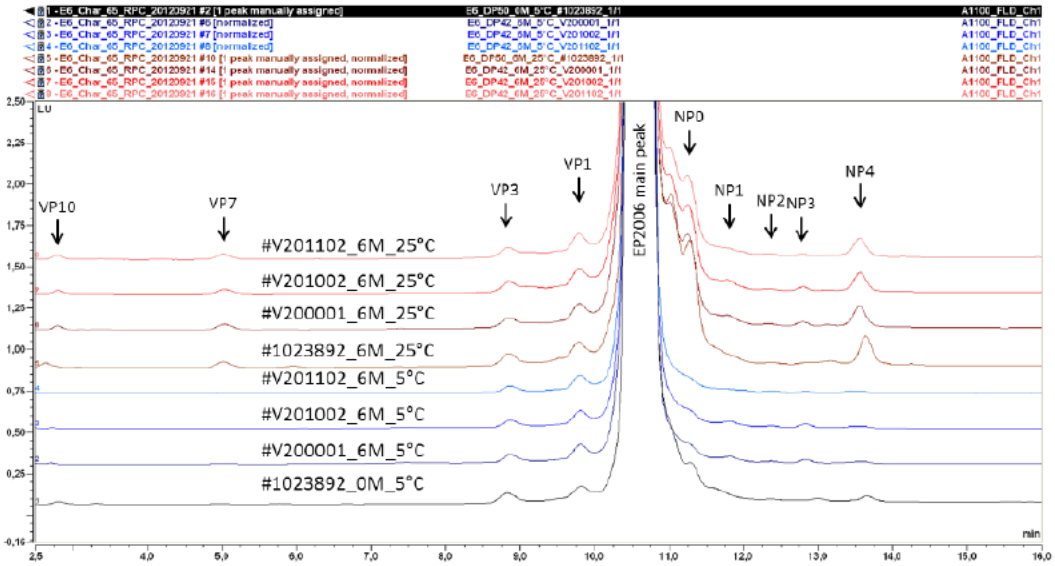
<u>Storage at 25± 2 ºC/60% ±5 RH (6 months study)</u>

A summary of the review of the stability data under these conditions is provided below

Reviewer's comments: *Under accelerated conditions, the main changes in quality attributes for the three products were detected by RP-HPLC, IEF, SDS-PAGE, and SEC.*

By RP-HPLC, at the 6 month time point, oxidized species VP10 (Met 122ox), succinimide species VP7 and deamidated species NP0 and NP4 were increased in EP2006, US-Neupogen (Figure 1-26) and EU-Neupogen (data not shown). All other species monitored by RP-HPLC were not altered upon accelerated storage of the three products.

Figure 1-26 Comparison of RP-HPLC patterns between EP2006 and Neupogen® 480 mcg/0.5 mL and 480 mcg/0.8 mL stored at accelerated conditions



Degradation rates of EP2006 and US-licensed Neupogen and EU-Neupogen were determined using total impurities measured by RP-HPLC. Visually, the degradation rate by means of the slope (% per month) for the three products is similar (Figures 1-28, 1-29 and 6-3).

Figure 1-28 Linear regression analysis of the sum of impurities by RP-HPLC for EP2006 drug product and Neupogen® batches of strength 480 mcg/0.8 mL

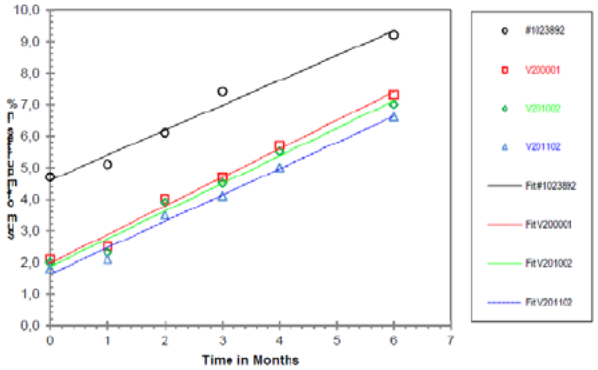


Figure 1-29 Linear regression analysis of the sum of impurities by RP-HPLC for EP2006 drug product and Neupogen® batches of strength 300 mcg/0.5 mL

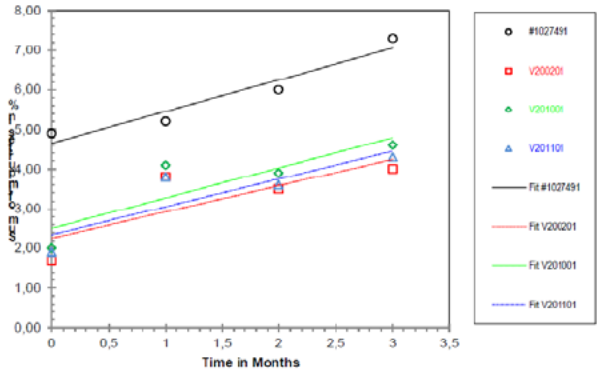
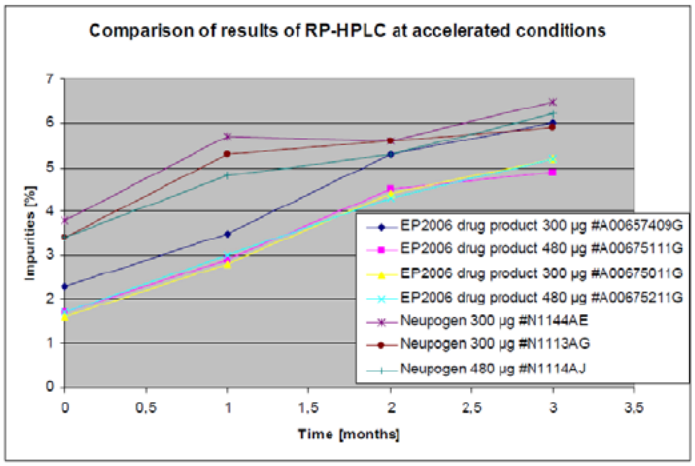


Figure 6-3 Comparison of RP-HPLC results of EP2006 drug product and Neupogen US after storage at 25°C ± 2°C/60%RH ± 5%RH (study 2)



Reviewer's note: There is a typo in Figure 6-3. The Neupogen lots listed correspond to EU-Neupogen

Table 1-33 Fit parameters of individual regressions of linear regression analysis of degradation kinetics of EP2006 drug product and Neupogen®

Batch 480 mcg/0.8 mL	slope [% per month]	Correlation Coefficient *r*	Batch 300 mcg/0.5 mL	slope [% per month]	Correlation Coefficient *r*
#1023892	0.7877	0.98861	#1027491	0.8000	0.96309
V200001	0.9029	0.99406	V200201	0.6600	0.80874
V201002	0.8743	0.99102	V201001	0.7600	0.86164
V201102	0.8314	0.99370	V201101	0.7000	0.86691

Table A. Regression linear analysis of degradation kinetics of EP2006 and EU-Neupogen[67]

Product	Lot #	Slope	Correlation	Lot #	Slope	Correlation
	480 mcg strength			300 mcg strength		
EP2006	A00675111G	1.12	0.9561	A00657409G	1.29	0.9757
	A00675211G	1.18	0.9932	A00675011G	1.24	0.9856
EU-Neupogen	N1114AJ	0.89	0.9642	N1144AE	0.8	0.825
				N1113AG	0.78	0.7984

Note: Table prepared by CMC reviewer

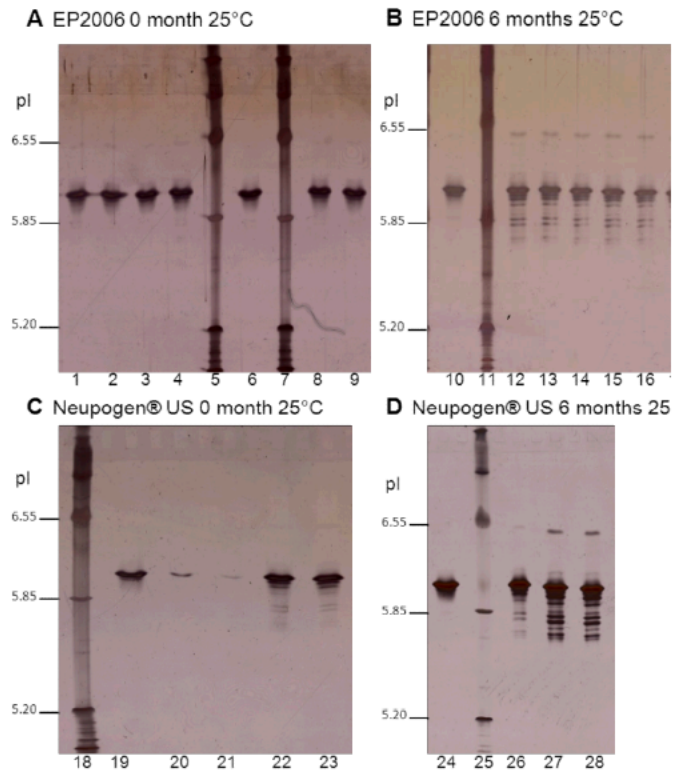
The slopes (% per month) summarized in Table 1-33 show slight differences between EP2006 (0.66-0.90%/month) and US-Neupogen (~0.8 % per month). Also, there appears to be slight differences in degradation rates between the two EP2006 strengths (~0.83-0.90% vs 0.66-0.76%). Similar results are observed between EP2006 and EU-Neupogen (Table A). This effect may be related to the difference in age of the products evaluated, the kinetics of degradation which may not be linear, the differences in formulation between EP2006 (glutamates, pH 4.4) and US-Neupogen and EU-Neupogen, and assay variability. Considering all these factors and the small differences in degradation rates, the degradation rates between the three products is considered similar. Additionally, both under real-time and accelerated conditions, the resulting degradation products are the same.

IEF evaluates charge variants mostly due to deamidation (acidic bands). Figure 1-25 provides a comparison of EP2006 and US-Neupogen. The IEF gels show a basic band (pI ~ 6.55) and ~9 acidic bands; these bands are present in both products at different amounts depending on the stability time point and age of the product.

In summary, EP2006 has the same number of bands in the same position as US-Neupogen, but in less abundance. Visually, the most abundant bands are positioned in the same location for the two products. The same applies for the comparison between EP2006 and EU-Neupogen (Figure 1-77). Based on the above, the three products are considered similar with respect to IEF.

[67] Regression analysis conducted by CMC reviewer

Figure 1-25 IEF gels of accelerated stability study



Lane	A&M Gel Nr.	Sample
1	Gel1 2012-02-15 bs	V201001_6months_2-8°C
2	Gel1 2012-02-15 bs	V201101_6months_2-8°C
3	Gel1 2012-02-15 bs	V200201_6months_2-8°C
4	Gel1 2012-02-15 bs	V200001_6months_2-8°C
5	Gel1 2012-02-15 bs	pI-Marker
6	Gel1 2012-02-15 bs	EP2006.15REF reference solution a
7	Gel1 2012-02-15 bs	pI-Marker
8	Gel1 2012-02-15 bs	V201002_6months_2-8°C
9	Gel1 2012-02-15 bs	V201102_6months_2-8°C
10	Gel1 2012-08-17 MKa	EP2006.15REF reference solution a
11	Gel1 2012-08-17 MKa	pI-Marker
12	Gel1 2012-08-17 MKa	V201001_6months_25/60
13	Gel1 2012-08-17 MKa	V201101_6months_25/60
14	Gel1 2012-08-17 MKa	V200201_6months_25/60
15	Gel1 2012-08-17 MKa	V200001_6months_25/60
16	Gel1 2012-08-17 MKa	V201002_6months_25/60
17	Gel1 2012-08-17 MKa	V201102_6months_25/60
18	Gel1 2011-12-06 Po	pI-Marker
19	Gel1 2011-12-06 Po	EP2006.15REF reference solution a
20	Gel1 2011-12-06 Po	EP2006.15REF reference solution b (5%)
21	Gel1 2011-12-06 Po	EP2006.15REF reference solution b (2%)
22	Gel1 2011-12-06 Po	1027491_0M_2-8°C
23	Gel1 2011-12-06 Po	1023892_0M_2-8°C
24	Gel1 2012-06-05 MKa	EP2006.15REF reference solution a
25	Gel1 2012-06-05 MKa	pI-Marker
26	Gel1 2012-06-05 MKa	1025277_6M_2-8°C
27	Gel1 2012-06-05 MKa	1027491_6M_25/60
28	Gel1 2012-06-05 MKa	1023892_6M_25/60

Figure 1-77 Example of an IEF gel (silver stained) of EP2006 drug product after three months, and Neupogen after two months of storage at 25°C ± 2°C/60%RH ± 5%RH (IEF Gel AL5- IEF 060519-A)

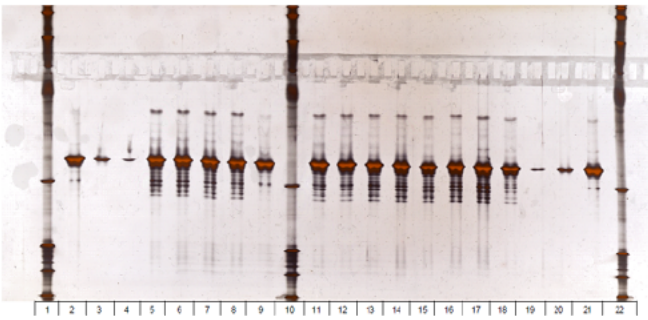


Table	Sample	Concentration of sample	Mass o sample
1	pI-Marker	/	/
2	E6WS14 Reference solution a	1.97 mg/mL	9
3	E6WS14 Reference solution b (5% band)	1.97 mg/mL	0.45
4	E6WS14 Reference solution b (2% band)	1.97 mg/mL	0.18
5	EP2006 drug product #000657509G 3m 25°C	0.96 mg/mL	9
6	EP2006 drug product #000657409G 3m 25°C	0.6 mg/mL	9
7	EP2006 drug product #000674911G 3m 25°C	0.6 mg/mL	9
8	EP2006 drug product # 000675011G 3m 25°C	0.6 mg/mL	9
9	E6WS14 Reference solution a	1.97 mg/mL	9
10	pI-Marker	/	/
11	Filgrastim #000675111G 3m 25°C	0.96 mg/mL	9
12	Filgrastim #000675211G 3m 25°C	0.96 mg/mL	9
13	Neupogen N1114AJ 2m 25°C	0.96 mg/mL	9
14	Neupogen N1113AG 2m 25°C	0.6 mg/mL	9
15	Neupogen N1144AE 2m 25°C	0.6 mg/mL	9
16-18	Filgrastim product not commercialized in the EU		

By SEC, HMW species increase up to 0.3-0.5% and potency decreased by ~10% from time zero to 6 months in the three products. The products are considered highly similar with respect to both attributes.

<u>High temperature stress</u>

A summary of the review of the stability data under these conditions is provided below

- *Storage at 40 ± 2 ºC (1.5 and 3 months data for the reference product and EP2006, respectively)*

Reviewer's comments: *Similar to accelerated conditions, the main changes in quality attributes for EP2006 and the reference product (the EU-Neupogen was not assessed in this study) were detected by RP-HPLC, IEF, SDS-PAGE, and SEC.*

Total impurities by RP-HPLC increased by ~7.9% and ~6.7% for EP2006 (at 2 months) and US-Neupogen (at 1.5 month), respectively. By IEF, the number of minor bands (2-5%) was in the range of 0-6 (at 3 months) for EP2006 and 1-7 for US-Neupogen (at 1.5 months) respectively. HMW species increased from ≤0.1 at the beginning of the study to ~2.5% for both products at the end of the study. Under these conditions, EP2006 and US-Neupogen have highly similar degradation profiles.

Mechanical stress

EP2006 and the reference product were evaluated in two formats:

1. PFS of both products were subjected to mechanical stress for 6h at 180 rpm at room temperature

2. The content of the PFS of the reference product and EP2006 was transferred into 6R glass vials and stressed mechanically by shaking at 180 rpm for 6 hours at room temperature.

A summary of the review of the stability data under these conditions is provided below

No significant change in quality attributes, including IEF was observed in the two formats. The observed changes are as follows:

Reviewer's comments: *Product related impurities by RP-HPLC appear to be unchanged when EP2006 was stressed in vials and PFS (Table 6-52). Conversely, the product-related impurities of US-licensed Neupogen appear to increase when it is stressed in vials. However, the expiration date of the lots evaluated in stressed vials are between 2012-2013, whereas expiration date of the lots evaluated in PFS is in 2015, which suggest that the difference observed is related to the age of the products.*

Table 6-52 Product related impurities (RP-HPLC) after mechanical stress

Batch	Vials[1)], not stressed	Vials[1)], stressed	PFS[1)], not stressed	PFS[1)], stressed
	sum / largest individual			
V200001, 480/0.8, PFS, GPG	n.a.	2.3 / 0.8	2.5 / 0.8	2.5 / 0.8
V201002, 480/0.8, PFS, GPG	n.a.	2.2 / 0.7	2.4 / 0.8	2.5 / 0.8
V201102, 480/0.8, PFS, GPG	n.a.	2.1 / 0.8	2.4 / 0.8	2.5 / 0.8
V201001, 300/0.5, PFS, GPG	n.a.	2.3 / 0.8	2.1 / 0.6	2.1 / 0.6
V201101, 300/0.5, PFS, GPG	n.a.	2.2 / 0.8	2.2 / 0.7	2.2 / 0.7
V200201, 300/0.5, PFS, GPG	n.a.	2.1 / 0.8	2.2 / 0.7	2.2 / 0.8
1014928, 480/0.8, PFS, N-US	n.a.	5.6 / 0.9	n.a.	n.a.
1025269, 480/0.8, PFS, N-US	n.a.	4.6 / 0.8	n.a.	n.a.
1020649, 300/0.5, PFS, N-US	n.a.	5.3 / 0.8	n.a.	n.a.
1021957, 300/0.5, PFS, N-US	n.a.	5.1 / 0.9	n.a.	n.a.
1036971, 480/0.8, PFS, N-US	n.a.	n.a.	2.7 / 1.1	2.9 / 1.2
1038184, 300/0.5, PFS, N-US	n.a.	n.a.	2.9 / 1.1	2.9 / 1.2
1035682, 480/0.8, PFS, N-US	n.a.	n.a.	2.8 / 1.1	2.8 / 1.1

n.a.: not applicable [1)] Container used for mechanical stress study

HMW impurities by SEC (Table 6-54) and SDS-PAGE (data not shown) increase significantly when EP2006 is stressed in vials. This result is contrary to that obtained for the reference product. Given that EP2006 is developed in PFS, the vial stress conditions are not relevant for the PFS presentation.

Table 6-54 High molecular weight variants by SE-HPLC after mechanical stress

Batch	Vials[1)], not stressed	Vials[1)], stressed	PFS[1)], not stressed	PFS[1)], stressed
	Area%			
V200001, 480/0.8, PFS, GPG	≤0.1	1.1	0.1	≤ 0.1
V201002, 480/0.8, PFS, GPG	≤0.1	1.7	0.2	0.1
V201102, 480/0.8, PFS, GPG	≤0.1	2.8	0.1	0.1
V201001, 300/0.5, PFS, GPG	≤0.1	2.9	0.2	0.3
V201101, 300/0.5, PFS, GPG	≤0.1	4.7	0.2	0.2
V200201, 300/0.5, PFS, GPG	≤0.1	2.9	0.2	0.1
1014928, 480/0.8, PFS, N-US	≤0.1	0.2	n.a.	n.a.
1025269, 480/0.8, PFS, N-US	≤0.1	0.1	n.a.	n.a.
1020649, 300/0.5, PFS, N-US	≤0.1	< 0.1	n.a.	n.a.
1021957, 300/0.5, PFS, N-US	≤0.1	< 0.1	n.a.	n.a.
1036971, 480/0.8, PFS, N-US	n.a.	n.a.	≤ 0.1	≤ 0.1
1038184, 300/0.5, PFS, N-US	n.a.	n.a.	≤ 0.1	≤ 0.1
1035682, 480/0.8, PFS, N-US	n.a.	n.a.	≤ 0.1	≤ 0.1

n.a.: not applicable [1)] Container used for mechanical stress study

Photostability

EP2006 (one manufacturing lot, IDT and one pilot scale lot) and EU-Neupogen (2 lots) were exposed to light (765 W) for 8 hours. DP samples were tested in original syringes with the label removed. The DP lot with the higher strength (16 syringes) was also emptied (under laminar flow) into a (b) (4) bottle (b) (4) used for DS (b) (4) also without any labels attached to it. As dark control, samples of each type wrapped in aluminum foil were stored for the same period of time in the testing unit. DS was also included in the study.

The test methods include appearance, pH, SEC, IEF, SDS-PAGE (reducing and non-reducing), AEX, RP-HPLC, and bioassay. The results of these studies can be found in the submission (Table 2-33 to 2-35, section 3.2R document entitled "Biosimilarity of EP2006 with the Reference Product Neupogen"). The results of the photostability studies showed the following:

Reviewer's comment: *Upon exposure to light, the degradation rate (% per hr) of EP2006 is similar to the EU-Neupogen, considering that the latter had already some degradation at time zero (Tables 2-12 to 2-14). No difference in degradation rate is observed between the 300 µg/0.5 ml and 480 µg/0.8 ml strengths in any of the products. No change in content was observed when EP2006 is stored in DS bottles (Table 2-14).*

Table 2-12 Results of content of EP2006 drug product (300 mcg/0.5 mL) and Neupogen (300 mcg/0.5 mL) measured by RP-HPLC after storage [mg/mL]

Hours	EP2006 drug product		Neupogen	
	030806	030806/ aluminum foil	N1204AJ	N1204AJ / aluminium foil
0	0.603	0.603	0.620	0.620
8	0.523	0.600	0.525	0.618
16	n.a.	n.a.	n.a.	n.a.
Span[%]	13.27	0.50	15.32	0.32
Slope [%/hour]	-1.6584	-0.0622	-1.9153	-0.0403

n.a.... not analyzed

Table 2-13 Results of content of EP2006 drug product (480 mcg/0.5 mL) and Neupogen (480 mcg/0.5 mL) measured by RP-HPLC after storage [mg/mL]

Hours	EP2006 Drug Product		Neupogen	
	060806	060806/ aluminum foil	N1213AH	N1213AH/ aluminium foil
0	0.971	0.971	0.997	0.997
8	0.840	0.970	0.832	0.990
16	n.a.	n.a.	n.a.	n.a.
Span[%]	13.49	0.10	16.55	0.70
Slope [%/hour]	-1.6864	-0.0129	-2.0687	-0.0878

n.a. ... not analyzed

Table 2-14 Results of content of EP2006 drug product in bottles (480 mcg/0.5 mL and EP2006 drug substance measured by RP-HPLC after storage [mg/mL]

Hours	EP2006 drug product in bottles		EP2006 drug substance	
	060806	060806/ aluminum foil	#48200407	#48200407/ aluminum foil
0	0.972	0.972	1.80	1.80
8	0.931	0.970	1.68	1.77
16	n.a.	n.a.	1.66	1.81
Span [%]	4.22	0.21	7.78	2.22
Slope [%/hour]	-0.5273	-0.0257	-0.4861	0.0347

n.a. … not analyzed

Purity by RP-HPLC decreases upon exposure to light (Figures 2-4 and 2-17) in both products. Based on the chromatogram (Figure 2-17), the same type and levels of impurities are induced upon exposure to light. Considering the difference in the age of the products, the degradation rates of EP2006 is considered similar to that of the EU-Neupogen (Tables 2-15 and 2-18).

Figure 2-4 Graphical presentation of results of purity detected by RP-HPLC (for results of regression see Table 2-15).

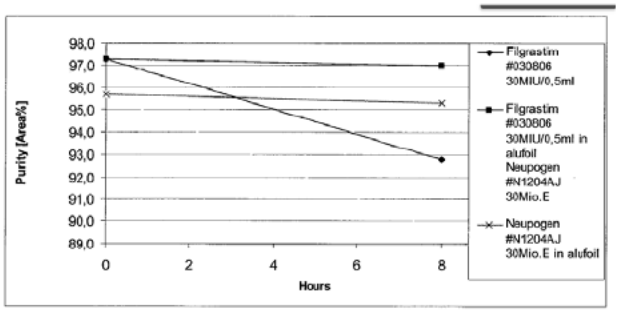
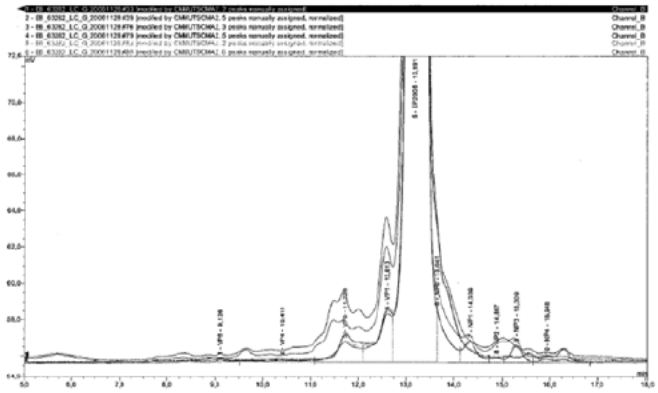


Figure 2-7 Comparison of RP-HPLC chromatograms of EP2006 drug product (batch 030806) and Neupogen (batch N1204AJ)



For comparison samples prior and after storage for 8 hours at 765 W are shown (fluorescence detection; black: Filgrastim Sandoz (t=0h); blue: Neupogen (t=0h); pink: Filgrastim Sandoz (t=8h); brown: Neupogen (t=8h); green: Filgrastim Sandoz in aluminum foil (t=8h); cyan: Neupogen in aluminum foil (t=8h)).

Table 2-15 Results of purity of EP2006 drug product (0.6 m/mL) and Neupogen (0.6 mg/mL) measured by RP-HPLC after storage [area%]

Hours	EP2006 drug product		Neupogen	
	030806	030806/ aluminum foil	N1204AJ	N1204AJ/ aluminum foil
0	97.3	97.3	95.7	95.7
8	92.8	97.0	89.5	95.3
16	n.a.	n.a.	n.a.	n.a.
Span [area%]	4.50	0.30	6.20	0.40
Slope [area%/hour]	-0.5625	-0.0375	-0.7750	-0.0500

n.a. ... not analyzed

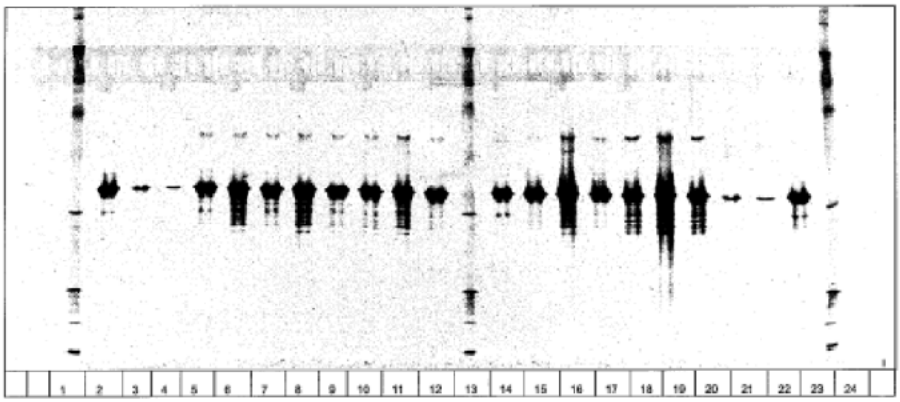
Table 2-18 Results of largest individual impurity of EP2006 drug product (300 mcg/0.5 mL) and Neupogen (300 mcg/0.5 mL) measured by RP-HPLC after storage [area%]

Hours	EP2006 drug product		Neupogen	
	030806	030806/ aluminum foil	N1204AJ	N1204AJ/ aluminum foil
0	0.8	0.8	1.2	1.2
8	2.1	0.9	2.5	1.3
16	n.a.	n.a.	n.a.	n.a.
Span [area%]	1.30	0.10	1.30	0.10
Slope [area%/hour]	0.1625	0.0125	0.1625	0.0125

n.a. ... not analyzed

Samples exposed to light induce the generation of additional acidic and basic bands detected by IEF. This is represented in Figure 2-10 where EP2006 and EU-Neupogen exposed to light (lane 16 and 19, respectively) have more bands than the corresponding non-exposed products (lanes 15 and 18, respectively). Based on EIF, the additional bands co-migrate in both products. Considering the difference in age of the products, the degradation profiles are considered similar.

Figure 2-10 IEF Gel of EP2006 drug product (batch 030806), drug substance and Neupogen (Batch N1204AJ) before and after storage at 765 W



Lane	Sample name	Concentration of sample	Concentration after dilution	Applied Volume [pl]	Applied Protein
1	pI-Marker	/	/	5	/
2	E6 WS14 Reference solution a	1.97 mg/mL	600 µg/mL	15	9
3	E6 WS14 Reference solution b (5% Bande)	1.97 mg/mL	30µg/mL	15	0.45
4	E6 WS14 Reference solution b (2% Bande)	1.97 mg/mL	30µg/mL	6	0.18
5	DS #48200407_TO AN:5-1006	1.82 mg/mL	600 µg/mL	15	9
6	DS #48200407_8h AN:5-1008	1.82 mg/mL	600 µg/mL	15	9
7	DS #48200407_8h in Aluminum foil AN:5-1008	1.82 mg/mL	600 µg/mL	15	9
8	DS #48200407_16h AN:5-1009	1.82 mg/mL	600 µg/mL	15	9
9	DS #48200407_16h in Aluminum foil AN:5-1009	1.82 mg/mL	600 µg/mL	15	9
10	DP in bottles #060806 480 mcg/0.5 mL_ TO AN: 5-1003	0.96 mg/mL	600 µg/mL	15	9
11	DP in bottles #060806 480 mcg/0.5 mL_ 8h AN: 5-1004	0.96 mg/mL	600 µg/mL	15	9
12	DP in bottles #060806 480 mcg/0.5 mL 8h in Aluminum foil AN: 5-1004	0.96 mg/mL	600 µg/mL	15	9
13	pI-Marker	/	/	5	/
14	E6 WS14 Reference Solution a	1.97 mg/mL	600 µg/mL	15	9
15	Filgrastim #030806 300 mcg/0.5 mL mL_ TO AN: 5-1003	0.60 mg/mL	600 µg/mL	15	9
16	Filgrastim #030806 300 mcg/0.5 mL _8h AN: 5-1005	0.60 mg/mL	600 µg/mL	15	9
17	Filgrastim #030806 300 mcg/0.5 mL _8h in Alufolie AN:5-1005	0.60 mg/mL	600 µg/mL	15	9
18	Neupogen 300 mcg/0.5 mL. #N1204AJ_TO AN: 5-1003	600 µg/mL	600 µg/mL	15	9
19	Neupogen 300 mcg/0.5 mL #N1204AJ_8h AN: 5-1005	600 µg/mL	600 µg/mL	15	9
20	Neupogen 300 mcg/0.5 mL #N1204AJ_8h in Alufolie AN: 5-1005	600 µg/mL	30µg/mL	6	0.18
21	E6 WS14 Reference Solution b (5% Band)	1.97 mg/mL	30µg/mL	15	0.45
22	E6 WS14 Reference Solution b (2% Band)	1.97 mg/mL	30µg/mL	6	0.18
23	E6 WS14 Reference Solution a	1.97 mg/mL	600 µg/mL	15	9
24	pI-Marker	/	/	5	/

Also, upon exposure to light, the biological activity of both products decreased by 20% (Table 2-32).

Table 2-32 Overview results of bioactivity [I.U./mg]

Sample	Batch	Comment	0 hours	8 hours	16 hours
EP2006 drug product	030806		1.1×10^8	n.a.	n.a.
	030806	Aluminum foil	1.1×10^8	n.a.	n.a.
	060806		1.1×10^8	0.9×10^8	n.a.
	060806	Aluminum foil	1.1×10^8	1.2×10^8	n.a.
Neupogen	N1204AJ		1.1×10^8	n.a.	n.a.
	N1204AJ	Aluminum foil	1.1×10^8	n.a.	n.a.
	N1213AH		1.1×10^8	0.9×10^8	n.a.
	N1213AH	Aluminum foil	1.1×10^8	1.2×10^8	n.a.
EP2006 drug product in bottles	060806		1.1×10^8	1.0×10^8	n.a.
	060806	Aluminum foil	1.1×10^8	1.2×10^8	n.a.
EP2006 drug substance	#48200407		1.1×10^8	1.1×10^8	n.a.
	#48200407	Aluminum foil	1.1×10^8	n.a.	n.a.

n.a. ... not analyzed

No additional bands were observed in reducing SDS-PAGE. However, in non-reducing SDS-PAGE, one additional band each was observed for EP2006 (1 lot) and both lots of EU-Neupogen.

There was no significant change in other quality attributes measured, including impurities by SEC and AEX.

In general, no change in quality attributes for samples wrapped in aluminum foil were observed. In addition, the results showed that the material of the container plays a role in protecting the product from degradation. When DP (both products) is poured in (b) (4) *DS bottles, degradation is slower. For example, content decreases ¼ to that of the samples exposed to light (Figure 2-14 above) and no additional bands by SDS-PAGE and IEF were observed.*

Overall, the EP2006, US-licensed Neupogen and EU-approved Neupogen have similar stability profiles under recommended storage, accelerated and stress conditions.

Reviewers' Conclusion on analytical similarity

The results discussed in this section indicate the following:

- ***Extent of analytical characterization of EP2006 and comparator products (US-licensed Neupogen and EU-approved Neupogen) is robust***

- ***Pair-wise analytical comparisons of EP2006, US-licensed Neupogen and EU-approved Neupogen support a scientific bridge based on the relatively simple structure of the protein, lack of post-translation modifications, and the robustness of the pair-wise analytical characterization***

- ***EP2006 (clinical and commercial product) is analytically highly similar to US-licensed Neupogen***

- ***Analytical similarity data do not raise residual uncertainties about the similarity of EP2006 and US-licensed Neupogen. The impact of the EP2006 formulation on PK/PD will be addressed in the non-clinical and clinical studies***

5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

Deferred to immunogenicity reviewers

97 Pages Have Been Withheld In Full As b4 (CCI/TS) Immediately Following This Page