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Analytical Method Validation for Testing of Limit of High Molecular Weight Proteins in Filgrastim Biopharmaceutical Products

Soraya Surasarang*, Chanida Karnpracha, Boontarika Boonyapiwat

Bureau of Drug and Narcotics, Department of Medical Sciences, Ministry of Public Health, Thailand

Abstract

Filgrastim is a biopharmaceutical drug used for treatment chemotherapy-induced neutropenia in cancer patients. High molecular weight proteins (HMWP) of filgrastim can lead to loss of efficacy and immunogenicity. Limit of HMWP is one of the test items showing quality of filgrastim products. However, a compendial method used for determination of these impurities in pharmaceutical products has not yet been available. In this study, a size-exclusion chromatographic (SEC-HPLC) method was validated for determination of HMWP of filgrastim. Method validation conducted parameters including specificity, limit of quantitation (LOQ) and precision. The results showed that the analysis of HMWP of filgrastim was not interfered by the excipients and other reagents in the analytical system. The method was sensitive with the lowest limit of quantitation of 0.0002 mg/mL. In addition, the percent relative standard deviation of repeatability and intermediate precision was in a range of 1.9-3.5% and 1.9-7.3%, respectively. These values were within the limits calculated from Horwitz's equation. Thus, the size-exclusion chromatography (SEC) method was specific, sensitive, precise and valid for determination of HMWP of filgrastim in pharmaceutical products. This validated method has been utilized as a standard method to quantify the HMWP of filgrastim in biopharmaceutical products collected from all over nation by the Thai FDA.

Keywords: filgrastim, high molecular weight proteins, aggregation, method validation, size exclusion chromatography

Corresponding author: Soraya Surasarang ; soraya.h@dmsc.mail.go.th

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Introduction

Biological products are used for diagnosis, prevention, and treatment of the diseases or medical conditions. Vaccines, blood and blood components, allergenics, tissues, monoclonal antibodies, and recombinant therapeutic proteins are some examples of the biological products (Pedersen-Bjergaard et al, 2019). Recently, the development of biological products has been greatly increasing. In the United States, biological products are growing fastest as compared with other therapeutic products (FDA, 20111). Top three largest segments of the biologics market are monoclonal antibodies, recombinant therapeutic proteins, and vaccines, respectively. These three segments are growing at an annual rate greater than 9%. However, their prices are notably more expensive than most of the small molecules (TBR, 2018). In most cases of the small molecule drugs, prices of the pharmaceutical products reduced by 50-80% after their original patent protection has expired due to the competition between multiple generic copies (Mulcahy et al, 2014). Unlike chemical drugs, molecular structure identity of biological products cannot generally be established due to the complexity of the production and minor natural variations in the molecular structure (Gámez-Belmonte et al, 2018). Generic products of the biologics launch after the patent of the originators have expired could be 'stand-alone biologicals' or 'biosimilars' (FDA, 2011; Calam PD, 2006). In general, stand-alone biologicals refer to the novel products with full clinical studies, which share the same international nonproprietary names. Whereas biosimilars are pharmaceutical products that are developed to have similar properties to an existing approved product and has no clinically significant differences (FDA, 2019). The amount of clinical data of the biosimilars needed are generally less than that required for a stand-alone products (Calam PD, 2006). This results in cost reduction of biosimilars as compared with that of the innovator products (Mulcahy et al, 2014). Biosimilars have been encouraged from the governments around the world to reduce the healthcare costs (TBR, 2018).

Both biological products and their biosimilars are inherently produced through biotechnology in a living system, such as a microorganism, plant or animal cells Jeske et al, 2013). They are generally large and more complex than small molecule drugs; therefore, characterizations of these drugs are more difficult. This leads to the challenging questions for the development and regulatory evaluation of the biosimilar products (Berkowitz et al, 2012). Regulatory agencies in several countries, starting with the European Medicines Agency, adopted the guidelines focusing on comparison issues and assurance quality, efficacy, and safety of the biosimilar products (Bennett et al, 2014). To monitor the critical quality attributes of drug substances and drug products, including biosimilars, a set of various analytical techniques are required to provide the complex information of the proteins (Driver et al, 2007; Boschetti et al, 2000). One of the characteristics according to the guidelines from the regulatory agency is the quantitative determination of the aggregation, including dimers and higher order aggregate of the active protein. There are number of analytical techniques used to characterize the aggregate such as light scattering techniques, analytical ultracentrifugation (AUC), filed-flow fractionation (FFF), gel electrophoresis, and size-exclusion chromatography (SEC) method (Hong et al, 2012; Philo et al, 2009). SEC has been a dominant favored method for aggregation analysis by far due to its speed, good sensitivity and reproducibility (Philo et al, 2009; Yu et al, 2008; Brange et al, 1992; Oliva etal, 2000). Moreover, SEC is able to separate both covalent and non-covalent dimers from the monomers (Watson et al, 1988).

Recombinant human granulocyte colony-stimulating factor (G-CSF) is a biopharmaceutical drug produced through genetic recombination (Souza et al, 1986; Lu et al, 1992). There are two types of G-CSF; glycosylated (lenograstim) and non-glycosylated (filgrastim) form produced by using the expression in mammalian cells and in *E. coli*, respectively (Vanz et al, 2008). Filgrastim, molecular weight of 18,800 daltons,

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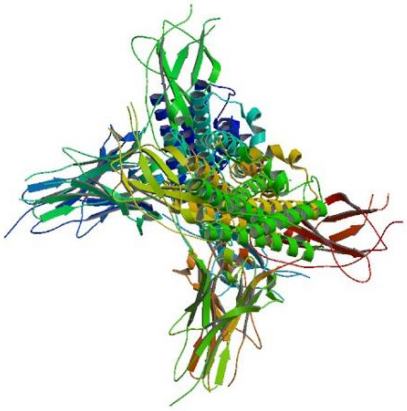
consists of 175 amino acids with an extra N-terminal methionine (Figure 1) (Vanz et al, 2008; FDA, 2012). A well-known and well-characterized biopharmaceutical drug as filgrastim has been approved for use in treatment of chemotherapy-induced neutropenia in cancer patients (Wang W, 1999; Beveridge et al, 1998). Pharmacodynamic of filgrastim is that the drug binds to G-CSF receptor and stimulates the production of neutrophils in bone marrow. G-CSF and its receptor are necessary for basal and stress-induced granulopoiesis, which forms neutrophils (Panopoulos & Watowich, 2008). The patents on Neupogen®, an originator product, expired in Europe and the US in 2006 and 2013, respectively (ICH, 2005). A number of filgrastim biosimilars have entered the market. In 2018, filgrastim products have been launched in Thai market under 10 brand names (18 registration numbers), which include 2 biosimilar products (Thai FDA, 2018). Analytical procedures are required to evaluate quality of the different products. Besides the potency assay, determination of high molecular weight proteins (HMWP) is the other significant test method to assess the characteristics of filgrastim (British Pharmacopeia, 2018; USP, 2017) since the HMWP of filgrastim may lead to multiple adverse effects, such as loss of efficacy and enhancement of immune responses to the monomeric form (Rosenberg AS, 2006). However, a compendial method for evaluating the HMWP of filgrastim in pharmaceutical products has not yet been available in any international pharmacopeias. The objective of this study is to validate the analytical method for determination of the HMWP of filgrastim in the pharmaceutical products. The size-based separation by SEC for separation of the different HMWP of filgrastim is studied.



MTPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWA
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QMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP

Figure 1. Structure (a) and amino acid sequence (b) of filgrastim (Filgrastim – DrugBank,2019)

Materials and Methods

Materials

USP high molecular weight filgrastim RS was purchased from the US Pharmacopeia, MD, USA. Ammonium hydrogen carbonate, sodium hydroxide, and acetic acid were acquired from Carlo Erba reagents, Italy. D-mannitol and sodium acetate were purchased from Sigma-Aldrich, MO, USA. Sorbitol, tween 80, and L-glutamic acid were purchased from TCI, Japan, Scharlau, Spain and Acros Organics, Belgium, respectively. Orthophosphoric acid was obtained from Merck, Germany. Water type I was produced from the Milli-Q® type I ultrapure water systems (Merck Ltd., Germany). All other chemicals and solvents used were American Chemical Society (ACS) reagent or high-performance liquid chromatography (HPLC) grade.

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Reagent preparation

Mobile phase

Ammonium hydrogen carbonate (7.9 g) was dissolved in 1000 ml of ultrapure water. The pH of the solution was adjusted by phosphoric acid to 7.0. The final volume was made up to 2,000 mL with ultrapure water (final concentration of 0.05 M). The solution was filtered by 0.2 µm of nylon membrane filter (National Scientific Supply Company, Inc., CA, USA).

Dissolve 7.9 g of ammonium hydrogen carbonate in 1,000 mL of water, and adjust with phosphoric acid to a pH of 7.0; dilute to 2,000 mL with ultrapure water, then filter with a nylon membrane filter, pore size 0.2 µm (National Scientific Supply Company, Inc.CA, USA)

Matrix solution

A mixed matrix solution contained all of the excipients often filgrastim brand names marketed in Thailand. The 100-mL of matrix solution contains 4 mg of tween 80, 60 mg of acetic acid, 6 mg of sodium hydroxide, 12.3 mg of sodium acetate, 500 mg of sorbitol, 500 mg of mannitol, and 147.2 mg of L-glutamic acid. The excipients were weighed and dissolved in water until a clear solution was obtained. The solution was sterile using membrane filtration technique (0. 2 µm cellulose acetate syringe filter, sterile Minisart® single use filter unit, Sartorius Stedim Biotech S.A., Germany).

Stock solution of high molecular weight (HMW) filgrastim (0.5 mg/mL)

USP HMW filgrastim RS is a mixture of filgrastim HMW including dimer, oligomer 1, oligomer 2, aggregate and filgrastim monomer. The contents of an entire vial of USP HMW filgrastim RS was reconstituted with the matrix solution to obtain a stock solution of HMW filgrastim (0.5 mg/mL).

Resolution solution

For system suitability testing, the resolution solution was prepared. The stock solution of HMW filgrastim was diluted with water to obtain a clear solution of the resolution solution (0.3 mg/mL).

Resolution solution in matrix solution

To determine the interferences from the excipients in all marketed products, the mixture of filgrastim and its HMWP in matrix solution was prepared. The stock solution of HMW filgrastim was diluted with matrix solution to obtain a clear solution of the resolution solution (0.3 mg/mL). This solution was used as a 'reference solution' for comparing the relative peak area of each impurity as well.

HPLC chromatographic condition

A Thermo Scientific high-performance liquid chromatography (HPLC), Ultimate 3000 module (Thermo Scientific, MA, USA) equipped with the PDA detector and Chromeleon 7 software was used for the analysis. The experiments were performed on a TSK gel SWxL G3000 (300 mm x 7.8 mm I.D.) size- exclusion column (Tosho Corporation, Japan). A security guard column with the same packing materials (Tosho Corporation, Japan) was used to protect the analytical column. The flow rate and column temperature were set at 0.5 mL/min and 30°C, respectively. A twenty µL of the solution was injected onto the column and chromatograms were acquired at a detection wavelength of 215 nm.

System suitability

To ensure the suitability of the analytical system, the resolution solution was firstly injected to the HPLC. Resolution between the peaks due to the filgrastim dimer and the monomer should be greater than 3 (British Pharmacopeia, 2018).

Method validation

The analytical method was adapted from the methods of the United States Pharmacopeia 40 National Formulary 35 (USP 40 NF 35) and the British Pharmacopoeia (British Pharmacopeia, 2018; USP, 2017). The validation procedures followed the International Conference of Harmonization (ICH) guidelines

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Q2(R1) validation of analytical procedures: text and methodology (ICH, 2005). This study evaluated the parameters beyond the guidelines to confirm that the method was sensitive and precise. Thus, the following parameters, specificity, limit of quantitation (LOQ), precision, and intermediate precision (inter-day precision and different analysts), were demonstrated.

Specificity

The specificity of the method for the filgrastim and its HMWP was established through the determination of interference of the excipients in matrix solution, diluent (water) and mobile phase. Peaks of any excipients and reagents used in the analytical method do not co-elute at the retention time of the interested peaks of the HMWP and filgrastim monomer.

Limit of quantitation (LOQ)

The predicted LOQ of each impurity was calculated based on the standard deviation of the response and slope. Firstly, a regression line of each impurity was generated by plotting peak area of the impurity as a function of concentration. The stock solution of HMW filgrastim (0.5 mg/mL) was diluted with water to obtain a series of solutions (concentrations 0.001-0.5 mg/mL). Then the mean peak area of each impurity from the triplicate determinations was plot against the concentration. The standard error of the predicted y-value for each x in the regression and slope of the regression line was evaluated. The predicted LOQ value of each impurity was calculated. Then the LOQ values were confirmed in the experimental test by preparing the triplicate LOQ solutions as calculated and injecting to the HPLC. The signal to noise ratio (S/N) of each HMWP of filgrastim at LOQ level was considered as not less than 10:1 (Shrivastava & Gipta, 2011). In case the 10:1 S/N of the predicted LOQ of any impurity did not reach, the LOQ value was obtained by diluting the solution until the S/N at least 10:1 was achieved.

Precision

The precision of the method was determined by repeatability and intermediate precision. Owing to the very small amount of impurity, Horwitz' s equation and Horwitz' s ratio (HORRAT) (Horwitz W, 2006) were applied to set the limit of the relative standard deviation (RSD) as the following equation;

$$RSD = 0.66 \times 2C^{-0.1505}$$

where C is the concentration ratio (no unit)

While the limit of HORRAT would not be more than 2. Calculation of the HORRAT was presented as follows;

$$HORRAT = \frac{RSD\ obs}{RSD\ expected}$$

where $RSD\ obs$ is the RSD calculated from the experiment
$RSD\ expected$ is the RSD calculated from Horwitz's equation

Repeatability (Intra-day precision)

Repeatability was examined by performing of 6 replicate injections of 6 determinations of HMW filgrastim solution at LOQ level. Percent relative area of any interested impurity (from the solution at LOQ level) was normalized as compared with the total peak area of the filgrastim and the other HMWP from the chromatogram of the reference solution of 0.3 mg/mL, excluding the peak area of that interested impurity (see the equation below). The RSD of % relative area of the HMW filgrastim from the replicate determinations expressed repeatability parameter.

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Intermediate precision

Intermediate precision of the method was assessed by inter-day precision and different analysts. Intermediate precision was also expressed as RSD. Limit of the RSD was also specified by the value obtained from Horwitz's equation and HORRAT.

Inter-day precision

The intra-day precision was repeatedly carried out on three non-consecutive days. The RSD of the data from 3-day experiments was calculated.

Different analysts

Two analysts were separately performed the experiments in the same manner. The RSD of the data from both analysts was calculated.

Results and Discussion

Method optimization

Two major concerns of the chromatographic method are stationary and mobile phases. Since the method needed to separate the molecular weight of filgrastim and its HMWP of approximately 18,800 Daltons and greater, the silicon-based size exclusion column was used as the stationary phase. The neutral buffer (pH 7.0, ammonium hydrogen carbonate solution) was selected as the mobile phase to separate the aggregates from the intact filgrastim since the neutral pH buffer trended to have lesser effect on breaking up the non-covalent aggregates than the acidic running buffer. The pH 7.0 was much closer to the isoelectric point of the filgrastim (pI = 5.65) [34] resulting in lacking of the formation of any potential components (USP, 2013). Besides, it seemed to be clearer from the interferences of the excipients, tween 80 in particular.

Method validation

Specificity

As reference standard of each filgrastim impurity is not available, the USP HMW filgrastim RS containing all major HMWP (dimer, oligomer 1, oligomer 2 and aggregate) and the monomer was used for method validation. Moreover, it was found that the smallest peak of HMWP X was also detected. Figure 2 shows chromatograms of diluent (water), mobile phase, resolution solution in matrix solution and matrix solution. The relative retention time (RRT) of aggregate, HMWP X, oligomer 1, oligomer 2, dimer and filgrastim monomer were 0.61, 0.78, 0.81, 0.84, 0.89, and 1.00, respectively. Resolution of the filgrastim dimer and monomer was not less than 3, which exhibited suitability of the system. There was no co-eluting peak from any excipients, diluent or mobile phase at the retention times of filgrastim and its HMWP peaks. According to the peak shape, it was noted that aggregate formed a broad and irregular shape while the other peaks were narrower and more symmetric.

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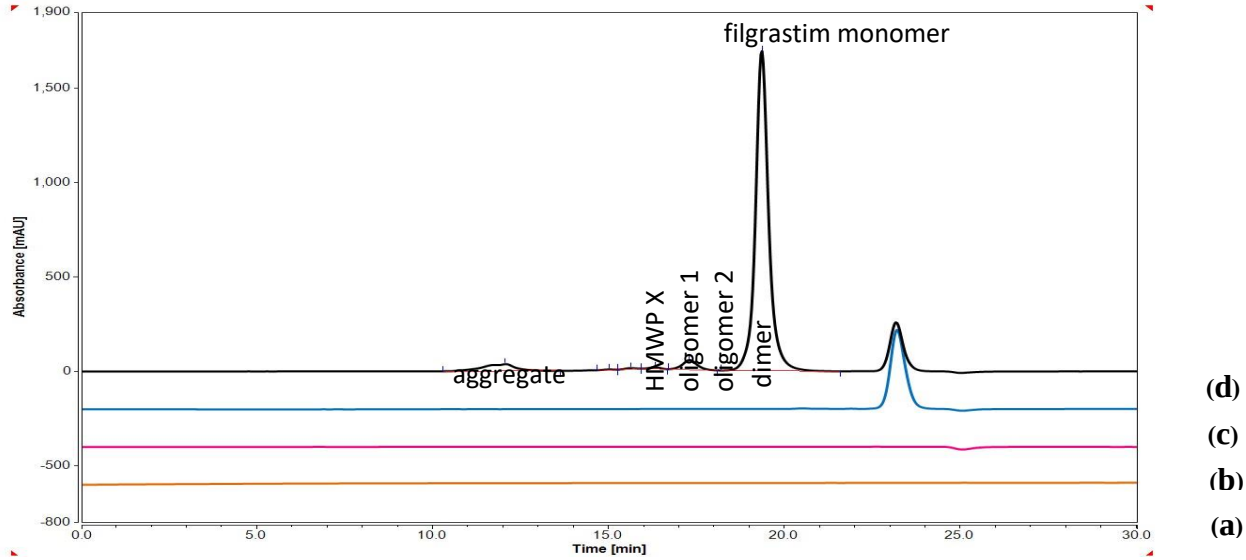

Figure 2. Chromatograms of mobile phase (a), diluent (water) (b), matrix solution (c), and resolution solution in matrix solution (d)

LOQ

The LOQ value shows how low the analyte compound can be reliably quantified [32]. There are various methods to obtain LOQ value. The mathematical calculation from the relationship between the standard deviation of the calibration curve and its slope using the multiplier suggested by the ICH guidelines was followed to determine the LOQ in this study [36]. As the USP HMW filgrastim RS used was the mixture of filgrastim and its HMWP, the initially calculated values were the concentrations of the USP HMW filgrastim RS solution, not the LOQ concentration of each HMWP.

A standard curve of each impurity was built from a series of the concentration of the USP HMW filgrastim RS solution varied from 0.001-0.5 mg/mL. Peak areas of each HMWP were plotted against the concentrations (Figure 2). All curves including oligomer 1 and oligomer 2 (data not shown) achieved the R^2 of ≥ 0.99, which indicated good linear relationship for analysis of impurity (Yin H, 2011). Slopes obtained from the regression lines and the STEYX calculated from the peak areas and concentrations were applied to determine the concentrations of the USP HMW filgrastim RS solutions as per the following equation;

$$concentration\ of\ the\ USP\ HMW\ filgrastim\ \ RS\ at\ LOQ\ level = \frac{10\ x\ (STEYX)}{Slope}$$

where $STEYX$ is the standard error of the predicted y-value for each x in the regression and $Slope$ is the slope of the regression line

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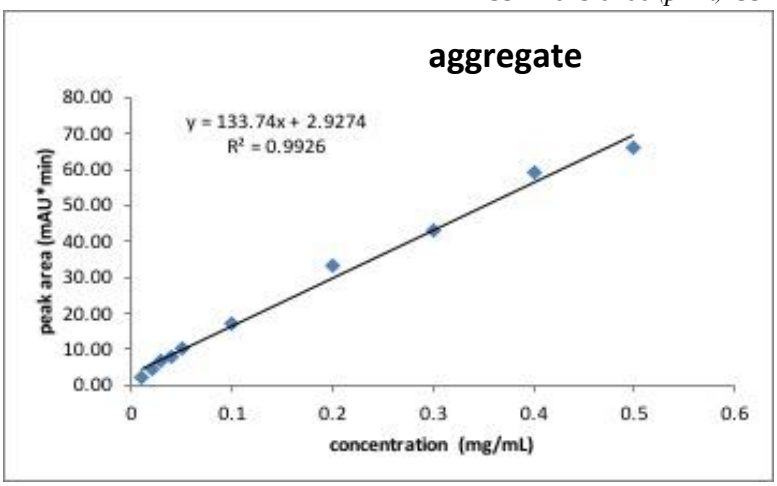

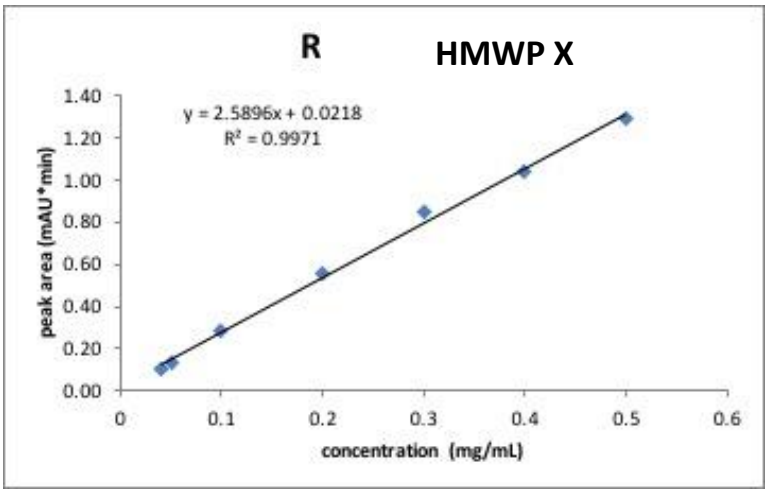

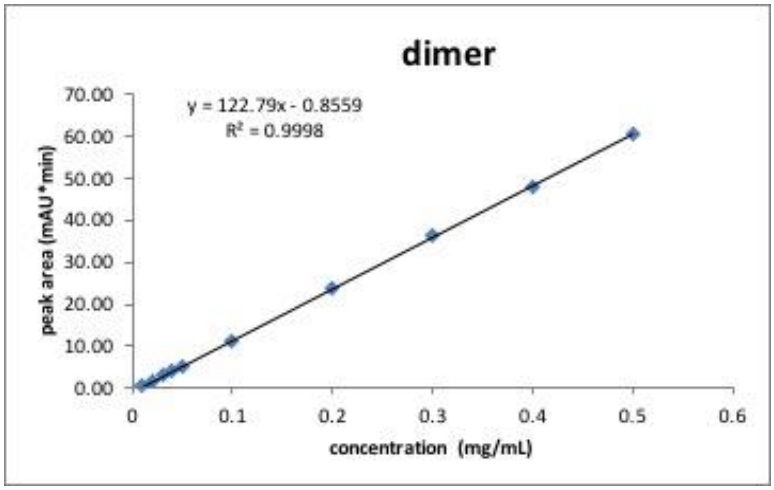

Figure 3. Linear curves between peak areas of HMWP of filgrastim and concentrations of the USP HMW filgrastim RS solutions

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Table 2. The concentration of the USP HMW filgrastim RS solution calculated based on standard deviation of the response (STEYX) and slope of each curve, experimental concentration, and S/N

Component	RRT	STEYX	Slope	Calculated concentration (mg/mL)	S/N	% relative area	LOQ (mg/mL)
aggregate	0.61	2.18	133.74	0.16	2,000:1	0.21	0.0006
HMWP X	0.78	0.03	2.5896	0.12	10:1	0.05	0.0002
dimer	0.89	0.29	122.79	0.02	10:1	0.28	0.0008

Table 2 shows the concentrations of the USP HMW filgrastim RS solutions calculated based on standard deviation of the response (STEYX) and its slope. The S/N ratio of each peak was verified and confirmed by the experiment. As mentioned earlier, S/N ratio of about 10:1 was expected to be obtained from the solution with concentration calculated based on the linear curve method. The peak of HMWP X was the smallest one adjacent to the peaks of oligomer 1 and 2. Thus, this peak was a representative in the evaluation of S/N ratio, % relative area and LOQ of these small peaks. The experimental results indicated that S/N ratios of HMWP X and dimer with the calculated concentrations were about 10:1 as predicted. However, S/N ratio of the aggregate peak (2000:1) was much greater than 10:1. The poor prediction accuracy of S/N ratio of the aggregate peak was because this peak composed of different sizes of groups of a number of filgrastim monomers leading to the irregular, broad peak shape (as shown in Figure 2). Particularly at low concentrations, the broad peaks resulted in lower accuracy of the prediction. This reflected on the R^2 of its regression line (0.9926) as compared with the other impurities (range of the R^2 from 0.9938 (oligomer 1) to 0.9998). To acquire S/N ratio of about 10:1 of the aggregate peak, the USP HMW filgrastim RS solutions were diluted and injected until the expected value was achieved. In summary, the 0.01, 0.12 and 0.02 mg/mL of USP HMW filgrastim RS solutions provided the acquired S/N ratio (10:1) of the aggregate, HMWP X and dimer peaks, respectively.

Then, % relative area of each peak was evaluated and used for calculation of LOQ. The USP HMW filgrastim RS solution of 0.3 mg/mL was used as the reference solution. The solution was appropriate for calculation of % relative area since this is the concentration of most filgrastim products and would be used as concentration of the test solution for this validated method. The peaks with S/N ratio of 10:1 obtaining from the USP HMW filgrastim RS solutions from the above were used for calculation of LOQ as 'Peak area A'. Peak area of the interested impurity on the chromatogram of the reference solution (0.3 mg/mL) was subtracted from total peak area of the same chromatogram. The 'Peak area A' was replaced by the subtracted area (Peak area B) and relative calculated as follows;

$$\%\ relative\ area = \frac{Peak\ area\ A}{(Total\ peak\ area\ from\ the\ ref.solution - Peak\ area\ B) + Peak\ area\ A}\ x\ 100$$

where $Peak\ area\ A$ is peak area of the interested impurity obtaining from the chromatogram of the USP HMW filgrastim RS solution providing peak with S/N of 10:1

$Total\ peak\ area\ from\ the\ ref.solution$ is total peak area of all peaks obtaining from the chromatogram of the 0.3 mg/mL reference solution

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$Peak\ area\ B$ is peak area of the interested impurity obtaining from the chromatogram of the 0.3 mg/mL reference solution

To calculate the LOQ of each HMWP of filgrastim, the following equation was applied.

$$LOQ\ of\ the\ imp.(mg/mL) = \frac{relative\ area\ (\%)\ x\ C_{ref.solution}(mg/mL)}{100}$$

where $relative\ area\ (\%)$ calculated from the above equation

$C_{ref.solution}$ = concentration of the reference solution = 0.3 mg/mL

The LOQ of aggregate, HMWP X, and dimer as 0.0006, 0.0002, and 0.0008 mg/mL, respectively (Table 2).

Precision and Intermediate Precision

The precision of the method was demonstrated in terms of repeatability and intermediate precision. A total of 36 injections (6 determinations x 6 injections) for each LOQ were analyzed per day for the repeatability study. The repeated experiments were carried out in three non-consecutive days to evaluate the inter-day precision. The intermediate precision was also demonstrated by two analysts, using the same HPLC system, performing in the same manner as for the first analyst. The RSD calculated from the Horwitz's equation and HORRAT of each impurity was presented in Table 3. The RSD between of the within-day replicate measurements was reported as 1.9-3.5%. Intermediate precision is the outcomes of the within-laboratory variations resulting from random situations, such as inter-day precision and different analysts [26]. Also, RSD of the inter-day precision were reported as and 3.3-6.1%. The RSD of the results of both analysts had to be lower than the RSD calculated from the Horwitz's equation. Table 3 shows the RSD of each impurity met the criteria. The RSD of 1.9-7.3% was also below the acceptance values (10.9-13.5%). This illustrated the good within-lab reproducibility of the analytical method. Therefore, this proposed SEC method was precise for analysis of HMWP of filgrastim.

Table 3. RSD, the acceptance criteria of RSD and HORRAT

Impurity	**RSD observed**			**Acceptance criteria**	
	repeatability	inter-day precision	different analysts	of RSD (RSD expected)	HORRAT (repeatability)
aggregate	1.9	6.1	7.3	11.4	0.2
HMWP X	2.2	3.3	1.9	13.5	0.2
dimer	3.5	4.1	5.3	10.9	0.3

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Conclusion

The results show the validation of SEC method was successful. The proposed method was specific, precise, and possesses excellent reproducibility attribute. Hence, this validated method was appropriate to use as a standard method to quantify the HMWP of filgrastim in various brands of biopharmaceutical products collected from multiple manufacturers by the Thai FDA.

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References

Bennett CL, Chen B, Hermanson T, Wyatt MD, Schulz RM, Georgantopoulos P, et al. Regulatory and clinical considerations for biosimilar oncology drugs. Lancet Oncol 2014;15: p. e594–605. doi:10.1016/S1470-2045(14)70365-1.

Berkowitz SA, Engen JR, Mazzeo JR, Jones GB. Analytical tools for characterizing biopharmaceuticals and the implications for biosimilars. Nat Rev Drug Discov 2012;11: p. 527–40. doi:10.1038/nrd3746.

Beveridge RA, Miller JA, Kales AN, Binder RA, Robert NJ, Harvey JH, et al. A Comparison of Efficacy of Sargramostim (Yeast-Derived RhuGM-CSF) and Filgrastim (Bacteria-Derived RhuG-CSF) in the Therapeutic Setting of Chemotherapy-Induced Myelosuppression. Cancer Invest 1998;16: p. 366–73. doi:10.3109/07357909809115775.

Biosimilars > Biosimilar and Interchangeable Products https://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapEUticBiologicApplications/Biosimilars/ucm580419.htm (accessed January 16, 2019).

Boschetti E, Jungbauer A. 15 Separation of antibodies by liquid chromatography. In: Ahuja S, editor. Sep. Sci. Technol., vol. 2, Academic Press; 2000: p. 535–632. doi:10.1016/S0149-6395(00)80062-8.

Brange J, Langkjaer L, Havelund S, Vølund A. Chemical stability of insulin. 1. Hydrolytic degradation during storage of pharmaceutical preparations. Pharm Res 1992;9: p. 715–26.

British Pharmacopoeia 2018. Filgrastim Concentrated Solution. British Pharmacopoeia 2018, vol. I, London: Stationery Office. p. I-1024–7.

Calam PD. WHO Informal Consultation on International Nonproprietary Names (INN) Policy for Biosimilar Products: p. 11.

Commentary Second Supplement USP 36-NF 31 https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/revisions/2013-10-25_usp36-nf31_2s_commentary_updated.pdf (accessed September 21, 2018).

Company TBR. Faster Drug Approval Process Will Boost the Biologics Market. Mark Res Blog 2018. https://blog.marketresearch.com/faster-drug-approval-process-will-boost-biologics-market (accessed March 9, 2019).

Driver JL, Raynie DE. 15 Method development for biomolecules. In: Ahuja S, Rasmussen H, editors. Sep. Sci. Technol., vol. 8, Academic Press; 2007: p. 425–39. doi:10.1016/S0149-6395(07)80021-3.

FDA. Biologics regulated products. Rockville, MD: FDA; 2009a. http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CBER/ucm123205.htm. (accessed July 20, 2011).

Filgrastim. U.S. Pharmacopeia-National Formulary [USP 40 NF35], vol. 1, Rockville, MD: United States Pharmacopeial Convention, Inc.; 2017, p. 4196–202.

Filgrastim - DrugBank. https://www.drugbank.ca/drugs/DB00099 (accessed June 12, 2019).

Gámez-Belmonte R, Hernández-Chirlaque C, Arredondo-Amador M, Aranda CJ, González R, Martínez-Augustin O, et al. Biosimilars: Concepts and controversies. Pharmacol Res 2018;133:251–64. doi:10.1016/j.phrs.2018.01.024.

ISSN 2673-0200 (print) ISSN 2673-0251 (online)

Hong P, Koza S, Bouvier ESP. Size-Exclusion Chromatography for the Analysis of Protein Biotherapeutics and their Aggregates. J Liq Chromatogr Relat Technol 2012;35: p. 2923–50. doi:10.1080/10826076.2012.743724.

Horwitz W, Albert R. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J AOAC Int. 2006;89: p. 1095–109.

International Conference on Harmonization (ICH). Analytical Procedure Development and Revision of Q2(R1) Analytical Validation 2005.

International Conference on Harmonization (ICH). International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: Text and Methodology. ICH-Q2B 1996.

Jeske W, Walenga JM, Hoppensteadt D, Fareed J. Update on the safety and bioequivalence of biosimilars – focus on enoxaparin. Drug Healthc Patient Saf 2013;5: p. 133–41. doi:10.2147/DHPS.S28813.

Lu HS, Clogston CL, Narhi LO, Merewether LA, Pearl WR, Boone TC. Folding and oxidation of recombinant human granulocyte colony stimulating factor produced in Escherichia coli. Characterization of the disulfide-reduced intermediates and cysteine-serine analogs. J Biol Chem 1992;267: p. 8770–7.

Mulcahy AW, Predmore Z, Mattke S. The cost savings potential of biosimilar drugs in the United States. Perspectives (Montclair) 2014.

Oliva A, Fariña J, Llabrés M. Development of two high-performance liquid chromatographic methods for the analysis and characterization of insulin and its degradation products in pharmaceutical preparations. J Chromatogr B Biomed Sci App 2000;749: p. 25–34. doi:10.1016/S0378-4347(00)00374-1.

Panopoulos AD, Watowich SS. Granulocyte Colony-Stimulating Factor: Molecular Mechanisms of Action During Steady State And 'Emergency' Hematopoiesis. Cytokine 2008;42: p. 277–88. doi:10.1016/j.cyto.2008.03.002.

Pedersen-Bjergaard S, Gammelgaard B, Halvorsen TG. Introduction to Pharmaceutical Analytical Chemistry. John Wiley & Sons; 2019: p. 460.

Philo JS. A critical review of methods for size characterization of non-particulate protein aggregates. Curr Pharm Biotechnol 2009;10: p. 359–72.

Rosenberg AS. Effects of protein aggregates: an immunologic perspective. The AAPS Journal 2006;8: p. E501–E507.

Shrivastava A, Gupta VB. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles of Young Scientists 2011;2: p. 21. doi:10.4103/2229-5186.79345.

Souza LM, Boone TC, Gabrilove J, Lai PH, Zsebo KM, Murdock DC, et al. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Science 1986;232: p. 61–5. doi:10.1126/science.232.4746.61.

Tange M, Matsumoto A, Yoshida M, Kojima H, Haraguchi T, Uchida T. Comparison of the Adsorption of Original and Biosimilar Preparations of Filgrastim on Infusion Sets and the Inhibition of Adsorption by Polysorbate 80. Chem Pharm Bull 2017;65: p. 36–41. doi:10.1248/cpb.c16-00478.

Thai Food and Drug Administration, Bureau of Drug Control. Searching and Statistic Information; Filgrastim. http://www.fda.moph.go.th/sites/Drug/ (accessed July 22, 2018).

U.S Food & Drug Administration. Neupogen® Filgrastim. https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/103353s5147lbl.pdf

ISSN 2673-0200 (print) ISSN 2673-0251 (online)

Vanz AL, Renard G, Palma MS, Chies JM, Dalmora SL, Basso LA, et al. Human granulocyte colony stimulating factor (hG-CSF): cloning, overexpression, purification and characterization. Microb Cell Factories 2008;7: p. 13. doi:10.1186/1475-2859-7-13.

Wang W. Instability, stabilization, and formulation of liquid protein pharmaceuticals. Int J Pharm 1999;185: p. 129–88. doi:10.1016/S0378-5173(99)00152-0.

Watson E, Kenney WC. High-performance size-exclusion chromatography of recombinant derived proteins and aggregated species. J Chromatogr A 1988;436: p. 289–98. doi:10.1016/S0021-9673(00)94586-2.

Yin H. Method and Validation basics-HPLC case study 2011.

Yu C-M, Mun S, Wang N-HL. Phenomena of insulin peak fronting in size exclusion chromatography and strategies to reduce fronting. J Chromatogr A 2008;1192: p. 121–9. doi:10.1016/j.chroma.2008.03.055.