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RESEARCH ARTICLE

CLEANING VALIDATION OF RECOMBINANT HUMAN GRANULOCYTE COLONY STIMULATING FACTOR (rHu-GCSF) IN MULTI PRODUCT MANUFACTURING FACILITY: A CASE STUDY

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ABSTRACT

Cleaning validation is an integral part of current good manufacturing practices in any Biopharmaceutical industry. Drug products usually get contaminate with residue of other drug products or cleaning agents manufactured in the same equipments, if the cleaning procedure is not proper. We studied the cleaning validation of GCSF Injection on three consecutive batches in multi product manufacturing facility. Various acceptance limits for product carryover was calculated based on 0.1 % of therapeutic dose, 10 ppm and 1/1000 Therapeutic daily dose of the drug. The stringent limit of maximum allowable carryover (MACO) of GCSF in other products was found to be 18 µg. The 0.5 M NaOH solution was used as a cleaning agent to clean the equipments and accessories used for bulk manufacturing of GCSF. Rinse and swab samples were collected from the cleaned equipments and accessories and tested for product carryover by sandwitch ELISA. The product carryover of GCSF in subsequent product was determined to be less than 0.1 µg and the level of cleaning agent was less than 10 ppm, thus indicating establishment of effective cleaning procedure for GCSF manufacturing.

INTRODUCTION

Contamination in drug products by other drug products, cleaning agents and micro-organisms remains the biggest concern by drug manufacturer throughout the world. Ineffective cleaning procedures may leave residues of the product or cleaning agents in the equipment. Due to these contaminations the purity and potency of the drug may be reduce and patients may show adverse drug reactions (Satu 2008). Cleaning validation studies are performed to establish documented evidence which demonstrates, with a high degree of assurance, that an equipment specific cleaning process will consistently yield results meeting specifications and quality attributes. Contamination can be controlled to an acceptable level through measures such as proper planning and implementation of cleaning processes. In validation, adequacy of each cleaning procedure requires demonstration that it can reliably and effectively remove or reduce residues to an acceptable level such that residues from the production of one product will not carry over in significant amounts to the next product. Regulatory scrutiny is more rigorous in a multi product manufacturing facility compared to a single product manufacturing. It is vital for a successful cleaning validation to have appropriate acceptance criteria. The acceptable limit for residue in the equipments is not established in the current regulations.

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Although various (FDA 1993; APIC 2000 and Health Canada 2000) regulatory guidance provide basis for cleaning validation, the scientific literature for conduct of validation is scarce. The present study demonstrate the cleaning validation of GCSF Injection, in a multi product facility with the associated therapeutic products such as Erythropoietin (EPO), Granulocyte Macrophage Colony Stimulating Factor (GMCSF), Tissue Plasminogen activator (TPA), Enoxaparin and PEG GCSF Injections.

MATERIALS AND METHODS

Chemicals and materials

Sodium hydroxide pellets were obtained from Merck specialities private Ltd, Human GCSF ELISA kit (Ray Bio®) obtained from Ray Biotech, Limulus Amoebocyte Lysate (LAL) kit (ENDOSAFE®) obtained from Charles river laboratories India pvt Ltd. Soya bean casein digest agar (SCDA) was obtained from Himedia®. Alpha swabs (Texwipe® 714A) were from Texwipe Co. High purified water was prepared by using Millipore (Milli-Q®) water purification system and three Glass plates (25cm^2) were used to simulate equipment surface.

Manufacturing and cleaning process

Formulated bulk of Granulocyte colony stimulating factor having the concentration of 300 µg / ml was prepared (5.28 L)

in 10 liter glass bottle. This bulk was filtered through 0.2 µ filter (Millipore) in another 10 L glass bottle and filled in depyrogenated vials with the help of filling machine in the same facility where other therapeutic products such as EPO, GMCSF, TPA, Enoxaparin and PEG GCSF Injection were manufactured. After filling, the left over GCSF was drained from the used equipments and accessories and flushed with purified water, followed by soaking in 0.5 molar sodium hydroxide solution for 1 hour. The soaked equipments and accessories were washed with purified water and finally flushed with water for injection (WFI) four times.

Sampling Techniques

The sampling techniques were based on Active Pharmaceuticals Ingradients committee Guidelines (APIC,2000). After completion of cleaning procedure, both swab and rinse methods were employed to collect the samples. The appropriate sampling method for various equipments and accessories used in the study are as given in Table 1. The maximum allowable carryover (MACO) was calculated based on 0.1 % therapeutic dose of drug, 10 ppm of drug and 1/1000 therapeutic daily dose of the drug (TDD) criteria as per APIC guidelines (2000).

Swab sampling

To mimic the swab sampling of equipment used in the manufacture of GCSF, three glass plates (25 cm^2) were contaminated with 1 ml of solution having 10 ppm of GCSF, then plates were dried in an oven at 40 ± 3°C. After drying, swab samples were collected with polyester swabs previously humidified with cold water for Injection (WFI). Briefly, swab was passed on the plate in zig-zag manner from right to left, returning from left to right, from top to bottom and returning upwards (Tatiana *et al.,* 2007). The collected swabs were rinsed in 5 ml cold WFI by gentle shaking and the resultant fluid was used for estimation of GCSF by sandwich ELISA test (Ray Biotech) as per manufactures instructions. The limit of Quantitation (LOQ) of GCSF was 1 pg/ml and the limit of detection (LOD) was less than 1 pg/ml as mentioned in user manual for GCSF sandwich ELISA kit.

The recovery factor of the swab was calculated using the Formula 1.
Recovery factor = Known concentration / Average estimated concentration.

The Maximum allowed product carryover (MACO) per cm^2 area (Target value) was calculated using Formula 2. Target value = MACO / Total Surface area in cm^2

The product carryover to be allowed in swab sample was calculated using Formula 3.
Swab limit (µg/ml) = Target value X Swabbed area (cm^2) / Volume of WFI used for swab.

Total product carryover by using swab result was calculated using Formula 4.
= Maximum product carry over per cm^2 X Total surface area.

Product carry over per cm^2 area was calculated using
Formula 5
Swab result (µg/ml) X volume of solvent used for swab / Swabbed area

Rinse sampling

After collecting the swab samples the equipments were rinsed each with 150 ml of WFI and the rinsate was collected in depyrogenated glass bottles. The rinsate samples were estimated for product carryover using sandwitch ELISA and also subjected to various quality determining parameter tests such as pH, conductivity, Total organic carbon (TOC), Bioburden and Bacterial Endotoxin test (BET). The GCSF to be allowed in rinse sample was calculated using the Formula 6.
Rinse limit (µg/ml) = Target value X Surface area of equipment / Volume of final rinse
Total product carryover by using rinse result was calculated using the Formula 7.
= ∑ (Obtained result of rinse µg/ml X total rinse volume + Product carryover of swabbed area)

Quality Determining Parameters

pH and conductivity: Ten milliliter of rinse samples were used to check the pH and conductivity. For preparing the standard curve, various concentrations of sodium hydroxide solutions ranging from 1 to 10 ppm were prepared and checked for pH and conductivity using Mettler Toledo pH and conductivity meter, model No. 8603 with glass electrode.

Total organic carbon (TOC): Total organic carbon in the rinse sample was determined as per Indian Pharmacopoeia (2007) using Shimadzu TOC analyzer (Japan).

Bacterial Endotoxin: The bacterial endotoxin test was performed by gel clot limit test method as per Indian Pharmacopoeia (2007) using lysate having the sensitivity of 0.125 Endotoxin units (EU) per milliliter. Briefly, 100 µl of rinse sample and 100 µl of lysate were added in the depyrogenated LAL test tube and incubated the tubes at 37±1°C for 60 minutes in a dry heating block along with positive and negative controls. After incubation the tubes were observed for intact gel by rotating the tubes in 180° angle in a smooth motion. The intact gel indicates that the endotoxin is more than or equal to 0.125 EU / ml.

Bioburden: one hundred milliliter of rinse samples were filtered using 0.45 µm cellulose nitrate membrane filter (mdi).The filter paper was placed on SCD agar plate and incubated the plate for 5 days at 30-35°C. At the end of incubation the bacterial colonies were counted.

RESULTS AND DISCUSSION

In recent years the subject of cleaning validation (CV) in active pharmaceutical ingredient manufacturing plants has received a high attention from regulators, companies and customers. Cleaning methods employed within a facility consistently controls potential carryover of product, cleaning agent and extraneous material into subsequent product to a level which is below predetermined limits (APIC 2000). The cleaning validation need to be performed on full scale commercial production and involves residue identification, residue detection, method selection, sampling techniques, setting residual acceptance criteria and recovery studies (Health Canada 2000).

The acceptance limit for product carryover are based on various criteria , such as i) Not more than 0.1 % of the normal therapeutic dose of the product to appear in the maximum daily dose of the next product, ii) Not more than 10 ppm of the product to appear in another product, iii) based on 1/1000 therapeutic daily dose (TDD). Along with these criteria, no quantity of residue should be visible on the equipment after cleaning procedures are performed (HSA 2008). In the present study, the limits for 0.1 % of therapeutic dose, 10 ppm and 1/1000 TDD were found to be 18µg, 12000 µg and 18µg respectively. Hence the stringent maximum allowable product carryover (MACO) was considered as 18µg described in Table 1.

Table 1: The theoretical calculation of MACO of GCSF using different criteria

Product name	0.1 % criteria	10 ppm criteria	1/1000 TDD
Erythropoietin Injection	80 µg	45,000 µg	75 µg
GMCSF Injection	50 µg	20,000 µg	45 µg
TNKtPA Injection	18 µg	12,000 µg	18 µg
Enoxaparin Injection	450 µg	30,000 µg	450 µg
PEG Filgrastim Injection	250 µg	10,000 µg	150 µg
Stringent limit	18 µg	12,000 µg	18 µg

Rinse and swab sampling are the two main sampling techniques available for cleaning validation. Sampling the rinse water is most useful in analyzing a large surface area or inaccessible areas, whereas the use of swabs (a direct method) can remove contaminants that may adhere to surfaces even following rinsing and it gives the exact leftover of product on the cleaned equipment, However, swab sampling is difficult to collect from the corners and inaccessible parts of equipments, in such cases rinse sampling is often followed (Health Canada 2000). In the present study rinse and swab sampling were performed as described in Table 2.

Table 2: Sampling methods for various equipments and accessories used for production of GCSF

Item	Description	Method of sampling	
		Swab	Rinse
1.	10 Liter Glass bottle used for formulation	√	√
2.	1 L measuring jar	√	√
3.	Silicon tube for filtration	NA	√
4.	Silicon tube for filling along with filling needles	NA	√
5.	10 Liter Glass bottle used filtration	√	√

The recovery studies are recommended in cleaning validation to show that the selected sampling procedure is capable of recovering the "seeded" drug substance from the surfaces cleaned (Li et al., 2007). Recovery efficiency is the fraction of material originally present on the test surface that is subsequently quantified by the analysis. The average recovery of GCSF from three glass plates (25cm^2) applied with 10 ppm was estimated as 8 ppm using sandwitch ELISA, indicating the recovery factor of swab sampling to be 1.25. Hence, the observed concentration of GCSF by swab sampling was multiplied with recovery factor to obtain the actual product carryover. Three consecutive batches of GCSF injection with standard batch size 5.28 liters were carried out. The total surface area of product contact parts was determined using mathematical formulas as 6497.84 cm^2. The theoretical maximum allowed product carryover per cm^2 area was calculated as 0.003 µg (Target value). The theoretical quantity of GCSF to be allowed in swab sample was calculated and was found to be 0.02 µg/ml. The estimated GCSF from swab sample were calculated using the recovery factor and was found to be in the range of 0.39 to 0.80 pg/cm^2.The estimated total product carryover of GCSF by swab method was 5198.3 pg or 0.005 µg.

The equipments were rinsed with 150 ml of cooled WFI by covering the total surface of the equipment after completion of swab sampling. The rinsate were collected in depyrogenated glass bottles. The theoretical acceptance limit of GCSF to be allowed in rinse sample of each item was calculated and the estimated product carryover of rinse sample for three consecutive batches were in the range of 997.5 pg to 1354.5 pg as given in Table 3. Total product carryover of GCSF by considering the correction factor due to sampling of swab in Batch no 1,2 and 3 were 1406.8 pg, 1334.8 pg and 1037 pg, respectively. The GCSF carryover into the subsequent product was observed to be less than 0.1µg using swab and rinse sampling techniques. Thus meeting the acceptance criteria. Hence, the described cleaning procedure would be useful for cleaning of equipments and accessories used for manufacturing of GCSF.

When detergents are used in the cleaning process, their composition should be known to the user and their removal should be demonstrated (Health Canada 2000). Sodium hydroxide is an alkali detergent, commonly used for cleaning in manufacturing facilities as it dissolves grease, oils, fats and protein based deposits on used equipments and is highly recommended for glass ware cleaning. (Hale et.al., 1994 and Zhe 2000). In the present study, 0.5M sodium hydroxide solution was used as a detergent to clean the glassware and accessories.

Table 3: Estimated Product carryover of rinse samples

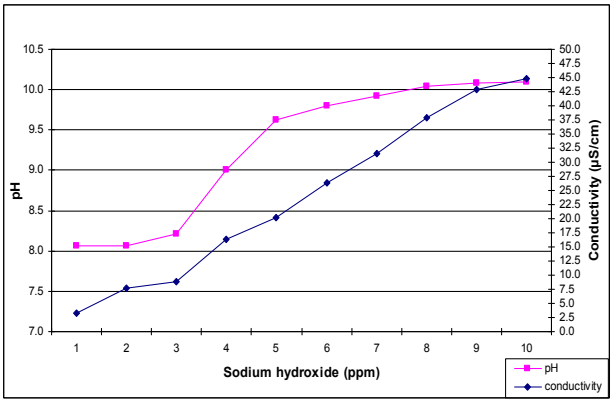
Item No	Description	Surface area in cm^2	Theoretical carry over in µg	Theoretical rinse limit pg/ml	Estimated product pg/150 ml		
					Batch 1	Batch 2	Batch 3
1	10 L Glass bottle used for formulation	2230	6.69	44600	315	261	334.5
2	1 L measuring jar	899.4	2.7	18000	268.5	274.5	246
3	Silicon tube for filtration	376.8	1.13	7533	216	198	207
4	Silicon tubes along with filling needle	761.64	2.28	15200	184.5	204	168
5	10 L Glass bottle used for filtration.	2230	6.69	44600	370.5	354	342
				Total product carryover	1354.5	1291.5	997.5

To estimate the presence of the detergent in rinse sample, various concentrations of sodium hydroxide solutions were prepared ranging from 1 to 10 ppm and checked for pH and conductivity. Standard curve of sodium hydroxide was

Table 4 : Quality determining parameters of rinse samples

ITEM →	Batch 1					Batch 2					Batch 3				
Parameters ↓	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
pH	6.42	6.38	6.74	6.66	6.29	5.97	6.54	6.66	6.48	6.53	6.45	6.55	6.71	6.21	6.38
Conductivity In µS/cm	0.79	0.64	0.54	0.76	0.77	0.84	0.62	0.51	0.62	0.71	0.64	0.55	0.52	0.63	0.74
TOC in ppb	87.4	74.2	66.8	54.2	89.1	88.2	59.6	72.3	62.5	83.7	75.4	71.2	68.5	56.8	74.6
Bioburden in CFU/100 ml	2	1	0	0	3	1	0	0	0	2	3	1	0	0	1
BET in EU/ml	Less than 0.125 EU/ml														

Fig.1: pH and conductivity of different concentration of sodium hydroxide solution



generated by plotting the concentration of NaOH on X axis and pH & conductivity on Y axis (Figure 1). Both pH and conductivity increased, with increase in concentration of NaOH, suggesting linear relationship between the parameters. The pH & conductivity of 10 ppm NaOH solution was found to be approximately 10 and 43 µS/cm respectively, which were much higher than the observed results of pH 5.0 – 7.0 and conductivity less than 1.3 µS/cm for final rinse samples.

There are many suitable physical, chemical and biological techniques available for validating and monitoring of cleaning, that include assays specific to particular molecules as well as many non specific analysis. A typical cleaning validation study involves pH, conductivity, TOC, detergent assays and product specific assays in multi product manufacturing facility (Destin 1998 and Derek *et al.,* 2007). The pH of rinse samples from three consecutive batches ranged from 6.21 to 6.74 and conductivity was not more than 0.9 µS/cm. The acceptable pH and conductivity limit for WFI to be used in manufacturing process should be 5-7 and not more than 1.3 µS/cm, respectively. The use of TOC analysis for cleaning validation has gained greater importance due to its low level detection, rapid analysis time, low cost and can detect all carbon based residues (Jenkins 1996). TOC is a non specific technique, the source of the contamination is insignificant so long as the TOC levels do not exceed established limits. Similarly, if residue limits are properly established, TOC is perfectly appropriate for use in cleaning validation applications (Jenkins 1996 and Jin *et al.,* 2001). The total organic carbon in rinse samples were less than 100 ppb, which was less than acceptable limit of 500 ppb for WFI (Indian Pharmacopoeia 2007). In addition to physical and chemical assays, microbiological evaluation of cleaned equipment is essential for parenteral drug manufacturing. Due to inherent disadvantage of using contact plates, bioburden of equipments used in validation were carried out by capturing microorganisms on membrane filters, which were subsequently incubated on SCDA plates at 30-35°C for 5 days. The number of colonies observed on the incubated membrane were in the range of 0 to 3 CFU / 100 ml, which were lower than the acceptable limits of 10 CFU /100 ml for WFI. Bacterial Endotoxin levels were less than 0.125 EU/ml, the acceptable limit of BET prescribed by Indian Pharmacopoeia 2007 is 0.25 EU/ml.

CONCLUSION

Cleaning validation was carried out for three consecutive batches of GCSF in multi product manufacturing facility. The used equipments and accessories were cleaned using 0.5 M sodium hydroxide solution and washed with purified water followed by four times water for injection rinsing. The described method of cleaning reduces the product carryover and detergent in the final rinse and swab samples to the acceptable level as described in the current regulatory guidelines.

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