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ORIGINAL ARTICLE

Development and Validation of Cleaning Process for Products Changeover Between Parental Dosage Forms of Cloxacillin Sodium 500 mg and Amoxycillin & Potassium Clavulanate 1.2 g

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ABSTRACT

Objective: Cross-contamination is a major problem in multiproduct manufacturing facilities. This can lead to major problems in a bulk drug manufacturing facility, as cross-contamination in one batch may end up in several batches. Hence, cleaning validation is of utmost importance in these facilities. The present study was undertaken to develop and validate the cleaning process for the changeover products between parental dosage forms of cloxacillin sodium and amoxycillin and potassium clavulanate where there is a risk of potential hazards of cross contamination

Method: Cloxacillin Sodium was selected using a cross-matrix approach with a 420 mg MACO value. The HPLC method was validated and recovery studies were conducted using swab sampling. The equipment was cleaned and samples were collected for chemical and microbial contamination.

Findings: Analytical method validation for Cloxacillin Sodium was carried out, and the data were found to be well within the limits. The swab recovery rate for Cloxacillin Sodium was found to be 89.67 % (Limit: NLT 70%). The swab & rinse method values were 5.6868 mg/swab and 28.14 mg / Rinse and the values were well within the acceptance limit of MACO (420 mg). The maximum total viable count was 0 cfu/swab (limit: 1 cfu /swab).

Novelty: All the chemical and microbiological results met the acceptance criteria. It can be concluded that the cleaning procedure used in manufacturing plants was satisfactory.

Keywords: Cleaning validation; Swab recovery method; Cloxacillin sodium; Crossmatrix approach

INTRODUCTION

Cleaning validation is a process that demonstrates the consistency and effectiveness of cleaning pharmaceutical production equipment. Validation detects and analyses the optimization potential and supports the implementation. Pharmaceutical manufacturers often create many product types in one facility. Often, different strengths are prepared for the same product. Cleaning processes and types of products can present a significant challenge for facilities given the multitude of procedures and equipment types involved. The variety of cleaning methods, assays, and equipment to be checked can be overwhelming [1].

Compliance with current good manufacturing practice (cGMP) regulations is crucial for ensuring quality of the product, with cleaning being a critical factor. In virtually all aspects of manufacturing, cleaning is a crucial process that extends from the initial bulk-production stage to the final form of dosage. The risk posed by cross-contamination is considered significant by the FDA and poses a serious threat to public health. The Food and Drug Administration (FDA) mandates that companies possess written guidelines known as standard operating procedures (SOPs) that outline the cleaning methods utilized for a range of equipment [2].

Amoxicillin is a type of antibiotic known as a semi-synthetic penicillin (β-lactam antibiotic), which is often combined with the beta-lactamase inhibitor potassium clavulanate (the potassium salt of clavulanic acid) to create a broad-spectrum agent that effectively targets a range of bacterial pathogens commonly found in general practice and hospitals. It prevents the formation of cross-links between linear peptidoglycan polymer chains, which are a signif-

icant component of the cell walls of both Gram-positive and Gram-negative bacteria[3,4]. Analytical techniques for potassium clavulanate and amoxicillin mixtures have been documented in USP and BP for both tablets and oral suspensions[5], whereas injections have only been reported for BP[6]. Potassium clavulanate and amoxicillin quantification in these medication formulations has been detailed in the pharmacopoeia. However, no related substance methods have been described for the determination of impurities in USP for both oral suspensions and tablets, where potassium clavulanate and amoxicillin were present in the mixtures. In BP, methods for co-amoxiclav drug formulations (oral suspension and tablets) were described using only the amount of amoxicillin dimer impurity (amox-imp J), which was determined by its relative retention time to amoxicillin. A different related substance approach was presented in the British Pharmacopoeia (BP) for co-amoxiclav injections, which involved the use of an amoxicillin impurity standard comprising penicilloic acid (amox-imp D) and amoxicillin dimer (amox-imp J) impurities[6].

Cloxacillin is a semi-synthetic antibiotic belonging to the class same as that of penicillin, and it is utilized for combating staphylococci that generate β-lactamase. This drug has less potent antibacterial properties compared to benzylpenicillin, and does not cause any significant toxicity, with the exception of allergic reactions. It is utilized to combat staphylococci that generate β-lactamases. From a literature survey, it was found that several procedures have been tested for both drugs individually, but no HPLC techniques have been reported for such a combination in any type of pharmaceutical dosage form thus far[6]. The present work depicts a precise, simple, and accurate reverse-phase HPLC approach for the simultaneous amoxycillin and cloxacillin estimation in combination with the dosage form[7].

Cleaning was the starting step of the manufacturing process. Validation is a process that involves providing evidence that is both documented and consistent, thereby ensuring that reproducible outcomes can be achieved. Cleaning validation shows that the cleansing process consistently produces residue levels that are well below the scientifically established acceptance criteria, which provide a higher degree of assurance in the effectiveness of the process. A comprehensively designed and rigorously tested cleaning process guarantees the eradication of contamination risks, thereby preserving patient security and ensuring product quality. Cleaning process validation is one of the long-standing Good Manufacturing Practice mandated by global regulatory authorities, and widely implemented by the pharmaceutical industry. The importance of cleaning validation has increased significantly because of the expansion and growth of multiproduct manufacturing facilities that use shared equipment for manufacturing.

In recent times, cleaning validation has emerged as a highly debated and evolving topic, raising a number of concerns and queries pertaining to various practices. The aim of this research is to offer recommendations for the best approaches related to cleaning validation based on the principles outlined in a range of global regulatory guidelines, including the practices that are commonly employed by leading Indian pharmaceutical companies[8]. The aim of this study was to verify the effectiveness of cleaning equipment used in manufacturing units by checking the residue level and to determine whether cleaning can limit the residue to a predetermined acceptance level for a continuous process.

The main aim of this work is to present conclusive evidence that the cleaning techniques utilized in a facility consistently keep the carryover of products, which also includes impurities and intermediates, as well as cleaning agents, and extraneous material into subsequent products to a level that falls below predetermined limits. In addition, there was no possibility of cross-contamination.

Broadly, this research endeavored to select a drug using a cross-matrix approach, and Cloxacillin Sodium was selected using this approach. Thus, the HPLC method for Cloxacillin Sodium was validated. A recovery study was conducted using a swab sampling method on a stainless-steel vessel, and the procedure was validated for cleaning the equipment used in manufacturing. The collected samples were analysed for chemical (swab and rinse) and microbial contamination, and the acceptance limit for drug carryover to the next batch was checked.

METHODOLOGY

Selection of drugs

Cleaning validation was performed in a parenteral manufacturing facility, and the drug was selected based on the human-to-human dosage form using a cross-matrix approach. Cleaning validation was performed for validating the cleaning procedure following the Standard Operating Procedure for cleaning the equipment used in the parenteral manufacturing facility; consistently and concurrently to yield results that did not exceed the predetermined limit of residues. The parameter difficulty of cleaning was somewhat subjective. This is arrived at by the number rating obtained by asking experienced operators to rate the difficulty of cleaning various products. The major concern regarding drug selection is its potency as per the MACO (maximum allowable carry over) values (MACO=Total concentration of product "A" in the final batch of product "B"). This only assumes that all of the residual of product "A" will be homogenously mixed within that specific batch of product "B."

Establishing limits and acceptance criteria

The medical dosage level is probably the most common basis for limit calculation in the pharmaceutical industry. The

MACO limit was calculated using the following formula:

$$MACO\ limit\ (as\ per\ 10\ ppm) = \frac{10\ X\ BS}{1000000}$$

Where, BS = Batch size of the previous product to be manufactured on the same Equipment.

$= \frac{10\ X\ 42000203}{1000000}$

Thus, MACO limit was found to be = 420 mg

Experimental Conditions

The experimental setup involved a binary gradient High-Performance Liquid Chromatography (HPLC) system equipped with a Photodiode Array (PDA) detector. Chromatographic separation was conducted on a ChromatopakC8Peerless® HPLC column with dimensions of 250 × 4.0 mm. The flow rate of 1.1 ml/min was maintained throughout the analysis. Compounds were detected at a wavelength of 225 nm using a PDA detector. The ambient temperature during the experiments was set at 27.0°C, while the room temperature was maintained at 25°C to ensure consistency. All the reagents used in the experiments were of HPLC grade to maintain the integrity and accuracy of the results.

Procedure for preparation of solution.

Std. solution preparation. 50 mg of cloxacillin std. in a 50 ml volumetric flask and dissolved in the mobile phase. The volume was made up using the mobile solvent. Five millilitres of the solution was pipetted into a 50 ml volumetric flask and made up to volume with the mobile phase.

Sample solution preparation. Rinse solution (filling hopper and filling wheel) was filtered the sample solution through 0.45μm filter & inject 50μl of solution was injected. The chromatograms were recorded.

Swab. Dissolve the swab sample in 10 ml of mobile phase. The solution through a 0.45μm. Filters and inject 50μl of the solution. The chromatograms were recorded.

Rinse solution. The sample solution through a 0.45μm filter & inject 50μl of the solution was injected. The chromatograms were recorded.

Cleaning procedure

The following procedure should be followed during the product-to-product changes.

Components of Filling Machine

After completing the filling activities, the contact parts of the filling machine, such as the powder-filling hopper assembly, fill wheel and accessories, piston, rubber stopper chute, bolts, and other relevant parts of the machine. Transfer all the above parts to the washing area through a wall hatch. All parts were washed thoroughly using purified water (four times) until they were free from the drug substance. Rinses thoroughly with purified water. Finally, they were rinsed with approximately 15 L of WFI (Water for Injection). During the final rinse, a sample of WFI was collected in a sterile conical flask. The washed machine parts are stored under a Laminar Air Flow (LAF) table of the machine parts assembling area until sterilization.

Sampling. Collect three samples of rinsed water from the final wash (15 liters) in sterile conical flasks, labelled, and sent to quality control for testing the absence of a previous product.

Sterile area cleaning. The spilled powder was removed from the surface of the filling table and guide rails with the help of a vacuum cleaner. Drug particles were removed from the floor using a non-fibre shedding hand brush. The walls and surface of the filling cabinet along with the table were thoroughly cleaned using WFI and a non-linting wet cloth (damped in WFI). Clean the sterile area walls and floor with WFI. The final rinse sample was used for the QC analysis.

Sampling. After completing the cleaning activities, swab sampling was performed at the filling cabinet platform, filling table, filling cabinet, and at the table surface near the filling wheel.

Sampling method. The following sampling methods were employed to confirm the validity of the cleaning procedure during the product changeover. Swab sampling, rinse sampling, and microbial sampling were employed for validating the cleaning procedure during the product change.

Sampling method and analysis

Sampling of the equipment for cleaning verification was performed after the equipment was visibly clean. All the equipment sampling locations selected were inclusive of "difficult to clean" areas.

Visual inspection

Visual cleanliness is the primary criterion for cleaning equipment. Visual inspection was performed after the completion of cleaning. After satisfactory observations, the equipment was sampled. The equipment's visual inspection was performed at a distance of less than 25 cm and lighting level of not more than 325lux.

Swab sampling

Swab samples were collected for chemical/microbiological analysis from locations specified as per the sampling locations in the cleaning validation test record. Before collection of the swab sample, the equipment's visual inspection was performed to check cleanliness. The selection of the sample position was based on the difficult-to-clean

equipment surface area. Swab samples were collected from 10×10 cm^2 (100 cm^2) for chemical analysis and from 5×5 cm^2 (25 cm^2) for microbiological analysis. The cotton tip of the swab was wetted with the respective solvents and squeezed the cotton tip by gently pressing against the wall of the test tube to remove excess solvent. A 100 cm2 area of the equipment's surface was thoroughly swabbed in a horizontal direction using one side of the swab and in a vertical direction using the other side of the swab, with a total of 10 strokes applied on each side. The swab was transferred to a clean borosilicate glass test tube and to a quality control laboratory in a dry state. Ten milliliters of the sample medium were poured into the test tube, and the residual drug from the swab in the sample medium was extracted by sonication of the test tube for approximately 5 min. The swab from the test solution was squeezed against the wall of the test tube and discarded. The test tubes were shaken to obtain a homogeneous solution. This solution was used as a test solution to analyze the drug swabbed from the equipment's surface. The calculation of quantity per swab was done with the help of the formula: -

$$\frac{Avg.\ area\ of\ sample}{area\ of\ std.\ sol} \times \frac{Std.\ wt}{50} \times dilutions\ made\ for\ sample \times purity\ factor$$

Rinse sampling

The sample was rinsed for large surfaces, equipment with hard-to-access locations, and unevenly sized equipment where swab sampling led to errors or where sampling was not possible. The equipment surface was finally rinsed with a given quantity of the mobile phase, and the sample was collected from the rinse. This solution was used as test solution to analyze the presence of the previous product. After cleaning, a 100 ml sample was drawn from the final rinse and sent for quality control of the residue content.

Microbial sampling

Carried out by using sterile soya bean casein digest agar medium.

Cotton swabs were fitted to swab samples (ear buds composed of a polypropylene stick with an absorbent extremity) in screw-capped polypropylene tubes and sterilized in an autoclave at 121 °C for 30 min. The swab was moistened with sterile distilled water and sampled from 24 to 30 cm^2 of the specified surface. The swab was rinsed with 100 ml of sterile saline (sodium hydroxide) and shaken thoroughly. One millilitre of the resultant solution was added to each of the two petri plates, and sterile soya bean casein digest agar medium was added to neutralize the effects of bacteriostatic and fungal static agents that could be picked up and transformed into the medium during sampling. The medium was allowed to solidify, invert, and incubated at 30-35 °c for 5 days. The colony forming units (cfu) per swab were counted which will give to estimate the microbial load using the surface method. The microbial count limit should not be more than 1cfu and fungi should be absent.

Recovery study of cloxacillin sodium

A recovery study was performed to ensure that after cleaning the manufacturing vessel, the result obtained by the swab method was acceptable, and the vessel was free from traces of cloxacillin sodium. The Recovery study of cloxacillin sodium using the swab method was performed on stainless steel manufacturing vessels.

Equipment used

A stainless steel vessel was used for the swab recovery of Cloxacillin Sodium to stimulate manufacturing equipment.

Procedure

This was performed to determine the efficiency of the swab testing procedure in terms of the quantity of drugs recovered from the surface.

$$\frac{The\ amount\ of\ recovered\ from\ each\ surface}{Amount\ seeded}$$

The measured concentration of the drug was added to the surface of a stainless steel sheet of approximately 10×10 sq.cm; the surface was swabbed as mentioned above. Ten milligrams of drug was accurately weighed, spread in 10 × 10 sq. cm, swabbed, and placed in a glass container containing 10 ml of WFI. The contents in the container were sonicated for 15 min, and the solution was passed through a membrane filter with a pore size of 0.45 micrometres. Subsequently, it is sent to the quality control department to determine the quantity of drugs that have been successfully retrieved from the surface.

Acceptance criteria. Each company is responsible for establishing limits that are practical, achievable, and have a scientific basis. Recovery was not less than 70%, and in case the recovery was not achievable, consistency of recovery was demonstrated**,** and all equipment was visually clean.

Chemical. Residual drug contamination was calculated by the said method and was not allowed to exceed 5.6868 mg/swab for the swab sample and 28.14 mg / rinse for the rinse sample of the next product using the same chain of equipment.

Microbiological. The total viable count did not exceed 1 CFU.

Swab Recovery. Recovery should not be < 70%.

RESULTS AND DISCUSSION

Cleaning validation studies were carried out at the parental department on the product change between Cloxacillin Sodium and Amoxicillin Potassium Clavulanate. The analytical method for Cloxacillin Sodium was validated by HPLC. The HPLC spectra of the standard and sample solutions

were recorded. The Accuracy of this method was determined using recovery studies. Recovery studies were performed, and the percentage recovery was calculated. The proposed method was found to be satisfactory in terms of its results, as demonstrated by the effectiveness of both cloxacillin and ampicillin. Ruggedness and Robustness were determined, and the % RSD values calculated from precision studies were less than + 2.0 results obtained were subjected to statistical analysis to determine standard deviation values. All validation parameters were within the limits.

The proposed High Performance Liquid Chromatography (HPLC) technique was simple and rapid. Hence, this procedure is appropriate for testing the quality of the raw materials and finished products. Recovery studies were performed on stainless-steel vessels using the swab technique. The amount recovered using the swab technique was > 70%. Therefore, the recovery study performed seemed to be suitable for the swabbing procedure followed for sample collection in cleaning validation studies of Cloxacillin Sodium. The outcome of the cleaning procedure used for chemical analysis was within the acceptance criteria (Table 1).

Cleaning validation for the equipment utilized in the manufacturing process was carried out for the last batch of Cloxacillin Sodium. (cloxacillin) to provide documented evidence ensuring that cleaning, as per the Standard Operating Procedure, yields concurrent and consistent results, which are well within the acceptance criteria. Cleaning validation for the manufacture of Cloxacillin Sodium was conducted for the last batch to ensure compliance with the Standard Operating Procedure. The collection of samples was done using swab and rinse methods, revealing a residual carryover of 5.6868 mg/swab (swab method) and 28.14 mg/rinse (rinse method) (Table 2 and Table 3).

Microbial swab analysis was also carried out to determine the growth of bacteria and fungi. The maximum total viable count for the last batch of Cloxacillin Sodium was noted to be 0cfu/swab. The outcomes met the specified acceptance criteria of 10 ppm and a MACO limit of 420 mg for chemical analysis and 1 cfu/swab for microbial analysis. With satisfactory completion of the cleaning validation, it was determined that the cleaning process followed was appropriate and could limit the drug residue to an intended level according to the company requirements.

CONCLUSION

The developed analytical method for Cloxacillin Sodium was validated and found to fall within the intended specifications. The acceptable limits for swab and rinse values were within MACO standards. The maximum total viable count for the last batch of 500 mg of Cloxacillin Sodium was found to be 0 cfu, which was well within the acceptance limit of 1 cfu/swab. All the chemical and microbiological results met the acceptance criteria. Hence, the cleaning procedure utilized for the entire equipment chain was satisfactory.

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Table 1: Results for swab recovery and cleaning validation of visual inspection

Equipment Name	Acceptance criteria	Results	Conclusion
Swab recovery on stainless steel vessel	The % recovery of cloxacillin sodium by swab method should be more than 70.00%.	The % recovery of cloxacillin sodium by swab method was 89.67 %.	The swab sampling method for cloxacillin sodium was found satisfactory
Cleaning validation of visual inspection			
✔ represents satisfactory		**☒ represents not satisfactory**	
Pre-filling turn table	Rotating platter	✔	
Filling machine	Hopper - 1	✔	The results of cleaning procedure which have been taken for visual analysis is found to be within acceptance limit. Hence the cleaning procedure was found to be validated.
	Hopper lid - 1	✔	
	Fill wheel -2	✔	
	Rubber stopper hopper	✔	
	Filling machine front platform (ss)	✔	
	Filling machine rear platform (ss)	✔	
	Convey belt	✔	
	Cabinet door	✔	
	Cabinet wall	✔	
Vacuum cleaner	Bag	✔	
	Hose pipe	✔	
Weigh balance table (ss)	Weigh balance platter	✔	
	Weigh bowl	✔	The results of cleaning procedure which have been taken for visual analysis is found to be within acceptance limit. Hence the cleaning procedure was found to be validated.
API storage LAF	Table platter	✔	
Sterile area	Filling room	✔	
	Filling cabinet	✔	
	Weigh balance table	✔	
	Machine parts assembly table	✔	
Hatch	Floor hatch	✔	
	Window hatch	✔	

Cleaning validation studies were conducted in the parental department, in which products from the same production line were used for different therapeutic purposes. Cloxacillin sodium which contains cloxacillin was found to be the product. The MACO value for cloxacillin was 420 mg for all the contacts.

Table 2: Validation results of swab cleaning method

Equipment	Equipment sub-component	Location	Chemical analysis results	Acceptance criteria limit
Pre-filling turn table	Rotating platter	Towards filling machine	2.31 mg/ cm^2	5.6868 mg / swab
Filling machine	Hopper 1	Inner surface	0.57 mg/ cm^2	
	Hopper lid 1	Inner surface	0.26 mg/ cm^2	
	Hopper 2	Inner surface	0.32 mg/ cm^2	
	Hopper lid 2	Inner surface	0.17 mg/ cm^2	
	Surface behind hopper 1 % hopper 2	-	0.86 mg/ cm^2	
	Rubber stopper hopper	Hopper	0.90 mg/ cm^2	
	Conc. Surface of rubber stopper	Outer surface	0.23 mg/ cm^2	
	SS platform opposite to rubber stopper hopper	-	0.27 mg/ cm^2	
	Rejected sample disk	-	0.27 mg/ cm^2	
	Post filling table	Towards vail filling area	0.90 mg/ cm^2	
	Filling machine front (ss)	Infront of the hopper	0.92 mg/ cm^2	
	Filling machine rear platform (ss)	Backside of the hopper	0.20 mg/ cm^2	
	Cabinet door	Inner surface	0.33 mg/ cm^2	
	Cabinet wall	In between hopper 1 & 2	0.87 mg/ cm^2	
Weigh balance (ss)	Weigh balance platter	Right portion of the table	0.23 mg/ cm^2	
API storage LAF	Table platter	Left portion of the table	0.69 mg/ cm^2	

Table 3: Validation results of rinse cleaning method

Equipment	Equipment sub-component	Location	Rinse analysis	Acceptance criteria limit
Filling machine	Miscellaneous parts	Hopper 1 & 2	0.09 mg/1000mL	28.14 mg/ rinse
	Fill wheel 1	Fill wheel 1	0.02 mg/1000mL	
	Fill wheel 2	Fill wheel 1	0.11 mg/1000mL	
Weigh balance table (ss)	Weigh bowl	Whole bowl	0.06 mg/1000mL	
Vacuum cleaner	Bag	0.05	0.05 mg/1000mL	
	Hosepipe	1.18	1.18 mg/1000mL	

Table 4: Validation results of cleaning of microbial sample by swab sampling method

Equipment	Equipment sub-component	Location	Microbial results	Acceptance criteria limit
	Rotating platter	Towards filling machine	0 cfu/swab	
	Hopper 1	Inner surface	0 cfu/swab	
	Hopper lid 1	Inner surface	0 cfu/swab	
	Hopper 2	Inner surface	0 cfu/swab	
	Hopper lid 2	Inner surface	0 cfu/swab	
	Hopper 1 & 2	Surface behind the hopper 1 & 2	0 cfu/swab	
Pre-filling turn table	Rubber stopper (inner)	Hopper	0 cfu/swab	
	Rubber stopper hopper (Outer)	Hopper	0 cfu/swab	
	Rubber stopper hopper	SS platform opposite to RSH	0 cfu/swab	
	Rejected sample disk	Opposite to hopper 2	0 cfu/swab	
	Filling machine front platform (ss)	Infront of hopper	0 cfu/swab	1 cfu
	Filling machine rear platform (ss)	Backside of hopper	0 cfu/swab	
	Cabinet door		0 cfu/swab	
	Cabinet wall	In between hopper 1 & 2	0 cfu/swab	
	Post filling turn table	Towards vial filling area	0 cfu/swab	
Weigh balance table (ss)	Weigh balance platter	Right portion of the table	0 cfu/swab	
API storage LAF	Table platter	Left portion of the table	0 cfu/swab	
		South east corner wall	0 cfu/swab	
		South door	0 cfu/swab	
Sterile area	Filling room	South west corner	0 cfu/swab	
		West wall	0 cfu/swab	
		North	0 cfu/swab	
		Outdoor	0 cfu/swab	
Floor hatch	Floor	Hatch platter	0 cfu/swab	
		Inner door	0 cfu/swab	