



Cleaning Validation of Cefalexin Capsule

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Abstract : Importance of cleaning validation in pharmaceutical industry is self-evident. It is enough to say that clean environment and clean operations are the heart of pharmaceutical activities. Cleaning is directly related to the safety and purity of pharmaceutical products and hence it becomes the most important and prime activity. The present topic deals with the cleaning validation of an oral antibiotic. The objective of the present research work is to study cleaning validation performed during changeover of Cefalexin product as oral formulation, determine the level detergent and microbial count at the manufacturing unit. For analysis of swab samples collected during cleaning validation of three different batches, many parameters were evaluated with particular acceptance limits. Among them the major evaluations such as UV analysis of drug residues and microbial count were carried out. In UV method the acceptance limit of previous product residues were calculated using maximum allowable carry over (MACO) technique. The samples were analyzed by the validated UV spectroscopic method at 262 nm. The detergent was analyzed by the validated UV spectroscopy at 650 nm. The results of analysis indicated that the levels of Cefalexin, detergent and microbial count were within the acceptance criteria. Hence it can be concluded that the cleaning methodology adopted for Cefalexin product does not leave any remains of previous batch which is confirmed by cleaning validation studies.

Key words : UV spectroscopy, Cefalexin, Microbial count, MACO.

Introduction

Validation is a quality system to assure that quality is designed in to a product or process. The FDA defines the term the validation as “establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce product meeting its Pre-determined specification and quality attributes”¹.

In terms of quality philosophy validation is defined as prevention based activity, meaning it is performed to ensure Product or Process integrity. The rationale being that if more effort is placed on development and validation at the beginning and then there will be no chance for failure during product life².

Cleaning Validation

To prevention of contamination is important in any industry producing high quality products, but is especially important in industries where the products are consumed, such as in food and drug industries. Contamination in manufacturing companies could come from many sources like-

- Contaminated starting material
- Poor cleaning of equipment
- Lack of training of operating staff
- Non-compliance with operating procedures.

The prevention of contamination through the use of control measures is important issue for manufacturing companies³.

Importance of cleaning validation^{4,5}.

- ✓ Pharmaceuticals can be contaminated by potentially dangerous substances so it is essential to establish adequate cleaning procedures
- ✓ Cleaning validation should be performed in order to confirm the effectiveness of a cleaning procedure
- ✓ The data should support a conclusion that residues have been reduced to an acceptable level (FDA)
- ✓ Particular attention should be accorded to the validation of cleaning procedures (WHO)

Cleaning validation protocol⁶⁻⁸

- ✓ The cleaning validation protocol should include the following data
- ✓ Objective of the validation
- ✓ Responsibility for performing and approving validation study
- ✓ Description of equipment to be used
- ✓ Interval between end of production and cleaning, and commencement of cleaning procedure
- ✓ Cleaning procedures to be used
- ✓ Any routine monitoring equipment used
- ✓ Number of cleaning cycles performed consecutively
- ✓ Sampling procedures used and rationale
- ✓ Sampling locations (clearly defined)

Materials & Methods

Materials

Product	Cefalexin
Generic name	Cefalexin monohydrate
Category	Anti-bacterial
Batch size	5000 No

List of equipment's

Table 1:List of equipment's used in Cefalexin capsule manufacturing

S.No	Equipment	Surface area in cm ²
	Dispensing Booth	5000
	Balance pan	2000
	Sifter	9200
	Multimill	6000
	Octagonal Blender	5310
	In process container	7000
	Capsule filling machine	25360
Total surface area of all equipment		59870 cm²

Cleaning agent

These are used in pre-wash and rinsing stage of the cleaning operation. They include material such as water, organic solvent like ethanol, isopropyl alcohol, complex detergent, oxidizing agents, organic and inorganic sequesters non foaming surfactants. Detergents are used for cleaning process, their composition should be known and acceptable limit of it should be defined after cleaning. Based on the cleaning requirement and the nature the concentration of cleaning agent must be decided [9]. The selection of cleaning agent is based on

- ✓ Mode and method of application
- ✓ Easy availability and economically affordable
- ✓ Compatibility with equipment surface
- ✓ Effective removal of dirt
- ✓ Non-toxic and easy to handle and remove

As the cefalexin capsule is soluble in SLS and slightly soluble in water. So water with SLS used as a cleaning agent with water¹⁰.

Equipment cleaning procedure^{11, 12}

Equipment cleaning procedure involves the different steps they are

- ✓ Removing of gross accumulation
- ✓ Equipment washing
- ✓ Initial rinse
- ✓ Final rinse

Method of equipment cleaning^{13, 14}

The Equipment cleaning methodology involves any one of the following types of cleaning process based on the manufacturing situation.

1. Batch to batch changeover of same product on different filling days
2. From one strength to different strength of same product on different filling days
3. One Product to other product changes over.

Sampling method¹⁵

Samples were collected from various equipments, by adopting cleaning methods as described in procedure for cleaning equipment. Swab and Rinse sampling methods were used for sampling purposes.

Rinsing¹⁵

Rinse Sampling involves passing water 10 liters (cleaning agent) over Surface area of equipment and analyzing the recovery solution.

Method of swabbing¹⁵

Swabbing was performed using Tex wipe 714 A swab, made of polypropylene tip of 1 cm² area. During the swabbing process, the operator used latex gloves. Handling of swab was done using tweezers. At no time, the operator touched the swab. The swab was moistened with appropriate cleaning agent (approx. 1ml). Moistened here means a sufficient amount of liquid to saturate the tip. Immediately after wetting the bud, the specific equipment or machine surface was swabbed. In each case the surface area swabbed was 10 cm². The diagram shown below specifies the method of swabbing the surface. Swabbing was done in a „painting“ motion across the surface. The swab was first applied in a North -South motion and then again in East - West motion (after rotating at 180⁰).

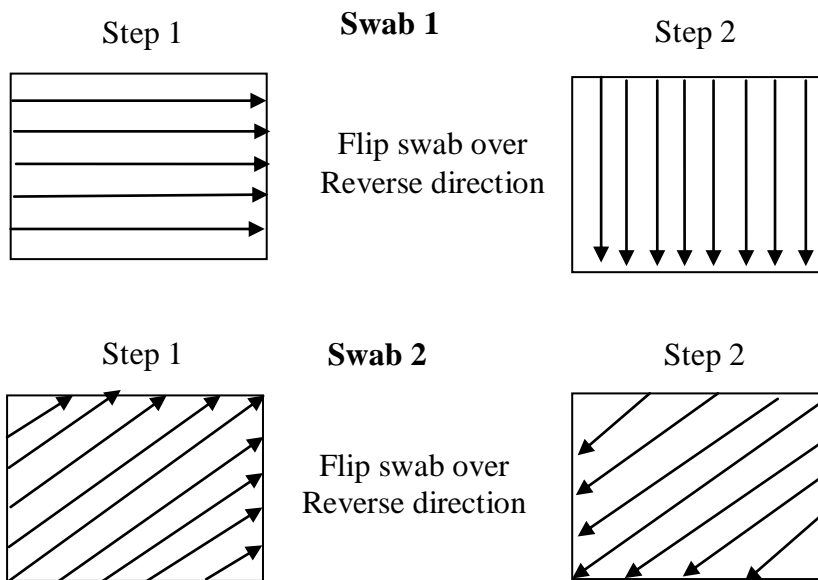


Figure 1: Structure of swabbing pattern

The swabbed swab was dipped in 10 ml of purified water, vortexed in a cyclo-mixer for 5 min. The resulting solution was analyzed by validated method of analysis. The concentration of drug was determined from the sample concentration and Standard concentration and the concentration of drug was calculated for each equipment or machine based on the surface area.

Results and Discussion

Determination of Acceptance Criteria

The principle is that the standard therapeutic daily dose of product B (Next product) may be contaminated by not more than 1/1000 of the TDD of the substance investigated in the cleaning validation (Product A or Previous product). It only applies when therapeutic daily dose is known. Establishing the acceptance limit by using the MACO shall involve the following equation¹⁶.

$$MACO = \frac{TDD\ Previous \times MBS}{SF \times TDD\ Next}$$

MACO = Maximum Allowable Carryover

TDD previous = Standard therapeutic dose of the investigated product

TDD next = Standard therapeutic dose of the daily dose for the next product

MBS = Minimum batch size for the next product

SF = Safety factor (normally 1000 is used in calculations based on TDD)

Table 2: MACO limits for Cefalexin capsules

	Product	API	Label Claim	Max.dose (No. of capsules/day)	Therapeutic daily dose	Minimum batch size
Previous Product	ProductA	Cefalexin	500 mg	2	1000mg	5000
Next Product	Product B	Cefuroxime Axe tile	500 mg	2	500 mg	4000

MACO calculation

According to the company MACO can be calculated based on the 10 ppm criteria

$$10 \text{ ppm} = \frac{R \times S \times U}{T}$$

R = 10 mg of active ingredient of product A/kg

S = Minimum batch size of product B (85kg)

T = Common surface area between product A and product B

U = Swab area (10 cm²)

$$\text{ppm} = \frac{10 \times 85 \times 10}{295490}$$

$$= 0.028\text{mg}$$

$$= 28.76 \text{ micrograms}$$

Acceptance limit: Not more than 28.76 µg of Cefalexin in next batch

Total Microbial Count: It was carried out by using soya bean casein digest agar media.

Procedure

1 ml of rinse sample was added to each of two Petri plates and sterile soya bean casein digest agar media was poured to which previously inactivated or neutralized the effect of bacteriostatic or fungi static which may present in swab sample solution. Tween and lecithin will inactivate the residue of disinfectants.

The medium was allowed to solidify and incubated the plate at $37 \pm 0.5^{\circ}\text{C}$ for 5 days.

The result was reported as colony forming units per 100 ml of rinse sample, which gave an estimate of the microbial load on that surface.

Acceptance criteria

Microbial count should be not more than 25 cfu / 100 ml

Acceptance limit for detergent

The toxicity data may be used for calculating MACO. The given equation is used for calculating the MACO from NOEL number (No Observable effect level)

NOEL = LD 50 × Empirical factor	
LD 50 of detergent	100
Empirical factor=	0.00005
NOEL = 100 × 0.00005 = 0.005	
ADI = NOEL × AAW × SF	
NOEL	0.005
AAW	70
SF	0.001
ADI = 0.005 × 70 × 0.001 = 0.00035	

$$\text{MACO} = \frac{\text{ADI} \times \text{MBS} \times \text{Swab area}}{\text{R} \times \text{common shared surface area}}$$

ADI	0.00035 mg
MBS	100000 no
Swab area	10 cm ²
Maximum daily dose	1000 mg
Common surface area	266710 cm ²

$$\text{MACO} = \frac{0.00035 \times 100000 \times 10}{1000 \times 295490} = 1.18 \text{ mg}$$

Acceptance limit

1. Not more than 1.18 mg/ swab of detergent in the next product
2. MACO acceptance limit for equipments based on 10 ppm = 28.76 µg
3. Microbial acceptance limit for equipments =25 cfu/100 ml
4. MACO acceptance limit for detergent = 1.18 mg/swab

Estimation of Cefalexin in Swab Sample¹⁷⁻¹⁹

This test is carried out during the product change over from Cefalexin to other product. This test estimates the amount of the drug in the swab sample.

Standard stock solution (500 ppm)

Weigh and transfer about 50.05 mg of Cefalexin in to 100 ml of clean and dry volumetric flask, add 60 ml of water and sonicate to dissolve and make up with water.

Standard solution (10 ppm)

Accurately 2 ml of standard stock solution was transferred in to 100 ml of volumetric flask and make up with water. The resulting solution was containing Cefalexin in the concentration of 10 ppm.

Blank solution

Transfer the 10 ml of diluents in to a test tube and place a clean swab into the test tube and sonicate for 3 minutes. Squeeze the swab and take it out.

Sample solution

Transfer the 10 ml of diluents in to a test tube and place a clean swab into the test tube and sonicate for 3 minutes. Squeeze the swab and take it out. Take the swabbing, place the swab into the test tube, sonicate for 3 minutes. Squeeze the swab and take it out.

Procedure

Measure the absorbance of standard solution and sample solution in 1cm cell on suitable uv spectrometer at 262 nm using water as a blank. Record the absorbance and calculate the content of Cefalexin by using the following formula.

$$\text{Amount of Cefalexin} = \frac{\text{Sam absorbance}}{\text{Standard absorbance}} \times \frac{\text{Standard weight}}{100} \times \frac{2}{100} \times \frac{\text{Potency}}{100} \times 1000$$

Estimation of Detergent in Swab Sample²⁰⁻²²

Preparation of standard solution

Weigh accurately 0.5 gm of sodium lauryl sulfate and transfer to a 500 ml volumetric flask. Dissolve it and dilute to a volume with distilled water. (Solution A)

Pipette 1.0 ml of solution A to a 100 ml flask and dilute to volume with distilled water (solution B)

Pipette 20 ml of solution B in to 250 ml separator add 80 ml of water and done the extraction collect the chloroform layer and make up to 100 ml with chloroform layer.

Preparation of blank solution

Pipette 100 ml of distilled water to a 250 ml separator and done the extraction, collect chloroform layer and make up to 100 ml with chloroform

Preparation of sample solution

Take the 100 ml of detergent sample to a 250 ml separator and done the extraction, collect chloroform layer and make up to 100 ml with chloroform.

Procedure

Measure the absorbance of the blank, standard, sample solution against chloroform at 650 nm in 1 cm cell.

Development and Validation of Analytical Method

UV Spectrophotometric method for the determination of Cefalexin in swab samples was developed and validated by determining the LOD and LOQ, precision, accuracy, linearity and recovery studies. Detection wavelength selected for analysis was at 262 nm.

Make: Shimadzu

Model: UV-1700 Series

Standard stock solution (500 ppm)

Weigh and transfer about 50.05 mg of Cefalexin in to 100 ml of clean and dry volumetric flask, add 60 ml of water and sonicate to dissolve and make up with water.

Standard solution (10 ppm)

Accurately 2 ml of standard stock solution was transferred in to 100 ml of volumetric flask and make up with water. The resulting solution was containing Cefalexin in the concentration of 10 ppm.

Blank solution

Transfer the 10 ml of diluents in to a test tube and place a clean swab into the test tube and sonicate for 3 minutes. Squeeze the swab and take it out.

Determination of absorption maxima

Standard solution 10 µg/ml were scanned between 200- 400 nm. Spectrum was recorded and the suitable absorption maxima selected was 262 nm.

Limit of detection and quantitation

Prepare sample solution of different concentration 0.05 µg/ml, 0.1 µg/ml, 0.5 µg/ml, 1.0 µg/ml, 2 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml. Run the solution and determine the lowest concentration of the active ingredient in the extraction solution, which can be quantitatively determined. Plot a calibration curve of concentration versus results.

Accuracy

Accuracy is measure for the difference between the average value found in the analysis and the theoretical value. It is often expressed as the percent recovery by the assay of the known, added amounts of the analyte to a blend of all excipients.

Standard stock solution (500 ppm)

Weigh and transfer about 50.05 mg of Cefalexin in to 100 ml of clean and dry volumetric flask, add 60 ml of water and sonicate to dissolve and make up with water.

Standard solution (10 ppm)

Accurately 2 ml of standard stock solution was transferred in to 100 ml of volumetric flask and make up with water. The resulting solution was containing Cefalexin in the concentration of 10 ppm.

Procedure for accuracy

The study is performed with solution of different concentration of active ingredient Cefalexin. Three different concentration of known concentration of solution across the range of 5 µg/ml, 10 µg/ml and 15 µg/ml (5 to 15 µg/ml of average limit which is 10 µg/ml).

- ✓ Record the absorbance readings in triplicate at 262 nm and note down absorbance reading at 262 nm.
- ✓ Record the absorbance readings in concentration of 80 %, 90 %, 100 %, 110 % and 120 % at 262 nm.

Acceptance criteria

Recovery from all levels should be between 98 - 102 %.

Precision

To determine the efficacy of cleaning from the surface of equipments, the process of cleaning was verified on plate of stainless steel. The process of cleaning was performed on the plate by swabbing.

Inter day precision

Inter day precision was found out by preparing 30 µg/ml equivalent concentration of formulation for three days and standard.

Dilution

Weigh and transfer about 50.05 mg of cefalexin in to 100 ml of clean and dry volumetric flask, add 60 ml of water and sonicate to dissolve and make up with water. From above solution 6 ml of standard stock solution was transferred in to 100 ml of volumetric flask and make up with water. The resulting solution was contains Cefalexin in the concentration of 30 ppm.

Acceptance criteria

RSD of the result of the determinations should not more than 2.0%.

Linearity

Accurately 2 ml of standard stock solution was transferred in to 100 ml of volumetric flask and make up with water. The resulting solution was containing Cefalexin in the concentration of 10 ppm. Aliquots from Linearity stock solution (10 ppm) were transferred into different sets of 100 ml volumetric flasks. The volumes were made up with water. To get concentration ranging from 10, 20, 30, 40 and 50ppm. These concentrations were labeled as Linearity Level 1, 2, 3, 4 and 5 respectively.

Acceptance criteria

The correlation coefficient and Regression coefficient must be greater than > 0.999 ($R^2 > 0.999$).

Recovery studies

To determine the efficacy of cleaning from the surface of equipments, the process of cleaning was verified on plate of stainless steel. The process of cleaning was performed on the plate by swabbing.

Sample solution

Prepare the standard solution of 50 µg/ml in water. Pipette 1 ml of this solution on cotton swab. Extract thrice with 30 ml of the water and transfer to 100 ml volumetric flask and add 10 ml of water to obtain 5 µg/ml.

Standard solution

Accurately 2 ml of standard stock solution was transferred in to 100 ml of volumetric flask and make up with water. The resulting solution was containing Cefalexin in the concentration of 10 ppm. The solution of 10 ppm is considered as reference solution and is considered to 100 % recovery.

Acceptance criteria

The recovery of the Swab should be greater than 75%.

The cleaning validation was performed. Cleaning validation was carried for various equipments used commonly during the production of Cefalexin capsules and the swab samples were collected and analyzed by the validated UV method for the content of Cefalexin.

Table 3:Cleaning validation report of detergent for 3Three Batches

S.No	Equipment	Location of swab area	Amount of Cefalexin in ppm		
			Batch 1	Batch 2	Batch 3
1	Dispensing Booth	Surface and Center	1.21	1.23	1.24
2	Sifter	Surface and Inner surface	2.16	2.15	2.14
3	Multi mill	Surface and Inner surface	1.42	1.43	1.41
4	Blender	Surface and Inner surface	2.23	2.21	2.21
5	In process container	Surface and contact surface	0.21	0.19	0.20
6	Capsule filling machine	Surface	1.83	1.85	1.82
7	Utensils	Surface	0.38	0.40	0.79

Amount of cefalexin was found within the limits for swab tests and MACO (Maximum allowable carry over) calculations have been performed and the results were found to be within the limits

Determination of Acceptance Criteria

The principle is that the standard therapeutic daily dose of product B (Next product) may be contaminated by not more than 1/1000 of the TDD of the substance investigated in the cleaning validation (Product A or Previous product). It only applies when therapeutic daily dose is known. Establishing the acceptance limit by using the MACO shall involves the following equation.

$$\text{MACO} = \frac{\text{TDD previous} \times \text{MBS}}{\text{SF} \times \text{TDD next}}$$

MACO =Maximum Allowable Carryover

TDD previous =Standard therapeutic dose of the investigated product

TDD next = Standard therapeutic dose of the daily dose for the next product

MBS =Minimum batch size for the next product

SF = Safety factor (normally 1000 is used in calculations based on TDD)

Table 4: MACO limits for Cefixin

MACO limits for Cefalexin capsules						
	Product	API	Lable claim	Max dose (No of Capsules/day)	Therapeutic daily dose	Minium batch size
Previous product	Product A	Cefalexin	500 mg	8	1500 mg	5000
Next product	Product B	Cefuroxime Axetile	500 mg	2	500 mg	3000

MACO calculation

According to the company MACO can be calculated based on the 10 ppm criteria

$$10 \text{ ppm} = \frac{R \times S \times U}{T}$$

R = 10 mg of active ingredient of product A/kg

S = Minimum batch size of product B (85kg)

T = Common surface area between product A and product B

U = Swab area (10 cm²)

$$10 \text{ ppm} = \frac{10 \times 85 \times 10}{295490}$$

Acceptance limit: Not more than 28.76 µg of Cefalexin in next batch

Total Microbial Count: It was carried out by using soya bean casein digest agar media.

Cleaning Method Validation by UV Spectrometry**Determination of wavelength maxima**

Working standard solution of the drug was scanned in the UV range of 200 to 400 nm, using water. The peak obtained was noted and the peak having highest absorbance was taken as wavelength maxima.

Report

The wavelength having maximum absorbance of Cefalexin was found to be 262 nm.

Limit of detection and quantitation

Absorbance of working standard solution of Cefalexin was taken at 262 nm by using water

Report

The Limit of detection and the limit of quantification were calculated for Cefalexin by visualization method.

✓ The limit of quantification is found to be 10 µg/ml.

✓ The limit of detection is found to be 0.1 µg/ml.

Accuracy

Absorbance readings in concentration of 80 %, 90 %, 100 %, 110 % and 120 % of Cefalexin was taken at 262 nm by using the water across the range of 5 µg/ml

10 µg/ml and 15 µg/ml (5 to 15 µg/ml of average limit which is 10 µg/ml)

Precision

Absorbance of samples of Cefalexin was taken at 262 nm by using water

Linearity determination

Five replicates of different concentration were studied. Absorbance of Linearity solutions of Cefalexin was measured at 262nm.

Acceptance Criteria: Correlation co-efficient should not be less than 0.999

Report

The linearity was found over a concentration range of 10 to 50 µg/ml at 262 nm, the correlation coefficient was found to be 0.999.

Recovery studies

The maximum absorbance of both standard and sample solution as given in each stage was measured at 262 nm using water as blank. Accordingly amount of analyte was calculated

Summary Table for Acceptance Criteria & Results

Table5: Acceptance criteria & results of Cefalexin

	Acceptance criteria	Results
Accuracy	Recovery from all level should be between 98% to 102%	The RSD for all active ingredients were found to be within 1%
Precision	RSD of 6 determinations should not be more than 2%	The RSD for all active ingredients were found to be within 1%
Limit of Detection	To determine the limit of detectable Limit	The LOD is found to be 0.1mcg/ml
Limit of Quantification	To determine the limit of Quantification concentration	The LOQ is found to be 0.1mcg/ml
Linearity	The correlation coefficient and Regression coefficient should not be less than 0.99 ($R^2 = \geq 0.99$)	The correlation coefficient is more than 0.99 for all the Concentration
<ul style="list-style-type: none"> • Recovery Studies • Recovery from Swab • Recovery from Plates • Recovery from Rinse 	The recovery of the Rinse, Spiked plates & Swab should be greater than 75%	The recovery of the swab was more than 80%

Result of Cleaning Validation of Cefalexin

The cleaning validation was performed at the Pharmaceutical Products manufacturing company Medreich limited, Hyderabad. Cleaning validation was carried for various equipments used commonly during the production of Cefalexin capsules. Swab samples were collected and analyzed by the validated UV method for the content of Cefalexin.

Table 6: Summarised results for cleaning validation batches

	Location of swab area	Amount of cefalexin(ppm)	Amount of Cephalexin(ppm)	Amount of cefalexin(ppm)
		batch -1	batch -2	batch -3
Equipment	Surface	1.31	0.23	0.22
	Surface	0.86	0.10	0.23
	Left	1.02	0.18	0.63
	Right	1.27	0.16	0.71
	Center	1.02	0.15	0.84
	Surface	1.14	0.14	0.12
	Surface	0.84	1.92	1.09
	Surface	0.84	1.92	1.92
	Surface	1.46	2.51	2.30
Vibro sifter	InnerSurface	1.34	2.38	1.38
	InnerSurface	1.38	1.17	1.07
	InnerSurface	1.30	1.71	1.51
	InnerSurface	1.21	1.92	1.22
	Surface	1.68	1.92	1.89
Octagonal blender	InnerSurface	1.59	1.75	1.76
Capsule filling Machine	ContactSurface	1	1	1
	Surface	1	1	1
	InnerSurface	1	1	1
	Surface	1	1	1
	InnerSurface	1	1	1
In process containers	Surface	1	1	1
	Surface	1	1	1
	Surface	1	2	1
Utensils	Surface	1	1	1

Summary & Conclusion

The conclusion drawn from the study indicates that the standard operating Procedures adopted for cleaning of the equipment and area are effective and consistent to reduce the level of cross contamination by active Pharmaceutical Ingredient (API), cleaning agent and bio load left over after cleaning to predetermined level of acceptance. This means that the next product can be manufactured in the equipment with no risk of contamination.

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