Research Article

Evaluation of cleaning method Validation techniques of ciprofloxacin

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ABSTRACT

Analytical methods used to determine the effectiveness cleaning were validated for Ciprofloxacin. The drug was selected on basis of solubility in water. Water is the major solvent used for cleaning of equipments. This drug is practically insoluble in water, so it may leave product residues even after cleaning of the equipments and require lot of water for cleaning. So, the analytical method used to determine the cleanliness should be effective. During analytical method validations following parameters were evaluated i.e. Linearity, Specificity, LOQ, LOD, and recovery studies by swab sampling and rinse sampling techniques. The visually cleanliness criteria was also evaluated by spiking known amount of samples on SS plates. From the results it was concluded that the developed analytical method was sensitive and accurate.Ciprofloxacin: UV Spectro-photometric analytical method was developed which showed an absorption maxima λmax at 282 nm wavelength and linearity range 2 to 12 µg/ml. Recovery of drug from Swab sampling, rinse sampling techniques were found satisfactory and within the acceptance criteria.

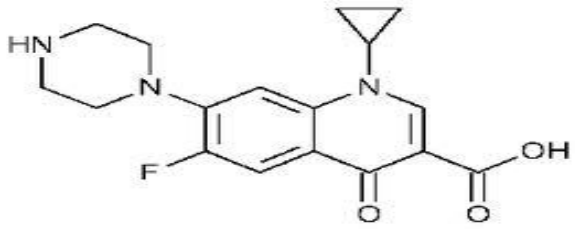
Keywords: Cleaning of equipment; analytical method validation; swab sampling.

INTRODUCTION

Pharmaceutical industries are vital segment of healthcare system. Manufactures of products which are life saving, life maintaining drugs. Not to fail its client by releasing substandard or adulterated drugs. Quality product should be maintained throughout its product lifecycle. It is essential to validate and maintain all the critical process parameters within the limit as per specification. So it is essential to establish adequate equipment cleaning procedures to prevent cross contamination due to remnants of product residues. **According to FDA-guideline:** "Process Validation is defined as establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes."[1] Equipment cleaning validation procedures are mainly used in pharmaceutical industries to prevent contamination of drug products; hence it is critically important as per federal and other standard regulations. The most important benefit of conducting validation study is identification and correction of potential problems that are previously unsuspected, which may compromise with quality of subsequent batches of drug product. The basic need of quality assurance that product produced must meet the quality requirements; i.e. Safety, Identity, Strength, Purity and Quality. The cleaning of "difficult to reach" surface is one of the most important consideration in equipment cleaning validation. Equipment cleaning validation in an API facility is extremely important as cross contamination is one of the pharmaceutical dosage forms, will multiply the problem. Therefore, it is important to do a step-by-step evaluation of API process to determine the most practical and efficient way to monitor the effectiveness of the cleaning process. It is necessary to validate cleaning procedure for the some reasons :(1) it is regulatory requirement in Active Pharmaceutical Ingredient product manufacture. (2) It is prime customer requirement since it ensures the purity and safety of the product. (3) It also assures the quality of the process through an internal control and compliance. [2,3]Now-a-days pharmaceutical industries are increasingly using the multipurpose equipment and automated clean-in-place procedures; it has become more important to establish evidence that cleaning procedure is effective. FDA has placed an increased emphasis on the cleanliness of the equipment to minimise the risk of cross contamination and adulteration of drug products made subsequently using the

same equipment. FDA"s July 1993 "Guide to Inspections, Validation of Cleaning processes requires companies to have "written general procedures on how cleaning processes will be validated, and these procedures" should address issues such as sampling procedures, analytical methods to be used, including the sensitivity of those methods. Analytical method should establish evidence that particular method will give expected and reproducible results up to acceptance or ppm levels. As per ICH Q2 guideline, typical validation parameters which should be considered are Accuracy, Precision, Linearity, Repeatability, Specificity, Detection Limit, Quantitation Limit. [4, 5, 6]

Ciprofloxacin is an antibiotic drug. It is soluble in water , ethanol and DMSO .[7, 8, 9]
Structure:



OBJECTIVE

The cleaning validation is to verify the effectiveness of the cleaning procedure for removal of product residues, degradation products, preservatives, excipients, and /or cleaning agents as well as the control of potential microbial contaminants. It is necessary to ensure that there is no risk associated with cross-contamination of active ingredients. Cleaning procedures must strictly follow carefully established and validated methods. The main objective of cleaning validation of equipment /utensils /components is to demonstrate sufficient documented evidence to ensure that the cleaning process can consistently remove residue of the subjected product below the established Acceptance Criteria.
The objectives of the study are
- Selection of Drug based upon the solubility of drug in water, because water is major solvent used for cleaning of equipment.
- Analytical method validation as per ICH Q2 guideline for selected API
- Validation sampling methods (swab and rinse sampling) which are usedCleaning validation study.
- The study also involves finding out criteria for visual cleanliness limit forselected drug by impregnating known amount of drug on SS plates.

EXPERIMENTAL

1) Solubility of ciprofloxacin

Solubility of Ciprofloxacin was checked by using different solvent.

2) Preparation of mixture

Mix 800.0 ml of acetonitrile and 200.0 ml of water to get (4:1) solvent mixture.

3) λmax OF CIPROFLOXACIN

Prepare 10µg/ml solution

Weighed accurately a quantity of 100 mg of pure ciprofloxacin and diluted to100.0 ml with solvent mixture. From that 1.0 ml of above solution was taken anddiluted to 100.0 ml by using solvent mixture to get a concentration of 10 µg/ml solution. Λmax were determined in the range of 200nm to 400nm in UVspectrophotometer by using solvent mixture as a blank.

Cleaning method validation

The following parameters shall be checked:
Linearity ,Specificity, Limit of detection, Limit of Quantitation, repeatability,reproducibility, Stability, Blank swab interference analysis, Recovery from swab sample, Recovery from S.S plate, Recovery from Rinse solution.

4)linearity of ciprofloxacin

Linearity assessed by analysing working standard solution of concentration in rangeof 0, 2,4,6,8 and 10 µg/ml. Measure the absorbance of the solution at 282 nm.Tabulate the results and plot the graph of absorbance v/s concentration and regressioncoefficient was calculated. The result was given in tablNo.1 and graph was given in Figure No.1.
Limit: The regression coefficient should not be less than 0.98.

5) Specificity and selectivity

Ciprofloxacin 10 µg/ml working standard solution was prepared in solvent mixtureand absorbance was measured at 282 nm by taking the solvent mixture as a blank. The absorbance of the same concentration solution was measured six times. The result was given in Table No.2.

6) Reproducibility of ciprofloxacin

Prepare 5 µg/ml solution of ciprofloxacin.
Absorbance was measured at 282 nm in UV spectrophotometer in two instrumentsthat is instrument -1 (Jasco) and Instrument-2(Shimadzu). This procedure was repeatedfor 10 µg/ml. Three readings were taken and mean and standard deviation wascalculated. The result was given in Table No.3

7) LOD AND LOQ

100 mg of Ciprofloxacin was accurately weighed and diluted to 100.0 ml with thesolvent mixture. From that 10.0 ml solution was taken and diluted to 100.0ml with the solvent mixture. From that 5 different concentration were prepared that is 2, 4, 6, 8and 10 µg/ml. Absorbance was measured at 282 nm by using solvent mixture as ablank. Six reading were taken for each concentration. Mean and standard deviationwas calculated. LOD and LOQ were calculated. The result was given in Table No.4 and graph was given in Figure No.2.

$$LOD = \frac{3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = standard deviation
s = slope of the calibration curve.

8) Repeatability

Standard solutions of ciprofloxacin (2, 4, 6, 8, and 10µg/ml) were prepared.Absorbance was measured at 282 nm by taking the solvent mixture as a blank. Theabsorbance of the same concentration solution was measured six times and RSD wascalculated.The result was given in Table No.5.

9) Stability of ciprofloxacin

Ciprofloxacin 10 µg/ml working standard solution was prepared in the solventmixture and absorbance was measured at time intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8 and24 hrs at 282 nm. The result was given in Table No.6.
Limit: RSD readings at different time interval should be less than 2%. The time atwhich the absorbance value does not vary by ± 5% to that of initial reading, will bethe time limit within which the standard solution can be used.

METHODS

10) Swab sampling method for ciprofloxacin

Blank swab absorbance:
Six polyurethane Texwipe swabs were washed with distilled water and dried.10.0 mlsolvent mixture was taken in test tube and that dried swab was dipped in solventmixture. Absorbance was measured at λmax 282 nm. This procedure was repeated forremaining swabs. Mean of the absorbance value were calculated.The result was given in Table No.7.

Recovery study from Swabs Spiked with Solution

Standard solution

Six different standard solutions i.e. 50, 75, 100,125, 150, and 200 µg/ml wereprepared in the solvent mixture from the ciprofloxacin working standard solution.From the above solution 0.5 ml was taken from and added directly into test tubecontaining 10.0 ml solvent mixture. Absorbance was measured at λmax 282 nm.The result was given in Table No.8.

Recovery study of swab sample

Polyurethane swabs were taken washed with distilled water and dried. 0.5 ml of 50,75, 100,125,150 and 200 µg/ml were taken and slowly delivered the solution with thehelp of graduated pipette on the tip of the swab so as to absorb the solution properlyby the swab. The spiked swabs were placed individually in test tube containing 10.0ml solvent mixture. Slowly stirred and allowed the bubble to subside before taking theabsorbance. Absorbance was measured at λmax 282 nm. From this absorbance valueBlank swab was subtracted to obtain corrected absorbance. Percentage recovery;mean percentage recovery was calculated. RSD for each set of swabs was calculated. The result was given in Table No.8 and graph was given in Figure No.3 and 4.

Plate recovery study from Swabs Spiked on SS plates

0.5 ml standard concentration was spreaded in 2 x 2 inch square area of S.S. plate anddried. Swab was Strikes on one side in a horizontal direction, and with the other sidein a vertical direction back and forth to cover the entire area. Swab was placed in testtube containing 10.0 ml of solvent mixture. Stirred and absorbance was measured.The experiment was repeated. Percentage recovery was calculated. The result was given in Table No.9 and graph was given in Figure No.5 and 6.

Limit: The minimum percentage recovery should be more than 90%. RSD should beless than 10%.

11) Rinse recovery from ss plate spiked with sample

Six different standard solutions that are 25, 50, and 75, 100, 125, 150µg/ml were prepared in the solvent mixture from the ciprofloxacin working standard solution. 0.5ml standard solution was dispersed in 2 x 2 inch square area on plate and dried.10.0mlsolvent mixture was added in S.S plate and it was rinsed. Collected Absorbance was measured at 282 nm in UV spectrophotometer. The experiment was repeated. The result was given in Table No.10 and graph was given in Figure No.7 and 8.

Limit: The minimum percentage recovery should be more than 85%. RSD should beless than 10%.

12) Visual clean method

Standard solutions of ciprofloxacin (25, 50, 75, 100, 200, 300 and 400 µg/ml) were prepared. 0.5 ml standard solution was taken and dispersed in 2 x 2 inch square areaof S.S. plate by using graduated pipette. It was properly dried. Check for the visibilityof the residue.The result was given in Table No.11 and 12.

RESULTS AND DISCUSSION

Results of evaluation of Cleaning method Validation techniques of ciprofloxacin are discussed below.

Sl. No.	Validation Parameter	Results
1	Solubility ofciprofloxacin	Slightly soluble in water.
2	Determination of λ max	Absorption maxima at λmax at 282 nm in solvent mixture (Acetonitrile : Water) (4:1)
3	Linearity and Range	Regression coefficient for linearity of the ciprofloxacinworking standard solution was found to be 0.994 in the range of 2-10 µg/ml.
4	Specificity and Selectivity	Method was selective, No interference of Solvent or Swab during analysis of drug.
5	Reproducibility or Ruggedness	Std. conc. solutions of 5 and 10µg/ml were checked by using two different make instruments. No significant difference in absorbance.
6	Repeatability	Different concentrations i.e. 2, 4, 6, 8, 10µg/ml were prepared no much variation in absorbance values for each concentration. The percentage RSD was found within the limit.
7	Limit of Detection Limit of Quantification	0.12 µg 0.4 µg
8	Stability of Pioglitazone Solution	The prepared standard solution can be used up to 24 hrs at room temperature
9	**Swab Sampling Method:** Blank swab absorbance (Interference):	No Interference of swab during analysis.
10	Recovery Study from Swabs Spiked with Std. Solutions	Percentage recovery from the swab samples were more than 90%. RSD was found to be less than 5%.
11	Plate Recovery Study from Swabs Spiked on SS Plates	Percentage of recoveries from the SS plate by using swab was more than 85%. RSD was found to be less than 5%.
12	Recovery from Rinse Samples.	Rinse recovery was found to be within limit i.e. more than 85%. Mean Rinse recovery was found to be 94.11%
13	Visual Clean Method	Above 40 µg/ml was Visible

CONCLUSION

Analytical methods for validation of cleaning procedures for Pioglitazone was developed. The validation study was carried out as per ICH Q2 guideline. Recovery studies were carried out by both Swab and Rinse sampling techniques. By employing visual examination of cleanliness by spiking known concentration drugs on SS plates were determined. This is first step to assess the cleanliness of equipment. If product residue visible, till how much visibility there and was evaluated and found out by swab and rinse sampling techniques. Swab sampling method: By this techniques recovery of drugs were found to be more than 90%. It is a direct method of sampling, rather than indirect such as rinse sampling. Advantages swab sampling techniques are that, residues that are insoluble can be sampled by physical removal and areas that are hardest to clean and which are reasonably accessible can be evaluated. It is expected the swab will pick up all the residues on the surface which can then be assayed. The reproducibility is suspect, because of the human involvement. Sampling spiked surfaces with known amounts is often served as a training method. The disadvantages of swabbing methods are a) Inability to access some areas; these are usually the most difficult to clean areas.

b) Assumes uniformity of contamination surface; invariably, contamination is not uniform, c) Must extrapolate sample area to whole surface; it can be difficult to estimate the total surface area of the equipment and the calculation also requires that the swab location be carefully measured and recorded. Rinse sampling method: By this techniques recovery of Pioglitazone was found to be more than 85%. The solubility of drug in solvent used sampling plays an important role. Rinse samples allow sampling of a large surface area, inaccessible systems or those that cannot be routinely disassembled. Volume of solvent used for rinsing is critical to ensure accuracy of results. Rinse samples on their own are insufficient evidence of cleaning and should be used in combination with other sampling methods. It should be used in combination with swabs; together they balance out the disadvantages.

Hence the Analytical method for validation of cleaning procedures for Ciprofloxacin was validated.

Table 1: Linearity of Ciprofloxacin Standard Solution at λmax 282nm

Concentration (µg/ml)	Absorbance
0	0.000
2 µg/ml	0.256±0.002
4 µg/ml	0.397±0.001
6 µg/ml	0.62±0.004
8 µg/ml	0.796±0.003
10 µg/ml	0.987±0.001

Table 2: specificity and selectivity of ciprofloxacin at λmax 282nm

Sl. No	Abs.
1	0.982±0.003
2	0.987±0.001
3	0.994±0.001
4	0.991±0.002
5	0.985±0.004
6	0.994±0.002

Table 3: Reproducibility of Ciprofloxacin at λmax 282nm

S. No.	Conc.(µg/ml)	Instrument 1 (Jasco)	Instrument 2 (Simadzu)	Inference
1	5	0.51	0.503	No significant difference
2		0.509	0.502	
3		0.512	0.504	
	Mean ±SD	0.510±0.001	0.503±0.001	
4	10	0.996	0.994	No significant difference
5		0.999	0.995	
6		0.998	0.992	
	Mean ±SD	0.996±0.001	0.993±0.001	

Table 4: Limit of Detection and Limit of Quantification of Ciprofloxacin at λmax 282nm

Conc. (µg/ml)	2	4	6	8	10
Mean ± SD	0.254±0.002	0.400±0.002	0.614±0.003	0.786±0.005	0.991±0.006

Table 5: Repeatability of Ciprofloxacin at λmax 282nm

Conc. (µg/ml)	2µg/ml	4µg/ml	6µg/ml	8µg/ml	10µg/ml
Abs	0.256	0.397	0.620	0.796	0.987
	0.250	0.402	0.609	0.776	1.005
	0.256	0.401	0.614	0.787	0.989
	0.253	0.400	0.612	0.784	0.987
	0.256	0.403	0.615	0.788	0.989
	0.254	0.402	0.614	0.785	0.992
Mean ± SD	0.254±0.002	0.400±0.002	0.614±0.003	0.786±0.005	0.991±0.006
CV= SD/Mean	0.0078	0.0050	0.0048	0.0063	0.0060
RSD	0.7874	0.5000	0.4885	0.6361	0.6054

Table 6: Stability of Ciprofloxacin Solution at λmax 282nm

Time (hrs)	Abs.
0	0.990±0.002
1	0.987±0.001
2	0.986±0.001
3	0.984±0.001
4	0.982±0.003
5	0.981±0.003
6	0.978±0.002
7	0.978±0.001
8	0.975±0.004
24	0.962±0.002

METHODS

Swab sampling method

Table 7: Blank swab absorbance (Interference) of Ciprofloxacin at λmax 282nm

Swab No.	1	2	3	4	5	6	Mean
Blank abs	0.016	0.018	0.019	0.017	0.019	0.017	0.018

Table 8: Recovery Study from Swabs Spiked with Std. Solutions

Concentration of the Solution used for spiking the swab (µg/ml)						
25		50	75	100	125	150
Concentration of the standard Solution (µg/ml)						
1.25		2.5	3.75	5	6.25	7.5
Absorbance of Standard Solutions						
0.268±0.002		0.352±0.005	0.489±0.002	0.639±0.006	0.740±0.001	0.968±0.004
Absorbance of Swabs samples Spiked with Std. Solutions						
Sl. No.	25	50	75	100	125	150
1	0.280	0.372	0.509	0.654	0.753	0.981
2	0.283	0.368	0.501	0.660	0.750	0.985
3	0.286	0.363	0.504	0.652	0.76	0.982
Mean Samp. abs	0.283±0.003	0.367±0.004	0.504±0.004	0.655±0.004	0.754±0.005	0.982±0.002
Corrected abs	0.265±0.003	0.349±0.005	0.480±0.001	0.637±0.004	0.736±0.005	0.965±0.002
RSD	1.13	1.43	0.21	0.63	0.68	0.21
% Recovery	98.88	99.14	98.15	99.68	99.45	99.69

Table 9: Plate Recovery Study from Swabs Spiked on SS Plates

Concentration of the Solution used for spiking the SS plate (µg/ml)						
25		50	75	100	125	150
Concentration of the standard Solution (µg/ml)						
1.25		2.5	3.75	5	6.25	7.5
Absorbance of Standard Solutions						
0.268±0.002		0.352±0.005	0.489±0.002	0.639±0.006	0.740±0.001	0.968±0.004
Absorbance of Swabs samples Spiked with Std. Solutions						
Sl. No.	25	50	75	100	125	150
1	0.261	0.340	0.450	0.592	0.681	0.904
2	0.259	0.341	0.452	0.594	0.680	0.902
3	0.260	0.338	0.457	0.590	0.677	0.905
Mean Samp. abs	0.260±0.001	0.339±0.001	0.453±0.001	0.592±0.002	0.679±0.002	0.903±0.001
Corrected abs	0.242±0.001	0.321±0.001	0.435±0.003	0.574±0.002	0.661±0.002	0.885±0.001
RSD	0.4132	0.3115	0.6896	0.3484	0.3025	0.1129
% Recovery	90.29	91.19	89.78	89.82	89.32	91.42

Table 10: Rinse Recovery from SS Plate spiked with sample

Concentration of the Solution used for spiking the SS plate (µg/ml)						
25		50	75	100	125	150
Concentration of the standard Solution (µg/ml)						
1.25		2.5	3.75	5	6.25	7.5
Absorbance of Standard Solutions						
0.092		0.111	0.206	0.247	0.361	0.488
Absorbance of Rinse samples with Std. Solutions						
Sl. No.	25	50	75	100	125	150
1	0.252	0.337	0.460	0.602	0.692	0.911
2	0.250	0.333	0.459	0.601	0.694	0.910
3	0.254	0.332	0.461	0.603	0.690	0.909
Mean Samp. abs	0.252±0.002	0.334±0.002	0.460±0.001	0.602±0.001	0.692±0.002	0.91±0.001
RSD	0.7936	0.5988	0.2173	0.1661	0.2890	0.1098
% Recovery	94.02	94.88	94.06	94.20	93.51	94.00

Table 11: Visual Clean Method of Ciprofloxacin at λmax 282nm

Conc.(µg/ml)	25	50	75	100	125	150
Inference	Not visible	Visible	Visible	Visible	Visible	Visible

Table 12: Visual clean criteria

Conc.(µg/ml)	**10**	**20**	**30**	**40**	**50**
Inference	Not visible	Not visible	Not visible	Visible	Visible

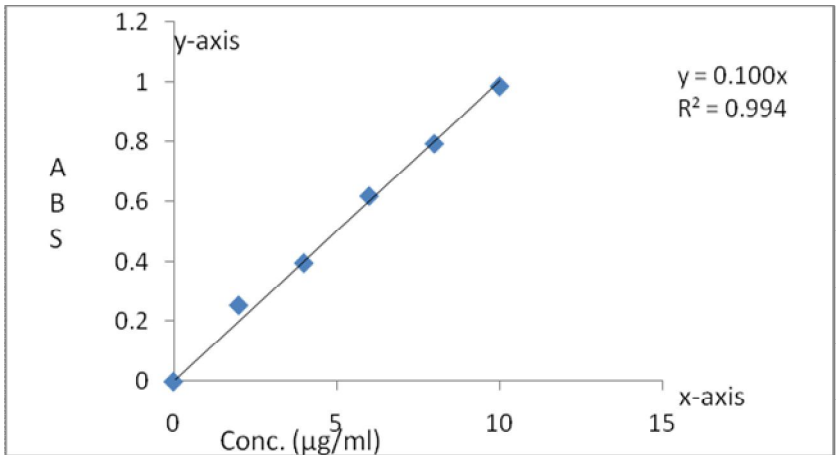


Fig. 1: Linearity of Ciprofloxacin Standard Solution at λmax 282nm

Acceptance criteria: The regression coefficient found to be greater than 0.98
Result: The regression coefficient for linearity of the ciprofloxacin working standard solution is found to be 0.994.

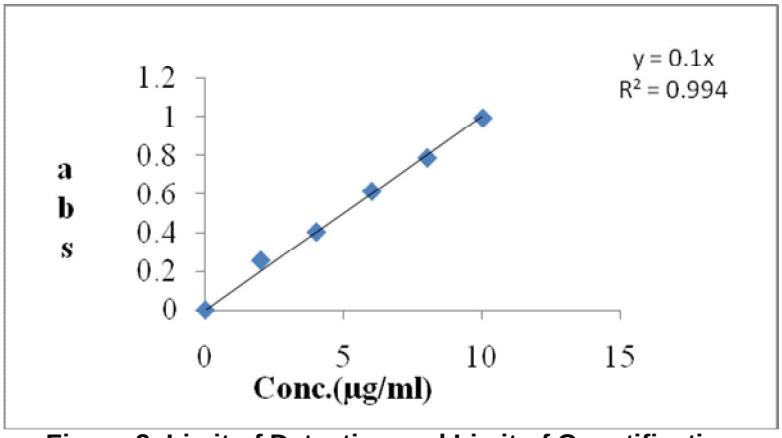


Figure 2: Limit of Detection and Limit of Quantification

Result: LOD was found to be 0.12µg and LOQ was found to be 0.4µg.

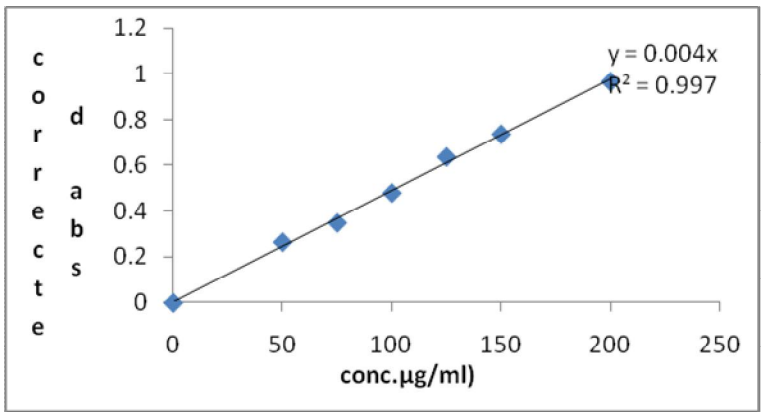


Fig. 3: Recovery Study from Swabs Spiked with Std. Solutions: Drug content per swab vs. corrected absorbance graph

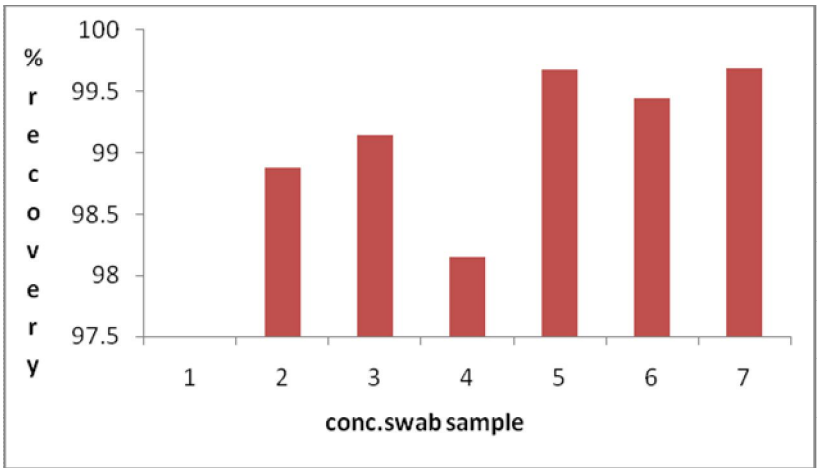


Fig. 4: Conc. swab sample Vs %Recovery graph

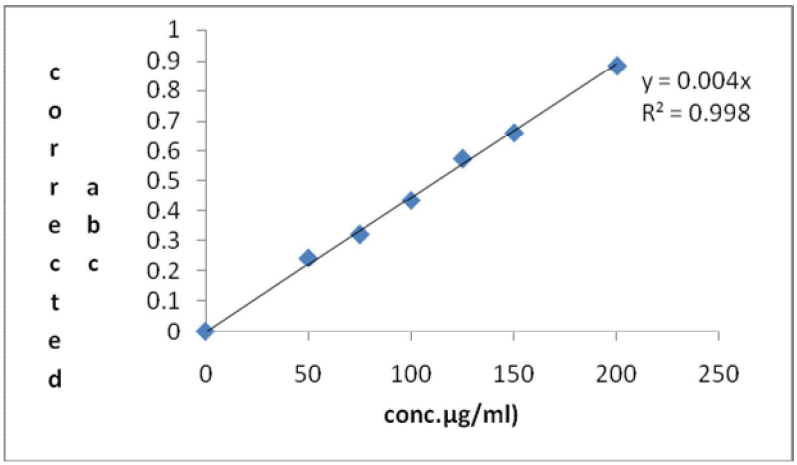


Fig. 5: Plate Recovery Study from Swabs Spiked on SS Plates: Conc. Vs corrected absorbance graph

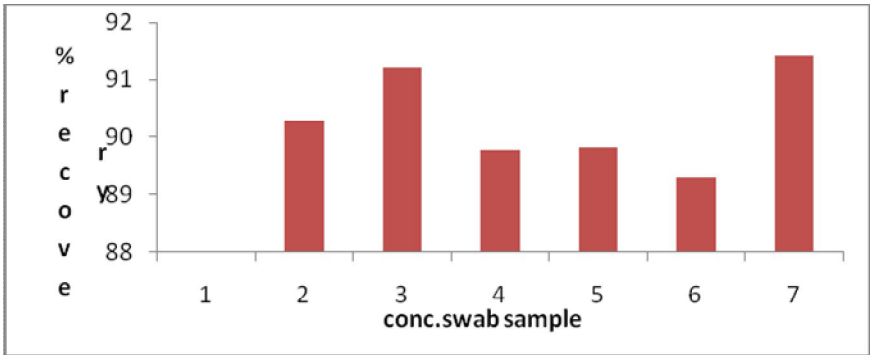


Fig. 6: Conc. swab sample Vs %Recovery graph

Result: The percentage recoveries from the swab spiked with SS plate were more than 85% for six different concentrations std. Solutions. Mean swab recovery was found to be 90.30%. RSD was found to be less than 5%. When graph was plotted drug content/swab vs. Mean recovery linear graph obtained with regression coefficient 0.998.

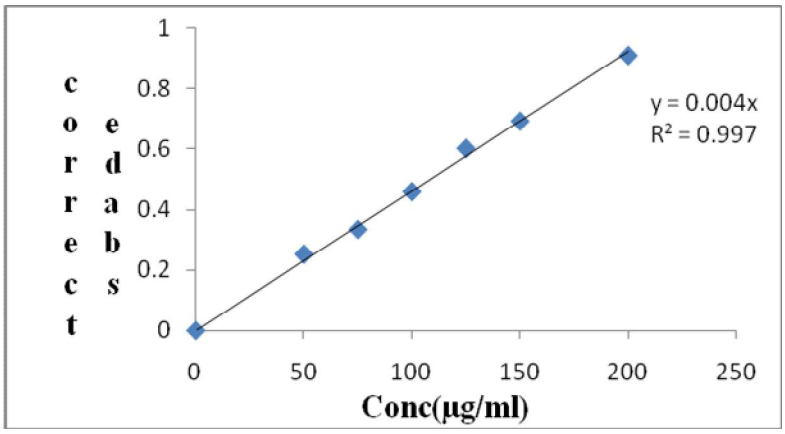


Fig. 7: Rinse Recovery from SS Plate spiked with sample: Drug Conc./spiked part Vs Rinse Sample absorbance

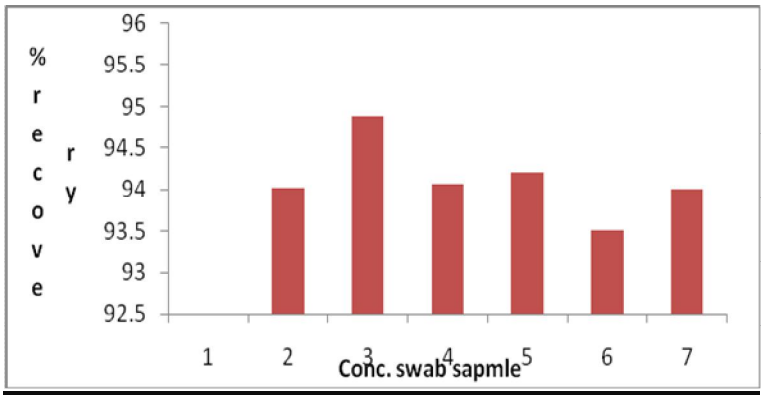


Fig. 8: Conc. Drug per Rinse sample Vs %recovery

Result: Six different concentrations were prepared and absorbance was measured**.** Rinse recovery was found to be within limit i.e. more than 85%. Mean Rinse recovery was found to be 94.11%.

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