Research Article

Evaluation of Cleaning Method Validation Techniques of Pioglitazone

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ABSTRACT

Analytical methods used to determine the effectiveness cleaning were validated for Pioglitazone. 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. Pioglitazone: UV Spectro-photometric analytical method was developed which showed an absorption maxima at λmax at 227 nm wavelength and linearity range 5 to 25 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.

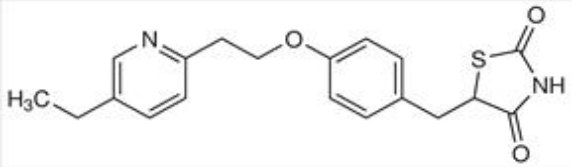
INTRODUCTION

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 increase demphasis 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 Q2guideline, typical validation parameters which should be considered are Accuracy, Precision, Linearity, Repeatability, Specificity, Detection Limit, Quantitation Limit.[4, 5, 6]

Pioglitazone is a Hypoglycemic agents. Pioglitazone is practically insoluble in water and insoluble in ether. Soluble in N, N-dimethyl formamide, slightly soluble in anhydrous ethanol, soluble in acetonitrile.[7]

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 used Cleaning validation study.
- The study also involves finding out criteria for visual cleanliness limit for selected drug by impregnating known amount of drug on SS plates.

EXPERIMENTAL

Performing solubility studies of drug:

The worst case selection based on solubility studies of active ingredients shall be doneto select a least soluble molecule (The lesser the solubility greater will be the difficulty to remove the residue from surface).The least soluble molecule is taken forthe analysis of cleaning validation because water is the major solvent used for cleaning.

Table 1: Solubility Index as Per Indian Pharmacopoiea

DESCRIPTIVE TERM	APPROX.VOL OF SOLVENT IN ml/gm SOLUTE
Very Soluble	Less than 1
Freely Soluble	From 1-10
Soluble	From 10-30
Sparingly Soluble	From 30-100
Slightly Soluble	From 100-1000
Very Slightly soluble	From 1000-10,000
Practically insoluble	More than 10,000
Partly Soluble	Mixture of which only some of the component dissolve

Pioglitazone solution was prepared by weighing 10 mg Pioglitazone drug and dilutedto 100 ml with water then dilute with water several times till it becomes clear solution and solubility factor was calculated.

Preparation of solvent mixture

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

DETERMINATION of λ max.

Preparation of 10µg/ml solution

Weighed accurately 100 mg of Pioglitazone and diluted to 100.0 ml solvent mixture.From this 1.0 ml of above solution was taken and diluted to 100.0 ml with the solventmixture to get a concentration of 10 µg/ml solution. λmax were determined in therange of 200 to 400 nm by UV spectrophotometer 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.

LINEARITY

Linearity assessed by analysing working standard solution of concentration in range of 0, 5, 10, 15, 20, 25 µg/ml. Absorbance was measured at 227 nm. Tabulate the results and plot the graph of absorbance v/s concentration and regression coefficien t 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.

SPECIFICITY AND SELECTIVITY

Pioglitazone 10 µg/ml working standard solution was prepared in solvent mixture and absorbance was measured at 227 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.

REPRODUCIBILITY

Prepare 10 µg/ml solution of Pioglitazone
Absorbance was measured at 227 nm in UV spectrophotometer in two instruments that is instrument -1 (Jasco) and Instrument-2(Simadzu). This procedure was repeated for 20 µg/ml. Three readings were taken and mean and standard deviation was calculated. The result was given in Table No.3.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

100 mg of Pioglitazone was accurately weighed and diluted to 100.0 ml by using the solvent mixture that is 1000 µg/ml. From that 10.0 ml solution was taken and diluted to 100.0 ml with the solvent mixture that is 100 µg/ml. From that 6 different concentration were prepared that is 5, 10, 15, 20, 25, and 30µg/ml. Absorbance was measured at 227 nm in UV spectrophotometer. Six reading were taken for each concentration. Standard deviation, LOD and LOQ was 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.

REPEATABILITY

Standard solutions of Pioglitazone (5, 10, 15, 20, 25, and 30µg/ml) were prepared. Absorbance was measured at 227 nm by taking the solvent mixture as a blank. The absorbance of the same concentration solution was measured six times and RSD was calculated. The result was given in Table No.5.

STABILITY OF PIOGLITAZONE

Pioglitazone 10 µg/ml working standard solution was prepared in solvent mixture and absorbance was measured at time intervals 1 hr that is 0, 1, 2, 3, 4, 5, 6, 7, 8 and 24hrs at 227 nm. The result was given in Table No.6.
Limit: RSD readings at different time interval should be less than 2%. The time at which the absorbance value does not vary by ± 5% to that of initial reading, will be the time limit within which the standard solution can be used.

METHODS

SWAB SAMPLING METHOD FOR PIOGLITAZONE

Blank swab

Six polyurethane Texwipe swabs were washed with distilled water and dried.10.0 ml solvent mixture was taken in test tube and that dried swab was dipped in solvent mixture. Absorbance was measured at λmax 227 nm. This procedure was repeated for remaining 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 50, 100, 200, 300, 400 and 500 µg/ml were prepared in the solvent mixture from the Pioglitazone working standard solution. From the above solution 0.5 ml solution was taken and added directly into test tube containing10.0 ml solvent mixture. Absorbance was measured at λmax 227 nm. The result was given in Table No.8.

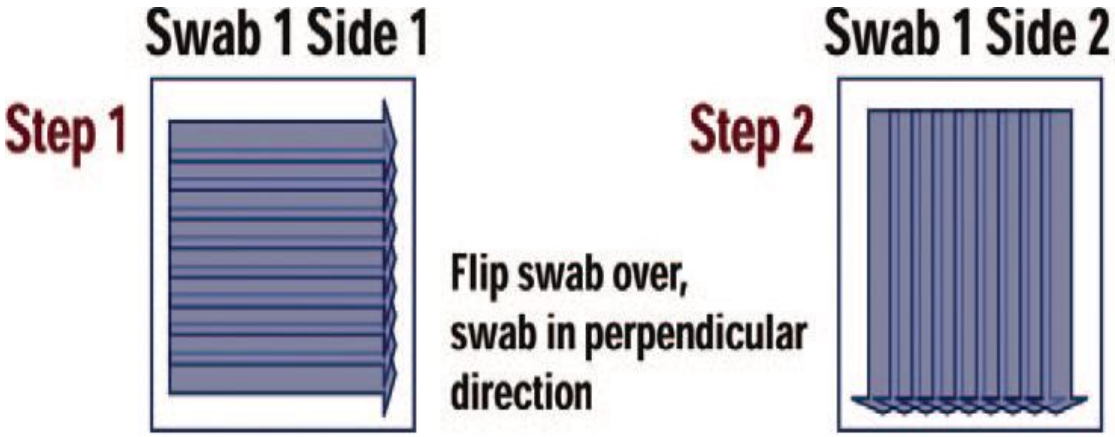
Recovery study of swab sample

Polyurethane swabs were taken washed with distilled water and dried. 0.5 ml of 50,100, 200, 300, 400 and 500 µg/ml were taken and slowly delivered with the help of graduated pipette on the tip of the swab so as to absorb the solution properly by the swab. The spiked swabs were placed individually in test tube containing 10.0 ml solvent mixture. Slowly stirred and allowed the bubble to subside before taking the absorbance. Absorbance was measured at

λmax 227 nm. From this absorbance value Blank 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 above standard concentration was spreaded in 2 x 2 inch square area of S.S.plate and dried. Swab was striked on one side in a horizontal direction, and with the other side in a vertical direction back and forth to cover the entire area. Swab was placed in test tube containing 10.0 ml of solvent mixture. Stirred and absorbance was measured at 227 nm. 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 be less than 10%.

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

RINSE RECOVERY FROM SS PLATE SPIKED WITH SAMPLE

Six different standard solutions that are 50, 100, and 200, 300, 400 and 500 µg/ml were prepared in the solvent mixture from the Pioglitazone working standard solution.0.5 ml standard solution was dispersed in 2 x 2 inch square area on plate anddried.10.0 ml solvent mixture was added on S.S plate and it was rinsed. Collected rinsed sample was measured at 227 nm in UV spectrophotometer. The experiment was repeated triplicate. Calculate the %Recovery. The result was given in Table No.10 and graph was given in Figure No.7 and 8.

VISUAL CLEAN METHOD

Standard solutions of Pioglitazone 25, 50, 75, 100, and 200, 300, 400 and 500 µg/ml were prepared. 0.5 ml standard solution was taken and dispersed in 2 x 2 inch square area of S.S. plate by using graduated pipette. It was properly dried. Check for the visibility of the residue.

The result was given in Table No.11 and 12.

RESULTS AND DISCUSSION

Results of evaluation of cleaning method Validation techniques of pioglitazone are discussed below.

S. No.	Validation Parameter	Results
1	Solubility of Pioglitazone	Practically Insoluble in water
2	Determination of λ max	Absorption maxima at λmax at 227 nm in solvent mixture (Acetonitrile : Water) (4:1)
3	Linearity and Range	Regression coefficient for linearity of the Pioglitazone working standard solution was found to be 0.998 in the range of 5-25 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 10 and 20µg/ml were checked by using two different make instruments. No significant difference in absorbance.
6	Repeatability	Different concentrations i.e. 5, 10, 15, 20, 25µg/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	0.405 µg 1.35 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 89.69%
13	Visual Clean Method	Above 80 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 Q2guideline. Recovery studies were carried out by both Swab and Rinse samplingtechniques.By employing visual examination of cleanliness by spiking known concentrationdrugs on SS plates were determined. This is first step to assess the cleanliness ofequipment. If product residue visible, till how much visibility there and was evaluatedand found out by swab and rinse sampling techniques. Swab sampling method: By this techniques recovery of drugs were found to be morethan 90%. It is a direct method of sampling, rather than indirect such as rinsesampling. Advantages swab sampling techniques are that, residues that are insolublecan be sampled by physical removal and areas that are hardest to clean and which arereasonably accessible can be evaluated. It is expected the swab will pick up all theresidues on the surface which can then be assayed. The reproducibility is suspect,because of the human involvement. Sampling spiked surfaces with known amounts isoften served as a training method. The disadvantages of swabbing methods area) Inability to access some areas; these are usually the most difficult to clean areas.

b) Assumes uniformity of contamination surface; invariably, contamination is notuniform, 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 thatthe 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 usedsampling 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 ontheir own are insufficient evidence of cleaning and should be used in combinationwith other sampling methods. It should be used in combination with swabs; togetherthey balance out the disadvantages.

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

Table 1: Linearity of Pioglitazone Standard Solution at λmax 227nm

Concentration (µg/ml)	Absorbance
0	0.000
5	0.221 ±0.005
10	0.409 ± 0.007
15	0.623 ± 0.003
20	0.808 ± 0.003
25	0.999 ± 0.004

Table 2: Specificity and Selectivity of Pioglitazone at λmax 227nm

Sl No.	ABS
1	0.411 ± 0.002
2	0.408 ± 0.001
3	0.405 ± 0.004
4	0.400 ± 0.004
5	0.414 ± 0.003
6	0.402 ± 0.001

Table 3: Reproducibility of Pioglitazone at λmax 227nm

S. No.	Conc.(µg/ml)	Instrument 1 (Jasco)	Instrument 2 (Simadzu)	Inference
1	10	0.409	0.406	No significant difference
2		0.407	0.402	
3		0.411	0.406	
	Mean	0.409 ± 0.002	0.404 ± 0.002	
4	20	0.809	0.805	No significant difference
5		0.808	0.802	
6		0.811	0.806	
	Mean	0.809 ± 0.001	0.804 ± 0.002	

Table 4: Limit of Detection and Limit of Quantification of Pioglitazone at λmax 227nm

Conc. (µg/ml)	5	10	15	20	25
Mean ±SD	0.218±0.007	0.406±0.004	0.618±0.005	0.804±0.002	0.991±0.009

Table 5: Repeatability of Pioglitazone at λmax 227nm

Conc. (µg/ml)	5	10	15	20	25
Mean ± SD	0.218±0.001	0.406±0.004	0.618±0.005	0.804±0.002	0.991±0.009
CV= SD/Mean	0.0045	0.0098	0.0080	0.0024	0.0090
%RSD	0.45	0.99	0.81	0.25	0.91

Table 6: Stability of Pioglitazone Solutionat λmax227nm

Time (hrs)	ABS
0	0.378 ± 0.002
1	0.375 ± 0.001
2	0.372 ± 0.002
3	0.370 ± 0.003
4	0.369 ± 0.001
5	0.366 ± 0.002
6	0.365 ± 0.002
7	0.361 ± 0.001
8	0.358 ± 0.001
24	0.360 ± 0.005

Table 7: Blank swab absorbance (Interference) of Pioglitazone at λmax 227nm

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)						
50		100	200	300	400	500
Concentration of the standard Solution (µg/ml)						
2.5		5	10	15	20	25
Absorbance of Standard Solutions						
0.115		0.221	0.409	0.623	0.808	0.999
Absorbance of Swabs samples Spiked with Std. Solutions						
Sl. No.	50	100	200	300	400	500
1	0.128	0.226	0.411	0.630	0.825	0.991
2	0.135	0.234	0.401	0.632	0.817	0.990
3	0.127	0.300	0.411	0.631	0.821	0.992
Mean Samp. abs	0.130 ±0.004	0.230 ±0.004	0.407 ±0.005	0.631 ±0.001	0.821 ±0.004	0.991 ±0.001
Corrected abs	0.112 ±0.006	0.212 ±0.004	0.389 ±0.003	0.613 ±0.003	0.803 ±0.002	0.973 ±0.002
RSD	3.07	1.73	1.22	0.15	0.48	0.10
% Recovery	97.39	95.92	95.11	98.39	99.38	97.39

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

Concentration of the Solution used for spiking the SS plate (µg/ml)						
50		100	200	300	400	500
Concentration of the standard Solution (µg/ml)						
2.5		5	10	15	20	25
Absorbance of Standard Solutions						
0.115		0.221	0.409	0.623	0.808	0.999
Absorbance of Swabs samples Spiked with Std. Solutions						
Sl. No.	50	100	200	300	400	500
1	0.124	0.216	0.390	0.587	0.760	0.945
2	0.121	0.215	0.387	0.582	0.759	0.943
3	0.119	0.217	0.388	0.579	0.752	0.941
Mean Samp. abs	0.121 ±0.002	0.216 ±0.001	0.388 ±0.001	0.584 ±0.004	0.757 ±0.004	0.943 ±0.002
Corrected abs	0.103 ±0.002	0.198 ±0.001	0.370 ±0.001	0.566 ±0.004	0.739 ±0.004	0.925 ±0.002
RSD	1.94	0.50	0.27	0.70	0.54	0.21
% Recovery	89.56	89.59	90.46	90.85	91.46	92.59

Table 10: Rinse Recovery from SS Plate spiked with sample

Concentration of the Solution used for spiking the SS plate (µg/ml)						
50		100	200	300	400	500
Concentration of the standard Solution (µg/ml)						
2.5		5	10	15	20	25
Absorbance of Standard Solutions						
0.115		0.221	0.409	0.623	0.808	0.999
Absorbance of Rinse samples with Std. Solutions						
Sl. No.	50	100	200	300	400	500
1	0.100	0.200	0.370	0.554	0.715	0.896
2	0.102	0.204	0.375	0.555	0.712	0.899
3	0.101	0.200	0.365	0.553	0.718	0.895
Mean Samp. abs	0.101 ±0.001	0.201 ±0.002	0.370 ±0.005	0.554 ±0.001	0.715 ±0.003	0.896 ±0.002
RSD	0.99	0.99	1.35	0.18	0.41	0.22
% Recovery	87.82	92.30	90.95	88.92	88.49	89.68

Table 11: Visual Clean Method of Pioglitazone at λmax 227nm

Conc.(µg/ml)	50	100	200	300	400	500
Inference	Not visible	Visible	Visible	Visible	Visible	Visible

Table 12: Visual clean criteria

Conc.(µg/ml)	50	60	70	80	90	100
Inference	Not visible	Not visible	Not visible	Visible	Visible	Visible

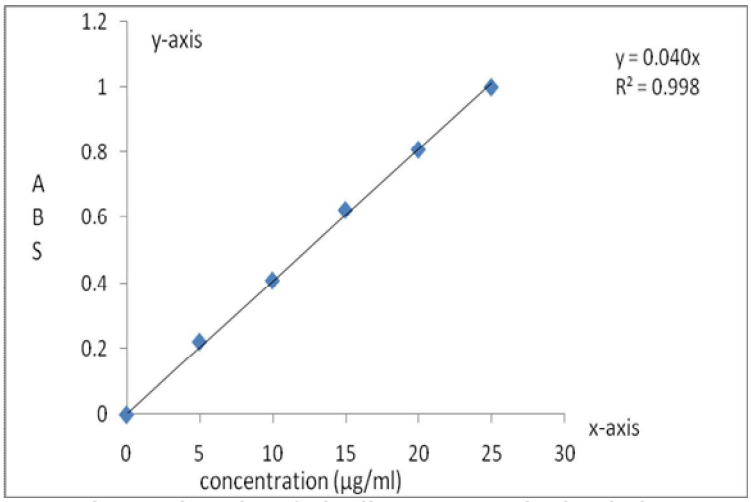


Fig. 1: Linearity of Pioglitazone Standard Solution

Acceptance criteria: The regression coefficient found to be greater than 0.98
Result: The regression coefficient for linearity of the Pioglitazone working standard solution is found to be 0.998.

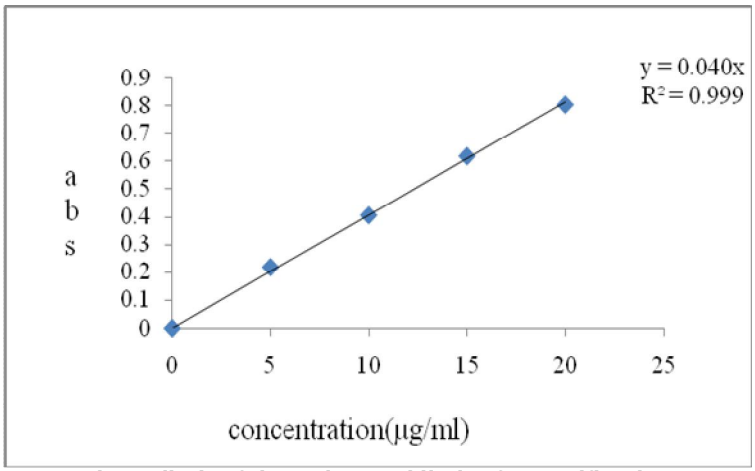


Fig. 2: limit of detection and limit of quantification

Result: LOD was found to be 0.405µg and LOQ was found to be 1.35µg.

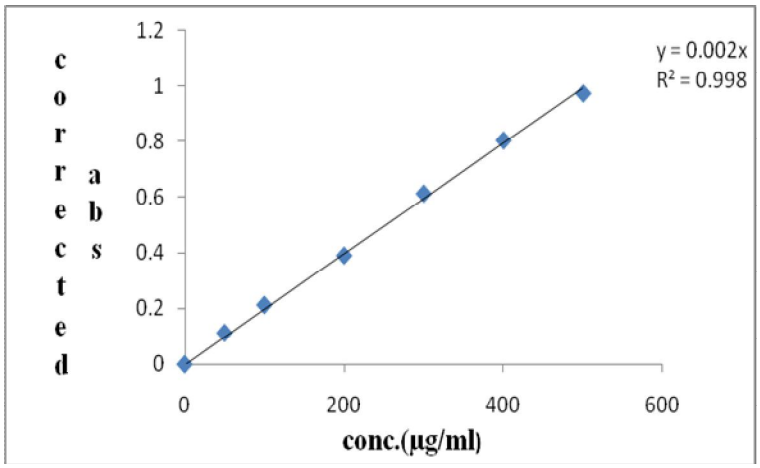


Fig. 3: recovery study from swabs spiked with std. Solutions: Drug content per swab vs. corrected absorbance graph

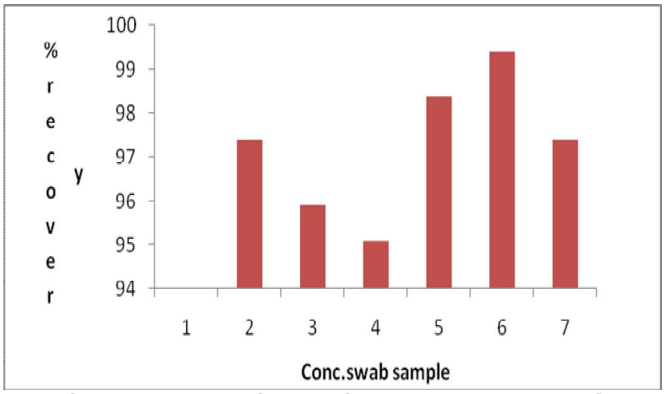


Fig. 4: conc. Swab sample vs %recovery graph

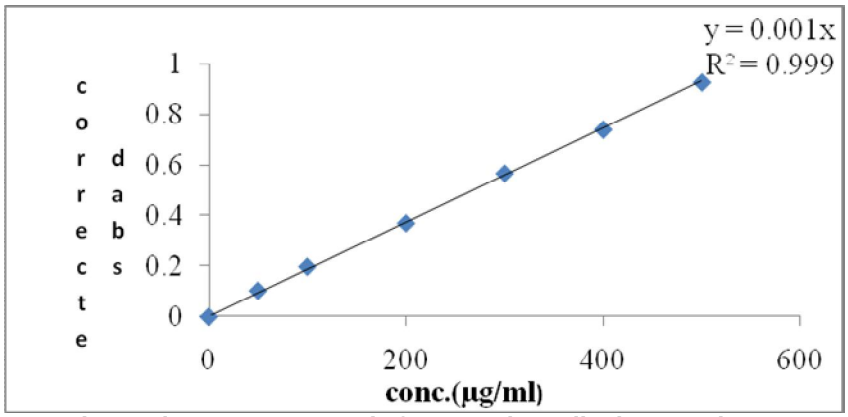


Fig. 5: plate recovery study from swabs spiked on ss plates: Conc. Vs corrected absorbance graph

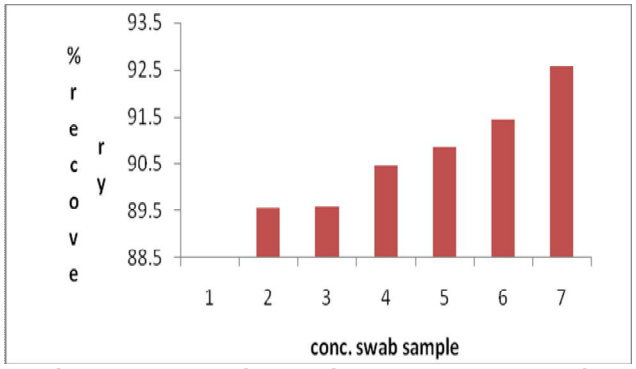


Fig. 6: conc. Swab sample vs %recovery graph

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 94.65%. RSD was found to be less than 5%. When graph was plotted drug content/swab vs. Mean recovery linear graph obtained with regression coefficient 0.999.

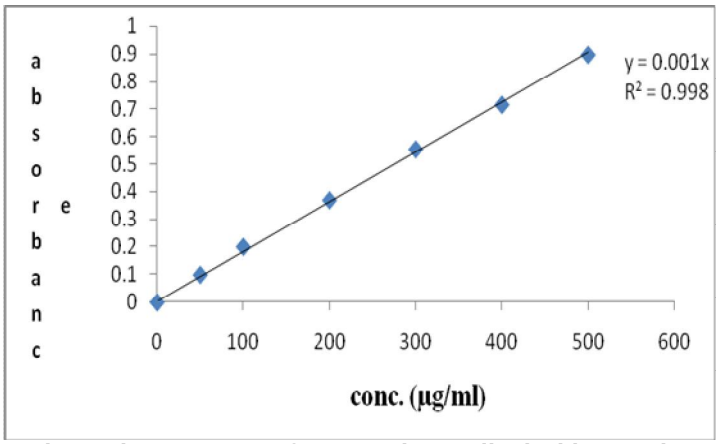


Fig. 7: rinse recovery from ss plate spiked with sample: Drug conc. /spiked part VS rinse sample absorbance

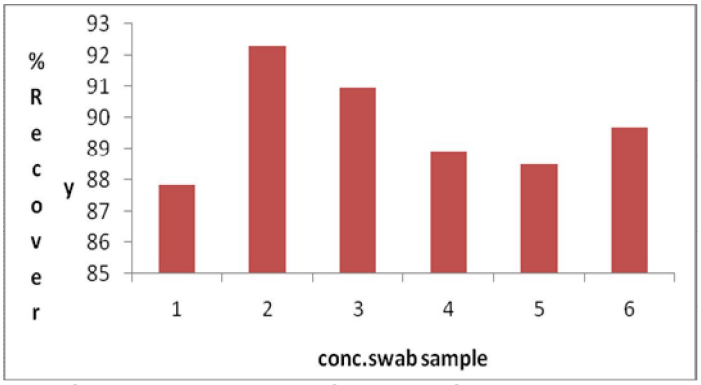


Fig. 8: conc. Drug per rinse sample vs %recovery

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 94.65%.

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