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Development and Validation of Analytical Method for Estimation of Cefixime in Swab Samples

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Abstract: The objective of the current study was to develop and validate simple and precise UV spectrophotometric method for estimation of Cefixime Trihydrate in the swab samples to validate cleaning procedure. The swabbing procedure was optimized in order to obtain a suitable recovery from stainless steel surface using Tex wipe polyurethane swab stick. Detection wavelength selected was 289 nm. The proposed method was validated in terms of Linearity, precision, accuracy, limit of detection and limit of quantitation. Linearity was studied over concentration range of 0.5 - 3 µg / ml and correlation coefficient was found to be 0.9998 for regression line. A mean recovery obtained was 86.55 %.
Key words: Cefixime trihydrate, Swab testing, Spectrophotometric, Cleaning validation.

Introduction and Experimental:

Introduction:

Cleaning Validation is a documented evidence that an approved cleaning procedure will provide equipment that is suitable for processing of pharmaceutical products or active pharmaceutical ingredients (APIs)[1]. The cleaning validation is to verify the effectiveness of the cleaning procedure for removal of product residues, degradation products, excipients, and cleaning agents as well as for the control of potential microbial contaminants. In addition one need to ensure that there is no risk associated with cross-contamination of active ingredients. The objective of the Cleaning Validation is the confirmation of a reliable cleaning procedure so that the analytical monitoring may be omitted or reduced to a minimum in the routine phase[1 - 6]. Cleaning validation has become a regulatory requirement now. 1963 GMP regulations (part 133.4) states that manufacturing equipments must be maintained in clean and orderly manner, all processing equipment should be specifically designed to facilitate cleanability and permit visual inspection and whenever possible, the equipment should be made of smooth surfaces of non-reactive materials. Critical areas (i.e., those hardest to clean) should be identified, particularly in large systems that employ semi-automatic or fully automatic CIP systems[2].

GMP states that in case of β lactam antibiotics there should be no traces of product after cleaning, therefore determining the sensitivity and LOD is of atmost importance[3, 7].

For validation of cleaning procedure three methods of sampling that are considered to be acceptable, namely direct surface sampling (swab method), indirect sampling (use of rinse solutions) and placebo sampling. A combination of the first two methods is generally the most desirable, particularly in circumstances where accessibility of equipment parts can mitigate against direct surface sampling. In Swab method the suitability of the material to be used for

sampling and of the sampling medium should be determined. The ability to recover samples accurately may be affected by the choice of sampling material. It is important to ensure that the sampling medium and solvent are satisfactory and can be readily used. Rinse samples allow sampling of a large surface area. In addition, inaccessible areas of equipment that cannot be routinely disassembled can be evaluated. However, consideration should be given to the solubility of the contaminant. A direct measurement of the product residue or contaminant in the relevant solvent should be made when rinse samples are used to validate the cleaning process. Placebo sampling method provides simulation of actual production of subsequent batches[1 - 9].

FDA has not published specific guidelines to set acceptance criteria or method for determining whether a cleaning process is validated because of wide variation in the equipment and process used throughout finished and bulk products .Therefore pharmaceutical companies are expected to establish acceptance criteria based on specific and logical rationale and these criteria should be practical, achievable and verifiable and scientifically sound. The analytical methods used to detect residuals or contaminants should be specific for the substance to be assayed and provide a sensitivity that reflects the level of cleanliness determined to be acceptable by the company. Important is to define the sensitivity of the analytical method in order to set their reasonable limits[2, 9]. The acceptance criteria vary with the varying products or drugs which are processed, equipments used for processing, the potency of the drug and toxicity levels[1].

Cleaning Validation Protocol is required to define how the cleaning process will be validated. It includes objective of the validation process, responsibilities for performing and approving the validation study, description of the equipment to be used, the interval between the end of production and the beginning of the cleaning procedure, the number of lots of the same product, which could be manufactured during a campaign before a full cleaning is done, detailed cleaning procedures to be used for each product, each manufacturing system or each piece of equipment, the number of cleaning cycles to be performed consecutively, any routine monitoring requirement, sampling procedures, including the rationale for why a certain sampling method is used, clearly defined sampling locations, data on recovery studies where appropriate, validated analytical methods including the limit of detection and the limit of quantitation of those methods, the acceptance criteria, including the rationale for setting the specific limits, other products, processes, and equipment for which the planned validation is valid according to a "bracketing" concept, change control and or re-validation[1, 2, 9].

Cefixime is an orally active third generation semisynthetic cephalosporin type of β lactam antibiotic. Chemically, Cefixime is, 5-Thia-1azabicyclo [4,2,0]oct-2-ene-2-carboxylicacid, 7-[[(2-amino-4-thiazolyl) [(carboxymethoxy) imino] acetyl]amino]-3-ethenyl-8 oxo, trihydrate. It is soluble in methanol and 0.1MNaOH, insoluble water and 0.1M HCl[10, 11]. Therefore the cleaning method was developed by using methanol as cleaning solvent. Cefixime is official in USP 2007, B.P. 2009. Literature survey revealed that only a few HPLC, HPTLC, Spectroscopic methods were reported for the estimation of Cefixime in the formulation and in bulk. No method is reported for estimation of cefixime in swab samples. Therefore UV method is developed for the same. The proposed analytical method has been validated with respect to linearity, precision, accuracy, LOD and LOQ. The present work focuses on development and validation of spectroscopic method for analysis of swab samples of cefixime[12].

Experimental:

Reagents and Chemicals

Cefixime Trihydrate working standard was obtained as the gift sample from Maxim Pharmaceuticals, Pune. All other reagents used were of analytical grade. Methanol (AR grade) was used as solvent for swab testing. The sample solution was passed through Whatman filter paper. Swab sampling was done by using Tip T $^{\times}$ $^{Tm.}$ 714 swabs from Tex Wipe Corporation, (Upper saddle River, NJ).

Equipments

Instrument used was UV-Visible spectrophotometer (Dual beam, Jasco) controlled by V550 software, AY-10 analytical balance (Schemadzu), Whatman filter paper. For the swab testing, instrument used was tablet compression machine (Remake, Mumbai).

Recovery Studies of Cefixime Trihydrate from Clean Tip Swabs and Stainless Steel Plate

Stainless steel plate (30cm × 15cm) was used for the surface testing. The spiking solution was prepared by dissolving 25mg Cefixime Trihydrate in 25ml methanol to get the concentration of 1000 µg / ml. This was further diluted to get 10 µg / ml.

Heads of the T^{XTM} 714 swab sticks were rinsed with methanol (AR grade). Using calibrated graduated pipette, 1.6 ml, 2ml and 2.2ml solution having concentration 10 µg / ml were transferred on the three specified areas of recovery plate. These solutions were spread on the recovery plate in the area of 5 cm ×5 cm and were allowed to dry. Swabs sticks previously

placed into glass test tube containing 5 ml of methanol (AR grade) were used for the swabbing the stainless steel plate. Swabbing was done first in horizontal and then in vertical direction. Finally swabs sticks were put again into glass test tube containing methanol and sonicated for 10 min at an ambient temperature and volume was made with the methanol (AR grade). Finally absorbance of these sample solutions were measured at the detection wavelength of 289nm.

Method for Cleaning the Instrument

Tablet compression machine was cleaned with dry cloth. To remove the traces of residue of drug, machine was then cleaned with 2% SLS solution twice and then wiped with methanol using cotton plug.

Method for Swab Testing

Critical sites were selected and marked with area as shown in Table I. Each swab was dipped in 5 ml methanol. Swabs were taken in selected area using separate swab for different area carefully. Swabbing is done first in horizontal and then in vertical direction. Then swabs were again dipped in 5 ml methanol contained in 10ml test tube. These Test tubes were then sonicated for 10 min and then volume was made. Resultant solutions were filtered using Whatman filter paper and analyzed at 289 nm.

Preparation of Standard Solution

Stock solution of Cefixime Trihydrate was prepared by dissolving 25mg of Cefixime Trihydrate in 25 ml methanol. This solution was further diluted suitably to get solution of concentration
10 µg / ml.

Determination of Absorption Maxima

Standard solution 10 µg / ml was scanned between 200 - 400 nm. Spectrum was recorded and the suitable absorption maxima selected was 289 nm.

Table I: Critical Sites and Area Selected for UV Readings

Critical sites selected	Areas for swab testing	Absorbance
Turret	2cm×2cm	Not detected
Upper punch (12.5mm)	1cm×1cm	Not detected
Lower punch (12.5mm)	1cm×1cm	Not detected
Die	1cm×1cm	Not detected
Upper camp tract	2cm×2cm	0.00048
Plat form	2cm×2cm	0.00036

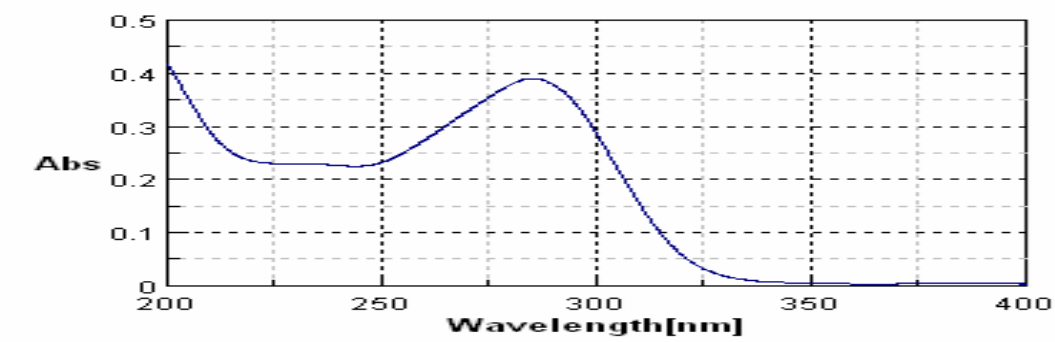

Fig. 1: Absorption maxima for Cefixime Trihydrate

Development and Validation of Analytical Method
Spectrophotometric method for the determination of Cefixime Trihydrate in swab samples was developed and validated by determining the linearity, precision, accuracy, LOD and LOQ. Detection wavelength selected for analysis was 289 nm.

Linearity
Linearity was studied over a small drug concentration range from 0.5 – 3 µg / ml. The correlation coefficient (R^2=0.9998) obtained for regression line showed excellent linearity relationship between absorbance and concentration of Cefixime Trihydrate [fig. 5]. Result of linearity studies are shown in Table II.

Precision
Precision of the method reported as % RSD, was estimated by repeatability, reproducibility and intermediate precision by measuring absorbance of six replicates of 2 µg / ml of Cefixime Trihydrate. % RSD values as in Table III is less than 2% that illustrate the good precision of the analytical method.

Accuracy
Accuracy of the procedure was determined by comparing the analytical amount determined Vs known amount spiked at 80%, 100% and 120% level of LOQ concentration with measurements for each concentration level achieved.

Limit of Detection and Quantitation
The LOD and LOQ of Cefixime Trihydrate were estimated from the standard deviation of the response and the slope of the calibration curve by using following formula.

$$LOD = \frac{3.3 \times \sigma}{S}$$

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

Where σ = the standard deviation of the response
S = the slope of the calibration curve

LOD and LOQ were found to be 0.0004714 µg / ml [0.4714 ng/ml] and 0.0014286 µg / ml [1.4286 ng/ml] respectively. And results are indicated in Table III.

Table II: Linearity for Cefixime Trihydrate

Sr. No.	Concentration (µg/ml)	Absorbance*
1	0.5	0.0261
2	1.0	0.0485
3	1.5	0.0743
4	2.0	0.1010
5	2.5	0.1242
6	3.0	0.1500

*Average of the five readings

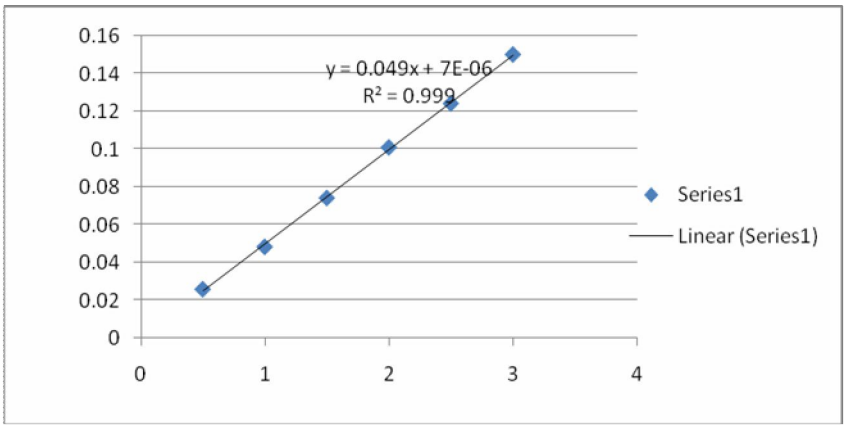

Fig. 2: Calibration Curve for Cefixime Trihydrate

Table III. Results for Validation Parameters

Sr. No.	Validation Parameter	Results
1	Linearity	R^2 = 0.9998
2	Precision A) Interday precision B) Intermediate precision C) Intraday precision	(%RSD) 1.28% 1.07% 1.17%
3	Accuracy 80% 100% 120%	Percentage recovery (%) 84.94 % 89.72 % 85.00 %
4	LOD	0.0004714 µg/ml
5	LOQ	0.0014286 µg/ml

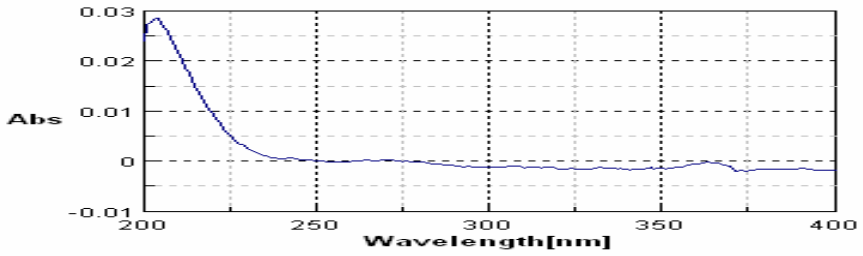

Fig.3 UV spectrum for LOD conc.

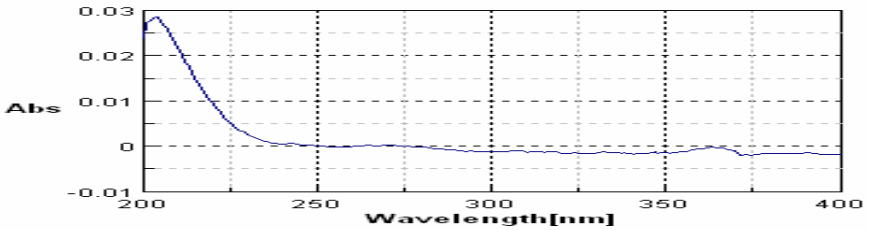

Fig.4. UV spectrum for LOQ conc. (0.0014286 µg/ml)

Results and Discussion:

Developed cleaning method removes even traces of residue of drug present on the instrument. Analytical method developed was found to be linear, precise, accurate and sensitive to detect even small quantity of drug residue.

Conclusion:

The proposed method is simple, rapid, sensitive and economic and hence can be used for the routine analysis of swabs.

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