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VALIDATION OF CLEANING PROCEDURE FOR ELIMINATION OF OFLOXACIN AND METRONIDAZOLE BENZOATE FROM MIXING EQUIPMENT BY USING UV SPECTROSCOPY

Nirav B. Patel*[1], Sneha K. Jansari [2], Alpesh C. Arvadiya [3] and Hemant T. Desai [1]

Nirlife Healthcare (Healthcare Division of Nirma), Sachana- 382 150, Ahmadabad, Gujarat, India
Department of Quality Assurance, Parul Institute of Pharmacy [2], Limda, TA, Waghodia, Vadodara, Gujrat-391760, India
Department of Pharmaceutics and Quality Assurance, R.C.Patel Institute of Pharmaceutical Education and Resaerch [3], Shirpur-425 405, Maharashtra, India

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Correspondence to Author:

Nirav B. Patel

QC Chemist, Nirlife Healthcare (Healthcare Division of Nirma), Sachana (382150), Ahemdabad, Gujrat, India

ABSTRACT

This research manuscript describes simple, sensitive, accurate, precise and repeatable UV spectroscopic method for the simultaneous determination of Metronidazole (MET) and Ofloxacin (OFL) in suspension dosage form). Metronidazole has absorbance maxima at 318.0 nm and Ofloxacin has absorbance maxima at 294 nm in Methanol and Water (50:50) solvent. The linearity was obtained in the concentration range of 1-13 µg/ml for Metronidazole and 1-13 µg/ml for Ofloxacin with mean accuracies 99.73 ± 0.05 and 99.13 ± 0.41 for Metronidazole and Ofloxacin, respectively. This paper presents a useful UV spectroscopic method for validating equipment cleaning procedures and verifying cleaning in a pharmaceutical plant. The study summarizes the initial steps that should be taken into account and focuses particularly on the solutions to some of the most critical considerations (e.g., detection and quantification limits, recovery). Cleaning validation is the process of assuring that cleaning procedures effectively remove the residue from manufacturing equipment/facilities below a predetermined level. This is necessary to assure the quality of future products using the equipment, to prevent cross-contamination, and as a World Health Organization Good Manufacturing Practices requirement. In this article we discuss the UV method that we developed for measuring residual of Ofloxacin and Metronidazole benzoate suspension contain ofloxacin (50mg/5ml) and metronidazole benzoate equivalent to metronidazole (100mg/5ml) on surface of mixing tank during manufacturing process. The method with correlation coefficient R^2 = 0.999 and method offers low detection capability and rapid sample analysis time. The accurate recovery values with method precision less than 2%RSD of precision, UV method is applicable for determining residual of suspension on pharmaceutical equipment surfaces and will be useful for cleaning validation.

INTRODUCTION: Metronidazole (MET), an antiprotozoal drug is widely used in treatment of invasive amoebiasis. Chemically it is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol (**fig. 1**) and Ofloxacin (OFL), an antimicrobial drug chemically is (RS)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-di hydro- 7H- pyrido[1, 2, 3, -de]-1, 4 benzoazeine-6-carboxylic acid (**fig. 2**),

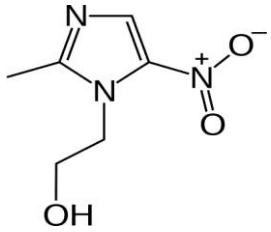

FIGURE 1: STRUCTURE OF METRONIDAZOLE

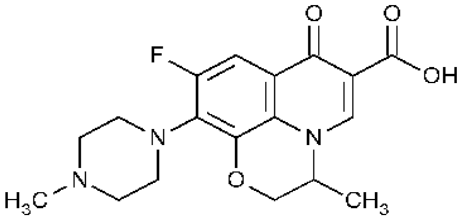

FIGURE 2: STRUCTURE OF OFLOXACIN

Both drugs are official in Indian pharmacopeia [1], British Pharmacopeia [2] and United States Pharmacopeia [3].The combination of MET and OFL is widely used in treatment of microbial infections. Literature search reveals that various analytical methods like UV-visible spectrophotometry [4, 5, 6], conductometry [7], HPLC [8-15] and LC-MS [16-17] have been reported for estimation of MET and OFL in their individual and combined dosage forms with other drugs. There is no reported method for Validation of Cleaning Procedure for elimination of Ofloxacin and Metronidazole benzoate from mixing equipment by using UV spectroscopy [18]. This prompted the present work.

Cleaning Validation:

MATERIALS & METHODS

Apparatus: A Shimadzu model 1700 double beam UV–visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells was used to measure absorbance. Mettler Toledo analytical balance CX-204 was used for weighing, Texwipe alpha swab polyester (Baxter Scientific Product, McGaw Park, IL) and an ultrasonic cleaner (Frontline FS 4) were used in the study.

Reagents and Materials: Metronidazole and Ofloxacin bulk powder was obtained from Nirlife, Healthcare Division of Nirma, Ahmadabad, India. The commercial fixed dose combination product was procured from the Nirlife. Methanol (Finar Reagent, Ahmadabad, India) used was of AR grade. Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Preparation of Standard Stock Solution: An accurately weighed Metronidazole (10 mg) and Ofloxacin (5 mg) were transferred into two different 100 ml volumetric flask , dissolved in 50 mL Methanol: Water (50:50) and sonicated after this diluted up to mark with same solvent to get concentration of Metronidazole (100µg/ml) and Ofloxacin (50µg/ml)

Preparation of Mixed Standard Working Solution: Accurately weighed Metronidazole (10 mg) and Ofloxacin (5 mg) were transferred to 100 ml volumetric flask, dissolved in 50 mL Methanol: Water (50:50) and diluted up to mark with same solvent to get concentration of Metronidazole (100µg/mL) and Ofloxacin (50µg/ml)

Preparation of Calibration Curve: Aliquots (0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3) of mixed standard working solutions (equivalent to 1, 3, 5, 7, 9, 11 and 13µg/ml of Metronidazole and(0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5µg/ml Ofloxacin, each) were transferred in a series of 10 ml volumetric flasks, and the volume was made up to the mark with solvent. Absorbance of each solution was recorded by UV. Calibration curves were constructed by plotting the peak areas versus the concentration (**fig. 3 and 4**), and the regression equations were calculated.

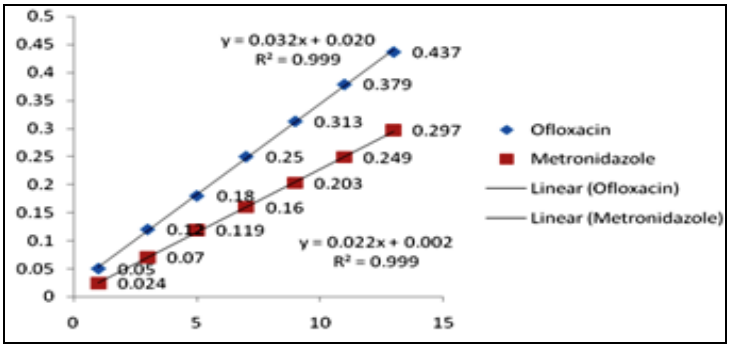

FIGURE 3: LINEARITY OF OFLOXACIN AND METRONIDAZOLE BENZOATE

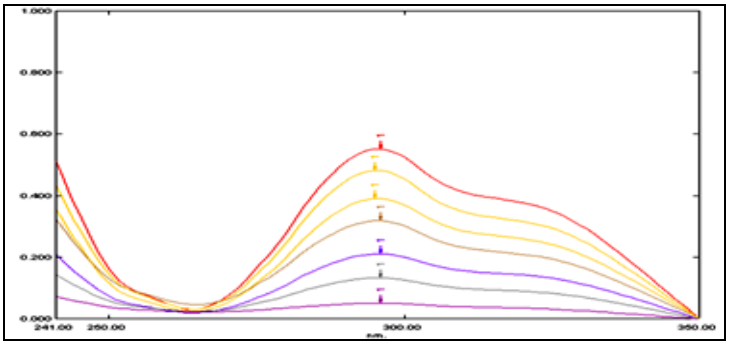

FIGURE 4: LINEARITY SPECTRA OF OFLOXACIN AND METRONIDAZOLE BENZOATE IN THEIR COMBINE FORM

Preparation of Marketed sample solution for Assay: For determination of the content of Metronidazole and Ofloxacin in marketed suspension (Label: MET-100mg/5ml and OFL-50mg/5ml). Take 0.5 ml solution from suspension and transferred to 100 ml volumetric flask, dissolved in Solvent and sonicated for 30 min. The solution was filtered through Whatman filter paper No. 41 and residue was washed with solvent. The solution was diluted up to the mark with solvent. Accurately measured 0.5 ml of solution was transferred to 10 ml volumetric flask, diluted up to the mark with mobile phase to get final working concentration of Metronidazole (5µg/ml) and Ofloxacin (2.5µg/ml) and absorbance were recorded.

Limit of Detection and Quantitation: Limit of detection and quantitation was measure by standard deviation method as par the guideline of ICH Q2B [19]: Validation of Analytical Procedure shown in **Table 1**.

TABLE 1 REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETER FOR THE PROPOSED UV METHOD

Parameters	RP-UPLC method	
	Metronidazole	Ofloxacin
Concentration range (µg/ml)	1-13	0.5-6.5
Slope	0.022	0.032
Intercept	0.002	0.02
Correlation coefficient	0.999	0.999
LOD[a] (µg/ml)	0.300	0.206
LOQ[b] (µg/ml)	0.909	0.625
Accuracy	99.73 ± 0.05	99.13 ± 0.41
Repeatability (% RSD n = 6)	0.229	0.414
Precision (%RSD)	--	--
Intraday (*n* = 3)	0.028-0.18%	0.078-0.529%
Interday (*n* = 3)	0.062-0.568%	0.148-0.244%

a=Limit of Detection, b=Limit of Quantitation, c=relative standard deviate

Swab Recovery: Stainless steel plates were used in the swab recovery test to simulate manufacturing equipment. One side of each plate was spiked with a solution of active substance Metronidazole (5µg/ml) and Ofloxacin (2.5µg/ml).The plates were allowed to dry completely overnight at room temperature. A Texwipe alpha swab was moistened with water and the spiked plate surface was swabbed both vertically and horizontally. The swab end was cut off, placed into a vial to which we added methanol: water (50:50). The vial was capped tight, vortexed, and allowed to stand for one hour prior to analysis. The same volume of each solution that was spiked onto the plates was separately spiked directly into 50-mL methanol: water (50:50). The percent recoveries of substances are listed in (**Table 2**) Reported values are the average of three individual swab samples for each substance. The swab recoveries varied between 98.67%-100.66%

Substance	ppm of spiked (Standard solution)	ppm of spiked recovered active substances on Plate	%Recovery	%RSD
Ofloxacin	2.5	2.46	98.4	1.28
Metronidazole benzoate	5	4.86	97.2	1.5

n=3 average

Application of this method to the Cleaning Process of Mixing Tank Vessel: This method was applied on the cleaning process of mixing tank where ofloxacin and metronidazole benzoate were mixed to manufacture suspension. For applying this method select sampling place in mixing tank (bottom site) having area 10cm^2 and swab it by using Texwipe alpha swab was moistened with water and the spiked plate surface was swabbed both vertically and horizontally. The swab end was cut off, placed into a vial to which we added 50-mL methanol: water (50:50). The vial was capped tight, vortexed, and allowed to stand for one hour prior to analysis. The same volume of each solution that was spiked onto the plates was separately spiked directly into 50-mL of 50-mL methanol: water (50:50) and analyzed by UV and result was shown in **Table 3.**

TABLE 3: ANALYSIS OF CLEANING PROCESS SAMPLE

Test	Result (Active Drug substance in ppm)	Complies with USP limit (Less than 10 ppm)
Cleaning Process Sample	3.25	Yes
Individual (Ofloxacin)	1.16	Yes
Individual (Metronidazole benzoate)	2.09	Yes

n=3 average

RESULTS AND DISCUSSION: From this study we measure the concentration of Residual substance with linear Correlation Coefficient which is 0.999 and Residual recovery(Swab recovery) ranged between 98.67%-100.66% and lower detection limit was found 0.3 ppm of metronidazole and 0.206 ppm of Ofloxacin and %RSD less than 2 for method precision and method also apply to cleaning process where we found 3.25 ppm concentration of active drug substance (ofloxacin 1.16 ppm and metronidazole benzoate 2.09 ppm)which is complies USP limit(less than 10ppm) for cleaning validation. All this indicate the accuracy and precision of proposed methods

CONCLUSION: This study demonstrates that the UV Spectroscopy method is suitable for measuring organic residues on stainless steel surfaces for cleaning validation, and that it is a reliable tool for cleaning validation. The UV Spectroscopy method offers low limits of detection, excellent linearity, precision, and accuracy. All of these UV results indicate that this technology is of low cost, simple and less time consuming alternative for cleaning validation.

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