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DEVELOPMENT AND VALIDATION OF CLEANING PROCEDURE OF MIXING EQUIPMENT USED FOR MANUFACTURING CIPROFLOXACIN HCL AND TINIDAZOLE TABLET BY USING UV SPECTROSCOPY

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ABSTRACT
Two simple, sensitive, rapid, accurate and economical methods were developed for the estimation of Ciprofloxacin HCl and tinidazole in two components tablet dosage form. First method is based on the simultaneous equation and second method is based on Q-analysis (absorbance ratio method). Ciprofloxacin HCl has absorbance maxima at 279.0 nm and tinidazole has absorbance maxima at 317 nm in Phosphate Buffer: Acetonitrile (80:20) solvent. The linearity was obtained in the concentration range of 1-13 µg/ml for Ciprofloxacin HCl and 1.2-15.6 µg/ml for tinidazole. In the first method, the concentrations of the drugs were determined by using simultaneous equations and in second method, the concentration of the drugs were determined by using ratio of absorbance at isoabsorptive point and at the λ-max of one of the drug. The results of analysis have been validated statistically and by recovery studies. 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 procedure validation 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 powder mixer on pharmaceutical equipment surfaces and will be useful for cleaning validation.
Key words: Ciprofloxacin HCl, tinidazole UV–visible spectrophotometer, equipment surface (mixing tank)

INTRODUCTION

Ciprofloxacin HCl (CH), an antibacterial drug is widely used to treat a number of infections including: infections of bones and joints, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, chancroid among others. Chemically it is the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. And Tinidazole (TZ), an anti-parasitic drug chemically is 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole. Both drugs are official in Indian pharmacopeia, British Pharmacopeia and United States Pharmacopeia. The combination of CH and TZ is widely used in treatment of microbial infections. Literature search reveals that various analytical methods like UV-visible spectrophotometry[1, 2]; Differential Pulse Polarography[3]; HPLC[4,5,6,7,8]; have been reported for estimation of CH and TZ in their individual and combined dosage forms. There is no reported developed method of cleaning validation for Ciprofloxacin HCl and Tinidazole by using UV spectroscopy. This prompted the present work. The aim of the present work is to develop and validate simple, rapid, accurate and precise method of cleaning validation for Ciprofloxacin HCl and Tinidazole by using UV spectroscopy. For simultaneous estimation of CH and TZ in their marketed formulation which is more rapid, sensitive, accurate and precise method than the RP-HPLC method. The chemical structure of Ciprofloxacin HCl and Tinidazole are shown in fig.1 and fig. 2.

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 and an ultrasonic cleaner (Frontline FS 4) were used in the study.

Reagents and materials

CH and TZ working standards were obtain from Nirlife-healthcare division of Nirma ltd, Sachana, Gujarat, India. The commercial fixed dose combination of CH and TZ (5:6) was procured from local market. Acetonitrile (spectroscopic grade) and KH_2PO_4 obtained from Finar chemicals, India was used for the study.

Preparation of solvent

Take 0.68 gm Dihydrogen orthophosphate and dissolve in 100 ml water in 1000ml volumetric flask, add 200 ml of Acetonitrile and mix it, make up the volume up to mark with water.

Preparation of standard stock solution

An accurately weighed CH (10 mg) and TZ (12 mg) were transferred into two different 100 ml volumetric flask, dissolved in 50 ml of solvent and sonicated, after this diluted up to mark with solvent to get concentration of CH (100µg/ml) and TZ (120µg/ml).

Method I: Simultaneous Equation Method

Working standard solutions (10 µg/ml) of CH and (12 µg/ml) of TZ were scanned in range of 200-400 nm to determine the λ-max of both drugs. The λ-max of CH and TZ were found to be 279.0 nm and 317.0 nm respectively. Six standard solutions having concentration 1, 3, 5, 7, 9, 11 and 13 µg/ml for CH and 1.2, 3.6, 6, 8.4, 10.8, 13.2 and 15.6 µg/ml for TZ were prepared by appropriate dilutions from their respective standard stock solutions. The absorbance of resulting solutions were measured at 279.0 nm and 317.0 nm and absorptivity coefficients were calculated using Beer Lambert law. The graph of absorbance Vs concentration was plotted at each wavelength and regression coefficients were calculated. The concentration of both drugs was calculated by solving these simultaneous equations.

$C_x = (A_1 a_{Y_2} - A_2 a_{Y_1}) / (a_{X_1} a_{Y_2} - a_{X_2} a_{Y_1})$…… ….. (1)

$C_y = (a_{X_1} A_2 - a_{X_2} A_1) / (a_{X_1} a_{Y_2} - a_{X_2} a_{y_1})$…… …… (2)

Where Cx & Cy are concentrations of CH and TZ respectively in gm/100 ml in the sample solution.

A_1& A_2 are the absorbance of the mixture at 297.0nm & 317.0 nm respectively

aX_1 and aX_2 =Absorbitivity of CH at 297.0nm and 317.0nm

aY_1 and aY_2=Absorbitivity of TZ at 297.0nm and 317.0nm

Method II: Absorbance ratio method (Q-Analysis)

Absorbance ratio method uses the ratio of absorbance at two-selected wavelength one which is an "isoabsorptive point" and other being the λ-max one of the two components from the overlay spectra of two drugs. It is evident that CH and TZ showed an isoabsoptive point at 297 nm. The second wavelength selected as 279.0 nm, λ-max of CH. Six standard solutions having concentration 1, 3, 5, 7, 9, 11 and 13 µg/ml for CH and 1.2, 3.6, 8.4, 10.8, 13.2 and 15.6 µg/ml for TZ were prepared by appropriate dilutions from their respective standard stock solutions. The absorbance of resulting solutions was measured at 297.0 nm and 279.0 nm and absorptivity coefficients were calculated using Beer Lambert law. The graphs of absorbance Vs concentration were plotted at each wavelength and regression coefficients were calculated.

The concentration of two drugs in the mixture can be calculated using equations

$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1 / aX_1$ .....(3)

$C_Y = (A_1/aX_1) - C_X$ ..... (4)

Where,

$$Q_M = \frac{A2}{A1} \qquad Q_X = \frac{ax_2}{ax_1} \qquad Q_Y = \frac{ay_2}{ay_1}$$

1 designates isoabsorptive point and 2 designates λ-max of OFL

ax_1 and ax_2 is absorptivity of CH at 1 and 2 wavelength respectively

$ay_1$and ay_2 is absorptivity of TZ at 1and 2 wavelength respectively

$A_1$and A_2 are absorbances of the mixture at 1 and 2 wavelength respectively

Analysis of marketed formulation:

Simultaneous estimation of CH and TZ in marketed tablet dosage form containing label claim of CH-500 mg and TZ-600mg was carried out. In this assay procedure, 20 tablets of formulation were crushed and ground to a fine powder. Powder equivalent to 10 mg of CH and 12 mg of TZ was transferred to a 100 ml volumetric flask containing about 75 ml of solvent and dissolved and sonicated for 30 min. The solution was diluted up to the mark with solvent. The solution was filtered through Whatmann filter paper No. 41 and residue was washed with solvent. Accurately measured 1.0 ml of solution was transferred to 10 ml volumetric flask, diluted up to the mark with solvent to get final working concentration of CH (10 µg/ml) and TZ (12 µg/ml). For Method I, the absorbance of the sample solution i.e. A_1 and A_2 were recorded at279.0 nm and 317.0 nm respectively and concentration of two drugs in the sample were determined using above equation (1) and (2). For method II, the absorbances of the sample solution i.e. A_1 and A_2 were recorded at 297.0 nm (isoabsorptive point) and 279.0 nm (λ-max of Ciprofloxacin HCl) respectively and ratio of absorbance were calculated i.e. A_2/A_1. Relative concentration of two drugs in the sample was calculated using above equation (3) and (4). The result of analysis of marketed formulation is shown in (Table 1).

VALIDATION OF DEVELOPED METHOD

The proposed method has been statistically validated for linearity, accuracy, precision, repeatability and reproducibility, limit of detection (LOD) and limit of quantification (LOQ) as per ICH Q2 guidelines.[3] Linearity was observed in range of 1-13 µg/ml for CH (fig.4) and 1.2-15.6 µg/ml for TZ (fig.5) for precision (intra day and inter day), R^2 value of CH and TZ were found to be 0.998 and 0.999 respectively from calibration curve (fig 3). Absorbances of three different concentrations were measured for three times within day and for three consecutive days respectively for both developed methods. The % RSD values were found to be less than 2%. To perform repeatability, absorbance of concentration nearer to assay concentration was measured for six times consecutively and %RSD was calculated. Reproducibility of the developed method was established by calculating %RSD values of the absorbance measured by different analyst. LOD and LOQ values were determined from mathematical equations. Accuracy (recovery) was determined by spiking different concentrations of pure Ciprofloxacin HCl and Tinidazole standard (80%, 100% and 120%) in pre-analyzed samples. Absorbance was measured at 279 nm and 317 nm for simultaneous equation method; and 297 nm and 279 nm for absorbance ratio method. From this % recovery was calculated. (Table 2)

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 CH (5µg/mL) and TZ (6µg/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 containing Phosphate Buffer: Acetonitrile (80:20). 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 Phosphate Buffer: Acetonitrile (80:20). The percent recoveries of substances are listed in (Table 4). Reported values are the average of three individual swab samples for each substance. The swab recoveries varied between 98.67%-100.66%

n=3 avarage

Application of this method to the cleaning process of mixing tank vessel

This method was applied on the cleaning process of mixing tank where CH and TZ were mixed for manufacturing. 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 containing Phosphate Buffer: Acetonitrile (80:20). 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 Phosphate Buffer: Acetonitrile (80:20). And analyzed by UV and result was shown in (Table 5)

RESULTS AND DISCUSSION:

The first method employing simultaneous equation is a very simple method and can be employed for routine analysis of these two drugs. Once the absorptivity values are determined very little time is required for analysis, as would require determination of absorbances of the sample solution at two selected wavelength and few simple calculations. In absorbance ratio method (Method II), the primary requirement for developing a method for analysis is that the entire spectra should follow the beer's law at all the wavelength. This requirement was fulfilled in spectra of both the drugs. In this method the calculations have been minimized by taking one of the measurements as an isoabsorptive point i.e. at 297 nm. As ratio is fixed for a specific mixture, the degree of dilution of two substances does not alter the value of ratio (A_2/A_1). Moreover, the value of standard deviation and coefficient of variation were satisfactory and recovery studies ranging from 99.35%-99.61% (CH) and 99.45%-99.77% (TZ) were indicative of the accuracy and precision the proposed methods. This method also apply to cleaning process where we found 3.25 ppm concentration of active drug substance (CH 1.16 ppm and TZ 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 and the developed simultaneous equation method and Q-ratio method could be used for routine analysis of CH and TZ in their combined dosage forms. 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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Table 1: Analysis of marketed formulation

Tablet	Label claim (mg)		Amount found (mg)		% Label claim* ± S. D.	
	CH	TZ	CH	TZ	CH	TZ
I	500.0	600.0	496.7	596.7	99.35±0.35	99.45±0.18
II	500.0	600.0	498.0	598.6	99.61±0.32	99.77±0.08

*Mean of five determination, I = simultaneous equation method, II = absorbance ratio method

Table 2: Recovery studies

Method	Amount of sample taken (µg/ml)		Amount of standard spiked (%)		Mean % Recovery ± S.D.	
	CH	TZ	CH	TZ	CH	TZ
I	10	12	80%	80%	99.07±0.100	99.28 ± 0.376
I	10	12	100%	100%	99.75 ± 0.484	99.43 ±0.107
I	10	12	120%	120%	99.24 ± 0.151	99.65 ±0.145
II	10	12	80%	80%	99.58 ± 0.99	99.85 ± 0.65
II	10	12	100%	100%	99.31 ± 0.75	99.68 ± 0.39
II	10	12	120%	120%	99.95 ± 1.2	99.79 ± 0.46

*n=3, I= simultaneous equation method, II= absorbance ratio method

Table 3: Summary of validation parameters

Parameters	Ciprofloxacin HCl	Tinidazole
Linearity range (n=6)	1-13 µg/ml	1.2-15.6 µg/ml
Equation	Y = 0.11x - 0.011	Y=0.030x + 0.012
R^2	0.998	0.999
Mean % recovery	99.35%-99.61%	99.45%-99.77%
Intraday precision (%RSD) (n=3)	1.02-1.25	0.41-0.96
Inter day precision (%RSD) (n=3)	1.14-1.60	1.22-1.47
Repeatability (%RSD) (n=6)	1.38	0.92
Reproducibility (%RSD) (n=6)	1.12	0.83
LOD	0.33 µg/ml	0.22 µg/ml
LOQ	1.0 µg/ml	0.67 µg/ml

Table 4: Swab Recovery (Residual Recovery)

Drug Substance	ppm of spiked Standard solution	ppm of spiked recovered active substances on Plate	%Recovery	%RSD
CH	5	4.92	98.4	1.28
TZ	6	5.83	97.2	1.5

Table 5: 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 (CH)	1.16	Yes
Individual(TZ)	2.09	Yes

n=3 average

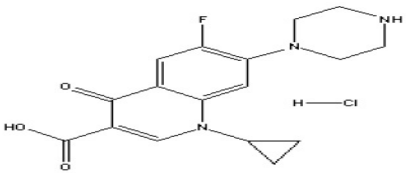

Figure 1: Structure of Ciprofloxacin HCl

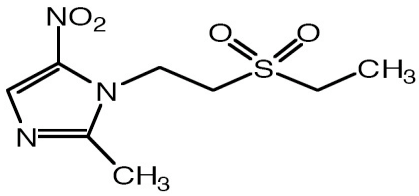

Figure 2: Structure of Tinidazole

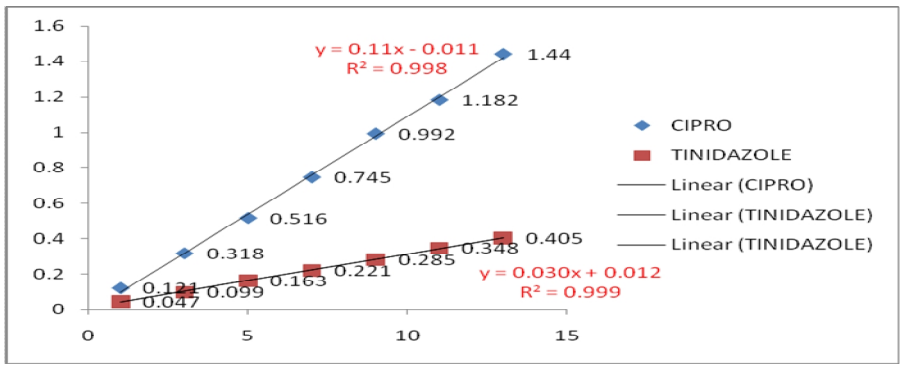

Figure 3: Calibration curve of CH at 297.0 nm and of TZ at 317 nm.

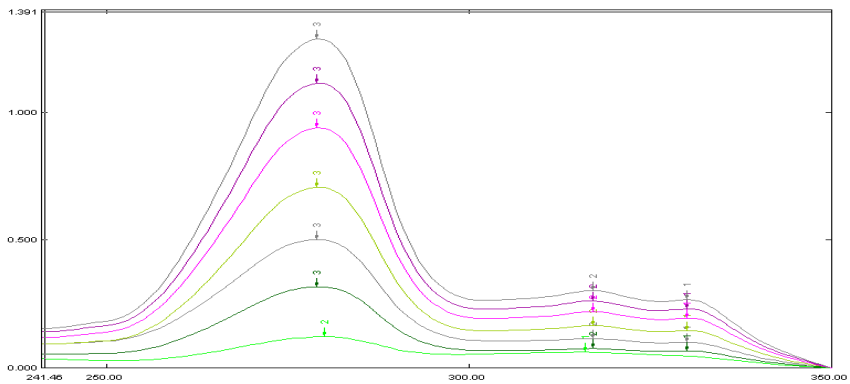

Figure 4: Linearity spectra of Ciprofloxacin HCl

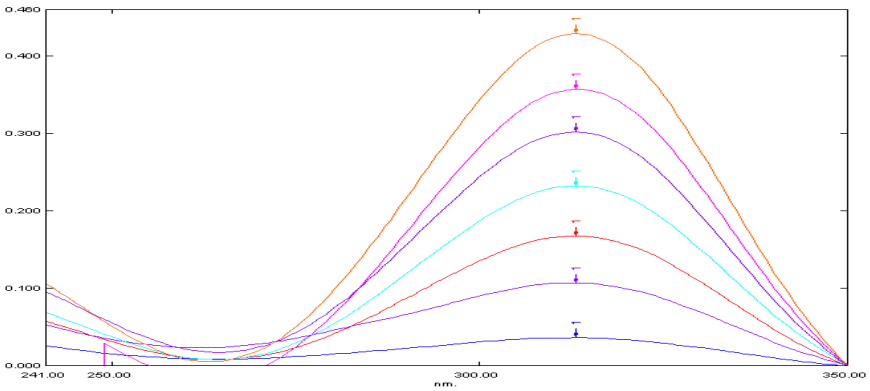

Figure-5 Linearity spectra of Tinidazole