Development of validated Spectrophotometric Methods: Simultaneous Equation Method and Q-Absorption Ratio method and for Simultaneous Estimation of Secnidazole and Ciprofloxacin

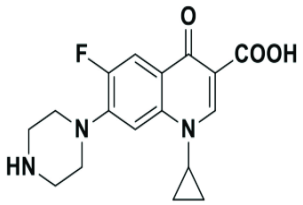
Megha Patel[*1], Kiran Suthar[1], Vishal Chudasama[1], Bharat Rajpurohit[1], Ashok Patel[2]
[*1:] *M N College of Pharmacy, B D Rao College campus, Khambhat*
[2] *B K Modi Govt. Pharmacy College, Rajkot*

***Abstract*: Two simple, accurate, sensitive and economical spectrophotometric methods have been developed and subsequently validated for determination of Ciprofloxacin and Secnidazole in bulk. For the simultaneous equation method, the estimation of Secnidazole and Ciprofloxacin was carried out at 319 nm (λ max of Secnidazole) and 271.2 nm (λ max of Ciprofloxacin). For Q - absorption ratio method, estimation of Secnidazole and Ciprofloxacin at 282 was carried out at 319 nm (λ max of Secnidazole) and 282 nm (isosbestic point of both drugs). Calibration curves of Secnidazole and Ciprofloxacin were found to be linear in the concentration ranges of 1-25 μg/mL, with their correlation coefficient values (r^2) being more than 0.995. In the precision study, the % RSD value was found within limits (RSD < 2%). The percentage recovery for Secnidazole at various concentration levels varied from 100.14 to 102.14 % and 1.1.2 to 102.13 by Method I and II respectively, While The percentage recovery for Ciprofloxacin varied from 100.52 to 101.51 and 100.3 to 101.33 by Method I and II respectively confirming that the projected methods are termed as an accurate and it can be applied successfully for the simultaneous estimation of Secnidazole and Ciprofloxacin in pure combined form.**

Key Words: Ciprofloxacin, Q-Absorption Ratio method, Secnidazole, Simultaneous, Simultaneous Equation method, Validation.

INTRODUCTION

Ciprofloxacin is a synthetic second-generation fluoroquinolone antibiotic used to treat various susceptible bacterial infections [1]. Ciprofloxacin is commonly used for infections caused by certain bacteria like Urinary tract infections (UTIs) and bladder infections, Prostate infections, Lung infections (e.g., bronchitis, pneumonia), Sinus infections, Skin infections, Bone and joint infections, Abdominal infections, Infectious diarrhoea, Typhoid fever etc [2]. Ciprofloxacin acts on bacterial topoisomerase II (DNA gyrase) and topoisomerase IV. Ciprofloxacin's targeting of the alpha subunits of DNA gyrase prevents it from supercoiling the bacterial DNA which prevents DNA replication [3-4]. Ciprofloxacin (Fig 1) is chemically described as 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.



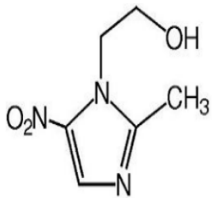


Fig 1: Ciprofloxacin Fig 2: Secnidazole

Secnidazole is a second-generation 5-nitroimidazole antimicrobial agent [5]. Secnidazole is a broad-spectrum antimicrobial drug. selective against many anaerobic Gram-positive and Gram-negative bacteria and protozoa. Secnidazole is indicated for treatment of Amoebiasis, Trichomoniasis in Women and Men, Bacterial Vaginosis and other infective diseases. [6-7]

Secnidazole enters the bacterial cell as an inactive prodrug where the nitro group is reduced by bacterial enzymes to radical anions. It is believed that these radical anions interfere with bacterial DNA synthesis of susceptible isolates [8]. Secnidazole (Fig 2) is chemically described as 1-(2-hydroxypropyl)-2- methyl-5-nitroimidazole.

Estimation of drugs by spectrophotometric methods rarely involves samples containing only one absorbing substances. Analysts have modified simple procedures in spectrophotometry to eliminate certain sources of interferences, or alternately to detect the amount of the other absorbing substances, resulting in to methods like 1) Simultaneous

equations method, 2) Absorbance ratio method (Q-Absorbance method), 3) Derivative spectroscopy, 4) Dual wavelength method, 5) Difference spectroscopy etc. Analyst has proposed spectroscopic method for simultaneous estimation of various drug combinations using these methods. Simultaneous equation method for simultaneous determination of rabeprazole sodium and aceclofenac from the combined capsule dosage form, Lansoprazole and Domperidone, etoricoxib and thiocolchicoside [9-11]. Q-absorbance ratio method for simultaneous determination Naproxen and Paracetamol, Prednisolone and 5-Amino Salicylic Acid [12-13]. Derivative spectroscopic method for simultaneous determination of Salicylic acid and Acetylsalicylicacid, Aspirin and Atorvastatin calcium [14-15]. Dual wavelength method for simultaneous determination of Gatifloxacin Sesquihydrate and Prednisolone Acetate, Atorvastatin and Ezetimibe [16-17]. Difference Spectroscopy method for simultaneous determination of Moxifloxacin and Cefixime Trihydrate, zidovudine and lamivudine [18-19].

Method I (Simultaneous equation method): Simultaneous equation method is also termed as Vierordt's method. It is possible to quantify both drugs using the simultaneous equation method if a sample contains two absorbing drugs (x and y), each of which absorbs at the wavelength maxima of the other. Secnidazole and Ciprofloxacin both can be absorbs at the wavelength maxima of each other.

Absorptivity = Absorbance/Concentration……. Eq. (1)

By using the below equations, the concentrations in the samples were obtained.

$Cx = a^1yA^2 – A^1a^2y / a^2xa^1y – a^1xa^2y$ …Eq. (2)

$Cy = a^2xA^1 – A^2a^1x / a^2xa^1y – a^1xa^2y$ …Eq. (3)

Where,

A^1, A^2 = absorbances of the mixture at λ1 and λ2 respectively

a^1_x and a^2_x = absorptivities of X at λ1 and λ2, respectively

a^1_y and a^2_y = absorptivities of Y at λ1 and λ2, respectively

Cx and Cy = concentration of the drug x and drug y respectively

Method II (Q-Absorbance ratio method): Q-Absorbance ratio method is a modification of simultaneous equation method. This method also called as Absorption ratio method. According to this method, the ratio of absorbance at any two wavelengths for a substance, which obeys Beer's law, is a constant value independent of the concentration and path length. This constant is termed as Q-value or "Hufner's Quotient". This method uses the ratio of absorbance at two selected wavelengths, one being the λ max of one of the components and the other being a wavelength of equal absorptivity of the two components, i.e., an iso-absorptive point.

Concentrations in the samples were obtained by using following equations:

Cx = (Qm-Qy / Qx-Qy) × A1 / ax1 …….. Eq. (4)

Cy = (Qm-Qx / Qy-Qx) × A2 / ay1 …….. Eq. (5)

Where,

A1 and A2 are the absorbances of mixture at λ max of one of the components and at iso-absorptive point

ax1 and ax2 are absorptivities of X at λ max of one of the components and at iso-absorptive point respectively

ay1and ay2 = absorptivities of y at λ max of one of the components and at iso-absorptive point respectively

Qm = A2/A1, Qy = ay2/ay1 and Qx = ax2/ax1

Cx and Cy represents concentration of the drug x and drug y respectively

By using the above two methods, estimation of both drugs by UV spectrophotometry has been done.

Several methods have been reported for estimation of Secnidazole [20-22] and Ciprofloxacin [23-25] as single component as API or in formulations. Several methods have also been reported for estimation of Secnidazole [26-28] and Ciprofloxacin [29-31] in combination with other drugs in different formulations. Literature survey revealed that no method has been reported for Simultaneous Estimation of Secnidazole and Ciprofloxacin. Hence attempt was made develop and validate rapid, cost-effective, simple, accurate, precise Spectrophotometric Methods: Simultaneous Equation Method and Q-Absorption Ratio method and for Simultaneous Estimation of Secnidazole and Ciprofloxacin.

MATERIAL AND METHOD

Since Method I and Method II basically differs in application of mathematical or statistical theories, Same material and methodology was used for both methods. As these methods differs in selectin of wavelength (Method I uses wavelength maxima of both drugs while Method II uses wavelength maxima of one drug and another wavelength used is

Isoabsorptive point). Both methods were validated in same terms and methodology.

Chemicals and Reagents:
Secnidazole and Ciprofloxacin powders were kindly gifted by Alembic Pharmaceuticals Ltd., Baroda, Gujarat. Double distilled water was used in the study. Combined tablets of Secnidazole and Ciprofloxacin were prepared in the laboratory.

Instruments and apparatus:
A Shimadzu model 1601 double beam UV–visible spectrophotometer with a pair of 10 mm matched quartz cells was used to measure absorbance of the resulting solutions.
A Shimadzu AX 200 analytical balance (Shimadzu, Japan) was used for accurately weighing.
An ultra-sonic cleaner (Frontline FS 4) was used for sonication. Volumetric flasks Borosil (10 ml, 50ml, 100 ml) was used for making solution and dilution,

Preparation of solutions
Standard Secnidazole and Ciprofloxacin stock solution (100 µg/ml) were prepared by accurately weighing and transferring 10 mg each drug separately to 100 ml volumetric flasks Both drugs were dissolved and diluted to 100 ml with water to obtain 100 µg/ml concentration in each volumetric flask. Combined Solution was prepared by transferring accurately weighed Secnidazole (10 mg) and Ciprofloxacin (10 mg) in to a 100 ml volumetric flask. Drugs were dissolved and diluted to 100 ml with water. The solution (0.5 ml) was transferred to a 10 ml volumetric flask and diluted to the mark with water to obtain final solution with Secnidazole (5 µg/ml) and Ciprofloxacin (5 µg/ml). Sample solution was prepared as follow: synthetic mixture was prepared in the laboratory comprising of Secnidazole and Ciprofloxacin in the proportion of 1:1 (w/w). A quantity of powder equivalent to 10 mg of Secnidazole and Ciprofloxacin was transferred to a 100 ml of volumetric flask and mixed with water (50 ml) and sonicated for 20 minutes and diluted to the mark with water. The solution (0.5 ml) was transferred to a 10 ml volumetric flask and diluted to the mark with water to obtain final solution with Secnidazole (5 µg/ml) and Ciprofloxacin (5 µg/ml).

Method 1: Simultaneous equation method
Determination of wavelength of maximum absorbance:
The standard stock solutions of Secnidazole and Ciprofloxacin were scanned in the range of 200 to 400 nm against water as a blank Maximum absorbance was obtained at 319 nm and 271.2 nm for Secnidazole and Ciprofloxacin, respectively (Fig 3).

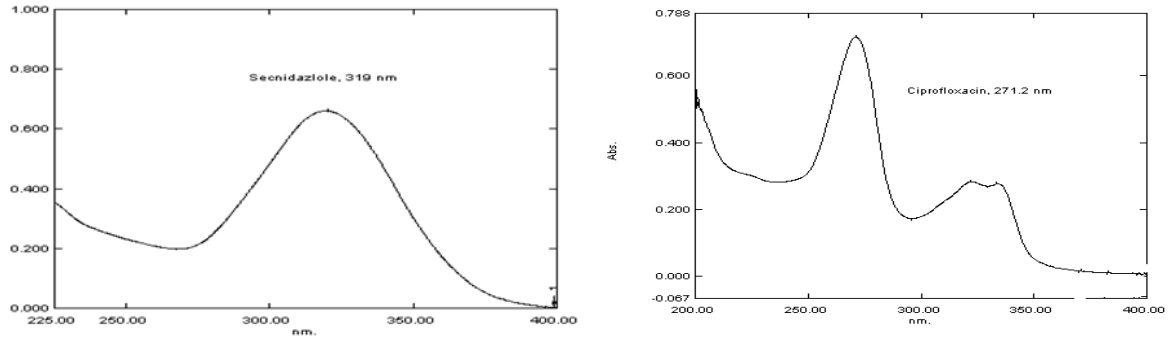


Fig 3: Spectra of Secnidazole (3.1). and Ciprofloxacin (3.2). showing wavelength maxima

Validation of method I: The developed methods were validated in accordance with the ICH Q2 (R1) guideline.

1. Linearity and range (Calibration curve):

A calibration curve was plotted over a concentration range of 1-25 µg/ml for Secnidazole and Ciprofloxacin in respectively. Accurately measured standard stock solutions of Secnidazole and Ciprofloxacin (0.1, 0.3, 0.5, 0.8, 1.0, 1.2, 1.5, 1.8, 2.0, 2.5 ml) were transferred in to a separate series of 10 ml volumetric flasks. The volume was adjusted to the mark with water and mixed. The absorbance of each solution was measured at 271.2 nm and 319 nm against water as a blank. Calibration curves were constructed for Secnidazole and Ciprofloxacin by plotting absorbance versus concentrations at both wavelengths. Each reading was average of five determinations.

2. limit of detection (LOD) & limit of quantitation (LOQ):

LOD and LOQ LOD and LOQ were calculated by assessing signal to noise ratio according to equations

stated in ICH Q2 (R1) Following equations were used:

LOD = 3.3 × σ/S …….. Eq. (6)

LOQ = 10 × σ/S …….. Eq. (7)

Where, σ=Standard deviation of response, S = Slope of calibration curve

3. *Precision (repeatability, Inter-day & Intra-day):*

The precision of developed methods was performed in terms of repeatability, intra-day, and inter-day precision. Repeatability measurement was carried separately for 6 times by analyzing solutions containing 10 µg/mL of Secnidazole and 10 µg/mL of Ciprofloxacin. Intraday and interday precision was determined with standard solution of Secnidazole 10 µg/mL and Ciprofloxacin 10 µg/mL for 5 times. Percentage Relative Standard Deviation (RSD) was calculated. Percentage Relative Standard Deviation (RSD) should be less than 2%.

4. *Accuracy (Recovery study):*

Accuracy was determined in terms of percent recovery. The proposed method was applied to determine Secnidazole and Ciprofloxacin in powder mixture. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the powder mixer with three different concentrations of standards. Standard addition at three addition levels i.e. 50% Low Concentrations (LC), 100% Intermediate Concentrations (IC) and 150% High Concentrations (HC) were carried out as per ICH quality guidelines. Percent recoveries were calculated as per following formula:

% Recovery=[Conc.(spiked)-Conc.(unspiked) × 100] / Conc. (added) …….. Eq. (8)

5. *Robustness:*

The robustness of an analytical technique is an indicator of its ability to remain unchanged by limited, yet deliberate changes in process parameters and demonstrates its reliability during daily use. Robustness of analytical method was determined by analyzing 10µg/ml solution of both drug at different wavelengths i.e. Wavelength maxima ±5 nm and temperatures i.e. 30±5° C.

6. *Specificity:*

Specificity of the method was judged by measuring the absorbance of both drugs individually at their maxima wavelength against the blank and synthetic excipients and their absorbance was compared with the blank and synthetic excipients.

Estimation in combination of and in bulk Powder with excipient:

The proposed validated method was applied to determine Secnidazole and Ciprofloxacin in bulk powder mixture. Absorbance of solution of the bulk powder mixture containing excipient was measured at wavelength maxima. Absorptivity and conc. of each drug in mixture was calculated using equations 1, 2 and 3.

Method 2: Q-absorbance ratio method

Determination of iso-absorptive point and wavelength of maximum absorbance:

The standard stock solutions of Secnidazole and Ciprofloxacin were scanned in the range of 200 to 400 nm against water as a blank. Iso-absorptive point was found at 282 nm (Figure 4). Another wavelength used is 319 nm (λ-max of Secnidazole,) Fig 3.1.

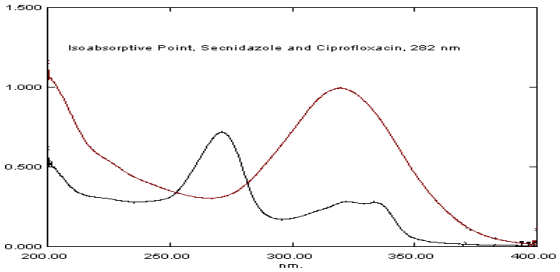


Figure 4: Overlain spectra of Secnidazole and Ciprofloxacin showing iso-absorptive point

Validation of method II:

The developed methods were validated in accordance with the ICH Q2 (R1) guideline.

Methodology and parameter used for validation of Method II were same as method I except the absorbance were measured at Iso-absorptive point i.e. 282 nm and 319 nm λ-max of Secnidazole. Conc. of each drug in drug mixture was calculated using equations 4 and 5.

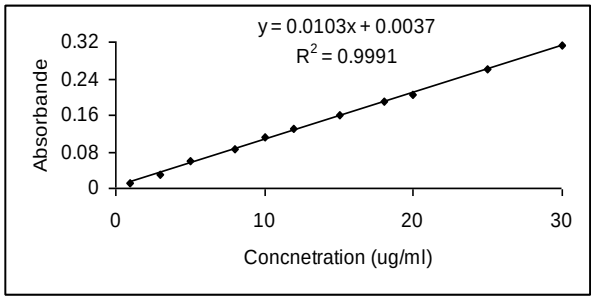
RESULT AND DISCUSSION

METHOD I:

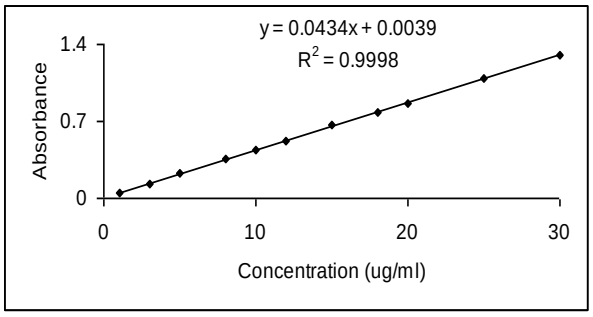
The proposed methods have been statistically validated in terms of linearity and range, limit of detection (LOD) and limit of quantification (LOQ) accuracy, precision (repeatability and reproducibility), robustness, specificity as per ICH Q2(R1) guidelines

1. *Linearity and range:*

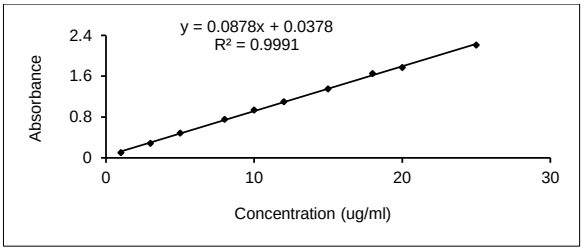
Calibration curve Correlation coefficients values were observed close to value 1. Linearity was observed in the range of 1-25 µg/mL both drugs with correlation coefficient values (r^2) more than 0.999. Result with other optical and regression characteristic are presented in Table 1.



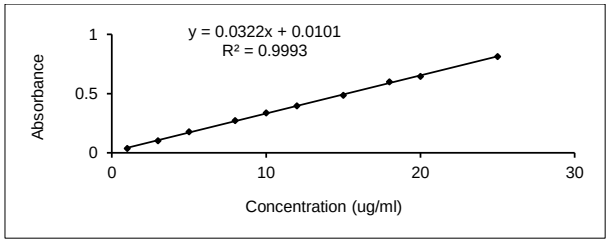
Calibration curve of Secnidazole at 271.2 nm



Calibration curve of Secnidazole at 319 nm



Calibration curve of Ciprofloxacin at 271.2 nm



Calibration curve of Ciprofloxacin at 319 nm

Figure 5 Calibration curves for Secnidazole and Ciprofloxacin at both wavelengths.

Table 1: Optical and Regression characteristics and validation parameters of simultaneous equations method for analysis of Secnidazole and Ciprofloxacin

Parameters	Secnidazole		Ciprofloxacin	
Wavelength	271.2nm	319nm	271.2nm	319nm
Beer's Law Limit (µg/ml)	1-25	1-25	1-25	1-25
Molar Absorptivity ($1\ mole^{-1}\ cm^{-1}$)	1.907×10^3	8.037×10^3	2.909×10^4	1.067×10^4
Sandell's sensitivity ($\mu g/cm^2$/0.001 absorbance unit)	0.0909	0.0226	0.0106	0.0297
Regression equation (y* = a + by)				
Slope (b),	0.0103	0.0434	0.0878	0.0322
Intercept (a)	0.0037	0.0039	0.0378	0.0101
Correlation Coefficient(r^2)	0.9986	0.9997	0.9991	0.9993
Standard Deviation (S.D)	0.0014	0.0087	0.0169	0.0062
Relative Standard Deviation (RSD or %CV)	1.157	1.532	1.539	1.493
Standard Error of Mean (S.E.M)	0.00043	0.00275	0.0053	0.00196
LOD (µg/ml)	0.65	0.35	0.25	0.55
LOQ (µg/ml)	1.00	1.00	1.00	1.00
Precision				
Repeatability (n=6) (% CV)	0.97-1.12	1.08-1.24	1.02-1.15	1.11-1.27
Intra-day (n=5) (% CV)	0.66-1.38	1.30-1.73	1.31-1.65	1.04-1.69
Inter-day (n=5) (% CV)	1.22-1.61	1.49-1.79	0.92-1.55	0.44-1.78
y* = a + by Where, 'c' is the concentration and 'y' is the absorbance				

2. LOD and LOQ were determined using mathematical equations 6 and 7. The results are presented in Table 1.

3. The results of Precision study in terms of repeatability, intra-day and inter-day precision are depicted in Table 1. % CV <2% indicated method is precise.

4. Accuracy was determined in terms of recovery study using std addition method. Result depicted in table indicated % Recovery between 98-102, which assures the developed method is accurate and can estimate the drugs successfully in presence of excipients.

Table 2: Data of recovery study of Secnidazole and Ciprofloxacin by Simultaneous Equations Method

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=5)
Secnidazole	6	3	9.046	100.14 ± 0.91
	6	6	1.2186	101.84 ± 0.86
	6	9	15.253	102.14 ± 0.71
Ciprofloxacin	6	3	9.105	100.52 ± 1.39
	6	6	12.085	101.51 ± 1.76
	6	9	15.078	100.98 ± 1.05

5. Specificity: Specificity of the method was judged by measuring the absorbance of Secnidazole and Ciprofloxacin individually at 319 nm, and 271.4 nm with the blank and synthetic excipients and their absorbance was compared with the blank and synthetic excipients. No interference was observed at 319 nm, and 271.4 nm indicating that the method is specific.

6. Robustness: The robustness of analytical method was determined by analyzing the Secnidazole (10µg/ml) and Ciprofloxacin (10µg/ml) solutions at different wavelengths i.e. 319±5 nm, 271.4±5 nm respectively and temperatures i.e. 30±5ºC. RSD <2% Indicated method is robust.

Estimation in combination of and in bulk Powder mixture: The proposed validated method was applied to determine Secnidazole and Ciprofloxacin in bulk powder (API). Absorbance of combined API solution was recorded at 319 nm and 271.4 nm is presented in (Table 3).

The content of Secnidazole and Ciprofloxacin in Mixture with other excipient are 99.67-101.3 % indicates no interference from the common excipients present.

Table 3: Data of estimation of Secnidazole and Ciprofloxacin by simultaneous equations method in bulk powder containing excipients

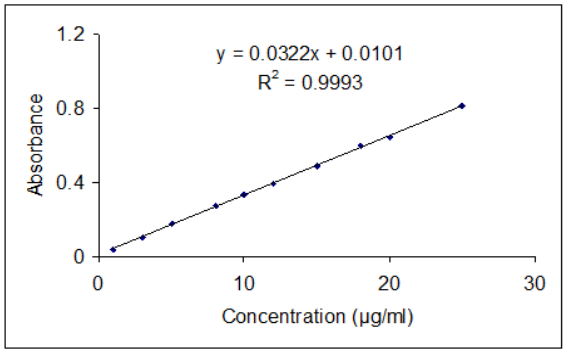
Formulation	Drug	Labelled/taken amount (mg)	Amount found(mg)	% Amount found ± S.D (n=3)
Bulk powder	Secnidazole	20	19.92	99.67 ± 0.99
	Ciprofloxacin	20	20.38	101.93 ± 1.92

METHOD II

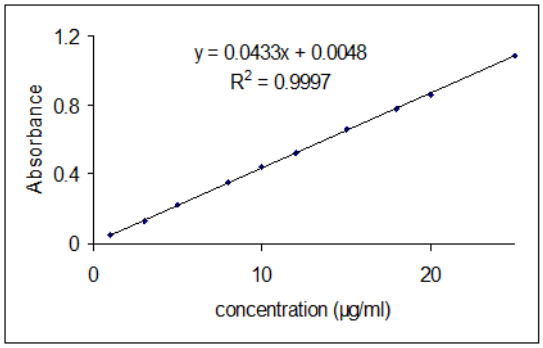
The proposed methods have been statistically validated in terms of linearity and range, limit of detection (LOD) and limit of quantification (LOQ) accuracy, precision (repeatability and reproducibility), robustness, specificity as per ICH Q2(R1) guidelines

1. *Linearity and range*:

Calibration curve Correlation coefficients values were observed close to value 1. Linearity was observed in the range of 1-25 µg/mL both drugs with correlation coefficient values (r^2) more than 0.999. Result with other optical and Regression characteristic are presented in Table 4.



Calibration curve of Ciprofloxacin at 319 nm



Calibration curve of Secnidazole at 319 nm

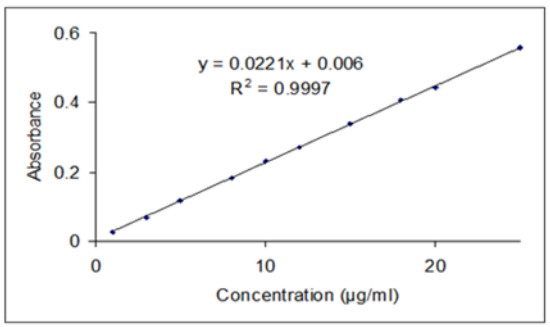


Fig 4. Calibration curve of Secnidazole and Ciprofloxacin at 282 nm (iso-absorptive point)

Table 4: Optical and regression characteristics and validation parameters of Q-absorbance ratio method for analysis of Secnidazole and Ciprofloxacin

Parameters	319 nm		282 nm
	Secnidazole	Ciprofloxacin	Secnidazole and Ciprofloxacin
Beer's Law Limit (µg/ml)	1-25	1-25	1-25
Molar Absorptivity ($1 mole^{-1} cm^{-1}$)	$8.0368 X 10^4$	$1.0669 X 10^4$	$4.0924 X 10^4$ $7.3226 X 10^4$
Sandell's sensitivity ($\mu g/cm^2$/0.001 absorbance unit)	0.0226	0.02976	0.0435
Regression equation (y* = a +by) Slope (b), Intercept (a)	 0.0434 0.0039	 0.0322 0.0101	 0.0221 0.006
Correlation Coefficient(r^2)	0.9998	0.9993	0.9997
Standard Deviation (S.D)	0.00874	0.00624	0.00429
Relative Standard Deviation (RSD or %CV)	1.532	1.493	1.402
Standard Error of Mean (S.E.M)	0.002764	0.001974	0.00136
LOD (µg/ml)	0.35	0.55	0.30
LOQ (µg/ml)	1.00	1.00	1.00
Precision Repeatability (n=6) (% CV) Intra-day (n=5) (% CV) Inter-day (n=5) (% CV)	 1.08-1.24 1.30-1.73 1.49-1.79	 1.11-1.27 1.04-1.69 0.44-1.78	 0.98-1.28 1.09-1.70 0.79-1.79
y* = a + by Where, 'c' is the concentration and 'y' is the absorbance			

2. LOD and LOQ were determined using mathematical equations 6 and 7. The results are presented in Table 1.

3. *The results of Precision study* in terms of repeatability, intra-day and inter-day precision are depicted in Table 1. % CV <2% indicated method is precise.

4. Accuracy was determined in terms of recovery study using std addition method. Result depicted in table indicated % Recovery between 98-102, which assures the developed method is accurate and can estimate the drugs successfully in presence of excipients.

Table 5: Data of recovery study of Secnidazole and Ciprofloxacin by Q-absorbance ratio method

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=5)
Secnidazole	6	3	9.17	102.13 ± 1.18
	6	6	12.12	101.20 ± 1.69
	6	9	15.25	102.08 ± 1.78
Ciprofloxacin	6	3	9.07	100.88 ± 1.57
	6	6	12.03	100.30 ± 1.72
	6	9	15.16	101.33 ± 1.39

5. Specificity: Specificity of the method was judged by measuring the absorbance of Secnidazole and Ciprofloxacin individually at 319 nm, and 282 nm with the blank and synthetic excipients and their absorbance was compared with the blank and synthetic excipients. No interference was observed at 319 nm, and 282 nm indicating that the method is specific.

6. Robustness: The robustness of analytical method was determined by analyzing the Secnidazole (10µg/ml) and Ciprofloxacin (10µg/ml) solutions at different wavelengths i.e. 319±5 nm, 282±5 nm respectively and temperatures i.e. 30±5°C. RSD <2% Indicated method is robust.

Estimation in combination of bulk Powder and excipient mixture: The proposed validated method was applied to determine Secnidazole and Ciprofloxacin (API) in Mixture containing both drugs and excipient powder. Absorbance of combined API solution was recorded at 319 nm and 282 nm is presented in (Table 6).

The content of Secnidazole and Ciprofloxacin in Mixture containing both drugs and excipient powder are 100.06-100.24 % indicates no interference from the common excipients present.

Table 6: Data of estimation of Secnidazole and Ciprofloxacin by Q-absorbance ratio method in Mixture containing both drugs and excipient powder

Formulation	Drug	Labeled/taken amount (mg)	Amount found (mg)	% Amount found ± S.D (n=5)
Bulk powder	Secnidazole	20	20.01	100.24 ± 1.21
	Ciprofloxacin	20	20.09	100.06 ± 1.47

CONCLUSION

All factors together point to the conclusion that both the Simultaneous Equation Method and the Q-Absorbance Ratio Method are rapid, cost-effective, simple, accurate, precise for the estimation of Secnidazole and Ciprofloxacin simultaneously in the mixture. Hence, the proposed method can be recommended for simultaneous determination of Secnidazole and Ciprofloxacin in routine quality control analysis.

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