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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CIPROFLOXANCINE, TINIDAZOLE AND DICYCLOMINE HCL

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Abstract:

A Simple, accurate and precise UV Spectrophotometric method has been developed for the simultaneous estimation of Ciprofloxacin, Tinidazole and Dicyclomine Hcl in tablet. The method is based upon formation of simultaneous equation of these drugs and detections were carried out at wavelength of maximum absorbance of drugs; viz, 271nm, 317nm and 212nm for Ciprofloxacin,Tinidazole & Dicyclomine Hcl respectively. The linearity was found to be in the concentration range of 2-10 µg/ml for Ciprofloxacin,2-20 µg/ml for Tinidazole and 100-600 µg/ml for Dicyclomine Hcl respectively. The results of tablet analysis were found to be 99.98% for Ciprofloxacin and 100.05% for Tinidazole, 99.92% for Dicyclomine Hcl.The proposed methods can be effectively applied for the routine analysis of Ciprofloxacin, Tinidazol and Dicyclomine Hcl in bulk and combined dosage form.

Keywords: *UV Spectrophotometric method, Ciprofloxacin, Tinidazole, Dicyclomine Hcl, Simultaneous equation method.*

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INTRODUTION:

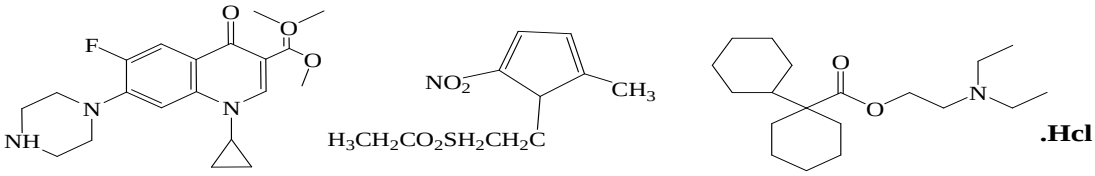
Ciprofloxacin (CPX) is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride monohydrate [1].It is a fluoroquinolone derivativeand has potent antimicrobial activity against a broad spectrum of bacteria [2]. Tinidazole(TNZ) is 1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitroimidazole [3].It is effective against protozoa and obligate anaerobic bacteria [4], amoebic and parasitic infections [5]. Dicyclomine Hcl (DIC) is 2-diethylaminoethylbicyclohexyl-1-carboxylate hydrochloride [6]. It is an antispasmodic and ant cholinergic agent [7] used as smooth muscle relaxant [8]. The structures of these three drugs are shown in Figure 1.

All these drugs were found to be soluble in water, methanol, ethanol etc. Hence distilled water is selected as solvent for simultaneous estimation of all these drugs.

The present method is relying on the use of simple chemicals than reported and technique provides sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC and HPTLC.

Literature survey revealed UV-Visible spectrophotometric methods such as simultaneous equation method [9-12]. Q-Analysis, Absorbance Correction method, multicomponent method, Differential Derivative Method, and RP-HPLC, HPTLC & CFI Analyzer for the estimation of Ciprofloxacin,Tinidazole,and Dicyclomine Hcl alone or in combination with other drugs. Fixed dose combination containing CPX, TNZ and DIC in tablet dosage form is recently available in the market. No method has been reported for this combination using Distilled water and by Simultaneous equation method. The present work therefore emphasizes on the quantitative estimation of CPX, TNZ and DIC in bulk and in their combined dosage form by UV spectroscopy. Tablet Gastrogyl plus contain Ciprofloxacin(250mg) Tinidazole (600mg) and Dicyclomine (10 mg) were analyzed for assay study.

The final stock solution of the formulation mixture contained very less concentration of Dicyclomine HCL, and contains less chromophorehence Assay was performed by Standard addition method [13].



Ciprofloxacine **Tinidazole** **Dicyclomine HCl**

Figure 1: Structure of CPX, TNZ, and DIC

SPECTROPHOTOMETRIC METHOD

Instruments [14]

Absorbance measurements were made on Shimadzu 1800 UV/Visible spectrophotometer with a pair of matched quartz cells of 1 cm width. Ultrasonicator of leelasonic instruments was used for sonication of the standard and sample solution.

Reagents and Materials

Bulk Drug of Ciprofloxacin and Tinidazole were obtained as gift samples from Aurbindo Laboratories, Hyderabad India and Dicyclomine Hcl was obtained as gift samples from Hindustan Laboratories Pvt. Ltd. Palghar. Mille Q water was used throughout the process. Combined tablets of ciprofloxacin, Tinidazole, and Dicyclomine Hcl (Gastrogyl plus) were purchased from local market.

Selection of solvent

The solvent was selected based on solubility and stability of drugs in it, as well as extraction of drug from its formulation. All these drugs were freely soluble in water and organic solvents, Hence distilled water was selected as solvent for UV Spectrophotometric determination.

Preparation of stock solution

An accurately weighed quantity of CPX (50mg), TNZ (50mg) and DIC (50mg) was transferred separately in 50 ml volumetric flask and dissolved in distilled water to get the final concentration of 1000µg/ml of each drug.

Preparation of working standard solution

From the above stock solution, 1ml of stock solution of CPX and TNZ was transferred to separate 50 ml volumetric flask and volume was made up to 50 ml with distilled water to get the final concentration as 20µg/ml of CPX and TNZ and 1ml of stock solution of DIC was transferred to separate 10 ml volumetric flask and volume was made up to 10 ml with distilled water to get the final concentration as 100 µg/ml DIC.

Determination of analytical wavelength

The working standard solution of CPX, TNZ and DIC were scanned in UV range from 200-400nm. The λ_{max} of each drug was selected as analytical wavelength i.e., as 271nm (CPX), 318nm (TNZ) and 212nm (DIC) respectively.

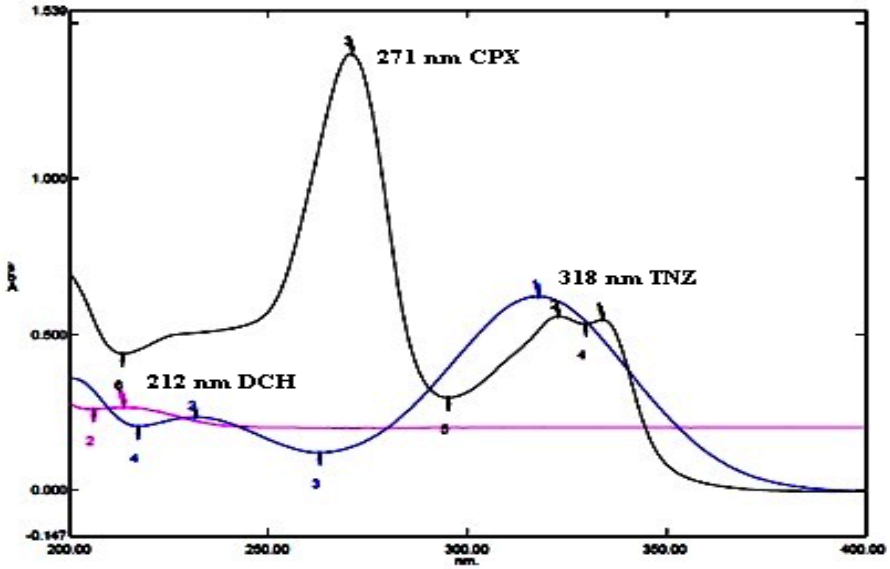


Figure 2: Overlain spectra of CPX, TNZ and DIC

Simultaneous equations method [15]

For the development of the simultaneous equations, the absorbances of CPX, TNZ and DIC were measured at selected wavelength and the absorptivity values E (1%, 1cm) were determined.

The concentrations of three drugs in mixture canbe calculated using the following Eqns,

$$C_{TNZ} = \frac{(A1(ay2az3 - az2ay3) - ay1(A2az3 - az2A3) + az1(A2ay3 - a\,y\,2\,A\,3)}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - y2ax3)} \ldots (1)$$

$$C_{TNZ} = \frac{(ax1(A2az3 - az2A3) - A1(ax2az3 - az2ax3) + az1(ax2A3 - A2ax3)}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - y2ax3)} \ldots (2)$$

$$C_{DCH} = \frac{(ax1(ay2A3 - A2ay3) - ay1(ax2A3 - A2ax3) + A1(ax2ay3 - ay2ax3)}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - y2ax3)} \ldots (3)$$

where, C_{CPX}, C_{TNZ} and C_{DIC} are the concentrations of CPX, TNZ and DIC, respectively in mixture and insample solutions, A1, A2 and A3 are the absorbances of sample at 271, 318 and 212nm,respectively,ax1, ax2 and ax3 are the absorptivity of CPX at 271, 318 and 212, respectively, ay1, ay2 and ay3 arethe absorptivity of TNZ at 271, 318 and 212 nm respectively, az1, az2 and az3 are the absorptivity DIC at 271, 318 and 212 nm.

Construction of calibration curve

A powder quantity equivalent to 50 mg Ciprofloxacin 60 mg Tinidazol and 1 mg Dicyclomine Hcl was accurately weighed and transferred to volumetric flask of 100ml capacity and for Dicyclomine Hcl 10 ml capacity and volume was made up to the mark with distilled water. From this solution 1 ml was transferred to volumetric flask of 10 ml capacity,

since the concentration of Dicyclomine Hcl is very less 2 ml having concentration of 200µg/ ml is added to the secondary stock solution. Volume was made up to the mark to give a solution containing 50µg/ml of ciprofloxacin 60µg/ml of Tinidazol and 200µg/ml of Dicyclomine Hcl . The concentrations of all drugs Ciprofloxacin, Tinidazol and Dicyclomine Hcl were determined by measuring the absorbance of the prepared mixture at 271nm, 318 nm and 212nm respectively for UV-Spectrophotometric method. From these absorbance values, the calibration curves of Ciprofloxacin, Tinidazol and Dicyclomine Hcl were constructed in UV- spectrophotometric method the results were shown in table 1.

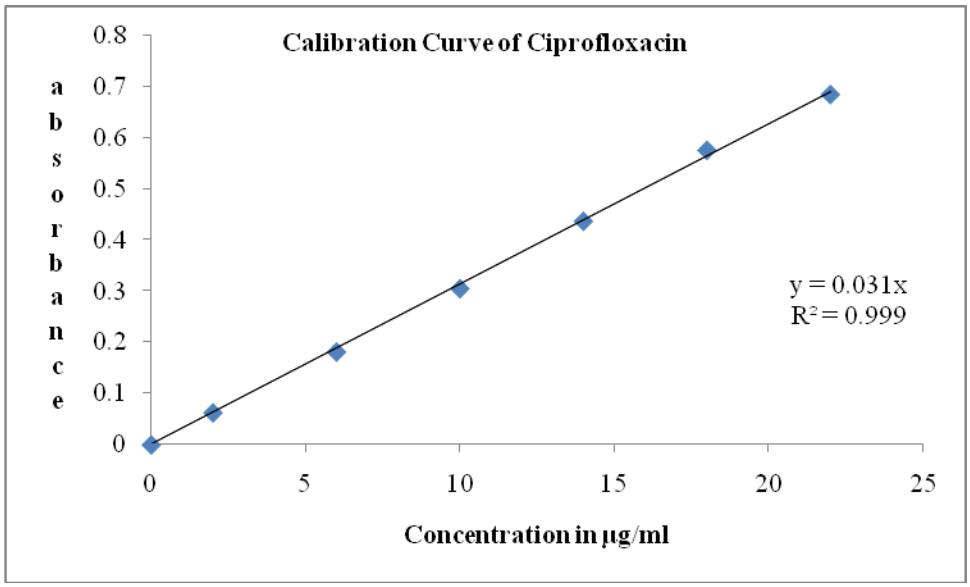


Figure 3: calibration curves Ciprofloxacin

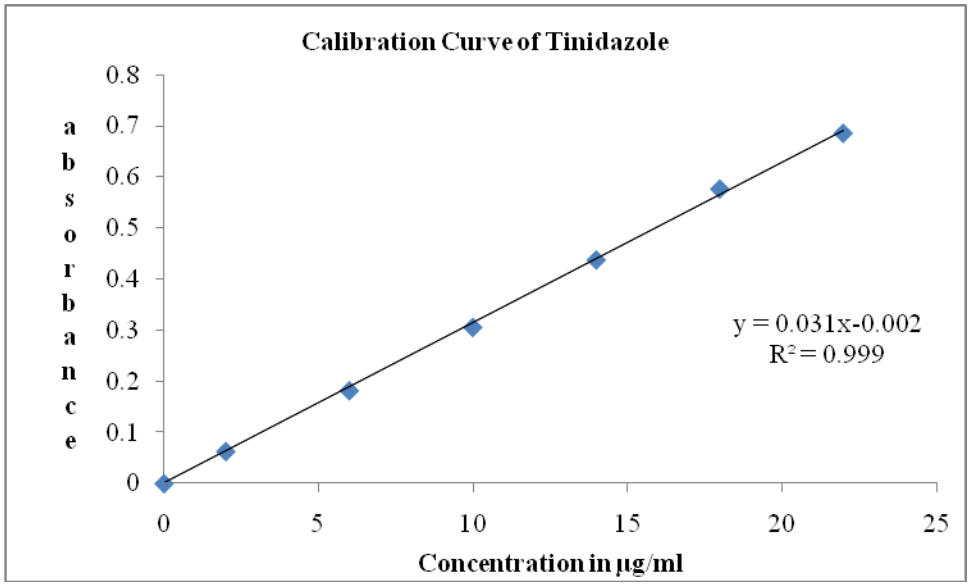


Figure 4: calibration curves Tinidazol

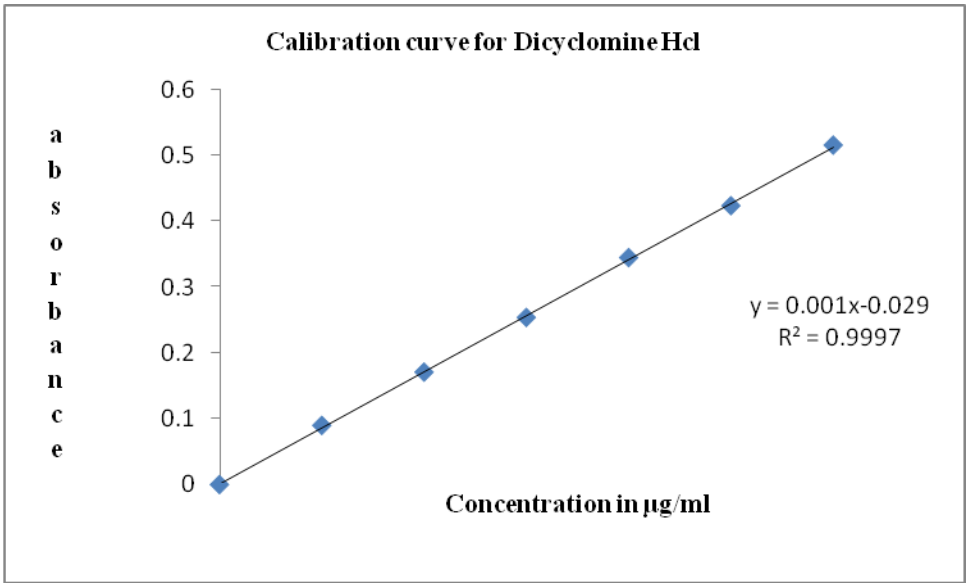


Figure 5: calibration curves Dicyclomine Hcl

Table 1: Optical characteristics and other Parameters

Parameters	Method		
	CPX	TNZ	DIC
Λmax	271	318	212
Linearity (µg/ml)	2-10	4-18	100-600
Regression Equation (y=ax + b)	y = 0.069x - 0.001	y = 0.031x - 0.002	y = 0.001x-0.035
Slope(b)	0.069	0.031	0.001
Intercept(a)	0.001	0.002	0.029
Correlation coefficient	0.998	0.999	0.997
LOD	0.047 µg/mL	0.21 µg/mL	6.2 µg/mL
LOQ	0.14 µg/mL	0.64 µg/mL	21.02 µg/mL

*y = mx + c; when x is the concentration in µg/mL and y is absorbance unit.

Method Validation

The developed method was validated as per ICH Guidelines[16,17].

Linearity

Linearity was checked by diluting standard stock solution at different concentrations. The linearity of Ciprofloxacin, Tinidazol and Dicyclomine Hcl response was evaluated from the range of 2-10µg/ml, 4-18µg/ml and 100-600µg/ml and showed a good correlation coefficient. To assess linearity, the standard curves Ciprofloxacin, Tinidazol and Dicyclomine Hcl were constructed by plotting concentration (µg/ml) verses absorbance.

Accuracy

To check the accuracy of the developed methods and to study the interference of formulation excipients, analytical recovery experiments were carried out by using three different concentrations (n=3). From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The %RSD was calculated. The results were shown in table 2.

Table 2: Results of Accuracy Study

Percentage Level	Drugs		
	CPX	TNZ	DIC
80%	99.56	99.86	99.65
100%	99.89	99.90	99.82
120%	99.78	99.79	99.78
Mean	99.74	99.85	99.75
S.D. *	0.168	0.055	0.073
% RSD*	0.168	0.056	0.073

*Average of three replicate at each level of recovery S.D –Standard deviation , % RSD-Relative standard deviation.

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation, repeated for six times with the same concentration. The amount of each drug present in the tablet formulation
was calculated. The %RSD was calculated. The Intermediate precision of the methods was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated. The results were shown in the table 3

Table 3: Results of Precision Study

	CPX	TNZ	DIC
	%Amount Found	%Amount Found	%Amount Found
Intraday Precision	99.89	99.87	99.65
Interday Precision	100.07	99.79	99.85
Mean	99.98	99.83	99.75
S.D*.	0.127	0.057	0.141
% RSD*	0.127	0.057	0.142

CPX- Ciprofloxacin, TNZ-Tinidazole , DIC –Dicyclomine Hcl , *Avarage of three replicate at each level of recovery, S.D –Standard deviation , % RSD-Relative standard deviation.

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations; LOD =3.3σ/s and LOQ = 10σ/ s, where σ is standard deviation of y-intercept of calibration curve (n = 3) and s is slope of regression equation. The results are shown in Table 1

RESULTS AND DISCUSSION:

The method discussed in the present work provide a convenient and accurate way for simultaneous analysis of Ciprofloxacin, Tinidazole and Dicyclomine Hcl In simultaneous equation method wavelength selected for Quantization were 271.0 nm for CPX318nm (TNZ) and 212nm (DIC) respectively . Linearity for detector response was observed in the concentration range of 2-10µg/ml for CPX ,4-18 TNZ and 100-600 . In this method , concentration of individual drug present in the mixture was determined against calibration curve in Quantitation mode .In Percent label claim for analysis of CPX,TNZ and DIC in tablet by this method was found in the range of 99.98% to 100.05% and Standard deviation and coefficient of variance for three determination of tablet sample was found to be less than ± 2.0 indicating precision of the method. The result of analysis shows that the developed method is accurate, precise, reproducible and economical and can be employed for routine quality control analysis of Ciprofloxacin, Tinidazole and Dicyclomine Hcl in combined dose formulation.

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