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RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN, TINIDAZOLE AND DICYCLOMINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Background: Ciprofloxacin Hydrochloride (CPX) is a fluoroquinolone antibacterial. Tinidazole (TNZ) is a nitroimidazole antiprotozoal while dicyclomine hydrochloride (DIC) is an anticholinergic antispasmodic agent. The three drugs in their fixed dose combination are frequently administered in typhoid fever and infections of mixed origin. **Purpose:** The aim of the present research was to develop a reversed-phase high-performance liquid chromatography method for simultaneous estimation of CPX, TNZ and DIC in bulk and combined tablet formulation. **Methodology:** HPLC system used was Thermo Finnigan coupled to a variable wavelength UV detector and was operated in isocratic mode. The data acquisition was carried out using Chrome Quist software. The separation was achieved using HiQ Sil C_{18} column having dimensions 250 x 4.6 mm i.d. with a particle size of 5 µm. The mobile phase consisted of buffer pH 4.0 and methanol combined in ratio of 60:40 v/v. The flow rate and UV detector was set at 1 ml/min and 218 nm respectively. **Findings:** The order of elution of all three drugs was found to be TNZ (5.42 min), DIC (6.96 min) and CPX (8.04 min). The linearity was established in the range of 5-30 µg/ml (CPX and TNZ) and 250-650 µg/ml (DIC). The method was validated in accordance with ICH guidelines. **Conclusion:** From this study it was concluded that the proposed RP-HPLC method is accurate, reproducible and precise. Application: The developed method was then successfully applied for simultaneous estimation of these drugs in marketed tablet dosage form.

Key words: *Ciprofloxacin Hydrochloride; Tinidazole; Dicyclomine Hydrochloride; RP-HPLC; isocratic.*

INTRODUCTION

High-performance liquid chromatography is still a popular analytical method for quantization of drugs in their dosage forms in spite of tremendous advances in analytical separations in recent years. The analysis of fixed dose combinations always poses challenge to the analyst. Hence analytical tools such as HPLC are often required for their simultaneous estimation as accurate analysis of drugs can be performed in a single run. One such fixed dose combination prescribed frequently for treatment of typhoid fevers and infectious diarrhea of mixed origin is ciprofloxacin, tinidazole and dicyclomine. Ciprofloxacin hydrochloride (CPX), a second generation fluoroquinolone is a broad spectrum antibiotic. Its IUPAC name is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (Fig.1).[1] Tinidazole (TNZ), a nitroimidazole antiprotozoal is also active against gram negative anaerobes. Its IUPAC name is 1-(2-ethylsulfonylethyl)-2-methyl-5-nitroimidazole (Fig.2).[2] Dicyclomine (DIC) is an antispasmodic agent and is frequently used to relieve spasms of gastrointestinal tract (Fig.3).[3] In IUPAC system it is named as 2-(diethylamino)ethyl-1-cyclohexylcyclohexane carboxylate.

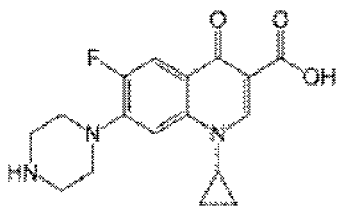

***Fig.1 :** Structure of Ciprofloxacin*

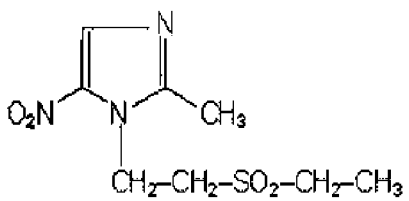

***Fig.2 :** Structure of Tinidazole*

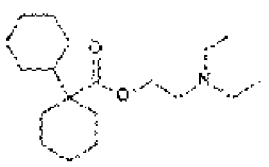

***Fig.3 :** Structure of Dicyclomine*

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A thorough literature survey was carried out to review the analytical methods reported for assay of CPX, TNZ and DIC alone and in their fixed dose combinations with other drugs. The survey revealed that CPX and TNZ have been analyzed using analytical tools such as UV spectrophotometry, RP-UPLC, RP-HPLC and differential pulse polarography.[4-9] DIC has been estimated in its combined dosage forms by UV spectrophotometry, RP-HPLC and HPTLC.[10-13] The fixed dose combination of CPX, TNZ and DIC has recently been analyzed by UV spectrophotometry.[14,15] But the analysis of CPX, TNZ and DIC in its fixed dose combination in bulk and tablet dosage form has not been carried out yet by RP-HPLC. The present work was therefore aimed at developing a simple and reliable method for quantitative estimation of CPX, TNZ and DIC in bulk and their combined tablet dosage form by RP-HPLC.

MATERIALS AND METHODS

The preparation of mobile phase was done using HPLC grade methanol and water. CPX, TNZ and DIC were obtained as gift samples from Hindustan Laboratories Pvt. Ltd. Palghar. Shimadzu digital balance (model AUY 200) was used for weighing the samples. The pH of the buffer was adjusted using Eutech digital pH meter. A Thermo Finnigan Isocratic HPLC system fitted with a HiQ Sil C_{18} column (250 x 4.6 mm, i.d., 5 µm particle size) and coupled to a UV detector was used for chromatographic separation. The mobile phase consisted of buffer pH 4.0 and methanol (60:40v/v). All chromatographic runs were carried out in isocratic elution mode with a flow rate of 1 ml/min and detection wavelength of 218 nm. The sample injection volume was kept fixed at 20 µl. A run time of 15 minutes was found to be suitable for achieving separation. Marketed tablet formulation Gastrogyl plus® containing CPX (250mg), TNZ (600 mg) and DIC (10 mg) was purchased from local pharmacy. For preparation of buffer solution, 2.5 ml of Triethylamine was transferred to a 500 ml volumetric flask, volume was made up with HPLC grade water and pH was adjusted to 4.0 with orthophosphoric acid. The buffer solution was filtered through 0.45 µm membrane filter and degassed before use. The buffer solution and methanol were pre-mixed together in 60:40 proportions and employed as mobile phase. The pre-mixed mobile phase was also used as diluent for preparing drug solutions.

Standard addition method was followed for estimation of DIC to improve its absorbance and peak area. An accurately weighed quantity of 325 mg of DIC was transferred to 50 ml volumetric flask. After adding 20 ml of diluent, the solution was sonicated to dissolve the drug. Volume was made up with diluent and mixed well. This gave a solution having concentration of DIC as 6500 µg/ml.

Accurately weighed quantity of CPX and TNZ (25 mg) and DIC (10 mg) was transferred to 25 ml volumetric flask separately. After dissolving the drug in about 15 ml of diluent by sonication, volume was made up with same diluent (stock 1). From this solution, 5 ml of each solution was pipetted out and transferred to 100 ml volumetric flask separately, volume was made up with diluent and mixed well (stock 2). Further dilutions of stock 2 solution was made to obtain 20 µg/ml solution of CPX and TNZ and 8 µg/ml solution of DIC separately. For estimation of DIC, standard addition method was followed by adding 1 ml of DIC solution having concentration 6500 µg/ml to the working standard solution of DIC. The concentration of DIC in the resulting solution was 650.8 µg/ml.

The sample solution for analysis was prepared by accurately weighing twenty tablets of Gastrogyl plus® (manufactured by Biological E. Ltd. Hyderabad) and grinding to a fine powder. A quantity of tablet powder equivalent to 25 mg of CPX, 60 mg of TNZ and 10 mg of DIC was weighed accurately and transferred to 25 ml volumetric flask. After adding 15 ml of diluent, the solution was sonicated for complete extraction of all three drugs. The volume was made up with same diluent and solution was filtered through 0.2 µm membrane filter. This solution was diluted further by transferring to 100 ml volumetric flask and making up the volume with same diluent. From this solution, 4 ml was pipetted out and transferred to 10 ml volumetric flask. After adding 1 ml of DIC solution having concentration 6500 µg/ml, the volume was made up with diluent. The resulting solution was injected in to HPLC system under the developed chromatographic conditions. The procedure for sample preparation was carried out six times by weighing a separate quantity of tablet powder each time.

RESULTS

The RP-HPLC method was initially developed using different mobile phases and chromatographic conditions to achieve separation of all three drugs with proper resolution. The conditions found to be optimal for achieving proper separation is shown in Table 1. The use of triethylamine was necessary to obtain good peak shapes with proper symmetry and to reduce tailing. The pH of the buffer was kept in the acidic range for separation of CPX. The wavelength of the detector was set at 218 nm as it was found optimum for separation of DIC. At this wavelength, proper separation of CPX and TNZ was also achieved. The standard solutions of the individual drugs were injected followed by injection of mixed standard solution of all three drugs five times. Using these conditions, the retention time for CPX, TNZ and DIC were obtained as 8.04 min, 5.42 min and 6.96 min. The diluent comprising of mobile phase was injected in to HPLC system and the chromatogram did not show any peaks at the retention time of CPX, TNZ

Table 1: *Optimum conditions for separation of CPX, TNZ and DIC by RP-HPLC*

Mobile Phase	Buffer pH 4.0: Methanol (60:40 v/v)
Stationary Phase	HiQ Sil C_{18} column (250 x 4.6 mm, i.d., 5 µm particle size)
Flow Rate	1 ml/min
Column Temperature	26 ° C
Injection volume	20 µl
Detection wavelength	218 nm
Run time	15 min
Retention time of CPX, TNZ and DIC	8.04 min, 5.642 min and 6.96 min

and DIC. The excipients used in the formulation did not interfere in the separation indicating the specificity of the proposed method for simultaneous estimation of CPX, TNZ and DIC (Figs. 4-9).

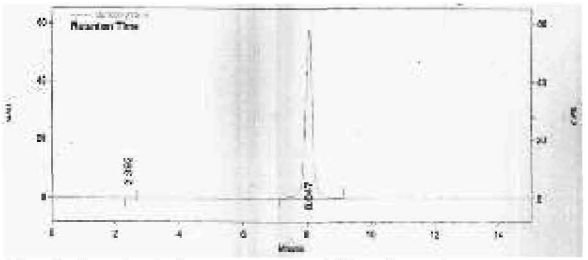

Fig. 4: *Standard chromatogram of Ciprofloxacin*

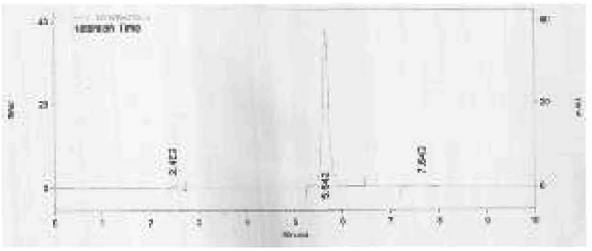

Fig. 5: *Standard chromatogram of Tinidazole*

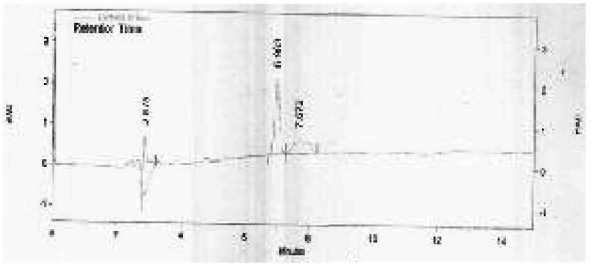

Fig. 6: *Standard chromatogram of Dicyclomine*

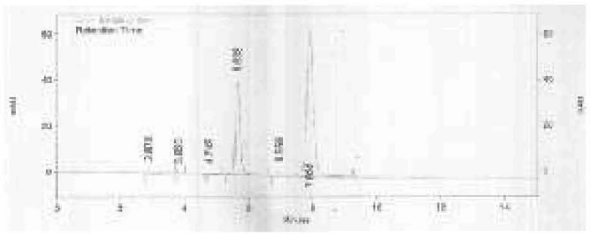

Fig. 7: *Chromatogram of mixed standard solution of CPX, TNZ and DIC*

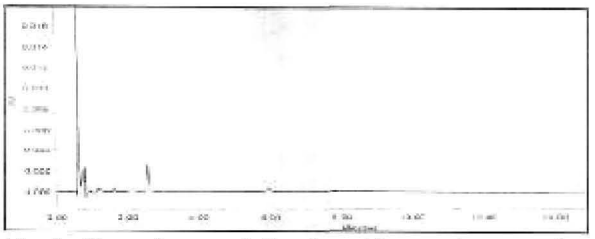

Fig. 8: *Chromatogram of diluent used for sample preparation*

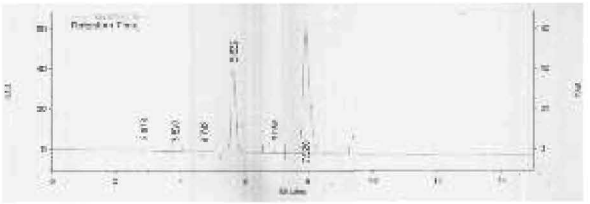

Fig. 9: *Chromatogram of sample solution of CPX, TNZ and DIC from tablet dosage form*

The analytical performance of the developed RP-HPLC method was assessed by carrying out its validation for parameters described in ICH guidelines.[16,17] For evaluation of linearity, standard solutions of CPX, TNZ and DIC were diluted to obtain different concentration of each drug. CPX and TNZ showed linearity in range of 5-30 µg/ml and DIC obeyed Beer's law in range of 250-650 µg/ml for DIC (Figs. 10-12). The correlation coefficient was found to be greater than 0.99 for all three drugs.

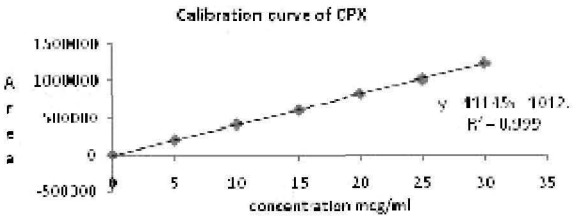

Fig. 10: *Calibration curve of CPX*

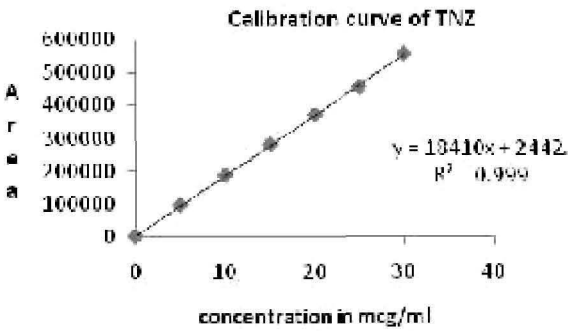

Fig. 11: *Calibration curve of TNZ*

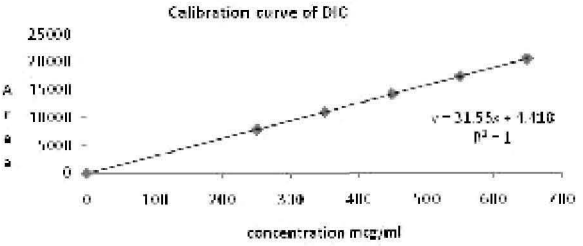

Fig. 12: *Calibration curve of DIC*

Recovery experiments were carried out to determine accuracy of method. The solutions employed for carrying out recovery studies had concentrations of CPX and TNZ as 16 µg/ml (80 %), 20 µg/ml (100 %) and 24 µg/ml (120 %). For DIC, the solutions employed had concentration 6.4 µg/ml, 8 µg/ml and 9.6 µg/ml at level of

80 %, 100 % and 120 %. The results are shown in table 2. Percent recovery value obtained was found to be 100.11 % for CPX, 102.86 % for TNZ and 98.90 % for DIC. The precision studies were performed by analyzing repeatability (intraday) and intermediate precision (inter-day). For this, six replicated injections of the sample solutions were made and % RSD was calculated. The results of precision studies are summarized in table 3. RSD(%) values for all three drugs were found to be less than 2 % and within the acceptance limit. This indicated the high precision of developed RP-HPLC method.

Table 2: *Accuracy study*

Recovery level	Mean Percent recovery*			S.D.*			% RSD*		
	CPX	TNZ	DIC	CPX	TNZ	DIC	CPX	TNZ	DIC
80 %	99.92	102.72	99.09	0.01	0.005	0.497	0.01	0.005	0.5
100 %	100.11	102.86	98.90	0.138	0.015	0.05	0.138	0.014	0.05
120 %	100.17	102.99	98.72	0.005	0	0.03	0.005	0	0.03

Table 3: *Method precision*

Injection No.	Intraday Precision Peak Area			Inter-day Precision Peak Area		
	CPX	TNZ	DIC	CPX	TNZ	DIC
1	823510	381210	20540	823510	381210	20540
2	823545	381225	20546	823488	381215	20488
3	823495	381278	20560	823475	381145	20475
4	823499	381199	20551	823460	381177	20499
5	823450	381145	20475	823499	381146	20465
6	823475	381175	20557	823451	381188	20525
Average	823480.5	381180.2	20498.67	823480.5	381180.2	20498.67
SD	22.75742	30.26164	29.01494	22.7575	30.26164	29.01494
% RSD	0.002	0.007	0.141	0.003	0.007	0.141

The LOD and LOQ for CPX, TNZ and DIC were determined by calculating the signal to noise ratio (s/n is 3.3 for LOD and 10 for LOQ). From the calibration curve the standard deviation of Y-intercepts and slope of regression line were used. LOD values were found to be 0.00080 µg/ml (CPX), 0.00328 µg/ml (TNZ) and 0.00157 µg/ml (DIC) and LOQ values for CPX, TNZ and DIC were found to be 0.00244 µg/ml, 0.00996 µg/ml and 0.00478 µg/ml. To ascertain the robustness of the method, small but deliberate changes in the optimized chromatographic conditions like mobile phase pH, volume of mobile phase, flow rate, and detection wavelength was done. The system suitability parameters were studied with six replicates of mixed standard solution of all three drugs and the results are tabulated in table 4.

Table 4: *System Suitability Parameters*

System suitability parameters	Observation		
	CPX	TNZ	DIC
Retention time (min)	7.920	5.632	6.955
Theoretical plates	7546	8569	10122
USP tailing	1.107	1.175	0
Area	823570	381210	20560
Resolution	3.240	12.94	5.250
Range (µg/ml)	5-30	5-30	250-650
Correlation coefficient	0.9998	0.9997	1
LOD	0.00080	0.00328	0.00157
LOQ	0.00244	0.00996	0.00478

DISCUSSION

The estimation of CPX, TNZ and DIC as reported in literature by UV spectrophotometry involves tedious calculations. Hence the aim of this study was to develop and validate a novel RP-HPLC method according to ICH guidelines for the analysis of all three drugs in tablet dosage form. A precise, sensitive and specific isocratic HPLC method was developed for assay of CPX, TNZ and DIC using a C-18 column with shorter run times, good peak resolution and acceptable tailing factor. The method was also found to be quite robust with no significant changes in retention times, peak shape and resolution with small changes in operating conditions. % RSD for validation parameters such as accuracy and precision were within acceptable limits of ± 2.0 % RSD. This indicates that the proposed method is highly precise and accurate. The method suitability was assessed by preparing sample solution of marketed formulation Gastrogyl plus® containing all three drugs in fixed dose. The percent recovery value obtained was 99.28%, 98.37% and 97.24% for CPX, TNZ and DIC. The values obtained for the assay of three drugs in marketed formulation was found to be in accordance with label claim. This confirmed that the method is quite suitable for analysis of CPX, TNZ and DIC in combined tablet dosage form.

CONCLUSION

The proposed RP-HPLC method for simultaneous estimation of ciprofloxacin, tinidazole and dicyclomine in bulk and in fixed dose combination enabled simple and rapid separation and quantization of all three drugs in a single run. The resolution for the drugs was found to be adequate with an analysis time of not more than 15 mins per injection. Moreover, the method was found to conform to the acceptance criteria of validation parameters as per ICH guidelines. Thus the method can be applied for routine quality control of ciprofloxacin, tinidazole and dicyclomine in combined dosage form.

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