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Method Development And Validation For The Simultaneous Estimation Of Ciprofloxacin And Tinidazole In Tablet Dosage Form By IN – VITRO Dissolution Profile And RP-HPLC

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ABSTRACT

To quantify ciprofloxacin and tinidazole together at the same time, a straightforward reverse phase liquid chromatographic method has been created and proven to work. Simultaneous ciprofloxacin estimation was another study focus. And tinidazole utilising the dissolution profile obtained in vitro.The study's findings demonstrated the simplicity, speed, precision, and accuracy of the suggested RP-HPLC method, making it a valuable tool for routinely determining the bulk drug concentrations of ciprofloxacin & tinidazole as well as their pharmaceutical dosage forms.

Keywords: *Ciprofloxacin, tinidazole, RP-HPLC, in-vitro dissolution profile.*

1. INTRODUCTION

Simple, affordable, repeatable, and appropriate analytical techniques are needed for the simultaneous determination of pharmaceutical multicomponents and routine quality control.[1] High-performance liquid chromatography (HPLC), which offers better separation and selectivity, is used in pharmaceutical analysis.. However, limitations include the need for specialized knowledge, expensive equipment prices, time-consuming procedures, and solvent use that restrict its accessibility. As a practical and environmentally friendly alternative to HPLC, UV spectrophotometry helps to overcome these issues.[2, 3] Although a large number of medications show appropriate UV light absorption, direct measurement can be difficult due to some drugs' overlapping spectra.[4]Together, spectrophotometric and chromatographic methods yield new hyphenated approaches that are helpful for impurity profiling and simultaneous estimation.[5] The determination of the compounds in the pharmaceutical formulation is made certain and particular by the simultaneous analytical examination.[6]

The market offers combined tablet dosages of ciprofloxacin and tinidazole, which are becoming more and more popular for treating bacterial, protozoal, and diarrhea, infections (figure 1). [7]

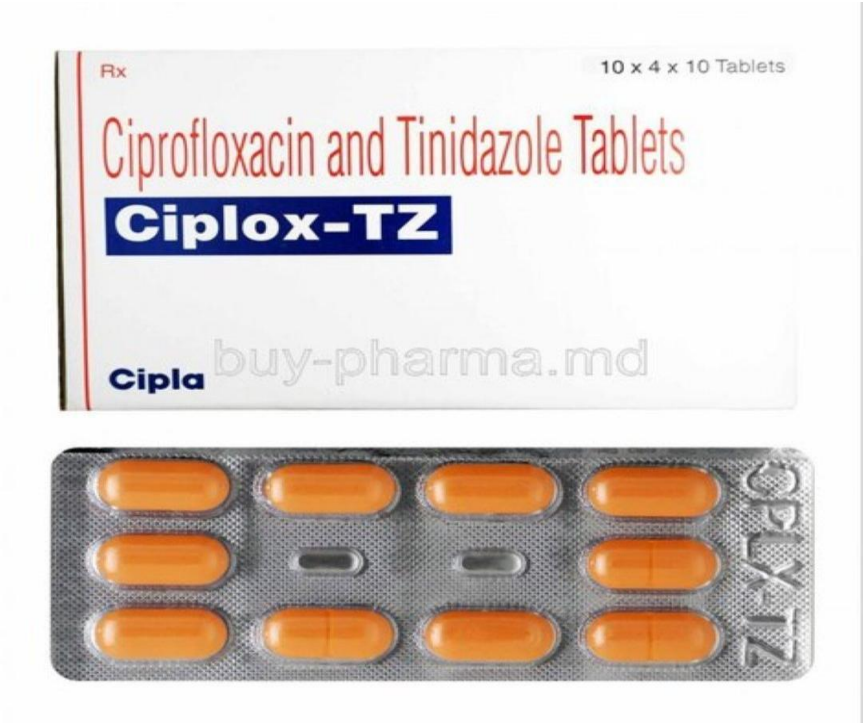

Figure 1: Combined tablet dosages of ciprofloxacin and tinidazole [8]

Tinidazole with ciprofloxacin It is used to treatparasite and bacterial infections. A combination of the antibiotics ciprofloxacin and tinidazole is known as ciprofloxacin + tinidazole. By stopping bacterial cells from proliferating and mending, ciprofloxacin kills the germs. By causing damage to their DNA, tinidazole eliminates anaerobic bacteria and parasites that cause illnesses. [9]

Two straightforward, precise, and repeatable spectrophotometric techniques for measuring ciprofloxacin and tinidazole simultaneously in tablet dose form are presented in this study. Since there is currently no published technique for estimating the combined dosage form of ciprofloxacin and tinidazole using HPLC, we set out to create an easy-to-use, precise, and reasonably priced analytical method.In this work, A description is given of a validated RP-HPLC for the simultaneous measurement of trimethoprim and ciprofloxacin in combination.

The method involves utilizing a 70:30v/v ratio of ortho phosphoric acid-adjusted pH 3.5 acetonitrile and 2 mm phosphate buffer. Phenomenex C18 was the column that was used. It used PDA identification at 293 nm and had a flow rate of 1 ml per minute. This study also provided an explanation for the in vitro dissolution profile-based simultaneous estimate of ciprofloxacin and tinidazole.

Ciprofloxacin, also known as 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)- quinoline-3-carboxylic acid, is a fluoroquinolone belonging to the second generation (figure 2).. A search of the literature yields a number of techniques for estimating the concentration of ciprofloxacin both alone and in combination using UV, high performance liquid chromatography, and in vitro dissolution profiles.[10]

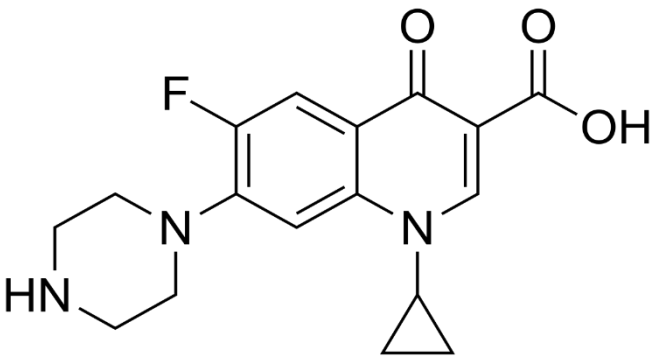

Figure 2: Chemical Structure of 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid [11]

The free nitro radical created by this reduction is assumed to be the source of the antiprotozoal action.

These harmful free radicals bond to DNA covalently, causing damage to the molecule and ultimately cell death. Although it is unknown, tinidazole's action against the Giardia and Entamoeba species is most likely comparable. A literature search reveals a number of techniques for measuring tinidazole alone and in combination using UV, in vitro dissolution profile, and high performance liquid chromatography. [12]

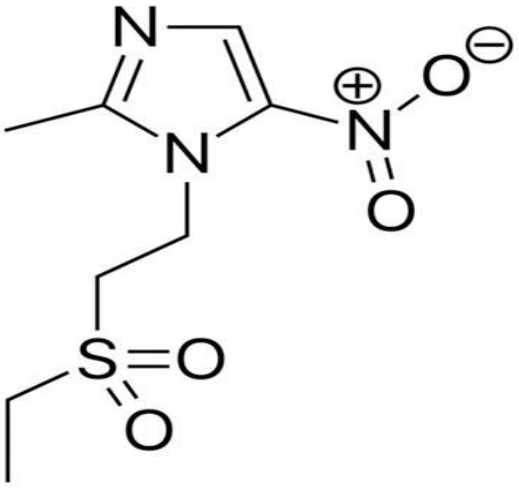

Figure 3: Chemical structure of 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole[13]

2. EXPERIMENTAL

Chemicals, reagents and Instrumental:

Operating guidelines fortinidazole and ciprofloxacin were acquired from India's Alkem Laboratories. AR for orthophosphoric acid grade was bought at a nearby market. HPLC standard. We bought acetonitrile, methanol, and water as solvents from the market.

A pharmaceutical dosage form named Alcipro-TN 500 mg/600 mg (Cipla) was sold on a tablet that was purchased from a nearby pharmacy. It comprised 600 mg of tinidazole and 500 mg of ciprofloxacin.

Instrumentation:

A reverse phase Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) was used for the analysis, and a Shimadzu UV-1800 double-beam ultraviolet visible spectrophotometer was used to quantify absorbance.

Standard preparation:

A 25 millilitre volumetric flask was filled with precisely 30 milligrammes of tinidazole and 25 milligrammes of CIP. It was sonicated for two minutes after 20 mL of diluents were added, and then the mobile phase was used to boost the volume to 25 mL. Then,
In accordance with the formulation composition, the mixture was diluted and filtered through a 0.45 µ membrane filter to produce the stock solution, which included 1000 µg of CIP and 1200 µg of tinidazole per millilitre.

Calibration plotpreparation:

The mobile phase has been used to create standard stock solutions with 25–125 µg/mL of CIP and 30–150 µg/mL of TNI. After sonication, the solutions were put into a sequence of volumetric flasks that held them after being passed through 0.45 µ membranes aliquots of 10 ml apiece for every component. Following the previously mentioned chromatographic conditions, each solution was injected three times. Plotting standard peak area ratios against the respective medication concentrations produced linear connections.

Preparation of sample:

We utilized and powered twenty commercial formulation tablets (AlciproTN) containing ciprofloxacin and tinidazole. A stock solution containing 1,000 and 1,200 µg/mL of CIP and TINI, respectively, was created by sonicating the mixture for 30 minutes after dissolving the powder equivalent to ten milligrams and twelve milligrams of each in 10 ml of diluent.

Whatman filter paper number four was used to filter this mixture. In order to conduct the analysis, 0.5 mL of the filtrate was extracted and subsequently diluted using diluent containing 50 and 60 µg/mL of tinidazole and ciprofloxacin, respectively, up to 10 ml.

Method validation:

Accuracy and precision:

The usual addition method was used in recovery tests to assess the approach's accuracy. Three injections of the solutions were made, and the percentage of recovery was computed.[14]

Linearity:

The method's linearity was assessed by examining various drug concentrations.

The International Conference on Harmonization (ICH) mandates the use of a minimum of five concentrations. Five concentrations of ciprofloxacin and tinidazole, ranging from 25 to 125 and 30 to 150 µg/mL, respectively, were selected for the current investigation

Specificity:

By determining if excipients found in pharmaceutical formulations affected the analysis, the specificity of the approach was assessed. [15]To create a placebo, the excipients that were used in every tablet were combined, and solutions were made by following the steps outlined in the sample preparation section.

Robustness:

The robustness of analytical techniques is a measure of their capacity to remain unaffected by deliberate but small changes in the working environment. This was explored by adjusting the pH of the mobile phase via 0.2, the mobile phase's buffer content by 2%, and the wavelengths of the detectors by 2 nm.

LOD and LOQ:

The standard deviation of the response and the slope of the calibration graphs were used to calculate the detection and quantification limits in compliance with ICH guidelines. [(regression slope)/(standard deviation of repeatability)] The LOD and LOQ values were calculated by dividing by 10 and 3.3, respectively, using equation).

Statistical analysis:

The results were described using mean ± SD, percentage RSD, and, if appropriate, a statistical analysis of the data using the t-test with Microsoft Excel 2007; data were deemed substantially different at the 5% significance level of probability ($p < 0.05$).

In vitro dissolution studies:

The USP Dissolution Testing Apparatus #2 (Paddle Method) was used for the procedure. To perform the dissolution test, 900 millilitres of 0.1 N HCl were pounded for two hours at 37 ± 0.5 °C at 50 rpm. Ten millilitres of the resultant solution are sampled every hour. were removed from the dissolving apparatus and swapped out with fresh dissolving media. Following their passage through a 0.45 µm membrane filter, the samples were diluted.

3. RESULTS

Linearity:

The calibration curves were generated by graphing the substance's peak regions against concentration; the ranges for CIP and tinidazole, respectively, had been linear or range between 25 to 125 and 30 to 150 µg/mL.Using a minimum of squares linear regression approach in the concentration levels and peak area ratios, correlation coefficients and the equation's calibration were ascertained. The average regression formulas for tinidazole and ciprofloxacin were determined to be $y = 10534x + 14849$ and $r^2 = 0.9990$, respectively, and $y = 14562x + 21425$, respectively. The linearity equation, $y = an\ x + b$, states that "y" represents the maximum area ratio of medicines, "a" denotes the slope, "b" denotes the intercept, and "x" represents the measured solution's concentration in g/mL.The outcome demonstrates that the peak ratio of area & medication concentration within the measured range have a strong association.

LOD & LOQ:

Tinidazole and ciprofloxacin had respective LODs of 0.9425 and 0.0427 µg/mL.

The quantification limits for ciprofloxacin and tinidazole were found to be 0.2111 µg/mL and 2.921 µg/mL, respectively.

Accuracy and precision:

Intraday accuracy was measured using the relative standard deviations of five successive assays for each sample at the three

concentration levels. The interday precision was obtained by analysing samples from five different days. The RSD demonstrated a high degree of precision. levels, which were found to be 0.211–1.726 percent for CIP and 0.390–1.452 percent for tinidazole, in that order.

Statistics:

Tinidazole and ciprofloxacin had respective probability (P) values of 0.216 and 0.211 at the 5% significance level. There was no discernible difference between the precision values obtained over two consecutive days, as indicated by the p value of > 0.05.

Recovery:

Recovery studies utilising the traditional addition method were conducted to assess the procedure's accuracy. With average percent recoveries ranging from 98.17 to 99.97, the method's great accuracy is demonstrated.

Specificity:

The full separation between ciprofloxain and tinidazole, as illustrated in Figure 4 and 5, was used to calculate the specificity along with metrics like tailing factor (T), resolution (Rs), and retention time (Rt).The obtained peaks demonstrated the absence of blank & placebo interferences from the primary peaks.

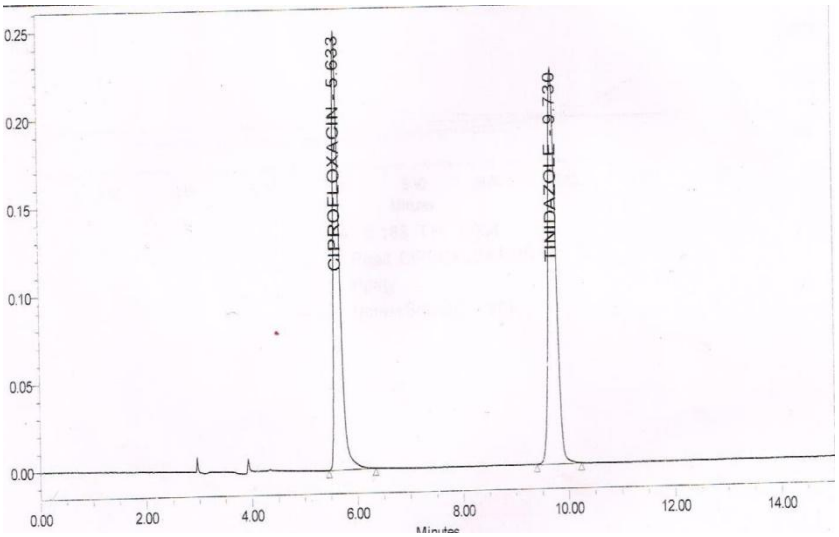

Figure 4: HPLC chromatogram of pure ciprofloxacin and tinidazole

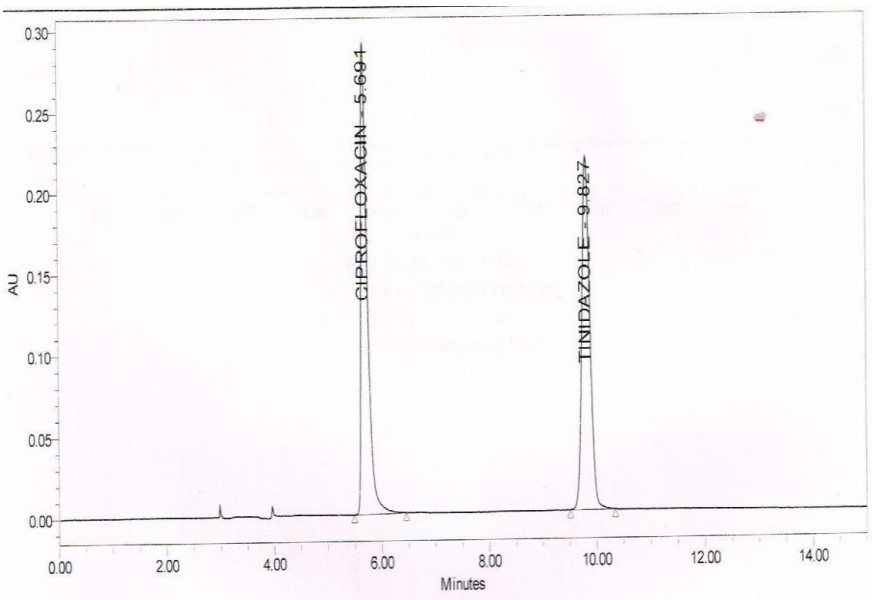

Fig. 5: HPLC chromatogram for tablet formulation

Robustness:

It is crucial to show the robustness of the HPLC method in order to guarantee that it is insensitive to even the smallest modifications in the experimental conditions.

Assay:

The assay results of (Alcipro-TN) were displayed in Table 1.

Table 1: Results of assay

Drug	Claimed (mg)	Found ± SD (mg)
Ciprofloxacin	500	496.31±0.12
Tinidazole	600	598.32±0.15

In vitro dissolution studies:

A double-beam Ultraviolet visible spectrophotometer was used to measure the absorption of these solutions at the λmax of both medicines in that media [Figure 3]. After two hours, the medium used for dissolution was swapped out for phosphate buffer pH 6.8, the process was repeated for three hours, and finally, dissolution was completed in the pH 7.4 phosphate buffer [Table 2].

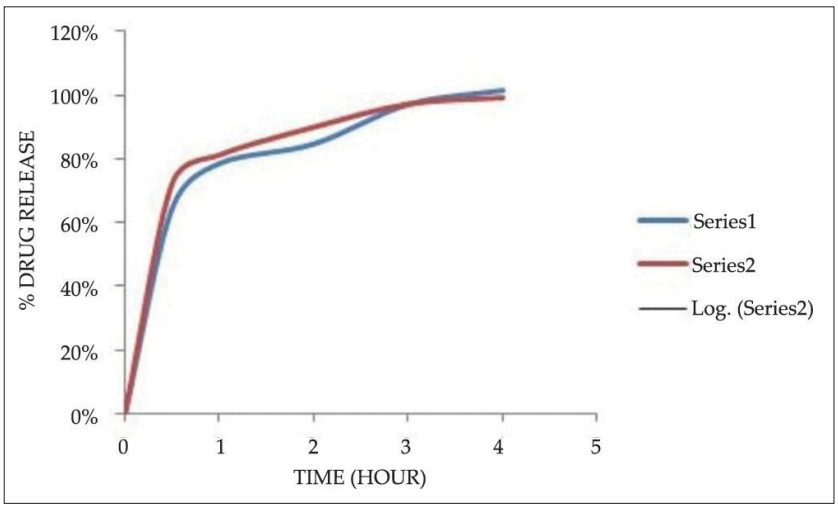

Figure 6: Dissolution profile of the marketed preparation

Table 2: Dissolution profile

Time (hr)	Percentage of CIP release	Percentage of TINI release
0	0	0
0.5	60	69
01	75	78
02	80	85
03	94	96
04	101	99

4. DISCUSSION

The outcomes show how accurate the suggested approach is: Tinidazole and CIP recovery rates were 101.44 and 99.94%, respectively; both medications' regression coefficient values of 0.999 show a linear response; the repeatability of the procedure and The resolution between the two peaks is consistently greater than two; The lowest values of LOD and LOQ obtained from the recommended method demonstrate the sensitivity of the procedure; the resolution between the two peaks is consistently greater than two; It was found that intermediate precision values fell within reasonable bounds; Both drugs remained stable throughout the day, per the results of the solution stability testing; Variations in temperature, flow rate, and mobile phase composition had no discernible effects on the results. It was discovered that the suggested technique for routinely simultaneous measurement of ofloxacin and ornidazole in various dissolving media was straightforward, accurate, and repeatable. Since there is a more than 20 nm difference in the λmax of these two medications, the simultaneous equation method was attempted for their simultaneous determination in formulation in separate media. This approach is particularly helpful to investigate the release tendency of a combination of both medications because there is currently no way for determining the dosage forms of ornidazole and ofloxacin when conducting a dissolution study.

5. CONCLUSION

The precision, repeatability, and accuracy of the developed method were confirmed. A strong linear correlation was noted between tinidazole and CIP. For the simultaneous detection of tinidazole and ciprofloxacin, either independently or in combination, a simple, fast, and precise HPLC method has been established.. The technique offers a number of benefits, such as enhanced sensitivity, quick analysis, an easy-to-use mobile phase, and straightforward sample preparation. Unlike earlier techniques, it is appropriate for the analysis of these medications in their binary compositions in one isocratic run. It was discovered that the suggested technique for routinely simultaneous measurement of ciprofloxacin and tinidazole in various dissolving media was straightforward, accurate, and repeatable.

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