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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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The simultaneous estimation of ciprofloxacin and tinidazole in combination has been achieved through the development and validation of a straightforward reverse phase liquid chromatographic method. This study also focused on the simultaneous estimation of ciprofloxacin and tinidazole by using in vitro dissolution profile. 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] Pharmaceutical analysis uses high-performance liquid chromatography (HPLC), which provides superior selectivity and separation. 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]

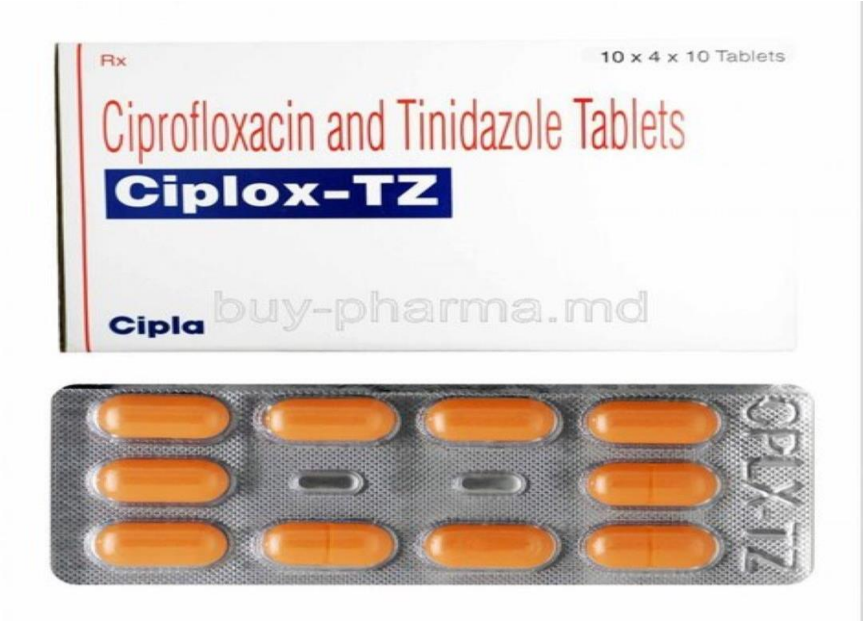

Figure 1: Combined tablet dosages of ciprofloxacin and tinidazole [8]

Tinidazole with ciprofloxacin is used to treat parasite 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, validated RP-HPLC for the simultaneous measurement of ciprofloxacin and trimethoprim in combination is described.

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 had a flow rate of 1 ml per minute and employed PDA identification at 293 nm. This study also explained the simultaneous estimation of ciprofloxacin and tinidazole by in vitro dissolution profile.

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]

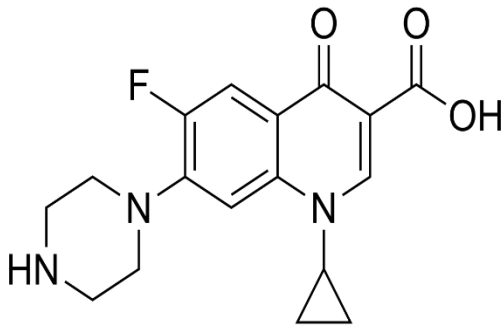

Figure 2: Chemical Structure of 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid [11]

This reduction produces a free nitro radical, which is thought to be the cause of the antiprotozoal activity.

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]

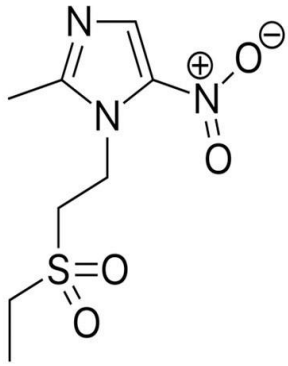

Figure 3: Chemical structure of 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole [13]

2. EXPERIMENTAL

Chemicals, reagents and Instrumental

Operating guidelines for tinidazole 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.

Tablet obtained from a local drug store sold a pharmaceutical dosage form called Alcipro-TN 500 mg/600 mg (Cipla), which contained 500 mg of ciprofloxacin and 600 mg of tinidazole.

Instrumentation

The analysis was conducted using a reverse phase Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) HPLC & absorbance measurement by Shimadzu UV-1800 double-beam ultraviolet visible spectrophotometer.

Standard preparation

A precise amount of 30 mg of tinidazole and 25 milligrams of CIP were added to a 25-milliliter volumetric flask. After adding 20 mL of diluents, it was sonicated for two minutes, and then the volume (25 mL) was increased using the mobile phase. After that, the mixture was diluted and filtrated via a 0.45 µ membrane filter to create the stock solution, which contained 1200 µg of tinidazole and 1000 µg of CIP per milliliter, in line in the formulation composition.

Calibration plot preparation

Standard stock solutions containing 25–125 µg/mL of CIP and 30–150 µg/mL of TINI have been produced using the mobile phase. Following sonication, the solutions were passed by 0.45 µ membranes and placed into a series of volumetric flasks holding 10 ml aliquots for each compound. 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 ability of analytical methods to stay unaffected by slight but intentional variations in the operating environment is measured by their robustness. This was investigated by adjusting the pH of the mobile phase through 0.2, the mobile phase's buffer content by 2%, and the detector wavelengths by 2 nm.

LOD and LOQ

The approach based on the response's standard deviation & the slope of the calibration graphs was utilized to ascertain the detection and quantification limits in compliance with ICH guidelines. [(SD of repeatability)/(slope associated with the regression equation)] was used to estimate the LOD and LOQ values. simply dividing by 3.3 and 10, in that order.

Statistical analysis

Results were reported as Mean ± SD, % RSD, and, when appropriate, data were statistically evaluated using the t-test with the use of Microsoft Excel 2007; data were deemed substantially different at the 5% significance level of probability ($p < 0.05$).

In vitro dissolution studies

It was carried out using USP Dissolution Testing Apparatus #2 (Paddle Method). 900 milliliters of 0.1 N HCl were pounded at 50 rpm for two hours at 37 ± 0.5 °C in order to conduct the dissolution test. Every hour, a sample ten millilitres of the resulting solution were obtained out of the dissolving equipment and replaced with brand-new dissolving media. The samples were diluted after being filtered through a 0.45 µm membrane filter.

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

The LOD for tinidazole and ciprofloxacin was 0.9425 and 0.0427 µg/mL, respectively. The limits of quantifying for tinidazole and ciprofloxacin was determined to be 2.921 µg/mL and 0.2111 µg/mL, respectively.

Accuracy and precision

Relative standard deviations of 5 consecutive assays for each sample at the 3 levels of concentration was used to measure intraday precision. The analysis of five distinct days' worth of samples yielded the interday precision. Good precision was shown by the RSD values, which were determined to be 0.211 – 1.726 % for CIP and 0.390 – 1.452 % for tinidazole, respectively.

Statistics

At the 5% significance level, the probability (P) values for ciprofloxacin and tinidazole were 0.211 and 0.216, respectively. The p value were > 0.05, indicating that no significant disparity between the carried out precision results out for two days in a row.

Recovery

Recovery studies using the standard addition method were conducted in order to assess the accuracy of the approach. The method's good accuracy is indicated by the average percent recoveries obtained, which range from 98.17 to 99.97

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 & placeb o interferences from the primary peaks.

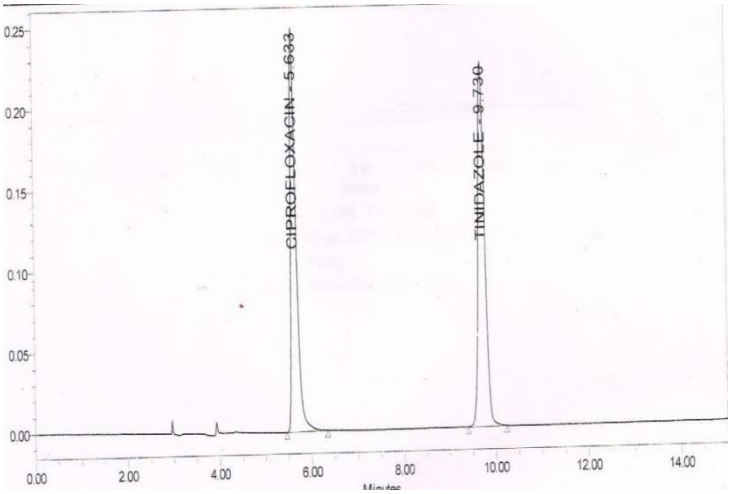

Figure 4: HPLC chromatogram of pure ciprofloxacin and tinidazole

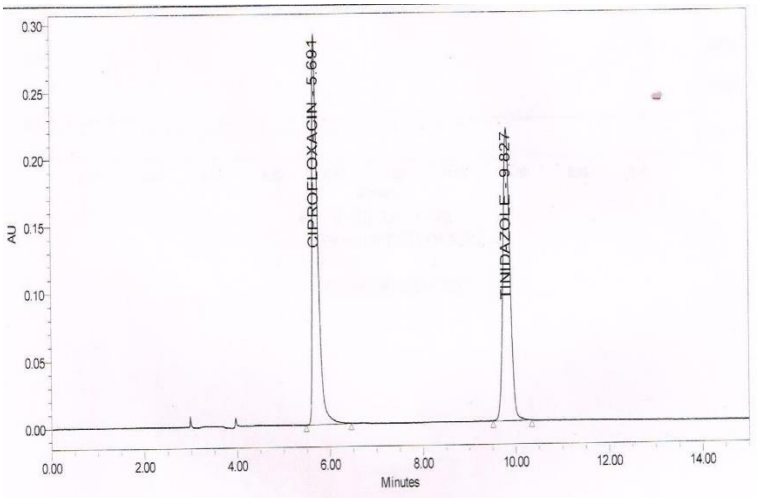

Fig. 5: HPLC chromatogram for tablet formulation

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].

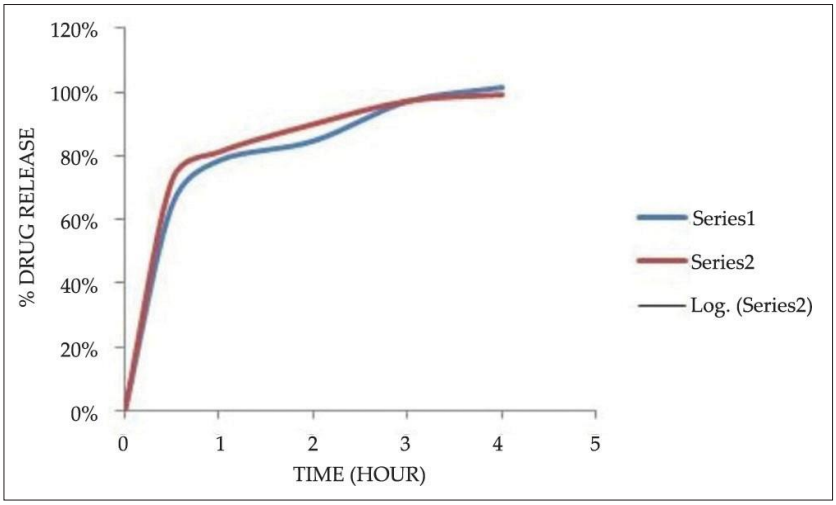

Figure 6: Dissolution profile of the marketed preparation

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 results demonstrate the accuracy of the proposed method: recovery of a drug called CIP and tinidazole was 101.44 and 99.94%, respectively; both drugs' regression coefficient values of 0.999 indicate a linear response; the method's repeatability and intermediate

precision values were found to be within acceptable bounds; the resolution between the two peaks is always greater than two; the lowest values of LOD and LOQ obtained from the proposed method demonstrate the method's sensitivity; the solution stability studies show that both drugs were stable for a full day; changes in flow rate, temperature, and mobile phase composition did not significantly alter 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. A straightforward, quick, and accurate HPLC technique has been developed for the simultaneous measurement of tinidazole and ciprofloxacin, either separately or in combination. 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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