International Journal of Chemistry and Pharmaceutical Sciences

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# Method Development and Validation for Simultaneous Estimation of Ciprofloxacin and Tinidazole in Bulk and Pharmaceutical Dosage Forms

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**A B S T R A C T**

A simple, rapid, sensitive reverse-phase high-performance liquid chromatography method was developed and validated for simultaneous estimation of ciprofloxacin and tinidazole, at single wavelength of 346nm.chromotographic separation was performed on an enable agilent zorabax (thermo) column (250nmx4.6mm ID particle size 5 um) and a mobile phase consisting of buffer and methanol(70:30v/v) at a flow rate of 1.0 ml/min. The calibration curve was linear($r^2$≥0.0999) over the concentration range 0.040-0.064 µg/ml.the limit of quantification was 9.737 µg/ml for ciprofloxacin and 9.36 µg/ml for tinidazole no interference was found by the excipients in the synthetic mixture. The proposed methods were validated for international conference on harmonization guidelines for linearity, accuracy, precision, and robustness for estimation of ciprofloxacin and tinidazole in bulk and synthetic mixture, and the results were found to be satisfactory.

**Keywords:** ciprofloxacin, tinidazole, RP-HPLC Validation.

**A R T I C L E I N F O**

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**Article History: Received** 14 January 2016, **Accepted** 28 February 2016, **Available Online** 27 March 2016

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Manuscript ID: IJCPS2909

**PAPER-QR CODE**

**Citation:** C. Rambabu, *et al.* Method Development and Validation for Simulataneous Estimation of ciprofloxacin and Tinidazole in Bulk and Pharmaceutical Dosage Forms. *Int. J. Chem, Pharm, Sci.*, 2016, 4(3): 144-149.

## 1. Introduction

Ciprofloxacin [1] Figure.1, 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxilic acid, is a third generation fluoroquinolone antibacterial agent used in the treatment of various bacterial infections caused by gram-positive and gram-negative microorganisms. Tinidazole [2] Figure.2, [1-(2-ethylsulfonyethyl)-2-methyl-5- nitroinidazole] is a 5-nitroimidazole derivative, used against protozoan infections. It is also used in the treatment of a variety of amoebic and parasitic infection. These two drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin. Literature survey revealed that several papers [3-15] have been reported for have been reported for estimation of the above selected drugs in single or in combination forms. However, only one stability indicating method [16] has been yet reported for the simultaneous determination of ciprofloxacin and tinidazole in combined dosage form. In the present project an attempt have been made to develop a RP-HPLC method for assay of ciprofloxacin and tinidazole and in combined dosage form and was validated following ICH guidelines.

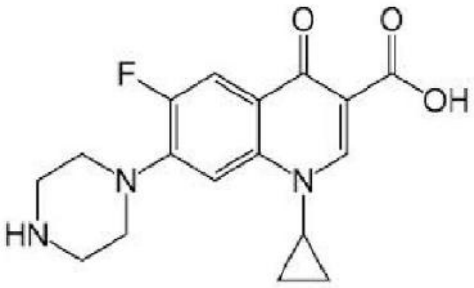


**Figure 1:** Structure of Ciprofloxacin

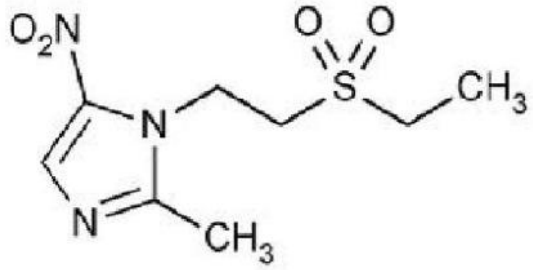


**Figure 2:** Structure of Tinidazole

## 2. Experimental

**a) Reagents & Materials:**

Ciprofloxacin and tinidazole standards (99.9% pure) was provided by Aarti Drugs Ltd., Boisar (India). Ciprofloxacin and tinidazole tablets containing 50 mg of ciprofloxacin and 20 mg of tinidazole together with inactive ingredients were obtained from local pharmacy. Milli-Q Water, Methanol (HPLC Grade) and Orthophosphoric acid (GR Grade) was obtained from Qualigens Ltd., Mumbai (India)

**b) Instrumentation:**

The chromatographic system used to perform development and validation of this assay method was comprised of a waters LC system equipped with 2695 pump and 2996 photodiode array detector connected to a multi-instrument data acquisition. A Reverse phase HPLC column [Aligent, Zorbax (Thermo); 250 mmx4.6 mm I.D; particle size 5 µm)] was used in the present assay. The output signal was monitored and integrated using Waters Empower 2 software.

**c) Mobile Phase Preparation:**

The mobile phase consisted of buffer and methanol in the ratio of 70:30 v/v respectively. The buffer solution used in the present assay was prepared by dissolving 1.0mL of ortho phosphoric acid in 1000 mL of Milli-Q Water. The mobile phase was filtered through a 0.45µm membrane filter (Millipore Pvt. Ltd. Bangalore, India) and degassed using an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai).

**d) Diluent Preparation:**

Mobile phase was used as a diluent in the present assay.

**e) Standard Preparation:**

Ciprofloxacin standard stock solution containing 1.0mg/mL was prepared in a 25mL volumetric flask by dissolving 25mg of ciprofloxacin and then diluted to volume with diluent. Further dilute this stock solution in 10mL volumetric flask and make up to mark with diluent (this standard solution of 1000-3000µg/mL). Tinidazole standard stock solution containing 1.0 mg/mL was prepared in a 25 mL volumetric flask by dissolving 25 mg of tinidazole and then diluted to volume with diluent. Further dilute this stock solution in 10mL volumetric flask and make up to mark with diluent (this standard solution of 1200-3600 µg/mL) and sonicated to dissolve make up to mark with diluents.

**g) Sample Preparation:**

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 mL volumetric flask. About 50 mL of diluent was added and sonicated for a minimum 30 minute with intermittent shaking. Then content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45 µm membrane filter. Further dilute aliquots of this stock solution in 50 ml of volumetric flask and make up to mark with diluent. The concentrations obtained were 1000-3000 for ciprofloxacin and 1200-3600 µg/mL of tinidazole.

**h) Chromatographic Conditions:**

Chromatographic analysis was performed on a HPLC column [Aligent, Zorbax (Thermo); 250 mmx4.6 mm I.D; particle size 5 µm))] at ambient temperature. The flow rate of the mobile phase was adjusted to 1.0mL/min and the injection volume was 10µL. Detection was performed by photodiode array detector at a wavelength of 283nm and the chromatographic runtime was 6 minutes for the analysis

## 3. Results and Discussion

**Development and optimization of the HPLC method:**

The analytical conditions for the proposed method were selected, basing on the chemical nature of ciprofloxacin and tinidazole. Initial spectroscopic analysis of compounds showed that ciprofloxacin and tinidazole showed a maximum UV absorbance ($\lambda_{max}$) at 340nm, 349nm respectively. Therefore, the chromatographic detection was performed at 346nm using a photo diode array detector as both the compounds showed good response at this wave length. The development trials of each component were

carried, by keeping them in various extreme conditions. The column selection has been done on the basis of back pressure, resolution, peak. Thirdly the selection of buffer based on chemical nature of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates and peak resolution between ciprofloxacin and tinidazole peak. After evaluating all these factors, Aligent, Zorbax (250 mm x 4.6 mm I.D; particle size 5 µm) was found to be suitable as it gave satisfactory results. The selection of buffer based on chemical nature of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates and peak shape of both components. Best results were obtained with 1.0% orthophospharic acid buffer for ciprofloxacin and tinidazole. Methanol was chosen as organic constituent of mobile phase, to reduce the longer retention time and to attain good peak shape. Preliminary trials using different composition of mobile phases consisting of buffer and methanol in the ratio of 500:500 v/v and 650:400 v/v, did not give good peak shape for ciprofloxacin and tinidazole.

Finally, the best separation and resolution of ciprofloxacin and tinidazole is achieved by fixing mobile phase composition consisting of a mixture of buffer and methanol in the ratio of 70:30 v/v achieved. Under these conditions ciprofloxacin and tinidazole were eluted at 4.91 and 3.72, minutes respectively with a run time of 6 min. Optimized mobile phase proportion provided good resolution between ciprofloxacin and tinidazole. The chromatogram for simultaneous estimation of ciprofloxacin and tinidazole standard by using the aforementioned mobile phase from 10µL of the proposed method is represented in Figure 3 and the results of the developed method are presented in Table1.

**Table 1:** System suitability parameters for ciprofloxacin and tinidazole by the proposed method

| Name of the Compound | Retention Time | Theoretical Plates | Tailing Factor | USP Resolution |
|---|---|---|---|---|
| Ciprofloxacin | 2.750 | 10227 | 2.151 | - |
| Tinidazole | 4.356 | 5591 | 1.467 | 9.180 |

**Table 2:** Method Precision (Inter and Intraday) Studies for Ciprofloxacin Andtinidazole by the Proposed Method

| Method Precision by Proposed Method | | |
|---|---|---|
| For ciprofloxacin | | For Tinidazole |
| Method Precision (Inter & Intra Day) | | Method Precision (Inter & Intra Day) |
| Set-1 | 4416852 | 5643562 |
| Set-2 | 4414994 | 5644336 |
| Set-3 | 4411891 | 5642624 |
| Set-4 | 4416117 | 5647978 |
| Set-5 | 4415773 | 5644366 |
| Set-6 | 4418450 | 5646346 |
| Over All Avg. | 4415680 | 5644869 |
| Over All Std Dev. | 214.23 | 1956.159 |
| Over All %RSD | 0.049 | 0.0346 |

**Method Validation:**

**a) System Suitability:**

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate, resolution and % RSD of peak area were determined for same. Acceptance criteria for system suitability, Asymmetry not more than 2.0, theoretical plate not less than 2000 for ciprofloxacin and 5000 for tinidazole and the % RSD of peak area not more then 2.0, were full fill during all validation parameter.

**Blank and Placebo Interference:**

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution Fig: 3 showed no peaks at the retention time of ciprofloxacin and tinidazole peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of ciprofloxacin and tinidazole and Ciprofloxacin tablets. Similarly Chromatogram of Placebo solution Fig: 4 showed no peaks at the retention time of ciprofloxacin and tinidazole peak revealing that the placebo used in sample preparation do not interfere in estimation of ciprofloxacin and tinidazole in formulations.

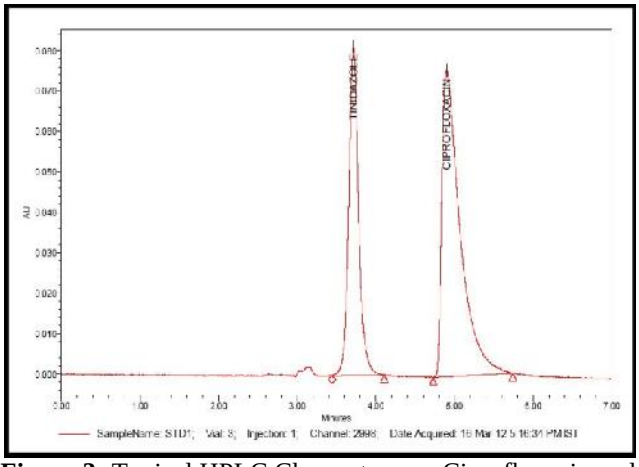


**Figure 3:** Typical HPLC Chromatogram Ciprofloxacin and Tinidazole

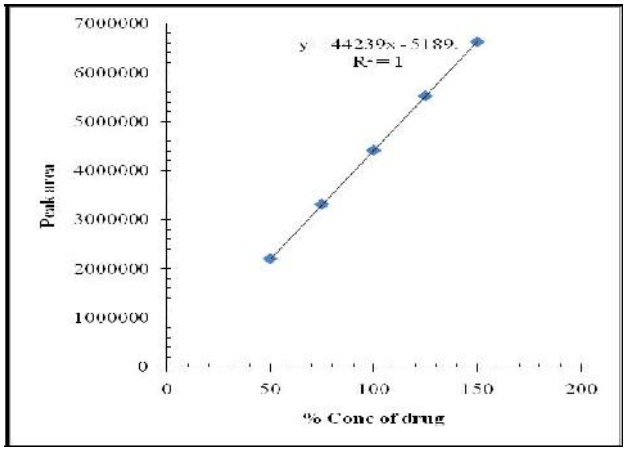


**Figure 4 A:** Linearity curve of ciprofloxacin

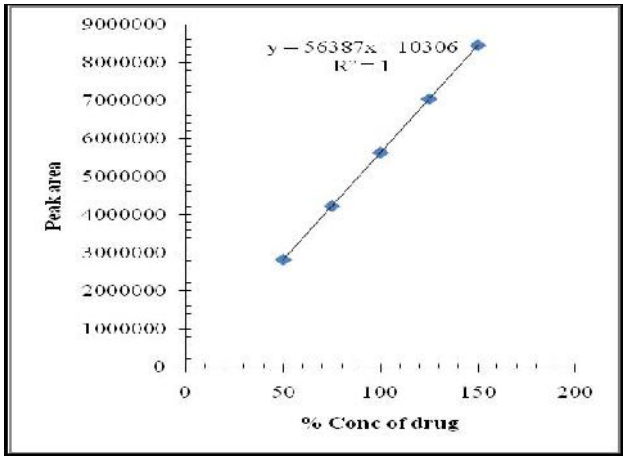


**Figure 4 B:** Linearity Curve of Ttinidazole

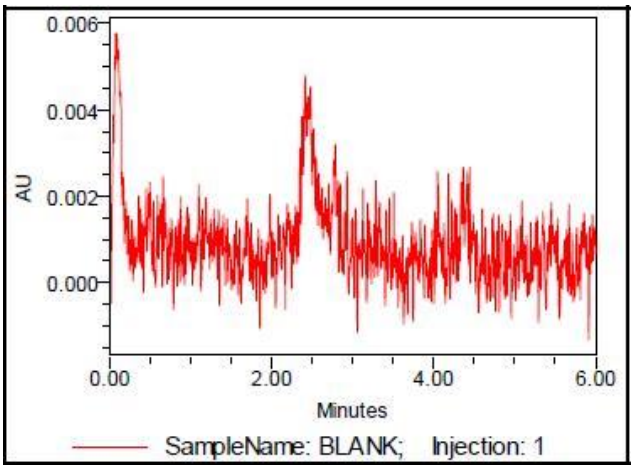


**Figure 5:** A Typical HPLC Chromatogram Showing No Interference of Diluent for Ciprofloxacin and Tinidazole

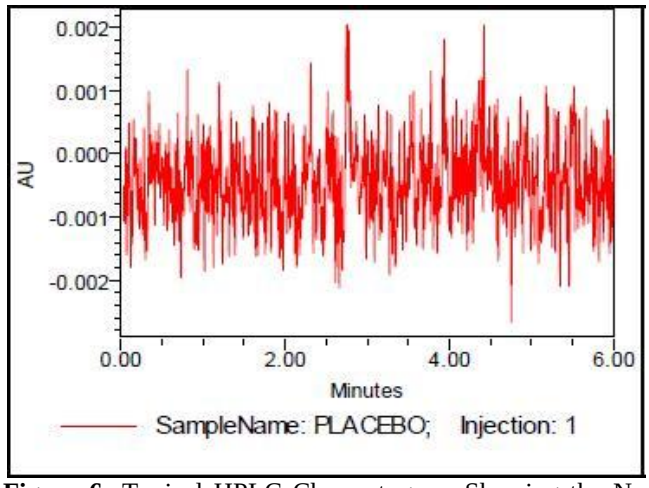


**Figure 6:** Typical HPLC Chromatogram Showing the No Interference of Placebo for Ciprofloxacin and Tinidazole

**b) Linearity:**

For linearity seven points calibration curve were obtained in a concentration range from 0.040-0.160 mg/ml for ciprofloxacin and 0.016-0.064 mg/ml for tinidazole. Chromatograms obtained during linearity study were shown in Figure 5. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for ciprofloxacin was y = 44239x – 5189 with correlation coefficient 1.00 [Figure.5] and for tinidazole was y=56387x+10306with correlation coefficient 1.00 Figure.6. The regression results of ciprofloxacin and tinidazole are reported in Table 3 respectively. The LOD value for ciprofloxacin and tinidazole were found to be 2.9218 µg/mL and 2.809 µg/mL, respectively and the LOQ value 9.737 µg/mL and 9.36 µg/mL, respectively.

**c) Precision:**

Intraday and inter day precision studies for ciprofloxacin and tinidazole are given respective chromatograms and in Table 2.03 respectively. The RSD values for intraday precision and interday precision studies were < 2.0 % for ciprofloxacin and tinidazole confirming that the developed method was precise.

**d) Accuracy:**

Recovery studies of ciprofloxacin and tinidazole were determined at three different concentration levels. Chromatograms obtain during accuracy study were shown in the mean recovery for ciprofloxacin and tinidazole was 100 % respectively (Table.5&6). These results indicated that the proposed method is accurate. Assay method for ciprofloxacin and tinidazole were established in all variance conditions. Assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory and in Table 1; hence, the analytical method would be concluded as robust.

**f) Assay in formulations:** The validated method was applied to the determination of ciprofloxacin and tinidazole in commercially available Acmebid-Tz tablets. The observed concentrations of ciprofloxacin and tinidazole were found to be 600mg/mL (Mean) and 499.84mg/mL respectively. The results of the assays (n = 6) undertaken yielded 100.03% (%RSD = 0.0237%) and 99.96% (%RSD = 0.0849%) of label claim for ciprofloxacin and tinidazole, respectively. The results of this assay (Table 2.06) revealed that the developed RP-HPLC method is selective for the analysis of both ciprofloxacin and tinidazole without interference from the excipients.

**e) Robustness:** The robustness study of the developed

**Table 3:** Linearity Studies for Ciprofloxacin by the Proposed Method

| **Linearity Study for Ciprofloxacin** | | |
|---|---|---|
| **% Level (Approx.)** | **Conc. µg/mL** | **Area** |
| 50 | 1000 | 2204508 |
| 75 | 1500 | 3315112 |
| 100 | 2000 | 4418619 |
| 125 | 2500 | 5526536 |
| 150 | 3000 | 6628635 |
| Slope | | 44239 |
| Intercept | | -5189 |
| RSQ(r2) | | 1.000 |
| LOD (µg/mL) | | 2.921 |
| LOQ (µg/mL) | | 9.737 |

**Table 4:** Linearity Studies for Tinidazole by the Proposed Method

| Linearity Study for Tinidazole | | |
|---|---|---|
| **% Level (Approx.)** | **Conc. µg/mL** | **Area** |
| 50 | 1200 | 2829953 |
| 75 | 1800 | 4239236 |
| 100 | 2400 | 5649443 |
| 125 | 3000 | 7056630 |
| 150 | 3600 | 8469585 |
| Slope | | 56387 |
| Intercept | | 10306 |
| RSQ(r2) | | 1.000 |
| LOD (µg/mL) | | 2.809 |
| LOQ (µg/mL) | | 9.36 |

## 4. Conclusion

The present work involves the development and validation of a simple, accurate and precise RP-HPLC method for the assay of ciprofloxacin and tinidazole in combined dosage forms. Ciprofloxacin and tinidazole were estimated on Aligent, Zorbax C18 column using buffer: Acetonitrile (70: 30v/v) as mobile phase and detection was carried out at 346 nm. The linearity range was found to be 1000-3000 µg/ml for ciprofloxacin and 1200-3600 µg/ml for tinidazole. The co-relation coefficient was found to be 1.00 for ciprofloxacin and tinidazole respectively. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination The %RSD was also less than 2% showing high degree of precision of the proposed method. The proposed method can be used as alternative method for routine analysis of ciprofloxacin and tinidazole.

**Table 5:** Recovery Studies for Ciprofloxacin by the Proposed Method

| Spiked Level | Sample Weight | Sample Area | µg/ml added | µg/ml found | % Recovery | % Mean |
|---|---|---|---|---|---|---|
| 50% | 674.85 | 2203569 | 991.000 | 988.25 | 100 | 100 |
| 50% | 674.85 | 2206780 | 991.000 | 989.69 | 100 | |
| 50% | 674.85 | 2203927 | 991.000 | 988.41 | 100 | |
| 50% | 674.85 | 2202229 | 991.000 | 987.65 | 100 | |
| 50% | 674.85 | 2205956 | 991.000 | 989.32 | 100 | |
| 50% | 674.85 | 2205014 | 991.000 | 988.90 | 100 | |
| 100% | 1349.70 | 4415764 | 1982.000 | 1980.38 | 100 | 100 |
| 100% | 1349.70 | 4414427 | 1982.000 | 1979.78 | 100 | |
| 100% | 1349.70 | 4415226 | 1982.000 | 1980.13 | 100 | |
| 150% | 2024.60 | 6624519 | 2973.073 | 2970.95 | 100 | 100 |
| 150% | 2024.60 | 6624623 | 2973.073 | 2971.00 | 100 | |
| 150% | 2024.60 | 6621244 | 2973.073 | 2969.49 | 100 | |
| 150% | 2024.60 | 6628448 | 2973.073 | 2972.72 | 100 | |
| 150% | 2024.60 | 6623817 | 2973.073 | 2970.64 | 100 | |
| 150% | 2024.60 | 6622003 | 2973.073 | 2969.83 | 100 | |

[a]Average of three determinations

**Table 6:** Recovery Studies for Tinidazole by the Proposed Method

| Spiked Level | Sample Weight | Sample Area | µg/ml added | µg/ml found | % Recovery |
|---|---|---|---|---|---|
| 50% | 674.85 | 2203569 | 991.000 | 988.25 | 100 |
| 50% | 674.85 | 2206780 | 991.000 | 989.69 | 100 |
| 50% | 674.85 | 2203927 | 991.000 | 988.41 | 100 |
| 50% | 674.85 | 2202229 | 991.000 | 987.65 | 100 |
| 50% | 674.85 | 2205956 | 991.000 | 989.32 | 100 |
| 50% | 674.85 | 2205014 | 991.000 | 988.90 | 100 |
| 100% | 1349.70 | 4415764 | 1982.000 | 1980.38 | 100 |
| 100% | 1349.70 | 4414427 | 1982.000 | 1979.78 | 100 |
| 100% | 1349.70 | 4415226 | 1982.000 | 1980.13 | 100 |
| 150% | 2024.60 | 6624519 | 2973.073 | 2970.95 | 100 |
| 150% | 2024.60 | 6624623 | 2973.073 | 2971.00 | 100 |
| 150% | 2024.60 | 6621244 | 2973.073 | 2969.49 | 100 |
| 150% | 2024.60 | 6628448 | 2973.073 | 2972.72 | 100 |
| 150% | 2024.60 | 6623817 | 2973.073 | 2970.64 | 100 |
| 150% | 2024.60 | 6622003 | 2973.073 | 2969.83 | 100 |

**Table 7:** Robustness Studies of the Proposed RP-HPLC Method

| Robust conditions | | Ciprofloxacin | | | Tinidazole | | |
|---|---|---|---|---|---|---|---|
| | | Theoretical plates | Rt | Peak area | Theoretical plates | Rt | Peak area |
| Flow Rate | 0.8 ml/min | 4218 | 3.063 | 5230641 | 6555 | 3.618 | 6568898 |
| | 1.2 ml/min | 3815 | 3.057 | 5224994 | 5982 | 3.611 | 6575829 |
| Temp. | $40^{O}$C | 4137 | 3.057 | 5264291 | 6279 | 3.612 | 6635470 |
| | $45^{O}$C | 3863 | 3.052 | 5235848 | 5923 | 3.615 | 6604124 |

**Table 8:** Analysis of Marketed Tablets by the Proposed Method

| Drug | Label claim | Quantity found* | %RSD | %assay |
|---|---|---|---|---|
| Ciprofloxacin | 600 | 600.05 | 0.0237 | 100.03 |
| Tinidazole | 500 | 499.84 | 0.0849 | 99.96 |
| | ***Average of six determinations** | | | |

## 5. Acknowledgments

We would like thank to Department of Chemistry, Acharya Ngarjuna University, Guntur, A.P, India, for giving good support.

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