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# Simultaneous Estimation of Ciprofloxacin and Tinidazole by U.V Spectrophotometer using a Hydrotropic Solubilization Technique

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**Advanced, easy and accurate UV spectroscopy technique (I & II) was developed for estimation of Ciprofloxacin and Tinidazole in bulk and tablet dosage form and validated using hydrotropic solubilization techniques. In Method I we focused on solving the simultaneous equation on the basis of absorbance measurement at 270nm and 320nm wavelength of Ciprofloxacin and Tinidazole individually in 2M urea solution which results in enhancement of solubility of CPH and TNZ to about 15 folds as compared to distilled water.Strategy II is lay out idea of Q-analysis in which absorbance was estimated at 288nm (iso-absorptive point) 270nm (?max of CPH) in 2M urea. Ciprofloxacin and Tinidazole display linearity at chose frequencies and comply with Beer's regulation in the focus scope of 0.2-1.2$\mu$g/mL and 10 to 60$\mu$g/mL separately. Recovery reads up for CPS and TNZ were completed and rate recuperation of the medications gotten in the scope of 99.2 - 99.6% (Method A) and 98.0-100.8% (Method B) affirming the exactness of the proposed strategy. Both the techniques showed great reproducibility and recuperation with %RSD less than 2. Factual approval of the information shows that the proposed strategies can be effectively applied for the standard examination of medications in business tablets.**

**Keywords:** absorbance ratio method; hydrotropic agent; Simultaneous estimation method.

Hydrotropic is solubilization process, where the adding of huge quantity of a subsequent solute outcome in the rise of aqueous solubility of insoluble and slightly solvable drugs.A poorly water- soluble drug refers to a "practically insoluble"[1]. Poor solubility of drugs is a crucial factor in dosage form designing and due to which drugs show low bioavailability. Hydrotropic agent like, nicotinamide, lysine, ß-cyclodextrinand urea,can be utilized for the solvency upgrade[2]. synthetically ciprofloxacin be in to the gathering of manufactured fluoroquinolone anti-infection agents with strong action against wide range microscopic organisms. Tinidazole is dynamic against both gram+ and gram - microbes.

## MATERIAL AND METHODS

### Chemical and Reagent

Analytical grade of reagents and solvents take place used for the quantitative estimation of Ciprofloxacin and Tinidazole from Himgiri traders, whereas gift sample of Ciprofloxacin

and Tinidazole was obtained from east African overseas. 2.0M urea was used as hydrotropic agent.

**Instrumentation**

Double beam UV-Visible Spectrophotometer of Agilent Technologies (Model No- G6860A) take placed. All weighing done on electronic balance.

**Selection of solvent**

Solubilityof the two not set in stone at 28 ± 2C. An immense measure of medications was added to the recepticle containing different hydrotropic arrangement. The measuring glasses were shaken precisely for 12 hrs.' at 280C ± 10 C in a mechanical stirrer. Sample were permitted to get stable within 24 hrs. and afterward agitate for 5min at 2000 rpm. The supernatant fluid taken for proper dilution after separated through filter paper (whatmann) and investigated spectrophotometric partner against reference. After examination it was observed that the expansion in the dissolvability of CPH and TNZ was viewed as in excess of 15 folds in 2.0M urea arrangement when contrasted with solvency in refined water[5,6].

**Preparation of Stock Solution (1000ìg/ml)**

Accurately weigh 50mg CPH and TNZ independently and moved to two separate 50ml volumetric, dissolve with the 2M urea arrangement and last volume left up to mark with distilled water get stock soln. ofCPH (1000µg/ml) and TNZ (1000µg/ml).

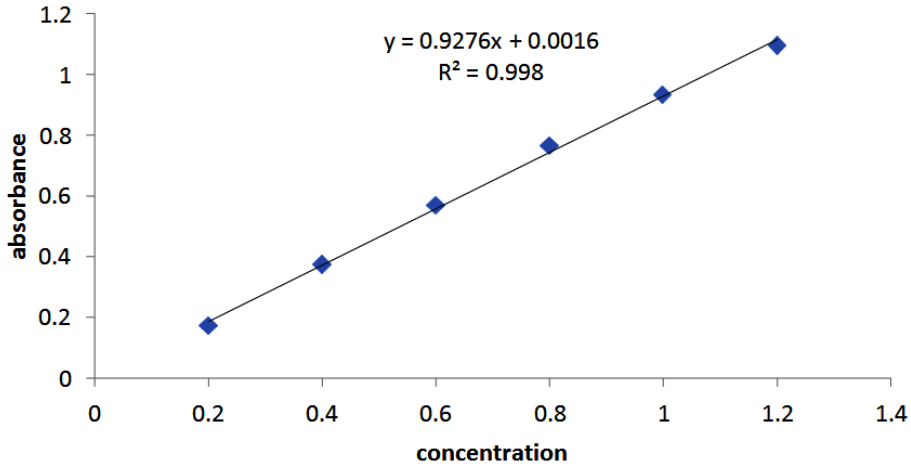


**Fig. 1.** Calibration curve of ciprofloxacin at 270nm

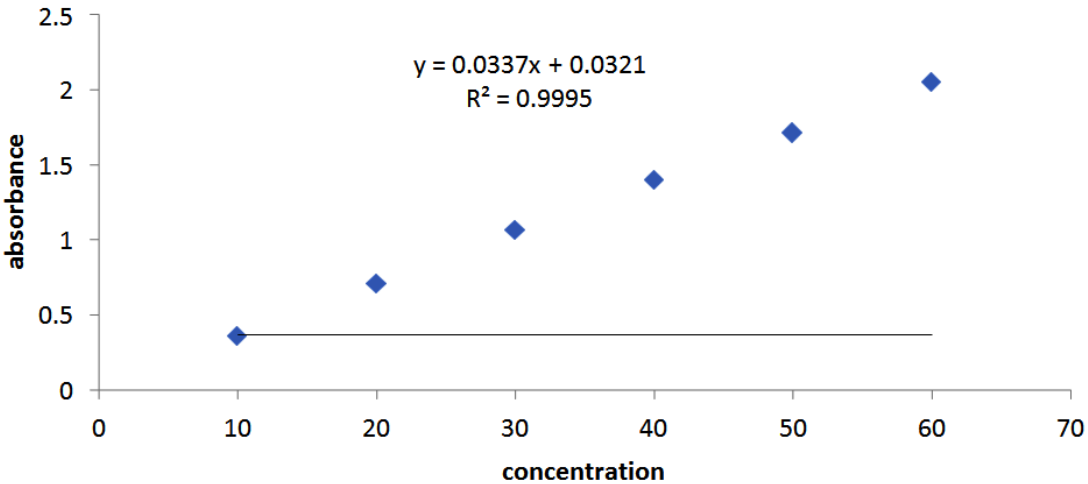


**Fig. 2.** Calibration curve of Tinidazole at 320nm

**Preparationof Working Standard (100µg/ml)**

From the above stock solution, 5ml every one of CPH and TNZ was taken more time, to isolate 50ml volumetric carafe and volume was up to 50ml D.W water to acquire working norm of CPH(100µg/ml) and TNZ(100µg/ml).

**Method I: Simultaneous Equation Method**

Assuming an example contain two absorbing drug (X and Y) all absorbed at different ëmax. it very well may be practicable to choose the two drugs by the simultaneous equation[7,8].

**Determination of wavelength of maximum absorbance of Ciprofloxacin and Tinidazole**

From the working standard, 10µg/ml sol[n.] of CPH and TNZ independentlyprepared in D.W and the solutions were scanned under spectrum mode for 200-400nm wavelength range against blank to analyses the ëmax .Sharp peaks were detected at 270nm for CPH and 320nm for TNZ.

**Preparation of Calibration curve of CPH and TNZ**

From the working norms of CPH(50µg/ml) 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and TNZ(50µg/ml) 10, 20, 30, 40, 50, 60 ml were moved to 10ml volume made up in volumetric flask to mark with D.W. Calibration curve of absorbance versus concentration was plotted and the regression coefficient was determined as shown in fig 1 and 2.

Both the drugs obey's Beer-Lambert's law in the conc. of0.2- 1.2µg/ml for Ciprofloxacin and 10-60µg/ml for Tinidazole. The correlation coefficient were found to be $R^2$>0.999. The absorptivity values of CPH and TNZ are calculated at their respective wavelengths and presented in table 1.

Derivation of equation [9]: from the absorptivity values obtained for CPH and TNZ,

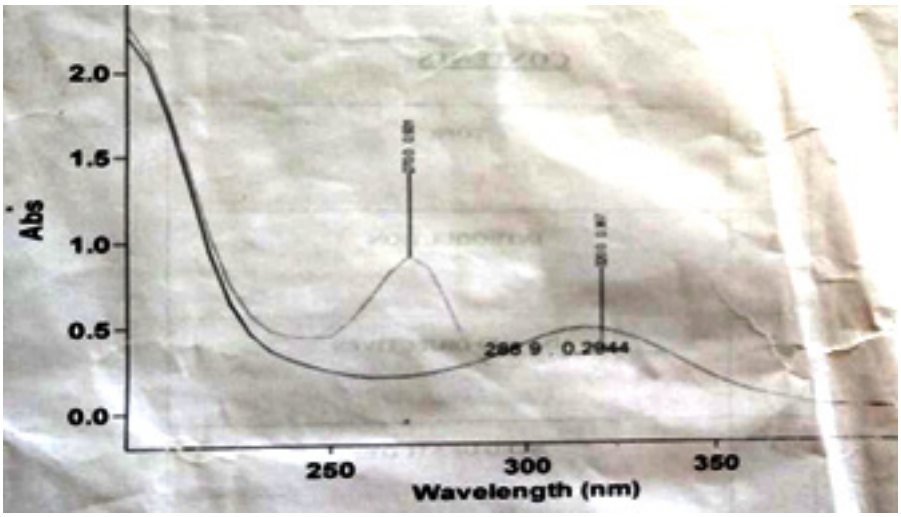


**Fig. 3.** Overlay spectra of ciprofloxacin and tinidazole

**Table 1.** Absorptivity values (A 1 %, 1 cm) of CPH and TNZ by method A and B

| | Method I | | | | | | Method II | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. | CPH | | Conc. | TNZ | | Conc. | CPH | | Conc. | TNZ | |
| µg/ml | 270nm | 320nm | µg/ml | 270nm | 320nm | µg/ml | 270nm | 288nm | g/ml | 270nm | 288nm |
| 0.2 | 865 | 471 | 10 | 354 | 347 | 0.2 | 771 | 180 | 10 | 431 | 161 |
| 0.4 | 680 | 397 | 20 | 226 | 315 | 0.4 | 646 | 197 | 20 | 256 | 132 |
| 0.6 | 646 | 433 | 30 | 188 | 262 | 0.6 | 590 | 170 | 30 | 223 | 108 |
| 0.8 | 614 | 467 | 40 | 163 | 207 | 0.8 | 578 | 133 | 40 | 195 | 146 |
| 1.0 | 598 | 454 | 50 | 159 | 188 | 1.0 | 564 | 100 | 50 | 168 | 194 |
| 1.2 | 578 | 469 | 60 | 149 | 204 | 1.2 | 562 | 167 | 60 | 150 | 183 |
| mean | 662 | 445 | | 206 | 253 | mean | 618 | 157 | mean | 237 | 154 |

simultaneous equation is derived for determination of these two drugs in combination in their pharmaceutical formulations.

A1 = 662CCPH + 206CTNZ A2 = 445CCPH + 253CTNZ

A1 and A2 are the absorbance of mixture at chosen wavelength 270nm and 320nm[10].

**Method II: Absorbtion Ratio Method**

**Selection of Iso-absorptive point**

From the overlay spectrum of CPH and TNZ two wavelength were selected, one at 288nm (ë1) isoabsorptive point for both drugs and other at 270nm(ë2) wavelength of ciprofloxacin for the study. Overlay spectrum is shown in figure 2 and the absorptivity values for CPH and TNZ at selected wavelength are presented in table 1.

Derivation of equation[13]: In Q analysis method, from overlay spectra of CPH and TNZ absorbances were detected at chosen wavelength i.e. at 270nm (ëmax of CPH ) and 288nm (isoabsorptive point). The concentration of each drug in the tablet is determined by substituting the absorbances and absorptivity in the equation

CCPH = (1.97-1.33)/ (4.21-1.337) x A1/ax1 CTNZ = (1.97-4.21)/ (1.337-4.21) x A2/ ay1

A1 and A2 absorbance of samples at 270nm and 288nm. ax1 and ay1 are the absorptivity of CPH at 270nm and TNZ at 288nm.

**Table 2.** Determination of accuracy of CPH and TNZ

| Drug | Level of recovery (%) | Quantity taken (µg/ml) | Quantity added (µg/ml) | Total (µg/ml) | Quantityrecovered Method I | Metho d II | % Recovery(n=3) Method I | Method II |
|---|---|---|---|---|---|---|---|---|
| CPH | 80 | 5 | 4 | 9 | 9.1 | 9.2 | 101.1 | 102.2 |
| | 100 | 5 | 5 | 10 | 9.8 | 9.6 | 98 | 96 |
| | 120 | 5 | 6 | 11 | 10.8 | 10.5 | 98.1 | 95.45 |
| TNZ | 80 | 6 | 4.8 | 10.8 | 10.7 | 10.6 | 99.07 | 98.1 |
| | 100 | 6 | 6 | 12 | 12.2 | 12.1 | 100 | 100.8 |
| | 120 | 6 | 7.2 | 13.2 | 13.0 | 13.1 | 98.4 | 99.2 |

**Table 3.** Analysis of tablet formulation for method A & Method

| Method | Label Claim CPH | TNZ | Amount Found CPH | TNZ | % Recovery CPH | TNZ |
|---|---|---|---|---|---|---|
| A | 500 | 600 | 496 | 598 | 99.2 % | 99.6 % |
| B | 500 | 600 | 490 | 605 | 98 % | 100.8 % |

**Table 4.** Optical characteristics Data

| Parameters | Method A CPH | TNZ | Method B CPH | TNZ |
|---|---|---|---|---|
| Analyticalwavelength | 270nm and 320nm | | 270nm and 288nm | |
| Beer's range | 0.2 –1.2µg/ml | 10–60µg/ml | 0.2 –1.2µg/ml | 10-60µg/ml |
| Slope | 0.52 | 0.01 | 0.51 | 0.009 |
| Intercept | 0.06 | 0.023 | 0.05 | 0.06 |
| Correlationcoefficient | 0.9995 | 0.997 | 0.999 | 0.998 |
| Intra-dayprecision | 0.366 | 0.951 | 0.350 | 0.421 |
| Inter-day precision | 0.433 | 0.853 | 0.465 | 0.243 |
| LOD | 0.310 | 0.180 | 0.101 | 0.212 |
| LOQ | 0.718 | 0.821 | 0.103 | 0.991 |
| Tablet assay | 99.2% | 99.6% | 98% | 100.8% |

**Anaysis of the Tablet Formulation**

Onconcurrent evaluation of CPH and TNZ in retail tablet dosage form (CIPLOX-TZ) containing retailproduct of CPH-500 mg and TNZ-600mg was completed. In this assay methodology, 20 tablets were squashed and ground to a fine powder. Powder comparable to 25 mg of CPH and 30 mg of TNZ was moved to a 50 ml volumetric flask containing around 50 ml of urea solution broke up and sonicated for 10 min. The solution was sifted through Whatmann filter paper. The solution was additionally dilution with D.W to get conc. of 10µg/ml for ciprofloxacin and 12µg/ml for tinidazole. Absorbance of these solution were estimated at 270nm(ciprofloxacin) and 320nm(tinidazole) as ë1 and ë2 and centralization of these two medications in the tablet were determined. The consequences of investigation were in table 3.

**Validation Of Developed Method [14]**

The developed U.V spectrophotometric was validated as per (International council for harmonization) guidelines in terms of linearity, range, specificity, precision, Accuracy, LOD & LOQ for estimation of ciprofloxacin and tinidazole in bulk & tablet dosage form.

*Linearity*[15]: From working standard of CPH(50µg/ml) 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and TNZ(50µg/ml) 10, 20, 30, 40, 50, 60 ml transferred to 10ml volumetric flask and volume was made up to mark with distilled water. These solution were then scanned in the range of 400- 200nm against solvent as blank at their respective wavelength and then calibration curve was plotted.

Precision[16] Repeatability (inter day) and (intraday). In precision for interday we are required to analyses three samples per day for consecutive three days and for intraday one sample was analysed for three days.

*Accuracy*[17]: Known amount of standard of CPH and TNZ were added to the pre-analyzed sample solutions of tablet dosage forms. Results are shown in table 2.

*LOD and LOQ*[18,19]: LOD and LOQ can be calculated as: LOD = 3.3ó/S

LOQ = 10ó/S

## RESULT AND DISCUSSION

Solvency of ciprofloxine and Tinidazole was viewed as in excess of 15 folds in 2.0M urea solution when compared with dissolvability in D.W. It is apparent from table 3 that the percent drug assessed in tablet definition was 99.2% for CPH and 99.6% for TNZ by technique An and 98% for CPH and 100.8% for TNZ by method IIThe optical characteristic such as, t , regression equation,beer's range and Correlation coefficient also determined in table 4.

## CONCLUSION

The analytical study of Ciprofloxacin and Tinidazole by U.V Visible Spectrophotometry was successfully performed. Developed method enhances solubility of both water insoluble drugs and here was no involvementgenerated by urea in the estimation, therefore we analyzed that two UV spectroscopytechniques were established to be easy, accurate, inexpensive and speedy for concurrent estimation of CPH and TNZ in bulk and tablet dosage forms. So different dosage form containing CPH and TNZ can be easily analyzed by utilizing these techniques of spectroscopy. Ciprofloxine belongs to Class IV and Tinidazole belongs to Class II of BCS Classification.

**Conflict of Interest**

There is no conflict of interest

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