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# Simultaneous Spectrophotometric Estimation of Norfloxacin and Tinidazole in Two Component Tablet Formulations

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Quantitative estimation of poorly water-soluble drugs involves use of organic solvents. Major drawbacks of organic solvents include high cost, volatility and toxicity. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubilities of poorly water-soluble drugs norfloxacin and tinidazole in two component tablet formulation for simultaneous spectrophotometric determination. Three simple, accurate and economical procedures employed are program in the multi-component mode of analysis of the instrument used, graphical absorbance ratio method and simultaneous equation method. All methods utilize 8.0 M urea solution as hydrotropic solubilizing agent. In the urea solution, norfloxacin and tinidazole show maximum absorbance at a wavelength of about 274 and 320.5 nm respectively and isobestic point is observed at 297.5 nm. The hydrotropic agent and additives used in the manufacture of tablets did not interfere in the analysis. The results of analysis have been validated statistically and by recovery studies.

**Key Words: Norfloxacin, Tinidazole, Urea, Hydrotropy, Spectrophotometry.**

## INTRODUCTION

The norfloxacin and tinidazole combination has an extended range of antimicrobial spectrum and is effective in mixed gut infections, diarrhoea and dysentery. Chemically, norfloxacin is 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid and tinidazole is 1-[2-(ethylsulphonyl) ethyl]-2-methyl-5-nitro-imidazole. The term 'hydrotropy' has been widely used to designate the increase in solubility of various poorly water-soluble compounds due to the presence of a large amount of additives. A large number of poorly water-soluble drugs have been solubilized using various hydrotropic solutions[1–18]. Sodium benzoate, sodium salicylate, urea, nicacinamide, sodium ascorbate and sodium citrate are the popular examples of hydrotropic agents. Maheshwari *et al.* have developed new analytical methods based on hydrotropic solubilization phenomenon for poorly water-soluble cefixime[1], frusemide[2], ketoprofen[3,5], salicylic acid[3], tinidazole[4], ofloxacin[6], metronidazole[7], nor-

floxacin[7], nalidixic acid[7], tinidazole[7], aceclofenac[8] and hydrochlorothiazide[9]. Extensive literature survey revealed HPLC and spectrophotometric methods[19, 20] for simultaneous and separate estimation of norfloxacin and tinidazole in binary tablet formulation which require use of costlier and toxic organic solvents but no method has been reported using hydrotropic solubilization. The aim of the present work was to develop a simple, rapid, precise, reproducible and economical method for the simultaneous estimation of the binary drug formulation using multi-component mode of analysis of the instrument, graphical absorbance ratio method and simultaneous equation method.

## EXPERIMENTAL

A Shimadzu UV-Visible recording spectrophotometer (model 160A) with 1 cm matched silica cells was used for spectrophotometric analysis. Commercial two-component tablets of norfloxacin and tinidazole (formulation I, Normax-TZ of Ipca Labs. Ltd. and formulation II, Enteroflox-T of Dey's Medical Pvt. Ltd.) were procured from the local market. Norfloxacin and tinidazole were gift samples by Alkem Labs. Ltd., Mumabi.

### Preliminary solubility study of norfloxacin and tinidazole

Solubilities of norfloxacin and tinidazole were determined at 28 ± 1°C in distilled water and 8.0 M urea solution. Enhancement in solubilities of norfloxacin and tinidazole in 8.0 M urea solution were found to be more than 10 and 105 folds respectively (as compared to their solubilities in distilled water).

### Preparation of standard norfloxacin and tinidazole solutions

Pure 50 mg of norfloxacin 75 mg of tinidazole were dissolved in 40 mL of 8.0 M urea solution separately and stirred for 15 min and the final volume of both solutions was made up to 100 mL with distilled water. The solutions were filtered through Whatmann filter paper No. 41 and first few mL were rejected. 20 mL of stock solutions were further diluted to 100 mL with distilled water to get working concentrations of 100 μg/mL and 150 μg/mL of norfloxacin and tinidazole respectively.

### Preparation of mixed standards

Six mixed standards containing 2, 4, 6, 8, 10 and 12 μg/mL of norfloxacin and 3, 6, 9, 12, 15 and 18 μg/mL of tinidazole each were prepared from their respective standard solutions.

### Method I: Employing multi-component mode

From the overlain spectra of the two drugs (Fig. 1), the two wavelengths selected as sampling wavelengths were 274 and 320.5 nm which are absorbance maxima for norfloxacin and tinidazole respectively. Now the sampling wavelengths and concentrations of the two components in each of the mixed standards were fed to the instrument using multi-component mode of the instrument and all the six mixed standards were scanned in the range of 400 to 200 nm. The instrument collects and compiles the spectral data from the mixed standards and

gets ready for the quantitative analysis of the samples. An overlain spectrum of six mixed standards of norfloxacin and tinidazole in their concentration ratio of 2 : 3 is shown in Fig. 2.

**Procedure for the analysis of tablet formulation**

20 Tablets were weigh and ground to a fine powder. Tablet powder equivalent to 50 mg norfloxacin (75 mg tinidazole) was weighed and transferred to a 100 mL volumetric flask. 40 mL of 8.0 M urea soiution was added to the flask and stirred for 15 min to dissolve the drug and the final volume was made up to 100 mL with distilled water. The solution was filtered through Whatmann filter paper No. 41 and the first few mL were rejected. The filtrate was diluted suitably with distilled water to get 6 μg/mL norfloxacin and 9 μg/mL tinidazole. The solution was then analyzed in the multicomponent mode of the instrument. The concentration of each component in the final dilution made from the tablet solution was printed out by the instrument. From these concentrations, the composition of the tablet was obtained. The results of analysis and statistical validation obtained from two different brands of tablet formulations were recorded in Table-1. After 48 h, the solutions were reanalyzed to determine chemical stability and precipitation, if any. The recovery studies carried out gave satisfactory results in the range 90 to 101%.

**Method II: Employing graphical absorbance ratio method (Q-analysis)**

This method depends upon the property that, for the substance that obeys Beer's law at all wavelengths, the ratio of absorbances at any two wavelengths are constant values, independent of concentration or path length. In the USP, this ratio is referred to as Q value.

One of the two selected wavelengths is an isoabsorptive point, if possible, and the other is the wavelength of maximum absorption of one of the two components. A simple straight line graph can be drawn to show the relationship between abosrbance ratio and the fraction of relative concentration of the two components. From the overlain spectra of the two drugs (Fig. 1) it is evident that norfloxacin and tinidazole show isoabsorptive point at 297.5 nm. Norfloxacin shows absorbance maxima at 274 nm. Therefore, the two wavelengths selected are 274 and 297.5 nm. The ratio of absorbance at 274 and 297.5 nm were plotted against the relative concentration of six mixed standards to obtain the calibration curve. This curve shows linearity in the concentration range of 0–18 μg/mL for both the drugs.

**Procedure for the analysis of tablet formulation**

Tablet solution was prepared as described in method I and suitably diluted to obtain 6 μg/mL norfloxacin and 9 μg/mL tinidazole. The ratio of absorbances at 274 and 297.5 nm from the spectra of sample solution were measured and the amount of drug present in the sample solution was obtained from the calibration curve. The results of analysis and statistical validation obtained from two different brands of tablet formulations were recorded in Table-1. The recovery studies carried out gave satisfactory results in the range of 99–101%.

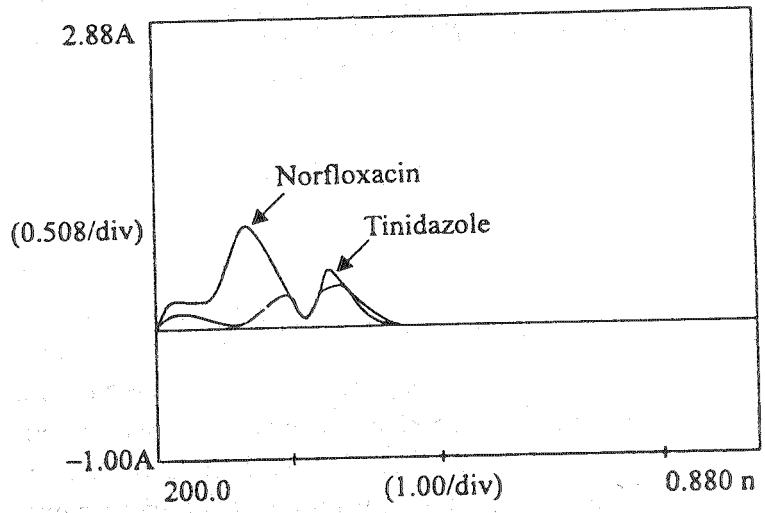


Fig. 1. Overlain spectra of pure drugs norfloxacin and tinidazole in urea solution

TABLE-1
RESULTS OF ANALYSIS OF COMMERCIAL TABLET FORMULATION WITH STATISTICAL EVALUATION

| Method | Tablet formulation | Label claim | | Per cent label claim estimated* (mean ± S.D.) | Per cent coefficient of variation | Standard error |
|---|---|---|---|---|---|---|
| | | Drug | mg/tablet | | | |
| I | 1 | NF | 400 | 100.13 ± 0.133 | 0.132 | 0.768 |
| | | TZ | 600 | 102.29 ± 0.836 | 0.817 | 0.483 |
| | 2 | NF | 400 | 100.53 ± 0.589 | 0.586 | 0.340 |
| | | TZ | 600 | 99.93 ± 0.533 | 0.533 | 0.308 |
| II | 1 | NF | 400 | 98.32 ± 0.481 | 0.489 | 0.278 |
| | | TZ | 600 | 102.29 ± 0.464 | 0.453 | 0.268 |
| | 2 | NF | 400 | 99.78 ± 1.043 | 0.104 | 0.602 |
| | | TZ | 600 | 99.69 ± 0.412 | 0.413 | 0.238 |
| III | 1 | NF | 400 | 98.73 ± 0.418 | 0.423 | 0.241 |
| | | TZ | 600 | 101.61 ± 0.810 | 0.797 | 0.468 |
| | 2 | NF | 400 | 99.47 ± 0.370 | 0.372 | 0.214 |
| | | TZ | 600 | 100.60 ± 0.546 | 0.543 | 0.315 |

*Average of 3 determinations. NF: Norfloxacin, TZ: Tinidazole.

### Method III: Employing simultaneous equation method

If the sample contains two absorbing drugs, each of which absorbs at the wavelength maximum of the other, it may be possible to determine the concentrations of both drugs by the technique of simultaneous estimation (Vierordt's method). From the overlain spectra of the two drugs (Fig. 1), it is evident that norfloxacin and tinidazole show absorption maxima at 274 and 320.5 nm respectively. Therefore, the two wavelengths selected are 274 and 320.5 nm. Standard solutions of 6 μg/mL norfloxacin and 9 μg/mL tinidazole from their respective working solutions were prepared separately and their absorbances were obtained at 274 and 320.5 nm to calculate their molar absorptivities.

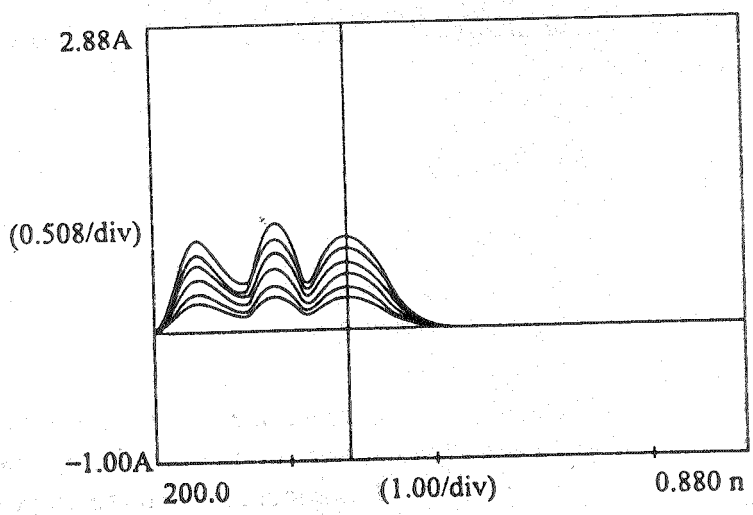


Fig. 2. Overlian spectra of six mixed standards of norfloxacin and tinidazole in urea solution

**Procedure for analysis of tablet formulation**

Tablet solution was prepared as decribed earlier and suitably diluted with distilled water to obtain final concentration of 6 μg/mL norfloxacin and 9 μg/mL tinidazole. The absorbances at 274 nm and 320.5 nm on the spectra of sample solutions were measured and the amount of drug present in the sample solution was obtained from the simultaneous equation described in literature[21]. The results of analysis and statistical validation obtained from two different brands of tablet formulations were recorded in Table-1. The recovery studies carried out gave satisfactory results in the range of 99 to 101%.

## RESULTS AND DISCUSSION

Results of solubility studies indicated that enhancement in aqueous solubilities of norfloxacin and tinidazole in 8.0 M urea solution were more than 10 and 105 folds, respectively as compared to their solubilities in distilled water. Therefore, this solution was employed to extract norfloxacin and tinidazole from the fine powder of tablet formulation. It is evident from Table-1 that the values of standard deviation, per cent coefficient of variation and standard error for all three methods are satisfactorily low showing accuracy of the proposed methods. Recovery studies were further performed to confirm the accuracy, precision and reproducibility of the proposed methods. Percentage recovery values ranged from 99 to 101% with very low values of standard deviation, coefficient of variation and standard error. The hydrotropic agent (urea) and excipients used in the manufacture of tablet did not interfere in the analysis. Drug content in the extract of 8.0 M urea solution was same within 48 h and also there was no precipitation of drug. This indicates that the extract can be analyzed within 48 h at least with sufficient accuracy.

The first method employing multi-component mode is very simple but requires sophisticated instruments with sufficient memory to store the spectral data obtained from the scans of the mixed standard while the other two methods, graphical absorbance ratio method and simultaneous equation method, are simpler. They employ the absorbance values at two selected wavelengths and thus

can be used for the routine analysis of the two drugs in combined dosage form using simple instruments.

## Conclusion

Ethanol, methanol, dimethyl formamide, chloroform, carbon tetrachloride, toluene, cyclohexane, diethyl ether, acetone have been employed to solubilize poorly water-soluble drugs for their spectrophotometric analysis. Most of the organic solvents are toxic, costlier and are pollutants. Inaccuracy due to voltatility is another drawback of organic solvents. Using norfloxacin and tinidazole as a model poorly water-soluble drug combination, the authors want to emphasize on the use of hydrotropic solutions as solubilizing agents for simultaneous spectrophotometric estimation. Urea does not interfere in the spectrophotometric estimation of drugs having $\lambda_{max}$ above 250 nm. It is concluded that the proposed methods are new, simple, accurate, safe, precise, economical, free from pollution and can be successfully employed in the routine simultaneous estimation of norfloxacin and tinidazole in two-component tablet formulation.

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