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Derivative Spectrophotometric and Graphical Absorbance Ratio Method for Simultaneous Estimation of Norfloxacin and Tinidazole in two-component tablet formulations

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Norfloxacin (NF) has an absorbance maxima at 275 nm and tinidazole (TZ) has an absorbance maxima at 317.8 nm in 10% v/v N,N-dimethylformamide. In derivative spectrophotometry, absorbance of norfloxacin was measured at 264.2 nm which is the zero crossing point on the first derivative spectra of tinidazole. Similarly, the absorbance of tinidazole was measured at 306.2 nm which is the zero crossing point of norfloxacin. The Beer's law is obeyed in the concentration range of 0-24 µg/ml for norfloxacin and 0-36 µg/ml for tinidazole. In the graphical absorbance ratio method the concentration of norfloxacin and tinidazole was determined using the ratio of absorbances at 275 nm and 300.4 nm for each drug. The calibration curves were linear in the concentration range of 0-20 µg/ml for both drugs. The methods have been validated statistically and by recovery studies.

The USP[1] described a HPLC method for the analysis of norfloxacin (NF) tablet formulation and IP[2] suggests a spectrophotometric method for the analysis of tinidazole (TZ) tablets formulations. The HPLC and spectrophotometric methods that have been reported for the analysis of norfloxacin[3-6] and tinidazole[7-10], from dosage forms, cannot be used for the simultaneous estimation of both drugs. However, one spectrophotometric method using multicomponent mode and two wavelength calculations has been reported for simultaneous estimation of these two drugs[11]. This paper describes the development of two methods for simultaneous estimation. Both the methods are simple, accurate, reproducible, economic and can be used for routine analysis of two component tablet formulations.

EXPERIMENTAL

A Shimadzu UV/Visible recording spectrophotometer (Model : UV 160A) was employed with spectral bandwidth (resolution) of 3 nm, wavelength accuracy of ± 0.5 nm (with automatic wavelength correction) and a pair of 10 mm matched quartz cells.

Materials and Reagents

Norfloxacin U.S.P., tinidazole I.P., N-N-dimethyl formamide (S.D. Fine Chemicals, A.R. grade) and double distilled water were used in the study. Two- component tablet formulations were procured from the local market.

Method 1 : Derivative Spectrophotometry[12,13]

Considering all the overlain derivative order spectras of NF and TZ from first to fourth derivative, the first derivative order spectra with d(N)=5 was found suitable. The overlain derivative spectra of two drugs reveals that NF and TZ show zero absorbance at 306.2 nm and 264.2 nm respectively (Fig.1). As at the zero crossing point on the first derivative spectra of one drug, the other drug shows substantial absorbance, these two wavelengths can be employed for estimation of TZ and NF respectively without any interference from the other drug in combined formulations. Six mixed standards having concentrations of 4,8,12,16,20 and 24 µg/ml of NF and 6,12,18,24,30 and 36 µg/ml of TZ respectively were prepared in 10% v/v N,N-dimethyl formamide (DMF). The calibration curves of NF

Table -1 : Results of Analysis of Commercial Tablets by Proposed Methods

Commercial Tablet Samples			Method-I First Derivative Spectrophotometry				Method-II Graphical Absorbance Ratio Method			
Brand	Label Claim (mg/tab)		Percent Estimated*	S.D.	C.O.V.	S.E.	Percent Estimated*	S.D.	C.O.V.	S.E.
I	NF	400	103.23	0.211	0.094	0.204	102.64	0.426	0.190	0.415
	TZ	600	101.10	0.918	0.439	0.4428	98.23	0.238	0.127	0.288
II	NF	400	102.26	0.183	0.084	0.383	100.90	0.439	0.196	0.435
	TZ	600	99.32	0.837	0.374	0.843	99.39	0.293	0.131	0.295

*Mean of five estimations, NF-Norfloxacin, TZ-Tinidazole, S.D.=Standard Deviation, C.O.V.-Coefficient of Variance, S.E.-Standard Error

and TZ were prepared from the absorbances measured at 264.2 nm and 306.2 from the first derivative spectra of these mixed standard solutions. Beer's law is obeyed in the concentration range of 0-24µg/ml for NF and 0-36 µg/ml for TZ.

Fig -1 : Overlain First Derivative Spectra of Norfloxacin (NF) and Tinidazole (TZ)

Preparation and Analysis of Tablet Sample Solution

Twenty tablets were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 40 mg of NF and 60 mg of TZ was transferred to a 100 ml volumetric flask. The powder was dissolved in N,N-dimethylformamide by intermittant shaking for about 20 minutes and the resultant solution was filtered through Whatman filter paper No.41. Aliquot of solution was diluted to get final concentration equivalent to 16 µg/ml of NF. The solution was scanned in the spectrum mode against a blank of 10% v/v DMF and absorbances of the sample solution were recorded at 264.2 nm and 306.2 nm from the first derivative spectra and the amount of drugs present in the sample solution were obtained from the calibration curves. The results of analysis and statistical validation obtained from two different brands of tablet formulations are recorded in Table 1. The results of recovery studies, conducted by addition of different amounts of pure drug (s) to a pre-analysed tablet solution, were found to be satisfactory in the range of 98-102%.

Method II : Graphical Absorbance Ratio Method[14]

Graphical absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one of which is

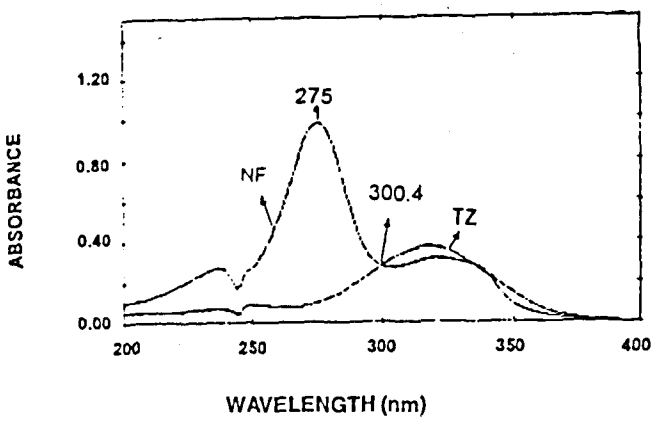

Fig -2 : Overlain Spectra of Norfloxacin (NF) and Tinidazole (TZ)

an 'Isoabsorptive point'[15] and other being the wavelength of maximum absorption of one of the two components. From the overlain spectra of two drugs (Fig.2) it is evident that NF and TZ show isoabsorptive point at 300.4 nm. At 275 nm the absorbance of NF is maximum. Therefore, the two wavelengths selected are 275 nm and 300.4 nm.

Six mixed standard solutions with concentrations of 0,4,8,12,16 and 20 µg/ml NF and 20,16,12,8,4 and 0 µg/ml of TZ respectively were prepared in 10% V/V DMF and the ratio of absorbances at 275 nm and 300.4 nm were plotted against the relative concentration to obtain the calibration curves. The curves show linearity in the concentration range of 0-20 µg/ml for both the drugs.

Preparation and Analysis of Tablet Sample Solutions

Tablet sample solution was made as described in method-I and solution was diluted to get a final concentration equivalent to 8 µg/ml of NF and 12 µg/ml of TZ. The ratio of absorbance at 275 nm and 300.4 nm on the spectra of sample solution were measured and the amount of drugs present in the sample solution were obtained from the calibration curves. 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%.

RESULTS AND DISCUSSION

The two methods developed were found to be accurate, simple and rapid for routine simultaneous analysis of the formulations containing NF and TZ. The values of standard deviation were satisfactorily low [0.211 and 0.426 for NF, 0.938 and 0.238 for TZ for both the methods]. The recovery studies were 99.79% to 100.78% for NF and 98.7% to 101.3% for TZ for both the methods. It indicates the accuracy and reproducibility of both the methods.

The first method requires spectral data processing and, hence, can be performed only on recording spectrophotometers with such facilities. First derivative spectrophotometry was employed to totally eliminate the spectral interference from one of the two drugs while estimating the other drug. This was achieved by selecting the zero-crossing point on the derivative spectra of each drug as the wavelength for estimation of the other drug.

The second method, also called Q-analysis, is simpler. It employs the absorbance ratio at two selected wavelengths and can be employed for routine analysis of the two drugs in combined dosage forms using simple instruments. Once the absorbance ratio is determined, the next step is determining the concentration of the drugs from the calibration curves. This method is less time consuming and can be performed on simpler instruments unlike the first method which requires more sophisticated instruments.

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