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Simultaneous determination of tinidazole, furazolidone and diloxanide furoate in a combined tablet preparation by second-derivative spectrophotometry

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Abstract

A second-derivative spectrophotometric procedure has been developed for the simultaneous determination of tinidazole (TD), furazolidone (FD) and diloxanide furoate (DF) in a commercial preparation. The method consists of the utilization of second-derivative absorption spectra of tablet extract in distilled water and then determination of the analyte concentration in the mixture was carried out using zero-crossing (ZC) and ratio-compensation (RC) techniques. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of TD (5–20 μg ml^{-1}), FD (2.5–10 μg ml^{-1}) and DF (7.5–15 μg ml^{-1}). The results were found to be accurate and free from interference. The details of the statistical treatment of analytical data are also presented.

Keywords: Tinidazole; Furazolidone; Diloxanide furoate; Simultaneous determination; Derivative spectrophotometry

1. Introduction

A combination of tinidazole (TD), furazolidone (FD) and diloxanide furoate (DF) in the form of a tablet preparation is used for treating diarrhoea associated with bacterial growth, amoebiasis and giardiasis. The official monographs describe the procedure for individual assay of DF [1,2], FD [1,2] and TD [2]. There are many reports available for the determination of TD either in combination with FD [3–5] or in combination with DF [6–9]. However, there has been no such report available for the simultaneous determination of these drugs from their combined formulation.

In our previous work, we have described the successful application of zero-crossing (ZC) [5,10–13] and ratio-compensation (RC) [5,10,11] techniques for the simultaneous estimation of various drugs from their combined formulations. In the present study, a second-derivative spectrophotometric method has been developed for the simultaneous estimation of TD, FD and DF in the presence of each other as well as of the excipients.

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2. Experimental

2.1. Standard solutions

The stock solutions of pure TD, FD and DF were prepared by dissolving 5 mg of each of the pure drugs in 10 ml of dimethyl formamide (DMF; analytical grade). Appropriate volume aliquots of the stock solutions were transferred to 10 ml volumetric flasks. The volumes were made up with distilled water to give a series of solutions containing TD (5–20 μg ml^{-1}), FD (2.5–10 μg ml^{-1}) and DF (7.5–15 μg ml^{-1}). Similarly, three series of 10 ml of each mixture solution were also prepared in distilled water from the stock solutions. The first series contained a constant concentration of FD (2.5 μg ml^{-1}), and DF (12.5 μg ml^{-1}) and a varying concentration of TD (5–20 μg ml^{-1}). The second series contained a constant concentration of DF (12.5 μg ml^{-1}), and TD (10 μg ml^{-1}) and a varying concentration of FD (2.5–10 μg ml^{-1}). The third series contained a constant concentration of TD (10 μg ml^{-1}), and FD (2.5 μg ml^{-1}) and a varying concentration of DF (7.5–15 μg ml^{-1}). Finally, a binary mixture solution containing TD (10 μg ml^{-1}) and FD (2.5 μg ml^{-1}) was also prepared. The solutions were stable through out the study.

2.2. Sample preparation

Twenty tablets of Amicline Plus (Franco-Indian, India) labelled to contain 300 mg of TD, 75 mg of FD, 375 mg of DF and excipients were accurately weighed and powdered. The powder weight equivalent to 10 mg of TD (corresponds to 2.5 mg of FD and 12.5 mg of DF) was dissolved in DMF by thorough mixing and made up to volume in a 50 ml volumetric flask. The sample was filtered through Whatman filter paper No. 1. The first and last 5 ml of the filtrate was discarded. Appropriate volume aliquots of filtrate were diluted with distilled water to give samples with a concentration of 10 μg ml^{-1} of TD and corresponding amounts of FD (2.5 μg ml^{-1}) and DF (12.5 μg ml^{-1}).

2.3. Procedure

2.3.1. Zero-crossing (ZC) technique

The absorbances of standard and sample solutions were recorded against a blank solution from 260 to 450 nm with a Jasco 7800 UV-visible spectrophotometer. The second-derivative (D_2) spectra for each set of solutions were subsequently recorded by Savitzky–Golay [14] parameter of $\Delta = 25$ points and further the derivative spectra were smoothed using $\Delta = 30$ points to eliminate the noise produced above 350.0 nm in the recorded spectra of FD. Ordinate maxima and minima were adjusted to the magnitude of derivative values. The solutions were measured at the selected zero-crossing points (ZCPs) of the other two drugs in combination for each instance.

2.3.2. Ratio-compensation (RC) technique

The sample cell contained the mixture of sample and standard solutions containing TD (10 μg ml^{-1}), FD (2.5 μg ml^{-1}) and DF (12.5 μg ml^{-1}) while the reference cell contained a binary mixture solution having TD (10 μg ml^{-1}) and FD (2.5 μg ml^{-1}). The second-derivative spectrum was recorded in each instance using appropriate set parameters as mentioned in Section 2.3.1 and a ratio between wavelength maxima to wavelength minima was also calculated. At the balance point, the concentrations of two of the analyte components of a mixture become equal to that of the reference solution [5,10,11]. The calculated ratio when compared with the ratio of pure DF solutions recorded against a blank solution should be equal.

3. Results and discussion

The zero-order spectra of pure drugs were found to be overlapping (Fig. 1), making their simultaneous determination difficult. The second-derivative spectrophotometric method was considered to be ideal for resolving the overlap over its first-derivative spectra to facilitate the quantitative determination of all the three drugs of interest simultaneously. It was observed during initial study that the first-derivative spectra have ideal

zero-crossing points (ZCPs) for the estimation of TD and FD, but lack such ideal point for simultaneous estimation of DF. Further, the pure DF spectra do not have wavelength minima to assist its determination by the RC technique. The ratio calculated between chosen wavelength maxima and minima becomes the basis for adopting such technique. However, this particular draw back was overcome during the spectral processing in second-derivative mode (Fig. 2). It can be observed from second-derivative spectra (Fig. 2) that DF does not show any appreciable absorbance above 320.0 nm thus the problem is overcome for two-component mixtures in this region of spectral measurement (i.e. 320–450 nm). The various useful ZCPs observed for estimation were at 335.4 and 397.4 nm for TD and at 340.8 nm for FD. In addition, FD estimation would also be possible at its wavelength maxima of 420.0 nm due to non-interference from other combined drugs at this wavelength. To determine quantitatively TD and FD from the solutions only those wavelengths at which greater absorbance values would be measured were considered. Thus, TD was estimated at 335.4 nm and FD at 420.0 nm. The determination of DF was carried out by calculating the ratio between 286.4 nm (λ_{max}) and 262.4 nm (λ_{min}) as explained under Section 2.3.2.

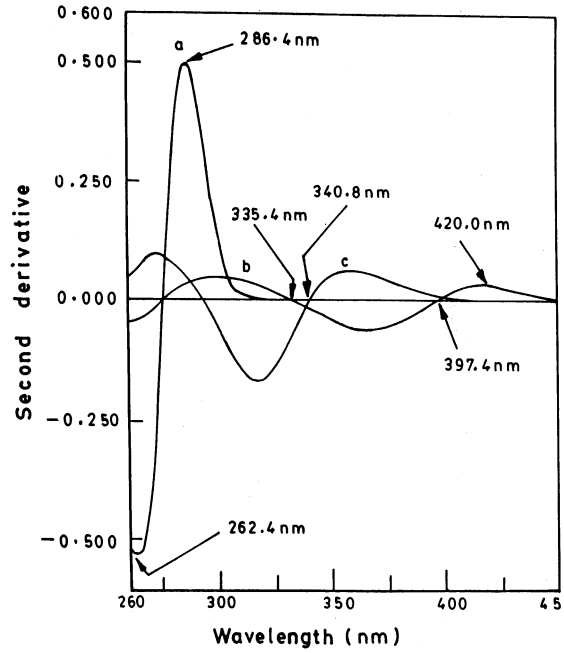

Fig. 2. Second-derivative spectra of (a) diloxanide furoate (12.5 μg ml^{-1}); (b) furazolidone (2.5 μg ml^{-1}) and (c) tinidazole (10 μg ml^{-1}) in distilled water.

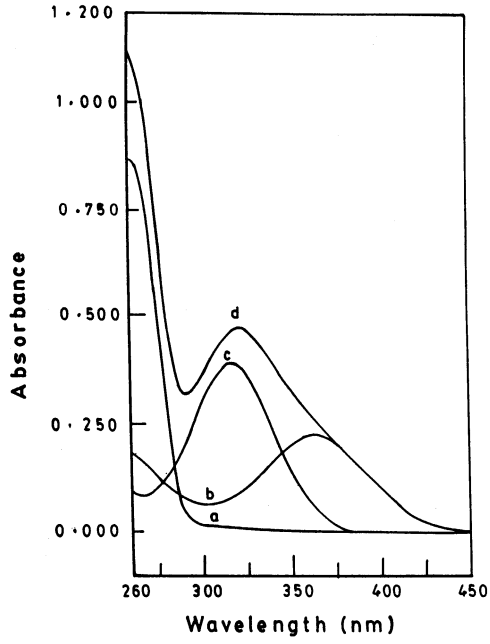

Fig. 1. Absorption spectra of (a) diloxanide furoate (12.5 μg ml^{-1}); (b) furazolidone (2.5 μg ml^{-1}); (c) tinidazole (10 μg ml^{-1}) and (d) their mixture in distilled water.

The proportionality between measured D_2 values and concentrations was found by measuring earlier prepared series of pure drug and mixture solutions at their selected wavelengths of estimation. The selection of ideal concentration ranges for different pure drugs and their mixtures was found based on a well-planned mixture interaction study. The study was conducted by considering a mixture of two drugs with a constant concentration as a single component and the other as the second component with a varying concentration as shown in Fig. 3. It was evident from results obtained that the non-interference zone for varying concentrations of FD and TD existed up to 25 and 50 μg ml^{-1} respectively at 335.4 nm and for DF up to 20 μg ml^{-1} at 286.4 nm. Thus, the proposed linear concentration ranges for standard and sample solutions were

appropriate in terms of their successful determination from combined formulation.

The D_2 spectra obtained for the earlier prepared mixture solutions are presented in Figs. 4–6. The presence of distinct isosbestic points at 340.8 nm (Fig. 4), 335.4 and 397.4 nm (Fig. 5) and followed by a constant absorption pattern around 420.0 nm (Fig. 4) and after 320.0 nm (Fig. 6) suggested no interference in the estimation of one drug in the presence of other drugs. It was also observed that measured values were proportional to the concentration of standard mixtures.

In the ratio-compensation (RC) method, the second-derivative spectra of DF from its pure and mixture solution were recorded according to the earlier mentioned procedure by assuming that

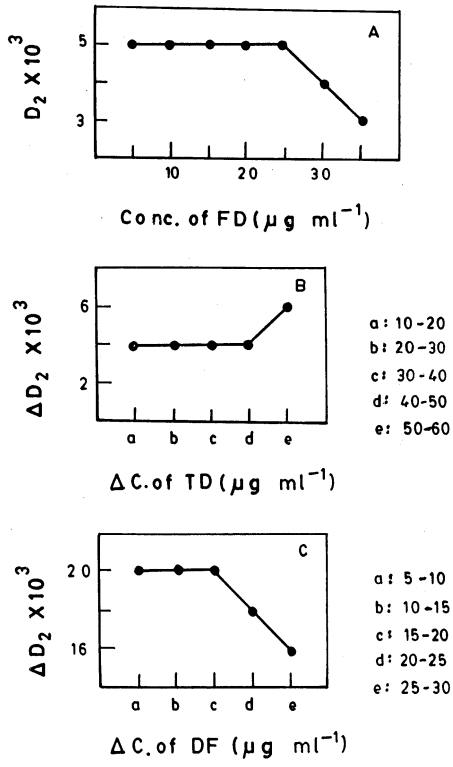

Fig. 3. Second-derivative interaction graphs for (A) diloxanide furoate (12.5 μg ml^{-1}) and tinidazole (10 μg ml^{-1}) in a mixture with furazolidone (at 335.4 nm); (B) furazolidone (2.5 μg ml^{-1}) and diloxanide furoate (12.5 μg ml^{-1}) in a mixture with tinidazole (at 335.4 nm) and (C) tinidazole (10 μg ml^{-1}) and furazolidone (2.5 μg ml^{-1}) in a mixture with diloxanide furoate (at 286.4 nm) in distilled water.

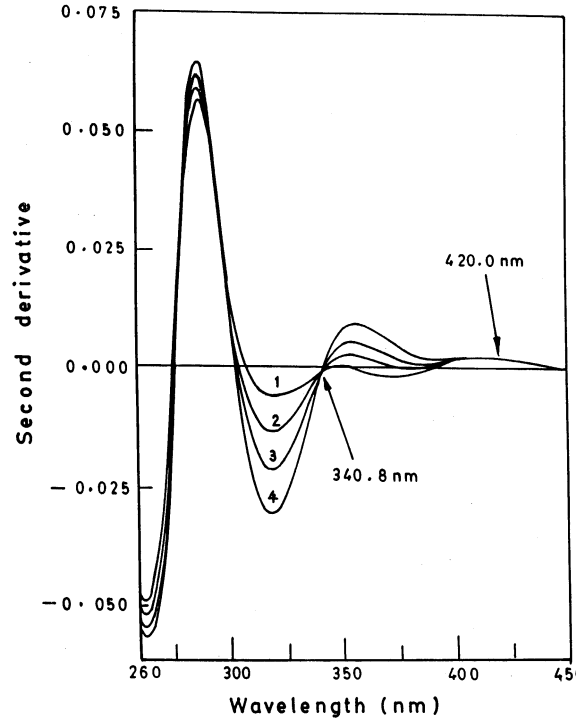

Fig. 4. Second-derivative spectra of tinidazole (5, 10, 15 and 20 μg ml^{-1}; curves 1–4) with a constant concentration of furazolidone (2.5 μg ml^{-1}) and diloxanide furoate (12.5 μg ml^{-1}) in distilled water.

there was no interference among the three-components of a mixture. This was confirmed from the average ratio value obtained for 10 measurements between the absorbance values (D_2) at 286.4 and 262.4 nm of pure drug (0.943 ± 0.001) and mixture (0.944 ± 0.003) solutions which were found to be similar.

The linear regression equations together with correlation coefficients, variance, standard error of slope and intercept [15] and detection limits (DL) [16] obtained for each drug are shown in Table 1. The similarity observed between regression equations of pure drug and mixture solutions suggested no interferences in the estimation of one drug in the presence of the others. The linearity of regression equations and negligible scatter of points was demonstrated from the correlation coefficients and variance.

The D_2 values of standard solutions of TD (10 μg ml^{-1}), FD (2.5 μg ml^{-1}) and DF (12.5 μg

Table 1
Statistical analysis of the determination of tinidazole, furazolidone and diloxanide furoate in standard solutions by second-derivative spectrophotometry[a]

Sample	Composition of solutions (μg ml^{-1})			Regression equations (at 335.4 nm for TD; at 420.0 nm for FD; at 286.4 nm for DF)	Corr. coeff. (r)	Variance (S^2)	Standard error		Detection limit (μg ml^{-1})
	TD	FD	DF				Intercept	Slope	
Series A	5–20	0	0	$D = 4.07E-04.C + 9.72E-04$	0.9998	4.62E−09	8.34E−05	6.09E−06	0.50
Series B	5–20	2.5	12.5	$D = 4.06E-04.C + 8.95E-04$	0.9999	1.29E−09	4.40E−05	3.21E−06	0.27
Series C	0	2.5–10	0	$D = 1.16E-03.C + 1.15E-04$	0.9998	5.09E−09	8.74E−05	1.28E−05	0.18
Series D	10	2.5–10	12.5	$D = 1.19E-03.C - 5.40E-05$	0.9999	1.22E−09	4.27E−05	6.23E−06	0.09
Series E	0	0	7.5–15	$D = 3.97E-03.C + 4.60E-04$	0.9999	1.43E−09	6.52E−05	5.63E−06	0.03
Series F	10	2.5	7.5–15	$D = 4.02E-03.C - 3.40E-04$	0.9999	9.98E−10	7.33E−05	3.15E−04	0.02

[a] Number of samples, $n = 10$; C, concentration of varying drug in μg ml^{-1}.

Table 2
Results of the assay of pure drug admixture and commercial formulation of tinidazole, furazolidone and diloxanide furoate by second-derivative spectrophotometry

Sample	Label claim (mg/tablet)			Recovery (%)[a]			Student's *t*-test for mean recovery			
							Calc.			Crit[b]
	TD	FD	DF	TD (33.4 nm)	FD (420.0 nm)	DF(286.4/262.4nm)	TD	FD	DF	(TD/FD/DF)
Pure drug admixture	–	–	–	100.03 ± 0.45	99.85 ± 0.60	100.11 ± 0.27	0.48	0.69	0.64	2.18
Amicline Plus	300	75	375	100.16 ± 0.54	100.09 ± 0.68	99.98 ± 0.49				

[a] Mean ± S.D. for seven determinations.
[b] Theoretical value of '*t* (two-sided)' at $P = 0.05$ level of significance with 12 df.

ml^{-1}) at the selected wavelengths were used in the determination of analytes from their standard and sample preparations. The percentage recovery values obtained are given in Table 2. It was confirmed from the reported Student's *t*-test [15] values (Table 2) that there were no significant differences between the mean recoveries of standard solution and commercial preparation. Further, it can also be observed that the assay results for each of the active substances are in good agreement with the stated content of the formulation and therefore suggested the non-interference from the formulation matrix with the proposed derivative technique.

4. Conclusions

The proposed method of determination is accurate, simple and reproducible. The lack of any suitable report at present for their simultaneous determination makes it possible to adopt the proposed method for routine analysis. The above results demonstrated that the complex problem of quantitating mixtures of three components with overlapping spectra can be easily resolved by this technique.

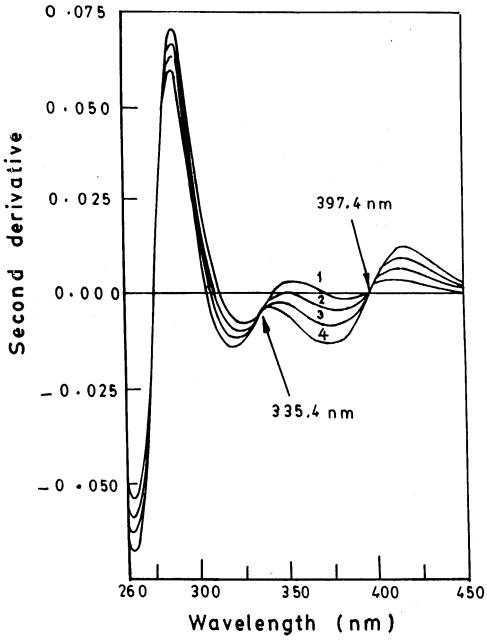

Fig. 5. Second-derivative spectra of furazolidone (2.5, 5, 7.5 and 10 μg ml^{-1}; curves 1–4) with a constant concentration of tinidazole (10 μg ml^{-1}) and diloxanide furoate (12.5 μg ml^{-1}) in distilled water.

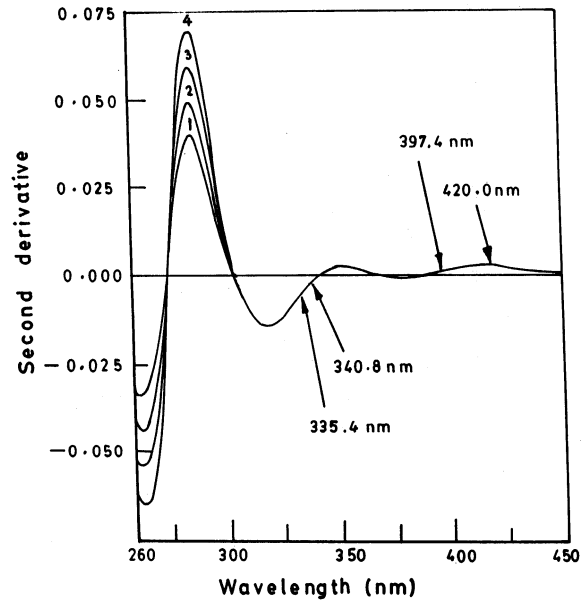

Fig. 6. Second-derivative spectra of diloxanide furoate (7.5, 10, 12.5 and 15 μg ml^{-1}; curves 1–4) with a constant concentration of tinidazole (10 μg ml^{-1}) and furazolidone (2.5 μg ml^{-1}) in distilled water.

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