RESEARCH ARTICLE

Advancements in HPTLC Method for Ciprofloxacin and Tinidazole

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ABSTRACT
Now a days multicomponent formulations are becoming more popular in order to ensure timely and complete medication in multidrug therapy and enhancement of patient compliance.
Through, analytical methods for single drug are extensively available, still due to complexity in multicomponent formulation, method development for the individual active component is sort of challenge for the analytical chemist. Information in the books also do not provide methods of simultaneous estimation of the newly developed and marketed formulations. Most of the methods are available after separation of active components, which become tedious, time consuming, and lack of accuracy.Therefore, the attention in the present project was focused on to foster simple easy economical reproducible analytical method for the assessment of Ciprofloxacin (CP) and Tinidazole (TZ) in combinations by using HPTLC Method.
Keywords: HPTLC method development, Simultaneous estimation, Ciprofloxacin (CP), Tinidazole (TZ), Combined dosage form analysis
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INTRODUCTION

High-performance thin-layer chromatography (HPTLC) is a versatile and efficient analytical technique widely used for the qualitative and quantitative estimation of various pharmaceutical compounds. It offers significant advantages, including high resolution, sensitivity, and the ability to simultaneously analyze multiple samples with minimal solvent and sample requirements. The method involves several key steps: sample preparation, chromatographic development, detection, and data analysis. Samples are typically applied onto a pre-coated HPTLC plate as small spots or bands. The plate is then placed in a chamber filled with solvent vapor, which facilitates the separation of the components based on their interactions with the mobile phase. Detection is performed using UV light or specific chemical reagents, and the separated components are quantified using densitometric analysis. This study aims to develop and validate an HPTLC method for the concurrent determination of ciprofloxacin and tinidazole, two frequently prescribed drugs in combination therapy. Ciprofloxacin, a fluoroquinolone antibiotic, and tinidazole, an antiprotozoal agent, are frequently co-prescribed for treating bacterial and protozoal infections. Developing a reliable and reproducible analytical method for these drugs is essential for ensuring quality and efficacy in pharmaceutical formulations. The optimized HPTLC method described here uses a carefully selected solvent system to achieve clear resolution of ciprofloxacin and tinidazole, providing a robust tool for routine analysis in pharmaceutical quality control.

Wavelength Selection for densitometric evaluation of separated spots

This study utilizes the CAMAG LINOMAT IV, an automated sample applicator, to ensure accurate and reproducible application of samples onto the HPTLC plate.

MATERIALS AND METHODS

Analysis of laboratory mixture

Figure 4. shows the densitogram for the standard and laboratory (sample) mixture. The amounts of CP and TZ in the standard solution were input into the computer. The sample solution amounts were calculated & was estimated using a specific formula.

$$\%\,Estimated = \frac{Mean\,Height / Area \times DilutionFactor}{Amount\,applied \times Wt.taken} \times 100$$

Results showed accurate estimation. The method was considered suitable for application to marketed formulations.

Utilization of the proposed method in commercial formulations.

Marketed Formulation A

Trade name: Zoxan-tz
Mfg.:FDC Ltd.
Composition:CP (500 mg) : TZ (600 mg)

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Average Wt.: 1322.1 mg
Marketed Formulation B
Trade name:CITIZOL
Mfg.:Aristo Pharmaceuticals Ltd.
Composition: CP (500 mg) : TZ (600 mg)
Average Wt.: 1368.7 mg
Chromatograms for both the standard and the sample were analyzed, with the densitograms depicted in Figures 5 and 6. The amounts of CP and TZ in the standard solution were entered into the computer. Drug amounts in the sample solution were determined. The CP and TZ content per tablet (average weight) was calculated using a specific formula.

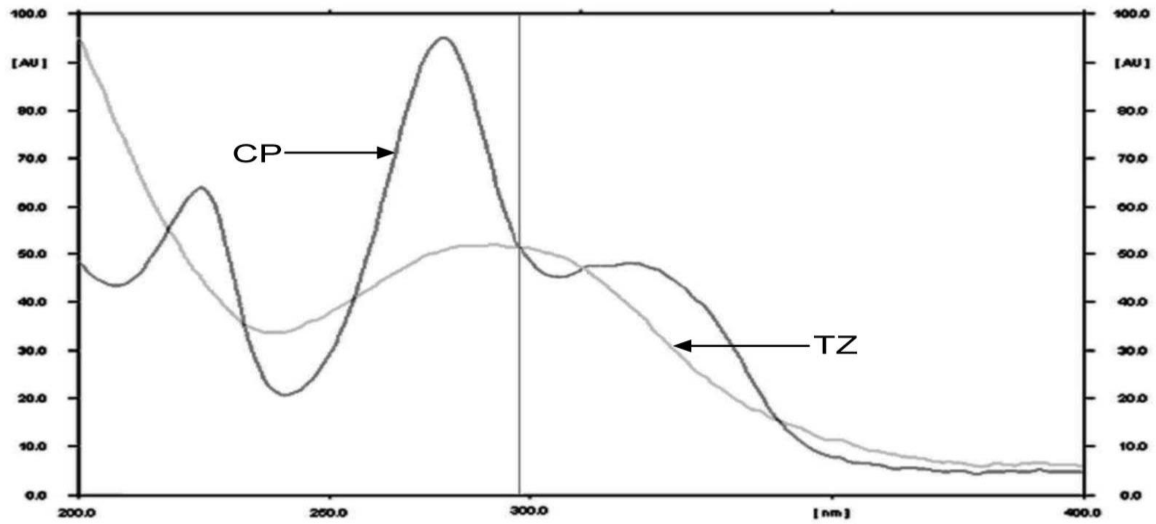


Figure 1: Overlain Spectrum of CP and TZ

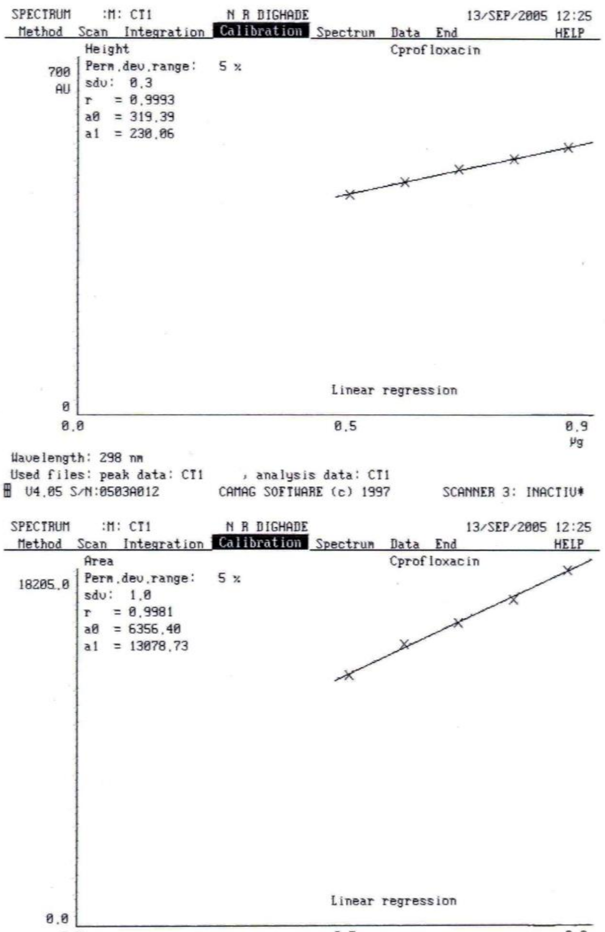


Figure 2: Calibration Curve for CP

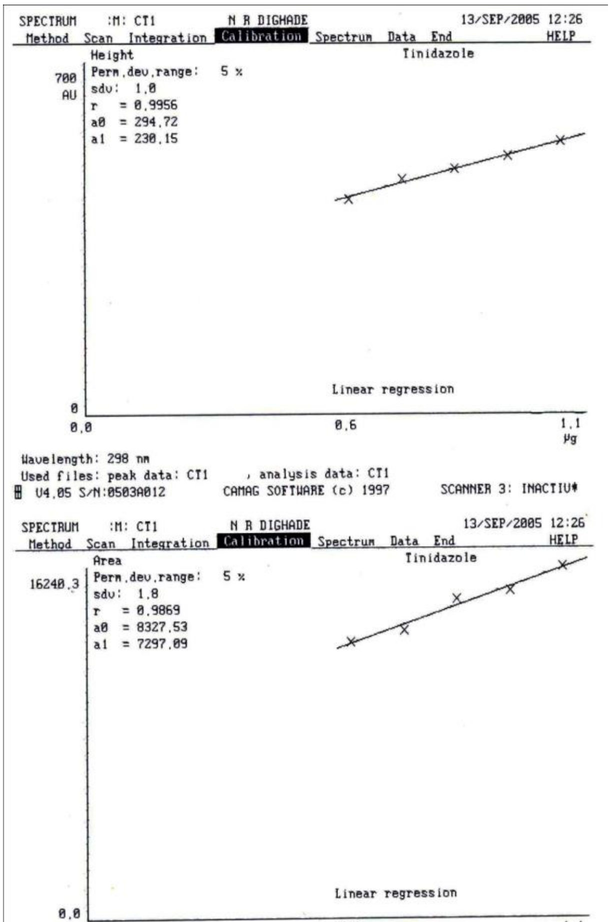


Figure 3: Calibration Curve for TZ

$$\text{Amt. of Drug estimated in avg. wt. of tablet} = \frac{\text{Amt. estimated} \times \text{Avg. Wt. of tablet}}{\text{Weight taken}}$$

$$\%\text{Labeled Claimed} = \frac{\text{Amt. estimated in Avg. Wt. of tablet}}{\text{Labeled Claim}} \times 100$$

Recovery Studies

The results of recovery studies are shown in Table No. 4 and 5.

Validation : Results of the accuracy studies are provided in Tables 4 and 5. Results of precision are presented in Tables 2 and 3.

Specificity: Specificity assessed by deliberate degradation of the tablet sample.

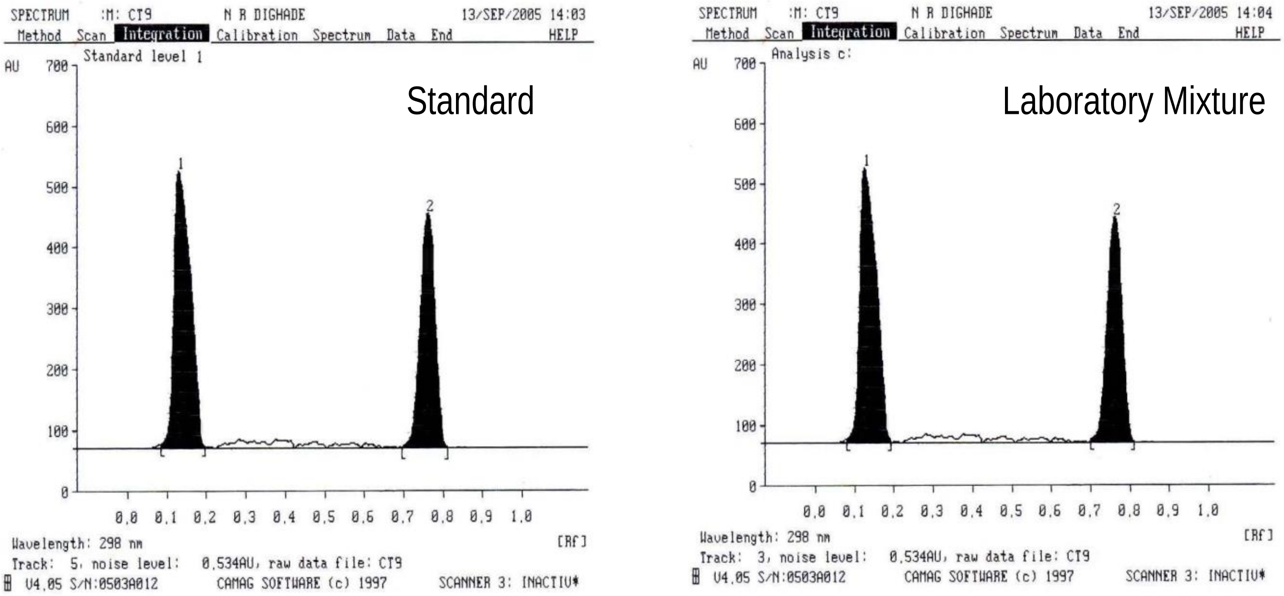


Figure 4: Densitogram of Ciprofloxacin (1) and Tinidazole (2) for Standard and Laboratory Mixture

Table 1 : Results estimation of CP and TZ

Sr. No.	Amount of drug taken (mg)		Percent estimation			
	CP	TZ	CP		TZ	
			In terms of height	In terms of area	In terms of height	In terms of area
1	24.6	29.7	99.76	98.07	100.9	98.98
2	24.95	29.8	100.07	98.89	101.18	99.51
3	25.3	30.45	98.98	101.67	98.17	100.28
4	24.7	24.75	100.73	98.91	100.58	99.67
5	24.5	24.40	98.90	99.10	99.95	99.12
		Mean	99.68	99.32	100.15	99.51
		± SD	0.7679	1.3680	1.2008	0.5127
		CV	0.7703	1.3772	1.1990	0.5152
		RSD	0.0077	0.0137	0.0119	0.0051

Table 2 : Results of estimation of CP and TZ in Marketed Formulation

ZOXAN-TZ		Average Weight = 1322.1 mg			
Sr. No.	Weight of tablet powder taken (mg)	Percent label claim			
		CP		TZ	
		By height	By area	By height	By area
1	64	98.16	98.46	101.97	99.33
2	67.2	98.22	101.91	99.77	99.94
3	66.1	98.12	100.56	99.47	98.29
4	66	99.94	100.12	99.42	101.30
5	68	98.48	100.79	101.38	102.12
	Mean	98.58	100.37	100.40	100.20
	±SD	0.7709	1.2545	1.1882	1.5304
	CV	0.7820	1.2499	1.1835	1.5274
	RSD	0.0078	0.0125	0.0118	0.0153

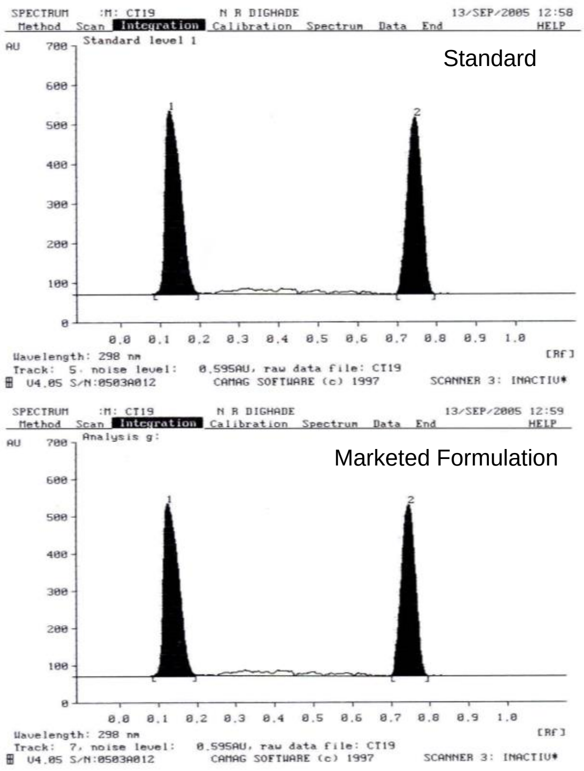


Figure 5: Densitogram of Ciprofloxacin, Tinidazole and ZOXAN-TZ

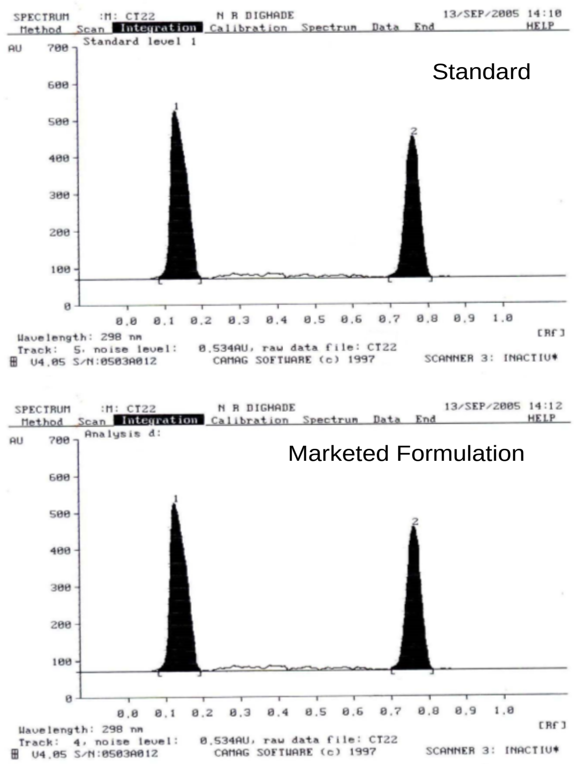


Figure 6: Densitogram of Ciprofloxacin, Tinidazole and CITIZOL

Table 3: Estimation results of CP and TZ in the marketed formulation.

CITIZOL		Average Weight = 1368.7 mg			
Sr. No.	Weight of tablet powder taken (mg)	Percent label claim			
		CP		TZ	
		By height	By area	By height	By area
1	69.65	98.05	98.20	100.15	100.46
2	67.7	99.38	100.94	101.42	100.53
3	70.2	98.30	99.47	100.38	100.35
4	68.7	98.08	98.39	100.12	100.20
5	68.5	98.21	98.09	100.28	101.40
	Mean	98.40	99.02	100.47	100.59
	±SD	0.5548	1.2066	0.5412	0.4707
	CV	0.5638	1.2185	0.5387	0.4680
	RSD	0.0056	0.0122	0.0054	0.0047

Table 4: Results of Recovery Studies (Zoxan-TZ)

Sr. No.	Wt. of tablet powder taken (mg)	Amount of drug added (mg)		Percent recovery			
		CP	TZ	CP		TZ	
				By height	By area	By height	By area
1	58.168	3	3.6	98.33	99.33	100.55	99.16
2	50.236	5.4	6.48	99.81	100.37	100.3	99.53
3	42.124	8.7	10.5	99.42	98.85	100.00	100.85
4	34.372	12.6	15.12	99.60	100.47	100.33	99.80
5.	26.44	13.88	16.5	99.92	98.55	100.3	101.03
			Mean	99.41	99.51	100.29	100.07
			±SD	0.6368	0.8733	0.1957	0.825
			CV	0.6406	0.8775	0.195	0.8243
			RSD	0.0064	0.0087	0.0019	0.0082

Table 5: Results of Recovery Studies (CITIZOL)

Sr. No.	Wt. of tablet powder taken (mg)	Amount of drug added (mg)		Percent recovery			
		CP	TZ	CP		TZ	
				By height	By area	By height	By area
1	60.22	2.9	3.48	98.27	99.31	100.5	101.43
2	50.01	5.8	6.95	101.72	98.44	102.3	98.56
3	43.8	8.6	10.4	100.93	98.95	100.09	102.4
4	35.59	11.1	13.7	99.09	98.19	101.24	101.75
5.	27.38	14.76	17.4	98.17	98.37	98.33	100.68
			Mean	99.63	98.65	100.49	100.96
			±SD	1.6070	0.4635	1.4717	1.4796
			CV	1.6129	0.4699	1.4645	1.4654
			RSD	0.0161	0.0047	0.0146	0.0146

Table 6: Results of Specificity Studies

Sr. No.	Stress condition	Percent label claim			
		CP		TZ	
		By height	By area	By height	By area
1	Acid	101.03	101.63	100.41	98.04
2	Alkali	80.60	60.34	79.87	72.46
3	Oxide	98.17	98.37	94.90	93.67
4	Heat	99.60	98.70	101.44	101.60
5	UV	95.38	96.77	98.33	100.68

Table 7: Results of Linearity Studies

Drug	Linearity range (µg)		Coefficient correlation		Slope		Y-Intercept	
	By Height	By Area	By Height	By Area	By Height	By Area	By Height	By Area
CP	0.5 to 0.9		0.9993	0.9981	230.06	13078.73	319.39	6356.40
TZ	0.6 to 1.1		0.9956	0.9869	230.15	7297.09	294.72	8327.53

Table 8: Results of Ruggedness Studies for different times

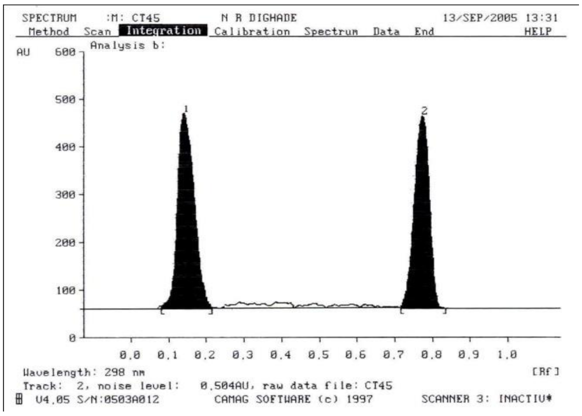
Sr No.	Sample		Percent label claim			
			CP		TZ	
			By Height	By Area	By Height	By Area
1	Morning		99.38	100.94	100.42	100.53
2	Afternoon		98.30	99.47	100.38	100.35
3	Evening		98.08	98.39	100.12	100.2
		Mean	98.59	99.60	100.31	100.36
		±SD	0.6958	1.2800	0.1629	0.1652
		CV	0.7058	1.2851	0.1624	0.1646
		RSD	0.0071	0.0129	0.0016	0.0016

Table No. 9: Results of Ruggedness Studies for different days

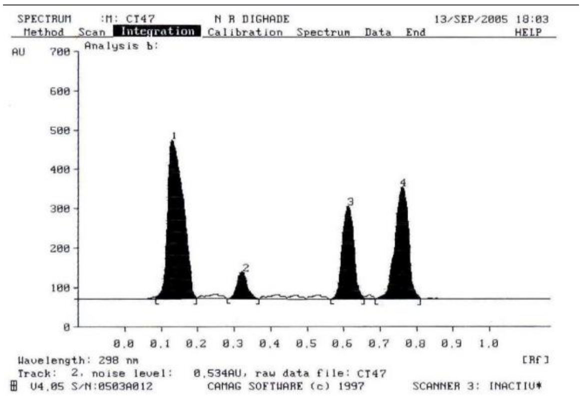
Sr No.	Days		Percent label claim			
			CP		TZ	
			Peak height	Peak area	Peak height	Peak area
1	Day-1		98.22	101.91	99.77	99.94
2	Day-2		98.12	100.56	99.47	98.29
3	Day-3		99.94	100.12	99.42	101.30
		Mean	98.22	101.91	99.77	99.94
		±SD	1.0231	0.9327	0.1893	1.5073
		CV	1.0359	0.9247	0.1901	1.5096
		RSD	0.0103	0.0092	0.0019	0.0151

Table 10: Results of Ruggedness Studies for different analysts

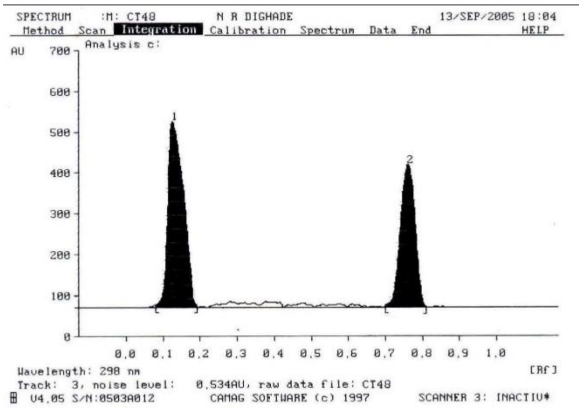
Sr. No.	Analysts		Percent label claim			
			CP		TZ	
			Peak height	Peak area	Peak height	Peak area
1	Analyst – 1		98.81	98.26	100.98	100.90
2	Analyst – 2		98.65	100.05	99.88	98.39
3	Analyst – 3		100.03	100.83	100.58	99.23
		Mean	99.16	99.71	100.48	99.51
		±SD	0.7548	1.3177	0.5568	1.2777
		CV	0.7612	1.3215	0.5541	1.2840
		RSD	0.0076	0.0132	0.0055	0.0128



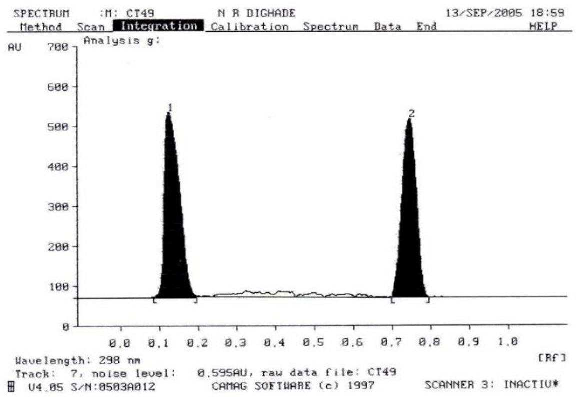
A : Stress : Acidic medium



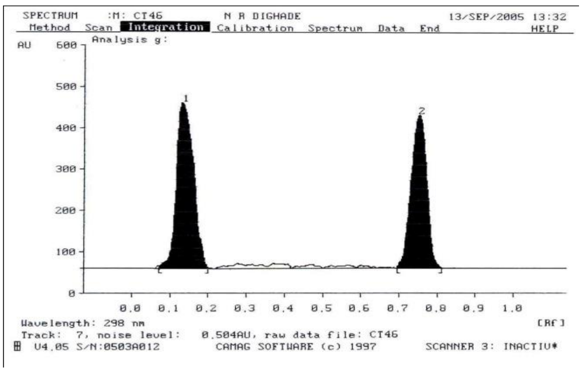
B : Stress : Alkali medium



C : Stress : Oxidation



D : Stress : Heat (60º)



E : Stress : UV Light

Figure 7 (A-E). Densitograms of specificity study of CP and TZ

Ruggedness: Proposed method was assessed by testing the marketed formulation under three varying conditions: at different times of the day on the same day, on separate days, and by different analysts.

RESULTS

The results are shown in Table No. 8 for different times, Table No. 9 for different days and Table No. 10 for different analyst. The results from testing the proposed formulation and its validation confirm that the method is both accurate (providing correct results) and precise (consistent results across repeated measurements). Therefore, this method can be reliably used for the routine evaluation of Ciprofloxacin (CP) and Tinidazole (TZ) in marketed preparations, ensuring the quality and consistency of the drug products in regular testing.

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