ORIGINAL ARTICLE

Derivative Spectrophotometric Methods for the Determination of Tilorone - An Antiretroviral Drug

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Abstract

Introduction: New spectrophotometric methods have been developed for the determination of Tilorone in pharmaceutical formulations. Spectrophotometric techniques have been proposed for the determination of Tilorone in sodium acetate buffer (pH 4), borate buffer (pH 9), and phosphate buffers (pH 2.0 and pH 5.0). **Materials and Methods:** Shimadzu UV-1800 model ultraviolet-visible spectrophotometer double beam was used for the present study. Three different spectrophotometric techniques were developed using sodium acetate buffer (pH 4), borate buffer (pH 9), and phosphate buffers (pH 2.0 and pH 5.0). **Results and Discussion**: Tilorone has shown absorption maxima at 270 nm, and linearity 0.4–14 µg/mL in all the three methods in all the four buffer solutions and all the methods were validated as the per ICH guidelines. **Conclusions:** These methods are simple, economical and can be successfully applied for the estimation of tilorone in pharmaceutical dosage forms.

Key words: First-order derivative spectroscopy (D_1), ICH guidelines, second-order derivative spectroscopy (D_2), tilorone, validation, zero order (D_0)

INTRODUCTION

Tilorone is a new class of anti-viral drug, approved by the FDA. Tilorone is an orally active interferon inducer.[1] It specifically induces a delayed and unusual prolonged interferon response which is not common in comparison to other synthetic inducers.[2-5] Tilorone is an orange colored powder and soluble freely in methanol, water and sparingly soluble in ethanol, dimethyl sulfoxide, and dimethylformamide. Chemically, it is 2, 7-bis-[2(dimethylamino)-ethoxy] - fluorene-9-one with molecular formula $C_{25}H_{34}N_2O_3$ (molecular weight: 410.55 g/mol). There is not even a single spectrophotometric method in the literature for the determination of tilorone in the literature and only a single LC-MS/MS method[6] was reported for its quantification in human blood. In the present study, the authors have proposed four zero order (A), four first-order derivative (B), and four second-order derivative (C) methods for the assay of tilorone in various buffer solutions and all the methods were validated as the per ICH guidelines [Figure 1].[7]

MATERIALS AND METHODS

Model No. UV-1800 double beam ultraviolet (UV)-visible spectrophotometer (Shimadzu) with quartz cells is used for the entire study, and all the solutions were scanned 200–400 nm.

Preparation of solutions

Buffer solutions such as sodium acetate (pH 4), borate buffer (pH 9), phosphate buffer pH 2.0, and phosphate buffer pH 5.0 were prepared as per the IP 2010. Stock solution of tilorone was prepared by dissolving 25 mg of tilorone in 25 mL volumetric flask with methanol (1000 µg/mL), and further

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working standard solutions (100 µg/mL) were prepared by diluting the stock solution with respective buffers as per the requirement for the proposed methods. Tilorone is available as film-coated tablets with brand name LAVOMAX (Label claim: 60 mg). Tablet formulation is not available in India, and hence tablet formulations were developed in our laboratory with the available excipients and assay was performed.

Method validation

Linearity

A. Zero-order spectroscopy (D0)
   A series of tilorone solutions 0.4–14 µg/mL were prepared from the stock solution on dilution with sodium acetate buffer pH 4.0 (Method I) and scanned (200–400 nm) against reagent blank. The zero-order spectrum so obtained has shown maximum absorbance (λ_{max}) at 270 nm. The absorbance of all the solutions was noted at λ_{max}, and a calibration curve was drawn by taking the concentration on the X-axis and the corresponding absorbance on the Y-axis. Similarly, the same procedure was repeated with other buffer solutions also, i.e., borate buffer (pH 9), phosphate buffer pH 2.0, and phosphate buffer pH 5.0 for Method II-IV, respectively.
B. First-order derivative spectroscopy (D1)
   The individual zero-order absorption spectra of tilorone (0.4–14 µg/mL) so obtained in Method I-IV were converted into their first-order derivative spectra with the help of inbuilt software of the instrument in all the buffers and the resultant derivative spectra have shown minima and therefore minima has been chosen against the concentration to plot the calibration curves for Method I-IV.
C. Second-order derivative spectroscopy (D2)
   The four zero-order spectra (D_0) obtained in four reagents such as sodium acetate (pH 4) (Method I) borate (pH 9) (Method II), phosphate (pH 2) (Method III), and phosphate buffer pH 5.0 (Method IV) were transformed in to second-order derivative (D_2) spectra by the inbuilt software of the instrument. The derivative spectra so obtained have shown maxima and minima, and therefore the amplitude has been chosen against the concentration to plot the calibration curves for Method I-IV.

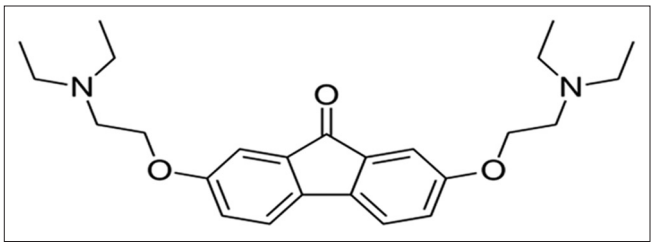

Figure 1: Chemical structure of tilorone

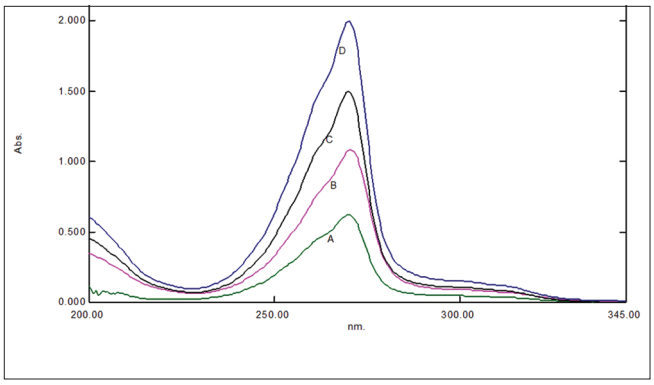

Figure 2: Absorption spectrum of tilorone (D_0) (a) sodium acetate buffer (4 µg/mL), (b) Borate buffer (8 µg/mL), (c) phosphate buffer pH 2.0 (10 µg/mL), and (d) phosphate buffer pH 5.0 (14 µg/mL)

Precision and accuracy studies

The intraday and interday precision studies were performed at three different concentration levels (3, 6, and 12 µg/mL) and accuracy studies were carried out by standard addition method (50%, 100%, and 150%). The percentage recovery was calculated for all three techniques A, B, and C.

Assay of tilorone

A total of 20 tilorone laboratory prepared tablets were taken, weighed and powdered, and powder equivalent to 25 mg of tilorone was extracted with methanol in a 25 mL volumetric flask and dilutions were made with sodium acetate buffer pH 4, borate buffer pH 9, and phosphate buffers (pH 2 and 5). The assay was carried out using the analytical techniques A, B, and C as per the procedure.

RESULTS AND DISCUSSION

Three different analytical techniques (A) zero order, (B) first-order derivative spectroscopy, and (C) second-order derivative spectroscopy have been developed for the determination of tilorone (tablets).

D. Zero-order spectroscopy (D0)
   The overlay absorption spectrum obtained in (A) zero-order spectrophotometric technique in sodium acetate (pH 4) (Method I), borate buffer (pH 9) (Method II), phosphate (pH 2) (Method III), and phosphate buffer pH 5.0 (Method IV) was shown in Figure 2. Tilorone has shown absorption maxima at 270 nm and obeys Beer-Lambert's law over the concentration range 0.4–14 µg/mL [Table 1] in all the four buffer solutions with linear regression equations y = 0.150x - 0.013 (R^2 = 0.999), y = 0.129x - 0.009 (R^2 = 0.999) and y = 0.149x - 0.022 (R^2 = 0.999), and y=0.144x-0.0007 (R^2 = 0.999) for Method I-IV [Figure 3], respectively. The optical characteristics were given in Table 2. The percentage RSD in precision and accuracy studies was

Table 1: Linearity of tilorone (a) zero-order spectroscopy

Concentration (μg/mL)	Absorbance			
	Method I	Method II	Method III	Method III
0.4	0.077	0.054	0.038	0.050
0.5	0.091	0.066	0.045	0.066
0.8	0.139	0.085	0.074	0.10
2	0.312	0.25	0.259	0.306
3	0.455	0.37	0.398	0.43
4	0.622	0.521	0.571	0.558
5	0.811	0.64	0.741	0.731
6	0.905	0.761	0.871	0.861
7	1.067	0.85	1.041	1.02
8	1.202	1.02	1.191	1.15
9	1.397	1.14	1.32	1.297
10	1.508	1.305	1.452	1.425
12	1.785	1.53	1.762	1.725
14	2.12	1.785	2.074	2.03

Table 2: Optical characteristics of tilorone in (A) zero-order spectroscopy

Parameters	Method			
	I	II	III	IV
Linearity range (μg/mL)	0.4–14	0.4–14	0.4–14	0.4–14
λ_{max} (nm)	270	270	270	270
Molar extinction coefficient (liter/mole/cm)	6.15825×10^4	5.357677×10^4	5.961186×10^4	5.850337×10^4
Sandell's sensitivity (μg/cm²/0.001 absorbance unit)	0.0066	0.00766	0.00688	0.00701
Slope	0.150	0.129	0.149	0.144
Intercept	0.013	0.009	0.022	0.00
Correlation coefficient	0.999	0.999	0.999	0.999
Precision (%RSD)	0.45–1.49	0.80–1.53	0.45–1.49	0.64–1.05
Accuracy (%RSD)	0.62–1.419	0.76–1.90	0.58–1.6	0.58–1.34
Assay (%)	98.90	98.54	98.71	98.86

found to be <2 in all the four methods indicating that the methods are precise and accurate.

First-order derivative spectroscopy (D_1)

The overlay first-order derivative spectra of tilorone in sodium acetate buffer pH 4, borate buffer pH 9, phosphate buffer pH 2, and phosphate buffer pH 5, i.e., for Method I–IV, respectively, were shown in Figure 4, and the spectral characteristics observed were shown in Table 3. Tilorone follows Beer-Lambert's law [Figure 5] over the concentration range 0.4–14 μg/mL in all Methods I-IV and the percentage RSD values in precision and accuracy studies were found to be <2 indicating that all the methods are precise and accurate [Table 4].

Second-order derivative spectroscopy (D_2)

The overlay second-order derivative spectra of tilorone in sodium acetate buffer pH 4, borate buffer pH 9, phosphate buffer pH 2, and phosphate buffer pH 5, i.e., for Method I–IV, respectively, were shown in Figure 6, and the spectral characteristics observed were shown in Table 5a and b. Tilorone follows Beer-Lambert's law [Figure 7] over the concentration range 0.4–14 μg/mL in all Methods I-IV and the percentage RSD values in precision and accuracy studies were found to be <2 indicating that all the methods are precise and accurate [Table 6].

Table 3: Linearity of tilorone (B) first-order derivative spectroscopy

Concentration (μg/mL)	Method I	Method II	Method III	Method IV
	Minima	Minima	Minima	Minima
0.4	0.006	0.005	0.004	0.006
2	0.03	0.018	0.027	0.031
3	0.044	0.035	0.04	0.041
4	0.063	0.048	0.058	0.057
5	0.079	0.063	0.074	0.074
6	0.091	0.072	0.084	0.085
7	0.108	0.084	0.104	0.103
8	0.119	0.095	0.113	0.112
9	0.137	0.106	0.127	0.131
10	0.149	0.12	0.141	0.141
12	0.179	0.144	0.17	0.171
14	0.209	0.242	0.206	0.201

Table 4: Characteristics of tilorone (B) first-order derivative spectroscopy

Parameters	Method I	Method II	Method III	Method IV
Linearity range (μg/mL)	0.4–14	0.4–14	0.4–14	0.4–14
Accuracy % recovery (%RSD)	99.1–100.25 (1.13)	99.9–100.26 (1.29)	99.5–99.93 (1.40)	99.9–100.43 (1.19)
Precision intraday (% RSD)	0.7–1.40	0.66–1.65	1.00–1.094	0.60–1.51
Interday (% RSD)	0.45–1.1	0.95–1.94	0.82–1.15	0.45–1.20

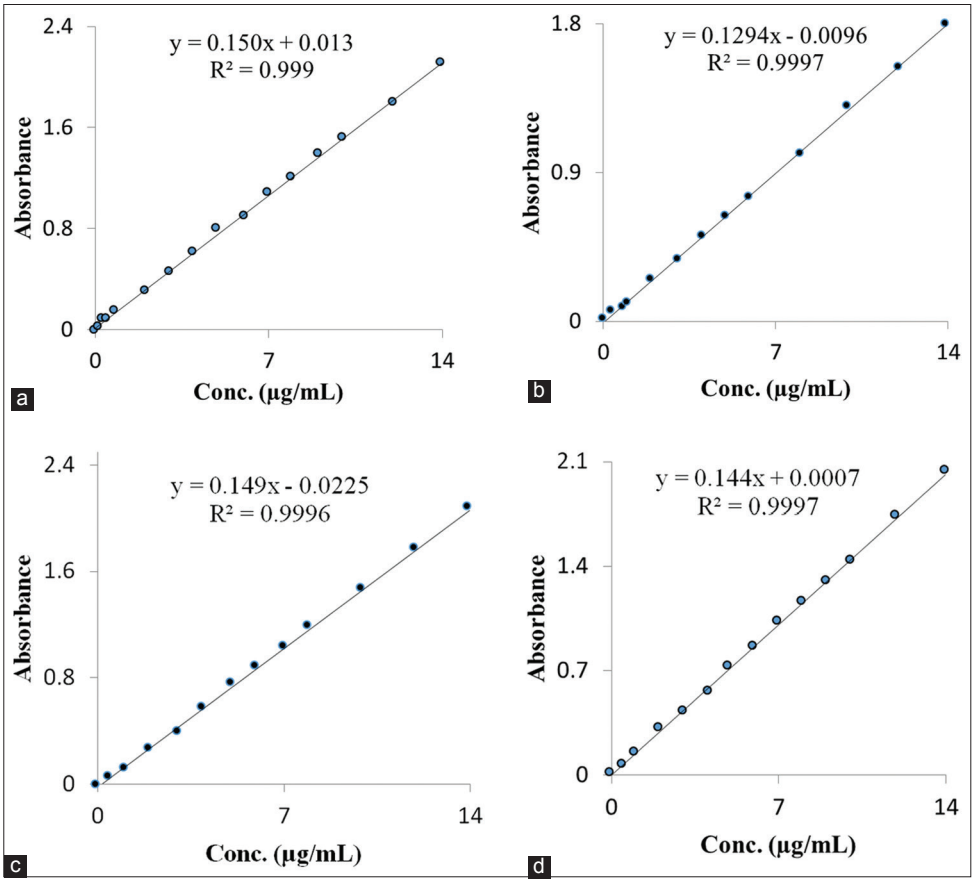

Figure 3: Calibration curves of tilorone (D_0) in (a) sodium acetate buffer (b) borate buffer, (c) phosphate buffer pH 2, and (d) phosphate buffer pH 5

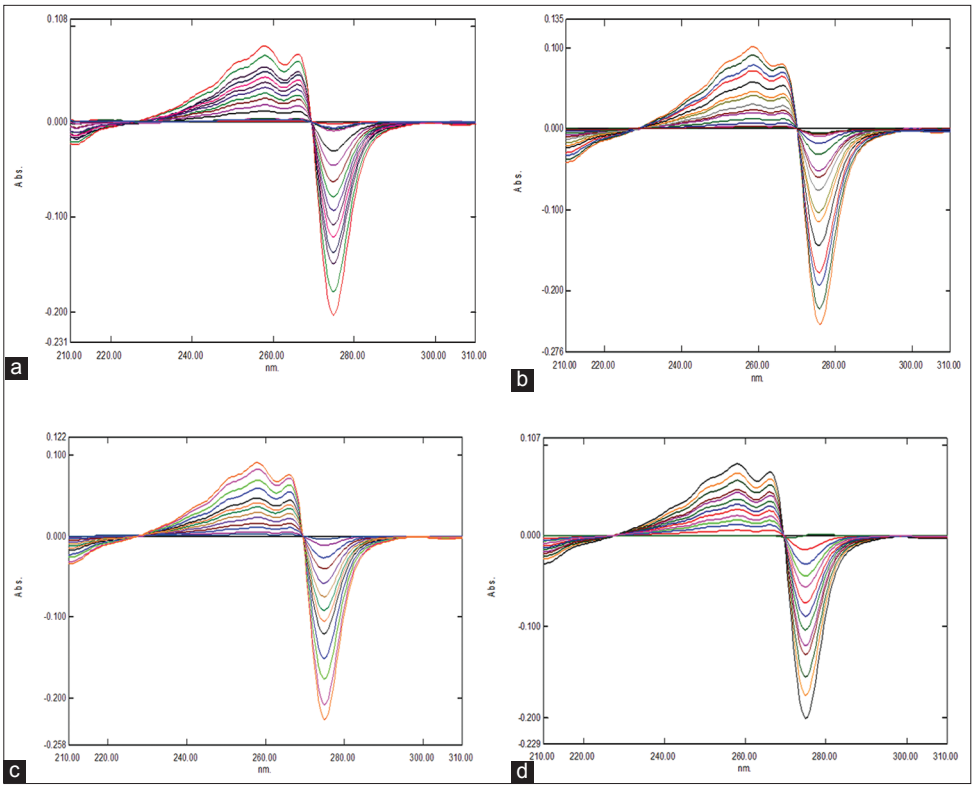

Figure 4: Overlay first derivative spectrum (D1) of tilorone in (a) sodium acetate buffer, (b) borate buffer, (c) phosphate buffer pH 2.0, and (d) phosphate buffer pH 5.0

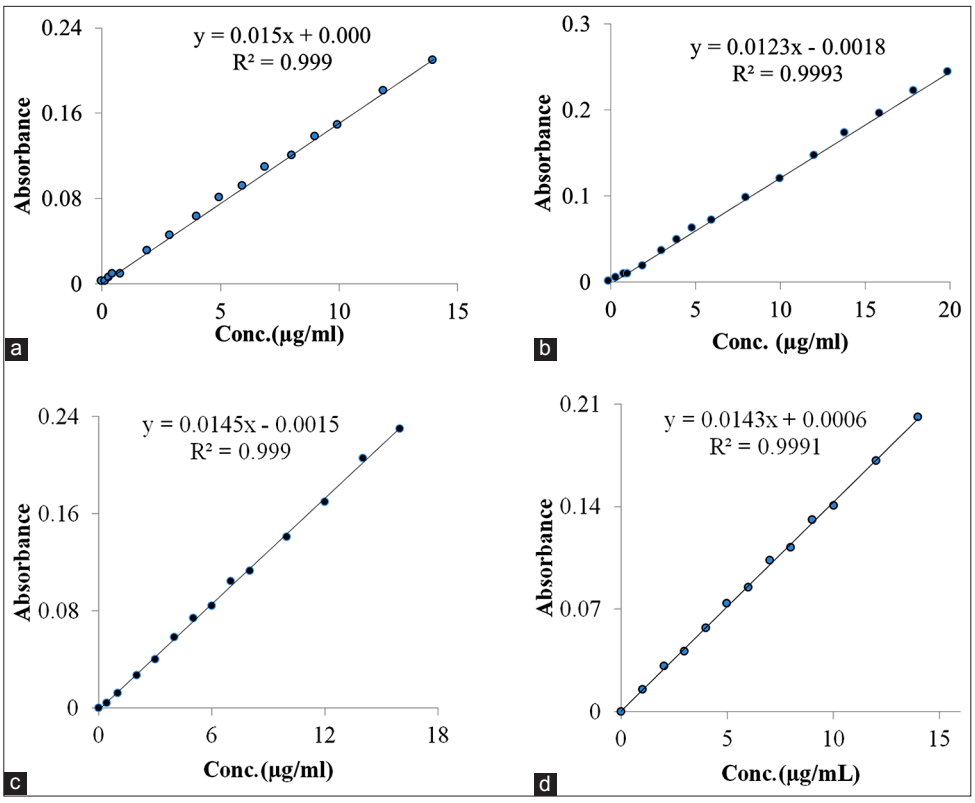

Figure 5: Calibration curves of tilorone (D_1) in (a) sodium acetate buffer, (b) borate buffer, (c) phosphate buffer pH 2, and (d) phosphate buffer pH 5

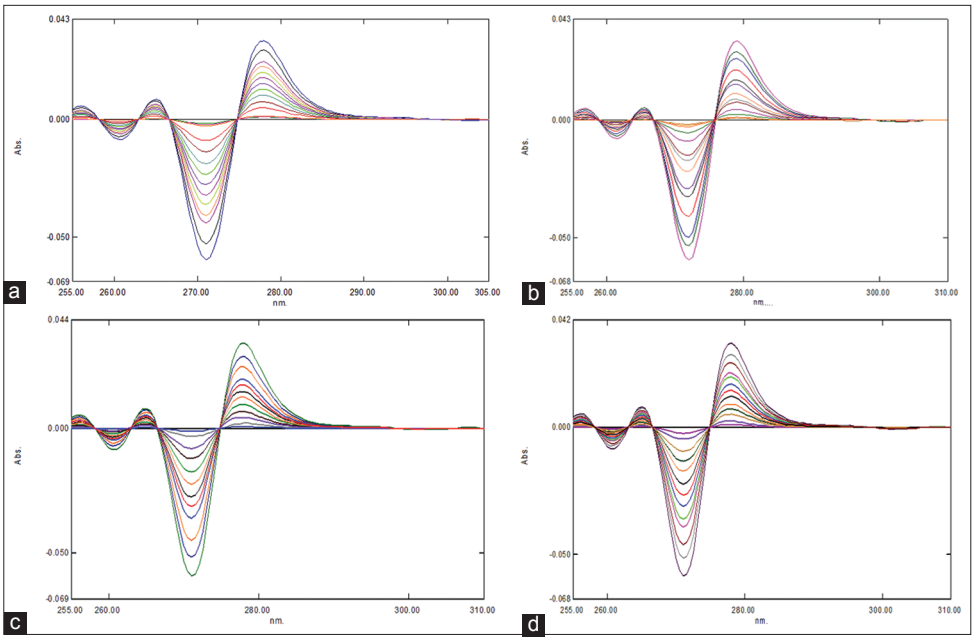

Figure 6: Overlay second derivative spectrum (D_1) of tilorone in (a) sodium acetate buffer,(b) borate buffer, (c) phosphate buffer pH 2.0, (d) phosphate buffer pH 5.0

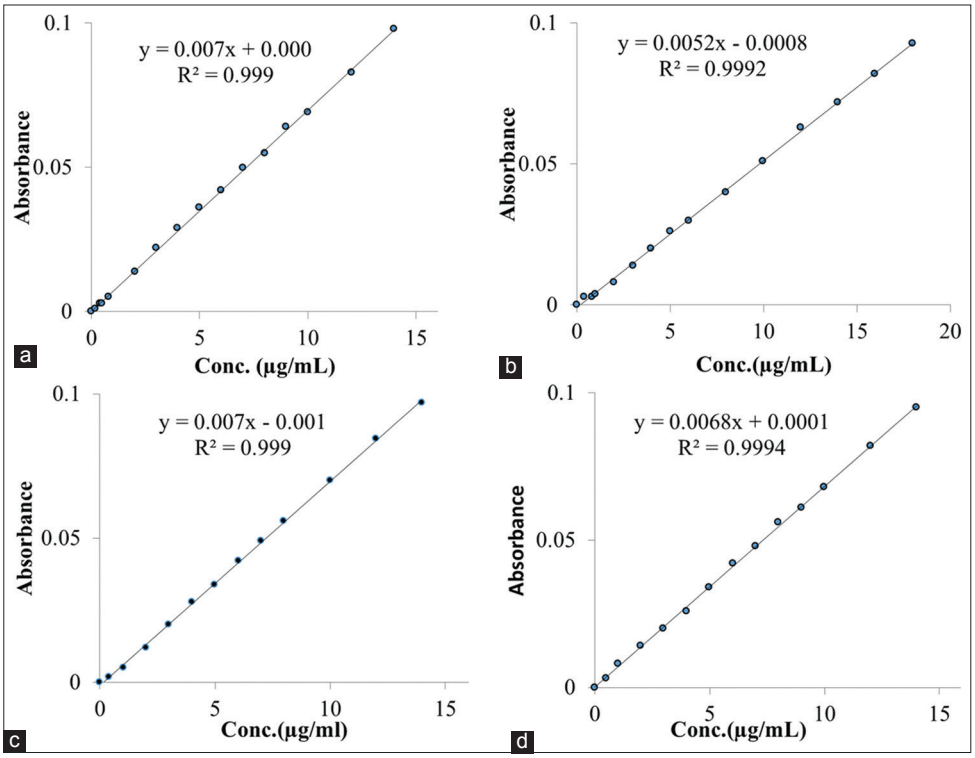

Figure 7: Calibration curves of tilorone (D_2) in (a) sodium acetate buffer, (b) borate buffer, (c) phosphate buffer pH 2.0, and (d) phosphate buffer pH 5.0

CONCLUSION

The three validated spectrophotometric techniques were found to be simple, precise, accurate, and economical and can be used for the routine analysis of Tilorone in pharmaceutical formulations.

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Table 5a: Linearity of tilorone (C) second-order derivative spectroscopy

Concentration (μg/mL)	Method I sodium acetate buffer pH 4			Method II borate buffer pH 9		
	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude
0.4	0.001	0.002	0.003	0.001	0.002	0.003
2	0.005	0.009	0.014	0.003	0.005	0.008
3	0.008	0.014	0.022	0.005	0.009	0.014
4	0.010	0.019	0.029	0.008	0.012	0.02
5	0.013	0.023	0.036	0.009	0.017	0.026
6	0.015	0.027	0.042	0.011	0.019	0.030
7	0.018	0.032	0.05	0.012	0.024	0.036
8	0.02	0.035	0.055	0.015	0.025	0.040
9	0.023	0.041	0.064	0.016	0.030	0.046
10	0.025	0.044	0.069	0.017	0.036	0.051
12	0.029	0.054	0.083	0.022	0.041	0.063
14	0.033	0.065	0.098	0.026	0.046	0.072

Table 5b: Linearity of tilorone (C) second-order derivative spectroscopy

Concentration (μg/mL)	Method III phosphate buffer pH 2			Method IV phosphate buffer pH 5		
	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude
0.4	0.001	0.001	0.002	0.001	0.002	0.003
2	0.004	0.008	0.012	0.005	0.009	0.014
3	0.007	0.012	0.019	0.007	0.013	0.02
4	0.010	0.018	0.028	0.009	0.017	0.026
5	0.012	0.022	0.034	0.012	0.022	0.034
6	0.015	0.028	0.042	0.015	0.027	0.042
7	0.017	0.032	0.049	0.017	0.031	0.048
8	0.020	0.036	0.056	0.020	0.036	0.056
9	0.022	0.042	0.064	0.022	0.039	0.061
10	0.025	0.045	0.07	0.026	0.042	0.068
12	0.029	0.056	0.084	0.029	0.053	0.082
14	0.034	0.063	0.097	0.033	0.062	0.095

Table 6: Characteristics of tilorone (C) second-order derivative spectroscopy

Parameters	Method I	Method II	Method III	Method IV
Linearity range (μg/mL)	0.4–14	0.4–14	0.4–14	0.4–14
accuracy (% recovery) (% RSD)	99.7–100.4 (1.42)	98.86–99.83 (1.53)	99.96–100.1 (1.68)	99.4–99.6 (0.69)
Precision				
Intraday (% RSD)	1.35–1.74	1.12–1.53	0.79–1.34	0.60–1.15
Interday (% RSD)	0.46–1.71	0.99–1.61	0.67–1.29	0.47–1.10
Assay (%RSD)	98.87	98.68	98.71	98.80

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