Determination of Spiramycin and Oxytetracycline in binary mixtures by novel spectrophotometric methods with and without regression equation

Original Article

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ABSTRACT

Spiramycin (SPI) and oxytetracycline (OTC) are widely used antibacterial drugs. Thus, a great attention has been focusing on the development of a simple and selective analytical procedure for fast analysis of these compounds in a binary pharmaceutical mixture. Herein, we report four simple and reproducible spectrophotometric methods for determination of SPI and OTC in a binary mixture and in veterinary pharmaceutical formulations. A ratio difference (RD) based on the difference in the amplitude of the ratio spectra and dual wavelength methods were proposed for sensitive and selective determination of SPI in presence of OTC. Furthermore, Constant Value and Concentration Value based spectrophotometric methods were suggested for simultaneous determination of SPI and OTC in a binary mixture. The methods were successfully applied for the determination of SPI and OTC over the concentration range from 4.0 to 36.0 µg mL^{-1} and from 2.0 to 32.0 µg mL^{-1}, respectively. The proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines. The methods are accurate (100±1%), precise (%RSD ≤1.5) and cost-effective for determination of SPI and OTC in binary mixtures.

Key Words: Concentration value, Constant value, Dual wavelength, Oxytetracycline, Ratio difference, Spectrophotometry, Spiramycin.

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INTRODUCTION

Spiramycin (Figure 1) is a macrolide antibacterial drug which is used to treat susceptible bacterial infections. It has also been used in the protozoal infections cryptosporidiosis and toxoplasmosis[1]. Spiramycin is a mixture of basic antimicrobial substances produced by the growth of Streptomyces ambofaciens and consistes of spiramycin I ($C_{43}H_{74}N_2O_{14}$, MW= 843.1) (about 63%), spiramycin II ($C_{45}H_{76}N_2O_{15}$, MW=885.1) (about 24%) and spiramycin III ($C_{46}H_{78}N_2O_{15}$ MW = 899.1) (about 13%)[2]. Oxytetracycline, on the other hand, is a tetracycline derivative which has a pharmacological action similar to that of tetracycline (Figure 1)[1]. Although spiramycin is produced by some pharmacopeias as a reference standard[3], there is no pharmacopeial method that can be followed for determination of spiramycin in veterinary dosage forms.

Several non-official analytical methods, however, were reported for the assay of SPI and OTC. These methods include liquid chromatography[4-8] and spectrophotometric methods[9,10] for the assay of SPI; and liquid chromatography[11-17], thin layer chromatography[18,19], spectrophotometry[20,21] and electrochemical methods[22] for the assay of OTC. A densitometic method was reported for simultaneous determination of SPI and OTC in a binary mixture[23]. The proposed method uses a mobile phase which consists of environmentally unfavourable solvents. Additionally, the desitometric chromatogram that shows semiltaneous separation and determination of SPI and OTC was not displayed.

In this his work, we report new simple and reliable spectrophotometric methods for the determination of SPI and OTC in a mixture without the need of separation or sophisticated manipulation steps. The Constant Value and Concentration Value methods use a normalized divisor which could be calculated mathematically to get a plateau region through which the compound can be determined and not affected by any change in the wavelength. Concentration value is considered as a new approach in spectrophotometry because it is the first spectrophotometric method that determines the concentration of the drugs

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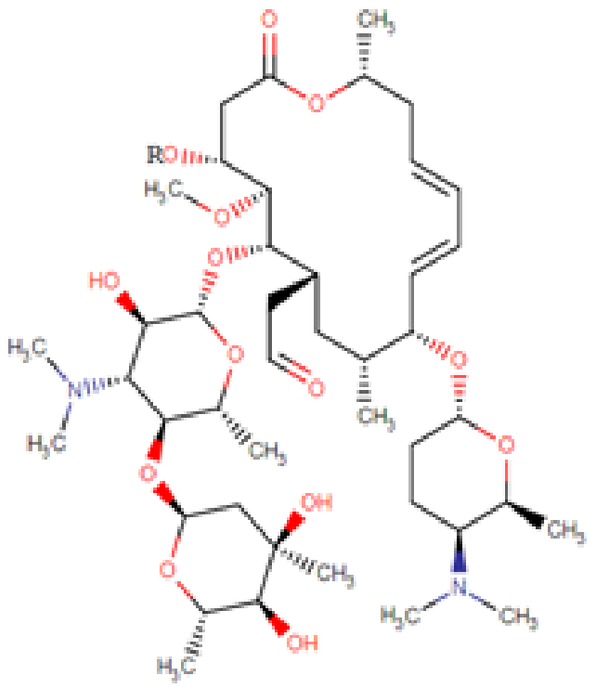
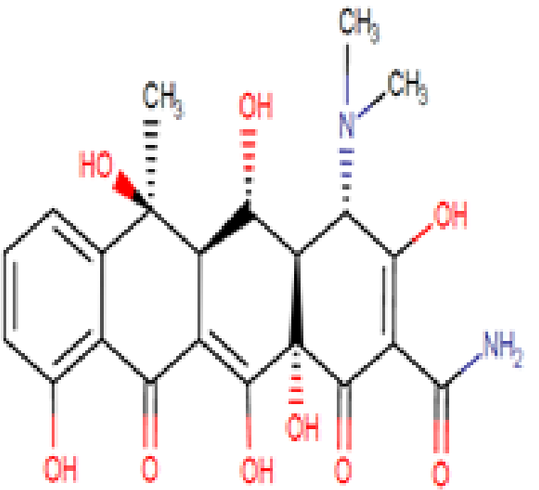
without using regression equations, where the analysis solely depends on the spectral representation rather than the calibration curves[24]. Also, in the ratio difference method, the difference in the amplitude between two points on the ratio spectra of a mixture was found to be directly proportional to the concentration of the component of interest; independent of the interfering component. Additionally, a dual wave length method was used for the determination of one component in presence of the other.

The proposed methods were successfully applied for the simultaneous determination of SPI and OTC in laboratory prepared mixtures and in pharmaceutical dosage form.



Spiramycin I R=H
Spiramycin II R=COCH3
Spiramycin III R=COCH2CH3
(SPI)



(OTC)

Fig 1: Chemical structure of Spiramycin (SPI) and Oxytetracycline (OTC).

2 Experimental

2.1 Apparatus

All spectrophotometric measurements were carried out using Shimadzu UV-1601 PC spectrophotometer (Kyoto, Japan) provided with two matched 1-cm quartz cells and spectroscopy software version (2.21). The spectral band width is 0.1 nm and the scanning speed is 2800 nm/min.

2.2 Materials

Pure standards

Spiramycin (purity=98.80%) was obtained from Henan Topfond Pharmaceuicals Co., Ltd (Zhumadian, China). Oxytetracycline HCl (purity=97.60%) was obtained from Pharma Swede, 10th of Ramadan, Egypt.

Pharmaceutical formulation

Arcospirox® powder (batch number: 100716/A) contains 107.9 mg (OTC) and 71.8 mg (SPI) per 1.0 gram was obtained from Arco Pharma, Cairo, Egypt.

2.3 Working solutions

All preparations were carried out using double distilled water, previously filtered through a 0.45 µm filter paper.

Stock standard solutions

An accurately weighed 10.0 mg of SPI and OTC were dissolved each in 100 mL water to give stock standard solutions with a concentration of 100.0 µg mL^{-1} for each compound.

Linearity solutions

Linearity solutions covering the concentration range from 4.0-36.0 and 2.0-32.0 µg mL^{-1} for SPI and OTC, respectively, were prepared by appropriate dilution from the corresponding stock standard solutions. The spectrum of each individual solution was recorded in the range from 200 to 400 nm and stored in the computer.

METHODS

2.4.1. Linearity and Construction of Calibration Curves

2.4.1.1 Ratio difference method (RD)

The recorded spectra of SPI are divided by the recorded spectrum of 32.0 µg mL^{-1} OTC and the difference in the amplitude of the ratio spectra (SPI/OTC) at 232 and 292 nm ($\Delta P_{232-292}$) is plotted against the corresponding concentrations of SPI.

2.4.1.2 Dual wavelength method (DWL)

The SPI spectra are recorded and the difference between the absorbance at 232 and 375.5 nm was plotted against the corresponding concentration. Note: the difference between the absorbance at 232 and 375.5 nm is zero for OTC.

2.4.1.3 Constant Value (CV) and Concentration Value (Conc. Value) of Oxytetracycline:

In these methods the zero-order spectrum of the more extended component (OTC) was divided by the zero order normalized spectrum divisor of OTC (1 µg mL^{-1}); the constant was obtained from the plateau region which is related to the concentration of OTC. In the CV method the regression equation was computed relating the constant to OTC concentration, while for the Conc. Value there was no need for regression equation. The concentration is equal to the constant in the plateau region can be directly obtained from the graphical representation.

2.4.1.4 Ratio Subtraction Constant Value (RS-CV) and Ratio Subtraction Concentration Value (RS-Conc. Value) of Spiramycin:

The spectra of pure SPI were divided by its normalized spectrum divisor of SPI; the constant was obtained from the plateau region which is related to the SPI concentration. The calibration curve was constructed between the concentration and the corresponding constant for the (CV) method. While for (Conc. Value) the concentration can be directly obtained from the graphical representation.

2.4.2 Application of the proposed methods for the determination of SPI and OTC in laboratory prepared mixtures

Into a series of 10-mL volumetric flasks, aliquots of SPI and OTC were accurately transferred from the stock standard solutions with the ratios of 1:8, 1:4, 1:1, 8:1 and 4.5:8 for SPI and OTC, respectively. Afterwards, each flask was completed to the mark with water. The spectra of the prepared mixtures were recorded and stored in the computer.

For the Ratio Subtraction Constant Value (RS-CV) and Ratio Subtraction Concentration Value (RS-Conc. value) of Spiramycin, the recorded zero order spectra of the laboratory prepared mixtures were divided by the absorption spectrum of standard OTC (32.0 µg mL^{-1}). Then, the amplitudes in the plateau region at λ 274-284 nm (the constant) were recorded and subtracted from the obtained ratio spectra, respectively. Then, multiplying the obtained spectra by OTC (32.0 µg mL^{-1}) to get the zero spectra of SPI and application of Constant Value (CV) and Concentration Value (Conc. value) methods.

For the other methods, applying the proposed methods, the concentrations of SPI and OTC in the prepared mixtures are calculated from the corresponding equations.

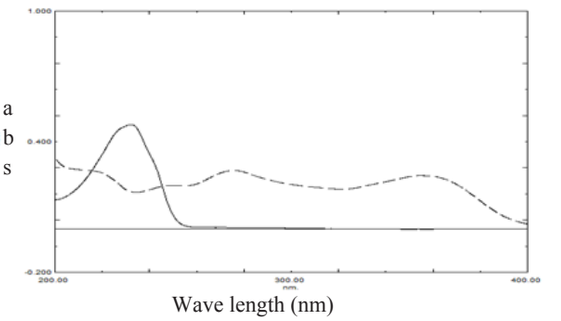
2.4.3 Application of the proposed methods for the determination of SPI and OTC in pharmaceutical formulation

An Accurately weighed 92.7 mg of the Arcospirox powder equivalent to 10.0 mg of OTC and 6.65 mg SPI was transferred into 100- mL volumetric flask, dissolved in 50 mL water and sonicated for 15 min. Then, the flask was completed to the mark with water. Further dilution was carried out by transferring 1, 2 and 3 mL into a series of 10-mL volumetric flasks.

The solutions were subjected to the analytical methods which are described previously. The concentrations of SPI and OTC were calculated from the corresponding regression equations. For the (RS-CV) and (RS-Conc.V) methods, the procedure mentioned under analysis of laboratory prepared mixtures was used. The validity of the methods was assessed using the standard addition technique.

RESULTS AND DISCUSSION

SPI and OTC are widely used antibacterial veterinary. Most of antibacterial veterinary dosage forms are produced with combination of SPI and OTC. Direct spectrophotometric determination of SPI and OTC in a binary mixture is difficult due to the sever overlap of the spectra of the two compounds (Figure 2). Therefore, this work was developed to enable simultaneous determination of SPI and OTC in veterinary dosage forms.



Wave length (nm)

Fig 2: Zero order spectra of 20 µg mL^{-1} SPI (-) and 8 µg mL^{-1} OTC (---) recorded against water as blank.

3.1 Method development and optimization

Ratio difference method (RD)

The major factors affecting the ratio difference[25] are the divisor concentration and the selected wavelength. The selected value for the divisor is a compromise between minimal noise and maximum sensitivity. Therefore, different concentrations including 4.0, 16.0 and 32.0 µg mL^{-1} of OTC as a divisor were tested. Satisfactory recovery, repeatability and signal to noise ratio were obtained with 32.0 µg mL^{-1} of OTC as a divisor. The second factor is the wavelengths at which the measurements are performed. Any two wavelengths can be selected provided that they exhibit difference in the amplitude of the ratio spectrum, and a good linearity is present at each wavelength individually.

The values of amplitude at 232 and 292 nm were chosen for determination of SPI in presence of OTC. The best results were obtained with OTC concentration of 32.0 µg mL^{-1} as a divisor (Figure 3).

A linear relationship is obtained by plotting the differences in the amplitude at 232 and 292 nm against the corresponding concentration of SPI, The linear regression equation (1) is found to be

$$\Delta P = 0.0337\ C - 0.0005, \qquad r = 0.9997 \qquad (1)$$

Where ΔP is the difference in the amplitude at 232 and 292 nm, C is the concentration of SPI in µg mL-1 and r is the correlation coefficient.

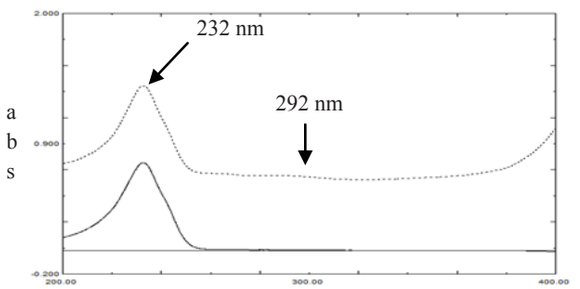


Fig. 3: Ratio spectra of 20.0 µg mL^{-1} SPI (-), and a binary mixture of 20.0 µg mL^{-1} SPI and 20 µg ml-1OTC (---) using 32.0 µg mL^{-1} OTC as a divisor. The arrows showing the selected wavelengths.

Dual wavelength method (DWL)

The dual wavelength method depends on the difference in the absorbance at two wavelengths on the spectra which is directly proportional to the component of interest and independent of the interfering component.

The requirement for dual wavelength method is the selection of two wavelengths where the interfering component shows the same absorbance while the component of interest shows significant difference in absorbance. (Figure 4) shows that OTC has the same absorbance at 232 and 375.5 nm, whereas, SPI has significant difference in the absorbance at these two wavelengths. Calibration graph was constructed and the concentration of SPI can be calculated from the following regression equation (2):

$$\Delta A = 0.0242\ C - 0.001, \qquad r = 0.9998 \qquad (2)$$

Where ΔA is the absorbance difference at 232 and 375.5 nm, C is the concentration of SPI in µg mL^{-1} and r is the correlation coefficient.

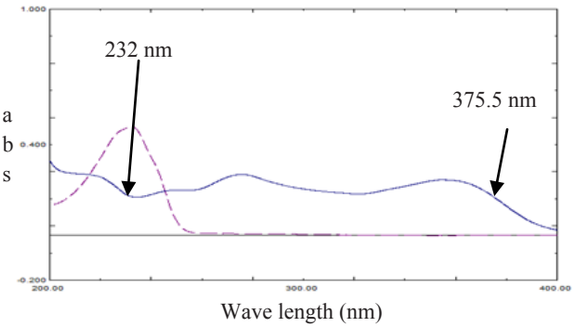


Fig. 4: Zero order absorption spectra of 20 µg mL^{-1}(---) SPI, and 8 µg mL^{-1} OTC (—). The arrows showing the selected wavelengths for dual wavelength calculations.

Constant Value and Concentration Value for Oxytetracycline

OTC spectrum is extended more than SPI as shown in (Figure 2). So that when the spectra of mixtures of OTC and SPI are divided by the spectrum of normalized divisor of OTC, a constant at the plateau region in the extended part of the spectrum (250 - 400 nm) was obtained as shown in (Figure 5).

This constant is equivalent to the concentration of OTC as it is resulting from dividing the spectra of the mixture by the normalized spectra of OTC of 1 µg mL^{-1}.

$$(SPI + OTC) / OTC' = SPI/ OTC' + OTC/ OTC'$$

$$(SPI + OTC) / OTC' = SPI/ OTC' + constant$$

Where OTC′ is a normalized divisor with concentration of 1 µg mL^{-1}.

For constant value method, a calibration curve between the constant and the corresponding concentration is constructed, where the concentration of OTC is obtained from regression equation (3):

$$Const. = 1.0001\ C - 0.062, \qquad r = 0.9999 \qquad (3)$$

Where Const. is the constant at the plateau region (250-400 nm), C is the concentration of OTC in µg mL^{-1} and r is the correlation coefficient.

While for Concentration Value method, the concentration was directly obtained from the spectra plateau region using that constant. The results of both methods were compared and both have good recoveries.

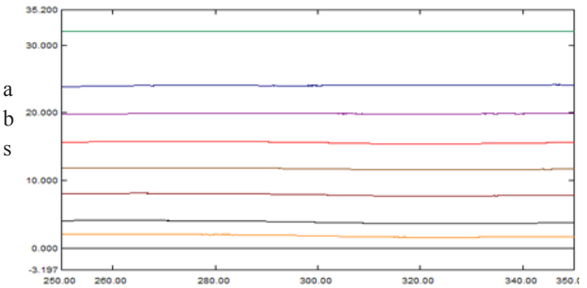


Fig. 5: The constant value obtained after the division of the zero order spectra of OTC (2.0–32.0 µg mL^{-1}) by the spectrum of normalized 1.0 µg mL^{-1} OTC as divisor.

Ratio Subtraction (RS) Constant Value and Ratio Subtraction Concentration Value for Spiramycin:

SPI was resolved from OTC by RS according to the following equations:

(SPI + OTC) / OTC ′ = SPI/ OTC ′ + OTC/ OTC ′

SPI/ OTC ′ + constant – constant = SPI/ OTC ′

SPI/ OTC ′ x OTC ′ = SPI

Then, the obtained spectra were divided by a normalized spectrum divisor of SPI. So, the constant obtained in the plateau region is related to SPI concentration as shown in (Figure 6).

For Constant Value, a calibration curve between that constant and the corresponding concentration was constructed and the concentration of SPI was obtained from the regression equation (4)

Const. = 1.0263 C – 0.1277, r = 0.9997 (4)

Where Const. is the constant at the plateau region (225-245 nm), C is the concentration of SPI in µg.mL^{-1} and r is the correlation coefficient.

While for Concentration Value, the concentration is directly obtained from the spectra plateau region using that constant. The results of the two methods were compared and showed good recoveries.

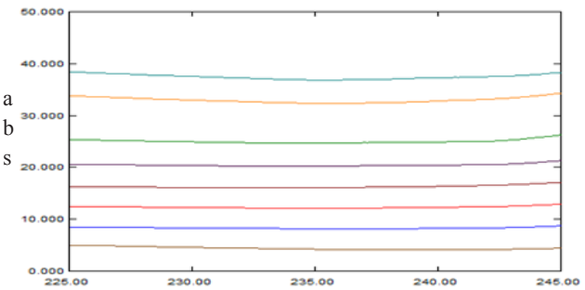


Fig. 6: The constant value obtained after the division of the zero order spectra of SPI (4.0–36.0 µg mL^{-1}) by the spectrum of normalized 1.0 µg mL^{-1} SPI as divisor.

3.2 Method validation

Methods validation has been performed according to ICH guidelines[26].

Linearity

The linearity of the proposed methods is evaluated by analyzing eight different concentrations of standard solutions ranging from 4.0-36.0 µg mL^{-1} for SPI and from 2.0-32.0 µg mL^{-1} for OTC in triplicates. The values of correlation coefficients are close to unity indicating good linearity, the regression equations are summarized in (Table 1).

Range

The calibration range was established by considering of the practical range necessary to Beer's law and the concentration of SPI and OTC present in the pharmaceutical preparations to give satisfactory accuracy, precision and linearity (Table 1).

Table 1: Validation resultsof the proposed spectrophotometric methods for the determination of Spiramycin (SPI) and Oxytetracycline HCl (OTC).

Parameters	(RD)	(DWL)	(CV)		(Conc.value)	
	SPI		SPI	OTC	SPI	OTC
Concentration range (µg mL^{-1}) linearity	4.0 – 36.0		4.0 – 36.0	2.0 – 32.0	4.0 – 36.0	2.0 – 32.0
Slope	0.037	0.024	1.026	1.000	---	---
Intercept	0.0005	0.001	0.128	0.062	---	---
Correlation coefficient (r)	0.9997	0.9998	0.9997	0.9999	---	---
Accuracy (mean ± S.D.)	100.33± 0.87	99.95± 1.16	100.64 ± 1.12	100.39 ± 1.09	101.19 ± 0.63	99.68± 0.99
Repeatability[a]	1.51	1.54	0.61	0.35	0.61	0.68
Intermediate precision[b]	0.57	1.12	0.87	1.66	1.11	1.33

a The intraday (n = 3), average of three different concentrations repeated three times within day.
b The interday (n = 3), average of three different concentrations repeated three times in three successive days.

Selectivity

The selectivity of the proposed methods is assessed by the analysis of six different laboratory prepared mixtures containing different ratios of SPI and OTC; satisfactory results were obtained and presented in (Table 2).

Table 2: Determination of Spiramycin (SPI) and Oxytetracycline HCl (OTC) in laboratory prepared mixtures by the proposed methods.

No. of mixtures	Claimed conc. taken in µg mL^{-1}		(RD) % Recovery*	(DWL % Recovery*	(CV) % Recovery*		(Conc.value) % Recovery*	
	SPI	OTC	SPI	SPI	SPI	OTC	SPI	OTC
1	4.0	32.0	98.31	101.24	100.24	100.36	101.87	100.18
2	8.0	32.0	100.17	101.23	100.10	99.99	101.13	99.81
3	16.0	16.0	101.47	99.10	101.52	98.88	100.05	98.5
4	20.0	20.0	101.96	98.69	100.49	99.42	100.25	99.13
5	32.0	4.0	99.79	98.85	99.15	100.11	101.36	98.58
6	36.0	8.0	100.04	99.68	100.85	99.14	100.14	98.37
	Mean ± SD		100.29 ± 1.29	99.79 ± 1.16	100.39± 0.79	99.65 ± 0.59	100.80± 0.76	99.09 ± 0.75

* Average of three determinations.

Accuracy

The accuracy of the results was studied by applying the proposed methods for determination of five different samples of SPI and OTC. The accuracy is presented in (Table 1).

To ascertain the accuracy of the proposed methods, recovery studies are carried out by standard addition technique at three different levels (Table 3).

Precision

The precision was studied by applying the proposed methods for determination of three different concentrations of each of SPI and OTC, which were analyzed three times intra-daily for repeatability and inter-daily on three different days for intermediate precision. The precision results are summarized in (Table 1).

Table 3: Determination of Spiramycin (SPI) and Oxytetracycline HCl (OTC) in their pharmaceutical formulation by the proposed methods and application of standard addition technique

Pharmaceutical formulation	%Found* ± S.D.						Added µg.mL^{-1}	Added µg.mL^{-1}	Standard addition technique %Recovery*					
	RD	DWL	CV		Conc. value				RD	DWL	CV		Conc. value	
	SPI	SPI	SPI	OTC	SPI	OTC	SPI	OTC	SPI	SPI	SPI	OTC	SPI	OTC
Arco spirox® WSP batch number: 100716/A	99.80 ±1.05	101.11 ± 0.44	100.11 ±1.69	101.30 ±0.79	100.27 ±0.87	100.96 ±1.02	4.0	4.0	100.75	101.88	99.38	99.51	98.80	101.83
							8.0	8.0	101.38	101.04	99.48	101.48	100.50	101.45
							16.0	16.0	101.00	99.78	99.70	101.26	101.53	99.61
			Mean ± S.D						101.04 ± 0.32	100.90 ± 1.06	99.52 ±0.16	100.75 ± 1.08	100.28 ±1.38	100.96 ± 1.19

Average of three determinations.*

Statistical analysis

Results obtained by the proposed methods for determination of pure SPI and OTC are statistically compared with those obtained by applying the reported methods[10, 13]. The calculated t- and F-values are found to be less than the theoretical ones, confirming accuracy and precision at 95% confidence level (Table 4).

Table 4: Statistical comparison of the results obtained by applying the proposed methods and published methods

Parameters	RD	DWL	CV		Conc. value		Reported methods[10, 13]	
	(SPI)	(SPI)	(SPI)	(OTC)	(SPI)	(OTC)	(SPI)[b]	(OTC)[c]
Mean%	100.33	99.95	100.64	100.39	101.19	99.68	100.09	99.48
S.D.	0.87	1.16	1.12	1.09	0.63	0.99	1.03	0.88
n								
Variance	0.76	1.35	1.25	1.18	0.39	0.98	1.06	0.77
Student's t-test (2.132)[a]	0.69	0.84	0.21	0.29	0.16	0.75	-	-
F-test (6.39)[a]	1.39	1.27	1.17	1.53	2.72	1.27	-	-

a The values in parenthesis are the corresponding theoretical values of t and F at ($P = 0.05$).
b charge transfer complex between SPI and quinalizarin producing charge transfer complex in methanolic medium $\Delta\lambda$ = 568 nm.
c HPLC method using mobile phase; methanol-0.01 M oxalic acid, pH 3.0 (30:70, v/v) at 250 nm using a Silasorb C8 column.

CONCLUSION

This work provides four different spectrophotometric methods for the simultaneous analysis of SPI and OTC in veterinary dosage forms. The developed Concentration Value method could determine both drugs without the use of regression equations; this was attained directly from the graphical representation. The proposed methods do not need any sophisticated apparatus or any preliminary separation steps and can be easily applied in quality control laboratories for the determination of SPI and OTC in presence of each other in veterinary pharmaceutical formulation.

CONFLICT OF INTEREST

There are no conflicts of interest.

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