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Universal procedures for spectrophotometric determination of anticoccidial drugs; application to multi-ingredient veterinary formulation and computational investigations for multivariate analysis

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RESEARCH ARTICLE

ABSTRACT

Simple, accurate, and eco-friendly spectrophotometric procedures were proposed and implemented for simultaneous determination of anticoccidial drugs from three different classes namely, amprolium hydrochloride (AMP), sulfaquinoxaline sodium (SQX) and diaveridine hydrochloride (DVD). Dual wavelength in ratio spectra procedure was proposed where the difference in amplitudes (ΔP) in the ratio spectra at 264 nm and 301.9 nm ($\Delta P_{264\&301.9\ nm}$) corresponded to AMP with mean percentage recovery 100.00±0.923%, while ($\Delta P_{250.9\&279\ nm}$) and ($\Delta P_{218\&243.5\ nm}$) corresponded to SQX and DVD with mean percentage recoveries 99.31±1.083 and 100.64±1.219%, respectively. The dual wavelength in ratio spectra procedure was validated according to the ICH guidelines and accuracy, precision and repeatability were found to be within the acceptable limit. Multivariate chemometric approaches, namely, partial least-squares (PLS-2) and principal component regression (PCR) were also proposed with mean percentage recoveries 99.31±0.769, 98.91±1.192 and 99.04±1.245% for AMP, SQX and DVD, respectively, in PLS-2 and 99.63±1.005, 99.11±1.272 and 98.93±1.338% for AMP, SQX and DVD, respectively, in PCR. These procedures were successfully applied to the multi-ingredient veterinary formulation with mean percentage recoveries 100.75±1.238, 99.29±0.875 and 99.34±0.745% for AMP, SQX and DVD, respectively, in dual wavelength in ratio spectra procedure and 101.03±1.261, 101.48±0.984 and 101.10±1.339% for AMP, SQX and DVD, respectively, in PLS-2 and 100.22±1.204, 101.10±0.546 and 100.91±0.677% for AMP, SQX and DVD, respectively, in PCR.

1. Introduction

Coccidiosis is a term sometimes applied to infections with protozoa of the order *Eucoccidiorida.* Coccidian protozoa, primarily *Eimeria*, cause economically important infections in domesticated animals [1]. Anticoccidial drug combinations proved to be very effective as prophylactic and treatment of coccidiosis in poultry because of the different mechanisms of action of the drugs being used in combination.

A multi-ingredient veterinary formulation composed of three anticoccidial drugs of three different classes was investigated. Amprolium hydrochloride (1-[(4-amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride hydrochloride) (Figure 1) is a thiamine analogue which inhibits the uptake of thiamine by second-generation schizonts of *Eimeria tenella* and so prevents formation of thiamine coenzyme which is required for many essential metabolic reactions [2]. Sulfaquinoxaline sodium (4-amino-*N*-2-quinoxalinyl benzene sulfonamide monosodium salt) (Figure 1) is an important member of the sulfonamides class and it interferes with the early phases of folate synthesis. Sulfonamides are often used in combination with dihydrofolate reductase inhibitors (DHFRI) such as diaveridine hydrochloride (2,4-diamino-5-(3,4-dimethoxybenzyl) pyrimidine hydrochloride) (Figure 1) which belongs to diaminopyrimidines class because of the observed synergistic effects due to activity at two places in folate biosynthesis [3].

Literature survey revealed many reported methods for the determination of AMP, SQX, and DVD either alone or with other drugs in different matrices such as veterinary formulation, surface water, eggs, chicken muscles, chicken plasma, chicken liver and chicken feed. These methods include HPLC/UV [4-6], LC/MS [7-12], spectroscopic methods [13-20] and electrochemical method [21]. To the best of our knowledge, the ternary mixture of AMP, SQX, and DVD has not been investigated spectrophotometrically. So, the main point of the current work was to develop simple, accurate and eco-friendly spectrophotometric procedures for the simultaneous determination of the three drugs in their multi-ingredient veterinary formulation.
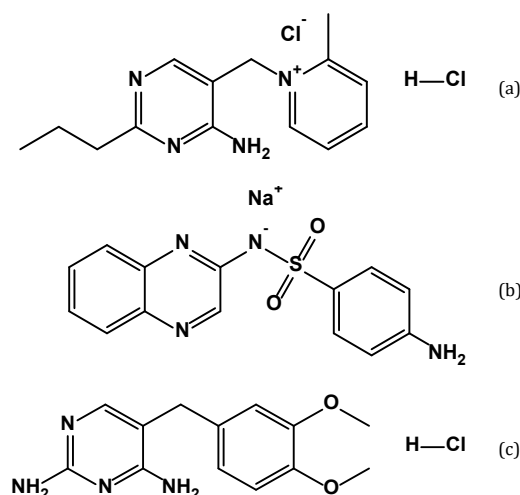
Figure 1. Structural formulas for (a) amprolium hydrochloride, (b) sulfaquinoxaline sodium, and (c) diaveridine hydrochloride.

2. Experimental

2.1. Instrumentation

The UV absorption spectra were recorded using a Shimadzu UV-1601 dual beam UV-visible spectrophotometer using 1-cm matched quartz cells. The spectral bandwidth was 0.1 nm with wavelength scanning speed of 2800 nm/min. Instrument software (version 3.91) was used to process the absorption. Matlab® (version 7.0.1.24704) was used to process the spectral data for multivariate analysis.

2.2. Materials

2.2.1. Standards

Amprolium hydrochloride, sulfaquinoxaline sodium, and diaveridine hydrochloride were kindly supplied by Pharma Swede Pharmaceutical Company, 10th of Ramadan City, Egypt. Their purities were found to be 99.22, 99.77, and 99.26%, respectively, according to the reported spectrophotometric methods [13-15], respectively. Analytical grade methanol was used throughout the work (ADWIC, Egypt). Standard stock solutions of AMP, SQX, and DVD (100 μg/mL) were prepared in methanol.

2.2.2. Veterinary formulation

A multi-ingredient veterinary formulation (Tricure®, Batch No. 0821/15) is a premix with 20 g amprolium hydrochloride, 25 g sulfaquinoxaline sodium and 6.4 g diaveridine hydro chloride per 100 g, produced by Arabcomed Co. S.A.E. Obour City, Egypt.

2.3. Procedures

2.3.1. Methods

2.3.1.1. Dual-wavelength-in-ratio spectra method

2.3.1.1.1. For determination of AMP

Various aliquots equivalent to 10-200 μg of AMP were taken from its stock standard solution (100 μg/mL) and completed to volume with methanol in a series of 10-mL volumetric flasks to obtain final concentrations of 1-20 μg/mL. The spectra of these solutions were scanned from 200-400 nm using methanol as a blank. AMP spectra are divided by the spectrum of 2 μg/mL of DVD. The amplitudes of the ratio spectra were obtained at 264 nm and 301.9 nm. A calibration curve was constructed that relates the differences in the amplitudes in the chosen wavelength couple $\Delta P_{264\&301.9\ nm}$ to the corresponding concentration of AMP [22,23].

2.3.1.1.2. For determination of SQX and DVD

Various aliquots equivalent to 5-100 μg of SQX and 5-190 μg of DVD were taken from their stock standard solutions (100 μg/mL) and completed to volume with methanol in two separate series of 10-mL volumetric flasks to obtain final concentrations of 0.5-10 μg/mL and 0.5-19 μg/mL, respecttively. The spectra of these solutions were scanned from 200-400 nm using methanol as a blank. The stored spectra were divided by the spectrum of 3 μg/mL of AMP. The amplitudes of the ratio spectra were obtained at 250.9, 279.0, 218.0, and 243.5 nm. Calibration curves for both SQX and DVD were constructed by plotting the differences in the amplitudes at the chosen wavelength couple $\Delta P_{250.9\&279\ nm}$ and $\Delta P_{218\&243.5\ nm}$ for SQX and DVD, respectively, versus the corresponding concentration [22,23].

2.3.1.2. PLS-2 and PCR

For each of PLS-2 and PCR, a calibration set consisting of twenty laboratory-prepared mixtures of AMP, SQX, and DVD in different proportions (Table 1) was obtained by the use of a multilevel multifactor experimental Brereton design [24] where levels (L) were the concentrations used and the experiments' number was L^2. The mixtures were prepared by taking various aliquots from AMP, SQX and DVD stock standard solutions (100 μg/mL) into a group of 10 mL volumetric flasks with ranges of concentrations 3-11, 1-9, and 3-15 μg/mL for AMP, SQX and DVD, respectively.

The spectra of the prepared mixtures were recorded in the range 210-400 nm with 0.2 nm intervals, transferred to MATLAB software producing 951 data points/spectrum, so the produced spectral data matrix composed of 25 rows representing various samples and 951 columns representing wavelengths. For each of PLS-2 and PCR, the calibration model was made after mean centering as a pre-processing step, while the cross-validation method was "random" for PLS-2 and "leave one out" for PCR.

Table 1. Calibration and validation set for the PLS-2 and PCR methods.

Mixture no	AMP	SQX	DVD
1	7	5	9
2	7	1	3
3	3	1	15
4	3	9	6
5	11	3	15
6	5	9	9
7	11	5	6
8	7	3	6
9	5	3	12
10	5	7	15
11	9	9	12
12	11	7	9
13*	9	5	15
14	7	9	15
15	11	9	3
16	11	1	12
17	3	7	3
18	9	1	9
19*	3	5	12
20*	7	7	12
21*	9	7	6
22	9	3	3
23	5	1	6
24	3	3	9
25*	5	5	3

* Validation set.

The suitable selection of the factors' number to be employed for constructing the model was essential for accomplishing the right quantification in both PLS-2 and PCR calibrations. To choose the ideal number of significant latent variables, F statistics were applied [24].

The validation set is composed of 5 laboratory-prepared mixtures containing different proportions of AMP, SQX, and DVD (Table 1). In addition, the concentrations of AMP, SQX, and DVD in the veterinary formulation extract were calculated using the optimized PLS-2 or PCR calibration model after being recorded in the same specified lambda range [25-33].

2.3.2. Application of the proposed procedures for determination of AMP, SQX, and DVD in laboratory-prepared mixtures

Solutions containing various proportions of the ternary mixture of AMP, SQX, and DVD were prepared. Zero-order absorption curves of these mixtures were recorded using methanol as a blank. By applying the proposed procedures, the concentrations of the drugs in the prepared mixtures were deduced.

2.3.3. Application of the proposed procedures for the determination of AMP, SQX, and DVD in veterinary formulation

A portion of the Tricure® premix containing 2 mg of AMP, 2.5 mg of SQX, and 0.64 mg of DVD was accurately weighed, sonicated in 25 mL methanol for 2 min and filtered into a 100 mL volumetric flask. The residue was washed three times, each with 5 mL of methanol, and then completed to volume with methanol. The procedures mentioned above in Sections 2.3.1.1 and 2.3.1.2 were applied and the concentrations of the drugs were deduced. The validity of the methods was tested using the standard addition technique.

3. Results and discussion

Three anticoccidial drugs, namely, amprolium hydro chloride, sulfaquinoxaline sodium, and diaveridine hydro chloride from three different classes; thiamine analogues, sulfonamides and dihydrofolate reductase inhibitors, respect-tively, were investigated to be analyzed in their multi-

ingredient veterinary formulation by simple, accurate and eco-friendly spectrophotometric procedures. Zero-order absorp-tion spectra (D_0) of the ternary mixture (AMP, SQX, and DVD) show severe overlap, which made the analysis of each drug in the presence of the other challengeable (Figure 2). By applying the proposed procedures, this overlapping can be resolved, allowing the quantitative analysis of the components of the mixture.

3.1. Method development and optimization

3.1.1. Dual-wavelength-in-ratio spectra method

This method can be applied to determine the ternary mixture of the investigated compounds whose spectra show a severe overlap [22,23]. It is based on the principles of both the ratio difference method [34-36] and the dual wavelength method [37-40]. The proposed method can determine a ternary mixture, such that every compound can be selectively deter-mined after total elimination of the impediment caused by the other two compounds. The developed method can be regarded as a dual wavelength in the ratio spectrum where the difference should be considered at certain two wavelengths showing equal peak amplitudes for the interfering component in its ratio spectrum [38,39]. After dividing by the spectrum of 2 μg/mL DVD, DVD was cancelled, while SQX showed equal amplitudes at 264.0 and 301.9 nm. Therefore, the difference in amplitudes at these two wavelengths $\Delta P_{264\&301.9\,nm}$ corresponded to AMP concentration (Figure 3).

Similarly, after dividing by the spectrum of 3 μg/mL AMP, SQX, and DVD were determined in the ratio spectra at $\Delta P_{250.9\&279\,nm}$ and $\Delta P_{218\&243.5\,nm}$, respectively, as shown in Figure 4.

The linear regression equations were found to be the following:

$$\Delta P_{AMP} = 1.4271 \times C - 1.1461 \quad r = 0.9998 \tag{1}$$

$$\Delta P_{SQX} = 0.7811 \times C - 0.0569 \quad r = 0.9998 \tag{2}$$

$$\Delta P_{DVD} = 0.8451 \times C + 0.0751 \quad r = 0.9998 \tag{3}$$

where C is the μg/mL concentration of AMP, SQX or DVD, ΔP is the difference in amplitudes at the two wavelengths and *r* is the correlation coefficient.
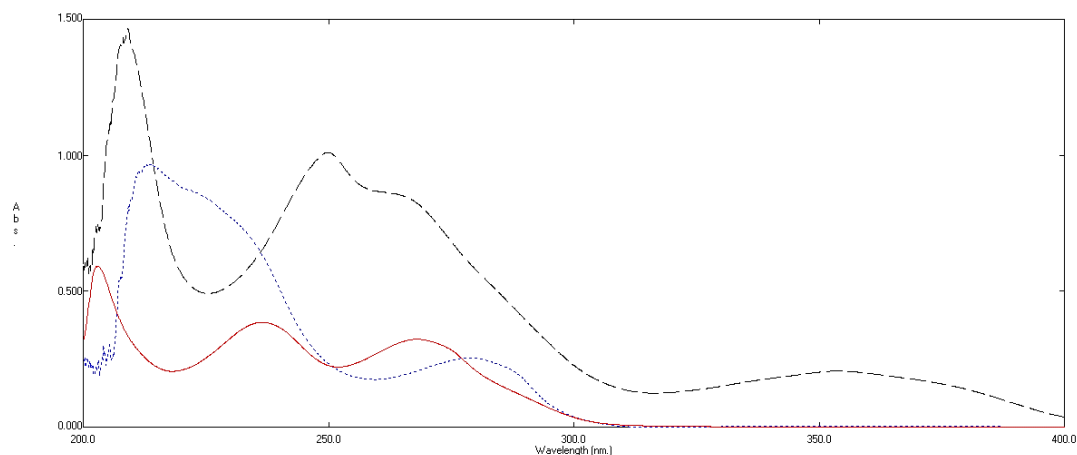
Figure 2. Zero order absorption spectra of 10 µg/mL AMP (-), 10 µg/mL SQX (- - -), and 10 µg/mL DVD (......) using methanol as blank.
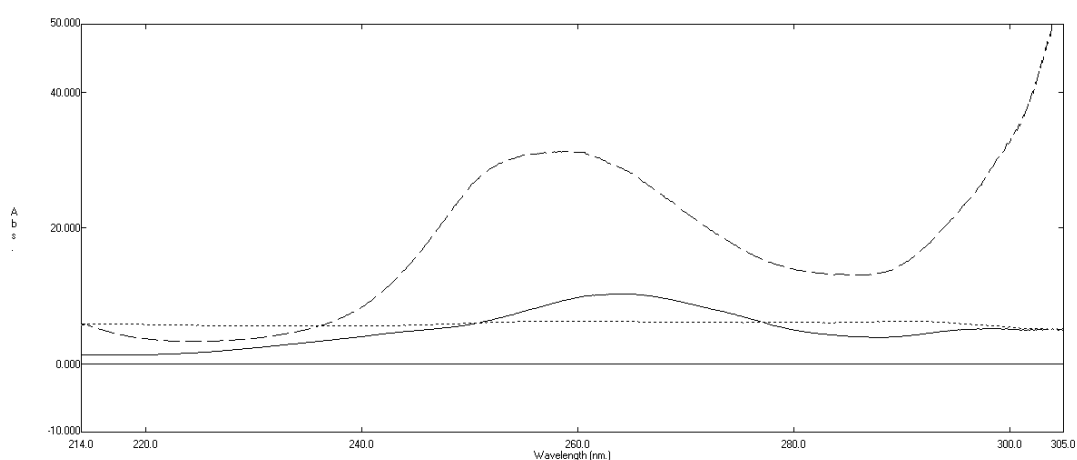

Figure 3. Ratio spectra of 10 µg/mL AMP (—), 10 µg/mL SQX (- - -), and 10 µg/mL DVD (.....) using 2 µg/mL DVD as a divisor and methanol as blank.
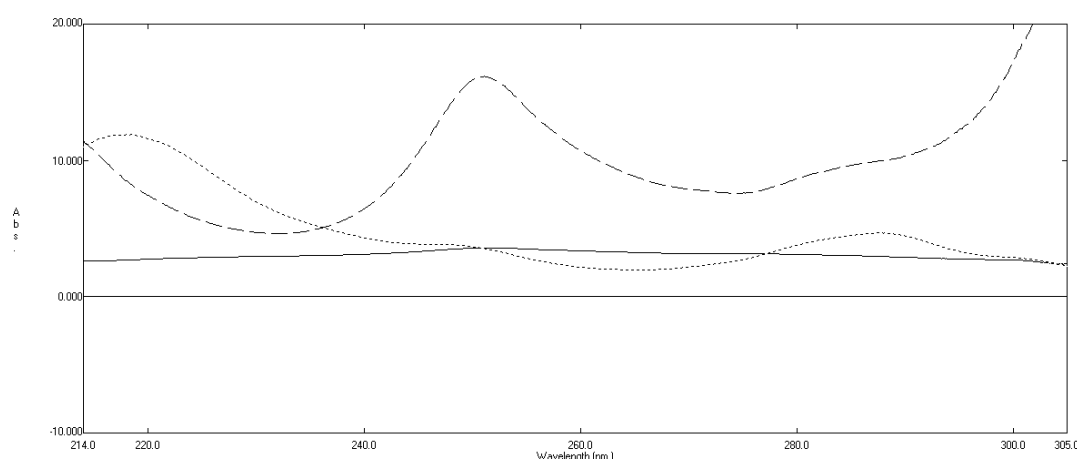

Figure 4. Ratio spectra of 10 µg/mL AMP (—), 10 µg/mL SQX (- - -) and 10 µg/mL DVD (.....) using 3 µg/mL AMP as a divisor and methanol as blank.

3.1.2. Multivariate chemometric methods: (PLS-2) and (PCR)

The optimum number of latent variables (LVs) described by the constructed models was found to be five factors for both PLS-2 and PCR as shown in Figures 5 and 6.

The predictive ability of the developed models was assessed using the validation set by plotting known versus predicted concentrations for each drug. A good linearity was observed for each drug, as indicated by the correlation coefficients 0.9997 ($y = 0.9987x$ - 0.0380), 0.9981 ($y = 0.9437x$ + 0.2559) and 0.9999 ($y = 0.9803x$ + 0.0646) for AMP, SQX and DVD, respectively, in PLS-2, and 0.9995 ($y = 1.0122x$ - 0.0965), 0.9964 ($y = 0.9531x$ + 0.2082) and 0.9999 ($y = 0.9804x$ + 0.0552) for AMP, SQX and DVD, respectively, in PCR.

Figure 5. RMSECV plot of the cross-validation results of the training set as a function of the number of principal components used to construct the PLS-2 calibration, using zero-order absorption spectra of AMP, SQX and DVD.
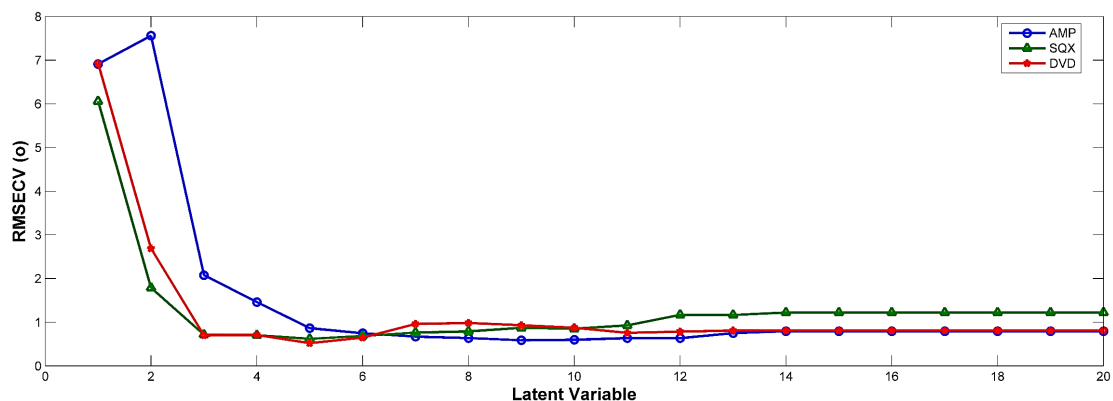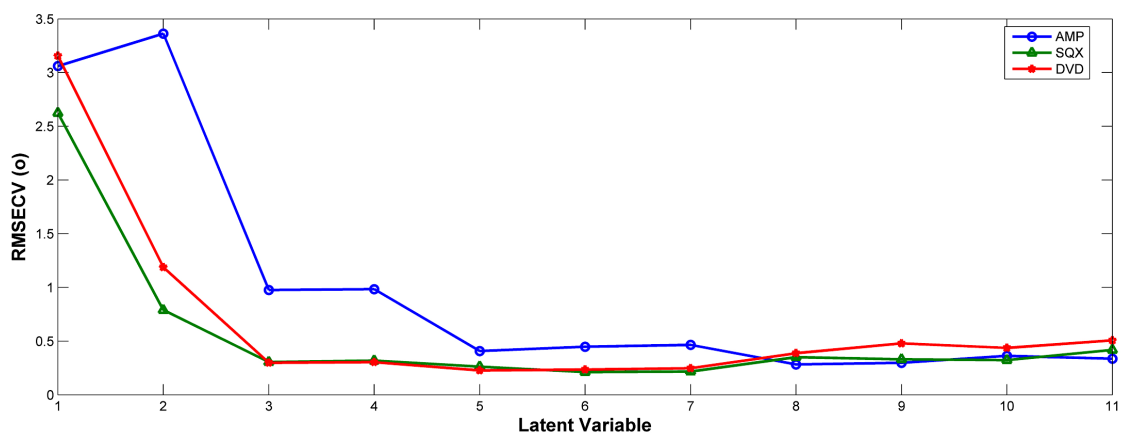

Figure 6. RMSECV plot of the cross-validation results of the training set as a function of the number of principal components used to construct the PCR calibration, using zero-order absorption spectra of AMP, SQX and DVD.
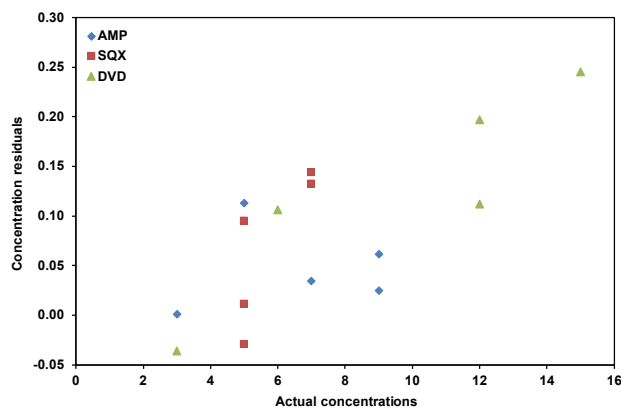

Figure 7. Residual versus actual concentration (µg/mL) plot for AMP, SQX and DVD in the validation set, using the PLS-2 method.

For both PLS-2 and PCR, the concentration residuals were plotted against the actual concentrations of the prepared mixtures (Figures 7 and 8), and the residuals for all samples were found to be randomly distributed around zero [25-33]. The average recoveries of each drug using PLS-2 and PCR methods are summarized in Table 2.

The following equation gives the RMSEP:

$$RMSEP = \sqrt{\frac{\varepsilon(y_r - y_p)^2}{n}} \qquad (4)$$

where y_r and y_p are the true and predicted values, respectively, and (n) is the number of samples used in validation. Statistical

parameters of each drug, using the optimized PLS-2 and PCR methods are shown in Table 3.

The procedures were applied for determination of the studied drugs in their laboratory-prepared mixtures with average percentage recovery as given in Table 4.

The proposed procedures were found to be valid and applicable for the analysis of the drugs in their multi-ingredient veterinary formulation with acceptable mean percentage recoveries. Furthermore, the standard addition technique was performed to assess the accuracy of the proposed methods. The obtained results revealed that there was no interference from excipients as shown in Table 5.

Table 2. Percent recoveries of AMP, SQX and DVD in the validation set, using PLS-2 and PCR methods.

Mixture number	Using zero order spectra					
	Found, %					
	AMP		SQX		DVD	
	PLS-2	PCR	PLS-2	PCR	PLS-2	PCR
13	99.32	99.81	98.10	98.00	98.37	98.26
19	99.97	99.74	99.77	100.54	99.07	99.12
20	99.51	99.84	98.11	98.45	98.36	98.33
21	99.73	100.77	98.00	98.12	98.23	97.80
25	98.00	98.00	100.59	100.44	101.19	101.17
Mean	99.31	99.63	98.91	99.11	99.04	98.93

Table 3. Statistical parameters for simultaneous determination of AMP, SQX, and DVD, using optimized PLS-2 and PCR methods.

Parameters	Using zero-order spectra					
	AMP		SQX		DVD	
	PLS-2	PCR	PLS-2	PCR	PLS-2	PCR
Concentration range (µg/mL)	3-11	3-11	1-9	1-9	3-15	3-15
Number of factors	5	5	5	5	5	5
Root mean square error of calibration	0.1831	0.2751	0.1627	0.1966	0.1459	0.1629
Root mean square error of prediction	0.06057	0.060526	0.098217	0.096624	0.157454	0.166605
Root mean square error of cross-validation	0.8633	0.4075	0.6176	0.2622	0.5189	0.2276
Intercept [a]	-0.0380	-0.0965	0.2559	0.2082	0.0646	0.0552
Slope [a]	0.9987	1.0122	0.9437	0.9531	0.9803	0.9804
(r^2) [a]	0.9997	0.9995	0.9981	0.9964	0.9999	0.9999

[a] Data of the straight line plotted between predicted concentrations of each component versus actual concentration.

Table 4. Determination of amprolium hydrochloride, sulfaquinoxaline sodium, and diaveridine hydrochloride in laboratory prepared mixtures by the proposed dual wavelength in ratio spectra spectrophotometric method.

No of mixtures	Claimed concentration taken (µg/mL)			Dual wavelength in ratio spectra method		
				% Recovery [a]		
	AMP	SQX	DVD	AMP	SQX	DVD
1	4	5	1.3	100.72	101.97	98.57
2	11	3	15	100.32	98.98	99.95
3	3	9	6	99.77	99.19	99.29
4	5	7	15	101.57	100.41	98.95
5	7	3	6	99.96	98	100.72
Mean				100.47	99.71	99.50
SD				0.715	1.527	0.852
RSD%				0.712	1.531	0.856

[a] Average of three determinations.
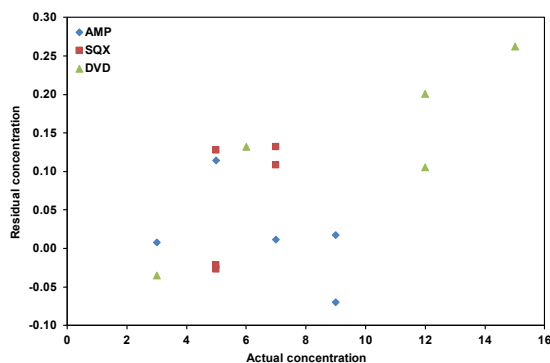
Figure 8. Residual versus actual concentration (µg/mL) plot for AMP, SQX, and DVD in the validation set, using the PCR method.

3.2. Method validation

Dual wavelength in ratio spectra method validation has been performed according to ICH guidelines [41].

3.2.1. Linearity

It was assessed by analysing different concentrations of standard solutions of each drug. The values of the correlation coefficients were close to unity indicating good linearity, the characteristic parameters for the constructed equations are summarized in Table 6.

3.2.2. Range

The calibration range was established depending on the practical range according to adherence to Beer's law and the concentration of the tested compounds present in their multi-ingredient veterinary formulation to obtain accurate, precise and linear results (Table 6).

3.2.3. Specificity

Laboratory-prepared mixtures of the tested drugs were analysed. Satisfactory results were obtained and presented in Table 4.

3.2.4. Accuracy

The accuracy of the results was checked by applying the proposed procedures for determination of different concent-rations for each drug within its linearity range (Tables 2 and 6). To assure the accuracy of the proposed method, a standard addition technique was applied (Table 5).

Table 5. Determination of amprolium hydrochloride, sulfaquinoxaline sodium, and diaveridine hydrochloride in multi-ingredient veterinary formulation by the proposed procedures.

Dosage form	Drug	Dual-wavelength-in-ratio spectra method				
		Taken (µg/mL)	Found±S.D. (%) [a]	Added (µg/mL)	Found (µg/mL)	% Recovery [a]
Tricure® contains 20 g AMP, 25 g SQX and 6.4 g DVD per 100 g	AMP	3.2	100.21±0.815	4	4.068	101.70
				5	5.060	101.2
				6	5.961	99.35
		Mean±S.D.				100.75±1.238
	SQX	4.0	101.12±1.290	3	2.963	98.77
				4	3.952	98.80
				5	5.015	100.30
		Mean±S.D.				99.29±0.875
	DVD	4.0	99.61± 1.600	4	3.956	98.90
				5	5.010	100.20
				6	5.935	98.92
		Mean±S.D.				99.34±0.745
PLS-2 method						
Tricure® contains 20 g AMP, 25 g SQX and 6.4 g DVD per 100 g	AMP	3.2	99.43±0.154	4	4.096	102.40
				5	5.038	100.76
				6	5.995	99.92
		Mean±S.D.				101.03±.261
	SQX	4.0	97.97±0.866	3	3.076	102.53
				4	4.053	101.33
				5	5.029	100.58
		Mean±S.D.				101.48±0.984
	DVD	4.0	101.09±0.333	4	4.090	102.25
				5	5.071	101.42
				6	5.978	99.63
		Mean±S.D.				101.10±1.339
PCR method						
Tricure® contains 20 g AMP, 25 g SQX and 6.4 g of DVD per 100 g	AMP	3.2	98.67±0.065	4	3.954	98.85
				5	5.055	101.10
				6	6.043	100.72
		Mean±S.D.				100.22±1.204
	SQX	4.0	101.18±0.448	3	3.040	101.33
				4	4.060	101.50
				5	5.024	100.48
		Mean±S.D.				101.10±0.546
	DVD	4.0	100.37±0.282	4	4.007	100.18
				5	5.076	101.52
				6	6.061	101.02
		Mean±S.D.				100.91±0.677

[a] Average of three determinations.

Table 6. Assay parameters and method validation for the determination of pure samples of the studied drugs using the proposed dual wavelength in ratio spectra method.

Parameters	Dual-wavelength-in-ratio spectra method		
	AMP	SQX	DVD
λ (nm)	ΔP 264 and 301.9	ΔP 250.9 and 279	ΔP 218 and 243.5
Concentration range (µg/mL)	1-20	0.5-10	0.5-19
Linearity			
Slope	1.4271	0.7811	0.8451
Intercept	-1.1461	-0.0569	0.0751
Correlation coefficient (r)	0.9998	0.9998	0.9998
Accuracy (mean±S.D.)	100.00±0.923	99.31±1.083	100.64±1.219
Specificity	100.47±0.715	99.71±1.527	99.50±0.852
Precision (%RSD)			
Repeatability [a]	1.261	0.365	0.630
Intermediate precision [b]	0.643	0.257	0.334
LOD (µg/mL) [c]	0.210	0.102	0.184
LOQ (µg/mL) [c]	0.635	0.309	0.557

[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.
[c] Limit of detection and limit of quantitation.

3.2.5. Precision

It was tested by determining three concentrations for each compound within its linearity range, where the concentrations were analyzed three times, each intraday (for repeatability) and on three successive days (for intermediate precision). The concentrations were obtained from the corresponding regression equation then the percentage recoveries and %RSD values are calculated (Table 6).

3.2.6. Detection and quantitation limits

They were calculated from the standard deviation (σ) of the response and the slope of the calibration curve (S) according to the following equations: LOD = 3.3 (σ/S) and LOQ = 10 (σ/S). Results presented in Table 6 indicate that the proposed procedure is sensitive for determination of the studied drugs.

3.3. Statistical analysis

Results obtained by the proposed procedures for determination of the studied drugs were statistically compared with those obtained by applying the reported spectrophotometric methods [13-15]. The calculated t- and F-values were found to be less than the theoretical ones, confirming accuracy and precision at 95% confidence level, as shown in Table 7.

Table 7. Statistical comparison of the results obtained by applying the proposed method and the reported methods for the analysis of pure AMP, SQX and DVD.

Value	Dual wavelength in ratio spectra method			PLS-2			PCR			Reported methods		
	AMP	SQX	DVD	AMP	SQX	DVD	AMP	SQX	DVD	AMP [13] b	SQX [14] c	DVD [15] d
Mean	100.00	99.31	100.64	99.31	98.91	99.04	99.63	99.11	98.93	99.22	99.77	99.26
SD	0.923	1.083	1.219	0.769	1.192	1.245	1.005	1.272	1.338	0.992	0.548	1.701
RSD%	0.923	1.091	1.211	0.774	1.205	1.257	1.009	1.283	1.352	1.000	0.549	1.714
N	5	5	5	5	5	5	5	5	5	5	5	6
Variance	0.852	1.173	1.486	0.591	1.421	1.550	1.010	1.618	1.790	0.984	0.300	2.893
Student's t-test a	1.284 (2.306)	0.851 (2.306)	1.508 (2.262)	0.144 (2.306)	1.456 (2.306)	0.241 (2.262)	0.643 (2.306)	1.064 (2.306)	0.351 (2.262)	_	_	_
F value a	1.153 (6.388)	3.907 (6.388)	1.948 (6.256)	1.661 (6.388)	4.732 (6.388)	1.865 (6.256)	1.028 (6.388)	5.390 (6.388)	1.616 (6.256)	_	_	_

a The values in parentheses are the corresponding theoretical values of t and F at $p = 0.05$.
b Ratio difference method for determination of AMP at 239 nm and 310 nm.
c First derivative spectrophotometry at 268.2 nm for SQX.
d Colorimetric determination of DVD at 460 nm via charge transfer complex formation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) reagent.

The proposed work has many points of strength in accordance with the principles of green chemistry [42] such as the usage of methanol as an environmentally preferable green solvent throughout the whole work [43]. Also, the proposed procedures offer better sensitivity and simpler data manipulation as compared to the tedious colorimetric methods [15,44,45], i.e. all the proposed methods avoid unnecessary colorimetric derivatization because such steps require additional reagents and can generate waste. Moreover, the adoption of chemometrics spectrophotometry (calculation spectrophotometry) which is a combination of chemometrics with analytical chemistry for the reduction of data dimensionality, grouping of variables, and processing of analytical signals. In this way, the analysis time, consumption of solvents or reagents, can be minimized [46]. The proposed procedures have been applied successfully on the multi-ingredient veterinary formulation and the good recovery and accuracy make them applicable in QC laboratories without the difficulties of HPLC. As compared to some recently published research articles [17-20], the proposed work shows better sensitivity for the determination of the three anticoccidial drugs as shown in Tables 3 and 6.

4. Conclusion

The proposed methods are simple, accurate, sensitive, and specific. They can be used for the routine analysis of the chosen drugs in their available multi-ingredient veterinary formulation in quality control labs lacking HPLC instruments. In addition, the proposed work conforms to the principles of green chemistry because of the use of a green solvent, simple data manipulation, and the elimination of time-consuming derivatization steps.

Disclosure statement DS

Conflict of interests: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement CR

Conceptualization: Amira Mabrouk El-Kosasy; Methodology: Mahmoud Mohamed Abbas; Software: Mahmoud Mohamed Abbas; Validation: Mahmoud Mohamed Abbas; Formal Analysis: Mahmoud Mohamed Abbas; Investigation: Mahmoud Mohamed Abbas; Resources: Lobna Abdel-Aziz Hussein; Data Curation: Mahmoud Mohamed Abbas; Writing - Original Draft: Mahmoud Mohamed Abbas; Writing - Review and Editing: Nancy Magdy Hanna; Visualization: Mahmoud Mohamed Abbas, Nancy Magdy Hanna; Funding acquisition: Mahmoud Mohamed Abbas, Amira Mabrouk El-Kosasy, Lobna Abdel-Aziz Hussein, Nancy Magdy Hanna; Supervision: Amira Mabrouk El-Kosasy; Project administration: Lobna Abdel-Aziz Hussein.

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