

## PAPER DETAILS

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Research Article

## Application of PCR and PLS Tools for the Simultaneous Quantification of Praziquantel and Ivermectin Binary Mixtures in Veterinary Tablets

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### ABSTRACT

Two chemometric calibration methods, principal component regression (PCR) and partial least squares regression (PLS) were proposed for the simultaneous spectrophotometric determination of praziquantel (PRA) and ivermectin (IVE) in a marketed veterinary formulation without using preliminary separation step. UV spectra of calibration set and samples including PRA and IVE were recorded in the spectral region of 225-315 nm. PCR and PLS algorithms were applied to absorbance data matrix and concentration set including PRA and IVE in the linear concentration range of 20.0-160.0 µg/mL for PRA and 2.0-44.0 µg/mL for IVE. The capability of the PCR and PLS methods were validated by analyzing validation samples. Assay results showed that both PCR and PLS approaches provided an opportunity for quantifying PRA and IVE in veterinary tablet formulation.

**Keywords:** PCR and PLS calibrations, Simultaneous quantification, Praziquantel, Ivermectin, Veterinary tablet preparation

## Veteriner Tabletlerinde Prazikuantel ve İvermektinin Eş Zamanlı Ölçümü için PCR ve PLS Yöntemlerinin Uygulanması

### Öz

Ticari bir veteriner formülasyonunda ön ayırma adımı kullanılmadan prazikuantel (PRA) ve ivermektinin (IVE) eş zamanlı spektrofotometrik tayini için iki kemometrik kalibrasyon yöntemi, temel bileşen regresyonu (PCR) ve kısmi en küçük kareler regresyonu (PLS) önerilmiştir. Kalibrasyon setinin UV spektrumları ve PRA ve IVE içeren numuneler 225-315 nm spektral bölgede kaydedildi. PRA için 20.0-160.0 µg/mL ve IVE için 2.0-44.0 µg/mL lineer konsantrasyon aralığında PRA ve IVE içeren absorbans veri matrisi ve konsantrasyon setine PCR ve PLS algoritmaları uygulandı. PCR ve PLS yöntemlerinin kapasitesi, doğrulama örnekleri analiz edilerek doğrulandı. Değerlendirme sonuçları, hem PCR hem de PLS yaklaşımlarının, veteriner tablet formülasyonunda PRA ve IVE'yi ölçmek için bir fırsat sağladığını gösterdi.

**Anahtar Kelimeler:** PCR ve PLS kalibrasyon, Eşzamanlı miktarsal ölçüm, Prazikuantel, Ivermectin, Veteriner tabletleri



Nowadays, principal component regression (PCR) and partial least squares regression (PLS) as chemometric tools are two most commonly used techniques in the quantification of active compounds in samples. Previous studies showed that the implementation of the PCR and PLS to the overlapping spectral bands or the overlapping chromatographic signals provided desirable outcomes for the quantitation of analytes in combined marketed veterinary formulation without using a preliminary separation step or requiring elution of analytes in a chromatogram [19-21].

In this paper, the multicomponent analysis of PRA and IVE in a marketed veterinary formulation was accomplished for first time by the applying PCR and PLS algorithms to UV spectral data sets. Both PCR and PLS were validated and implemented for the analysis of mixture and marketed veterinary preparation consisting of the related drugs.

## **II. MATERIALS AND METHODS**

### **A. 1. Instrumentation and Software**

UV Spectrophotometric analysis was made by means of a Shimadzu 2550 UV spectrophotometer. The UV spectrum of analysis samples were plotted from 225 to 315 nm. After that, the data of UV spectra were transferred into Microsoft Excel software to process. The PCR and PLS treatments of the spectral records were done by MATLAB 7.0 and Microsoft EXCEL was utilized for the traditional calibration and prediction procedures.

### **A. 2. Preparation of Stock and Standard Solutions**

The stock solutions of ivermectin and praziquantel was prepared by dissolving 20 mg of analytes in 100 ml of methanol. A calibration series including PRA and IVE in the linear concentration range of 20.0-160.0 µg/mL and 2.0-44.0 µg/mL was built up from the above stock solution, respectively. By using the same stock solutions, the validation samples of PRA and IVE in the working ranges as well as calibration were prepared for the capability and validity working of the applied chemometric tools. Also, intra-day and inter-day sample sets were prepared in three concentration levels of 20, 60 and 100 µg/mL for PRA and 2, 14 and 24 µg/mL for IVE. Standard addition test samples were arranged four different concentrations by apposition by adding rising quantities of PRA and IVE. All concentration level was prepared in triplicates.

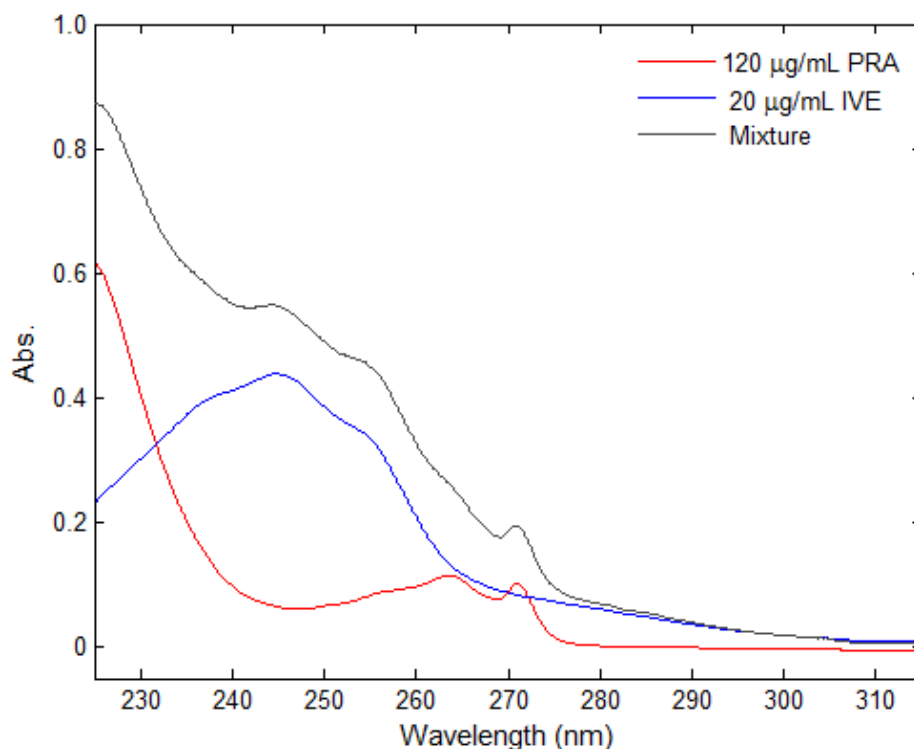
### **A. 3. Commercial Tablet Formulation**

Dicromec tablets containing 10 mg ivermectin and 250 mg praziquantel (Anatolia Medicine & Chemical Industry Co., Konya, Turkey) was obtained from a local pharmacy market. Active standard compounds, PRA and IVE were friendly denominated from the national Pharm. Industry firms, Turkey. Before the analyzing commercial tablets, sample package and labelling of commercial veterinary preparation were controlled. Ten tablets were weighed and powdered on the mortar mixed. An amount equivalent one tablet accurately was weighed and transferred into 100 mL calibrated flask. Then the volume was made up to the mark with methanol. Afterword, the sample solution in the calibrated flask was sonicated for 25 min. For the spectral registration, the resulting solution was diluted with methanol into working concentration range of PRA and IVE.

## **III. RESULTS AND DISCUSSION**

In this work, the UV spectrum of PRA and IVE and binary mixtures were recorded in the spectral region of 225-315 nm as shown in Figure 3. As seen in the Figure 3, the simultaneous analysis of PRA and IVE was impossible by direct spectrophotometric measurements due to the overlapping spectral bands of the

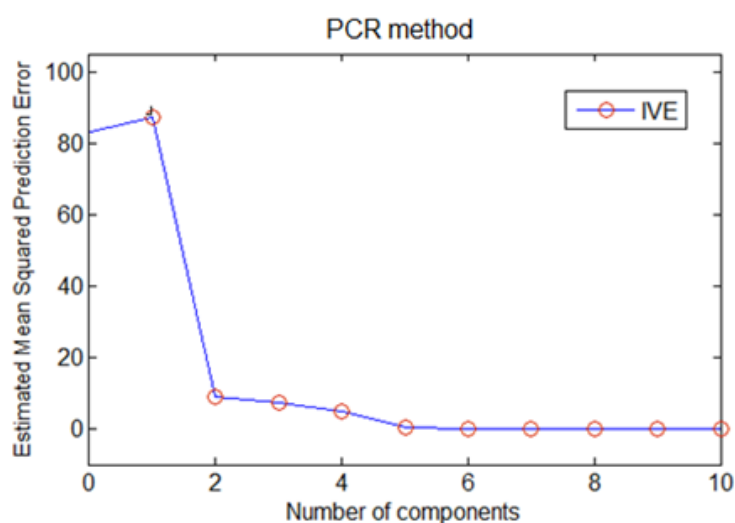
analytes in the identical spectral area. In order to eliminate the mentioned trouble, we deduced that PCR and PLS methods were suitable tools for the quantification of PRA and IVE in marketed veterinary tablets.



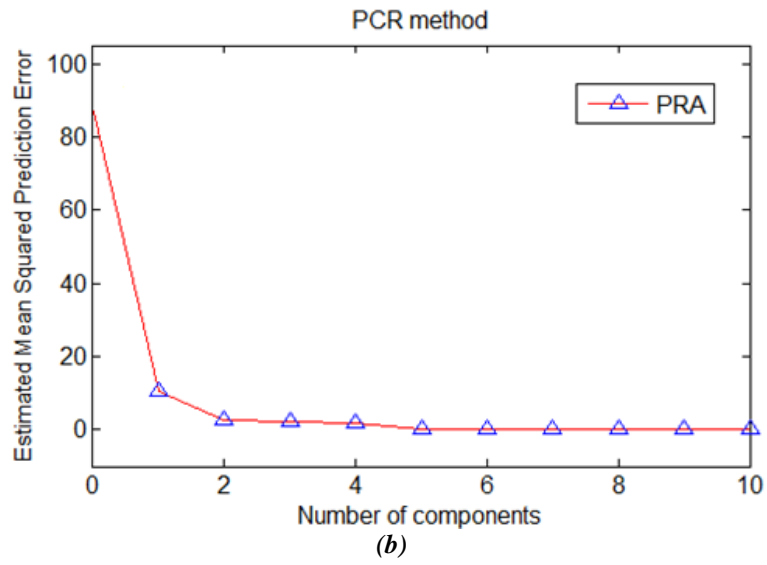
**Figure 3.** UV absorption spectra of the PRA and IVE substances, and their binary mixture.

## B.1 PLS and PCR Applications

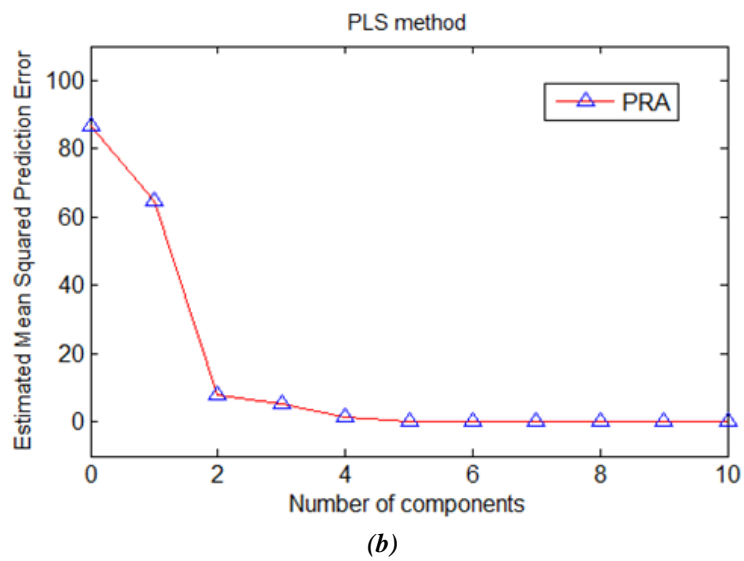
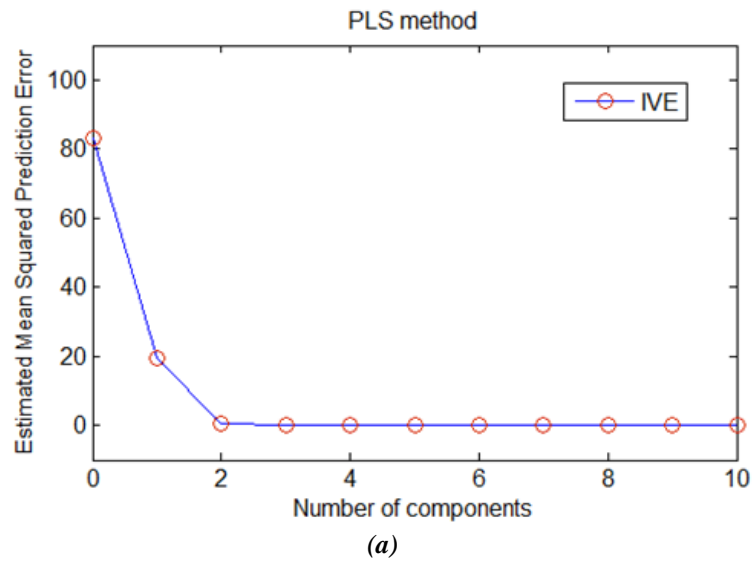
The PCR and PLS models were built up by using mathematical relationship between independent variable (concentration set) and dependent variable (absorbance data set). The applied PCR and PLS tools were validated by analyzing the independent validation samples. When first four factors were taken into account the calibration process, the minimum root mean square error of cross-validation was reported for both PCR and PLS models (Figure 4a-b and Figure 5a-b, respectively).



(a)

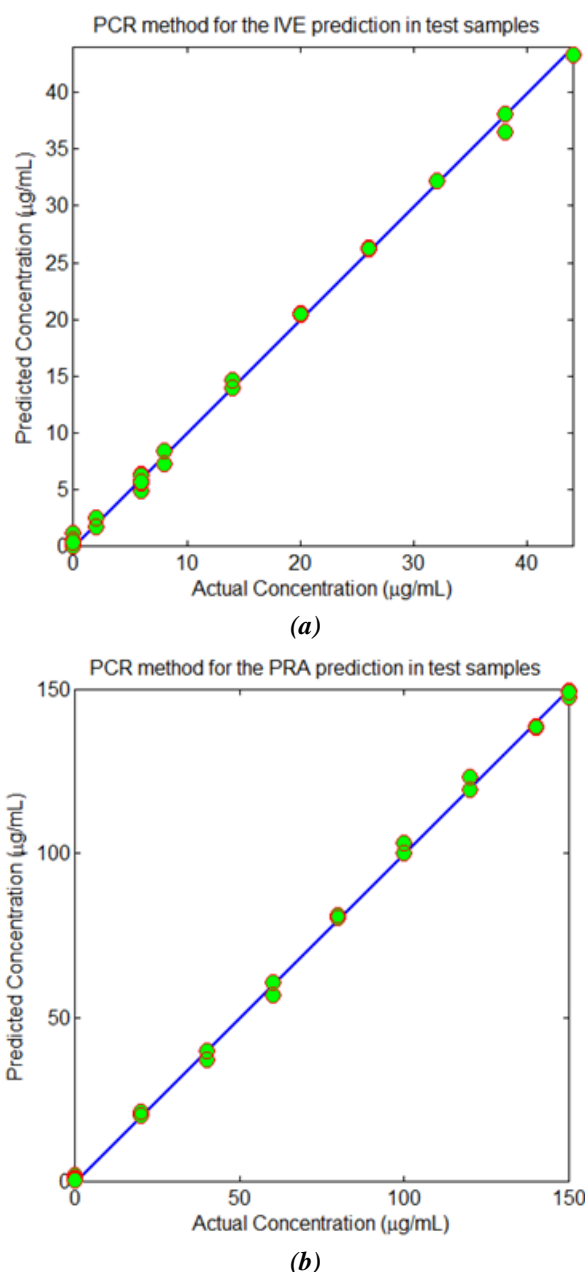


**Figure 4.** Estimated mean squared prediction error of PCR method for IVE (a) and PRA (b)

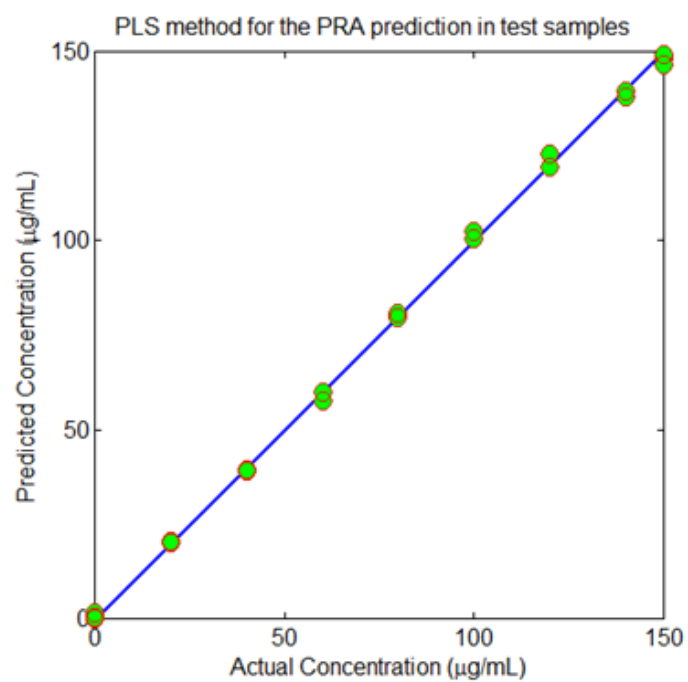


**Figure 5.** Estimated mean squared prediction error of PLS method for IVE (a) and PRA (b)

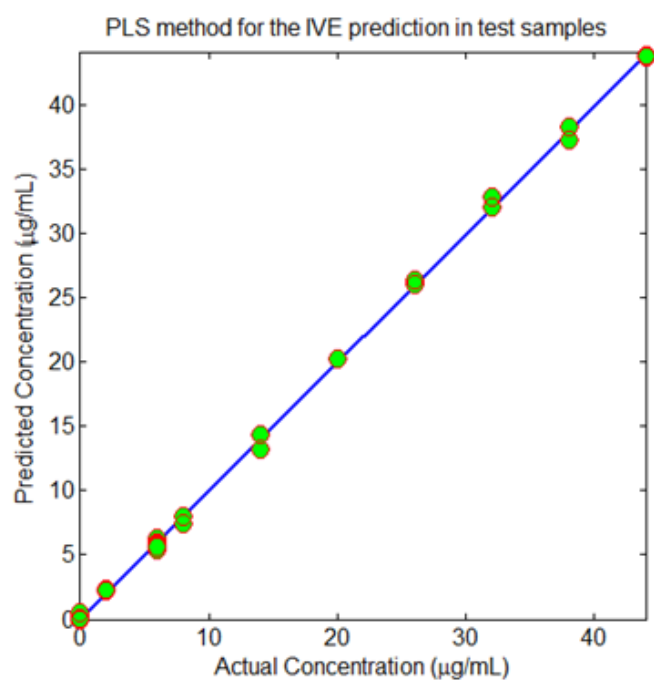
A calibration set of the mixtures containing PRA and IVE in the calibration range of 20.0-160.0  $\mu\text{g/mL}$  and 2.0-44.0  $\mu\text{g/mL}$  was made ready and presented in Table 1, respectively. The calibration set was used as y-block, which was named as the concentration matrix. The UV spectra of the concentration set were registered in the spectral region 225-315 nm (0.1 nm releasement). The spectral data matrix of the concentration set was arranged as x-block, which named as the absorbance data matrix. In the calibration step, the algorithms of PLS and PCR models were applied to the relationship between the calibration data matrix and the absorbance data matrix. In the following step, the content of PRA and IVE in the marketed veterinary tablets and related samples was estimated by using building PCR and PLS tools. In a condition of the PLS calibrations, the actual and predicted concentrations for PRA and IVE were plotted and shown in Figure 7. In a similar manner, for the PCR calibration, actual and predicted concentrations for PRA and IVE were graphically given in Figure 6. As can be seen in the mentioned figures, good correlation coefficients were stated. In the prediction steps of the applied PLS and PCR methods, Figure 7 and 6, respectively indicates the plots of the actual and predicted concentrations in the implementation of PLS and PCR, respectively.



**Figure 6.** Plot of the actual and predicted concentrations in the calibration step by using the PCR method with four components for (a) IVE and (b) PRA drugs



(a)



(b)

**Figure 7.** Plot of the actual and predicted concentrations in the calibration step by using the PLS method with four components for (a) PRA and (b) IVE drugs

**Table 1.** Calibration set model for the Uv-Vis spectrophotometric analysis of PRA and IVE.

PRA		IVE		PRA	
Set No.	( $\mu\text{g/mL}$ )	Set No.	( $\mu\text{g/mL}$ )	Set No.	( $\mu\text{g/mL}$ )
1	20.0	0.0	17	0.0	2.0
2	20.0	6.0	18	150.0	2.0
3	40.0	0.0	19	0.0	8.0
4	40.0	6.0	20	150.0	8.0
5	60.0	0.0	21	0.0	14.0
6	60.0	6.0	22	150.0	14.0
7	80.0	0.0	23	0.0	20.0
8	80.0	6.0	24	150.0	20.0
9	100.0	0.0	25	0.0	26.0
10	100.0	6.0	26	150.0	26.0
11	120.0	0.0	27	0.0	32.0
12	120.0	6.0	28	150.0	32.0
13	140.0	0.0	29	0.0	38.0
14	140.0	6.0	30	150.0	38.0
15	160.0	0.0	31	0.0	44.0
16	160.0	6.0	32	150.0	44.0

## B. 2. Validation of Chemometric Calibration Methods

The validation samples, synthetic mixtures, intra-day, inter-day, and standard addition samples were studied for the validation of the applied PCR and PLS methods. The recovery results were obtained from the implementation of the PCR and PLS methods for the analysis of synthetic mixtures were listed in Table 2 with standard deviation (SD) and relative standard deviation (RSD). Recovery results obtained by PCR method were get to be 100.1 % and 100.8 % for PRA and IVE, respectively. In case of PLS method, the recoveries were computed as 100.0 % and 99.8 % for PRA and IVE, respectively. From the recovery results in Table 2, it was observed the low amounts of SD and RSD. This showed that the methods provided acceptable accuracy and precision for the analysis. For the accuracy and precision of PCR and PLS methods, the intra-day and inter-day samples were analyzed. The obtained results were indicated in Table 3. Successful results were reported for the PCR and PLS methods with high recoveries, low amount of RSD, and relative standard error (RSE). To determine the presence and absence of the excipient effect on the quantitation of the PRA and IVE in commercial veterinary tablet samples, the standard addition method was implemented. The results of these experiments were presented in Table 4. As it can be understood from Table 4, no interference was reported. After method validation processes, these proposed PLS and PCR approaches were attentively validated and implemented to the analysis of the real marketed veterinary tablet samples including PRA and IVE substances.

**Table 2.** The Uv-Vis spectrophotometric analysis results of calibration set.

Mix	Added		Found		Recovery		Found		Recovery	
	PRA	IVE	PRA	IVE	PRA	IVE	PRA	IVE	PRA	IVE
	(µg/µL)		(µg/µL)		(%)		(µg/µL)		(%)	
No.										
M1	20	6	20.2	6.2	100.8	102.5	20.1	6.2	100.6	102.8
M2	40	6	39.7	5.9	99.2	98.1	39.5	5.9	98.9	98.3
M3	60	6	60.6	6.1	101.0	101.0	60.1	6.1	100.1	102.0
M4	80	6	80.3	5.8	100.4	97.4	79.7	6.1	99.6	101.8
M5	100	6	103.4	5.9	103.4	98.1	102.3	6.1	102.3	101.5
M6	120	6	119.4	5.9	99.5	98.2	119.4	5.9	99.5	98.0
M7	140	6	138.7	6.2	99.1	103.9	139.8	5.9	99.8	97.8
M8	160	6	158.6	6.0	99.1	99.2	158.7	5.9	99.2	98.5
M9	150	2	151.3	2.1	100.9	103.6	151.7	1.9	101.1	96.5
M10	150	8	149.5	8.2	99.7	102.2	150.4	7.8	100.3	97.2
M11	150	14	147.5	14.7	98.4	104.7	148.1	14.4	98.7	102.8
M12	150	20	22.3	20.4	14.9	102.2	24.1	20.2	16.1	101.1
M13	150	26	17.9	26.2	11.9	100.8	21.7	26.4	14.5	101.4
M14	150	32	18.6	32.2	12.4	100.7	22.2	32.9	14.8	102.7
M15	150	38	45.7	37.4	30.5	98.4	48.9	38.2	32.6	100.4
M16	150	44	47.3	44.7	31.5	101.6	49.9	43.7	33.3	99.3
M17	150	6	47.7	6.0	31.8	99.7	50.3	6.2	33.5	103.8
				Mean	100.1	100.8			100.0	99.8
				SD	1.38	2.70			1.04	2.41
				RSD	1.38	2.68			1.04	2.41

SD: Standard deviation

RSD: Relative standard deviation

**Table 3.** Analysis result acquired from Inter-day and intra-day samples with PCR and PLS.

Added			Found			
			PCR		PLS	
	PRA	IVE	PRA	IVE	PRA	IVE
<i>(μg/μL)</i>			<i>(μg/μL)</i>			
Inter-day	20	2	19.95	2.03	20.45	1.98
	60	14	60.87	14.20	61.37	14.00
	100	24	103.61	26.54	100.48	27.95
Intra-day	20	2	19.62	2.03	21.02	2.00
	60	14	60.87	14.20	62.37	13.90
	100	24	103.61	26.54	103.81	26.75
Recovery						
			PCR		PLS	
	PRA	IVE	PRA	IVE	PRA	IVE
<i>(%)</i>						
Inter-day			99.7	101.7	102.2	99.0
			101.4	101.4	102.3	100.0
			103.6	102.1	100.5	107.5
Intra-day			98.1	101.7	105.1	99.9
			101.4	101.4	103.9	99.3
			103.6	102.1	103.8	102.9
RSD						
			PCR		PLS	
	PRA	IVE	PRA	IVE	PRA	IVE
<i>(%)</i>						
Inter-day			1.77	1.91	1.58	1.77
			1.71	0.69	2.29	0.68
			0.97	0.75	1.03	0.63
Intra-day			12.17	1.91	5.08	3.73
			1.71	0.69	3.00	0.68
			0.97	0.75	1.39	2.05
RSE						
			PCR		PLS	
	PRA	IVE	PRA	IVE	PRA	IVE
<i>(%)</i>						
Inter-day			-0.25	1.68	2.25	-0.98
			1.45	1.41	2.28	0.00
			3.61	2.09	0.48	7.50
Intra-day			-1.92	1.68	5.08	-0.15
			1.45	1.41	3.94	-0.71
			3.61	2.09	3.81	2.89

RSD = Relative standard deviation

RSE = Relative standard error

**Table 4.** Assay results of standard addition samples with PCR and PLS methods.

	Added		Found			
			PCR		PLS	
	PRA	IVE	PRA	IVE	PRA	IVE
	(µg/µL)		(µg/µL)			
Formulation	20	5	20.33	4.99	20.90	4.73
Formulation	40	10	40.91	10.28	41.70	10.01
Formulation	60	20	60.49	20.78	60.87	20.95
Formulation	80	30	80.34	30.66	81.00	29.31
Recovery						
			PCR		PLS	
			PRA	IVE	PRA	IVE
(%)						
			101.6	99.7	104.5	94.5
			102.3	102.8	104.2	100.1
			100.8	103.9	101.5	104.7
			100.4	102.2	101.3	97.7
RSD						
			PCR		PLS	
			PRA	IVE	PRA	IVE
(%)						
			0.16	0.06	0.40	0.18
			1.27	0.33	0.88	0.36
			0.56	0.17	0.52	0.42
			1.16	0.95	1.81	0.64

RSD = Relative standard deviation

### B. 3. Commercial Veterinary Tablet Analysis

In the analysis of the marketed veterinary tablets including of PRA and IVE, the sample solutions were prepared as described in the A.3. sub-section "Commercial Tablet Formulation". Marketed veterinary tablet samples were quantified in ten times with the PCR and PLS methods. Basically, the UV spectra of the analyzed samples were processed by using the proposed PCR and PLS methods to obtain the simultaneous quantification of PRA and IVE in marketed veterinary tablets. The Assay results of marketed veterinary tablets were listed in Table 5.

**Table 5.** Quantitation estimation of commercial veterinary tablet by PCR and PLS methods.

ExpNo.	PCR		PLS	
	PRA	IVE	PRA	IVE
	(mg/mL)		(mg/mL)	
1	248.2	10.4	247.2	9.9
2	245.3	10.4	241.2	10.0
3	249.3	10.1	249.9	9.7
4	249.2	10.4	249.7	10.3
5	249.9	9.8	250.2	10.3
6	245.2	10.3	248.8	10.0
7	249.1	10.3	247.8	10.6
8	249.1	10.2	249.2	10.0
9	246.0	10.3	243.8	9.9
10	247.1	10.3	247.6	10.0
Mean	247.8	10.2	247.5	10.1
SD	1.78	0.20	2.90	0.26
RSD	0.72	1.91	1.17	2.57

SD : Standard deviation

RSD : Relative standard deviation

Label claim: 250.0 mg PRA and 10.0 mg IVE / per tablet.

## **IV. CONCLUSION**

In this investigation, new implementation of PLS and PCR tools were proposed for simultaneous quantitative resolution of binary mixtures and marketed veterinary samples containing PRA and IVE substances. In order to get rapid and inexpensive spectral simultaneous analysis of commercial veterinary tablets containing the studied drugs, PCR and PLS methodologies were applied to UV spectra data set of the calibration samples. Then these two chemometric calibrations were used for the estimation of the content of PRA and IVE in samples without using preliminary separation step. Assay results showed that the PCR and PLS applications to the UV spectral data were provided successful results for the quality control protocols and research test of the marketed veterinary formulation including of the studied compounds.

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