Düzce University Journal of Science & Technology

Research Article

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

Eda BÜKER[a,*], Erdal DİNÇ [b], Nagihan YAŞAR[b]

[a] *Department of Basic Pharmaceutical Science, Faculty of Pharmacy, Gazi University, Ankara, TURKEY*
[b] *Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, TURKEY*
* *Corresponding author's e-mail address: edabiyik@gazi.edu.tr*
DOI: 10.29130/dubited.1051389

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*

Received: 30/12/2021, Revised: 07/01/2021, Accepted: 13/01/2021

I. INTRODUCTION

Praziquantel is a pyrazinoisoquinoline used versus several parasite and trematode parasites, most clearly schistosomes for veterinary and human medicine [1]. Its anthelmintic efficiency is depend on muscle spasm and tegument harm, which consist of a remained influx of divalent calcium ions, pursued by spastic paralysis. Figure 1 shows the chemical structure of praziquantel (PRA).

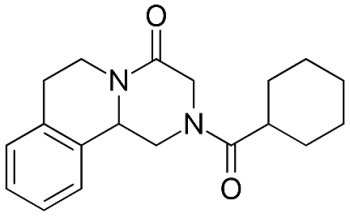


Figure 1. *Chemical structure of praziquantel.*

Ivermectin (IVE) has an effective macro-cyclic lactone triggering paralysis in many nematodes and arthropods through an influx of chloride ions across cell membranes. At this time, it is alternative drug for human onchocerciasis and displays forceful microfilaricidal activity contrary to the other main filarial parasites of humans (Wuchereria bancrofti, Brugia malayi, Loa ba and Mansonella ozzardi) but not against M. perstans. Ivermectin also has tremendous efficacy in both human stronglyoidiasis and cutaneous larva migrans for which beneficial different remedies have not been accessible; and it is as useful as currently accessible drugs beside the intestinal nematodes Ascaris lumbricoides, Trichuris trichiura and Enterobius vermicularis; against the human hookworms it indicates merely unfinished efficacy [2]. The molecular structure of IVE was presented in Figure 2.

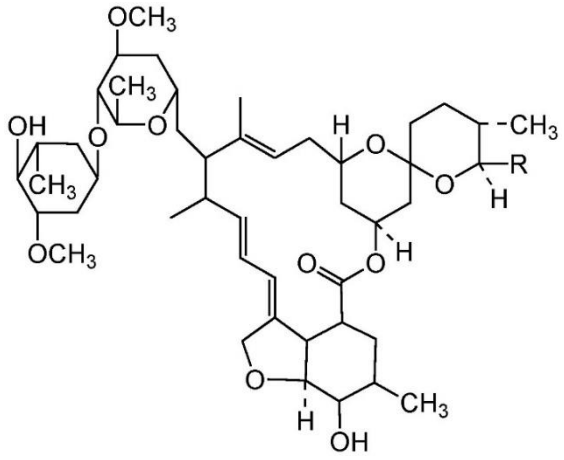


Figure 2. *Chemical structure of ivermectin.*

In the literature, the determination of PRA in its mixtures with the active compounds was carried out by several methods including spectrophotometry [3], HPLC [4-7]. In previous studies, ivermectin in samples with other active compounds was analyzed by using chromatography [8-14]. In the simultaneous analysis of PRA and IVE in commercial formulations, four different methods were reported, including HPLC [15, 16], LC-MSMS [17, 18].Simultaneous determination of analytes in the same sample requires the separation procedure or pretreatment based on the use of chromatographic methods. However, the analysis with these separation methods is high cost and time consuming to find optimal experimental conditions. To overcome these drawbacks, the use of UV-Spectrophotometry combined with PCR and PLS multivariate technique is very promising approaches for solving complex mixture of two or more component systems.

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.

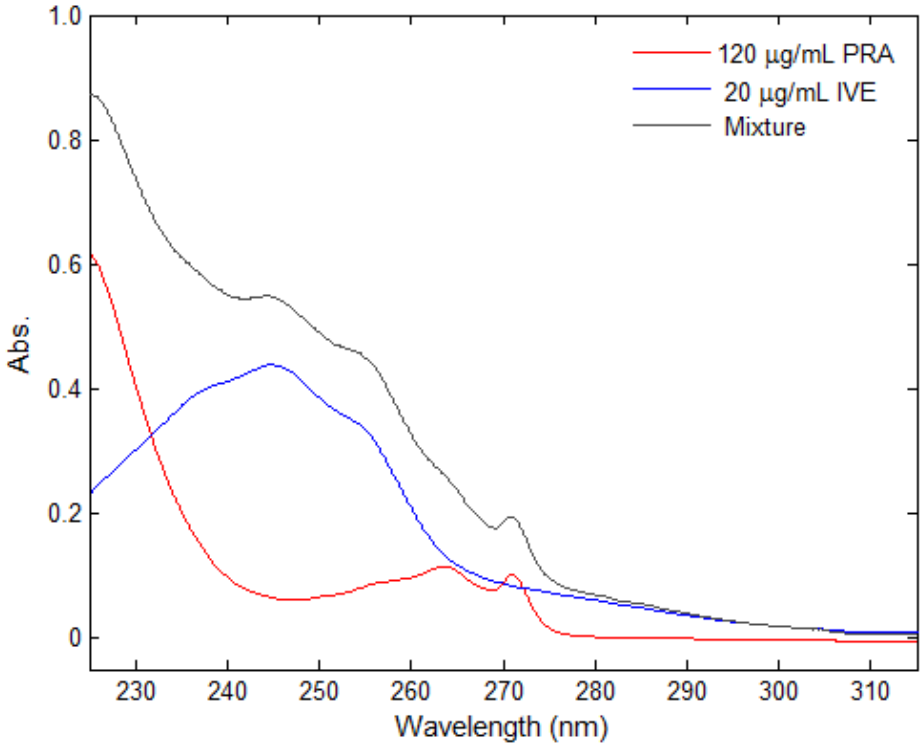
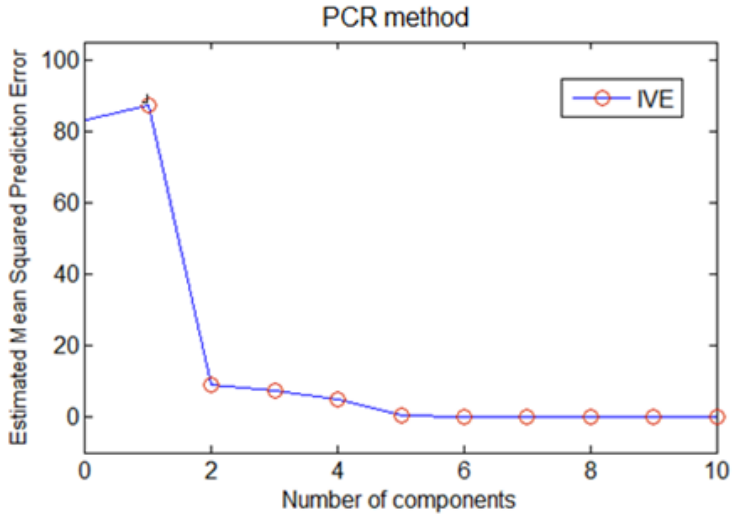


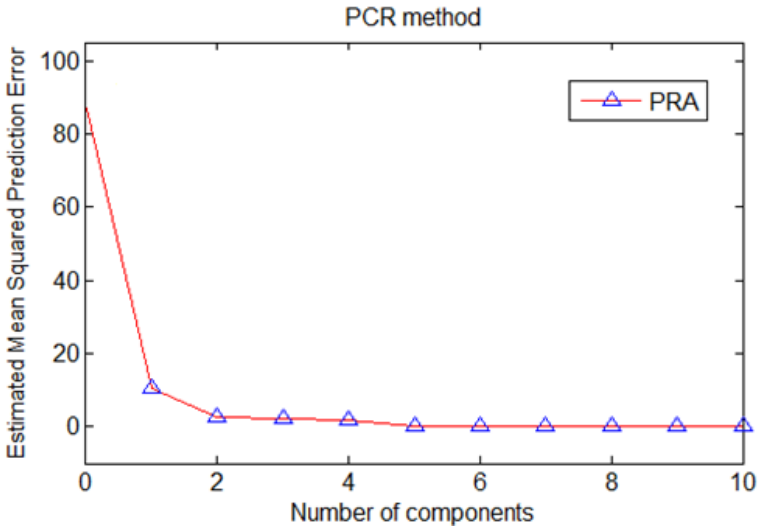
Figure 3. *UV absorption spectra of the PRA and IVE substances, and their binary mixture.*

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).

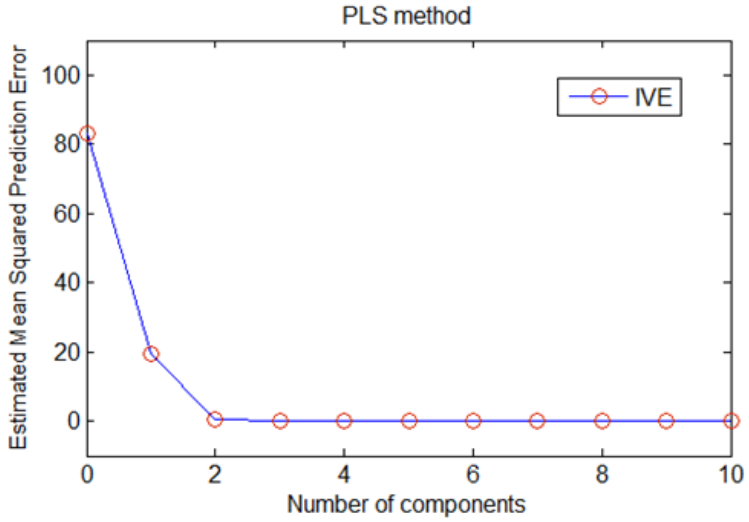


(a)

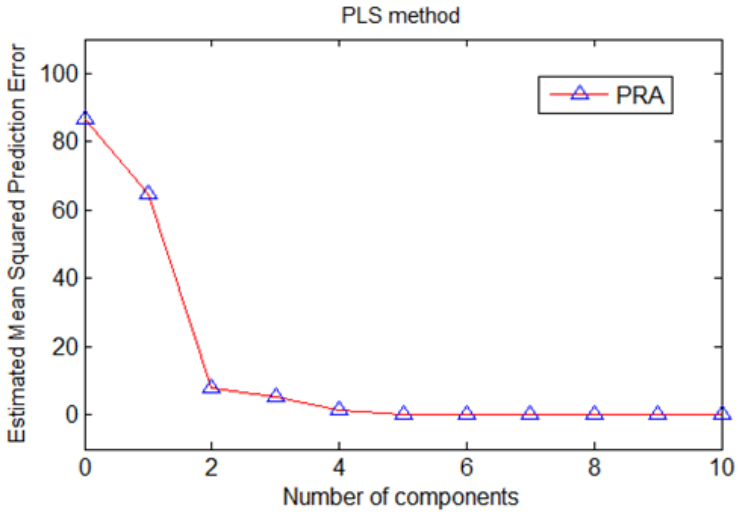


(b)

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



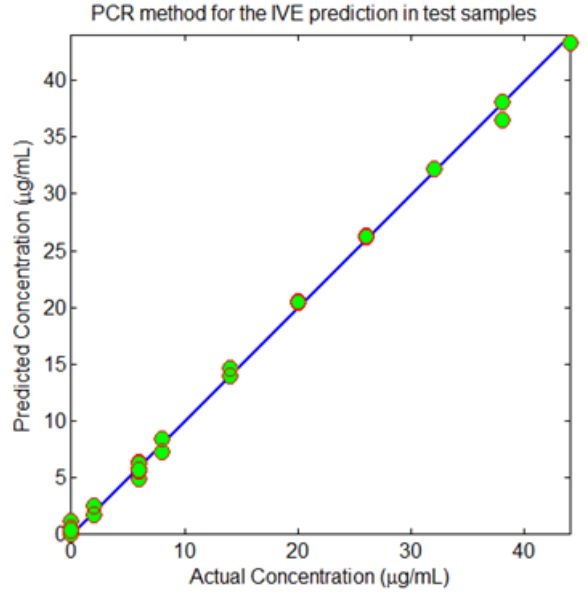
(a)



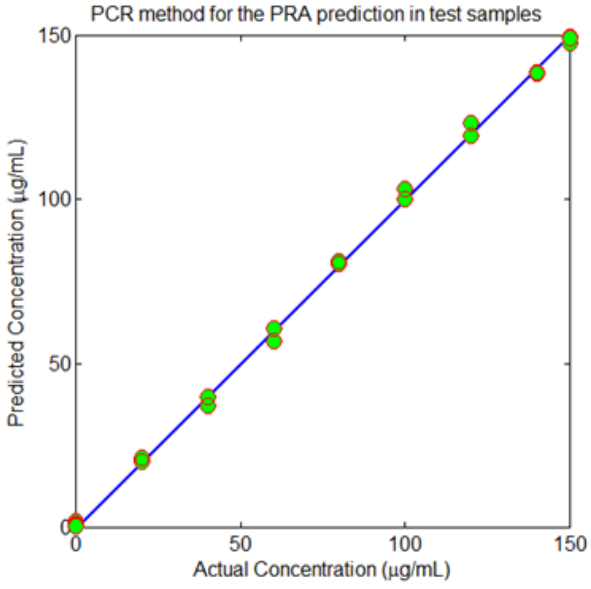
(b)

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 µg/mL and 2.0-44.0 µg/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 registrated 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.

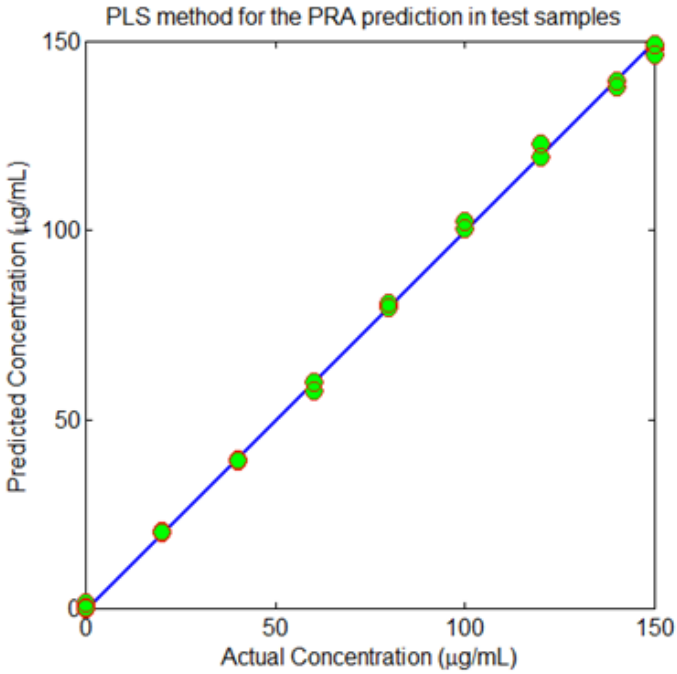


(a)

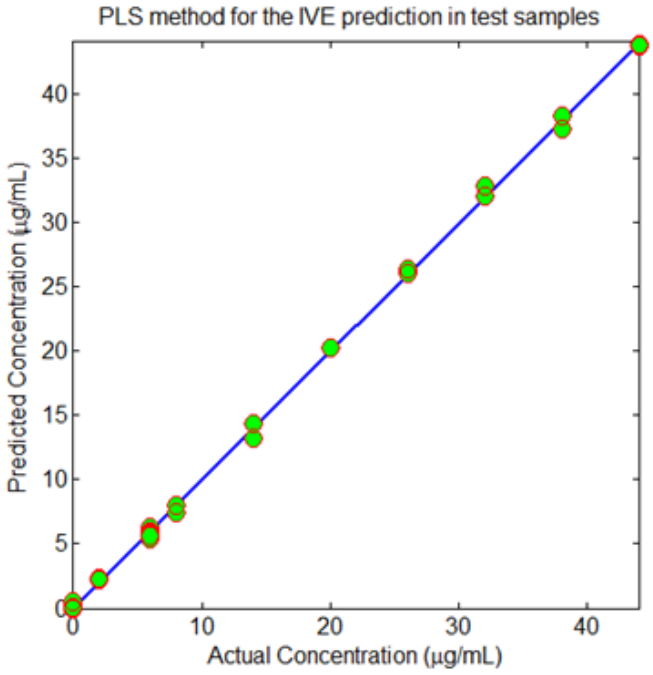


(b)

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.*

Set No.	PRA *(µg/mL)*	IVE	Set No.	PRA *(µg/mL)*	IVE
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**
No.	***(µg/µL)***		***(µg/µL)***		***(%)***		***(µg/µL)***		***(%)***	
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
	(µg/µL)		*(µg/µL)*			
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
			(%)			
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
			(%)			
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
			(%)			
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.*

	PRA	IVE	PRA	IVE
	PCR		PLS	
ExpNo.	*(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 ser 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 seperation 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.

V. REFERENCES

[1] A. Dayan, "Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics," *Acta Tropica*, vol. 86, pp. 141–159, 2003. https://doi.org/10.1016/S0001-706X(03)00031-7

[2] Eric A. Ottesen, and W. Campbell, "Ivermectin in human medicine," *Journal of Antimicrobial Chemotherapy*, vol. 34, no. 2, pp. 195–203, 1994.

[3]. M. S. Piantavini, F. L. Pontes, L. X. Weiss, M. M. Senab, and R. Pontarolo, "Comparison between ultraviolet and infrared spectroscopies for the simultaneous multivariate determination of pyrantel and praziquantel," *J Braz Chem Soc*., vol. 26, pp. 1387-95, 2015. doi:10.5935/0103-5053.20150107

[4] H. M. Phatak, V. V. Vaidya, M. S. Phatak, and D. Rajeghadge, "A rapid high performance liquid chromatography method for simultaneous quantification of praziquantel, ivermectin and abamectin from veterinary formulations: Development, validation and application," *Int J Pharm Res Scholars*, vol. 5, pp. 57-65, 2016.

[5] S. Rupali, B. Shyamala, K. Kulesh, and K. J. Shailendra, "Simultaneous estimation of pyrantel pamoate, praziquantel & febantel by high performance liquid chromatography using dual wavelength," *J Appl Pharm Res*., vol. 2, pp. 32-43, 2014.

[6] R. Rajesh, and J. J. Jithu, "A validated RP-HPLC method for simultaneous estimation of pyrantel pamoate and praziquantel in bulk and pharmaceutical dosage form," *Int J Pharm Pharm Sci*, vol. 11, pp. 62-67, 2019. doi:10.22159/ijpps.2019v11i5.30488

[7] W. Hanpitakpong, V. Banmairuroi, B. Kamanikom, A. Choemung, and K. Na-Bangchang, "A high-performance liquid chromatographic method for determination of praziquantel in plasma*," Journal of Pharmaceutical and Biomedical Analysis*, vol. 36 , pp. 871–876, 2004.

[8] G. Vijay Kumar, B. Sravanthi, and A. Praveen, "Analytical method development and validation for ivermectin and albendazole in combine dosage form by RP-HPLC," *Int J Curr Trends Pharma Res.*, vol. 7, pp. 7-12, 2019.

[9] B. Bhavya, P. Nagaraju, V. Mounika, and G. I. Priyadarshini, "Stability indicating RP-HPLC method development and validation for simultaneous estimation of albendazole and ivermectin in pharmaceutical dosage form," *Asian J Pharm Anal*., vol. 7, pp. 6-14, 2017. doi:10.5958/2231-5675.2017.00002.3

[10] A.K. Patel, H.V. Joshi, and J.K. Patel, "Development and validation of stability indicating RP-HPLC method for estimation of ivermectin and albendazole in pharmaceutical dosage form," *Indian J. Drugs*, vol. 3, pp. 57–70, 2015.

[11] L. K. Vegad, B. D. Paranjape, A. D. Shah, D. E. Patel, K. Y. Patel, and R. K. Patel, "Development and validation of RP-HPLC method for simultaneous estimation of ivermectin and clorsulon in ivercam injection," *Indo Am. J. Pharm. Res*., vol. 7, pp. 523–531, 2017.

[12] M. Shurbaji, M. H. Abu Al Rub, M. M. Saket, A. M. Qaisi, M. L. Salim, and E. S. Abu-Nameh, "Development and validation of a new HPLC-UV method for the simultaneous determination of triclabendazole and ivermectin B1a in a pharmaceutical formulation," *J. AOAC Int.*, vol. 93, pp. 1868–1873, 2010.

[13] N. Salem Rasheda, S. Zayedb, A. Abdelazeema, and F. Fouada, "Development and validation of a green HPLC method for the analysis of clorsulon, albendazole, triclabendazole and ivermectin using monolithic column: Assessment of the greenness of the proposed method," *Microchemical Journal*, vol. 157, no. 105069, pp. 1-10, 2020. https://doi.org/10.1016/j.microc.2020.105069

[14] E. Felici, C. Casado, C.C. Wang, J. Raba, and M.R. Gomez, "A green alternative method for analysis of ivermectin and moxidectin in environmental water samples using automatized preconcentration previous MEEKC," *Electrophoresis*, vol. 37, pp. 2977–2985, 2016.

[15] N. V. Suresh Kumar Devaka, and V. Madhusudhan Rao, "Chromatographic Quantification of Ivermectin and Pranziquantel in the Tablets Using Stability Indicating RP-HPLC Method," *Pharmaceutical Sciences*, vol. 25, pp. 254-261, 2019. doi: 10.15171/PS.2019.41

[16] A. Kulik, A. Szczotkowska, W. Bia£Ecka, M. Podolska, B. Kwiatkowska-Puchniarz and A. Mazurek, "Determination of Active Substances in Binary Mixture Antiparasitic Veterinary Formulations By Hplc," *Acta Poloniae Pharmaceutica in Drug Research*, vol. 68, no. 4, pp. 467-472, 2011.

[17] F. L. Degaut Pontes, R. Pontarolo, F. R. Campos, J. C. Gasparetto, M. A. Cardoso, M. S. Piantavini, and A. C. Leal Badaró Trindade, "Development And Validation of An Hplc-Ms/Ms Method For Simultaneous Determination of Ivermectin, Febantel, Praziquantel, Pyrantel Pamoate And Related Compounds in Fixed Dose Combination for Veterinary Use," *Asian J Pharm Clin Res*, vol. 6, no. 2, pp. 191-200, 2013.

[18] F. L. Pontes, R. O. Pontarolo, F. R. Campos, J. C. Gasparetto, M. A. Cardoso, M. S. Piantavini, et al., "Development and validation of an HPLC-MS/MS method for simultan ous determination of ivermectin, febantel, praziquantel, pyrantel pamoate and related compounds in fixed dose combination for veterinary use," *Asian J Pharm Clin Res*.," vol. 6,pp. 191-9, 2013.

[19] E. Dinç, A. Ozdemir, H. Aksoy, and Ö. Üstündağ, "Chemometric Determination of Naproxen Sodium and Pseudoephedrine Hydrochloride in Tablets by HPLC," *Chem. Pharm. Bull.*, vol. 54, pp. 415-421, 2006.

[20] E. Dinç, Ö. Üstündağ, and D.Baleanu, "Simultaneous chemometric determination of pyridoxine hydrochloride and isoniazid in tablets by multivariate regression methods," *Drug Testing and Analysis*, vol. 2, pp. 383-387, 2010.

[21] E. Dinç, and D. Baleanu, "Chemometric Simultaneous Determination of Atorvastatin and Amlodipine in Tablets by PCR and PLS Calibrations," *Revista de Chimie -Bucharest- Original Edition*, vol. 2, pp. 127-130, 2009.