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Research Article

Simultaneous Determination of Albendazole and Praziquantel Using Zero Order Spectrophotometry Absorbance correction Methods in Veterinary Pharmaceutical Formulation

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ABSTRACT

The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of albendazole (ABZ) and praziquantel (PZQ) by absorbance correction method. The simultaneous determination of albendazole (ABZ) and praziquantel (PZQ) was performed by absorbance correction method using two different wavelengths i.e. 217nm & 295.4nm. Both the drugs were dissolved in methanol for estimation. A linear response was observed in the range of 4-14µg/ml with a regression coefficient of 0.999. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. The detection limits (LOD = 4 µg/ml for both the drugs) for absorbance correction method were determined and presented the best analytical features. The recoveries of ABZ and PZQ from the synthetic samples were near to 100 ± 5%. The methods were applied in veterinary pharmaceutical formulation whose mass ratio ABZ: PZQ is 12:1; the results obtained were according to nominal content.

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1. Introduction

The antiparasitic drugs are widely used in veterinary practice and human therapy. The antihelminthics are an important type of these drugs used in cattle and pets, bringing benefit and improved quality of life to such animals.[1] An important example of a disease treated with these drugs is helminthiases, a common parasitic disease of great economical and public health importance. Since the antihelminthic spectra of most drugs used for treatment is limited, combinations of more than one active ingredient are required to control helminthic infections effectively. In this context, albendazole, methyl 5-(propylthio)-2- benzimidazole carbamate (ABZ) (Fig. 1a), is an antihelminthic drug, it is active against most of the nematode worms and some of the cestode worms in humans and animals, and it acts by inhibiting fumarate reductase and the microtubular polymerization of the parasite.[2] Praziquantel, 2-cyclohexylcarbonyl-1,2,3,6,7,11bhexahydro- 4h-pyrazino(2,1-a) isoquinolin-4-one (PZQ) (Fig. 1b), acts on the parasite, increasing the permeability to calcium ions causing contractions and vacuolization. The mix of both drugs is efficiently used in veterinary treatment of parasitic diseases. Many publications describe the determination of ABZ and PZQ in pharmaceutical formulations. Individual and simultaneous determination with other drugs are possible by FIA using UV-detection and HPLC method in suspensions,[3] adsorptive stripping voltammetry (ASV) and linear sweep voltammetry (LSV), square-wave voltammetry (SWV), differential pulse voltammetry (DPV),[4-5] Vis-spectrophotometry,[6–9] HPLC,[10,11] derivative UV-spectrophotometry,[12-13] GLC[14] and PMR spectrometry.[15] In biological samples for individuals, simultaneous determinations with other drugs and metabolites have been reported by: HPLC,[16–22] LC-TMS,[23-24] LC-ES-SM,[25] nonaqueous capillary electrophoresis,[26] IR[27] and fluorometry.[28-29]

A newer dose regiment was developed in combination of ABZ and PZQ for treatment of helminthes and the estimation was also been performed by RP-HPLC in rat plasma.[30] Classical least squares (CLS) analysis is one of the simplest multivariate methods and is easy to perform, although its results are not very accurate in the quantification of mixtures when the analyzed spectra have significant overlapping.[31]Second derivative spectroscopic method was developed for the estimation in Veterinary Pharmaceutical Formulation .[32] On the

other hand, PCR and PLS regression have been used more successfully in quantification of those types of samples, although their use presents more complications than CLS and second derivative spectrophotometry (SDS). The aim of this work is to develop different mathematical approaches for the simultaneous determination of ABZ and PZQ in veterinary pharmaceutical formulation.

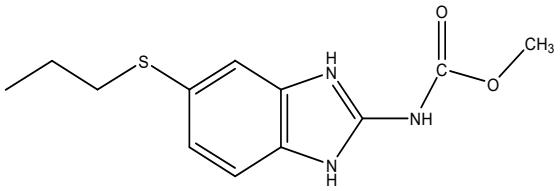

Fig. 1.Chemical structures of Albendazole

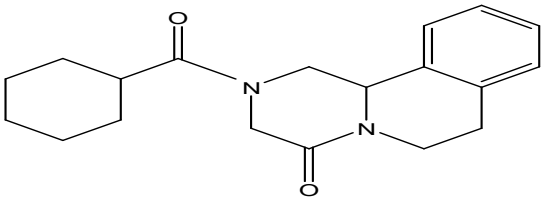

Fig. 2.Chemical structures of Praziquantel

2. Experimental

2.1. Materials and Solutions

Albendazole (Fig. 1) was obtained from Mercury Pharmaceutical Ltd, Vadodara, Gujarat, India. Praziquantel (Fig. 2) was obtained from Micro Labs Ltd., Goa. The solvent used was methanol which was of AR grade, purchased from SD Fine Chemicals Limited, India and double distilled water.

2.2. Instrumentation:

The instrument used for the present study was a UV-Vis double beam spectrophotometer (model 2080, Analytical Technological Limited) with 1cm matched pair quartz cell.

2.3. Method:

2.3.1. Solubility Test:

Solubility test for the drug Albendazole and Praziquantel was performed by using various solvents. The solvents include water, methanol, ethanol, acetonitrile, 0.1N hydrochloric acid (0.1N HCl), 0.1N sodium hydroxide (0.1N NaOH), DMF, and chloroform.

Solvent: Methanol

2.3.2. Preparation of stock solution of Albendazole and Praziquantal (1000µg/ml): 25 mg of both the drugs were weighed and transferred to a 25ml volumetric flask. 15 ml of diluent was added and the solution is sonicated for 15 min. Volume is made up to the mark with diluent to obtain stock solution of 1000µg/ml.

2.3.3. Preparation of working standard solution Albendazole and Praziquantal (100µg/ml): 2.5 ml of stock solution was withdrawn and transferred to 25 ml volumetric flask, volume is made upto the mark with Solvent to get the working standard solution of 100 µg/ml.

2.3.4. Absorbance Correction Method:

Each working standard solution was scanned between the range 200-400 nm in 1cm cell against blank and the overlain spectra was recorded (Fig.3). Albendazole shows two absorbance maxima (λmax) at 295.4 nm and 215.8 nm wavelengths, whereas Praziquantel shows no absorbance maxima (λmax), but it shows Absorbance at 217.0 nm. (Fig.3). Overlain spectra of Albendazole (ABZ) and Praziquantel (PRQ). The calibration curves for ABZ and PRQ were prepared with in the concentration range of 4-14 µg/ml at selected wavelengths by diluting aliquot portions of standard stock solution of each drug. The plots of Beer's law limit are shown in (Fig.4, 5, & 6)

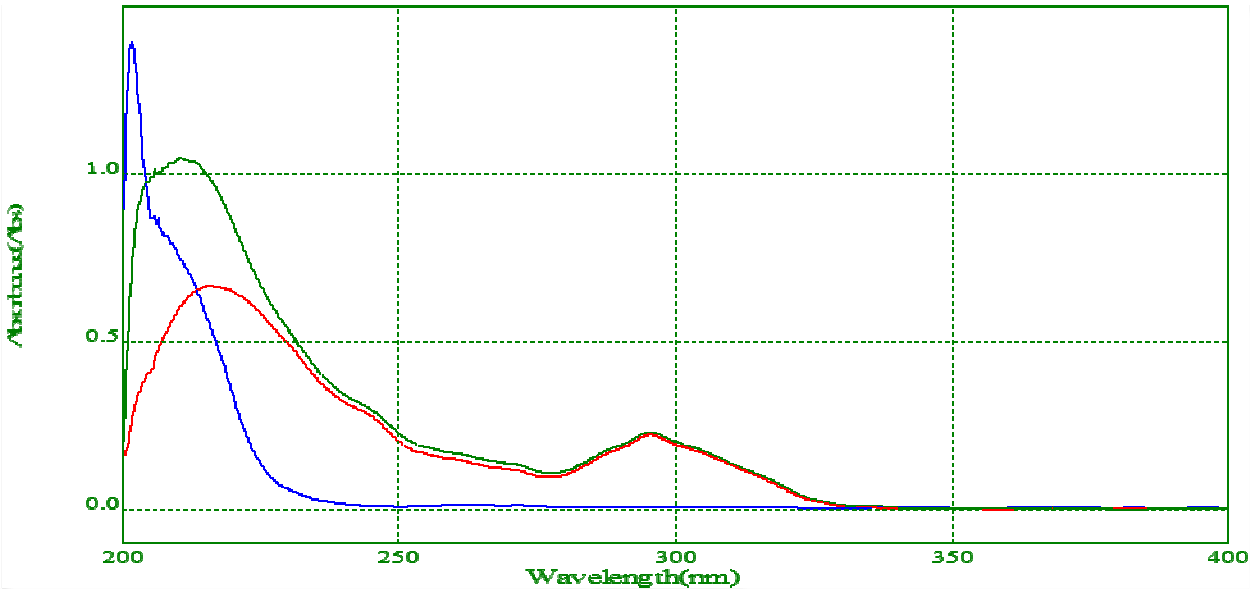

Fig: 3- Selection of detection wavelength for ABZ & PRQ at 295.4nm and 217.0nm respectively; ____: Albendazole (ABZ); ____: Praziquantel (PRQ); ____: Mixture

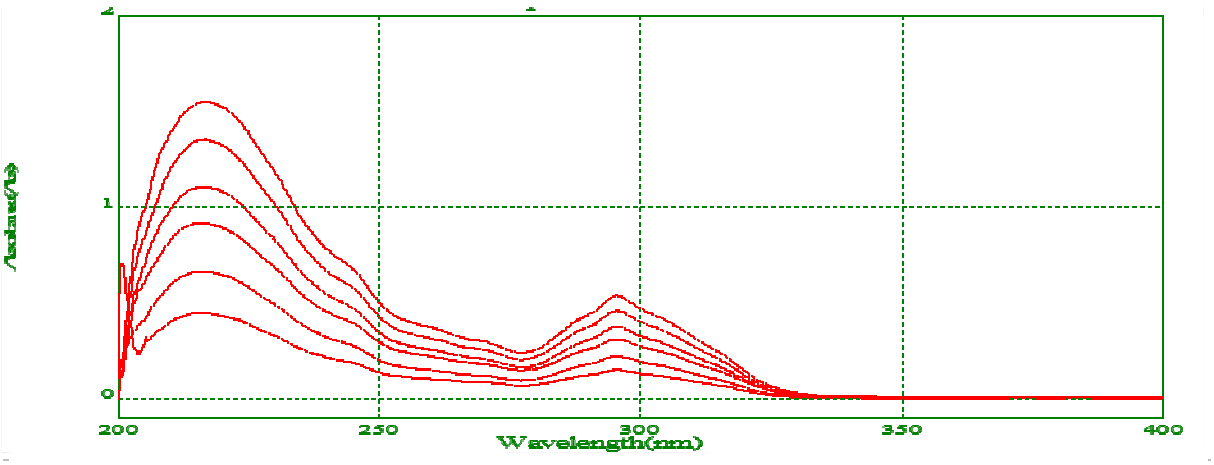

Fig: 4- Overlay Spectra of linearity *of Albendazole*

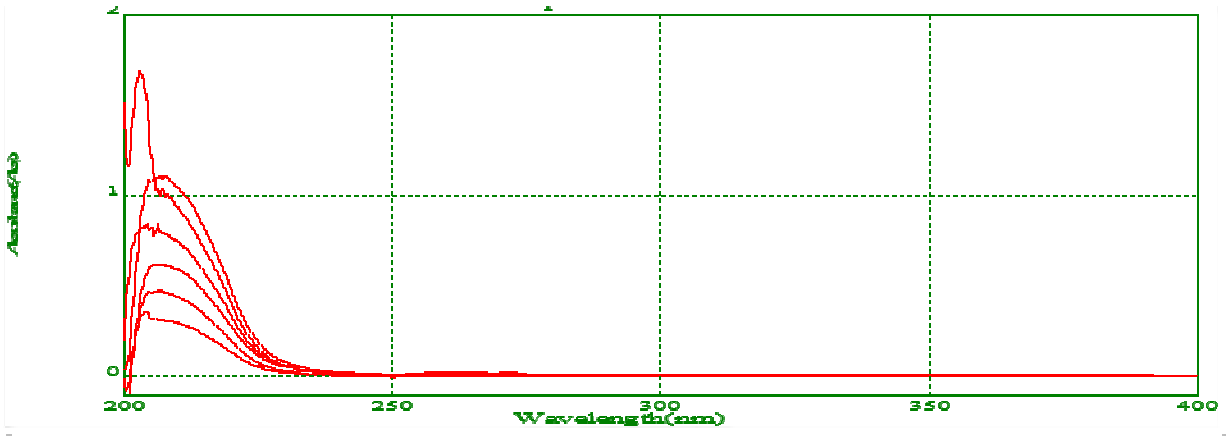

Fig: 5-Overlay Spectra of linearity of Praziquantel

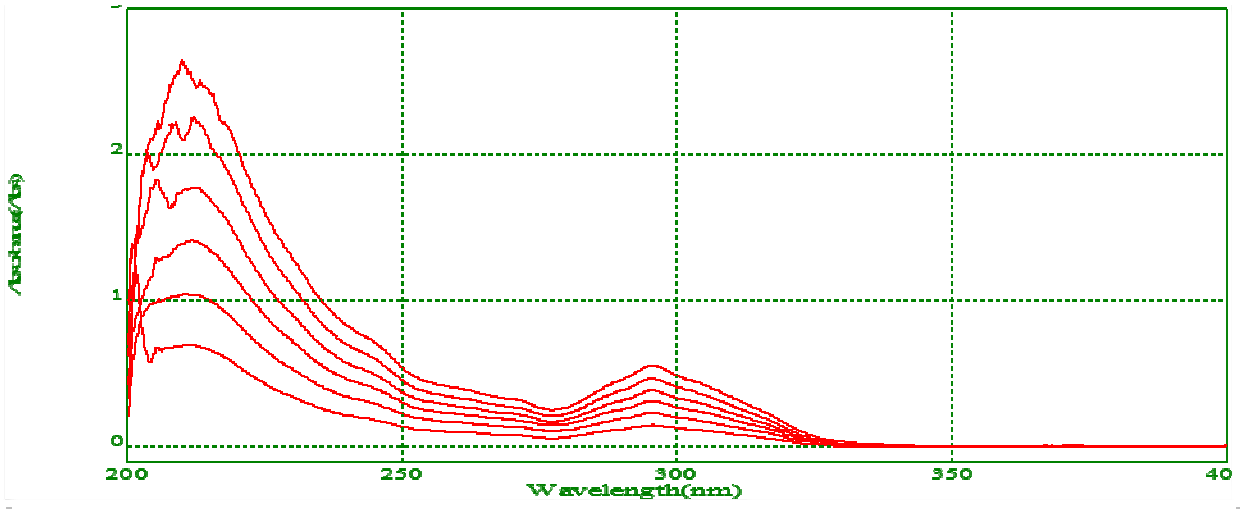

Fig: 6- Overlay Spectra of linearity of Albendazole and Praziquantel

2.4. UV Method Development

2.4.1. Preparation of Stock Solution:

Standard stock solution of Albendazole and praziquantel was prepared by dissolving individually both the drug (10mg) of in 10ml of Methanol which gives 1000µg/ml. One ml of this stock Solution was taken and was diluted up to 10ml by using methanol (solvent) to produce a concentration of 100µg/ml solution.

2.4.2. Preparation of Working Solution:

From the above stock solution 1ml was transferred into 10ml volumetric flask and volume was made up to the mark with methanol to make 10µg/ml.

2.4.3. Determination of λmax:

Then the above working sample solution (10µg/ml) was scanned with UV-Vis Spectrophotometer in the range 400-200nm against Methanol as blank and the wavelength corresponding to absorbance was noted For Albendazole at 295.4nm and Praziquantel at 217nm for Absorbance Correction Method. (Fig-3)

2.4.4. Preparation of Calibration Curve:

From the above stock solution (100µg/ml) further dilution were made and the volume was make up to 10ml using Methanol to produce 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml 12 µg/ml and 14µg/ml solutions respectively. Then the construction of calibration curve was showed a straight line (Fig- 7, 8, 9, 10 & 11). The correlation coefficient was found to be 0.999 for Absorbance correction method.

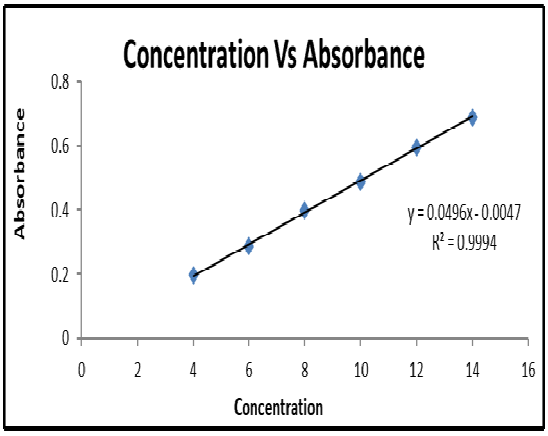

Fig: 7- Calibration Curve of "Praziquantel" at 217nm

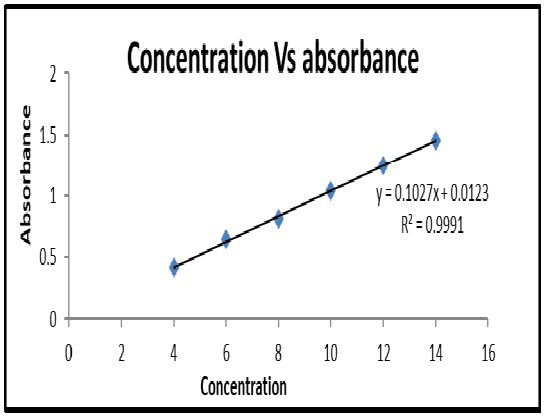

Fig: 8- Calibration Curve of "Albendazole" at 217nm

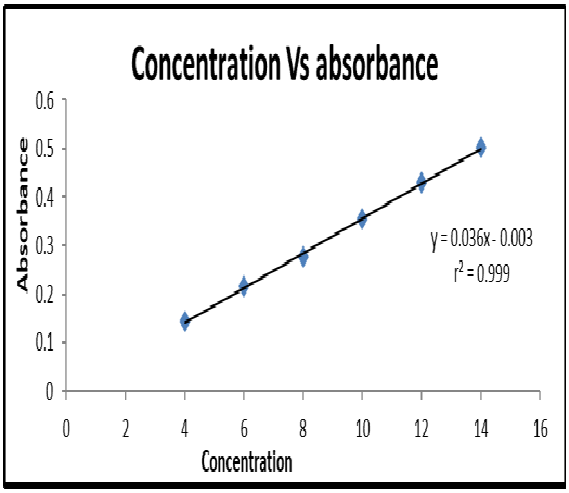

Fig: 9- Calibration Curve of "Abendazole" at 295.4nm

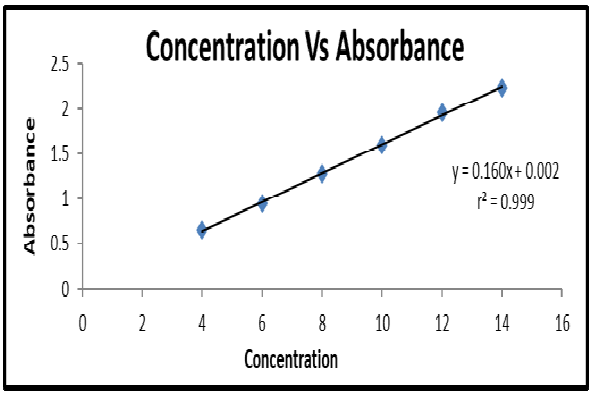

Fig: 10- Calibration Curve of "Mixture" at 217.0nm

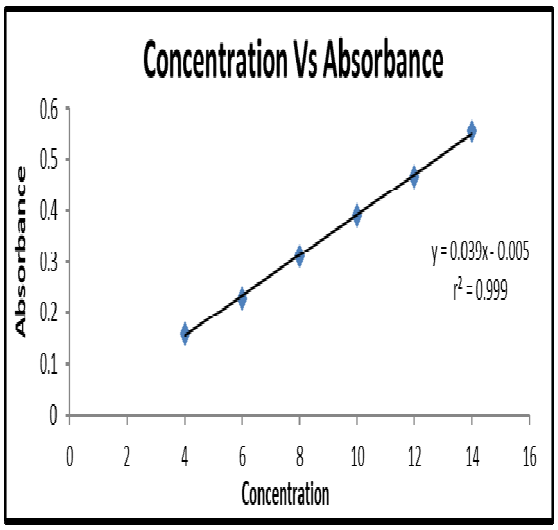

Fig: 11- Calibration Curve of "Mixture" at 295.4nm

2.5. Method Validation:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The validation for UV method development was performed using parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, and Limit of detection (LOD), Limit of quantification (LOQ)[33]

2.5.1. Linearity

The linearity was tested for the concentration range of 4, 6, 8, 10, 12, 14µg/ml for ABZ & PRQ. The calibration curve was constructed and evaluated by its coefficient of determination (r^2). The calibration plot (concentration of PRQ versus Absorbance of PRQ at 217nm & concentration of ABZ versus Absorbance of ABZ at 295.4nm.) was generated by replicate analysis (n = 10) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program. For minimum error with precise, concise and accurate data 10 different concentration were been taken which gave a wide range for linearity. The coefficient of determination (r^2) for ABZ & PRQ were 0.999 & 0.999 for ABZ & PRQ and given in (Fig.10, 11).

2.5.2. Accuracy:

Accuracy of the method was determined by replicate analysis of three sets of samples spiked with three different levels of ABZ & PRQ in level 80%, 100% & 120% and comparing the difference between spiked value (theoretical value) and that actually found value.

2.5.3. Precision:

The precision of the method based on within-day repeatability was determined by replicate analysis of three sets of samples spiked with three different concentrations of ABZ & PRQ (4, 10and 14µg/ml). The reproducibility (day-to-day variation) of the method was validated using the same concentration range as described above, but only a single determination of each concentration was

made on three different days. Relative standard deviation (R.S.D.) were calculated from the ratios of standard deviation (S.D.) to the mean and expressed as percentage.

2.5.4. Specificity:

Specificity study was performed by analyzing standard solution in the presence of an excipient to find was there any interference of excipients in % recovery of ABZ & PRQ. Amount of ABZ & PRQ was spiked with 50%, 100%, and 150% of excipient (talc) and the sample was analyzed for ABZ & PRQ recovery UV-VIS Spectrophotometer.

3. Result and Discussion

3.1. Calibration Curve:

Linearity of response for ABZ and PRQ for determination of both of them in Single as well as synthetic mixture, by preparing a stock solution and suitably diluted to achieve concentration of about 4, 6, 8, 10, 12, 14µg/ml. Value of Coefficient of determination (r^2), slope and intercept were y = 0.049x - 0.004, R^2 = 0.999 at 217.0nm for PRQ in bulk, y = 0.102x + 0.012, R^2 = 0.999 at 217.0nm for ABZ in bulk, y = 0.036x - 0.003, R^2 = 0.999 at 295.4nm for ABZ in bulk, y = 0.160x + 0.002, R^2 = 0.999 at 217.0nm in synthetic mixture, y = 0.039x - 0.005, R^2 = 0.999 at 295.4nm in synthetic mixture. The linear regression data from the calibration curve indicate that the response is linear over the concentration range studied for both the drug. So it can be apply for determination of ABZ and PRQ in synthetic mixture.

3.2. Analytical method validation:

3.2.1. Linearity:

The linearity was tested for the concentration range of 4, 6, 8, 10, 12, 14µg/ml. and the calibration curve was constructed and evaluated by its correlation coefficient. The linear relationship was evaluated using the least square method within Microsoft Excel® program. The coefficient of determination (r^2) for both ABZ and PRQ was 0.999 given in (Table 1, 2, 3, 4, 5, & 6) and (Fig. 3, 4, & 5)

Table No.: 1: Linearity data of Praziquantel at 217.0

Conc. (µg/ml)	*Abs. (n=5)*	*Conc. found*	*Conc. gm/100ml*	*Absorptivity gm/100ml*	*Avg. absorptivity*	**Slope:**

	(nm)				*gm/100ml*	0.0496
4	0.1958	4.0416	0.00040	484.455		
6	0.2874	5.8884	0.00058	488.075		**Intercept:**
8	0.3968	8.0940	0.00080	490.235	489.811	0.0046
10	0.4882	9.9368	0.00099	491.304		
12	0.5959	12.1081	0.00121	492.146		**r^2 :**
14	0.687	13.9448	0.00139	492.653		0.9994

Table No.: 2: Linearity data of Albendazole at 217.0nm

Conc. (µg/ml)	*Abs. (n=5) (nm)*	*Conc. found (µg/ml)*	*Conc. gm/100ml*	*Absoptivity gm/100ml*	*Avg. absorptivity gm/100ml*	*C alz*	**Slope:** 0.1110
4	0.447	3.986	0.00039	1122.793		0.0004	**Intercept**
6	0.662	5.923	0.00059	1118.609		0.0005	**:**
8	0.912	8.172	0.00081	1116.240		0.0008	0.0051
10	1.103	9.893	0.00098	1115.154	1116.777	0.0009	
12	1.351	12.12	0.00121	1114.204		0.0012	**r^2 :**
14	1.551	13.92	0.00139	1113.661		0.0013	0.9990

Table No.: 3: Linearity data of Albendazole at 295.4nm

Conc. (µg/ml)	*Abs. (n=5) (nm)*	*Conc. found (µg/ml)*	*Conc. gm/100ml*	*Absoptivity gm/100ml*	*Avg. absorptivity gm/100ml*	*C alz*	**Slope:** 0.039
4	0.1493	4.0336	0.00040	370.15		0.0003	**Intercept:**
6	0.2215	5.8918	0.00058	375.94		0.0005	-0.0074
8	0.3106	8.1853	0.00081	379.45		0.0008	**r^2 :**
10	0.3754	9.8532	0.00098	380.98	378.68	0.0009	0.9990
12	0.4591	12.007	0.00120	382.33		0.0012	
14	0.5377	14.030	0.00140	383.22		0.0014	

Table No.: 4: Linearity data of Mixture at 217.0 nm

Conc. (µg/ ml)	*Abs. (n=5) (nm)*	*Conc. Found (µg/ml)*	*Conc. gm/100 ml*	*Absor-ptivity*	*Avg. absor-ptivity*	*Alz-217 (absorptivit y*Conc.)*	*Cprq= (abs-alz _absorptivity * Conc.at217) /prz_absorptivi ty at 217nm*	**Slope:** 0.160 **Intercept:** 0.002 **r^2 :** 0.999
4	0.654	4.06	0.0004	1611.2		0.447	0.00042	
6	0.953	5.92	0.00059	1609.6		0.662	0.00059	
8	1.281	7.96	0.00079	1608.6		0.912	0.00075	
10	1.601	9.95	0.00099	1608.1	1608.8	1.103	0.00101	
12	1.959	12.1	0.00121	1607.7		1.351	0.00124	
14	2.235	13.9	0.00139	1607.5		1.551	0.00139	

Table No.: 5: Linearity data of Mixture at 295.4 nm

Conc. (µg/ml)	*Abs.(n=5) (nm)*	*Conc. found*	*Conc. gm/100ml*	*Absorptivity*	*Avg. absorptivity*	*Calz*	**Slope:** 0.160 **Interce pt:** -0.005 **r^2 :** 0.9990
4	0.1592	4.142	0.00041	384.30		0.00040	
6	0.2296	5.915	0.00059	388.14		0.00058	
8	0.3104	7.950	0.00079	390.43		0.00079	
10	0.3893	9.937	0.00099	391.76	390.10	0.00099	
12	0.4678	11.91	0.00119	392.65		0.00119	
14	0.5565	14.14	0.00141	393.35		0.00142	

Table No.: 6: Spectral and statistical data for determination of Albendazole and Praziquantel by Absorbance Correction Method

Parameters	**Drug (n=5)**	
	Albendazole	**Praziquantel**

Absorption Wavelength	295.4nm	217.0nm
Linearity	y = 0.039x - 0.005	y = 0.160x + 0.002
Precision (%RSD, NMT 2)	0.2-0.9	0.2-0.5
Repeatability	0.9	0.5
Intermediate precision	0.2-0.7	0.2-1.7
Accuracy (%recovery± SD*)n=3	99.79-101.95	99.09-102.19
LOD (µg/ml)	1.32	1.31
LOQ (µg/ml)	4.0	4.0
Specificity	100.74-101.24	100.15-102.49
Assay (%w/w± SD*) n=6	100.176 ± 0.03	100.17 ± 0.060

3.2.2. Limit of detection and limit of quantification:

A limit of detection (LOD) and a limit of quantification (LOQ) were established based on the calibration curve parameters, according to the formula:

LOD = 3.3*S.D/Slope and

LOQ = 10*S.D/Slope. (Table 7)

Table No.: 7: Determinations of LOD and LOQ.

Conc. (µg/ml)	***Abs. at 217(nm)***	***Abs. at 295.4(nm)***	***At 217nm***	***At 295.4nm***
4	0.6499	0.1542	***Avg.:*** 0.6456	***Avg.:*** 0.1555
4	0.6307	0.1569	***S.D*:*** 0.0105	***S.D*:*** 0.0024
4	0.6339	0.1565	***%R.S.D:***1.6344	***%R.S.D:***
4	0.6505	0.1542	***LOD:*** 1.31	1.51476
4	0.6539	0.1592	***LOQ:*** 4.0	***LOD:*** 1.32
4	0.6549	0.1525		***LOQ:*** 4.0

3.2.3. Precision:

Precision was measured in terms of repeatability of measurement, performed by injecting the standard solution six times and measure the peak area. The RSD was found to be less than 2.0% for both ABZ & PRQ. This shows that Precision of the method is satisfactory which is shown in (Table 8, 9, 10.)

Intermediate Precision: Intermediate Precision with expected results and express as percentage.

Table No.: 8: Determination of Repeatability

Conc. (µg/ml)	*Abs at 217.0nm*	*Avg.:* 1.616	*Abs at 295.4nm*	*Avg.:* 0.387
10	1.605	***S.D*:*** 0.009	0.3839	***S.D*:*** 0.003
10	1.608	***%R.S.D.:*** 0.5854	0.3889	***%R.S.D.:*** 0.9012
10	1.6236		0.3833	
10	1.6299		0.3892	
10	1.614		0.3922	
10	1.6192		0.3855	

Table No.: 9: Determination of Intraday Precision

CONC.(µg/ml)	*Abs at 217.0nm*		*Abs at 295.4nm*	
4	0.3607	***Avg:***0.6383	0.1469	***Avg:***0.1475
4	0.6339	***S.D:***0.010	0.1465	***S.D:***0.001
4	0.6505	***%R.S.D:***1.66	0.1492	***%R.S.D:***0.98
10	1.608	***Avg:***1.6152	0.3839	***Avg:***0.3853
10	1.6236	***S.D:***0.007	0.3889	***S.D:***0.003
10	0.6141	***%R.S.D:***0.48	0.3833	***%R.S.D:***0.79
14	2.2356	***Avg.:*** 2.2271	0.5565	***Avg.:***0.5568
14	2.2005	***S.D:***0.023	0.5579	***S.D:***0.0009
14	2.2453	***%R.S.D:***1.05	0.5562	***%R.S.D:***0.16

Table No.: 10: Determination of Interday Precision

DAY	***AT 217.0nm***				***AT 295.4nm***			
	CONC. (MG/ML)	**Avg.**	**S.D* (n=3)**	**%RSD**	**CONC. (MG/ML)**	**Avg.**	**S.D* (n=3)**	**%RSD**
1.	4	0.7759	0.0085	1.095	4	0.2030	0.0005	0.280
	10	1.8008	0.0069	0.387	10	0.4761	0.001	0.366
	14	2.4166	0.0403	1.670	14	0.6826	0.003	0.477
2.	4	0.7769	0.0020	0.262	4	0.2111	0.001	0.521
	10	1.8510	0.0068	0.368	10	0.4774	0.001	0.346
	14	2.4672	0.0111	0.452	14	0.6728	0.002	0.311
3.	4	0.7950	0.0140	1.763	4	0.2241	0.001	0.449
	10	1.8760	0.0111	0.593	10	0.4961	0.004	0.941
	14	2.5131	0.0100	0.399	14	0.6895	0.005	0.744

3.2.4. Accuracy:

The accuracy of the method was determined by the recovery study carried out using standard addition method at three different concentration levels 80%, 100% & 120%. Resulting spiked sample solutions were assayed in triplicate and the result obtained were compare with

expected result and express as percentage. The mean % recovery of ABZ and PRQ was found to be in the range 99.79-101.95 & 98.63-101.68 % within the acceptance limit which is shown in (Table 11).

Table No.: 11: Accuracy (% Recovery Study)

Analytes	No. of Obs.	Amt. Synthetic Mix. Add. (µg/ml)	% of Pure Drug Added	Amt. Pure Drug Added (µg/ml)	Total Amt. Found (µg /ml)	%Rec.	Mean %Rec± S.D	%RSD
ABZ (295.4nm)	S1	10	80%	8	18.15	101.95	100.84 ± 1.08	1.07
	S2	10	80%	8	17.98	99.79		
	S3	10	80%	8	18.06	100.79		
	S1	10	100%	10	20.13	101.36	101.02 ± 0.33	0.33
	S2	10	100%	10	20.07	100.7		
	S3	10	100%	10	20.1	101		
	S1	10	120%	12	22.18	101.5	101.0 ± 0.43	0.43
	S2	10	120%	12	22.08	100.69		
	S3	10	120%	12	22.09	100.89		
PRQ (217.0nm)	S1	10	80%	8	17.95	98.63	100.81 ± 1.37	1.36
	S2	10	80%	8	18.06	98.89		
	S3	10	80%	8	18.17	100.43		
	S1	10	100%	10	20.12	100.18	100.34 ± 0.76	0.76
	S2	10	100%	10	19.98	100.1		
	S3	10	100%	10	19.99	101.68		
	S1	10	120%	12	21.89	99.76	100.03 ± 1.23	1.22
	S2	10	120%	12	21.95	98.96		
	S3	10	120%	12	22.17	101.51		

3.2.5. Specificity:

Specificity study was performed by analyzing standard solution in the presence of an excipient (talc). 10mg each ABZ & PRQ were spiked with 50% (5mg), 100% (10mg), and 150% (15mg) of talc and the samples were analyzed for ABZ & PRQ recovery by UV-Spectrophotometer. Acceptance criteria for % interference <0.5%. Interference was found to be 100.74-101.24% & 100.15-102.49% for ABZ and PRQ respectively which is within the acceptance limit. Hence the excipients do not interfere with the estimation of drug which is shown in (Table 12).

Table No.: 12: Determination of % Specificity

Analyte	No. of Obs.	Excipient amount added (mg)	Abs. (nm)	% Recovery	Avg.% Recovery	S.D*	% R.S.D
Praziquantel (10mg)	S1	5	1.2681	99.82			
	S2	5	1.2838	101.24	100.15	0.962	0.96
	S3	5	1.2634	99.40			
	S1	10	1.3002	102.72			
	S2	10	1.3034	103.00	102.49	0.660	0.64
	S3	10	1.2894	101.74			
	S1	15	1.2645	99.50			
	S2	15	1.294	102.16	100.16	1.758	1.75
	S3	15	1.2571	98.83			
Albendazole (10mg)	S1	5	0.3254	102.46			
	S2	5	0.3239	101.96	101.24	1.702	1.68
	S3	5	0.3159	99.3			
	S1	10	0.3259	102.63			
	S2	10	0.3216	101.20	101.15	1.500	1.48
	S3	10	0.3169	99.63			
	S1	15	0.3241	102.03			
	S2	15	0.3193	100.43	100.74	1.164	1.15
	S3	15	0.3173	99.76			

3.2.6. Sample Stock Solution for Assay

Ten tablets equivalent powdered were mixed in ratio ABZ: PRQ (300mg: 25mg). A quantity of synthetic mixture powder equivalent to 65mg was taken in a 100 ml volumetric flask and diluent was added up to the mark. The solution was sonicated for 5 min. This solution was further diluted to obtain a concentration 12µg/ml for ABZ & 5µg/ml for PRQ the result is summarized in (Table 13) and UV Spectra shown in (Fig 12).

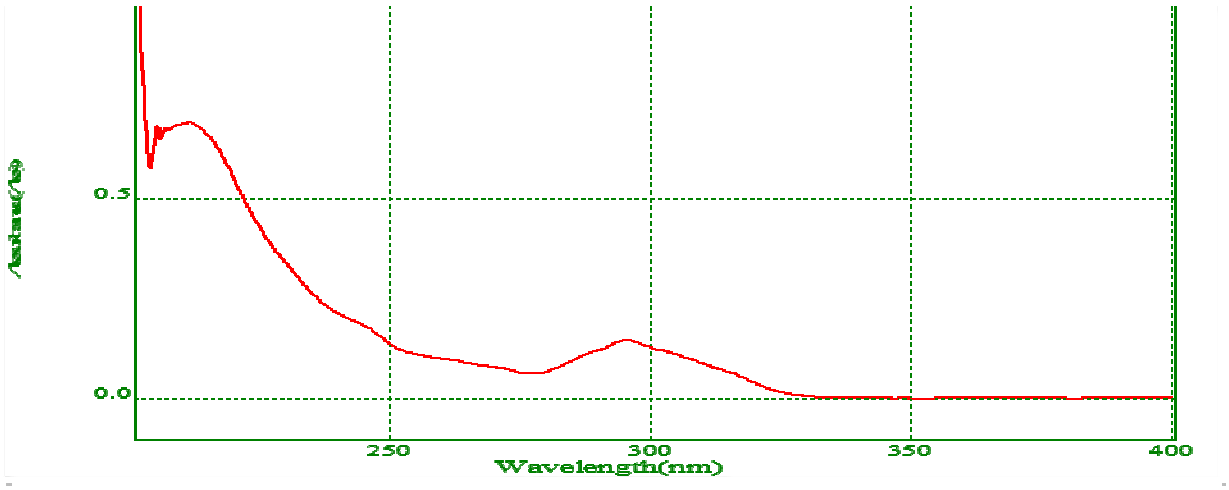

Fig: 12- Assay of synthetic mixture.

Table No.: 13: Determination of % Assay

Synthetic Mixture	Drug	Label claim mg/tablet	Conc. estimated (mg)	Mean Conc. Estimated (mg)	%assay (w/w)± S.D*	% RSD
Albendazole + Praziquantel	Albendazole	300mg	298.79 305.59 301.24 302.75 298.03 301.24	301.27	100.42 ± 0.03	0.902
	Praziquantel	25mg	24.46 24.58 25.23 25.36 25.40 25.21	25.044	100.17 ± 0.067	1.639

4. Conclusion:

Linearity was determined at different concentration ABZ and PRQ shows linearity in the concentration range of 4-14 µg.mL-1 for ABZ and PRQ both. The percent recovery both the drugs are within the rage 95-105% which indicates the method is accurate. The % RSD values for precision are <2.0%. Method shows positive response to all validation parameter. The results of the synthetic mixture were found to be 100.42 ± 0.03% and 100.17 ± 0.067% for ABZ and PRQ respectively. The proposed Absorbance Correction method found to be simple, rapid and sensitive. Therefore, validated UV spectrophotometric method will play a role for determination of ABZ and PRQ in their combined dosage formulation.

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