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Development and Validation of Albendazole and Praziquental

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Abstract

A simple, precise and accurate RP-HPLC method has been developed for simultaneous estimation of Albendazole and Praziquental. A mixture of 20 volumes of phosphate buffer pH 4.0:70 volumes of acetonitrile: 10 volumes of methanol were selected as mobile phase and that itself used as solvent also which gives good resolution and good peak shape for Albendazole and Praziquental. The flow rate was set at 1.0 ml/min, and the detection was carried out with UV detector at 221 nm. Inertsil ODS 3V 250×4.6 mm ID, 5 µm Particle size column was used for separation. At the optimum conditions mentioned above, the total run time required was 6minutes. The linearity and range was established over the range of 60-180 µg/ml and 5-15 µg/ml concentration range of Albendazole and Praziquental respectively. The correlation coefficient of Albendazole and Praziquental was found to be 0.990. The method validation data showed excellent results for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness. The present method can be successfully used for routine quality control and stability studies of Albendazole and Praziquental in bulk and pharmaceutical dosage form.

Keywords: Albendazole, Praziquental, RP-HPLC, Validation.

Introduction

Albendazole (Fig. 1) is chemically methyl N-[6-(propylsulfanyl)-1*H*-1,3-benzodiazol-2-yl]carbamate is a anthelmintic, that causes degenerative alterations in the tegument.[1]

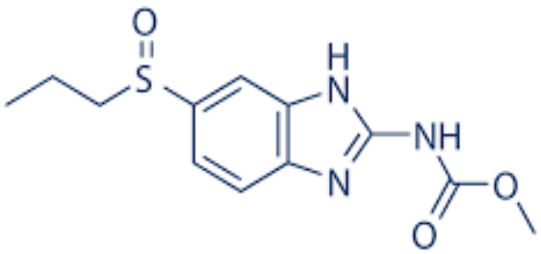

Fig. 1: Molecular structure of Albendazole

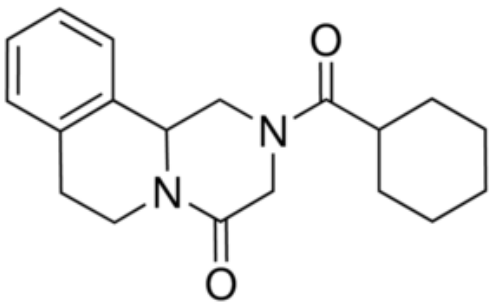

Fig. 2: Molecular structure of Praziquental

Praziquental (Fig. 2) is chemically 2-cyclohexanecarbonyl-1*H*, 2*H*, 3*H*, 4*H*, 6*H*, 7*H*, 11b*H*-piperazino[2, 1-a] isoquinolin-4-one is a anthelmintic, that causing severe spasms and paralysis of the worms' muscles.[2]

Albendazole and Praziquental is the most prompt drug of choice for neurocysticercosis[3] which is a widely spreading disease nowadays because of changing food habits of this social era.

Literature survey reveals that there is only one HPLC method was reported for simultaneous estimation of Albendazole and Praziquental in pharmaceutical formulations.[4] Therefore, an attempt has been made to develop a novel, rapid, accurate and precise RP-HPLC method for simultaneous estimation of Albendazole and Praziquental in tablet dosage form and validated in accordance with ICH guidelines.[5]

Materials and Methods

Instrumentation: To develop a high performance liquid chromatographic method for simultaneous estimation of Albendazole and Praziquental using Shimadzu (LC 20 AT VP) HPLC system on Inertsil ODS C18 (250 mm ×4.6 mm ID, 5 µm particle size) column was used. The instrument is equipped with an auto sampler and UV-Visible detector.[6] A 20 µl rheodyne injector port was used for injecting the samples. Data was analyzed by using Spin chrome software. A Global digital pH meter was used for pH measurements.

Chemicals and solvents: The working standards of Albendazole and Praziquental were provided as gift samples from Chandra Labs, Hyderabad, India. The marketed formulation of Albendazole and Praziquental tablets (Albendazole 300 mg and Praziquental 25 mg) were procured from local market. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai, India. Methanol and potassium dihydrogen phosphate of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai, India.

Chromatographic conditions: phosphate buffer, acetonitrile and methanol (20:70:10, v/v/v) was found to be the most suitable mobile phase for ideal chromatographic separation for simultaneous estimation of Albendazole and Praziquental. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 ml/min. Injection volume was 20 µl and the column was maintained at a temperature of 27^0C. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 221 nm. The run time was set as 6 minutes.

Preparation of phosphate buffer pH 4.0: 2.7 gm of potassium dihydrogen phosphate (KH_2PO_4) was weighed and dissolved in water and volume was made up to 1000 ml with water. Adjust the pH to 4.0 using ortho phosphoric acid. The buffer was filtered through 0.45 µ filters to remove all fine particles and gases.

Preparation of mobile phase and diluent: A mixture of 20 volumes of phosphate buffer pH 4.0, 70 volumes of acetonitrile and 10 volumes of methanol was prepared. The mobile phase was sonicated for 10 min to remove gases.

Preparation of standard solution: Accurately weighed and transferred 300 mg of Albendazole and 25 mg of Praziquental in to 100 ml volumetric flask and was dissolved in sufficient mobile phase. After that filtered the solution using 0.45 µ syringe filter and sonicated for 5 minutes and dilute to 50 ml with mobile phase. Further dilutions of 120 µg/ml of Albendazole and 10 µg/ml of Praziquental were made by adding 1 ml of stock solution to 50 ml of mobile phase.

Preparation of sample preparation: 20 tablets (each tablet contains 300 mg of Albendazole and 25 mg of Praziquental) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Albendazole and Praziquental were prepared by dissolving weight equivalent to 300 mg of Albendazole and 25 mg of Praziquental and dissolved in sufficient mobile phase. After that filtered the solution using 0.45 µ syringe filter and sonicated for 5 minutes and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 120 µg/ml of Albendazole and 10 µg/ml of Praziquental was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Procedure

The column was maintained at a temperature of 27^0C. The run time was set at 6 minutes. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solutions. Inject 20 µl of the standard and sample solutions six times into the chromatographic system at a flow rate of 1.0 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed.

Method Validation

Linearity: Several aliquots of standard solutions of Albendazole and Praziquental were taken in six different 10 ml volumetric flasks and diluted up to the mark with diluent such that the final concentrations were in the range of 60-180 µg/ml for Albendazole and 5-15 µg/ml for Praziquental. The above solutions were injected into the HPLC system keeping the injection volume constant. The drugs were eluted with UV detector at 221 nm, peak areas was recorded for all the peaks. The linearity curves were constructed by plotting concentration of the drugs against peak areas. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of drugs in tablet dosage forms.

Precision: Precision for Albendazole and Praziquental was determined in terms of intra-day precision and inter-day precision. Every sample was injected three times. The measurements for peak areas were expressed in terms of %RSD.

Accuracy: The accuracy of the method was assessed by recovery studies of Albendazole and Praziquental at three concentration levels 75%, 100% and 125%. Fixed amount of pre-analyzed sample was spiked with known amount of Albendazole and Praziquental. Each level was repeated three times. The %Recovery of Albendazole and Praziquental were calculated.

System suitability: The system suitability parameters like retention time, theoretical plates and tailing factor were evaluated by six replicate analyses of Albendazole and Praziquental and compared with standard values. The acceptance criteria are %RSD of peak areas not more than 2%, theoretical plates numbers (N) at least 2000 per each peak and tailing factors not more than 2.0 for Albendazole and Praziquental.

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions of Albendazole and Praziquental using the developed HPLC method. LOD and LOQ were estimated from signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ/s and 10 σ/s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

Robustness: The robustness of the method was determined by making small deliberate changes in method like variation of flow rate, mobile phase ratio and temperature.

Assay: Standard preparations are made from the bulk drug and sample preparations are made from commercial formulation. Both standard and sample solutions were injected in six homogeneous samples. 20 µl of sample solution was injected and from the peak

areas of Albendazole and Praziquental, amount of each drug in the sample were computed. The results were compared with the label claim of Albendazole and Praziquental in tablet dosage forms. From the results the average %Assay was calculated.

Results and Discussion

The HPLC procedure was optimized with a view to develop an accurate, precise and reproducible method for simultaneous estimation of Albendazole and Praziquental in tablet dosage form using Shimadzu (LC 20 AT VP) HPLC system on Inertsil ODS C18 (250 mm ×4.6 mm ID, 5 µm Particle size) column in isocratic mode with mobile phase composition phosphate buffer pH 4.0, acetonitrile and methanol (20:70:10, v/v/v) resulted in peak with maximum separation, good shape and resolution. Flow rates between 0.8 to 1.2 ml/min were studied. A flow rate of 1.0 ml/min gave an optimum signal-to-noise ratio with reasonable separation time, the retention times for Albendazole and Praziquental were found to be 2.713 minutes and 4.770 minutes respectively. Total run time was 6 minutes. The drug components were measured with UV detector at 221 nm. The results of optimized chromatographic conditions were shown in Table 1.

Table 1: Optimized chromatographic conditions

Mobile phase	Phosphate buffer: acetonitrile: methanol (20:70:10, v/v/v)
Column	Inertsil ODS 3V, 250×4.6 mm ID, 5 µm Particle size
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25ºC)
Sample temperature	Room temperature(20-25ºC)
Wavelength	221 nm
Injection volume	20 µl
Run time	6 minutes
Retention time	2.713 min for Albendazole and 4.770 min for Praziquental

Linearity was obtained in the range of 60-180 µg/ml for Albendazole and 5-15 µg/ml for Praziquental. The correlation coefficient (r^2) was found to be 0.991 and 0.990 for both Albendazole and Praziquental respectively. The regression equation of the linearity plot of concentration of Albendazole over its peak area was found to be y = 23.341x + 891.11, where x is the concentration of Albendazole (µg/ml) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Praziquental over its peak area was found to be y = 59.397x + 206.07, where x is the concentration of Praziquental (µg/ml) and y is the corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity results were shown in Table 2 and the calibration curves were shown in Fig. 3 and Fig. 4.

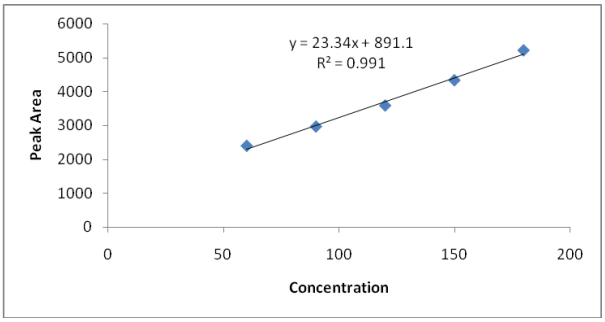

Fig. 3: Calibration curve of Albendazole

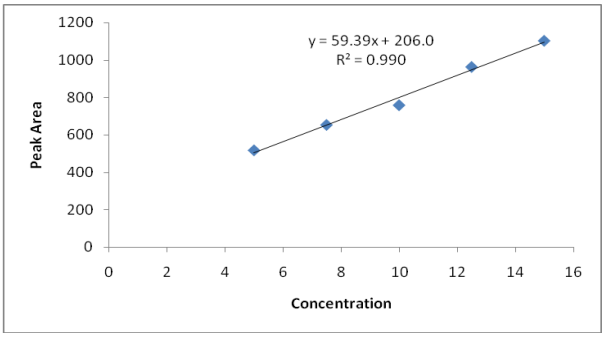

Fig. 4: Calibration curve of Praziquental

Table 2: Linearity results of Albendazole and Praziquental

S. No.	Albendazole		Praziquental	
	Concentration (µg/ml)	Mean peak area	Concentration (µg/ml)	Mean peak area
1	60	2389.85	5	517.72
2	90	2958.94	7.5	653.78
3	120	3578.64	10	759.27
4	150	4324.48	12.5	964.66
5	180	5208.23	15	1104.74

The %RSD for intra-day precision and inter-day precision for Albendazole and Praziquental were found to be 0.407% and 0.351% respectively (limit %RSD<2.0%) and hence the method is precise. The precision data of Albendazole and Praziquental were furnished in Table 3.

Table 3: Precision data of Albendazole and Praziquental

S. No.		Albendazole		Praziquental	
		Rt (min.)	Area	Rt (min.)	Area
Intra day	1.	2.633	3408.658	4.610	727.074
	2.	2.653	3435.285	4.637	756.095
	3.	2.633	3435.649	4.610	740.222
Inter day	1.	2.630	3487.630	4.603	753.700
	2.	2.620	3403.080	4.587	734.433
	3.	2.633	3408.658	4.610	727.074

Avg.	2.634	3429.827	4.610	739.766
S.D	0.011	31.675	0.016	12.741
%RSD	0.407	0.924	0.351	1.722

The %Recovery of the drugs Albendazole and Praziquental were found to be 99.11 to 101.49% and 98.68 to 99.48% respectively and the high percentage of recovery of Albendazole and Praziquental indicates that the proposed method is highly accurate. The results of accuracy studies of Albendazole and Praziquental were shown in Table 4.

The retention times for the drugs Albendazole and Praziquental was 2.719 minutes and 4.770 minutes respectively. The number of theoretical plates calculated for Albendazole and Praziquental was 2219 and 2942 respectively. The tailing factor for Albendazole and Praziquental was 1.671 and 1.436 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantification (LOQ) for Albendazole were found to be 6.71 µg/ml and 20.32 µg/ml; 0.22 µg/ml and 0.67 µg/ml for Praziquental respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 5.

Table 4: Accuracy studies of Albendazole and Praziquental

% Concentration level	Albendazole			Praziquental		
	Conc. added (µg/ml)	Conc. found (µg/ml)	% Recovery	Conc. added (µg/ml)	Conc. found (µg/ml)	% Recovery
75%	90	89.20	99.11%	7.5	7.44	99.22%
100%	120	120.76	100.63%	10	9.95	99.48%
125%	150	152.24	101.49%	12.5	12.33	98.68%

Table 5: System suitability parameters of proposed method

S. No.	Parameters	Albendazole	Praziquental
1	Linearity (µg/ml)	60-180	5-15
2	Correlation coefficient	0.991	0.990
3	Retention time (min.)	2.719	4.770
4	Resolution	-	6.917
5	Tailing factor	1.671	1.436
6	Theoretical plates (N)	2219	2942
7	LOD (µg/ml)	6.71	0.22
8	LOQ (µg/ml)	20.32	0.67

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the % RSD for replicate injections of Albendazole and Praziquental were found to be within the acceptable limits.

Validated method was applied for the simultaneous estimation of Albendazole and Praziquental in commercial tablet dosage forms. The %Assay of Albendazole and Praziquental were found to be 98.59% and 97.88% respectively. The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Albendazole and Praziquental by the proposed HPLC method. The assay results are shown in Table 6.

Table 6: Assay results of marketed formulations

	Albendazole		Praziquental	
	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	3554.425	3340.267	736.722	723.473
Injection-2	3570.129	3599.278	751.855	714.675
Injection-3	3532.134	3518.764	745.268	751.016
Injection-4	3565.519	3543.510	755.989	753.849
Injection-5	3554.757	3543.172	762.105	751.651
Average Area	3552.229	3508.998	750.388	738.933
Tablet avg. weight	300		25	
Standard weight	349.65		349.65	

Sample weight	349.65	349.65
Label amount	300	25
Std. purity	99.8	99.4
Amount found (mg)	295.76	24.47
Assay (%purity)	98.59	97.88

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Albendazole and Praziquental standard were shown in Fig. 5.

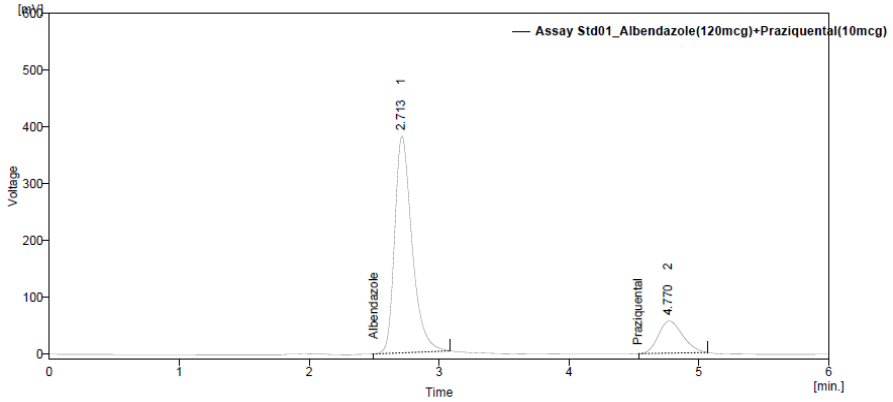

Fig. 5: Typical chromatogram of Albendazole and Praziquental

Conclusion

The proposed HPLC method is rapid, sensitive, precise and accurate for the simultaneous estimation of Albendazole and Praziquental and can be reliably adopted for routine quality control analysis of Albendazole and Praziquental in its tablet dosage forms.

Source of support: None

Conflict of interest: Nil

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