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"Development and validation of an RP-HPLC method for the simultaneous estimation of praziquantel and ivermectin in bulk and combined tablet dosage form."

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A simple, rapid, accurate, and cost-effective RP-HPLC method was devised to estimate praziquantel and ivermectin in combination tablet dosage form. Chromatography was conducted using a Cosmosil C18 column (250 mm x 4.6 mm, particle size: 5 microns), with a mobile phase consisting of methanol and HPLC grade water in a ratio of 80:20 v/v, along with O-phosphoric acid to adjust the pH to 3. The flow rate was set at 1.0 ml/min, and the detection was done at 223 nm. The retention times were found to be 4.757 ± 0.10 min for praziquantel and 6.751 ± 0.10 min for ivermectin. The method shows a linear response when the concentration is between 25-125µg/ml for Praziquantel and 1-5µg/ml for Ivermectin. The method was validated using parameters such as accuracy, precision, linearity, limit of detection and quantitation as well as robustness.

Keywords: RP-HPLC method, Praziquantel, Ivermectin, Validation

1. Introduction

Ivermectin (IVE) is a macrocyclic lactone that has been identified as a powerful, effective, and safe antiparasitic agent.[1] It is widely used as an antiparasitic medication in domestic animals and is the preferred treatment for lymphatic filariasis and river blindness (onchocerciasis) in humans.[2] Ivermectin exhibits antiviral effectiveness against a variety of viruses, even when used in conjunction with additional antiviral medications.[3] Ivermectin has been shown to have the ability to prevent the SARS-CoV-2 virus from replicating in vitro.[4] Ivermectin is the structurally related 22,23-dihydro derivative of avermectin. The chemical name of ivermectin is 22,23-dihydroavermectin B1a + 22,23-dihydroavermectin B1b (Figure 1).

Praziquantel (PRQ) is a compound derived from pyrazinoisoquinoline, characterized by the IUPAC name [2-(cyclohexyl carbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino [2,1-a]isoquinoline-4-one]. Known for its potent efficacy and low toxicity, PRQ finds extensive application in treating various diseases, including human trematode and cestode infections, schistosomiasis, and other pathogenic conditions in humans.[5] Praziquantel is given to treat pancreas fluke, blood fluke, and tapeworm infections in dogs, horses, and cats.[6-8]

Literature states that there is a single existing method for estimating these drugs in tablet combinations using quantitative analytical liquid chromatography.[9] Similarly, only one HPLC method has been documented for concurrently quantifying ivermectin and praziquantel in Equimax paste.[10] Additionally, previous reports have outlined HPLC techniques for determining these active pharmaceutical ingredients (APIs) individually or in

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combination with other drugs.[11-19] The International Conference on Harmonization (ICH) guidelines were followed during the validation process of proposed method.

2. Materials and methods:

2.1. Chromatographic conditions

A prominence HPLC Binary Gradient System (Analytical Technologies Ltd HPLC 3000 Series with UV-3000-M detector) column Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron). The sample was injected using a 20µl syringe. Methanol and water in the ratio of (80:20 v/v) by adjusting pH to 3 with O-phosphoric acid were used for the preparing the mobile phase. The solvents were passed through a 0.45µ membrane filter and sonicated prior to utilization. The mobile phase flow rate was set at 1mL/min, and detection was carried out at 223 nm using a UV detector.

2.2. Chemicals and Reagents

HPLC grade Water, HPLC grade Methanol, Orthophosphoric acid, Ivermectin and Praziquantel.

2.3. Instrumentation:

The HPLC system (Model-3000 series) consisted of a P-3000-M Reciprocating pump. The detector consisted of UV-3000-M operated at a wavelength of 223nm.Data was integrated using HPLC Workstation software. The column used was Cosmosil C18 (250mm × 4.6ID,5µ) and the injection volume was 20 µL.

2.4. Method development

2.4.1. Mobile phase preparation

The mobile phase used was 80:20 v/v combination of methanol and water. Its pH was adjusted to 3 using O-phosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter and then sonicated before use.

2.4.2 Standard solution preparation

Accurately weighed 10mg of Praziquantel & 10 mg of Ivermectin were dissolved in 10 ml of solvent. The resulting solution was then marked and labeled as the Stock solution.

2.4.3 Sample solution preparation

Weigh 20 tablets individually and then calculate their average weight. Triturate 20 tablets to a fine powder. Weighed accurately equivalent to 50mg of Praziquantel and 2 mg of Ivermectin, dissolve in 10ml of solvent. This was marked and labelled as sample solution.

3. RESULTS AND DISCUSSION

3.1. Linearity

The linearity of the method for Praziquantel and Ivermectin was evaluated across concentration ranges of 25-125 µg/ml and 1-5 µg/ml, respectively, by plotting calibration curves. The results demonstrated a strong correlation between the peak area and the concentration of the pure drug across the concentration range, as illustrated in Figures 3 and 4. The correlation coefficient (r^2) were calculated and found to be 0.999 for Praziquantel and 0.9992 for Ivermectin as presented in Table:1.

3.2. Precision

Precision of the method was evaluated through inter-day and intra-day variation studies. In the intra-day and inter-day studies, six replicated injections of standard solutions containing concentrations of 75µg/ml for Praziquantel and 3µg/ml for Ivermectin were performed.

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Subsequently, the response factors of the drug peaks and the percentage relative standard deviations (% RSD) were calculated and are presented in Table 1. The chromatogram is presented in Fig 10. Based on the data collected, the devised method has demonstrated precision.

3.3. Accuracy

A recovery study was carried out on Praziquantel and Ivermectin using standard solution at three different spike levels: 50%, 100%, and 150%. The samples were prepared by adding active pharmaceutical ingredients of Praziquantel and Ivermectin to achieve concentrations equivalent to the intended initial levels of these substances. The percentage recovery data were tabulated in Table 2. The mean recoveries of Praziquantel from spiked samples fell within the range of 99.32% to 99.73%. The mean recoveries of Ivermectin from spiked sample fell within the range of 99.01 % to 99.67%.

3.4. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the formula mentioned below:

LOD = 3.3× S.D/Slope and

LOQ = 10× S.D/Slope. (Table 1)

3.5. Robustness

The method's robustness was assessed by varying method parameters, including adjusting the wavelength by ± 2 nm and altering the pH of the mobile phase by ± 2. The solution containing 50µg/ml PRA and 2µg/ml IVE was injected under the varied conditions and change in the responses of PRA and IVE were noted (Table no.3).

3.6. Ruggedness

The ruggedness of method was proven by evaluating the sample with multiple analysts under the same operational conditions.

3.7. System suitability

System suitability tests are essential in method development to ensure that the chromatographic system meets the required standards. Tailing factor (T), number of theoretical plates (N) and Retention time (Rt) were determined by three repeated injections of mixed standard solution. The results are given in Table 4.

4. FORCE DEGRADATION STUDIES

4.1. Acid degradation

To the combination of praziquantel and ivermectin 0.1N HCl added within a round-bottom flask equipped with a reflux condenser. Subsequently, the solution underwent reflux at 60ºC for a duration of one hour. Following this reflux period, the solution was allowed to cool to room temperature. The final solution was injected under optimal chromatographic conditions.

4.2. Base degradation

To the praziquantel and ivermectin combination 0.1N NaOH solution added within a round bottom flask. The solution underwent reflux at 60ºC for one hour. After completion of the reflux process, the solution was allowed to cool to room temperature. The final solution was injected under optimal chromatographic conditions.

4.3. Oxidative degradation

To the mixture of praziquantel and ivermectin 0.3% hydrogen peroxide solution is added in a round bottom flask equipped with a reflux condenser. This solution is then heated under

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reflux conditions for 24 hours. After the reflux period, the solution is cooled to room temperature. Finally, the resulting solution is injected under optimal chromatographic conditions.

4.4. Thermal degradation

A mixture of praziquantel and ivermectin was prepared in a suitable solvent. The mixture was transferred into a round-bottom flask. The flask was placed in a temperature-controlled heating mantle set to 60°C for 24 hours. After the degradation period, the flask was cooled to room temperature, and the solution was transferred to a suitable container. This solution was injected to generate the chromatogram.

4.5. Photolytic degradation

A mixture of praziquantel and ivermectin was prepared using a suitable solvent. The prepared mixture was transferred into a round-bottom flask. The flask placed under a light source for 24 hours. After the degradation process, the solution was further injected under optimal detecting conditions to analyse the components.

Conclusion

The proposed reverse phase RP-HPLC method has been developed for the simultaneous analysis of PRA and IVE in their tablet formulation. The validation of the procedure followed ICH guidelines. The results of the validation demonstrate the precision, linearity, robustness, and accuracy of the methods, thereby establishing the reliability of the proposed method. In the short chromatographic run time, every analyte was successfully separated and resolved. In terms of sensitivity, time savings, and minimal detection limits, it offers clear advantages over other current approaches.

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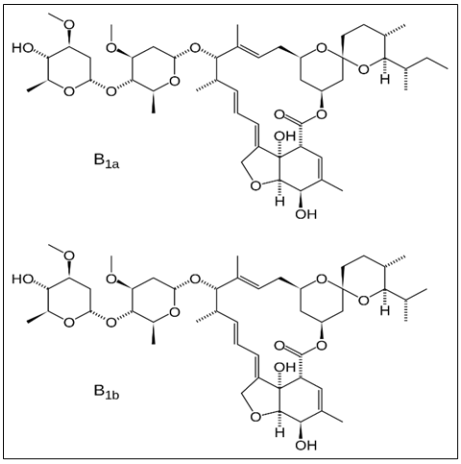

Fig. 1 Structure of ivermectin

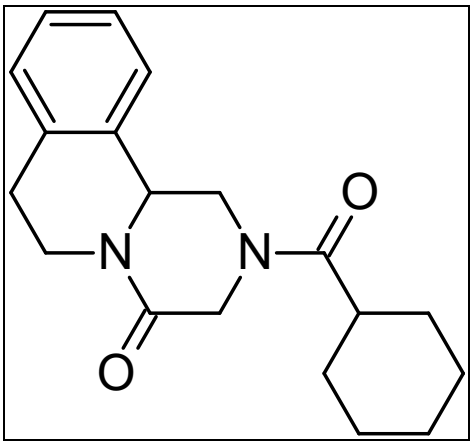

Fig. 2 Structure of praziquantel

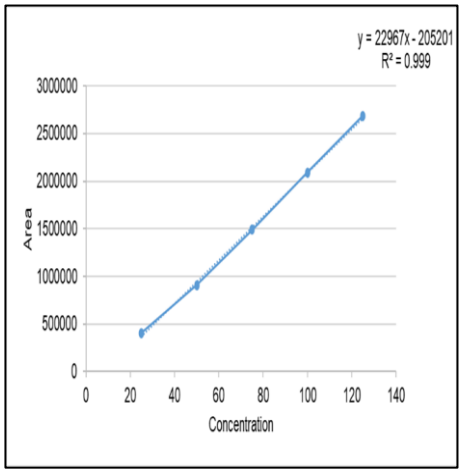

Fig. 3 Linearity of praziquantel

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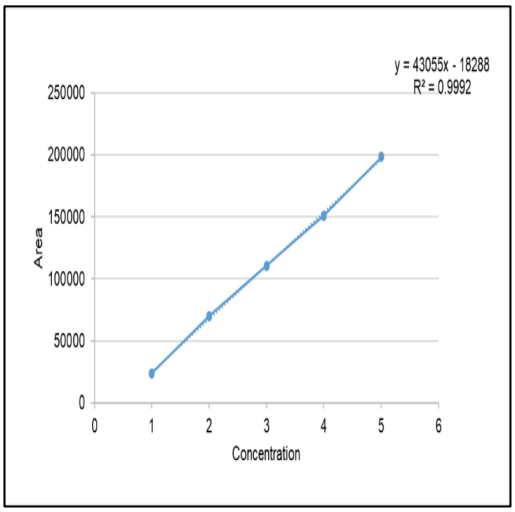

Fig. 4 Linearity of ivermectin

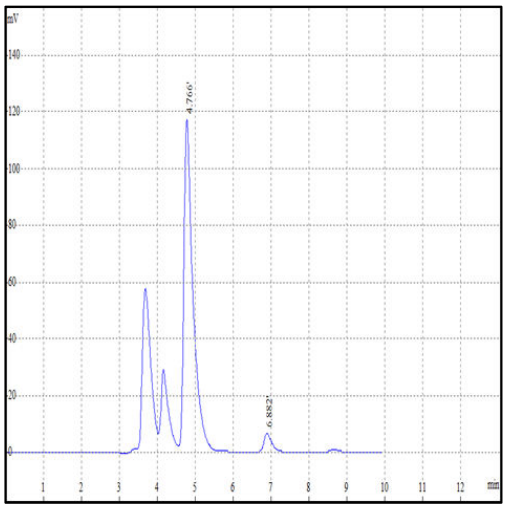

Fig. 5 Sample treated with 3% H_2O_2 for 24 hours

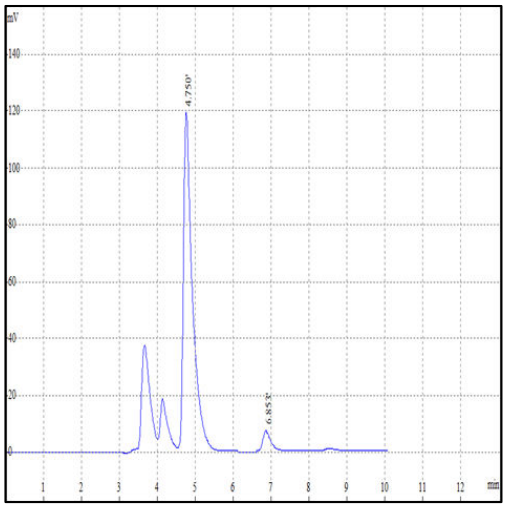

Fig. 6 Sample treated with 0.1N HCl for one hour at 60ºC

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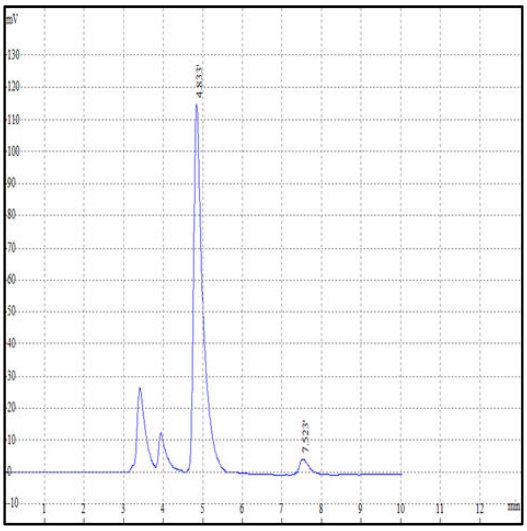

Fig. 7 Sample treated with 0.1N NaOH for one hour at 60ºC

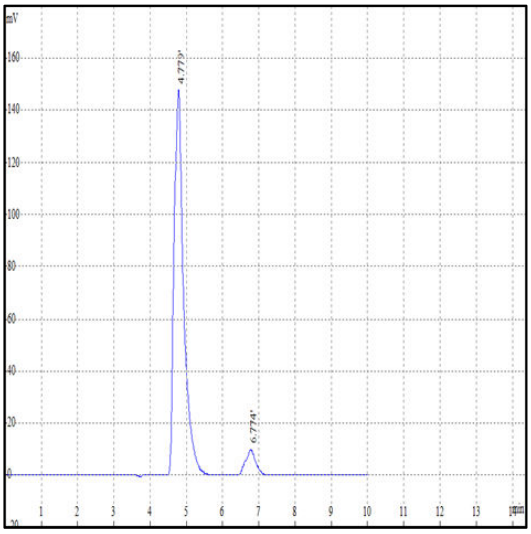

Fig. 8 stability of sample in sunlight after 24 hours

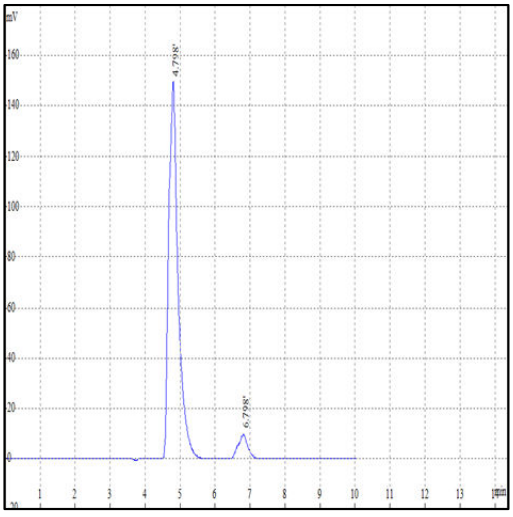

Fig. 9 Thermal degradation

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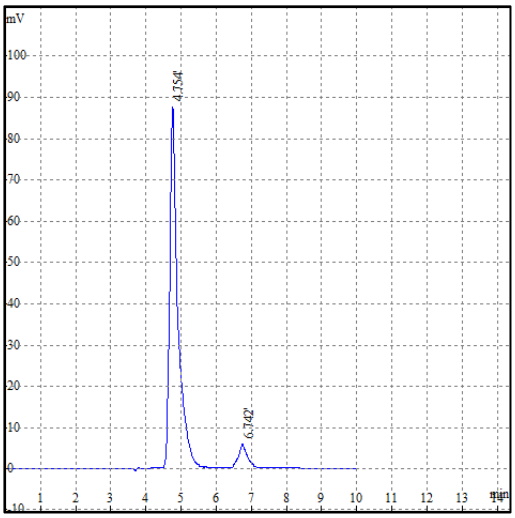

Fig. 10 chromatogram of standard

	Parameter	**Praziquantel**	**Ivermectin**
1	Linearity(µg/ml)	25-125(µg/ml)	1-5(µg/ml)
2	Regression Equation	y=22967x-205201	Y=43055x-18288
3	Slope(m)	22967	43055
4	Intercept(c)	205201	18288
5	Correlation coefficient	0.999	0.9992
6	LOD(µg/ml)	0.062394814	0.004609116
7	LOQ(µg/ml)	0.18907519	0.01396702
8	Intra-day precision (%RSD, n=6)	0.036560916	0.147572692
9	Inter-day precision (%RSD, n=6)	0.143214332	0.023679648
n=number of determinations			

Table 1 Statistical parameters for the calibration graph of praziquantel and ivermectin by RP-HPLC method.

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Drug	Level	Amount of test taken	Amount of standard added	Total conc. found	% recovery	Mean % recovery ±SD
Praziquantel	50%	50	25	74.49	99.32	99.29±0.137
	100%	50	50	99.73	99.73	99.75±0.052
	150%	50	75	124.22	99.37	99.37±0.009
Ivermectin	50%	2	1	2.97	99.01	99.03±0.360
	100%	2	2	3.98	99.67	99.58±0.223
	150%	2	3	4.95	99.01	99.04±0.230

Table 2 Results of recovery study of praziquantel and ivermectin by RP-HPLC method. RSD: relative standard deviation.(n=3)

Parameter	Modification/Level	Retention time(min)		Accuracy (%)		Asymmetry	
		PRQ	IVE	PRQ	IVE	PRQ	IVE
wavelength	+2	4.718	6.710	99.52	101.27	2.27	1.26
	-2	4.718	6.689	98.44	101.47	2.28	1.26
pH	+0.2	4.724	6.710	99.19	100.89	1.26	1.27
	-0.2	4.713	6.697	100.80	101.25	2.27	1.25

Table 3 Results of robustness of praziquantel and ivermectin by RP-HPLC method.

Parameter	Praziquantel	Ivermectin
Peak area	1488625	110957
Theoretical plates	8618	8389
Tailing factor	1.27	1.28
Retention time	4.728	6.716
Resolution	4.46	
%RSD	0.264	0.354

Table 4 System suitability parameter of RP-HPLC method.

Agent	Exposure	Condition	Total % degradation	
			Praziquantel	Ivermectin
0.1N HCl	1 hr	Heat at 60°C	13.2818	15.7848
0.1N NaOH	1 hr	Heat at 60°C	14.3271	16.3464
3%H_2O_2	24 hr	At RT	3.4680	3.1748
Dry heat	24 hr	Heat at 60°C	2.0062	2.1523
Sunlight	24 hr	At RT	1.0696	0.6800

Table 5 Results of force degradation study