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Research Article

Analytical Method Development and Validation for the Simultaneous Estimation of Praziquantel and Ivermectin in Its Bulk and Pharmaceutical Dosage Forms

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Abstract

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Praziquantel and Ivermectin was done by RP-HPLC. The Phosphate buffer was p^H3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Praziquantel and Ivermectin were found to be from 100-500µg/ml of Praziquantel and 1-5µg/ml of Ivermectin. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Praziquantel and Ivermectin. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Inertsil C18, Praziquantel and Ivermectin, RP HPLC

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Contents

1. Introduction

Praziquantel is an anthelmintic medication used to treat a number of parasitic worm infections such as schistosomiasis. Ivermectin is a semi-synthetic antiparasitic medication derived from avermectins, a class of highly-active broad-spectrum antiparasitic agents isolated from the fermentation products of *Streptomyces avermitilis*. Ivermectin itself is a mixture of two avermectins, comprising roughly 90% 5-O-demethyl-22,23-dihydroavermectin A_{1a} (22,23-dihydroavermectin) and 10%

5-O-demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl)avermectin. Ivermectin is mainly used in humans in the treatment of onchocerciasis, but may also be effective against other worm infestations (such as strongyloidiasis, ascariasis, trichuriasis and enterobiasis). Applied topically, it may be used in the treatment of head lice infestation.

2. Methodology

Instrumentation

The instrument used was HPLC Waters model No. 2695 separation module.2487 UV detector, Empower software. The stationary phase used was Inertsil C_{18} column C18 (4.6x150mm, 5µm). Digital weighing balance-Model number Afcoset ER-200A, Sonicator (Enertech)-SE60US, pH meter Model number Adwa–AD 1020, UV/VIS spectrophotometer LABINDIA UV 3000^{+}.

Materials and reagents

Praziquantel and Ivermectin were gift samples supplied by Mylan and Cipla labs respectively Acetonitrile for HPLC was supplied by MolychemKH_2PO_4 was supplied by Finer chemical LTD Water and Methanol for HPLC was supplied by Lichrosolv.

Method development

Six trials were made by changing the mobile phase ratios and solvents ACN: water (30:70 v/v) water: Methanol P^H 2.5 (30:70 v/v) ACN: Water (80:20) v/v Phosphate buffer P^H 4.5: Methanol (35:65 v/v) Phosphate buffer: Methanol P^H 4.5 (65:35 v/v) Phosphate buffer: Methanol P^H 4.5(20:80 v/v). Finally, the mobile phase optimized was 30% buffer 70% Methanol.

Chromatographic conditions

The Phosphate buffer was p^H3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min.

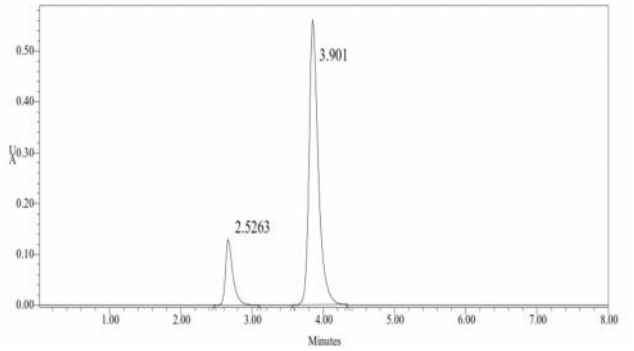

Figure 1 chromatogram for system suitability

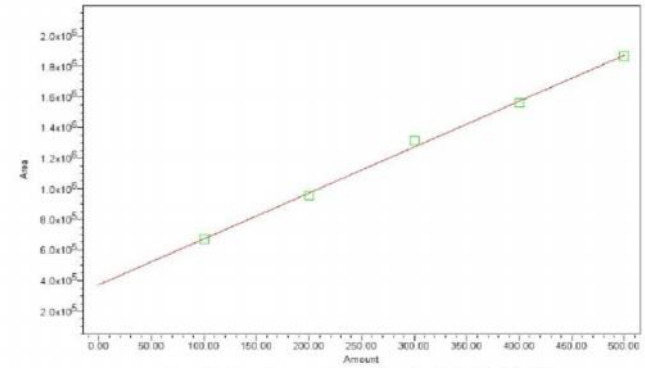

Figure 2 calibration graph for Praziquantel

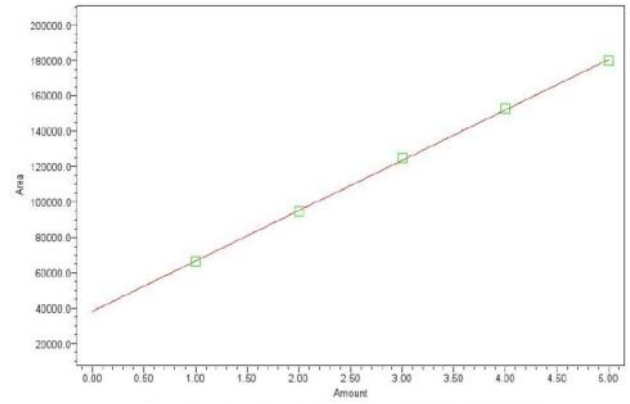

Figure 3 calibration graph for Ivermectin

3. Results and discussion

Table 1: Results of system suitability parameters for Praziquantel and Ivermectin

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Praziquantel	2.5	124505	213642		1.2	4673.4
2	Ivermectin	3.9	1308495	154566	6 0	1.3	6090.3

Table 2: Results of method precision for Praziquantel

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759

Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 3: Results of method precision for Ivermectin

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Table 4: Results of Intermediate precision for Praziquantel

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 5: Results of Intermediate precision for Ivermectin

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Table 6: accuracy (recovery) data for Praziquantel

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 7: accuracy (recovery) data for Ivermectin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table 8: Area of different concentration of Praziquantel

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table 9: Area of different concentration of Ivermectin

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Table 10 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Praziquantel	52	522	10.03
Ivermectin	52	524	10.1

Table 11: Flow Rate (ml/min) data for Praziquantel

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 12: flow rate (ml/min) data for Ivermectin

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 13: Change in Organic Composition in the Mobile Phase for Praziquantel

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 14: Change in Organic Composition in the Mobile Phase for Ivermectin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

This High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Praziquantel and Ivermectin was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5μm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Praziquantel and Ivermectin were found to be from 100-500 μg/ml of Praziquantel and 1-5μg/ml of Ivermectin. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Praziquantel and Ivermectin. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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