WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Analytical Method Validation of Ivermectin and Praziquantel in Bulk and Pharmaceutical Dosage Form by UPLC

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A B S T R A C T

A new simple, accurate, precise, and robust Ultra performance liquid chromatography (UPLC) was developed and validated for simultaneousestimation of Ivermectin and Praziquantel. The separation was achieved by using the combination of Acetonitrile: water: methanol in ratio (40:30:30) in zodiac C18 columnC18 (250×4.6 ID× 5µm) with a Flow rate of 1.0 ml/min at a wavelength of 244nm. Ivermectin and Praziquantel were eluted at the retention time of 2.3 and 4 mins respectively. System suitability parameters are found to be within limits. Method produces linear responses within the concentration range 1535µg/ml for Ivermectin and 40-120µg/ml for Praziquantel. The method was found to be accurate as the percentage recovery was 99.6 % and 98 % for ABM and PRQ and was within the limits. LOD was found to be 0.21µg/ml for Ivermectin and 2.09µg/ml for praziquantel LOQ was found to be 0.65µg/ml for Ivermectin and 6.35µg/ml for praziquantel. Proposed method was found to be simple, accurate, precise, and quick could be used for regular analysis.

Keywords*:* UPLC, Ivermectin Praziquantel.

1. Introduction

Ivermectin is effective against nematodes and arthropods, but not against cestodes and trematodes. This is because Ivermectin acts as a GABA receptor agonist, and cestodes and trematodes lack a GABA system. IUPAC name of Ivermectin is a Mixture of (1R,4S,5'S,6R,6'R,8R,10E,12S,13S,14E,16E,20R,21R,24S)-6'-[(2S)-butan-2-yl]-21,24-dihydroxy-12-[(2R,4S,5S,6S)-5-[(2S,4S,5S,6S)-5-hydroxy-4-methoxy-6-methyloxan-2-yl]oxy-4-methoxy-6-methyloxan-2-yl]oxy-5',11,13,22-tetramethylspiro[3,7,19-trioxatetracyclo[15.6.1.14,8.020,24] pentacosa- 10,14,16,22-tetraene-6,2'-oxane]-2-one .

In mammals, a GABA system is present only in the CNS, and Ivermectin exerts toxicity by blocking the post-synaptic transmission of nerve impulses by potentiating the release and binding of GABA, thus blocking GABA-mediated transmission.

Experimental evidence indicates praziquantel increases the permeability of the membranes of schistosome cells towards calcium ions. The drug thereby induces contraction of the parasites, resulting in paralysis in the contracted state. The dying parasites are dislodged from their site of action in the host organism and may enter systemic circulation or may be destroyed by host immune reaction (phagocytosis). Additional mechanisms including focal disintegrations and disturbances of oviposition (laying of eggs) are seen in other types of sensitive parasites. IUPAC name of praziquantel is (RS)-2-(cyclohexylcarbonyl)- 1,2,3,6,7,11b-hexahydryo-4h-pyrazino-(2,1-a)is 0 quinoline -4- one

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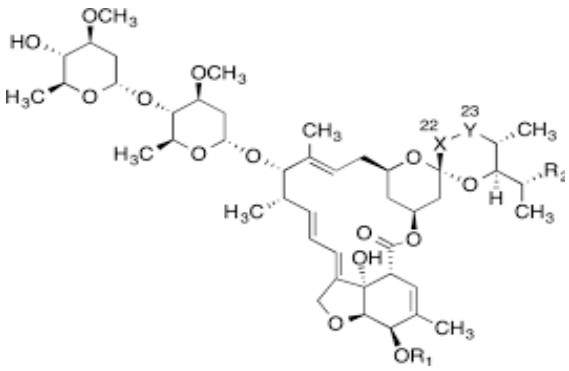
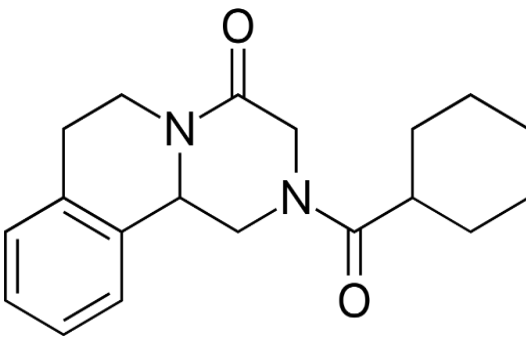
Structures



Structure of Ivermectin



structure of praziquantel

2. Materials and Method

Instrument used

UV-Visible Spectrophotometer-Thermo Electron co-orporation, UPLC- Agilent Infinity 1290, Ultra Sonicator- Citizen, Digital Ultrasonic Cleaner, pH meter- Thermo, Electronic balance- Mettler Toledo, UPLC ColumnZodiac column,C18(150x4.6 ID) 5µm.

Drug sample

Ivermectin and praziquantel bulk drugs as Gift samples obtained from cipla pharmaceuticals and marketed product Ivec-Plus from REMPEX pharmaceutical.

Reagent and Solutions

Methanol, water (uplc grade), Potassium Dihydrogen ortho Phosphate, Acetonitrile

Determination of working wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

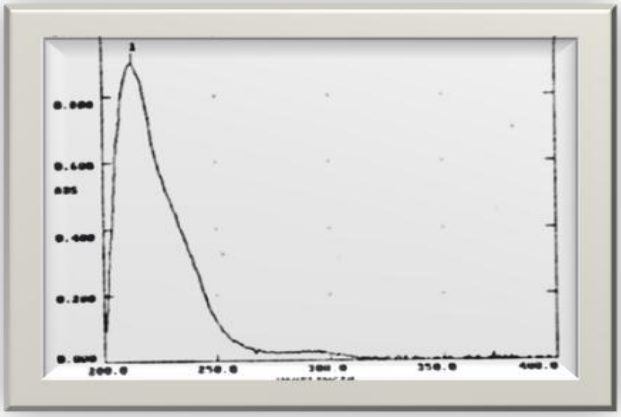
Preparation of standard stock solution of Ivermectin

50 mg of ABAMECTINwas weighed and transferred in to 500ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

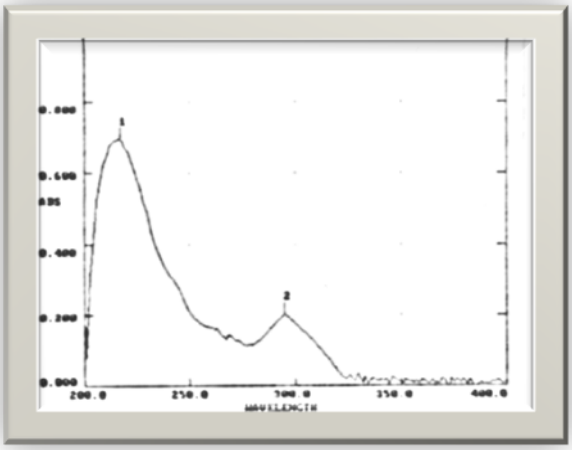
Preparation of standard stock solution of praziquantel

50mg of PRAZIQUANTELwas weighed in to 500ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

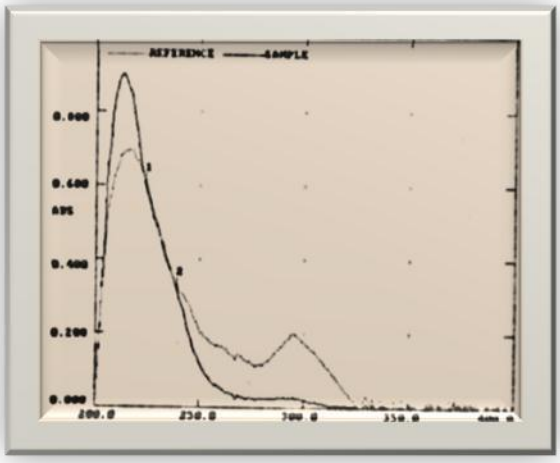
The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 8.1,
8.2 and 8.3 and the absorption curve shows characteristic absorption maxima at 221 nm for
Ivermectin 231 nm for PRAZIQUANTEL and 244nm for the combination.



UV-VIS spectrum of Ivermectin



UV-VIS spectrum of PRAZIQUANTEL.



UV-VIS spectrum of Ivermectin and PRAZIQUANTEL and the isosbestic point was 244nm

3. Result and Discussion

Method development

Optimised method: Trials were performed for the method development and the best peak with least fronting factor was found to be with RT=2.350 min for Ivermectin and 4.055 min for PRAZIQUANTEL.

Table 1: Optimized chromatographic conditions.

Mobile phase	Acetonitrile: Methanol :Water(50:30:20)
pH	4.0
Column	Zodiac, C18 (250×4.6 ID× 5µm)
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	244nm
Injection volume	20 µl
Run time	10 min
Retention time	About 2.350 min for Ivermectin and 4.055 min for PRAZIQUANTEL

Validation parameters

Specificity

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

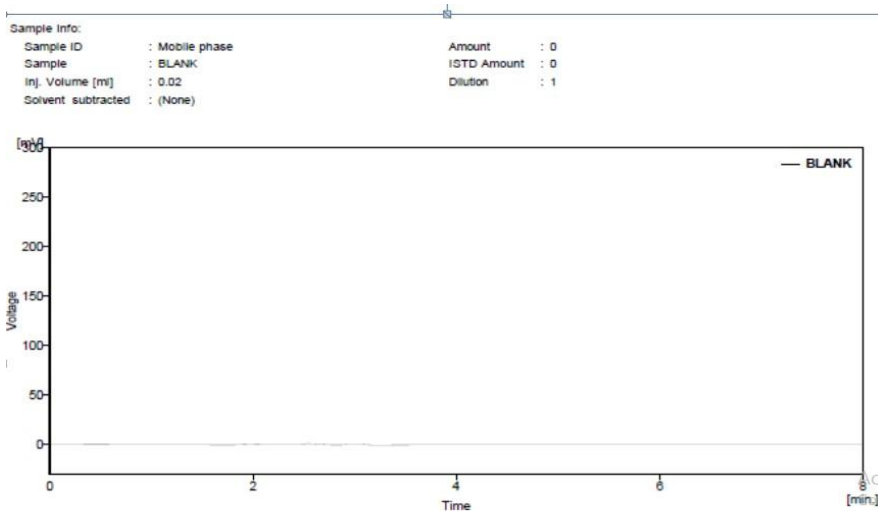


Fig. 1: Chromatogram of blank.

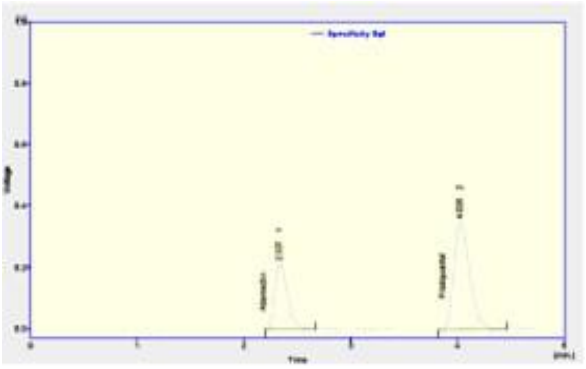


Fig. 2: Chromatogram For Specificity of Ivermectin and Praziquantel Standard.

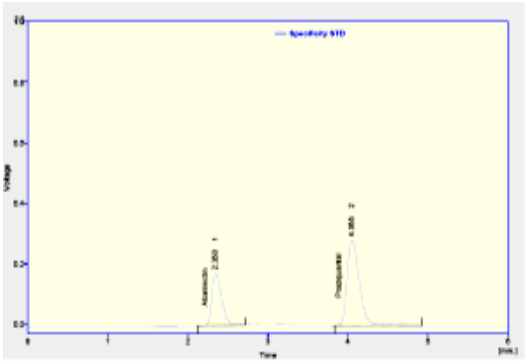


Fig. 3: Chromatogram for Specificity of Ivermectin and Praziquantel sample.

RESULTS

It is observed from the above data, diluent or excipient peaks are not interfering with the Ivermectin and Praziquantel peaks.

System suitability

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 30 µg/ml of Ivermectin and 80 µg/ml of Praziquantel were injected six times and the chromatograms were recorded for the same.

Results for system suitability of Ivermectin

Injection	Retention time	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.460	3598.217	6841	1.16	8.99
2	4.457	3540.341	6882	1.15	8.91
3	4.457	3545.924	6830	1.11	8.92
4	4.450	3605.390	6841	1.16	8.98
5	4.458	3585.636	6811	1.15	8.84
6	4.457	3592.572	6895	1.17	8.80
MEAN	4.457	3578.013	-	-	-
SD	0.003	27.845	-	-	-
%RSD	0.076	0.778	-	-	-

Table 2: Results for system suitability of praziquantel.

Injection	Retention time	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.460	3598.217	6841	1.16	8.99
2	4.457	3540.341	6882	1.15	8.91
3	4.457	3545.924	6830	1.11	8.92
4	4.450	3605.390	6841	1.16	8.98
5	4.458	3585.636	6811	1.15	8.84
6	4.457	3592.572	6895	1.17	8.80
MEAN	4.457	3578.013	-	-	-
SD	0.003	27.845	-	-	-
%RSD	0.076	0.778	-	-	-

Linearity and Range

Preparation of mixed standard solution

weigh accurately 150 mg of Ivermectin and 400 mg of PRAZIQUANTEL in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.

Table 13: linearity of Ivermectin

S. no.	Conc.(µg/ml)	Area
1	15	868.602
2	20	1202.205
3	25	1622.818
4	30	1996.496

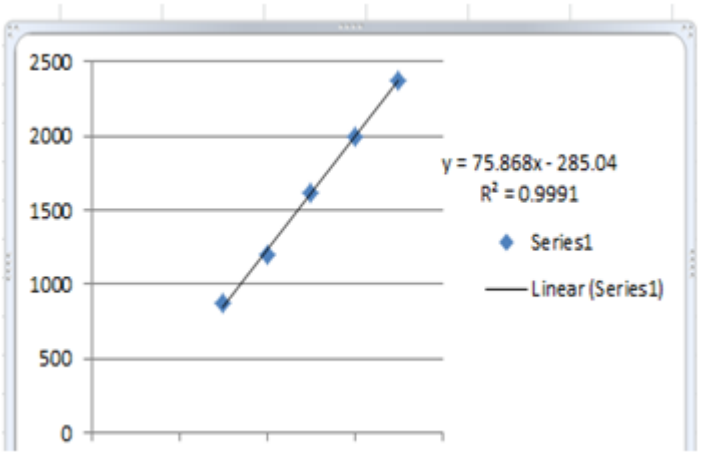


Fig. 13: Linearity graph of Ivermectin

Table 14: linearity of praziquantel.

S. no.	Conc.(µg/ml)	Area
1	40	1679.729
2	60	2554.729
3	80	3344.737
4	100	4306.953

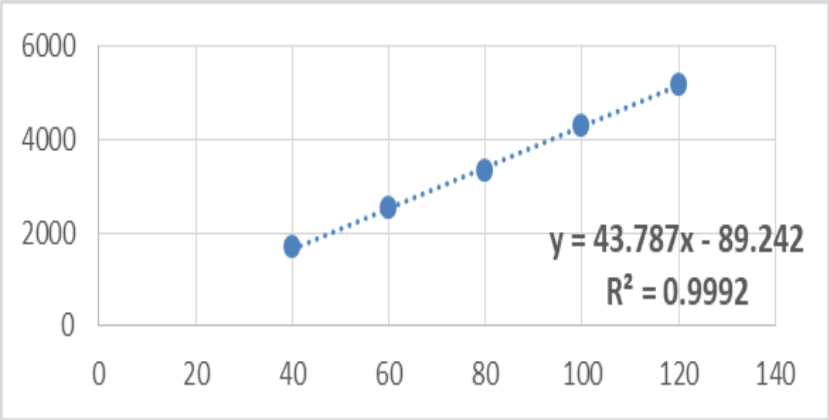


Fig. 14: Linearity graph of Praziquantel.

RESULTS

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Ivermectin and Praziquantel is 0.9991 and 0.9992. The relationship between the concentration of Ivermectin and Praziquantel and area of Ivermectin and Praziquantel is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Precision Method precision

Prepared sample preparations of Ivermectin and Praziquantel as per test method and injected 6 times in to the column.

Table 17: Results for method precision of Ivermectin and Praziquantel.

Ivermectin			**Praziquantel**		
S. no.	**Rt**	**Area**	**S. no.**	**Rt**	**Area**
1	2.357	1768.188	1	4.060	3628.245
2	2.337	1767.595	2	4.028	3600.321
3	2.337	1757.733	3	4.028	3595.941
4	2.357	1772.345	4	4.060	3625.390
5	2.342	1769.771	5	4.035	3605.636
avg	2.3460	1767.126	avg	4.042	3611.107
stdev	0.0102	5.564	stdev	0.016	14.782
%RSD	0.44	0.31	%RSD	0.41	1.56

RESULTS

Test results for Ivermectin and Praziquantel are showing that the %RSD of Assay results are within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre- analyzed sample solution at three different levels 75%, 100% & 125%.

Table 15: Recovery results for Ivermectin

Recovery level	Amount taken(mcg/ml)	Area	Amount recovered (mcg/ml)	%Recovery
75%	20	1664.824	19.8	99.45
100%	25	1670.898	24.82	99.41
150%	30	1656.472	29.57	100.19
Average		1664.065		99.66
Standard deviation		7.242914		
%RSD		0.435254		

Table 16: Recovery results for praziquantel.

Recovery level	Amount taken (mcg/ml)	Area	Amount recovered (mcg/ml)	%Recovery
75%	60	3493.162	59.34	98.90
100%	80	3486.782	78.70	98.37
150%	100	3411.275	98.92	98.92
Average		3463.74		98.73
Standard deviation		45.54758		
%RSD		1.314983		

RESULTS

The percentage mean recovery of Ivermectin and Praziquantel is 99.66% and 98.73% respectively.

Limit of detection (LOD)

Ivermectin = (3.3)*(4.979)/75.868 = 0.21µg/

ml

Praziquantel = (3.3)*(27.845)/43.787

= 2.09µg/ml

Where, σ = the standard deviation of the response S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOD for this method was found to be 0.21µg/ml (Ivermectin and 2.09µg/ml (praziquantel)

Limit of quantification (LOQ)

Ivermectin = (10)*(4.979)/75.868

= 0.65µg/ml Praziquantel = (10)*(27.845)/43.787

= 6.35µg/ml

Where

σ = the standard deviation of the response S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

OBSERVATION

The LOD for this method was found to be 0.65µg/ml (Ivermectin and 6.322µg/ml (praziquantel).

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Table 18: Result of robustness study.

Parameter	**Ivermectin**		**PRAZIQUANTEL**	
	Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow				
0.8ml/min	2.817	1.585	4.860	1.585
1.0ml/min	2.350	1.473	4.055	1.456
1.2ml/min	2.022	1.574	3.487	1.574

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Wavelength				
242nm	2.367	1.571	4.080	1.571
244nm	2.350	1.344	4.055	1.478
246nm	2.367	1.535	4.082	1.535

RESULTS

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Table 19: Results for ruggedness.

Ivermectin	**%Assay**	**Praziquantel**	**%Assay**
Analyst 01	97.99	Analyst 01	99.96
Analyst 02	98.37	Analyst 02	97.59
%RSD	0.27	%RSD	1.69

RESULTS

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

4. Conclusion

- The present work focuses on developing of stability indicating method development and validation for Ivermectin and Praziquantel as per ICH guidelines.
- The present work reveals that the optimization is achieved by using the combination of Acetonitrile: Water: Methanol in the Ratio (40:30:30) in Column Zodiac, C18 (250×4.6 ID× 5µm) with a Flow rate of 1.0 ml/min at a wavelength of 244nm
- The detection wavelength for uplc method of the drug is selected as 244nm
- The developed method has shown acceptable precision, accuracy, and adequate sensitivity demands to be use for further studies.
- System suitability parameters are found to be within limits.

- Method produces linear responses within the concentration range 15-35µg/ml for
- Ivermectin and 40-120µg/ml for Praziquantel.
- %RSD for retention time and peak area was less than 2%
- LOD was found to be 0.21µg/ml for Ivermectin and 2.09µg/ml for praziquantel
- LOQ was found to be 0.65µg/ml for Ivermectin and 6.35µg/ml for praziquantel
- The validation parameters such as precision, accuracy, limit of detection, limit of quantification, ruggedness of this method are found to be within the acceptable limits
- No significant interferences were found

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