International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

R E S E A R C H A R T I C L E

Analytical Method Development and Validation for Ivermectin and Albendazole in Combine Dosage Form by RP-HPLC

Dr. Gampa Vijay Kumar [1*], B. Sravanthi [2], A. Praveen[3]

[1]*Professor and Head, Dept. of Pharmacy, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*
[2,3]*KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*

A B S T R A C T

New method was established for simultaneous estimation of Ivermectin and Albendazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ivermectin and Albendazole by using ACE C18 column (4.6×150mm) 5µ, flow rate was 1.2 ml/min, mobile phase ratio was (70:30 v/v) methanol: Phosphate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.344 mins and 3.284 mins. The % purity of Ivermectin and Albendazole was found to be 101.27% and 99.97% respectively. The system suitability parameters for Ivermectin and Albendazole such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Ivermectin and Albendazole was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.2 and 0.2, % RSD for intermediate precision was 0.2 and 0.1respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ivermectin and Albendazole in API and Pharmaceutical dosage form.

Keywords: ACE C18 column, Ivermectin and Albendazole, RP-HPLC

A R T I C L E I N F O

Corresponding Author
Dr. Gampa Vijay Kumar
Professor and Head, Dept. of Pharmacy,
KGR Institute of Technology and Management,
Rampally, Kesara, Rangareddy, Telangana, India.
MS-ID: IJCTPR3765

PAPER QR-CODE

Article History: Received 02 November 2018, **Accepted** 07 December 2018, **Available Online** 15 January 2019

Citation: Dr. Gampa Vijay Kumar, *et al.* Analytical Method Development and Validation for Ivermectin and Albendazole in Combine Dosage Form by RP-HPLC.*Int. J. Curnt. Tren. Pharm, Res., Res.*, 2019, 7(1): 07-12.

C O N T E N T S

1. Introduction

Ivermectin is a medication that is effective against many types of parasites. It is used to treat head lice, scabies, river blindness, strongyloidiasis, and lymphatic filariasis, among others. It can be either applied to the skin or taken by mouth. The eyes should be avoided Common side effects include red eyes, dry skin, and burning skin. It is unclear if it is safe for use during pregnancy, but is likely acceptable for use during breastfeeding. It is in the avermectin family of medications and works by causing an increase in permeability of cell membrane resulting in paralysis and death of the parasite.

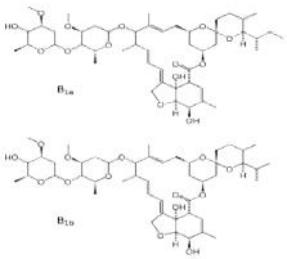

Figure 1: Structure of Ivermectin

Albendazole, also known as albendazolum, is a medication used for the treatment of a variety of parasitic worm infestations. It is useful for giardiasis, trichuriasis, filariasis, neurocysticercosis, hydatid disease, pinworm disease, and ascariasis, among others. It is taken by mouth. Common side effects include nausea, abdominal pains, and headaches. Potentially serious side effects include bone marrow suppression which usually improves on stopping the medication. Liver inflammation has been reported and those with prior liver problems are at greater risk. It is pregnancy category C in the United States and category D in Australia, meaning it may cause harm if taken by pregnant women. Albendazole is a broad-spectrum antihelminthic agent of the benzimidazole type.

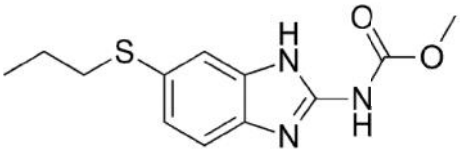

Figure 2: Structure of Albendazole

2. Materials and Methods

Instrumentation

HPLC- Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution, U.V double beam spectrometer UV 3000+ U.V win software Lab India Digital weighing balance(sensitivity 5mg) pH meter Sonicator.

Chemicals

Ivermectin and Albendazole, Ortho phosphoric acid, KH2PO4, K2HPO4, Acetonitrile, Methanol, Water.

Chromatographic Conditions:

Column	: ACE C_{18} Column (4.6mm x 150mm) 5µm.
Column temperature	:Ambient
Wavelength	:240 nm
Mobile Phase	:70:30 Methanol: Phosphate buffer
Flow rate	:1.2ml/min
Auto sampler Temperature	:Ambient
Injection volume	:10µl
Run time	:10min

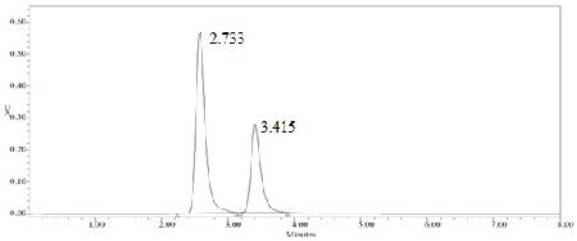

Figure 3: Optimized Chromatogram

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Preparation of the individual Ivermectin standard preparation:

10 mg of Ivermectin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Albendazole standard preparation:

10 mg of Albendazole working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to Dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 3 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the Ivermectin and Albendazole standard and sample solution

Sample solution preparation:

10 mg of Ivermectin and 1 mg Albendazole tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

10 mg Ivermectin and 1 mg Albendazole working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml

volumetric flask and was diluted up to the mark with diluent.

Method Validation

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity:

10 mg of Ivermectin and 1 mg of Albendazole working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Range:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 50µg/ml-250µg/ml and 5µg/ml-25µg/ml of Ivermectin and Albendazole respectively.

Accuracy:

10mg of Ivermectin and 1mg of Albendazole working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent

Precision:

Repeatability:

Preparation of stock solution:10 mg of Ivermectin and 1 mg of Albendazole working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

IntermediatePrecision/Ruggedness: To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Limit of detection (LOD):

LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Limit of quantification:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness:

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

System suitability:

10 mg of Ivermectin and 1 mg of Albendazole working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent

3. Results and Discussion

Linearity:

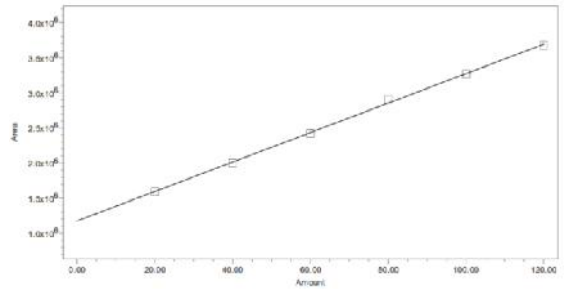

Figure 4: Showing Calibration graph Ivermectin

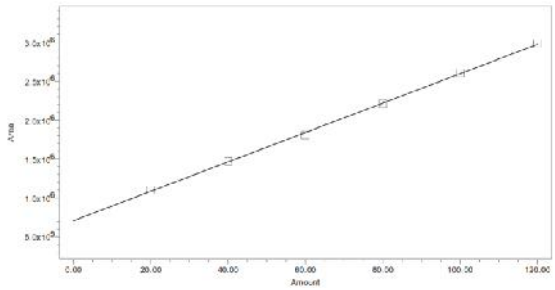

Figure 5: Showing calibration graph for Albendazole

Precision:

The Method precision study was performed for the %RSD of Ivermectin and Albendazole was found to be 0.2and 0.2(NMT 2).The intermediate precision was performed for %RSD of Ivermectin and Albendazole was found to be 0.2and 0.1 respectively (NMT 2).

Robustness:

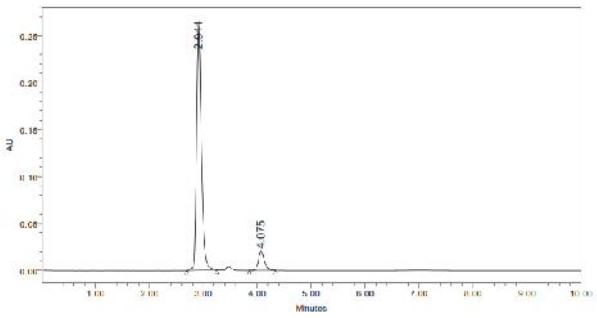

Figure 6: Chromatogram showing less flow rate 0.8ml/min

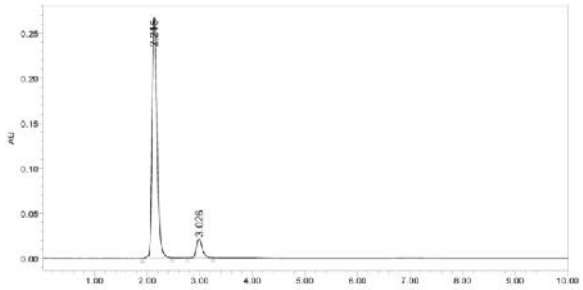

Figure 7: Chromatogram showing more flow rate 1.2 ml/min

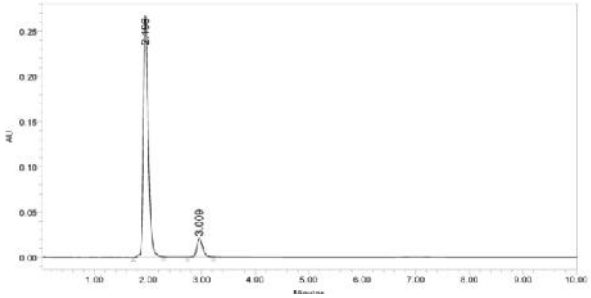

Figure 8: Chromatogram showing more organic phase ratio

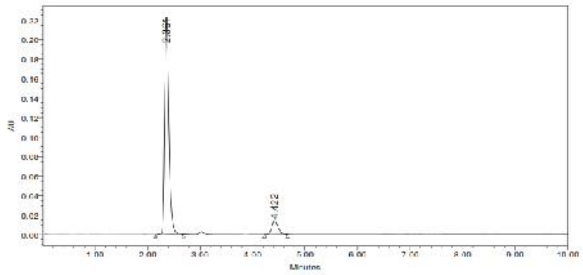

Figure 9: Chromatogram showing less organic phase ratio

Table 1: Linearity Results for Ivermectin

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	471543
2	II	100 ppm	656277
3	III	150 ppm	794999
4	IV	200 ppm	946124
5	V	250 ppm	1002139
Correlation Coefficient			0.999

Table 2: Linearity Results for Albendazole

S.No	Linearity Level	Concentration	Area
1	I	5ppm	56472
2	II	10 ppm	73841
3	III	15ppm	92655
4	IV	20ppm	111541
5	V	25ppm	130567
Correlation Coefficient			0.999

Table 3: Showing accuracy results for Ivermectin

%Concentration (at specification level)	Average area	Amount added(mg)	Amount Found(mg)	% Recovery	Mean recovery
50%	656659	5	4.96	99.91%	99.56%
100%	1304258	10	9.98	99.18%	
150%	1854608	15	15.02	99.60%	

Table 4: Showing accuracy results for Albendazole

%Concentration (at specification level)	Average area	Amount added(mg)	Amount found(mg)	% Recovery	Mean recovery
50%	65312	0.5	0.99	99.53%	99.47%
100%	124509	1.0	1.05	99.38%	
150%	178517	1.5	1.495	99.52%	

Table 5: Showing% RSD results for Ivermectin and Albendazole

Peak Name: Ivermectin

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Ivermectin	2.343	1302729	248455
2	Ivermectin	2.344	1309759	248699
3	Ivermectin	2.344	1302947	249526
4	Ivermectin	2.345	1303977	246695
5	Ivermectin	2.345	1303236	250012
Mean			1304529.8	
Std. Dev.			2961.1	
% RSD			0.2	

Peak Name: Albendazole

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Albendazole	3.285	124263	19458
2	Albendazole	3.287	124487	19634
3	Albendazole	3.287	124175	19600
4	Albendazole	3.288	124894	19327
5	Albendazole	3.288	124495	19540
Mean			124462.7	
Std. Dev.			278.6	
% RSD			0.2	

Table 6: Showing results for intermediate precision of Ivermectin and Albendazole

Peak Name: Ivermectin

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Ivermectin	2.342	1305937	247870
2	Ivermectin	2.343	1306476	246764
3	Ivermectin	2.344	1304520	245696
4	Ivermectin	2.344	1300148	247140
5	Ivermectin	2.345	1308271	247280
Mean			1305070.2	
Std. Dev.			3061.8	
% RSD			0.2	

Peak Name: Albendazole

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Albendazole	3.278	122962	19165
2	Albendazole	3.281	122487	19115
3	Albendazole	3.281	122632	19073
4	Albendazole	3.281	122626	19003
5	Albendazole	3.283	122702	19123
Mean			122681.8	
Std. Dev.			174.8	
% RSD			0.1	

Table 7: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Ivermectin	382625.50	572175863	3.17
Albendazole	5862.40	467579210	0.0172

Table 8: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Ivermectin	381727.80	583265980	5.80
Albendazole	5681.30	469828490	0.212

Table 9: Showing system suitability results for Ivermectin

S.No	Flow rate (ml/min)	System suitability	
		USP Plate count	USP Tailing
1	0.8	5339	1.4
2	[1]	4668	1.3
3	1.2	5216	1.4

Table 10: Showing system suitability results for Albendazole

S.No	Flow rate (ml/min)	System suitability	
		USP Plate count	USP Tailing
1	0.8	7036	1.3
2	[1]	6089	1.2
3	1.2	6998	1.3

Table 11: Showing system suitability results for Ivermectin

S.No	Change in organic composition in mobile phase	System suitability	
		USP Plate count	USP Tailing
1	5% less	6232	1.4
2	Actual [*]	4668	1.3
3	5% more	6387	1.4

Table 12: Showing system suitability results for Albendazole

S.No	Change in organic composition in mobile phase	System suitability	
		USP Plate count	USP Tailing
1	5% less	5437	1.3
2	Actual [*]	6089	1.2
3	5% more	4817	1.2

4. Conclusion

A new method was established for simultaneous estimation of Ivermectin and Albendazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ivermectin and Albendazole by using ACE C18 column (4.6×150mm) 5µ, flow rate was 1.2 ml/min, mobile phase ratio was (70:30 v/v) methanol: Phosphate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm.

The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.344 mins and 3.284 mins. The % purity of Ivermectin and Albendazole was found to be 101.27% and 99.97% respectively. The system suitability parameters for Ivermectin and Albendazole such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Ivermectin and Albendazole was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.2 and 0.2, % RSD for intermediate precision was 0.2 and 0.1respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ivermectin and Albendazole in API and Pharmaceutical dosage form.

5. References

[1] B. Saidulu et al, Analytical Method Development and Validation for Simultaneous Estimation of Albendazole and Ivermectin Tablet of Dosage Form by RP-HPLC. International Journal of Chemistry and Pharmaceutical Sciences

[2] Limbani Rajen K. Et Al, Method Development and Validation Of Ivermectin And Clorsulon In Their Combined Dosage Form. International Bulletin of Drug Research., 4(6): 140-147, 2014

[3] Elena Gabriela Oltean et al, Development and Validation of a RP-HPLC Method for the Quantitation Studies of Ivermectin In Solutions Dosage Forms. Medicamentul Veterinar / Veterinary Drug Vol. 5 (2) 2011, December.

[4] R. S. Chandan et al, Development and Validation of the Determination of Albendazole Residues Pharmaceutical Manufacturing Equipment Surfaces. International Journal of Medical, Health, Biomedical and Pharmaceutical Engineering Vol:7, No:12, 2013.

[5] Nica A.et al, Development and validation of a RP-HPLC method for the quantization studies of ivermectin in solutions dosage forms. OALib Journal ISSN: 2333-9721

[6] Flávia Lada Degaut Pontes Et Al, Development And Validation Of An HPLC-MS/MS Method For Simultaneous Determination Of Ivermectin, Febantel, Praziquantel, Pyrantel Pamoate And Related Compounds In Fixed Dose Combination For Veterinary Use. Asian Journal of Pharmaceutical and Clinical Research Vol 6, Issue 2, 2013