A NOVEL METHOD FOR SIMULTANEOUS ESTIMATION OF IVERMECTIN AND ALBENDAZOLE BY USING RP-HPLC

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Article History: **Received:** 03.12.2022 **Revised:**15.01.2023 **Accepted:** 20.02.2023

Abstract: The simultaneous measurement of ivermectin and albendazole by RP-HPLC method has been established as a new unique method. The chromatogram conditions were successfully generated utilising an ACE C18 column (4.6150mm) 5, a flow rate of 1.2 ml/min, a mobile phase of (70:30 v/v) methanol: phosphate buffer pH 3 (pH was altered with ortho phosphoricacid), and a lamba max of 240nm. WATERS HPLC Sampler and a PDA detector are the tools utilised. 2.3 and 3.3 minutes were discovered to be the RT. Ivermectin and albendazole were found to be respectively 101.3% and 99.9% pure. The resolution was 6.0, and the system suitability parameters for ivermectin and albendazole were determined to be 4668, 1.3, and 6089, and 1.2, respectively. The analytical technique was ICH-validated.

Keywords: Ivermectin, Albendazole, RP-HPLC

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DOI: 10.31838/ecb/2023.12.2.005

INTRODUCTION

There is a 22,23-dihydroavermectin B1a + 22,23-dihydroavermectin B1b in it. a broad-spectrum anti-parasite drug that is effective against different worm infestations as well as onchocerciasis.

A methyl N-[6-(propylsulfanyl)-1H-1,3-benzodiazol-2-yl]carbamate, albendazole causes degenerative changes in the worm's tegument and intestinal cells by binding to tubulin, which is colchicine-sensitive, and preventing it from polymerizing or assembling into microtubules.

METHODS

Phosphate buffer preparation: 2.58 grammes of KH2PO4 were added to a 1L beaker, dissolved, and diluted to 1L with HPLC water. The orthophosphoric acid pH was then raised to 3 to complete the process.
Take 30 ml of the aforementioned buffer and 70 ml of HPLC grade Methanol (70%) to prepare the mobile phase. Degas the mixture in an ultrasonic water bath for five minutes. Apply a 0.22 filter to it.
Mobile phase served as the diluent throughout preparation.
Ivermectin standard preparation for each unique preparation: It is necessary to accurately weigh 10 mg of Ivermectin standard before transferring it to a 10 ml volumetric flask, adding 2 ml of diluent, and sonicating it until it is completely dissolved. Pipette 1.5 ml more from the stock solution mentioned above into a volumetric flask with a 10 ml liner.Preparation of the individual Albendazole standard preparation

Correctly weighed 10 mg of albendazole standard was transferred to a 10 ml volumetric flask, mixed with 2 ml of diluent, fully sonicated, and made to the appropriate volume. Pipette 3 ml more of the stock solution mentioned above into a volumetric flask with a 10 ml capacity.

the preparation of the standard solution, sample, and Ivermectin and Albendazole standards
It is weighed out and transferred to a 10-ml dry volumetric flask together with a standard tablet containing 1 mg of albendazole and 10 mg of ivermectin. A 2-ml diluent is then added to completely dissolve the tablets, and the mixture is then made up to 100 ml (Stock solution). Pour 10ml of the solution into a 100ml volumetric flask starting from stock.
Procedure
10µL of blank, standard and sample were injected into the injection port and areas for both drugs was calculated.

OPTIMIZED METHOD

Chromatographic conditions

ACEC18 (4.6 x 150 mm) column 5.0µm
cellular phase ratio Phosphate buffer: Methanol (70:30% v/v)
Maximum detection lamba: 240 nm
The flow rate is 1.2 ml/min.
10 l of injection volume
Temperature of the heating column: ambient
Ambient temperature for the auto sampler
Runtime: 8 minutes
Time spent in retention: 2.449 and 3.191 **minutes**

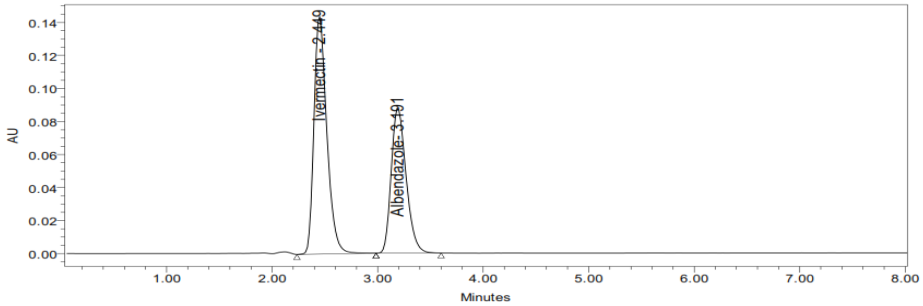


Figure 1. Chromatogram showing trial-1 injection

METHOD VALIDATION

SPECIFICITY

If there was any interference from any impurities in the RT of the analytical peak, the system appropriateness and specificity were assessed. By injecting a blank, the study was carried out.

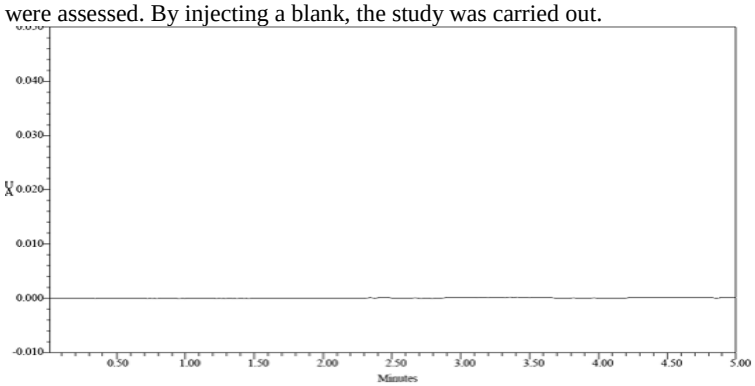


Figure 2. Graph showing blank (mobile phase preparation)

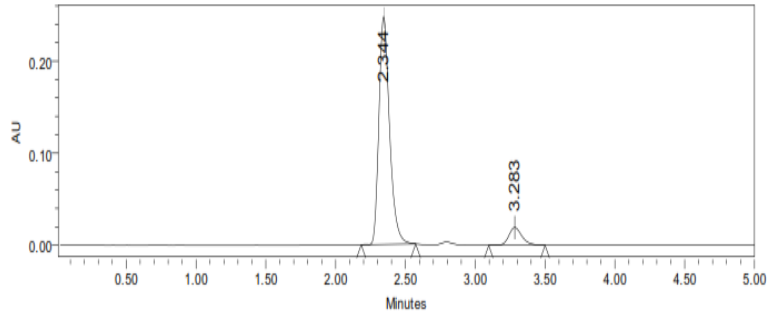


Figure 3. Chromatogram showing standard injection

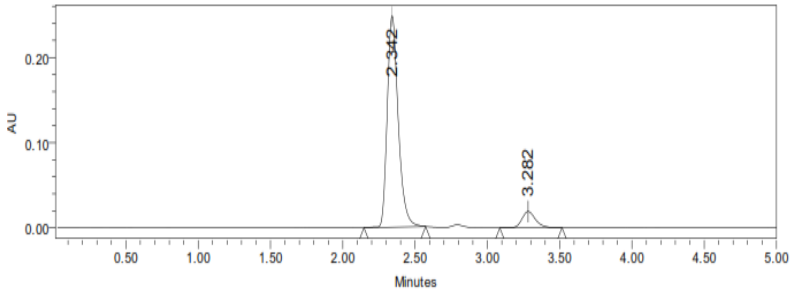


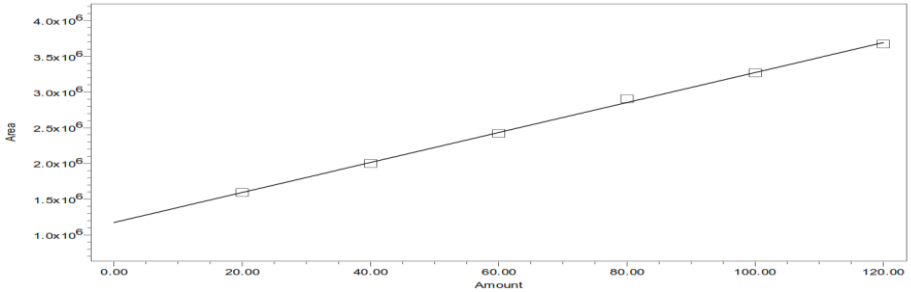
Figure 4. Chromatogram showing sample injection

LINEARITY

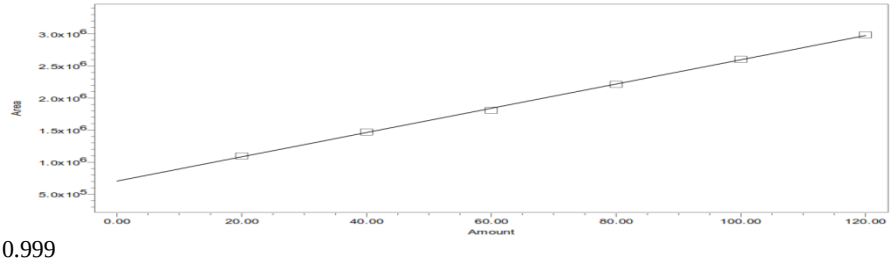
The linearity test was conducted at levels of 5 ppm to 25 ppm and 50 ppm to 250 ppm. The injection port received each range. It is employed in the estimation of the correlation coefficient.

S.No	Linearity Level	Concentration	Area
1	1	50	4715
2	2	100	6562
3	3	150	7949
4	4	200	9461
5	5	250	10021
R^2			0.999

Table 1. Linearity Results for Ivermectin and Albendazole:



Ivermectin $r^2 = 0.999$



Albendazole $r^2 = 0.999$

ACCURACY

For both medications, the accuracy was 50%, 100%, and 150%. Three injections of each level were made into the injection port. The area's percentage recovery was calculated. Table 2-3 lists the outcomes\

.**Table 2.** Showing accuracy results for Ivermectin

%Concentration	Average area	Drug added (mg)	Drugfound (mg)	% Recovery	Mean
50%	656659	5	4.96	99.91%	99.56%
100%	1304258	10	9.98	99.18%	
150%	1854608	15	15.02	99.60%	

Table 3. Showing accuracy results for Albendazole

%Concentration	Average area	Drug added (mg)	Amount found (mg)	% Recovery	Mean
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%	

PRECISION

Table 4. % RSD results for Ivermectin

	Peak name	RT	Area	Height
1	Ivermectin	2.343	1302729	248455
2	Ivermectin	2.343	1309759	248699
3	Ivermectin	2.343	1302947	249526
4	Ivermectin	2.343	1303977	246695
5	Ivermectin	2.343	1303236	250012
Mean			1304529.8	
Std.Dev.			2961.1	
%RSD			0.2	

Table 5. %RSD results for Albendazole

	Peak name	RT	Area	Height
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**	

INTERMEDIATE PRECISION/RUGGEDNESS

Table 6. Results for intermediate precision of Ivermectin

	Peak name	RT	Area	Height
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**	

Table 7. Results for intermediate precision of Albendazole

	Peak name	RT	Area	Height
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			1226818.8	
Std.Dev.			174.8	
%RSD			**0.2**	

LOD

Table 8. Results for LOD

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

LOQ

Table 9. Results for LOQ

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

ROBUSTNESS

It is carried out by changing the mobile phase ratio from a higher to a lower organic phase ratio, as well as the flow rate, which ranges from 0.4 to 0.6 ml per minute. Only at low flow conditions and with changes of less than 5% in the mobile phase can the approach be considered robust.

Table 10. Reults of suitability for Ivermectin

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

Table 11. system suitability results for Albendazole

S. No	Mobile flow rate(ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	1	6089	1.2
3	1.2	6998	1. 3

Showing system suitability results for Ivermectin

S. No	composition of organic solvents	System suitability results	
		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	composition of organic in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	*Actual	6089	1.2
3	5 % more	4817	1.2

ASSAY CALCULATION FOR IVERMECTIN AND ALBENDAZOL

Table 13. Showing assay results

S.No	Name of compound	Label claim	Amount taken	%purity
1	Ivermectin	500	754.7	99.24
2	Albendazole	2.5	735.6	101.04

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