Simultaneous Estimation Of Ivermectin, Albendazole & Diethylcarbamazine Citrate By Using Hplc

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Abstract

Background: For the simultaneous assessment of the drugs Ivermectin (IVR), Albendazole (ALB), and Diethylcarbamazine Citrate (DEC) in combination, a new, straightforward, accurate, and stability-indicating HPLC (High-Performance Liquid Chromatography) approach was developed and validated.

Results: The method was developed using a Zorbax SB Phenyl column (150 mm×4.6 mm, 3.5 µ) with gradient elution. 5 mM ammonium acetate with 0.1% Acetic Acid buffer and methanol with acetonitrile (50:50 v/v) was used as mobile phase with 1 mL per min flow rate at room temperature. The detection wavelength was fixed at 210 nm for DEC and 245 nm for ALB and IVR; the run time was within 15 min. The method was validated in terms of linearity, accuracy, robustness, and reproducibility. Calibration plots were linear over the 0.49-62.5 µg/ml range for IVR, 7.8-1000 µg/ml for ALB, and 15.625-2000 µg/ml for DEC. Recovery was in the 80-120% range with a relative standard deviation of less than 2% for both drugs. The limit of detection and the limit of quantification for the IVR were found to be 0.225 and 0.49 µg/ml respectively, for ALB it was 0.9 and 7.8 µg/ml and for DEC it is 7.8 and 15.63 µg/ml respectively.

Conclusions: The combination of IVER, ALB, and DEC is used as mass drug administration for Lymphatic filariasis recommended by WHO. The developed HPLC method for the simultaneous estimation of Ivermectin, Albendazole, and Diethyl carbamazine is very robust and rugged thus this method can be used in the future for qualitative as well as quantitative analysis.

Keywords: HLPC analysis; Ivermectin, Albendazole, Diethylcarbamazine Citrate

Background

Lymphatic filariasis, also known as elephantiasis, is a disease caused by filarial parasites. This parasite is a nematode (roundworm) that belongs to the filariodidea family. They are of three types. Wulcheriria

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Ban Croftia is responsible for near about 90% of the cause, Burgia Malai is responsible for the rest of the transmission, Burgia fimoria also causes disease. Recent proof, [Ramaswamy Kalyanasundaram et al (2016), Ottesen, E. A. (2006), Edward K Thomsen (2016), Peter U et al. (2017), C. Ediet al (2019), P. Jambulingam et al. (2021), C.L. King et al (2018), M. Hardy et al (2020)B. Tripathi et al.(2022)] indicate that a triple-drug remedy, a mixture of IVER, ALB & DEC, is extra effective. So, it's miles now encouraged for MDA (Mass drug management) regime in nations without onchocerciasis i.e., IVER 200 µg, ALB 400 mg, and DEC 6 mg per kg in certain settings are administered.

Drug Profile:

IVER result in an influx of chloride ions through the invertebrate's cell membrane as it activates specific ivermectin-sensitive ion channels. Mechanism of action involves binding to specific receptors and channels on parasites' nerve and muscle cells, leading to increased permeability of cell membranes to chloride ions. This increased chloride influx hyperpolarizes the parasite's cells, causing paralysis and ultimately death of the parasite.

ALB's mechanism of action involves disrupting the microtubule structure in the cells of parasites, leading to impaired glucose uptake, inhibition of microtubule polymerization, and interference with various cellular processes.

DEC's mechanism of action involves a combination of immune modulation, interference with arachidonic acid metabolism, and direct effects on the parasites' musculature and reproductive systems. However, the precise details of these mechanisms and their interactions are still being investigated.

Not many validated methods are available for quantifying all three drugs together in the HPLC. Thus, an HPLC method has been developed and validated in this work for the quantification of ivermectin albendazole & DEC for further studies.

Fig 1: Structure of IVER

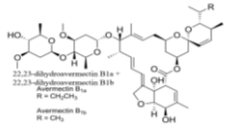
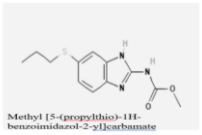
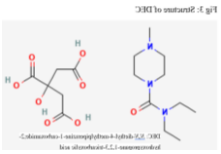


Fig 2: structure of ALB





EXPERIMENTAL

Material

IVER and ALB standards were obtain from Vivan life science where DEC standard was procured from Shubham Pharmachem Pvt Ltd, Acetic acid from Sigma and Ammonium Acetate and Acetonitrile were

purchased from Merck and Milli-Q water with 0.45 micron filter from Millipore. Waters HPLC was used for analysis.

Instrumentation and chromatographic conditions

The experiment was performed on Alliance 2695 HPLC combined 996 PDA Detector. The analytical Zorbax SB Phenyl column (150 mm×4.6 mm, 3.5 µ) was used for chromatic separations. 5 mM ammonium acetate with 0.1% Acetic Acid buffer (channel A) and combination of methanol and acetonitrile (50:50 v/v) (channel B) was used as mobile phase with 1 mL min^{-1} flow rate at ambient temperature. Gradient elution was carried out. It started with 20% B for 2 min, concentration increased to 95% B till 5 min retain for 12 min and returned to initial concentration in 13 min and ended in 15 min. The total run time was 15 mins. The injection volume was 5µL. After every injection, the needle was washed with 50: 50 composition Acetonitrile and Methanol (needle wash solution). The temperature was maintained at 10^{o}C for the autosampler.

All acquisition data were controlled by Empower software buildup 1154.

Preparation of stocks and working solutions

All primary stocks were prepared by dissolving the standard in 5% Hydrochloric acid (HCl) in methanol. Further, the stock solution was serially diluted with 1:1 dilution with same diluent to prepare working solutions for all the drugs. The working standards range of Ivermectin (IVER), Albendazole (ALB) & Diethylcarbamazine Citrate (DEC) were 0.49 µg/mL to 62.5µg/ mL, 7.8 µg/mL to 1000µg/ mL and 15.6 µg/mL to 2000µg/ mL respectively. All the stocks were stored at 4˚C and brought to ambient temperature for use. In order to determine the method's precision and accuracy calibration curve standards were made. A calibration curve (CC) of IVER (0.45, 0.90,1.80, 3.9, 7.8, 15.6, 31.25 and 62.5 µg/mL), ALB (7.8, 15.6, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL) and DEC (15.6, 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg /mL) was prepared by spiking the appropriate volume of working solutions.

RESULTS

Specificity and Carryover

There was no interference of blank with the analyte as well as all analytes are compatible with each other hence not influencing each other response then it shows there is no carry-over. The retention time for DEC was 3.1 min., ALB was 7.05min., and IVER was 8.28 min. Purity

passed in each case for the main peaks, as shown in Fig 4. Thus, the method passed the specificity parameter.

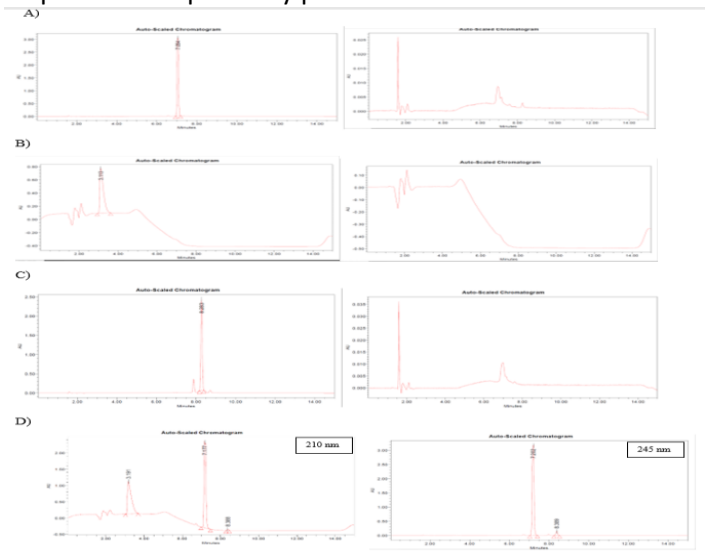


Fig. 4: Chromatogram: - (A) Albendazole & blank at 245 nm, (B) Diethyl carbamazine citrate & blank at 210 nm, (C) Ivermectin & blank at 245 nm, (D) Co-injection of all analytes at 210 nm and 245 nm.

Calibration curve and linearity

A specific quantification range was chosen for the experiment and calibration standards concentrations were prepared based on the expected concentration range. The calibration curves for IVER, ALB and DEC were consistent and repeatable. The calibration curve was created by graphing the ratio of the analyte's peak area against the standards' concentration.

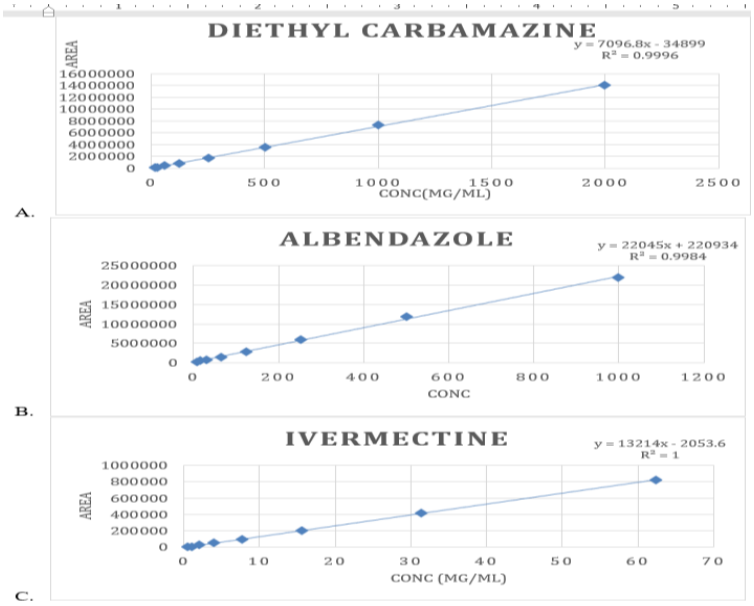


Fig. 5: Calibration graph of A) DEC, B) ALB and C) IVER respectively.

Accuracy: All analytes were within their specified criteria i.e., 85-115%
Cal 8: DEC: - 2000µg/ml, ALB: 1000µg/ml, IVER: 62.5 µg/ml
Cal 7: DEC: - 1000µg/ml, ALB: 500µg/ml, IVER: 31.25 µg/ml
Cal 6: DEC: - 500µg/ml, ALB: 250µg/ml, IVER: 15.63 µg/ml

Table 1: Accuracy

	DEC			ALB			IVER		
	Cal_8	Cal_7	Cal_6	Cal_8	Cal_7	Cal_6	Cal_8	Cal_7	Cal_6
1	14650100	8286373	3494955	21618390	13519866	6015385	820120	463775	175143
2	14704884	8278649	3484004	21498483	13406064	5990731	815581	468717	176158
3	14569956	8371719	3575832	21026291	13835293	6113766	830292	464478	177091
Avg	14641647	8312247	3518264	21381055	13587074	6039961	821998	465657	176131
Std	67860.0	51648.9	50155.4	313029.3	222367.0	65095.1	7533.1	2673.5	974.3
%RSD	0.46	0.62	1.43	1.46	1.64	1.08	0.92	0.57	0.55
Accuracy	104.03	114.27	102.94	97.58	106.1	99.28	99.81	112.82	86.7

Precision: As per ICH guidelines Q2(R1) % RSD should be ≤ 2% for repeatability and intermediate precision it should be ≤ 5%. All results are showing a % RSD below 2%.

Table 2: Precision of DEC, ALB, IVER

Precision	DEC	ALB	IVER
	%RSD	%RSD	%RSD
Repeatability	0.81	1.85	1.36
Intermediate (day)	1.82	1.83	1.75
Intermediate (instrument)	1.25	1.31	1.72
Intermediate (analyst)	0.71	0.92	0.73

LOQ: On the bases of visual evaluation LOQ for Ivermectin was found to be 0.49g/ml, for Albendazole it was 7.8 µg/ml and for Diethylcarbamazine, it was 15.63µg/ml.

LOD: On the bases of visual evaluation LOD for Ivermectin was found to be 0.24µg/ml, for Albendazole it was 0.9 µg/ml and for Diethylcarbamazine, it was 7.8µg/ml**.**

Robustness: the ICH Q2(R1) recommends that the % RSD should be less than or equal to 2.0% for changes in method parameters that are likely to occur during routine use and less than or equal to 5.0% for changes in method parameters that are unlikely to occur during routine use. All the parameters of robustness are passing i.e., % RSD is below 2%.

Table 3: Robustness of DEC, ALB, IVER

Robustness	DEC	ALB	IVER
	%RSD	%RSD	%RSD
column	1.00	1.65	1.16
pH±0.2	1.81	1.97	1.63
mobile phase + 2%	0.74	1.41	0.78
mobile phase - 2%	1.08	1.92	1.46
system	1.06	1.87	1.93

Discussion:

Due to the solubility limitation for DEC, 5% HCl in methanol was used. IVER was unstable if kept for more than two days in the above solution, thus, before analysis sample was prepared freshly.

Conclusion

A combination of all three drugs, Ivermectin, Albendazole, and diethyl carbamazine citrate is under MDA trial for a treatment for Lymphatic filariasis. This method of HPLC quantification analysis of DEC, ALB and IVER is highly sensitive, concise, and precise. The above validated method can be successfully applied to pharmaceutical analysis.

Abrevations:

Ivermectine: IVER
Albendazole: ALB
Diethyl carbamazine: DEC
Fig: figure
HPLC: high performance liquid chromatography
LOD: limit of detection
LOQ: limit of quantification

Std: standard deviation
AVG: Average
% RSD: Percentage relative standard deviation
µg: micro gram
ml: micro litre
mg: milli gram
MDA: mass drug administration

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Figure legend:

Fig 1**:** Structure of IVER

Fig 2: structure of ALB

Fig 3: Structure of DEC

Fig 4: Chromatogram: - (A) ALB & blank at 245 nm, (B) DEC & blank at 210 nm, (C) IVER & blank at 245 nm, (D) Co-injection of all analytes 210 nm and 245 nm.

Fig 5: Calibration graph of A) DEC, B) ALB and C) IVER respectively.