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Research

Rp Hplc Method Designing And Assessment To Analyze Albendazole And Closantel Amine In Its Formulation

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Abstract

A simple, precise, and accurate method was developed to estimate ALB and CLS in bulk and tablet dosages. Chromatogram was produced using Agilent Zorbax C18 250 x 4.6 mm, 5mm column and 50:50 v/v Acetonitrile and KH2PO4 mobile phase at 0.8 ml/min at 220nm at 30°C column temperature. The retention times for ALB and CLS are 2.189 and 3.079 minutes. The ALB and CLS regression coefficients were 0.9917 and 0.9984. % We recovered 100.14% and 99.98% for ALB and CLS. The ALB and CLS regression equations yielded LOD and LOQ values of 0.08, 0.25, and 0.04, 0.12. Due to reduced retention and run times, the recommended method was simple and cost-effective for routine quality control laboratory testing.

Keywords: Albendazole, Closantel amine, RP-HPLC

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for separating, identifying, and quantifying components in a mixture. It operates on the principle of liquid-phase chromatography, where a liquid mobile phase carries the analytes through a stationary phase (column), leading to differential retention and separation based on chemical properties such as polarity, molecular weight, and interaction with the stationary phase.[1]

A benzimidazole derivative called albendazole is used to treat gastrointestinal helmentiasis in humans. In the larval and adult stages of sensitive parasites, it reduces the production of adinosine triphosphate (ATP) and depletes glycogen stores by blocking microtubule and glucose uptake. Albendazole (ALB) is a broad-spectrum anti-helminthic with a molecular weight of 265.331 g/mol, a monoisotopic mass of 265.088498 g/mol, and the molecular formula $C_{12}H_{15}N_3O_2S$. Its primary mechanism of action involves inhibiting tubulin

polymerization, leading to the loss of cytoplasmic microtubules in the intestines of nematode worms. This disruption depletes the parasite's energy reserves, ultimately causing its death. ALB is marketed under the brand name BENDEX-400. [2,3]

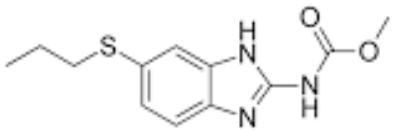

Fig 1: Structure of ALB

Closantel amine (CLS) is mainly used to treat and manage adult and juvenile liver flukes, hemophagous nematodes, and some arthropod larvae in cattle and sheep. With a molecular weight of 663.074 g/mol and the molecular formula $C_{22}H_{14}Cl_2I_2N_2O_2$, CLS is soluble in acetone and should be stored in a refrigerator. Its IUPAC name is rac-N-[5-chloro-4-[(R)-(4-chlorophenyl)(cyano)methyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide. The mechanism of action involves the inhibition of succinate dehydrogenase and fumarate reductase enzymes, leading to mitochondrial oxidative phosphorylation decoupling, which disrupts ATP generation and ultimately results in the parasite's death. Additionally, CLS has been found to block chitinase in Onchocerca volvulus, a filarial worm responsible for human river blindness. Since chitinase is essential for larval molting, its inhibition prevents the worms from maturing into adults, disrupting their lifecycle. CLS is marketed under the brand name Tricloz oral solution. [4]

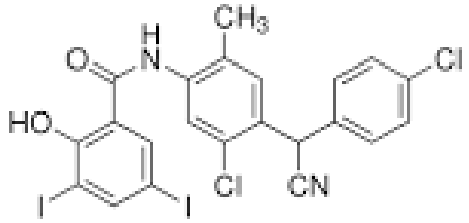

Fig 2: CLS structure

Ali et al. (2022) developed an HPLC-DAD method for analyzing Q-DRENCH, a multi-drug oral suspension for sheep GIT worms, demonstrating its greenness and efficacy in detecting degradation products. [5] Saleh et al. (2023) used HPLC to study closantel enantiomers in goat tissues, showing stereoselective distribution with half-lives of 9–14 days. [6] Basavaiah et al. (2003) developed titrimetric and spectrophotometric methods for albendazole using chloramine-T. [7] Nikale & Gadikar (2019) created an RP-HPLC method for albendazole impurity analysis. [8] Rao et al. (2014) established an RP-HPLC method for mebendazole and levamisole in tablets. [9] Maingi et al. (1997) demonstrated superior worm control using albendazole and closantel in sheep. [10] Literature survey showed no HPLC method for ALB and CLO. Hence the present research was undertaken. The main purpose of this work is to establish an ICH-compliant approach for simultaneous analysis of pharmaceutical dosage forms of albendazole (ALB) and closantel amine (CLS) in bulk and veterinary products.

MATERIALS AND METHODS

Chemicals and Solvents

HPLC-grade water, acetonitrile, 5mM potassium dihydrogen phosphate buffer, and ALB and CLS APIs were used. Commercial tablets containing 300 mg ALB and 100 mg CLS (Hebei New Century Pharmaceutical Co., Ltd) were prepared. CLS (working standard) was obtained from Vetindia Pharmaceuticals Ltd., Hyderabad.

Instrumentation

HPLC analysis was performed using Shimadzu LC 2030C 3D Plus HPLC (Prominence-i series) with Empower-2 software. The Agilent Zobrax C18 column (250 × 4.6 mm, 5µm) was used for separation.

Preparation of Solutions

Buffer: 680 mg potassium dihydrogen phosphate dissolved in 1L HPLC water, pH adjusted to 3.5 with orthophosphoric acid.

Mobile Phase: 50% buffer and 50% acetonitrile, degassed for 5 minutes and vacuum filtered.
Diluent: Standard solution containing 300 µg/ml ALB and 100 µg/ml CLS was prepared and diluted to obtain 90 µg/ml ALB and 30 µg/ml CLS.
Sample Solution: 20 tablets were weighed, the average weight determined, and dissolved in a 10 mL volumetric flask, sonicated, and diluted to achieve 300 µg/ml ALB and 100 µg/ml CLS.

Standard Graph Preparation

Volumes of 1, 2, 3, 4, and 5 ml were extracted from primary solutions of 300 µg/ml ALB and 100 µg/ml CLS, and transferred to a 10 ml volumetric vessel. The final volume was adjusted with diluent to achieve concentrations of 10 - 50 µg/ml for both ALB and CLS. For each step, 10 µl of each individual sample was loaded three times, and a calibration curve was constructed using peak area versus target molecule concentration.

Assay

Tablets labelled ALB 300 mg and CLS 100 mg were manufactured in our laboratory. The punched formulation was utilised for the experiment.

Method Validation

System suitability was assessed by injecting standard solutions of 30 µg/ml ALB and 10 µg/ml CLS five times to evaluate the tailing factor, area, and USP plate count, with an RSD of less than 2%. Specificity was confirmed as no interfering peaks were observed at the drug retention times. Accuracy and linearity were evaluated by analyzing ALB and CLS at concentrations ranging from 10–50 µg/ml, with a calibration curve confirming linearity and a recovery rate of 98–102%. Precision was assessed using spiked solutions at 50%, 100%, and 150% levels. Robustness was tested by varying the flow rate and mobile phase composition, with %RSD remaining within acceptable limits. Sensitivity was confirmed through LOD and LOQ studies, with sample dilutions of 0.25 ml and 0.3 ml demonstrating reliable detection.

RESULTS AND DISCUSSION

The optimized chromatographic conditions involved a mobile phase of 5 mM Acetonitrile: KH_2PO_4 (50:50 v/v) with a flow rate of 0.8 mL/min. Separation was performed using an Agilent Zobrax C18 column (4.6 × 250 mm, 5 µm) at a column temperature of 30°C. Detection was carried out at a wavelength of 220 nm, with an injection volume of 10 mL and a total run time of 10 minutes. Methanol was used as the diluent, and the experiment was considered optimized as all peaks were clearly visible. ALB and CLS were eluted at 2.182 minutes and 3.091 minutes, respectively (Fig. 3). The method was optimized and evaluated for tailing factor and plate count, ensuring both values remained within prescribed limits. System suitability parameters were assessed following ICH guidelines, confirming that all parameters were within the acceptable range (Table 1).

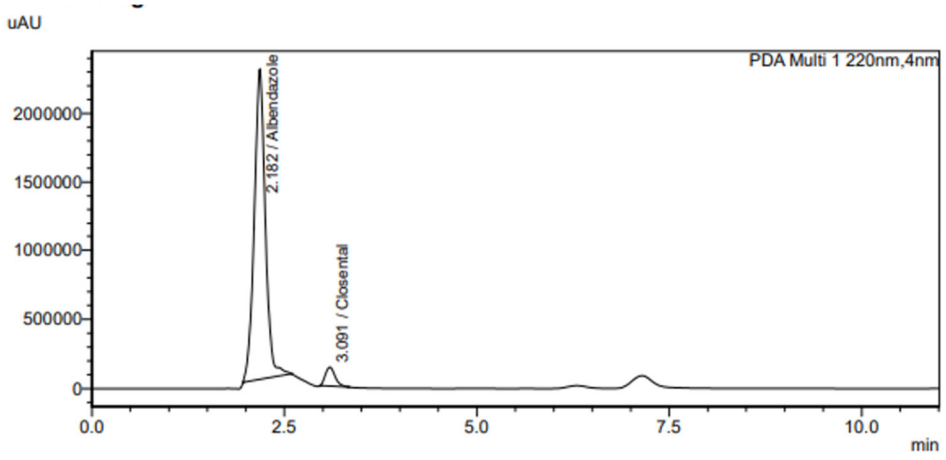

Fig 3: Chromatogram (Optimised)

The following table 2 and Figs. 4 and 5 shows the calibration data of ALB and CLS. Correlation coefficients obtained were 0.9917 for ALB and 0.9984 for CLS.

Table 1: Linearity data of ALB and CLS

Conc (µg/ml)	ALB (Peak Area)	CLS (Peak Area)
10	3084945	114981
20	9730023	465094
30	20669018	884754
40	32859263	1270548
50	42960086	1722551

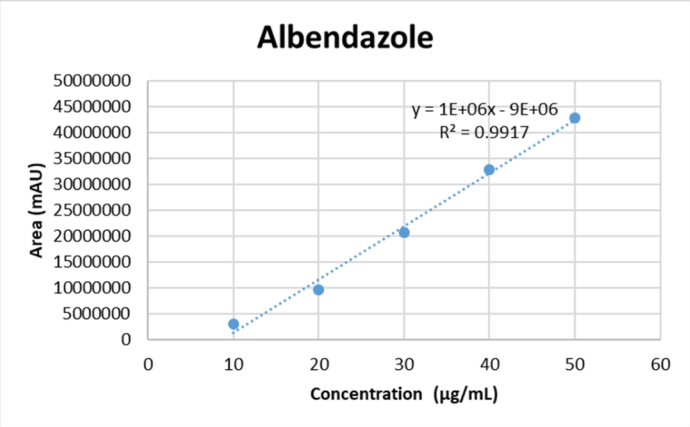

Fig 4: Calibration graph for ALB

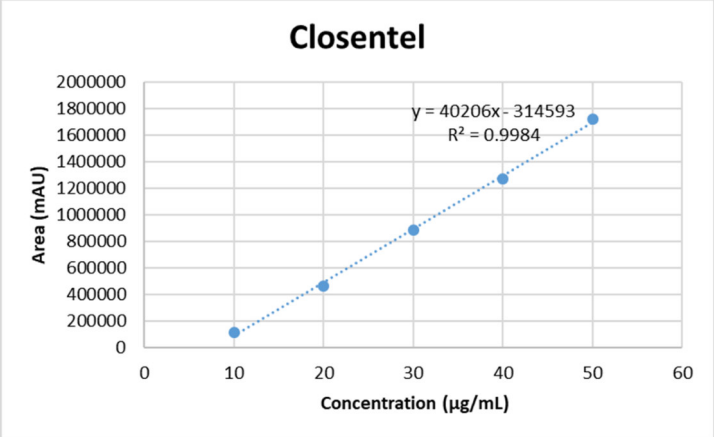

Fig 5: Calibration graph for CLS

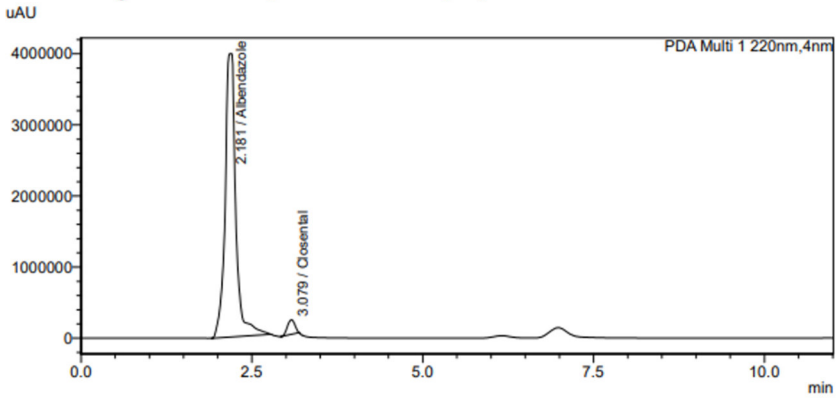

Fig 6: Chromatogram of Assay

The assay results for CLS showed an average assay value of 100.01%, with a %RSD of 0.6, indicating high accuracy and precision (Fig. 6). Similarly, the assay results for ALB demonstrated an average assay value of 99.99%, with a %RSD of 0.5, confirming consistency and reliability in drug quantification. The standard and sample areas for both compounds were closely matched, ensuring the method's robustness. The low %RSD values (≤ 1.0%) further validate the precision of the analytical method, making it suitable for routine quality control analysis.

Table 2: Method validation parameters

Parameters		**ALB**	**CLS**	**LIMITS**
Linearity Range		10-50µg/ml	10-50 µg/ml	R< 1
Regression coefficient		0.9917	0.9984	
Assay		99.99%	100.01%	90-110%
Specificity		unique	unique	Interfering peaks were absent
System precision		0.9	0.6	%RSD≤ 2.0
Method precision		0.9	0.5	%RSD≤2.0
Accuracy		100.14%	99.98%	98-102%
Limit of Detection		0.08	0.04	3:1 S/N
LOQ		0.25	0.12	10:1 S/N
Robustness	**FM**	0.7	1.1	%RSD NMT 2.0
	FP	1.2	1.7	
	MM	0.6	0.4	
	MP	1.2	1.6	

The method demonstrated good linearity for both ALB (R^2 = 0.9917) and CLS (R^2 = 0.9984) within the 10-50 µg/mL range, ensuring reliable quantification. The assay values for ALB (99.99%) and CLS (100.01%) were within the acceptable limits (90-110%), confirming accuracy in drug content determination. Specificity testing showed no interfering peaks, ensuring the method is unique for both compounds.

System precision (%RSD ≤ 2.0) was 0.9% for ALB and 0.6% for CLS, while method precision was 0.9% and 0.5%, respectively, indicating high reproducibility. Accuracy values of 100.14% (ALB) and 99.98% (CLS) were within the acceptable range of 98-102%, confirming method reliability.

The Limit of Detection (LOD) was 0.08 µg/mL for ALB and 0.04 µg/mL for CLS, with an S/N ratio of 3:1, ensuring sensitivity. The Limit of Quantification (LOQ) was 0.25 µg/mL (ALB) and 0.12 µg/mL (CLS), with an S/N ratio of 10:1, confirming the method's ability to quantify low concentrations.

Robustness testing under different conditions (Flow rate (FM), Flow precision (FP), Mobile phase (MM), and Mobile phase precision (MP)) showed %RSD values below 2.0, indicating the method remains reliable under minor variations. These results confirm the method's accuracy, precision, specificity, sensitivity, and robustness, making it suitable for routine analysis of ALB and CLS.

CONCLUSION

An accurate, precise, robust, and cost-effective method was developed to estimate ALB and CLS in tablets. ALB held for 2.182 and CLS for 3.091 minutes. The ALB and CLS recovery rates were 99.99% and 100.01%. Regression equation yielded LOD and LOQ values of 0.08, 0.25, and 0.04, 0.12. CLS' regression coefficient is 0.9984, ALB's 0.9917. The shortened retention and run lengths made the system cost-effective and easy to utilise for drug testing lab sample analysis.

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