INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DEVELOPMENT AND VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF ALBENDAZOLE AND CLOSANTEL FROM VETERINARY FORMULATION

M. SPhatak*, VV. Vaidya and H. MPhatak

Department of Chemistry, Ramnarain Ruia College, Matunga,
Mumbai-400019, India.

ABSTRACT

A simple and rapid Reverse Phase HPLC method has been developed for the simultaneous quantification of Closantel and Albendazole from veterinary anthelmintic formulation. HPLC analysis was performed on C18 column maintained at 30ºC using a simple mixture of acetonitrile, distilled water and methanol as isocratic mobile phase at a flow rate of 1.8ml per minute at detection wavelength of 254nm. The method was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with International Conference on Harmonization guidelines. Good linear correlation coefficients (r2>0.999) were obtained for calibration plots in the range of 50–150µg/ml for Closantel and 25 – 75 µg/ml Albendazole. Intraday and Interdayprecision of retention times and peak areas were less than 2.0%. Accuracy of the method was between 99.35% and 99.86% for Closantel and 99.58% and 100.16% for Albendazole. Validation revealed the method to be specific, accurate, precise, reliable and reproducible. The method was successfully used for quantitative analysis of these analytes from marketedVeterinary anthelmintic formulation.

Keywords: Simultaneous determination; RP-HPLC; Albendazole; Closantel; veterinary formulation.

INTRODUCTION

Closantel is a salicylanilide class of anthelmintic agent (Figure 1). It is a broad-spectrum antiparasitic agent used against several species and developmental stages of trematodes, nematodes and arthropods. The anti-trematode activity of closantel is mainly used against liver fluke. The anti-nematode and anti-arthropod activity is especially used against those species which feed on blood or plasma. Closantel has shown beneficial effect when used in humans for the treatment of infections causing “River blindness”[1]. Closantel is available commercially as oral treatments in the form of tablets and drench formulation alone or in combination with other anthelmintic agents for veterinary use. There are a few methods available for the quantification of Closantel from biological matrix[2] or pharmaceutical presentation[3]. The analytical methods used were LC MS/MS and HPLC.

Albendazole is a benzimidazole class of drug being used in the treatment for parasitic infections (Figure 2). It is a broad spectrum anthelmintic effective against roundworms, tapeworms, and flukes of domestic animals and humans[4]. Albendazole is available commercially in the form of tablets for human use and tablets and oral drench for veterinary use. Albendazole is used alone or in combination with other anthelmintic drugs for better efficacy. Various analytical method have been published for quantification of Albendazole[5,6,7].

There is no method available currently for the determination of Closantel and Albendazole from a veterinary formulation. In the present work the development and validation of a simple and rapid method for the simultaneous

quantification the two drugs from an oral drench formulation.

MATERIALS AND METHODS

Instruments

The HPLC system included Agilent Technologies 1200 HPLC model with photo diode array detector, Rheodyne injector having 20µL loop volume. Separation was carried out using Thermo Electron Corporation, Hypersil BDS C18 (150 x 4.6 mm, 5µ) column. Detection was carried out using UV-visible detector. The flow rate was kept at 1.8 ml/ min and the column oven was maintained at 30 ºC. The total chromatography run time was 4 minutes.

Reagents

All chemicals and solvents used were of HPLC grade. Distilled water generated from TQA water purification system was used. Closanteland Albendazole were provided as gift sample by Cipla Ltd., India. Veterinary anthelmintic formulation Closal drenchwas purchased from local market.

Standard Solutions

Stock solutions, of 1 mg/ml of Closantel and 0.5 mg/ml of Albendazole were freshly prepared individually in methanol. Aliquots of Closantel (50-150µg/mL) and Albendazole (25-75 µg/mL) were prepared by subsequent dilutions of the stock solutions in the mobile phase.

Sample Preparation

The Closal drench is available as a formulation containing 19 g/L of Albendazole and 37.5 g/L of Closantel. The sample for analysis was prepared bydiluting 1.1 ml of the formulation to 100 ml with mobile phase. Further, 5 ml of this solution is diluted to 20 ml to give the sample concentration injected into the chromatographic system. The resultant sample solution has a concentration of 103.1 µg/mLof Closantel and 52.3 µg/mLof Albendazole.

Preparation of Mobile Phase

The mobile phase is prepared by mixing acetonitrile, distilled water and methanol. In a volumetric cylinder 600 ml of acetonitrile is taken to which 300 ml of distilled water and 100 ml of methanol is added. The mobile phase is sonicated for 2 minutes and filtered through 0.45 µm Teflon filter before using in the HPLC system.

Calibration Curve

Standard solutions of 50 - 150 µg/mL of Closantel and 25-75 µg/mL of Albendazole were analyzed to check the linearity range.

Specificity

The specificity of the method was ascertained by analysing the standards and the samples. The peaks of Closantel and Albendazole in sample were confirmed by comparing the retention time and spectra of the standards[8].

Precision

Precision was evaluated by injecting six injections at the working level concentrations of Closantel (100µg/mL) and Albendazole (50µg/mL) were analysed to examine the precision of the method. Intraday precision and interday precision for the developed methods were measured in terms of %RSD. The % RSD of the six replicates was considered for intraday precision and % RSD of six replicate injections, injected on two different days were considered for inter day precision. The concentration values for both intraday precision and interday precision were calculated and percent relative standard deviation were calculated using following formula[8].

% RSD = [S/X] 100,

Where;
S is standard deviation and
X is mean of the sample analyzed.

Accuracy

Accuracy of the method was determined by recovery experiments. Recovery experiments were carried out by the standard addition method. This study was performed by diluting adding 10 ml, 10 ml and 20 ml each of the of standard solution of Closantel (1000 µg/ml) and Albendazole (500 µg/ml) to three different volumetric flasks containing the 1.1 ml of sample. The resultant sample solutions were correspondingly diluted to provide 50%, 100% and 150% of Closantel and Albendazole concentration compared to the standard solution. The amounts of standard recovered were calculated in terms of mean recovery with the upper and lower limits of percentage relative standard deviation[8].

RESULTS AND DISCUSSION

Optimization of the method was carried out using various concentrations of acetonitrile,distilled water and methanol. A solvent combination of acetonitrile: D.W.: methanol (60:30:10) gave a satisfactory separation of the Closantel and Albendazole. This optimized mobile phase separated

Albendazole at 1.47 min and Closantel at 2.21 min respectively. The column efficiency, resolution and peak asymmetry and resolution were calculated for the standard solutions and are presented in Table 1.Increasing the concentration of distilled water in the solvent system resulted in deterioration of the peak shape and retention of peaks on the column, resulting is longer analysis time. On the other hand increasing the organic phase caused the Closantel peak to elute very close to the placebo peaks. The calibration curves of Closantel and Albendazole were linear in the range of 50- 150µg/mL for Closantel and 25-75 µg/mL for Albendazole. The linearity experiment observations and the slope, y-intercept and regression coefficient (r^2) are shown in Table 2.

Accuracy rom sample matrices was between 99.52% &100.10% and 99.67% and 99.98% respectively for Closantel and Albendazole. The individual standard chromatogram for Closantel and Albendazoleare shown in figure 3 and 4, while the chromatogram of sample solution is shown in figure 5. Even though Closantel and Albendazole show common therapeutic activities, they are entirely different in their chemical nature. Good resolution of Closantel and Albendazole in a simple isocratic mobile phase were achieved. The analysis for commercialised formulation sample purchased were done and the results demonstrated consistency between different samples and analysis conducted on different days.

CONCLUSION

Based on the above results it can be concluded that the developed method can be used for the simultaneous quantification of Albendazole and Closantel for routine quality control analysis.

ACKNOWLEDGEMENT

We would like to thank Cipla Ltd., India for providing the analytical standards for the research work.

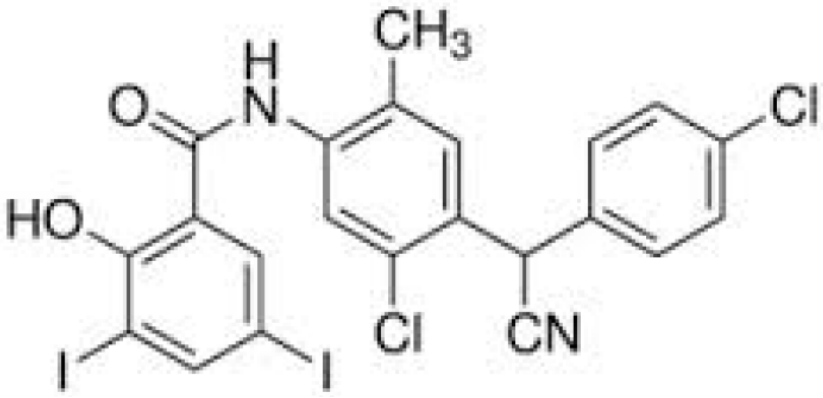

Fig. 1: Structure of Closantel

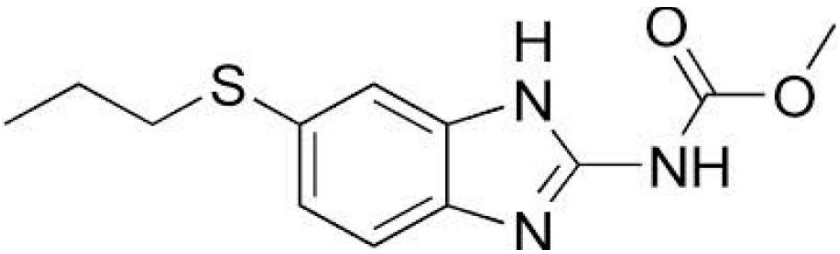

Fig. 2: Structure of Albendazole

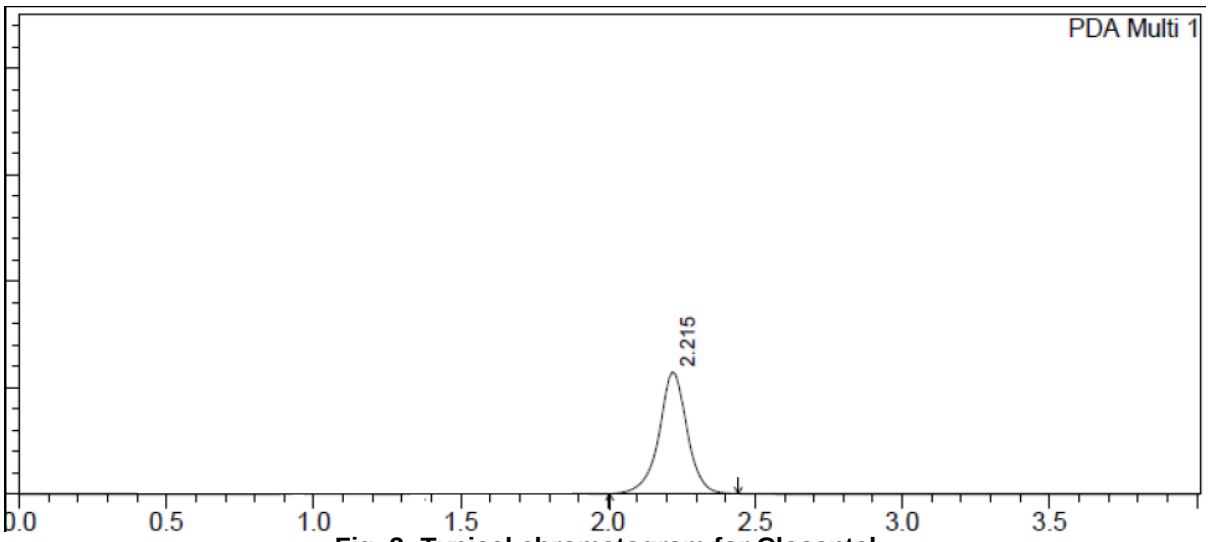

Fig. 3: Typical chromatogram for Closantel

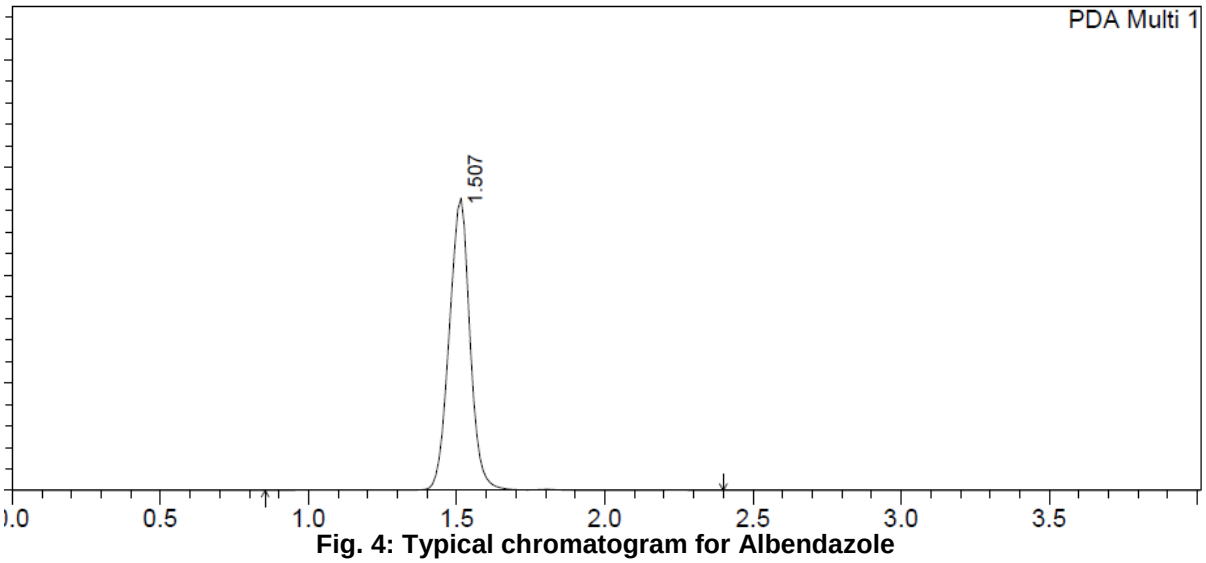

Fig. 4: Typical chromatogram for Albendazole

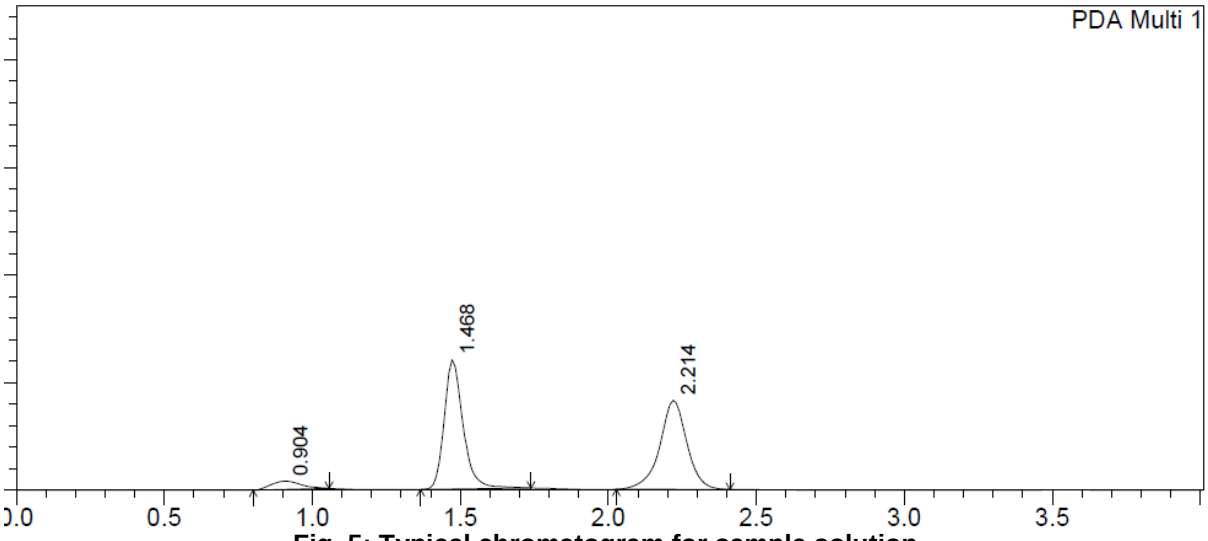

Fig. 5: Typical chromatogram for sample solution

Table 1: Chromatographic peak characteristics

Parameter	Albendazole	Closantel
Retention time	1.47	2.21
Theoretical plates	5376	5238
Asymmetry	1.09	0.92
Resolution	---	4.49

Table 2: Linearity experiment observations

	Albendazole		Closantel	
Linearity level	**Concentration**	**Peak area**	**Concentration**	**Peak area**
Level 1	25	267645	50	271960
Level 2	30	323242	60	324742
Level 3	40	429433	80	430452
Level 4	50	543644	100	539221
Level 5	60	643918	120	651024
Level 6	70	754010	140	762586
Level 7	75	817875	150	814923
Slope	10896		5452	
y-intercept	4832		3129	
r^2 value	0.9996		0.9998	

REFERENCES

1. Christian Gloeckner. Repositioning of an existing drug for the neglected tropical disease Onchocerciasis, Proceedings of National Academy of Science of the United States of America. 107(8):3424–3429.
2. Hanwen Sun. Validated method for determination of ultra-trace Closantel residues in bovine tissues and milk by solid-phase extraction and liquid chromatography–electrospray ionization-tandem mass spectrometry. Journal of Chromatography A. 2007;1175:227–233.
3. Gayatri S. Method development and validation for estimation of Closantel in tablet dosage form by RP-HPLC method. International Journal of Biomedical Research. 2011;4:246-251.
4. Theodorides VJ, Gyurik RJ, Kingsbury WD and Parish RC. Antihelmintic Activity of AlbendazoleAgainst Liver Flukes, Tapeworms, Lung and Gastrointestinal Roundworms. Experientia. 1976;32(8):702–703.
5. Kitzman D, Cheng KJ and Fleckenstein L. HPLC assay for albendazole and metabolites in human plasma for clinical pharmacokinetic studies. J Pharm Biomed Anal. 2002;15;30(3):801-13.
6. Anil Waldia, Shubash Gupta, RoshanIssrani and BadriNagori. Validated liquid chromatographic method for simultaneous estimation of albendazole and ivermectin in tablet dosage form, Indian J ChemTechnol. 2008;15:617-620.
7. Shreya Shah, Dey S, Prasanna Pradhan, Jain HK and UmeshUpadhyay. Method development and validation for simulataneous estimation of albendazole and praziquantel in bulk and in a synthetic mixture, Journal of Taibah University for Science. 2014;8:54–63.
8. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline-Validation of Analytical Procedures: Text and Methodology Q2 (R1), Current Step 4 version, London, 2005.