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Research Article

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A simple and rapid chromatographic technique for the quantification of Albendazole from marketed solid dosage forms

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ABSTRACT

The current study presents a simple, rapid, accurate and precise chromatographic technique for the quantification of Albendazole from marketed formulation. A dual lambda UV-visible detector is used for the quantification purpose. The analysis is carried out on a C18 column (250mm × 4.6mm; 5µm) at a mobile phase flow rate of 0.7 ml/min. The method is found to be linear in a concentration range of 10.61µg/ml to 63.65µg/ml. The method is validated as per ICH Q2 guidelines. The mean recovery is 99.99% and was found to be precise on the basis of inter-day and intra-day precision studies. The limit of detection and limit of quantification is found to be 0.04 µg/ml and 1.67µg/ml respectively explaining the high sensitivity of the method. This chromatographic method is found to be suitable for the regular analysis of Albendazole from bulk drug and marketed formulations.

Keywords: Albendazole; HPLC; UV-Vis detection; oral formulations; tablets.

INTRODUCTION

Helminths constitute a group of parasitic worms affecting about two million people worldwide, mainly all developing tropical and subtropical countries [1]. Albendazole (ANZ) is a drug of choice for the treatment of tissue nematodes and intestinal nematodes. It is a broad spectrum antihelmintic drug mainly recommended for the treatment of flatworm and nematodes infections like Enterobiusvermicularis, Trichinelaspiralis, Ancylostomabraziliense, Taeniasolium and Echinococcusgranulosus [1]. Chemically it is methyl-5-polythio-2-benzimidazolecarbamate (Fig. 1). It acts through inhibition of formation of microtubules by binding with β-tubulin monomers, thereby preventing tubuline polymerization leading to loss of cytoplasmic microtubules [2].

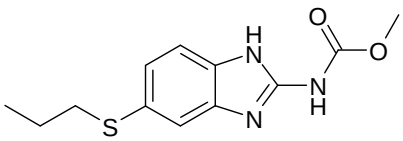

Fig. 1 Chemical structure of Albendazole

On reaching blood ANZ is transported to liver where it is metabolized to its corresponding sulfoxide which is responsible for its systemic antihelmintic activity [3]. ANZ suffers from a number of side effects like nausea, abdominal pain and headaches. Other potential side effects include bone marrow suppression and liver damage

which are not common and a few critical cases are registered [4, 5]. In India, infections caused by soil borne helminthes are quite common and ANZ has been the drug of choice in most of the cases [6-8]. Several types of ANZ formulations are available in the Indian market. Therefore, a proper quantification of ANZ from these formulations is necessary. Literature reports present a number of different liquid chromatographic mass spectroscopic techniques for the quantification of ANZ and their corresponding metabolites from different matrices [9-14]. Other reported techniques involved solid phase extraction, voltametric techniques and high performance liquid chromatoghraphic techniques [15, 16] and from formulations in combination with other active pharmaceutical ingredients [17,18]. Most of the reported methods presented the use of liquid chromatographic techniques coupled with mass spectroscopic detectors. These techniques are therefore costly and time consuming for regular quality control analysis of formulations as well as quantification of ANZ from environmental samples. In the current study we reported a simple, rapid precise technique for the quantification of ANZ from bulk and marketed formulation. The current method has been validated for the accurate quantification of ANZ following a simple technique for sample preparation in presence of different excipients used in formulation of the drug delivery systems.

EXPERIMENTAL SECTION

Materials

The analytical method was validated on a Waters Alliance e2695 separation module. The analysis was carriedout on a C18 column (250 mm × 4.6 mm, 5 µm). Chromatographic detection was made with Waters 2489 dual lambda absorbance detector. Data analysis and quantification was carried out using Empower-3 software.

ANZ was received as a gift sample from Dey's Medical Stores (Mfg.) Ltd. The analysis, recovery study was carried out using marketed oral formulation (Nowarm; 400mg tablet;AlkemUlticare, Mumbai, India Batch no. 4133062 ; Mfg. 11/2014, Exp. 10/2017). All reagents of chromatography grade were purchased from Spectrochem India Ltd. (Mumbai, India). The C18 column was purchased from Waters (Millford, USA).

Methods:

Chromatographic Conditions:

The analysis was carried out following isocratic elution mode with a mobile phase flow rate 0.7ml/min. The mobile phase consisted of a mixture of 30%,5.3mMphosphate buffer of pH 2.8 ± 0.2, 20% acetonitrile and 50% methanol. The pH of the buffer was maintained using 0.1(M) orthophosphoric acid.The injection volume was 20µL. The column was maintained at 25ºC. The detection wavelength was 292nm.

Preparation of standard and sample solutions:

The buffer solution was used as diluents for the preparation of standard and stock solution. The standard stock solution was prepared by accurately weighing ANZ working standard in a 25ml volumetric flask (about 26.42mg). To it diluents was added followed by 2 to 3drops of concentrated sulfuric acid. Suitable dilution of this stock solution was made for the preparation of the respective standard solutions using the mobile phase as diluents in this step. Altogether five different solutions of varying concentrations of ANZ were prepared as presented in Table 1.The final volume was made up with mobile phase.

Table 1: Preparation of standard solutions

Solution number	Concentrations (µg/ml)	Average peak area of standard solutions*
1	10.61	651998
2	21.22	1301897
3	42.43	2663803
4	53.04	3419129
5	63.65	3914912
**Three replicate injections of each solution were carried out.*		

The sample solutions were prepared by dissolving suitable weights of the powder sample containing ANZ equivalent to 25mg and finally diluting the stock in the concentration range of 40µg/ml. Each of these solutions was filtered through 0.45µm membrane filter before each injection. Each injection was carried out in three replicates and the mean area was used for the quantification purpose.A representative chromatogram is presented in Fig. 2 and the result for the quantification of ANZ from formulation is presented in Table 2.

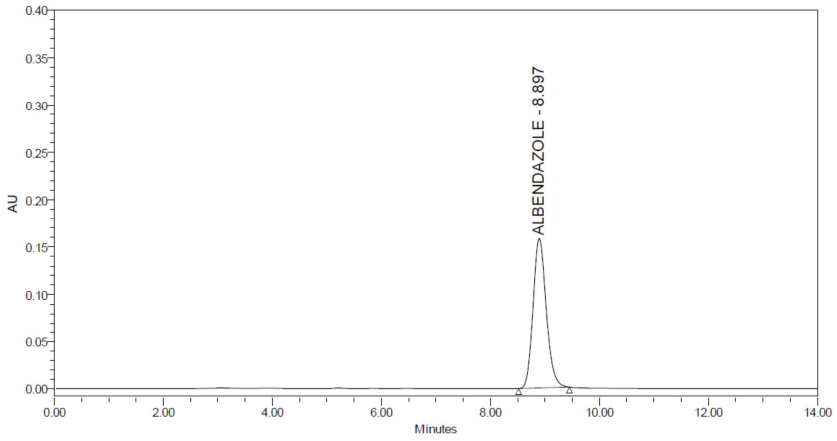

Fig. 2Representative chromatogram for Albendazole (ANZ)

Method validation:

The proposed method was validated as per USP and ICH Q2 guidelines in terms of system suitability parameters, linearity, intraday and interday precision, accuracy, robustness, ruggedness, LOD and LOQ [19,20].

Table 2: Sample formulation

Formulation	Drug	Amount of Drug (mg/tab)		% of Label Claim	% RSD
		Labelled	Estimated*		
Nowarm; 400mg tablet;AlkemUlticare, Mumbai, India Batch no. 4133062; Mfg. 11/2014, Exp. 10/2017	ANZ	400	399.99	99.99	0.00

** Mean from three replicate analyses.*

RESULTS AND DISCUSSION

The current method describes a validated chromatographic technique for the quantification of ANZ from marketed formulations as discussed earlier.Initially the mobile phase combination and the pH of the buffer were optimized through trial and error method. After optimization the sample was analyzed using the proposed method. The amount of drug in the formulation was 399.99 mg/tablet against a labeled claim of 400mg (Table 2). The retention time of the analyte peak was 8.89 min (Fig. 2). The final method was validated on the basis of the following parameters.

System Suitability:

The study was carried out to study whether the high performance liquid chromatography system used for the analysis of ANZ was suitable for the quantification of the same from their respective formulations available in the market. For this study six replicate injections of the standard solution was carried out and column efficiency (Theoretical plates), %RSD of retention time and peak area, tailing factor were determined (Table 3).The results presents minimal deviation of retention time and peak area from the mean and an acceptable theoretical plate count. The peak was found to be symmetrical and the tailing factor was only 0.1099. The results thus present the system to be suitable for regular analysis of ANZ using the proposed method.

Table 3: System suitability parameter

Parameters	ANZ
Wavelength maxima (nm)	292
Retention Time (mins)*	8.901±0.1
Peak Area*	2663803±0.0
Tailing factor	0.1099
Theoretical Plate	4000.5
**Presented as Mean ± %RSD (% Relative Standard Deviation)*	

Study of linearity and Limits of Detection and Quantitation:
Linearity was determined by taking five different concentrations of ANZ in triplicate and calibration curve wasplotted in the concentration range 10.61µg/ml and 63.65µg/ml (Fig. 3, Table 1). The linearity was evaluated by linear regression analysis by least square method [20]. The linear regression co-efficient was found to be0.998 (Table 4). The limit of detection(LOD)and the limit of quantitation (LOQ) were found to be 0.04µg/ml and 1.67 µg/ml respectively. This thus presents the technique to be linear within the range of analysis under consideration.

Table 4: Linearity parameters

Parameters	Albendazole
Linearity range (µg/ml)	(10.61 - 63.65)µg/ml
Regression coefficient	0.998
Intercept	10491
Slope	62866
LOD (µg/ml)	0.04
LOQ (µg/ml)	1.67

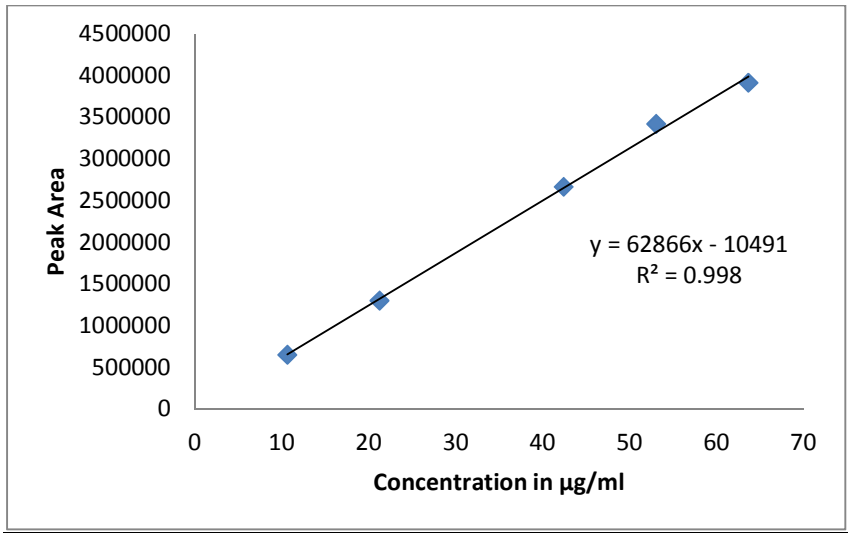

Fig. 3 Peak area verses concentration linearity curve

Precision:
The precision of the analytical method was determined on the basis of intra-day and inter-day precision studies. The experiments were repeated three times a day for intra-day precision and three consecutive days for inter-day precision. The %RSD with respect to the peak area, peak retention time and the amount were calculated for each case and the results were deputed in Table 5. The study presents a good agreement between the individual test results.

Table 5: Precision parameters

Parameters	Intra-day	% RSD	Inter-day			
			Day1	Day2	Day3	% RSD
Peak Area	2663803	0.10	2661997	2663941	2655147	0.002
Peak RT	8.908	0.00	8.891	8.897	8.889	0.73
Amount (mg/tab)	399.99	0.00	399.72	400.01	398.69	0.21

Accuracy:
The accuracy of the proposed method was determined on the basis of recovery study. The experiment was carried out on by added a known quantity of the standard into the sample solution and estimating the amount of the ANZ recovered with respect to the known amount of standard added to sample solution [20]. Sample solutions were prepared at three different concentration levels 80%, 110% and 120%. The percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table 6. The results present high accuracy of

the method within the limits of the studied parameters and can be used for the regular quantification of ANZ from different marketed formulations.

Table 6: Accuracy parameters (recovery study)

Formulation	Drug	Labeled Amt. (mg/tab)	Assay amount (mg/tab)	% label claim (n =3)	Recovery Studies (n = 3)				
					Total Amt. after spiking (mg)	Amt recovered (mg) Mean ± SD	% Recovery	% Mean Recover	% RSD
Nowarm; 400mg tablet;AlkemUlticare, Mumbai, India Batch no. 4133062 ; Mfg. 11/2014, Exp. 10/2017	ANZ	400.00	399.99	99.99	360 440 480	359.81±0.21 440.12± 0.11 479.99 ± 0.56	99.95 100.03 99.99	99.99	0.20

Sensitivity:

Sensitivity of the method was determined by calculating LOD (limit of detection) and LOQ (limit of quantification)using the equation: LOD = 3.3 σ/s and 10 σ /s where 'σ' was the standard deviation of response (y intercept) and 's' denotes the slope of the calibration curve. The results are found to be 0.04µg/ml and 1.67µg/ml, respectively (Table4).

Selectivity:

The selectivity of the method was determined on the basis of chromatographic purity by using PDA detector (Waters 2996 PDA; Waters USA) in place of UV detector. The peak purity angle was found to less than the peak purity threshold signifying the peak to be chromatographically pure and free from any interference from the sample matrix. More over the average recovery was 99.99% (Table 6), which presents an allowable interference of theexcepients with the analytepeak.

CONCLUSION

A rapid, accurate, precise and selective liquid chromatographic technique has been developed for the quantification of albendazole form marketed tablet formulation. The developed method is simple and cost effective for the quantification ofAlbendazole from bulk and marketed formulations in both small laboratories as a dual lambda UV-Vis detector was used and is also suitable for quantification purpose in pharmaceutical manufacturing units dealing with albendazole.

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