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Simultaneous UV Spectrophotometric Method for Estimation of Albendazole and Levamisole Hydrochloride in Tablet Dosage Form

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ABSTRACT

A simple and sensitive spectrophotometric method for the determination of albendazole and levamisole hydrochloride in pharmaceutical dosage forms has been developed. The absorption maxima were found at 295nm for albendazole and 213 nm for levamisole hydrochloride using ammonium dihydrogen phosphate: methanol as solvent. Beer's law was obeyed for both the drugs in the concentration range of 5-25 for albendazole with correlation coefficient 0.998 and 2-10 for levamisole hydrochloride with correlation coefficient 0.997. The limits of detection for albendazole and levamisole hydrochloride were found to be 0.305µg/mL and 0.732µg/mL respectively and the limits of quantitation were 0.924µg/mL and 2.2µg/mL respectively. Accuracy of the method verified by performing recovery using simultaneous equation method and found to be 98 to 99.5%w/w for albendazole and 97.97 to 99.55 %w/w for levamisole hydrochloride. %RSD of repeatability and intermediate precision studies were found to be <2 for both the drugs. Ruggedness of the method was checked by changing analyst and instrument used. In both the cases, the %RSD was found to be less than 2.

Keywords: Simultaneous, Spectrophotometry, albendazole, levamisole hydrochloride, Beer's law.

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INTRODUCTION

Albendazole is a broad spectrum anthelmintic. It is used for the treatment of threadworm, hookworm and tapeworm[1]. Chemically, Albendazole is Methyl 5-propylthio-1H-benzimidazol-2-ylcarbamate (British Pharmacopoeia, 2001)[2]. Albendazole is a benzimidazole which acts primarily through selective degeneration of parasite cytoplasmic microtubules, leading to decreased ATP formation and energy depletion. It also has antimitotic activity (binds to tubulin), disrupting cell division, and causes loss of trans- port of secretory vesicles alter glucose intake, leading to parasite starvation[3].

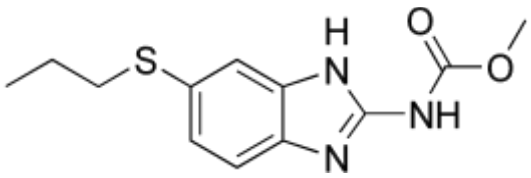

Figure 1: Chemical Structure of Albendazole

IUPAC name of Levamisole Hydrochloride is (6S)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-b] thiazole hydrochloride. Levamisole was originally used as an anthelmintic to treat worm infestations in both humans and animals. Levamisole works as a nicotinic acetylcholine receptor agonist that causes continued stimulation of the parasitic worm muscles, leading to paralysis.

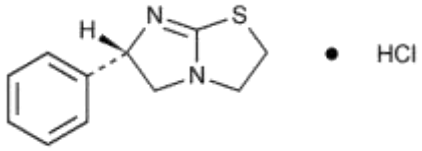

Figure 2: Chemical Structure of Levamisole Hydrochloride

Levamisole has been used to treat a variety of dermatologic conditions, including skin infections, leprosy, warts, lichen planus, and aphthous ulcers[4]. Literature survey reveals that some analytical methods are available for the determination of Albendazole and Levamisole Hydrochloride individually and in combination with other drugs, which include UV spectrophotometry[5-12], HPLC[13-23], RP-HPLC[24-30], LC-MS[31-33], GC[34], GLC[35] and UP-HPLC[36]. For the combination of Albendazole and Levamisole Hydrochloride Neither spectrophotometric nor HPLC methods have been developed. Hence, an attempt has been made to develop simultaneous UV method for its estimation in pharmaceutical dosage formulations with good accuracy, simplicity and sensitivity.

MATERIALS AND METHOD

Instrument

A Shimadzu UV-1800 UV/VIS Spectrophotometer was used with 1cm matches quartz cell.

Materials

Albendazole and Levamisole Hydrochloride were purchased from active product manufacturing industry. Tablets containing both drugs i.e. Albendazole and Levamisole Hydrochloride were purchased from local pharmacy of commercial brand Wormis-L.

Solvent used: Mixture of ammonium dihydrogen phosphate : methanol (30:70)

Preparation of Standard Solution

Accurately weigh and transfer about 25mg of Albendazole and 9.37mg of Levamisole Hydrochloride into 25 mL volumetric flasks. Dissolve and make up the volume with methanol (1000 and 374.8µg/mL). Take 2.5mL of above solution and it was transferred into a 25mL volumetric flask and diluted up to the mark with ammonium dihydrogen phosphate: methanol (30:70) (100 and 37.48µg/mL). Take 2.5mL of above solution and transfer into a 25mL volumetric flask and dilute up to the mark with ammonium dihydrogen phosphate :methanol (30:70) (10 and 3.748µg/mL).

Preparation of Sample Solutions

20 tablets were accurately weighed and average weight of tablets was determined. The tablets were crushed to fine powder using mortar and pestle. Accurately weighed and transferred 43.7mg of powder, equivalent to 25mg of Albendazole in 25 mL volumetric flask and diluted up to the mark with methanol (1000µg/mL). 2.5mL of the above solution was pipetted out into a 25 mL volumetric flask and diluted up to the mark with ammonium dihydrogen phosphate: methanol (30:70) (100µg/mL).

Preparation for Analysis of Tablet Formulation

Marketed tablet formulation (Wormis-L, Mfg in India by-AARON HEALTHCARE & EXPORT PVT. LTD.) containing 400mg of Albendazole and 150mg of Levamisole Hydrochloride was analyzed by this method. 20 tablets were accurately weighed and powdered and an amount equivalent to 25 mg was taken and dissolved in 25mL of methanol and necessary dilutions were made with ammonium dihydrogen phosphate: methanol (30:70) to give final concentration of 10 and 3.748µg/mL of Albendazole and Levamizole Hydrochloride. The concentration of each component was obtained by the spectral data of the sample solution with reference to that of the mixed standard.

Validation

The methods were validated with respect to accuracy, linearity, sensitivity and Limit of detection(LOD) and limit of quantification (LOQ). Validation of the proposed method was performed according to the ICH Q2 (B) guidelines.

Accuracy

The accuracy of the method was determined by recovery studies using standard addition method. The recovery study was performed to determine if there was any positive or negative interference from excipients present in the formulation. From 100µg/mL of standard stock of Albendazole 2, 2.5, 3 mL of Albendazole was taken and added to three 25 mL volumetric flasks which were labelled as 80%, 100%, 120%. To flasks were added 2, 2.5, 3 mL of Levamisole Hydrochloride from 100ug/mL. 2.5 mL of sample solution was taken and added to three flasks. The final volume was made up to 25 mL with ammonium dihydrogen phosphate: methanol (30:70) and absorbance of the above solutions was noted against blank. The concentrations of the above solutions were determined by substituting the absorbance values in simultaneous equation method.

$Cx= (A_1a_{y2}-A_2a_{y1}) / (a_{x1}a_{y2}-a_{x2}a_{y1})$

$Cy= (A_2a_{x1}-A_1a_{x2}) / (a_{x1}a_{y2}-a_{x2}a_{y1})$

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Albendazole and Levamisole Hydrochloride. For both the method, the Beer-Lambert's concentration rangr was found to be 5-25 µg/ml and 2-10 µg/ml for Albendazole and Levamisole hydrochloride respectively.

Sensitivity

High Molar absorptivity and low sandell's sensitivity for the respective method reveals that all these methods are highly sensitive.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of Albendazole and Levamisole hydrochloride were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines.

Precision

Repeatability

Six replicates of the 10ug/mL of Albendazole and Levamisole Hydrochloride were prepared and the absorbance was noted. The precision is reported in terms of %RSD.

Intermediate Precision

Intermediate precision expresses within laboratories variations i.e., different days, different analysts, different equipment's, etc. It was done by measuring absorbance of the same solution on the different day.

Robustness and Ruggedness

The sample solution was prepared and then analyzed with change in the typical analytical conditions like stability of analytical solution and for ruggedness, the same procedure was carried out by a different analyst.

RESULTS AND DISCUSSION

The wavelength of maximum absorbance was found to be at 295nm for Albendazole and at 212nm for Levamisole Hydrochloride. As a result, the wavelength was selected for quantitative analysis. The developed method was applied for estimation of Albendazole and Levamisole Hydrochloride in tablet formulation. Albendazole found to be linear in the concentration range of 5-25ug/mL and Levamisole Hydrochloride found to be linear in the concentration range of 2-10mL. The results obtained are shown in Table 6. Linear regression data is shown in Figure 4 and Figure 5. The correlation coefficients calculated from the calibration curves were 0.998 for Albendazole and 0.997 for Levamisole Hydrochloride. The limits of detection for Albendazole and Levamisole Hydrochloride were found to be 0.305µg/mL and 0.732µg/mL and the limits of quantitation were 0.924µg/mL and 2.2µg/mL respectively. Accuracy of the method was verified by performing recovery studies with solutions prepared by addition method. The percent recovery was found to be 98 to 99.5%w/w for Albendazole and 97.97 to 99.55 %w/w for Levamisole Hydrochloride, which indicates the accuracy of the method (Table 2). %RSD of repeatability and intermediate precision studies were found to be <2 for both the drugs, which indicates that the method was precise (Table 3). Ruggedness of the method was checked by changing the analyst worked and also the instrument used. In both the cases, the %RSD was found to be <2(Table 4). Marketed formulation was analyzed with the proposed method and the percentage purity of Albendazole was found to be 98.87%w/w and for Levamisole Hydrochloride, it was found to be 98.57%w/w (Table 6).

Table 1: Linearity Data of Albendazole and Levamisole Hydrochloride

Sr No	Concentration (µg/ml)	Absorbance of Albendazole (at 295nm)	Concentration (µg/ml)	Absorbance of Levamisole Hydrochloride (at 213nm)
1	5	0.2040	2	0.1703
2	10	0.3999	4	0.3375
3	15	0.5963	6	0.4832
4	20	0.7860	8	0.6142
5	25	0.9743	10	0.8089

Table 2: Recovery Study Data of Albendazole and Levamisole Hydrochloride

Drug Substance (mg)	Percent recovery level (%)	Amount added (µg/ml)		Amount found (µg/ml)	Percent recovery (%)
		Test	standard		
Albendazole 400	80	10	8	17.84	98%
	100	10	10	19.9	99%
	120	10	12	21.94	99.5%
Levamisole Hydrochloride 150	80	3.75	3	6.689	97.97%
	100	3.75	3.75	7.49	99.97%
	120	3.75	4	8.23	99.55%

Table 3: Statistical Data of Repeatability for Albendazole and Levamisole Hydrochloride

Concentration (µg/ml)	Absorbance of Albendazole (at 295nm)		Absorbance of Levamisole Hydrochloride (at 213nm)	
	Intraday	**Interday**	**Intraday**	**Interday**
10	0.3999	0.3988	0.8089	0.8154
	0.3980	0.3959	0.8000	0.8349
	0.3989	0.3899	0.8145	0.8077
	0.4000	0.3950	0.8005	0.8009
	0.3950	0.4000	0.8122	0.8265
	0.3900	0.3990	0.8079	0.8409
Mean*	0.3969	0.3964	0.8073	0.8211
SD**	0.003873	0.003739	0.005971	0.01569
%RSD***	0.98	0.94	0.74	1.09

*mean of six determinations, **standard deviation, ***relative standard deviation

Table 4: Data of Robustness of Albendazole and Levamisole Hydrochloride

Time (min)	Absorbance	
	Albendazole (at 295nm) (10 µg/ml)	**Levamisole Hydrochloride (at 213nm) (10 µg/ml)**
0	0.3950	0.8005
15	0.3980	0.8349
30	0.3988	0.8000
45	0.3899	0.8077
60	0.3950	0.8009
75	0.4000	0.8154
Mean*	0.3961	0.8099
SD**	0.00366	0.01362
%RSD***	0.92%	1.6%

*mean of six determinations, **standard deviation, ***relative standard deviation

Table 5: Data of Ruggedness of Albendazole and Levamisole Hydrochloride

Concentration (µg/ml)	Absorbance of Albendazole				Absorbance of Levamisole Hydrochloride			
	Instrument (1)	**Instrument (2)**	**Analyst (1)**	**Analyst (2)**	**Instrument (1)**	**Instrument (2)**	**Analyst (1)**	**Analyst (2)**

10	0.3999	0.3988	0.3999	0.3950	0.8089	0.8154	0.8145	0.8265
	0.3980	0.3959	0.4000	0.3999	0.8000	0.8349	0.8089	0.8089
	0.3989	0.3899	0.3959	0.4000	0.8145	0.8077	0.8122	0.8079
	0.4000	0.3950	0.3988	0.3990	0.8005	0.8009	0.8349	0.8349
	0.3950	0.4000	0.3999	0.3950	0.8122	0.8265	0.8079	0.8265
	0.3900	0.3990	0.3999	0.3999	0.8079	0.8409	0.8154	0.8089
Mean*	0.3969	0.3964	0.3964	0.3981	0.8073	0.8212	0.8156	0.8189
SD**	0.00387	0.00374	0.0037	0.0025	0.0059	0.016	0.0099	0.012
%RSD***	0.98	0.94	0.40	0.62	0.74	1.90	1.2	1.44

*mean of six determinations, **standard deviation, ***relative standard deviation

Table 6: Assay of Albendazole and Levamisole Hydrochloride

Drug Substance	Label Claim (mg)		Percentage Purity (%w/w)
	Taken	Found	
Albendazole	400	396	99%
Levamisole Hydrochloride	150	148.6	99.05%

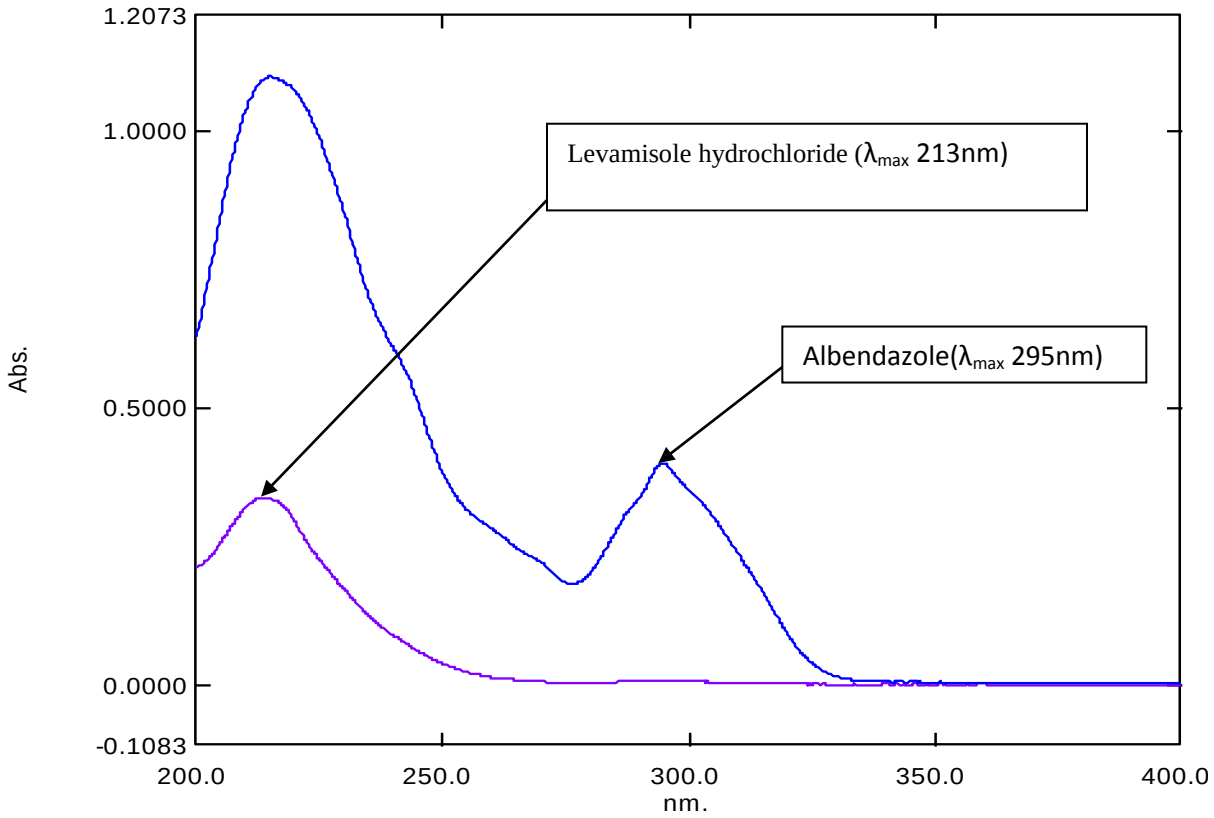

Figure 3: Overlain spectra of Albendazole and Levamisole Hydrochloride in ammonium dihydrogen phosphate: methanol (30:70) solvent.

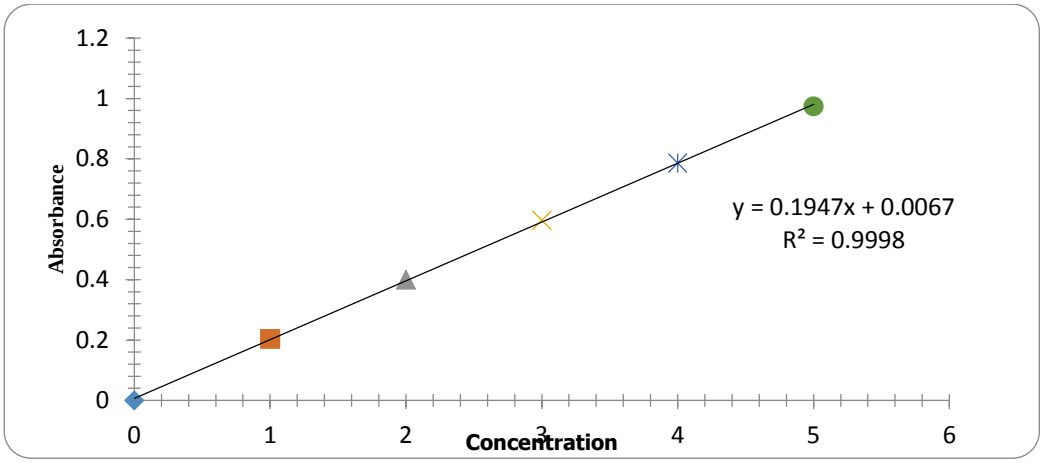

Figure 4: Linearity graph of Albendazole

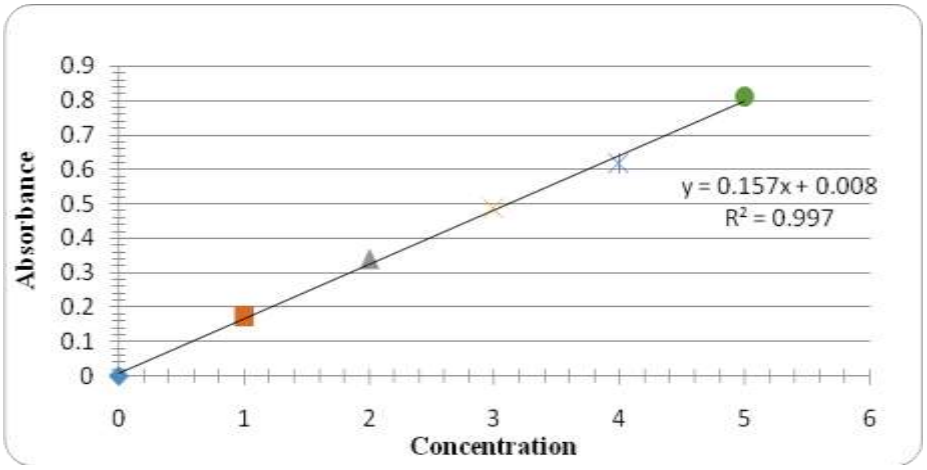

Figure 5: Linearity Graph of Levamisole Hydrochloride

CONCLUSION

In the present investigation a simple, sensitive and economical analytical method was developed for the assay of the Albendazole and Levamisole Hydrochloride by UV spectrophotometry. The results of analysis of tablet formulation and recovery studies obtained by spectrophotometric method were statistically validated and the high percentage of recovery studies suggest that the developed method was free from interference of excipients generally used in tablet formulations. The developed method was statistically validated in terms of accuracy, precision, linearity and reproducibility. Hence, above method can be employed in quality control to estimate the amount of Albendazole and Levamisole Hydrochloride in commercial formulations.

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