DEVELOPMENT AND VALIDATION OF DERIVATIVE SPECTROSCOPIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF MEBENDAZOLE AND LEVAMISOLE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, novel, sensitive and precise validated spectrophotometric method was developed for simultaneous determination of Mebendazole (MBZ) and Levamisole Hydrochloride (LVM) in its tablet formulation. 1% H_2SO_4 in methanol was selected as a common solvent for estimation of MBZ and LVM. For first order derivative method, estimation of MBZ was carried out at 307nm (ZCP of LVM) and of LVM at 232.6nm (ZCP of MBZ). The linearity was obtained in the concentration ranges of 2-6 µg/mL for MBZ and 3-9 µg/mL for LVM with correlation coefficient(r^2) value greater than 0.995 . The % RSD value for intraday & interday precision were less than 2. The detection limit and quantification limit were found to be 0.44 µg/mL and 1.34 µg/mL for MBZ and 0.14 µg/mL and 0.42 µg/mL for LVM, respectively. All the validation parameters were performed as per the ICHQ2 (R1) guidelines. The recovery study was carried out, results were 98.82 – 101.93 % for MBZ and 100.05-101.42% for LVM. So, the developed method could be applied for the routine quality control analysis of MBZ and LVM in combined tablet formulation.

Keywords: *Mebendazole, Levamisole Hydrochloride, First Order Derivatives, ICH guideline, Validation*

INTRODUCTION

Mebendazole is methyl (5-benzoyl-1H-benzimidazol-2-yl) carbamate.It is soluble in formic acid [Figure 1(a)]. Levamisole Hydrochloride(LVM) is (S)-6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b] thiazole hydrochloride [Figure 1(b)]. It is soluble in methanol, ethanol and water. MBZ and LVM are official in Indian Pharmacopoeia[1], British Pharmacopoeia[2], European Pharmacopoeia[3] and United States Pharmacopoeia[4]. Both drugs are used as anthelmintic[5].

Literature review revealed that various analytical methods like Spectrophotometry[6-7], Spectrofluorimetry[8], HPLC[9-13] and LC-UV/LC-MS/MS[14-17] have been reported for estimation of MBZ and LVM either individually or in combination with other drugs. However, derivative spectrophotometric method has not been reported for the simultaneous estimation of MBZ and LVM in their combined dosage formulation. Hence, it was proposed to develop simple, rapid, accurate and precise derivative UV-visible spectrophotometric method for simultaneous estimation of MBZ and LVM in their marketed formulation.

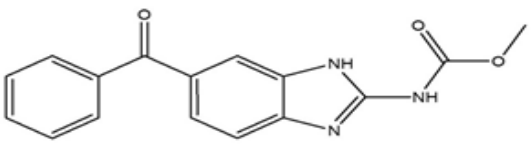

Figure 1 (a): Structure of Mebendazole

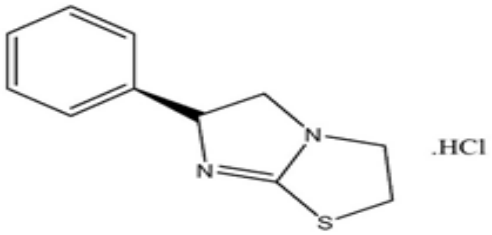

Figure 1 (b): Structure of Levamisole Hydrochloride

MATERIALS AND METHODS:

Apparatus:

Shimadzu model 1800 double beam UV–visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells was used to measure absorbance. The instrument was equipped with Shimadzu UV-Probe 2.34 version software. Shimadzu AUX 220 analytical balance was used for weighing.

Reagents and chemicals:

The MBZ reference standard was gifted by Baroque Pharmaceuticals Pvt. Ltd. (Cambay, Gujarat - India) and LVM reference standard was received as a gift sample from Shree Pharma (Gujarat, India).The commercial fixed dose formulation containing 100 mg MBZ and 150 mg of LVM, RAID Tablet was procured from the local market. Analytical grade Methanol and H_2SO_4 were purchased from Loba Chemicals.

Preparation of Stock Solution and working standard solutions

Accurately weighed 10mg each of MBZ and LVM were transferred to two different 10 mL volumetric flasks. The volume was made up to the mark with 1 % v/v H_2SO_4 in methanol to obtain stock solutions of MBZ and LVM having concentration 1000 µg/mL of each.

From the above stock solutions, aliquots of 1 mL were pipetted out and placed into two different 10mL volumetric flasks. The volume was made up to mark with 1 % v/v H_2SO_4 in methanol to give solutions containing 100 µg/mL of MBZ and LVM.

Preparation of Binary mixture stock solution and working standard solutions:

Binary mixture which contains standard MBZ and LVM was prepared by transferring 10 mg MBZ and 15 mg LVM to a 10 mL volumetric flask,the volume was made up to the mark with 1 % v/v H_2SO_4 in methanol. It produced solution containing 1000 µg/mL MBZ and 1500 µg/mL LVM.

Working solution containing 100 µg/mL MBZ and 150 µg/mL LVM was prepared by transferring 1 mL of binary mixture stock solution to 10mL of volumetric flask and the volume was made up to the mark with 1 % v/v H_2SO_4 in methanol.

First order derivative Method[18-19]

The absorption spectra of working binary mixtures of 3 µg/mL of MBZ and 4.5µg/mL of LVM were scanned in range of 200-400 nm. Using the UV probe software the spectra were transformed to 1st order derivative spectra with smoothing factor (Δλ) =8 and multiplying the entire spectra by a constant scaling factor 1 to obtain zero crossing points (ZCP) for simultaneous estimation of MBZ and LVM (Figure 2). The ZCP for MBZ and LVM were obtained at 232.6 nm and 307 nm, respectively. So, estimation of MBZ is carried out by measuring amplitudes at 232.6 nm and for LVM at 307 nm. Optimized parameters for first order derivative method is reported in Table 1. Aliquots of working binary mixture solution of MBZ and LVM (0.2,0.3,0.4,0.5 and 0.6) were pipetted out in 10mL volumetric flask and further diluted to attain concentration of about 2,3,4,5,6 µg/mL concentration for MBZ and 3,4.5,6,7.5,9 µg/mL concentrations for LVM. The absorbance of resulting solutions were measured at 232.6 nm (ZCP of MBZ) and 307 nm (ZCP of LVM). The graph of absorbance vs concentration was plotted at each wavelength and regression coefficients (r^2) were calculated (Figure 3).

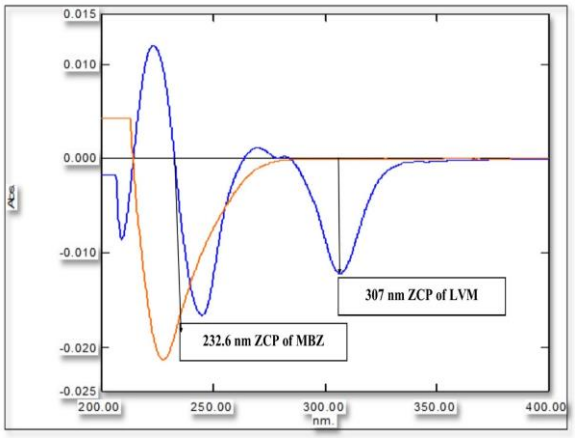

Figure 2: First order derivative spectra of MBZ and LVM for selection of analytical wavelength

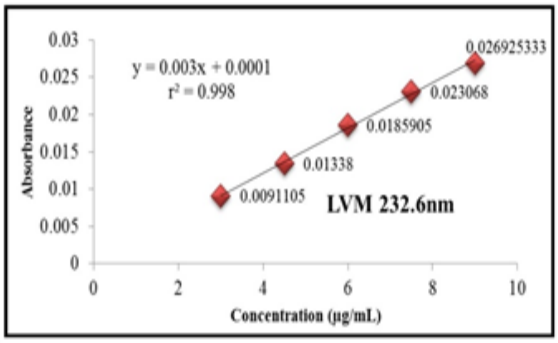

Figure 3(b): Calibration Curve of MBZ at 232.6nm

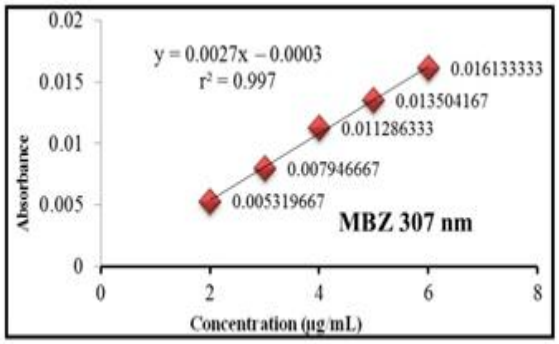

Figure 3(a): Calibration Curve of MBZ at 307 nm

VALIDATION OF DEVELOPED METHODS

The proposed methods have been validated in terms of linearity, accuracy, precision, repeatability and reproducibility, limit of detection (LOD) and limit of quantification (LOQ) as per ICH Q2(R1) guidelines.[20]

Linearity:

Linearity was observed in the range of 2-6 µg/mL for MBZ and 3-9 µg/mL for LVM, respectively with r^2 value of greater than 0.995.

Precision:

Variation of results within the same day (Intraday) and between days (interday) were analyzed. The Intraday and Interday precision was determined by analyzing three different concentrations of MBZ (2, 4 and 6 µg/mL) and LVM (3, 6 and 9 µg/mL), obtained by dilution from stock solutions for three times in a day (Intraday) and for three consecutive days (Interday). The low %RSD value suggests that the developed method is precise. The results are reported in Table 4

Accuracy (Recovery):

The accuracy of the developed methods was determined by calculating % recovery at three different levels (80%, 100% and 120%) in pre analyzed samples using standard addition method. The results of recovery studies are reported in Table 2. The % recovery for MBZ and LVM are within 98.82% - 101.93%, assuring that the both the developed method can estimate the drugs successfully in presence of excipients.

LOD and LOQ:

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined using mathematical equations. The results are reported in Table 4.

LOD = 3.3 X σ/S and LOQ = 10 X σ/S

Where, σ = Standard deviation of the response

S = Slope of the calibration curve.

Analysis of marketed formulation:

20 tablets were weighed and powdered. A quantity of powder equivalent to 10 mg of MBZ and 15 mg of LVM was weighed and transferred into 10 mL volumetric flask .The volume was made by using 1 % v/v H_2SO_4 in methanol. Solution was sonicated for 30 minutes and resulting solution was filtered. The filtered solution (1 mL) was transferred to 10 mL volumetric flask and diluted with 1 % v/v methanolic H_2SO_4. From this, 1 mL solution was taken and diluted to 10 mL with 1 % v/v methanolic H_2SO_4 to get a solution containing 10 µg/mL MBZ and 15 µg/mL of LVM. The result is reported in Table 3.

RESULTS AND DISCUSSION

The standard solutions of MBZ and LVM were scanned separately in the UV range and First-order spectra for MBZ and LVM were recorded. The first order derivative absorption at 232.6 nm (zero cross point for MBZ) was used for LVM and 307 nm (zero cross point for LVM) was used for MBZ shown in (Figure 2). These two wavelengths can be employed for the determination of MBZ and LVM without any interference from the other drug in their combined dosage form.

Standard calibration curves for MBZ and LVM were linear with Correlation coefficients (r^2) values in the range of 0.997 and 0.998, respectively (Figure 3) at all the selected wavelengths and the values were average of five readings and statistical data is shown in (Table 4).

The developed method was found to be precise as the %RSD values for the intermediate precision studies were < 2 %. (Table 4)

Accuracy of the proposed method was ascertained by recovery studies and the results were expressed as percent recovery and were found in the range of 98.82% – 101.93 % %.Values of standard deviation and coefficient of variance was satisfactorily low indicating the accuracy of the method. (Table 2)

The influence of excipients was studied by mixing two drugs with excipients as per the ratio. LOD and LOQ were found to be 0.44 µg/mL and 1.34 µg/mL for MBZ and 0.14 µg/mL and 0.42 µg/mL for LVM. Table 4 shows the summary of all validation parameters.

The assay results of tablet formulation are shown in Table 3, which show good agreement with the labelled claim. So it proved that no interference was observed from the presence of excipients in the amounts, which are commonly present in tablet formulation.

Table 1: Optimized parameters for first order derivative spectroscopic method

Sr. no	Method Parameters	Optimized parameters
1	Scanning range	200-400 nm
2	Scan speed	Fast
3	Scaling factor for MBZ and LVM	1
4	Smoothing factor for MBZ and LVM	8
5	Analytical wavelength for Determination of MBZ	307 nm
6	Analytical wavelength for Determination of LVM	232.6 nm

Table 2: Accuracy data of MBZ and LVM (n=3)

Levels (n=3)	Target sample solution (µg/mL)	Amt. of std. Added (µg/mL)	Average amount recovered ± SD (µg/mL)	% Recovery	%RSD
MBZ					
80	2	1.6	3.59± 0.0009	99.89	1.03
100		2	4.07 ± 0.0007	101.93	0.66
120		2.4	4.34 ± 0.0005	98.82	0.42
LVM					
80	3	2.4	5.42 ± 0.0001	100.31	0.94
100		3	6.03 ± 0.0004	100.05	0.46
120		3.6	6.69 ± 0.0002	101.42	0.32

Table 3: Assay of synthetic mixture of MBZ and LVM (n=6)

Actual content (mg)		Content Estimated ± SD (mg)		% Assay		%RSD	
MBZ	LVM	MBZ	LVM	MBZ	LVM	MBZ	LVM
10	15	9.99 ± 0.0008	15.3 ± 0.0001	99.67	102.29	1.01	1.28

Table 4: Summary of validation parameters

Parameters	MBZ	LVM
Analytical Wavelength	307 nm	232.6 nm
Linearity (µg/mL) (n=6)	2-6	3-9
Regression equation(n=6)	y = 0.0027x – 0.0003	y = 0.003x + 0.0001
Correlation co-efficient (r^2)	0.997	0.998
% Recovery (n=3)	98.82-101.93%	100.05-101.42%
Intraday precision (%RSD, n=3)	0.12-0.79	0.31-0.74
Interday precision (%RSD, n=3)	0.28-0.74	0.68-0.76
LOD (µg/mL)	0.43	0.13
LOQ (µg/mL)	1.33	0.41

CONCLUSION

A simple, accurate and precise first order derivative UV spectrophotometric method was developed for quantitative determination of MBZ and LVM in combined tablet formulation. The results of validation parameters revealed that the proposed method could be successfully applied for the routine quality control analysis of tablets containing Mebendazole and Levamisole hydrochloride.

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