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UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF LEVOCETIRIZINE AND IVERMECTIN IN BULK AND COMBINED DOSAGE FORM

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Abstract

Two simple, rapid, precise and reproducible UV spectroscopic methods has been developed for simultaneous estimation of two component drug mixture of Levocetirizine(LEVEC) and Ivermectin(IMEC) in bulk and combined tablet dosage form. The method employs the application of simultaneous equation. All these methods utilize 1:1 of acetonitrile and water as a solvent. LEVEC shows maximum absorbance at a wavelength of 230 nm and IMEC at 245 nm, where the linearity ranges for LEVEC and IMEC were 1.0-6.0 µg/ml and 1.2-7.2 µg/ml, respectively. The procedures were successfully applied for the simultaneous determination of both the drugs in laboratory prepared mixtures and in tablet preparation. The accuracy of the methods was found to be ranging from 97.75-100.9% for LEVEC and 98.85-99.43% for IMEC respectively, the relative standard deviation was found to be 0.5974 and 0.4096 with excellent precision and accuracy. Assay results were in good agreement with label claim.

Keywords: Simultaneous determination, Levocetirizine, Ivermectin.

Introduction

LEVEC, chemically is [2-[4- [(r)-(4-chlorophenyl) phenylmethyl]-1- piperazinyl] ethoxy] acetic acid is a third generation non-sedative antihistamine, developed from the second generation antihistamine cetirizine. It is the L-enantiomer of the cetirizine racemate. LEVEC works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents it binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area and provides relief from the typical symptoms of hay fever (Grant *et al.,* 2002). IMEC chemically (22,23-dihydroavermectin B_{1a} + 22,23-dihydroavermectin B_{1b}) is produced by fermentation of actinomycete Streptomyces avermiltilis[1-2]. IMEC has broad spectrum activity against arthropod parasites located in the different layers of skin and nematode parasites located in gastrointestinal and pulmonary tracts[2-3]. It exerts its action by opening γ-aminobutyric acid (GABA) channel-I and thus widely employed in the treatment of scabies, ascariasis, onchocerciasis, trichuriasis

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strongyloidiasis, and enterobiasis[2-4]. Literature review reveals that some analytical methods have been reported for LEVEC alone and in biological fluids or in combination with other drugs in pharmaceutical dosage forms[5]. High-performance Liquid chromatography[6,7], liquid chromatography with electrospray ionization mass spectrometry[8,9] for determination of IMEC as a bulk drug and in dosage forms, biological fluids as well as in human and animal body tissues has been reported in the literature. However, no published analytical technique focuses on simultaneous estimation of LEVEC and IMEC. The present work aimed at developing a simple, sensitive, accurate, and precise UV simultaneous method for routine analysis. The proposed method was validated according to ICH guidelines (ICH, 2005).

Materials and Methods

Instrumentation

A dual-beam Shimadzu UV-visible spectro - photometer 1700 Pharmaspec was used.

Reagents and Chemicals

Gift samples of LEVC and IMEC were procured from CHANDRA LABS pvt ltd. Kukatpally, Hyderabad. According to the solubility characteristics, the common solvent for the two drugs was found to be 1:1 of acetonitrile and water.

Preparation of standard stock solution

Standard stock (100µg/ml) of LEVEC and (100 µg/ ml) of IMEC were prepared in 1:1 of acetonitrile and water. The aliquot portions (1.0, 2.0, 3.0, 4.0, 5.0, 6.0) from the 100 µg/ ml LEVC and (1.2, 2.4, 3.6, 4.8, 6.0, 7.2) from the 100µg/ml working IMEC solutions were accurately transferred to 10ml volumetric flasks, the volume was completed with distilled water The absorption spectra between 200-400 nm of all solutions of LEVC and IMEC were measured at 230 nm (λ_max for LEVC), 245 nm (λmax for IMEC).

Simultaneous Equation Method

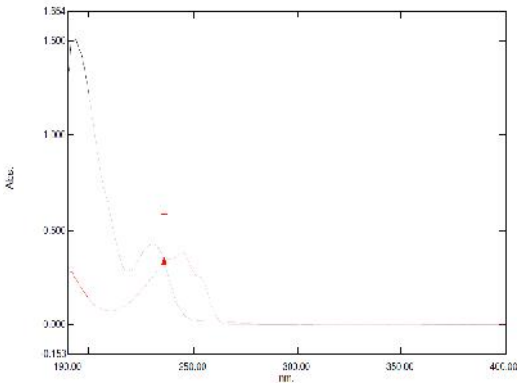

Fig. No. 01:
Overlain spectra of LEVEC & IMEC

Table No. 01: Value from Overlain spectra of LEVEC & IMEC

S.No	Wavelength (nm)	Absorbance
1.	236.00	0.332

From the overlain spectra of LEVEC(1µg/ml) and IMEC (1.2µg/ml) in 1:1 of acetonitrile and distilled water, wavelengths 230nm (λmax of LEVC) and 245nm (λmax of IMEC) were selected for the formation of Simultaneous equation method. From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 1 – 6.0 µg/ml of LEVC and 1.2-7.2 µg/ml of IMEC. Absorbances of these solutions were recorded in the respective wavelengths. Both the drugs were linear in the concentration range of 1–6.0µg/ml of LEVC and 1.2-7.2µg/ml of IMEC and Calibration curves [n=6] were plotted between concentration and absorbances of drugs with

correlation coefficient value not less than 0.999. E (1%, 1cm) is determined for Levocetirizin at 230nm and 245nm were 0.182 and 0.06382 while respective values for IMEC are 0.2446 and 0.225. These values are the mean of six independent determinations.

The simultaneous equations formed were,

At λ_1 $A_1 = a\,x_1 bc_x + ay_1 bc_y$ ------ (1)

$A1 = 0.182C_X + 0.06382C_Y$ -----(2)

At λ $A_2 = a\,x_2 bc_x + ay_2 bc_y$ ------- (3)

$A2 = 0.2446C_X + 0.225C_Y$ ------ (4)

$$Cx = \frac{A_2\, a_{y1} - A_2\, a_{y2}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

Where A_1 and A_2 are the absorbances of sample solution at 230nm and 245nm respectively. Cx and C_Y are the concentration of LEVEC and IMEC respectively (µg/ml) in sample solution. The absorbances [$A_{1\&}$ A_2] of the sample solution were recorded at 230 and 245nm respectively and concentration of both the drugs were calculated using above mentioned equation (2 &4).

Analysis of tablet formulation

Twenty tablets were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 5 mg of LEVEC was transferred in to 100ml volumetric flask, sufficient distilled water was added and the solution was sonicated for 15 minutes and diluted to the mark with distilled water. It was filtered through Whatmann filter paper no: 41, filtrate was suitably diluted to get final concentration of 1µg/ml of LEVEC and 1.2µg/ml of IMEC with distilled water. The absorbance of sample solution was measured at all selected wavelengths. The content of LEVEC and IMEC in sample solution of tablet was calculated. This procedure was repeated for six times.

Table No. 02:

Parameters of tablet formulation

Parameters	LEVC	IMEC
Labeled claim (mg)	5mg	6mg
% Assay*	100.13%	99.88%
SD	0.003312	0.002582
%RSD	0.49	0.31

***=average of 6 determinations**

Results

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation and it was repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % Relative standard deviation (RSD) were calculated which is less than 2%.

Table No. 03: Precision Data's

Drug	Intra day Precision		Inter-day Precision	
	S.D*	% RSD	S.D*	% RSD
LEVEC	0.003312	0.49	0.003311	0.48
IMEC	0.002582	0.31	0.002583	0.31

***= average of six determinations**

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at 100% level at 3 different standard concentrations. The recovery samples were prepared in before mentioned procedure three different concentrations of the samples were prepared for each recovery level. The solutions were then analyzed and the results of recovery studies were found to be satisfactory and the results are presented in table.

Table No. 04: Accuracy Data's

S.no	% spike	Amount recovered	% recovery
		LEVEC	
1.	50%	48.87*	97.75
2.	100%	99.55*	99.55
3.	150%	151.34*	100.9
		IMEC	
1.	50%	49.43*	98.85
2.	100%	98.85*	98.85
3.	150%	149.14*	99.43

***= average of six determinations**

Linearity

The linearity of the response of the drugs was verified at 0-100ug/ml concentrations, the calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis(table no.) The equation of the calibration curve for LEVEC and IMEC obtained Y=0.016x+0.011 and Y=0.042x + 0.016, the calibration curve were found to be linear in therefore mentioned concentrations. The correlation co-efficients (r^2) for LEVEC and IMEC were determined by 0.9991 and 0.999.

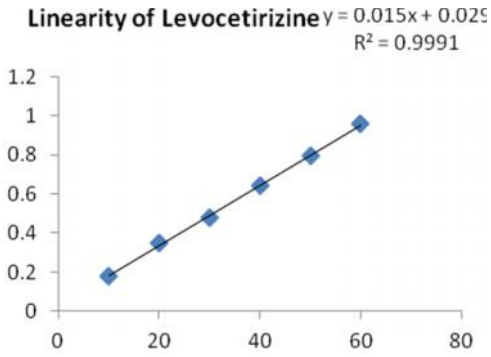

Fig. No. 02

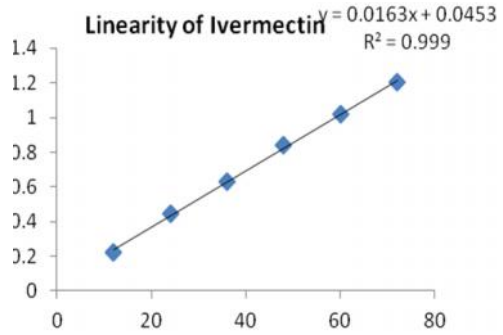

Fig. No. 03

Table No. 05

S.No	Parameter	LEVEC	IMEC
1.	Linearity Range(µg/ml)	1-6	1.2-7.2
2.	Slope(m)	0.015	0.029
3.	Intercept	0.029	0.045
4.	Correlation Co-efficient(R^2)	0.9991	0.999

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

The LOD and LOQ of the LEVEC and IMEC were determined by using standard deviation of the response and slope approach as defined in ICH guidelines. The LOD and LOQ was found to be as in table.

Table No. 06

Drug	LOD	LOQ
LEVEC	0.7286	0.5325
IMEC	2.2742	1.6621

Discussion

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 1-6 µg/ml and 1.2-7.5 µg/ml for LEVEC and IMEC, respectively with co-efficient of correlation, (r2)=0.9991 and (r2) = 0.999 for LEVEC and IMEC, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. This method can be adopted as an alternative to the existing methods. Analysis of authentic samples containing LEVEC and IMEC showed no interference from the common additives and excipients. The method can be used for the routine analysis of the LEVEC and IMEC in combined dosage form without any interference of excipients.

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