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DEVELOPMENT AND VALIDATION OF TWO UV SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF ILAPRAZOLE, AND LEVOSULPIRIDE IN TABLET DOSAGE FORM

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ABSTRACT

Two simple, rapid and precise UV spectroscopic methods namely First order Derivative (Method I), and Ratio first order derivative (Method II) have been developed for the simultaneous determination of Ilaprazole (ILA) and Levosulpiride (LEVO). The first order derivative absorption at 305.51 nm (zero cross point of Ilaprazole) was used for quantification of Levosulpiride and 321.88 nm (zero cross point of Levosulpiride) for quantification of Ilaprazole, linear concentration range for ILA and LEVO was 2-12 µg/ml and 15-90 µg/ml with regression coefficient 0.9994 and 0.9990 respectively. The second method is based on ratio first order derivative spectrophotometry, at 310.70 nm and at 277.73 nm, with regression coefficient 0.9992 and 0.9997 respectively. The proposed methods were validated according to ICH guidelines for evaluation of accuracy, precision, sensitivity etc. The obtained relative standard deviation i.e. less than 2% demonstrated that proposed methods were accurate and precise and can be employed for routine analysis in quality control laboratories eliminating the need of prior separation of the pharmaceutical mixtures.

KEYWORDS: Ilaprazole, Levosulpiride, First order derivative method, Ratio first order derivative method, validation.

1. INTRODUCTION

Ilaprazole [ILA];- 2[(4-methoxy-3-methyl-2-pyridinyl)-methyl]sulfinyl]-5(1H-pyrrol-1-yl) 1H-Benzimidazole (Fig. 1) is a the latest generation of proton pump inhibitor It belongs to class of antisecretory compound and widely used in various acid related gastrointestinal diseases. It is a new candidate drugs that suppresses gastric acid secretion by specific inhibition of the H^+/K^+-ATPase in the Gastric Parietal Cell. This enzyme system is regarded as the acid i.e. proton pump within the parietal cell, Ilaprazole has been characterize as gastric acid pump inhibitor, in that it blocks the final step of acid production.

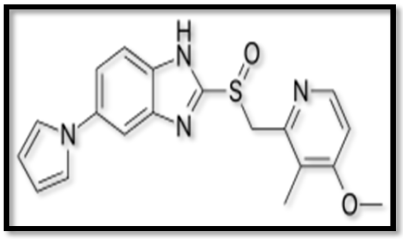

Fig. 1: Chemical Structure of Ilaprazole.

Levosulpiride [LEVO];- (1)-N-[[(S)-1-Etyl-2-pyrrolidinyl]methyl]-5sulfamoyl Benzamide is the levo enantiomer of sulpiride (Fig. 2) It is a substituted benzamide which is used for several indications like depression, psychosis, somatoform disorders, emesis and dyspepsia. It is a substituted benzamide antipsychotic, reported to be a selective antagonist of dopamine D2 receptor activity on both central and peripheral levels. It acts by inhibiting the receptor (D2 and D3) found in the brain and subsequently decreasing the level of chemical compound (dopamine) present in the brain.

Scientific literature reports that there are many methods reported for the determination of ILA individually and in combination with other drugs like Domperidone based on reversed-phase HPLC method.[12,13,14] For the determination of LEVO either alone or in combination with other drugs several analytical methods were reported includes UV spectroscopic method, HPLC, HPTLC, UPLC. ILA is official in IP. To the best of our knowledge, no simple, rapid, spectrophotometric method has been reported for the simultaneous estimation of ILA and LEVO in marketed formulation. Therefore, the goal of present work is to develop a simple procedure that could be applied in quality control laboratories for the simultaneous determination of both drugs. Hence, This work aims to present simple, accurate and precise First order derivative and ratio-derivative spectrophotometric methods for the simultaneous determination of ILA and LEVO in solid dosage form.

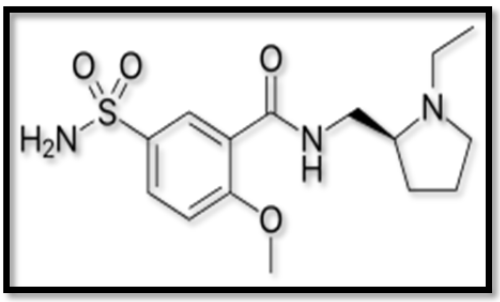

Fig. 2: ChemicalStructure of Levosulpiride.

2. MATERIALS AND METHODS

2.1 Instruments and software

A Shimadzu double beam UV-Visible Spectrophotometer (UV-1800 Shimadzu Corporation, Kyoto, Japan)was used for all absorbance measurements with a quartz cell of 1 cm path length and fixed slit width 2 nm.UV probe software version 2.34 (Shimadzu 1800, Japan); Electronic analytical balance (AUX-220, ShimadzuCorp., Japan) were used in the study.Calculations were performed with the help of Microsoft Excel 2010 software (Microsoft Corporation, USA).

2.2. Chemicals and materials

Analytical pure samples of ILA and LEVO were obtained as a gift samples from ZydusCadila, Ahmedabad and Precise Pharmacare, Ankleshwar. These samples were used without further purification. tablet dosage form "Blokcid-L" manufactured by Ipca Laboratories Ltd. Mumbai, was purchased from the local market containing ILA (10 mg) and LEVO (75 mg). Analytical grade methanol purchased from Merck, Mumbai was used throughout the study.

Drugs/Chemicals/Reagents	Manufacturer/ Supplier
Ilaprazole	Zydus Cadila Private Limited, Ahmedabad
Levosulpiride	Pure Chem. Private Limited, Ankleshwar, Gujrat
Methanol (AR Grade)	Merck Specialties Private Limited, Mumbai
Blokcid-L	Ipca Laboratories Ltd. Mumbai

2.3 Preparation of solutions

2.3.1 Preparation of standard solutions

A stock solution of 1000 µg/ml of ILA, and LEVO were prepared by dissolving accurately weighed 10 mg of stated drugs into 10 ml volumetric flask with methanol separately.The working standard solutions (100 µg/ml) of mentioned drugs were obtained by dilution of the respective stock solution in methanol. For verification of Beer's law, a series of dilutions in

the concentration range of 2-12 µg/ml for ILA and 15-90 µg/ml for LEVO were prepared separately to establish calibration curve.

2.3.2 First order derivative method (Method I)

2-12 µg/ml solutions of ILA and 15 - 90 µg/ml solutions of LEVO were prepared in methanol by appropriate dilution and spectrum was recorded between 200-400 nm, All zero order spectrums (D^0) were converted to first derivative spectrum (D^1) using delta lambda 2.0 and scaling factor 10.0. The overlain first derivative spectra of ILA and LEVO at mentioned concentration were recorded figure 3 (a) and (b). The zero crossing point (ZCP) of ILA was found to be 305.51 nm as wavelength maxima(λmax)and ZCP of LEVO was found to be 321.88 nm as wavelength minima (λmin) was selected for the simultaneous determination of ILA and LEVO in marketed tablet dosage form, respectively.

2.3.3 Ratio first order derivative method (Method II)

The method involves dividing the spectrum of formulation by the standardized spectra of each of the analyte and deriving the ratio to obtain spectrum that is dependent of concentration of analyte used as a divisor. Using appropriate dilutions of standard stock solution, the standard solutions of ILA (2-12 µg/ml) and LEVO (15-90 µg/ml) were prepared and their zero order spectra recorded over the range 200-400 nm using methanol as blank. The ratio spectra of different ILA standards at increasing concentrations were obtained by dividing each with the stored zero order spectrum of standard solution of LEVO (45 µg/ml) and the first derivative of these spectra traced with the interval of Δλ= 2 nm, illustrated in figure 4(c). Similarly, the ratio derivative spectra of the solutions of LEVO at different concentrations were obtained by dividing each with the stored zero order spectrum of standard solution of ILA (4 µg/ml) and the first derivative of these spectra traced with the interval of Δλ= 2 nm, illustrated in figure.4.c and figure.4.f From figure.4.b and figure.4.e, 310.70 nm and 277.73 nm as wavelength maxima (λmax) was selected for the simultaneous determination of ILA and LEVO in marketed solid dosage form, respectively.

2.4 Validation of methods

The proposed methods were validated with respect to linearity, sensitivity, accuracy and precision in accordance with ICH Q2 (R1) guideline.[9]

2.4.1 Linearity

The linear relationship between absorbance and concentration were evaluated over the concentration range of ILA (4-12µg/ml), and LEVO (15-90µg/ml) for both methods by making five replicate measurements. The homoscedasticity of the variances along the regression line of each drug was verified using the Bartlett's test.

2.4.2 Sensitivity

Limit of detection and quantification of the proposed methods were calculated from thestandard deviation of the response (σ) and slope of the calibration curve (S) of each drug using the formula, LOD = 3.3 x σ/s;LOQ =10 x σ/s.

2.4.3 Precision

Repetabilityand intermediate precision were performed in three replicates using ILA (4, 8 and 12 µg/ml), and LEVO (30, 60 and 90 µg/ml) concentration for both drugs in method I and II.

2.4.3 Accuracy

The accuracy of both the methods was ascertained by performing recovery study at three levels (50%, 100% and 150%). Recovery studies for ILA, and LEVO were carried out by spiking three different amount of the standard to the dosage form. Recovery studies were performed in triplicate. The amounts of ILA, and LEVO were estimated by ordinary linear regression equation. by adding known amount of ILA and LEVO standard to the sample of known concentration and calculating the recovery and % RSD for both the drugs. Recovery studies were carried out by spiking three different amount of ILA standard (2, 4, 6 µg/ml) to the dosage form (4 µg/ml) and LEVO standard (15, 30 and 45µg/ml) to the dosage form (30µg/ml) by standard addition method.

2.5 Analysis of formulation

Twenty tablets of Blokcid-L were weighed and finely powdered. From tablet powder, an amount equivalent to 10 mg of ILA and 75 mg LEVO was accurately weighed and taken into the 100 ml volumetric flask. About 30 ml of methanol was added and the sample solution was sonicated for 15 minutes. The mixture was diluted to volume with methanol, mixed well and filtered to obtain the sample stock solution of 100 µg/ml of ILA and 750 µg/ml LEVO. From the above solution, 0.4 ml withdrawn and transferred into a 10 ml volumetric flask and the volume was adjusted up to the mark with methanol to attain final concentration 4µg/ml of ILA and 30 µg/ml of LEVO selected. The resultant solutions were then used to estimate both

the drugs at selected λ_{max} in both methods. In Method I, final spectra was converted in first order and then amplitude of that spectra was taken at ZCP of ILA and LEVO.

Similarlyin Method II, final spectra of sample solution was divided by divisor concentration of LEVO 45µg/ml and 4 µg/ml of ILA. Then final spectra were converted into the first order spectra and amplitude was taken at particular wavelength of ILA and LEVO. The analysis was performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Method development and selection of wavelength

The selection of optimum wavelength of first order derivative spectra method is based on the fact that the absolute value of the total derivative spectrum at the selected wavelength has the best linear response to the analyte concentration. Moreover, it is not affected by the concentration of any other component (Method I). For the ratio first derivative spectra several amplitude peaks were observed for both drugs spectra but the previous wavelength showed minimum noise and best recovery for ILA and for LEVO (Method II). The linear regression parameters like intercept, corelation coefficient and slope were tested to check the effect of divisor concentration. The selected divisor concentrations shows good results for these parametrs. The wavelength selected for the estimation of ILA (305.51 nm), and LEVO (321.88 nm) gavegood correlation coefficient for method I (figure.3b). Similarly, acceptable correlation coefficient was obtained for the wavelength selected for the estimation of ILA (310.70 nm) and LEVO (277.73 nm) using method II in figure.4c and figure.4f respectively.

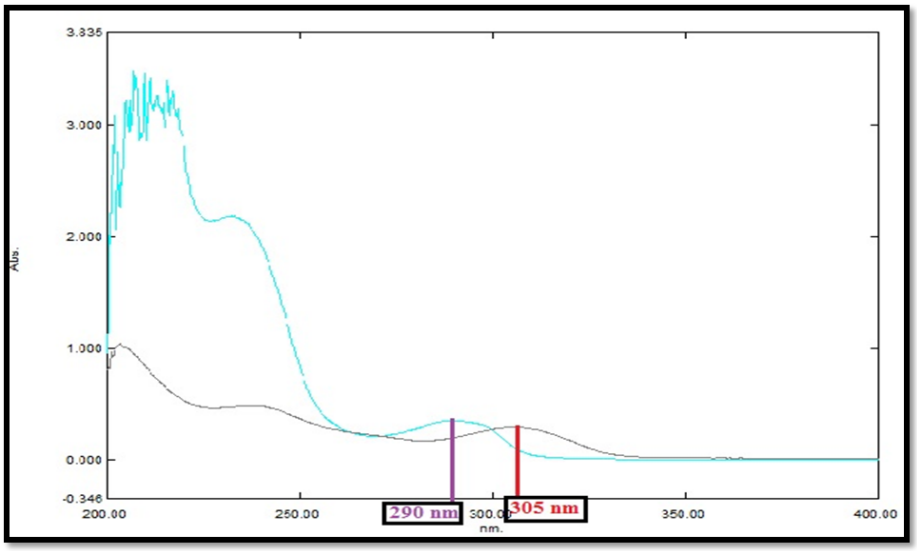

Fig.3(a): Zero order derivative overlay spectra of Ilaprazole and Levosulpiride.

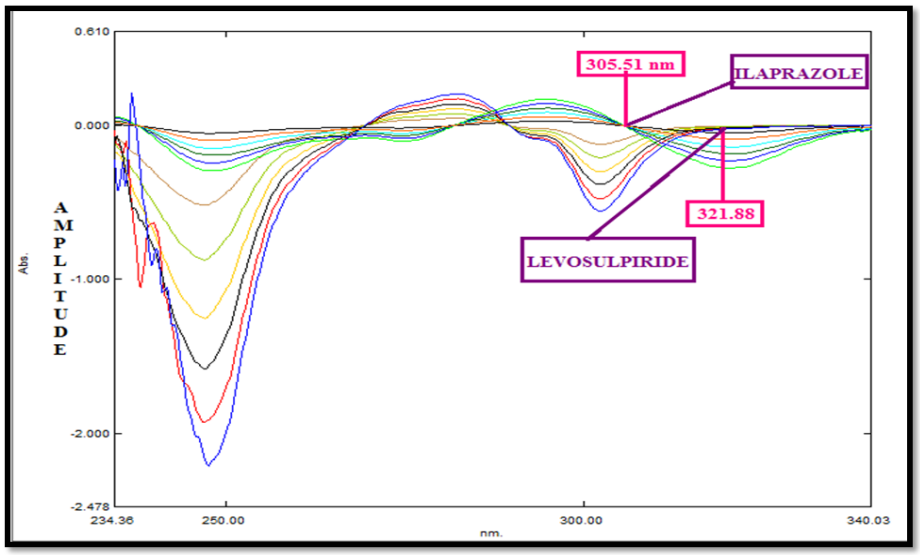

Fig.3(b): First order derivative overlay spectra of Ilaprazole and Levosulpiride.

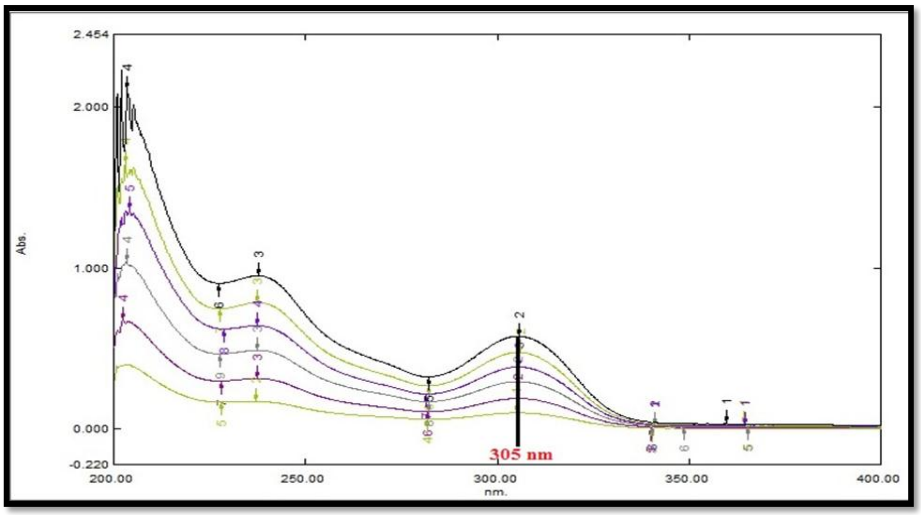

Fig. 4(a): Zero order spectra of Ilaprazole.

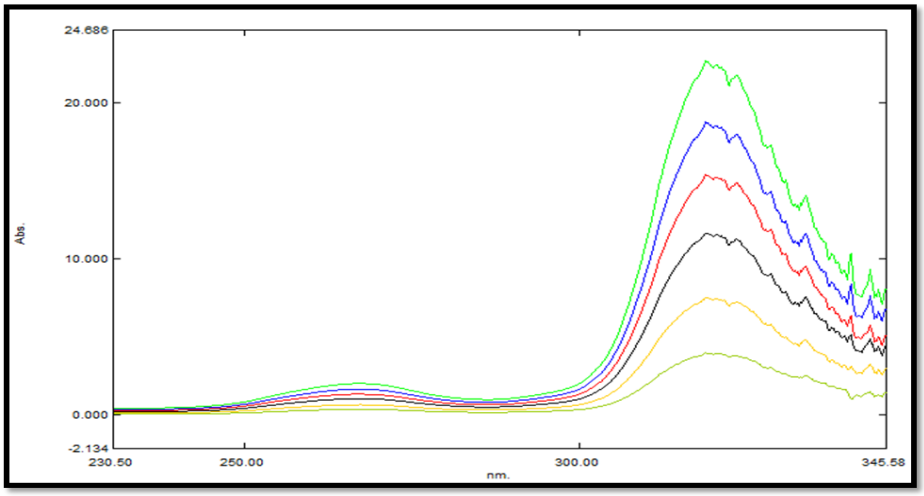

Fig. 4(b): Division of the zero order spectra of ILA using (45 µg/ml) LEVO as divisor.

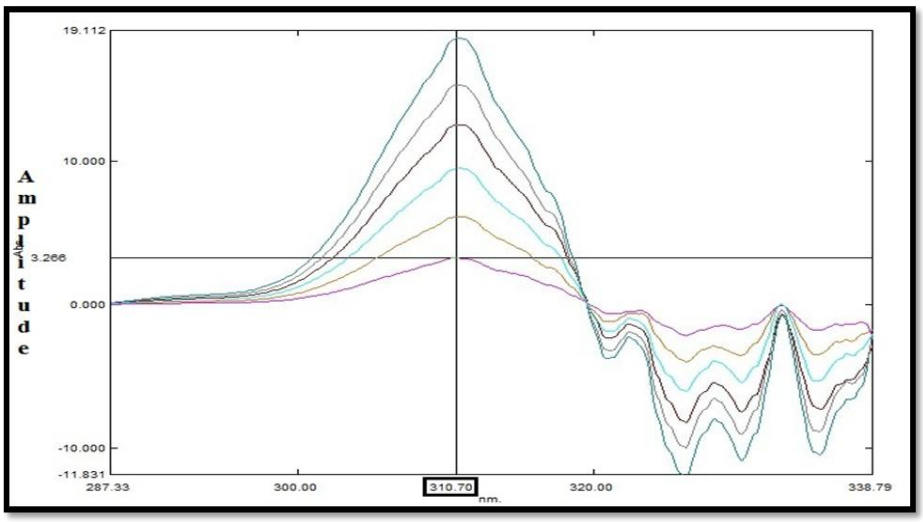

Figure 4(c): Ratio first order derivative absorbance spectra of ILA (2-12 µg/ml).

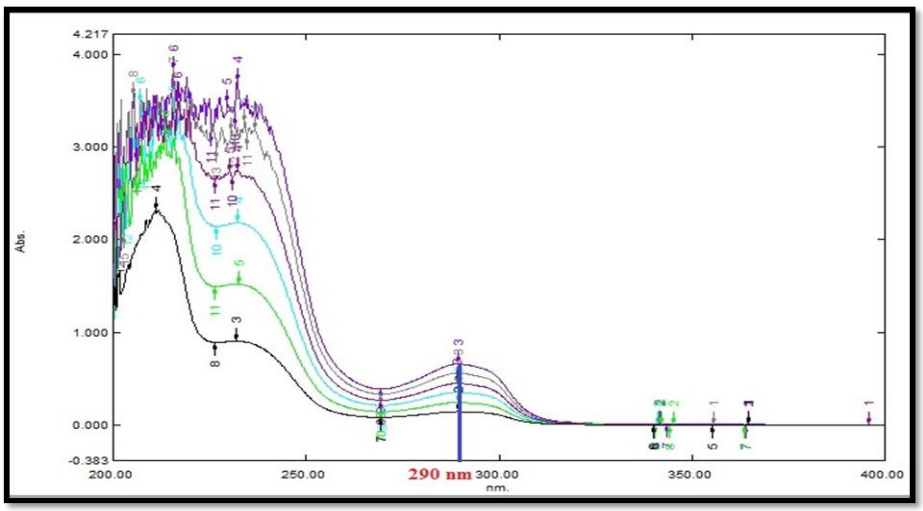

Figure 4(d): Zero order spectra of LEVO (15–90 µg/ml).

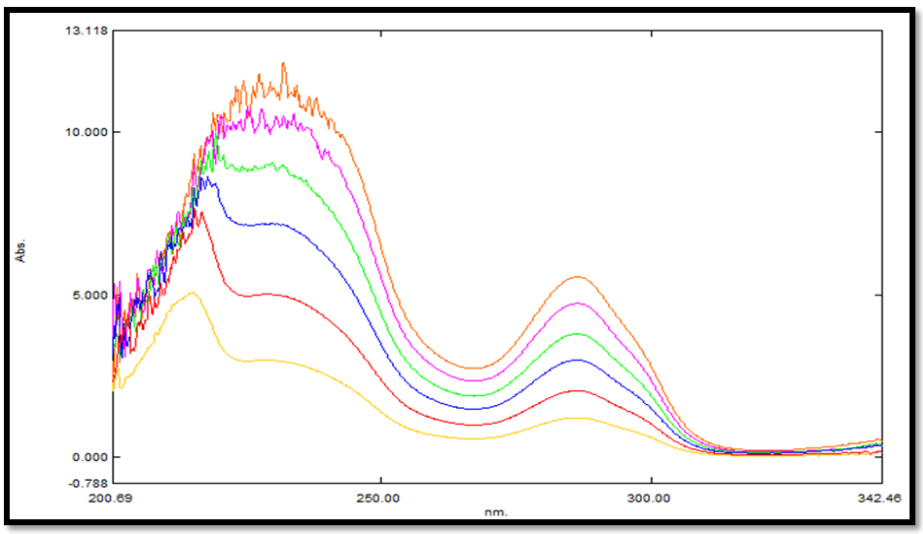

Figure 4(e): Division of the zero order spectra of LEVO using (4 µg/ml) ILA as divisor.

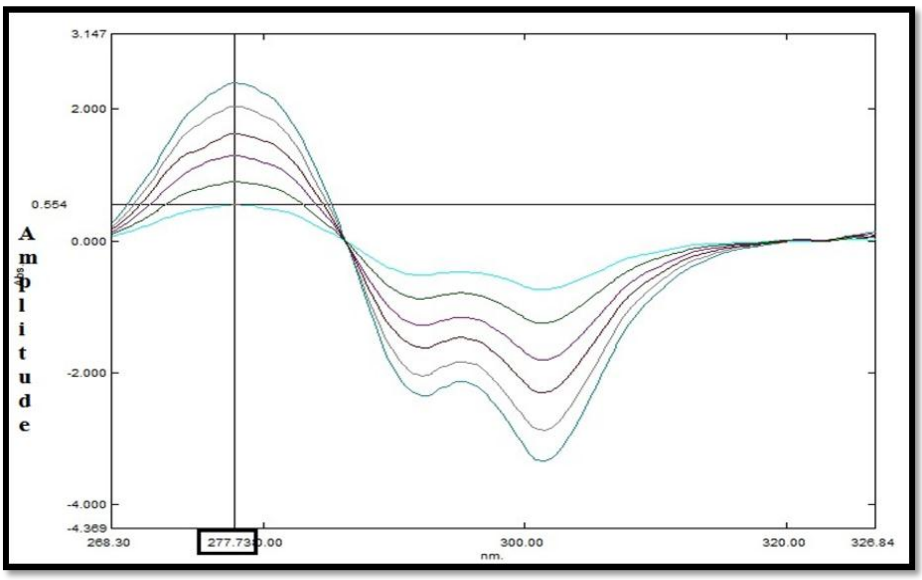

Figure 4(f): Ratio first derivative absorbance spectra of LEVO (15-90 µg/ml).

3.2 Validation of methods

The proposed methods had been validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ), as per ICH guidelines.The calibration curves were constructed for the proposed methods according to their respective concentration ranges and were found to be linear over the concentration range for ILA and LEVO with acceptable regression coefficient as evident from Table I. Furthermore, the homoscedasticity of the calibration standards was verified using a Bartlett's test before performing regression. The results showed that the calculated $\chi 2$ value is less than the critical value at 95% confidence interval, $\chi 2$ (0.05, 5) = 9.488; thus proving the homogeneity of the variances in both methods.

Table I: Linear regression parameters of ILA and LEVO for proposed methods.

Parameters	Method I		Method II	
	ILA	LEVO	ILA	LEVO
Linearity range (µg/ml)[a]	2 -12	15-90	2 -12	15-90
Correlation coefficient(r^2)	0.9994	0.9990	0.9992	0.9997
Slope ± SD	0.0222 ±7.4200	0.0044 ±3.558	1.5387±0.0002	0.0245±0.0004
Confidence limit of slope[b]	0.0222-0.0224	0.0043-0.0044	1.5384-1.5390	0.0238-0.0248
Intercept ±SD	0.0032 ±0.0004	0.0243±0.0013	0.0912±0.0040	0.1745±0.0135
Confidence limit of intercept[b]	0.0024-0.0036	0.0222-0.0256	0.0855-0.0961	0.1619-0.1970
Limit of detection (µg/ml)	0.0725	1.0295	0.0088	1.8027
Limit of quantitation (µg/ml)	0.2197	3.1198	0.0265	5.4627
Bartlett's test[C]	0.0004	0.0024	6.681	0.011

[a]n=5 replicates, [b]confidence interval at 95% confidence level and 5 degree of freedom (t=2.57), [c]$\chi 2$ critical value = 9.488 at α=0.05

Table I also depicts the LOD and LOQ value of ILA and LEVO. Results of Repetability and intermediate precision studies showed % RSD < 2, thus demonstrating precision of the proposed methods (Table II). Recovery study by spiking the standard at three concentration levels, 50, 100 and 150 %, showed % RSD of less than 2% with acceptable percent recovery, indicating that the proposed methods are accurate and can be applicable for routine analysis of formulation (Table III). In method I, LOD and LOQ for ILA were found to be 0.0725 µg/ml and 0.2197 µg/ml and for LEVO were found to be 1.0295 µg/ml and 3.1198 µg/ml respectively. Similarly in method II, LOD and LOQ for ILA were found to be 0.0088µg/ml and 0.0265µg/ml and for LEVO were found to be 1.8027µg/ml and 5.4626µg/ml respectively. F-ratio calculated at 95% confidence interval by F test and t test for comparison of results of analysis of formulation of all two developed methods showed values of **3 and 0.113** this $F_{cal}$was found to be lesser than the F-ratio for comparison of results of analysis of formulation of all two developed methods showed value of **3.640** this F_{cal} was found to be lesser than the tabulated value, F_{crit}**19**. Therefore, wecan conclude that there is no significant difference amongst the results of all two methods.

value, F_{stat} **0.5 and 1**. this t_{stat} was found to be lesser than the tabulated value, t_{crit}**12.70 and 4.30**. Therefore, wecan conclude that there is no significant difference amongst the results of all four methods.

Table II: Precision study for ILA and LEVO by proposed methods.

Method I				Method II		
Conc. (µg/ml)	Repeatability	Intermediate precision		Repeatability	Intermediate precision	
		Day 1	Day 2		Day 1	Day 2
	Amplitude[a]± %RSD (µg/ml)	Amplitude[a]± %RSD (µg/ml)	Amplitude[a]± %RSD (µg/ml)	Amplitude[a]± %RSD (µg/ml)	Amplitude[a]± %RSD (µg/ml)	Amplitude[a]± %RSD (µg/ml)
ILA						
4	0.092 ±1.086	0.092 ± 1.250	0.093 ± 0.616	6.065 ± 0.049	6.060 ±0.033	6.061± 0.041
8	0.184 ±0.543	0.184 ± 0.828	0.186 ± 0.537	12.502±0.018	12.505±0.007	12.506±0.007
12	0.275 ±0.363	0.276 ± 0.552	0.276 ± 0.552	18.526±0.018	18.517±0.013	18.518±0.005
LEVO						
30	0.149 ± 1.020	0.146 ± 1.041	0.148 ± 0.675	0.886 ± 0.172	0.887 ± 0.283	0.888 ± 0.112
60	0.286 ± 0.699	0.284 ± 0.704	0.286 ± 0.349	1.650 ± 0.229	1.656 ± 0.120	1.656 ± 0.034
90	0.418 ± 0.478	0.416 ± 0.240	0.419 ± 0.239	2.347 ± 1.982	2.351 ± 1.651	2.370 ± 0.421

[a]n=3 concentration/3 replicates**,** SD = standard deviation, % RSD = relative standard deviation

Table III: Recovery study at three concentration levels for ILA and LEVO by proposed methods.

Drug	% Spike level	Amount taken(µg/ml)	Amount of drug added(µg/ml)	Amount of drug recovered (µg/ml) Mean±SD	% Recovery	% RSD
	Method I					
ILA	50%	4	2	5.9443±0.3007	99.0823	0.3030
	100%		4	8.0133±0.6018	100.1488	0.6000
	150%		6	9.9344±0.4559	99.3452	0.4590
LEVO	50%	15	15	45.0832±0.7714	100.1851	0.7700
	100%		30	60.1590±0.7575	100.2651	0.7555
	150%		45	75.1590±0.6060	100.2121	0.6047
Method II						
ILA	50%	4	2	6.0708±0.5341	101.1818	0.5279
	100%		4	8.0633±0.8121	100.7927	0.8057
	150%		6	9.9995±0.8521	99.9961	0.8521
LEVO	50%	15	15	45.3534±0.8134	100.7855	0.8071
	100%		30	60.0188±0.2194	100.0313	0.2193
	150%		45	75.0550±0.0821	100.0734	0.0820

n= 3 concentration/ 3 replicates, SD = standard deviation, % RSD = relative standard deviation

Application of the proposed methods for the simultaneous determination of ILA and LEVO

When laboratory prepared mixture analyzed in triplicate using the developed methods, no interference of the added excipients was observed. The content of ILA was in the range of 99.161-99.866% and for LEVO in the range of 99.866%, which proves applicability of the developed methods in routine analysis of formulation (Table IV).

Table IV: Analysis of marketed formulation.

Drugs	Label claim	% Assay		% RSD	
		(Method I)	(Method II)	(Method I)	(Method II)
ILA	10 mg	99.161	99.250	0.524	0.251
LEVO	75 mg	99.866	99.866	0.222	0.033

4. CONCLUSION

Two novel and simple spectrophotometric methods namely First order derivative and Ratio first order derivative were developed for the determination of ILA, and LEVO in mixture and tablet formulation using methanol as a solvent without prior separation. From the results of validation parameters, it is proved that the method is simple, precise, reliable, sensitive and accurate. These methods showed good recovery for both drugs and hence can be used in the

routine quality control for the simultaneous determination of binary mixture and tablet dosage form.

4 ACKNOWLEDGEMENTS

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5 COPYRIGHT TRANSFER

The authors acknowledge that the content presented in this manuscript is entirely original in nature and not published anywhere else.

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