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Analytical Method Development and Validation for Simultaneous Estimation of Ilaprazole And Levosulpiride In Capsule

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed for the separation and quantification of Ilaprazole and Levosulpiride in pharmaceutical dosage form. The quantification was carried out using Hypersil BDS C_8 column (150x 4.6mm,5µm) and mobile phase comprised of methanol and phosphate buffer(pH 7.5 adjusted with 0.01 M NaOH) in proportion of ratio 70:30 degassed under ultra- sonication. The flow rate was adjusted to 1 ml/min and the effluent was monitored at 242nm using PDA detector. The method was validated in terms of linearity, precision, accuracy and specificity, limit of detection and limit of quantization. Linearity of Ilaprazole and Levosulpiride were in range of 10-60 µg/ml and 75-450 µg/ml respectively. The retention time of Ilaprazole and Levosulpiride were 6.52 and 2.02 respectively. The percentage recoveries of both drugs were found to be 100.03% and 100.18% for Ilaprazole and Levosulpiride respectively from the capsule formulation. The method was found to be precise, accurate and specific during the study. The proposed method enables rapid quantification and simultaneous analysis of both drugs from commercial formulations without any excipients interference. The method can be used for routine analysis of marketed product of Ilaprazole and Levosulpiride in combined capsule formulation.

Keywords: Ilaprazole, Levosulpiride, reverse-phase HPLC

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INTRODUCTION

Ilaprazole (ILA)[1-2] of 2-{(4-Methoxy-3-methyl-2-pyridinyl)methylsulfinyl}-5- (1H-pyrrol-1-yl)-1H-benzimidazole,represent the class of Anti-ulcer agents, Proton Pump Inhibitor(ppi. It exert its action by Specific Inhibition Of H^+/K^+- ATPase In The Gastric Parietal Cell by Suppresses Gastric Acid Secretion . By Acting Specifically On The Proton Pump, it Blocks The last Step In Acid Production, Thus Reduce Gastric Acidity. Chemical structure of ILA is in figure1 . Levosulpiride (LEVO)[3-5] of N-(1-ethylpyrrolidin-2-yl)methyl-2-methoxy-5-sulfamoylbenzamide, represent the class of Antipsychotic agents, Dopamine antagonist, Anti-depression agents. LEVO exert its action is a substituted benzamide, which have effective activity on both central and peripheral levels by antidopaminergic (selective dopamine D2 receptors) activity . It is an atypical neuroleptic and a prokinetic agent. The chemical structure of levosulpiride is in figure 2.

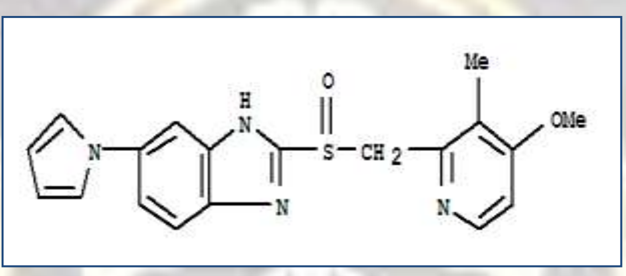

Figure 1 : Structure of ILA

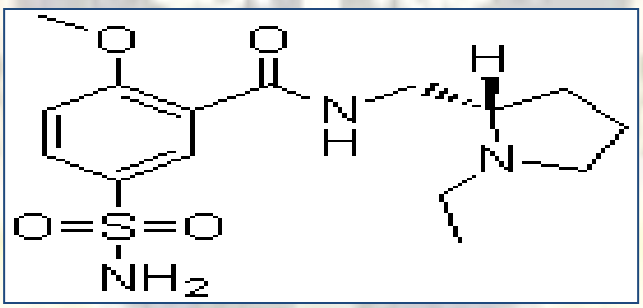

Figure 2: Structure of LEVO

The individual determination of Ilaprazole has been carried out in capsule by UV spectrophotometer[6], by UPLC [7], RP-HPLC [8-9] in bulk and solid dosage forms.Literature survey revealed that few analytical methods have been reported for the estimation of Levosulpiride include UV spectrophotometry [10-11], by RP-HPLC [12-13]. The analytical method for simultaneous determination of Ilaprazole with other combination has been reported by RP-HPLC[14-15].The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in capsule.

MATERIALS AND METHOD

Apparatus

A RP-HPLC instrument (YL-Instrument) equipped with UV-Visible detector(Shimadzu,model1800), manual injector with 20 µl loop, and Hypersil BDS C_8 column (150x 4.6mm,5µm)and YL-Clarity software were used. Analytical weight balance(Mettler Toledo, schwerzenbach, Switzerland), and ultra sonic cleaner (Branson ultrasonic corporation)) , pH meter (Systonic) used during the study.

Reagents and Material

ILA and LEVO were received as a gift sample from zydus cadilla and Intas pharmaceuticals , Acetonitrile of HPLC grade, Triethylamine of AR grade, Water of HPLC grade. The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India).

The optimized Chromatographic Conditions

Hypersil BDS C_8 column (150x 4.6mm,5µm) was used at ambient temperature. The mobile phase was consist of 0.05M Potassium dihydrogen phosphate (pH adjusted to 7.5±0.1 using orthophosphoric acid): methanol (70:30) at a flow rate of 1.0 ml/min. The mobile phase was filtered through a nylon 0.45 µm-47 mm membrane filter and degassed before use. The elution was monitored at 242nm, and the injection volume was 20 µl.

Preparation of Mobile phase:

Preparation of 0.1M NaOH solution:

Accurately weighed 1.00 gm of sodium hydroxide (NaOH) and transferred in to 250 ml volumetric flask dissolved and diluted up to mark with HPLC grade water, mix thoroughly.

Preparation of Buffer solution: (0.01M kH_2PO_4, pH 7.5 adjust with 0.1M NaOH)

Accurately weighed 1.36 gm Potassium dihydrogen phosphate (KH_2PO_4**)** and transferred in to 200 ml volumetric flask dissolved and diluted up to mark with HPLC grade water and adjusted pH 7.5 (± 0.05) with 0.1M NaOH solution, filtered and sonicated.

Preparation of Mobile phase: (Methanol : Buffer solution,70:30 %v/v)

Accurately measured 700 ml of Methanol and 300 ml of above prepared 0.01M Potassium dihydrogen phosphate (KH_2PO_4**),** adjusted pH 7.5 with 0.1M NaOH, Mix thoroughly and degassed by sonicator.

Preparation of stock solutions:

Standard stock solution of Ilaprazole (100 µg/ml):

Accurately weighed 5 mg of Ilaprazole and transferred in to 50 ml of amber color volumetric flask and diluted up to mark with diluent, mix thoroughly.

Standard stock solution of levosulpiride (1000 µg/ml):

Accurately weighed 50 mg of levosulpiride and transferred in to 50 ml volumetric flask and diluted up to mark with diluent, mix thoroughly.

Working standard solution of Ilaprazole and Levosulpiride (20 : 150 µg/ml):

Pipette out and mixed of both above prepared solution of 2 ml of standard solution of Ilaprazole (100 µg/ml) and 1.5 ml of Standard stock solution of Levosulpiride (1000 µg/ml) were transferred in to 10 ml of amber color volumetric flask and diluted up to mark with diluent, Mix thoroughly.

Working sample solution of Ilaprazole and Levosulpiride (20:150 µg/ml)

Twenty capsule were weighed. Weight the empty shell of capsule and average weight of capsules content was calculated. Capsules powder equivalent to 20 mg of Ilaprazole was transferred in 100ml volumetric flask. 50 ml of diluents was added and sonicated for 15 min. allowed it to cool at room temperature and diluted upto mark with diluents. 2 ml of resulting solution were transferred into 10ml volumetric flask and dissolved with diluents and volume made up to mark with diluent to obtain Ilaprazole (20µg/ml) and Levosulpiride (150 µg/ml)

Selection of wavelength for maximum absorbance:

Sample solutions of ILA and LEVO were separately prepared in mobile phase. Each solution was scanned between 200-400 nm in double beam UV-visible spectrophotometer (Shimadzu, model 1800) .wavelength was selected from the overlay spectra of ILA and LEVO .both the components show reasonably good response at 242 nm.

Method Validation [16]:

System suitability

System suitability tests are an integral part of liquid chromatography method. System suitability was checked on each day of validation to evaluate the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameter established are number of theoretical plates, resolution and tailing factor.

Linearity

The linearity of the proposed method was constructed for ILA and LEVO standard solution by plotting the concentration of the compound versus peak area response. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method

Accuracy and precision

The accuracy of the method was determined by recovery studies were carried on the selected drugs at three different concentration levels (50%, 100%.150%). The percentage recovery and standard deviation of the percentage recovery were calculated. The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra day studies , six repeated injection of standard and sample solution were made and the response factor of drug peaks and percentage factor of drug peaks and percentage relative standard deviation were calculated.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as composition of mobile phase ratio and temperature of the column, and studying its effects on the performance of the method.

LOD and LOQ

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentration of ILA and LEVO . the LOD and LOQ values were calculated by using the following formula

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response and S = Slope of calibration curve.

RESULTS AND DISCUSSION

System suitability studies

The column efficiency, resolution and tailing factor were calculated for the standard solutions (table 1). The values obtained demonstrated the suitability of the system for the analysis of the selected drug combinations. System suitability parameters may fall within 2% relative standard deviation range during routine performance of the method. The chromatogram is in the figure 4 and system suitability parameter is in the table 1.

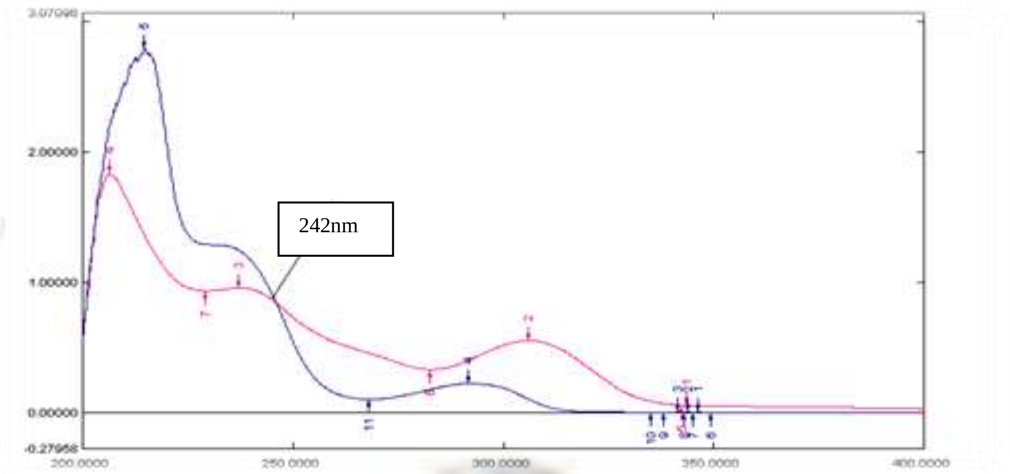

Figure 3: Wavelength selection overlain UV spectra of ILA and LEVO

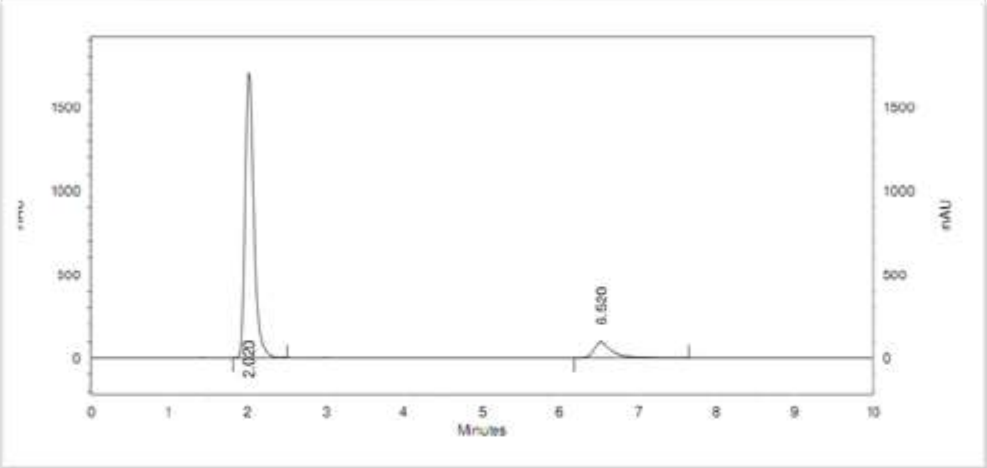

Figure 4 : Chromatogram of ILA (20µg/ml) and LEVO (150 µg/ml)in Methanol :0.05M phosphate buffer(pH-7.5) 70:30, flow rate- 1ml/min

Calibration curve (Linearity) and range

The linearity of the method was determined at six concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. ILA and LEVO exhibited linearity of the concentration range of 10-60 µg/ml) and (75-450µg/ml). Plot the graph for area Vs time to get calibration curve. The linearity of ILA in table 2 and calibration curve in figure 5, and for the levo the linearity is in table 3 and calibration curve in figure 6.

Table 1 : System Suitability

Parameters	Ilaprazole	Levosulpiride
Retention time (min)	6.520	2.027
Theoretical plate	9216	5475
Tailing factor	1.05	1.16
Resolution	24.16	

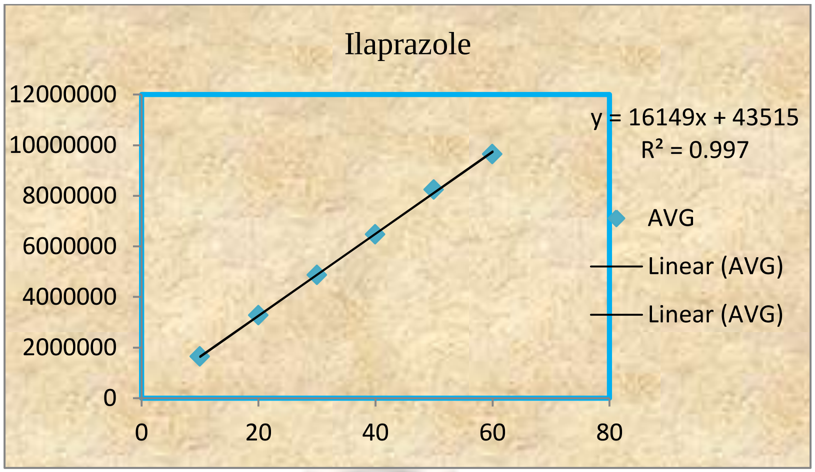

Figure 5: Calibration curve of ILA

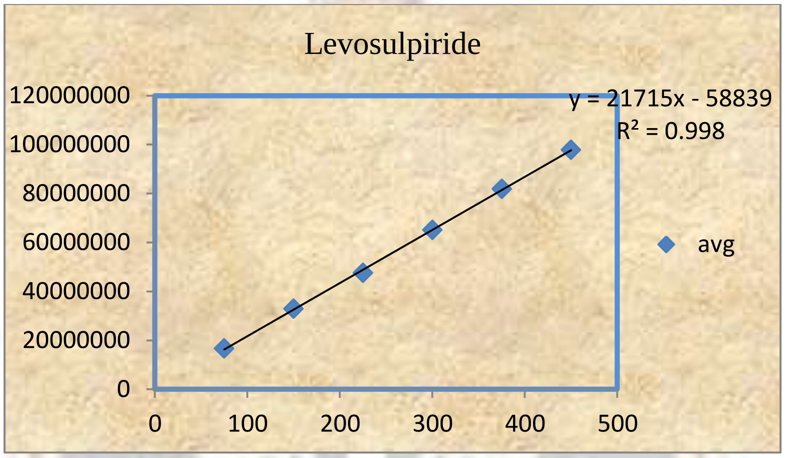

Figure 6 : Calibration curve of LEVO

Table 2: Linearity study of ILA

Concentration (µg/ml)	Peak Area (Mean±SD) (n=6)	%RSD
10	1642466±22825.86	1.38
20	3282573±16551.51	0.50
30	4874945±19405.36	0.39
40	6480385±154495.4	0.23
50	8248393±32909.13	0.39
60	9646666±24261.25	0.251

Table 3 : Linearity of LEVO

Concentration (µg/ml)	Peak Area (Mean±SD) (n=6)	%RSD
75	16604819± 164891.7	0.97
150	29806081± 165201.2	0.55
225	47560893±72336.73	0.15
300	65110185± 582780.4	0.89
375	81716837±228548.3	0.27
450	97761520±158707.6	0.16

Accuracy (% Recovery)

Accuracy of the methods was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (ILA 20 µg/ml and LEVO 150 µg/ml) with three different concentrations of standards (ILA 10,20,30 µg/ml and LEVO 75,150,225 µg/ml). The good recoveries with the standard addition method prove the good accuracy of the proposed methods. (Table- 4)

Table 4: Recovery data of ILA and LEVO

Drug	Accuracy Level (%)	Amount of drug taken (mg/ml)	Amt. of API added (mg/ml)	Total Amount found (mg/ml)± S.D.(n=3)	%Recovery ± SD(n=3)
ILA	50	20	10	29.76	99.20
	100	20	20	39.78	99.45
	150	20	30	50.47	100.94
LEVO	50	150	75	225.09	100.04
	100	150	150	301.99	100.66
	150	150	225	374.79	99.94

Method Precision:

The precision of the method was demonstrated by inter - day and intra- day variation studies. In the intra-day studies, 5 repeated injections of standard solution were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, 5 injection of standard solution were made for 5 consecutive days and response of drugs peaks and percentage RSD were calculated. From the data obtained , the developed RP-HPLC method was found to be precise. (Table 5)

Table 5: Repeatability data of ILA and LEVO

ILA		LEVO	
Conc (µg/ml)	**Area (n=5)**	**conc(µg/ml)**	**Area (n=5)**
20	3313801	150	26662124
20	3310731	150	26724431
20	3303203	150	26685811
20	3309428	150	26701919
20	3305878	150	26698088
20	3311494	150	26701952
Mean ±SD	3309089±19172.74	**Mean± SD**	26695721±81225.785
%RSD	0.57	**%RSD**	0.30

Intraday precision

Combine solution containing the mixture of ILA (20, 30, 40 µg/ml) and LEVO (150, 225, 300 µg/ml) were analyzed for 3 times on the same day at 2 hours time interval, peak areas were determined and % RSD was calculated in table 6

Table 6 : Intraday precision data of ILA and LEVO

ILA			LEVO		
Conc (µg/ml)	**Area ±SD (n=3)**	**%RSD**	**Conc (µg/ml)**	**Area ±SD (n=3)**	**%RSD**
20	3348920±16054.68	0.47	150	26857255±120074.2	0.45
30	4940152±28847.84	0.58	225	45476006±248306.3	0.54
40	6651544±30409.65	0.45	300	56691850±264530.6	0.46

Interday precision

Combine solution containing the mix of ILA (20,30,40µg/ml) and LEVO (70,75,80µg/ml) were analyzed for different 3 days , peak area were determined and % RSD was calculated In table 7.

Table 7 :Interday precision data of ILA and LEVO

Ilaprazole			Levosulpiride		
Conc. (µg/ml)	**Area ±SD (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Area ±SD (n=3)**	**%RSD**
20	3351255±16054.68	0.41	150	26798879±156803.3	0.58
30	4940633±24612.63	0.49	225	45760394±249203.2	0.54
40	6569971±22559.63	0.34	300	55741626±224531.6	0.40

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ that gives information about the sensitivity of the method. The LOD and LOQ values for the ILA and LEVO are presented table 8.

Table 8 : Result for LOD and LOQ

Sr.no.	Drug	LOD	LOQ
1	ILA	3.36	10.18
2	LEVO	33.12	37.04

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions such as wavelength, mobile phase composition pH of mobile phase. It was observed that there were no marked changes in the analytical performance of the method. The result shown in table 9. The results demonstrated that the proposed method is robust.

Table 9 : Robustness data of ILA and LEVO

Wavelength (±2nm)	**Value**	**Area**	
		ILA	**LEVO**
	240	3310374	26621134
	242	3290846	26685374
	244	3295975	26729393
	Mean Area± SD	3299065±10124.07	26678634±54443.34
	%RSD	0.30	0.20
pH(±0.1)	7.4	3315864	26649570
	7.5	3292972	26610671
	7.6	3296336	26737257
	Mean Area ±SD	3301724±12360.58	26665833±64841.03
	%RSD	0.37	0.24
Mobile phase composition(± 2)	68-32	3444669	27692601
	70-30	3416150	27638191
	72-28	3412007	27608488
	Mean Area ±SD	3424275±17782.5	27646427±42656.99
	%RSD	0.51	0.15

Analysis ILA and LEVO in Combined Dosage Forms

Pharmaceutical formulation of ILA and LEVO was purchased from local pharmacy. The responses of formulations were measured at 242 nm for quantification of ILA and LEVO by using RP-HPLC. The amounts of ILA and LEVO present in sample solution were determined by fitting the responses into the regression equation for ILA and LEVO in both the methods. . Results are given in Table 10.

Table 10: Analysis of marketed formulation of Ilaprazole and Levosulpiride by proposed method (n=3)

Ilaprazole			**Levosulpiride**		
Labeled Amount (mg)	**Amount found (mg)**	**%Assay**	**Labeled Amount(mg)**	**Amount found(mg)**	**%Assay**
10 mg	10.15	101.5	75 mg	75.56	100.74
	9.88	98.8		75.18	100.24
	10.06	100.6		74.69	99.58
Mean ± SD	10.03± 0.13	100.3± 1.37	**Mean ± SD**	75.14±0.43	100.18±0.58
%RSD	1.37		**%RSD**	0.58	

To optimize the RP-HPLC parameters, several mobile phase composition were tried. A satisfactory separation and good peak symmetry was found in a mixture of 0.05 M potassium dihydrogen phosphate (pH7.5 using 0.01 M NaOH):methanol(70:30) and 1ml/min flow rate proved to be better than the other mixture in terms of resolution and peak shape. The optimum wavelength for detection was set at 242nm at which much better detector responses for both drug were obtained as it was shown in fig the retention time was 6.52 for ILA and 2.02 min for LEVO the calibration graph for ILA and LEVO were constructed by plotting the peak area versus their corresponding concentration good linearity for both was found over the range ILA range (10-60 µg/ml) and LEVO range (75-450µg/ml). Result obtained by applying the RP-HPLC Method showed that the concentration of ILA and LEVO can be simultaneously determined in prepared mixtures. The result obtained indicate the additives present do not interfere with analysis of the studied mixtures. System suitability test parameters for ILA and LEVO for RP-HPLC method are reported in table 1 .

CONCLUSION

Results are in good agreement with label claim which indicate there is no interference of routinely used excipients. The proposed method is accurate and precise. Therefore proposed method can be used for routine analysis of ILA and LEVO in capsule

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