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DEVELOPMENT AND VALIDATION RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LEVOSULPIRIDE AND ILAPRAZOLE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

RP-HPLC method Chromatographic separation of LSP and ILA were performed by use of isocratic mobile phase prepared from Phosphate buffer: Methanol: ACN (pH 3.8 adjusts with 10% ortho phosphoric acid) (35:45:20). Absorbance is measured at wavelength 294nm. Flow rate is 1 ml/min and Run time is 10 min. The average retention time was found to be 2.353 min and 4.85min for LSP and ILA respectively. Calibration curve was linear in concentration range of 1-5μg/mL for ILA and 7.5-37.5μg/mL for LSP. %RSD for precision was found to be less than 2% and the % recovery was found to between 98-102%.

Keywords *– Ilaprazole (ILA), Levosulpiride (LSP), Reverse Phase High Performance Liquid Chromatography (RP-HPLC), Ortho Phosphoric Acid (OPA), Relative Standard Deviation (RSD)*

1. INTRODUCTION

Ilaprazole (ILA) 2-[[(4-Methoxy-3-methyl-2-pyridinyl)methyl]sulfinyl]-6-(1Hpyrrol-1-yl)-1H-benzimidazole (Fig 1) is a new proton pump inhibitor used in the treatment of peptic ulcer disease, dyspepsia, gastro esophageal reflux disease and duodenal ulcer which reduces acid secretion by inhibiting the parietal cell H^+/K^+ ATP pump[1].

Levosulpiride (LSP) is levo enantiomer of sulpiride. Chemically it is N - [[(2S) - 1 -ethylpyrrolidin-2-yl] methyl] - 2 - methoxy - 5 – sulfamoylbenzamide (Fig. 2). LSP is an atypical antipsychotic and a prokinetic agent. It is used in several indications like depression, psychosis, somatoform disorders, emesis and dyspepsia[2].

Levosulpiride and Ilaprazoleare available in combination in capsule dosage form that have been used for the treatment of gastro esophageal reflux syndrome and in treatment of psychic patient and to suppress acid secretion in stressed condition.Literature survey revealed that UV, HPLC and HPTLC methods are reported for the estimation of Ilaprazole[3-12] and Levosulpiride[13-19] either in alone or in combination with other drugs. However no methods are yet reported for the simultaneous estimation of Ilaprazole and Levosulpiride.

The present work is an attempt to develop and validate a simple and accurate method for simultaneous estimation of Levosulpiride and Ilaprazoleby RP-HPLC methods.

2. MATERIALS AND METHOD

2.1 Materials

Instrument used in current research HPLC (Shimadzu) (model SPD-20A, LC-20AD). Phenomenex Luna C18 (250 x 4.6mm, 5 µm) column was used. LSP was provided by Torrent pharma. Ahmedabad, India as a gift sample and ILA were obtained as paid sample from Precise Chemipharma Pvt. Ltd., Mumbai, India. LSP and ILA combination tablet (Iladac L, 75 mg LSP and 10 mg ILA, manufactured by Aeon Formulations Pvt Ltd, Puducherry, India) were purchased from local pharmacy.

2.2 Preparation of standard stock solution

An accurately weighed standard powder of 5 mg of ILA and LSP were transferred in 50 ml volumetric flask separately, dissolved and diluted up to the mark with methanol of HPLC grade, to get final concentration 100µg/ml of ILA and LSP.

From this standard stock solution, different aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with mobile phase. This solution was used as a working standard solution (WSS).

2.3 Selection of Detection Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 294 nm both drug give good peak height and shape (Fig.3). So, 294 nm was selected for simultaneous estimation of Ilaprazole and Levosulpiride in HPLC method.

2.4 Selection of mobile phase

Optimization can stared with minor change in mobile phase composition after getting a reasonable chromatogram that means more or less symmetrical peak on chromatogram detect all the compounds. An optimized chromatogram is one in which all the peaks are symmetrical and are well separated in less run time. Various mobile phases, such as Methanol: Water, Acetonitrile: Water, Methanol: ACN: water, Phosphate buffer: Methanol: Acetonitrile in different proportion was tried. The combination of 10mM Phosphate buffer: Methanol: Acetonitrile (pH=3.8) (35:45:20%v/v/v) provided optimum polarity for proper migration, separation and resolution of Ilaprazole and Levosulpiride. Under these conditions, the eluted peaks were well defined and resolved (Fig. 4).

Chromatographic separation of LSP and ILA were performed by use of isocratic mobile phase prepared from Phosphate buffer: Methanol: ACN (pH 3.8 adjusts with 10% ortho phosphoric acid) (35:45:20). Absorbance is measured at wavelength 294nm. Flow rate is 1 ml/min and Run time is 10 min.

2.5 Analysis of capsule dosage form by both the methods

For analysis of Ilaprazole and Levosulpiride in capsule, twenty capsules (Iladac L containing 10mg of Ilaprazole and 75mg of Levosulpiride) were accurately weighed and average weight was calculated. Pellets from Capsule were finely powdered and mixed thoroughly. Powder weight equivalent to 10 mg of drug containing Ilaprazole were dissolved in a 100 ml volumetric flask diluted with methanol up to the mark. It was sonicated followed by filtration through whatmann filter paper. The filtrate was diluted up to the mark with Methanol. The mixture contains 100 µg/ml of Ilaprazole and 750 µg/ml of Levosulpiride.

From above mixture solution pipette out 0.2ml and transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with Mobile phase to attain final concentration of Ilaprazole 2 µg/ml and Levosulpiride 15 µg/ml. Concentration of both the drug were determined at 294 nm. Theresult of formulation was calculated against the calibration curve in Quantitation mode.

2.6 Analytical method validation

The developed method was validated with respect to linearity, accuracy, precision, limit of detection and limit of quantification in accordance with the ICH Q2 (R1) guideline.

2.6.1 Linearity (calibration curve)

Concentration range of 1-5µg/ml for ILA and 7.5-37.5 µg/ml for LSP were prepared in mobile phase and detected at wavelength 294nm (Fig. 5) Linearity of both the drugs was checkedin term of slope, intercept and correlation coefficient. (Fig. 6 & 7)

2.6.2 Method precision (repeatability)

The precision of the instrument was checked by repeated analysis of absorbance of Solutions containing 4 µg/ml of Ilaprazole and 30 µg/ml of Levosulpiride were analyzed for six times and %R.S.D (relative standard deviation) was calculated for both the method (Table. 1).

2.6.3 Intermediated precision (Reproducibility)

Precision was studied to find out intra and inter-day variations in the test method of ILA and LSP. Calibration curves prepared in medium were run in triplicates on the same day and for three days at three different concentration levels(Table 1) and % RSD (relative standard deviation) was calculated.

2.6.4 Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of Ilaprazole and Levosulpiride by the standard addition method. Known amount of standard solution of Ilaprazole and Levosulpiride were added at 50, 100 and 150% level of prequantified sample solution of Ilaprazole and Levosulpiride (2µg/ml of ILA and 15 µg/ml of LSP). The amount of ILA and LSP were estimated by applying obtained value to the regression equation of the calibration curve. (Table 2)

2.6.5 Limit of detection and Limit of quantification:

Limit of detection (LOD) and limit of quantification (LOQ) of the drug were estimated from the standard calibration curve. The std. deviation of Y- intercept of regression line was used to calculate LOD and LOQ. (Table 1)

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

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

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

3. RESULTS AND DISCUSSION

For HPLC method various mobile phase compositions was tried to get adequate separation of eluted compound. Separation of LSP and ILA were performed by use of isocratic mobile phase prepared from Phosphate buffer: Methanol: ACN (pH 3.8 adjusts with 10% ortho phosphoric acid) (35:45:20) at detection wavelength 294nm with flow rate is 1 ml/min and run time is 10 min. the average retention time was found to b 2.353 min and 4.85min for LSP and ILA respectively. The calibration was linear in concentration range of 1-5μg/mL for ILA and 7-35 μg/mL for LSP. The low RSD (< 2%) value indicates that the method is precise. The recoveries for ILA and LSP were found to be in the range of 99-100%.

Table 1: Optical characteristic of ILA and LSP

Parameter	ILA	LSP
Wavelength	294nm	
Linearity range (µg/ml)	1-5	7.5-37.5
Regression reaction	y = 20716x + 69532	y = 2931.x + 92235
Correlation coefficient	0.998	0.997
Intraday precision (n=3) (% RSD)	0.70-0.79	0.65-0.76
Interday precision (n=3) (% RSD)	0.37-0.75	0.36-0.79
Repeatability(% RSD)	0.91	0.48
Detection limit	0.080	0.274
Quantitation limit	0.242	0.833

Table 2: Result of recovery study

Name of drug	% level	Amount of drug Taken (µg/ml)	Amount of drug added (µg/ml)	% Recovery ±SD (n=3)
ILA	50	2	1	99.37±1414.13
	100	2	2	99.5±799.52
	150	2	3	99.58±4725.45
LSP	50	15	7.5	99.98±1018.08
	100	15	15	99.51±1010.03
	150	15	22.5	99.37±1000.07

Table 3: Analysis of marketed formulation by proposed method

Drug formulation	Amount taken (mg)		Amount found (mg)		% drug found	
Capsule	ILA	LSP	ILA	LSP	ILA	LSP
	2	15	1.9	15.03	99.52±0.014	99.52±0.014

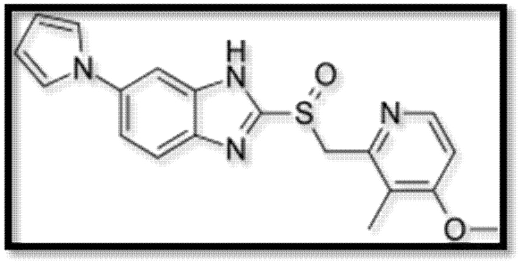

Figure 1: Chemical Structure of Ilaprazole

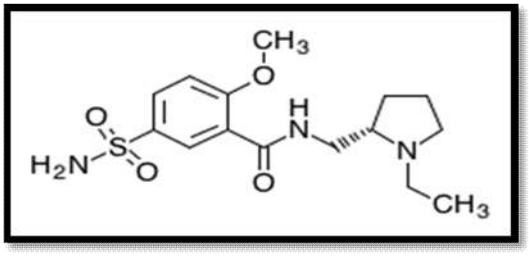

Figure 2: Chemical Structure of Levosulpiride

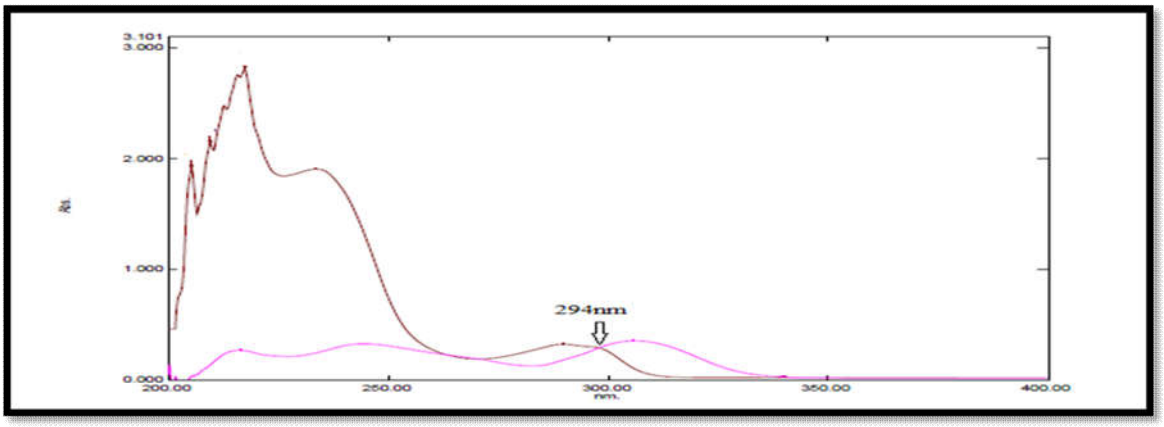

Figure 3: Selection of detection wavelength

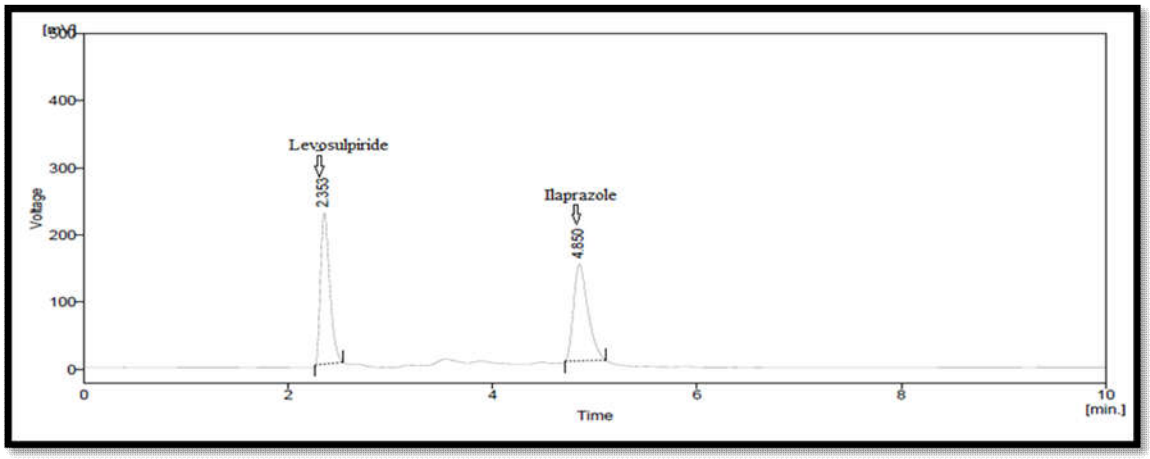

Figure 4: Chromatogram of Ilaprazole (4 µg/ml) and Levosulpiride (30µg/ml) in Phosphate buffer: Methanol: ACN (pH 3.80) (35:45:20 **%v/v)**

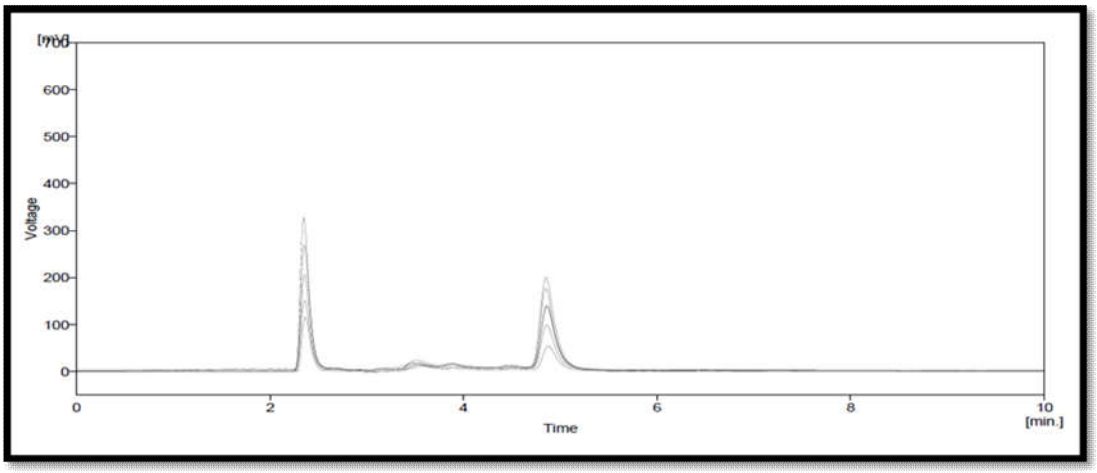

Figure 5: Overlay chromatogram of Ilaprazole (1-5µg/ml) and Levosulpiride (7.5-37.5 µg/ml)

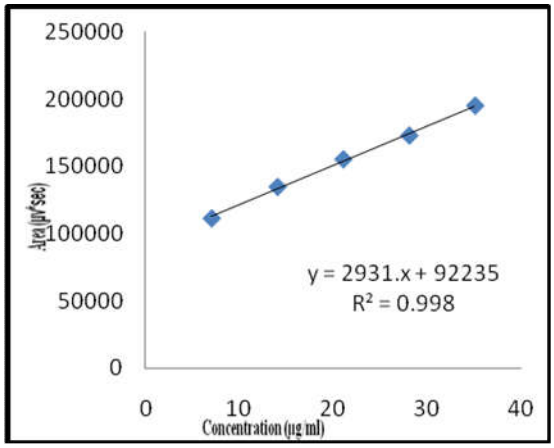

Figure 6: Calibration curve of Levosulpiride (7-35 µg/ml)

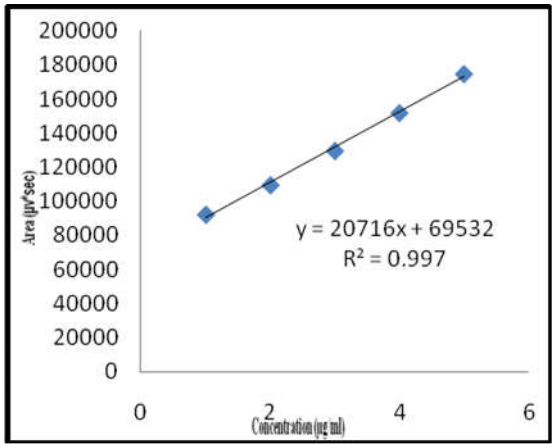

Figure 7: Calibration curve of Ilaprazole (1-5µg/ml)

4. CONCLUSION

Developed HPLC method was found to be simple accurate and precise quantitative analysis for simultaneous estimation of ILA and LSP in capsule. These methods can be successfully applied for the simultaneous of ILA and LSP in capsule dosage form without prior separation in quality control**.**

5. ACKNOWLEDGEMENT

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