ABSTRACT
Two precise, accurate, simple and cost-effective UV spectrophotometric methods have been developed and validated for simultaneous estimation of Levosulpiride and Ilaprazole in Capsule dosage form. Method I is estimation using simultaneous equation method at 231 nm (λmax of Levosulpiride) and 305.6 nm (λmax of Ilaprazole). Method II is Q-absorbance ratio method utilize absorbance of Ilaprazole and Levosulpiride at 219 nm which is the Iso-absorptive point and 305.6 nm the λmax of Ilaprazole. Both methods obey Beer's law in the concentration range of 2-40 μg /ml for Ilaprazole and Levosulpiride. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. The method was successfully applied to the determination of these drugs in pharmaceutical dosage form.

KEYWORDS: Levosulpiride, Ilaprazole, UV spectrophotometry, Simultaneous equation method, Q-absorbance ratio method.

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

Ilaprazole (ILA) 2-[(4-Methoxy-3-methyl-2-pyridinyl)methyl]sulfinyl]-6-(1Hpyrrol-1-yl)-1H-benzimidazole (Fig 2) 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.

Levosulpiride and Ilaprazole are available in combination in capsule dosage form that have been used for the treatment of gastroesophageal 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 and levosulpiride 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 ilaprazole by UV methods.

MATERIALS AND METHOD

Instruments

- UV spectrophotometric method was performed on double beam UV-visible spectrophotometer (Shimadzu, model 1700, Japan) having two matched quartz cells with 1 cm path length and UV – probe 2.31 software.
- Analytical balance (CP224S, Sartorius, Germany)
- Ultrasonic cleaner (Frontline FS 4, Mumbai, India)
- Corning volumetric flasks, beakers and pipettes of borosilicate glass were used in the experiment.

Materials and Reagents

- LSP and ILA were provided by Zydus Cadila, Ahmedabad, India as a gift sample. LSP and ILA combination capsule (Iladac L, 75 mg LSP and 10 mg ILA, manufactured by Aeon Formulations Pvt Ltd, Puducherry, India) were purchased from local pharmacy.
- Methanol AR grade as solvent. (S.D. Fine Chemical Ltd., Mumbai, India)
- Whatmann filter paper no. 41.
Preparation of standard stock solution

An accurately weighed standard powder of 10 mg of LSP and ILA were transferred in 100 ml volumetric flask separately, dissolved and diluted up to the mark with methanol, to get final concentration 100μg/ml of LSP and ILA. From this standard stock solution, different aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with methanol. This solution was used as a working standard solution (WSS).

Selection of analytical wavelength

10 μg /ml solution of LSP and ILA were prepared in methanol and spectrum was recorded between 200-400 nm. The overlain spectrum of both drugs were recorded.

Preparation for calibration curve

For construction of calibration curve, two series of concentration in range of 2-40 μg/ml for LSP and ILA were prepared in methanol from stock solution. These solutions were scanned in range of 200-400 nm and absorbances were measured at selective wavelength and calibration curve were plotted for absorbance vs. concentration.

Method I (Simultaneous Equation Method)

Two wavelengths selected for the method are 231 nm (λ1) and 305.6 nm (λ2) that are absorbance maxima of LSP and ILA respectively in methanol. Standard stock solution(s) of 100 μg/ml each of LSP and ILA were prepared separately in methanol. The stock solutions of both the drugs were further diluted separately with to get a series of standard solutions of 2-40 μg /ml of LSP and ILA. The absorbance was measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations:

\[ C_x = \frac{(A_1 a_{y2} - A_2 a_{y1})}{(a_{x1} a_{y2} - a_{x2} a_{y1})} \] and
\[ C_y = \frac{(a_{x1} A_2 - a_{x2} A_1)}{(a_{x1} a_{y2} - a_{x2} a_{y1})} \]

Cx and Cy = Concentration of LSP and ILA respectively (gm/100 ml)

ax1 and ax2= Absorptivity of LSP at λ1 and λ2 respectively

ay1 and ay2= Absorptivity of ILA at λ1 and λ2 respectively

A1 and A2= Absorbance of test at λ1 and λ2 respectively
Method II (Q- Absorbance Ratio Method)
In absorption ratio method, the absorbance of both the drugs was calculated at two selected wavelengths among which λ1 is the wavelength of isosbestic point (where both drugs show the same absorbance) and λ2 is the Amax of either drug among the drugs to be analyzed. From the overlain spectra, wavelength 219 nm (λ1, i.e., an isosbestic point) and 305.6 nm (λ2, i.e., absorbance maxima of Ilaprazole) were selected for analysis. The concentration of the individual drug is determined by equations written below.

\[ Cx = \frac{(Qm - Qy) \times A_1}{(Qx - Qy) \times ax_1} \]
\[ Cy = \frac{(A_1/ax_1)}{x (Cx)} \]

Cx: Concentration of LSP.
Cy: Concentration of ILA.
A1: absorbance of sample at λ1(isosbestic point).
A2: absorbance of sample at λ2 (λmax ILA)
ax1, ax2: Absorptivities of LSP at λ1 and λ2 respectively
ay1, ay2: Absorptivities of ILA at λ1 and λ2, respectively.

These ratio values are used in equations to calculate the Qm values to determine the assay values. The values are given in Tables 1 and 2.

Procedure for Analysis of Capsule Formulation
To determine the content of levosulpiride and ilaprazole simultaneously in capsule (label claim: 75 mg levosulpiride and 10 mg ilaprazole); twenty tablets were weighed and average weight was calculated. The capsules were crushed to obtain fine powder. Capsule powder equivalent to 75 mg of levosulpiride and 10 mg of ilaprazole was transferred to 100 ml volumetric flask and diluted to 10 ml with methanol. Sonication for 15 min and was diluted upto the mark. It was mixed and filtered the resulting solution with whatmann filter paper. 0.2 ml of resulting solution was taken and diluted to 10 ml with methanol mixed properly. The resulting solution appropriate diluted with methanol to obtain 15μg/ml of levosulpiride and 2 μg/ml of ilaprazole. The concentration of both levosulpiride and ilaprazole were determined by measuring the absorbance of the sample at 231.0 nm, 305.6 nm and 219 nm (i.e. iso-absorptive point). The results of the capsule analysis were calculated against the calibration curve in quantitation mode.
Method Validation
The proposed methods were validated according to ICH Q2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification. The results are shown in table 1.

Linearity & Range
The standard stock solution containing 100 μg/ml each of LSP and ILA were further diluted to get linearity conc. of 2-40 μg/ml of LSP and ILA respectively. Calibration curve was plotted by taking absorbance on Y –axis and concentration on X –axis the relation between drug and its absorbance is expressed by the equation Y = MX + C, where ‘M’ is slope and ‘C’ is intercept, linear regression equation.

Accuracy
Recovery studies were performed to validate the accuracy of developed method by adding an 80 %, 100 %, 120 %. of standard drug in pre-analyzed sample solution. These results summarized in table 2.

Precision (Repeatability)
Six dilution in three replicate of concentration were analyzed in same day for repeatability and result were found within acceptable limit (RSD < 2) as shown in table 3.

Intermediate Precision (Reproducibility)
Three dilutions in the three replicate were analyses on two different day, two analysts for day to day & analyst to analyst variation. All result were fall within acceptable limits (RSD < 2) as shown in table 3.

Limit of Detection and Quantification
The limit of detection and limit of quantification were estimated from the standard calibration curve. The residual standard deviation of regression line or standard deviation of Y – intercepts of regression lines was used to calculate LOD and LOQ. Here LOD = 3.3*D/S and LOQ = 10*D/S. Where D is the standard deviation of Y –intercept of regression line S is the slope of calibration curve table 1.

RESULT AND DISCUSSION
For both the methods linearity was observed in the concentration range of 2-40 μg/ml for both levosulpiride and ilaprazole. Marketed brand of capsule was analyzed and amount of
Drug determined by proposed methods ranges from 99.33 to 100.5 as shown in Table- 4. The proposed methods were validated as per ICH guideline. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 80,100 and 120 % level. The % recovery ranges from 99.24 to 99.98% for both the methods and are presented in Table 2. Precision was calculated as repeatability (% RSD is less than 1) and inter and intraday variations (%RSD is less than 1) for both drugs. The repeatability data are presented in Table-3. The proposed methods were found to be simple, accurate and rapid for the routine determination of levosulpiride and ilaprazole in capsule formulation. To study the validity and reproducibility of proposed methods, recovery studies were carried out. The methods were validated in terms of linearity, accuracy, precision and reproducibility. Both methods can be successfully used for simultaneous estimation of levosulpiride and ilaprazole in combined dosage form.

**Table 1: Summary of Linear regression analysis and optical characteristics of LSP and ILA.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wavelength</strong></td>
<td>LSP</td>
<td>ILA</td>
</tr>
<tr>
<td></td>
<td>231 nm</td>
<td>305.6 nm</td>
</tr>
<tr>
<td><strong>Linearity range (mcg/ml)</strong></td>
<td>2-40 μg /ml</td>
<td>2-40 μg /ml</td>
</tr>
<tr>
<td><strong>Regression equation</strong></td>
<td>$Y=0.0483x + 0.0472$</td>
<td>$Y=0.04x +0.0454$</td>
</tr>
<tr>
<td><strong>Correlation co-efficient</strong></td>
<td>0.9984</td>
<td>0.9986</td>
</tr>
<tr>
<td><strong>Intraday Precision (% RSD)</strong></td>
<td>0.2- 0.6 %</td>
<td>0.51- 0.75 %</td>
</tr>
<tr>
<td><strong>Interday Precision (% RSD)</strong></td>
<td>0.24- 0.51 %</td>
<td>0.51- 0.84 %</td>
</tr>
<tr>
<td><strong>Repeatability (% RSD)</strong></td>
<td>0.45 %</td>
<td>0.3 %</td>
</tr>
<tr>
<td><strong>Detection Limit (By calculation)</strong></td>
<td>0.199</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Quantification Limit (By calculation)</strong></td>
<td>0.60</td>
<td>0.61</td>
</tr>
</tbody>
</table>

**Table 2: Results of Recovery Study by developed method.**

<table>
<thead>
<tr>
<th>Name of drug</th>
<th>% level</th>
<th>Amount of drug Taken (µg /ml)</th>
<th>Amount of drug Added (µg /ml)</th>
<th>Method I (% Recovery)</th>
<th>Method II (% Recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSP</td>
<td>80</td>
<td>15</td>
<td>12</td>
<td>99.98 %</td>
<td>99.4%</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>15</td>
<td>15</td>
<td>99.51 %</td>
<td>99.6%</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>15</td>
<td>18</td>
<td>99.37 %</td>
<td>99.24%</td>
</tr>
<tr>
<td>ILA</td>
<td>80</td>
<td>2</td>
<td>1.6</td>
<td>99.37 %</td>
<td>99.86%</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2</td>
<td>2</td>
<td>99.5 %</td>
<td>99.41%</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>2</td>
<td>2.4</td>
<td>99.58 %</td>
<td>99.26%</td>
</tr>
</tbody>
</table>
Table 3: Result of Precision Study.

<table>
<thead>
<tr>
<th>Intraday (% RSD)</th>
<th>LSP and ILA (μg/ml)</th>
<th>Method I LSP</th>
<th>Method II At Iso-absorptive point (219 nm) ILA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>0.49%</td>
<td>0.51%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.6%</td>
<td>0.28%</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.21%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Interday (% RSD)</td>
<td>2</td>
<td>0.43%</td>
<td>0.56%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.51%</td>
<td>0.32%</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.24%</td>
<td>0.16%</td>
</tr>
</tbody>
</table>

Table 4: Analysis of marketed formulation by proposed method.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Iladac L label claim mg/capsule</th>
<th>Method I (% Assay)</th>
<th>Method II (% Assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSP</td>
<td>75</td>
<td>98.67%</td>
<td>99.33%</td>
</tr>
<tr>
<td>ILA</td>
<td>10</td>
<td>100.00%</td>
<td>100.5%</td>
</tr>
</tbody>
</table>

Figure 1: Chemical Structure of Levosulpiride.

Figure 2: Chemical Structure of Ilaprazole.

Figure 3: Calibration Curve of LSP (2-40 μg/ml) and ILA (2-40 μg/ml) for Simultaneous Equation Method.
CONCLUSION

All the validation parameters were studied as per the ICH guidelines. All the methods were found to be accurate, simple, precise, selective, specific and reproducible. Hence, the methods can be used for routine analysis of both the drugs in their combined solid dosage form.
ACKNOWLEDGEMENTS
We are very thankful to Zydus Cadila, Ahmedabad for providing standards of Levosulpiride and Ilaprazole respectively and to S. K. Patel College of Pharmacy, Ganpat University, Kherva for providing the necessary facilities and constant encouragement.

REFERENCES
11. S.Surve, J. Patel, Arpit Patwari, Mahesh Chhabaria, HPTLC and HPLC method development and validation for Simultaneous estimation of Rabeprazole Sodium and


