3.1 INTRODUCTION

Analytical method development is the process of selecting procedure which is suitable for its intended use. It is generally used to determine the composition of a formulation. It is the process of proving that an analytical method should be acceptable to measure the concentration of subsequent samples (1). The important parameters that may be considered during method development are linearity range, accuracy, precision, Limits of Detection (LOD) and Limit of Quantitation (LOQ) (1).

3.2 MATERIALS

Asenapine maleate and Lurasidone HCl were received as gift samples from Alembic Pharmaceuticals, Vadodara, India. Methanol, Acetonitrile (ACN) and water were of High-performance liquid chromatography (HPLC) grade and purchased from Merck Chemicals, India. Sodium dihydrogen phosphate and potassium dihydrogen phosphate were purchased from S.D. Fine Chemicals, India. All the other solvents and reagents used were of analytical grade and filtered through 0.22μ membrane filter before use.

3.3 ESTIMATION OF ASENAPINE MALEATE BY UV SPECTROSCOPY

3.3.1 Calibration plot in methanol

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of methanol (1000 µg/ml). Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 20-200 µg/ml. The absorbance of these solutions was measured using UV- Visible Spectrophotometer (UV-1800, Shimadzu). (2).

3.3.2 Calibration plot in chlorofom:methanol (1:1)

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of chloroform:methanol (1000 µg/ml). Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 20-140 µg/ml. The absorbance of these solutions was measured using UV- Visible Spectrophotometer.

3.3.3 Calibration plot in 0.1 N HCl

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of 0.1 N HCl (1000 µg/ml). Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 40-200 µg/ml. The absorbance of these solutions was measured using UV- Visible Spectrophotometer.

3.3.4 Calibration plot in phosphate buffer pH 6.8

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of phosphate buffer pH 6.8 (1000 µg/ml). From this stock solution, 5 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 500 µg/ml. Suitable aliquots of were measured and diluted accordingly to make different concentrations in the range of 40-320 µg/ml. The absorbance of these solutions was measured using UV- Visible Spectrophotometer.

3.3.5 Calibration plot in phosphate buffer pH 7.4

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of phosphate buffer pH 7.4 (1000 µg/ml). From this stock solution, 5 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 500 µg/ml. Suitable aliquots of were measured and diluted accordingly to make different concentrations in the range of 40-280 µg/ml. The absorbance of these solutions was measured using UV-Visible Spectrophotometer.

3.4 ESTIMATION OF ASENAPINE MALEATE USING HPLC

3.4.1 HPLC conditions

Quantitative estimation of Asenapine maleate was done by HPLC as described in literature with slight modifications (4,5).

The HPLC system (Shimadzu, Japan) composed of a UV-visible spectrophotometric detector. The separation was performed on a C-18 HPLC column (Phenomenex, Torrence, USA). A filtered and degassed mixture of buffer (phosphate buffer, 6.9 g sodium dihydrogen phosphate in 1000 ml of double distilled water, 1 ml Triethylamine and pH adjusted to 3.0 with ortho phosphoric acid) and acetonitrile (65:35) was used as mobile phase. The run time was 10 min and the retention time was 5.8 min. The mobile phase was delivered at a flow rate of 1 ml/min, the injection volume was 20 µl and the effluent was monitored at ultraviolet detection at 210 nm. Data processing was done using Spinchrom CFR (Spinchotech, Japan).

3.4.2 Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of asenapine maleate in 10 ml of Acetonitrile (1000 µg/ml). From this stock solution, 1 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 100 µg/ml.

3.4.3 Calibration plot of Asenapine maleate in mobile phase

Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 25-5000 ng/ml. These standards were analyzed by HPLC at UV detection wavelength 210 nm and mobile phase flow rate 1.0 ml/min. The calibration curve was plotted for the measured area against drug concentration.

3.5 HPLC METHOD FOR ESTIMATION OF ASENAPINE MALEATE IN PLASMA

Human Plasma was obtained from Suraktam Blood Bank, Vadodara, India. Calibration plot of Asenapine maleate in plasma was prepared in concentration in the range (25-5000 ng/ml). The blank plasma samples were spiked with stock solution prepared in acetonitrile (100 µg/ml) to get concentration in above range. The protein precipitation was carried out by addition of acetonitrile. For 0.5 ml of plasma sample, 1.5 ml of acetonitrile was used. The separation of precipitate from organic phase was achieved by centrifugation (4000 rpm X 15 min). The organic phase was separated, filtered using syringe filter and injected using Hamilton HPLC Syringe.

The mobile phase consisted of mixture of buffer (phosphate buffer, 6.9 g sodium dihydrogen phosphate in 1000 ml of double distilled water, 1 ml Triethylamine and pH adjusted to 3.0 with ortho phosphoric acid) and acetonitrile (65:35). The run time was 10 min and the retention time was 5.2 min. The mobile phase was delivered at a flow rate of 1 ml/min, the injection volume was 20 µl and the effluent was monitored at ultraviolet detection at 210 nm. Data processing was done using Spinchrom CFR (Spinchotech, Japan). Calibration curve was plotted against peak area of curve vs. drug concentration.

3.6 ESTIMATION OF LURASIDONE HCl BY UV SPECTROSCOPY

3.6.1 Calibration plot in methanol

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of methanol (1000 µg/ml). From this stock solution, 1 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 100 µg/ml. Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 5-30 µg/ml. The absorbance of these solutions was measured using UV-Visible Spectrophotometer. The calibration curve was plotted for the measured absorbance against drug concentration (6).

3.6.2 Calibration plot in chlorofom:methanol (1:1)

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of chloroform:methanol (1000 µg/ml). From this stock solution, 1 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 100 µg/ml. Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 10-70 µg/ml. The absorbance of these solutions was measured using UV- Visible Spectrophotometer.

3.6.3 Calibration plot in 0.1 N HCl

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of 0.1 N HCl (1000 µg/ml). From this stock solution, 1 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 100 µg/ml. Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 4-20 µg/ml. The absorbance of these solutions was measured using UV- Visible Spectrophotometer.

3.6.4 Calibration plot in phosphate buffer pH 6.8

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of phosphate buffer pH 6.8 (1000 µg/ml) containing 0.1% Sodium lauryl sulphate (SLS). From this stock solution, 1 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 100 µg/ml. Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 20-100 µg/ml. The absorbance of these solutions was measured using UV- Visible Spectrophotometer.

3.6.5 Calibration plot in phosphate buffer pH 7.4

Standard stock solution was prepared by dissolving 10 mg of drug in 10 ml of phosphate buffer pH 7.4 (1000 µg/ml) containing 0.1% SLS. From this stock solution, 1 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 100 µg/ml. Suitable aliquots were measured and diluted accordingly to make different concentrations in the range of 20-100 µg/ml. The absorbance of these solutions was measured using UV- Visible Spectrophotometer.

3.7 ESTIMATION OF LURASIDONE HCl USING HPLC

3.7.1 HPLC conditions

Quantitative estimation of lurasidone HCl was done by HPLC as described in literature with slight modifications (7).

The HPLC system (Shimadzu, Japan) composed of a UV-visible spectrophotometric detector. The separation was performed on a C-18 HPLC column. A filtered and degassed mixture of buffer (phosphate buffer, 1.36 g potassium dihydrogen phosphate in 1000 ml of double distilled water and pH adjusted to 3.0 with ortho phosphoric acid) and acetonitrile (55:45) was used as mobile phase. The run time was 10 min and the retention time was 6.1 min. The mobile phase was delivered at a flow rate of 1 ml/min, the injection volume was 20 µl and the effluent was monitored at ultraviolet detection at 235 nm. Data processing was done using Spinchrom CFR (Spinchotech, Japan).

3.7.2 Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of lurasidone HCl in 10 ml of Acetonitrile (1000 µg/ml). From this stock solution, 1 ml was transferred in volumetric flask and further diluted to 10 ml to produce concentration of 100 µg/ml.

3.7.3 Calibration plot of Lurasidone HCl in mobile phase

Suitable aliquots of were measured and diluted accordingly to make different concentrations in the range of 50-5000 ng/ml. These standards were analyzed by HPLC at UV detection wavelength 235 nm and mobile phase flow rate 1.0 ml/min. The calibration curve was plotted for the measured area against drug concentration. Analytical method was validated for linearity, precision, and accuracy as described in section 3.3.

3.8 HPLC METHOD FOR ESTIMATION OF LURASIDONE HCl IN PLASMA

Human plasma was obtained from Suraktam Blood Bank, Vadodara, India. Calibration plot of lurasidone HCl in plasma was prepared in concentration in the range of 50 to 5000 ng/ml. The blank plasma samples were spiked with stock solution prepared in acetonitrile (100 µg/ml) to get concentration in above range. The protein precipitation was done by addition of acetonitrile. For 0.5 ml of plasma sample, 1.5 ml of acetonitrile was used. The separation of precipitate from organic phase was

achieved by centrifugation (4000 rpm X 15 min). The organic phase was separated, filtered using syringe filter and injected using Hamilton HPLC Syringe.

The mobile phase consisted of mixture of buffer (phosphate buffer, 1.36 g potassium dihydrogen phosphate in 1000 ml of double distilled water and pH adjusted to 3.0 with ortho phosphoric acid) and acetonitrile (55:45). The run time was 10 min and the retention time was 6.1 min. The mobile phase was delivered at a flow rate of 1 ml/min, the injection volume was 20 μl and the effluent was monitored at ultraviolet detection at 235 nm. Data processing was done using Spinchrom CFR (Spinchotech, Japan). Analytical method was validated for linearity, precision, and accuracy as described in section 3.3.

3.9 ANALYTICAL METHOD VALIDATION

3.9.1 Linearity

It is defined as its ability to elicit tests that directly, or by a well defined mathematical transformations proportional to the concentration of analyte in samples within a given range. The linearity was determined by analyzing independent levels of calibration curve in the selected concentration range. Calibration curve of absorbance vs concentration was plotted and correlation coefficient and regression line equation for was determined.

3.9.2 Precision assay

It is defined as the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample. The precision of assay was determined by repeatability (intraday), intermediate precision (interday) and reported as the % relative standard deviation (%RSD). %RSD of the data obtained were calculated with the formula:

$$\%RSD = \frac{Standard\ deviation}{average} * 100$$

3.9.3 Accuracy

It is defined as the closeness of the test results obtained by that method to the true value. Accuracy was determined using following equation:

$$\%Accuracy = \frac{mean\ observed\ concentration}{Actual\ concentration} * 100$$

3.9.4 Limit of Detection (LOD)

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under standard experimental condition.

LOD was calculated using the following formula:

$$LOD = 3.3\,(\sigma|S)$$

Where σ is Standard deviation of the y- intercept and S is slope of the calibration curve.

3.9.5 Limit of Quantification (LOQ)

The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under standard experimental condition.

LOQ was calculated using the following formula:

$$LOQ = 10\,(\sigma|S)$$

Where σ is Standard deviation of the y-intercept and S is slope of the calibration curve.

3.10 RESULTS AND DISCUSSION

3.10.1 ESTIMATION OF ASENAPINE MALEATE BY UV SPECTROSCOPY

3.10.1.1 Calibration Curve in methanol

Asenapine maleate in methanol showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 270 nm in methanol and Beer's law was obeyed between 20-200 μg/ml (Table 3.1). The overlay plot of Asenapine maleate in methanol is shown in figure 3.1. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.005x + 0.0032$ and correlation coefficient (R^2) was found to be 0.9998 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.2).

Table 3.1: Standard Calibration data of Asenapine maleate in methanol

Sr. No.	Concentration (μg/ml)	Absorbance
1.	20	0.107±0.004
2.	40	0.206±0.004
3.	60	0.306±0.003
4.	80	0.406±0.005
5.	100	0.502±0.003
6.	120	0.607±0.007
7.	140	0.712±0.005
8.	160	0.808±0.006
9	180	0.905±0.005
10.	200	1.019±0.007
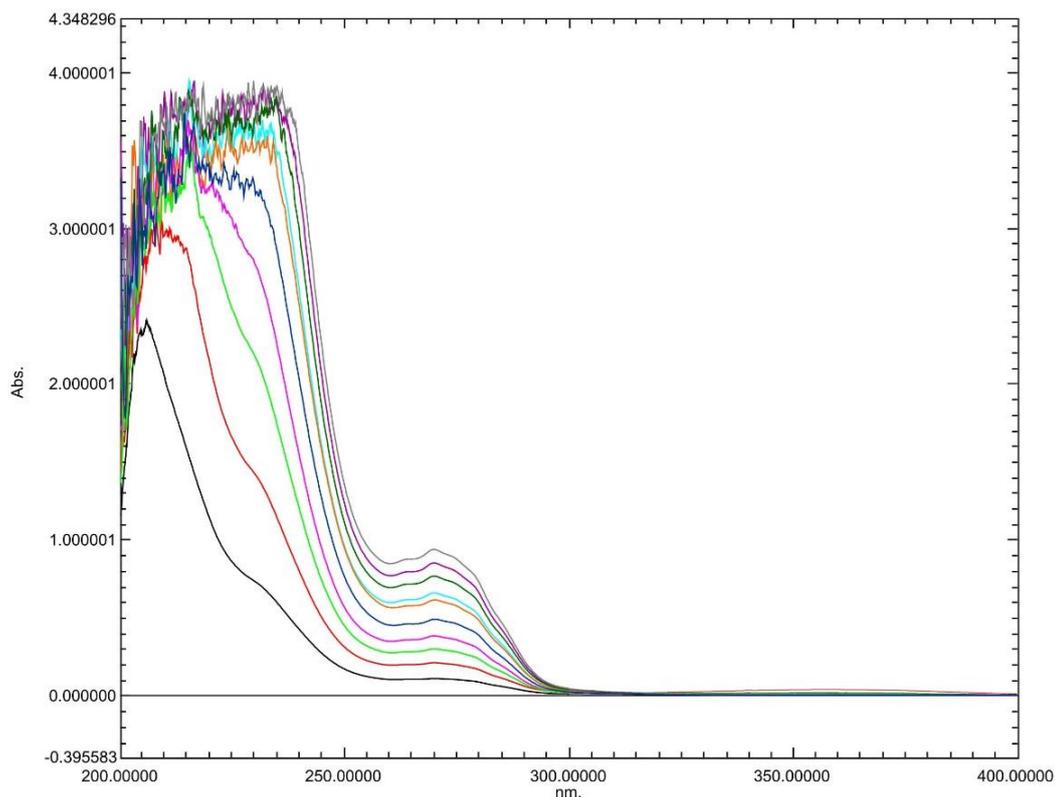
Figure 3.1: Overlay plot of Asenapine maleate in Methanol
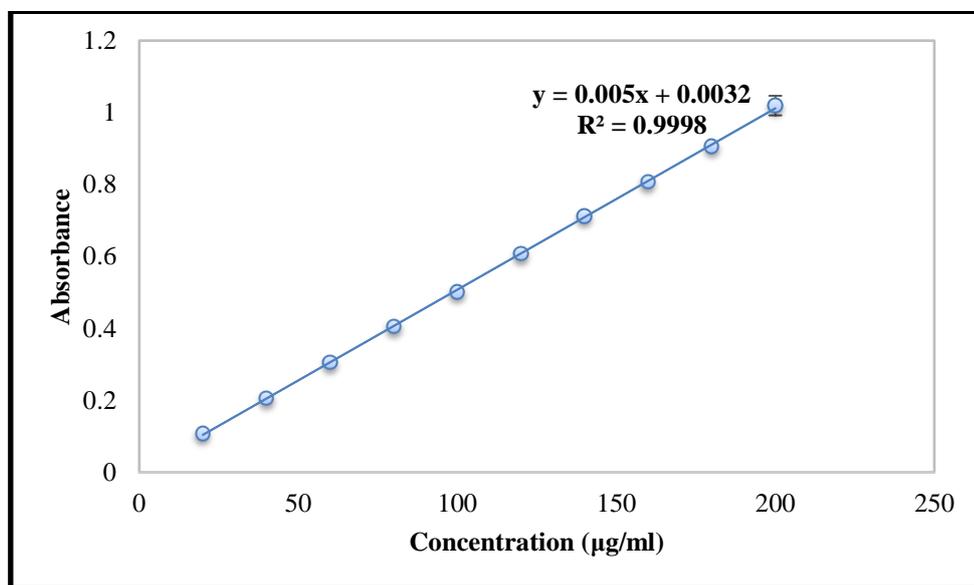

$y = 0.005x + 0.0032$
$R^2 = 0.9998$

Figure 3.2: Standard calibration curve of Asenapine maleate in Methanol

3.10.1.1.1 VALIDATION

The analytical method for estimation of Asenapine maleate in methanol was validated for different parameters of analytical method validation.

3.10.1.1.1.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Asenapine maleate in concentration range of 20 to 200 µg/ml for methanol. The summarized parameters for regression equation and correlation are given in Table 3.2.

Table 3.2: Regression analysis of Asenapine maleate in methanol

Parameters	Results
λ_{max}	270 nm
Linearity range	20 to 200 µg/ml
Regression equation (y = a + bc)	$y = 0.005x + 0.0032$
Correlation coefficient ($R^{2)}$	0.9998

3.10.1.1.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.3. The results reveal that the proposed method is accurate and precise.

Table 3.3: Accuracy and precision for Asenapine maleate in methanol

Standard concentration (µg/ml)		Precision		Accuracy
Actual	observed	Interday	Intraday	(%)
20	19.92±0.140	0.703	1.262	99.60
120	120.01±0.206	0.171	0.456	100.09
200	199.95±0.147	0.147	0.236	99.81

3.10.1.1.1.3 LOD and LOQ

The LOD and LOQ for Asenapine maleate in methanol was found to be 2.807 and 8.506 µg/ml respectively.

3.10.1.2 Calibration curve in chlorofrom:methanol

Asenapine maleate in chloroform:methanol showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 270 nm in methanol and Beer's law was obeyed between 20-140 µg/ml (Table 3.4). The overlay plot of Asenapine maleate in chloroform:methnol is shown in figure 3.3. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0059x + 0.00534$ and correlation coefficient

(R^2) was found to be 0.9991 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.4).

Table 3.4: Standard Calibration data of Asenapine maleate in chloroform:methanol

Sr. No.	Concentration (µg/ml)	Absorbance
1.	20	0.163±0.003
2.	40	0.284±0.004
3.	60	0.425±0.006
4.	80	0.534±0.004
5.	100	0.645±0.005
6.	120	0.767±0.005
7.	140	0.881±0.005
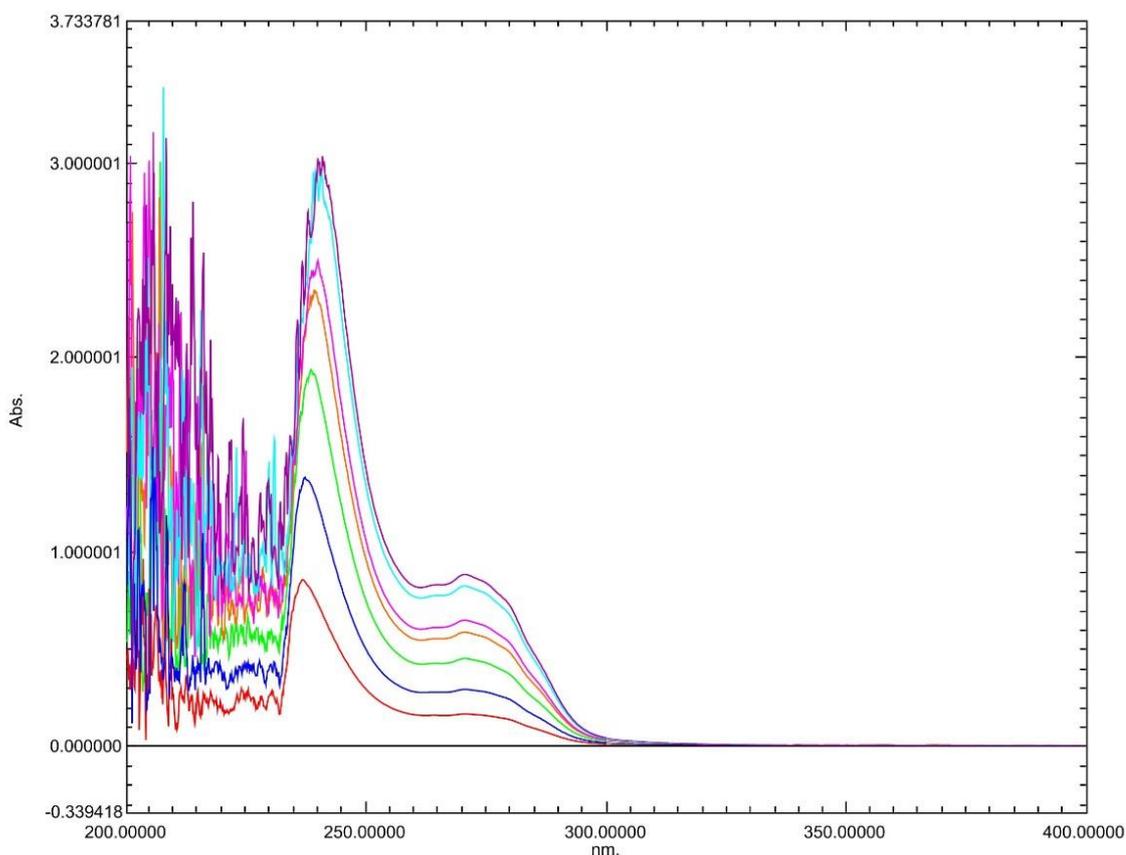
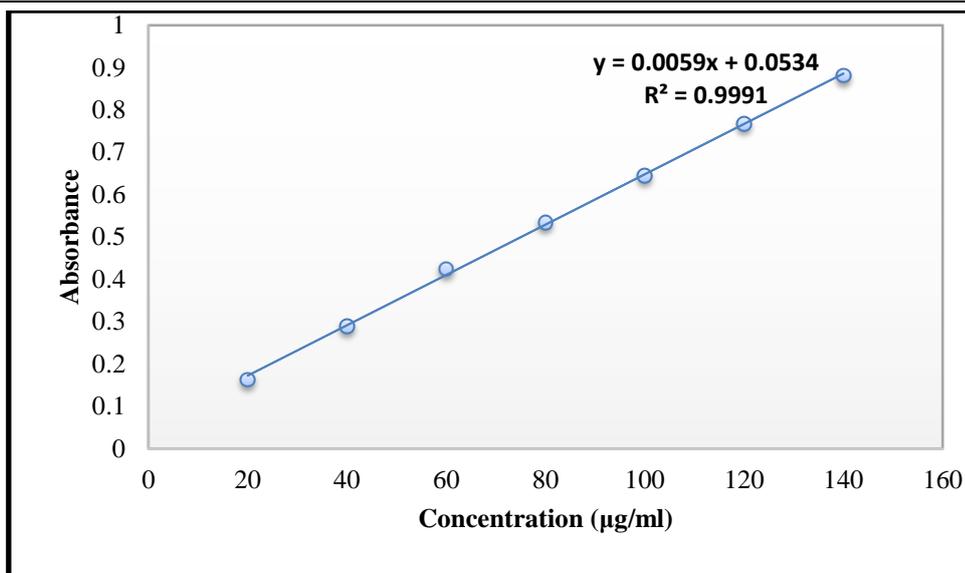**Figure 3.3: Overlay plot of Asenapine maleate in chloroform:Methanol**

Figure 3.4: Standard calibration curve of Asenapine maleate in Chloroform: Methanol

3.10.1.2.1 VALIDATION

The analytical method for estimation of Asenapine maleate in chloroform:methanol was validated for different parameters of analytical method validation.

3.10.1.2.1.1 Linearity

Linear correlation was obtained as per Beer- Lambert law between absorbance and concentrations of Asenapine maleate in concentration range of 20 to 140 μg/ml for chloroform:methanol. The summarized parameters for regression equation and correlation are given in Table 3.5.

Table 3.5: Regression analysis of Asenapine maleate in chloroform:methanol

Parameters	Results
λ_{max}	270 nm
Linearity range	20 to 140 μg/ml
Regression equation (y = a + bc)	$y = 0.0059x + 0.00534$
Correlation coefficient ($R^{2)}$)	0.9991

3.10.1.2.1.2 Accuracy and precision assay

The results of accuracy and precision are shown in table 3.6. The results reveal that the proposed method is accurate and precise.

Table 3.6: Accuracy and precision for Asenapine maleate in chloroform:methanol

Standard concentration (µg/ml)		Precision		Accuracy
Actual	observed	Interday	Intraday	(%)
20	19.98±0.314	1.573	1.193	99.90
80	79.68±0.496	0.622	0.834	99.60
140	140.23±0.229	0.163	0.726	100.16

3.10.1.2.1.3 LOD and LOQ

The LOD and LOQ for Asenapine maleate in chloroform:methanol was found to be 4.766 and 14.441 µg/ml respectively.

3.10.1.3 Calibration curve in 0.1 N HCl

Asenapine maleate in 0.1 N HCl showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 270 nm in 0.1 N HCl and Beer's law was obeyed between 40-200 µg/ml (Table 3.7). The overlay plot of Asenapine maleate in 0.1 N HCl is shown in figure 3.5. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0039x + 0.0022$ with Correlation coefficient (R^2) was found to be 0.9991 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.6).

Table 3.7: Standard Calibration data of Asenapine maleate in 0.1 N HCl

Sr. No.	Concentration (µg/ml)	Absorbance
1.	40	0.159±0.004
2.	60	0.233±0.007
3.	80	0.314±0.003
4.	100	0.362±0.007
5.	120	0.545±0.014
6.	140	0.555±0.005
7.	160	0.614±0.004
8.	180	0.696±0.006
9	200	0.782±0.007
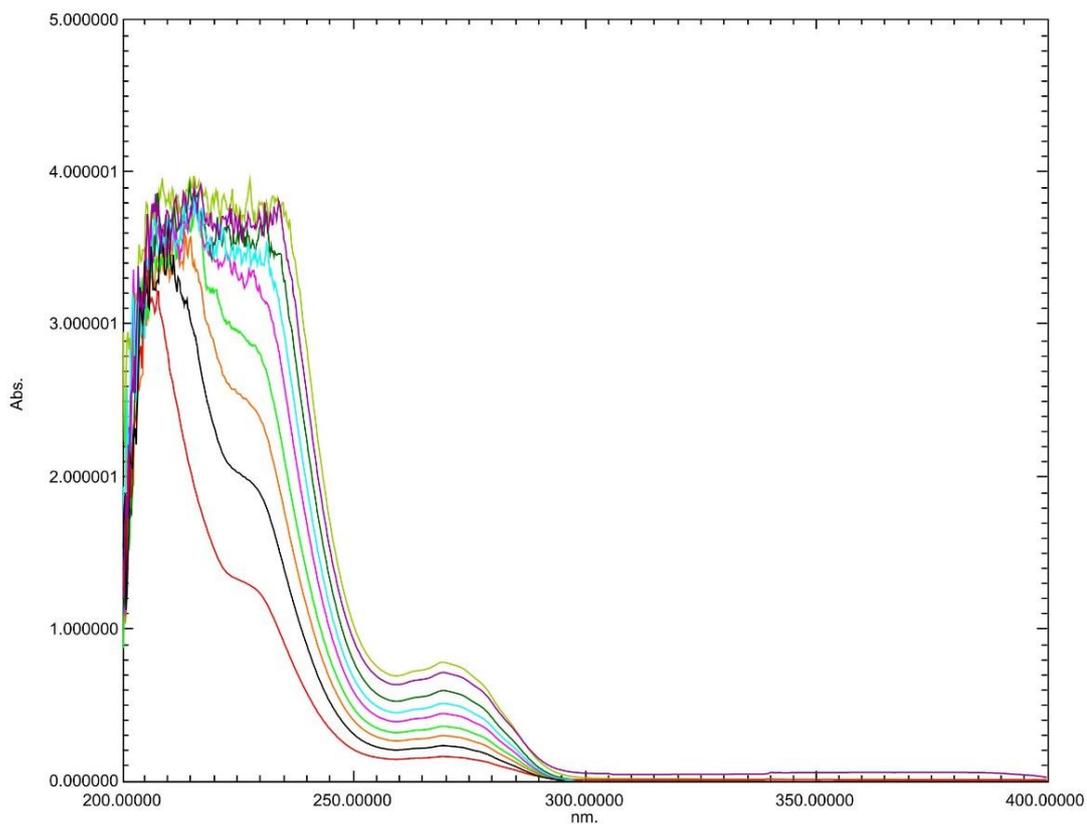
Figure 3.5: Overlay plot of Asenapine maleate in 0.1 N HCl
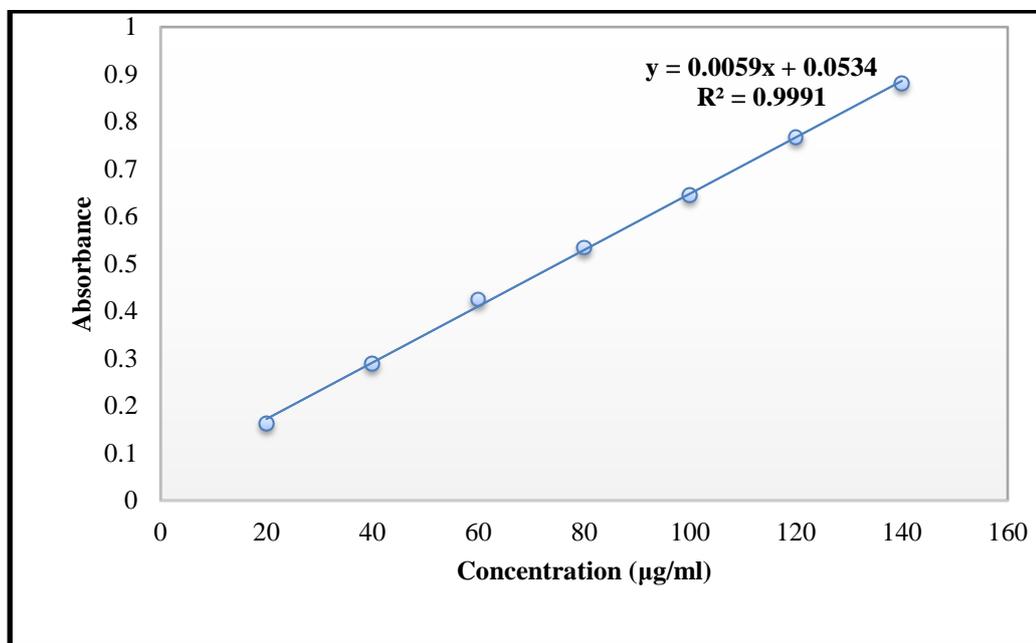

y = 0.0059x + 0.0534
R² = 0.9991

Figure 3.6: Standard calibration curve of Asenapine maleate in 0.1 N HCl

3.10.1.3.1 VALIDATION

The analytical method for estimation of Asenapine maleate in 0.1 N HCl was validated for different parameters of analytical method validation.

3.10.1.3.1.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Asenapine maleate in concentration range of 40 to 200 µg/ml for 0.1 N HCl. The summarized parameters for regression equation and correlation are given in Table 3.8.

Table 3.8: Regression analysis of Asenapine maleate in 0.1 N HCl

Parameters	Results
λ_{max}	270 nm
Linearity range	40 to 200 µg/ml
Regression equation (y = a + bc)	$y = 0.0039x + 0.0022$
Correlation coefficient ($R^{2)}$)	0.9991

3.10.1.3.1.2 Accuracy and precision assay

The results of accuracy and precision are shown in table 3.9. The results reveal that the proposed method is accurate and precise.

Table 3.9: Accuracy and precision for Asenapine maleate in 0.1 N HCl

Standard concentration (µg/ml)		Precision		Accuracy
Actual	observed	Interday	Intraday	(%)
40	39.87±0.160	0.402	0.820	99.68
120	120.46±0.350	0.291	0.339	100.38
200	202.14±1.040	0.514	0.840	101.07

3.10.1.3.1.3 LOD and LOQ

The LOD and LOQ for Asenapine maleate in 0.1 N HCl was found to be 5.692 and 17.250 µg/ml respectively.

3.10.1.4 Calibration curve in phosphate Buffer pH 6.8

Asenapine maleate in phosphate buffer pH 6.8 showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 269 nm in methanol and Beer's law was obeyed between 40-320 µg/ml (Table 3.10). The overlay plot of Asenapine maleate in phosphate buffer pH 6.8 is shown in figure 3.7. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0032x + 0.0019$ and correlation

coefficient (R^2) was found to be 0.9992 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.8).

Table 3.10: Standard Calibration data of Asenapine maleate in phosphate buffer pH 6.8

Sr. No.	Concentration (µg/ml)	Absorbance
1.	40	0.130±0.004
2.	80	0.257±0.006
3.	120	0.384±0.006
4.	160	0.501±0.007
5.	200	0.628±0.007
6.	240	0.759±0.005
7.	280	0.870±0.007
8.	320	1.027±0.005
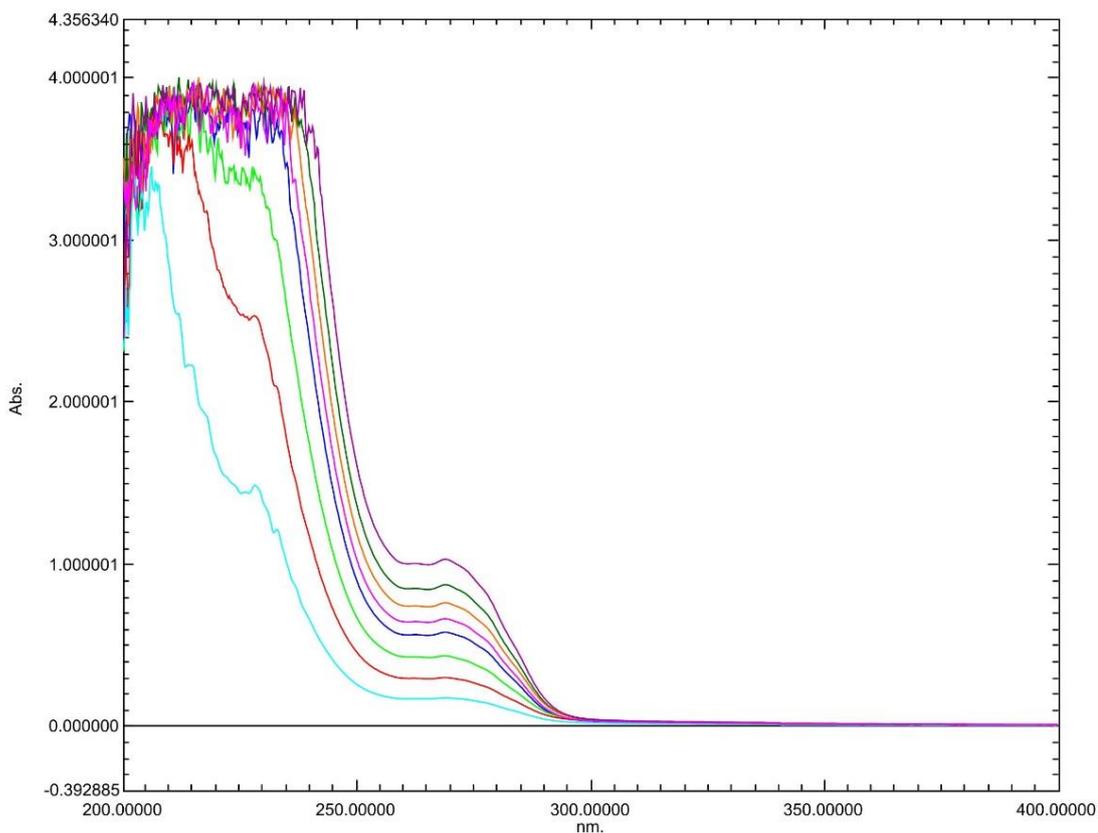
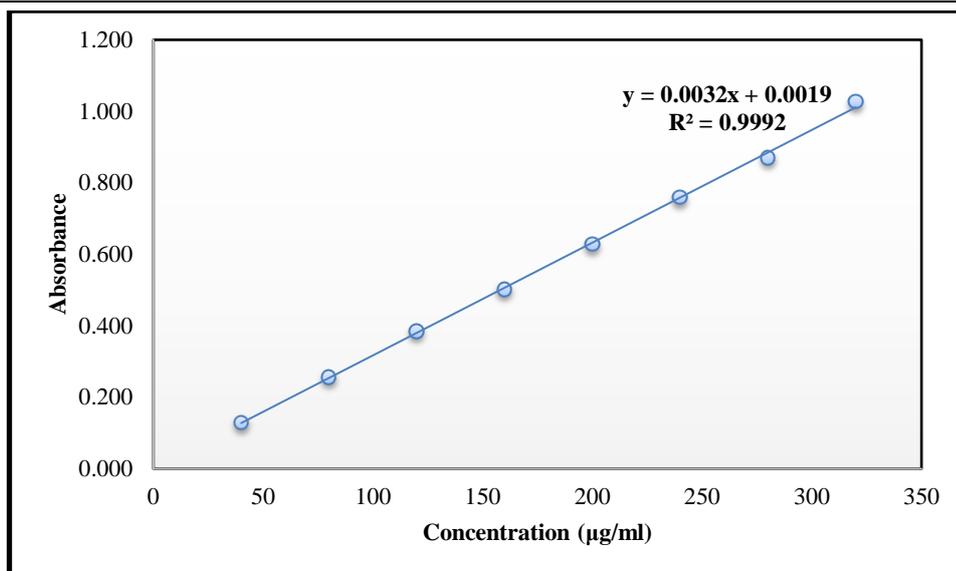**Figure 3.7: Overlay plot of Asenapine maleate in phosphate buffer pH 6.8**

Figure 3.8 Standard calibration curve of Asenapine maleate in phosphate buffer pH 6.8

3.10.1.4.1 VALIDATION

The analytical method for estimation of Asenapine maleate in phosphate buffer pH 6.8 was validated for different parameters of analytical method validation.

3.10.1.4.1.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Asenapine maleate in concentration range of 40 to 320 μg/ml for phosphate buffer pH 6.8. The summarized parameters for regression equation and correlation are given in table 3.11.

Table 3.11: Regression analysis of Asenapine maleate in phosphate buffer pH 6.8

Parameters	Results
λ_{max}	269 nm
Linearity range	40 to 320 μg/ml
Regression equation (y = a + bc)	$y = 0.0032x + 0.0019$
Correlation coefficient ($R^{2)}$)	0.9992

3.10.1.4.1.2 Accuracy and precision assay

The results of accuracy and precision are shown in table 3.12. The results reveal that the proposed method is accurate and precise.

Table 3.12: Accuracy and precision for Asenapine maleate in phosphate buffer pH 6.8

Standard concentration (µg/ml)		Precision		Accuracy (%)
Actual	observed	Interday	Intraday	
40	39.88±0.257	0.644	1.551	99.80
180	179.76±0.221	0.317	2.569	99.53
320	319.82±0.325	0.102	0.261	100.03

3.10.1.4.1.3 LOD and LOQ

The LOD and LOQ for Asenapine maleate in phosphate buffer pH 6.8 was found to be 10.047 and 30.447 µg/ml respectively.

3.10.1.5 Calibration curve in phosphate buffer pH 7.4

Asenapine maleate in phosphate buffer pH 7.4 showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 269 nm in methanol and Beer's law was obeyed between 40-280 µg/ml (Table 3.13). The overlay plot of Asenapine maleate in phosphate buffer pH 7.4 is shown in figure 3.9. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0032x - 0.0069$ and correlation coefficient (R^2) was found to be 0.9993 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.10).

Table 3.13: Standard Calibration data of Asenapine maleate in phosphate buffer pH 7.4

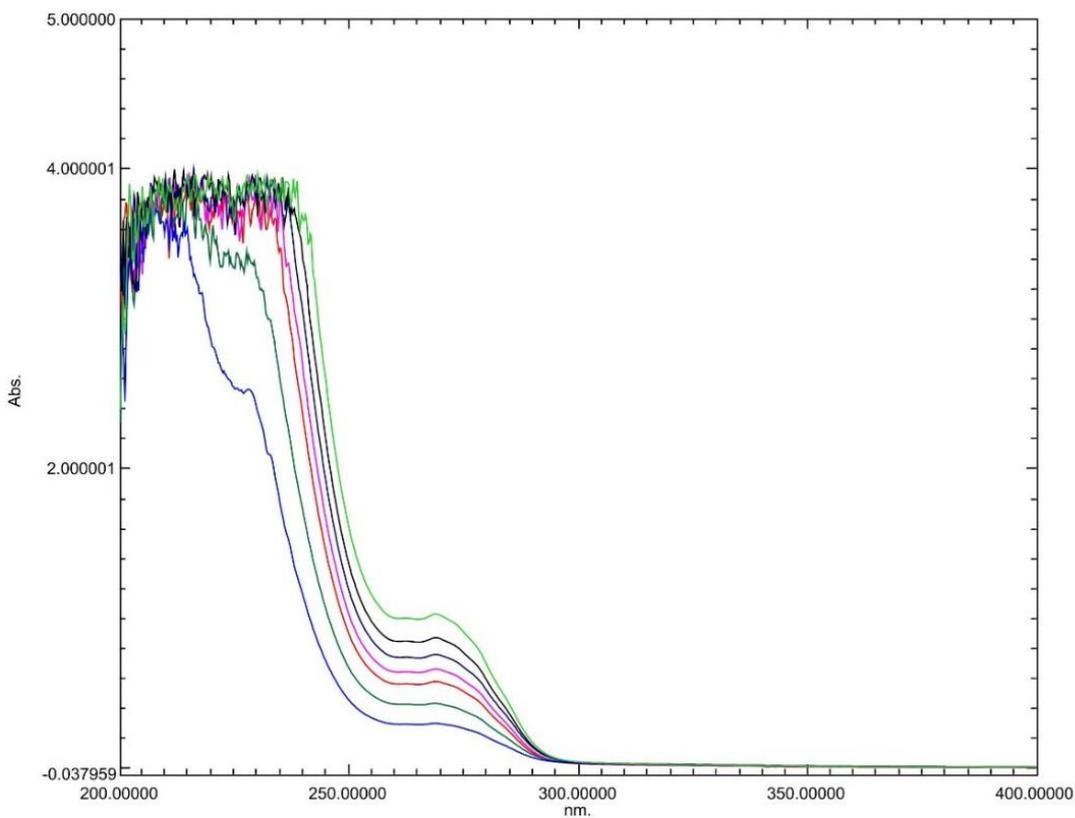| Sr. No.	Concentration (µg/ml)	Absorbance
1.	40	0.125±0.006
2.	80	0.250±0.005
3.	120	0.368±0.008
4.	160	0.495±0.004
5.	200	0.621±0.006
6.	240	0.745±0.009
7.	280	0.891±0.010

Figure 3.9: Overlay plot of Asenapine maleate in phosphate buffer pH 7.4
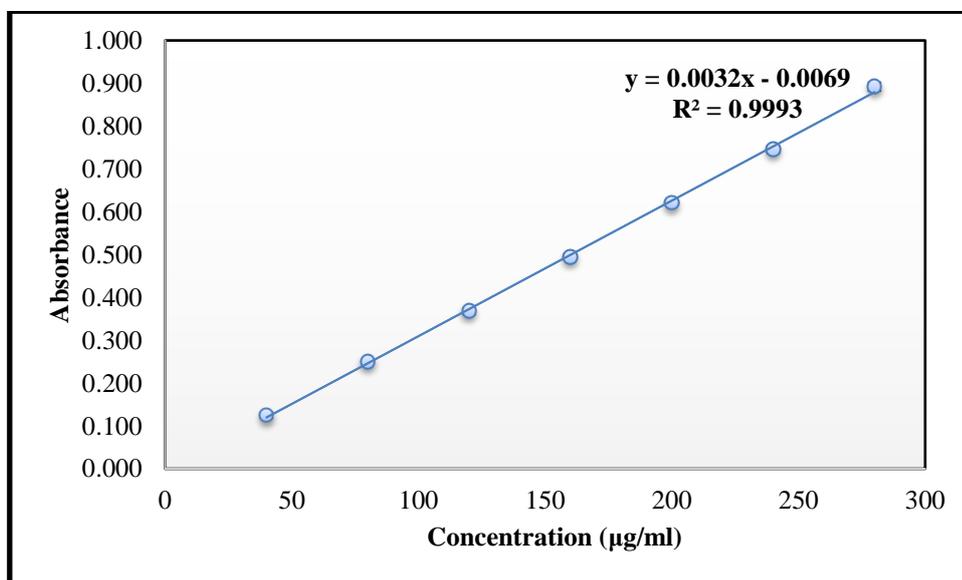

Figure 3.10: Standard calibration curve of Asenapine maleate in phosphate

buffer pH 7.4

3.10.1.5.1 VALIDATION

The analytical method for estimation of Asenapine maleate in phosphate buffer pH 7.4 was validated for different parameters of analytical method validation.

3.10.1.5.1.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Asenapine maleate in concentration range of 60 to 280 μg/ml for phosphate buffer pH 7.4. The summarized parameters for regression equation and correlation are given in table 3.14.

Table 3.14: Regression analysis of Asenapine maleate in phosphate buffer pH 7.4

Parameters	Results
λ_{max}	269 nm
Linearity range	40 to 280 μg/ml
Regression equation (y = a + bc)	$y = 0.0032x - 0.0069$
Correlation coefficient ($R^{2)}$	0.9993

3.10.1.5.1.2 Accuracy and precision assay

The results of accuracy and precision are shown in table 3.15. The results reveal that the proposed method is accurate and precise.

Table 3.15: Accuracy and precision for Asenapine maleate in phosphate buffer pH 7.4

Standard concentration (μg/ml)		Precision		Accuracy (%)
Actual	observed	Interday	Intraday	
40	39.84±0.322	0.809	1.551	99.05
160	159.76±0.570	0.357	0.848	99.47
280	278.82±1.705	0.611	1.455	100.04

3.10.1.5.1.3 LOD and LOQ

The LOD and LOQ for Asenapine maleate in phosphate buffer pH 7.4 was found to be 8.505 and 25.774 μg/ml respectively.

3.10.2 CALIBRATION CURVE OF ASENAPINE MALEATE IN PHOSPHATE BUFFER:ACN

The retention time of Asenapine maleate was 5.8 min. The standard plot of Asenapine maleate in buffer: ACN is shown in Table 3.16. The overlay plot of HPLC chromatogram is shown in figure 3.11. Regression equation for standard curve was $y = 0.1003x + 0.0313$ and correlation coefficient (R^2) was found to be 0.9998 signifying that a linear relationship existed between peak area and concentration of the drug (Figure 3.12).

Table 3.16: Standard Calibration data for estimation of Asenapine maleate in phosphate buffer:ACN

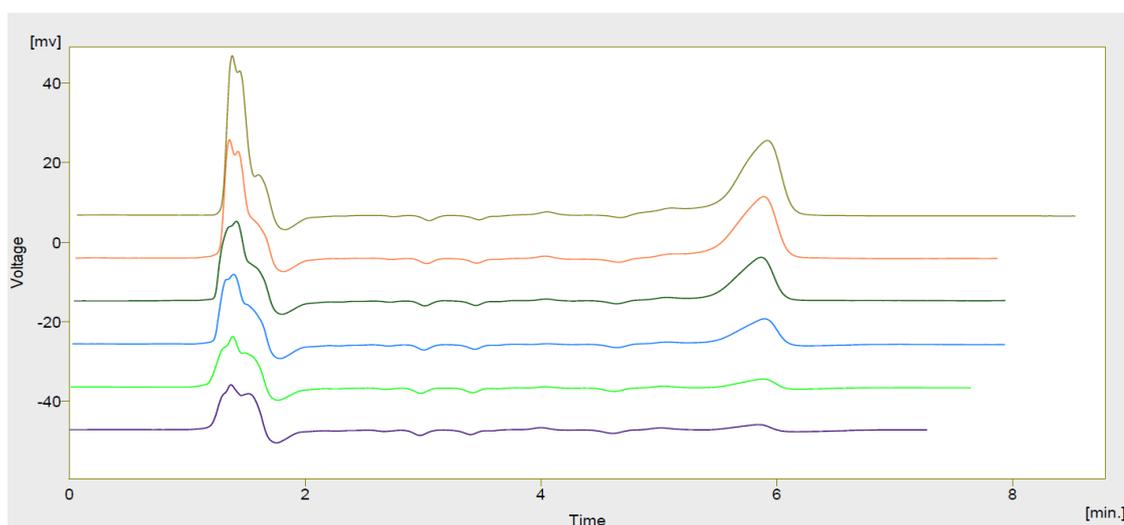| Sr. No.	Concentration (µg/ml)	Peak Area (mV.s)	Retention time (min)
1.	25	2.50±4.56	5.83
2.	100	10.07±3.12	5.84
3.	500	50.13±8.76	5.85
4.	1000	100.32±9.23	5.84
5.	2500	251.23±10.44	5.86
6.	5000	501.58±9.24	5.84

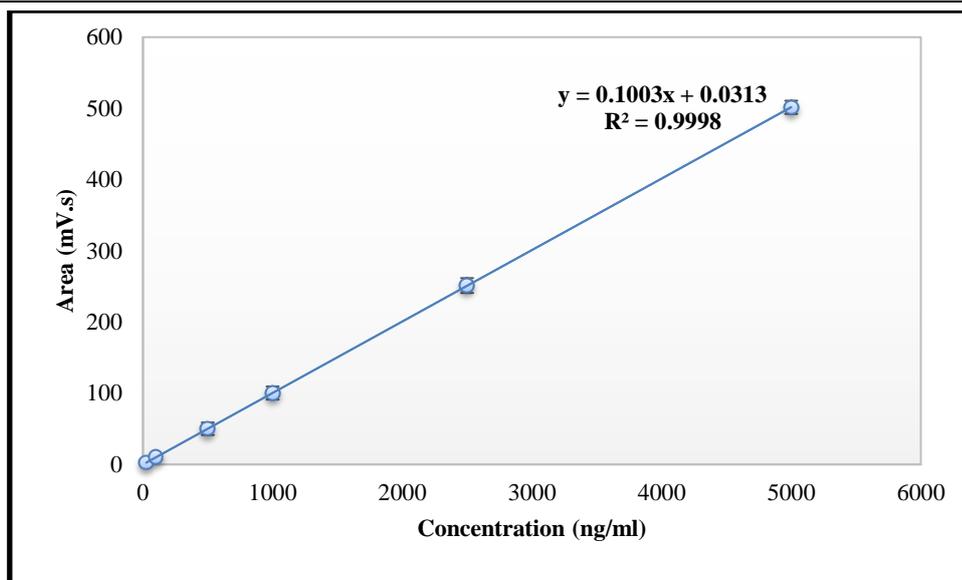**Figure 3.11: Overlay plot of Asenapine maleate in Phosphate buffer:ACN**

Figure 3.12: Calibration curve of Asenapine maleate in phosphate buffer:ACN

3.10.2.1 VALIDATION

The analytical method for estimation of Asenapine maleate in phosphate buffer: ACN was validated for different parameters of analytical method validation.

3.10.2.1.1 Linearity

Linear correlation was obtained for Asenapine maleate in concentration range of 25 to 5000 ng/ml. The summarized parameters for regression equation and correlation are given in table 3.17.

Table 3.17: Regression analysis of Asenapine maleate in phosphate buffer:ACN

Parameters	Results
Retention time (min)	5.8
Linearity range	25 to 5000 ng/ml
Regression equation (y = a + bc)	$y = 0.1003x + 0.0313$
Correlation coefficient (R^2)	0.9998

3.10.2.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.18. The results reveal that the proposed method is accurate and precise.

Table 3.18: Accuracy and precision for Asenapine maleate in phosphate buffer:ACN

Standard concentration (ng/ml)		Precision		Accuracy
Actual	observed	Interday	Intraday	(%)
25	24.79±0.115	0.465	1.828	99.16
1000	996.03±6.178	0.620	0.798	99.60
5000	4988.30±9.94	0.199	0.235	99.77

3.10.2.1.3 LOD and LOQ

The LOD and LOQ for Asenapine maleate in phosphate buffer:ACN was found to be 6.394 and 19.375 ng/ml respectively.

3.10.3 CALIBRATION CURVE FOR ESTIMATION OF ASENAPINE MALEATE IN PLASMA

The retention time of Asenapine maleate was 5.2 min. The standard plot of Asenapine maleate in is shown in figure 3.19. The overlay plot of HPLC chromatogram is shown in figure 3.13. Regression equation for standard curve was $y = 0.0888x - 0.0416$ with and correlation coefficient (R^2) was found to be 0.9999 signifying that a linear relationship existed between peak area and concentration of the drug (Figure 3.14).

Table 3.19: Standard Calibration data for estimation of Asenapine maleate in plasma

Sr. No.	Concentration (ng/ml)	Peak Area (mV.s)	Retention time (min)
1.	25	2.20±3.56	5.26
2.	100	8.92±4.17	5.26
3.	500	44.41±12.76	5.24
4.	1000	88.77±9.23	5.23
5.	2500	221.74±15.44	5.22
6.	5000	444.19±17.24	5.22
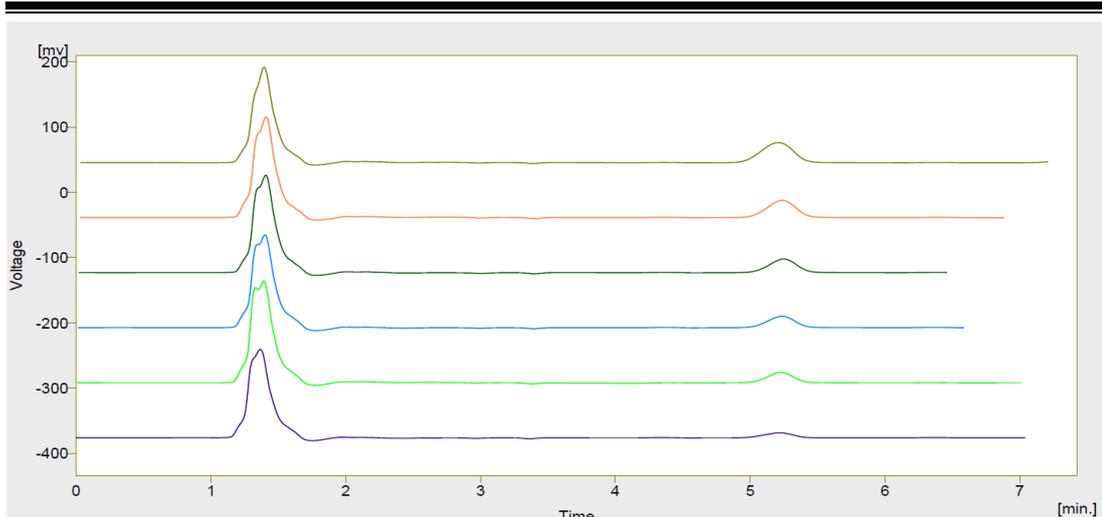
Figure 3.13: Overlay plot of Asenapine maleate in plasma

$$y = 0.0888x - 0.0416$$
$$R^2 = 0.9999$$

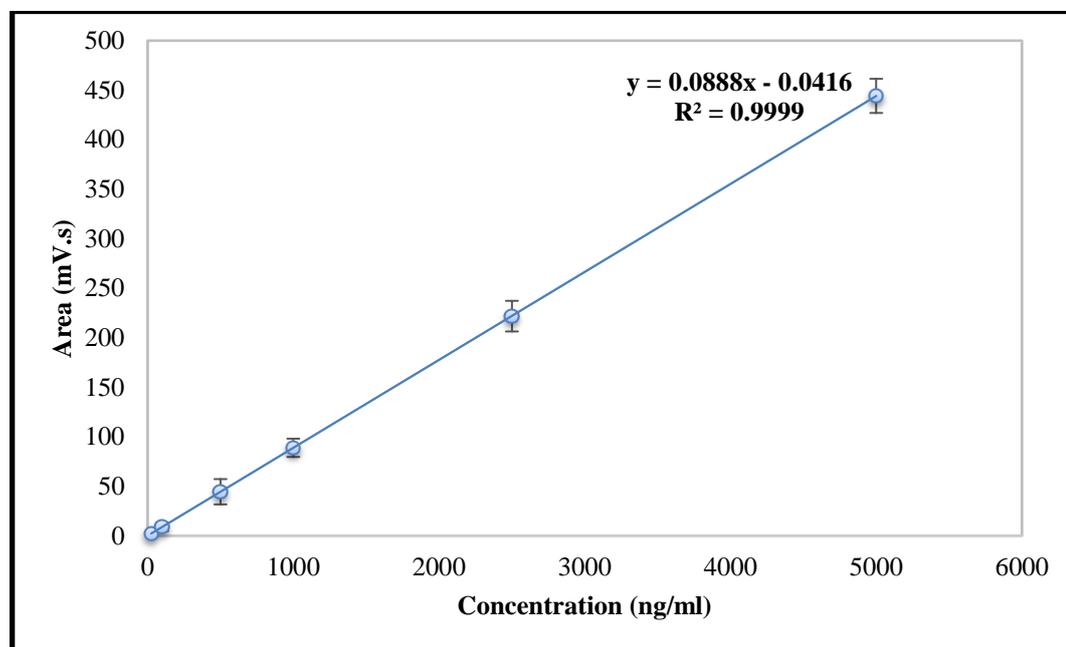**Figure 3.14: Calibration curve of Asenapine maleate in plasma**

3.10.3.1 VALIDATION

The analytical method for estimation of Asenapine maleate in plasma was validated for different parameters of analytical method validation.

3.10.3.1.1 Linearity

Linear correlation was obtained for Asenapine maleate in concentration range of 25 to 5000 ng/ml in plasma. The summarized parameters for regression equation and correlation are given in table 3.20.

Table 3.20: Regression analysis of Asenapine maleate in plasma

Parameters	Results
Retention time (min)	5.2
Linearity range	25-5000 ng/ml
Regression equation (y = a + bc)	$y = 0.0888x - 0.0416$
Correlation coefficient ($R^{2)}$)	0.9999

3.10.3.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.21. The results reveal that the proposed method is accurate and precise.

Table 3.21: Accuracy and precision for Asenapine maleate in plasma

Standard concentration (ng/ml)		Precision		Accuracy
Actual	observed	Interday	Intraday	(%)
25	24.59±0.373	1.518	2.188	98.36
1000	993.70±10.22	1.028	1.376	99.37
5000	4960.30±18.10	00.365	0.818	99.21

3.10.3.1.3 LOD and LOQ

The LOD and LOQ for Asenapine maleate in plasma was found to be 5.77 and 17.48 ng/ml respectively.

3.10.4 ESTIMATION OF LURASIDONE HCl BY UV SPECTROSCOPY

3.10.4.1 Calibration curve of Lurasidone HCl in methanol

Lurasidone HCl in methanol showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 230 nm in methanol and Beer's law was obeyed between 5-30 μg/ml (Table 3.22). The overlay plot of Lurasidone HCl in methanol is shown in figure 3.15. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0315x + 0.0082$ and correlation coefficient (R^2) was found to be 0.9996 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.16).

Table 3.22: Standard Calibration data of Lurasidone HCl in methanol

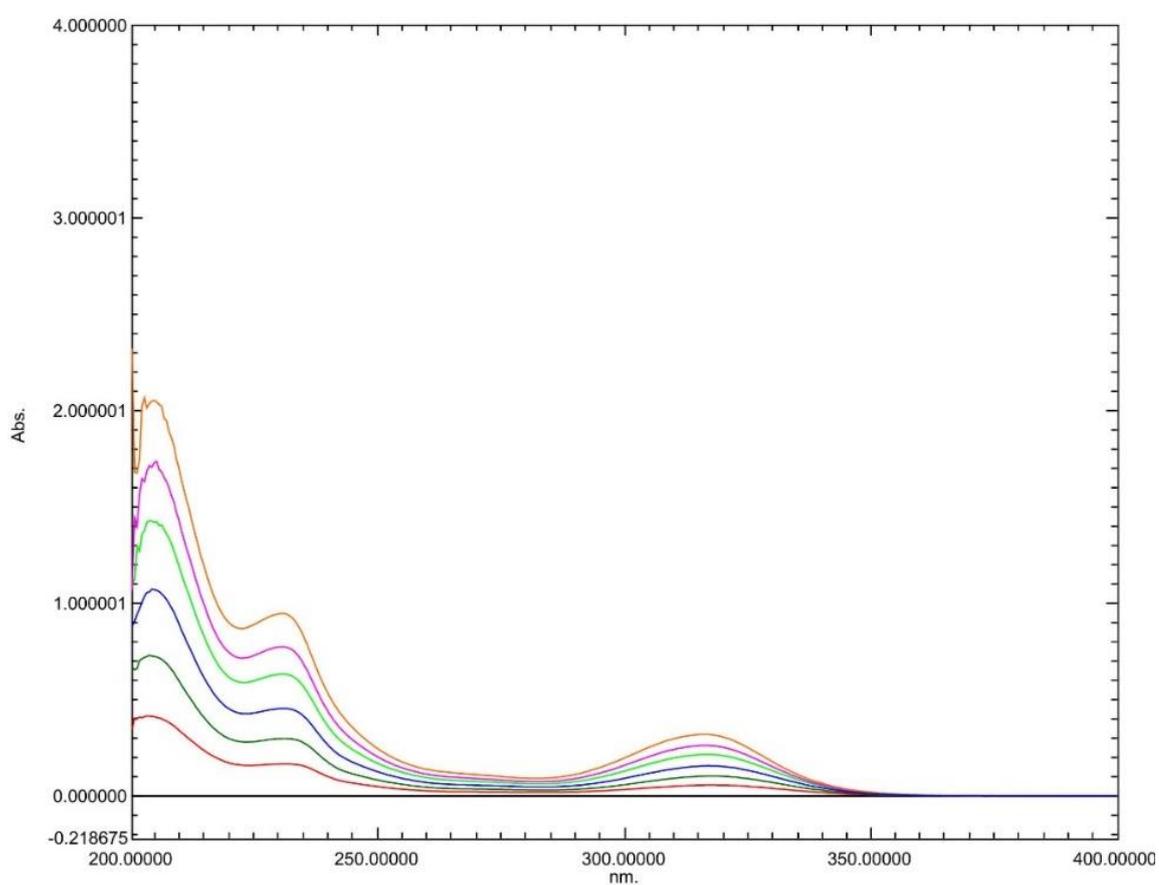| Sr. No.	Concentration (µg/ml)	Absorbance
1.	5	0.160±0.003
2.	10	0.330±0.005
3.	15	0.482±0.003
4.	20	0.632±0.003
5.	25	0.801±0.003
6.	30	0.949±0.004

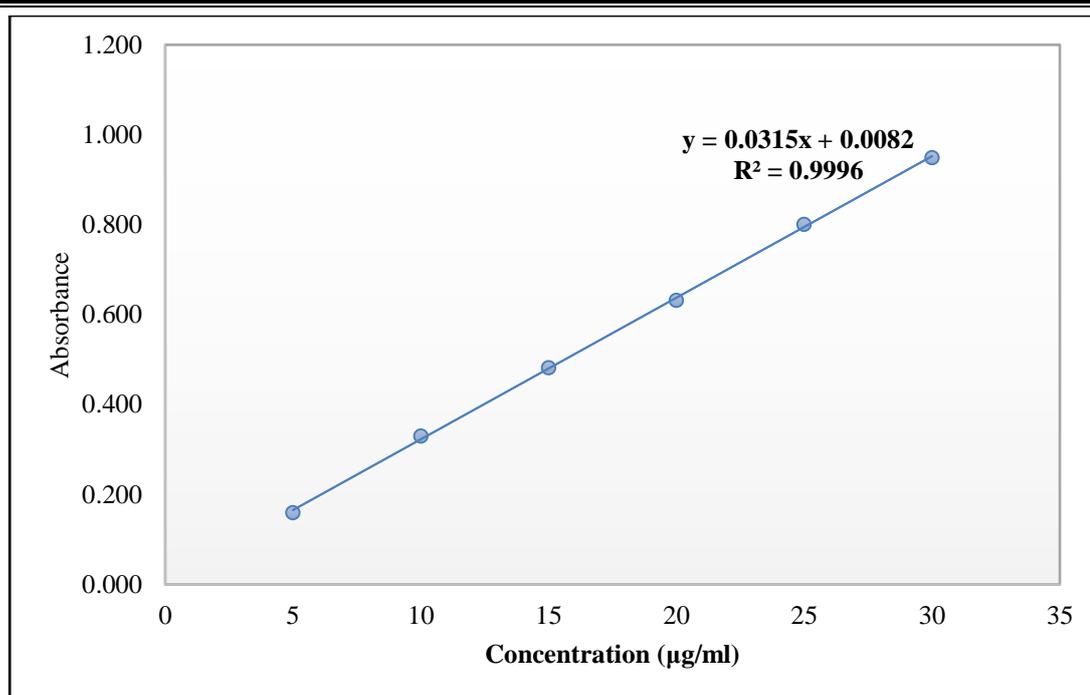**Figure 3.15: Overlay plot of Lurasidone HCl in methanol**

Figure 3.16: Standard calibration curve of Lurasidone HCl in methanol

3.10.4.1.1 VALIDATION

The analytical method for estimation of Lurasidone HCl in methanol was validated for different parameters of analytical method validation.

3.10.4.1.1.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Lurasidone HCl in concentration range of 5 to 30 μg/ml for methanol. The summarized parameters for regression equation and correlation are given in Table 3.23.

Table 3.23: Regression analysis of Lurasidone HCl in methanol

Parameters	Results
λ_{max}	230 nm
Linearity range	5 to 30 μg/ml
Regression equation (y = a + bc)	$y = 0.0315x + 0.0082$
Correlation coefficient ($R^{2)}$)	0.9996

3.10.4.1.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.24. The results reveal that the proposed method is accurate and precise.

Table 3.24: Accuracy and precision for Lurasidone HCl in methanol

Standard concentration (µg/ml)		Precision		Accuracy (%)
Actual	**observed**	**Interday**	**Intraday**	
5	4.94±0.067	1.349	2.718	98.73
15	14.89±0.064	0.432	1.335	99.29
30	30.74±0.653	2.125	2.125	102.48

3.10.4.1.1.3 LOD and LOQ

The LOD and LOQ for Lurasidone HCl in methanol was found to be 0.668 and 2.027 µg/ml respectively.

3.10.5 Calibration curve of Lurasidone HCl in chloroform: methanol

Lurasidone HCl in chloroform:methanol showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 318 nm in chloroform:methanol and Beer's law was obeyed between 10-70 µg/ml (Table 3.25). The overlay plot of Lurasidone HCl in chloroform:methanol is shown in figure 3.17. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0143x + 0.0183$ and correlation coefficient (R^2) was found to be 0.9987 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.18).

Table 3.25: Standard Calibration data of Lurasidone HCl in chloroform:methanol

Sr. No.	Concentration (µg/ml)	Absorbance
1.	10	0.167±0.003
2.	20	0.308±0.003
3.	30	0.435±0.004
4.	40	0.582±0.003
5.	50	0.747±0.005
6.	60	0.892±0.006
7.	70	1.012±0.003
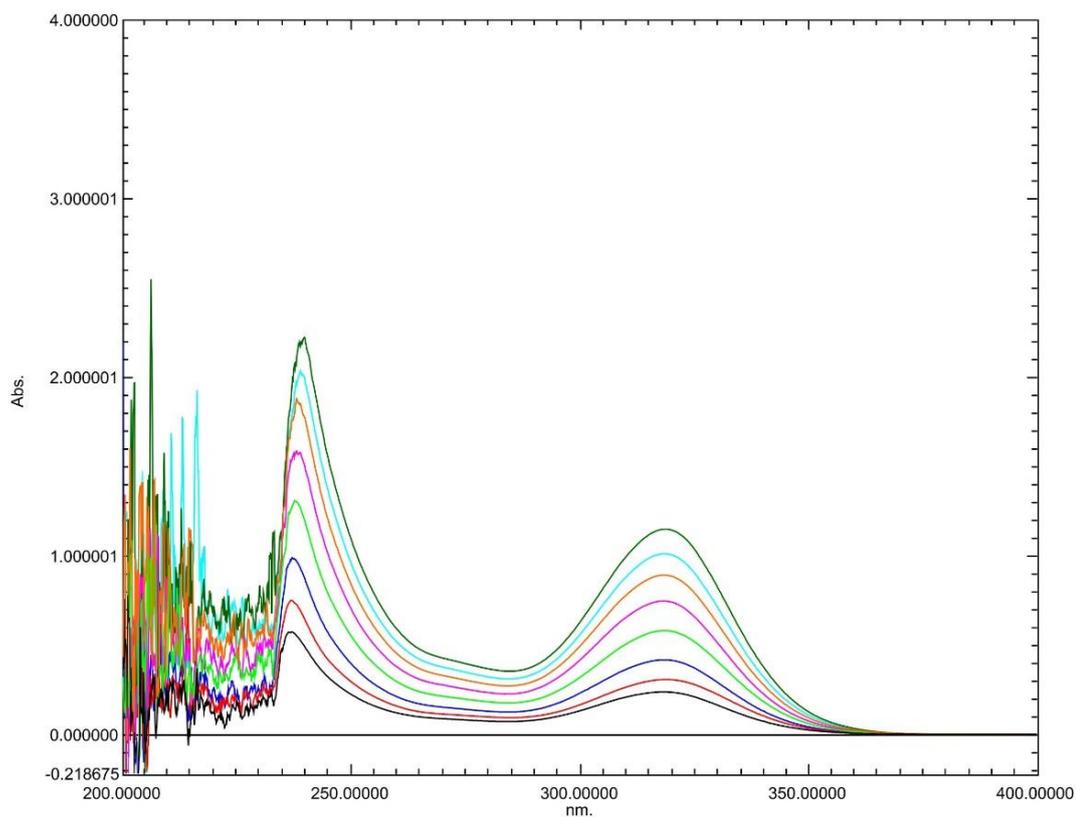
Figure 3.17: Standard calibration curve of Lurasidone HCl in chlorofom:methanol
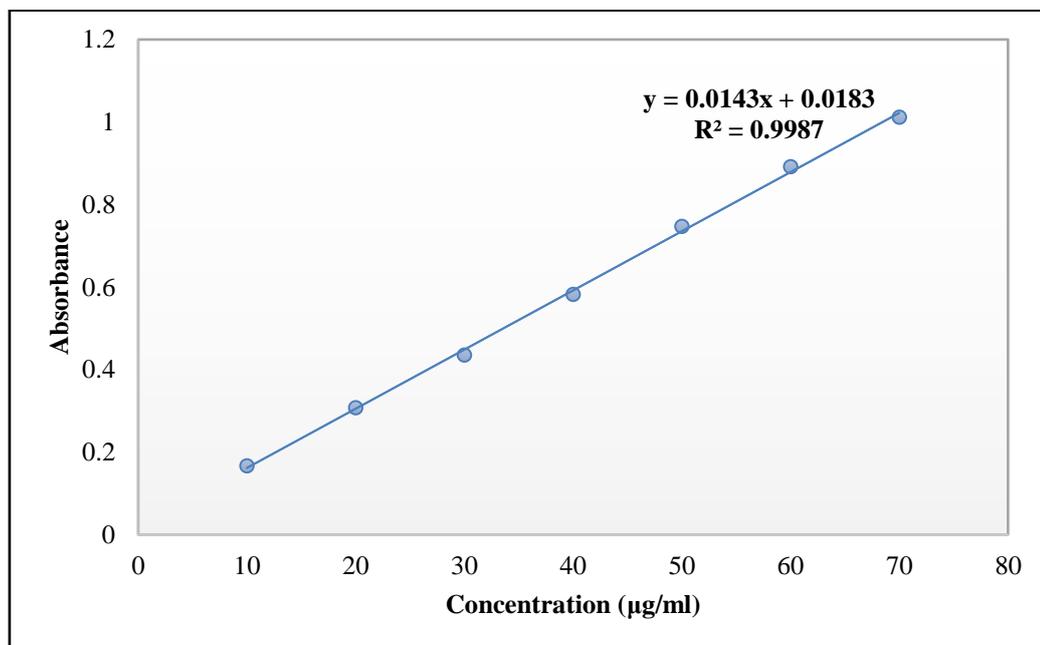

$y = 0.0143x + 0.0183$
$R^2 = 0.9987$

Figure 3.18: Standard calibration curve of Lurasidone HCl in chlorofom:methanol

3.10.5.1 VALIDATION

The analytical method for estimation of Lurasidone HCl in Chlorofom:Methanol was validated for different parameters of analytical method validation.

3.10.5.1.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Lurasidone HCl in concentration range of 10 to 70 µg/ml for chloroform:methanol. The summarized parameters for regression equation and correlation are given in table 3.26.

Table 3.26: Regression analysis of Lurasidone HCl in chlorofom:methanol

Parameters	Results
λ_{max}	318 nm
Linearity range	10 to 70 µg/ml
Regression equation (y = a + bc)	$y = 0.0143x + 0.0183$
Correlation coefficient (R[2)])	0.9987

3.10.5.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.27. The results reveal that the proposed method is accurate and precise.

Table 3.27: Accuracy and precision of Lurasidone HCl in chlorofom:methanol

Standard concentration (µg/ml)		Precision		Accuracy
Actual	observed	Interday	Intraday	(%)
10	9.97±0.070	0.704	2.251	99.73
40	40.29±0.817	2.027	2.568	100.73
70	69.85±0.159	0228	0.806	99.79

3.10.5.1.3 LOD and LOQ

The LOD and LOQ for Lurasidone HCl in chloroform: methanol was found to be 2.786 and 8.441 µg/ml respectively.

3.10.6 CALIBRATION CURVE OF LURASIDONE HCl IN 0.1 N HCl

Lurasidone HCl in 0.1 N HCl showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 230 nm in 0.1 N HCl and Beer's law was obeyed between 4-20 µg/ml (Table 3.28). The overlay plot of Lurasidone HCl in 0.1 N HCl is shown in figure 3.19. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0499x + 0.0243$ and correlation coefficient (R[2]) was found to be

0.9993 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.20).

Table 3.28: Standard Calibration data of Lurasidone HCl in 0.1 N HCl

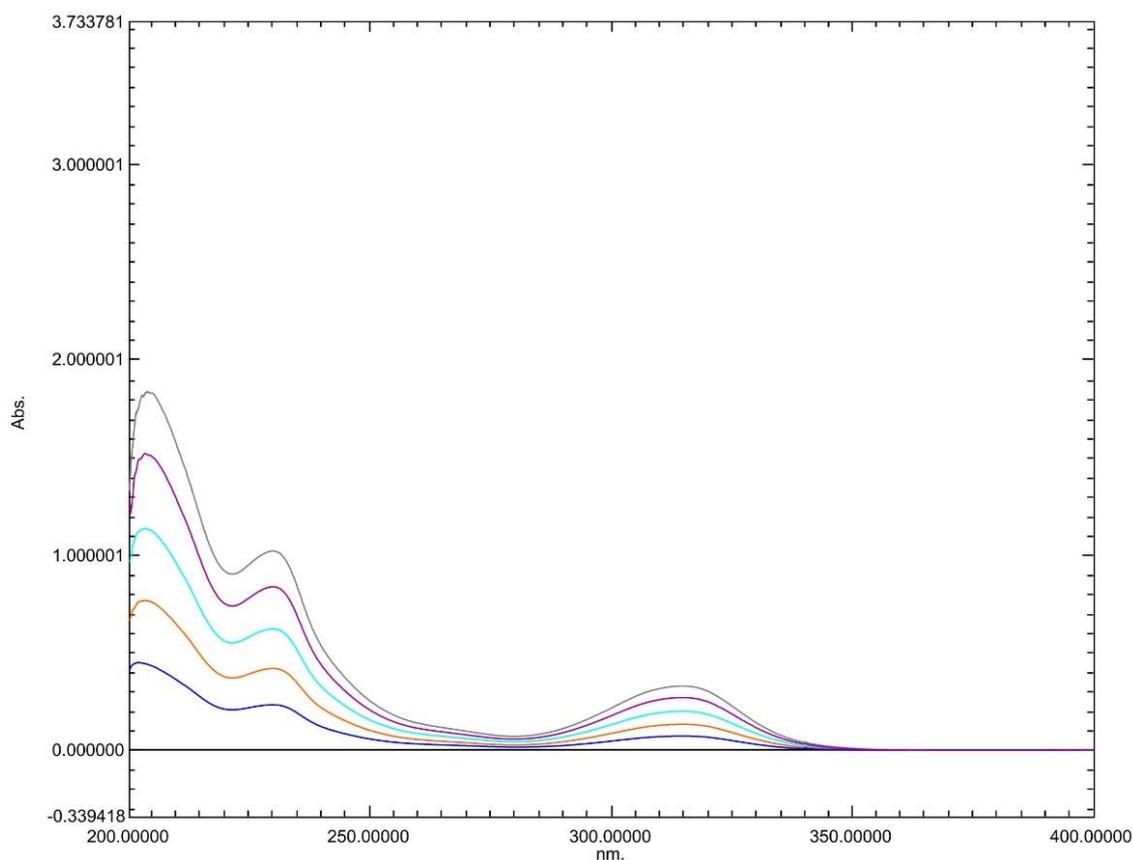| Sr. No.	Concentration (µg/ml)	Absorbance
1.	4	0.229±0.005
2.	8	0.416±0.004
3.	12	0.619±0.003
4.	16	0.835±0.004
5.	20	1.018±0.004

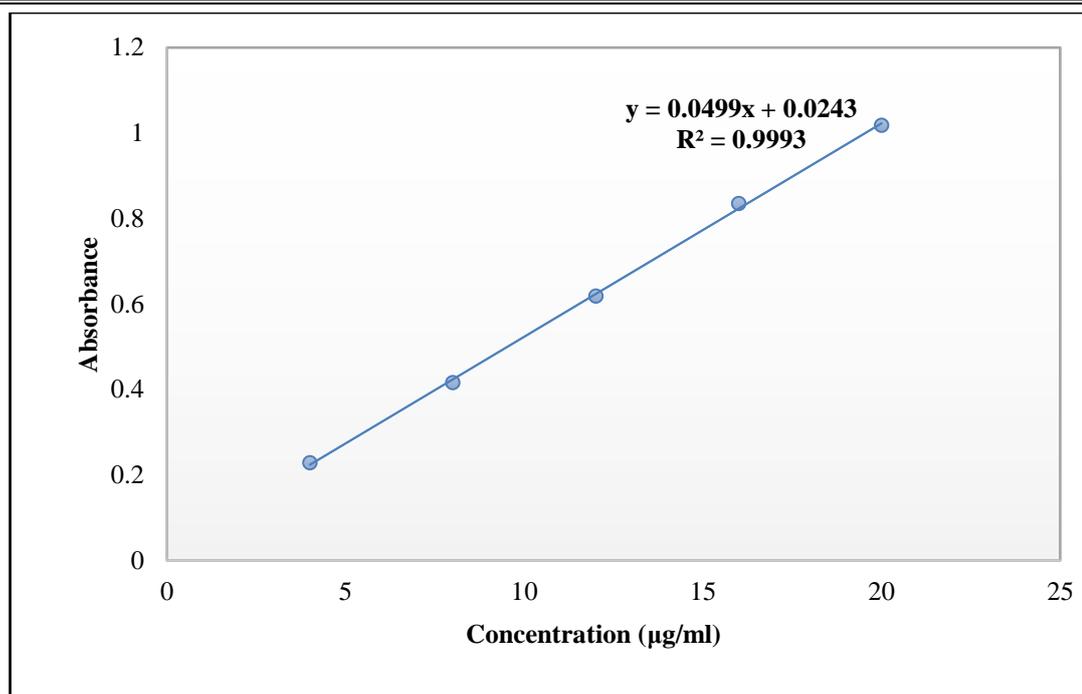**Figure 3.19: Overlay plot of Lurasidone HCl in 0.1 N HCl**

Figure 3.20: Standard calibration curve of Lurasidone HCl in 0.1 N HCl

3.10.6.1 VALIDATION

The analytical method for estimation of Lurasidone HCl in 0.1N HCl was validated for different parameters of analytical method validation.

3.10.6.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Lurasidone HCl in concentration range of 4 to 20 µg/ml for 0.1N HCl. The summarized parameters for regression equation and correlation are given in Table 3.29.

Table 3.29: Regression analysis of Lurasidone HCl in 0.1N HCl

Parameters	Results
λ_{max}	230 nm
Linearity range	4 to 20 µg/ml
Regression equation (y = a + bc)	$y = 0.0499x + 0.0243$
Correlation coefficient ($R^{2)}$)	0.9993

3.10.6.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.30. The results reveal that the proposed method is accurate and precise.

Table 3.30: Accuracy and precision for Lurasidone HCl in 0.1N HCl

Standard concentration (µg/ml)		Precision		Accuracy (%)
Actual	**observed**	**Interday**	**Intraday**	
2	2.01±0.053	2.633	3.521	100.50
10	10.00±0.051	0.513	1.032	100.00
20	20.03±0.085	0.427	1.161	100.15

3.10.6.3 LOD and LOQ

The LOD and LOQ for Lurasidone HCl in 0.1N HCl was found to be 0.625 and 1.894 µg/ml respectively.

3.10.7 CALIBRATION CURVE OF LURASIDONE HCl IN PHOSPHATE BUFFER pH 6.8

Lurasidone HCl in phosphate buffer pH 6.8 showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 233 nm phosphate buffer pH 6.8 and Beer's law was obeyed between 20 - 100 µg/ml (Table 3.31). The overlay plot of Lurasidone HCl in phosphate buffer pH 6.8 is shown in figure 3.21. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0116x - 0.0063$ and correlation coefficient (R^2) was found to be 0.9987 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.22).

Table 3.31: Standard Calibration data of Lurasidone HCl in phosphate buffer pH 6.8

Sr. No.	Concentration (µg/ml)	Absorbance
1.	20	0.221±0.002
2.	40	0.447±0.006
3.	60	0.709±0.004
4.	80	0.924±0.004
5.	100	1.140±0.053
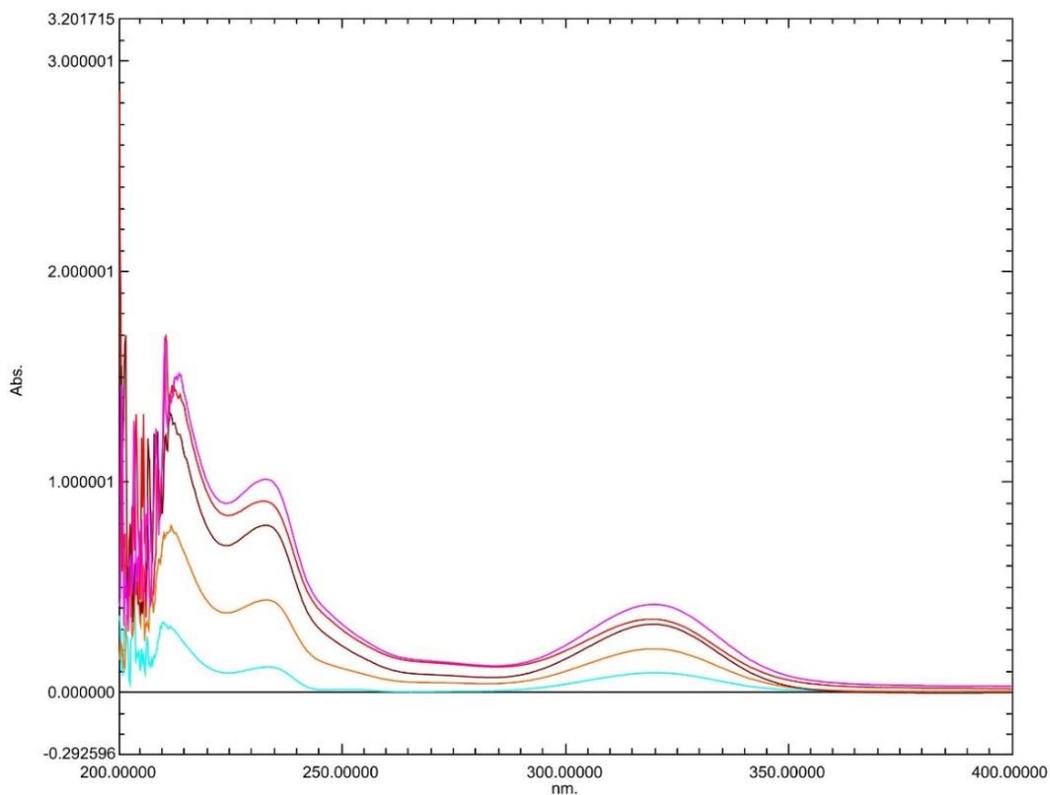
Figure 3.21: Overlay plot of Lurasidone HCl in phosphate buffer pH 6.8

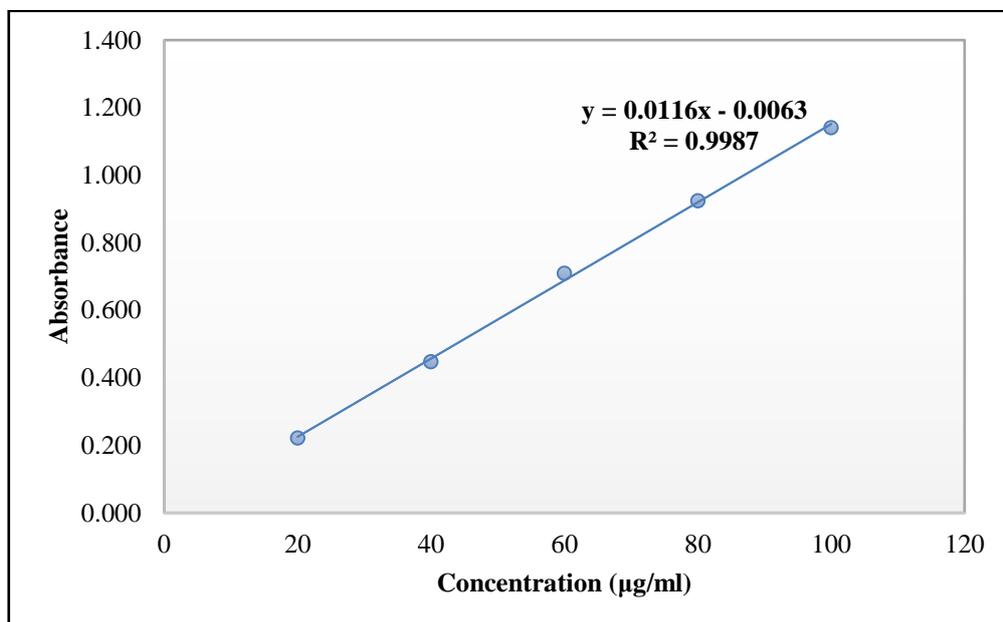y = 0.0116x - 0.0063
$R^2 = 0.9987$

Figure 3.22: Standard Calibration curve of Lurasidone HCl in phosphate buffer pH 6.8

3.10.7.1 VALIDATION

The analytical method for estimation of Lurasidone HCl in phosphate buffer pH 6.8 was validated for different parameters of analytical method validation.

3.10.7.1.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Lurasidone HCl in concentration range of 20 to 100 µg/ml for phosphate buffer pH 6.8. The summarized parameters for regression equation and correlation are given in table 3.32.

Table 3.32: Regression analysis of Lurasidone HCl in phosphate buffer pH 6.8

Parameters	Results
λ_{max}	232 nm
Linearity range	20 to 100 µg/ml
Regression equation (y = a + bc)	$y = 0.0116x - 0.0063$
Correlation coefficient ($R^{2)}$)	0.9987

3.10.7.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.33. The results reveal that the proposed method is accurate and precise.

Table 3.33: Accuracy and precision for Lurasidone HCl in phosphate buffer pH 6.8

Standard concentration (µg/ml)		Precision		Accuracy
Actual	Observed	Interday	Intraday	(%)
20	19.93±0.115	0.579	1.769	99.65
60	59.74±0.323	0.540	0.666	99.57
100	100.03±0.106	0.106	0.351	99.93

3.10.7.1.3 LOD and LOQ

The LOD and LOQ for in phosphate buffer pH 6.8 was found to be 4.327 and 13.112 µg/ml respectively.

3.10.8 CALIBRATION CURVE OF LURASIDONE HCL IN PHOSPHATE BUFFER pH 7.4

Lurasidone HCl in phosphate buffer pH 7.4 showed a characteristic spectrum when scanned in ultraviolet range between 200-400 nm. The absorption maxima (λ_{max}) was found at 232 nm phosphate buffer pH 7.4 and Beer's law was obeyed between 20 - 100 µg/ml (Table 3.34). The overlay plot of Lurasidone HCl in phosphate buffer pH 7.4 is shown in figure 3.23. Regression analysis was performed on the experimental data. Regression equation for standard curve was $y = 0.0113x - 0.0179$ and correlation coefficient (R^2) was found to be 0.9992 signifying that a linear relationship existed between absorbance and concentration of the drug (Figure 3.24).

Table 3.34: Standard Calibration data of Lurasidone HCl in phosphate buffer pH 7.4

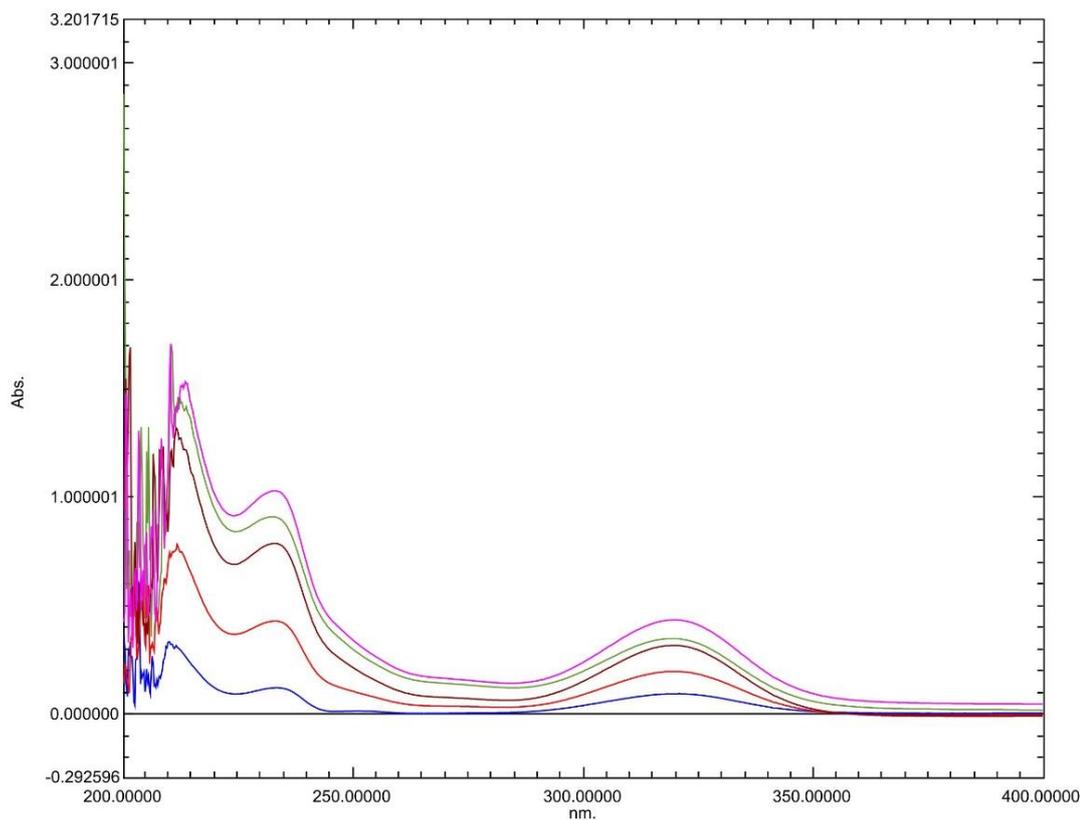| Sr. No.	Concentration (µg/ml)	Absorbance
1.	20	0.210±0.008
2.	40	0.437±0.013
3.	60	0.650±0.009
4.	80	0.904±0.016
5.	100	1.110±0.012

Figure 3.23: Overlay plot of Lurasidone HCl in phosphate buffer pH 7.4
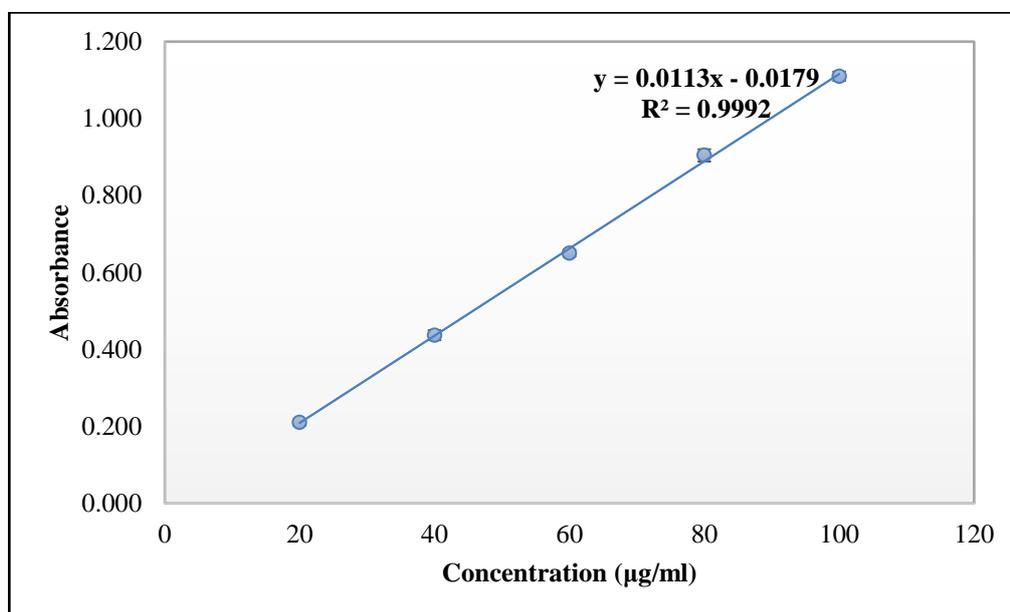

Figure 3.24: Standard Calibration curve of Lurasidone HCl in phosphate buffer

pH 7.4

3.10.8.1 VALIDATION

The analytical method for estimation of Lurasidone HCl in phosphate buffer pH 7.4 was validated for different parameters of analytical method validation.

3.10.8.1.1 Linearity

Linear correlation was obtained as per Beer-Lambert law between absorbance and concentrations of Lurasidone HCl in concentration range of 20 to 100 μg/ml for phosphate buffer pH 7.4. The summarized parameters for regression equation and correlation are given in table 3.35.

Table 3.35: Regression analysis of Lurasidone HCl in phosphate buffer pH 7.4

Parameters	Results
λ_{max}	232 nm
Linearity range	20 to 100 μg/ml
Regression equation (y = a + bc)	$y = 0.0113x - 0.0179$
Correlation coefficient ($R^{2)}$)	0.9992

3.10.8.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.36. The results reveal that the proposed method is accurate and precise.

Table 3.36: Accuracy and precision for Lurasidone HCl in phosphate buffer pH 7.4

Standard concentration (μg/ml)		Precision		Accuracy
Actual	observed	Interday	Intraday	(%)
20	19.90±0.147	0.741	1.957	99.52
60	59.58±0.525	0.882	0.904	99.57
100	99.77±0.229	0.229	0.354	99.93

3.10.8.1.3 LOD and LOQ

The LOD and LOQ for in phosphate buffer pH 7.4 was found to be 3.453 and 10.462 μg/ml respectively.

3.10.9 CALIBRATION CURVE OF LURASIDONE HCl IN PHOSPHATE BUFFER:ACN USING HPLC

The retention time of Lurasidone HCl was 6.1 min. The standard plot of Lurasidone HCl in phosphate buffer: ACN is shown in table 3.37. The overlay plot of HPLC chromatogram is shown in figure 3.25. Regression equation for standard curve $y = 0.1192x - 0.1048$ and correlation coefficient (R^2) was found to be 0.9999 signifying that a linear relationship existed between peak area and concentration of the drug (Figure 3.26).

Table 3.37: Standard Calibration data for estimation of Lurasidone HCl in phosphate buffer:ACN

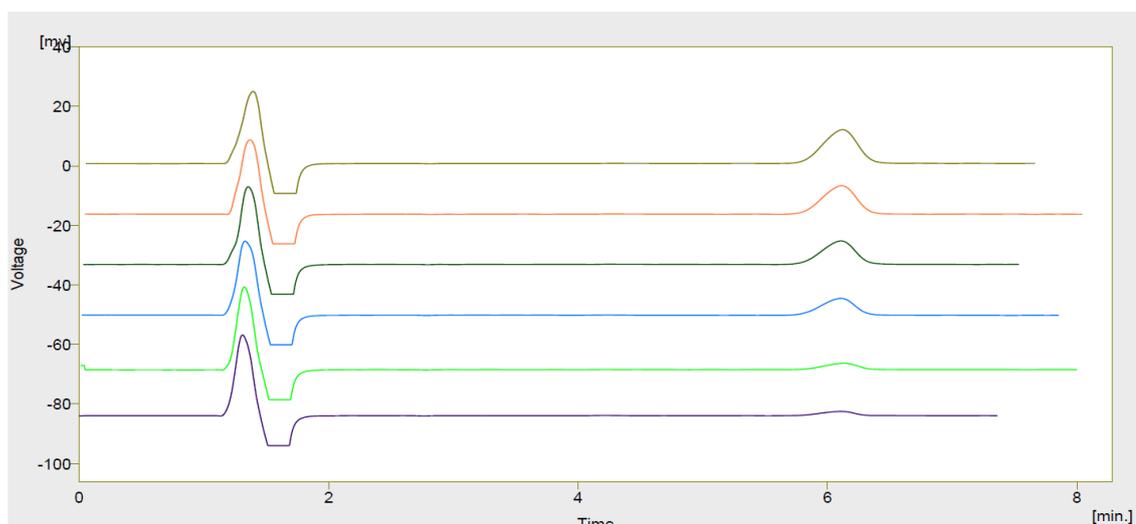| Sr. No.	Concentration (ng/ml)	Peak Area (mV.s)	Retention time (min)
1.	50	5.95±3.06	6.11
2.	100	11.96±4.59	6.12
3.	500	59.67±3.19	6.14
4.	1000	119.40±9.29	6.10
5.	2500	297.47±7.30	6.12
6.	5000	595.80±12.77	6.11

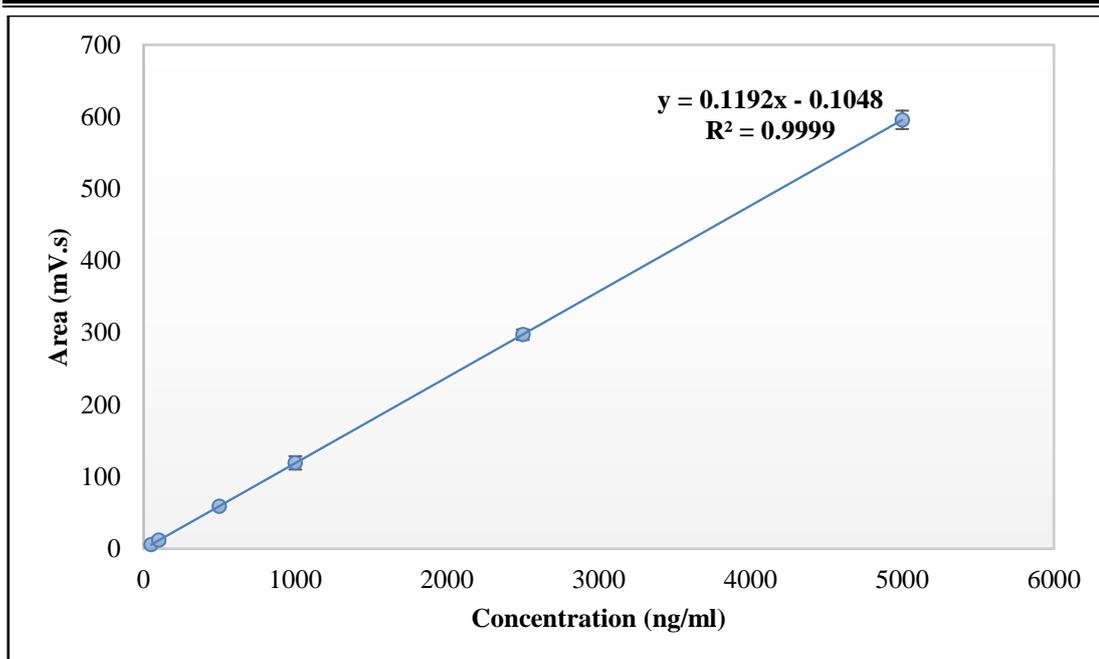**Figure 3.25: Overlay plot of Lurasidone HCl in phosphate buffer:ACN**

Figure 3.26 : Standard calibration curve of Lurasidone HCl in Phosphate buffer:ACN

3.10.9.1 VALIDATION

The analytical method for estimation of Lurasidone HCl in Phosphate buffer:ACN was validated for different parameters of analytical method validation.

3.10.9.1.1 Linearity

Linear correlation was obtained of Lurasidone HCl in concentration range of 50 to 5000 ng/ml for in Phosphate buffer:ACN. The summarized parameters for regression equation and correlation are given in table 3.38.

Table 3.38: Regression analysis of Lurasidone HCl in Phosphate buffer:ACN

Parameters	Results
Retention time (min)	6.1
Linearity range	50 to 5000 ng/ml
Regression equation (y = a + bc)	$y = 0.1192x - 0.1048$
Correlation coefficient ($R^{2)}$)	0.9999

3.10.9.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.39. The results reveal that the proposed method is accurate and precise.

Table 3.39: Accuracy and precision for Lurasidone HCl in Phosphate buffer:ACN

Standard concentration (ng/ml)		Precision		Accuracy
Actual	**observed**	**Interday**	**Intraday**	**(%)**
50	49.16±0.637	1.296	1.433	98.31
1000	987.37±7.60	0.770	2.332	98.74
5000	4984.97±7.12	0.143	0.763	99.70

3.10.9.1.3 LOD and LOQ

The LOD and LOQ for Lurasidone HCl in Phosphate buffer:ACN was found to be 9.597 and 29.081 ng/ml respectively.

3.10.10 CALIBRATION CURVE OF LURASIDONE HCl IN PLASMA

The retention time of Lurasidone HCl was 7.5 min. The standard plot of Lurasidone HCl in buffer: ACN is shown in Table 3.40. The overlay plot of HPLC chromatogram is shown in figure 3.27. Regression equation for standard curve $y = 0.083x + 0.3227$ and correlation coefficient (R^2) was found to be 0.9998 signifying that a linear relationship existed between peak area and concentration of the drug (Figure 3.28).

Table 3.40: Standard Calibration data for estimation of Lurasidone HCl in Plasma

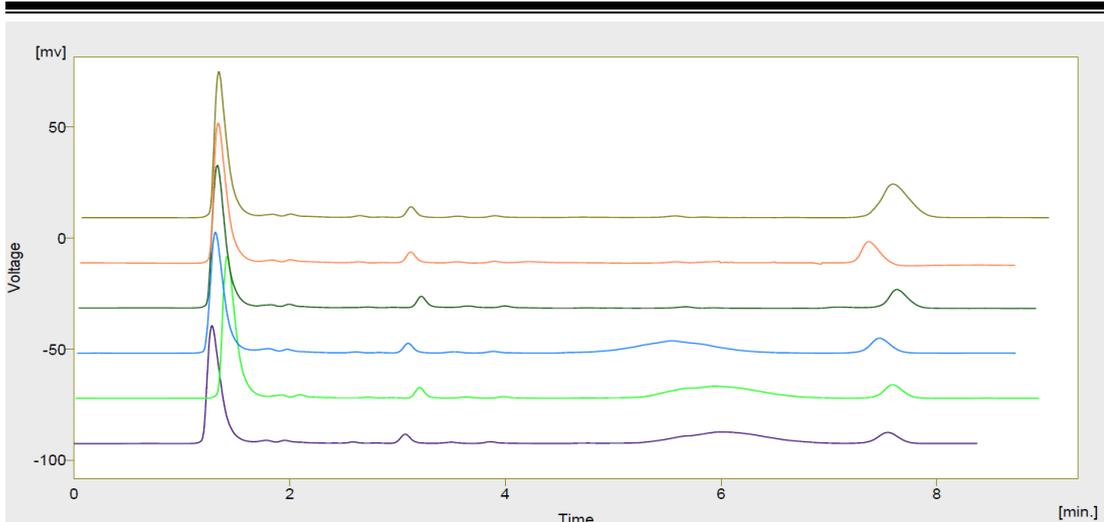| Sr. No.	Concentration (ng/ml)	Peak Area (mV.s)	Retention time (min)
1.	50	4.26±1.89	7.53
2.	100	8.42±2.56	7.50
3.	500	41.99±6.45	7.55
4.	1000	83.95±8.45	7.56
5.	2500	207.29±6.45	7.50
6.	5000	415.41±9.23	7.54

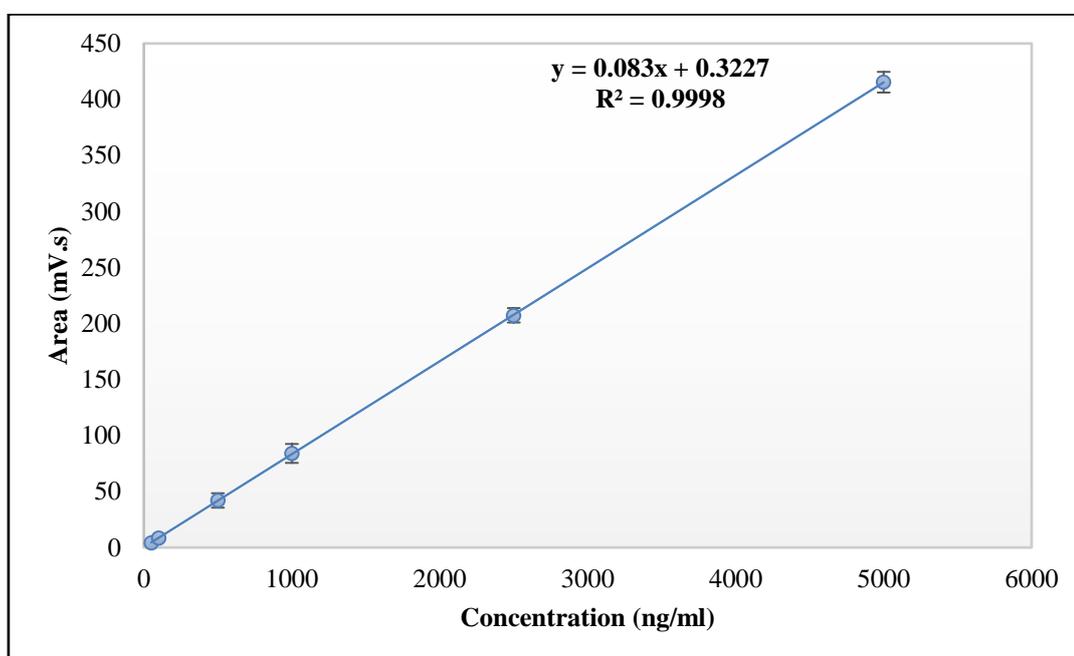**Figure 3.27: Overlay plot of Lurasidone HCl in Plasma**

Figure 3.28: Standard calibration curve of Lurasidone HCl in Plasma

3.10.10.1 VALIDATION

The analytical method for estimation of Lurasidone HCl in Plasma was validated for different parameters of analytical method validation.

3.10.10.1.1 Linearity

Linear correlation was obtained for Lurasidone HCl in Plasma in concentration range of 50 to 5000 ng/ml. The summarized parameters for regression equation and correlation are given in Table 3.41.

Table 3.41: Regression analysis of Lurasidone HCl in plasma

Parameters	Results
Retention time (min)	7.5
Linearity range	50 to 5000 ng/ml
Regression equation (y = a + bc)	$y = 0.083x + 0.3227$
Correlation coefficient ($R^{2)}$)	0.9998

3.10.10.1.2 Accuracy and Precision assay

The results of accuracy and precision are shown in table 3.42. The results reveal that the proposed method is accurate and precise.

Table 3.42: Accuracy and precision for Lurasidone HCl in plasma

Standard concentration (ng/ml)		Precision		Accuracy (%)
Actual	observed	Interday	Intraday	
50	49.29±0.338	0.686	0.815	98.58
1000	981.37±8.57	0.873	1.961	98.14
5000	4981.30±9.88	0.198	0.685	99.63

3.10.10.1.3 LOD and LOQ

The LOD and LOQ for Lurasidone HCl in Plasma was found to be 15.437 and 46.778 ng/ml respectively.

3.11 CONCLUSION

UV spectroscopic methods for Asenapine maleate (AM) and Lurasidone Hydrochloride (LH) were successfully developed and validated in different solvents such as Methanol, Chloroform:Methanol, 0.1 N HCl, Phosphate buffer pH 6.8 and pH 7.4. HPLC methods for AM and LH were developed and validated in mobile phase and plasma which can be utilized to determine in vivo results.

3.12 REFERENCES

1. Chauhan A, Mittu B, Chauhan P. Analytical Method Development and Validation: A Concise Review. J Anal Bioanal Tech. 2015;6(1):1-5.

2. Halima OA, Aneesh TP, Ghosh R, Thomas NR. Development and validation of UV spectrophotometric method for the estimation of asenapine maleate in bulk and pharmaceutical formulation. Der Pharma Chemica. 2012;4(2):644-649.

3. Gandhimathi R, Vijayaraj S, Jyothirmaie MP. Method development and validation of uv- spectroscopic method for estimation of asenapine maleate in bulk and tablet formulation. Int J Med Chem Anal. 2012;2(2):85-90.

4. Parthasarathi TR, Srinivas TS, Sri MV, Ram SS, Basha MM, Pudi R. Quantitative determination of asenapine maleate using reverse phase-high performance liquid chromatography. In J Pharm Bio Sci. 2012;3(4):360 – 366.

5. Govindarajan N, Koulagari S, Methuku A, Podhuturi S, Venkata RB. Method development and validation of RP-HPLC method for determination of new antipsychotic agent asenapine maleate in bulk and in pharmaceutical formulation. Der Pharmacia Lettre. 2012;4(6):1805-1810.

6. Mali N, Patel J, Patel M. Validated Spectrophotometric Methods for The Estimation of Lurasidone Hydrochloride in Bulk and Pharmaceutical Dosage Forms. Int J Res Pharm Sci. 2012;2(2):44-50.

7. Ravisankar P, Rajyalakshmi G, Dasu CD, Babu PS, Reddy PV. Novel analytical method development and validation for the quantitative analysis of lurasidone hydrochloride in bulk and pharmaceutical dosage forms by rp-hplc. World J Pharm Res. 2014;3(7):453-466.