Chapter 4

Analytical Profiles

4.1 ESTIMATION OF DRUGS USING UV- VISIBLE SPECTROPHOTOMETRY

4.1.1 Estimation of Ziprasidone

The UV-Visible method of estimation was developed based on the observation that ziprasidone base (ZB) showed strong absorbance in the UV region of the electromagnetic spectrum in various solvents like tetrahydronfuran (THF), pH 7.4 phosphate buffer (pH 7.4 PB) with 0.1% w/v tween 80 and pH 7.4 PB with 2% sodium lauryl sulphate (SLS).

Procedure for Calibration Plot

Stock solution of ziprasidone in the solvent in which the calibration plot is to be prepared (THF, pH 7.4 PB with 0.1% tween 80 and pH 7.4 PB with 2% SLS) was prepared by dissolving 10mg ZB in 100mL of the solvent. Suitable aliquots of the 100µg/mL stock solution of ZB were pipetted into 10mL volumetric flasks. The volume was made upto 10mL using the same solvent. The contents were shaken well and the absorbance was measured at 317nm using a SHIMADZU (Shimadzu, Japan) spectrophotometer against suitable blank. The above procedure was repeated six times and the mean absorbance value was determined. The absorbance values obtained in THF, pH 7.4 PB with 0.1% tween 80 and pH 7.4 PB with 2% SLS are tabulated in table 4.1, table 4.2 and table 4.3 respectively.

Stability and Selectivity

Solution stability of ZB in THF, pH 7.4 PB with 0.1% tween 80 and pH 7.4 PB with 2% SLS was studied by preparing the calibration curve at 317nm over a period of 72 hours. Further, ZB was estimated in the presence of the polymer (Poly lactide co-glycolide 50:50) in the same ratio as present in the formulation. This estimation was performed to get an idea about the selectivity of the developed UV – Visible analytical method and also to check the absence of interference of the polymer in the absorptivity of ZB.

Accuracy and Precision

In order to determine the accuracy and precision of the developed method, known amounts of ZB (10µg/mL, 20µg/mL and 25µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in table 4.4, table 4.5 and table 4.6 respectively for ZB in THF, pH 7.4 PB with 0.1% tween 80, and pH 7.4 PB with 2% SLS.

Concentration (µg/mL)	Mean Absorbance ± S.D
1	0.023 ± 0.002
2	0.045 ± 0.003
5	0.083 ± 0.006
10	0.172 ± 0.008
20	0.329 ± 0.017
25	0.417 ± 0.018
50	0.815 ± 0.016

Regression equation: y = 0.0162x + 0.0082 ; Correlation coefficient: $R^2 = 0.9998$

Table 4.1: Calibration Curve of Ziprasidone in Tetrahydrofuran (λ_{max}: 317nm)

Concentration (µg/mL)	Mean Absorbance ± S.D
1	0.0220 ± 0.004
2	0.0367 ± 0.005
5	0.0825 ± 0.007
10	0.1689 ± 0.007
20	0.3332 ± 0.012
25	0.4121 ± 0.016
50	0.8354 ± 0.022

Regression equation: y = 0.0167x + 0.0013 ; Correlation coefficient: $R^2 = 0.9998$

Table 4.2: Calibration Curve of Ziprasidone in pH 7.4 Phosphate buffer with 0.1% tween 80 (λ_{max}: 317nm)

Concentration (µg/mL)	Mean Absorbance ± S.D
1	0.023 ± 0.002
2	0.045 ± 0.003
5	0.083 ± 0.006
10	0.172 ± 0.008
20	0.329 ± 0.017
25	0.417 ± 0.018
50	0.815 ± 0.016

Regression equation: $y = 0.0159x + 0.0039$; Correlation coefficient: $R^2 = 0.9997$

Table 4.3: Calibration Curve of Ziprasidone in pH 7.4 Phosphate buffer with 2% Sodium lauryl sulphate (λ_{max}: 317nm)

Theoretical Concentration of ZB (µg/mL)	Determined Value (µg/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits*
10	10.09	4.25	0.32	10.09 ± 0.2133
20	19.83	2.76	0.41	19.83 ± 0.4095
25	25.24	4.24	0.75	25.24 ± 0.5185

Table 4.4: Evaluation of accuracy and precision of the estimation method of Ziprasidone in Tetrahydrofuran

** At 95% Confidence level; $t_{tab} = 2.78$ for 5 degrees of freedom*

Theoretical Concentration of ZB (µg/mL)	Determined Value (µg/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits*
10	10.26	1.62	0.11	10.09 ± 0.19
20	19.44	2.21	0.32	19.83 ± 0.39
25	24.75	1.17	0.20	25.24 ± 0.26

Table 4.5: Evaluation of accuracy and precision of the estimation method of Ziprasidone in pH 7.4 Phosphate buffer with 0.1%w/v tween 80

** At 95% Confidence level; t_{tab} = 2.78 for 5 degrees of freedom*

Theoretical Concentration of ZB(µg/mL)	Determined Value (µg/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits*
10	9.98	3.33	0.25	9.98 ± 0.21
20	19.85	1.72	0.25	19.85 ± 0.41
25	24.61	1.66	0.31	24.61 ± 0.51

Table 4.6: Evaluation of accuracy and precision of the estimation method of Ziprasidone in pH 7.4 Phosphate Buffer with 2.0%w/v Sodium Lauryl Sulphate

** At 95% Confidence level; t_{tab} = 2.78 for 5 degrees of freedom*

4.1.2 Estimation of Olanzapine

The UV-Visible method of estimation was developed based on the observation that olanzapine (OL) showed strong absorbance in the UV region of the electromagnetic spectrum in various solvents like 0.1N hydrochloric acid (0.1N HCl), methanol: chloroform (1:1 ratio) and pH 7.4 phosphate buffer (pH 7.4 PB).

Procedure for Calibration Plot

Stock solution of OL in the solvent in which the calibration plot is to be prepared (0.1N HCl and 1:1 ratio of methanol: chloroform) was prepared by dissolving 10mg OL in 100mL of the solvent. Stock solution of OL in pH 7.4 PB was prepared by dissolving 5mg of OL in 2 mL of methanol and making up the volume to 50mL with pH 7.4 PB. After ensuring that the drug has totally dissolved, suitable aliquots of the 100µg/mL stock solution of OL was pipetted into 10mL volumetric flasks. The volume was made upto 10mL using the same solvent. The contents were shaken well and the absorbance was measured at 258nm for OL samples in 0.1N HCl, 276nm for OL samples in methanol-chloroform mixture and 260nm for samples in pH 7.4 PB using a SHIMADZU (Shimadzu, Japan) spectrophotometer against suitable blank. The above procedure was repeated six times and the mean absorbance values were determined. The absorbance values obtained in 0.1N HCl, 1:1 ratio of methanol: chloroform and pH 7.4 PB are tabulated in table 4.7, table 4.8 and table 4.9 respectively.

Stability and Selectivity

Solution stability of OL in 0.1N HCl, 1:1 ratio of methanol: chloroform and pH 7.4 PB was studied by preparing the calibration curve at λ_{max} 276nm of OL solutions in 0.1N HCl, 258nm for OL solutions in 1:1 ratio of methanol: chloroform and 260nm for OL solutions in pH 7.4 PB over a period of 72 hours. Further, OL was estimated in the presence of the polymer (Glyceryl mono stearate, Glyceryl distearate, Glyceryl tristearate, Witepsol® E85) in the same ratio as present in the formulation. This estimation was performed to get an idea about the selectivity of the developed UV – Visible analytical method and also to check the absence of interference of the polymer in the absorptivity of OL.

Accuracy and Precision

In order to determine the accuracy and precision of the developed method, known amounts of OL (10µg/mL, 20µg/mL and 25µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in table 4.10, table 4.11 and table 4.12 respectively for OL in 0.1N HCl, 1:1 ratio of methanol: chloroform and pH 7.4 PB.

Concentration (µg/mL)	Mean Absorbance ± S.D
1	0.0745 ± 0.004
2	0.1517 ± 0.009
5	0.3849 ± 0.010
10	0.7492 ± 0.015
20	1.5004 ± 0.042
25	1.8126 ± 0.046

Regression equation: $y = 0.073x + 0.0111$; $R^2 = 0.9995$

Table 4.7: Calibration Curve of Olanzapine in 0.1N Hydrochloric Acid (λ_{max} = 258nm)

Concentration (µg/mL)	Mean Absorbance ± S.D
1	0.0775 ± 0.006
2	0.1267 ± 0.006
5	0.3091 ± 0.011
10	0.6091 ± 0.019
20	1.1719 ± 0.027
25	1.4721 ± 0.039
30	1.7738 ± 0.040

Regression equation: $y = 0.0584x + 0.0173$; $R^2 = 0.9995$

Table 4.8: Calibration Curve of Olanzapine in methanol – chloroform mixture (1:1 ratio) (λ_{max} = 276nm)

Concentration (µg/mL)	Mean Absorbance ± S.D
1	0.0621 ± 0.005
2	0.1392 ± 0.011
5	0.3812 ± 0.014
10	0.7474 ± 0.023
20	1.4335 ± 0.046
25	1.8180 ± 0.052

Regression equation: $y = 0.0724x + 0.003$; $R^2 = 0.9997$

Table 4.9: Calibration Curve of Olanzapine in pH 7.4 phosphate buffer (λ_{max} = 260nm)

Theoretical Concentration of OL (µg/mL)	Determined Value (µg/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits*
10	10.23	0.83	0.28	10.23 ± 0.43
20	20.17	0.91	0.59	20.17 ± 0.82
25	25.06	0.35	0.29	25.06 ± 0.77

Table 4.10: Evaluation of accuracy and precision of the estimation method of Olanzapine in 0.1N Hydrochloric Acid

** At 95% Confidence level; t_{tab} = 2.78 for 5 degrees of freedom*

Theoretical Concentration of OL (µg/mL)	Determined Value (µg/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits*
10	10.06	0.62	0.37	10.06 ± 0.75
20	19.73	0.31	0.48	19.73 ± 0.46
25	24.95	0.58	0.68	24.95 ± 0.83

Table 4.11: Evaluation of accuracy and precision of the estimation method of Olanzapine in 1:1 ratio of methanol: chloroform

** At 95% Confidence level; t_{tab} = 2.78 for 5 degrees of freedom*

Theoretical Concentration of OL (µg/mL)	Determined Value (µg/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits*
10	10.32	1.17	0.39	10.32 ± 0.93
20	20.03	2.24	0.46	20.03 ± 0.81
25	24.89	2.31	0.87	24.89 ± 1.04

Table 4.12: Evaluation of accuracy and precision of the estimation method of Olanzapine in pH 7.4 Phosphate Buffer

** At 95% Confidence level; t_{tab} = 2.78 for 5 degrees of freedom*

4.1.3 Estimation of Rose Bengal

The UV-Visible method of estimation was developed based on the observation that Rose Bengal (RB) showed strong absorbance in the visible region of the electromagnetic spectrum in distilled water.

Procedure for Calibration Plot

Stock solution of RB was prepared by dissolving accurately weighed 10mg of RB in 100mL of double distilled water. After ensuring that RB has totally dissolved, suitable aliquots of the

100µg/mL stock solution of RB was pipetted into 10mL volumetric flasks. The volume was made upto 10mL using the distilled water. The contents were shaken well and the absorbance was measured at 548nm suing a SHIMADZU (Shimadzu, Japan) spectrophotometer against suitable blank. The above procedure was repeated five times and the mean absorbance values were determined. The absorbance values obtained in distilled water are tabulated in table 4.13.

Stability and Selectivity

Solution stability of RB in distilled water was studied by preparing the calibration curve at 548nm over a period of 72 hours. This estimation was performed to get an idea about the stability of RB in distilled water.

Accuracy and Precision

In order to determine the accuracy and precision of the developed method, known amounts of RB (10µg/mL, 20µg/mL and 25µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in table 4.14.

Concentration (µg/mL)	Mean Absorbance ± S.D
0.5	0.0423 ± 0.006
1	0.0747 ± 0.010
2	0.1426 ± 0.011
5	0.3422 ± 0.014
10	0.6671 ± 0.019
20	1.3089 ± 0.035
25	1.6562 ± 0.040

Regression equation: $y = 0.655x + 0.0098$; $R^2 = 0.9999$

Table 4.13: Calibration Curve of Rose Bengal in distilled water (λ_{max} = 548nm)

Theoretical Concentration of RB (µg/mL)	Determined Value (µg/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits*
10	10.03	1.87	0.55	10.03 ± 0.83
20	19.96	0.77	0.45	19.96 ± 0.64
25	25.16	0.62	0.47	25.16 ± 0.96

Table 4.14: Evaluation of accuracy and precision of the estimation method of Rose Bengal in distilled water

** At 95% Confidence level; t_{tab} = 2.78 for 5 degrees of freedom*

4.2 FLUORIMETRIC ESTIMATION OF ZIPRASIDONE IN PLASMA, AND TISSUE HOMOGENATES

Instrumentation

All fluorimetric estimations were performed on a Shimadzu RF-540 spectrofluorometer (Shimadzu Coporation, Japan) equipped with a xenon lamp. The various experimental conditions like the slit-width for excitation (kept at 3) and emission (kept at 5) and the excitation and emission wavelengths ($\lambda_{excitation}$ = 334nm; $\lambda_{emission}$ = 404 ± 2nm) were optimized.

Preparation of Calibration Plot in acetonitrile: phosphate buffer pH 7.4 (30:70 ratio)

Stock solution of ZB in a mixture of acetonitrile: phosphate buffer pH 7.4 (in a ratio of 30:70) was prepared by accurately weighing 10mg of ZB in 100mL of solvent mixture. After ensuring that the drug has totally dissolved, suitable aliquots of the 100µg/mL stock solution of ZB was pipetted into 10mL volumetric flasks. The volume was made upto 10mL using the same solvent. The contents were shaken well and the relative fluorescence intensity was measured setting the $\lambda_{excitation}$ at 334nm and the corresponding $\lambda_{emission}$ peak intensity was measured at 404 ± 2nm (slit widths as mentioned above) using a Shimadzu RF-540 spectrofluorometer (Shimadzu Corporation, Japan) against suitable blank. The above procedure was repeated six times and the mean relative fluorescence intensity values were determined. The mean relative fluorescence intensity obtained tabulated in table 4.15. The $\lambda_{emission}$ peaks of ziprasidone in acetonitrile: phosphate buffer pH 7.4 (30:70) is shown in figure 4.1.

Estimation of Ziprasidone in Plasma and tissue homogenates

Blood Collection and tissue homogenate preparation

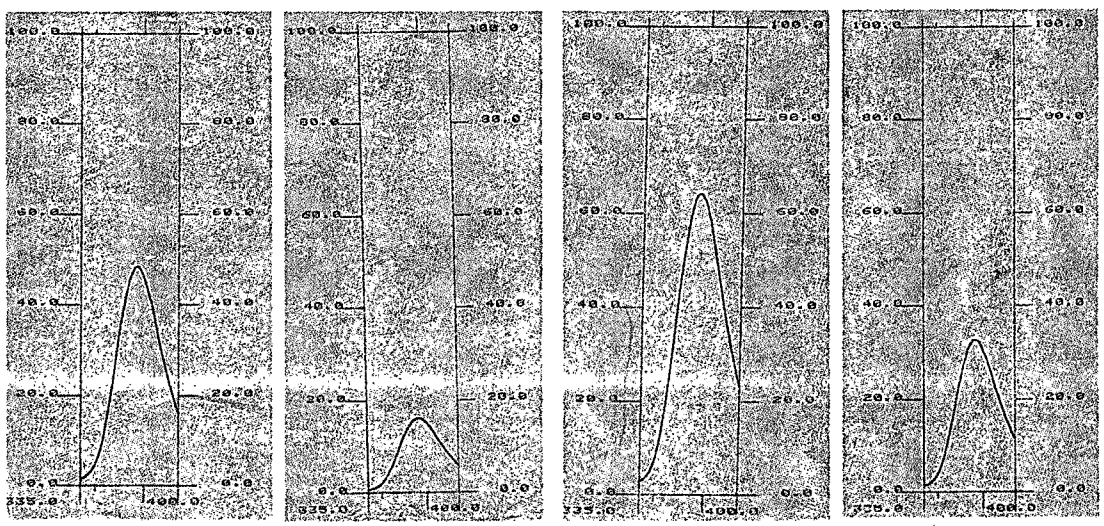
Albino rats were anaesthetized with chloroform and blood was collected from retro-orbital plexus of the eye using sterile glass capillary tube into glass vials containing 3%w/v sodium citrate solution as anticoagulant. The rats were sacrificed by cervical dislocation and dissected to collect the tissues such as brain, kidney, liver, lung, spleen and heart. The organs were then blotted using filter paper, weighed separately and homogenized, to a concentration of 10% w/v in water. The samples were centrifuged at 3000 rpm for 10 minutes and at 6000 rpm for 10 minutes at 4°C in a cooling centrifuge (Sigma, Osterode, Germany) to isolate the plasma and clear tissue homogenate respectively.

Construction of calibration plot in plasma and tissue homogenates

To 0.1 ml plasma or 1 ml tissue homogenate, was added the required quantity of drug solution (from a stock solution of ZB of 2µg/ml in pH 7.4 PB: acetonitrile; 70:30 ratio) to obtain the final concentrations of ZB ranging between 50 – 1000 ng/ml. The contents were gently mixed to ensure uniform mixing and kept aside for 30min at room temperature. To the samples was added 5.0 ml of methyl - *t* -butyl ether, mixed for 15 min followed by centrifugation at 1500 x *g* for 10 min. The supernatant (organic layer) was transferred into the glass tubes. The extraction procedure was repeated twice and the combined organic layers were evaporated to dryness. The resulting residue was reconstituted with acetonitrile: pH 7.4 PB (30:70). The fluorescence of extracted drug was measured in Shimadzu RF-540 spectrofluorophotometer (Shimadzu Corporation, Japan) at wavelengths of excitation at 334 nm and emission at 404 ± 2nm. All the estimations were carried out between 20°C - 27°C, and care was taken to prevent solvent evaporation at every stage of estimation. Calibration plots were constructed for the measured relative fluorescence intensity against drug concentration. Accuracy and precision of the method was determined by performing recovery studies after addition of ZB in plasma and tissues in triplicate.

Accuracy and Precision

In order to determine the accuracy and precision of the developed method, known amounts of ZB (100ng/mL, 200ng/mL and 500ng/mL) were subjected to recovery studies as per the procedure described earlier. The results obtained are tabulated in table 4.16.



A B C D

Figure 4.1: λ emission peaks at $\lambda_{emission}$ of 404 ± 2nm of Ziprasidone in plasma (A), rat brain homogenate (B), rat liver homogenate (C) and rat spleen homogenate (D)at $\lambda_{excitation}$ of 334nm.

Concentration (ng/mL)	Mean Relative Fluorescence Intensity ± S.D
10	4.38 ± 0.51
20	11.24 ± 0.79
30	16.28 ± 0.85
40	22.62 ± 0.79
50	38.51 ± 1.10
100	72.75 ± 1.21
200	142.43 ± 1.66
300	202.20 ± 2.27
400	269.04 ± 2.74
500	326.21 ± 3.01
750	486.64 ± 3.37
1000	641.15 ± 4.03

Regression equation: $y = 0.6441x + 3.558$; $R^2 = 0.9992$

Table 4.15: Calibration Curve of Ziprasidone in rat plasma ($\lambda_{excitation}$: 334nm; $\lambda_{emission}$: 404 ± 2nm)

Theoretical Concentration of ZB (ng/mL)	Determined Value (ng/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits*
100	107.45	2.64	0.86	107.45 ± 9.65
200	209.84	1.47	0.74	209.84 ± 17.51
500	492.13	1.62	0.89	492.13 ± 29.82

Table 4.16: Evaluation of accuracy and precision of the estimation method of Ziprasidone in rat plasma ($\lambda_{excitation}$: 334nm; $\lambda_{emission}$: 404 ± 2nm)

** At 95% Confidence level;*

Concentration (ng/mL)	Mean Relative Fluorescence Intensity ± S.D (in brain homogenate)	Mean Relative Fluorescence Intensity ± S.D (in Liver homogenate)	Mean Relative Fluorescence Intensity ± S.D (in lung homogenate)	Mean Relative Fluorescence Intensity ± S.D (in spleen homogenate)	Mean Relative Fluorescence Intensity ± S.D (in kidney homogenate)	Mean Relative Fluorescence Intensity ± S.D (in heart homogenate)
10	7.38 ± 0.76	15.12 ± 0.84	9.35 ± 0.64	10.31 ± 0.89	9.42 ± 0.56	6.35 ± 0.71
20	16.24 ± 0.77	30.26 ± 0.74	16.06 ± 0.44	18.69 ± 0.85	17.65 ± 1.00	14..33 ± 0.94
50	41.51 ± 1.04	80.21 ± 1.31	50.54 ± 1.09	54.64 ± 1.23	52.09 ± 1.51	38.64 ± 1.09
100	76.05 ± 1.19	164.08 ± 1.48	108.89 ± 1.54	115.85 ± 1.94	112.73 ± 1.84	73.41 ± 1.14
200	159.31 ± 1.53	319.25 ± 2.44	224.58 ± 1.39	234.31 ± 1.59	229.40 ± 1.67	150.67 ± 1.43
300	233.90 ± 2.54	478.34 ± 3.11	344.40 ± 2.83	359.47 ± 2.68	350.94 ± 2.07	225.79 ± 1.87
400	309.24 ± 3.01	604.53 ± 4.01	442.32 ± 3.32	459.63 ± 2.91	449.61 ± 2.01	299.23 ± 2.73
500	383.62 ± 2.64	755.26 ± 3.85	572.10 ± 3.10	598.49 ± 3.61	587.12 ± 3.52	368.50 ± 3.56

Regression equation for ZB in brain homogenate : $y = 0.7688x + 1.5741$; $R^2 = 0.9993$

Regression equation for ZB in liver homogenate : $y = 1.5128x - 6.175$; $R^2 = 0.9992$

Regression equation for ZB in lung homogenate : $y = 1.1447x - 5.0489$; $R^2 = 0.9994$

Regression equation for ZB in spleen homogenate : $y = 1.1688x - 4.717$; $R^2 = 0.9992$

Regression equation for ZB in kidney homogenate : $y = 0.7688x + 1.5741$; $R^2 = 0.9992$

Regression equation for ZB in heart homogenate : $y = 0.739x + 1.7935$; $R^2 = 0.9991$

Table 4.17: Mean Relative Fluorescence Intensity ± SD obtained from the calibration curve of fluorimetric estimation of ZB in tissue homogenates of rat (n= 3).

Theoretical Concentration of ZB (ng/mL)	Determined Value (ng/mL)	Coefficient of variance (CV)	Relative mean error	Confidence limits
Brain Homogenate				
100	105.85	3.60	0.99	105.85 ± 10.72
200	206.90	2.67	0.64	206.90 ± 12.96
500	495.53	2.92	0.83	495.53 ± 24.28
Liver Homogenate				
100	107.51	3.42	0.56	107.51 ± 9.20
200	210.33	2.84	0.95	210.33 ± 16.12
500	493.07	2.97	0.85	493.07 ± 22.01
Lung Homogenate				
100	106.15	2.84	1.05	106.15 ± 8.82
200	205.45	3.15	1.09	205.45 ± 17.10
500	513.19	3.45	0.88	513.19 ± 25.89
Spleen Homogenate				
100	110.61	2.55	0.99	110.61 ± 10.45
200	208.39	3.14	0.77	208.39 ± 13.94
500	490.78	3.29	1.06	490.78 ± 30.31
Kidney Homogenate				
100	95.62	2.46	0.94	95.62 ± 10.05
200	210.73	2.48	0.91	210.73 ± 15.40
500	492.01	3.19	0.89	492.01 ± 27.07
Heart Homogenate				
100	96.11	3.15	1.23	96.11 ± 8.07
200	208.30	2.94	0.89	208.30 ± 11.01
500	491.05	3.84	1.00	491.05 ± 25.09

Table 4.18: Determination of accuracy and precision of fluorimetric estimation of ZB in tissue homogenates of rat