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VALIDATED STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF OLANZAPINE AND SAMIDORPHAN IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of Olanzapine in combination with Samidorphan using reverse phase with column Azilent C18 150x 4.6mm, 5µm. UV detection at 226.0 nm. The mobile phase consisting of 50:50 % (V/V) Acetonitrile: 0.1% Orthophosphoric Acid and at a flow rate of 1.0 mL/min with run time 6 minutues. The method was linear over the concentration range for Olanzapine 15 µg/mL and for Samidorphan 10 µg/mL. The recoveries of active pharmaceutical ingredient (API) Olanzapine and Samidorphan were found to be in the range of 98.00-102.00% respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Olanzapine and Samidorphan in combined tablet dosage form.

Keywords: Olanzapine, Samidorphan, HPLC and Validation.

INTRODUCTION

Olanzapine is an antipsychotic drug used in the management of schizophrenia, bipolar 1 disorder, and agitation associated with these disorders. Olanzapine is a thienobenzodiazepine classified as an atypical or second-generation antipsychotic agent[1]. The second-generation antipsychotics were introduced in the 90s and quickly gained traction due to their impressive efficacy, reduced risk for extrapyramidal side effects and reduced susceptibility to drug-drug interactions[2]. The activity of olanzapine is achieved by the antagonism of multiple neuronal receptors including the dopamine receptor D1, D2, D3 and D4 in the brain, the serotonin receptors 5HT2A, 5HT2C, 5HT3 and 5HT6, the alpha-1 adrenergic receptor, the histamine receptor H1 and multiple muscarinic receptors [1,3]. Olanzapine was initially used orally and intramuscularly for the chronic treatment of schizophrenia in patients over 13 years old and other psychiatric disorders such as bipolar I disorder including mixed or manic episodes. Olanzapine presents a linear pharmacokinetic profile and, after daily administration, it reaches steady-state in about a week[4,5].

Samidorphan is a novel opioid-system modulator, similar to naltrexone, that functions primarily as a µ-opioid receptor antagonist in vivo and is used primarily in combination with antipsychotics to reduce their metabolic dysfunction-associated adverse effects[6]. Samidorphan belongs to a class of drugs known as opioid antagonists. It reduces the risk of weight gain caused by olanzapine. Samidorphan is a novel opioid antagonist structurally related to naltrexone, with a higher affinity for opioid receptors, more potent µ-opioid receptor antagonism, higher oral bioavailability, and a longer half-life, making it an attractive candidate for oral dosing.[7,8,9] Although antipsychotic-induced weight gain is incompletely understood, it is thought that the opioid system plays a key role in feeding and

metabolism, such that opioid antagonism may be expected to ameliorate these negative effects. Samidorphan has been shown in animal models and clinical trials to ameliorate olanzapine-induced weight gain and metabolic dysfunction [8,10].

EXPERIMENTAL

Chemicals and reagents

- Methanol (HPLC grade, Merck Ltd),
- Milli-Q water,
- Olanzapine and Samidorphan (Reference standard purchased from Alkemers, Dublin),
- Ortho phosphoric acid, Potassium dihydrogen phosphate (LR Grade, SD Fine Chem. Ltd).
- All other chemicals are of the highest grade commercially available unless otherwise specified.

Apparatus and chromatographic condition

The Analytical equipment consists of a WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sample injector. Data acquisition was done by using the software Empower 2.

The analytical C18 ace-EPS column (100 × 4.6 mm, 5µm) was used. The mobile phase consist of

40 parts of Acetonitrile is mixed with 60 parts of 0.1% Orthophosphoric acid to obtain 60:40 % (v/v) of 0.1% Orthophosphoric acid and Acetonitrile. The mixture is mixed well, sonicated in an ultrasonic bath for 20 min and then used for experiment. The solution is labeled and used within 7 days from the date of preparation. The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1 mL/min and the detector wavelength was set at 226nm. The injection volume was 10 µL. The mobile phase was used as diluent.

Preparation of Olanzapine and Samidorphan diluent, standard & sample solution

Diluent solution preparation

50% of Acetonitrile and 50% of milli-Q water were mixed well to obtain 50:50 % (v/v) of Acetonitrile and water. This mixture was mixed well and it is sonicated in an ultrasonic bath for 20 minutes and then used for the analysis. The solution is labeled and it is used within 7 days from the preparation date.

Standard solution preparation

Accurately weighed 7.5mg of Olanzapine, 5mg of Samidorphan and transferred to separate 50ml volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluent (50% Acetonitrile and 50% milli-Q Water) and labeled as Standard stock solution (150µg/ml of Olanzapine and 100µg/ml Samidorphan). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (15µg/ml of Olanzapine and 10µg/ml of Samidorphan).

Sample Solution Preparation

10 tablets were accurately weighed, powdered and then Weight equivalent to 1 tablet was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered (150µg/ml of Olanzapine and 100µg/ml Samidorphan). From the filtered solution 1 ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent (15µg/ml of Olanzapine and 10µg/ml of Samidorphan).

Procedure

Inject 10 µL of the standard, sample solution into the chromatographic system and measure the peak areas for Olanzapine and Samidorphan and calculate the % assay value.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines[12]. Obtained validation parameters are presented in Table 1.

Linearity

Linearity was performed by preparing the standard solutions of Olanzapine and Samidorphan in different concentration levels including working concentration mentioned in experimental condition i.e. 15 µg/mL for Olanzapine and 10 µg/mL for Samidorphan. 10 µL of each concentration was injected into the HPLC system. Linearity results were presented in Table 1.

Accuracy

Accuracy of the method can be determined by using standard addition method. Certain amount of pure drugs were taken in three different concentration levels in their solution and are been added to the pre analysed working standards of the respective drugs separately. These sample solutions were injected in triplicate to HPLC system for the analysis at the proposed conditions of the method. The individual % of recovery and % RSD for recovery at each level were calculated. A recovery ranged from 98.00 - 102.00% has been obtained by the method

indicates its accuracy. Results obtained for accuracy were given in table 3.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 45.7 µg/mL and 83.2 µg/mL for Olanzapine and 27.2 µg/mL and 42.4 µg/mL for Samidorphan. The LOD and LOQ showed that the method is sensitive for Olanzapine and Samidorphan.

System suitability test

System suitability study was performed by six replicate injections of the working standard solutions. The % RSD for the peak areas obtained was calculated. The system suitability data presented in the table 4 (% RSD < 2) which establishes reproducible performance of the instrument.

Precision

Precision is the measure of degree of repeatability or reproducibility of an analytical method under normal working conditions. The precision of the method is calculated in terms of repeatability (intra-day assay), Reproducibility and intermediate precision (inter-day assay).

Stress degradation studies

The stress studies involving acid, light (UV) and oxidation revealed that Olanzapine and Samidorphan were not completely degraded. In alkaline conditions (2N NaOH), the drugs were unstable and the degradation peak was eluted earlier followed with a peak distortion and increased tailing. Except in alkaline conditions, the drugs were indicating stability for all stress conditions within 95 - 105% and specificity of the analytical method to differentiate the degradation peaks.

Oxidative stress condition

To 1 ml of stock solution of (Olanzapine and Samidorphan) 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600C. For HPLC study, the resultant solution was diluted to obtain (15µg/ml and 10µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid stress conditions

To 1 ml of stock solution Olanzapine and Samidorphan 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. the resultant solution was diluted to obtain (15µg/ml and 10µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Base stress condition

To 1 ml of stock solution Olanzapine and Samidorphan 1ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0C. the resultant solution was diluted to obtain (15µg/ml and 10µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat stress condition

The standard drug solution was placed in oven at 1050C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain (15µg/ml and 10µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photolytic stress condition

The photochemical stability of the drug was also studied by exposing the (150µg/ml and 100µg/ml) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/min photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (15µg/ml and 10µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Stress condition

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to obtain (15µg/ml and 10µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Control sample

10 tablets were taken, weighed and made into a fine powder of uniform size by using mortar and pestle. From this the average weight of the tablet was calculated. Now from the accurately weighed portion 15 mg of Olanzapine and 10 mg of Samidorphan were taken and transferred to 100 mL volumetric flasks which contain 20 mL of Diluent. For complete solubility of the drugs, the contents of the flasks were sonicated for about 20 min

and made up the mark with diluent. Then the mixture is filtered by passing through 0.45 µ membrane filter. From this solution, 1 mL aliquot was taken into a separate 10 mL volumetric flask and made up to the mark with mobile phase and mixed well. The above solution (10 µL) was then injected eight times into the column. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated by the regression equation of the method. The % assay results are shown in the table .

CONCLUSION

This study presents a simple and validated stability indicating HPLC method for simultaneous estimation of Olanzapine and Samidorphan in the presence of degradation products. The developed method is specific, accurate, precise and robust. The method was linear response in stated range and is accurate and precise. All the degradation products formed during forced decomposition studies were well separated from the analyte peaks demonstrating that the developed method was specific and stability indicating**.** The method could be applied with success even to the analysis of marketed products of Olanzapine and Samidorphan combined tablet formulation, as no interference was observed due to excipients or other components present.

ACKNOWLEDGEMENTS

The authors are thankful to Aurobindo Pharma Ltd., Hyderabad, India for providing the gift samples Olanzapine and Samidorphan.

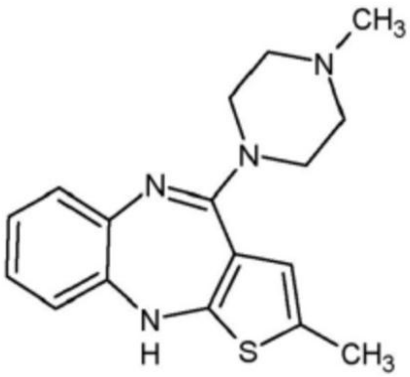


Fig. 1: Chemical structure of Olanzapine

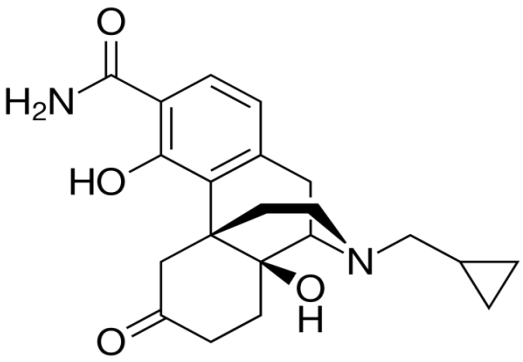


Fig. 2: Chemical structure of Samidorphan

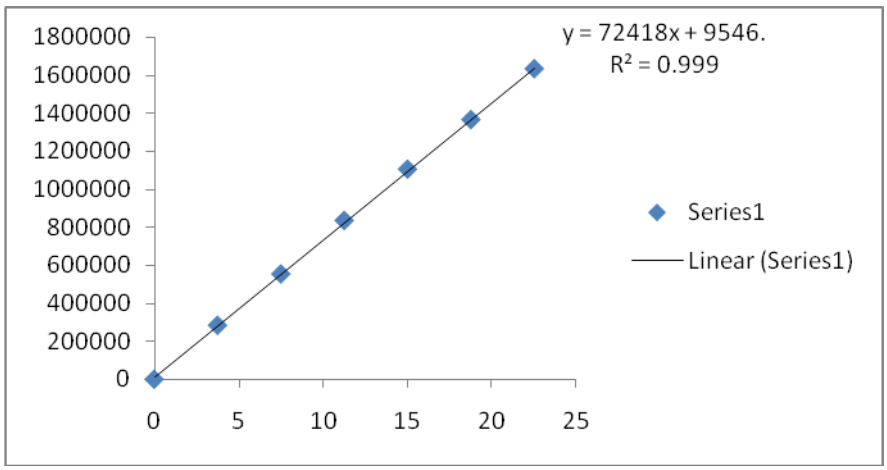


Fig. 2.6: Linearity curve of Olanzapine

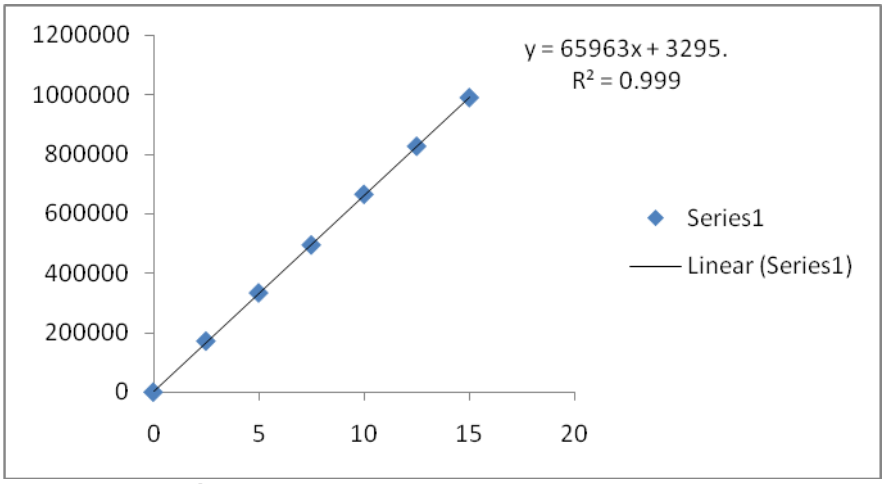
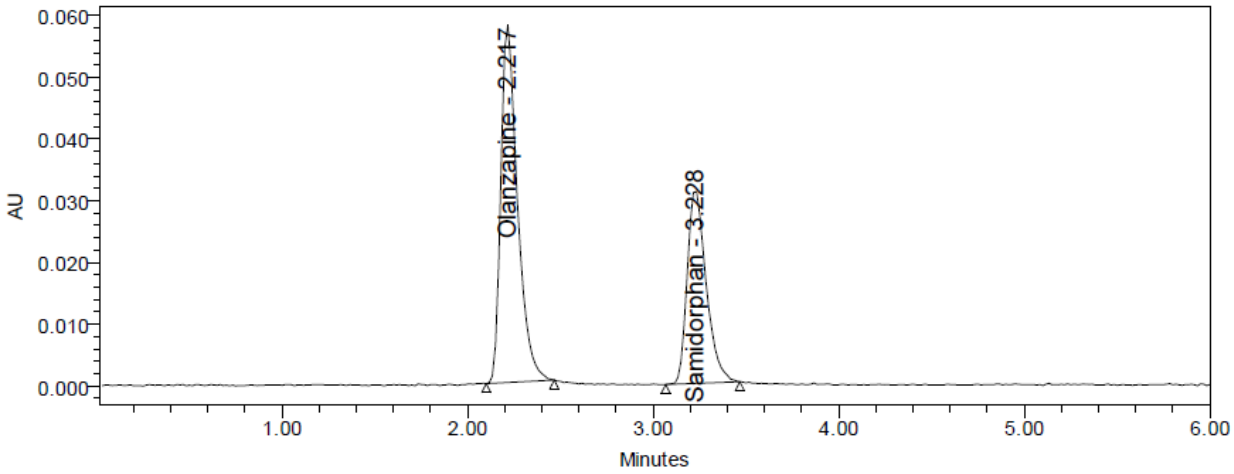


Fig. 2.7: Linearity curve of Samidorphan



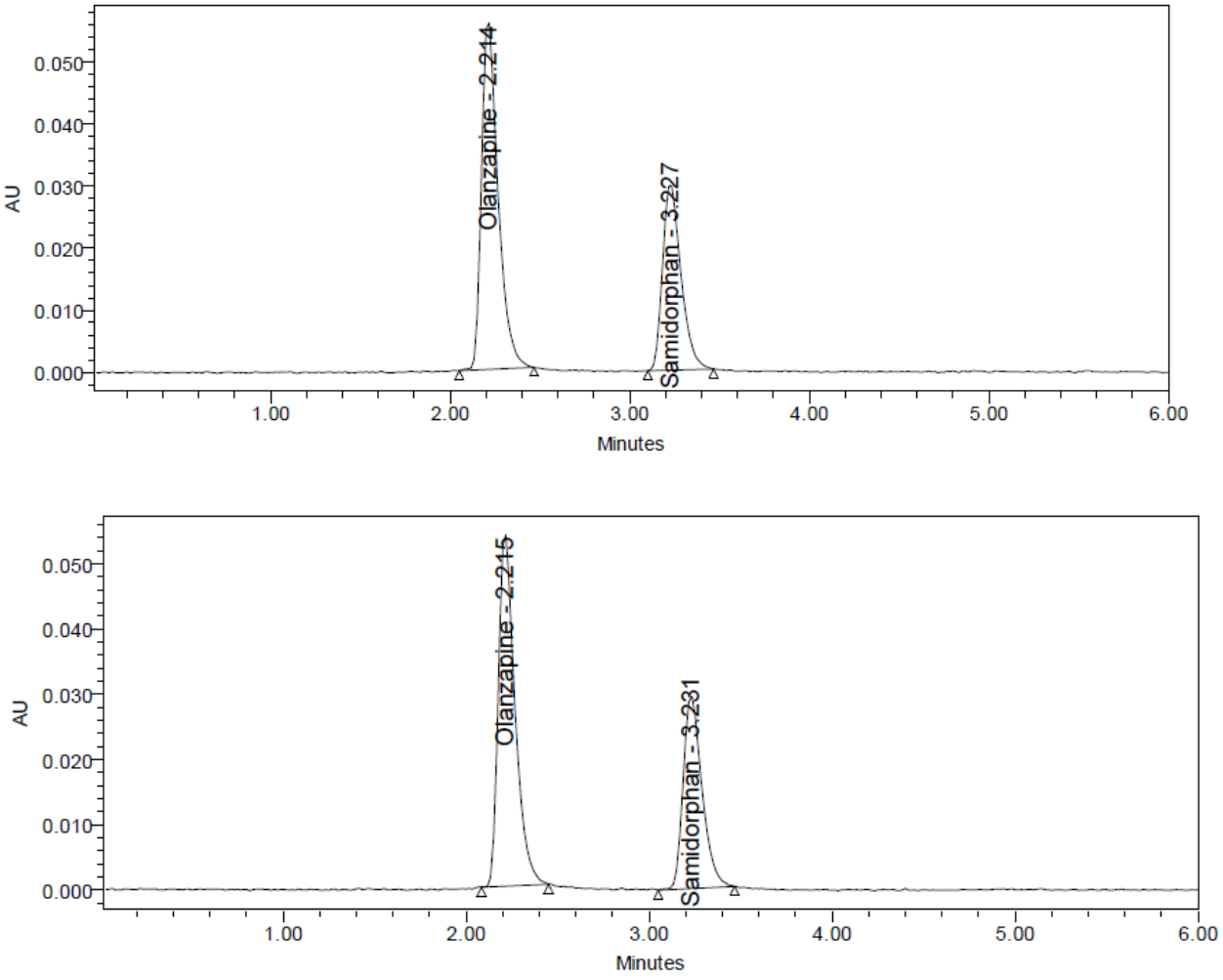
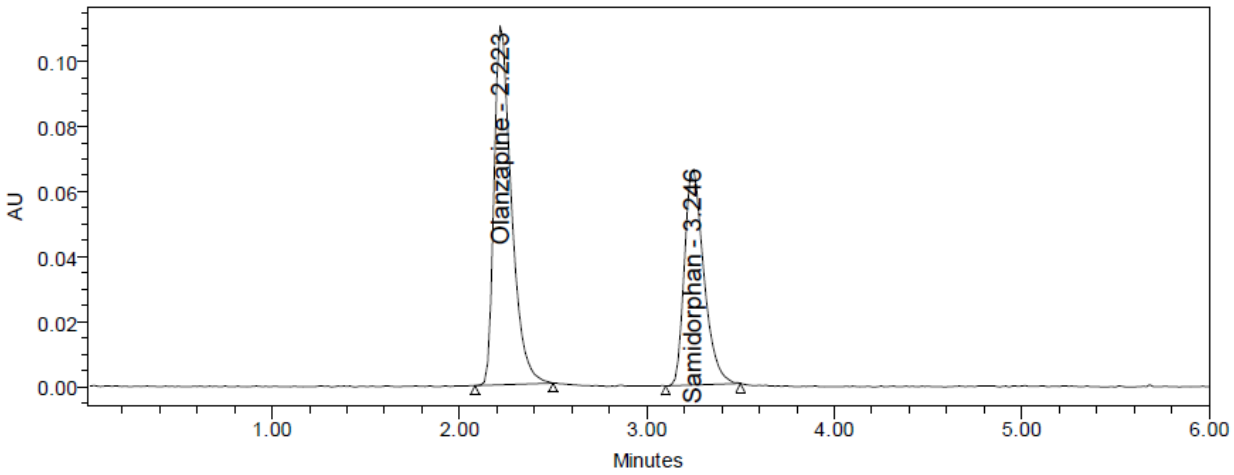


Fig. 2.8: Linearity 25% Chromatogram of Olanzapine and Samidorphan



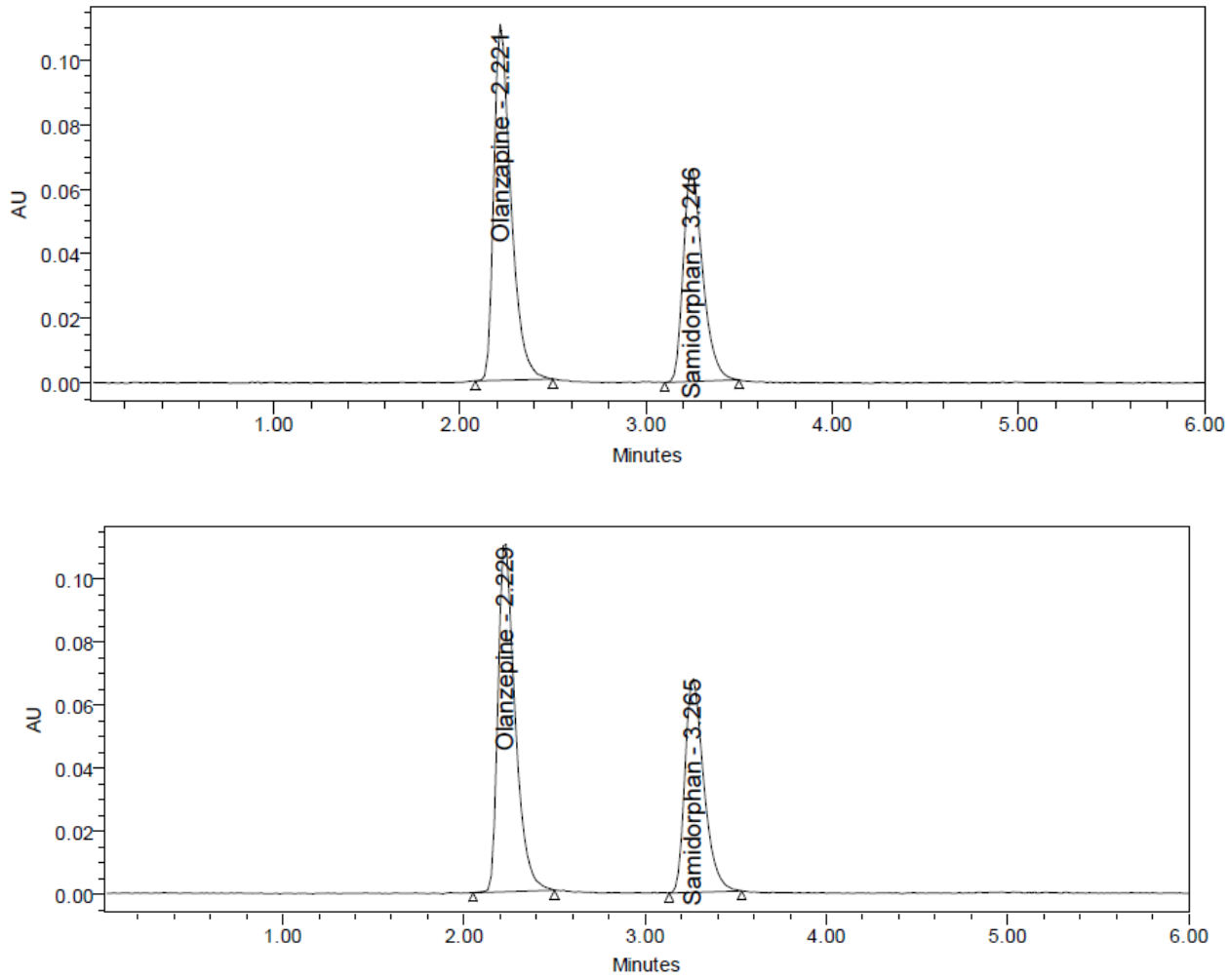
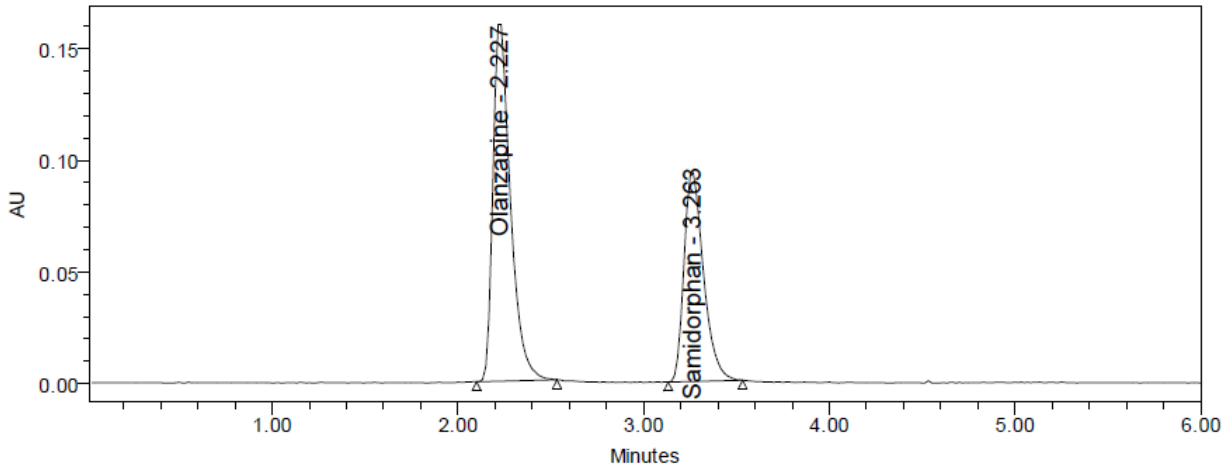


Fig. 2.9: Linearity 50% Chromatogram of Olanzapine and Samidorphan



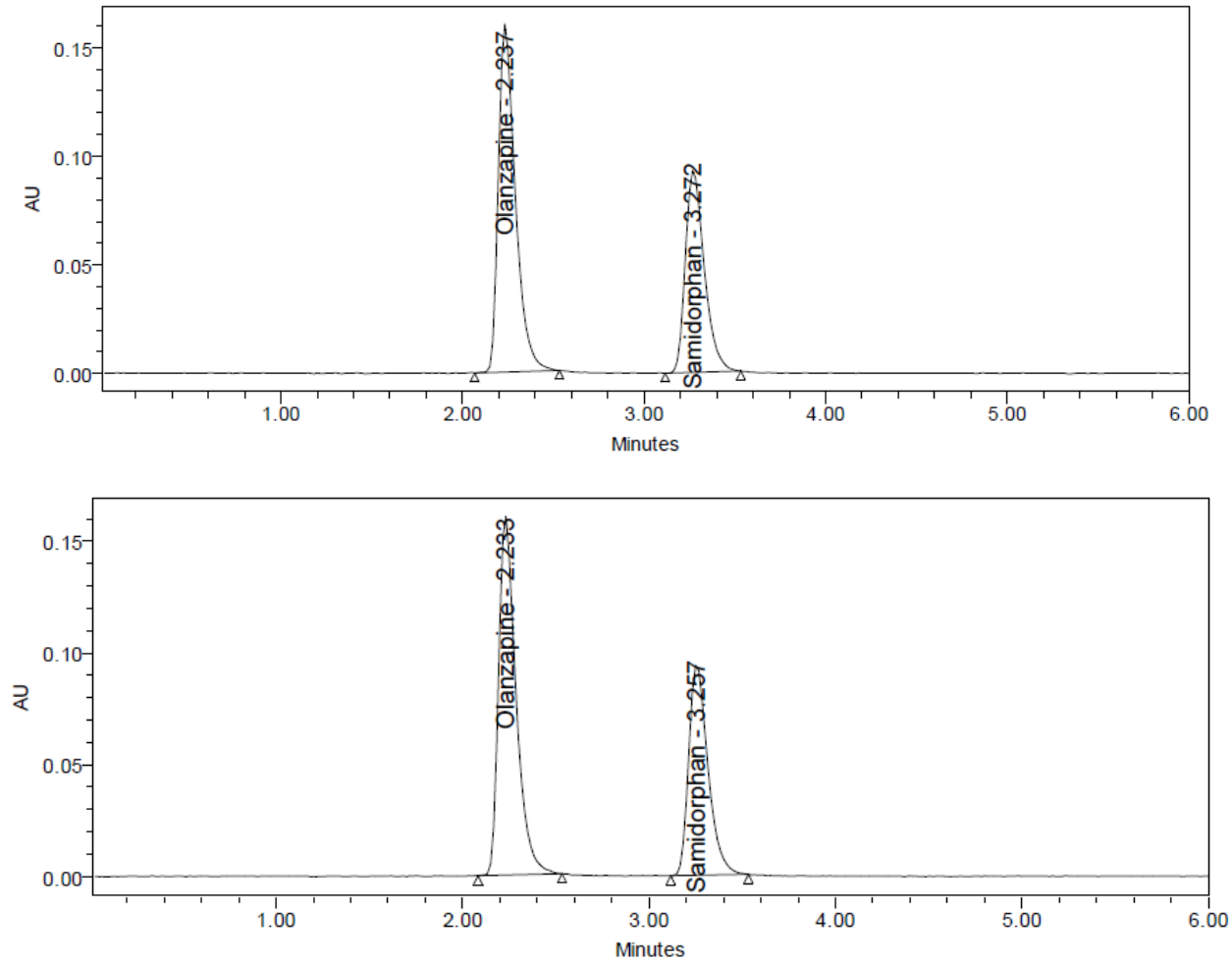
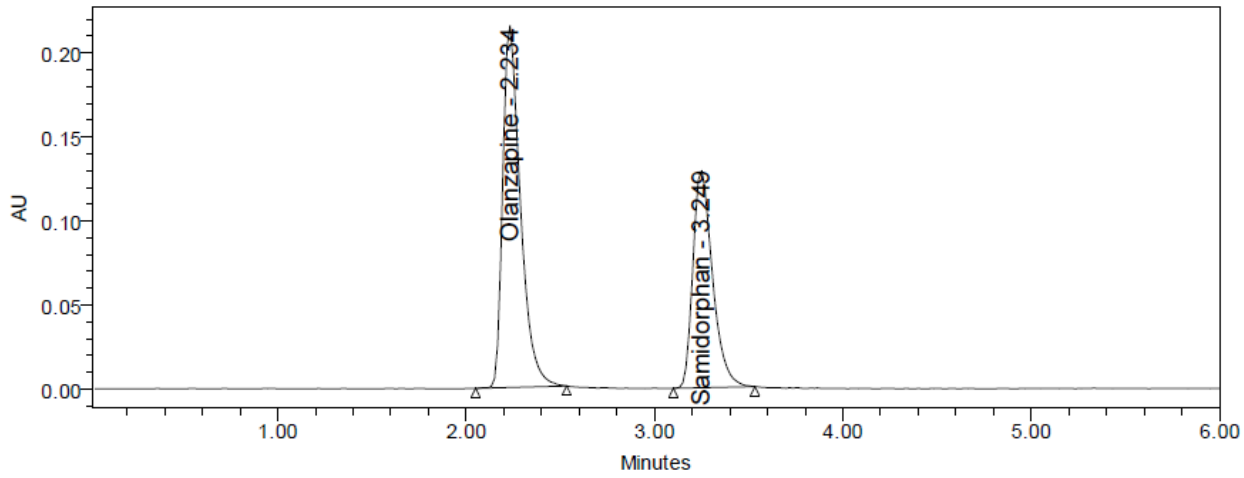


Fig. 2.10: Linearity 75% Chromatogram of Olanzapine and Samidorphan



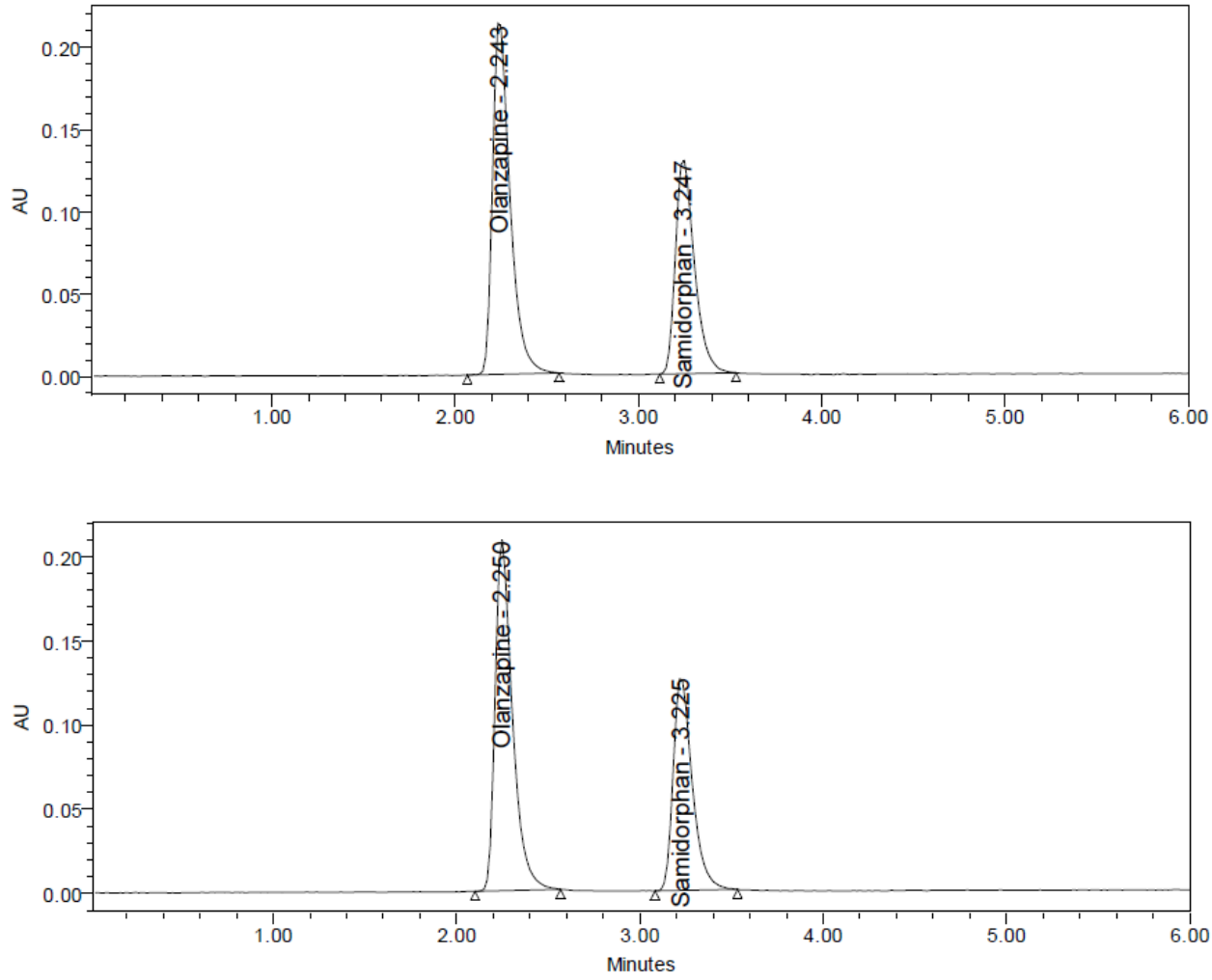
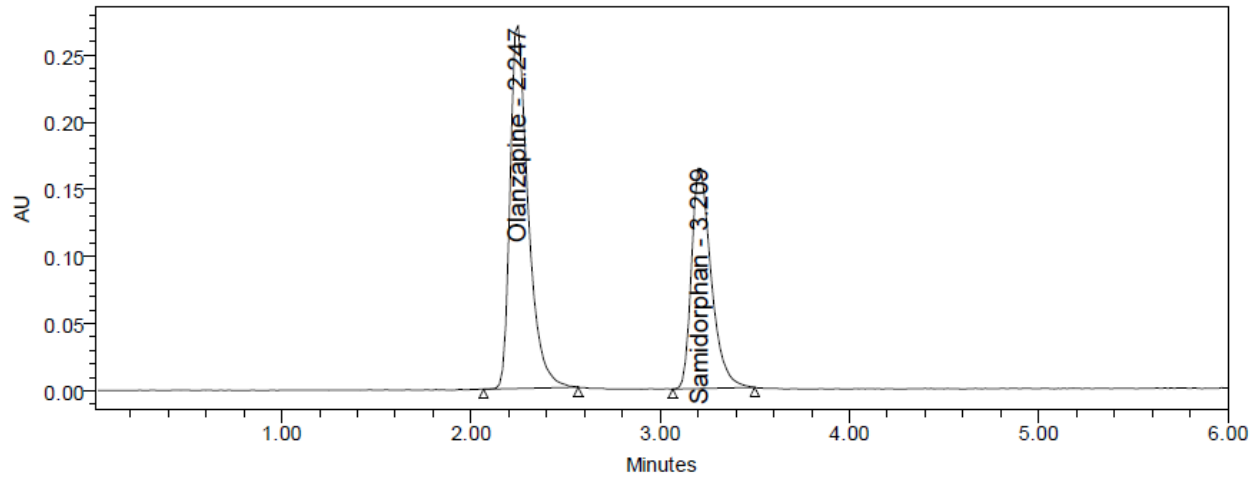


Fig. 2.11: Linearity 100% Chromatogram of Olanzapine and Samidorphan



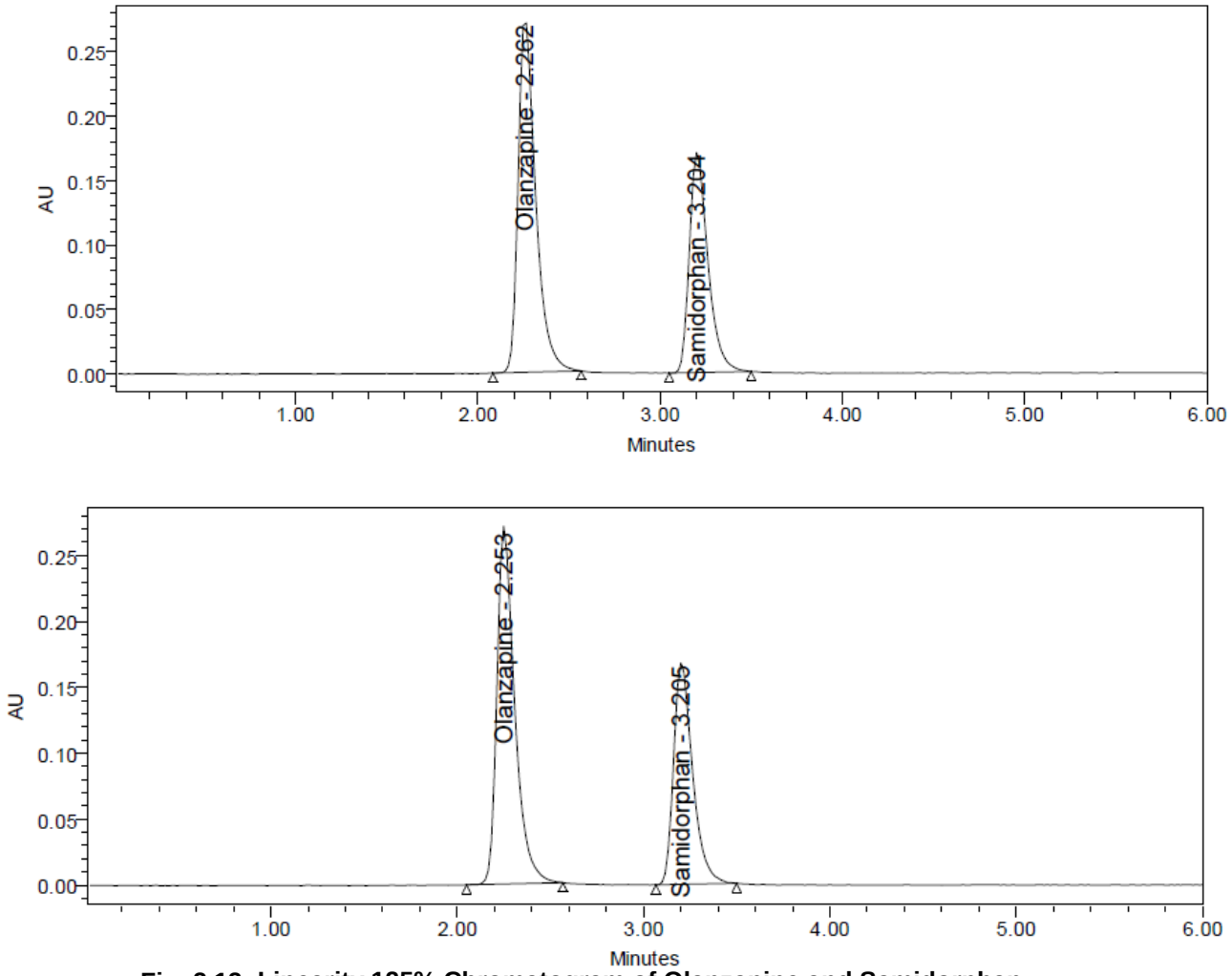
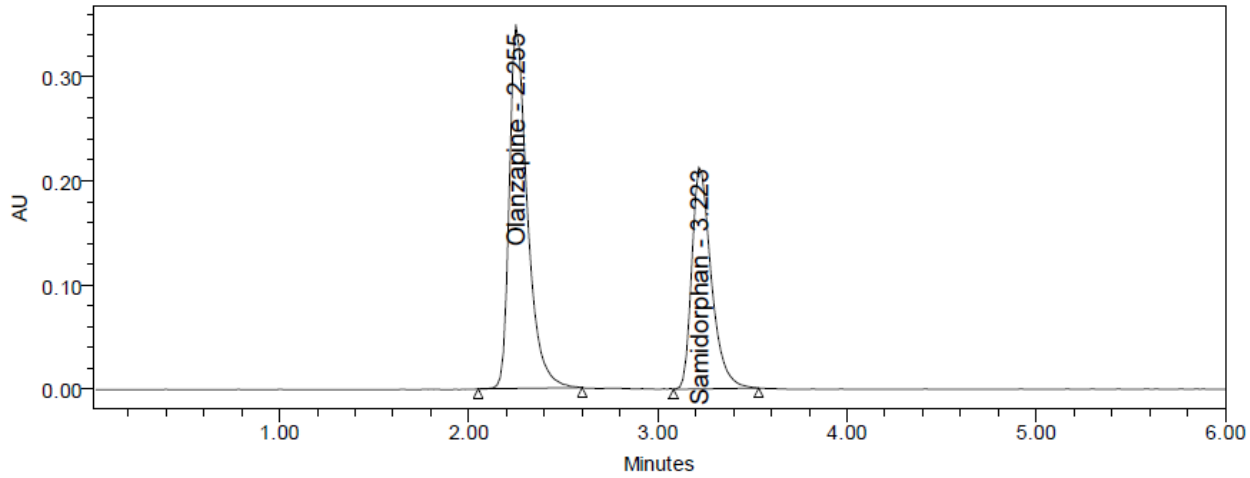


Fig. 2.12: Linearity 125% Chromatogram of Olanzapine and Samidorphan



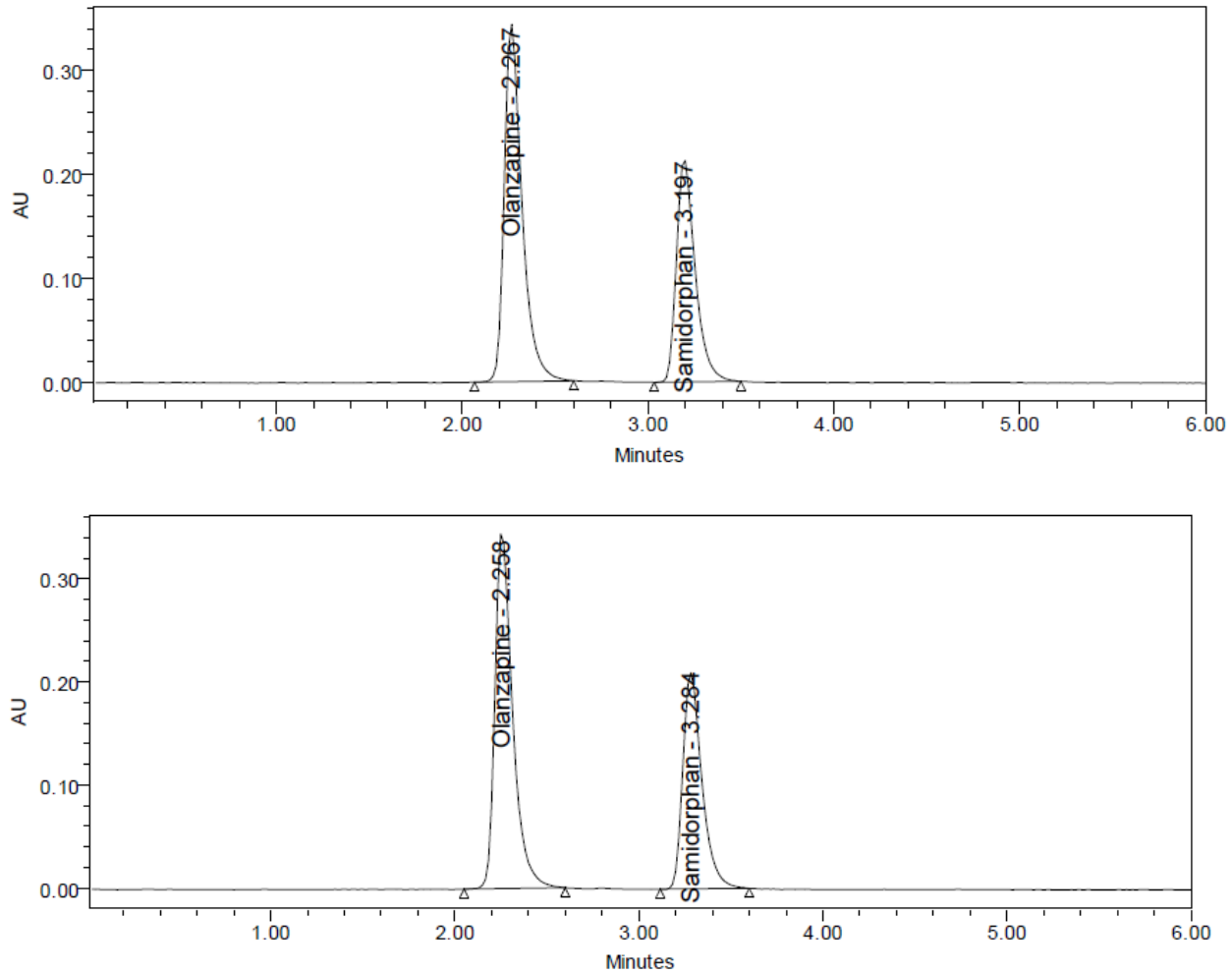
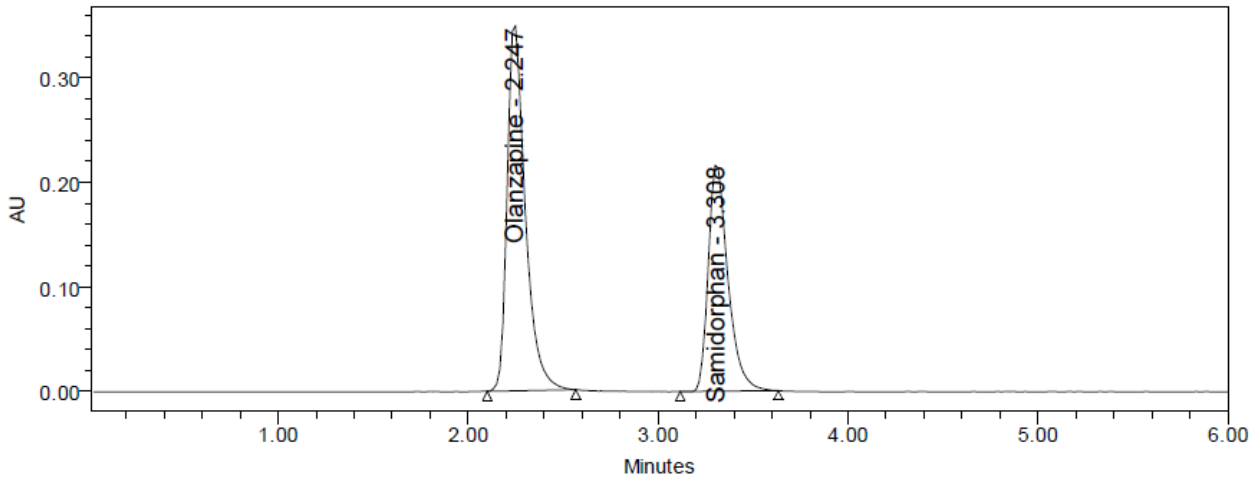


Fig. 2.13: Linearity 150% Chromatogram of Olanzapine and Samidorphan



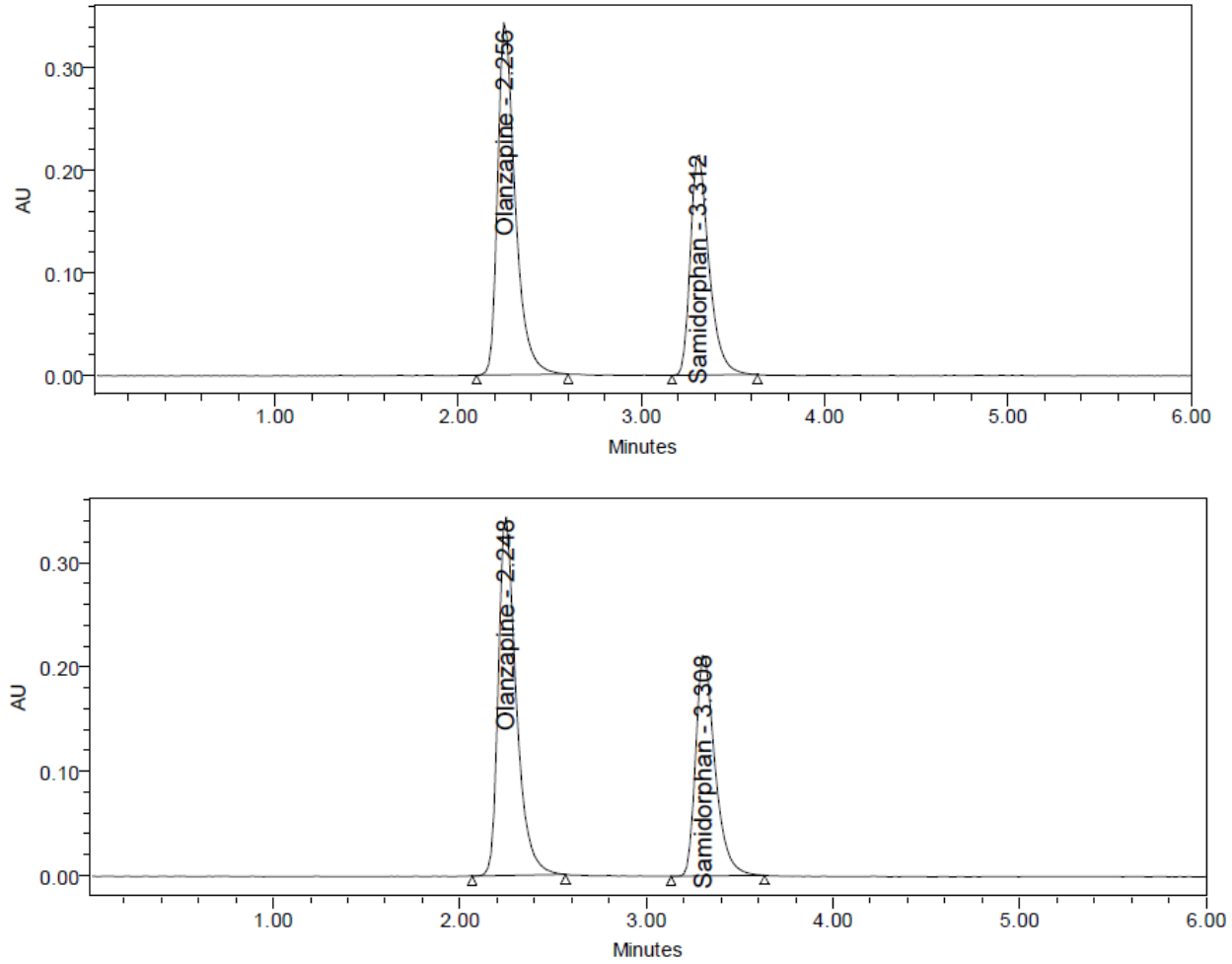
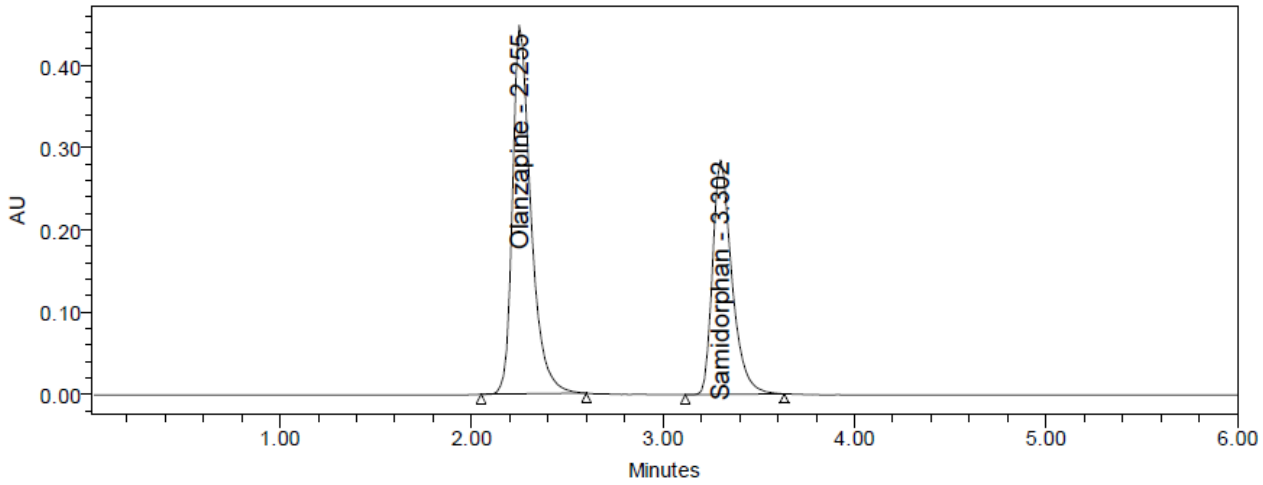


Fig. 2.14: Accuracy 50% Chromatogram Olanzapine and Samidorphan



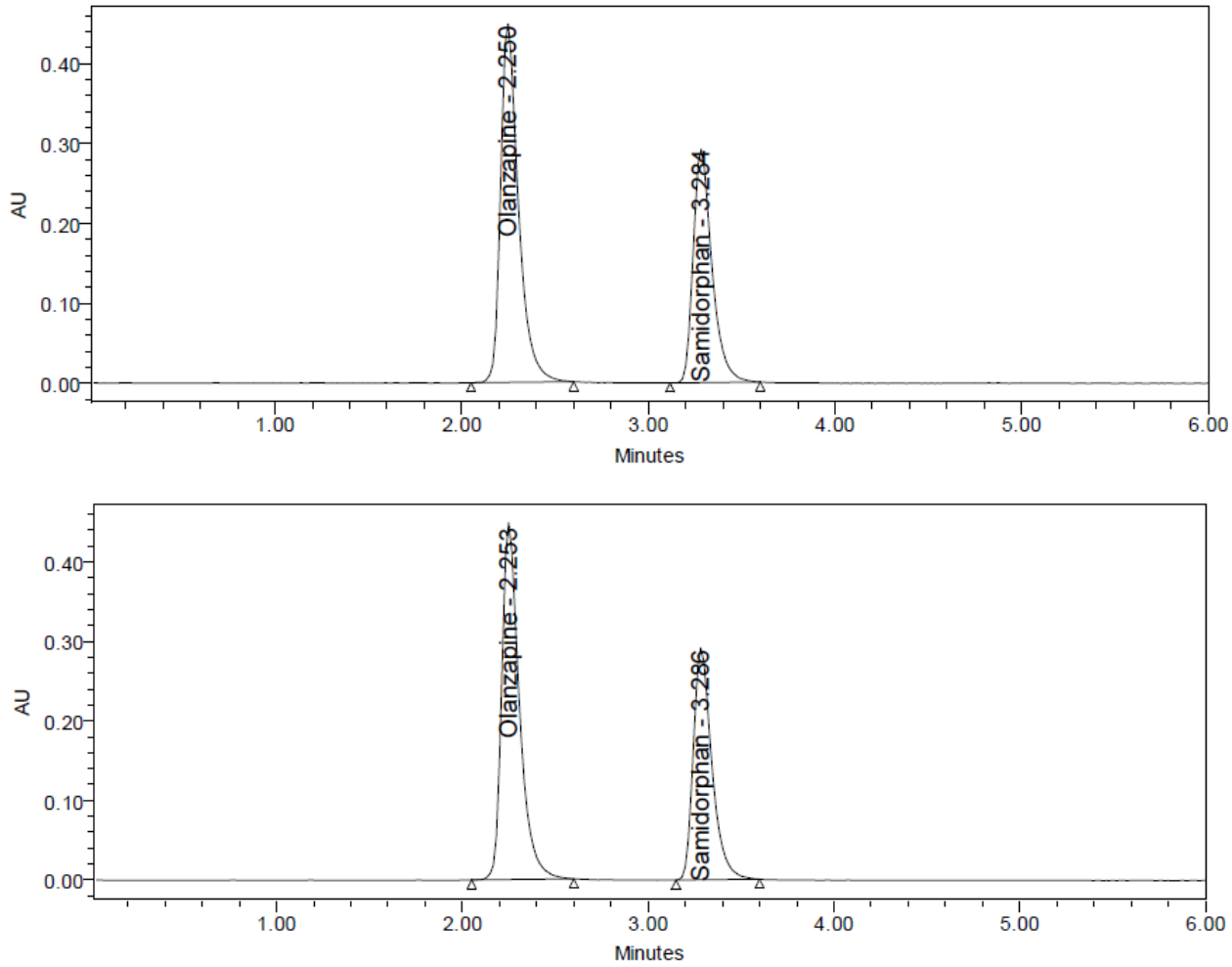
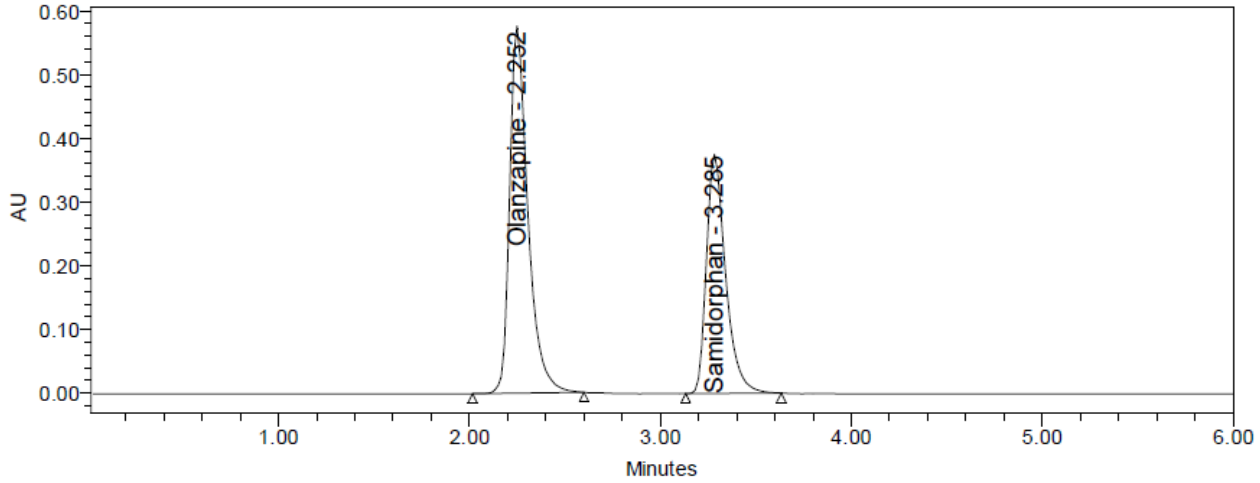


Fig. 2.15: Accuracy 100% Chromatogram of Olanzapine and Samidorphan



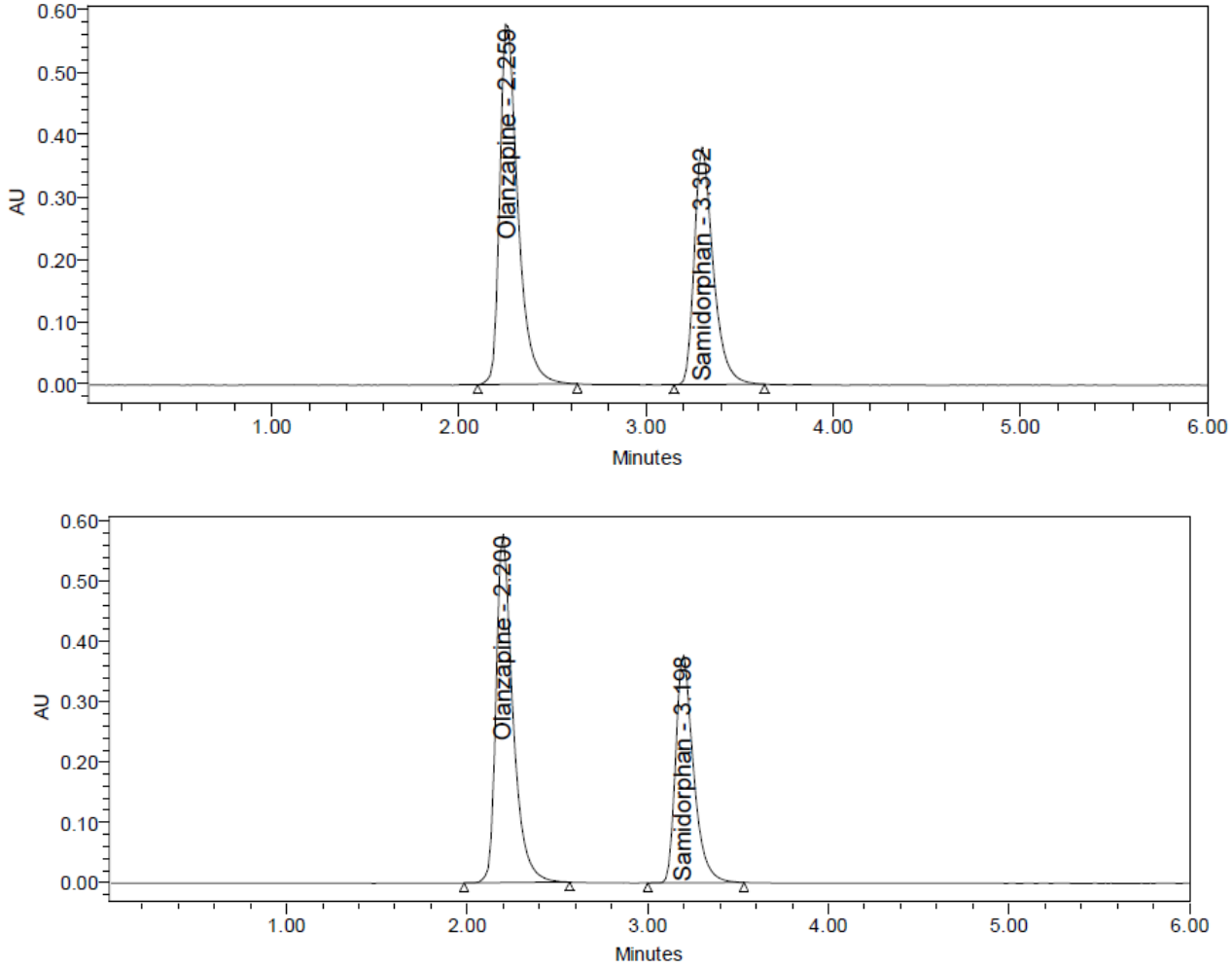
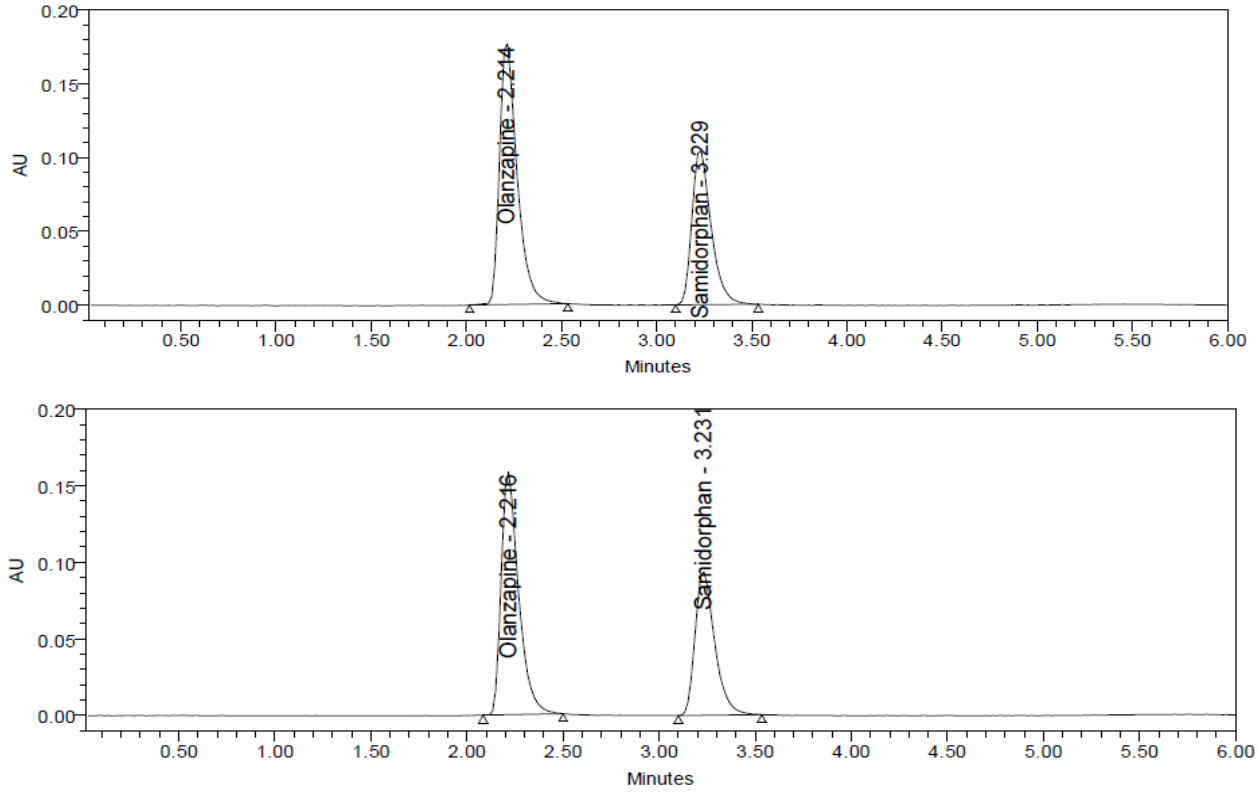


Fig. 2.16: Accuracy 150% Chromatogram Olanzapine and Samidorphan



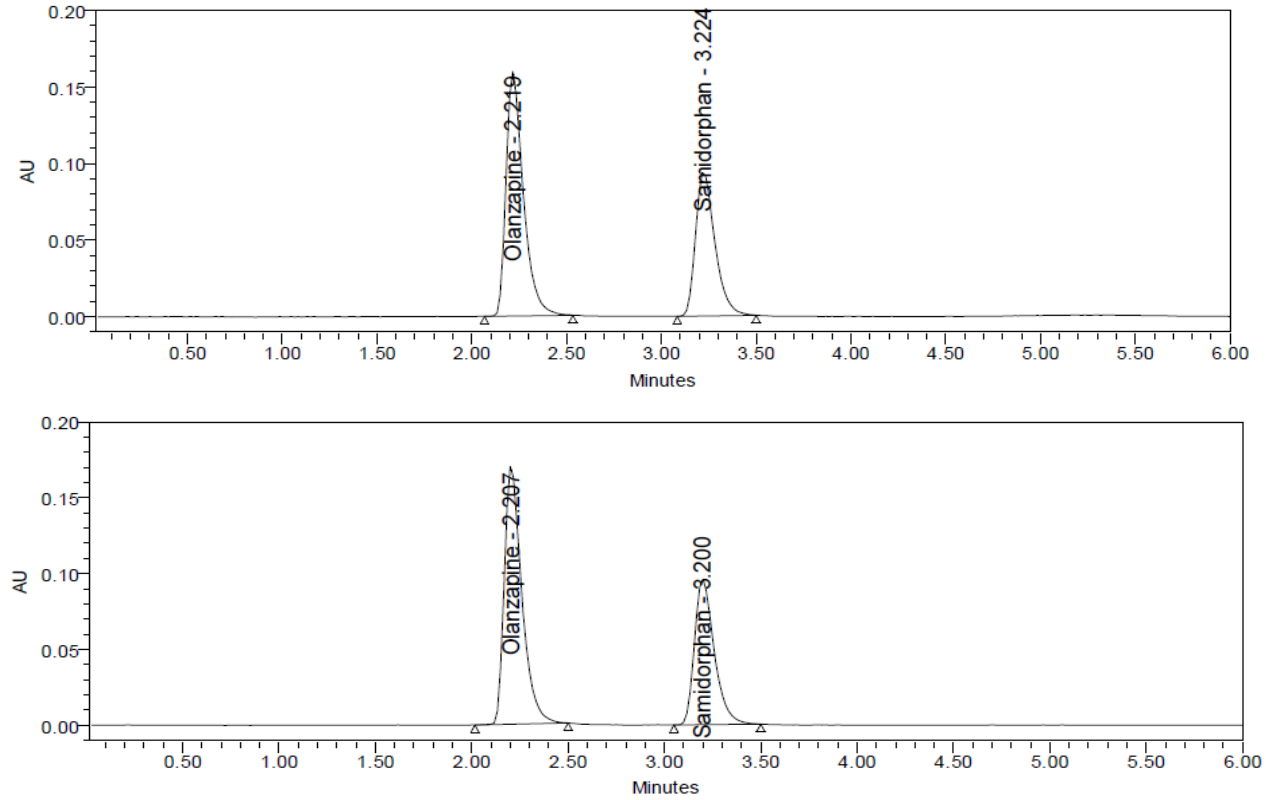


Fig. 2.18: Reproducibility Chromatogram of Olanzapine and Samidorphan

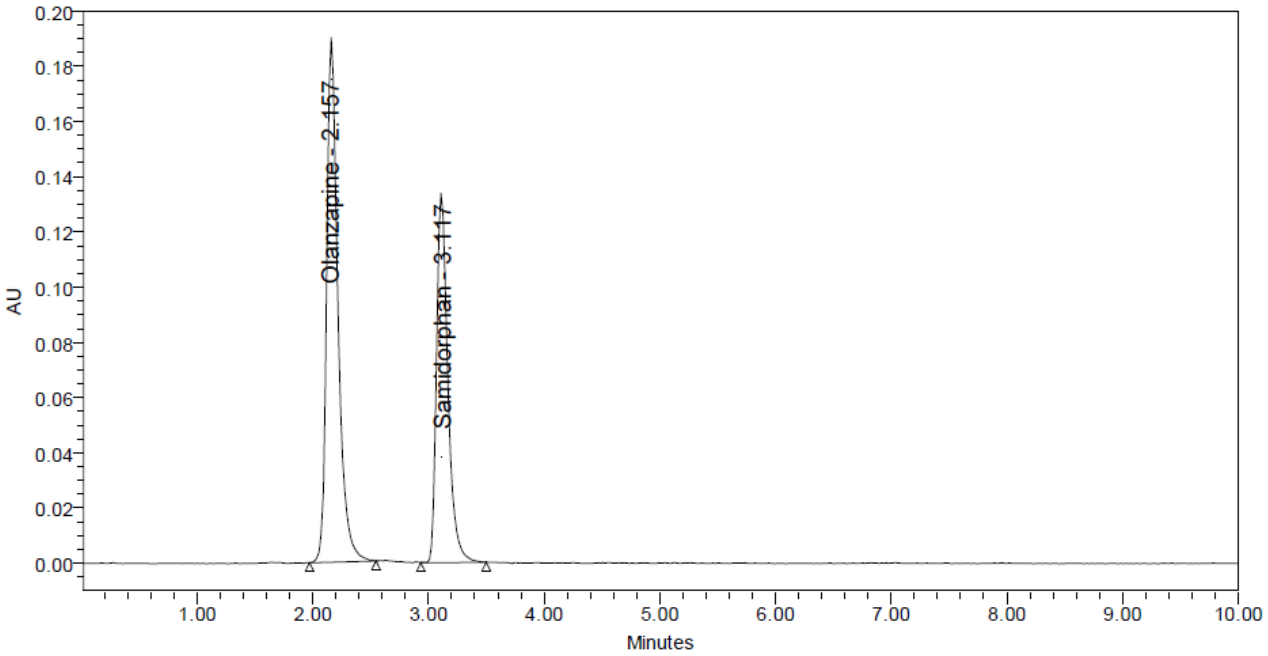


Fig. 2.26: Acid stress chromatogram of Olanzapine and Samidorphan

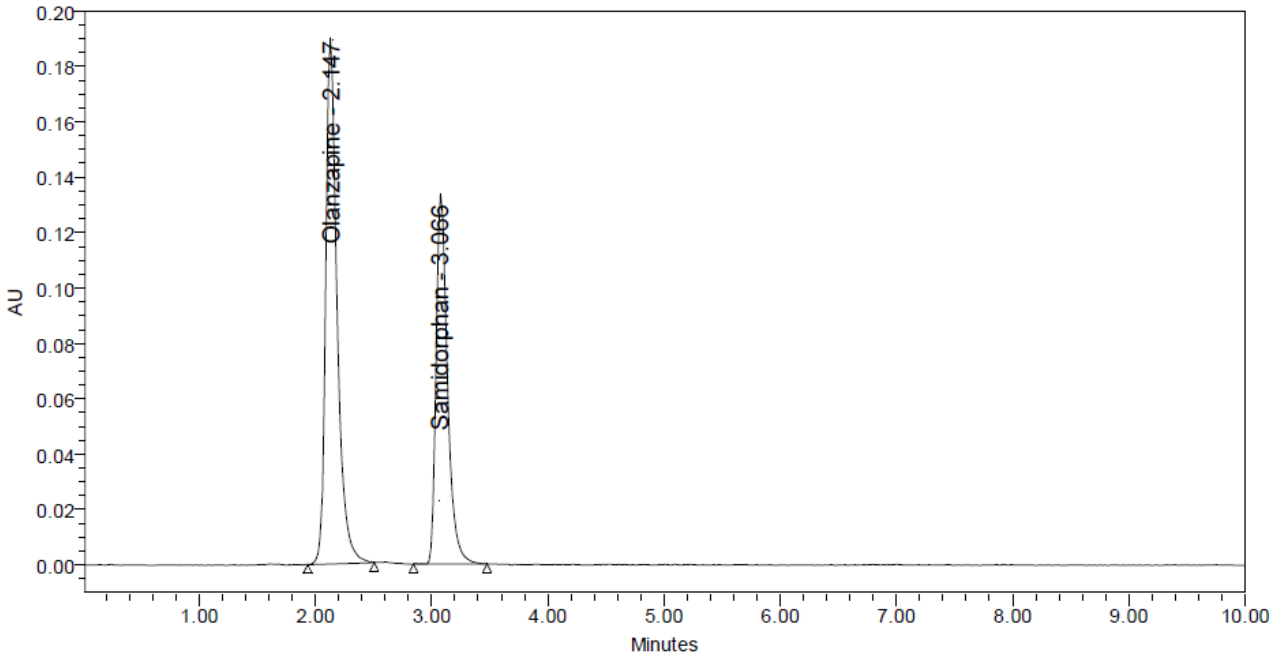


Fig. 2.27: Alkaline stress chromatogram of Olanzapine and Samidorphan

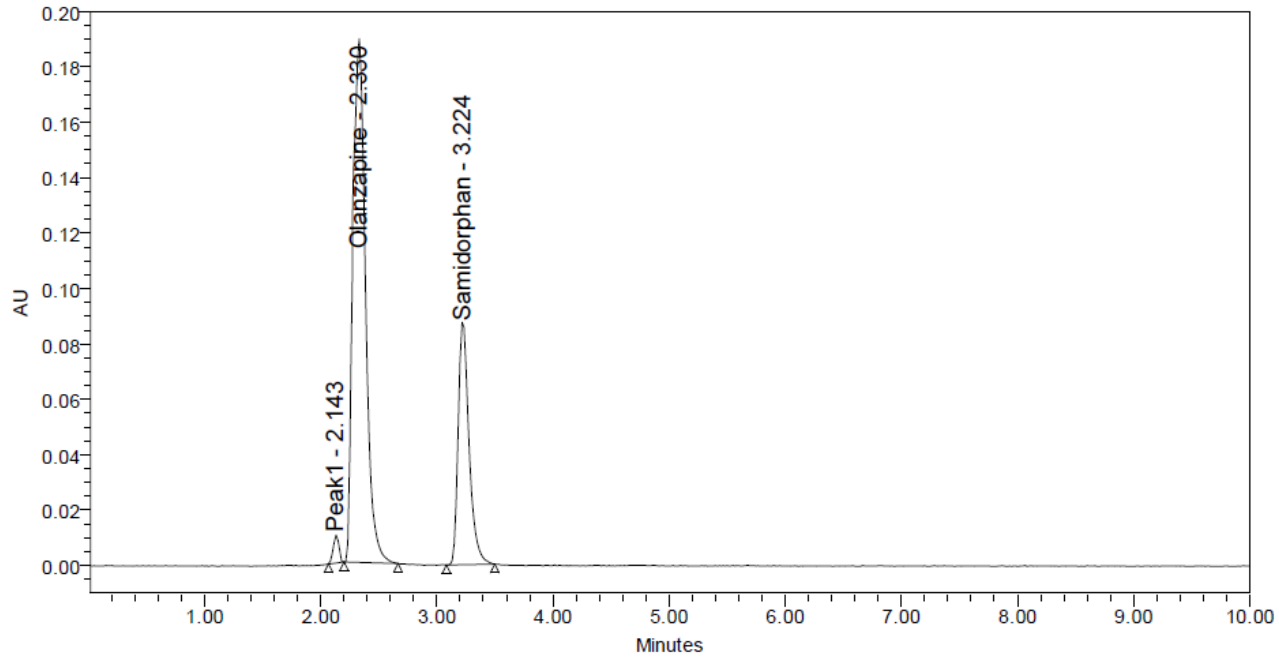


Fig. 2.28: Oxidative stress chromatogram of Olanzapine and Samidorphan

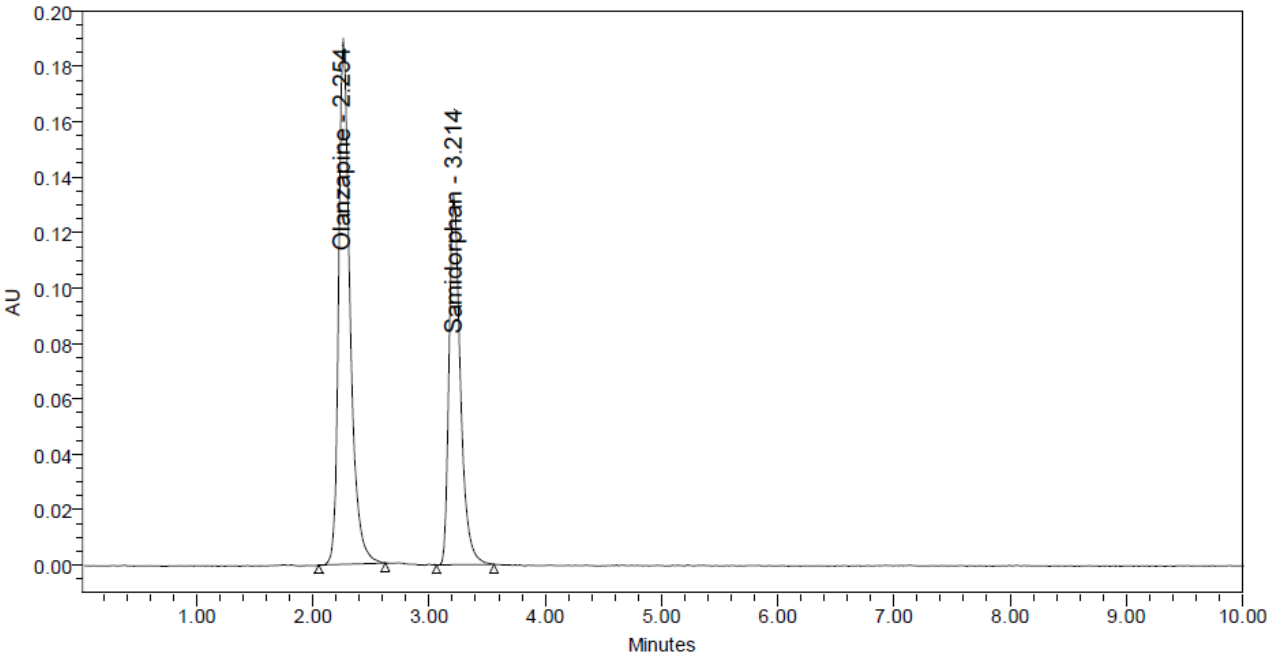


Fig. 2.29: Dry heat stress chromatogram of Olanzapine and Samidorphan

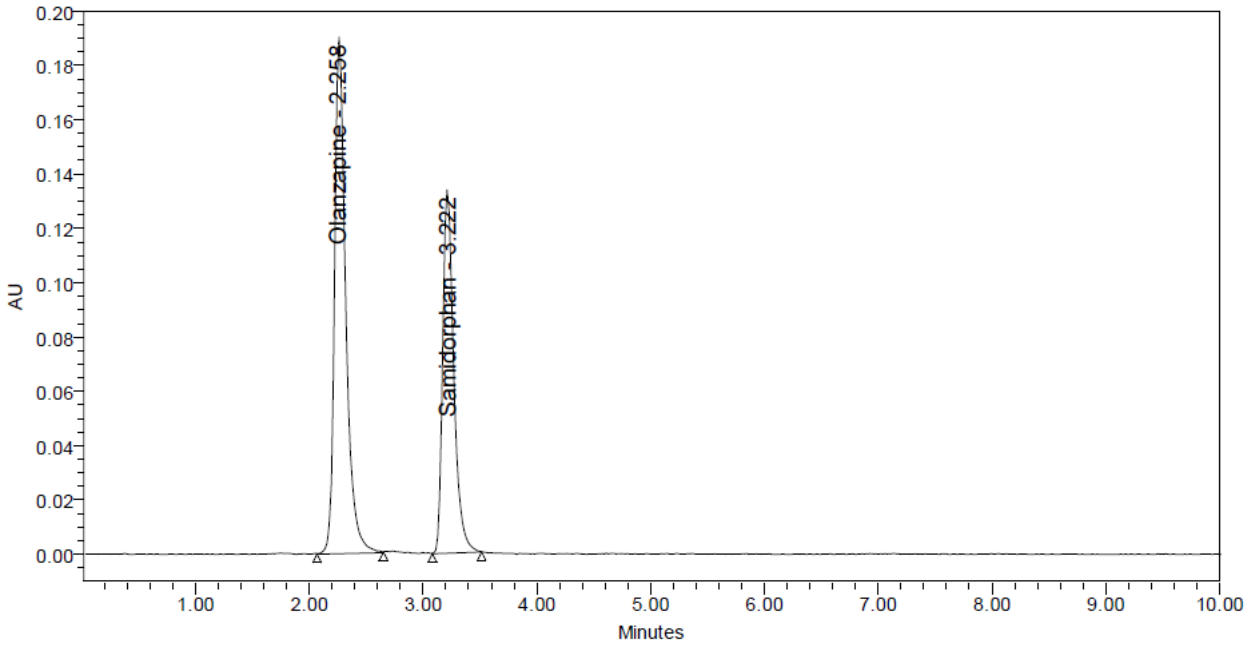


Fig. 2.30: Photolytic stress chromatogram of Olanzapine and Samidorphan

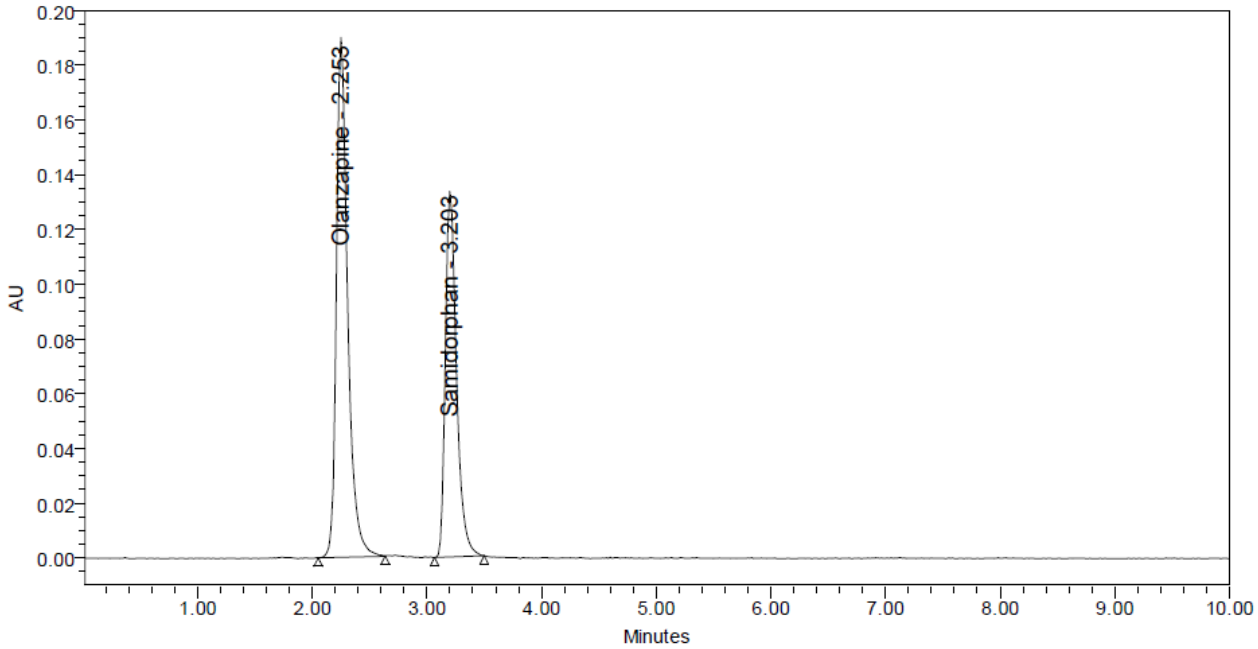


Fig. 2.31: Water stress chromatogram of Olanzapine and Samidorphan

Table 1: linearity results of olanzapine and samidorphan

%Level	Concentration Olanzapine (µg/ml)	Peak Response
25	3.75	283740
50	7.5	553176
75	11.25	834297
100	15	1103683
125	18.75	1363641
150	22.5	1631183

%Level	Concentration Samidorphan (µg/ml)	Peak Response
25	2.5	171860
50	5	334164
75	7.5	495276
100	10	665790
125	12.5	827592
150	15	991434

Table 2: calibration parameters of Olanzapine and Samidorphan

Best fit line parameters	Olanzapine (Mean ± Std. Deviation)	Samidorphan (Mean ± Std. Deviation)
Slope	72418x± 195.146	65963± 296.764
Intercept	9546.6± 3648.9	3295.9±1588.9
Correlation coefficient (r^2)	0.9998	0.9999

Table 3: Accuracy data results of Olanzapine

%Accuracy Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	7.5	7.5	100.5	99.83%
	7.5	7.4	99.3	
	7.5	7.6	100.9	
100%	15	15.0	100.2	
	15	14.7	98.1	
	15	15.1	100.7	
150%	22.5	22.3	99.0	
	22.5	22.5	100.0	
	22.5	22.4	99.7	

Accuracy obtained results of Samidorphan

%Accuracy Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.96	99.16	99.84%
	5	5.00	99.97	
	5	5.00	100.08	
100%	10	9.92	99.23	
	10	10.02	100.20	
	10	9.98	99.79	
150%	15	14.96	99.72	
	15	15.00	100.03	
	15	15.06	100.42	

Table 4: System suitability results of Olanzapine and Samidorphan

S.No.	Olanzapine		Samidorphan	
	Peak Retention Time	Peak Area	Peak Retention Time	Peak Area
1	2.195	1095197	3.219	664304
2	2.198	1099827	3.234	659676
3	2.200	1107811	3.245	658066
4	2.203	1087921	3.258	664819
5	2.204	1129729	3.275	663003
6	2.206	1101276	3.285	664958
Mean	**2.202**	**1103627**	**3.259**	**662471**
Std.dev	**0.003**	**14398.1**	**0.021**	**2917.4**
%RSD	**0.1**	**1.3**	**0.6**	**0.4**

Table 5: Intermediate precision Reproducibility results of Olanzapine and Samidorphan

S.No	Olanzapine Peak Area	Samidorphan Peak Area
1	1085051	661105
2	1109750	662449
3	1084589	659733
4	1094450	658963
5	1101426	664355
6	1105314	659829
AVG	**1096763**	**661072**
STDEV	**10528.6**	**2022.9**
%RSD	**1.0**	**0.3**

Table 6: Assay result of tablet dosage formulation

Brand Name	%Assay
LYBALVI	% Assay for Olanzapine = 99.28
	% Array for Samidorphan = 99.69

Forced degradation studies of Olanzapine and Samidorphan

Table 7:Degradation Data of Olanzapine

S.NO	Degradation Condition	Retention time	Peak Area	% Drug Remained	% Drug Degraded
1	Acid	2.157	1044612	94.56	5.44
2	Alkali	2.147	1056640	95.65	4.35
3	Oxidation	2.339	1061566	96.09	3.91
4	Thermal	2.254	1083216	98.05	1.95
5	UV	2.258	1089371	98.61	1.39
6	Water	2.253	1095143	99.13	0.87

Degradation Data of Samidorphan

S.NO	Degradation Condition	Retention time	Peak Area	% Drug Remained	% Drug Degraded
1	Acid	3.117	629721	94.96	5.04
2	Alkali	3.066	631767	95.27	4.73
3	Oxidation	3.224	637776	96.18	3.82
4	Thermal	3.214	647526	97.65	2.35
5	UV	3.222	654035	98.63	1.37
6	Water	3.203	658285	99.27	0.73

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