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A SIMPLE, ACCURATE, PRECISE METHOD WAS DEVELOPED FOR THE SIMULTANEOUS ESTIMATION OF THE OLANZAPINE AND SAMIDORPHAN IN TABLET DOSAGE FORM

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ABSTRACT
A simple, Accurate, precise method was developed for the simultaneous estimation of the Olanzapine and Samidorphan in Tablet dosage form. Chromatogram was run through Std zorbax eclipse xdb-c18(150 x 4.6 mm, 5m) Mobile phase containing Buffer Na2Hpo4: Acetonitrile taken in the ratio 70:30 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.01N Na2Hpo4 buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 226nm. Retention time of Olanzapine and Samidorphan were found to be 2.209 min and 3.196 min. %RSD of the Olanzapine and Samidorphan were and found to be 0.6 and 0.2 respectively. %Recovery was obtained as 99.79% and 99.60% for Olanzapine and Samidorphan respectively. LOD, LOQ values obtained from regression equations of Olanzapine and Samidorphan were 0.09, 0.28 µg/ml and 0.04, 0.13µg/ml respectively. Regression equation of Olanzapine is y =40559x + 8327, and y = 39599x + 4901 of Samidorphan. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Olanzapine, Samidorphan, RP-HPLC.

INTRODUCTION

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data [1]. Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.

Reversed Phase - High Performance Liquid Chromatography (RP-HPLC): As opposed to NP-HPLC, RP-HPLC employs mainly dispersive forces (hydrophobic or vanderwal's interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. RP-HPLC is by far the most popular mode of chromatography. Almost 90 % of all analyses of low-molecular-weight samples are carried out using RP-HPLC. Dispersive forces employed in this separation mode are the weakest intermolecular forces, thereby making the overall background interaction energy in the chromatographic system very low compared to other separation techniques. This low background energy allows for distinguishing very small differences in molecular interactions of closely related analytes. Adsorbents employed in this mode of chromatography are porous rigid materials with hydrophobic surfaces. The majority of packing materials used in RP-HPLC are chemically modified porous silica.

ANALYTICAL METHOD DEVELOPMENT (17-21):

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeias) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is

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intended to replace the existing procedure, comparative laboratory data including merits / demerits should be made available.

Steps involved in method development:

Analyte standard characterization [2]. Method requirements [3]. Literature search and prior methodology [4]. Choosing a method [5]. Instrumental setup and initial studies [6]. Optimization [7]. Documentation of analytical figures of merit [8]. Evaluation of method development with actual samples [9]. Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

METHOD DEVELOPMENT PROCEDURE [18]:

The wide variety of equipment's, columns, eluent and operation preparations involved high performance liquid chromatography (HPLC) method development seems complex. The processes influenced by the nature of analytes and generally follow the following steps

Steps: Step 1 - Selection of the HPLC method and initial system, Step 2 - Selection of initial conditions, Step 3 - Selectivity optimization, Step 4 - System optimization, Step 5 - Method validation. Depending on the overall requirements and nature of the sample and analytes, some of these steps will not be necessary during HPLC analysis. For example, a satisfactory separation may be found during step 2, thus steps 3 and 4 may not be required. The extent to which method validation (step 5) is investigated will depend on the use of the end analysis; for example, a method required for quality control will require more validation than one developed for a one-off analysis. The following must be considered when developing an HPLC method:

STABILITY INDICATING METHOD:

It is essential that the analytical methods developed for estimation of the purity and impurities are capable enough to separate all the desired and undesired components and devoid of any interferences from the formulation matrix. When analytical methods are able to precisely and accurately quantify without missing any impurities, without underestimation or over estimation, and detect all possible impurities and degradants those can form during stability studies with adequate sensitivity and exactly reflect the quality of drug substances and drug products (formulated products of drugs), those methods are called stability indicating methods.

A stability-indicating assay method should accurately measure the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities. If an industry uses a non-stability indicating analytical procedure for release testing, then an analytical procedure capable of qualitatively and quantitatively monitoring the impurities, including degradation products, should complement it. Analytical procedures for stability studies of assay should be stability indicating. As a result of stability testing a re-test period for the active substance or a shelf life for the pharmaceutical product can be established, and storage conditions can be recommended.

The ICH (International conference on Harmonization) guideline QIA on Stability Testing of New Drug Substances and Products emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy must be done by validated stability indicating testing methods. It is also mentioned that forced decomposition studies (stress testing) at temperatures in 10 °C increments above the accelerated temperatures, extremes of pH, under oxidative and photolytic conditions should be carried out on the drug substance and drug product so as to establish the inherent stability characteristics and degradation pathways to support the suitability of the proposed analytical procedures.

Structure of Samidorphan

Indication:

Samidorphan is indicated in combination with olanzapine for the treatment of bipolar I disorder, either as an adjunct to lithium or valproate or as monotherapy for the acute treatment of manic or mixed episodes or as maintenance therapy, and for the treatment of schizophrenia in adults.

MATERIALS AND METHODS

Materials:

Olanzapine and Samidorphan pure drugs **Lybalvi,** Combination Olanzapine and Samidorphan Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Instruments:

Electronics Balance-Denver,p^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, Waters HPLC System series with Binary pumps, Photo Diode array detector and manual sampler integrated with empower software, Lab india UV double beam spectrophotometer with UV win5 software was used for measuring absorbances of Olanzapine and Samidorphan solutions.

Methods: Diluent:

Based up on the solubility of the drugs, Diluent was selected, Buffer and Water taken in the ratio of 50:50

Preparation of Standard stock solutions:

Accurately weighed 15mg of Olanzapine and 10mg of Samidorphan and transferred to 50ml volumetric

flask. And 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (300µg/ml of Olanzapine and 200µg/ml of Samidorphan)

Preparation of Standard working solutions (100% solution):

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (30µg/ml Olanzapine of and 20µg/ml of Samidorphan)

Preparation of Sample stock solutions:

5 tablets were weighed and equivalent to 1 tablet is weighed and transferred to 50 ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made upto 50ml with diluents and filtered through 1 µm or finer porosity membrane filter (300µg/ml of Olanzapine and 200µg/ml of Samidorphan)

Preparation of Sample working solutions (100% solution):

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (30µg/ml of Olanzapine and 20µg/ml of Samidorphan)

Preparation of buffer: Buffer: 0.01N Sodium hydrogen phosphate:

Accurately weighed 1.42gm of Sodium hydrogen phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.5 with dil. Orthophosphoric acid solution.

Validation: System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Olanzapine (30ppm) and Samidorphan (20ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity:

Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Preparation of Standard stock solutions:

Accurately weighed 15mg of Olanzapine and 10mg of Samidorphan and transferred to 50ml volumetric flask. And 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (300µg/ml of Olanzapine and 200µg/ml of Samidorphan)

Preparation of Standard working solutions (100% solution1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (30µg/ml Olanzapine of and 20µg/ml of Samidorphan)

Preparation of Sample stock solutions:

5 tablets were weighed and equivalent to 1 tablet is weighed and transferred to 50 ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made upto 50ml with diluents and filtered through 0.45 µm or finer porosity membrane filter (300µg/ml of Olanzapine and 200µg/ml of Samidorphan)

Preparation of Sample working solutions (100% solution):

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (30µg/ml of Olanzapine and 20µg/ml of Samidorphan)

Linearity:

Preparation of Standard stock solutions:

Accurately weighed 15mg of Olanzapine and 10mg of Samidorphan and transferred to 50ml volumetric flask. And 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (300µg/ml of Olanzapine and 200µg/ml of Samidorphan).

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (7.5µg/ml of Olanzapine and 5µg/ml of Samidorphan)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Olanzapine and 10µg/ml of Samidorphan)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (22.5µg/ml of Olanzapine and 15µg/ml of Samidorphan)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (30µg/ml of Olanzapine and 20µg/ml of Samidorphan)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (37.5µg/ml of Olanzapine and 25µg/ml of Samidorphan)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml(45µg/ml of Olanzapine and 30µg/ml of Samidorphan)

Accuracy: Preparation of Standard stock solutions:

Accurately weighed 15mg of Olanzapine and 10mg of Samidorphan and transferred to 50ml volumetric flask. And 3/4 th of diluents was added to these flask and

sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (300µg/ml of Olanzapine and 200µg/ml of Samidorphan).

Preparation of 50% Spiked Solution:

0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution:

1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution:

1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102

Robustness:

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation:

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Olanzapine and Samidorphan solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation:

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Olanzapine and Samidorphan solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

DEGRADATION STUDIES:

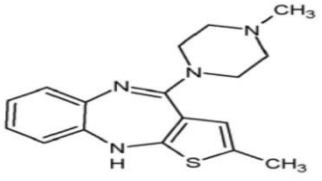
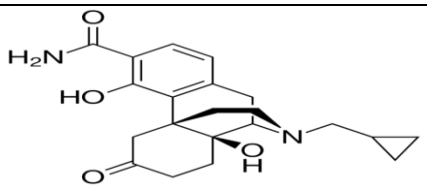
Oxidation:

Acid Degradation Studies, Alkali, Degradation. Studies, Dry Heat Degradation Studies, Photo stability studies: Neutral Degradation Studies

Table 1: Characteristics to be validated in HPLC

Characteristics	**Acceptance Criteria**
Accuracy/trueness	Recovery 98-102% (individual)
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity / Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10
Linearity	Correlation coefficient R^2 > 0.999
Range	80 –120 %

DRUG PROFILE: Olanzapine

Structure: **Structure of Olanzepine**	**Structure of Samidorphan**
	

S No	Olanzapine			Samidorphan			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.197	2774	1.44	3.058	4765	1.40	4.8
2	2.200	2740	1.43	3.219	4967	1.39	5.6
3	2.202	2670	1.44	3.234	5001	1.37	5.6
4	2.205	2696	1.41	3.245	4917	1.36	5.7
5	2.205	2756	1.45	3.275	4973	1.38	5.8
6	2.207	2628	1.46	3.285	4977	1.37	6.0

Table 2: Linearity table for Olanzapine and Samidorphan.

Olanzapine		Samidorphan	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
7.5	319448	5	208469
15	614834	10	412360
22.5	925182	15	593738
30	1227382	20	789227
37.5	1534804	25	981352
45	1824728	30	1207086

Table 3: System precision table of Olanzapine and Samidorphan

S. No	Area of Olanzapine	Area of Samidorphan
1.	1223199	837556
2.	1228898	839005
3.	1220332	833278
4.	1242530	839029
5.	1233107	841773
6.	1227376	831345
Mean	1229240	836998
S.D	7886.9	3925.8
%RSD	0.6	0.5

Repeatability:

Table 4: Repeatability table of Olanzapine and Samidorphan

S. No	Area of Olanzapine	Area of Samidorphan
1.	1223895	835558
2.	1222724	838993
3.	1223237	830463
4.	1227788	834109
5.	1224103	832093
6.	1229397	836704
Mean	1225191	834653
S.D	2727.7	3107.0
%RSD	0.2	0.4

Intermediate precision (Day_ Day Precision):

Table 5: Intermediate precision table of Olanzapine and Samidorphan

S. No	Area of Olanzapine	Area of Samidorphan
1.	1251965	830277
2.	1234853	839766
3.	1254472	836474
4.	1260066	832566
5.	1238849	834720
6.	1240840	834915

Mean	1246841	834786
S.D	10029.8	3253.6
%RSD	0.8	0.4

Table 6: Accuracy table of Olanzapine.

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	15	14.79	98.62	99.79%
	15	14.90	99.33	
	15	14.88	99.19	
100%	30	29.82	99.39	
	30	30.03	100.12	
	30	30.33	101.09	
150%	45	45.18	100.40	
	45	44.65	99.22	
	45	45.35	100.77	

Table 7: Accuracy table of Samidorphan

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	10	9.93	99.34	99.60%
	10	9.97	99.66	
	10	10.02	100.22	
100%	20	19.95	99.74	
	20	19.83	99.13	
	20	19.93	99.67	
150%	30	29.91	99.69	
	30	29.88	99.60	
	30	30.19	100.64	

Table 8: Sensitivity table of Olanzapine and Samidorphan.

Molecule	**LOD**	**LOQ**
Olanzapine	0.09	0.28
Samidorphan	0.04	0.13

Table 9: Robustness data for Olanzapine and Samidorphan.

S.No	**Condition**	**%RSD of Olanzapine**	**%RSD of Samidorphan**
1	Flow rate (-) 0.9ml/min	0.6	0.1
2	Flow rate (+) 1.1ml/min	0.9	0.2
3	Mobile phase (-) 75B:25A	0.9	0.7
4	Mobile phase (+) 65B:35A	0.7	0.3
5	Temperature (-) 25°C	1	1
6	Temperature (+) 35°C	0.8	0.6

Table 10: Assay Data of Olanzapine

S.no	**Standard Area**	**Sample area**	**% Assay**
1	1223199	1223895	99.17
2	1228898	1222724	99.07
3	1220332	1223237	99.11
4	1242530	1227788	99.48
5	1233107	1224103	99.18
6	1227376	1229397	99.61

Avg	1229240	1225191	99.27
Stdev	7886.9	2727.7	0.22
%RSD	0.6	0.2	0.2

Table 11: Assay Data of Samidorphan

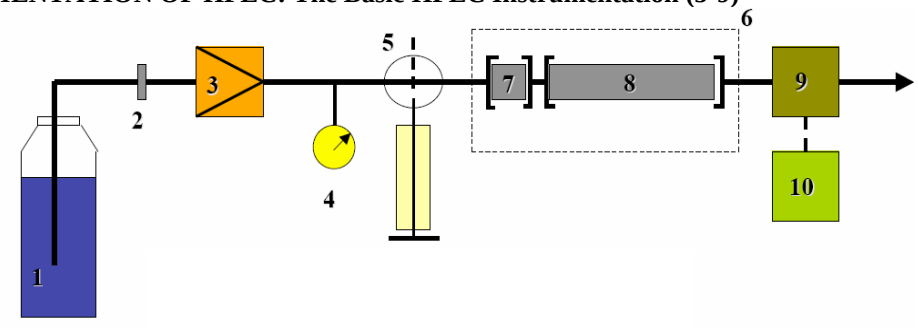
S.no	Standard Area	Sample area	% Assay
1	837556	835558	99.73
2	839005	838993	100.14
3	833278	830463	99.12
4	839029	834109	99.56
5	841773	832093	99.31
6	831345	836704	99.86
Avg	836998	834653	99.62
Stdev	3925.8	3107.0	0.4
%RSD	0.5	0.4	0.4

Table 12: Degradation data

Type of degradation	Olanzapine		Samidorphan	
	%RECOVERED	% DEGRADED	%RECOVERED	% DEGRADED
Acid	93.72	6.28	94.10	5.90
Base	95.61	4.39	95.67	4.33
Peroxide	95.46	4.54	95.57	4.43
Thermal	96.81	3.19	97.33	2.67
Uv	98.49	1.51	98.94	1.06
Water	99.42	0.58	99.59	0.41

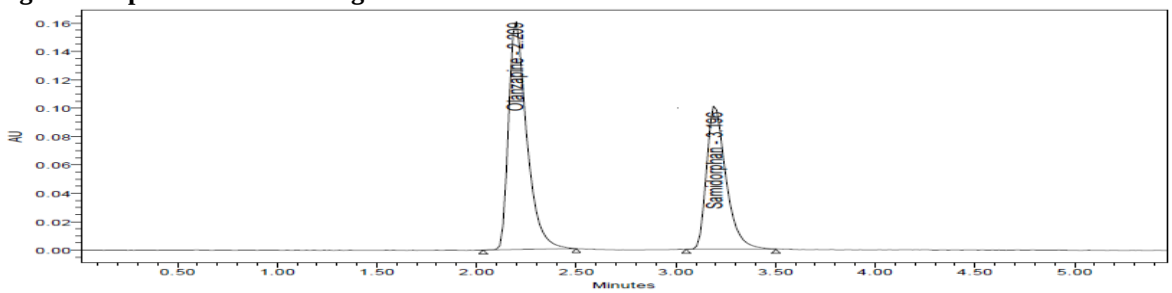
Parameters		Olanzapine	Samidorphan	LIMIT
Linearity Range (µg/ml)		7.5-45 µg/ml	5-30 µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		40559	39599x	
Intercept(c)		8327	4901	
Regression equation (Y=mx+c)		y = 40559x + 8327.	y =39599x + 4901.	
Assay (% mean assay)		99.27%	99.62%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.6	0.5	NMT 2.0%
Method precision %RSD		0.2	0.4	NMT 2.0%
Accuracy %recovery		99.79%	99.60%	98-102%
LOD		0.09	0.04	NMT 3
LOQ		0.28	0.13	NMT 10
Robustness	**FM**	0.6	0.1	%RSD NMT 2.0
	FP	0.9	0.2	
	MM	0.9	0.7	
	MP	0.7	0.3	
	TM	1	1	
	TP	0.8	0.6	

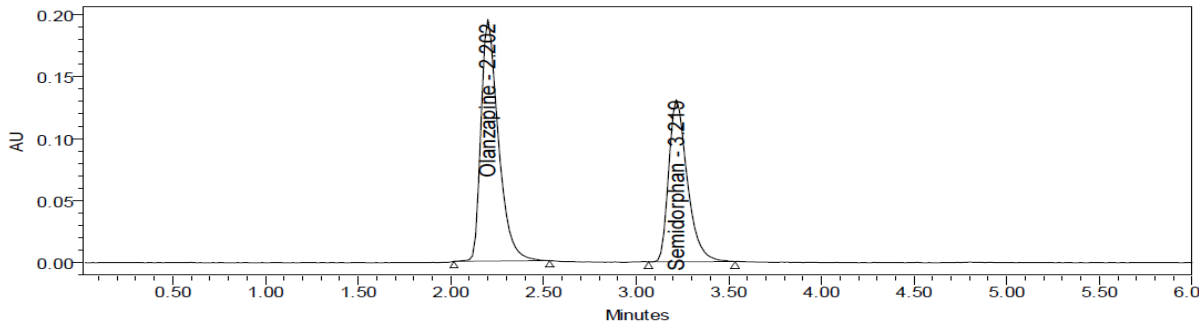
Figure 1: INSTRUMENTATION OF HPLC: The Basic HPLC Instrumentation (5-9)

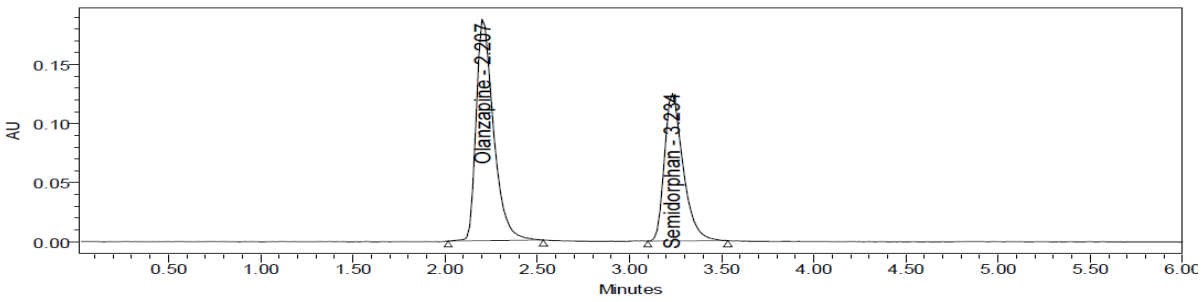


1 = eluent reservoir
2 = filter
3 = high pressure pump with pulse dampener
4 = pressure gauge
5 = sample injection valve with syringe
6 = column oven
7 = guard column
8 = column
9 = detector
10 = recorder (integrator, PC etc.)

Figure 2: Optimized Chromatogram







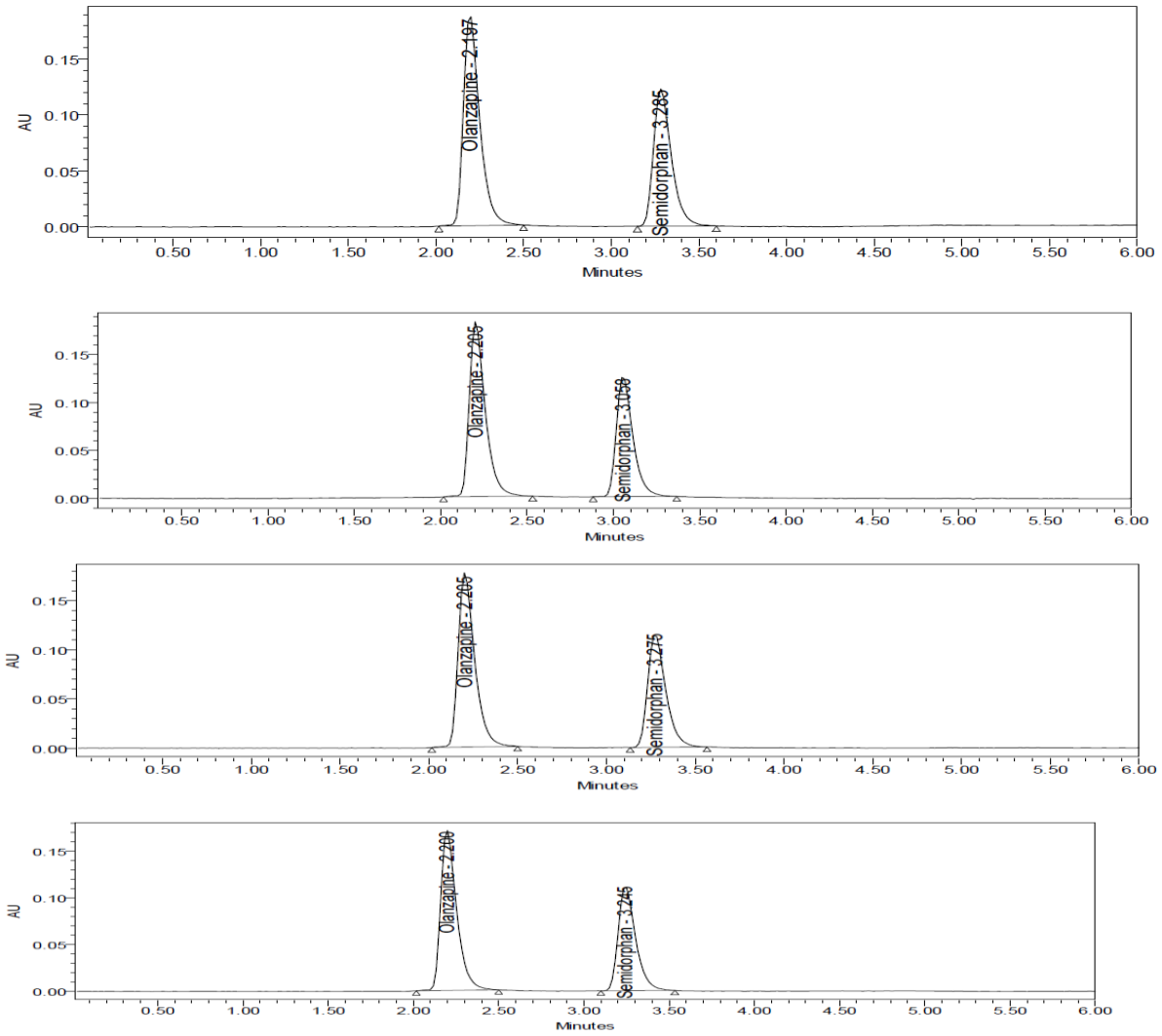


Figure 3: Chromatogram of blank.

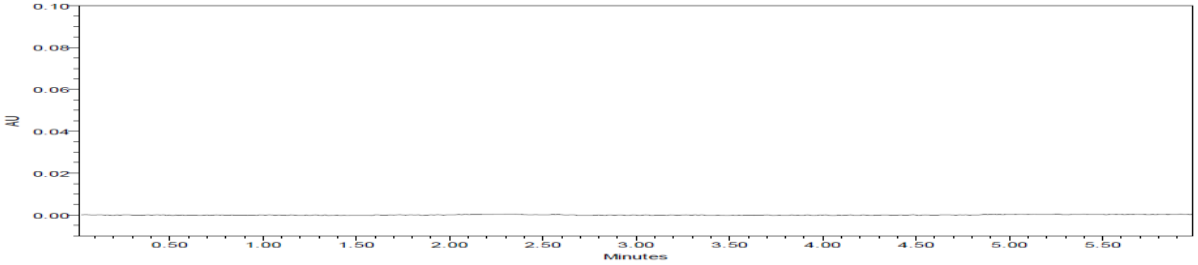


Figure 4: Chromatogram of placebo

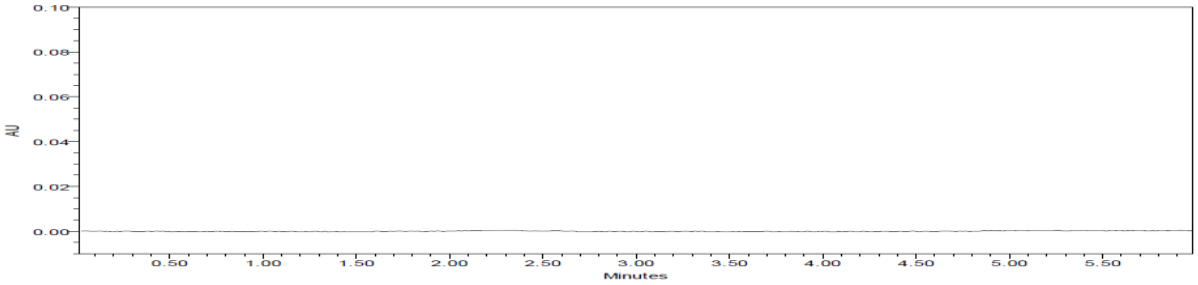


Figure 5: Typical Chromatogram

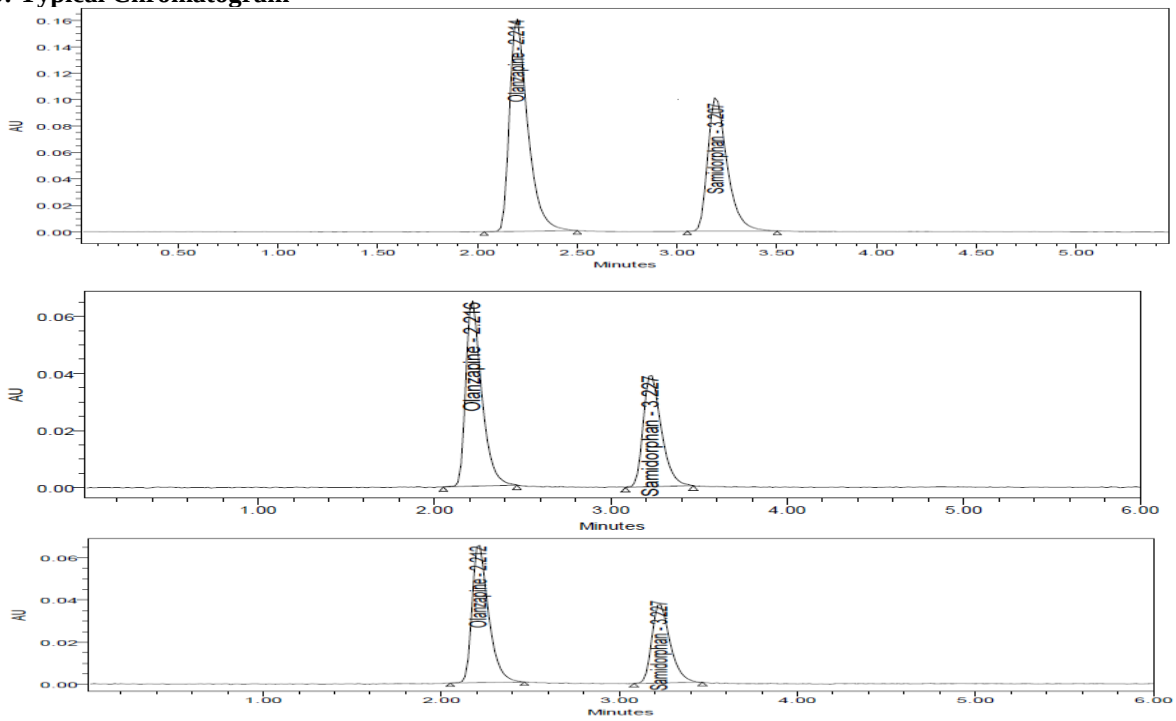


Figure 6: Calibration curve of Olanzapine

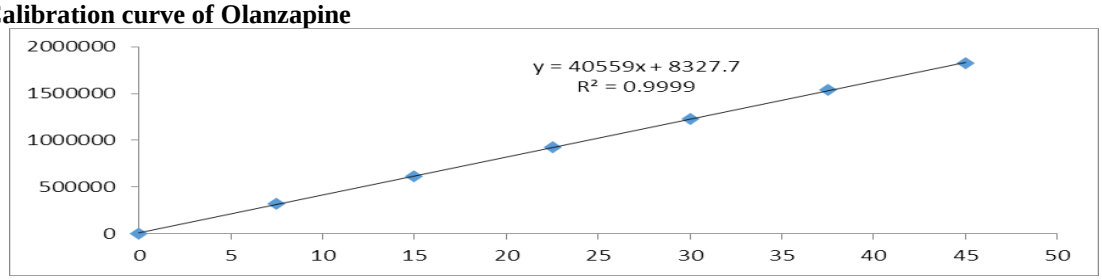


Figure 7: Calibration curve of Samidorphan

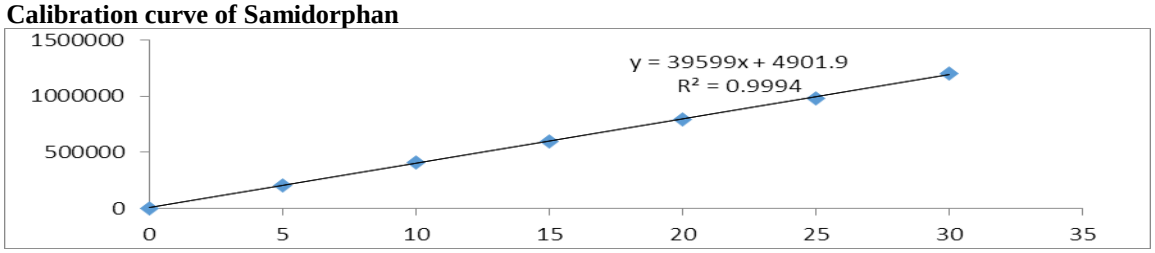
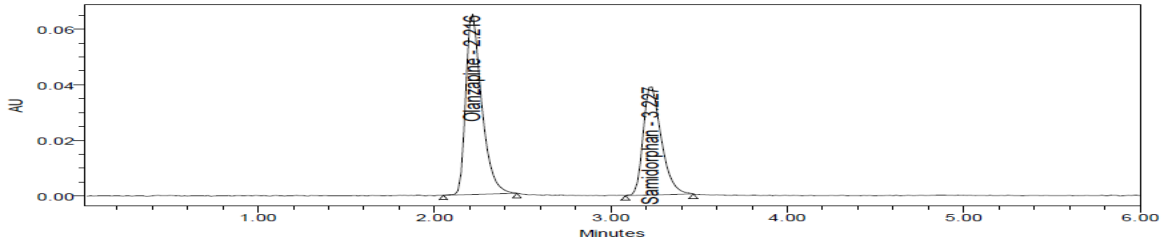


Figure 8: Linearity 25% Chromatogram of Olanzapine and Samidorphan



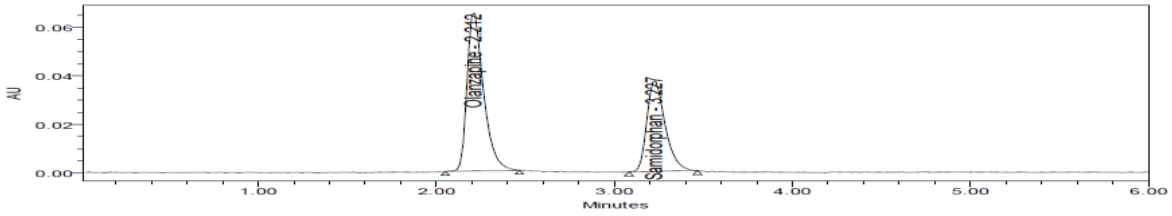


Figure 9: Linearity 50% Chromatogram of Olanzapine and Samidorphan

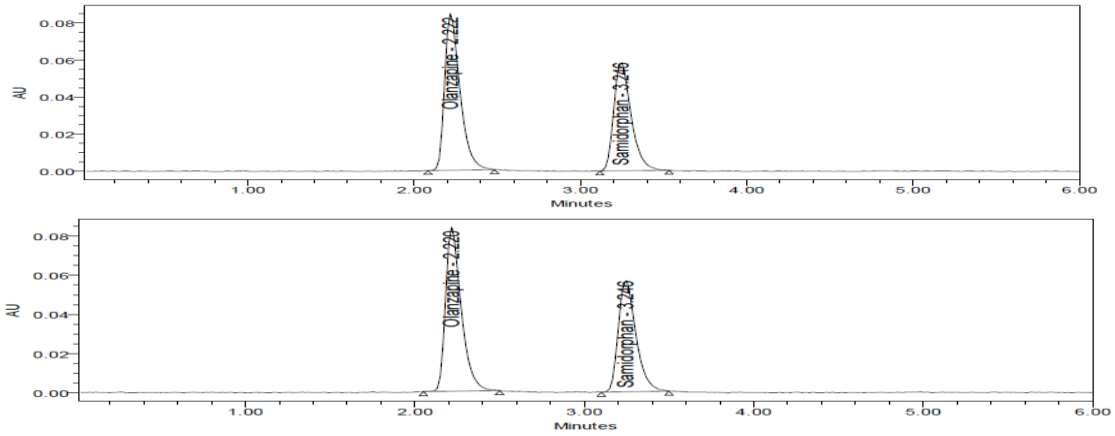


Figure 10: Linearity 75% Chromatogram of Olanzapine and Samidorphan

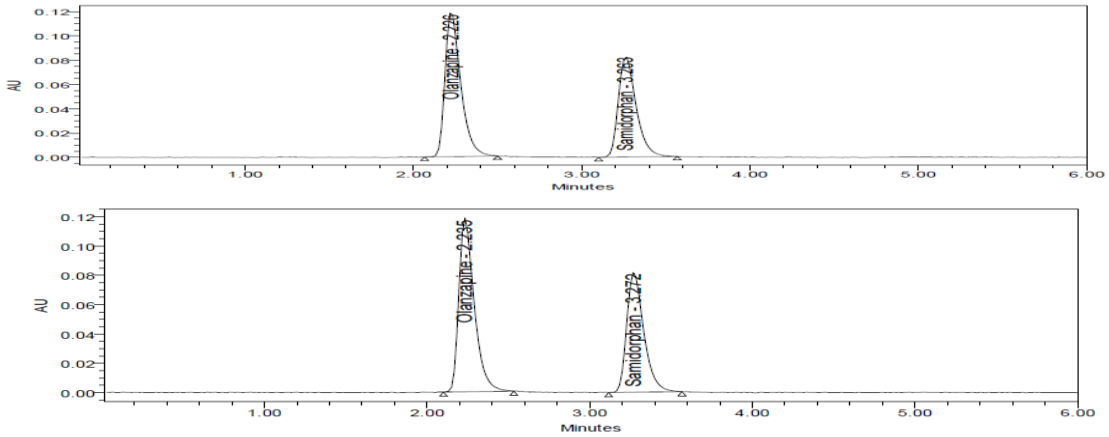


Figure 11: Linearity 100% Chromatogram of Olanzapine and Samidorphan

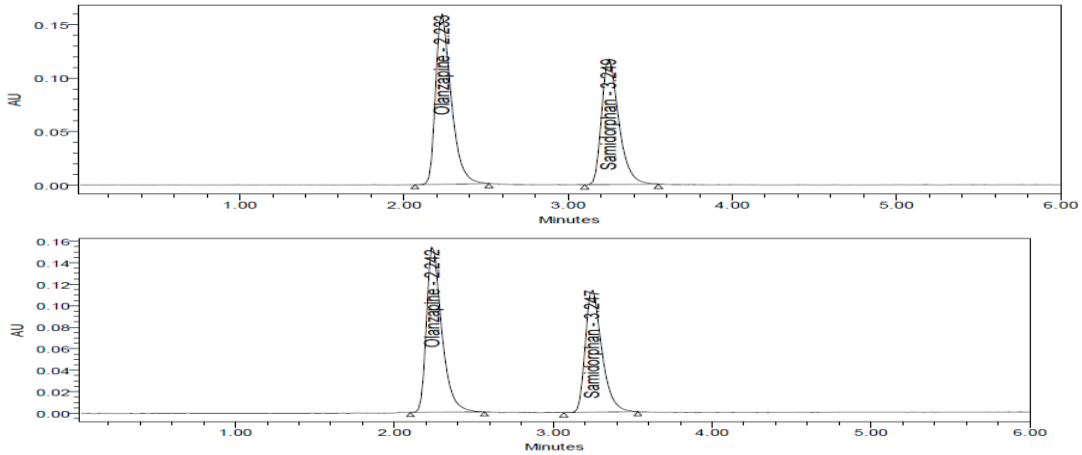


Figure 12: Linearity 125% Chromatogram of Olanzapine and Samidorphan

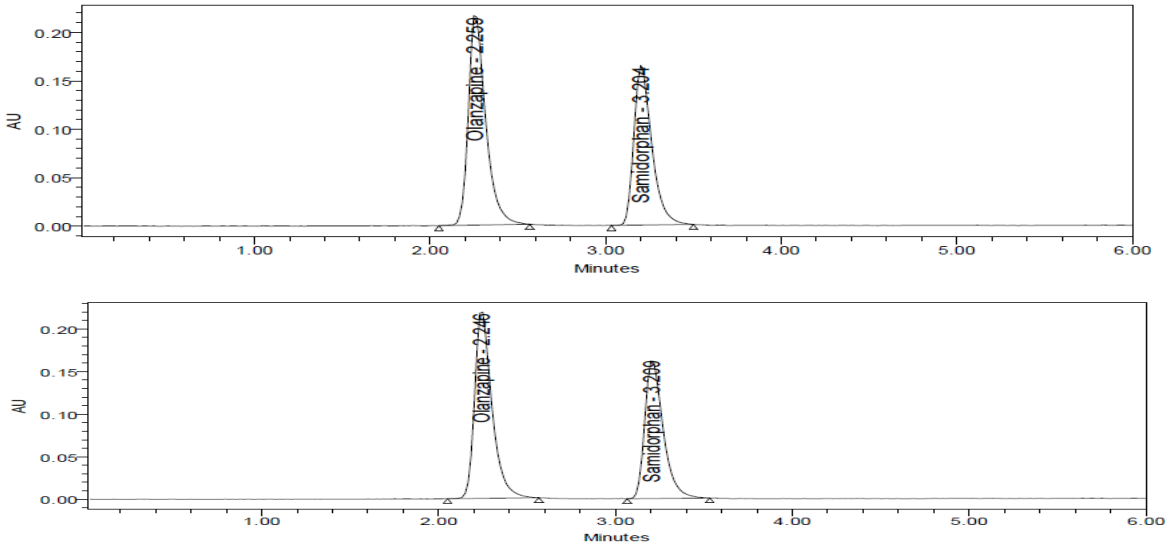


Figure 13: Linearity 150% Chromatogram of Olanzapine and Samidorphan

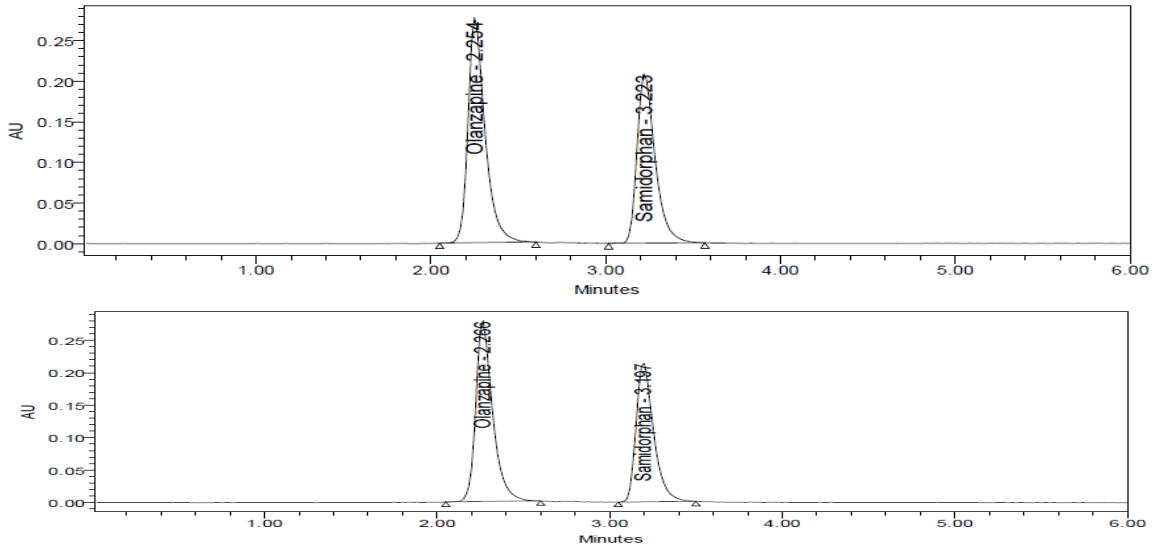
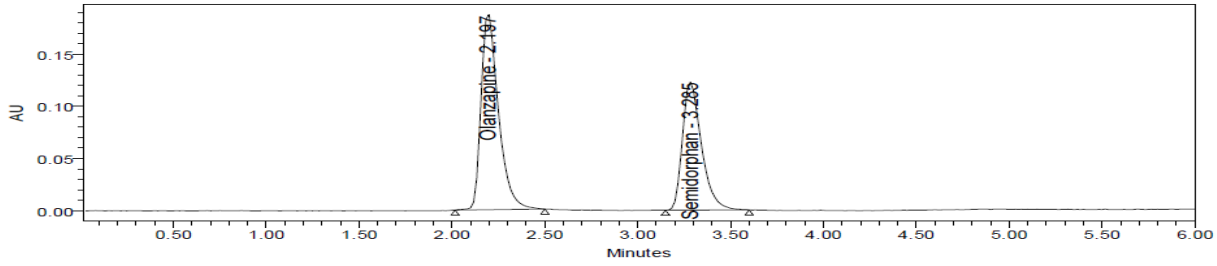


Figure 14: System precision chromatogram



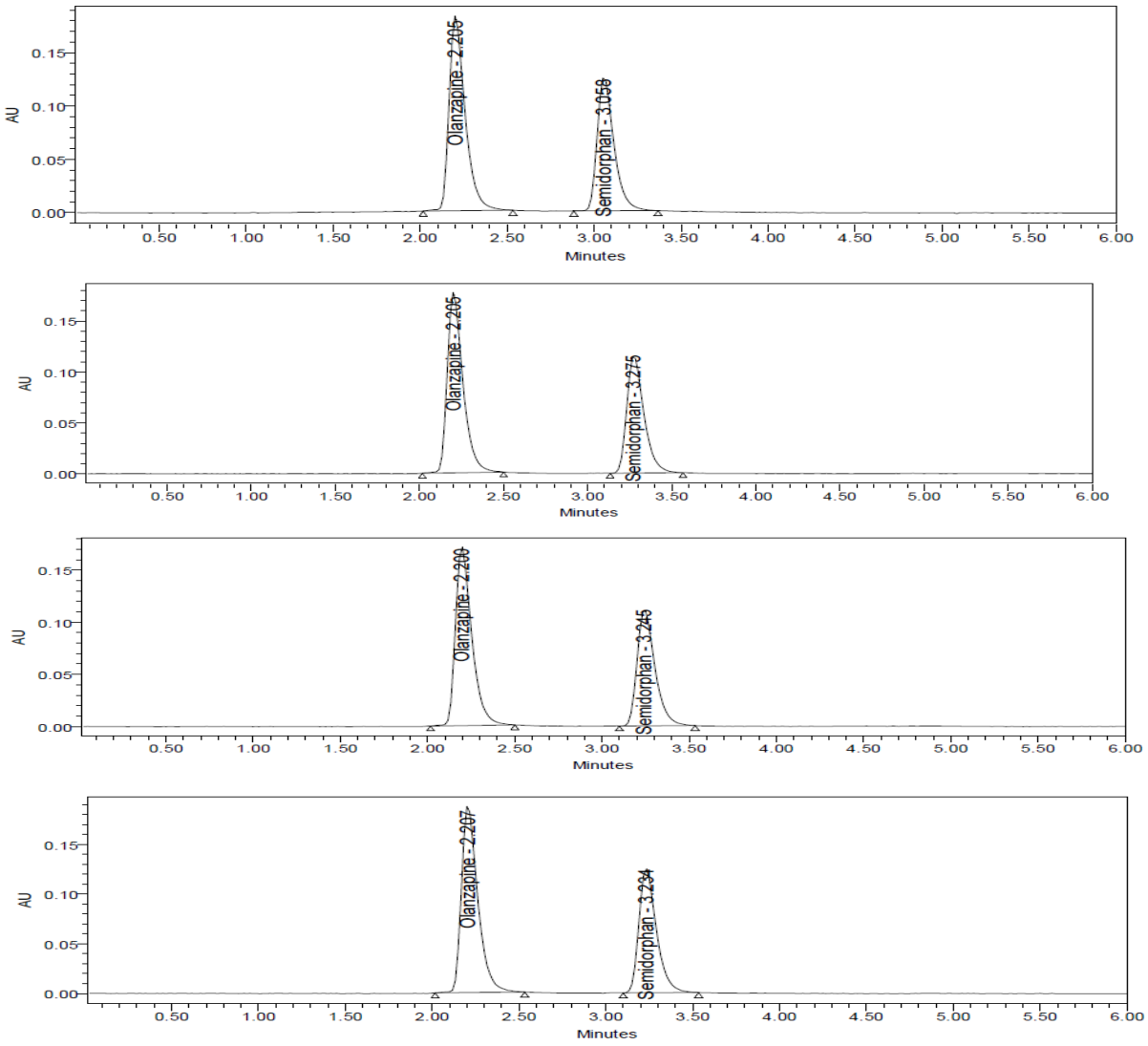
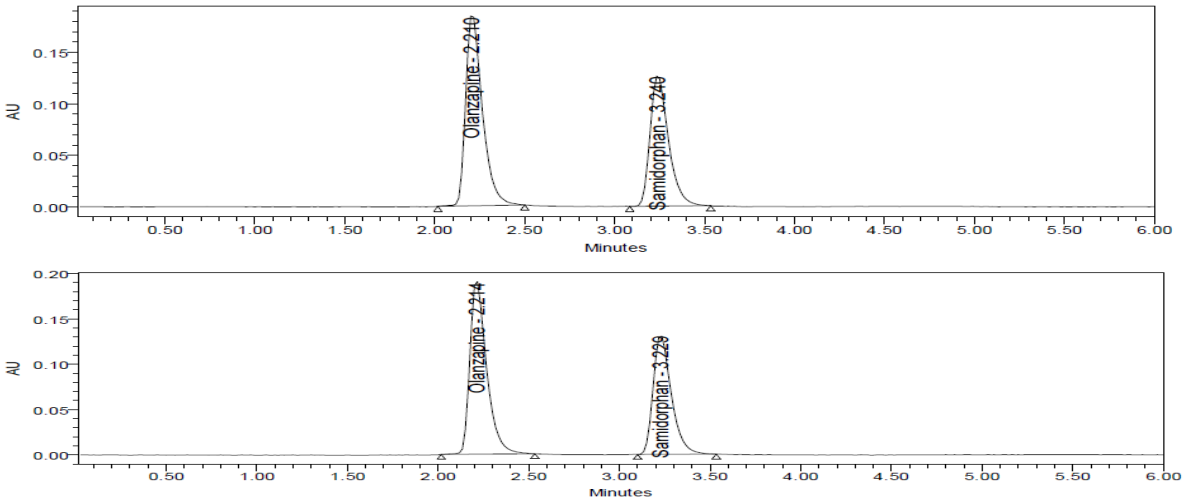


Figure 15: Repeatability chromatogram



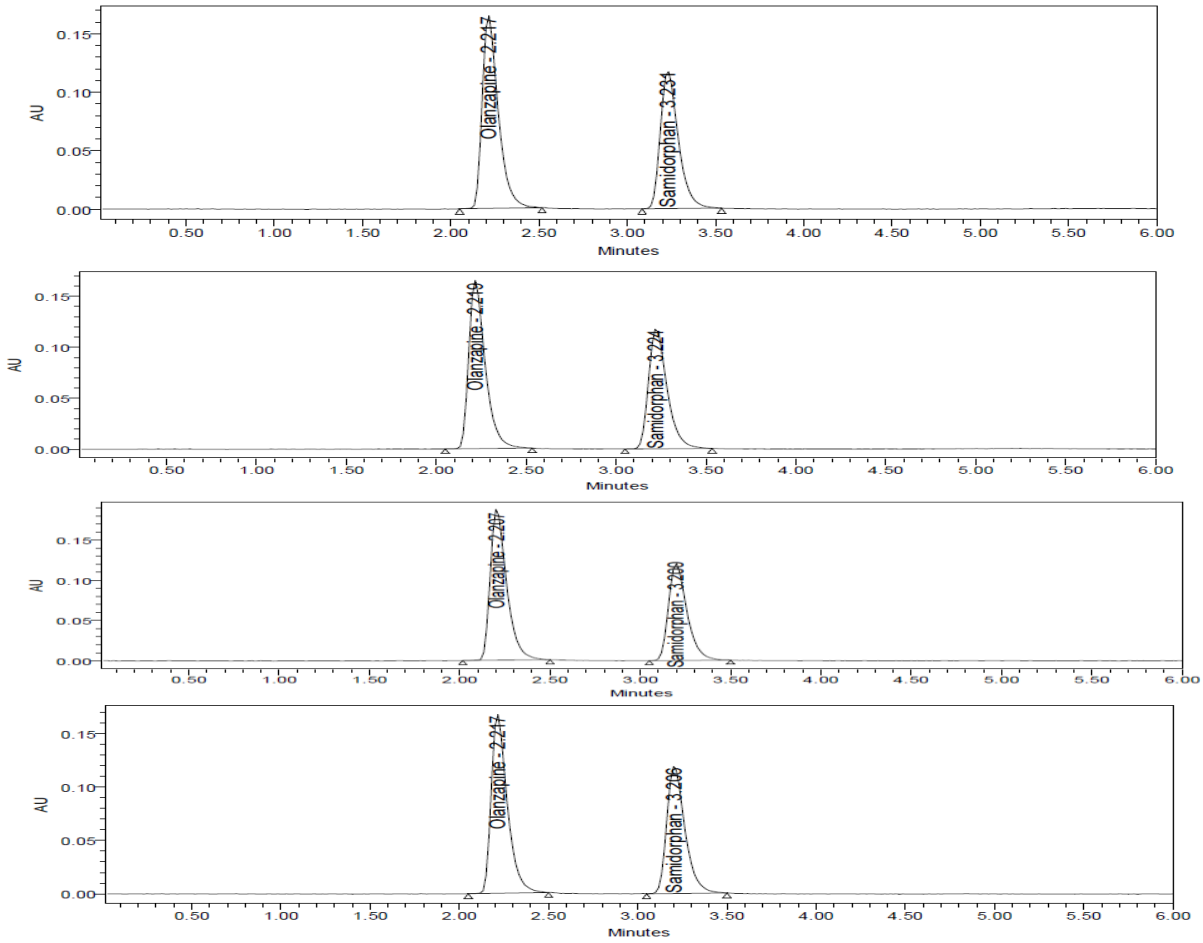
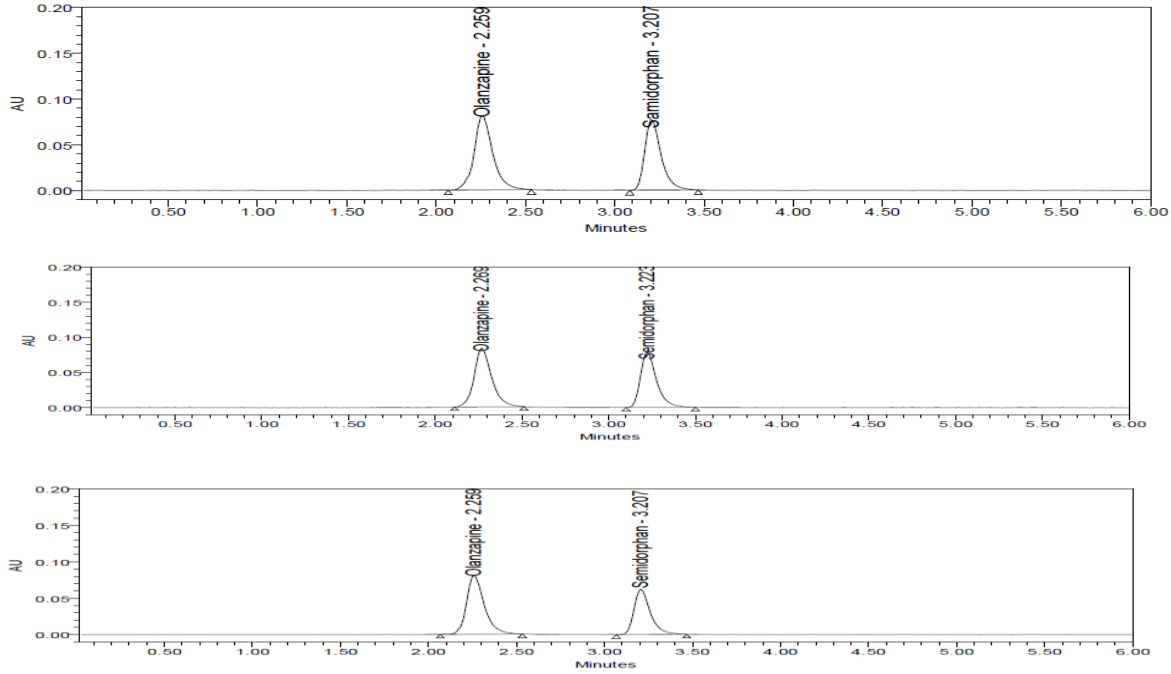


Figure 16: Inter Day precision Chromatogram



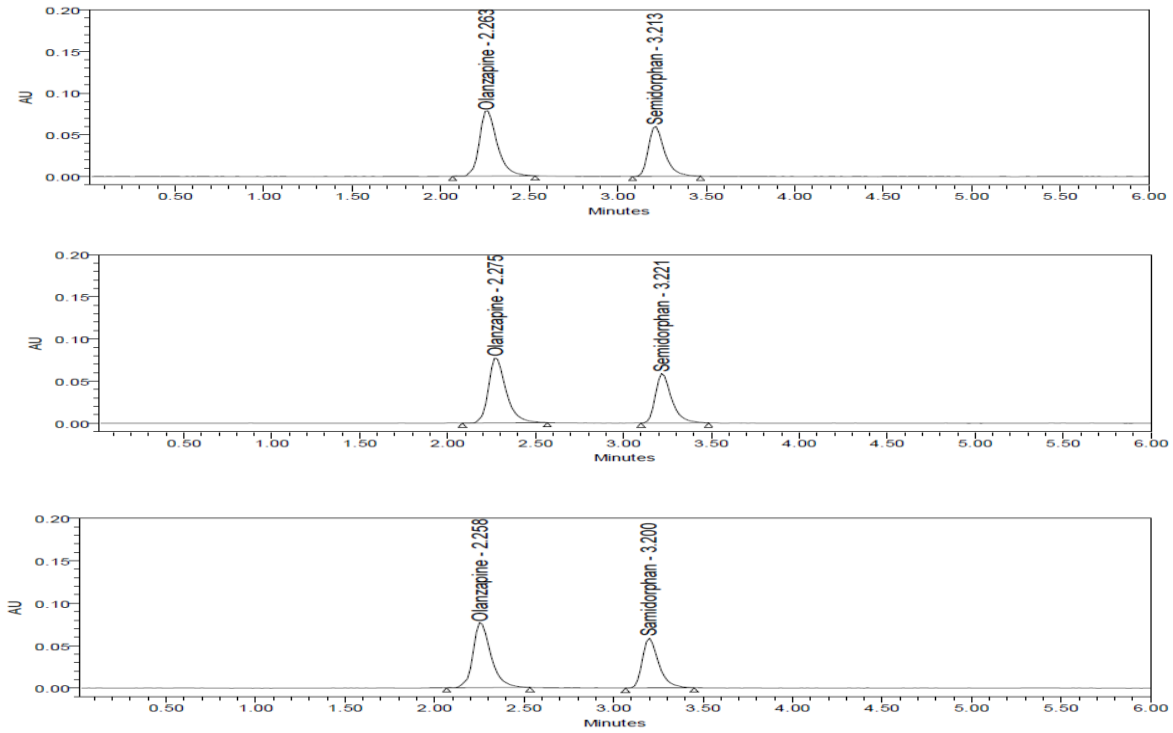


Figure 17: LOD Chromatogram of Standard

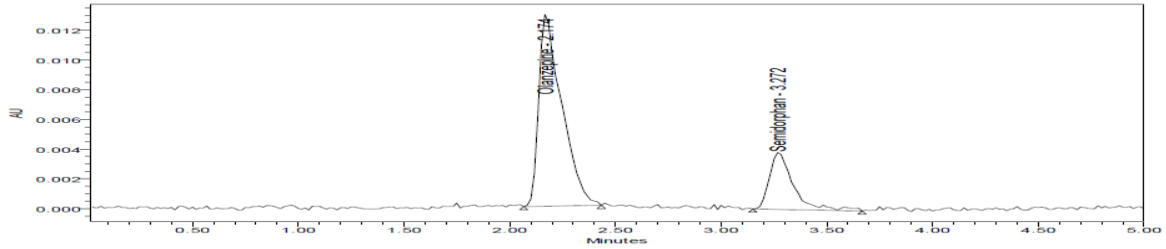


Figure 18: LOQ Chromatogram of of Standard

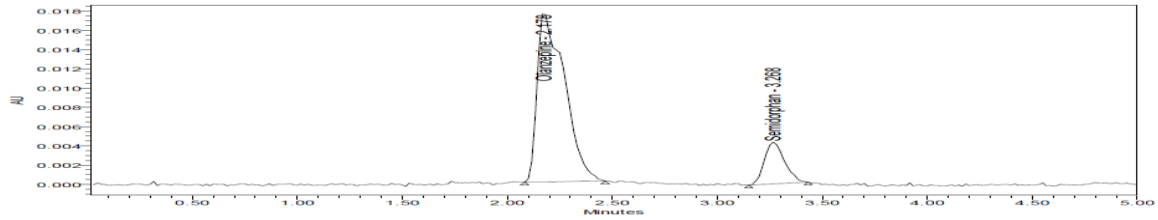


Figure 19: Chromatogram of working standard solution

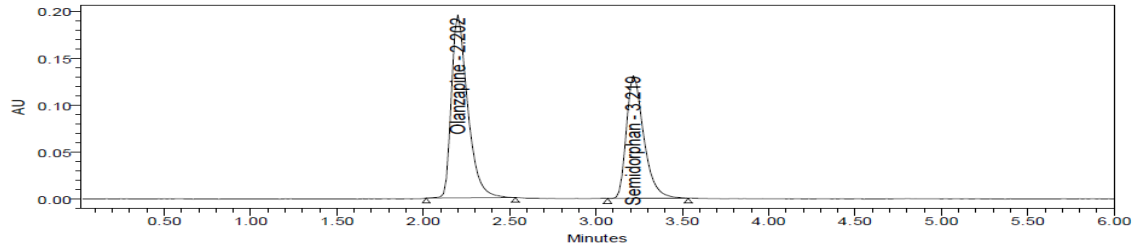
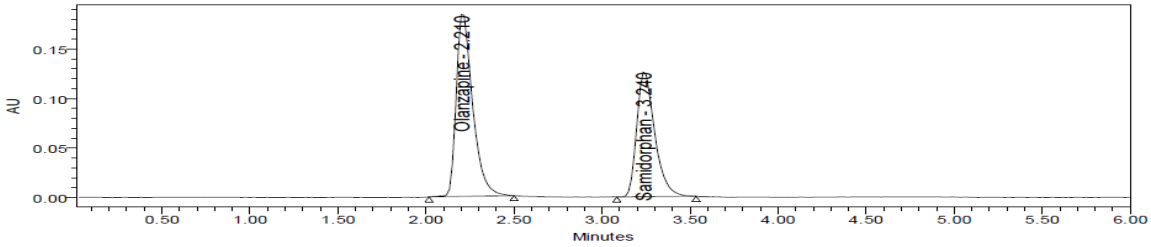


Figure 20: Chromatogram of working sample solution.



RESULTS AND DISCUSSION:

Method development:

Method development was done by changing various, mobile phase ratios, buffers etc.

Optimized method: Chromatographic conditions:

Mobile phase : 0.01N Na2HPO4 70%: 30% Acetonitrile
Flow rate : 1 ml/min
Column : Zorbax eclipse xdb-c18 (4.6 x 150mm, 5µm)
Detector wave length : 226nm
Column temperature : 30°C
Injection volume : 10µL
Run time : 6min
Results : Both peaks have good resolution, tailing Factor, theoretical plate count. So this can be consider as optimised

Observation:

Olanzapine and Samidorphan were eluted at 2.209min and 3.196 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability:

All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Discussion:

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation:

Discussion:

Retention times of Olanzapine and Samidorphan were 2.214min and 3.207 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Discussion:

Six linear concentrations of Olanzapine (7.5-45µg/ml) and Samidorphan (5-30µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Olanzapine was y = 40559x + 8327.and of Samidorphan was y = 39599x + 4901 Correlation coefficient obtained was 0.999 for the two drugs.

System Precision:

Discussion:

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.6% and 0.8% respectively for Olanzapine and Samidorphan .As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability:

Discussion:

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.2% and 0.4% respectively for Olanzapine and Samidorphan. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day_ Day Precision):

Discussion:

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.8% and 0.4% respectively for Olanzapine and Samidorphan. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy: Table 6.6 Accuracy table of Olanzapine
Discussion:

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.79% and 99.60% for Olanzapine and Samidorphan respectively.

Sensitivity:
Discussion:

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (65B:35A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay:

label claim containing Olanzapine 15mg + Samidorphan 10mg.Assay was performed with the above formulation. Average % Assay for Olanzapine and Samidorphan obtained was 99.27 and 99.62% respectively

CONCLUSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Olanzapine and Samidorphan in injection dosage form. Retention time of Olanzapine and Samidorphan were found to be 2.209 min and 3.196 min. %RSD of the Olanzapine and Samidorphan were and found to be 0.6 and 0.2 respectively. %Recovery was obtained as 99.79% and 99.60% for Olanzapine and Samidorphan respectively. LOD, LOQ values obtained from regression equations of Olanzapine and Samidorphan were 0.09, 0.28 µg/ml and 0.04, 0.13µg/ml respectively. Regression equation of Olanzapine is y =40559x + 8327, and y = 39599x + 4901 of Samidorphan. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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