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Development and Validation of New Analytical Method for the Determination of Olanzapine and Samidorphan in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

For the simultaneous estimate of olanzapine and samidorphan in tablet dose form, a straightforward, accurate, and exact approach has been established. Standard Agilent C18 (150 x 4.6 mm, 5 m) Mobile phase containing Buffer KH2PO4 was used to conduct the chromatogram. At a flow rate of 1 ml/min, acetonitrile in the proportion 60:40 was injected across the column. This approach made use of 0.1N KH2PO4 buffer. 30°C was kept as the temperature. The chosen optimized wavelength was 226 nm. Olanzapine and Samidorphan had retention times of 2.214 and 3.207 minutes, respectively. Olanzapine and Samidorphan were both found to have %RSD values of 0.8 and 0.4, respectively. % Olanzapine and Samidorphan both had recovery rates of 100.30% and 100.64%, respectively. Olanzapine and LOQ regression models' LOD and LOQ values were 0.05, 0.14, and 0.02, 0.07 g/ml of samidorphan, respectively. Olanzapine's regression equation is y = 81119x + 8327, whereas Samidorphan's is y = 79198x + 4901. As a result of shorter retention durations and shorter run times, the method was created to be straightforward and cost-effective, and it may be used for routine Quality Control Tests in Industries.

INTRODUCTION:

Drug combinations are often simultaneously estimated using chromatographic techniques including HPLC, GC, and HPTLC, among others. These techniques have good repeatability and accuracy, but the cost of analysis is extremely high because of the pricey equipment, reagents, and expertise required. Therefore, it is important to create a more straightforward and economical approach for a simultaneous estimate of medicines for regular formulation analysis. When a simultaneous estimate of the medication combination is necessary, spectrophotometric analysis satisfies this need with efficacy comparable to that of chromatographic approaches..[1,2]. The majority of medications in multicomponent dosage forms may be tested using the HPLC technique because of its many benefits, including speed, specificity, accuracy, precision, and simplicity of automation. The tedious extraction and isolation processes are eliminated by the HPLC approach. In RP-HPLC, the polarity of the mobile and stationary phases are reversed, resulting in a stationary phase with a hydrophobic surface and a polar mobile phase, which is used mostly with water-based solutions. The method of chromatography that is most loved is unquestionably reversed-phase HPLC. RP HPLC is used to analyze low-molecular-weight materials in about 90% of cases.[3,4].

Olanzapine is a benzodiazepine with the chemical name 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b][1,5]. It is an atypical antipsychotic that the U.S. Food and Drug Administration (FDA) has licensed for the treatment of bipolar disorder and schizophrenia. It falls under the thienobenzodiazepine subclass. Olanzapine is a type 1, type 2, and type 4 dopamine receptor antagonist. The antipsychotic action of olanzapine is caused by antagonistic activity at dopamine and serotonin type 2 receptors, with serotonin 5-HT 2 receptors showing more activity than dopamine type 2 receptors. Opposition at the muscarinic receptors Olanzapine also affects H1 receptors and alpha 1 receptors.[5,6,7].

Samidorphan (INN, USAN) (developmental code names ALKS-33, RDC-0313), also known as 3-carboxamido-4-hydroxynaltrexone is an opioid antagonist that preferentially acts as an antagonist of the μ-opioid receptor (MOR). It is under development by Alkermes for the treatment of major depressive disorder and possibly other psychiatric conditions. Samidorphan has been investigated for the treatment of alcoholism and cocaine addiction by its developer, Alkermes, showing similar efficacy to naltrexone but possibly with reduced side effects. However, it has attracted much more attention as part of the combination product ALKS-5461 (buprenorphine/samidorphan), where samidorphan is combined with the

mixed MOR weak partial agonist and κ-opioid receptor (KOR) antagonist buprenorphine, as an antidepressant. Buprenorphine has shown antidepressant effects in some human studies, thought to be because of its antagonist effects at the KOR, but has not been further developed for this application because of its MOR agonist effects and consequent abuse potential Samidorphan (INN, USAN) (developmental code names ALKS-33, RDC-0313), also known as 3-carboxamido-4-hydroxynaltrexone is an opioid antagonist that preferentially acts as an antagonist of the μ-opioid receptor (MOR). It is under development by Alkermes for the treatment of major depressive disorder and possibly other psychiatric conditions. Samidorphan has been investigated for the treatment of alcoholism and cocaine addiction by its developer, Alkermes, showing similar efficacy to naltrexone but possibly with reduced side effects. However, it has attracted much more attention as part of the combination product ALKS-5461 (buprenorphine/samidorphan), where samidorphan is combined with the mixed MOR weak partial agonist and κ-opioid receptor (KOR) antagonist buprenorphine, as an antidepressant. Buprenorphine has shown antidepressant effects in some human studies, thought to be because of its antagonist effects at the KOR, but has not been further developed for this application because of its MOR agonist effects and consequent abuse potential.

Samidorphan3-Carboxamido4-hydroxynaltrexone, commonly known as (INN, USAN) (developmental code names ALKS-33, RDC-0313), is an opioid antagonist that predominantly works as an antagonist of the -opioid receptor (MOR). Alkermes is developing it to treat the major depressive disorder and perhaps other mental diseases. Alkermes, the company that developed samidorphan, has studied it for the treatment of cocaine and alcohol addiction, finding that it had similar efficacy as naltrexone but perhaps fewer adverse effects. However, it has garnered much greater interest as a component of the combination drug ALKS-5461 (buprenorphine/samidorphan), which combines samidorphan with the opioid receptor (KOR) antagonist mixed MOR weak partial agonist buprenorphine as an antidepressant. In certain human investigations, buprenorphine has demonstrated antidepressant effects, which are assumed to be caused by its antagonist actions at the KOR. although due to its MOR agonist properties and potential for misuse, it has not been further researched for this application.[8]

There are some other RP-HPLC methods published[9,10,11,12,13].

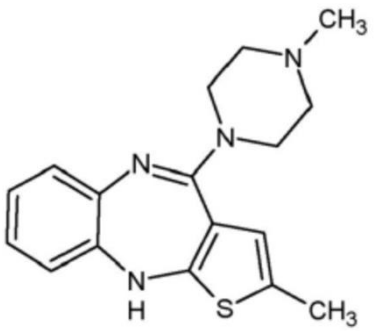

Figure No 1: Chemical structure of Olanzapine

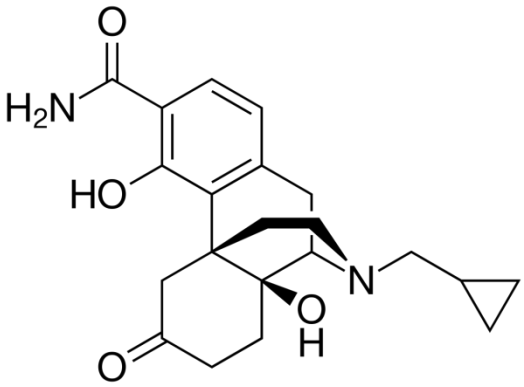

Figure no 2: Chemical Structure of Samidorphan

MATERIALS AND METHODS

Chemicals and reagents

• Olanzapine and Samidorphan Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: The instrument used in the study was HPLC (Waters 2695 with PDA detector 2996) was monitored and integrated using Empower 2 software. electronic balance, sonicator, hot air oven, digital pH meter, and UV-Visible chamber.

Preparation of Standard stock solution: Accurately weighed 7.5mg of Olanzapine and 5mg of Samidorphan and transferred them to a 50ml volumetric flask. And 3/4 th of the diluents was added to this flask and sonicated for 10 minutes. Flasks were made up of diluents and labeled as Standard stock solutions. (150µg/ml of Olanzapine and 100µg/ml of Samidorphan)

Preparation of Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (15µg/ml Olanzapine of and 10µg/ml of Samidorphan).

Preparation of Sample stock solution: 5 tablets were weighed and the equivalent of 1 tablet is weighed and transferred to a 50 ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made up to 50ml with diluents and filtered through 1 µm or finer porosity membrane filter (150µg/ml of Olanzapine and 100µg/ml of Samidorphan).

Preparation of Sample working solution:1ml of filtered sample stock solution was transferred to a 10ml volumetric flask and made up with diluent. (15µg/ml of Olanzapine and 10µg/ml of Samidorphan).

Chromatographic conditions:

Flow rate	**:** 1 ml/min
Column	**:** Azilent 150 (4.6 x 150mm, 5µm)
Wavelength	**:** 226 nm
Column temperature: 30°C	
Injection volume	**:**10.0µL
Run time	**:** 6 minutes
Diluent	**:** Water and Acetonitrile in a ratio of 50:50

Observation: Olanzapine and Samidorphan were eluted at 2.214min and 3.207 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Degradation: According to ICH recommendations and standard industry practice, forced deterioration is typically carried out in conjunction with a control sample under various stress conditions, including acid, alkali, peroxide, heat, and UV. Although there are no established standards for industrial degradation, it is recommended that 5 to 30 percent of degradation be reached under any of the applied stress conditions. The goal of the degradation to be accomplished by stress testing is to replicate the stability circumstances of the control room temperature[15]. To conduct the forced degradation experiment, standard stock solutions of Olanzapine and Samidorphan was exposed to various stress conditions, including 1 mL of 20% H_2O_2 (for oxidative degradation), 1 mL of 2N HCL (for acidic degradation), and 1 mL

of 2N NaOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30 minutes at 60ºC. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield 50µg/ml of Olanzapine and Samidorphan for degradation studies. To examine sample stability, 10µl samples were fed into the system, and chromatograms were obtained.

Method Validation: The method was validated following ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Olanzapine (15ppm) and Samidorphan (10ppm) 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 injection 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.

Linearity: Six linear concentrations of Olanzapine (3.75-22.5µg/ml) and Samidorphan (2.5-15µg/ml) were injected in a duplicate manner. Average areas were mentioned above and the linearity equations obtained for Olanzapine was y = 81119x + 8327.7.and of Samidorphan was y = 79198x + 4901.9 Correlation coefficient obtained was 0.999 for the two drugs.

Precision:

Repeatability: Multiple sampling from a sample stock solution was done and six working sample solutions of the 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 1.0% and 0.5% respectively for Olanzapine and Samidorphan. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate Precision: Multiple sampling from a sample stock solution was done and six working sample solutions of the 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: Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.30% and 100.64% for Olanzapine and Samidorphan respectively.

Robustness: 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) were maintained and samples were injected in a duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay: Azstarys, bearing the label claim containing Olanzapine 15mg + Samidorphan 10mg. Assay was performed with the above formulation. The average % Assay for Olanzapine and Samidorphan obtained was 100.01 and 99.6% respectively.

Degradation Studies: Degradation studies were performed with the stock standard solution and the degraded samples were analyzed using the proposed method. Assay % of Olanzapine and Samidorphan in the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in table 7.

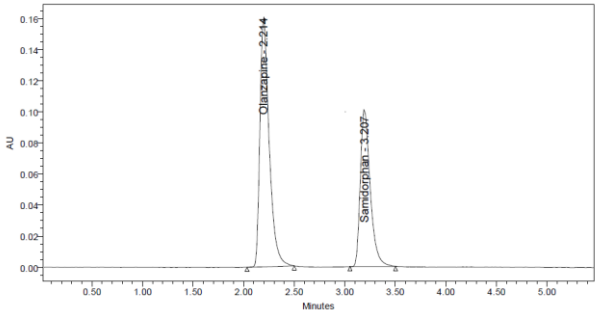

Figure No.3: Optimised Chromatogram

Table No.1: System suitability parameters

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

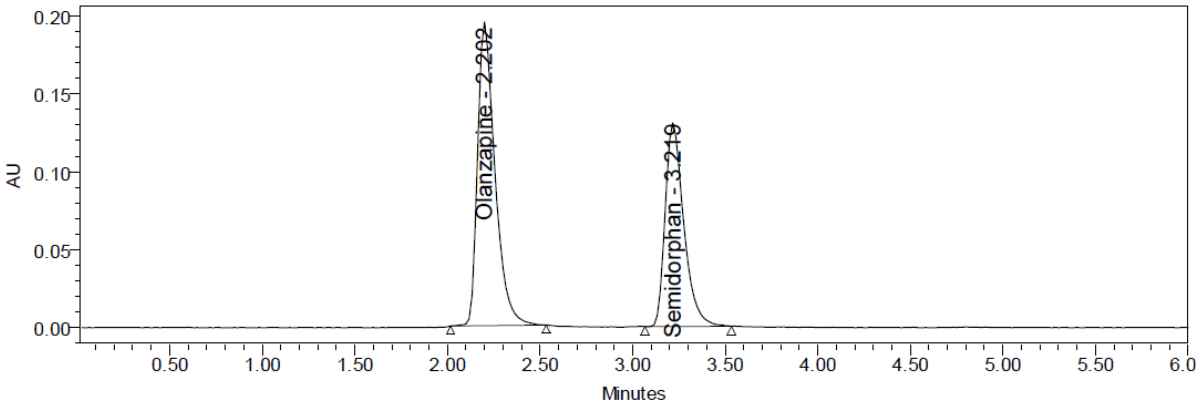

Figure No.4: Standard solution chromatogram

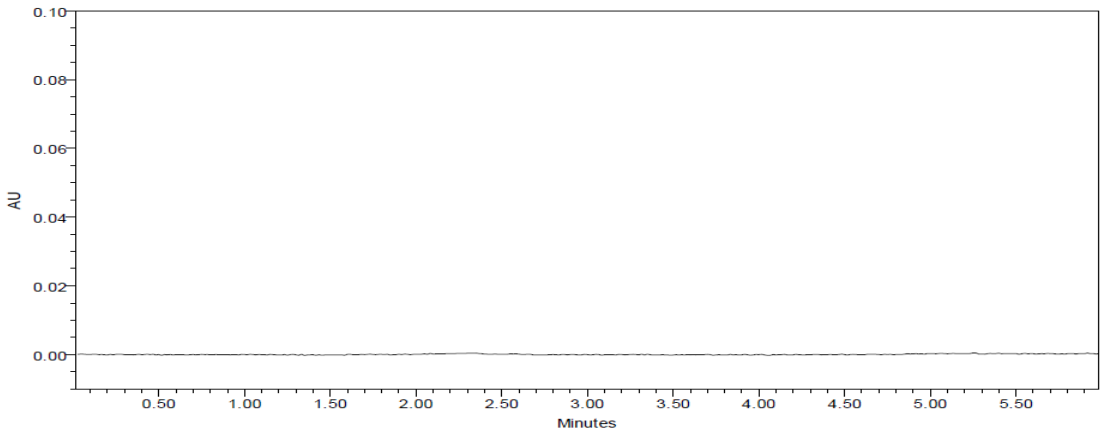

Figure No.5: Blank chromatogram

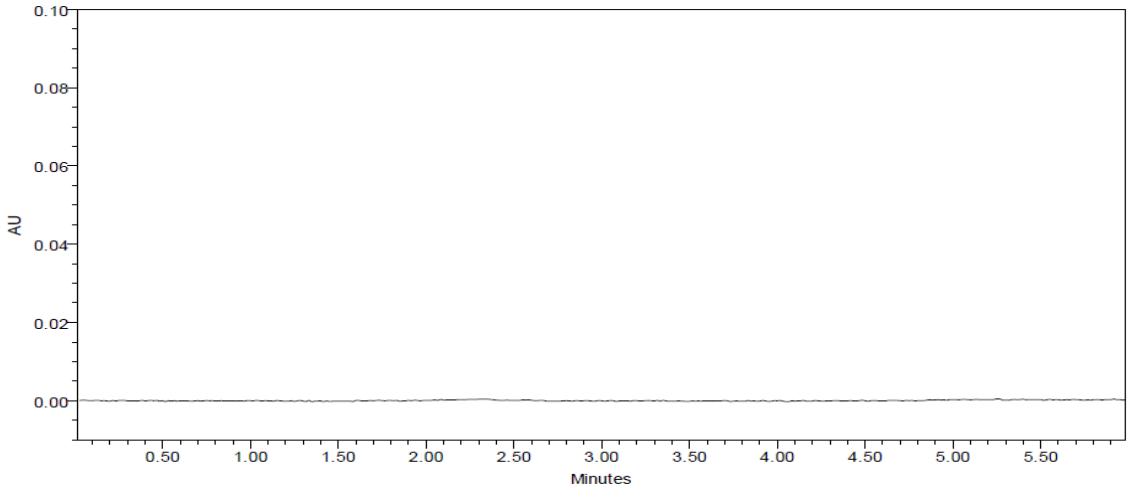

Fig No.6: Placebo chromatogram

Table No.2: Linearity table for Olanzapine and Samidorphan,

Olanzapine		Samidorphan	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
3.75	319448	2.5	208469
7.5	614834	5	412360
11.25	925182	7.5	593738
15	1227382	10	789227
18.75	1534804	12.5	981352
22.5	1824728	15	1207086

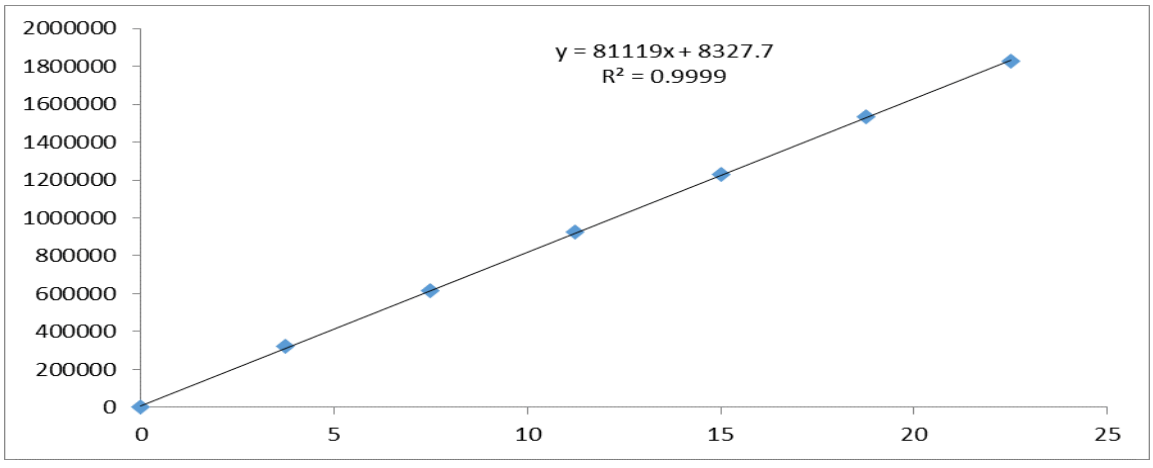

Figure No 7: Calibration curve of Olanzapine

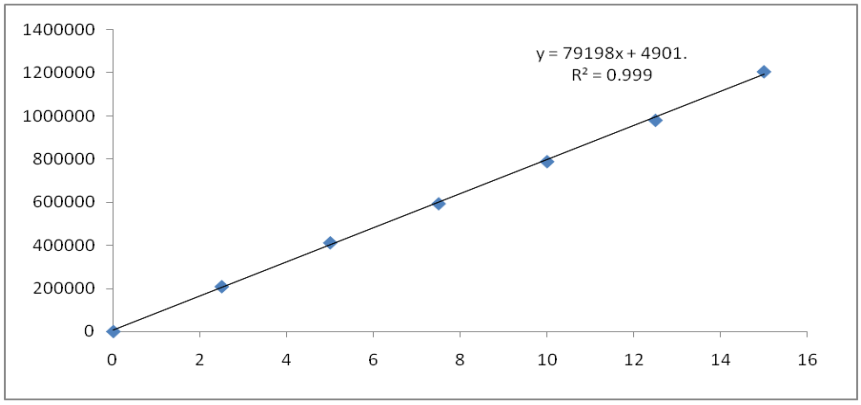

Figure no.8 Calibration curve of Samidorphan

Table No.3: Repeatability table of Olanzapine and Samidorphan

S. No	Area of Olanzapine	Area of Samidorphan
1.	1243895	835558
2.	1222724	841993
3.	1213237	830463
4.	1227788	834109
5.	1244103	832093
6.	1229397	836704
Mean	1230191	835153
S.D	12090.8	4044.9
%RSD	1.0	0.5

Table No.4: 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 No.5 Accuracy table of Olanzapine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	7.5	7.3964916	98.61989	100.30%
	7.5	7.4492289	99.32305	
	7.5	7.4388738	99.18498	
100%	15	15.28	101.8518	
	15	15.02	100.1129	
	15	15.16	101.0856	
150%	22.5	22.089769	98.17675	
	22.5	22.939509	101.9534	
	22.5	23.042949	102.4131	

Table no 5.1 Accuracy table of Samidorphan

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	5.09	101.86	100.64%
	5	4.98	99.66	
	5	5.01	100.22	
100%	10	10.10	101.00	
	10	10.04	100.39	
	10	10.09	100.93	
150%	15	15.08	100.53	
	15	15.07	100.44	
	15	15.10	100.64	

Table No.6: 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 No.7: Degradation Data

Type of degradation	Olanzapine		Samidorphan	
	%RECOVERED	% DEGRADED	%RECOVERED	% DEGRADED
Acid	93.91	6.09	94.01	5.99
Base	95.80	4.20	95.58	4.42
Peroxide	95.65	4.35	95.47	4.53
Thermal	97.01	2.99	97.23	2.77
Uv	98.69	1.31	98.85	1.15
Water	99.62	0.38	99.49	0.51

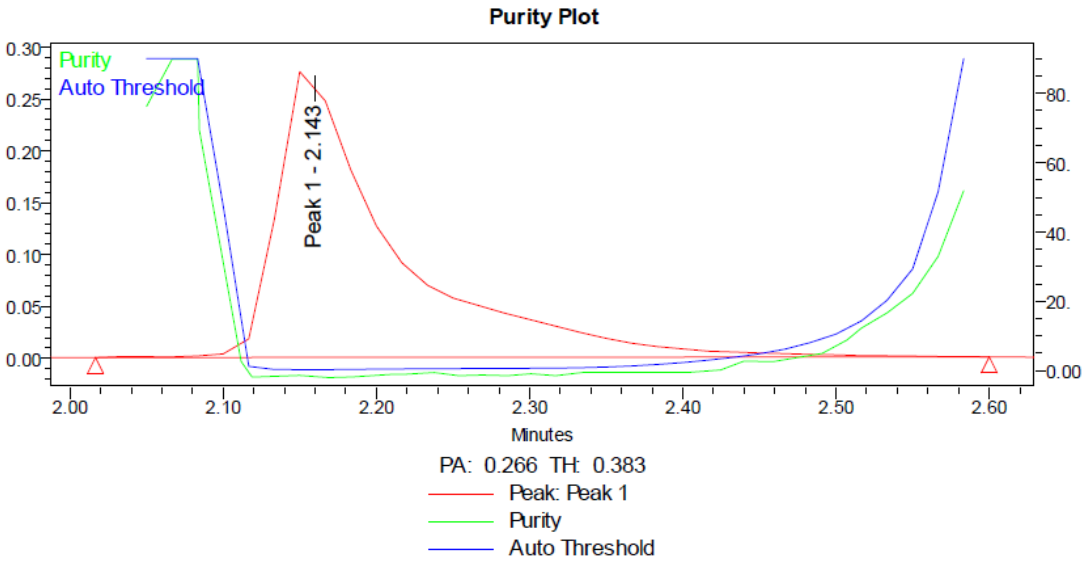

Figure No 9- purity plots

CONCLUSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Olanzapine and Samidorphan in the injection dosage form. The retention time of Olanzapine and Samidorphan was found to be 2.214 min and 3.207 min. %RSD of the Olanzapine and Samidorphan was found to be 0.8 and 0.4 respectively. %Recovery was obtained as 100.30% and 100.64% for Olanzapine and Samidorphan respectively. LOD and LOQ values obtained from regression equations of Olanzapine and Samidorphan were 0.05, 0.14 µg/ml and 0.02, 0.07µg/ml respectively. Regression equation of Olanzapine is y =81119x + 8327, and y = 79198x + 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 tests in Industries.

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