e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OLANZAPINE AND SAMIDORPHAN

Ganji Ravali*1, Dr. M. Ajitha*2

*1,2Department Of Pharmaceutical Analysis, Centre For Pharmaceutical Sciences, UCEST, JNTUH, Hyderabad, India.

ABSTRACT

An HPLC approach was devised to simultaneously determine the dose of olanzapine and samidorphan in tablet form. Chromatography made use of a Kromasil column with dimensions 150 mm x 4.6 mm and a particle size of 5 µm. The mobile phase was a 60:40 combination of acetonitrile and 0.01N potassium hydrogen phosphate. The flow rate was kept at one milli litre per minute while the temperature was kept constant at thirty degrees Celsius. A wavelength of 268.0 nm was determined to be the most effective for detecting purposes. The approach showed a high degree of linearity in the regression equations for Olanzapine (y = 52713x + 1796.2) and Samidorphan (y = 42791x + 1106.6), respectively, with retention times of 2.215 and 2.778 minutes at the time of administration. We can see that we were rather precise because the percentage RSD values for olanzapine and samidorphan were just 0.5% and 0.3%, respectively. Evidence that the approach might provide reliable findings was provided by the recoveries of 99.25% for olanzapine and 100.31% for samidorphan. The limits of detection (LOD) for Samidorphan were determined to be 0.03 µg/mL, while the limits of quantification (LOQ) were 0.08 µg/mL. However, Olanzapine had comparable values of 0.06 µg/mL and 0.19 µg/mL.

Keywords: Olanzapine And Samidorphan, RP- HPLC And Method Validation.

I. INTRODUCTION

Olanzapine and samidorphan (OLZ/SAM) is a combination of an atypical antipsychotic and an opioid receptor antagonist that is used to treat schizophrenia and bipolar disorder. 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 receptor. Samidorphan acts as an antagonist at the μ-opioid receptor when it signals through Gαi proteins, a partial agonist when the receptor signals through GαoA, GαoB, and Gαz proteins, and essentially lacks β-arrestin-mediated signalling; samidorphan also acts as a partial agonist at both the κ- and δ-opioid receptors. In this investigation a stability indicating RP-HPLC method for simultaneous determination of Olanzapine and Samidorphan.

II. MATERIALS AND METHODS

Materials:

Olanzapine and Samidorphan pure drugs (API), Combination Olanzapine and Samidorphan (**Lybalvi**), 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, Ultrasonicator-BVK enterprises.

WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.

UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Olanzapine and Samidorphan solutions.

Methodology:

Chromatographic conditions:

Mobile phase: Acetonitrile: 0.01N Kh2po4 (60:40)

Flow rate: 1 ml/min

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Column: Kromasil c18 (4.6 x 150mm, 5µm)

Detector wave length: 268nm

Column temperature: 30°C

Injection volume: 10mL

Run time: 10 min

Diluent: Water and Acetonitrile in the ratio 50:50

Results: Both peaks have good resolution, tailing factor, theoretical plate count and resolution.

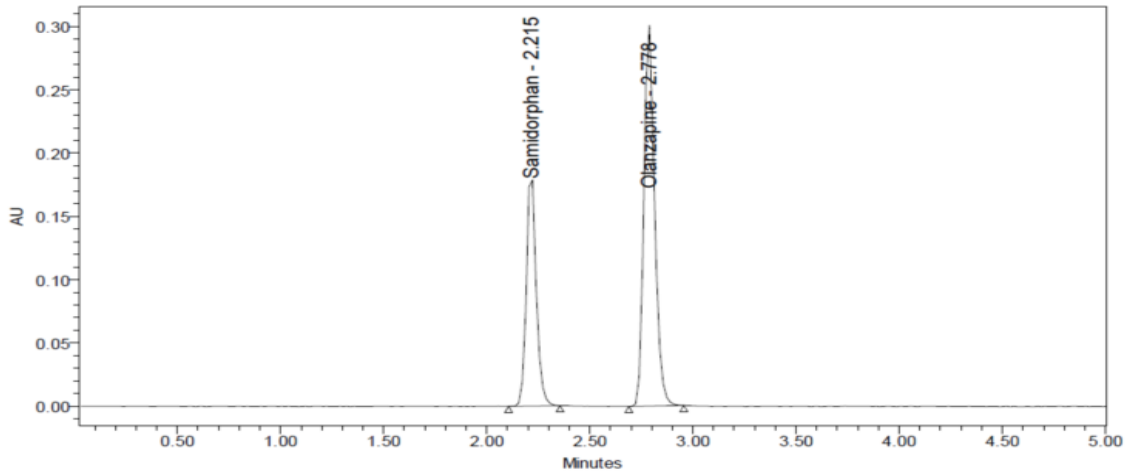

Fig 1: Optimized Chromatogram

Methods:

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

Preparation of Standard stock solutions: Accurately weighed 2.5 mg of Samidorphan, 5mg of Olanzapine and transferred to 25ml volumetric flasks and 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution. (100µg/ml of Samidorphan and 200µg/ml Olanzapine)

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. (10µg/ml of Samidorphan and 20µg/ml of Olanzapine)

Preparation of Sample stock solutions: 10 tablets were weighed and equivalent to 1 tablet is weighed and transferred to 50ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made upto 20ml with diluents and filtered through 0.45 µm or finer porosity membrane filter (200µg/ml of Samidorphan and 400µg/ml of Olanzapine).

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (10µg/ml of Samidorphan and 20µg/ml of Olanzapine)

Preparation of buffer:

0.1% OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

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 4.0 with dil. Orthophosphoric acid solution

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

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III. METHOD VALIDATION

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

Table 1: System suitability parameters for Olanzapine and Samidorphan

Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	RS
1	2.220	6650	1.08	2.784	13479	1.2	5.4
2	2.220	6939	1.11	2.785	13522	1.19	5.5
3	2.221	6644	1.07	2.787	13391	1.19	5.4
4	2.223	6465	0.99	2.793	13528	1.11	5.5
5	2.224	6551	1.04	2.793	13418	1.11	5.5
6	2.224	6784	1.07	2.794	13699	1.11	5.6

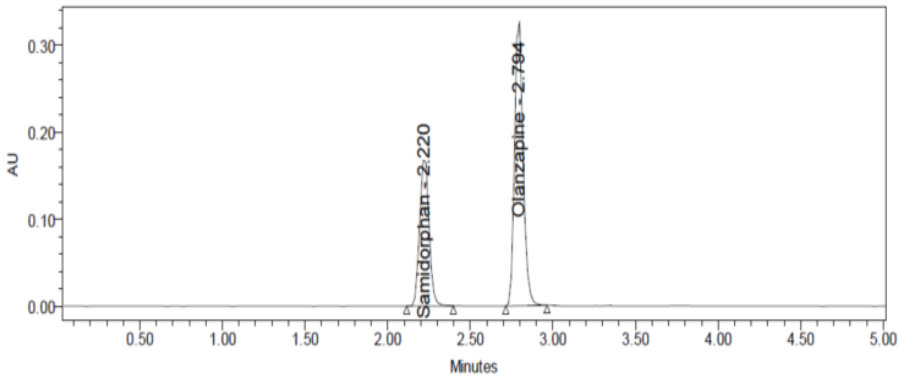

Fig 2: System Suitability Chromatogram of Olanzapine and Samidorphan

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.

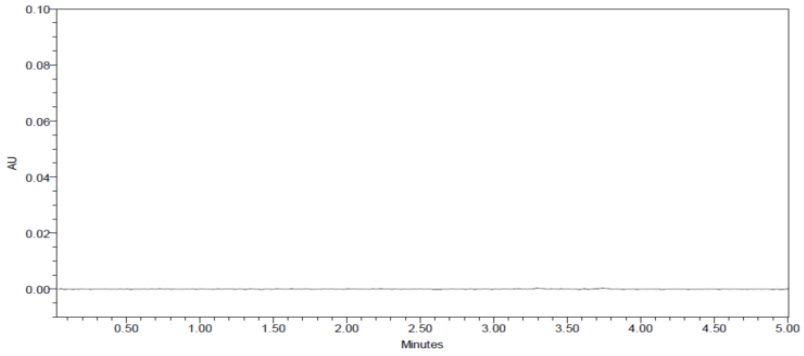

Fig 3: Specificity chromatogram

Table 2: Linearity table for Olanzapine and Samidorphan

Olanzapine		Samidorphan	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
5	262725	2.5	108571
10	534801	5	216501

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15	794931	7.5	324889
20	1047648	10	424072
25	1329500	12.5	535813
30	1577787	15	645447

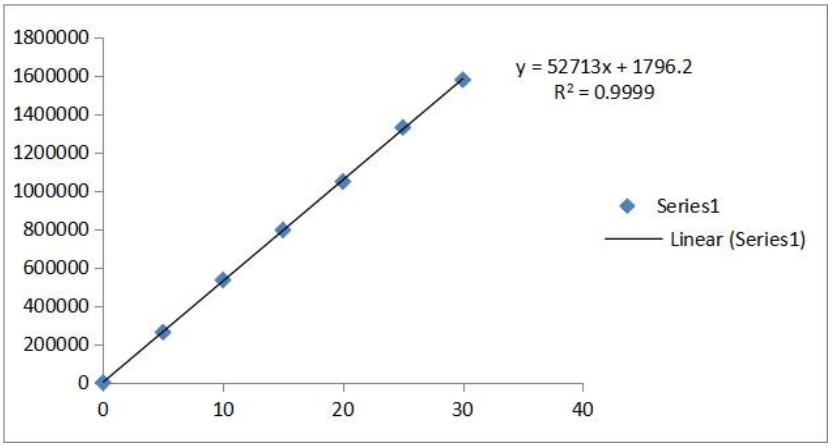

Fig 4: Calibration curve of Olanzapine

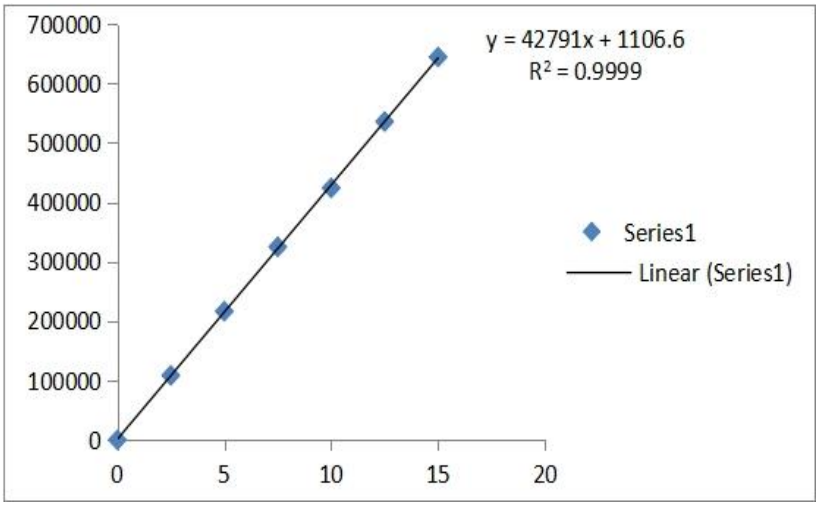

Fig 5: Calibration curve of Samidorphan

Accuracy: Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 3.

Table 3: Recovery data of Olanzapine and Samidorphan

	Olanzapine			Samidorphan		
% stage	Amount taken	recovered	% of recovery	Amount taken	recovered	% of recovery
50%	10	9.83	98.34	5	5.03	100.69
	10	9.88	98.79	5	5.09	101.73
	10	10.01	100.13	5	5.04	100.76
100%	20	19.62	98.12	10	9.98	99.82
	20	19.88	99.39	10	9.99	99.92
	20	19.82	99.11	10	10.05	100.51
150%	30	29.81	99.38	15	14.97	99.81
	30	29.73	99.10	15	14.98	99.88

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	30	30.27	100.90	15	14.95	99.67
Mean % recovery	99.25			100.31		

Precision:

System Precision: The% RSD was found to be 0.5 and 0.3%

Table 4: Olanzapine and Samidorphan System Precision data

S. No	Area Of Olanzapine	Area Of Samidorphan
1.	1069769	429897
2.	1056669	430788
3.	1069600	429977
4.	1069588	429590
5.	1068783	431546
6.	1061087	427628
Mean	1065916	429904
S.D	5638.2	1323.8
%RSD	0.5	0.3

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Olanzapine and Samidorphan were 0.5% and 0.4%.

Table 5: Inter day precision results for Olanzapine and Samidorphan

S. No	Area Of Olanzapine	Area Of Samidorphan
1.	1058799	428371
2.	1061483	431009
3.	1055214	432592
4.	1068369	432529
5.	1059715	430985
6.	1066758	432335
Mean	1061723	431304
S.D	4990.5	1613.3
%RSD	0.5	0.4

Intraday Precision: Intraday Precision was performed and % RSD for Nivolumab and Ipilimumab were found to be 0.5% and 0.5% respectively.

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Table 6: Repeatability results for Olanzapine and Samidorphan

S. No	Area Of Olanzapine	Area Of Samidorphan
1.	1033757	420452
2.	1046575	423934
3.	1034221	423899
4.	1043409	420099
5.	1038430	423149
6.	1042710	425307
Mean	1039850	422807
S.D	5232.6	2083.7
%RSD	0.5	0.5

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

Table 7: Robustness data of Olanzapine and Samidorphan method.

S.no	Condition	%RSD of Samidorphan	%RSD of Olanzapine
1	F (-) 0.9ml/min	0.3	0.2
2	F (+) 1.1ml/min	0.9	0.2
3	M (-)55B:45A	0.5	1.0
4	M (+)65B:35A	0.4	0.6
5	T (-) 25°C	0.5	0.8
6	T (+) 35°C	0.9	0.9

IV. DEGRADATION STUDIES

Table 8: Forced degradation conditions for Olanzapine and Samidorphan

Stress conditions	Solvent	Temperature(C°)	Exposed time
Acid	2N HCl	60°C	30min
Base	2N NaOH	60°C	30min
Oxidation	20% H_2O_2	60°C	30min
Thermal	Diluent	105°C	30min
Photolytic	Diluent	-	1 Day
Hydrolytic	Diluent	60°C	

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Table 9: Degradation Data of Olanzapine and Samidorphan

Type of degradation	Samidorphan			Olanzapine		
	Area	%Undegraded	% Degrade	Area	% Undegraded	% Degrade
Acidic	409001	94.76	5.24	997037	93.16	6.84
Alkali	400589	92.81	7.19	1016956	95.03	4.97
Oxidative	412903	95.66	4.34	1016013	94.94	5.06
Thermal	420903	97.51	2.49	1026013	95.87	4.13
Photolytic	425717	98.63	1.37	1046756	97.81	2.19
Neutral	427836	99.12	0.88	1066110	99.62	0.38

Assay: **(Lybalvi)** Bearing the label claims Samidorphan 10mg, Olanzapine 20mg. Assay was performed with the above formulation. Average % Assay for Olanzapine and Samidorphan obtained was 99.95%and 100.75% respectively.

Table 10: Assay of Samidorphan and Olanzapine

	Samidorphan			Olanzapine		
S.no	Std	Sample	% of Assay	Std	Sample	% of Assay
1	429897	428371	**99.24**	1069769	1058799	**98.93**
2	430788	431009	**99.86**	1056669	1061483	**99.19**
3	429977	432592	**100.22**	1069600	1055214	**98.60**
4	429590	432529	**100.21**	1069588	1068369	**99.83**
5	431546	430985	**99.85**	1068783	1059715	**99.02**
6	427628	432335	**100.16**	1061087	1066758	**99.68**
Avg	429904	431304	**99.92**	1065916	1061723	**99.21**
Stdev	1323.8	1613.3	**0.37**	5638.2	4990.5	**0.5**
%RSD	0.3	0.4	**0.4**	0.5	0.5	**0.5**

V. CONCLUSION

An accurate, reliable, and efficient technique for quantifying Olanzapine and Samidorphan in pharmaceutical formulations is the concurrent analysis of both components via HPLC. The validated method precisely measures the two substances without influence from degradation products or contaminants, as it effectively separates them with exceptional clarity. The mobile phase composition, flow rate, and detection wavelength have been optimized in the chromatographic parameters to provide enhanced sensitivity, accuracy, and repeatability. According to ICH standards, processes evaluated for linearity, precision, accuracy, and stability are relevant in both commercial and clinical settings.

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