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Research

Method Development and Validation for Simultaneous Estimation of Samidorphan and Olanzapine in pharmaceutical Dosage Form by RP- HPLC.

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Abstract

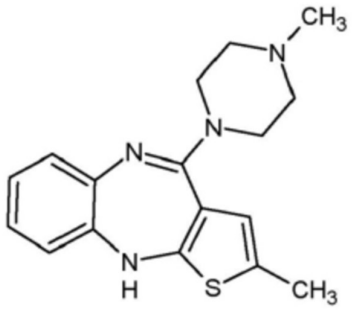
A simple, Accurate, precise method was developed for the simultaneous estimation of the Samidorphan and Olanzapine in bulk and pharmaceutical dosage form. Chromatogram was run through Std BDS 150 x 4.6 mm, 5µm. Mobile phase containing Buffer 0.01N Potassium dihydrogen phosphate: Methanol taken in the ratio 80:20 %v/v was pumped through column at a flow rate of 0.9 ml/min. Buffer used in this method was 0.01N Kh2po4 buffer. Temperature was maintained at 29°C. Optimized wavelength selected was 278.0 nm. Retention time of Samidorphan and Olanzapine were found to be 2.365 min and 3.108 min. %RSD of the Samidorphan and Olanzapine were and found to be 0.6% and 0.4% respectively. %Recovery was obtained as 100.29% and 100.46% for Samidorphan and Olanzapine respectively. LOD, LOQ values obtained from regression equations of Samidorphan and Olanzapine were 0.29, 0.89 and 0.04, 0.12 respectively. %Assay was obtained as 99.71% and 99.58% for Samidorphan and Olanzapine respectively. Regression equation of Samidorphan is y = 21331x + 3547.4, y = 29592x + 9783.5 of Olanzapine. 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: Samidorphan, Olanzapine, RP-HPLC

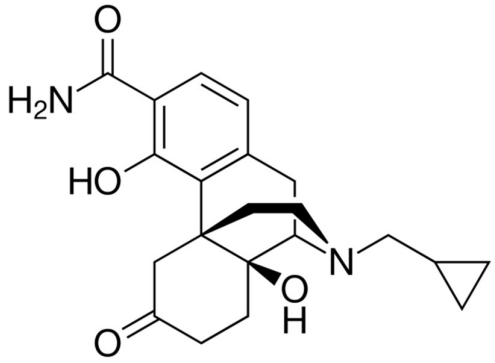
INTRODUCTION

Olanzapine/samidorphan, soldunder the brand nameLybalvi, is a fixed-dose combination medication for the treatment of schizophrenia and bipolar I disorder. It contains olanzapine, an atypicalantipsychotic, and samidorphan, an opioidantagonist. Samidorphanreduces the weight gain associatedwith olanzapine whilestillallowing olanzapine to exertitstherapeuticeffect. The formulation wasapproved for medical use in the United States in May 2021.Olanzapine and Samidorphan are combined to treat the signs and symptoms of schizophrenia in adults. It is also used to treat manic episodes in individuals with bipolar illness, either on its alone or in combination with other drugs. Olanzapine belongs to the group of drugs known as atypical antipsychotics. It functions by altering the activity of certain natural substances in the brain. An antagonist of opioids is samidorphan. It serves to lessen potential Olanzapine side effects, such as weight gain. (1) Olanzapine and

Samidorphan are chemically described as 17-(Cyclopropylmethyl)-4, 14-dihydroxy-6-oxomorphinan-3-carboxamide and 2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [benzodiazepine. The current study seeks to develop a rapid, stability-indicating RP-HPLC method for the simultaneous quantification of Olanzapine and Samidorphan in bulk pharmaceuticals and commercial dosage forms. This is accomplished by improving sensitivity and reducing elution times. The validation study was also finished in compliance with ICH Guidelines Q2 (International Conference on Harmonization) (R1).



Structure of Olanzapine



Structure of Samidorphan

MATERIALS AND METHODS:

Instrument used:

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- 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 Samidorphan and Olanzapine solutions.

Materials Used

Olanzapine and Samidorphanpure drugs (API), Combination Olanzapine and Samidorphan (Lybalvi), Distilledwater, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Methods

Preparation of buffer
Buffer:0.1N Potassium dihyrogen Ortho phosphate
Accurately weighed 1.36gm of Potassium dihyrogen Ortho 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 3.8 with dil. Orthophosphoric acid solution
0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Preparation of Standard stock solutions: Accurately weighed 10 mg of Samidorphan, 5mg of Olanzapine and transferred to 25ml 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. 400µg/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. (40µg/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 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 (200µg/ml of Samidorphan and 100µg/ml of Olanzapine).

Preparation of Sample working solutions (100% solution): 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (40µg/ml of Samidorphan and 20µg/ml of Olanzapine).

RESULTS ANDDISCUSSION

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

Table:6.1 Systemsuitability parameters forSamidorphan and Olanzapine

S no	Samidorphan			Olanzapine			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.365	6648	1.2	3.108	10574	1.3	6.1
2	2.365	6719	1.2	3.108	10582	1.3	6.1
3	2.366	6698	1.2	3.109	10550	1.3	6.1
4	2.368	6898	1.2	3.112	10442	1.3	6.1
5	2.370	6746	1.2	3.117	11192	1.3	6.1
6	2.379	6296	1.2	3.124	10672	1.3	6.2

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.

Linearity

Table 6.2 Linearity table for Samidorphan and Olanzapine

Samidorphan		Olanzapine	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
10	217770	5	156137
20	436203	10	307057
30	640186	15	457921
40	857192	20	602522
50	1071825	25	737663
60	1281215	30	904578

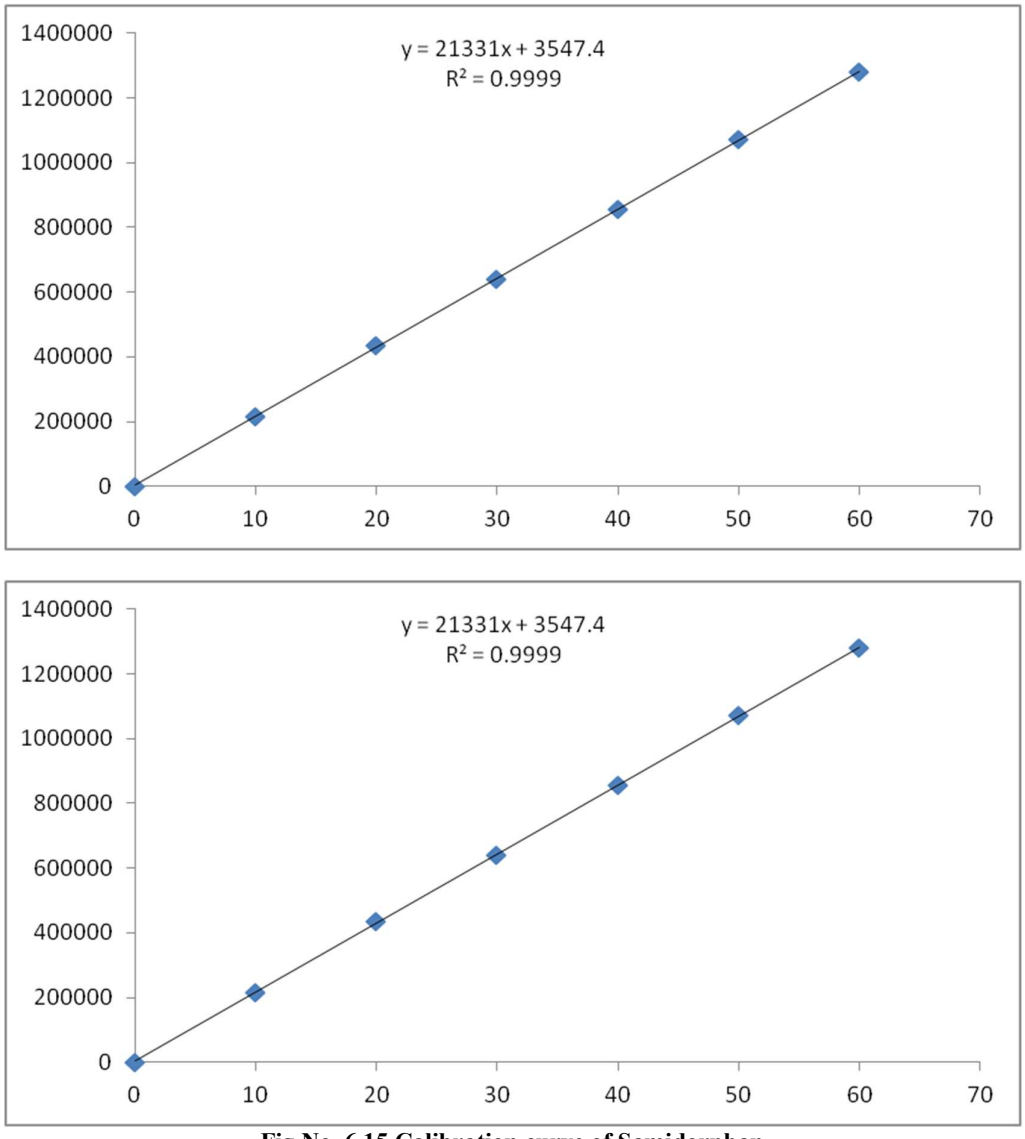


Fig No. 6.15 Calibration curve of Samidorphan

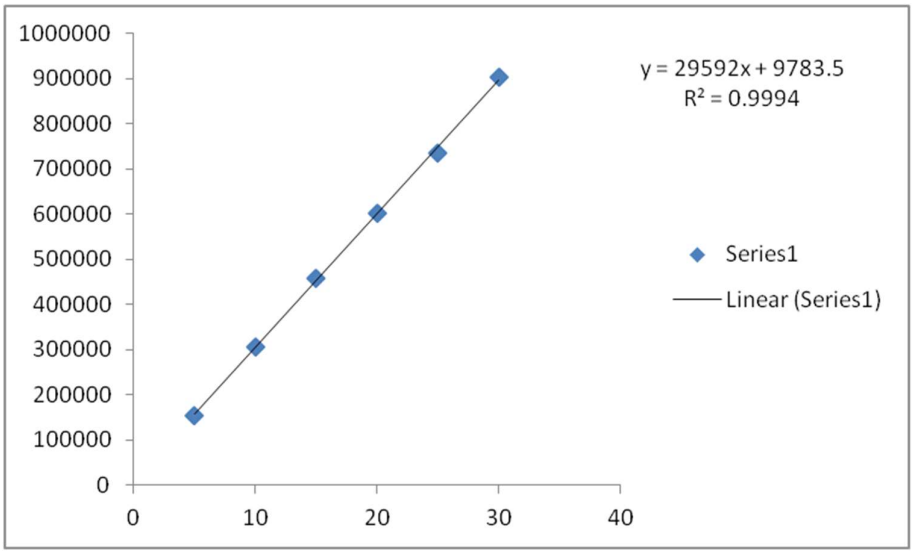


Fig No. 6.16 Calibration curve of Olanzapine

Discussion: Six linear concentrations of Samidorphan (10-60µg/ml) and Olanzapine (5-30µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Samidorphan was y = 21331x + 3547.4. And of Olanzapine was y = 29592x + 9783.5. Correlation coefficient obtained was 0.999 for the two drugs.

Precision

System Precision

Table 6.3 System precision table of Samidorphan and Olanzapine

S. No	Area of Olanzapine	Area of samidorphan
1.	604917	858528
2.	604839	857329
3.	604061	859978
4.	607995	865188
5.	601490	867777
6.	606964	868042
Mean	605044	862807
S.D	2282.5	4777.0
%RSD	0.4	0.6

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 0.4% respectively for Samidorphan and Olanzapine .As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability

Table 6.4 Repeatability table of Samidorphan and Olanzapine

S. No	Area of Olanzapine	Area of Samidorphan
1.	6759192	856383
2.	6701156	869602
3.	6758058	859348
4.	6717457	857318
5.	6690601	872024
6.	6694369	859983
Mean	6720139	862443
S.D	31199.7	6658.3
%RSD	0.5	0.8

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 0.8% and 0.5% respectively for Samidorphan and Olanzapine. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day_ Day Precision):

Table 6.5 Intermediate precision table of Samidorphan and Olanzapine

S. No	Area of Olanzapine	Area of Samidorphan
1.	606596	869985
2.	606119	861122
3.	607969	865485
4.	608532	862477
5.	604114	863040
6.	606934	874180
Mean	606711	866048
S.D	1552.8	5059.9
%RSD	0.3	0.6

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 0.6% and 0.3% respectively for Samidorphan and Olanzapine. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy

Table 6.6 Accuracy table of Samidorphan

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	20	20.15	100.75	100.29%
	20	20.04	100.19	
	20	19.99	99.97	
100%	40	40.35	100.87	
	40	40.39	100.98	
	40	39.87	99.68	
150%	60	60.34	100.57	
	60	59.97	99.95	
	60	59.79	99.65	

Table 6.6 Accuracy table of Olanzapine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	10.09	100.93	100.46%
	10	10.05	100.48	
	10	10.08	100.83	
100%	20	20.11	100.53	
	20	20.02	100.08	
	20	20.07	100.36	
150%	30	29.76	99.22	
	30	30.23	100.78	
	30	30.28	100.92	

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 100.29% and 100.46% for Samidorphan and Olanzapine respectively.

Sensitivity

Table 6.7 Sensitivity table of Samidorphan and Olanzapine

Molecule	LOD	LOQ
Samidorphan	0.29	0.89
Olanzapine	0.04	0.12

Robustness

Table 6.8 Robustness data for Samidorphan and Olanzapine.

S.no	Condition	%RSD of Samidorphan	%RSD of Olanzapine
1	Flow rate (-) 0.8ml/min	0.4	0.2
2	Flow rate (+) 1.0ml/min	1.0	0.5
3	Mobile phase (-) 75B:25A	0.1	0.2

4	Mobile phase (+) 85:15A	0.2	0.2
5	Temperature (-) 24°C	0.3	1.0
6	Temperature (+) 34°C	0.3	0.3

Discussion: Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus (75B:25A), mobile phase plus (85B:15A), temperature minus (24°C) and temperature plus (34°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.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Samidorphan and Olanzapine in bulk and pharmaceutical dosage form. Retention time of Samidorphan and Olanzapine were found to be 2.365 min and 3.108 min. %RSD of the Samidorphan and Olanzapine were and found to be 0.6% and 0.4% respectively. %Recovery was obtained as 100.29% and 100.46% for Samidorphan and Olanzapine respectively. LOD, LOQ values obtained from regression equations of Samidorphan and Olanzapine were 0.29, 0.89 and 0.04, 0.12 respectively. %Assay was obtained as 99.71% and 99.58% for Samidorphan and Olanzapine respectively. Regression equation of Samidorphan is y = 21331x + 3547.4, y = 29592x + 9783.5 of Olanzapine. 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.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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