WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

RP-HPLC Method Development, Validation and Stability Studies of Samidorphan and Olanzapine in API and Marketed Formulation.

Barla Karuna Devi*[1], Thumu Pranith[2], Soujanya Chaganti[1], Swathi Naraparaju[1], Pani Kumar D Anumolu[2], Ceema Mathew[2]

[1] Department of Pharmaceutical Chemistry, Gokaraju Rangaraju College of Pharmacy, Hyderabad 500 090, Telangana, India.
[2] Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Hyderabad-500 090, Telangana, India.

ABSTRACT :

A rapid and accurate HPLC method was developed for simultaneous estimation of samidorphan and olanzapine. The method utilized an Ascentis C18 column (150x4.6mm, 5µm) with a 1.0 mL/min flow rate at 30ºC. The mobile phase consisted of KH_2PO_4 buffer (0.01N) and acetonitrile (70:30) with pH adjusted using 0.1M orthophosphoric acid. Retention times were 2.258 min for samidorphan and 2.986 min for olanzapine at 250 nm. Linearity ranged from 2.5-15µg/mL (samidorphan) and 5-30µg/mL (olanzapine), with correlation coefficients of 0.9996 and 0.9998, respectively. Drug content in the marketed formulation was 99.95% (samidorphan) and 100.75% (olanzapine). Detection and quantification limits were 0.10, 0.30µg/mL (samidorphan) and 0.21, 0.64µg/mL (olanzapine), with degradation in the 1-6% range. The method is simple, precise, and suitable for quality control tests.

Keywords: Chromatography, Degradation, Samidorphan, Olanzapine.

INTRODUCTION

Olanzepine (OLA) is a benzodiazepine derivative (Figure. 1) where benzene is fused with diazepine ring, used for treating schizophrenia & bipolar I disorder. As it's associated with a side effect of weight gain samidorphan (SAM) an opioid receptor antagonist was added to overcome this side effect[1]. SAM is a polycyclic compound (Figure.2) containing phenanthrene nucleus. There are various analytical methods for estimation of drugs such as Spectroscopic, spectrofluorimetric, chromatographic methods, etc. Among all the above methods high performance liquid chromatographic methods are more advantageous in terms of precision, sensitivity[2], cost-effectiveness, speed, etc. Apart from pharmaceutical industry HPLC methods have several applications in other sectors encompassing forensic, clinical, and food industry[3]. HPLC is not only used for determination of drug substances but also for separation of isomers, determination of plant extracts [4], proteins, environmental pollutants, and in performing stability studies. Olanzepine and SAM are estimated by spectrofluorimetric method [5] and various HPLC methods. The HPLC methods developed so far used various mobile phase, buffer combinations such as 0.001N sodium hydrogen phosphate (Na_2HPO_4) and ACN (60:40)[6], (70:30)[7,8],10 mM ammonium acetate[9], 0.1 N KH_2PO_4 and ACN (60:40) [10], 0.01N ammonium acetate and ACN (60:40) [11] OPA and ACN (50:50)[12,13], (60:40)[14], formic acid and CAN (20:80)[15], OPA and methanol (40:60)[16]. The present method developed by using potassium hydrogen phosphate and ACN in 70:30 ratio. The method developed is simple, accurate, precise and exhibits reduced run-time along with retention time.

MATERIALS AND METHODS

Materials

Pure drug samples of samidorphan and olanzapine (API), a combined marketed formulation of samidorphan and olanzapine (Lybalvi) tablets, distilled water, methanol, ortho-phosphoric acid phosphate buffer, potassium dihydrogen orthophosphate buffer, and ACN. The reagents and solvents used were from SD-fine make. Instruments p^H meter (Elico, LI-120), Ultrasonicator (SONICA), and electronics balance (Shimadzu, AUX-220) were used. Shimadzu HPLC-LC-20AD series binary gradient pump with solution version software.

Method development

Diluent

ACN and Water mixture (50:50) were considered suitable based on the solubility studies.

Preparation of Standard stock solutions

Accurately measured SAM (5mg), & OLA (10mg) were shifted to 50mL standard flasks and diluted with ACN along with water, and then sonicated for 10min. Volume was adjusted to specific mark using diluent and were labeled as standard stock solutions to obtain SAM, OLA with (100µg/mL) &

(200µg/mL) concentrations respectively.

Preparation of Standard working solutions (100% solution)

An aliquot (1mL) from above stock solution had been transferred into 10mL standard flask and raised volume by a diluent to obtain 10µg/mL SAM along with 20µg/mL OLA.

Preparation of Sample stock solutions

Commercially available formulations of drugs Lybalvi 10tablets were weighed, & weight corresponding to one tablet had been measured and transferred to a 100mL volumetric flask, to which 5mL CAN had been added, followed by sonication. Diluent was added to achieve a final volume of 50mL and subsequently filtered using a 0.45µm membrane.

Preparation of Sample working solutions (100% solution)

*S*tock solution 1 mL had been transferred to a 10 mL standard flask, then diluted to achieve concentrations of 10µg/mL SAM along with 20µg/mL OLA.

Preparation of buffer

Buffer solution OPA (0.1%)

Orthophosphoric acid (1mL) had been transferred to 1000mL volumetric flask, then volume was raised using water of HPLC grade.

Buffer: 0.01N Potassium dihydrogen orthophosphate

Accurately measure (1.36g) KH_2PO_4 and add 900mL milli-Q water, followed by sonication. Adjust volume up to mark using water. 1mL of Triethylamine was added along with dil. OPA solution and adjusted the pH to 3.8.

Method validation

System suitability parameters

Standard solutions of SAM (10ppm) & OLA (20ppm) had been utilized for system suitability parameters determination. Parameters encompassing peak tailing (T), resolution (R), & United States Pharmacopoeia (USP) plate count (N) had been evaluated by injecting prepared standard solutions six times.

Specificity

Specificity in method was established by recording blank, placebo, and analyte chromatograms.

Linearity

A series of sample concentrations within range of 2.5µg/mL to 15µg/mL of SAM & 5 µg/mL to 30µg/mL of OLA a linearity plot showing concentration values on X-axis along with peak area values on Y- axis were plotted. Plot regression coefficient (r^2) was determined.

Accuracy

Sample solutions containing known amounts were spiked at three distinct levels (50, 100, and 150 %), each in triplicate, to evaluate method's accuracy. Percentage recovery at 3 distinct levels was calculated.

Precision

By injecting the 100 % level working standard concentrations for six times in a day system precision, repeatability (intraday), and intermediate (inter day) precision were performed. From the peak areas %RSD (percentage relative standard deviation) had been evaluated.

Sensitivity

Detection limit and quantification were calculated for both the drugs by standard deviation method.

Robustness

Method's robustness had been determined by making little conscious variations in a method encompassing flow rate, temperature, and mobile phase ratio along with no changes in results were found, and were consistent with ICH (International Conference on Harmonization) guidelines. Samples were injected in duplicate to evaluate robust conditions, including Flow plus (1.1mL/min), Flow minus (0.9mL/min), mobile phase plus (65B:35A), mobile phase minus (75B:25A), temperature plus (35°C), and temperature minus (27°C). There wasn't much effect on system suitability parameters and % RSD found to be within limit.

Forced degradation studies

Through forced degradation studies, drug was subjected to extreme circumstances encompassing base hydrolysis, acid, thermal degradation, oxidation, photostability, as well as neutral conditions.

Acid Degradation Studies

To SAM and OLA stock solutions of 1mL each, 2N HCl of 1 mL was added and boiled at 60°C for 30min by attaching condenser. The solution obtained had been diluted to get 10µg/mL & 20µg/mL solution. From the above solutions, 10µL solutions had been injected to record chromatograms.

Alkali Degradation Studies

To SAM and OLA stock solutions of 1 mL, 1 mL of 2N NaOH had been added as well as refluxed at 60°C for 30min. Solution obtained had been diluted for obtaining 10 µg/mL & 20 µg/mL solution. From the above solutions, 10µL solutions were injected to record chromatograms.

Oxidation

To SAM and OLA stock solutions of 1 mL, hydrogen peroxide (H_2O_2) 20% of 1 mL was added separately. Solutions were stored at 60◦C for 30min. Solution obtained was diluted for obtaining 10µg/mL & 20µg/mL solution. From the above solutions, 10µL solutions had been injected to record chromatograms.

Thermal Degradation Studies

Drug solution's thermal stability had been determined by keeping analyte solution in an oven for 1hr. at 105°C. Above solutions had been diluted for obtaining 10µg/mL as well as 20 µg/mL and respective chromatograms were recorded.

Photo Stability studies

The analyte solutions containing SAM (100µg/mL) along with OLA (200µg/mL) were kept for 1 day within UV Chamber. Diluted solutions of 10µg/mL & 20µg/mL were injected to record the chromatograms to assess their photostability.

Neutral Degradation Studies

Drug had been refluxed in water at 60ºC for 1hr for investigating stress testing under neutral conditions. Resultant solution had been diluted for achieving concentrations of 10µg/mL and 20µg/mL. 10ml of solutions mentioned above were introduced into system to evaluate stability of samples by monitoring chromatograms.

RESULTS

Method optimization:

The current HPLC method has been optimized by using different columns, mobile phase ratios, buffers along with wavelengths. The results obtained in various trials are represented in Table 1. The chromatographic conditions in Trial 5 were found to be optimum and the relevant chromatograms had been demonstrated in Figure3.

Method validation

System suitability

System suitability parameters such as retention time (RT), USP plate count (N), as well as USP tailing (T), were within range and the obtained values are shown in Table 2.

Specificity

SAM and OLA had retention times of 2.236min & 2.954min respectively. At retention times of these drugs, no interfering peaks (Figure.4) in blank along with placebo had been observed. Hence this approach was said to be specific.

Linearity

From linearity response (Fig. 5) of SAM and OLA. R^2 was calculated as 0.9997 and 0.9998. The linearity data is represented in Table3.

Accuracy

Correctness in method had been determined by injecting the analyte solutions at three different concentrations. Percentage mean recovery for each level was calculated and represented in Table 4.

Precision

% RSD values of peak area responses were found to be 1.5 and 0.35 respectively for system precision. For inter-day precision, % RSD values were obtained as 1.35 % & 0.5% respectively for SAM & OLA. For intraday precision % RSD values were achieved as 0.9% & 0.6% respectively for SAM & OLA. Obtained values are within the limits i.e., <2 %. The obtained results are shown in Table 5.

Sensitivity

Method's sensitivity had been determined by LOD(limit of detection) as well as LOQ(limit of quantitation) calculations. LOD & LOQ values were 0.10,0.30 and 0.21, 0.64 for SAM and OLA.

Robustness

The results suggested that minor variations in method conditions didn't impact system suitability parameters. The values are represented in the Table 6.

Degradation

The drug solutions were subjected to various degradation studies and the degraded samples were injected. The results are shown in Table 7. The chromatogram for acid degradation is represented in the Figure. 6.

Assay

(Lybalvi) The labelled claim for SAM and OLA were 10mg and 20mg. With the above formulation, assay had been conducted. Average % Assay values were 99.95 % & 100.75 % for SAM and OLA. The assay data of SAM and OLA is represented in Table8. The system suitability parameters of current method along with the earlier reported methods are represented in Table 9.

DISCUSSION

RP-HPLC method was developed as well as validated for SAM and OLA in API as well as tablet form. By performing various trials, the optimized conditions were ACN:buffer (30:70) at wavelength of 250nm with 1mL/min flow rate along with 5min run time. Run-time was found to be reasonable in comparison to previously reported method with good resolution for both the drugs. The linearity data for SAM was obtained at lower concentration range of 2.5-15µg/mL when compared to few already available literature methods. For OLA, the linearity had been achieved at concentration range of 5-30µg/mL. Precision data was obtained by calculating system-precision, Intraday-precision, and Interday-precision, and %RSD for both the drugs were within the specifications. By varying mobile phase ratio, flow rate, as well as temperature, method was found to be robust. Degradation studies had been performed by employing various degradation conditions such as acids, alkali, oxidation by using H_2O_2, and thermal stability determined by exposing the analyte to high temperature of 105ºC by placing it in oven. Photostability was determined by placing the analyte in UV chamber. The degradation percentages were within the limits for all the employed degradation criteria. The drug content in the marketed formulation was estimated as 99.95 % and 100.75 % for SAM and OLA respectively.

CONCLUSION

A rapid as well as accurate HPLC method for simultaneous quantification of the SAM as well as OLA in bulk and tablet form has been established. Optimization of the method was done by conducting various Trials. In comparison to literature-reported methods, currently developed method had reasonably low retention times with 2.258min for SAM and 2.986min for OLA. Developed method has been validated for accuracy, linearity, sensitivity, robustness as well as precision. All validated parameters meet criteria as per ICH specifications. % Assay values for the marketed tablet formulation had been achieved as 99.95% & 100.75% for SAM & OLA respectively. The results attained through the degradation studies were within the permissible limits. The present established method has decreased retention time along with run time. So, developed method may be implemented in standard quality control testing within industries as it's simple and economical.

FUNDING

No funding received for this research work.

AVAILABILITY OF DATA AND MATERIAL

All data and materials are available on request.

DECLARATIONS

ETHICS APPROVAL & CONSENT TO PARTICIPATE

Not applicable.

CONSENT OF PUBLICATION

Not applicable as our study does not include patients.

ACKNOWLEDGEMENT

The authors are grateful to Prof. Giriraj T Kulkarni, Principal, Gokaraju Rangaraju College of Pharmacy for providing necessary laboratory facilities. The authors are thankful to Gokaraju Rangaraju Educational Society for providing the adequate infrastructure facilities.

REFERENCES :

1. Sun, L.; Mills, R.; Sadler, B.M.; Rege, B., *J Cl Pharmacol*., **2021**, 61(11), 1430–41.
2. Kumar, S.D.; Harish, K. D., *IJPSR*, **2012**, 3(12), 4626–33.
3. Malviya, R.; Bansal, V.; Pal, O.; Sharma, P., *J. Glob. Pharma Technol.,* **2010,** 2 (5), 22-26.
4. Boligon, A.A.; Athayde, M.L., *Austin Chromatogr*., **2014**, 1(3), 2.
5. Salem, H.; Samir, E.; Mazen, D.Z.; Madian, H.; Elkhateeb, A.E.; Elaraby, M.; Rasekh, M.I.; Gamal, A., *Spectrochim Acta A Mol Biomol Spectrosc*., **2022,** 5, 274.
6. Blessing, R. D.J.; Asha, D. D., *YMER*, **2023**, 22 (3), 599-616.
7. Sravani, N., *IJMCA.*, **2023**,13(01), 1-18.
8. Kethavat, R.P.; Shobha, R.S., *World J Pharm Sci*., **2022**, 10(09), 52-60.
9. Pavani, B.; Malothu, N.; Prasanth, D.; Guntupalli, C., *Ind. J. Pharm. Edu. Res.*, **2023**, 57(3), 873–82.
10. Anusha, V.; Ramana, K.V., *IJPPR.*, **2022** Oct; 25 (03), 211-224.
11. Marakatham, S.; Shanmugapandiyan, P., *J. Xi'an Shiyou Univ. Nat. Sci*., **2020,** 18(2), 281-291.
12. Vinod, S.; Rajendra, Y., *IJRPC.*, **2022,** 12(2), 33–36.
13. Syed, I.B.; Nannapaneni, M., *J Pharm Res Allied Sci.*, **2022**, 11(4), 87–94.
14. Rafi, S.; Rambabu, K., *Biosci. Biotechnol. Res. Commun*., **2021**, 14(9), 198–204.
15. Padmavathi, S.; Sai, G.; Lakshmi, S.; Sai, K.; Bhavani, D.; Rahaman, S.A., *J. Pharm. Negat.* **2022**, 13(09), 8691-8713.
16. Rasheed, S.H.; Pavani, C.H.; Pranaya, P.; Rafay, A.; Praveena, S., *J. Pharm. Negat*., **2022,** 13 (06), 828-843.

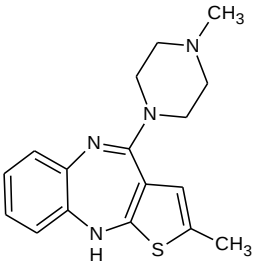

Fig. 1. Structure of Olanzepine

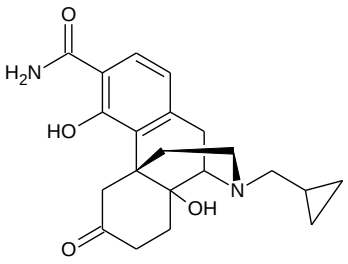

Fig. 2. Structure of Samidorphan

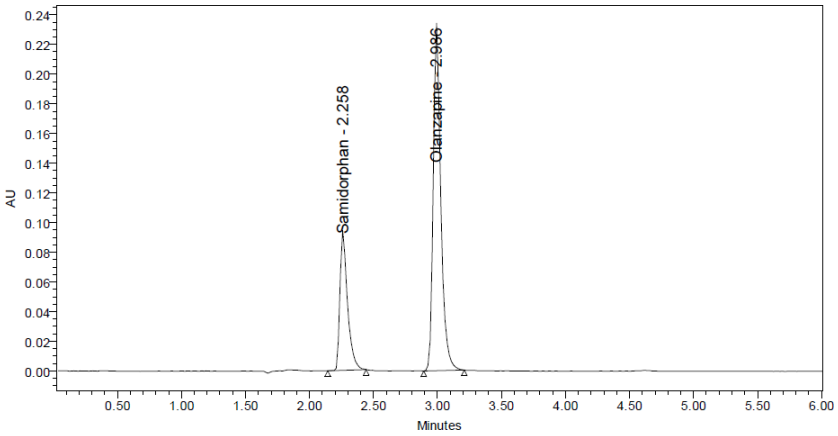

Fig. 3. Optimized Chromatogram of the method

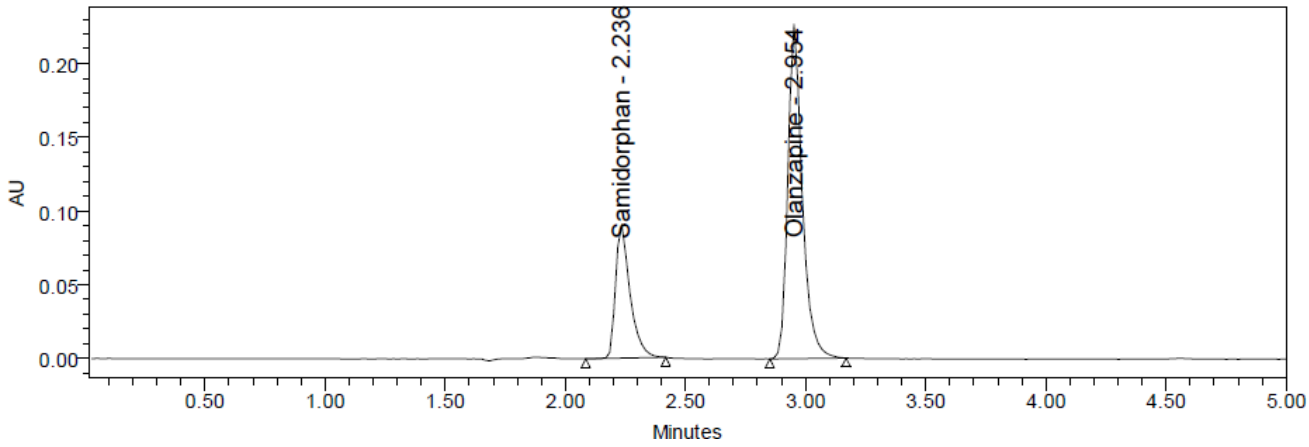

Fig. 4. Typical Chromatogram

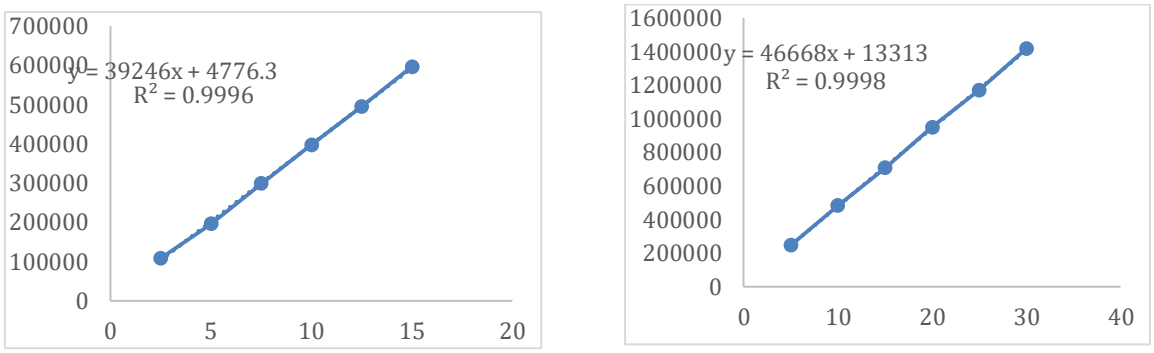

Fig.5. Calibration curves of Samidorphan and Olanzapine

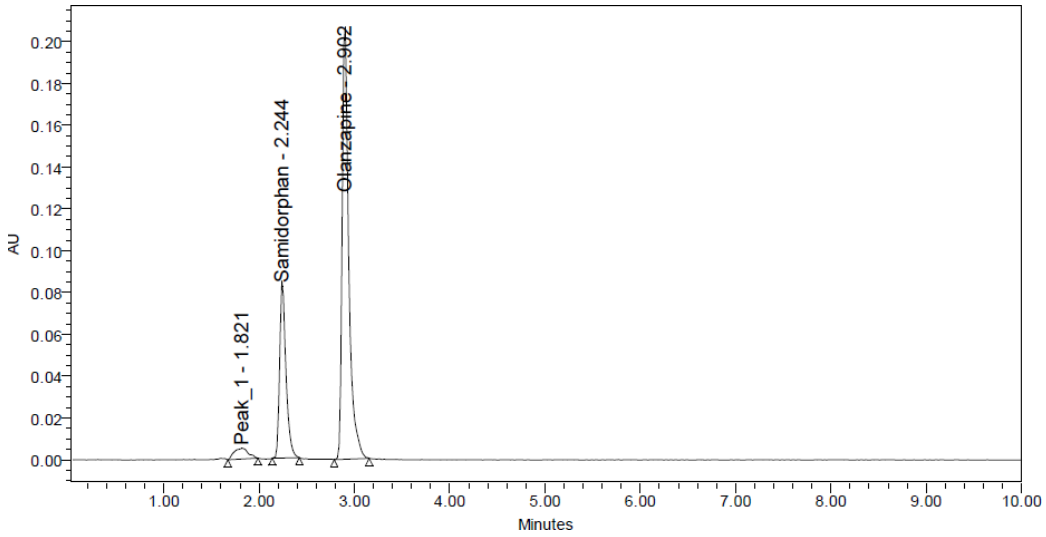

Fig. 6. Acid chromatogram of Samidorphan and Olanzapine

TABLE 1
Trials

Trial	Column	Buffer	Mobile phase	Wave length	Run time	Flow rate mL/min	Observation
1	STS Discovery 250 (4.6 x 150mm, 5µm)	0.1% OPA	Methanol: Buffer (50:50)	250 nm	10 min	1 mL/min	Peak splitting for both the drugs.
2	STD discovery 250 (4.6 x 150mm, 3µm)	0.01N KH2PO4	Methanol: Buffer (50:50)	250 nm	5 min	1 mL/min	Olanzapine has low USP plate count and broad peak.
3	STD discovery 250 (4.6 x 150mm, 5µm)	0.1% OPA	Acetonitrile: Buffer (40:60)	245 nm	6 min	1 mL/min	Broad peaks for both the drugs and peak splitting for samidorphan.
4	Ascentis C18 (4.6 x 150mm, 5µm)	0.1% OPA	Acetonitrile: Buffer (60:40)	250 nm	10 min	1 mL/min	Peak asymmetry for olanzapine
5	Ascentis C18 (4.6 x 150mm, 5µm)	0.01N KH_2PO_4	Acetonitrile: Buffer (30:70)	250 nm	5 min	1 mL/min	Good resolution, tailing factor Limit of Detection and theoretical plate count.

TABLE 2
System suitability parameters.

S. No	Samidorphan	Olanzapine

Inj	**RT (min)**	**USP Plate count (N)**	**USP Tailing (T)**	**RT (min)**	**USP Plate count (N)**	**USP Tailing (T)**	**RS**
1	2.236	6925	1.48	2.950	12155	1.29	6.4
2	2.244	6941	1.47	2.954	12328	1.30	6.3
3	2.256	6814	1.48	2.989	12341	1.31	6.4
4	2.257	7015	1.49	2.990	12252	1.31	6.3
5	2.257	6924	1.50	2.994	12273	1.31	6.3
6	2.260	7144	1.49	2.997	12304	1.29	6.4
Mean	2.252	6960.5	1.48	2.979	12275.5	1.30166	6.3
SD	0.00948	110.5238	0.0104	0.02114	67.731	0.0098	0.05477
% RSD	**0.421**	**1.58**	**0.706**	**0.709**	**0.551**	**0.755**	**0.862**

TABLE 3
Linearity data

Samidorphan		**Olanzapine**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
2.5	108032	5	246532
5	195721	10	483643
7.5	297837	15	708998
10	397246	20	950495
12.5	494621	25	1171425
15	595615	30	1418952

TABLE 4
Accuracy data

Samidorphan				
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean % Recovery
50%	5	5.00	100.03	100.29 %
	5	5.01	100.19	
	5	5.03	100.66	
100%	10	10.00	100.04	100.71 %
	10	10.13	101.32	

	10	10.08	100.77	
150%	15	14.9	99.3	99.4 %
	15	14.9	99.5	
	15	14.9	99.4	
Olanzepine				
50%	10	9.82	98.17	99.49 %
	10	9.81	98.06	
	10	9.92	99.24	
100%	20	19.85	99.27	99.9 %
	20	20.20	101.02	
	20	19.88	99.42	
150%	30	29.78	99.26	100.20 %
	30	30.27	100.91	
	30	30.13	100.44	

TABLE 5

Precision data

System Precision	**S. No**	**Peak area**	
		Samidorphan (10 (µg/mL)	**Olanzapine (20 (µg/mL)**
	1.	394487	944785
	2.	406231	944932
	3.	395210	943718
	4.	404770	940068
	5.	403194	939711
	6.	391849	936792
	Mean	399290	941668
	S.D	6140.6	3308.7
	%RSD	**1.5**	**0.35**
Repeatability (Intraday precision)	1.	400513	945219
	2.	394733	951849
	3.	395496	943336
	4.	408754	956445
	5.	404962	954394
	6.	399608	952517
	Mean	400678	950627
	S. D	5421.1	5206.0
	%RSD	**1.35**	**0.5**
Inter day pr	**S. No**	**Area of Samidorphan**	**Area of Olanzapine**

	1.	386053	938918
	2.	377571	939212
	3.	384985	938460
	4.	379509	925116
	5.	381749	932760
	6.	385661	929499
	Mean	382588	933994
	S. D	3536.9	5864.6
	%RSD	**0.9**	**0.6**

TABLE 6
Robustness data

S. No	**Condition**	**%RSD of**	
		Samidorphan	**Olanzapine**
1	Flow rate (-) 0.9ml/min	0.7	0.1
2	Flow rate (+) 1.1ml/min	0.7	0.3
3	Mobile phase (-) 65B:35A	0.6	0.7
4	Mobile phase (+) 55B:45A	0.9	0.4
5	Temperature (-) 25℃	0.4	0.1
6	Temperature (+) 35℃	0.1	0.2

TABLE 7
Degradation data

S. NO	**Degradation Criteria**	**% Drug Degraded**	
		Samidorphan	**Olanzapine**
1	Acid	6.16	5.45
2	Base	4.54	4.11
3	Oxidation	4.02	3.68
4	Thermal	2.54	2.99
5	UV	2.06	1.98
6	Water	1.17	0.95

TABLE 8
Assay Data

S. No	**Samidorphan**			**Olanzapine**		
	Standard Area	**Sample area**	**% Assay**	**Standard Area**	**Sample area**	**% Assay**
1	394487	400513	99.91	944785	945219	100.18
2	406231	394733	98.46	944932	951849	100.88
3	395210	395496	98.65	943718	943336	99.98
4	404770	408754	101.96	940068	956445	101.37
5	403194	404962	101.01	939711	954394	101.15

6	391849	399608	99.68	936792	952517	100.95
Avg	399290	400678	99.95	941668	950627	100.75
Std dev	6140.6	5421.1	1.35	3308.7	5206.0	0.55
%RSD	1.5	1.4	1.4	944785	945219	0.54
1	394487	400513	99.91	944932	951849	100.88
2	406231	394733	98.46	943718	943336	99.98

TABLE 9

System suitability parameters of Samidorphan and Olanzapine.

S No	**Methods available**	**Drug**	**Column Type and its Dimensions**	**Wavelength (nm)**	**Buffer**	**Mobile phase**	**Flow rate (mL/min)**	**Linearity range (µg/mL)**	**R^2**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**% assay**	**RT (min)**	**Run time (min)**
1	Current method	SAM	Ascentis (150 X 4.6 mm, 5 µm)	250	0.01N KH_2PO_4	Buffer: ACN 70:30	1.0	2.5 – 15	0.9996	0.10	0.30	99.95	2.258	5
		OLA						5 – 30	0.9998.	0.21	0.64	100.75	2.986	
2	[6]	SAM	standard C18 (Agilent) (15cm x 4.6 mm i.d, 5µm)	226	0.001N Na_2HPO_4	Buffer: ACN 60:40	1.0	2.5 -15	0.9994	0.02	0.07	100.01	3.207	-----
		OLA						3.75 - 22.5	0.9999	0.05	0.14	99.6	2.214	
3	[7]	SAM	zorbax eclipse xdb-C18 (150 x 4.6 mm, 5m)	226 nm	0.001N Na2Hpo4:	Buffer: ACN 70:30	1.0	5 - 30	0.9994	0.04	0.13	99.6	2.209	6
		OLA						7.5 - 45	0.9999	0.09	0.28	99.27	3.196	
4	[8]	SAM	Std Zorbax 150 x 4.6 mm, 5µm)	268	0.01N Sodium hydrogen phosphate	Buffer: ACN 60:40	1.0	2.5 to 15	0.9996	0.21	0.63	99.19	2.235	--
		OLA						5 to 30	0.9999	0.09	0.23	99.81	2.784	
5	[9]	SAM	Zorbax SB-C18 column (250 mm x 4.6 mm, 5µ)	270	10 mM ammonium acetate	Buffer: ACN 60:40	0.8	2.25-90	0.9991	-----	-------	----	3.894,	-----
		OLA						1-40	0.9991	-----	-------	----	9.572	
6	[10]	SAM	Standard Agilent C18 (150 x 4.6 mm, 5 m)	226.	0.1N KH_2PO_4	Buffer: ACN 60:50	1.0	2.5- 15	0.999	0.02	0.07	----	3.207	6
		OLA						3.75-22.5	0.999	0.05	0.14	-----	2.214	
7	[11]	SAM	Inertsil (250 4.6mm, 5µm)	228	0.01N Ammonium acetate	Buffer: ACN 60:40	1.0	2.75 to 275 ng/mL	0.9999	---------	----------	-------	2.909	8
		OLA						4.75 to 475 ng/mL	0.9992	------	----------	--------	3.408	
8	[12]	SAM	Azilent C18 (150x 4.6mm, 5µm)	226.0	0.1% OPA	Buffer: ACN 50:50	1.0	25 to 150	0.999	27.2	42.4	99.69	3.227	6
		OLA						25 to 150	0.999	45.7	83.2	99.28	2.216	
9	[13]	SAM	Inertsil ODS (250x4.6 mmx 5 µm)	261	0.1% OPA	Buffer: ACN 50:50	1.0	12.5 to 75	0.9996	5	5	----	7.255	----
		OLA						12.5 to 75	0.9998	1.6	1.6	------	3.007	
10	[14]	SAM	symmetry C18 column (150x4.6m	261	0.1% OPA	Buffer: ACN 60:40	1.0	1-15	0.9992	-----	------	-----	7.732	10
		OLA						2-30	0.9998	-----	------	------	4.363	

			m, 3.5 µm)											
11	[15]	SAM	Luna phenyl hexyl (250X4.6mm, 5µm)	280	formic acid	Buffer: ACN 20:80	1.0	2.5 to 15	0.99947	0.2	1	100.2	3.940	
		OLA						5 to 30	0.99986	0.6	2	99.4	2.054	
12	[16]	SAM	Xterra (4.6 x 150mm, 5µm)	220.0	OPA	Buffer: methanol 40:60	1.0	10 to 50	0.999	0.21	0.68	99.8	4.270	10
		OLA						5 to 25	0.999	0.20	0.66	99.72	3.124	