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A Novel RP- UPLC Technique for the Simultaneous Estimations of Olanzapine and Samidorphan in Bulk and Tablet Dosage Form

Y.Haritha Sri*, , M Raghu Prasad[1],Y. Rajendra Prasad[2], L. Surendra Babu[3], M. Sri Vinay Reddy[3], D. Ganesh[3], G. Satya Narayanna[3], P. Venkata Prasad[3], S. Likhitha[3]

[1*]Shri Vishnu College of Pharmacy, vishnupur,Bhimavaram, A.P, 534202India.
[2]AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India.
[3]Adikavi Nannaya University Campus, Tadepalligudem
*corresponding author:Dr. L. Surendra Babu
Address: Assistant Professor, AKNU college of pharmaceutical sciences, Tadepalligudem, A.P, 534202 India.
Email: lsbabu033@gmail.com

ABSTRACT

A convenient, precise, consistent, and robust quantitative approach was established for separation and simultaneous quantification of olanzapine and samidorphan in combination. The isocratic elution was attained by Hibar C18 (100 × 2.1mm, particle size 2µ) column at a flow rate of 0.3ml/min and temperature was maintained at 30°C. The system was operated with chromatographic conditions like Buffer(0.01N Kh2po4, PH 3.5) and acetonitrile (50:50 v/v) ratio and diluent is water: acetonitrile (50:50) with TUV detector. Olanzapine and samidorphan were eluted with retention time 1.678min and 2.262min at a wavelength of 317.8nm . system suitability parameters were analyzed by injecting six times standard solution and the results were within the acceptance criteria. The linearity was observed within a range of 5-30 µg/ml for olanzapine and samidorphan with correlation coefficient of 0.999 respectively. Accuracy was attained between 97.0% -100% and 98- 103.0% for olanzapine and samidorphan respectively. Results shows that the proposed method was suitable for the routine analysis.

Keywords: ICH guideline (Q2 R1), UPLC, Olanzapine, Samidorphan and Validation.

1. INTRODUCTION

Schizophrenia is a psychiatric issue that impacts over 21 million people across the world, placing it among the top ten physical illnesses (WHO). The current generation antipsychotic medicines have a lower incidence of schizophrenia. The first generation of antipsychotics, have extra pyramidal effects, are routinely often used to treat schizophrenia.[1,2] .Olanzapine, effective antipsychotic agent available for the treatment of schizophrenia, belongs to the atypical antipsychotics class of pharmaceuticals. Moreover, safety issues like excess weight and metabolic

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 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

impairments have surfaced. An alloy of olanzapine and samidorphan product is in design to treat of schizophrenia and bipolar I disorder. [2] Olanzapine is a typical antipsychotic and a second-generation neuroleptic drug which is approved by FDA .It is chemically (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno-[2,3b] [1,2 benzodiazepine[3].Samidorphan is an Opioid blocker. It is chemically (1R,9R,10S)-17-(cyclopropylmethyl)-3,10-dihydroxy-13-oxo-17-azatetracyclo [7.5.3.0^{1,10}.0^{2,7}] heptadeca-2,4,6-triene-4- carboxamide[3]. Literature survey reveals few method of LC [4], spectrophotometric studies[5] for determination of olanzapine and samidorphan alone. The objective of this study is to develop a novel, accurate and easy technique, so an attempt was made for simultaneous and method development of olanzapine and samidorphan in combination to develop a sensitive and reproducible method.

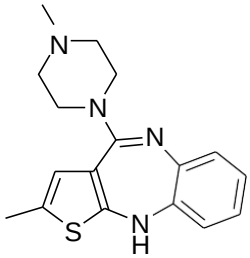


Fig 2 Olanzapine

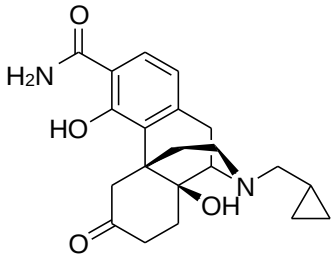


Fig 3 Samidorphan

2. Materials and methods

2.1 Chemicals used

UPLC grade water, acetonitrile, potassium dihydrogen orthophosphate purchased from Thermo Fischer Scientific Pvt.Ltd. The drug samples were attained as a gift sample from S.S labs Ltd.,India.

2.2 Instrument used:

The Acquity UPLC instrument 1200 series used was of WATERS with quaternary pumps, TUV detector and Auto sampler by Empower 2.0. UV-VIS spectrophotometric PG Instruments T60 with band width of 2 mm and 10 mm and coordinated quartz cells incorporated with the UV of 6. pH (Digisun Electronics India) was being used to disassociate the mixtures.

ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

2.3 Method development

2.3.1Mobile phase preparation:

0.01N Phosphate buffer (PH 3.5) and acetonitrile in the ratio of 70:30v/v was taken, filtered and sonicated for 10min and pH adjusted with dil. orthophosphoric acid .

2.3.20.01N phosphate Buffer

To 1000ml of volumetric flask 1.36 g of potassium dihydrogen phosphate was added. To this 900ml of milli-Q water was added .Then degassed, sonicated and volume was made up with water. Then pH was adjusted to 3.5 with dil.orthophosphoric acid .

2..3.3 Diluent

Acetonitrile: water in the ratio of 50:50v/v was used.

2..3.4 Preparation of standard solution

10mg of olanzapine and samidorphan working standard solution was weighed into 50ml volumetric flask. To this 10ml of diluent (water:ACN) was added and finally make up to volume by using diluent to get 200µg/ml of olanzapine and samidorphan. From this 1ml was taken in to 10ml volumetric flask and further made up to volume with diluent to get 20µg/ml of olanzapine and samidorphan.

2.3.5 Preparation of test solution

10 tablets of olanzapine and samidorphan was weighed and powdered. Then from the powder weight equivalent to 1 tablet was transferred into a clean 100ml volumetric flask, 50ml of diluents added and the solution was sonicated for 25 min. Finally made up volume to get 100µg/ml with diluent. To 10ml of volumetric flask, 2ml of above solution was added and finally volume was made to get 20µg/ml with diluent.

2..3.6 Chromatographic condition

In this method chromatographic separation was developed by using Hibar C18 (100 × 2.1mm, particle size 2µ) column. Mobile phase consisting of acetonitrile and phosphate buffer in the ratio of 50:50v/v was used. The flow rate and injection volume was maintained at 0.3ml/min and 1.0µL and absorbance was monitored at 317.8 nm.

ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

2.4 Method validation:

The current method was validated as per ICH guidelines (Q2 R1).The variables like linearity, precision, accuracy specificity LOD&LOQ, specificity, stress degradation studies[12] were performed.

2.4.1 System suitability

standard solutions of olanzapine and samidorphan were taken at a concentration of 20 µg/ml and solutions were injected six times and the chromatograms were recorded. Parameters like theoretical plates, retention time ,tailing factor was used to determine the system suitability of the method.

2.4.2 Specificity

Specificity was analyzed by injecting blank, and sample solution in order to scrutinize the excipients interference. Interference was not observed and the method was specific.

2.4.3 Accuracy

Accuracy was analyzed by spiking at three different levels 50%,100%,150% of the targeted concentration of drugs. From this mean percent recovery was calculated by comparing the difference between spiked value and actual value.

2.4.4 Precision

Intraday precision was analyzed by using same concentration on the same day for six times. Interday precision was analyzed by using three analysts for six times on different days. The relative standard deviation (%RSD)of olanzapine and samidorphan for six standard preparations should be not more than2.0% was caluculated for both drugs and the method is considered to be precised and rugged.

Linearity

Linearity was analyzed by series of standard six solutions over the range of 5-30 µg/ml of olanzapine and samidorphan respectively. A standard plot was constructed between concentration versus mean peak area in µg/ml to know the best fit line of both drugs.

ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

2.4.5 Robustness

Robustness was analyzed by altering analytical conditions like flow rate, mobile phase composition and column temperature and no change was identified in the method. %RSD was within the limits.

2.4.6 LOD&LOQ

Detection limit(LOD) and quantitation limit(LOQ) was calculated by formula according to ICH(Q2R1).

$$LOD = 3.3 \times \sigma / s, \quad LOQ = 10 \times \sigma / s$$

2.4.7 Forced degradation studies

100mg of standard drug of olanzapine and samidorphan was mixed with 100ml of 2N hydrochloric acid, 2N sodium hydroxide and 20% hydrogen peroxide for acidic, basic and oxidation studies respectively. For neutral study the solution was refluxed in water for 1hr at 60 ºC. For photolytic and thermal studies standard drug solutions were kept in UV for 1 day at 258nm and for heat in an oven 105^0C for 2hr.Percentage degradation was calculated by comparing the peak area response of both drugs with calibration curve results

3 RESULTS AND DISCUSSION

3.1 Method development for optimized chromatographic condition

Several compositions of mobile phase, various columns, PH, flow rates were scrutinized and the better result was accomplished by using Hibar C18 (100 × 2.1mm, particle size 2µ) column. 0.01N phosphate buffer and acetonitrile (50:50v/v) was used as mobile phase and water and acetonitrile (50:50v/v) as diluent was taken for preparing operational solutions of drugs. Absorbance was monitored at wavelength of 317.8nm with a run time of 3.0min and column temperature was maintained at 30°C.The optimized chromatogram and conditions were given in fig 3 and table1.

Tab 1: optimized conditions

optimized chromatographic conditions	
chromatographic parameters	**optimized condition**
Column	Hibar C18 (100 × 2.1mm, particle size 2µ)
Mobile phase	Buffer(0.01N Kh2po4) and ACN 50:50

ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

Mode	Isocratic
Flow rate	0.3ml/min
Wavelength	317.8 nm
Column temperature	30°C
Injection volume	1.0µL
Run time	3 .0min
Diluent	water:ACN (50:50)

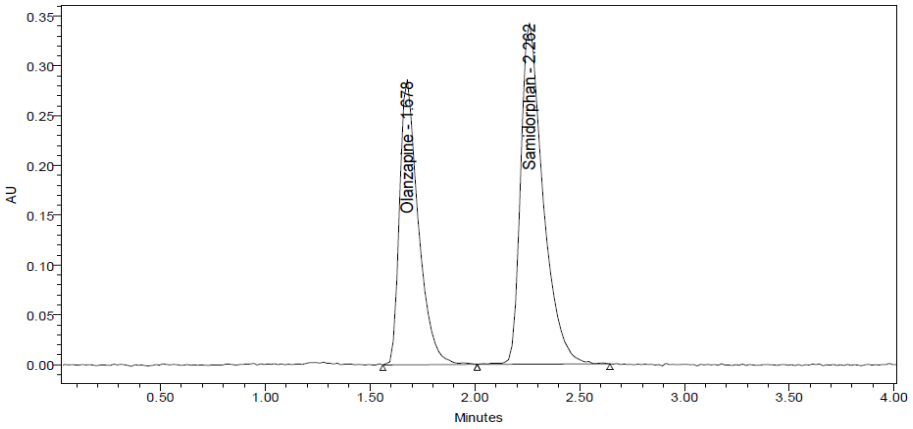


Fig 3: Optimized chromatogram

3.2 Method validation

3.2.1system suitability

System suitability was analyzed from standard solutions by injecting six times and chromatograms were recorded .From the chromatograms Retention time (Rt), number of theoretical plates (N) and tailing factor (T) are evaluated for six replicate injections The results were given in table 2.

Table:2 System suitability results

Parameters	Olanzapine	Samidorphan
Retention time	1.677	1.51
Theoretical plate	2676	2194
Tailing factor	1.51	1.53

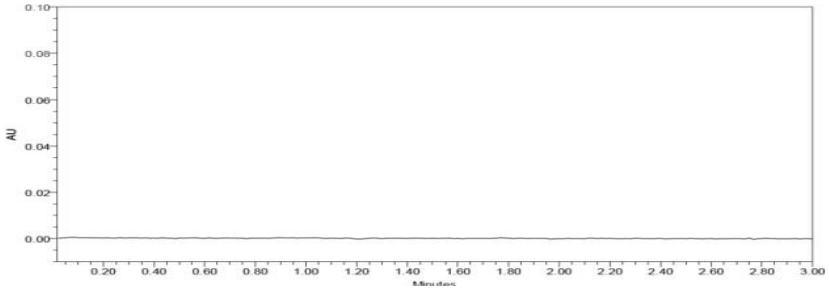
ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

3.2.2 Specificity

Interference is not observed with the standard peaks and the standard and sample chromatograms are identical with same retention time. The results were given in figure 4&5.

Fig: 4 blank chromatogram



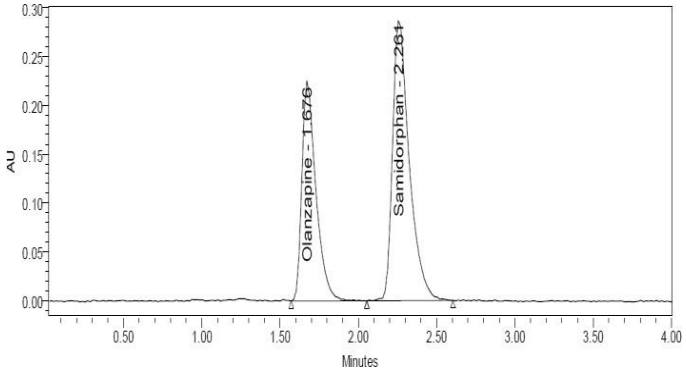


Fig 5: Standard chromatogram

3.2.3 Linearity

The linearity for olanzapine and samidorphan was analyzed in the range of 5-30 µg/ml and the correlation coefficient was found to be 0.999 for both drugs respectively. Linearity graph for both drugs was revealed in fig6,7 and table 3

ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

Table 3: Linearity data

Analyte	Linearity range (µg/ml	Calibration curve equation	Correlation coefficient
Olanzapine	5-30µg/ml	Y=88886.x+8791	0.999
Samidorphan	5-30µg/ml	Y=11102.x+9711	0.999

Fig 6: Linearity plot of Olanzapine

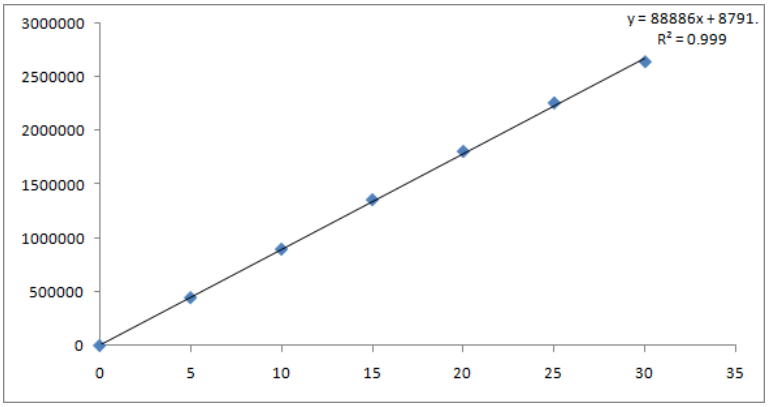
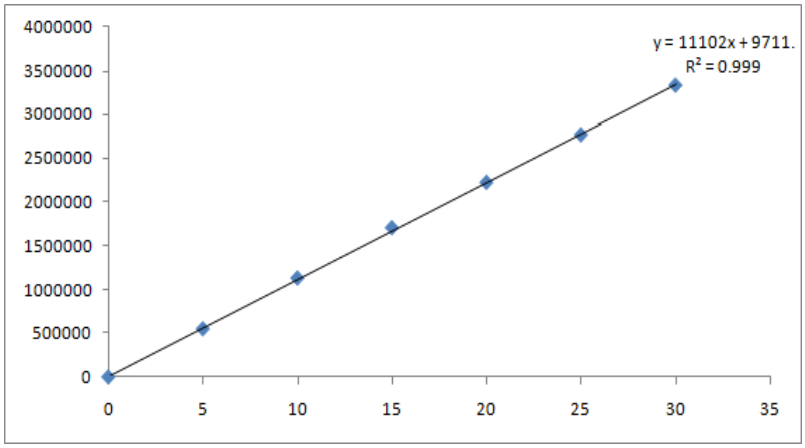


Fig 7: Linearity plot of samidorphan



ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

LOD&LOQ

In this method LOD&LOQ values were found to be 0.11µg/ml and 0.330µg/ml for olanzapine and 0.12µg/ml,0.36µg/ml for samidorphan respectively. The results were summarized in the tab 4.

Table 4: Results of LOD and LOQ

S.no	Drugs	LOD(µg/ml)	LOQ(µg/ml)
1	Olanzapine	0.11	0.33
2	Samidorphan	0.12	0.36

Precision

This method was precise as the %RSD was 2% for both interday and intraday precision. The results were summarized in the given tab 5.

Table 5: Results of Intraday and interday precision

S.no	Intra day precision		Interday precision	
	Olanzapine	**Samidorphan**	**Olanzapine**	**Samidorphan**
1	1790701	2206680	1786746	2192072
2	1786288	2155377	1779493	2128201
3	1798616	2181448	1767529	2166423
4	1801498	2214058	1774294	2194456
5	1789436	2189427	1769681	2142861
6	1760615	2158660	1765190	2163192
Mean	1787859	2184275	1773822	2164534
Std.Dev	14539.6	24144.4	8132.3	26263.4
%RSD	0.8	1.1	0.5	1.2

Accuracy

Recovery studies was performed by spiking at three different levels(50%,100%,150%).The percent recovery was found to be97.0% -100% for olanzapine and 98- 103.0% for samidorphan. Hence the developed method is accurate. The results were summarized in table 6.

ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

Table 6: Accuracy for olanzapine and samidorphan

Level	Amt added		Amt recovered		%recovery		Mean % recovery	
	OLZ	SMP	OLZ	SMP	OLZ	SMP	OLZ	SMP
	10	10	9.9	9.96	98.80	99.61		
50%	10	10	9.9	10.05	99.00	100.45	99.03	100.30
	10	10	9.9	10.08	99.30	100.83		
	20	20	19.9	20.05	99.70	100.24		
100%	20	20	20.2	19.85	100.90	99.26	100.20	99.65
	20	20	20.1	19.89	100.00	99.46		
	30	30	30.4	30	101.30	99.99		
150%	30	30	30.1	29.68	100.40	98.92	100.30	99.63
	30	30	29.8	29.99	99.20	99.98		

Robustness

By deliberating some changes in experimental conditions like flow rate, mobile phase composition, column temperature the %RSD was found to be not more than 2,hence the developed method specifies to be robust. The results were summarized in the table 7.

Tab 7 : Results of robustness

		OLZ		SMP	
S.no	Condition	Peak area	%RSD	Peak area	%RSD
1	FR 1	1912435	0.7	2337732	1.5
2	FR 2	1600043	0.3	2123770	0.3
3	MP 1	1769411	0.4	2235966	1.8
4	MP 2	1810576	0.4	2414442	0.3
5	CT 1	1823002	1.2	2357969	0.6
6	CT 2	1752716	0.6	2142624	1.6

.

FR1- flow rate minus(0.27ml/min),FR2- flow rate plus(0.33ml/min), MP1-mobile phase minus(buffer 75:25 ACN) ,MP2-mobile phase plus(buffer 65:35 ACN ,CT1-column temperature minus (27°C) , CT2-column temperature plus (33°C)

ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

Stress degradation studies

In this method various studies like acid, base, oxidation, thermal, and neutral investigations were carried out as per ICH Q2 R1 guidelines and all were within the acceptance criteria. The data was summarized in the tab 8.

Table 8:Results of degradation studies

Degradation condition	Olanzapine		Samidorphan	
	% of undegraded	% of degraded	% of undegraded	% of degraded
Acid	95.04	4.96	94.73	5.27
Base	95.57	4.43	95.43	4.57
Oxidation	96.58	3.42	96.4	3.6
Dry heat	97.51	2.49	97.59	2.41
UV	98.05	1.95	98.26	1.74
Water	99.45	0.55	99.32	0.68

Assay

The proposed UPLC method was pertained to simultaneous estimation of olanzapine and samidorphan in commercial tablets.. The amounts of olanzapine and samidorphan estimated were found to be 99.90% and99.80% .This shows that the developed method was accurate and also within the acceptable levels of 98% to 102% and results were given in table9.

Table 9: Assay calculation

S.no	Drugs	Marketed formulation	Label claim	% Assay
1	Olanzapine	OLZ&SMP	10mg&10mg	99.90%
2	Samidorphan			99.80%

CONCLUSION:

A specific ,precise and simple UPLC method has been developed for simultaneous estimation of olanzapine and samidorphan, which contributes to better resolution between both of the drugs.

ISSN PRINT 2319-1775 Online 2320-7876

 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, January 2023

The method was validated by analyzing linearity, specificity, accuracy ,precision, robustness etc and the obtained results were within the acceptance criteria as per ICH (Q2R1) guidelines. . The stress degradation studies were conducted for two drugs by using some degradation conditions like acidic,base,oxidation,thermal and photolytic conditions and the method was found to be stable. Stastical analysis confirms that the developed method was stable and effective for quantitation of both drugs in pharmaceutical analysis and routine quality control without any interference.

DISCLAMAR

In this research products used are commonly and predominantly used products in our area of research and country. There was no conflict of interest between the authors and producers of the products because we do not intend to use the products as an avenue for any litigation but the advancement of knowledge.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Visakhapatnam for providing necessary facilities for research work

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