BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SAMIDORPHAN AND OLANZAPINE USING HPLC IN HUMAN PLASMA

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ABSTRACT

A simple, precised, accurate method was developed for the estimation of Samidorphan and Olanzapine in human plasma using the Dolutegravir as internal standard by RP-HPLC (Reverse phase-High performance Liquid Chromatographic) technique. Chromatographic conditions used are stationary phase Inertsil 250 4.6mm, 5µm, Mobile phase 0.01N Ammonium acetate Buffer : Acetonitrile 60:40 and flow rate was maintained at 1.0ml/min, detection wave length was 228 nm, column temperature was set to 30ºC and diluent was mobile phase Conditions were finalized as optimized method. Retention time of Samidorphan and Olanzapine were found to be 2.909 min and 3.408min. %CV of theSamidorphan and Olanzapine was found to be 0.42% and 0.52%. %Recovery was obtained as 99.96% and 101.47% . The linearity concentration is in the range of 2.750-275.000 for Samidorphan and 4.750-475.000 for Olanzapine and linearity is (r2 = 0.999) . Further, the reported method was validated as per the ICH guidelines and found to be well within the acceptable range. The proposed method is simple, rapid, accurate, precise, and appropriate for pharmacokinetic and therapeutic drug monitoring in the clinical laboratories.

Key words: Samidorphan, Olanzapine, RP-HPLC

INTRODUCTION

Samidorphan is a 17-(Cyclopropylmethyl)-4,14-dihydroxy-6-oxomorphinan-3-carboxamide. Olanzapine is a 2-Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine drug used for the management of schizophrenia and bi polar disorders in patients

LITERATURE REVIEW

Various methods are reported in the literature for the estimation of Samidorphan and Olanzapine in human plasma. According to literature survey there is no method reported for the estimation of Samidorphan and Olanzapine in human plasma by RP-HPLC either in literature.

Materials:

Samidorphan and Olanzapine, Distilled water, Acetonitrile, Ammonium Acetatebuffer, Triethylamine,Orthophosphoric acid All the above chemicals and solvents are from Rankem

Instruments:

s.no	Instrument	Company name	Brand name
1	Electronic balance	Sartorious	Denver
2	pH meter	Metsar	BVK enterprises
3	Sonicator	Lab man	BVK enterprises
4	Centrifuge	Thermo Fisher	-
5	Vertex	Remi CM101	-
6	HPLC water	Alliance	Water HPLC 2695 SYSTEM

Methods:

Buffer: 0.01N Ammonium Acetate

Accurately weighed 0.77gm of **Ammonium Acetate** 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 3.0 with dil. Orthophosphoric acid solution**Mobile phase: A**cetonitrile, and Buffer (30:70)

Preparation of Samidorphan Stock solution (0.11 mg/ML):

Take 11 mg of Samidorphan in 100 ml volumetric flask and make the volume with diluent to produce 0.11 mg/ml

Preparation of Olanzapine Stock solution (0.19 mg/ML):

Take 19 mg of Olanzapine in 100 ml volumetric flask and make the volume with diluent to produce 019 mg/ml

Preparation of Samidorphan Spiking Solutions (2.75ng/ML to 275 ng/ML):

From the above Samidorphan stock solution 0.01ml, 0.02ml, 0.03ml, 0.2ml, 0.5ml, 0.6ml, 0.8ml and 1.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 110 **ng/ML,** 220 **ng/ML,** 330 **ng/ML,** 2200 **ng/ML,** 5500 **ng/ML,** 6600 **ng/ML,** 8800 **ng/ML** and 11000 **ng/ML.**

Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 2.75 ng/ML, 5.5 ng/ML, 8.25 ng/ML, 55 ng/ML, 137.5 ng/ML, 165ng/ML, 220ng/ML and 275 ng/ML.

Preparation of Olanzapine Spiking Solutions (4.75 ng/ML to 475 ng/ML):

From the above Olanzapine stock solution 0.01ml, 0.02ml, 0.03ml, 0.2ml, 0.5ml, 0.6ml, 0.8ml and 1.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 190 **ng/ML,** 380 **ng/ML,** 570 **ng/ML,** 3800 **ng/ML,** 9500 **ng/ML,** 11400 **ng/ML,** 15200 **ng/ML** and 19000**ng/ML.**

Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 4.75 ng/ML, 9.5 ng/ML, 14.25 ng/ML, 95 ng/ML, 237.5 ng/ML, 285 ng/ML, 380 ng/ML and 475 ng/ML.

Preparation of internal standard Solution (675 ng/ml):

Take 10 mg of Dolutegravir in 10 ml volumetric flask and make up the volume with diluent. From that stock solution take 1 ml of solution into 10 ml volumetric flask &make up the volume with diluent, from this solution take 0.3ml of solution into 10ml volumetric flask and make up the volume with diluent to produce 3000 ng/ml solutions.

Finally internal standard sample was prepared by spiking blank plasma to produce 675ng/ml.

(optimized method):

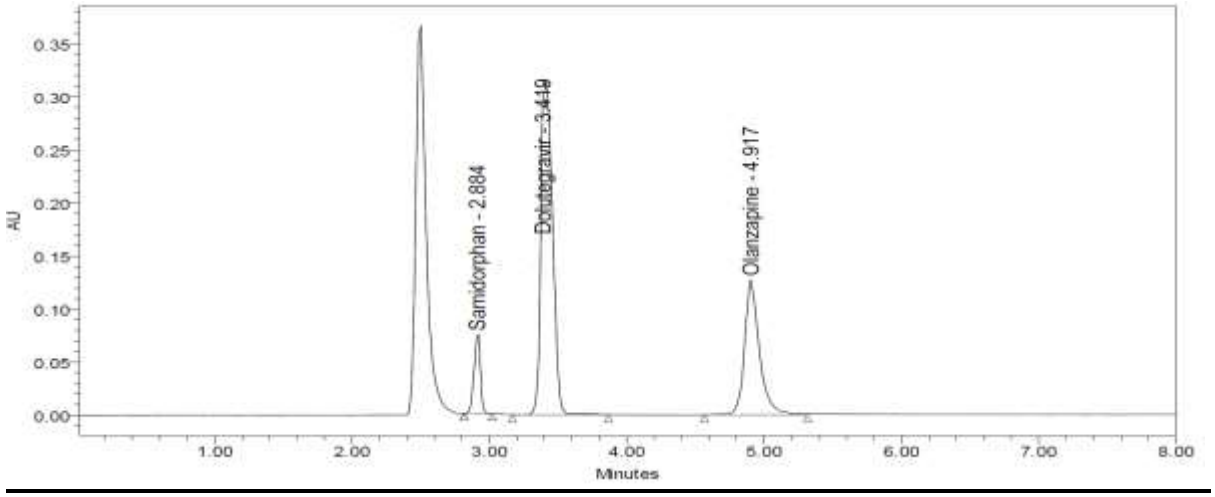
Column	:Inertsil 250 4.6mm, 5µm
Mobile phase composition	: 0.01N Ammonium acetate Buffer : Acetonitrile 60:40
Flow rate	: 1 ml/min
Injection volume	: 10 µl
Run time	: 8min

Detection wavelength : 228nm

Column temperature : 30 ºc

Sample temperature : 5 ºc

Diluent : water : Acetonitrile 50:50



Validation:METHOD VALIDATION

System suitability of

Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area Ratio
MEAN			5.234 6.631		3.595 3.625	0.19683 0.50444
SD			0.0221 0.0344		0.0347 0.0143	0.001367 0.011593
%CV			0.42 0.52		0.96 0.39	0.69 2.30

Table no 1: System Suitability ofSamidorphan and Olanzapine

Matrix factor evalution of Samidorphan and Olanzapine

Acquisition Batch ID		Date	
S. No.	**Plasma Lot No.**	**HQC**	**LQC**
		Nominal Concentration (µg/mL)	
		220.000 380.000	8.250 14.250
		187.000-253.000 323.000-437.000	7.013-9.488 12.113-16.388
		Calculated Concentration (µg/mL)	
n		18	18
Mean		215.9681 381.7575	8.1357 14.2163
SD		3.57836 4.63303	0.15681 0.36203
% CV		**1.66** **1.21**	**1.93** **2.55**
% Mean Accuracy		**98.17** **100.46**	**98.61** **99.76**
No. of QC Failed		**0**	**0**

Table no 2: Matrix factor evaluation of Samidorphan and Olanzapine

2. Linearity of Samidorphan and Olanzapine

Linearity of Samidorphan

Acquisition Batch ID	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	Nominal Concentration (ng/mL)							
	2.750	5.500	8.250	55.000	137.500	165.000	220.000	275.000
	Nominal Concentration Range (ng/mL)							
	(2.200-3.300)	(4.675-6.325)	(7.013-9.488)	(46.750-63.250)	(116.875-158.125)	(140.250-189.750)	(187.000-253.000)	(233.750-316.250)
	Back Calculated Concentration (ng/mL)							
P&A1	2.792	5.386	8.240	54.690	134.750	159.312	216.045	272.429
P&A2	2.781	5.414	8.181	54.711	136.153	164.011	212.501	272.150
P&A3	2.678	5.384	8.257	52.727	134.756	162.610	218.447	276.452
n	3	3	3	3	3	3	3	3
Mean	2.7503	5.3947	8.2260	54.0427	135.2197	161.9777	215.6643	273.6770
SD	0.06288	0.01677	0.03989	1.13945	0.80830	2.41247	2.99122	2.40727
%CV	**2.29**	**0.31**	**0.48**	**2.11**	**0.60**	**1.49**	**1.39**	**0.88**
% Mean Accuracy	**100.01**	**98.08**	**99.71**	**98.26**	**98.34**	**98.17**	**98.03**	**99.52**

Table no 3: Linearity of Samidorphan

Linearity of Olanzapine

Acquisition Batch ID	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	Nominal Concentration (ng/mL)							
	4.750	9.500	14.250	95.000	237.500	285.000	380.000	475.000
	Nominal Concentration Range (ng/mL)							
	(3.800-5.700)	(8.075-10.925)	(12.113-16.388)	(80.750-109.250)	(201.875-273.125)	(242.250-327.750)	(323.000-437.000)	(403.750-546.250)
	Back Calculated Concentration (ng/mL)							
P&A1	4.83	9.56	14.630	95.66	236.77	286.84	380.21	475.56
P&A2	4.82	9.49	14.310	94.36	239.89	285.07	379.20	473.70
P&A3	4.86	9.49	13.980	94.71	231.17	285.25	380.36	476.79
n	3	3	3	3	3	3	3	3
Mean	4.8367	9.5133	14.3067	94.9100	235.9433	285.7200	379.9233	475.3500
SD	0.02082	0.04041	0.32501	0.67268	4.41839	0.97411	0.63090	1.55567
%CV	**0.43**	**0.42**	**2.27**	**0.71**	**1.87**	**0.34**	**0.17**	**0.33**
% Mean Accuracy	**101.82**	**100.14**	**100.40**	**99.91**	**99.34**	**100.25**	**99.98**	**100.07**

Table no 4: Linearity of Olanzapine

Fig no 1: calibration curve of Samidorphan

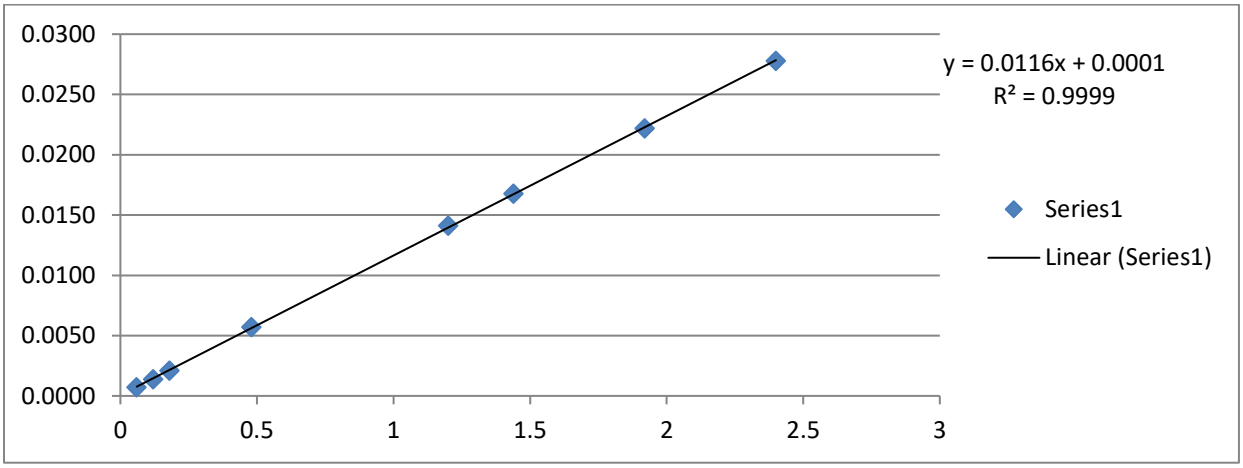
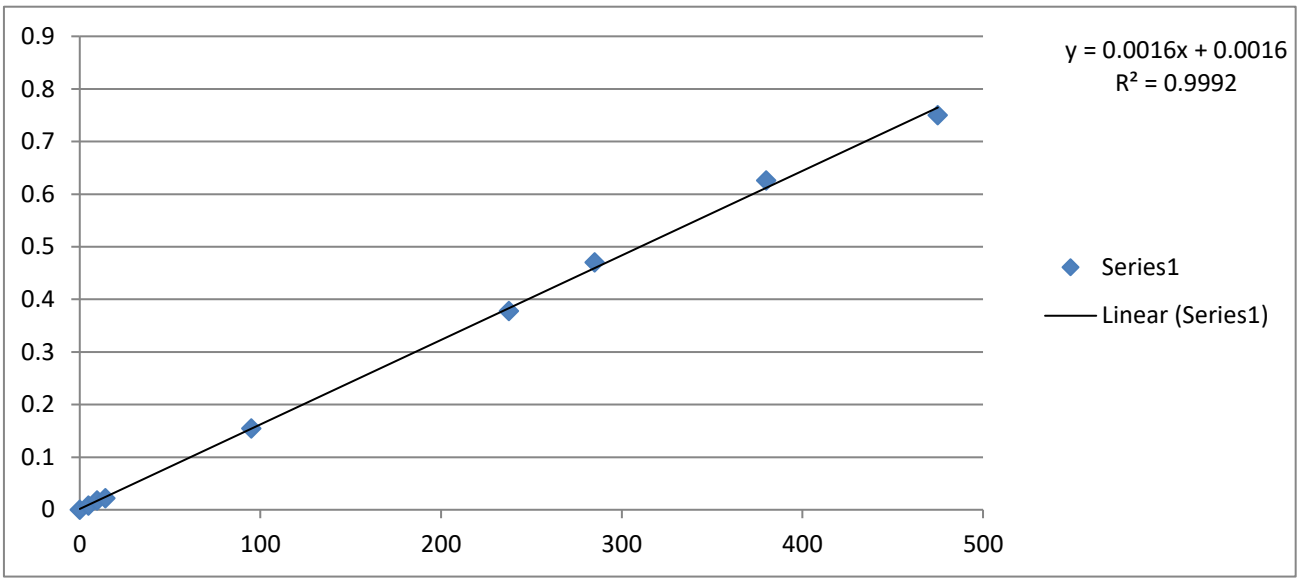


Fig no 1: calibration curve of Olanzapine



3.Precision&Accuracy (intra-day runs ofSamidorphan and Olanzapine)

Acquisition Batch ID	Date	HQC	MQC1	LQC	LLOQ QC
		Nominal Concentration (µg/mL)			
		220.000 380.000	**137.500 237.500**	**8.250 14.250**	**2.750 4.750**
		Nominal Concentration Range (µg/mL)			
		187.000-253.000 323.000-437.000	116.875- 158.125 201.875- 273.125	7.013-9.488 12.113-16.388	2.200-3.300 3.800-5.700
		Back Calculated Concentration (µg/mL)			
n		**6**	**6**	**6**	**6**
Mean		**216.0887 380.0500**	**134.7637 237.9117**	**8.1468 14.2200**	**2.7480 4.8333**
SD		**2.83324 0.98801**	**1.37909 1.37149**	**0.21096 0.64817**	**0.05969 0.17614**

%CV	**1.31** **0.26**	**1.02** **0.58**	**2.59** **4.56**	**2.17** **3.64**
% Mean Accuracy	**98.22** **100.01**	**98.01** **100.17**	**98.75** **99.79**	**99.93** **101.75**
Between Batch Precision and Accuracy				
n	**18**	**18**	**18**	**18**
Mean	**216.0402** **380.1672**	**135.0419** **238.1989**	**8.1536** **14.3072**	**2.7489** **4.8200**
SD	**2.21548** **0.61811**	**1.21511** **1.30376**	**0.14300** **0.48711**	**0.04444** **0.14046**
%CV	**1.03** **0.16**	**0.90** **0.55**	**1.75** **3.40**	**1.62** **2.91**
% Mean Accuracy	**98.20** **100.04**	**98.21** **100.29**	**98.83** **100.40**	**99.96** **101.47**

Table no 4: precision data for intra-day runs of **Samidorphan and Olanzapine**

4.Recovery

Recovery of **Samidorphan and Olanzapine**

Acquisition Batch ID						
Replicate No.	**HQC**		**MQC1**		**LQC**	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
n	6	6	6	6	6	6
Mean	145225 285798	141948 282178	90636 173602	90092 175377	5472 10169	5431 10174
SD	2693.95 2251.13	1305.82 1450.77	783.27 3118.55	404.06 2376.43	40.95 35.26	31.66 25.90
% CV	**1.86** **0.79**	**0.92** **0.51**	**0.86** **1.80**	**0.45** **1.36**	**0.75** **0.35**	**0.58** **0.25**
% Mean Recovery	97.74 98.73		99.40 101.02		99.26 100.05	
Overall % Mean Recovery	98.801 99.934					
Overall SD	0.9185 1.1488					
Overall % CV	**0.93** **1.15**					

Table no 5: Recovery of Dolutegravi

Recovery - Internal standard

Acquisition Batch ID	Date	
S.No.	Un extracted Area Ratio	Extracted Area Ratio
n	6	6
Mean	468166.7	461813.0
SD	4430.31	2344.72
% CV	0.95	0.51
% Mean Recovery	98.64	

Table no 6: Recovery of Lumacaftor (IS)

5. Stabilities

Long term stock solution stability at Zero Samidorphan and Olanzapine

Acquisition Batch ID	Date	
Replicate No.	**HQC**	**LQC**
	Nominal Concentration (µg/mL)	
	220.000 380.000	8.250 14.250
	Nominal Concentration Range (µg/mL)	
	187.000-253.000 323.000-437.000	7.013-9.488 12.113-16.388
	Calculated Concentration (µg/mL)	
n	6	6
Mean	216.1487 380.5250	8.1423 14.3217
SD	2.83580 1.17716	0.11637 0.21414
% CV	**1.31** **0.31**	**1.43** **1.50**
% Mean Accuracy	**98.25** **100.14**	**98.69** **100.50**

Table no 7: Long term stock solution stability at Zero Samidorphan and Olanzapine

CHAPTER-8 : SUMMARY AND CONCLUSION

Parameters	**SAMIDORPHAN**	**OLANZAPINE**	**LIMIT**
Linearity Range(ng/ml)	2.750-275.000ng/ml	4.750-475.000ng/ml	$R^2 < 1$
Regression coefficient	0.999	0.999	
Slope(m)	0.0116	0.0001	
Intercept(c)	0.0001	0.001	
Regression equation (Y=mx+c)	y = 0.0116x + 0.0001	y = 0.001x + 0.001	
Specificity	Specific	Specific	No interference of any peak
System precision %CV	1.31	0.26	NMT 15.0%
Method precision %CV	1.03	0.16	NMT 15.0%
Accuracy %recovery	99.96%	101.47%	80-120%

Table no 1**0: summary for Samidorphan and Olanzapine**

Conclusion

A simple, accurate, precise method was developed for the estimation of the Samidorphan and Olanzapinein human plasma using theDolutegravi as internal standard. Retention time of Samidorphan and Olanzapinewere found to be2.909 min and 3.408min. %CV of Samidorphan and Olanzapinethe was found tobe0.42% and 0.52%. %Recovery was obtained as99.96% and 101.47%. The linearity concentration is in the range of 2.750-275.000 for Samidorphan and 4.750-475.000 for Olanzapine and linearity is (r^2 = 0.999) Further, the reported method was validated as per the ICH guidelines and found to be well within the acceptable range.The proposed method is simple, rapid, accurate, precise, and appropriate for pharmacokinetic and therapeutic drug monitoring in the clinical laboratories.

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