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Analytical Method Development and Validation of Olanzapine and Samidorphan in Pure and Pharmaceutical Dosage Forms by RP-HPLC

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ABSTRACT

A straightforward, rapid, precise, sensitive, and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Olanzapine and Samidorphan in pharmaceutical dosage forms. The chromatographic separation of Olanzapine and Samidorphan was performed using a WATERS HPLC 2695 SYSTEM with Column C18(100 ×4.6 mm, 5µm). The mobile phase comprised Acetonitrile and Mono basic potassium phosphate in a ratio of 50:50% v/v. The flow rate was maintained at 1.0 mL/min, and detection was conducted at 280 nm using a photodiode array detector at ambient temperature. The %RSD (Relative Standard Deviation) was 0.60 for Olanzapine and 0.3 for Samidorphan. Linearity was established by preparing various concentrations ranging from 5 ppm to 30 ppm, yielding regression coefficients of 0.9998 for Olanzapine and 0.9999 for Samidorphan. Precision was confirmed using six dilutions at 20 µg/mL, with a %RSD of 2. Accuracy was assessed at 50%, 100%, and 150% concentration levels, resulting in mean recoveries of 99.83% for Olanzapine and 99.84% for Samidorphan. The limits of detection (LOD) and quantitation (LOQ) were determined to be 0.6 µg/mL and 0.3 µg/mL for Olanzapine, and 2 µg/mL and 1 µg/mL for Samidorphan, respectively. The %RSD of peak areas for all measurements was consistently below 2.0%. The method was validated according to ICH guidelines, including assessment of degradation under thermal, photochemical, and other stress conditions, confirming that the method is simple, cost-effective, precise, accurate, robust, and suitable for the quantitative analysis and stability studies of Olanzapine and Samidorphan.

Key-words: Olanzapine, Samidorphan, Validation, Stability and HPLC

INTRODUCTION:

Chromatographic techniques like HPLC, GC, and HPTLC are commonly used for simultaneously estimating drug combinations due to their high repeatability and accuracy. However, the cost of analysis is significant, driven by the expensive equipment, reagents, and specialized expertise required. To address this, a more cost-effective and simpler method is needed for routine formulation analysis. Spectrophotometric analysis offers an efficient alternative for simultaneous drug estimation with comparable efficacy to chromatographic methods [1-2]. Among these, HPLC is widely favored for analyzing multicomponent dosage forms due to its speed, specificity, accuracy, precision, and ease of automation, eliminating the need for complex extraction and isolation processes. Reversed-phase HPLC (RP-HPLC) is particularly popular, with about 90% of analyses involving low-molecular-weight substances using this method, where a hydrophobic stationary phase and a polar mobile phase are employed, typically with water-based solutions [3]. The high-performance liquid chromatography (HPLC) method is widely recognized for its rapid analysis, superior sensitivity, enhanced resolution, suitability for low volatility substances, and excellent reproducibility. HPLC detectors offer features such as high sensitivity, a broad linear dynamic range, applicability to a wide range of solutes, no contribution to band broadening, non-destructive analysis, and quick response times [4]. The objective of this study was to validate a stability-indicating reverse-phase HPLC method for the simultaneous estimation of Olanzapine and Samidorphan in bulk and pharmaceutical formulations.

Olanzapine:

Mechanism of Action: Olanzapine is an atypical antipsychotic that works by modulating neurotransmitter activity in the brain. It primarily blocks serotonin type 2 (5-HT2A) and dopamine type 2 (D2) receptors. This dual antagonism helps to balance the neurotransmitter activity, which is believed to be disrupted in conditions like schizophrenia and bipolar disorder. Olanzapine also

has affinities for other receptors, including histamine (H1), muscarinic acetylcholine (M1), and alpha-adrenergic receptors, contributing to its therapeutic effects and side effect profile.

Uses: Olanzapine is an atypical antipsychotic medication primarily used to treat schizophrenia and bipolar disorder. It is also sometimes used in combination with other medications to treat depression.

Chemical formula: C17H20N4S

Molecular weight: 312.44 g/mol

IUPAC Name: 2-Methyl-4-(4-methyl-1-piperazinyl)-10Hthieno[2,3b][1,5]benzodiazepine

Samidorphan:

Mechanism of action: Samidorphan is an opioid receptor modulator with antagonist activity at the mu-opioid receptor (MOR) and partial agonist activity at the kappa-opioid receptor (KOR) and delta-opioid receptor (DOR). Its primary action is to block the effects of opioids at the MOR, thereby reducing the risk of opioid abuse and dependence. This makes it useful in combination therapies to mitigate the addictive potential of certain drugs, such as in combination with buprenorphine or as part of the drug ALKS 3831 (a combination of samidorphan and olanzapine).

Uses:

Molecular Weight: 366.44 g/mol

Chemical Formula: C22H30N2O4

IUPAC Name: 17-cyclopropylmethyl-4,5α-epoxy-3,14-dihydroxymorphinan-6β-carboxamide

AIM:

The aim is to design and validate a novel isocratic Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantification of Olanzapine and Samidorphan in tablet formulations.

OBJECTIVE:

- The objective of validation of analytical procedure is to demonstrate that it is suitable for intended purpose.
- To validate the method with a focus on key parameters such as linearity, precision, and accuracy.

MATERIALS AND METHODS:

Materials required Instruments employed:

- Digital balance: LCGC
- pH meter: Digital pH meter digisun model 2001
- Sonicator: Sonica-spinco biotech
- Membrane filter: Nylon membrane filter (0.45µ)
- HPLC : WATERS HPLC 2695 SYSTEM

a. Software used: Empower 2

b. Detector: UV detector

C. Analytical column: Column C18 (100 ×4.6 mm, 5µm)

Tablets brand used: LYBALVI

Chemical Required:

- Acetonitrile: HPLC grade
- Monobasic potassium phosphate: HPLC grade

Optimization of mobile phase:

Separation of both the drugs was tried using the following combination of mobile phases. The table gives the details of the same.

Table no.1 Method development trails

Serial No	Mobile phase	Ratio(v/v)	Elution of peak
1	Acetonitrile : Mono basic potassium phosphate	30: 70	Not proper separation (Theoretical plate LT 2000& Split peak)
2	Acetonitrile : Mono basic potassium phosphate	40:60	Not proper separation (Peak eluted & more noise peak)
3	Acetonitrile : Mono basic potassium phosphate	60:40	Not proper separation (Broad& fronting peak)
4	Acetonitrile : Mono basic potassium phosphate	50:50	Good separation

Out of 4 trails was selected for further studies because when compared to other trails 4 th trails was found less in retention time due to the ratio or organic solvent in mobile phase.

Selection of wavelength:

The solvent used Acetonitrile - Mono basic potassium phosphate in the ratio of 50:50. It was seen that220nm both compounds have very good absorbance, which can be used for the estimation of compounds by HPLC. Selection of Chromatographic Method Proper selection of the method depends on nature of the sample (ionic or ionisable or neutral molecules), its molecular weight, pKa value and stability. The Drugs selected in the present study are polar and so reversed phase or ion exchange chromatography can be used. The reversed phase HPLC was selected for the initial separation because of its simplicity and suitability. From the literature survey and with the knowledge of properties of the selected drugs, Column C18 (100 ×4.6 mm, 5µm) was chosen as stationary phase and mobile phase with different compositions such as Acetonitrile and monobasic potassium phosphate was used. From all the data observed, obtained, available the initial separation conditions were set to work around.

Effect of Ratio of Mobile Phase:

Under the chromatographic conditions mentioned above, the different ratios of mobile phase were tried. The chromatograms were observed for each of the trails, out of which Acetonitrile - Mono basic potassium phosphate in the ratio of 50:50 was selected as the separation was achieved in minimum retention time.

Effect of pH of mobile phase:

Several trials were made using different pH range. The best separation was achieved with pH to 3.0. Effect of flow rate on separation The mobile phase consisting of Acetonitrile : Mono basic potassium phosphate in the ratio of 50:50 and the chromatograms were recorded at flow rates of 1ml to 2ml. The sharp peaks were obtained with 1 ml flow rate.

Effect of column (Stationary phase) on separation:

At the chromatographic conditions of mixed solutions, combinations of Olanzapine and Samidorphan were injected and chromatograms were obtained using C- 18 column, so C-18 was preferred for further studies.

Reference Standards:

Keeping the all above fixed conditions External standard was used.

Optimized Conditions:

The following optimized parameters were used as a final method for the Simultaneous estimation of Olanzapine and Samidorphan.

Table number 2: Optimized Conditions

Instrument	**WATERS HPLC 2695 SYSTEM**
Column	Column C18(100 ×4.6 mm, 5µm)
Column Oven Temperature	30°C
Wave length	220nm
Flow rate:	1ml/min
Injection Volume	10µl
Runtime	6 minutes
Mode of Operation	Isocratic
Mobile Phase	Acetonitrile and Mono basic potassium phosphate
Solvent ratio	50:50

METHOD VALIDATION BY– HPLC SYSTEM

SUITABILITY: System suitability of the method was performed by calculating the parameter namely, resolution, tailing and number of theoretical plates on the five replicate injection of standard solution.

Table No.3: system suitability date

System suitability Parameters	**Olanzapine**	**Samidorphan**
Resolution	7.68	
Tailing factor	1.21	1.16
No. of Theoretical Plates	2085	3852

Acceptance criteria:

- Resolution should be NLT 2.
- Tailing factor should be NMT 2.
- No. of theoretical plates should be NLT 2000.

The system suitability parameter and % RSD for peak areas for six replicate injection of standard solution was found to be within limits.

QUANTITATIVE ESTIMATION:

Assay preparation: Pipette out 5 ml from sample stock solution into 50 ml volumetric flask and made up to the volume of 25 ml with mobile phase. Inject the replicate six preparations and record the peak area response and calculate.

Table no.4: Assay preparation

Sample	Label claim	Peak area*	Amount obtained*	Percent label claim% w/w*	SD	%RSD
Olanzapine	20 mg	2534187	19.87	99.49%	0.26	0.3
Samidorphan	10mg	1319542	10.02	100.2%	0.4	0.4

LINEARITY:

Appropriate aliquots of two drug combination were pipette out from the stock solution into a series of 50ml volumetric flaks. The volume was made up the mark with HPLC water to obtain a concentration of Olanzapine and Samidorphan (25%, 50%, 75%, 100%, 125% and 150%µg/ml). Inject 10µl of each concentration into HPLC system and chromatographed under the optimized conditions. Evaluation was performed with the UV detector set at 220 nm and the peak areas were recorded.

Procedure for preparation of standard stock solution:

Weigh and transfer accurately 7.5 mg of Olanzapine working standard and 5 mg of Samidorphan standard into 50 ml of standard measuring flask with 25ml of mobile phase, sonicate for 15 min, cooled to room temperature and diluted to 50ml with diluents.

OLANZAPINE:

Table no.5: Linearity of Olanzapine

SI.No	Levels	Conc. (µg/ml)	Area of Olanzapine
1	Level 1(25%)	3.75	283740
2	Level 2(50%)	7.5	553176
3	Level 3(75%)	11.25	834297
4	Level 4(100%)	15	1103683
5	Level 5(125%)	18.75	1363641
6	Level 6(150%)	22.5	1631183
SD			195146
Intercept			9546
Slope			72418
Correlation coefficient			0.9998

Acceptance criteria:

The correlation coefficient should be NLT 0.9998 for Olanzapine

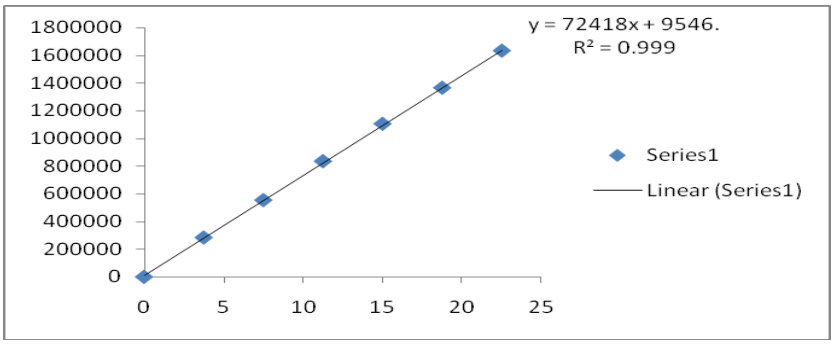

Figure number 1: Linearity curve of Olanzapine

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LINEARITY OF OLANZAPINE:

SAMIDORPHAN:

Table no.6: Linearity of Samidorphan

SI.No	Levels	Conc (µg/ml)	Area of Olanzapine
1	Level 1(25%)	2.5	171860
2	Level 2(50%)	5	334164
3	Level 3(75%)	7.5	495276
4	Level 4(100%)	10	665790
5	Level 5(125%)	12.5	827592
6	Level 6(150%)	15	991434
SD			296764
Intercept			3295.9
Slope			65963
Correlation coefficient			0.9999

Acceptance criteria:

The correlation coefficient should be NLT 0.9999 for Olanzapine

LINEARITY OF SAMIDORPHAN

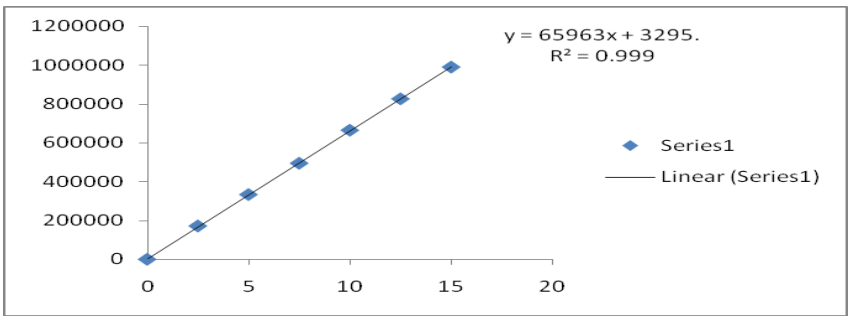

Figure number 2: Linearity curve of Samidorphan

PRECISION:

System precision:

System precision was done by using Olanzapine and Samidorphan combination. prepared six times and injected into the HPLC system under the optimized conditions.

Table no.7: System precision data

SI.No	Area of Olanzapine	Area of Samidorphan
1	178876	349453
2	177224	347162
3	179055	349458
4	178739	348377
5	176699	348482
6	179220	349771
Mean	178302.2	348783.8
S.D	1064.1	976.1
%RSD	0.6	0.3

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Acceptance criteria:

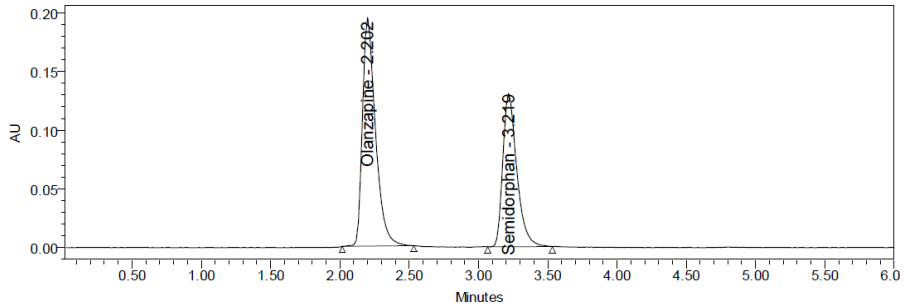
The % RSD should be NMT 2.

Standard Chromatogram:

Chromatogram 1 :

chromatogram

OLANZAPINE + SAMIDORPHAN

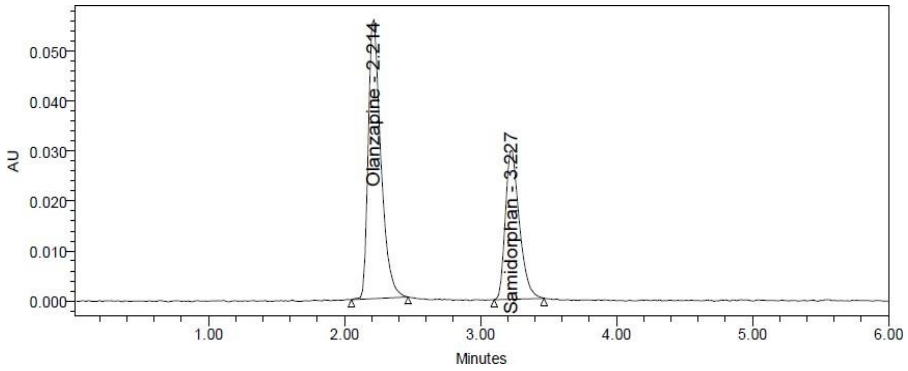


Peak Table

Peak #	Ret.Time	Area	% Area	Therotical.plate	Resolution	Taling.factor
OZP	2.202	1668462	40.500	2085	0.00	1.21
SMP	3.219	1995210	50.300	3852	7.68	1.61
Total		3763672	100.000			

Chromatogram 2 :

OLANZAPINE + SAMIDORPHAN



Peak Table

Peak #	Ret.Time	Area	% Area	Therotical.plate	Resolution	Taling.factor
OZP	2.214	283740	35.500	2085	0.00	1.21
SMP	3.227	835558	64.500	3852	7.68	1.61
Total		1119298	100.000			

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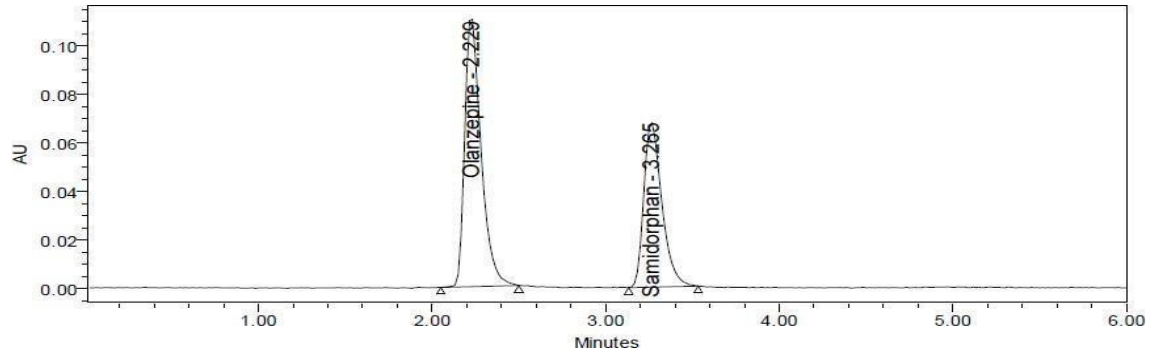
Chromatogram 3 :

OLANZAPINE + SAMIDORPHAN

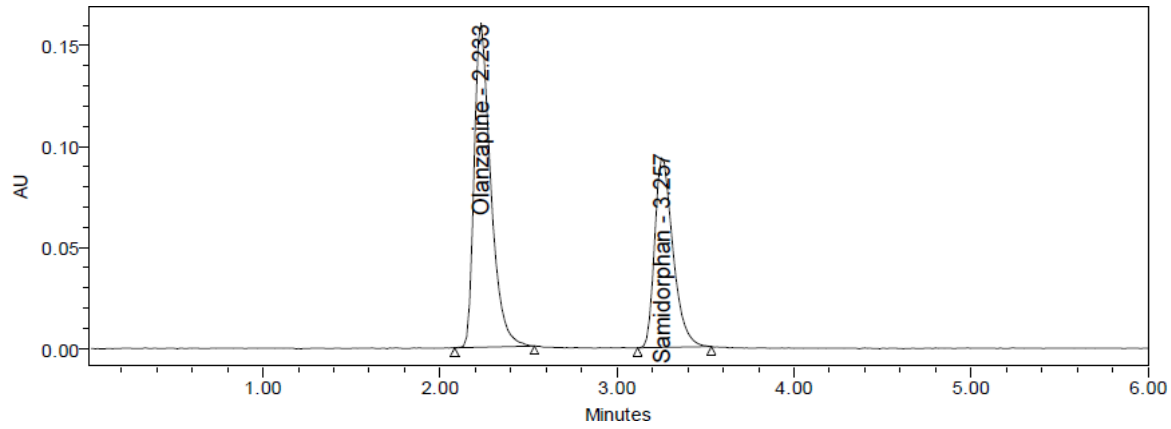


Peak Table

Peak #	Ret.Time	Area	% Area	Therotical.plate	Resolution	Taling.factor
OZP	2.229	208469	35.000	2085	0.00	1.21
SMP	3.265	725182	65.000	3852	7.68	1.61
Total		933651	100.000			

Chromatogram 4 :

OLANZAPINE + SAMIDORPHAN



Peak Table

Peak #	Ret.Time	Area	% Area	Therotical.plate	Resolution	Taling.factor
OZP	2.233	254882	45.700	2085	0.00	1.21
SMP	3.257	131921	54.300	3852	7.68	1.61
Total		386804	100.000			

SYSTEM PRECISION:

Peak #	Ret.Time	Area	% Area	Therotical.plate	Resolution	Taling.factor
OZP	2.207	178739	41.14	2085	0.00	1.21
SMP	3.200	348377	58.86	3852	7.68	1.61
Total		527116	100.000			

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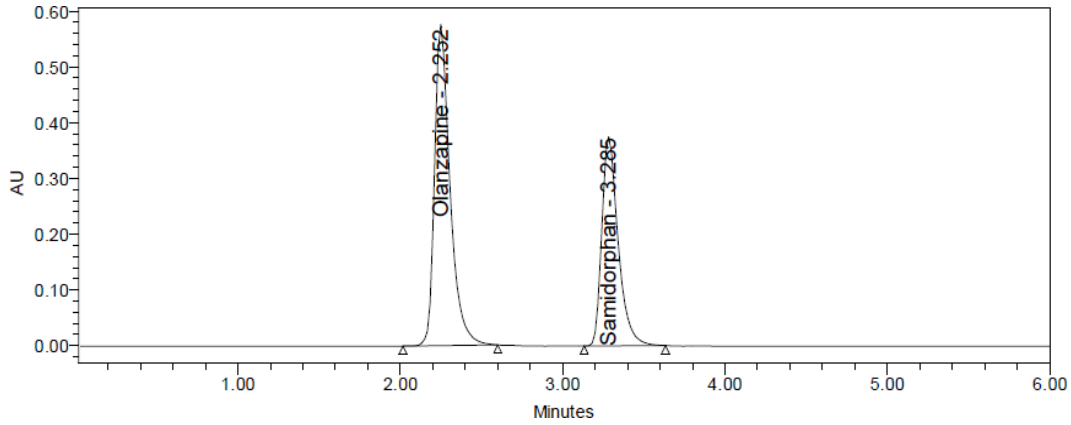
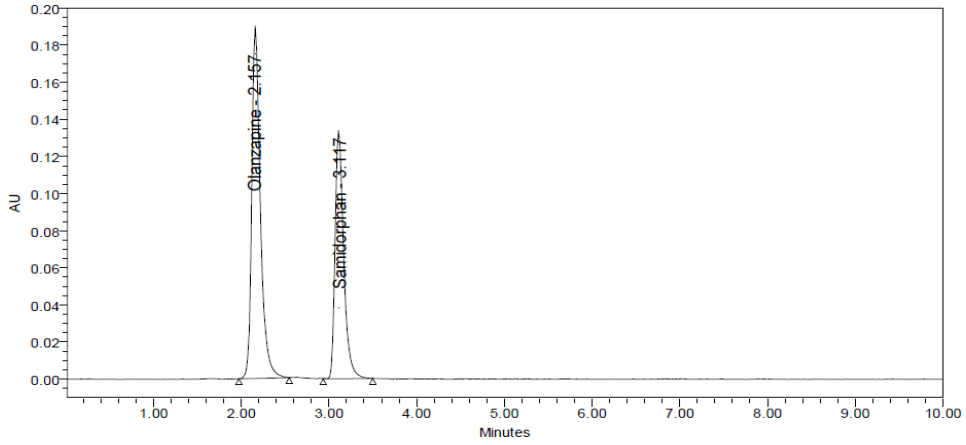
LIMIT OF DETECTION:

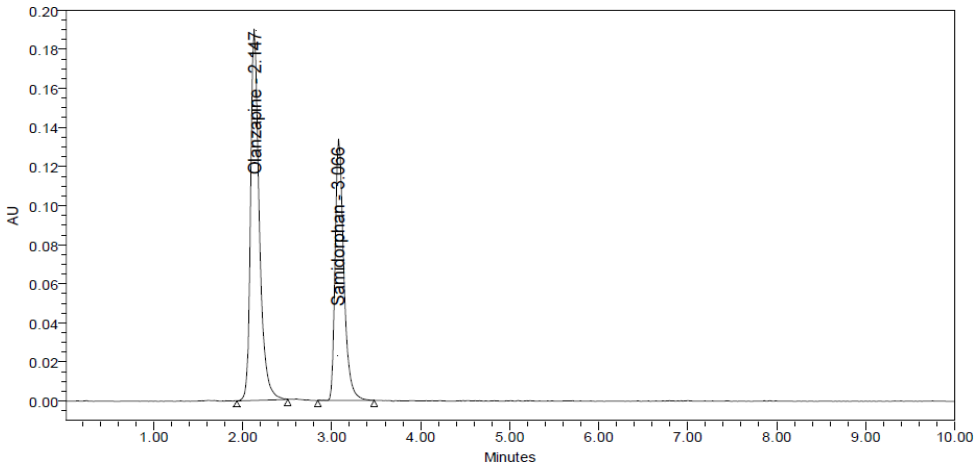


FORCED DEGRADATION:

Acid stress chromatogram

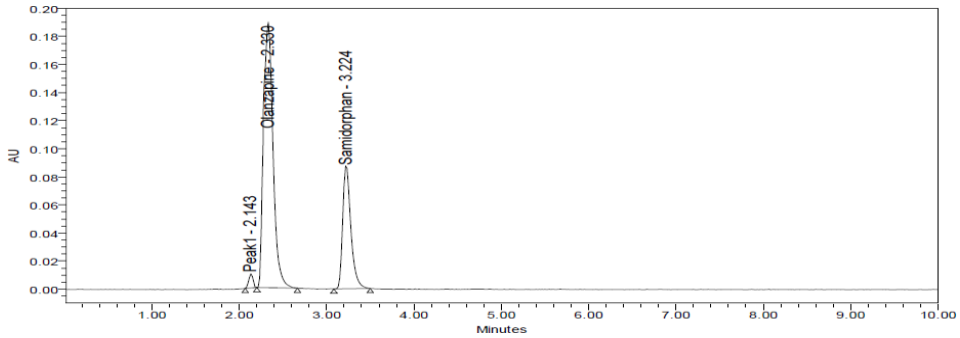


Alkaline stress chromatogram

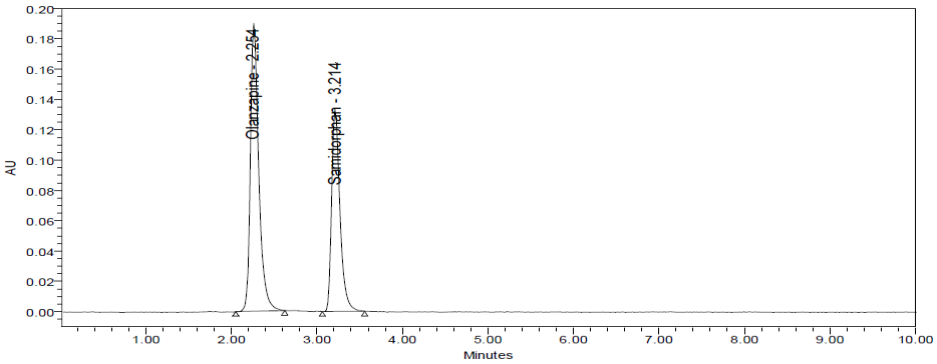


1 Det.A Ch1/220nm

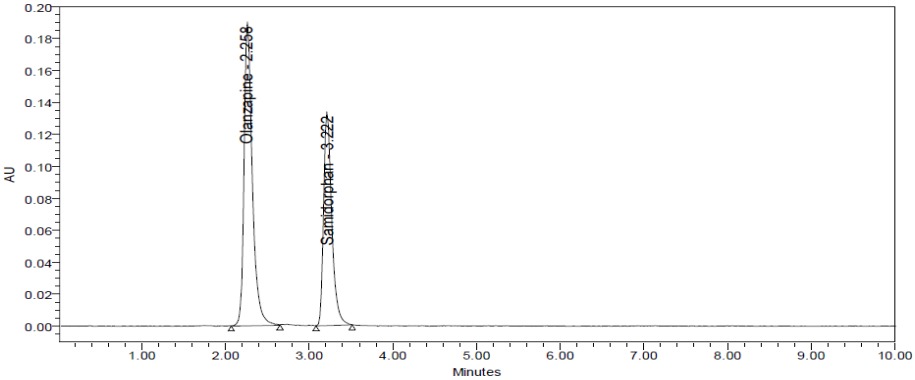
Oxidative stress chromatogram



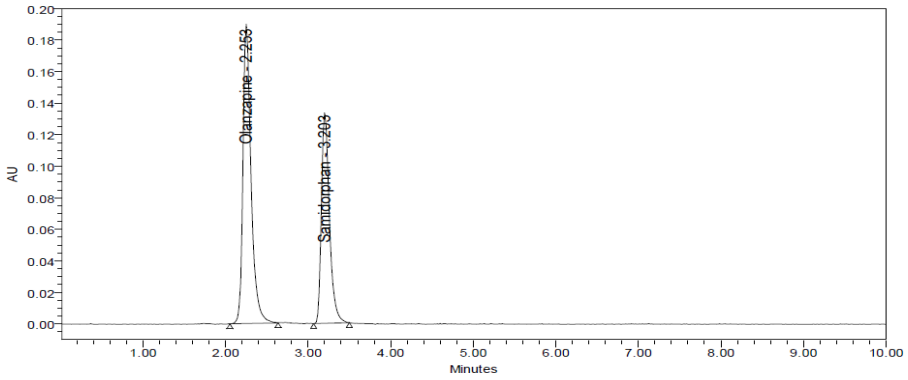
Dry heat stress chromatogram



Photolytic stress chromatogram



Water stress chromatogram



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RESULTS AND DISCUSSION:

A reverse phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the analysis of Olanzapine and Samidorphan in pharmaceutical formulations. The separation was achieved using a mobile phase composed of Acetonitrile and Monobasic Potassium Phosphate in a 50:50 ratio, adjusted to pH 3. Detection was performed with a UV-Visible detector set at 220 nm.

Chromatographic separation was carried out on a C18 column (100 × 4.6 mm, 5 µm) with a flow rate of 1.0 ml/min. The retention times for Olanzapine and Samidorphan were approximately 2.2 minutes, with asymmetry factors (tailing factors) of 1.21 and 1.16, respectively, indicating symmetrical peak shapes. The number of theoretical plates for Olanzapine and Samidorphan was found to be 2085 and 3852, respectively, demonstrating the column's efficiency.

These parameters confirm the specificity of the method. Linearity studies showed that Olanzapine and Samidorphan were linear over a concentration range of 2.5 to 23 µg/ml, corresponding to 25% to 150% of the target concentration, with correlation coefficients of 0.9998 and 0.9999, respectively. Method validation was further confirmed by system precision and method precision tests using RP-HPLC, along with recovery studies. The percentage recovery was found to be within acceptable limits. Limits of Detection (LOD) and Limits of Quantification (LOQ) were also established. Robustness was evaluated by varying flow rate and temperature, and the method was further validated through forced degradation studies, which also yielded satisfactory results.

The method met all the criteria specified by ICH guidelines, ensuring its reliability and accuracy for the intended analytical purposes.

S. no	Parameters	Limit	Observation		Result
			OZP	SMP	
1.	System suitability	Resolution NLT 2	7.68		Passes
		Tailing factor NMT 2.0	1.21	1.16	
2.	Precision	%RSD NMT 2.0			
	System precision		0.61	0.30	Passes
	Intermediate precision		1.0	0.3	Passes
3.	Linearity	Correlation coefficient(R^2)NLT0.99	0.9998	0.9999	Passes
4.	Recovery	98.0% to 102.0%	99.83%	99.84%	Passes
5.	Robustness	%RSD NMT 2.0			
	Change in flow rate -0.2 ml +0.2 ml		0.20 0.30	1.83 0.05	Passes
	Change in temperature -0.2 +0.2		0.17 0.18	0.15 0.036	Passes
6.	Limit On Detection		0.6 mcg/ml	0.3 mcg/ml	Passes
7.	Limit On Quantification		2 mcg/ml	1 mcg/ml	Passes

CONCLUSION:

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the analysis of Olanzapine and Samidorphan in both pure and tablet dosage forms, adhering to ICH guidelines. The method utilized a UV-visible detector and a C18 column (100 × 4.6 mm, 5µ). A 10 µl sample was injected and eluted using a mobile phase consisting of Acetonitrile and monobasic potassium phosphate in a 50:50 ratio, with a flow rate of 1.0 ml/min at a detection wavelength of 220 nm. The peaks of Olanzapine and Samidorphan were well separated within 6 minutes. The method was validated according to ICH guidelines, evaluating parameters such as system suitability, linearity, system precision, intermediate precision, recovery, robustness, limits of detection (LOD), limits of quantification (LOQ), and forced degradation studies. The validation results demonstrated that the RP-HPLC method is reliable and can be effectively employed for routine pharmaceutical analysis of Olanzapine and Samidorphan.

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Conflict of Interest Statement: All authors have nothing else to disclose.