

## A Review on Various HPLC Methods for Estimation of Olanzapine and Samidorphan

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### ABSTRACT

Olanzapine is an anti-psychotic agent, which works on dopamine D2 receptors in the mesolimbic pathway as an antagonist, blocking dopamine from potential action at the post synaptic receptor. Samidorphan is an opioid antagonist, it act as partial agonist at k-opioid receptors. High performance liquid chromatography is analytical chemistry technique applied to separate identify and quantify semi and non-volatile compounds in liquid samples. This technique involves two phases stationary and mobile phase. The method was validated for precision recovery, robustness, specificity, detection and quantification limits. Liquid chromatography is a popular analytic technique used for separation, identification and quantification of each constituent of mixture. This articles about the strategies and the issues pertinent to designing HPLC method development and validation. The test results are directly proportional to the concentrate analyte. The accuracy results are from 98-102%. The percentage RSD values are less than 2 in the review articles of Olanzapine and Samidorphan.

**KEYWORDS:** HPLC, Method development, Method validation, Olanzapine and Samidorphan.

### I. INTRODUCTION

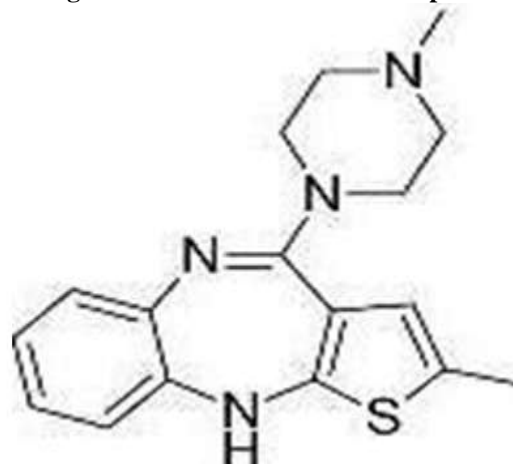
Pharmaceutical analysis is the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. This is mainly used for the separation of components from the mixture and determine structure of compounds. It involves assessing the quality and purity of drugs. Techniques like chromatography and spectroscopy are commonly used to analyze substance in pharmaceutical formulations. It ensures products meet regulatory standards and are safe for consumption. It plays a crucial role in research, manufacturing within the pharmaceutical industry. It includes various

methods like HPLC, mass spectrometry etc<sup>[1]</sup>.

### OLANZAPINE:

Olanzapine is an a typical anti psychotic medication, commonly prescribed for conditions like schizophrenia and bipolar disorder. It belongs to the thienobenzodiazepine class and is known for its efficacy in managing symptoms such as hallucinations, delusions and mood fluctuations. Involves antagonizing dopamine and serotonin receptors in the brain. Specifically, it blocks dopamine receptors [D2 type] and serotonin receptors [5-HT<sub>2A</sub> and 5-HT<sub>2C</sub>], leading to a balancing effect on neurotransmitter activity. This modulation helps alleviate symptoms of psychosis, schizophrenia and bipolar disorder. Olanzapine is primarily used to treat various mental conditions including schizophrenia, bipolar disorder, agitation in dementia and resistant depression<sup>[2]</sup>.

Fig1. chemical structure of Olanzapine



IUPAC Name: 2-Methyl-4-[4-methyl-1-piperazinyl]-10H-thieno[2,3b][1,5]benzodiazepine.

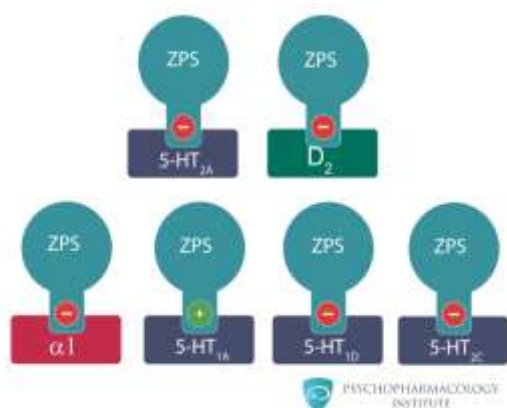


Fig2.Mechanism action of Olanzapine

### SAMIDORPHAN:

It is indicated in combination with olanzapine for the treatment of bipolar disorder either as an adjunct to lithium or valproate or as mono therapy for the acute treatment of manic or mixed episodes or as maintenance therapy, and for the treatment of schizophrenia in adults<sup>[3]</sup>.

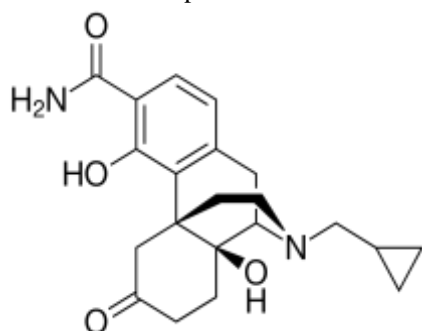


Fig .chemical structure of Samidorphan

IUPAC name: 17-(Cyclopropyl methyl)-4,14-dihydroxy-6-oxomorphenon-3-carboxamide. Samidorphan, 3-carboxamide -4-hydroxy naltrexone, is a relatively new opioid system modulator that as a high' affinity for binding with  $\mu$ -opioid, K-opioid, and  $\delta$ -opioid receptors while

acting as an antagonist at  $\gamma$ -opioid receptors. It act as a partial agonist at  $\kappa$ - opioid and  $\sigma$ -opioid receptors.[6] It is used to treat mental/mood conditions. This product can help to decrease hallucinations and help you to think more clearly and positively about yourself, feel less agitated, and take a more active part in every day life<sup>[4]</sup>.

### II. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY(HPLC):

HPLC is a type of column chromatography commonly used for the separation, identification and quantification of active compounds in analytical chemistry .HPLC typically uses a column that transfers the mobile phase (molecules) through the column and a detector that displays the retention time of the molecules. The retention time depends on the interactions of the stationary phase with analyte, and the solvents used. The sample is introduced in small volumes to the stream of the mobile phase<sup>[5]</sup>.

### PRINCIPLE:

This principle based on the process involves injecting a solution of sample into a column of porous material and pumping a liquid through the column at High pressure. The separation of sample is determined by the migration rates through the column resulting from the different partitioning of the sample from stationary phase to the mobile phase, depending on the partition behavior of the different components, elution occurs at different time.

It is well known that the resolution of chromatographic columns increases with the length of the column and the theoretical number of units per unit length, although there are limitations to the column length due to the issue of peak expansion. The smaller the size of the particles in the stationary phase, the higher the resolution<sup>[6]</sup>.

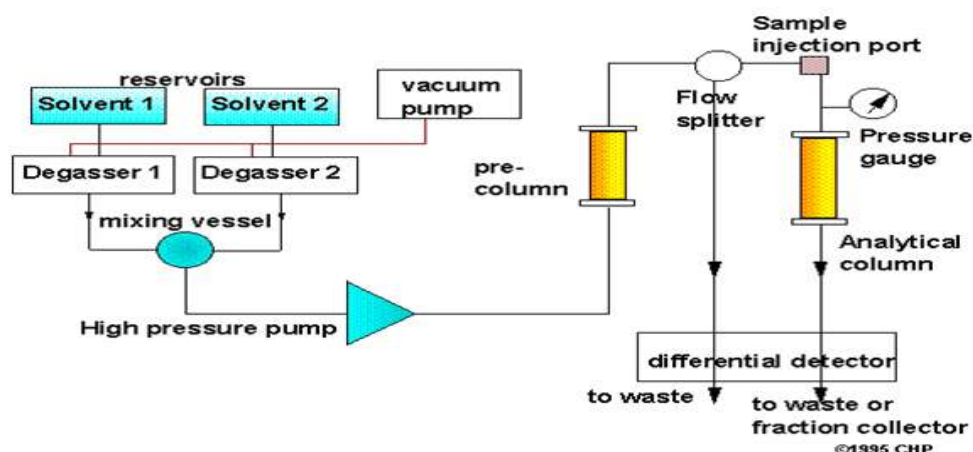


Fig4.Schematic representation of HPLC

**INSTRUMENTATION:**

HPLC essentially comprises of the following main components namely.

- 1.Solvent reservoir and mixing system
- 2.High pressure pumps
- 3.Sample inlet pump
- 4.Columns
- 5.Detectors
- 6.recorder

**Solvent reservoir:** The reservoir holds the solvent , this solvent is referred to as the mobile phase because it moves. Usually, there are a minimum of two reservoir in the system, in there each reservoir holds up to 1000cc of solvent and it is usually fitted with a gas diffuser through which helium can be bubbled. In there the mixture is dissolved in a fluid solvent known as the mobile phase, which carries through a system (a column, a capillary tube, a plate, or a sheet) which material known as the stationary phase is fixed<sup>[7]</sup>.

The mixing system is responsible for accurately mixing the solvent there by creating the desired mobile phase composition. The precision of the mixing is crucial for achieving reproducible and reliable chromatographic results<sup>[8]</sup>.

**High pressure pumps:** A pump suction the versatile stage from the dissolvable reservoir and drives it through the frameworks column and detector. Normal flow rate of in HPLC is in the 1-2 mL/min range. During the chromatographic process, a pump can provides either a stable mobile phase composition<sup>[10]</sup>.

**Types of pumps:**

- 1.Constant flow reciprocating pump

- 2.Syringe type pump
- 3.Pneumatic pump

**Sample inlet pump:** The liquid sample is inserted into a stream of solvent(mobile phase)flowing through the column packed with a separation medium(stationary phase).The sample components are separated from one another by the process of differential migration as they flow through the column. And the injector will be placed next to the pump<sup>[11]</sup>.

**Column:** This is the most important part of the HPLC system, the column produces a separation of analytes in the mixture.A column is where the mobile phase interacts with the stationary phase to form an interface with the large surface area, most of the development of chromatography in recent went towards the design of many different ways to enhance this interfacial contact. The column length varies from 5cm-30cm,The column diameter ranges from 2mm-50mm<sup>[12]</sup>.

**Detectors:** The chromatographic detectors is a device used in liquid chromatography to analyse components of a mixture being eluted off the chromatography column. Normally there are two types detectors these are the first one destructive and second one non-destructive. The universal detector is defined as the one which can respond to every component in the column effluent except the mobile phase 2.In a contrast, selective detectors are defined as detectors which respond to a related group of sample in the column effluent<sup>[13]</sup>.

**Recorder:**The signal emerging from the detector of a HPLC is recorded continuously as function of time most commonly with the help of a potentiometric recorder.

### TYPES OF CHROMATOGRAPHY:

**Normal phase chromatography:** It involves the retention of the sample components on the stationary phase. This is due to the interaction between permanent dipoles on one component and permanent dipoles on a stationary phase this adsorption mechanism results in the formation of a general class of chromatographic adsorption methods.

**Reverse phase chromatography:** In this type the stationary phase of RP - HPLC is a non-polar phase of HPLC, while the mobile phase is an aqueous and slightly polar phase. As a consequence of the repulsive interaction between a polar solvent, a relatively non- polar solvent, and the non- polar stationary analyte, RP - HPLC works on the hydrophobic interaction theory<sup>[14]</sup>.

### III. METHOD DEVELOPMENT

Method development refers to the process of determining whether an analytical method can be used to measure the active ingredient concentration a particular compounded

dosage, so that a simplified approach can be used to determine if an analysis procedure will reliable and consistently measure the active ingredient concentration of compounded preparation. The primary goal of this study is to create and validate a simple, accurate, reliable, efficient, faster, more precise and least time consuming HPLC method for the co-administration of Olanzapine and Samidorphan in API and various formulation according to ICH guidelines<sup>[15]</sup>. The analytical method development should consist of

1. It is a technique in analytical chemistry used to separate the components in a mixture to identify each component and to quantify each component.
2. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material.
3. The various components in the sample exhibit distinct interaction with the adsorbent material, resulting in varying flow rates. Consequently these components pass through the column they undergo separation<sup>[16]</sup>.

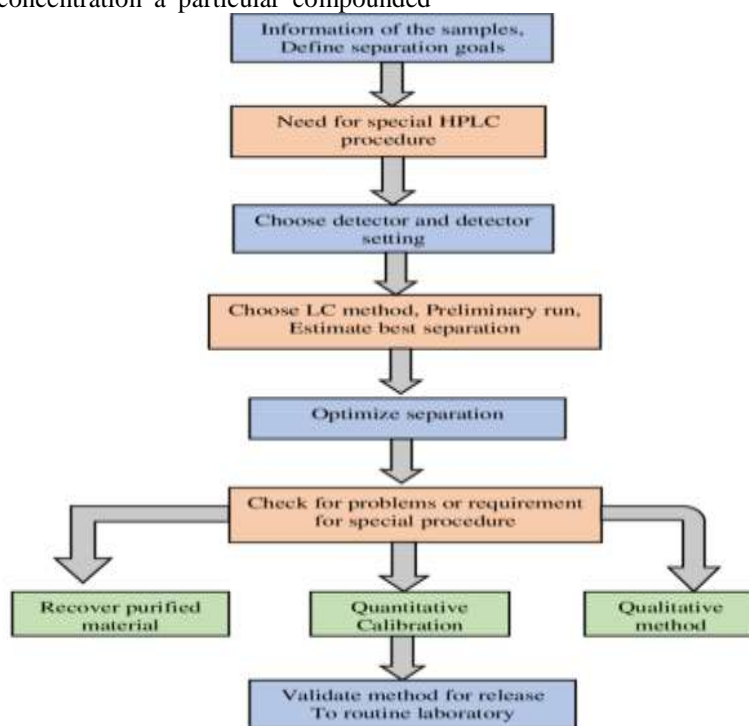


Fig 5. Method development of HPLC

### IV. METHOD VALIDATION

Method validation is the process of proving that an analytical method is acceptable for its intended purpose.

Steps in method validation

1. Develop a validation protocol or operating procedure for the method of validation
2. Define the application, purpose and scope of the method
3. Define performance parameters and acceptance

- criteria
4. Define validation experiments
  5. Verify relevant performance characteristics of equipment
  6. Qualify materials, eg. standards and reagents
  7. Perform pre-validation experiments
  8. Adjust method parameters and acceptance criteria
  9. Perform full validation experiments
  10. Develop SOPs for executing method in nature
  11. Define criteria for revalidation
  12. Define type and frequency of system suitability tests for routine
  13. Document validation experiments and results of validation<sup>[17]</sup>.

#### COMPONENTS OF METHOD VALIDATION:

Linearity  
Specificity  
Accuracy  
Precision  
Limits of detection  
Limits of quantification  
Robustness  
Range  
System suitability

**Linearity:** Linearity of a method is its ability to obtain test results that are directly proportional to sample concentration over a given range. It is evaluated by visual inspection of a graph plot of signal as a function of analyte concentration<sup>[18]</sup>.

**Specificity:** It is the study of the chromatography which is performed by the separation of the analyte from the other potential components such as impurities, degradation or excipients. In addition forced degradation studies are carried out to challenge the method<sup>[19]</sup>.

**Accuracy:** The accuracy of an analytical procedure express the degree of agreement between the value acknowledged as a conventional true value and the value discovered. The accuracy is defined as closeness of a measured value to the true value or accepted value.

**Precision:** Precision of an analytical method expresses closeness agreement between a series of

measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

It includes three levels

1. Repeatability
2. Intermediate precision
3. Reproducibility

#### Limits of Detection

The limit of detection of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantified as an exact value.

The detection limit expressed as ;

$$LOD=8.8\sigma/S$$

Where S=slope of the calibration curve  
 $\sigma$  =standard deviation if the response<sup>[20]</sup>.

#### Limit of quantification:

The quantification limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. Based on the standard deviation of the response and slope it is calculated by the formula;

$$LOQ=10\sigma/S$$

Where  $\sigma$  =standard deviation of the response  
S=slope of the calibration curve

**Robustness:** The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

**Range:** The method range is the interval between an analyte upper and lower levels obtained with appropriate precision, accuracy, and linearity<sup>[21]</sup>.

**System suitability:** It is an integral part of many analytical procedures the tests are based on the concept that the equipment analytical operation and samples to be analyzed constitute an integral system that can be evaluated. The process involves making five injections of a standard solutions and evaluating several chromatographic parameters such as resolution, area % reproducibility, number of theoretical plates and tailing factor.

**Table: Literature review of Olanzapine:**

S.no	Drug name	Mobile Phase	Column	RT	Results	Reference
01.	Olanzapine	Methanol(70.30v/v)	Intersil C18	3.447 min	Linearity:0.9999 Accuracy:99.5% precision:<2%	Anna Krebs, BarbaraStarzewski2006
02.	Olanzapine	Acetonitrile(35:65v/v)	Inert sil ODS3v		Linearity:0.9999 accuracy:0.87-2.800 precision:0.15-0.46%	K. Basaraiah N.Rajendraprasad 2014 <sup>[22]</sup> .

**Table Literature review combination of Olanzapine and samidorphan:**

S.no	Drug Name	Mobile Phase	Column	Retention Time	Results	Reference
01.	Olanzapine and Samidorphan	Acetonitril(60;40)	Symmetry c18	Olanzapine-4.63 mins	Linearity; Olanzapine 2-30µg/ml Samidorphan;1-15µg/ml Accuracy; Olanzapine;99.5-99.9% Samidorphan;98-100% Precision;<2%	Syed Rafi KantiPudi Rambabu2021
02.	Olanzapine and Samidorphan	Ammonium formate/Formic acid(20:80v/v)	Water alliance 2995	Olanzapine;2.054 mins Samidorphan; 3.940mins	Linearity; 5-30µg/ml Samidorphan; 2.50-15µg/ml Accuracy; Olanzapine; 100.6-100.7% Samidorphan; 100.1-101.0% Precision; Olanzapine; 2.050% Samidorphan; 3.942%	Dr.Sakinala Padmavathi, Gassisaisn Laxshmi, k.Sai durga bhavani 2022 <sup>[23]</sup> .
03.	Olanzapine and Samidorphan	Acetonitril/ 0.1% ortho Phosphoric acid(50;50v/v)	Inertisil ODS column		Linearity; Olanzapine: 15-225µg/ml Samidorphan: 4-60µg/ml Accuracy; Olanzapine; 99-101.2% Samidorphan: 100.1-100.2% Precision;<2%	Ibrahim Baje Syed,Madhavi Nannapaneni 2022
04.	Olanzapine and	Buffer 0.1N Sodium	Intersil	Olanzapine; 2.235mins	Linearity; Olanzapine:	Kethavi Raja, Priya

	Samidorphan	hydrogen phosphate		Samidorphan; 2.785mins	0.9999 Samidorphan: 0.9996 Accuracy; Olanzapine: 99.49% Samidorphan; 99.49% Precision; Olanzapine: 0.3% Samidorphan: 0.4%	saha,Shobha Rani 2022
05.	Olanzapine and Samidorphan	Buiffer ortho Phophoric acid 40%,60% Methanol	Vterra 4.6* 150nm	Olanzapine: 2.05mins Samidorphan: 3.06mins	Linearity; Olanzapine: 0.9999 Samidorphan: 0.9999 Precision; Olanzapine: 0.6µg/ml Samidorphan: 0.3µg/ml Accuracy; Olanzapine: 98.9%-100.1% Samidorphan: 99.5%-101.5%	Shaik Harun Raseed, Ch.Pavani, P.Pranaya, Md.Abdul Rafay,S.Pravee na. <sup>[23]</sup>

## V. CONCLUSION

This review on HPLC method for estimating Olanzapine and Samidorphan suggests that a reliable technique for accurate quantification of the compounds. The method development for quantitative analysis of Olanzapine and Samidorphan in pharmaceutical formulations is precise and accurate. The accuracy, precision, linearity, LOD, LOQ, Repeatability were verified using this technique. The RSD values for all the parameters were found to be less than 2%, which indicates the validity of the method and results obtained by this method. Hence these can be used for routine analysis for Olanzapine and Samidorphan.

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