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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF OLANZAPINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Background: Spectrophotometric analysis fulfils requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods. Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is advisable to develop simpler and cost-effective method for simultaneous estimation of drugs for routine analysis of formulation.

Methods: A descriptive study design was used and information was obtained through various literature reviews. RP-HPLC method was used and data was analyzed.

Conclusion: The developed stability indicating HPLC method for quantitative estimation of olanzapine in bulk and pharmaceutical dosage forms is fast, simple, accurate, and more precise. Validation of this method was accomplished, getting results meeting all requirements. Thus, the developed HPLC method can be used for routine quality control test.

Keywords: Chromatography, RP-HPLC, Spectrophotometric, Simultaneous estimation.

INTRODUCTION

Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is advisable to develop simpler and cost-effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods [1].

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for the quantitation of the drug in biological fluids may not be available.
- Analytical methods for a drug in combination with other drugs may not be available.
- The existing analytical procedures may require expensive reagents and solvents. It may also grip cumbersome extraction and separation procedures and these may not be reliable [1].

THEORY OF SPECTROPHOTOMETRY [1]

Lambert's Law

when a beam of light is allowed to pass through a transparent medium the rate of decrease of intensity with the thickness is directly proportional to the intensity of light of incident light.

Beer's law

The intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substance. The two separate laws prevailing absorption are

usually known as Lambert's law & Beer's law, Mathematically, the radiation concentration and radiation path length relation can be expressed:

$$A = -\log T = -\log (I_t / I_0) = \log (I_0 / I_t) = \varepsilon .b .c$$

Where I_0 = incident light intensity, A = absorbance

b= cell path length in cm

c= solution concentration in M/L, and ε = molar absorptivity

INSTRUMENTATION

It consists of a dual light source viz., tungsten lamp for visible range and deuterium lamp for ultraviolet region, grating monochromatic, photo-detector, mirrors and glass or quartz cells. For measurements to be made under visible region both glass and quartz cells can be used. For the measurements under ultraviolet region, only quartz cell should be used, since, glass cells absorb ultraviolet rays.

There are two types of instrumental designs for this technique as single beam and double beam spectrophotometers. However double beam spectrophotometers are generally used and following is the sketch out of the instruments.

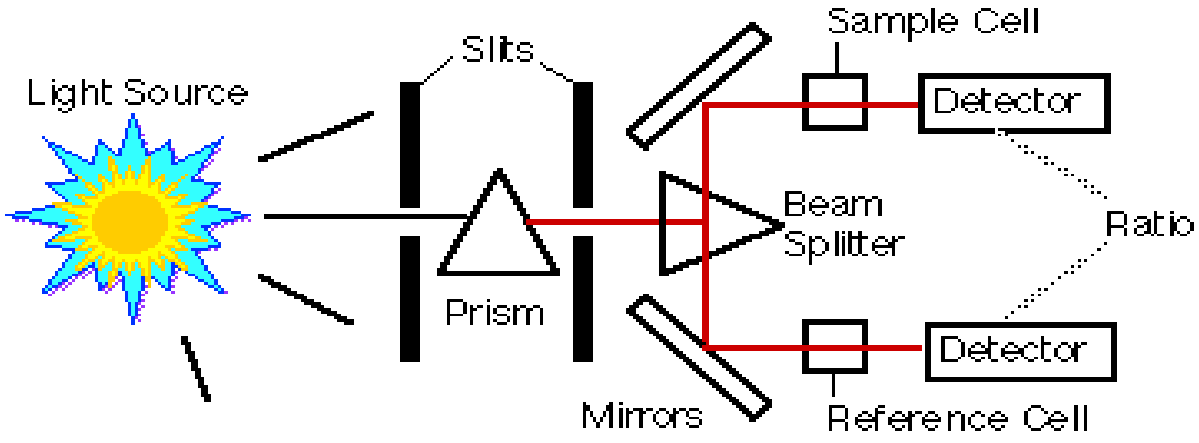


Figure 1. Schematic diagram of double beam spectrophotometer

Disadvantages

Samples should be in solution. Mixture of substances poses tricky to analyze and requires prior separation. Interference from the sample's matrix makes the measurement hard.[1]

Derivative Spectroscopy

For the purpose of spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which combination contribute interfering absorption, a method of manipulating the spectral data is known as derivative spectroscopy. In the perspective of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order, or D^0 spectrum. The first derivative D^1 spectrum is a plot of the rate of change of absorbance with wavelength against wavelength i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ vs. λ. The maximum positive and maximum negative slopes respectively in the D spectrum correspond with a maximum and a minimum respectively in the D^1 spectrum. The λmax in D spectrum is a wavelength of zero slope and gives $dA/d\lambda = 0$ in the D^1 spectrum.

The second derivative D^2 spectrum is a plot of the curvature of the D spectrum against wavelength or a plot of $d^2A/ d\lambda^2$ v/s. λ. The maximum negative curvature in the D spectrum gives a minimum in the D^2 spectrum, and the maximum positive curvature in the D spectrum gives two small maxima known as satellite bands in the D^2 spectrum.

Advantages:

- Derivative spectrum shows better resolution of overlapping bands than the fundamental spectrum.
- Permit the accurate determination of the λmax of the individual bands.

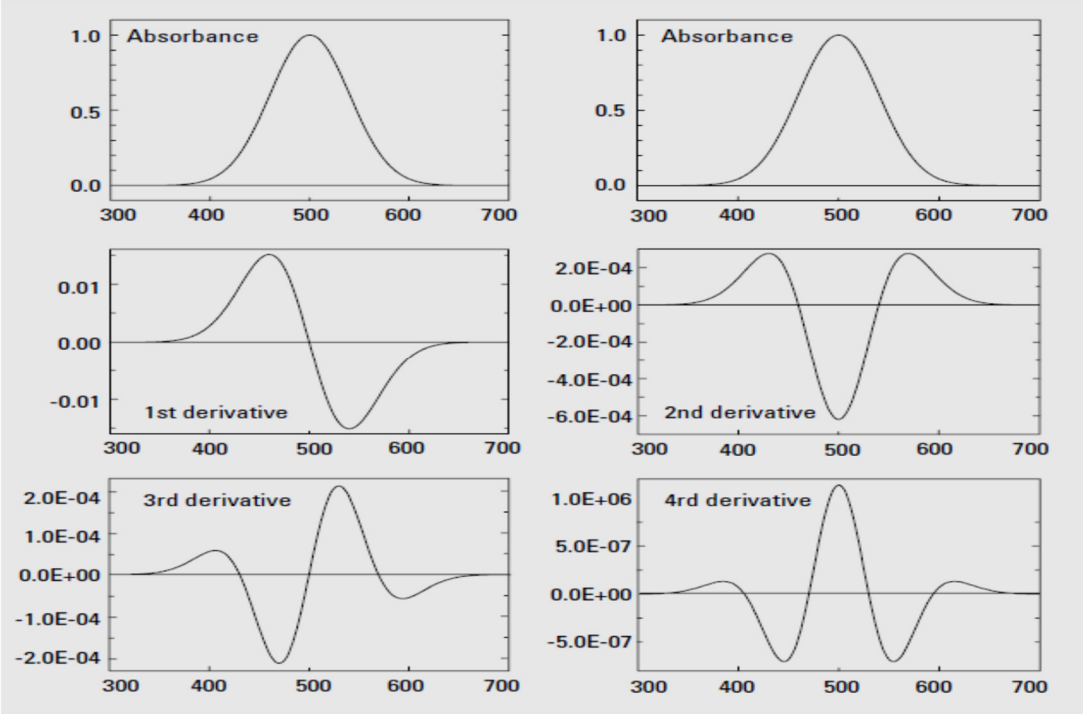


Figure 2. Different types of derivative spectrum

Following drugs have been reported to be estimated simultaneously by the Derivative spectroscopy method [2, 3, 4, 5].

- Estimation of Propranolol and Hydrochlorothiazide.
- Estimation of Phenylpropanolamine, Chlorpheniramine and Bromhexine.
- Estimation of Naphazoline hydrochloride and Chlorpheniramine maleate.

COMPARISON OF DS WITH HPLC METHODS

Although, chromatographic methods are characterized by high selectivity and sensitivity, and are giving reliable results, they need classy equipment and demand highly skilled operating personnel. The HPLC method has been highly used in the quality control of drugs for the reason that of its sensitivity, reproducibility and specificity. On the other hand, the DS method is very simple, rapid, and economical and allows the determination of drugs with sufficient reliability. DS method is selective, accurate, precise and excellent alternative to the HPLC method. spectrophotometry is a very good tool for fine resolution of spectra. It provides much improved fingerprints than traditional absorbance spectra. It is able to stifle matrix effects.

Dual wavelength method

In dual wavelength method from the overlain spectra for estimation of a drug two wavelengths are chosen in such a way where the absorbance of other drug (interfering compound) is similar, so that the absorbance difference at these wavelengths are directly proportional to the concentration of that drug [6].

Following drugs have been reported to be estimated simultaneously by the Dual wavelength method.

- Estimation of Famotidine and Domperidone [6].
- Estimation of Simvastatin and Ezetimibe [7].
- Estimation of Atorvastatin and Amlodipine Besylate [8].

METHOD DEVELOPMENT

Five stages are to be taken into consideration when starting new HPLC method.

1) Instrumentation
2) Determination of molecular characteristics of sample
3) Selection of column
4) Selection of mobile phase
5) Selection of detector

HPLC INSTRUMENTATION [6, 7, 8, 9]

Liquid chromatography (LC) is a physical separation procedure conducted in the liquid phase. A sample is separated into its constituent components (or analytes) by distributing among the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). HPLC is a modern form of LC that uses small-particle columns through which the mobile phase is pumped at high pressure.

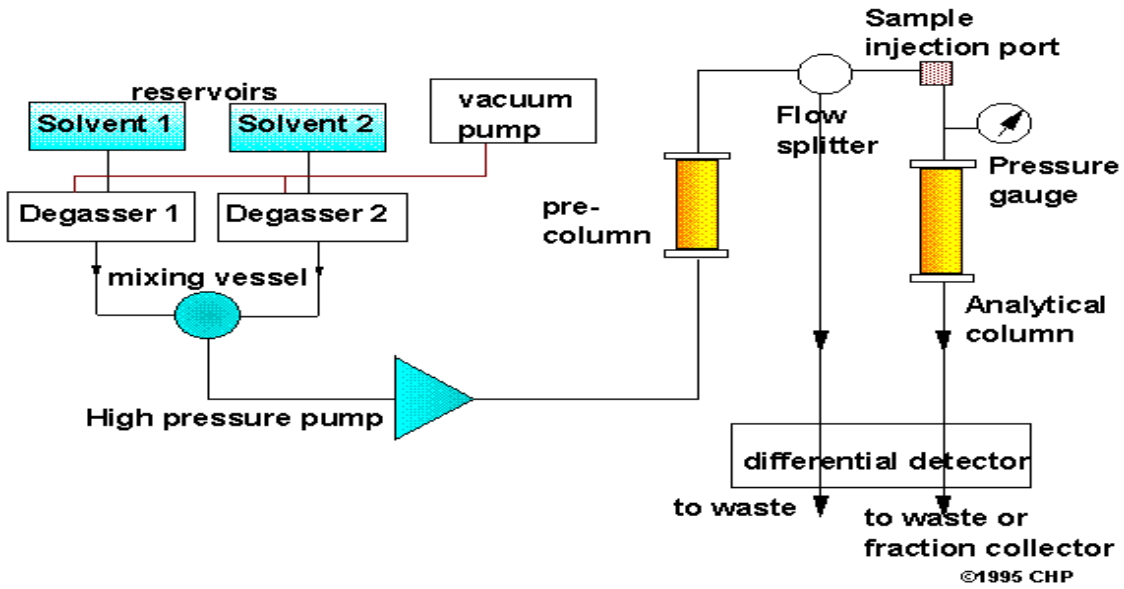


Figure 3. HPLC Instrumentation.

SEPARATION MECHANISM

Compounds are separated since the molecules travel at different rates in the column. Due to different interaction between stationary phase and different sample, the molecules move at different rate, therefore separation can be done.

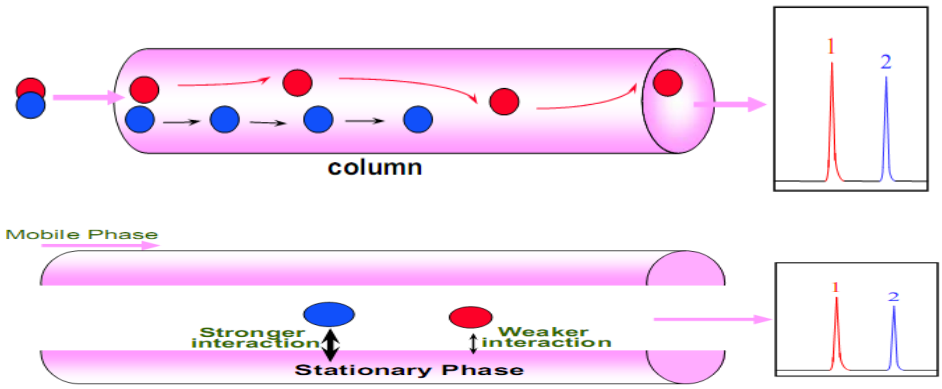


Figure 4. HPLC Separation mechanism

Types of HPLC

I Based on modes of chromatography

a) Normal-Phase Chromatography (NP HPLC)

In NP HPLC, stationary phase is polar in nature and mobile phase is non polar solvents (such as hexane, heptane, etc.), In this technique, non-polar compound travel faster and are eluted first. This is because less affinity among solute and stationary phase. Polar compounds are

retained for longer time in column because of more affinity towards stationary phase. NP uses mainly non polar solvents and it is the method of preference for highly hydrophobic compounds.

b) Reversed-Phase HPLC (RP HPLC or RPLC)

As opposed to normal-phase HPLC, reversed-phase chromatography is based mainly dispersive forces (hydrophobic or Vander Waals interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. Reversed-phase HPLC is by far the most admired mode of chromatography. Almost 90% of all analyses of low-molecular-weight samples are performed out by using RP HPLC. Less polar (more hydrophobic) analytes are more attracted and spend more time linked with the hydrophobic bonded phase, therefore, they are eluted at last.

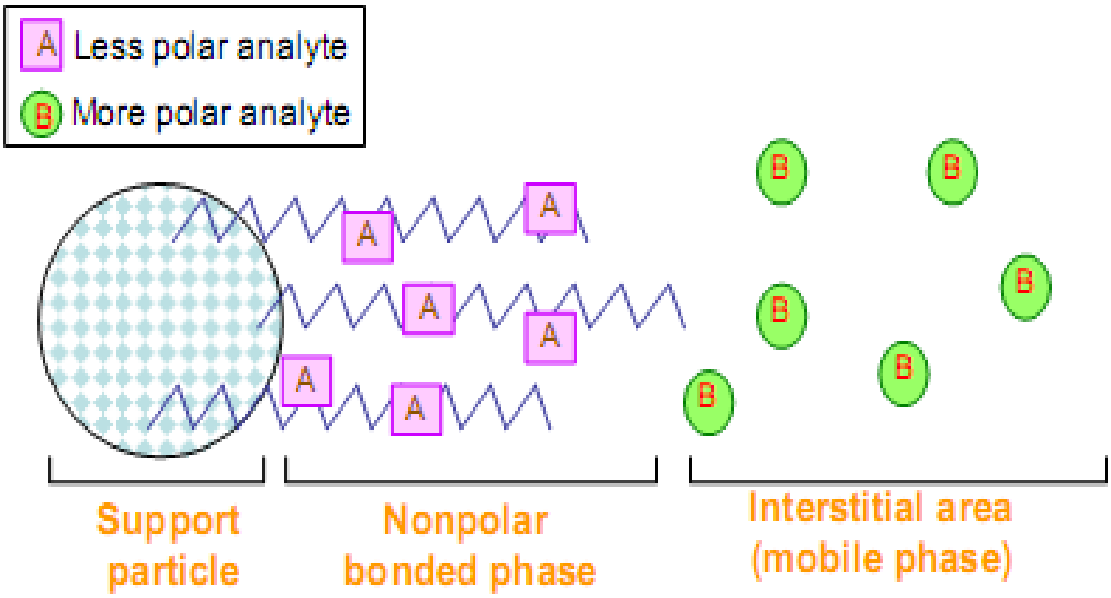


Figure 5. RP- HPLC Separation mechanism

II Based on elution technique

a) Isocratic elution: A separation in which the mobile phase composition remains unchanged. The mobile phase may comprise of a single solvent or a pre-mixed mixture of solvents.

b) Gradient elution: The composition of the mobile phase is changed during the separation. Two or three solvents that differ in polarity are employed. After sample introduction, the ratio of these solvents is planned to vary either continuously or in steps, ensuing in enhanced separation efficiency. The terms 'binary gradient', ternary gradient', and quaternary gradient' refer to the use of 2, 3, and 4 solvents, respectively, to make up the mobile phase composition in a gradient elution method.

DETERMINATION OF MOLECULAR CHARACTERISTICS OF SAMPLE

Methods for analyzing drugs in multicomponent dosage forms can be developed, provided one has knowledge about the nature of the sample, namely, its molecular weight, polarity, ionic character and the solubility parameter [10].

Solubility in different organic or aqueous solvents determines the best composition of the sample solvent. P^{Ka} determine the pH in which the analyte will exist as a neutral or ionic species. When the pH = pKa, for the analyte, at this concentration equal proportion ionic and non-ionic species exists. As a general rule pH caused changes in retention occurs within ±1.5 units above or below the pKa to ensure practically100% unionization for retention reason. This information will smooth the progress of an efficient sample extraction scheme and determine the optimum pH in mobile phase to achieve good separations [11].

SELECTION OF COLUMN

Column is the heart of HPLC system. Good silica and bonding process will provide the reproducible and symmetrical peaks necessary for exact quantification [12].

Column selection basics (Rule of thumb)

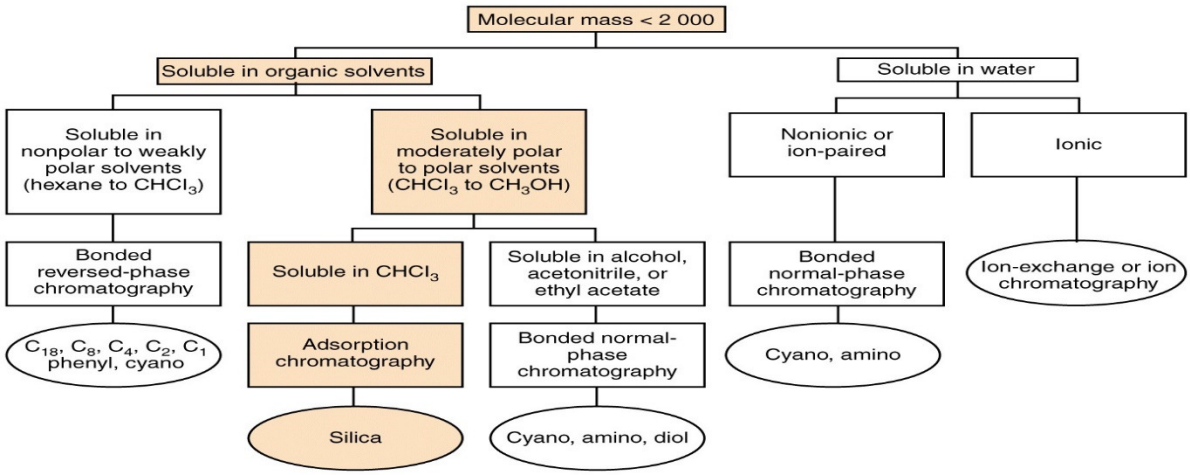


Figure 6. (a) Selection of column in HPLC.

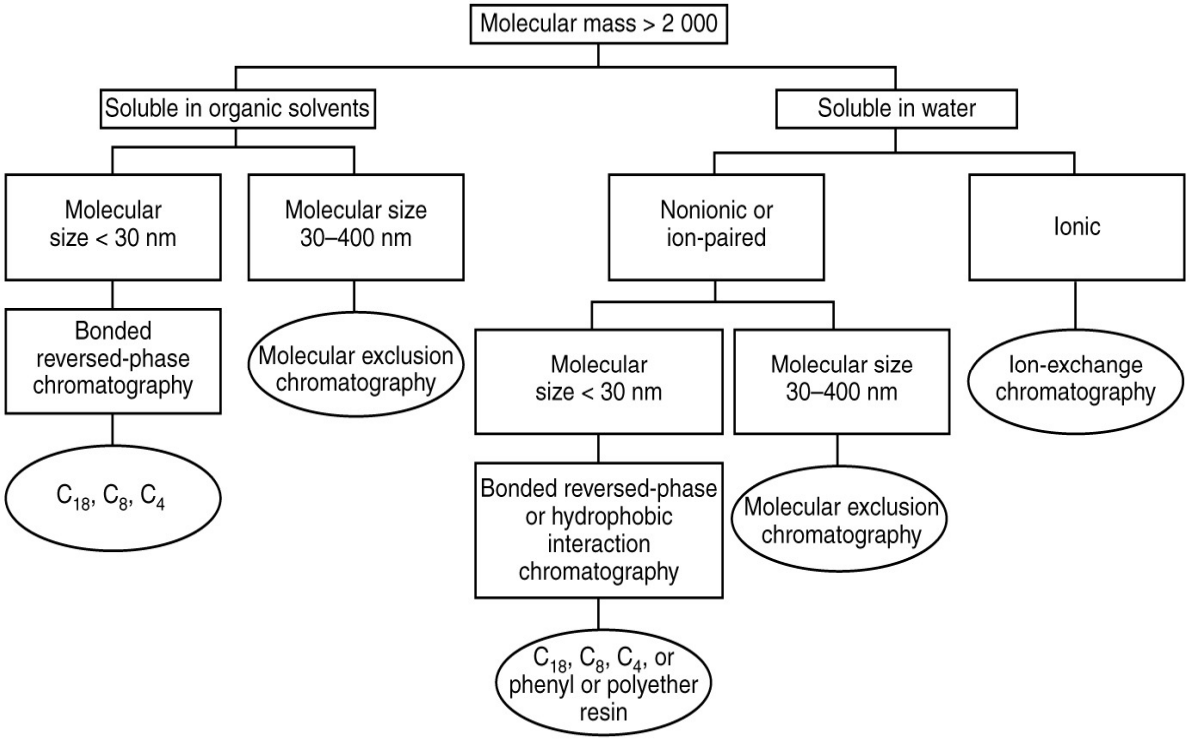


Figure 6. (b) Selection of column in HPLC.

SELECTION OF MOBILE PHASE

The selection of mobile phase based on the nature and physicochemical properties of the analytes to be determined. Since the mobile phase governs solute-stationary phase interactions, its choice is decisive.

- Practical considerations utter that it should not degrade the equipment or the column packing. For this reason, strong acids, bases and halide solutions should be avoided.
- Viscosity should be less than 0.5 centipoise; otherwise higher pump pressures are required and mass transfer between solvent and stationary phase will be reduced. For LC/MS only volatile buffers are used.
- The mobile phase should have a pH 2.5 and 7.0 to maximize the lifetime of column.
- Reduce cost and toxicity of the mobile phase by means of methanol instead of acetonitrile when possible.

SELECTION OF DETECTOR

There is little use in running a separation if the detector one uses cannot "see" all the components of interest, or conversely, if it"sees" too much. UV-vis detectors are the most popular as they can detect a broad range of compounds and have a fair degree of selectivity for some analytes. Unfortunately, UV-vis detectors are not universal detectors so it is worthwhile to look at the chemical structure of the analyte to see if it has suitable chromaphores, such as aromatic rings, for UV-vis detection [13]. Some of the detectors are described below in table.

Table 1. Detectors

Detector	Analytes	Solvent Requirements	Comments
UV-Visible	Any with chromophores	UV-grade non-UV absorbing solvents	Has a degree of selectivity and useful for many HPLC applications.
Fluorescence	Fluorescent compounds	UV-grade non-UV absorbing solvents	Highly selective and sensitive and used to analyze derivatized compounds.
Refractive Index (RI)	Compounds with a different RI to the mobile phase	Cannot run mobile phase gradients	Virtually a universal detector but has limited sensitivity
Conductivity	Charged or polar compounds	Mobile phase must be conducting	Excellent for Ion Exchange methods

Electrochemical	Readily oxidized or reduced compounds	Mobile phase must be conducting	Very Selective and sensitive
Evaporative Light Scattering (ELSD)	Virtually all compounds	Must use volatile solvents and volatile buffers	A universal detector which is highly sensitive and non-selective
Mass Spectrometer (MS)	Broad range of compounds	Must use volatile solvents and volatile buffers	Highly sensitive and is a powerful 2nd dimensional analytical tool. Many modes are available.

ANALYTICAL METHOD VALIDATION

The process of establishing documented evidence which provides a high degree of assurance that the method does what it is intended to do.

Positioning of validation in method development process

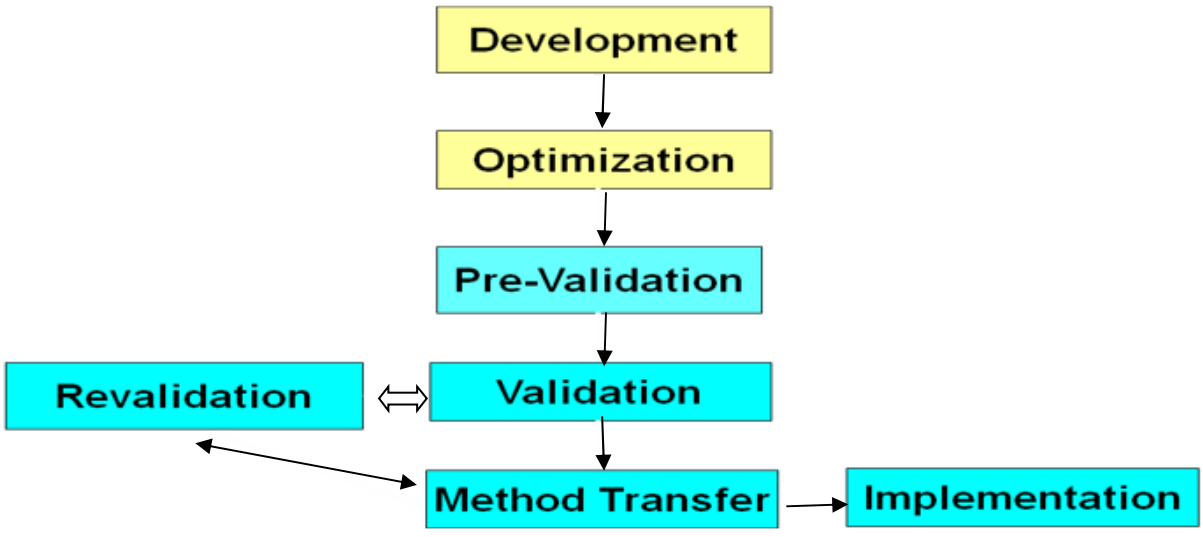


Figure 7. Positioning of validation in method development process.

STABILITY STUDIES

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to sustain its identity, strength, quality, and purity all over the retest or expiration dating periods [14].

Stability plays an important role in the drug development process. It explains several factors that influence the expiration dating of drug products, including the chemical and physical stability during the pre-clinical formulation stages, process development, packaging development, and post-marketing life [16].

Stability testing allows the establishment of suggested storage conditions, retest periods, and eventually product shelf-life and expiry dating. In pharmaceutical field stability studies finds an application in the following areas of drug development program [17, 18, 19, 20].

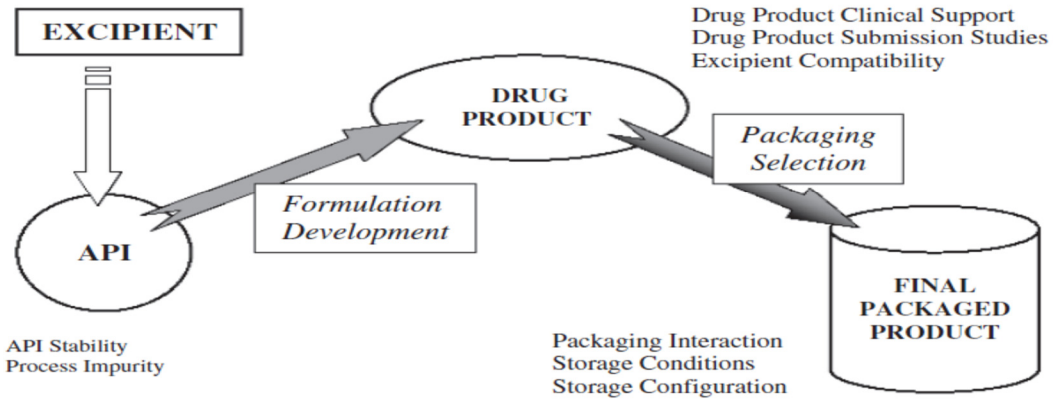


Figure 8. Stability studies to support development of new drug product components of a film-coating or capsule shell. For combination parenteral or aerosol products, the guidance recommends an exploration of the chemical compatibility or stability of multiple actives that will be combined before administration [24, 25].

Study Protocol [21, 22, 23]

A general protocol for conducting forced degradation studies, shown in Table.

Table 2. General protocol for stress testing of drug substances and drug products

Condition	Drug Substance		Drug Product	
	Solid	Solution/ Suspension	Solid (Tablets, Capsules, Blends)	Solution (IV, Oral Suspension)
Acid/base		√		X
Oxidative	X	√	√	√
Photostability	√	X	√	√
Thermal	√		√	√
Thermal/humidity	√		√	

√= recommended; X = optional, suggested for some compounds [26-29].

Conditions for Stress Testing

Specific parameters for stress testing of drug substance and drug product are shown in tables, respectively, describing the different stress conditions and range of exposure times.

The desired target extent of degradation is approximately 5–20%. This is achieved by varying the stress conditions, for example, exposure time, temperature, or concentration of stressing agent (acid, base, oxidizer, etc.). Overstressing may destroy the compound or may lead to further degradation of the relevant primary degradants. Under-stressing may fail to create important degradation products.

The degradation studies should be terminated after the maximum recommended time/stress conditions, even if sufficient degradation has not been achieved. It is unnecessary and even imprudent to try to degrade the drug at all cost as it would only increase the complexity of the method development with little or no benefit in the quality of the data generated by the method. The concentration of drug in the stressed sample solution may affect the target level of degradation that is eventually achieved.

Therefore, lowering the drug concentration may help to increase degradation when necessary. Additional recommendations for preparation of the stressed samples follow [30]:

If the solid drug substance is unstable to thermal stress at high temperature due to melting, decomposition, use a lower temperature with longer stress time.

Reaction Mechanism/Degradation Pathway

The common reaction mechanisms of chemical degradation of pharmaceutical compounds consist of hydrolysis, oxidation, isomerization/epimerization, decarboxylation, rearrangement, dimerization/polymerization, photolysis, and reactions with excipients and salt forms [31, 32].

Table 3. Common degradation routes for functional group

Functional group	Degradation route	Degradants
Acetals	Hydrolysis	Ketones /aldehydes /alcohols
Esters/lactones		Acids/alcohols
Amides/lactams		Amines/acids
Alkenes		Alcohols
2◦ and 3◦ Amines	Oxidation (radical, light, metal, peroxide mediated)	N-oxide, hydroxylamine
Thiols		Disulfide
Thioethers		Sulfoxide, sulfone
Alkenes		Epoxide
Allylic Alcohols		α, β, unsaturated ketones
Aldehyde		Acids
Alcohol		Ketones, acids
Oxazoles/imidazoles		Various products
Dienes (able to aromatized)		Aromatic rings
Benzyl/Allylic groups		Benzylic/allylic alcohols

Stability Indicating Assay Methods (SIAM)

ICH defines SIAM as quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product and that will differentiate each active ingredient from its degradation products so that the active ingredient content can be accurately measured.

USFDA defines SIAM as validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be perfectly measured without interference [33, 34, 35].

Specific SIAM is defined as 'a method that is able to measure unambiguously the drug(s) in the presence of all degradation products, excipients and additives, expected to be present in the formulation'.

Selective SIAM is defined as 'a method that is able to measure unequivocally the drug(s) and all degradation products in the presence of excipients and additives, expected to be present in the formulation'.

Stability-indicating method development

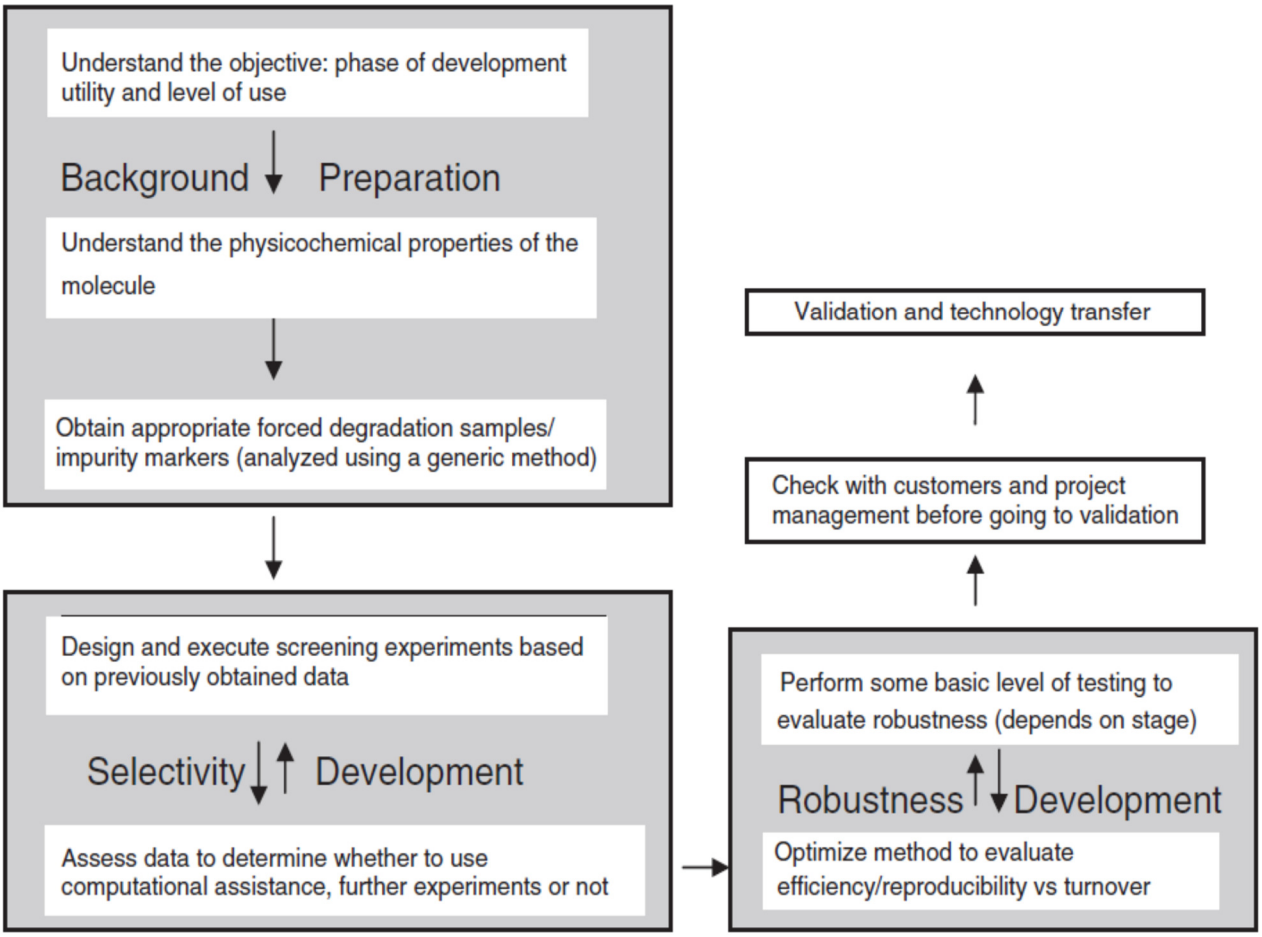


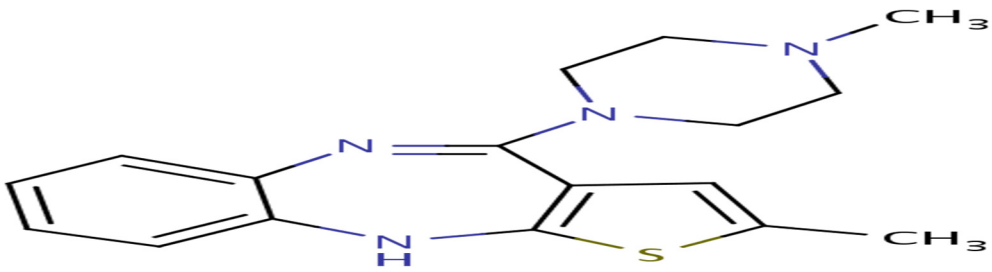
Figure 9. Stability-indicating method development process

DRUG PROFILE

OLANZAPINE

Olanzapine is an atypical antipsychotic, approved by the U.S. Food and Drug Administration (FDA) for the treatment of schizophrenia and bipolar disorder. Olanzapine is structurally similar to clozapine and quetiapine, but is classified as a thienobenzodiazepine. The olanzapine formulations are manufactured and marketed by the pharmaceutical company Eli Lilly and Company; the drug went generic in 2011.

Structural formula of Olanzapine



Chemical Name	2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5] benzodiazepine
Molecular Formula	$C_{17}H_{20}N_4S$
CAS no.	132539-06-1
Molecular Weight	312.4
Description	A yellow crystalline powder
Solubility	Freely soluble in dichloromethane, insoluble in water
Bioavailability	93%
Half life	21-54 hrs
Melting Point	195°C (383°F)
U V max.	259 nm
Category	Anti-psychotic

MECHANISM OF ACTION

Olanzapine is an antagonist at types 1, 2 and 4 dopamine receptors. Olanzapine's antipsychotic effect is due to antagonism at dopamine and serotonin type 2 receptors, with

greater activity at serotonin 5-HT 2 receptors than at dopaminetype 2 receptors. Antagonism at muscarinic receptors. H1 receptors, and alpha (1) - receptors also occurs with olanzapine.

USES

- Oral formulation used in acute and maintenance treatment of Schizophrenia in adults, acute treatment of manic or mixed episodes associated with Bipolar I Disorder (monotherapy and in combination with lithium or valproate).
- Intramuscular formulation used in acute agitation associated with Schizophrenia and Bipolar I Mania in adults.
- Oral formulation combined with fluoxetine used in acute treatment of depressive episodes associated with Bipolar I Disorder in adults, or acute treatment of treatment resistant depression in adults.

SIDE EFFECTS

Side effects seen with olanzapine include

- akathisia (an inability to sit still)
- constipation
- dizziness
- drowsiness
- insomnia
- dry mouth
- orthostatic hypotension

ANALYTICAL METHOD DEVELOPMENT BY HPLC [36,37]

Mobile Phase Selection

Initially to estimate Olanzapine number of mobile phases in different ratio were tried. Results were shown in table.

Taking into thought the system suitability parameter like RT, Tailing factor, no. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 1.75 gm KH_2PO_4 in 1000 ml of water add 1 ml of TEA and adjust the pH 6 with OPA .The mobile phase was filtered through 0.45 µm filter paper to remove particulate matter and then degassed. Flow rate employed for analysis was 1.0 ml/min.

Selection of wavelength

100 mg of Olanzapine was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile phase. From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at λmax 257.0.

Preparation of Standard Stock Solution

10mg of Olanzapine was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the Water : Acetonitrile (50:50 v/v) to give a stock solution of 1000ppm.

Preparation of Working Standard Solution

From stock solutions of Olanzapine 1 ml was taken and diluted up to 10 ml from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with Water : ACN (50:50 v/v) , gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

ANALYSIS OF TABLET FORMULATION

Assay of tablet formulation

For analysis of the tablet formulation, weight equivalent to weight 10 mg of Olanzapine was transferred to 10 ml volumetric flask and dissolved in mobile phase. The solution was shaking vigorously for 20 mins and filtered through Whatman filter paper no. 41, then volume was made up to mark with mobile phase. From the above solution 1 ml of solution was taken and

diluted to 10 ml with mobile phase to get a solution containing 100 µg/ml. From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 10 µg/ml of Olanzapine. The amounts of Olanzapine in tablet formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation.

VALIDATION

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration was recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in Figure 8.9. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration [38,39].

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

a) Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. (Table no. 8.11). Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

b) Intermediate Precision

I. Day to Day

The statistical analysis method was carried out and the data is presented in table.

II. Analyst to Analyst

The intermediate precision expresses with in laboratories variation (different days, different analysts, different equipment etc). The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Olanzapine was studied.

Forced Degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on olanzapine powder and the analysis was carried out by HPLC with a U.V. detector.

Acid degradation:

50 mg of olanzapine sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Olanzapine.

Base degradation

50 mg of olanzapine sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Olanzapine.

Hydrolytic degradation

50 mg of olanzapine sample was taken into a 50 ml round bottom flask, 50 ml of water was added and the contents were mixed well and kept for constant stirring for 48 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Olanzapine.

Oxidative degradation:

50 mg of olanzapine sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Olanzapine.

Thermal degradation

50 mg of olanzapine sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks [40,41].

RESULTS AND DISCUSSION

Result of FTIR of Olanzapine

FTIR Spectrum

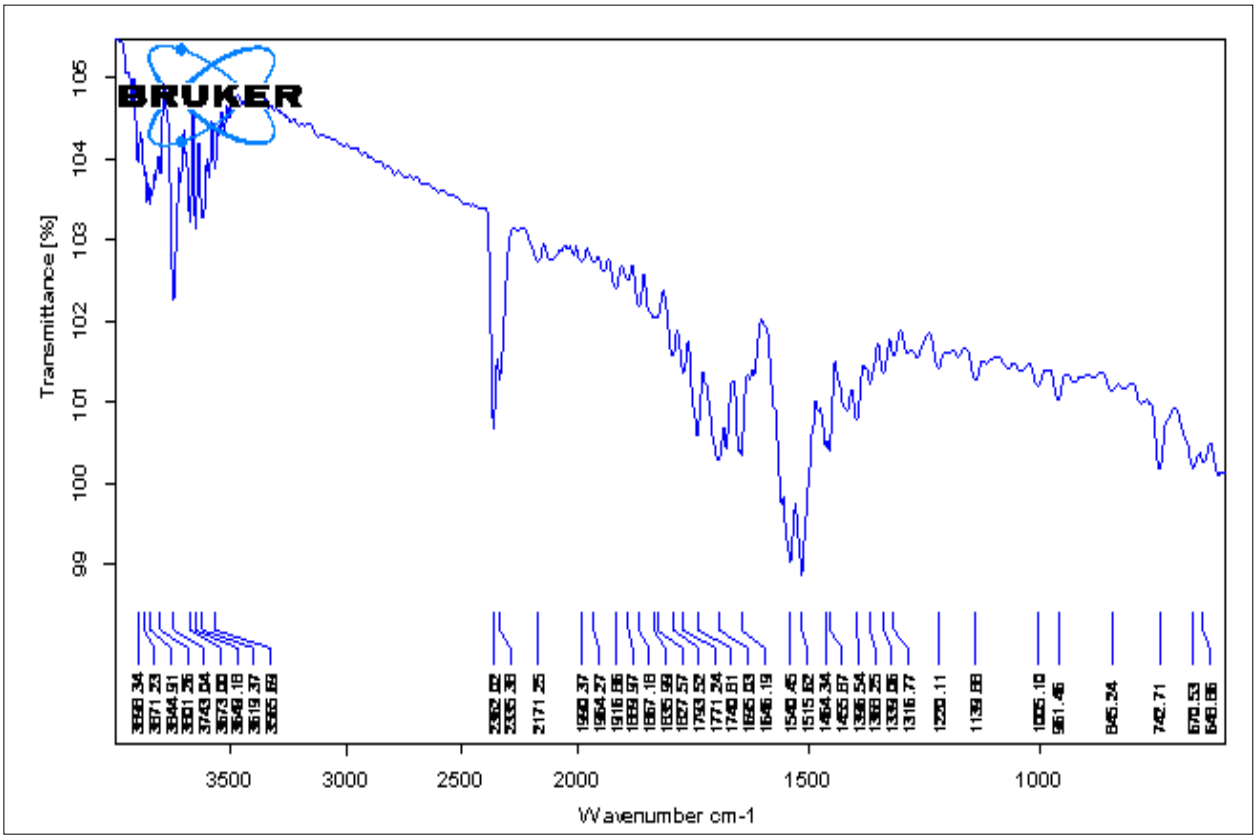


Figure 10. FTIR spectra of Olanzapine

Interpretation of FTIR

Table 4. Interpretation of FTIR spectra of Olanzapine

S.No.	Functional Group	Experimental peak No.(cm^{-1})	Theoretical peak No.(cm^{-1})
1	OH- stretching vibration single bridge	3565.69	3570-3450
2	-N=C=N- stretching vibrations	2171.25	2175-2130
3	-C-C- multiple bond stretching	1964.27	1960
4	Anhydride stretching vibration, saturated 5-membered ring	1889.97	1890-1750

5	Anhydride stretching vibrations, saturated acyclic	1771.24	1790-1740
6	-C=N- stretching vibration	1646.19	1660-1630
7	N-H bending vibration, secondary amides	1515.62, 1540.45	1550-1510
8	Sulfur compound	1139.06	1200-1050

Results of Solubility Study

Table 5. Solubility of Olanzapine

S. No.	**Solvent**	**Solubility**
1	Water	Insoluble
2	0.1 N HCl	Freely soluble
3	Methanol	Freely soluble
4	Ethyl alcohol	Slightly soluble
5	0.1 N NaOH	Insoluble
6	Acetonitrile	Freely soluble
7	Phosphate buffer pH 7.4	Insoluble
8	Benzene	Freely soluble

Determination of λ_{max} of Drug: - 257.0 nm

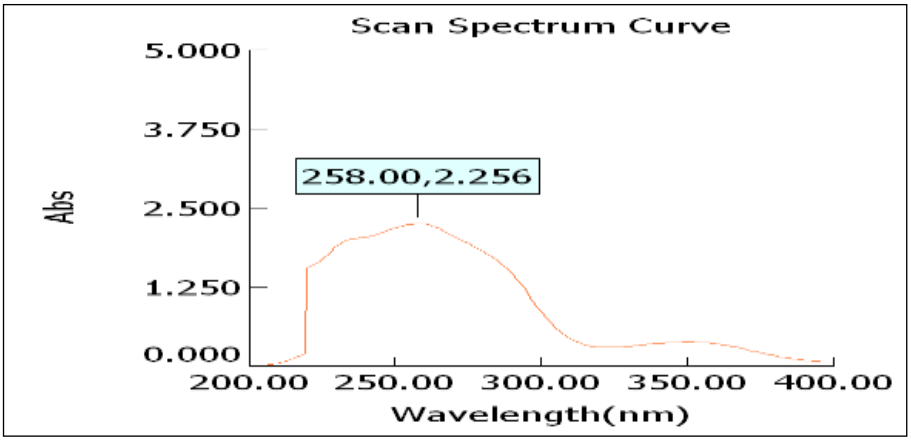


Figure 11. Selection of λ_{max} of Olanzapine

Results of RP-HPLC Method

System Suitability Parameters

Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Buffer	60
ACN	40
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	257.0 nm
Retention time Olanzapine	3.476 $\pm$ 0.3 min

Table 6. Result of System Suitability Parameters for Olanzapine

System suitability Parameter →	RT	AUC	Theoretical plates	Tailing factor
Rep-1	2.375	1251.23	3078	1.18
Rep-2	2.374	1250.458	3056	1.20
Rep-3	2.375	1256.658	3098	1.15
Mean	2.374667	1252.782	3077.333	1.176667
S.D.	0.000577	3.378835	21.00794	0.025166

Linearity and Calibration Graph

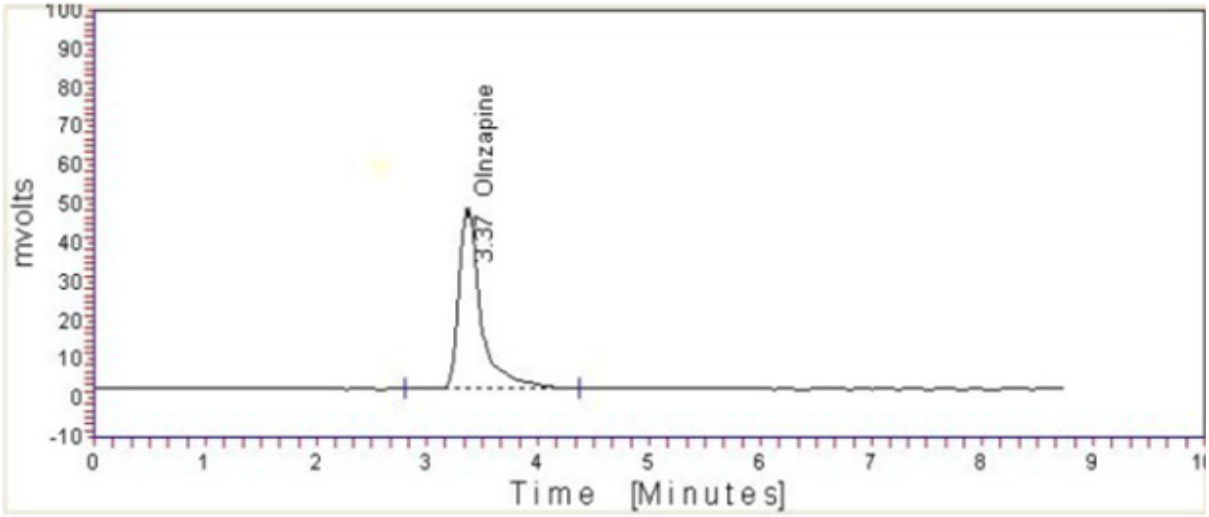


Figure 12. Chromatogram of 5 rpm

Assay of tablet Formulation

Table 7. Result of Analysis for Olanzapine in tablet Formulation

Std Conc. µg/ml	**Olanzapine**
	10
Rep-1	10.10
Rep-2	10.05
Rep-3	10.08
% found *	
Rep-1	101
Rep-2	99.504
Rep-3	100.298
Mean	10.076
SD	0.747
% RSD	0.742

*Each reading is mean reading of three batch of formulation

Validation of Developed Method

Linearity

Table 8. Response Ration Data for Linearity of Olanzapine

Replicates	Concentration (µg/ml)	Mean AUC	Response Ratio
Rep-1	5	611.774	122.3548
Rep-2	10	1251.23	125.123
Rep-3	15	1875.978	125.0652
Rep-4	20	2483.22	124.161
Rep-5	25	2982.129	119.2852
Mean		123.197	
S.D.		2.456	
R.S.D.		1.994	

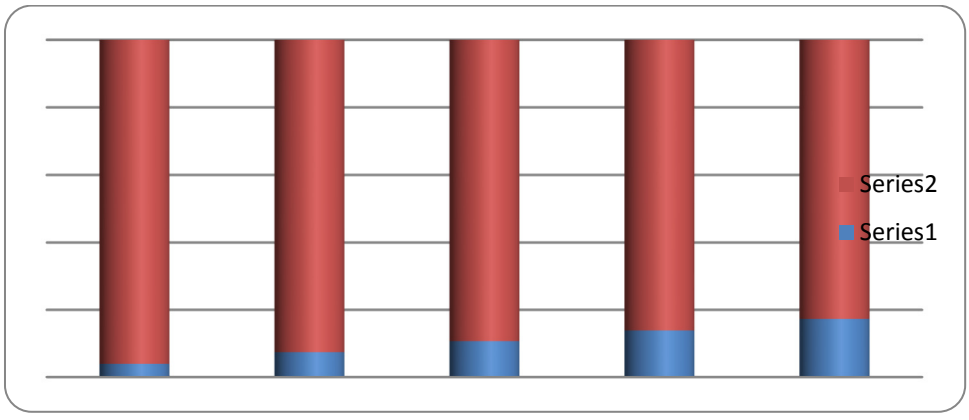


Figure 13. Response Ratio Curve of Olanzapine

Result of Accuarcy

Table 9. Recovery Studies of Formulation

Level of Recovery (%)	**80**	**100**	**120**
	Olanzapine	**Olanzapine**	**Olanzapine**
Amount present (mg)	10 10 10	10 10 10	10 10 10
Amount of Std. added (mg)	8 8 8	10 10 10	12 12 12
Amount recovered (mg)	7.98 8.01 8.00	10.05 10.00 9.98	11.95 12.01 11.95
% Recovery	99.75 100.12 100.00	100.50 100.00 99.80	99.58 100.08 99.58

Table 10. Statistical Validation of Recovery Studies

Level of Recovery (%)	Drug	% Recovery	Standard Deviation*	% RSD
80	Olanzapine	99.958	0.191	0.191
100	Olanzapine	100.100	0.361	0.360
120	Olanzapine	99.750	0.289	0.289

*Denotes average of three determinations

Result of precision

(A) Repeatability

Table 11. Results of analysis Data of Tablet Formulation

Drug	Label claim	Amount found*	Label claim (%)	S.D.	% RSD
Olanzapine	10 mg	9.95	99.50	0.254	0.125

(B) Intermediate Precision- (Inter-day and Intra-day Precision)

Table 12. Intra-day and Inter-day Precision

Intra-day Precision		Inter-day Precision	
	% Label Claim		% Label Claim
	Olanzapine		Olanzapine
After 1hr	99.20	First day	97.50

After 2hr	99.10	Second day	97.00
After 3hr	99.00	Third day	96.80
After 4hr	98.85		
After 5hr	98.70		
After 6hr	98.30		
Mean	98.97	Mean	97.1
SD	0.198746	SD	0.360555
% RSD	0.200814	% RSD	0.371324

Result of Robustness

Table 13. Result of Robustness of Formulation

Compound	**% RSD in Normal**	**Changed Condition n= 6**	
Temperature		- 5 ^{o}C	+ 5 ^{o}C
Olanzapine	0.54	0.69	0.52
Flow rate		(-10%)	(+10%)
Olanzapine	0.41	0.48	0.89
Mobile phase ratio		- 2 %	+ 2 %
Olanzapine	0.31	0.77	0.15

Forced Degradation Studies

Table 14. Forced degradation studies of Olanzapine

Stress conditions	**Drug recovered (%)**	**Drug decomposed (%)**
Standard drug	99.90	0
Acidic hydrolysis	83.26	16.64
Alkaline hydrolysis	89.65	10.25
Oxidative degradation	91.23	8.67
Thermal degradation	98.89	1.01

CONCLUSION

The developed stability indicating HPLC method for quantitative estimation of olanzapine in bulk and pharmaceutical dosage forms is fast, simple, accurate, and more precise. Validation of this method was accomplished, getting results meeting all requirements. Thus, the developed HPLC method can be used for routine quality control test.

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