DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING HPLC METHOD FOR AMLODIPINE BESYLATE: INSIGHTS FROM FORCED DEGRADATION AND QUALITY ASSURANCE PERSPECTIVE.

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Abstract

Amlodipine besylate is a dihydropyridine calcium channel blocker widely used in the treatment of hypertension. Evaluating its stability and degradation behaviour is essential for ensuring pharmaceutical quality, safety, and efficacy. This article focuses on the development and validation of a stability-indicating high-performance liquid chromatography (HPLC) method for amlodipine besylate, emphasizing forced degradation studies and quality assurance. The degradation behaviour of the drug under acidic, alkaline, oxidative, thermal, photolytic, and humidity stress conditions is discussed. Reversed-phase HPLC (RP-HPLC) with a C18 column and UV detection is considered for separating the active pharmaceutical ingredient from impurities and degradation products. Method validation parameters, including specificity, accuracy, precision, linearity, robustness, and system suitability, are examined in accordance with ICH guidelines. The study highlights the importance of forced degradation and validated analytical procedures in supporting stability assessment, impurity control, and reliable pharmaceutical quality assurance.

Keywords*: Amlodipine Besylate, Stability-Indicating HPLC, Forced Degradation Studies, Method Development, Analytical Method Validation, RP-HPLC, Quality Assurance, Degradation Products, Pharmaceutical Stability, ICH Guidelines*.

1.INTRODUCTION:

Amlodipine besylate is a long-acting dihydropyridine calcium channel blocker widely used in the management of hypertension and cardiovascular diseases. It acts by inhibiting the influx of calcium ions into vascular smooth muscle cells, resulting in vasodilation and a reduction in peripheral vascular resistance. Maintaining the quality, purity, potency, and stability of amlodipine besylate is essential to ensure its therapeutic effectiveness and pharmaceutical safety.[1]

Stability studies play a significant role in evaluating the effects of environmental factors, including temperature, humidity, light, and chemical stress, on pharmaceutical products. Amlodipine besylate may undergo degradation under acidic, alkaline, oxidative, and photolytic conditions, potentially resulting in the formation of degradation products that can affect drug quality. Therefore, reliable analytical techniques are necessary to determine the active pharmaceutical ingredient in the presence of impurities and degradation products.[1]

High-performance liquid chromatography (HPLC), particularly reversed-phase HPLC (RP-HPLC), is widely employed in pharmaceutical analysis because of its separation efficiency, sensitivity, and reproducibility. Developing a stability-indicating HPLC method involves optimizing chromatographic conditions to achieve adequate separation between the intact drug and its degradation products. Forced degradation studies are essential for evaluating the specificity and stability-indicating capability of the analytical method under different stress conditions.[1]

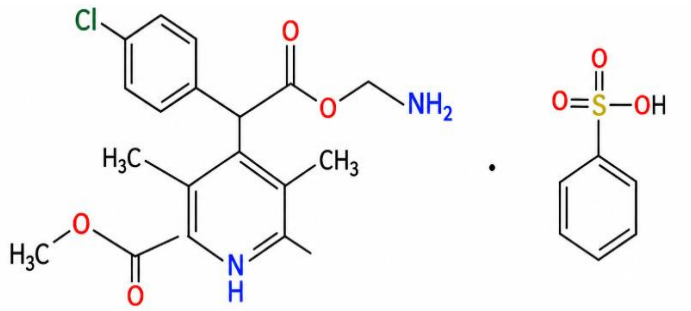
Analytical method validation is another important aspect of pharmaceutical quality assurance. Parameters such as specificity, accuracy, precision, linearity, range, robustness, and system suitability help establish whether an analytical procedure is appropriate for its intended purpose. International Council for Harmonisation (ICH) guidelines provide a scientific framework for stability testing and analytical method validation.[1]

Therefore, this article focuses on the development and validation of a stability-indicating HPLC method for amlodipine besylate, with particular emphasis on forced degradation studies, degradation-product assessment, chromatographic separation, regulatory requirements, and quality assurance in pharmaceutical analysis.[1]

2. DRUG PROFILE:

2.1 chemical structure, molecular formula, pKa, solubility:

Amlodipine besylate is the besylate salt of amlodipine belonging to the dihydropyridine class of calcium channel blockers. Amlodipine besylate appears to be a white crystalline powder and is slightly soluble in water and sparingly soluble in ethanol. Its chemical name is 3-ethyl-5-methyl (±) -2- [(2-aminoethoxy) methyl] -4-(2-chlorophenyl) -1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulfonate with a molecular formula of C20H25ClN2O5 ·C6H6O3S and molecular weight of 567.1 g/mol.[1]



Amlodipine (free base) Benzenesulfonic acid (besylate)

Molecular formula: $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$

Molecular weight: 567.06 g/mol

Amlodipine has a 1,4-dihydpopyridine ring, two ester groups, an aminoethoxy side chain, and a chlorophenyl substituent. All of these characteristics are considered during the physicochemical behavior and possible ways of degradation. For example, the presence of ester fragments highlights the possibility of hydrolytic degradation of the drug under certain conditions.[1]

Amlodipine is an ionizable compound with a pKa of approximately 8.6. Therefore, in solutions with a pH close to physiological, this substance is predominantly ionized. This property affects the solubility of amlodipine and its ability to adhere to a stationary phase during high-performance liquid chromatography.[1]

Drug profile information:

PARAMETERS	AMLODIPINE BESYLATE
Drug class	Dihydropyridine calcium channel blocker.
Chemical form	Besylate salt
Molecular weight	567.1 g/mol
Molecular formula	$C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$
pKa	Approximately 8.6
Appearance	White crystalline powder

Water solubility	Slightly soluble
Therapeutic use	Antihypertensive

The drug's physicochemical characteristics are relevant to the development of a suitable chromatographic method because pH, mobile-phase composition and organic solvent proportion can influence peak shape, retention and separation efficiency.

2.2 Mechanism of action and therapeutic use:

Amlodipine is a long-acting dihydropyridine calcium channel blocker that works by inhibiting the transmembrane influx of calcium ions (Ca(2+)) into vascular smooth muscle and cardiac muscle cells through voltage-gated calcium channels. This agent is more selective for vascular smooth muscles than cardiac muscles. [2]

The blockade of Ca(2+) entry into vascular smooth muscle cells results in decreased intracellular concentration of Ca(2+), which in turn causes vasodilation effects. The decrease in peripheral vascular resistance ultimately leads to reduced blood pressure, making this drug a good candidate for treating hypertension and associated cardiovascular diseases. Amlodipine's vascular selectivity and slow onset of action make it a preferred medication in the management of hypertension.[2]

From a pharmaceutical-quality standpoint, the stability of amlodipine is critical during the production and storage processes. The degradation of this substance could reduce the amount of the active ingredient that becomes available for medicinal use and may generate other chemical species as well.[2]

2.3 known degradation pathways reported in literature(hydrolytic, oxidative, photolytic, thermal):

The degradation behaviour of amlodipine has been investigated under a variety of stress conditions including acidic hydrolysis, alkaline hydrolysis, neutral hydrolysis, oxidation, photolysis and thermal stress. Published LC–MS/MS investigations demonstrated that amlodipine was particularly susceptible to acidic and alkaline hydrolysis whereas comparatively greater stability was observed under some neutral, oxidative, light and thermal conditions in the specific experimental system studied. Nine degradation products were characterized in that investigation.[2]

More recent work specifically examining amlodipine besylate reported degradation under base and photolytic stress whereas lower levels of degradation were found under acidic and oxidative conditions and no significant degradation under the thermal and thermal/humidity conditions used in that study. The differences between studies highlight the importance of experimental conditions such as drug form, stress intensity, solvent, pH, temperature and exposure time when interpreting degradation behaviour.[2]

A. Hydrolytic degradation:

Hydrolysis involves the reaction of susceptible chemical groups with water under acidic, neutral or alkaline conditions. The ester-containing structure of amlodipine provides potential sites for hydrolytic transformation. Experimental studies have demonstrated that amlodipine can undergo degradation under both acidic and alkaline stress conditions with alkaline conditions producing substantial degradation in some investigations.[2]

B.Oxidative degradation:

Oxidative stress is often imposed as a part of forced degradation studies to evaluate the tendency of the active pharmaceutical ingredient (API) to undergo oxidation. In most cases, amlodipine exhibited a

relatively low level of degradation when exposed to oxidative conditions, which was reported by the authors of one of the studies. Nonetheless, the degree of oxidation is closely related to the specific experimental conditions.[2]

C. Photolytic degradation:

Exposure to light can induce photochemical transformations of the molecule, which should also be taken into account when studying the behavior of a drug under stress conditions. Amlodipine was the subject of extensive research aimed at understanding its photochemistry. According to one of the studies, photoirradiation can lead to aromatization of the dihydropyridine ring to pyridine. Another study reports that amlodipine undergoes photo-induced transformations, resulting in the formation of various photoproducts, one of which was identified by using UHPLC-QTOF-MS.[2]

D. Thermal degradation:

Forced degradation under heating conditions can provide invaluable information about the thermal stability of the drug. The data reported by the authors of one of the studies suggest that amlodipine besylate might be regarded as thermally stable, as no substantial degradation was observed in most cases. However, this can only be true for a particular set of experimental conditions, as the authors stated that amlodipine besylate displayed limited thermostability in some cases.[2]

Reported degradation behaviour:

STRESS CONDITION	REPORTED BEHAVIOUR OF AMLODIPINE
Acid hydrolysis	Degradation reported
Base hydrolysis	Significant degradation reported
Neutral hydrolysis	Relatively stable in some studies
Oxidative stress	Generally limited degradation in some studies
Photolytic stress	Photodegradation reported
Thermal stress	Relatively stable under some conditions

2.4 Marketed formulation and dosage forms (tablet, combination products eg., amlodipine+atorvastatin/telmisartan):

Amlodipine is commercially available as an oral tablet preparation. Amlodipine besylate tablets are commonly supplied containing 2.5 mg, 5 mg and 10 mg of amlodipine. The tablets comprise the active pharmaceutical ingredient plus suitable excipients to give the required manufacturing, mechanical and drug-release properties.[2]

Amlodipine is also available in combination preparations, where it is combined with another cardiovascular drug. Examples include combinations with atorvastatin, angiotensin-receptor blockers and other antihypertensive agents. Combination therapy can result in complementary effects and improve convenience of therapy.[2]

From a quality-assurance standpoint, both single- and combination preparations require appropriate analytical procedures capable of accurately determining amlodipine in the presence of excipients, other active ingredients and potential degradation products. Stability-indicating RP-HPLC methods have therefore been reported for amlodipine alone and a combination such as amlodipine–atorvastatin.[2]

3. REGULATORY FRAMEWORK FOR STABILITY-INDICATING METHODS:

The development of a stability-indicating analytical method is an essential aspect of pharmaceutical quality control and stability testing. Regulatory guidelines provide a scientific basis for the evaluation of the stability of drug substances and products, investigation of degradation trends, assessment of photostability, and analytical validation. For the case of amlodipine besylate, such principles are especially significant because the analytical procedure should allow the determination of the drug in the presence of its degradation products and other formulation components.[2]

3.1 ICH Q1A(R2)-stability testing of new drug substance and products:

The ICH Q1A(R2) guideline Stability Testing of New Drug Substances and Products sets out the stability data package for new drug substances and drug products. The objective of stability testing is to obtain information on how the quality of a drug substance or product varies with time under the influence of environmental factors such as temperature, humidity and light. The resulting stability information is used to set storage conditions, retest periods and shelf life.[1]

In terms of analytical method development, stability studies are required to ensure that the analytical procedure is able to detect meaningful changes in the quality attributes of the drug during storage. In general, stability testing will evaluate attributes such as assay, degradation products, physical characteristics and other quality parameters. A suitable stability-indicating method should therefore be able to distinguish between the unchanged drug substance or product and any degradation products formed during storage or stress.[1]

In the context of amlodipine besylate, the Q1A(R2) guideline provides the regulatory basis for assessing the stability of the drug substance and finished dosage form. The information obtained will provide the basis for determining suitable storage conditions and shelf life and the appropriate specifications for the product.[1]

3.2 ICH Q1B-photostability testing:

ICH Q1B, Photostability Testing of New Active Substances and Medicinal Products, provides guidelines for the testing of drug substances and medicinal products under the influence of light. Photostability testing aims to identify the impact of light exposure on drug properties, such as quality, potency, or purity.[2]

Photostability evaluation usually involves exposing the drug substance and/or product to defined light conditions and conducting subsequent analytical tests. The analytical procedure should allow for differentiating between the parent compound and photodegradation products, which is critical for compounds with complex structures.[2]

Amlodipine is known to undergo photochemical degradation; therefore, photostability testing is a critical step in the development of the stability-indicating method. Thus, photolytic stressing can be

implemented in the forced degradation experiments to evaluate the ability of the developed HPLC method to separate amlodipine from its photodegradation products.[2]

Importance for amlodipine besylate:

Photostability testing is important as degradation due to light exposure could alter the amount of amlodipine and lead to the formation of more degradation products. An appropriate stability indicating HPLC method would separate amlodipine from these additional products which allows the evaluation of photostability.[2]

3.3 ICH Q2(R1)/Q2(R2)-validation of analytical procedures:

The ICH Q2 guideline gives the regulatory framework for establishing that an analytical procedure is suitable for its intended use. The current guideline, ICH Q2(R2), applies to analytical procedures used for release and stability testing of drug substances and drug products. It includes analytical procedures for analytical purposes including assay, potency, purity, impurity determination and identity.[3]

The aim of analytical procedure validation is to demonstrate that the procedure is fit for its intended purpose. Depending on the analytical use intended, validation may involve assessment of characteristics such as specificity/selectivity, accuracy, precision, response/linearity, range, detection limit, quantitation limit and robustness. On the other hand, system suitability is an integral part of analytical procedures and is generally established during method development.[3]

For a stability-indicating HPLC method, the specificity/selectivity is particularly important. The method should be able to determine amlodipine without interference from degradation products, impurities, excipients or other components that can be present in the sample. This ability is generally demonstrated using stressed samples generated during forced degradation studies. [3]

Major validation characteristics relevant to the present method to below the tabular colums;

VALIDATION PARAMETERS	AMLODIPINE HPLC METHOD
Accuracy	Demonstrates closeness of measured value to the true value.
Precision	Demonstrates reproducibility of analytical results.
Specificity	Demonstrates separation of amlodipine from degradation products and impurities.
Linearity	Demonstrates appropriate relationship between concentration and analytical response.

Range	Establish the concentration interval over which the method performs suitably.
Limit of detection (LOD)	Determines the lowest level of analyte/degradation products that can be detected, when applicable.
Limit of quantification (LOQ)	Determines the lowest level that can be quantitatively measured with suitable performance.
Robustness	Evaluates the effects of deliberate variation in analytical conditions.
System suitability	Confirms satisfactory performance of the chromatographic system before/while analysis.

3.4 USFDA guidance on forced degradation studies:

Forced Degradation or Stress Testing is applied to identify the inherent stability of the drug and possible degradation pathways. According to the guidelines, forced degradation can be utilized to generate information needed to develop and validate a stability-indicating analytical procedure. The stress conditions can involve exposing the drug to higher-than-normal temperatures and humidity, oxidation, photodegradation, and hydrolysis in different pH environments.[4]

The FDA guideline states that stress testing is particularly valuable in demonstrating the ability of the analytical procedure to distinguish between the active ingredient and its degradation products and impurities. Stress testing the drug substance and, where relevant, the drug product should be performed to a sufficient degree to generate information about the degradation pathways. It should consider the degradation mechanisms anticipated, the knowledge available, and the capability of the analytical procedure.[4]

Typical stress conditions which are considered during forced degradation consists of:

- Acid hydrolysis
- Alkali hydrolysis
- Neutral hydrolysis
- Oxidative degradation
- Thermal degradation
- Photochemical degradation
- Stress due to humidity if applicable

The objective of these studies is not to induce maximum degradation, but to generate significant degradation products so that the analytical method's ability to indicate specificity and its tendency to

indicate stability can be demonstrated. Forced degradation studies can also elucidate potential degradation mechanisms and pathways.[4]

Application to amlodipine besylate:

For the given drug substance, forced degradation studies can include the exposure to acidic, alkaline, oxidative, thermal, and photolytic conditions. After the degradation, it will be possible to analyze the mixture using the described HPLC method, which should allow assessing whether the peak of amlodipine besylate is well separated from the degraded product peaks. This fact will confirm the suitability of the method for stability testing.[4]

3.5 pharmacopoeial requirements (USP, EP, IP) for amlodipine besylate assay:

Pharmacopoeias set out official standards of quality for pharmaceutical substances and dosage forms. United States Pharmacopeia (USP), European Pharmacopoeia (EP) and other official pharmacopoeias contain monographs and general analytical requirements which facilitate the quality assessment of pharmaceutical products.[4]

For an amlodipine-containing pharmaceutical product, pharmacopoeial quality control may comprise tests related to identification, assay, impurities/degradation products, dissolution and other applicable quality attributes. The pharmacopoeial analytical procedures are important references for routine quality control and may be used as compendial methods where applicable.[4]

The assay of amlodipine in pharmaceutical formulations is of particular importance since it determines whether the dosage form contains the active ingredient within the specified quality requirements. However, in the case of stability studies an assay method alone may not be sufficient if not able to distinguish amlodipine from its degradation products. A stability-indicating analytical procedure should therefore demonstrate adequate specificity for the intact drug in the presence of degradation products and other potential interferences.[4]

From a quality-assurance perspective, the requirements from pharmacopoeias and the ICH/FDA guidance complement each other. The pharmacopoeial monographs set the official quality requirements, whereas the ICH and regulatory guidance provide the scientific framework for the stability studies, forced degradation and analytical procedure validation.[4]

4. FORCED DEGARDATIONB STUDIES:

4.1 purpose: Demonstrating specificity and method suitability:

Forced degradation studies (also known as stress testing or forced decomposition studies) are used to assess the intrinsic stability of a drug substance or drug product under deliberately severe conditions. These studies are key to the pharmaceutical development process as they will help surface degradation pathways, identify degradation products and understand the susceptibility of the drug to environmental and chemical stress. ICH Q1A(R2) suggests that stress testing can help identify potential degradation products, degradation pathways and drug substance intrinsic stability. Also, it assists in developing and validation of stability indicating analytical procedures.[5]

Forced degradation is not its sole aim to produce the highest amount of degradation, but to create degradation products under controlled and scientifically valid circumstances. The resulting stressed samples are then tested by a suitable analytical method (RP-HPLC) to assess the ability of the analytical method to baseline separate the drug from its degradation products and impurities. This will give the proof of specificity and stability indicating capacity of the method.[5]

For amlodipine besylate, the forced degradation studies are significant because published studies have shown it to be degraded under a number of stress conditions, particularly in hydrolytic and photolytic

conditions. The comprehensive study of amlodipine involved acidic, neutral and alkaline hydrolysis, oxidation, photolysis and thermal stress and revealed several degradation products by LC–MS/MS.[5]

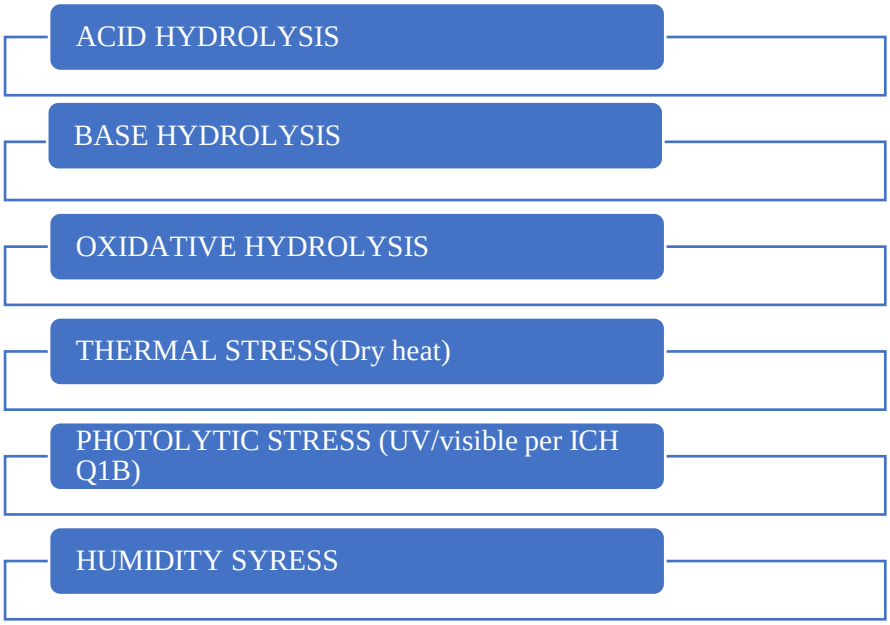
The forced degradation study from a Quality Assurance (QA) point of view would give scientific proof that the analytical method can detect the change in the drug quality and differentiate the active pharmaceutical ingredient from the degradation products. This information can then be used for stability specifications, for impurity control and routine quality control testing.[5]

4.2 stress condition applied:

The aim of forced degradation studies is usually to test the susceptibility of a drug to various chemical and physical stresses. The following major stress factors for amlodipine besylate are considered: acid hydrolysis, base hydrolysis, oxidative degradation, thermal stress, photolytic stress and humidity stress.[5]

Forced degradation studies are recommended to be performed for elevated temperature, humidity (if applicable), oxidation, photodegradation and hydrolysis at a variety of pH to address ICH stability guidance. The conditions, and the length of exposure, or both, should be scientifically justified, depending on the sensitivity of the drug.[5]

Stress conditions are:



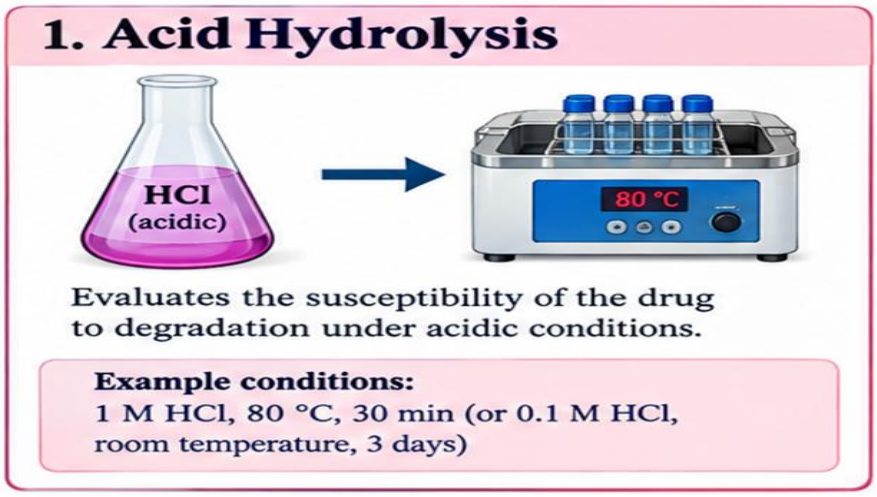
ACID HYDROLYSIS:

A chemical degradation test, acid hydrolysis is conducted to study the degradation susceptibility of the drug in the acid environment. Drug substance is generally treated with an acidic reagent (e.g., hydrochloric acid) followed by controlled heating or incubation. At the end of the predetermined stress period, the sample can be neutralized or sufficiently diluted prior to chromatographic analysis.[6]

Amlodipine has been found to degrade in acid solution. Amlodipine was subjected to stress conditions in one comprehensive study with 1 M HCl at 80°C for 30 minutes, which caused degradation under acid conditions.[6]

In a more recent study, a stability indicating HPLC of amlodipine besylate was conducted using 0.1M HCl at ambient temperature for three days and about 1% degradation of the drug was observed under the conditions of the study.This difference in the observations clearly shows that the degradation is

strongly dependent on the concentration of acid, temperature, time, and concentration of the drug and experimental conditions. Literature conditions must not be considered as universal specifications. [6]



BASE HYDROLYSIS:

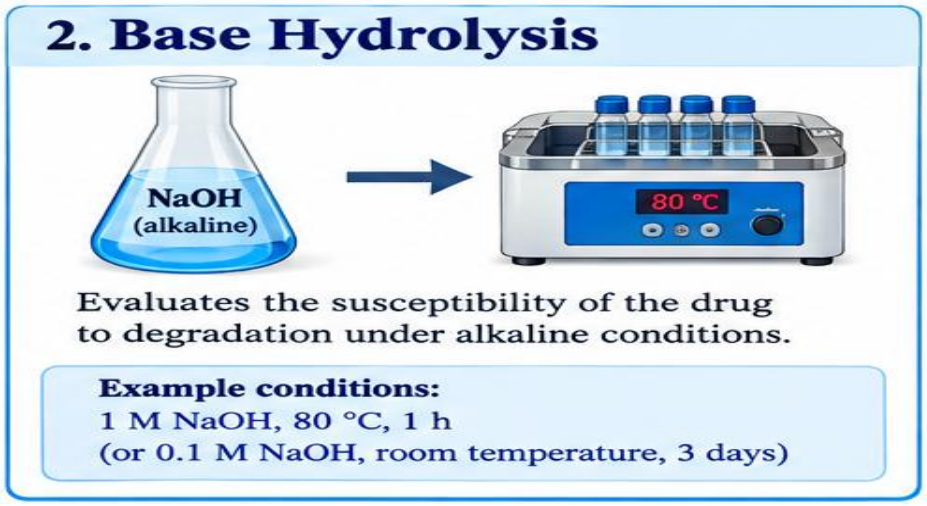
Base hydrolysis is a test for the sensitivity of the drug to alkaline conditions. The most common stressing agent is sodium hydroxide (NaOH) or another appropriate alkaline solution.[6]

Amlodipine has been found to be highly alkaline degradable. A single publication reported that amlodipine was significantly degraded under the conditions of 1 M NaOH at 80°C for 1 hour.[6]

A similar study on amlodipine besylate showed that the compound was highly sensitive to alkaline stress as 0.1 M NaOH at ambient temperature degraded it by about 43% after three days.[6]

The amlodipine molecule is also prone to alkaline hydrolysis and this aspect must be taken into consideration when formulating a HPLC method for amlodipine stability studies as the alkaline

Hydrolysis product should be separated from amlodipine peak. [6]



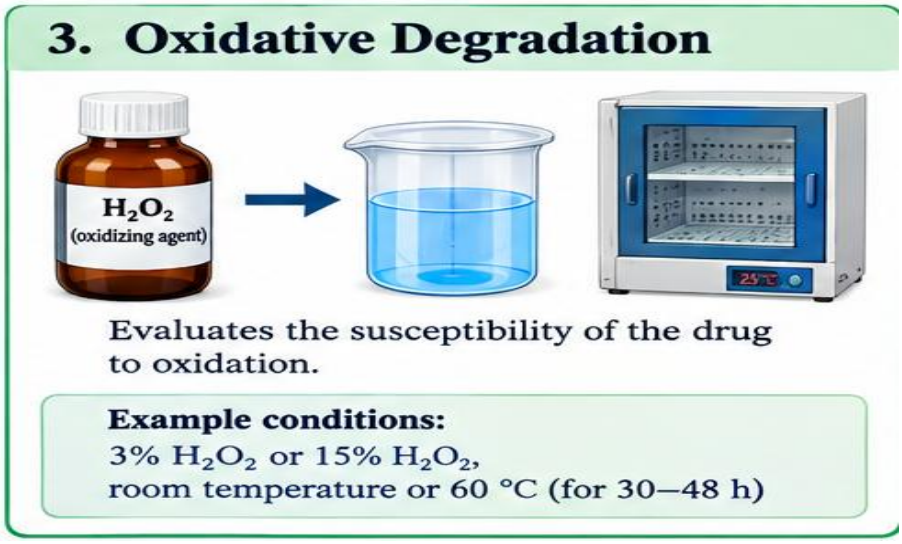
OXIDATIVE DEGRADATION (H_2O_2):

susceptibility to oxidation of a drug substance is determined by means of oxidative stress. Hydrogen peroxide (H_2O_2) can be routinely used as an oxidizing agent, but the concentration and exposure time depend on the sensitivity of the drug.[6]

The degradation of amlodipine was investigated in detail by exposing it to 15% H_2O_2 for 48 h at room temperature. In the present study, the stability of amlodipine under oxidative stress conditions was greater than that under acidic and alkaline hydrolysis conditions.[6]

A similar study of amlodipine besylate found only partial oxidation in the presence of 3% H_2O_2.[6]

Even if a limited degradation is not found, oxidative stress is extremely important for establishing the drug degradation profile and indicates if the analytical method can detect low level oxidative degradation products.[6]



THERMAL STRESS (DRY HEAT):

Thermal degradation studies determine the stability of a drug exposed to high temperatures. Thermal stress can give some insights into the temperature sensitivity of the drug to any potential chemical changes and it can help understand any potential degradation that might be occurring during manufacturing, processing or storage.[6]

ICH guidance suggests that evaluating elevated temperatures could be a part of stress testing and that the specific temperatures used depend on the properties of the drug substance and the purpose of the study.[6]

The thermal stability of amlodipine has been different under various conditions. One study found that amlodipine did not degrade significantly when exposed to heat at 50°C for 48 hours. Also, in a subsequent investigation of amlodipine besylate, the drug was subjected to 105°C for 3 days and no significant changes were observed under the above experimental conditions.[6]

From these observations, it is concluded that the thermal stability observed for one condition is not definitive for all thermal conditions and times of exposure.[6]

PHOTOLYTIC STRESS (UV/VISIBLE PER ICH Q1B):

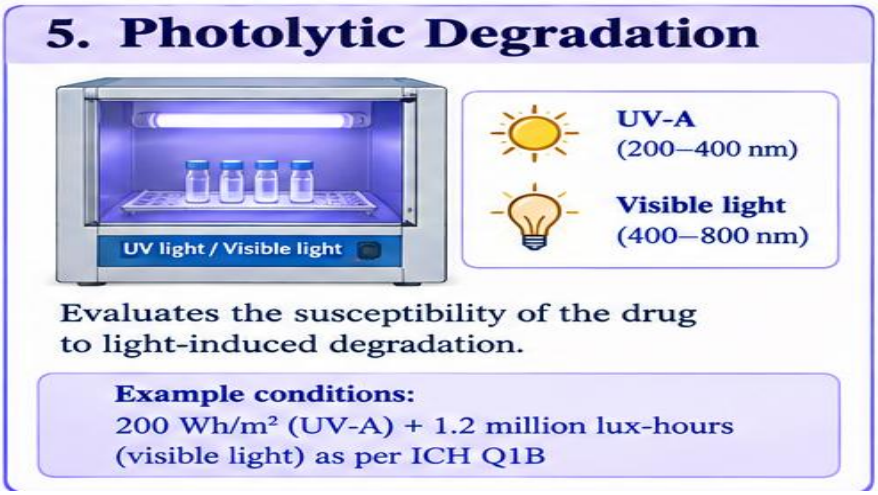
Photolytic or photochemical stress is carried out to test the susceptibility of the drug to degradation under the influence of light. Photostability is one of the important aspects of stability assessment, and ICH Q1B offers specific guidance for photo stability testing.[6]

Amlodipine has a 1,4-dihydropyridine ring that can undergo a photochemical transformation. The oxidative aromatization of dihydropyridine to pyridine has been shown to be a major degradation pathway of amlodipine and can be accelerated by light.[6]

The comprehensive stress study used fluorescent and UV-A light under ICH Q1B related conditions and the degradation products of amlodipine were investigated by LC–MS/MS.

A later amlodipine besylate study utilized 200 Wh/m² of UV-A light followed by 1.2 million lux-hours of visible light and saw about 5% degradation under those given conditions.[6]

Hence, the issue of photolytic stress is of special significance for amlodipine as it can give information on its photosensitivity and potential photodegradation pathway.[6]



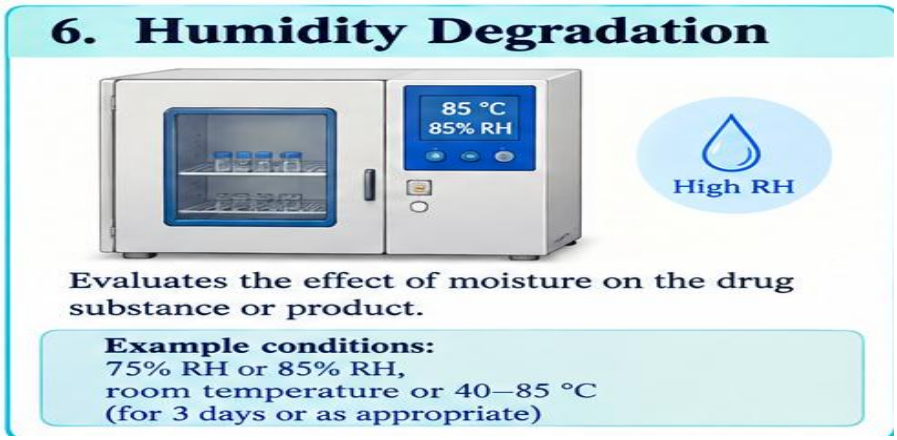
HUMIDITY STRESS:

Humidity stress evaluates the effect of elevated relative humidity on the chemical and physical stability of a drug substance or drug product. Moisture can influence hydrolytic degradation and may also affect physical properties such as appearance, crystallinity and moisture content.[6]

ICH stability guidance recommends considering humidity, such as 75% relative humidity (RH) or greater where appropriate, during forced degradation studies. However, the exact humidity and exposure duration should be scientifically justified based on the nature of the drug and dosage form.[6]

For amlodipine besylate, thermal-humidity stress has been investigated experimentally. One study exposed the drug to 85°C/85% RH for three days and reported no significant degradation under those particular conditions.[6]

Humidity studies are particularly useful for drug products because moisture can interact with both the API and excipients and may influence the overall stability of the formulation.[6]



4.3 Acceptable degradation range (typically 5-20%) and rationale:

One of the critical factors to be taken into account for forced degradation studies is the level of degradation. One of the most popular development strategies is to aim for about 5-20% degradation of the active, but this is not a regulatory requirement. The degradation level should be scientifically substantiated according to the stability properties of the drug and the aim of the study.[6]

The aim of controlled degradation is to create degradation products in sufficient amounts to test the analytical method, but not so many that could result in formation of metabolites which would not be relevant under normal storage conditions. ICH indicates that stress conditions and exposure time should be matched to the sensitivity of the product and excessive decomposition should be avoided.[6]

In an example amlodipine degradation study to be published, for instance, the stress conditions were optimized with the goal of a degradation of about 15-20%. Under other conditions, however, they have been able to obtain a degradation of about 43% under alkaline stress, demonstrating that degradation extent is very dependent on the stress protocol.[6]

As such, the degradation target should be viewed as a method-development strategy and not a regulatory limit.[6]

QA significance

A controlled degradation process helps the analyst to prove that:

4.4 Identification /characterization of degradation products (mass balance, peak purity via PDA/LC-MS):

The identification and characterization of degradation products are key items in an extensive forced degradation study. While HPLC chromatograms may at first illustrate the creation and separation of extra peaks, the chromatographic separation may not be enough to identify the chemical structure of an unidentified degradation product.[6]

So, multiple analytical tools can be used. The UV spectral characteristics can be used for evaluation by photodiode array (PDA/DAD) detection and the purity of the peaks can be checked, while the molecular-mass and fragmentation information can be useful for structural characterization by LC–MS or LC–MS/MS.[6]

A study of degradation products by LC–MS/MS has been shown to be useful in the case of amlodipine. Nine degradation products were identified in a comprehensive study under various stress conditions and LC–MS/MS and MS^n fragmentation studies were conducted to examine the structures and degradation pathways.[6]

The study of the major degradation products of amlodipine besylate by HPLC with mass spectrometry indicated that the amlodipine peak did not contain interfering impurities under the various stress conditions tested.[6]

Peak Purity (PDA):

The UV spectra are obtained throughout the chromatographic peak using PDA/DAD analysis. Comparisons of spectra for the leading, apex and trailing portions of a peak may give clues to the spectral homogeneity.[6]

The PDA peak-purity analysis in the reported amlodipine besylate HPLC method has been carried out between 200 and 450 nm and the amlodipine peak has been seen to meet the peak-purity criteria of the study for the stressed samples. An additional investigation was conducted for possible co-elution by LC/MS analysis.[6]

However, PDA peak purity should not be taken as an absolute indication of chemical purity due to the possibility of similar UV spectra for related compounds and the need for additional evidence. Orthogonal techniques such as LC–MS can provide additional evidence when the characterization of degradation products is required.[6]

LC–MS/MS Characterization:

The analysis technique of LC–MS/MS combines chromatographic separation with mass spectrometric detection. Chromatographic retention times provide separation information, while mass-to-charge ratio and fragmentation patterns provide structural information.[6]

Analysis of amlodipine utilizing LC–MS/MS has been successful in investigating degradation products formed by acidic and alkaline hydrolysis and other stress conditions, and mass information and fragmentation patterns have been used to propose structures and establish degradation pathways.[6]

In addition, an acid degradation impurity of amlodipine besylate has also been isolated and characterized using complementary techniques, including Q-TOF, NMR, IR and single-crystal X-ray analysis, highlighting the need for orthogonal analytical techniques to provide definitive structural identification when needed.[6]

4.5 Degradation pathway summary and proposed degradation scheme:

The final stage in forced degradation assessment is to integrate the chromatographic, spectroscopic and mass-spectrometric findings together into a proposed degradation pathway for the drug.[7]

For amlodipine, published investigations have shown that the drug can degrade along different pathways depending on the stress condition, with hydrolytic degradation occurring under acidic and alkaline conditions, and photochemical/oxidative transformation of the dihydropyridine moiety resulting in formation of a corresponding pyridine-type degradation product.[7]

A proposed degradation scheme should therefore include:

Amlodipine besylate

↓

Acid hydrolysis → Acid degradation products

↓

Base hydrolysis → Alkaline degradation products

↓

Oxidative stress → Oxidation products

↓

Photolytic stress → Photodegradation products / aromatization products

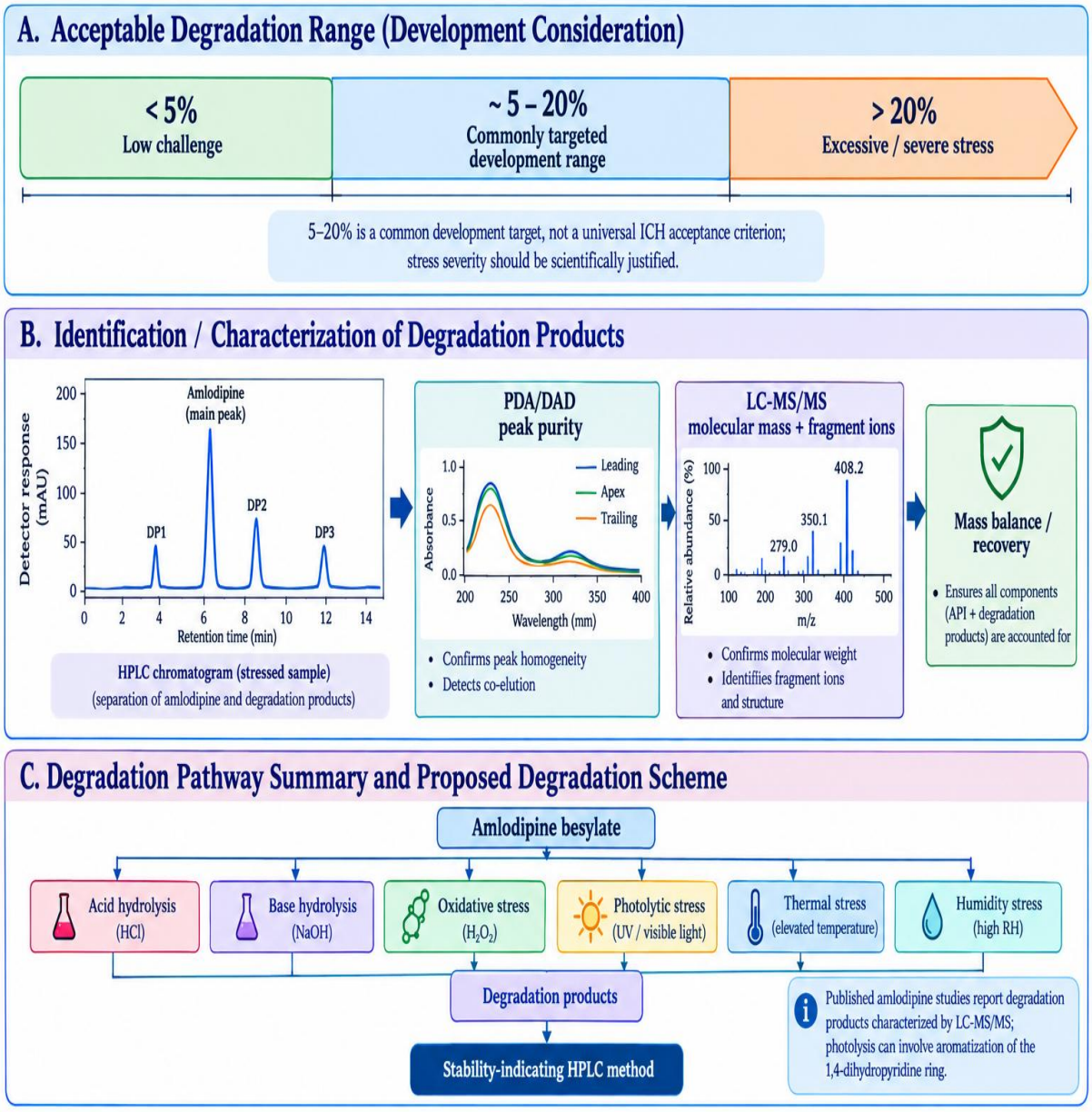
↓

Thermal stress → Thermal degradation products, if formed

↓

Humidity stress → Hydrolytic/physical changes, if observed

The individual structures of degradation products should only be assigned on adequate analytical justification. Published LC–MS/MS studies have provided evidence for multiple amlodipine degradation products and proposed pathways based on molecular masses and fragmentation behavior.[7]



1.1 Schematic diagram for acceptable degradation, Identify and characters of degradation products, degradation pathway summary and proposed degradation scheme.

5. HPLC METHOD DEVELPOMENT:

High Performance liquid chromatography (HPLC) has become one of the most popular analytical methods for the development of stability indicating methods for pharmaceutical substances and products. A HPLC method development is necessary to ensure the separation of the active pharmaceutical ingredient (API), process-related impurities, degradation products and other potentially interfering components for amlodipine besylate. The analytical procedure should accordingly be selective and selective enough for its intended application and be robust consistent with the principles outlined in ICH Q2(R2).[6]

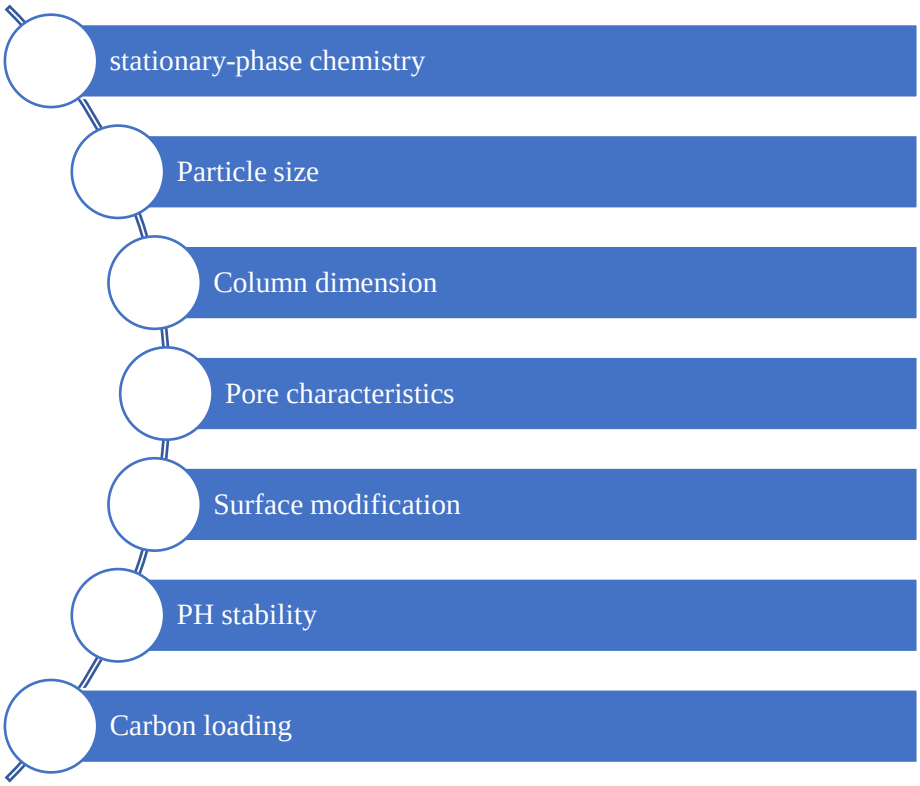
In general, a stability indicating HPLC method must be systematically developed by choosing the stationary phase, mobile phase, detection wavelength and the chromatographic mode, and optimizing critical chromatographic parameters. The third approach should give acceptable peak shape, good resolution, a suitable analysis time and reproducible performance.

5.1 Selection of stationary phase, mobile phase, detection wavelength:

Selection of stationary phase:

An adequate separation between amlodipine besylate and related substances/degradation products would be obtained by employing the stationary phase. Amlodipine can be analysed by reversed-phase HPLC (RP-HPLC) with octadecylsilane (C18/ODS) columns, as these columns are versatile and have the advantage of efficient separation of moderately lipophilic pharmaceutical compounds.[6]

Column characteristics such as:

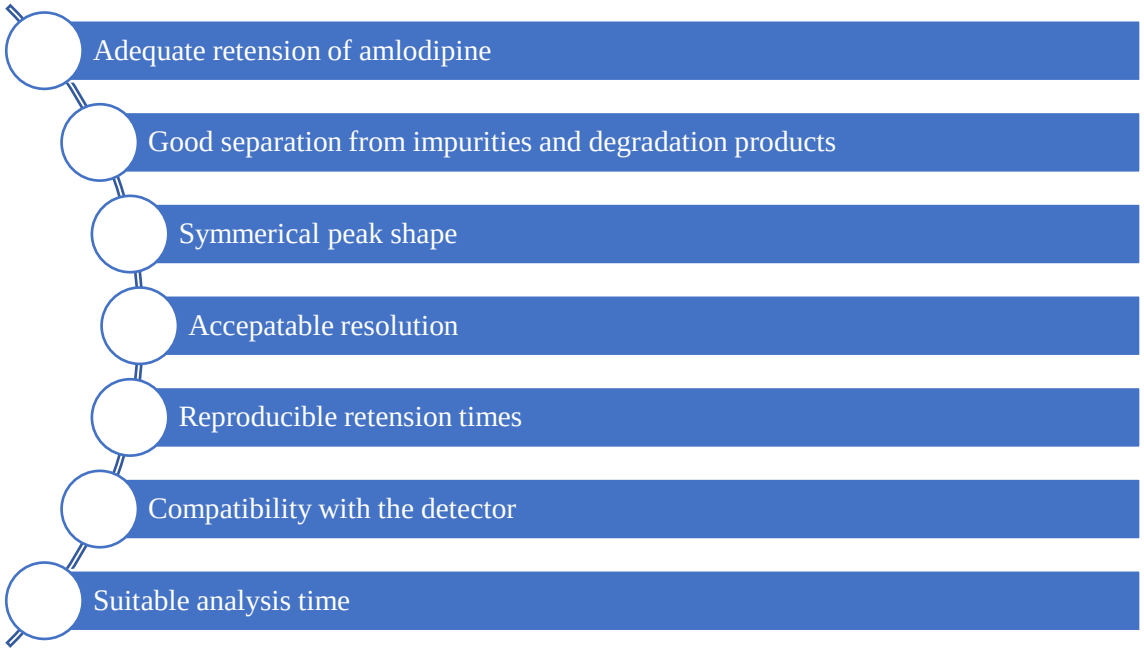


The pKa of Amlodipine is about 8.6 (basic compound). As a result, the chromatographic behavior of this substance can be greatly affected by the pH and the stationary-phase characteristics of the mobile phase. A high-pH compatible Kinetex EVO C18 column was used in a recent stability indicating method to achieve better peak shape and separation of amlodipine and its impurities.[6]

For instance, Jeelani and Kouznetsova devised an effective stability-indicating technique using a 100 × 4.6 mm, 2.6 µm C18 column, which could successfully resolve amlodipine from seven organic impurities and degradation products in 15 min run.[6]

Selection of mobile phase:

Mobile-phase selection is another critical aspect of HPLC method development. The mobile phase should provide:



For amlodipine besylate, commonly investigated mobile-phase components include aqueous buffers, ammonium hydroxide, acetonitrile and methanol.[6]

Because amlodipine is a basic compound, controlling the mobile-phase pH can significantly affect its ionization and chromatographic behavior. In the 2023 stability-indicating method, 0.4% ammonium hydroxide in water and methanol were used in gradient mode. Increasing ammonium hydroxide concentration from 0.1% to 0.4% improved amlodipine peak shape, with a tailing factor of not more than 1.5 reported for the optimized method. [6]

An alternative stability-indicating RP-HPLC method for amlodipine/valsartan tablets used phosphate buffer containing triethylamine at pH 3.0 together with methanol-based mobile phase, demonstrating that different chromatographic systems can be suitable depending on the analytical objective and formulation. [6]

Therefore, the mobile phase should not simply be selected from a previously published method; it should be optimized according to the drug substance, impurities, degradation products and required analytical performance.[6]

Selection detection wavelength:

The selection of the detection wavelength depends on the UV absorption properties of the analyte and whether the mobile phase or the formulation components will affect the sensitivity.[6]

A wavelength of 237 nm is an important analytical wavelength for amlodipine besylate and has been included in stability indicating HPLC procedures. According to the authors, the HPLC-UV method used

the wavelength of 237 nm, which is the UV maximum of amlodipine and is also recommended by the USP and European Pharmacopoeia methods.[6]

Thus, wavelength 237 nm can be used as the initial wavelength of development, but the final wavelength of detection needs to be experimentally substantiated for the specific method.[6]

5.2 Method optimization strategy (isocratic vs gradient, QbD-based DoE approach if applicable):

After the selection of stationary phase, the mobile phase and the detection wavelength, the chromatographic conditions are optimized systematically.

- Important variables include:
- Mobile-phase composition
- Mobile-phase pH
- Organic solvent type
- Organic solvent percentage
- Flow rate
- Column temperature
- Injection volume
- Gradient program
- Detection wavelength
- Run time

The aim is to achieve acceptable peak symmetry and good resolution of the amlodipine, its related substances and degradation products in a reasonable analysis time.[6]

Isocratic and gradient elution:

In isocratic elution, the composition of the mobile phase remains the same during the chromatographic separation. It can be beneficial for analyte retention and/or when reasonably short separation times can be achieved.[6]

In a gradient elution, the composition of the mobile phase is varied during the run instead. When the sample consists of compounds that have significantly different retention times, such as the parent drug and many impurities and degradation products.[6]

For instance, the 2023 stability indicating method of amlodipine employed 0.4% ammonium hydroxide and methanol gradient program and separated amlodipine and seven impurities in 15 minutes.[6]

Quality-by-Design / DoE approach:

Another alternative, if multiple chromatographic variables are interacting, is to use a systematic or Design of Experiments (DoE) approach. These methods enable the systematic study of the effect of variables (like the composition of the mobile phases, their pH, flow rate and temperature) without having to adjust one variable at a time.[6]

ICH Q2(R2) focuses on the need to establish the fitness of analytical procedures for their intended use, and permits scientifically sound methods for analytical procedure development and validation.[6]

5.3 System suitability parameters (theoretical plates, tailing factor, resolution):

System suitability testing (SST) is used to run before or during the chromatographic analysis to determine if the HPLC system and analytical procedure are working properly for the intended analysis. System suitability testing (ICH Q2(R2)) is defined as a method of ensuring that the system and analytical operations are sufficient and are used to identify potential failures.[7]

Several parameters for the chromatography are important:

Theoretical plates (N):

Theoretical plate number is an indicator of column efficiency. Theoretical plate number is a good measure of the efficiency of the column and sharper the peak, the higher the theoretical plate number.

The number of theoretical plates can be calculated using:

$$N = 16(t_r/W)^2$$

where:

N = number of theoretical plates

The time for which the analyte is retained in the column. Retention time of the analyte (t_r).

W = highest width at the base

The acceptance criterion should be defined based on the quantitative analytical method or the relevant pharmacopoeial requirement used, and not a single value.[7]

Tailing factor:

The tailing factor is related to peak symmetry. Too much tailing will have negative impact on integration, resolution and quantitative accuracy.

The simplified expression is:

$$T = W_{0.05} / 2f$$

where:

$W_{1/2}$ = width at half height

f = distance between the peak maximum and the front of the 5% height.

The published stability indicating method for amlodipine optimized the concentration of ammonium hydroxide, and the amlodipine peak tailing factor from this method was not more than 1.5.[7]

Resolution:

Resolution is a measure of separation of two adjacent chromatographic peaks.

For a stability-indicating HPLC method, it is especially crucial to have a good separation of the amlodipine peak from degradation products and related substances.

The standard equation used is:

$$R_S = 2(t_{R2} - t_{R1)} / W1 + W2$$

where:

Rs = resolution

tR1 and tR2 = retention times of two adjacent peaks

W1 and W2 = corresponding peak widths.

The typical resolution value greater than or equal to 2.0 is usually regarded as good separation of critical peak pairs but for specific methods this value should be scientifically justified.[7]

For the amlodipine method of the 2023, the resolution between the peak of amlodipine and the peak of the adjacent peaks was not less than 2.0; the resolution of the impurities from each other was not less than 2.0.[7]

Retention time:

Retention time gives information of the chromatographic behaviour of the analyte and helps in identification of the peak. But retention time should not be the only criterion used for identification of a degradation product. Further confirmation can be obtained by orthogonal methods, e.g. by comparing the PDA spectra or by LC-MS.[8]

This is especially crucial in stability-indicating studies in which degradation products may be present that are unknown.[8]

5.4 Chromatographic conditions summary table:

Parameter	Optimized/example condition for amlodipine SI-HPLC	Scientific purpose
HPLC mode	RP-HPLC	Separation of API and impurities
Stationary phase	C18	Provides suitable retension /selectivity
column	Kinetex EVO C18,100*4.6MM,2.6m	Efficient separation
Mobile phase A	o.4 % ammonium hydroxide in water	Controls retension /peak shape
Mobile phase B	methanol	Organic modifier
Elution	gradient	Separation of multiple impurities / degradants
Detection	UV/PDA	Qualitative detection
Detection wavelength	237mm	Suitable UV response for amlodipine
Flow rate	1.0ml/min	Controls analysis efficiency
Column temperature	35^0C	Improves reproducibility
Injection volume	20µL	Sample introduction
Run time	15 min	Rapid separation
Critical resolution	≥2.0 reported	Separation of critical peak pairs
Peak tailing	≤1.5 reported for amlodipine	Peak symmetry
application	Assay +related substances + degradation products	Stability indicating analysis

6. ANALYTICAL METHOD VALIDATION (ICH Q2(R_1)):

The validation of a pharmaceutical analytical method is a crucial part of pharmaceutical quality assurance which indicates that an analytical method is fit-for-purpose. Stability indicating HPLC method validation is especially of importance as the method needs to correctly quantify the drug substance or drug product in the presence of impurities, process related substances and degradation products.[9]

ICH Q2(R2) states that the analytical procedure validation is a procedure that gives documented evidence of the analytical procedure's fitness for its intended purpose. Specificity/selectivity, accuracy, precision, response/linearity, range, detection limit, quantitation limit and robustness may be evaluated as part of the validation, depending on the analytical application.[9]

The amlodipine besylate HPLC stability indicating method should, therefore, provide sufficient resolution with regards to its degradation products and related impurities and acceptable analytical performance over the desired concentration range. A newly published stability-indicating HPLC method was validated to demonstrate specificity, linearity, accuracy, precision, LOQ and robustness for amlodipine besylate and showed separation of amlodipine from seven impurities.[9]

1. Specificity/selectivity (peak purity, resolution from degradants)
2. Linearity and range
3. Accuracy (% recovery)
4. Precision (repeatability, intermediate, reproducibility precision)
5. LOD and LOQ
6. Robustness (deliberate variations)
7. System suitability testing
8. Solution stability

1.Specificity/selectivity (peak purity, resolution from degradants):

Specificity of an analytical procedure is its capacity to accurately identify or quantify the analyte in the presence of other components that might be expected to be present such as degradation products, excipients, impurities, and components of the matrix. Specificity is one of the most important method validation parameters for a stability indicating HPLC method since degradation products should not interfere with the quantification of the parent drug.[9]

Specificity can be shown by analysing:

- Blank/diluent
- Amlodipine reference standard
- Unstressed sample
- Individual impurity solutions
- Sample with known contaminants
- Acid-stressed sample
- Base-stressed sample
- Oxidative-stressed sample
- Thermal-stressed sample
- Photolytically stressed sample
- Humidity-stressed sample

The peaks should be well resolved and there should be no significant peak overlaps at the amlodipine retention time.[9]

Peak purity analysis:

If available, the use of a PDA/DAD detector can give further proof of the spectral homogeneity of the amlodipine peak, when performing peak purity analysis. The calculated purity angle is lower than the purity threshold, as set by software criteria, then the peak is spectrally pure.[9]

PDA peak purity analysis was carried out for the stressed samples in the published HPLC stability indicating method for Amlodipine Besylate and the amlodipine peak was found to be free from co-eluting degradation products. There was also no co-elution detected in the LC/MS analysis.[9]

Resolution from degradants:

Capable of resolving degradation products. Another factor that is of importance in consideration of specificity is the chromatographic resolution.

$$R_s = 2(t_{R2} - t_{R2}) / w_1 + w_2$$

where:

tR2 = retention time of second peak
tR2= retention time of second peak
W1and W2 = corresponding peak widths.

The resolution between adjacent impurity peaks and the separation of amlodipine from the adjacent peaks was greater than 2.0 in the reported amlodipine method indicating the specificity of the method.[9]

2.linearity and range:

Linearity is the property of an analytical procedure that gives a response that is directly proportional to the concentration of analyte in a given range of concentration.

The relationship can be typically represented by:

$$y = mx + c$$

where:

The detector response or peak area is equal to y.
x = analyte concentration
m = slope
c = intercept.

Linearity is usually determined by making a series of concentrations in the range of the intended analytical range and graphing the analytical response against concentration. Then regression analysis is done.[9]

ICH Q2(R2) suggests that the relationship between the analytical response and the concentration of the analyte should be evaluated over the working range by suitable statistical evaluation.[9]

Application to amlodipine besylate

The range might be appraised around the nominal concentration for an assay method, while, for an impurity method, the concentration range is likely to be lower, and close to the specification level.

With respect to HPLC method published in one:

- Linearity of the assays was conducted over the range of 50–150% of the nominal concentration.
- The correlation coefficient was more than 0.999.
- Impurity linearity was assessed for the range of 0.05–1.0% depending upon the impurity.
- The correlation coefficients of the impurities evaluated were generally above 0.99.

Range:

The range is the range of concentration within which the analytical procedure is shown to have suitable qualities with respect to the parameters considered, such as accuracy, precision and response.[9]

The range to be used for the proposed stability-indicating method should be justified based on whether the method is to be used for:

- Assay determination,
- Related substances/impurity determination,
- Degradation-product determination, or
- A combination of these applications.[9]

3. Accuracy (% recovery):

Accuracy is a statement of how close the value obtained from the analytical procedure is to the accepted/ reference value.[9]

In HPLC, the accuracy is usually determined by recovery tests that involve the injection of known amounts of analyte into the sample matrix and then analyzing the resulting sample on the HPLC system with the method developed.

The percentage recovery can be determined by:

The percentage of recovery is calculated as follows:

$$\% \text{ recovery} = \text{amount recovered} / \text{amount added} * 100$$

The accuracy should be determined over the range of concentrations intended for the analysis with a suitable number of concentration levels and replicate determinations.[9]

Accuracy for amlodipine assay

Typical levels for an assay method are:

- 80%
- 100%
- 120%

Other levels may be justified, based on the intended method and validation protocol.

Typically, recovery is determined at concentrations that are relevant to the specification and reporting level when determining impurities.[9]

The published HPLC stability study of amlodipine stability indicated acceptable results at the assay levels of 80%, 100%, and 120%. The same study was conducted to evaluate the recovery of the impurities in the impurity-specific concentration ranges.[9]

Accuracy shows that the method is capable of measuring the amount of amlodipine in the sample without introducing significant amounts of bias from the sample matrix or other constituents.[9]

4. Precision (repeatability. Intermediate, reproducibility precision):

The closeness of agreement between individual measurements obtained from several samples of a homogeneous sample taken under specified conditions is called precision.[9]

There are three levels of precision:

Repeatability:

Repeatability is precision performed within the same operating conditions in a short time period.

It may involve:

- Same analyst
- Same instrument
- Same laboratory
- Same method

Multiple replicate preparations/injections.

This is often reported as %RSD.

%RSD = Standard deviation / Mean * 100

Intermediate Precision:

Intermediate precision assesses the differences between the results obtained from the various settings of the same instrument in the same laboratory, which can include:

- Different analysts
- Different days
- Different instruments
- Different column lots

As appropriate, a variety of environmental conditions.[9]

Reproducibility:

Reproducibility normally relates to precision between laboratories and is especially important for collaborative studies or method transfer.

Repeatability and intermediate precision were tested for the published HPLC method for amlodipine. The repeatability of the impurities and the assay precision were both less than 3% RSD for the impurities evaluated. Another analyst, a different HPLC model, another day, and a different lot of column was also used to evaluate intermediate precision.[9]

5. limit of detection (LOD) and limit of quantification (LOQ):

Limit of detection (LOD):

The amount of a chemical that can be detected twice in a sample.

The lowest concentration of the analyte that can be measured but not necessarily be quantified with adequate accuracy and precision in the sample is the limit of detection (LOD).

One of the most popular signal-to-noise techniques is to assume the signal is about three times as strong as the background noise:

$$S/N \approx 3:1$$

Limit of Quantification (LOQ):

The smallest quantity of an analyte that can be measured with the required accuracy and precision.

A common signal to noise approach is to think about:

$$S/N \approx 10:1$$

LOQ is especially relevant for chromatographic impurity methods in which the degradation products can be present at very low levels.[9]

Alternatively, if the standard deviation of the response and the slope of the calibration curve can be estimated, then LOD and LOQ can be estimated statistically.

LOD = 3.3 X / S

LOQ = 10 X / S

where:

X = sample mean of the response

S = Slope of the calibration curve.

The chosen method should be scientifically justified using the analytical procedure and appropriate validation approach.[9]

6. Robustness:

The property of an analytical procedure, which means that it is not substantially affected by small but deliberate variations in its method parameters.[9]

The parameters that can be assessed in an RP-HPLC method are:

- Mobile-phase composition
- Buffer concentration
- Mobile-phase pH
- Flow rate
- Column temperature
- Detection wavelength
- Organic solvent composition
- Injection volume
- Type/column lot (as applicable).

7. system suitability:

System suitability testing (SST) is carried out before or during chromatographic analysis to verify that the HPLC system and chromatographic conditions are capable of delivering acceptable result.[9]

Important parameters include:

Theoretical Plates:

Theoretical plate number indicates column efficiency.

One commonly used formula is:

$N = 16(t_R / W)$

where:

N = theoretical plate number

tR = retention time

W = peak width.

Higher theoretical plate numbers mean higher column efficiency, in general.

Tailing Factor:

Tail factor provides information about peak symmetry.

Excessive tailing can deteriorate the ability to quantify and separate peaks

Resolution:

Resolution defines the degree of separation between two adjacent peaks.

$$R_S = 2 (t_{R2} – t_{R1}) / W1 = W2$$

For a stability-indicating method, critical pairs such as amlodipine and a nearby degradation product/impurity should have sufficient resolution.

Retention:

Retention time should remain sufficiently consistent during analysis.

In the published amlodipine stability-indicating method, system suitability included chromatographic performance criteria, and the method achieved resolution of at least 2.0 between relevant peaks with acceptable peak shape.[9]

9.Solution stability:

Solution stability is a measure of whether the prepared standard and sample solutions are chemically and analytically stable to the time of analysis.

This is important, since the degradation of the standard or sample solution in the interim period could result in inaccurate assay or impurity results.[9]

Solution stability may be assessed by comparing:

- Freshly prepared solution
- Stored solution after a specified period
- Peak area
- Assay result
- Impurity/degradation-product profile
- % difference or %RSD.

Typical storage conditions may include:

- Room temperature

- Refrigerated conditions
- Protected from light, if applicable.

The actual storage time should be experimenter-derived, rather than assumed.[9]

For example, in the published stability-indicating HPLC method for amlodipine, the sensitivity and spiked solutions were evaluated for 24 hours at room temperature, and were found to be stable for the duration of the study based on the defined peak-area criterion.[9]

7. QUALITY ASSURANCE PERSPECTIVES:

Quality assurance (QA) is a critical function to ensure that the analytical method(s) employed for pharmaceutical testing are scientifically valid, validated, scientifically controlled and provide reliable data throughout the product lifecycle. For a stability-indicating HPLC method for amlodipine besylate, QA also involves control of analytical procedures in the routine batch release, stability testing, investigation of degradation trends, change control, data integrity, method transfer, and method lifecycle.[10]

ICH Q10 outlines a pharmaceutical quality system which is applicable throughout the pharmaceutical product lifecycle and ICH Q14 is a science- and risk-based approach for the development and maintenance of analytical procedures for release and stability testing**.**[10]

7.1 Role of stability indicating methods in batch release and shelf life determination:

A stability-indicating analytical method is an analytical procedure that can be used to accurately measure the API in the presence of potential degradation products, process-related impurities and other components that may be formed during storage.[10]

A stability-indicating HPLC method would be of special significance for amlodipine besylate, because it can be degraded under various stress conditions such as hydrolytic, oxidative, thermal, and photolytic. Thus, the method used for the stability studies should be able to separate the drug and its degradation products. Rise to the occasion Batch release.[10]

Validated HPLC method can be used during batch release testing to determine:

- Amlodipine Besylate Assay
- Substances that may be related or impurities
- Degradation products
- Chromatographic purity
- Meets specified requirements.

The method should be sufficiently specific and reproducible so as to not being rejected or accepted incorrectly due to analytical interference in a batch.

Role in stability testing:

Stability testing assesses the ability of the drug substance or drug product to withstand changes over time and under environmental conditions (such as temperature, humidity and light). The data obtained are used to determine storage conditions and shelf life/retest period.[11]

Amlodipine besylate stability indicating HPLC testing can be used to monitor:

Amlodipine concentration ↓

Degradation products ↑

Related substances ↑

Chromatographic purity ↓

A proper stability indicating method is thus readily available as an analytical tool for chemical stability monitoring during the intended shelf life.[11]

7.2 Integration with change control and CAPA when degradation trends shift:

Change control:

For controlled manufacturing, packaging, storage, and/or analytical change that may impact product quality identified during an investigation, the change needs to be controlled under the site's approved change control system.[12]

ICH Q10 requires the performance of change management based on quality risk management, consideration of changes in relation to the product/process knowledge and regulatory commitments, engagement of relevant expertise and assessment of the change following implementation to ensure the change's aims were met and that there were no adverse effects on product quality.[12]

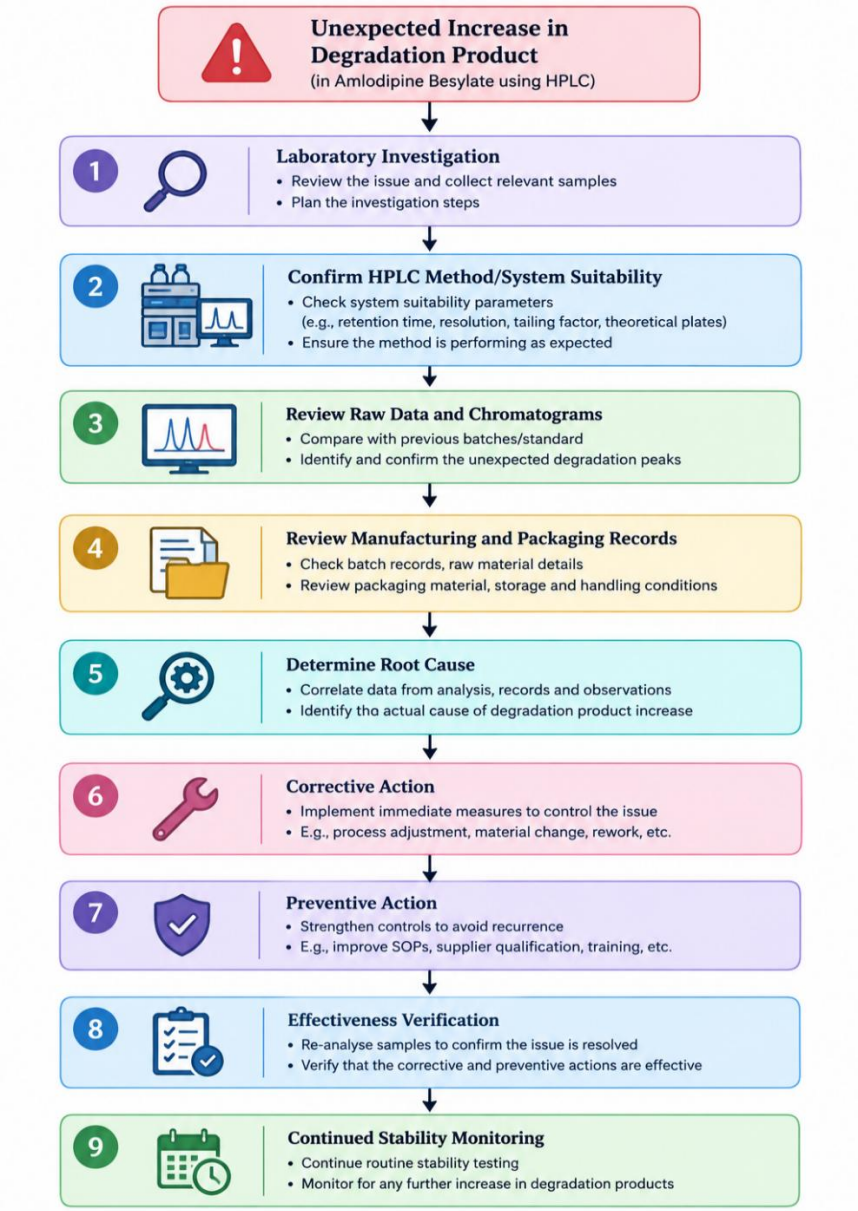
Several factors may contribute to an unexpected change, including:

- ★ Raw-material variability
- ★ Manufacturing-process variation
- ★ Change in formulation composition
- ★ Change in manufacturing equipment
- ★ Container-closure problems
- ★ Increased exposure to moisture
- ★ Temperature excursion
- ★ Light exposure
- ★ Packaging changes
- ★ Analytical method variation
- ★ Instrument performance problems
- ★ Column or reagent variability.

CAPA (corrective and preventive action):

CAPA may be initiated when an investigation identifies a confirmed or recurring cause.

Example:



CAPA investigations

7.3 Out of specification (OOS) and out of trend (OOT) handling in stability studies:

OOS investigation in stability studies:

When OOS result are noted during a stability test, the result must be recorded and investigated following an approved procedure. The investigation will be used to identify if the outcome is a laboratory error, analytical method error or a product quality problem.[13]

The following steps are typically part of an investigation:

1. Initial laboratory investigation:

The original analytical data, chromatograms, calculations, sample preparation records and instrument records should be reviewed by the laboratory analyst and Quality Assurance (QA) personnel. For HPLC,

system suitability, peak integration, retention time, baseline behaviour, and analytical system performance should be covered.[13]

2. Analytical method/reagent performance review:

The analyst should verify if the HPLC method was carried out correctly. This is done by verifying the mobile-phase preparation, standard and sample preparation, column condition, detector condition, injection sequence, and system suitability results. All documented instrument malfunction and/or deviation from procedure should be assessed.[13]

3. Investigation of possible laboratory errors:

Possible laboratory sources of error are weighing error, wrong dilution, contamination, wrong filtration, wrong calculations, or instrument failure. Any potential cause should be based on evidence and not on assumption.[13]

4. manufacturing and packaging investigation:

If the laboratory investigation fails to produce an assignable analytical cause then the investigation should be expanded, as appropriate, to manufacturing and packaging records. Some of these factors can be raw material differences, manufacturing variations, moisture, packaging, and storage.[13]

5. root cause identification and corrective action:

The investigation should determine the most likely cause with supporting evidence. Then, corrective and preventative action (CAPA) should be taken and the effectiveness of these actions should be measured. [13]

OOT investigation in stability studies:

OOT investigations are directed towards the detection of any surprise changes in the stability data prior to the results being outside of the approved specifications. These investigations are especially useful in monitoring degradation products, assay values, dissolution results, water content, and other parameters that are critical for the stability of the formulation.[14]

Stability testing of amlodipine besylate tablets, for instance, could reveal the presence of a degradation product which increases in concentration at each testing time point by HPLC analysis. Any increase in the levels above the approved maximum concentration, but within the approved limit should be considered under the organisation's OOT procedure.[14]

An OOT investigation can include the following activities:

a. Trend review: Review the current stability result compared to past stability results from the same batch and, if scientifically sound, with similar batches.
b. Chromatogram Review: Peaks, areas, retention times, shapes, integration and new peaks (causing degradation).
c. Analytical review: Ensure system suitability, instrument performance, sample preparation and compliance with validated analytical method.
d. Environmental Review: Review the temperature and humidity records from the stability chamber, stability chamber excursions, and stability chamber interruptions.
e. Packaging review: Determine if there is a need for container closure integrity review or if there is moisture and light protection issue.
f. Quality assessment: Identify if the trend is likely to impact on product quality, shelf life or specification.[14]

Where a probable cause or quality risk is found, take appropriate action and observe subsequent stability results: CAPA and follow-up.[14]

8.REGULATORY AFFAIRS TOUCHPOINTS:

8.1 stability data requirements in regulatory submissions:

Stability studies are a critical component of a pharmaceutical regulatory submission as they give scientific evidence of the change in the quality of a drug substance or drug product during storage in a defined environment. These studies facilitate the proper storage conditions, retesting time for the drug substance, and the drug product shelf life.[15]

Stability data is used by regulatory agencies like the United States Food and Drug Administration (USFDA), the European Medicines Agency (EMA), and the Central Drugs Standard Control Organisation (CDSCO) in India to assess the proposed shelf life and storage conditions of the product based on the evidence provided.[15]

Types of Stability Studies:

There are various types of stability studies that can be performed on pharmaceutical products.

1. Long-term stability studies:

Long term studies test the product for the recommended storage conditions for the proposed shelf-life. The studies are the main source of establishing or confirming the retest period and/or shelf life.[15]

2. Accelerated stability studies:

Accelerated studies introduce the product to high temperature and/or humidity to assess environmental stress effects when applicable. They could be relevant for degradation properties and to support shelf life assessment (if applicable) in accordance with relevant regulatory guidance.[15]

3. Intermediate stability studies:

Accelerated testing may result in a significant change for which the applicable guideline specifies further testing under intermediate conditions, in which case intermediate studies may be required.[15]

4. Photostability studies:

The photostability studies assess the impact of light on the drug substance or drug product. ICH Q1B is a guideline aimed at the photostability testing of new drug substances and new drug products.[15]

5. Forced degradation studies:

Forced degradation studies are conducted with a drug substance or a drug product under conditions of acid, base, oxidation, heat, or light to assess degradation pathways and to determine if the analytical procedure differentiates the drug substance from those of the degradants.

Forced degradation should not be mistaken for routine long term or accelerated stability testing and is used to support the development of analytical methods.[15]

HPLC in the role of stability indicating HPLC.Stability-Indicating HPLC in Regulatory Submissions:

Stability indicating HPLC method is used as an analytical tool to track the content of the assay and related degradation products during the stability studies.

- An appropriate method, which is well developed and validated, can assist with:
- Determination of the drug content at different stability intervals.
- Analysis of relevant degradation products to detect and quantify.

- Comparing the chromatographic profile over time.
- Tests of impurities during storage.
- Analysis of unusual stability findings.
- Establishment of dependable analytical data for shelf-life.[15]

8.2 comparison of stability / shelf life filing expectations: USFDA, EMA and CDSCO:

Regulatory aspects	USFDA	EMA	CDSCO
Main regulatory role	Regulates pharmaceutical products in US	Coordinates scientific evaluation of medicines in EU	Performs central drug regulatory functions
Stability framework	Applicable FDA guidance	Applicable EU and ICH stability guidance	Applicable CDSCO guidance
Shelf life justification	Based on appropriate stability data and scientific evaluation	Based on appropriate stability data and scientific evaluation	Based on data and requirements applicable to submissions
Analytical procedure suitability	Suitable for intended purpose	Suitable for intended purpose	Suitable for intended purpose
Post approval stability	Depends on products and proposed change	Variation specific stability guidance applies	Applicable post approval change guidance
Stability protocol changes	Assess and report to applicable requirements	Assess and classify applicable requirements	Assess and report to applicable CDSCO requirements
Main consideration	Evidence supporting product quality and proposed shelf life	Evidence supporting product quality and proposed shelf life	Evidence supporting product quality and relevant Indian regulatory application

8.3 Post approval variations linked to analytical method changes:

A post-approval change may involve the analytical method, analytical instrument, chromatographic column, sample preparation procedure, acceptance criteria, stability protocol, manufacturing process, or packaging system. Since these changes can affect the reliability of analytical results or the interpretation of stability data, they must be scientifically evaluated and appropriately documented.[16]

The regulatory requirements depend on the nature and potential quality impact of the change. FDA guidance addresses post-approval changes to chemistry, manufacturing, and controls (CMC) information, while European requirements classify changes according to the applicable variation framework. CDSCO also provides guidance addressing specified post-approval changes.[16]

There are several common types of analytical method changes the following are typical analytical method changes:

For several reasons, a change in analytical methods might be implemented.

1.Modification of chromatographic conditions:

These may encompass alterations in the composition of the mobile phase, in the flow rate, in column temperature or in the gradient programme. These changes can affect retention time, peak shape, resolution and detection of impurities.[16]

2. Change in the column:

Column dimension change, particle size, stationary phase chemistry, and column manufacture may impact chromatographic selectivity or separation. The level of evaluation needed will depend on the change and how the method is to be used.[16]

3. Replace analytical method:

A current method can be replaced with another method e.g. a new HPLC method or another analytical method. The replacement method should be shown to be appropriate for its intended use, and to give the required performance for the specific quality attributes.[16]

4. Changes to sample preparation:

A change in extraction solvents, dilution, filtering materials or the extraction conditions can result in a change in analyte recovery, impurity measurement and reliability of the results.[16]

5. Changes to the stability-testing protocol:

A change can include the introduction or withdrawal of tests, changes in sampling time or changes in storage conditions. These changes must be backed by scientific evidence and may need regulatory notification or approval and/or stability data.[16]

The regulatory classification should not be determined simply from a change description, but should be based on the applicable requirements.[16]

9.APPLICATIONS:

9.1 Routine quality control testing:

Routine quality control (QC) testing is a crucial part of pharmaceutical manufacturing that helps ensure that pharmaceutical products comply with the required quality standards before being released and during their shelf life period. For amlodipine besylate, regular QC testing aids in the evaluation of the identity, potency, purity and quality of the Active Pharmaceutical Ingredient (API) and finished drug products.[18]

High-Performance Liquid Chromatography (HPLC) is applied broadly in quality control in the pharmaceutical industry where it can separate, identify and quantify the active pharmaceutical ingredient from other impurities and components. An adequately developed and validated stability indicating HPLC method can also be useful in the monitoring of possible degradation products which can be generated during manufacture or storage.[18]

ICH guideline Q1A(R2) defines stability testing as the testing that is carried out to establish evidence of the change in the quality of a drug substance and/or drug product when exposed to environmental conditions like temperature, humidity and light over time. The analytical procedures employed in stability studies should be appropriate for identifying changes of significance in the quality of the product.[18]

Applications of HPLC in Routine Quality Control are explained:

1. Assay determination

The amount of amlodipine besylate in a sample is measured by assay testing. HPLC is used to quantify the amount of active ingredient by comparing the response of the drug with an appropriate reference standard.[19]

2. Impurity profiling

Impurity profiling is useful for the identification and quantification of relevant process-related impurities and degradation products. It is important to monitor these substances because the concentration of these substances may change during manufacturing and storage.[19]

3. Stability-indicating analysis

A stability indicating HPLC method should be able to identify amlodipine (M) from the amlodipine degradation products and other materials that it may interact with and interfere in its determination. This is also valuable for assessing samples taken in stability studies.[19]

4. Batch release testing

Validated analytical procedures give data for assessing if a batch conforms to its approved specifications. The analytical results are discussed with other relevant quality criteria prior to making a batch-release decision.[19]

5. An investigation of unexpected results

HPLC chromatograms and quantitative results may be used to assist in investigations of out-of-specification (OOS) and/or out-of-trend (OOT) results. These studies can be used to identify if an out-of-range outcome is due to analytical error, manufacturing error, or a true change in product quality.[19]

9.2 stability studies (real - time and accelerated):

Real time stability studies:

Real-time stability studies are conducted to determine the stability of a pharmaceutical product under the recommended long-term storage conditions for the proposed shelf life.

Samples are collected and tested according to suitable analytical procedures at predetermined intervals. The outcomes are evaluated to see if key quality attributes are within acceptable limits.[20]

A complete blood count (CBC), which measures blood cell levels:

- Analysis of the active pharmaceutical ingredient.
- Determination of the amounts of specified and other relevant degradation products.
- Dissolution testing.
- Water content (if applicable).
- Dosage-form specific physical appearance and other dosage-form quality attributes.
- If applicable to product: Microbiological testing.[20]

Specific testing program will be dictated by the dosage form, formulation, packaging and regulatory requirements.

Accelerated stability studies:

The accelerated stability studies are the studies conducted on the pharmaceutical product at the elevated storage conditions to study the effect of environmental stress on product quality.[20]

In the case of conventional drug products, ICH Q1A(R2) specifies the following commonly accepted accelerated storage condition of 40°C ± 2°C and 75%RH ± 5%RH, if applicable. Depending on the product type and applicable guideline, the appropriate conditions should be chosen.[20]

Accelerated testing can be used to gain insight in possible degradation/changes in the properties of the product of possible packaging issues in less time than long-term testing.

In case of amlodipine besylate, an HPLC analysis of accelerated stability samples can assist in the detection of:

- A reduction in the amount of drug in the sample.
- An increase in relevant degradation products.
- Variations in the impurity profile of the chromatogram.
- Stability differences between formulations and/or packaging systems.

Accelerated stability results should NOT be used as a stand alone to predict the exact shelf life in every situation, however. The results need to be considered in conjunction with real-time stability data and other available scientific information.[20]

9.3 Analytical challenges in combination products:

1. Differences in chemical properties

There may be differences between the APIs in the combination product regarding polarity, ionisation behaviour, solubility and chemical stability. Such variations can affect the retention time and separation in HPLC.[21]

2. Overlapping chromatographic peaks

Peak peaks may be obscured if the components and/or degradation products have similar retention times. This may introduce a degree of inaccuracy and lack of specificity to the assay.[21]

3. Different degradation pathways

The responses of each API to acid, base, oxidation, heat or light can be different. The analytical method should then be tested to see if it can distinguish the relevant degradation products of the components being measured.[21]

4. Formulation interference

Other formulation components can interfere with the measurement of the analytes. The results should be robust and unaffected by these components, as demonstrated by appropriate method development and validation.[21]

5. Simultaneous quantification

The method should be accurate and precise for the range of concentrations tested for each API. All analytes for which the method is designed should be able to be analyzed under the same conditions.[21]

10. CHALLENGES AND FUTURE PERSPECTIVES:

10.1 Green analytical chemistry consideration in method development:

1. Reduction of organic solvent consumption

HPLC commonly uses organic solvents such as methanol or acetonitrile. Reducing their consumption can decrease solvent waste and lower the environmental burden of analytical testing.

However, solvent selection must also consider analyte solubility, chromatographic selectivity, pressure, detection, and the separation of relevant degradation products.[22]

2. Optimisation of analytical run time

A shorter run time may reduce solvent consumption and increase laboratory throughput. Nevertheless, reducing run time should not compromise chromatographic resolution or the detection of important impurities.[22]

3. Miniaturisation of analytical procedures

Where suitable equipment and validated procedures are available, reducing sample volume and solvent use may improve resource efficiency.[22]

4. Selection of safer solvents

Potentially less hazardous solvents may be considered during method development. Any replacement should be evaluated scientifically because changing the solvent can affect retention, selectivity, peak shape, and sensitivity.[22]

5. Reduction of analytical waste

Appropriate preparation of mobile phases, efficient sample preparation, and avoidance of unnecessary repeat analyses can reduce laboratory waste.[22]

6. Use of experimental design

Design of Experiments (DoE) can help identify the effects of critical chromatographic variables and their interactions. This approach may reduce the number of experiments needed to optimise a method and help identify operating conditions that provide reliable performance.[22]

10.2 Use of UPLC / hyphenated techniques for degradant characterization:

Ultra-Performance Liquid Chromatography (UPLC) and hyphenated analytical techniques also offer opportunities for the separation, detection and characterisation of pharmaceutical degradation products.[23]

Traditional HPLC is applied routinely for quality control and stability testing. However, if there is a more complex impurity profile, better separation in the chromatograph or more information about the molecular nature of the degradation products will be necessary.[23]

UPLC employs chromatographic systems optimized to be performed in the presence of more suitable columns and stationary-phase particles which are smaller. UPLC can offer superior performance in terms of efficiency, analysis time, or resolution depending on the method and instrument configuration.[23]

Hyphenated techniques: usually a chromatographic separation is used in conjunction with another analytical detector. Examples include:

- ★ LC–MS: Liquid chromatography combined with Mass spectrometry.
- ★ LC–MS/MS: Liquid chromatography tandem mass spectrometry.
- ★ LC–NMR: Liquid chromatography coupled with nuclear magnetic resonance spectroscopy.
- ★ LC–PDA/DAD: Liquid chromatography–photodiode-array or diode-array detector.

These techniques are complementary methods for the study of pharmaceutical impurities and degradation products.[23]

Role of UPLC in Degradation Analysis:

UPLC can be useful when complex mixtures contain closely eluting components or when improved chromatographic efficiency is needed.[23]

Potential advantages include:

1. Improved chromatographic efficiency

Columns with smaller particles can provide high separation efficiency when used with an appropriate instrument and method.[23]

2. Shorter analysis time

UPLC methods may reduce analysis time compared with some conventional HPLC methods, depending on the chromatographic conditions.[23]

3. Improved separation of complex mixtures

UPLC may help resolve components with similar retention characteristics when the stationary phase and mobile-phase conditions are appropriately selected.[23]

4. Reduced solvent consumption in suitable methods

Shorter or miniaturised procedures may reduce solvent use, although the actual benefit depends on the complete method and its operating conditions.[23]

5. Support for impurity profiling

UPLC can help separate the active ingredient from relevant impurities and degradation products, providing a more detailed chromatographic profile.[23]

11. CONCULSION:

The development and validation of a stability-indicating high-performance liquid chromatography (HPLC) method for amlodipine besylate is essential for ensuring pharmaceutical quality, safety, and efficacy. The review highlights the importance of forced degradation studies in evaluating the stability of amlodipine besylate under acidic, alkaline, oxidative, thermal, photolytic, and humidity stress conditions. Among these conditions, alkaline hydrolysis and photolytic stress are particularly important considerations because degradation has been reported under these conditions. Reversed-phase HPLC using a suitable C18 column and appropriate mobile-phase conditions provides a valuable approach for separating the active pharmaceutical ingredient from impurities and degradation products. Validation parameters, including specificity, accuracy, precision, linearity, robustness, and system suitability, are essential for establishing analytical reliability in accordance with applicable ICH guidelines. Furthermore, integrating validated analytical procedures with quality assurance practices supports routine quality control, stability monitoring, impurity management, and shelf-life assessment. Overall, a scientifically developed and appropriately validated stability-indicating HPLC method contributes significantly to maintaining the quality and consistency of amlodipine besylate pharmaceutical formulations.

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