Sudan University of Science and Technology

College of Graduate Studies

Stability Study of Amlodipine Besylate 5mg in Different Test Conditions

دراسه استقرار حبوب الاملودبين بيسايليت 5ملجم فى ظروف اختبار مختلفه

A Thesis Submitted in the Fulfillment for the Requirement of the Master Degree in Chemistry

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الإستهلال

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

(سورة المجادله)

الايه 11

Dedication

To my parents

(Sisters, brothers, friends)

And

Comrades in the revolution

Acknowledgments

I would like to gratefully acknowledge my supervisor Dr. Mohamed Suleiman Altoum for his guidance and extended inspiration added great values to my study. He always encourages me to work and solve problems easily. My acknowledgment goes to the Climax pharmaceutical factory staff for their technical support I will never forget the tremendous moral support I received from my friends.

Abstract

This research aimed to study the stability of amlodipine besylate (5mg) tablet under different condition. According to united state pharmacopeia (USP monograph).

The *melting* point of amlodipine besylate was found 200C and agreed with results melting point found in the literature. Whereas, the characteristic bond of amlodipine besylate was showed clearly by IR spectroscopy absorption. The assay of active ingredient of (amlodipine besylate) was carried out using HPLC with *(Column: 3.9mm* 150mm 5µm packing L1)* and (Acetonitrile**,** methanol, buffer (15:35:50)) was used as a mobile phase in different conditions (ongoing and accelerated). The results were found in ongoing between (108.17% to 103.76%). and accelerated results between (107.32% to 102.51%).

The finding of assay in the range of acceptance value (90 -110 %). In addition, other physical test (hardness, Diameter, friability, thickness and disintegration) was measured and results was pass.

Therefore, microbiological tests in ongoing and accelerated condition the results were pass. The results of analysis(chemical, physical, microbial) in ongoing and accelerated condition were found in the acceptance limit. That main drug is stable in those conditions.

المستخلص

هذا البحث هدف الى دراسه استقرار حبوب الاملودبين بيسايليت (5ملجرام) تحت ظروف اختبار مختلفه وفق دستور الادويه الامريكى . من الدراسه وجد ان درجه انصهار الاملودبين بيسايليت (5ملجرام)تساوى 200درجه مئويه وهى متوافقه مع نتائج درجه الانصهار له الموجوده في المراجع العلميه ايضا تم التعرف على الروابط المميزه للاملودبين عن طريق جهاز مطيافيه الاشعه تحت الحمراء . ثم تم ايجاد تراكيز الماده الفعاله باستخدام (جهازكروماتوغرافيا السائل عالى الدقه) باستخدام عمود فصل بمواصفات معينه والطور المتحرك المناسب ووجد ان نتائج التحليل في الفتره الزمنيه للدراسه ان تراكيز الماده الفعاله فى الظروف العاديه تتراوح بين (103.76%_108.17%)وفى الظروف المسرعه تقع بين (102.51%_107.17%) هذه التنائج تقع في المدى المقبول (90%_110%). بالاضافه لذلك تم اجراء الاختبارات الفيزيائيه (زمن التحلل _التفتت_ القطر_السمك _الصلابه) تم الحصول على نتائج تقع المدى المقبول.ايضا تم اجراءالاختبارات الميكروبيه فى ظروف الدراسه ووجد ان النتائج تقع فى المدى المقبول. نتائج التحليل(الكيميائيه _الفيزيائيه _الميكروبيه) فى الظروف العاديه والمسرعه تقع فى المدى المسموح به وهذا يعنى ان حبوب الاملودبين بيسايليت مستقرفى هذه الظروف .

List of Contents

N0.	Title	Page
	الاستهلال	I
	Dedication	II
	Acknowledgements	III
	Abstract	IV
	المستخلص	V
	List of Contents	VI
	List of Tables	X
	List of Figures	XI
	Chapter one	
1	Introduction	1
1.1	Historical background	3
1.2	Types_of_stability_study	3
1.2.1	Physical stability	3
1.2.1.1	Crystal formation in pharmaceutical	3
1.2.1.2	Loss of volatile substances	2
1.2.1.3	Loss of water	3
1.2.1.4	Absorption of water	3
1.2.1.5	Change in crystalline form	4
1.2.2	Chemical stability	4
1.2.2.1	Solvolysis	4
1.2.2.2	Oxidation	4
1.2.2.3	Photolysis	4
1.2.2.4	Polymerization	5
1.2.2.5	Optical isomerization	5
1.2.3	Microbial stability	5

1.3	Importance of stability testing	5
1.4	Stability testing methods	6
1.5	Factors affecting drug stability	7
1.5.1	Oxygen	7
1.5.2	Water	7
1.5.3	Temperature	7
1.5.4	pH	8
1.5.5	Moisture	8
1.5.6	Light	9
1.5.7	Concentration	9
1.6	Definition of Terms	9
1.6.1	Drug Substance	9
1.6.2	Dosage Form	9
1.6.3	Batch Lot	9
1.6.4	Excipients	9
1.6.5	Climatic Zones	10
1.6.6	Mean Kinetic Temperature	10
1.6.7	Real Time (Long-term) Studies	11
1.6.8	Shelf life	11
1.6.9	Stability	11
1.6.10	Stability Test	11
1.7	Drug Product	11
1.7.1	In the Developing Phase	11
1.7.2	Design of Stability Testing	12
1.7.3	Shelf life	13
1.8	Drug substance	14

1.8.1	Stress Testing	14
1.8.2	Testing frequency	15
1.8.3	Storage Conditions	15
1.8.4	Stability Commitment	16
1.9	Introduction of amlodipine besylate	17
1.9.1	Pharmaceutical information of amlodipine	17
1.9.1	Pharmaceutical information of amlodipine	18
1.9.1	structural formula amlodipine	19
1.9.3	Mechanism of Action	19
1.9.4	Pharmacokinetics	20
1.9.4.1	Absorption	20
1.9.4.2	Metabolism	20
1.9.4.3	Excretion	20
1.9.5	Amlodipine tablets with food and drink	20
1.9.6	How to take Amlodipine tablets	21
1.9.6.1	Adults	21
1.9.6.2	Use in Children and adolescents	21
1.9.7.1	Pregnancy and breast-feeding	21
1.9.7.2	Breast-feeding	21
1.10	Objectives of study	22
1.10.1	Objectives	22
	Chapter two	
2	Materials and methods	23
2.1	Chemicals	23
2.2	Instruments	23
2.3	Equipment	24

2.4	Methods of analysis	24
2.4.1	Melting point Determination	24
2.4.2	Infra-red spectroscopy	24
2.4.3	Assay of Amlodipine besylate	24
2.4.3.1	Buffer	24
2.4.3.2	Mobile phase	24
2.4.3.3	Standard solution	25
2.4.3.4	Sample solution	25
2.4.3.5	Chromatographic system	25
2.4.3.6	System suitability	25
2.4.3.7	Suitability requirement	25
	Chapter three	
3	**Results and discussion**	27
3.1	Identification of amlodipine besylate	28
3.2	Physical stability	29
3.3	Chemical stability	30
3.3.1	High performance liquid chromatography	30
3.3.2	Results of ongoing condition	30
3.3.3	Result of acceralated condition	34
3.4	Microbial stability	38
3.5	Conclusion	39
3.6	Recommendation	40
4	References	41

List of Tables

1.1	Climatic Zones	10
1.2	Table testing plant	13
1.3	Storage condition	16
1.4	Pharmaceutical information	17
3.1	Melting point	28
3.2	IR frequency of Amlodipine besylate	28
3.3	Result of ongoing condition _ Physical stability	29
3.4	Result of acceralated condition_ Physical stability	29
3.5	Acceptance criteria to Physical parameter according USP	29
3.6	Results of ongoing condition_ chemical stability	31
3.7	Result of acceralated condition_ chemical stability	34
3.8	Results of Ongoing amlodipine(5mg) microbiological analysis	38
3.9	Results Acceralated (5mg) microbiological analysis	39
3.10	Acceptance limit OF microbiological analysis According to(USP, BP and JP)	39

List of figures

1.1	structural formula amlodipine	19
3.1	HPLC data for amlodipine besylate in zero time in ongoing condition	32
3.2	HPLC data for amlodipine besylate in weak one in ongoing condition	33
3.3	HPLC data for amlodipine besylate in weak second in ongoing condition	33
3.4	HPLC data for amlodipine besylate in weak third in ongoing condition	34
3.5	HPLC data for amlodipine besylate in zero time in acceralated condition	35
3.6	HPLC data for amlodipine besylate in weak one in acceralated condition	36
3.7	HPLC data for amlodipine besylate in weak second in acceralated condition	37
3.8	HPLC data for amlodipine besylate in weak third in acceralated condition	37
3.9	HPLC data for standard amlodipine besylate	38

Chapter one

1 Introduction

Stability is defined as the capacity of a drug substance or drug product to remain within the established specifications which maintains its identity, strength, quality and purity throughout the retest or expiration dating period. The objective of stability study is to determine the shelf life, namely the time period of storage at a specified condition within which the drug product still meets its established specifications. (Gokani 2012)

Stability is an essential factor of quality, safety and efficacy of a drug product. A drug product, which is not of sufficient stability, can result in changes in physical (like hardness, dissolution rate, phase separation etc) as well as chemical characteristics (formation of high risk decomposition substances). The Chemical stability of drug is of great importance since it becomes less effective as it undergoes degradation. Also drug decomposition may yield toxic byproducts that are harmful to the patient. Microbiological instability of a sterile drug product could also be hazardous. Stability evaluation of drug substance or drug product is the key to drug quality as it determines the efficacy of any drug or dosage form. Stability assessment of drug products and drug substances are mandated by regulatory agencies across the globe. In fact, stability-testing issues are responsible for a number of audit findings by regulatory agencies. (Gokani 2012) Stability testing problems are regularly cited in warning letters and sometimes results in costly product recall. Stability testing provides evidence that the quality of drug substance or drug product changes with time under the influence of various `stability study consists of a series of tests in order to obtain an assurance of stability of a drug product, namely maintenance of the drug product packed in it

specified packaging material and stored in the established storage condition within the determined time period. (Gokani 2012)

1.1 Historical Background

Jordan was the one to give the name office organized a workshop for validation of expiry dates of drug in Amman .The workshop ordered medical authority to collaborate with every pharmaceutical company to guide them about the importance of drug stability and expiry date. Thus International Conference for Stability testing in the pharmaceutical companies. The need arose when regional on Harmonization thus took a step to implement these guidelines. FDA issued its first stability guidance in 1987.Considerable efforts were taken, to harmonize the stability practices within the ICH region then after in the early 1990. As a result to the efforts, International Conference on Harmonization (ICH) was established in 1991 and various guidelines for drug substance and drug product came into existence regarding their quality, safety and efficacy. These guidelines are called as quality, safety, efficacy and multi-disciplinary (also called as Q, S, E and M) guidelines (Bhuyian 2015). Work on stability of pharmaceutical products was initiated by the WHO in 1988 and the WHO Guidelines on Stability Testing for Well Established Drug Substances in Conventional Dosage Forms were adopted in 1996 by the WHO Expert Committee on Specifications for Pharmaceutical Preparations following extensive consultation. In 2000, discussions began between the International on Harmonization (ICH) expert working group Q1 (stability) and the WHO to harmonize the number of stability tests and conditions employed worldwide.(Bhuyian 2015)

1.2 Types of stability study

1.2.1 Ph*ysical* stability

1.2.1.1 Crystal Formation in pharmaceutical Preparations:

Causes of crystal formation in pharmaceutical formulations are like Polymorphism phenomena which are seen in Chloramphenicol, while in Saturated solution by different temperature precipitation of solute may occur and in suspension when very fine powder is used a part of suspending agent which dissolve and precipitated as crystals.

1.2.1.2 Loss of volatile substances: Loss of Volatile substance from pharmaceutical dosage forms like Aromatic waters, Elixirs, Spirits and some types of tablets which contain aromatic water cases physical instabilities.

1.2.1.3 Loss of water: This can be seen in the Dosage forms like, in saturated solution, by loss of water they become supersaturated and precipitate as crystals is formed. In emulsions, loss of water leads to separation of the two phases and change to other type while, in creams especially oil/water, they become dry by loss of water.

1.2.1.4 Absorption of water

In the different type of dosage form the absorption of water cause physical instability by different mechanism. In Powders type of dosage form Liquefaction and degradation may occur as a result of absorption of water while in case of Suppositories which base made from hydrophilic substances as Glycerin, Gelatin, poly ethylene glycol, the consistency of these forms becomes jelly-like appearance and causes physical instabilities in pharmaceutical preparations.

1.2.1.5 Change in crystalline form:

Change in crystalline forms changes physical properties like solubility, melting point, bioavailability so change in crystalline form case physical instability. Example: Cocoa butter which is capable of existing in four polymorphic forms. Cocoa butter can crystallize in to six polymorphic forms designated as i-vi according to their stability and different physical characteristics. The chemical composition is identical in all forms; only the arrangement of the lipid molecules varies. The diverse polymorphs are formed under different crystallization condition. (Gokani 2012)

1.2.2 Chemical stability

Degradation product occurs during storage under shelf-life testing should be identified. These products should be toughly investigated, and evaluated for safety and toxicology purpose. The presence of degradation product may require additional safety and efficiency studies.

1.2.2.1Solvolysis

Solvolysis involves the degradation of drug or excipients through reaction with the solvent present in the formulation. Most of Solvolysis reactions involve in the degradation of drug and excipients involve labile carbonyl compound, such as ester, amides, and lactones and lactates, (Diana 1990)

1.2.2.2 Oxidation

Oxidation is an extremely common cause of drug and excipients degradation. This method of degradation occurs in, Water and oil-soluble drugs, as well as fixed and volatile oils.

1.2.2.3 Photolysis

Degradation of drug or excipients molecules can be brought about light, either room light or sunlight such reactions are termed photolysis and light sensitive drugs and known as photo labile.

1.2.2.4 Polymerization

Polymerization involves the combination of two or more identical molecules to form a much larger and complex molecule. Degradation of pharmaceutical products by polymerization is not very common and such reactions may occur after the initial degradation of the drug.

1.2.2.5 Optical isomerization

A change in the optical activity of a drug may result in a change in its biological activity. Racemization is mean type of optical Isomerization which effects drug molecules and this occur when the optically active form of drug is converted to its enantiomorphism. In most cases the enantiomorphism has less therapeutic effect than the original drug (Nunes 1974).

1.2.3 Microbial stability

Contamination from microorganisms is a big problem for all formulations containing moisture but it can be a bother in solid dosage forms also if some natural polymers are used because many natural polymers are fertile sources of microorganisms. In the type of hygienic manufacture carried out today where "Quality Assurance" is a prerequisite as per the GMP procedures, there are definite procedures to prevent microbial contamination in all formulations.

1.3 Importanc*e* of stability testing

The primary reason for stability testing is the concern for the well-being of the patient suffering from the disease for which the products is designed. Apart from degradation of the unstable product into toxic decomposition products, loss of activity up to a level of 85% of that claimed on the label may lead to failure of the

therapy resulting in death e.g. nitroglycerine tablets for angina and cardiac arrest. Because of this concern, it has become a legal requirement to provide data for certain types of stability tests for the regulatory agencies before approval of a new product. Second important concern is to protect there putation of the manufacturer by assuring that the product will retain fitness for use with respect to all functionally relevant attributes for as long as they are on the market. Other benefits of stability studies at the developmental stage or of the marketed products are to provide a database that may be of value in selection of adequate formulations, Excipients and container closure systems for development of a new product, to determine shelf life and storage conditions for development of a new product, preparation of registration dossier, to substantiate the claimed shelf life for the registration dossier and to verify that no change.

1.4Stability testing methods

Stability testing is a routine procedure performed on drug substances and products and is employed at various stages of the product development. In early stages, accelerated stability testing (at relatively high temperatures and/or humidity) is used in order to determine the type of degradation products which may be found. After long-term storage. Testing under less rigorous conditions i.e. those recommended for long-term shelf storage, at slightly elevated temperatures is used to determine a product's shelf life and expiration dates. The major aim of pharmaceutical stability testing is to provide reasonable assurance that the products will remain at an acceptable level of fitness/quality throughout the period during Which they are in market place available for supply to the patients and will be fit for their consumption until the patient uses the last unit of the product (Kommanaboyin 1999). Depending upon the aim and steps followed, stability testing procedures have been categorized into the following four types

1.5 Factors affecting drug stability

1.5.1Oxygen

Oxidation is the most important Part of the drug degradation. Oxygen is present everywhere in the atmosphere and exposure to oxygen will decompose the drug substance that is not in their most oxidized state through autoxidation. Oxygen is a di radical and most auto oxidation is a free radical reactions. Oxidation/reduction reaction involves transfer of electron, oxygen or hydrogen in substances. The process involves a radical chain reaction between molecular oxygen and the pharmaceutical drug candidate, a process known as auto-oxidation. The free radical process of auto-oxidation consists of a chain sequence involving three distinct types of reactions: initiation, propagation, and termination. The initiator produce a free radical to begin the chain initiator is used to accelerating auto-oxidation. For example, diazine. Oxidation of diazine occurs by N-oxidation. (Gokani 2012).

1.5.2 Water

The word hydrolysis literally means “splitting by water”. Drug substance having ester & amide functional groups within their structure undergoes hydrolysis reactions. For example solutions of sodium acetate produce acetic acid and hydroxide ions. The reaction involving lactam group are fastest and are those followed by ester, amides and imides in that order and follows first order. These reactions are catalyzed by divalent metal ions, heat, light and high drug concentrations. (Gokani 2012).

1.5.3 Temperature

Temperature has a high degree of influence on all variety of chemical reactions and usually they are accelerated by raise in temperature. To evaluate stability utilizing elevated temperatures and stress conditions are selected based on the Arrhenius expression a quantitative relationship of reaction rate and temperature. Based on this estimate, a 10◦C increase in temperature results in a doubling of the reaction rate and a decrease in the reaction time by a factor of 2. (Gokani 2012)

1.5.4 PH

PH influences the rate of decomposition of most drugs. Most of the drugs are stable between pH 4 and 8. Weekly acidic and basic drugs show good solubility when they are ionized and they also decompose faster when they are ionized. So if the pH of a drug solution has to be adjusted to improve solubility and the resultant pH leads to instability then a way out of this tricky problem is to introduce a water miscible solvent into the product. It will increase stability by suppressing ionization, reducing the extreme pH required to achieve solubility, enhancing solubility and reducing the water activity by reducing the polarity of the solvent .For example, 20% propylene glycol is placed in chlordiazepoxide injection for this purpose. Reactions catalyzed by pH are monitored by measuring degradation rates against pH, temperature, ionic strength and solvent concentration constant. Some buffers such as acetate, citrate, lactate, phosphate and ascorbate buffers are utilized to prevent drastic change in pH. Sometimes pH can have a very serious effect on decomposition. As little as 1 pH unit change in pH can cause a change of tenfold in rate constant. So when formulating a drug into a solution we should carefully prepare a pH decomposition profile and then formulate the solution at a pH which is acceptable physiologically and stability-wise also (Gokani 2012).

1.5.5 Moisture

Water catalysis chemical reactions as oxidation, hydrolysis and reduction reaction, it also promotes microbial growth.

1.5.6 Light

Affects drug stability through its energy or thermal effect which lead to Oxidation.

1.5.7 Concentration

Rate of drug degradation is constant for the solutions of the same drug with different concentration. So, ratio of degraded part to total amount of drug in diluted solution is bigger than of concentrated solution. (Gokani 2012).

1.6 Definition of Terms

1.6.1 Drug Substance

It is the unformulated drug substance that may subsequently be formulated with excipients to produce the dosage form**. (**Guideline 2003)

1.6.2Dosage Form

It is a pharmaceutical product type (e.g. tablet, capsule, cream, etc.,) that contains a drug substance. (Patrekar and Mali 2014)

1.6.3Batch Lot

A defined quantity of starting material, packaging material or product processed in one process or series of processes so that it could be expected to be homogeneous. In the case of continuous manufacture, the batch must correspond to a defined fraction of the production, characterized by its intended homogeneity, (it may be necessary to divide a batch into a number of sub-batches) which are later brought together to form a final homogeneous batch (. Engisch and Muzzio 2016)

1.6.4Excipients

Any ingredient other than the drug substance in dosage form (Chaudhari and Patil 2012).

1.6.5 Climatic Zones

The concept of dividing the world into five zones on defining the prevalent annual climatic conditions. Five climatic zones can be distinguished for the purpose of worldwide stability testing, Table (1.1) (Grimm 1998).

Table (1.1)climatic zones

Climatic Zone	Definition	Criteria Mean annual temperature measured in the open air/Mean annual partial water vapor pressure	Long-term testing conditions
I.	Temperate Climate	≤ 15°c /≤ 11hPa	21° C /45% RH
II.	Subtropical and Mediterranean Climate	> 15 to 22°C / > 11 to 18 hPa	25°C / 60% RH
III.	Hot and dry Climate	> 22°C/ ≤ 15hPa	30°C /35% RH
IVA	Hot and humid Climate	> 22°C / > 15 to 27 hPa	30°C /65% RH
IVB	Hot and very humid climate	> 22°C / 27hPa	30°C / 75% RH

Sudan confirmed long-term stability testing condition 30°C /65% RH (zone IVA), (ICH, 2004).

1.6.6 Mean Kinetic Temperature

A single derived temperature which if maintained over a defined period of time, would afford the same thermal change to a drug substance or drug product as would have been experienced over a range of both higher and lower temperature for an equivalent defined period. The mean kinetic temperature is higher than the arithmetic mean temperature and takes into account the Arrhenius equation. (ICH, 2004).

1.6.7Real Time (Long-term) Studies

Studies designed to evaluate the physical, chemical, biological and microbiological characteristic of a drug substance or drug product, during the expected time of shelf life and storage of samples at expected storage conditions in the intended market. The results are used to establish shelf life, confirm projected shelf life and recommend storage conditions. (Haynes .1971).

1.6.8 Shelf life

The time interval that drugs product is expected is to expect to remain within the approved specification, provided that is stored under conditions, stated on the label of the containers (AUPAM, 1995).

1.6.9 Stability

The ability of a drug substance or drug product to retain its chemical, physical, microbiological, and biological properties within specified limits throughout its shelf life. (Rodes, 1984).

1.6.10 Stability Test

Stability tests are a series of tests designed to obtain information on the stability of a drug substance or drug product in order to define its shelf life and utilization period for the product under specified packaging and storage conditions.

1.7 Drug Product

1.7.1. In the Developing Phase

Accelerated stability test are carried out to compare alternative formulations, packing materials, and the manufacturing process in short-term experiments. As soon as the final formulation and manufacturing process have been established, the manufacturing will carry out a series of accelerated studies which will permit production of the stability, and predetermine the shelf life and storage conditions of the drug product. Real-time studies must be started at the same time for conformation purposes. Suitable measures should be taken for the establishment of the utilization period for preparations in multi-does containers.

1.7.2. Design of Stability Testing

The design of stability studies for the product should be based on the knowledge of properties and stability characteristic of drug substances. The design of the stability testing program needs to take into consideration the intended market and the climatic conditions of the area in which the drug products will be based For Sudan where certain regions are situated in zone IVA, and also with the view to global market, it is recommended that the stability testing program be based on conditions corresponding to climatic zone III and IVA.

A stability study is based on varying degrees of temperature, time, humidity, light intensity and partial vapor pressure and their effect on the product in question. It should be pointed out that the effective or mean kinetic temperature reflects actual situation more precisely than measured mean temperature, i.e. there is a difference between a products being kept for one month at 30°C. Moreover, storage conditions

often represent a higher temperature than average meteorological data indicated for a country.

For some dosage forms, especially liquid and semi-solid dosage forms, the study design may also need to consider low temperatures, e.g. below zero - 10°C to 20°C (freezer), the cycles and temperatures between 2°C to 8°C (refrigerator). For certain preparations it is important to observe effect caused by their exposure to light. Photo stability testing should be an essential part of the stability design (ICH, 2004).

Table (1.2) testing plan

1.7.3 Shelf life

Storage condition	Storage Time (month)							
	0	3	6	9	12	18	24	36
Accelerated 40 ± 2°C /75±5% RH	X	X	X					
Long-term 30±2°C /65% RH	X	X	X	X	x	x	X	X
Long-term 25± 2°C /60± 5%RH								

Shelf life always determined in relation to storage conditions. If batches of a product demonstrate different stability profiles, the shelf life proposed should be based on the stability of the at least stable. A Tentative shelf life of 24 months may be established. However, the proposed shelf life could be 12 months longer than the period covered by long-term data. This should be based on the duration and evaluation of the data submitted and provided the following conditions are satisfied:

A_ The active ingredient is considered to be stable (not easily degradable).

B_ Stability studies as previously outlined have been performed with no significant changes.

C_ Supporting data indicated that similar formulations in the same packaging container and closure system have been assigned a shelf life of 24 months or more

D_ The manufacturer will continue to perform the real-time studies until the proposed shelf life is covered.

1.8. Drug substance

Information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation for drug product.

1.8.1. Stress Testing

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the individual drug substance. Stress testing is likely to be carried out on a single batch of the drug substance. The testing should include the effect of temperatures (in 10C, increments (e.g.50C, 60C) above that accelerated testing), humidity (e.g. 75 percent relative humidity or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photo stability testing should be an integral part of stress testing. Examining degradation products under stress conditions is useful in establishing degradation pathway, in developing and validating suitable analytical procedures. However, such examination may not be necessary for certain degradation products if it has been demonstrated that they are not formed under accelerated or long-term storage conditions. Results from those studies will form an integral part of information provided to regulatory authorities.

1.8.2. Testing frequency

Frequency of testing should be sufficient to establish the stability profile of the drug substance. The frequency of testing at the long-term storage conditions should normally be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed retest period. At the accelerated storage conditions for a 6 months study a minimum of three times points is

recommended including the initial and final time points (e.g. 0, 3, and 6 months). Where an expectation (based on development experience) exists that the results from accelerated studies are likely to approach significant change criteria, increased testing should be conducted either by adding samples at the final time point or including a fourth time point time in the study designed,(WHO,2006).

1.8.3. Storage Conditions

In general, a drug substance should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and if applicable, its sensitivity to moisture and light. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment and subsequent use. The long-term testing should cover a minimum of 12 months duration on at least three primary batches at the time of submission and should be continued for a period of time sufficient to cover the proposed retest period. Additional data accumulated during assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition can be used to evaluate the effect of short-term excursions outside the label storage conditions (such as might occur during shipping). (WHO, 2007).

Table (1.3) storage condition

Study	Storage condition	Minimum time to recover by data at submission
Long- term	30°C±2°C 65%RH ±5%	12 months
Accelerate	40°C ±2°C 75%RH ±5%	6 months

If it cannot be demonstrated that the drug substance will remain within its acceptance criteria when stored at 30°C ± 2°C / 65% RH ±5RH for the duration of the proposed shelf life, the following options should be considered:

1) Reduced retest period.
2) A more protective container closure system.
3) Additional cautionary statements in the labeling.

1.8.4. Stability Commitment

When available long-term stability data on primary batches do not cover the proposed retest granted at the time of approval, a commitment should be made to continue the stability studies post approval to firmly establish the retest period. Where the submission include long-term stability data on three production batches covering the proposed retest period, a post approval commitment is considered unnecessary. Otherwise, one of the following commitments should be made:

- If the submission includes data from stability studies on at least three production batches, a commitment should be made to continue these studies through the proposed retest period.
- If the submission includes data from stability studies on fewer than three production batches, a commitment should be to continue these studies through the proposed retest period and to place additional production batches, to a total of at least three, on long-term stability studies through the proposed retest period.
- If the submission does not include stability data on production batches, a commitment should be made to place the first three production batches on long-term stability studies through the proposed retest period (ICH, 2004).

1.9 Introduction of amlodipine besylate

1.9.1 Pharmaceutical Information

Table (1.4) Pharmaceutical Information for Amlodipine besylate

Proper Name	Amlodipine besylate
Chemical Name	3-Ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl3,5-pyridinedicarboxylate benzenesulphonate (Mushashi, 2016.)
Molecular formula and molecular mass	C20H25 ClN2O5.C6H6O3S and 567.1 (Mushashi, 2016.)

Physicochemical properties Description	Amlodipine besylate is a white crystalline substance, slightly soluble in water and sparingly soluble in ethanol. (Mushashi, 2016.)
Inactive ingredients	Micro crystalline cellulose, dibasic calcium phosphate anhydrous, sodium starch glycolate, and magnesium stearate. (Mushashi, 2016.)
DOSAGE forms	2.5, 5, and 10 mg Tablets (Angina, C.S., Angina, V. and CAD 1987)
User	used to treat: • high blood pressure (hypertension). • angina pectoris (pain in the chest caused by blockages in the arteries leading to the heart) or chest pain classed as vasospastic angina pectoris (or Prinzmetal's angina). (Milic, and Ziegler, 2006.)

1.9.2Structural formula amlodipine

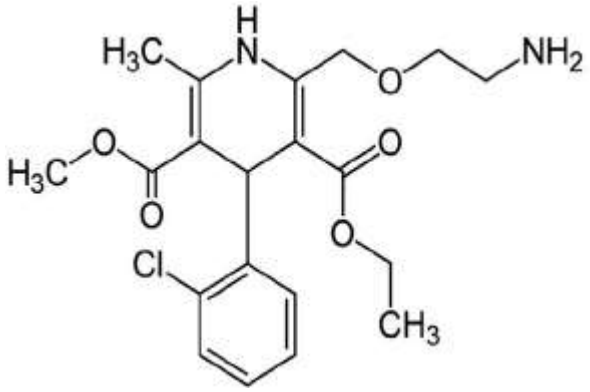


Figure (1.1) structural formula amlodipine

(Ananchenko, Novakovic, and Lewis, 2012)

1.9.3 Mechanism of Action

Amlodipine besylate is a calcium ion influx inhibitor (calcium entry blocker or calcium ion antagonist). Amlodipine is a member of the dihydropyridine class of calcium antagonists. The therapeutic effect of this group of drugs is believed to be related to their specific cellular action of selectively inhibiting transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. The contractile processes of these tissues are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. Serum calcium concentration is not affected by amlodipine. Within the physiologic pH range, amlodipine is an ionized compound and its kinetic interaction with the calcium channel receptor is characterized by the gradual association and dissociation with the receptor binding site. Experimental data suggest that amlodipine binds to both dihydropyridine and nondihydropyridine binding sites. (Mushashi, 2016.)

1.9.4 Pharmacokinetics

1.9.4.1 Absorption

After oral administration of therapeutic doses of amlodipine, absorption occurs gradually with peak plasma concentration reached between 6 and 12 hours. Absolute bioavailability has been estimated to be between 64 and 90%. The bioavailability of amlodipine is not altered by the presence of food. (Mushashi, 2016)

1.9.4.2 Metabolism

Amlodipine is metabolized through the cytochrome P450 system, mainly via CYP 3A4 isoenzyme. Amlodipine is extensively (about 90%) converted to inactive metabolites (via hepatic metabolism) with 10% of the parent compound and 60% of the metabolites excreted in the urine. Ex vivo studies have shown that approximately 93% of the circulating drug is bound to plasma proteins in hypertensive patients.

1.9.4.3 Excretion

Elimination from the plasma is biphasic with a terminal elimination half-life of about 35-50 hours. Steady state plasma levels of amlodipine are reached after 7 to 8 days of consecutive daily dosing. (Mushashi, 2016)

1.9.5 Amlodipine tablets with food and drink

Grapefruit juice and grapefruit should not be consumed by people who are taking Amlodipine tablets. This is because grapefruit and grapefruit juice can lead to an increase in the blood levels of the active ingredient amlodipine, which can cause an

unpredictable increase in the blood pressure lowering effect of Amlodipine tablets .(Milic, and Ziegler 2006.)

1.9.6 How to take Amlodipine tablets

Always take this medicine exactly as your doctor has told you. Check with your doctor or pharmacist if you are not sure. The recommended dose is:

1.9.6.1 Adults (including the elderly and children 18 years or over): 5mg once a day, up to a maximum of 10mg a day as a single dose depending on your response. If you are elderly, your doctor will closely monitor your response to any dose increase.

1.9.6.2 Use in Children and adolescents (6 -17 years old): the recommended usual starting dose is 2.5mg a day. The maximum recommended dose is 5mg a day. Amlodipine 2.5mg is not currently available and the 2.5mg dose cannot be obtained with Amlodipine tablets 5mg as these tablets are not manufactured to break into two equal halves. (Milic and Ziegler 2006.)

1.9.7 Pregnancy and breast-feeding

1.9.7.1 Pregnancy

The safety of Amlodipine in human pregnancy has not been established. If you are pregnant, think you might be pregnant, or are planning to get pregnant, doctor are must told by pregnancy before you take Amlodipine tablets.

1.9.7.2 Breast-feeding

Amlodipine has been shown to pass into breast milk in small amounts. If you are breast-feeding or about to start breast-feeding you must tell your doctor before taking Amlodipine tablets.(Milic, and Ziegler 2006).

1.10 Objective of study

To study stability testing and provide Amlodipine besylate information on how the quality of varies with time under the influence of a variety of environmental factors such as temperature, humidity, and concentration.

1.10.1 Objectives

1. To study stability of amlodipine besylate in different condition (temperature an humidity)

2. To characterize final products by physiochemical methods.

3. To compare the finding of the study with previous studies

Chapter TWO

2. Materials and methods

2.1Chemicals

- Trimethylamine (laba chime www.labachemie .com)
- Phosphoric acid.(cdh)
- Acetonitrile.(duksan pure chemicals)
- Methanol. (duksan pure chemicals)
- Amlodipine besylate.
- Distilled water

2.1.2 Instruments

- High performance liquid chromatography(SHIMADZU (JAPAN)
- FT-IR spectrometer
- Disintegration tester
- Hardness tester
- Friability tester
- Thickness tester
- Diameter tester
- Colony counter
- Laminar air flow
- Incubator
- Autoclave
- Sensitive balance (METLER TOLEDO GERMANY)
- Melting point apparatus **:**(Electrical type Galen Kamp. England, Serial NO.SG93105/05/680)

2.1. Equipment

- Volumetric flask 10ml grade A.
- Pipette1ml grade A .
- Measuring cylinder class A.
- Filtration unites.
- Volumetric flask 1000ml.

2.4 Methods of analysis

2.4.1. Melting point Determination

Small portions (0.25g) of Amlodipine besylate finely shaped pure were transferred into dry capillary tubes .The powder was packed by tapping the tube on a hard surface to obtain about 5mm of the drug in the tube. These were heated slowly in a Gallenkamp Melting point apparatus. The temperature at which melting of the substance occurred was noted and recorded.

2.4.2. Infra-red spectroscopy

The KBr disc was prepared by finely grinding 1part of Amlodipine besylate with about 250 parts of dried potassium bromide, the mixture was compressed under 10 tons in vacuum pressure Perkin Elmer (FT-IR) computerized spectrometer was used to obtain the IR spectrum of The Amlodipine besylate result obtain are shown in Table (3.2)

2.4.3 Assay of Amlodipine besylate

2.4.3.1 Buffer: 7.0 ml of tri ethyl amine were added in to 1000 mL volumetric flask containing 900 mL of water. Then Adjusted PH solution with phosphoric acid to PH of 3.0 ± 0.1. The solution was Diluted with water to volume and mixed well.

2.4.3.2 Mobile phase: Acetonitrile**,** methanol, buffer (15:35:50).

2.4.3.3 Standard solution: 0.0275mg/mL of USP Amlodipine besylate RS and 0.0025mg/ml of Amlodipine related compound A RS weighted in mobile phase.

2.4.3.4 Sample solution

Five tablets (25mg) placed in suitable volumetric flask and added sufficient mobile phase to disintegrate the tablets, shacked for 30 minute and diluted with mobile phase to volume. The sample was passed through a syringe tip filter 0.45-µm pore size. The first few mL Discarded of the filtrate.

2.4.3.5 Chromatographic system

- Detector: UV 237nm.
- Column**:** 3.9mm* 150mm 5µm packing L1.
- Mode: LC.
- Flow rate: 1mL/min.
- Injection volume: 50µl.
- Run time: NLT 3 times of retention time of Amlodipine peak.

2.4.3.6 System suitability

- Sample**:** standard solution.

The relative retention times for Amlodipine and Amlodipine related compound A are about 1.0 and 0.5 respectively.

2.4.3.7 Suitability requirement

- Resolution**:** NLT 8.5 between Amlodipine and Amlodipine related compound A.
- Tailing factor**:** NLT 2.0 for both Amlodipine and Amlodipine related compound A.
- RSD: NM T 5% for Amlodipine related compound A.

- (Peak response of sample (r_u) / peak response of standard (r_s))*(concentration of Standard (C_s) / concentration of sample (C_u)) * (M_{r1}/M_{r2}) *100
- Peak response of sample (r_u) =……..
- Peak response of standard (r_s) =…….
- Concentration of Standard (C_s) =……..
- Concentration of sample (C_u) =…….
- M_{r1} = molecular weight of Amlodipine 408.88 g/ mole
- M_{r2} = molecular weight of Amlodipine besylate 567.05 g/ mole

Chapter three

3 Results and discussion

Amlodipine content 97 percent to 102 percent (anhydrous substance) and appearance white or almost white powder and slightly soluble in water, freely soluble in methanol, sparingly soluble in anhydrous ethanol slightly soluble in 2_propanole. The period of stability of a pharmaceutical product is the time from the date of manufacture of formulation until its chemical or bioactivity is not less than 95% of labeled potency and its physical characteristics have not changed appreciably or deleteriously (Olaniyi, 2000).

The quality of pharmaceutical product may deteriorate with time and upon standing along the chain of the drug supply system under the prevalent conditions of storage and transportation in Sudan.

results of study show that the stability of amlodipine besylate tablet (5mg)to define suitable storage and shelf life .the study include chemical ,physical and microbiology tests according to official monographs condition of study was ongoing (real time) and acceralated,

Results in ongoing of assay tests of amlodipine besylate was found to ranging between (108.17% to 103.76%). Also results in accceralated condition of assay tests ranging between (107.32%to 102.51%). This results in acceptance value (90% to 110) according to USP. Results of physical tests to amlodipine besylate (5mg) in on going and acceralated condition was pass according to USP also the results of microbiological tests was pass According to(USP, BP and JP). the other factors may be effect of stability on amlodipine besylate was pH and sun light .in agreement with studies related to (Gul, Basheer, Karim, and Ayub,2015) (Jakimska, Śliwka-Kaszyńska, Nagórski, Namieśnik, and Kot-Wasik, 2014)

3.1 Identification of amlodipine besylate

Melting point

Show results melting point of amlodipine besylate in Table (3.*1)*

Table (3.1) Melting point of Amlodipine besylate

Melting point	200 ºC

Infra-red analysis

The FTIR spectra presented frequencies of the modes are fully consistent of amlodipine besylate and with the vibrational data reported inTable(3.2)

Table (3.2) IR frequency of Amlodipine besylate

Show the FTIR for vibration data Amlodipine besylate

Functional group	Band frequency cm^{-1} of Reference standard Amlodipine besylate	Band frequency cm^{-1} of Amlodipine besylate sample
OH stretching of SO_3	3157.47	3528.80
NH stretching of primary amino group	3298.28	3379.29
CH stretching of benzene ring	2985.52	2906.73
C=O stretching of carbonyl group	1685.79	1670.35
C=C stretching of carbonyl group	1614.42	1431.18

3.2 Physical stability

in this study amlodipine besylate tablet (5mg) measured Physical test include (diameter , thickness , friability, friability, disintegration time) during Period of analysis then recorded results in Table(3.4)

Table (3.3) Result of ongoing condition

Period of analysis	diameter	Thickness	friability	Hardness	disintegration time
Zero	8.08	3.16	0.03	5.76	20 second
Second	8.06	3.17	0.02	6.50	12 second
Third	8.05	3.20	0.04	5.80	6 second
Fourth	8.06	3.22	0.05	6.00	30 second

Table(3.4) Results of acceralated condition

Period of analysis	Diameter	Thickness	friability	Hardness	disintegration time
Zero	8.05	3.18	0.02	6.70	8 second
Second	8.09	3.30	0.04	6.50	10 second
Third	8.07	3.25	0.02	5.80	7 second
Fourth	8.04	3.20	0.12	5.0	5 second

Table (3.5) Acceptance criteria to Physical parameter according USP

Parameter	Diameter	Thickness	Friability	Hardness	disintegration time
Acceptance value	8.00+_0.20	3.5+_0.50	NMT1.0%	NLT4.0 Kg/cm^2	NMT15 Minutes

This results in Ongoing and acceralated Condition Found within acceptance criteria according USP.

3.3 Chemical stability

3.3.1. High performance liquid chromatography

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through pressure and makes it faster. It's also allows the use of smaller particle size for a column packing materials which gives a much greater surface area for interaction between the stationary phase and the molecules flowing past it .this allows a much better separation of the components of the mixture.

Amlodipine analyzed by HPLC, it is possible to determine the degradation of amlodipine .HPLC has the advantage of rapid precise quantitative analysis, automated separation, high sensitivity detection, quantitative sample recovery and it is amenable to diver's samples. It can be used to assess the purity and/or to determine the content of many pharmaceutical substances. it can also be used to determine enantiomer composition, using suitably modified mobile phase or chiral stationary phases. Individual separation mechanisms of isolation, since several principles act together, (Michael. 2006), (international pharmacopoeia, 2012).

Figures (3.1 to 3.9) shows the results of (HPLC) to amlodipine besylate (5mg)

3.3.2 Results of ongoing condition

Table (3.6) show the HPLC analysis of amlodipine besylate (5mg) in ongoing

Table (3.6) Results of ongoing condition

Period of analysis	Standard area	Sample area	Assay%
Zero	5034.976	6591.786	103.83
Second	5034.976	6599.757	103.95
Third	5034.976	6587.326	103.76
Forth	5034.976	6867.728	108.17

Amlodipine besylate in tablet (5mg) form when it kept in stability chamber at determinative condition (30c +_2 temperature and 60+_5 humidity) this causes degradation for a pharmaceutical ingredient in amlodipine tablet .the concentration of active pharmaceutical ingredient in amlodipine tablets from zero time to forty weaks was recorded in Table(3.6) from Figure (3.1 to 3.4).this results show be affected active pharmaceutical ingredient(amlodipine besylate) in ongoing condition .this noticeable in the concentration amlodipine besylate in period of the analysis but this results found in acceptance value according USP.

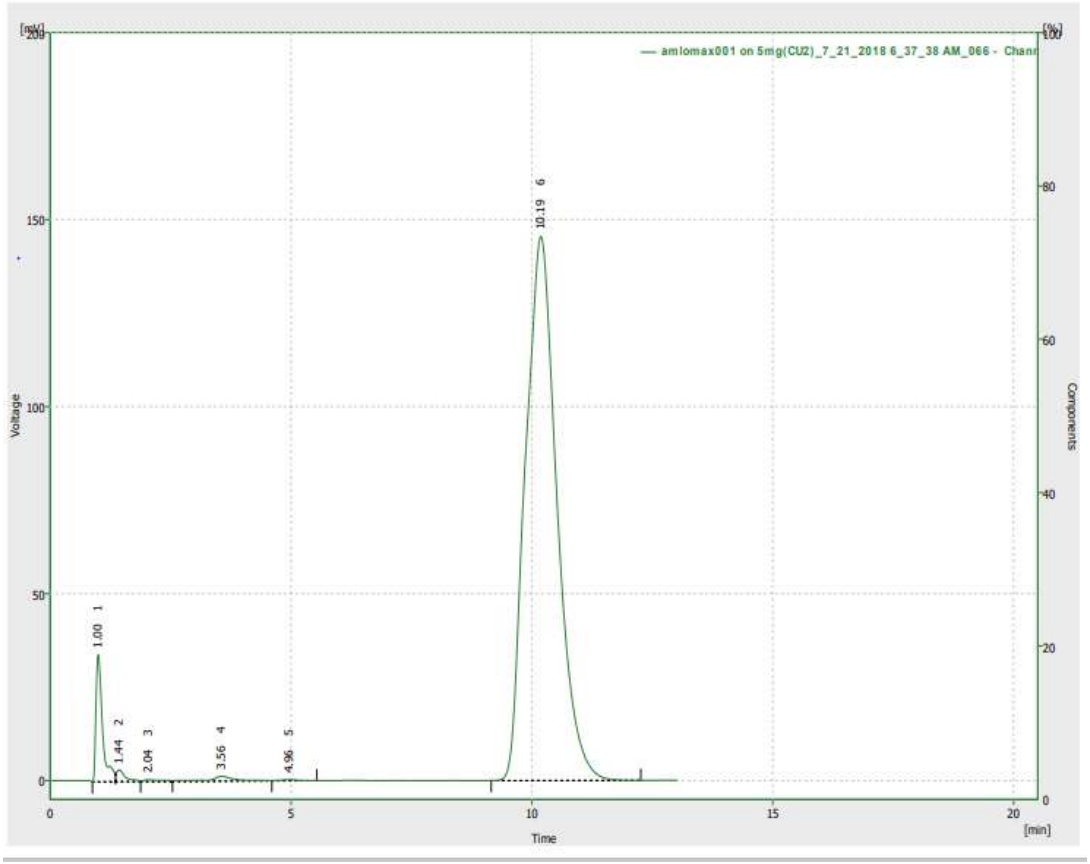


Figure 3.1 HPLC chromatogram for amlodipine besylate in zero time

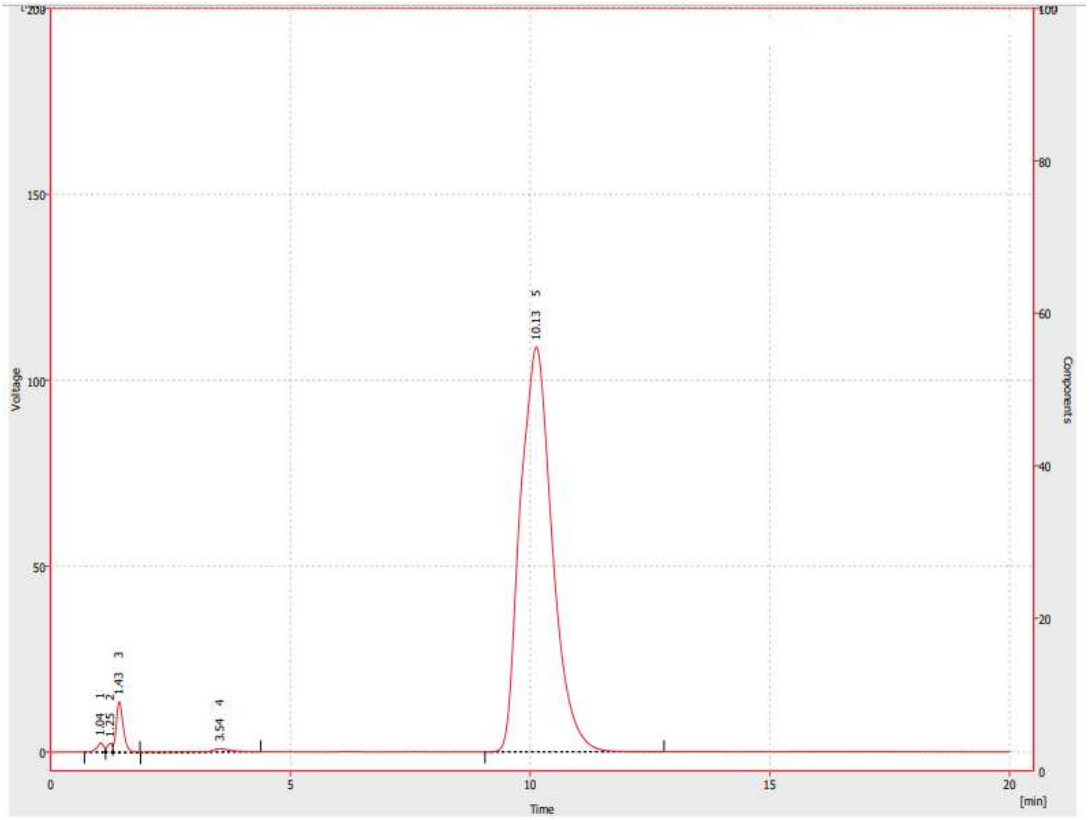
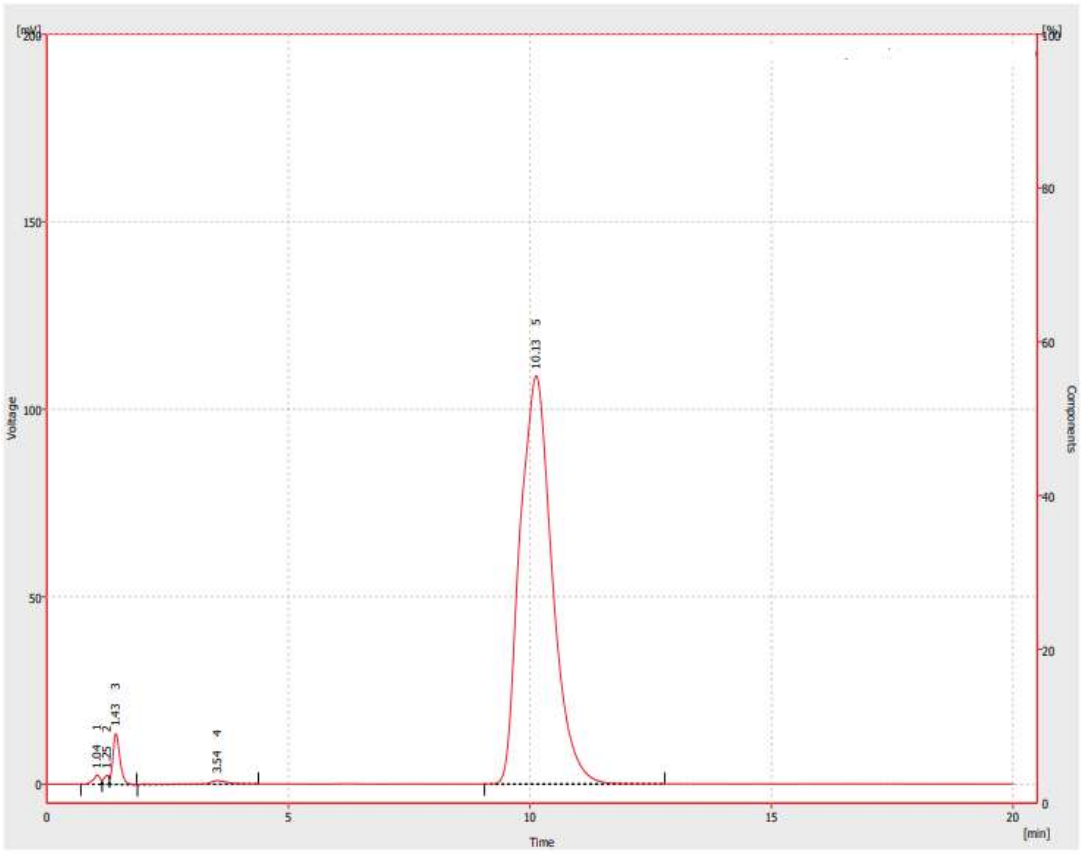

Voltage
Components
Time
[min]
1.04 1
1.25 2
1.43 3
3.54 4
10.13 5
0
50
100
150
200
0
20
40
60
80
100
0
5
10
15
20

Figure 3.2 HPLC chromatogram for amlodipine besylate in weak



one

Figure 3.3 HPLC chromatogram for amlodipine besylate in weak second

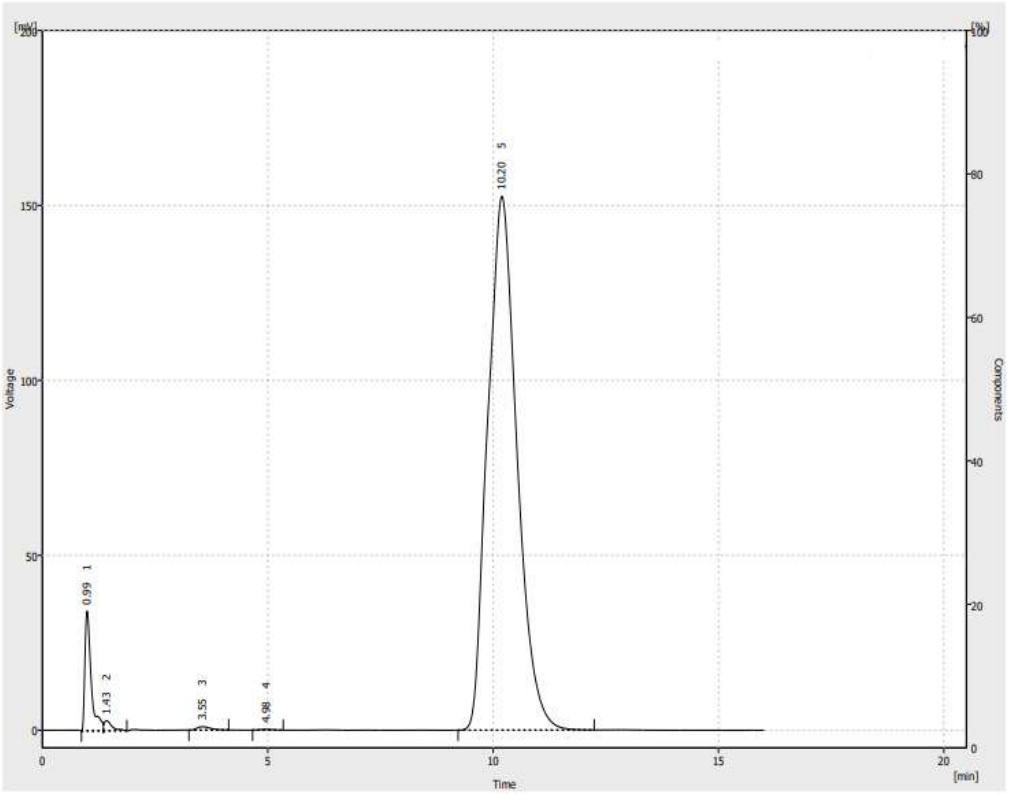


Figure 3.4 HPLC chromatogram for amlodipine besylate in weak third

3.3.3 Results of acceralated condition

Table (3.7) show the HPLC analysis of amlodipine besylate (5mg) in acceralated

Table(3.7)Results of acceralated condition

Period of analysis	Standard area	Sample area	Assay%
Zero	5034.976	6508.091	102.51
Second	5034.976	6618.819	104.25
Third	5034.976	6564.466	103.40
Forth	5034.976	6813.811	107.32

Amlodipine besylate in tablet (5mg) form when it kept in stability chamber at determinative condition (40 +_2 temperature and 70+_5 humidity) this causes degradation for a pharmaceutical ingredient in amlodipine tablet .the concentration of active pharmaceutical ingredient in amlodipine tablets from zero time to forty weeks was recorded in Table(3.7). from Figure (3.4 to3.8).this results show be affected active pharmaceutical ingredient(amlodipine besylate) in acceralated condition .this noticeable in the concentration amlodipine besylate in period of the analysis but this results found in acceptance value according USP.

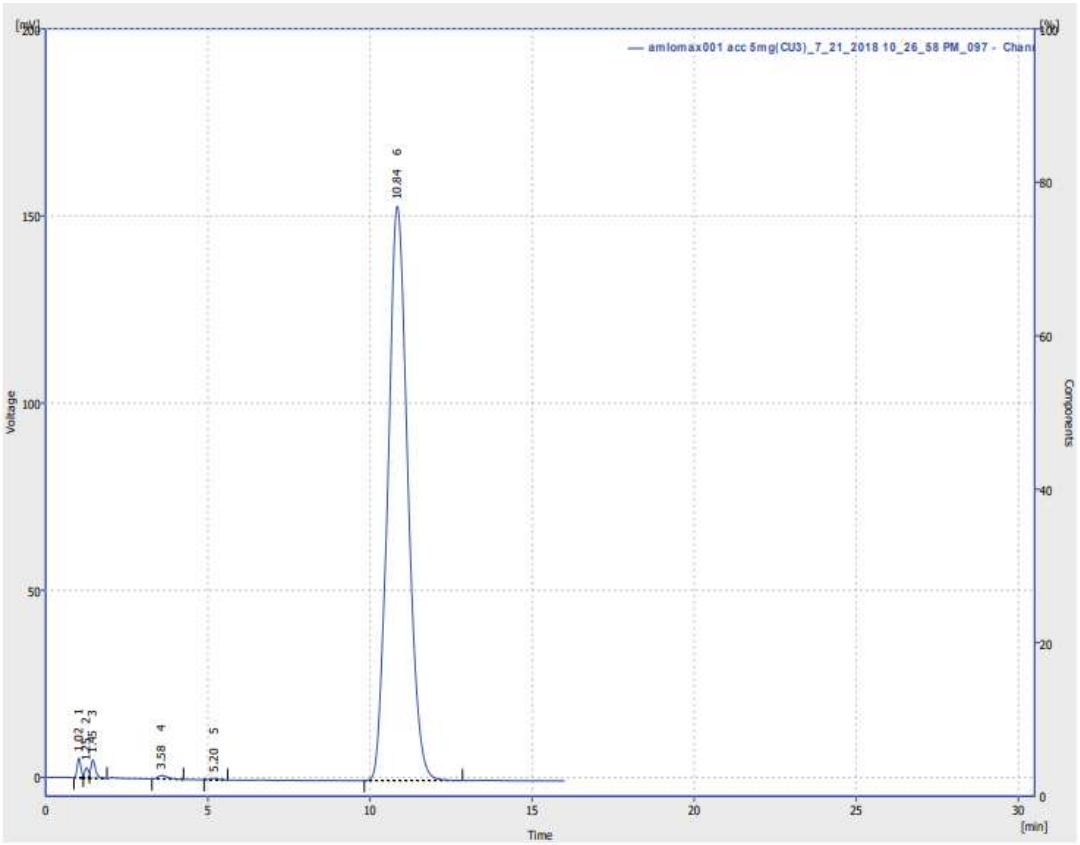


Figure 3.5 HPLC chromatogram for amlodipine besylate in zero time

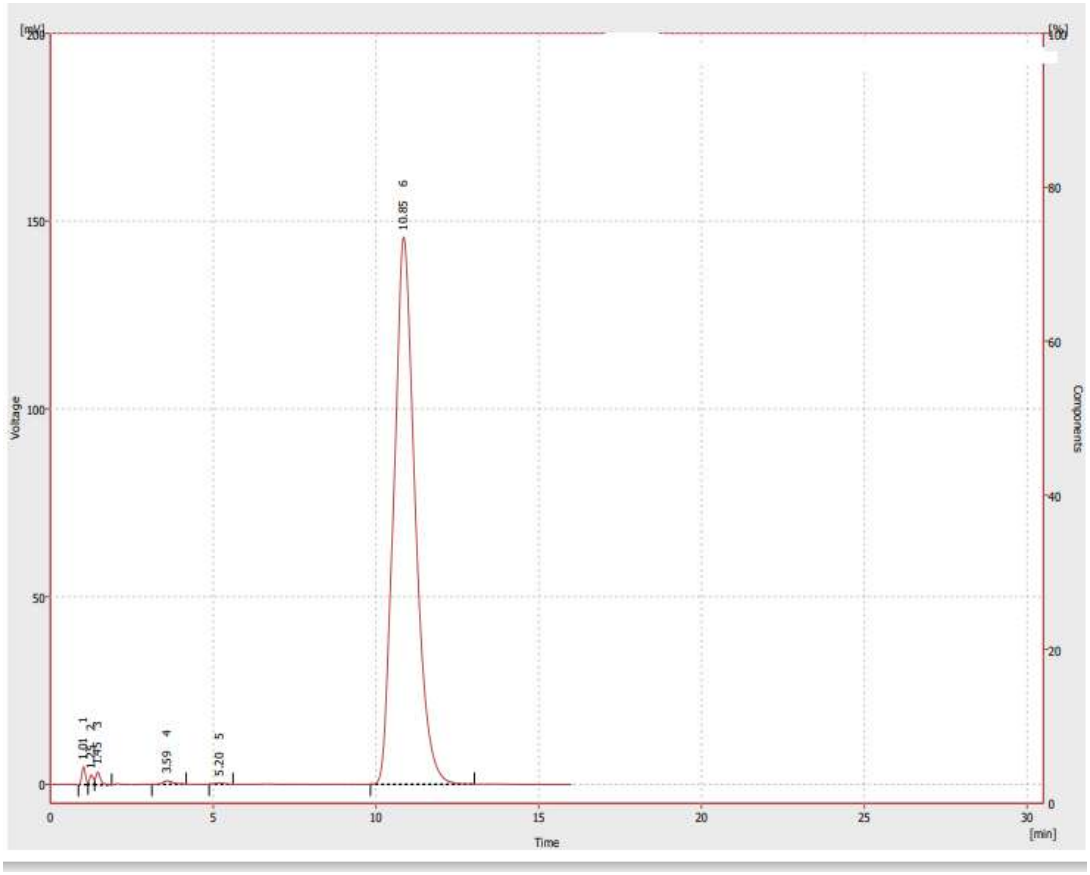


Figure 3.6 HPLC chromatogram for amlodipine besylate in one weak

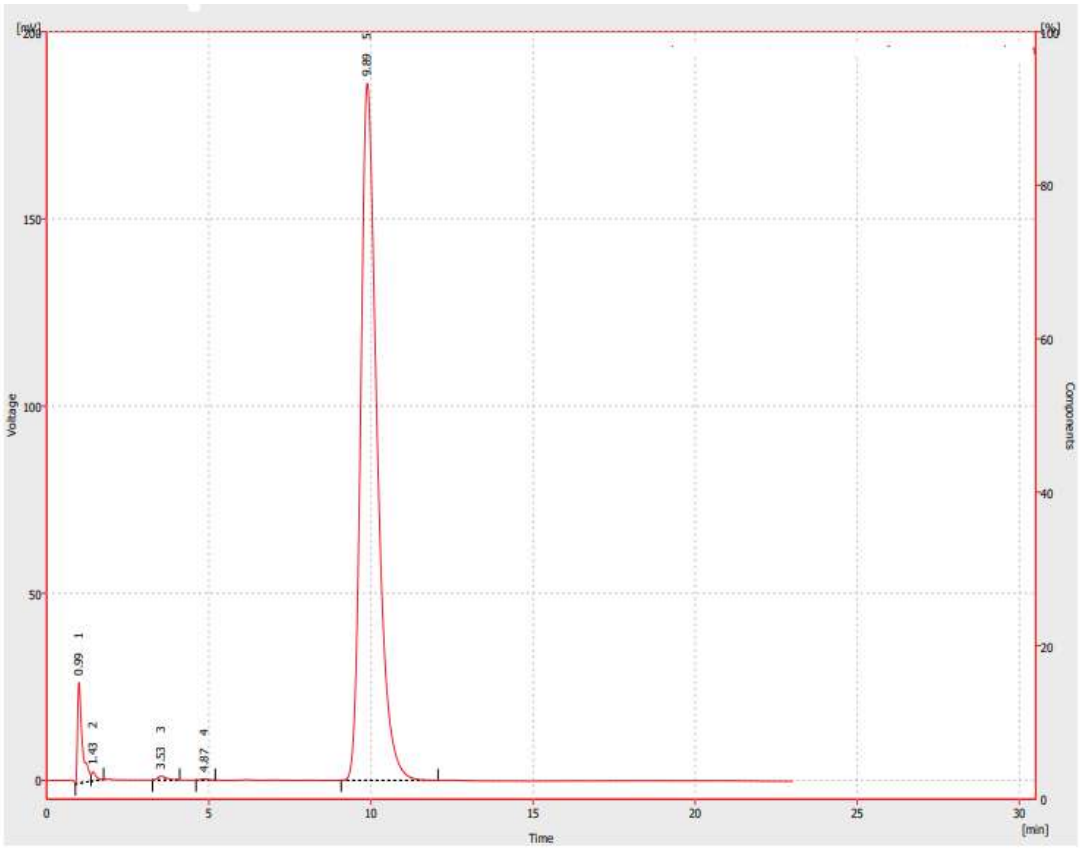


Figure 3.7 HPLC for a chromatogram amlodipine besylate in second weak

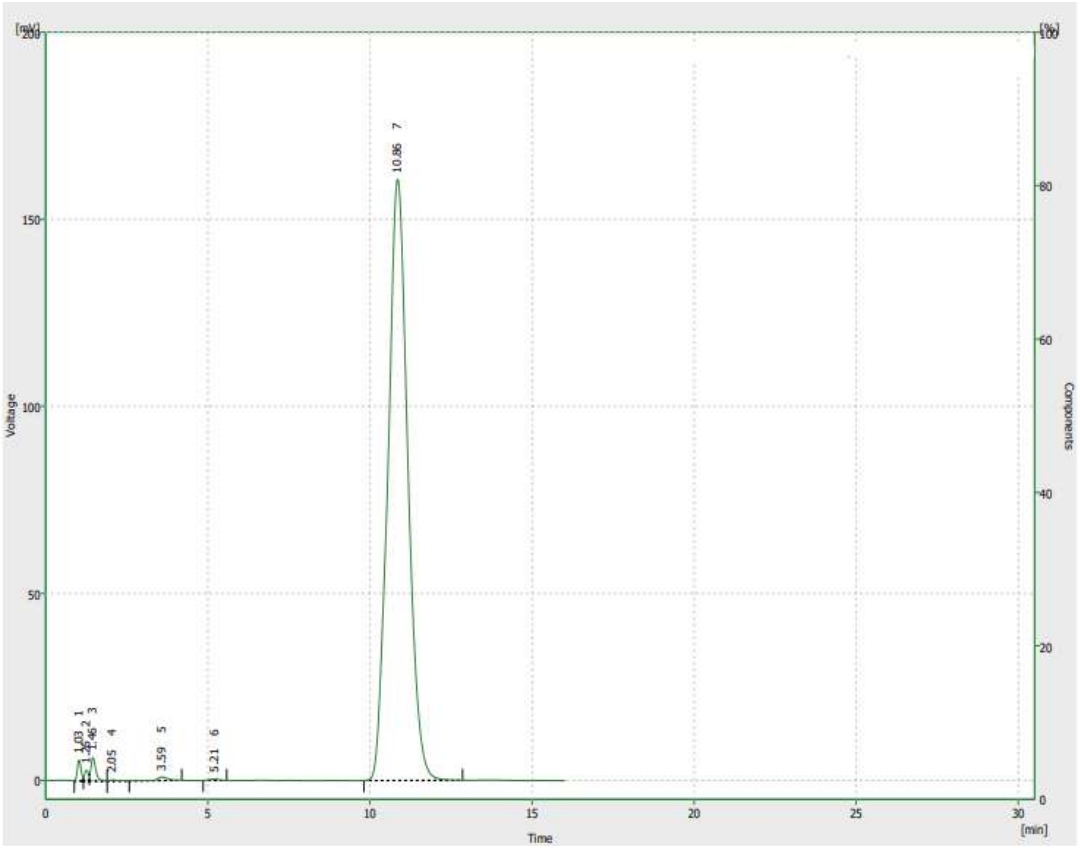


Figure (3.8) HPLC for chromatogram amlodipine besylate in third weak

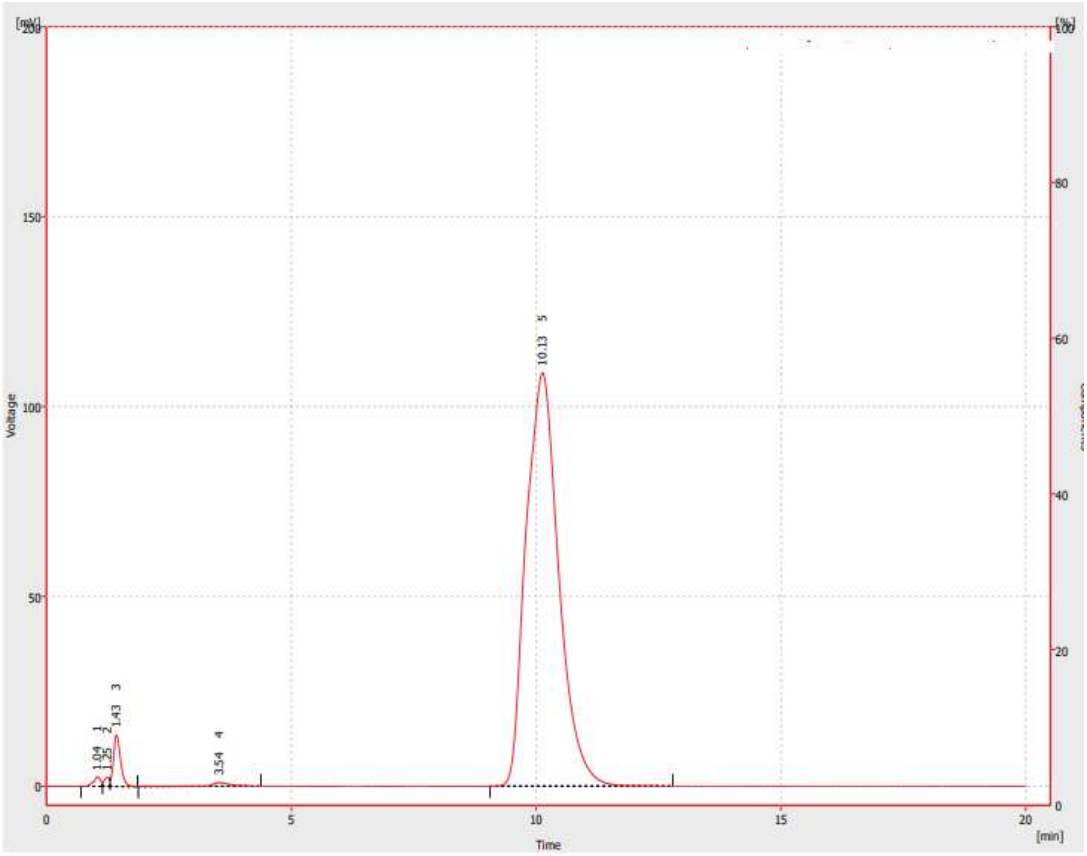


Figure 3.9 HPLC chromatogram for standard amlodipine besylate

3.4 Microbial stability

In this study amlodipine in tablet(5mg) it was microbiological tests in different condition then results record in Tables below :

Table (3.8) Show results in Ongoing amlodipine(5mg) microbiological analysis

Analysis period	TAMC	TYMC
Zero stage	Zero	Zero
After weak	Zer0	Zero
After 15 days	Zero	Zero

Table (3.9) Show results in Acceralated (5mg) microbiological analysis

Analysis period	TAMC	TYMC
Zero stage	Zero	Zero
After weak	Zero	Zero
After 15 days	Zero	Zero

Table (3.10) Acceptance limit OF microbiological analysis According to(USP, BP and JP)

Type microbiological	TAMC	TYMC
Acceptance limit	NMT 10^3	NMT 10^2

TAMC= Total Aerobic Microbial Count TYMC= Total Yeast Molds Count

The Results in Table (3.8 and 3.9) found within acceptance value according USP

3.5 Conclusions

After analysis it was found Amlodipine besylate tablets (5mg) with this formula was stable in ongoing temperature and humidity(30c+-2,60+_5%) and acceralated temperature and humidity (40c+_2,70+_5%) according to results. And this conclusion support amlodipine besylate should be note store at higher temperature and humidity and under light.

3.6 RECOMMENDITION

The study recommend for further analytical studies such as: study effect of temperature and humidity of different areas in Sudan .and study the impact of the PH of water that use for taking the drug that possible effect on absorbance drug.

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