Chapter One

1. Introduction

1.1General principles

1.1.1 Definitions

The purpose of stability testing is to provide evidence of how the quality of an active pharmaceutical ingredient (API) or finished pharmaceutical products (FPP) varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. The stability programme also includes the study of product-related factors that influence its quality, for example, interaction of API with excipients, container closure systems and packaging materials. In fixed-dose combination (FDCs) FPPs the interaction between two or more APIs also has to be considered.

Stability studies of generic pharmaceutical products differ from studies of products containing new chemical entities; the manufacturer of generic product will have access to a body of experience with the original product, and may therefore concentrate his attention on the assessment of its stability.

Tests must also be made to study the possible interaction of the drug product with packaging material in which it will be delivered, transported and stored throughout its shelf-life. The drug regulatory authority in this circumstance should be able to establish a tentative shelf-life on the basis of the initial stability data. These however, have to be confirmed by data generated through an on-going stability programme as required by good manufacturing practice (GMP) rules. The shelf-life should be established with due regard to climatic zone in which the product is to be marketed.

For certain preparation, the shelf-life can only be guaranteed if specific storage instructions are complied with.

For the safety of the patients and rational management of drug supplies, it is important that the expiry date, and when necessary the storage conditions, are indicated on the label.

Certain terms are routinely used, these include, accelerated stability testing, batches, active pharmaceutical ingredients, climatic zones, container and closure system, excipients, expiry date, ongoing stability, specifications, shelf-life, significant change, and utilization period. (WHO 2009 a)

1.1.2 Stability indicating methods

Stability indicating method is one of validated analytical procedures that can detect the changes with time in the chemical, physical or microbiological properties of the API or FPP, and that are specific so that the content of the API, degradation products, and other components of interest can be accurately measured without interference.(Monika *etal* 2002)

1.1.3 Stress testing

Stress testing of the API can help identify the likely degradation products, which, in turn, can help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual API and the type of FPP involved.

For an API the following approaches may be used:

When available, it is acceptable to provide the relevant data published in the scientific literature to support the identified degradation products and pathways.

When no data are available, stress testing should be performed. Stress testing may be carried out on a single batch of the API. It should include the effect of temperature (in 10 °C increments (e.g. 50 °C, 60 °C, etc.) above the temperature used for accelerated testing), humidity (e.g. 75%)Relative humidity (RH) or greater) and, where appropriate, oxidation and photolysis on the API. The testing should also evaluate the susceptibility of the API to hydrolysis across a justified range of pH values when in solution or suspension.

Assessing the necessity for photostability testing should be an integral part of a stress testing strategy. Results from these studies will form an integral part of the information provided to regulatory authorities. (WHO 2009 b***)***

1.1.4 Storage and stability of dispensed products:-

It is true to say that no pharmaceutical product is stable indefinitely and certainly the majority of products are stable only for a limited time. The instability of pharmaceutical products may be demonstrate as dry or excipients degradation. All instability is thermodynamic in nature, but poor formulation, poor packing, and poor storage conditions may exacerbate this inherent instability. (WHO 2009c)

1.1.5 Possible results of pharmaceutical product instability:-

The pharmaceutical products instability may result in.

a- Loss of active drugs (e.g. aspirin hydrolysis, oxidation of adrenaline).

b- Loss of vehicle (e.g. evaporation of alcohol from alcoholic mixture, evaporation of water from oil-in-water creams).

c- Loss of content uniformity (e.g. impaction of suspension, creaming of emulsions).

D- Reduction of bioavailability (e.g. aging of tablets resulting in change in dissolution profile).

e- Loss of pharmaceutical elegance (e.g. fading of coloured solutions and tablets).

f- Production of potentially toxic materials (e.g. breakdown Products from drug degradation). (Shaikh and Sial 1996)

1.1.6 Chemical degradation

There are number of chemical degradation that can happen to the pharmaceutical products, these include solvolysis, oxidation, photolysis, polymerization and optical isomerization. (Jens *et al, 1991*)

1.1.7 Physical degradation

A lot of physical degradation could happen to pharmaceutical products through polymorphism, vaporization, water loss, absorption of water and partial sedimentation. (Jens *et al, 1991*)

1.1.8 Temperature

An increase in temperature generally increases the rate of reactions and thus the degradation of pharmaceutical products is generally increased with an increase in temperature. The Arrhenius equation quantitatively describes the relationship between reaction (or degradation) rate (k) to temperature (T)

$$k = A\exp(-Ea/RT)$$

Where (A) is the frequency factor, (R) is the gas constant, (Ea) is the activation energy, and (T) is the temperature in degrees absolute. (Ertel and Carstensen, 1990.)

1.1.9 Solvent

Drugs may by place in variety of solvents in order to produce a suitable dosage form. However, the different solvents used may influence the degradation of the drug. Solvents alter the activity coefficients of the reactant molecules, but the change in solvent can also cause a change in (pK_a), surface tension, or viscosity, thus affecting the reaction rate. (Jens *et al, 1991*)

1.1.10 Moisture (atmospheric)

Moisture or humidity can adversely affect the stability of dry drug formulations, such as tablets and capsules. (Jens *et al, 1991*)

1.1.11 Additives

1.1.11.1Buffer salts

These may be used to adjust and maintain the pH of a formulation. (Golightly *et. al, 1988).*

1.1.11.2 Surfactants

Surfactants may be added to formulation solubilizers or emulsifier. However, the presence of the surfactants may increase, decrease or leave unchanged the degradation of a drug or the excipients. (Golightly *et. al 1988*).

1.1.12 Selection of batches

Data from stability studies on at least three primary batches of the API should normally be provided. The batches should be manufactured to a minimum of pilot scale by the same synthesis route as production batches, and using a method of manufacture and procedure that simulates the final process to be used for production batches. The overall quality of the batches of API placed on stability studies should be representative of

the quality of the material to be made on a production scale (WHO 2009d*)*

1.1.13 Testing frequency

For long-term studies, frequency of testing should be sufficient to establish the stability profile of the API. For APIs with a proposed re-test period or shelf-life of at least 12 months, the frequency of testing at the long-term storage condition should normally be every three months over the first year, every six months over the second year, and annually thereafter throughout the proposed re-test period or shelf-life. At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g. 0, 3 and 6 months), from a six month study is recommended. (WHO 2009e*)*

1.1.14. Evaluation

The purpose of the stability study is to establish, based on testing a minimum of the number of batches, unless otherwise justified and authorized, of the API and evaluating the stability information (including, as appropriate, results of the physical, chemical, biological and microbiological tests), a re-test period applicable to all future batches of the API manufactured under similar circumstances. The degree of variability of individual batches affects the confidence that a future production batch will remain within specification throughout the assigned re-test period. (WHO 2009f*)*

1.2 Analytical method validation

Validation should be performed in accordance with the validation protocol. The protocol should include procedures and acceptance criteria for all characteristics. The results should be documented in the validation report. (BP 2010a)

1.2.1 Justification

Should be provided when non-pharmacopoeial methods are used if Pharmacopoeial methods are available. Justification should include data such as comparisons with the pharmacopoeial or other methods (BP2010a).

1.2.2 Standard test methods

Standard test methods should be described in detail and should provide sufficient information to allow properly trained analysts to perform the analysis in a reliable manner. As a minimum, the description should include the chromatographic conditions (in the case of chromatographic tests), reagents needed, reference standards, the formulae for the calculation of results and system suitability tests. (BP 2010b)

1.2.3. Characteristics of analytical procedures

The characteristics of analytical procedure include the following Accuracy, precision, repeatability, intermediate precision, reproducibility, robustness or ruggedness, linearity, range, specificity, detection limit, and quantitation limit. (BP 2010a)

1.2.4 System suitability testing

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters that need to be established for a particular procedure depend on the type of procedure being evaluated, for instance, an HPLC procedure should establish plate number, tailing factor or asymmetry, retention time, resolution, RSD% (relative standard deviation) and C.V (coefficient of variation). (BP 2010)

1.3 Atenolol

Atenolol is a drug used in the treatment of hypertension, angina pectoris and acts as an anti-arrhythmic to regulate the heartbeat in the prevention of myocardial infractions. It is more commonly known as a beta-blocker.

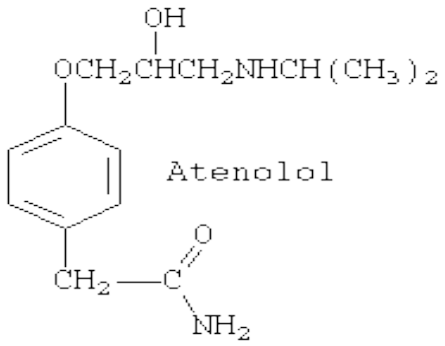
More specifically it acts as a beta-1 (ß-1) cardio selective adrenoreceptor blocking agent, whose fundamental objective is to control the heart. Atenolol does so by restricting certain nerve impulses, thereby controlling the rate and force of contraction, consequently reducing blood pressure. In addition, its use in the treatment of Angina Pectoris makes it an invaluable drug in industry. On the outset, Atenolol actively reduces the heart rate, in turn decreasing systolic and diastolic blood pressures. The net effect of both the heart rate and blood pressure being controlled is the reduction in myocardial work and oxygen requirement which reduce cardiovascular stress, thereby preventing arrhythmia and anginal attacks. (Neutel et. *al*, 1990*)*.

1.3.1 Brief history.

Atenolol was discovered by imperial chemical industries (ICI) in 1976, whilst searching for a specific Beta-1 cardioselective adrenoreceptor blocking agent. Though imperial chemical industries (ICI's) research was invaluable, Atenolol may be seen as a drug evolved from the series of research being conducted into beta receptors during the late nineteen fifties. The first development of a chemical that acted to inhibit beta receptors was discovered by Slater, Powell and co-workers at Lilly in 1958. However the compound, 3,4-dichloro isoproterenol only acted as a partial agonist that produced marked stimulation of cardiac beta receptors before inhibition (Neutel *et. al*, 1990).

1.3.2 Characteristics:

Atenolol chemical structure.



(RS)-4-(2-hydroxy-3-(isopropyl amino) propoxy) phenyl) acetamide or 2-4-(2-hydroxy-3-isopropylaminopropoxy) phenyl) acetamide or 4-(2-hydroxy-3-((1-methlethyl) amino) prpoxy) benzeneacetamide.

C_{14} H_{22} N_2 O_3. Relative molecular mass is 266.3. Melting point is 152-154 ^oC and it has two enantiomers. Atenolol is soluble in water (0.3mg/ml) and very soluble in ethanol (3.4 mg/ml) and DMSO (18 mg/ml) .practically insoluble in ether. Industrially, Atenolol is produced as a racemic mixture of the two enantiomers. Conveniently both forms are bioactive in treating hypertension, angina and arrhythmia, which makes it a truly versatile drug. Albeit, recent studies have shown that the S-Atenolol isomer was found to avoid the occasional side effect of an excessively lowered heart rate sometimes encountered with the racemate. (Bevinakatti and. Banerji, 1992)

1.3.3 Spectral data of Atenolol

1.3.3.1 IR spectroscopy

A key observation which is raised in the IR spectrum of atenolol is the level of hydrogen bonding. By virtue of the electronegative nitrogen atom and the even more electronegative oxygen atom, the IR spectra indicate that intermolecular H-bonding may be present. This is demonstrated by

the IR frequency bands of the -OH and H-N and groups having stretched at 3368cm^{-1} and 3198-3071 cm^{-1} respectively. (de Castro 2007 *et. al*).

1.3.3.2 Mass Spectra of Atenolol

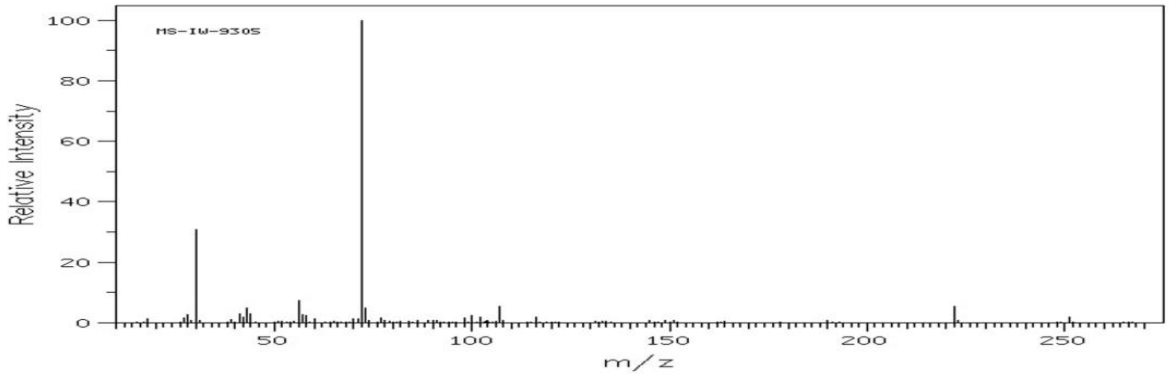


Fig 1.1 Atenolol mass spectrum

The molecular ion was determined at 266 da

1.3.3.3 ^{1}H NMR of Atenolol

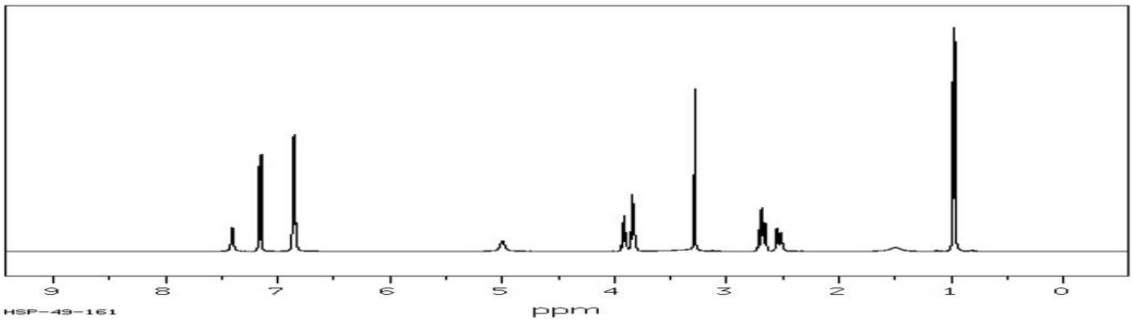


Fig 1.2 ^{1}H NMR of Atenolol

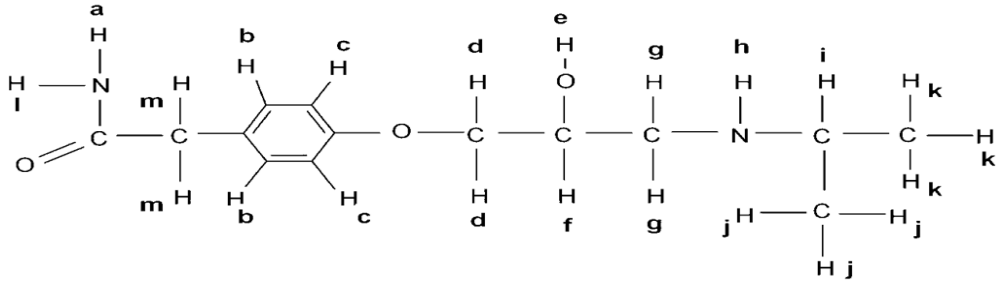


Table 1.1 protons ^{1}H NMR of Atenolol

proton	a	b	c	d	e	f	g	h	i	j	k	l	m
Chemical shift ppm	7.41	7.19	6.86	3.84	5.0	3.91	2.54	1.5	2.7	0.97	0.97	6.85	3.3

^{1}H NMR proton labels

There are a few key inferences that can be made from the data acquired. Proton a is bonded to Nitrogen, a fairly potent electronegative atom (3.04 Pauling units). The N-H bond is thus slightly polarised with the Nitrogen atom drawing the hydrogen's electron towards it. The nature of this bond means that Proton a become deshielded thereby making it extremely acidic. The net effect is that the 1-H nmr displays the proton vastly downfield in relation to its counterparts. For comparative purposes, it is clear that Proton's j and k are shielded rather well by virtue of their extreme upfield positions. They exhibit integration, being in the same environment shown as the largest peak on the spectra. As well as Proton a, Proton c experiences deshielding even though not are directly bonded to the electronegative species. The atom responsible is now the extremely electronegative Oxygen atom (3.44 Pauling units). Despite its distance, the pair of protons experience deshielding by virtue of oxygen's potent electron withdrawing affect. As predicted, Proton e is isolated as a single broad peak not coupling with neighboring atoms, being attached to the electronegative Oxygen atom. Coupling is displayed, pronounced for Protons i whereby it is able to couple with Protons k and j, evolving a doublet of quartets (Monika et. *al*, 2011)

1.3.3.4 ^{13}C NMR of Atenolol

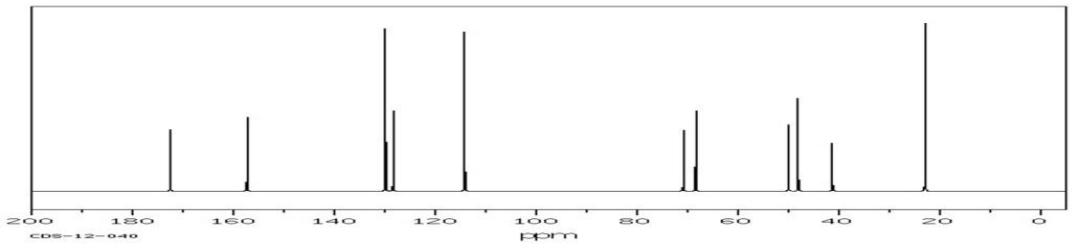


Fig 1.3 ^{13}C NMR of atenolol

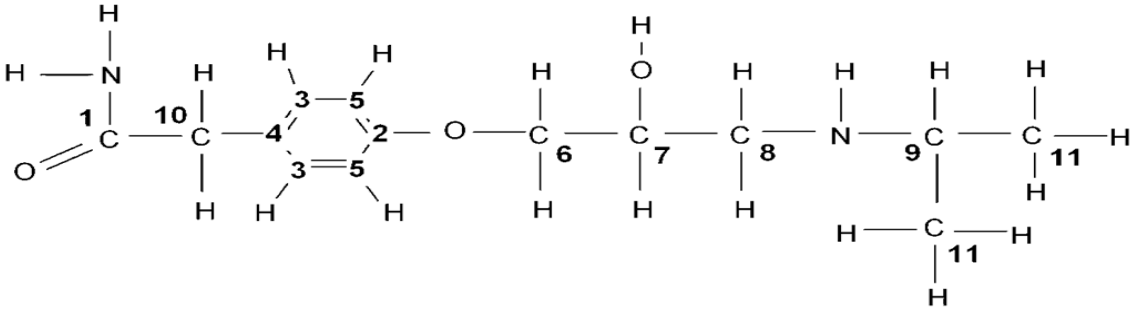


Table 1.2 protons ^{13}C NMR of atenolol

carbon	1	2	3	4	5	6	7	8	9	10	11
Chemical shift	175.52	157.27	129.88	128.31	114.17	70.81	68.35	49.96	48.09	41.31	22.88
intensity	366	440	967	477	946	361	478	394	553	285	1000

^{13}C NMR carbon labels

Similar inferences can be made for the ^{13}Carbon NMR, with the electronegative oxygen showing obvious electronegative potency with Carbon 1 being shifted extremely downfield this time being assisted with the largely electronegative nitrogen atom as well. The net effect is a highly polarized delta positive carbon atom. (Monika et. *al*, 2011)

1.4.1 Stability of Atenolol

Atenolol is unstable in solutions and therefore the development of a liquid dosage form is a significant challenge. Studies showed that the degradation rate of Atenolol is dependent on the temperature, indicating higher stability at 4 degrees C. Atenolol syrup is stable for 9 days, with

acceptable appearance. A second order model adequately described Atenolol decomposition when stored as syrup. A stability-indicating method was developed and validated in order to evaluate these studies. (Foppa et. *al, 2007*,)

There is a possibility that lower air, moisture and light protection could impact on physico-chemical stability of medicines tablets atenolol at room temperature in original packaging, in compliance aids and Petri dishes remained the same in appearance and passed physico-chemical tests. Tablets exposed to 40°C with 75% relative humidity in compliance aids passed tests for uniformity of weight, friability and chemical stability but became pale and moist, softer (82 newtons ± 4; $p < 0.0001$) than tablets in the original packaging (118 newtons ± 6), more friable (0.14% loss of mass) compared with other tablets (0.005%), and failed the tests for disintegration (>15 minutes) and dissolution (only 15% atenolol released at 30 minutes).(Chan, *et. al*, 2007).

It was found that Atenolol is compatible with starch, Sat-Rx, Primojel, Avicel pH, Ac-Di-Sol, cross-linked PVP, magnesium stearate, calcium sulphate dihydrate, dicalcium phosphate and icing sugar. Interactions of atenolol with PVP, lactose and the lubricant stearic acid were found, although it cannot be conlusively stated that interaction incompatibilities will occur during storage at room temperature. (Botha and. Lötter, 1990)

The stability of atenolol solutions was evaluated under accelerated isothermal degradation conditions at 90 ºC. A specific and sensitive HPLC method was adapted to study the pH dependence of the stability. The maximum stability of atenolol was achieved at pH 4 with a k value of 1.1 × 10(-3) h-1. The degradation of atenolol followed first-order kinetics at above mentioned conditions (Hatem, *et. al*, 1996).

The photostability of the beta-blocker drug Atenolol was evaluated at pH 9, 7.4 and 4.0. The drug was exposed to UVA-UVB radiations and the photoproducts were detected by reversed phase LC methods. The photodegradation was found to increase with the pH value decreasing. The major photodegradation product at pH 7.4 was identified as 2-(4-hydroxyphenyl) acetamide. The LC method developed for routine analyses (column: C-18 Alltima; mobile phase: TEA acetate (pH 4; 0.01 M)-acetonitrile 96:4) was found to be suitable for the stability indicating determination of Atenolol in pharmaceutical dosage forms. (Andrisano 1999 *et. al*).

1.5 Aims and objectives

The climatic conditions (temperature, sunlight and humidity) affected the stability of many formulated drugs. Under such conditions the instability may produce toxic materials and/or decrease the amount of active Ingredients,

Atenolol, a widely used drug in Sudan, may raises questions concerning its Stability under such conditions.

Therefore this project aims to study the stability of this drug in its pure and formulated forms, at the same time the project aims to establish analytical method to analyze Atenolol in the presence of its decomposed products.

The project aims to study the compatibility of excipients with Atenolol and their effect on Atenolol stability.

It also aims to study the possibility of liquid formulation of Atenolol like syrup, suspension and injection and their stability.

Chapter Two

2 Materials and methods:

2.1 Chemicals

2.1.1 Atenolol raw material manufacturer (Ipca, India).

2.1.2 Atenolol (BPRCS) British Pharmacopoeia Reference Chemical Substance Lot No BP 492-HA71AY

2.1.3 1-pentanesulphonic acid sodium salt Analar (AppliChem, Germany).

2.1.4 Dibasic potassium phosphate Analar (Scharlau, Spain).

2.1.5 Dibutylamine GPR (Scharlau ,Spain).

2.1.6 Orth-phosphoric acid Analar (Scharlau, Spain)

2.1.7 Hydrochloric acid Analar (Sharlau ,Spain).

2.1.8 Methanol HPLC grade (Scharlau ,Spain).

2.1.9 Acetonitrile HPLC grade (Scharlau , Spain).

2.1.10 Sodium hydroxide pellets Analar (Scharlau ,Spain).

2.1.11 Butan-2-ol GPR(Scharlau , Spain)

2.1.12 Propan-2-ol GPR (Scharlau , Spain)

2.1.13 Propanol Analar (Scharlau. Spain).

2.1.14 Ethanol GPR

2.1.15 n-Hexan (Scharlau, Spain).

2.1.16 Dichloromethane Analar (Scharlau, Spain).

2.1.17 Dimethylformamide GPR (BDH ,England).

2.1.18 Ethyl acetate (BDH ,England).

2.1.19 Dioxan Analar (BDH , England).

2.1.20 Working standard (Amipharma labs)

2.2 Instruments :

2.2.1.1 High Performance Liquid Chromatograph (HPLC) isocratic

LC delivery isocratic system (pump) model S1122 with on-line degassing.

Manufactured by Sykam (Germany) 2008 configured with automatic Sample injector model S5200 and Diode Array Detector model (DAD) S3210 the data processed by Peak simple chromatography data system software.

2.2.1.2 High Performance Liquid Chromatograph (HPLC) gradient

LC delivery gradient system (pump) model S1132 with on-line degassing, stainless steel analytical head, flow rate up to 10 ml/min increment 0.1 ml/min.

Manufactured by Sykam (Germany) 2010 configured with automatic Sample injector model S5200 and Diode Array Detector model (DAD) S3210 the data processed by chromo star software.

2.2.2 UV / Vis. Spectrophotometer

UV /Vis double beam spectrophotometer model 1800 scanning range 190-1100 nm ,band width 1 nm, with UV probe software, manufactured by Shimadzu. Japan 2010.

2.2.3 FT-IR Spectrophotometer

Fourier transform double beam infrared spectrometer. Model 4100 manufactured by Jasco. Japan 2011

2.2.4 Automatic Polarimeter

Digital automatic polarimeter Model DR-188D manufactured by Time Star India. 2011.

2.2.5 Melting point apparatus

Digital melting point apparatus manufactured by Gallenkamp England 1990.

2.2.7 Disintegration tester

Three stations disintegration tester model DZ-3 manufactured by Erweka Germany 1990.

2.2.8 Hardness Tester.

Hardness tester with magazine for automatic movement of samples model TBH 30

Manufactured byErweka Germany 1990.

2.3 Methods

2.3.1 Identification tests

2.3.1.1 Melting point

The melting point was determined using digital melting point apparatus, the sample was placed in one closed end capillary tube and inserted in the instrument and watched from eye view window until it was melted and the temperature was recorded.

2.3.1.2 UV/Vis scanning of atenolol

Dilute solution of Atenolol having the concentration of 0.01% in methanol was scanned between 230 to 350 nm using UV / Vis spectrophotometer. The instrument parameter was set to medium scan

speed, the wavelength range (nm) was 210–300 and mode was set to absorbance and the slit width was set to 1 nm.

2.3.1.3 IR of atenolol

The sample was prepared in potassium bromide disc by mixing Atenolol with potassium bromide in the ratio of 1:300 and grinded to fine powder with agate mortar and pestle then pressed under vacuum by hydraulic press and examined by FT-IR in form of transparent disc the spectrum was compared with BPCRS.

2.3.1.4 Optical rotation of atenolol

0.1 gm of Atenolol was dissolved in 10 ml of water and the angle of rotation was measured using automatic polarimeter. The tube used was 1dc.and the optical rotation was calculated.

2.3.2 High Performance Liquid Chromatography (HPLC) method

This is USP method was used for assaying Atenolol and its preparation, the instrument used (2.2.1).

The chromatographic system:

Mobile phase was prepared by dissolving 1.1 gm of 1-pentanesulphonic sodium salt and 0.7 gm of anhydrous dibasic potassium phosphate in 700 ml of water.2ml of dibutylamine was added and the pH was adjusted to 3 by 0.8M phosphoric acid. 300 ml of methanol HPLC grade was added, then filtered through 0.45 micron membrane filter, The solvent delivery system was isocratic, Column type ODS 150 mm length, 4.6 mm diameter (Waters).Detector diode array (spectrometric) wave length 225 nm. Flow rate 1ml / minute. Injected volume 20 micro lire. Column temperature is room temp.

2.3.3Accelerated stability studies of Atenolol (effect of temperature and humidity).

The accelerated stability was done on tablets sample (Amitenol 100 mg from Amipharma laboratories, the sample was placed in stability chamber adjusted at 40 C and 75% RH. Testing time protocol was made and the samples analysis done in intervals of one, two, three, and six months. The tests include chemical assay which was done by HPLC method and other physical tests including appearance, hardness done by hardness tester and disintegration done by disintegration tester. (Table3.1)

2.3.4 The effect of sunlight on Atenolol solution

0.1% solution of Atenolol was prepared in water in volumetric flask with stopper and placed in direct sunlight and assayed each 1 hour for 5 hours by HPLC method. Dilution was made by taking 1 ml from the solution that placed in the sunlight in 100 ml volumetric flask and completed tothe mark by mobile phase then 20 µl was injected in the HPLC system. (Table3.2)

2.3.5 The effect of solvents on Atenolol UV spectrum.

A solution having concentration of 10 micro gram / ml of Atenolol was prepared using different types of solvents and examined by scanning with UV spectrometer in the range of 230 nm to 350 nm.The solution was prepared by dissolving 0.1 g Atenolol in 100 ml of the solvent under test then further 1 ml was diluted to 100 ml with the same solvent. (Table3.3)

2.3.6 The effect of pH on Atenolol UV spectrum

Atenolol solutions (50 micro gram) were prepared in different ranges of phosphate buffers from pH less than 1 up to approximately 14 starting with orthophosphoric and ending with potassium hydroxide. The

solutions were scanned in the range of 230nm to 350 nm by UV spectrometer. (Table 3.4)

2.3.7 The effect of UV light on Atenolol

0.1% solution of Atenolol in water was exposed to UV light for 24 hours and analyzed after 1hour, 2 hours, 10 hours and 24 hours. The analysis was done by HPLC method .The solution was kept under UV 254 nm, and 316 nm, 1 ml was diluted to 100 ml with mobile phase and 20 µl was injected each time interval. (Table 3.5)

2.3.8 The effect of excipients

100 mg of Atenolol plus individual excipients that used in formula of Amitenol. In the ratio of their presence was dissolved in water and placed in water bath at 70 ^{o}C analysis was done after 7 hours by HPLC method by diluting 1 ml of each solution to 100 ml with mobile phase and 20 µl was injected on the HPLC system. Table (3.6)

2.3.9 Acidic and Alkaline hydrolysis of Atenolol

Three 0.1% solutions Atenolol were prepared in water, 1M HCl and 1M NaOH The solutions were placed in two water baths one adjusted at 50 ^{o}C and the other at 70 ^{o}C. Samples were taken every 30 min neutralized and analyzed simultaneously by (HPLC) and UV spectrometer taking the absorption at 275 nm and scanned between 230 to 350 nm. Tables (3.7, 3.8, 3.9, 3.10, and 3.11)

2.3.10 Analytical method validation

The procedure is as follow.

The mobile phase was prepared by dissolving 0.7 gm of disodium hydrogen orthophosphate in 700 ml of water; 0.5 gm of 1-heptanesulphonic acid sodium salt and 2 ml of dimethylamine, the volume completed to 1 litre with distilled water and the pH was adjusted

to 4.5 with phosphoric acid. 850 ml of this buffer and 150 ml acetonitile were mixed together and used as mobile phase. The system was isocratic pump. The flow rate was adjusted to 1 ml/ min. The column ODS 250mm × 4.6 mm. The injected volume 20 µl using auto injector. The spectrometric detector was adjusted to230 nm.The column temp was room temp.The software used to collect the data was Chromostar.The standard prepared by dissolving 0.100 gm of Atenolol working standard in 100 volumetric flasks with water and completed to volume with the same solvent, further dilution by pipetting 1 ml into 25 volumetric flasks and completed with mobile phase.

The protocol of validation was done by preparing five concentrations of Atenolol from 80% to 120% in step of 10% each level was injected three times and used for calculation of accuracy and linearity .The 100% concentration was injected six times for calculation the precision and intermediate precision which was done by three different analysts using two different instruments for robustness. Placebo (all the excipients except the API) was prepared and injected for specificity.

Chapter Three

Results and discussion

3.1 Identification tests of atenolol

3.1.1 Melting point

The melting point of Atenolol was found to be 153 ^{o}C and as stated by British Pharmacopoeia (BP) in between (152 ^{o}C - 155 ^{o}C).

3.1.2 UV /Vis spectrophotometer scan identification.

When an Atenolol solution of 10 µg concentration in methanol was scanned by UV/Vis spectrophotometer in the range of UV from 230 nm to 350 it showed two absorption peaks at 275 nm and 282 nm fig (3.1) and it is complying with (BP) British Pharmacopoeia.

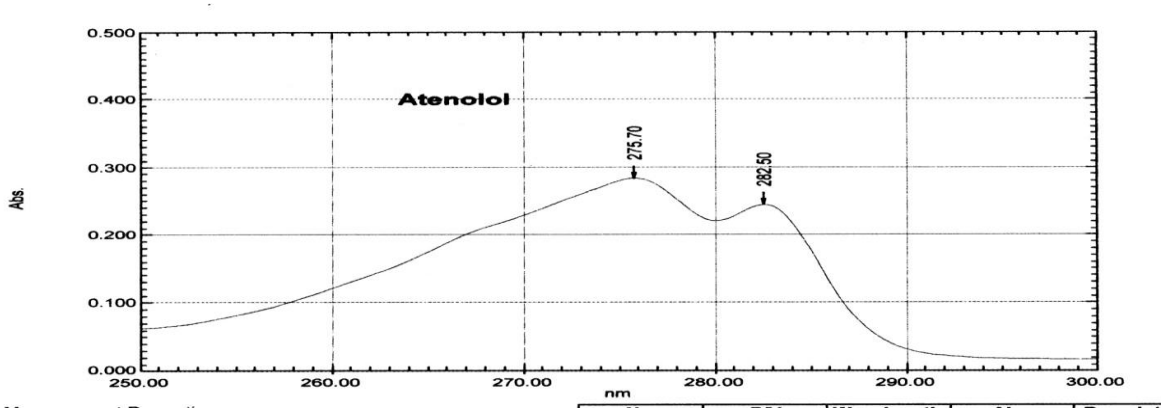


Fig (3.1) UV spectrum of 10 µg Atenolol in methanol

3.1.3 Infrared (FT-IR) Identification

Atenolol powder was prepared in potassium bromide disc and examined by FT-IR instrument in the range from (400 nm to 4000 nm) the spectrum Fig. 3.2 and 3.3) was compared with (BPRCS) spectrum which was prepared and scanned in the same conditions it was found that the two spectrums were identical.

It is clear that Atenolol possesses a multitude of component substituents. The main "bulk" on the molecule is attached to a bulky structure itself, namely the benzene ring, such a molecule can be described as a benzeneacetamide by the $O=C-NH_2$ amide group extruding from a benzene ring. As well as the amide functional group, the conjugating C=C bond in the benzene ring, the methine (CH), methylene (CH_2), methyl (CH_3) and -OH functional group were distinctive on the IR spectra (amide criteria 1650cm^{-1}; CH criteria 2880-2900cm^{-1}; CH_2 criteria 2916-2936 cm^{-1}; CH_3 criteria 2850 cm^{-1}; conjugating C=C criteria 1640-1610; -OH criteria 3200-3550cm^{-1}).

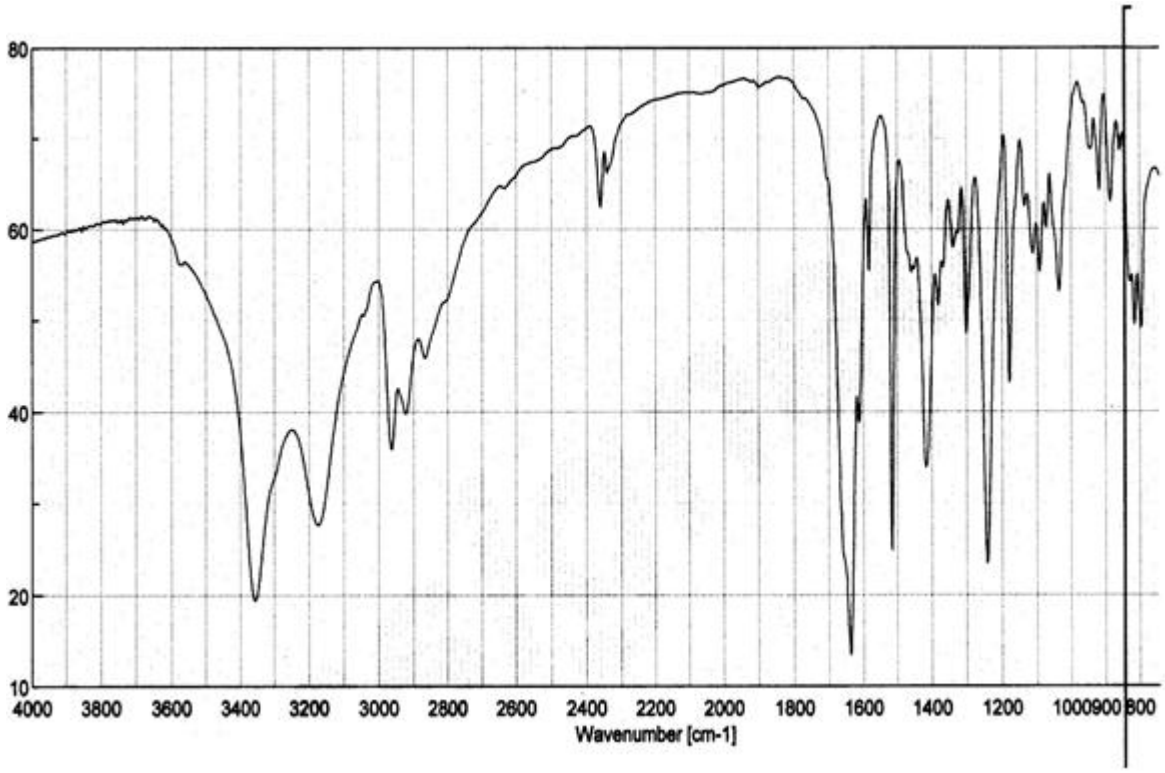


Fig (3.2) IR Spectrum ofAtenolol in KBr disc

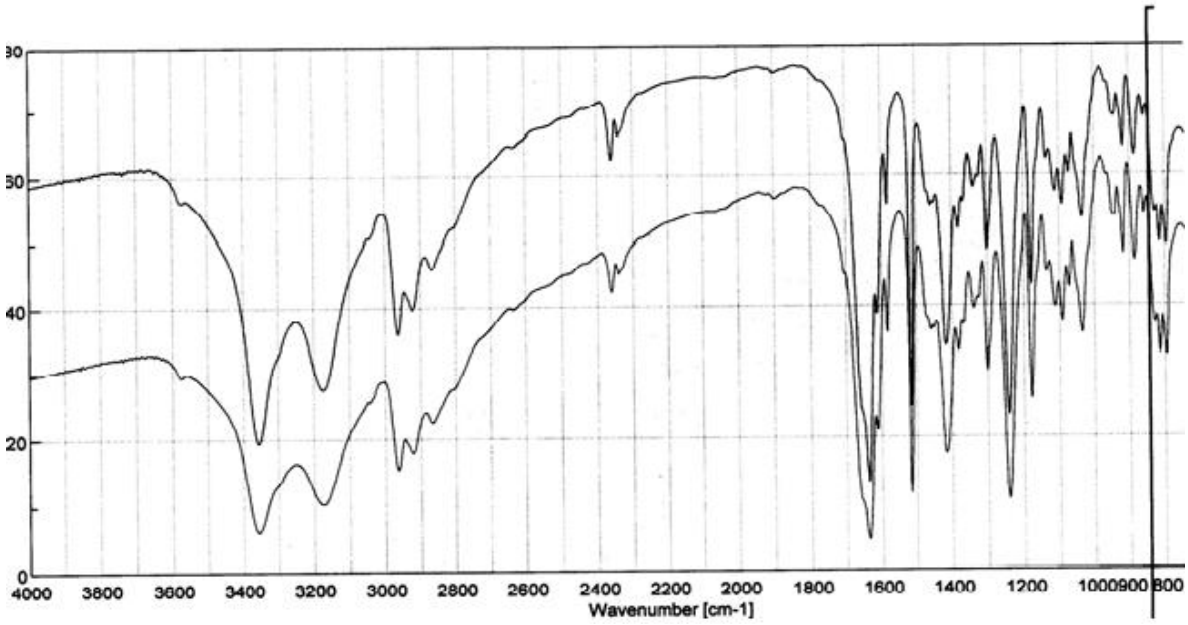
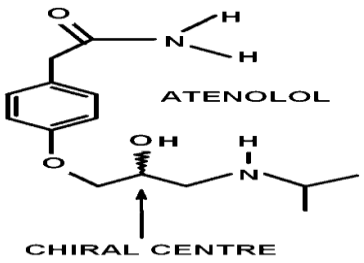


Fig (3.3) comparison of Atenolol standard and sample

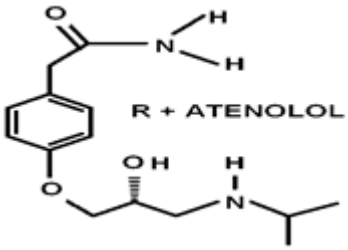
3.1.4 Optical rotation of atenolol

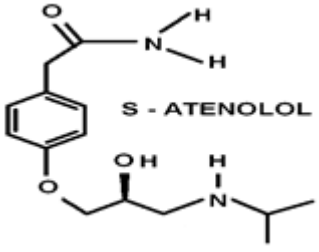
Atenolol has two optical isomers. These mirror images are labeled the R (+) and S (-) enantiomers.

This is due to Atenolol chiral center as showed below:



Chirality of the carbon, the molecule is able to exhibit optical isomerism. These are in the forms of the stereoisomer R+ Atenolol and S- Atenolol





Industrially, Atenolol is produced as racemic mixture of the two enantiomers. So that when measure the optical rotation by automatic polarimeter it was found 0.04 (BP 2010)

3.2 Chemical assay and system suitability

The chemical assay was done by HPLC.0.1 g accurately weight was taken from working standard No (WS/AMI/BP492) and dissolve in 100 ml mobile phase, further dilution 1ml to 100 ml with mobile phase the final concentration was 0.01mg/ml. The sample was prepared by the same manner.

20µl of standard and sample were injected on the same system and the results were shown in the chromatograms. Fig (3.4).

The theoretical plate (column efficiency) is 5624 the required value not less than 5000,the tailing factor (asymmetry at 5%) is 1.65 the required value is not more than 2.0,the relative standard deviation (RSD) is 0.619% the required value is Not More Than 2.0

(Ru / Rs) × (Cs /Cu) ×100 = Chemical assay

Ru = peak response from sample solution.

Rs = peak response from Atenolol working standard.

Cs =concentration of Atenolol in the standard solution (mg / ml)

Cu =concentration of Atenolol in the sample solution.

From the chromatogram the average peak area of standard =1617283. The average peak area of sample are =1611189

$$\frac{1611189 \times\ 0.01 \times 100}{1617282 \times\ 0.01} = 99.62\%$$

The acceptance limit from 98 to 102 %. (United States pharmacopoeia 2010)

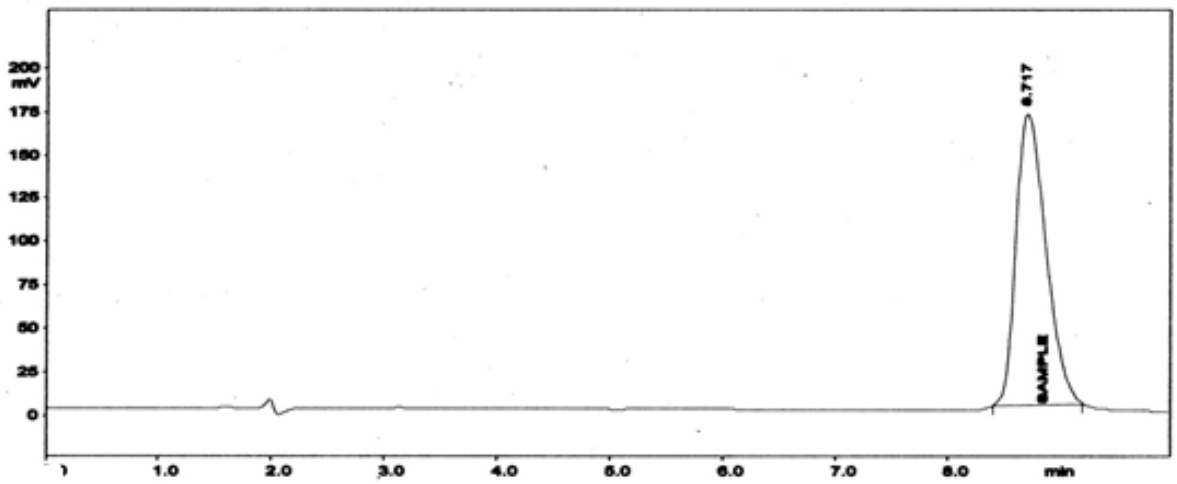


Fig (3.4).Atenolol sample for assay

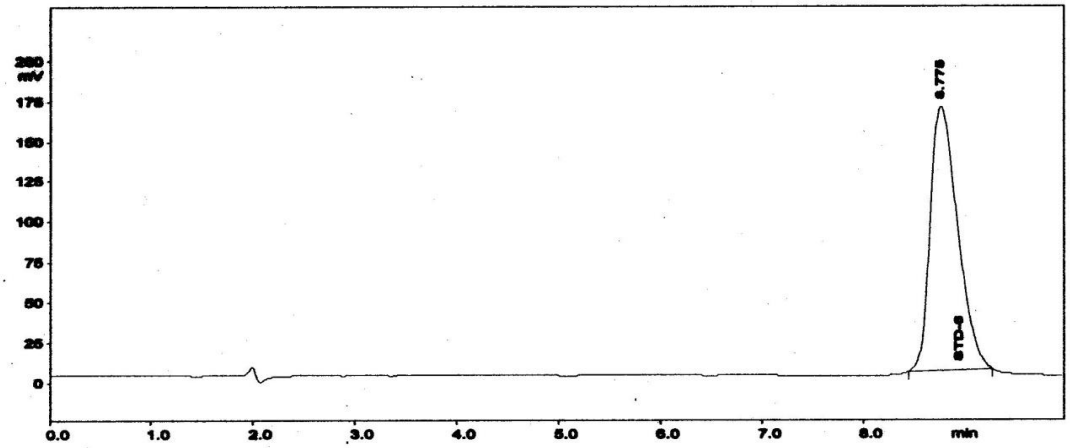


Fig (3.5).Atenolol standard for assay

3.3 Accelerated stability of atenolol

The tests carried out included chemical assay, appearance, hardness and disintegration time. The assay was done by HPLC stability indicating method, the hardness was done by hardness tester and the disintegration time was done by disintegration tester instrument.

The results in (table 3.1) and the chromatogram (Fig 3.6).

Product Name: Amitenol tablets 100 mg which is produced by Amipharma laboratories ltd.

Description and Packaging: an orange, circular coated tablet, blistered in aluminum foil and PVC.

Condition: - Temp: 40±2 °C, Relative Humidity: 75±5 %RH

Table (3.1) accelerated stability study of atenolol

Test	appearance	hardness	disintegration	assay
Specifications	Orange coated tablets	NLT 70 N	NMT 30 min	90-110%
Zero time	Orange coated tablets	104.16	7 min	100.31%
After 1 months	Orange coated tablets	104.05	7 min	100.17%
After 2 months	Orange coated tablets	103.35	8 min	100.04%
After3months	Orange coated tablets	102.65	8 min	99.97%
After 6 months	Orange coated tablets	101.79	9 min	98.0%

- NLT (not less than) * NMT (not more than)

The drop in the active pharmaceutical ingredient (API) is less than 5% and the physical parameters were not changed and no formation of degraded product is detected.

Amitenol tabs 100 mg considered stable product and can safely distributed and marketed in Sudan and all Atenolol products in tablets form of the similar formulation.

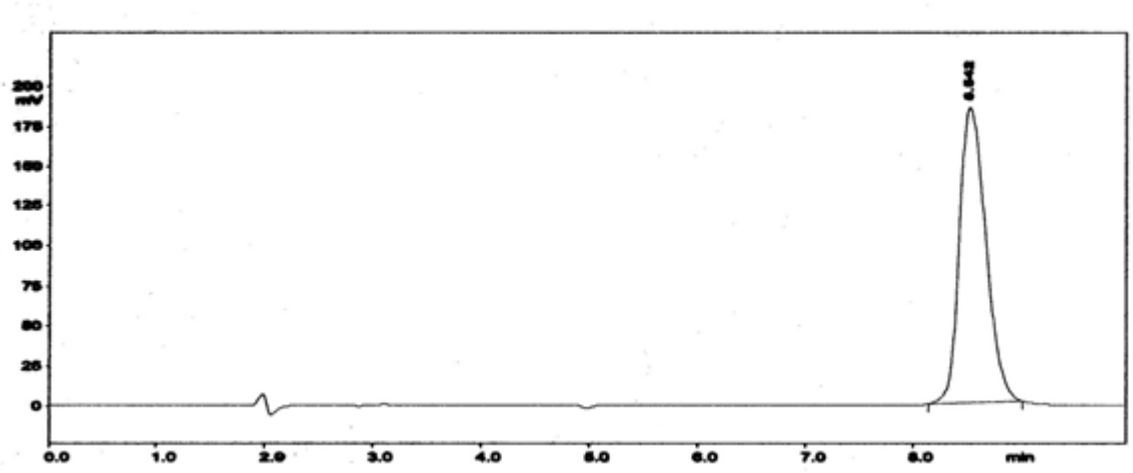


Fig (3.6) Amitenol after 6 months on accelerated stability

3.4 The effect of sunlight on Atenolol in solution form

Atenolol solution of 0.1% concentration in water was placed in direct sunlight and analyzed by HPLC in intervals of one hour up to five hours.

The results in table (3.2) and chromatograms Fig (3.7and 3.8)

Table 3.2 Effect of sunlight on Atenolol solution.

Time intervals	Peak area	Content %	log	1/log
zero time	815.46	100%	2.0000	0.50000
After 1 hour	816.44	100.12%	2.0005	0.49987
After 2 hours	821.82	100.77	2.0033	0.49918
After 3 hours	825.31	101.21%	2.0052	0.49870
After 4 hours	827.49	101.47%	2.0063	0.49843
After5 hours	830.56	101.85%	2.0080	0.49801

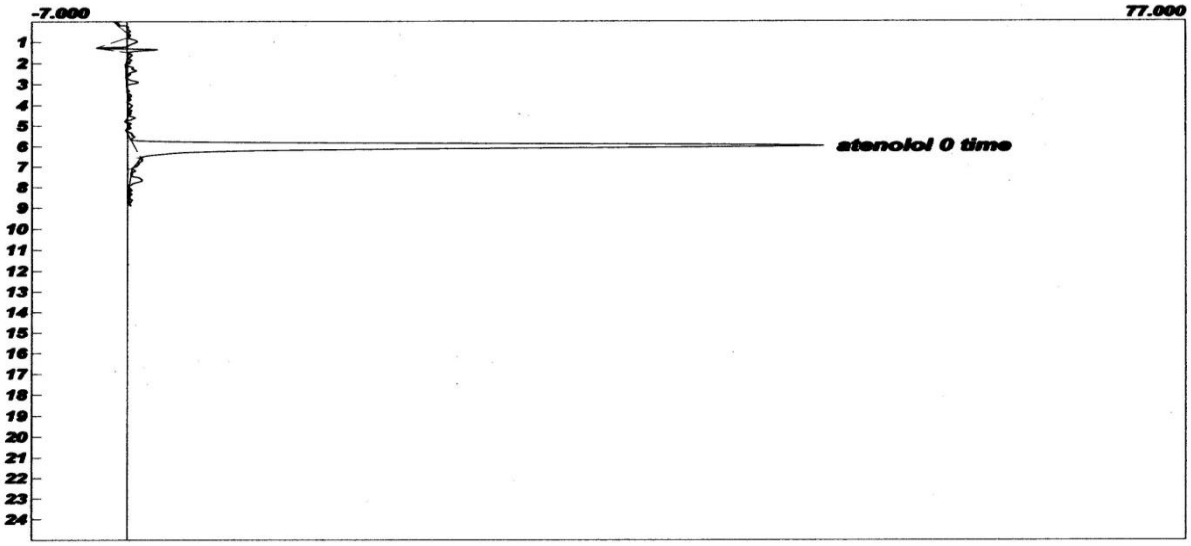


Fig (3.7) Atenolol solution before placing in sunlight

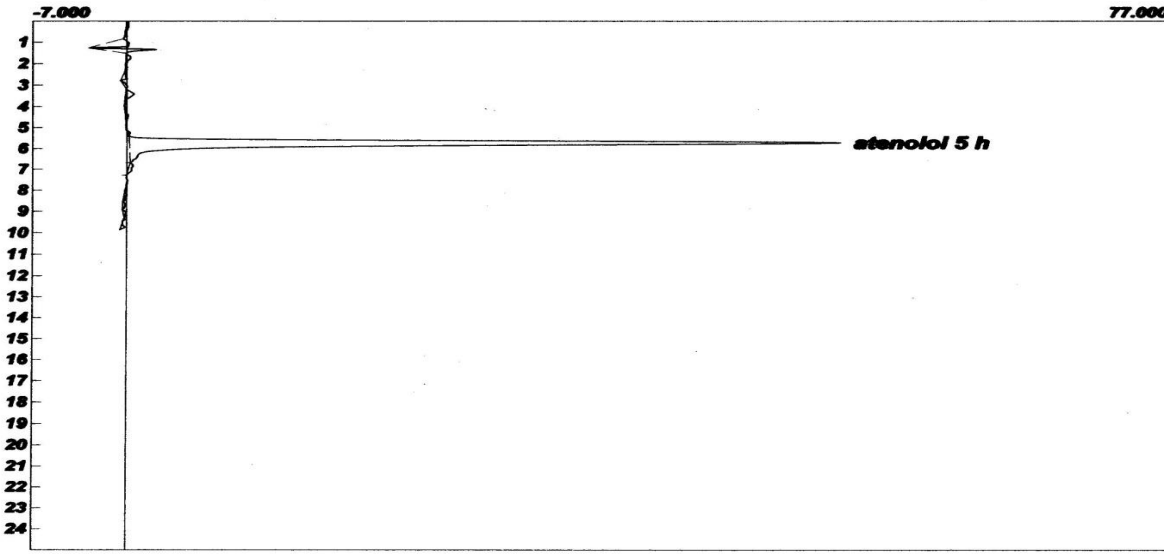


Fig (3.8) Atenolol solution 5 hours in the sunlight

The result showed that there was no significant change in concentration and no any secondary beak appeared on the chromatogram Atenolol was considered stable at the concentration of 1mg/ml in water up to 5 hours in the sunlight.

3.5 The effect of solvents on Atenolol

A solution having concentration of 10 µg / ml of Atenolol was prepared using different types of solvents and examined by scanning with UV

spectrometer in the range of 230 nm to 350 nm. The results shown in table (3.3) and Fig (3.9 and 3.10)

Table (3.3) Solvents effect on Atenolol spectrum

Solvent	Peak1	abs	Peak2	abs	Peak3	abs
Methanol	282.2	0.0444	275.7	0.0519	226.4	0.3606
Butan-2-0l	283.1	0.0486	276.6	0.0563	227.2	0.3662
Propanol	283.3	0.0427	276.5	0.0504	227.5	0.3614
Ethanol	282.3	0.0347	275.8	0.0402	226.3	0.3197
Water	280.2	.0364	273.7	.0445	224.2	0.3362
n-Hexan	-	-	274.5	0.0043	223.5	0.0303
Dichloromethane	283.6	0.0343	276.6	0.0440	-	-
Dimethyl formamide	284.5	0.0648	277.6	0.0770	-	-
Ethyl acetate	284.1	0.0106	277.0	0.0139	-	-
Dioxane	284.1	0.0140	277.4	0.0126	225.7	0.2459
Acetonitrile	283.2	0.0465	276.2	0.0558	228.4	0.3153

It was noticed that there were three peaks one at 282±2. The_second at 275 ± 2 and the third at226 ± 2. Most of the solvents have the three peaks and some have showed only two peaks with low absorbance and that could be due to solute -solvent interaction. The solvents has no significant effect on Atenolol UV spectroscopy and every solvent have absorbance at λ_{max} 275 $\pm$2 because of that it was used for analyzing Atenolol by spectrophotometer.

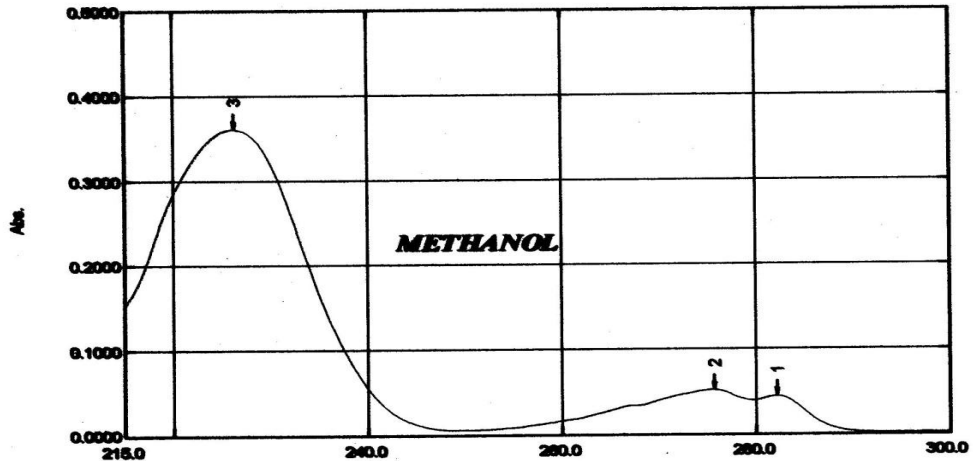


Fig (3.9) Atenolol spectrum 10µg in methanol

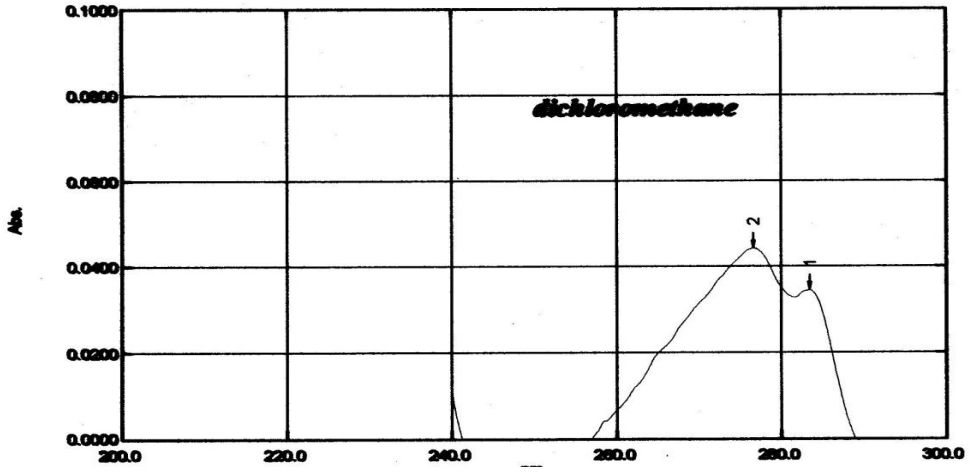


Fig (3.10) Atenolol spectrum 10µg in dichloromethane

3.6 The effect of pH on Atenolol

A solution having concentration of 50 µg / ml of Atenolol was prepared in different ranges of buffers and examined by scanning with UV spectrometer in the range of 230 nm to 350 nm The results were shown in the table (3.4) and the spectrum Fig (3.11and 3.12)

Table (3.4) pH effect on Atenolol spectrum

Buffer PH value	Peak1	abs	Peak2	abs	Peak3	abs
Buffer PH 0.32	277.6	0.1417	271.3	0.1652	222.0	1.2987
Buffer PH 2.0	280.1	0.2341	273.7	0.2777	224.2	1.7717
Buffer PH 4.0	280.2	0.2264	273.7	0.2695	224.7	1.7962
Buffer PH 6.0	280.2	0.2562	273.8	0.2562	224.5	1.7553
Buffer PH 7.0	280.2	0.1940	273.8	0.2341	224.4	1.6997
Buffer PH 8.0	280.2	0.1979	273.7	0.2386	224.4	1.7316
Buffer PH 10	280.3	0.1910	273.9	0.2320	224.9	1.7414
Buffer PH 14	-	-	274.9	0.2422	226.4	2.3703

there were three peaks at 277nm, 271 and 222 nm these peaks were detected on all the range of pH but it was noticed that there was a shift in position and as the pH increased the λ_{max} increased as shown on the table above.

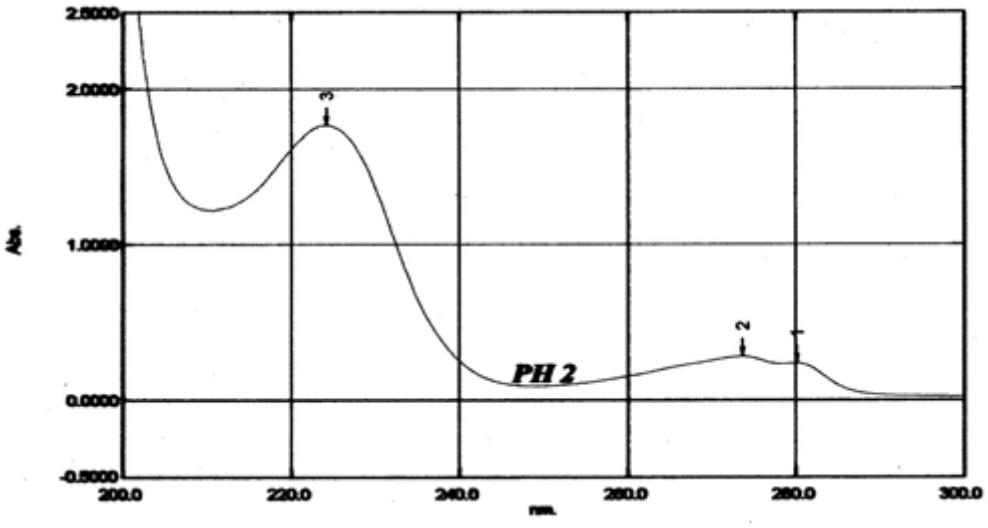


Fig (3.11) Atenolol 50µg / ml in phosphate buffer pH 2

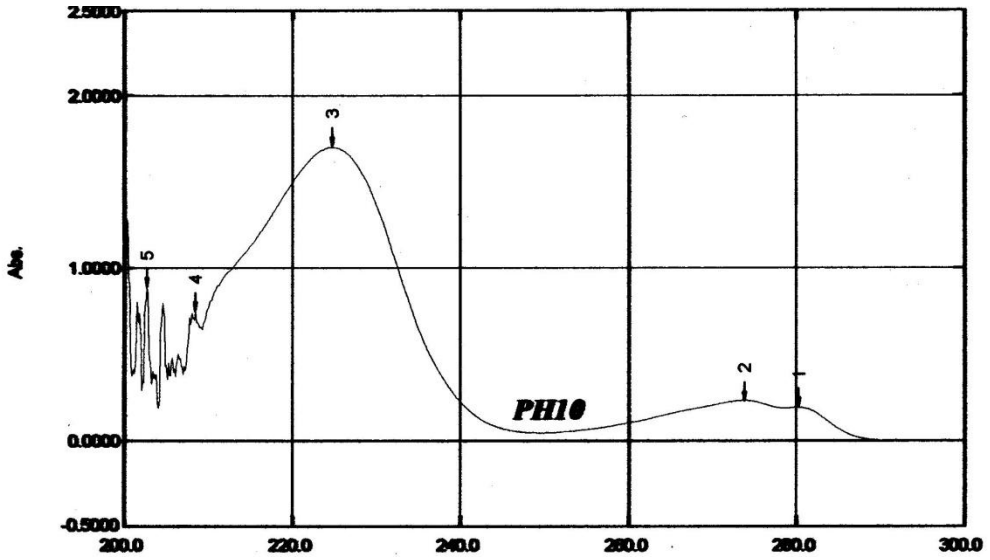


Fig (3.12) Atenolol 50µg / ml in phosphate buffer pH 10

3.7 The effect of UV light on Atenolol in solution form

0.1% solution of Atenolol in water was exposed to UV light for 24 hours and analyzed after 1hour, 2 hours, 10 hours and 24 hours table (3.5) and the chromatograms Fig (3.13 and 3.14)

Table (3.5) Effect of UV light on Atenolol solution

Time intervals	Atenolol content%	Log content
Zero time	100.00%	2.0000
After one hour	99.64	1.9984
After two hours	99.19%	1.9965
After ten hours	97.63%	1.9896
After 24 hours	94.779%	1.9767

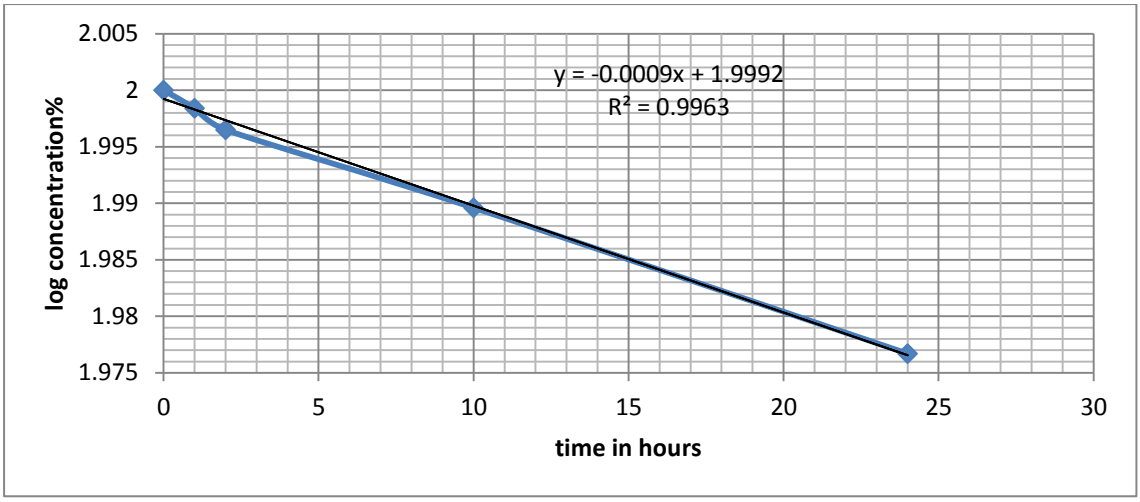


Fig (3.13) log conc. against time for the UV effect on Atenolol

It was found that Atenolol is not stable in UV light and there was significant drop in the content and there was degradation when plotting log concentration against time it gives linear relation (fig 3.13) with R^2 = 0.9963 so the reaction follow first order reaction. Atenolol is not stable towards UV light in solution form. (Andrisano. V. *et. al*, 1999)

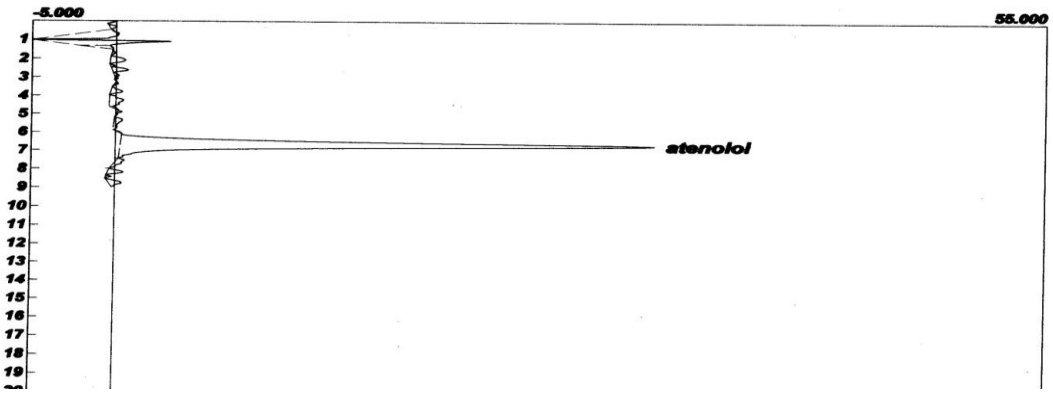


Fig No (3.14) Atenolol before exposing to UV light

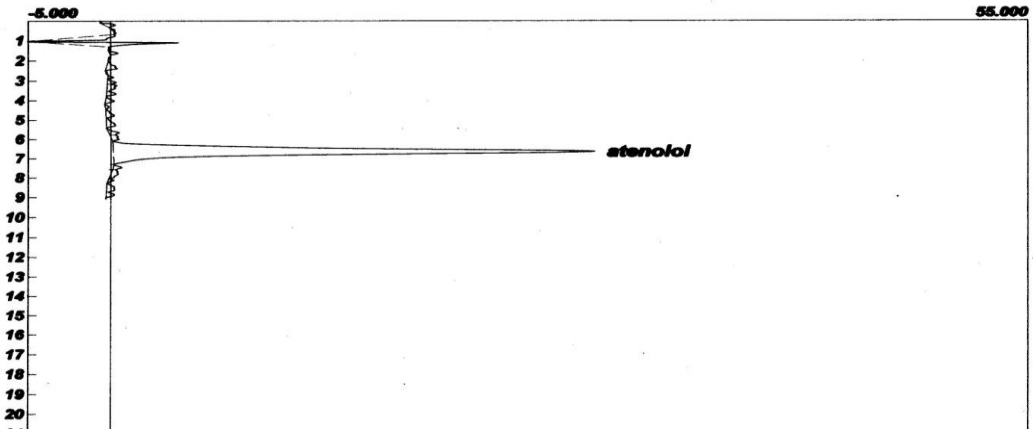


Fig (3.15) Atenolol after exposing to UV light for 24 hours

3.8 The effect of excipients on Atenolol

0.1 g of Atenolol with some excipients were prepared in water kept at room temp, and analyzed after seven hours table (3.6) and the chromatograms Fig (3.15 and 3.16).

Table (3.6) Effect of some excipients on atenolol

Excipients name	Content after 7 hours
Magnesium stearate	100.61%
Maze starch	99.02%
Micro crystalline cellulose	101.9%
Sodium lauryl sulphate	101.55%
Talcum powder	99.74%
lactose	101.42%
Povidone (p.v.p k30)	99.87%

It can be judged that all the above excipients which were used in the formulation of tablets were compatible with Atenolol and nor individual excipients neither in combined have any effect on Atenolol as it appeared

in the accelerated stability study and there is no drop in API exceed 5% and no secondary peak was detected.(Botha and Looter 1990)

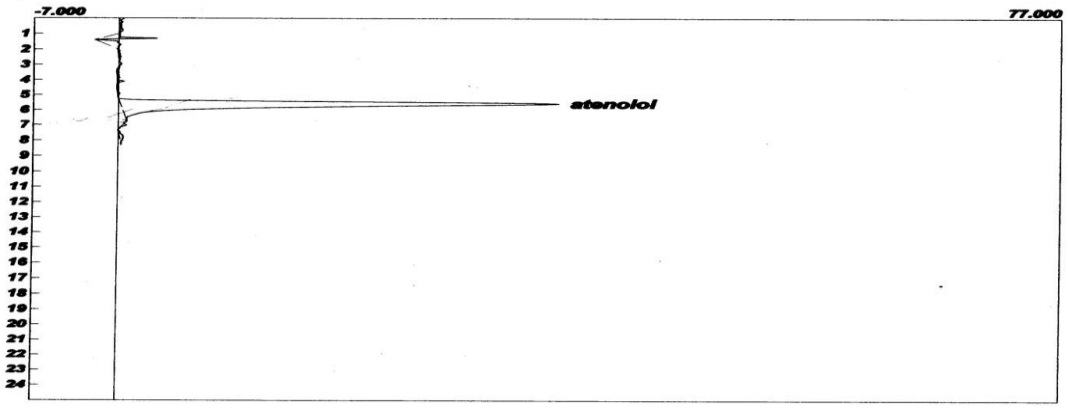


Fig (3.16) Atenolol + starch at zero time

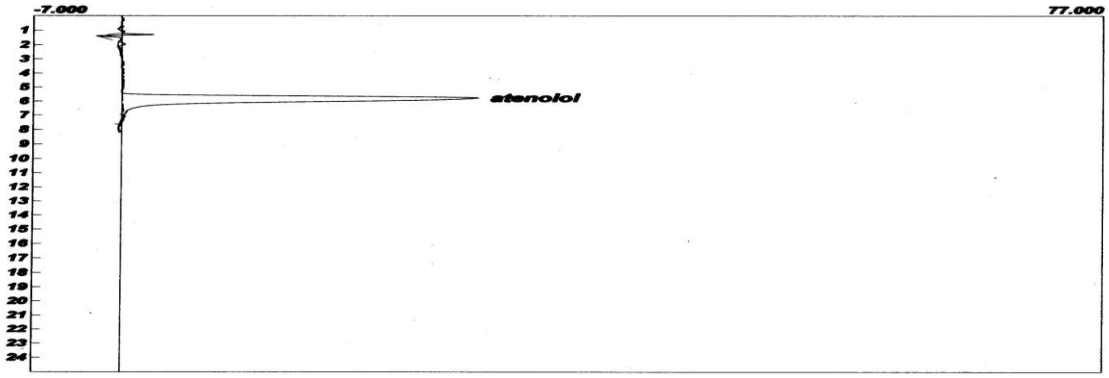


Fig (3.17) Atenolol + starch after 7 hours

3.9 Acidic and Alkaline hydrolysis of Atenolol at 50 °C

3.9.1 Atenolol dissolved in water at 50°C

The Atenolol was found to be stable in water at 50°C up to 3 hours.

Table (3.7) and the chromatograms Fig (3.17). Simultaneously the absorbance of the solution was measured at 276 nm and recorded on the same table.

Table (3.7) Atenolol dissolved in water at 50° C.

Time intervals	Atenolol content%	Log content.	Abs at 276
0 min	100.0%	2.00	0.0925
30min	100.02%	2.0001	
60 min	99.94%	1.9997	0.0920
90min	99.77%	1.9990	
120min	99.69%	1.9987	0.0915
150min	99.68%	1.9986	
180min.	99.66%	1.9985	0.0916

3.9.2 Acidic hydrolysis of atenolol at 50°C

Atenolol hydrolyzed in acid media at 50 ° C and by plotting log concentration against time it gives linear co-relation (R^2 = 0.9955) so Atenolol hydrolysis in acid flows first order reaction table (3.8) and the chromatograms Fig (3.19, 3.20 and 3.21).simultaneously the absorbance of the solution was measured at 276 nm and recorded on the same table. (Hatem *et. al* 1996).

Table (3.8) Atenolol acid hydrolysis at 50 °C

Time intervals	Atenolol content%	Log content.	Abs at 276
0 min	100.0%	2.00	0.0801
30min	90.57%	1.96	
60 min	73.49%	1.87	.0933
90 min	61.09%	1.78	
150min	51.31%	1.61	0.104
210min	27.87%	1.45	0.0912

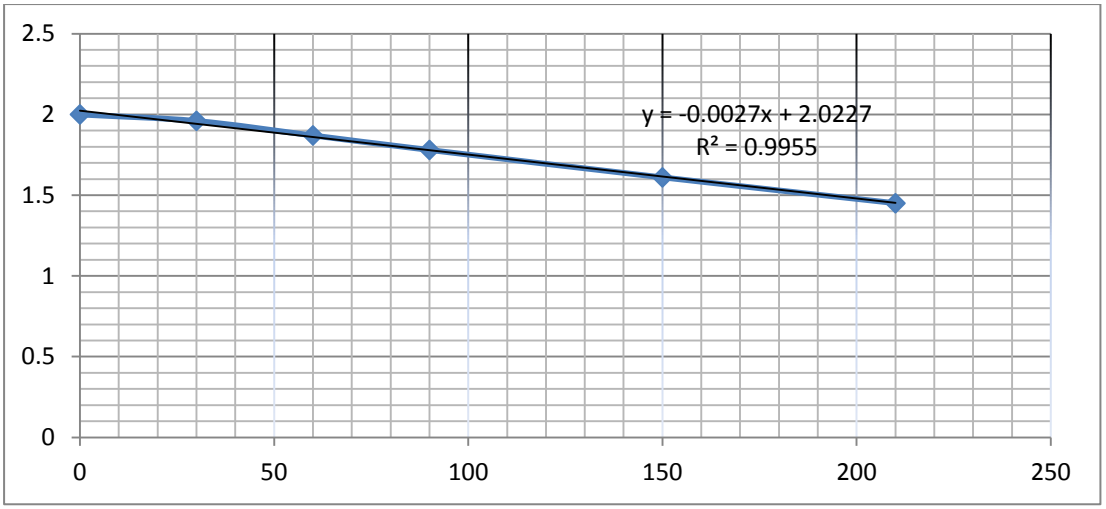


Fig (3.18) log conc. against time of atenolol acid hydrolysis at 50 °C

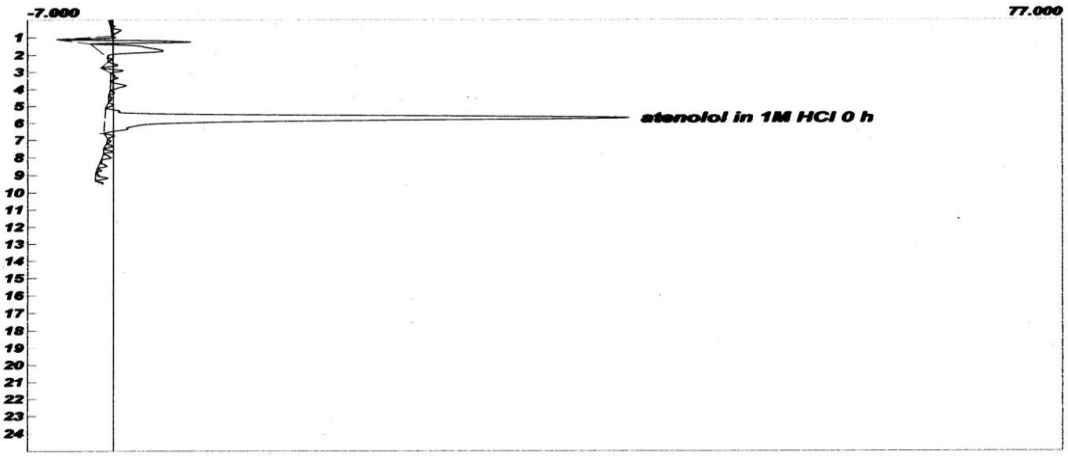


Fig (3.19).Atenolol in IM HCl at 50 °C zero time

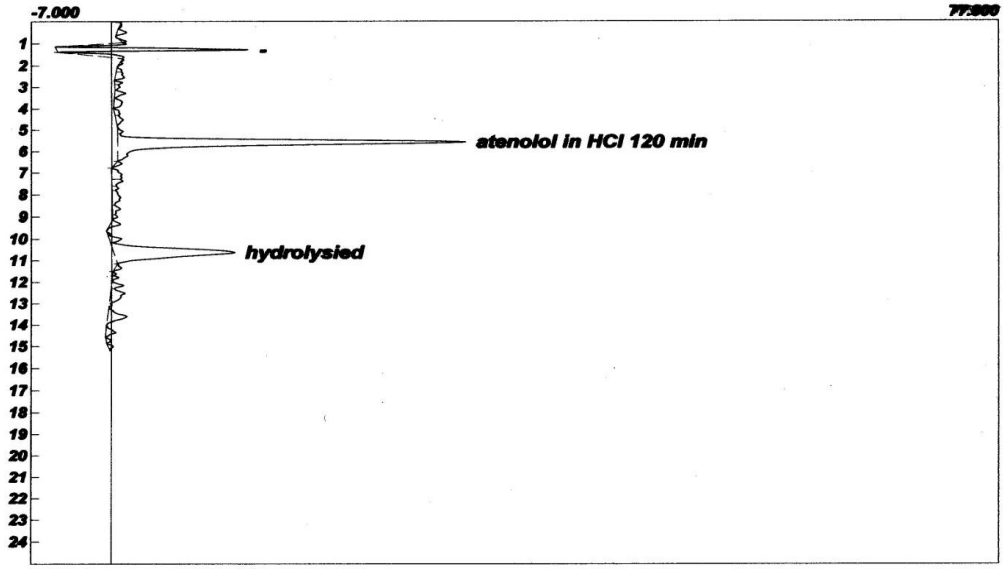


Fig (3.20). Atenolol in IM HCl at 50 °C after 120 min

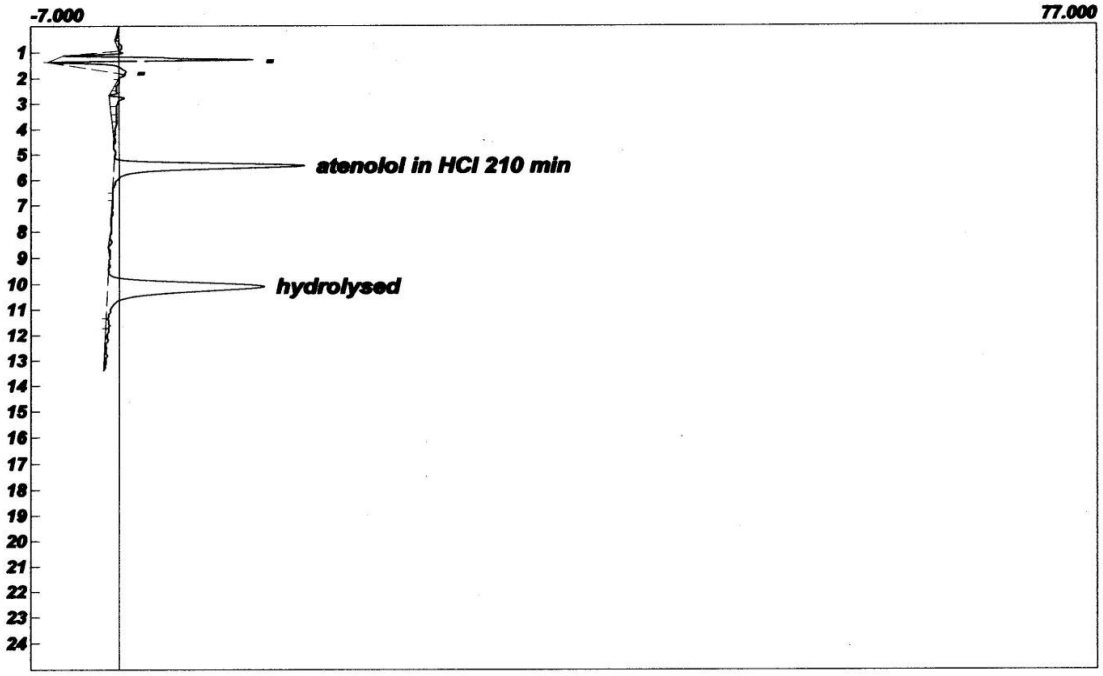


Fig (3.21) Atenolol in IM HCl at 50 °C after 210 min

3.9.3 Atenolol alkaline hydrolysis at 50 °C

Atenolol hydrolyzed in alkaline media and by plotting of log concentration of Atenolol against time gave straight line with R^2 =0.9935 and prove Atenolol hydrolyzed in alkaline media flows first order reaction. Table (3.9) and the chromatograms Fig (3.23, 3.24 and 3.25). Simultaneously the absorbance of the solution was measured at 276 nm and recorded on the same table. (Hatem A *et. al*, 1996)

Table (3.9) Atenolol alkaline hydrolysis at 50 °C

Time intervals	Atenolol content%	Log content.	Abs at 276
0 min	100.0%	2.000	0.0842
30min	69.82%	1.844	
60 min	49.78%	1.697	0.0883
90min	27.0%	1.431	
120min	17.85%	1.251	0.0947
150min	10.4%	1.056	0.0841

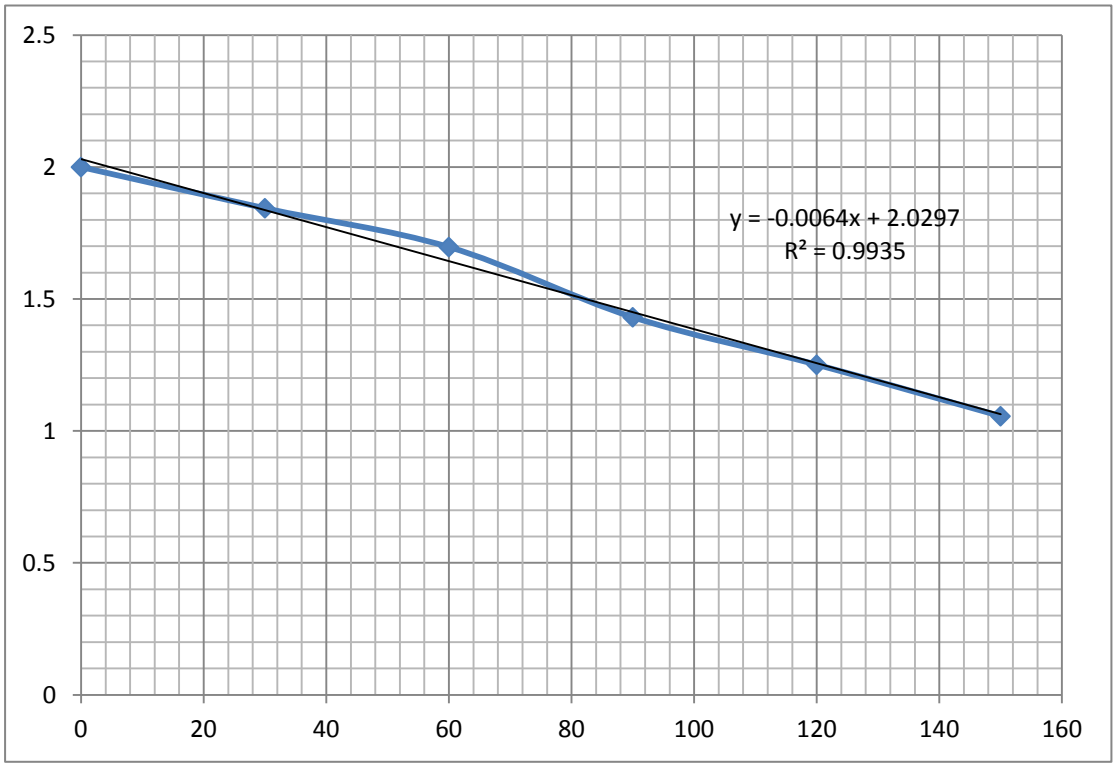


Fig (3.22) log conc. against time of atenolol alkaline hydrolysis at 50 °C

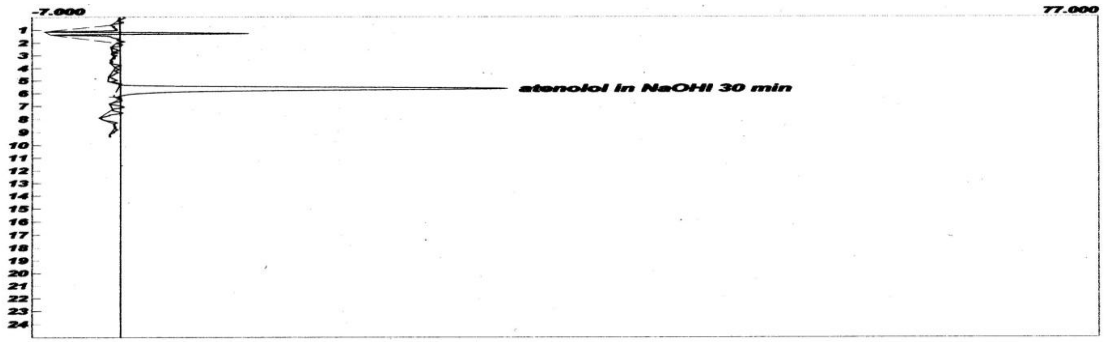


Fig (3.23) Atenolol in IM NaOH at 50 °C after 30 min

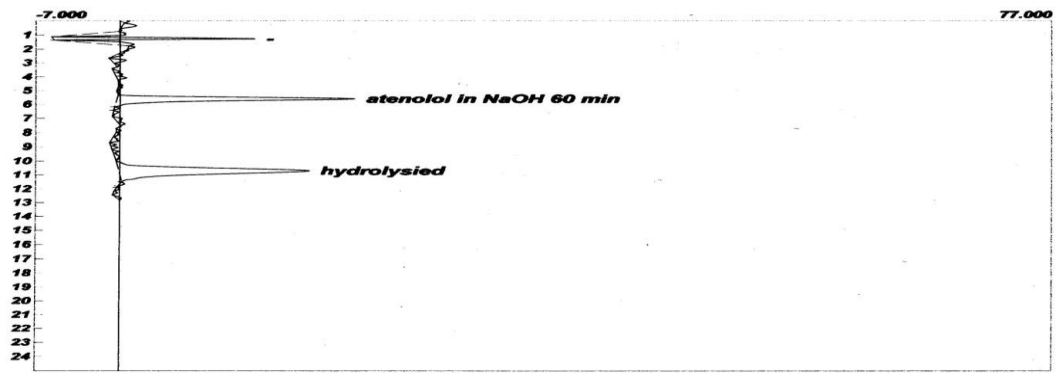


Fig (3.24). Atenolol in IM NaOH at 50 °C after 60 min

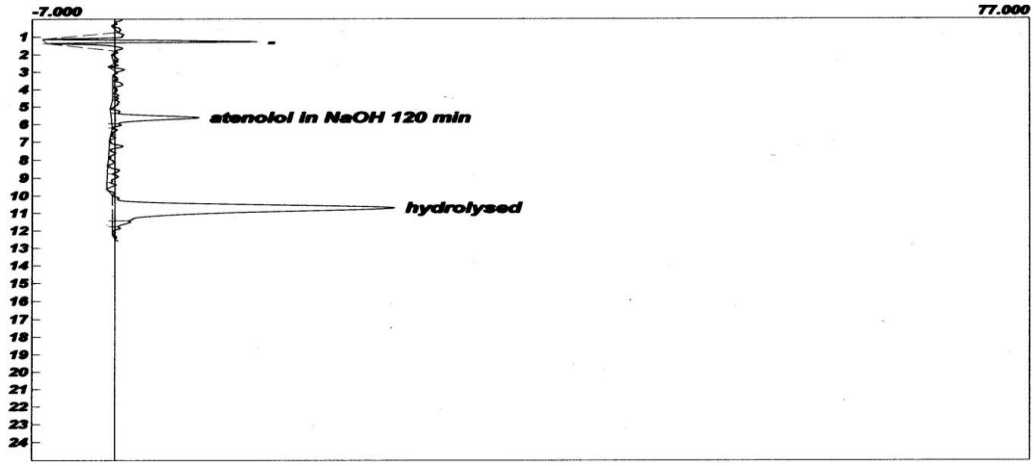


Fig (3.25) Atenolol in IM NaOH at 50 °C after 120 min

3.10. Acidic and Alkaline hydrolysis of Atenolol at 70 °C

3.10.1 Atenolol dissolved in water at 70 °C

There was no significant drop in the content exceeded 5% and no formation of secondary beaks in the chromatograms .Atenolol was found to be stable at 70 °C up to 150 min. simultaneously the absorbance of the solution was measured at 276 nm and recorded on the same table. (3.10) and the chromatograms Fig (3.26).

Table (3.10) Atenolol dissolved in water at 70 °C.

time intervals	Atenolol content%	Log Atenolol content	ABS at 275 nm
0 min	100%	2.00	0.468
30 min	100.5%	2.005	0.488
60 min	100.5%	2.005	0.477
90 min	101.3%	2.006	0.490
120 min	99.0%	1.996	0.485
150 min	98.32%	1.988	0.477

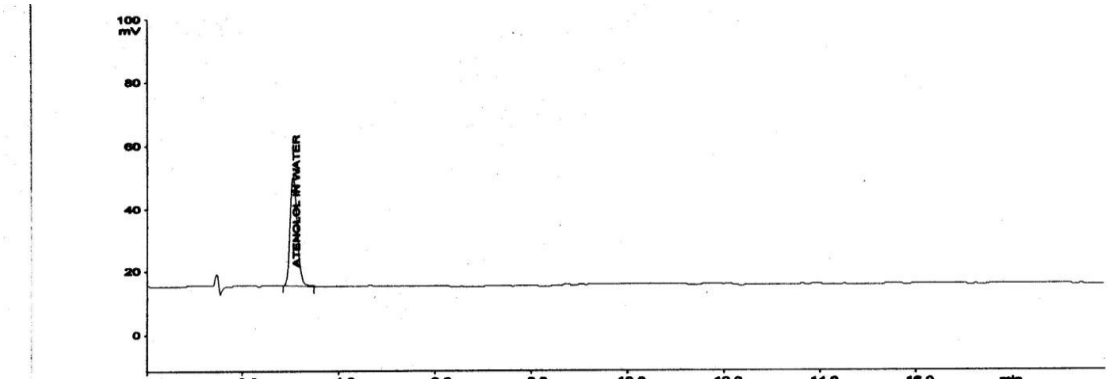


Fig (3.26) Atenolol in water at 70 °C after 150 min

3.10.2 Acidic hydrolysis of atenolol at 70°C

Atenolol hydrolyzed in acidic media at 70 °C and by plotting log concentration against time it gives linear co-relation (R^2=0.994) so Atenolol hydrolysis in acid flows first order reaction table (3.11) and the chromatograms Fig (3.28, 3.29 and 3.30). Simultaneously the absorbance of the solution was measured at 276 nm and recorded on the same table. (Hatem *et. al*, 1996).

Table (3.11) Atenolol acid hydrolysis at 70 °C

time intervals	Atenolol content	Log Atenolol content	ABS at 275 n
0 min	100%	2.00	0.467
30 min	56.96	1.76	0.460
60 min	27.90%	1.45	0.458
90 min	13.32%	1.12	0.456
120 min	6.42	0.81	0.462
150 min	2.46%	0.39	0.445

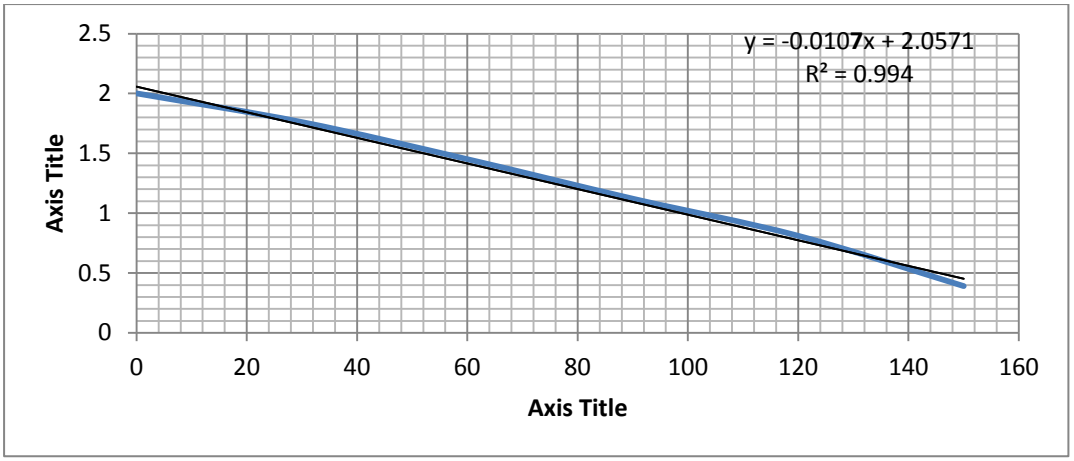


Fig (3.27) log conc against time of atenolol acid hydrolysis at 70 °C

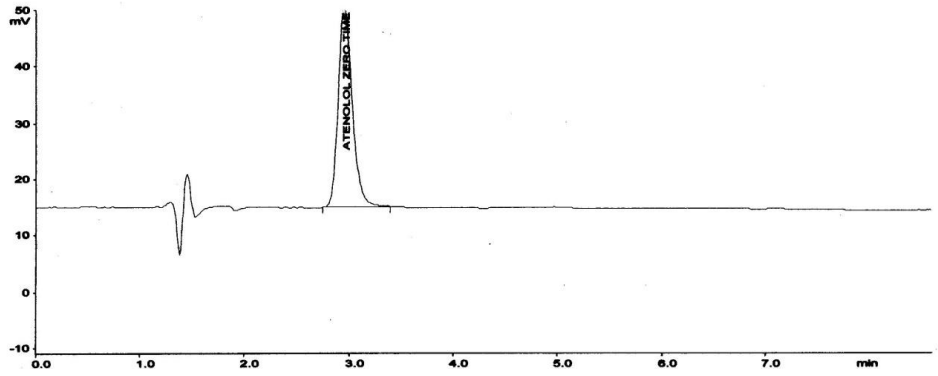


Fig (3.28) Atenolol in 1 M HCl at zero time

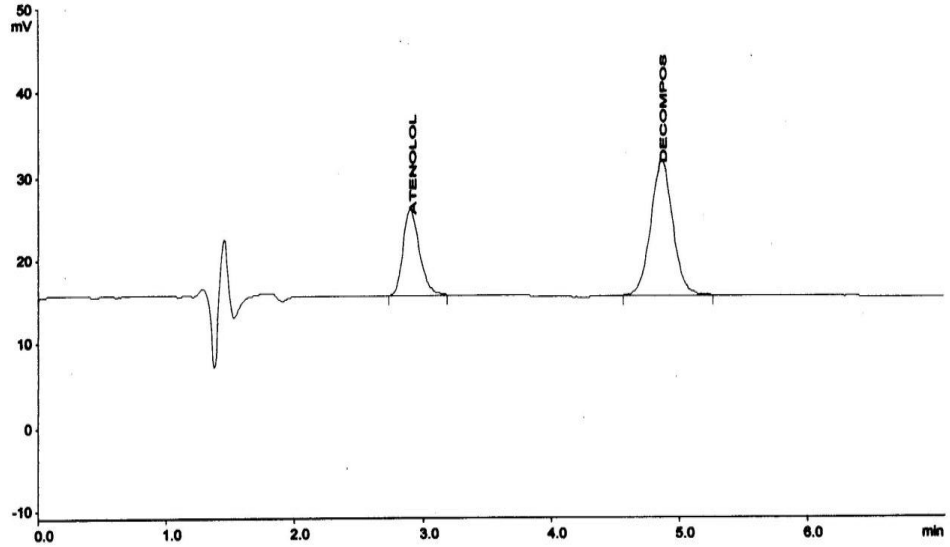


Fig (3.29) Atenolol in 1 M HCl at 70 °C after 60 min

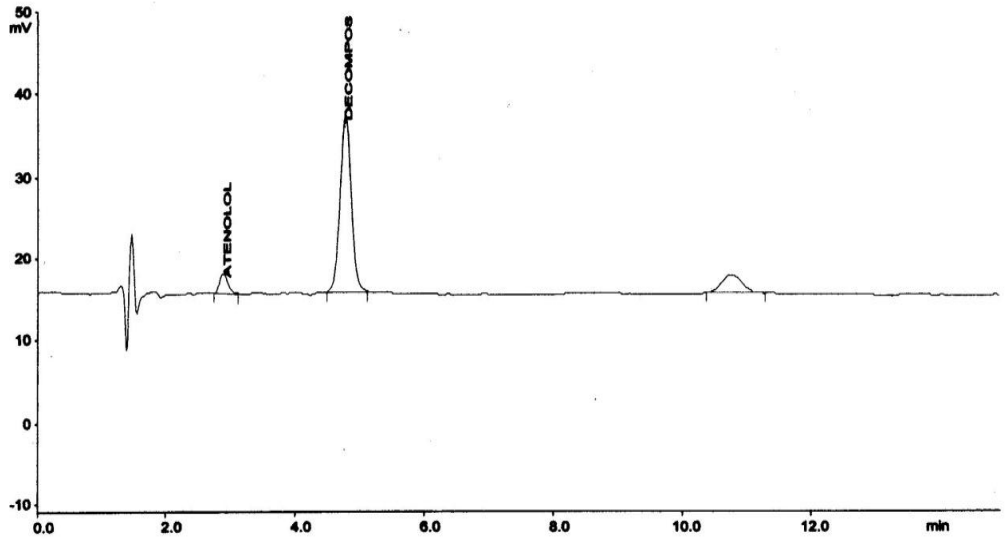


Fig (3.30) Atenolol in 1 M HCl at 70 °C after 120 min

3.10.3 Atenolol alkaline hydrolysis at 70 °C

Atenolol hydrolyzed in alkaline media at 70 °C and by plotting log concentration against time it gives linear co-relation (R^2=0.9852) so Atenolol hydrolyzed flowing First order reaction. Table (3.12) and the chromatograms fig (3.32, 3.33 and 3.34). Simultaneously the absorbance of the solution was measured at 276 nm and recorded on the same table (Hatem *et. al* 1996).

Table (3.12) Atenolol alkaline hydrolysis at 70 °C

time intervals	Atenolol content%	Log Atenolol content	abs at 275 nm
0 min	100%	2.00	0.473
30 min	43.02%	1.63	0.473
60 min	12.18%	1.08	0.474
90 min	*4.06%*	*0.61*	*0.474*
120 min	0.73%	-0.13	0.475

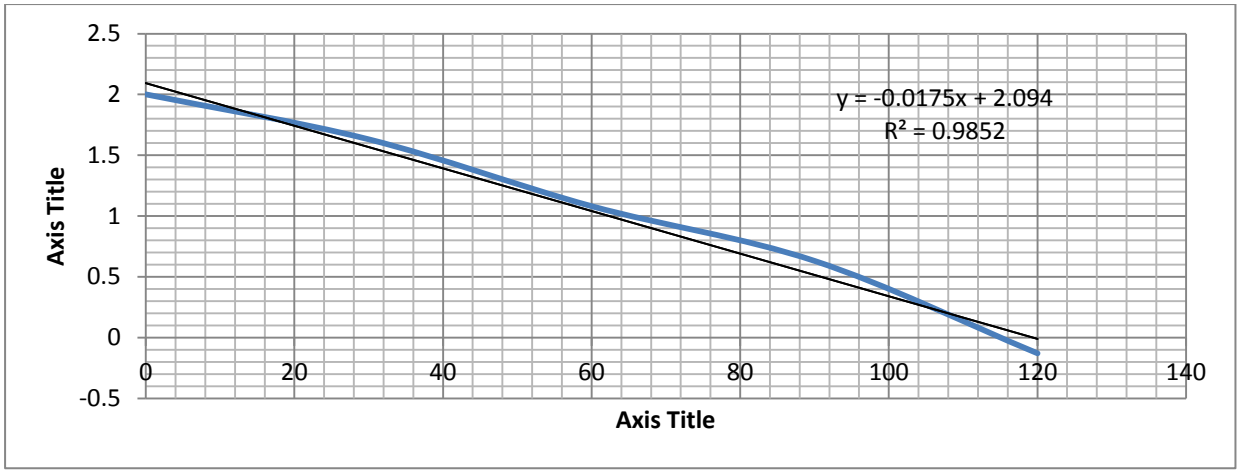


Fig (3.31) log conc. against time of atenolol alkaline hydrolysis at 70 °C

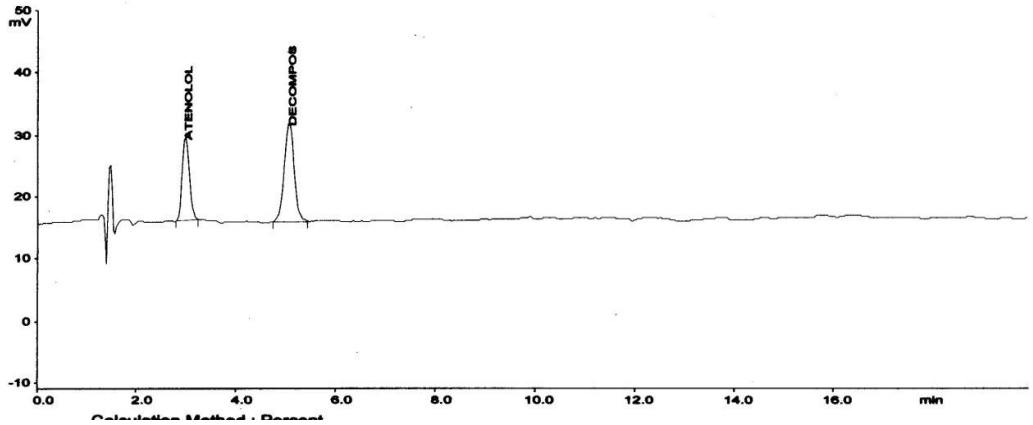


Fig (3.32) Atenolol in 1M NaOH at 70 °C after 30 min

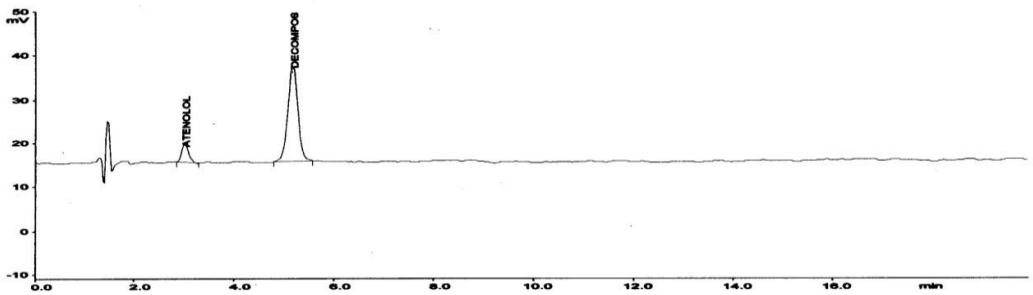


Fig (3.33) Atenolol in 1M NaOH at 70 °C after 60 min

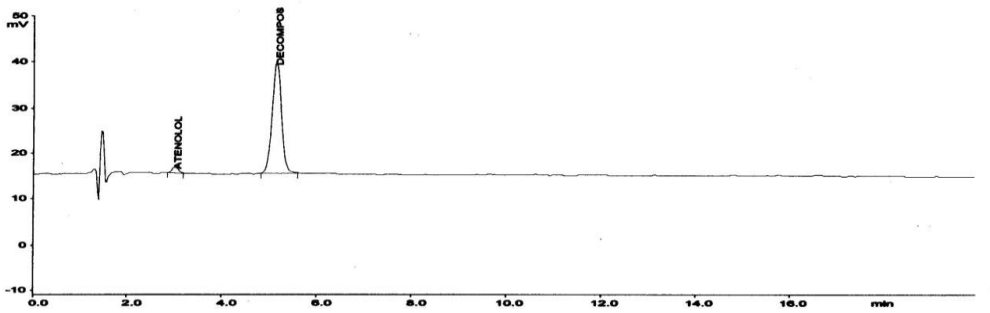


Fig (3.34) Atenolol in 1M NaOH at 70 ^{o}C after 90 min

3.11-Analytical method validation

There are three methods for assaying Atenolol .The first one is the non-aqueous titration which is used for assaying the raw material. The second one is UV spectrophotometer which is used by BP (British pharmacopoeia) and IP (Indian Pharmacopoeia) for assaying the formulated Atenolol tablets. The third one is HPLC which is used by USP for assaying raw material and formulated Atenolol. The justification for validation of HPLC method is to be use as alternative method for assaying formulated Atenolol in (BP and IP) they are using spectrometric method which is not stability indicating method.

3.11.1 Parameter required for analytical method validation.

3.11.2 Accuracy.

Expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

$$content = \frac{Peak\ area\ of\ assay\ preparation \times Purity\ of\ STD}{Peak\ area\ of\ standard\ preparation}$$

Determination of actual assay for accuracy in a concentration of 80%, 90%, 100%, 110% and 120%. According to the procedure table (3.13)

Table (3.13) actual assay

Theoretical Assay%	Actual assay% of Atenolol
80	80.03
80	80.42
80	80.57
90	90.57
90	90.69
90	90.65
100	100.43
100	100.36
100	100.59
110	110.01
110	110.22
110	110.49
120	120.48
120	120.71
120	120.46

$$Recovery\ \% = \frac{Theoretical\ assay}{Actual\ assay} \times 100$$

Relative standard deviation (R.S.D)

$$\frac{S.D \times 100}{\overline{X}} = C.V\%$$

Determination of recovery in concentration 80%, 90%, 100%, 110% and 120% table (3.14)

Table (3.14) recovery %

No.	Theoretical Assay%	Actual assay%	% Recovery	R.S. (C.V %)
1	80	80.03	99.96	0.348
2	80	80.42	99.48	
3	80	80.57	99.29	
4	90	90.57	99.37	0.067
5	90	90.69	99.24	
6	90	90.65	99.28	
7	100	100.43	99.57	0.118
8	100	100.36	99.64	
9	100	100.59	99.41	
10	110	110.01	99.99	0.216
11	110	110.22	99.80	
12	110	110.49	99.56	
13	120	120.48	99.60	0.116
14	120	120.71	99.41	
15	120	120.62	99.62	

Mean Over All Percent Recovery = Accuracy

$$\frac{(99.96+99.48+99.29+99.37+99.24+99.28+99.57+99.64+99.41+99.99+99.8+99.56+99.6+99.41+99.62)}{15}$$

Accuracy =99.55

3.11.3 Precision: (Repeatability).

Expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Expresses the precision under the same operating conditions over a short interval of time. Using a concentration 100% with six replicates. Table (3.15)

Table (3.15) Precision for six replicates

No.	Actual assay % of Atenolol
1	100.38
2	100.64
3	100.96
4	100.66
5	100.16
6	100.30

Table (3.16) relative standard deviation (R.S.D)

No.	X	$(X-\overline{X})$	$(X-\overline{X})^2$
1	100.38	.0.14	0.0196
2	100.64	0.12	0.0144
3	100.96	0.44	0.1936
4	100.66	0.14	0.0196
5	100.16	-0.36	0.1296
6	100.30	-0.22	0.0484
$\overline{X}$=100.52%	Sum. $(X-\overline{X})^2$=0.4252		

S.D=$\sqrt{\Sigma(X-\overline{X})^2/n-1}$. $=\sqrt{(0.4252 / 5)} = 0.2915$

R.S.D=C.V%=S.D×100/$\overline{X}$ $= \frac{0.1503 \text{ X } 100}{100.52} = 0.29\%$

R.S.D = C.V% = 0.29%

Repeatability: C.V%=0.29%

3.11.4 Intermediate Precision:

Expresses within – laboratory variations: different days, different analysts or equipment, etc.

One concentration (100%) with three different analysts.X, Yand Z

X-Analyst

Table (3.17) X-analyst C.V%

No.	X	$(X-\overline{X})$	$(X-\overline{X})$ 2
1	100.91	0.47	0.2209
2	100.83	0.39	0.1521
3	99.58	-0.86	0.7396
$\overline{X}$= 100.44		Sum$(X-\overline{X})^2$=1.1126	

S.D=$\sqrt{\Sigma(X-\overline{X})^2/n-1)}$ =0.7458

R.S.D=C.V%=S.D×100/$\overline{X}$

$$= \frac{0.0.7458}{100.44} X100$$

R.S.D = C.V% = 0.742%

Y-Analyst

Table (3.18) Y-analyst C.V%

No.	X	$(X-\overline{X})$	$(X-\overline{X})^2$
1	100.21	-0.19	0.0361
2	100.72	0.32	0.1024
3	100.26	-014	0.0196
$\overline{X}$=100.465		Sum$(X-\overline{X})^2$=0.01481	

S.D=$\sqrt{\Sigma. (X-\overline{X})^2/n-1)}$ =0.3606

R.S.D=C.V%=S.D×100/$\overline{X}$

$$= \frac{0.3606 \times 100}{100.465}$$

R.S.D=C.V%=0.359%

Z-Analyst

Table(3.19) Z-analyst C.V%

No.	X	$(X-\overline{X})$	$(X-\overline{X})^2$
1	99.99	-0.04	0.0016
2	99.82	-0.21	0.0441
3	100.27	0.24	0.0576
$\overline{X}$=100.026		Sum$(X-\overline{X})^2$=0.1033	

S.D=$\sqrt{\Sigma. (X-\overline{X})^2/n-1)}$ = 0.2272

R.S.D=C.V%=S.D×100/$\overline{X}$

$$= \frac{0.2272 \times 100}{100.026}$$

RSD=CV% =0.2272%

*Intermediate Precision: - X Analyst = 0.742%

Y Analyst = 0.359%

Z Analyst = 0.227 %

3.11.5 Linearity

The ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Table (3.20) Fig (3.35)

Correlation coefficient (r) from the plot of theoretical Assay% against actual Assay%

The theoretical assay plotted against actual assay Fig (3.35) Chromatogram Fig (3.36, 3.37 and 3.38)

Table (3.20) theoretical and actual assay

Theoretical Assay %(X-axis)	80	90	100	110	120
Actual Assay%(y-axis)	80.34	90.64	100.46	110.33	120.55

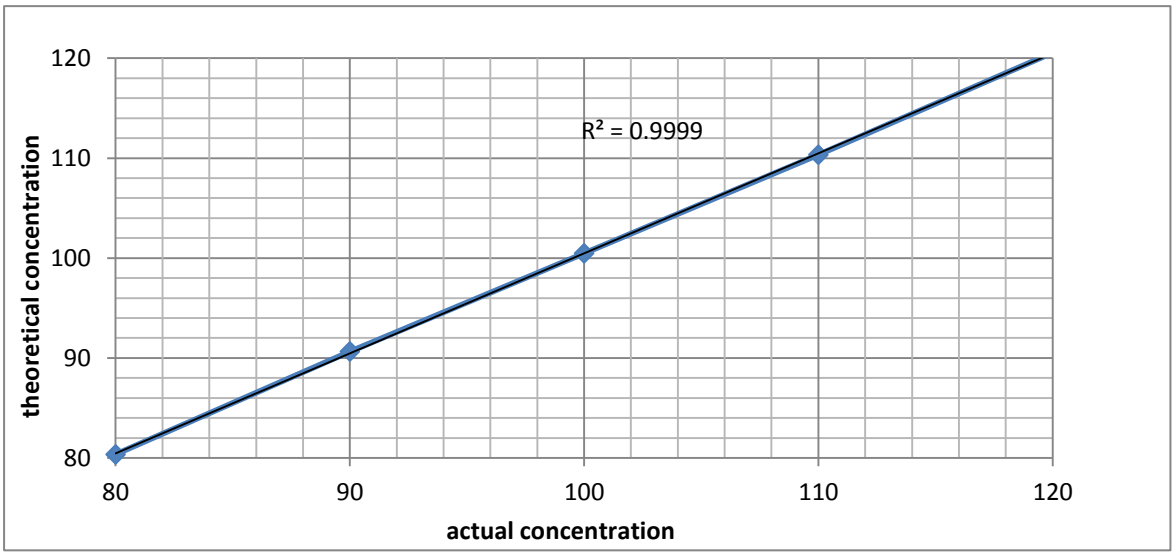


Fig (3.35) the theoretical assay against actual assay

Correlation coefficient (r^2) from the plot of theoretical Assay% against actual Assay%=0.9999

Linearity = $(0.999)^2$x100

Linearity=Fit%=99.99%

3.11.6 Range:

The interval between the upper and lower concentration (amounts) of analyte (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. 80%_____120 %

3.11.7 Specificity:

The ability to assess unequivocally the analyte in the presence of components that may be expected to be present. It`s specific method because there is no interference from the excipients.

3.11.8 Robustness:

The measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The difference of mean assay value between three analyte. = 0.515%

3.11.9 The Data elements required for the assay validation of Atenolol

1. Accuracy = %Recovery = 99.55%
2. Precision (Repeatability): C.V% = 0.29 %

(Intermediate Precision: C.V).

X analyst = 0.742%

Y analyst = 0.359%

Z analyst = 0.227%

3. Robustness: The difference between analysts =0.515%

4. Linearity=Fit%=99.99%

5. Range: 80%_____120%.

6. Specificity: Conforms.

The method was validated and conforms to all parameter required and can be used as alternative method for assay analysis of Atenolol raw material and finished products.

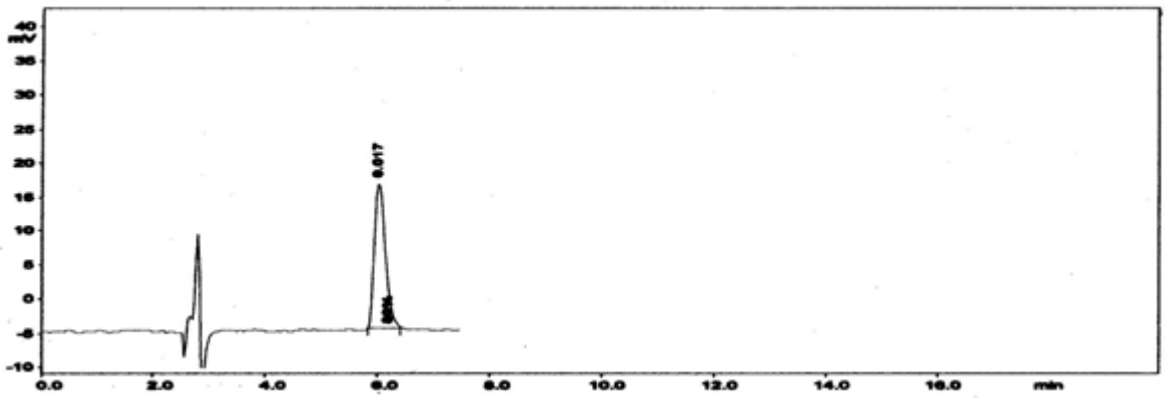


Fig (3.36) chromatogram of Atenolol 80%.

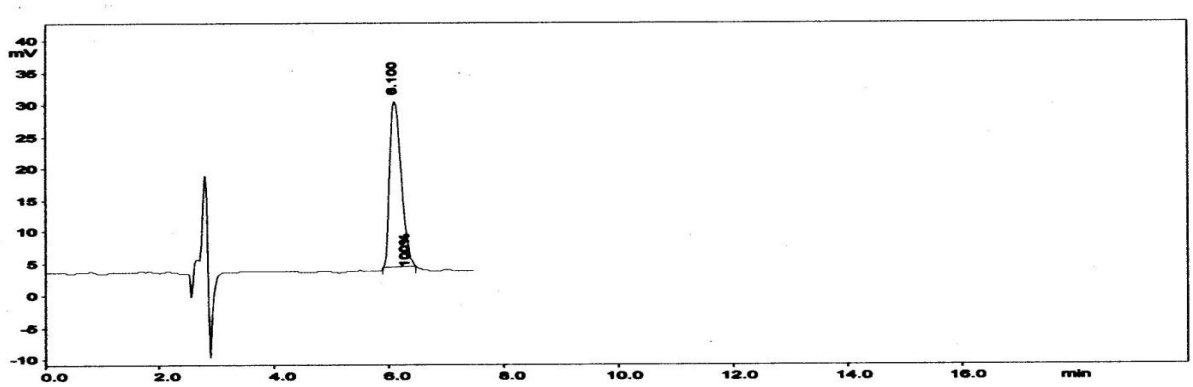


Fig (3.37) chromatogram of Atenolol 100%.

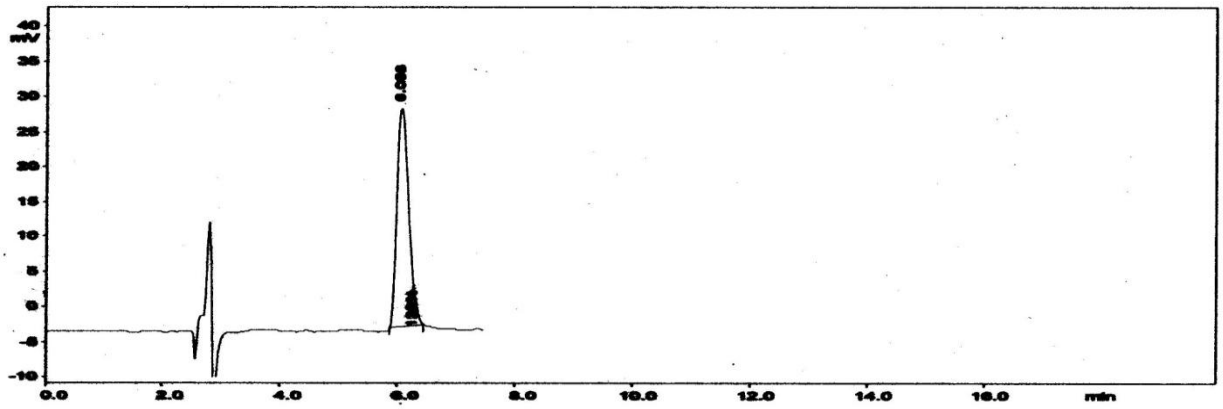


Fig (3.38) chromatogram of Atenolol 120%.

3.12 Comparison of the validated method and the USP method.

The comparison was done between the two methods by analyzing one sample using the two different methods.

A 0.1 gm of Atenolol sample and 0.1 gm of Atenolol working standard were dissolved in separate 100 ml volumetric flask using distilled water. 1 ml was pipetted twice from each flask into separate four 100 ml volumetric flasks. Each two flask sample and standard completed to the mark with the mobile phase related to each method ,20µl was injected on HPLC ,first with USP method and then with in-house validated method. The results of comparison were shown on table (3.13).chromatograms Fig (3.39, 3.40) (3.41,3.42) the method was checked for capability in monitoring and detecting the degradation and/or related substances by heating Atenolol solution to 50 ^{o}C in sodium hydroxide for 30 min and 1ml was taken and neutralized with hydrochloric acid and 20 µl was injected Fig(3.43).

The resolution between Atenolol and the hydrolyzed compound was 10.38 this indicate this method was capable to detect and monitor the related substances of Atenolol.

Table (3.21) Comparison between USP method and validated method

Parameter	USP method	validated method
Chemical assay	100.37%	100.10%
Coefficient of variation	0.51%	0.53%
Total plates	3125	3325
Asymmetry at 5%	1.11	1.20
Retention time	3.17 min	3.27 min

From the table of comparison especially on the parameter of system suitability the two methods were almost the same which indicates the validated method as efficient as USP method.

Fig (3.39) atenolol sample analyzed by USP method.

Fig (3.40) atenolol stanard analyzed by USP method.

Fig (3.41) atenolol stanard analyzed by validated method.

Fig (3.42) atenolol sample analyzed by validated method

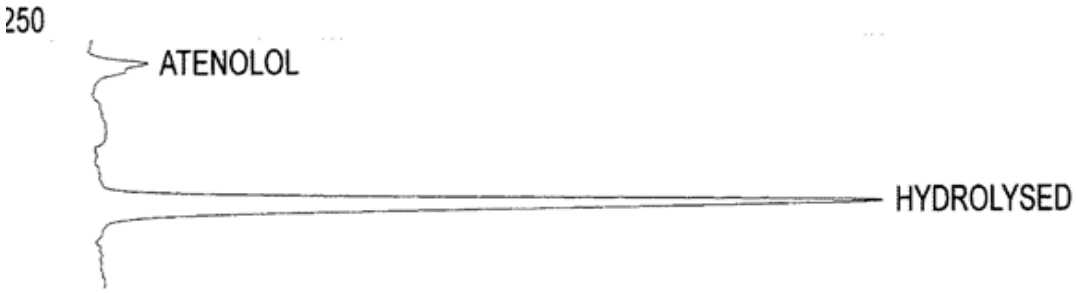


Fig (3.43) atenolol hydrolyzed analyzed by validated method

Conclusion and Recommendations

Atenolol was stable towards heat up to 40 ^{o}C and relative humidity up to (RH) 75% in tablets form.

0.1% solution of Atenolol was stable up to five hours in sun light. The solvents have no significant effect on Atenolol UV Spectroscopy. The pH has an effect on Atenolol spectroscopy and the λ_{max} increase as the pH value increased.

Atenolol was not stable towards UV light and it degradated flowed first order reaction with linear relation of R^2= 0.9963.

The common excipients used in tablets formulation have no effect on Atenolol stability.

Atenolol solution in water was stable towards heat up to70^{o}C.

Atenolol hydrolysis in acidic and alkaline media. The hydrolysis follows first order reaction. From the result the formed compound from both mediums have the same retention time on the chromatograms. The constant rate of the reaction depends on the medium and temperature.

The analysis was done simultaneously by HPLC and spectrometric and found that the result of the spectrometric remained unchanged. The spectrometric method was not capable to monitor any degradation or hydrolysis of Atenolol molecule which means that it is not stability indicating method.

Comparison of validated method and USP method indicates that the validated method was capable to monitor and detect the degraded or hydrolyzed compounds and accurately quantifies Atenolol, the method can be used as alternative method for analysis of Atenolol as raw material or formulated product.

The recommendations are that: Atenolol should be marketed and distributed in coated tablets form to be protected from UV light. When formulated atenolol as syrup, suspension and injection should be in reconstitution powder forms because Atenolol was not stable in solution form and the pH was critical parameter to be monitored.

HPLC method should be used for stability study of Atenolol because the UV spectrophotometer is not stability indicating method.

References

Andrisano V, Gotti R, Leoni A, Cavrini V. (1999)Photodegradation studies on Atenolol by liquid chromatography, *J Pharm Biomed Anal.* Dec; 21(4):851-7.

Bevinakatti H. S. and A. A. Banerji 1992,Lipase Catalysis in Organic Solvents. Application to the Synthesis of (R)-and (S)-AtenololJ . *Org. Chem.* Volume 57, No. 22, 6003-6005

Botha S. A. and A. P. Lötter (1990)Compatibility Study Between Atenolol and Tablet Excipients Using Differential Scanning Calorimetry *Research Institute for Industrial Pharmacy*.. 16, 12, 1945-1954

BP (2010b) Chromatographic system suitability.*British Pharmacopoeia* 20210 volume IV page A174-181.

BP (2010a) Validation of analytical procedure, *British Pharmacopoeia* 20210 volume IV page A586-588.

Chan, K., Swinden, J. and Donyai, P. (2007*)* Pilot study of the short-term physico-chemical stability of atenolol tablets stored in a multi-compartment compliance aid. *European Journal of Hospital Pharmacy Science*, 13 (3). pp. 60-66.

De Castro RA, Canotilho J, Barbosa RM, Redinha JS (2007) Infrared spectroscopy of racemic and enantiomeric forms of Atenolol.*Spectrochim Acta A Mol Biomol Spectrosc.* Aug; 67(5):1194-200.

Ertel, K. D., and Carstensen, J. T. (1990). Examination of a modified Arrhenius relationship for pharmaceutical stability prediction. *International Journal of Pharmaceutics*, 61, 9-14

Foppa T, Murakami FS, Silva MA(2007) Development, validation and stability study of pediatric atenolol syrup..*pharmazie.* Jul;62(7):519-21

Golightly LK, Smolinske SS, Bennett ML, Sutherland EW , Rumack BH (1988) Pharmaceutical excipients. Adverse effects associated with inactive ingredients in drug products *Med Toxicol Adverse Drug Exp.* 3(2):128-

Hatem A, Marton S, Csóka G, Rácz I (1996) Preformulation studies of atenolol in oral liquid dosage form. I. Effect of pH and temperature. *Acta Pharm Hung.* 66(4):177-80.

Jens, T.Carstensen,.Marcel Dekker.and James W.Mc Ginity(1991).Drug stability.Princible and practice, *Journal of pharamaceutical science*, 80, 1, 98.

Monika Bakshi, Saranjit Singh(2002)Development of validated stability-indicating assay methods., *Journal of Pharmaceutical and Biomedical Analysis.*Volume 28, Issue 6, 15 June 2002, Pages 1011–1040

Monika Baksh, Baljinder ,Amarjit and Saranjit (2001) stress degradation studies on ornidazole and development of a validated stability-indicating assay. *Pharmaceutical and Biomedical Analysis* 26, I 5–6, 891–897.

Monika Agnieszka, Zielińska-Pisklak, Dariusz Maciej Pisklak, Iwona Wawe (2011) H and ^{13}C NMR characteristics of β-blockers .*Magnetic Resonance.in.chemistry*, 49, 5, 284–290.

Neutel JM, Schnaper H, Cheung DG, Graettinger WF, Weber MA (1990). "Antihypertensive effects of beta-blockers . *Am. Heart J.* 120 (1): 166–71.

Shaikh Riaz Hussin and Ali Akbar Sial (1996) Stability of Pharmaceutical Formulations.*Pakistan Journal of Pharmaceutical Sciences*.9 (2).83-86

World Health Organization (2009a). Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. *Annex 2 WHO Technical Report Series*, No. 953, 1.3

World Health Organization (2009b) Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. *Annex 2 WHO Technical Report Series*, No. 953, 2.1.2

World Health Organization (2009c) Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. *Annex 2 WHO Technical Report Series*, No. 953, 2.1.7

World Health Organization (2009d) Stability testing of active pharmaceutical ingredients and finished pharmaceutical products *Annex 2 WHO Technical Report Series*, No. 953, 2.2.2

World Health Organization.(2009e) Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. *Annex 2 WHO Technical Report Series*, No. 953, 2.2.5

World Health Organization (2009f) Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. *Annex 2 WHO Technical Report Series*, No. 953, 2.2.8