CHAPTER ONE

1. Introduction

1.1 Definition of validation

Validation is verifying and documenting with a high degree of assurance that specific equipment will perform consistently according to predetermined specifications. Also validation means to check that something is officially true and acceptable , especially in order to approve it , the test results have been validated by independent experts to validate asystem requirements is to make sure its contents translate correctly and to validate the design of a system is to demonstrate that it satisfies itssystem requirements,(Hucker and Axel ,2001).

The guidelines on general principles of validation process,(Lang and Bolton ,1991).

It is also defined as an action of approving, in accordance with the principle of good manufacturing practice, that any procedure, process, equipment, material, activity or system actually leads to the expected result.

The use of Medical devices by human beings includes diagnosis, prevention, monitoring, treatment or alleviation of disease, an injury or a handicap, investigation, replacement or modification of the anatomy of a physiological process and control of conception. The potential benefit from the use of medical device ranges from relieving minor irritations to correcting life threatening conditions. If the device design and manufacturing processes are done adequately, there is a high probability that the device will perform as desired at the time it is manufactured. However, there are many naturally occurring factors that can affect how long after manufacturing the device will maintain the ability to fully perform the intended function.

Shelf life is the term or period during which a commodity remains suitable for the intended use. To determine if a particular device requires a shelf life and assign an expiration date, there are a number of l different parameters that must be considered.

The device must be analyzed to determine if it is susceptible to degradation that may lead to functional failure and the level of risk that the failure can cause. For some devices, e.g., tongue depressors, it is not reasonable to assign a shelf life because of the small likelihood of time-dependant product degradation and the lack of serious consequences if it did fail to perform as designed (FDA, 1990). For certain devices are susceptible to degradation that intended to treat life-threatening conditions, e.g., pacemakers, the failure rate should approach zero within the labeled shelf life.

The medical devices include disposable plastic syringes, breast implants, hip joint prostheses and contact lenses.

1.2 Syringes as medical devices

Disposable syringe manufacturing is a very complicated process, beginning with raw material medical grade with very high purity to finished product.

The process has different sections beginning with plastic molding and sterilization section with ethylene oxide 30% and carbon dioxide 70%, (British pharmacopeia,2016).

Plastic syringes are classified as Class IA medical devices which are frequently used for the parental administration of drugs.

Syringes and their materials have to fulfill tests like transparency, water vapor permeability, leakage and cytotoxicity. These are standard tests set in

pharmacopoeia and conducted to test the suitability of the product. Also they obey to some requirements such as electrical properties, sterility, chemical resistance and extractable/ leachable.

Sterilization of plastic syringes with ethylene oxide or radiation and their effects of on the plastic syringe materials are explained. Finally, several studies done on the possible interaction between plastic materials of syringes and some chemical solutions have been summarized.

1.2.1 Components of syringe

Although a lot of syringes have been designed until, all types of syringe basically have the same parts sour as plunger, barrel, needle and cap as shown in Figure 1 (How Products are made volume syringe*)*.

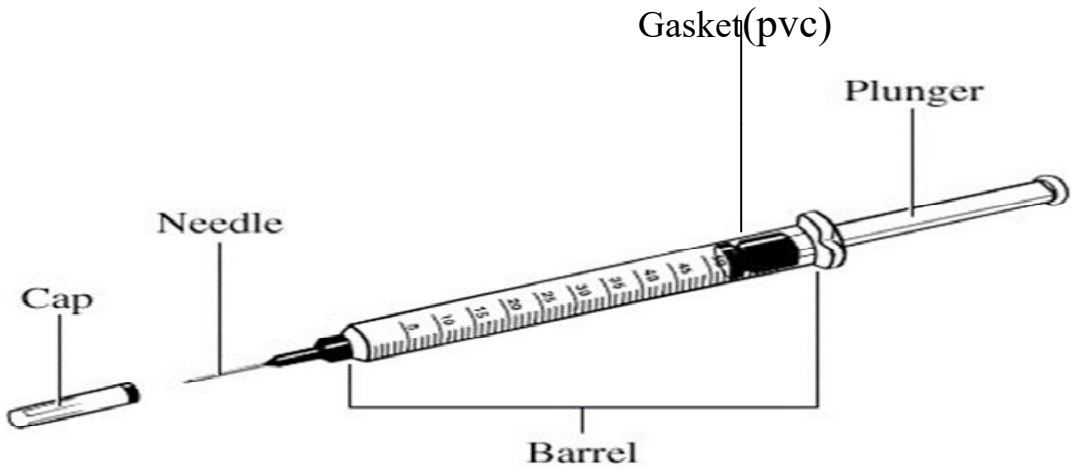


Figure 1.1 Syringe parts

If the syringe needle is made of stainless steel, it is hypodermic type needle. There are some methods for measuring the diameter of the syringe needle such as French Catheter Gauge, Metric Sizes in Millimeters, Stubs Wire Gauge. Among them Stubs Wire Gauge method is the most frequently used for medical catheters

and equipment worldwide. In accordance with this system, if the aperture of pinhole is 0.134 inch it is called 10 gauge and is 0.035 inch for the 20 gauge.

Syringe plunger, barrel and cap are made of polypropylene (PP) or polyethylene (PE) plastic materials. These materials must be of medical grade because of their medical use. For this purpose, some essential tests are applied to the plastic materials and the materials are characterized by their composition. The most important properties of the produced syringes are mechanical- thermal and electrical properties, sterility, chemical resistance and extractable/ leachable, biocompatibility, hemocompatibility and stability.

Biocompatibility and hemocompatibility are not very important because of no contact in between the syringe barrels and skin or blood (Sastri, 2014).

Some test methods, like combustion, extractable substances, fine particles, transparency, water vapor permeability, leakage and cytotoxicity, are stated in pharmacopeias. Depending on the results of these test methods, the requirements for PP and PE containers, which are used for aqueous injection, are indicated.

The conditions in the Japanese Pharmacopeia include transparency when it is tested as defined at pharmacopoeia, material transmittance is not less than 55%.

At sensory test, turbidity is not more than 20% in water loaded container and also being turbid is not more than 80% in the suspension loaded container.

Appearance of materials must not contain cracks or bubbles, the pH difference in between the blank and test solution is not more than 1.5.

UV Spectrum in 220-240 nm it must not be more than 0.08 and in 241-350 nm it must not be more than 0.05. The conditions in the European Pharmacopeia 2008 include appearance of standard solution, which is prepared with sufficient number of syringes, must be clear and colorless, acidity or alkalinity when 0.1 ml

bromothymol blue added to 20 ml S solution is titrated with 0.01 M HCl or NaOH, the amount of acid or alkali must not be used more than 0.3 mL. Absorbance in between 230-306 nm, it must not be more than 0.2 and reducing agents when the standard solution and blank solution (fitting to pharmacopeia requirements) are titrated with 0.01 M sodium thiosulfate solution, the difference between the volumes should not be more than 1.5 mL.

1.6. Types of syringes

Syringes are named according to the volume and the usage purpose and they are classified into disposable syringes and non-disposable syringes. In Table 1, types of syringes and the commercial samples are summarized. Disposable *syringes* are sterile, packed, and ready for use, non-toxic, non-pyrogenic and have lower risk in transmitting diseases such as AIDS or Hepatitis B than non-disposable syringes hence they are, mostly, preferred. Non-disposable syringes are, generally, made of heat-resistant glass such as borosilicate and they are not often used. There are two types of syringes according to intended use include oral and hypodermic. Oral syringes are used efficiently in the administration of drugs by oral or enteral route and the preparation of drugs of very small volume (Grissinger, 2013).Hypodermic syringes are calibrated by cubic centimeter (cc), mililitre (mL) or units. Small volume syringes, which have 1 to 3 mL volumes, are used in the administration of the intramuscular or subcutaneous injections. Syringes which have larger volumes from 5 to 12 mL, are used in with drawing blood draw from patients or administrating intravenous drugs injections and syringes, which have volume larger than 20 ml, are used in the injections of larger volume, sterile solutions. (British pharmacopeia, 2007).

In accordance with the special intended use, there are 3 types of syringes include Insulin, Tuberculin and Pre-filled Syringes. Insulin syringes are used in the injection of insulin hormone, for the treatment of Insulin dependent diabetes mellitus and they are unit calibrated.

Tuberculin syringes which are used in the diagnosis of tuberculosis. Their volumes are 1 mL and subdivided into 0.01 mL each. This type of syringes are used for intra dermal (i.d.) injection of very small volume drugs which are used in tube culosis and allergy tests. They are, also, preferred for injection of drugs in volumes lower than 1 mL.

Pre-filled syringes, which are used for the administration of the various drugs (such as insulin or vaccines), are single dose cartridges that have fixed needles on it (Dunne and Whitaker, 2016).

There are a lot of advantages of pre-filled syringes over traditional vials and ampoules for the patients and health workers (Yoshino *et al.*,2014).

Minimizing of the drug waste, Extra time for the drug shelf life, Being effective, safe and useful, Providing of the presic dose drug administration rapidly, Minimizing of dose mistakes and risk of contamination, allowing the patients to administer drugs by themselves

Pre-filled insulin syringes are especially recommended for patients, who use insulin in diabetes treatment, because of adsorption effect of plastic syringes.

In the production of this type of syringes, Class I borosilicate glass for syringe barrel; stainless steel or elastomer for needle; elastomer for plunger and cap and plastic materials for the other pieces of the syringe are used, respectively.

In the sterilization of these types of syringes, autoclave or radiation can be used, but the main sterilization method is gamma radiation sterilization (Makwana *et al.*, 2011).

Table: 1.2 Types of syringes

According to Material	1.PlasticsyringesPolypropylene 2.Polyethylene. 3.Glasssyringes.
According to Administration Route	1.Hypodermic syringes. 2.Oral *syringes.*
According to Special Intended Use	1.Tuberculin syringes. 2.Insulin syringes. 3.Pre-filled syringes.

1.7 Syringes production

Along the process of syringe production, some tests are applied to syringes in order to understand whether the requirements were provided or not. This tests and requirements are stated in national and international standards which are summarized in Table 1.3 below.

When the syringe pieces (plunger, barrel, needle and cap) are investigated in between 300 and 700 lux-light without magnifying glass, the syringe surfaces which are directly contact with injection liquids, must not include particles or impurities.(ISO 7886-1 (1998), TS EN ISO 7886-2 (2006), TS EN ISO 7886-4 (2007).

Table 1.3 National and international standards for syringes

TS EN ISO 7886-2 Sterile hypodermic syringes for single use - Part 2: Syringes for use with power-driven syringe pump
TS EN ISO 8537 Sterile single-use syringes, with or without needle, for insulin
TS EN ISO 21533/AC Dentistry - Reusable cartridge syringes intended for intraligamentary injections
TS EN ISO 7886-1 Syringes-Hypodermic-Single use, sterile Part1: Syringes-Manual
TS EN ISO 7886-3 Sterile hypodermic syringes for single use - Part 3: Auto-disable syringes for fixed-dose immunization
TS EN ISO 7886-4 Sterile hypodermic syringes for single use - Part 4: Syringes with re-use prevention feature
TS ISO 11040-3 Prefilled syringes - Part 3: Seals for dental local anesthetic cartridges
TS 3592 Needles for Syringes
TS 4021 Ear Syringe- Metal
TS 5031 Insulin Syringes-Reusable
TS 5462 Tuberculin Syringes
TS EN ISO 9997 Dental Cartridge Syringes

For the determination of pH and amount of extractable metals, at least 3 syringes are filled to nominal capacity line with distilled water which is suitable for the third degree in TS ISO 3696 and they are kept for 8 hours at 37 C°. In order to determine the needle of the syringe pH, 25 needles are immersed in distilled water and kept for 60±2 minutes. The content was decanted into a borosilicate glass containered and compared with control solutions (distilled water) the pH must not be higher 1 unit than control solution.

Total amount of lead, tin, zinc and iron must not be higher than 5 $mg.L^{-1}$ and amount of cadmium must be less than 0.1 $mg.L^{-1}$.

If any lubricant is used for the inner surfaces of syringe barrels and needles, particle of lubricant, which is in droplet form, must not be seen at visual inspection. For three piece syringes, polydimethylsiloxane must be used as lubricant and its amount must not be higher than 0.25 mg. Also, in two piece syringe samide of erucic and oleic acids must be used as lubricant and the amount must not exceed 6% ($m.m^{-1}$) the mass of the cylinder.The pinpoint of syringe must be sharp and smooth.

Syringes mist an identical scale which is calibrated in one or more interval and the volume of the syringe must be shown at the syringe barrel (British Pharmacopoeia 2007).

The length of syringe barrel is suitable to provide maximum usable capacity which must be at least 10 % more than the nominal capacity. Syringes must have finger grips which prevent rotation more than 180 degrees when syringes are placed horizontally at flat which is angled 10 degree with horizontal plane. In addition, finger grips must be in appropriate shape, measure and resistance and they must not have sharp edge or bulge.

When the barrel of a syringe is held in one hand, the plunger can be pushed by the same hand. For this purpose, syringe nozzle is connected to reference steel cone and the syringe is filled with water. While negative pressure is applied from the nozzle, possible disconnection between piston and the body of piston is checked.

1.8 Objectives of the study

The main objectives of this research are:

1. To determine shelf – life for hypodermic plastic disposable Syringes.

2. To validate ethylene oxide gas cycle for the sterilization of hypodermic plastic disposable Syringes.

3. To verify that no changes have occurred in hypodermic plastic disposable syringes that can adversely affect the stability study.

CHAPTER TWO

2. Literature Review

2.1 Stability study

The United States Pharmacopoeia (USP, 2009) defines stability as "the extent to which a product retains, within specified limits, and throughout its period of storage and use, i.e., its shelf life. The same properties and characteristics that it possessed at the time of manufacture."

There is no one exhaustive set of criteria that would apply equally to all medical devices. The USP section **(**1191**)** entitled "Stability Considerations in dispensing Practicing that supplies general information on this topic.(Magari, 2003).

Lists of five sets of criteria for acceptable levels of stability for drug products are chemical, physical and microbiological.

Although this set of criteria applies specifically to the evaluation of drug product stability, it is useful as a starting point in developing a set of criteria to evaluate the stability of medical devices.

2.1.1 Real - time stability

In the real – time stability testing, a product is stored at recommended storage conditions and monitored until it fails the specifications.(Magari, 2003).

2.1.2 Accelerated stability

Accelerated shelf-life studies may be run by storing samples at higher temperatures for a shorter period of time and mathematically correlating increased temperature with time:

2.2 Climatic zones for stability

These zones table 2.1 and 2. 2are created due to the difference in temperature and humidity in the different parts of the world.

Table 2.1Long Term conditions

Climatic zone	Temperature	Humidity
Zone I	21C º± 2C º	45% RH ± 5% RH
Zone I I	25C º± 2C º	60% RH ± 5% RH
Zone I II	30C º± 2C º	35% RH ± 5% RH
Zone I Va	30C º± 2C º	65% RH ± 5% RH
Zone I Vb	30C º± 2C º	75% RH ± 5% RH

Table 2.2 Accelerated and intermediate conditions

Climatic zone	Temperature	Humidity
Accelerated Ambient	40C º± 2C º	75% RH ± 5% RH
Accelerated Refrigerated	25C º± 2C º	60% RH ± 5% RH
Accelerated Frozen	5C º± 3C º	No Humidity
Intermediate	30C º± 2C º	65% RH ± 5% RH

2.3 Materials used for plastic syringes manufacturing:

2.3.1Polypropylene

Polypropylene (PP) is a linear hydrocarbon polymer, expressed as C_nH_{2n}. PP, like polyethylene (High Density Poly Ethylene or Low Density Poly Ethylene) and poly butene (PB), is a polyolefin or saturated polymer. Polypropylene is one of those most versatile polymers available with applications, both as a plastic and as

a fiber, in virtually all of the plastics end-use markets.(British Standard and British pharmacopeia ,2016).

Polypropylene (PP) , also known as poly propene, is a thermo plastic polymer used in a wide variety of applications including packaging and labeling, textiles (e.g., ropes, thermal underwear and carpets), stationery, plastic parts and reusable containers of various types, laboratory equipment, loudspeakers, automotive components, and polymer banknotes. In addition polymer made from the monomer propylene, it is rugged and unusually resistant to many chemical solvents, bases and acids,(Gran well,2012).

Following the work of Ziegler in Germany, the process for producing "stereo regular" polymers was perfected by Professor Giulio Natta in Italy. Natta produced the first polypropylene resin in Spain in 1954. Natta utilized catalysts developed for the polyethylene industry and applied the technology to propylene gas.These new polymers with their ability to crystallize soon became popular and polypropylene is now a very successful product in many areas.

The plastic materials used in the production are cyclo olefin polymers (COP) or cyclic olefin copolymers (COC). Cycloolefin-copolymers (COCs) are clear amorphous copolymers based on cyclic and linear olefins. These materials form a family of engineering resins that exhibit a unique combination of properties, including high transparency, low density, excellent moisture barrier capabilities, and resistance to aqueous and polar organic media. COC is being used for pre-filled syringes, needleless injectors and other drug delivery systems. (Oktay, Ş., and Kayaalp, O., 2012).

Commercial production began in 1957 and polypropylene usage has displayed strong growth from this date. Oriented polypropylene (OPP) has seen considerable growth, having replaced cellophane in, virtually, all applications.

Polypropylene Figure 2.3.1.3 and Figure 2.3.1.4 is the material of choice in medical application not only because of its light weight, low cost but also due to high chemical and bacterial resistance.

Medical vials, cylinders of syringes, diagnostic devices, Petri dishes, intravenous bottles, specimen bottles, food trays, pans, and amber-colored small containers that hold pills and capsules are also of PP products. 'Sharp containers' used to collect used syringes and other contaminated medical wastes are also PP products.

2.3.1.1 Specification of polypropylene medical grade:

Polypropylene medical grade is a transparent homo polymer that features high flow ability and balanced mechanical properties, suitable for injection molding applications .For manufacturing hypodermic sterile plastic syringes

2.3.1.2 Recycling

Polypropylene is recyclable and has the number "5" as its resin identification code as 

2.3.2 Poly vinyl chloride (PVC) medical grade

Black granules based on plasticized poly vinyl chloride.For manufacturing gaskets of hypodermic sterile plastic syringes.(Gran well ,2012)

PVC is useful because it resists to fire and water.(Fenichell , Stephen , 1996)

PVC is a polymer similar to poly ethylene which is produced by free radical polymerization of vinyl chloride.

2.3.3 Silicon oil specifications

Silicon oil is a poly(dimethylsiloxane) obtained by hydrolysis and poly condensation of dichloromethylsiloxane. Different grades exist which are characterized by anumber indicating the nominal.

Silicon oil used as a lubricant. It is colorless , transparent fluid and odorless , Insoluble in water, Insoluble in methanol and very slightly soluble in ethanol

2.4 Syringes sterilization process

According to the old definition, sterilization is the elimination of all live organism, bacteria and fungi spores from the contents of a products.

Recently, sterilization is defined as the presence probability of viable microorganism in one million product.

Theoretically, in order to designate a medical device as sterile, the probability level should be lower than one in a million (Perçin, 2014).

Sterility Assurance Level (SAL) is the term used for indicating that sterility has reached desired level. In other words, SAL is a term which can be defined as probability of presence non-sterile products or survival of microorganisms in the samples.

For medical devices SAL level is very low 10^{-6} (Turker, 2009a.; Sterility Assurance Level). Among the main sterilization methods, there are several

sterilization methods such as dry air ,oven sterilization , sterilization with pressurized steam in autoclave at 121C°, UV radiation sterilization, ethylene oxide (EO) sterilization, ionized radiation.

sterilization which uses gamma rays or electron beam (e-beam) and sterilization in aseptic conditions using membrane filters which have pores in 0.22 µm diameter (Moraes *et al.*, 2014). Syringes have to be sterile because of their use in the administration of sterile and a pyrogenic drugs by parenteral route. Sterilization of products is possible in the terminal packages. Microbial death/ bioburden control are evaluated by this method and has the lowest risk. Because of these reasons, terminal sterilization method is recommended (Japenese Pharmacopoeia, 2011b). Among the sterilization methods, there are two methods used for the sterilization of syringes. One of them is EO sterilization which has been used since the 1950s. This method is especially suitable for heat-sensitive and moisture-sensitive materials. Pure EO gas is flammable and explosive, also according to Environmental Protection Agency (EPA) it is a toxic and carcinogenic gas. So EO gas is diluted with some agents like hydro chlorofluoro carbon (HCFC) and carbon dioxide. There are some disadvantages of this method; for instance it is complex because of depending on some critical parameters such as temperature (30-65C°), relative humidity (30-99%), EO concentration (250-1500 mg,mL^{-1}), overall exposure time (1-30 hours), type of microorganisms, product and load density, and gas permeability factors. In addition, due to the formation of EO decomposition products after sterilization process it requires aeration .(Ozer, 2009).

Other sterilization method is ionized radiation sterilization which uses gamma rays or e-beam. In order to apply this sterilization method in practice, the first national draft law has been published in 1965. now a days.

Food and Drug Administration (FDA,USA) and Department of Health and Social Services (DHSS, UK) for checking the suitability of facilities with laws and regulations. In addition, Occupational Safety and Health Administration (OSHA) and Environmental Protection Agency (EPA), established in 1997 within the frame work of US.

2.5. Ethylene oxide (oxirane)

Is an organic compound with the formula C_2H_4O.It is a cyclic ether.It was first reported in 1859 by the French chemist Charles-AdolpheWurtz, who prepared it by treating 2-chloroethanol with potassium hydroxide (David L. Seitelman , 1988) :$Cl–CH_2CH_2–OH + KOH \rightarrow (CH_2CH_2)O + KCl + H_2O$

Ethylene oxide is a colorless flammable gas at room temperature, with a faintly sweet odor; it is the simplest epoxide: a three-membered ring consisting of one oxygen atom and two carbon atoms. Because of its special molecular structure, ethylene oxide easily participates in addition reactions; e.g., opening its ring and thus easily polymerizing.

Ethylene oxide is industrially produced by direct oxidation of ethylene in the presence of silver catalyst. It is extremely flammable and explosive and is used as a main component of thermobaric weapons;(Juran& Frank ,1980).

Therefore, it is commonly handled , shipped as arefrigerated liquid. As a poison gas that leaves no residue on items it contacts, pure ethylene oxide is a disinfectant that is, widely, used in hospitals and the medical equipment industry to replace steam in the sterilization of heat-sensitive tools and equipment, such as disposable plastic syringes

Ethylene oxide is important or critical to the production of detergents, thickeners, solvents, plastics, and various organic chemicals such as ethylene glycol, ethanol amines, simple and complex glycols, polyglycol ethers and other compounds. Aqueous solutions of ethylene oxide are rather stable for a long time without any noticeable chemical reaction, but adding a small amount of acid, such as strongly diluted sulfuric acid, immediately leads to the formation of ethylene glycol, even at room temperature:

$(CH_2CH_2)O + H_2O \rightarrow HO–CH_2CH_2–OH.$

CHAPTER THREE

3. Materials and methods

3.1 Collection of samples

300 Pieces of disposable plastics syringes as raw material were collected from Avamed medical industries factory in Khartoum - Sudan after sterilization process and then were transferred to Accelerated stability study chamber in the hard conditions (Temperature 40C° & Humidity 70%) for 6 months, (British pharmacopeia, 2016).

3.2 Methods of analysis

3.2.1 Chemical tests

A. Preparation of sodium hydroxide solution 0.01M

(0.04 g) of sodium hydroxide is weighed, dissolved and diluted to 100 mL of distilled water to prepare 0.01M sodium hydroxide.

B. Preparation of sodium thiosulfate

(0.6205 g) of sodium thiosulfate is weighed and dissolved in 100 mL of distilled water to prepare 0.02 M sodium thiosulfate.

C. Preparation of Potassium Permanganate

(0. 079 g) of Potassium permanganate is weighed and dissolved in 100 mL of distilled water to prepare 0.02 M $KMnO_4$

D. Preparation of starch as indicator

(1.0 g) of soluble starch is dissolved in 5mL of distilled water.

Added while continuously, stirring to 100 mL of boiling water containing 10 mg of mercury iodide.

E. Preparation of promothymol as indicator

(50mg) of bromothymol blue is dissolved in a mixture of 4 mL of 0.02 M sodium hydroxide and 20 mL of ethanol 96 % .

F. Preparation of standard solution

The solution is prepared taking care to avoid contamination by foreign particles

Using a syringes sufficient number of syringes to produce 50 mL volume of test solution.

The syringes are filled to their nominal volume with distilled water.

Maintained at 37 C° for 24 hours in stability chamber .(Sastri, V.R., 2014).

The contents of the syringes are emptied in ,suitable, borosilicate – glass containers.

G. Reducing substances test

(20 ml) of solution were added to 2 ml of sulfuric acid ® and 20 ml of 0.002M potassium permanganate, boiled for 3 min and immediately Cooled.

(1 g) of KI (R) is added and immediately titrated with0.02 M sodium thiosulfate using 0.25 ml of starch solution R as indicator.

A blank titration is carried out using 20 ml of water.

The difference between the titration volumes is not greater than 3.0 ml.

H. Blank test

The consumed volume of sodium thiosulfate to complete titration of distilled water which has PH between 5.5 to 6.5 read from burette is called the blank .

using starch as indicator to determine end point , the final result appears color changing from black to colorless. The same steps which is carried out to read blank result is applied to all samples.

The difference between the blank result and sample result gives amount of reduced substances in polypropylene samples. Final result should be not more than 3ml according to British pharmacopeia 2007.

I. Acidity or alkalinity test

(20 ml) of standard solution were added to0.1 ml of bromthymol blue solution and**.**Titrated with 0.01M sodium hydroxide or 0.01M hydrochloric acid

Not more than 0.3 ml of 0.01M sodium hydroxide or 0.01M hydrochloric acid is required to change the color of the indicator.

J. IR identification of syringe tube

Disposable syringes (Tubes) made of poly propylene (Medical grade) were cut to small piece about 0.25 g .

Small pieces of cylinder are placed into bottom flask and toluene was added as solvent into reflux condenser under heating for fifteen minutes for completed solubility. Drops of the hot solution were placed on sodium chloride disc and the solvent was evaporated in an oven at 80C°.The disc was examined by infrared absorption spectrophotometer.

The spectrum obtained a certain number of maxima, at 1375 cm^{-1}, 1170 cm^{-1} ,995 cm^{-1} and 970 cm^{-1} .

The spectrum obtained is identical to the spectrum obtained with the material selected for the type sample.

If the material to be examined is in the form of sheets , the identification may be performed directly on a cut piece of suitable size .

K. Absorbance by UV-Vis spectrophotometer for standard solution

Distilled water was used as blank solution to adjust the reading of the spectrophotometer. The absorbance of solution (S) was Measured from 220 nm to 360 nm.Absorbance of solution (S) does not exceed 0.4.

L. Blank test

The cells were filled with distilled water and put it in UV instrument

Clicked on the Base line the result should be zero, blank is distilled water.

One of cell is filled with sample and another is already filled with distilled water.

Click on measure, the difference between them appears in the peak form between wave length 220 nm to 360 nm , the reading result should not exceed 0.2.

M. Determination of silicon oil residues.

The internal surface area of a syringe is Calculated in square centimeter using the following expression:

$$2\sqrt{V.\pi.h}$$

V = nominal volume of the syringe. In cubic centimeters.

h = height of the graduation, in centimeters.

Sufficient number of syringes were Taken to give an internal surface area of 100 cm^2 to 200 cm^2.Volume of methylene chloride into each syringe was aspirated to half the nominal volume and made up to the nominal volume with air.

The internal surface corresponding to the nominal volume with the solvent was rinsed by inverting the syringe ten times in succession with the needle fitting

closed by a finger covered by plastic film inert to methylene chloride.The tarred dish was Weighed (empty) .The extract into a tarred dish was expelled and repeated the operation. The combined extract was evaporated to dryness on a water bath and dried at 100 C° to 105 C° for 1 hour. The residue weigh should not exceed 0.25 mg per square centimeter of internal surface area.

4. Physical tests

4.1. Volume and dead space of syringe test.

The empty syringe is weighed (a) grams. The syringe was filled to the nominal graduated capacity with distilled water was weighed (b) grams, taking care to expel all air bubbles and to ensure that the level meniscus of the water coincides with the end of the nozzle lumen. The water was expelled by fully depressing the plunger , surface of the syringe was wiped and dried the outer, weighed (c).The difference between (c) and (a) grams was calculated. Expressed in milliliters which is the dead space or residual volume of the syringe was recorded.
The difference between (c) and (b) grams was calculated. Expressed in milliliters which is the nominal volume of the syringe was recorded.

4.2. Liquid leakage test

The disposable syringe is drawn up with water completely affixed with needle, all air expelled and with the aid of suitable device, the water is expelled within 30 seconds at constant pressure.3 bars pressure applied there by the distal ring of the plunger tip moved along the whole scale from top to zero mark. Constant liquid over pressure at 3 bars was maintained. Water leakage was observed through piston and nozzle joining the hub of needle. Part 1: ISO 7886 – 1

4.3. Air leakage test

Into a syringe a volume was drawn of water should not less than 25% of the nominal graduated capacity. The syringe nozzle was connected to the reference. The vacuum pump was switched on with the air bleed control open.

The bleed control was adjusted so that a gradual reduction in pressure is obtained and manometer reading of 66 cm/Hg is reached. The syringe was examined for leakage of piston. The syringe and manometer were isolated and assembled by means of vacuum tight valve. The manometer reading was observed for 60 seconds and any fall in the reading was observed. (Part 1: ISO 7886 – 1)

5. Microbiological tests

5.1 Medium preparation

(29.75 grams) of thioglycollate were weighed and diluted with 1000 ml distilled water, 30 grams of Casein Soya Bean Digest Medium per litre of distilled water , 15mls of the Broth was boiled. After boiling the culture media was distributed in 100 ml borosilicate screw cap containers and sterilized by Autoclave for 20 minutes at 121C°. One positive and one negative control bottle were prepared. The Bacillus subtilis and candida albicans were used for positive control to show positive growth in thioglycollate medium and Casein Soya bean respectively digest medium.

5.2. Sterility test

Syringes were stated to be sterile only internally comply with the test for sterility carried out as follows:

Laminar Flow equipment was switched on at least an hour before the test. UV lights was switched on. 50 mls of inoculation medium were used for each test syringe. The needle protector was removed using aseptic technique. The needle in the culture medium was Submerged.

The syringe was Flushed five times by withdrawing the plunger to its fullest extent .

Thioglycollate medium was Incubated at 30C° - 35 C° and Casein Soya bean digest medium at 20 C° - 25C° both should not less than 14 days. The boyyles was daily observed up to 14 days. The test results was Interpreted, if no growth up to 14 days as passed. If growth was observed within 14 days the test should be repeated .(British Pharmacopoeia, 2016).

5.3. Determination of bacterial endotoxin by laminar air follow

Shake each dilution on vortex mixer for 30 seconds. Keep the endotoxin solution in refrigerator for 7 days. Before using the prepared endotoxin dilution, shake on vortex mixer for 1 minute at room temperature . 0.1 mL of the reconstituted lysate was added (keep on ice throughout the test) to each tube. Each test tube ,gently , was swirled , mixed and placed in the incubating block , maintained at 37C° ± 1 C° for 1 hour ± 2 minutes. Each tube was examined for gel formation and results was recorded. The positive control must always gel and the test sample and negative control must not gel. (British Pharmacopoeia 2007).

CHAPTER FOUR

4.1 Results and discussion

Results during the period of time are six months for hypodermic plastic disposable syringe in harsh conditions (Temperature over 30C^{o} and Humidity over 60% as standard in BP 2007) were as below:

Table 4.1.1 Absorption results

Absorption	At zero time	After first month	After second month	After third month	After 6 th month
Reading In 220 nm	0.002	0.077	0.054	0.046	0.159
Reading In 360 nm	0.001	0.003	0.005	0.008	0.066
Standard range	NMT 0.4				

Table 4.1.1 and Figure 4.1.2, 4.1.3, 4.1.4, 4.1.5 and 4.1.6 show the effect of UV absorption during this stability study in 220 nm and 360 nm , all the result were the range of limit and should not exceed 0.4.

The results explain that no changes occurred and no more impurities in the solution, so the chemical properties are stable.

Table 4.1.2 Silicon oil residues result at zero time

No of syringe	Surface Area	Volume taken	Total volume	Weight 1 before	Weight 2 After	Silicon oil
6	100cm^2	2.5 mL	15 mL	45.600	45.610	0.01

Silicon oil per mg / cm^2= $\frac{0.01}{100} X\ 1000 = 0.1\ mg\ /cm2$

Table 4.1.3 Silicon oil residues result after first month

No of syringe	Surface Area	Volume taken	Total volume	Weight 1 before	Weight 2 After	Silicon oil
6	100cm^2	2.5 mL	15 mL	40.110	40.126	0.016

Silicon oil per mg / cm^2= $\frac{0.016}{100} X\ 1000 = 0.16\ mg\ /cm2$

Table 4.1.4 Silicon oil residues result after second month

No of syringe	Surface Area	Volume taken	Total volume	Weight 1 before	Weight 2 After	Silicon oil
6	100cm^2	2.5 mL	15 mL	40.13	40.12	0.01

Silicon oil per mg / cm^2= $\frac{0.0157}{100} X\ 1000 =\ mg\ /cm2$

Table 4.1.5 Silicon oil residues result after third month

No of syringe	Surface Area	Volume taken	Total volume	Weight1 before	Weight 2 After	Silicon oil
6	100cm^2	2.5 mL	15 mL	46.0601	46.0775	0.0174

Silicon oil per mg / cm^2= $\frac{0.0174}{100} X\ 1000 = 0.174\ mg\ /cm2$

Table 4.1.6 Silicon oil residues result after six month

No of syringe	Surface Area	Volume taken	Total volume	Weight 1 before	Weight 2 After	Silicon oil
6	100cm^2	2.5 mL	15 mL	39.2610	39.2791	0.0181

Silicon oil per mg / cm^2= $\frac{0.0181}{100} X\ 1000 =\ \ 0.181\ mg\ /cm2$

Table 4.1.2 , 4.1.3 ,4.1.4 ,4.1.5 and 4.1.6 show all the results of silicon oil residue in the syringe that were the range of the limit and acceptance criteria should not exceed 0.25mg / cm^2 and there is no change in the silicon oil residues.

Table 4.1.7 reducing substances results

Reducing substance	Zero time 24 hrs	First month	Second month	Third month	6^{th}month
Blank result(B)	17.5 mL	15.4 mL	15.5 mL	15.7 mL	20.6 mL
Sample result	15.4 mL	14.5 mL	15.3 mL	13.2 mL	19.4 mL
Result = Difference between (S) and (B)	2.1 mL	0.9 mL	0.2 mL	2.5 mL	1.2 mL
Standard result	NMT 3 mL				

Table 4.1.7 shows the effect of reduced substances in the solution on the chemical properties such as the purity of the material, but all the results were within the range of the limit, should not exceed 3.0 ml.

Table 4.1.8 Alkalinity results

Alkalinity result	At zero time	After one month	After two month	After three month	After six month
Standard result : Not More Than 0.3 mL	0.13 mL	0.08 mL	0.24 mL	0.19 mL	0.16 mL

Table 4.1.8 shows the effect of alkalinity of the solution on the syringe stability that all above the results during this study were within the range of the limit, should not exceed 0.3 ml.

4.2 Physical tests results

Table 4.1.9 Volume and dead space results at zero time

No	**(A)**	**(B)**	**(C)**	**(B - C)**	**(C - A)**
	Empty	**With water**	**without water**	**Volume**	**Dead space**
1	3.93 gm	8.90 gm	3.95 gm	4.95 gm	0.05 gm
2	3.87 gm	8.92 gm	3.88 gm	5.04 gm	0.04 gm
3	3.90 gm	8.91 gm	3.91 gm	5.00 gm	0.08 gm
4	3.92 gm	8.95 gm	3.94gm	5.03 gm	0.05 gm
5	3.90 gm	8.90 gm	3.91 gm	4.99 gm	0.05 gm
Total Average				24.98 mg	0.27 mg
				4.99ml	0.054 ml

Table 4.1.10 Volume and dead space results after first month

No	(A)	(B)	(C)	(B - C)	(C - A)
	Empty	With water	without water	Volume	Dead space
1	3.92 gm	8.88 gm	3.97 gm	4.91 gm	0.05 gm
2	3.90 gm	8.89 gm	3.94 gm	4.95 gm	0.04 gm
3	3.88 gm	8.91 gm	3.96 gm	4.95 gm	0.08 gm
4	3.91 gm	9.04 gm	3.96 gm	5.08 gm	0.05 gm
5	3.89 gm	8.88 gm	3.94 gm	4.94 gm	0.05 gm
Total Average				24.83 mg	0.27 mg
				4.96 ml	0.054 ml

Table 4.1.11 Volume and dead space results after second month

No	(A)	(B)	(C)	(B - C)	(C - A)
	Empty	With water	without water	Volume	Dead space
1	3.91 gm	8.97 gm	3.94 gm	5.03 gm	0.03 gm
2	3.91 gm	8.97 gm	3.95 gm	5.02 gm	0.04 gm
3	3.87gm	8.89 gm	3.91 gm	4.98 gm	0.04 gm
4	3.91 gm	8.96 gm	3.95 gm	5.01 gm	0.04 gm
5	3.90 gm	8.83 gm	3.95 gm	4.88 gm	0.05 gm
Total Average				24.92 mg	0.20 mg
				4.98ml	0.04 ml

Table 4.1.12 Volume and dead space results for third month

No	(A)	(B)	(C)	(B - C)	(C - A)
	Empty	**With water**	**without water**	**Volume**	**Dead space**
1	3.88 gm	8.99 gm	3.92 gm	5.08 gm	0.04 gm
2	3.86 gm	8.96 gm	3.91 gm	4.99 gm	0.05 gm
3	3.93 gm	9.00 gm	3.97 gm	5.03 gm	0.04 gm
4	3.88 gm	8.77 gm	3.94 gm	4.83 gm	0.06 gm
5	3.92 gm	8.97gm	3.95 gm	5.02gm	0.03 gm
Total Average				24.95 mg	0.22 mg
				4.99ml	0.044 ml

4.1.13 Volume and dead space results after six month

No	(A)	(B)	(C)	(B - C)	(C - A)
	Empty	**With water**	**without water**	**Volume**	**Dead space**
1	3.92 gm	8.87 gm	3.93 gm	4.94 gm	0.05 gm
2	3.90 gm	8.89 gm	3.92 gm	4.97 gm	0.04 gm
3	3.89 gm	8.78 gm	3.91 gm	4.87 gm	0.08 gm
4	3.89 gm	8.91 gm	3.90 gm	5.01 gm	0.05 gm
5	3.91 gm	8.88 gm	3.93 gm	4.95 gm	0.05 gm
Total Average				24.74mg	0.27 mg
				4.948ml	0.016ml

Tables 4.1.9, 4.1.10, 4.1.11, 4.1.12 and 4.1.13 show the effect of high temperature and humidity on the dimensions of syringe contents in the volume and dead space volume results. But all the results were within the tolerance ± 0.2 ml for the volume result and should not exceed 0.075 ml for dead space volume.

Table 4.1.14 5Liquid/Air leakage results

Sample No.	Quantity of Syringes	Liquid / Air Leakage Test	Month	Remarks
1	5 Pieces	√	At zero time	pass
2	5 Pieces	√	After one month	pass
3	5 Pieces	√	After two month	pass
4	5 Pieces	√	After three month	pass
5	5 Pieces	√	After six month	pass

Table 4.1.14 shows that all results were positive and no liquid and air leakage and the all dimensions were controlled. The goal of these tests is to be sure that there is no leakage between the syringe contents according to the dimensions during this period of time and high temperature and humidity (40 C° and 70%) .

4.3 Microbiological tests results

Table 4.3.1 Sterility test at zero time

Days	Test Control		Test Results		Remarks
	Positive	Negative	FTM	TSB	
1	+ ve	- ve	- ve	+ ve	negative
2	+ ve	- ve	- ve	+ ve	negative
3	+ ve	- ve	- ve	+ ve	negative
4	+ ve	- ve	- ve	+ ve	negative
5	+ ve	- ve	- ve	+ ve	negative
6	+ ve	- ve	- ve	+ ve	negative
7	+ ve	- ve	- ve	+ ve	negative
8	+ ve	- ve	- ve	+ ve	negative
9	+ ve	- ve	- ve	+ ve	negative
10	+ ve	- ve	- ve	+ ve	negative
11	+ ve	- ve	- ve	+ ve	negative
12	+ ve	- ve	- ve	+ ve	negative
13	+ ve	- ve	- ve	+ ve	negative
14	+ ve	- ve	- ve	+ ve	negative

Table 4.3.2 Sterility tests after one month

Days	Test Control		Test Results		Remarks
	Positive	Negative	FTM	TSB	
1	+ ve	- ve	- ve	+ ve	negative
2	+ ve	- ve	- ve	+ ve	negative
3	+ ve	- ve	- ve	+ ve	negative
4	+ ve	- ve	- ve	+ ve	negative
5	+ ve	- ve	- ve	+ ve	negative
6	+ ve	- ve	- ve	+ ve	negative
7	+ ve	- ve	- ve	+ ve	negative
8	+ ve	- ve	- ve	+ ve	negative
9	+ ve	- ve	- ve	+ ve	negative
10	+ ve	- ve	- ve	+ ve	negative
11	+ ve	- ve	- ve	+ ve	negative
12	+ ve	- ve	- ve	+ ve	negative
13	+ ve	- ve	- ve	+ ve	negative
14	+ ve	- ve	- ve	+ ve	negative

Table 4.3.3 Sterility test after two month

Days	Test Control		Test Results		Remarks
	Positive	Negative	FTM	TSB	
1	+ ve	- ve	- ve	+ ve	negative
2	+ ve	- ve	- ve	+ ve	negative
3	+ ve	- ve	- ve	+ ve	negative
4	+ ve	- ve	- ve	+ ve	negative
5	+ ve	- ve	- ve	+ ve	negative
6	+ ve	- ve	- ve	+ ve	negative
7	+ ve	- ve	- ve	+ ve	negative
8	+ ve	- ve	- ve	+ ve	negative
9	+ ve	- ve	- ve	+ ve	negative
10	+ ve	- ve	- ve	+ ve	negative
11	+ ve	- ve	- ve	+ ve	negative
12	+ ve	- ve	- ve	+ ve	negative
13	+ ve	- ve	- ve	+ ve	negative
14	+ ve	- ve	- ve	+ ve	negative

Table 4.3.4 Sterility test after three month

Days	Test Control		Test Results		Remarks
	Positive	Negative	FTM	TSB	
1	+ ve	- ve	- ve	+ ve	negative
2	+ ve	- ve	- ve	+ ve	negative
3	+ ve	- ve	- ve	+ ve	negative
4	+ ve	- ve	- ve	+ ve	negative
5	+ ve	- ve	- ve	+ ve	negative
6	+ ve	- ve	- ve	+ ve	negative
7	+ ve	- ve	- ve	+ ve	negative
8	+ ve	- ve	- ve	+ ve	negative
9	+ ve	- ve	- ve	+ ve	negative
10	+ ve	- ve	- ve	+ ve	negative
11	+ ve	- ve	- ve	+ ve	negative
12	+ ve	- ve	- ve	+ ve	negative
13	+ ve	- ve	- ve	+ ve	negative
14	+ ve	- ve	- ve	+ ve	negative

Table 4.3.5 Sterility test after six month

Days	Test Control		Test Results		Remarks
	Positive	Negative	FTM	TSB	
1	+ ve	- ve	- ve	+ ve	negative
2	+ ve	- ve	- ve	+ ve	negative
3	+ ve	- ve	- ve	+ ve	negative
4	+ ve	- ve	- ve	+ ve	negative
5	+ ve	- ve	- ve	+ ve	negative
6	+ ve	- ve	- ve	+ ve	negative
7	+ ve	- ve	- ve	+ ve	negative
8	+ ve	- ve	- ve	+ ve	negative
9	+ ve	- ve	- ve	+ ve	negative
10	+ ve	- ve	- ve	+ ve	negative
11	+ ve	- ve	- ve	+ ve	negative
12	+ ve	- ve	- ve	+ ve	negative
13	+ ve	- ve	- ve	+ ve	negative
14	+ ve	- ve	- ve	+ ve	negative

Also all the results were within the limit according to the sterility and pyrogen test there is no any growth of bacteria and fungi after 14 days from the sterilization process by the ethylene oxide 30% and Carbon dioxide 70% mixed in the one cylinder. Stability study validation for the 5 ml hypodermic disposable plastic syringes gave good results in the limit, so this study will help in extension of the shelf life of the plastic syringe for three years according to the good manufacturing practices, world health organization and British pharmacopeia 2007 for disposable syringes plant.

Conclusion

Accelerating stability study shows the validity or shelf life of the products with reference standards (British Standards) for quality, found that samples of product (5 ml hypodermic disposable plastic syringes) complied with standard after passing all the tests even in the hard conditions with no change in the properties during six months in the hard controlled conditions (Temperature over 30C° about 40C° & Humidity over 60% about 70%).

Recommendations:

Compliance with standards and results of quality for hypodermic plastic disposable syringes make safety and effect.

From any new products especially which affect the human health like syringes, drugs and medical devices must to be check the accelerated stability tests to determine the validly or shelf life in the hard conditions (after six months) so to see how the product able to maintain the chemical, physical and microbiological properties in the standards limits.

Also I recommend to take care with this industry (plastic disposable syringe plant) and encourage government and business men to invest in it because for its importance in the medical field and should be to promote it.

Good conditions should be done and prepared for manufacture and distribution of this product. Finally from this study, I recommend using Gama radiation source in the medical devices sterilization process so, it is high safety, easy in the process, little cost and does not take time for sterilization process comparing with the Ethylene oxide gas sterilization.

Appendix

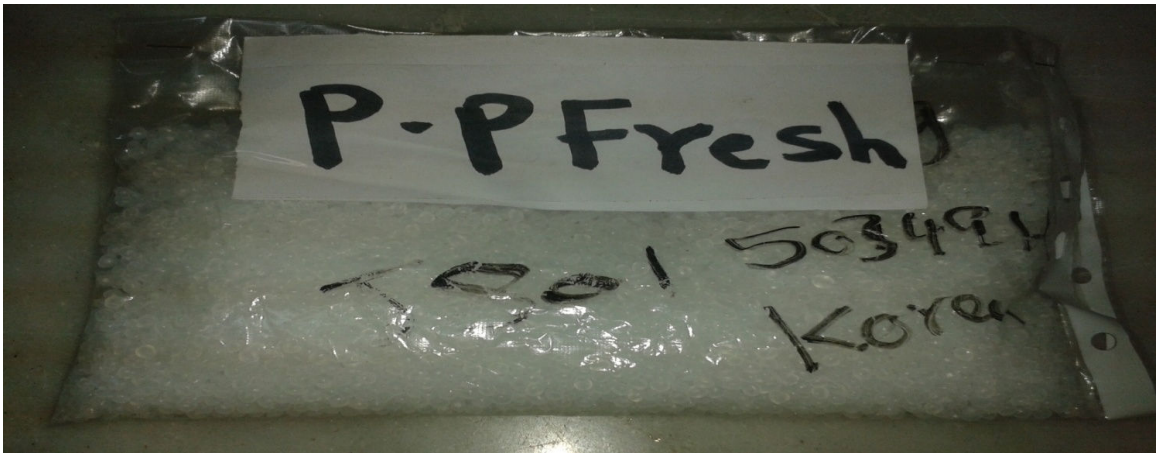


Figure: 2.3.1.1 white fresh granules of PP

$(C_3H_6)_n$

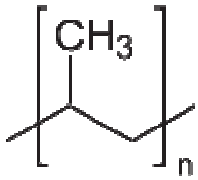


Figure 2.3.1.2

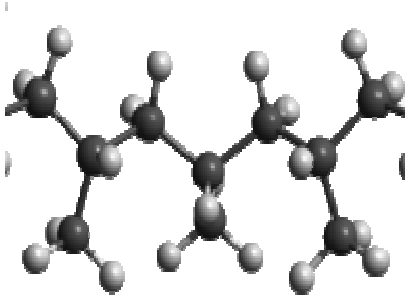


Figure 2.3.1.3

Chemical structure of polypropylene

Figure: 2.3.2.1 black fresh granules of PVC

Figure 4.1.1 Stability chamber or incubator (37C°).

Figure 4.1.2. IR spectrophotometer

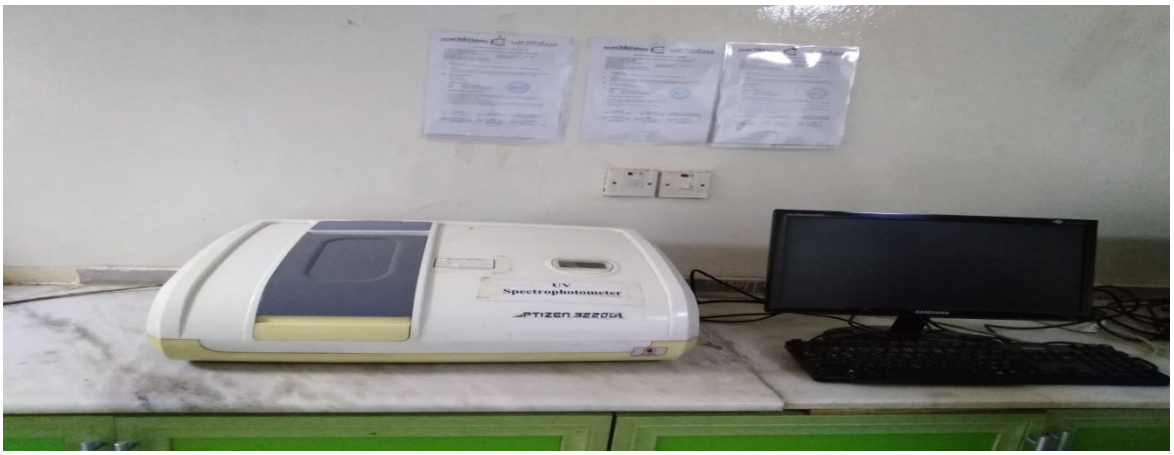


Figure 4.1.3 UV spectrophotometer (shimadzu 3220)

Figure 4.1 Liquid Leakage tester

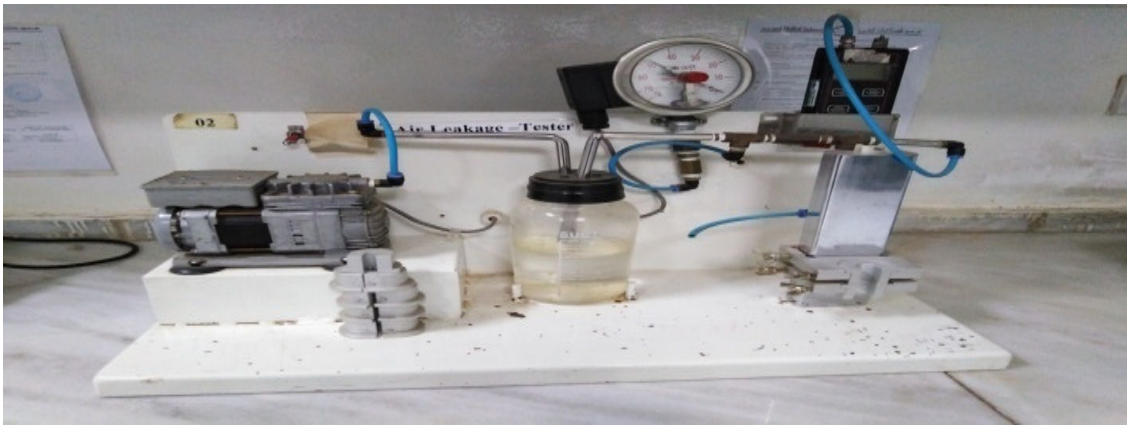


Figure 4.2 Air Leakage tester

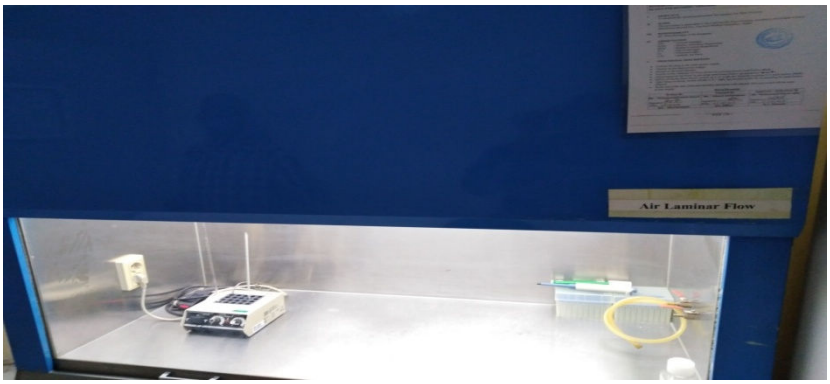


Figure 5.1.1 Laminar Air Follow

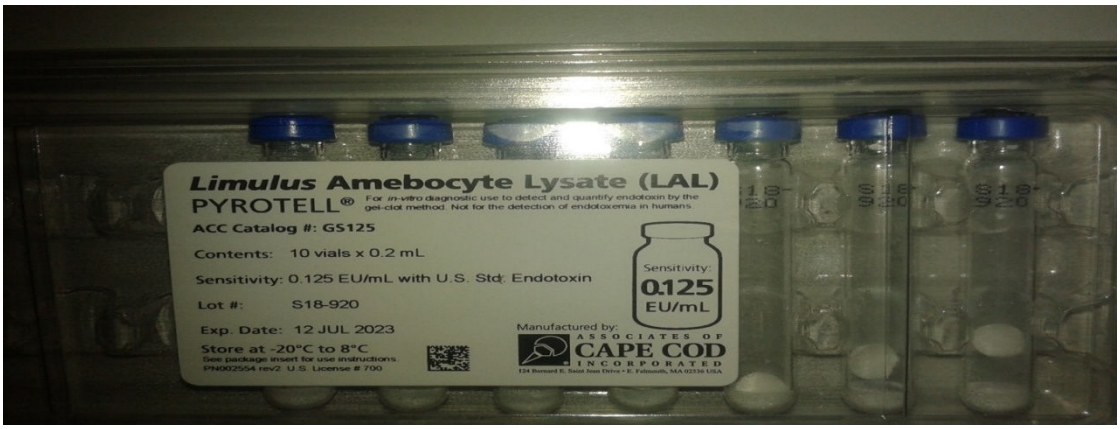
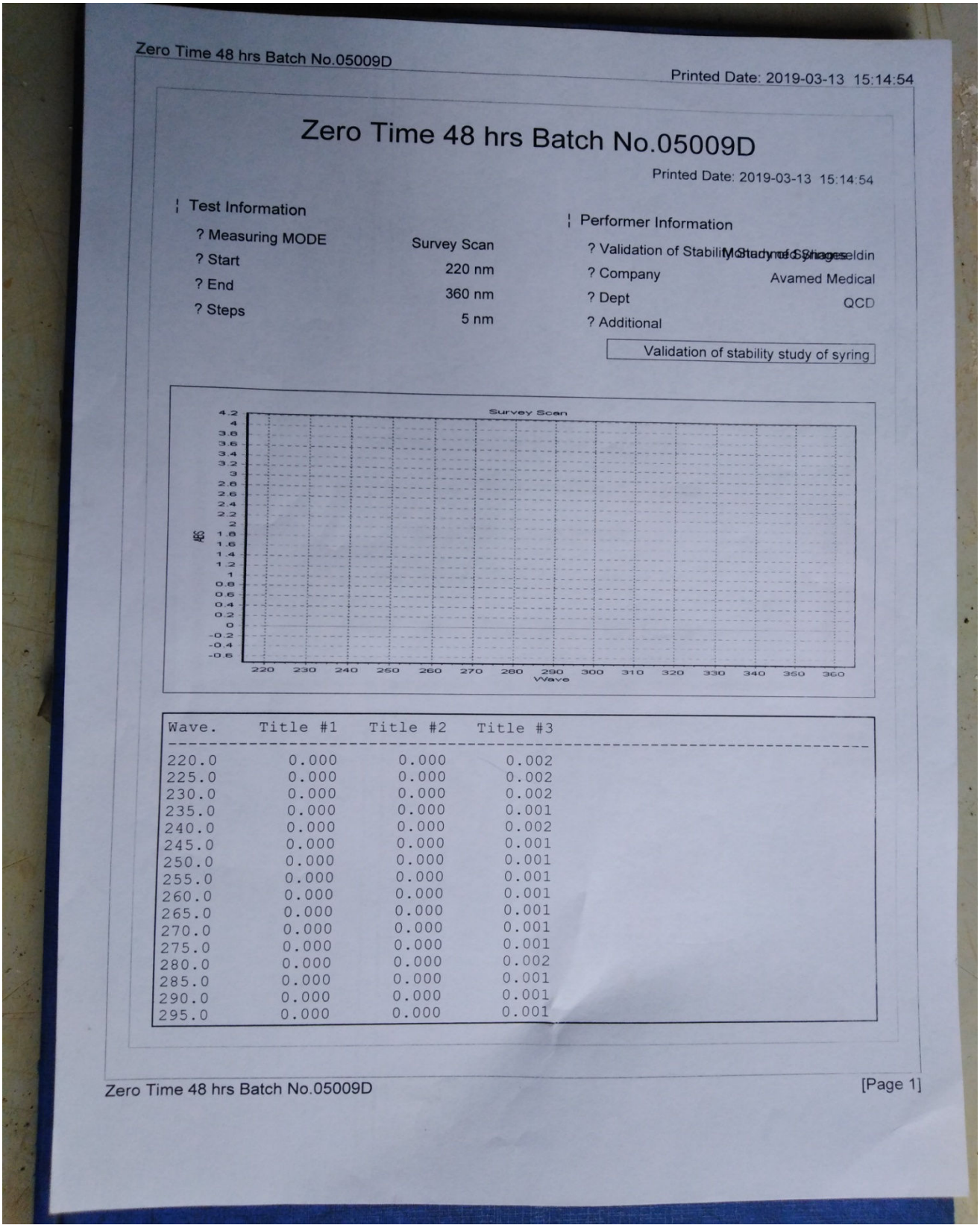


Figure: 5.1.2 LAL Pyrotell.



Zero Time 48 hrs Batch No.05009D

Printed Date: 2019-03-13 15:14:54

Zero Time 48 hrs Batch No.05009D

Printed Date: 2019-03-13 15:14:54

Test Information

? Measuring MODE	Survey Scan
? Start	220 nm
? End	360 nm
? Steps	5 nm

Performer Information

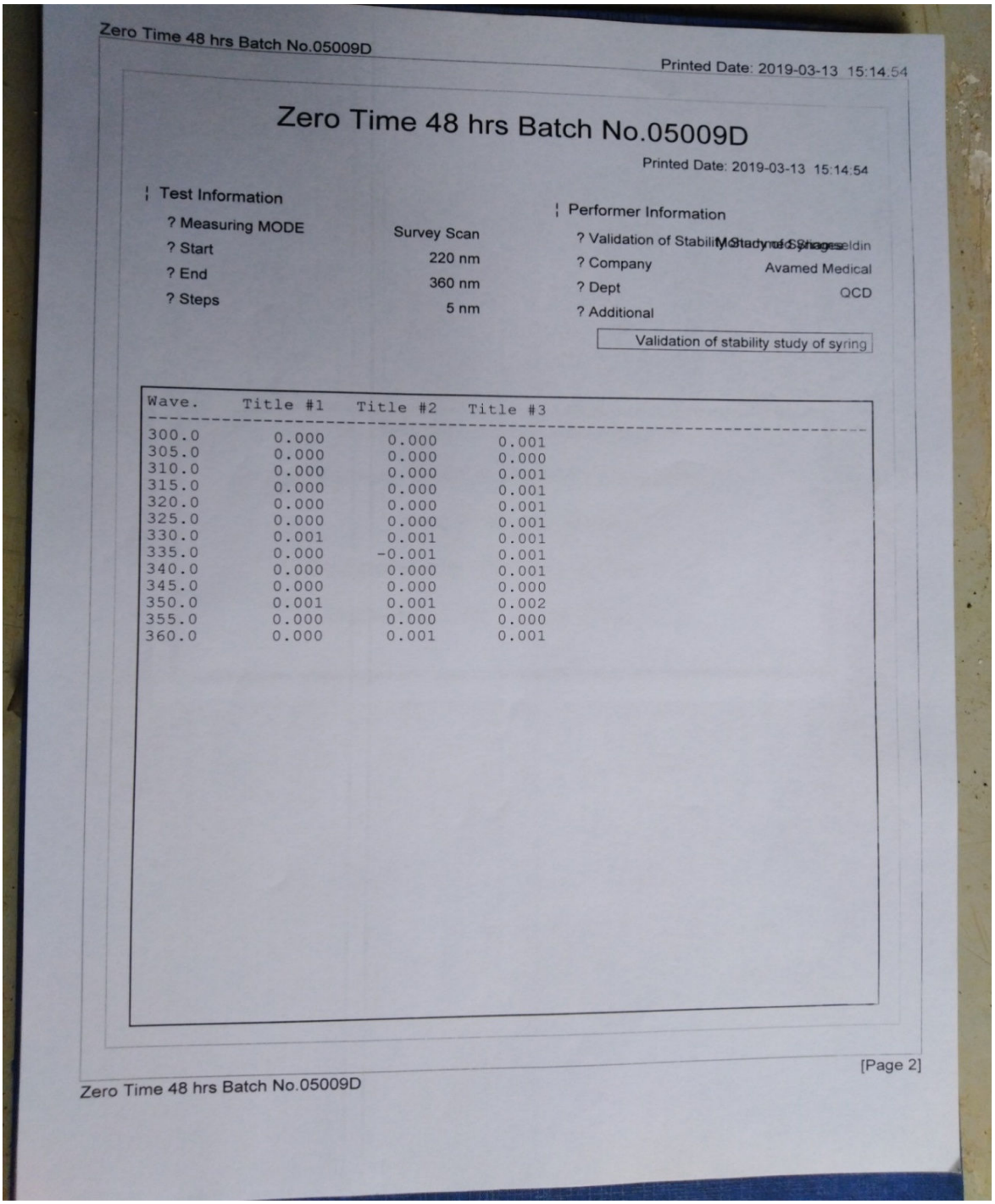
? Validation of Stability Study of Syringes	Mohamed Shageeldin
? Company	Avamed Medical
? Dept	QCD
? Additional	

Validation of stability study of syring

Wave.	Title #1	Title #2	Title #3
220.0	0.000	0.000	0.002
225.0	0.000	0.000	0.002
230.0	0.000	0.000	0.002
235.0	0.000	0.000	0.001
240.0	0.000	0.000	0.002
245.0	0.000	0.000	0.001
250.0	0.000	0.000	0.001
255.0	0.000	0.000	0.001
260.0	0.000	0.000	0.001
265.0	0.000	0.000	0.001
270.0	0.000	0.000	0.001
275.0	0.000	0.000	0.001
280.0	0.000	0.000	0.002
285.0	0.000	0.000	0.001
290.0	0.000	0.000	0.001
295.0	0.000	0.000	0.001

Zero Time 48 hrs Batch No.05009D [Page 1]

Figure UV Spectral of sample solution (S) at zero time.



Zero Time 48 hrs Batch No.05009D

Printed Date: 2019-03-13 15:14:54

Zero Time 48 hrs Batch No.05009D

Printed Date: 2019-03-13 15:14:54

Test Information

? Measuring MODE	Survey Scan
? Start	220 nm
? End	360 nm
? Steps	5 nm

Performer Information

? Validation of Stabili	Study of Syringeldin
? Company	Avamed Medical
? Dept	QCD
? Additional	

Validation of stability study of syring

Wave.	Title #1	Title #2	Title #3
300.0	0.000	0.000	0.001
305.0	0.000	0.000	0.000
310.0	0.000	0.000	0.001
315.0	0.000	0.000	0.001
320.0	0.000	0.000	0.001
325.0	0.000	0.000	0.001
330.0	0.001	0.001	0.001
335.0	0.000	-0.001	0.001
340.0	0.000	0.000	0.001
345.0	0.000	0.000	0.000
350.0	0.001	0.001	0.002
355.0	0.000	0.000	0.000
360.0	0.000	0.001	0.001

[Page 2]

Zero Time 48 hrs Batch No.05009D

Figure UV Spectral of sample solution (S) at zero time.

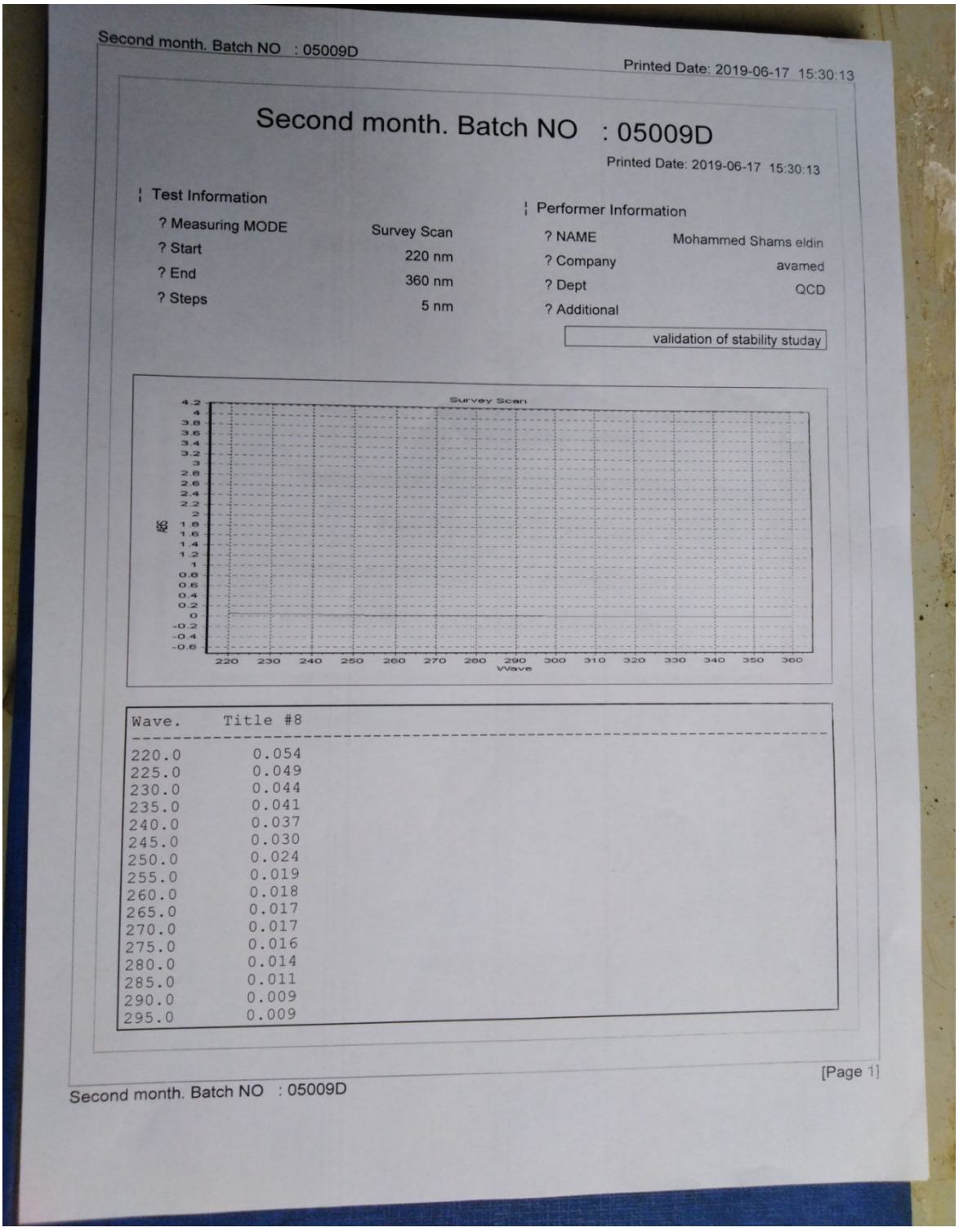
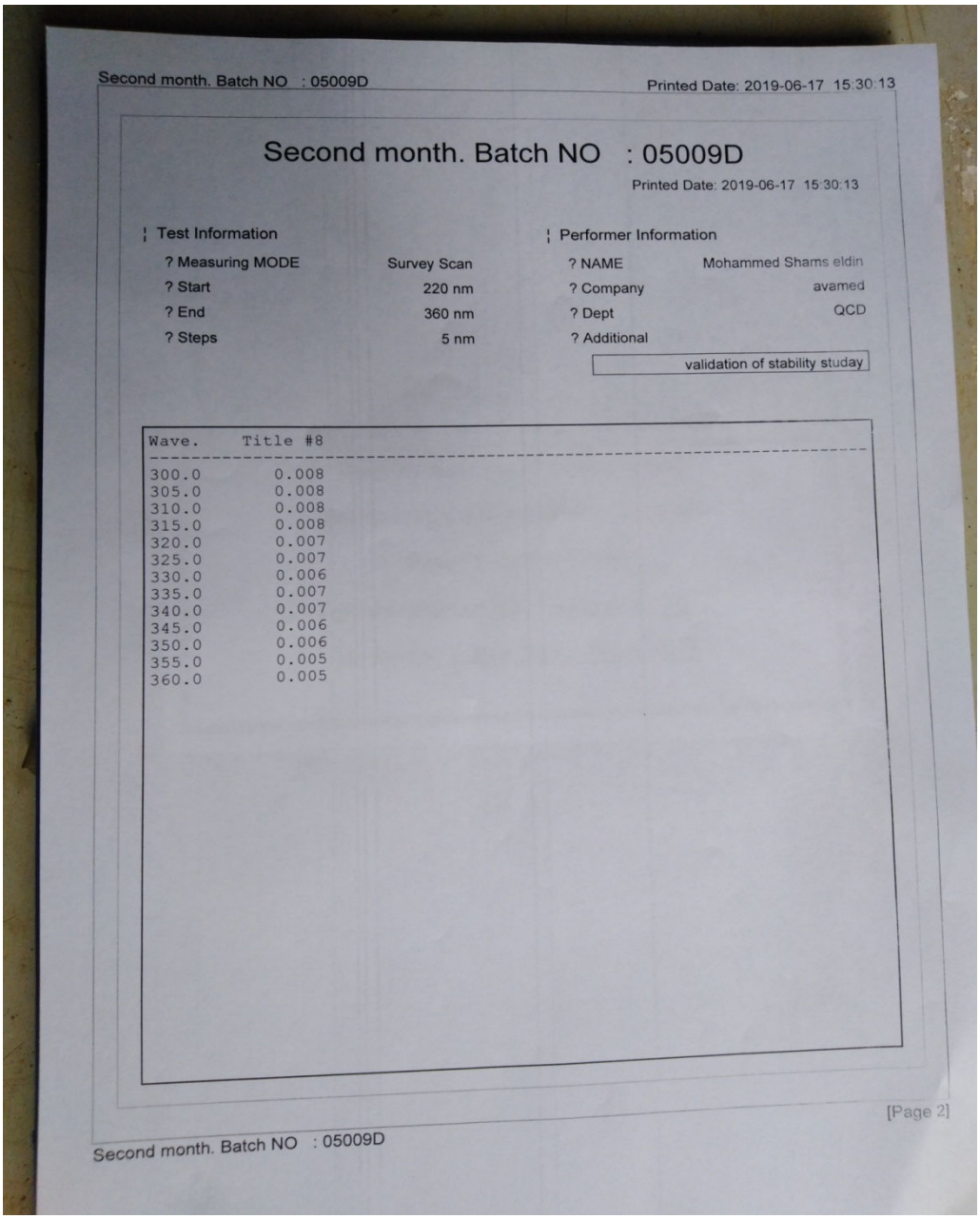


Figure UV Spectral of sample solution (S) after second month



Second month. Batch NO : 05009D

Printed Date: 2019-06-17 15:30:13

Second month. Batch NO : 05009D

Printed Date: 2019-06-17 15:30:13

Test Information		Performer Information	
? Measuring MODE	Survey Scan	? NAME	Mohammed Shams eldin
? Start	220 nm	? Company	avamed
? End	360 nm	? Dept	QCD
? Steps	5 nm	? Additional	validation of stability studay

Wave.	Title #8
300.0	0.008
305.0	0.008
310.0	0.008
315.0	0.008
320.0	0.007
325.0	0.007
330.0	0.006
335.0	0.007
340.0	0.007
345.0	0.006
350.0	0.006
355.0	0.005
360.0	0.005

[Page 2]

Second month. Batch NO : 05009D

Figure UV Spectral of sample solution (S) after second month

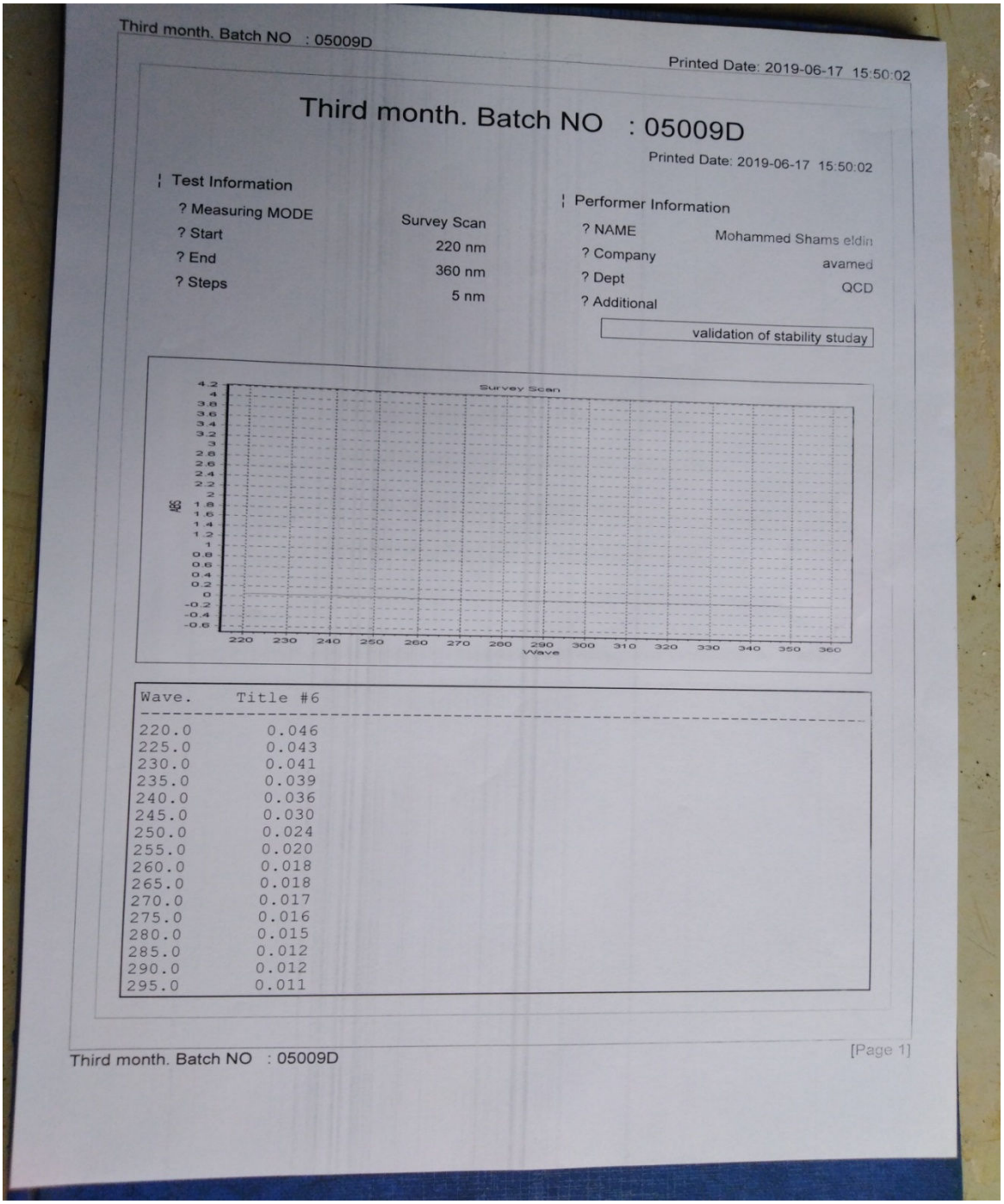
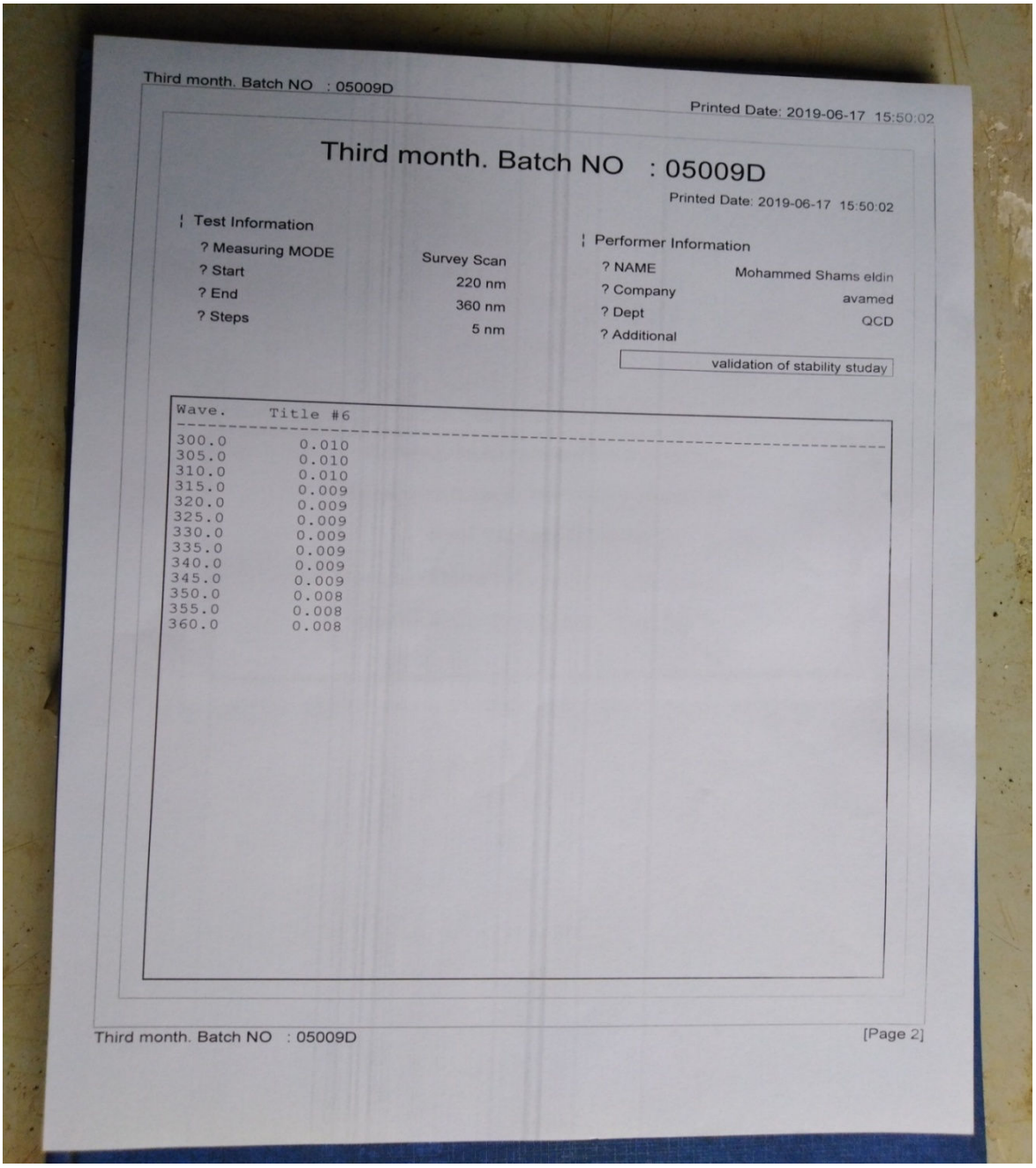


Figure UV Spectral of sample solution (S) after third month



Third month. Batch NO : 05009D

Printed Date: 2019-06-17 15:50:02

Third month. Batch NO : 05009D

Printed Date: 2019-06-17 15:50:02

Test Information

? Measuring MODE	Survey Scan
? Start	220 nm
? End	360 nm
? Steps	5 nm

Performer Information

? NAME	Mohammed Shams eldin
? Company	avamed
? Dept	QCD
? Additional	validation of stability studay

Wave.	Title #6
300.0	0.010
305.0	0.010
310.0	0.010
315.0	0.009
320.0	0.009
325.0	0.009
330.0	0.009
335.0	0.009
340.0	0.009
345.0	0.009
350.0	0.008
355.0	0.008
360.0	0.008

Third month. Batch NO : 05009D [Page 2]

Figure UV Spectral of sample solution (S) after third month.

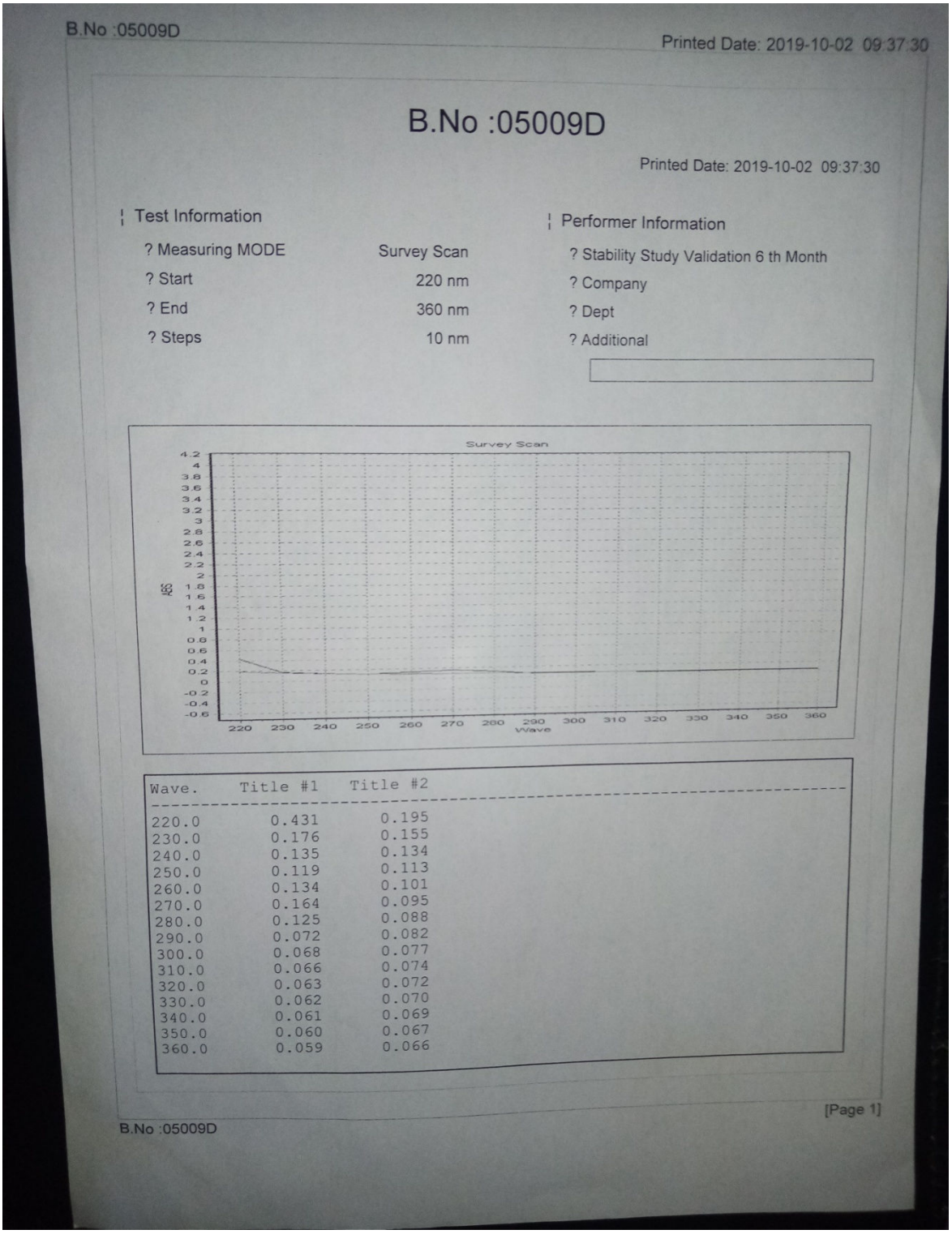


Figure UV Spectral of sample solution (S) after six month.

References

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