INDUSTRIAL ATTACHMENT

(Horticulture Work Experience)

(HMP – 400)

Himalaya™

WELLNESS SINCE 1930

Carried out at

Himalaya Drug Company, Clemment town, Dehradoon

Under Supervision of

Dr. Z Mahmood, Sr. Manager Q.A/Q.C

(19-10-2015 to 30-11-2015)


Himalaya
HERBAL HEALTHCARE

ACKNOWLEDGMENT

- The Work embodied in this project was carried out at laboratory of Himalaya Drugs Company,Dehradun and a well known herbal industry. I take this opportunity to express my deep sense of gratitude to **Dr. S. Farooq**. President of Dehradun Unit for providing the opportunity to avail all necessary facilities towards the completion of this project.
- I express my deepest gratitude and veneration to Dr. Zafar Mahmood, Senior Manager of QC/QA Department for the skillful discussion and Mr. Akbar Ali for providing their valuable guidance, continuous motivation and genuine suggestion. I also express my sincere thanks to Miss shazia and Miss HumaKhan for their contribution in the completion of my project work.
- I thank to the management of the Himalaya Drug Company, Dehradun who provided me with this great opportunity for working and preparing this project report. I also my sincere gratitude towards Dr. Ajaya Paliwal,Dr. SC Pant and college staff to provide academic help on every step and special thank and deepest gratitude is given to **Proff. B.P Nautiyal**, Dean college of horticulture, bharsar for providing us opportunity to work in this globally known industry.
- I thank my family, who have believed in my work and let us use so much time, which otherwise I would have spent with them.

RESEARCHING NATURE • ENRICHING LIFE • SINCE 1930

THE HIMALAYA DRUG COMPANY
Makali Post, Bangalore - 562 123
Karnataka, India

Phone:
+91 80 2839 4201
+91 80 2839 4202
+91 80 2839 4203

Facsimile:
+91 80 2839 4204

Website:
www.himalayahealthcare.com

C.S.T. No. DD 5892126 DT. 1-4-80
U.A.S.T. No. DD 0148151 DT. 1-4-80

Himalaya
HERBAL HEALTHCARE

To Whom It May Concern

This is to certify that **Mr. Arvind Sharma** has worked as a trainee in our Quality Control Department from 20.10.2015 to 30.11.2015.

He was always enthused to work and completed all project assignments during the training period with great dedication.

We wish him all the success in his future career.

(Dr. Z. Mehmood)
Sr. Manager QA/QC

December 01, 2015

INTRODUCTION

Industrial attachment is a component of horticulture work experience . it consists 30 working days for which students may approach industrial units as per their interest to get hands on training and understanding of various aspects of industrial activities. They have accept the work offered by the Units as per their terms and conditions.

Industrial attachment was introduced to inspire the students with practical technical skill as a partial fulfillment award of bachelor degree and to introduced the students into working life.

It provide students with valuable insights

Objectives

- . To develop skills in the application of theory to practical work situations.
- To develop skills and techniques directly applicable to their careers.
- Internships will increase a student's sense of responsibility and good work habits.
- To expose students to real work environment experience gain knowledge in writing report in technical works/projects
- . To enhance the ability to improve students creativity skills and sharing ideas.
- To build the strength, teamwork spirit and self-confidence in students life.
- The student will be able instilled with good

COMPANY PROFILE

The Himalaya Drug Company was founded in **1930 by Mr. M. Manal** Eighty one years ago, on a visit to Burma, Mr. Manal saw restless elephants being fed with a root to pacify them. The plant from which this was taken is ***Rauwolfia serpentina***. Fascinated by the plant's effect on elephants, he had it scientifically evaluated. After extensive research, **Serpinaâ,** the world's first anti-hypertensive drug, was launched in 1934.

In 1955 Himalaya introduced **Liv52**, a liver formulation that ensures option liver function . The product soon become Himalaya flagship brand herbal medicine.

Today. Himalaya product have been endorsed by 400.000 doctor around the globe and consumer 90 countries rely on Himalayas for their health and personal care needs. Starting off operations in Dehradun way back in the 1930s, the company later spread its wings to Mumbai and across the country. In 1975, the company set up an advanced manufacturing facility in Makali, Bangalore, India. In 1991, the company relocated its R&D facility to Bangalore. Himalaya Global Holdings Ltd. (HGH) is the global headquarters of all Himalaya subsidiaries.

Some of the products manufactured in Himalaya Drug Company

Global Appearance of Himalaya

EMPLOYEE STRENGTH

- 5000 (INCLUDING HEADCOUNT)

ANNUAL TURNOVER OF HIMALAYA

- 2008- INR 300 crore
- 2015-INR 1500 crore
- Target 2020- INR 3000 crore(domestic market)

% SHARE OF DIFFERENT PRODUCTS IN TOTAL BUSINESS

- Pharmaceuticals – 38%
- Personal care products- 38%
- Baby care- 8%
- Animals health care- 4%
- Overseas business – 12%

RESEARCH AND DEVELOPMENT AT BANGLURU BASED UNIT

- Himalaya conduct at least 10years of research before introducing any new drug or a product
- There are 270 scientists engaged in R&D work
- At least 80 new products are in research pipeline
- It has advanced stage of conducting clinical trials in the area of oncology, type-ii diabetes and women's disorders,

QUALITY CONTROL

This age of cutthroat competition and large scale production, only that manufacturer can survive who supplies better quality goods and renders service to-the consumers. In fact quality control has become major consideration before establishing an industrial undertaking. Proper quality control ensures most effective utilization of available resources and reduction in cost of production.

Objective

- To establish the desired quality standards which are acceptable to the customers?
- To discover flaws or variations in the raw materials and the manufacturing processes in order to ensure smooth and uninterrupted production.
- To evaluate the methods and processes of production and suggest further improvements in their functioning.
- To study and determine the extent of quality deviation in a product during the manufacturing process.
- To analyse in detail the causes responsible for such deviation.
- To undertake such steps which are helpful in achieving the desired quality of the product.

ANALISIS OF VARIOUS MATERIAL IN QC LAB

- **SAMPLES**

RAW MATERIALS	POWDER	GRANULE	EXTRACT	OIL
Description	Description	Description	Description	Description
LOD	LOD	LOD	TS	RI
Sieve analysis	BD	BD	UDS	pH
BD	pH	pH	TDS	Acid value
pH	Total ash	Total ash	Active marker	Iodine value
Total ash	Active marker	Active marker	Ph	TVAC
Active marker	TLC fingerprints	TLC fingerprints	TLC fingerprints	
TLC fingerprints	Heavy metal	Heavy metal	Heavy metal	
			TVAC ,TVMC ,	
			Pathoge	

- **SAMPLES DISTRIBUTED TO DIFFERENT SECTIONS**

ANALYTICAL LAB	INSTRUMENTATION LAB	MICROBIOLOGY LAB
Ph	HPLC	TVAC
G|F ratio	HPTLC	TYMC
LOD	UV	Pathogen
Ash content	ICP-MS	
BD	AAS	
RI		
TS		
UDS		
Specific gravity		

PHARMACOGNOSY

- **Pharmacognosy** is the study of medicinal drugs derived from plants or other natural sources. The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the from natural sources It is also defined as the study of crude drugs.
- **Introduction** **T**he term comes from two Greek words: "pharmakon" meaning drug or medicine, and "gnosis" meaning knowledge. The defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drug from natural sources". The term "pharmacognosy" was used for the first time by the Austrian physician Schmidt in 1811 and 1815 by Crr. Anotheus Seydler in a work titled *Analecta Pharmacognostica*.

- Originally—during the 19th century and the beginning of the 20th century—"pharmacognosy" was used to define the branch of or commodity sciences (*Warenkunde* in German) which deals in their crude, or unprepared, form. are the dried, unprepared material of plant, animal or mineral origin, used for medicine. The study of these materials under the name *pharmakognosie* was first developed in German-speaking areas of Europe, while other language areas often used the older term *materia medica* taken from the works of .In German the term *drogenkunde* ("science of crude drugs") is also used synonymously.
- As late as the beginning of the 20th century, the subject had developed mainly on the botanical side, being particularly concerned with the description and identification of drugs both in their whole state and in powder form. Such branches of pharmacognosy are still of fundamental importance, particularly for pharmacopoeial identification and quality control purposes,

Parameters of Pharmacognosy

- Macroscopic Study
- Shape
- Size
- Color
- Odor
- Taste
- Foreign matter

- Microscopic Study
- Histology(Arrangement of tissue in TS&LS)

- Analytical Study
- Total Ash
- Acid insoluble ash
- pH value
- Alcohol soluble extractive
- Water soluble extractive

Plants Chosen for Pharmacognosy

- *Rauwolfia serpentina* Arvind Sharma
- *Solanum surattense* Shikha
- *Asparagus racemosus* Babita Bahuguna

MATERIALS AND METHODS

- MATERIALS REQUIRED
- **Table no.1:** Following materials used in the experimental study.

S. No.	CHEMICALS	SOURCE
1	Alcohol	Changshu hongsheng fine chemical Co.Ltd.
2	Chloroform	Merck specialities Pvt.Ltd.
3	Ammonia	Merck specialities Pvt.Ltd.
4	Iodine	Himedia laboratories Pvt.Ltd.
5	Hydrochloric Acid	Merck specialities Pvt.Ltd.

- EQUIPMENTS REQUIRED
- **Table No. 2:** Following equipment used in study.

S. No.	EQUIPMENTS	SOURCE
1	Rectangular (12 hole) water bath	Navang Scientific work Pvt. Ltd.
2	Hot air oven	Ambassader
3	Muffle furnace	Ambassader
4	Wrist action shaker	-
5	pH meter	LABINDA

Methods

- **Microscopic-** the plant sample was dipped in alcohol (70%) and kept for few days for softening. Then a thin piece of sample was taken and very fine section was cut using a sharp blade.the cut section was soaked for few minutes in watch glass containing safranine solutionfor staining.then the section was transferred to glass slide and 1-2 dropes of glycerine was dropped over it. Lastly the slide was observed under microscope
- **Determination of Total Ash**
- Incinerate about 2 to 3g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450degree until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust Calculate the percentage of ash with reference to the air-dried drug.

- **Determination of Acid-Insoluble Ash**
- Boil the ash obtained in for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or on as cashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.
- **Determination of Water-Soluble Ash**
- Boil the ash or 5 minutes with 25ml of water; collect insoluble matter in a Gooch crucible, or *on an* ashless filter paper, wash fir 15 minutes at a temperature not exceeding 450degree. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

- **Determination of Alcohol-Soluble Extractive**
- Macerate 5g of the air dried drug, coarsely powdered, with 100ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105degreem to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.
- **Determination of Water-Soluble Extractive**
- Proceed as directed for the determination of Alcohol-soluble extractive, using chloroform water instead of ethanol.

- **Determination of alkaloid**
- Take 10g sample in 500ml iodine flask
- Add solvent mixture 200ml to it (diethyl ether 483ml chloroform 180ml ethanol 52.5ml)
- Add 7ml of dilute ammonia shake it for 1hr
- Keep flask overnight add 10ml purified water next day and filter it through cotton plug 100ml filtrate
- Extract with successive quantities 35ml, 25ml , 20ml sulfuric acid.
- Reject the upper layer and collect the bottom layer transfer it to clean separating funnel
- Add 10 ml concentrated ammonia
- fractionate the alkaline solution with chloroform in successive quantities of 35ml, 25ml, 20ml, in separating funnel reject the upper layer and collect the chloroform layer transfer it to clean separating funnel
- add 10ml distilled water shake slightly
- filter chloroform layer with wattsman no. 1 filter paper into a weighed dish .
- allow evaporation of chloroform on water bath flask containing residue keep in oven 105^0c for 30 min
- calculate alkaloid content.

Result and description
Rauwolfia serpentina

- **Macroscopic** – Roots are sub cylindrical to tapering, tortuous or curved, rarely branched. Occurs as segments usually from 5to15 cms in length and 3to 20 mm in diameter. Externally grayish yellow to brown, wood pale yellow. Roots tough with longitudinal marking S slightly wrinkled surface. When scraped, bark separates readily form the wood. Fracture is short and irregular.
- **Microscopic** –In transverse section, cork cells in 2to8 alternation bands of radically narrow and broader cells, thin, lignified up to 75 um in tangential width, broader cells up to about 90 um in radial length, phelloderm, tangentially elongated to isodimetric parenchyma cells containing starch and short latex cells with brown resinous matter secondary cortex consists of parenchyma cells, heavily packed with starch grains secondary phloem contains phloem parenchyma and sieve elements, parenchyma contains starch and angular crystals of calcium oxalate 3to20 um in length. Xylem is about 4/5 of the diameter of the root, wood is transverse by medulla rays 1to5 cells in width. Xylem consists of essay tracheas S wood fibers. Xylem

Results of physicochemical parameters (*Rauwolfia serpentina*)

S. no.	Parameters	Specification	Results
1	Total ash	Not more than 8%	3.31%
2	Acid insoluble ash	Not more than 2%	.367%
3	Alcohol soluble extractive	Not less than2%	6.8%
4	Water soluble extractive	Not less than 5%	23.192%
5	Alkaloid content		0.41%
6	pH	6.5-7.5	6.7

METHODS

- **Microscopic**
- Collection of plant material and cut in a small pieces, dipped in 70% alcohol until they become soft. When they become soft cut in fine and thin section and dipped in a iodine dye or safranin dye for clear vision, take fine section in a slide and observe under microscope.
- Analytical
- **Determination of Total Ash**
- Incinerate about 1g accurately weighed,of the sample in a crucible at a temperature not exceeding 450°c until free from carbon,cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450 °c.
- **Determination of Acid-Insoluble Ash**
- Boil the ash obtain from total ash for 5 minute with 25ml of dilute Hydrochloric acid, collect the insoluble matter in crucible or on a ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

- **Determination of Alcohol- Soluble Extractive**
- Weigh about 5g of the sample with 100ml of Alcohol of the specified strength in a closed flask for 24 hours, shaking frequently during 6 hour and allowing to stand for eighteen hours. Filter rapidly, taking precaution against loss pf solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish and at 105 °c. to constant weight. Calculate the percentage of alcohol soluble extractive with reference to the air dried drug.
- **Determination of water soluble extractive**
- Weigh about 5g of the sample with 100ml water in a closed flask for 24 hours, shaking frequently during 6 hour and allowing to stand for eighteen hours. Filter rapidly, taking precaution against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish and at 105 °c. to constant weight. Calculate the percentage of water soluble extractive with reference to the air dried drug.
- **Determination of moisture content (Loss on Drying)**
- Weigh about 1g sample in a loss on drying bottle and kept in oven at 105 °c for one hour. Calculate the moisture content with reference to the air dried drug.

- **Determination of alkaloid**
- **Reagent**
- Solvent mixture: Measure 322ml of solvent ether and add 120ml of chloroform water and 35ml ethyl alcohol.
- Dilute ammonia : Measure 40ml ammonia and make a volume 100ml
- Dilute Sulphuric acid: Measure 14ml H_2SO_4 and make volume 1000ml
- **Procedure –**
- Weigh about 10g sample
- Add 200ml of solvent mixture and 6ml of dilute ammonia.
- Shake for a 1hr in shaker leave for overnight.
- Next day add 10ml water and filter it
- Take a 100ml filtrate sample in a separating funnel by adding dilute H_2SO_4 and shake it separate the layer, this process repeat three times by adding 35ml, 25ml, 20ml, dilute H_2SO_4 and separate the layer.
- Take separated material in a separating funnel by adding chloroform and shake it separate the layer, this process repeat three times by adding 35ml, 25ml, 20ml chloroform and separate the layer.
- Take separated material and add 10ml distilled water in a separating funnel and filter the separated material by filter paper no.1
- Filtrate material kept in a water bath at 80-100 °c for evaporate solvent.
- After evaporate the solvent sample kept in oven for 1hr
- After 1hr whenever it comes in room temperature take a weight
- Calculate the alkaloid percentage.

OBSERVATIONS AND RESULTS *Solanum surattense*

- In this project work we were studied Pharmacognostical studies on Solanum surrattence under different parameters, like microscopic, physiochemical analysis
- **Macroscopic-** Diffuse herb with prickly stem, leaves and calyx. Root almost cylindrical and tapering. Fracture , short. Test bitter with no characteristic odor .Leaves ovate oblong, acute, pinnately 7-11 lobed sparsely stellate pubescent. Odour and test not distinct. Stem nodes and internode prominent. Fracture short to slightly fibrous. Flower purple in few flowered axillary cymes. Fruit a glabrous, globular berry, green and white stripes when young, yellow when mature. Seeds smooth compressed, reniform. Taste bitter.
- **Microscopic-** Transverse section of stem consists of layer of cork of thin walled somewhat rectangular cell; epidermis remains intact for a long time; secondary cortex consists of layer of parenchymatous cells. Vascular bundles radially arranged. Central region occupied by large pith

Physicochemical parameter

- Table no. 1- Results of physicochemical parameter of Solanum surrattence

S.No.	Parameters	Specifications	Results
1	Foreign matter	Not more than 2%	1%
2	Total ash	Not more than 9%	8.98%
3	Acid – insoluble ash	Not more than 3%	2.417%
4	Alcohol – soluble extractive	Not less than 6%	5.897%
5	Water – soluble extractive	Not less than 16%	23.1793%
6	pH value	_	6.02
7	Moisture content	_	2.3469%

- Active marker

1	Alkaloid	-	.26%

Methods (*Asparagus racemosus*)

- **Microscopic-** the plant sample was dipped in alcohol (70%) and kept for few days for softening. Then a thin piece of sample was taken and very fine section was cut using a sharp blade.the cut section was soaked for few minutes in watch glass containing safraninesolutionforstaining.then the section was transferred to glass slide and 1-2 dropes of glycerine was dropped over it. Lastly the slide was observed under microscope.

Physicochemical parameters *Asparagus racemosus*

- **Determination of total Ash**
- Incinerate about 2 to 3g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450^0c until free from carbon, cool and weight. If a carbon free ash can not be obtained in this way,exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness,and ignite at a temperature not exceeding 450^0c
- **Determination of acid insoluble ash**
- Boil the ash obtain from total ash for 5 minutes with 25ml o dilute hydrochloric acid collect the insoluble matter in crucible, or on an ashless filter paper, wash with hot water and ignite to constant weight .calculate the percentage o acid insoluble ash.

- **Determination of water-soluble ash**
- Boil the ash obtain from total ash for 5 minutes with 25ml of water collect the insoluble matter in crucible, or on an ashless filter paper, wash with hot water and ignite to constant weight .calculate the percentage o water soluble ash.
- **Determination of alcohol soluble extractive**
- Macerate 5g air dry drug, with 100ml alcohol shaking frequently during 6hr and allowing to stand or 18hr. filter rapidly, evaporate 25ml of the filtrate to dryness and dry at 105^0c, to constant weight and weigh. Calculate the percentage of alcohol soluble extractive.

- **Determination of water soluble extractive**
- Macerate 5g air dry drug, with 100ml chloroform water. I shaking frequently during 6hr and allowing to stand or 18hr. filter rapidly, evaporate 25ml of the filtrate to dryness and dry at 105^0c, to constant weight and weigh. Calculate the percentage of water soluble extractive.
- **Determination of moisture content**
- Weigh 1g sample in a loss on drying bottle and kept in hot air oven for 1hr at 105^0c, to constant weight and weigh.

- **Determination of Active marker**
- Weigh 5g sample
- Add 50ml alcohol (90%)
- Reflex the sample in water bath at 80^0c temperature for 30min
- After reflex filter the sample in conical flask and evaporate
- At room temperature add 25ml petroleum ether and kept in water bath or 30min
- After that add 25 ml chloroform and kept in water bath or 30min after that remove the chloroform
- Than add 25ml ethyl acetate and kept in water bath for 30 min and remove ethyl acetate
- And then kept in oven or 30 min after that add 25mi methanol (90%) and kept in sonicater for dissolving and filter it
- After that add 125ml acetone and shake by glass rod and kept for overnight after that separate the Saponin and calculate the % of saponin

Result and description

Asparagus racemosus

- Root tuberous, 10 to 30cm in length and 0.1to0.5cm thick, tapering at both endswith ends with longitudinal wrinkles, colour cream, taste sweetish.
- **Microscopic:**
- An outer layer of piliferouscells,ruptured at places,composed of small,thinwalled,rectangular asymmetrical cells,a number of cells elongated to form unicellular root hairs.cortex comprises of 25 to 29 layers,distinct in two zones,outer and inner cortex; outer cortex consists of 6 or 7 layers, compactly arranged, irregular to polygonal ,thick walled ,lignified cells; inner cortex comprise of 21 to 23 layers,oval topolygonal, thin walled, tangentially elongated cells with intercellular spaces; stone cells ,either singly or in groups, from a discontinuous to continuous ring in the upper part of this region .endodermis composed of thin walled parenchymatous cells. Xylem consist of vessels, tracheidsandparenchyma.pith composed of circular to oval parenchymatous cells.

Results of physicochemical parameters (*Asparagus racemosus*)

S. no.	Parameters	Specification	Results
1	Total ash	Not more than 5%	5%
2	Acid insoluble ash	Not more than 0.5%	0.5%
3	Alcohol soluble extractive	Not less than10%	12.2208%
4	Water soluble extractive	Not less than 45%	49.4361%
5	Moisture content		3.55%
6	pH		5.94

Working in Instrumentation Lab.

- HPTLC (Make : CAMAG) : Is an enhanced form of (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements.

- Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. One recent approach to automation has been the use of piezoelectric devices and inkjet printers for applying the sample

- The spot capacity (analogous to peak capacity) can be increased by developing the plate with two different solvents,. The procedure begins with development of sample loaded plate with first solvent. After removing it, the plate is rotated 90° and developed with a second solvent.

SCHEMATIC PROCEDURE FOR HPTLC METHOD DEVELOPMENT

Sample and standard preparation

Selection of chromatographic layer

Layer pre-washing

Layer pre-conditioning

Linomat 5 Applicator

Application of sample and standard

Chromatographic development

Twin trough Chamber

Detection of spots, scanning and documentation

Scanner

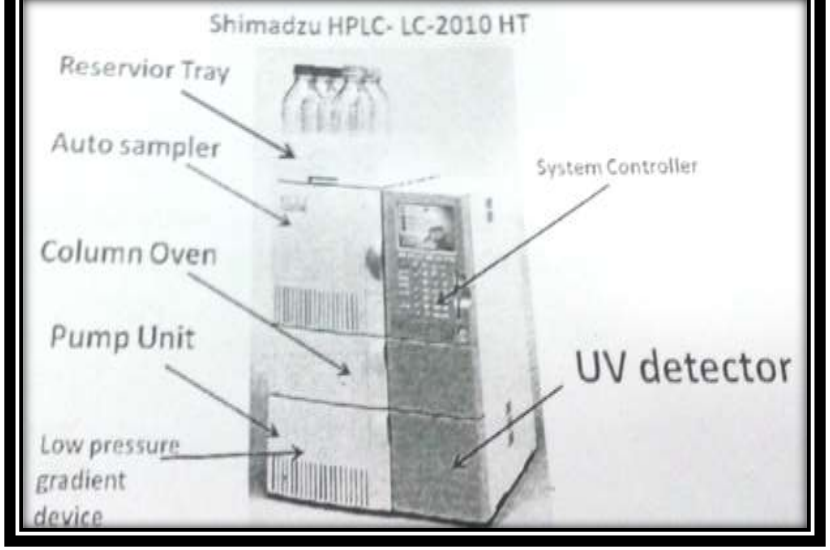
Photo documentation with digital camera

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) Make : SHIMADZU

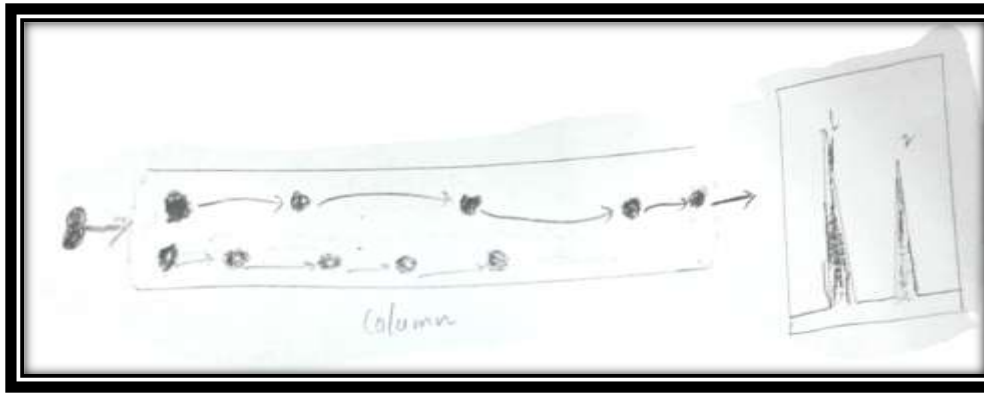
- **High-performance liquid chromatography** (sometimes referred to as high-pressure liquid chromatography),HPLC, is a chromatography technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying and purifying the individual components of the mixture. HPLC is also considered an instrumentation technique of analytical chemistry, instead of a gravimetric.

- HPLC relies on the pressure of mechanical pumps on a liquid solvent to load a sample mixture onto a chemistry column , in which the separation occurs .A HPLC separation column is filled with solid particles (e.g. silica,polymers or sorbents) and the sample mixture is separated into compounds as it interacts with the column particles.HPLc separation is influenced by the liquid solvents's condition (e.g. pressure ,temperature),chemical interactions between the sample mixture and the liquid solvent(e.g. hydrophobicity,protonation etc) and the chemical interactions between the sample mixture and the solid particles packed inside of the separation column (e.g. ligand affinity ,ion exchange etc…)

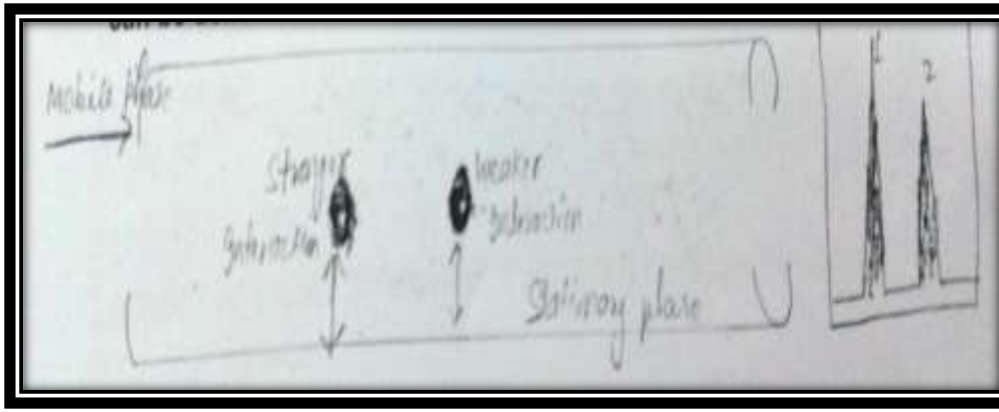
- The main purpose of chromatography is to separate and quantify the target sample in the matrix.



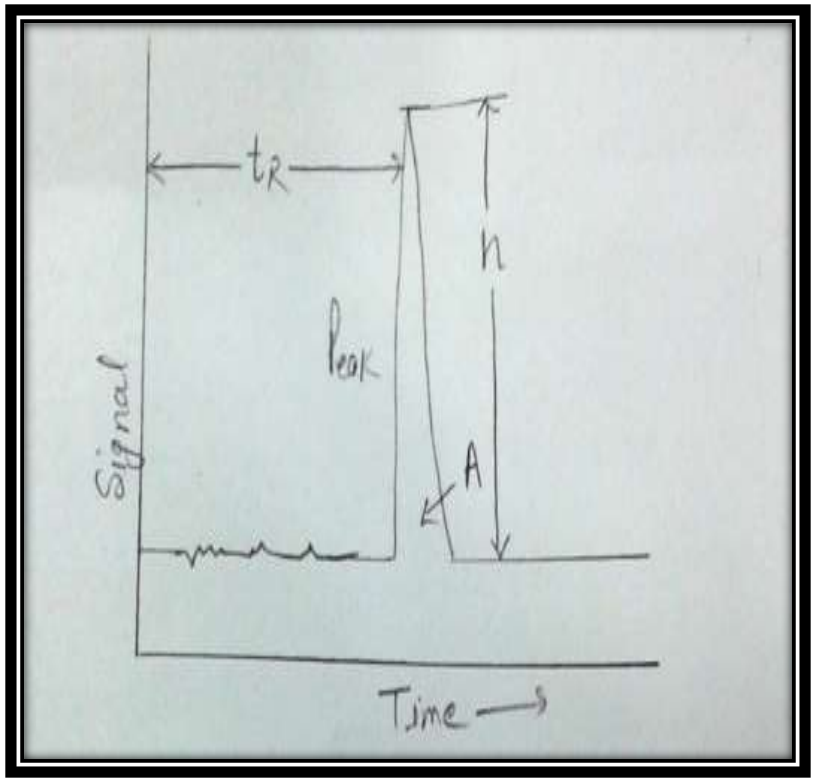
- **Separation mechanism**
- Compounds are separated because the molecules move at different rates in the column



- Due to Duedifferent interaction between stationary phase and different sample, the molecules moves at different rate, therefore separation can be done Chromatogram



- A plot of detector signal output versus time or elution volum
- Tr retention time: the time taken by the analyte peak to reach the detector after sample injection
- A : area
- H height
- **Qualitation :** an analysis processwhich is design to identify the components of a substance or mixture.
- **Quantitation :** an analysis process which is designed to determine the amounts
- Proportion of the components of a substance



TISSUE CULTURE

- Plant tissue culture is the propagation of plants through "cloning" an asexual method of reproduction. A portion (explant) of a desired plant is cultured in vitro ("glass") on a defined medium, which promotes rapid multiplication of cells. The new plants are removed from the culture and transferred to a standard potting medium.
-
- Tissue culture is based on the theory of totipotency; that is, the genetically based ability of a nonembryonic organ or cell to develop along a pathway similar to that of a zygote, leading to the formation of a new entire plant identical to the original. Currently, tissue culture is being used in both research and commercial applications. Tissue culture not only provides a method of mass propagation, but also makes possible the production of disease-free plants, mutants, and secondary plant products. A new and important use is in the genetic engineering of plants.
-

- **STAGE I PREPARATION OF EXPLANT**
- Select the younger leaves near the center of the plant, as their cells will be more likely to have retained their totipotency. Remove the young leaves, leaving a length of petiole (leaf stalk) attached to each.
-
- 2.) Disinfestations process: Sterilize the leaves by immersing them into the 10% bleach (sodium hypochlorite) solution to which one drop of dish washing detergent has been added. Put the top on and shake the solution with the leaves in it for 10 minutes. (The detergent acts as a wetting agent and allows the entire surface of the leaf will be exposed to the sodium hypochlorite. This process will remove surface contaminants such as: exterior bacteria, fungi, spores, mites, or small insects.)
-
- 3.) Make sure all items that will be needed are sprayed with ethanol and placed in the sterile area.
-
- **STAGE II MULTIPLICATION**
- In a few weeks small pimplelike buds should appear on or near cut surfaces. You may need a microscope to see these structures as they begin to grow, but after 5- 6 weeks they should be visible to the unaided eye. If a sterile environment was not maintained, contamination will be obvious within 3-4 days. Materials contaminated by fungus will have a fuzzy growth on them. Materials contaminated by bacteria will have a slimy growth on them.
-

- **STAGE THREE: ROOTING**
- Once a sufficient number of shoots have been generated, portions of explants that contain one or more shoots could be transferred to a medium that contains a higher concentration of the hormone auxin, resulting in root production.

- 1) In the transfer box, under sterile conditions, remove the para-film and cap from the test-tube and use the forceps to carefully remove the explant from the medium. Place the explant inside a sterile petri dish. Remove the old test tube from the box.
- 2) Using a sterile scalpel carefully remove or cut plantlets away from the explant.
- 3) While maintaining sterile conditions, carefully remove the cap off of the new
- 4) Carefully cap your test tube, write your initials and date on the cap and return them to the rack for 2- 4 weeks under low lights. During this time, the shoots will continue to grow, however, most of the plants energy will be focused into producing roots.

- **STAGE FOUR: ACCLIMATIZATION**
- Once roots are visible, plantlets need to be moved from the medium to soil.
- 1.) Gently wash all medium from the leaves and roots with distilled water
- 2.) Transplant plantlets into small clean pots.

Class by Dr. Mayaram Uniyal

- A globally known herbalist of uttarakhand Dr. Mayaram uniyal shared knowledge of medicinal and aeromatic plants available in himalaya herbal garden.
- He took the classes and shared his research, adminstrative and academic knowledge.
- The plants visited with him was Ashwgandha, Sarpgandha, Shatawari, Geloy, Arjun, Tulsi, Bhangra, Haldi, Zinger, Lemon Grass, Gurbhachh, Tejpaat, Aloevera, Brahmi, Allspice, Thyme, Stevia, Touch Me Not, Jamun, Punarnava etc.

Day by Day Schedule of different work done at Himalaya Drug Ltd. Dehradoon

- 19/10/2015 – reporting at company.
- 20/10/2015 – joining of company and interview with President Dr. S. Farooq.
- 20 /10 - 31/10/2015 – sent us to different laboratories for learning standerd operating procedure (SOPs) of different equipments.
- Boys were sent in analytical lab. And girls in Domestic lab.
- 02/11/2015 – 06/11/2015 – students were exchanged, (boys in domestic and girls in analytical lab.)
- 7/11/2015 – allocation of plants for pharmacognostical studies.
- 7/11/2015- 19/11/2015 – pharmacognostical study on plants like *Rauwolfia serpentina, Solanum surattense, Asparagus racemosus.*

- 20/11/2015- 21/11/2015 - Working in instrumentation lab. It includes high performance liquid Chromatography and High Performance Thin Layer Chromatography.

- 23/11/2015 – 24/11/2015 – working on micropropagation of *Withania somnifera*.

- 25/11/2015-27/11/2015 – classes taken by Dr. Mayaram Uniyal, a renowned herbilist on identifcation, uses and characteristics of different medicinal plants at Himalaya Herbal Garden.

- 28/11/2015 – prepared project report at Himalaya Library and submitted it to Dr. Zafar Mahmood Sr. Manager Qua. Control and Qua. Assurance.

- 30/11/2015 – Visited Himalya Musieum.
- 1/12/2015 – Received Certificate

GLIMPSES OF HIMALAYA PRESIDENT (DOON & DELHI UNIT) DR. S. FAROOQ

CONCLUSION

- We learned about various steps of herbal industry set up and its well functioning. Pharmacognosical study was main part of our work. We learned about quality control and quality assurance of semi finished products. We also came to know about very small but vital things i.e.; instrument handling, cleanliness, glassware washing and maintaining laboratory decorum. The main thing we came to know was that a industry is being recognized not on the base of Capital but how it target on people's heart and it needs continuous maintenance of quality of a product.

Thank You

- www.k8449r.weebly.com
- www.anilrana13014.weebly.com