Hello students, today we're going

to look at Unit 4 chromatography,

model number 11 that is

principle and application HPLC.

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Khandola. So the outline,

HPLC, the principle, working

components and applications.

So the learning outcomes of this will be

the student will be able to understand

the principle of chromatography,

describe the components and working

of HPLC and state its applications.

So liquid chromatography in this, the

sample is loaded on the stationary

phase and separated one by one.

So slow separating technique

performed in vertical columns

and under gravitational flow.

So improvements in liquid chromatography,

increase speed by pumping the

solution through a column at a

pressure of up to more than 10,000

PSI. Versatility by using column

particles of smaller diameter and

surface area and suitable packing

structure provides about 7 times

more resolving power than a normal

liquid chromatographic technique.

This improved technique is known as

high performance liquid chromatography.

This is the machine.

HPLC.

The characteristic features are sensitivity,

ready adaptability to accurate

quantitative determination and

suitability for separating non volatile

species or thermally fragile species.

Rapid results HPLC allows separation

and measurements to be made in minutes.

Other names for this are high

pressure liquid chromatography,

high speed liquid chromatography.

So the working. Solvent reservoir is

attached to a pressure pump which

pumps the mobile phase into the column

through an injector with high pressure.

Molecules are separated.

They are detected by the detector and

peaks are observed on the computer.

Pek is plotted against retention

time an interpreted with the help

of standard plots for identification

of compounds. So that we can see

the flow diagram of this process.

The solvent is first degassed and

then pumped into the column by the

simple injector and then detected

in the form of pictures on the computer.

Solvent reservoir. HPLC contains one

or more glass or steel reservoirs.

Isocratic elution separation by

HPLC employing a single solvent

of constant composition.

Gradient elution two or more

solvent system of significant

differences and polarity are used.

HPLC often consist of devices

which introduce solvents from two or

more reservoir into a mixing chamber.

Reservoirs possess dissolved gases removal.

This means oxygen and nitrogen

interfere by forming bubbles in

the column and detector system,

which lead to spreading of bands.

Bubbles are removed either by heating or

passing the solvent through a multipore

filter and vacuum before introducing

the solvent into the solvent reservoirs.

Components of HPLC. HPLC system

consist of four major components.

Pump, injector, Column and detector.

Delivery pump it delivers a steady

stream of solvent from the reservoir

to the detector through the column.

It should have sufficient pressure, resistant,

Less pulsation and discharge

of constant volume.

Can deliver solvent at a

pressure up to 10,000 PSI with

the flow rate of 50ML per minute.

Components of HPLC.

The types of pumps are reciprocating pump

widely used consist of a small chamber in

which the solvent is pumped by a back and

forth motion of a motor driven piston.

Displacement pump consist of a large syringe,

like a chamber equipped with a plunger which

is activated by a screwdriver mechanism

through a motor. Pneumatic pump contains a

mobile phase which is contained in a

collapsible container and placed in a vessel.

Second is a sample injection unit, provides constant volume injection of the sample into the mobile phase stream. Volume of the sample used ranges from 0 to 500 microliters and since the system operates at high pressure, injection process is complicated. So the injection method. First one is syringe injection. Simplest means of sample introduction using micro syringes designed to withstand pressure of up to 1500 PSI through a self-sealing elastomeric septum. Stopped flow injection. No septum is used. Flow of the solvent is momentarily stopped and the sample is directly injected on the head of the column. Sampling valve. Sample loop of a fixed capacity is connected to a high pressure valve and the sample is filled into the sample loop through a syringe.

Sampling loops permit introduction of

the sample at pressure up to 7000 PSI.

Thirdly,

is the column. A stainless steel tube packed

with a stationary phase which differs

in dimensions depending on its application.

Column size ranges in length

from 5 to 30 centimeters,

with the inner diameter between

4 to 10 millimeters.

Micro columns as short as 3 to 7.5

centimeters with an inner diameter

ranging from 1 to 4.6 millimeters.

Mostly columns are used at room

Temperature. Since temperature affects

speed of affinity,

and diffusion between the sample

and the column and solubility of the

sample and viscosity of the solvent,

use of column in the thermostatic oven is

recommended that is either jacket

method or air circulating method.

So column packing.

Three types of particles used for

Packing. Pellicular particles consist

of glass beads whose surface are

coated with a thin silica layer.

Microporous particles composed of silica,

alumina,

or ion exchange resin.

Macroporous particles containing high

porosity of several 100 angstroms. So

forth and the last one detector.

Detector gives a specific response for

the component separated by the column,

and provides the required sensitivity.

Independent of any changes in

mobile phase composition.

Mostly used are UV-VIS detection,

followed by IR, mass spectrometer.

Aplications.

Preparative HPLC process of isolation and

purification of compounds. Analytical HPLC

obtain information about sample compounds.

Identification,

quantification and resolution of compounds.

Applicable for thermally unstable

substances as it carries out at room

temperature. So the applications,

pharmaceutical applications,

tablet dissolution

study of pharmaceutical dosage form,

identification of active ingredients,

and shelf life

determination of pharmaceutical products.

Next,

the environmental applications of

this pharmaceutical quality control.

Detection of phenolic compounds

in drinking water. Identification

of diphenhydramine in

sediment samples.

Biomonitoring of pollutants.

The forensic applications

of this. Quantification of

the drug in biological samples.

Identification of anabolic steroids

in serum, urine, sweat and hair.

Forensic analysis of textile dyes.

Determination of cocaine

and metabolites in blood.

So the next, quantification of

ions in human urine. Analysis of

antibiotics in blood. Detection

of endogenous neuropeptides in

extracellular fluids of the brain.

These are the clinical applications.

And finally, food and flavor.

Quality of soft drinks and drinking water.

Analysis of beer.

Sugarless is in fruit juices.

So to summarize.

High pressure liquid chromatography

is an improved column chromatography

technique with higher speed of

separation and better versatility.

The components of HPLC

are, solvent reservoir,

delivery pump,

sample injection unit, column and

detector. Solvent reservoir is

attached to a pressure pump which

pumps the mobile phase into the column

through an injector with high pressure,

thus, separating molecules which can

be detected as peaks on the computer.

Due to its high speed and versatility,

HPLC finds its application in

various fields like pharmaceutical

forensics, clinical etc.

These are your references, thank you.