

High Performance Liquid Chromatography (HPLC)

Principal and working of HPLC: The separation principle of HPLC is based on the distribution of an analyte between a mobile phase and a stationary phase. Different constituents of a sample are eluted at different times, resulting in the separation of the sample ingredients.

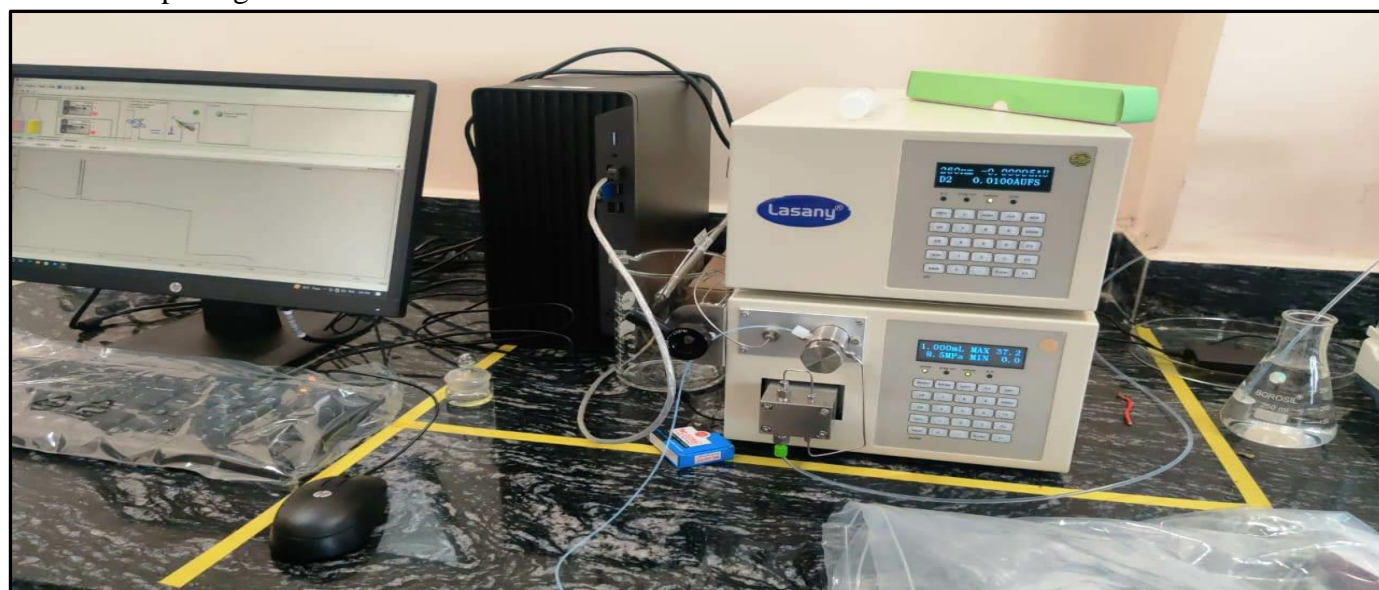


Figure 1 HPLC in the laboratory

Applications of HPLC in the analysis of various food constituents

Food Constituents	Examples	Separation mode	Method of detection
Mono & oligosaccharides	Fructose, Raffinose, sucrose, sorbitol	Ion exchange; normal or reversed phase	Electrochemical; refractive index; post column analysis
Vitamins	Fat-soluble vitamins, such as vitamins E, D, and A, and water-soluble vitamins, such as vitamins C, B6, B2, B1, and B12	Normal or reversed phase	Fluorescence; electrochemical; UV
Amino acids	Isoleucine, Leucine, Lysine, Threonine, Histidine, Valine, Methionine, Phenylalanine, Tryptophan, and Tyrosine	Ion exchange; reversed phase	Post- or pre-column derivatization
Protein separation	Bovine serum albumin, Insulin	Ion exchange, reversed-phase, affinity, hydrophobic interaction	UV
Phenolics	Rutin, Gallic acid, Quercetin	Reversed phase	UV
Pesticides	DDT, Urea, Phosphine	Normal or reversed phase	UV; fluorescence; mass spectrometry
Mycotoxins	aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone and nivalenol/deoxynivalenol	Reversed phase; Immuno-affinity	UV; fluorescence

Other applications

I. Applications of HPLC in pharmaceuticals

- The analysis of pharmaceutical drug formulations using HPLC is both qualitative and quantitative
- For the separation of complex molecules, high-performance liquid chromatography (HPLC) is used.
- High-performance liquid chromatography is used to purify massive amounts of substances.
- It is utilized to test the purified compounds for the presence of minute contaminants.
- HPLC is utilized in the analysis of pharmaceutical products in order to determine their bioavailability.

II. Applications of HPLC in environmental science

- HPLC is utilized in the water quality analysis.
- To detect trace amounts of pollutants like PCBs in pesticides and used oil.

III. Applications of HPLC in forensic science

- HPLC is used to detect drug traces in urine, from doping agents to opioids, cocaine, LSD, cannabis, and ketamine.

IV. Applications of HPLC in medical diagnosis

- High-performance liquid chromatography is also useful for analyzing nutrients in blood and other medical samples.