International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1294-1302 www.ijprajournal.com ISSN: 2249-7781

HPLC's Importance in the Pharmaceutical production

Hitesh Kumar
V. P. O – Bakana, Radaur, Yamunanagar
Department of pharmacy (Pharmaceutical Quality Assurance), Maharishi Markandeshwar college of pharmacy (MMDU), Mullana, Ambala, Haryana, India.

Submitted: 17-01-2023 Accepted: 27-01-2023

ABSTRACT
Long-performance liquid chromatography (HPLC) is a major instrument for the divorce and inspection of pharmaceutical medications, drug monitoring, quality assurance, and life science inquiry. Non-volatile compound dissolution and evaluation need HPLC. We have received many demands for routine practice in the pharmaceutical business, reasoning workshops, experimental workshops, and more colleges because of the flexibility of this technology.To analyze the chemical's character and conduct a quantitative examination of the compound found in pharmaceutical stocks like aspirin, ibuprofen, and paracetamol, sodium chloride, and potassium phosphate are examples of salts. Many common productions, such as ginseng, herbal medicines, plant extracts, and unstable compounds, such as trinitrotoluene (TNT), as well as objective management HPLC, is an accepted system. Organic chemicals include polymers (such as polystyrene, and polyethylene), proteins like egg white or blood protein, heavy hydrocarbons like asphalt or motor oil, and many natural substances In actuality, this summarizes an evaluation of many significant HPLC functions for the pharmaceutical trade.These functions contain specific chromatographic limits and HPLC performances. The name of the medication, the static state, and the mobile state, the composition of the mobile point, the flow rate, and the radar are all crucial pieces of information.

Keywords: static phase, pharmaceutical quality control, impurity profiling in pharmaceuticals, and HPLC studies.

I. INTRODUCTION

Early in the 20th century, colored compound departure was one of the first uses of liquid chromatography as a diagnostic approach. This is where the name "chromatography"—where "chroma" stands for "color" and "graph" for "writing"—was derived [1]. A process in an analytic system known as high-performance liquid chromatography (likewise seen as high-pressure liquid chromatography, or HPLC) is applied to break up, recognize, and quantify each part in a mixture [2].Many intrinsic properties of reproducibility ease of selectivity change, and excellent recoveries handle HPLC's immense success. The most notable aspect is the exceptional resolution that may be got under a variety of circumstances for both separate molecules and molecules that are related [3] [4].A liquid (mobile time) is pushed through the list at high pressure while I injected a solution to the problem into a porous column (static phase). Based on variations in the sample's barrier between the static and mobile phases, they separated the situation based on differences in the rates of migration across the file. Elution occurs at various times, depending on how distinct components behave during segregation. Compared to compounds with lesser affection, which lead quicker and farther, the fact compound with higher devotion to the stagnant layer will proceed more slowly and over a shorter distance [5].Someone often exposes an eluting fragment to UV photons produced by the sonar connected to the HPLC machine, which has a certain wavelength. The eluting species' molecules are stimulated by absorbing UV ray energy; as they de-excite, heat is released that the sonar measures [6] [7].

Principle

Long-performance liquid chromatography's guiding principle is to partition solute on stationary phase based on affinity towards stationary phase and separate by absorption, ion exchange, and other means [8]. Normally, a column's solvent flows through it with the help of gravity, but HPLC procedures force the solvent under high pressure of up to 400 atmospheres. HPLC pumps will be used to move the pressured liquid solvent and sample combination into a column made of solid adsorbent material. For

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1294-1302 www.ijprajournal.com ISSN: 2249-7781

instance, the mobile phase includes substances like water, acetonitrile, and methanol [9]

Types of HPLC

I.BASED ON THE MODE OF SEPARATION

1. **Normal phase chromatography:** By using polarity, this technique divides analytes. Both the stationary phase and the mobile phase in NP-HPLC are non-polar. So, typically, silica serves as the stationary phase, whereas hexane, methylene chloride, chloroform, diethyl ether, and their combinations serve as the typical mobile phases. As a result, polar samples stay on the polar surface of the column packing longer than less polar substances [10].

2. **Reverse phase chromatography (RP-HPLC or RPC**): In reversed phase, there is an aqueous, moderately polar mobile phase as well as a non-polar stationary phase. RPC functions based on hydrophobic interactions, which are caused by repulsive forces between a polar eluent, the comparatively non-polar analyte, and the non-polar stationary phase. When the analyte associates with the ligand in the aqueous eluent, the contact surface area around its non-polar segment determines how much of the analyte will bind to the stationary phase [11].

II. BASED ON THE PRINCIPLE OF SEPARATION

1. **Size exclusion chromatography:** is a type of chromatography that primarily uses gel permeation or gel filtration to separate particles based on their sizes. Additionally, it helps figure out the quaternary and tertiary structures of proteins and amino acids. The molecular weight of polysaccharides can be determined using this technique [12].
2. **Ion exchange chromatography:**Retention in ion-exchange chromatography is based on the attraction of solute ions to charged sites bound to the stationary phase. Ions with the same charge are not included. This type of chromatography is frequently employed in the purification of water, ligand-exchange chromatography, ion-exchange chromatography of proteins, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, etc [13].
3. **Bio-affinity chromatography:** A division based on a particular, reversible interaction between a protein and its ligand. Proteins that interact with the column-bound ligands are retained because they are covalently bonded to solid supports on a bio-affinity matrix. There are two methods for eluting proteins coupled to a Bio-affinity column: A free ligand that competes with column-bound ligands is included in the elution buffer for bio-specific elution.

Atypical elution: pH, salt, etc. changes that reduce the strength of the protein-substrate interaction.

Bio-affinity chromatography can produce extremely high purity in a single stage because of the specificity of the contract (10 - 1000-fold) [14].

III. BASED ON ELUTION TECHNIQUE

1. **Isocratic elution:** A separation method that uses one solvent or a solvent mixture with a fixed composition.
2. **Gradient elution:** In this case, two or more solvent systems are used, each of which has a very different polarity. The ratio of the solvents is designed to change after elution has started, sometimes continuously and other times in a series of phases. Gradient elution significantly improves separation efficiency.

PARAMETERS

- **Internal circumference:**An HPLC column's internal diameter (ID), which also affects sensitivity, is a crucial factor in determining how many analytes can be placed onto the column. Larger columns are typically found in industrial settings, such as when a medicine product is being purified for future use. Low ID columns have reduced solvent use and increased sensitivity at the cost of loading capacity [15].
- **Pumping force:** Although pumps come in a variety of pressure capacities, their effectiveness is determined by their capacity to produce a predictable and constant flow rate. Modern HPLC systems have been enhanced to operate at far greater pressures, allowing for the use of columns with particles as fine as 2 micrometers [16].
- **Particle size**: In most instances of conventional HPLC, tiny, spherical silica particles are used as the stationary phase, which is attached to their exterior. The most popular size of these silica beads is 5µm; however, they come in a variety of sizes. The pressure needed to achieve the best linear velocity is inversely proportional to particle

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1294-1302 www.ijprajournal.com ISSN: 2249-7781

diameter squared; hence smaller particles often offer more surface area and better separations. For non-HPLC applications like solid-phase extraction, larger particles are used in preparative HPLC where column diameters range from 5 cm to >30 cm [16][17].

- **Pore diameter:**The column's pore size determines how well analyte molecules can interact with the particle's inner surface by penetrating within the particle. Many stationary phases are porous to increase their surface area. Although smaller pores offer a greater surface area, larger holes offer improved kinetics, especially for larger analytes. How well analyte molecules can interact and penetrate the particle depends on the size of the pores [18].

Instrumentation

The separation process used in HPLC instrumentation involves injecting a solution (the stationary phase) into a porous column of material and pumping a liquid phase (the mobile phase) through the column at a higher pressure [19].

a) **Solvent reservoir:** Glass container contains the contents of the mobile phase. A mixture of polar and non-polar liquid components makes up the mobile phase, also known as the solvent, in HPLC. The polar and non-polar solvents will be changed depending on the sample's composition [20].

b) **Pump:**To generate and measure a specific flow rate of mobile phase, generally millilitres per minute, a high-pressure pump is utilized (also known as a solvent delivery system or solvent manager). The mobile phase is forced into a column by the pump and then sent on to the detector after being drawn from the solvent reservoir. The column's size, particle makeup, flow rate, and operating pressure are all affected by these factors. In HPLC, 1 to 2 ml/min is considered the standard flow rate. Pressures between 6000 and 9000 psi are what most pumps can achieve (400-to 600-bar)[21]. Column size, particle size, flow rate, and mobile phase composition all affect pump pressure[22].

c) **Sample Injector:**The injector's purpose is to add the liquid sample to the mobile phase's flow stream. The typical sample volume ranges from 5 to 20 microliters(l). The liquid system's high pressures must be handled by the injector as well. When a user has a lot of samples to analyze or when manual injections are impractical, an auto sampler is an automatic version that can be used.Numerous tools are available for manual or automatic injection[23].

- **Rheodyne injector (loop valve type):**The most common kind, it has a set volume loop of 20 to 50 l or more. The injector has two modes: load position for when the sample is put into the loop and inject mode for when the sample is injected. The injector may be a computerized infusion system or a single injection. An injector for an HPLC framework should deliver an infusion of the fluid specimen inside the range of 0.1 ml to 100 ml of volume with excellent repeatability and under high pressure (up to 4000 psi).
- **Automatic injector:** It is a computerized variation of the manual universal injector. Normally, the auto-injector tray can accommodate up to 100 samples. The system's parameters, including flow rates, injection volume, gradient, run time, etc., are chosen, stored in memory, and then systematically carried out on subsequent injections[24].

d) **Column:**The stationary phase of the column, which is referred to as the "heart of the chromatograph," uses a variety of physical and chemical factors to separate the important sample components. At typical flow rates, the high back pressure is brought on by the tiny particles inside the column. Due to the pump's exerted effort to force the mobile phase through the column, the chromatograph is under high pressure[23]. Uses strong pressure produced by the tiny particles to provide separation. Stainless steel or glass tubes with an inner diameter of 1 to 10 mm and a length of 5 to 50 cm are used as analytical columns (most frequently)[25].Depending on the nature of the pharmaceutical component and the column separation capability, various types of HPLC columns are utilized in the analysis. The separation of the components of the sample is done by the column, which is why columns are the primary component in HPLC. As the mobile phase is present in the column, the sample moves through it and separates into its constituent parts when it exits.

Due to its particle size and porosity, which aid in component separation, and because it is an inert substance that does not react with mobile phases, silica gel is typically used as filler in high-performance liquid chromatography columns. As a result, silica columns can be utilized to analyze

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1294-1302 www.ijprajournal.com ISSN: 2249-7781

substances with various chemical properties. A stationary phase is a substance that is placed inside the HPLC columns.

e) Detector: The detector can identify each molecule that elutes (comes out) from the column. For the chemist to quantitatively analyze the sample components, a detector measures the quantity of those molecules. The liquid chromatogram, or graph of the detector response, is produced by a recorder or computer using an output from the detector[23]**.**There are primarily two categories of detectors used in HPLC:

I. Selective detectors: These detectors should be mobile phase-independent and react to a specific physical or chemical feature of the solute.

- Absorbance detectors
- Fluorescence detectors
- Electrochemical detectors
- Mass spectrometric detectors[26]

II. **Universal detectors**: Measure the variation in a solute's physical properties between the mobile phase and the solute alone. They often have weak sensitivity and a small operating range despite their widespread application. Gradient elution techniques cannot be used with such detectors since they are typically impacted by even little changes in the mobile-phase composition.

f) **Data collection device:** The ability to analyze, store, and reprocess chromatographic data as well as the multifaceted quality of the signals from the detector might vary widely between graph recorders and electronic integrators. Additionally, it establishes the analysts' elution time. A chromatograph that requires little interpretation is created by the PC, which also coordinates the indicator's response to each component[27]. The chromatogram is drawn using computer software or a printer using the data that the detector collected. Then, using specialized software employed in the techniques intended to separate a certain compound from a mixture, this chromatogram can be examined manually or automatically. Specifically, this system just produces data as output[28].

HPLC industry Applications[29]

The process of developing a novel drug involves a wide range of applications, from drug discovery to the production of prepared goods that will be given to patients.

There are 3 basic steps in the process of developing a new medicine:

- Drug discovery
- Drug development
- Drug manufacturing

The greatest tool for identifying and characterizing compounds is LC-MS. As a measurement tool for high throughput screening, it is possible to use it. To isolate and purify hits and lead compounds as needed, preparative HPLC is also used.

Pharmaceutical applications[30-33]

- Study on the pharmaceutical dosage form's tablet dissolving.
- Drug stability control and shelf-life estimation.
- Identifying the active components.
- Supervised pharmaceutical quality.
- Pharmaceutical dosage form dissolving in tablet form.

Analysis of food & flavour[34]

- Quick component screening and analysis for nonalcoholic beverages.
- Evaluation of the purity of water and soft medicines.
- Analysis of sugar in fruit juices.
- Vegetable polycyclic compound analysis.
- Analysis of preservation.
- LC triples quadrupole MS multi-residue analysis of many pesticides in food samples.

Application in Environment [35-38]

- Diphenhydramine identification in sedimented samples.
- Pollution bio-monitoring.
- HPLC allows for the quick isolation and identification of carbonyl compounds.
- Pharmaceuticals and personal care items can be detected using an HPLC/MS/MS solution in water, sediment, soil, and bio solids.

Forensics applications[39-41]

- Biological sample quantification for drugs.
- Finding anabolic steroids in urine, sweat, serum, and hair.
- Fabric dye forensic analysis.
- Measuring the presence of cocaine and other illicit narcotics in urine, blood, etc.
- LC/MS is used to find benzodiazepines in oral fluid.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1294-1302 www.ijprajournal.com ISSN: 2249-7781

Therapeutic application[42-45]

- Dopamine and other catecholamines, including epinephrine, are crucial for a variety of biological processes. It is possible to diagnose conditions including Parkinson's disease, heart disease, and muscular dystrophy by analyzing their precursors and metabolites.
- Analysis of blood plasma for the presence of antibiotics and ion concentrations in human urine.
- Estimation of bilirubin and biliverdin in blood plasma when hepatic diseases are present.
- Finding endogenous neuropeptides in the brain's extracellular fluids.

Examination of the Pharmaceutical impurities

- Impurity structure elucidation using LC/MS.
- A quick LC technique for greater sample throughput.

Pharmaceutical drug discovery analysis

- The creation of a quick, general technique for liquid chromatography with quadrupole MS detection.Drug analysis is possible using a quick, generic LC/MS approach in less than a minute.

Recent applications

Key components of every pharmaceutical development program are the development and validation of analytical methods[46-54]. To identify, quantify, or purify desired substances, HPLC analysis methods are created. Drug formulation stability studies benefit greatly from the use of HPLC. Studies on the stability of atropine, antibiotics, and biotechnology-based medications like insulin and streptokinase benefit greatly from the use of HPLC.

i. Inorganic chemistry uses it to separate anions and cations.
ii. It is employed in forensic science to separate phenyl alkylamines from blood plasma (including morphine and its metabolites) and to find poisons or intoxicants such as alcohol, carbon monoxide, cholinesterase inhibitors, heavy metals, hypnotics, etc.
iii. It is employed in environmental research to determine the number of pesticides in drinking water.
iv. It is used in food analysis to distinguish between vitamins that are fat- and water-soluble from a variety of food products, fortified foods, and animal feed.
v. It is also used to determine whether a food contains antioxidants and preservatives.
vi. For the assay and quality control of different cosmetics like lipsticks, creams, ointments, etc., it is utilized in the cosmetics sector.
vii. It is employed for isolating distinct plant products' constituents that have structural similarities.
viii. In the agrichemical sector, it is used to separate herbicides.
ix. It is utilized in agrichem. It is used to separate and analyze steroid hormones, lipids, proteins, carbohydrates, and amino acids.
x. It is employed to separate finished coal and oil from their raw materials.
xi. It is used to separate and identify psychotropic medicines such as benzodiazepines, butyrophenones, neuroleptics, phenothiazines, and antidepressants.
xii. It can be applied to assess the stability of different medicines. Analyzing the drug's breakdown products is used to achieve this. Example: Atropine stability studies.
xiii. It can be used in bioassays for substances including sulphonamides, penicillins, peptide hormones, and chloramphenicol.
xiv. It is utilized to regulate the microbiological procedures that are involved in the manufacturing of certain antibiotics, including chloramphenicol, tetracyclines, and streptomycin.
xv. It is employed for both isolating reaction products and tracking the progress of chemical synthesis.
xvi. It provides information on the pharmacokinetics of the medications as well as the biopharmaceutical characteristics of a dose form. In designing dosage forms, it is utilized.
xvii. For both natural and manufactured pharmaceuticals, it serves as an analytical technique. It is applied at various pharmacy and pharmacology levels.

IV. CONCLUSION

The best method for analyzing traces of organic and inorganic chemicals is high-performance liquid chromatography. Since even a tiny molecule can be hazardous or detrimental, determining trace substances is crucial for pharmacological, biological, toxicological, and

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1294-1302 www.ijprajournal.com ISSN: 2249-7781

environmental studies. In analytical chemistry, pharmaceutical and drug science, clinical sciences, food technology, consumer products, combinatorial chemistry, polymer chemistry, environmental chemistry, and green chemistry, HPLC is used to determine the molecular weight of molecules.Particularly during preformulation, process development, formulation development, drug discovery, and to confirm drug purity, HPLC plays a critical role in the pharmaceutical sector. High-performance liquid chromatography, also known as GC, is used routinely and quickly every day to carry out all the work that needs to be done in pharmaceutical substances, the preparation of pure compounds, trace analysis, and food safety, where we have to analyze for pesticides and toxic chemicals found in food and food products.We are so dependent on chemicals in today's world, mostly synthetic chemicals created by chemists that have a mixed blessings, primarily pesticides that are very good for agriculture but very harmful for humans if they happen to ingest these pesticides. Chromatography plays an important role in the QC of the quality of foods but also of drugs controlling the raw materials and control the finished products ensuring the safety of the people. Therefore, HPLC is the optimum separation method for quantifying trace levels of dangerous chemicals[55], and impurities, manufacturing highly pure products, therapeutic applications, and research purposes.

REFERENCES

[1]. JosephC. Touchstone (2006), History of Chromatography, Journal of liquid chromatography, volume 8 (16), 1647-1665. https://doi.org/10.1080/10826079308021679.

[2]. Sánchez J. L., Agnes I. B. L., Antonio S. K., Carretero S. (2018), Chromatographic Technique: High-Performance Liquid Chromatography (HPLC), Modern Techniques for Food Authentication (Second Edition).

[3]. Ravisankar P., Anusha S., Kommanaboina S., Kumar U. A. (2019), Fundamental Chromatographic Parameters, Int. J. Pharm. Sci. Rev. Res, 55(2) 46 - 50.

[4]. Ravisankar P, Geethika S. A, Rachana G, Srinivasa B. P, Bhargavi J (2019), Bioanalytical method validation: A comprehensive review, Int. J. Pharm.Sci. Rev.Res., 56(1), 50-58.

[5]. Neue U. D. (1997), Wiley Analytical Science, HPLC Columns: Theory, Technology, and Practice.

[6]. Karatepe M. (2004), Simultaneous Determination of Ascorbic Acid and Free Malondialdehyde in Human Serum by HPLC–UV, LCGC NORTH AMERICA, (22). 362-365.

[7]. Dinçer Z., Basan H., Göger N. G. (2003), Journal of Pharmaceutical and Biomedical Analysis, Quantitative determination of ambroxol in tablets by derivative UV spectrophotometric method and HPLC 5 (31), 867-872. https://doi.org/10.1016/S0731-7085(02)00664-7.

[8]. Santosh Kumar Bhardwaj a, b *, K. Dwivedi and D. D. Agarwala a School of Studies in Chemistry, A Review: HPLC Method Development and validation, Jiwaji University, Gwalior, India.

[9]. A. Venkata Suresh, Babu Research Scholar, HPLC, and LCMS-A Review a recent update College of Pharmacy, Sri Satya Sai University Technology and Medical Sciences, Opp.Oiled Plant, Bhopal-Indore Road, Sehore, Madhya Pradesh 466001, India.

[10]. Hamilton J.G., Comai K. (1988), Separation of neutral lipid, free fatty acid and phospholipid classes by normal phase HPLC. Lipids, (23). 1150–1153. https://doi.org/10.1007/BF02535282.

[11]. Horvath C., Melander W. (1977), Liquid Chromatography with Hydrocarbonaceous Bonded Phases; Theory and Practice of Reversed Phase Chromatography, Journal of Chromatographic Science, 9 (15), 393–404, https://doi.org/10.1093/chromsci/15.9.393.

[12]. Oeyen E., Mol K. V., Baggerman G., Willems H., Boonen K., Rolfo C., Pauwels P. Jacobs A., Schildermans K., Cho W., Mertens. I. (2018), Ultra-filtration and size exclusion chromatography combined with asymmetrical-flow field-flow fractionation for the isolation and characterization of extracellular vesicles from urine. Journal of Extracellular Vesicles, 1-7. https://doi.org/10.1080/20013078.2018.1490143.

[13]. Gallant S.R., Vunnum S., Cramer S.M. (1996), Optimization of preparative ion-

exchange chromatography of proteins: linear-gradient separations, Journal of Chromatography A, 2 (23), 295-314. https://doi.org/10.1016/0021-9673(95)00909-4.

[14]. Malviya R, Bansal V, Pal O.P. and Sharma P.K. (2010), High-Performance Liquid Chromatography: A Short Review, Journal of Global Pharma Technology 2(5), 22-26.

[15]. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. John Wiley & Sons. New York. 2011.

[16]. Xiang Y, Liu Y, Lee ML. Ultrahigh-pressure liquid chromatography using elevated temperature. Journal of Chromatography. 2006; 1104(1): 198-202.

[17]. Horvath CG, Preiss BA, Lipsky SR. Fast liquid chromatography. Investigation of operating parameters and the separation of nucleotides on pellicular ion exchangers. Analytical chemistry, 1967; 39(12): 1422-1428.s

[18]. Malviya R, Bansal V, Palo P, and Sharma PK. High-Performance Liquid Chromatography: A Short Review. Journal of Global Pharma Technology. 2010; 2(5):22-23.

[19]. Yogesh Kumar, Sayed Md Mumtaz, Mushtaq Ahmad, search Scholar, Pharmacology Department, HPLC: Principle and Maintenance with Application, Delhi Pharmaceutical Sciences, and Research University Delhi, Delhi, India

[20]. MukthiThammana*, Department of Pharmacy, A Review on High-performance Liquid chromatography(HPLC), Vignan Institute of Pharmaceutical Technology, Visakhapatnam, Andhra Pradesh, India.

[21]. Gita Chawla1*, Krishna Kr. Chaudhary Professor, 1-2Dept. of Pharmaceutical c chemistry, A review of HPLC techniques its pharmaceutical, environmental, forensic, clinical, and other applications, School of Pharmaceutical Education and Research, JamiaHamdard(Deemed to be University), Delhi, India.

[22]. Devyani M. Rode, NutanN.Rao*, Department of Pharmaceutical Chemistry and Quality Assurance, A Review on Development and Validation of Stability Oriental indicating HPLC Methods For Analysis of Acidic Drugs, College of Pharmacy, Sector 2, Sanpada West, Mumbai, Maharashtra, India.

[23]. Fundamentals of HPLC, polymer.ustc.edu.cn

[24]. Shine sudev, Dr.SreeJanardhanan. V, Research scholar, Review on HPLC Method Development validation and Optimization, Shri JagdishprasadJhabarmalTibrewala University, Vidyanagari, Jhunjhunu, Rajasthan, India.

[25]. Rohini S. Koli*, Aslam S. Patel, Kamlesh N. Chaudhari, Khushbu R. Patil, A Review on HPLC and Its New Trends, SES Arunamai College of Pharmacy, Mamurabad, Jalgaon-425001 [M.S.] India.

[26]. Taniya Biswas, M.Pharm, Detectors Used in High-Performance Liquid Chromatography, Pharmaceutical analysis, Bengal school of Technology.www.slideshare.net

[27]. More Siddhant*, GhodekarSmita, JadhavVaishali, Jain Ashish, HPLC&UPLC-Chromatographic system-A Review On Modern Liquid chromatography, Shri. D.D.Vispute College of Pharmacy and Research Centre, Panvel, Maharashtra, INDIA.

[28]. Hayder obeys has him, Ph.D., University of Babylon, chromatography and HPLC principles.

[29]. Bailey F, Applications of high-performance liquid chromatography in the pharmaceutical industry, Journal of Chromatography A, 122, 1976, 73-84.

[30]. Bergh J. J., Breytenbach, J. C. Stability-indicating High-performance Liquid-chromatographic Analysis of Trimethoprim in Pharmaceuticals. J. Chromatogr. 1987; 387: 528-531.

[31]. Stubbs C., Kanfer, I. Stability indicating High-performance Liquid-chromatographic Assay of Erythromycin Estolate in Pharmaceutical Dosage Forms. Int. J. Pharm. 1990; 3(2): 113-119.

[32]. MacNeil L., Rice J. J., Muhammad N. Laubach R. G. Stability-indicating Liquid-chromatographic Determination of Cefapirin, Desacetylcefapirin and Cefapirin Lactone in Sodium Cefapirin Bulk and Injectable Formulations. J. Chromatogr. 1986; 361: 285-290.

[33]. Bonine J. P., Tardif B., Beltran P. Mazzo D. J. High-performance Liquid-chromatographic Stability-indicating Determination of Zopiclone in Tablets. J. Chromatogr. 1994; 677(1): 87-93.
[34]. Ma Bokai, Gou XinLei, Zhao XinYing, Application of high-performance liquid chromatography in food and drug safety analysis, Journal of Food Safety and Quality, 7, 2016, 4295- 4298.
[35]. Lauback R. G., Rice J. J., Bleiberg B., Muhammad N., Hanna, S. A. 1984. Specific High-performance Liquid-chromato- graphic Determination of Ampicillin in Bulks, Injectables, Capsules, and Oral Suspensions by Reversed-phase Ion-pair Chromatography. J. Liq. Chromatogr. 1984; 7(6): 1243-1265.
[36]. Wiklund A E., Dag B., Brita S. Toxicity evaluation by using intact sediments and sediment extracts. Marine Pollution Bulletin (2005); 50(6): 660-667.
[37]. Kwok Y. C., Hsieh D. P. H., Wong P. K. Toxicity identification evaluation (TIE) of pore water of contaminated marine sediments collected from Hong Kong waters. Marine Pollution Bulletin. 2005; 51(8-12): 1085-1091.
[38]. Hongxia Yu., Jing C., Cui Y., Shang H., Ding Z., Jin H. Application of toxicity identification evaluation procedures on wastewaters and sludge from a municipal sewage treatment works with industrial inputs. Ecotoxicology and Environmental Safety. 2004; 57(3): 426-430.
[39]. Ayrton J. Assay of ceftazidime in biological fluids using high-pressure liquid chromatography. J. Antimicrob. Chemother. 1981; 8: 227-231.
[40]. Bowden R.E., Madsen P.O. High-pressure liquid chromatographic assay of sulbactam in plasma, urine, and tissue. Antimicrob. Agents Chemother. 1986; 30: 31-233.
[41]. Haginaka J., Yasuda H., Uno T., Nakagawa T. Alkaline degradation and determination by high-performance liquid chromatography. Chem. Pharm. Bull. 1984; 32: 2752-2758.
[42]. Fredj G., PailletAussel M. F., Brouard A., Barreteau H., Divine C., Michaud M. Determination of sulbactam in biological fluids by high-performance liquid chromatography. J. Chromatogr. 1986; 383: 218-222.
[43]. Rodenas V., Garcia M.S., Sanchez-Pedro C., Albero M.I. Flow-injection spectrophotometric determination of frusemide or sulphathiazole in pharmaceuticals. J. Pharm. Biomed. Anal. 1997; 15: 1687-1693.
[44]. Shah A.J., Adlard M.W., Stride J.D. A sensitive assay for clavulanic acid and sulbactam in biological fluids by high-performance liquid chromatography and pre-column derivatization. J. Pharm. Biomed. Anal. 1990; 5: 437-443.
[45]. Abidi S.L. High-performance liquid chromatography of phosphatidic acids and related polar lipids. J.Chromatogr. 1991; 587: 193-203.
[46]. Ravisankar P, Gowthami S, and Devala Rao G, A review on analytical method development, Indian journal of research in pharmacy and biotechnology, 2 (3), 2014, 1183-1195.
[47]. Shivani Sharma, SwapnilGoyal, Kalindi Chauhan, A review on analytical method development and validation, International Journal of Applied Pharmaceutics, 10(6), 2018, 8-15.
[48]. RavisankarPanchumarthy, Naga NavyaCh, Pravallika D, Navya Sri D, A review on step-by-step analytical method validation, IOSR Journal of Pharmacy, 5 (10), 2015, 07-19.
[49]. SiroPassi, Paolo Casella, Marcella Nazzaro - Porro, et al, An application of high-Performance liquid chromatography to analysis of lipids in archaeological samples, Journal of lipid research, 22(5), 1981, 778 - 784.
[50]. Mohammed Ashraf Ali et al, Application of High-Performance Liquid Chromatography for hydrocarbon group type analysis of Crude Oils, Fuel Science and Technology International, 12, 1994, 21 -23.
[51]. Ravisankar P, Swathi V, SrinivasaBabu P, ShaheemSulthana Md, Gousepeer SK: Current trends in performance of forced degradation studies and stability indicating studies of drugs. IOSR Journal of Pharmacy and Biological Sciences 12(6), 2017, 17-36.
[52]. Cavazzini A, Pasti L, Massi A, Marchetti N, Dondi F., Recent applications in chiral high-performance liquid chromatography, Anal ChimActa, 706(2), 2011, 205-22.

[53]. PanchumarthyRavisankar, S. Anusha, KommanaboinaSupriya, U. Ajith Kumar, Fundamental Chromatographic Parameters, Int. J. Pharm. Sci. Rev. Res, 55(2), 2019, 46 - 50.
[54]. Ravisankar P, Sai Geethika A, Rachana G, SrinivasaBabu P, Bhargavi J, Bioanalytical method validation: A comprehensive review, Int.J.Pharm.Sci. Rev.Res., 56(1), 2019, 50-58.
[55]. Francois-LudovicSauvage, Franck-Saint-Marcoux, BenedicteDuretz, Didier Deporte, Gerard Lachatre, Pierre Marquet, Screening of drugs and toxic compounds with liquid chromatography- Linear ion trap tandem mass spectrometry, Clinical Chemistry, 52(9), 2006, 1735-1742.