International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 1s, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Tandem Mass: The Creation and Appropriation of a Liquid Chromatography

Smruti Ranjan Dash[1], Mahendra Kumar Sahu[2]

[1]Assistant Professor, Department of Pharmacy, Kalinga University,Raipur, India.
[2]Research Scholar,Department of Pharmacy, Kalinga University,Raipur, India.

Received: 15.05.2024 Revised: 18.06.2024 Accepted: 24.07.2024

ABSTRACT
The remarkable method for chemical and pharmaceutical analysis that can separate, analyse, and/or purify any material is liquid chromatography. For the purpose of separating drug particles and performing their metabolite bioanalysis, a variety of analytical techniques are employed, including mass spectrometry (MS), capillary electrophoresis (CE), gas chromatography (GC), radioimmunoassay (RIA), fluorescence, and refractive index. The most popular ones are chromatography-based. The ability of chromatography to separate materials and mass spectrometry (MS) has completely changed the way chemical analysis is conducted today. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) equipment is highly versatile and selective. Because of its outstanding selectivity, high sensitivity, and rapid analysis, LC-MS/MS is a commonly used and ideal method for evaluating medicines and their metabolites. Even at lower concentrations, analytes that are easily separated by liquid chromatography can be identified by MS/MS detection due to a variety of ionisation techniques, including electro-spray ionisation (ESI), atmospheric pressure photo ionisation (APPI), and air pressure chemical ionisation (APCI). A fingerprint mass spectrum is produced by MS and includes information on the molecular weight and fragmentation ions peculiar to a given structure. The primary flaw of MS is its inability to handle compound mixes. Tandem mass spectrometry (MS/MS) can assist with this problem to some extent. Therefore, when HPLC and MS are interfaced, the very sensitive and structure-specific detection capabilities of MS and the high-resolution separation skills of HPLC benefit each other. MS functions as an all-purpose LC detector nearly flawlessly.

Keywords: Liquid Chromatography, Tandem Mass Spectrometry.

1. INTRODUCTION

The first type of chromatography to be identified was liquid chromatography (LC), which was first applied in the late 1890s by Russian botanist Tswett as a liquid-solid chromatography (LSC) method to isolate and separate different plant pigments. His creation of coloured bands on the adsorbent bed gave rise to the word "chromatography," which means "colour writing," to describe this kind of separation.A form of liquid-liquid chromatography was created by Martin and Synge in the late 1930s and early 1940s. They used it to support the stationary phase—in this case, water—on silica gel in the form of a packed bed in order to segregate specific acetyl amino acids. They presented their findings in 1941 and recommended that, in order to promote quicker phase transfer and increase separation efficiency, the liquid mobile phase be replaced with a suitable gas. Thus, gas chromatography as a notion came to be[19]. Martin and Synge proposed in the same 1941 publication that high pressures and small particles be used in liquid chromatography (LC) to enhance separation. These two crucial suggestions ultimately led to the invention of high-performance liquid chromatography. There are six essential components of a basic liquid chromatograph. These include the pump and programmer, the data processor, the column, the detector, the mobile phase supply system, and the sample injection valve[1].High Performance Liquid Chromatography (HPLC) is a crucial technology for the pharmaceutical and chemical exploration and analysis of drugs and their metabolites in biological fluid samples and formulations[2] [5]. The primary goals of the HPLC process are sample isolation, inspection, analysis, and purification. Due to varying substance partition coefficients, analytes are separated based on variations in the relative velocities of the mobile and stationary phases through the column. Reverse phase-high performance liquid chromatography (RP-HPLC) is generally utilised for most compounds[20]. Reverse phase, high performance liquid chromatography combined with mass spectrometry is increasingly used for compound analysis[10] [24]. Liquid chromatography-mass spectrometry (LC-MS/MS) has become an effective and frequently used technique in the research of pharmaceuticals and the products of their metabolism because it offers good selectivity, sensitivity, and a fast rate of analysis [18]. Even at lower fixation levels, substances that need to be examined in liquid chromatography can be readily separated and found using LCMS/MS detection[12] [23]. This can be accomplished using a variety of ionisation processes, including air pressure-chemical ionisation (APCI), electro-spray, and atmospheric

pressure-photo ionisation[8].

Fig. 1: Block Diagram of the Basic Liquid Chromatograph

LC-MS/MS, is a combination of two selective techniques that makes it possible to separate and analyse the analytes of interest in extremely complex mixtures: LC (HPLC, UPLC, or UFLC) and MS (mass spectrometer). Today, LC-MS/MS has enormous potential for advancements in laboratory medicine and pharmaceutical fields, particularly in the areas of therapeutic drug monitoring, endocrinology, toxicology, and metabolic analyses[17]. As a result, new tools such as hyphenated techniques are being used to develop quick and affordable analytical methods[16]. Since the 1990s, one of the most popular hyphenated techniques, LC-MS/MS, has significantly advanced the field of quantitative bioanalysis due to its inherent specificity, sensitivity, and speed. It is currently generally accepted as the preferred technique for measuring small molecule drugs, other xenobiotic biomolecules, and metabolites in biological matrices.[3][11]

2. LITERATURE REVIEW

Recovery is a tool that gives the analyst an assessment of how well the procedure cleaned and sufficiently determined the analyte from the biological specimen, according to Barggio et al. (1996). He provided examples of how the recovery should be adequate at all concentration levels, from lower to higher.

Many medicinal substances can be quantified using very affordable photometric and potentiometric techniques.These techniques are used in the diagnostic sector, however method development and validation are still costly, time-consuming, and labour-intensive processes.Chromatographic assay design is simpler, however HPLC procedures are typically less practical and produce less accurate results[4]. Despite these limitations, High Performance LLC–MS/MS seems to be a dependable technique. Sample analysis during drug discovery and other phases of drug development can be made easier by developing LC-MS/MS methods quickly, validating the methods, and then analysing research samples[13] [15]. With numerous uses in pharmacological, biological, clinical, food, environmental, and molecular weight measurement, LC-MS/MS is a potent analytical method [17].Throughout the whole drug development process, including drug discovery, preclinical development, clinical development, and manufacturing, this technique has been extensively utilised for the quantification of numerous pharmaceutical drug products (Bansal S. Et al., 2007; Ardrey E. Robert, 2003; Mike S. Et al.). According to a comparative study of several analytical instruments used in bioanalysis by Bruins et al. (1987) and Fenn et al. (1989), LC-MS/MS equipment with their varied models are best suited to meet the needs and satisfy the intended expectations of bioanalysis[6].

According to Dadgar et al. (1995), tandem mass spectrometry is superior to single quadrupole[14]. They said that compared to LC-MS analysis, the LC-MS/MS method of detection offers greater selectivity in detection. The creation of methods, which entails assessing and standardising experimental parameters at various phases of sample preparation (clean-up), chromatographic separation, detection, and quantification, is an essential initial step towards the quantification of pharmaceuticals and their metabolites in biological matrix[7] [22]. Furthermore, it is necessary to investigate and reduce the effects of different circumstances and procedures encountered during sample collection, sample pre-treatment, storage, and analysis on drug/metabolite quantification (Shah et al. 1992). An estimate of the nature of the analytical problem, the required level of accuracy, the available sample volume, the required concentration range, potential interfering substances, the number of samples, the physicochemical properties of the analyte of interest, and the sample matrix are among the crucial factors that must be taken into account when beginning the development of a method (Skoog et al. 2007). According to Pranay Wal et al. (2010), developing a method necessitates a thorough review of the literature to learn about the physiochemical characteristics of the drug (analyte/metabolite), including its molecular weight, molecular formula, functional group, polarity, pKa, LogP, ionic character, and solubility. Setting up chromatographic settings and an extraction process that can separate a drug of interest from biological matrix components is the

primary goal of method development[9]. Significant peak resolution, a high signal to noise ratio, precision, accuracy, specificity, and sensitivity are the goals of analytical techniques (USFDA, 2005). There are multiple processes involved in the quantitative determination of pharmaceuticals and their metabolites in biological matrices, from sample collection to the final report of results[21]. These procedures often involve preparing the sample, storing it, identifying it, and quantifying the analyte. (ICH Q2 B, Geneva, 1996).
The process of creating recorded proof of a technique's accuracy, precision, and suitability for its intended use is known as bioanalytical method validation, or BMV[8]. Method of Bioanalysis The creation, assessment, and interpretation of results from studies on bioavailability, bioequivalence, pharmacokinetics, and toxicokinetics are greatly influenced by the validation used for the quantitative determination of pharmaceuticals and their metabolites in biological fluids. Regulatory filings are often supported by these findings. As a result, it is critical that pre-established guidelines for the validation of analytical procedures be shared with the pharmaceutical community(Taleuzzaman M. et. al., 2015).

3. Methodology Of Development Of A Lc- Ms/Ms Method

Sample Preparation –Because biological matrix samples are usually not immediately suitable for LC-MS/MS analysis, sample pretreatment is required. This has traditionally been achieved through the use of solid-phase extraction (SPE), liquid-liquid extraction (LLE), or protein precipitation (PPT) techniques. A crucial component of quantitative bioanalysis is adequate sample preparation, which frequently results in bottlenecks during high-throughput analysis.

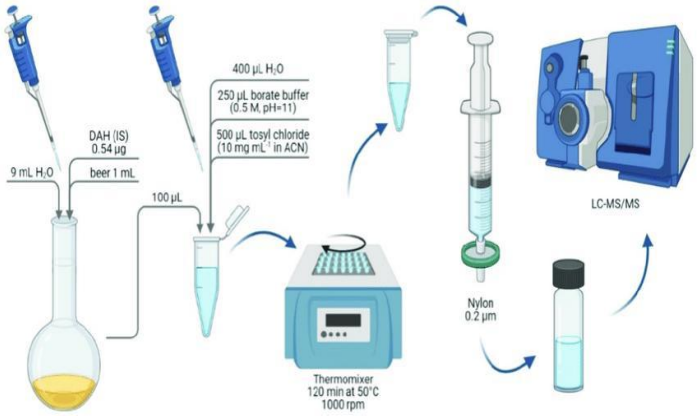

Fig. 2: Schematic Representation of Sample Preparation

Protein Precipitation Technique (PPT): Plasma sample bioanalysis commonly uses PPT sample preparation. The process of extracting proteins from biological matrix is very straightforward. Proteins in biological matrix are precipitated using organic solvents, inorganic acids, trichloroacetic acids, perchloric acids, acetonitrile, and methanol. After that, the mixture is centrifuged to get rid of the denatured proteins. Clear supernatant is either directly put into LC-MS/MS after centrifugation or after drying and reconstitution.While the extraction process is efficient and fast, there are numerous matrix interferences in the sample that can build up in the column and cause obstructions. Periodic system cleaning and ion augmentation or suppression are also necessary. Nevertheless, when supernatant from a plasma sample is examined using PPT, endogenous material and salts are still present. Ion suppression or enhancement follows from this, increasing sample-to-sample variation.
Liquid Liquid Extraction (LLE): The process of separating compounds in two distinct immiscible liquids—usually water and an organic solvent—based on their relative solubility and differential polarity is known as liquid liquid extraction, or LLE. Since the drug must be in a unionised condition for extraction to take place, the pH of the sample must be changed.A solvent's ability to dissolve in water is a critical component used in this application. The most common solvents used to increase solvent strength include ethyl acetate, methylene chloride, pentane, methyl tertiary butyl ether (MTBE), butyl chloride, hexane, petroleum ether, and chloroform. The actual sequence could vary depending on the parameters used to calculate solvent strength. To extract the medication from the aqueous phase without eliminating closely related endogenous chemicals, the solvent's polarity should ideally be sufficient. It's also critical to take solvent toxicity, density, and volatility into account.To get rid of interferences from the sample, it may occasionally be necessary to back extract the chemicals or perform several extractions. Although it is less expensive than solid phase extraction, the process is laborious and time-consuming because it necessitates drying and then reconstitution. For the separation and concentration of comparatively hydrophobic substances, LLE is an easy-to-use and effective technique. Certain polar chemicals cannot be obtained as a clean, matrix-free sample using this extraction procedure.
Solid Phase Extraction (SPE): Analytes are bonded to solid materials in SPE cartridges, interferences are removed, and the analyte is selectively eluted using this selective method of sample preparation. Due to many

distinct possibilities of sorbents, SPE is a very effective approach. Conditioning the cartridge, loading the sample, cleaning the cartridge, and then eluting the analyte are the four processes of SPE. To moisten the packing material, activate functional groups, and remove air and contaminants from the cartridge, the SPE cartridge is first conditioned by running a solvent through the sorbent. Acetonitrile and methanol are typical solvents used in reversed-phase SPE. A buffer is added after the organic solvent to ensure compatibility with aqueous samples. Next, the cartridge is filled with the sample that contains the analyte. The analyte and some matrix elements are kept in this stage, while other elements flow through. Interferences are eliminated during the wash stage, but the analyte is left behind. Lastly, a solvent that can sabotage the contacts between the analyte and the sorbent is added to elute the analyte from the sorbent. The eluent should ideally be free of any interferences. Frequently, evaporation and reconstitution are carried out to concentrate the analyte and move it into a solvent that is better suited for the chromatographic conditions.

Chromatography: In essence, it is the process of separating compounds based on variations in the equilibrium distribution, or distribution coefficient, of sample components between two distinct phases. There are two stages involved, one of which is movable and the other stationary. Liquid chromatography (LC) and liquid chromatography combined with other techniques like mass spectrometry (LC-MS) are the two types of liquid phase chromatography procedures.

Mass Spectrometer: The mass spectrometer is a widely used analytical instrument with exceptional sensitivity and selectivity. Ionising molecules is the fundamental process of a mass spectrometer. The molecules are then sorted and identified according to their mass-to-charge (m/z) ratios. The three primary components of a mass spectrometer are detectors, mass analysers, and ion sources.

Ion Source: Ions are created at the interface between LC and MS, which is referred to as the "ion source," when the LC eluent evaporates. Many interface types are commonly used in mass spectrometers, including thermo spray ionisation, electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI), atmospheric pressure photo ionisation (APPI), and atmospheric pressure ionisation (API).

Mass Analyser: After ionisation in the ion source, the ions are sorted in a mass analyser based on their mass to charge (m/z) ratio. The Fourier transfer ion cyclotron resonance (FTICR), quadrupole mass analyser, time of flight, and ion trap are the most widely used mass analysers.

Detectors: After being screened by the mass spectrometer and produced in the ion source, the ions must be identified and converted into a signal. The detector is an essential part of a mass spectrometer because it generates current proportional to the number of ions that hit it.

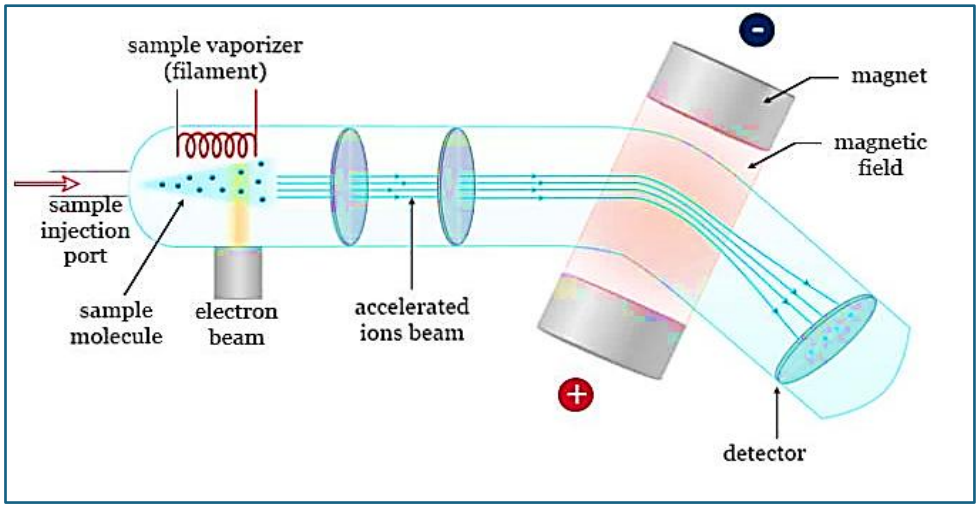

Fig. 3: Diagram of Mass Spectrometry

The analytical method was validated in compliance with various regulatory guidelines, confirming its selectivity, sensitivity, specificity, matrix effect, Ion suppression and enhancement through infusion, carry over, linearity and goodness-of-fit, accuracy & precision, blood stability, stability in plasma and solutions, recovery, reinjection reproducibility, and dilution integrity.

Selectivity and Isotopic Interference: The capacity to distinguish and measure the analytes in the presence of other sample constituents is known as selectivity. Proof that the material being quantified is the intended analyte must be shown. Analyses of blank samples of the pertinent biological matrix, such as urine, plasma, or another matrix, should be available from at least six sources. Each blank sample must be examined for interference, and selectivity at the lower limit of quantification (LLOQ) must be ensured.

Ion Suppression and Enhancement Through Infusion: The matrix is made up of a variety of substances (such as proteins, ions, and amino acids). Because of different endogenous chemicals, the LC eluent entering the

MS can suppress and enhance the MS signal (ion suppression and enhancement).As a result, ion suppression and enhancement brought on by an ineffective extraction technique may obstruct the identification of the analytes in samples. It is necessary to ascertain the impact of the extracted various blank matrix lots (for the matrix under analysis) on the variability of the electrospray ionisation response during the analyte and internal standard retention times.

Matrix Effect: It is analysed to determine whether the matrix has any unfavourable effects that could result in chromatographic peak tailing, retention time drift, ion suppression or enhancement, sensitivity changes, elevated baseline, or imprecise results. The matrix factor, or the ratio of the peak response with matrix ions present to the peak response without them, was used to compute quantitative estimates of the matrix effect.Check for contamination and carry-over to assess the possibility of carry-over through autosampler and in-process contamination, the chromatographic system (i.e., washing solution) was examined for the likelihood of in process contamination (i.e., from mobile phase, processing solution, chemicals, and apparatus). The top limit of the quantitation sample was injected both before and after the blank sample.

Sensitivity: The method's sensitivity is the lowest analyte concentration that can be measured with a sufficient degree of accuracy and precision.

Linearity and Goodness-of-fit: The estimation of goodness of fit was performed using the three batches of precision and accuracy. The best fit for the regression was found by taking into account the concentrations of calibration curve standards that were back-calculated using 1/x and 1/x2 weighing. The calibration curve's linearity was demonstrated using a correlation coefficient (r2) as a standard.

Re-injection Reproducibility: The purpose of the experiment was to prove that the repeatability of the results is unaffected by the reinjection of samples that were maintained in the auto sampler at a regulated temperature. Re-injection repeatability was achieved by reinjecting an entire precision and accuracy batch after it had been stored in the auto sampler for the predetermined amount of time at the predetermined temperature after the original batch's last injection.

4. Research Opportunities And Recommendations

LC-MS/MS is evolving at a rapid pace, with sensitivity almost doubling annually. The advent of novel ion sources or faster and more sensitive techniques for gathering many SRMs in a single run for triple quads are the two factors contributing to this increase in sensitivity, which will lead to better analysis. Novel features such as ion mobility are expected to yield improved detection specificity. It is reasonable to expect significant improvements in both sensitivity and specificity from more sophisticated sample preparation techniques (like IAC and MIP-SPE) or multi-tagging and plexing strategies to increase throughput, in addition to the increase in sensitivity and selectivity brought about by instrumental innovation. Clinical laboratories are expected to use LC-MS/MS due to the availability of kits with pre-measured and ready-to-use chemicals. The majority of LC–MS/MS experiments currently focus on a single component. Enhancements in sample preparation and increased sensitivity would greatly enhance our comprehension of pathophysiological processes by allowing for the profiling of metabolically linked chemicals.

5. CONCLUSION

The advent of novel ion sources or faster and more sensitive techniques for gathering many SRMs in a single run for triple quads are the two factors contributing to this increase in sensitivity, which will lead to better analysis. Novel features such as ion mobility are expected to yield improved detection specificity. It is reasonable to expect significant improvements in both sensitivity and specificity from more sophisticated sample preparation techniques (like IAC and MIP-SPE) or multi-tagging and plexing strategies to increase throughput, in addition to the increase in sensitivity and selectivity brought about by instrumental innovation. Clinical laboratories are expected to use LC-MS/MS due to the availability of kits with pre-measured and ready-to-use chemicals. Currently, most LC-MS/MS experiments concentrate on a single component. Improvements in sample preparation and sensitivity would enable the profiling of molecules connected to metabolism, which would significantly improve our understanding of pathophysiological processes.

REFERENCES

1. Fanali, Salvatore, Paul R. Haddad, Colin Poole, and Marja-Liisa Riekkola, eds. Liquid chromatography: applications. Elsevier, 2017.
2. Yıldız, H., &Miçooğulları, Ü. (2022). Effects of Propolis on Serum Biochemical Parameters in Azaserine Treated Rats. Natural and Engineering Sciences, 7(2), 89-96.
3. Murray, Kevin J., Peter W. Villalta, Timothy J. Griffin, and Silvia Balbo. “Discovery of Modified Metabolites, Secondary Metabolites, and Xenobiotics by Structure-Oriented LC–MS/MS.” Chemical research in toxicology 36, no. 11 (2023): 1666-1682.

4. Rahimi, Faraz, Stelios Chatzimichail, Aliyah Saifuddin, Andrew J. Surman, Simon D. Taylor-Robinson, and Ali Salehi-Reyhani. “A review of portable high-performance liquid chromatography: the future of the field?.” Chromatographia 83 (2020): 1165-1195.
5. Ozyılmaz, G., Dönmez, M., &Üstükarcı, H. (2022). The Effect of Enzyme and Sonicator Application of Biochemical Properties of Raw and Ripe Myrtus communis L. Juice. Natural and Engineering Sciences, 7(3), 271-283.
6. Meng, Min, Laixin Wang, Troy Voelker, Scott Reuschel, K. C. Van Horne, and Patrick Bennett. “A systematic approach for developing a robust LC–MS/MS method for bioanalysis.” Bioanalysis 5, no. 1 (2013): 91-115.
7. Swartz, Michael E., and Ira S. Krull. Analytical method development and validation. CRC press, 2018.
8. Danková, Z., Štyriaková, I., Kovaničová, Ľ., Čechovská, K., Košuth, M., Šuba, J., Nováková, J., Konečný, P., Tuček, Ľ., Žecová, K., Lenhardtová, E., & Németh, Z. (2021). Chemical Leaching of Contaminated Soil – Case Study. Archives for Technical Sciences, 1(24), 65–72.
9. Saadawi, EnasMagdi, Abdelaziz Said Abohamama, and Mohammed FathiAlrahmawy. "IoT-based Optimal Energy Management in Smart Homes using Harmony Search Optimization Technique." (2022).
10. Lindegardh, N., A. Annerberg, N. J. White, and N. P. J. Day. "Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of piperaquine in plasma: stable isotope labeled internal standard does not always compensate for matrix effects." Journal of Chromatography B 862, no. 1-2 (2008): 227-236.
11. French, Deborah, Kirk K. Sujishi, Janel R. Long-Boyle, and James C. Ritchie. "Development and validation of a liquid chromatography–tandem mass spectrometry assay to quantify plasma busulfan." Therapeutic drug monitoring 36, no. 2 (2014): 169-174.
12. Nair, J. G., Raja, S., &Devapattabiraman, P. (2019). A Scientometric Assessment of Renewable Biomass Research Output in India. Indian Journal of Information Sources and Services, 9(S1), 72–76.
13. Nász, Szilárd, Lajos Debreczeni, Tamás Rikker, and Zsuzsanna Eke. "Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of eleven coccidiostats in milk." Food chemistry 133, no. 2 (2012): 536-543.
14. Craddock, H., Konudula, L. P., & Kul, G. (2019). A Survey on the Challenges of Implementing Physical Memory Pools. Journal of Internet Services and Information Security, 9(2), 57-71.
15. Vita, Marina, Patrik Skansen, Moustapha Hassan, and Mohamed Abdel-Rehim. "Development and validation of a liquid chromatography and tandem mass spectrometry method for determination of roscovitine in plasma and urine samples utilizing on-line sample preparation." Journal of Chromatography B 817, no. 2 (2005): 303-307.
16. Muralidharan, J. "Advancements in 5G Technology: Challenges and Opportunities in Communication Networks." Progress in Electronics and Communication Engineering 1.1 (2024): 1-6.
17. Ratnadewi, R., Hangkawidjaja, A.D., Prijono, A., &Pandanwang, A. (2022). Design of Robot Plotter Software for Making Pattern with Turtle Graphics Algorithm. Journal of Wireless Mobile Networks, Ubiquitous Computing, and Dependable Applications, 13(4), 137-154.
18. Adaway, Joanne E., Brian G. Keevil, and Laura J. Owen. “Liquid chromatography tandem mass spectrometry in the clinical laboratory.” Annals of clinical biochemistry 52, no. 1 (2015): 18-38.
19. Kurshid, Bimer, et al. "The Potential of Ultra-Wideband Printed Rectangular-Based Monopole Antennas." National Journal of Antennas and Propagation 5.2 (2023): 14-20
20. P. Vijayakumar, Sivasubramaniyan, G., & Saraswati Rao, M. (2019). Bibliometric Analysis of Indian Journal of Nuclear Medicine (2014 – 2018). Indian Journal of Information Sources and Services, 9(1), 122–127.
21. Tiwari, Gaurav, and Ruchi Tiwari. “Bioanalytical method validation: An updated review.” Pharmaceutical methods 1, no. 1 (2010): 25-38.
22. Culpa, E. M., Mendoza, J. I., Ramirez, J. G., Yap, A. L., Fabian, E., & Astillo, P. V. (2021). A Cloud-Linked Ambient Air Quality Monitoring Apparatus for Gaseous Pollutants in Urban Areas. Journal of Internet Services and Information Security, 11(1), 64-79.
23. Lindegårdh, N., W. Hanpithakpong, Y. Wattanagoon, P. Singhasivanon, N. J. White, and N. P. J. Day. "Development and validation of a liquid chromatographic–tandem mass spectrometric method for determination of oseltamivir and its metabolite oseltamivir carboxylate in plasma, saliva and urine." Journal of Chromatography B 859, no. 1 (2007): 74-83.
24. Ludhiani, Simran, Rajesh Kumar Maheshwari, and . 2022. NOVEL APPLICATION OF MIXED SOLVENCY CONCEPT TO DEVELOP AND FORMULATE LIQUISOLID SYSTEM OF A POORLY WATER SOLUBLE DRUG, FUROSEMIDE AND THEIR EVALUATIONS. International Journal of Pharmacy Research & Technology, 12 (1), 28-57. doi:10.31838/ijprt/12.01.05

25. Tanjo, T., Minami, K., Mano, K., & Maruyama, H. (2014). Evaluating data utility of privacy-preserving pseudonymized location datasets. Journal of Wireless Mobile Networks, Ubiquitous Computing, and Dependable Applications, 5(3), 63-78.
26. Beccaria, Marco, and Deirdre Cabooter. "Current developments in LC-MS for pharmaceutical analysis." Analyst 145, no. 4 (2020): 1129-1157.
27. De Ruyck, Hendrik, Els Daeseleire, Herman De Ridder, and Roland Van Renterghem. "Development and validation of a liquid chromatographic–electrospray tandem mass spectrometric multiresidue method for anthelmintics in milk." Journal of Chromatography A 976, no. 1-2 (2002): 181-194.
28. Janssen, Julie M., Niels de Vries, Nikkie Venekamp, Hilde Rosing, Alwin DR Huitema, and Jos H. Beijnen. "Development and validation of a liquid chromatography-tandem mass spectrometry assay for nine oral anticancer drugs in human plasma." Journal of Pharmaceutical and Biomedical Analysis 174 (2019): 561-566.