

MODERN PHARMACEUTICAL ANALYTICAL TECHNIQUES-RECENT ADVANCEMENTS AND APPLICATIONS

Abstract

Modern Pharmaceutical Analytical Techniques have revolutionized the drug development process, enabling the identification, quantification, and characterization of pharmaceutical compounds with unprecedented accuracy and sensitivity. These techniques play a crucial role in ensuring the quality, safety, and efficacy of pharmaceutical products. Recent advancements in pharmaceutical analytical techniques have led to the development of new and more powerful methods, such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy. These techniques have significantly expanded the range of analytes that can be detected and quantified, and have improved the sensitivity and selectivity of analysis. Modern pharmaceutical analytical techniques play a critical role in ensuring the safety, efficacy, and quality of pharmaceutical products. The continuous development of new analytical methods and technologies is essential for advancing drug discovery and development, ultimately leading to better treatments for patients.

Keywords: HPLC, LC-MS, NMR, HPTLC, HTE

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I. INTRODUCTION

For the separation, estimation, and quantification of chemical substances received from both natural and artificial sources, analytical chemistry uses drug analysis. These substances typically consist of one or more chemical substances. Qualitative and quantitative analysis are the first two main categories in the analytical chemistry process. Only the samples that can be obtained are estimated in qualitative analysis, and the total number of elements in a compound should be identified in quantitative analysis. For instance, because it considers life, the examination of a wide range of chemicals or products is beneficial for the analysis of medications. The market has seen the introduction of a significant number of medications, and demand for them is rising quickly.

The recently developed pharmaceuticals are either a brand-new variety or a modified version of ones that are already on the market. These medications are described with reference to the commercially available medications and pharmacopeial scenarios. In order to report on the better therapeutic agents for withdrawal that are currently on the market, pharmacopoeia have to be used in the medication development process. The analytical profile of a drug may occasionally not be present in pharmacopoeias during drug development. In that situation, it is required to prepare the crucial analytical methods for the creation of new medications. Many compounds are created by inventors during the medication development process, and they can simply assess their structure, behaviour, and help detect impurities in a molecule. The drug's bioassays will be conducted to determine if all the parameters have been set up to target it.

High throughput experimentation (HTE) is a notion that several industries and scientific fields have embraced recently in order to accommodate the rising number demand for speedier report creation or acceleration of the stages of product development. Regarding HTE, used in the pharmaceutical industry for a variety of purposes, including the discovery of biomarkers and as a technique to speed up the characterization of novel pharmaceutical compounds and the creation of small-molecule chemical processes, new chemical entities in drug discovery, in the analysis of bio-therapeutics, therapeutic peptides' forced degradation investigations and the certification of analytical techniques.

High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Liquid Chromatography-Mass Spectrometry (LC-MS), and other techniques are suitable for the analysis of these large or small compounds. These analytical methods are frequently employed for the detection of substances using mass spectrometry and the other methods previously discussed. HPLC, a highly helpful method, was a key and improved method for drug analysis. The liquid chromatography-mass spectrometry approach was also crucial for the analysis of pharmaceutical medications and helpful for research into the metabolism of drugs.

These methods can also be used to analyze, estimate, and identify pharmaceutical products that contain impurities, products that have undergone degradation, or products that are utilized to separate out and characterize a drug's potential from diverse natural and synthetic sources.

The following requirements are important for the analyst to build the best, appropriate, simple, and accurate method:

- To address any analytical challenge, data is necessary.
- It's crucial to work accurately and with sensitivity.
- The ideal range for drug analysis
- When developing a procedure, accuracy is necessary.

The method validation process, in which the documents are checked as part of any method development process, is also included in the method development process. In order to analyze the method, the many needs for the validation of documents include:

- Quality control
- Approval by the specified international organizations for product development
- Registration of pharmaceutical or pesticide products should be necessary.
- Only when acceptance is accomplished through testing does the validation procedure take place.
- The product should also be validated after the quality control division completes its required tasks.

If analytics are not to be the bottleneck, highly automated systems that can quickly perform several experiments in parallel also need to boost the speed at which the needed analytical information can be produced and analyzed.

For the majority of analytical procedures, A compromise is made between the quality and accuracy of the information and speed at which it can be generated. Therefore, the HTE workflow, it is important to achieve the perfect harmony between these two elements. High throughput analysis (HTA) approaches that may generate datasets in as little Considering that a minute or two are essential for HTE workflows, even though conventional analytical tools like HPLC or NMR spectroscopy normally must measure periods per sample, on the order of several minutes.

Therefore, HTA is a crucial part of HTE, and the effectiveness of any HTE platform depends on the technique and implementation choices made. Even though chromatography-based techniques offer a wealth of data, their throughput is occasionally insufficient to accommodate HTE setups for a particular experiment, such as when several 96-, 384-, or even 1536-well microplates need to be analyzed in a single day.

The analytical cycle time has already decreased significantly in recent years, making analysis may be performed now or tomorrow, for the majority of HTE operations, is necessary. To prevent analytics from becoming a time-limiting factor, additional work will need to be done on developing analytical methodologies to support future HTE configurations. Along with efficiency, choosing the right analytical tools should take into account a technique's applicability across the board and its capacity to produce "quantitative" data (absolute concentrations). The core of an HTA setup frequently consists of chromatography-based techniques due to its adaptability and selectivity because NMR is the best method for quantification since relative concentrations may be determined without the

use of standards and absolute concentrations of components within a mixture can be established with a single standard. The typical time required for one sample using NMR is several minutes. Due to the combination of its high selectivity and quick sample throughput (a few samples per second), MS has become widely used in HTA workflows. The drawback of MS-based approaches is that they cannot easily give precise quantification and may experience problems such as ion suppression and other matrix effects.

Previous evaluations of HTA have been released, covering the period up to 2019. A comprehensive evaluation of the material that was published between January 2019 and September 2020 is what this study intends to do. Survey on overview of the quickly developing realm of HTA, encompassing both existing chromatographic techniques and recently emerging spectroscopic and microfluidics-based strategies; the study was organized appropriately. The emphasis was on HTA platforms and methods in the fields of small molecule drug discovery and chemical and pharmaceutical development.

II. CHROMATOGRAPHIC TECHNIQUES

- 1. High Performance Thin Layer Chromatography (HPTLC):** This method was employed all around the world to identify, estimate, and verify the analytical profile of pharmacological compounds. It is a very sophisticated method that will be acknowledged as a key instrumental method for drug analysis. In the pharmaceutical industry, it is able to test a variety of medicinal components because of its quick separation action and adaptable nature. The key benefit of this technique is the ability to quickly test drugs while also making crude drug sample handling and cleaning simple. This method enables us to characterise the chromatogram for a wide number of parameters without time constraints.
- 2. High Performance Liquid Chromatography (HPLC):** One important technology for separating complicated mixtures of chemicals and their molecules is High performance liquid chromatography. The biological elements and chemical molecules can be encountered by this method quite effectively. Due to the adoption of HPLC, this method, which was developed in the year 1980, became the first to analyse the assay of bulk drug materials from the USP-1980.

Prior to the examination of medicines, the HPLC method called for beginning their process in terms of accuracy, precision, and a wide variety of samples were analysed before doing the HPLC. An UV detector was utilised to estimate samples using HPLC, and it was successful in determining the sample's wavelength. UV detector operation won't begin until many wavelength scanning programmes have been used.

- 3. Thin Layer Chromatography (TLC):** A very old method for analysing chemicals in medicines is called thin layer chromatography. In this method, two phases—one known as the mobile phase and the other as the stationary phase—were used. Solid phase, adsorbent, and a thin layer of silica gel were distributed on a glass plate while carrying an aluminium support during the sample preparation process. For both inorganic and organic chemical analyses, this method is employed frequently. Due to its benefit over minimal cleaning, various mobile phase selections, flexibility, capacity to load large numbers of

samples, and lower cost, TLC was chosen to investigate the chemicals. This approach was particularly useful for the examination of bulk drug components.

4. Liquid Chromatography (LC): Liquid chromatography is a crucial analysis method in many HTE analytical procedures. Reducing the measurement time per sample is the simplest technique to increase the analytical platform's throughput. Many methods have been used to reduce the length of the LC analysis, including applying high temperatures using monolithic columns or using parallel segmented flow columns. The pace of the analysis is increased by these modifications, but only modestly.

- **Ultrahigh-Pressure LC:** Although major efforts are currently being made to speed up and boost the throughput Ultrahigh-pressure LC (UHPLC) apparatus and sub-2 μm diameter stationary phase particles were created a number of years ago for the LC technique for HTA applications. Armstrong and co-workers have lowered rates of separation in the sub-second time frame (Figure 1) and are getting close to sensor-like throughput by using specially constructed machines with the use of signal processing technologies, optimised geometries, and relatively short bed lengths.

Although there has been significant development in commercially available column technology and instrumentation, more improvements that reduce the peak dispersion introduced by frits, tubing, and other instrumentation components are needed to make such performance improvements available to users in the industry. When systems are able to operate at pressures up to 3000 bar and when using columns filled with 1 μm diameter particles, the separation speed can be doubled, according to recent studies on the gain that may be anticipated from extremely high-pressure separations (Figure 2).

The "rule of diminishing returns," which states that more work is required to create progressively smaller or less meaningful improvements, appears to govern every breakthrough in this subject. This is shown by the forecast that a two-fold improvement in separation speed can be expected vs a three-fold rise in pumping pressure. The development of uniform, mechanically strong 1- μm particles, the creation of uniform 1- μm particles, the mass production of well-packed 1-1.2 μm internal diameter columns, the design of instruments and detectors with significantly reduced overall dispersion, the development of pressure-tolerant column housings, connectors, and valves, and sufficiently precise pressure-compliant flow metres are all things that the authors noted as being necessary for further advancements. Employing currently available/commercially available equipment and columns, it has been possible to accelerate by utilising extremely short columns loaded with microscopic particles and high flow rates, LC analysis can be completed in less than a minute.

While achiral analysis quickly adopted the use of small particle sizes, chiral analysis lagged before sub-2 μm immobilised chiral stationary phases (CSPs) became a commercially viable product in 2016, the field lagged substantially. Since then, several research teams have looked into using sub-2 μm fully porous particles (FPPs) for rapid enantioselective LC methods, which has allowed the analysis time to be cut down to a few minutes (Figure 3).

High back pressures and mobile phase velocities are used in columns filled with extremely tiny particles to create peak broadening because of rubbing between the moving and still phases. Lesko et al., who recently addressed this issue, demonstrated that the use of a stationary phase made of diamond that conducts heat better, might be used to reduce the influence of viscous heating.

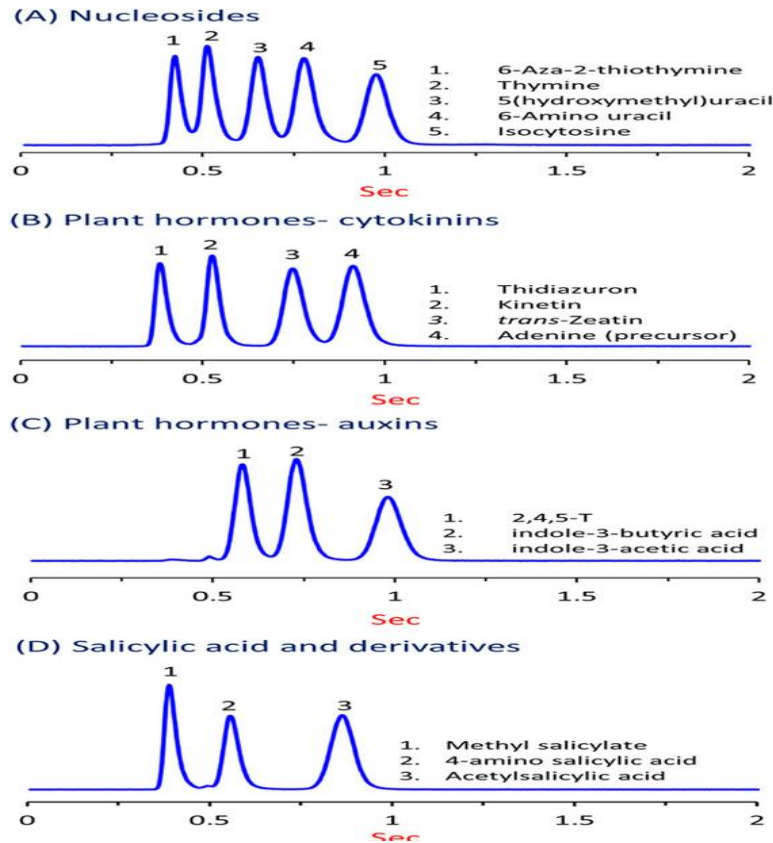


Figure 1

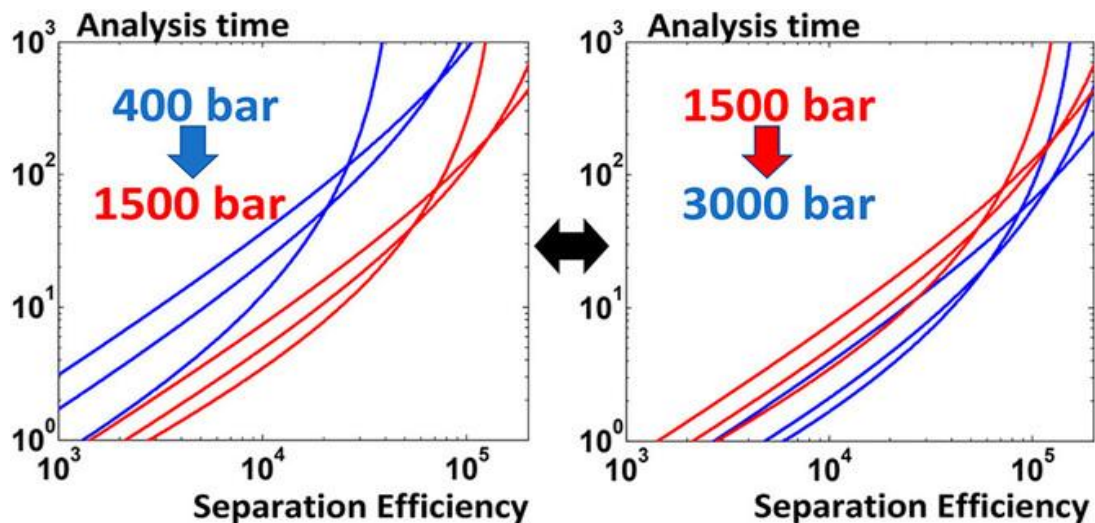


Figure 2

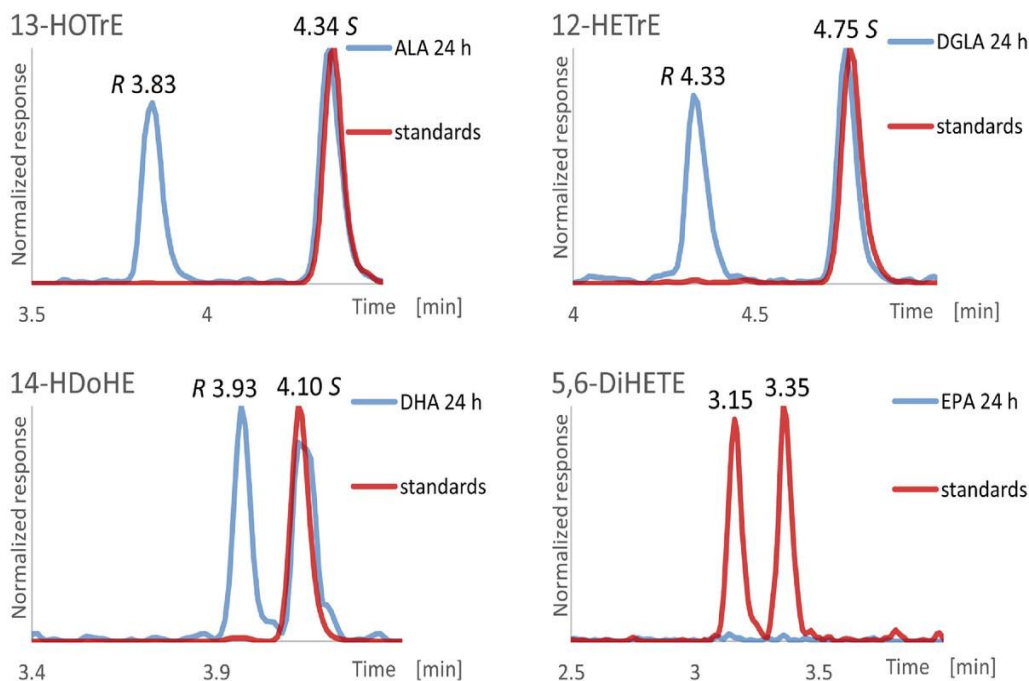


Figure 3

- Superficially Porous Particles:** In contrast to using small-diameter particles, which needs equipment capable of operating at high pressures (> 1000 bar), the use of superficially porous particles (SPPs), also known as core-shell particles, has been a popular technique for doing quick analyses. The benefit of SPP columns is that, as a result of a shortening of the analyte diffusion route, the plate heights of the stationary phase particles are significantly reduced and are comparable to those of sub-2 μm particles. The latter has an impact on the van Deemter equation's terms A and C (eddy diffusion) (barrier to mass transfer). Without using high column pressures, the better separation efficiency is achieved due to the bigger total particle diameter.

Modern SPPs were initially proposed in the 1970s, but they weren't commercially released until 2006 with 2.7 μm Halo particles that had a 1.7 μm nonporous silica core and a 0.5 μm thick porous silica shell.

Most researchers have discovered that SPPs provide greater chances for higher throughput than FPPs when contrasting particles with FPP and SPP. Columns with SPP particles out performed FPP columns when the chromatographic performance was assessed using van Deemter curves. SPP columns are the best pressure decrease, enabling for separations of less a minute with no compromise in terms of speed, effectiveness, with substantially smaller pressurized dips, according to a kinetic plot-based study.

SPPs were investigated by Godinho et al in regard to researching polycyclic aromatic hydrocarbons. They discovered that both fully porous 1.8 μm particles and superficially porous 2.7 μm particles had the same levels of selectivity and performed equally well, while the latter produced less force applied to the SPP column's back.

Throughput was enhanced by using the SPP column, which allowed them in just one minute, they were able to separate a 16-component sample combination (Figure 4). SPP columns advantages for supercritical fluid chromatography (SFC) separations have also been noted.

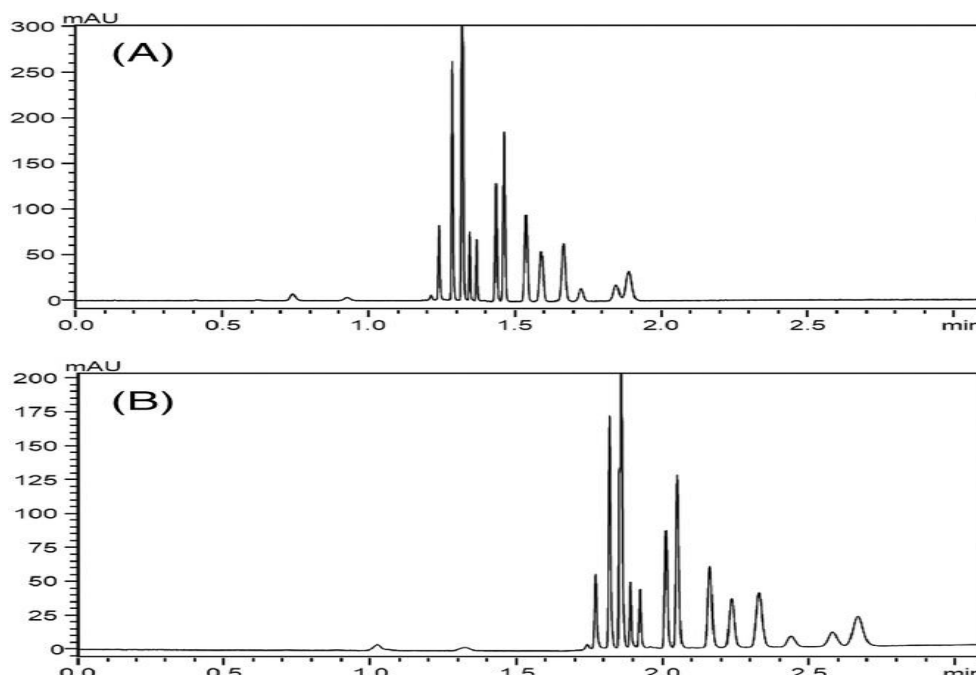


Figure 4

- **Open Tubular Columns:** Another strategy for cutting down on the common packed-bed arrangement employed in LC will be replaced during analysis time with the open tubular (OT) column style, which is typically connected to GC. The OT format, which was first presented in the 1970s, supposedly results in lower plate heights because eddy dispersion is not present, and it may also provide improved kinetic performance. Due to the trade-off between the limiting mass load capacity of such small internal diameter columns and the great efficiency (using very narrow capillaries), which causes problems with recognition, the format never gained popularity.

Calculations by Causon et al. gave advice on the design of OT columns by balancing their kinetic performance and loadability. By applying thin, porous (octadecylsilylated) silica films to the inner surface of an OT capillary column, it has been possible to increase the loadability of OT columns, leading to efficiency gains of around 15%.

Since then, only a few writers have shown that OT columns can be used for rapid LC separations. Recently, Xiang et al. used an OT column that was 2.7 cm long and 2 μ m broad to illustrate the theory. Six peptides could be resolved in 0.7 seconds using this downsized Trypsin-digested cytochrome C separations could take 10–50 seconds, and they might require a column and a better laser-induced fluorescence detection technique (Figure 5).

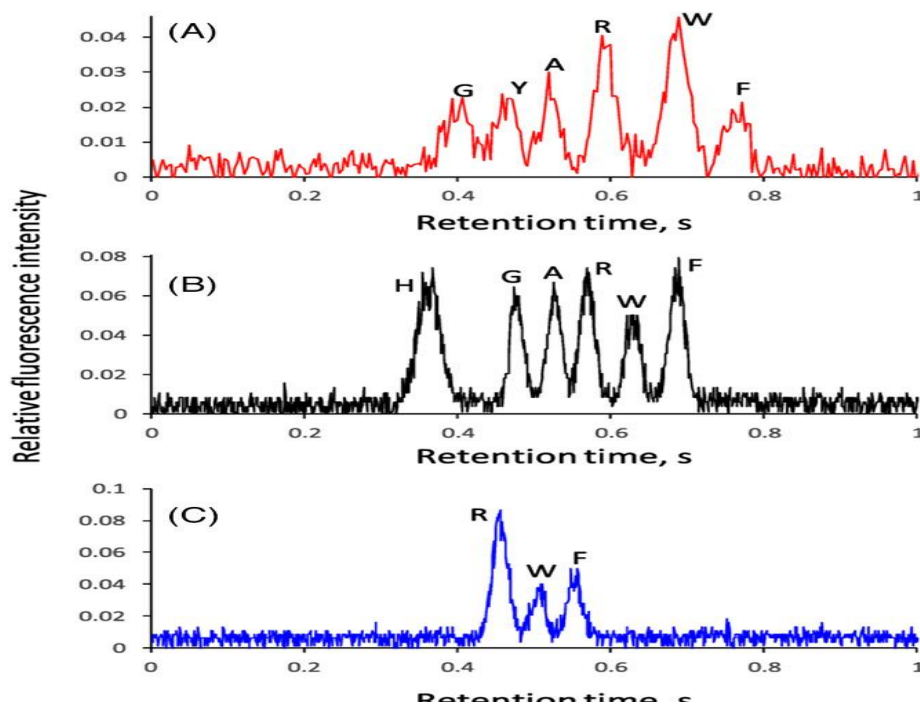


Figure 5

5. Supercritical Fluid Chromatography (SFC): The usage of SFC has frequently been linked to quick or ultrafast separations, and it is possible to achieve analysis times on the order of minutes or even seconds. SFC-based techniques typically produce superior throughput compared to LC-based ones. Armstrong and his colleagues used high-efficiency, narrow particle-size distribution silica packed in short columns and extremely high flow rates to demonstrate separations that took only a few seconds (19 mL min⁻¹). The researchers did observe that, as the analysis duration decreases in the order of seconds, various unanticipated characteristics that are not present in ultrafast LC are observed and have an impact on the system's apparent efficiency. Such effects are caused by the mobile phase's compressibility, and careful consideration must be given to the system's design, tubing selection, and back-pressure regulator designed to fully take use of the benefits of ultrafast SFC separations. There seems to be a broad understanding that instrumentation capabilities are currently outpacing column technology, and that any future improvements in performance would probably require greater instrument optimization.

In order to address the necessity to lessen extra-column dispersion, Berger published a report on the quick SFC separation of achiral solutes using 20–30 mm-long columns that were half-full of sub-2 μm particles. By reducing the system's extra-column dispersion from 80 to 5 μL², he was able to get decreased plate heights as low as 2.2 and resolve 7 solutes on a 2 cm long column in less than 8 seconds. The injection solvent and volume used for the experiment both had a significant impact on efficiency.

Similar to the advantages for LC mentioned above, utilizing in SFC mode, SPPs have been shown to be advantageous. In a study that looked at Hellinghausen et al.

"chiral's study of pesticides and their stereoisomers" stated that the majority of the chemicals they looked at employing 2.7 μm SPPs separated in less than a minute. Roy and Armstrong used high-efficiency 2.7 μm chiral SPPs, exceptionally high flow rates (up to 14 mL/min), and the low viscosity of the CO_2/MeOH mobile phase to accomplish chiral separations in 13 seconds.

The authors claim that if advancements in SFC instrumentation could address some problems with extra-column effects and pressure limitations, even quicker separations might be feasible. With such developments, the full potential of SPPs and other small particle supports would be unlocked. Recently, reports on the use of SPPs in chiral analysis under SFC conditions have also been made by other organizations.

With either 2.0 μm SPPs with a wide particle-size distribution or 1.9 μm FPPs with a tight particle-size distribution as the bonding substrate for a chiral selector based on teicoplanin, Mazzocanti et al. studied a collection of 31 derivatized amino acid racemates, with the SPP phase showing superior performance to the FPP version. Similar teicoplanin-based SPP-packed chiral columns have been used by Folprechtova et al. for SFC separations of phytoalexins, substituted tryptophans, and ketamine derivatives. Galietti et al. accomplished chiral compound screening and rapid separations using OT columns in SFC mode with turbulent flow.

To create turbulent flow conditions, the authors employed GC OT columns and CO_2 at a relatively high flow rate. They saw a decrease in plate height as the flow regime changed from laminar to turbulent, which increased peak capacity by almost three times. The observation was explained by the faster analyte dispersion over the OT column and the greater mass transfer efficiency in the mobile phase as a result of a flatter flow profile. It was possible to isolate using turbulent flow conditions in OT columns, four polycyclic aromatic hydrocarbons were detected in 2.2 seconds. (Figure 6).

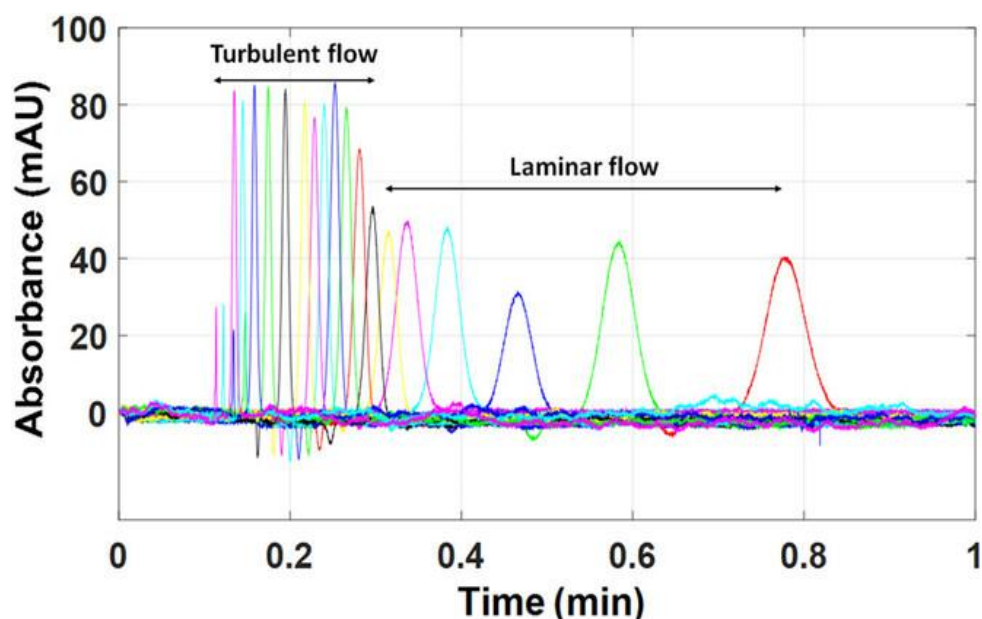


Figure 6

6. Multiple Injections In A Single Experimental Run Chromatography (MISER):

Christopher Welch created the so-called "Multiple Injections in a Single Experimental Run" (MISER) technique, while he was employed by Merck & Co. It is a well-liked and straightforward technique for high throughput monitoring of a wide range of studies by LC or LC-MS.

The use of MISER chromatography, which depends on successive sample injections and little chromatographic separation of the desired analytes from undesirable compounds, is well suited for kinetic analysis and profiling. A MISER run's solvent concentration is adjusted so that since the stationary phase interacts with the analyte(s) less, the analyte(s) can travel along the column more quickly. The purpose is to resolve matrix or interfering peaks and minimize or eliminate any possible matrix effects such as ion-suppression rather than totally separate all peaks. After the mobile phase has been tuned, the autosampler's injection rate is practically the sole factor limiting how quickly samples may be evaluated.

When comparing comparable samples of the same subject matter of interest were produced under various conditions, MISER analysis is frequently utilised. A "misergram" (Figure 7) is a compilation of results that enables quick analysis of the data. The method has been highly effective in the field of catalyst discovery, where a huge number of reactions must be screened. Although SFC- and GC-based MISER procedures have also been documented, LC or LC-MS is the method most frequently employed with them. A 96-well microplate can be analyzed using a MISER-GC-MS setup, such as the one described by Knorrscheidt et al., in 60 minutes.

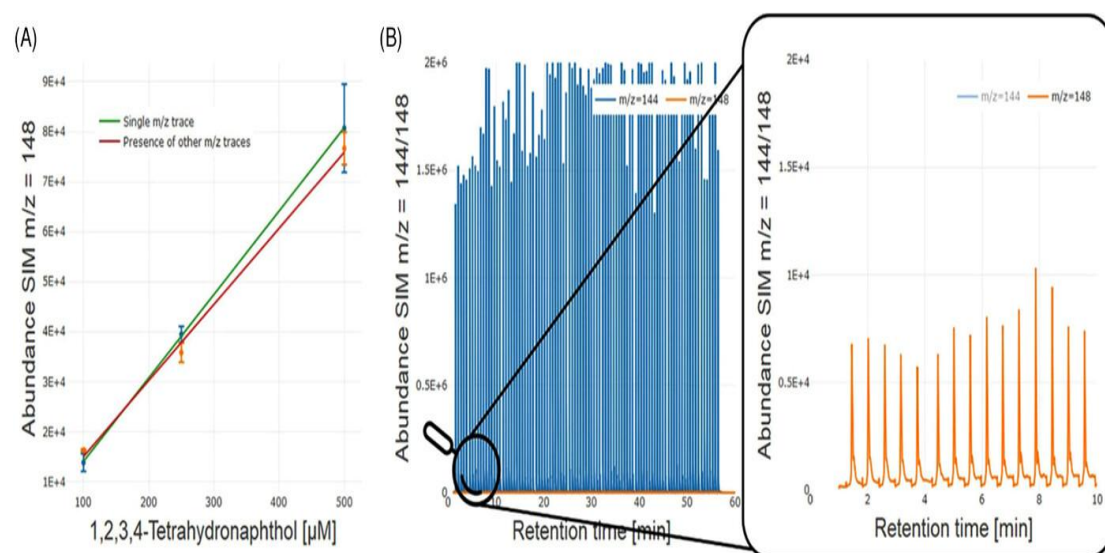


Figure 7

7. On-Chip Chromatography: One of the most important factors impacting separation efficiency and, as a result, speed and throughput, is the detrimental effect of so-called extra-column volumes (such as tubing, detectors, etc.) on peak broadening. With the aid of microfluidic systems, in which each component is located on a separate chip, is one technique to lessen these effects. Significant improvements in chip design and fabrication

have been accomplished ever since the initial LC on a microfluidic chip demonstration. The performance of these devices has been greatly improved by the application of increased strains and distinct on-chip injection and detecting methodologies. The development of fabrication techniques like 3D printing has also cleared the path for the affordable production of such devices. The enhanced heat transmission provided by the planar chip format is one of its advantages. In both isocratic and gradient HPLC modes, this characteristic has been used to separate analytes at high temperatures in under 30 seconds.

Ion mobility spectrometer (IMS) the use of a chip-based chromatographic separation device to quickly link to an IMS as a new detection method for chip-based HPLC was shown. Using a TOF-MS coupled to a chip-based LC, Zheng et al. were successful in automating a high throughput drug screening platform used in the pharmaceutical industry.

Throughput can be raised by constructing devices with numerous, parallel separation channels in addition to using downsizing to accomplish quicker separations. Recently, Komendova et al. published a study on a titanium device with four parallel channels that was 3D-printed and contained an electrochemical detector that could analyse dopamine precursors and metabolites at the same time.

- 8. GAS Chromatography (GC):** In the field of HTA, gas chromatography (GC) is a widely used pharmaceutical analytical technique which is particularly helpful in the examination of more unstable chemicals. Although traditional GC systems don't have the necessary speed to handle HTE operations, certain strategies that allow for faster analysis has been developed. Recently, Fialkov et al. combined low-pressure (vacuum outlet) GC-MS with low thermal mass (LTM) resistive heating for rapid heating and cooling of the capillary column to achieve reasonably acceptable separations with cycle periods of less than 1 min. They inserted the analytical column into an "LTM Fast GC" module mounted to a normal GC system's detector port. This module contained LTM thin-walled metal tubing.

The GC injector and MS transfer line were linked to the column's inlet and exit, respectively (Figure 8). There is a vacuum in the analytical column while the inlet runs at standard GC pressures, increasing the ideal helium carrier gas flow rate and the speed of full range separations while preserving a maintainable level of chromatographic separation quality. In some common applications, the LTM-LPGC-MS combo could reduce the analysis time from minutes to seconds while providing a 64-fold increase in analysis speed compared to ordinary GC-MS (Figure 9).

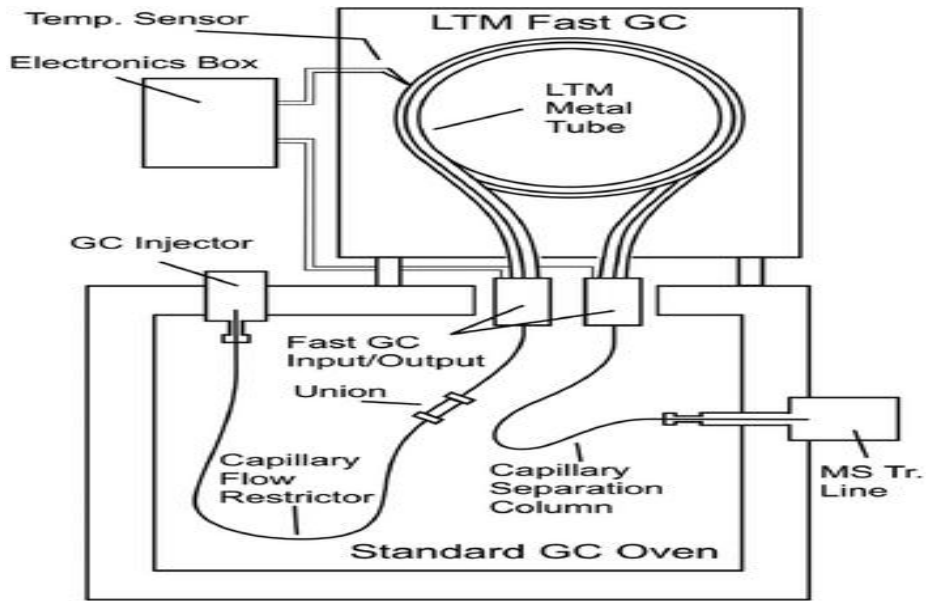


Figure 8

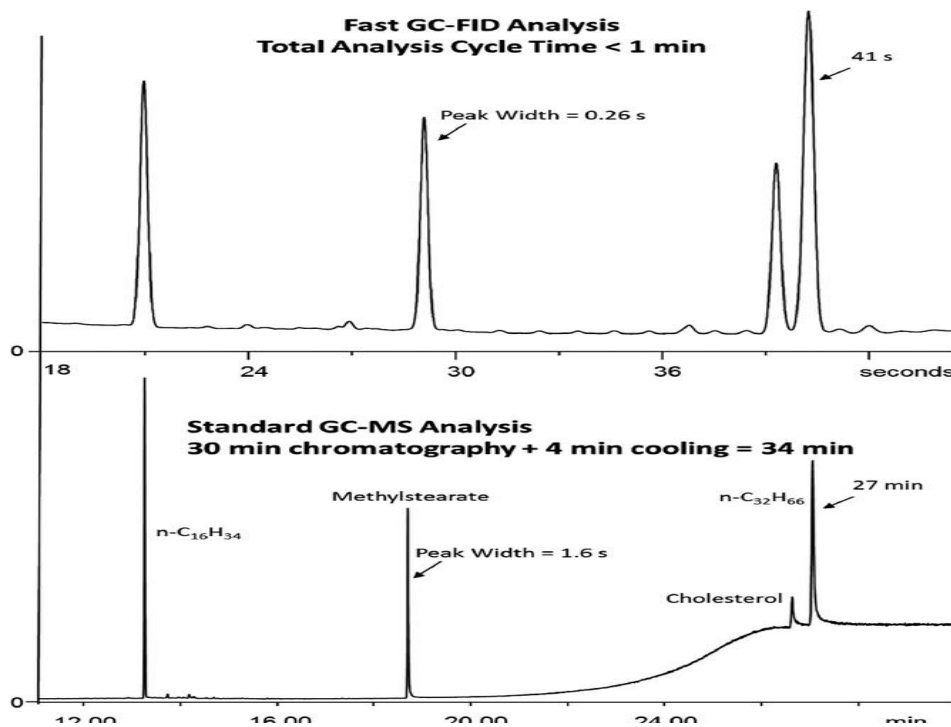


Figure 9

III. ANALYTICAL METHOD DEVELOPMENT TECHNIQUES

Various techniques were utilized in analytical chemistry for the quantitative and qualitative determination of pharmaceuticals with their precision for developing methods.

Spectroscopic Methods: The most crucial technology for the method development

procedure was spectroscopic technique. In our pharmacopoeias This method is based on the organic UV light absorption as well as other chemical processes. The entire foundation of spectroscopy is the quantitative measurement, transmission characteristics, and wavelength function. This strategy has been quite helpful for saving time, or the exertion of labour. Moreover, this approach has excellent accuracy and precision. This is a pharmacological analysis a unique methodology was used to examine the dose forms in the pharmaceutical industry has consistently grown. Additionally, there are a few aspects for colorimetric techniques include:

- Complex reaction formation
 - Oxidation and reduction processes
 - The catalytic ions impact
- 1. Ultraviolet (UV) Spectroscopy:** The energy, radiation, or excitation of electrons is the foundation of the UV visible spectroscopy technique. In the UV-Visible technique, the energy light used to excite electrons is what determines the sample's wavelength. The absorbance ranges from 200 to 800 nm. Only when conjugated pielectrons are present does the absorption take place.
 - 2. Ftir Spectroscopy:** A few atoms and molecules vibrate or are excited when infrared spectroscopy causes the absorption to move into its lower energy state. This technique helped scientists create a novel way by identifying the functional group and the original peaks with relation to the molecule.
 - 3. Mass Spectroscopy (MS):** When it comes to HTA, MS-based analysis offers advantages that are extremely desirable, such as fast speed and the capacity to detect and quantify the chemicals in a combination. For example, strike and run detection or the capacity to precisely monitor the mass of a single target is very useful for chemical reaction screening. These benefits have accelerated the industry's acceptance and creation of MS-based HTE solutions operations and have encouraged university research efforts to create novel methodologies. Utilizing automated sample preparation processes and quick, optimized UHPLC techniques, considerable increases in analysis speed have been made in standard LC-MS setups that rely on ionization techniques like ESI, APCI, or APPI.

Pu et al and Kempa et al. have provided more detailed reviews of cutting-edge MS-based HTA tools and methods that were more focused on sample preparation processes that are automated or eliminated in strike and run generation or response monitoring and improvement.

Agilent's "RapidFire MS," which combines a commercially available MS system for HTA is one that uses SPE-based sample-cleanup robotics workflow with ESI-MS detection and provides analysis speeds as low as 5-10 s per sample. When a throughput of up to 5000 samples per day is required for screening or chemical profiling operations, such platforms are frequently used. The technique, however, cannot handle the requirements of screening tests, which call for the daily analysis of tens of thousands to millions of samples.

These requirements have led to the development of techniques like surface-based MS and microfluidics devices, which require little to no sample preparation and speed up analysis time into the sub-second range. For instance, the HTA field soon embraced direct infusion (DI) MS techniques or flow-injection (FI) MS, in which materials are immediately injected into the ionization source. The introduction of samples without prior separation or purification has the disadvantage of perhaps causing ion competition, reducing sensitivity, or obscuring the outcome. In metabolomics and lipidomics analyses of serum samples, we investigated the distribution of ion m/z values and computationally generated a series of optimum scan ranges. Sarvin et al. described a method to get around this impact.

The sample rate, which is typically significantly slower than the actual collection of MS data, is the most frequent bottleneck in DI or FI analysis. In order to effectively use the MS system's characteristics, the instrument needs to be quickly fed with small amounts of material. New methods of sample introduction were created since the rate at which an autoinjector may sample each experiment and introduce it into the MS ionization chamber naturally limits the speed of classic LC-MS methodologies. Due to this, the term "ambient ionization MS" (AIMS) refers to a novel family of ionization procedures in which samples are ionized at air pressure.

In order for quick in situ analysis to be possible with some surface/plate-based procedures, materials must first be either incorporated into a suitable matrix or placed onto a suitable carrier. Because AIMS approaches provide throughputs orders of magnitude greater than those of conventional LC-MS apparatus, reaching analytical speeds that are feasible with the use of fluorescent markers is not necessary when using fluorescence-based approaches. These advancements have completely transformed the HTA field. Many surface-based MS methods, including self-assembled monolayers coupled with desorption/ionization, matrix-assisted laser desorption/ionization (MALDI), direct analysis in real-time (DART), desorption electrospray ionization (DESI), secondary ion mass spectrometry (SIMS), and others, can directly ionize analytes with little to no sample preparation (SAMDI).

Kuo et al. have given a thorough analysis on the numerous AIMS strategies that have been described up to this point. We'll concentrate on a few of the more popular methods down below. Some AIMS methods, such as surface-assisted laser desorption/ionization mass spectrometry (SALDI), have been employed for ultrafast photocatalyst screening and online monitoring of photocatalytic activities. In this investigation, the substrate for starting and monitoring the processes simultaneously was a photocatalytic nanomaterial. The tests demonstrated a reaction acceleration effect, which is interesting for ultrafast reaction screening because interfacial reactions occurred in seconds as opposed to conventional reactions in the bulk phase lasting hours.

- **Matrix-Assisted Laser Desorption/Ionization:** MALDI-MS is one of the first and most established types of ambient, surface-based MS. Prior to being subjected to UV laser light, the analyte is co-crystallized on a surface with the suitable matrix, causing the analytes to desorb and ionise. For ultrahigh-throughput screening of big molecules, Combining MALDI and time-of-flight (MALDI-TOF) MS has been used extensively. There are numerous examples in the literature of how to identify

pharmacological targets using proteomics and how to analyse DNA/RNA, lipids, oligosaccharides, and synthetic polymers. due to matrix interferences and the lower mass range organic matrix molecules' fragmentation, conventional MALDI-TOF-MS has a number of disadvantages when investigating tiny compounds.

As a result, its application to the study of tiny molecules has not been explored as thoroughly. However, other teams have shown that MALDI may be used even for the fast examination of reaction mixtures containing catalysts, salts, and bases in small molecule applications (for example, 1536 reactions in under 10 minutes). Park et al. with the use of a parylene-matrix chip and MALDI-TOF-MS for the high throughput quantification and analysis of small-molecule cancer biomarkers. Blincoe et al. has published a practical guide for bench chemists on how to develop and evaluate high throughput MALDI-TOF-MS procedures for the screening of chemical processes on well plates without utilising any MALDI plate modification or product tagging (Figure 10).

Simon and colleagues demonstrated the effectiveness of automated, direct MALDI-TOF-MS as a readout strategy for prolonged drug discovery HTS operations. They swiftly, robustly, and precisely identified inhibitors of human cyclic GMP-AMP synthase using a screening technique using a 1536-well format and MALDI-TOF.

Different MS ionization techniques have difficulty analyzing or assaying chemicals in biological matrices. The problem could be caused by the matrix's ability to suppress ion signals or by an element that is generally incompatible with the MS technique, such as the presence of nonvolatile salts or, in the case of MALDI, a matrix crystallization inhibitor. In a study published in 1987, Krenkel et al. looked at the application in complicated biological fluids using liquid support matrices for the MALDI-MS identification of peptides, antibiotics, and lipids.

The intricate makeup of the sample and the present hardware constraints were shown to be the key determinants of throughput and robustness. It was shown that standard label-based, non-MS assays could be changed to accommodate MALDI-MS analysis and that the addition of a second MALDI spot washing phase could lower buffer concentrations and allow the use of MALDI-MS for more studies. The liquid atmospheric pressure MALDI is a handy way to combine the analytical speed of conventional solid-state AP-MALDI and the adaptability of ESI.

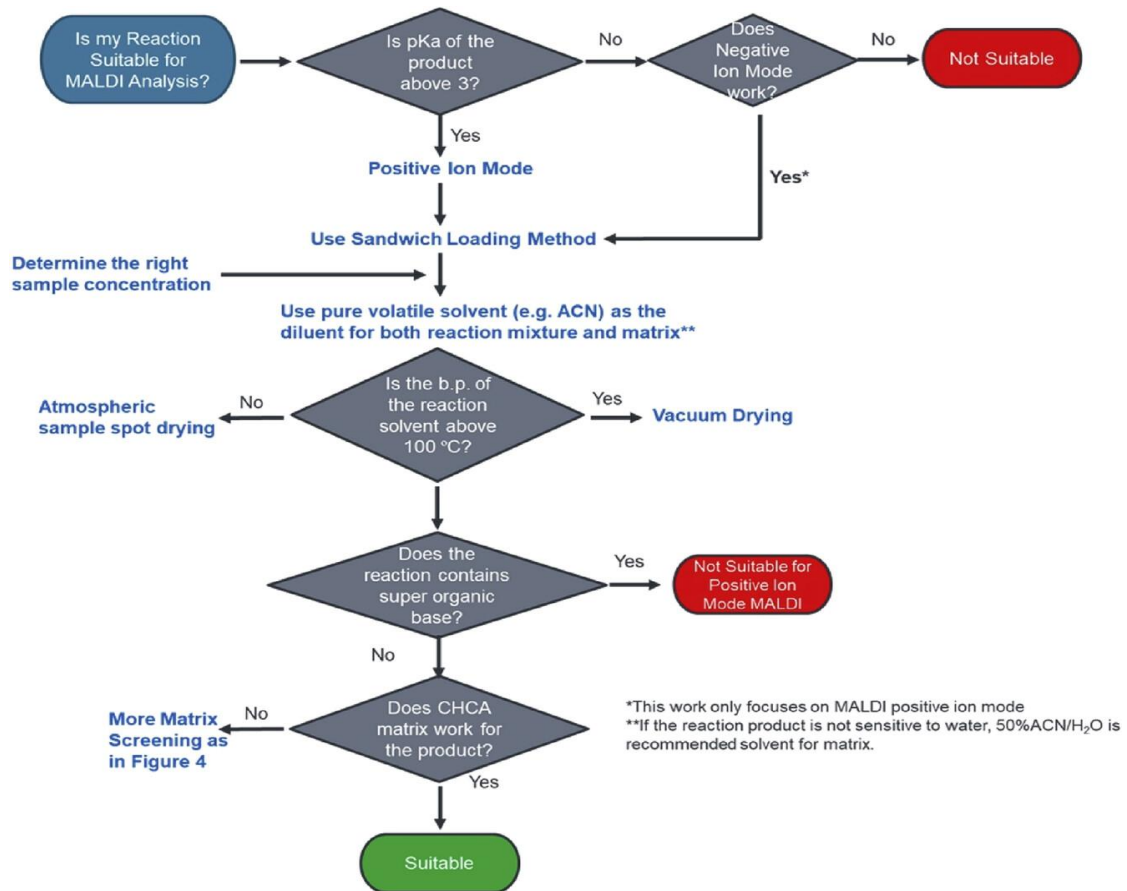


Figure 10

The authors' calculations show that liquid AP-MALDI could possibly process more than it is a very competitive technology, processing 10–20 samples per second, when compared to conventional label-free MS technologies (Figure 11).

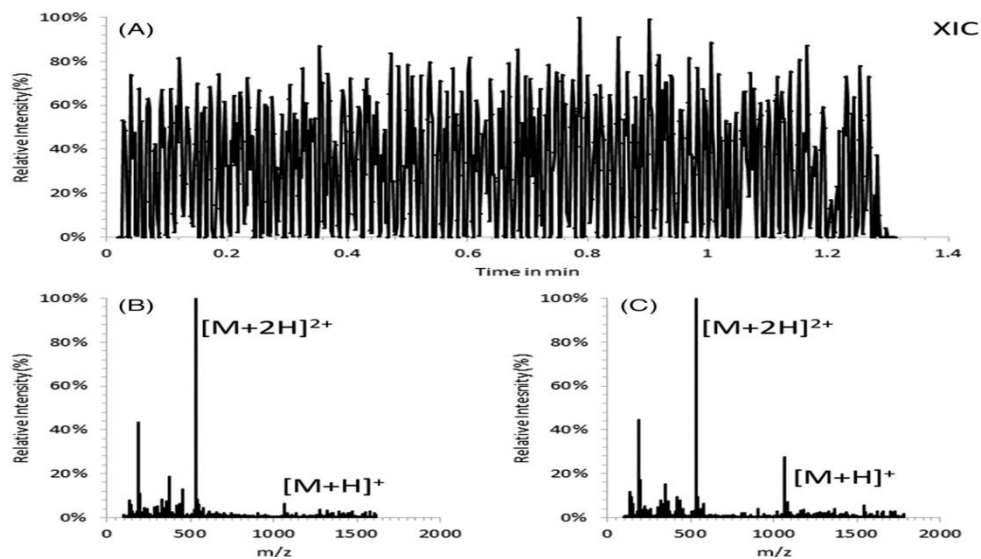


Figure 11

- **Direct Analysis in Real Time/Desorption Electrospray Ionization:** other MS techniques, like desorption electrospray ionisation (DESI) and direct analysis in real time (DART), were developed from the MALDI approach. DART has gained popularity as a tool in fields like food safety monitoring, environmental applications, and the detection of tainted or counterfeit drugs due to its ability to rapidly screen analytes in complicated matrices with little sample preparation. For the purpose of screening multiresidue pharmaceutical medicines in samples of bovine tissue, the HTS methodology used by Khaled et al. is based on SPME-DART. They demonstrated that DART could efficiently ionise and quantify at the required level 53 percent of the 98 target analytes, and that overall analysis times might be as little as one minute per sample thanks to the fully automated sample preparation approach. Their study highlights the potential benefit of SPME-DART-MS/MS as a tool for fast analysis in food safety detector applications, even if DART demonstrated limited capabilities in terms of analyte coverage.

DESI is a different ambient ionisation method that has gained a lot of interest. It has been a hot research issue in the HTA field ever since its start in 2004. An analyte coated on an insulating surface or an aqueous spray directed onto an insulating sample, such as polytetrafluoroethylene, is used in the simplest DESI-MS setup (PTFE). The DESI-MS inlet can then be moved across the surface using an x-y stage, creating a 2D chemical information map in the form of full mass spectra.

The DESI approach has two advantages over MALDI-MS: it doesn't require a matrix to conduct the experiment, and it can produce multiply charged ions, which extends the mass range of the detector needed for large molecules or biological materials. The desorbed ions are sampled using a commercial ion trap mass spectrometer with an air interface connected to a long, ideally flexible ion transfer line made of metal or an insulator. Peptides and proteins that are found on surfaces made of metal, polymer, and minerals have all been effectively ionised using DESI. The capacity to record mass spectra of samples in their natural environment enables extraordinarily rapid analysis with high sensitivity and high chemical specificity, traits that are widely wanted for HTA. This eliminates the need for sample preparation by generating ions outside the instrument. In recent years, the HTA for small drug response screening and optimization has significantly utilised the DESI technique. If the DESI spray/analysis solvent is methanol, for instance, Cooks and colleagues optimised amine alkylation processes on PTFE membrane substrates.

Reagents that can only react with particular functional groups present in the mixture are added to the spray solvent in one type of DESI, known as "reactive DESI," which is used to screen chemical reactions. Microdroplet-surface collisions can produce more reactions than bulk solutions do.

In order to qualitatively predict the outcomes of rapid screening of N-alkylation processes based on microfluidics, Loren et al. used DESI-MS as a tool. The number of critical reaction factors, such as the kind of solvent, could be quickly reduced by using DESI-MS HTA. Fedick et al. published similar research with a focus on the Reductive amination and Suzuki-Miyaura cross-coupling reactions. These were able to test numerous reagents, bases, and stoichiometries using their

DESI-MS technology, which used microdroplet-based reaction acceleration, at rates that were almost ten thousand reaction mixtures each hour (i.e., approximately 3 Hz).

Thompson, Cooks, and colleagues used HTE in flow settings to optimise nucleophilic aromatic substitution processes. A system with a DESI-MS module and a liquid handling robot for preparing the reaction mixture allowed them to analyse 3072 different reactions at a rate of about 3.5 seconds per reaction. Microtiter arrays were used to carry out the reactions. Heat maps were created from the MS data using proprietary algorithms, allowing for quick examination and selection of the most favourable situations (Figure 12). Similar methods were employed by similar research group authors to screen 3840 different reductive amination reactions.

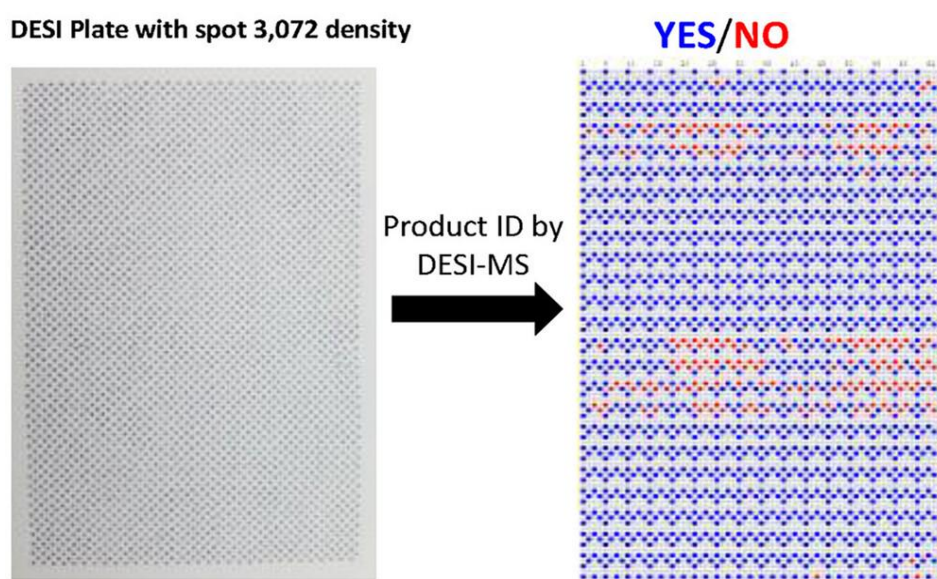


Figure 12

N-alkylation, N-acylation, and N-sulfonylation processes were studied, Sobreira and co-workers achieved analytical times up to one combination reaction per second using DESI-MS. The measurements revealed strong reproducibility (94-97%) and an about 6% rate of false negatives (depending on the chosen noise threshold).

For enzymatic experiments, which are typically carried out using labelled chemicals and plate readers, Cooks and colleagues employed DESI-MS. The performance of analyses straight from the bioassay matrix was made possible by DESI-MS, which had an effective analysis time of 0.3 s per sample. After the external calibration, the substrate and product were monitored to study the enzymatic process.

- **Acoustic Droplet Ejection:** Recent advancements in acoustic sample handling and the introduction of MS interfaces have considerably reduced the entire analysis time by eliminating the requirement for spotting samples onto a surface or plate prior to the actual analysis. These acoustic ejection MS (AEMS) systems are now commercially available, such as the (AMI) Acoustic Mist Interface from LabCyte or Waters and the Echo MS system from SCIEX. AEMS uses an open-port interface for

continuous fluid transfer (OPI) to acoustically dispense (i.e., eject in a contactless manner) sample droplets of femto- to nanoliter volume for subsequent ionisation at air pressure. Electrically charged droplets are directly expelled from well plates into a specialised transfer interface in the AMI format, where they undergo ESI mode desolvation and ionisation.

In the area of drug discovery, researchers from AstraZeneca have discussed the creation and use of an AMI interface for the metabolic profiling and triage assays. This technology could transmit three samples maximum into a mass detector at a rate of three nanoliters per second without making contact. A single mass spectrometer was able to process 100,000 samples per day thanks to automated plate handling. AstraZeneca's collection of 2 million small compounds was utilised to find inhibitors of a human histone deacetylase as a proof of concept. A study on the use of an ultrahigh-throughput reader platform based on ADE-OPI-MS for reaction screening in the context of drug development was just recently published by DiRico and colleagues at Pfizer (Figure 13).

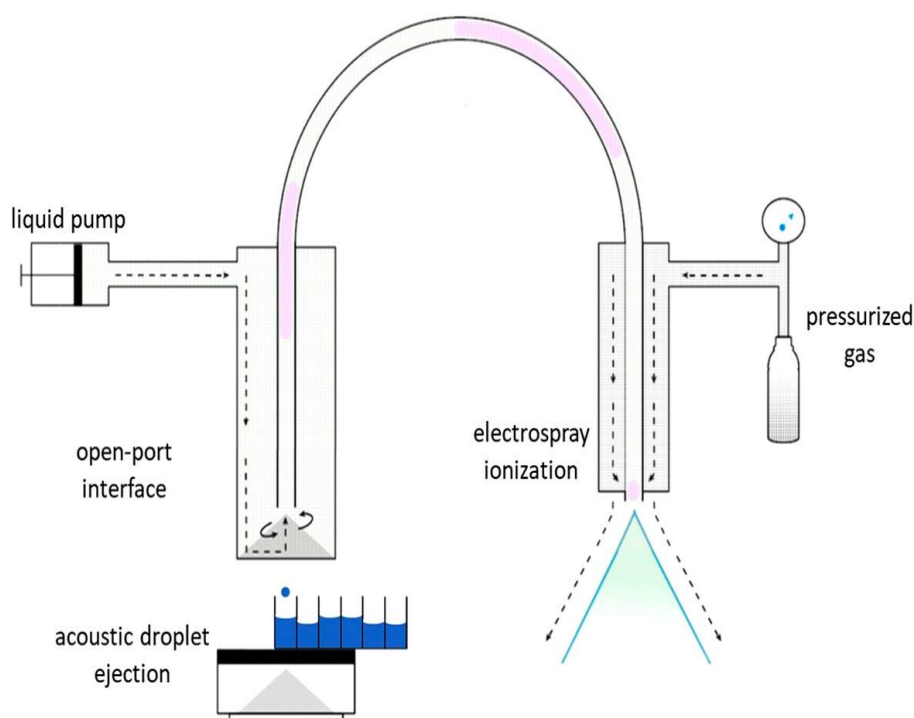


Figure 13

AEMS has also been used to analyse polar analytes in the field of bioanalysis. It is challenging to measure these analytes in biological matrices using conventional LC-MS/MS because of their poor chromatographic retention and the ion suppression brought on by co-eluting matrix components. An AEMS-based technology for performing Polar compound bioanalysis with ultrahigh throughput and without chromatography was introduced by from Bristol-Myers Squibb, Wagner et al. Results from genuine test samples acquired by AEMS were compared to those from the quickest previously published LC-MS/MS technology, and a 15-fold speed advantage

and nearly 500-fold lower sample consumption were observed. Boehringer Ingelheim's Häbe et al. published a study on a system using AEMS, it was used in HTS and was capable of sampling at a maximum rate of 6 Hz.

AEMS-based platforms have recently been the subject of a number of intriguing articles that highlight their exciting screening and profiling possibilities for HT compounds. In order to help with technique confirmation and adjustment of the surface tensions and viscosities of the samples, and of the acoustic dispensing parameters, Liu et al. proposed a new approach to measure the volume of individually dispersed droplets. Additionally, they assessed a system that was developed in-house for various important performance indicators, including well-to-well sampling speed, droplet volume calibration, precision, and reproducibility. They talked about the different operating modes and showed how a 1536-well plate format was used for the first time in AEMS. A "continuous infusion" mode that delivered an enhanced ion statistic, a higher signal-to-noise ratio, and a maintained steady-state signal for analyte detection optimization was also presented. The OPI and ESI electrodes in their design had a significantly reduced transmission distance for maximum throughput, and it was capable of sampling at speeds of up to 6 Hz.

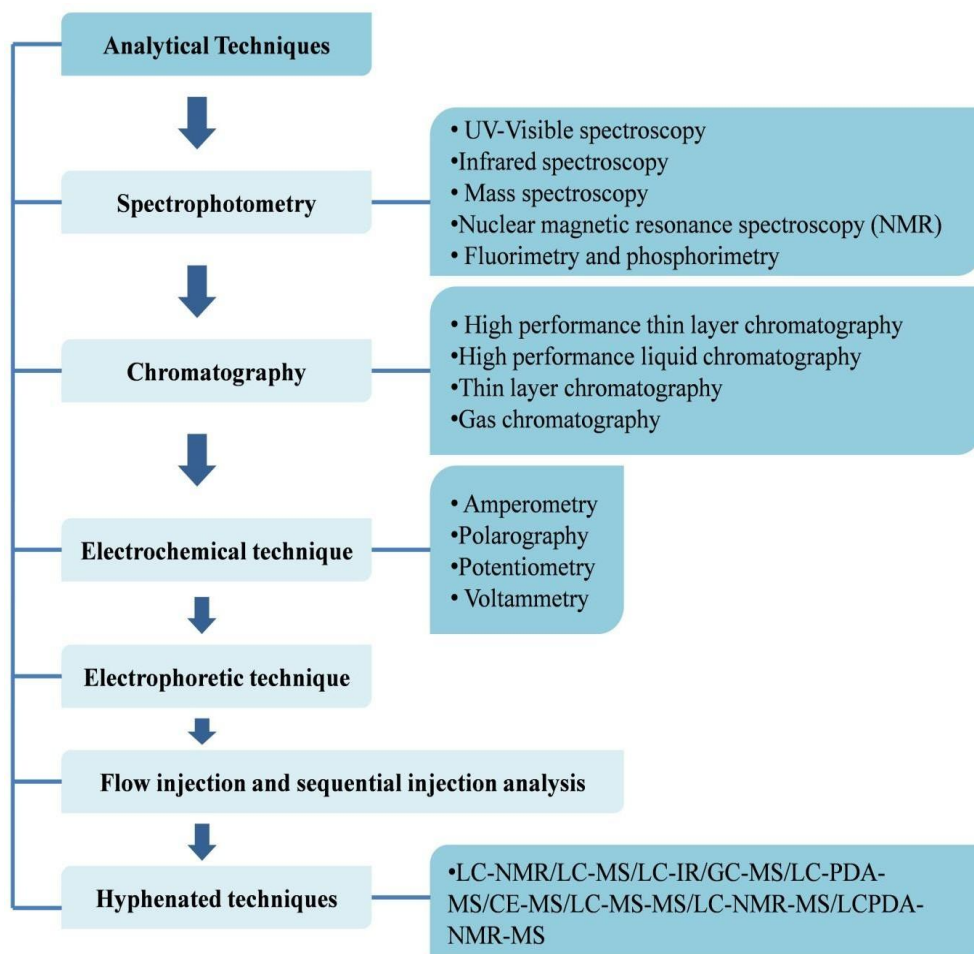


Figure 14: Method Development Techniques At Hand

- **Nuclear Magnetic Resonance Spectroscopy (NMR):** In the recent years, scientists have developed a variety of ways to solve the analysis issues with novel pharmacological compounds. For the creation of pharmaceuticals, the nuclear magnetic resonance spectroscopy method was extensively used. This method helped with drug identification and quantitative drug analysis to identify compounds in the medications. Additionally, this method's technique helped describe the chemical products, identify the medications employed in pharmaceutical formulations, and identify biological fluids.
- **Phosphorimetry and Fluorimetry:** Fluorimetry and phosphorimetry techniques were continuously improving in our pharmaceutical sectors for the study of tiny samples. Fluorimetry allows for the analysis of highly sensitive systems without sacrificing method specificity or precision. In earlier investigations, a steady increase in the number of applications for fluorometry or phosphorimetry was seen. These methods for estimating various medications quantitatively that are available in the form of biological fluids have been used for some time.
- **Electrophoretic Technique:** Capillary electrophoresis is the correct name for this method, which is crucial for drug analysis in the pharmaceutical industry (CE). The entire basis of the capillary electrophoresis technology is the electromagnetic field applied to electric charge ions. For the separation and analysis of medication components, this method was helpful. The area of traversing the components of a particular peak during the electrophoresis process is directly proportional to the compound concentration, and because of this phenomenon the quantitative analysis of samples were done using this practical technique.
- **Flow Injection Analysis:** Ruzicka and Hansen introduced the flow injection analysis technique (FIA) in the US and Denmark. This method is based on the automatic chemical experiments. Therefore, the study's authors claimed that FIA has a strong interest in automating chemical analysis. Additionally, it is the primary tool utilised for chemical analysis or measurement when there is chemical and physical equilibrium.
- **Electrochemical Techniques:** Currently, there is a greater need for electrochemical methodology in the pharmaceutical industry than there was in the past for the analysis of medicinal molecules. Additionally, a variety of samples are provided for both the quantitative examination of pharmaceutical ingredients as well as drug analysis. Recent advancements in electrochemical techniques have made it possible to analyze medications like trimipramine, desipramine, and imipramine, among others, using amberlite XAD-2, titanium dioxide nanoparticles, and carbon plates containing glassy carbon. The following methods, including chronocoulometry, cyclic voltammetry, electrochemical impedance spectroscopy, and adsorptive strip pulse voltammetry, were employed to ascertain the electrochemical behaviour of these compounds

Table 1: Drug Detection Using a Range of Electrochemical Methods

Technique	Drug	Electrode nature
Polarography	Ciclopirox olamine	Static mercury drop electrode (SMDE) or Dropping mercury electrode (DME)
	Anti-cancer drug, Vitamin K3	Polished glassy carbon electrode (GCE)
Potentiometry	Pentoxifylline	Multi walled carbon nanotube paste electrode
	N-acetyl-L- cysteine	Mercury film electrode
Amperometry	Verapamil	Dropping mercury electrode
	Diclofenac	Carbon paste electrode
Voltammetry	Leucovorin	Silver solid amalgam electrode
	Dopamine	Differential pulse stripping Voltammetry

- **Kinetic Analysis Technique:** The kinetic approach was created in 1950 and is utilised in automated devices for the analysis of numerous pharmaceutical components. The notion of kinetic methodology, which aids scientists in chemical instrumentation processes or is highly relevant in pharmaceutical drug analysis, data analysis, and method creation, was the major implementation made. Because the available procedures for drug analysis could halt their flow system and adding reagent continuously was slow, this method was entirely dependent on an automatic system.

- **Hyphenated Techniques:** A new approach for drug analysis known as hyphenated techniques will be developed using the separation technique based on the coupling of offline and online separation. This method has been used extensively throughout the years in analytical research to progress, develop, and apply pharmaceuticals in pharmaceutical analysis. The primary analysis phase for the development of novel pharmaceuticals and drug products is the determination of the medication's base material from biological sources. The following hyphenated strategies were employed to boost the possibility of drug analysis:
 - Liquid chromatography-Nuclear magnetic resonance (LCNMR)
 - Liquid chromatography
 - Mass spectrometry (LC-MS)
 - Liquid chromatography-Infrared spectrometry (LC-IR)
 - Gas chromatography-Mass spectrometry (GS-MS)
 - Capillary electrophoresis-Mass spectrometry (CE-MS)
 - Liquid chromatography-Photodiode array-Mass spectrometry (LC-PDA-MS)
 - Liquid chromatography-Mass spectrometry-Mass spectrometry (LC-MS-MS)
 - Liquid chromatography-Nuclear magnetic resonance Mass spectrometry (LC-NMR-MS)

- Liquid chromatography photodiode array-Nuclear magnetic resonance-Mass spectrometry (LCPDA-NMRMS)
- **Microfluidics:** The application of microfluidics for ultrahigh-throughput testing and analysis is another area that has experienced tremendous progress, particularly in the last ten years. Similar to acoustic ejection technology, using microfluidic devices and droplet producers eliminates the need to deposit samples onto a substrate before inserting them into the MS detector. With the use of this technology, it is possible to precisely alter sample quantities in the microliter to femtoliter range, which is valuable for automating routine laboratory tasks in a droplet-based, miniature format. Chemical reactions, for instance, can be carried out inside isolated droplets rather than individual wells employing segmented flow and two immiscible fluids. Each droplet can then be subjected to direct coupling to MS detection for analysis.

A multiwell plate workflow and droplet microfluidics technology were coupled to MS detection utilizing both ESI and MALDI, as described by Sun and Kennedy in 2014. They developed segmented flow microdroplets from a 384-well plate using a multichannel syringe pump and capillary tubing in their initial, quite basic setup. A single channel's individual droplets were fed into an ESI-MS detector at a rate of 0.58 Hz. They were able to achieve an overall analytical rate of 4.5 Hz in ESI mode by using eight parallel channels. In a more recent publication, utilizing a "nanoESI" emitter at nL/min flow rates, the reaction volume was further reduced to a few nL or even the pL range, producing throughputs of up to 10 droplets per second. For as long as 2.5 hours, or the analysis of more than 20 000 samples, continuous infusion of droplets into the nanoESI emitter for monitoring in-droplet enzymatic reactions was demonstrated. Additionally demonstrated were a sample-to-sample carryover of 3% and a linear concentration-based response. A method known as "mass activated droplet sorting" was employed in an even more advanced setup to sort nanoliter droplets in a high throughput manner using direct ESI-MS detection (MADS). The produced droplets are divided into two sections, unlike fluorescence-activated droplet sorting (FADS), one for MS detection and the other for sorting and collection. The range of detectable analytes can now include almost any molecule that can be ionised via ESI when MS is used in place of fluorescence-based detection. The stated setup had a throughput of 0.7 samples/sec, although it was determined that speed might be increased by addressing current MS system restrictions. In applications where sample preparation and analysis are bottlenecks, such as in the directed evolution of enzymes, the ability of microfluidic to produce, analyze, and sort microscale samples is appealing. Traditional well-plate methods cannot compare in speed or volume to the high throughput screening and selection of water- in-oil microreactors made possible by microfluidic droplet sorting

IV. MODERN ANALYTICAL TECHNIQUES FOR PHARMACEUTICAL DRUG DEVELOPMENT

1. **High Performance Thin Layer Chromatography (HPTLC) Automated Development:** The advanced method of improving Thin Layer Chromatography is called High Performance Thin Layer Chromatography (HPTLC). With the aid of a thin layer

chromatography plate, automation in the HPTLC technique helps to overcome the size of the droplets and the applied location of the sample. Due to its benefits over the reliability for the quantitative estimation of some analytes in microgram, and nanogram amount, this technique has become the most efficient tool in recent times.

- 2. Reverse Phase-High Performance Liquid Chromatography Development (RP-HPLC):** This method is easy to use and effective for identifying the enzymes ATP, AMP, ADP, NADP⁺, NAD⁺, NADPH, NADH in human erythrocytes. Reverse phase-high performance liquid chromatography was used to analyse these enzymes using supecosil LC-18 columns of 5 μ m, and the enzymes were detected using ultraviolet visible spectroscopy. Reverse phase-high performance liquid chromatography and reverse phase chromatography both use a polar mobile phase or a stationary phase that is non-polar and aqueous in nature.
- 3. Parallel Analysis:** The development and validation of the TLC Densitometric method for the simultaneous measurement of Bergenin, (+)-Catechin, Galicin, and Gallic Acid as well as the quantification of β -Sitosterol by HPTLC are described. A technique for microbial detection known as bioautography is connected to flat chromatography methods. It is mostly based on the tested compounds antimicrobial or antifungal capabilities. LC-MS Approach: Sensitivity, selectivity, speed of analysis, and cost-effectiveness make LC/MS procedures useful for a variety of pharmaceutically relevant substances. Tools are now simpler to use and more dependable as a result of ongoing improvements to these analytical functions.
- 4. Automation of the Injection Process:** As strict Good Laboratory Practice (GLP) and Manufacturing Practice (GMP) regulations demand in-depth analysis of large quantities of samples during all stages of the process, and manufacturing of a pharmaceutical formulation, automation is a key requirement in modern pharmaceutical analysis and quality control.

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