Blood pressure in blood vessels reaching 140/90 mmHg or above is diagnosed as hypertension. Blood pressure is a representation of the systolic and diastolic measurements. Contraction of the heart results in a certain pressure in the blood vessels, which is measured as the systolic number, and the diastolic number is a measurement of the pressure in the vessels when the heart rests between beats. The complexity of hypertension includes primarily heart damage and other conditions such as angina, heart attack, and heart failure (Figure 1). Globally, 1.28 billion individuals (30–79 years) have hypertension.

The pathophysiology of hypertension still has a lot of ambiguity. The factors contributing to hypertension include cardiac output and peripheral resistance, autonomic nervous system, renin-angiotensin system, insulin sensitivity, endothelial dysfunction, vasoactive substance, genetic factors, hypercoagulability, diastolic dysfunction, and so on. Other annotations of pathophysiology include renal pressure natriuresis impairment, where renal function impairment, sympathetic nervous system inappropriate activation, and improper salt and water excretion regulation by the kidneys.

A chronic metabolic disease typified by heightened blood glucose levels is diabetes. Type 1 (T1DM) and type 2 diabetes (T2DM) are the two main forms of the disease. Juvenile diabetes, commonly known as type 1 diabetes or insulin-dependent diabetes mellitus, is the most common form of diabetes in young people wherein the pancreas is not able to produce even little insulin or no insulin production. In contrast, type 2 diabetes mellitus is exemplified by the individual body resisting insulin over time or not producing insulin. Hyperglycaemia is the most common outcome of uncontrolled diabetes. Straightaway worldwide, 700 million adults are expected to have diabetes by 2045, as per the statistics of the international diabetes federation. Apart from T1DM and T2DM, the other two categories are gestational diabetes and secondary diabetes (caused by unknown conditions) (Figure 2). Autoantibodies ally with immune-mediated β-cell destruction, which is the main parameter of autoimmune type 1 diabetes mellitus. Insulin resistance and β-cell dysfunction are the two main insulin-related aberrations characterizing T2DM. With regards to gestational diabetes it is a condition of insulin resistance mainly observed during the onset of the 2nd and 3rd trimesters of pregnancy.

These patients often have insulin resistance and beta-cell disorders, two major insulin-related abnormalities. The impairment of many cellular processes leads to less insulin-
Responsive or insulin-sensitive peripheral tissue cells. This is especially true for the liver, muscle, and adipose tissue. Initially, in the progression of diabetes, impaired insulin sensitivity causes beta-cells to hyperfunction, leading to an increased production of insulin to restore and sustain normal blood sugar levels. Hyperinsulinemia, or increased amounts of insulin in the blood, helps keep blood sugar levels normal. However, over time, the steady decline in insulin sensitivity cannot be adequately compensated for by the enhanced insulin production of these cells. Insulin insufficiency develops when beta-cell function declines and as beta-cell dysfunction progresses. Because of this, normal blood sugar levels are unable to be maintained any longer, and hyperglycemia sets in.

Liquid Chromatography-Tandem Mass Spectrometry
The coupling of online spectrophotometry and a separation technique is known as hyphenation. The added advantages of hyphenated techniques are accurate analysis, faster processing, automated methods, and good reproducibility. One such hyphenated analytical technique is liquid chromatography-tandem mass spectrometry (LC-MS/MS), which is when liquid chromatography is coupled with tandem mass spectrometry. Electron spray ionization (ESI) is the most commonly used interface that transfers the eluents from the LC system to the MS system in a suitable form. LC is high-performance liquid chromatography (HPCL) and instrumentation encompasses of the pump, sample injector, column, detector, and recorder. Schematic diagram of LC-MS/MS as shown in Figure 3. Mass spectrometry contains the following instrumentation parts: Ionization sources and interfaces, mass analyzers, and detectors. MS/MS works out by collision-induced disassociation (CID) where the collisions by other molecules break down the ions. Mass analyzer single quadrupole is extensively used along with the ESI for the CID spectra. Diversified applications of LC-MS are molecular pharmacognosy, quantitative and qualitative analysis, and clinical chemistry and toxicology.

Sample preparation for LC-MS/MS
Based on the LC-MS/MS selectivity and the compatibility with the interface, automated and less time-consuming techniques such as simple protein precipitation and solid phase extraction are used.

- **Solid phase extraction**

SPE is a technique of specific sample preparation in which the analyte is attached to a solid substance inside SPE cartridges, interferences are cleared, and the analyte is then eluted only in the intended way. SPE is a particularly potent method because of the multitude of sorbent options. It is a 4-step process that starts with preparing the cartridge, moves on to feeding the sample, cleans the cartridge, and ends with eluting the analyte. In order to dampen the packing material’s active moiety and eliminate contaminants in addition to any air that may be trapped in the cartridge, the cartridge must first be washed by running a solvent through the sorbent. Common products used in reversed-phase SPE include acetonitrile and methanol. A buffer is placed after the extraction liquid to ensure compatibility with the liquid solution. The cartridge is subsequently filled with the sample that contains the analyte. The analyte and certain matrix elements are kept in this stage, whereas others flow by. Interferences are eliminated during a wash stage while keeping the analyte intact. Lastly, a solvent that may sever analyte-sorbent connections is added for eluting the analyte from the sorbent.

- **Liquid extraction**

It is a technique employed for separating molecules in two distinct, incompatible liquids, often water and an organic solvent, according to their respective solubility and unequal polarity. Since the component must be unionized before extraction, the sample’s pH must be adjusted. When removing interferences from a sample, it may be necessary to re-extract the chemicals or do several extractions. While it is more time and labor-intensive than SPE since drying and reconstitution are required, but it is cost-effective. LLE is a quick and effective way to concentrate and separate somewhat hydrophobic substances. With this extraction method, obtaining a matrix-free clean sample of certain polar compounds is not always feasible.

- **Protein precipitation**

In many cases, plasma sample bioanalysis uses sample preparation using PPT. The technique has been expanded...
Antidiabetic and Antihypertensive Drugs

to include evaluating drug and byproduct levels in whole blood. PPT provides a simple, quick, and automatatable sample preparation method.23 Proteins may be taken out of the biological matrix using this simplest method.24 The proteins are precipitated using an acid or any organic solvent, including perchloric acid, TCA, formic acid, ACN, or MeOH. After that, denatured proteins are extracted by centrifuging the mixture. Collect supernate is immediately put into LC-MS/MS following centrifugation, or it may be reconstituted and dried beforehand. While it is a quick and economical extraction process, the sample it yields has a lot of external interference that may build up in columns, obstruct them, inhibit or promote ions, and need regular system cleaning.25

High-Performance Liquid Chromatography
It is a type of liquid chromatography. As the name suggests, high-pressure, the solvent moves down the column up to a pressure of 400 atmospheres. The sample is separated based on the relative affinities towards the column and mobile phases. The high-performance liquid chromatography (HPLC) instrumentation contains a solvent handling system, degasser, injector, pump, column, and detector.26

Sample preparation for HPLC
The main purpose of the sample preparation technique is to obtain the sample in the best form to yield better analytical results. The sample must be compatible with the mobile phase, dissolve in it, and be ideal with the column. The sample preparation procedure must not influence the sample retention time. Sample preparation techniques such as filtration, centrifugation, and sonication are looked into for insoluble components. Syringe filters must be competent in removing the interferences and contaminants.26

Validation components for HPLC
The validation components contain accuracy, precision, the limit of detection and quantification, linearity, range, specificity, and robustness.26

- Selectivity
When additional components are also present in the sample, the method's selectivity determines its capacity to identify and estimate the analyte. For selectivity, a minimum of six distinct laboratories must perform studies on blank samples of the relevant matrix. Selectivity must be guaranteed at the lower limit of quantitation (LLoQ), and every blank sample must be examined for interference, which may come from concurrent medications, indigenous matrix proteins, chemicals, and degradation products. Every analyte must be evaluated to ensure that no interference exists if the technique is meant to quantify multiple analytes.27

- Accuracy
The accuracy of a given analytical technique refers to how closely mean test findings generated by the technique match the nominal quantity of the analyte. Repeated analyses of samples with established analyte concentrations are used to evaluate accuracy. Accuracy must be evaluated for each concentration with not less than five readings. Using at least three levels that fall within the predicted concentration limit is advised. Excluding LLoQ, when it must not differ by over 20%, the average value ought to fall below 15% of true value. Accuracy is assessed using the SD of the mean of the real values.28

- Precision
When a technique is performed on many serial dilutions of a homogenous matrix volume, the technique's precision refers to how closely separate measurements of an analyte are to one another.29 For each concentration, at least six readings are required to estimate precision. Using at least three concentrations that fall within the predicted concentration range is advised. Excluding LLoQ, when it must not differ by over 20%, the mean ought to fall below 15% of true value.30 The subcategories of precision include within-run, intra-batch precision, which evaluates precision throughout a single analysis run, and among different runs, inter-batch precision, which evaluates precision across batches and includes several analysts, tools, reagents, and labs.

- Recovery
In an analysis, an analyte's recoveries are measured by the difference between the response of the real quantity of the clear original standard and that of an analyte that has been introduced or removed from the sample matrix. Recovery is concerned with an analyzing technique's capacity to retrieve data effectively within its variability range. Analyte recovery does not have to be 100% accurate, yet it ought to be constant, exact, and repeatable in terms of how much of the IS is recovered.31 In recovery trials, the analytical findings for recovered materials at LQC, MQC, and HQC must be compared with un-extracted standards, which indicate 100% recovery.32

- Lower limit of quantification
The Lower limit of quantification (LLoQ) is a minimal analyte quantity at which measurements may be made with respectable accuracy and precision. If the requirements listed below are satisfied, the minimum standard on the calibration graph must be considered as LLoQ. The analyte response
- Needs to be a minimum 5X greater than the blank response.
- Must be repeatable with an accuracy and precision of 80 to 120%.

- Stability
It is important to ascertain if the analyte is stable in the given matrix at the specified preservation temperatures. Stability tests should assess the analytes' stability throughout sample gathering and processing, as well as during long-term,33 short-term, and upon undergoing freeze-thaw sessions and analysis.34 Stability tests need to be performed in circumstances similar to those that would be present while handling and analyzing actual samples. Analyte stability in standard solutions assessment should also be part of the process.35

To ascertain the stability of an analyte, a collection of samples derived from a newly manufactured stock solution is required at all times.36 The maximum values of the
validated range for the analyte and IS ought to correspond to the concentration of the working solution. Stock solutions for stability assessment need to be made in an apt solvent.\textsuperscript{38} If the diluant differs from the diluant in the standard solutions, it’s probably necessary to assess the stability of working solutions at greater and lesser concentrations in the approved range.

- **Matrix effect**
Blank matrices obtained from 6 separate lots, including a hemolytic and a lipemic plasma, were spiked with analyte at low and high concentration levels.\textsuperscript{39,40} Then three samples from each lot at each level should be processed as per extraction method and analyzed with fresh calibration standards.

- **Ruggedness**
This should be done with many columns of the same size and design. For maximum precision and accuracy, injecting a batch \textit{via} a separate column is recommended. To gauge the robustness of the extraction approach, it is proposed that the precision and accuracy batch be evaluated by separate analysts.\textsuperscript{41,42}

**System suitability parameters**
The analysis process sometimes includes a system suitability test (SST). The analyses are predicated on the idea that all the parts of the system—the instruments, the electronics, the analytical procedures, and the samples to be analyzed—are interconnected and should be assessed collectively.\textsuperscript{43} The kind of process being tested ought to dictate the parameters of the SST to be created for that operation.\textsuperscript{44,45}

**Ultra-Performance Liquid Chromatography**
Ultra-performance liquid chromatography (UPLC) technology facilitates the improvement of resolution sensitivity and speed without compromise. Schematic diagram of UPLC is shown in Figure 4. The main goal of ultra-performance liquid chromatography is separation with a faster analysis.\textsuperscript{46} UPLC columns contain ethylene bridged hybrid structure that has good efficiency, superior mechanical strength, and hydrophilic lipophilic interactions. The ethylene bridged hybrid particles are manufactured in a multistep process, where initially pure monomers of tetraethoxysilane and 1,2- bis(triethoxysilyl) ethane are mixed in a molar ratio of 4:1, resulting in water-immiscible poly ethoxy oligosilane polymer further are emulsified in water to form highly spherical oil droplets.\textsuperscript{47}

**List of Recently Approved Antihypertensive Drugs (Table 1)**

\textbf{Selexipag}
On 31.12.2021, selexipag is a drug that has been authorized to manage pulmonary arterial hypertension (PAH) to slow the course of the condition and lessen the likelihood of hospitalization due to PAH. \textit{RP-HPLC\textsuperscript{48} and LC-MS/MS approach\textsuperscript{49,50} were devised to quantify process-related impurities in selexipag API.}

\textbf{Azelnidipine}
On 04.03.2020, azelnidipine was approved for the treatment of stage I hypertension and on 16.10.2020, for managing stage II hypertension. Reversed-phase high performance liquid chromatography (RP-HPLC) method\textsuperscript{51} and UV spectrophotometry\textsuperscript{52} were employed to quantify azelnidipine alongside telmisartan in tablet form. Azelnidipine and olmesartan medoxomil was analyzed in human plasma using the RP-HPLC method. The procedure was approved for use in biological fluid analyses after being verified per CDER guidelines.\textsuperscript{53}

\textbf{Netarsudil}
On 14.10.2020, netarsudil was approved to lessen higher intraocular pressure in those with open angle glaucoma or ocular hypertension. Ophthalmic solution dosage form containing netarsudil and latanoprost were quantified using HPLC and the results revealed that the method developed was simple and economical.\textsuperscript{54-56}

\textbf{Ripasudil hydrochloride hydrate}
On 20.11.2019, when conventional glaucoma medications were ineffective or contraindicated, ripasudil hydrochloride hydrate was officially authorized to be employed as a treatment. A UV-spectrophotometric approach may determine its concentrations in pure and ophthalmic preparations.\textsuperscript{57} Its impurities were separated and identified using an RP-HPLC approach, including precolumn derivatization.\textsuperscript{58}

\textbf{Riociguat}
On 16.04.2018, riociguat was licensed to increase exercise capacity and World Health Organization (WHO) function in patients with chronic thromboembolic pulmonary hypertension (CTEPH) that has persisted after surgical therapy or is inoperable. UPLC–MS/MS approach was designed for pharmacokinetic studies and the quantitation of riociguat in patients with pulmonary hypertension.\textsuperscript{59} In another study, one unique approach to its detection in human serum was devised\textsuperscript{60} using capillary electrophoresis coupled with mass spectrometry (CE-MS).\textsuperscript{60} RP-HPLC was used effectively for routine assessment of Riociguat in bulk and formulations.\textsuperscript{51}

- **Azilsartan**
On 09.07.2018, azilsartan, combined with chlorthalidone, for managing hypertension in adults. Azilsartan medoxomil was quantified using RP-HPLC\textsuperscript{62} and HPTLC.\textsuperscript{63} Azilsartan

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure_4.png}
\caption{Schematic diagram of UPLC}
\end{figure}
medoxomil and chlorthalidone were quantified using UV and HPTLC methods.\textsuperscript{64} 

**Fimasartan potassium trihydrate**

On 22.11.2018, The use of fimasartan potassium trihydrate in the management of moderate hypertension was officially authorized. HPLC was used to determine its concentrations in bulk and medicinal doses.\textsuperscript{65-67}

**Telmisartan**

On 14.05.2013, telmisartan was approved for the treatment of hypertension as second-line therapy. Its quantitation was done using UV-spectroscopic methods.\textsuperscript{68} Using the HPTLC method, simultaneous estimation of telmisartan (TMS) and gallic acid (GA) was carried out.\textsuperscript{69} In another study, photometrix may be utilized as a substitute to UV for routine assessment of TMS and extractive colorimetric techniques have been established for estimating telmisartan in bulk and pharmaceutical dose form.\textsuperscript{70}

**List of Recently-Approved Antidiabetic Drugs (Table 2)**

- Saxagliptin
- Ertugliflozin
- Ozempic (Semaglutide)

**Saxagliptin**

The simplest and most sensitive direct HPLC approach was used to isolate and quantify six sulfonate esters.\textsuperscript{71} Spectrophotometric techniques for determining saxagliptin concentration in pharmaceutical formulations through charge transfer complexation and Schiff’s base formation were developed using a design of experiments.\textsuperscript{72} A novel HPLC technique determined saxagliptin and dapagliflozin concentrations in rat plasma.\textsuperscript{73}

**Ertugliflozin**

Ertugliflozin and Metformin tablets were tested together using the HPLC method.\textsuperscript{74} The stability-indicating RP-UPLC method \textsuperscript{75} and LC-MS/MS \textsuperscript{76} were created to simultaneously determine the concentrations of both sitagliptin and ertugliflozin.

**Ozempic (Semaglutide)**

The RP-HPLC method was developed to determine Semaglutide concentrations in drug substances and finished products.\textsuperscript{77} Lee et al., developed a novel Using LC-MS/MS, the pharmacokinetics and cerebral circulation of semaglutide in mice were studied.\textsuperscript{78} Using UV-visible spectrophotometric method, the determination of semaglutide was successfully performed and a deeper comprehension of semaglutide’s impact mechanism may be aided by this analytical approach and pharmacokinetic data.\textsuperscript{79}

**Challenges and Future Perspectives**

- Antihypertensive and antidiabetic medications contribute significantly to monitoring the patient’s level of blood pressure and glucose in the blood.\textsuperscript{80} Careful and accurate monitoring ensures patient safety and satisfactory results.
- Develop accurate and precise analytical methods and extraction procedures, hands-on accurately quantifying the medication levels, and further proper management of the conditions.

### Table 1: Recently approved antihypertensive drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Approval Date</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omidenezep Isopropyl</td>
<td>9/26/2022</td>
<td>Topical Ocular Hypotensive Agent</td>
</tr>
<tr>
<td>Selexipag</td>
<td>31/12/2021</td>
<td>Prostacyclin Receptor Agonist</td>
</tr>
<tr>
<td>Azelnidipine</td>
<td>04/03/2020</td>
<td>Calcium Channel-Blocking Agents.</td>
</tr>
<tr>
<td>Ripasudil Hydrochloride Hydrate</td>
<td>20/11/2019</td>
<td>Rho Kinase Inhibitor.</td>
</tr>
<tr>
<td>Riociguat</td>
<td>16/04/2018</td>
<td>Soluble Guanylate Cyclase Stimulator.</td>
</tr>
<tr>
<td>Azilsartan</td>
<td>09/07/2018</td>
<td>Angiotensin-Receptor Blocking (ARB)</td>
</tr>
<tr>
<td>Fimasartan Potassium Trihydrate</td>
<td>22/11/2018</td>
<td>Angiotensin II Receptor Antagonist</td>
</tr>
<tr>
<td>Netarsudil</td>
<td>12/18/2017</td>
<td>Rho Kinase Inhibitor.</td>
</tr>
<tr>
<td>Latanoprostene Bunod</td>
<td>11/2/2017</td>
<td>Prostaglandin F2a Analog.</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>14/05/2013</td>
<td>Angiotensin II Receptor Antagonist</td>
</tr>
</tbody>
</table>

### Table 2: Recently approved antidiabetic drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Approval Date</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sotagliflozin</td>
<td>5/26/2023</td>
<td>SGLT2 Inhibitor</td>
</tr>
<tr>
<td>Bexagliflozin</td>
<td>1/20/2023</td>
<td>SGLT2 Inhibitor</td>
</tr>
<tr>
<td>Saxagliptin (Second Tentative Approval)</td>
<td>17/2/2023</td>
<td>(DPP-4) Inhibitors</td>
</tr>
<tr>
<td>Lantidra</td>
<td>6/28/2023</td>
<td>Pancreatic Islet Cellular Therapy</td>
</tr>
<tr>
<td>Tirzepatide</td>
<td>5/13/2022</td>
<td>GLP-1 Agonists</td>
</tr>
<tr>
<td>Tepiluzamab</td>
<td>9/17/2022</td>
<td>Anti-CD3 Monoclonal Antibody</td>
</tr>
<tr>
<td>Ozempic (Semaglutide)</td>
<td>3/28/2022</td>
<td>GLP-1 Agonists</td>
</tr>
<tr>
<td>Imeglimin Hydrochloride</td>
<td>2021</td>
<td>Aminotriazines</td>
</tr>
<tr>
<td>Finerenone</td>
<td>7/9/2021</td>
<td>Mineralocorticoid Receptor (MR) Antagonists</td>
</tr>
<tr>
<td>Ertugliflozin</td>
<td>12/19/2017</td>
<td>SGLT2 Inhibitor</td>
</tr>
</tbody>
</table>

- Although the scope of a couple of the uses of these methods presented in this study is quite narrow, they all have some value in their respective fields and add to the overall significance of analytical assays in present-day drug analysis.
- Since this is a burgeoning field, several novel analytical techniques for antihypertensive medications may become available in the not-too-distant future.
- With the emergence of new antidiabetic and antihypertensive treatments, analytical methods are required for pre-clinical and clinical studies.
- Each of the aforementioned methods has its own set of benefits and drawbacks, so it’s important to choose the right one for the task at hand, and preferably employ a combination of methods to get the greatest results.
• In addition, it is crucial to give careful consideration to proper sample handling, particularly when dealing with biological material, since even the most advanced bioanalytical technology would be unable to retrieve analytes that were wasted while processing the sample.

• Currently, a large number of medicinal drugs undergo quantitative bioanalysis using LC-MS, making it the technique of choice. Nevertheless, many more innovations remain on the horizon, including the creation of high-throughput studies, the improvement of the sensitivity and durability of mass spectrometers, and the optimization of the connected LC systems.

• Quantitative isotope measurements with rapid MS might also become more commonplace. Using this method, even trace amounts of radioactive material may be detected in very tiny sample volumes, making it ideal for detecting uncommon and long-lived isotopes. It shows promise as a useful method for analyzing low concentrations of analyte in difficult samples, such as those seen in phase I ADME studies of novel drugs.

• Patients on several medications may benefit from simultaneous multi-drug assessment approaches due to the time and money saved during analysis.

• Due to the sensitivity of metal-binding to biomolecules towards variations in pH, buffer makeup, and ion concentration, typical circumstances should be maintained throughout metallomic research.

• Further, novel electrochemical techniques are in development and may eventually play an important role in analytical and biological studies.

CONCLUSION

A lot of effort has been put into studying how to make drugs more effective and safer to use so that people may have higher quality of life. Consequently, these goals need the use of very sensitive and precise analytical techniques. The purpose of this study was to provide some background and instances of how contemporary analytical techniques have been used to better understand the efficacy of antihypertensive and antidiabetic medicines in various dose forms and in biological materials. Analysis technique design and modification facilitate accurate drug monitoring and personalized treatment strategies for patients with diabetes and hypertension. This paper might serve as a starting point for a movement toward standardizing the use of HPLC and UPLC techniques for determining the appropriate dosages of newly authorized antidiabetic and antihypertensive medications.

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