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Review on Method Development and Validation of Metformin, Empagliflozin and Glimepiride Using UPLC

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Abstract:- UPLC, also known as ultra performance liquid chromatography, was developed by Waters in 2004 and represents an advanced form of HPLC(1). This method has revolutionized liquid chromatography by catering to particles smaller than 2 µm, leading to enhanced sensitivity, speed, and resolution. Many experts believe that UPLC will eventually supplant traditional HPLC methods(2). The Van Deemter equation underpins this transformative process by establishing a connection between linear velocity and plate height.(1) Operating with UPLC on smaller particles necessitates a higher pressure limit compared to standard HPLC, which typically operates at 6000 psi. In comparison to HPLC, this method reduces mobile phase volume usage by at least 80% and shortens the runtime to approximately 1.5 minutes(3,4). UPLC, a modified version of HPLC, takes advantage of advancements in particle chemistry performance, system architecture, detector design, data processing, and control, resulting in significant enhancements in resolution, sensitivity, and efficiency(3). This also leads to quicker results and reduced solvent usage, making the technology more cost-effective and eco-friendly(4)

Keywords:- *UPLC, Metformin, Glimepiride, Empagliflozin, Diabetes.*

I. INTRODUCTION

The primary concept behind the UPLC method is established by the Van Deemter relationship. This explains the connection between flow rate and plate height, and it is well-known that the van der Waals equation illustrates that, for optimal results, the flow range for smaller particles is significantly larger than that for larger particles.(1)

The Van Deemter equation can be represented as follows: H=A+ B/V + Cv(5)

Where:

H represents the height equivalent to a theoretical plate (HETP).
A, B, and C are constants.
V is the flow rate of the carrier gas.

The lower particle diameter can significantly reduce HETP, thereby enhancing column efficiency.(6) Eddy mixing, denoted by the term A, remains unaffected by velocity. When columns are filled with uniformly sized small particles, the quantity is diminished. The term B represents the tendency of particles' natural diffusion(1). It is divided by v, as its impact decreases at higher flow rates. Kinetic resistance during the separation process is indicated by the term C(1). Kinetic resistance refers to the temporal lag resulting from the transition between the mobile phase and the stationary phase. UPLC application has facilitated the identification of drug metabolites and improved the quality of separation spectrum.(7)

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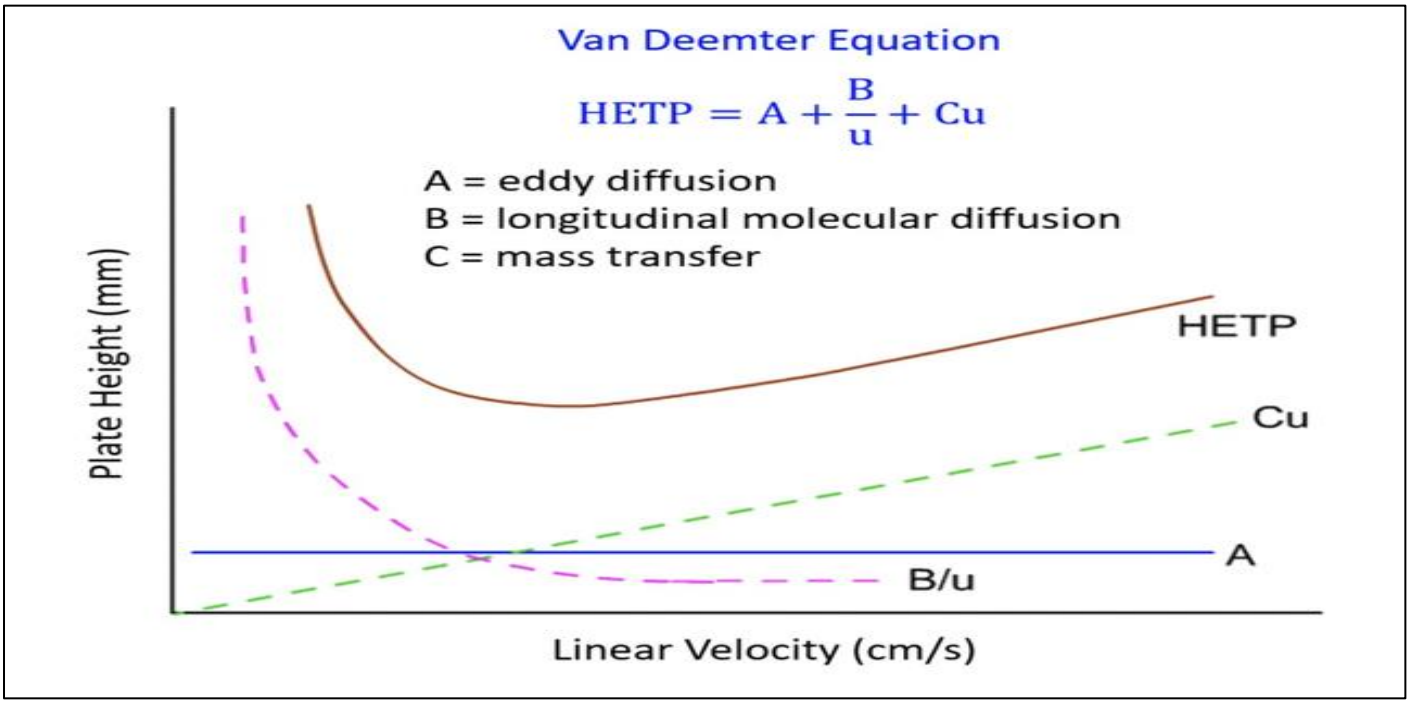

Fig 1: Van Deemter Plot

➢ *Instrumentation:*

The setup includes sensors, UPLC tubes, and sample injection.(8)

➢ *Injectable Sample:*

Sample injection is essential in UPLC. An injector is utilized to introduce a small quantity of sample solution into the mobile phase(9). A standard injection valve can be programmed or operated by hand. A brief injection cycle time is required to achieve the highest speed that the UPLC process provides. Minimal transfer and low-volume injections are necessary to enhance sensitivity.(1)

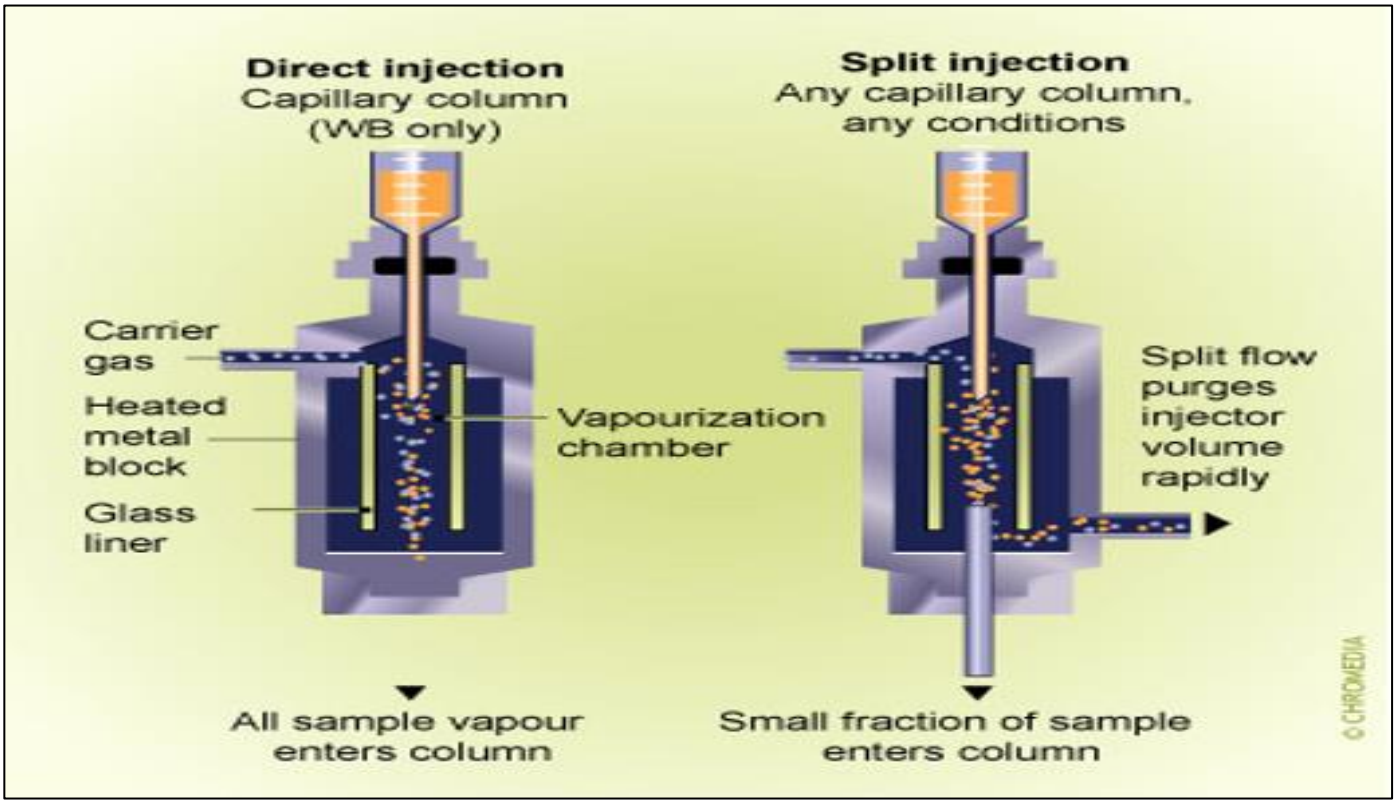

Fig 2: Sample Injection

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➢ *UPLC Columns:*

The production of different types of UPLC columns is carried out by several companies.

- Vanguard precolumns and Waters-Acquity UPLC columns have been produced by various manufacturers.
- Agilent technology is known for providing high-quality columns that deliver fast and consistent results.
- Altech has an affiliation with the production of UPLC columns.(1,3,4)
- UPLC utilizes a variety of column types created through different technologies, including Peptide Separation Technology, Ethylene Bridged Hybrid Particle Technology, High strength Silica Particle Technology, and Charged Surface Hybrid Particle Technology. (2)

➢ *Vanguard Precolumns and Water-Acquity UPLC Columns:*

VanGuard Column Protection products help improve the performance of analytical columns by removing chemical and particle contamination from the mobile-phase stream, thus preserving separation efficiency and resolution. The reduced dead volume design minimizes chromatographic peak dispersion and waste to maintain optimal performance. (10)

When using ACQUITY UPLC separations, the low dispersion, one-piece patented design of the VanGuard Pre-column enables higher efficiency, improved resolution, and increased throughput. The pre-column is compatible with all stationary phases of the ACQUITY UPLC Column and directly connects to the column's inlet. (10,11)

➢ *Waters-Acquity UPLC Columns:*

These includes following:

- ACQUITY UPLC BEH C18 and C8 columns: For a broad pH range, these straight alkyl chains are the most commonly utilized.
- ACQUITY UPLC BEH phenyl columns: These feature a tri-functional C6 alkyl ethyl between the phenyl rings and silyl functionality.
- ACQUITY UPLC BEH Amide columns: The remarkable column life time is achieved by combining the tri functionally bound amide phase with BEH tiny particles. They make it easier to use a large range of phase pH, from 2 to 11. (12,13)

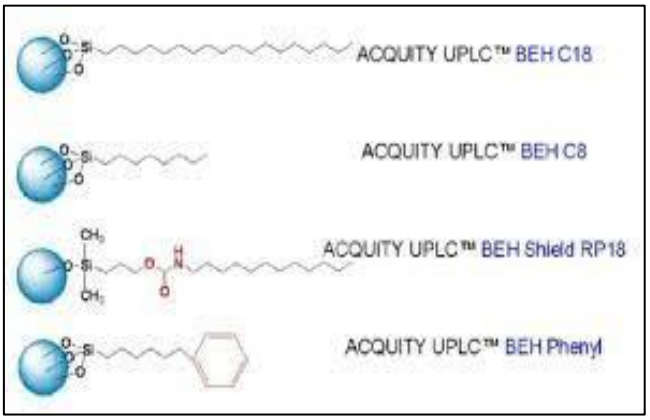

Fig 3: Acquity Columns

➢ *Agilent Technology Columns:*

Agilent Technologies offers a wide range of UPLC columns that are appropriate for different types of analytical applications, such as:

- Small molecule separations: A variety of substances can be successfully separated using their HPLC and UHPLC columns, including the InfinityLab Poroshell 120 and the ZORBAX, Polaris, and Pursuit series.
- Biomolecule separations: Biomolecules can be isolated using Agilent's LC columns.
- Glycan mapping: Agilent's LC columns are also used for this purpose.
- Protein analysis is made easier by Agilent's LC columns.
- mAb analysis: Monoclonal antibody (mAb) analysis is intended to be performed on their LC columns.(11,13)


Fig 4: Agilent Column

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➢ *Altech Columns:*
These columns includes following

➢ *Monoliths*
Monoliths, unlike discrete particles, are formed as continuous homogeneous columns (similar to concrete in a mold). The potential for monolithic columns to offer a stable, easily replaceable column for analytical and preparative separations is significant.(14) Numerous research projects have examined monoliths constructed from polymers and silica. Because a monolithic column is packed with a single piece of porous material, it reduces back pressure and particle size simultaneously. When operated at the same pressure as a particle-packed column, it allows for a faster flow rate of the mobile phase.(15)

➢ *Hydrophilic interaction liquid chromatography(HILIC) Column:*
This column increases the time retention of polar compound and provide separation method for mixtures of ionizable/polar compounds.

- Example: Separation of different classes of lipids.
- Combination study: Ultra Performance Liquid Chromatography coupled with photodiode and mass spectroscopy which can give rapid identification of compound along with sensitivity 49 . The coupling of UPLC with other devices different techniques is convenient and economical as compared to HPLC50-51. (16)

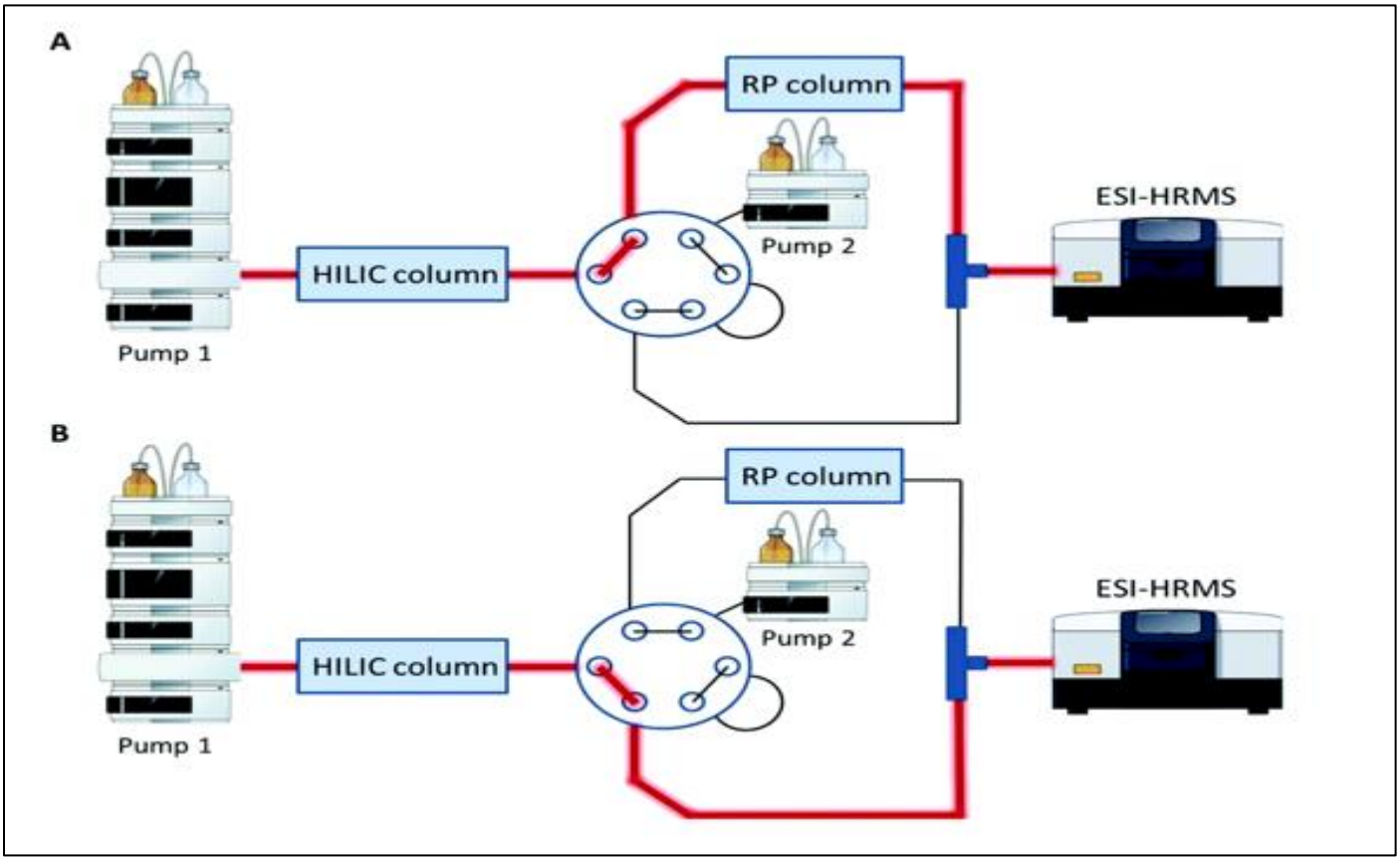

Fig 5: HILIC Column

➢ *Detectors:*
Detectors play a crucial role in reducing the separated solute on the column by providing a high sampling rate with small attainable peaks and low peak dispersion in UPLC. UPLC utilizes Tunable Vis-UV and Acquity photodiode array detectors. The Tunable Vis-UV detector employs Teflon AF to eliminate internal absorption, creating an internally reflective surface that enhances light transmission.(17)

➢ *Acquity Photodiode Array:*
The Acquity UPLC photodiode array (PDA) detectors provide exceptional trace impurity detection and quantitation capabilities, as well as advanced spectral analysis capabilities. This makes it an ideal detector for various laboratory applications, such as technique development and compound identification.(18) The user-friendly AQUITY UPLC PDA detector is reliable and perfect for everyday analysis, with enhanced software control enabling simultaneous 2D and 3D operations.(19)

➢ *Tunable Vis- UV:*
The Adjustable Vis-UV devices are linked to an Acquity photodiode array.(20)

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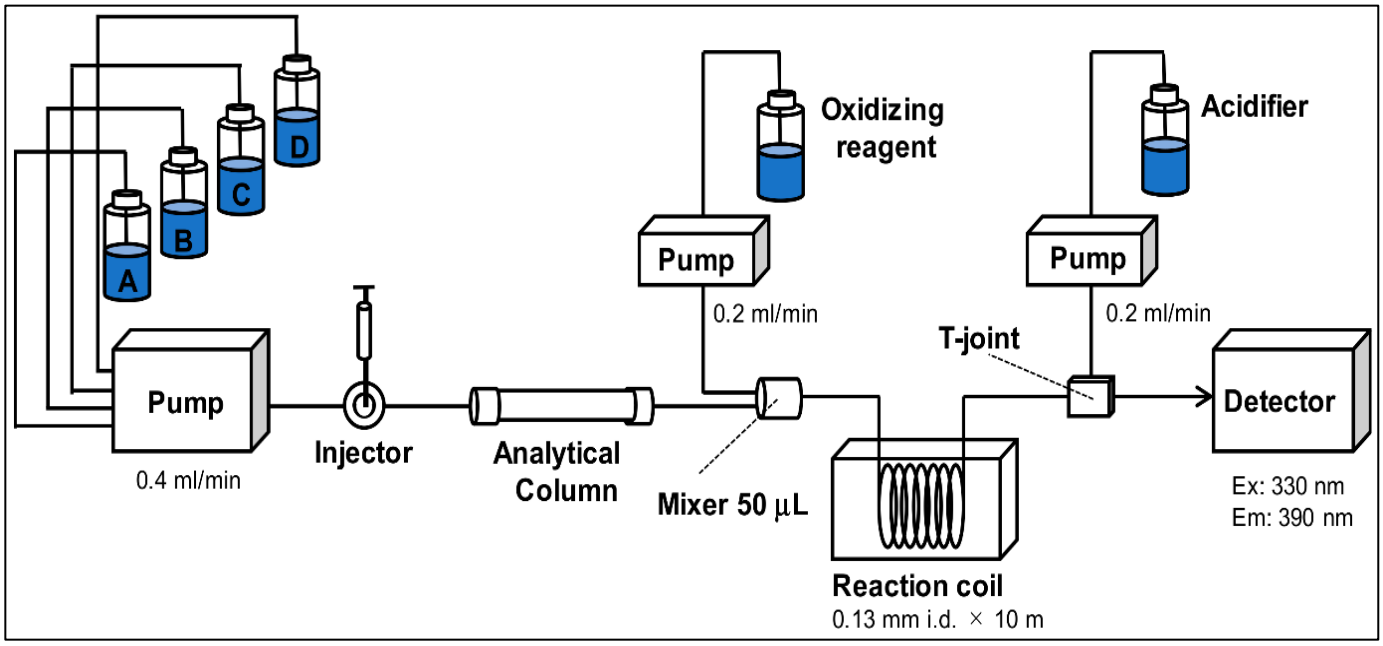

Fig 6: UPLC Detector Flow Cell

II. APPLICATIONS

A. Pharmaceutical Applications:

UPLC is employed in the development and quality assurance of pharmaceuticals, as well as in research on bioequivalence, pharmacokinetics, drug metabolism, and the purity and stability of medications. (21)

B. Food Safety and Quality Control:

UPLC is utilized for the examination of pesticide residues, the identification of contaminants like heavy metals, the analysis of food additives and preservatives, the profiling of nutrients, and the detection of allergens to ensure food safety and quality control. (22)

C. Forensic analysis:

UPLC is involved in toxicology screening, DNA analysis, crime scene investigation, and the identification of poisons in forensic analysis. (23)

D. Biotechnology Applications:

Biotechnology applications of UPLC include the production of vaccines, research on gene therapy, peptide mapping, and the analysis and purification of proteins.(23)

E. Environmental Monitoring:

UPLC is used for environmental monitoring in research on climate change, air quality assessment, analysis of industrial waste, and water pollution analysis. (24)

F. Cosmetics Industry:

It is employed to ensure that cosmetics comply with safety regulations and to monitor quality control.(25)

➢ *Advantages*

- Enhanced Resolution: UPLC enhances separation efficiency and enables better compound resolution by utilizing smaller particles and higher pressure. (26)
- Quicker analysis: UPLC methods require less time for analysis compared to HPLC.
- Increased sensitivity: UPLC's improved capacity for separation and detection can lead to higher sensitivity and lower detection limits. (27)
- Enhanced data quality: The outcome of increased resolution and efficiency is higher-quality data.(28)
- Improved reproducibility: UPLC systems deliver more consistent and repeatable results due to their advanced technology. (29)
- Reduced solvent consumption: Less solvent is utilized because the analysis takes less time. (27)

➢ *Disadvantages*

- Expense: UPLC equipment and columns are pricier when compared to HPLC systems.
- Complexity: UPLC processes may require specialized expertise and experience, potentially making them more intricate. (30)
- Lifespan of columns: UPLC columns have a shorter longevity in contrast to HPLC columns. (31)
- Suitability: UPLC may not be suitable for all types of sample or solvent combinations. (32)

III. DIABETES

Diabetes is a chronic health condition that affects the body's ability to process glucose, also known as blood sugar. The root cause of diabetes is typically related to problems with insulin, a hormone produced by the pancreas that regulates blood sugar levels. (33)

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- **Type 1 diabetes:** develops when the immune system attacks the insulin-producing cells in the pancreas, leading to reduced or absent insulin production. This type of diabetes usually begins in early adulthood or during childhood. (34)
- **Type 2 Diabetes:** It is characterized by insulin resistance, occurs when the body is unable to effectively utilize insulin, eventually leading to insufficient production of the hormone by the pancreas to maintain normal blood glucose levels. (35)

✓ **Gestational Diabetes:** Primarily caused by pregnancy, typically resolves after childbirth.(36)

➢ *Pathophysiology:*

The pathophysiology of diabetes can be categorized into two main groups:

- *Type 1 Diabetes:*

✓ Autoimmune destruction: In Type 1 diabetes, an autoimmune disease, the body's immune system attacks and eliminates the beta cells in the pancreas responsible for producing insulin.

✓ Insulin deficiency: The loss of beta cells leads to an inadequate supply of insulin, which is essential for cells to uptake glucose and regulate blood sugar levels.

✓ Hyperglycemia: Without insulin production, glucose accumulates in the body, causing high blood sugar levels.(37)

- *Type 2 Diabetes:*

✓ *Insulin Resistance:*

Reduced response to insulin by the body's cells causes glucose to remain in the blood rather than reaching cells effectively.

✓ *Beta Cell Dysfunction:*

The pancreas's inability to produce sufficient insulin to counter this resistance leads to an insulin deficiency.

Both types of diabetes can lead to long-term complications affecting various organs, including neuropathy, retinopathy, and cardiovascular disease. (38)

➢ *Antidiabetic Agents:*

Anti- diabetic agents are the medications used to manage diabetes by lowering the blood glucose levels.

➢ *Classification*

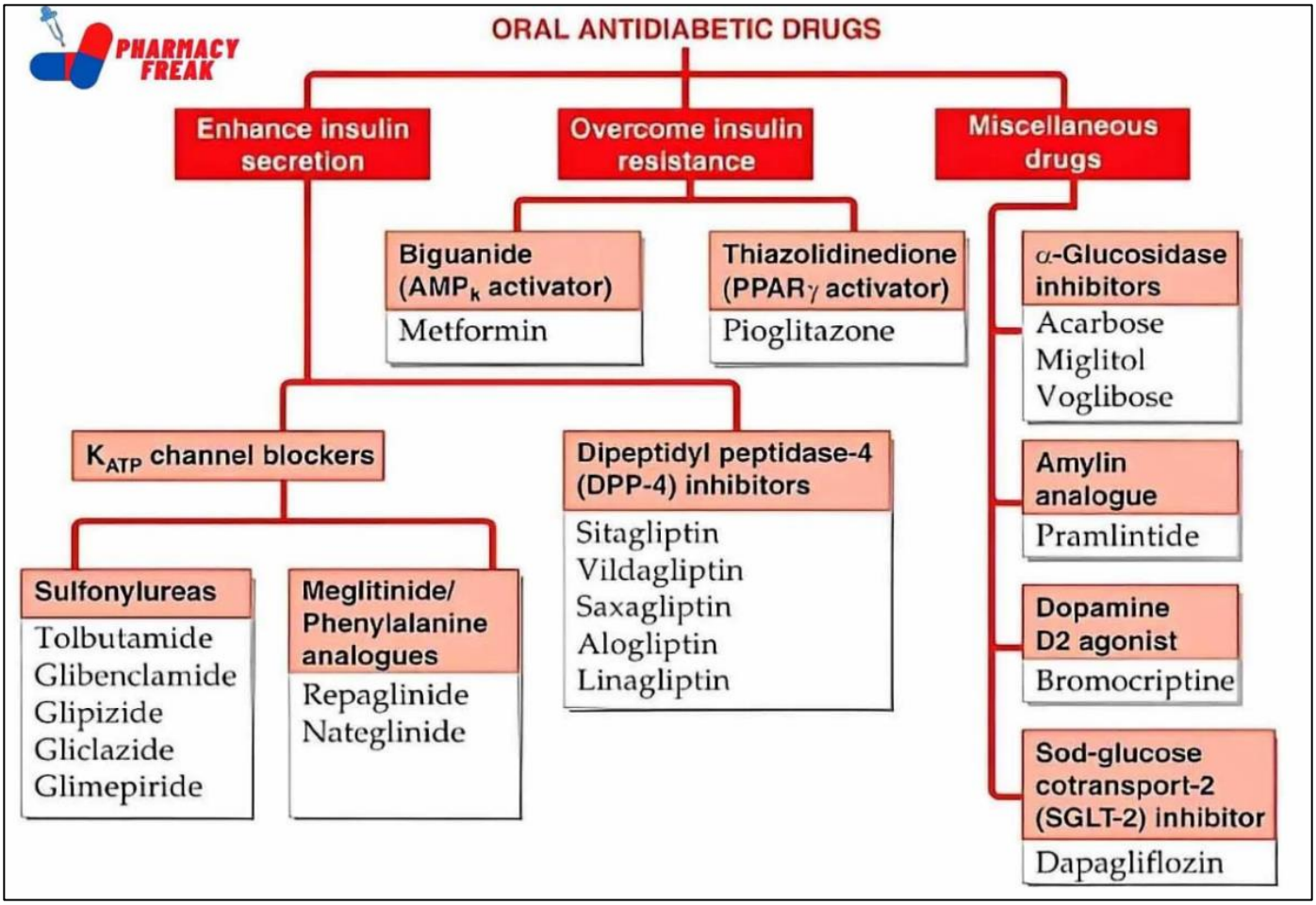

Fig 7: Classification of Anti- Diabetic Drugs

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➢ *Drug Profile*

- *Glimepiride*

Glimepiride, an oral medication, is utilized for the treatment of type 2 diabetes and belongs to the sulfonylurea drug class. The IUPAC designation for sulphonyl ureas is 4-ethyl-3-methyl-N-[2-[4-[(4methylcyclohexyl) carbamoyl sulfamoyl] phenyl] ethyl] and its chemical formula is C24H34N4O5S with a molecular weight of 490.63 g/mol. With a melting point of 207 °C, the crystalline powder of glimepiride appears white to yellowish-white and is poorly soluble in water. (39) It is insoluble in diethyl ether, but readily soluble in methanol and ethanol, and somewhat soluble in chloroform. Glimepiride has a pKa of approximately 4.5, indicating the pH at which half of the molecules are protonated and the other half are deprotonated. The mechanism of action of glimepiride involves stimulating the pancreas to release more insulin, leading to a reduction in blood glucose levels.(40)

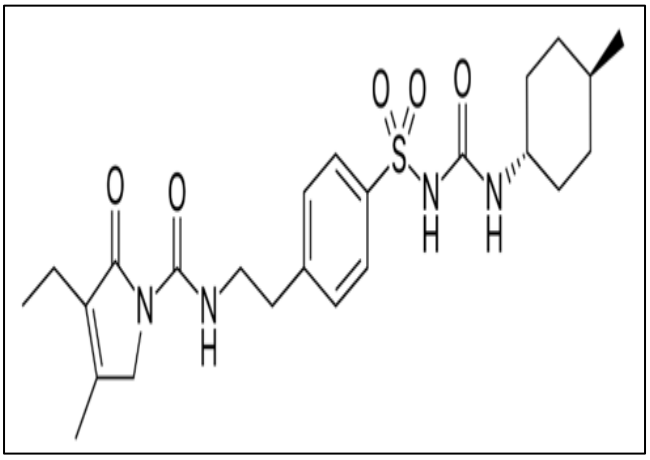

Fig 8: Chemical Structure of Glimepiride

Table 1: Chromatographic Conditions of Glimepiride

Stationary Phase (Column)	**Phenomenex luna C1 (250×4.5 mm) packed with 5µm particles**
Mobile phase	Acetonitrile,0.2 M phosphate buffer (pH 7.4) 40:60 (V/V)
Detection wavelength (nm)	228
Run time (min)	10
Flow rate (ml/min)	1
Volume of injection loop (µL)	20
Column temperature	Ambient
Glimepiride $\boldsymbol{R_t}$ (min)	3.543

- *Empagliflozin*

Empagliflozin is a new oral medication for diabetes that works by blocking the selective sodium-glucose transport protein 2 (SGLT2). This medication comes in the form of a film-coated pill containing either 10 mg or 25 mg of empagliflozin as the active ingredient. (40)The United States Food and Drug Administration (USFDA) gave its approval for this medication in 2014. Empagliflozin is also referred to as D-Glucitol,1,5-anhydro-1-C-[4-chloro-3-[[4-[(3S)-tetrahydro-3-furan-3-yloxy]benzyl}phenyl]methyl]phenyl] or (1S)-1,5-an-hydro-1-(4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy]benzyl}phenyl)-D-glucitol.+(1S).(41)

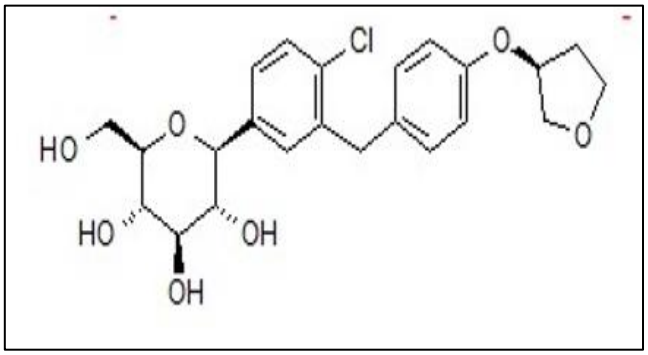

Fig 9: Chemical Structure of Empagliflozin

The effects are nullified by empagliflozin through the inhibition of SGLT-2 receptors. It also decreases the preload (through diuresis) and afterload (by lowering blood pressure and arterial stiffness), leading to a significant reduction in CV mortality. The pharmacokinetic characteristics of empagliflozin in both healthy individuals and T2DM patients receiving empagliflozin monotherapy have been examined (42)Empagliflozin fights diabetes by preventing over 90% of glucose from entering the bloodstream and excreting it through urine.

➢ *Instrumentation*

The wavelength was selected using a UV-Visible Spectrophotometer (Shimadzu-1800, Japan) with compatible 10 mm quartz cells. (43)

All weight measurements were conducted using a Shimadzu model AUX 220 electronic balance, and sonication was performed using an Ultra Sonicator.(44)

The Cyber Lab HPLC (Model: LC-100B) binary gradient system utilized a Neosphere C18 column (4.6 X 250 nm, 5 µm), a gradient mixer (GM-100), a DAD detector, a 772Si Rheodyne injector (20 µl), and a DS-100 control data system.(45)

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Table 2: Chromatographic Conditions of Empagliflozin

Mobile phase	**Orthophosphoric acid buffer and acetonitrile (70: 30% V/V) pH adjusted to 2.7**
Column	ODS C18, 250×4.6 mm, 5µ
Wavelength	230 nm
Flow rate	0.5 mL/min
Injection volume	10µL
Run time	7 min
Diluent	50 mL of acetonitrile

➢ *Metformin*

Metformin, a biguanide antihyperglycemic medication, is commonly used as the first line of treatment for type 2 diabetes(46). It is also observed to be used off-label for insulin resistance in Polycystic Ovary Syndrome (PCOS).(47) With over 48 million generic formulation prescriptions in the US alone, it is one of the most widely used oral antidiabetic drugs globally. In 1972, it was first authorized in Canada, and it was approved by the FDA in the USA again in 1995. (48)Metformin's IUPAC designation is N,N-dimethyltriimidodicarbonicdiamide, also known as 3-(diaminomethylidene)-1,1-dimethylguanidine. It has a molecular weight of 129.1636 and a formula of C_4 H_11 N_5(49). Metformin has various brand names, such as Riomet, Kazano, and Zituvimet.(50)

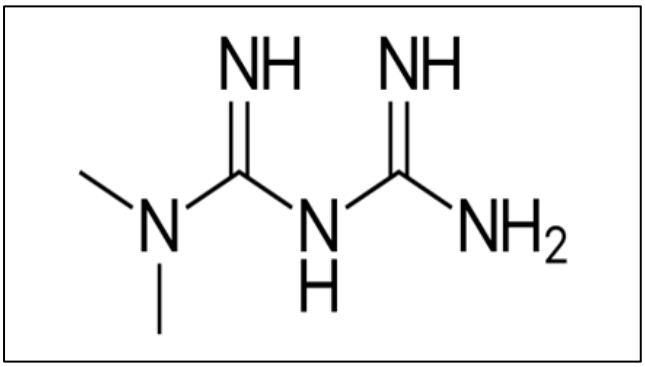

Fig 10: Chemical Structure of Metformin

Metformin is often called an "insulin sensitizer" because it lowers insulin resistance and fasting insulin levels in a manner that is clinically significant.(51)

Table 3: Chromatographic conditions of Metformin

Instrument	**ACQUITY UPLC H- Class configured with CM- A, CM- AUX, SSV, PDA**
Buffer	20 Mm potassium phosphate, pH 2.3
Mobile phase	80: 20 acetonitrile: buffer
Separation mode	Isocratic
Detection	UV at 218nm
Column	ACQUITY UPLC BEH Amide, 2.1150nm,186004802
Sample diluent	70: 30 acetonitrile: water
Flow rate	0.5 mL/min
Column temperature	40°c
Injection volume	1.0 L

Table 4: Summary of Literature Review

S.NO	**Author name**	**Journal name**	**Title name**	**Analytical conditions**
	Ms. Seju Patel, Ms. Janki Patel, Dr. Vanita Marvaniya, Dr. L.M Prajapati	International Journal of Novel Research and Development	Development and Validation of RP-HPLC method for simultaneous estimation of Glimepiride and Linagliptin from their synthetic mixture	Column: Hibar ODS C18 Dimensions: 250*4.6mm Mobile phase: Methanol: Acetonitrile: Water(35:35:30 v/v/v) Run time: 15 min Flow rate: 1ml/min Retention time: 3.198 min
	Tarekegn Tadesse Unade, A. Krishnamanjari Pawar	International Journal of Applied Pharmaceutics	A new stability indicating UPLC method for the determination of two Antidiabetic drugs in combination: Applications to bulk and tablet formulation	Column: Lunna C18 Dimensions: 100*2.6mm Mobile phase: 0.1% TFA buffer: Acetonitrile (70:30) Flow rate: 1.0ml/min Temperature: 25°C Run time: 3.5 min
	Abdul Bari Mohd, Krishna Sanka, Nalini Shastri	Journal of Analytical Science and Technology	Development and Validation of RP-HPLC method for Glimepiride and its applications for a novel self-	Column: Phenomenex Luna C18 Dimensions: 250*4.6mm

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			nano emulsifying powder(SNEP) formulation analysis and dissolution study	Mobile phase: Acetonitrile: 0.2M Phosphate buffer (40:60 v/v) Flow rate: 1ml/min
	Gadapa Nirupa, Upendra M. Tripathi	Journal of Chemistry	RP-HPLC analytical method development and validation for simultaneous estimation of three drugs: Glimepiride, Pioglitazone, Metformin and its pharmaceutical dosage form	Column: Inertsil ODS-3V Dimensions: 250*4.6mm Mobile phase: Buffer: Acetonitrile: Tetrahydrofuran (40:50:10) Flow rate: 1.7ml/min Temperature: 25°C
	Dhanshri Nandre, Aejaz Ahmed, Khan GJ	Asian Journal of Pharmaceutical and Clinical Research	Stability indicating HPLC method development and validation for simultaneous estimation of Dapagliflozin and Metformin tablet dosage form	Column: C18 column Dimensions: 250*4.6mm Flow rate: 1ml/min Mobile phase: Methanol: Water (75:25)
	Gita Chawla and Chanda Ranjan	Open Chemistry Journal	Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography.	Column: Analytical UPLC BEH C18 Dimensions: 150×3.2 mm Flow rate:3.0 ml/min
	Michael E. Swartz	Journal of Liquid Chromatography and Related Technologies.	UPLC: An Introduction and Review.	Column: Acquity UPLC BEH C18 Dimentions: 2.1 by 30mm 1.7µm Flow rate: 0.86mL/min Mobile phase: 0.1% formic acid: acetonitrile.
	K. G. Sheliya, K.V. Shah	An International Journal of Pharmaceutical Sciences.	Ultra Performance Liquid Chromatography (UPLC): A Modern Chromatography Technique.	Column: Acquity UPLC BEH T M C18 Dimensions: 150×2.1 mm Flow rate: 0.6 ml/min Mobile phase: Acetonitrile: Water (0.53ml:0.66ml).
	Haritha G, Shanmugasundaram P	International Journal Of Research In Pharmaceutical Sciences>	Stability indicating RP-UPLC method development and validation for the simultaneous determination of ertugliflozin and metformin in plasma	Column: Acquity BEH-C18 Dimensions: 100×2.1 mm Flow rate: 0.29mL/min Mobile phase: NaH_2PO_4: Methanol: acetonitrile (50:10:40)
	Mokhtar,m.mashoruk suzan, m.soliman, heba	Springer link	A UPLC method for simultaneous determination of empliflozin in spiked human plasma.	Column used : C_{18} column Dimensions: 50 mm × 2.1 mm i.d, 1.7 µm particle Mobile phase: aqueous trifluoroacetic acid (0.1%, pH 2.5): acetonitrile Flow rate : 0.5 mL/min
	RajendraD.Dighe1, PrasannaA.Amrutkar, GaneshB.Sonawane, Vinod A. Bairagi	African journal of biological science	Development and validation of stability indicating RP HPLC method for estimation of epaglitiflozin in bulk drug	Column used: C_{18} column Dimensions: 150mm x 4.6 mm, 5 µm Mobile phase : Methanol and water containing 0.1%formic acid Rate flow 0.8 ml/min.
	Vinayak A. katekar , Prafful P. Kothari , Swapnil S. Kawarkhe , Manish P. Surung and Vaishnavi B. Akotkar	Review on method development and validation of stability indicating RP HPLC Method	SC Biological and Pharmaceutical Sciences	Column used : kromasil c_{18} Dimensions : (150 mm x 4.6 mm, 5 µm) Flow rate : 1ml/min

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		for metformin and Empagliflozin		Mobile phase : Acetonitrile and buffer (1.0 % orthophosphoric acid)
	N.madana gopal, c.sridhar international	Journal of applied pharmaceutics	A validated stability indicating UPLC method for simultaneous determination of mrtformin Hcl and empaligliflozin in bulk drug and tablet dosage form	Column used : dikma c_{18} Dimensions : 150 mm x 2.1mm Mobile phase : phosphate buffer : methanol (30:70) Flow rate : 10mL/min Wavelength : 240nm
	Manjiri Gundle, Kishor Danao*, Ruchi Shivhare, Vijayshri Rokde, Amol Warokar, Debarshi Mahapatra	Journal of medical pharmaceutical and allied science	Methodical insights into reported sophisticated analytical techniques for the determination of anti-diabetic drug empagliflozin in various pharmaceutical products	Column used: acclaim RLCS 120 C_{18} Dimensions: 150 mm x 2.1mm Mobile phase : K_2HPO_4:Methanol Flow rate : 0.4Ml/min Wavelength : 225nm

IV. CONCLUSION

UPLC builds on established HPLC technology, offering improvements and expanded uses over traditional HPLC, which many researchers have encountered challenges with in terms of separations. UPLC provides faster analysis, greater sensitivity, and improved resolution compared to HPLC, allowing for more comprehensive data acquisition. Additionally, UPLC requires less solvent and analysis time compared to alternative chromatographic methods. However, UPLC also shares similarities with HPLC in terms of resolution, tailing factors, and reproducibility of peak area and retention time. Analysis suggests that UPLC (Ultra-Performance Liquid Chromatography) will play a vital role in increasing the productivity of research scientists and improving the quality of pharmaceutical analyses.

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