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A Comparative Review of the HPLC Methods for the Analysis of Metformin and its Combination

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ABSTRACT:

High-Performance Liquid Chromatography (HPLC) is one of the most reliable and widely applied techniques in analytical chemistry, particularly in pharmaceutical, clinical, food, forensic, and environmental fields. It allows precise qualitative and quantitative evaluation of drugs and related compounds. This review outlines the basic principle, instrumentation, applications, and process of method development and validation in HPLC, with special focus on metformin, the first-line drug for type 2 diabetes. Various reported HPLC methods for metformin and its combinations are compared, emphasizing differences in mobile phase composition, retention time, sensitivity, linearity, and overall performance.

KEYWORDS: HPLC, Method development, Validation, Metformin.

INTRODUCTION

High-performance liquid chromatography is the most powerful tool in analytical chemistry. Analytical chemistry is used to determine the qualitative and quantitative contents of materials being studied. Both of these elements are required to understand the sample material. High Performance Liquid Chromatography (HPLC) evolved from traditional column chromatography and is now one of the most essential analytical chemistry methods available. Chromatography is used to analyze and separate active chemicals. The HPLC column retains the stationary phase, the mobile phase is pumped through the column, and the detector measures the drug molecule's retention time. Based on migration rates from the sample to stationary and mobile phases. Retention time varies based on interactions.The stationary phase, the chemicals being studied, and the solvent are all interdependent. Compounds with higher affinity to the stationary layer travel more slowly and over shorter distances, while those with lower affinity travel quicker and for longer distances. The sample chemical is injected in a tiny volume into the mobile phase stream and densely interacts with the stationary phase. Retardation is influenced by the analyte and the stationary/mobile phase composition. The retention time refers to how long an analyte takes to elute from a column. Solvents often consist of miscible water or organic liquids, such as methanol and acetonitrile. Gradient elution involves varying the mobile phase composition throughout analysis. Choose the appropriate solvents based on the stationary phase and analyte.[1][2]

PRINCIPLE:

The HPLC principle involves injecting a sample solution into a porous column (the stationary phase) and pumping the liquid phase (the mobile phase) at high pressure through it. The column. The separation principle involves adsorbing solutes on stationary phases based on their affinity. HPLC is a type of column chromatography where the mobile phase is pushed through the column at high speed. As a result, the analysis time decreases by 1-2 orders. Compared to traditional column chromatography, using smaller adsorbent or support particles can significantly increase column efficiency. [3]

CLASSIFICATION OF HPLC:

Based on modes of chromatography

1. Normal phase chromatography
2. Reverse-phase chromatography

Based on the principle of separation

- Adsorption chromatography
- Partition chromatography

- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Based on the elution technique

- Isocratic separation
- Gradient separation

Based on the scale of operation

- Analytical HPLC
- Preparative HPLC[1]

INSTRUMENTATION :

1. Solvent reservoir
2. Pump.
3. Sample injector.
4. Column.
5. Detector.
6. DATA collector.[1]

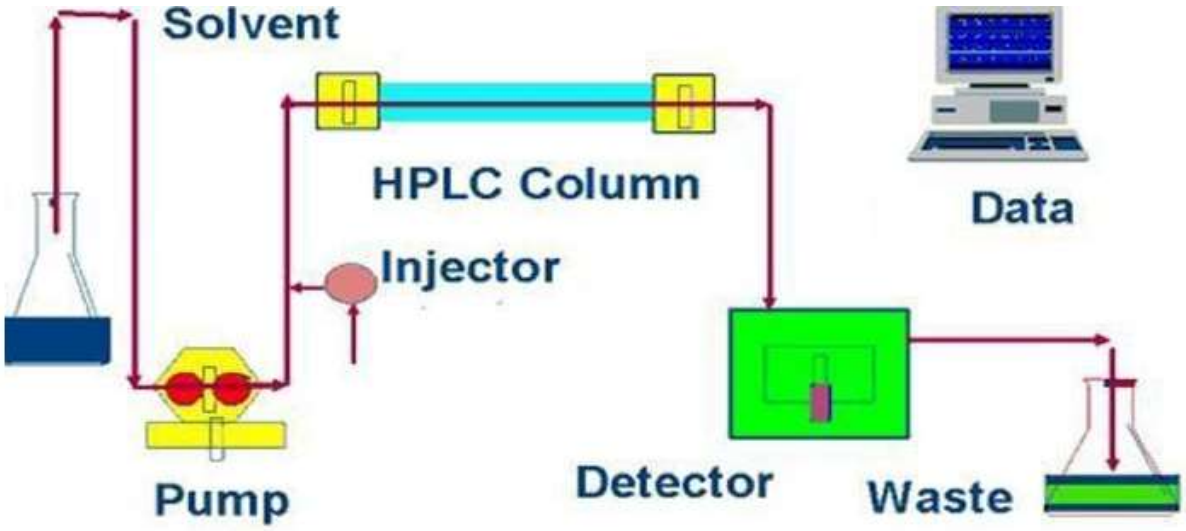


Fig.1: Flow Diagram of HPLC

APPLICATION OF HPLC:

HPLC has several applications in the fields of pharmacy, forensic, environmental, and clinical. It also helps in the separation and purification of a compound.

1) Pharmaceutical Applications: The pharmaceutical applications include controlling drug stability, dissolution studies, and quality control.

2) Environmental Applications: Structure elucidation and Monitoring of unknown pollutants and detecting components of drinking water.

3) Forensic Applications: Analysis of textile dyes, quantification of drugs and steroids in biological samples.

4) Food and Flavour Applications: Sugar analysis in fruit juices, detecting polycyclic compounds in vegetables, and analysis of preservatives.

5) Clinical Applications: Detecting endogenous neuropeptides, analysis of biological samples like blood and urine. [4]

METHOD DEVELOPMENT:

The invention and validation of analytical methods play crucial roles in the analysis, including the creation, development, and manufacturing of drugs and pharmaceuticals. A product that contains one or more drugs is referred to as a combination product. [5]

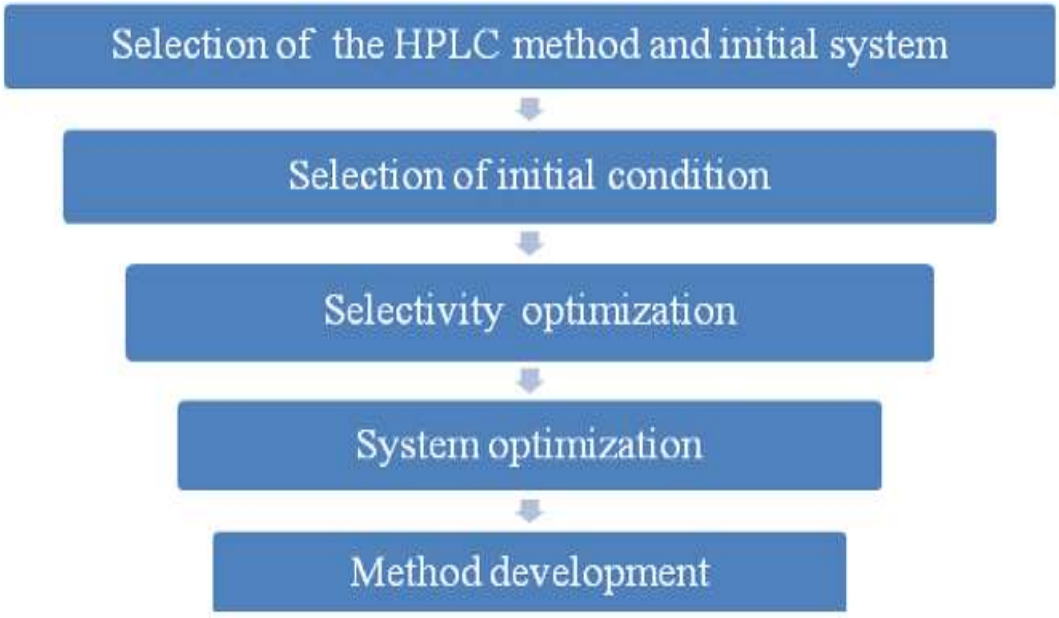


Fig.No:02

Steps in method development:

1. Understanding the physicochemical properties of drug molecule.
2. Selection of chromatographic conditions.
3. Selection of Column.
4. Selection of Chromatographic mode.
5. Optimization of Mobile phase.
6. Buffer Selection.
7. Effect of pH.
8. Effect of organic modifier.
9. Selection of detector and wavelength.
10. Developing the approach for analysis.
11. Sample preparation.
12. Method optimization. [1]

METHOD VALIDATION:

Validation of an analytical technique involves laboratory tests to ensure that the method's performance meets the requirements for its intended use. The validation process for analytical processes.

The applicant collects validation data systematically to assist analytical techniques. Analytical procedures for clinical samples must be validated. Validation of analytical procedures follows ICH criteria.

VALIDATION PARAMETERS:

The following are typical analytical performance characteristics which may be tested during methods validation:

1. Accuracy
2. Precision
3. Repeatability
4. Intermediate precision
5. Linearity
6. Detection limit
7. Quantitation limit
8. Specificity
9. Range
10. Robustness
11. System suitability determination
12. Forced degradation studies
13. Stability studies [(1)]

INTRODUCTION TO DRUG PROFILE: METFORMIN

Diabetes mellitus is a metabolic illness characterized by elevated blood glucose levels caused by insulin deficiency. Symptoms of diabetes include polyuria, polydipsia, and polyphagia. It'll help. The goal is to improve insulin sensitivity, lower blood sugar levels, and prevent diabetes complications. Metformin is an effective and safe treatment for type 2 diabetes, with positive cardiovascular and metabolic effects. The American Diabetes Association recommends using metformin as the first line of treatment for diabetic individuals. [(6)]

PHARMACODYNAMICS:

Metformin, an oral antihyperglycemic medication, improves glucose tolerance in individuals with NIDDM by reducing both baseline and postprandial plasma glucose. Metformin is distinct from other oral antihyperglycemic medicines in terms of both chemical and pharmacological properties. Metformin, unlike sulfonylureas, does not cause hypoglycemia in NIDDM patients or healthy individuals, nor does it cause hyperinsulinemia. Metformin doesn't impact insulin secretion. [(6)]

PHARMACOKINETICS:

ABSORPTION:

Under fasting conditions, bioavailability ranges between 50 and 60% for absorption over 6 hours. Administration with meals slows absorption [35]. After taking an oral dose of GLUMETZA after a meal, the maximum plasma metformin concentration is reached in about 7 - 8 hours**.**

DISTRIBUTION:

Metformin has very little affinity for plasma proteins. Metformin partitions into erythrocytes, most likely over time. Following IV injection, the volume of distribution ranges from 63-276 L, indicating lower binding in the GI tract.

METABOLISM AND EXCRETION:

Metformin is not metabolized by the liver and is eliminated unaltered in the urine, according to intravenous single-dose investigations in healthy individuals. Within the first 24 hours, 90% of the medication is cleared by the kidneys, while the remaining 10% is eliminated through the plasma. The elimination half-life is approximately 6.2 hours.

MECHANISM OF ACTION:

Metformin lowers blood glucose levels via reducing hepatic glucose synthesis, decreasing intestinal glucose absorption, and boosting insulin sensitivity through increased peripheral glucose uptake and utilization. The effects are mediated by the initial stimulation.

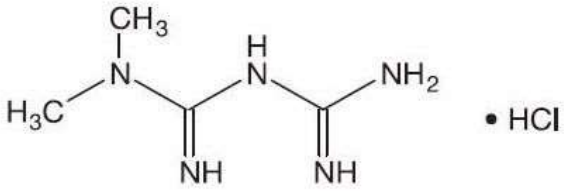
Metformin inhibits the liver enzyme AMP-activated protein kinase (AMPK), which regulates insulin signaling, energy balance, and glucose and fat metabolism. Metformin's ability to suppress glucose synthesis by liver cells requires activation of AMPK. Improved insulin binding to insulin receptors may contribute to increased glucose consumption in the peripheral tissues. Metformin treatment enhances AMPK activation in skeletal muscle. AMPK activates GLUT4 in the plasma membrane, leading to insulin-independent glucose absorption [33-34]. Lactic acidosis, an uncommon adverse effect, may result from decreased liver uptake of serum lactate, a substrate for gluconeogenesis. [6]

ADVERSE EFFECT:

Abdominal pain, Cough, decreased appetite, diarrhea, Breathlessness, Fever, Muscle pain, Painful urination, Sleepiness and Lactic acidosis. [6]

DRUG PROFILE: Metformin

Molecular formula	$C_4H_{11}N_5$
Molecular weight	129.164 g/mol
Chemical name	N, N-dimethylimidodicarbonimidic diamide hydrochloride
Description	Metformin is a first-line biguanide antihyperglycemic drug that lowers blood glucose mainly by reducing hepatic gluconeogenesis and improving insulin sensitivity.
Melting point	223-226°C
Solubility	Freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride.
Adverse effects	Abdominal pain, Cough, decreased appetite, diarrhea, Breathlessness, Fever, Muscle pain.
Bioavailability	oral bioavailability of 40 to 60%
Category	Biguanides
PKa	2.8- 11.5



Metformin Hydrochloride

Fig.no: 03 Chemical structure of metformin

S.no	Methods	Short-term introduction	Ref
1.	Development and validation of an RP-HPLC method for the analysis of metformin.	**Mobile Phase:** Methanol: Water(30:70v/v)	7
		Column: OD-5-100, C18 µ-bonda pack column (0.4×25cm) with 0.5 µm particle size	
		Detector: UV- detector	
		Flow rate: 0.5 ml/min	
		Wave length: 233 nm	
		Linearity: 0.312 -5 µg/ml	
		LOD & LOQ: 0.1& 0.3 µg/ml	
		Retention time: -	
2.	Analytical method development and validation of metformin hydrochloride by using RP-HPLC with ICH guidelines.	**Mobile Phase:** 70:30 (Methanol: Phosphate buffer PH -3	8
		Column: Cosmosil C18 (250mm × 4.61D, Particle size :5 micron)	
		Detector: UV -3000-M	
		Flow rate: 1 ml/ min	
		Wave length: 238 nm	
		Linearity: 10-50 µg/ml	
		LOD & LOQ: 4.2 s/s -9.8 s/s	
		Retention time: Approx 4.2 min	
3.	Method development and validation of metformin HCL and dapagliflozin by using RP -HPLC.	**Mobile Phase:** Phosphate buffer (PH 6.8): acetonitrile (45:55v/v)	9
		Column: CDS version 2.4. C18 column (250mm × 4.6 mm; 5µm)	
		Detector: -	
		Flow rate: 1.0 ml /min	
		Wave length: 220 nm	
		Linearity: 50 -250 µg/ml	
		LOD & LOQ: -	
		Retention time: -	
4.	Development and validation of stability- indicating RP-HPLC method for simultaneous determination of metformin HLC and glimepiride in fixed - dose combination.	**Mobile Phase:** Aqueous phase (20 mM Phosphate buffer, PH 3.0) and organic phase (Methanol: Acetonitrile;62.5:37.5) ratio 80:20	10
		Column: JASCO C18 column (4.6 ×250 mm; 5 µm particle size)	
		Detector: -	
		Flow rate: 1.0 ml/min	
		Wave length: 230 nm	
		Linearity: 5- 30 µg/ml	
		LOD & LOQ: 0.73-0.24 µg/ml	
		Retention time: 2.8-5.8 min	
		Mobile Phase: Acetonitrile: Phosphate buffer (0.01 M, PH 2.5): 0.3% Sodium heptane (60:20:20 v/v/v)	
		Column: Inertsil ODS column (250mm,4.6,5µm)	

S.no	Methods	Short-term introduction	Ref
5.	Experimental design methodology for optimization and robustness determination in ion pair RP- HPLC method: Application for the simultaneous determination of metformin hydrochloride, alogliptin benzoate and repaglinide in tablets.	**Detector:** UV detector	11
		Flow rate: 1ml/min	
		Wave length: 220 nm	
		Linearity: 25-625 µg	
		LOD & LOQ: 0.391& 1.185	
		Retention time: 1.925 min	
6.	Development of a sustainable High-performance liquid chromatography (HPLC) Method for Quantifying metformin, sitagliptin, and empagliflozin in type 2 -diabetes treatment.	**Mobile Phase:** Phosphate buffer (20 mM), Methanol, acetonitrile (65:30:5 v/v/v)	12
		Column: Isocratic elution on C10 column (4.6×250 mm,5µm)	
		Detector: UV spectroscopy	
		Flow rate: 1.0 ml/min	
		Wave length: 208 nm	
		Linearity: 0.08-0.13 mg/ml	
		LOD & LOQ: 0.0266 &0.0807 mg/ml	
		Retention time: 3.0 min	
7.	Bioanalytical RP-HPLC method development and validation for the determination of metformin hydrochloride in spiked human plasma.	**Mobile Phase:** ZPRBAX Eclipse plus C18 (4.6×100mm, 3.5µm)	13
		Column: sodium dodecyl sulphate: acetonitrile (60:40% v/v)	
		Detector: PDA detector	
		Flow rate: 2.0 ml/min	
		Wave length: 235 nm	
		Linearity: 1-6 µg/ml	
		LOD & LOQ: -	
		Retention time: 4.152 min	
8.	Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms.	**Mobile Phase:** Acetonitrile: Ammonium acetate buffer (PH-3) ratio (42:58, v/v)	14
		Column: phenomenex C18 reverse phase column (150× 4.6 mm,5 µm)	
		Detector: UV- visible detector	
		Flow rate: 0.3 ml/min	
		Wave length: 255 nm	
		Linearity: 0.5-50µg/ml	
		LOD & LOQ: 0.003&0.0061 µg/ml	
		Retention time: 5.16 min	
9.	Development and validation of a new analytical HPLC method for simultaneous determination of the	**Mobile Phase:** Ammonium formate buffer (20mM) PH 3.5 and acetonitrile ratio (45:55v/v)	
		Column: Alltima CN column (250mm× 4.6 mm×5µ)	
		Detector: UV detector	
		Flow rate: 6.9 min	

S.no	Methods	Short-term introduction	Ref
	antidiabetic drugs, metformin and gliclazide.	**Wave length:** 227 nm	15
		Linearity: 2.5-150µg/ml	
		LOD & LOQ: 0.8-2.45 µg/ml	
		Retention time: 6.9 min	
10.	Development and validation of RP-HPLC method for simultaneous determination of metformin and miglitol in bulk and pharmaceutical formulation.	**Mobile Phase:** Methanol and potassium dihydrogen orthophosphate buffer (0.01M, PH 5.3 using potassium hydroxide) in ratio(40:60v/v)	16
		Column: Kromosil C18 column (250× 4.6mm;5µ)	
		Detector: UV detector	
		Flow rate: 0.9 ml/min	
		Wave length: 238 nm	
		Linearity: 200-500µg/ml	
		LOD & LOQ: 30µg/ml&0.25µg/ml	
		Retention time: 3.86 min	

CONCLUSION:

HPLC continues to be the most versatile and dependable method for pharmaceutical analysis and drug quality assurance. The comparative overview of different methods for metformin shows that appropriate optimization of chromatographic conditions enhances precision, accuracy, and robustness, making it ideal for routine analysis. With ongoing improvements in analytical techniques, HPLC plays a crucial role in monitoring drug stability, therapeutic efficacy, and safety, thereby contributing significantly to pharmaceutical research and development.

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