CURRENT REVIEW ON METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SOME ANTIDIABETIC DRUGS (METFORMIN, SAXAGLIPTIN, AND DAPAGLIFLOZIN)

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ABSTRACT

Analytical procedures play an important part in delivering solutions such as development. The rapid growth of pharmaceutical industries and medication production in many parts of the world has increased the demand for innovative analytical techniques in the pharmaceutical industries. As a result, developing analytical methodologies has become a vital aspect of research. This review paper provides a description of HPLC Method Development and Validation Procedures for Anti-Diabetic Drugs, with a particular emphasis on Metformin, Saxagliptin, and Dapagliflozin. Drug analysis is critical to the advancement of medicine, its manufacture, and its therapeutic application. A variety of appropriate techniques, including as uv spectrophotometers and HPLC, are used for the simultaneous estimate of medicine included in dosage forms. These are tough, forceful tactics that are also extremely particular, accurate, correct, linear, and fast. Quantitative chemical analysis is used in the pharmaceutical sector to ensure that the raw materials utilised and the final product obtained fulfil the specified specifications.

Keywords: HPLC, Antidiabetic drugs, Metformin, Saxagliptin, Dapagliflozin, Method development, Validation

INTRODUCTION

Diabetes is the most prevalent endocrine condition. It is a collection of disparate illnesses that frequently manifest as periods of glucose intolerance and hyperglycemia. This condition is caused by insulin insufficiency or inefficient insulin synthesis by the pancreas. It has been discovered to harm several biological systems, including the kidneys, heart, eyes, blood vessels, and nerves. Diabetes increases the risk of several consequences, including peripheral vascular disease, cardiovascular disease, stroke, renal failure, neuropathy, amputations, blindness, retinopathy, and so on. According to reports, 366 million individuals had diabetes in 2011, and 4.6 million died as a result of the disease. The number of individuals with
diabetes is expected to reach 552 million by 2030. The prevalence of type 2 diabetes (T2DM) is increasing in all nations, with low- and middle-income countries accounting for 80% of those affected (Shikata *et al*., 2013; Herman *et al*., 2017).

Drugs are generally utilised in diabetes to preserve lives and treat symptoms. Secondary goals of diabetes drugs include preventing long-term diabetic problems and increasing lifespan by removing various risk factors. All of these goals are met by the blood glucose-lowering capabilities of diabetes medications. Except for insulin, pramlintide, liraglutide, and exenatide, all diabetes medications are taken orally and are hence referred to as oral hypoglycemic agents or oral antihyperglycemic medicines. There are various types of diabetes medications, and their use is determined by the type of diabetes, the person's age and circumstances, as well as other consideration

(Padhi *et al*., 2020. Contreras *et al*., 2000).

Established Drug Classes for the Treatment of Diabetes

Alpha-Glucosidase Inhibitors

Alpha-glucosidase is a glucosidic bond cleaving enzyme that is extensively expressed. Inhibiting alpha-glycosidase inhibits complex carbs from being broken down into monosaccharides in the small intestine. As a result, these compounds function as pseudo-carbohydrates (substrate mimics), inhibiting digestive enzymes and preventing oligo- and polysaccharide catabolization to monomers. This results in less sugar being absorbed, lowering postprandial glucose levels and lowering hyperglycemia. Because AGIs have been demonstrated to be equally effective as metformin, they are frequently recommended as first-line therapy or in combination with other antidiabetics (Bischoff, 1994; Lebovitz *et al*., 1997).

Sodium-Glucose Cotransporter Type 2 Inhibitors

SGLT2 inhibitors are the most recent and promising therapeutic class. Canagliflozin and dapagliflozin were the first SGLT2 inhibitors licenced in 2013, followed by other monotherapy drugs empagliflozin in 2014 and ertugliflozin in 2017. Furthermore, SGLT2 inhibitors are commonly used in combination regimens with metformin and DPP4 inhibitors, as well as combinations of all three plus TZD medications. After incretin treatments, SGLT2 inhibitors are the second largest group of antidiabetic medicines in clinical trials (12%). Other regulatory agencies have previously approved three of the twelve medications in phase II, III, and IV clinical trials. Five other medications are in phase III trials, indicating that new SGLT2 inhibitors may be approved in the near future (Milder *et al*., 2018).

Sulfonylureas (SU)

Sulfonylurea (SU) molecules bind to sulfonylurea receptors (SURs) on the surface of beta cells, inhibiting ATP-dependent inward-rectifier potassium ion channels. As a result, the intracellular concentration of potassium cations rises, resulting to depolarization of the plasma membrane. These conditions cause voltage-gated calcium channels to open, and an increase in cytosolic calcium cation concentration causes an increase in insulin secretion. For well than 50 years, SU medications have been widely used to treat T2DM. SUs are well-tolerated, and their popularity may be due to their low cost and ability to be used as a monotherapy or in conjunction with metformin (Costello *et al*., 2022; Kalra and Gupta,2015).

Thiazolidinediones

Thiazolidinediones (TZDs) are insulin sensitizers that activate peroxisome proliferator-activated receptors (PPARs), a nuclear receptor family. TZD molecules can interact with PPAR- and PPAR- isoforms, which are mostly found in fatty tissues and skeletal muscle. This activates these receptors and stimulates complexation with another important component, the retinoid X receptor. The triple complex has the ability to bind selectively to DNA via peroxisome proliferative response elements (PPRE) and operate as a target gene promoter, thereby promoting gene expression. This therapeutic technique raises adiponectin levels while decreasing gluconeogenesis and increasing glucose absorption in muscle and fat. Adiponectin is an adiponectin-secreted hormone that controls glucose concentration by enhancing insulin sensitivity (Henry,1997; Schoonjans and Auwerx,2000).

Meglitinides

Meglitinides work in the same way as sulfonylureas in that they enhance insulin secretion in the pancreas. They attach to SURs in pancreatic beta cells, although at a different binding location than SUs, and initiate the same reaction cascade that results in insulin production. In contrast to SUs, meglitinides, particularly nateglinide, have a glucose-sensitive effect, which means that their potency increases with greater glucose concentrations.

Meglitinides have a shorter half-life and are associated with reduced risks of hypoglycemia, weight gain, and chronic hyperinsulinemia than sulfonylurea medications). Other research has shown that meglitinides may be related with an increased risk of hypoglycemia in diabetic patients with severe chronic renal disease (Black *et al*., 2017; Pfeiffer *et al*., 2016).

Drug Combinations

The number of different types of approved oral combinations has gradually expanded, as has the fraction of approved combinations in comparison to monotherapies. Combination regimens account for over 40% of all authorised diabetes medications. FDA-approved antihyperglycemic medication combinations are classified into two generations. First-generation combinations were mixes of various insulin isoforms that differed in preparation process, natural source, duration of action, or concentrations. By 2004, the proportion of medication combinations had increased significantly as second-generation antihyperglycemic combinations became available. They mostly consisted of medications that had to be taken orally, and metformin was usually one of them. There are now 23 distinct antihyperglycemic medication combinations. In 2019, the first triple combination regimen of metformin, saxagliptin, and dapagliflozin was approved. In 2020, metformin, linagliptin, and empagliflozin were approved in another triple combination. This trend emphasises the importance of utilising all available techniques for the entire treatment of T2DM. (Ramzan *et al*., 2018; Wilkinson, *et al*., 2017).

Analytical chemistry

Analytical chemistry has never been more important than it is today. The need in modern cultures for a diverse range of healthy meals, affordable consumer goods, plentiful energy, and labor-saving technologies places a significant strain on the environment. In addition to the desirable chemicals, all chemical manufacture produces waste products, and waste disposal has not always been done carefully. It is critical in pharmaceutical sciences. It delivers precise and reliable data to enhance drug research and development operations. Examples include drug substance purity during synthesis, pharmacokinetic investigations, drug stability, elucidation of drug metabolic pathways, drug-protein interactions, and so on (Skoog *et al*., 2013).

Pharmaceutical medicines for therapeutic use are either manufactured or naturally derived from plants. To ensure that these pharmaceuticals serve their purpose, various analytical methods for drug quantification are created at regular intervals. These procedures include bulk drug analysis, intermediates, drug products, drug formulations, contaminants, and biological fluid analysis. Some analytical methods for the qualitative and quantitative analysis of pharmaceuticals in environmental samples, such as wastewater and forensic toxicology samples, have also been developed. Despite the availability of diverse instrument characteristics, there are numerous problems in developing precise and dependable methods. These issues include selecting an effective sample preparation strategy for separating a selection of analytes from the matrix and determining the target compounds based on their physicochemical properties (Christian *et al*., 2013).

Sophisticated analytic instruments, frequently linked with computers, have increased chemists' ability to identify chemicals and lowered detection limits. Gas chromatography is a common analytical technique that separates the distinct components of a gaseous mixture by passing the mixture through a long, narrow column of absorbent but porous material. Different gases interact differently with this absorbent material and move at different rates through the column. As the various gases exit the column, they can be fed into another analytical equipment known as a mass spectrometer, which separates compounds based on the mass of their constituent ions. A combination gas chromatograph-mass spectrometer can quickly detect individual components of a chemical mixture with concentrations as low as a few parts per billion. Under suitable conditions, techniques such as atomic absorption, polarography, and neutron activation can yield similar or even higher sensitivities. Because of the rapid rate of

instrumental invention, analytic instruments are frequently rendered outdated within ten years of their introduction. Newer instruments are more accurate and faster, and they are frequently used in environmental and pharmaceutical chemistry(Harvey,2000).

Method development

Analytical technique development and validation play critical roles in pharmaceutical discovery, development, and manufacturing. These techniques are used to assure the identification, purity, potency, and safety of pharmaceutical items. The HPLC method's purpose is to isolate and quantify the main active medication, any reaction contaminants, any possible synthesis intermediates, and any degradants (Sahu *et al*., 2018).

Need for developing a method:

- The available approach may be excessively expensive, time consuming, or energy heavy, or it may be difficult to automate.
- Existing methods may be prone to inaccuracy, contamination, or be unreliable.
- There may be a need for an alternative method to confirm analytical data obtained by existing procedures for legal or scientific reasons.
- There may be no viable method for a given analyte in the sample matrix.
- The existing method may not be sensitive enough.
- It is required for regulatory purposes.
- Newer instrumentation and techniques may have evolved, allowing for superior methods such as improved analyte identification or limit of detection, more accuracy, or a higher return on investment(Singh,2013).

Steps for the development of the method

The development procedure is followed with proper documentation. All data from these experiments must be recorded in a laboratory notebook or an electronic database.

a) All known important information about the analyte and its structure is gathered, including physicochemical parameters such as solubility, optical isomerism, and so on.

c) The pure standard analyte is obtained. A perfect storage setup (refrigerator, desiccators, and freezer) must be created.

c) When many components are to be analysed in the sample matrix, the number of components is indicated duly presenting the data and the accessibility of standards is evaluated.

d) Methods such as spectroscopy, HPLC, GC, MS, and others are considered when matched with sample stability. (Vidushi *et al*., 2017).

Preparation of sample solutions for method development

The drug substance being tested should be stable in solution (diluent). Preparations of the solutions in amber flasks should be undertaken during initial method development until it is verified that the active component is stable at room temperature and does not degrade under typical laboratory settings. The sample solution should be filtered; normally, a 0.22 or 0.45 m pore-size filter is recommended for particle removal. Filtration is an HPLC analysis preventative maintenance tool. The analyst must explore sample preparation as a vital phase in method development. The capacity of syringe filters to remove contaminants/insoluble components without leaching unwanted artefacts (i.e., extractables) into the filtrate determines their effectiveness. If there are any additional peaks in the filtered samples, the diluent must be tested to see if a leachable component is coming from the syringe filter housing/filter (Sowjanya *et al.,* 2015).

Method optimization:

The compositions of the mobile and stationary phases must be considered. Optimisation of mobile phase

parameters is always prioritised over optimisation of stationary phase parameters since it is considerably easier and more comfortable. Only the parameters that are likely to have a substantial effect on selectivity in the optimisation must be studied to reduce the number of trial chromatograms involved.

Method Validation

Analytical technique validation is defined as "documented proof that a particular process will consistently produce a product that meets its set criteria and quality features." (Chawla *et al*., 2019).

Accuracy

The term "truth" is used to characterise it. Accuracy is a measure of how close the measured and true values are to each other. When measured against the reference standard, The analyte was spiked into a blank matrix before being recovered using the standard addition method. The reported accuracy limitations for drug substances and goods are 98.0 - 102.0% and 97.0 - 103.0%, respectively. Impurity determination may withstand an average recovery range of 50 to 150%.

Precision

The accuracy of an analytical procedure is defined as the degree of agreement between a series of measurements acquired from multiple samplings of the same homogeneous sample within the specified parameters of repeatability, intermediate precision, and reproducibility.To ensure method precision for important analytes, RSD should be 2%. RSD of 5-10% is normally considered acceptable for low-level pollutants.

- **Repeatability:** The term "repeatability" relates to how well a procedure performs in a single lab and on a single equipment on a given day. The 'Repeatability limit' calculation is useful. It is necessary to prepare a minimum of six determinations at 100% of the test concentration.
- **Reproducibility:** Reproducibility refers to how well a method works from lab to lab, day to day, analyst to analyst, and instrument to instrument, in both qualitative and quantitative terms. It is useful to calculate the reproducibility limit,' R.
- **Intermediate precision:** The percentage RSD of tests measures how well a method works qualitatively and quantitatively inside a single lab, but also from instrument to instrument and day to day. Linearity B. The capacity of an analytical technique to produce test results that are directly proportional to the concentration of analyte in the sample is referred to as its linearity. Statistical approaches should be used to evaluate test results. For example, using the least squares approach to calculate a regression line

Detection Limit:

The detection limit of an individual analytical method is the smallest amount of analyte in a sample that can be detected but not necessarily quantified as an exact number. LOD is calculated using the formula LOD= 3.3 /S. Where = standard deviation of calibration curve intercepts (Yadav and Bharkatiya,2017).

S = the slope of linearity plot. Visually, the detection limit can be identified. ratio of signal to noise the response's standard deviation and the slope

Quantitation Limit:

The quantitation limit of a specific analytical technique is the smallest amount of analyte in a sample that can be quantitatively quantified with adequate precision and accuracy. The quantitation limit is a quantitative test parameter for determining contaminants and degradation products in sample matrices with low concentrations of chemicals. It can be determined by following formula, $LOQ = 10 \times \delta/S$ Where, δ = standard deviation of response.

S = Mean of slopes of the calibration curves.

Specificity

Specificity refers to the ability to measure the desired analyte in the presence of components that are expected to be

present. It is common to find impurities, degradants, matrix, and other components. The peak purity value must be more than 0.999 (for Agilent systems) or the purity angle must be less than the threshold in all scenarios (for Waters systems).

Range

An analytical technique's range is the distance between the sample's upper and lower analyte concentrations for which the analytical procedure has been proved to have a suitable level of precision, accuracy, and linearity.

Robustness

The robustness of an analytical procedure is a measure of its capacity to withstand minor but deliberate changes in method parameters, and it reflects its dependability in routine use. Variable process factors in HPLC include flow rate, column temperature, sample temperature, pH, and mobile phase composition (Ravisankar, 2014).

System suitability

As a routine practise, liquid chromatographic operations include system suitability checks. They are used to ensure that the detection sensitivity, resolution, and reproducibility of the chromatographic system are adequate for the analysis. The tests are based on the premise that the equipment, electronics, analytical processes, and samples to be examined are all components of a bigger system that may be evaluated. The approach's adequacy was determined by measuring characteristics such as peak resolution, theoretical plate count, peak tailing, and capacity (ICH,1996)

Force Degradation

Force degradation of therapeutic material can help identify the most likely degradation products, which can subsequently be used to determine the molecule's degradation pathways and inherent stability. The parts of force degradation are as follow:

- Acidic hydrolysis
- Alkaline hydrolysis
- Hydrolytic
- Oxidative degradation
- Solid state stability
- Thermal degradation
- Photolytic degradation (Reynolds *et al*., 2002).

Advantages of Analytical Method Validation

- It instills trust in the developer as well as the user.
- Although the validation exercise appears to be costly and time consuming, it ultimately shows to be inexpensive by reducing unpleasant repetitions and leads to better time management.
- Method validation absorbs the shock of varying analytical conditions and pays for itself more than once.

The development of analytical methods for drug identification, purity evaluation, and quantification has gained a lot of attention in the field of pharmaceutical analysis in recent years. Prior to developing any HPLC process, it is critical to understand the physiochemical parameters of the main molecule. The makeup of the buffer and mobile phase (organic and pH) has a significant impact on separation selectivity. Finally, the gradient slope, temperature, and flow velocity, as well as the kind and concentration of mobile-phase modifiers, can be optimised. The optimised method is validated using several criteria (such as specificity, precision, accuracy, detection limit, linearity, and so on) in accordance with ICH requirements (Azim *et al*., 2013).

Table 1: Method development & validation studies on Metformin, Saxagliptin & dapagliflozin

Name of drugs	Method developed	References
Saxagliptin and dapagliflozin	The procedure was carried out on a Youglin-HPLC machine -SP930D. The analyte separation was accomplished using a Hypersil BDS C18 (250mm x 4.6mm, particle size: 5 um) column with a mobile phase of (Methanol: Acetonitrile: Water: Trifluloroaceticacid, 50:25:25:0.1) with a flow rate of 1.0 mL/min.	(Nagulkar and Waghulkar,2023)
Metformin, dapagliflozin, and saxagliptin	The suggested approach employs a Kromasil C18 column (150 4.6 mm, 5 m) with a column oven temperature of 30°C and a mobile phase composed of 60% phosphate buffer (pH = 3) and 40% acetonitrile. The injection volume was 10 L, and the flow rate was set to 1.0 mL/min. The detection was performed at 230 nm.	(Vankalapati *et al*., 2022)
Dapagliflozin and Saxagliptin	The BDS C18 (150 x 4.6mm, 5.0) column was employed in an isocratic mode with mobile phase comprising an Ammonium acetate buffer: ACN (40: 60%v/v) for efficient chromatographic separation. The mobile phase flow rate was set at 1.0mL/min with a column temperature of 30oC and a wavelength detection of 220nm.	(Manasa and Aanandhi,2021)
Saxagliptin, Dapagliflozin and Metformin	Agilent C18 150 x 4.6mm, 5| was used. For the chromatogram development, a mobile phase containing 0.1% OPA:Acetonitrile in a 50:50 ratio was injected through the column at a flow rate of 1.0 ml/min. The buffer in this approach was 0.1% OPA. The	(Kandula and Sundararajan,2021)

	temperature was kept at 30°C. The optimal wavelength chosen was 260nm.	
Saxagliptin and Metformin	Younglin (S.K) isocratic System UV Detector C18 column (150 mm 4.6 mm) was used. The mobile phase in this method was a 25:75 mixture of Potassium Acetonitrile: Pot. Phosphate Buffer (ph 3.2) and the process was verified according to ICH guidelines with a flow rate of 1.0 ml/min (UV detection at 212 nm).	(Mittal *et al*., 2021)
Metformin Hydrochloride, Saxagliptin, Dapagliflozin	Aluminium HPTLC sheets covered with silica gel 60 F254 were used for separation, using a mobile phase of acetonitrile: 1% w/v ammonium acetate in methanol (9: 1, v/v). Scanning was done at 210 nm.	(Abdelrahman *et al*., 2020)
Dapagliflozin Propanediol and Metformin Hydrochloride	Column C8 Thermoquest, hypersil division of dimension 250 4.60 mm, particle size 5 micron was used. The mobile phase employed in the procedure was a 30:65:05 mixture of 10 mM ammonium acetate buffer (pH-4), methanol, and acetonitrile.	(Patel and Patel, 2020)
Saxagliptin and Dapagliflozin	The discovery C18 column (250 mm, 4.6 mm, and 5 m) was used. The mobile phase was a 50:50 combination of acetonitrile and 0.1% orthophosphoric acid. A wavelength of 210 nm was chosen for detection, and both medicines responded well.	(Gundala *et al*., 2019)
Saxagliptin hydrochloride and Dapagliflozin	Grace C18 (250mm x 4.6 ID, particle size: 5micron) was used to elute the materials. At 225nm, methanol: water (pH adjusted to 3 with potassium dihydrogen orthophosphate) (80: 20).	(Godge *et al*., 2019)
Dapagliflozin and	The mobile phase was buffer and	(Singh *et al*., 2018)

Saxagliptin	acetonitrile (53:47 v/v) in an Xterra C-18 analytical column (150 mm 4.6 mm i.d., particle size 3.5). The buffer employed in mobile phase comprised 20 mM sodium dihydrogen phosphate, and its pH was adjusted with orthophosphoric acid to 5.5 0.02. The flow rate of the apparatus was set to 1.2 mL min1 at room temperature, and the wavelength of the UV-visible detector was 230 nm.	
Empagliflozin, Canagliflozin, Dapagliflozin and Metformin	The separation was carried out on a C18 column (2504.6 mm-5m p.s) Inertsil® ODS utilising isocratic elution with acetonitrile and 0.05 M potassium dihydrogen phosphate buffer PH 4 at a ratio of [65:35, v/v] as the mobile phase at a flow rate of 1ml/min. UV detection was carried out at 212 nm.	(Khalil *et al*., 2018)
Metformin and Dapagliflozin	The determination was performed using an HPLC of waters (Model: Alliance 2695) with a Phenomenex Luna C18 (4.6mm I.D. 250mm, 5m) column for chromatographic separation. It has a water injector as well as a PDA detector (Deuterium). Acetonitrile: Water (75:25% v/v) was used in the mobile phase, and the flow rate was set to 1ml/min. The detecting wavelength was 285nm, and the injection volume was 10 microliter.	Urooj *et al*., 2017
Saxagliptine and Dapagliflozin	Separation takes place on an XTerra C 18 column (150mm x 4.6mm x 5m particle size). The optimised mobile phase is made up of phoaphate buffer (pH 4) and acetonitrile (50:50v/v) at a flow rate of 1ml/min with UV detection at 225mn.	Kommineni *et al*.,2017

Conclusion

The development of analytical methods for drug identification, purity evaluation, and quantification has gained a lot of attention in the field of pharmaceutical analysis in recent years. This paper discusses HPLC technique development and validation for diabetes medicines in general, with an emphasis on Metformin, Saxagliptin, and Dapagliflozin in particular. A general and extremely easy technique to developing HPLC methods for chemical separation was outlined. Prior to developing any HPLC process, it is critical to understand the physiochemical parameters of the main molecule. The makeup of the buffer and mobile phase (organic and pH) has a significant impact on separation selectivity. Finally, the gradient slope, temperature, and flow velocity, as well as the kind and concentration of mobile-phase modifiers, can be optimised. The optimised method is validated using several criteria (such as specificity, precision, accuracy, detection limit, linearity, and so on) in accordance with ICH requirements.

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