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Various Method Development and Validation, Impurity Profiling, Toxicological Studies of Metformin in Combined Dosage Form -A Review

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ABSTRACT:
Metformin is a most common drug used to treat type 2 diabetes mellitus.The hydrophilic cation metformin has the ability to bind to metals. In contrast to oral treatment, intravenous metformin injection did not result in any immediate changes in glucose metabolism. For the determination of metformin in bulk and pharmaceutical dosage forms, a number of analytical techniques likeSpectrofluorimetry, UV-visible spectrophotometry, RP-HPLC, HPTLC, and UPLCMS approaches were established. It is appropriate for standard quality control assays of pharmaceutical formulations and the medication in pure forms. As Metformin toxicity research and impurity profiling were developed.

KEY WORDS: Metformin, RP-HPLC, UPLCMS, HPTLC, Spectro-fluorimetry, UV–visible spectrophotometry, Impurity profiling, and Metformin toxicology investigations.

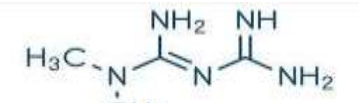
I. INTRODUCTION:

Diabetes mellitus is a group of metabolic disorders in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. Diabetes mellitus can be classified into two,

1)Type 1 diabetes mellitus
2)Type 2 diabetes mellitus

Metformin is a medicine used to treat type 2 diabetes and gestational diabetes.Metformin HCL is 1,1-dimethylbiguanide hydrochloridechemically, and it has antidiabetic effects. It serves as the initial line of treatment for type 2 diabetes mellitus. There are over 380 million diabetic patients worldwide, of whom about 120 million intake metformin.Metformin is a drug derived from the guanidine in Gallego officinalis.

METFORMIN



Chemical Formula - $C_4H_{11}N_5$
Molecular Weight - 129.16 g/mol
Melting Point - 221
P ka Value - 12.4

Metformin, a biguanide, lowers blood sugar levels by inhibiting the liver from making glucose. Adults with type 2 diabetes mellitus are prescribed metformin, either alone or in conjunction with insulin, other antidiabetic drugs, and dietary changes.

Metformin helps persons with type 2 diabetes mellitus lower their fasting plasma glucose levels and blood glucose levels after eating, according to a thorough investigation. Because it is quickly absorbed from the small intestine, metformin improves lipid and glucose metabolism and decreases glycaemic index. In around two hours, the peak plasma concentration is completely absorbed. According to a meta-analysis, metformin reduces glycated haemoglobin levels by about 1% or more and Fasting Plasma Glucose levels by at least 4.00 mmol/l. It reduces gluconeogenesis, stimulates glycolysis, and inhibits glycogenolysis. Metformin also improves blood free fatty acid levels, reduces them, and cures dyslipidemia. The preventive properties of metformin may be advantageous for pancreatic islet cells.

The diabetes prevention study discovered that over the course of three years, metformin enhanced beta cell function in persons with poor glucose tolerance and reduced the progression of diabetes by 31%. Metformin, however, only has an effect when endogenous insulin is available, which means it only has an effect while some functional pancreatic islet cells are still alive. Metformin does not directly stimulate insulin secretion, but it does increase insulin sensitivity because it improves

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glucose absorption in peripheral tissues and glucose utilization in muscular tissues. As a result, the enterocytes' lactate concentration rises. Metformin has been reported to have less of an impact on dipeptidyl peptidase-4 while increasing plasma levels of glucagon-like peptide-1. Metformin has been shown to be both effective and safe, and it is typically well tolerated. Due to the drugsadverse effects, many people cannot tolerate them. Oral metformin pills may have mild to severe adverse effects.

The typical adverse reactions are heartburn, headache, agitation, chills, dizziness, nausea, stomach bloating, flatulence, vomiting, diarrhoea or constipation, diarrhoea or constipation, upper respiratory tract infection, and an altered or metallic taste. Although the liver is one of the drug's primary sites of action, recent studies have shown that metformin also has an impact on the gut because of its connection to the gut-brain-liver axis.

The majority of gastrointestinal and digestive tract problems are seen after taking metformin. Metformin causes the bile acids in the colon to increase, which can impact the microbiome and influence glucagon-like peptide-1 secretion, cholesterol levels, and stool consistency. Diagemet, bolamyn, glucophage, metabet, glucient, fortamet, and glumetza are a few of the brand names for metformin that are available. The medication known by its generic name, metformin, comes in a variety of dosage forms including tablets, capsules, oral suspensions, oral solutions, and tablets with a modified release.

By inhibiting mitochondrial glycerophosphate dehydrogenase, metformin acts through both an adenosine monophosphate-activated protein kinase dependent and independent mechanism. It may also act through an inhibition of mitochondrial respiration and a lysosomal mechanism.

The brand name glucophage is used for the rapid release metformin pill. The commercial name for liquid medium is Riomet. Children under the age of 10 are not allowed to use metformin.

The type of metformin that is used in pharmaceutical formulations is the hydrochloride salt, which has an absolute oral bioavailability of 40–60%, gastrointestinal absorption that seems to be finished within 6 hours of consumption, is rapidly distributed, does not bind to plasma proteins, does not go through liver metabolism, and has not been linked to any metabolites or metformin conjugates.

Clinically severe drug interactions involving metformin are uncommon. A few drugs may prevent metformin from working properly. Pre-existing diabetes and gestational diabetes are both treatable during pregnancy with metformin. Metformin can be taken safely throughout pregnancy, either alone or in combination with insulin.

If cimetidine, frusemide, or nifedipine are also provided simultaneously, the concentration of metformin may rise. Metformin should be used with caution in patients who have trauma, fever, congestive heart failure, surgery, renal or hepatic impairment, or in the elderly, and metformin treatment should be stopped before any type of surgery. Dosages should be adjusted if a patient is taking frusemide, prednisolone, oestrogen, progesterone, testosterone, contraceptive pills, or other diabetes medications, and blood glucose levels may need to be checked more frequently.

Women who have polycystic ovarian syndrome can also utilize it. Metformin may improve fertility and regularize menstrual periods. The term "insulin sensitizer" is also used to describe metformin. On cancer cells from the colon, pancreas, breast, ovary, prostate, and lung, metformin exhibits potent antiproliferative actions. Metformin obtained Food and drug administration approval in the US in 1995 after originally receiving permission in Canada in 1972.

In comparison to other glucose-lowering medications, metformin is related with a better short- and long-term outcome in patients with acute coronary syndrome. Patients taking metformin showed lower levels of holotranscobalamin and serum B12 than normal.

Metformin should be prescribed with caution to patients who have type 2 diabetes mellitus and chronic kidney disease since it can impair kidney function, necessitating continuous monitoring of renal functions.

Metformin can aid the body in preventing high blood sugar-related issues like retinopathy, diabetic neuropathy, and kidney damage. It properly breaks down food and improves the body's capacity to react to insulin. Because of its inexpensive price and strong safety record, metformin is advised.

Through skin absorption, ingestion, or inhalation, metformin hydrochloride may be hazardous. It irritates the upper respiratory tract, mucous membranes, and eyes in addition to causing skin and eye discomfort. Although it has a stated Lethal

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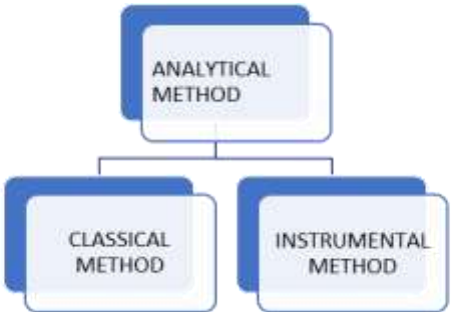
Dose 50 of 1g/kg in rats, it is thought to be of low order of toxicity.

In comparison with sulphonylureas, metformin is found to be ineffective in hypoglycaemic condition.

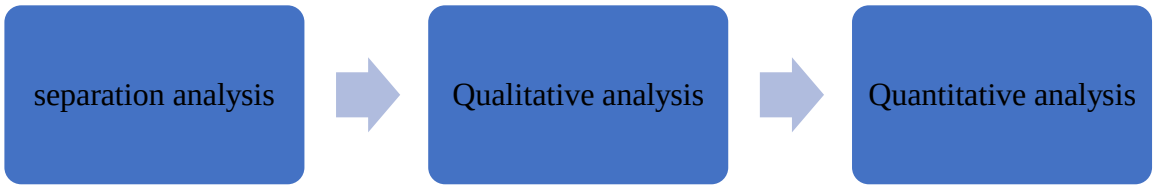
II. ANALYTICAL METHOD

"Analytical method" refers to a set of steps and a technique that are employed in the qualitative, quantitative, or structural analysis of a sample for one or more analytes.

Spectral, chromatographic, electrochemical, hyphenated, or other analytical methods are all possible. Development of analytical methods is the process of choosing an exact assay method to a formulation composition. Analytical techniques are primarily divided into two categories.



The term "classical method" refers to a method in which the signal is inversely proportional to the absolute amount of the analyte.Three categories of traditional techniques are



A technique where the relationship between the signal and analyte concentration is inverse. This is called as instrumental technique, it can be categorized into four categories:The following methods are available:

a) Spectroscopic Methods,
b) Electrochemical Methods,
c)Chromatographic Methods
d) Other Techniques.

2.1METHOD DEVELOPMENT AND VALIDATION

The development of a method is the process of deciding on an appropriate analysis approach to manage the preparatory arrangement. These techniques are used to verify the authenticity, openness, potency, and safety of pharmaceutical medicines. The goal of an analytical chemist is to be able to offer data that is accurate, dependable, and consistent.

The protocols and acceptance criteria outlined in the International Council for Harmonisation guidelines must be implemented when developing analytical method development techniques for use in Good manufacturing practices and Good Laboratory practices environments. Qualified and calibrated instruments, documented techniques, trustworthy reference standards, qualified analysts, sample selection and integrity, and change control are requirements for method development.

The following are the typical steps in the method development process:

1)Characterization of Standard Analyte
2) Required Methods
3) Literature
4) Method
5) Instrumentation and Initial Research

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6) Parameter optimization
7)Documentation
8) Evaluation of the Method Development Using the Sample
9) Calculating the Sample Percent Recovery
10) Quantitative Sample Analysis Demonstration

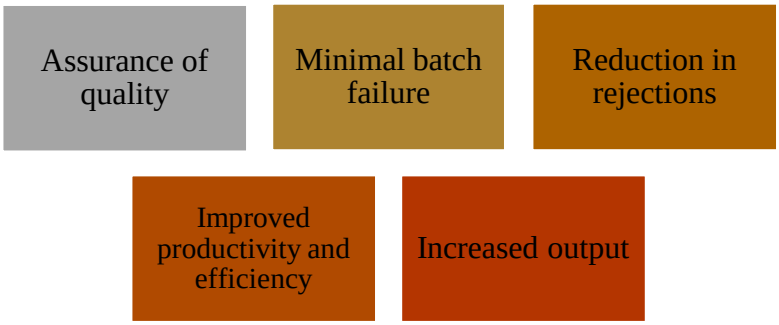
VALIDATION

The written proof that offers a high level of assurance to a desired result with planned conformity is the validation process. In the pharmaceutical industry, the phrase "validation" is frequently employed. The word "validation," which meaning "legally defined," is where the phrase "validation" originates.

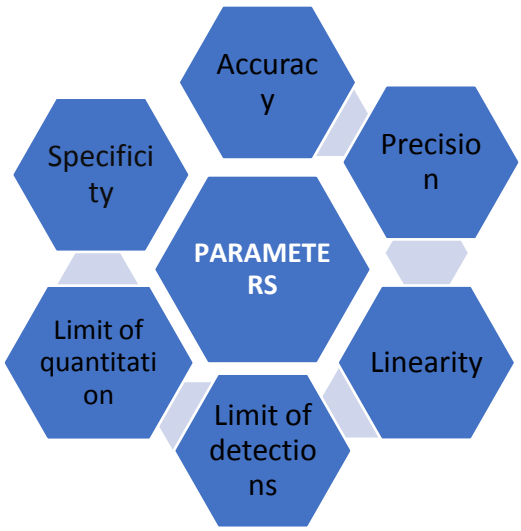
The "process of establishing documented evidence" known as "method validation" offers a high level of assurance that the equipment will satisfy the demands of the planned analytical applications.

Confidence in the validated approach is low. After method creation, it must be validated in accordance with requirements to provide a level of assurance for use as intended.

IMPORTANCE OF VALIDATION



PARAMETERS OF METHOD VALIDATION



Method accuracy is defined as the test result generated by an analytical method match the actual value. The sample matrix of interest is spiked with an analyte standard at a predefined concentration and evaluated using the "method being validated" in order to ascertain accuracy. A method precision is the degree of consistency between individual test results when a technique is applied repeatedly to different samples. Precision is determined by injecting a number of standards or by analysing a number of samples from various samplings from a homogeneous batch.The smallest concentration at which the method can accurately detect the analyte in the matrix is known as the

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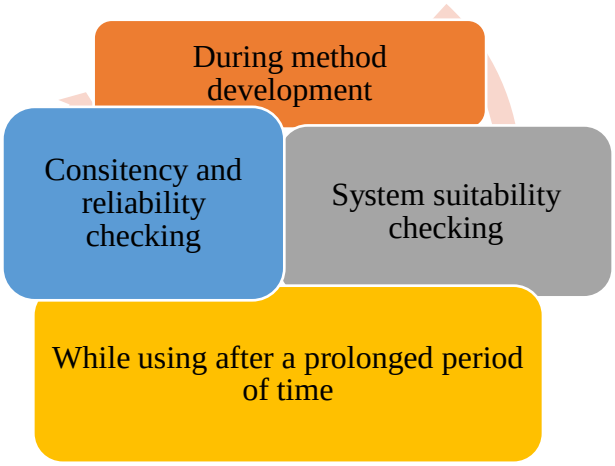
Limit of Detection. The limit of quantitation is the lowest analyte concentration that can be quantitatively determined with a specific level of accuracy and precision. Specificity is the ability of an analytical method to measure an analyte precisely even when interferences that might be present in the sample matrix are expected.

CONDITIONS FOR VALIDATION



2.2. UV-VISIBLE SPECTROSCOPY

A quick, dependable, and effective approach was created and validated for the determination of metformin hydrochloride. Metformin concentration was discovered to be more than 7 g/mL in accordance with Beer's law. The investigation of analytical method validation involves the determination of metformin by UV in tablet formulation.

Metformin stock solution was diluted to a concentration of not more than 100 g/ml in order to accept the analytical wavelength selection. From the overlapping spectra, the wavelength range of less than 400 nm was scanned before choosing the wavelength for the creation of the calibration curve. Different metformin concentration ranges that were discovered to be not more than 13 g /ml were developed from the working standard solutions. The drug absorptivities were calculated at 233 nm in wavelength. The computedresults were based on the mean of six determinations.System specificity, appropriateness, limit of quantification, linearity of response, limit of detection, precision, accuracy, and robustness were utilized as parameters to examine the validity.

The Ultra-violet spectrophotometric approach was straightforward with less time. Less reagent and ingredients were required for this procedure. UV spectroscopic methods were used in the pharmaceutical sectors to conduct quality checks. The response of the detector was measured throughout a range of less than 13 g/ml. The UV-spectroscopy technique was assessed for the correlation coefficient and intercept value.

The accuracy is assessed through experimentation. The recovery studies were carried out nine times. The percentage relative standard deviation and recovery was estimated, and it was discovered that the standard drugs yield similar result to that of test.

The inter-day and intra-day precision for metformin were found to be roughly more than 0.093. It was determined that a UV-spectrophotometric approach was reliable and accurate. Recovery experiments that were approved demonstrate that excipient influence is non - existent.

The limits of quantification and detection of the UV-spectrophotometric method were used to calculate a series of escalating concentrations of the standard solution. The signal to noise ratio was used to establish the Limit of detection, or lowest detectable dose, of the analyte. The signal to noise ratio was used to quantify the Limit of quantitation, which is the lowest concentration, with the necessary accuracy and precision.

Limit of detection and Limit of quantitation for metformin were both determined to be less than 3.0

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g/ml, respectively. Changes in the proportion of organic material, the mobile phase's pH, and the wavelength were used to calculate the method's validity.

The absorbance did not significantly alter using this procedure. It demonstrated the consistency with which the UV-spectrophotometric approach was created. The UV-spectrophotometric approach is straightforward and precise, and it is also simple to use. Samples were quickly analysed using the UV-spectrophotometric technique. When the low limit of detection and low limit of quantification make this approach appropriate, it is employed in quality control testing.

2.3. UPLCMS METHOD

The ultra performance liquid chromatography-tandem mass spectroscopy technique is a quick, reliable, easy-to-use approach. By employing tolazamide as an internal standard, the Ultra-performance liquid chromatography-tandem mass spectroscopy approach was used to analyse and verify glimepiride, metformin, and pioglitazone. Acetonitrile was employed as the mobile phase in a 1.8 m, 2.1x50 mm column to achieve separation in chromatography.

Trifluoroacetic acid is used in this approach to modify the ammonium acetate buffer in gradient mode. Glimepiride, metformin, and pioglitazone were separated using the chosen chromatographic conditions. The flow rate for this approach was discovered to be roughly not less than 0.4ml/minute.

Acuity Waters The validation assay was carried out using an Ultra performance liquid chromatographyMicromesh Quattro Micro API mass spectrometer, auto sample, Evaporating light scattering detector, photodiode array, fitted with a binary solvent delivery system. On a C18column, the Ultra performance liquid chromatography-tandem mass spectroscopy procedure was used, with acetonitrile serving as the mobile phase. Trifluoroacetic acid was used to alter the buffer pH in the Ultra performance liquid chromatography condition, and a flow rate of more than 0.4 ml/minute was maintained at room temperature.

The necessary amount of glimepiride, metformin, pioglitazone, and tolazamide were prepared as a stock solution, which was then liquefied in methanol and reduced with acetonitrile. The developed stock solutions were kept in temperature-neutral storage.

Standard solutions were obtained from the stock solution by diluting it with acetonitrile, glimepiride, and metformin. Each solution was provided in triplicate and chromatographed in accordance with the chromatographic condition. Utilizing tolazamide as an internal reference, the combination of metformin, glimepiride, and pioglitazone concentrations were measured.

The tablet was roughly weighed and then finely pulverized. The amount of powder, which was equal to 1 tablet, was transferred to the measuring flask together with the necessary doses of metformin, glimepiride, and pioglitazone. This flask was filled with the appropriate amount of methanol, and the mixture was periodically shaken while briefly being sonicated. It was cooled until the combination was at room temperature.Acetonitrile was then added to the volume, and it was centrifuged for a short time at a speed of more than 8,000 rpm.

The centrifuged solution was filtered through Nylon filters with a maximum pore size of 0.45 m. Aliquots of the appropriate volume from the filtered solution were transferred to the volumetric flasks and diluted to volume with acetonitrile to provide the concentration range.

The method linearity was evaluated using the various concentrations in the glimepiride, metformin, and pioglitazone ranges. Glimepiride was found to have a concentration larger than 2ng/ml, pioglitazone was found to have a concentration not greater than 90ng/ml, and metformin was found to have a concentration less than 3,000ng/ml. Recovery tests using the traditional addition method were done to assess the accuracy of the approaches. The intra-day and inter-day experiments involved the evaluation of standard and sample solutions in triplicate on the same day.

Using a technique based on the response of standard deviation and the slope of the calibration plots, the detection was established in line with internationalconference on Harmonisation requirements withLimit of detection and Limit of quantitation values.

Robustness is the capacity of analytical techniques to remain unaffected by minute but deliberate alterations to the working environment.

Three columns were utilised to determine the medicines simultaneously. Using the C18 column gave us the best resolution and peak shape with minimal tailing.Acetonitrile and 1% of ammonia acetate buffer in the mobile phase provided the best resolution with adequate retention duration. pH was adjusted appropriately using trifluoroacetic acid in gradient elution mode at a

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flow rate was found to be approximately greater than 0.1ml/min. To maximise Patent ductus arteriosusdetection sensitivity at a low wavelength, 1%concentration was primarily used.

Mass spectroscopy characteristics were used to create and validate a quick, selective test method for glimepiride, metformin, and pioglitazone determination in pharmaceutical dosage form. By injecting a reference analyte solution into the mass spectrometer using an atmospheric pressure electrospray as the ionisation source, the mass spectroscopy parameters were optimised.The method is suitable as evidenced by the Relative standard deviation values of peak area and retention time for medicines and internal standard is being within 2%.

The peak areas of the medications were plotted against internal standard, which was linear in the designated range, to create the calibration curves.

Recovery studies using the usual addition method were conducted in order to evaluate the accuracy of method. Additional standard recovered percentage of the assay samples was calculated from

The complete separation of glimepiride, metformin, pioglitazone and internal standard using the parameters of retention duration, resolution, and tailing factor served as the basis for determining the specificity of the Ultra- Performance Liquid Chromatography Mass Spectroscopy approach.It is crucial to show the method's robustness in order to guarantee that the High-Performance Liquid Chromatographic method is insensitive to minute modifications in the experimental circumstances.

The resolution between the medicines and internal standard, peak area Relative standard deviation, peak width, or theoretical plates were not significantly altered by any of the modifications.

2.4. RP-HPLC METHOD

Utilizing a C18 analytical reverse-phase column, the reversed-phase high performance liquid chromatographic technology has been expanded to quantify metformin hydrochloride in raw materials and pharmaceutical formulations. In the Reverse Phase-High Performance liquid chromatography method, diazepam serves as the internal standard, while the mobile phase is made up of methanol and water flowing at the right flow rate and temperature. At 233 nm in the ultraviolet, metformin hydrochloride was found. The findings indicated that the declared contents in pharmaceutical formulations were in good accord with the findings.

Because of its sensitivity and reproducibility, the reverse phase high performance liquid chromatographic method was used to undertake a quantitative analysis of the metformin in Neodipar pills. Reverse phase high performance liquid chromatography is a quick, precise, and selective process.

Typically, the Reverse-phase high performance liquid chromatography process uses an n-alkyl silicate-based sorbent. The analysis was built on an 0d-5-100, c18-bond pack column with particles that were at least 0.5 m in size and at room temperature. Using a suitable sample loop and a Rheodyne injector valve, the sample was introduced into the chromatographic.

Different metformin concentrations were made using stock solution. Metformin in the needed quantity was dissolved in water or methanol, then transferred to a measuring flask to create the stock solution. The material was thoroughly dissolved by shaking in an ultrasonic bath. The mixture was then thoroughly combined, brought up to the required volume using diluents, and filtered using membrane filters.

The tablet was mixed and powdered. The material was dissolved using an ultrasonic bath and a quantity of metformin that was equal to what was needed. Water was then diluted with methanol to obtain dilutions with defined concentrations. The solutions after adding diazepam were inserted onto the C18 analytical reverse-phase column.

To get the best results, the solvent system and column requirements were employed. As an internal standard, diazepam was used, and it had no negative effects on our analysis. The duration of separation was shorter, and the declared peak symmetry was better. Retention times that were ideal were achieved

To evaluate the accuracy of the intra-day and inter-day measurements, complete metformin reference standards in mobile phase were injected.

Accuracy and precision for the sample were computed during the intra-day and inter-day runs. The intra-day range accuracy was found to be approximately more than -0.04%. All of the data satisfied the acceptance criteria, and the intermediate precision values were designated as good method precision.

An indicator of the system suitability was given by the retention time of medication as well as the peak area% coefficient of variation. Utilizing the five replicate experiments of the medication at the

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required concentration, the appropriateness of the was evaluated.

The smallest amount of an analyte in a sample may be analysed with the required precision and accuracy, albeit it cannot be mechanically quantitated. It was decided what the quantitation limit. The limit of the quantification range was discovered to be no larger than 6 g/ml by employing the five concentrations to construct linearity.

Reverse-Phase High Performance Liquid Chromatography is the exact method for quantifying metformin in dosage form. The dosage of the drug was estimated using this technique. Reverse phase high performance liquid chromatography is a sensitive technique.

2.5.HPTLC METHOD

High performance thin layer chromatography is a reliable, definite, straightforward, and exact technique. High performance thin layer chromatography was created for the measurement of sitagliptin phosphate and metformin hydrochloride in tablet dosage forms. Acetonitrile and phosphate buffer serve as the mobile phase in this procedure and the Phenomenex C18 column is operating in gradient mode.

At 218nm, emission was seen. Orthophosphoric acid is used in High performance liquid chromatography to modify the pH. High performance thin layer chromatographic separation was accomplished by employing the silica gel High performance thin layer chromatographic plates with the mobile phase such as water, methanol, and ammonium phosphate. The methodologies linearity, accuracy, and precision were all confirmed. Utilizing anhighperformance thin layer chromatographic technique, sitagliptin phosphate and metformin hydrochloride in tablet dose form were thoroughly assessed

The High-performance thin layer chromatographic method analysis was carried out using the Cyberlab UV-100 UV-VIS detector, Cyberlab LC-100B HPTLC equipped with a Cyberlab LC-100B, Cyberlab GM-100 gradient mixer, Cyberlab DS-100 HPTLC control data software, and redone injector with the proper loop volume. The Phenomenex C18 column and mobile phase were utilized for separation. Orthophosphoric acid is used to modify the pH at the proper flow rate, with detection at 218nm.

The mobile phase, which included water, methanol, and ammonium sulphates, was added to aluminium backed silica gel High-performance liquid chromatographic plates to create the high-performance thin layer chromatography.

By dissolving the necessary quantity of the drug in ammonia, the standard stock solutions of sitagliptin phosphate and metformin hydrochloride were prepared separately. The volume was then brought up to the desired level with the use of methanol.

Take the necessary amount of tablets, and then finely powder them. Add powder to the measuring flask in an amount that is similar to the recommended dosage of sitagliptin phosphate and metformin hydrochloride in the tablet formulation. The necessary amount of ammonia was added to the solution and mixed with the methanol in High-performance liquid chromatographic method. The Whatmann filter paper was used to complete the filtration process. The filtrate was properly diluted to achieve the desired concentration of sitagliptin phosphate and metformin hydrochloride, which is needed for analysis.

The High-performance liquid chromatography method uses a stock solution of sitagliptin phosphate and metformin hydrochloride. The methanol was mixed with the necessary quantity of stock solution, and the mixture was dot-tested for metformin hydrochloride. The plate was enlarged and looked at in the developed conditions. Sitagliptin and metformin hydrochloride amounts in the formulation were measured using the calibration plots, and desigrams were recorded.

The enhanced high-performance liquid chromatography method was validated using concepts including linearity, precision, inter-day and intra-day, accuracy, limit of quantification, and limit of detection.

Sitagliptin phosphate, Rf value was discovered to be roughly less than 0.68, while metformin hydrochloride, Rf value was discovered to be roughly larger than 0.59. The moderate improvement for sitagliptin phosphate was found to be not less than 99% and the moderate improvement for metformin hydrochloride was found to be greater than 100%, demonstrating the absence of excipients from the formulation in the approach. Low values of the standard deviation and coefficient of variation at every level are said to be indicative of the high precision of the approach. The high-performance thin layer chromatography method was validated using the ICH criteria, and it was successfully used for routine quality analysis of tablet formulation.

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2.6. SPECTROFLUORIMETRIC METHOD

In this study, two sensitive spectrofluorimetric methods for the simultaneous measurement of amlodipine and metformin in bulk and synthetic mixtures are developed. These methods are simple, quick, accurate, and affordable.

After excitation at 345 nm, the medications react with 1-dimethylaminonaphthalene-5-sulphonyl chloride to produce a highly fluorescent derivative that is detected at 450 nm and 520 nm for amlodipine and metformin, respectively. A spectrofluorimetric analysis is based on this process. Thorough study and optimisation were performed on the many experimental factors that affected the reaction product growth and stability.

By transferring various aliquots of required amount of metformin and amlodipine solution into a calibrated volumetric flasks and binary mixture solutions containing the amlodipine and metformin were created. In each flask add acetone and 1 M sodium carbonate solvent. Dansyl chloride solution, 0.1% w/v, was added in an appropriate amount. After sealing the flasks, the contents were well mixed, allowed to stand for sometimes, and then finished to the proper strength with acetone. For few minutes, the reaction mixture was let to stand. For metformin and amlodipine, the final concentrations were found to be approximately less than 2 µg/ml and greater than 1µg/ml.The fluorescence intensity was estimated for the reaction product.

While metforminmaximum absorbance was found to be approximately greater than 300 nm and amlodipine maximum absorbance was found to be greater than 400 nm. One iso-absorptive point was visible at 256 nm in the overlaid absorbance spectra,this wavelength was chosen for the simultaneous estimation of amlodipineandmetforminusing the Q-absorbance ratio approach, whereas the maximum wavelengths of 238 nm and 361 nm were chosen for the simultaneous estimation of metforminand amlodipine using the simultaneous equation method. The amlodipine and metformin in bulk and synthetic mixtures could thus be determined satisfactorily using the suggested approved procedures.

Dansyl chloride, also known as 1-dimethylaminonaphthalene-5-sulphonyl chloride, for the purpose of creating fluorescent albumin conjugates. Since then, it has been widely utilized as a fluorescent reagent for peptides and proteins. It is discovered that under the right circumstances, primary and secondary amines, imidazoles, and phenols quantitatively react with Dansyl chloride to form the matching sulphonamides or phenolic esters.For the measurement of a few primary, secondary, imidazoles, and phenols, dansyl chloride was used.

According to the results of the current spectrofluorimetric approach of amlodipine and metformin react with Dansyl chloride atappropriate pH to produce a highly fluorescent derivative with maximal emission at 450 nm and 520 nm following excitation at 345 nm.

The pH, dansyl chloride concentration, temperature, reaction duration, and dilution time are a few of the experimental parameters that may influence the growth of the reaction product and its stability. These parameters were adjusted separately while the others were held constant.

The effect of pH on the reaction product's fluorescence intensity was researched. Acetone and a 1M sodium carbonate solution mixture produced the highest level of fluorescence. In the reaction mixture, appropriate pH was determined.

The low fluorescence intensity of the reagent, however, indicates that there was no dansyl hydroxide interference under the suggested selected circumstances and wavelengths used.

Various volumes of the 0.1% w/v solution of dansyl chloride were used to study the impact of the dansyl chloride concentration. It was discovered that the addition of required amount of dansyl chloride solution in the presence of sodium carbonate caused the reaction of dansyl chloride with medicines to begin. The volume was observed to enhance fluorescence intensity, after which it remained constant. Therefore, 0.1% w/v dansyl chloride solution in a volume was found to be greater than 8 ml and it is the ideal amount of the reagent.

At temperatures higher than room temperature, the same derivatization process was performed. The fluorescence intensity did not significantly vary with rising temperature, according to the results.

To determine when the solution reaches its peak fluorescence intensity, various time intervals were investigated. The reaction product was discovered to attain its peak fluorescence intensity after sometimes and to hold steady at room temperature for few min.

Throughout the study, various solvents were tried to dilute the reaction mixture; it was found that acetone gave the highest fluorescence

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intensity, while acetonitrile and isobutyl methyl ketone produced relatively low fluorescence intensity. The increase in fluorescence obtained with acetone was caused by lowering the fluorescence of the blank reagent.

To determine when the solution reaches its peak fluorescence, dilution times were tried. It was discovered that the reaction product attained its peak fluorescence intensity after 10 minutes of dilution with acetone.

Recovery studies using the conventional addition technique were carried out in order to determine the precision and validity of the suggested procedures. Pure amlodipine and metformin were added to a pre-analysed drug mixture at three different degrees of spike, and the total amount of drugs was calculated using the suggested procedures. The quantitative recoveries of the pure pharmaceuticals added were shown in Table 3 and shown that experimental and common additions like talc, flour, hydroxyl propyl methyl cellulose, magnesium stearate, and calcium dihydrogen orthophosphate did not affect the determination.The intra-day, inter-day, and repeatability precision of the suggested approaches were examined. Six analyses of the same drug concentration were done to test for repeatability. The outcomes indicated that the suggested strategies were best received when used regularly. By analysing aliquots from homogenous slots by various analysts under identical operational and environmental conditions, the robustness of the suggested approach was assessed.

The proposed methods were evaluated in the assay of synthetic mixtures. This method shows the results obtained by the application of the proposed models on the synthetic samples. Six replicate determinations were carried out on each experiment. These results confirm satisfactory to the drug content and indicate the high precision and accuracy of the proposed methods when applied to synthetic mixture.

III. IMPURITY PROFILING ON METFORMIN HYDROCHLORIDE IN DOSAGE FORM:

For the impurity profiling in the tablet containing metformin hydrochloride and teneligliptin hydrobromide hydrate, an exact, precise, and reliable analytical approach was devised. The gradient was adjusted using C18, which was run at 35 °C, for better impurity separation. The phosphate buffer and octane sulfonic acid. The chromatogram was seen at 210 nm, and the gradient was adjusted for improved resolution.

For the development and validation investigations, standards for the drugs metformin (99.8% purity) and teneligliptin (99.6% purity) were prepared. For the production of the diluent and mobile phase, High performance liquid chromatography grade methanol, acetonitrile, potassium dihydrogen phosphate, sodium salt of octane sulfonic acid, and orthophosphoric acid were utilized. The pH was measured using a calibrated seven-piece portable pH meter. On a C18 column, all the contaminants were successfully separated.

From Limit of Quantitation level to 150%, the observed percentage of teneligliptin and metformin hydrochloride recovery was above 90%. For metformin hydrochloride, teneligliptin, melamine, cyanocobalamin, and teneligliptin impurity A, the correlation coefficient was 0.999, while for teneligliptin impurity, it was 0.998. During the robustness research, it was discovered that the methodwas unaffected by changes in method variance. Maximum degradation was seen with peroxide during the stress assay with acid, base, peroxide, and temperature, suggesting the sensitivity of the molecule against oxidative stress.

According to the findings of the validation investigation, the created approach is exact, accurate, linear, and robust. The technique yields extremely accurate and exact results when used to investigate the stability of formulation products. Key factors in determining the effectiveness and safety of any drug or drug product are impurity isolation, detection, separation, and quantification. Due to the significant market demand for this combination, the contaminants were properly extracted from both medications using a single process, ensuring that the patient would receive a safe and effective medication. As a result, the suggested approach helps researchers cut down on development time and costs.

The analysis did not involve the use of any potentially harmful compounds, and the approach uses little in the way of organic solvents, making it both secure and environmentally benign. During the commercial manufacturing of batches, the established approach can be utilized with effectiveness for routine testing of contaminants in pharmaceutical goods.

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IV. TOXICOLOGICAL STUDIES OF METFORMIN HYDROCHLORIDE IN DOSAGE FORM

Glibenclamide and metformin hydrochloride are used to treat type 2 diabetes. This investigation examined the effects of metformin and glibenclamide toxicity on the testicles, kidneys, and liver of rats. Three groups of the required number of rats were created. The findings demonstrate that neither metformin nor glibenclamide therapy affected the rats' capacity to put on weight. Furthermore, rats treated with metformin and glibenclamide showed no treatment-related alterations in the absolute and relative weights of the liver, kidney, and testis compared to controls. The ratio of live to dead sperm was unaffected, but testicular lipid peroxidation significantly increased and was accompanied by a decline in epididymal sperm motility and count.

Metformin and glibenclamide in particular reduced sperm count and motility. In rats treated with metformin and glibenclamide, serum superoxide dismutase and catalase activities, alkaline phosphatase, lactate dehydrogenase, and alanine aminotransferase activities were not significantly changed, but metformin treatment significantly increased testicular serum superoxide dismutase, catalase, glutathione, serum aspartate aminotransferase, and conjugated bilirubin activities. According to histological results, the testis of rats given metformin displayed considerable necrosis, degeneration of seminiferous tubules, and defoliation of spermatocytes. Metformin and Glibenclamide changed the seminal quality, boosted lipid peroxidation, and decreased antioxidant status when used in conjunction. These medications may interfere with normal biochemical processes in the rat testes and liver.

Both metformin hydrochloride and glibenclamide, were acquired. The following items were hydrogen peroxide, 5,5'-dithios-bis-2-nitrobenzoic acid and epinephrine and all additional chemicals were provided by the approved supplier.

Catalase and glutathione in metformin and the weight increase and relative weights of liver, GB-treated rats were not substantially affected by the administration of Glibenclamide and Metformin at dosages of 5 and 30 activities of testicular superoxide dismutase, mg/kg body weight.the levels of hepatic and renal, superoxide dismutase, and catalase in Metformin and Glibenclamide treated rats were not significantly different from controls. Slides from control animals demonstrated the tissues' typical morphology. Organ tissues from animals treated with Metformin and Glibenclamide showed mild necrosis and deformation. Metformin therapy resulted in significant testicular necrosis, seminiferous tubule degeneration, and spermatocyte defoliation. This study, to our knowledge, is the first to document the direct impact of metformin hydrochloride and glibenclamide on the male reproductive system, particularly on the quality of sperm. Together, the findings of our study imply that MET and GB may lead to testicular dysfunction and exacerbate diabetes problems. This study implies that persons receiving metformin and glibenclamide therapy need to be closely monitored.

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