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A Review of Analytical Methods for Determination of Oral Hypoglycaemic Agents

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ABSTRACT:
Diabetes mellitus is the most common multisystem disorder and occurs primarily due to insulin deficiency or its function affecting carbohydrate, protein, and fat metabolism. It is classified into insulin-dependent diabetes mellitus, non-insulin-dependent diabetes mellitus, and gestational diabetes. Oral hypoglycemic agents play a major role in controlling the glycemic index of people with non-insulin-dependent diabetes mellitus, along with dietary modifications and physical exercise. This study is a review of various analytical methods used for the determination of oral hypoglycemic agents reported in the literature. This review encompasses methods such as spectrometry (UV), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), thin-layer chromatography (TLC), High-performance thin-layer chromatography (HPTLC), liquid chromatography-mass spectrometry (LC-MS), and gas chromatography (GC). It was found that all the above-mentioned methods were used for the estimation of oral hypoglycemic agents. UV spectrometry and HPLC were the most commonly used.

KEYWORDS: Diabetes mellitus, Oral hypoglycemic drugs, Analytical methods, validation parameters.

I. INTRODUCTION:

Diabetes mellitus is caused by the insufficient secretion of insulin. It is considered a metabolic disorder due to the long-term tissue or organ damage. It may also occur due to damage to the kidney, vascular system, or nervous system. In diabetes, blood sugar levels are increased for a prolonged period due to a group of metabolic diseases. Diabetes is also characterised by a reduction in protein, carbohydrate, and fat metabolism due to insulin deficiency, causing hyperglycemia. [1]

The normal range of DM is

Mg/dl	BEFORE EATING	AFTER EATING	2-3HOURS AFTER EATING
Normal	80-100	170-200	120-140
Impaired glucose	101-125	190-230	140-160
Diabetes	126+	220-300	200+

TYPES:

1. Type-I diabetes: Insulin or synthetic insulin is administered according to the weight, age, and sex of the patient.
2. Type II diabetes: Metformin is the most preferred drug for this type of diabetes. Angiotensin-converting enzyme inhibitors are also used for the treatment of type 2 diabetes. [2,3]

TYPE-II DIABETES MELLITUS:

Type 2 diabetes mellitus is a syndrome characterised by relative insulin deficiency, insulin resistance, and increased hepatic glucose output. Oral hypoglycemic agents are a group of drugs used to help reduce the amount of sugar present in the blood. They are not insulin-dependent, but they stimulate the pancreas to produce insulin. The most effective management of diabetes mellitus demands an interprofessional approach involving both lifestyle modifications with diet and exercise and

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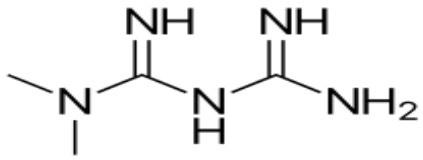
pharmacologic agents combined with oral pharmacologic agents for optimal glycemic control, particularly as type 2 diabetes mellitus progresses with continued loss of pancreatic beta-cell function and insulin production. Type 2 diabetes affects approximately 200 million people worldwide, including more than a quarter of the elderly living in developed countries. Diet and exercise are first-line treatments, along with oral hypoglycaemic drugs, to achieve the goal of improving glycaemic control and preventing both microvascular and macrovascular complications. There are seven distinct classes of hypoglycemic agents: biguanides, sulfonylureas, meglitinides, thiazolidinediones, -glucosidase inhibitors, incretin mimetics, and DPP-4 inhibitors.[4}

1. BIGUANIDES
METFORMIN HYDROCHLORIDE:



.HCL

IUPAC name: 3-(diaminomethylidene)-1,1-dimethylguanidinehydrochloride
Molecular formula: $C_4H_{12}ClN_5$
Molecular weight: 165.2g/mol
Physical properties
Appearance: white to off-white crystalline compound
Solubility: freely soluble in water, slightly soluble in water and practically insoluble in acetone and in methylene chloride.
Boiling point: 224.1˚C
Melting point: 222-226˚C
Pharmacological action: Metformin inhibits complex I (NADPH:biquinone oxidoreductase) of the mitochondrial respiratory chain, thereby increasing the cellular AMP to ATP ratio, leading to activation of AMP-activated protein kinase (AMPK), and regulating AMPK-mediated transcription of target genes. This eventually prevents hepatic gluconeogenesis, enhances insulin sensitivity and fatty acid oxidation, and ultimately leads to a decrease in glucose levels. Metformin may exert antineoplastic effects through AMPK-mediated or AMPK-independent inhibition of the mammalian target of rapamycin, which is up-regulated in many cancer tissues. Furthermore, this agent also inhibits tumour cell migration and invasion by inhibiting matrix metalloproteinase-9 (MMP-9) expression, which is mediated through the suppression of transcription activator protein-1 (AP-1) activation. [5]

ANALYTICAL METHODS:
HPLC:

The chromatographic separation was performed on an Analytical Technologies HPLC-3000 series compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with a 20-l fixed loop. A reverse-phase C18 [Cosmosil C18 (250mm x 4.6 ID, Particle size: 5 micron)] was used. Model: UV 2012 double beam UV visible spectrophotometer and Wenser High Precision Reagents and Chemicals A pharmaceutical-grade, pure metformin hydrochloride sample was procured. HPLC-grade Methanol and HPLC-grade Water were used. C18 [Cosmosil C18 (250mm x 4.6 ID, Particle size: 5 micron)] was used for the chromatographic separation at a detection wave length of 238nm. Methanol and phosphate buffer pH 3 in a ratio of 70:30 v/v were selected as the mobile phase for elution, and the same mixture was used in the preparation of standard and sample solutions. The elution was monitored by injecting 20 l, and the flow rate was adjusted to 1 ml/min. The mean retention time for metformin hydrochloride was found to be approximately 4.2. [6]

Table.1: Results of HPLC system suitability parameters for Biguanides.

S.NO	Parameters Drug name	Linearity		Accuracy		Precision		LOD	LOQ (µg/ml)
		Conc. (µg/ml)	r^2	Conc. (µg/ml)	%SD (min-max)	Conc. (µg/ml)	%RSD (min-max)		
1.	Metformin hydrochloride	10-50	0.9986	10-50	0.4350946-0.2256536	10-50	0.435094036-0.22565365	0.1502	0.4553

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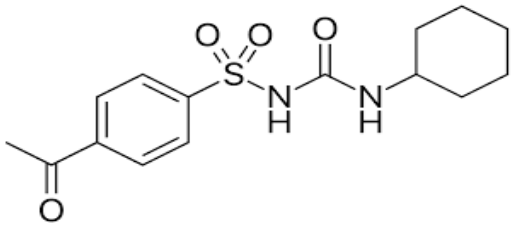
UV-SPECTROSCOPY:

A simple, rapid, accurate, economic, precise, and robust UV method for estimation of metformin HCl in bulk and tablet dosage forms using water as the solvent A Shimadzu UV-1800 240V UV/VIS spectrophotometer with two matched 1 cm quartz cells was used. Metformin Hydrochloride showed maximum absorbance at 234 nm. The percentage recoveries for Metformin Hydrochloride were found to be in the range of 99–10%. The method was quantitatively evaluated in terms of linearity, accuracy, precision, ruggedness, robustness, and recovery. [7]

Table.2: Results of UV system suitability parameters for Biguanides.

S.NO	Parameters Drug name	Linearity		Accuracy		Precision		
		Conc. (µg/ml)	r^2	Conc. (ppm)	%RSD	Conc. (ppm)	Intraday precision Average %RSD	Interday precision %RSD
1.	Metformin hydrochloride	10-50	0.9998	80% 120% 100%	0.34 0.41 1.17	20 20 20	1.08	0.66 0.38 0.29

2. SULFONYL UREAS:
FIRST GENERATION:
ACETOHEXAMIDE:



IUPAC name: 1-(4-Acetyl phenyl) sulfonyl-3-cyclohexylurea
Molecular formula: $C_{15}H_{20}N_2O_4S$
Molecular weight: 324.4g/mol
Physical properties:
Appearance: white to pale Red
Solubility: Practically insoluble in water and soluble in Alcohol and Chloroform
Boiling point: 528.1˚C
Melting point: 188- 190˚C [8]
Mechanism of action:
Acetohexamide (trade name Dymelor) is a first-generation sulfonylurea medication used to treat type 2 diabetes mellitus. Acetohexamide binds to the ATP-Kinase K^- (K_{ATP}) channel on the cell membrane of pancreatic -cells. This inhibits the outflow of potassium, which causes the membrane potential to become more positive. This depolarization, in turn, opens the voltage-gated calcium channels. The rise in the level of intracellular calcium leads to increased fusion of insulin granules with the cell membrane and, therefore, increased insulin secretion. [9]

ANALYTICAL METHODS:
THIN LAYER CHROMATOGRAPHY:

The TLC method is used for the identification, assay, and purity determination of the drug and other hypoglycemic agents in powder or tablet forms. The drug was detected by dissolving powder tablets in a dichloromethane-acetone mixture. Chromatographing on the solution of the Silica Gel F_{254} plates with cyclohexane, chloroform, acetic acid, and ethanol in a ratio of 10:7:2:1 For the Quantitative determination, the spots were developed, separated, and eluted with methanolic HCL, and the absorbance was measured. The spots were located by viewing them in 254nm radiation. [10]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

In HPLC, the recovery of drugs from inert tablets by this method is near 100%. A column (100 cm X 2.1 mm) packed with 1% ethylene-propene copolymer on Zipax was used with a mobile phase of 0.01 M disodium hydrogen citrate containing 15% methanol (pH 4.4). Detection was carried out at 254 nm, and pack areas were integrated. [10]

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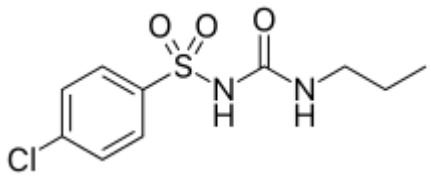
CHLORPROPAMIDE:



IUPAC name: 4-chloro-N-(propyl carbamoyl)benzenesulfonamide
Molecular formula: $C_{10}H_{13}ClN_2O_3$
Molecular weight: 276.74g/mol
Physical properties:
Appearance: White crystalline powder
Solubility:soluble in ethanol,chloroform,acetone.
Boiling point: 302˚C
Melting point: 128˚C [11]

Mechanism of action:

Diabinese (chlorpropamide) is an oral blood-glucose-lowering drug of the sulfonylurea class. The Sulfonylureas increase both basal insulin secretion and meal-stimulated insulin release. Sulfonylureas such as chlorpropamide bind to ATP-sensitive potassium channels on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis, of insulin. [12]

ANALYTICAL METHODS:
UV-SPECTROSCOPY:

By Using this UV spectrophotometry method, which is used to determine the maximum wavelength and absorbance of chlorpropamide, The main purpose of this study was to determine chlorpropamide in a bulk formulation. A spectrophotometer with UV-visible light (Shimadzu UV-1800) was used. The necessary quantity of chlorpropamide was dissolved in methanol to prepare a stock solution with a concentration of 100 mcg/ml. By using a UV/Visible spectrophotometer, the baseline was corrected with a blank. The solution's absorbance was then compared to a blank sample at 580 nm.[13]

Table.3: Results of UV system suitability parameters for Chlorpropamide.

S.NO	Parameters / Drug name	Linearity		Accuracy		Precision			LOD	LOQ	Absorbance (nm)	
		Conc. (µg/ml)	r^2	Conc. (ppm)	%RSD	Conc. (ppm)	Intraday precision Average %RSD	Inter-day precision Average %RSD			Min	Max
1.	Chlorpropamide	10-60	0.9919	10 30 50	< 2	10 30 50	1.35	0.57	2.99g	8.89g	580	710

HPLC:

The assay was performed on an Inertsil ODS 3V (150mm × 4.6mm; 5 m particle size) column using a mixture of phosphate buffer (pH 4.5), methanol, and acetonitrile (30:63:7 v/v/v) as mobile phases at a flow rate of 1 mL min-1 and with UV detection at 254nm. The column temperature was 30°C, and the injection volume was 20 L.The retention behaviour of CLP as a function of mobile phase pH, composition, and flow rate was carefully studied, and chromatographic conditions, yielding a symmetric peak with the highest number of theoretical plates, were optimised. The calibration curve was linear (r = 0.9999) over the concentration range 0.5–300 gmL-1. The limits of detection (LOD) and quantification (LOQ) were found to be 0.1 and 0.3 gmL-1, respectively. Both intra-day and inter-day precisions determined at three concentration levels were below 1.0%, and the respective accuracies expressed as %RE were 1.10%. Assays were performed under slightly altered chromatographic conditions, and the results were not significantly different from those obtained under optimal conditions, reflecting the robustness of the method. Inter-equipment and inter-analyst deviations were insignificant, testifying to the ruggedness of the method. The method was validated for selectivity via placebo blank and synthetic mixture analyses.[14]

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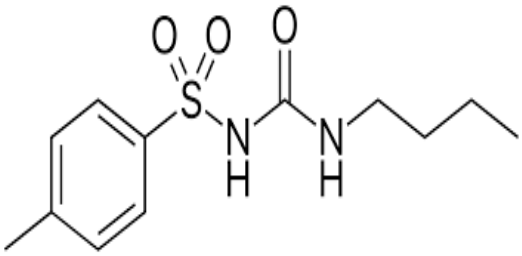
Table.4: Results of HPLC system suitability parameters for Chlorpropamide.

S.NO	Parameters Drug name	Linearity		Accuracy		Precision			LOD (µg/ml)	LOQ (µg/ml)
		Conc. (µg/ml)	r^2	Conc. (µg/ml)	%RE	Conc. (µg/ml)	%RSD			
							Intraday	Inter-day		
1.	Chlorpropamide	0.5-300	0.9999	100-300	≤1.1 %	100-300	0.27-0.63	0.30-0.45	0.10	0.30

TOLBUTAMIDE:



IUPAC name:N-[(butylamino)carbonyl]-4-methyl benzenesulfonamide
Molecular formula: $C_{12}H_{18}N_2O_3S$
Molecular weight: 270.35g/mol
Physical properties
Appearance: White crystalline powder
Solubility: soluble in Ethanol, Dimethyl formamide.
Boiling point: 430˚C
Melting point:128.5-129.5˚C [15]

Mechanism of action:

Tolbutamide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It is structurally similar to acetohexamide, chlorpropamide, and tolazamide and belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating the cells of the pancreas to release insulin. Sulfonylureas increase both basal insulin secretion and meal-stimulated insulin release. Medications in this class differ in their dose, rate of absorption, duration of action, route of elimination, and binding site on their target pancreatic β cell receptor. Sulfonylureas also increase peripheral glucose utilisation, decrease hepatic gluconeogenesis, and may increase the number and sensitivity of insulin receptors. Sulfonylureas are associated with weight gain, though less so than insulin. Due to their mechanism of action, sulfonylureas may cause hypoglycemia and require consistent food intake to decrease this risk. The risk of hypoglycemia is increased in elderly, debilitated, and malnourished individuals. Tolbutamide appears to be metabolised in the liver. Tolbutamide and its metabolites are excreted in urine (75–85%) and faeces.[16]

ANALYTICAL METHODS:
RP-HPLC:

The RP-HPLC method was developed for the determination of Tolbutamide in pure and pharmaceutical formulations. Analysis was carried out on a Zodiac C18 column (250 mm × 4.6 mm × 5 µ particle size) using Methanol: 0.1% Orthophosphoric acid: Acetonitrile (10: 30: 60) as mobile phase. Detection was carried out by UV at 231 nm. The proposed method obeyed linearity in the range of 20–120 µg/mL and met all specifications as per ICH guidelines. [17]

Table.5: Results of HPLC system suitability parameters for Tolbutamide.

S.NO	Parameters Drug name	Linearity		Accuracy		Precision			LOD (µg/ml)	LOQ (µg/ml)
		Conc. (µg/ml)	Area	Conc. (µg/ml)	%Recovery	Conc. (µg/ml)	%RSD			
							Intraday	Inter-day		
1.	Tolbutamide	20-120	57867-369780	50% 100% 150%	99%-101%	60ppm	1.61	1.79	5	1.54

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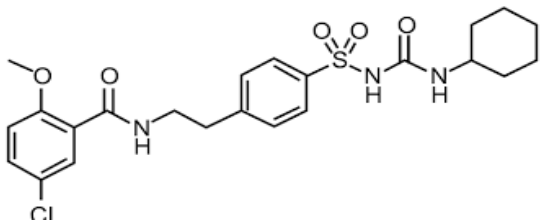
UV- SPECTROPHOTOMETRY:

In this method, the absorption maxima were scanned from 200 to 400 nm, and the λmax was found to be 248 nm was selected for analysis of Tolbutamide. An ELICO model SL210 double-beam UV/Visible spectrophotometer with a matched pair of 10nm quartz cells are used for experimental purposes. Linearity was observed in the concentration range 1-12µg/ml (r2 =0.9992) for the method. The % assay for the marketed formulation for absorption maxima and area under the curve method was found to be 100.49% and 100.78%, respectively. The methods were validated with respect to linearity, precision, and accuracy studies. Recovery studies for absorption maxima and area under the curve were found to be 99.52% and 100.64%, respectively. The developed methods were validated for linearity, precision, accuracy, LOD, and LOQ as per ICH guidelines. The method was found to be linear within the concentration range of 1-12µg/ml for Tolbutamide. [18]

Table.6: Results of UV system suitability parameters for Tolbutamide.

S.NO	Parameters / Drug name	Linearity		Accuracy		Precision			LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2	Conc. (µg/ml)	%RSD	Conc. (ppm)	Intraday precision Average %RSD	Inter-day precision Average %RSD			
1.	Tolbutamide	1-12	0.9992	8-12	< 1	10	0.23146	0.298973	0.19+	0.627	248

SECOND GENERATION GLIBENCLAMIDE:



IUPAC name:5-chloro-N-[2-[4-(cyclohexyl carbamoylsulfamoyl)phenyl]ethyl]-2-methoxy benzamide
Molecular formula: $C_{23}H_{28}N_2O_4S$
Molecular weight:494.0g/mol
Physical properties
Appearance: crystalline powder
Solubility:practically insoluble in water, Ether and soluble in chloroform, methanol.
Boiling point:705.7˚C
Melting point:169˚C [19]

Mechanism of action:

Glyburide is a widely used medicament that belongs to a class of drugs known as sulfonylureas. treat type2 2 diabetes mellitus These drugs act by closing ATP-sensitive potassium channels in pancreatic beta cells. The ATP-sensitive potassium channels on beta cells are known as sulfonylurea receptor 1 (SUR1). Under low glucose concentrations, SUR1 remains open, allowing for potassium ion efflux to create a -70 mV membrane potential. Normally, SUR1 closes in response to high glucose concentrations, the membrane potential of the cells becomes less negative, the cell depolarizes, voltage-gated calcium channels open, calcium ions enter the cell, and the increased intracellular calcium concentration stimulates the release of insulin-containing granules. Glyburide bypasses this process by forcing SUR1 to close and stimulating increased insulin secretion. [20]

ANALYTICAL METHODS:
RP-HPLC:

The reverse-phase HPLC method was developed and validated for simultaneous estimation of glibenclamide in tablet dosage form. The method employed for analysis uses methanol as a solvent. The wavelength UV 300 nm was selected for estimation, and linearity was observed in the concentration range of 160–240 - 240µg/ml for glibenclamide, respectively. The recovery studies ascertained the accuracy of the proposed method, and the results were validated as per ICH guidelines. The method can be employed for the estimation of pharmaceutical dosage formulations with no interference from any other excipients and diluents. The resolution between the closest peaks

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of glibenclamide was more than 1.5, gives a linear response (r2 > 0.999). [21]

Table.7: Results of HPLC system suitability parameters for Glibenclamide:

S.NO	Parameters Drug name	Linearity		Accuracy		Precision		
		Range	r^2	Conc. (µg/ml)	%RE	Conc. (µg/ml)	%RSD	
							Intraday	Inter-day
1.	Glibenclamide	40−300 mV/spot	0.296	100-300	≤1.1%	80-120	5.607	0 ≤ 2%

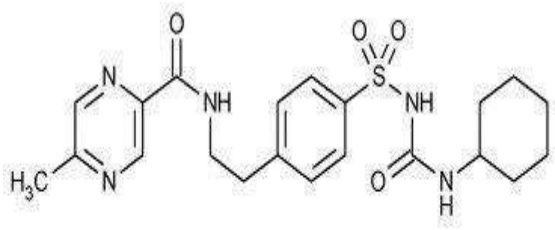
UV SPECTROSCOPY:

Following the ICH guidelines, a simple, accurate, and precise method has been developed using a UV spectrophotometer for the determination of GLB in both bulk and pharmaceutical dosage forms. 0.1 N NaOH was used as a solvent medium in the entire method. Wavelength selection is done by running a stock solution of 50 µg mL−1 between the range of 200 and 400 nm, and GLB showed the maximum absorbance at 227 nm, which is further used in the entire analysis. The proposed method is linear (R2 > 0.999) with the range 5–25 µg mL−1, accurate (99.60%), precise (inter and intraday variation 0.241 and 0.019%, respectively), and robust (<1%). The quantification and detection limits were 1.46 and 0.48µg/ml, respectively. [22]

Table.8: Results of UV system suitability parameters for Glibenclamide.

S.NO	Parameters Drug name	Linearity		Accuracy		Precision			LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2	Conc. (µg/ml)	%RSD	Conc. (ppm)	Intraday precision	Inter-day precision			
							Average %RSD	Average %RSD			
1.	Glibenclamide	5-25	0.999	0.8-1.2	< 1	579	0.19	0.241	0.48	1.46	227

GLIPIZIDE:



IUPAC Name: 1-cyclohexyl-3-[[p-[2-(5-methylpyrazin carboxamide)ethyl]phenyl] sulfonyl]urea
Molecular formula: $C_{21}H_{27}N_5O_4S$
Molecular weight: 445.5g/mol
Physical properties
Appearance:white powder
Solubility: Insoluble in water and alcohol soluble in 0.1N NaOH and DMF
Boiling point: 676˚C
Melting point:203-208˚C [23]

Mechanism of action:

Glipizide undergoes entero-hepatic circulation and acts by stimulating the release of insulin from the pancreases and also reducing blood glucose levels in human beings. GLP binds to KATP channels on the cell membrane of pancreatic β cells of the islets of Langerhans. This leads to increased fusion of insulin granules with the cell membrane and, therefore, increased insulin secretion. [24]

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ANALYTICAL METHODS:

HEAD SPACE -GAS CHROMATOGRAPHY:

An analytical methodology was developed for the quantification of residual solvents in Glipizide using headspace gas chromatography (HSGC) with the help of a flame ionisation detector (FID). Methanol, acetone, and dimethylformamide as residual solvents were determined in Glipizide. Analysis was performed by the headspace GC/FID method on the auto system, HS40. Nitrogen was used as a carrier gas, and the separation of residual solvents was achieved by a DBWax 0.25mm, 0.3 mcm column. The thermostat temperature was 115 °C for 40 minutes for each vial. %RSD for nine injections obtained is within acceptance criteria. The correlation coefficient R2 obtained greater than 0.99. The method parameters were validated includes specificity, limit of detection and quantification, accuracy, linearity, precision, and robustness. [25]

HPLC:

A simple, sensitive, and selective HPLC method with UV detection for the determination of Glipizide in human plasma was developed. The liquid-liquid extraction method was used to extract the drug from the plasma samples. Chromatographic separation of Glipizide was achieved using a C18 column (ZORBAX ODS 4.6x150 mm). The mobile phase was comprised of 0.01 M potassium dihydrogen phosphate and acetonitrile (65:35, v/v) adjusted to pH 4.25 with glacial acetic acid. The analysis was run at a flow rate of 1.5 mL/min with an injection volume of 20 ml. The detector was operated at 275 nm. The calibration curve was linear over a concentration range of 50–1600 ng/ml. Intra-day and inter-day precision and accuracy values were below 15%. The limit of quantification was 50 ng/mL [26]

Table.9: Results of HPLC system suitability parameters for Glipizide.

S.NO	Parameters Drug name	Linearity		Accuracy		Precision			LOD (ng/ml)
		Range (ng/ml)	r^2	Conc. (ng/ml)	%RE	Conc. (ng/ml)	%RSD		
							Intraday	Inter-day	
1.	Glipizide	50-1600	0.9989	50-1600	<15%	50 150 850 1300	8.14 8.18 4.21 6.17	10.69 7.45 6.81 4.59	50

UV SPECTROSCOPY:

A simple and sensitive ultraviolet spectrophotometric method for quantitative estimation of glipizide in the presence of lipid turbidity is described to avoid false estimation due to diffraction by turbidity. UV detection was performed at 230nm and 225 nm, and the calibration curve was plotted between the resultant of the absorbance of [230 nm-(225 nm + 235 nm)/2] and the concentration of the analyte. The calibration curve was linear over the concentration range tested (1-20µg/mL) with a limit of detection of 0.27µg/mL and a limit of quantification of 0.82µg/ml. Percent relative standard deviations and percent relative mean error, representing precision and accuracy, respectively, for clear as well as turbid solutions, were found to be within acceptable limits, that is, always less than 0.69 and 0.41, respectively, for clear solutions and 0.65 and 0.47, respectively, for turbid solutions. [27]

Table 10: Results of UV system suitability parameters for Glipizide

S.NO	Parameters Drug name	Linearity		Precision			LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2	Conc. (ppm)	Intraday precision Average %RSD	Inter-day precision Average %RSD			
1.	Glipizide	1-20	0.99929	5 10 15	<0.69	<0.41	0.27	0.82	225&230

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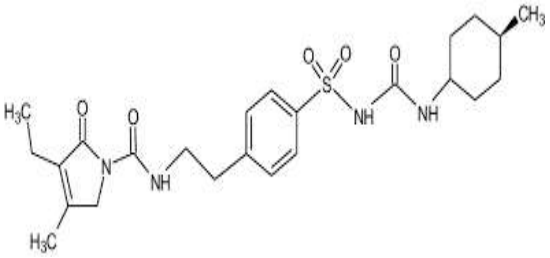
GLIMEPRIDE:



IUPAC name: 3-Ethyl-4-methyl-N-[2-(4-{[(trans-4-methyl cyclohexyl)carbamoyl]sulfamoyl}phenyl)ethyl]-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide
Molecular formula: $C_{24}H_{34}N_4O_5S$
Molecular weight: 490.6g/mol
Physical properties
Appearance: white colour compound
Solubility: Insoluble in water, but soluble in DMF and slightly soluble in Methylene chloride and methanol
Boiling point: 677°C
Melting point: 207°C [28]

Mechanism of action:

Glimepride is administered orally and is completely absorbed by the gastrointestinal tract. Thus, it stimulates insulin release by potentially binding with ATP-sensitive potassium channel receptors on the pancreatic beta cell surface, dropping a potassium conductance across the membrane and causing depolarization of the membrane, which stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin. It can be employed for the treatment of noninsulin-dependent (type II) diabetes mellitus. [29]

ANALYTICAL METHODS:
HPLC:

High-performance liquid chromatographic method for Glimepiride Tablets from their dosage forms. The detection was carried out at 230 nm using a Shimadzu UV-visible detector HPLC system. The accuracy and precision were determined and validated statistically. The linearity was observed in the range of 15–120 µg/mL with a correlation coefficient of 0.999. The limits of detection and the limit of quantification were found to be 4 ng and 10 ng, respectively. A Lichrosorb (RP-18) column with a mobile phase consisting of acetonitrile, water, and glacial acetic acid (550:450:0.6 v/v) was used. The flow rate was 1 mL/min. The linear coefficient of 0.999 was determined from the linearity curve in the concentration range of 15–120 µg/ml. The method was validated in terms of linearity, precision, accuracy, limit of detection, and limit of quantification.[30]

Table.11: Results of HPLC system suitability parameters for Glipizide.

S.NO	Parameters Drug name	Linearity		Precision			LOD (ng/ml)	LOQ (ng/ml)
		Range (ng/ml)	r^2	Conc. (ng/ml)	%RSD			
					Intraday	Inter-day		
1.	Glimepride	15-120	0.999	30 60 90	1.0385 0.7543 0.6934	1.4123 1.6638 1.2371	4	10

UV-SPECTROSCOPY:

A simple, sensitive, and accurate UV spectrophotometric method to be applied for the quantification of Glimepiride in tablets. The spectrophotometer used was a Spectro 2080plus (double-beam UV-visible spectrophotometer). An absorption maximum was found to be at 249 nm with the solvent system of chloroform. The drug follows Beer's law limits in the range of 5–30 µg/ml with a correlation coefficient of 0.999732. The analysis was validated for accuracy, precision, and LOD.[31]

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Table.12: Results of UV system suitability parameters for Glimepride.

S.NO	Parameters Drug name	Linearity		Accuracy		Precision			LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2	Conc. (%l)	%RSD	Conc. (µg/ml)	Intraday precision Average %RSD	Inter-day precision Average %RSD			
1.	Glimepride	5-30	0.999 732	80 100 120	< 1	15	0.257 0.259 0.262	0.261 0.260 0.258	0.4	1.2	249

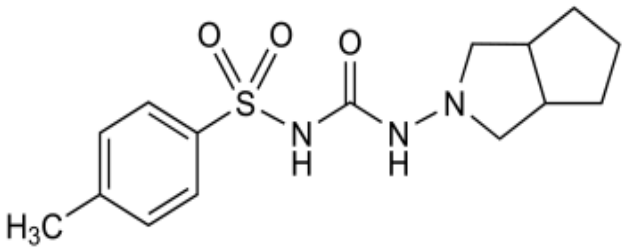
GAS CHROMATOGRAPHY:

It is the suitable derivatization technique. The liquid-liquid extraction method was employed by using 1-butanol:hexane (50:50, v/v) under an alkaline medium, and then back extraction was done via acetic acid. The analysis is carried out on a Perkin Elmer Clarus 500 GC. Elite 5MS of 30m × 0.32 mm, 0.25 µm and 1,4-bis-9-dimethylsiloxy) phenylene and dimethyl polysiloxane columns is used. Helium gas was employed as the carrier gas with a flow rate of 1.2 ml/min. The injecting volume of the sample was 2 µl. Distinct derivatization techniques were employed for the sample preparation for GC-MS analysis, i.e., silylation and acylation. Derivatization approaches were optimised under different parameters, i.e., reaction temperature and reaction time. N-Methyl-N-(trimethylsilyl) trifluoroacetamide [MSTFA] was found to be the best derivatization reagent for the GC-MS analysis of glimepiride. The total ion current (TIC) mode was selected for the monitoring of ions of the trimethylsilyl (TMS) derivative of glimepiride with an m/z ratio of 256. Distinct parameters like specificity, carryover, stability, precision, and accuracy were evaluated for validating the identification method. The GC-MS method is found to be linear and illustrated within the range of 500 to 2500 ng/ml, with the value of R2 (coefficient of determination) at 0.9924. The stability of the extracted and derivatized glimepiride was assessed with regard to processed/extracted sample conditions and autosampler conditions, respectively. The accuracy at each concentration level was within +/- 15% of the nominal concentration. Precision (%) for the inter-day and intraday analysis was found to be in the range of 0.70 to 1.003 and 0.74 to 0.99.[32]

Table.13: Results of GC system suitability parameters for Glimepride.

S.NO	Parameters Drug name	Linearity		Precision		
		Range (ng/ml)	r^2	Conc. (ng/ml)	%RSD	
					Intraday	Inter-day
1.	Glimepride	500-2500	0.9924	500-2500	0.74-0.99	0.70-1.003

GLICLAZIDE:



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IUPAC name: N-(hexahydrocyclopenta[c]pyrrol-2(1H)-ylcarbamoyl)-4-methylbenzenesulfonamide
Molecular formula: $C_{15}H_{21}N_3O_3S$
Molecular weight: 323.4g/mol
Physical properties
Appearance: White to off white crystalline powder
Solubility: practically insoluble in water and freely soluble in methylene chloride, sparingly soluble in acetone and slightly soluble in ethanol.
Boiling point: 180-182˚C
Melting point: 163-172˚C [33]

Mechanism of action:

It is under the brand name Diamicron, which is mostly suitable for the use of diabetic patients with renal impairment. The drug Gliclazide binds to the β -cell sulfonyl urea receptor (SUR1). This binding subsequently blocks the ATP-sensitive potassium channels. The binding results in the closure of the channels and leads to a decrease in potassium reflux, which leads to depolarization of the β cells. This opens voltage-dependent calcium channels in the β cell, resulting in calmodulin activation, which in turn leads to the exocytosis of insulin-containing secretory granules. [34]

ANALYTICAL METHODS:
RP-HPLC:

A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for the rapid assay of Gliclazide in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.2 ml min-1 was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of methanol and phosphate buffer 50:50 (V/V). The UV detection wavelength was 210 nm. Linearity was observed in the concentration range of 1–100 µg/ml. The retention time for Gliclazide was 3.25 minutes. The method was validated as per the ICH guidelines. [35]

Table.14: Results of HPLC system suitability parameters for Gliclazide.

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)
		Range (µg/ml)	r^2	Conc. (µg/ml)	%RSD Intraday		
1.	Gliclazide	1-100	0.999	50	0.01	0.003	0.01

UV-SPECTROPHOTOMETRY:

A simple and sensitive ultraviolet spectrophotometric method for quantitative estimation of gliclazide. A Shimadzu UV 1800 ultraviolet-visible spectrophotometer was used. The UV detection was performed at 226 nm, 221 nm, and 231 nm, and the calibration curve was prepared between the resultant of absorbance at these three different wavelengths (226 nm, 221 nm, and 231 nm) and the concentration of gliclazide. The calibration curve was found to be linear over the concentration range tested (04–28 µg/ml) having a limit of detection of 0.45 µg/ml and a limit of quantification of 1.36 µg/ml. Percent relative standard deviations, representing precision, for pure as well as impure solutions were found to be within acceptable limits.[36]

Table.15: Results of UV system suitability parameters for Gliclazide

S.NO	Parameters Drug name	Linearity		Precision			LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2	Conc. (µg/ml)	Intraday precision Average %RSD	Inter-day precision Average %RSD			
1.	Gliclazide	4-28	0.999732	11.2 14 16.8	1.575	1.85	0.45	1.36	226

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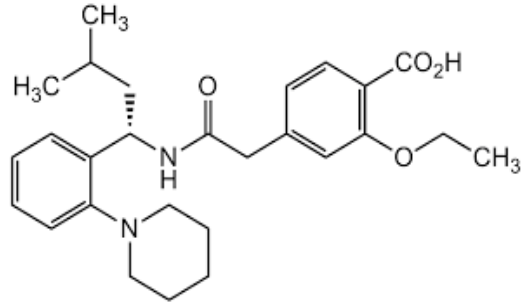
MEGLITINIDES:
REPAGLINIDE:



IUPAC name: (S)-2-Ethoxy-4-(1-[2-{piperidin-1-yl}phenyl]-3methylbutylcarbamoylmethyl)benzoic acid
Molecular formula: $C_{27}H_{36}N_2O_4$
Molecular weight: 452.6g/mol
Physical properties
Appearance:white to off white colour
Solubility: slightly soluble in water and soluble in ethanol, DMSO, DMF.
Boiling point: 672˚C
Melting point: 126-128˚C [37]

Mechanism of action:

Meglitinides work by triggering the production of insulin. This particular class of medication is meant to help lower blood sugar after meals. Repaglinide activity is dependent on the presence of functioning β cells and glucose. In contrast to sulfonylurea insulin secretagogues, repaglinide has no effect on insulin release in the absence of glucose. Rather, it potentiates the effect of extracellular glucose on the ATP-sensitive potassium channel and has little effect on insulin levels between meals and overnight. As such, repaglinide is more effective at reducing postprandial blood glucose levels than fasting blood glucose levels and requires a longer duration of therapy by stimulating beta cells to produce more insulin, allowing the body to better process glucose, thereby lowering blood sugar levels. [38]

ANALYTICAL METHODS:
HPLC-MS

A rapid and sensitive method based on high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed for the determination of repaglinide in human plasma. The analyte and internal standard (I.S.), diazepam, were extracted from plasma (25 mL) by liquid-liquid extraction with diethyl ether-dichloromethane (60:40, v/v) and separated on a XDB-C18 column using acetonitrile-ammonium acetate buffer (pH 6.8, 0.01 mol/L) as mobile phase. The retention times of repaglinide and I.S. were 1.95 and 2.35 min, respectively. Detection was carried out using an API 4000 mass spectrometer with an ESI interface operating in the multiple reaction monitoring (MRM) mode. The assay was linear over the concentration range of 0.050–50 ng/mL with a limit of detection (LOD) of 0.010 ng/mL. Intra- and inter-day precisions (as relative standard deviation, R.S.D.) were r5.07% and r11.2%, respectively, and accuracy. [39]

Table.16: Results of HPLC-MS system suitability parameters for Repaglinide:

S.NO	Parameters Drug name	Linearity		Precision		LOD (ng/ml)	LOQ (ng/ml)
		Range (ng/ml)	r^2	Conc. (ng/ml)	%RSD Intraday		
1.	Repaglinide	0.050-50	0.9982	50	0.01	0.010	0.01

UV & RPHPLC:

UV spectrophotometric and reversed-phase high-performance liquid chromatography (RP-HPLC) methods were developed for the determination of repaglinide. The UV spectrum recorded between 200 and 400 nm using methanol as solvent and the wavelength 241 nm were selected for the determination of repaglinide. RP-HPLC analysis was carried out using an Agilent TC-C18 (2) column and a mobile phase composed of methanol and water (80:20 v/v, pH adjusted to 3.5 with orthophosphoric acid) at a flow rate of 1.0 ml/min. Parameters such as linearity, precision, accuracy, recovery, specificity, and ruggedness are studied. The developed methods illustrated excellent linearity (r2 > 0.999) in the concentration range of 5–30 µg/ml and 5–50 µg/ml for UV spectrophotometric and HPLC methods,

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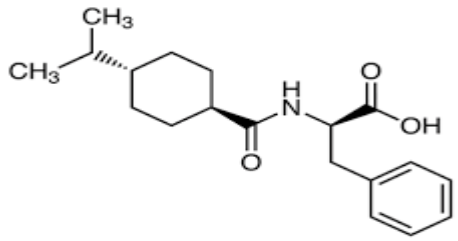
respectively. Precision (%R.S.D. 1.50) and mean recoveries were found in the range of 99.63–100.45% for the UV spectrophotometric method and 99.71–100.25% for the HPLC method, which shows the accuracy of the methods. [40]

Table.17: Results of UV with HPLC system suitability parameters for Repaglinide:

S.NO	Parameters Drug name	Linearity		Precision			LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2	Conc. (µg/ml)	Intraday precision Average %RSD	Inter-day precision Average %RSD			
1.	Repaglinide	UV-5-30 HPLC-5-50	0.999	50	0.2576 0.4971	0.1569 0.4001	1.15 3.48	0.73 2.21	241

NATEGLINIDE:



IUPAC name: 2R)-2-({[trans-4-(1-methylethyl)cyclohexyl]carbonyl}amino)-3-phenylpropanoic acid
Molecular formula: $C_{19}H_{27}NO_3$
Molecular weight: 317g/mol
Physical properties
Appearance: Off white to white powder
Solubility: freely soluble in ethanol,methanol and chloroform,soluble in ether and sparingly soluble in acetonitrile and octanol, practically insoluble in water.
Boiling point: 527.6˚C
Melting point: 137-141˚C [41]

Mechanism of action:

Nateglinide activity is dependent on the presence of functioning β cells and glucose. In contrast to sulfonylurea insulin secretagogues, nateglinide has no effect on insulin release in the absence of glucose. Rather, it potentiates the effect of extracellular glucose on the ATP-sensitive potassium channel and has little effect on insulin levels between meals and overnight. As such, nateglinide is more effective at reducing postprandial blood glucose levels than fasting blood glucose levels and requires a longer duration of therapy (approximately one month) before decreases in fasting blood glucose are observed. The insulinotropic effects of nateglinide are highest at intermediate glucose levels (3 to 10 mmol/L), and it does not increase insulin release already stimulated by high glucose concentrations (greater than 15 mmol/L). Nateglinide appears to be selective for pancreatic β cells and does not appear to affect skeletal or cardiac muscle or thyroid tissue. [42]

ANALYTICAL METHODS:
HPLC:

A rapid, simple, and sensitive HPLC method with UV detection was developed and validated for the determination of nateglinide (NTG) from rabbit plasma. The retention behaviour of NTG and gliclazide (GLZ, internal standard IS) as a function of mobile phase pH, composition, and flow rate was investigated. Separation was developed on a reverse-phase C_{18} column (250 mm × 4.6 mm i.d.,5µm particle size), using a mixture of acetonitrile (ACN):10 mM phosphate buffer (PBS, pH 3.0) in the ratio of 70:30 (%v/v) at a flow rate of 1.0 ml/min with UV detection at 203 nm within 8 min, and quantified based on drug/IS peak area ratios. The plasma samples were prepared by simple deproteinization with a mixture of methanol and acetonitrile, yielding more than 97.86% extraction efficiencies. The calibration curve was linear (correlation coefficient of 0.9984) in the concentration range of 10–2500 ng/ml. The limit of detection (LOD) and limit of Quantification (LOQ) were found to be 2.91 and 9.70 ng/ml, respectively. Both the intra-day and inter-day precisions at the four tested concentrations were below 1.32% R.S.D. The present method was selective enough to

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analyse NTG in rabbit plasma without any tedious sample clean-up procedure and was successfully applied for estimating the pharmacokinetic parameters of NTG following oral administration of a single 15 mg dose of NTG to white albino rabbits. [43]

Table.18: Results of HPLC system suitability parameters for Nateglinide :

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)
		Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday		
1.	Nateglinide	10-2500	0.9984	50	1.32	2.91	9.70

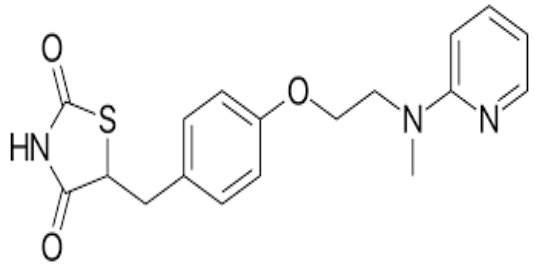
UV-SPECTROSCOPY:

Two methods that are simple, rapid, and cost-effective are presented for the determination of NTG in bulk and tablets using UV spectrophotometry. Methods are based on measurement of the absorbance of drug solution either in 0.1M NaOH at 210 nm (the NaOH method) or in 0.1 M HCl at 270nm (the HCl method). Beer's law is obeyed over concentration ranges of 3-54 and 4-72 µg/mL for the NaOH method and the HCl method, respectively, and corresponding molar absorptivity values are 4.09 $\times 10^3$ and 3.04 $\times 10^3$ L/mol/cm. Calculated Sandell sensitivities are 0.0776 and 0.0995 µgcm^{-2} with NaOH and HCl as diluents, respectively. Limits of detection (LOD) and quantification (LOQ), calculated according to the ICH guidelines, are 0.91 and 2.73 µg/mL (NaOH method) and 0.72 and 2.16 µg/mL (HCl method). Intra-day and inter-day accuracy and precision, determined by replicate analyses at three concentration levels, were =2% (%RE) and =1.63% (%RSD), respectively. Method robustness was assessed by making small changes in the measurement wavelength, whereas the ruggedness was tested by inter-analyst and inter-cuvette variations. [44]

Table.19: Results of UV system suitability parameters for Nateglinide:

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday			
1.	Nateglinide	4-28	0.999732	50	1.63	0.45	1.36	210&270

THIAZOLIDINEDIONES:
ROSIGLITAZONE:



IUPAC name: (RS)-5-[4-(2-[methyl(pyridin-2-yl)amino]ethoxy)benzyl]thiazolidine-2,4-dione
Molecular formula: $C_{22}H_{23}N_3O_7S$
Molecular weight: 357.428g/mol
Physical properties
Appearance: white to off white
Solubility: soluble in organic solvents such as DMSO, DMF, Ethanol.
Boiling point: 585.5˚C
Melting point: 122-123˚C [45]

Mechanism of action:

Rosiglitazone acts as a highly selective and potent agonist at peroxisome proliferator-activated receptors (PPAR) in target tissues for insulin action, such as adipose tissue, skeletal muscle, and the liver. Activation of PPAR-gamma receptors regulates the transcription of insulin-

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responsive genes involved in the control of glucose production, transport, and utilisation. In this way, rosiglitazone enhances tissue sensitivity to insulin. Apart from its effect on insulin resistance, it appears to have an anti-inflammatory effect. Nuclear factor kappa-B levels fall and inhibitor levels increase in patients on rosiglitazone. [46]

ANALYTICAL METHODS:

UV-SPECTROPHOTOMETRY:

A simple derivative method with reliable, sensitive, and reproducible UV Spectroscopic results has been developed for the estimation of Rosiglitazone Maleate in bulk and tablet formulation. A Shimadzu 1700 UV-Visible spectrophotometer with 1cm-matched quartz cells and 0.1 N NaOH as solvent were employed in both the methods. The first method is the zero-order UV spectrophotometric method, which is based on the measurement of absorbance at 245 nm, and the second method is the derivative spectrophotometric method, in which the derivative amplitude was measured at 218nm. The correlation coefficients for the first and second methods were 0.99992 and 0.99995, respectively. The recovery rates from pharmaceutical formulations were 99.577 and 100.33 for the first and second methods, respectively. [47]

Table.20: Results of UV system suitability parameters for Rosiglitazone:

S.NO	Parameters Drug name	Linearity		LOD (µg/ml)	LOQ (µg/ml)	Absornace (nm)
		Conc. (µg/ml)	r^2			
1.	Rosiglitazone	04-40 08-40	0.99992 0.99995	0.35 0.40	1.0 1.0	245 218

RP-HPLC:

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous determination of Rosiglitazone (ROS) and Glimepiride (GLM) in combined dosage forms and human plasma. The separation was achieved using a 150 mm × 4.6 mm i.d., 5 µm particle size Symmetry. C18 column. A mobile phase containing a mixture of acetonitrile and 0.02 M phosphate buffer of pH 5 (60:40, V/V) was pumped at a flow rate of 1 mL/min. UV detection was performed at 235 nm using nicardipine as an internal standard. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The developed and validated method was successfully used for the quantitative analysis of tablets. The chromatographic analysis time was approximately 7 min per sample with complete resolution of ROS (tR = 3.7 min.), GLM (tR = 4.66 min.), and nicardipine (tR = 6.37 min.). Validation studies performed according to ICH Guidelines revealed that the proposed method is specific, rapid, reliable, and reproducible. The calibration plots were linear over the concentration ranges 0.10-25 µg/mL and 0.125-12.5 µg/mL with an LOD of 0.04 µg/mL for both compounds and limits of quantification of 0.13 and 0.11 µg/mL for ROS and GLM, respectively. [48]

Table.21: Results of HPLC system suitability parameters for Rosiglitazone and Glimepride:

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)
		Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday		
1.	Rosiglitazone and Glimepride	0.1.-25 0.125-12.5	0.9984	50	1.32	0.04	0.13 0.11

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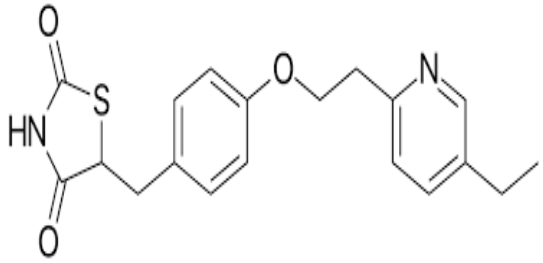
PIOGLITAZONE:



IUPAC name: (RS)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione
Molecular formula: $C_{19}H_{20}N_2O_3S$
Molecular weight: 356.4g/mol
Physical properties
Appearance: white
Solubility: soluble in water, ethanol, methanol, DMF, DMSO.
Boiling point: 575.4˚C
Melting point: 183-184˚C [49]
Mechanism of action:

Pioglitazone is a selective agonist at peroxisome proliferator-activated receptor-gamma (PPAR) in target tissues for insulin action, such as adipose tissue, skeletal muscle, and the liver. Activation of PPAR increases the transcription of insulin-responsive genes involved in the control of glucose and lipid production, transport, and utilisation. Through this mechanism, pioglitazone both enhances tissue sensitivity to insulin and reduces the hepatic production of glucose (i.e., gluconeogenesis); insulin resistance associated with type 2 diabetes mellitus is therefore improved without an increase in insulin secretion by pancreatic beta cells. [50]

ANALYTICAL METHODS:
RP-HPLC:

A simple, precise, and rapid RP-HPLC method was developed and validated for the estimation of pioglitazone hydrochloride. Separation was achieved by using a Phenomenex Luna C18 column (250x4.6mm, 5 µm) with a mixture of methanol and water in the ratio 75:25 and a flow rate of 1 ml/min. The analyte was monitored using a UV detector at 268nm. The retention time was found to be 3.28 minutes. The proposed method showed linearity for a concentration range of 10–18 µg/ml with a correlation coefficient of r2 = -0.999. The proposed method showed good recovery. This method is accurate, precise, and linear and can be used for routine analysis of Pioglitazone Hydrochloride. [51]

Table.22: Results of HPLC system suitability parameters for Pioglitazone:

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)
		Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday		
1.	Pioglitazone	10-18	0.999	50	1.32	0.005	0.001

UV-SPECTROPHOTOMETRY:

A simple UV-spectrophotometric method has been developed for the quantitative estimation of pioglitazone in bulk and pharmaceutical dosage forms. A Shimadzu UV-Visible double beam spectrophotometer (Model 1700) with 1 cm-matched quartz cells was used for the absorbance measurements. Pioglitazone hydrochloride has absorption maxima at 224.4 nm in ethanol and obeys Beer's law in the concentration range of 5–25 µg/mL.. The proposed UV spectrophotometric method is simple, sensitive, accurate, economical, and precise and can be utilised for the routine determination of pioglitazone hydrochloride in bulk and pharmaceutical dosage forms. [52]

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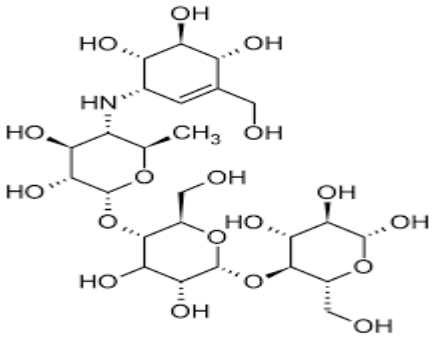
Table.23: Results of UV system suitability parameters for Pioglitazone:

S.NO	Parameters Drug name	Linearity		LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2			
1.	Pioglitazone	5-25	0.999732	0.45	1.36	224.4

ALPHA-GLUCOSIDASE INHIBITORS:
ACARBOSE:



IUPAC name: O-4,6-Dideoxy-4-[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose
Molecular formula: $C_{25}H_{43}NO_{18}$
Molecular weight: 645.6g/mol
Physical properties
Appearance: white to off white powder or to light yellow
Solubility: soluble in organic solvents such as DMSO & DMF.
Boiling point: 971.6˚C
Melting point: 165-170˚C [53]

Mechanism of action:

Acarbose is a complex of oligosaccharides that act as competitive, reversible inhibitors of pancreatic alpha-amylase and membrane-bound intestinal alpha-glucoside hydrolase. Pancreatic alpha-amylase hydrolyzes complex carbohydrates into oligosaccharides in the small intestine. Intestinal alpha-glucosidase hydrolase breaks down oligosaccharides, trisaccharides, and disaccharides into monosaccharides in the brush border of the small intestine. By delaying the digestion of carbohydrates, acarbose slows glucose absorption, resulting in a reduction in postprandial glucose blood concentration. [54]

ANALYTICAL METHODS:
HPLC:

Chromatographic analyses were performed using a Waters HPLC system. The chromatographic separation was achieved on a Lichrospher-100-NH2, 5 µm, 250 × 4.6 mm i.d. column using a mobile phase consisting of acetonitrile-0.007 M phosphate buffer (pH 6.7) (750:250, v/v) at a flow rate of 2 mL/min and UV detection at 210 nm. The column was maintained at 35 °C, and an injection volume of 10 µL was used. The linearity of the developed method was investigated in the range of 2.5–20 mg/mL (R2 = 0.9995). [55]

Table.24: Results of HPLC system suitability parameters for Acarbose:

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)
		Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday		
1.	Acarbose	2.5-20	0.9995	50	1.32	2.91	9.70

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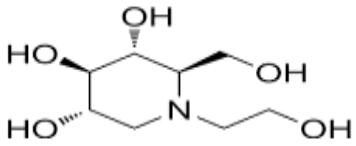
TLC:

TLC technique that was sensitive and accurate. The media were Silica TLC plates (TLC Silica Gel 60 F254, 0.20 mm thickness), Glucose, sucrose, maltose, glycerol, monosodium glutamate, acetone, n-propanol, diphenylamine, aniline, and phosphoric acid as analytical reagents, and agar as medium. An appropriate amount (5 uL) of each fermentation broth was spotted onto a 10×10 cm silica gel 60 F254 layer. The developing solvent was n-propanol:water (8:2, v/v). Acetone containing 10% (v/v) phosphoric acid, 2% (v/v) aniline, and 2% (w/v) diphenylamine was used as a colour developer. Acarbose on the TLC plate was visualised by the colour developer through a fine spray, followed by heating at 110°C for 10 min. The linearity of the acarbose in this way was good within the range from 2 to 10 µg (r2 = 0.9997). To determine the accuracy of the TLC method developed in this study. [56]

MIGLITOL:



IUPACname: (2R,3R,4R,5S)-1-(2-Hydroxyethyl)-2-(hydroxymethyl) piperidine-3,4,5-triol
Molecular formula: $C_8H_{17}NO_5$
Molecular weight: 207.22g/mol
Physical properties
Appearance: white to off white solid
Solubility:soluble in water, slightly soluble in ethanol, methanol
Boiling point:453.7˚C
Melting point: 143-145˚C [57]

Mechanism of action:

The reversible inhibition of membrane-bound intestinal glucoside hydrolase enzyme Membrane-bound intestinal a-glucosidases hydrolase oligosaccharides and disaccharides to glucose and others monosaccharides in the brush border of the small intestine in diabetic patients. This enzyme inhibition results in delayed glucose absorption and lowering of postprandial hyperglycemia. [58]

ANALYTICAL METHODS:
HPLC:

A selective and sensitive, stability-indicating reverse-phase high-performance liquid chromatography method has been first developed and validated for the estimation of miglitol in bulk and tablet dosage forms. Samples were separated on a prepacked Inertsil amino C_{18} column (150×4.6 mm i.d.) using a mobile phase comprised of acetonitrile and monobasic sodium phosphate pH 7.5 (80:20, v/v) delivered at a 1.5 ml/min flow rate. Detection was performed on a SPD-20A prominence UV/Vis detector at 220 nm. The retention time for miglitol was 13.93 ± 0.0367. The method was validated in terms of linearity, precision, accuracy, ruggedness, specificity, limit of detection, and limit of quantification. The linearity (r) and percentage recoveries of miglitol were 0.9986 and 99.85%, respectively. [59]

Table.25: Results of HPLC system suitability parameters for Miglitol:

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)
		Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday		
1.	Miglitol	10-2500	0.9984	50	1.32	2.91	9.70

UV-SPECTROPHOTOMETRY:

A simple, accurate, economical, and reproducible UV spectrophotometric method for simultaneous estimation of Miglitol in tablet dosage forms In this method. A Shimadzu UV/Visible double-beam spectrophotometer Model 1700 with 1cm-matched quartz cells was used. component spectroscopy using 300nm, 270nm, 240 nm, and 210nm as wavelengths for estimation. Miglitol was found to be linear in the concentration range of 0.2–1.2 µg/ml. The assay was found to be in the range of 99.27–99.92%. [60]

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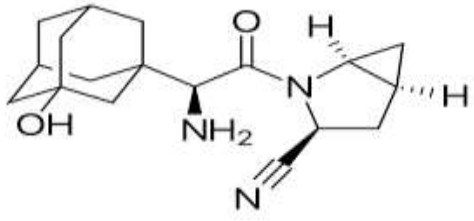
Table.26: Results of HPLC system suitability parameters for Miglitol:

S.NO	Parameters Drug name	Linearity		Precision			LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2	Conc. (µg/ml)	Intraday precision Average %RSD	Inter-day precision Average %RSD			
1.	Miglitol	5-25	0.999732	50	1.32	1.35	0.45	1.36	225

DPP4-INHIBITORS:
SAXAGLIPTIN:



IUPAC name: (1S,3S,5S)-2--2-azabicyclo, hexane-3-carbonitrile
Molecular formula: $C_{18}H_{25}N_3O_2$
Molecular weight: 315.4 g/mol
Physical properties
Appearance: pale yellow to light yellow
Solubility: soluble in water and slightly soluble in DMSO and sparingly soluble in methanol.
Boiling point:548.7˚C
Melting point:96-102˚C [61]

Mechanism of action:

Saxagliptin works by affecting the action of natural hormones in the body called incretins. Incretins decrease blood sugar by increasing consumption of sugar by the body, mainly through increasing insulin production in the pancreas, and by reducing production of sugar by the liver. [Bristol-Myers Squibb Press Release] DPP-4 is a membrane-associated peptidase that is found in many tissues, lymphocytes, and plasma. DPP-4 has two main mechanisms of action: an enzymatic function and another mechanism where DPP-4 binds adenosine deaminase, which conveys intracellular signals via dimerization when activated. Saxagliptin forms a reversible, histidine-assisted covalent bond between its nitrile group and the S630 hydroxyl oxygen on DPP-4. The inhibition of DPP-4 increases levels of active glucagon-like peptide 1 (GLP-1), which inhibits glucagon production from pancreatic alpha cells and increases insulin production of insulin from pancreatic beta cells. [62]

ANALYTICAL METHODS:
HPLC& UV-SPECTROPHOTOMETRY:

A simple, precise, and rapid high-performance liquid chromatography method with UV detection for the determination of saxagliptin in bulk An Agilent Zorbax CN (250 × 4.6 mm I.D., 5 µm) column was used with a mobile phase mixture of methanol and 50 ml phosphate buffer (pH 2.7) in a gradient elution mode at a flow rate of 1.0 ml min-1. The analytes were detected at 225 nm, and the total run time for the method was 7 min. The calibration graphs were linear in the range of 5.00–125.00 µg ml-1 for saxagliptin. [63]Table.27: Results of HPLC with UV system suitability parameters for Saxagliptin:

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday			
1.	Saxagliptin	5.00-125.0	0.9976	50	1.32	0.45	1.50	225

UV-SPECTROPHOTOMETRY:

UV spectrophotometric method for the estimation of saxagliptin A systronic UV-visible double beam spectrophotometer (2201) was used for measuring the absorbance. A standard solution of 50 µg/ml of Saxagliptin was scanned in the 200–400 nm range in a UV-visible spectrophotometer. The Maximum absorbance was found to be 213

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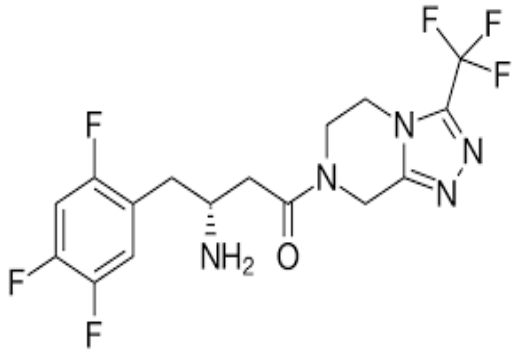
nm. The method was validated for linearity, range, accuracy, precision, robustness, LOD, and LOQ. The linearity regression coefficient was found to be 0.996 in the concentration range of 10–60 µg/ml. Accuracy was determined using a recovery study. The amount of drug recovered was found to be in the range of 99.01–100.1%. The LOD was found to be 0.3967 (µg/ml). The LOQ was found to be 1.202 (µg/ml). [64]

Table.28: Results of UV system suitability parameters for Miglitol:

S.NO	Parameters Drug name	Linearity		LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2			
1.	Miglitol	10-60	0.996	0.3967	1.202	213

SITAGLIPTIN:



IUPAC name: (R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro [1,2,4] triazolo[4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine
Molecular formula: $C_{16}H_{15}F_6N_5O$
Molecular weight: 407.31g/mol

Physical properties
Appearance: White to off white crystalline
Solubility: soluble in water
Boiling point: 529.9˚C
Melting point: 120.2 ˚C [65]

Mechanism of action:

Inhibition of DPP-4 by sitagliptin slows DPP-4-mediated inactivation of incretins like GLP-1 and GIP. Incretins are released throughout the day and upregulated in response to meals as part of glucose homeostasis. Reduced inhibition of incretins increases insulin synthesis and decreases glucagon release in a manner dependent on glucose concentrations. These effects lead to an overall increase in blood glucose control, which is demonstrated by reduced glycosylated haemoglobin. [66]

ANALYTICAL METHODS:
HPLC:

A new stability-indicating high-performance liquid chromatographic method for the analysis of sitagliptin C_8 (Qualisil BDS, 250×4.6 mm, 5 µ) column with PDA detection and a UV-Visible double beam spectrophotometer with a fixed slit width of 1nm and 1cm matched quartz cells was used. The mobile phase consisted of methanol and water (70:30, v/v), with 0.2% of n-heptane sulfonic acid adjusted to pH 3.0 with orthophosphoric acid. The Retention time of sitagliptin was 4.3 min with a flow rate of 1 ml/min, and the injection volume was 20 µl. The eluents were monitored at 253 nm using PDA detection. The linear regression analysis data for the linearity plot showed a correlation coefficient value of 0.9998 with respective concentration ranges of 20–150 µg/ml. The relative standard deviation for inter-day precision was lower than 2.0%. The limit of Detection (LOD) for SIT was 0.829 ng/ml, and the limit of Quantification (LOQ) was 2.509 ng/ml. [67]

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Table.29: Results of HPLC system suitability parameters for Sitagliptin:

S.NO	Parameters	Linearity		Precision			
	Drug name	Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday	LOD (µg/ml)	LOQ (µg/ml)
1.	Sitagliptin	20-150	0.9998	20-150	>2%	0.829	2.509

RP-HPLC:

A Simple, accurate, and precise method for estimation of the reversed phase The HPLC method has been developed for the simultaneous determination of sitagliptin and Metformin by using a Hypersil BDS C18 (100 x 4.6 mm, 5 µm particle size) column and a mobile phase at 215 nm. A mobile phase has a composition of potassium dihydrogen orthophosphate and methanol (50:50 v/v), a pH of 8.5 with O-phosphoric acid, and a flow rate of 1.0 ml/min. The retention times of Sitaglipitin were 2.3 min. The linearity coefficient was 0.999 for Sitagliptin. The %RSD for Sitagliptin is 0.29. [68]

Table.30: Results of HPLC system suitability parameters for Sitagliptin:

S.NO	Parameters	Linearity		Precision			
	Drug name	Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday	LOD (µg/ml)	LOQ (µg/ml)
1.	Sitagliptin	10-2500	0.999	50	0.29	2.91	9.70

UV-SPECTROSCOPY:

An attempt was made to develop a UV Spectroscopic method that is economical, accurate, precise, and sensitive for the determination of metformin and sitagliptin in combined dosage form. The spectroscopic conditions were optimised. Metformin was found to be linear between the concentrations of 20 to 80 µg/ml for Sitagliptin. Their correlation coefficients were found from the linear graph to be 0.99977 for Metformin and sitagliptin, respectively. The limits of Detection and quantification were calculated from the regression lines using their standard deviation and slope. LOQ is 3.3 s/S, and LOD is 10 s/S. The precision of the method was determined for one lot of combined dosage forms by considering intraday and interday measurements. The accuracy of the method was checked by performing recovery studies. [69]

Table.31: Results of UV system suitability parameters for Sitagliptin:

S.NO	Parameters	Linearity		Precision			
	Drug name	Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday	LOD (µg/ml)	LOQ (µg/ml)
1.	Sitagliptin	20-80	0.99977	5	1.636	0.01693	0.6839

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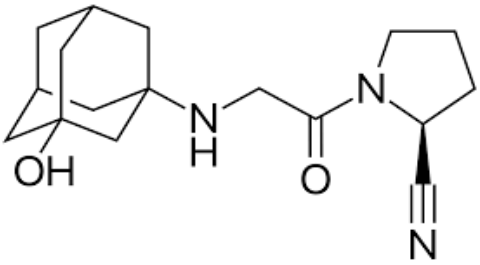
HPTLC:

A simple, sensitive, and accurate HPTLC method was developed by Chirag Patel et al. for the simultaneous determination of MET and SITA in marketed formulations. The mobile phase used was 1% w/v ammonium acetate in methanol. The detection of spots was carried out densitometrically using a UV detector at 257 nm in absorbance mode. The Rf value for MET and SITA were found to be 0.43 ± 0.009 and 0.60 ± 0.013, respectively. The calibration curve was found to be linear between 600 to 2000 and 1000 and 7000 ng/spot for MET and SITA, respectively. The limits of detection and quantitation were found to be 76.257 and 231.083 ng/spot, respectively, for MET and 65.080 and 197.212 ng/spot, respectively, for SITA. The authors demonstrated that the proposed HPTLC method was highly reproducible and reliable. [67]

VILDAGLIPTIN:



IUPAC Name:(S)-1-[2-(3-Hydroxyadamantan-1-ylamino) acetyl]pyrrolidine-2-carbonitrile
Molecular formula:$C_{17}H_{25}N_3O_2$
Molecular weight: 303.4g/mol
Physical properties
Appearance: White to light yellow or light brown
Solubility: soluble in water
Boiling point: 548.7˚C
Melting point: 96-102˚C [70]

Mechanism of action:

Vildagliptin is a selective, reversible, and competitive inhibitor. Inhibition of dipeptidyl peptidase-4 (DPP-4) by vildagliptin prevents degradation of glucagon-like peptide-1 (GLP-1) and reduces glycemia in patients with type 2 diabetes mellitus with low risk for hypoglycemia and no weight gain. Vildagliptin binds covalently to the catalytic site of DPP-4, eliciting prolonged enzyme inhibition. This raises intact GLP-1 levels, both after meal ingestion and in the fasting state. Vildagliptin has been shown to stimulate insulin secretion and inhibit glucagon secretion in a glucose-dependent manner. At hypoglycemic levels, the counterregulatory glucagon response is enhanced relative to baseline by vildagliptin. Vildagliptin also inhibits hepatic glucose production, mainly through changes in islet hormone secretion, and improves insulin sensitivity, as determined by a variety of methods. These effects underlie the improved glycaemia with low risk for hypoglycaemia. Vildagliptin also suppresses postprandial triglyceride (TG)-rich lipoprotein levels after ingestion of a fat-rich meal and reduces fasting lipolysis, suggesting inhibition of fat absorption and reduced TG stores in non-fat tissues. The large body of knowledge on vildagliptin regarding enzyme binding, incretin and islet hormone secretion, and glucose and lipid metabolism is summarised, with discussion of the integrated mechanisms and comparison with other DPP-4 inhibitors and GLP-1 receptor activators. [71]

ANALYTICAL METHODS:

RP-HPLC:

Vildagliptin is a potent dipeptidyl peptidase IV inhibitor used for the treatment of diabetes. Reverse phase separation was obtained within 4 minutes. Separation was performed on a C18 column using a mixture of pH 8.2 buffer, acetonitrile, and methanol as the mobile phase, along with indirect UV detection at 254 nm. The retention time was found to be Rt = 3.9 ± 0.1 min. The validation of this method included the determination of its linearity range of 50–90 µg/mL (r2 = 0.999), specificity, accuracy, precision, linearity, LOD, LOQ, and robustness. The LOD was 2.98 g/mL, and The LOQ was 9.94 g/mL. [72]

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Table.32: Results of HPLC system suitability parameters for Vildagliptin:

S.NO	Parameters Drug name	Linearity		Precision		LOD (µg/ml)	LOQ (µg/ml)
		Range (ng/ml)	r^2	Conc. (µg/ml)	%RSD Intraday		
1.	Vildagliptin	50-90	0.999	50	0.1	2.98	9.94

UV-SPECTROSCOPY:

The method employs measurement of absorbance at the wavelength of maximum absorption of vildagliptin using water as a solvent. A UV-visible 1601 Shimadzu double-beam spectrophotometer was used to measure spectra. About 100 ppm of vildagliptin solution was accurately prepared in water. These solutions were scanned in the 200–400 nm UV regions. The wavelength maxima (λmax) were observed at 244 nm, and this wavelength was adopted for absorbance measurement. The calibration curve was linear in the concentration range of 12.5-200 micrograms per ml µg/ml for vildagliptin, with a correlation coefficient of 0.985. [73]

Table.33: Results of UV system suitability parameters for Vildagliptin:

S.NO	Parameters Drug name	Linearity		Precision			LOD (µg/ml)	LOQ (µg/ml)	Absorbance (nm)
		Conc. (µg/ml)	r^2	Conc. (µg/ml)	Intraday precision Average %RSD	Inter-day precision Average %RSD			
1.	Vildagliptin	12.5-200	0.985		1.23	2	0.055	0.166	244

II. CONCLUSION:

In this literature study, the most popular Spectrophotometric and Chromatographic analytical procedures have been listed. The development of these methods involved the use of analytical equipment such as UV-Visible Spectrophotometers, High-Performance Liquid Chromatography, Reversed-Phase High-Performance Liquid Chromatography, Thin Layer Chromatography, and Ultra-High-Performance Liquid Chromatography. It was developed to describe particular oral hypoglycaemic drugs. Biguanides, sulfonyl ureas, meglitinides, thiazolidinediones, alpha-glucosidase inhibitor in their pure form, human plasma, and other biological fluids.

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