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"INNOVATIVE APPROACHES IN ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR EVOGLIPTIN TARTRATE AND ITS MULTI-DRUG COMBINATIONS"

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Abstract:
As pharmaceutical formulations become more complex—especially in the management of type 2 diabetes—there is a growing need for advanced analytical techniques to accurately assess drug content. This work presents innovative strategies for the development and validation of analytical methods tailored to Evogliptin tartrate, both as a standalone compound and in fixed-dose combinations with other antidiabetic agents. Due to the absence of official pharmacopoeial methods for newer drugs like Evogliptin, novel approaches utilizing UV spectrophotometry, high-performance liquid chromatography (HPLC), and LC-MS were investigated. The methods were developed following ICH guidelines and rigorously validated for parameters such as specificity, linearity, accuracy, precision, and sensitivity. These validated methods are designed to ensure reliable quality control and regulatory compliance for Evogliptin-containing formulations, ultimately supporting the safety and therapeutic effectiveness of modern antidiabetic treatments.

Key Words: Diabetes mellitus, Evogliptin Tartrate, Metformin Hydrochloride, Analytical methods (HPLC, UV, HPTLC),

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Introduction:

Diabetes, or diabetes mellitus, refers to a group of conditions in which the body cannot effectively regulate blood glucose levels, resulting in persistent high blood sugar.[1]

The main role of insulin, which is synthesized in the pancreas, is to help transport blood glucose into the cells for energy production. In type 2 diabetic patients, the pancreas may not generate adequate insulin, or the tissues may not utilize it well due to insulin resistance.[2]

Evogliptin was first approved in South Korea for managing blood glucose levels among individuals with type 2 diabetes.[3] Many different health complications can develop due to diabetes. Heart disease, stroke, chronic renal disease, foot ulcers, nerve damage, vision problems, and cognitive impairment are all serious long-term complications.[4]

22 oct 2018 approved by CDSCO.[5]

Evogliptin tartrate is a salt form of Evogliptin. Evogliptin tartrate is a targeted inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4), developed to treat type 2 diabetes mellitus. By blocking DPP-4, it

prevents the breakdown of incretin hormones like glucagon-like peptide-1 (GLP-1), which stimulates insulin secretion and inhibits glucagon release, thus aiding in effective blood sugar regulation.[6]

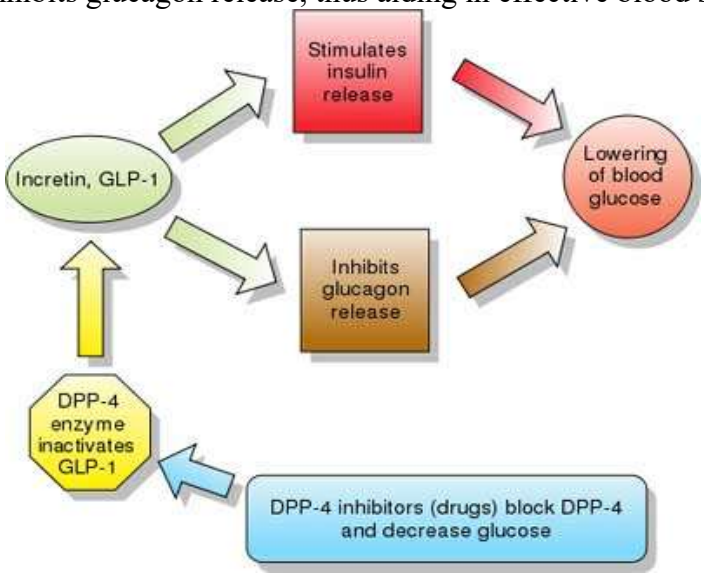

FIGURE 1: Mechanism of DPP-4 inhibitors

Drug profile of Evogliptin Tartrate:

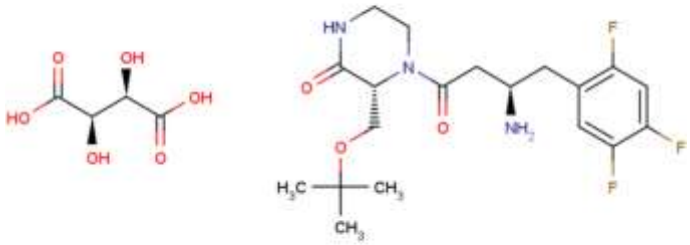

FIGURE 2: Structure of Evogliptin Tartrate

TABLE 1: Drug profile of Evogliptin Tartrate [7]

IUPAC Name	2-Piperazinone, 4-((3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl) butyl)-3-((1,1-dimethylethoxy)methyl)-, (3R)-, (2R,3R)-2,3-dihydroxybutanedioate (1:1)
Chemical Formula	$C_{23}H_{32}F_3N_3O_9$
Molecular Mass	551.5 g/mole
Physical State	Solid
Solubility	Soluble in Water and Methanol
Therapeutic Use	Used to reduce hyperglycaemia in Type II Diabetes mellitus
pKa	Acidic: 13.69 Basic: 8.78
Parent Compound	Evogliptin
Component Compound	L- Tartaric Acid Evogliptin
Chemical Class	DPP-4 inhibitor Class

Metformin, a biguanide antidiabetic medication, acts by suppressing hepatic gluconeogenesis, enhancing tissue responsiveness to insulin, and increasing the uptake of glucose by muscles and fat tissues. It traces back to the natural compound from Galega officinalis and has since evolved into the front-line oral medication for type 2 diabetes due to its robust glucose-lowering effects and favourable safety characteristics.[8]

Drug profile of Metformin Hydrochloride:

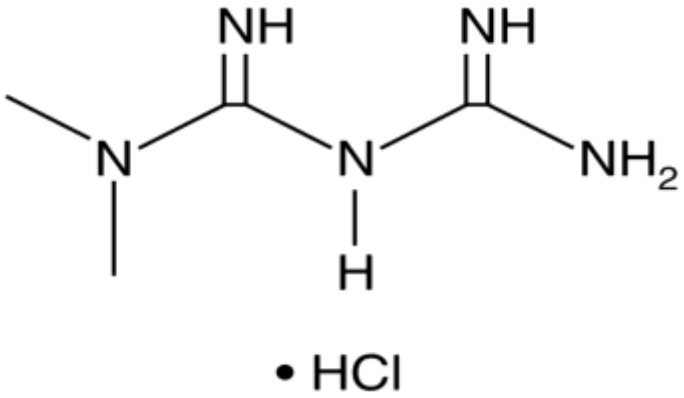

FIGURE 3: Structure of Metformin Hydrochloride

TABLE 2: Drug profile of Metformin Hydrochloride [9]

IUPAC Name	hydrogen 1-[(diaminomethylidene)amino]-N,N-dimethylmethanimi damide chloride or 3-(diaminomethylidene)-1,1-dimethyl guanidine ;hydrochloride
Molecular Formula	$C_4H_{12}ClN_5$
Molecular Mass	165.62 g/mole
Physical State	Powder
Solubility	Freely soluble in water, slightly soluble in alcohol, practically insoluble in acetone and in methylene chloride.
Therapeutic Use	To treat high blood glucose level in Diabetes mellitus
pKa	Acidic : 12.4
Parent Compound	Metformin
Component Compound	Hydrochloric Acid Metformin
Chemical Class	Biguanide Class

Pharmacology:

Pharmacokinetics:

1. Absorption: When taken orally, Evogliptin is absorbed into the body at a rate higher than 50%. Eating food at the same time does not change how well it is absorbed. In healthy individuals, a single dose of 1.25–60 mg generally leads to peak blood levels within 3 to 5.5 hours.

In healthy subjects, a 5 mg oral dose of Evogliptin produced a Cmax of 5.6 ± 1.3 μg/L. Both Cmax and AUC_{last} increased in a dose-dependent manner. Steady-state levels were reached by the third day of once-daily dosing at 5–20 mg, with peak concentrations occurring 4–5 hours post-dose.

2. Distribution: In a first-in-human clinical trial, Evogliptin demonstrated good tolerability in healthy individuals following repeated once-daily dosing. The drug showed a terminal elimination half-life ranging between 33 and 39 hours, indicating sustained presence in the body. Peak plasma concentrations (Tmax) were observed approximately 4 to 5 hours after administration. Additionally, Evogliptin exhibited an absolute bioavailability of roughly 50%, suggesting that half of the administered oral dose successfully reached systemic circulation.[10]

3. Metabolism: In vitro studies indicate that Evogliptin neither induces nor inhibits cytochrome P450 (CYP) enzymes. Its metabolism is primarily carried out by CYP3A4, resulting in the formation of metabolites M7 and M8, whose pharmacological activities remain unknown. Conversely, pioglitazone undergoes extensive metabolism mainly via CYP2C8, CYP3A4, and CYP2C9 enzymes, producing active metabolites M3 and M4. Clinical data show no significant CYP enzyme induction or inhibition by pioglitazone in vivo.

Evogliptin has distinct elimination pathways with minimal overlap, their pharmacokinetic interaction is unlikely, as supported by current study findings.[11]

4. Excretion: In studies involving healthy adult volunteers, approximately 46.1% of the administered Evogliptin dose was eliminated through urinary excretion, while about 42.8% was excreted via the feces, encompassing both the parent compound and its metabolites. These findings suggest that Evogliptin undergoes dual routes of elimination through renal and hepatic pathways. Additionally, Evogliptin had no significant effect on the pharmacokinetics of commonly co-prescribed antidiabetic medications such as glimepiride, pioglitazone, and Metformin. This indicates a low likelihood of pharmacokinetic drug-drug interactions when Evogliptin is used in combination therapy for the management of type 2 diabetes.[12]

Interaction of Evogliptin Tartrate with other drugs:

Metformin: The pharmacokinetics of Evogliptin 5 mg and twice daily Metformin 1,000 mg (an OCT1 and OCT2 substrate) did not improve clinically meaningfully until a stable state was achieved.[13]

Clarithromycin: Single administration of Evogliptin at a dose of 5 mg resulted in a 2.17-fold increase of Evogliptin Cmax and a 2.02-fold increase in Evogliptin AUC compared to multiple administration of potential CYP3A4 inhibitor clarithromycin at a daily dose of 1,000 mg until the steady concentration was achieved. Precautions should be taken since the pharmacokinetic parameters of Evogliptin can increase if it is administered with a CYP3A4 inhibitor.[14]

Advance Combination Therapy in T2DM:

In the initial stages of type 2 diabetes, many patients still retain sufficient β-cell function to respond effectively to glucagon-like peptide-1 (GLP-1) stimulation. In such cases, DPP-4 inhibitors can play a valuable role by enhancing the body's endogenous incretin response, thereby improving insulin secretion and reducing glucagon levels in a glucose-dependent manner. Evidence from the landmark UK Prospective Diabetes Study (UKPDS) has shown that long-term glycemic control is rarely sustained through monotherapy alone. Over time, the progressive nature of β-cell dysfunction in type 2 diabetes typically necessitates the introduction of additional glucose-lowering agents. [15]

Evogliptin Tartrate Monotherapy in Patients with Type II DM:

This clinical trial was conducted to evaluate both the efficacy and safety profile of a once-daily 5 mg oral dose of Evogliptin, with the additional goal of identifying the most appropriate dose and dosing regimen for patients with type 2 diabetes mellitus (T2DM) who were unable to achieve adequate Glycemic control through lifestyle modifications such as diet and exercise alone. The primary endpoint of the study was the change in glycated Haemoglobin (HbA1c) levels from baseline to week 24. Treatment with Evogliptin resulted in a statistically significant reduction in HbA1c compared to placebo, with a mean difference of –0.28% ($p < 0.0001$), demonstrating its effectiveness in improving long-term Glycemic control in this patient population.[16]

Evogliptin Tartrate in Combination with Metformin Hydrochloride in Patients with Type II DM:

The study was conducted to evaluate and compare the therapeutic efficacy and safety of Evogliptin in combination with Metformin against that of sitagliptin plus Metformin in patients with type 2 diabetes mellitus (T2DM) who exhibited insufficient glycemic control on Metformin monotherapy. After 24 weeks of treatment, the primary efficacy endpoint—change in HbA1c from baseline was assessed. The adjusted mean difference in HbA1c between the Evogliptin and sitagliptin groups was 0.06%, with the upper bound of the 95% confidence interval measured at 0.22%. This value remained below the predefined non-inferiority threshold of 0.35%, thereby meeting the criteria for non-inferiority. These results demonstrate that Evogliptin is comparably effective to sitagliptin in improving glycemic control when used alongside Metformin, offering a viable alternative treatment option for patients with inadequately controlled T2DM. [17]

Analytical methods:

Analytical method development and validation are essential in pharmaceutical research and manufacturing, ensuring accurate measurement of the active ingredient (API) in specific dosage forms. This process confirms that the method is suitable, reliable, and fit for its intended purpose.

With the continual rise in the number of new drugs entering the market each year—either as novel chemical entities or modified versions of existing compounds—there is often a lack of official analytical methods available in pharmacopoeias for these substances. As a result, the development of new analytical techniques becomes essential. Official methods are primarily used in quality control laboratories to verify the identity, purity, potency, and overall performance of pharmaceutical products. A variety of analytical techniques can be employed for this purpose, including UV spectrophotometry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), and liquid chromatography (LC), among others. [18-20]

After conducting a literature survey on Analytical method development and validation for Metformin Hydrochloride and Evogliptin Tartrate it was found that till date there has been not any method reported for this combination.

Official method for estimation of Evogliptin Tartrate:

There is no official method for Evogliptin tartrate in any pharmacopoeia.

Reported method of estimation of Evogliptin Tartrate:

TABLE 3: Reported method of estimation of Evogliptin Tartrate

Sr. No	Drug	Detection Method	Description	Reference No
1.	Evogliptin Tartrate	HPLC	Solvent:- Methanol : Evogliptin Tartarate (10:50%v/v) R^2 : 0.9926 Limit of detection : 1.08 µg/mL Limit of Quantitation: 3.27 µg/mL	[21]
3.	Evogliptin Tartrate	UV	UV visible double beam Model: Shimadzu 2600 Solvent: Water Wavelength: 275 to 277nm	[22]

Official method of estimation of Metformin Hydrochloride:

TABLE 5: Official method of estimation of Metformin Hydrochloride

Sr. No.	Official Method	Detection Methods	Description	Reference no.
1.	Indian Pharmacopoeia 2018	Liquid Chromatography	Stationary Phase: stainless steel column with octadecylsilane bonded to porous silica	[23]

			Mobile Phase: A solution containing 0.087 % w/v of Sodium pentane sulphonate and 0.12% w/v of sodium chloride, adjusted to pH 3.5 using 1% v/v solution of orthophosphoric acid. Flow rate: 1 ml/min. Wavelength: 218 nm Injection volume: 20µl	
2.	British Pharmacopoeia 2003	Liquid Chromatography	Stationary phase: irregular, porous silica gel Mobile phase: 17 g/l solution of ammonium dihydrogen phosphate R adjusted to pH 3.0 with phosphoric acid Flow rate: 1 ml/min Wavelength: 218 nm Injection volume: 20µl	[24]

Reported method of estimation of Metformin Hydrochloride:

TABLE 4: Reported method of estimation of Metformin Hydrochloride

Sr. No	Drug	Detection Method	Description	Reference No
1.	Metformin in bulk and tablet dosage form	UV	UV visible double beam Model: Shimadzu 1800 Solvent: Water Wavelength: 234nm Correlation coefficient: 0.9998 Linearity: 10-50 µg/ml	[25]
2.	Metformin in tablet dosage form	UV	UV visible double beam Model: Shimadzu 1700 Solvent: NaOH Wavelength: 233nm Linearity: 1-25 µg/ml	[26]
3.	Metformin Hydrochloride	RP-HPLC	Stationary phase: Cosmosil C_{18} Mobile phase: Methanol: Phosphate buffer (70:30 %v/v) Flow rate: 1 ml/min Wavelength: 238 nm Linearity: 10-50 µg/ml Retention time: 4.2 min	[27]
4.	Metformin Tablet dosage form	RP-HPLC	Stationary phase: Hypersil C18 Mobile phase: Acetonitrile: Phosphate buffer (65:35 %v/v) Flow rate: 1.0 ml/min Wavelength: 233 nm Linearity: 50-150 µg/ml Retention time: 7.168 min	[28]

Reported methods for estimation of Metformin Hydrochloride and Evogliptin Tartrate with different combinations:

TABLE 6: Reported methods for estimation of Metformin Hydrochloride and Evogliptin Tartrate with different combinations

Sr. No	Drug	Detection Method	Description	Reference No
1.	Evogliptin Tartrate + Metformin Hydrochloride	RP-HPLC	Stationary Phase:- C_{18} Mobile Phase:- Water: Acetonitrile (25:75%v/v)	[29]

			Flow Rate : 1.2 mL/min %RSD: Metformin Hydrochloride was 0.18, Evogliptin Tartrate was 0.20 Wavelengths: Metformin Hydrochloride 210nm, Evogliptin Tartrate 230 nm Loss of Detection: Metformin Hydrochloride 0.55 µg/mL, Evogliptin Tartrate 0.006 µg/mL Limit of Quantitation: Metformin Hydrochloride 1.67 µg/mL, Evogliptin Tartrate 0.017 µg/mL R^2: Metformin Hydrochloride 1, Evogliptin Tartrate 0.9997	
2.	Metformin Hydrochloride + Glipizide	UV	Model: Shimadzu 1800 Solvent: Distilled water Wavelength: Metformin Hydrochloride 272 nm, Glipizide: 232 nm Linearity: Metformin Hydrochloride 5-25 µg/ml, Glipizide 20-50 µg/ml	[30]
3.	Metformin Hydrochloride + Alogliptin	UV	Model: Shimadzu 1800 Solvent: Methanol Wavelength: Metformin Hydrochloride 232nm, Alogliptin Benzoate 277nm Linearity: Metformin Hydrochloride 1-10 µg/ml, Alogliptin 5-25 µg/ml	[31]
4.	Metformin Hydrochloride + Saxagliptin	RP-HPLC	Stationary phase: C_8 Mobile phase: Methanol : Phosphate buffer (70:30 %v/v) Flow rate: 1 ml/min Wavelength: 228 nm Linearity: Metformin Hydrochloride 250-1250µg/ml, Saxagliptin 2.5-12.5µg/ml Retention time: Metformin Hydrochloride 2.8min, Saxagliptin 4.9min	[32]
5.	Metformin Hydrochloride and Glibenclamide	HPTLC	Stationary phase: Silica Gel 60 F_{254} Mobile Phase: Methanol: Water: Sodium Sulphate in Water (7:5:11 %v/v/v) Wavelength: Metformin Hydrochloride 232 nm, Glibenclamide 238 nm Linearity: Metformin Hydrochloride: 250-1750µg/ml Glibenclamide: 250-1750µg/ml	[33]

			Rf value: Metformin Hydrochloride 0.27, Glibenclamide 0.80	
6.	Metformin Hydrochloride + Sitagliptin Phosphate	HPTLC	Stationary phase: Silica gel 60 F254 Mobile Phase: Butanol: Water: Glacial Acetic Acid (6:2:2 %v/v/v) Wavelength: 227 nm Linearity: Metformin Hydrochloride 500-10000µg/ml Sitagliptin Phosphate 50-1000µg/ml Rf value: Metformin Hydrochloride 0.35, Sitagliptin phosphate 0.75	[34]

Validation Parameters: [35]

Accuracy:

The accuracy of the proposed method was checked using recovery studies. Tablet powder equivalent to 2.5 mg of drug was taken, and known amounts of standard drug were added at three levels (50%, 100%, and 150%). These solutions were analyzed again using the developed method. Each level was tested three times to ensure repeatability. The results confirmed that the method is accurate.

Precision:

Precision means how close the results are when the same sample is tested multiple times under the same conditions. It is checked in two ways:

• Intra-day precision: The sample (20 µg/mL) was tested three times in the same day.
• Inter-day precision: The same sample was tested on five different days.

Ruggedness:

Ruggedness checks if the method gives consistent results even when tested by different people or on different instruments. For this, Dapagliflozin (20 µg/mL) was analyzed by two analysts using two UV-spectrophotometers (Jasco V-630 and Shimadzu-1700) under the same conditions.

Linearity:

Linearity shows if the test results increase in direct proportion to the concentration of the drug. For this, five solutions of Dapagliflozin (80–120% of label claim) were prepared and analyzed, and the results were used to plot calibration curves.

Limit of detection (LOD) and limit of quantification (LOQ):

The LOD and LOQ were calculated using following equations as per International Conference on Harmonization guideline for DAPA.

LOD= 3.3 x σ/S

LOQ= 10 x σ/S

Where σ is standard deviation of the response and S is the standard deviation of y-intercept of regression lines.

Conclusion:

The development and validation of innovative analytical methods for Evogliptin tartrate and its multi-drug combinations represent a significant advancement in pharmaceutical analysis, especially in the management of type 2 diabetes mellitus. With the increasing demand for precise and reliable analytical tools, a wide range of modern techniques—such as HPLC, HPTLC, LC, UV- Vis spectrophotometry, and —have been explored and optimized to achieve accurate quantification and characterization of Evogliptin, both alone and in fixed-dose combinations. The innovative analytical

techniques reviewed not only contribute to the robust analysis of Evogliptin tartrate and its combinations but also serve as a valuable framework for future research and method development. They offer a strategic foundation for pharmaceutical scientists aiming to develop reliable, scalable, and compliant methods that support both clinical and commercial needs in diabetes therapy.

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