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CHARACTERIZATION, DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF SITAGLIPTIN, DAPAGLIFLOZIN &VILDAGLIPTIN

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ABSTRACT
Diabetes mellitus is a rapidly growing global health concern, necessitating the development of reliable analytical methods for the quality control of antidiabetic drugs. The present study aims to develop and validate a simple, precise, accurate, and robust Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the estimation of antidiabetic drugs in bulk and pharmaceutical dosage forms.

The chromatographic separation was achieved using a C18 reverse phase column with a suitable mobile phase consisting of acetonitrile and phosphate buffer in an optimized ratio, delivered at a constant flow rate. Detection was carried out using a UV detector at an appropriate wavelength based on the drug's absorption maxima. The method was optimized to obtain well-resolved peaks with acceptable retention times and minimal tailing.

The developed method was validated according to ICH guidelines for parameters such as linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). The results demonstrated excellent linearity over the selected concentration range with high correlation coefficient values. Accuracy studies showed satisfactory recovery, while precision studies indicated low %RSD values, confirming the reliability of the method. The method was also found to be specific and robust under varied conditions.

Keywords: *RP-HPLC, Impurities, API's, Hazardous,LOQ,LOD*

INTRODUCTION
Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from defects in insulin secretion, insulin action, or both. The global prevalence of diabetes has increased significantly, making the effective management and quality control of antidiabetic drugs critically important.

Antidiabetic drugs such as sitagliptin, dapagliflozin, vildagliptin, metformin, and others are widely used either alone or in combination therapy. Ensuring the quality, safety, and efficacy of these pharmaceutical formulations requires reliable analytical methods for their accurate estimation in bulk drugs and dosage forms.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is one of the most widely employed analytical techniques in pharmaceutical analysis due to its high sensitivity, accuracy, precision, and reproducibility. RP-HPLC utilizes a non-polar stationary phase and a relatively polar mobile phase, allowing effective separation of compounds based on hydrophobic interactions.

Method development involves selecting suitable chromatographic conditions such as column type, mobile phase composition, flow rate, detection wavelength, and temperature to achieve optimal separation. Method validation, as per ICH guidelines, ensures that the developed method is suitable for its intended purpose by evaluating parameters such as accuracy, precision, specificity, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

The present study focuses on the development and validation of a simple, precise, accurate, and reproducible RP-HPLC method for the estimation of antidiabetic drugs in pharmaceutical formulations, ensuring compliance with regulatory standards.

MATERIALS AND METHOD:
Pure drug samples of antidiabetic agents (e.g., Sitagliptin, Dapagliflozin, Vildagliptin, etc.)

Pharmaceutical dosage forms (tablets)

HPLC grade solvents:

Methanol

Acetonitrile

Water (HPLC grade)

Analytical grade reagents:

Potassium dihydrogen phosphate

Orthophosphoric acid

Membrane filters (0.45 µm)

Chromatographic Conditions:
Column: C18

Mobile phase: Acetonitrile: Phosphate buffer (60:40 v/v)

Flow rate: 1.0 mL/min

Detection wavelength: 210–260 nm (depending on drug)

Injection volume: 20 µL

Run time: 10–15 minutes

Temperature: Ambient

RESULT AND DISCUSSION:
Method development and validation

Forced degradation studies

Regulatory readiness documentation

All studies were performed in accordance with:
- ICH Q2(R1) – Analytical Validation
- ICH Q3A(R2) – Impurity Guidelines

A stability-indicating RP-HPLC method was developed for Vildagliptin and its related impurities.

Optimized Conditions:
- C18 reversed-phase column (4.6 × 250 mm, 5 µm; Agilent)
- Mobile phase: Acetonitrile and 0.1% orthophosphoric acid in water (80:20, % v/v)
- Flow rate: 0.8 mL/min
- Detection: 209 nm

Key Achievements:
- Successful separation of API and impurities
- Resolution > 2.0 between all peaks
- Theoretical plates > 4000
- Asymmetry factor within limits

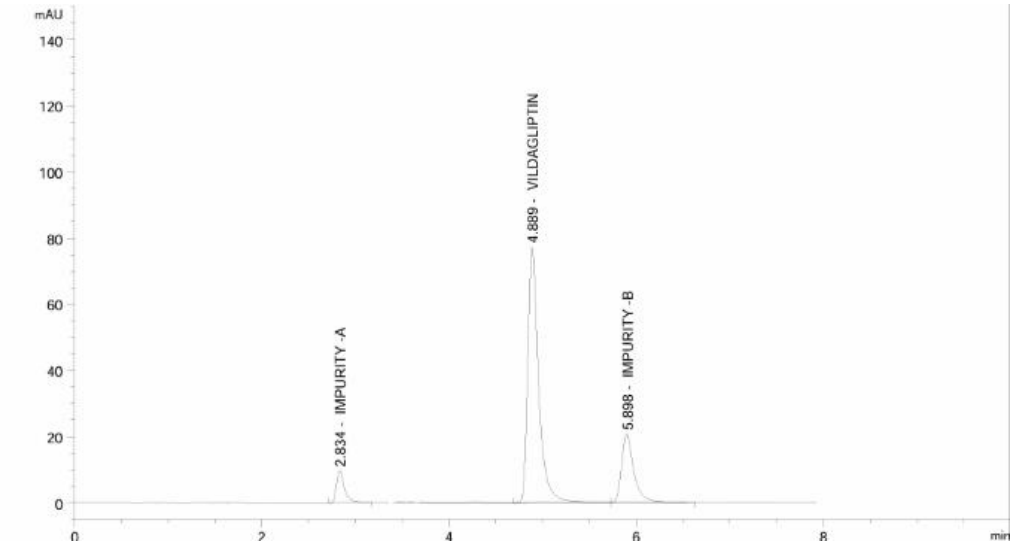


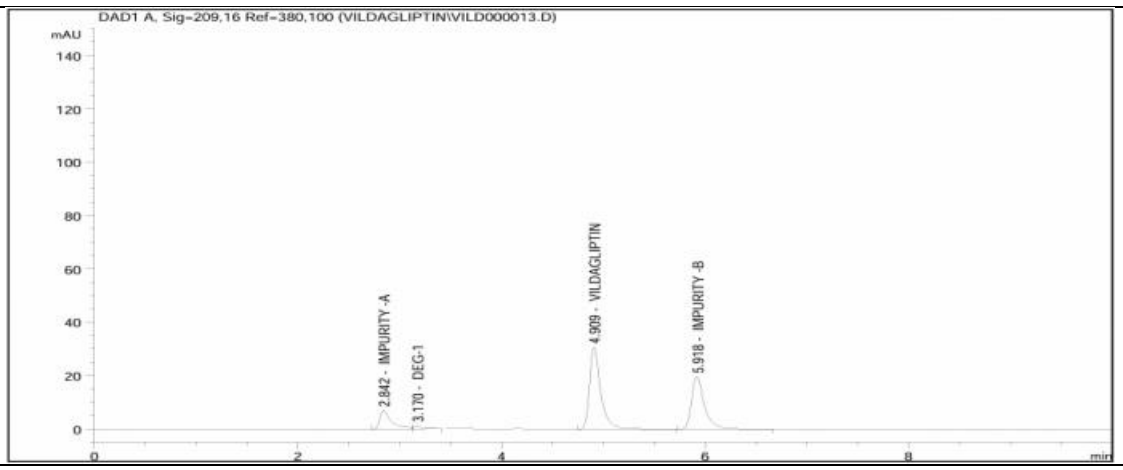
Figure 1: Chromatogram showing the separation of VILDA, IMP-1 and IMP-2

ISSN 2322 - 0899

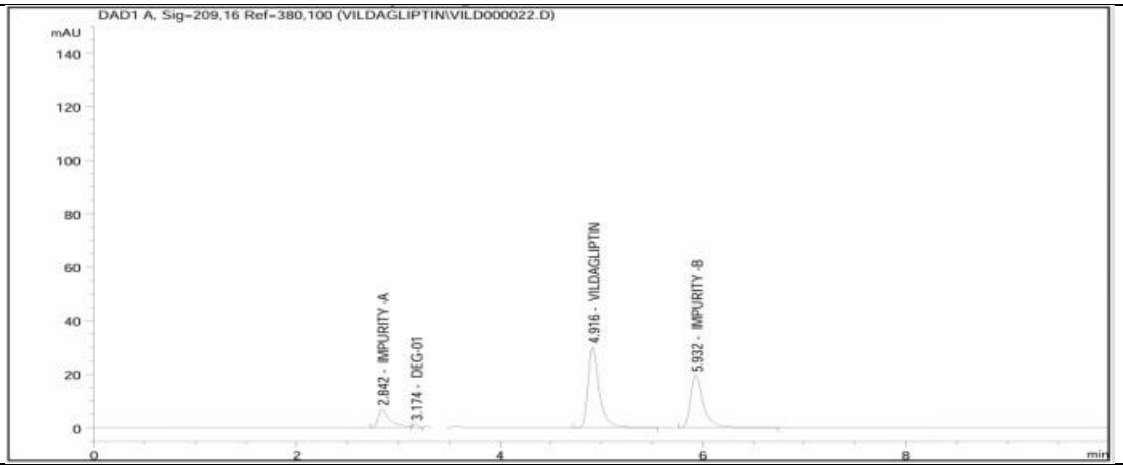
Validation Summary:

Parameter	Outcome
Linearity	$r^2 > 0.999$
Precision	% RSD < 2%
Accuracy	98–102% recovery
LOD/LOQ	Within ICH limits
Robustness	No significant variation
Forced Degradation	Stability-indicating confirmed

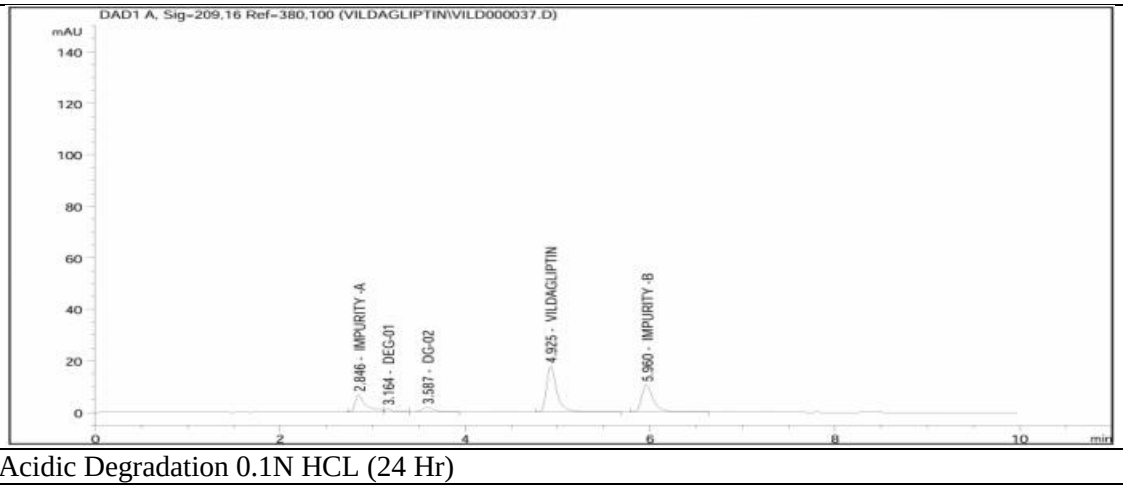
Major Finding:
Vildagliptin showed maximum degradation under alkaline stress conditions, confirming susceptibility to base hydrolysis.



Acidic Degradation 0.1N HCL (1 Hr)



Acidic Degradation 0.1N HCL (2 Hr)



Acidic Degradation 0.1N HCL (24 Hr)

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A precise and robust RP-HPLC method was developed for Sitagliptin in presence of two related impurities (IMP-1 and IMP-2).

Optimized Conditions:

- C18 column (4.6 × 250 mm, 5 µm)
- Mobile phase: Methanol : 0.1% OPA (80:20 % *v/v*)
- Flow rate: 1.0 mL/min
- Detection: 266 nm

Key Achievements:

- Successful separation of API and impurities
- Resolution > 2.0 between all peaks
- Theoretical plates > 5000
- Asymmetry factor within limits

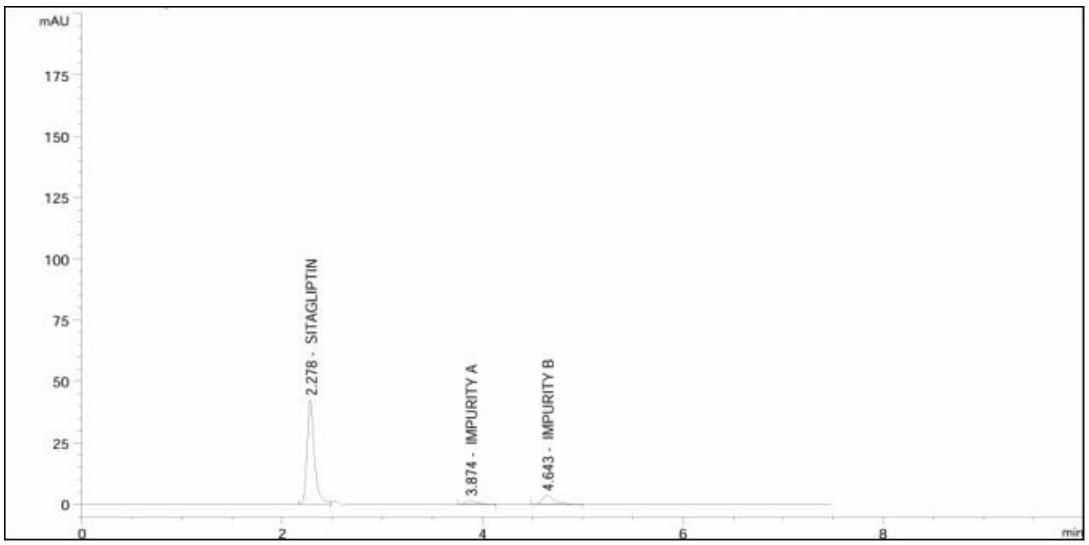

Figure 3: Chromatogram showing the separation of SITA, IMP-1 and IMP-2

System Suitability:

Compound	Resolution	Plates
SITA vs IMP-1	> 10	> 9000
SITA vs IMP-2	> 4	> 5000

Validation Summary:

Parameter	Result
Linearity	$r^2 = 0.9993$
Precision	%RSD < 2%
Accuracy	98–103%
Robustness	Acceptable
Ruggedness	Analyst-independent

Forced Degradation Findings:

- Highest degradation under alkaline condition (~51%)
- Oxidative degradation significant (~28%)
- Acid degradation moderate (~18%)
- Method proved stability-indicating

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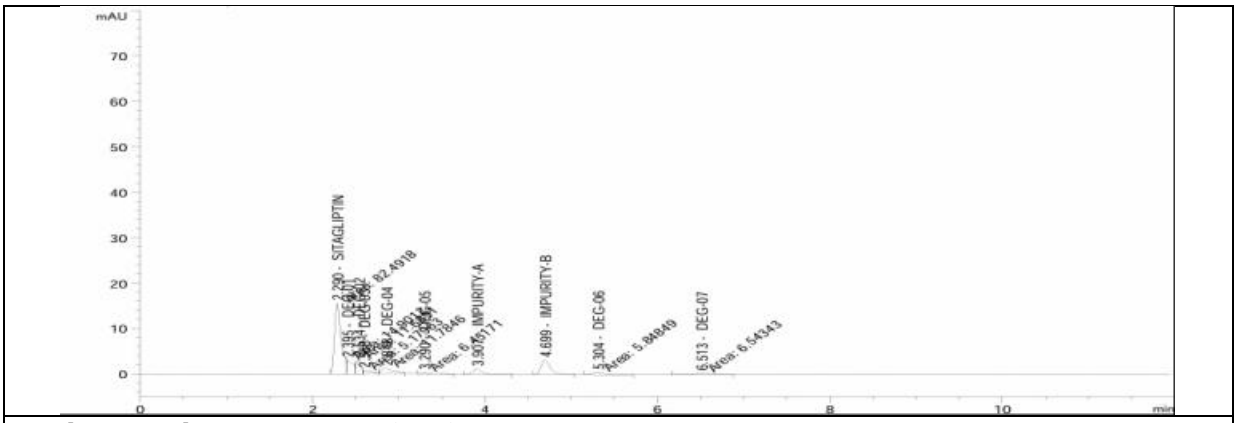
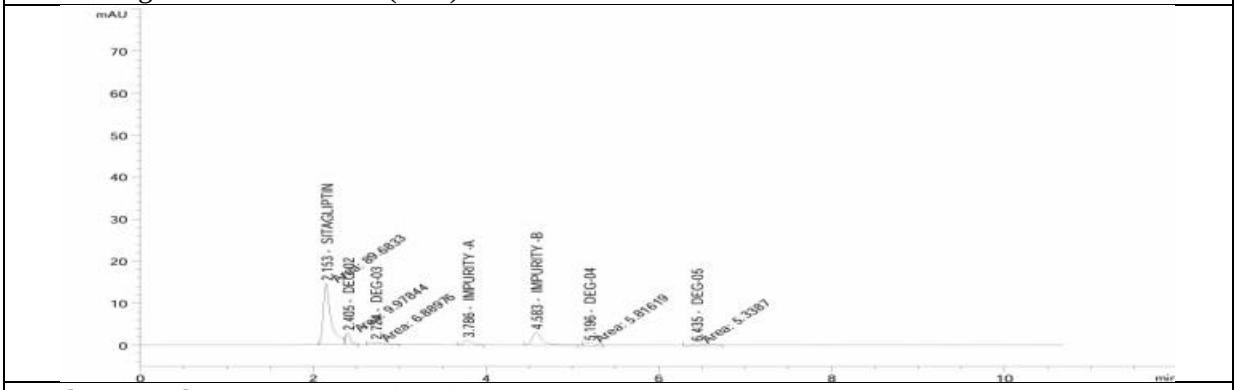
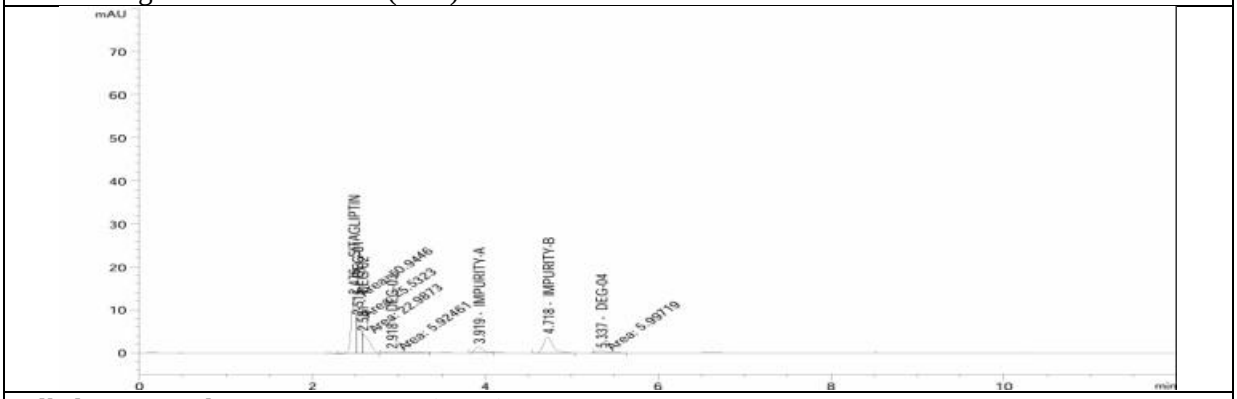


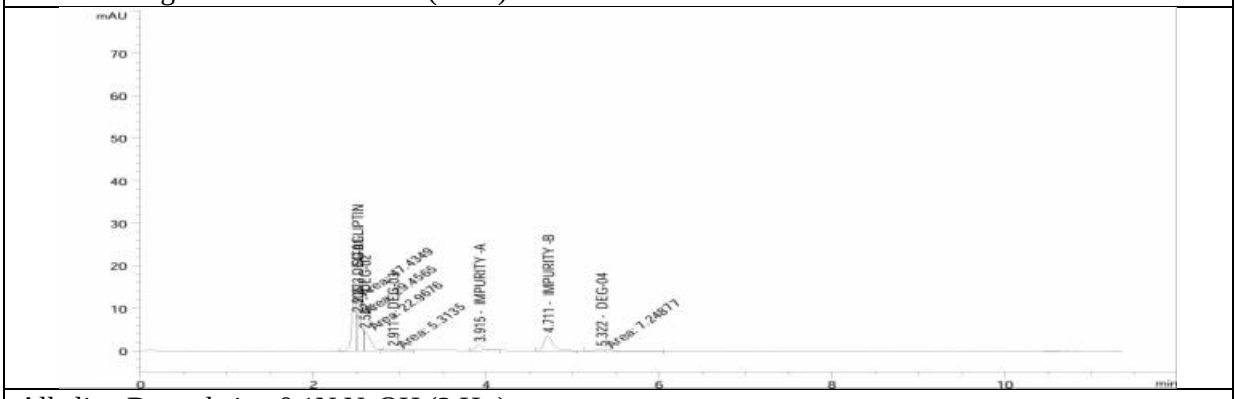
Acidic Degradation 0.1N HCL (1 Hr)



Acidic Degradation 0.1N HCL (2 Hr)



Alkaline Degradation 0.1N NaOH (1 Hr)



Alkaline Degradation 0.1N NaOH (2 Hr)

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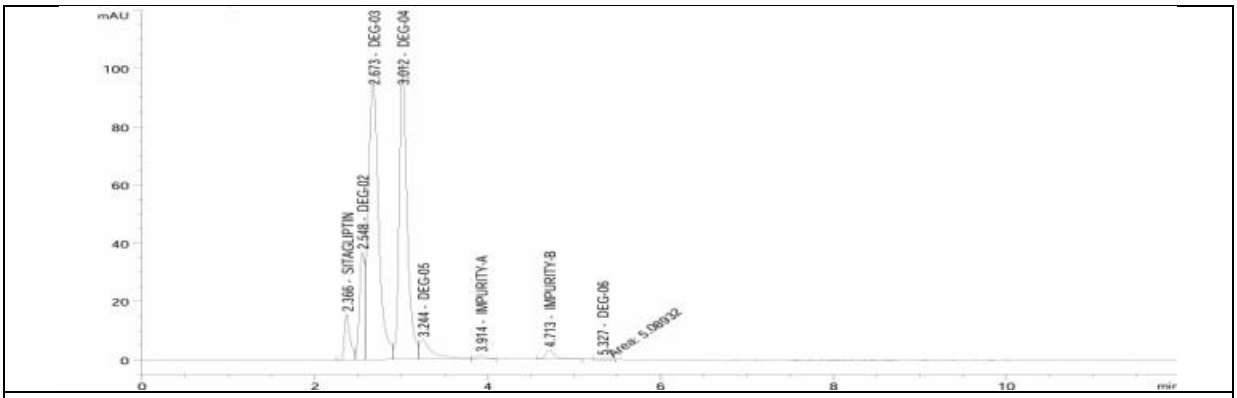
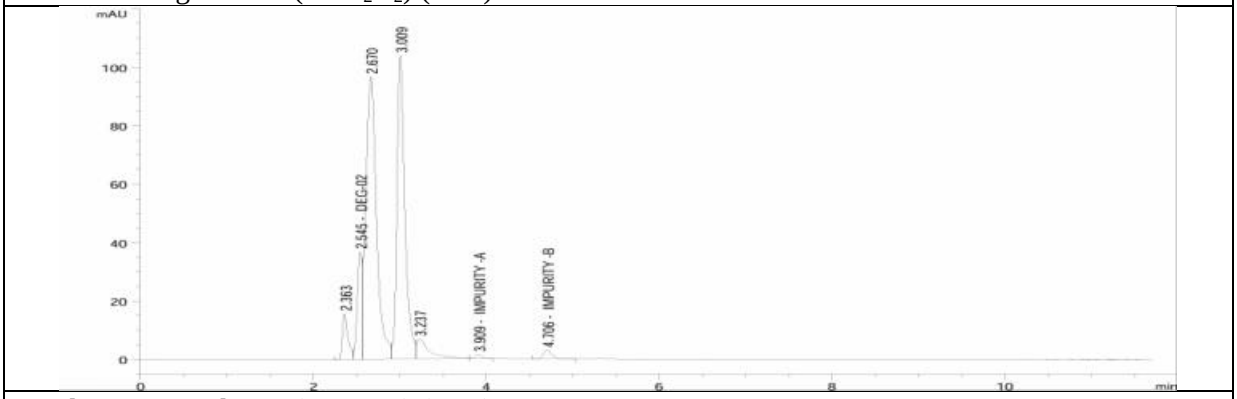
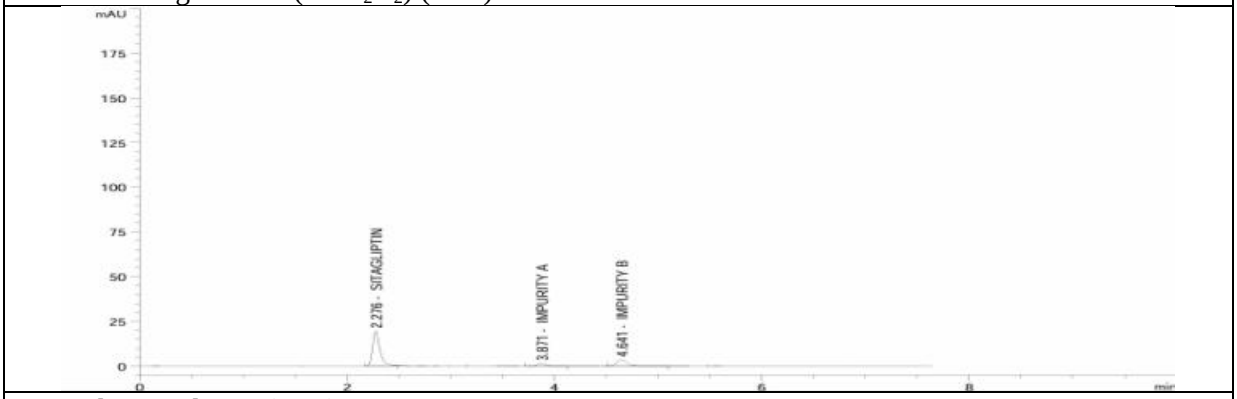


Oxidative Degradation (3%H_2O_2) (1 Hr)



Oxidative Degradation (3%H_2O_2) (2 Hr)



Neutral Degradation (1 Hr)

Figure 4: Forced degradation study of SITA in the sample matrix

A stability-indicating RP-HPLC method was developed for Dapagliflozin and its related impurities.

Optimized Chromatographic Conditions:

- C18 column
- Acetonitrile : Buffer system
- Flow rate: 1.0 mL/min
- UV detection at optimized wavelength: 225 nm

Key Achievements:

- Successful separation of API and impurities
- Resolution > 2.0 between all peaks
- Theoretical plates > 3000
- Asymmetry factor within limits

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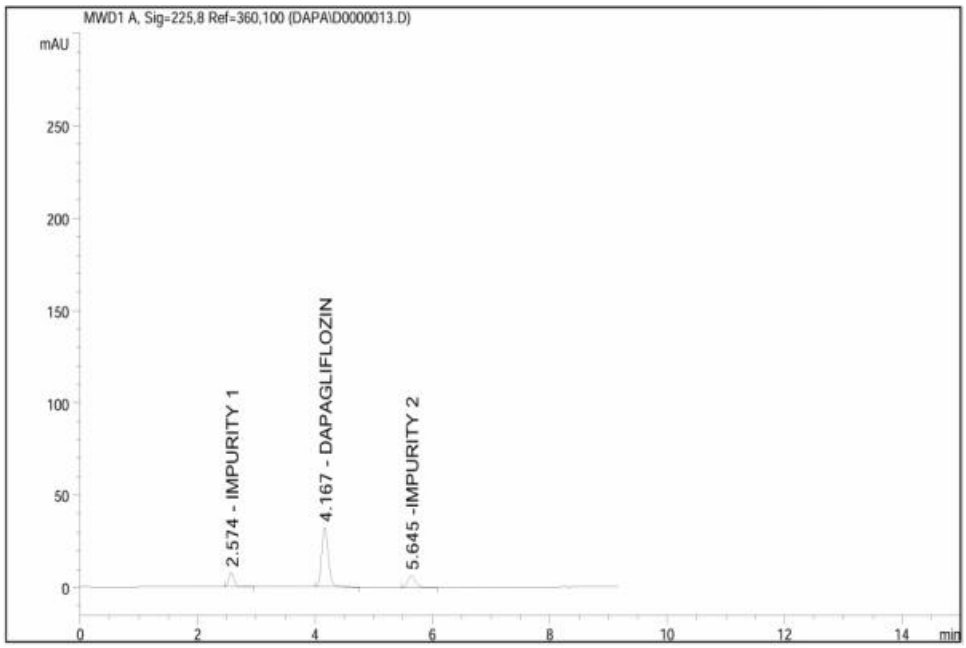


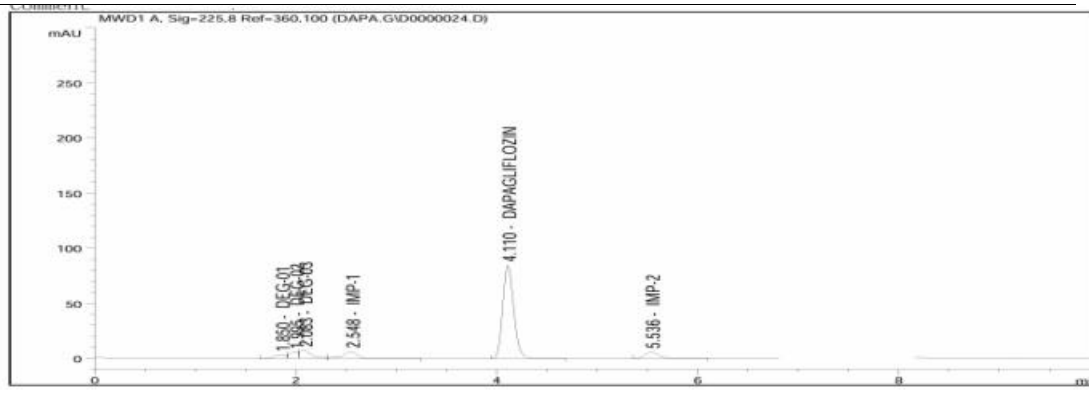
Figure 5: Chromatogram showing the separation of DAPA, IMP-1 and IMP-2

Validation Highlights:

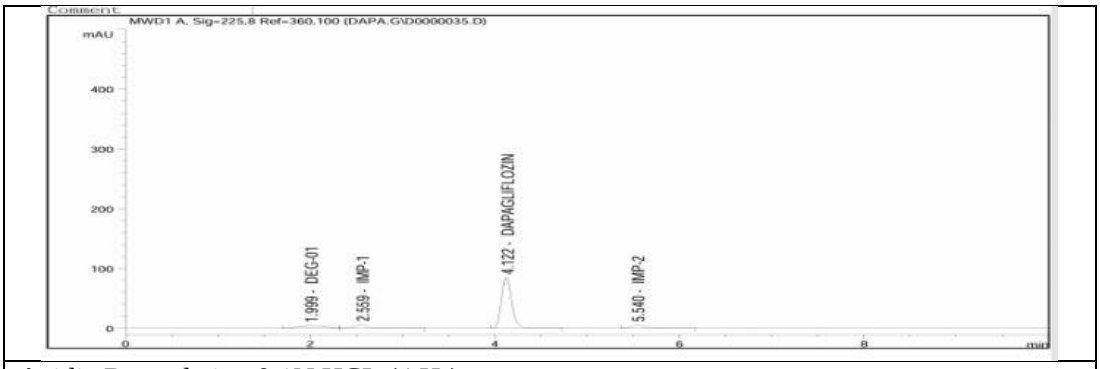
Parameter	Outcome
Linearity	$r^2 > 0.999$
Precision	%RSD < 2%
Accuracy	99–101%
Robustness	Within limits
Specificity	No interference

Forced Degradation Results:

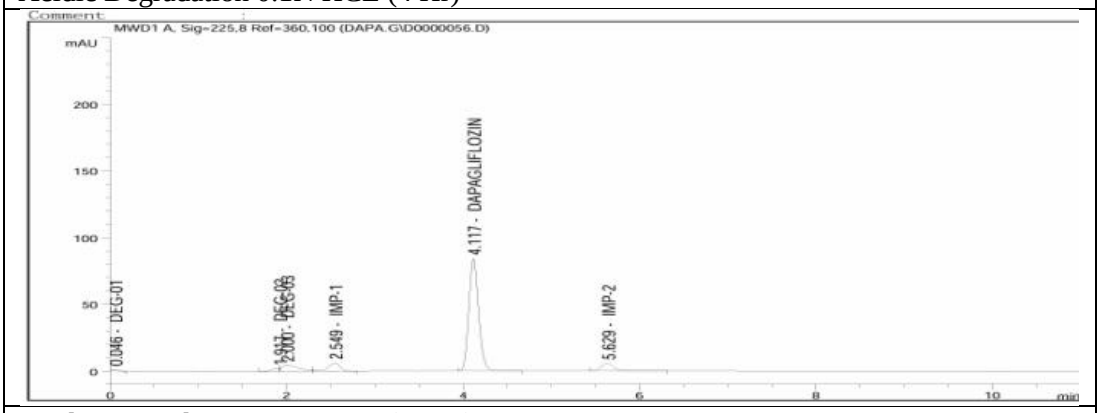
- Significant degradation under oxidative stress
- Moderate degradation under acidic and alkaline conditions
- Thermal and photolytic stress showed minimal degradation
- Clear separation of degradation products



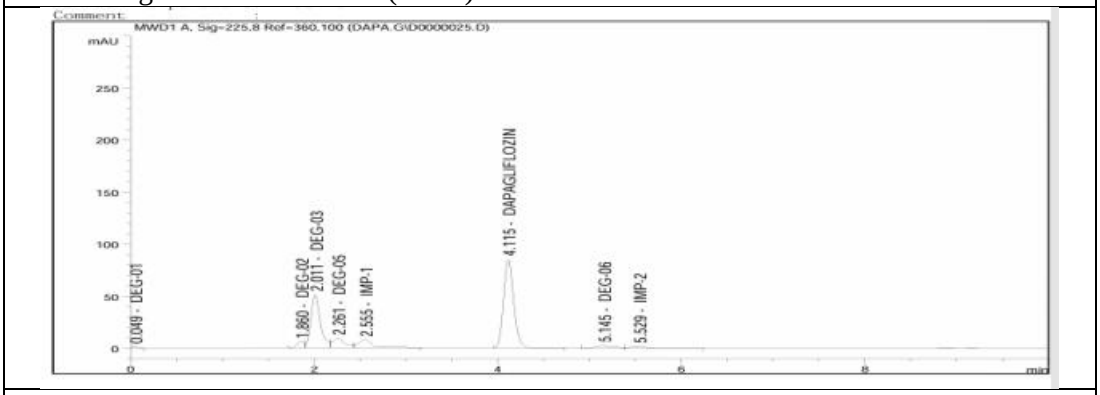
Acidic Degradation 0.1N HCL (2 Hr)



Acidic Degradation 0.1N HCL (4 Hr)



Acidic Degradation 0.1N HCL (24 Hr)



Alkaline Degradation 0.1N NaOH (2 Hr)

Comparative Evaluation of All three Drugs

Parameter	Vildagliptin	Sitagliptin	Dapagliflozin
Stability-Indicating	Yes	Yes	Yes
r^2	>0.900	0.9223	>0.990
Precision	<2%	<2%	<2%
Accuracy	98–100%	98–103%	99–101%
Alkaline Degradation	High	Very High	Moderate
Oxidative Degradation	Moderate	High	High

CONCLUSION

In the present study, a simple, rapid, precise, and accurate RP-HPLC method was successfully developed for the estimation of antidiabetic drugs in bulk and pharmaceutical dosage forms. The method showed good chromatographic separation with well-resolved peaks, acceptable retention times, and minimal tailing, indicating its suitability for routine analysis.

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