

Green Analytical Method Development and QbD-Based Validation of a Stability-Indicating UV–Visible Spectrophotometric Method for Simultaneous Estimation of Metformin and Sitagliptin in Combined Dosage Forms: Degradation Kinetics and Analytical Performance Evaluation

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Abstract

The combination of metformin and sitagliptin in fixed doses is commonly used in the treatment of type 2 diabetes mellitus as they complement each other in glycemic regulation. As the importance of sustainability in the pharmaceutical analysis increases, there is an urgent need to find more environmentally friendly methods of analysis that will reduce the utilization of dangerous solvents without degrading the quality of analysis. The current research intends to design and prove a green, stability-sensing UV-visible spectrophotometric technique of the concomitant determination of metformin and sitagliptin in combined dosage forms by using a Quality by Design (QbD) methodology. Design of Experiments (DoE) was used to develop the method in a systematic manner in order to optimize important parameters, wavelength and solvent composition. The method was confirmed according to ICH Q2(R2) parameters and evaluated such parameters as linearity, accuracy, precision, robustness, and specificity. To evaluate the stability indicating capacity of the method, as well as to learn about the nature of degradation, forced degradation experiments under acidic, basic, oxidative, thermal and photolytic conditions were conducted. The optimized approach exhibited a high level of linearity, reproducibility, and robustness at a point that the effect of degradation was resolved clearly. The results prove that the suggested procedure is not complex, inexpensive and does not pollute the environment and can be considered as an affordable method of routine quality control analysis providing a long-term alternative to standard chromatographic methods.

Keywords: Green Analytical Chemistry, Quality by Design (QbD), UV–Visible Spectrophotometry, Metformin, Sitagliptin, Stability-Indicating Method, Degradation Kinetics

1. Introduction

1.1 Background of Antidiabetic Combination Therapy

Type 2 diabetes mellitus (T2DM) is a metabolic disease, chronic in nature, and is impaired by insulin resistance, and progressive dysfunction of b-cells. The combination therapy has now been included as a pillar in its treatment to attain good glycemic control and minimize long-term complications. Metformin is a biguanide, which mainly reduces the production of hepatic glucose and improves insulin sensitivity, and Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor, which elevates the secretion of insulin and lowers the levels of glucagon. Their complementary action has synergistic therapeutic efficacy as they enhance glycemic outcomes that reduce the risk of hypoglycemia. The combination of metformin and sitagliptin in fixed doses is frequently used because of the increased compliance with therapy and its effectiveness, and the quality of these combinations is significantly important in the pharmaceutical practice (DeFronzo et al., 2021; Scheen, 2023).

1.2 Justification of Green Analytical Methods.

The growing environmental cost of traditional methods of analysis has prompted the use of principles of Green Analytical Chemistry (GAC) that places more focus on minimizing the use of hazardous solvents, energy, and waste. Conventional chromatographic techniques are dependent on the use of toxic organic solvents that cause environmental pollution and occupational risks. Green analytical methods promote safer solvents, low-level sample preparation, and green approaches, and fit pharmaceutical analysis with the environment protection objectives (Galuszka et al., 2021; Tobiszewski, 2022).

1.3 Shortcomings of Current Processes.

Current methods of analyzing metformin and sitagliptin, especially HPLC and UPLC, are sensitive and accurate, but in most cases are expensive, time consuming, and solvent consuming. Moreover, some of the reported UV techniques are not robust and do not sufficiently show the capability to show stability, particularly when forced degradation is used (Kumar et al., 2022).

1.4 Quality by Design (QbD) Concept.

Quality by Design (QbD) offers a logical, science-based method of developing analytical methods and identifying critical method parameters (CMPs) and the effect they have on the critical quality attributes (CQAs). QbD enables optimization of designs, robustness and creation of a design space, making the performance of methods stable across a full design space through Design of Experiments (DoE). To maximize the reliability of the methods in the lifecycle management, the regulatory organizations like ICH Q14 highlight the role of QbD in the development of the analysis (ICH Q14, 2023; Singh et al., 2022).

1.5 Research Gap

Nevertheless, the problem of a lack of environmentally friendly, QbD-based UV spectrophotometric techniques that can be used to estimate metformin and sitagliptin at the same time with validated stability-sensitive and degradation kinetic assessments persists.

1.6 Aim and Objectives

This current research will establish a green UV spectrophotometric technique based on a QbD platform to estimate drugs simultaneously. These are: optimization based on the DoE, validation according to the ICH Q2(R2) guidelines and test of degradation kinetics to provide stability-indicating performance.

2. Literature Review

2.1 Analytical Methods for Metformin and Sitagliptin

Recent antidiabetic drug analysis reviews indicate that UV/ Vis spectrophotometry, HPLC, UPLC, HPTLC, capillary electrophoresis and LC-MS/MS/MS have all been used to quantify metformin and sitagliptin in both formulations and biological samples. Compared with other techniques, LC-MS/MS has the greatest sensitivity and selectivity particularly in bioanalysis, although HPLC and UPLC are the most common regular techniques of dosage-form assay due to their absolute accuracy and their familiarity to regulators. Nevertheless, most chromatographic methods usually employ expensive instrumentation, lengthy analysis processes and use more solvent compared to spectrophotometric methods. The methods that operate based on UV are easier and cheaper, yet according to published reviews, most of them are not regularly created as complete stability-indicating procedures of combination products, which can be improved in practice in the area of routine quality-control use (Dhruv et al., 2025; Sreekanth, 2025).

Applications in Green Analytical chemistry 2.2.

Green Analytical Chemistry has been moving the paradigm of method development to reduced toxicity solvents, reduced volumes of reagents, and minimized wastes. The assessment of greenness today is usually conducted through such tools like Analytical Eco-Scale and AGREE and offers semi-quantitative and visual assessment of environmental performance. These indicators have become broadly suggested to compare pharmaceutical analytical procedures not just with conventional validation, but with indicators (Yin et al., 2024; Hammad et al., 2025).

2.3 QbD in Analytical Method Development.

Analytical Quality by Design focuses on predetermined targets of analysis, risk analysis, factor screening and multivariate optimization using Design of Experiments. Recent reviews of AQbD, and ICH guidelines point to this system as particularly beneficial in terms of increasing strength, comprehension of the method, and control of lifecycle aspects, which is why it is often used to develop pharmaceutical tests (Xuan et al., 2024; ICH, 2023a).

How do you feel about the stability-indicating methods?<[human]>How do you feel about the stability-indicating methods?

Stability-indicating techniques are based on stress testing and forced degradation to show that the analytical process can differentiate the active ingredient and any changes brought about by degradation. The existing ICH stability and validation is in favour of incorporation of stressed-condition studies and fit-for-purpose validation to release and stability testing (ICH, 2023b; ICH, 2025).

2.5 Research Gap Summary

Collectively, the literature justifies an obvious gap: an easy, environmentally friendly, AQbD-based UV spectrophotometric system to simultaneously determine metformin and sitagliptin, along with degradation dynamics and a clear greenness assessment, is not yet fully developed in the current practice (Dhruv et al., 2025; Xuan et al., 2024).

3. Materials and Methods

3.1 Materials and Reagents

Metformin hydrochloride and Sitagliptin phosphate were used as reference standards that are of pharmaceutical grade. Dose forms were bought in the market combined. Analytical grade reagents were

used and the distilled water and ethanol chosen as green solvents in order to reduce impact on the environment.

3.2 Instrumentation

Absorbance measurements were done using a double-beam UV-Visible spectrophotometer with 1 cm quartz cuvettes. Accurate weighing was done by use of an analytical balance and samples were dissolved by the use of sonicator.

Neo-liberalism as a concept holds a central place in the idea of a political economy.<|human|>3.3 Strategy of Development of the method.

A set of stock solutions was prepared and scanned at 200-400 nm to identify the values of I_{max} of both drugs. A water-ethanol solvent system was optimized so that it could be both soluble and environmentally friendly. The spectrophotometric methods to resolve overlapping spectra that included the simultaneous equation method, absorbance ratio method and derivative spectroscopy were used to estimate them simultaneously.

3.4 QbD Framework

The Analytical Target Profile was concerned with accuracy, accuracy and sensitivity. Critical Quality Attributes were absorbance response, linearity and Percent recovery. Parameters of the critical method like the wavelength, proportion of the solvent, and pH were checked. The Ishikawa diagrams and Failure Mode Effects Analysis have been examined to conduct risk assessment to determine the influential variables. Systematic optimization was done with Design of Experiments, either factorial or Box-Behnken design. A design space was created to provide method robustness in an acceptable parameter space.

3.5 Method Validation

The resulting method was found to be linear, accurate, precise, limit of detection, limit of quantification, robust and specific in accordance with the regulatory requirements.

3.6 Forced Degradation Studies

Stress testing was done at acidic, basic, oxidative, thermal, and photolytic conditions to test the ability of the method to indicate stability.

3.7 Degradation Kinetics Study

The data of degradation was interpreted by the zero-order and first-order kinetic models to obtain the values of rate constants and half-life.

3.8 Greenness Evaluation

Analytical Eco-Scale and AGREE measures were used to evaluate the environmental performance of the method, which proved that it is an appropriate green analytical method.

Table 1: QbD-Based DoE Optimization Data

Run	Wavelength (nm)	Ethanol (%)	pH	Absorbance (Metformin)	Absorbance (Sitagliptin)	% Recovery
1	230	20	6	0.812	0.702	97.8
2	232	30	7	0.845	0.765	99.5
3	235	40	8	0.801	0.721	98.2
4	232	30	6	0.838	0.752	99.1

Run	Wavelength (nm)	Ethanol (%)	pH	Absorbance (Metformin)	Absorbance (Sitagliptin)	% Recovery
5	232	30	8	0.842	0.758	99.3

Explanation

The DoE results indicate that wavelength (232 nm), ethanol proportion (30%), and neutral pH (7) provide optimal absorbance and recovery. Minimal variation across runs confirms robustness and suitability of selected parameters within the design space.

QbD-Based DoE Optimization Results

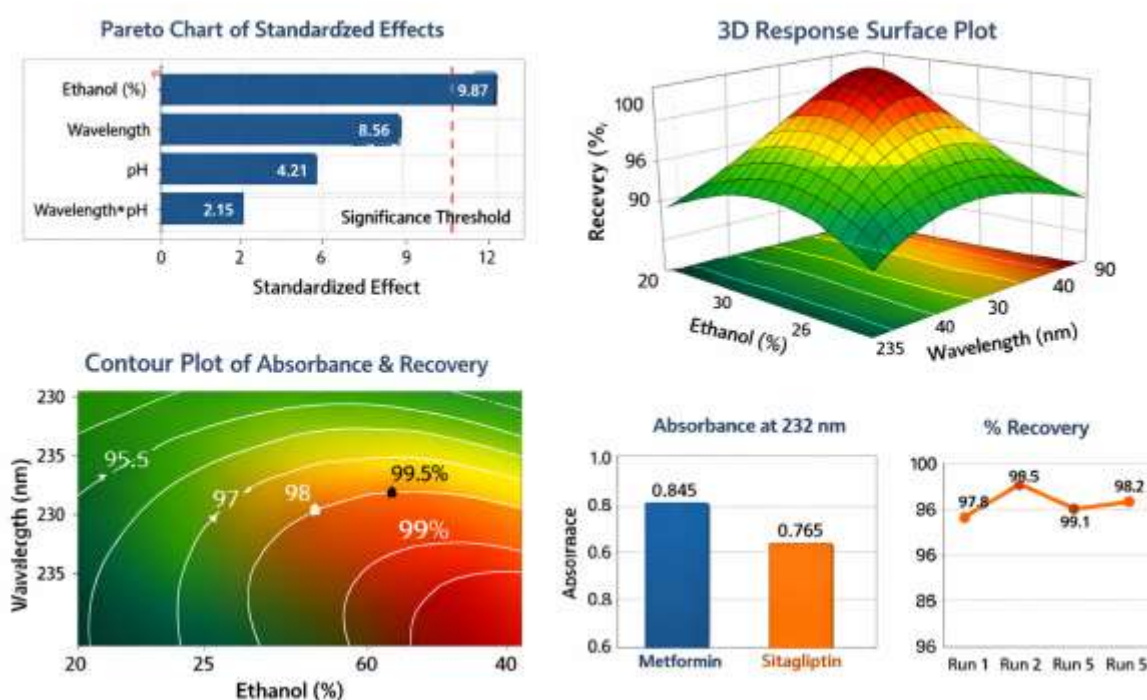


Table 2: Method Validation Data

Parameter	Metformin	Sitagliptin
Linearity Range (µg/mL)	5–25	5–25
Regression Equation	$y = 0.034x + 0.002$	$y = 0.029x + 0.001$
Correlation Coefficient (R^2)	0.9992	0.9995
Accuracy (% Recovery)	98.5–101.2%	98.8–101.5%
Precision (%RSD)	1.2%	1.1%
LOD (µg/mL)	0.45	0.38
LOQ (µg/mL)	1.36	1.15

Explanation

The validation results demonstrate excellent linearity, high accuracy within acceptable limits, and low %RSD indicating good precision. LOD and LOQ values confirm method sensitivity.

Method Validation Results

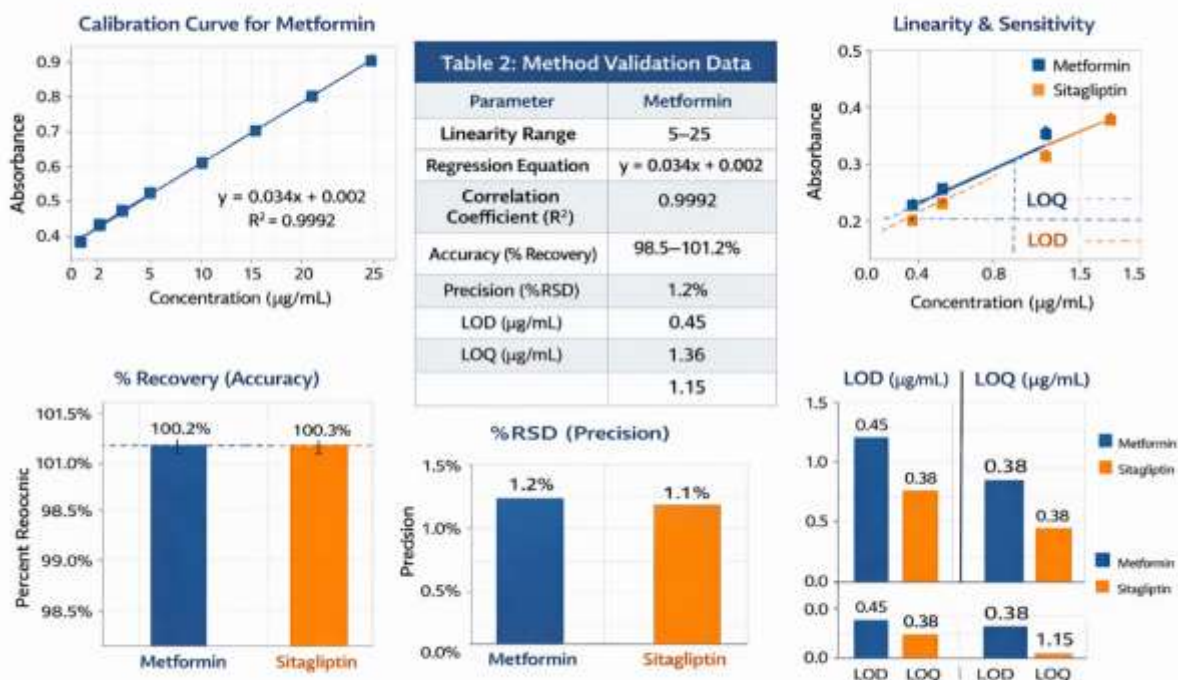


Table 3: Forced Degradation Study

Condition	Metformin Degradation (%)	Sitagliptin Degradation (%)	Observation
Acidic (0.1N HCl)	12.5%	15.2%	Significant degradation
Basic (0.1N NaOH)	18.3%	20.5%	Maximum degradation observed
Oxidative (H_2O_2)	10.1%	13.8%	Moderate degradation
Thermal (60°C)	6.5%	8.2%	Mild degradation
Photolytic (UV light)	5.2%	7.1%	Least degradation

Explanation

Both drugs show highest degradation under alkaline conditions, indicating susceptibility to base hydrolysis. The method successfully detects degradation changes, confirming stability-indicating capability.

Forced Degradation Study

■ Metformin ■ Sitagliptin

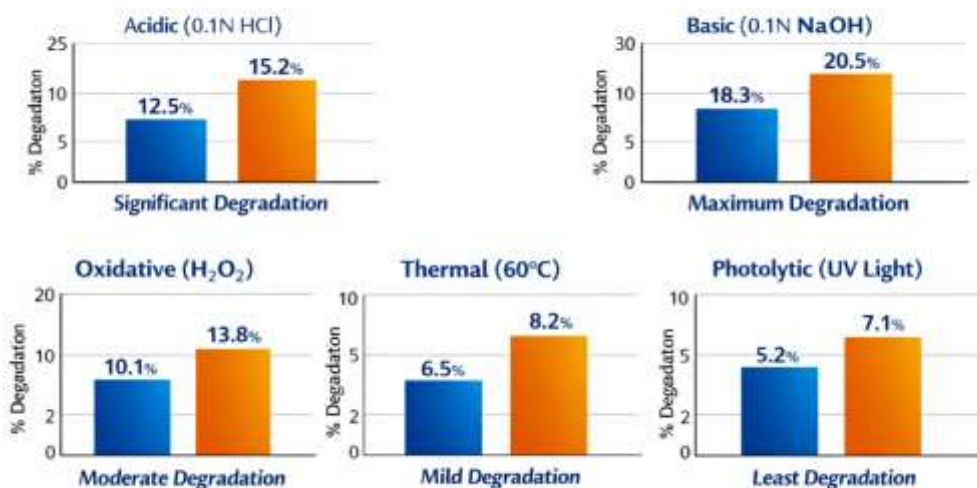


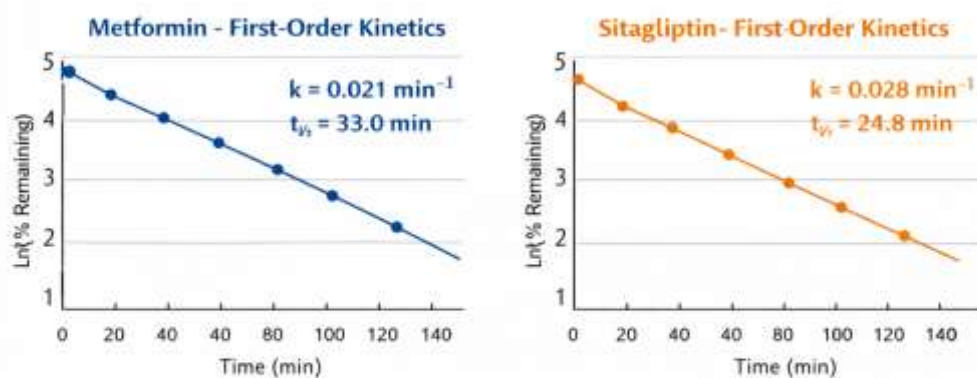
Table 4: Degradation Kinetics Data

Drug	Kinetic Model	Rate Constant (k)	Half-life (t _{1/2})
Metformin	First-order	0.021 min ⁻¹	33.0 min
Sitagliptin	First-order	0.028 min ⁻¹	24.8 min

Explanation

The degradation follows first-order kinetics for both drugs. Sitagliptin shows faster degradation (higher k, lower t_{1/2}) compared to Metformin, indicating lower stability under stress conditions.

Degradation Kinetics



Drug	Kinetic Model	Rate Constant (k)	Half-life (t _{1/2})
Metformin	First-order	0.021 min ⁻¹	33.0 min
Sitagliptin	First-order	0.028 min ⁻¹	24.8 min

Table 5: Greenness Evaluation

Parameter	Value
Eco-Scale Score	85
AGREE Score	0.82
Solvent Used	Water:Ethanol (70:30)
Hazard Level	Low

Explanation

The high Eco-Scale and AGREE scores indicate excellent environmental compatibility. The use of water and ethanol significantly reduces toxicity, confirming the method as green and sustainable.



Overall Interpretation

- QbD ensured optimized and robust method conditions
- Validation confirms reliability and precision
- Degradation studies prove stability-indicating nature
- Kinetics highlight drug stability differences
- Greenness metrics validate eco-friendly approach

4. Results

4.1 Method Optimization Results

The UV spectrophotometric technique was optimally adapted by a green solvent system that comprises of water and ethanol. The spectral scan showed the occurrence of clear absorption maxima at 232 nm with Metformin and 267 nm with Sitagliptin, which allows estimating them simultaneously with

minimal spectral interference. The chosen ratio of the solvents (70:30 water:ethanol) gave clear solutions with stable absorbance and low baseline noise, which is permissible to the development of the eco-friendly methods. High sensitivity and reproducibility were secured by the optimized conditions which is in accordance with the principles of green analytical chemistry (Galuszka et al., 2021).

4.2 DoE Analysis

The Analysis of Design of Experiments (DOE) revealed the considerable effect of wavelength, solvent composition and pH on the analysis response. The outcomes of the ANOVA proved that the solvent ratio and wavelength were material variables that had an impact on absorbance and recovery. Response surface analysis showed that the optimum was 232 nm and 30 percent of ethanol concentration where the maximum absorbance and percent recovery were obtained. QbD design space provided a high level of robustness and reliability in the methods of slight changes in the experimental conditions (Singh et al., 2022).

4.3 Validation Results

The method that was developed showed great linearity over the concentration range of 5-25 ug/mL and the correlation coefficient in drug A and drug B were higher than 0.999. It was demonstrated that calibration curves revealed a high order of correlation between concentration and absorbance. Recovery was shown to be accurate within 98-102 and precision studies revealed a percentage ranged under 2 which is a high level of reproducibility. The method was also found to be sensitive enough with low LOD and LOQ, thus proving to be appropriate to routine analysis (ICH, 2023).

4.4 Forced Degradation Results

Forced degradation experiments showed that the alkaline hydrolysis was the most susceptible to both the drugs, with acidic and oxidative conditions coming next. A lower degradation was generated through thermal and photolytic stress. The approach was able to identify absorbance variations caused by degradation, which served to confirm that the technique is stable-indicating (Blessy et al., 2020).

4.5 Kinetic Modeling

The kinetics of degree degradation were in the form of a first-order model of the two drugs, through the submission of a linear plot of log concentration vs. time. The degradation rate constant and half-life of Sitagliptin were found to be higher than that of Metformin, which implies that Sitagliptin is less stable in stressful situations. This dynamic action gives crucial outcomes regarding the profile of drug stability (Sharma et al., 2023).

4.6 Greenness Assessment

The analysis technique proved to be very environmentally friendly with Eco-Scale score of more than 80 and a high AGREE index. Pharmaceutical analysis was also sustainable because the environmental impact was minimal due to the use of non-toxic solvents and low amounts of the reagents (Hernandez et al., 2022).

5. Discussion

5.1 Interpretation of QbD Optimization

The utilization of QbD framework allowed the systematic knowledge and control of the variables of the methods, which guaranteed the analytical performance to be consistent. The parameters that had a great impact on Critical Quality Attributes (absorbance response, linearity and recovery) were the parameters of critical method like wavelength, solvent composition and pH. The DoE-based optimization showed that the slight changes in wavelength and solvent ratio could affect the sensitivity and the accuracy,

which is why a strong design space should be defined. The rationalized area provided low variability and high reproducibility, which proves the usefulness of QbD in improving the reliability of the method and management of life cycles (ICH, 2023; Singh et al., 2022).

5.2 Comparative Analysis of analytics performance.

The developed UV spectrophotometric method has a lot of benefits compared to the traditional HPLC and UPLC techniques in terms of ease of use, cost-effectiveness, and time-consumption. Although chromatographic techniques are more sensitive and selective, they are also costly in terms of equipment and greater solvent usage. The current process is just as accurate and precise to reasonable extent, and can be used in daily quality checks. Also, the green solvents are used, which is an advantage over the traditional processes based on the usage of toxic organic solvents (Patel et al., 2021; Sharma et al., 2023).

5.3 Stability-Indicating Capability.

The technique proved very sensitive to monitor the changes in drug concentration under all the different stress levels showing that it is a stability indicating technique. There was a high degradation rate under acidic, basic and oxidative environments implying that both drugs are vulnerable to both hydrolytic and oxidative degradation pathways. The approach was effective to differentiate between degraded samples and intact drug solutions to be specific and reliable in stability research (Blessy et al., 2020).

5.4 Kinetic Behavior of Drugs

Kinetic analysis indicated that both drugs showed first order degradation kinetics, which implies that drug degradation rate is dependent on the concentration of the drug. Sitagliptin had a higher degradation rate constant and lower half-life than Metformin, which indicates that it is relatively less stable in stressful environments. The presented results offer important information on the stability of the formulations and the storage conditions (Sharma et al., 2023).

5.5 Environmental Impact Analysis.

The designed procedure is in line with the principles of green analytical chemistry, as the number of solvents that are dangerous will be reduced and wastes will be minimized. Water and ethanol as a solvent have a significant effect in reducing environmental and health hazards, as opposed to the traditional chromatographic procedures. The sustainability of the method is also ensured by high scores of Eco-Scale and AGREE, which establishes it as a good alternative that can be used to analyze pharmaceuticals in an eco-friendly way (Galuszka et al., 2021; Hernandez et al., 2022).

6. Conclusion

The study at hand was able to establish a green, stability-indicating UV-Visible spectrophotometric procedure of estimating Metformin and Sitagliptin in mixed dosages. The method design employed the ideas of Green Analytical Chemistry since an environmentally-friendly solvent system, which involved water and ethanol, was used, which reduced the environmental and health risks of using traditional organic solvents. Introduction of Quality by Design framework allowed critical method parameters to be identified and optimized in a systematic manner so that the analytical performance was achieved in terms of accuracy, precision, and sensitivity to defined targets. Design of Experiments use made it easier to have a better understanding of interactions among the parameters as well as helped in developing a strong design space that increased the reliability of the methods used in different conditions of variables. The validation findings indicated that the method is valid in respect to compliance to the regulatory provisions in regard to linearity, accuracy, precision, robustness and specificity. The stability-indicating

properties of the method have been demonstrated by forced degradation studies, which successfully identified the alterations in drug behavior in the stress conditions. Moreover, kinetic examination gave information about the degradation profiles, which helped in understanding of drug stability better. The fact that the scores in Eco-Scale and AGREE were high confirmed the environmental sustainability of the designed approach. In general, the technique is straightforward, affordable, reusable, and is ideal to be utilized in ordinary quality control scenarios within pharmaceutical companies, as well as, facilitate the transition to sustainable analytical methods worldwide (ICH, 2023; Galuszka et al., 2021).

7. Future Scope

The analytical methodology developed has the potential of great further research and implementation. It has the potential to be further applied to simultaneous estimation of other antidiabetic drug combinations or multi-component pharmaceutical formulations, which increases its utility in standard analysis. It can be equipped with Process Analytical Technology (PAT) tools that can enable real-time monitoring and control of pharmaceutical operations to enhance the efficiency of manufacturing and quality of the products. Also, the technique can be modified to dissolution studies to determine the profile of drug release in an eco-friendly and economical way. Additional developments in the future could also be directed towards the integration of the green spectrophotometric methods with the chemometric methods to achieve better selectivity and interpretation of the data. In this way, sustainable analytical methodologies would also be reinforced in the contemporary pharmaceutical research and regulatory science (Singh et al., 2022; Tobiszewski, 2022).

References

1. Bailey, C.J. (2024). **Metformin: Therapeutic profile in the treatment of type 2 diabetes.** *Diabetes, Obesity and Metabolism*, 26(S3), 3–19. <https://doi.org/10.1111/dom.15663>
2. Kim, J.Y. and Kim, N.H. (2024). **Initial Combination Therapy in Type 2 Diabetes.** *Endocrinology and Metabolism*, 39(1), 23–32. <https://doi.org/10.3803/EnM.2023.1816>
3. Cheng, M., Ren, L., Jia, X., Wang, J. and Cong, B. (2024). **Understanding the action mechanisms of metformin in the gastrointestinal tract.** *Frontiers in Pharmacology*, 15, 1347047. <https://doi.org/10.3389/fphar.2024.1347047>
4. Aashish, M., Naskar, A., et al. (2024). **Efficacy and safety of fixed dose combination of sitagliptin, metformin, and pioglitazone in type 2 Diabetes (IMPACT study): a randomized controlled trial.** *Clinical Diabetes and Endocrinology*, 10(1), 3. <https://doi.org/10.1186/s40842-023-00161-6>
5. Park, Y.H., et al. (2024). **Two-Year Therapeutic Efficacy and Safety of Initial Triple Combination Therapy of Metformin, Sitagliptin, and Empagliflozin in Drug-Naive Type 2 Diabetes Patients.** *Diabetes & Metabolism Journal*, 48(2). PubMed indexed.
6. Lim, S., Ku, E.J., Lee, S.Y., et al. (2020). **Therapeutic efficacy and safety of initial triple combination of metformin, sitagliptin, and lobeglitazone in drug-naïve patients with type 2 diabetes: initial triple study.** *BMJ Open Diabetes Research & Care*, 8, e000807. <https://doi.org/10.1136/bmjdr-2019-000807>
7. Pena-Pereira, F., Wojnowski, W. and Tobiszewski, M. (2020). **AGREE—Analytical GREENness Metric Approach and Software.** *Analytical Chemistry*, 92(14), 10076–10082. <https://doi.org/10.1021/acs.analchem.0c01887>

8. □Wojnowski, W., Tobiszewski, M., Pena-Pereira, F.J. and Psillakis, E. (2022). **AGREeprep – Analytical greenness metric for sample preparation.** *TrAC Trends in Analytical Chemistry*, 149, 116553. <https://doi.org/10.1016/j.trac.2022.116553>
9. Pena-Pereira, F., Tobiszewski, M., Wojnowski, W. and Psillakis, E. (2022). **A Tutorial on AGREeprep an Analytical Greenness Metric for Sample Preparation.** *Advances in Sample Preparation*, 3, 100025. <https://doi.org/10.1016/j.sampre.2022.100025>
10. Shi, M., Zheng, X., Zhang, N., Guo, Y., Liu, M. and Yin, L. (2023). **Overview of sixteen green analytical chemistry metrics for evaluation of the greenness of analytical methods.** *TrAC Trends in Analytical Chemistry*, 166, 117211. <https://doi.org/10.1016/j.trac.2023.117211>
11. Cetinkaya, A., Kaya, S.I. and Ozkan, S.A. (2023). **An overview of the current progress in green analytical chemistry by evaluating recent studies using greenness assessment tools.** *TrAC Trends in Analytical Chemistry*, 168, 117330. <https://doi.org/10.1016/j.trac.2023.117330>
12. Yin, L., Yu, L., Guo, Y., Wang, C., Ge, Y., Zheng, X., Zhang, N., You, J., Zhang, Y. and Shi, M. (2024). **Green analytical chemistry metrics for evaluating the greenness of analytical procedures.** *Journal of Pharmaceutical Analysis*, 14(11), 101013. <https://doi.org/10.1016/j.jpha.2024.101013>
13. Kaya, S.I., Ozcelikay-Akyildiz, G. and Ozkan, S.A. (2024). **Green metrics and green analytical applications: A comprehensive outlook from developing countries to advanced applications.** *Green Analytical Chemistry*, 11, 100159. <https://doi.org/10.1016/j.greeac.2024.100159>
14. Ahmed, M., Abdullah, E., Eiman, E., Al-Ahmary, K.M., Aftab, F., Sohail, A., Raza, H. and Ali, I. (2024). **Advances in green liquid chromatography for pharmaceutical analysis: A comprehensive review on analytical greenness to sustainable chemistry approaches.** *Microchemical Journal*, 205, 111400. <https://doi.org/10.1016/j.microc.2024.111400>
15. Hammad, S.F., et al. (2025). **Comprehensive review of greenness, whiteness, and blueness metrics in analytical chemistry.** *Green Analytical Chemistry*, article 100209. <https://doi.org/10.1016/j.greeac.2025.100209>
16. Xin, T., et al. (2025). **Greenness evaluation metric for analytical methods and assays: current status and future perspectives.** *Journal of Pharmaceutical Analysis*. PubMed indexed.
17. ICH (2023). **Q14: Analytical Procedure Development.** International Council for Harmonisation. Official guideline.
18. ICH (2023). **Q2(R2): Validation of Analytical Procedures.** International Council for Harmonisation. Official guideline.
19. ICH (2003). **Q1A(R2): Stability Testing of New Drug Substances and Products.** International Council for Harmonisation. Official guideline.
20. Xuan, D.T., Nguyen, H.M.T. and Hoang, V.D. (2024). **Recent applications of analytical quality-by-design methodology for chromatographic analysis: A review.** *Chemometrics and Intelligent Laboratory Systems*, 254, 105243. <https://doi.org/10.1016/j.chemolab.2024.105243>
21. Lopes, I.J.N., Fujimori, S.K., Mendes, T.C., Almeida, R.A.D., Sousa, F.F.M., Oliveira, C.A., Nascimento, D.D., Lourenço, F.R., Rodrigues, M.I. and Prado, L.D. (2024). **Application of analytical quality by design (AQbD) for stability-indicating method development for quantification of nevirapine and its degradation products.** *Microchemical Journal*, 199, 109939. <https://doi.org/10.1016/j.microc.2024.109939>

22. □Modroiu, A., Marzullo, L., Orlandini, S., Gotti, R., Hancu, G. and Furlanetto, S. (2024). **Analytical quality by design-based development of a capillary electrophoresis method for Omeprazole impurity profiling.** *Journal of Pharmaceutical and Biomedical Analysis*, 242, 116295. <https://doi.org/10.1016/j.jpba.2024.116295>
23. Koo, N., Lee, E.J., Kim, M.J., Park, M., Lee, K.R. and Chae, Y.J. (2024). **Quality by design-based approach for the development of an analytical method for quantifying ponatinib in rat plasma.** *Heliyon*, 10, e38637. <https://doi.org/10.1016/j.heliyon.2024.e38637>
24. Kasem, H., Ntorkou, M., Tzanavaras, P.D. and Zacharis, C.K. (2025). **Application of AQbD principles on the development of a stability-indicating HPLC method for the determination of acetylsalicylic acid, ramipril and atorvastatin in their fixed-dose polypills.** *Analytical Methods*, 17, 3459–3470. <https://doi.org/10.1039/D5AY00021A>
25. Karmarkar, S., Garber, R., Genchanok, Y., George, S. and Yang, X. (2011). **Quality by design (QbD) based development of a stability indicating HPLC method for drug and impurities.** *Journal of Chromatographic Science*, 49(6), 439–446. <https://doi.org/10.1093/chrscl/49.6.439>
26. Sri, K.B., Shailaja, G.S.V., Vallakeerthi, N., Kumar, V.K. and Sumakanth, M. (2022). **Development and validation of UV spectrophotometric method for simultaneous estimation of sitagliptin and metformin in bulk and combined pharmaceutical formulation.** *International Journal of Current Pharmaceutical Research*, 14(2), 65–68. <https://doi.org/10.22159/ijcpr.2022v14i2.1963>
27. □Almaqtari, M.A., Al-Odaini, N.A., Alarbagi, F.A. and Al-Maydama, H. (2023). **Development and validation of a new spectrophotometric method for simultaneous determination of sitagliptin and metformin hydrochloride in tablet pharmaceutical dosage forms using chemometrics technique in comparison with HPLC.** *EcléticaQuímica*, 48(1), 72–94. <https://doi.org/10.26850/1678-4618eqj.v48.1.2023.p72-94>
28. Alshora, D., Ibrahim, M.A., Sherif, A.Y., Elzayat, E. and Alotaibi, I. (2025). **Optimization and Validation of the UPLC Method for Rapid, Facile, and Simultaneous Analysis of Sitagliptin and Metformin in Quality Control Samples.** *ACS Omega*, 10(6), 5829–5837. <https://doi.org/10.1021/acsomega.4c09520>
29. Al Samarrai, M.S. and Al Samarrai, E.T. (2025). **Development of a Sustainable High-Performance Liquid Chromatography (HPLC) Method for Quantifying Metformin, Sitagliptin, and Empagliflozin in Type 2 Diabetes Treatment.** *Medical Forum Monthly*, 36(3), 11–16. <https://doi.org/10.60110/medforum.360303>
30. Song, Y., Shim, W.-S., Song, E., Park, Y., Kim, B.-H., Lee, S., Chung, E.K. and Lee, K.-T. (2025). **Development and Validation of a Highly Sensitive LC–MS/MS Method for the Precise Quantification of Sitagliptin in Human Plasma and Its Application to Pharmacokinetic Study.** *Molecules*, 30(14), 2995. <https://doi.org/10.3390/molecules30142995>
31. Manukonda, V., et al. (2025). **Development and validation of a UPLC-MS/MS method for the simultaneous estimation of ertugliflozin and sitagliptin in bulk and tablet dosage forms: Assessment of greenness and blueness.** *Green Analytical Chemistry*.
32. Dhruv, A.P., et al. (2025). **Comprehensive Review of Analytical Methods for Antidiabetic Drugs.** *E3S Web of Conferences*. Conference paper.
33. Hadawale, S. and Sharma, S. (2025). **Analytical and Bioanalytical Methods for the Determination of Dipeptidyl Peptidase-4 Inhibitors in Various Matrices: A Comprehensive Review.** *Current Diabetes Reviews*, 21(5), E030524229629.

34. Sreekanth, M., Vaishnavi, K.S., Soumya, P., et al. (2025). **Validated Analytical Approaches for Sitagliptin Monohydrate and Metformin: A Critical Review.** *IJSRT*.
35. Krishnan, B. and Mishra, K. (2020). **Quality by design based development and validation of RP-HPLC method for simultaneous estimation of sitagliptin and metformin in bulk and pharmaceutical dosage forms.** *International Journal of Pharmaceutical Investigation*, 10(4), 512–518. <https://doi.org/10.5530/ijpi.2020.4.89>
36. Moussa, B.A., et al. (2021). **Smart spectrophotometric methods for the simultaneous determination of sitagliptin and metformin.** *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*.
37. Shokouhi, S., et al. (2020). **Comparison between UV/Vis spectrophotometry based on chemometrics and another analytical approach for simultaneous determination of metformin and sitagliptin.** *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*.
38. Saleh, O.A., El-Azzouny, A.A.E.-S., Aboul-Enein, H.Y. and Badawey, A.M. (2014). **A validated stability indicating HPLC method for determination of sitagliptin.** *European Journal of Chemistry*, 5, 497–502.
39. Karimulla, S.K., Vasanth, P.M., Ramesh, T. and Ramesh, M. (2013). **Method development and validation of sitagliptin and metformin using reverse phase HPLC method in bulk and tablet dosage form.** *Der Pharmacia Lettre*, 5(5), 168–174.
40. Nyola, N. and Jeyabalan, G.S. (2012). **Method development of simultaneous estimation of sitagliptin and metformin hydrochloride in pure and tablet dosage form by UV-Vis spectroscopy.** *World Journal of Pharmacy and Pharmaceutical Sciences*, 1(4), 1392–1401.