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Eco-Friendly First-Derivative UV-Spectrophotometric Method for Simultaneous Estimation of Dapagliflozin and Eplerenone in Bulk and Synthetic Mixture

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Abstract

Background: Dapagliflozin and Eplerenone are commonly used together for the management of diabetes-related cardiovascular and renal disorders, necessitating a reliable analytical approach for their combined analysis.

Objective: The present work describes the development and validation of an environmentally benign first-order derivative UV-spectrophotometric method for the simultaneous determination of Dapagliflozin and Eplerenone in a synthetic mixture, in accordance with ICH Q2(R2) guidelines.

Methods: First-order derivative spectra were employed to resolve spectral overlap between the two drugs. Dapagliflozin exhibited a zero-crossing point at 232 nm, while Eplerenone showed a zero-crossing at 254 nm, enabling selective quantification without mutual interference.

Results: The method demonstrated linearity over concentration ranges of 1–6 µg/mL for Dapagliflozin and 2.5–15 µg/mL for Eplerenone, with correlation coefficients approaching unity. Validation studies confirmed acceptable precision, accuracy, specificity, robustness, and sensitivity. No interference from excipients was observed, and the limits of detection and quantification were suitable for routine analysis.

Conclusion: Overall, the proposed method is simple, accurate, cost-effective, and eco-friendly, making it suitable for routine quality control of pharmaceutical preparations containing these drugs.

NOVELTY STATEMENT

A routine quality control first order eco spectrophotometric of UV derivative for reliable simultaneous measurement of Dapagliflozin and Eplerenone in synthetic mixtures and no complex chromatographic systems is presented within the work. The method also improves zero crossing analysis of spectral resolution and simultaneous measurement of components. Rapid analysis, low consumption of reagents, and simplicity of the method make the approach alternative for sustainable analysis in laboratory systems for quality control.

INTRODUCTION

Dapagliflozin (DAPA) is a selective sodium-glucose co-transporter-2 (SGLT2) inhibitor, widely prescribed in the management of type 2 diabetes mellitus and chronic heart failure. By inhibiting renal glucose reabsorption, it effectively lowers blood glucose levels and provides cardiovascular and renal protection. [1] Eplerenone (EPL) is a selective aldosterone antagonist indicated for hypertension and post-myocardial infarction heart failure. It blocks aldosterone binding at mineralocorticoid receptors, thereby reducing blood pressure and preventing cardiac remodeling.[2]

The therapeutic combination of DAPA and EPL is gaining clinical importance due to their synergistic action in cardio-renal disease management. Consequently, the availability of a reliable analytical method for their simultaneous estimation in combined formulations or synthetic mixtures is of great significance for routine quality control and pharmaceutical analysis.[2] Chemical structure of Dapagliflozin and Eplerenone is shown in Fig. 1, 2.

Several analytical techniques, such as HPLC, LC-MS, and HPTLC, have been reported for individual or combined estimation of related drug classes. However, these techniques are expensive, time-consuming, and require complex instrumentation. In contrast, UV-spectrophotometry offers a rapid, simple, and economical alternative. The challenge in simultaneous UV estimation arises from spectral overlap between the two drugs at their absorption maxima (232 nm for DAPA and 254 nm for EPL), making direct quantification difficult. Derivative spectrophotometry is a powerful approach to overcome such limitations. By converting normal spectra to their derivative forms, it enhances selectivity, minimizes background interference, and enables accurate quantification at zero-crossing points. Among the different orders, the first order derivative method is widely used due to its simplicity, reproducibility, and suitability for routine applications. The present study focuses on the Eco-friendly UV-spectrophotometric method Development and validation for the simultaneous estimation of Dapagliflozin and Eplerenone in a synthetic mixture.

Validation Studies [10]

The method was validated as per ICH Q2(R2) guidelines, evaluating parameters such as linearity, accuracy, precision, sensitivity, and robustness. This proposed method offers a cost-effective and reliable tool for quality control analysis of pharmaceutical formulations containing these drugs.

Green Analytical Method [11, 12]

Green first-derivative UV analytical method development focuses on creating an environmentally sustainable, highly selective, and reagent-free spectroscopic approach. By eliminating toxic solvents, reducing sample volume, and relying solely on UV absorbance behavior, this technique minimizes chemical waste and energy consumption. The use of first-derivative processing enhances resolution between overlapping spectra, allowing accurate quantification without complex extractions or chromatographic systems. This eco-friendly strategy supports rapid, cost-effective, and precise analysis while aligning with the core principles of Green Analytical Chemistry and sustainable laboratory practices.12 principle of Green Analytical method was shown in Fig. 3.

MATERIALS AND METHODS

Materials

Apparatus and Instruments

Double beam UV-1780 Shimadzu, Japan

Analytical Balance: Shimadzu, Japan Model AP225WD

Ultrasonicator: FS 4 Frantline, India

Volumetric Flasks: 10ml, 50ml, 100ml

Pipettes: 1ml, 2ml, 5ml

Beaker: 50ml, 100ml, 250ml, 500ml

Reagent and Chemicals

Dapagliflozin

Alembic Research Center.

Eplerenone

Triveni Chemicals pvt, Ltd.

UV Spectroscopic Conditions

Solvent: Methanol

Mode: Spectrum

Scanning range: 200-400nm

Baseline correction: Methanol

Diluent: Methanol

Standard Solution Preparation

Dapagliflozin Standard stock solution (100 µg/ml) Preparation

After being carefully weighed, 10mg of Dapagliflozin had been transferred to 100ml volumetric flask. Methanol was incorporated into the flasks, the volume was suitably adjusted, and the flask were subjected to a 15-minute sonication. After the sonication make up the volume with methanol.

Eplerenone Standard stock solution (100 µg/ml) Preparation

Eplerenone 10mg had been carefully weighed as well as then transferred to various 100ml volumetric flask. Following the addition of methanol and appropriate volume adjustment, the flask were sonicated for 15 minutes. After the sonication make up the volume with methanol.

Analytical wavelength Selection for Dapagliflozin and Eplerenone

From the Standard stock solution of Dapagliflozin and Eplerenone: 10ml from the drug solution are transferred into 100ml volumetric flasks separately, then completed to volume with methanol. From the solution obtained above prepare the working standards of Dapagliflozin (1–6 µg/ml) and Eplerenone (2.5–15 µg/ml) in 10 ml volumetric flask using methanol as solvent. They were scanned from 200 to 400 nm and D_0 spectra is collected using UV spectrophotometer. All D_0 spectra of Dapagliflozin and Eplerenone were converted to the D_1 spectrum using UV probe 2.70 software. Comparison of D_1 spectra of Dapagliflozin and Eplerenone A). D_0 and D_1 spectra were overlaid to confirm the position.

D_0 spectrum

- This is the zero-order spectrum (normal UV spectrum).
- It's just the absorbance vs wavelength (A–λ plot).
- Peaks are broad, as per Beer–Lambert law.

D_1 spectrum

- This is the first derivative spectrum.
- It represents the slope of the D_0 spectrum (dA/dλ vs λ).
- Instead of normal peaks, you see positive and negative bands crossing the baseline.
- The zero-crossing point of one analyte can be used to selectively measure another analyte in mixtures → this is why first-order derivative UV is useful for simultaneous estimation.

In summary:

- D_0 = zero-order = normal absorbance spectrum.
- D_1 = first-order derivative = slope spectrum (dA/dλ vs λ), useful for resolving overlapping peaks.

Dapagliflozin and Eplerenone synthetic Mixture Preparation:

To create the final concentration of Dapagliflozin (1µg/ml) along with Eplerenone (2.5µg/ml), aliquots of 1ml from Standard solution of Dapagliflozin (10µg/ml), 2.5ml from the Standard solution of eplerenone

(10µg/ml), had been placed into a common volumetric flask, and make up the volume with methanol up to 10ml.

Validation of the First order derivative UV Spectroscopic Method

Specificity

Dapagliflozin and Eplerenone both alone and in combination. No interference from excipients or other formulation components was observed at the selected analytical wavelengths, indicating that the method is specific for the drugs under study.

Linearity and Range

Calibration studies were conducted by the preparation of various concentrations of solutions. The response of Dapagliflozin was linear in the concentration range of 1–6 µg/mL, whereas Eplerenone was linear over a concentration range of 2.5–15 µg/mL. The calibration curves were found to be well correlated with the correlation coefficients of nearly one, and %RSD values less than 2% indicated that the method is linear in these ranges.

Accuracy

Accuracy

The reliability of the method was determined by performing recovery experiments in three different level: 80, 100 and 120% of target concentration. Synthetic mixtures were compound by adding known amounts of the standards. The percentage recoveries were found between 98 and 102%, thus demonstrating that the method is accuracy and matrix effect free.

Precision

Intraday precision: Three concentrations of Dapagliflozin (2, 4, and 6 µg/mL) and Eplerenone (5, 10, and 15 µg/mL) were injected in triplet for the same day.

Interday precision: Three concentrations were measured for 3 days in a row.

In both cases, the %RSD values were lower than 2%; hence this modality delivers replicable results.

Detection and Quantitation Limits [10]

The sensitivity of the method relates to the limit of detection (LOD) and limit of quantitation (LOQ) that were derived from three calibration curves created to establish method linearity. These values were determined based on the standard deviations of the intercepts of the calibrations and the slopes of the calibration curves by applying the following equations:

LOD = 3.3 × (SD / Slope)

LOQ = 10 × (SD / Slope)

The values obtained show that the method is sensitive enough to detect and quantify low amounts of both drugs.

Robustness

To determine robustness, slight changes were made to the experimental conditions, such as minor changes to the detecting wavelength (± 2 nm). As these changes did not lead to significant changes in the attained results, this demonstrated that the method is robust and reliable.

Assay

The validated method was successfully used to estimate both Dapagliflozin and Eplerenone in mixture. The results of the assay were satisfactory, confirming that the method is applicable for routine quality control analysis.

RESULTS

Figure 4 indicate Selecting an appropriate wavelength in Eco-friendly first-order derivative UV spectrophotometry, is essential for achieving spectral clarity while maintaining sustainability by avoiding chemical reagents. The derivative spectra of Dapagliflozin and Eplerenone provided distinct zero-crossing points, enabling selective measurement of each drug without mutual spectral overlap which is shown in Fig. 5 and Fig. 6 and synthetic mixture first order derivative spectra shown in Fig. 7 The corresponding calibration plots demonstrated excellent linearity between derivative amplitude and concentration, confirming adherence to the Beer–Lambert law within the optimized green analytical range. Specificity experiments showed that common excipients and matrix components did not interfere at the chosen zero-crossing wavelengths, reflecting the selectivity of this solvent-free approach.

The correlation coefficients in Tables 1 in the reports confirmed that **linearity** was achieved over from 1 to 6 µg/ml for Dapagliflozin and 2.5 to 15 µg/ml for Eplerenone. And their calibration curve is shown in Figs. 8 and 9 respectively

Table 1
Calibration Response of Dapagliflozin & Eplerenone

Conc. (µg/ml)	Dapagliflozin Mean Absorbance ± SD	%RSD	Conc. (µg/ml)	Eplerenone Mean Absorbance ± SD	%RSD
1	0.0050 ± 0.0000158	0.316	2.5	0.0130 ± 0.0000380	0.292
2	0.0076 ± 0.0000381	0.501	5	0.0249 ± 0.0000450	0.181
3	0.0112 ± 0.0000277	0.247	7.5	0.0378 ± 0.0000320	0.084
4	0.0149 ± 0.0000500	0.335	10	0.0480 ± 0.0000400	0.083
5	0.0181 ± 0.0000400	0.221	12.5	0.0610 ± 0.0000180	0.029
6	0.0220 ± 0.0000339	0.154	15	0.0727 ± 0.0000550	0.076
Linearity range (µg/ml)	1–6		Linearity range (µg/ml)	2.5–15	
Regression equation	Y = 0.0034x − 0.0011		Regression equation	Y = 0.0048x − 0.0012	
Correlation coefficient (R^2)	0.9975		Correlation coefficient (R^2)	0.9994	
Slope	0.0034		Slope	0.0048	
Intercept	−0.0011			−0.0012	

Calibration curves were constructed by plotting first-derivative absorbance against concentration over the ranges of 1–6 µg/ml for dapagliflozin and 2.5–15 µg/ml for eplerenone. Results are expressed as mean ± SD (n = 3), with corresponding %RSD values indicating precision. The regression equations and high correlation coefficients confirm excellent linearity and proportional analytical response for both analytes.

Accuracy, evaluated by standard-addition techniques, yielded recoveries of 98.50–99.10% for Dapagliflozin and 99.10–99.70% for Eplerenone showed in Table 2, indicating strong trueness of the green method.

Table 2
Recovery assessment for Dapagliflozin & Eplerenone

Drug	Recovery level (%)	Target conc. (µg/ml)	Added (µg/ml)	Final conc. (µg/ml)	Mean %Recovery ± SD	%RSD
Dapagliflozin	80	1	0.8	1.8	98.88 ± 0.554	0.56
	100	1	1.0	2.0	98.50 ± 1.00	1.02
	120	1	1.2	2.2	98.93 ± 0.693	0.70
Eplerenone	80	2.5	2.0	4.5	99.25 ± 0.342	0.34
	100	2.5	2.5	5.0	99.33 ± 0.305	0.31
	120	2.5	3.0	5.5	99.33 ± 0.695	0.70

Accuracy of the proposed method was evaluated at 80%, 100%, and 120% levels by standard addition. Results are expressed as mean % recovery ± SD (n = 3) with %RSD. Recovery values close to 100% with low variability confirm the accuracy and reliability of the method for both analytes.

Table 3
Assessment of method precision within the same day & different day

Drug	Precision type	Conc. (µg/ml)	Mean response	SD	%RSD
Dapagliflozin	Intra-day	1	0.0069	0.0000079	0.114
		2	0.0082	0.0000367	0.447
		3	0.0114	0.0000158	0.139
	Inter-day	1	0.0065	0.0000790	0.112
		2	0.0079	0.0000177	0.224
		3	0.0118	0.0000367	0.311
Eplerenone	Intra-day	2.5	0.0146	0.0000367	0.251
		5	0.0258	0.0000750	0.291
		7.5	0.0445	0.000224	0.503
	Inter-day	2.5	0.0158	0.0000316	0.200
		5	0.0235	0.0000381	0.162
		7.5	0.0437	0.0000791	0.181

Precision of the proposed method was evaluated at multiple concentration levels under intra-day (repeatability) and inter-day (intermediate precision) conditions. Results are expressed as mean absorbance ± SD (n = 3) with corresponding %RSD values. The low %RSD observed across all concentration levels indicates excellent precision and reproducibility of the method for both analytes.

Table 4
Wavelength variation effect on Dapagliflozin & Eplerenone

Drug	Sr. No.	Nominal wavelength response	+ 2 nm response	−2 nm response
Dapagliflozin (232 nm)	1	0.00667	0.00663	0.00669
	2	0.00668	0.00665	0.00670
	3	0.00667	0.00664	0.00668
	Mean	0.006673	0.00664	0.00669
	SD	0.00001	0.00001	0.00001
	%RSD	0.1498	0.1506	0.1495
Eplerenone (254 nm)	1	0.0138	0.0135	0.0139
	2	0.0135	0.0133	0.0137
	3	0.0137	0.0136	0.0140
	Mean	0.0137	0.0135	0.0139
	SD	0.000158	0.000158	0.000158
	%RSD	1.15	1.17	1.14

Robustness of the proposed method was evaluated by measuring derivative response at the selected wavelengths (232 nm for dapagliflozin and 254 nm for eplerenone) and at ± 2 nm variations. Results are expressed as mean ± SD (n = 3) with %RSD values. Minimal variation in analytical response indicates that the method is robust and unaffected by small changes in wavelength.

Table 5
Detection and Quantitation limits of the proposed method

Name of Drug	Limit of Detection(µg/ml)	Limit of quantitation(µg/ml)
Dapagliflozin	0.0153	0.0465
Eplerenone	0.0380	0.0792

Limits of detection (LOD) and quantitation (LOQ) for dapagliflozin and eplerenone were determined based on the standard deviation of the response and the slope of the calibration curve, in accordance with ICH guidelines. The low LOD and LOQ values indicate high sensitivity of the proposed method for the quantification of both analytes.

Table 6
Assay results for Synthetic Mixture analysis

Theoretical Amount (mg)		Measured Amount (mg)		Assay value (%)	
Dapagliflozin	**Eplerenone**	**Dapagliflozin**	**Eplerenone**	**Dapagliflozin**	**Eplerenone**
1	2.5	0.99	2.49	98.67 ± 0.0057	99.06 ± 0.611
1	2.5	0.98	2.46		
1	2.5	0.99	2.48		

Assay of dapagliflozin and eplerenone in synthetic mixture was performed to evaluate the accuracy of the proposed method. Results are expressed as mean assay values ± SD (n = 3). The assay values close to 100% indicate the suitability of the method for accurate quantification of both analytes in combined formulations.

Overlay UV spectra showing the selection of analytical wavelength based on optimal absorbance and suitability for simultaneous estimation.

Precision studies, including both intraday and interday precision Tabulated in Table no 3, produced low %RSD values, confirming the method's reproducibility without requiring hazardous chemicals.

Minor, intentional variations in analytical conditions (± 2 nm wavelength changes) had minimal impact on results, demonstrating **robustness** and operational stability showed in Table no 4. The sensitivity parameters **detection limit (DL) and quantitation limit (QL)** showed in Table no 5 which indicate that the method can efficiently detect and quantify low analyte levels while maintaining environmental friendliness.

Table no 6 showed **Assay** results of the synthetic mixture further validated the approach, providing purity values of 98.67 ± 0.0057% for Dapagliflozin and 99.06 ± 0.611% for Eplerenone. Overall, the findings confirm that the developed first-order derivative UV method is precise, accurate, selective, and inherently green, supporting its suitability for routine eco-friendly analytical applications. Figure 10 shows the overall green analytical assessment of the UV spectrophotometric method evaluated using the AGREE software.”

DISCUSSION

The Successful development and validation of a green first-order derivative UV spectroscopic method are crucial for ensuring accurate, sustainable, and reliable quantification of drug combinations with overlapping spectral features. This technique inherently supports green analytical principles by eliminating the need for hazardous solvents and reducing overall resource consumption. The use of zero-crossing points significantly enhanced selectivity, allowing clear distinction between Dapagliflozin and Eplerenone without chemical separation steps. Carefully selected wavelengths based on derivative maxima and zero-crossing behavior provided optimal sensitivity for both analytes, contributing to strong analytical performance. The calibration data exhibited excellent linearity across the validated

concentration ranges, fulfilling the expectations outlined in ICH guidelines. The high correlation coefficients confirm that the derivative approach maintains a proportional response, a key requirement for dependable quantitative analysis. Accuracy was further validated through recovery studies, which yielded values close to 100%, demonstrating that the method can accurately determine each analyte even in the presence of formulation excipients. These findings align with literature emphasizing accuracy as a cornerstone of pharmaceutical quality control. Precision, assessed through repeatability and intermediate studies, produced consistently low %RSD values, reinforcing the method's reproducibility. Robustness testing indicated that small, deliberate variations in analytical conditions—such as minor wavelength shifts—did not significantly impact results, demonstrating that the method is stable, practical, and suitable for routine use. The sensitivity parameters (DL and QL) reflected the method's capability to detect and quantify low analyte levels, making it advantageous for impurity monitoring and low-dose formulations. Assay values consistent with pharmacopeial specifications further validate the applicability of this green derivative method for active pharmaceutical ingredient determination in synthetic mixtures. Overall, the method meets all essential ICH validation criteria, including specificity, linearity, accuracy, precision, robustness, and sensitivity. Its simplicity, minimal environmental impact, and reliable analytical performance make it a strong candidate for eco-friendly routine quality assessment. Future work may incorporate chemometric techniques to enhance spectral resolution and further broaden the scope of green UV derivative applications.

CONCLUSION

An Eco-friendly reliable and straightforward simplified spectrum derivative for the first order of the UV for the method of spectrophotometry was developed and validated for the first time for the simultaneous measurement of Dapagliflozin and Eplerenone in a synthetic mixture. The method attained the ICH Q2 (R2) validation levels for the analysis of pharmaceuticals in the fields of accuracy, precision, specificity, and robustness. The method was reproducible, not interfering at the predetermined wavelengths. The method is suitable for routine pharmaceutical quality control due to the low sensitivity and the straightforward nature of the method to use.

Declarations

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Author's contributions

Prexa Tandel performed the experimental work and drafted the manuscript. Hiralben Mehta & Ratna Musale supervised the study and critically revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethics declaration and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

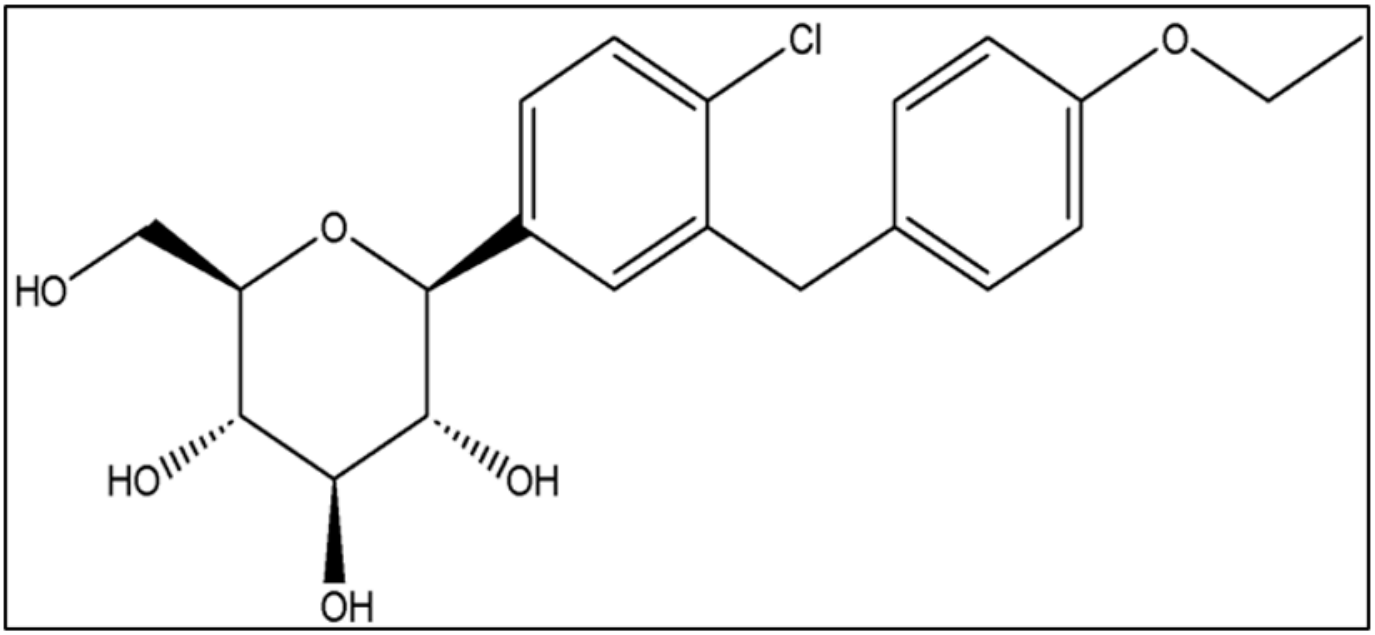


Figure 1

Chemical structure of Dapagliflozin

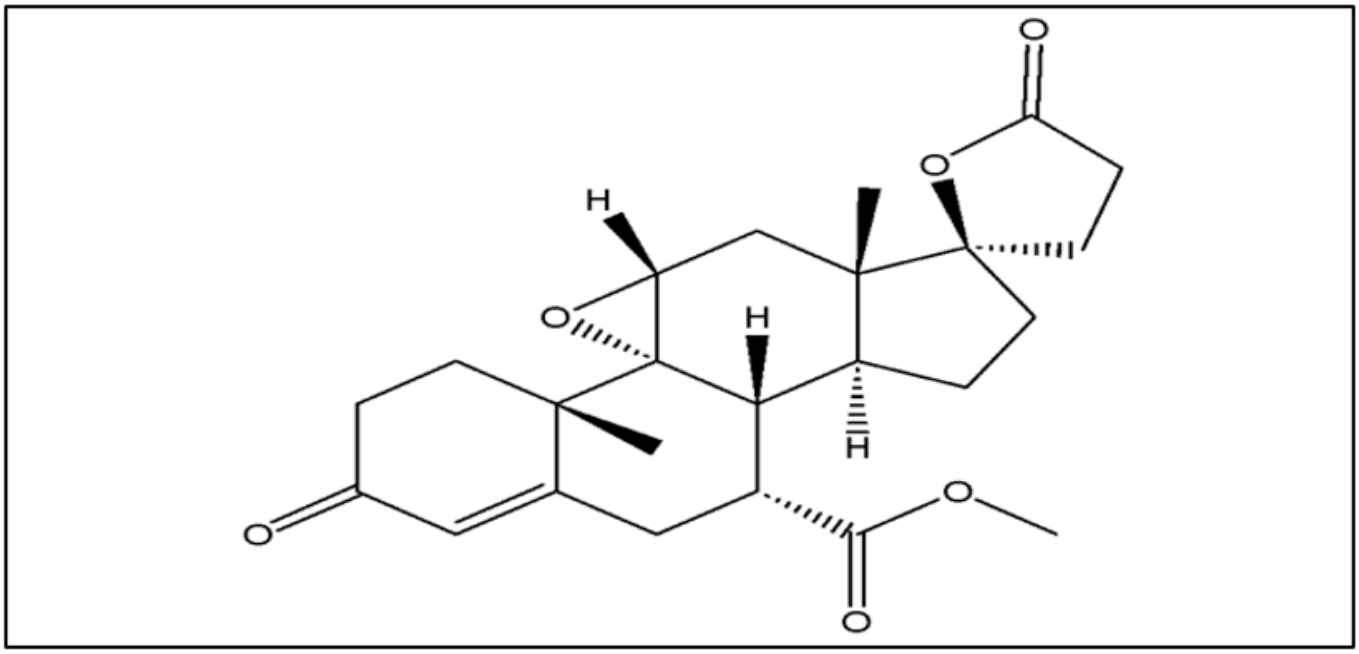


Figure 2

Chemical structure of Eplerenone



Figure 3

12 Principle of Green Analytical Method

Illustration of the key principles of green analytical chemistry, highlighting strategies such as reduction of solvent consumption, energy efficiency, waste minimization, and safer analytical practices applied during method development.

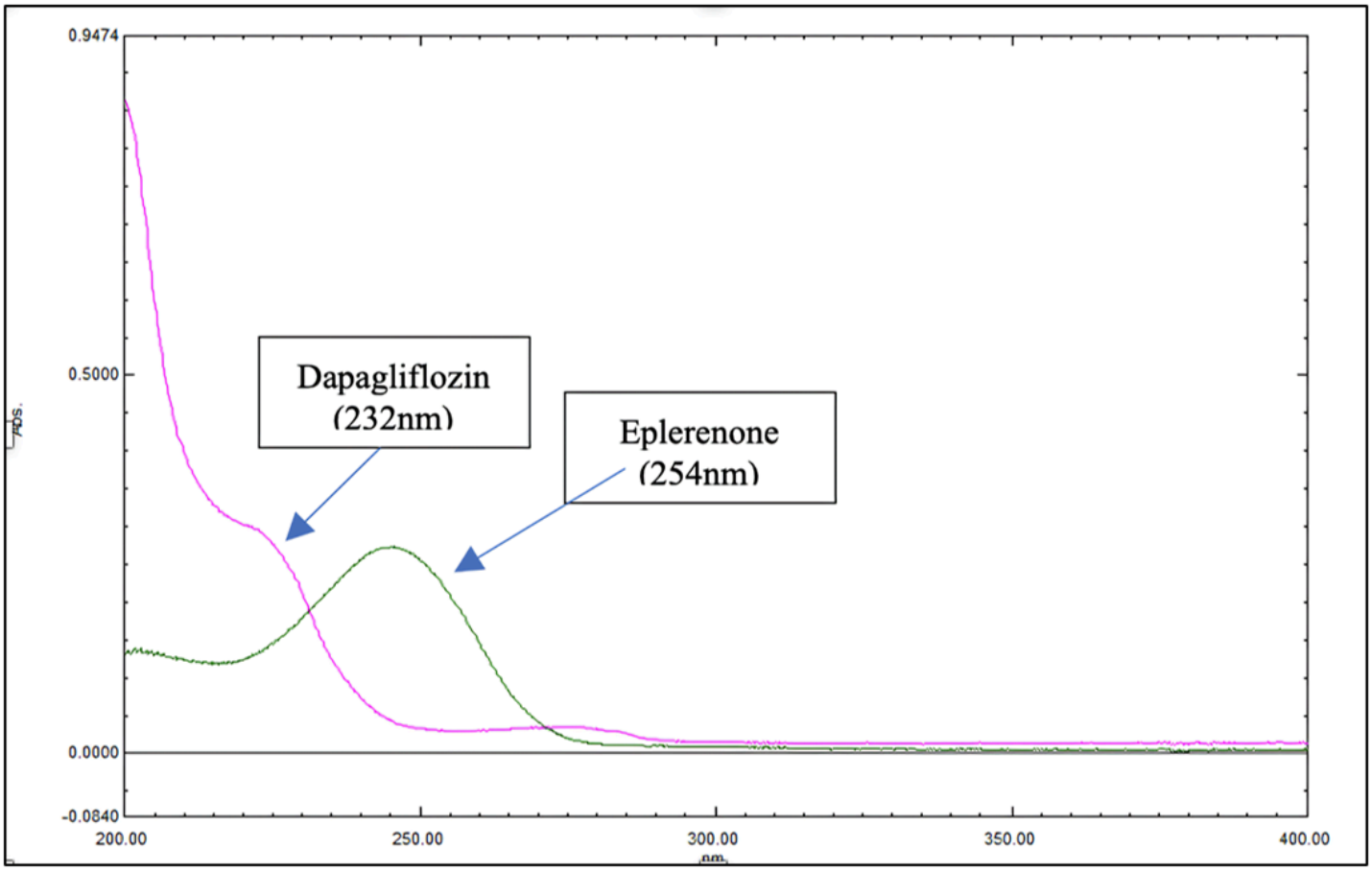


Figure 4

Selection of Wavelength

Overlay UV spectra showing the selection of analytical wavelength based on optimal absorbance and suitability for simultaneous estimation.

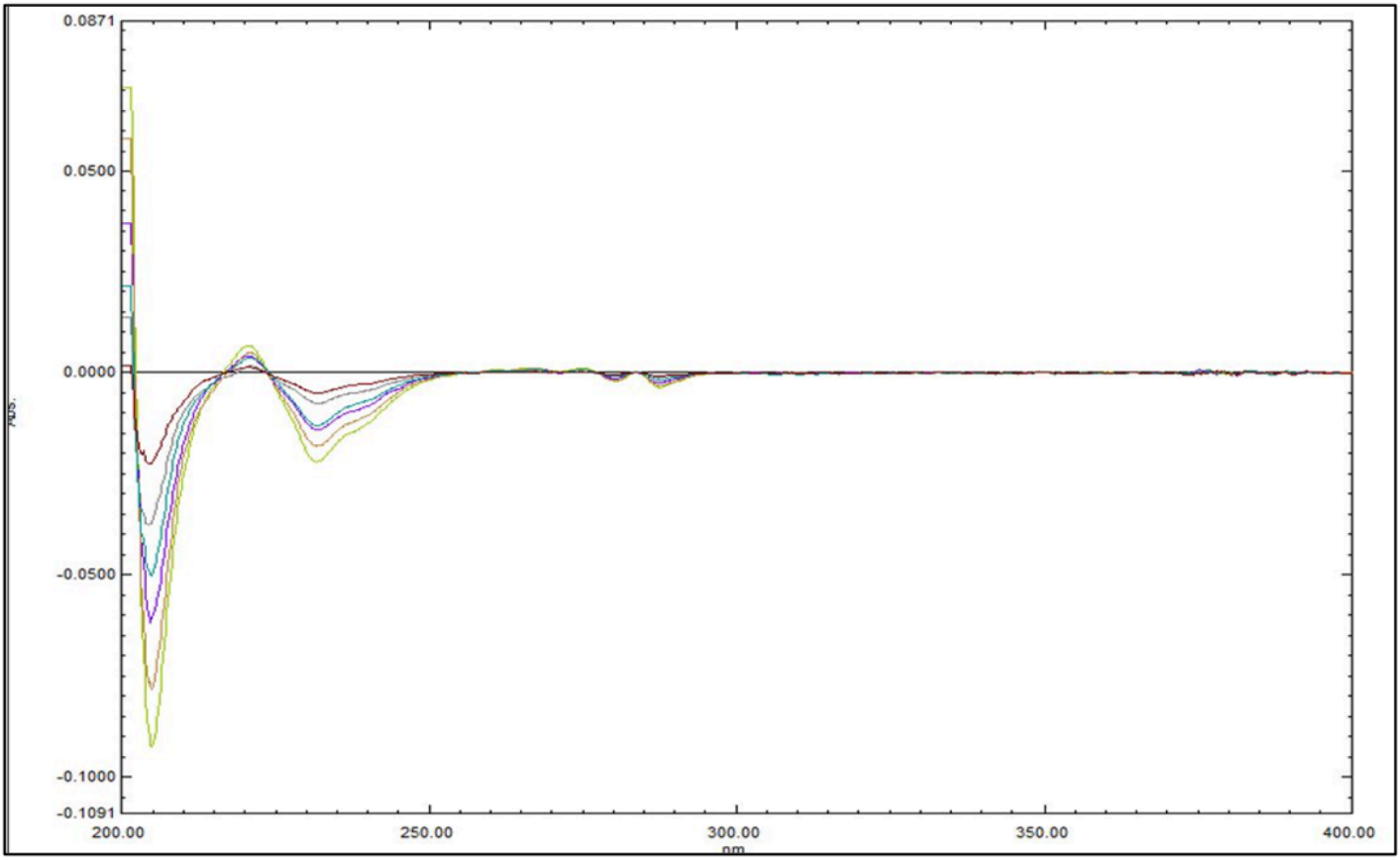


Figure 5

First order spectra of Dapagliflozin.

First-order derivative UV spectrum of dapagliflozin showing characteristic peaks and zero-crossing points used for its selective quantification in the proposed method.

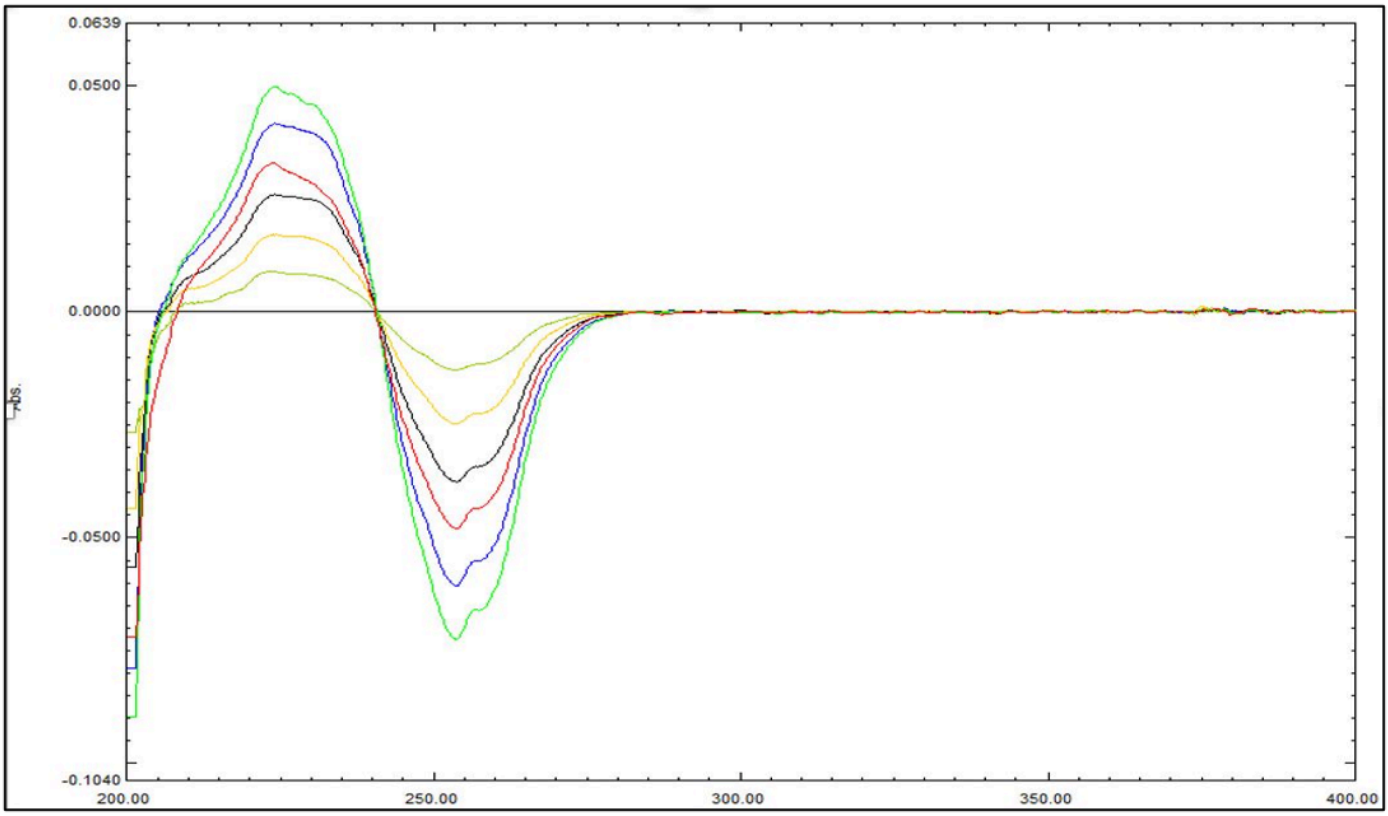


Figure 6

First order spectra of Eplerenone.

First-order derivative UV spectrum of eplerenone illustrating distinct spectral features enabling its accurate determination without interference.

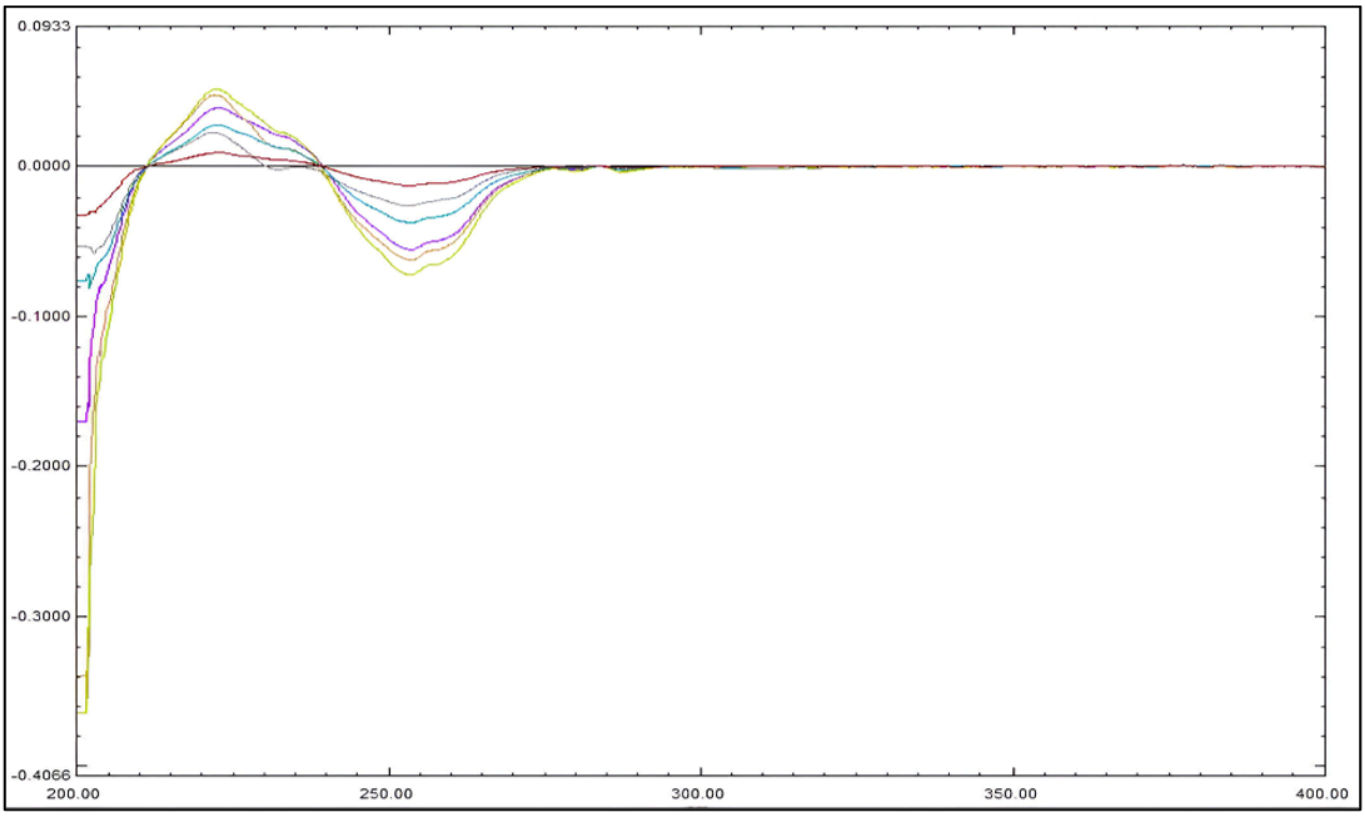


Figure 7

First order spectra of Mixture.

Overlay first-order derivative spectra of dapagliflozin and eplerenone in combined form, demonstrating the absence of spectral interference and suitability for simultaneous estimation.

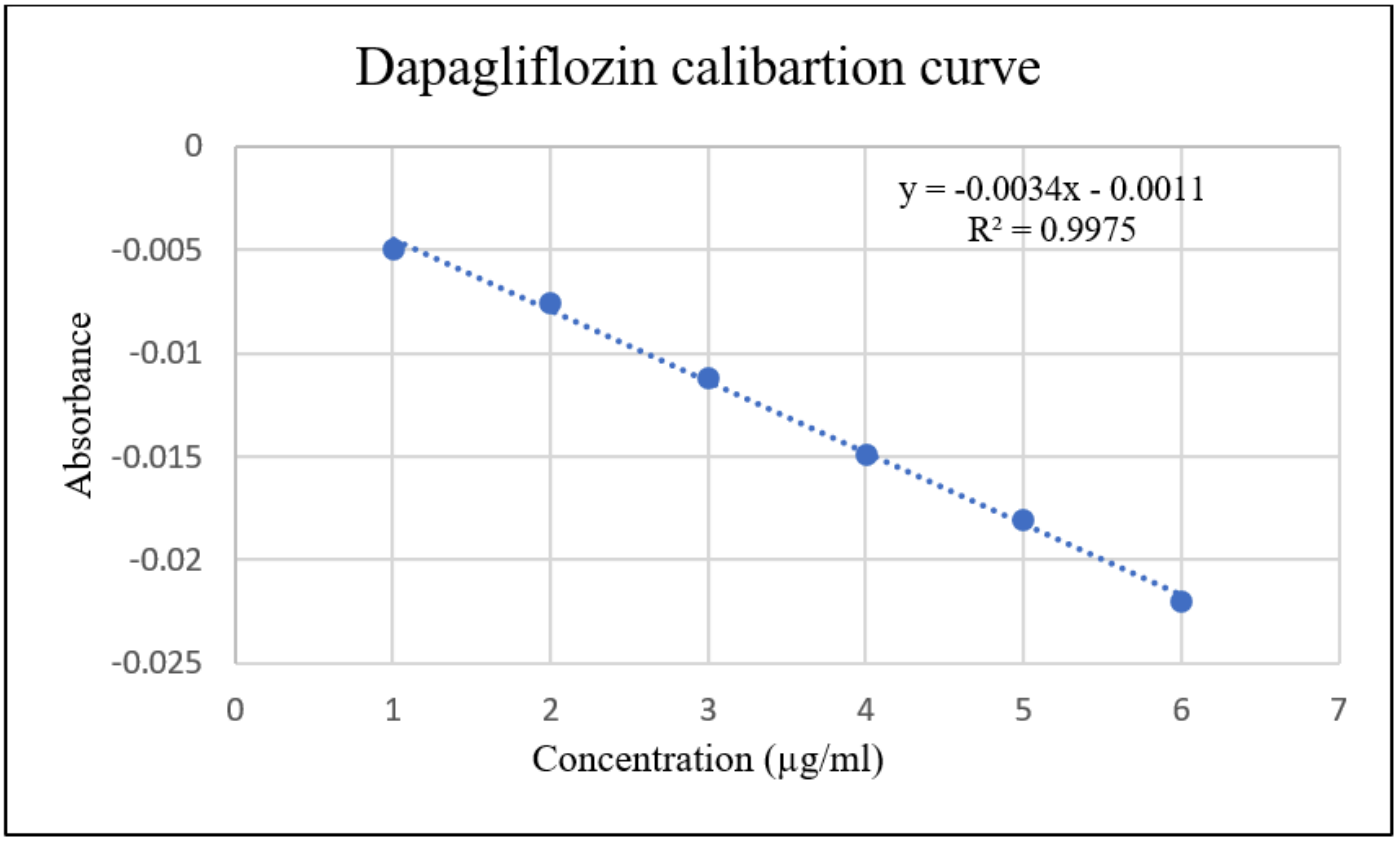


Figure 8

Calibration Curve of Dapagliflozin

Calibration curve obtained by plotting first-order derivative absorbance against concentration for dapagliflozin, showing a linear relationship within the studied range with high correlation.

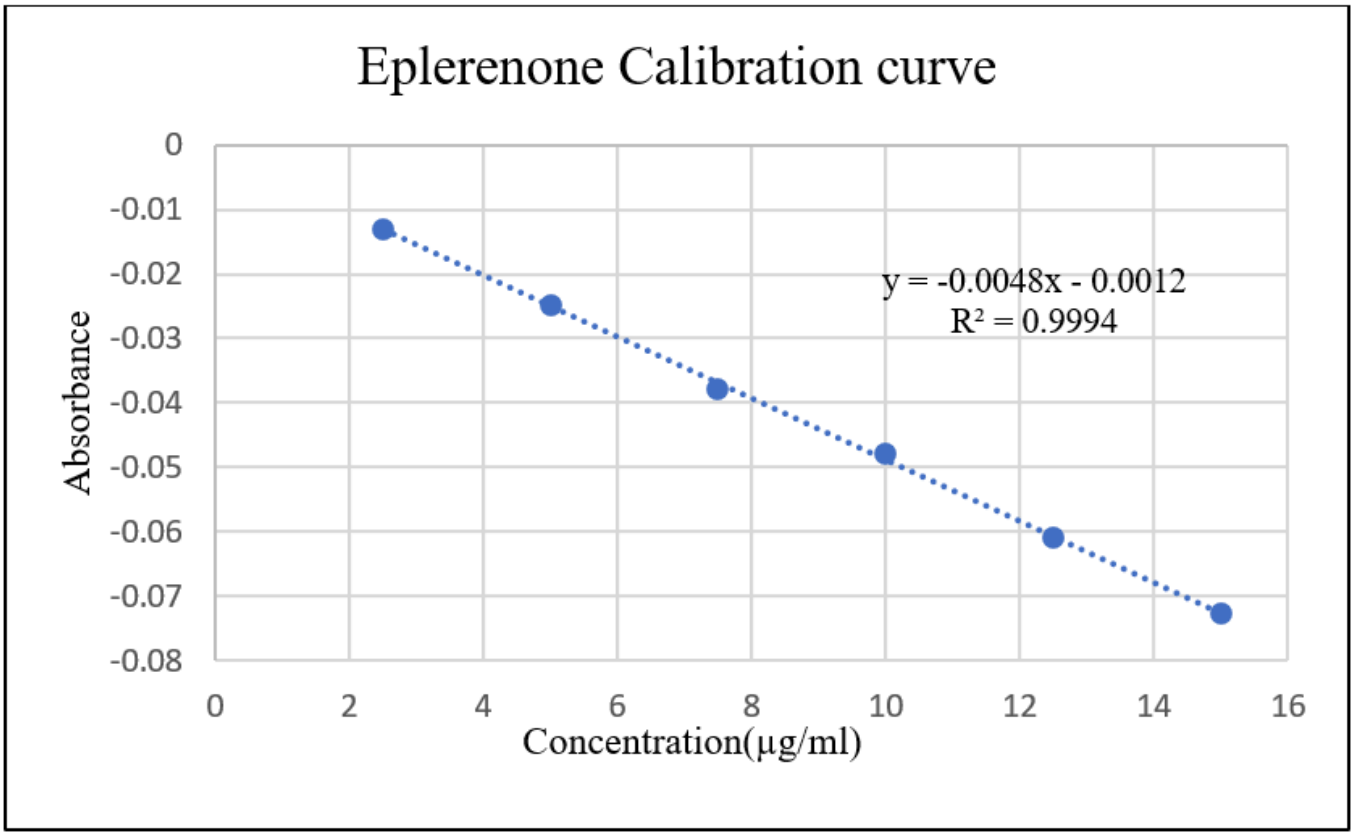


Figure 9

Calibration Curve of Eplerenone

Calibration curve of eplerenone representing the linear relationship between derivative absorbance and concentration, confirming the method's reliability for quantitative analysis.

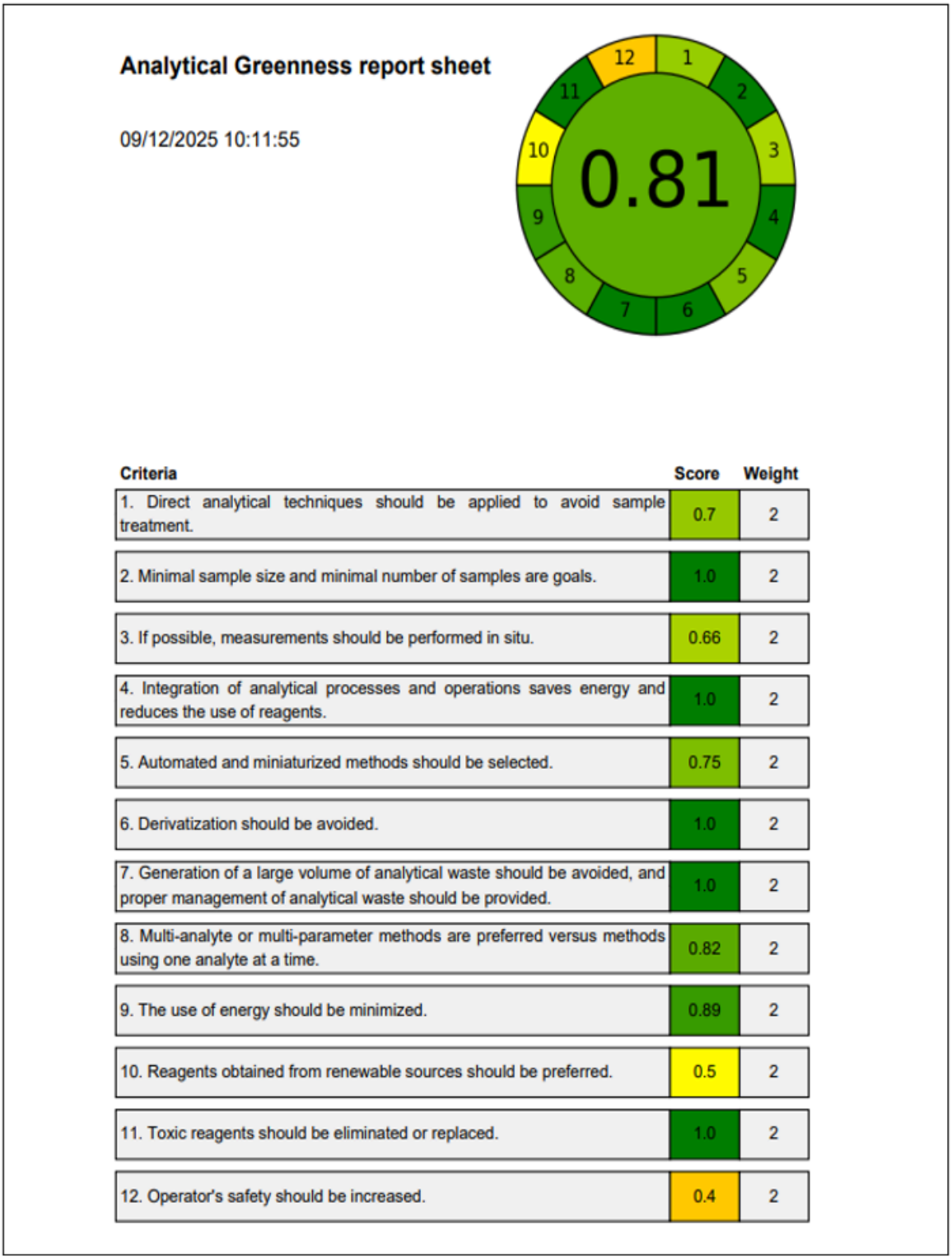


Figure 10

Result of Green Analysis of the Method (Analysis was perform using **AGREE** Software) Graphical representation of the greenness assessment of the proposed analytical method evaluated using AGREE (Analytical GREEnness) software. The circular diagram summarizes compliance with the 12 principles of green analytical chemistry, with the overall score indicating the environmental sustainability of the

method. The high AGREE score reflects minimal solvent usage, reduced waste generation, and energy-efficient analytical conditions, confirming the eco-friendly nature of the developed method.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.docx