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Utilization of Green Analytical Chemistry Principles for the Simultaneous Estimation of Dapagliflozin, Sitagliptin Phosphate, and Metformin HCl by UV and RP-HPLC Method



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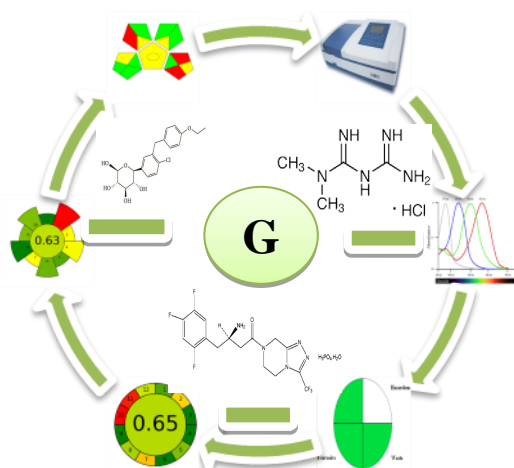
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Keywords: Dapagliflozin (DAP), Sitagliptin Phosphate (SIT), Metformin HCL(MET), UV- Spectrophotometry, RP-HPLC, Green analytical chemistry, National Environmental Methods Index (NEMI), Analytical Eco-scale (ESA), Green Analytical Procedure Index (GAPI), AGREE metrics.

ABSTRACT

The Drug Controller General of India (DCGI) recently approved the use of metformin hydrochloride, sitagliptin phosphate, and dapagliflozin together for the treatment of type 2 diabetes mellitus. There was no way to estimate metformin HCL (MET), sitagliptin phosphate (SIT), and dapagliflozin (DAP) simultaneously, according to the literature review. The current work describes a multicomponent UV Spectrophotometric & RP-HPLC method for the determination of Dapagliflozin, Sitagliptin phosphate, and Metformin, based on the principles of green analytical chemistry. Prominent research labs and pharmaceutical companies use green analytical methodologies to develop environmentally friendly analytical procedures. Methanol and distilled water were used as diluents in the initial UV method. For DAP, SIT, and MET, the chosen wavelengths for the analysis were 223 nm, 267 nm, and 232 nm, respectively. Acetonitrile and buffer have been used as diluents in the development of the Second RP – HPLC method. Drugs were successfully separated on a C18 column Sun fire (250x4.6mm, 5µm) with a flow rate of 1 mL/min using acetonitrile (70:30, % v/v) as the mobile phase and potassium dihydrogen orthophosphate, pH 4.3. 259 nm was the detection wavelength. The developed methods were validated in compliance with the ICH Q2 (R1) guideline. For the UV and RP-HPLC methods, the calibration curve had a r² of 0.999 and was linear over the concentration range of 0.1-0.5µg/mL (DAP), 1-5µg/mL (SIT), and 10-50µg/mL (MET), respectively. Recovery studies were used to determine the accuracy of the methods, which ranged from 98% to 102%. The entire procedure was validated by the principles of green chemistry, with the percent RSD values for all the validation parameters being less than 2.0% for both approaches. The UV spectrophotometric method and the developed RP-HPLC method were successfully used to quantify the drugs in question in pharmaceutical dosage form.



INTRODUCTION

The aim of "green analytical chemistry" is to diminish the negative effects that chemical analysis's analytical techniques have on the environment. Its main objective is to create and apply methods that minimize the use of dangerous chemicals, energy, and resources while preserving or enhancing analytical performance. This includes using solvents that are less harmful to the environment, producing less waste, utilizing renewable energy sources, and improving analytical techniques to make them more sustainable. The objective is to lessen the environmental impact of chemical analysis procedures while still obtaining accurate and trustworthy results.

DAP, or dapagliflozin (Fig. -1) The first SGLT2 inhibitor to be approved, known as (1S)-1, 5-anhydro-1-C-{4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-D-glucitol, is an inhibitor of the sodium-glucose co-transporter 2 (SGLT2). Indicated for the treatment of type 2 diabetes. Phosphatase Sitagliptin (SIT) (Fig-1) chemically, 3-(trifluoromethyl)-3R)-3-amino-1-[3--6,8-dihydro-5H-triazolo[1,2,4][4,3-a][pyrazin-7-yl] An oral dipeptidyl peptidase-4 (DPP-4) inhibitor called -4-(2,4,5tri fluoro phenyl) butane-1-one;phosphoric acid is used in combination with diet and exercise to help patients with type 2 diabetes mellitus improve their glycemic control. First-line pharmacotherapy for the treatment of type II diabetes involves the use of metformin HCL (MET) (Fig. 1), a biguanide antihyperglycemic agent that is chemically represented as 3-(diamino methylidene)-1, 1-dimethylguanidine hydrochloride. Since metformin reduces blood glucose levels in type II diabetes without producing hypoglycemia, it is referred to as an anti-hyperglycemic medication.

Since there is no official method for the simultaneous estimation of Dapagliflozin, Sitagliptin Phosphate, and Metformin HCL in any kind of dosage form, the combination of DAP, SIT, and MET is not recognized in any Pharmacopoeias, which makes the current work novel. According to a review of the literature, several techniques using UV-visible spectrophotometry, HPLC, and HPTLC have been reported for the estimation of these three drugs either alone or in combination with other drugs. According to the literature, there is no analytical technique for estimating Dapagliflozin, Sitagliptin Phosphate, and Metformin HCL in tablet dosage form simultaneously. The current work describes a simple, economical, and eco-friendly multicomponent UV& RP-HPLC Spectrophotometric method for the simultaneous calculation of dapagliflozin, sitagliptin phosphate, and metformin. This technique is based on the concepts of green analytical chemistry.

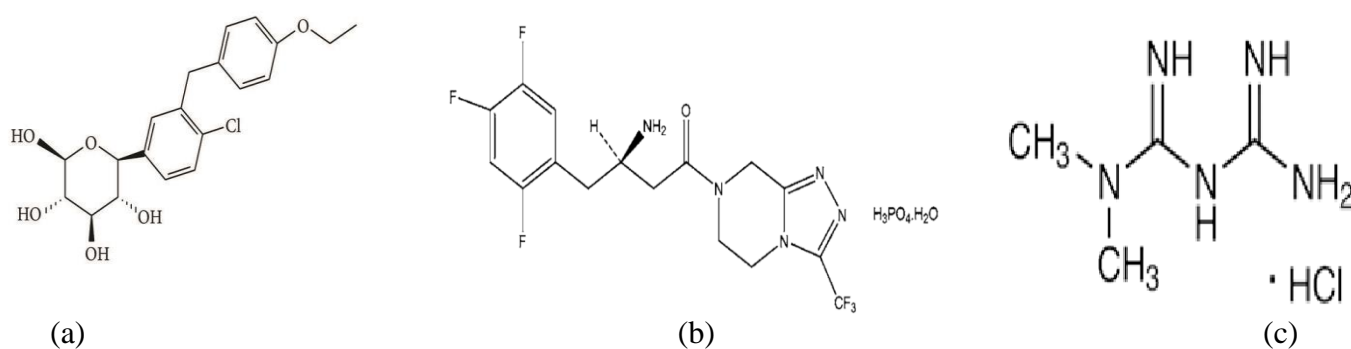


Figure 1 : (a) Dapagliflozin, (b) Sitagliptin Phosphate, (c) Metformin HCL

MATERIALS AND METHODS

Instrument

Spectral measurements were performed using a double-beam UV-visible spectrophotometer (Shimadzu, model 1650PC, Japan) with two matching quartz cells and a 1 cm light path. The UV-visible spectrophotometer was configured with UV probe 2.42 software. A comprehensive HPLC system, Shimadzu Corporation's LC 20AR. A paired inclination siphon, a manual sampler, and a photodiode exhibit locator made up the HPLC framework. The samples were weighed using a Shimadzu AUX 220 electronic weighing balance. Using the Millipore Water Purification System (DQ5), type I - HPLC grade water was obtained.

Reagents and chemicals

Purchased from carbanion were the active pharmaceutical ingredients: metformin hydrochloride standard (bulk), sitagliptin phosphate, and dapagliflozin. The GLUCRETA SM extended-release tablet, produced by Torrent Pharmaceuticals Ltd., was bought from the local market. It contained 10 mg of dapagliflozin, 100 mg of sitagliptin phosphate, and 500/1000 mg of metformin hydrochloride. Arrow Chemicals in Coimbatore provided the HPLC grade methanol and acetonitrile that was purchased. Analytical-grade chemicals were used for all other purposes.

UV Method

Experimental condition

Methanol was discovered to be the common solvent for all three medications based on their solubility characteristics. Therefore, methanol was used to prepare the stock solution, and distilled water was used to make further dilutions.

Standard stock solution preparation

To set up the standard stock arrangement of DAP, SIT, and MET (1000 μ g/ml), 10 mg of each medication was definitively broken up into 10 ml of methanol until it was totally disintegrated. To find the assimilation maxima, the functional standard arrangements of 10 μ g/ml of DAP, SIT, and MET were made and checked in the 200-400 nm range. The overlay spectra that resulted from the absorption maxima of DAP, SIT, and MET at 223,267, and 232 nm, respectively, are displayed in figure 2.

Evaluation of marketed formulation

Twenty tablets were taken, and the average weight of the tablets was estimated, to quantify the drugs in the marketed formulation. They crushed the tablets into a fine powder. Weighed samples having 10 mg of dapagliflozin, 100 mg of sitagliptin phosphate, and 1000 mg of metformin HCL were mixed with methanol to dissolve the equivalent of 10 mg of DAP, SIT, and MET. Sonicate for twenty minutes, then use the same to reach the mark. A Whatman channel paper was utilized to channel the mixture. A 1 ml aliquot of this arrangement was taken out and weakened with water to the proper level in a 10 ml volumetric jar. DAP, SIT, and MET at 10 μ g/ml are available in the arrangements. Three absorbances were estimated at

223 nm, 232 nm, and 267 nm, separately. Figure 2 displays the overlay spectra that were produced.

Chromatographic condition

Experimental condition

The buffer (potassium dihydrogen orthophosphate) and acetonitrile were mixed in a 70:30 v/v ratio, and orthophosphoric acid (OPA) was used to adjust the pH to 4.3. Sonication was utilized to filter and degas the mobile phase through a membrane filter with a porosity of 0.45 μm . The Sun Fire HPLC analytical C18 column (250x4.6mm, 5 μm) was used for the separation process, and isocratic elution was used. The finder was aligned at 259 nm, and the stream rate was 1 mL/min. Twenty microliters (μl) of the example arrangement were infused. The investigation was finished at a temperature of 25 °C.

Analysis of marketed formulation

Weighing the GLUCRETA SM extended-release tablet, which contained 10 mg of dapagliflozin, 100 mg of sitagliptin phosphate, and 1000 mg of metformin hydrochloride, the equivalent of 10 mg of dapagliflozin, 10 mg of sitagliptin phosphate, and 10 mg of metformin hydrochloride was transferred into a 100 mL volumetric flask that was kept dry and clean. Sonication was used to dissolve the powder in a large enough amount of mobile phase. The whatman filter paper was used to filter the suspension that resulted. With a mobile phase concentration of 100 $\mu\text{g/ml}$, the filtrate volume was increased to 100 mL. To get the last grouping of 10 $\mu\text{g/ml}$, 1 milliliter of the resultant arrangement was taken and weakened with versatile stage. In the wake of infusing this arrangement into the logical section, the chromatogram that is shown in Fig. 4b was recorded.

RESULTS AND DISCUSSIONS

Optimization of Spectrophotometric parameters

The suggested approach uses distilled water and methanol as solvents and is based on the simultaneous equation method for the simultaneous estimation of DAP, SIT, and MET in the UV region. Fig. 2 displays the overlaid spectra of the formulation, DAP, SIT, and MET.

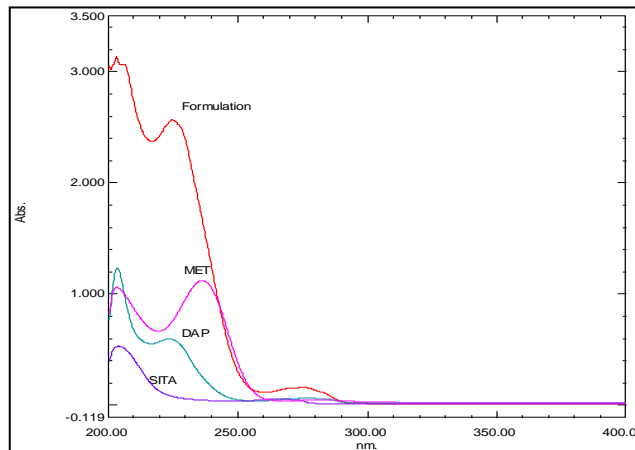


Fig 2: Overlay spectra of DAP, SIT, MET, and formulation

The drugs' absorbance at their mutual absorption maximum—DAP, SIT, and MET—was what the simultaneous equation method relied upon. The λ max selected drugs were at 223, 232, and 267 nm, which were three wavelengths assigned for the advancement of the simultaneous equations. At each of the three chosen wavelengths, the absorbance's of DAP, SIT, and MET were measured, and the absorptivity values were computed. An average of five evaluations produced these results. To determine the concentration of three medications, create the simultaneous equation shown below.

$$C_x = \frac{A_1(ay_2az_3 - az_2ay_3) - ay_1(A_2az_3 - az_2A_3) + az_1(A_2ay_3 - ay_2A_3)}{ax_1(ay_2az_3 - az_2ay_3) - ay_1(ax_2az_3 - az_2ax_3) + az_1(ax_2ay_3 - ay_2ax_3)}$$

$$C_y = \frac{ax_1(A_2az_3 - az_2A_3) - A_1(ax_2az_3 - az_2ax_3) + az_1(ax_2A_3 - A_2ax_3)}{ax_1(ay_2az_3 - az_2ay_3) - ay_1(ax_2az_3 - az_2ax_3) + az_1(ax_2ay_3 - ay_2ax_3)}$$

$$C_z = \frac{ax_1(ay_2A_3 - A_2ay_3) - ay_1(ax_2A_3 - A_2ax_3) + A_1(ax_2ay_3 - ay_2ax_3)}{ax_1(ay_2az_3 - az_2ay_3) - ay_1(ax_2az_3 - az_2ax_3) + az_1(ax_2ay_3 - ay_2ax_3)}$$

Where,

C_x , C_y and C_z are the concentrations of DAP, SIT and MET respectively.

A_1, A_2 , and A_3 are the absorbance of formulation at 223nm, 232nm and 267nm respectively.

ax_1, ax_2 and ax_3 are the absorptivities of DAP, at 223nm, 232nm and 267nm respectively.

ay_1, ay_2 and ay_3 are the absorptivities of SIT at 223nm, 232nm and 267nm respectively.

az_1, az_2 and az_3 are the absorptivities of MET at 223nm, 232nm, and 267nm respectively.

The absorptivity of each was calculated by using the following formula,

$$\text{Absorptivity} = \frac{\text{Absorbance}}{\text{Concentration}}$$

Optimization of chromatographic conditions

We performed a wavelength scan of the DAP, SIT, and MET standard solution between 200 and 600 nm. Just as shown in Figure 2. The frequency maxima of DAP (223 nm), SIT (267 nm), and MET (232 nm) are isolated from each other by a critical distance, and an isosbastic point was recognized at 259 nm. The separation of the relative multitude of pinnacles was first inspected utilizing switched stage Sun fire HPLC scientific C18 (250x4.6mm, 5 μ m) molecule size segments with isocratic elution. While choosing the versatile stage, the number of hypothetical plates, top immaculateness list, top evenness, and best goal was considered. As the portable stage, acetonitrile (70:30% v/v) at pH 4.3 was picked. (Figure 3):

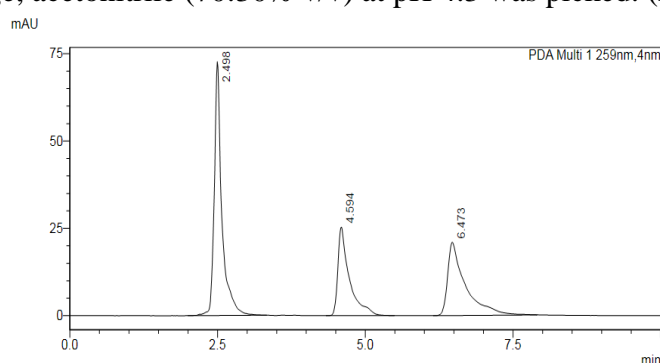


Fig. 3: Mixture (DAP+SIT+MET) HPLC chromatogram under finally optimized conditions at 259 nm

Method validation

Accuracy, robustness, linearity, sensitivity [limit of detection (LOD) & limit of quantitation (LOQ), repeatability, precision [repeatability & intermediate precision], and system suitability were all confirmed for the developed and optimized method by ICH Q2 (R1) guidelines.

System Suitability (for RP-HPLC)

Accuracy, robustness, linearity, sensitivity [limit of detection (LOD) & limit of quantitation (LOQ), repeatability, precision [repeatability & intermediate precision], and system suitability were all confirmed for the developed and optimized method according to ICH Q2 (R1) guidelines.

Table 1: system suitability parameters for DAP, SIT and MET using RP-HPLC

Parameter	DAP	SIT	MET
Retention time	6.607	4.895	2.513
Peak area	95428	158983	587945
Tailing factor	1.89	1.67	1.24

*Mean of six determinations

Specificity

By contrasting the UV and RP-HPLC spectra and chromatograms of the norm and test arrangements of DAP, SIT, and MET, the explicitness of the cycle was discovered. The HPLC top virtue list for each medication in the example arrangement was more like 1. Results acquired under ideal circumstances show that normal toxins and excipients in tablets don't cause obstruction. The outcomes (Fig. 2 and 4a-4b) show how explicit the method is.

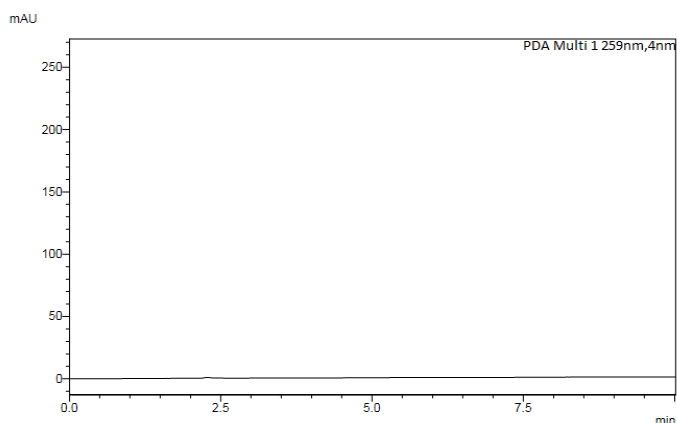


Figure 4a: Blank chromatogram of mobile phase

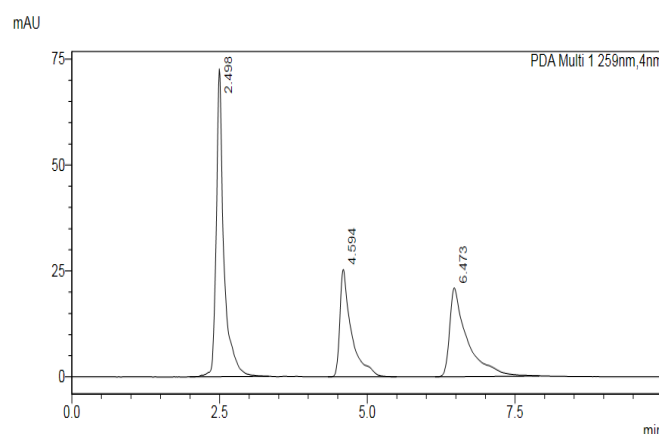


Figure 4b: Chromatogram of formulation

Sensitivity

The following formulas were used to calculate the limit of quantification (LOQ) and limit of detection (LOD) to measure the sensitivity of the analytical method. Table 2 shows the results of the sensitivity analysis.

$$\text{LOD} = 3.3 \sigma / S \text{ and } \text{LOQ} = 10 \sigma / S$$

Where, σ the standard deviation of y-intercept of calibration curve ($n = 6$)

S is the slope of a regression equation.

Table 2: LOD&LOQ of DAP, SIT and MET by proposed techniques

Parameters	UV			HPLC		
	DAP	SIT	MET	DAP	SIT	MET
LOD $\mu\text{g/ml}$	0.006	0.112	1.161	0.12	0.71	1.81
LOQ $\mu\text{g/ml}$	0.020	0.340	3.517	0.39	2.16	5.51

Linearity

Linearity was confirmed by dilution of the standard stock solution at six concentrations. Analyte concentration was plotted against absorbance (UV) and peak area (HPLC) to produce a linear regression analysis with correlation coefficients (r^2) more than 0.997. The statistical outcomes, including the slope, intercept, and correlation coefficient (r^2), are shown in Table 3.

Table 3: Data from linear regression for the calibrating curve

Parameters	UV			HPLC		
	DAP	SIT	MET	DAP	SIT	MET
Concentration range ($\mu\text{g/ml}$)	0.1-0.5	1-5	10-50	0.1-0.5	1-5	10-50
Correlation coefficient (r^2)	0.999	0.999	0.998	0.999	0.999	0.999
Intercept	0.00109524	0.00714286	0.0508571	81034	50709	48082
Slope	0.160286	0.00951429	0.0589657	14761	10981	10524

Precision

The precision of the technique was upheld by its repeatability and middle-of-the-road precision. The ability to communicate precision throughout a brief time frame under steady working circumstances is known as repeatability. To test the repeatability of the plan, the detailing examination was performed multiple times at a similar fixation. How much each medication in the detailing was determined and given as a rate RSD. Table 4 introduced the repeatability result.

Table 4: outcomes of the DAP, SIT, and MET repeatability studies

Parameters	UV			HPLC		
	DAP	SIT	MET	DAP	SIT	MET
Concentration($\mu\text{g/ml}$)	0.3	3	30	0.3	3	30
SD*	0.00516	0.0004	0.01032	2410.902	5644.001	4358.87
%RSD*	0.55	1.19	0.34	1.93	1.47	0.54

*Mean of six determinations

The technique's transitional accuracy was upheld by both intraday (variety of results around the same time) and interday (variety of results between days) investigation. The proposed strategies were assessed for both intraday and interday accuracy utilizing three unique convergences of standard tertiary blend arrangements. The comparing reactions were assessed multiple times around the same time for intraday accuracy and throughout the span of three days for interday accuracy. The outcomes were communicated as a level of the general standard deviation (% RSD). The rate RSD was determined following the utilization of every focus in three-fold. The information from the accuracy reads up for DAP, SIT, and MET are shown in Tables 5, 6, and 7, separately.

Table 5: Findings from DAP studies on intraday and interday precision

Parameters		UV			HPLC		
Concentration ($\mu\text{g/ml}$)		0.3	0.4	0.5	0.3	0.4	0.5
Intra day precision	SD*	0.001	0.001	0.001	133.50	53.67	72.91
	%RSD*	1.07	1.56	1.23	0.10	0.03	0.047
Inter day Precision	SD*	0.001	0.005	0.01	133.50	109.6	112.1
	%RSD*	1.61	0.60	1.02	0.10	0.07	0.81

*Mean of three determinations

Table 6: Findings from SIT intraday and interday precision studies

Parameters		UV			HPLC		
Concentration (µg/ml)		3	4	5	3	4	5
Intra day precision	SD*	0.0005	0.0005	0.0005	177.84	347.26	137.6
	%RSD*	1.68	1.49	1.23	0.041	0.07	0.023
Inter day Precision	SD*	0.005	0.01	0.005	82.58	85.76	89.13
	%RSD*	1.26	1.65	1.76	0.021	0.042	0.093

*Mean of three determinations

Table 7: Findings from MET intraday and interday precision studies

Parameters		UV			HPLC		
Concentration (µg/ml)		30	40	50	30	40	50
Intra day precision	SD*	0.017	0.017	0.017	83.21	87.6	94.84
	%RSD*	0.57	0.71	0.34	0.74	1.58	1.34
Inter day Precision	SD*	0.07	0.07	0.07	153.2	180	201.3
	%RSD*	1.12	0.66	1.63	0.97	1.71	1.60

*Mean of three determinations

Accuracy

The systemic error involved in a method determines its accuracy. It is the degree to which test results produced using that approach resemble the actual value. Three levels of the sample's working concentration—80%, 100%, and 120%—were used to test the method's accuracy. The standard solutions of Dapagliflozin, Sitagliptin Phosphate, and Metformin HCL were prepared to levels 80, 100, and 120 percent of the working concentration by adding sample solution to a calculated amount of standard solution. The percentage recovery was computed based on the total amount of drugs discovered. Each concentration saw three iterations of this process. A percentage RSD was computed. The outcomes are displayed in Table 8.

Table 8: The outcomes of the DAP, SIT, and MET recovery studies

Level	UV						HPLC					
	DAP	SIT	MET	DAP	SIT	MET	DAP	SIT	MET	DAP	SIT	MET
	%RSD			%Recovery			%RSD			%Recovery		
80	0.23	0.50	0.75	97.1	98	99.3	0.71	0.90	0.56	99.5	98	100.1
100	0.41	0.13	0.12	99.6	100.3	97.6	0.13	0.74	0.39	101.6	100.8	99.8
120	0.14	0.72	0.81	100.9	99.2	101.7	0.39	0.12	0.84	100.7	101.8	99.5

*Mean of three determinations

Robustness (for RP-HPLC)

An analytical procedure's robustness indicates how reliable it is under typical operating conditions and can withstand intentional, small variations in method parameters. The robustness of the technique was assessed by varying the mobile phase ratio, pH, wavelength of detection, and small flow rate. A new flow rate of 0.9 ± 1.0 mL/min was implemented. The three components' mobile phase ratios were adjusted to $\pm 2\%$ each. The detection wavelength was adjusted to 259 ± 2 nm. The pH was altered by 0.2. It was discovered that the approach remained resilient to variations in the applied circumstances. The robustness result is displayed in Table 9.

Table 9: Results of the DAP, SIT, and MET robustness study

Chromatographic parameter	Actual condition	Change condition	%RSD		
			DAP	SIT	MET
pH ± 0.2	4.3	4.1	1.131	0.808	0.491
		4.5	0.323	0.512	1.032
Flow rate $\pm 10\%$	1	0.9	0.549	0.541	1.036
		1.1	0.324	0.543	0.996
Wavelength ± 2 nm	259	257	0.921	0.996	0.935
		261	0.231	0.542	0.264
Variation in mobile phase ratio $\pm 2\%$	100%	98%	0.201	0.043	1.572
		102%	1.394	0.618	1.039

*Mean of three determinations

EVALUATION OF GREENESS PROFILE

The green analytical strategy expects to limit or annihilate any potential dangers connected with synthetic cycles. Lately, the evaluation of insightful strategies for their greenness profile has acquired importance, at last prompting the production of a positioning framework for greenness profiles.

The Analytical Eco Scale (AES), National Environmental Method Index (NEMI), and Green Analytical Procedure have been utilized in the improvement of the clever UV Spectrophotometric & RP-HPLC strategy. The Green Insightful Method List (GAPI) and the product-based logical greenness record (Concur) are the two apparatuses. To discover the level of eco-cordiality of an item, utilize insightful investigation of the greenness trademark parts of any method.

National Environmental Method Index (NEMI)

The NEMI evaluation tool offers a thorough conclusion about the environmental effects of the researched approach that is easily understood by simply looking at the pictogram, thanks to its user-friendly design. These four terms—hazardous, corrosive, waste, persistent, bioaccumulative, and toxic—define the criteria for the profile. Whether a quadrant is blank or green depends on how well the approach fits that criterion. A green-filled quadrant (allude to tables 10 and 11) shows that the technique meets the quadrant's choice rules. The two quadrants are subsequently green when the general profile of the proposed UV Spectrophotometric & RP-HPLC strategy is analyzed. Since acetonitrile for HPLC and methanol for UV spectroscopy are not PBT list classification synthetic substances and the created squander is under 50 g or ml for each example, the proposed method passes the two quadrants of the greenness profile.

Analytical Eco scale

The Eco-Scale is a semi-quantitative tool that the authors introduced to measure the environmental friendliness of analytical methods. Based on variables such as energy consumption, trash production, and the quantity and hazard of chemical compounds, this tool assigns penalty points. Next, by subtracting the total number of penalty points from 100, the best green analysis is displayed. A score of 50 to 75 is considered average, and a score of more than 75 is exceptional green. The investigation's criteria included solvents, instruments,

and waste volume. According to Tables 10 and 11, the UV Spectrophotometric method scored 82, while the HPLC method received an 80.

Analytical Eco scale=100- Total penalty points

Green Analytical Procedure Index (GAPI) & Complex GAPI


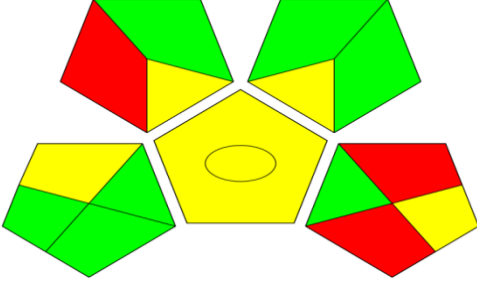
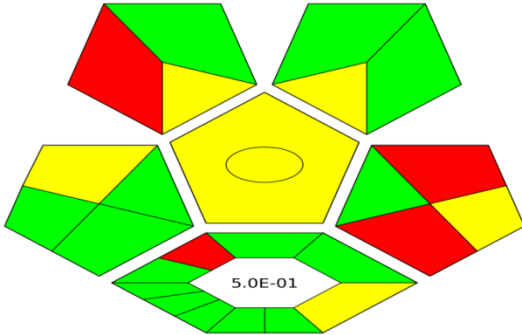
The GAPI greenness matrix is a well-liked and frequently used tool for assessing how analytical methods impact the environment. This quick, simple, and reliable tool offers useful details regarding how ecologically friendly a method is. GAPI is a great semi-quantitative tool for both teaching and lab practice. Using the GAPI diagram, the environmental effects of each step of an analytical process are assessed and quantified. The results of the GAPI diagram are shown in Tables 10 and 11. It is composed of five pentagrams that stand for different phases in the analytical approach, such as equipment, chemicals and solvents used, sample preparation and collection, and the goal of the analytical method. Three red pentagrams indicate areas of concern, while seven green and five yellow pentagrams are produced by the UV Spectrophotometric approach in its GAPI index. Three red pentagrams indicate areas of concern, while five green and seven yellow pentagrams are produced by the recommended green HPLC method. The yield result in complex GAPI shows eight green, one yellow, and one red, with the E factors for UV being 0.5 and 0.3.

AGREE & AGREE prep

The foundation of this approach is AGREE & AGREE prep, a state-of-the-art tool for assessing the greenness profile. The application generates a clockwise circular diagram in which the 12 philosophies of green analytical chemistry are represented by multiple numbers 1 through 12 around the periphery. For every segment of the 12 principles, the inputs and their corresponding weights were provided, and the results were aggregated on a scale from 0 to 1. The amount of the 12 standards and the Centerpiece of the AGREE diagram (tables 10 and 11) address the score. The variety range goes from red to yellow to green contingent upon the upsides of the outcomes that fall somewhere in the range of 0 and 1. The variety is red when the score values are near 0, and dim green when they are near 1. The result of the AGREE diagram programming was 0.65 & 0.72 for UV and HPLC, while the consequences of the Concur prep metric programming were 0.63 & 0.62 for UV for UV and HPLC. A score of more than 0.5 demonstrates further developed wellbeing and security, a decrease in the

utilization of unsafe materials, and the presence of protections against negative natural impacts.

Table 10: Evaluation of greenness profile for UV

ASSESSMENT TOOL	RESULT
NEMI	
Eco Scale Assessment(ESA)	<p>Chemical –Methanol (3 pictogram * 2 danger* 2 (1-100ml)=12 Energy used=0 Waste=3 Occupational hazard=3 Total penalty points =18 ESA=100-18=82</p>
GAPI	
Complex GAPI	

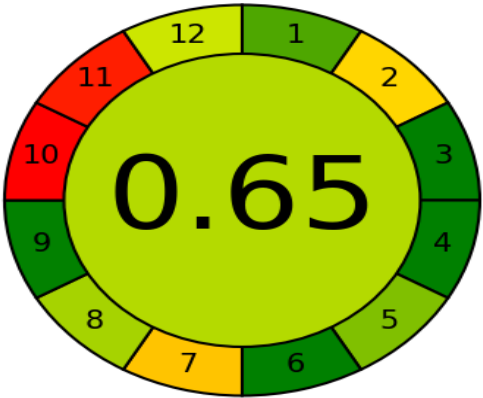
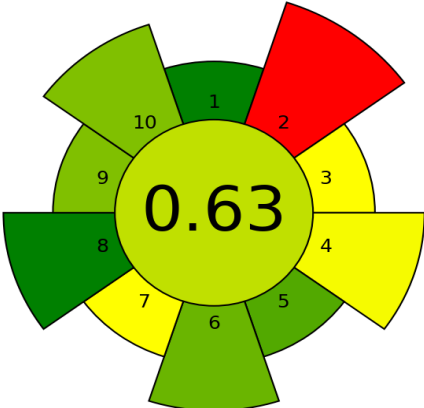
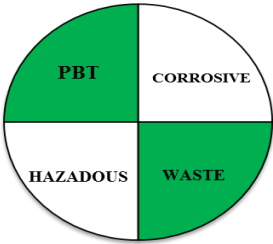
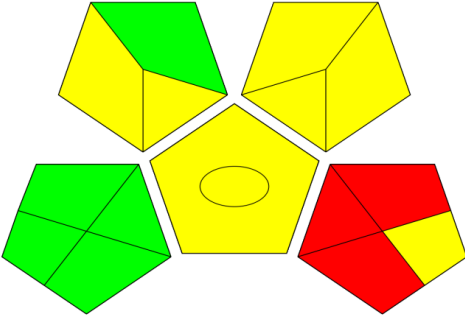
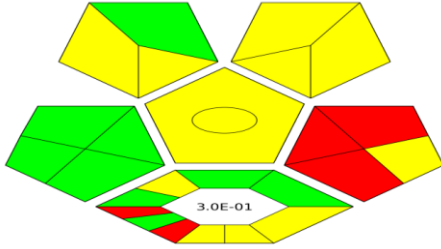


<p>AGREE</p>	
<p>AGREE on prep</p>	

Table 11: Evaluation of greenness profile for HPLC

Assessment Tool	Result
<p>NEMI</p>	
<p>Eco Assessment(ESA) Scale</p>	<p>Chemical –Acetonitrile (2 pictogram * 2 danger* 2 (1-100ml)=8 OPA (2 pictogram * 2 danger* 2 (1-100ml)= 4 KH₂PO₄ (No pictograms)=0 Energy used=0 Waste=5 Occupational hazard=3 Total penalty points =20 ESA=100-20=80</p>

<p>GAPI</p>	
<p>Complex GAPI</p>	
<p>AGREEE</p>	 

CONCLUSION

The development and validation of UV Spectrophotometric (Simultaneous Equation Method) and RP-HPLC methods enabled the successful simultaneous determination of DAP, SIT, and MET. The procedures that were developed were found to be precise, accurate, sensitive, and robust. This implies that the recommended UV and HPLC techniques can be used to perform quality control analysis on DAP, SIT, and MET in the combined pharmaceutical formulation. Overall, green analytical chemistry can lessen the environmental impact of analytical techniques while maintaining analytical performance, as demonstrated by the protocols for the UV Spectrophotometric and RP-HPLC methods. The created RP-HPLC and UV techniques were suitable for routine pharmaceutical dosage form analysis as well as bulk forms of metformin HCL, dapagliflozin, and sitagliptin phosphate. They were also ecologically friendly.

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