Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

https://doi.org/10.36673/AJRCPS.2024.v12.i04.A16

VALIDATION AND QUANTIFICATION OF SIMPLE PRECISE UV METHOD FOR DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE IN BULK AND MARKETED FORMULATIONS

Anand Kumar Yegnoor[*1] and Srushti[1]

[1*]Department of Pharmaceutics, V.L. College of Pharmacy, Raichur, Karnataka, India.

ABSTRACT

A simple, rapid, accurate and reproducible UV method was developed for quantification of Dapagliflozin propanediol monohydrate (DP) in bulk and marketed tablets utilizing Methanol: Water (1:3) and Methanol: Water (3:1) as solvent blends. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 2-10µg/ml, correlation coefficient was found to be 0.9998 and 0.9995 for DP in two solvent blends. The LOD and LOQ for DP were found to be 0.049 to 0.0452µg/ml and 0.149 to 0.137µg/ml respectively for two solvent blends. The proposed method shows good percentage recovery of DP i.e., 99 to101 from marketed tablets in two solvent blends for DP, which indicates that the proposed method was highly accurate. The specificity of the method shows good correlation between recoveries of sample solution by standard addition method. Therefore, the proposed method specifically quantify the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

KEYWORDS

Dapagliflozin, UV, Accuracy, Robustness and Quantification.

Author for Correspondence:

Anand KumarY,
Department of Pharmaceutics,
V.L. College of Pharmacy,
Raichur-584103, Karnataka, India.

Email: anand241586@gmail.com

INTRODUCTON

World Health Organization (WHO), describes Diabetes mellitus (DM)[1] is a persistent metabolic disorder marked by heightened blood glucose levels, resulting in long-term damage to the heart, blood vessels, eyes, kidneys and nerves. Type-2 DM, constituting over 90% of DM cases and is characterized by insufficient insulin secretion from pancreatic islet β-cells, tissue insulin resistance (IR) and an inadequate compensatory insulin secretory response. Hyperglycemia occurs in diabetes when

the pancreas fails to produce sufficient insulin. Dapagliflozin propanediol monohydrate (DP) functions as a selective and reversible sodium-glucose cotransporter-2 (SGLT2) inhibitor. DP is D-glucitol 1, 5-anhydro-1-C-[4-chloro-3-[(4ethoxyphenyl) methyl] phenyl]-, (1S)-, compounded with (2S)-1, 2-propanediol, hydrate (1:1:1) Figure No.1. The determination of DP alone or in combination with other drugs was performed using (UV) spectrometry[2-4], reverse phase high performance liquid chromatography (RP-HPLC)[5-6], high performance liquid chromatography (HPLC)[7-11] and LC-mass spectrometry (LC-MS)[12-14]. The reported methods were expensive and time consuming, this necessitates the requirement for the development of simple, rapid with economical methods for quantification of DP in bulk and marketed tablets and it can be used for routine analysis. The present investigation aimed to develop and validate simple method for estimation of DP in pharmaceutical dosage forms by UV.

MATERIAL AND METHODS

Chemicals and Reagents

Dapagliflozin propanediol monohydrate (DP) obtained as gift sample from Caplin Point Laboratories, Chennai, India. Dapagain-10 and Cipla Dapagliflozin-10 marketed tablets were procured from local community pharmacy. All reagents, solvents used were of analytical grade (SD Fine Chemicals, Bangalore, India).

Instruments and apparatus

The analysis was performed using a double beam UV-Visible Spectrophotometer (SHIMADZU, Japan Model, UV-1900 Pharma Spec), connected to a compatible computer and supported with UV Probe software, the absorbances recorded using matched quartz cells of 1 cm path length. For precise measurements Anamed weighing balance was used. All glassware were meticulously cleaned with double-distilled water, and dried before use.

Preparation of stock solution and working solution for wavelength selection

Stock solutions of DP were prepared separately by dissolving 25mg in 25mL Solvent blend-1(1:3 Methanol: Distilled water) and Solvent blend-2 (3:1 Methanol: Distilled water) in volumetric flask, resulting in a concentration of 1000µg/mL. The solutions were sonicated for 5 mins to ensure complete dissolution. From each stock solution, 2.5mL was withdrawn using a calibrated glass pipette and diluted to 25mL with Solvent blend-1 and Solvent blend-2 to achieve a concentration of 100µg/mL for DP. Further dilutions were performed using solvent blends to obtain final concentrations of 10µg/mL for DP. Both the solutions were scanned individually in the ultraviolet range (400nm to 200nm) to determine their maximum absorbance wavelengths (λmax).

Validation of methods

The ICH Q2 (R1) guidelines were adopted in the validation of the outlined processes. Linearity, range, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ) were among the validation aspects assessed[15,16].

Analysis of tablet dosage form

Dapagain-10 and Cipla Dapagliflozin-10 were commercially available. In each case 20 tablets were weighed and flattened into powder. The powder corresponding to 25mg of DP were accurately weighed and transferred separately to a 25mL volumetric flask. The volume was made up to the mark with solvent blends under the study and the solutions were sonicated for 5 min to ensure uniform mixing. Aliquots of this solution were diluted further to obtain 10µg/mL of DP respectively.

RESULTS AND DISCUSSION

Selection of wavelengths

The λmax of DP were identified at 223 and 224nm respectively for solvent blend 1 and 2 (Figure No.2).

Validation parameters

Linearity and range

DP concentrations of 2µg/mL to 10µg/mL, were prepared in both solvent blends separately. To determine linearity and range, calibration curve and overlay plots for DP in both solvent blends at their respective wavelength maxima (λmax) such as 223 and 224nm were plotted and the data was shown in

Table No.1 and plots in Figure No.3 and Figure No.4. Absorbance and concentration was subjected to least squares linear regression analysis to calculate the calibration equations and other statistical parameters and were given in Table No.2. Correlation coefficient (R^2) values were observed to be close to 1, indicating excellent linearity. For DP, the R^2 values at 223 and 224nm were 0.9998 and 0.9995 respectively for solvent blend 1 and 2.

LOD and LOQ

The ICH Q2 (R1) guidelines formulas were used to determine the limits of detection (LOD) and quantification (LOQ) based on the signal to noise ratio. The LOD and LOQ values for DP in both solvent blends were found to be 0.049 and 0.0452 LOD; 0.149 and 0.137µg/ml LOQ indicating the sensitivity of the method.

Precision

Repeatability, intra-day precision, and inter-day precision were used to assess the precision of the developed procedures. Six analyses of a solvent blends containing 10µg/mL of DP were performed to evaluate repeatability. Intra-day and inter-day precision studies were conducted using standard solutions of DP (10, 15µg/mL) three times within a single day and on three different days, respectively. The repeatability (% RSD), intra-day and inter-day precision results are presented in Table No.3. The results were expressed in terms of % RSD values and were below 2 %, meeting ICH Q2 (R1) criteria.

Accuracy

The accuracy of the developed method were determined by standard addition method for both solvent blends. Recovery studies were carried out by spiking known amounts of DP (10µg/ml) at three concentration levels such as 40% (low concentration), 80% (intermediate concentration) and 120% (high concentration). The percentage recovery values for both solvent blends were within the acceptable range of 99% to 101%, as per ICH Q2 (R1) guidelines (Table No.4).

Assay of marketed formulations

The newly developed method with two solvent blends were successfully applied to the marketed tablet dosage form, demonstrating its effectiveness for the accurate estimation of DP in marketed tablets (Table No.5). The assay results suggest DP content in two marketed products determined by solvent blends under the study was in good agreement with the label claim with % RSD values less than 2.

Robustness and Ruggedness

Change in λ max of ± 5nm to the actual λ max in robust analysis results significant in the percentage recovery in both solvent blends indicates the methods were robust with % RSD less than 2. In ruggedness, analysis by different analyst and change of instrument indicates the proposed solvent systems were significantly rugged with % RSD less than 2.

Stability studies

The stability study was carried out for DP content in both solvent blends at room temperature for 72 hrs. The % recovery from the bulk and marketed tablets were found to be 99.78 to 100.01 % with % RSD less than 2, indicate DP in solvent blends under the study were stable over the period of 72 hr.

Table No.1: Calibration curve data of DP in both solvent blends

S.No	Concentration µg/ml	Absorbance Mean±SD n=6	
		Solvent blend -1	Solvent blend -2
1	2	0.075±0.000577	0.077±0.000523
2	4	0.153±0.000567	0.153±0.000561
3	6	0.227±0.000537	0.232±0.000577
4	8	0.303±0.000557	0.308±0.000513
5	10	0.378±0.000577	0.387±0.000522

Table No.2: Regression and statistical data of DP in solvent blend 1 and 2

S.No	Particulars	Solvent blend-1	Solvent blend-2
1	Absorption maxima	223 nm	224 nm
2	Linearity and Range	1-30 µg/ml	1-30 µg/ml
Regression values			
3	Regression equation	Y = 0.03833*X - 0.002974	Y = 0.03867*X - 0.0005360
4	SD of slope	0.0001258	0.0001528
5	% RSD of slope	0.3293	0.3950
6	Variance of slope	7.26×10^{-5}	8.819×10^{-5}
7	SD of intercept	0.02151	0.03553
Best-fit values			
8	Slope	0.03816	0.03867
9	Y-intercept	-0.001324	-0.0005360
10	X-intercept	0.03469	0.01386
11	1/slope	26.20	25.86
12	R square	0.9998	0.9995

Table No.3: Repeatability, Intra-day and Inter-day data of DP in solvent blends

S.No	Precision	Mediums	Concentrations µg/ml	Amount recovered µg/ml (n=6)	SD	% RSD	% Recovery
1	Repeatability	Solvent blend-1	10	10.08	0.05099	0.5059	100.8
		Solvent blend-2	10	10.05	0.03011	0.2997	100.5
2	Intra-day	Solvent blend-1	10	10.08	0.02517	0.2471	100.8
			15	15.02	0.02887	0.1859	100.13
		Solvent blend-2	10	10.04	0.01732	0.1725	100.4
			15	14.99	0.01732	0.1155	99.99
3	Inter-day	Solvent blend-1	10	10.03	0.02517	0.2471	100.3
			15	15.01	0.02887	0.1877	100.06
		Solvent blend-2	10	10.04	0.03606	0.3591	100.4
			15	14.96	0.05292	0.3537	99.73

Table No.4: Accuracy data of DP in both solvent blends by standard addition method

S.No	Accuracy	Mediums	Concentrations µg/ml	Amount recovered µg/ml (n=6)	SD	% RSD	% Recovery
1	40%	Solvent blend-1	4	4.03	0.0115	0.2774	100.75
		Solvent blend-2	4	3.96	0.0173	0.4374	99.00
2	80%	Solvent blend-1	8	7.973	0.02511	0.1485	99.66
		Solvent blend-2	8	7.953	0.05774	0.7259	99.41
3	120%	Solvent blend-1	12	11.89	0.01155	0.7985	100.3
		Solvent blend-2	12	11.95	0.04041	0.3420	99.58

Table No.5: Analysis of marketed tablet dosage forms

S.No	Mktd tablets	Mediums	Concentrations µg/ml	Amount recovered µg/ml (n=6)	SD	% RSD	% Recovery
1	Dapagain-10	Solvent blend-1	10	10.02	0.04041	0.3420	100.2
		Solvent blend-2	10	9.96	0.03155	0.4875	99.6
2	Cipla Dapagliflozin-10	Solvent blend-1	10	9.98	0.02155	0.1485	99.8
		Solvent blend-2	10	10.03	0.02155	0.2734	100.3

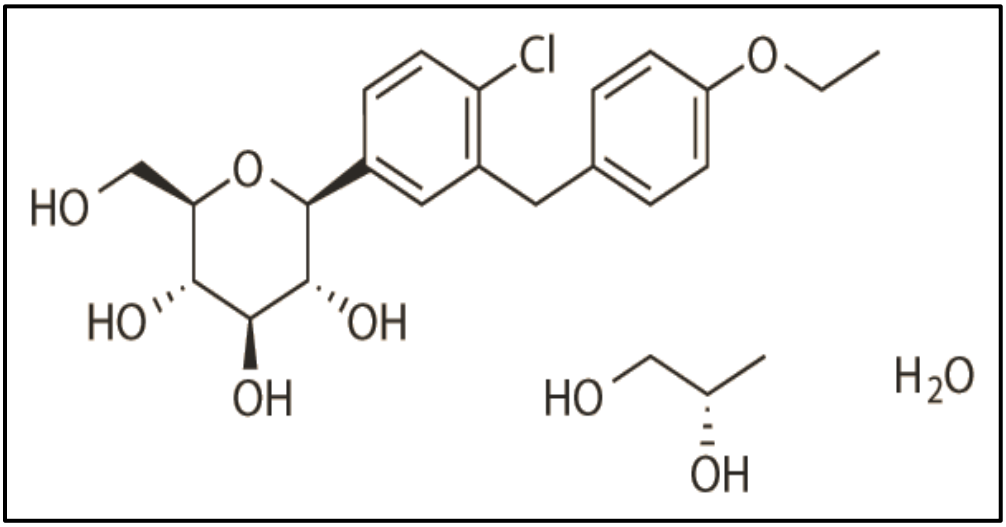

Figure No.1: Chemical structure of dapagliflozin propanediol monohydrate

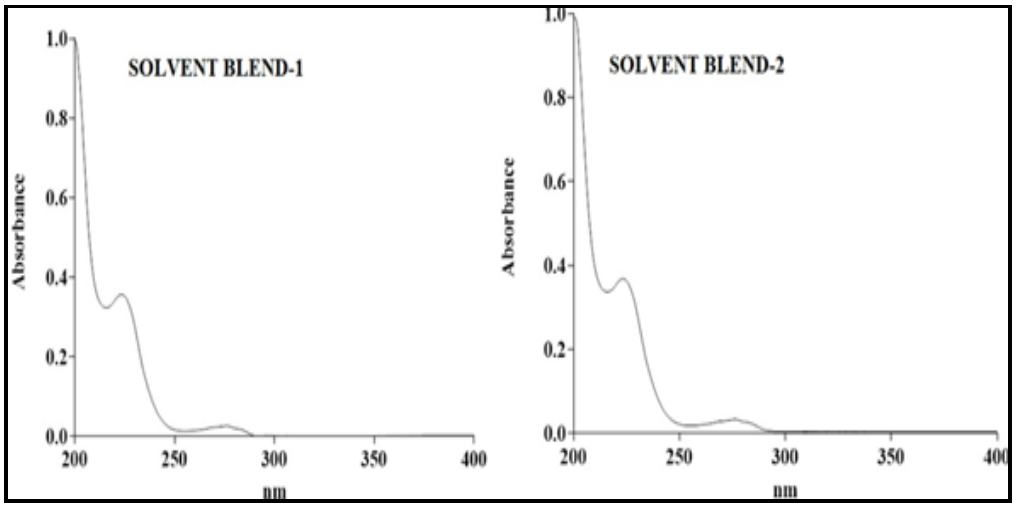

Figure No.2: Absorption maxima of DP in Solvent blend-1 and Solvent blend-2

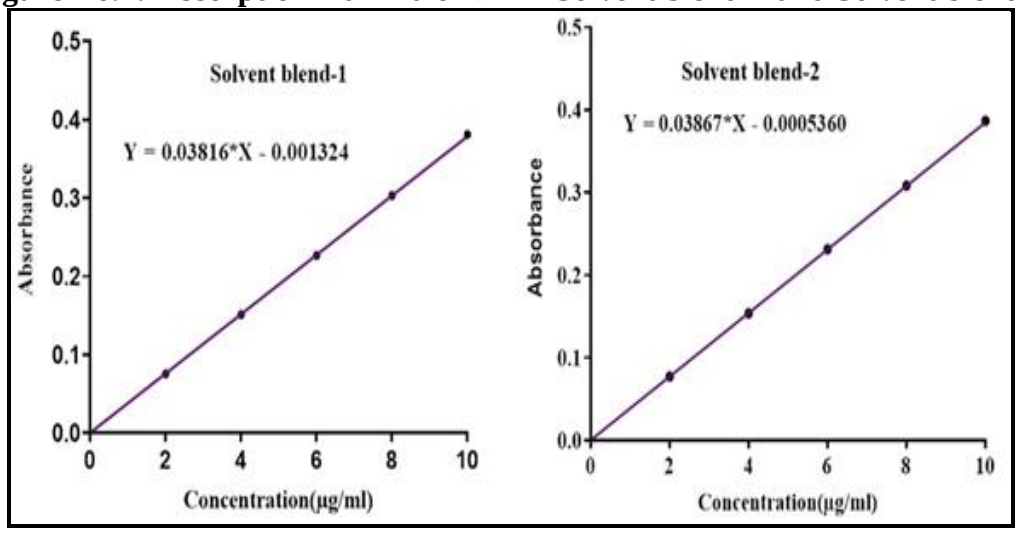

Figure No.3: Calibration curve plots of DP in Solvent blend-1and 2

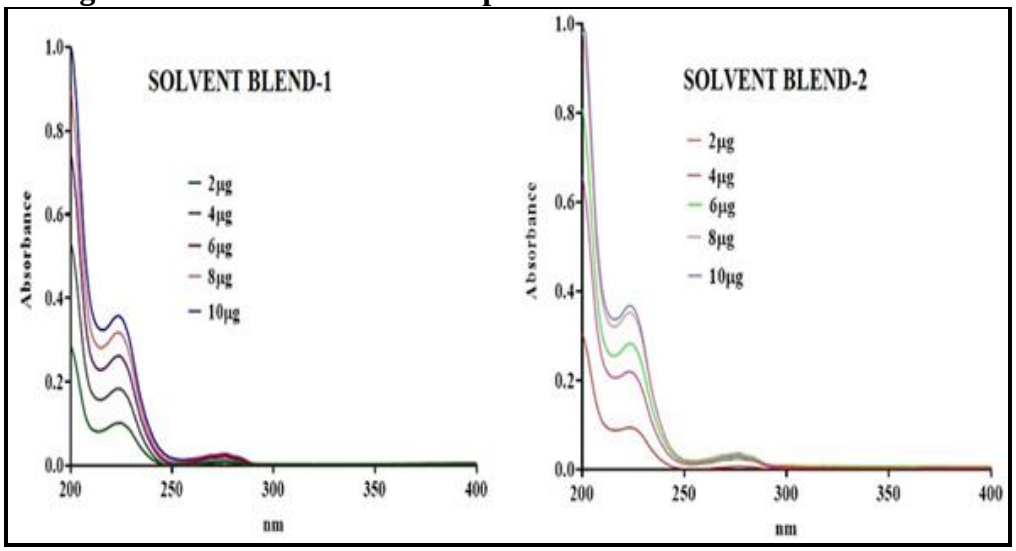

Figure No.4: Overlay absorption maxima plots of DP concentrations in Solvent blend-1 and 2

CONCLUSION

The present study was aimed to develop and validate simple UV spectroscopic method for the estimation of Dapagliflozin Propanediol Monohydrate in bulk and marketed tablets. The proposed method comprising Methanol: Distilled water (1:3) as Solvent blend-1 and Methanol: Distilled water (3:1) as Solvent blend-2. The accuracy and precision results suggest the method was found to be reproducible. The statistical parameters and the recovery data reveal high precision and accuracy of the methods besides being robust and rugged. Further in conclusion, the proposed UV spectrophotometric methods offer a simpler and equally reliable alternative to conventional chromatographic techniques, making them suitable for routine pharmaceutical analysis, particularly in resource-limited settings.

AUTHORSHIP CONTRIBUTION STATEMENT

Srushti

Methodology, Software, Writing Formal analysis, Validation;

Anand Kumar Yegnoor

Supervision, Formal analysis, Writing original draft, Review and Editing, Data curation.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest related to the publication of this manuscript. Both the authors have made equal contributions to the research and manuscript preparation and affirm that no financial or personal relationships exist that could improperly influence the content of this work.

ACKNOWLEDGEMENT

The authors express their gratitude toward Principal and Management of V.L. College of Pharmacy, Raichur and Karnataka, India for providing a platform to conduct the research work. The authors also express sincere thanks to Sri Umesh Shetty, Senior Vice President-R and D, Caplin Point, Laboratories, Chennai, for providing the gift sample of Dapagliflozin Propanediol Monohydrate.

BIBLIOGRAPHY

1. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea Sebal A, Siddiqi H, Uribe K B *et al*. Pathophysiology of type 2 diabetes mellitus, *Int J Mole Sci*, 21(17), 2020, 62-75.
2. Tambe V B, Tajane P S, Godge R K. Analytical method development and validation of dapagliflozin by UV-spectroscopy, *Int J Food Nutri Sci,* 11(1), 2022, 709-716.
3. Bodade B J, Kanade D A, Chaudhari S S. Quantitative estimation of dapagliflozin in blood plasma by using UV spectroscopy, *Pharm Anal Acta,* 10(2), 2019, 1-3.
4. Ganorkar S B, Sharma S S, Patil M R, Bobade P S, Dhote A M, Shirkhedkar A A. Pharmaceutical analytical profile for novel SGL-2 inhibitor: Dapagliflozin, *Crit Revi Anal Chem*, 51(8), 2020, 835-847.
5. Sravanthi S, Ramya Krishna D, Manjeera A. A new analytical method development and validation for the estimation of Dapagliflozin by using reverse phase-high performance liquid chromatography, *Int J Adv Res Med Pharm Sci,* 6(4), 2021, 13-20.
6. Ashok Kumar A. Analytical method development and verification of Dapagliflozin related substances estimation in Dapagliflozin tablets by RP-HPLC, *World J Pharm Pharm Sci,* 13(11), 2024, 1916-1929.
7. Suma B V, Deveswaran R, Shenoy P. A new high performance thin layer chromatographic method development and validation of Dapagliflozin in bulk and tablet dosage form, *Int J Pharm Pharm Sci,* 11(8), 2019, 58-63.
8. Caroline A, Grace A, Prabha T, Sivakumar T. Development and validation of high performance liquid chromatographic method for determination of Dapagliflozin and its impurities in tablet dosage forms, *Asian J Pharm Clin Res,* 12(3), 2019, 447-453.
9. Verma V M, Patel M M. Development and stability indicating HPLC method for Dapagliflozin in API and pharmaceutical dosage form, *Int J App Pharm,* 9(5), 2017, 33-41.

10. El-Shoubashy O H, Beltagy Y A, Issa A E, El-Kafrawy D S. Comparative study of HPLC-DAD and HPTLC for the simultaneous determination of a new multitarget antidiabetic ternary mixture in combined tablets, *J Planar Chromat*, 33, 2020, 59-52.
11. Game M D, Bopudi N. Development and validation of stability indicating HPLC method for estimation of Dapagliflozin in marketed formulation, *Int J Pharm Pharm Res*, 12(3), 2018, 123-144.
12. Phanindra A, Kumar Y S. Development and validation of sensitive LC-ESI-MS/MS method for the simultaneous estimation of Dapagliflozin and Saxagliptin in human plasma, *Int J Pharm Pharm Sci*, 11(4), 2019, 55-59.
13. Devrukhakar S P. Shankar S M. Degradation pathway proposal, structure elucidation, and in silico toxicity prediction of Dapagliflozin propane diol hydrolytic degradation products, *Chromatorphia*, 83, 2020, 1233-1245.
14. Annemarie B V, Wesselsa M A, Heerspinka H J L, Touw J D. Simple, fast and robust LC-MS/MS method for the simultaneous quantification of Canagliflozin, Dapagliflozin and Empagliflozin in human plasma and urine, *J Chromatogr B*, 1152, 2020, 122257.
15. ICH, International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for Human use ICH Harmonised tripartite guideline validation of analytical procedures: Text and Methodology Q2 (R1), *Fed Regist*, 62(96), 1997, 27463-27467.
16. Youssef R M, Korany M A, Afify M A. Development of a stability indicating HPLC DAD method for the simultaneous determination of mometasone furoate and salicylic acid in an ointment matrix, *Anal Method*, 6(10), 2014, 3410-3419.

Please cite this article in press as: Anand Kumar Yegnoor and Srushti. Validation and quantification of simple precise UV method for dapagliflozin propanediol monohydrate in bulk and marketed formulations, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, 12(4), 2024, 132-139.