Journal of Current Pharma Research (JCPR)

Volume 21, Issue 2, February 2025 **jcpr.humanjournals.com** ISSN: 2230-7842, 2230-7834

Green Spectrophotometric Method Development for the Estimation of Dapagliflozin and Glimepiride from the Pharmaceutical Dosage Form

Akash T. Kesare [1], Shubham S. Ghogare [1], Kshitij J. Salve [1], Vilas B. Pawar [1], Gurappa K. Dyade [1], Rajendra S. Bandal [1], Amit S. Lunkad [2]

[1] Dept of Post Graduate in Pharmaceutical Quality Assurance, SVPM'S College of Pharmacy, Malegaon (BKII)-413115 Baramati Dist Pune, Maharashtra, India

[2] Dept of Pharmaceutical Chemistry, Sitabai Thite College of Pharmacy, Shirur (Ghodnadi)-412210 Dist Pune, Maharashtra, India

Received: 2025-2-02 Revised: 2025-2-15 Accepted: 2025-2-21

Abstract

The aim of the present research was to develop analytical method utilizing sustainable simple solvents which enhances solubility of analyte, sensitivity of the method etc. An absorption spectrophotometric method was developed for the estimation of dapagliflozin (DGZ) and Glimepiride (GMP) by using aqueous 0.1 N NaOH. Wavelengths 228 nm and 267 nm were selected for absorbance measurement of GMP and DGZ respectively. Effect of input variables on spectrum characteristics were studied for selection of critical parameters and developed method was validated as per ICH Q 2 R1 regulatory guidelines. Linearity of the drugs was ascertained over the conc range 20 to 200µg/ml and 1 to 24µg/ml for DGZ and GMP respectively. The accuracy was found within acceptable limit with standard deviation 2.6152 to 3.71141for DGZ and 0.1155 to 1.6971for GMP; and the assay study data was found 101.37 % for DGZ and 101.09 % for GMP. The stability of the method was studied by minor variation in the wavelength, scan speed. The developed method is rigid, robust and efficient for the estimation of DGZ and GMP from their combination. The effort was made to develop sustainable green analytical method utilising commonly available solvent for dapagliflozin and water insoluble drug glimepiride.

Keywords : Sustainable method, Dapagliflozin, Glimepiride, green solvent, absorption spectroscopy

INTRODUCTION

Sustainable solvents are a topic of growing interest in both the research community and the chemical industry due to a growing awareness of the impact of solvents on pollution, environment. The prime objective of the present research was to use ecologically suitable solvent and to enhance the solubility of analyte. Aqueous solubility of a therapeutically active substance is a key property as it governs dissolution, absorption and thus the efficacy in vivo; and also restricts use of organic solvent in method development [1]. Articles [2, 3] are deliberately shows significance of agents like hydrotropes in solubilisation of very poor water soluble drug. The development of eco-friendly method by avoiding organic solvent could be termed as economical green method [4]. There is consistently pressure from environmental department to minimise hazardous and volatile solvent content in the waste which seriously affects environment. Use of hydrotropic solutions, supercritical fluids in the organic synthesis curbs use of organic solvent in view point of green chemistry [5]. Hydrotropes are capable of increasing the solubility of organic compounds up to 200 times in water [6]. In literature review it is revealed that green FT-IR method [7], eco-friendly method [8] is suitable for analytical purpose; and green analytical methods are preferred over analytical methods using harmful organic solvent for environment [9, 10]. Drugs dapagliflozin and glimepiride were selected for these research purpose studies due to wide difference in water solubility.

Dapagliflozin (DGZ) is a (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol [11] antidiabetic and pharmacologically sodium-glucose co-transporter 2 inhibitor that enhances urinary excretion of glucose by suppressing renal glucose reabsorption. It is used as monotherapy or with other antidiabetic treatment, including insulin, in the management of type 2-diabetes [12].

Various analytical methods have been reported for the estimation of DGZ alone or in combination with other anti-diabetic agents in pharmaceutical dosage form includes lonely UV spectroscopic method [13], with other drug UV spectroscopic method [14-17], RP-HPLC methods [18, 19] , stability indicating HPLC methods [20-23], bio analytical HPLC technique [24, 25], UHPLC bio analytical [26],

kinetic study UHPLC method [27], stability indicating UPLC method [28], UPLC DAD bio analytical [29], LC-MS/MS bio analytical method [30, 31] and critical review on bio analytical [32].

Glimepiride (GMP) is chemically 1-[[4-[2-(3-ethyl-4 methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulphonyl]-3-*trans*-(4-methyl cyclo hexyl) urea [11, 33, 34] and is oral anti-diabetic II generation sulfonylurea class of drug promotes insulin release at ATP-sensitive potassium channels on pancreatic β-cells via binding to a 65 kDa subunits of the sulfonyl urea receptor [12]. It is used for the treatment of type 2 diabetes mellitus and has duration of action of up to 24 Hrs [12].

Various analytical methods have been reported for the estimation of GMP alone or in combination with other anti-diabetic agents in pharmaceutical dosage form includes lonely UV spectroscopic methods [35-38], Stability indicating RP-HPLC method [39], RP-HPLC methods [40-44], bio analytical HPLC chromatographic methods [45-46], LC-MS/MS analytical technique [47], Bio-analytical LC-MS/MS methods [48-54], clinical study of glimepiride [55], efficacy of drug [56], clinical study of drug [57, 58], efiicacy of drug along with insulin[59], The drug Glimepiride is official in both pharmacopoeia IP and BP[33, 34]. Chemical structure of both the drugs is shown in Fig No 1.

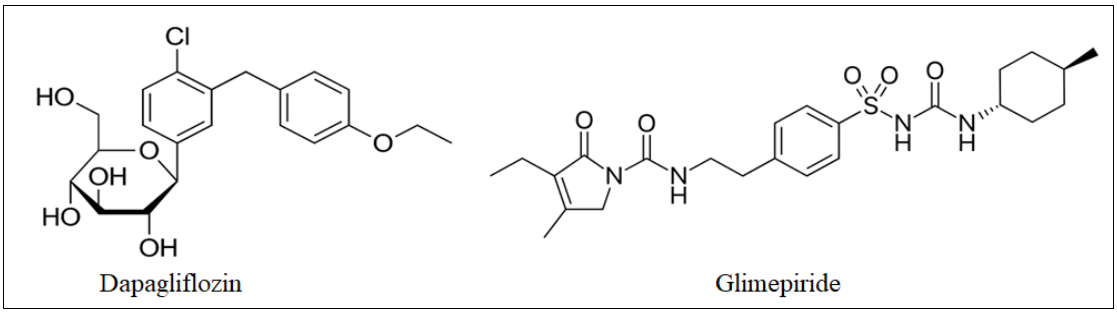

Fig 1: Chemical structure of Drug molecule

For analytical method validation ICH Q2 (R1) has given various method performance characteristics [60, 61].

MATERIALS AND METHODS

Instrumentation

Analysis was performed with a UV-1900i Shimadzu Double beam spectrophotometer (Shimadzu, Kyoto, Japan) with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with 10 mm matched Quartz cells was used. Drugs were weighed on electronic balance 'Afcoset' (The Bombay Burmah Trading corpo Ltd) with accuracy ± 0.1 mg Model No. ER 200A and Digital Ultrasonic cleaner 1.8 Ltr (Labman scientific Instruments Chennai) was used for degassing the solutions.

Reagents and Chemicals

Pharmaceutically pure samples of Dapagliflozin from Akums Drugs and Pharmaceuticals Ltd Haridwar Uttarakhand and Glimepiride from Zim Laboratories, Kalmeshwar Dist. Nagpur were procured as a gift samples and the commercial pharmaceutical formulation containing DGZ and GMP was purchased from the local Pharmacy; and NaOH AR grade, laboratory distilled purified water were utilised for making the solvent.

Solvent selection

Research article [62] was focused on techniques to be adopted while selection of suitable solvent. DGZ is freely soluble in alkaline, acidic solutions and soluble in water; also it is freely soluble in ethanol, DMSO and DMF. Similarly GMP is freely soluble in DMF, DMSO, dichloromethane, and chloroform; soluble in ethanol, slightly soluble in methanol, acetone, acetonitrile and dil alkali, dil acids, practically insoluble in water [12]. Although solubility of the procured drug was studied in 0.1 N HCL, water and 0.1 N NaOH; and to understand characteristic nature of spectra each solution of known conc of analyte was scanned in UV range. The recorded spectra in these solvents are shown in (Fig No 2 and 3). It was found that 0.1 N NaOH is suitable on the basis of common solubility and minimum/negligible interference at each other's wavelength.

Journal of Current Pharma Research (JCPR)

Volume 21, Issue 2, February 2025 **jcpr.humanjournals.com** ISSN: 2230-7842, 2230-7834

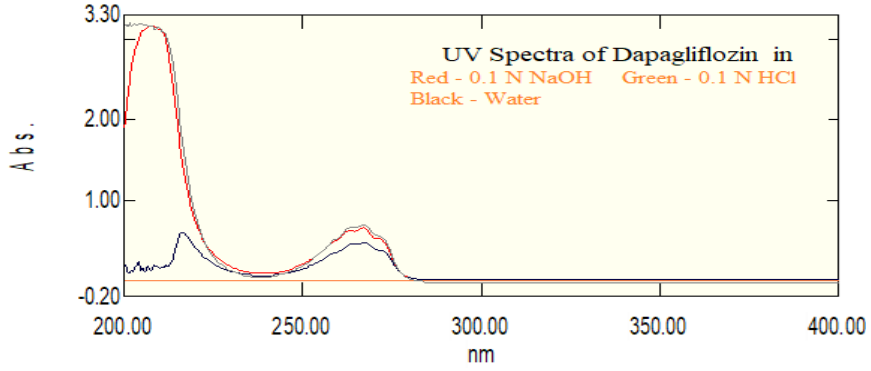

Fig 2: UV-VIS spectra of Dapagliflozin in selection of solvent study

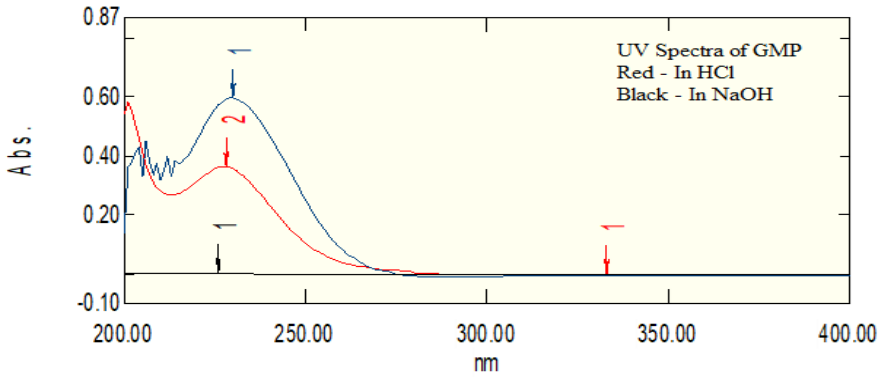

Fig 3: UV-VIS spectra of Glimepiride in selection of solvent study

Selection of wavelength and conc range

From UV spectra it was found that DGZ and GMP have measurable absorbance at 267 and 228 nm respectively. From the nature of spectra working conc range 20 to 200µg/ml and 1 to 24µg/ml was selected in solvent for DGZ and GMP respectively.

Above discussed observations was guided to select critical parameters listed in Table No 1 and by using these; method was validated as per ICH guidelines and by analysing marketed preparations.

Preparation of stock solutions and standard solutions

10 mg of pure drug DGZ and GMP were accurately and separately weighed; and transferred into separate 25 ml volumetric flask. Dissolved into 0.1 N NaOH solution and volume was made to 25 ml with solvent. Working standard solution of DGZ 100 µg/ml and GMP 10 µg/ml was obtained by diluting aliquot of stock solution.

Table No 1: Selected critical parameter for UV-VIS analytical method of DGZ and GMP

Parameter	Selected variables For DGZ	Selected variables For GMP
Wavelength range	400-200 nm	400-200 nm
Wavelength	267 nm	228 nm
Solvent	0.1 N NaOH	0.1 N NaOH
Scan speed	Fast	Fast
Sampling interval	± 0.2 nm	± 0.2 nm

Experimental Method for estimation

From the overlain spectra it was found that many approaches of multicomponent analysis are applicable for simultaneous estimation of both these drugs. Among of this simultaneous equation method and absorption correction method was selected for estimation of DGZ and GMP from the combined dosage form.

Journal of Current Pharma Research (JCPR)

Volume 21, Issue 2, February 2025 **jcpr.humanjournals.com** ISSN: 2230-7842, 2230-7834

Method-I: Simultaneous equation method

DGZ was shown absorbance at (λ_{max}) 267 nm and GMP has maximum absorbance (λ_{max}) at 228 nm. The wavelength 228 and 267 nm was considered as 1 (λ_1) and 2 (λ_2) respectively. The equation A= abc was applied for x (GMP) and y (DGZ) determination. On rearranging the 2 generated equations, the conc of x and y was calculated by following formula. Working standard solutions of GMP of conc 10 µg /ml and DGZ of conc 100 µg /ml were separately prepared and used for the method.

$$C_x = \frac{A_2 . a_{y_1} - A_1 . a_{y_2}}{a_{x_2} . a_{y_1} - a_{x_1} . a_{y_2}}$$

$$C_y = \frac{A_1 . a_{x_2} - A_2 . a_{x_1}}{a_{y_1} . a_{x_2} - a_{y_2} . a_{x_1}}$$

Where CX and Cy = Conc of GMP and DGZ in sample solution

A_1 and A_2 = absorbance of sample solution at 1 and 2 wavelength

ay_1 and ay_2 = absorptivity of DGZ at 1 and 2 wavelength of standard solution

ax_1 and ax_2 = absorptivity of GMP at 1 and 2 wavelength of standard solution

Method-II Absorbance correction method

DGZ was shown maximum absorbance at 267 nm; and GMP has maximum absorbance at 228. At 267 nm negligible absorbance of GMP was found hence this wavelength was suitable for exclusive measurement of DGZ; and 228 nm where DGZ shows moderate interference, which was corrected by absorbance correction. DGZ of different conc was made and absorbance measured, from 3 sets of measurement absorptivity of DGZ decided. The equation A= abc was applied for x (GMP) and y (DGZ) determination. Working standard solutions of GMP and DGZ having conc 10mcg/ml and 100mcg/ml respectively were separately prepared and used for the method.

At 267 nm $A = a_{y2} .b.c_y$

Where A = absorbance of sample at 267 nm

a_{y2} = absorptivity of drug at 267 nm

b = Pathlength of solution 1 cm

C_y = Conc of DGZ in sample

$$C_Y = \frac{A_s - aX_2}{aY_2}$$

At 228 nm $A_s = A_1 + A_2$

$A_s = a_{X1} .b. C_x + a_{y1} .b. C_y$

On rearranging equation

$$C_X = \frac{A_s - aY_1\ C_Y}{aX_1}$$

A_s = absorbance of sample containing GMP and DGZ at 228 nm

a_{X1} = absorptivity of GMP at 228 nm

a_{y1} = absorptivity of DGZ at 228 nm

b = Pathlength of solution 1 cm

C_x = Conc of GMP in sample

Validation of the Method

Selected critical parameters should meet the performance characteristics of the analytical method so as to attain analytical target profile of the method. An ICH guideline Q2 R1 was applied to study methods performance with critical parameters in order to implement part of AQbD approach. The method was validated as per ICH guidelines.

System suitability

System suitability is studied to demonstrate the suitability of the developed procedure under consideration for the analytical method. Six replicates of working standard solutions with conc12 mcg/ml of GMP and 120 mcg/ml of DGZ were prepared separately and absorbance was recorded, and SD and % RSD of the response was calculated.

Linearity

The linearity of an analytical method is its ability to obtain response i.e. absorbance which is directly proportional to the conc of analyte. Series of working standard solutions were prepared in conc. range of 1-24µg/ml for GMP and 20-200µg/ml for DGZ and scanned in 200 to 400 nm range in spectrum mode of the spectrophotometer, absorbance of the standard solutions were recorded at their respective wavelength; i.e. 228 for GMP and 267 nm for DGZ in spectrum order. Microsoft office excel software tool was used to obtain the standard regression curve and its analysis as slope, intercept, and correlation coefficient.

Assay of formulation

Assay was carried out by proposed methods and assay was validated by statistical parameters.

Estimation of combination by simultaneous equation method

Powder equivalent to 1 mg GMP and 10 mg DGZ was weighed and transferred into 25 ml volumetric flask. Dissolved into 0.1 N NaOH and volume was made to 25ml with solvent. Solution was filtered through what man filter paper and aliquots of solution were further diluted to obtain tablet solution. Solution was scanned in the range of 200 to 400 nm to obtain absorbance of tablet solution at 228 and 267 nm in spectrum order. Obtained absorbance were utilised to estimate unknown conc of formulation; and results were statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

Accuracy and Precision

The accuracy of an analytical method expresses the closeness of an agreement between test result and true result. Accuracy study was performed by recovery study i.e. standard addition method; diluted standard solutions of GMP and DGZ were prepared and standard solutions added in 80,100 and 120% proportionate to the tablet solution. Three replicates at each of these three levels were prepared and measured and % of conc, SD and RSD were calculated.

The precision study was carried out by performing assay of tablet six times; also the reproducibility in result was studied by inter day and intraday precision.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of GMP and DGZ by the proposed method were determined using calibration graph method and calculated as 3.3σ/s and 10 σ/s for LOD and LOQ respectively; σ is the standard deviation of calibration curve and s is the slope of regression line.

Robustness and Ruggedness

It is measure of capacity of analytical procedure to remain unaffected by small but deliberate variations in method parameter.

RESULTS AND DISCUSSION

Method development comprises numerous steps; of which solvent selection, method for measurement selection are significant one. Uses of eco-friendly solvents have got remarkable weightage due to low cost, readily available and environmentally sound. Drugs underlying analysis must have appreciable solubility in the selected solvent. Chemical structure of the drug and physico-chemical properties available in the literature guides about use of appropriate solvent in the method. Solubility of GMP and DGZ was studied in each solvent; and in 0.1 N NaOH solvent both drugs were shown maximum and consistent absorbance as compare to other solvent.

System Suitability

The absorbances of six replicates of standard solutions) are reported in Table No 2 and overlaid spectra of both drug is shown in Fig No 4 (120 and 12 µg/ml. The SD was found for DGZ and GMP within acceptable limit and meets the system suitability requirements indicates method was suitable for analysis.

Table No 2: System suitability study of DGZ and GMP

S N	Conc in µg /ml	Absorbance of DGZ	Conc in µg /ml	Absorbance of GMP
1	120 µg /ml	0.464	12 µg /ml	0.582
2	120 µg /ml	0.488	12 µg /ml	0.572
3	120 µg /ml	0.453	12 µg /ml	0.571
4	120 µg /ml	0.454	12 µg /ml	0.590
5	120 µg /ml	0.484	12 µg /ml	0.577
6	120 µg /ml	0.467	12 µg /ml	0.592
	SD	0.01478	SD	0.008936

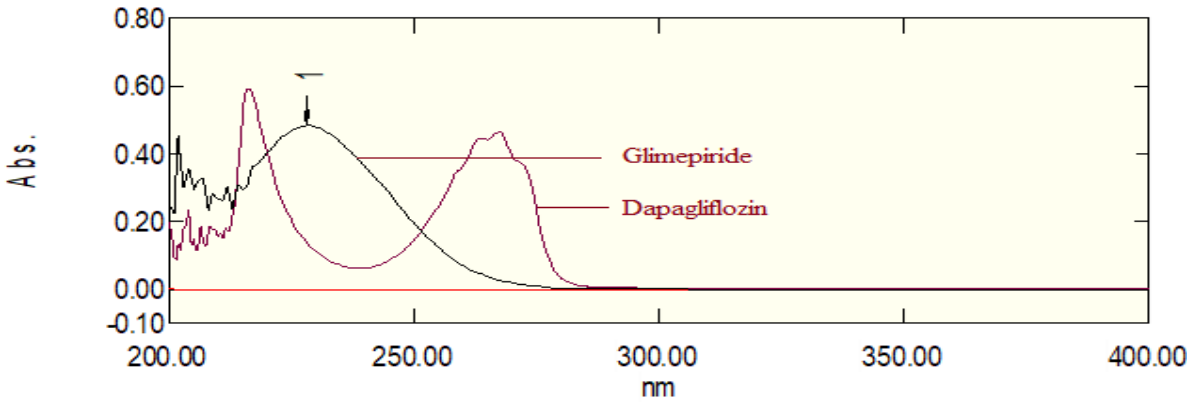

Fig No 4: Overlain spectra of GMP and DGZ in spectrum mode

Linearity

The overlay spectra obtained in linearity study was shown in Fig No 5 and 6 and the obtained calibration curve of both analytes was found to be linear in the selected conc range as shown in Fig No 7. The regression equation of line and its parameters slope, r^2 value and intercept are tabulated in Table No 3, which proved the linear relationship between conc and obtained response.

Journal of Current Pharma Research (JCPR)
Volume 21, Issue 2, February 2025 **jcpr.humanjournals.com** ISSN: 2230-7842, 2230-7834

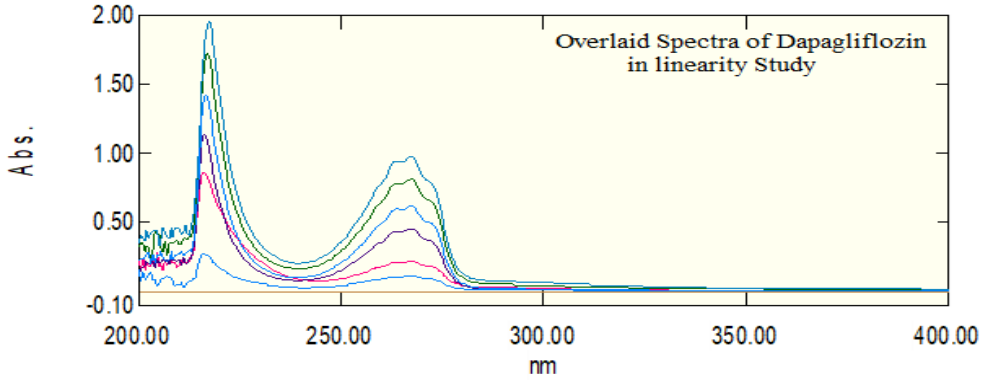

Fig 5: UV-VIS overlain spectra of DGZ in linearity study

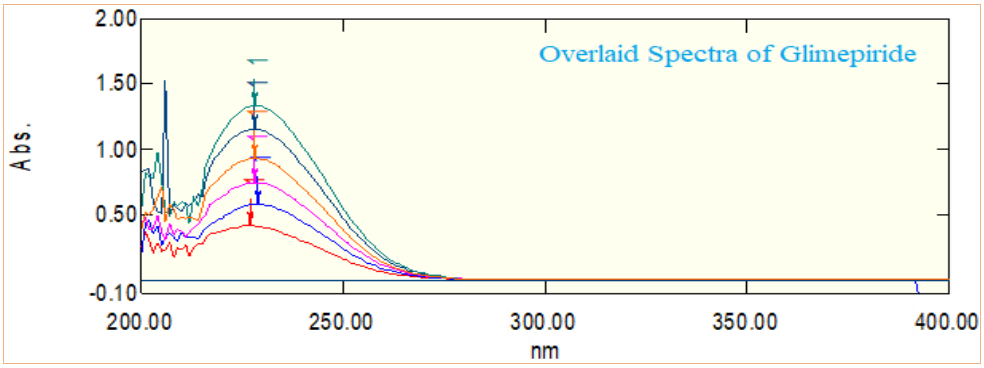

Fig 6: UV-VIS overlain spectra of GMP in linearity study

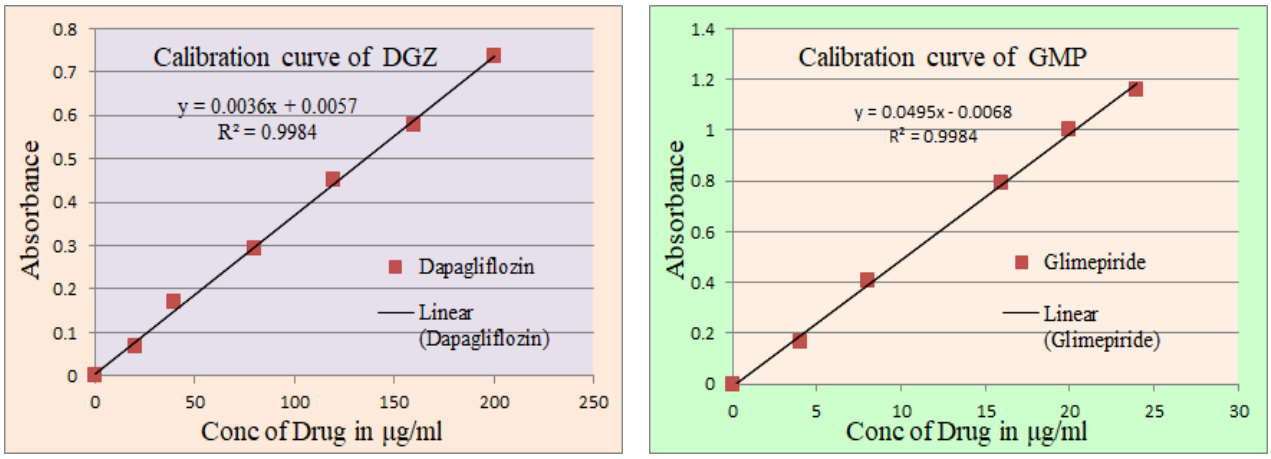

Fig 7: Calibration curve of Dapagliflozin and Glimepiride

Table No 3: Parameters of regression equation obtained in Microsoft excel office

Parameters	**DGZ**	**GMP**
Detection wavelength	267 nm	228 nm
Beer's law limit (µg/ml)	20-200 µg/ml	1-24 µg/ml
Correlation coefficient (r^2)	0.9934	0.9984
Regression equation (y = mx + c)	y=0.0036x+0.0057	y=0.0495x-0.0068

Assay

The assay was carried out by both the methods. The spectra of formulation was obtained (Shown in Fig No 8) and calculated % of nominal conc and SD, data was found within acceptable limits are summarized in Table No 4. The results indicated applicability of the method for estimation of formulation.

Journal of Current Pharma Research (JCPR)
Volume 21, Issue 2, February 2025 jcpr.humanjournals.com ISSN: 2230-7842, 2230-7834

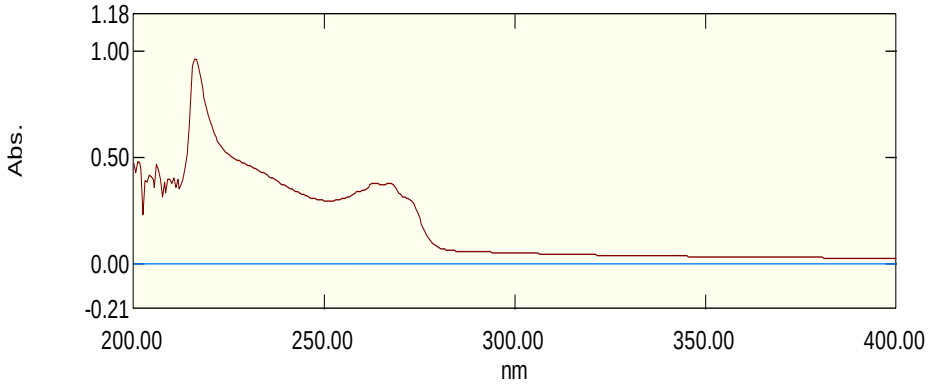

Fig 8: UV-VIS spectra of Formulation

Table No 4: Results of assay of formulation by proposed method

Name	Drug	Label Claim (mg/Tablet)	Amount found/mg; n=6	Drug Content %	Std Deviation	% RSD
Formulation	DGZ	10 mg	10.13 mg	101.37	2.3624	1.9418
	GMP	1 mg	1.012 mg	101.09	4.6703	4.5792

Accuracy and Precision

The results of accuracy are summarised in Table No 5a of method I and 5b of method II, the obtained results were within acceptable limit; and methods accuracy was justified by calculating % drug content. The precision study was carried out by performing assay of solutions; further the reproducibility in result was studied by interday and intraday precision. The values obtained SD and % RSD was shown methods precision and are summarised in Table No 5a and 5b.

Table No 5a: Results of accuracy and precision for method -I

Sr No	Parameter	Level of study	Data Title	Obtd. Data	S.D.	RSD
1 (Method –I)	Precision study of DGZ	Intraday Precision	Mean of Abs n= 6	106.89	3.5101	3.2901
		Interday precision		98.37	2.0615	2.0956
	Precision study of GPE	Intraday Precision	Mean of Abs n= 6	98.87	1.5003	1.5173
		Interday precision		98.02	1.3715	1.3991
2 (Method –I)	Accuracy study of GPE	80%	% Purity found	99.96	0.1155	0.1556
		100%		106.33	0.7560	0.7115
		120%		106.61	1.6971	1.5919
	Accuracy study of DGZ	80 %	% Purity	99.34	3.1961	3.2172
		100 %		102.66	3.7141	3.6176
		120 %		102.81	2.6152	2.5441

Table No 5b: Results of accuracy and precision for method-II

Sr No	Parameter	Level of study	Data Title	Obtd. Data	S.D.	RSD
1 (Method-II)	Precision study of DGZ	Intraday Precision	Mean of Abs n= 6	102.174	1.6959	1.6598
		Interday precision		101.391	2.4661	2.4322
	Precision study of GPE	Intraday Precision	Mean of Abs n= 6	102.527	1.8334	1.7931
		Interday precision		98.388	0.8273	0.8409
2 (Method-II)	Accuracy study of GPE	80%	% Purity found	97.04	1.7607	1.8143
		100%		99.16	2.1709	2.1892
		120%		96.93	6.0677	6.2595
	Accuracy study of DGZ	80 %	% Purity	99.68	3.1475	3.1575
		100 %		102.18	2.1808	2.1343
		120 %		100.04	4.2002	4.1985

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of DGZ and GMP by the proposed method were found within acceptable limit.

Robustness and Ruggedness

Robustness was studied and capacity of analytical procedure to measure analyte was remain unaffected by small but deliberate variations in method parameter like variation in the wavelength ± 1 nm, variation in the solvent strength by ± 0.1 %. The analytical method was found rugged during development; similarity the result was produced by performing the analysis by different analyst.

CONCLUSION

Both the methods were developed with eco-friendly, readily available and economical aqueous 0.1 N NaOH solvent for the estimation of Dapagliflozin and Glimepiride from the combination and obtained results were found satisfactory. The simultaneous equation method was given reproducible results as compare to absorption correction method; however obtained results of both the methods were within acceptable limits given in the pharmacopoeia. The validated method is economical, precise, accurate, robust and reproducible hence can be routinely used for estimation of both the drugs concurrently.

CONFLICT OF INTEREST

All Authors declared that there is no conflict of interest.

ACKNOWLEDGEMENT

Authors are thankful to Akums Drugs and Pharmaceuticals Ltd Haridwar Uttarakhand for providing pure drug dapagliflozin and to Zim Laboratories, Kalmeshwar Dist. Nagpur for providing Glimepiride as a gift sample. Moreover Authors are thankful to Management, Principal SVPM'S College of Pharmacy Malegaon BKII Baramati Dist. Pune, Maharashtra for providing facilities for research.

REFERENCES

1) Coby J. Clarke, Wei-Chien Tu, Oliver Levers, Andreas Bröhl, and Jason P. Hallett. Green and Sustainable Solvents in Chemical Processes. Chemical Reviews 2018 118 (2), 747-800
DOI: 10.1021/acs.chemrev.7b00571.
2) Sabitha Reddy P, Swetha C , Ravindra Reddy K. Effect of Hydrotropes and Physical Properties on Solubility of Glibenclamide. Research J. Pharma. Dosage Forms and Tech. 2011; 3(6): 294-297.
3) Nilesh S. Kulkarni, Sanghamitra B. Ghule, Shashikant N. Dhole. A Review on Hydrotropic Solubilization for Poorly Water Soluble Drugs: Analytical Application and Formulation Development. Research J. Pharm. and Tech. 2019; 12(7):3157-3162. Research J. Pharm. and Tech. 2019; 12(7):3157-3162.
4) Gurumurthy V., Deveswaran R., Bharath S., Basavaraj B.V., Madhavan V. Application of Hydrotropic Solubilisation in Simultaneous Estimation of Atenolol and Amlodipine Besylate. Asian J. Research Chem. 2012; 5(1): 57-60.
5) Ceema Mathew, Sunayana Varma. Green Analytical Methods based on Chemometrics and UV spectroscopy for the simultaneous estimation of Empagliflozin and Linagliptin. Asian Journal of Pharmaceutical Analysis. 2022; 12(1):43-48.
6) Reem H. Obaydo, Amir Alhaj Sakur. A Green Analytical Method using Algorithm (PCCA) for Extracting Components' Contribution from Severely Overlapped Spectral Signals in Pharmaceutical Mixtures. Research J. Pharm. and Tech 2019; 12(9):4332-4338.
7) Parixit Prajapati, Chandni Chandarana. Fourier Transform Infrared Spectrophotometry: An Eco-friendly green tool for quantification of Omeprazole in Pharmaceutical formulation. Research Journal of Pharmacy and Technology. 2022; 15(8):3531-4.
8) Haripriya A., Sirisha N., Vishali S., Ramakrishna K., Panikumar A.D.. Validated Eco - Friendly Derivative Spectrophotometric Method for Valsartan and Hydrochl**o**rothiazide Combined Tablet Dosage Form. Asian J. Research Chem. 2012; 5(8): 1074-1077.
9) Remi. S. L, Joyamma Varkey, R. K. Maheshwari, A. Jayakumaran Nair. A Novel Ecofriendly, Cost effective mobile phase for HPLC- Simultaneous estimation and Validation of Paracetamol and Diclofenac sodium in Bulk and Pharmaceutical Formulation by RP-HPLC using Hydrotropic Solution as Mobile phase. Asian J. Pharm. Res. 2020; 10(3):163-170.
10) Yashwant S. Surve, Dharmesh G. Panchal, R.S. Lokhande. A novel methodology for the synthesis of teriflunomide using hydrotropes as a reaction media. Research J. Pharm. and Tech. 2015; 8(9): 1247-1249.
11) The Merck Index, An Encyclopaedia of chemicals, drugs and Biological, 15th edition, the royal society of chemistry Cambridge UK, 2013, pp. 506, 820.
12) Alison Brayfield, Martindale (The complete drug reference), 39th edition, Pharmaceutical press London, 2017, A: pp. 476, 480.

13) Mante G V, Gupta K R, Hemke A T. Estimation of Dapagliflozin from Its Tablet Formulation by UV-Spectrophotometry. Pharm Methods. 2017; 8(2):102-107.
14) Jani B R, Shah K V, Kapupara P P. Development and Validation Of UV Spectroscopic Method For Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride an Synthetic Mixture. International Journal of Research and Development In Pharmacy & Life Sciences. 2015; 4(3):1569-1576.
15) Sen A K, Khatariya S B, Sen D B, Maheshwari R A, Zanwar A S, Velmurugan R. Various Innovative UV Spectroscopic Methodologies For Concurrent Estimation Of Dapagliflozin And Vildagliptin In Combined Tablet. Journal of Applied Pharmaceutical Science. 2023; 13(9):213-23.
16) Bhavyasri K, Surekha T, Sumakanth M. A Novel Method Development And Validation Of Dapagliflozin And Metormin Hydrochloride Using Simultaneous Equation Method By UV–Visible Spectroscopy In Bulk And Combined Pharmaceutical Formulation Including Forced Degradation Studies. Journal Of Pharmaceutical Sciences And Research. 2020; 12(8):1100-5.
17) Barbude P, Tawar M, Burange P. Method Development Using A UV Visible Spectrophotometer For The Simultaneous Estimation Of Metformin (Met), Saxagliptin (Sxg), And Dapagliflozin (Dgf) In Marketed Formulation. Asian Journal of Pharmaceutical Analysis. 2022; 12(4): 243-247.
18) Rao B R, Rao V V, Venkateswarlu B S. Rp-Hplc Method For Simultaneous Estimation of Dapagliflozin And Saxagliptin In Bulk Samples. Journal of Pharmaceutical Sciences and Research. 2019; 11(1):254-257.
19) Debata J, Kumar S, Jha S K, Khan A. A New RP-HPLC Method Development and Validation of Dapagliflozin in Bulk and Tablet Dosage Form. Int J Drug Dev Res. 2017; 9(2):48-51
20) Deepan T, Dhanaraju M D. Stability Indicating Hplc Method For The Simultaneous Determination Of Dapagliflozin And Saxagliptin In Bulk And Tablet Dosage Form. Current Issues In Pharmacy And Medical Sciences. 2018; 31(1):39-43.
21) Kommineni V, Chowdary K P, Prasad S V. Development Of A New Stability Indicating Rp-Hplc Method For Simultaneous Estimation Of Saxagliptine and Dapagliflozin and its Validation as Per ICH Guidelines. Indo American journal of pharmaceutical sciences. 2017; 4(09):2920-32.
22) Singh N, Bansal P, Maithani M, Chauhan Y. Development and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Determination of Dapagliflozin and Saxagliptin in Fixed-Dose Combination. New Journal of chemistry. 2018; 42(4):2459-66.
23) Manoharan G, Ismaiel A M, Ahmed Z M. Stability-Indicating Rp-Hplc Method Development for Simultaneous Determination and Estimation Of Dapagliflozin In Raw And Tablet Formulation. Chem Res Journal. 2018; 3(2):159-64.
24) Ameeduzzafar, et al. Quality By Design (Qbd) Based Development And Validation Of Bioanalytical Rp-Hplc Method For Dapagliflozin: Forced Degradation And Preclinical Pharmacokinetic Study. Journal of Liquid Chromatography & Related Technologies. 2020; 43(1-2):53-65.
25) Kazi M, Alqahtani A A, Alsaadi B S, Alkholief M, Alanazi F K. UHPLC Method Development for Determining Sitagliptin and Dapagliflozin in Lipid-Based Self-Nanoemulsifying Systems as Combined Dose in Commercial Products and its Application to Pharmacokinetic Study of Dapagliflozin in Rats. Pharmaceutical Chemistry Journal. 2019; 53:79-87.
26) Zaghary WA, Mowaka S, Hendy M S. Kinetic Degradation Study of Dapagliflozin Coupled with UHPLC Separation in the Presence of Major Degradation Product and Metformin. Chromatographia. 2019; 82:777-89.
27) Bueno L, Manoel J W, Koetz M, Henriques A T, Steppe M, Schapoval E E. Simultaneous Analysis of Dapagliflozin and its Three Related Impurities by Stability-Indicating UPLC Method and in Vitro Toxicity Evaluation. Drug Analytical Research. 2022; 6(2):27-37.
28) Mabrouk M M, Soliman S M, El-Agizy H M, Mansour F R. A UPLC/DAD Method for Simultaneous Determination of Empagliflozin and Three Related Substances in Spiked human Plasma. BMC Chemistry. 2019; 13:1-9.
29) Chan-Jiang E, Godoy R, Mennickent S, Vergara C, De Diego M. Determination of the Chemical Stability of Dapagliflozin by LC/DAD and MS/MS Methods. Journal of Chromatographic Science. 2022; 60(8):741-9.
30) Aubry A F, et al. Validated LC–MS/MS Methods for the Determination of Dapagliflozin, a Sodium-Glucose Co-Transporter 2 Inhibitor in Normal and ZDF Rat Plasma. Bioanalysis. 2010; 2(12):2001-2009.
31) Goday S, Shaik A R, Avula P. Development and Validation of a LC-ESI-MS/MS Based Bioanalytical Method for Dapagliflozin and Saxagliptin in Human Plasma. Indian Journal of Pharmaceutical Education and Research. 2018; 52(4):S277-86.
32) Desai S, Maradia R B, Suhagia B N. A Comprehensive and Critical Review on Analytical and Bioanalytical Methods for Metformin Hydrochloride, Dapagliflozin, and Saxagliptin. Current Pharmaceutical Analysis. 2023; 19(1):20-50.
33) British Pharmacopoeia, Medicines and Healthcare products regulatory agency London, 2019, I: 1157.
34) Indian Pharmacopoeia, Govt of India, ministry of Health and family welfare, 8th edition, The Indian pharmacopoeia commission Ghaziabad, 2018, II: pp. 2177.
35) Sacide Altinoz, Dilara Tekel. Analysis of glimepiride by using derivative UV spectrophotometric method. Journal of Pharmaceutical and biomedical analysis. 2001; 24 (3): 507-515.
36) Rudy Bonfilio, Magali B de Araújo, Hérida Salgado. Development and validation of an UV-derivative spectrophotometric method for determination of glimepiride in tablets. Journal of the Brazilian Chemical Society. 2011; 22(2): 292-299.
37) Ozadheoghene E Afieroho, Ogbonna Okorie, Tochukwu J N Okonkwo. An ultraviolet-spectrophotometric method for the determination of glimepiride in solid dosage forms. Diabetes Technology & Therapeutics. 2011; 13 (6): 671-674.

38) Sakala Bhargavi, Gopisetty Suryasagar, Dantu Krishna Sowmya, Kota Ashok, Sreekanth Nama. UV spectrophotometric method for determination of glimepiride in pharmaceutical dosage forms. International Journal of Pharmaceutical Science Review and Research. 2013; 21 (2): 131-133.
39) Rudy Bonfilio, Carolina Peres, Herida R N Salgado, Magali B De Araujo, Cesar R T Tarley. Multivariate development and validation of a stability-indicating HPLC method for the determination of glimepiride in tablets. Journal of AOAC International. 2013; 96 (5): 960-967.
40) Petra Kovarikova, Jiri Klimes, Jiri Dohnal, Lucie Tisovska. HPLC study of glimepiride under hydrolytic stress conditions. Journal of Pharmaceutical and Biomedical Analysis. 2004; 36 (1): 205-209.
41) Abdul Bari Mohd, Krishna Sanka, Rakesh Gullapelly, Prakash V Diwan, Nalini Shastri. (2014).Development and validation of RP-HPLC method for glimepiride and its application for a novel self-nano emulsifying powder (SNEP) formulation analysis and dissolution study. Journal of Analytical Science and Technology, 2014; 5(27): 1-8
42) Abdul Bari Mohd, Krishna Sanka, Rakesh Gullapelly, Prakash V Diwan, Nalini Shastri. Development and validation of RP-HPLC method for glimepiride and its application for a novel self-nanoemulsifying powder (SNEP) formulation analysis and dissolution study. Journal of Analytical Science and Technology. 2014; 5(27): 1-8.
43) Rajendra Narayan Dash, Habibuddin Mohammed, Touseef Humaira. An integrated Taguchi and response surface methodological approach for the optimization of an HPLC method to determine glimepiride in a supersaturatable self-nanoemulsifying formulation. Saudi Pharmaceutical Journal. 2016; 24 (1): 92-103.
44) Khan I U, Aslam F, Ashfaq M, Asghar M N. Determination of glimepiride in pharmaceutical formulations using HPLC and first-derivative spectrophotometric method. Journal of Analytical Chemistry. 2009; 64: 171-175.
45) Sujatha Samala, Sandhya Rani Tatipamula, Ciddi Veeresham. Determination of glimepiride in rat serum by RP-HPLC method. American Journal of Analytical Chemistry. 2011; 2 (2): 152-157.
46) Rita Abi Daoud, Chawki Atallah, Antoine Zoghbi. A simple and sensitive method for determination of glimepiride in human serum by HPLC. Journal of liquid Chromatography and Related technologies. 2005; 28 (20): 3255-3263.
47) Gulshan Bansal, Manjeet Singh, Jindal K C, Saranjit Singh. LC–UV–PDA and LC–MS studies to characterize degradation products of glimepiride. Journal of Pharmaceutical and Biomedical Analysis. 2008; 48 (3): 788-795.
48) Constantinos Pistos, Maria Koutsopoulou, Irene Panderi. Improved liquid chromatographic tandem mass spectrometric determination and pharmacokinetic study of glimepiride in human plasma. Biomedical Chromatography. 2005; 19 (5): 394-401.
49) Hohyun Kim, Kyu Young Chang, Chang Hun Park, Moon Sun Jang e*t.al.* Determination of glimepiride in human plasma by LC-MS-MS and comparison of sample preparation methods for glimepiride. Chromatographia, 2004; 60: 93-98.
50) N Yuzuak, T Ozden, S Eren, S Ozilhan. Determination of glimepiride in human plasma by LC–MS–MS. Chromatographia. 2007; 66: 165-168.
51) Yun-Kyoung Song, Jeong-Eun Maeng, Hye-Ryung Hwang, Jeong-Sook Park, Bae-Chan Kim, Jin-Ki Kim, Chong-Kook Kim. Determination of glimepiride in human plasma using semi-microbore high performance liquid chromatography with column-switching. Journal of Chromatography B. 2004; 810 (1): 143-149.
52) Lagishetty Chakradhar, Rajareddy Kallem, Arumugam Karthik, Bala Tripura Sundari . *et. al*. A rapid and highly sensitive method for the determination of glimepiride in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry: application to a preclinical pharmacokinetic study. Biomedical Chromatography. 2008; 22 (1): 58-63.
53) Isam Ismail Salem, Jafer Idrees, Jaafar I Al Tamimi. Determination of glimepiride in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry. Journal of Chromatography B. 2004; 799 (1): 103-109.
54) Yannis Dotsikas, Constantinos Kousoulos, Georgia Tsatsou, Yannis L Loukas. Development of a rapid method for the determination of glimepiride in human plasma using liquid-liquid extraction based on 96-well format micro-tubes and liquid chromatography/tandem Mass Spectrometry. Rapid communication Mass Spectrometry. 2005; 19 (14): 2055-2061.
55) Xiaoqin Zhao, Ping Huang, Jie Yuan. Influence of glimepiride plus sitagliptin on treatment outcome, blood glucose, and oxidative stress in diabetic patients. American Journal of Translational Research. 2022; 14 (10): 7459-7466.
56) Subodh Kumar, Anuj Kumar Pathak, Dibyajyoti Saikia, Amish Kumar. Efficacy, safety and treatment satisfaction of glimepiride vs sitagliptin in combination with metformin in type 2 diabetes mellitus. Journal of Clinical and diagnostic research: JCDR 2015; 9 (12), FC07.
57) Preeti Singh, Ruchi Choudhary, V K Singh, Prithpal S Matreja. Comparison of metabolic effects of glimepride and sitagliptin with metformin in patients suffering from type 2 diabetes mellitus in a tertiary care hospital. International Journal of Basic Clinical Pharmacology. 2019; 8(7): 1467-1472.
58) Devarajan T V, Venkataraman S, Narayanan Kandasamy, Abraham Oomman, Hari Kishan Boorugu, Karuppiah S K P, Dushyant Balat.Comparative evaluation of safety and efficacy of glimepiride and sitagliptin in combination with metformin in patients with type 2 diabetes mellitus: Indian multicentric randomized trial-start trial. Indian Journal of Endocrinology and Metabolism. 2017; 21 (5): 745-750.
59) Xue Chen, Sheng Kang, Zeqing Bao. Effects of Glimepiride Combined with Recombinant Human Insulin Injection on Serum IGF-1, VEGF and TRACP-5b Oxidative Stress Levels in Patients with Type 2 Diabetes Mellitus. Evidence-Based Complementary and Alternative Medicine. 2022; doi: 10.1155/2022/4718087.
60) ICH Expert working group. ICH Harmonized tripartite Guideline-Pharmaceutical Quality system Q 10. In current step 4Th version. 2008; p. 1-21.

61) ICH Expert working group. ICH Harmonized tripartite Guideline-Validation of analytical procedures: Text and methodology Q 2 R1. In current step 4 version. 2005; p. 1-17.
62) Byrne, F.P., Jin, S., Paggiola, G. et al. Tools and techniques for solvent selection: green solvent selection guides. Sustain Chem Process. 2016; 4(7): 1-24. Doi.org/10.1186/s40508-016-0051-z.

How to cite this article:
Akash T. Kesare et al. Jcpr.Human, 2025; Vol. 21 (2): 1-12

Conflict of Interest Statement:
The authors have no conflicts of interest to declare.