Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 13 [3] February 2024 : 228-234

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Exploration and Validation of a Spectrophotometric Q-Absorbance Ratio Approach for Concurrent Determination of Sitagliptin Phosphate and Empagliflozin

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ABSTRACT

A new modest, exact, precise, sensitive and inexpensive UV spectrophotometric absorbance ratio method was established and simultaneously authenticated for the concurrent assessment of sitagliptin phosphate and empagliflozin in bulk form. The technique involves intricate Q-absorbance ratio study using two wavelengths, the isobestic point of both drugs (274.5 nm) and the λmax of sitagliptin phosphate (267nm) and the other being. Methanol:Water (5:5) has been employed as common solvent for the proposed method. The standardization plot was ranked to be linear between 50-250 µg/ml for sitagliptin phosphate and empagliflozin with R^2= 0.9957 and 0.9981 respectively. Process validation was accomplished as per ICH requirement for linearity, correctness, meticulousness, system appropriateness, sturdiness, sensitivity and specificity. The proposed approach was modest, exact, delicate, precise, rapid and appropriate for repetitive quality scrutiny of sitagliptin phosphate and empagliflozin in bulk and commercial formulations encompassing combination of these two drugs in the future.

Keywords: *Sitagliptin phosphate, Empagliflozin, Q-absorbance ratio method, UV spectroscopy, Isobestic point, ICH guidelines.*

Received 12.11.2023 Revised 23.12.2023 Accepted 14.02.2024

INTRODUCTION

Sitagliptin phosphate (SP) is an orally existing, competitive, beta-amino acid derived inhibitor of DPP-IV (dipeptidyl peptidase -IV) employed in the management of Type-2 Diabetes mellitus (T2DM). The suppression of DPP-IV induces the augmented levels of GIP (glucose dependent insulinotropic polypeptide) and GLP-1 (Glucagon like peptide-1) incretin hormones which ultimately reduces the blood glucose levels. Sitagliptin phosphate monohydrate[1,2] chemically is, (3R)-3-amino 1-[3-(trifluoromethyl)-5,6-dihydro [1,2,4] triazolo[4,3-α] pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one phosphate monohydrate. Fig. 1 depicts the Sitagliptin Phosphate's chemical structure. Empagliflozin (EMP)[3,4] is an obstructor of sodium glucose co-transporter-2 (SGLT-2), through which renal reabsorption of glucose is carried out. It is used clinically as an assistant to food régime and workout, frequently in blend alongside alternative drug therapies in the management of T2DM. Among other commercially available gliflozins, empagliflozin has the maximum discernment for SGLT-2 (2500-fold) as compared to SGLT-1. Empagliflozin is chemically (1S)-1,5-anhydro-1-(4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy] benzyl} phenyl)-D-glucitol, also known as D-Glucitol,1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3 furanyl] oxy] phenyl] methyl] phenyl]-(1S). The chemical structure is presented in Fig. 2. Literature survey exposed that numerous methods of analysis have been documented for sitagliptin phosphate and empagliflozin estimation independently or in blend with other drugs.[5-15] Although no UV analytical technique is stated for concurrent estimation[16] of sitagliptin phosphate and empagliflozin, as no such combination is commercially available till date. Hence an endeavor has been undertaken to forge and authenticate an easy, exact, meticulous, specific UV technique for concurrent quantification of SP and EMP in combination. The method was successfully established and validated in harmony with ICH guidelines.

MATERIAL AND METHODS

Instrumentation

For all spectral measurements shimadzu 1800 UV/VIS double beam spectrophotometer (1 cm accorded quartz cubicles) was utilized.

Reagents and chemicals

Sitagliptin phosphate (SP) and empagliflozin (EMP) were furnished by Intas pharmaceutical Ltd., (Ahmedabad, Gujarat, India) as gift samples. Methanol AR grade (Merck chemicals) and double distillation water were utilized for the research.

Method development

Selection of common solvent

Methanol:water (5:5) was selected as common solvent for investigation after so many trials with different ratio of both the solvents.

Assortment of appropriate wavelengths for investigation

Solutions comprising suitable concentration of SP and EMP in methanol:water (5:5) were perused in spectrum mode through the instrument spanning from 200-400 nm against methanol:water (5:5) as reference and superimposed bands were recorded. Through the overlain ranges of both the substances' investigative wavelengths for recognition were nominated as isosbestic point.

Spectroscopical settings

Following spectroscopical settings were fine-tuned for scrutiny:

Solvent: Methanol:water (5:5), Measurement configuration: Spectrum, Scanning span: 200-400 nm

Absorbance series: 0.0-4.0 ABS unit, Scanning haste: Intermediate, Finding wavelengths: 267 nm (λmax for SP) and 274.5 nm (Isosbestic point)

Standard stock solutions

SP and EMP were weighed (100 mg each) individually and shifted to two 100 ml of volumetric flasks and made solubilized by using a blend of methanol and water (5:5) and then volume was attuned to 100 ml using the similar solvent to obtain the stock solution with 1000 µg/ml strength.

Working standards

Figurals from the standard solutions of SP and EMP were suitably diluted with methanol:water (5:5) to attain employed standards of SP and EMP.

Absorbance ratio Method

The absorbance ratio method is a technique employed to concurrently determine the quantities of two components. It relies on the fact that the ratio of absorbances remains constant at any 2 wavelengths regardless of the pathlength or concentration of the substances.

Two wavelengths λ_1 267 nm (λmax of SP Fig. 3) and λ_2 274.5nm (isobestic point, selected from an overlain spectra, Fig. 4) were selected as detection wavelengths for this method. The absorbances of both drugs were quantified at 267nm and 274.5nm in the solutions prepared with working standard (methanol:water 5:5). The absorbances and absorptivities at these specific wavelengths were replaced in the ensuing equations to derive the concentration of both the drugs:

$$C_{SP} = (Q_M - Q_Y / Q_X - Q_Y) \times A_1 / a_{x1}$$

$$C_{EMP} = (Q_M - Q_X / Q_Y - Q_X) \times A_1 / a_{Y1}$$

Where, $Q_M = A_2 / A_1$, $Qx = a_{x2} / a_{x1}$, $Q_y = a_{y2} / a_{y1}$

A1 and A2 are the absorbances of the mixture at 267nm and 274.5 nm respectively.

a_{x1} and a_{y1} are absorptivities of SP and EMP respectively at 267nm.

a_{x1} and a_{y1} are absorptivities of SP and EMP respectively at 274.5 nm.

Method validation

Validation of developed technique was performed in accord with the ICH guidelines for validation of systematic procedures Q2 (R1).

Linearity:

The linearity of extent was assessed by scrutinizing varying concentration of standard solution of SP and EMP at detection wavelengths 267nm and 274.5 nm individually as well as for the laboratory mixture of drugs (5:1 ratio) at both the detection wavelengths. The calibration curves were plotted between mean absorbance of six replicate analyses and concentrations. The calibration plot was deemed to be linear between 50-250 µg/ml. The calibration plots are represented in Fig. 5 and 6.

Precision:

Meticulousness of the projected technique was established by two ways-repeatability and intermediary measurement. Repeatability was measured by scrutinizing numerous replicates samples of SP and EMP.

Intermediate precision was accomplished by Intra-day and Inter day precision.

For **Intra-day precision**, mixture comprehending 100µg/ml SP and 100µg/ml EMP was analyzed for 6 different times within the identical day. For **Inter-day precision**, mixture solution comprising 100 µg/ml SP and 100 µg/ml EMP was replicated for 6 dissimilar days. The outcomes were computed as to % relative standard deviation (%RSD).

Accuracy:

Establishment of accuracy of the proposed technique was done in terms of recovery studies. The accuracy was assessed by computing recoveries of SP and EMP using the conventional adds technique at 3 distinct levels 80, 100, 120%. Total 9 determinations were assessed over these 3 concentration levels for both drugs. At each stage, the average recovery percentage along with its corresponding standard deviation (SD) and RSD was determined.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD is prescribed as the lowermost quantity of a substance that can be sensed despite not essentially as an accurate worth. LOQ is described as lowermost quantity of an analyte that may be quantitively estimated with appropriate exactness and accurateness. LOD and LOQ were derived utilizing the standardization graph's slope and Y-intercept's SD of regression line. The formula of LOD and LOQ is:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, S is calibration graph's slope and σ is the intercept's SD

RESULTS AND DISCUSSION

Superimposed spectra of SP and EMP illustrated manifestation of isobestic point at 274.5 nm as shown in Figure 4. Several experimental settings were attempted to attain a good absorbance and peak profile for SP and EMP by optimizing the UV parameters. Numerous solvents of diverse compositions were endeavored to offer adequate discernment towards the drugs. Methanol and water lead to better responsiveness. The technique deliberated in the contemporary work offer an expedient and precise manner for Q-absorbance scrutiny of SP and EMP. In Q-absorbance ratio technique, analysis employed the wavelengths 267 nm and 274.5 nm (Fig. 3 and 4). Selected method's linear relationship was evident within 50-250 µg/ml concentration range for mixture. The concentration of distinct drug in this technique was estimated by resolving the equations of Q-absorbance ratio method, which were found Cx=108.6µg/ml and Cy=107µg/ml and are in harmony with the ICH guideline. Calibration curves' linearity was authenticated by the correlation coefficients of the regression (r^2), which was found 0.999 for the combination. The outcomes of linearity are listed in Table I and found in harmony with the ICH guideline. The intraday and interday precision outcomes are outlined in Table II in which the value of % RSD was determined to be < 2, demonstrating that the established technique is exact. The accuracy studies were conducted using the conventional addition procedure. The % recovery for SP was determined in the range of 99.83% to 100.79% and for EMP it was found in the range of 99.08% to 100.66%, which is in accordance with ICH guideline as according to the guideline the acceptance range for % recovery is 70-120%. Table III displays the recovery percentage numbers. The LOD values of SP and EMP were 0.68 µg/ml and 1.06µg/ml and LOQ values were found as 2.08µg/ml and 3.21 µg/ml for SP and EMP correspondingly, which demonstrates the detectability of the established technique. Table IV represents precis of regression features and validation constraints for the developed method.

Table I: Linearity results of SP and EMP

Conc. (µg/ml)	At 267 nm						At 274.5 nm					
	SP		EMP		SP+EMP		SP		EMP		SP+EMP	
	Mean ABS±SD (n=6)	% RSD	Mean ABS±SD (n=6)	% RSD	Mean ABS±SD (n=6)	% RSD	Mean ABS±SD (n=6)	% RSD	Mean ABS±SD (n=6)	% RSD	Mean ABS±SD (n=6)	% RSD
0	0	0	0	0	0	0	0	0	0	0	0	0
50	0.2575± 0.0052	2.0196	0.1210± 0.0005	0.39601	0.3879±0.0005	0.1393	0.1486± 0.0002	0.1423	0.1565± 0.0005	0.3435	0.2946± 0.0037	1.287834
100	0.4285± 0.0008	0.1871	0.2324± 0.0036	1.56111	0.7339±0.0007	1.0458	0.2558± 0.002	0.7847	0.2893± 0.0003	0.0966	0.6356± 0.005	0.798757
150	0.6593± 0.0096	1.4489	0.3558± 0.0008	0.21748	1.0395±0.04303	4.1398	0.3815± 0.0003	0.0851	0.4524± 0.0003	0.0781	0.8933± 0.0021	0.24181
200	0.8229± 0.0067	0.8147	0.4671± 0.0005	0.10661	1.3087±0.0012	0.0925	0.4825± 0.0002	0.0527	0.5916± 0.0003	0.0540	1.2099± 0.0029	0.242124
250	1.0227± 0.0305	2.9827	0.5735± 0.0003	0.058	1.5989±0.0005	0.0316	0.5991± 0.0003	0.0644	0.7014± 0.0003	0.0517	1.5455± 0.0042	0.274865

Table II: Intraday and Interday Precision results of SP and EMP

Precision	% Assessment of SP±SD (n=6)	% RSD	% Assessment of EMP±SD (n=6)	% RSD
Intraday at 267nm	99.90±0.0008	0.187	99.39±0.0036	1.561
Intraday at 274.5nm	99.58±0.0022	0.859	99.82±0.0003	0.096
Interday at 267nm	99.70±0.0006	0.130	99.13±0.0042	1.806
Interday at 274.5nm	99.00±0.0006	0.244	99.28±0.0003	0.097

Table III: Accuracy (% recovery) results of SP and EMP

Drug	%Level	Quantity used ((µg/ml)	Quantity incorporated (µg/ml)	Cumulative quantity (µg/ml)	Mean ABS±SD (n=3)	Quantity identified (µg/ml)	%RSD	%Recovery
SP	50	50	5	55	0.1486±0.0002	54.91	0.13	99.83
	100	100		105	0.2708±0.002	105.83	0.73	100.79
	150	150		155	0.3895±0.0003	155.29	0.07	100.18
EMP	50	50	5	55	0.1625±0.0005	54.93	0.3	99.87
	100	100		105	0.3011±0.0003	104.03	0.09	99.08
	150	150		155	0.4456±0.0003	156.03	0.06	100.66

Table IV: Precis of regression characteristics and Validation constraints

Parameters	SP		EMP	
	267 nm	274.5 nm	267 nm	274.5 nm
Linearity range ((µg/ml)	50-250	50-250	50-250	50-250
Regression Equation (y=mx+c)	y = 0.004x + 0.0289	y = 0.0024x + 0.0168	y = 0.0023x + 0.0038	y = 0.0028x + 0.0087
Slope (m)	0.004	0.0024	0.0023	0.0028
Intercept (c)	0.0289	0.0168	0.0038	0.0087
Correlation coefficient (r^2)	0.9964	0.9972	0.9995	0.9981
Standard Deviation (SD)	0.0087	0.0005	0.0009	0.0009
Intraday Precision (% RSD) (n=6)	0187	0.859	1.561	0.096
Interday Precision (% RSD) (n=6)	0.130	0.244	1.806	0.097
% Recovery	99.83 to 100.79		99.08 to 100.66	
LOD (µg/ml)	0.68 µg/ml		1.06 µg/ml	
LOQ (µg/ml)	2.08 µg/ml		3.21 µg/ml	

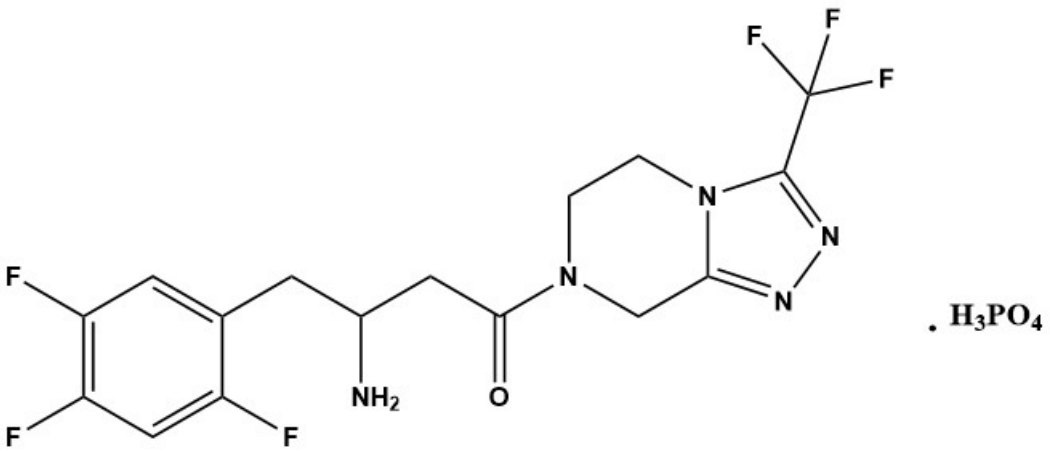


Fig 1: Chemical structure of Sitagliptin phosphate (SP)

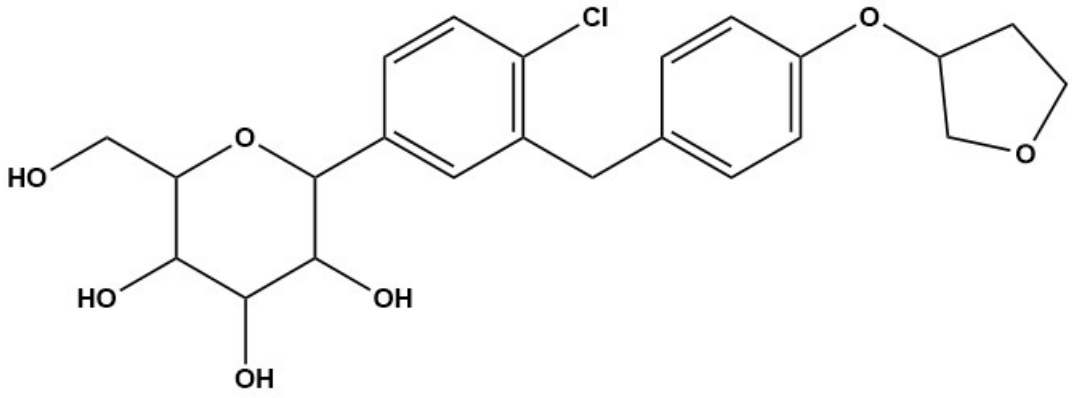


Fig 2: Chemical structure of Empagliflozin (EMP)

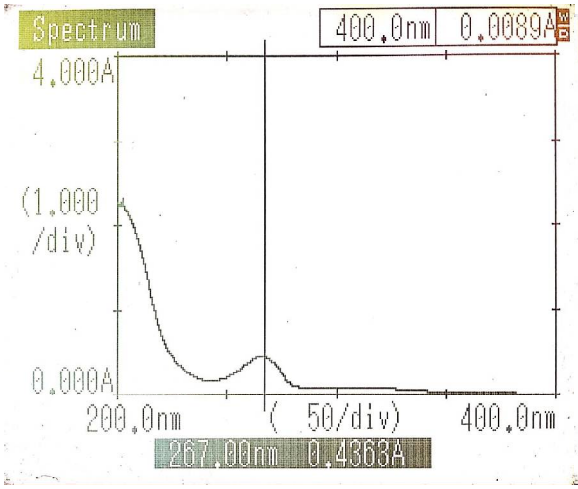


Fig. 3: λmax of SP

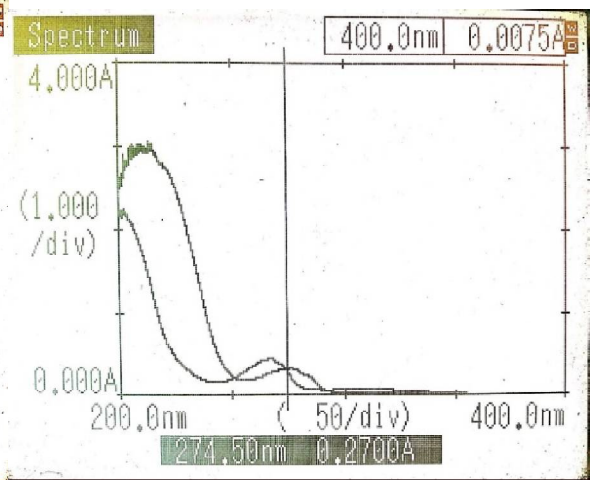
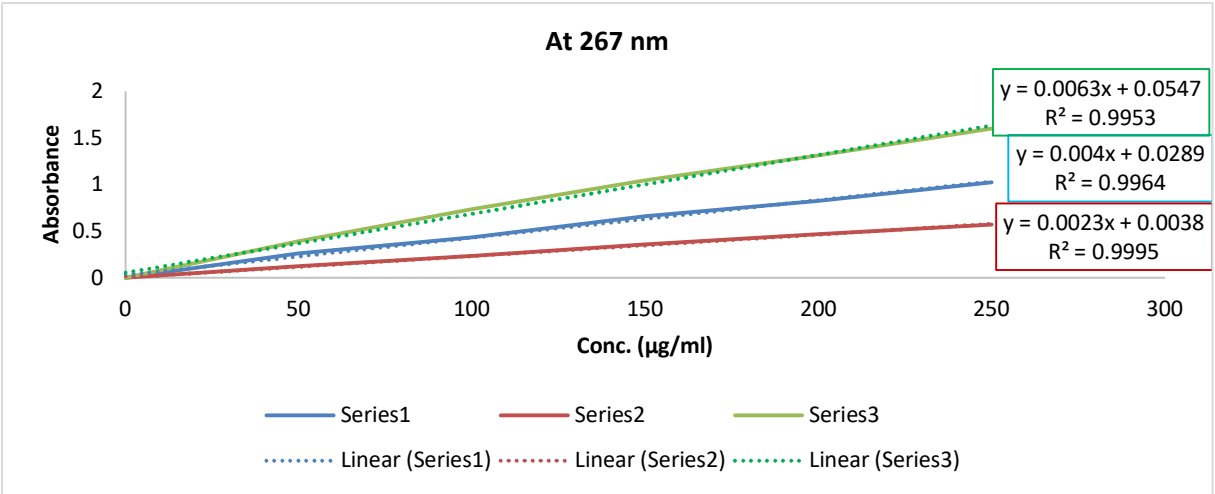
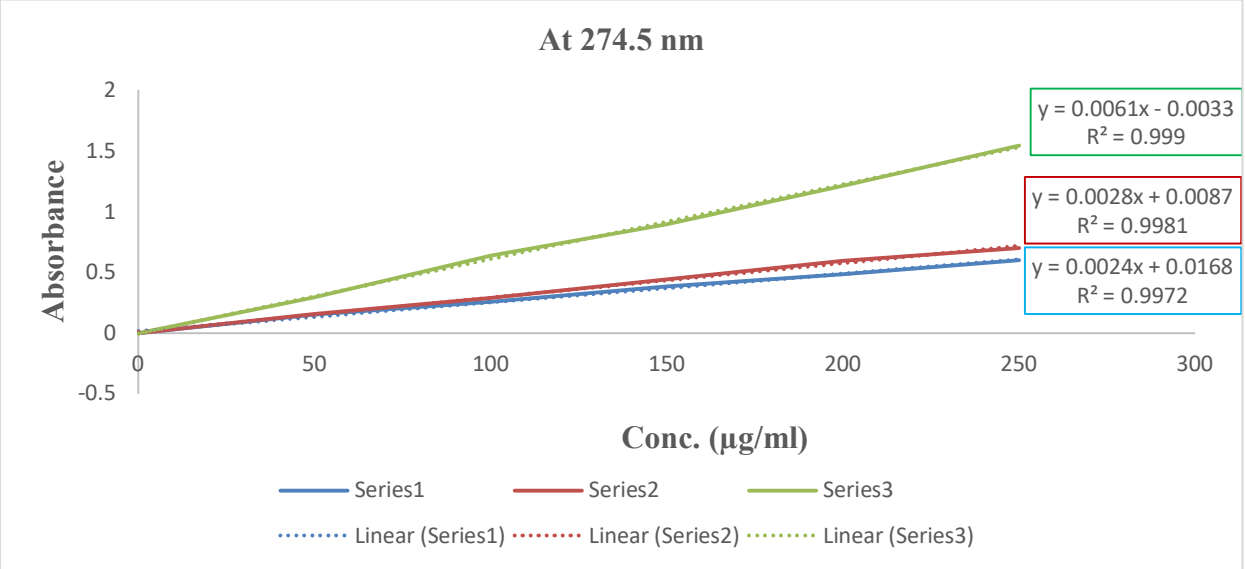


Fig. 4: Overlain spectra of SP and EMP



series 1- SP, series 2- EMP, series 3- SP+EMP

Fig. 5: Standardization curve of SP, EMP and (SP+EMP) at 267 nm



series 1- SP, series 2- EMP, series 3- SP+EMP

Fig. 6: Standardization curve of SP, EMP and (SP+EMP) at 274.5 nm

CONCLUSION

Entirely the results persuaded to the decision that the projected technique is straightforward, exact, particular, sensitive, and consistent with the ICH recommendations and might be functional effectively for assessment of SP and EMP in bulk drug formulation.

ACKNOWLEDGEMENTS

The authors are obliged to Intas Pharmaceuticals, Ahemdabad (Gujarat) for furnishing gift samples of Sitagliptin Phosphate and Empagliflozin for the research work.

CONFLICT OF INTEREST

Authors have no conflict of interest.

AUTHOR'S CONTRIBUTION

Akankha Dwivedi- Idea of the research and drafted the manuscript
Rajesh Sharma- Final proof reading of the manuscript

FUNDING

Not applicable.

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CITATION OF THIS ARTICLE

Akanksha Dwivedi, Rajesh Sharma. Exploration And Validation of a Spectrophotometric Q-Absorbance Ratio Approach for Concurrent Determination of Sitagliptin Phosphate and Empagliflozin. Bull. Env. Pharmacol. Life Sci., Vol 13 [3] February 2024: 228-234