Available online at www.jmpas.com

ISSN NO. 2320 – 7418
DOI:10.22270/jmpas.v9i3.952

Research Article

METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN FIXED-DOSE COMBINATION AND VALIDATION ON UV SPECTROSCOPIC

Patel Aneesh*, Dr. Omray Lavakesh, Soni Pushpendra

Radharaman Institute of Pharmaceutical Sciences, Bhopal, Madhya Pradesh, India

Correspondence
Aneesh Patel*
Radharaman Institute of Pharmaceutical Sciences, Bhopal, Madhya Pradesh, India
✉ aneeshpatelpatel@gmail.com

Keywords
Dapagliflozin, Saxagliptin, Spectrophotometric analysis, Simultaneous equation method

Received
10/07/2020
Reviewed
16/07/2020
Revised/ Accepted
20/07/2020

ABSTRACT

Simple, precise, and accurate UV-Spectrophotometric simultaneous equation method for estimation of Dapagliflozin (DAPA) and Saxagliptin (SAXA) was developed and validated as per ICH guidelines. This Method involves solving simultaneous equations based on the measurement of absorbance at two wavelengths 222 nm and 276 nm (λmax of SAXA and DAPA) in phosphate buffer pH 6.8. Both the drugs obey Beer's law in the concentration ranges 5-25µg/ml. % Recovery for both the drugs was in the range of 98.44-99.05% indicating excellent accuracy. The methods were precise, with a relative standard deviation of less than 2% for both drugs. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analyses were found to be in good accordance with the prescribed values. Thus, the method can be used for routine monitoring of drugs in the industry for the assay of bulk drugs and commercial formulation (QTERN tablets, Astra Zeneca).

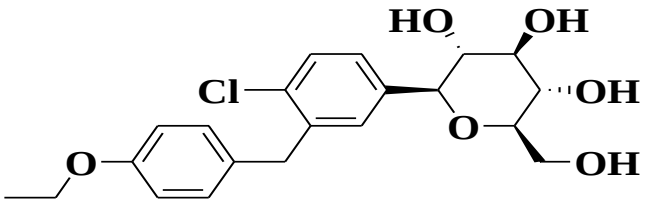
INTRODUCTION

Saxagliptin is chemically called as (1S, 3S, 5S)-2-[(2S)-2-Amino-2-(3 hydroxy tricyclo [3.3.1.13, 7] dec-1-yl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile (Figure 1A). It is the oral hypoglycemic (antidiabetic) agent, class of dipeptidyl peptidase (DPP-4) inhibitor [1]. Saxagliptin was inhibiting the activity of dipeptidyl peptidase-4(DPP-4) enzyme by increasing the insulin production in response to a meal and decreasing the gluconeogenesis rate in the liver, in blood glucose regulation is thought to be through degradation of GIP [2] and the degradation of GLP-1[3]. Saxagliptin was used for the treatment of type-2 diabetics in the form of mono or combination of other drugs. Dapagliflozin is chemically called as (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxy phenyl) methyl] phenyl]-D-glucitol (Figure 1B). It is a highly selective, sodium-Glucose Co-Transporter 2 (SGLT2).

DOI: 10.22270/jmpas.v9i3.952

Dapagliflozin blocking the activity of the sodium-glucose transport proteins, which is regulated for at least 90% of the glucose reabsorption in the kidney and obstructs the transporter mechanism causes blood glucose to be removed through the urine. Dapagliflozin is improved glycemic control in patients with type 2 Diabetes Mellitus [4]. A survey of literature revealed the availability of several analytical methods for the quantitative determination of DAPA and SAXA alone or combination with other drugs. [5] The reported methods were mainly based on liquid chromatographic estimation using UV-VIS, fluorescence, electrochemical, or mass spectrometry detectors. [6] The available methods are based on spectrophotometry, thin layer chromatography, or high-performance liquid chromatography [5-27]. However, no UV-Spectrophotometric simultaneous equation method is available for the simultaneous determination of the DAPA and SAXA in the combined P'ceutical dosage form. [7-8] In the present study, an attempt was made to develop a simple, precise, and accurate method for the simultaneous estimation of these drugs in combined P'ceutical dosage form and validate as per (ICH) guidelines[9-10].

(A)



(B)

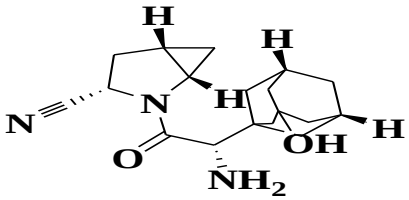


Figure 1 Chemical structure of (A) Saxagliptin and (B) Dapagliflozin

MATERIAL AND METHOD

Reagents and Chemicals

DAPA and SAXA standards were obtained from Alembic Pharmaceutical (Baddi). Methanol, acetonitrile was procured from Rankem, RFCL Limited, New Delhi, India. Ammonium acetate AR, sodium dihydrogen phosphate AR and ortho-phosphoric acid AR grade were procured from Central Drug House (P) Limited, New Delhi, India. The 0.45- mm pump nylon filter was obtained from Advanced Microdevices (Ambala Cantt, India). HPLC grade water was used throughout the study. Other chemicals used were of analytical or HPLC grade. [11-12]

Instrument

In the UV-spectrophotometric method, Lab India model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

METHOD DEVELOPMENT

Standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80ml phosphate buffer pH 6.8 in a 100 ml volumetric flask. The flask was sonicated for about 10 min to solubilizing the drug and the volume was made up

DOI: 10.22270/jmpas.v9i3.952

to the mark 100ml with phosphate buffer pH 6.8 to get a concentration of 1000 µg/ml (Stock-A) for both drugs. [13-14]

Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of SAXA and DAPA and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with phosphate buffer pH 6.8 that gave a concentration of 100 µg/ml (Stock-B). [15]

Determination of λ_{max}

10 µg/ml standard solutions of both SAXA and DAPA were prepared from respective sub-stock solutions. Both the solutions were scanned in the wavelength region of 200-400 nm and the λmax was found to be 222 nm and 276 nm for SAXA and DAPA respectively. [16] They were scanned in the wavelength range of 200-400 nm and the overlain spectrum was obtained (Figure 2-4).

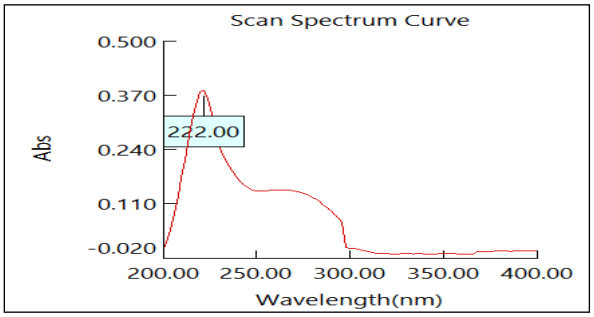


Figure 2 Determination of λ_{max} of SAXA

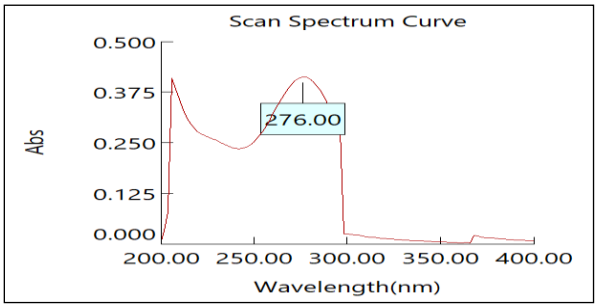


Figure 3 Determination of λ_{max} of DAPA

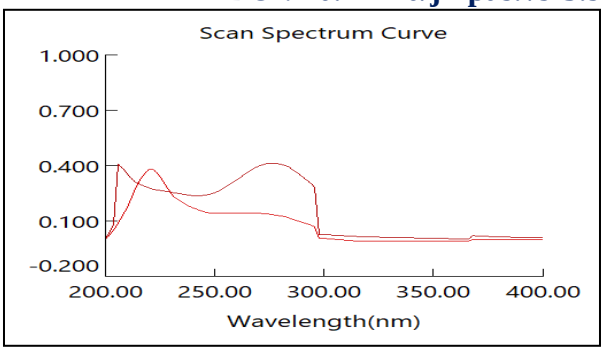


Figure 4 overlay spectra of SAXA and DAPA

Preparation of calibration curve

From the standard stock solution of each drug, appropriate aliquots were pipette out into a series of 10 ml volumetric flasks. The volume was made up to the mark with phosphate buffer pH 6.8 to get a set of solutions having a concentration range of 5-25µg/ml for both drugs. Triplicate dilutions of each drug solutions were prepared separately.

The prepared working solutions of SAXA and DAPA were scanned 222 nm and 276 nm, respectively. [17-18] the absorbance's were recorded and were plotted against the concentrations to obtain their respective calibration curves.

Simultaneous equation method (Vierordt's)

Working standard solution from the standard stock solution prepared in concentration 40µg/ml of SAXA and 2µg/ml of DAPA were scanned in the spectrum mode over the range of 200-400 nm against phosphate buffer pH 6.5 as blank and the overlain spectra of the two were recorded. [19] SAXA showed an absorbance peak at 222.0 nm, whereas DAPA at 276.0 nm. The overlain spectra also showed iso absorptive points at 252.0 nm. Due to differences in absorbance maxima and having no interference with each other so both drugs can be

DOI: 10.22270/jmpas.v9i3.952

simultaneously estimated by the simultaneous equation method.

The simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. [20] Two wavelengths selected for the method are 222.0 nm and 276.0 nm that are λ_{max} of SAXA and DAPA respectively. The absorbance was measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as the mean of five independent determinations. [21] Concentrations in the sample were obtained by using the following equations

$$C_{saxa} = \frac{A_1 ay_2 - A_2 ay_2}{ax_1 ay_2 - ax_2 ay_1} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. (1)}$$

$$C_{dapa} = \frac{A_1 ax_2 - A_2 ax_1}{ax_1 ay_2 - ax_2 ay_1} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. (2)}$$

Where, A_1 and A_2 are the absorbance of mixture at 222.0 nm and 276.0 nm respectively, ax_1 and ax_2 are absorptivities of SAXA at λ_1 (222.0 i.e. λ_{max} of SAXA) and λ_2 (276.0) λ_{max} of DAPA) respectively and ay_1 and ay_2 are absorptivities of DAPA at λ_1 and λ_2 respectively. C_{DAPA} and C_{SAXA} are concentrations of SAXA and DAPA respectively. Figure 4 represents the overlain spectra of both the drugs in 2:40 ratio and the criteria for obtaining maximum precision [absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the SAXA and DAPA [21-23].

METHODS VALIDATION

Validation of the method was carried out following the International Conference on Harmonization Q2B guidelines 2005.

LINEARITY

The linearity of the analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of an analyte in the sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results were recorded. The results of linearity are reported in Table 1.

ACCURACY

The validity and reliability of the proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100%, and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD, and % RSD are less than 2 indicate the accuracy of the method. Result of the recovery study shown in Table 2.

PRECISION

Precision was determined by repeatability and Intermediate precision of drugs. Repeatability result indicates the precision under the same operating condition over a short interval time. The intermediate precision study is expressed within laboratory variation on different days and analysts to analyst variation by different analysts. The value of SD and %RSD is less than 2 indicate the precision of the method. Result of precision shown in Table 3.

DOI: 10.22270/jmpas.v9i3.952

ASSAY OF TABLET FORMULATION

Mixed Blends of SAXA and DAPA were weighed and ground to a fine powder; the amount equal to 10mg of DAPA was taken in a 10 ml volumetric flask. The present in this amount of marketed tablets (QTERN tablets, Astra Zeneca) was 5mg SAXA. Then 5ml of phosphate buffer pH 6.8 was added and the flask was sonicated for about 10 min to solubilizing the drug present in tablet formulation and the volume was made up to the mark with Buffer. After sonication filtration was done through Whatman filter paper No. 41. The filtrate was collected and further diluted with buffer to get the final concentrations of both drugs in the working range. The absorbance of final dilutions was observed at selected wavelengths and the concentrations were obtained from the simultaneous equation method. [24-26] the procedure was repeated five times in Table 4.

Table 1 Results of Linearity of Saxagliptin and Dapagliflozin

PARAMETER	SAXA	DAPA
Concentration (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.037	0.038
Intercept (c)*	0.000	-0.002

*value of three replicate

Table 2 Results of Recovery Study

% LEVEL	% MEAN±SD*	
	SAXA	DAPA
80%	98.736±1.076	98.549±1.875
100%	98.870±0.951	99.059±0.358
120%	98.449±0.211	99.000±0.465

***** Value of three replicate and five concentrations.

Table 3 Results of Precision

PARAMETER	% MEAN±SD*	
	SAXA	DAPA
Repeatability	99.086±0.084	98.860±0.064
Intermediate precision		
Day to day precision	99.071±0.070	99.070±0.107
Analyst-to-Analyst	98.816±0.107	99.345±0.087
Reproducibility	98.956± 0.114	99.090±0.141

***** Value of five replicate and five concentrations

Table 4 Assay of Tablet Formulation

Conc. Present (µg/ml)		% Conc. Found	
SAXA	DAPA	SAXA	DAPA
5	5	99.00	97.00
10	10	98.50	96.50
15	15	97.93	99.00
20	20	99.75	74.25
25	25	83.28	98.60

*Average of three replicate and five concentrations

RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost-effective and time-saving as compared to the HPLC method of analysis [30]. Thus, for estimation of the routine sample of drugs simple, rapid, sensitive, and accurate analytical UV methods were utilized which reduces unnecessary tedious sample preparations and the use of costly materials. To develop suitable methods of analysis, various solvents were studied. Based on the sensitivity of the method and non-toxic behavior phosphate buffer pH 6.8 was selected as a solvent for the methods. Overlain spectra (Figure 4) shows that at λ_{max} of SAXA (222 nm) interference of DAPA and at λ_{max} of DAPA (276nm) interference of SAXA occurs which suggested the development

DOI: 10.22270/jmpas.v9i3.952

of simultaneous equation method. The optimized methods showed good reproducibility and mean recovery with 98.956± 0.114 (SAXA), 99.090±0.141 (DAPA) and 98.870±0.951 (SAXA), 98.549±1.875 (DAPA) respectively. The standard deviation, coefficient of variance, and standard error were obtained for SAXA and DAPA was satisfactorily low. The result of precision at different levels was found to be within acceptable limits (RSD < 2). Thus, the method provides a simple, convenient, rapid, and accurate way to determine SAXA and DAPA simultaneously.

CONCLUSION

A new, simple, sensitive, and economical UV spectrophotometric method was developed for the simultaneous estimation of SAXA and DAPA in their tablet formulation. Validation of developed methods was performed according to ICH guidelines. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Vierordt's method has the advantage of being simple, economic, rapid, and subsequently not required sophisticated technique, instrument, and costly solvents. Thus, the proposed methods can be successfully applied for the determination and dissolution testing of SAXA and DAPA in a commercial formulation (Qtern).

REFERENCE

1. Deacon CF, Holst JJ, 2009. Saxagliptin is a new dipeptidyl peptidase-4 inhibitor for the treatment of type 2 diabetes. Adv Ther 26: 488-499.
2. Mentlein R, Gallwitz B, Schmidt WE, 1993. Dipeptidyl peptidase IV hydrolyzes gastric inhibitory polypeptide, glucagon-like peptide-1(7-36) amide, peptide histidine methionine, and is responsible for their degradation in human serum. Eur J Biochem 214: 829-835.
3. Ahren B, Landin Olsson M, Jansson PA, Svensson M, Holmes D, 2004. Inhibition of dipeptidyl peptides-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab 89: 2078-2084.
4. Vithoba MG, Krishna RG, Hemke AT, 2017. Estimation of dapagliflozin from its tablet formulation by UV-spectrophotometry. Pharm Methods 8: 102-107.
5. BR Jani, VK Shah, PP Kapupara, 2015. Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture. J Bioequiv. 1(1) 102.
6. M Sanagapati, K Dhanalakshmi, NG Reddy, S Sreenivasa, 2014. Method Development and Validation of Dapagliflozin in API by RP-HPLC and UV-Spectroscopy. Int J Pharm Sci and drug Res. 6(3) 250-2.

7. PC Karuna, E China, MV Basaveswara Rao, 2015. Unique UV spectrophotometric method for reckoning of Dapagliflozin in bulk and pharmaceutical dosage forms. J Chem Pharm Res. 7(9) 45-9.
8. DJ Augeri, JA Robl, DA Betebenner, DR Magnin, A Khanna, 2005. Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. J Med Chem. 48 5025-5037.
9. R. Kalaichelvi, E. Jayachandran, 2011. Validated Spectroscopic method for the estimation of Saxagliptinin pure and from tablet formulation. Int J Pharm Pharm Sci. 3, 179-180.
10. N Nicola, G S Jeyabalan, 2012.Development and validation of UV-VIS spectroscopy method for simultaneous estimation of saxagliptin hydrochloride and metformin hydrochloride in active pharmaceutical ingredient, J Pharm Educ Res. 3, Issue. 2.
11. P Patil Prafulla, Kalkotwar Ramesh.S, VV Patil, VB Jadhav, N Patil, 2012. A new RP – HPLC Method for determination of metformin HCl and saxagliptin in tablet dosage form, IJPBS 2(4) 161-167.
12. NVMS Bhagavanji, 2012. Development and validation of stability-indicating LC method for the simultaneous estimation of metformin and saxagliptin in combined dosage form, VSRD International Journal of Technical &Non-Technical Research, Vol. 3 No. 11.
13. S Inturi, R Inturi, IK Tagaram, 2011. Validated novel LC determination of saxagliptin in pure bulk & p'ceutical dosage forms, IJPRD, V. 3(8): (45-52).
14. R Kalaichelvi, E Jayachandran, 2011 Validated El Bagary, I Ramzia, Elkady F, Ehab, Ayoub, M Bassam, 2012. Spectrophotometric methods based on charge transfer complexation reactions for the determination of saxagliptin in bulk and pharmaceutical preparation. International Journal of Biomedical Science. 8, 204-208.
15. Akash C Nandanikar, Mohsin J Jamadar, Raj H Shaikh, Sanaulla A Rahim, Rohit R Shah, Shrinivas K mohite, Javeed Manure, 2016. Formulation design and evaluation of chewable Tablet containing sucralfate and Diclofenac. Journal of Medical Pharmaceutical and Allied Sciences, 198-224.
16. X Xu, R Demers, H Gu, L Christopher, H Su, L Cojocaru. 2012. Liquid chromatography and tandem mass spectrometry method for the quantitative determination of saxagliptin and its major pharmacologically active 5-monohydroxy metabolite in human plasma, Journal of Chromatography B, 889 77-86.
17. R Kalaichelvi, E Jayachandran, 2011. Validated spectroscopic method for the estimation of saxagliptin in pure and from tablet formulation; International Journal of Pharmacy and Pharmaceutical Science. 3, 179-180.

18. Manoj G Bajait, R Ghatmale, B mundhey, 2019. Comparative pharmaceutical study of some branded and generic tablet formulations, Journal of Medical Pharmaceutical and Allied Sciences, V8-I6, 876, 2392-2402.
19. RP Kumar, M Vasudevan, a Deecaraman, 2012. Validated RP-HPLC method for simultaneous estimation of metformin and saxagliptin in tablets. Rasayan Journal of Chemistry. 5, 137-114.4.
20. PS Rao, D Rama Chandran, K Murali, S Srinivasu, 2013. Stability Indicating Isocratic Reverse Phase HPLC Method with PDA Detector for the Estimation of Saxagliptin in Bulk Drugs and Its Formulation. International Journal of Pharma Sciences. 3 333-342.
21. PP Prakash, RS Kalkotwar, VP Vikas, BJ Vijay, PP Nilesh, 2012.A new RP-HPLC method for the determination of Metformin HCl and Saxagliptin in the tablet dosage form. International Journal of Pharmacy and Biological Sciences. 2, 161-167.
22. M Sarat, P Murali Krishna, C Rambabu, 2012. RP-HPLC method for simultaneous estimation of saxagliptin and pioglitazone in tablets. International Research Journal of Pharmacy. 3, 399-402.
23. MM Abdul Azim, EF Elkady, MA Fouad, 2012. Development and validation of a reversed-phase column liquid chromatographic method for the simultaneous determination of two novel gliptins in their binary mixtures with metformin. European Journal of Chemistry. 3, 152-155.
24. Beckett AH, Stanlake JB. 1997. Practical Pharmaceutical Chemistry, fourth ed., part 2. CBS Publishers and Distributors, New Delhi.
25. ICH Guidelines, 2005 Validation of Analytical Procedures: Text and Methodology Q2 (B).
26. Laxman R, Acharya A, Jain V, Bhardwaj S, Jain D, 2010. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods simultaneous determination of spironolactone and torsemide in the pharmaceutical dosage form. Int J Res Ayurveda Pharm; 1(2):459-467.