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RP-HPLC method for the simultaneous estimation of Saxagliptin and Dapagliflozin in combined pharmaceutical dosage form

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of Saxagliptin and Dapagliflozin in Tablet dosage form. Chromatogram was run through standard altima C18 column (150 ×4.6 mm, 5µ) The Mobile phase containing Phosphate buffer: Acetonitrile in the ratio 60:40 v/v. The solution was pumped through the column at a flow rate of 1 ml/min. The column temperature was maintained at 30°C. Optimized wavelength selected was 222 nm. Retention time of Saxagliptin and Dapagliflozin were found to be 2.18 min and 2.74 min. % RSD of the Saxagliptin and Dapagliflozin were found to be 0.7 and 0.7 respectively. % Recovery was obtained as 99.19% and 99.06% for Saxagliptin and Dapagliflozin respectively. LOD, LOQ values obtained from regression equations of Saxagliptin and Dapagliflozin were 0.01, 0.03 and 0.04, 0.13 respectively. Regression equation of Saxagliptin is y = 14803x + 786.4 and y =13944x+4370.2 of Dapagliflozin. So the developed method was simple and economical that can be applied successfully for simultaneous estimation of both Saxagliptin and Dapagliflozin in bulk and combined tablet formulation.

Keywords: Saxagliptin, Dapagliflozin, RP-HPLC, Validation, Simultaneous estimation.

INTRODUCTION

Saxagliptin [1] (Fig:1) is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile and molecular formula $C_{18}H_{25}N_3O_2$ and mass 315.41 g/mol. It is White solid powder and soluble in Soluble in PEG-400, acetone, acetonitrile, ethanol, isopropyl alcohol, methanol; sparingly soluble in water and slightly soluble in ethyl acetate and half-life is 2.5-3.1 hours. It is a highly potent, reversible, competitive dipeptidyl peptidase-4 inhibitor indicated for the treatment of patients with type 2 diabetes.

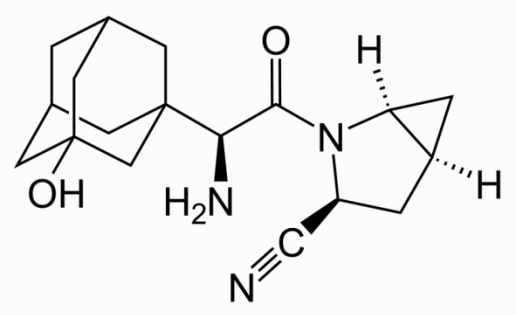

Figure 1: Chemical structure of Saxagliptin

Dapagliflozin [2] (Fig:2) is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl) methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol and molecular formula $C_{21}H_{25}ClO_6$ and mass 408.9 g/mol. It belongs to the class of medications called sodium-glucose co-transporter 2 (SGLT2) inhibitors. It is White or off white crystalline solid and soluble in organic solvents like ethanol, DMSO, Dimethyl formamide and sparingly soluble in aqueous buffers (0.173 mg/mL) and half-life is 12.9 h. It is used along with diet and exercise, and sometimes with other medications, to lower blood sugar levels in patients with type 2 diabetes.

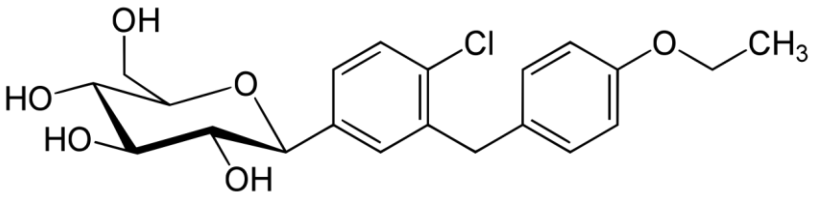

Figure 2: Chemical structure of Dapagliflozin

LITERATURE

Many HPLC methods have been reported for the determination of Saxagliptin [3-5] and Dapagliflozin [6-7] in pharmaceutical dosage forms and biological samples. Some analytical methods using chromatography [8-39] and spectroscopy [40-46] have been reported for the simultaneous determination of saxagliptin and dapagliflozin in combined dosage forms.

MATERIALS AND METHODS

Materials

The reference samples of saxagliptin and dapagliflozin (API) were obtained from M/s. Aurobindo Pharmaceuticals, Hyderabad, India. The branded formulation (tablets) (**Qtern**tablets containing 5mg of saxagliptin and 10 mg of dapagliflozin) manufactured by M/s. Astrazeneca Pharmaceuticals, Chennai was procured from the local market. HPLC grade acetonitrile, methanol and analytical grade potassium dihydrogen phosphate, orthophophoricacid were obtained from M/s. Rankem Chemicals Ltd, Mumbai, India. Milli-Q water dispensed through a 0.22 µ filter of the Milli-Q water purification system (Millipore, Merck KGaA, Darmstadt, Germany) was used throughout the study.

Instrumentation

The analytical method was performed by using the HPLC system Shimadzu (SPD-AT20) equipped with auto sampler, UV and PhotoDiode Array (PDA) detector, Rheodyne injector with 20 µl loop volume, analytical balance (Model AX200), pH analyser (Chemiline CL 180 based pH meter) and Toshcon Ultra Sonicator.

Methods

Preparation of phosphate buffer solution (pH 3.0)

About 1.36 gm potassium dihydrogenphosphate was weighed and transferred into a 1000 mL flask and 400mL of Milli-Q water was added and mixed well. Then volume was made upto 1000mL, sonicated for five minutes and cooled to room temperature. The pH of above buffer solution was adjusted to 3.0±0.05 with orthophosphoric acid solution and then filtered through a 0.45 µ membrane filter.

Preparation of the mobile phase

A 60:40v/v mixture of the above phosphate buffer (pH 3.0) and acetonitrile was prepared and used as the mobile phase in the study.

The diluent

A 50:50 v/v mixture of water and acetonitrile was prepared and used as the diluent in the preparation of drug dilutions.

Preparation of mixed standard and tablet solution

About 25 mg of saxagliptin and 50 mg of dapagliflozin were accurately weighed and transferred into a 50 mL clean dry volumetric flask containing 30 mL of the diluent. The solution was sonicated for 5 min and then volume was made up to the mark with a further quantity of the diluent to get a concentration of 500 µg/mL of saxagliptin and 1000 µg/mL of dapagliflozin (Stock solution). A mixed working standard solution was further prepared by diluting the above stock solution to obtain a concentration of 50 µg/mL of saxagliptin and 100 µg/mL of dapagliflozin.

Twenty tablets of the commercial sample of 'Qtern' were weighed and finely powdered. An accurately weighed portion of powdered sample equivalent to 25 mg of saxagliptin and 50 mg of dapagliflozin was transferred into a 50 mL volumetric flask containing 30 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drugs and the volume made up with a further quantity of the diluent. Then, this mixture was filtered through a 0.45 µ membrane filter. Further, 1 mL of the above stock solution was pipetted into a 10 mL volumetric flask and the volume was made up with the diluent.

METHOD DEVELOPMENT

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Water and Acetonitrile as mobile phases, in which the drug did not respond properly. The organic content of the mobile phase was also investigated to optimize the elution of the drug. To improve the tailing factor, the pH of mobile phase becomes an important factor. Thereafter, Phosphate buffer and Acetonitrile were taken in isocratic ratio 60:40 v/v and with a flow rate of 1.0 ml/min were employed. Altima C18 column (150 ×4.6 mm, 5µ) was selected as the stationary phase to reduce the tailing of the peak. 222 nm was selected as the detection wavelength for PDA detector. The retention time was found to about 2.18 min for Saxagliptin and 2.74 min. for Dapagliflozin. The results were shown in table 1 and fig.3.

Table 1: Optimized chromatographic conditions for simultaneous estimation of saxagliptin and dapagliflozin in combined tablet dosage form

Column	**:**	Altima C18 column(150 mm x 4.6 mm, 5 µm)
Elution mode	**:**	Isocratic
Mobile phase	**:**	Phosphate buffer:acetonitrile = 60:40 v/v
pH of Buffer	**:**	3.0±0.05
Column Temp	**:**	30^0 C
Wavelength	**:**	222 nm
Injection Volume	**:**	20 µL
Flow rate	**:**	1 mL/min
Run time	**:**	5 min

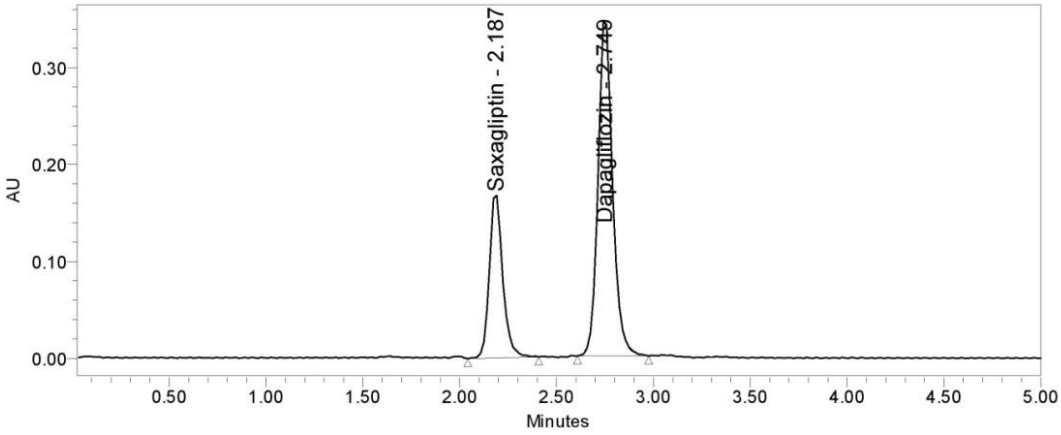

Figure 3: Chromatogram of standard solution of saxagliptin and dapagliflozin

Method Validation

The method was validated by determining system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness by analyzing saxagliptin and dapagliflozin. The analytical method validation was carried out as per ICH method validation guidelines [47, 48].

System Suitability

A system suitability test was performed to evaluate the chromatographic parameters (retention time, number of theoretical plates, capacity factor and asymmetry factor) before the validation runs. The results of system suitability parameters were given in table 2.

Table 2: System suitability of saxagliptin and dapagliflozin

S. No.	Saxagliptin			Dapagliflozin		
	Area	USP Plate Count	USP tailing	Area	USP Plate Count	USP tailing
1	74018	5135	1.18	145393	7317	1.14
2	74880	5417	1.15	145648	7236	1.14
3	74418	5150	1.17	147415	7127	1.14
4	74589	5162	1.16	146112	6947	1.14
5	74784	5126	1.16	147197	6980	1.12
6	73576	5453	1.15	147630	7219	1.13
Mean	74378			146566		
Std. Dev.	497.1			967.0		
% RSD	**0.7**			**0.7**		

Linearity and Range

The linearity of saxagliptin and dapagliflozin were evaluated at six concentration levels by diluting the standard stock solution to give solutions of saxagliptin and dapagliflozin in the concentration range from 1.25 – 7.5 µg/ml and 2.5 - 15 µg/ml. The regression analysis was carried out for the slope, intercept and correlation coefficient. The results were given in table 3, 4 and fig. 4 - 6.

Table 3: Linearity data of saxagliptin and dapagliflozin

Saxagliptin		Dapagliflozin	
Concentration (µg/ml)	**Mean Peak area (n=3)**	**Concentration (µg/ml)**	**Mean Peak area (n=3)**
1.25	18538	2.5	39673
2.5	37271	5	74086
3.75	58267	7.5	107357
5	74379	10	145670
6.25	94074	12.5	177551
7.5	110756	15	213955

Table 4: Regression Analysis of Calibration Curve

Parameters	**Saxagliptin**	**Dapagliflozin**
Slope (m)	14803	13944
Intercept (c)	786.4	4370
Correlation coefficient (R2)	0.998	0.999

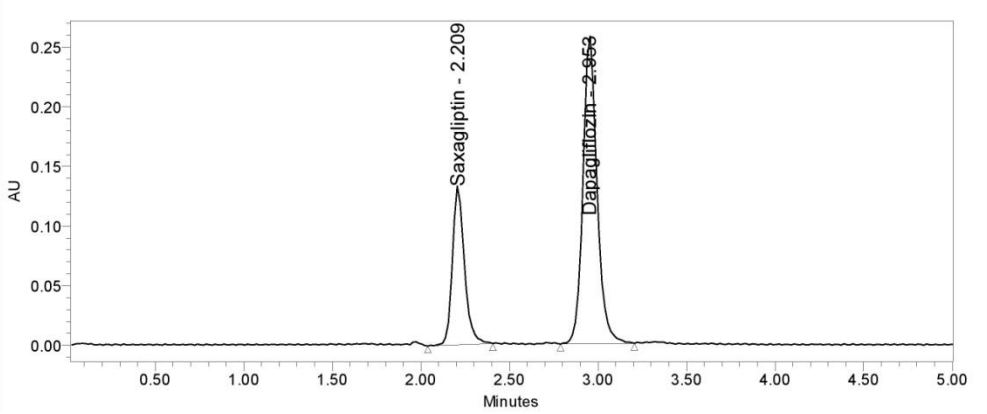

Figure 4: Linearity Spectra of Saxagliptin and Dapagliflozin at 222 nm

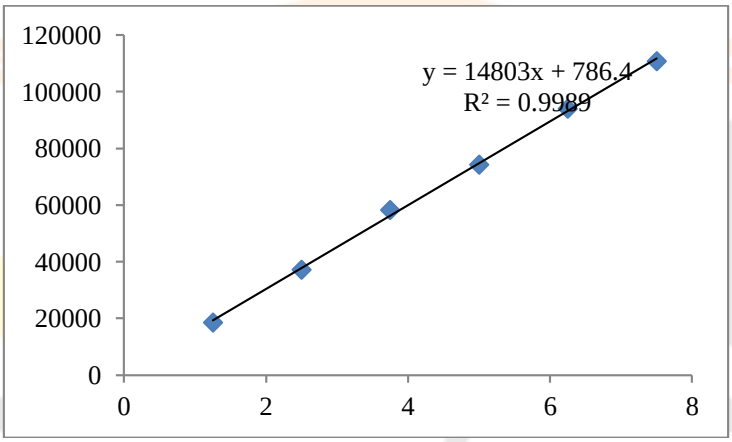

Figure 5: Calibration curve for Saxagliptin

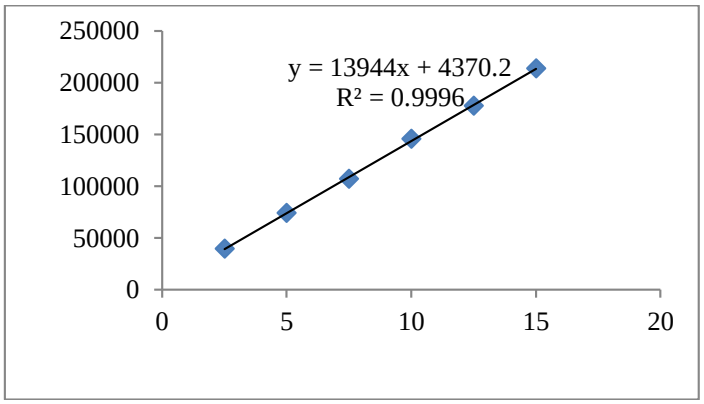

Figure 6: Calibration curve for Dapagliflozin

Accuracy

The accuracy of the method was determined by the standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of standard solution. The solutions were analyzed in triplicate at each level as per the proposed method. The corresponding results were recorded in table 5.

Table 5: Results of recovery experiments of saxagliptin and dapagliflozin

Preanalysed amount (µg/ml)		Spiked amount (µg/ml)		% Recovered	
Saxagliptin	**Dapagliflozin**	**Saxagliptin**	**Dapagliflozin**	**Saxagliptin**	**Dapagliflozin**
2.5	5	5	10	99.65	98.69
2.5	5	5	10	99.51	99.15
2.5	5	5	10	98.83	98.88
5	10	5	10	99.43	99.38
5	10	5	10	99.50	98.76
5	10	5	10	99.47	99.11
7.5	15	5	10	98.95	98.85
7.5	15	5	10	98.66	99.29
7.5	15	5	10	98.67	99.45
			MEAN	99.19	99.06
			SD	0.401	0.278
			%RSD	**0.4**	**0.28**

Precision

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Saxagliptin and Dapagliflozin test solution in the equipment. Record the chromatogram. The results were shown in table 6.

Table 6: Results of repeatability of saxagliptin and dapagliflozin

S. No.	Saxagliptin			Dapagliflozin		
	Area	**USP Plate Count**	**USP Tailing**	**Area**	**USP Plate Count**	**USP Tailing**
1	74018	5814	1.16	145393	7307	1.15
2	74880	5818	1.16	145648	7315	1.11
3	74418	5207	1.14	147415	6989	1.10
4	74589	5192	1.17	146112	6984	1.13
5	74784	5014	1.18	147197	7325	1.13

6	73576	5001	1.19	147630	7320	1.12
MEAN	74378			146566		
SD	497.1			967.0		
% RSD	**0.7**			**0.7**		

Intermediate Precision:

Six replicate injections of the same dilution were analyzed on two different days by different analyst for verifying the variation in the precision. The % RSD of the results for saxagliptin and dapagliflozin were found to be 0.4 and 0.7 respectively, which are within acceptable limit of ≤2. Hence, the method is reproducible on different days. This indicates that the method is precise. The results are shown in the Table 7a and 7b.

Table 7a: Results of Intermediate Precision of saxagliptin

S. No.	Average area (n=6)	USP Plate Count	USP Tailing
Day 1	73351	5315	1.28
Day 2	73349	5298	1.24
Overall average	73350		
SD	269.8		
% RSD	**0.4**		

Table 7b: Results of Intermediate Precision of dapagliflozin

S. No.	Average area (n=6)	USP Plate Count	USP Tailing
Day 1	148386	6656	1.14
Day 2	148384	6545	1.08
Overall average	148385		
SD	999.1		
% RSD	**0.7**		

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. The parameters included slight variation in flow rate of the mobile phase, composition of the mobile phase and column temperature. The robustness study was performed by slight modifications in flow rate of the mobile phase, composition of the mobile phase and column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed was robust. The results of robustness study are shown in Table 8 and 9.

Table 8: Robustness study for saxagliptin

Condition	Mean area	% assay	% difference
optimised	796701	99.65	-----
Flow rate at 0.9 mL/min	792534	99.01	0.64
Flow rate at 1.1 mL/min	790125	99.85	0.20
Mobile phase:			
• Buffer-acetonitrile(55:45)	785981	100.06	0.41
• Buffer-acetonitrile(65:35)	745869	100.12	0.47
Column Temperature:			
• at 25°C	789586	99.05	0.60
• at 35°C	785241	99.67	0.02

Table 9: Robustness study for dapagliflozin

Condition	Mean area	% assay	% difference
optimised	1783758	99.84	----
Flow rate at 0.9 mL/min	1801204	100.09	0.25
Flow rate at 1.1 mL/min	1795871	99.99	0.15
Mobile phase:			
• Buffer-acetonitrile(55:45)	1789854	99.67	0.17
• Buffer-acetonitrile(65:35)	1786549	99.25	0.59
Column Temperature:			
• at 25°C	1775847	99.01	0.83
• at 35°C	1785846	100.02	0.18

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

LOD and LOQ were determined from the standard deviation of y-intercept of the regression line and slope method as per ICH guidelines. LOD and LOQ values for saxagliptin were 0.01 and 0.03µg/ml respectively and those for dapagliflozin were 0.04 and 0.13µg/ml respectively. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive.

Analysis of Marketed Formulation by Developed Method

Assay of marketed tablet formulation containing 5 mg of Saxagliptin and 10 mg of Dapagliflozin was carried out by using this validated RP-HPLC method. Three injections of prepared sample and standard solutions were injected. The estimated values of the labeled claim of saxagliptin and dapagliflozin in the Qtern tablets (Saxagliptin – 5 mg and Dapagliflozin – 10 mg) were 99.38±0.5 % and 99.19±0.66 % respectively. RSD values for both saxagliptin and dapagliflozin are within limit of ≤2 and the results are shown in Fig.7 and Table 10.

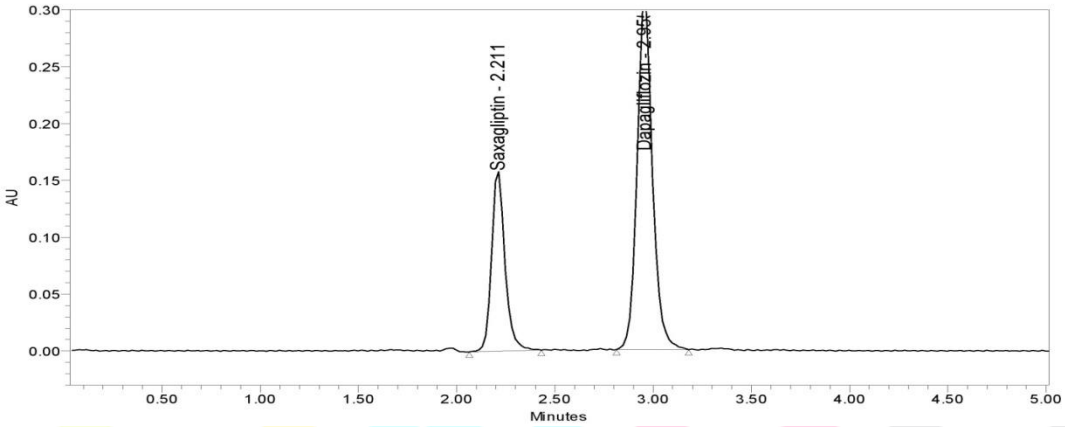

Figure 7: chromatogram showing separation of saxagliptin and Dapagliflozin from tablet formulation

Table 10: Analysis of Tablet dosage form

S.No.	Drug Name	Labeled amount (mg)	Amount found (mg)	% recovery ± SD*
1	Saxagliptin	5	4.95	99.0 ± 1.04
2	Dapagliflozin	10	9.89	98.9 ± 0.68

* n=6 for each parameter

DISCUSSION ON RESULTS

An Altima C18 column (4.6x150mm; 5µm) was selected as the stationary phase for separation of both drugs and detection was carried out at 222 nm. Initially, reverse phase liquid chromatography separation was attempted using various ratios of methanol and water and acetonitrile and water as the mobile phases, in which both the drugs were not eluted properly, and the resolution was also poor. Further, trials were also performed to optimize the organic

content of mobile phase using phosphate buffer. The retention times were found to about 2.187 min and 2.749 min for saxagliptin and dapagliflozin respectively.

CONCLUSION

The observations and result obtained from this study, including system suitability, linearity and range, accuracy, precision, robustness lie well within acceptable criteria. From the experimental studies, it can be concluded that the proposed method can be adopted for the routine analysis of Saxagliptin and Dapagliflozin in their combined dosage form.

REFERENCES

1. Dhillon S, Saxagliptin: A review in type 2 diabetes, Drugs 2015; 75:1783-1796.
2. Singhal M, Rasania S, Gaur A, Vishnu VM, Raju SV,Upadhyay Y, Overview on sodium glucose transport inhibitors as a therapeutic tool against diabetes mellitus, Global Journal of Pharmacology 2012; 6:86-93.
3. Abdalla SA, Validation of stability indicating RP-HPLC method for the estimation for saxagliptin in tablet formulations,Indo American Journal of Pharmaceutical Research 2014; 4:3550-3358.
4. Sravana kumara K,Sailaja B, Analytical Method Development and validation for estimation of dipeptidyl peptidase-4 inhibitors: A Review,International journal of current research in chemistry and pharmaceutical sciences 2015; 2:83–98.
5. Daswadkar C, Deshpande SV, Roy MA, Shubhan,Development and validation of uv- spectrophotometric method for estimation of saxagliptin in bulk and pharmaceutical dosage form,International journal of pharmaceutics and drug analysis2016;4:30-34.
6. Mante GV, Gupta KR, Hemke AT, Estimation of Dapagliflozin from its Tablet Formulation by UV-Spectrophotometry,Pharm Methods 2017; 1:102-107.
7. Debata J, Kumar S, Jha SK, Khan A, A New RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form,International journal of Drug Development and research 2017;9:48-51.
8. Deepan T, Dhanaraju MD, 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;31:39-43.
9. Vinutha K, Chowdary KP, Prasad SV, 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; 4:2920-32.
10. Patel AB, Patel DR, Shah Z, Development and validation of stability indicating method for the simultaneous estimation of saxagliptin hydrochloride and dapagliflozin using RP-HPLC method in tablet dosage form,World journal of pharmacy and pharmaceutical sciences2017;6:444-458.
11. Madhavi S,Prameela Rani A, Development and Validation of a Method for Simultaneous Determination of Dapagliflozin and Saxagliptin in a Formulation By RP-UPLC,World journal of pharmaceutical research2017;6:904-916.
12. SharmilaDonepudi, SuneethaAchanta, Simultaneous Estimation of Saxagliptin and Dapagliflozin in Human Plasma by Validated HPLC-UV Method,International journal of life science and pharmaceutical research 2018.
13. Bansal NSP,Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of dapagliflozin and saxagliptin in fixed-dose combination,New journal of chemistry2018;4.
14. Narendra N,Govindasamy J, Simultaneous estimation of saxagliptin hydrochloride and metformin hydrochloride in active pharmaceutical ingredient by RP-HPLC, Asian Journal of Pharmaceutical Research and Health Care2012;4:70-77.
15. Navneetkumarupadhyay, Charulrathore, Novel RP-HPLC method development and validation for the simultaneous estimation of Saxagliptin and Glimepiride, International journal of applied pharmaceutical sciences 2018;10:151-156.
16. DeepanT,BasaveswaraRao MV,Dhanaraju MD,Development of Validated Stability Indicating Assay Method for Simultaneous Estimation of Metformin and Dapagliflozin by RP- HPLC,European Journal of Applied Sciences2017; 9:189-199.

17. Mohammad Yunoos, GowriSankar D,A Validated stability indicating High-Performance liquid chromatographic method for simultaneous determination of metformin HCl and dapagliflozin in bulk drug and tablet dosage form,Asian Journal of Pharmaceutical and clinical research2015; 8:320-326.
18. Lakshmi Prameela K, Rama Krishna veni P, Satyanarayana PVV, Haribabu B,Development and validation of stability indicating reversephase high performance liquid chromatography method withphotodiode array detection for the simultaneous estimation of hypoglycemic agents: Dapagliflozin and Metformin,International journal of pharmaceutical biological sciences2017;8:328-336.
19. Ashishpatel, Dilipmaheshwari, Development and Validation of UV spectrophotometric method and RP-HPLC method for simultaneous estimation of dapagliflozinpropanediol and glimepiride in synthetic mixture,European journal of pharmaceutical and medical research 2017; 4:416-434.
20. Sherif A, Abdel-gawad, Abdul Malik S, Al-tamimi, Chromatographic simultaneous quantification of Dapagliflozin and Metformin hydrochloride in presence of their degradation products,International journal of biology, pharmacy and allied sciences 2017;6:2007-2021.
21. Patel PD,Pandya SS, Validated RP - HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin Hydrochloride in Tablet Dosage Form, International journal of pharmaceutical research scholars2018;7:9-15.
22. Shyamala, Nidhi B, Validated RP-HPLC Method for Simultaneous Estimation Of Metformin Hydrochloride and Dapagliflozin in Tablet Dosage Form,American Journal of Biological and Pharmaceutical Research. 2015; 2:09-113.
23. Syed Abdul Hadi, BharathRathna Kumar P,Simultaneous estimation of Metformin andDapagliflozin tablets by RP-HPLC, World journal of pharmacy and pharmaceutical sciences. 2017;6:1188-1199.
24. Phani RSCH, Prasad KRS,A Study of new method development, validation and forced degradation for simultaneous analysis of dapagliflozin and saxagliptin in pharmaceutical dosage form by HPLC Method,Der PharmaChemica2017;9: 96-103.
25. Pravincumar R,VasudevanM, A Validated RP-HPLC method for simultaneous estimation of metformin and saxagliptin in tablets, Rasayan Journal of Chemistry2012; 5: 137-141.
26. SarifNiroushKonari, Jane T. Jacob, Stability indicating validated RP-HPLC technique for the analysis of multicomponent anti-diabetic drug combos in pharmaceutical dosage forms, KarbalaInternational Journal of Modern Science2015;1: 39-48.
27. Hanan A.Merey, Nesrin K. Ramadan, Chromatographic methods for the simultaneous determination of binary mixture of SaxagliptinHCl and Metformin HCl, Bulletin of Faculty of Pharmacy2017;55:311–317.
28. NyolaNarendra,GovindasamyJeyabalan, Simultaneous Estimation of Saxagliptin Hydrochloride and Metformin Hydrochloride in active pharmaceutical ingrident by RP-HPLC,Asian journal of pharmaceutical research and Health care2014;4:70-77.
29. Asiya begum, shilpa K,Development and validation of stability indicating RP-HPLC method for saxagliptin and metformin in tablet dosage form,International journal of pharmacy2014; 4:271-279.
30. Prasanna ACK,PriyankaKanuri, Method development and validation of simultaneous determination of metformin and saxagliptin in Pharmaceutical Dosage form by RP-HPLC.International Journal of Pharmaceutical, Chemical and Biological Sciences2015;5:381-387.
31. PrasadPBN, Satyanaryana K, Simultaneous Determination of Metformin, Linagliptin In Jentadueto and Metformin, Saxagliptin in Kombiglyze by LC-MS Method,International Journal of Pharmacy and Pharmaceutical Sciences. 2018; 10:110-116.
32. Kumara Swamy G,Shruthi S, A New stabilty indicating RP-HPLC method for simultaneous determination of saxagliptine and dapagliflozin in bulk and combined tablet dosage forms,Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry2017;5:113-121.
33. PrasadPBN, Satyanaryana K,Development and validation of a method for simultaneous determination of metformin and saxagliptin in a formulation by RP-HPLC, American Journal of Analytical Chemistry2015;6:841-850.
34. Prakash PP, Ramesh KS,A New RP-HPLC Method for determination of Metformin HCl and Saxagliptin in Tablet Dosage Form, International Journal of Pharma and Bio Sciences2012;2:161-167.
35. Bhagavanji NVMS,Satyanarayana PVV, Development and validation of stability indicating liquid Chromatographic method for the simultaneous estimation of Metformin and saxagliptin in combined dosage form,International Research Journal of Pharmaceutical and Applied Sciences2013;3:37-42.

36. Ramesh J, Senthil Kumar N, A Validated High Performance Liquid Chromatography Method for the Determination of Saxagliptin and Metformin in Bulk and Pharmaceutical Dosage Form, a Stability Indicating Study,Journal of Pharmacy and Biological Sciences2016; 11:92-100.
37. Mohammad Yunoos,GowriSankar D, Stability indicating quantitative RP-HPLC method development and validation for simultaneous determination of metformin hydrochloride and saxagliptin in bulk and combined tablet dosage form,Journal of Chem. Pharm2015;7:346-35.
38. Salwa R. El-Shaboury, Samia M. El-Gizawy,Validated Spectrodensitometric Method for Simultaneous Estimation of Sofosbuvir, Ribavirin and Saxagliptin in their Pure and Pharmaceutical Dosage Forms,Current Pharmaceutical Analysis2018;14:212-218.
39. Eman I. El-Kimary,Hamdy Dalia A, Mourad Sara S, Barary Magda A, HPTLC determination of three gliptins in binary mixtures with metformin,Journal of Chromatographic Science2016; 54:79–87.
40. Nyola. Narendra, Jeyabalan,Development and validation of UV-Vis spectroscopy method for simultaneous estimation of saxagliptin hydrochloride and metformin hydrochloride in active pharmaceutical ingredient,Journal of Pharmaceutical Education and Research2012;3:19-23.
41. Pranali SS,RajHA,Simultaneous determination of Saxagliptin Hydrochloride and Glibenclamide in Synthetic Mixture using Spectrophotometric technique (First order Derivative Method).
42. Jani BR, Shah KV, Kapupara PP, Development and Validation of UV Spectroscopic method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture Journal of Bioequivalence Studies 2015; 1:1-9.
43. Raveendra BG, KumarRA,Shaheen SD, A Novel Stability-Indicating Method for the Simultaneous Estimation of Saxagliptin and Dapagliflozin in Rat Serum by Using UV Spectroscopy,PharmaceuticaAnalyticaActa2018;9:1-5.
44. Jani BR, Shah KV,Kapupara PP, Development and Validation of UV spectroscopic method for simultaneousestimation of Dapagliflozin and Metformin hydrochloride in syntheticMixture,International Journal of Research and Development in Pharmacy & Life Sciences2015; 4:1569-1576.
45. Sutharasim M, Laxman MP, Estimation of Saxagliptin hydro chloride and Dapagliflozinpropendiol monohydrate in combined dosage form,Journal of Innovations in Applied Pharmaceutical Science2018; 3:01-07.
46. Sawant RL,Mhaske SM, Analytical method development for simultaneous estimation of saxagliptin and methyldopa,Asian Journal of Pharmaceutical Research2014;4:134-140.
47. ICH guideline Q2 (R1). (1996). Validation of analytical procedures: text and methodology. Geneva. 12. Guidance for industry. (2001).
48. Bioanalytical method validation. Rockville, Maryland: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM).