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Research article

Estimation of Saxagliptin hydrochloride and Dapagliflozin propendiol monohydrate in combined dosage form

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Keywords: Dapagliflozin propendiol monohydrate, saxagliptin hydrochloride, UV- spectroscopy, dual wavelength.

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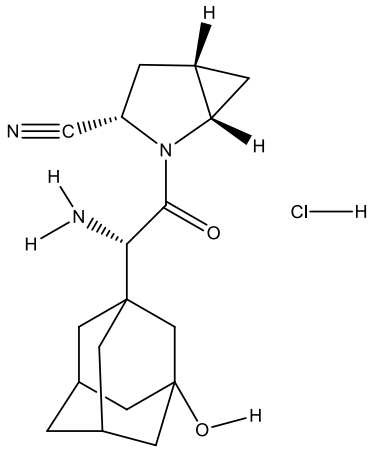
Abstract

Aim of present study is to develop simple, precise, and accurate method for simultaneous quantitative estimation of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate in pharmaceutical tablet dosage form. The method was based on determination of saxagliptin hydrochloride at an absorbance difference between 214.40 nm - 220.0 nm and sapagliflozin propendiol monohydrate at an absorbance difference between 208.0 nm - 209.0 nm. The linearity was obtained in the concentration range of 4-16 µg/ml and 10-22 µg/ml for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate respectively. The suitability of this method was proved by validation in accordance with ICH Q2 (R1) guidelines. The method was found to be accurate with percent recovery 99.30-99.86 % and 100–100.31% for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate respectively. The proposed method was found to be simple and sensitive for routine quality control application of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate used in pharmaceutical tablet dosage form.

Introduction

Chemically Saxagliptin hydrochloride (SAXA) is (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile; hydrochloride [1]. (Figure 1). Saxagliptin hydrochloride is a dipeptidyl peptidase-4(DPP-4) inhibitor used as an oral hypoglycaemic agent. It is used in combination with diet and exercise in the therapy of type 2 diabetes, either alone or in combination with other oral hypoglycemic agents [2] (Figure 1).



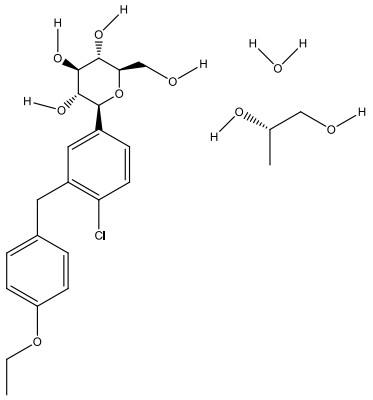


Figure 1. Structures of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate.

Dapagliflozin propendiol monohydrate (DAPA) is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-6-(hydroxymethyl) oxane-3, 4, 5-triol; propane-1, 2-diol; dehydrate [3] (Figure 1). Dapagliflozin propendiol monohydrate is indicated for the management of diabetes mellitus type-2 to improve glycemic control in adults when combined with diet and exercise. Dapagliflozin propendiol monohydrate is a sodium-glucose co-transporter-2 inhibitor, which prevents glucose re-absorption in the kidney [4]. Combination therapy is mainly indicated as an adjunct to diet and exercise to

improve glycemic control in adults with type-II Diabetes [5].

Saxagliptin hydrochloride and dapagliflozin propendiol monohydrate both are not official in any pharmacopoeia. As literature review reveled that there are reported methods available for saxagliptin hydrochloride alone and in combination with other drugs [6-11]. There is couple of methods reported for dapagliflozin propendiol monohydrate either single or in combination with other drugs [12-15]. Literature survey also revealed that several analytical methods have been reported for the estimation of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate in combination, all are being HPLC [17-20]. No UV-spectrophotometric method has been reported for quantitative estimation of saxagliptin hydrochloride and dapagliflozin propendiol from combined dosage form

Therefore, it seemed necessary to develop a UV-spectrophotometric method for the simultaneous estimation of saxagliptin hydrochloride and sapagliflozin propendiol monohydrate in tablet dosage form. The present investigation was conducted with the goal of establishing a simple, rapid and robust dual wavelength UV-spectrophotometric method for the simultaneous estimation of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate in pharmaceutical tablet dosage form.

Experimental

Materials and method

Apparatus and instruments

Double beam UV- visible spectrophotometer (Shimadzu, model 1601) having two matched quartz cells with 1 cm light path.

Digital analytical balance (Shimadzu ATX 224)

Volumetric flask 10-100ml

Pipettes-1, 2, 10 ml

Calibrated Glassware was used throughout the work.

Reagent and materials

Pure drug samples of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate were obtained from Advanced Analytical Lab, Ahmadabad

Methanol AR was used as solvent.

Preparation of standard stock solution (100 µg/ml)

Accurately weighed quantity of saxagliptin hydrochloride (10mg) and dapagliflozin propendiol monohydrate (10mg) was transferred to two separate 100 ml volumetric flask, dissolved in little amount of methanol (sonicate at 37 ºC for 5 min as necessary) and diluted to the mark with methanol.

Determination of Analytical wavelength

Working standard solution of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate were scanned in range of 200-400 nm for the determination of wavelength. From the overlain spectra (figure 2), four wavelength 214.40 nm, 220.0 nm, 208.0 nm and 209.0 nm were selected for quantization of both the drug by proposed dual wavelength spectrophotometry method. The quantitative determination of saxagliptin hydrochloride is carried out by measuring the absorbance difference value at 214.40 nm and 220.0 nm, where dapagliflozin propendiol monohydrate has same absorbance at both the wavelength. As a same way quantitative determination of dapagliflozin propendiol monohydrate is carried out by measuring the absorbance difference value at 208.0 nm and 209.0 nm where saxagliptin hydrochloride has same absorbance at both the wavelength. Considering above facts, four wavelength 214.40 nm, 220.0 nm, 208.0 nm and 209.0 nm were selected for quantization of both the drug by proposed dual wavelength spectrophotometry method. The overlain spectra are shown in figure 2.

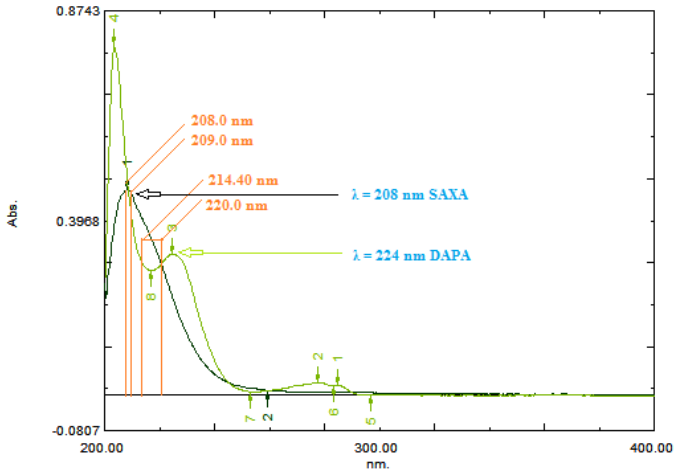


Figure 2. Overlain spectra of saxagliptin hydrochloride (6µg/ml) and dapagliflozin propendiol monohydrate 18µg/ml).

Preparation of calibration curve

Calibration curve for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate consisted of different concentration of standard saxagliptin hydrochloride solution ranging from 4-16 µg/ml and dapagliflozin propendiol monohydrate solution ranging from 10-22 µg/ml. These concentrations were prepared by pipetting out appropriate volumes of aliquots from the stock solution and dilute with methanol in 10 ml volumetric flask. The absorption spectra of above solutions were recorded in the range of 200-400nm. The increasing absorbance difference between 214.40nm and 220.0nm is directly proportional to concentration of saxagliptin hydrochloride, where dapagliflozin propendiol monohydrate has same absorbance at the both wavelength. Same as the increasing absorbance difference between 208.0nm and 209.0nm is directly proportional to concentration of dapagliflozin propendiol monohydrate,

where saxagliptin hydrochloride has same absorbance at the both wavelength. The absorbance differences of resulting solutions were plotted against their respective concentration.

Method validation

The developed method was validated according to ICH guidelines Q2 (R1) for parameters such as linearity, repeatability, precision, accuracy and, limit of detection and limit of quantification [21].

Linearity and range

The calibration curve was plotted over a concentration range of 4-16 µg/ml and 10-22 µg/ml for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate respectively. Accurately measured standard solution of saxagliptin hydrochloride (100µg/ml) (1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 ml) and dapagliflozin propendiol monohydrate (100µg/ml) (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of the solution was measured at 214.40nm, 220.0 nm, 208.0 nm and 209.0 nm against methanol as blank. The calibration curves were constructed by plotting absorbance differences versus concentrations and the regression equations were calculated.

Precision

Repeatability

Standard solutions of saxagliptin hydrochloride (6 µg/ml) and dapagliflozin propendiol monohydrate (18 µg/ml) were prepared and spectra were recorded. Absorbance was measured using methanol as a blank. The absorbances of the same concentration solution were measured five times and % RSD was calculated.

Intraday & interday

The intraday and Inter day precision of the proposed method was determined by analyzing the corresponding response 5 times on the same day and different day for three concentrations of saxagliptin hydrochloride (6, 8, 10 µg/ml) and dapagliflozin propendiol monohydrate (10, 12, 14 µg/ml). The result was reported in terms of relative standard deviation (RSD).

Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of Quantitation (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by international conference on harmonization (ICH) guidelines

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

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

Where, σ= the standard deviation of the response
S= slope of the calibration curve

Accuracy

The accuracy of the method was determined by calculating recoveries of saxagliptin hydrochloride (4.5µg/ml) and dapagliflozin propendiol monohydrate (9µg/ml) by the spiked standard addition method. The experiment was repeated three times.

Assay

Twenty tablets were triturated to fine powder, An Accurately weighed powder equivalent to 5 mg saxagliptin hydrochloride and 10 mg dapagliflozin propendiol monohydrate in to a 100ml volumetric flask. Add 30 ml of methanol and sonicate it for min and volume was made up to 100ml. and filter it through whatman filter paper. Transfer to final volume of filtrate (0.5ml) into 10 ml volumetric flask diluted to mark with methanol to get the concentration of 5 µg/ml saxagliptin hydrochloride and 10 µg/ml of dapagliflozin propendiol monohydrate.

Results and discussion

Linearity and range

The calibration curve was plotted over a concentration range of 4-6 µg/ml and 10-22 µg/ml for SAXA and DAPA respectively (Figure 3 and 4).

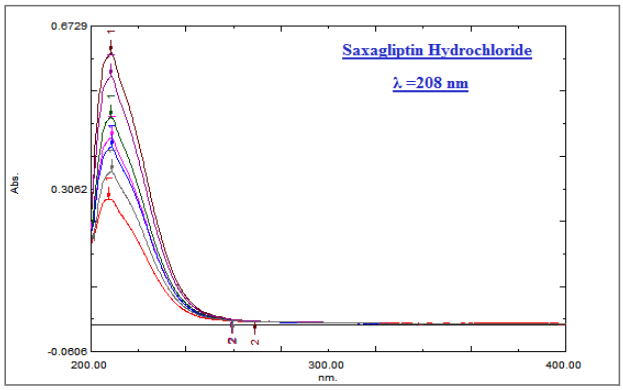


Figure 3. Linearity spectra of Saxagliptin hydrochloride (4µg/ml to16 µg/ml).

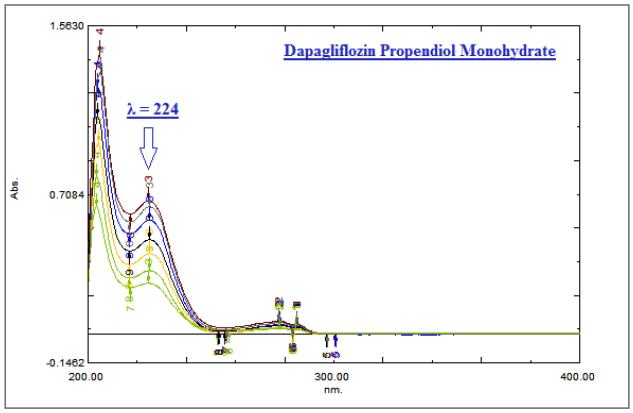


Figure 4. Linearity spectra of dapagliflozin propendiol monohydrate (10µg/ml to 22 µg/ml).

Beers law was obeyed over this concentration range. Linearity data for SAXA and DAPA are presented in Table 1. The regression equation was found to be Y=0.004x – 0.001 for SAXA and Y =0.006x + 0.0023 for DAPA. The correlation coefficient (r^2) of the standard curve was found to be 0.996 and 0.995 for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate respectively. Calibration curve is presented in the (Figure 5 and 6).

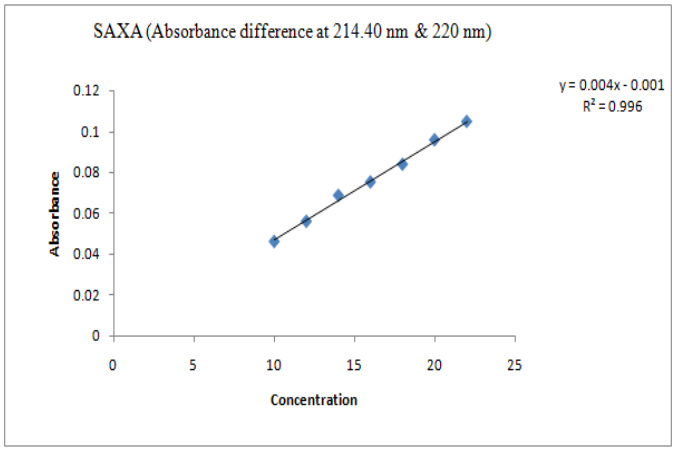


Figure 5. Calibration curve Saxagliptin hydrochloride (SAXA) (4 µg/ml to 16µg/ml) in methanol.

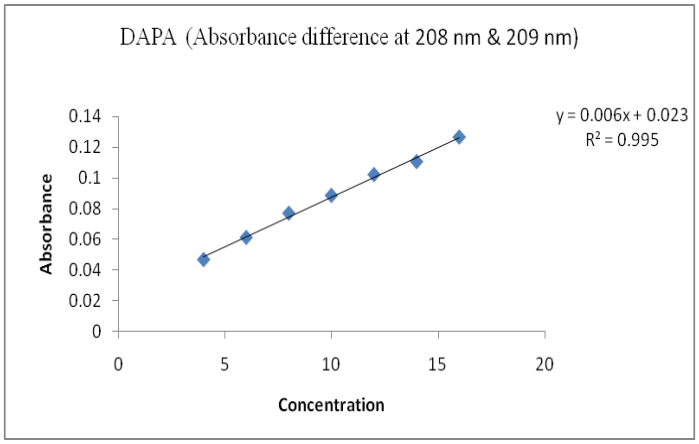


Figure 6. Calibration curve dapagliflozin propendiol monohydrate (DAPA) (10 µg/ml to 22 µg/ml) in methanol.

Table 1. Linearity data of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate.

Dapagliflozin propendiol monohydrate			Saxagliptin hydrochloride		
Conc. (µg/ml)	**Absorbance difference at 208 nm & 209 nm**	**Correlation coefficients r^2**	**Conc. (µg/ml)**	**Absorbance difference at 214.40 nm & 220 nm**	**Correlation coefficients r^2**
10	0.0471		4	0.0463	
12	0.0614		6	0.0561	
14	0.0771		8	0.0687	
16	0.0886	0.995	10	0.0752	0.996
18	0.1021		12	0.0838	
20	0.1105		14	0.0957	
22	0.1264		16	0.1046	

Repeatability

Results of Repeatability studies expressed in % RSD follows ICH guideline acceptable limits, which indicate that the proposed method is repeatable (Table 2).

Table 2. Repeatability data of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate.

Sr. No.	Absorbance of Saxagliptin hydrochloride (6 µg/ml)	Absorbance of Dapagliflozin propendiol monohydrate (18 µg/ml)
1	0.0841	0.0618
2	0.0839	0.0624
3	0.0846	0.0611
4	0.0840	0.0605
5	0.0839	0.0621
6	0.0837	0.0609
Mean	0.0841	0.06158
SD	0.000292	0.000773
% RSD	0.34666	1.25352

Precision

Variation of results within the same day (intra- day), variation of results between days (interday) were analyzed.

For intra-day (n=3) % RSD was found to be 0.56 - 0.82 and 0.33-1.21 for Saxagliptin and Dapagliflozin respectively (Table 3). For interday (n=3) % RSD was found to be 0.91 - 1.43 and 0.52-1.02 for Saxagliptin and Dapagliflozin respectively (Table 4). The low values of % RSD for intraday and interday precision suggest that method is precise.

Limit of detection and limit of quantitation

The developed method was found to be sensitive which was evaluated in terms of LOD and LOQ. Limit of detection and limit of quantitation for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate are depicted in table 5.

Accuracy

The accuracy of the method was determined by calculating recoveries of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate by the spiking standard addition method. The obtained results for % recovery of to be within the acceptable range (% RSD<2), thus providing evidence that the proposed method is accurate (Table 6).

Assay of marketed formulation

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation QTERN. The results are shown in table 7 and 8.

Table 3. Intraday precision data for estimation of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate.

Saxagliptin hydrochloride			Dapagliflozin propendiol monohydrate.		
Conc. (µg/ml)	Absorbance difference between (214.40nm & 220.0nm) ±SD	% RSD	Conc. (µg/ml)	Absorbance difference between (208.0nm & 209.0nm) ±SD	%RSD
6	0.0461±0.0003	0.7716	10	0.0618±0.0007	1.2183
8	0.0564±0.0003	0.5606	12	0.0772±0.0002	0.3375
10	0.0679±0.0005	0.8234	14	0.0886±0.0003	0.3776

Table 4. Interday precision data for estimation of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate.

Saxagliptin hydrochloride			Dapagliflozin propendiol monohydrate		
Conc. (µg/ml)	Absorbance difference between (214.40nm & 220.0nm) ±SD	%RSD	Conc. (µg/ml)	Absorbance difference between (208.0nm & 209.0nm) ±SD	%RSD
6	0.0398±0.0005	1.436	10	0.0718±0.0007	1.028
8	0.0521±0.0005	1.140	12	0.0849±0.0007	0.6342
10	0.0659±0.0006	0.9134	14	0.0987±00005	0.5290

Table 5. Limit of detection and limit of Quantitation data for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate.

Parameters	Saxagliptin hydrochloride	Dapagliflozin propendiol monohydrate
LOD	0.2409	0.4235
LOQ	0.73	1.28

Table 6. Recovery data for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate. (n=3)

Drug	Accuracy	Amount				% Recovery	Mean % Recovery
		Taken	Added	Total	Found		
Dapagliflozin propendiol monohydrate	80%	9	7.2	16.2	16.19	99.93	
					16.24	100.24	100
					16.17	99.81	
	100%	9	9	18	17.82	99.0	
					18.25	101.38	100.314
					18.1	100.55	
	120%	9	10.8	19.8	19.85	11.252	
					19.79	99.94	100.269
					19.92	100.60	
Saxagliptin hydrochloride	80%	4.5	3.6	8.1	8.2	101.234	
					7.98	98.51	99.300
					7.95	98.148	
	100%	4.5	4.5	9	9.1	101.111	
					8.89	98.77	99.81
					8.96	99.95	
	120%	4.5	5.4	9.9	9.85	99.49	99.86

Table 7. Analysis of marketed formulation.

Tablet Brand Name	Label claim (mg)		% Assay	
QTERN	Dapagliflozin propendiol monohydrate	Saxagliptin hydrochloride	Dapagliflozin propendiol monohydrate	Saxagliptin hydrochloride
	10	05	101%	101.5%

Table 8. Summary of optical characteristics and other parameters.

Parameters		Saxagliptin hydrochloride	Dapagliflozin propendiol monohydrate
Linearity range(µg/ml)		4-16µg/ml	10-22µg/ml
Regression line equations		Y=0.004x – 0.001	Y =0.006x + 0.0023
Correlation coefficient (R^2)		0.996	0.995
Limit of detection (µg/ml)		0.2409	0.4235
Limit of quantification (µg/ml)		0.73	1.28
Intraday (%RSD) (n=3)		0.7716 0.5606 0.8234	1.2183 0.3375 0.3776
Inter day (%RSD) (n=3)		1.4368 1.140 0.9134	1.028 0.6342 0.5290
Repeatability(%RSD) (n=6)		0.3469	1.2570
Accuracy (% recovery) (n=3)	80% 100% 120%	99.30 99.81 99.86	100 100.31 100.26
% Assay		101.5%	101%

Conclusion

The results and the statistical parameters demonstrate that the proposed dual wavelength UV spectrophotometric method is found to be simple, sensitive, accurate, precise, reproducible, and relatively inexpensive. These levels of accuracy and precision obtained indicate suitability of the developed method for the quality control analysis. So, the developed method can be recommended for the routine of the saxagliptin hydrochloride and dapagliflozin propendiol monohydrate in pharmaceutical tablet dosage form

References

1. Scott AS, Gregory SJ, Sergei K, Shelly AR, Truc V, Robert E. *et al.* Preparation of Saxagliptin, a Novel DPP-IV Inhibitor. Org Process Res Dev. 2009; 13(6):1169–76.
2. Frederich R, Alexander JH, Fiedorek FT, Donovan M, Berglind N, Harris S *et al.* A systematic assessment of cardiovascular outcomes in the saxagliptin drug development program for type 2 diabetes. Postgrad Med. 2010; 122(3):16-27.
3. Wei M, Bruce AE, Alexandra AN, Peggy JM, Manorama P, Ravindar NG. et al. A Potent, Selective Renal Sodium-Dependent Glucose Cotransporter 2 (SGLT2) Inhibitor for the Treatment of Type 2 Diabetes J Med Chem. 2008; 51(5):1145–49.
4. Chao EC, Henry RR. SGLT2 inhibition—a novel strategy for diabetes treatment. Nat Rev Drug Dis. 2010; 9(7):551–59.
5. Rosenstock J, Hansen L, Zee P, Li Y, Cook W, Hirshberg B. *et al.* Dual add-on therapy in type 2 diabetes poorly controlled with metformin monotherapy: a randomized double-blind trial of saxagliptin plus dapagliflozin addition versus single addition of saxagliptin or dapagliflozin to metformin. Diabetes Care 2015; 38(3):376-83.
6. Chhabda P, Balaji M, Srinivasarao V, Ramakrishna K, Apparao KM. Devolpment And Validation Of Simple Stability Indicating Rp-Hplc Method For Analysis of Saxagliptin And Its Forced Degradation Impurities In Bulk Drug And Pharmaceutical Dosage Form. Int J Res Dev Pharm Life Sci. 2014; 3(3):993-1003.
7. Shubhangi CD, Madhumita AR, Sanjay GW, Mahendra CB. Quality By Design Approach For The Development And Validation Of Saxagliptin By RP-HPLC With Application To Formulated Forms. Int J Pharm Sci Res. 2016; 7(4): 1670-77.
8. Saiful Md, Taleb H, Sukalyan K, Abdul Md, Rafiquzzaman Md. Development and validation of RP-HPLC method for determination of saxagliptin hydrochloride in bulk and tablet dosage form. World J Pharm Pharm Sci. 2016; 5(5): 107-19.
9. Prasad PBN, Satyanaryana K, Krishnamohan G. Development and Validation of a Method for Simultaneous Determination of Metformin and Saxagliptin in a Formulation by RPHPLC. Am J Analyt Chem. 2015; 6(11): 841-50.
10. Kalaichelvi R, Jayachandran E. Validated spectroscopic method for estimation of saxagliptin in pure and from tablet formulation. Int J Pharm Pharm Sci. 2011; 3(3):179-80.
11. Sawant RL, Mhaske SM. Analytical method development for simultaneous estimation of saxagliptin and methyldopa. Asian J. Pharm. Res. 2014; 4(3):134-40.
12. Sanagapati M, Dhanalakshmi K, Nagarjunareddy G, Sreenivasa S. Development and validation of a RP-HPLC method for the estimation of dapagliflozin in API. Int J Pharm Sci Res. 2014; 5(12):5394-97.
13. Shyamala, Nidhi B, Kavitha M, Pooja, Sharma JVC. Validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and dapagliflozin in tablet dosage form. American J of Bio and Pharma Res. 2015; 2(2):109-13.
14. Karuna P, Chitra M, China E, Basaveswara MV. Unique UV spectrophotometric method for reckoning of dapagliflozin in bulk and pharmaceutical dosage forms. Journal of Chem and Pharma Res. 2015; 7(9):45-49.

15. Gajanan VM, Krishna RG, Atul TH. Estimation of Dapagliflozin from its Tablet Formulation by UV-Spectrophotometry. Pharm Methods 2017; 8(2): 102-107.
16. Gandla S, Shruthi S, Rajkumar M, Sudheer D. A new stabilty indicating RP-HPLC method for simultaneous determination of saxagliptine and dapagliflozin in bulk and combined tablet dosage forms. Asian J of Pharma Ana and Med Chem. 2017; 5(3):113-21.
17. Sarath N, Seshagiri Rao J V L N. A Stability Indicating RP-HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in Combined Tablet Dosage Forms. Inventi Rapid: Pharm Analysis & Quality Assurance 2017; (4):1-11.
18. Advaita B, Deepa R, Zarna S. 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 J Pharm Pharm Sci. 2017; 6(10):444-58.
19. Patel PD, Pandya SS. Validated RP - HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin Hydrochloride in Tablet Dosage Form. Int. J. Pharm. Res. Scholars. 2018; 7(1): 9-15.
20. Jani BR, Shah KV, Kapupara PP. Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture. Journal of Bioequivalence Studies. 2015; 1(1):1-8.
21. International Conference on Harmonisation. Guideline. ICH. Validation of analytical procedures: text and methodology. Q2 (R1). Geneva: ICH; 2005. 17.
22. Naved Ahmed, Obaid Shaikh, Aateka Barrawaz, Sarfaraz Khan, Zahid Zaheer. Development and validation of rapid HPLC method for determination of Aripiprazole in bulk drug and pharmaceutical formulation. Journal of Innovations in Pharmaceutical and Biological Sciences 2017; 4(3): 15-19.
23. Sushil D. Patil, Sunil V. Amurutkar, Chatpalliwar V. A., Chandrashekhar D. Upasani. Development and validation of RP-HPLC method for Empagliflozin and Metformin HCL. Journal of Innovations in Pharmaceutical and Biological Sciences 2017; 4(4): 185-189.
24. Haritha Gali, Vishwanadham Yerragunta. Development and validation of RP-HPLC method for simultaneous estimation of Naproxen and Esomeprazole in pharmaceutical dosage form. Journal of Innovations in Pharmaceutical and Biological Sciences 2016; 3(3): 39-42.
25. Desabattina Sai Jahnavi, Desabattina Venkata Narayana Rao, Yeluri Ramachandra Reddy. RP-HPLC method for the quantification of Metformin and Rosiglitazone in bulk and combined tablet dosage form: method development and validation. Journal of Innovations in Pharmaceutical and Biological Sciences 2016; 3(4): 55-60.
26. Zahid Zaheer, Furquan Nazimuddin Khan, Darakhshan Afreen Shaikh, Sarfaraz Khan, Mirza Shahed Baig, Moizul Hasan. Zero order derivative spectroscopic estimation of olopatadine hydrochloride from eye drops. Journal of Innovations in Pharmaceutical and Biological Sciences 2016, 3(2): 103-107.