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RESEARCH ARTICLE

A NOVEL ANALYTICAL METHOD FOR SIMULTANEOUS QUANTIFICATION OF DAPAGLIFLOZIN AND SITAGLIPTIN BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT
The Reverse phase HPLC method was developed for simultaneous determination of Dapagliflozin and Sitagliptin in single analytical method. Chromatographic separation was achieved on a Hypersil BDS C18 (250mmx4.6mm, 5µm) column applying an gradient elution based on potassium phosphate monobasic buffer pH (3.0) as mobile phase A while methanol and acetonitrile in the ratio of (60:40 v/v) as a mobile phase B with gradient program Time/Mobile phase A%/Mobile phase B% is as 0 min./55/45, 3 min./55/45, 9 min./20/80, 13 min.20/80, 15 min./55/45, 20 min./55/45. Validation parameters specificity, linearity, accuracy, precision and robustness have been observed to be desirable over the concentration ranges of 50-150 µg/ml for Dapagliflozin and Sitagliptin respectively. All the variables have been studied to optimize the chromatographic conditions. The optimized approach verified through validation and confirmed to be intended purpose for the quality control of the mentioned drugs, as per ICH guidelines. For simultaneous quantification of Dapagliflozin and Sitagliptin, the developed method was found to be genuinely exact precise, accurate, linear, fast and cost effective.

KEYWORDS: Sitagliptin, Dapagliflozin, High-performance liquid chromatography, Validation.

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INTRODUCTION
Many multi components drugs have been developed by pharmaceutical industries. Analyst can separate and quantify them with more effective method.(1) Nowadays regulatory agencies and industries required faster drug product development and validation in a shorter time. Therefore pharmaceutical analysts have pressure to deliver accurate and precise analytical data in a potential time.(2) LC is the most abundant analytical technique in pharmaceutical industry for qualitative and quantitative estimation of analyte and their impurities in production batches.(3)

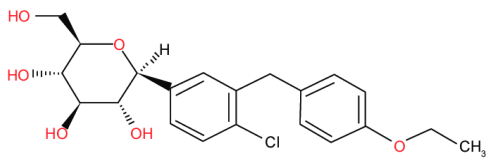
For achieving drug discovery, development and their production in pharmaceutical industries, method development and method validation play an important role. In current scenario one or more drugs formulated in one product, typically referred to as combination products to increase therapeutic effects. Combination products are challenging to pharmaceutical analyst for developing and validating a simple, precise, accurate, reproducible, robust method in single analytical method to achieve timeline and cost effective to commercialize. (4)

A detailed literature review shown that individual analytical HPLC method is available for the determination of Dapagliflozin and Sitagliptin.(5, 6) But so far there is no single analytical HPLC method is available for simultaneous estimation of Dapagliflozin and Sitagliptin. The goal of this study is to establish a simple, reproducible, linear, precise single analytical method for simultaneous quantification of Dapagliflozin and Sitagliptin.

Dapagliflozin improved glycemic control by inhibiting glucose resorption in the proximal tubule of nephron.(7,8) When combination with diet and exercise, Dapagliflozin suppressed sodium-glucose co transporter 2 and controlled diabetes mellitus type 2 in adults.(9) FDA approved Dapagliflozin on Jan 2014.(10)

Dapagliflozin IUPAC Name: (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol

Structure:



Molecular Formula: $C_{21}H_{25}ClO_6$

Molecular Weight: 408.9 g/mol

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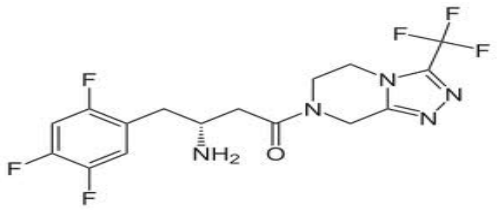
Sitagliptin is used to treat high blood sugar levels caused by type 2 diabetes. Sitagliptin acts by preventing incretin hormones from being rapidly broken down. This improves insulin sensitivity and decreases blood sugar levels. Sitagliptin belongs to the dipeptidyl peptidase-4 (DPP-4) inhibitors class of drugs. It works by increasing the amounts of certain natural substances that lower blood sugar when it is high.[11] Sitagliptin was granted FDA approval on October 16, 2006. [12]

Sitagliptin IUPAC Name: (3R)-3-amino-1-[3-(trifluoro-methyl)-6,8-dihydro-5*H*-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one

Structure:



Molecular Formula: $C_{16}H_{15}F_6N_5O$

Molecular Weight: 407.31 g/mol

METHOD AND MATERIALS

The liquid chromatography consisted of a Shimadzu HPLC SYSTEM Model LC 2010 High-Performance Liquid Chromatography. For the RP-HPLC system, a Hypersil BDS (make: thermo) C18 (250 mm x 4.6 mm, 5 µm) column was used. A Photodiode array detector (PDA) with an automated sample injector integrated with the system. Empower software was used to monitor and integrate the output signal. Digital pH meter was used to adjust and determine the hydrogen ion concentration (pH) of the buffer solutions. Active Pharmaceutical ingradient (API) of Dapagliflozin and Sitagliptin Phosphate were supplied as gift from Aspire Lifesciences Pvt Ltd, Mumbai. HPLC grade Methanol, Acetonitrile, Water, Ortho-Phosphoric acid (OPA) and Potassium Phosphate monobasic of analytical grade were obtained from Finar Chemicals Ltd.

Mobile Phase and Chromatographic conditions

Potassium phosphate monobasic buffer solution was prepared by dissolving 2.72 gm of potassium phosphate monobasic in 1000 ml of HPLC grade water. The solution pH was adjusted to 3.0 with orthophosphoric acid (OPA). Use buffer solution pH 3.0 as a mobile phase A. Prepared by mixing methanol and acetonitrile in the ratio of 60:40 v/v respectively and used as mobile phase B. Diluent used mixture of Milli-Q water and methanol in the ratio of 50:50 v/v.

Standard Preparation

Standard stock solutions of Dapagliflozin were prepared by dissolving about 30 mg of Dapagliflozin propanediol monohydrate working standard equivalent to about 25 mg of Dapagliflozin into a 50 mL volumetric flask add about 30 mL of methanol sonicate to dissolve and make up to volume to 50 mL with diluent.

Standard stock solutions of Sitagliptin were prepared by dissolving 80 mg of Sitagliptin Phosphate working standard equivalent to about 62 mg of Sitagliptin into a 50 mL volumetric flask add about 30 mL of methanol sonicate to dissolve and make up to volume to 50 mL with diluent. Transfer 5 mL of Dapagliflozin standard stock solution and 20 mL of Sitagliptin standard stock solution to a 50 mL volumetric flask, dilute to volume with diluent and mix.

Sample Preparation

Accurately 20 tablets were weighed and the average weight was calculated. The tablets were crushed and a quantity of powder equivalent to 50 mg of Dapagliflozin and 500 mg of Sitagliptin was transferred into 200 mL volumetric flask and diluent was added about 140 ml. The solution was sonicate for 10 min. It was diluted to volume with diluent and mixed well. A portion of the solution was filtered with the 0.45µm membrane filter and transferred 5 ml of filtered solution into a 25 ml volumetric flask and made up to the mark with diluent and mixed well.

Chromatographic Conditions

Conditions of chromatography Photodiode array detector (PDA), with an automated sample injector integrated with Shimadzu, Model: LC 2010. Empower software was used to monitor and integrate the output signal. On a Hypersil BDS C18 (250x4.6mm, 5µm) column, the separation was obtained. The eluent was monitored at 215 nm using a PDA detector while the column temperature was kept at 35°C with 10 µl injection volume. Phosphate monobasic buffer pH (3.0) as mobile phase A while methanol and acetonitrile in the ratio of (60:40 v/v) as a mobile phase B at a flow rate of 1.5 mL/min was used with gradient program Time/Mobile phase A%/Mobile phase B% is as 0 min./55/45, 3 min./55/45, 9 min./20/80, 13 min.20/80, 15 min./55/45, 20 min.55/45.

Development and Optimization of method

The main objective of chromatographic method is to develop a single RP-HPLC method for accurate quantification of Dapagliflozin and Sitagliptin. After detailed literature survey the pKa value of Dapagliflozin was found 12.57 and pKa value of Sitagliptin was 8.78. Dapagliflozin is soluble in organic solvents,[13] whereas Sitagliptin phosphate is active form to take orally and it is soluble in water. Therefore method development started with different ratio of water and methanol as mobile phase on zorbax C18 (250x4.6 mm, 5 µm) with 1.0 mL/min. flow rate. But chromatography was not achieved as desired. Therefore 0.1 M potassium phosphate monobasic buffer (pH 3.0) used as mobile phase A and mixture of methanol and acetonitrile in different ratio is taken as mobile phase B on Hypersil BDS C18 (250x4.6 mm, 5 µm) with 1.5 mL/min. flow rate.[14] Peaks of both drugs were found in this chromatography. But for shorter run time, Gaussian

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peak shape and better resolution gradient profile is used for optimization of method.

After optimization of method the final chromatographic conditions are Hypersil BDS C18 (250x4.6 mm, 5µm) column as stationary phase. Mobile phase 0.1 M potassium phosphate monobasic buffer (pH 3.0) as mobile phase A and mixture of methanol and acetonitrile in the ratio of (60:40 v/v) as mobile phase B pumped at 1.5 mL/min. with gradient profile as mentioned Time/Mobile phase A%/Mobile phase B% is as 0 min./55/45, 3 min./55/45, 9 min./20/80, 13 min. 20/80, 15 min./55/45, 20 min. 55/45, column oven compartment temperature kept at 35°C. The injection volume is 10 µL.

METHOD VALIDATION

Method validation parameters performed as per ICH guidelines.

Specificity

The specificity was established by injecting blank, placebo, sample, spiked sample and individual impurity into the system. All samples solutions were prepared as per developed method. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of the analyte. No interference was observed from the blank, placebo & known impurity at the retention time of Dapagliflozin and Sitagliptin peak. As purity angle was found less than purity threshold for Dapagliflozin and Sitagliptin peak (Table 1). Blank and standard solution chromatograms are shown in Figure 1 & Figure 2. Peak purity graphs of Dapagliflozin and Sitagliptin gave in Figure 3 & Figure 4 respectively.

Table1: Specificity: Peak purity data of Standard and Sample solution

	Dapagliflozin		Sitagliptin	
	Purity angle	Purity threshold	Purity angle	Purity threshold
Standard solution	0.227	0.921	0.354	1.028
Sample solution	0.238	0.869	0.324	1.111
Spiked Sample solution	0.235	0.874	0.311	1.254

Figure 1: Blank Solution

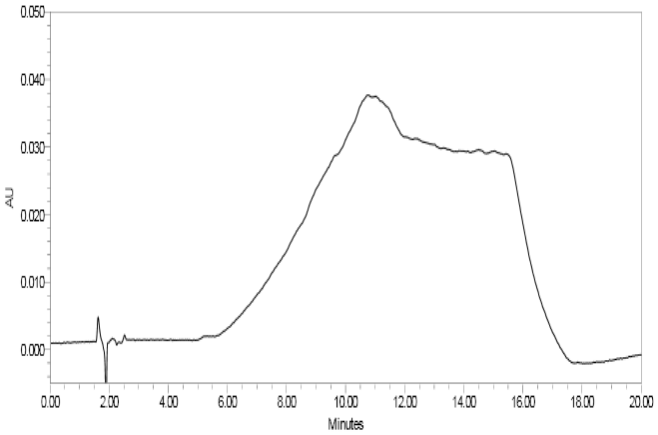


Figure 2: Standard Solution

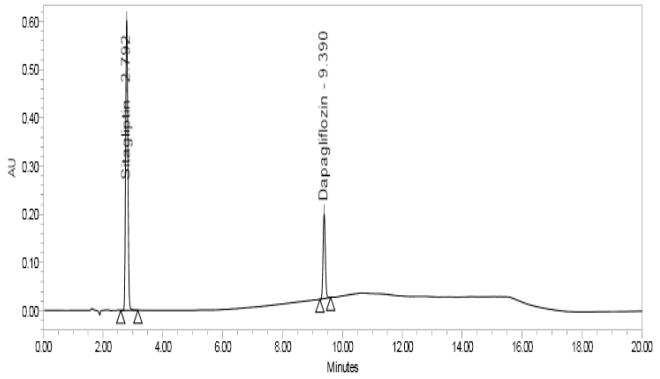


Figure 3: Peak purity graph of Dapagliflozin

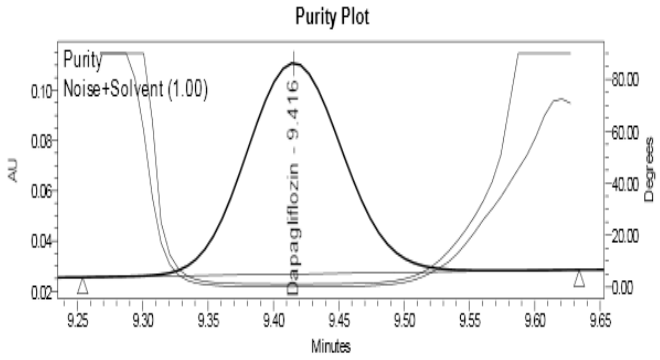
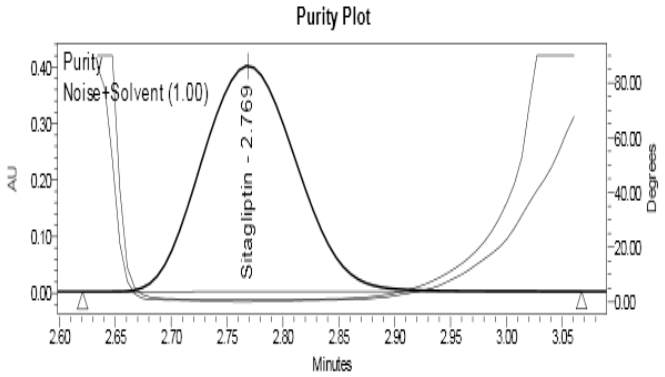


Figure 4: Peak purity graph of Sitagliptin



Precision

The system precision was verified by injecting six replicate injections of standard solutions. Calculated the mean assay and percent relative standard deviation (%RSD) of area counts of Dapagliflozin and Sitagliptin peak (Table 2). The method precision was verified by injecting six replicate injections of sample solution. Calculated the mean assay and percent relative standard deviation (%RSD) of area counts of Dapagliflozin and Sitagliptin peak (Table 3). The intermediate precision was verified by injecting six replicate injections of sample solution on a different day by a different analyst and analyse on a different instrument using the column of different serial number. Calculated the mean assay and percent relative standard deviation (%RSD) of area counts of Dapagliflozin and Sitagliptin peak (Table 3).

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Table 2: Summary of system precision data

Injection No.	Dapagliflozin Peak Area	Sitagliptin Peak Area
1	1013567	3551571
2	1025874	3564075
3	1019452	3567457
4	1028586	35698/5
5	1015762	3562247
Mean	**1020648**	**3561338**
SD	**6437.525**	**6859.395**
%RSD (≤2%)	**0.63**	**0.19**

Table 3: Summary of method precision and intermediate precision data

Sample No.	Dapagliflozin Assay (% of label claim)		Sitagliptin Assay (% of label claim)	
	Method Precision	Intermediate Precision	Method Precision	Intermediate Precision
1	100.4	100.2	99.8	99.5
2	98.9	99.8	100.1	100.3
3	100.2	99.8	100.6	99.8
4	100.1	100.7	100.7	99.6
5	99.5	99.9	99.8	99.8
6	100.0	100.5	100.9	100.2
Overall Mean	100.0		100.1	
Overall SD	0.481		0.454	
Overall %RSD (≤2%)	0.48		0.45	

Linearity

In the linearity parameter, concentration of Dapagliflozin and Sitagliptin response were determined in the range of 50%-150% of standard solution. Calibration curves were plotted between analyte concentration and peak response. The slope, intercept, and coefficient of correlation were calculated using MS-Excel. The calibration data of Dapagliflozin and Sitagliptin is given in Table 4, while Figure 5 & Figure 6 represents calibration curve of both drugs respectively

Table 4: Summary of Linearity data

Dapagliflozin		Sitagliptin	
Concentration (µg/mL)	**Mean Area**	**Concentration (µg/mL)**	**Mean Area**
76.01	1542989	743.45	5342007
60.81	1226391	619.54	4479236
50.68	1020648	495.63	3561338
30.41	618195	309.77	2259618
25.30	513324	247.82	1770663
Peak Name	**Correlation Coefficient**	**Intercept**	**Slope**
Dapagliflozin	0.9999	412.43785	20227.36906
Sitagliptin	0.9999	9558.60714	7186.89165

Figure 5: Linearity graph of Dapagliflozin

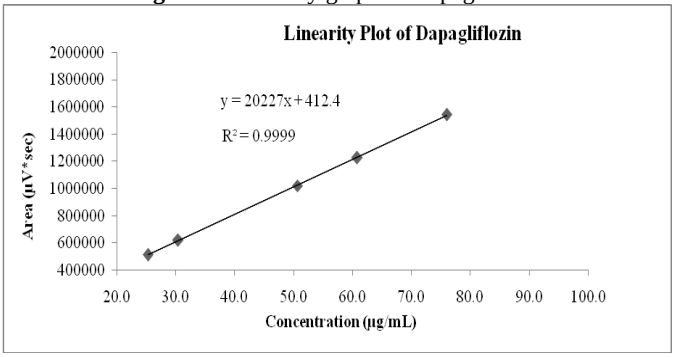
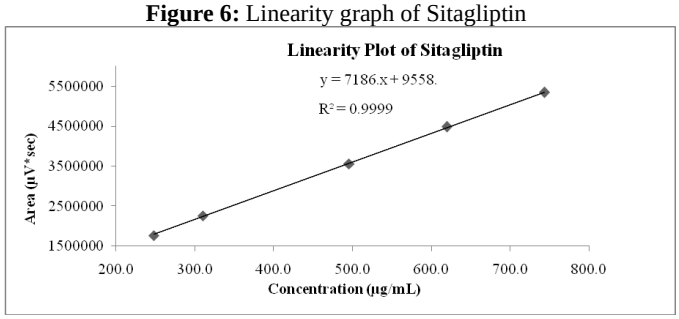


Figure 6: Linearity graph of Sitagliptin



Accuracy

The accuracy was determined by adding known quantities of the analyte to the placebo. A 3-fold measurement at 50% (Level 1), 100% (Level 2), and 150% (Level 3) of sample concentration respectively (9 Determinations in total) was carried out. Data for Dapagliflozin and Sitagliptin are shown in Table 5-6 respectively.

Table 5: Summary of Accuracy results of Dapagliflozin

Level	Sample	Actual Amount added (mg)	Amount Recovered (mg)	% Recovery (98.0% - 102.0%)	% Mean Recovery
50%	1	25.239	25.340	100.4	100.4
	2	25.332	25.329	100.0	
	3	25.517	25.683	100.7	
100%	1	51.777	51.598	99.7	99.7
	2	52.055	51.843	99.6	
	3	51.870	51.807	99.9	
150%	1	76.181	75.995	99.8	100.0
	2	76.366	76.462	100.1	
	3	76.273	76.393	100.2	
Overall % Mean Recovery (Between 98.0% -102.0%)					100.0
Overall Standard deviation (SD)					0.35
Overall %Relative standard deviation (%RSD) (≤2%)					0.4

Table 6: Summary of Accuracy results of Sitagliptin

Level	Sample	Actual Amount added (mg)	Amount Recovered (mg)	% Recovery (98.0% - 102.0%)	% Mean Recovery
50%	1	201.026	200.831	99.9	99.8
	2	199.939	199.569	99.8	
	3	199.861	199.245	99.7	
100%	1	397.003	396.147	99.8	100.1
	2	398.091	399.484	100.3	
	3	397.974	398.372	100.1	
150%	1	602.146	600.180	99.7	99.8
	2	601.913	601.915	100.0	
	3	601.137	599.179	99.7	
Overall % Mean Recovery (Between 98.0% -102.0%)					99.9
Overall Standard deviation (SD)					0.21
Overall %Relative standard deviation (%RSD) (≤2%)					0.2

Robustness

Robustness of the method shall be demonstrated by deliberately changing the chromatographic parameters and monitoring system suitability parameters under each condition. Prepared standard solutions as described in method to be injected under each of the variable conditions such as wavelength of detection by ± 3 nm, flow rate by ± 10%, pH of buffer by ±0.2 unit and column oven temperature by ± 5°C. Data shown in Table 7.

Table 7: Summary of Robustness results of Dapagliflozin and Sitagliptin

Variation in chromatographic	Observed system suitability parameters in standard	
	USP Tailing (≤2.0)	%RSD (≤2%)

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condition	Dapagliflozin	Sitagliptin	Dapagliflozin	Sitagliptin
Method Precision	1.01	1.03	0.63	0.19
Column oven temperature (+5°C)	1.06	1.12	0.73	0.23
Column oven temperature (-5°C)	1.08	1.06	0.75	0.25
Buffer pH (+0.2 unit)	0.98	1.04	0.56	0.33
Buffer pH (-0.2 unit)	1.01	1.06	0.57	0.38
Wavelength (+3 nm)	1.00	1.15	0.65	0.24
Wavelength (-3 nm)	1.03	1.06	0.69	0.19
Flow rate (+10%)	1.05	1.14	0.98	0.53
Flow rate (-10%)	0.95	1.13	0.95	0.58

RESULTS AND DISCUSSIONS

Specificity: There was no interference of blank and placebo at the retention time of Dapagliflozin and Sitagliptin peak. Also purity angle was found less than from purity threshold for both drugs. Hence the method was found to be specific.

Linearity and Range: The correlation coefficients for Dapagliflozin and Sitagliptin were found to be 0.9999 between 50%-150% range of the target concentration of analyte.

Precision: The % RSD was found 0.63 and 0.19 for system precision and 0.48 and 0.45 was found for repeatability study for Dapagliflozin and Sitagliptin respectively.

Accuracy: Percentage recovery for Dapagliflozin was found to be 100.4%, 99.7% and 100.0% whereas for Sitagliptin it was found to be 99.8%, 100.1% and 99.8% at three levels (50%, 100%, and 150%).

Robustness: All system suitability criteria were found within acceptance limit during small but deliberately changes in chromatographic conditions that indicates developed method is robust.

Stability of Analytical Solution: Prepare the standard and sample solution as per developed method, kept the solutions at 25°C. Inject at different time intervals. Solution stability of standard and sample solution was found for 24 hours.

CONCLUSION

Above results concluded that developed method is specific, precise, linear, reproducible and rugged. This method is validated according with ICH guideline. During analytical method validation, results were found satisfactory. Simultaneously quantification of Dapagliflozin and Sitagliptin in single analytical method with shorter run time shows this method cost-effective, time saving and can be used for routine analysis in industries.

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REFERENCES

1. Zimmermann, G. R., Lehár, J. & Keith, C. T., 2007. Multi-target therapeutics: when the whole is greater than the sum of the parts. Drug Discov. Today 12, 34–42. DOI: 10.1016/j.drudis.2006.11.008.
2. Paul, S. M., 2010. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat. Rev. Drug Discov. 9, 203–214.
3. Nageswara Rao, R. & Nagaraju, V., 2003. An overview of the recent trends in development of HPLC methods for determination of impurities in drugs. J. Pharm. Biomed. Anal. 33, 335-377. DOI:10.1016/s0731-7085(03)00293-0.
4. Shabir, G. A., John Lough, W., Arain, S. A. & Bradshaw, T. K., 2007. Evaluation and Application of Best Practice in Analytical Method Validation. J. Liq. Chromatogr. Relat. Technol. 30, 311–333.
5. Suma, B. V. an. R. D., 2019. An verview on analytical methods for Dapagliflozin – An antidiabetic drug. Int. J. Pharm. Sci. Res. 10, 2688-92. DOI: 10.13040/IJPSR.0975-8232.10(6).2688-92.
6. Balamurugan K, K. M. and S. R., 2018. Sitagliptin: A literature review on analytical and bioanalytical methods. Pharma Innov. 7, 357-361.
7. Joubert, M., 2017. The sodium-glucose cotransporter 2 inhibitor dapagliflozin prevents cardiomyopathy in a diabetic lipodystrophic mouse model. Diabetes 66, 1030–1040. DOI: 10.2337/db16-0733.
8. Defronzo, R. A., 2013. Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. Diabetes Care 36, 3169–3176. DOI: 10.2337/dc13-0387.
9. Mosley, J. F., Smith, L., Everton, E. & Fellner, C., 2015. Sodium-glucose linked transporter 2 (SGLT2) inhibitors in the management of type-2 diabetes: A drug class overview. P T 40, 451–462.
10. Dhillon, S., 2010. Sitagliptin: a review of its use in the management of type 2 diabetes mellitus. Drugs 70, 489–512. DOI: 10.2165/11203790-000000000-00000.
11. Drucker, D., Easley, C. & Kirkpatrick, P., 2007. Sitagliptin. Nat. Rev. Drug Discov. 6, 109–110. DOI: 10.1038/nrd2245.
12. Jitendra Debata, Sundeep Kumar, S. K. J. and A. K., 2017. A New RP-HPLC Method Development and Validation of Dapagliflozin in Bulk and Tablet Dosage Form. Int. J. Drug Dev. Res. 9, 48–51.
13. Sankar, A. S. K., Sythana, S., Jhansi, A., Shanmugasundharam, P. & Sumithra, M., 2013. Development and validation for simultaneous estimation of sitagliptin and metformin in pharmaceutical dosage form using RP-HPLC method. Int. J. PharmTech Res. 5, 1736–1744.
14. Snyder L. R., Glajch, J. L. & Kirkiland J. J. (Jack) 2012. Practical HPLC Method Development, Second edition, pp. 7-17 and 402-414.

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