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Research Article

Reverse Phase High Performance Liquid Chromatographic Method for Separation and Estimation of Impurities Present in Pharmaceutical Formulation of Dapaglifozin

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Keywords Dapaglifozin; SGLT-2 Inhibitor; Zorbax Bonus RP-18; Stress condition; Impurities; ICH Q2 (R1)

Abstract

Dapaglifozin is a Sodium-glucose co-transporter-2 inhibitors work by inhibiting SGLT2, to prevent re-absorption of glucose and facilitate its excretion in urine. Impurities in pharmaceuticals which are unwanted chemicals that remains with the Active Pharmaceutical Ingredients (APIs), or develop during stability testing, or develop during formulation or upon aging of both API and formulation. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. It is necessary to estimate related impurities present in this drug. A simple and very sensitive method developed for estimation of impurities present in Dapagifozin formulation by Reverse Phase High Performance Liquid Chromatographic method. Method is capable to detect impurities in very low level (0.1µm/mL). Chromatographic separation of six different impurities was achieved on Agilent Zorbax Bonus RP-18 (250 x 4.6) mm, 5µm column using gradient elution method at 30°C column temperature and the detection was carried at 230 nm at a flow rate of 1.2mL/min. The method was validated as per ICH Q2 (R1) guideline along with stress studies.

Introduction

Dapaglifozin is a Sodium-glucose co-transporter-2 inhibitors work by inhibiting SGLT2 in the PCT, to prevent re-absorption of glucose and facilitate its excretion in urine. As glucose is excreted, its plasma levels fall leading to an improvement in all glycemic parameters. This mechanism of action is dependent on blood glucose levels and, unlike the actions of thiazolidinediones (mediated through GLUTs), is independent of the actions of insulin. Thus, there is minimal potential for hypoglycemia, and no risk of overstimulation or fatigue of the beta cells. Because their mode of action relies upon normal renal glomerular-tubular function, SGLT2i efficacy is reduced in persons with renal impairment (Figure 1).

Due to lots of advantages of Dapaglifozin, it is necessary to estimate related impurities present in these drug. So present investigation involve the development of RP-HPLC related substances method for pharmaceutical dosage form for Dapaglifozin. Possibly six impurities identified base on API source so separation was done on this impurities and validate developed method [1-6].

Impurities Details

(Tables 1 and 2)

Material and Methods

Reagents and Chemicals

Dapaglifozin and its impurities were the generous gifts from MSN Laboratories PVT Ltd, India. HPLC grade Acetonitrile was procured from Merck. Potassium di-hydrogen phosphate (KH_2PO_4) and ortho-phosphoric acid (H_3PO_4) were purchased from Merck. All other chemicals and solvents used were of analytical grade. Water used in the HPLC analysis was prepared by the water purifier (Merck Millipore Milli-Q). The mobile phase and all the solutions were filtered through a 0.45µm Merck HV membrane filter. Sample was filtered through 0.45µm Millipore PVDF syringe filter.

Instruments

HPLC system (Waters system, USA, e2695) with a PDA detector, equipped with a quaternary pump, auto sampler, column compartment and empower software was employed during this study.

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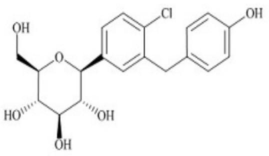
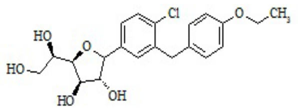
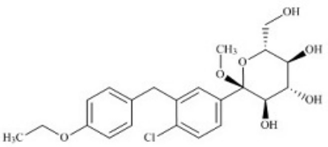
Table 1: Details of Impurity-I to III.

Impurity Name	Impurity-I	Impurity-II	Impurity-III
Chemical Structure			
Chemical Formula	$C_{19}H_{21}ClO_6$	$C_{21}H_{25}ClO_6$	$C_{22}H_{27}ClO_7$
Chemical Name	(2S,3R,4R,5S,6R)-2-(4-Chloro-3-(4-Hydroxy benzyl phenyl)-6-(hydroxylmethyl) tetrahydro-2H-pyran-3,4,5-triol	(3R,4R,5R)-2-(4-Chloro-3-(4-ethoxybenzyl phenyl)-5-((R)-1,2-dihydroxyethyl) tetrahydofuran-3,4-diol	(3R,4S,5S,6R)-2-(4-Chloro-3-(4-ethoxybenzyl phenyl)-6-(hydroxyl methyl)-2-methoxy tetrahydro-2H-pyran-3,4,5-triol
Molecular Weight	380.82	408.88	438.9

Chromatographic condition

Chromatographic separation was achieved at 30°C column temperature and the detection was carried at 230nm at a flow rate of 1.2mL/min. Run time was kept at 80 min. Prior to the injection of drug solution, column was equilibrated for 60 min with the mobile phase flowing through the system. The injection volume was 25µL. The analysis has been performed by using Agilent ZORBAX Bonus RP-18 (250 × 4.6 mm, 5µ). The mobile phase A containing 50mM phosphate buffer adjusted pH 2.2 with ortho phosphoric acid solution and mobile phase B contains Acetonitrile and Methanol in ratio of (70:30)%v/v using following gradient (Table 3).

Standard Preparation

The standard stock solutions 200ug/ml of Dapaglifozin was prepared by dissolving working standards in Diluent and diluting with the same solvent to obtain final concentration 4µg/mL.

Sample Preparation

Twenty tablets were weighed and finely powdered. Powder equivalent to 40mg Dapaglifozin was accurately weighed into a 100ml volumetric flask, 50ml of diluent was added and sonicated for 15min with intermittent shaking, made up to the volume with diluent and mixed. Filter the solution through 0.45µm Millipore PVDF syringe filter.

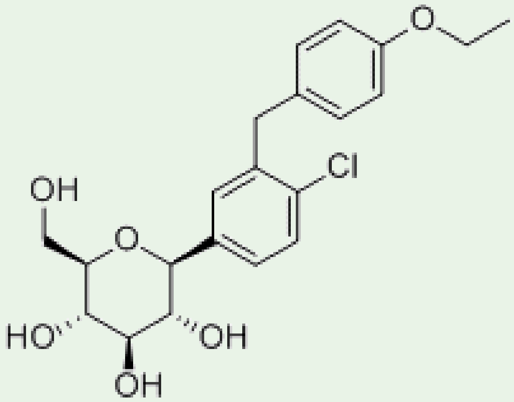
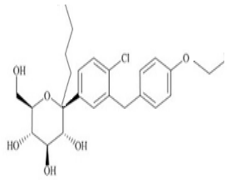
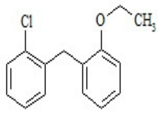
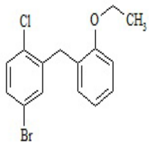


Figure 1: Chemical Structure of Dapaglifozin.

Table 2: Details of Impurity-IV to VI.

Impurity Name	Impurity-IV	Impurity-V	Impurity-VI
Chemical Structure			
Chemical Formula	$C_{25}H_{33}ClO_6$	$C_{15}H_{15}ClO$	$C_{15}H_{14}BrClO$
Chemical Name	(2S,3R,4S,5S,6R)-2-butyl-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol	1-chloro-2-(4-ethox y benzyl)benzene	4-bromo-1-chloro-2-(4-ethoxybenzyl)benzene
Molecular Weight	464.98	246.73	325.63

Citation: Patel N and Patel S. Reverse Phase High Performance Liquid Chromatographic Method for Separation and Estimation of Impurities Present in Pharmaceutical Formulation of Dapaglifozin. Ann Chromatogr Sep Tech. 2018; 4(1): 1033.

Table 3: Gradient Programme.

Time (Min)	% Mobile Phase-A	% Mobile Phase-B
0	80	20
2	80	20
22	60	40
40	40	60
60	20	80
70	20	80
75	80	20
80	80	20

Table 4: System suitability Result.

Injection No	Peak Area of Dapagliflozin	Theoretical Plates	Tailing Factor
1	116319	89802	1.1
2	117895	89682	1.1
3	116874	89005	1.1
4	115682	90105	1.1
5	116475	89744	1.1
6	116258	88975	1.1
Mean	**116583.8**		
SD	**749**		
%RSD	**0.6**		

Method Validation

After method development, validation of the current test method for Dapaglifozin tablets was performed in accordance with United States Pharmacopeia requirements/ICH guidelines for related substance method the parameter includes precision, accuracy, linearity, LOD and LOQ, precision and accuracy at LOQ level, selectivity, specificity includes blank, placebo, known impurity interference and interference of degradants by degradation study. Robustness was also performed [7-13].

Specificity

To assess the method specificity, tablet powder without Dapaglifozin was prepared with the same excipients as those in the commercial formulation. For RP-HPLC, the solution was prepared using the same procedure as for the analytical sample. Placebo solution was injected into the HPLC system following test conditions, the chromatogram was recorded and the responses of the peaks if any measured. Chromatogram of the placebo has not shown any interference at the retention time of both Dapaglifozin and its impurities. Blank, placebo, and impurity spiked sample preparation and impurities mixture chromatogram shown in Figures 2-6.

System Suitability: 25μL of standard solution six times injected into HPLC and recorded the chromatogram, % RSD of Dapaglifozin, area was within the limit of 5.0%. The results summarized in Table 4 and standard solution chromatogram shown in Figure 7.

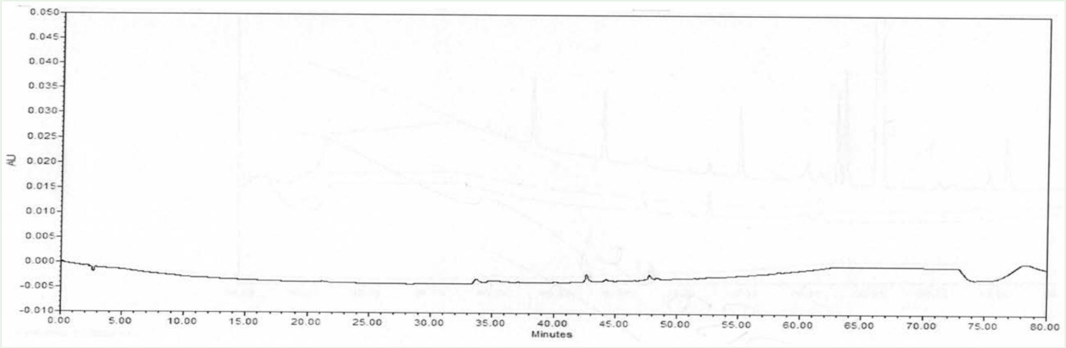


Figure 2: Chromatogram of Diluent.

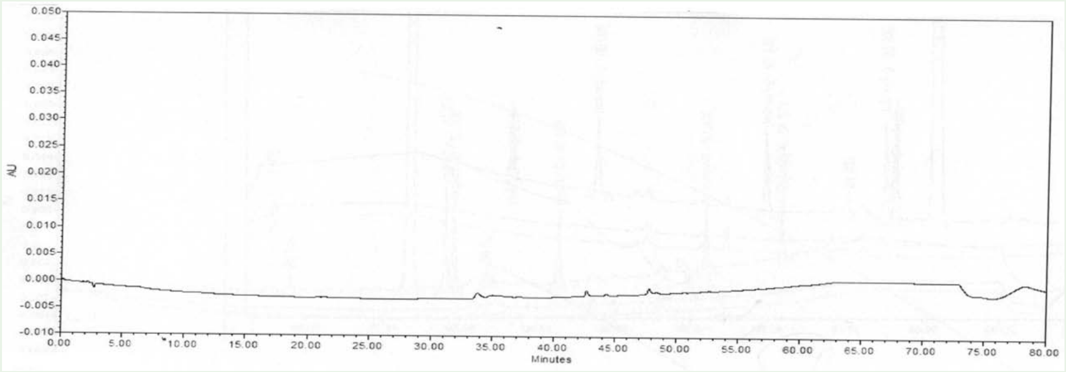


Figure 3: Chromatogram of Placebo.

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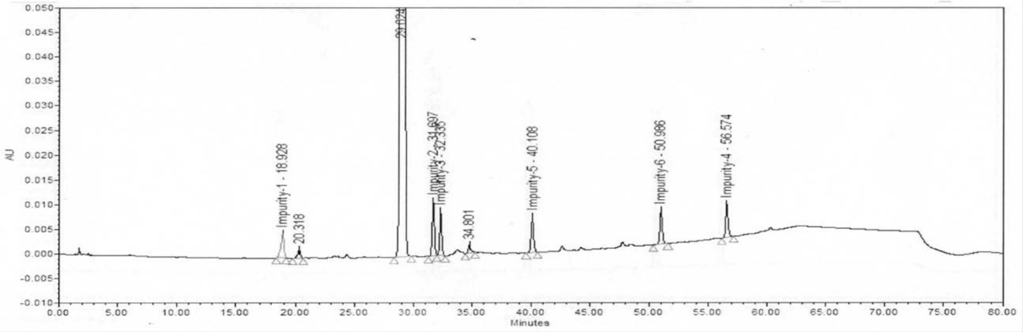

Figure 4: Chromatogram of Impurity spiked sample.

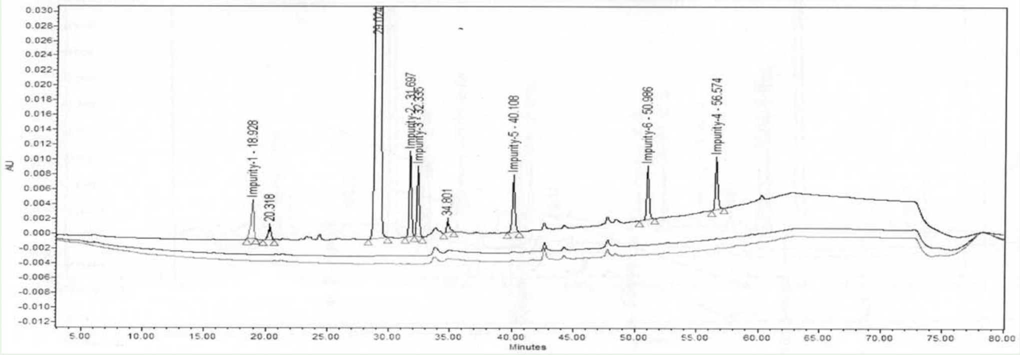

Figure 5: Chromatogram of Overlay of Diluent, Placebo and Impurity spiked sample.

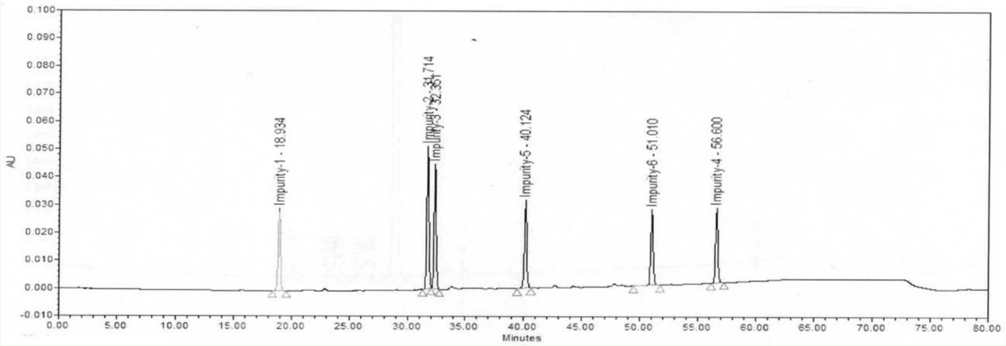

Figure 6: Chromatogram of Impurity mixture Solution.

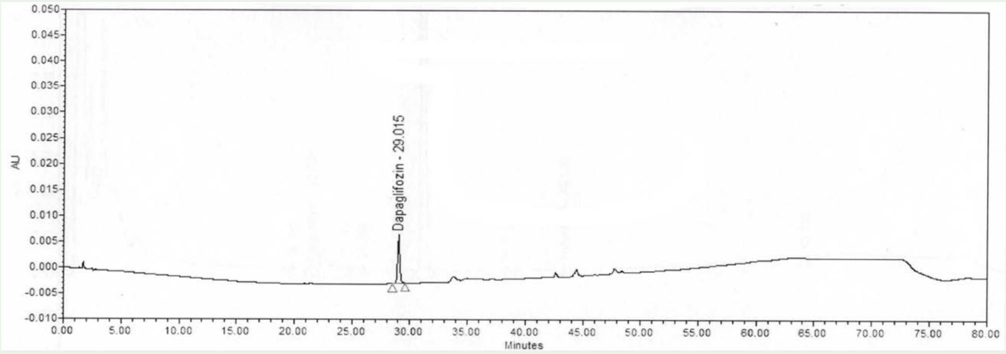

Figure 7: Chromatogram of Standard Solution.

Citation: Patel N and Patel S. Reverse Phase High Performance Liquid Chromatographic Method for Separation and Estimation of Impurities Present in Pharmaceutical Formulation of Dapagliflozin. Ann Chromatogr Sep Tech. 2018; 4(1): 1033.

Table 5: Method Precision.

SNo	Impurity-I	Impurity-II	Impurity-III	Impurity-IV	Impurity-V	Impurity-VI	Single Max Unk	Total Imp
1	0.52	0.92	0.75	0.59	0.67	0.60	0.00	4.05
2	0.52	0.92	0.75	0.59	0.67	0.60	0.00	4.05
3	0.52	0.93	0.75	0.58	0.66	0.60	0.00	4.04
4	0.51	0.92	0.75	0.59	0.67	0.60	0.00	4.04
5	0.52	0.92	0.75	0.60	0.67	0.61	0.00	4.07
6	0.52	0.91	0.75	0.59	0.67	0.61	0.00	4.05
Mean	**0.52**	**0.92**	**0.75**	**0.59**	**0.67**	**0.60**	**0.00**	**4.05**
SD	**0.004**	**0.006**	**0.000**	**0.006**	**0.004**	**0.005**	**0.00**	**0.01**
%RSD	**0.8**	**0.7**	**0.0**	**1.1**	**0.6**	**0.9**	**0.0**	**0.3**

Table 6: Intermediate Precision.

S.No	Impurity-I	Impurity-II	Impurity-III	Impurity-IV	Impurity-V	Impurity-VI	Single Max Unk	Total Imp
1	0.56	0.71	0.62	0.51	0.47	0.62	0.00	3.49
2	0.56	0.71	0.62	0.51	0.46	0.62	0.00	3.48
3	0.55	0.71	0.62	0.50	0.46	0.62	0.00	3.46
4	0.56	0.71	0.62	0.51	0.47	0.62	0.00	3.49
5	0.56	0.71	0.62	0.51	0.47	0.62	0.00	3.49
6	0.56	0.72	0.62	0.51	0.47	0.64	0.00	3.52
Mean	**0.56**	**0.71**	**0.62**	**0.51**	**0.47**	**0.62**	**0.00**	**3.49**
SD	**0.004**	**0.004**	**0.000**	**0.004**	**0.005**	**0.008**	**0.00**	**0.02**
%RSD	**0.7**	**0.6**	**0.0**	**0.8**	**1.1**	**1.3**	**0.0**	**0.6**

Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application (System precision). Precision study of Dapaglifozin and its impurities were carried out by spiking known concentration in sample and calculating % recovery of impurities in sample. Intermediate precision carried out using same manner but on other day using different column and HPLC. The results summarized in Tables 5 and 6.

Linearity

To evaluate linearity of the method, six levels calibration curve made includes LOQ level. Signal to noise ratio was observed. The linearity of method is obtained by preparation of the calibration curve. The calibration curve for Dapaglifozin and its impurities were obtained by plotting the peak area of Dapaglifozin versus concentration of Dapaglifozin over the range of 0.30-16 µg/ml. The results are summarized and overall linearity graph for Dapaglifozin and its impurities was shown in Figures 8-14.

Accuracy

Accuracy of the method was studied for three levels from 50% to 150% by spiking 0.05% for LOQ and 0.25% for 50% level from the target concentration of Dapaglifozin impurities and 0.5%, 0.75% for 100%, 150% level from the target concentration of Dapaglifozin impurities spiked in sample preparation and analyzed with unspiked sample preparation, recorded the chromatogram. Six preparation for 50%, 150% level and triplicate preparation of median level concentration were done. Results are summarized in Table 7.

Table 7: Result of Recovery of LOQ to 150%.

Name	Level	%Recovery	%RSD
Impurity-I	LOQ	98.6	2.9
	50%	92.2	3.6
	100%	97.6	0.7
	150%	94.1	3.5
Impurity-II	LOQ	100.0	1.5
	50%	113.9	2.1
	100%	110.2	0.8
	150%	90.9	0.8
Impurity-III	LOQ	113.1	1.8
	50%	111.5	1.6
	100%	104.6	0.8
	150%	96.3	1.1
Impurity-IV	LOQ	105.1	5.3
	50%	118.9	1.5
	100%	114.8	0.6
	150%	93.1	0.7
Impurity-V	LOQ	110.4	1.2
	50%	110.1	1.0
	100%	100.1	1.1
	150%	93.9	2.1
Impurity-VI	LOQ	119.1	3.4
	50%	112.8	0.3
	100%	113.3	1.8
	150%	93.2	1.9

Citation: Patel N and Patel S. Reverse Phase High Performance Liquid Chromatographic Method for Separation and Estimation of Impurities Present in Pharmaceutical Formulation of Dapaglifozin. Ann Chromatogr Sep Tech. 2018; 4(1): 1033.

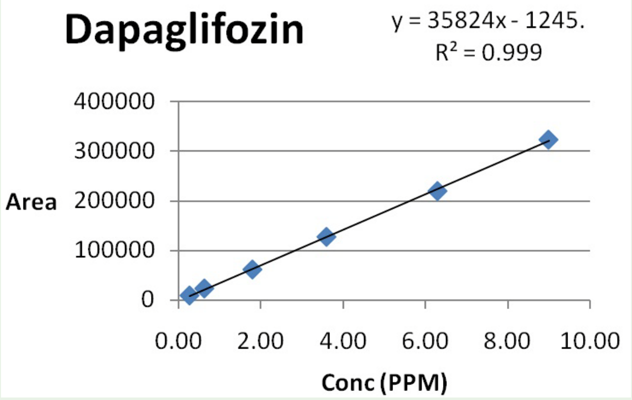

Figure 8: Linearity curve of Dapaglifozin.

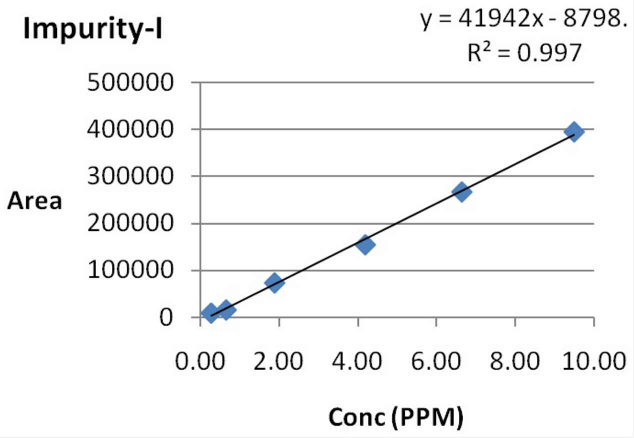

Figure 9: Linearity curve of Impurity-I.

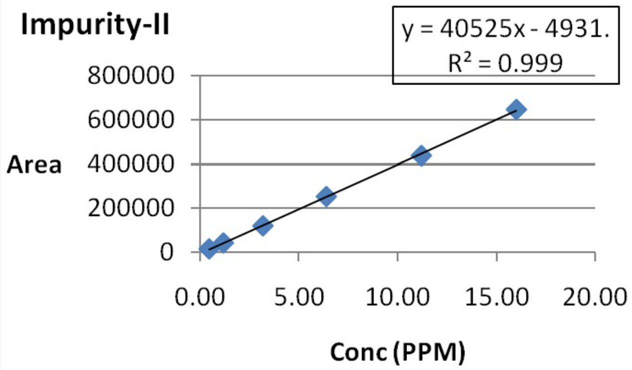

Figure 10: Linearity curve of Impurity-II.

Robustness

Robustness of the current method was investigated by analyzing the standard solution and established system suitability with the deliberate variation of flow rate, column temperature and pH of Buffer at 10 percentage level from the original value. RSD of six replicate injections of standard solution was found below 5.0% for all the chromatographic condition and all peaks in standard solutions.

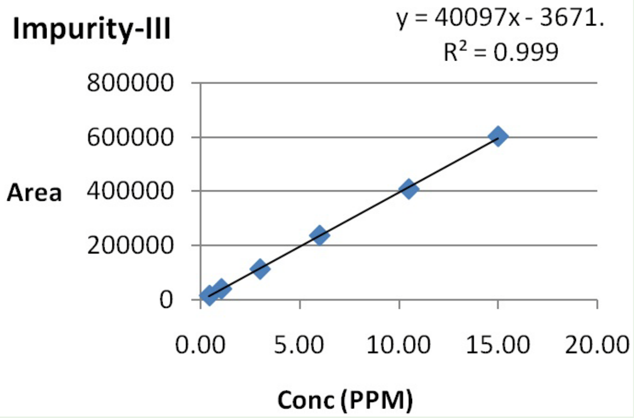

Figure 11: Linearity curve of Impurity-III.

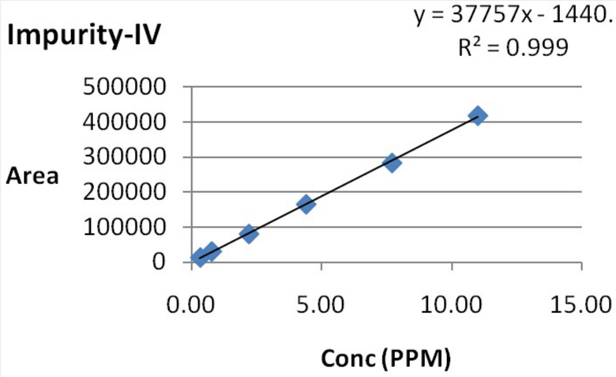

Figure 12: Linearity curve of Impurity-IV.

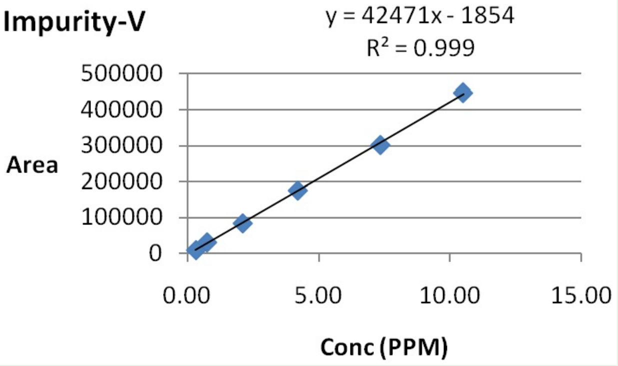

Figure 13: Linearity curve of Impurity-V.

The conditions with the variation and the results are presented in Tables 8-10.

LOD & LOQ

LOD and LOQ were calculated by using the formula 3.3S.D/S and 10S.D/S where S.D is the standard deviation of Y-intercept and S is the slope of the calibration curve (Table 11).

Force Degradation

(Tables 12 and 13) and (Figures 15-20).

Citation: Patel N and Patel S. Reverse Phase High Performance Liquid Chromatographic Method for Separation and Estimation of Impurities Present in Pharmaceutical Formulation of Dapaglifozin. Ann Chromatogr Sep Tech. 2018; 4(1): 1033.

Table 8: Robustness (Column Temperature).

	Normal Condition		Column Tem +		Column Tem -	
Name	**RRT**	**Resolution**	**RRT**	**Resolution**	**RRT**	**Resolution**
Impurity-I	0.65	-	0.66	-	0.66	-
Dapaglifozin	1.00	30.5	1.00	30.1	1.00	30.7
Impurity-III	1.09	7.9	1.11	7.3	1.09	7.9
Impurity-II	1.11	1.9	1.13	1.8	1.11	1.9
Impurity-V	1.38	22.0	1.38	21.7	1.37	21.9
Impurity-VI	1.74	28.7	1.76	28.4	1.74	28.9
Impurity-IV	1.93	15.0	1.95	15.2	1.91	15.4

Table 9: Robustness (Flow Rate).

	Normal Condition		Flow +		Flow -	
Name	**RRT**	**Resolution**	**RRT**	**Resolution**	**RRT**	**Resolution**
Impurity-I	0.65	-	0.66	-	0.67	-
Dapaglifozin	1	30.5	1	29.6	1	30.7
Impurity-III	1.09	7.9	1.1	7.5	1.09	7.8
Impurity-II	1.11	1.9	1.12	1.8	1.11	1.9
Impurity-V	1.38	22	1.39	22.2	1.38	22.2
Impurity-VI	1.74	28.7	1.77	28.6	1.76	28.3
Impurity-IV	1.93	15	1.94	15.1	1.9	15.6

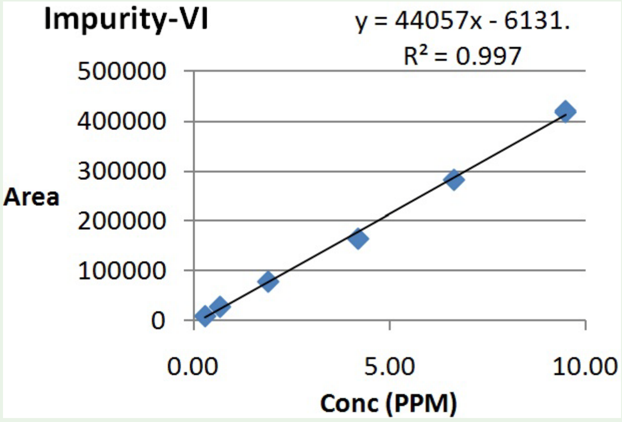

Figure 14: Linearity curve of Impurity-VI.

Table 10: Robustness (pH of Buffer).

	Normal Condition		pH +		pH -	
Name	**RRT**	**Resolution**	**RRT**	**Resolution**	**RRT**	**Resolution**
Impurity-I	0.65	-	0.66	-	0.64	-
Dapaglifozin	1.00	30.5	1.00	30.6	1.00	30.2
Impurity-III	1.09	7.9	1.10	7.9	1.08	7.8
Impurity-II	1.11	1.9	1.12	1.9	1.10	1.8
Impurity-V	1.38	22.0	1.38	22.1	1.37	21.9
Impurity-VI	1.74	28.7	1.76	28.9	1.71	28.6
Impurity-IV	1.93	15.0	1.94	14.6	1.88	15.0

Table 11: LOD and LOQ of Dapaglifozin and its Impurities.

Name	LOD	LOQ
Dapaglifozin	0.02%	0.05%
Impurity-I	0.02%	0.05%
Impurity-II	0.02%	0.05%
Impurity-III	0.02%	0.05%
Impurity-IV	0.02%	0.05%
Impurity-V	0.02%	0.05%
Impurity-VI	0.02%	0.05%

Table 12: Force Degradation sample condition.

Sample	Condition
Acid	5N HCL (5mL) 80°C, 18Hrs
Base	1N NaOH (5mL) 80°C, 18Hrs
Peroxide	30%H_2O_2(1mL) 80°C, 8Hrs
Thermal	80°C, 24hrs
Photo	Sun Tester, 1.2 Lux/Hrs, 24Hrs
Humidity	75%RH, 24Hrs

Solution Stability

Solution stability optimized by injected standard solution at different time interval and calculated % deviation against initial area of standard solution. It was found that standard and sample were stable up to 72 hrs. Results are presented in Table 14.

Results and Discussion

The main objective of the chromatographic method development was to separate Dapaglifozin from the impuities were carried out for accurate and precise method development and impurities were coeluted. After using several columns and buffers, suitable column

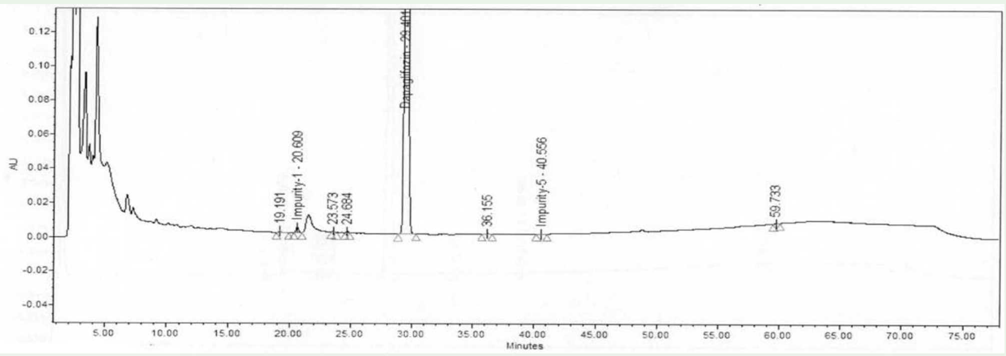

Figure 15: Chromatogram of Base Degradation sample.

Citation: Patel N and Patel S. Reverse Phase High Performance Liquid Chromatographic Method for Separation and Estimation of Impurities Present in Pharmaceutical Formulation of Dapaglifozin. Ann Chromatogr Sep Tech. 2018; 4(1): 1033.

Table 13: Result of Force Degradation study.

Sample	Imp-I	Unk-1	Unk-2	Unk-3	Unk-4	Unk-5	Total Imps	Assay	Mass Balance	Purity Angle	Purity Threshold
Acid	0.1	5.8	4.2	0.03	0.07	0.11	10.31	89.7	100.2	0.214	0.296
Base	0.08	0.12	0.06	0.03	0.08	0.09	0.46	99.0	99.6	0.140	0.393
Peroxide	0.18	0.02	0.05	0.04	0.07	0.09	0.47	99.5	100.1	0.443	0.505
Thermal	0.12	0.02	0.04	0.03	0.07	0.05	0.34	100.2	100.7	0.410	0.531
Photo	0.03	0.09	0.05	0.06	0.1	-	0.33	98.2	98.7	0.370	0.507
Humidity	0.02	0.11	0.03	0.08	0.11	0.02	0.37	98.9	99.5	0.380	0.484

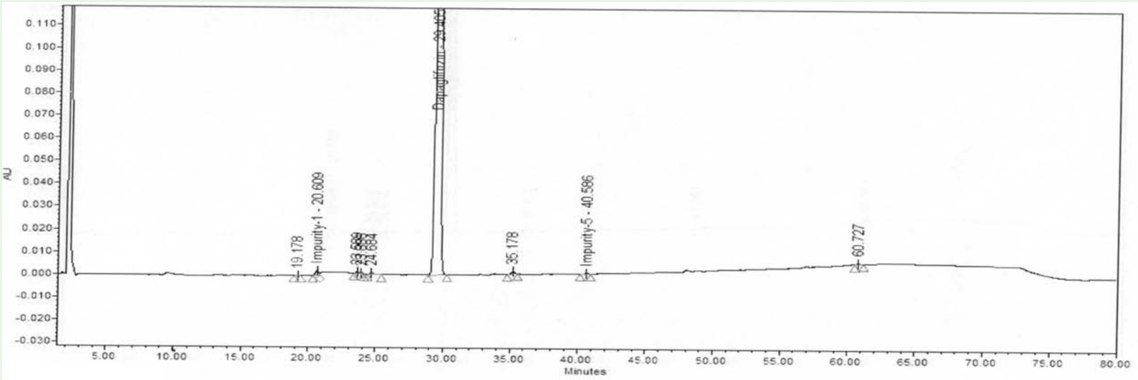

Figure 16: Chromatogram of Peroxide Degradation sample.

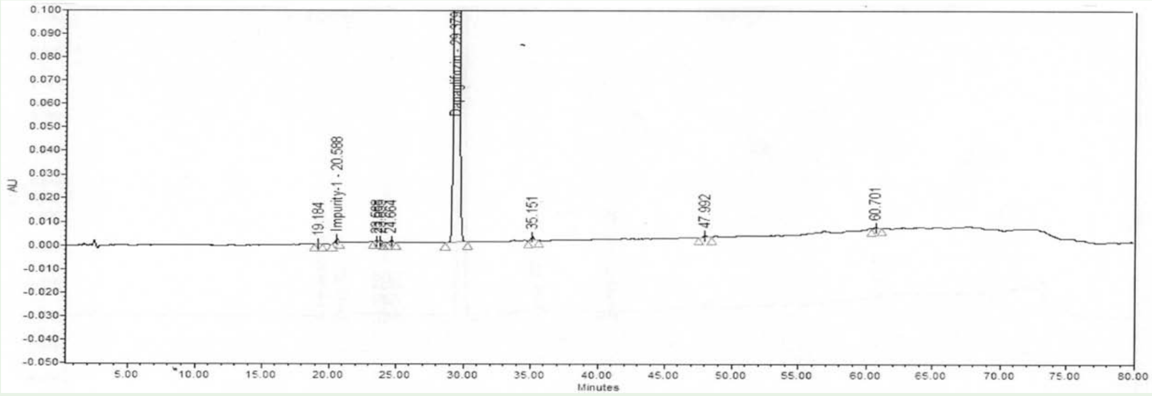

Figure 17: Chromatogram of Thermal Degradation sample.

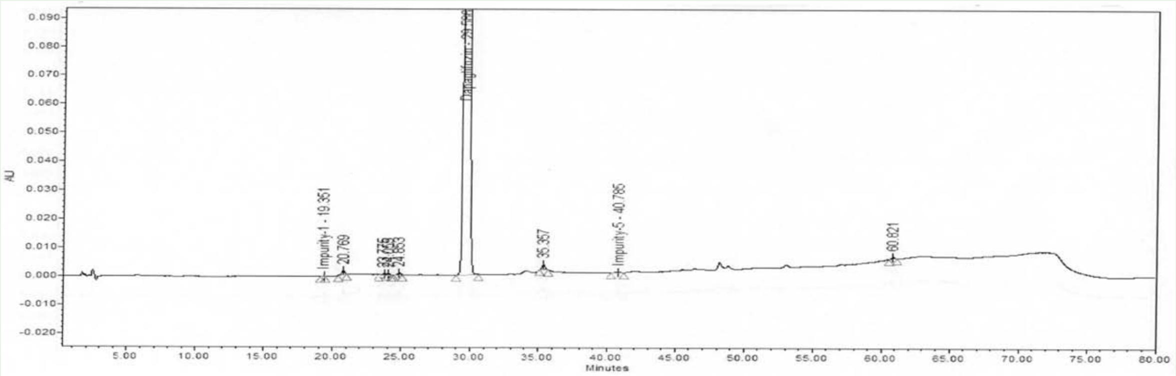

Figure 18: Chromatogram of Photo Degradation sample.

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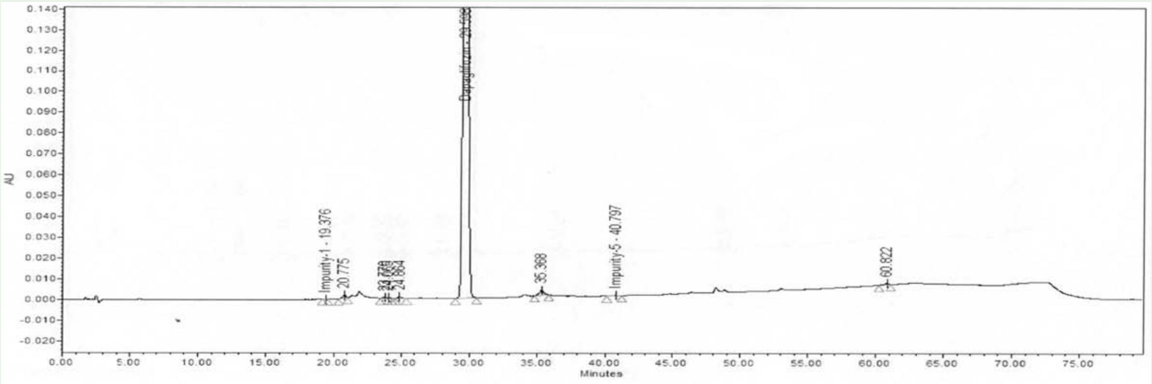

Figure 19: Chromatogram of Humidity Degradation sample.

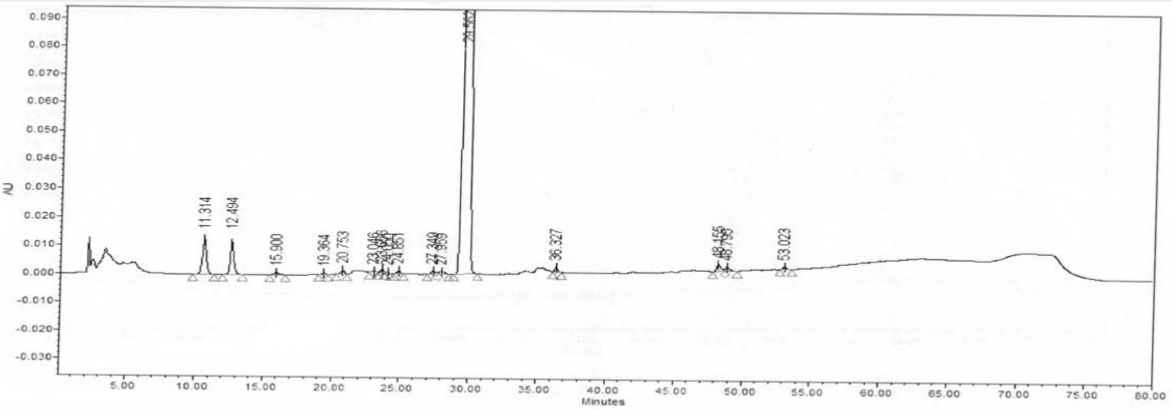

Figure 20: Chromatogram of Acid Degradation sample.

Table 14: Result of Stability of Standard Solution.

Standard Solution		
Time (Hrs)	**Area**	**% Deviation**
Initial	116589	-
26	116874	0.24
36	117058	0.40
45	117985	1.20
60	118054	1.26
72	119524	2.52

chemistry and good peak shape were obtained with Zorbax Bonus RP-18 (250×4.6)mm 5μ particle size, column temperature was adjusted at 30°C, with gradient mobile phase system consisting the mobile phase A containing 50mM phosphate buffer adjusted pH 2.2 with ortho-phosphoric acid solution and mobile phase B contains Acetonitrile and Methanol in ratio of (70:30)%v/v using above mentioned gradient.

HPLC method has been development and validated for determination of related substances of Dapaglifozin in tablets with gradient eluation. The method is selective, because we have very good separation between impurities. The method described in this study is suitable to determine impurities at very low level. These parameters showed a good linearity with correlation coefficients. We have shown that the method is robust with little change critical chromatographic parameters. Validation parameters have proved that our method can used as stability indicating method for determination of related substances of Dapaglifozin in tablet.

Conclusion

A novel, reverse phase liquid chromatographic method has been developed and validated for the estimation of Dapaglifozin and its impurities with a very recent and advanced HPLC method. The proposed method is found to be simple, accurate, precise, sensitive, specific and robust. Hence, it can be successfully used for the routine analysis of Dapaglifozin in pharmaceutical dosage forms.

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