A QBD Approach Based RP-HPLC Method Development and Validation for Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate and Sacubitril-Valsartan Sodium Complex Salt in Its Synthetic Mixture

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ABSTARCT

A robust, sensitive, and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated using a Quality by Design (QbD) approach for the simultaneous estimation of dapagliflozin propanediol monohydrate and sacubitril–valsartan sodium complex in a synthetic mixture. Critical method parameters were systematically optimized through experimental design to ensure optimal separation, peak symmetry, and resolution. The chromatographic separation was achieved using a suitable C18 column with an optimized mobile phase composition under isocratic conditions. The method demonstrated excellent linearity, precision, accuracy, and specificity within the tested concentration ranges. Validation was performed in accordance with regulatory guidelines, confirming the reliability of the method for routine analysis. The QbD framework enabled a thorough understanding of method variability and robustness, ensuring consistent performance. This developed method can be effectively applied for quality control and simultaneous quantification of these drugs in combined pharmaceutical formulations.

Keywords: Quality by Design (QbD), RP-HPLC, Dapagliflozin propanediol monohydrate, Sacubitril–Valsartan sodium complex, Method development, Method validation, Simultaneous estimation, Analytical optimization, Robustness, Pharmaceutical analysis

INTRODUCTION

The increasing use of combination therapies in the management of cardiovascular and metabolic disorders has created a need for reliable analytical methods for simultaneous drug estimation. Dapagliflozin propanediol monohydrate, a sodium–glucose co-transporter 2 (SGLT2) inhibitor, and sacubitril–valsartan sodium complex, an angiotensin receptor–neprilysin inhibitor (ARNI), are widely prescribed for the treatment of heart failure and related conditions. Accurate quantification of these drugs in combined formulations is essential to ensure quality, safety, and therapeutic efficacy.

Reverse-phase high-performance liquid chromatography (RP-HPLC) is commonly employed for such analyses due to its precision and sensitivity. Incorporating a Quality by Design (QbD) approach enhances method development by systematically evaluating critical factors affecting performance. This study focuses on developing a robust and validated RP-HPLC method using QbD principles for the simultaneous estimation of these drugs in a synthetic mixture.

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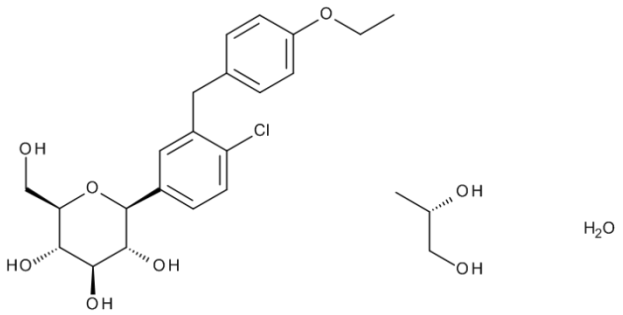

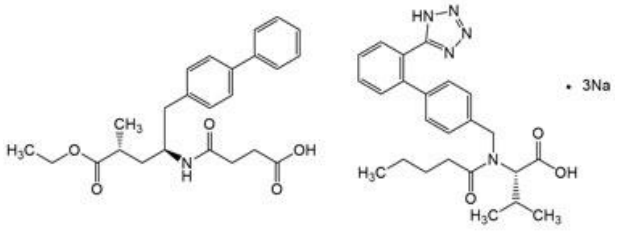

Figure1: Structure of Dapagliflozin Propanediol Monohydrate Figure 2: Stucture of Sacubitril-Valsartan

MATERIALS AND METHODS

Materials:

Dapagliflozin propanediol monohydrate (API), Sacubitril–valsartan sodium complex salt (API), HPLC grade methanol, acetonitrile, water, analytical gradents.

Analytical grade reagents Buffer Preparation:

A 0.02 M Potassium Dihydrogen Phosphate buffer was prepared by dissolving 2.72 g of potassium dihydrogen orthophosphate in 1 L of Milli-Q water using sonication. The pH was adjusted to 2.5 with diluted orthophosphoric acid.

Instrumentation:

Chromatographic analysis was carried out using a Shimadzu HPLC system (LC-2010 CHT) equipped with a 100 µL fixed loop injector and LC Solution software. Spectral analysis was performed using a Shimadzu UV-1800 double-beam UV–Visible spectrophotometer with UV Probe software. Additional instruments included a Sartorius analytical balance, Lab India digital pH meter, ultrasonic bath (Athena Technology), hot air oven (Patel Scientific), and micropipettes (Eppendorf).

Chromatographic Conditions:

Separation was achieved on a Cosmosil C18 column (250 mm × 4.6 mm, 5 µm) using a mobile phase of Buffer: ACN (40:60 % v/v) at a flow rate of 1.0 mL/min. Detection was performed at 233 nm, the injection volume was 30 µL, and the column temperature was maintained at 25 °C.

Preparation of Solutions

Dapagliflozin Stock solution (50 µg/ml): 5 mg working standard of dapagliflozin propanediol monohydrate was transferred into 100 ml flask. 30 ml methanol added. Sonicated for 5 minutes. Volume made with methanol. Further 5 mL diluted to 50 mL using methanol.

Sacubitril-Valsartan stock solution (500 µg/ml):50 mg working standard of sacubitril-valsartan sodium complex salt was taken in 100 ml volumetric flask. Approximately 50 ml methanol added. Sonicated for 5 minutes. Volume made with methanol.

Mixed standard solution (5 µg/ml Dapagliflozin + 50 µg/ml Sacubitril-Valsartan): 1 ml dapagliflozin stock solution + 1 ml sacubitril-valsartan stock were taken into 10 ml volumetric flask. Volume made with diluent.

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Sample Preparation:

Sample Stock: Synthetic mixture 200 mg (equivalent to 5 mg dapagliflozin +50 mg Sacubitril-valsartan) was weighed on butter paper. Powder was then transferred to 50 mL volumetric flask. To this 30 mL methanol was added and active contents were extracted in sonication for 15 minutes. The flask was allowed to cool down on room temperature and then the volume was made with methanol. The solution was then filtered through 0.45 µ Whatman PVDF syringe filter.

Sample (5 µg/ml Dapagliflozin + 50 µg/ml Sacubitril-valsartan):1 mL of clear filtrate was then further diluted to 10 ml with diluent and used as a sample solution.

RESULTS AND DISCUSSION

IR identification and wavelength selection

The individual standard drugs Dapagliflozin propanediol monohydrate &Sacubitril-valsartan were mixed with KBr and KBr pallets were prepared. These KBr pallets of drugs were used for FTIR analysis. And then FTIR spectra were interpreted and results were co-related with M.P., UV spectra and solubility to confirm identity of individual drugs. Wavelength was selected from the overlay spectra of above solutions.

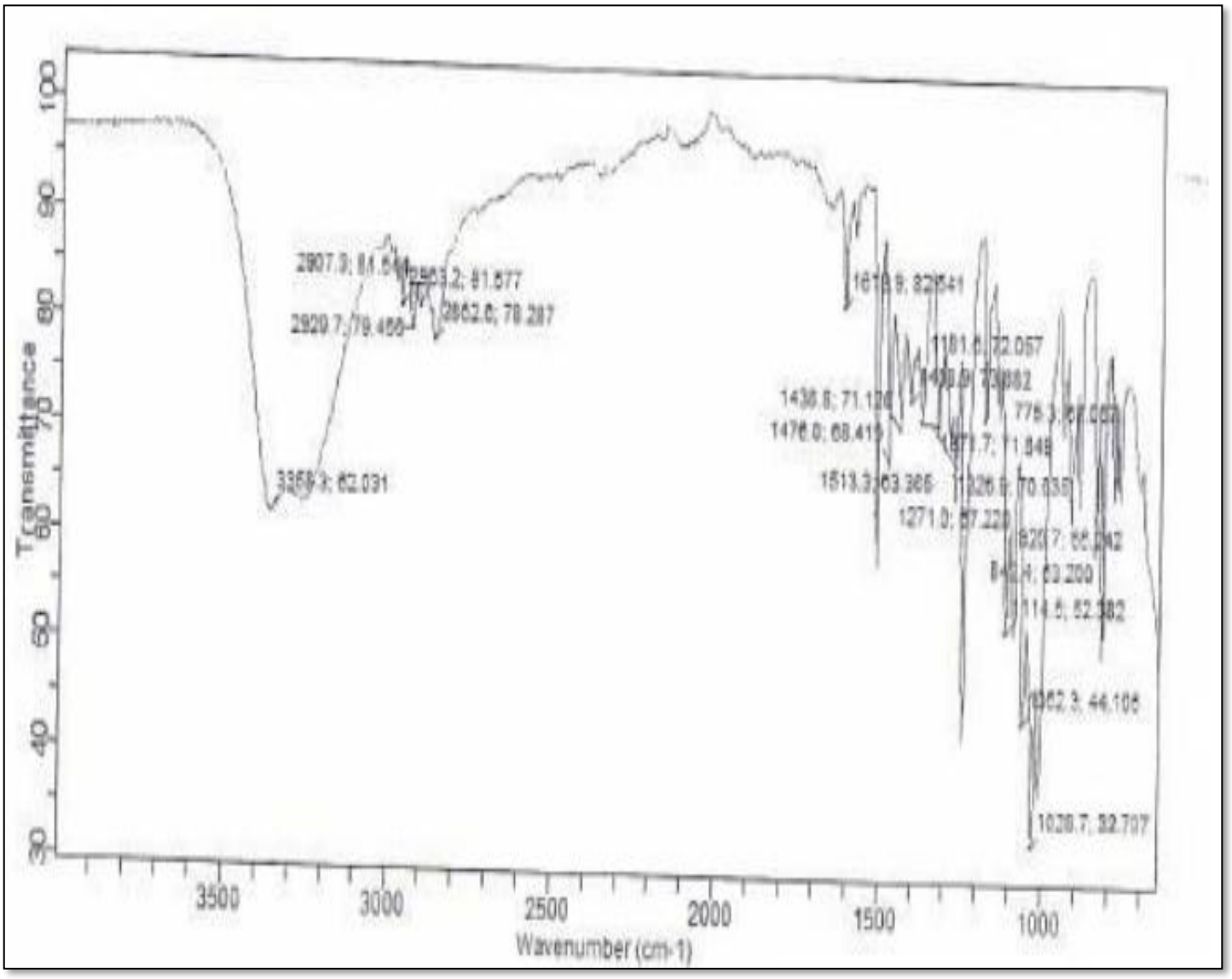

Figure 3:IR spectrum of Dapagliflozin (API)

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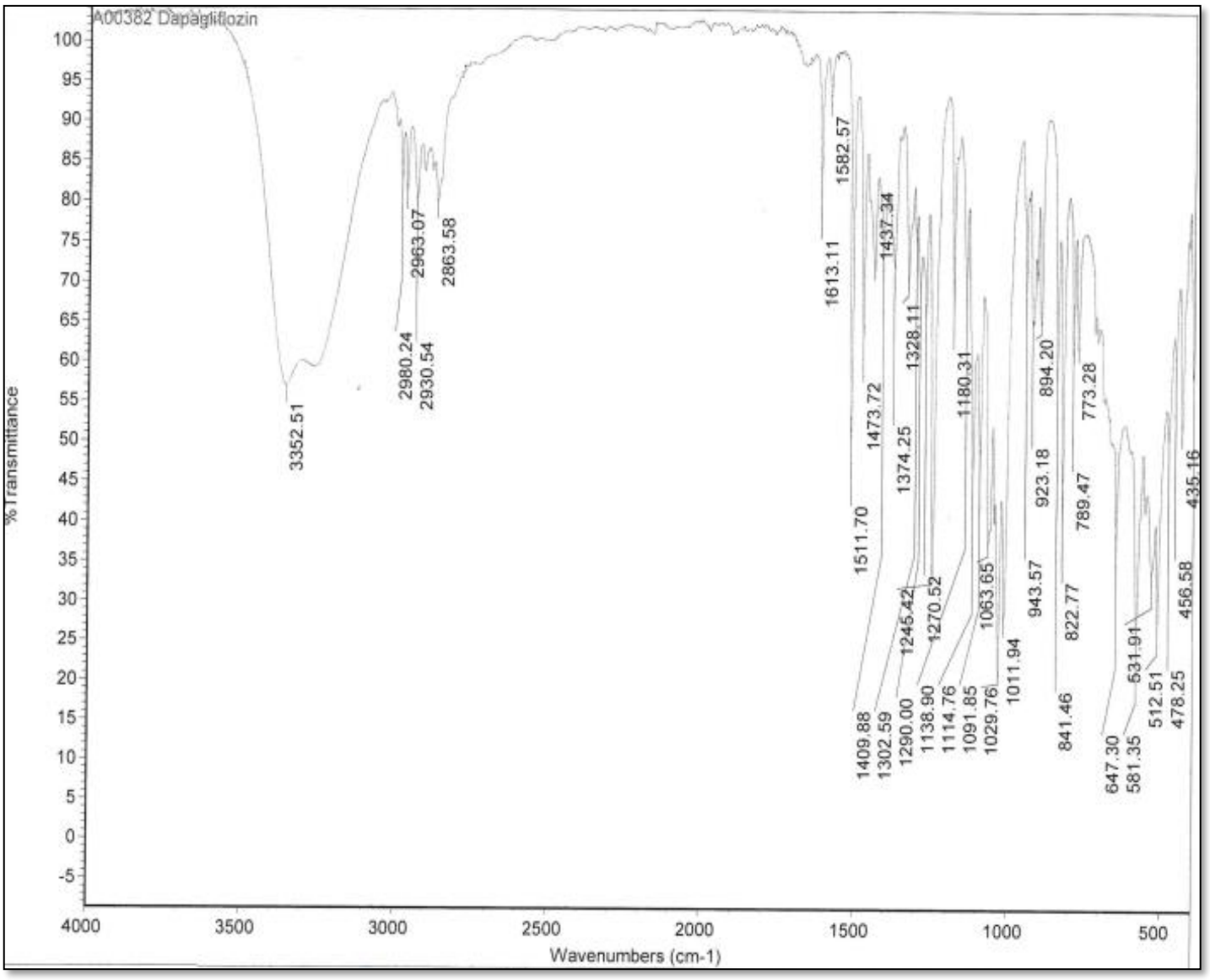

Figure 4:IR Spectrum of Sacubitril-Valsartan (API)

Table: 3.1 IR spectrum of Dapagliflozin

SR.NO.	Functional group	Observed value	Standard value
1	O–H stretching vibration	3352.51	3500–3100
2	Aliphatic C–H stretching vibration	2980.24, 2930.54, 2863.58	2900–2700
3	C–O–C stretching vibration	1138.90	1150–1085
4	C–Cl stretching vibration	841.46	850–550

Table:3.2 IR spectrum of Sacubitril-Valsartan

Sr. No.	Functional group	Observed value	Standard value
1	O–H stretching vibration	3057.01	3600–3000
2	Aliphatic C–H stretching vibration	2954.85	2960–2850

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3	Carbonyl (C=O) stretching vibration	1710.71	1750–1600
4	Aromatic C=C stretching vibration	1636.53	1660–1450

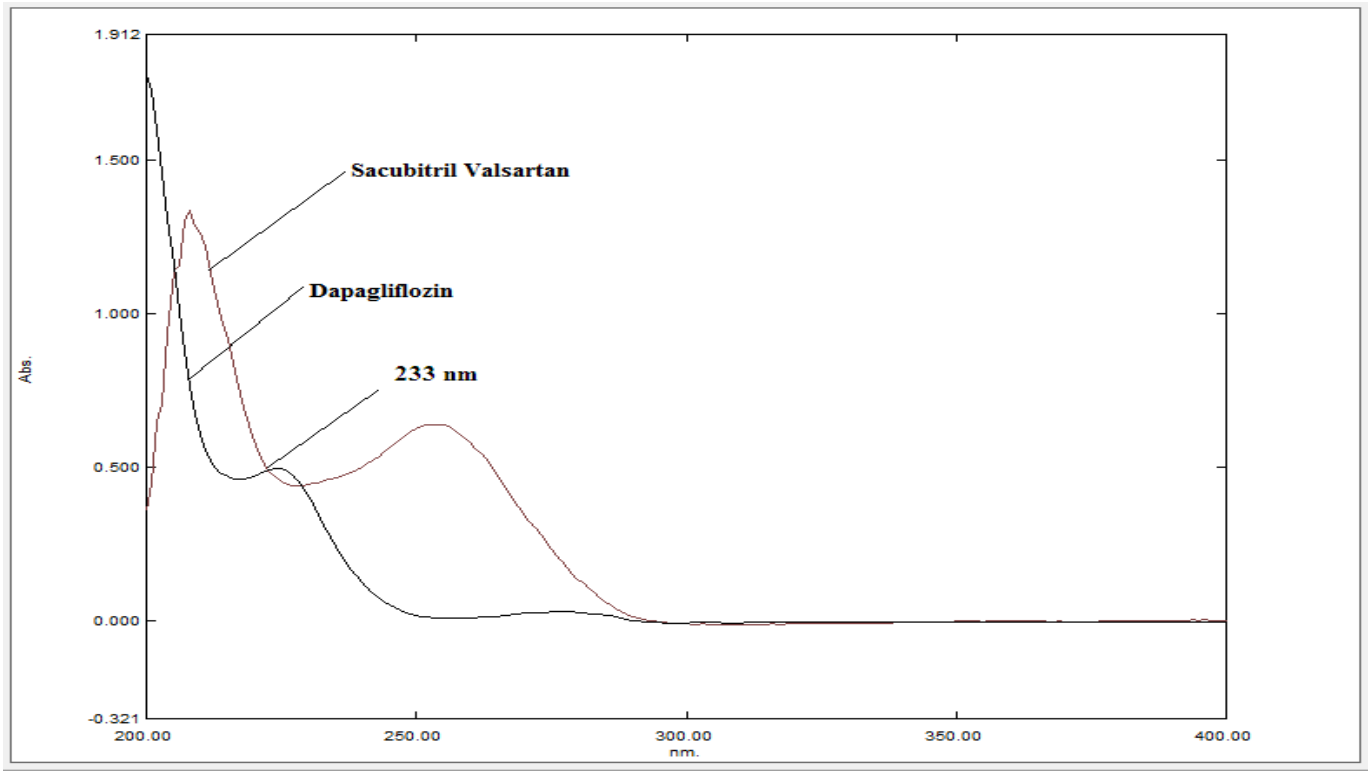

Figure 5: Determination of wavelength maxima(233nm)

Method Development

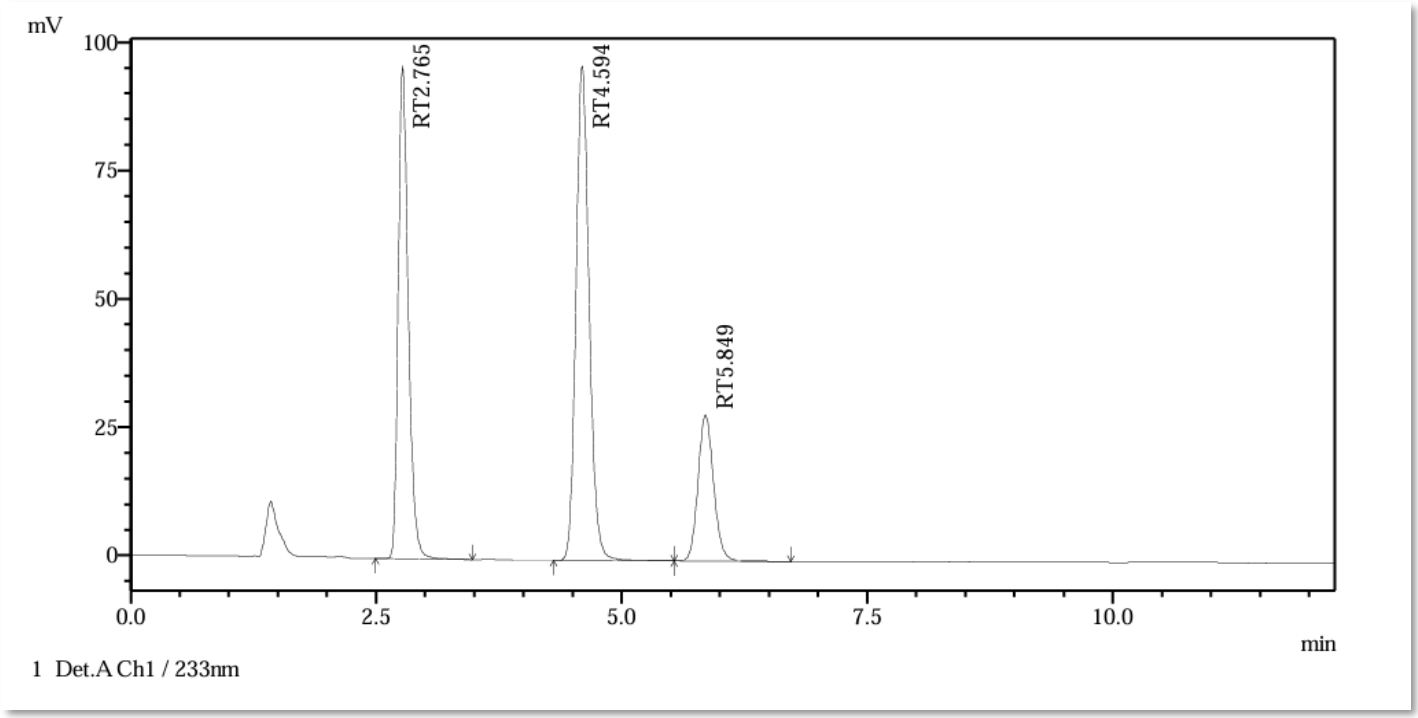

Fig 6: Final HPLC Chromatogram of 5 µg/mL Dapagliflozin + 50 µg/mL Sacubitril–Valsartan

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QBD APPROACH TO RP-HPLC METHOD DEVELOPMENT

Analytical Target Profile (ATP)

The Analytical Target Profile (ATP) for the present study was established to develop a robust, accurate, and reproducible RP-HPLC method for the simultaneous quantitative estimation of Dapagliflozin propanediol monohydrate and Sacubitril–Valsartan sodium complex salt in synthetic mixture

Selection of Critical Analytical Attributes (CAA)

The Critical Analytical Attributes (CAA) identified for this method included:

Retention time of Dapagliflozin and Sacubitril–Valsartan

Resolution between adjacent peaks

Theoretical plate count

Tailing factor

These attributes were selected because they directly reflect chromatographic efficiency, peak symmetry, selectivity, and reproducibility, which are essential for the accurate and precise quantification of both analytes in a combined formulation.

Selection of Critical Method Variables (CMV)

Based on preliminary chromatographic trials and a review of relevant literature, the following parameters were identified as Critical Method Variables (CMVs):

Mobile phase composition

Percentage of organic modifier (acetonitrile)

Flow rate

Buffer pH

Risk Identification

Risk identification was carried out using an Ishikawa (fishbone) diagram to systematically evaluate potential sources of variability that could affect the critical analytical attributes. The impact and criticality of each input factor were assessed based on prior literature, experimental knowledge, and structured brainstormi

ANOVA Interpretation for Dapagliflozin and Sacubitril and Valsartan

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	12.58	9	1.40	1963.3	< 0.0001	significant
A-MP Ratio	0.0014	1	0.0014	1.93	0.2076	
B-Column Temperature	7.254E-07	1	7.254E-07	0.0010	0.9754	
C-Flow Rate	0.3269	1	0.3269	459.30	< 0.0001	

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AB	0.0011	1	0.0011	1.52	0.2575	
AC	0.1791	1	0.1791	251.65	< 0.0001	
BC	0.0005	1	0.0005	0.6971	0.4313	
A^2	0.0336	1	0.0336	47.24	0.0002	
B^2	0.0005	1	0.0005	0.7356	0.4195	
C^2	0.2169	1	0.2169	304.75	< 0.0001	
Residual	0.0050	7	0.0007			
Lack of Fit	0.0050	5	0.0010	80.39	0.0123	significant
Pure Error	0.0000	2	0.0000			
Cor Total	12.58	16				

Table 3.3 ANOVA Table for reduced linear model of **Rt** of Dapagliflozin

ANOVA Table

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Remark**
Model	0.0695	3	0.0232	5.15	0.0145	Significant
A – MP Ratio	0.0689	1	0.0689	15.31	0.0018	Significant
B – Column Temperature	0.0002	1	0.0002	0.0555	0.8174	Not Significant
C – Flow Rate	0.0004	1	0.0004	0.0800	0.7818	Not Significant
Residual	0.0585	13	0.0045	—	—	—
Lack of Fit	0.0576	11	0.0052	12.09	0.0788	Not Significant
Pure Error	0.0009	2	0.0004	—	—	—
Cor Total	0.1280	16	—	—	—	—

Table 3.4: ANOVA Table for reduced linear model of Tf of Dapagliflozin

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	92.35	9	10.26	707.73	< 0.0001	significant
A-MP Ratio	0.0193	1	0.0193	1.33	0.2870	
B-Column Temperature	0.0010	1	0.0010	0.0694	0.7998	

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C-Flow Rate	0.4766	1	0.4766	32.87	0.0007	
AB	0.0102	1	0.0102	0.7003	0.4303	
AC	2.71	1	2.71	186.98	< 0.0001	
BC	0.0020	1	0.0020	0.1391	0.7203	
A^2	0.5543	1	0.5543	38.23	0.0005	
B^2	0.0022	1	0.0022	0.1519	0.7083	
C^2	1.09	1	1.09	75.30	< 0.0001	
Residual	0.1015	7	0.0145			
Lack of Fit	0.1011	5	0.0202	91.73	0.0108	significant
Pure Error	0.0004	2	0.0002			
Cor Total	92.45	16				

Table 3.5: ANOVA Table for reduced linear model of **Rt** of Sacubitril

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	1.284E+07	3	4.281E+06	8.16	0.0026	significant
A-MP Ratio	2.778E+06	1	2.778E+06	5.29	0.0386	
B-Column Temperature	4.376E+05	1	4.376E+05	0.8338	0.3778	
C-Flow Rate	9.628E+06	1	9.628E+06	18.34	0.0009	
Residual	6.823E+06	13	5.249E+05			
Lack of Fit	6.462E+06	11	5.875E+05	3.26	0.2583	not significant
Pure Error	3.607E+05	2	1.804E+05			
Cor Total	1.967E+07	16				

Table 3.6: ANOVA Table for reduced linear model of Theoretical Plate Count of Dapagliflozin

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Remark**
Model	0.0350	3	0.0117	15.21	0.0002	Significant
A – MP Ratio	0.0319	1	0.0319	41.55	< 0.0001	Significant
B – Column Temperature	0.0017	1	0.0017	2.20	0.1617	Not Significant

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C – Flow Rate	0.0014	1	0.0014	1.87	0.1949	Not Significant
Residual	0.0100	13	0.0008	—	—	—
Lack of Fit	0.0099	11	0.0009	33.06	0.0297	Significant
Pure Error	0.0001	2	0.0000	—	—	—
Cor Total	0.0450	16	—	—	—	—

Table 3.7 ANOVA Table for reduced linear model of Tailing Factor of Sacubitril

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Remark**
Model	3.231E+07	3	1.077E+07	6.28	0.0072	Significant
A – MP Ratio	7.756E+06	1	7.756E+06	4.52	0.0532	Not Significant
B – Column Temperature	1.603E+05	1	1.603E+05	0.0935	0.7647	Not Significant
C – Flow Rate	2.439E+07	1	2.439E+07	14.22	0.0023	Significant
Residual	2.230E+07	13	1.715E+06	—	—	—
Lack of Fit	2.175E+07	11	1.977E+06	7.22	0.1278	Not Significant
Pure Error	5.477E+05	2	2.738E+05	—	—	—
Cor Total	5.460E+07	16	—	—	—	—

Table 3.8 ANOVA Table for reduced linear model of Theoretical Plate Count of Sacubitril

ANOVA Interpretation for Valsartan

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	49.89	9	5.54	570.88	< 0.0001	Significant
A – MP Ratio	0.0043	1	0.0043	0.4395	0.5286	Not Significant
B – Column Temperature	0.0004	1	0.0004	0.0372	0.8525	Not Significant
C – Flow Rate	0.4796	1	0.4796	49.39	0.0002	Significant
AB	0.0020	1	0.0020	0.2044	0.6649	Not Significant
AC	1.26	1	1.26	129.85	< 0.0001	Significant
BC	0.0031	1	0.0031	0.3214	0.5885	Not Significant
A^2	0.2264	1	0.2264	23.31	0.0019	Significant

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B^2	0.0045	1	0.0045	0.4598	0.5195	Not Significant
C^2	0.7083	1	0.7083	72.95	< 0.0001	Significant
Residual	0.0680	7	0.0097	—	—	—
Lack of Fit	0.0677	5	0.0135	111.95	0.0089	Significant
Pure Error	0.0002	2	0.0001	—	—	—
Cor Total	49.96	16	—	—	—	—

Table 3.9 ANOVA Table for reduced linear model of Rt of Valsartan

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	3.292E+07	3	1.097E+07	27.60	< 0.0001	Significant
A – MP Ratio	8.477E+06	1	8.477E+06	21.33	0.0005	Significant
B – Column Temperature	1.308E+06	1	1.308E+06	3.29	0.0928	Not Significant
C – Flow Rate	2.313E+07	1	2.313E+07	58.19	< 0.0001	Significant
Residual	5.168E+06	13	3.975E+05	—	—	—
Lack of Fit	4.655E+06	11	4.232E+05	1.65	0.4371	Not Significant
Pure Error	5.127E+05	2	2.564E+05	—	—	—
Cor Total	3.808E+07	16	—	—	—	—

Table 3.10 ANOVA Table for reduced linear model of Theoretical Plate Count of Valsartan Fit Summaries of selected models

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	
Linear	< 0.0001	0.0002	0.9332	0.8952	
2FI	0.3595	0.0002	0.9361	0.7399	
Quadratic	**< 0.0001**	**0.0123**	**0.9991**	**0.9963**	**Suggested**
Cubic	0.1345	0.0164	0.9997	0.9077	**Aliased**

Table 3.11 Response 1: **Rt** of **Dapagliflozin**

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	**Remark**
Linear	0.0145	0.0788	0.4374	0.0822	Suggested
2FI	0.1012	0.1032	0.5968	-0.1727	—

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Quadratic	0.8885	0.0715	0.4709	-1.0593	—
Cubic	0.0137	0.7306	0.9611	0.1492	Aliased

Table 3.12 Response 2: **Tf** of **Dapagliflozin**

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	
Linear	**0.0026**	**0.2583**	**0.5730**	**0.3517**	**Suggested**
2FI	0.3795	0.2550	0.5864	-0.3594	
Quadratic	0.6205	0.2100	0.5341	-1.1270	
Cubic	0.0993	0.4848	0.8668	-9.5395	**Aliased**

Table 3.13 Response 3: Theoretical Plates of Dapagliflozin

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	**Remark**
Linear	< 0.0001	0.0004	0.9141	0.8574	—
2FI	0.1255	0.0005	0.9354	0.7300	—
Quadratic	< 0.0001	0.0108	0.9975	0.9911	Suggested
Cubic	0.0057	0.1340	0.9999	0.9773	Aliased

Table 3.14 Response 1: Retention Time of Sacubitril

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	
Linear	**0.0002**	**0.0297**	**0.7271**	**0.6181**	**Suggested**
2FI	0.6397	0.0255	0.6980	0.2849	
Quadratic	0.9571	0.0167	0.5864	-0.4395	
Cubic	0.0303	0.0641	0.9479	-12.5751	**Aliased**

Table 3.15 Response 2: Tailing Factorof Sacubitril

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	**Remark**
Linear	0.0072	0.1278	0.4975	0.3443	Suggested
2FI	0.9810	0.0963	0.3577	-0.4922	—
Quadratic	0.4448	0.0870	0.3589	-1.1280	—
Cubic	0.0923	0.1677	0.8259	-34.8922	Aliased

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Table 3.16 Response 3: **Theoretical Plates** of **Sacubitril**

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.0004	0.9194	0.8682	
2FI	0.1633	0.0005	0.9358	0.7387	
Quadratic	**< 0.0001**	**0.0089**	**0.9969**	**0.9890**	**Suggested**
Cubic	0.0070	0.0934	0.9999	0.9645	**Aliased**

Table 3.17 Response 1: **Retention Time** of **Valsartan**

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	Remark
Linear	0.0002	0.0606	0.7092	0.5800	Suggested
2FI	0.4919	0.0557	0.6997	0.2625	—
Quadratic	0.9283	0.0374	0.5964	-0.4646	—
Cubic	0.0226	0.1887	0.9583	-7.1582	Aliased

Table 3.18 Response 2: Tailing Factor of Valsartan

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.4371**	**0.8330**	**0.7804**	**Suggested**
2FI	0.9174	0.3559	0.7931	0.4884	
Quadratic	0.4142	0.3393	0.7985	0.3653	
Cubic	0.1405	0.8407	0.9263	0.4236	**Aliased**

Table 3.19 Response 3: Theoretical Plates of Valsartan

Three-dimensional response surface plots

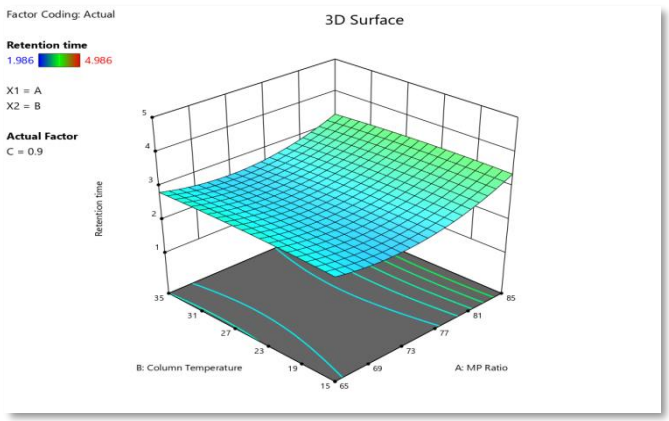

Fig 7: 3D plots Dapagliflozin (Rt)

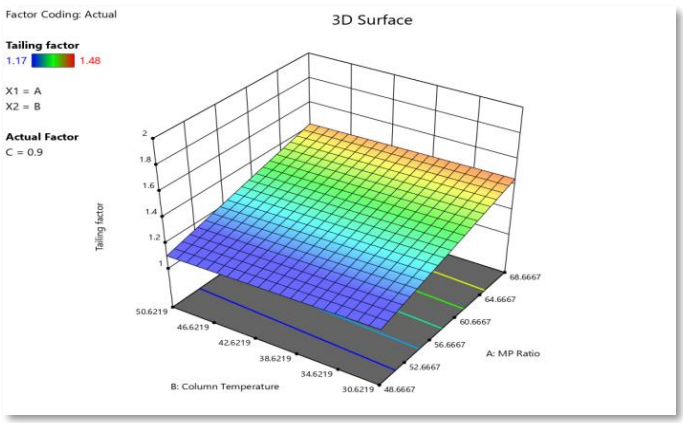

Fig 8: 3D plots Dapagliflozin (Tf)

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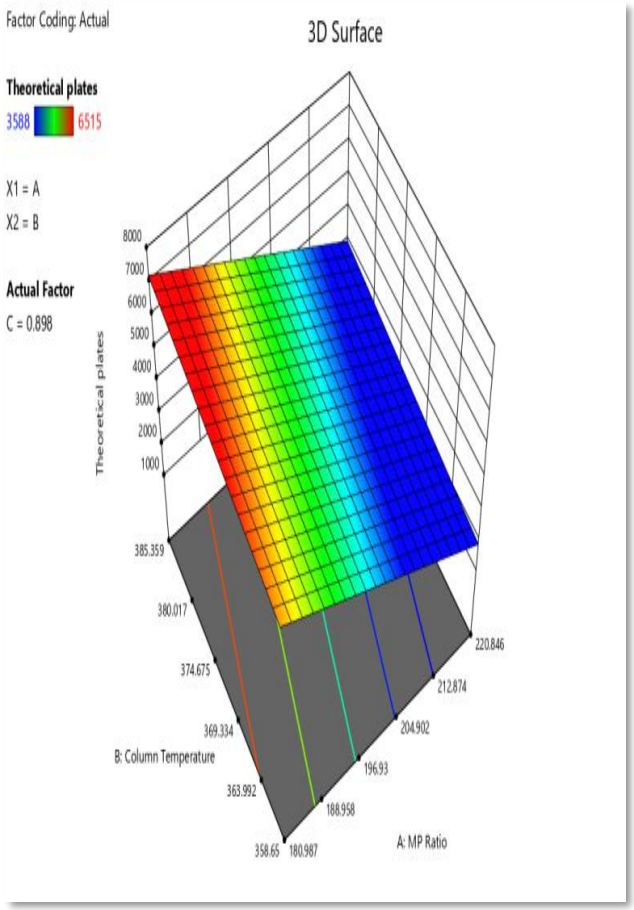

Fig 9: 3D plots Dapagliflozin (Theoretical plate)

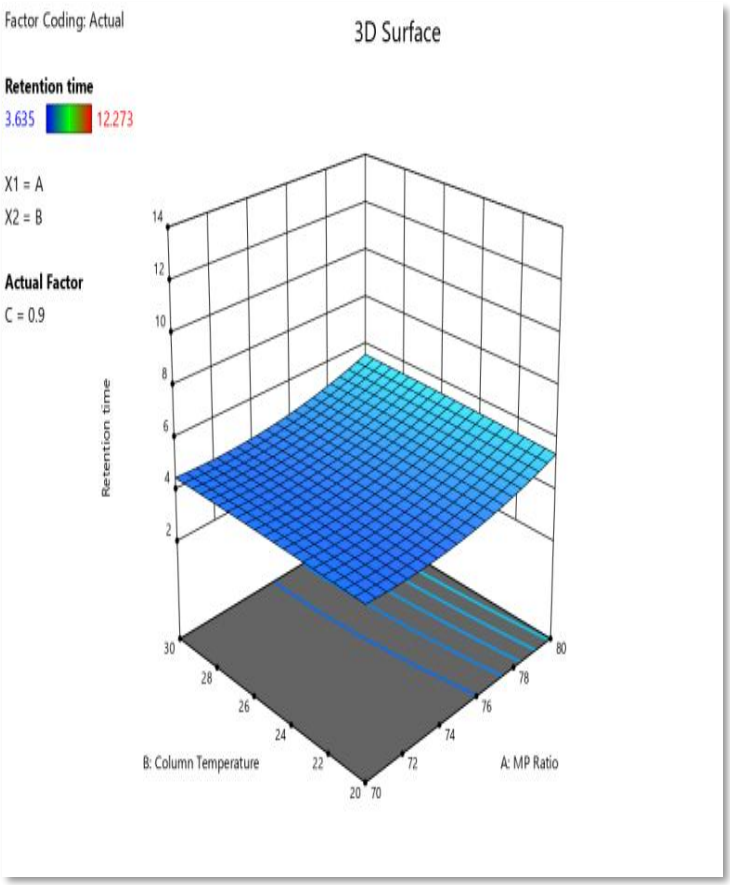

Fig 10: 3D plots **Sacubitril (Rt)**

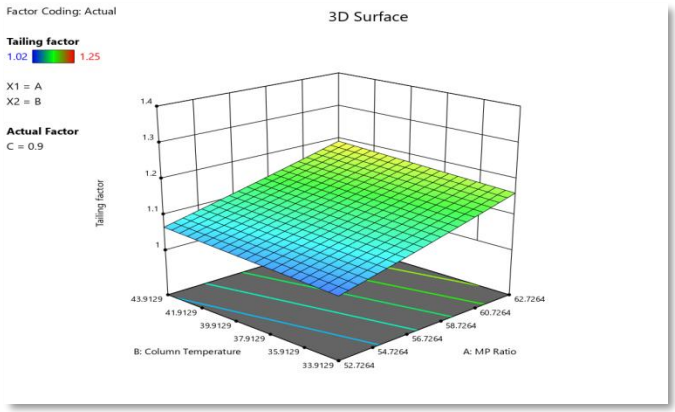

Fig 11:3D plots Sacubitril (Tf)

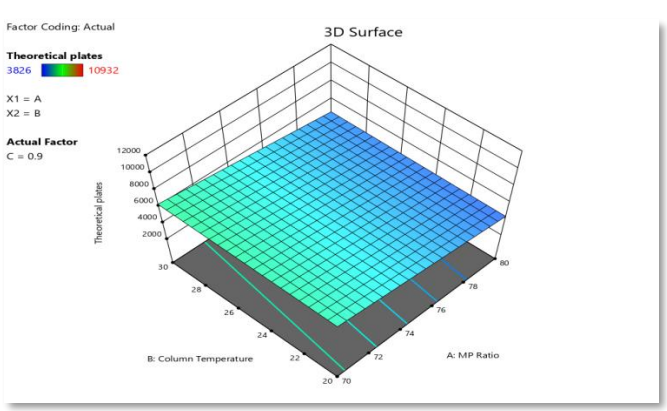

Fig 12: 3D plots Sacubitril (Theoretical plate)

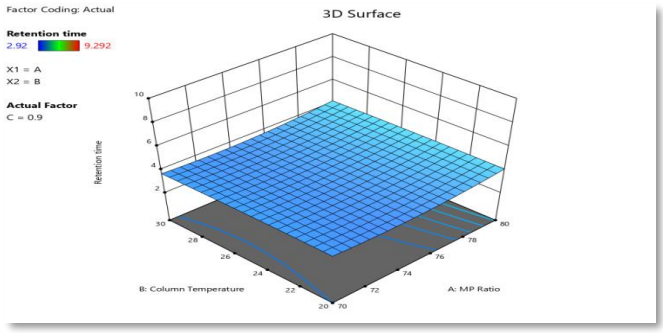

Fig 13: 3D plots Valsartan (Rt)

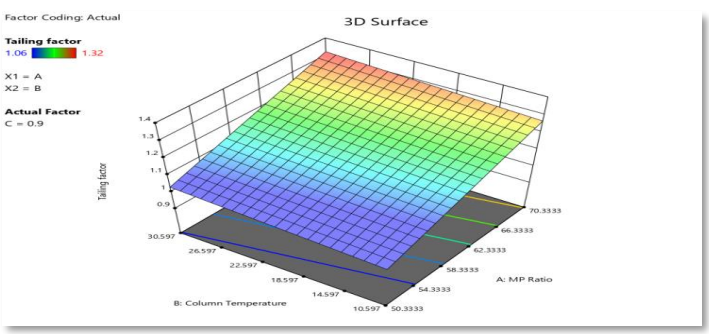

Fig 14: 3D plots Valsartan (Tf)

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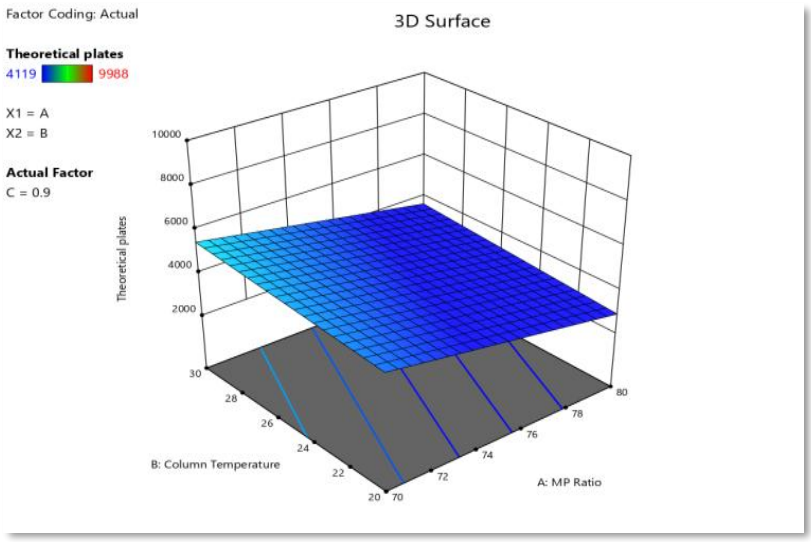

Fig 15: 3D plots Valsartan **(**Theoretical plate)

3.3.7 Contour plots

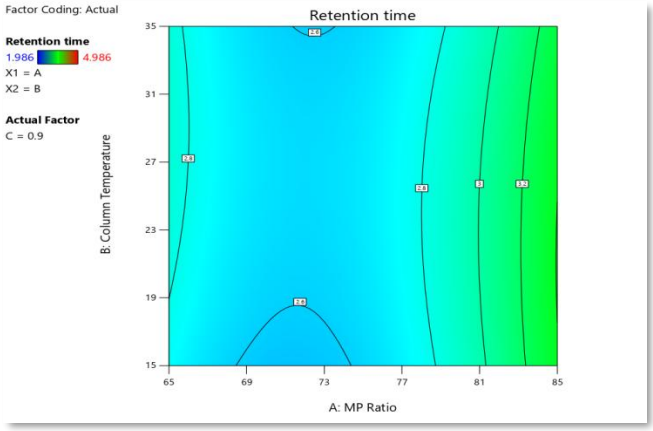

Fig 16: Contour plots Dapagliflozin (Rt)

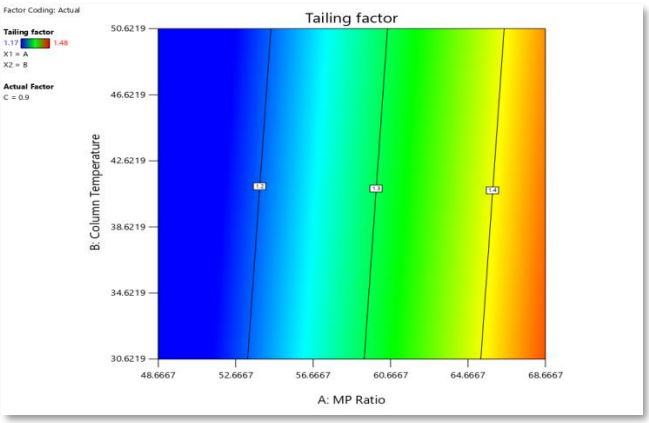

Fig 17: Contour plots Dapagliflozin (Tf)

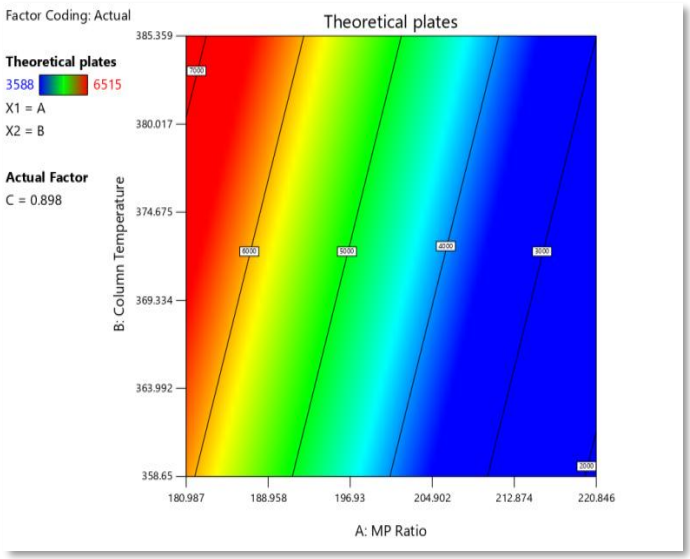

Fig 18: Contour plots Dapagliflozin (Theoretical Plate)

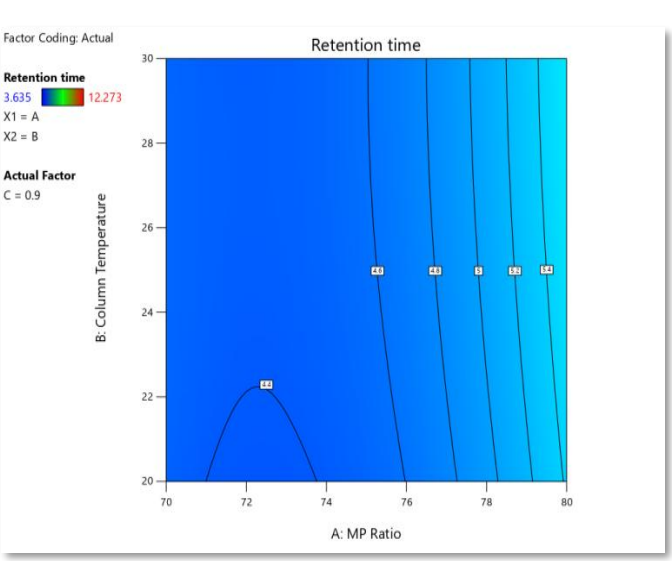

Fig 19: Contour plots Sacubitril (Rt)

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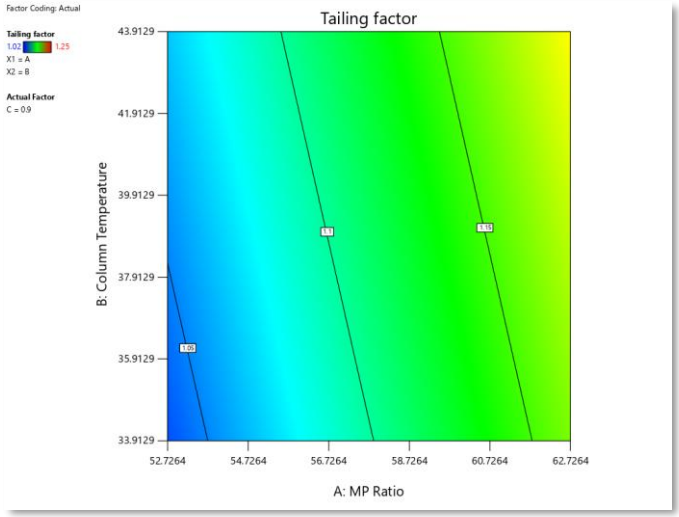


Fig 20: Contour plots Sacubitril **(**Tf)

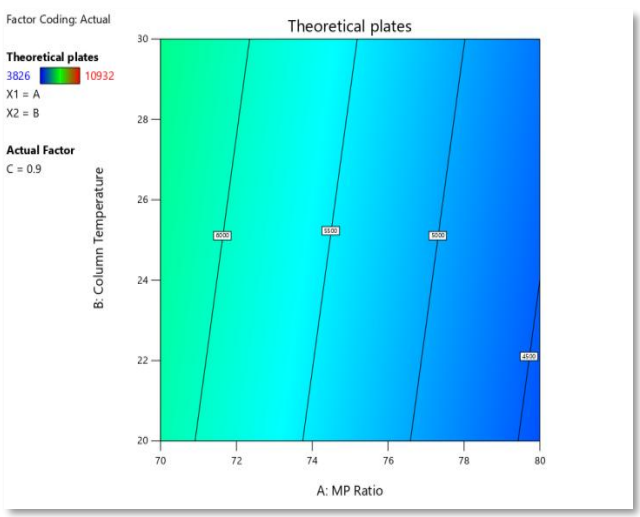
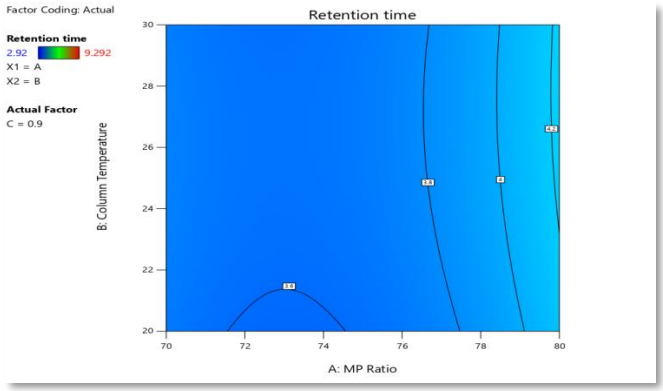


Fig 21: Contour plots Sacubitril (Theoretical Plate)



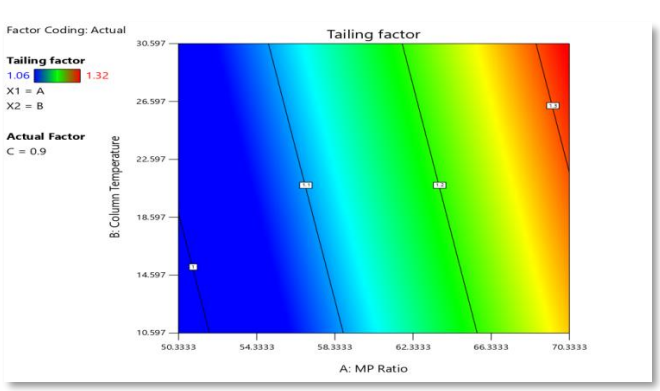


Fig 22: Contour plots Valsartan (Rt)Fig 23: Contour plots Valsartan (Tf)

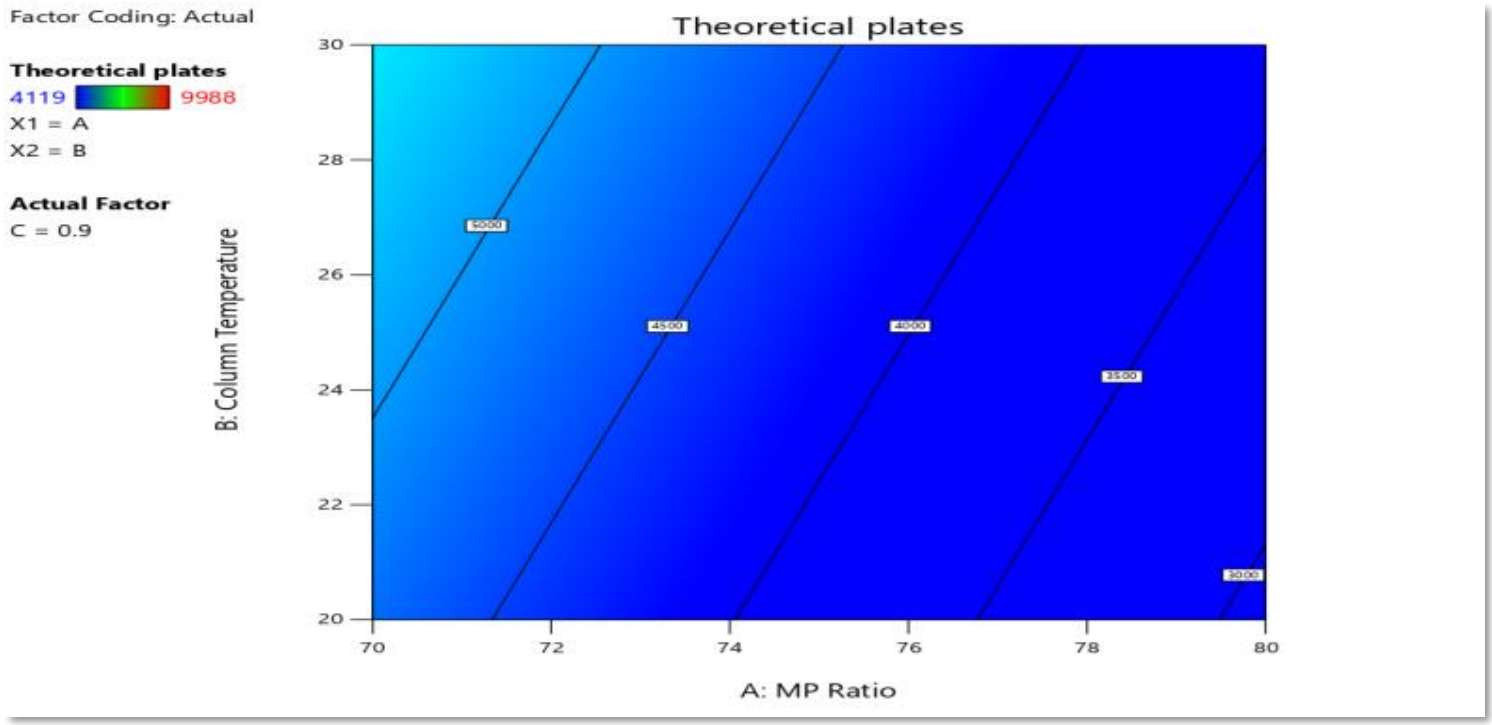


Fig 23: Contour plots Valsartan (Theoretical Plate)

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Desirability Function

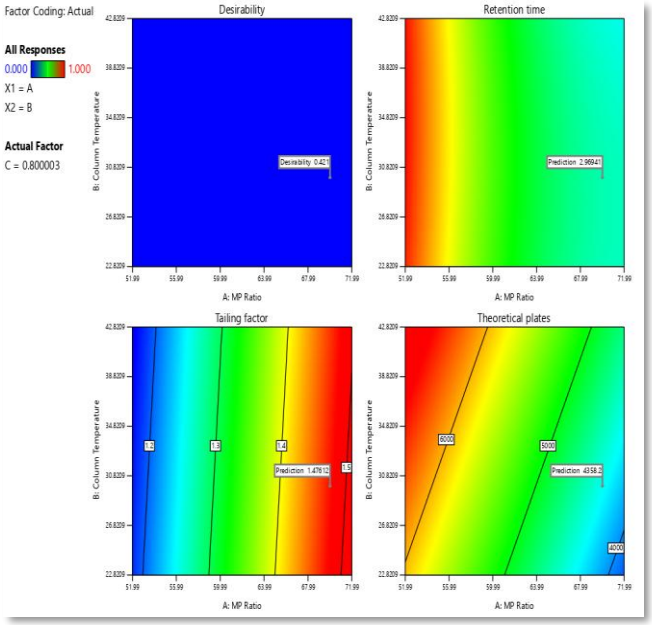

Fig 24: Desirability Function Dapagliflozin

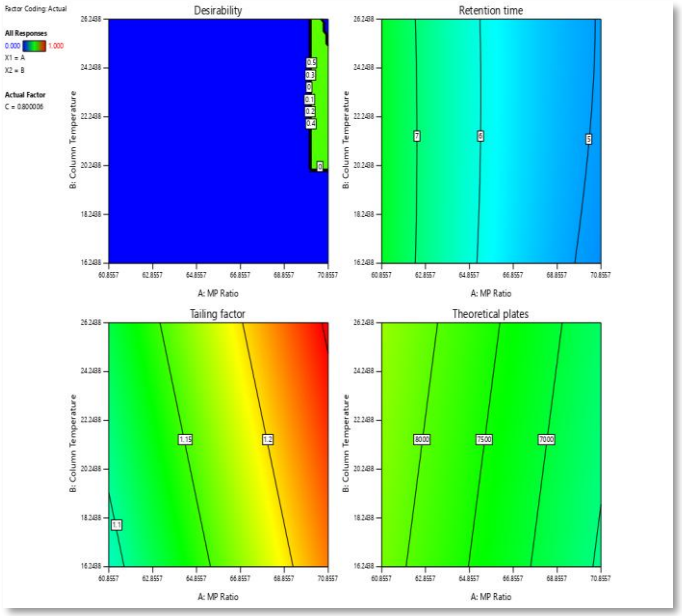

Fig 25: Desirability Function Sacubitril

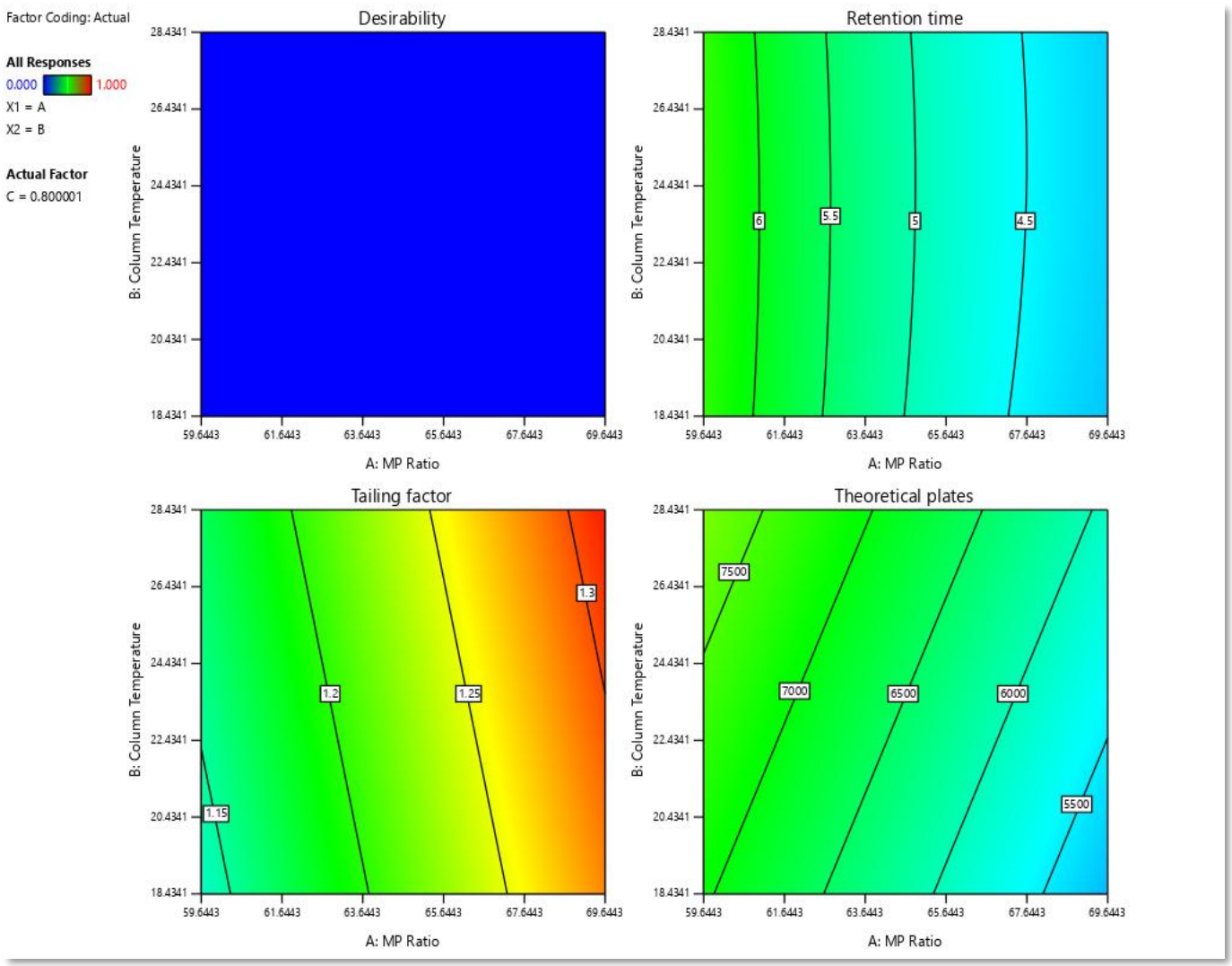

Fig 26: Desirability Function Valsartan

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Based on Overlay Plot:

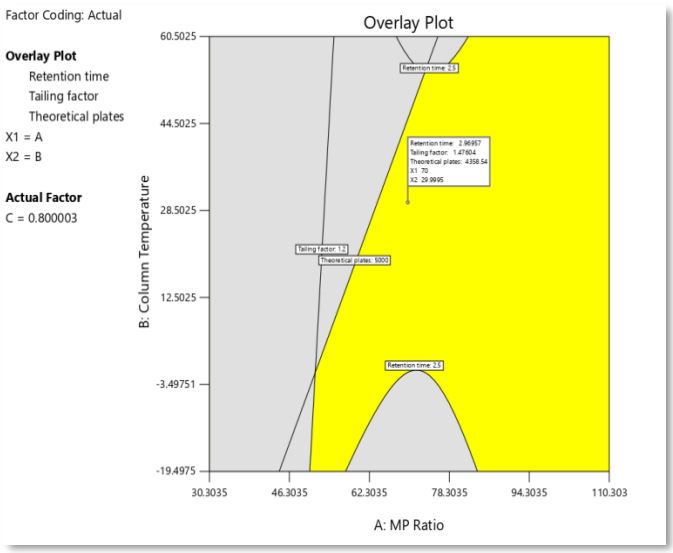

Fig 27: Overlay plot analysis Dapagliflozin

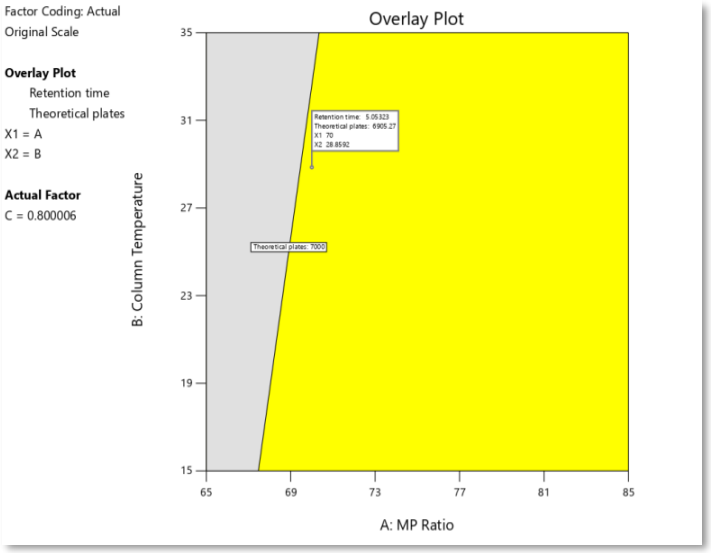

Fig 28: Overlay plot analysis Sacubitril

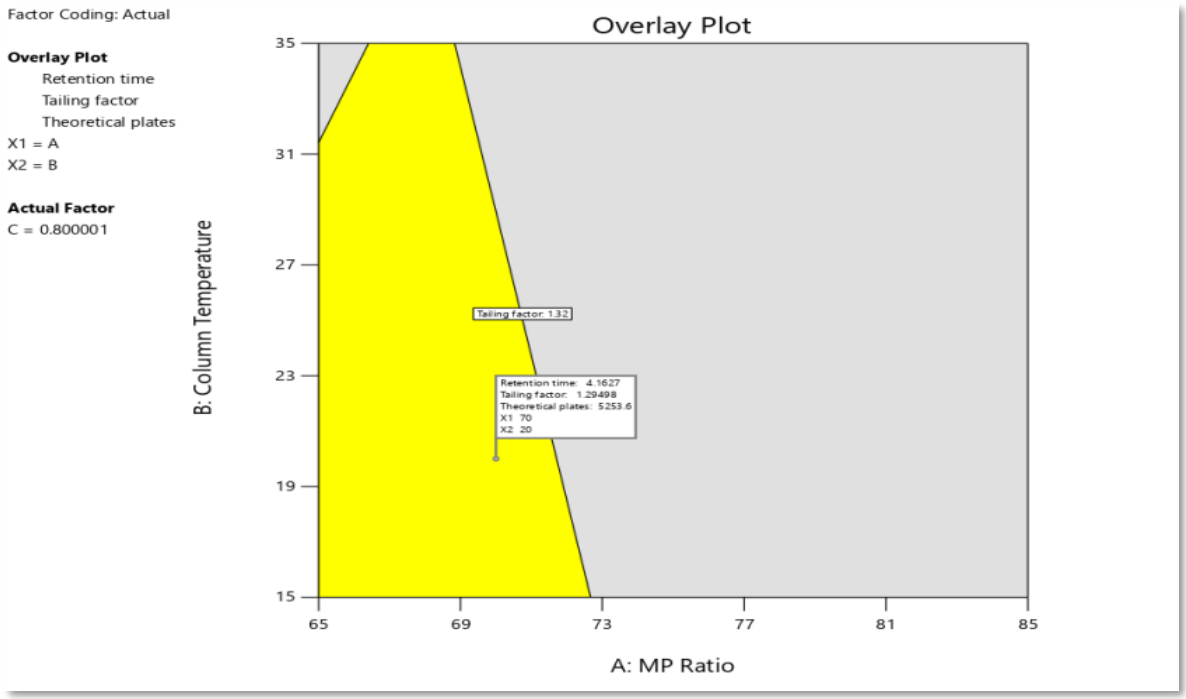

Fig 29: Overlay plot analysis Valsartan

Analytical Method Validation

System Suitability:

System suitability was evaluated using a mixed standard solution containing 5 µg/mL of Dapagliflozin Propanediol Monohydrate and 50 µg/mL of Sacubitril–Valsartan Sodium Complex Salt prepared in the diluent. The solution was injected six consecutive times into the RP-HPLC system.

Chromatograms were assessed for parameters such as retention time, peak area, theoretical plates, tailing factor, and resolution between the analyte peaks**.** The percentage relative standard deviation (%RSD) of peak areas was

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calculated for both analytes and was found to be within the acceptable limit of **≤ 2.0%**, indicating proper performance of the chromatographic system.

Table 3.20: System suitability study

Sr. No.	**System Suitability Parameter**	**Dapagliflozin Mean ± SD**	**%RSD**	**Valsartan Mean ± SD**	**%RSD**	**Sacubitril Mean ± SD**	**%RSD**
1	Retention Time (min)	2.908 ± 0.0039	0.13	4.767 ± 0.0040	0.08	6.145 ± 0.0043	0.07
2	Theoretical Plates (N)	5194 ± 52.18	1.00	7110 ± 61.23	0.86	8299 ± 70.47	0.85
3	Tailing Factor	1.29 ± 0.006	0.46	1.13 ± 0.005	0.44	1.08 ± 0.004	0.37
4	Resolution	—	—	9.61 ± 0.07	0.73	5.55 ± 0.05	0.90

Specificity:

Specificity of the developed method was examined by injecting blank (diluent), standard solution, and sample solution individually into the chromatographic system.

The obtained chromatograms were carefully evaluated to confirm that no interfering peaks were observed at the retention times corresponding to Dapagliflozin Propanediol Monohydrate and Sacubitril–Valsartan Sodium

Complex Salt, demonstrating the selectivity of the method for the analytes.

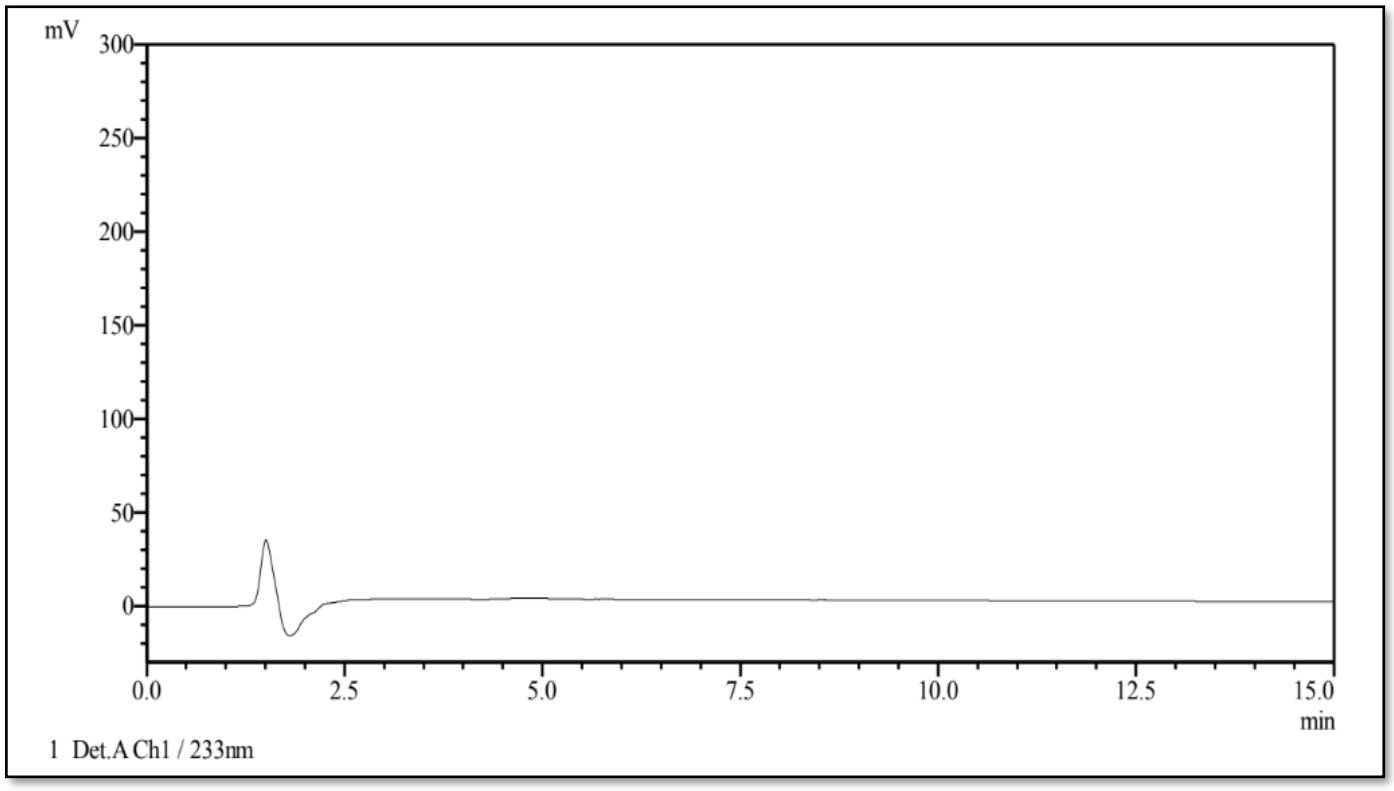

Fig 30: Chromatogram of Diluent

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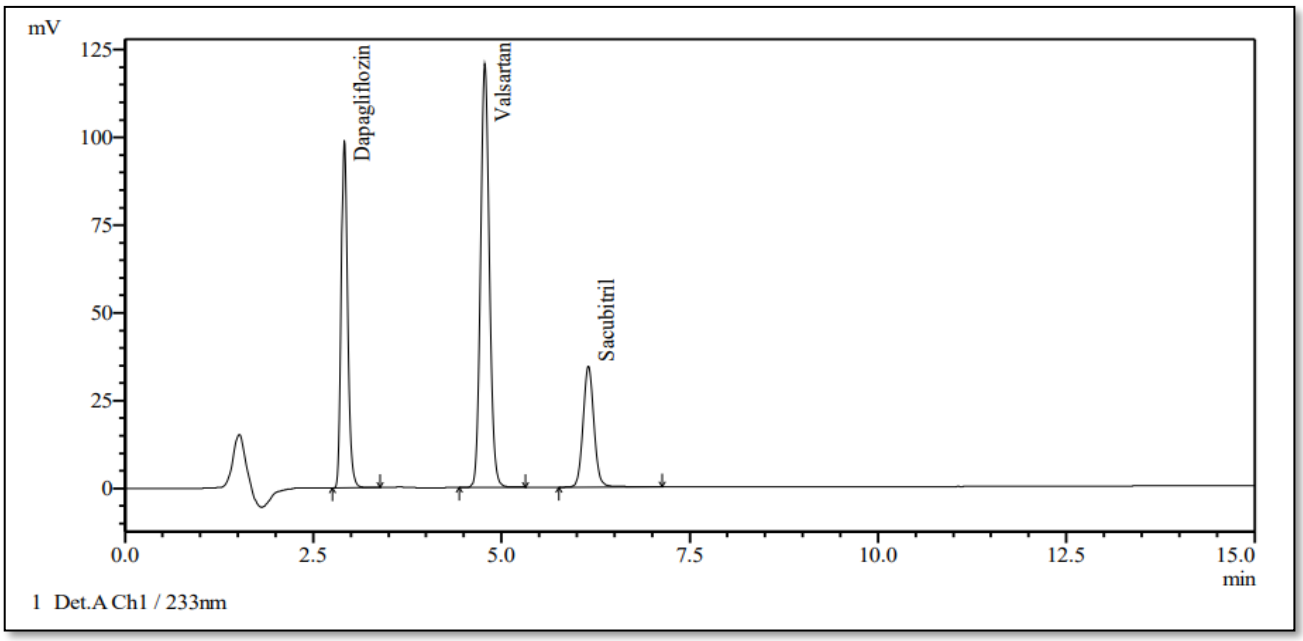

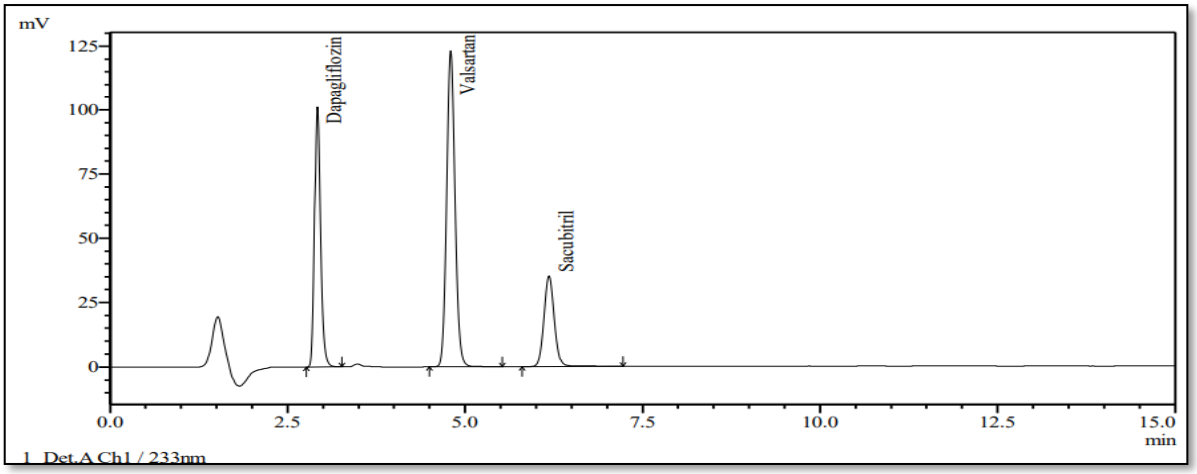

Fig 31: Standard of Dapagliflozin and Sacubitril–Valsartan **Fig 32: Sample of** Dapagliflozin and Sacubitril–Valsartan

Linearity

Linearity of the method was determined by analyzing five concentration levels ranging from 50% to 150% of the working concentration for both analytes.

Each level was injected three times, and calibration plots were constructed by plotting peak area versus concentration. The correlation coefficient (r^2) values were found to be greater than 0.999, confirming excellent linearity over the selected concentration range.

Sr. No.	Conc. (µg/mL)	Mean Area (n=3)	SD	% RSD
1	2.50	298358	338	0.11
2	3.75	432349	141	0.03
3	5.00	567766	326	0.05

4	6.25	716437	405	0.05
5	7.50	865624	1282	0.14

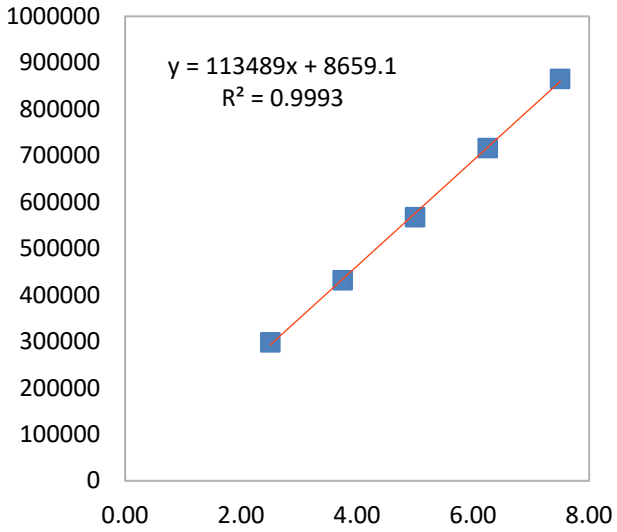

Fig 33: Calibration Curve of Dapagliflozin

Table 3.21: Linearity Data for Dapagliflozin

Sr. No.	Concen. (µg/mL)	Mean Area (n=3)	SD	% RSD
1	25.00	687446	463	0.06
2	37.50	983545	3243	0.32
3	50.00	1341881	1017	0.07
4	62.50	1668541	915	0.05
5	75.00	2008874	118	0.005

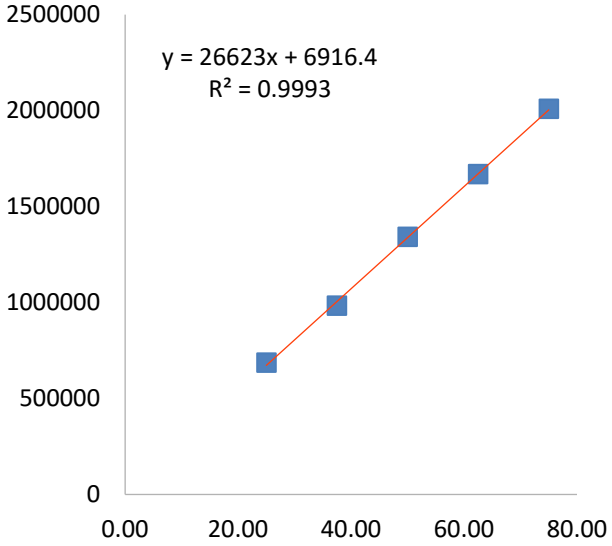

Fig 34: Calibration Curve of Dapagliflozin

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Table 3.22: Linearity Data for Sacubitril–Valsartan

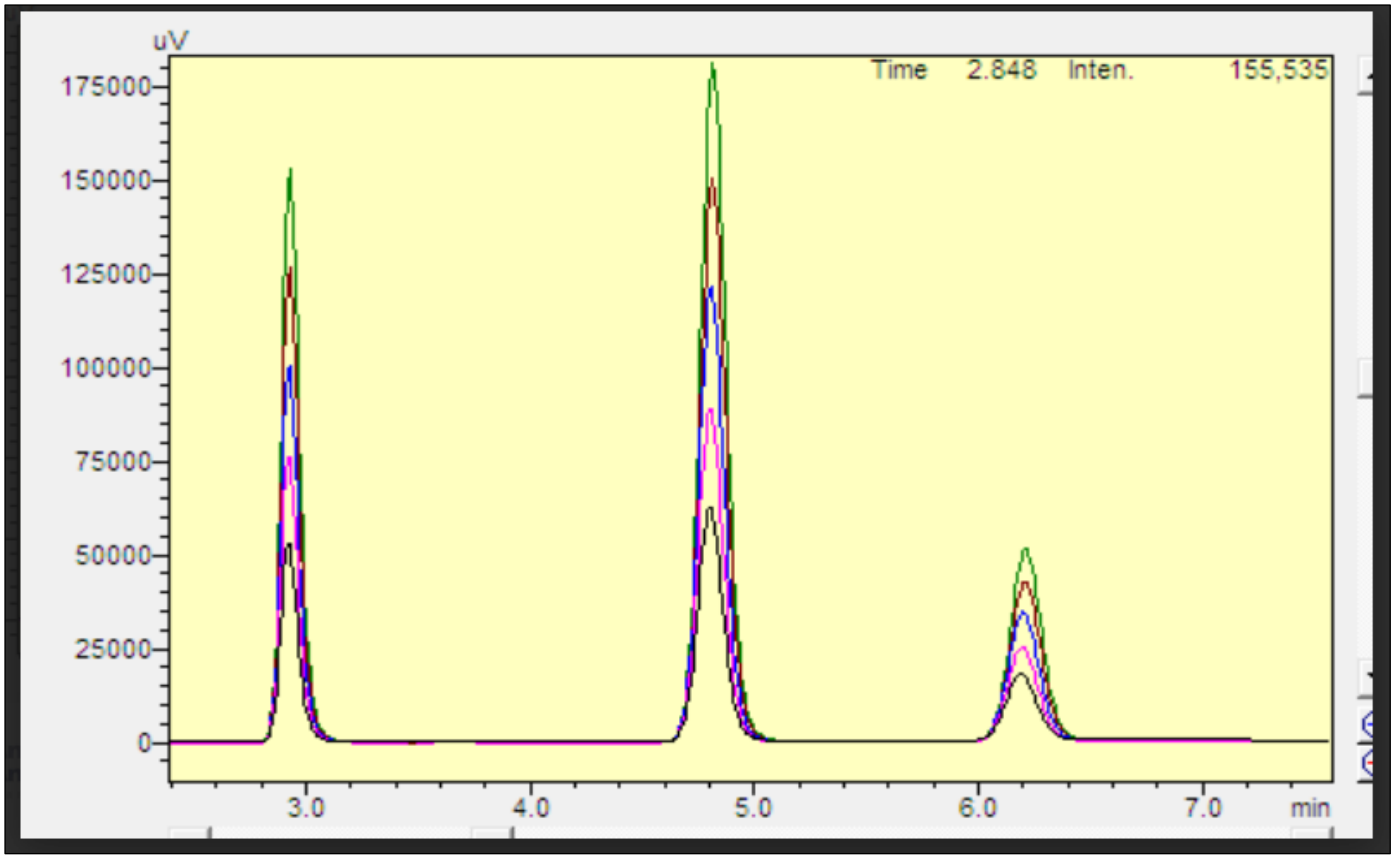

Fig 35: Linearity Chromatogram of Dapagliflozin and Sacubitril–Valsartan

Precision

Precision of the developed method was evaluated in terms of repeatability, intraday precision, and inter day precision for both analytes.

Repeatability (n = 6)

Table3.23: Repeatability Data of Dapagliflozin and Sacubitril–Valsartan

Conc of Dapagliflozin	**Peak area**	**Conc of Sacubitril–Valsartan**	**Peak area**
5 µg/ml	566908	50 µg/ml	1333371
	566827		1348843
	567200		1347447
	567273		1350239
	566868		1350657
	567295		1352234
Mean	567062	Mean	1347132
SD	216.5321377	SD	6935.435745
% RSD	0.03	% RSD	0.51

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Intraday Precision

Table 3.24 Intraday Precision

Drug	Concentration (µg/mL)	Mean Peak Area ± SD (n=3)	%RSD
Dapagliflozin	2.5	288922 ± 628.70	0.22
	5	569028 ± 1611.43	0.28
	7.5	864205 ± 447.03	0.05
Sacubitril–Valsartan	25	664669 ± 1908.54	0.29
	50	1343329 ± 7193.18	0.54
	75	2004894 ± 3784.72	0.19

Inter day Precision

Table 3.25: Inter day Precision

Drug	Concentration (µg/mL)	Mean Peak Area ± SD (n=3)	%RSD
Dapagliflozin	2.5	292836 ± 4006.42	1.37
	5	573882 ± 2961.20	0.52
	7.5	871510 ± 3307.61	0.38
Sacubitril–Valsartan	25	669175 ± 7055.57	1.05
	50	1350839 ± 8496.08	0.63
	75	2017251 ± 7452.69	0.37

Limit of Detection and Limit of Quantitation

The **LOD and LOQ** values for Dapagliflozin Propanediol Monohydrate and Sacubitril–Valsartan Sodium Complex Salt were calculated from the calibration curve data.

The calibration curves were prepared in triplicate, and the **standard deviation of the intercept (σ)** and **slope (S)** were determined. The values were calculated using the following equations:

- LOD = $\mathbf{3.3 \times \sigma / S}$
- LOQ = $\mathbf{10 \times \sigma / S}$

The obtained values confirmed that the developed method possesses adequate sensitivity for detection and quantification of both analytes.

Drugs	LOD (µg/mL)	LOQ (µg/mL)
Dapagliflozin	0.45	1.36
Sacubitril–Valsartan	4.36	13.2

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Table 3.26: Limit of Detection and Limit of Quantitation data for Dapagliflozin and Sacubitril–Valsartan

Accuracy

Accuracy was evaluated using the standard addition method at three levels: 50%, 100%, and 150**%** of the target concentration.The pre-analyzed synthetic mixture solution was spiked with known quantities of the standard drugs.

Each level was prepared in triplicate and analyzed using the optimized chromatographic conditions. The percentage recovery was calculated, and the mean recovery values for both drugs were found within **98–102% with %RSD < 2%**, confirming good accuracy of the method.

Table 3.27: Accuracy Data Dapagliflazoin & Sacubitril–Valsartan

Level %	**Set**	**Dapagliflazoin**					**Sacubitril–Valsartan**				
		Amount Added (µg)	**Amount Found (µg)**	**%Recovery**	**Mean % Recovery**	**%RSD**	**Amount Added (µg)**	**Amount Found (µg)**	**%Recovery**	**Mean % Recovery**	**%RSD**
50%	1	2.500	2.490	99.60	99.2	0.4	25.000	24.830	99.30	98.8	0.5
50%	2	2.500	2.470	98.80			25.000	24.600	98.40		
50%	3	2.500	2.480	99.20			25.000	24.670	98.70		
100%	1	5.000	4.930	98.60	98.8	1.1	50.000	49.520	99.00	99.9	0.9
100%	2	5.000	5.000	100.00			50.000	50.340	100.70		
100%	3	5.000	4.890	97.80			50.000	50.040	100.10		
150%	1	7.500	7.390	98.50			75.000	74.370	99.20		
150%	2	7.500	7.390	98.50			75.000	74.370	99.20		
150%	3	7.500	7.290	97.20	98.1	0.8	75.000	72.890	97.20	98.5	1.2

Robustness:

Robustness of the developed method was studied by introducing small, deliberate changes in chromatographic parameters.

The following variations were applied:

- Flow rate: ±0.1 mL/min from the optimized value
- Column temperature: ±5 °C
- Organic phase composition: ±2% of the mobile phase ratio

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A standard solution containing 5 µg/mL of Dapagliflozin and 50 µg/mL of Sacubitril–Valsartan was injected under each modified condition. Chromatograms were evaluated for system suitability parameters such as retention time, peak area, theoretical plates, and tailing factor.

The %RSD values obtained under all conditions were within acceptable limits, indicating that the method is robust and reliable.

Para meter	**Drug**	**Area at Tem. (-5°C)**	**Area at Tem. (+5°C)**	**Area at Flow (-1%ml/min)**	**Area at Flow (+1%ml/min)**	**Area at Organic Phase-2%**	**Area at Organic Phase+2%**
Mean Peak Area	**Dapagliflozin**	569439	571227	633750	517748	569588	569026
	Sacubitril–Valsartan	1347018	1344871	1490324	1232106	1345127	1384884
% RSD	**Dapagliflozin**	0.1	0.0	0.1	0.3	0.1	0.1
	Sacubitril–Valsartan	0.1	0.5	0.3	1.1	0.0	0.6
Theoretical Plates	**Dapagliflozin**	5079	5266	5868	4853	5404	5044
	Sacubitril	7845	8265.00	8971	7983	8362	7884
	Valsartan	6858	7198.00	7766	6815	7305	6685
Tailing Fator	**Dapagliflozin**	1.12	1.13	1.12	111	1.12	1.12
	Sacubitril–	1.07	1.09	1.08	1.08	1.05	1.09
	Valsartan	1.11	1.14	1.14	1.14	1.10	1.14

Table 3.28: Robustness Data Dapagliflazoin & Sacubitril–Valsartan

Assay of Synthetic Mixture

Table 3.29: Assay of Synthetic Mixture

Drug	**Label Claim (mg)**	**Mean Amount Found (mg)**	**Assay (% Mean ± SD)**	**% RSD**
Dapagliflozin	5.0	5.05	101 ± 1.450	1.44
Sacubitril–Valsartan	50.0	50.8	101.6 ± 1.759	1.73

CONCLUSION

The present study successfully developed and validated a QbD-based RP-HPLC method for the simultaneous estimation of Dapagliflozin Propanediol Monohydrate and Sacubitril–Valsartan Sodium Complex Salt in a synthetic mixture. The application of the QbD approach allowed systematic optimization and identification of a robust design space, ensuring consistent chromatographic performance.

The validated method demonstrated excellent linearity, precision, accuracy, sensitivity, and robustness, with all validation parameters meeting acceptable analytical criteria. The overlay plot confirmed a stable method operable design region, indicating that the method can tolerate small variations in chromatographic conditions without affecting performance.

Therefore, the developed RP-HPLC method is simple, rapid, precise, and reliable, making it suitable for routine quality control analysis and simultaneous estimation of Dapagliflozin and Sacubitril–Valsartan in pharmaceutical formulations or synthetic mixtures.

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