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A New RP-HPLC Technique That Can Simultaneously Measure Dapagliflozin ,Vildagliptin and Metformin in Formulation And Bulk Materials

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Abstract: In this work, we present a novel method for determining the prescribed dosages of Dapagliflozin (DAPA), Vildagliptin (VIL), and Metformin (MET) all at once. The goal of this research is to create and test a new RPHPLC technique that can simultaneously measure DAPA, VIL, and MET in formulation and bulk materials.The goal of this research is to create and test a new RP-HPLC technique that can simultaneously measure DAPA, VIL, and MET in formulation and bulk materials. An isocratic elution method was used with a flow rate of 1.0 ml min-1 and a diode array detector operating at 261nm to perform the chromatographic separation on a kromasilC18 column (4.5 x 250mm; 5µm). Using orthophosphoric acid to get the pH down to 3.5, the mobile phase consisted of a combination of 0.05 mmol potassium dihydrogen phosphate buffer and acetonitrile in an 80:20 v/v ratio. With concentrations ranging from 0.1-1.0µg/ml and 225µg/ml, as well as DAPA, VIL, and MET values from 10 to 120µg/ml, the calibration curve displayed linearity. The research found that DAPA had a limit of detection and quantification of 0.0122µg/ml while VIL had a limit of 0.0323µg/ml.

Key words: Dapagliflozin (DAPA), Vildagliptin (VIL), and Metformin (MET) , RP-HPLC.

INTRODUCTION:

Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data. The quality and safety of a drug is generally assured by monitoring and controlling the assay and impurities effectively. While assay determines the potency of the drug and impurities will determine the safety aspect of the drug. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients.Developing analytical methods for diverse drugs is challenging due to their varying properties. Achieving selectivity, speed, cost-effectiveness, simplicity, sensitivity, reproducibility, and accuracy is crucial for addressing pharmaceutical and chemical industry needs. Physicochemical methods, including optical, photometric, and chromatographic techniques, are commonly employed to study chemical reactions' physical phenomena. Furthermore, Nuclear Magnetic Resonance (NMR), Para Magnetic Resonance (PMR), and mass spectrometry (MS) coupled with gas chromatography are increasingly favored techniques in drug analysis.Employing HPLC eliminates repetitive extraction and isolation processes.

MATERIALS AND METHOD

- Metformin, Dapagliflozin and Vildagliptin pure drugs are procured from spectrum laboratories. Pvt.Ltd, Hyderabad.
- Materials and Instruments: The following materials used were either AR/LR grade or the best possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

Instruments:

1. Electronics Balance-Denver
2. p^H meter -BVK enterprises, India
3. Ultrasonicator-BVK enterprises
4. WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
5. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Metformin, Dapagliflozin and Vildagliptin solution.

Table 6.1: List of drug and marketed formulation used in research

List of drugs	Procured from	Marketed formulation
Metformin, Dapagliflozin and Vildagliptin	Spectrum laboratories. pvt. limited, Hyderabad	Dapavryl-VM500

METHOD DEVELOPMENT TRAILS

The primary objective of this experiment was to enhance the assay method for accurately determining the concentration of Metformin, Dapagliflozin and Vildagliptin. The mentioned trials illustrate the process of optimization carried out during the study.

Table 6.2: Final Selected Factors and Responses

Factor 1 (F1)	Flow Rate
Factor 2 (F2)	Mobile phase, (v/v)
Factor 3 (F3)	Temperature, ℃
Response 1 (R1)	Retention of Vildagliptin RT_1;
Response 2 (R2)	Retention of Metformin RT_2;
Response 3 (R3)	Retention of Dapagliflozin $RT_{3;}$
Response 4 (R4)	Resolution between Vildagliptin & Metformin RS_1
Response 5 (R5)	Resolution between Metformin & Dapagliflozin RS_1
Response 6 (R6)	Number of theoretical plates (NTP1)
Response 7 (R7)	Number of theoretical plates (NTP2)
Response 8(R8)	Number of theoretical plates (NTP3)

Optimization of Chromatographic conditions using Quality by Design (QbD) approach

RESULTS AND DISCUSSION

According to the ICH guidelines, the RP-HPLC method was developed and validated for the Metformin, Dapagliflozin and Vildagliptin estimation. Metformin, Dapagliflozin and Vildagliptin was estimated using RP-HPLC with the following parameters: stationary phase: SunFire C18 Column (3.5 µm, 4.6 mm X 250 mm); Mobile phase containing 0.01N KH_2PO_4 and Acetonitrile in the ratio of 67:33 v/v; and selected for

the wavelength of 220.0 nm. QbD applied for the development of most accurate, reliable and stable method. The method was validated for system suitability, specificity, precision, accuracy, linearity, LOD, LOQ and robustness studies. The values obtained for the proposed method are within the specified limit according to the system suitability parameter. %RSD of system precision for Metformin, Dapagliflozin and Vildagliptin. were and found to be 0.5%, 0.9% and 0.6% respectively. % RSD of method precision for Metformin, Dapagliflozin and Vildagliptin were and found to be 0.7%, 0.6% and 0.2 %respectively. It shows how exact the method was precise.

Table 7.1.2: Results of regression analysis of models for response 1

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.0189	0.9882	0.9860	0.9795	0.0100	
2FI	0.0195	0.9898	0.9852	0.9755	0.0120	
Quadratic	0.0073	0.9989	0.9979	0.9923	0.0038	Suggested
Cubic	0.0050	0.9997	0.9990	0.9685	0.0154	Aliased

Table 7.1.3: Results of regression analysis of models for response 2

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.0238	0.9868	0.9843	0.9777	0.0154	
2FI	0.0244	0.9888	0.9836	0.9717	0.0195	
Quadratic	0.0113	0.9981	0.9965	0.9856	0.0099	Suggested
Cubic	0.0047	0.9998	0.9994	0.9890	0.0076	Aliased

Table7.1.4: Results of regression analysis of models for response 3

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.0777	0.9532	0.9444	0.9142	0.1772	
2FI	0.0520	0.9830	0.9751	0.9418	0.1201	Suggested
Quadratic	0.0483	0.9887	0.9785	0.9138	0.1780	
Cubic	0.0228	0.9985	0.9952	0.7123	0.5938	Aliased

Table 7.1.5: Results of regression analysis of models for response 4

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.3123	0.8435	0.8141	0.7150	2.84	
2FI	0.2386	0.9258	0.8915	0.6361	3.63	Suggested
Quadratic	0.2367	0.9438	0.8932	0.5540	4.45	
Cubic	0.0848	0.9957	0.9863	0.3747	6.23	Aliased

Table 7.1.6: Results of regression analysis of models for response 5

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.3230	0.8901	0.8694	0.7956	3.10	
2FI	0.1480	0.9813	0.9726	0.9249	1.14	Suggested
Quadratic	0.1598	0.9832	0.9681	0.8751	1.90	
Cubic	0.0432	0.9993	0.9977	0.9573	0.6479	Aliased

Table 7.1.7: Results of regression analysis of models for response 6

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	292.86	0.6283	0.5586	0.3792	2.292E+06	
2FI	319.18	0.6412	0.4757	0.1868	3.002E+06	
Quadratic	108.35	0.9682	0.9396	0.7591	8.895E+05	Suggested
Cubic	7.92	0.9999	0.9997	0.9998	640.73	Aliased

Table 7.1.8: Results of regression analysis of models for response 7

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	210.30	0.8674	0.8425	0.7560	1.302E+06	
2FI	157.83	0.9393	0.9113	0.8343	8.844E+05	
Quadratic	96.63	0.9825	0.9668	0.8729	6.785E+05	Suggested
Cubic	88.65	0.9912	0.9720	-0.7365	9.267E+06	Aliased

Table7.1.9: Results of regression analysis of models for response 8

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	524.59	0.8086	0.7727	0.6683	7.630E+06	Suggested
2FI	545.84	0.8316	0.7539	0.5923	9.379E+06	
Quadratic	446.79	0.9132	0.8351	0.4047	1.369E+07	Suggested
Cubic	433.54	0.9510	0.8448	-7.5927	1.977E+08	Aliased

Response 1: Retention time of Vildagliptin

Table 7.2.1: ANOVA table for Retention time using CCD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.4875	9	0.0542	1003.15	< 0.0001	Significant
A-FR	0.4782	1	0.4782	8854.74	< 0.0001	
B-MP	0.0034	1	0.0034	63.85	< 0.0001	
C-Temp	0.0007	1	0.0007	13.49	0.0043	
AB	0.0005	1	0.0005	8.33	0.0162	
AC	0.0002	1	0.0002	4.08	0.0709	
BC	0.0001	1	0.0001	2.08	0.1795	
A^2	0.0042	1	0.0042	77.76	< 0.0001	
B^2	0.0000	1	0.0000	0.5944	0.4586	
C^2	8.886E-06	1	8.886E-06	0.1646	0.6935	
Residual	0.0005	10	0.0001			
Lack of Fit	0.0005	5	0.0001	5.92	0.0366	Significant
Pure Error	0.0001	5	0.0000			
Cor Total	0.4881	19				

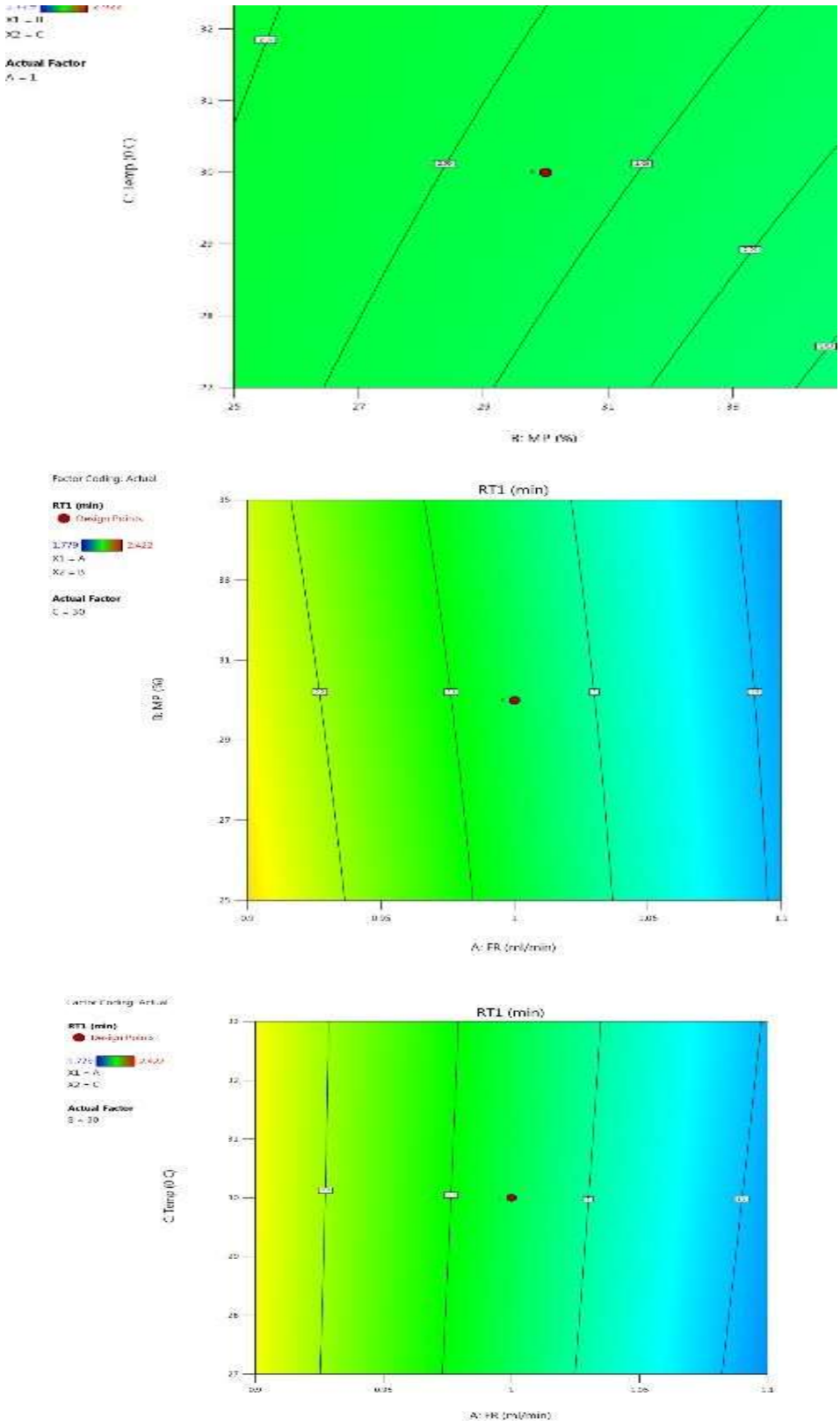


Fig 7.1.3 2D contour plots of retention time as a function of Column temperature, Flow rate and organic ratio.

In order to understand the results, contour plots and 3D plot were generated after processing all data using the Design Expert® software. It shows the two-dimensional contour plot as a function of Column temperature, Flow rate and organic ratio. Based on the color code, the working region can be easily identified. Retention time maps represent the value of the retention time, with warm "red" colors indicating

larger retention time, cold "blue" colors lower and light green to yellow color represent intermediate retention time.

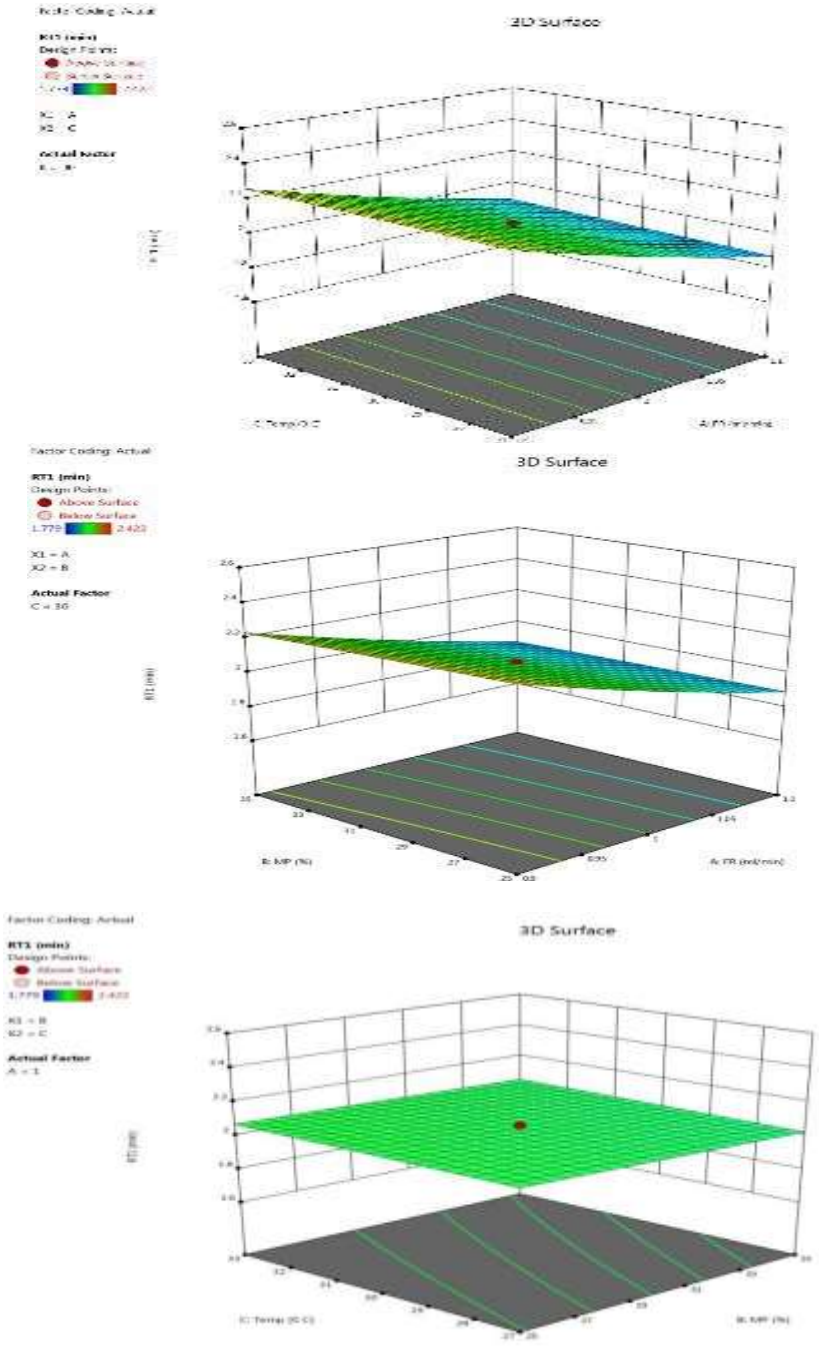


Fig 7.1.4: 3D contour plots of retention time as a function of Column temperature, Flow rate and organic **ratio.**

Response 2: Retention time of Metformin

Table 7.2.2: ANOVA table for Retention time using CCD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.6878	9	0.0764	595.60	< 0.0001	Significant
A-FR	0.6423	1	0.6423	5006.04	< 0.0001	
B-MP	0.0364	1	0.0364	284.04	< 0.0001	

C-Temp	0.0012	1	0.0012	9.43	0.0118	
AB	0.0005	1	0.0005	3.74	0.0817	
AC	0.0008	1	0.0008	6.55	0.0284	
BC	0.0000	1	0.0000	0.3156	0.5866	
A^2	0.0064	1	0.0064	49.52	< 0.0001	
B^2	0.0002	1	0.0002	1.24	0.2919	
C^2	2.192E-08	1	2.192E-08	0.0002	0.9898	
Residual	0.0013	10	0.0001			
Lack of Fit	0.0012	5	0.0002	11.98	0.0082	Significant
Pure Error	0.0001	5	0.0000			
Cor Total	0.6891	19				

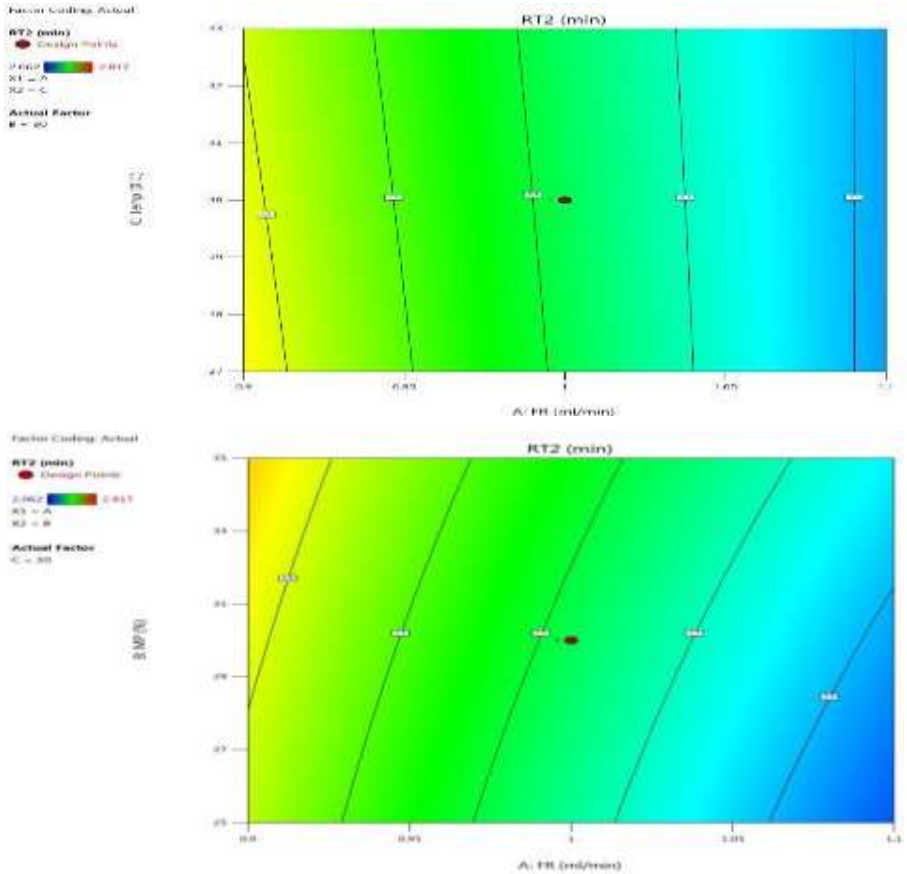
Factor coding id **coded**

Sum of squares is Type III - Partial

The **Model F-value** of 595.60 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AC, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 11.98 implies the Lack of Fit is significant. There is only a 0.82% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit.



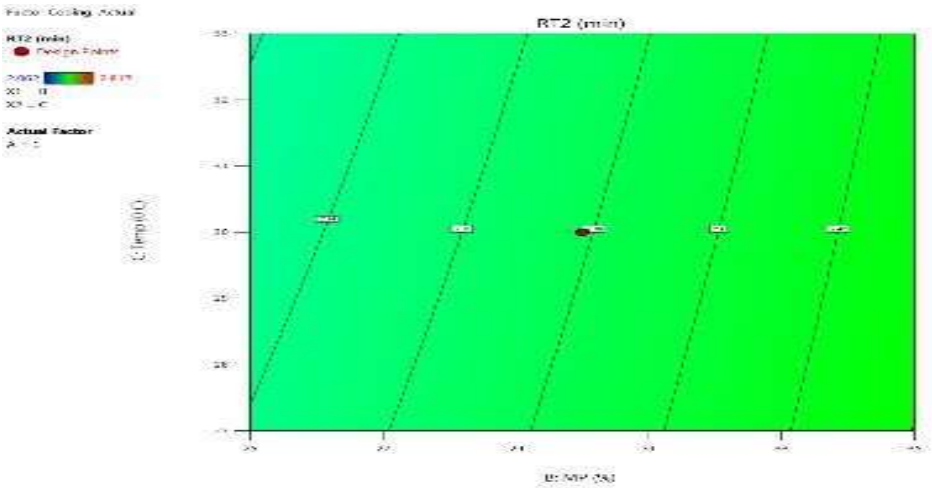
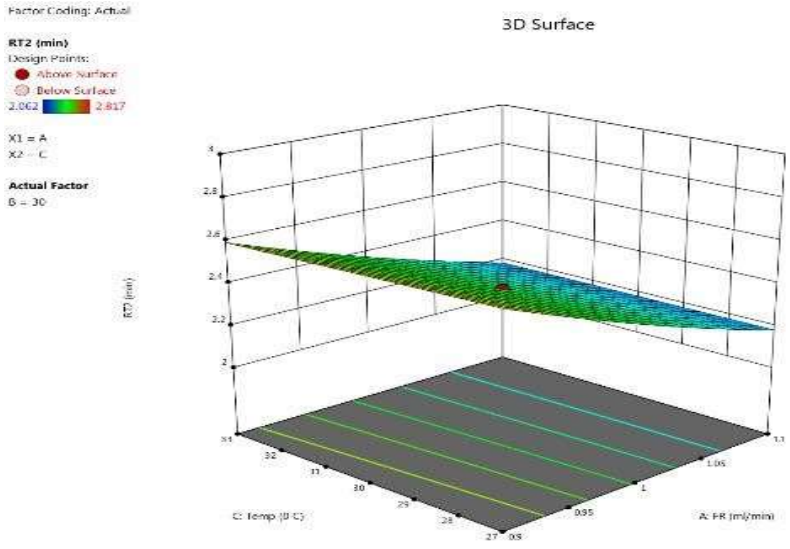
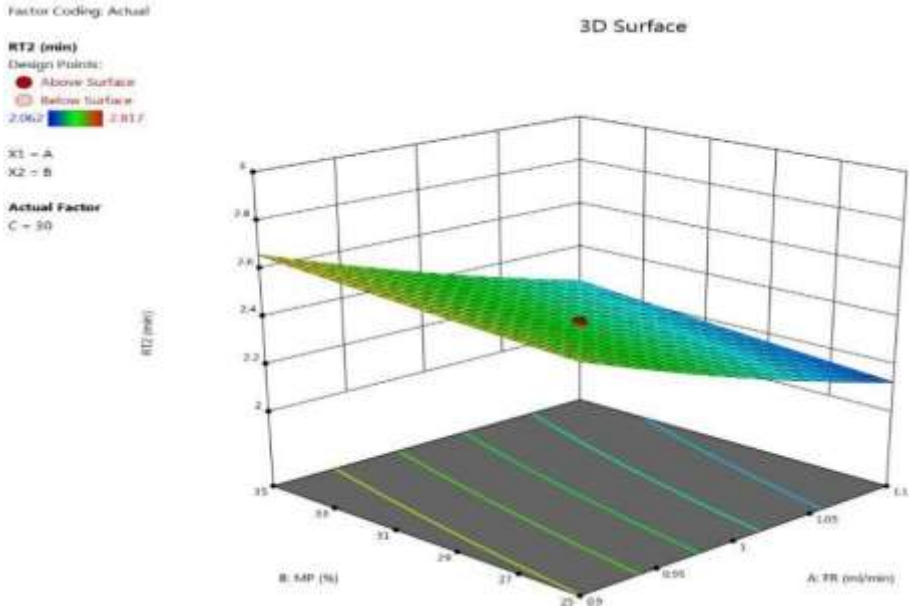


Fig 7.1.5 2D contour plots of Retention time as a function of Column temperature, Flow rate and organic ratio.





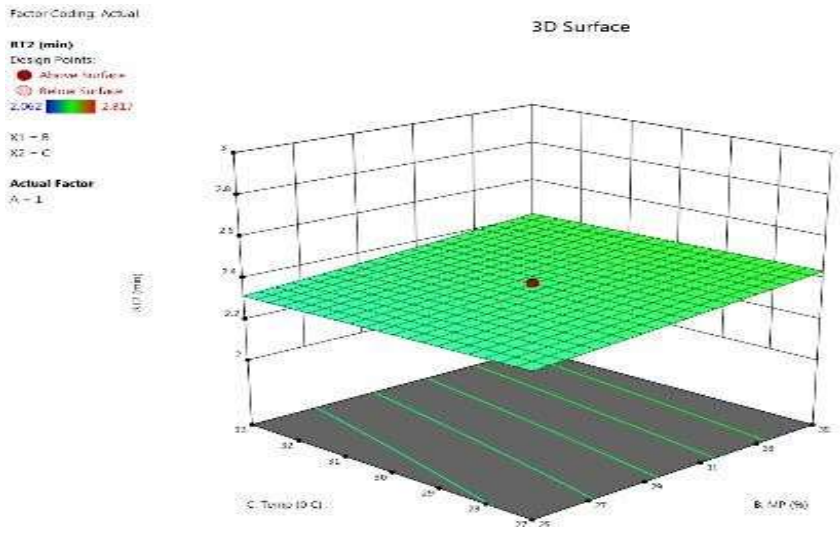
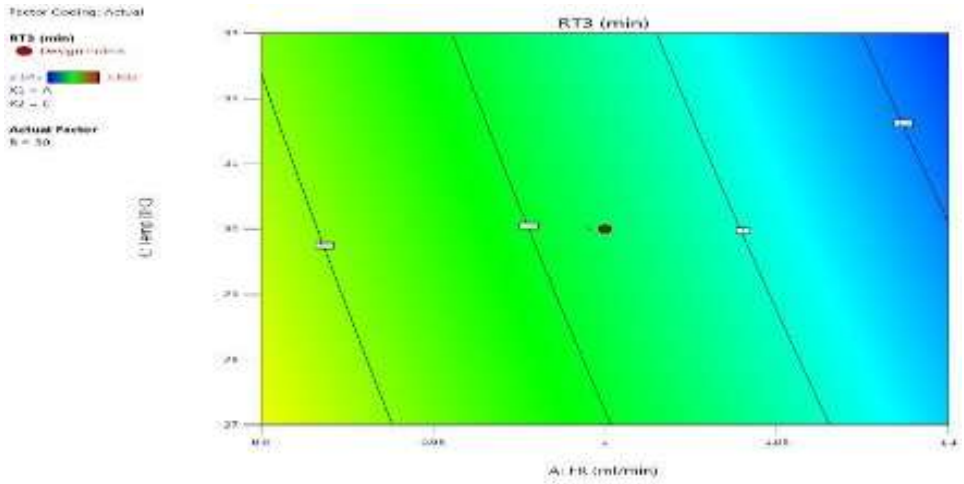


Fig 7.1.6: 3D contour plots of Retention time as a function of Column temperature, Flow rate and organic ratio.

Response 3: Retention time of Dapagliflozin

Table 7.2.3: ANOVA table for Retention time using CCD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.03	6	0.3382	125.16	< 0.0001	Significant
A-FR	1.44	1	1.44	534.43	< 0.0001	
B-MP	0.4413	1	0.4413	163.32	< 0.0001	
C-Temp	0.0822	1	0.0822	30.43	< 0.0001	
AB	0.0348	1	0.0348	12.90	0.0033	
AC	0.0009	1	0.0009	0.3421	0.5686	
BC	0.0258	1	0.0258	9.53	0.0086	
Residual	0.0351	13	0.0027			
Lack of Fit	0.0347	8	0.0043	50.49	0.0002	Significant
Pure Error	0.0004	5	0.0001			
Cor Total	2.06	19				



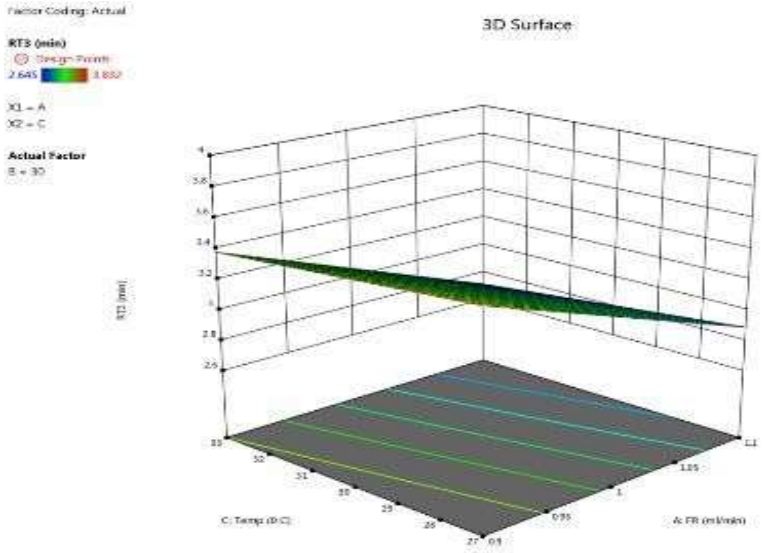
Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 125.16 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 50.49 implies the Lack of Fit is significant. There is only a 0.02% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit.



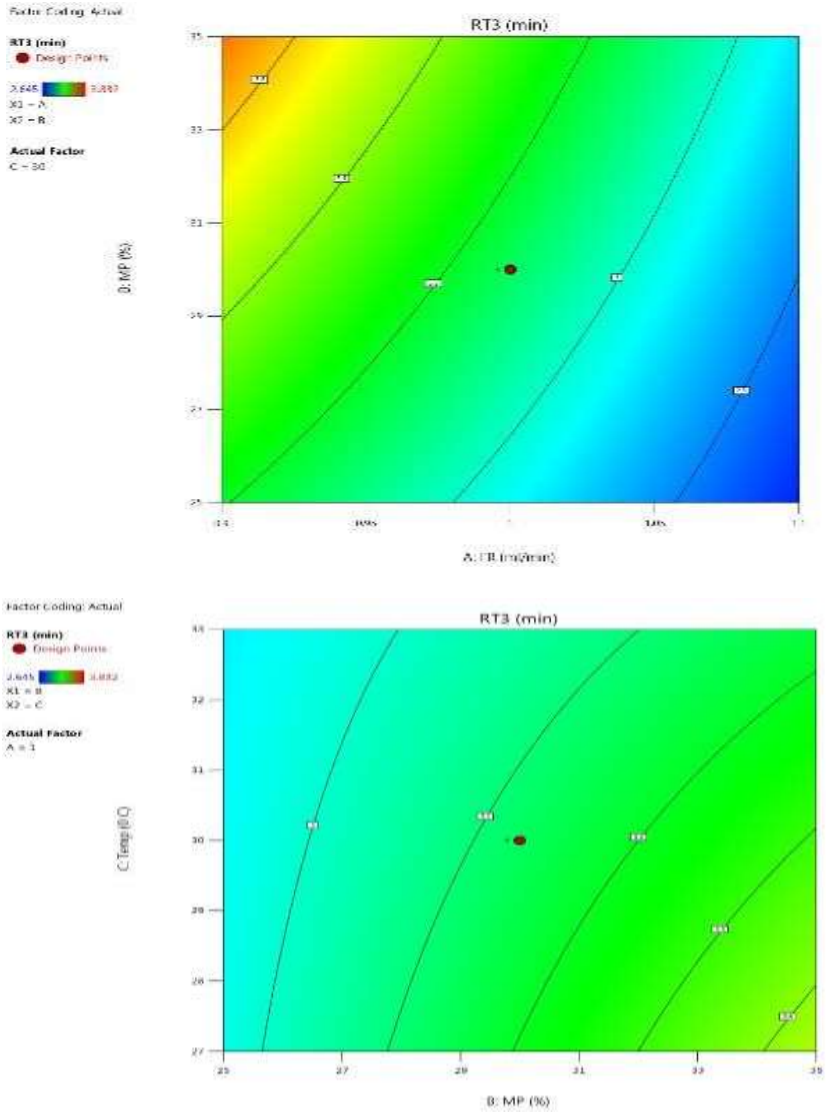


Fig 7.1.7: 2D contour plots of Theoretical plates as a function of Column temperature, Flow rate and organic ratio.

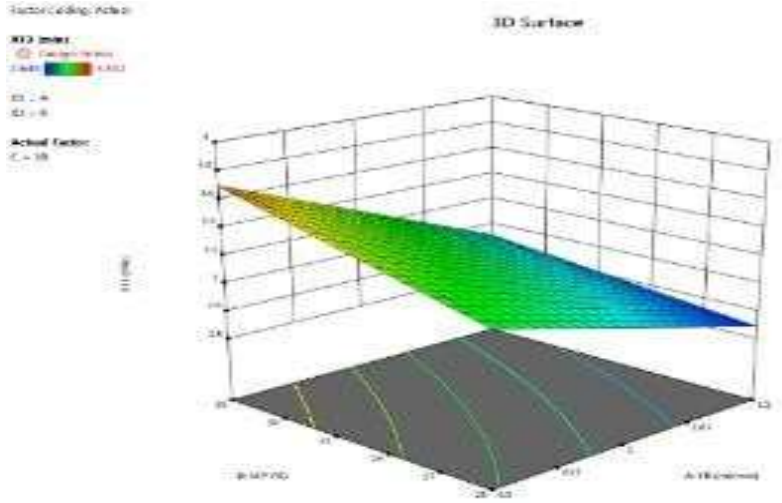


Fig 7.1.8 3D contour plots of Theoretical plates as a function of Column temperature, Flow rate and organic ratio.

Resolution of Metformin

Table 7.2.4: ANOVA table for Resolution using CCD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.23	6	1.54	27.01	< 0.0001	Significant
A-FR	0.5483	1	0.5483	9.63	0.0084	
B-MP	6.39	1	6.39	112.22	< 0.0001	
C-Temp	1.47	1	1.47	25.83	0.0002	
AB	0.3200	1	0.3200	5.62	0.0339	
AC	0.1800	1	0.1800	3.16	0.0988	
BC	0.3200	1	0.3200	5.62	0.0339	
Residual	0.7401	13	0.0569			
Lack of Fit	0.7251	8	0.0906	30.21	0.0008	Significant
Pure Error	0.0150	5	0.0030			
Cor Total	9.97	19				

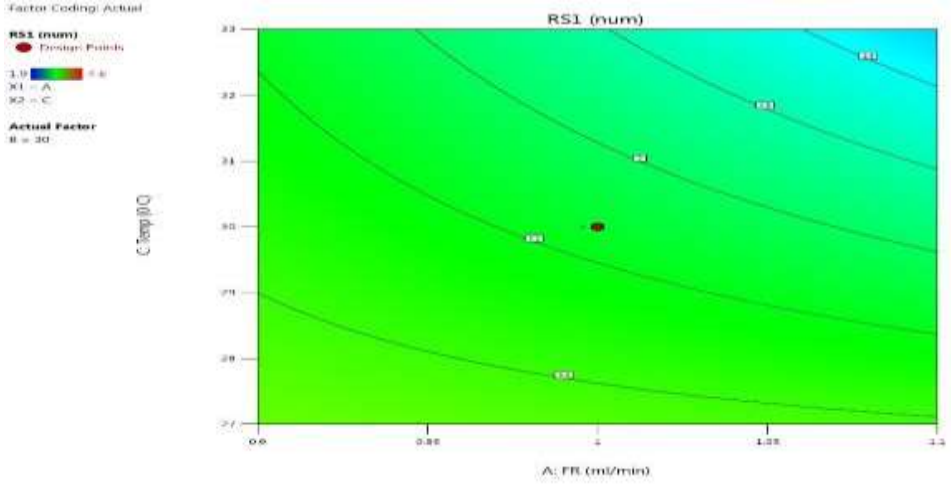
Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 27.01 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 30.21 implies the Lack of Fit is significant. There is only a 0.08% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad – we want the model to fit.



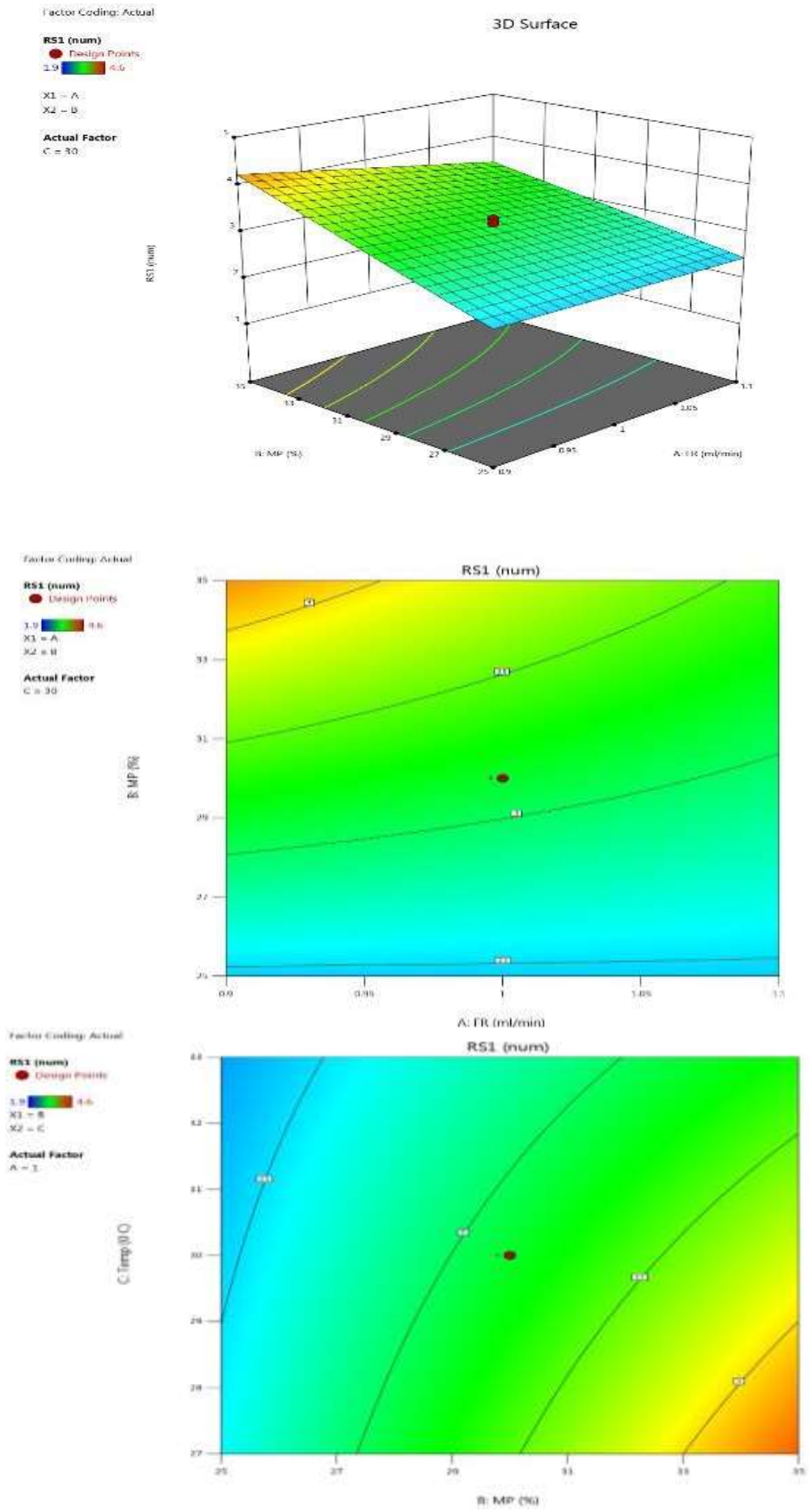


Fig 7.1.9: 2D contour plots of Resolution as a function of Column temperature, Flow rate and organic ratio.

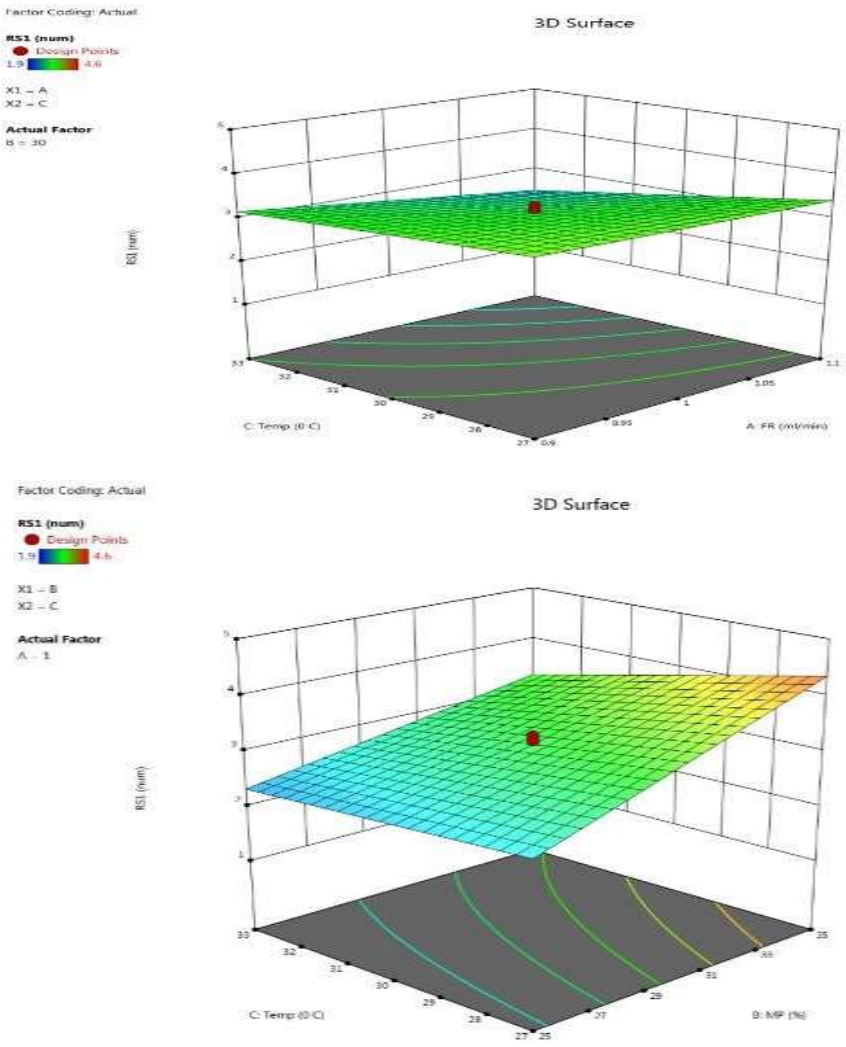


Fig 7.1.10 3D contour plots of Resolution as a function of Column temperature, Flow rate and organic ratio.

Response 5: Resolution of Dapagliflozin

Table 7.2.5: ANOVA table for Resolution using CCD

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	14.90	6	2.48	113.44	< 0.0001	significant
A-FR	2.86	1	2.86	130.65	< 0.0001	
B-MP	8.93	1	8.93	408.06	< 0.0001	
C-Temp	1.72	1	1.72	78.68	< 0.0001	
AB	1.12	1	1.12	51.39	< 0.0001	
AC	0.0800	1	0.0800	3.65	0.0782	
BC	0.1800	1	0.1800	8.22	0.0132	
Residual	0.2846	13	0.0219			
Lack of Fit	0.2763	8	0.0345	20.72	0.0020	Significant

Pure Error	0.0083	5	0.0017
Cor Total	15.19	19	

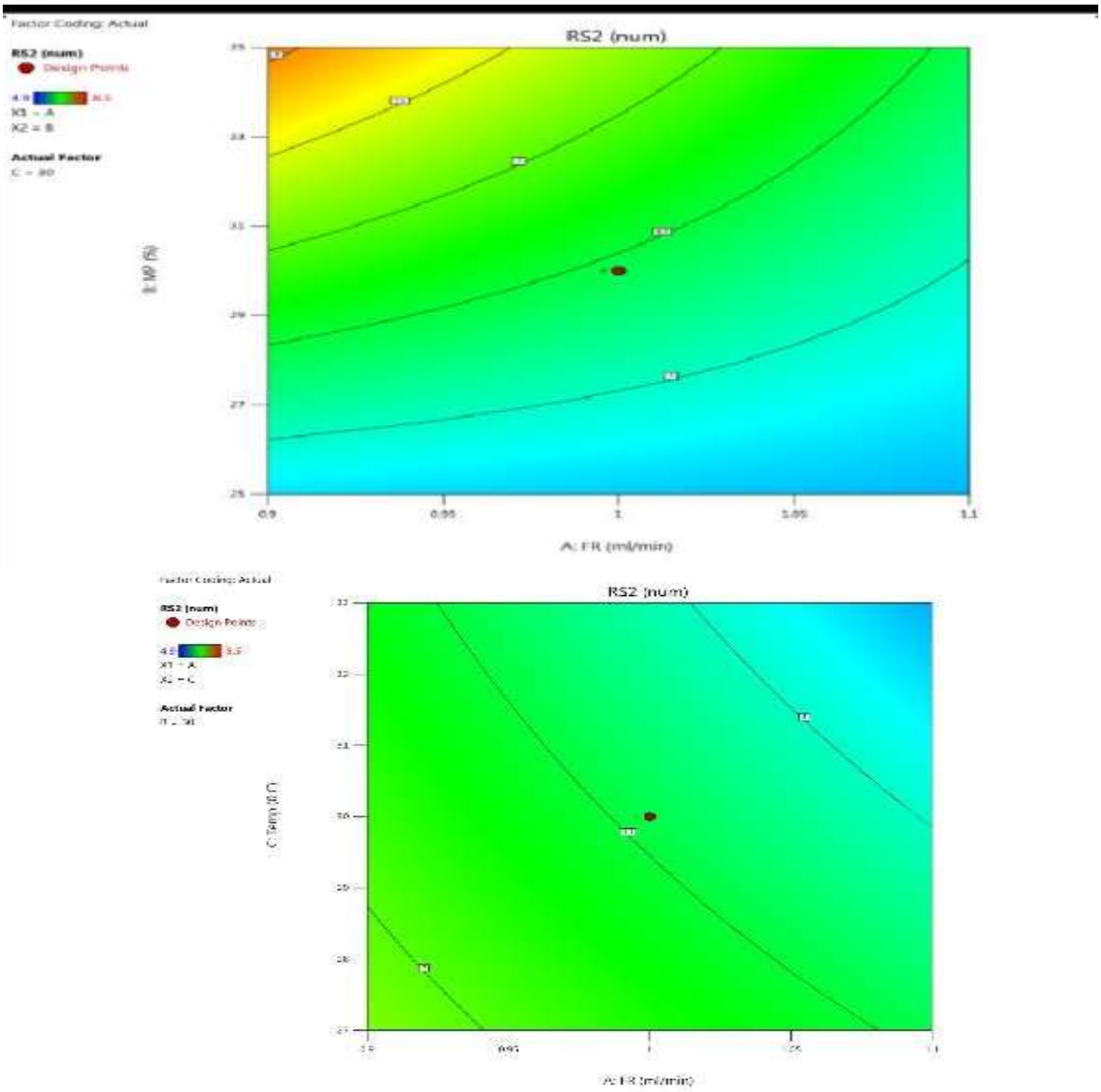
Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 113.44 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 20.72 implies the Lack of Fit is significant. There is only a 0.20% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit.



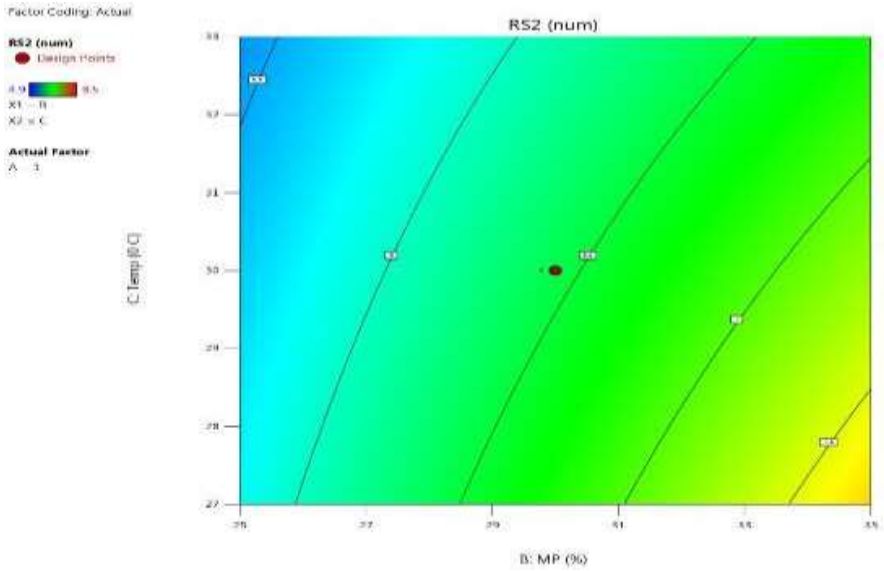
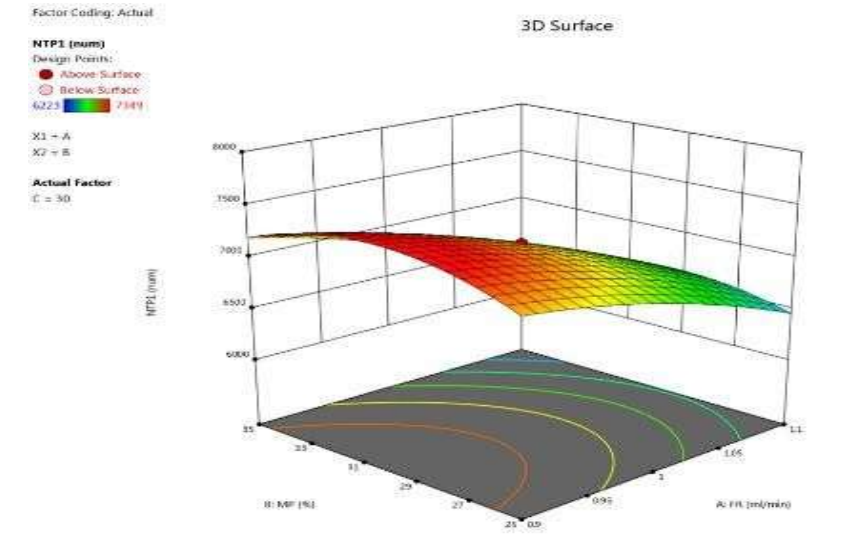


Fig 7.1.11 2D contour plots of Resolution as a function of Column temperature, Flow rate



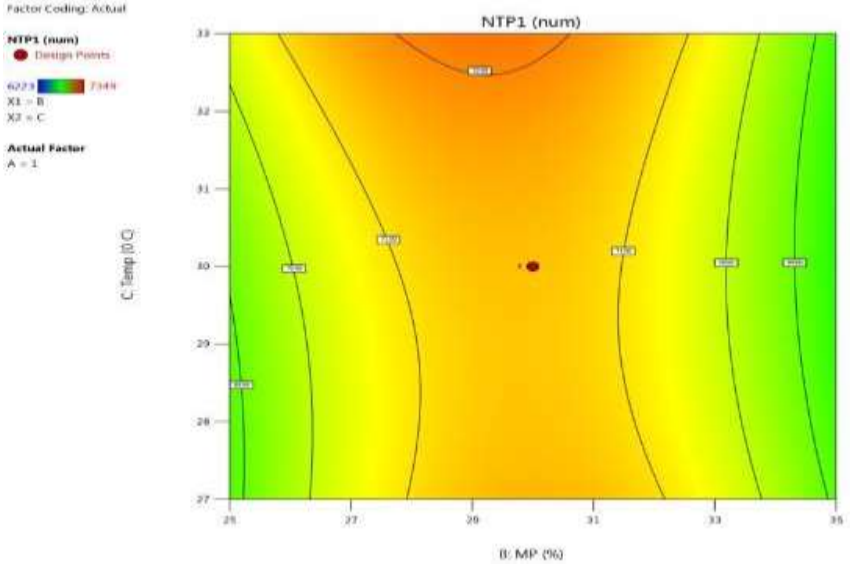


Fig 7.1. 13: 2D contour plots of asymmetry as a function of Column temperature, Flow rate and organic ratio.

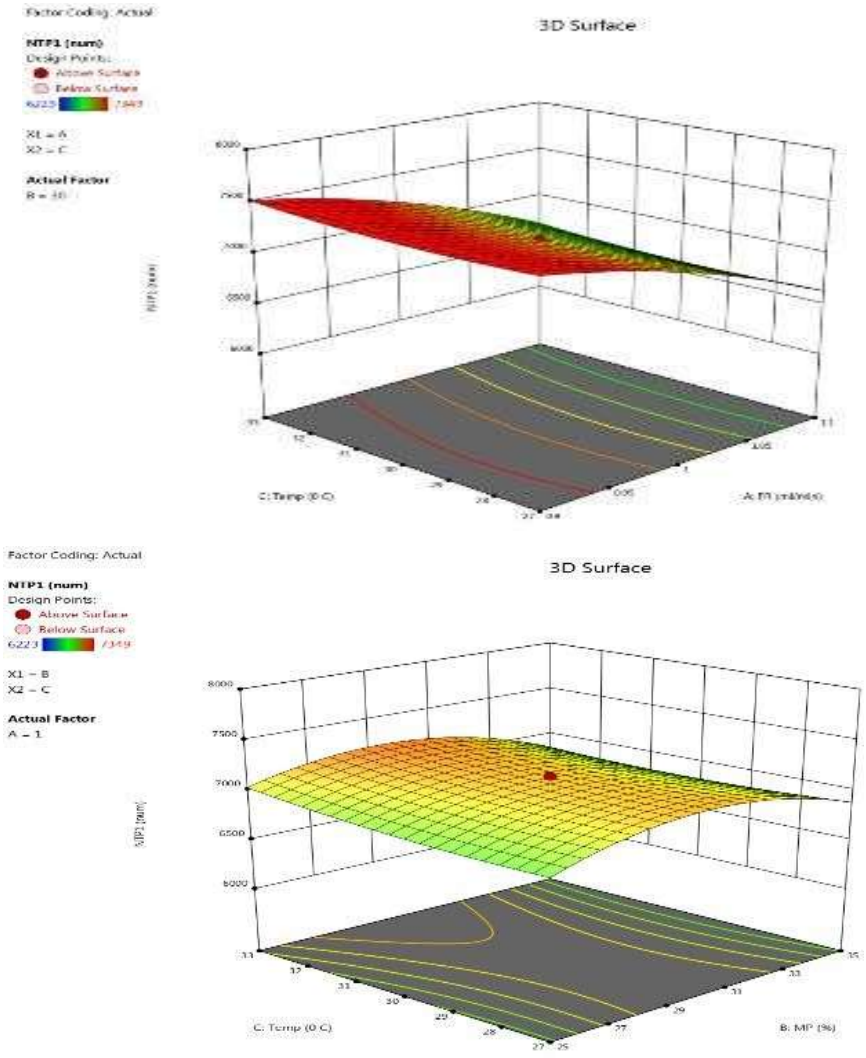


Fig 7.1.14: 3D contour plots of asymmetry as a function of Column temperature, Flow rate and organic ratio.

Response 7: Theoretical plate count

ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5.243E+06	9	5.826E+05	62.40	< 0.0001	Significant
A-FR	4.573E+06	1	4.57	489.83	< 0.0001	
B-MP	11171.21	1	11171.21	1.20	0.2997	
C-Temp	44522.32	1	44522.32	4.77	0.0539	
AB	1.306E+05	1	1.306E+05	13.98	0.0038	
AC	2.067E+05	1	2.067E+05	22.14	0.0008	
BC	46512.50	1	46512.50	4.98	0.0497	
A^2	66555.82	1	66555.82	7.13	0.0235	

B^2	1.395E+05	1	1.395E+05	14.94	0.0031	
C^2	4199.94	1	4199.94	0.4498	0.5176	
Residual	93365.58	10	9336.56			
Lack of Fit	88220.24	5	17644.05	17.15	0.0036	significant
Pure Error	5145.33	5	1029.07			
Cor Total	5.337E+06	19				

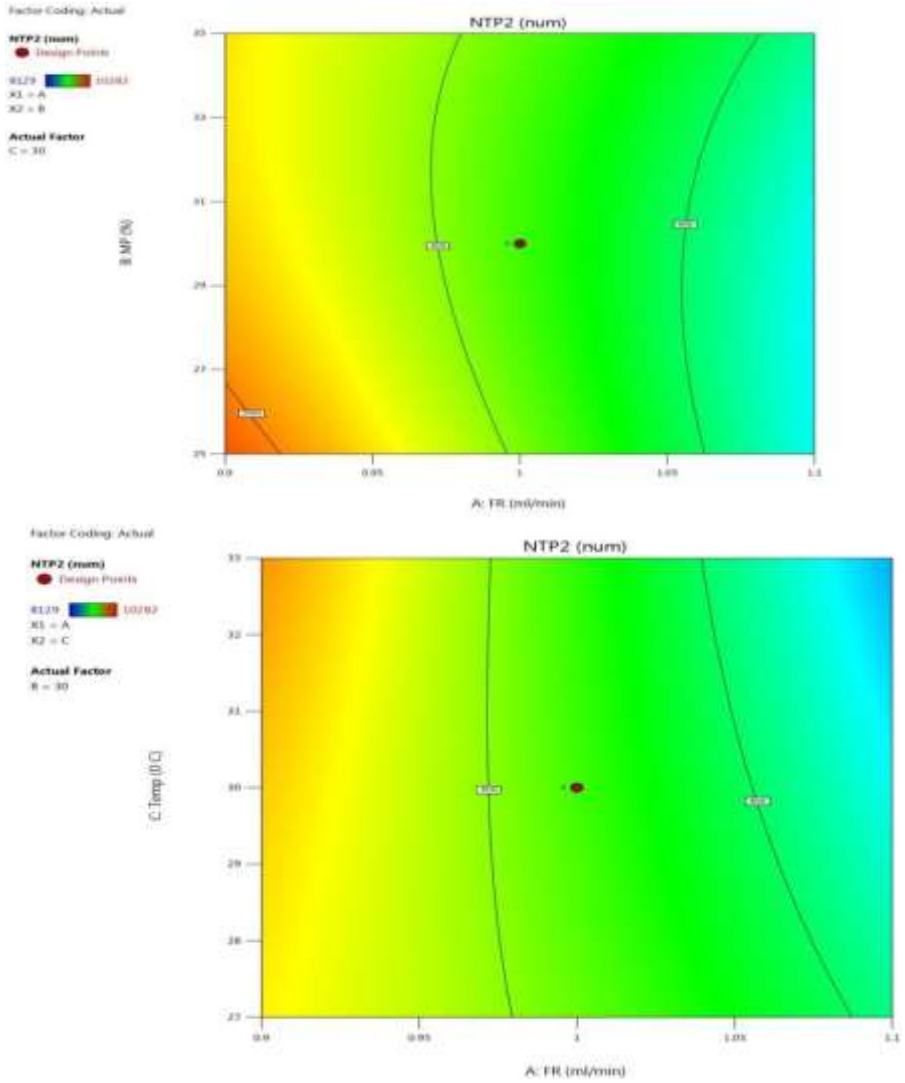
Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 62.40 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, AB, AC, BC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 17.15 implies the Lack of Fit is significant. There is only a 0.36% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit.



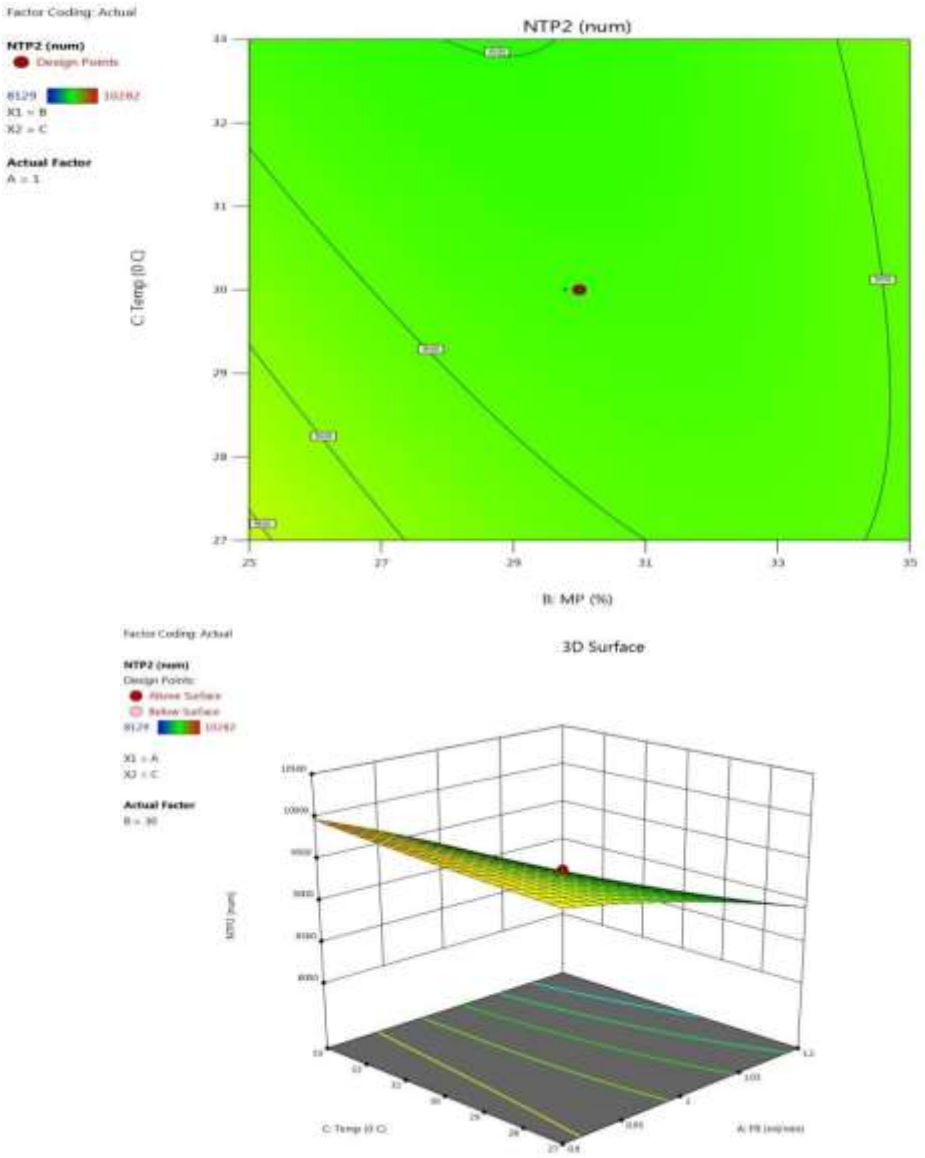
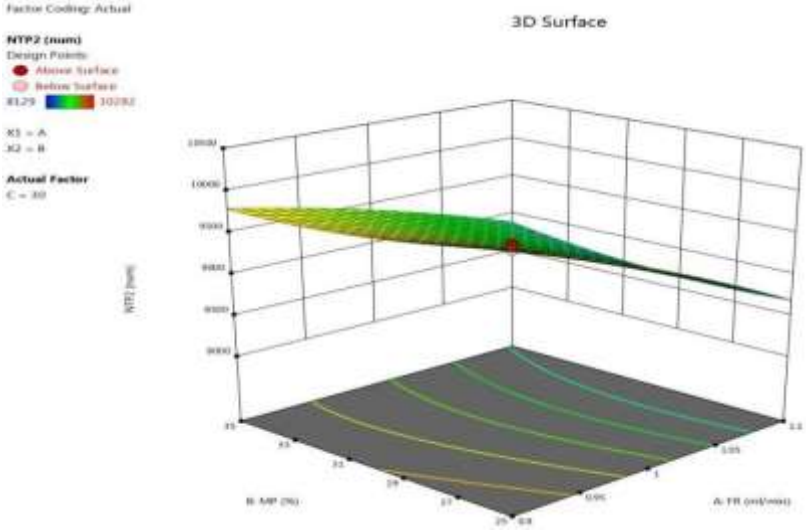


Fig 7.1.15 2D contour plots of asymmetry as a function of Column temperature, Flow rate and organic ratio.



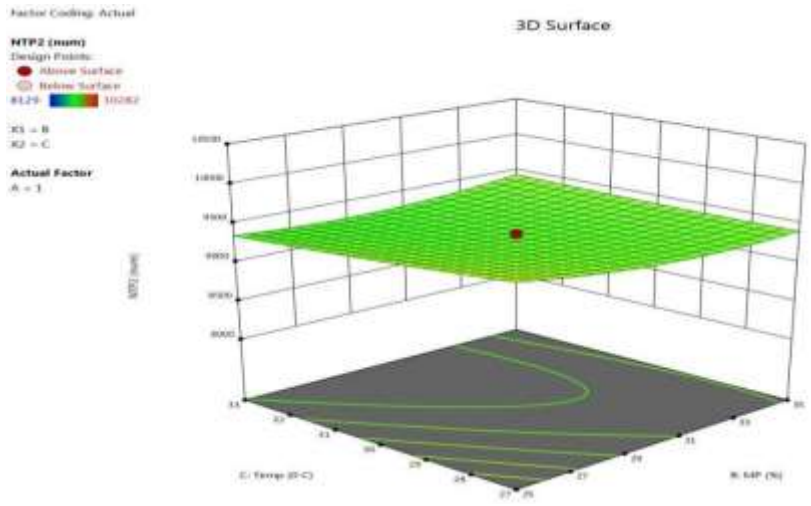


Fig 7.1.16 3D contour plots of asymmetry as a function of Column temperature, Flow rate and organic ratio

Response 8:Theoritical plate count

SUMMARY AND CONCLUSION

METHOD DEVELOPMENT:

The analytical method was developed by investigating various parameters to optimize the performance. A common wave length of 220nm was selected to ensure excellent peak purity. An injection volume of 20ul was chosen to achieve a desirable peak area. The SunFire C18 Column 3.5 µm, 4.6 mm X 250 mm was utilized for its ability to provide satisfactory peak shape. A flow rate of 1.05ml/min was fixed to obtain optimal peak area, retention time, and resolution. After studying the mobile phase ratio, a composition of 0.01N KH_2PO_4 : Acetonitrile (67:33 v/v) was deemed suitable due to its ability to generate symmetrical peaks and good resolution, making it the preferred mobile phase for proposed method

METHOD OPTIMISATION:

The method was optimized using central composite design (CCD). The initial trials are needed to optimize the final method. Three factors viz; % Organic concentration, Flow rate and column temperature were needed to be optimized. So CCD was used to optimize these parameters which were varied over three levels (high, mid and low). different ranges of three parameters ranging from 21.5-38.41%, 0.01N potassium dihydrogen orthophosphate, column temperature 24.9 and 35.0^0C and 0.83-1.17ml/min flow rate respectively were taken and contour and 3D contour anSdur3fDace plot showing the effect of each parameter on Retention Time, Area, Theoretical plates and Asymmetry (CQA) were generated. A desirability function was applied to the optimized conditions to predict retention time, asymmetry, theoretical plates and peak area.

Conclusion

A simple, accurate, precise method was developed for the simultaneous estimation of the Metformin, , Dapagliflozin and Vildagliptin in Tablet dosage form. Retention time of Metformin, Dapagliflozin and Vildagliptin were found to be 2.181 min, 2.561 min and 2.981 min. %RSD of system precision for Metformin, Dapagliflozin and Vildagliptin were and found to be 0.5, 0.9 and 0.6 respectively. %RSD of method precision for Metformin, Dapagliflozin and Vildagliptin were and found to be 0.7, 0.6 and 0.2 respectively. % recovery was obtained as 99.95%, 99.65% and 99.71% for Metformin, Dapagliflozin and Vildagliptin respectively. LOD values are obtained from regression equations of Metformin, Dapagliflozin and Vildagliptin were 0.52ppm, 0.018ppm, 0.12pm and LOQ values are obtained from regression equations of Metformin, Dapagliflozin and Vildagliptin were 1.58ppm, 0.053ppm ,0.36ppm respectively. Regression equation of Metformin was y = 10407x + 1862.5. Dapagliflozin was y = 51736x + 319.29. and of Vildagliptin was y = 11818x + 417.71 Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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