Advances in Bioresearch
Adv. Biores., Vol 1 (1) January 2025: 122-131

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.1.92102

Advances in Bioresearch

ORIGINAL ARTICLE

QbD Approach to Validated Stability Indicating RP-HPLC Method Development of Umifenovir

[1]Namita A. Joshi, [2]Sunil V. Amrutkar, [3]Chandrashekhar D. Upasani
[1,3] SNJB's Shriman Sureshdada Jain College of Pharmacy, Chandwad 423101, Maharashtra, India.
[2]Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nasik 422005, Maharashtra, India.
Correspondence author's email: joshinamitaa@gmail.com

ABSTRACT
The QbD paradigm was employed to develop a selective stability-indicating analytical LC method for estimation of Umifenovir in bulk and dosage form. From the literature it was evident that the QbD technique was not used for analysis of Umifenovir. HPLC method was developed as per Box-Behnken experimental design involving statistics supported by Design Expert software. Critical method parameters were identified from risk assessment and varied. The 3D response graphs were used to study the impact of interaction of process variables on the key quality characteristics. Statistical values and plots were used to decipher the optimum LC separation conditions aided by statistical software. The scientific QbD approach allows risk assessment and enables creation of a design space, thereby making the method highly robust unlike the conventional method development. Chromatographic system comprised of Cosmosil C-18 column, simple mobile phase composition consisting of methanol:water at acidic pH and UV detection at wavelength 258 nm. Validation and successful application of the method on dosage form and stress degradation studies was performed. The analytical QbD method provides scope for perpetual method improvement through a lifecycle approach.
Keywords: *Box-Behnken design, Forced degradation, Quality-by-design, Umifenovir, Validation.*

Received 21.11.2024 Revised 29.12.2024 Accepted 12.01.2025

How to cite this article:
Namita A. J, Sunil V. A, Chandrashekhar D. U. QbD Approach to Validated Stability Indicating RP-HPLC Method Development of Umifenovir. Adv. Biores. Vol 16 [1] January 2025. 122-131

INTRODUCTION

Quality-by-design is a scientific approach to method development encompassing critical method parameters, risk identification and generates a robust method operable design region or a design space with the goal of quality enhancement [1]. The term Analytical Quality-by-Design or AQbD is used in the context of analysis [2-5]. Since AQbD bestows enormous advantages of design space, easy regulatory approvals and constant method monitoring till the product lifecycle; it has drawn attention of pharmaceutical industries. Methods developed by conventional trial and error one-factor-at-a-time (OFAT) approach are less robust, time consuming, require lot of experimental trials and fail during method transfer [6-9].

The world witnessed devastation of lives with the advent of Covid-19 virus pandemic [10]. Antiviral agents were being sought globally against Corona. Umifenovir (UMF), was one such agent used for prevention as well as therapy of influenza, and tried against Corona. Chemically, UMF is 6-bromo-4-[(dimethylamino)methyl]-5-hydroxy-1-methyl-2-[(phenylthio)methyl]-1H-indole-3-carboxylic acid, ethyl ester (fig.1). It is white amorphous powder, soluble in ethanol and slightly soluble in water. It's pKa values are 6.01 and 9.87. Umifenovir combines with influenza viral surface glycoprotein namely haemagglutinin. This inhibits its binding to sialic acid, thereby preventing its entry into the host [11].

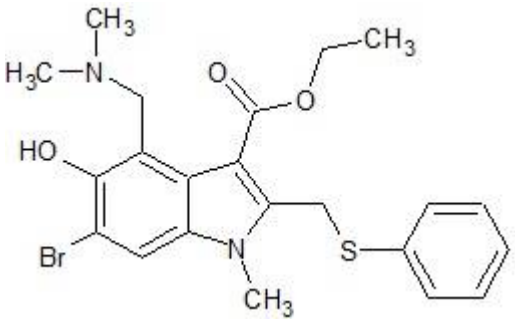


Fig. 1: Structure of Umifenovir

In the pharmaceutical companies, reverse-phase High Performance Liquid Chromatography (RP-HPLC) is a highly popular analytical technique. Its quality is greatly enhanced by QbD. Literature reveals very few analytical HPLC methods for estimation of Umifenovir [12-15]. Analytical HPLC methods utilizing the AQbD technique are not available for the estimation of Umifenovir. Reported methods also use quite complicated, costly and non-eco-friendly mobile phase compositions. Recently, the design of experiment (DoE) approaches have been used efficiently for development of cost-effective liquid chromatographic methods for drug analysis [16].

Stability study is an indispensable portion of drug development. The degradation products and reduction of drug content, over the shelf life of drug product can be determined from SIAM or stability-indicating assay methods [17]. There are not many analytical HPLC-SIAM methods for determination of UMF and there is no complete assessment of drug stability covering all its factors [18, 19]. Moreover, existing methods do not focus on evaluation of risk, robustness and DoE approach. Therefore, the goal of present study was to establish a quick, economic RP-HPLC method incorporating stability and high on robustness, for determination of UMF in drug and dosage form via AQbD technique.

MATERIAL AND METHODS

Glenmark Pharmaceuticals Ltd. Provided the gift sample of Umifenovir drug. Chemical requirements for analysis were AR grade and solvents HPLC grade.

Equipment:

HPLC instrument consisted of Analytical Technologies P-3000-M reciprocating pump and UV-3000-M detector. Refer table 6 for chromatographic details.

Standard UMF solution:

Umifenovir 25 mg was weighed into a 25 ml volumetric flask to produce a 1000 ppm standard stock solution. Mobile phase/diluent was used to make up the volume. From this stock solution, a working standard of 100 ppm was prepared.

AQbD mediated HPLC method development

Initially, the analytical target profile (ATP) was defined as HPLC method development using AQbD technique, determination of Umifenovir, method validation and stress studies for assessment of stability [20]. The critical method parameters of flow rate, ratio of mobile phase composition, pH, tailing factor, number of theoretical plates (TPN), peak area, and retention time (R_t) were identified from risk assessment [21].

Design of experiment and MODR

The λ_{max} of Umifenovir was found by performing UV scan of standard solution from 200–400 nm. Statistical model selected for the design of experiment was Box-Behnken design (BBD). It requires selection and optimization of its factors (critical process parameters, CPP) from the corresponding responses for critical quality attributes (CQA). Table 1 shows low, centre and high levels for BBD and table 2 shows DoE for BBD. The desirability function of Design Expert (free version 10) software enables selection of final chromatographic conditions. Software also generates a method operable design region (MODR, synonymous with design space), which is a region that provides flexible movement for the method parameters confined in the space. No regulatory approval is required for movement in the approved design space, making the enhanced AQbD approach robust in the real sense. Refer fig. 6. The QbD technique also provides a lifecycle approach for the product favouring continuous method monitoring and improvement based on scientific knowledge, unlike the conventional method development approach [22].

Validation of AQbD Method

According to ICH Q2 (R1) requirements, the AQbD technique was verified on a number of factors listed in table 5 for reliability of the method. [23].

Assay

Total 20 tablets of umifenovir were weighed and finely powdered. The powder equivalent to 50 mg was extracted using the eluent, followed by sonication and filtration. A 30 ppm standard and sample solution each were prepared for assay.

Forced degradation

A 50 ppm standard was subjected to individual treatments of hydrolysis with acid/base, oxidation, thermal and UV radiation followed by introduction into the LC system to evaluate the degradation.

RESULTS AND DISCUSSION

AQbD Method Optimization

Initial experiments to optimize the percent eluent composition and flow rate consisted of variations in methanol: water at 80:20, 70:30, 60:40, 65:35 at 0.8 and 0.9 ml/min. Simple mobile phase was employed for generation of a cost-effective method.

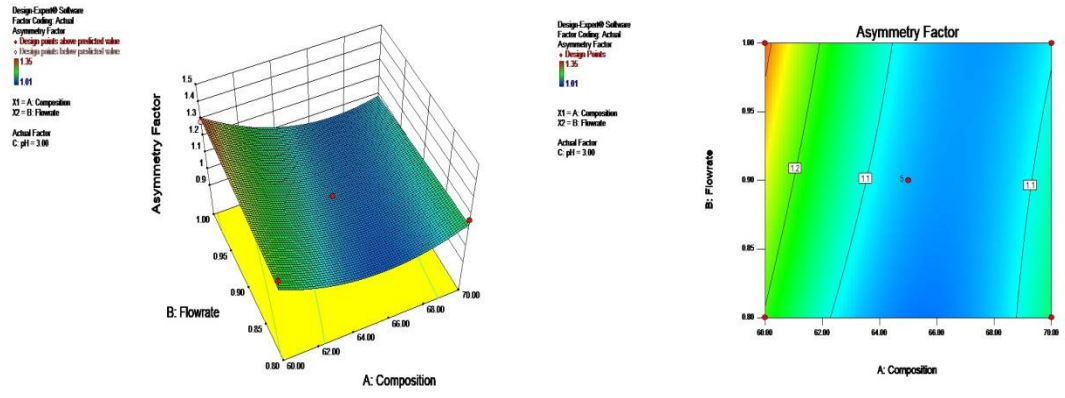
Statistical analysis

The responses were generated using BBD multi-variable quadratic response surface model. The data was statistically analyzed by analysis of variance (ANOVA) to check how the factors and their interactions affected peak tailing. The ratio of mobile phase composition, flow rate and pH were independent factors chosen for the BBD model. Table 3 shows the statistical values generated by the software.

The parameters % composition, pH, (% composition x pH) and (% composition x % composition) all had p-values < 0.05 and model F-value (6.0585) implied significant for optimization. It is possible to predict a positive correlation between these variables and how their interactions affect peak asymmetry. “Adeq Precision” indicates signal to noise (S/N) ratio. S/N value above 4 is recommended. A sufficient signal was suggested by S/N ratio of 7.476. The equation shows that composition and pH had a negative effect, whereas flowrate had a positive effect on Asymmetry factor of UMF. Interaction of composition and flowrate, flowrate and pH, and flowrate^2 had a negative effect, whereas interaction of composition and pH, composition^2, and pH^2 had a positive effect on the asymmetry factor (Refer table 4). These values indicated that the model suited for optimization.

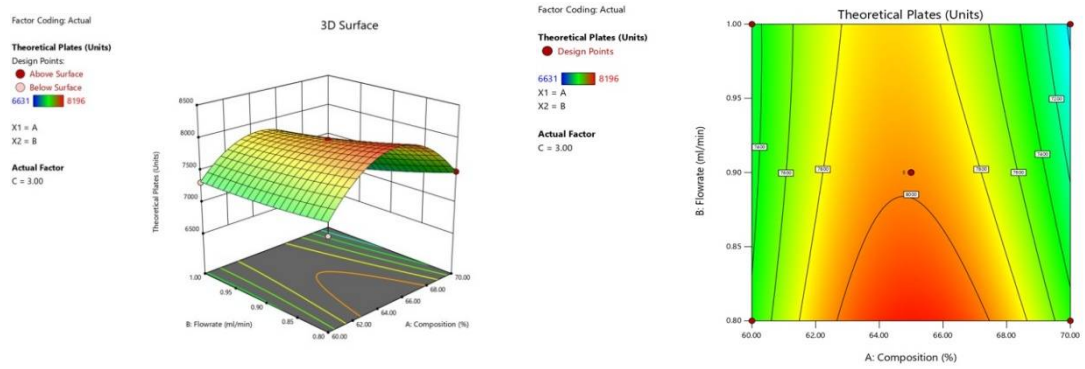
The contour and 3D plots (fig. 2-5) serve as additional tools for studying how the factors and their interactions affected the responses; as well as for creation of MODR or design space to generate a robust analytical QbD method.

The software provided a high desirability value for the set of chromatographic conditions shown in table 5. Thus, these were chosen as optimized conditions for the LC method by AQbD technique. Fig. 7 shows a representative chromatogram for the optimized chromatographic conditions.



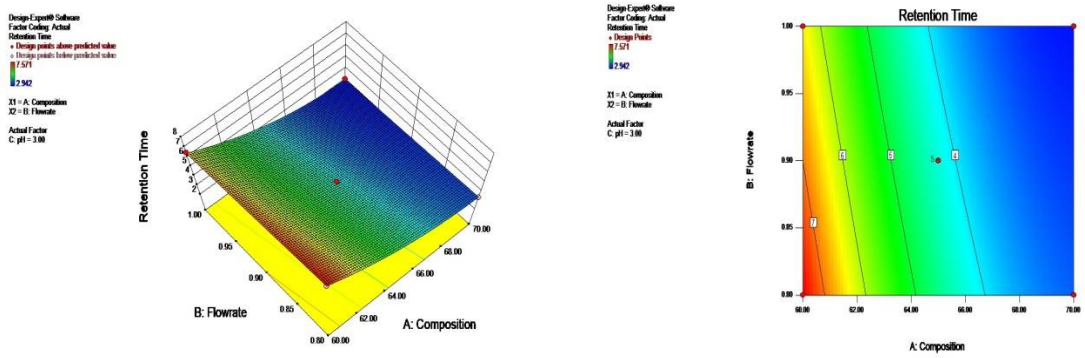
a b

Fig. 2(a) 3D Response surface plot (b) Contour plot for peak asymmetry response as a function of composition and flow rate (constant pH 3.0).



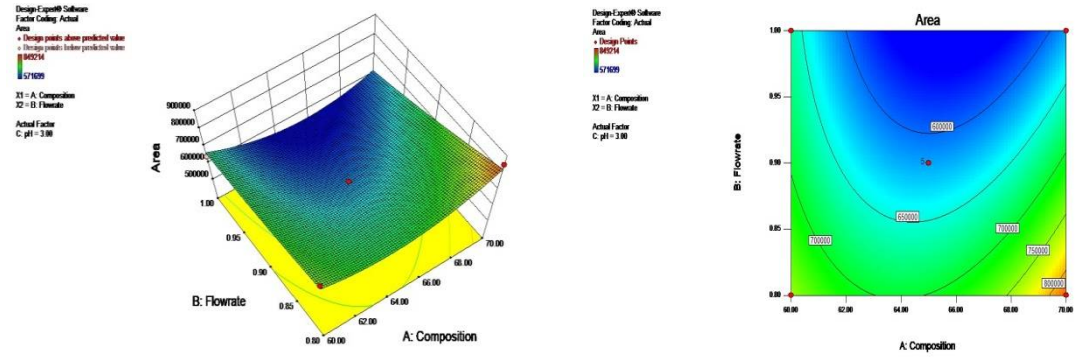
a b

Fig. 3(a) 3D Response surface plot (b) Contour plot for TPN response as a function of composition and flow rate (constant pH 3.0).



a b

Fig. 4(a) 3D Response surface plot (b) Contour plot for R_t response as a function of composition and flow rate (constant pH 3.0).



a b

Fig. 5(a) 3D Response surface plot (b) Contour plot for area response as a function of composition and flow rate (constant pH 3.0).

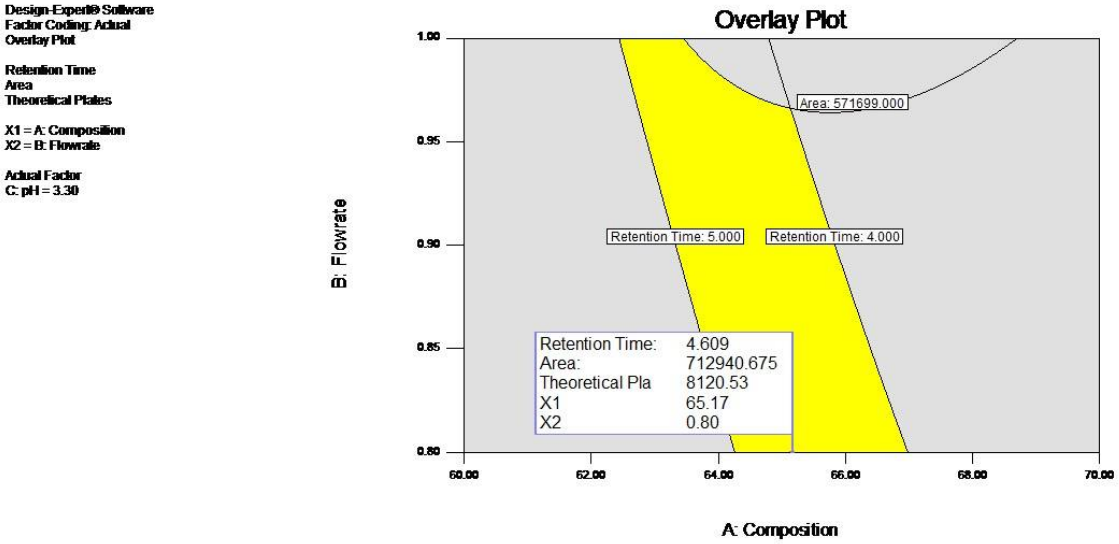


Fig.6 MODR/Design space for UMF

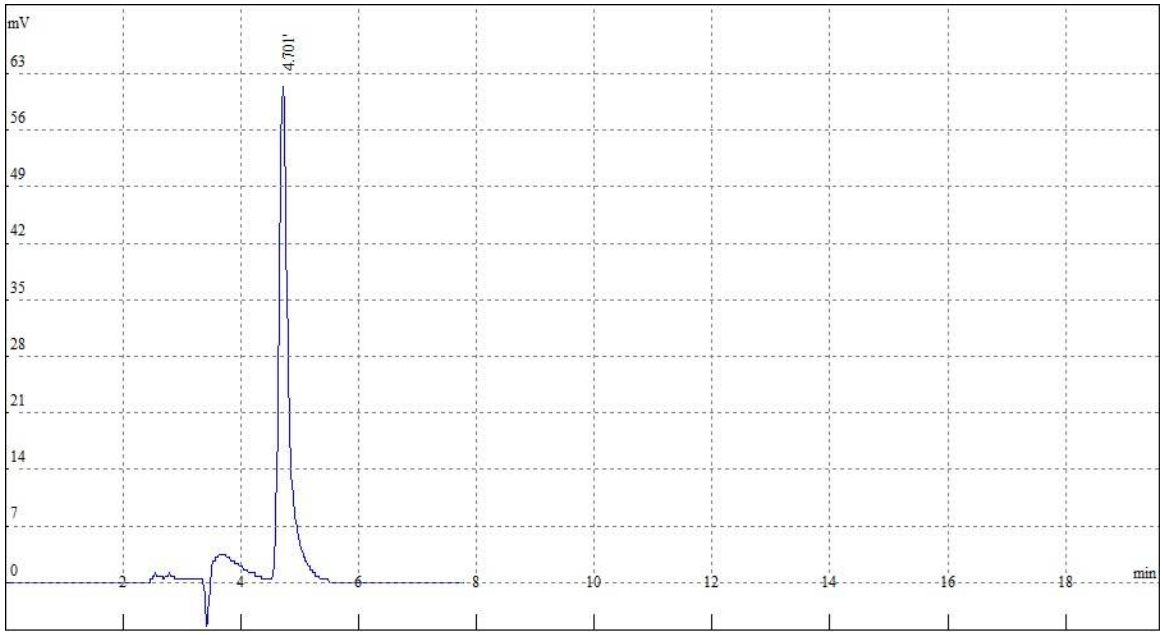


Fig. 7: Representative chromatogram for optimized chromatographic conditions

Method Validation

Linear relationship for UMF was perfectly achieved with the correlation coefficient r^2 close to 1 for the given concentration range (refer fig. 8 and table 6). For testing intraday precision, concentration of 30 ppm standard solution was injected three times each in the morning and evening each; whereas the solution was injected thrice on two different days for interday precision. Accuracy was calculated by percent recovery of known added amount of standard to the sample at 80%, 100% and 120%. Three readings were taken at each level. Robustness involved deliberately making minor alterations to parameters of mobile phase pH and detection wavelength. Percent RSD < 2% assured precision, accuracy and reliability of the method. Limit of detection (LOD) and limit of quantitation (LOQ) were computed from the formulae, LOD = 3.3 x standard deviation/slope and LOQ = 10 x standard deviation/slope.

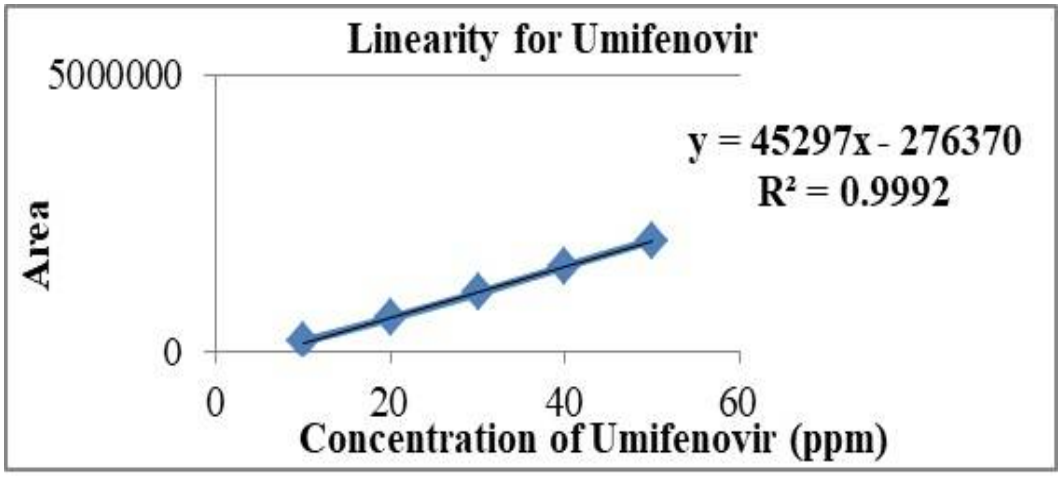


Fig. 8 Linearity for Umifenovir

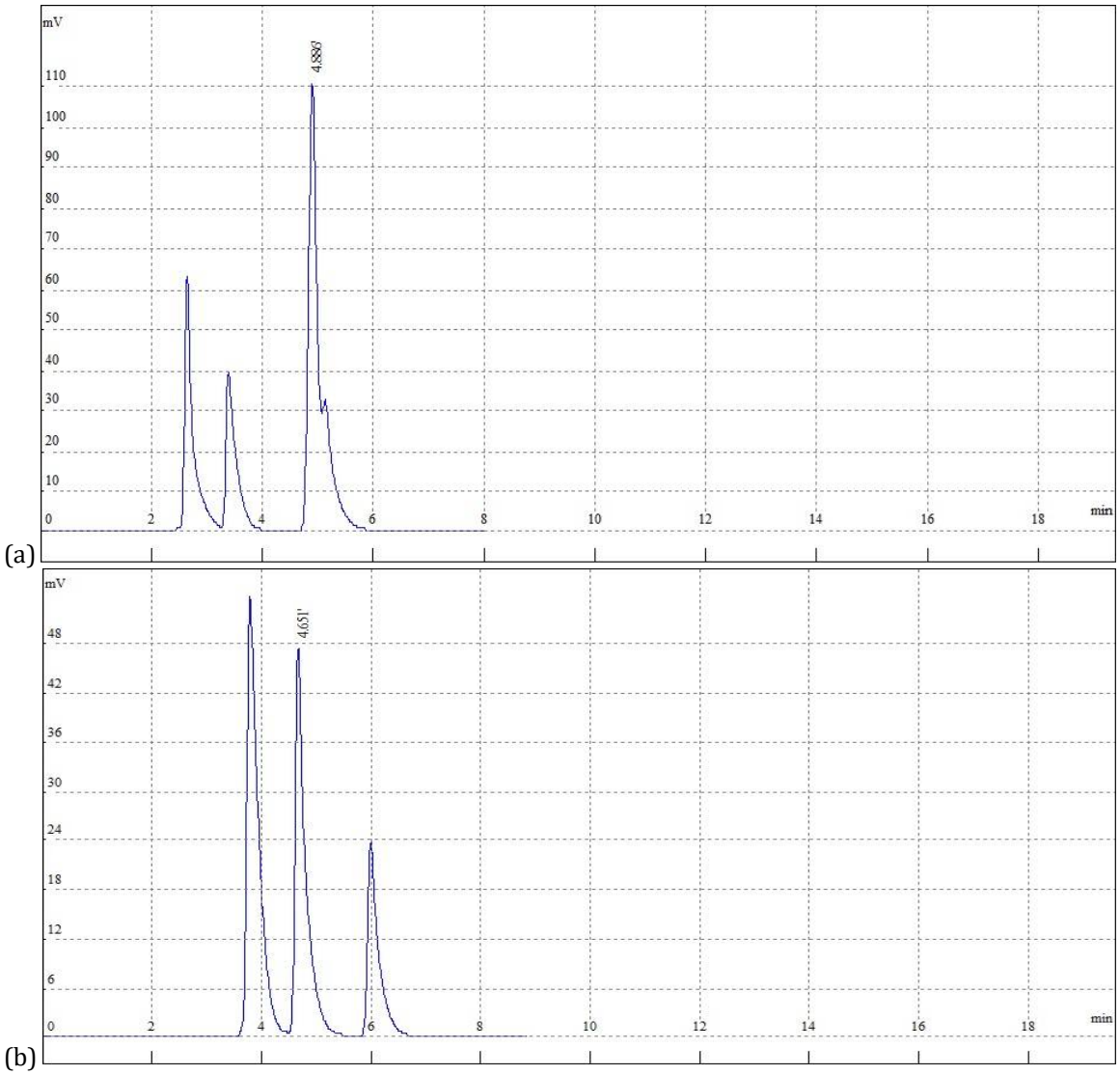


Fig. 9 Chromatogram of Umifenovir showing peroxide degradation in 3 % H_2O_2 at R.T. for (a) 6 hr and (b) 24 hr

Forced degradation

Umifenovir showed relative stability under stress degradation conditions of heat and UV light (Refer table 7). UMF degraded to some extent in acid-base hydrolysis. Two degradant peaks along with UMF were

observed in peroxide degradation (See figure 8). The degradation percentage indicated that the stability of drug was considerably affected by oxidation, followed by alkaline and acid hydrolysis stress conditions.

Table 1. Levels for Box-Behnken Design

LC Separation	**Levels**		
Condition	**Low (-)**	**Center (0)**	**High (+)**
% Mobile phase system	60	65	70
Flow rate (ml/min)	0.8	0.9	1
pH (units)	2.5	3.0	3.5

Table 2. Box-Behnken Design showing factors and responses for 17 QbD trials

Run	**Variable 1**	**Variable 2**	**Variable 3**	**Response 1**	**Response 2**	**Response 3**	**Response 4**
	A:Mobile phase system	**B:Flowrate**	**C:pH**	**Retention Time (Rt)**	**Area**	**Plate Number (TPN)**	**Peak Asymmetry**
	Units - **(%)**	**(ml/min)**		**(min)**	**(Area Unit)**		
1	70	1	3	3.045	596186	7166	1.04
2	60	1	3	6.493	640155	7314	1.29
3	60	0.9	3.5	7.066	722517	7317	1.27
4	65	0.9	3	4.228	615920	7973	1.07
5	65	0.9	3	4.228	615920	7973	1.07
6	65	0.9	3	4.228	615920	7973	1.07
7	60	0.9	2.5	6.897	668119	7255	1.3
8	70	0.9	3.5	3.481	687363	7192	1.35
9	65	1	3.5	3.848	574792	8130	1.17
10	65	0.9	3	4.228	615920	7973	1.07
11	70	0.9	2.5	2.942	718436	6631	1.01
12	60	0.8	3	7.571	770913	7238	1.25
13	70	0.8	3	3.318	849214	7486	1.17
14	65	1	2.5	3.817	571699	7068	1.17
15	65	0.9	3	4.228	615920	7973	1.07
16	65	0.8	2.5	4.677	652116	7982	1.02
17	65	0.8	3.5	4.672	698852	8196	1.09

Table 3. ANOVA (partial sum of squares) for asymmetry factor

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value**	**Remark**
Model	0.169812	9	0.018868	6.058524	0.0134	Significant
A - Composition	0.03645	1	0.03645	11.70413	0.0111	Significant
B - Flowrate	0.00245	1	0.00245	0.786697	0.4045	
C - pH	0.01805	1	0.01805	5.795872	0.0469	Significant
AB	0.007225	1	0.007225	2.319954	0.1715	
AC	0.034225	1	0.034225	10.98968	0.0129	Significant
BC	0.001225	1	0.001225	0.393349	0.5504	
A^2	0.059375	1	0.059375	19.06537	0.0033	Significant
B^2	6.58E-06	1	6.58E-06	0.002113	0.9646	
C^2	0.008059	1	0.008059	2.58782	0.1517	
Residual		7	0.003114			
Lack of Fit		3	0.007267			
Pure Error		4	0			
Cor Total		16				
ANOVA Summary						
Std. Dev.	0.0558	PRESS	0.3488	Adeq Precision	7.4764	
R^2	0.8862	Adj R^2	0.74	Pred R^2	-0.8203	

Table 4. Polynomial equation for asymmetry factor

Std. Dev.	Mean	C.V. %	PRESS	R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision
0.055806	1.145882	4.870115	0.3488	0.886228	0.73995	-0.82035	7.476446
Regression equation for the quadratic model							
Equation for asymmetry factor = + 24.345 -0.6655 * Composition + 6.975 * Flowrate -3.045 * pH -0.085 * Composition * Flowrate + 0.037 *Composition*pH -0.35 * Flowrate * pH+ 0.00475 * Composition^2 -0.125 * Flowrate^2 + 0.175 * pH^2							

Table 5. AQbD mediated optimized chromatographic conditions

% Composition	Flowrate (ml/min)	pH	R_t	TPN	Area	Asymmetry Factor	Desirability
65	0.8	3.0	4.609	8121	712947	1.11	0.785

Table 6: System suitability parameters and validation results	
Parameter	**Result**
Chromatographic column	Cosmosil C-18 column (250 x 4.6 mm x 5µ)
Mobile phase	methanol:water (65:35 % v/v) at pH 3.0 with OPA
Flow rate	0.8 ml/min
Injection volume	20 µl
Detection wavelength	UV detection at 258 nm
Retention time	4.6 min
Tailing factor	1.1
Theoretical plates	> 8000
Area	712947
Linearity	R^2 = 0.9992
Range	10-50 µg/ml
Precision	Intraday %RSD: 0.58%, Interday %RSD: 0.46%
Accuracy	%RSD < 2.0%
Percent Recovery	100.77%
Assay (%)	99.51%
LOD	0.04 µg/ml
LOQ	0.13 µg/ml
Robustness, pH	%RSD: 0.06%
Robustness, Wavelength	%RSD: 0.13%

Table 7. Degradation percentage of Umifenovir under various stress conditions

Sr. No.	Degradation Parameter	Condition Employed	% Assay After Degradation	% Degradation
1	Acid	0.1N HCl for 2 hours at 60°C	89.35	10.65
2	Base	0.1N NaOH for 2 hours at 60°C	87.81	12.19
3	Peroxide	3 % H2O2 for 6, 24 hours at R.T.	85.96	14.04
4	Photolytic	UV light, 24 hours at R.T	98.83	1.17
5	Thermal	60°C for 24 hours	98.37	1.63

CONCLUSION

The AQbD research was successful in developing a linear, accurate, rapid, cost-effective and selective liquid chromatographic method for determination of UMF in drug and dosage form. The method also generated a method operable design region for providing regulatory flexibility and enhanced robust characteristics. Moreover, it is stability-indicating as evident from forced degradation studies. Hence, the

AQbD technique for UMF can be employed in pharmaceutical industries and quality control laboratories routinely for analysis.

ACKNOWLEDGEMENTS
The authors are grateful to Glenmark Pharmaceuticals Ltd. for providing gift sample of Umifenovir and RAP lab for providing the necessary facilities for analysis.

COMPETING INTERESTS
The authors have declared that no competing interest exists.

REFERENCES
1. Guideline, I. H. T. (2009). Pharmaceutical development Q8 (R2). In: International Conference on Harmonisation, 2009, Geneva, Current Step 4 Version. https://database.ich.org/sites/default/files /Q8%2 8R2%29% 20 Guideline.pdf
2. Ramalingam, P., & Jahnavi, B. (2019). QbD considerations for analytical development. In: Pharmaceutical Quality by Design, Academic Press, pp. 77-108. https://doi.org/10.1016/B978-0-12-815799-2.00005-8
3. Urmi, KF, Nawaz M, Islam SM (2022). Analytical quality by design approach to RP-HPLC method development and validation for simultaneous estimation of esomeprazole and naproxen in modified-release dosage form. Futur J Pharm Sci, 8(1):1-16. https://doi.org/10.1186/s43094-021-00396-z
4. Kumar, L. (2023). Quality-by-design driven analytical method (AQbD) development and validation of HPLC-UV technique to quantify rivastigmine hydrogen tartrate in lipidic nanocarriers: Forced degradation, and assessment of drug content and in vitro release studies. Microchem. J., 108944. https://doi.org/10.1016/j.microc.2023.108944
5. Nagar, P., Garg, M., Chauhan, C., Kumar, R., & Chaudhary, A. K. (2022). Analytical Quality by Design (AQBD) Approach for HPLC Method Development, Method Optimization and Validation. Quality Assurance, 13(2): 103-110. DOI: 10.25258/ijpqa.13.2.2
6. Volta e Sousa, L., Gonçalves, R., Menezes, J. C., & Ramos, A. (2021). Analytical method lifecycle management in pharmaceutical industry: A review. Aaps Pharmscitech, 22: 1-14. https://doi.org /10.1208/s12249-021-01960-9
7. Alhakeem, M. A., Ghica, M. V., Pîrvu, C. D., Anuța, V., & Popa, L. (2019). Analytical quality by design with the lifecycle approach: A modern epitome for analytical method development. Acta Marisiensis Seria Med., 65(2): 37-44. https://doi.org/10.2478/amma-2019-0010
8. Varnekar DS., & Sanjay, N. T. (2023) Quality by Design Approach-To Analytical Method Validation. IJFMR., 5(2). https://doi.org/10.36948/ijfmr.2023.v05i02.1903
9. Mahapatra, A., & Meyyanathan, S. N. (2022). Approach of analytical quality by design and regulatory need. IJHS., 6(S5): 2572–2592. https://doi.org/10.53730/ijhs.v6nS5.9208
10. Sanders, J. M., Monogue, M. L., Jodlowski, T. Z., & Cutrell, J. B. (2020). Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. Jama, 323(18): 1824-1836. doi:10.1001/ jama.2020.6019
11. Mathada, B. S., & Somappa, S. B. (2022). An insight into the recent developments in anti-infective potential of indole and associated hybrids. J. Mol. Struct., 1261: 132808. https://doi.org/10.1016/j.molstruc.2022.132808
12. Iqbal, M., Imam, F., Ali, E. A., Kalam, M. A., Alhudaithi, S. S., & Anwer, M. K. (2023). A Validated UPLC-MS/MS Method for Rapid Quantification of Umifenovir in Plasma Samples and Its Greenness Assessment. Separations, 10(7): 379. https://doi.org/10.3390/separations10070379
13. Angirekula, N. (2018). Quantification of Arbidol by RP-HPLC with photo diode array detection. AJP., 12(02). https://doi.org/10.22377/ajp.v12i02.2391
14. Da Ruos, J., Baldo, M. A., & Daniele, S. (2022). Analytical methods for the determination of major drugs used for the treatment of COVID-19. A review. Critical Reviews in Analytical Chemistry, 1-35. https://doi.org/10.1080/10408347.2022.2039094
15. Damle, M. C., Phadtare, S. A., Raskar, H. D., Gadge, K. G., Mehendre, R., & Bothara, K. G. (2010). A Validated HPTLC Method for Determination of Arbidol from Pharmaceutical Formulation. Int. J. Chemtech Res., 2(2): 1042-1046. https://europepmc.org/article/pmc/pmc3262966
16. Stojanovic, J., Krmar, J., Protić, A., Svrkota, B., Djajić, N., & Otašević, B. (2021). DoE Experimental design in HPLC separation of pharmaceuticals; a review. Archives of Pharmacy, 71(4): 279-301. https://doi.org/10.5937/arhfarm71-32480
17. Guideline, I. H. T. (2003) Stability testing of new drug substances and products. Q1A (R2). In: International Conference on Harmonisation, 1993, current step 4: 1-24. https://database. ich.org/sites/default /files/Q1A%28R2%29%20Guideline.pdf
18. Annapurna, M. M. (2018). New stability indicating ultrafast liquid chromatographic method for the determination of umifenovir in tablets. IJGP., 12(1). https://doi.org/10.22377/ijgp.v12i01.1619
19. Surabhi, S. R., & Jain, D. N. (2021). Validated stability indicating method for determination of umifenovir-remdesivir in presence of its degradation products. Int. J. Dev. Res, 11(4): 46227-46232. https://doi.org/10.37118/ijdr.21666.04.2021

20. Sawarkar, K. T., Asnani, A. J., Chaple, D. R., & Ikhar, J. S. (2022). Quality by Design (QbD): Building and Enhancing Quality of Pharmaceuticals. ***Int. J***. Pharm.Res. Appl., 7(3): 1738-1748. DOI: 10.35629/7781-070317381748
21. Dewi, M. K., Pratama, R., Arifka, M., & Chaerunisaa, A. Y. (2022). Quality By Design: Approach to Analytical Method Validation. Sciences of Pharmacy, 1(1): 33-40. https://doi.org/10.58920/sciphar01010033
22. Tandel, F.B., Khandai, M., Shah, P., Panda, D.S., Patel, V.P. (2024). Analytical Quality by Design. In: Jain, N.K., Bajwa, N. (eds) Introduction to Quality by Design (QbD), Springer, Singapore, p. 91-118. https://doi.org/10.1007/978-981-99-8034-5_4
23. Guideline, I. H. T. (2005). Validation of analytical procedures: text and methodology. Q2(R1). In: International Conference on Harmonisation, 1996, 1(20): 05 https://databa se.ich.org/sites/ default /files/Q2% 28R1%29%20Guideline.pdf