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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF RITONAVIR AND NIRMATRELVIR IN PHARMACEUTICAL DOSAGE FORM BY UV-SPECTROSCOPY

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Keywords:

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ABSTRACT: The present study describes the development and validation of a simple, economical, accurate, and precise UV spectrophotometric method for the simultaneous estimation of Ritonavir and Nirmatrelvir in active pharmaceutical ingredients and tablet dosage forms, in accordance with ICH guidelines. Ritonavir, an HIV protease inhibitor, and Nirmatrelvir, a SARS-CoV-2 protease inhibitor, were selected for their combined therapeutic significance in COVID-19 antiviral therapy. Solubility profiling revealed that Ritonavir was freely soluble in acetonitrile and methanol, while Nirmatrelvir was soluble in methanol. Considering cost and solubility, acetonitrile and methanol were used as solvents for Ritonavir and Nirmatrelvir, respectively. The λmax values were found to be 239 nm for Ritonavir and 228 nm for Nirmatrelvir. Linearity was established in the ranges of 20–80 µg/mL for Ritonavir and 2–18 µg/mL for Nirmatrelvir, with excellent correlation coefficients (r^2 = 0.999 and 0.9998, respectively). The method was validated for accuracy, precision, and sensitivity. LOD and LOQ were 0.01285 µg/mL and 0.03894 µg/mL for Ritonavir, and 0.13419 µg/mL and 0.40662 µg/mL for Nirmatrelvir. Recovery studies confirmed accuracy within 98.06–102.56%, and %RSD values were within acceptable limits (<2%), indicating good precision. The assay of marketed tablets showed drug contents of 99.39% for Ritonavir and 99.41% for Nirmatrelvir. Thus, the proposed UV spectrophotometric method is reliable, cost-effective, and suitable for routine quality control analysis of both drugs in bulk and tablet forms.

INTRODUCTION:

Virus: A virus is a microscopic infectious agent. It consists of a segment of nucleic acid (either DNA or RNA) surrounded by a protein coat. Viruses can infect hosts such as humans, animals, and plants. They cannot reproduce without a host [1].

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Antiviral drugs assist your body in fending off specific viruses that might lead to illness. Additionally, antiviral medications are preventative. In other words, they can shield you from contracting a virus or infecting others. Antiviral therapy is what medical professionals sometimes refer to as antiviral medication [2].

Analytical Method Development: The present work is to develop and validate UV Spectroscopic method for the determination of Ritonavir **Fig. 1** and Nirmatrelvir **Fig. 2** in API and pharmaceutical dosage formas per ICH Guidelines for solubility, selection of solvent, selection of Wavelength,

Linearity, Range, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantification (LOQ) were assessed [3]. Ritonaviris an HIV protease inhibitor [4] and Nirmatrelvir is an orally bioavailable inhibitor of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [5].

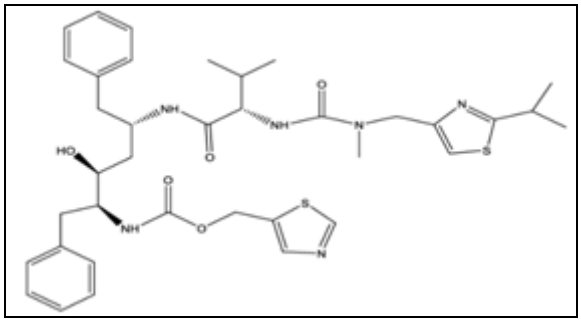

FIG. 1: THE STRUCTURE OF RITONAVIR1,3-THIAZOLE-5-YLMETHYL N-[(2S, 3S, 5S)-3-HYDROXY-5- [[(2S)-3METHYL-22 [[METHYL-[(2-PROPAN-2-YL-1, 3-THIAZOL-4YL) METHYL] CARBAMOYL] AMINO] BUTANOYL] AMINO]1,6-DIPHENYLHEXAN 2-YL] CARBAMATE [4]

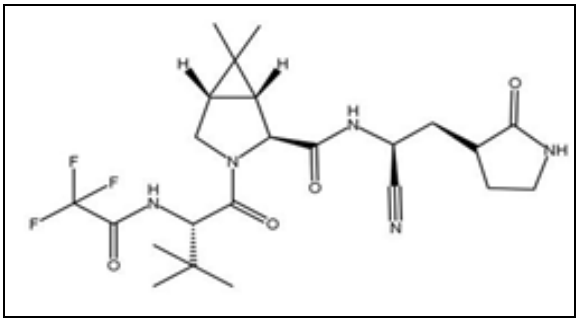

FIG. 2: THE STRUCTURE OF NIRMATRELVIR(1R,2S,5S)-N-[(1S)-1-1CYANO-2-[(3S)-2-OXOPYRROLIDIN-3-YL] ETHYL] 3-[(2S)-3,3-DIMETHYL-2-[(2,2,2-TRIFLUOROACETYL) AMINO] BUTANOYL] 6,6-DIMETHYL-3-AZABICYCLO [3.1.0] HEXANE-2-CARBOXAMIDE [5]

MATERIALS AND METHODOLOGY:

Materials: The Glassware's and Apparatus used for the Research work were made up of borosilicate and that were calibrated before used in the research. For weighing the required chemicals in the range of (10 mg –700 mg)a calibrated digital weighing balance (sartorious-TE-214S) was utilized (max capacity – 200 gm)A Shimadzu (UV-1700Pharmaspec) double beam UV-Visible Spectrophotometer with spectral width of 2mm, Wavelength Accuracy of 0.5nm and a pair of 10mm matched quartz cell were used. A Shimadzu (FTIR-8400S) was used for determining FT-IR range for the APIs with palletization sampling technique using KBR (AR Grade). Ultra-Sonicator (RC system-MU1700) was used for getting the uniform mixture of solvent and the sample. Ritonavir API and Nirmatrelvir API were purchased from Dhamtec Pharma Consultants Navi Mumbai, Maharashtra. India. Tablets of brand Combovir (Nirmatrelvir 150mg and Ritonavir 100mg) were purchased from Bharat Pharma New Delhi, India.

Methodology:

Solubility: 10 mg of RTV was weighed and added to 3 different 10 ml volumetric flasks and add the small quantity of Water, Methanol and Acetonitrile respectively and shaken for 1-2 min and then made up the volume with respective solvents where results were found that the drug RTV was insoluble in water and freely soluble in Methanol and Acetonitrile.

10 mg of NMV was weighed and added to 3 different 10 ml volumetric flasks and add the small quantity of Water, Methanol and Acetonitrile respectively and shaken for 1-2 min and then made up the volume with respective solvents where results were found that the drug NMV was insoluble in water and freely soluble in Methanol.

Selection of Solvent: RTV was soluble in both Acetonitrile and Methanol so in the present research work Acetonitrile was selected as a solvent to make the method economical (i.e. cheaper than the methanol).

NMV was soluble in Methanol so in the present research work Methanol was selected as a solvent.

Selection of Wavelength: The standard stock solution- 1 of Ritonavir (1000µg/ml) was prepared by dissolving 10 mg of pure drug in 10 ml of Acetonitrile , from the 1st stock solution transfer 1 ml of solution to a 10 ml volumetric flask and made up the volume with water and resulting solution with the concentration (100µg/ml i.e. stock solution -2), then the final solution was prepared by taking 1 ml of from the 2nd stock solution to the 10 ml volumetric flask and made up the volume to 10 ml with water and the resulting solution of 10µg/ml. The solution (10µg/ml) was scanned from 200 to 400nm and selected 239nm with the absorbance value of 1.023 for RTV. The standard stock solution of Nirmatrelvir (1000µg/ml) was prepared by dissolving 10 mg of API in 10 ml of Methanol from the 1st stock transfer 1 ml of solution to a 10 ml volumetric flask and make up the volume with water making the solution concentration (100µg/ml), the final solution was prepared by taking 1 ml of solution from the 2nd stock to 10 ml volumetric flask and make up the volume to 10 ml with water to obtain the final concentration of 10µg/ml. The solution was scanned from 200 to 400nm and found 228nm with absorbance value of 0.948 for NMV.

Selection of Analytical Concentration Range:

Selection of Analytical Concentration Range for RTV: 10 volumetric flasks (each 10 ml) were taken and labelled as C1-C10. From the 2nd stock solution 10-90 µg/ml conc's were prepared and made up the volume with water and all the solutions were scanned from the 200nm - 400nm then the absorbancewas measured at 239nm and calibration curve was plotted for Abs v/s Conc with sextuplicate results **Table 1** and **Fig. 3.**

Selection of Analytical Concentration Range for NMV: 10 volumetric flasks (each 10 ml) were taken and labelled as C1-C10. From the 2nd stock solution 2-14 µg/ml conc's were prepared and made up the volume with water and all the solutions were scanned from the 200nm - 400nm then the absorbance was measured at 228nm and calibration curve was plotted for Abs v/s Conc with sextuplicate results **Table 2** and **Fig. 4.**

Analysis of Tablet Formulation:

Analysis of Ritonavir Tablets (100 mg): Twenty Tablets of RTV (Combovir) were weighed, average weight was calculated crushed using mortar and pestle to obtain fine powder of RTV. Accurately weighed tablet powder equivalent to 10mg of powder was transferred to 25 ml volumetric flask with 10 ml of ACN and sonicated for 20 min at 26 ^{0}C for the extraction of drug and volume was made up to 25 ml with ACN. Then resulting solution was filtered through Whatman filter paper no. 41, where initial filtrate drops were discarded and rest of the solution was used as Sample stock A (400µg/ml). From the stock A 1 ml of the aliquot was pipetted out and transferred to 10 ml volumetric flask and made up the volume with water to obtain final concentration of 40µg/ml. The resulting solution was scanned from 200-400nm and absorbance was measured at 239nm [9].

Analysis of Nirmatrelvir Tablets (150mg): Twenty Tablets of NMV (Combovir) were weighed, average weight was calculated crushed using mortar and pestle to obtain fine powder of RTV. Accurately weighed tablet powder equivalent to 10mg of powder was transferred to 25 ml volumetric flask with 10 ml of Methanol and sonicated for 20 min at 26 ^{0}C for the extraction of drug and volume was made up to 25 ml with Methanol. Then resulting solution was filtered through Whatman filter paper no. 41, where initial filtrate drops were discarded and rest of the solution was used as Sample stock A (400µg/ml). From the Stock Solution A, 1 ml of the aliquot was pipetted out and transferred to 10 ml volumetric flask and made up the volume with water to obtain final concentration of 40µg/ml. The resulting solution was scanned over 200-400nm and absorbance was measured at 228nm [10].

Validation of Spectrophotometric Method:

Accuracy Studies of Ritonavir and Nirmatrelvir:

Ritonavir: To study the accuracy assay of synthetic mixture was carried out. Recovery studies were caried out by Standard addition method by adding the known amount of RTV (Std) separately to the pre-analyzed sample at three different concentration levels i.e., 80%, 100%, and 120% of assay concentration and percentage recovery was calculated **Table 3.**

Nirmatrelvir: To study the accuracy assay of synthetic mixture was carried out. Recovery studies were caried out by Standard addition method by adding the known amount of NMV (Std) separately to the pre-analyzed sample at three different concentration levels i.e., 80%, 100%, and 120% of assay concentration and percentage recovery was calculated **Table 4.**

Precision Studies of Ritonavir and Nirmatrelvir: The precision of an analytical method was studied by performing intermediate precision and Repeatability.

Precision Study of Ritonavir:
Intermediate Precision:
Intra-day Precision: Variation of results were analyzed by measuring the Abs of Std solution of RTV (40,50,60 µg/ml Conc's) at 239nm on three different time intervals (0hr, 2hr, 4hr) of same day. The % RSD was calculated by using the following formula: **Table 5.**

$$\%RSD = (SD/mean) \times 100$$

Inter-day Precision: Variation of results were analyzed by measuring the Abs of Std solution of RTV (40,50,60 µg/ml Conc's) at 239nm on same time of three consecutive days. The % RSD was calculated using the following formula: **Table 7.**

$$\%RSD = (SD/mean) \times 100$$

Repeatability: Standard solution of 40µg/ml of RTV was prepared in triplicate and absorbance was measured at 239nm. %RSD was calculated **Table 9.**

Limit of Detection (LOD) and Limit of Quantification (LOQ): Detection limit and quantification limit were calculated from the standard intercept of y- intercepts of six calibration curves and average slope of six calibration curves. The formula is given as follows:

LOD = 3.3 x (standard Deviation of y-intercepts of six calibration curves) / (Average Slope of six calibration curves)

LOQ = 10 x (Standard Deviation of y-intercepts of six calibration curves) /Average Slope of six calibration curves

Precision Study of Nirmatrelvir:
Intermediate Precision:
Intra-day Precision: Variation of results were analyzed by measuring the Abs of Std solution of NMV (40,50,60 µg/ml Conc's) at 228nm on three different time intervals (0hr, 2hr, 4hr) of same day. The % RSD was calculated using the following formula: **Table 6.**

$$\%RSD = (SD / mean) \times 100$$

Inter-day Precision: Variation of results were analyzed by measuring the Abs of Std solution of NMV (40,50,60 µg/ml Conc's) at 228nm on same time of three consecutive days. The % RSD was calculated using the following formula: **Table 8.**

$$\%RSD = (SD/mean) \times 100$$

Repeatability: Standard solution of 40µg/ml of NMV was prepared in triplicate and absorbance was measured at 228nm. %RSD was calculated using the formula: **Table 10.**

Limit of Detection (LOD) and Limit of Quantification (LOQ): Detection limit and quantification limit were calculated from the standard intercept of y- intercepts of six calibration curves and average slope of six calibration curves. The formula is as follows [6]:

LOD =3.3 x (Standard Deviation of y-intercepts of six calibration curves) / (Average Slope of six calibration curves)

LOQ = 10 x (Standard Deviation of y-intercepts of six calibration curves) / Average Slope of six calibration curves

RESULTS:

Linearity studies of Ritonavir and Nirmatrelvir:
Ritonavir:

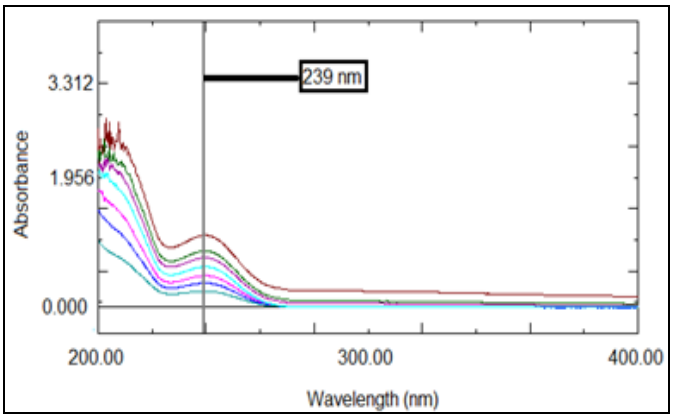

FIG. 3: THE LINEARITY OF THIS METHOD WAS DETERMINED AT RANGES FROM 20-80 MG/ML FOR RITONAVIR

TABLE 1: LINEARITY RANGE OF RITONAVIR

Conc µg/ml	Average Absorbance	SD	% RSD
20	0.00207	0.00207	0.92367
30	0.00557	0.00557	1.60473
40	0.00343	0.00343	0.72039
50	0.00829	0.00829	1.37498
60	0.00163	0.00163	0.22147
70	0.00117	0.00117	0.13512
80	0.00476	0.00476	0.46691

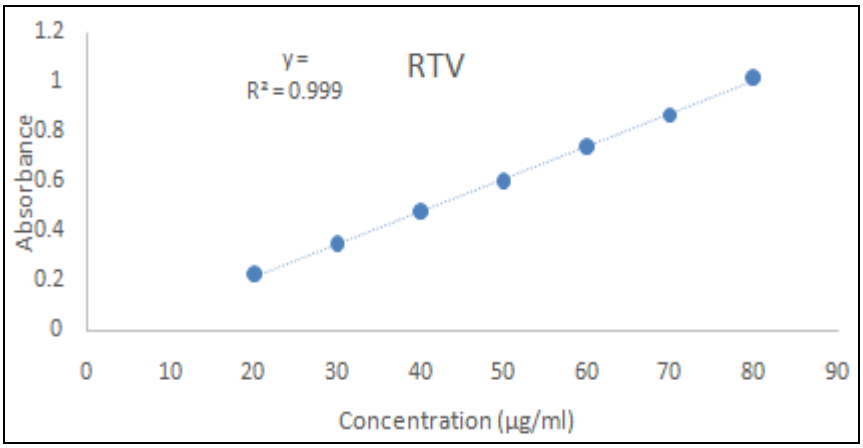

FIG. 4: CALIBRATION CURVE OF RITONAVIR. [The Linearity for Ritonavir was found to be linear in the range of 20-80 µg/ml with R^2= 0.999 and the straight-line equation as Y=0.0132x + 0.0472 and R^2=0.999.]

Nirmatrelvir:

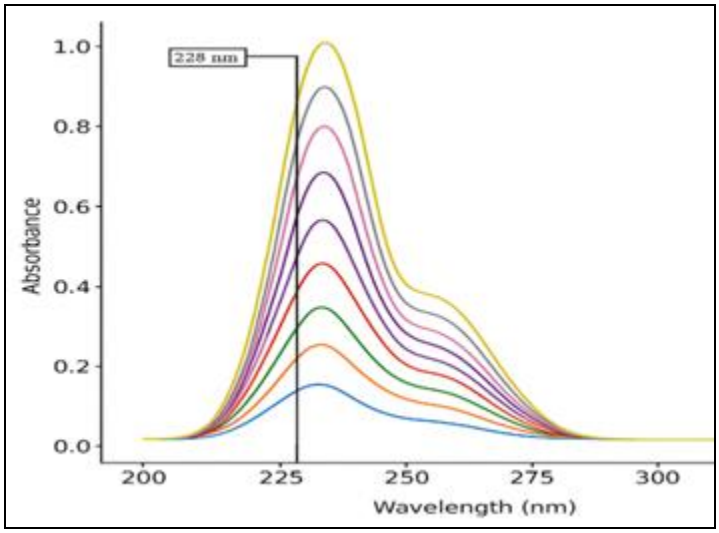

FIG. 5: THE LINEARITY OF THIS METHOD WAS DETERMINED AT RANGES FROM 2-18MG/ML FOR NIRMATRELVIR

TABLE 2: LINEARITY RANGE OF NIRMATRELVIR

Conc. µg/ml	Average Absorbance	SD	% RSD
2	0.13567	0.00121	0.89267
4	0.24467	0.00294	1.20324
6	0.37467	0.00151	0.40184
8	0.50483	0.00279	0.55204
10	0.6345	0.00266	0.41995
12	0.759	0.00303	0.39962
14	0.87633	0.00151	0.1718
16	1.00833	0.00378	0.37459
18	1.13417	0.00319	0.28113

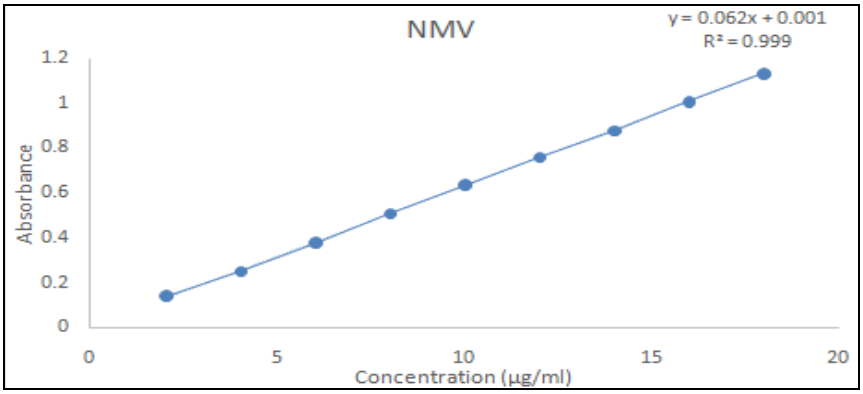

FIG. 6: CALIBRATION CURVE OF NIRMATRELVIR. [The Linearity for Nirmatrelvir was found to be linear in the range of 2-18 µg/ml with R^2= 0.9998 and the straight-line equation as Y=0.0629x + 0.0017 and R^2=0.9998.]

Accuracy Studies of Ritonavir and Nirmatrelvir: The accuracy of analytical method for Ritonavir and Nirmatrelvir was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 239nm for Ritonavir and 228nm for Nirmatrelvir. And results were expressed in terms of % studies.

Ritonavir:

TABLE 3: ACCURACY STUDIES OF RITONAVIR

Level of Recovery	Absorbance	% Recovery
80%	0.989	99.09 %
100%	1.09	98.75 %
120%	1.23	101.001 %

Nirmatrelvir:

TABLE 4: ACCURACY STUDIES OF NIRMATRELVIR

Sl. no.	Level of Recovery	Absorbance	% Recovery
1	80%	1.112	98.06 %
2	100%	1.255	99.62 %
3	120%	1.550	102.56 %

Precision Studies of Ritonavir and Nirmatrelvir: In Precision the measurement of intraday, interday, repeatability and results were showed good reproducibility with the present relative standard deviation (% RSD) was below 2.0%. This indicated that this method was highly precise [7].

TABLE 5: INTRADAY PRECISION OF RITONAVIR

Conc. µg/ml	Absorbance					
	0 hour	2 hours	4 hours	Average	SD	% RSD
40	0.476	0.474	0.472	0.474	0.002	0.421941
50	0.61	0.599	0.596	0.601667	0.007371	1.225116
60	0.733	0.729	0.726	0.729333	0.003512	0.48152

TABLE 6: INTRADAY RESULTS OF NIRMATRELVIR

Conc. µg/ml	Absorbance					
	0 hour	2 hours	4 hours	Average	SD	% RSD
6	0.387	0.385	0.384	0.38533	0.00153	0.39642
10	0.637	0.635	0.634	0.63533	0.00153	0.24043
14	0.886	0.885	0.884	0.885	0.001	0.11299

TABLE 7: INTERDAY RESULTS OF RITONAVIR

Conc. µg/ml	Absorbance					
	Day 1	Day 2	Day 3	Average	SD	% RSD
40	0.476	0.47	0.468	0.471333	0.004163	0.883309

50	0.593	0.589	0.584	0.588667	0.004509	0.766011
60	0.709	0.702	0.699	0.703333	0.703333	0.729612

TABLE 8: INTERDAY RESULTS OF NIRMATRELVIR

Conc µg/ml	Absorbance					
	Day 1	Day 2	Day 3	Average	SD	% RSD
6	0.382	0.383	0.384	0.383	0.001	0.2611
10	0.63	0.628	0.629	0.629	0.001	0.15898
14	0.88	0.878	0.882	0.88	0.002	0.22727

Repeatability:

TABLE 9: REPEATABILITY OF RITONAVIR

Conc µg/ml	Average Absorbance	SD	%RSD
40	0.571667	0.001528	0.267206

TABLE 10: REPEATABILITY RESULTS OF NIRMATRELVIR

Conc µg/ml	Average Absorbance	SD	%RSD
10	0.627333	0.000577	0.092032

Acceptance Criteria:

TABLE 11: ACCEPTANCE CRITERIA FOR ALL THE PARAMETERS

Sl. no.	Parameters	Limit
1	Linearity Range (%RSD)	<2
2	Correlation Coefficient (r^2)	≤1
3	Analysis of Tablets (Mean % Assay)	98-105%
4	Accuracy(%Recovery)	98-105%
5	Intra Day Precision (%RSD)	<2
6	Inter Day Precision (%RSD)	<2
7	Repeatability (%RSD)	<2

CONCLUSION: The present study successfully developed and validated a simple, precise, accurate, and cost-effective UV spectrophotometric method for the quantitative determination of Ritonavir (RTV) and Nirmatrelvir (NMV) in bulk APIs and tablet formulations, following ICH guidelines. The method showed excellent linearity, precision, accuracy, and sensitivity. The results confirmed that both drugs could be accurately and reliably estimated in bulk and pharmaceutical dosage forms. The low %RSD values indicated good repeatability and intermediate precision, while the high recovery rates confirmed the accuracy of the method. Thus, this UV method can be effectively employed for routine quality control analysis of Ritonavir and Nirmatrelvir in pharmaceutical industries.

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CONFLICT OF INTEREST: The authors declare that no conflicts of interest exist related to this research. The study was carried out independently as part of an academic research project at the Department of Pharmaceutical Quality Assurance, Nargund College of Pharmacy, Bengaluru. No external funding, financial assistance, or commercial sponsorship was received for conducting or publishing this work. All materials and facilities were provided by the institution for academic purposes only. The authors alone are responsible for the content and writing of this manuscript.

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