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Research Article

Method Development and Stress Degradation Profile of Umifenovir by UV Spectrophotometry

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Abstract

A simple, selective, and economical UV spectrophotometric method has been developed using ethanol as solvent (50%) to determine the umifenovir content in bulk and pharmaceutical dosage formulations. At a pre-determined λ max of 260 nm, it has shown linear in the 10-50 µg/mL range and exhibited a good correlation coefficient (R^2 = 0.9927) and excellent mean recovery (98.00–104%). The method was validated statistically, and recovery studies were done for linearity, precision, and accuracy. The obtained results proved that the method can be employed for the routine analysis of umifenovir in bulks and commercial formulations.

Keywords: UV spectrophotometry, Umifenovir, Validation, Method development, Stress Degradation

INTRODUCTION

The chemical name for Umifenovir is ethyl 6-bromo4-[(dimethylamino)methyl]-5-hydroxy-1-methyl-2-(phenylsulfanylmethyl)indole-3-carboxylate. It has a molecular formula of $C_{22}H_{28}BrClN_2O_4S$ and a molecular weight of 513.9 g/mol. Arbidol, or Umifenovir, is an antiviral medication used in Russia and China to treat influenza and COVID infections.

The chemical structure of broad-spectrum Arbidol is given below:

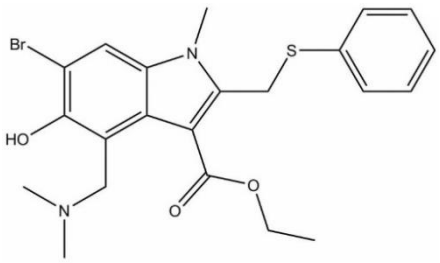

Figure 1: Chemical structure of Umifenovir.

Umifenovir is an indole-based, hydrophobic, dual-acting direct antiviral/host-targeting agent used for treating and prophylaxis influenza and other respiratory infections.

Umifenovir is currently licensed in China and Russia for the prophylaxis and treatment of influenza and other respiratory viral infections.

Umifenovir (arbidol) has been investigated in the past as a potential drug for SARS and MERS. Its mechanism of action is similar to Imatinib, an Abelson kinase inhibitor (Abl), the anchor drug in treating Chronic Myeloid Leukaemia. Both molecules prevent the virus from binding to the cell membrane. A trial on 33 adults with laboratory proven COVID-19, who had not been invasive, has reached encouraging favorable results 4- joint therapy of umifenovir and lopinavir/ritonavir was more efficacious than lopinavir/ritonavir only [1]. Patients treated not only with protease inhibitor but also with umifenovir became sooner SARS-CoV-19- negative (nasopharyngeal specimens), and more of them were found to improve radiologically, according to CT scans. As reported, umifenovir might decrease both the risk of

SARS-CoV-19 transmission and the risk of acute respiratory distress syndrome (ARDS)[2]. Other studies on the effectiveness of umifenovir showed its superiority in comparison with lopinavir/ritonavir, potency to reduce COVID-19 symptoms and accelerate recovery time.

The literature survey revealed that a few analytical methods, such as HPLC [3,4], UPLC [5], HPTLC [6], and LC-MS [7], procedures for determining Arbidol have been reported. Hence, the present work aims to develop a simple, precise, and accurate method for estimating Arbidol Hydrochloride in pharmaceutical dosage form using the UV method and validate the developed method per ICH guidelines.

MATERIALS AND METHODS

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu) loaded with spectra manager software UV Probe was employed with a spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. The 200-400 nm wavelength range was selected for scanning with medium scanning speed.

Chemicals and reagents

Analytical grade solvents and chemicals used included methanol (CH_3OH), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), and distilled water. Umifenovir was obtained from our research collaborators.

Selection of solvent

The solubility of Umifenovir was determined in various solvents as pharmacopeia standard. Solubility test was carried out in different solvent like distilled water, methanol, ethanol and chloroform. Both the actives showed good solubility profile in methanol which is inexpensive and thus it was chosen for the study.

Preparation of phosphate buffer 6.8 pH

About 2.8g of disodium hydrogen phosphate and 1.145g of potassium dihydrogen phosphate were taken and dissolved in sufficient water to produce 100.

Preparation of standard stock solution (1000µg/ml)

10 mg of umifenovir was weighed and placed into a 10 mL volumetric flask. Afterward, methanol was added to dissolve umifenovir, obtaining a 1000 µg/mL concentration. Further, the dilutions were made from this solution; the 0.3ml was made up to 10 ml using the phosphate buffer to obtain the final concentration of 30 µg/mL.

Determination of λ max

The standard solution of Umifenovir (30 µg/ml) was scanned in the wavelength region of 200 to 400 nm, and the spectrum was recorded. Phosphate buffer (pH 6.8) was used as blank. It was observed that the maximum wavelength was to be 260 nm by plotting a spectrum between absorbance and wavelength.

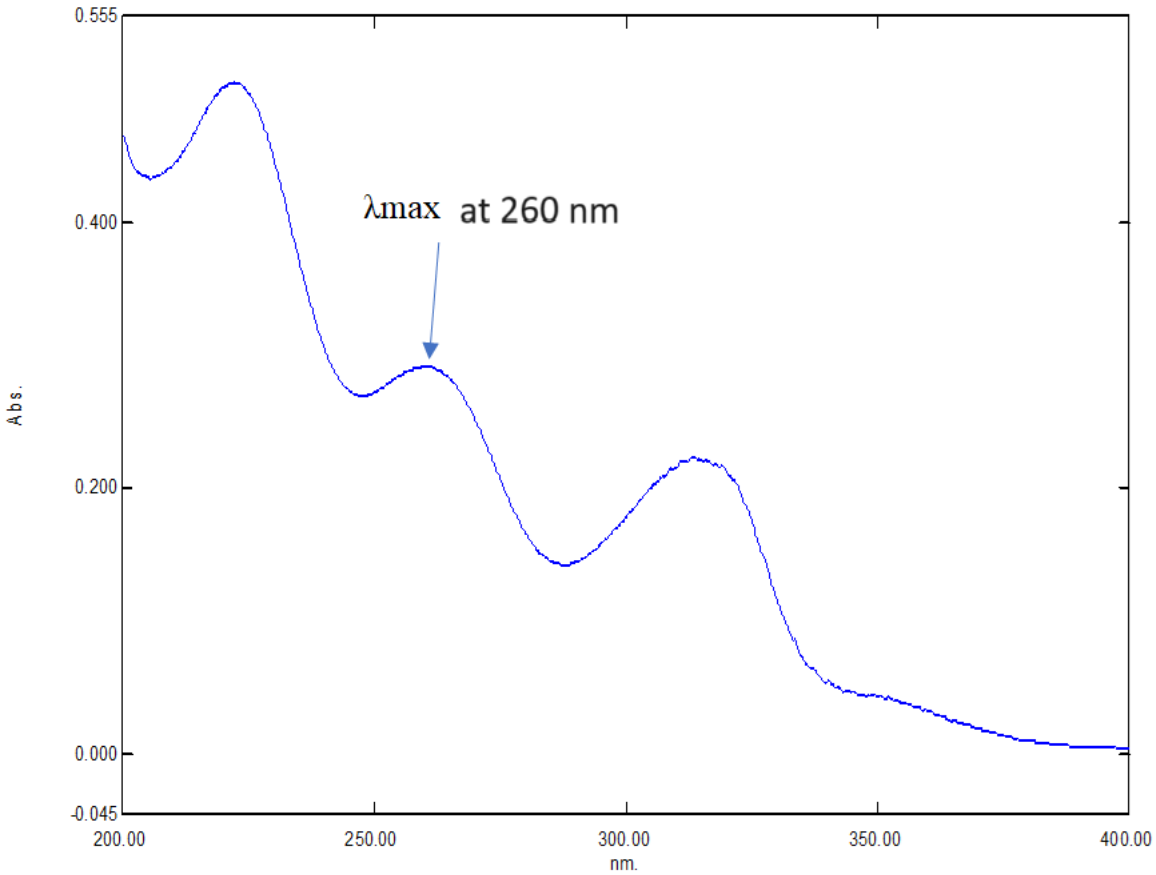

Figure 2: Spectrum of Umifenovir at 30 µg/mL

METHOD VALIDATION

The method developed was according to International Council of Harmonization (ICH) Q2(R1) guidelines. The determined parameters are linearity, accuracy, stress degradation, quantification limit, and detection limit [8, 9].

Linearity

The linearity was assessed by analyzing standard solutions having concentrations (10 -50µg/mL). A plot was constructed for a concentration between absorbance. Standard solutions of umifenovir at different concentrations were prepared. Calibration curves were constructed by plotting the absorbance vs concentration.

Precision

It is usually expressed as the closeness of agreement between a series of measurements obtained. It is generally expressed as the relative standard deviation of a series of measurements. Intra-day and inter-day studies demonstrated the precision of the method. In the intra-day study, three solutions of the same concentrations (30 µg/mL) were prepared and analyzed thrice daily (morning, afternoon, and evening). In the inter-day variation study, the solutions of the same concentration (30µg/ml) were prepared and analyzed daily for three consecutive days, and the absorbance was recorded. The precision, intra-day and inter-day study results are shown in Tables 3 and 4.

Accuracy

This study was carried out using pre-formulated granules containing pure umifenovir and common excipients. Calculation was done from the label claim and the average weight of the final product. Dilution pattern was followed for the granules to obtain three concentrations—50%, 100%, and 150% of reference solution.

Limit of Detection (LOD)

Limit of Detection (LOD) is the lowest possible concentration at which the method can detect the analyte within the matrix . It is also defined as the lowest concentration that can be separated from a background noise with some reliability.

It was found to be 0.5864 µg/ml which is calculated from the following formula,

$$LOD=3.3\ \sigma/S$$

Where,

σ=Standard deviation of the response of the analyte,

S=Slope of the linearity plot of the analyte.

Limit of Quantification (LOQ)

Quantitation limit is the concentration that can be quantitated reliably with a specified level of accuracy and precision. It was found to be 1.7769. Limit of Quantitation can be calculated from the following formula,

$$LOQ =10\sigma/S$$

Where,

σ=Standard deviation of the response,

S=Slope of the linearity plot of the analyte.

STRESS DEGRADATION STUDIES

The ICH guideline Q1A (R2) emphasizes the importance of stress testing for an established analytical method. The stress testing of a drug molecule helps to interpret the API's innate stability properties and validate the analytical process's stability-indicating power. In the current study, stress degradation studies on active samples of moclobemide were performed under several stress environments like acidic, alkali, oxidative, thermal, and photolytic conditions.

Acid Hydrolysis

Acid degradation was performed by taking 1 mL of the stock solution and treating it with 1 mL of 0.1 N HCL. The volume was made up to 10 mL of methanol and stored at room temperature for 3 hours. Aliquots(1.0mL) of the sample were drawn after 60 min, neutralized with NaOH, and diluted appropriately to get a solution of 10µg/mL and analyzed at 260 nm against a blank (solvent without compound). The samples were scanned in the UV region.

Alkaline stress

The procedure described above for the acid hydrolysis was used here for studying alkaline stress in 0.1N NaOH solution.

Pipette 1 mL of sample solution from the mixed

solution into a 10 mL volumetric flask with precision.

For 2 hours, it w

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solution into a 10 mL volumetric flask with precision.

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Thermal stress

The Umifenovir sample was taken in a Petri plate and exposed to thermal energy in a hot air oven at a temperature of 45°C for 30 minutes. After 48 hours, 10 mg of the sample was diluted with 10 mL of methanol. From this solution, dilutions were carried out to achieve the appropriate concentration (30 µg/mL), the solution was taken in the cuvette for the UV analysis, and the degradation percentage was calculated.

Photolytic stress

The compound was subjected to various photolytic conditions. Briefly, the sample (1.0mg/µg/mL) was exposed to UV irradiation for 2 hours under a UV lamp at 365 nm. Pure methanol was also exposed to the same conditions. The sample solution was diluted appropriately to get the final 30 µg/mL concentration and analyzed at 260 nm. The solution was also scanned in the UV region to compare the spectrum with the unexposed compounds.

RESULTS AND DISCUSSION

Method development and optimization

Umifenovir (hydrochloride) is soluble in organic solvents such as ethanol, DMSO, and dimethylformamide. It is sparingly soluble in aqueous buffers. Identifying the wavelength of maximum absorbance is a prerequisite for quantitative UV analysis. A solution representing an absorbance value less than one is generally considered suitable for determining the wavelength of maximum absorbance. Considering the prerequisite and the suitability, the maximum wavelength for the Umifenovir solution (30 µg/mL) was determined using a UV-visible spectrophotometer's full scan mode. The full scan was processed using UV software, and the λmax was identified with the help of software. It was found to be 260 nm for Umifenovir.

METHOD VALIDATION

Linearity

The calibration curve (figure 2) obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 10-50 µg/mL (Table 1) was linear with a correlation coefficient (R^2) of 0.9927. The LOD and LOQ were calculated as 0.5864 mg/mL and 1.7769 mg/mL respectively.

Precision

The developed method's intra-day and inter-day precision study (Tables 3 and 4) confirmed adequate sample stability and method reliability where all the RSDs were <2%.

Accuracy

Results within the range of 99.00–100.07% ensure an accurate method (Table 5) and indicate non-interference with the excipients of the formulation.

Table 1: Linearity for Umifenovir

Concentration (µg/mL)	Absorbance
10	0.2951
15	0.3413
20	0.5253
25	0.7425
30	0.9189
35	1.0583
40	1.2121
45	1.4010
50	1.4995

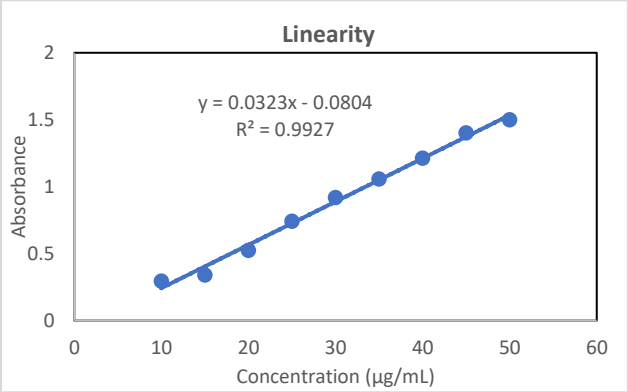

Figure 2: Calibration curve of Umifenovir

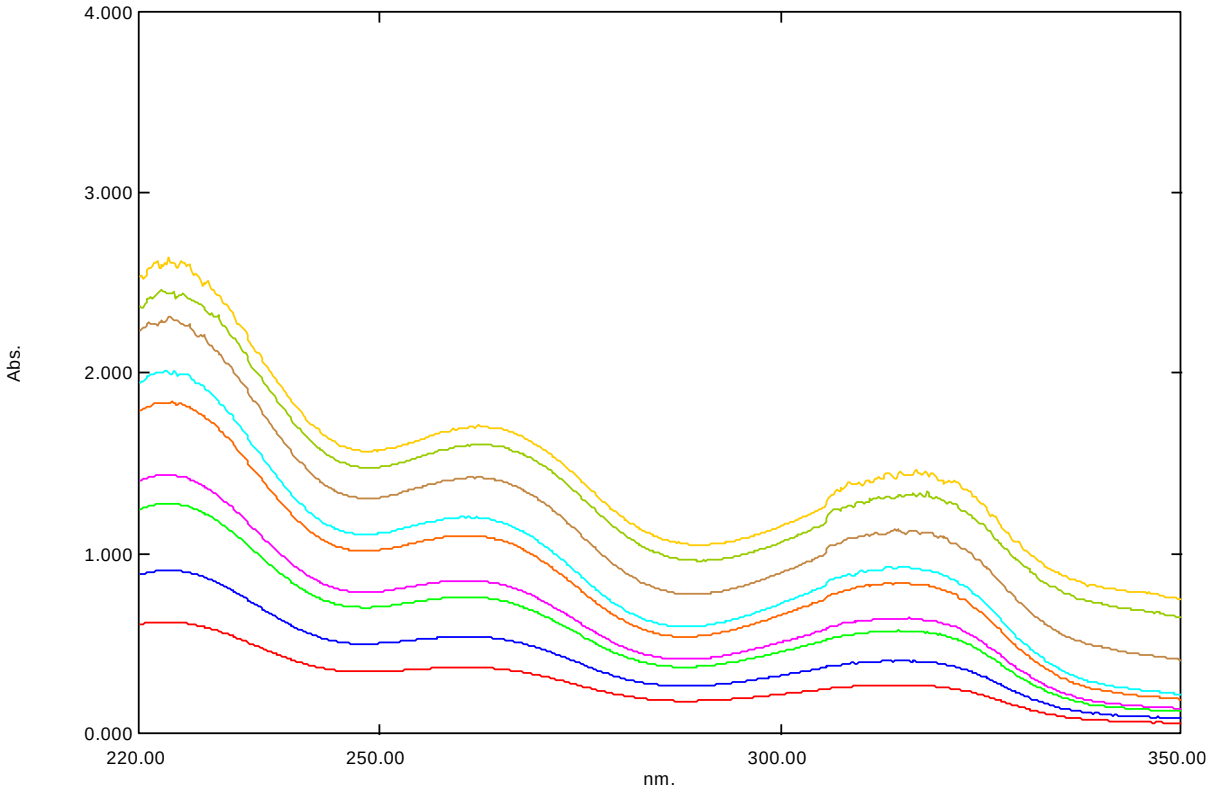

Figure 3: Overlay spectrum of Umifenovir at 10-50 µg/mL

Table 2: Optical characteristics and other parameters:

Parameters	**Method**
Reagent	Phosphate buffer pH 6.8
Linearity (µg/mL)	10-50
Max(nm)	260
Molar extinction (litre/mole/cm)	$1.455x10^{3}$
Sandell's sensitivity (µg/cm2/0,001 absorbance unit)	0.0326
Slope	0.0323
Intercept	0.0804
Correlation coefficient	0.9927
Precision (% RSD) Intra-day	0.86 -1.3
Inter-day	0.86 – 1.7
Accuracy(%Recovery)	98.61-104.37

Table 3: Intraday precision

Morning

Conc (µg/mL)	**Absorbance**	**Obs. conc**	**Accuracy%**
30	0.933	31.37	104.58
30	0.921	30	103.34
30	0.914	30.78	102.62
30	0.919	30.94	103.13
30	0.923	31.06	103.55
30	0.934	31.40	104.68
MEAN	**0.924**		
STD. DEV	**0.007**		
%RSD	**0.8603**		

Afternoon

Conc (µg/mL)	**Absorbance**	**Obs. conc**	**Accuracy%**
30	0.912	30.27	102.41
30	0.932	31.34	104.47
30	0.927	31.18	103.96
30	0.906	30.53	101.79
30	0.902	30.41	101.38
30	0.924	31.09	103.65
MEAN	0.9171		
STD. DEV	0.0122		
%RSD	1.3307		

Evening

Conc (µg/mL)	**Absorbance**	**Obs.conc**	**Accuracy%**
30	0.933	31.37	104.58
30	0.926	31.15	103.85
30	0.922	31.03	103.44
30	0.916	30.84	102.82
30	0.908	30.60	102.00
30	0.911	30.69	102.31
MEAN	**0.9193**		
STD. DEV	**0.009**		
%RSD	**1.0288**		

Table 4: Inter day precision

Day – 1

S. No	**Conc (µg/mL)**	**Absorbance**	**Obs.conc**	**Accuracy%**
1	30	0.931	31.31	104.37
2	30	0.926	31.15	103.85
3	30	0.927	31.18	103.96
4	30	0.917	30.87	102.93
5	30	0.922	31.03	103.44
6	30	0.909	30.63	102.10
MEAN		0.922		
STD.DEV		0.008		
%RSD		0.862		

Day – 2

S. No	**Conc (µg/mL)**	**Absorbance**	**Obs.conc**	**Accuracy%**
1	30	0.932	31.34	104.47
2	30	0.933	31.37	104.58
3	30	0.911	30.69	102.31
4	30	0.932	31.34	104.47
5	30	0.916	30.84	102.82
6	30	0.907	30.56	101.89
MEAN		**0.922**		
STD.DEV		**0.012**		
%RSD		**1.286**		

Day – 3

S. No	**Conc (µg/mL)**	**Absorbance**	**Obs.conc**	**Accuracy%**
1	30	0.935	31.43	104.78
2	30	0.9	30.35	101.78
3	30	0.921	31.00	103.34
4	30	0.946	31.77	105.92
5	30	0.929	31.25	104.16
6	30	0.918	30.91	103.03
MEAN		**0.925**		
STD.DEV		**0.016**		
%RSD		**1.707**		

Table 5: Accuracy for Umifenovir

Level	Added amount (µg/mL)	Absorbance	Amount found(µg/mL)	%Recovery	Mean recovery	S.D.	%RSD
50	15	0.415	15.33	102.24	101.69	0.630	0.620
	15	0.409	15.15	101.01			
	15	0.413	15.27	101.83			
100	30	0.931	31.31	104.37	103.44	0.881	0.852
	30	0.914	30.78	102.62			
	30	0.921	31.00	103.34			
150	45	1.395	45.67	101.50	99.39	1.847	1.859
	45	1.345	44.13	98.06			
	45	1.353	44.37	98.61			

Table 6: Stress degradation studies

Stress Condition	Reagent	Time(min)	%Assay	%Degradation
Acid Hydrolysis	0.1N HCL	180	95.60	4.39
Alkaline	0.1N NaOH	180	77.18	22.81
Oxidative	1% H_2O_2	60	77.97	22.02
Thermal	45^0C	30	93.54	6.45
Photolytic	365nm	120	86.04	13.95

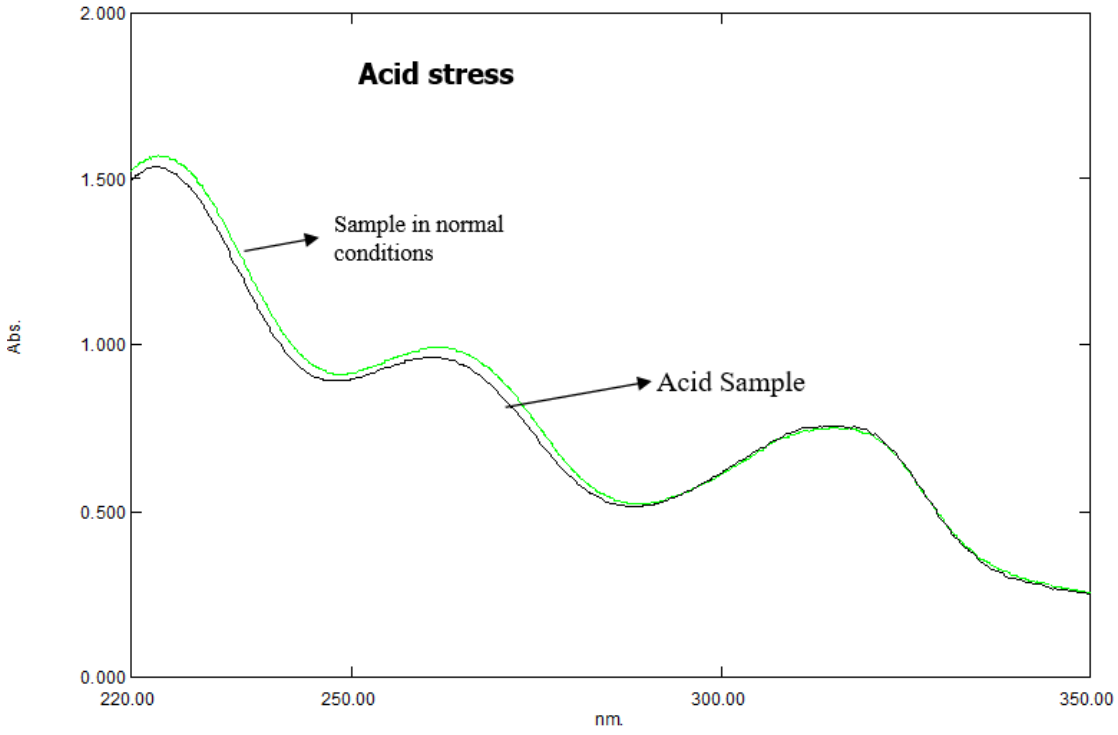

Figure 4(a): Acid Stress degradation studies

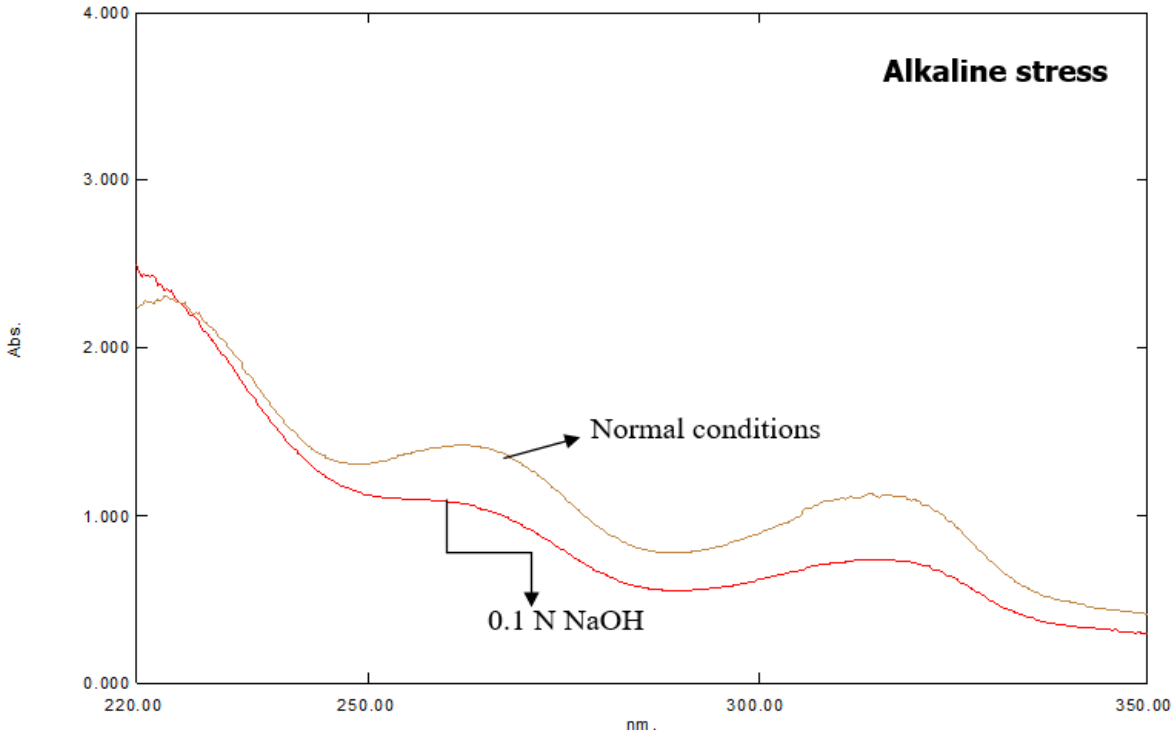

Figure 4(b): Alkaline Stress degradation studies

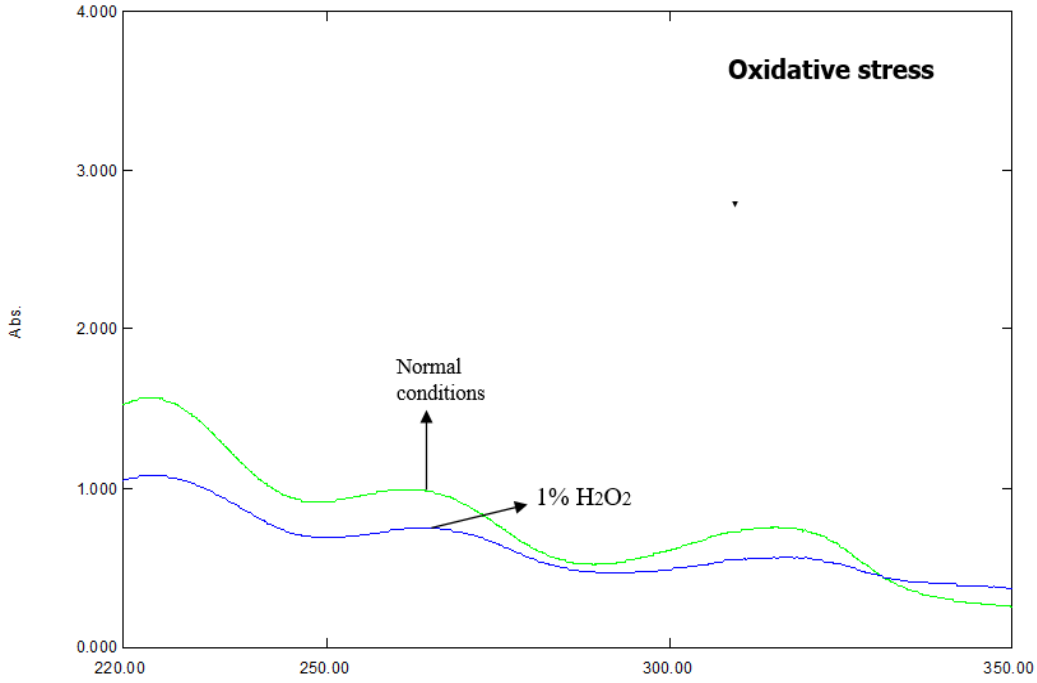

Figure 4(c): Oxidative Stress degradation studies

CONCLUSION

The proposed UV- Spectrophotometric method for estimation of Umifenovir in API and its validation was carried out per ICH guidelines. By studying various parameters, we finally conclude that the process is simple, precise, sensitive, economical, and specific and can be applied to determine Umifenovir in quality control laboratories. All the required validation parameters were estimated and were found to be within the limits.

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Authors contribution

RBN designed the study concept and corrected the manuscript. AH assisted in the data collection, analysis, and interpretation of results and manuscript preparation. RP helped carry out the analysis and manuscript preparation.

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Competing interests/Conflicts of interest

All the authors declared that they have no conflicts of interest

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