Research Article

Development and Validation of UV Method for Quantitative Estimation of Umifenovir in Pharmaceutical Dosage Form

P. Ravisankar*, Chowdary Reddy. D, Sk. Sohail, B. Divya, V. Baji Kumar, N. Vamsi Krishna, P. Srinivasa Babu
Department of Pharmaceutical Analysis, Vignan Pharmacy College, Vadlamudi, Guntur, A.P, India.
***Corresponding author's E-mail:** banuman35@gmail.com

Received: 06-11-2022; **Revised:** 23-01-2023; **Accepted:** 29-01-2023; **Published on:** 15-02-2023.

ABSTRACT

To develop and validate a simple, sensitive, precise, rapid, and cost-effective method for the determination of Umifenovir in bulk and pharmaceutical formulations as per ICH Guidelines. Methods: A simple double-beam UV Spectrophotometric method has been developed and validated with different parameters such as Linearity, Precision, Limit of Detection (LOD), Limit of Quantification (LOQ), Accuracy, Robustness, and Ruggedness. Umifenovir in methanol shows maximum absorbance at 223 nm. Beer's law was obeyed in the concentration range of 2-10 µg/mL, The LOD and LOQ were found to be 0.1510 mcg/ml and 0.4576 µg/mL respectively. The % recovery was found to be in the range lies between 99.3 - 99.5 %. The percentage assay of Umifenovir capsules was found to be more than 99.7 %. Conclusion: The proposed method is precise, accurate, and reproducible and can be used for routine analysis of Umifenovir in bulk and pharmaceutical dosage forms.

Keywords: Umifenovir, Method development, Validation, Ultraviolet Spectroscopy.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2023.v78i02.002

DOI link: http://dx.doi.org/10.47583/ijpsrr.2023.v78i02.002

INTRODUCTION

The chemical name for Umifenovir is ethyl 6-bromo-4-[(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, also known as Umifenovir. Arbidol, is an antiviral medication for the treatment of influenza and COVID infections used in Russia and China. Umifenovir inhibits membrane fusion of the influenza virus. The broad spectrum Umifenovir is rapidly absorbed following oral administration, with an estimated Tmax between 0.65-1.8 hours[1-4]. The chemical structure of broad-spectrum Arbidol is shown in Figure 1.

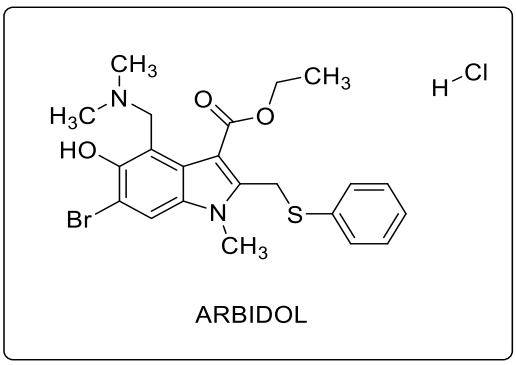

Figure 1: Chemical Structure of Umifenovir

The literature survey revealed that a few analytical methods such as UV[5], HPLC[6-11], UPLC[12], HPTLC[13], LC-MS[14], methods for the determination of Arbidol have been reported. Hence the present work aims to develop a simple, precise, and accurate method for the estimation of Arbidol Hydrochloride in pharmaceutical dosage form by using RP- HPLC method and to validate the developed method as per ICH guidelines.

MATERIALS AND METHODS

Instruments

A double beam ELICO SL 210 UV spectrophotometer containing two matched quartz cells with a one cm light path was taken for measuring of absorbance of Umifenovir. Essae vibra AJ (0.1 mg sensitivity) balance was used for weighing. Ultra Sonicator bath Model no - 91250, PCI Ltd., Mumbai was used in this present study.

Chemicals and reagents

An analytically pure sample of Umifenovir was obtained from Hetero Drugs Ltd., Hyderabad, and Telangana, India. Umifenovir capsules containing 100 mg labeled claim. Umifenovir capsules were used for this study. ACN and CH_3OH were procured from E. Merck specialties, private Ltd., Mumbai, India.

Selection of the solvent

Plentiful trials were executed to find out the suitable solvent system for dissolving the Umifenovir. The solvents such as acetonitrile, methanol, and triple distilled water were tried based on the solubility of the drug. Umifenovir is soluble in solvents such as methanol and Acetonitrile.

Selection of detection wavelength

Umifenovir 6 µg/mL of working standard solution was

scanned between 200 nm to 400 nm and showed maximum absorbance at 223 nm by UV spectrophotometer. To confirm the following analysis, an overlay spectrum using different concentrations was plotted (Figure 2).

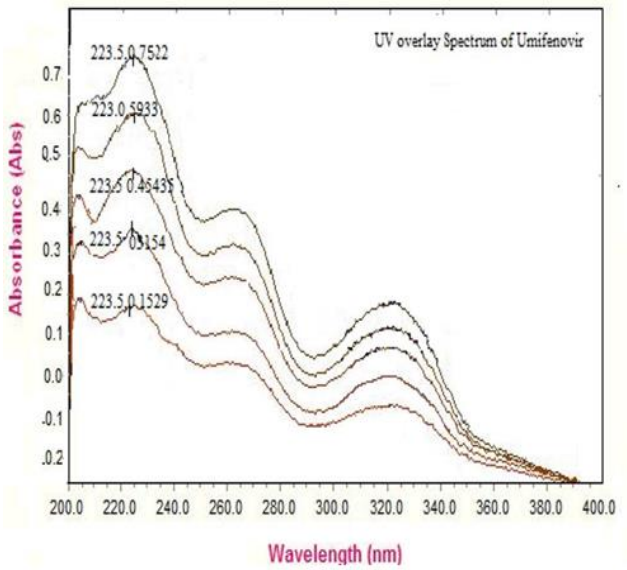

Figure 2: Overlay spectrum of the standard Umifenovir at different concentrations.

Preparation of stock and working standard solution

10 mg of Umifenovir was accurately weighed and taken in a 10 ml clean and dry volumetric flask. The drug was dissolved and diluted up to the mark using methanol. This was considered the standard stock solution (1000 µg/ml). 10 ml of the stock solution was pipette out and made up to 100 ml to get a concentration of 100 µg/ml and was treated as the working standard.

Preparation of calibration curve

From this stock solution, appropriate dilutions were made to get a final concentration of 2, 4, 6, 8, and 10 µg/ml and absorbance was taken at λmax 290 nm. (Table 1) Averages of such 5 sets of values were taken for the standard calibration curve, and the Overlay spectrum of the standard Umifenovir at different concentrations is shown in fig 3.

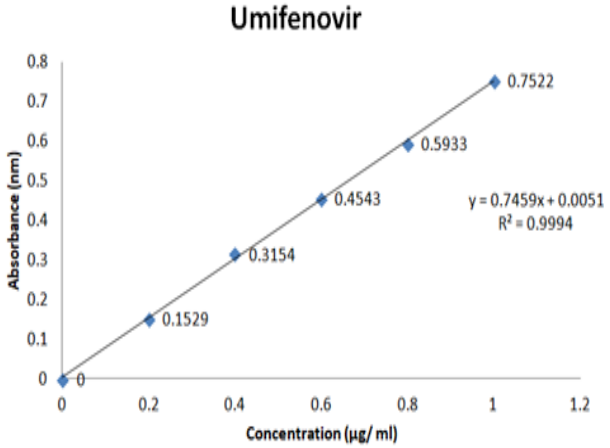

Figure 3: Linearity graph of Umifenovir

Table 1: Calibration data of Umifenovir

Concentration (µg/ml)	Absorbance
2	0.1529
4	0.3154
6	0.4543
8	0.5933
10	0.7522

Table 2: Linear regression data of Umifenovir

Parameter	Results
Detection wavelength (λ max)	223 nm
Beer's law limits (µg/ml)	2-10
Molar absorptivity (L. mole^{-1} cm^{-1})	7885
Sandel's sensitivity (µg/cm^2/0.001 absorbance unit)	0.00126
Regression equation (Y = mx+ c)	0.7459x-0.0051
Slope (m)	0.07459
Intercept (c)	0.0051
The standard error of slope (S_m)	0.000683706
The standard error of intercept (S_c)	0.004140045
Standard error of estimate (S_e)	0.00572029
Correlation coefficient (r^2)	0.9994

SUMMARY OUTPUT

Regression Statistics

Multiple R	0.999811127
R Square	0.999622289
Adjusted R Square	0.999527862
Standard Error	0.005720294
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.346396366	0.3463964	10586.116	5.3506E-08
Residual	4	0.000130887	3.272E-05		
Total	5	0.346527253			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-0.004861905	0.004140045	-1.17436	0.3053933	-0.01635651	0.0066327	-0.01635651	0.006632702
X Variable 1	0.070345714	0.000683706	102.88885	5.351E-08	0.06844744	0.07224399	0.068447442	0.072243986

Figure 4: Summary output of Umifenovir (ANOVA)

RESULTS AND DISCUSSION

Method development & Validation

Different types of solvents were tested for solubility for Umifenovir solvents such as Methanol,acetonitrile, and distilled water at 10 µg/ml concentrations. Nevertheless, Umifenovir was soluble and stable in methanol for a minimum of 24 hours at room temperature. Therefore, methanol solvent was used for the detection of wavelength and preparation of standard and working concentration. To ensure the planned method of the pharmaceutical formulation, an assay of Umifenovir 100 mg capsule was utilized at working concentration. An assay for the working concentration of the sample at 223.5 nm was analyzed. A UV spectrophotometric method

is validated according to ICH Q2 (R1) guidelines for the validation of analytical procedures. The process was validated for parameters such as precision, accuracy, specificity, linearity, ruggedness, robustness, LOD, and LOQ.

Precision

Precision studies were carried out to check the reproducibility of the method. Repeatability was determined by six replicates of the same concentration ofthe sample and by measuring the absorbance. The intra-day precision study was carried out by analyzing the prepared drug solution at three different times (8-hour samples) in a day. The same procedure was followed for three different days to determine inter-day precision.

Method precision

Method precision was estimated by conducting the assay of a sample under the test of repeatability (intraday precision) and intermediate precision performed during three successive days three times. Eventually, the mean, SD, and % Relative standard deviation were determined. Intraday and Inter-day precisions are shown in Tables 3 & 4 respectively.

Table 3: Results of method precision (Inter-day precision)

Concentration (µg/mL)	Inter-day Precision		
	Day 1	Day 2	Day 3
6	0.5933	0.5929	0.5930
6	0.5933	0.5932	0.5931
6	0.5932	0.5931	0.5932
6	0.5931	0.5930	0.5932
6	0.5930	0.5931	0.5929
6	0.5929	0.5930	0.5930
Statistical validation data of Inter-day precision			
SD	0.00016	0.00010	0.00012
Mean	0.5931	0.5930	0.5930
% RSD	0.0275	0.0176	0.0204

Table 4: Results of method precision (Intraday precision)

Concentration (µg/mL)	Absorbance			
	0hr	2hr	4hr	6hr
6	0.5933	0.5932	0.5932	0.5931
6	0.5935	0.5934	0.5931	0.5929
6	0.5930	0.5932	0.5929	0.5926
6	0.5931	0.5931	0.5932	0.59333
6	0.5932	0.5932	0.5933	0.5929
6	0.5932	0.5931	0.5930	0.5931
Statistical validation data of Intraday precision				
SD	0.00017	0.00014	0.00010	0.00024
Mean	0.5932	0.5932	0.5931	0.5929
% RSD	0.0290	0.0248	0.0176	0.0418

Accuracy (recovery studies)

Recovery studies of Umifenovir were carried out by utilizing a standard addition method in which estimation of % mean recovery of the sample by % method at 3 different levels (80 %, 100 %, and 120 %, i.e., 4 µg/ml, 6 µg/ml, 8 µg/ml). These 80 to 120 levels of the sample solutions were prepared as per the procedure given in the methods from the dilutions used for linearity (6 µg/ml). At each level, three analyses were performed. % Mean recovery was calculated as shown in table 7. The accepted limits of recovery are 98 % - 101 %. In fact, the amount of Umifenovir was found, and %recovery was estimated. The accuracy results of Umifenovir is shown in Table 5.

Ruggedness

Ruggedness is done by performing the proposed method on different instruments. In addition to that, this method is carried out by two various analysts performing the technique on different days to check the reproducibility Results of ruggedness are shown in table 6.

Table 5: Accuracy results of Umifenovir

Recovery level	Amount of standard drug added (µg/mL)	Amount of test added (µg/mL)	The total amount recovered (µg/mL)	% Recovery
80 %	0.2	0.2	0.398	99.5%
100 %	0.4	0.2	0.596	99.3%
120 %	0.6	0.2	0.795	99.3%
Mean Recovery: 99.3 - 99.5 %				

Table 6: Results of ruggedness

S. NO	Absorbance for 6 µg/mL			
	Analyst-1	Analyst-2	Instrument-1	Instrument-2
1.	0.5933	0.5932	0.5931	0.5932
2.	0.5932	0.5930	0.5933	0.5930
3.	0.5931	0.5931	0.5932	0.5933
4.	0.5929	0.5932	0.5934	0.5934
5.	0.5934	0.5928	0.5930	0.5935
6.	0.5931	0.5932	0.5932	0.5931
Statistical validation data of Ruggedness				
SD	0.000175	0.00016	0.000141	0.000187
Mean	0.593167	0.593083	0.5932	0.59325
%RSD	0.029523	0.027013	0.02384	0.031535

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is the lowest amount of analyte in the sample that can be detected. LOQ is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOD and LOQ were determined by the following equation. LOD = 3.3σ/S, LOQ= 10 σ/S. where σ is the standard deviation of the Y-intercept of the calibration curve and S is the slope of the regression equation. The LOD and LOQ values were found to be in Table 7.

Table 7: LOD & LOQ Values of Umifenovir

Parameter	Result
Limit of detection (µg/mL)	0.1510
Limit of quantitation (µg/mL)	0.4576

Robustness

Analysis was carried out using a concentration of 10 µg/ml standard at two different wavelengths, and room temperature to determine the robustness of the method, and the respective absorbance was measured. the results were indicated as % RSD in Table 8.

Table 8: Robustness studies of Umifenovir

S.NO	Absorbance at 223.5 nm	Absorbance at 221 nm
1	0.454	0.456
2	0.453	0.454
3	0.452	0.455
Mean	0.453	0.455
Total SD	0.001	0.001
Total % RSD	0.22	0.21

Solution Stability

The Solutions of Umifenovir (Concentration 10 µg/ml) were tested for their stability at ambient temperatures. The absorbance values for 8 hrs, 16 hrs, 24 hrs, 32 hrs, and 48 hrs, were reproducible, and absorbance variation was found to be less than 2 % in both conditions. Solution Stability studies of Umifenovir are shown in Table 9.

Table 9: Solution Stability studies of Umifenovir

Time (hrs)	Absorbance (10 µg/ml)
0	0.7522
8	0.7525
16	0.7529
24	0.7531
32	0.7526
48	0.7535

Analysis of marketed formulation

The developed method was applied to analyze commercially available Umifenovir. The tablet was having the content of Umifenovir equivalent to 100 mg. Ten tablets were weighed and a weight equivalent to 100 mg was dissolved in methanol. By frequent shaking, volume was made up to mark with methanol. The solution was then filtered through Whatman filter paper #41. This filtrate was diluted suitability with solvent to get a solution of 5 µg/ml concentration. The absorbance wasmeasured against the solution blank. The amount of Umifenovir was calculated from the calibration curve. The readings were taken in triplicate. The assay results are shown in Table 10.

Table 10: Result of assay of the pharmaceutical formulation of Umifenovir

Formulation	Amount present (mg)	Amount obtained* (mg)	% Purity (% w/w)
Arbidol (Capsule)	100	99.7	99.7

DISCUSSION

The ultraviolet spectra of Umifenovir were scanned in the region between 200-400 nm. The overlay spectra of Umifenovir at different concentrations have absorbed a maximum of 223 nm, which was selected as the detection wavelength. The response of the Umifenovir was found to be linear in the concentration range of 2-10 µg/ml with a good correlation coefficient of r^2 = 0.999. The system precision, and intermediate precision results, i.e., inter-day and intra-day precision of Umifenovir, are tabulated in tables 3 & 4 respectively. The % RSD was less than 2 in all precision results which indicates that the method was precise. In this recovery, study accuracy was carried out by using a standard addition method at three different concentration levels (80 %, 100 %, and 120 %). The mean percentage recovery at each level should be 99.3-99.5 %. All the results are well within the acceptance criteria, and

the results indicate that the method is accurate. Ruggedness was performed to check the reproducibility which showed a % RSD less than 2 which indicates that the method was rugged. The developed method was eventually applied for the quantification of Umifenovir in tablets. The mean % assay values were found to be 99.7 %. The amount of the drug in the tablet sample was in good agreement with the labeled claim of the formulation.

CONCLUSION

The UV method was developed for the estimation of Umifenovir. In this study, the precision and accuracy % RSD was < 1 % in all cases. This method provides reproducible results with high precision, and accuracy, and was capable of analyzing Umifenovir in low concentrations. The results proved thatthis method is successfully feasible for routine quality control testing of Umifenovir in tablet formulation.

Acknowledgment: The authors are thankful to Hetero Labs for providing the sample of Umifenovir. We are profoundly grateful to Dr. L. Rathaiah, honorable chairman, of the vignan group of institutions, Vadlamudi, Guntur for providing the necessary laboratory services to carry out the present investigation

REFERENCES

1. Deng P, Zhong D, Yu K, Zhang Y, Wang T, Chen X,et al. Pharmacokinetics, metabolism, and excretion of the antiviral drug arbidol in humans, Antimicrob Agents Chemother, 2013;57:1743-55.
2. Boriskin YS, Leneva IA, Pécheur EI, Polyak SJ. Arbidol: A broad-spectrum antiviral compound that blocks viral fusion. Curr Med Chem 2008;15:997-1005.
3. Blaising J, Polyak SJ, Pecheur EI. Arbidol as a broad-spectrum antiviral: an update. Antiviral Res 2014;107:84–94.
4. Brooks MJ, Burtseva EI, Ellery PJ, et al. Antiviral activity of arbidol, a broad-spectrum drug for use against Respiratory viruses, varies according to test conditions. J Med Virol 2012;84:170–81.
5. Panchumarthy Ravisankar, A Niharika, H Anusha, V Himaja, SK Afzal Basha, A simple validated UV spectrophotometric method for quantitative analysis of levetiracetam in pharmaceutical dosage form, Indian Journal of Research in Pharmacy and Biotechnology 2015; 3(5):2320-3471.
6. K.C. Gowridevi, S. Rubesh kumar, Manasa. K. Simple validated RP-HPLC method for estimation of Arbidol hydrochloride in bulk and pharmaceutical tablet formulations. Journal of Global Trends in Pharmaceutical sciences. 2014; 5(3): 1854-1857.
7. Xin Xiong, Suodi Zhai. Method for the determination of Arbidol in Human Plasma. Journal of AOAC International, 2011; 94(4): 1100-1105.
8. Liu X, Chen XH, Zhang YY, Liu WT, Bi KS, Determination of arbidol in rat plasma by HPLC-UV using cloud-point extraction, Journal of chromatography. B, Analytical Technologies in the Biomedical and Life Sciences, 2007; 856(1-2):273-277.
9. Angirekula Narendra, Mukthinuthalapati Mathrusri Annapurna. Quantification of Arbidol by RP-HPLC with photo diode array detection. Asian journal of Pharmaceutics. 2018; 12(2): S553-S557.
10. Ravi Sankar P, Swathi Vaka, Srinivasa Babu Puttagunta, Shaheem Sulthana Md, Gousepeer SK, Current trends in performance of forced degradation studies and stability indicating studies of drugs, 2017;12:17-36.
11. P. Ravisankar, G. Rajyalakshmi, Ch. Devadasu, G. Devala Rao. Instant tips for right and effective approach to solve HPLC troubleshooting, Journal of chemical and pharmaceutical sciences. 2014;7:191-206.
12. Mukthinuthalapati Mathrusri Annapurna, Duvvi Sri Valli, Sunkara Mrunal Chaitanya. New stability indicating ultrafast liquid chromtographic method for the determination of umifenovir in tablets. 2018; 12(1): S194-S199.
13. M. C. Damle, S.A.Phadtare, H.D.Raskar, K.G. Gadge, Ratnakar Mehendre, and K.G. Bothara, A Validated HPTLC Method for Determination of Arbidol from Pharmaceutical Formulation, International Journal of ChemTech Research 2010;2(2):1042-1046.
14. Yuya Wang, Xiaoyan Chen, Qiang Li, Dafang Zhong. Metabolite identification of Arbidol in human urine by the study of CID fragmentation pathways, 2008;12(1):1099-1109.
15. Adiki SK. International Conference on Harmonization (ICH) harmonized tripartite guide guidelines of analytical procedures: text and methodology. 2005;Q2:1–13
16. Ravisankar P, Gowthami S, Devala Rao G., A review on analytical method development, IJRPB, 2014;2(3):1183-1195.
17. Ravi Sankar P, Sai Geethika A, Rachana G, Srinivasa Babu P, Bioanalytical method validation: A comprehensive review, Int. J. Pharm. Sci. Rev. Res, 2019;56(1):50-58.
18. Ravisankar Panchumarthy, Naga Navya Ch, Pravallika D, Navya Sri D, A review on step-by-step analytical method validation, IOSR journal of pharmacy, 2015;5:07-19.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.