Research Article

RP-HPLC Method Development and Validation for the Estimation of Umifenovir in Oral Dosage Form

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ABSTRACT

An accurate, sensitive, precise, quick, and isocratic reverse phase HPLC (RP-HPLC) method has been developed and validated for quantification of Umifenovir in bulk and pharmaceutical tablet dosage forms. With acetonitrile as the organic solvent, the best separation was achieved on a 250 mmx 4.6 mm i.d, 5µ-particle size Inertsil®-Octadecyl-silyl-3V-Reverse-Phase-C18-column with 0.02M Dipotassium hydrogen Orthophosphate and 0.02M Potassium Di hydrogen Orthophosphate in water: Acetonitrile (40:60v/v) in the isocratic mode of elution as mobile phase solvent at a speed of 1.0 mL.min−1. UV detection was at 265 nm. Retention time of Umifenovir was 5.6 minutes. With a correlation coefficient of about 0.998, peak-response was obtained as function of concentration over the range of 40 to 240 µg/ mL for Umifenovir. Umifenovir was shown to have a percentage assay of 109.47 %. Umifenovir had a limit of detection of 0.1 mcg/ mL and a limit of quantification (LOQ) of 0.3 mcg/ mL. The presence of excipients in the formulation had no effect on the assay method. The procedure is appropriate for use in QC- laboratories since it is economical and precise.

Keywords: Umifenovir, Arbidol, RP-HPLC, Isocratic, Acetonitrile.

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INTRODUCTION

Umifenovir is an indole-based (Fig 1), potent, orally active broad-spectrum dual direct-acting antiviral (DAA) and host-targeting agent used in the treatment and prophylaxis of influenza and other respiratory viruses[3]. It exerts its antiviral effects through multiple pathways resulting in virucidal activity against variety of enveloped and non-enveloped RNA and DNA viruses.[9] It is a hydrophobic molecule capable of forming aromatic stacking interactions with certain amino acid residues (e.g., tyrosine, tryptophan), which contributes to its ability to directly act against viruses.[4]

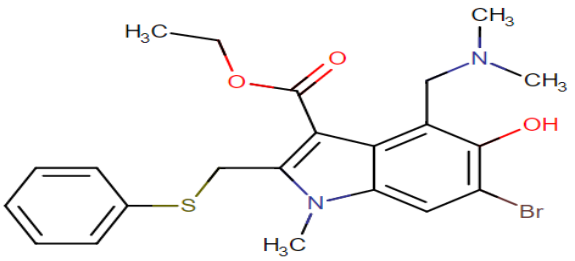

Figure 1: Structure of Umifenovir

Its molecular formula and molecular weight are $C_{22}H_{25}BrN_2O_3S$ and 477.4 g/ mol respectively. IUPAC Name of Umifenovir is ethyl 6- bromo- 4- [(dimethyl amino) methyl]-5- hydroxy-1-methyl- 2- (phenyl sulfanyl methyl) indole-3-carboxylate. It also shows anti-inflammatory activity and is sold under the brand name Arbidol[10,11], as an antiviral medication for the treatment of influenza and COVID infections.[8] It also has been screened for compensation of oxidative stress that is caused by a viral disease and the therapeutic effect of the drug.[7] Hardly very few techniques for the determination of Umifenovir in oral fixed dosage form have been published[1,2,6]. Furthermore, no official or preliminary monograph on this analyte has been published in any of the compendial pharmacopoeias. The goal of this study was to develop an accurate and efficient RP-HPLC method to estimate Umifenovir in unit dosage forms for oral administration. The validation of the devised approach is also addressed in this study, as per ICH standards.[5]

MATERIALS AND METHODS

Chemicals and Reagents:

- 99%, Umifenovir pure was acquired from Srikem labs Pvt Ltd, Mumbai, India.
- Rankem-Fine-Chemicals of HPLC- Grade- Acetonitrile
- *ortho*- H_3PO_3, 85% (v/v), Qualigen-Fine chemicals.
- Dipotassium hydrogen Orthophosphate, Qualigen-Fine chemicals.
- Potassium Di hydrogen Orthophosphate, Qualigen-Fine chemicals.
- HPLC Grade water, Rankem-Fine chemicals.

Chromatographic-Instrument:

Quantitative RP- HPLC was carried out on a Waters 2996 high-performance liquid chromatograph with a PDA detector module, which included an automated injector with a 20 microliters injection volume and a quadra-pump. The column utilized was a Reverse Phase Inertsil Octa Decyl-S-3V-C_{18} column (250mmx 4.6 mm internal diameter with particle size 5µm). EMPOWER Software was installed on the HPLC equipment. The column temperature was adjusted to 25^0C and eluted over 15.0 minutes at a mobile solvent speed of 1.0 mL.min^{-1} under isocratic conditions. The mobile phase used was 0.02M dipotassium hydrogen orthophosphate and 0.02M potassium di hydrogen orthophosphate in water: Acetonitrile (40:60 v/v). It was degassed and filtered via 0.45µm Nylon membrane filters before use. For the analyte, UV detection at 265 nm was used as wavelength of detection with a PDA detector. CH_3CN:H_20 in a ratio of 40:60 (v/v) was used as diluent to make the standard dilutions. Umifenovir was eluted at 5.7 minutes.

Preparation of the Primary Standard Drug solutions: To make the primary standard stock solution, 200 mg of Umifenovir was dissolved in a volumetric flask (100mL) and diluted with the diluent (40:60 v/v H_2O: CH_3CN), sonicated for 15 minutes and diluted up to 100mL with the diluent to get the primary standard stock solution containing 2000µg-mL^{-1}of Umifenovir.

Preparation of Working Standard Drug Solution: After adding 5 ml of the primary working standard solution to the 50-mL volumetric flask, the flask was filled with 50 ml of the diluent. This resultant mixture, which includes 200 ug/mL of Umifenovir, was suitable for use as a working standard solution. The stock solutions were kept in a cool, dark place that was controlled to be four degrees Celsius.

Sample Preparation: After measuring the weight of each individual tablet, the average weight of twenty Arbidol® pills was calculated. Crushing the tablets into a powder form obtained a sample containing 200-mg of Umifenovir, which was then weighed, shifted to a 100mL pre-calibrated-measuring flask, and dissolved in a blend of acetonitrile and aqueous media with a volumetric ratio of 60:40 (v/v). After being sonicated in diluent and strained via Whattman 41 filter paper, the resultant primary working sample solution has 2000 mcgs-mL^{-1} of Umifenovir. After quantitatively transferring 5mL of the filtrate to a 50-mL pre-calibrated-measuring flask, the diluents were added to bring the volume of the solution to 50 mL. This served as a working testing solution having 200 mcgs- mL^{-1} of Umifenovir. The stock solution was kept in a dark place at 4 degrees centigrade.

RESULTS AND DISCUSSION

The purpose of this research was to create a chromatographic technique for the quantifiable determination of fixed-dose of Umifenovir.

Optimized Chromatographic Conditions:

Elution solvents: 0.02M Dipotassium hydrogen orthophosphate and 0.02M Potassium di hydrogen orthophosphate in water: Acetonitrile (40:60) v/v

Elution mode: Isocratic

Column: Inertsil ODS C-18-3V (250 x 4.6mm, 5µm particle size)

Flow rate: 1.0 ml/ min

Injection volume: 20 µl

Detector: Photo diode array (PDA)

Wavelength (λ_{max}): 265nm

Column temperature: Ambient

Diluent: CH_3CN and 0.02M Di potassium hydrogen Orthophosphate and 0.02M Potassium Di hydrogen Orthophosphate in water in the ratio of 60:40 (v/v)

Run time: 15minutes

Retention time: 5.7 mins

Linearity: Aliquots of Umifenovir working stock solutions was placed in various 10mL volumetric flasks and the volume was made up to the 10mL with the mobile phase, yielding in final strengths of 80-240 µg. mL^{-1}(Table 2). The peak areas and retention times of each of these drug solutions (loaded at 20µL) were measured thrice in the column. Using a PDA-detector set at 265 nm, a linearity-graph was generated by plotting peak areas-vs- Umifenovir concentrations in µg-mL^{-1}.

Accuracy: The accuracy of the method was found by evaluating the drug recovery using the standard-spiking method. To assess if the analyte contained in the formulation caused positive or negative interventions, known amounts of the drug equivalent to 12 percent standard drug solution was added to 80 percent, 100 percent and 120 percent of the target test concentrations of the formulation. Each set-of-addition was replicated thrice at each dilution level. The results were compared to a competent reference standard after extraction of sample preparation. The percentage of analyte recovered by the assay was used to assess the accuracy. Table-3 shows the results of accuracy investigations on standard solution and process-related impurity; recovery measurements suggest that the procedure was accurate.

Precision: Quality-control samples in 100 % (w/v) dilution were used to assess intraday and inter-day precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability was indicated by the low RSD value (1%). (Table-4)

Limits of Detection and Quantification: The method's LOD was set at the lowest concentrations of active

pharmaceutical component with a signal-to-noise (S/N) ratio of around 3. (LOD). The lowest active therapeutic medication concentration that can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ) was also determined.

Method Applicability: The newly created method was evaluated by applying it to pharmaceutical tablets for the estimation of Umifenovir.

Optimization of Chromatographic Conditions:

An isocratic RP- HPLC procedure for assaying the active ingredients was developed due to lack of an easy, economical, reproducible, and quick-to-use method for the determination of Umifenovir concentrations in formulary matrices. The effect of various HPLC technique variables was examined on the result of the study to optimize the chromatographic parameters, various proportions of CH_3CN-KH_2PO_4, CH_3CN-H_2O, and CH_3CN: *O*-H_3PO_3 buffer were tested. After several early investigatory tests, CH_3CN: KH_2PO_4 *O*-H_3PO_3 and K_2HPO_4 *O*-H_3PO_3 (0.1%) binary system at the proportion of 60:40 (v/ v) was chosen over other mobile phases because it resulted in improved resolution of active component. This procedure gave the good detection of analyte after multiple exploratory & investigatory trail runs. The active pharmaceutical analyte had excellent UV sensitivity and was interference-free at 265 nm. The analyte peak was highly defined and showed no incidence of tailing under these conditions. The set of conditions previously noted in this article were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Method Validation Tests:

Method precision (RSD percent), method accuracy (recovery percent & % RSD,), linearity range (r^2) and LOD & LOQ were explored as recommended method validation characteristics.

Linearity: With a correlation coefficient of 0.994, the graph of chromatographic-peak areas of the analyte versus respective concentration was shown to be linear in the band of 80-240 µg. mL^{-1} for Umifenovir (Table 2). The least square fit data of linear regression analysis derived from the measurements is given in Table 1. Umifenovir is y = 44103x. Table 1 presents the regression parameters for this technique that include slope, intercept, and % RSD. These findings suggest that there was a significant correlation (Fig 3).

Accuracy: Individual recovery of analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 94.41 percent to 97.08 percent for Umifenovir demonstrating the method's accuracy. The % RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results (Table 3)

Precision: Table 4 summarizes the intraday and interday fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value (less than-1%). These results show that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs.

Limit-of-Detection & Limit-of-Quantifications:

Umifenovir has a limit of detection of 0.1 mcg /mL and a limit of quantification (LOQ) of 0.3 mcg/ mL. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can be used to detect and quantify the analyte over a wide concentration range.

Specificity: The Retention time for Umifenovir was determined to be 5.7 minutes, according to the representative chromatogram given in Figure 2. When the pharmaceutical tablet matrices were evaluated, no indication of excipient interference signal was observed in the respective retention time of the chromatogram. It indicates that the analyte was not disturbed of probable merging peaks. As a result, this technique can be employed with certainty.

Table 1: Regression analysis & Operating-System Suitability Results:

Study-Parameter	Umifenovir
Retention Time (min)	5.7
Peak areas	8685882
Percentage of peak areas	99.99
USP-Tailing	1.19
Theoretical Plates	29192.04
Resolution	3.58
Linear range in (µg/mL)	80-240
Limit-of-Detection in µg. mL^{-1}	0.1
Limit-of-Quantification in µg. mL^{-1}	0.3
Correlation-Coefficient (r^2)	0.994
Assay-in-Percentage (%)	109.47

Table 2: Summary of the standard calibration Curve for Linearity experiment

Calibration Standard Dilution Level	Concentration of Umifenovir (µg/ mL)	Peak Area
40 %	80	3604230
60 %	120	5313447
80%	160	7049452
100 %	100	9161739
120 %	240	10495301

Table 3: Accuracy evaluation by Spike-analysis method

Accuracy study at 80% target level	**Injection Number**	**Umifenovir**	
		Standard Soln.	**Spiked Soln.**
Arbidol-® tablet dosage form solution at 80% level was spiked with 10% of standard solution of API	1	6989438	7857146
	2	6920416	7810912
	3	6937420	7892541
	Mean area	6949864	7848847
	Std. Dev	29820	26971.00
	% RSD	0.42	0.31
	% Recovery		94.41
80% of the target concentration is equivalent to 160 µg/ mL in acetonitrile: water 60:40 v/v as diluent.			
Accuracy study at 100% target level	**Injection Number**	**Umifenovir**	
		Standard Soln.	**Spiked Soln.**
Arbidol-® tablet dosage form solution at 100% level was spiked with 10% of mixed standard solution of API's	1	8839915	9631950
	2	8824191	9707560
	3	8808437	9631970
	Mean area	8821431	9658540
	Std. Dev	8468	17962.00
	% RSD	0.11	0.18
	% Recovery		89.33
100% of the target concentration is equivalent to 200 µg/ mL in acetonitrile: water 60:40 v/v as diluent.			
Accuracy study at 120% target level	**Injection Number**	**Umifenovir**	
		Standard Soln.	**Spiked Soln.**
Arbidol-® tablet dosage form solution at 120% level was spiked with 10% of mixed standard solution of API's	1	10413805	11285991
	2	10411309	11308956
	3	10417553	11295053
	Mean area	10413979	11296788
	Std. Dev	32622	11792.00
	% RSD	0.28	0.19
	% Recovery		97.08
120% of the target concentration is equivalent to 240 µg/mL in acetonitrile:water 60:40 v/v as diluent.			

Table 4: Evaluation of precision with-in-day and day-to-day analysis

Intra-Day Precision study of 100% standard dilution containing 200 µg/ mL of Umifenovir			**Inter-Day Precision study of 100% standard dilution containing 200 µg/ mL of Umifenovir**	
S. No	**Umifenovir**		**Umifenovir**	
	Ret. time	**Peak area**	**Ret. time**	**Peak area**
1	5.718	8659365	5.791	8751687
2	5.713	8620201	5.759	8738443
3	5.712	8589662	5.772	8693353
4	5.707	8572795	5.749	8748581
5	5.698	8672245	5.740	8708626
6	5.702	8574168	5.732	8705573
Average	5.709	8617196	5.757	8724533
Std. Dev	0.007	16998	0.022	24838
% RSD	0.13	0.19	0.38	0.29

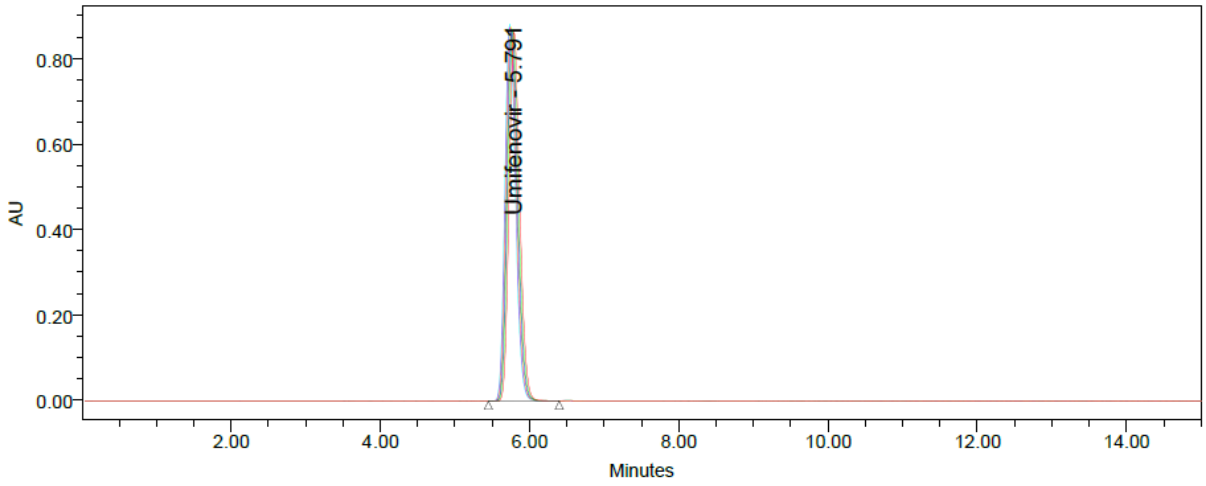

Figure 2: Chromatogram of Umifenovir 200µg/mL analyzed by optimized Isocratic RP-HPLC method.

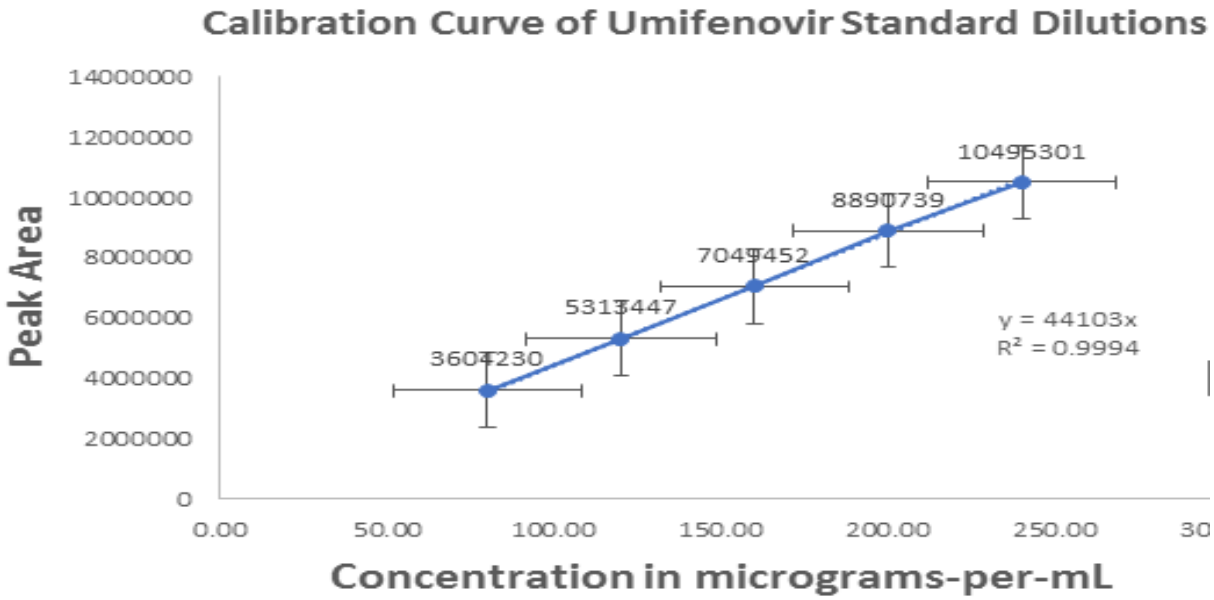

Figure 3: Linearity graph of Umifenovir standard solution

CONCLUSION

In this study, an economical, efficient and commonly available HPLC method for the analysis of Umifenovir in pharmaceutical matrices was devised. This method's key advantages are its significantly reduced cost, ease of use, reduced run time and ease of operation. All these features are critical in operation, especially when analyzing a large number of samples. The validation experiments demonstrated that the procedural approach has a large calibration concentration range, adequate precision & accuracy, and practically reliable sensitivity. The method can be used for regular analysis in formulation QC-studies and allows for a straightforward, selective, sensitive, and specific assessment of Umifenovir.

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