ORIGINAL ARTICLE

New stability indicating ultrafast liquid chromatographic method for the determination of umifenovir in tablets

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Abstract

Introduction: A new stability indicating RP-UFLC method was proposed for the determination of Umifenovir in bulk and its tablet dosage forms. **Materials and Methods**: Chromatographic separation was achieved through C18 Agilent column(150 mm × 4.6 mm i.d., 3.5 µm particle size) using acetonitrile: 0.1% triethylamine (pH adjusted to 3.2 with orthophosphoric acid) mixture as the mobile phase. The UFLC system was monitored at 223 nm on isocratic mode with flow rate 0.6 mL/min and the total run time is 10 min. The method was validated, and forced degradation studies were performed. **Results and Discussion**: Umifenovir has obeyed Beer-Lambert's law over a concentration range 0.05–50 µg/mL with correlation coefficient 0.9997. The limit of detection and limit of quantification are found to be 0.0156 and 0.0421 µg/mL, respectively. Umifenovir was found to be highly sensitive toward alkaline conditions. **Conclusions**: It is observed that this reverse phase UFLC method is accurate, precise, sensitive, and reproducible for the estimation of Umifenovir in tablets. The method was validated as per the ICH guidelines and very much specific as the degradants were well separated without interfering the drug peak.

Key words: ICH guidelines, stability indicating, UFLC, Umifenovir

INTRODUCTION

Umifenovir [Figure 1] is an antiviral drug used for influenza infection is an indole derivative.[1] The drug inhibits viral entry into target cells and also stimulates the immune response. It inhibits membrane fusion and prevents contact between virus and target host cells.[2-6] Umifenovir is manufactured and made available as both tablets and capsules This drug has also been investigated for its usage in treatment of hepatitis C virus. So far no literature has been found regarding this drug. Wang et al., identified the metabolites in human urine and studied the fragmentation pathways using HPLC coupled with ion trap mass spectrometry.[7] The authors have approached to develop a stability indicating liquid chromatographic method and validated as per ICH guidelines.[8] Forced degradation studies were also performed to study the stability[9] of Umifenovir in different environments.

MATERIALS AND METHODS

Chemicals and Reagents

Umifenovir was procured from HONOURS Labs Ltd. (India). Umifenovir is available as tablets (Arpeflu; Label claim: 50 mg; 100 mg) and capsules (Arbidol; Label claim: 100 mg) The formulation is not available in India. Hence, tablets are prepared with the available excipients in the laboratory and extracted with the mobile phase during the study. All other chemicals are of AR grade, and all solvents are of HPLC grade. The analysis of Umifenovir was performed using Shimadzu Model CBM-20A/20 Alite UFLC system

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(Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence photodiode array detector.

Optimized Chromatographic Conditions

C_{18} column (Agilent) (150 mm × 4.6 mm i.d., 3.5 µm particle size) was employed for the chromatographic study. Chromatography work was performed on isocratic mode using a mixture of 0.1% triethylamine (TEA) and acetonitrile (40: 60, v/v) as mobile phase with flow rate 0.6 mL/min (ultraviolet detection at 223 nm). The overall run time was 10 min and the study was observed at ambient temperature (25°C±2°C).

Preparation of Drug Solutions

Accurately 25 mg of Umifenovir was taken in a 25 mL volumetric flask, and volume is made up to the mark with HPLC grade acetonitrile (1000 µg/mL), diluted with mobile phase and filtered through a membrane filter.

Method Validation

The method was validated by evaluating linearity, recovery, precision, accuracy, system suitability, solution stability, limit of detection (LOD), limit of quantification (LOQ), and robustness as per the ICH guidelines for the determination of Umifenovir.

Linearity, Precision, Accuracy, and Robustness Studies

Different diluted solutions (0.05–50 µg/mL) were prepared from the stock, and 20 µL of each solution was injected into the UFLC system, and the peak area of the chromatogram was noted. A graph was plotted using concentration on the X-axis and the mean peak area on the Y-axis. Intraday and interday precision was studied using three different concentrations of Umifenovir on the same day and 3 consecutive days, respectively. The accuracy of the method was proved by the standard addition method, and the recovery values were determined. The robustness of an analytical procedure indicates its ability to remain unaffected by small and deliberate changes in method parameters and provides an assurance of its reliability for routine analysis. The proposed method was checked for the robustness by slightly changing the optimized conditions such as flow rate (± 0.1mL), mobile phase composition (± 2%), pH (± 0.2 units), and detection wavelength (228 nm and 218 nm).

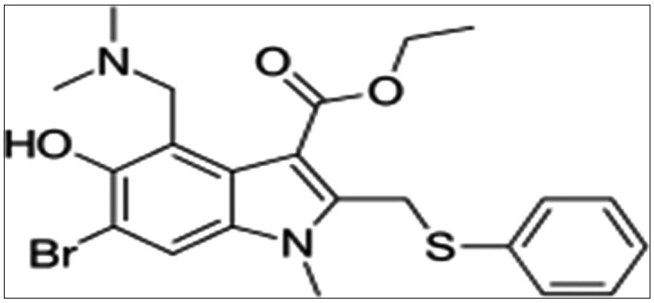

Figure 1: Chemical structure of Umifenovir

Assay of Commercial Formulation

The tablets prepared laboratory was powdered and powder equivalent to 25 mg Umifenovir was extracted using the mobile phase. The solution was sonicated for half an hour and filtered through 0.45 mm membrane filter. 20 µL of this solution was injected into the UFLC system, and the peak area was noted from the respective chromatogram.

Stability Studies

Forced degradation studies were performed to determine the ability of the drug to withstand its properties in the applied stress conditions. Umifenovir was exposed to different stress conditions such as acidic hydrolysis, basic hydrolysis, oxidation, and hydrolysis treatment. Degradation studies were performed by heating the solutions on a water bath for 30 min at 80°. In case of acidic degradation, the drug solution was treated with 0.1N HCl, heated, cooled, and neutralized with 0.1N sodium hydroxide solution and the solution was made up to volume to the required concentration with the mobile phase. Similarly, the alkaline degradation was performed by treating the drug solution with 0.1 N NaOH, heated, neutralized with hydrochloric acid and diluted with mobile phase. Oxidative degradation was performed by treating the drug solution with 30% v/v H_2O_2, heated, cooled, and diluted with mobile phase. Hydrolysis was performed by treating the solution with HPLC grade water, heated, and the solution was made to the required concentration with the mobile phase. All the solutions were filtered through Whatman membrane filter No. 45 and 20 µL of each solution was injected into the UFLC system, and the peak area was noted from the corresponding chromatogram.

RESULTS AND DISCUSSION

A simple stability indicating reverse phase ultrafast liquid chromatographic method has been developed for the determination of Umifenovir in active pharmaceutical ingredient and its tablet dosage forms using C18 Agilent column and a mixture of acetonitrile and 0.1% Triethylamine as the mobile phase.

Method Development and Optimization

UFLC system was initially optimized using C8 phenomenex column with 0.1% formic acid and acetonitrile (50: 50, v/v) as the mobile phase. A low concentration of Umifenovir was injected into the system, and a quite broad chromatogram was eluted with very low theoretical plates (i.e., <2000). Trials were made with different mobile compositions, columns, flow

Table 1: Method optimization (Trial runs)

Column	Mobile phase (v/v)	Flow rate (mL/min)	Rt (min)	Comments	Figure
C8 phenomenex	Formic acid: ACN (50:50)	0.6	4.893	Theoretical plates<2000 Tailing factor>1.5	1(A)
Luna phenyl-hexyl	Phosphate buffer: ACN (30:70)	0.5	3.974	Fronting	1(B)
Luna phenyl-hexyl	Phosphate buffer: ACN (20:80)	0.5	3.509	Fronting	1(C)
C18 Agilent	ACN: TEA (0.1%) (50:50)	0.6	4.203	Fronting	1(D)
C18 Agilent	ACN: TEA (0.1%) (35:65)	0.8	2.976	Fronting	1(E)
C18 Agilent	ACN: TEA (0.1%) (40:60)	0.6	3.003	Method optimized	1(F)

Table 2: Optimized conditions for determination of Umifenovir

Parameter	Optimized chromatographic conditions
Mobile Phase	0.1% Triethylamine and Acetonitrile (40: 60, v/v)
Stationary Phase	C18 Agilent column (150 mm×4.6 mm i.d., 3.5 µm particle size)
Flow Rate	0.6 mL/min
Detection Range	223
Column temp.	(25°±2°C)
Injection Volume	20 µL
Detector	SPD M20A prominence photodiode array detector
Elution	Isocratic mode
Total run time	10 min
Retention time	3.003±0.02 min

Table 3: Linearity of Umifenovir

Conc.(µg/mL)	*Mean peak area	% RSD
0.05	6685.3	0.47
1	132829.3	0.12
5	6681573.3	1.01
10	1323628	0.42
15	1963248	1.43
20	2635125	0.84
25	3294769	0.36
30	3865085	0.73
50	6432712	1.66

*Mean of three replicates. RSD: Relative standard deviation

rates, and the observations were shown in Table 1 and Figure 2. C18 Agilent column (150 mm × 4.6 mm i.d.3.5 µm particle size) with mobile phase composition 0.1% triethylamine: Acetonitrile (40: 60, v/v) and flow rate 0.6 mL/min was found to be more appropriate to satisfy the system suitability parameters and the method was optimized [Table 2] where a sharp drug peak was eluted at 3.003 ± 0.02 min [Figure 3].

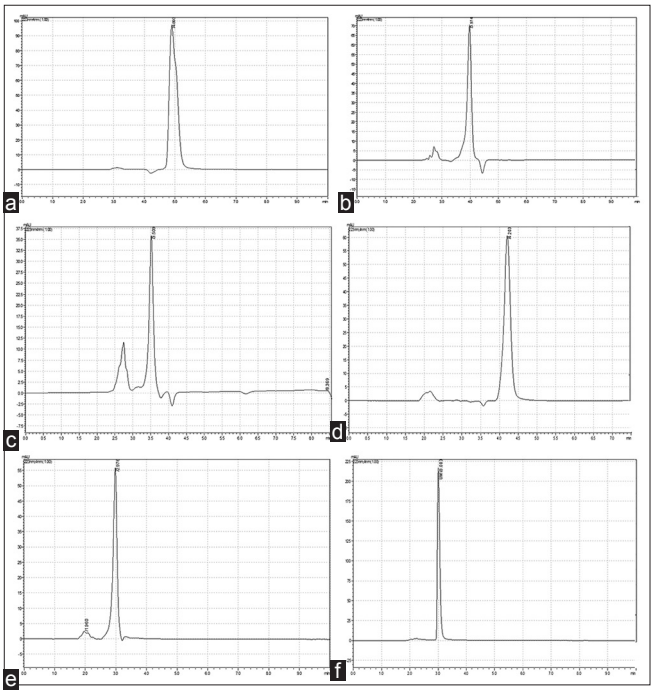

Figure 2: (a-f) Chromatograms observed during method optimization of Umifenovir (Trial runs)

Umifenovir has shown 99.50 recovery in the formulation developed in our laboratory and the chromatogram obtained in the formulation was shown in Figure 3c.

Method Validation

The proposed method was validated by linearity, precision, accuracy, and robustness as per the ICH guidelines for the Umifenovir determination. The calibration curve was drawn taking a concentration of Umifenovir on X-axis and the corresponding mean peak area values on the Y-axis. Umifenovir obeys Beer Lambert's law over the concentration range 0.05– 50 µg/mL [Table 3] with linear regression equation y = 12916x + 23282 (correlation coefficient 0.999 [Figure 4]. The LOD and LOQ are found to be 0.0156 µg/mL and 0.0421 µg/mL, respectively.

Intraday and interday precision was studied using three different concentrations of Umifenovir on the same day

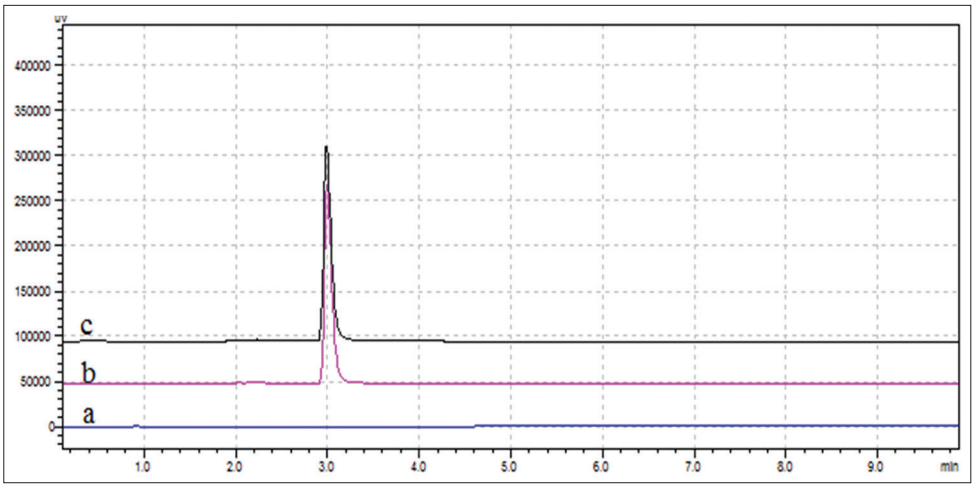

Figure 3: Typical chromatograms (a) Blank, (b) Umifenovir standard (10 µg/mL), and (c) Umifenovir tablets (10 µg/mL)

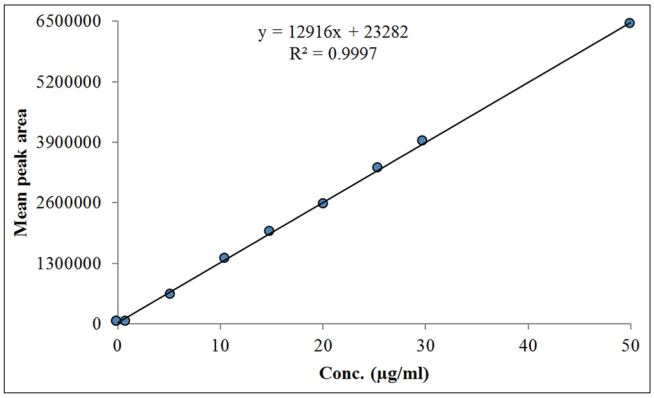

Figure 4: Calibration curve of Umifenovir

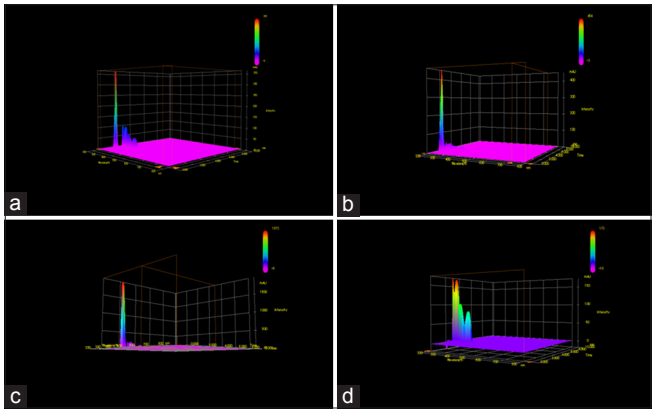

Figure 5: (a) Typical chromatograms of Umifenovir Acidic hydrolysis, (b) alkali hydrolysis, (c) oxidation, and (d) hydrolysis

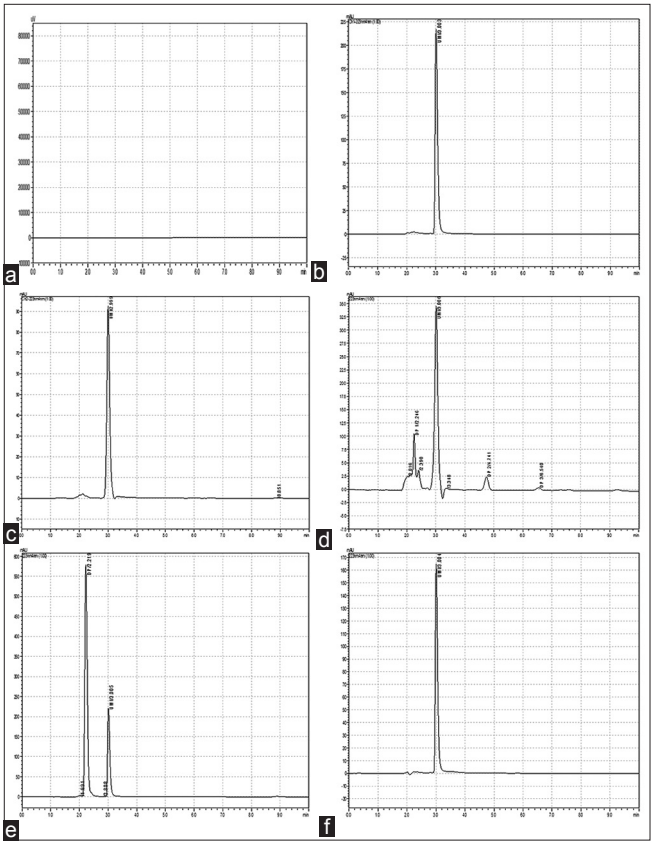

Figure 6: Typical chromatograms of Umifenovir (10 µg/mL) (a) blank, (b) standard, (c) acidic hydrolysis, (d) alkaline hydrolysis, and (e) oxidation (f) hydrolysis

and on 3 consecutive days, respectively. The percentage standard deviation (RSD) was found to be 0.42–0.84 and 0.12–0.54, respectively (<2.0%), demonstrating that the method is precise [Tables 4 and 5]. The accuracy of the method was proved by the standard addition method, and the recovery values were determined. The percentage recovery of Umifenovir and its results of the method are reported in Table 6. The percentage RSD was found to be 0.36–1.45 (<2.0%) with a recovery of 98.80–101.68 %. The percentage RSD was found to be 0.47–0.88 (<2.0%) in robustness study.

The system suitability and solution stability were evaluated, and the percentage RSD was <2%.

Stress Degradation Studies

Umifenovir was exposed to various stress conditions such as acidic, oxidative, hydrolysis, and alkaline hydrolysis. In acidic hydrolysis, Umifenovir was eluted at 3.003 min. About 50.92% of degradation was observed, and it may be due to the

Table 4: Intraday precision study of Umifenovir

Conc. (µg/mL)	*Mean peak area	Statistical analysis
		*Mean±SD (% RSD)
10	1328645	132829.33±5658.89 (0.42)
10	1317494	
10	1324746	
20	2610109	2635125±22370.81 (0.84)
20	2653210	
20	2642056	
30	3895919	3865085±28498.56 (0.73)
30	3839712	
30	3859625	

*Mean of three replicates. RSD: Relative standard deviation, SD: Standard deviation

Table 5: Interday precision study of Umifenovir

Conc. (µg/mL)	*Mean peak area			*Mean±SD(% RSD)
	Day 1	Day 2	Day 3	
10	1322640	1321953	1334746	1326446±7195.925 (0.54)
20	2600105	2610215	2613615	2607978±7027.33 (0.26)
30	3894102	3899654	3890268	3894675±4719.93 (0.12)

*Mean of three replicates. RSD: Relative standard deviation, SD: standard deviation

Table 6: Accuracy study of Umifenovir

Conc (µg/mL)			*Mean Conc. (µg/mL) ±SD (%RSD)	% Recovery
Formulation	Pure drug	Total		
10	5	15	15.01±0.21 (1.45)	101.68
10	5	15		99.88
10	5	15		98.80
10	10	20	20.22±0.173 (0.85)	100.13
10	10	20		98.87
10	10	20		101.37
10	15	25	25.32±0.092 (0.36)	100.54
10	15	25		99.49
10	15	25		100.88

*Mean of three replicates. RSD: Relative standard deviation, SD: Standard deviation

Table 7: Stress degradation studies of Umifenovir

Stress condition Medium/temp./duration	Rt (min)	% Recovery	% Drug degradation	Theoretical plates	Tailing factor
Standard drug	3.003	100	-	5003.976	1.714
Acidic hydrolysis 0.1 N HCl/80°C/30 min	2.999	49.90	50.92	3570.849	1.248
Alkaline hydrolysis 0.1 N NaOH/80°C/30 min	3.006 2.246 4.741 6.540	22.39	77.60	3468.12	1.332
Oxidation 30%H_2O_2/80°C/30 min	3.005 2.219	94.78	5.21	5560.21	1.68
Hydrolysis Water/80°C/30 min	3.004	75.59	24.41	5347.22	1.19

amino moiety attached to the phenoxy phosphoryl part of the Umifenovir drug molecule. Most of the drug has been degraded in alkaline hydrolysis (77.60%) and degradant peaks at 2.246, 4.741, and 6.540 min. The instantaneous degradation of Umifenovir may be definitely due to the propionic acid moiety present in it. Due to oxidation degradation peak at 2.219 min has been observed along with the drug peak at 3.005 min (drug degradation 5.21%). In hydrolysis, the drug peak was eluted at 3.004 min, and no degradants were reported. It is confirmed that the drug is sensitive toward acidic conditions and more sensitive toward alkali. In all the degradation studies, it was found that the drug peak was well separated among the degradants indicating that the method is selective and specific. The system suitability parameters were well in the acceptance criteria [Table 7]. The 3D as well as the typical chromatograms obtained during degradation studies were shown in Figures 5 and 6.

CONCLUSION

The validated stability indicating method developed for the determination of Umifenovir is specific and selective and more economical. Umifenovir is known to be more sensitive toward basic environment. This method can be excellently applied for the determination of Umifenovir in tablets.

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