RESEARCH ARTICLE

Quantification of Arbidol by RP-HPLC with photo diode array detection

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Abstract

Introduction: A new liquid chromatographic method has been developed for the determination of Arbidol in capsules. Arbidol is the brand name for the anti-viral drug Umifenovir. Umifenovir is used for the treatment for influenza infections. **Materials and Methods:** SHIMADZU HPLC system with Agilent column (150 mm × 4.6 mm i.d., 3.5 µm particle size) was used for the chromatographic study of Umifenovir on isocratic mode. A mixture of 10 mM tetra butyl ammonium hydrogen sulphate and acetonitrile (50: 50, v/v) was used as mobile phase with flow rate 0.8 mL/min. for the chromatographic separation of Umifenovir. PDA detector was used (UV detection: at 223 nm) and the total run time was 10 mins. **Results and Discussion:** Umifenovir has obeyed Beer-Lambert's law 0.5-50 µg/mL with correlation coefficient 0.9999. The LOD and LOQ are found to be 0.1581 µg/mL and 0.4829 µg/mL respectively. **Conclusions:** The method is simple, precise and accurate and can be used for the determination of Umifenovir. The method was validated as per ICH guidelines.

Key words: Arbidol (Umifenovir), eplerenone (internal standard), high-performance liquid chromatography, ICH guidelines, validation

INTRODUCTION

Arbidol (Umifenovir [UMI]) is used for the treatment of hepatitis C virus [Figure 1]. It is also used as an antiviral drug for influenza infection. UMI was currently given the license for the prevention and treatment of influenza. It belongs to the category of fusion inhibitors.[1-4] It inhibits membrane fusion and prevents contact between virus and target host cells.[5,6] Annapurna *et al*. established stability indicting ultrafast liquid chromatographic (LC) method for the determination of UMI in tablets[7] and Wang *et al*. identified the metabolites present in human urine with the help of fragmentation patterns with LC–mass spectrometry LC-MS.[8] In the present study, the authors have developed a validated LC method for the quantification of UMI in the presence of Eplerenone as internal standard (IS). In the present study, a new LC method has been developed for the quantification of UMI [Figure 1], and the method was validated as per the ICH guidelines.[9]

MATERIALS AND METHODS

Chemicals and reagents

UMI is available as capsules with brand name Arbidol. UMI was obtained from HONOURS (India). As the formulation is under development in India, its capsule formulation was prepared in the laboratory with commonly available excipients. High-performance LC (HPLC) grade (Merck) solvents and AR grade chemicals are used. Shimadzu Model CBM-20A/20 Elite HPLC system (Shimadzu Co., Kyoto, Japan) was used with SPD M20A prominence photodiode array (PDA) detector.

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Stock solutions of UMI and Eplerenone were prepared by dissolving 25 mg of Eplerenone and UMI separately in two 25 mL volumetric flasks with HPLC grade methanol (1000 µg/mL), diluted with mobile phase and filtered. Eplerenone (20 µg/mL) was used as an IS throughout the study.

Chromatographic conditions

The chromatographic system (PDA detector) was optimized with Agilent column (150 mm × 4.6 mm i.d., 3.5 µm particle size) using a mixture of 10 mM tetra butyl ammonium hydrogen sulfate and acetonitrile (50: 50, v/v) (isocratic mode). The same mobile phase mixture but with different composition was used as a diluent (50:50, v/v). Eplerenone (20 µg/mL) was used as an IS throughout the analysis. The system was monitored at 223 nm with flow rate of 0.8 mL/min and the overall run time was 10 mins, and the study was observed at ambient temperature (25°C ± 2°C). All the drug solutions were periodically monitored for their stability on daily basis.

Method validation

0.5–50 µg/mL UMI drug solutions were prepared from the stock solution and 20 µg/mL eplerenone was added to them. 20 µL of each solution was injected into the HPLC system, and the peak areas of UMI and eplerenone were noted from the respective chromatograms. Calibration graph was drawn by plotting the concentration of UMI on the X-axis and the corresponding mean peak area ratio (UMI/EPL) on the Y-axis. Precision and accuracy were studied in the presence of IS. The proposed method was checked for the robustness by varying the optimized chromatographic conditions.

Assay of commercial formulations

UMI capsules were prepared in the laboratory with the available excipients and powder equivalent to 10 mg UMI was extracted with acetonitrile and later diluted with mobile phase. 20 µL of this UMI solution was injected along with the IS and the peak area ratio was determined from the respective chromatogram from the linear regression equation.

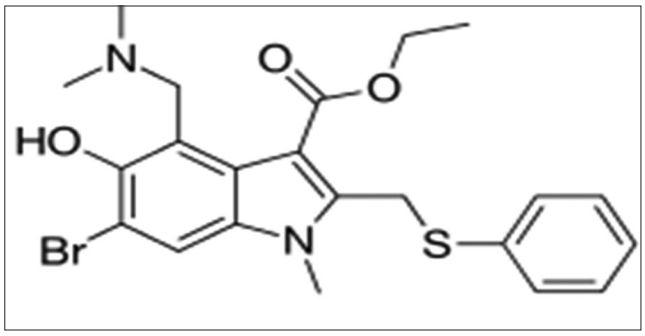

Figure 1: Chemical structure of Arbidol

Table 1: Optimized chromatographic conditions

Parameter	Optimized chromatographic conditions
Detector	SPD M20A prominence photodiode array detector
Flow rate	0.8 mL/min
Ultraviolet detection	223 nm
Column temperature	(25°±2°C)
Injection volume	20 µL
Elution	Isocratic mode
Mobile phase	Tetra butyl ammonium hydrogen sulfate: Acetonitrile (50: 50 v/v)
Column particulars	Agilent column (150 mm×4.6 mm i.d., 3.5 µm particle size)
Retention time	2.175±0.02 min (Umifenovir) 3.420±0.02 min (Internal standard)
Total runtime	10 min

UMI: Umifenovir

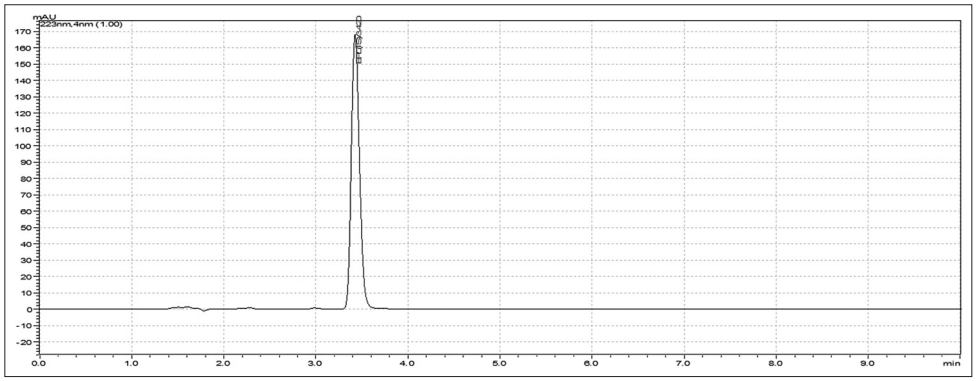

Figure 2: Typical chromatogram Eplerenone (internal standard) (20 µg/mL) C (Rt 3.420 min)

RESULTS AND DISCUSSION

A simple LC method has been developed for the determination of Arbidol capsules in the presence of IS [Figure 2], and the optimized conditions were shown in Table 1. UMI was eluted at 2.175 ± 0.02 mins and Eplerenone at 3.437 [Figure 3].

Method validation

UMI obeys Beer-Lamberts law (0.5–50 µg/mL) [Table 2] with linear regression equation, y = 0.0362 × −0.0039 [Figure 4], and correlation coefficient 0.9999. The limit of detection and limit of quantitation are found to be

Table 2: Linearity of UMI in the presence of Eplerenone (IS)

Conc. (µg/mL)		*Mean peak area		Peak area ratio (UMI/EPL)	% RSD
UMI	EPL	UMI	EPL		
0.5	20	35922	1980550	0.018	0.21
5	20	359326	1981059	0.17	0.24
10	20	719053	1982047	0.36	0.39
15	20	1079657	1981054	0.54	0.34
20	20	1398104	1971098	0.70	0.26
25	20	1788639	19806940	0.90	0.28
30	20	2158149	1980157	1.08	0.30
40	20	2867214	1983526	1.44	0.25
50	20	3594275	1980143	1.81	0.22

*Mean of three replicates. IS: Internal standard, UMI: Umifenovir, % RSD: % relative standard deviation

Table 3: Intraday precision study of UMI in the presence of IS

Conc. (µg/mL)	*Mean peak area			Statistical analysis *Mean±SD (% RSD)
	UMI	EPL	UMI/EPL	
10	719053	1981124	0.362952	0.36±0.0001 (0.05)
10	719765	1981113	0.363314	
10	719562	1981021	0.363228	
20	1438104	1981048	0.725930	0.72±0.0025 (0.35)
20	1437821	1981053	0.725786	
20	1429102	1981041	0.721389	
30	2158149	1980998	1.089425	1.08±0.0001 (0.01)
30	2157622	1981013	1.089150	
30	2157431	1981002	1.089060	

*Mean of three replicates. IS: Internal standard, UMI: Umifenovir, % RSD: % relative standard deviation

Table 4: Interday precision study of UMI in the presence of IS

Conc. (µg/mL)	*Mean peak area			Statistical analysis *Mean±SD (% RSD)
	UMI	EPL	UMI/EPL	
10	719062	1981220	0.362938	0.36±0.0002 (0.05)
10	719758	1981045	0.363322	
10	719062	1981106	0.362959	
20	1429101	1981054	0.721384	0.72±0.0025 (0.37)
20	1437818	1981062	0.725781	
20	1438102	1981045	0.725931	
30	2157430	1981302	1.088895	1.08±0.0003 (0.02)
30	2157628	1981510	1.088880	
30	2158145	1981023	1.089409	

*Mean of three replicates. IS: Internal standard, UMI: Umifenovir, % RSD: % relative standard deviation

Table 5: Accuracy study of UMI in the presence of IS

Conc. (µg/mL)			*Mean±SD (%RSD)	% Recovery
Formulation	Pure drug	Total		
10	5	15	15.01±0.28 (1.49)	100.99
10	5	15		99.78
10	5	15		99.90
10	10	20	20.22±0.172 (0.82)	100.13
10	10	20		100.87
10	10	20		99.80
10	15	25	25.01±0.072 (0.36)	100.25
10	15	25		99.49
10	15	25		100.79

*Mean of three replicates. IS: Internal standard, UMI: Umifenovir, % RSD: % relative standard deviation

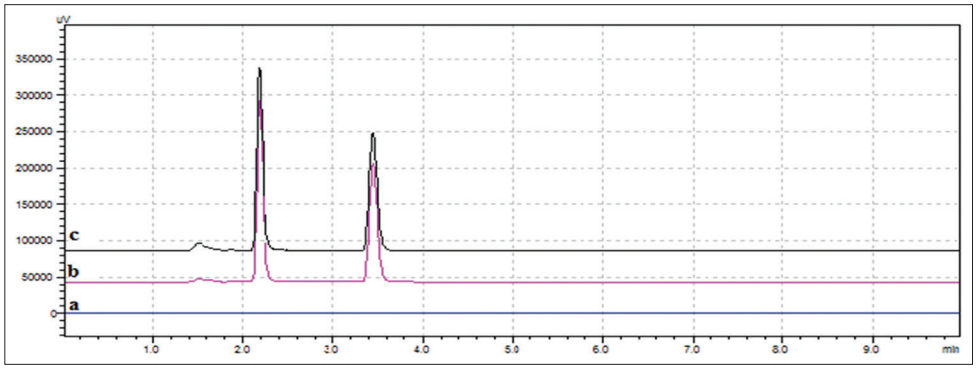

Figure 3: Typical chromatogram of (a) blank (b) Umifenovir with an internal standard (IS) (c) formulation (capsule) in the presence of IS

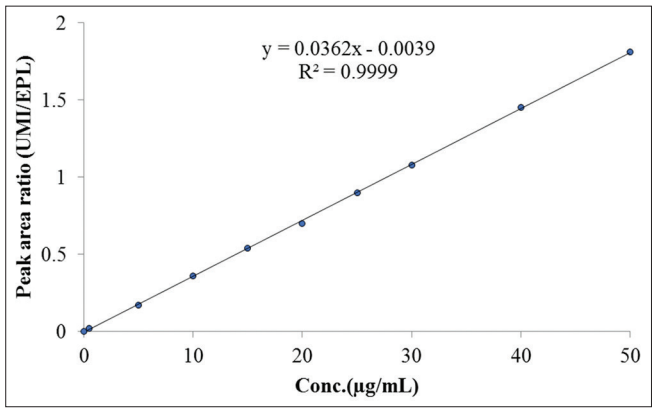

Figure 4: Calibration of Umifenovir in the presence of internal standard

0.1581 µg/mL and 0.4829 µg/mL, respectively. The percentage relative standard deviation (% RSD) was found to be 0.01–0.35 (intraday) [Table 3] and 0.02–0.37 (interday) [Table 4], respectively (<2.0%), in precision study indicating that the method is precise. In accuracy study, the recovery values were found to be 99.49–100.99% [Table 5]. The percentage RSD in accuracy study and robustness study were found to be 0.36–1.49 and 0.91–1.56 which is <2.0. The above results indicate that the method is precise, accurate, and robust. The theoretical plates were found to be 5885 for the IS and 3675 for UMI (>2000), and the tailing factor was found to be 1.246 for the IS, and 1.407 for UMI (<1.5) and the resolution is more than 2.

Assay of commercial formulations

UMI has shown 99.59% recovery in the laboratory-prepared capsule formulation in the presence of IS. The recovery was calculated from the linear regression equation. The chromatogram obtained during the assay was shown in Figure 3, and there is no interference of the excipients.

CONCLUSIONS

The proposed LC method was validated as per the ICH guidelines and can be used for the quantification of Umifenovir in pharmaceutical industries.

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