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Research Article

Stability Indicating LC-APCI-MS Methods for the Quantification of Trifluridine by Tandem Triple Quadrupole Mass Spectrometry

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Abstract

Trifluridine is an anti-viral drug. A new stability indicating isocratic LC-MS method has been developed and validated for the quantification of Trifluridine as per ICH guidelines. Thermo scientific-TSQ Quantis with Vanquish HPLC coupled with MS was employed for the present study. Trifluridine and its degradation products were separated using Shimpack C18 (250 mm x 4.6 mm x 5 µm) column using 0.1% acetic acid: methanol as mobile phase at 1.0 ml/min flow rate, 10 µL injection volume and 259 nm detection wavelength. Four major acid degradation products and two alkaline degradation products were identified and characterized by liquid chromatography-APCI mass spectrometry (LC-APCI-MS). The plausible mechanism for the formation of degradation products was identified based on the fragmentation pattern of Trifluridine. The proposed method is simple, precise, accurate, robust and used for the routine analysis of marketed formulations of Trifluridine.

Keywords: Trifluridine, HPLC, LC-APCI-MS, Forced degradation studies, Validation

Introduction

Trifluridine (Figure 1), chemically 1- [(2R, 4S, 5R)-4-hydroxy-5 (hydroxyl methyl) oxolan-2-yl]-5-(tri Fluoro-methyl) pyrimidine-2, 4-dione ($C_{10}H_{11}F_3N_2O_5$) is used for the treatment of viral infections of eye [1]. It acts on viral DNA synthesis by forming the defective proteins that intensifies the mutation rate [2]. Sai Pavan Kumar., *et al*. developed a stability indicating RP-UFLC method [3] for the estimation of Trifluridine using C_{18} Shim-pack GWS HPLC packed column with mobile phase mixture consisting of acetonitrile: 10 mM potassium dihydrogen phosphate buffer (pH adjusted to 3.5 with dilute tri fluro acetic acid) (70:30) with 1.0 ml/min flow rate (Detection wavelength 272 nm) on an isocratic mode. In this method Trifluridine obeys Beer-Lambert's law over the concentration range 0.1-120 µg/ml. Spandana Yasaswini., *et al*. developed a stability indicating RP-UFLC method [4] for the estimation of Trifluridine using acetonitrile: water (50:50) as mobile phase with UV detection at 261 nm and the linearity was observed as 1-100 µg/ml. Sai Gnaneswari and Annapurna developed a stability indicating RP-HPLC method [5] for the quantification of Trifluridine using formic acid: methanol (45:55) as mobile phase with UV detection at 259 nm and the linearity was observed as 0.5-120 µg/ml. Mohammad., *et al*. developed a LC-MS/MS technique [6] for the quantification of Trifluridine in presence of an internal standard, *β*-thymidine in human plasma. Mobile phase mixture consisting of acetonitrile: methanol: ammonium formate (45:40:15) with flow rate 0.8 ml/min was chosen for the study and the linearity was observed over the concentration range 0.005-2.0 µg/ml. Some of the heterocyclic ring systems were established

to show the anti-viral[7] and anti-oxidant [8,9] properties. In the present study a new stability indicating HPLC method coupled with mass spectrometry has been proposed for the quantification of Trifluridine and the method was validated as per ICH guidelines.

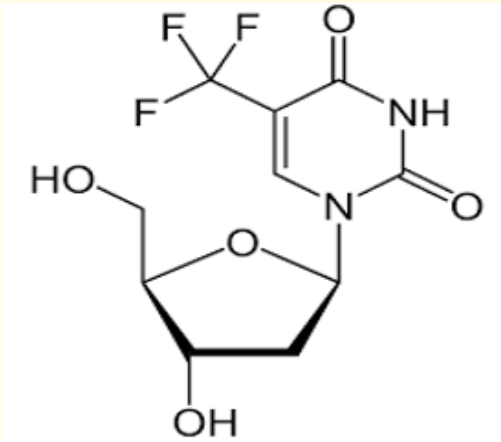

Figure 1: Structure of Trifluridine ($C_{10}H_{11}F_3N_2O_5$).

Materials and Methods

Instrumentation

HPLC conditions

TSQ scientific Quantis LCMS with Thermo Vanquish model HPLC with PDA detector and Simpack C18 (250 mm x 4.6 mm x 5 µm) column was employed for the present study. The injection volume was 10 µL and the total run time was 25 mins (Detection wavelength 259 nm). 0.1% Acetic acid: Methanol (75: 25, v/v) was used as mobile phase with flow rate was 1 ml/min on isocratic mode.

MS conditions

Ion Source type: APCI

Spray Voltage: Static

Positive Ion (V): 3500

Negative Ion (V): 2500

Sheath Gas (Arb): 45

Aux Gas (Arb): 10

Sweep Gas (Arb): 2

Ion transfer tube temperature: 275

Vaporizer temperature: 400

Scan mode: Full scan Q1

Scan Range: 50-2000 m/z (positive mode)

: 50-2000 m/z (Negative mode)

Preparation of stock solution

50 mg of Trifluridine API was weighed accurately and transferred carefully into a 50 ml volumetric flask and was dissolved in HPLC grade methanol (1000 µg/ml) and the resulting solution was sonicated for 30 mins and dilutions were made further with the mobile phase and all the solutions were filtered.

Method validation [10]

Linearity, Precision, Accuracy and Robustness

0.2-100 µg/ml Trifluridine solutions were prepared from the stock solution (1000 µg/ml) and each solution was injected (n=3) into the LC system and the average peak area from the respective chromatograms was calculated. A calibration graph was drawn by plotting the concentration of the drug solutions on the x-axis and the corresponding peak area of the chromatograms on the y-axis. The intraday precision studies were conducted on the same day at different equal time intervals and the interday precision studies were conducted on three successive days (Day 1, Day 2 and Day 3) and the % RSD was calculated. Accuracy studies were performed by spiking the formulation solution with 50, 100 and 150% API solution and thereby the percentage recovery was calculated with the help of regression equation. Robustness of the method was performed by incorporating small changes in the chromatographic conditions. The percentage relative standard deviation was calculated in all the validation parameters.

Assay of trifluridine ophthalmic solution (1%)

Pfizer Laboratories (India) and Sandoz Falcon Pharmaceuticals (India) supply 1% Trifluridine ophthalmic solution in the pharmaceutical market. Two different brands of Trifluridine were procured and Trifluridine was extracted with methanol after

sonication with the mobile phase. The resulting solution was filtered through membrane filter and 10 µL of these formulation solutions were injected in to the HPLC system. The peak area of the chromatogram (n =3) was noted and the percentage purity was determined.

Forced degradation studies [11]

During the acidic degradation study Trifluridine drug solution was treated with 0.1N HCl and immediately neutralized with 1ml 0.1N NaOH solution. The contents were diluted with mobile phase and the resultant solution was injected into HPLC and LCMS system and the peak area as well as the mass spectrum was recorded. During the thermal degradation study Trifluridine drug solution was heated at 60ºC with 0.1N HCl for about 30 mins and then neutralized with 1ml 0.1N NaOH solution. The contents were diluted with mobile phase and the resultant solution was injected into HPLC and LCMS system and the peak area as well as the mass spectrum was recorded. During the basic degradation study Trifluridine drug solution was treated with 0.1N NaOH for about 30 mins and then neutralized with 1ml 0.1N HCl solution. The contents were diluted with mobile phase and the resultant solution was injected into HPLC and LCMS system and the peak area as well as the mass spectrum was recorded. During the oxidative degradation study Trifluridine drug solution was treated with hydrogen peroxide for about 30 mins and then diluted with mobile phase and the resultant solution was injected into HPLC and LCMS system and the peak area as well as the mass spectrum was recorded.

Results and Discussion

A new stability-indicating LC-MS method has been developed for the quantification of Trifluridine. The earlier reported methods were discussed with the present proposed method and the details were given in table 1. Mobile phase consisting of 0.1% Acetic acid: Methanol (75:25, v/v) (Isocratic mode) with 1.0 ml/min flow rate (Detection wavelength 259 nm) are the optimized chromatographic conditions. Trifluridine was eluted at Rt 8.017 min with theoretical plates more than 2000 and tailing factor less than 1.5. The HPLC and LC-MS chromatograms and mass spectra of Trifluridine obtained in the optimized chromatographic conditions were shown in figure 2.

Table 1: Literature survey.

Method	Reagent/Mobile phase (v/v)	Linearity (µg/ml)	Ref
RP-UFLC	Acetonitrile: Potassium dihydrogen phosphate buffer (pH adjusted to 3.5 with TFA) (70:30)	0.1-120	3
RP-UFLC	Acetonitrile: Water (50:50)	1-100	4
RP-HPLC	Formic acid: Methanol (45:55)	0.5-120	5
LC-MS/MS (Human plasma) (Internal standard: β-thymidine)	Acetonitrile: Methanol: 5 mM Ammonium formate (45:40:15)	0.005-2.0	6
HPLC and LC-APCI-MS	0.1% Acetic acid: Methanol (75: 25)	0.01-100	Present method

Figure 2: Representative chromatograms and mass spectra of Trifluridine (API).

Linearity, precision, accuracy and robustness

Trifluridine obeys Beer-Lambert's law over the concentration range 0.2-100 µg/ml (Table 2) and the linear regression equation was found to be y = 2104.7x + 462.99 (R^2 = 0.9999) Figure 3). The LOD and LOQ values were found to be 0.1872 µg/ml and 0.6154 µg/ml respectively. The % RSD in intraday precision (0.0482), interday precision (0.0066-0.385) (Table 3) was found to be less than 2.0% stating that the method is precise. In the accuracy study the % RSD was found to be 0.81-1.01 (<2) (Table 4) with a recovery of 99.19-99.81 and the % RSD in robustness study was found to be 0.0207-0.0754 (Table 5) (<2.0) indicating that the method is accurate and robust.

Table 2: Linearity.

Conc. (µg/ml)	*Mean peak area
0	0
0.2	441.95
0.5	1089.18
1	2197.36
2	4391.28
5	10922.93
10	21924.28
20	42831.95
40	86729.24
60	127489.70
80	168311.81
100	209999.30

*Mean of three replicates

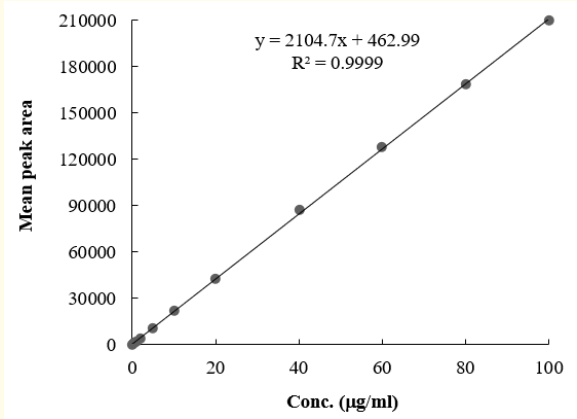

Figure 3: Calibration curve.

Assay of trifluridine

The assay of Trifluridine was performed using the proposed liquid chromatographic method with the optimized chromatographic conditions. The percentage of purity of Trifluridine was found to be 99.51-99.76 (Table 6).

Table 3: Precision study.

Intraday precision study				
Conc. (µg/ml)	**Mean peak area**	**Statistical analysis *Mean peak area ± SD (% RSD)**		
10	21924.28	21915.85 ± 10.56029 (0.0482)		
10	21904.36			
10	21911.58			
10	21903.89			
10	21927.37			
10	21923.63			
Interday precision study				
Conc. (µg/ml)	**Day 1**	**Day 2**	**Day 3**	**Statistical analysis *Mean peak area ± SD (% RSD)**
5	10922.93	10918.63	10914.89	10916.76 ± 2.6446 (0.0242)
10	21924.28	21936.19	21919.87	21926.78 ± 8.4423 (0.385)
20	42831.95	42827.71	42833.11	42830.923 ± 2.8426 (0.0066)

*Mean of three replicates

Table 4: Accuracy study.

Spiked conc. (µg/ml)	Formulation (µg/ml)	% Recovery	% RSD
10 (50%)	20	99.19	0.81
20 (100%)	20	99.24	0.91
30 (150%)	20	99.81	1.01

*Mean of three replicates.

Table 5: Robustness study (10 µg/ml).

Parameter	Condition	*Mean peak area	*Mean peak area ± SD (RSD)
Flow rate (± 0.1 ml/min)	1.1	21901.17	21919.57 ± 16.53 (0.0754)
	1.0	21924.28	
	0.9	21933.19	
Detection wavelength (± 2 nm)	261	21915.22	21919.55 ± 4.54 (0.0207)
	259	21924.28	
	257	21919.14	
Mobile phase composition 0.1% Acetic acid: Methanol (± 5%)	80: 20	21912.22	21917.60 ± 6.13 (0.0279)
	75: 25	21924.28	
	70: 30	21916.31	

*Mean of three replicates.

Table 6: Assay of Trifluridine ophthalmic solution.

S. No.	Brand name	Label claim (1%)	*Observed amount (gm/100 ml)	% Recovery*
1	Brand I	1 gm/100 ml	0.9951	99.51
2	Brand II	1 gm/100 ml	0.9976	99.76

*Mean of three replicates.

Forced degradation studies

Trifluridine (100 µg/ml) was exposed to different stress conditions under the optimized chromatographic conditions and then diluted to get 10 µg/ml. During the acidic degradation, it was observed that Trifluridine was eluted at Rt 8.117 min and the entire drug has undergone decomposition with degradant peaks at 3.342, 3.967 and 7.392 min. From the mass spectrum (Figure 4) it was observed that at Rt 4.07 min Trifluridine has undergone degradation showing a degradant with m/z 272.21 corresponding to the molecular formula, $C_{10}H_{12}N_2O_7$. The plausible degradation pathway was shown in Scheme 1.

Figure 4: Representative chromatograms and mass spectra of Trifluridine during acidic degradation.

Scheme 1: Plausible degradation pathway of Trifluridine during acidic degradation.

During thermal degradation Trifluridine was eluted at Rt 8.050 min and about 23.93 of the drug has undergone decomposition and the corresponding mass spectrum was shown in figure 5.

During the basic degradation, it was observed that Trifluridine was eluted at Rt 8.125 min and the entire drug has undergone decomposition with degradant peaks at 3.342, 3.967 and 7.392 min. From the mass spectrum (Figure 6) it was observed that at Rt 4.20 min Trifluridine has undergone degradation showing a degradant with m/z 272.08 corresponding to the molecular formula, $C_{10}H_{12}N_2O_7$. The plausible degradation pathway was shown in scheme.

Mass spectrum of Trifluridine during thermal degradation (MH^+ m/z = 297.11)
Figure 5: Representative chromatograms and mass spectra of Trifluridine during thermal degradation.

Figure 6: Representative chromatograms and mass spectra of Trifluridine during basic degradation.

During the oxidative degradation, it was observed that Trifluridine was eluted at Rt 8.100 min and about 44.12% drug has undergone decomposition with a very small degradant peaks at 3.283, 4.206, 5.475, 5.983 and 6.442 min within a run time of 25 mins and the mass spectrum was shown in figure 7.

The details of the forced degradation studies of Trifluridine were shown in table 7. It is observed that Trifluridine is highly sensitive towards acidic and basic degradation conditions and a major degradant of about m/z 272 was observed which is a potential impurity.

Table 7: Forced degradation studies.

Condition	R_t (min)	*Mean peak area	% Recovery*	% Drug degradation
Standard drug	8.017	21924.28	100	-
Acidic hydrolysis 0.1N HCl/30 min	8.117 3.342 3.967 7.392	663.21	3.03	96.97
Thermal degradation 60°C/30 min	8.050	16676.82	76.07	23.93
Alkaline hydrolysis 0.1N NaOH/60°C/30 min	8.125 3.475 3.992 4.233 7.567 9.150	253.44	1.16	98.84
Oxidative degradation H_2O_2/80ºC/30 min	8.100	12252.01	55.88	44.12

*Mean of three replicates.

Conclusion

The new validated stability indicating RP-HPLC coupled with APCI and triple quadrupole analyser has been developed and validated for the estimation and identification of the stress degradation behaviour of Trifluridine. The method is simple, precise, accurate and robust and is very much useful for the regular

analysis of Trifluridine in pharmaceutical formulations and also for the impurity profiling study in pharmaceutical industries. The method is selective and specific and no interference of excipients was observed during the assay.

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