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Original Article

STABILITY INDICATING RP-HPLC AND HPTLC METHODS FOR THE DETERMINATION OF ZANAMIVIR IN BULK AND DOSAGE FORM

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ABSTRACT

Objective: To develop a simple, precise, accurate, validated stability indicating reverse phase high-pressure liquid chromatography and high-performance thin layer chromatography method for the determination of zanamivir in bulk and capsule dosage form.

Methods: The high-pressure liquid chromatography separation was achieved on Agilent TC C18 (2) 250 x 4.6 mm, 5 µ column using mobile phase composition of methanol-0.02 M phosphate buffer, pH 3.5, 50:50 (v/v). Flow rate was maintained at 1 ml/min at an ambient temperature. Quantification was achieved with ultraviolet detection at 230 nm. The retention time obtained for zanamivir was at 3.6 min. The method employed thin layer chromatography aluminum plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of chloroform: methanol: acetic acid (4.5:0.5:0.3*v/v*) and then scanned. The system was found to give a compact spot for zanamivir (Rf value of 0.29±0.02).

Results: In HPLC,the result obtained with the detector response was found to be linear in the concentration range of 2-12 µg/ml. In HPTLC, the linear regression analysis data for the calibration plots showed a good relationship with r^2=0.9999±0.0001 in the concentration range 500-3000 ng/spot. The reliability and analytical performance of the proposed methods, including linearity, range, precision, accuracy, detection and quantitation limits, were statistically validated. When zanamivir was subjected to different stress conditions; the proposed methods could effectively separate the drug from its degradation products, and were thus considered as good stability-indicating procedures.

Conclusion: It is concluded that this method can be applied for routine quality control of zanamivir in dosage forms as well as in bulk drug.

Keywords: Zanamivir, Reverse phase high-performance liquid chromatography, High-pressure thin layer chromatography, Virenza capsule, Method development and validation, Stability indicating

INTRODUCTION

The chemical name of zanamivir (ZMV) is 5-(acetylamino)-4-[(amino imino methyl)-amino]-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonic acid. It has a molecular formula of $C_{12}H_{20}N_4O_7$ and a molecular weight of 332.3[1, 2] as shown in fig. 1. The proposed mechanism of action of zanamivir is via inhibition of influenza virus neuraminidase with the possibility of alteration of virus particle aggregation and release [3, 4]. Mechanism of action has on viral neuraminidase catalyzes cleavages of terminal sialic acid residues attached to glycoprotein and glycolipids, a process necessary for release of virus from host cell surfaces [5].

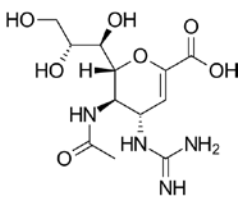

Fig. 1: Chemical structure of Zanamivir

A literature survey reveals there are few analytical methods such as ultraviolet(UV) method [6],HPLC method [7, 8] and high-throughput HILIC–MS/MS study[9] have been reported for estimation of ZMV, but there is no stability indicating method by reverse phase high-pressure liquid chromatography (RP-HPLC), and high-performance thin-layer chromatography (HPTLC) was reported for ZMV. It was felt necessary to develop a stability indicating RP-HPLC and HPTLC methods for the determination of ZMV as bulk and in pharmaceutical dosage form. According to the stability test guidelines issued by ICH [10-14], in the present study, the stress induced stability studies were carried out for ZMV to establish its stability characteristics and also an analytical method develop by RP-HPLC and developed method was validated

MATERIALS AND METHODS

Materials

A pharmaceutical grade of zanamivir kindly supplied as a gift sample by Cipla Ltd., Mumbai, India. All chemicals and reagents used were of HPLC of analytical grade and were purchased from Merck Chemical, India. Analytical grade sodium hydroxide, hydrochloric acid, and 10% hydrogen peroxide were used.

Instrumentation and chromatographic conditions

For HPLC

Agilent technologies 1260 LC system with a gradient pump connected to DAD UV detector, LC-GC AGN204PO balance was used for all weighing an Agilent zorbax eclipse C18 column (150 mm × 4.6 mm i.d., 5 μm) was maintained at 30 °C. The mobile phase was composed of a mixture of methanol-0.02 M phosphate buffer, pH 5, 50:50 (V/V). The flow rate of the mobile phase was set at 1 ml/min Measurements were made with 20 µl of injection volume. The retention time obtained for zanamivir was at 3.6 min. For the analysis of forced degradation samples, the photodiode array detector was used in scan mode with a scan range of 200–400 nm.

For HPTLC

HPTLC has performed on 20 cm x 10 cm aluminum plates coated with silica gel aluminum plate 60F-254 (0.2 mm thickness E. Merck, Germany). ZMV was spotted in the form of bands of width 6 mm with Camagmicrolitre syringe using CamagLinomatV (Switzerland). A constant application rate of 150 nl/sec was employed, and space between two bands was 15 mm. The slit dimension was kept 6 mm x

0.45 mm micro, 20 mm/s scanning speed was employed. The mobile phase consisted of Chloroform: methanol: acetic acid (4.5:0.5:0.3*v/v*). The chromatogram was developed in twin trough glass chamber saturated with mobile phase using the linear ascending technique. The optimized chamber saturation time for mobile phase was 25 min at room temperature. The length of chromatogram run was approximately 80 mm. The system was found to give a compact spot for zanamivir (Rf value of 0.29±0.02). Subsequent to the development; TLC plates were dried in current of air with the help of an air dryer. Densitometric scanning was performed using Camag TLC scanner III in the absorbance mode at 230 nm. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum in the range of 200-400 nm.

Preparation of standard solution and calibration graphs

For HPTLC densitometry

Accurately weighed the quantity of ZMV (10 mg) was transferred to 10.0 ml volumetric flask. Then small amount methanol was added, and ultrasonicated for 5 min and diluted up to the mark with methanol (Concentration: 1000 µg/ml). Different volume of stock solution 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 µl were spotted in six replicates on TLC plates to obtain concentration of 500, 1000, 1500, 2000, 2500,3000 ng/spot of zanamivir respectively

The plate was developed on previously described mobile phase. The peak areas were plotted against corresponding concentrations to obtain the calibration graphs

For HPLC

Stock solutions of ZMV (100µg /ml) were prepared by transferring 10 mg each of ZMV standard in separate 100 mL volumetric flasks, dissolved in 50mL of methanol and made up to volume using the same. ZMV (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml) were transferred to a series of 10 ml volumetric flasks and diluted up to the mark with mobile phase. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.

Preparation of sample solution

For HPTLC densitometry

Twenty tablets were accurately weighed, and average weight per tablet was determined. Tablets were ground to fine powdered and weighed tablet powder equivalent to one tablet of ZMV was transferred to 100 ml volumetric flask. The powder was dissolved in 30 ml methanol by intermittent shaking, and the volume was made up to the mark with methanol. The solution was then filtered through Whatman filter paper no.45. 1 µlof above solution was spotted on TLC plate. The analysis was repeated for six times. ZMV gave sharp and well-defined peaks at Rf 0.29, when scanned at 230 nm.

For HPLC

An accurately weighed 5 mg of ZMV was transferred into 100 ml volumetric flask containing 25 ml of methanol; volume was made up to the mark with methanol and solution was filtered using 0.45 µm filter (Mill filter, Milford, MA). From filtrate, 2 ml of solution was transferred into 10 ml volumetric flask and the volume was made up to mark with mobile phase to obtain the final concentration 10 µg/ml was subjected to propose a method and the amount of ZMV were determined. The assay procedure was repeated for six times.

Method validation

The method was validated for its linearity range, accuracy, precision, sensitivity, and specificity. Method validation is carried out as per ICH guidelines.

Precision

Intra-and inter-day precisions of the methods were determined by performing replicate (n=3) analyses of standards and samples. This procedure was replicated on different days (n=3). Recovery studies by standard addition method were performed in view of justifying the accuracy of the proposed methods. Previously analyzed samples containing ZMV was spiked with standard ZMV, and the mixtures were analyzed in triplicate (n=3) by proposed methods. Precision was calculated from percentage relative standard deviation (RSD %) for repeated measurements, whereas accuracy was expressed as % of recovery.

Robustness and ruggedness of the method

For HPTLC–densitometric method

Robustness was studied in six replicate at the concentration level of 1000 ng/spot. In this study, seven parameters (mobile phase composition, mobile phase volume, development distance, relative humidity, duration of saturation, time from spotting to chromatography and chromatography to spotting) were studied, and the effects on the results were examined.

The ruggedness of the proposed method was studied by two different analysts using the same experimental and environmental conditions. The spots 1000 ng/band of ZMV was applied on TLC plates. This procedure was repeated in triplicates.

For HPLC method

Robustness of the method was studied by making deliberate variations in the chromatographic conditions and effects on the peak areas were recovered. Different chromatographic parameters such as variations in flow rate, column oven temperature, mobile phase composition and change in pH of mobile phase were made. It was performed using 10 µg/ml of ZMV and the effects on the peak areas were recorded. The each parameter was repeated for six times. The ruggedness of the method was studied by analyzing 10 µg/ml of ZMV by two different analyst using same operational and environmental conditions.

Limit of detection and limit of quantitation

For HPTLC–densitometric method

The sensitivity of the proposed method was estimated in terms of the limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by the use of the equation LOD = 3.3 X ASD/S and LOQ = 10 X ASD/S; where, 'ASD' is Average standard deviation of the peak height and area of the drugs, taken as a measure of noise, and 'S' is the slope of the corresponding calibration curve. The different volume of stock solution in the range 1000-1500 ng/band was spotted on TLC plate. The procedure was repeated in triplicate.

For HPLC method

The sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). For the study of the sensitivity of the method lower part of the linearity, the curve was selected. From the stock solution, six different concentrations in the range of 2, 2.4, 2.8, 3.2, 3.6 and 4.0 µg/ml were prepared and injected into the column. Each concentration was injected into a column for three times, and peak area was recorded. The average standard deviation of peak area is 3460.69.

The LOD was calculated using formula LOD = 3.3 ASD/S and LOQ = 10 ASD/S, where ASD is the average standard deviation and S is the slope of the corresponding calibration curve line.

Specificity

For HPTLC–densitometric method

To confirm the specificity of the proposed method, ZMV sample was spotted on TLC plate. The mobile phase designed for the method resolved ZMV very efficiently**.** The peak purity of ZMV was tested by correlating the spectra of ZMV extracted from tablets and standard ZMV at the peak start (S), peak apex (A) and at the peak end (E) positions. Correlation between these spectra indicated purity of ZMV peak {correlation r (S, M) = 0.9994, r (M, E) = 0.9998}.

For HPLC method

Specificity is a procedure to detect the analyte quantitatively in the presence of component that may be expected to be present in the

sample matrix, while selectivity is the procedure to detect the analyte qualitatively in the presence of components that may be expected to be present in the sample matrix.

The method is quite selective. There was no other interfering peak around the retention time of ZMV; also the baseline did not show any significant noise.

Recovery studies

Recovery experiments were performed at three different levels i.e. 80, 100 and 120 %. To the pre-analysed sample solutions, a known amount of standard drug solution of ZMV was over spotted at three different levels.

Forced degradation studies

A stock solution containing 10 mg for ZMV in 10 ml methanol was prepared. This solution was used for forced degradation to provide an indication of the stability-indicating property of the method.

Acid degradation

In HPTLC methanolic solution of the drug (10 mg) was separately dissolved in 10 ml of 1M HCl and these solutions were kept for 8 h at room temperature in dark in order to exclude the possible degradative effect of light. The solutions (1 ml) were taken and neutralized and then diluted up to 10 ml with methanol. The resultant solutions were applied on TLC plate in triplicate (10 µL each, i.e. 1000 ng/band). The plate was chromatographed as described above.

In HPLC ZMV solution treated with 1 ml of 1 M methanolic HCl. The solutions were kept at room temperature for 8 hr. The solution was diluted with the mobile phase to reach a final concentration of 10 µg/ml of ZMV and injected to the column.

Base degradation

In HPTLC methanolic solution of the drug (10 mg) was separately dissolved in 10 ml of 1 M NaOH solution. These solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The solutions (1 ml) were taken and neutralized and then diluted up to 10 ml with methanol. The resultant solutions were applied on TLC plate in triplicate (10 µL each, i.e. 1000 ng/band). The plate was chromatographed as described above.

In HPLC ZMV solution treated with 1 M methanolic NaOH. The solutions were kept at room temperature for 8 hr. The solution was diluted with the mobile phase to reach a final concentration of 10 µg/ml of ZMV and injected.

Oxidative degradation

In HPTLC, the drug (10 mg) was dissolved in 10 ml of a methanolic solution of hydrogen peroxide (30% v/v) and kept for 8 h at room temperature in the dark, to exclude the possible degradative effect of light. The solution (1 ml) was then diluted to 10 ml with methanol and treated as described for acid and base-induced degradation.

In HPLC, ZMV solution was treated with 1 ml of hydrogen peroxide 10%. The solution was kept at room temperature for 8hr. Both solutions were kept protected from light. After the specified time intervals, the solutions were diluted with the mobile phase to reach a final concentration of 10 µg/ml of ZMV. After the previous treatments, the solutions were filtered with a 0.45-µm filtration disc prior to injection to the column

Photochemical degradation

The drug solution was left in sunlight for 8h. The resultant solution was treated as described for hydrogen peroxide-induced degradation.

Dry heat degradation

The powdered drug was stored for 3h. Under dry heat conditions at 55 °C. In HPTLC, a solution of the treated powder was then prepared and 1000 ng/band was applied to a plate in triplicate. The plate was then chromatographed and treated as described above.

In HPLC, the solution was diluted with the mobile phase to reach a final concentration of 10 µg/ml of ZMV. The chromatograms were run by injecting the sample in the column.

RESULTS AND DISCUSSION

Optimization of procedures

Optimization of HPTLC–densitometric method

The TLC procedure was optimized with a view to develop a stability indicating assay method. Both the pure drug and the degraded products were spotted on the TLC plates and run in different solvent systems. Initially chloroform: methanol in varying ratios was tried. The mobile phase chloroform: methanol 4.5:0.5 (*v/v*) was tried. The developed spot was diffused. To the above mobile phase, 0.1 ml acetic acid was added. Both the peaks were symmetrical in nature, and tailing was observed. To overcome this problem, the volume of acetic acid was increased to 0.3 ml, gave good resolution, sharp and symmetrical peak with Rf value of 0.29 for ZMV **(**fig. **2).** Also, the spot for ZMV was compact and not diffused. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 15 min ensure good reproducibility and peak shape of ZMV.

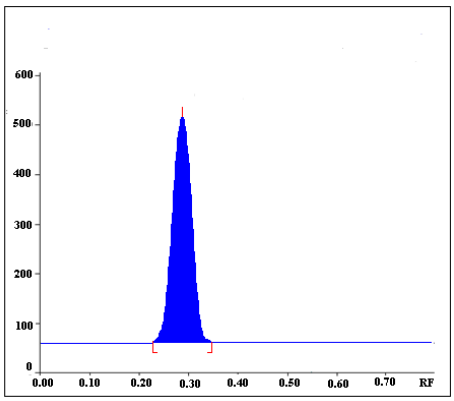

Fig. 2: Densitogram of standard ZMV (Rf=0.29)

Optimization of HPLC method

Initially, a combination of methanol and water (50:50 *v/v*) was tried as a mobile phase, but a broadening of the peak was observed. Then, pH of the mobile phase was adjusted to 3.5; the chromatogram showed fronting and splitting. To overcome the problem combination of methanol and phosphate buffer (0.02 M) in the ratio of 50:50 % *v/v* was tried for resolution of ZMV. Good resolution and the symmetric peak were obtained for ZMV when the pH of the mobile phase was adjusted to 5.0 and column oven temperature was kept at 30 °C. Under these optimum chromatographic conditions, the retention time for ZMV was found to be 3.6±0.02 min (fig. 3), at a flow rate of 1.0 ml/min. The detection was carried out at 230 nm.

Linearity

In case of HPLC, linearity was studied by injecting six concentrations of standard ZMV (2, 4,6,8,10,12 µg/ml) in triplicate. In HPTLC, a series of dilutions and standard curves were prepared over a concentration range from 500-3000 ng/band of ZMV from the stock solution. In both methods, peak area versus concentration data was performed by least square linear regression analysis, whereby slope, intercept, and correlation coefficient were determined.

Precision

For HPTLC–densitometric method

Precision was studied as intra-day and inter-day variations. An Intra-day variation was assessed by analyzing three different

concentrations 1000, 1500 and 2500 ng/band of ZMV for three times within a day.

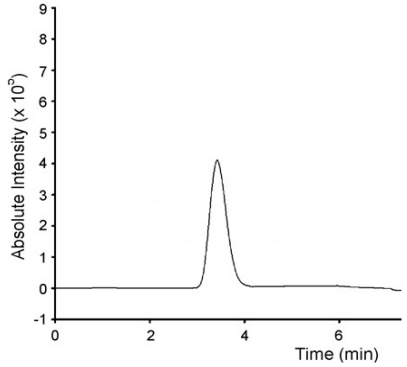

Fig. 3: Typical chromatogram of ZMV at 230 nm (Rt=3.6)

Inter-day variation was assessed using same concentration of drug (mentioned above) and analyzing it for three different days, over a period of a week. The results are as shown in table 1.

For HPLC method

The precision of the method was studied by intra-day and inter-day variations. Intra-day variation of the method was performed by analyzing, the three different concentrations 4 µg/ml, 6 µg/ml and 8 µg/ml of ZMV, for three times in the same day. Inter-day variations of the method were performed by analyzing the same concentrations for the period of the three consecutive days over a period of a week. The results are as shown in table 2**.**

Robustness and ruggedness of the method

For HPTLC–densitometric method

The standard deviation of peak areas was calculated for each parameter. The low %R. SD indicates the robustness of the method. The results are as shown in table 3.

Table 1: HPTLC intra-day and Inter-day precision studies

Drug	Conc. [ng/band]	Intra-day amount found [ng] mean±SD [*n* = 3]	% RSD	Inter-day amount found [ng] mean±SD [*n* = 3]	% RSD
ZMV	1000	994.78±6.56	0.70	1002.09±17.56	1.75
	1500	1501.37±24.42	1.61	1502.63±22.97	1.52
	2500	2497.49±19.92	0.79	2491.84±34.57	1.38

Table 2: HPLC intra-day and inter-day precision studies

Drug	Conc. [µg/ml]	Intra-day amount found [µg/ml] Mean ± SD [n= 3]	% RSD	Inter-day amount found [µg/ml] Mean ± SD [n= 3]	% RSD
ZMV	4	3.98 ± 0.03	0.79	3.97 ± 0.05	1.32
	6	5.99 ± 0.04	0.67	5.99 ± 0.03	0.62
	8	8.002 ± 0.02	0.32	8.033 ± 0.04	0.12

Table 3: HPTLC robustness studies

Parameters	SD of peak height and area [n = 6]	% RSD
Mobile phase composition		
Chloroform: methanol: acetic acid (8.0:2.0:0.3*v/v*).	34.21	0.56
Chloroform: methanol: acetic acid (7:3:0.3*v/v*).	25.76	1.04
Mobile phase volume (ml)		
10	27.82	0.45
12	24.81	0.78
Development distance (cm)		
7	36.87	0.37
7.5	23.45	1.31
8	41.56	1.81
Relative humidity		
55	27.67	1.27
65	43.56	1.63
Duration of saturation (min)		
20	23.56	0.22
25	42.02	0.69
30	27.64	1.27
Activation of prewashed TLC plates (min)		
8	19.94	0.45
10	27.83	0.37
12	17.93	1.76
Time from spotting to chromatography (0, 20 and 40 min)	37.45	0.19
Time from chromatography to scanning (0, 20 and 40 min)	23.19	0.67

The ruggedness of the proposed method was evaluated by two different analysts. The results for ZMV were found to be 99.15% and 99.27% respectively.

For HPLC method

The standard deviation of peak areas was calculated for each parameter and % R. SD was found to be less than 2%. The low % R. SD values as shown in table 4 indicated the robustness of the method.

LOD and LOQ

For HPTLC–densitometric method

The LOD and LOQ were found to be 36.40 ng and 110.30 ng, respectively.

For HPLC method

LOD and LOQ were found to be 0.15 µg and 0.46 µg, respectively.

Recovery studies

For HPTLC–densitometric method

To the pre-analysed 1000 ng/band solutions, a known amount of standard drug solutions of ZMV was over spotted at 80 %,100 % and 120 % levels i.e.800,1000 and 1200 ng/band. The chromatogram was developed and scanned. The result of % recovery was found to be 0.52-1.27 indicated accuracy of the method. The results are as shown in table 5.

For HPLC

To the preanalysed 4µg/ml solutions a known amount of standard drug solutions of ZMV at 80 %,100 % and 120 % levels i. e 3.2,4.0,4.8 µg/ml.% RSD of recovery study was found to be 0.58 to 1.13, which indicated that the method is accurate. The results are as shown in table 6.

Table 4: HPLC robustness studies

Parameter	± SD of peak area	% RSD
Mobile phase composition		
a) Methanol: Buffer (51: 49 *v/v*)	5163.12	0.68
b) Methanol: Buffer (49: 51 *v/v*)	3820.70	0.50
pH of mobile phase		
a) 4.1	5460.07	0.72
b) 3.9	4481.01	0.59
Change in flow rate		
a) 0.9	5795.81	0.77
b) 1.0	5504.32	0.73
Change in column temperature		
a) 25 °C	2937.58	0.39
b) 30 °C	2182.78	0.28

The ruggedness of the proposed method was evaluated by two different analysts. The results for ZMV were found to be 99.78% and 99.72% respectively

Table 5: Recovery studies

Drug	Initial amount [ng/band]	Amount added [ng/band]	Amount recovered ±SD [ng, *n* = 3]	% Recovered	% RSD
ZMV	1000	800	800.37±4.63	100.04	0.57
	1000	1000	1007.43±12.80	100.74	1.27
	1000	1200	1199.93±14.01	99.99	1.16

Table 6: Recovery studies

Drug	Initial amount [µg/ml]	Amount added [µg/ml]	Amount recovered±SD [µg/ml, n = 3]	% Recovery	% RSD
	4.0	3.2	3.18±0.03	99.47	1.13
ZMV	4.0	4.0	3.98±0.04	99.62	1.24
	4.0	4.8	4.81±0.02	100.30	0.58

System suitability

System suitability tests were also carried out to verify reproducibility. The system suitability was assessed in HPTLC by using six replicate analysis of drugs at concentration of 1000 ng/spot of ZMV respectively.% RSD was found to be 1.45 which was less than 2 indicated the method is reproducible. In HPLC the parameters such as capacity factor (K), injection repeatability tailing factor (T), theoretical plate number (N) and resolution (Rs) for the principal peak and its degradation product were tested on a 10 µg/ml sample of ZMV to assist the accuracy and precision of the developed HPLC system. The results are as shown in table 7.

Table 7: System suitability

System suitability parameters	Proposed method
Retention time (T_R)	6.64
Capacity factor (K)	0.76
Theoretical plate (N)	7974
Tailing factor (T)	1.03

Stability-indicating property

For HPTLC–densitometric method

The chromatogram of samples degraded with acid, base, hydrogen peroxide and light showed well-separated spots of pure ZMV as well as some additional peaks at different Rf values. Degradation of ZMV in acid, base, H_2O_2, dry heat and light heat are as shown in fig. **4**. The content of ZMV remained, and percentage recovery was calculated and listed in table 8.

For HPLC method

The chromatogram of samples degraded with acid, base, hydrogen peroxide and light showed well-separated spots of pure ZMV as well as some additional peaks at different Retention time. ZMV was successfully separated from all the degradation products as confirmed by the resolution values calculated for each chromatogram. The number of degradation product with their retention time values are as shown in fig. **5**. The content of ZMV remained, and percentage recovery was calculated and listed in table 9.

Table 8: HPTLC forced degradation

Sample exposure condition	Number of degradation products [R_f values]	ZMV remained [1000 ng/spot]	SD	Recovery [%]
1 M HCl, 8h,RT	4(0.03,0.14,0.20,0.40)	829.9	12.98	82.99
1 M NaOH, 8h, RT[a]	2 (0.13,0.20)	869.6	10.40	86.96
10 % H_2O_2, 8h, RT[a]	3 (0.16,0.22,0.27)	853.1	7.40	85.31
Heat, 3h, 55 °C	4 (0.16,0.22,0.27)	833.1	4.98	83.31
Photo, 8 h	No degradation	996.5	2.56	99.65

[a]RT = Room Temperature

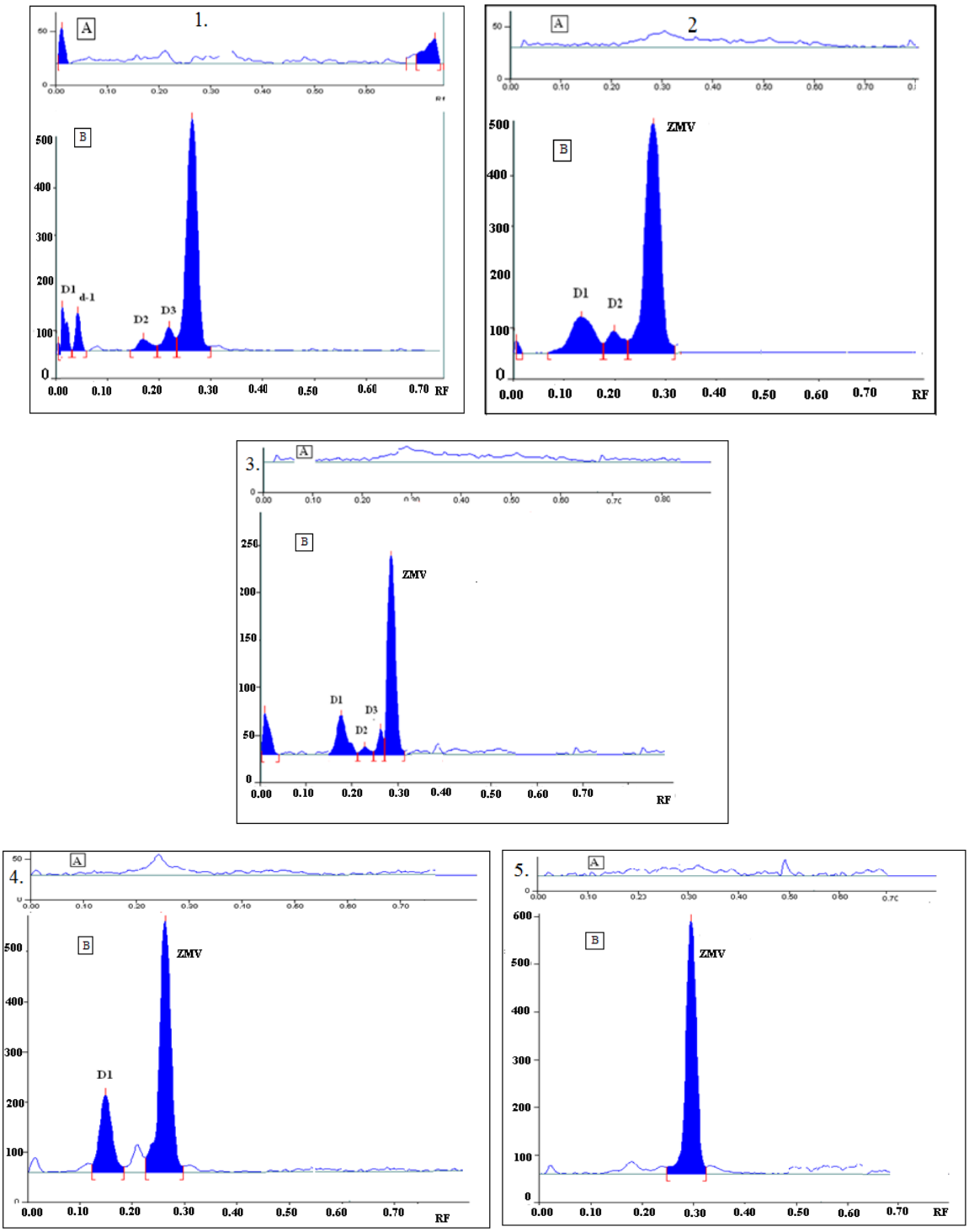

Fig. 4: Forced degradation of Zanamivir by HPTLC 1. A-Blank HCl, B-Sample treated with HCl 2. A-Blank NaOH, B-Sample treated with NaOH 3. Blank H2O2, B-Sample after oxidation 4. Blank MeOH, B-Sample after exposing to heat 5. A-Blank MeOH, B-Sample after exposing to light

Table 9: HPLC forced degradation studies

	Number of degradation products [Rt values]	ZMV remained [10ug/ml]	SDSD	Recovery [%]
1 M HCl, 8h,RT[a]	1 (2.7)	8.971	9.36	89.71
1M NaOH,8h, RT[a]	2 (1.9,2.8)	8.713	11.23	87.13
30%H_2O_2,8h,RT[a]	1(1.1)	9.6.7	4.42	96.07
Heat, 3H, 55 °C	1(1.9)	9.821	3.96	98.21
Photo, 8 h	No degradation	100.13	1.56	100.13

[a]RT = Room Temperature

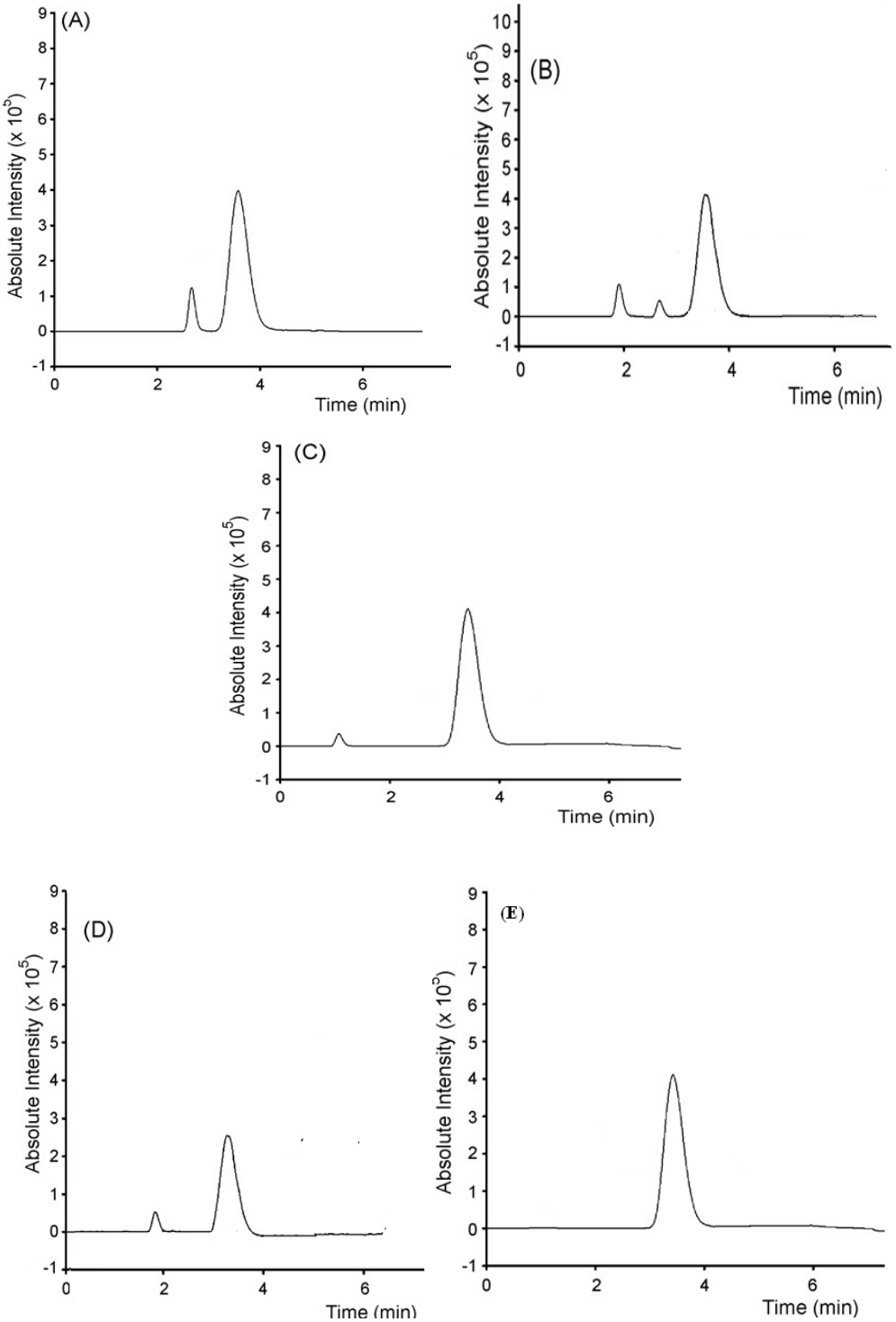

Fig. 5: Forced degradation of Zanamivirby HPLC: A) 1N HCl+zanamivir; B) 1N NaOH+zanamivir; C) 10% H_2O_2+zanamivir; D) Dry heat zanamivir; E) Light heat zanamivir

Analysis of the marketed formulation

Six replicate determinations were performed on the commercially available tablet. For ZMV recovery was found to be 100.23±0.38% for HPLC and for HPTLC the spots of Rf 0.29 was observed in chromatograms obtained from drug samples extracted from tablets and recovery was found to be 99.28±0.605%. There was no interference was observed from the excipients commonly present in the tablets. It may, therefore, be inferred that degradation of ZMV had not occurred in the marketed formulations analyzed by this method. The low RSD indicated that the method is suitable for routine estimation of ZMV in pharmaceutical dosage forms.

CONCLUSION

The proposed HPTLC and HPLC methods provide simple, accurate and reproducible quantitative analysis for determination of ZMV in tablets. The method was validated as per ICH guidelines. As the method could effectively separate the drugs from their degradation products; therefore, it can be employed as a stability indicating study.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests

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