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STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE IN PHARMACEUTICAL DOSAGE FORM

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Keywords:

Doravirine, Lamivudine, Tenofovir Disoproxil Fumarate, Stability indicating RP-HPLC, Novel anti-viral drugs, Delstrigo

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ABSTRACT: Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate belong to the class of anti-viral drugs and have been widely used in the treatment of HIV-I infection. Doravirine is a novel non-nucleoside reverse transcriptase inhibitor used in the combination of Lamivudine and Tenofovir Disoproxil Fumarate to effectively treat HIV/AIDS by overcoming the common resistance mutations without any possible drug interactions when co-administered. A literature review has reported few methods for the simultaneous estimation of Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate in combination with other antiviral drugs. The present investigation aimed to develop a simple, rapid, economical and stable indicating RP-HPLC method for the simultaneous estimation of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate in the pharmaceutical dosage form. The chromatographic separation was achieved on Kromasil C18 column with the dimensions of 250 x 4.6 mm, 5µm particle size, and maintained at 30 ˚C at a wavelength of 260nm. A combination of buffer (0.1% OPA) and Acetonitrile as mobile phase has optimized the method for efficient separation of drugs. Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate were eluted at 2.431, 3.187min, and 4.338, respectively, with good resolution. The linear regression coefficient for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate was 0.999 across the concentration range of 12.5-75µg/mL (Doravirine), 37.5-225 µg/mL (Lamivudine and Tenofovir Disoproxil Fumarate respectively). The proposed drugs were subjected to forced degradation studies and further validated as per ICH Guidelines. No interferences were observed, and the peak purity index was found to be within limits.

INTRODUCTION: Doravirine [1, 4] (DOR, MK-1439) is a novel non-nucleoside reverse transcriptase inhibitor helpful in the treatment of HIV-I infection. DOR was first approved by the US Food and Drug Administration (FDA) in two formulations – as a complete once-daily dose regimen in combination with two NRTIs, Lamivudine [2] and Tenofovir Disoproxil Fumarate [3] (DOR/3TC/TDF, Delstrigo [Merck & Co. Inc., Kenilworth, NJ]) and as a single tablet of 100 mg DOR (Pifeltro [Merck & Co. Inc.]) to be used in combination with other active antiretroviral drugs both for the treatment of adults living with HIV [5, 6, 7].

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DOR is an HIV-1 pyridone non-NNRTI. The chemical name for DOR is 3-chloro-5-[[1-[(4,5-dihydro – 4 – methyl – 5 – oxo - 1H-1, 2, 4 -triazol-3-yl)methyl]-1,2-dihydro-2-oxo-4(trifluoromethyl)-3-pyridinyl] oxy] benzonitrile. Hinging on the literature, only a few methods were established to estimate Doravirine, Lamivudine and Tenofovir

Disoproxil Fumarate by HPLC-DAD [8], UPLC [9] in plasma samples [10]. Accordingly, the present research aimed to develop a cost-efficient, specific and precise method for the determination of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate in its marketed tablet dosage form.

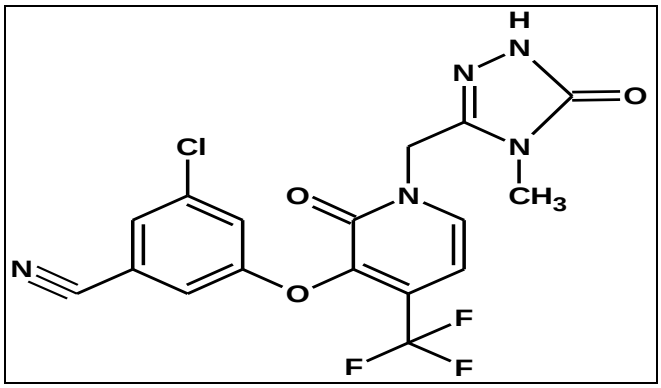


FIG. 1: CHEMICAL STRUCTURE OF DORAVIRINE

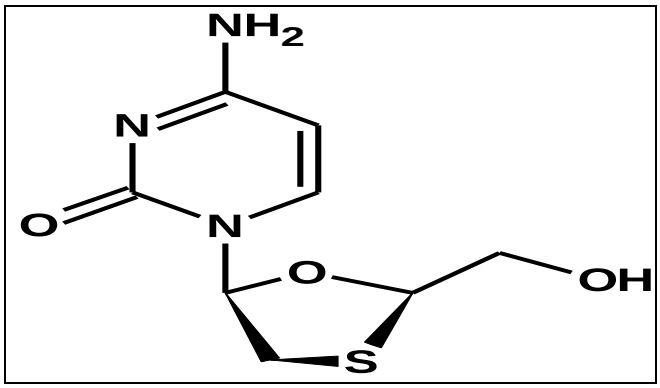


FIG. 2: CHEMICAL STRUCTURE OF LAMIVUDINE

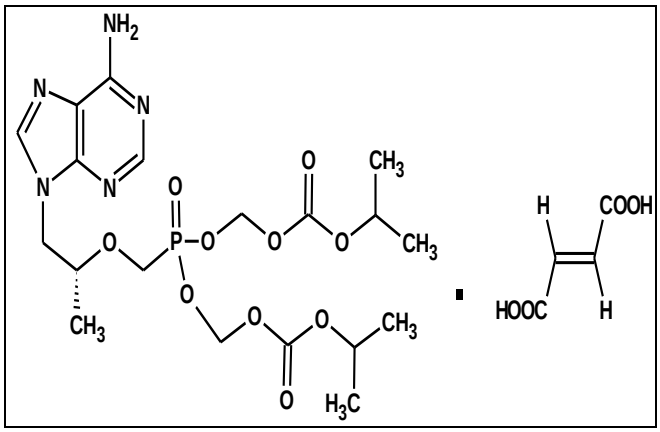


FIG. 3: CHEMICAL STRUCTURE OF TENOFOVIR DISOPROXIL FUMARATE

MATERIALS AND METHODS:

Materials: Pure drugs were obtained from Spectrum pharma research solutions Pvt. Ltd., Hyderabad and the tablet dosage form Delstrigo was manufactured by Abiba Pharmacia Pvt. Ltd., Chandigarh, India (Label Claim: Doravirine 100mg, Lamivudine 300mg, Tenofovir Disoproxil Fumarate 300mg) was procured from the local market. All the solvents and reagents used for the method are of HPLC grade and obtained from Ranchem Pvt. Ltd.

Instrumentation and Chromatographic Conditions: RP- HPLC analysis was performed on WATERS HPLC 2695 series solvent delivery system equipped with Quaternary pumps, photodiode array (PDA) detector, Autosampler integrated with EMPOWER^{TM} software solutions for the analysis of data. Eluates were separated on Kromasil C18 column with dimensions of 250 x 4.6 mm and 5µm particle size and monitored at 260 nm. The mobile phase consisting of 0.1% OPA and Acetonitrile in the ratio of 60:40 % v/v was prepared and degassed using an Ultra Sonicator, BVK enterprises, and pumped at a flow rate of 1mL/min. The chromatographic separation was achieved at a temperature of 30˚C with an injection volume of 10 µL.

Preparation of Standard Stock Solutions: 25mg of Doravirine, 75mg of Lamivudine and 75mg of Tenofovir Disoproxil Fumarate were accurately weighed and transferred to 50 mL volumetric flask separately. Approximately 20 mL of diluent (50:50 % of water: acetonitrile) was added and allow to dissolve the contents. The solutions were then sonicated for about 10 minutes and final volume was made up to the mark with diluent. 1ml from each stock solution was pipetted out, taken into a 10ml volumetric flask, and made up with diluent. The resultant concentrations were found to be 50 µg/mL, 150 µg/mL, 150 µg/mL for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate respectively.

Preparation of 0.1% OPA (Buffer) Solution: 1mL of Orthophosphoric acid solution was pipetted out in a 1000ml of the volumetric flask, about 100ml of milli-Q water was added, and a final volume make up to 1000 ml with milli-Q water. The pH of the resulting buffer solution was observed to be 2.5.

Preparation of Sample Stock Solutions: 10 units of the dosage form were weighed and made to a fine powder. The equivalent weight of the powder was accurately weighed and transferred to 50 mL volumetric flask.

Approximately 20 mL of diluent (50:50 % of 0.1% OPA: Acetonitrile) was added and allowed to dissolve the contents. The solutions were then sonicated for about 10 min and the final volume was made up to the mark with diluent. The solution was then filtered, and 1mL of the filtrate was diluted to 10mL with diluent.

Method Validation:

Linearity: The linear response for the proposed method was explained by preparing three series of solutions of individual drugs from the standard stock solutions in the range of 12.5-75µg/mL (Doravirine), 37.5-225 µg/mL (Lamivudine and Tenofovir Disoproxil Fumarate. The peak area of the standard calibration solutions was plotted against the concentration, and the linear response was compared by the linear regression equation and regression coefficient values.

Preparation of Calibration Standards: From the standard stock solution of Doravirine (500 µg/mL), Lamivudine (1500 µg/mL), and Tenofovir Disoproxil Fumarate (1500 µg/mL) 25µL, 50µL, 75µL, 100µL, 125µL,150µL were pipetted out into a series of volumetric flasks and made to 10mL with the diluent. The resultant calibration standard solutions range from 12.5-75µg/mL for Doravirine, 37.5-225 µg/mL for Lamivudine and 37.5-225 µg/mL for Tenofovir Disoproxil Fumarate.

System Suitability: The system suitability parameters like peak tailing, resolution, USP count, *etc.*, were determined by preparing the standard stock solutions of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate. The solutions were injected six times, and the acceptance limits for the parameters were observed.

Precision:

Method Precision: Method precision can be defined as the repeatability of the proposed method. Six sample injections were injected onto the column; the parameters like peak area, standard deviation, relative standard deviation, and % assay were calculated.

System Precision: System precision can be determined by injecting six replicates of the standard solutions of the analytes. The parameters like peak area, standard deviation, relative standard deviation and % assay were calculated, and the results were compared with the acceptable limits.

Intermediate Precision: The intermediate precision is determined by injecting six standard working solutions and six sample injections by different operators or by different instruments on different days of analysis. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay was calculated.

Accuracy: The accuracy of the method was ascertained by performing recovery studies. Recovery studies were carried out by the addition of standard drug solution to pre-analyzed tablet sample solution at three different concentrations levels (50%, 100% and 150%) within the linearity range. The percentage recoveries were calculated from the results obtained.

Limit of Detection and Limit of Quantification: The method's sensitivity can be explained by the limit of detection and the limit of quantification values. The limit of detection and limit of quantification was calculated for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate from the chromatograms by observing the signal to noise (s/n) ratio.

Robustness: The robustness of the method can be explained by slightly varying the LC parameters like flow rate of mobile phase, detection wavelength, temperature, and percent of organic phase in the mobile phase composition.

Assay: Five units of the tablet dosage form (DELSTRIGO) with the label claim of Doravirine 100mg, Lamivudine 300mg, and Tenofovir Disoproxil Fumarate 300mg were weighed and

made to a fine powder. The equivalent weight of the powder was weighed accurately and transferred to a 100mL volumetric flask. An aliquot of diluent was added and finally made up to the mark with the diluent. The solution was then filtered through a nylon disc filter and 1 mL of the filtered solution was then diluted to 10 mL with the same diluent.

Forced Degradation Studies:

Oxidation: 1mL of the sample stock solution was taken, and 1mL of hydrogen peroxide solution was added. The resulting mixture was placed at 60˚C for 30 minutes and sufficiently diluted to get 50, 150, and 150 µg/mL of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate. 10 µL of this solution was then injected onto the chromatographic column.

Acid Degradation Studies: 1mL of the sample stock solution was taken, and 1mL of 2N Hydrochloric acid solution was added to it. The resulting mixture was refluxed at 60°C for 30 minutes and sufficiently diluted to get 50, 150, and 150 µg/mL of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate. 10 µL of this solution was then injected onto the chromatographic column.

Alkali Degradation Studies: 1mL of the sample stock solution was taken and 1mL of 2N Sodium hydroxide solution was added to it. The resulting mixture was refluxed at 60˚C for 30 minutes and sufficiently diluted to get 50, 150, and 150 µg/mL of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate. 10 µL of this solution was then injected onto the chromatographic column.

Dry heat Degradation Studies: The standard drug solution was placed in hot air oven at a temperature of 105˚C for a period of 6 h, and the resultant solution was diluted to produce 50, 150, and 150 µg/mL of Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate. 10 µL of this solution was then injected on to the chromatographic column.

Neutral Degradation Studies: Neutral degradation studies were performed by refluxing the dg solution in water for a period of 6 hours at 60°C and sufficiently diluted to get 50, 150, and 150 µg/mL of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate. 10 µL of this solution was then injected onto the chromatographic column.

Photo Stability Studies: The photostability study was performed by placing the drug solution in the photostability chamber comprising a UV chamber from which the drug solution was exposed to UV light for 7 days or 200-Watt hours/m^2. The resultant solution was diluted to produce 50, 150, and 150 µg/mL of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate. 10 µL of this solution was injected onto the chromatographic column.

RESULTS AND DISCUSSION:

Method Development and Optimization of RP-HPLC Method: The present investigation aimed to develop a stability-indicating RP-HPLC method for the simultaneous determination of Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate in the tablet dosage form (DELSTRIGO). The method development was initiated based on the solubility and the pKa of active pharmaceutical ingredients. This was ensured by solubility studies in various different solvents like water, methanol, ethanol, acetonitrile, a combination of pure solvents, and buffers like acetate and phosphate buffers. Finally, the combination of Doravirine, Lamivudine, and TenofovirDisoproxil Fumarate was found to be satisfactorily soluble in 1:1 ratio of 0.1% OPA and Acetonitrile. Therefore 1:1 ratio of 0.1% OPA and Acetonitrile was chosen as the diluent for the analysis. The trials were performed initiating with 60: 40 % v/v Acetonitrile and 0.1% OPA solution as the mobile phase composition on the Symmetry C18 column (150 x 4.6; 5 µm particle size). The peaks were eluted at void volume therefore, the trails were further continued. In the next trial the mobile composition was altered to 50:50 % v/v acetonitrile and 0.1% OPA, Doravirine was eluted at the void volume.

In the further trail, the stationary phase was alerted by changing the column to Discovery reverse phase column (250 x 4.6; 5 µm particle size), Doravirine and Lamivudine were eluted with no proper resolution. Therefore, the next trail was performed by altering the buffer from 0.1% OPA to potassium dihydrogen phosphate buffer, and the column used was Discovery (250 x 4.6; 5 µm particle size).

The peaks were eluted, but the retentions times were found to be too long. Therefore, the trails proceeded further by considering the 50: 50 % v/v

Acetonitrile and 0.1% OPA solution as the mobile phase composition on the ODS C18 column (150 x 4.6; 5 µm particle size). The peaks were eluted, but the frosting was observed with one of the peaks. Hence the column was changed in the preceding trial. The trial was carried out with a Kromasil C18 column (250 x 4.6; 5 µm particle size) with a mobile phase composition of 60: 40 % v/v Acetonitrile and 0.1% OPA and the peaks were eluted with great resolution and short retention times. The retention times for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate were found to be 2.431 min, 3.187min, and 4.338 min, respectively, with good resolution. The column temperature was maintained at 30°C, and the eluates were monitored at 260nm with a mobile phase flow rate of 1 mL/min. Consequently, the method was optimized with these LC conditions, degradation studies were performed, and were further validated as per ICH guidelines. The chromatogram for Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate with the optimized LC conditions is as follows:

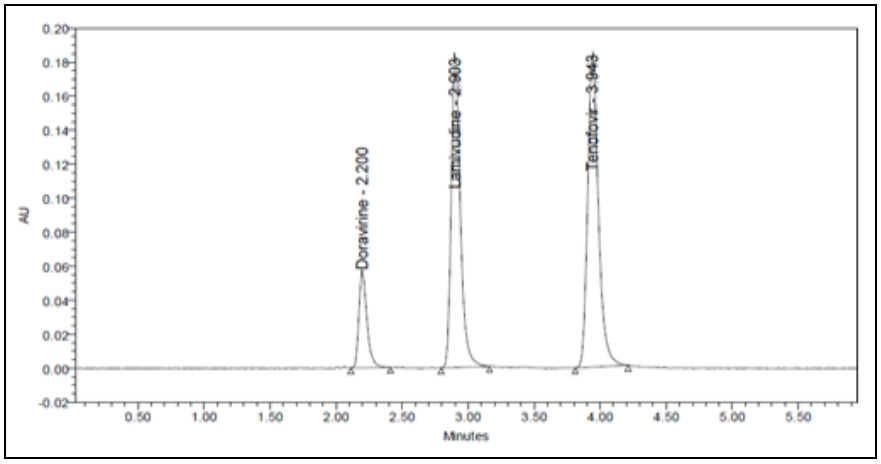


FIG. 4: THE OPTIMIZED CHROMATOGRAM FOR DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE

System Suitability Parameters: The System suitability parameters were evaluated prior to validation of optimized LC conditions. The retention times for Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate were found to be 2.431 min, 3.187min, and 4.338 min, respectively. The theoretical plate count for all the peaks was more than 2000 and was within the acceptance criterion. The tailing factor was also observed to be less than 2 for all the three drugs and % RSD for peak areas of all the six injections of the standard was found to be ≤ 2% **Table 1.**

TABLE 1: SYSTEM SUITABILITY PARAMETERS

S. no.	Doravirine			Lamivudine				Tenofovir Disoproxil Fumarate			
Injection #	Rt (min)	TP	Tailing	Rt (min)	TP	Tailing	RS	Rt (min)	TP	Tailing	RS
1	2.492	4354	1.20	3.252	9295	1.34	5.1	4.399	12261	1.28	7.7
2	2.492	4662	1.19	3.252	9608	1.30	5.3	4.403	12697	1.30	7.6
3	2.494	4706	1.19	3.253	9403	1.35	5.3	4.403	12364	1.28	7.7
4	2.495	4680	1.19	3.254	9302	1.34	5.2	4.405	12598	1.30	7.7
5	2.495	4120	1.17	3.254	9778	1.35	5.2	4.407	12542	1.30	7.6
6	2.495	4256	1.16	3.255	9740	1.35	5.3	4.411	12325	1.28	7.8

Linearity: The linearity range for Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate was determined based on the label claim of the marketed pharmaceutical dosage form. The concentration range of calibration standard solutions of Doravirine (12.5-75 µg/mL), Lamivudine (37.5-225µg/mL) and Tenofovir Disoproxil Fumarate (37.5-225µg/mL) has shown a good linear response with the regression equations y (Doravirine) = 20057x + 3689.5 **Fig. 5**. Y (Lamivudine) = 20006x + 7422.1 **Fig. 6**. y (Tenofovir Disoproxil Fumarate) = 20160x + 14417 **Fig. 7.** The results in **Table 2** were analyzed with appropriate statistical methods and the regression coefficient (R^2) values were observed to be 0.9999, 0.9998 and 0.9995, respectively.

TABLE 2: LINEARITY

Doravirine		Lamivudine		Tenofovir Disoproxil Fumarate	
Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
12.5	252513	37.5	755959	37.5	754633
25	507137	75	1515551	75	1564593
37.5	756438	112.5	2277285	112.5	2272186
50	1019338	150	3016311	150	3089292
62.5	1253457	187.5	3707451	187.5	3735855
75	1501991	225	4534235	225	4560223

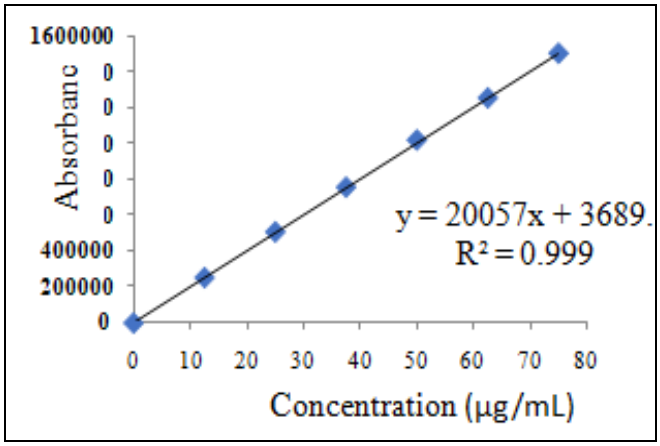


FIG. 5: CALIBRATION CURVE FOR DORAVIRINE

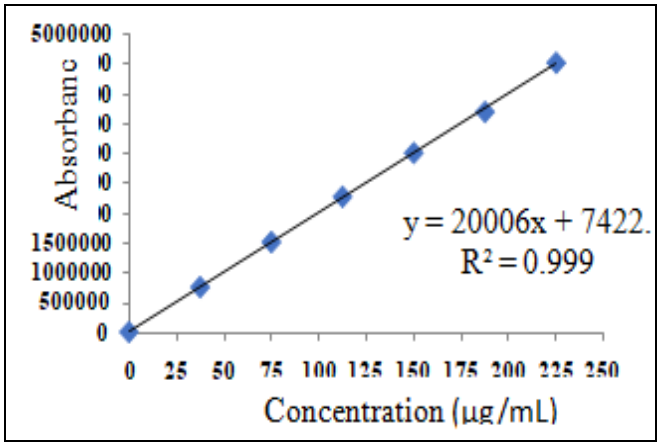


FIG. 6: CALIBRATION CURVE FOR LAMIVUDINE

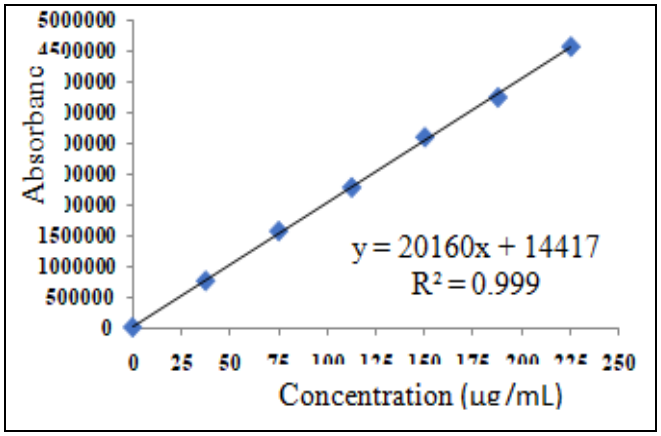


FIG. 7: CALIBRATION CURVE FOR TENOFOVIR DISOPROXIL FUMARATE

Precision: The proposed LC conditions optimized for the estimation of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate were observed to be precise as the % RSD values for six replicate injections in method precision and variations in reproducibility for inter-day and intraday analysis was noted to be within the acceptable limits (% RSD ≤ 2%).

TABLE 3: METHOD PRECISION AND INTERMEDIATE PRECISION

Active pharmaceutical ingredient	Method Precision			Intermediate Precision		
	Peak area	STD.DEV	% RSD	Peak area	STD.DEV	% RSD
Doravirine	1021164	70822.2	0.7	9275228	179806	0.2
Lamivudine	3062149	27664.0	0.9	2935306	13946.3	0.5
TenofovirDisoproxil Fumarate	3039913	30483.0	1.0	2906275	19032.1	0.7

Accuracy: The accuracy of the method was determined based on the recovery results of the sample stock solution. Standard addition method was followed to spike the sample solution with the standard stock solution. This method was done at three different levels of 50%, 100%, and 150%; the recovery results were found to be 99.37%, 100.51% and 99.61% for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate, respectively **Table 4.**

TABLE 4: ACCURACY (RECOVERY STUDIES)

S. no.	Active pharmaceutical ingredient	% Recovery ± Std. Dev	% RSD
1	Doravirine (n=3)	99.37 ± 0.72	0.72
2	Lamivudine (n=3)	100.5 ± 0.7	0.7
3	Tenofovir Disoproxil Fumarate (n=3)	99.61 ± 0.53	0.53

Limit of Detection and Limit of Quantification: Sensitivity of the optimized LC conditions for the estimation of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate can be determined by the Limit of Detection (LOD) and Limit of Quantification (LOQ) values obtained by the signal to noise ratio.

The LOD values for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate were observed to be 0.19 µg/mL, 0.95 µg/mL and 1.21 µg/mL respectively. The LOQ values for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate were observed to be 0.57 µg/mL, 2.88 µg/mL and 3.67 µg/mL, respectively **Table 5.**

TABLE 5: LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

S. no.	Active pharmaceutical ingredient	LOD (µg/ml)	LOQ (µg/ml)
1	Doravirine	0.19 µg/ml	0.57 µg/ml
2	Lamivudine	0.95 µg/ml	2.88 µg/ml
3	Tenofovir Disoproxil Fumarate	1.21 µg/ml	3.67 µg/ml

Robustness: The robustness of the method was determined by results obtained after making small, deliberate changes in the optimized LC conditions. Parameters like organic phase composition, column temperature, and mobile phase flow rate were altered. The results of system suitability parameters were obtained with negligible changes compared with the proposed method.

Assay: Delstrigo was the marketed tablet dosage form with the label claim of 100 mg of Doravirine, 300mg of Lamivudine, and 300 mg of Tenofovir Disoproxil Fumarate. The assay was performed for this pharmaceutical dosage form using the optimized LC conditions, and the sample peak areas were compared with the standard.

The percent assay values were calculated and reported as 100.32 % for Doravirine, 100.81 for Lamivudine, and 99.31% for Tenofovir Disoproxil Fumarate. The % RSD values were within limits ≤ 2.

TABLE 6: ASSAY

S. no.	Active pharmaceutical ingredient	% Assay ± Std. Dev	% RSD
1	Doravirine (n=6)	100.32 ± 0.696	0.7
2	Lamivudine (n=6)	100.81 ± 0.911	0.9
3	Tenofovir Disoproxil Fumarate (n=6)	99.31 ± 1.00	1.0

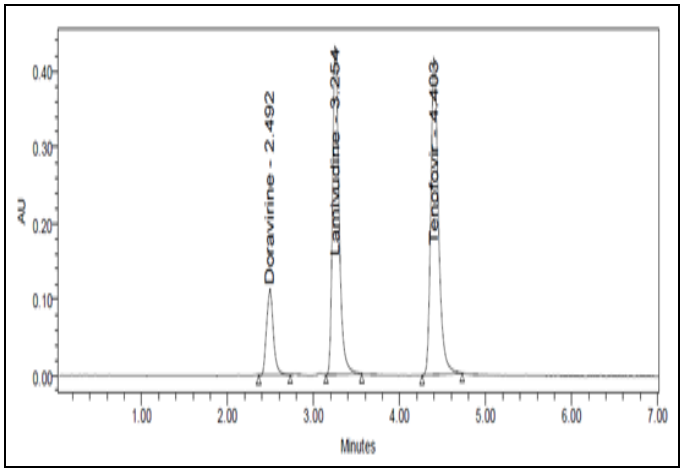


FIG 8: CHROMATOGRAM OF STANDARD (DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE)

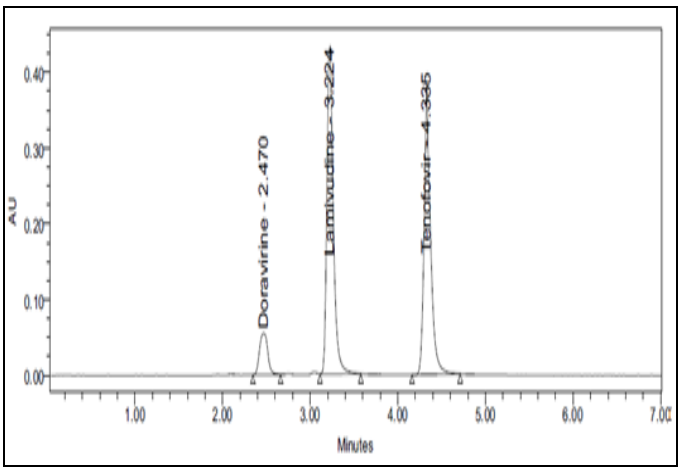


FIG 9: CHROMATOGRAM OF SAMPLE (DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE)

Stability Studies: The stability of active pharmaceutical ingredients was demonstrated by performing forced degradation studies as per the ICH guidelines. The stress testing of API included acid degradation, alkali degradation, and peroxide degradation studies. The dry heat degradation and photodegradation studies were also performed to declare the stability of Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate. The retention times for the degradation products were found at 2.816 minutes and 3.388 minutes, respectively. Therefore, the degradation products were not interfered with the active pharmaceutical ingredients in the alkali degradation and peroxide degradation studies. In acid degradation, thermal degradation, neutral degradation, and photolytic degradation, the active pharmaceutical ingredients were not degraded and were proved to be stable under stress conditions. There were no changes in the system suitability parameters which indicates that the proposed stability indicates the RP-HPLC method for estimating Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate is highly specific. It can be stated that the drug peaks for Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate, when analyzed under the PDA detector, were homogenous and pure under the forced degradation conditions.

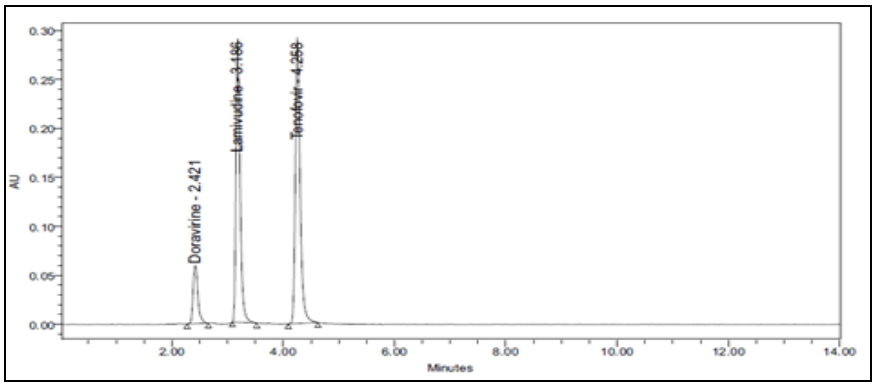


FIG. 10: ACID DEGRADATION STUDIES (DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE)

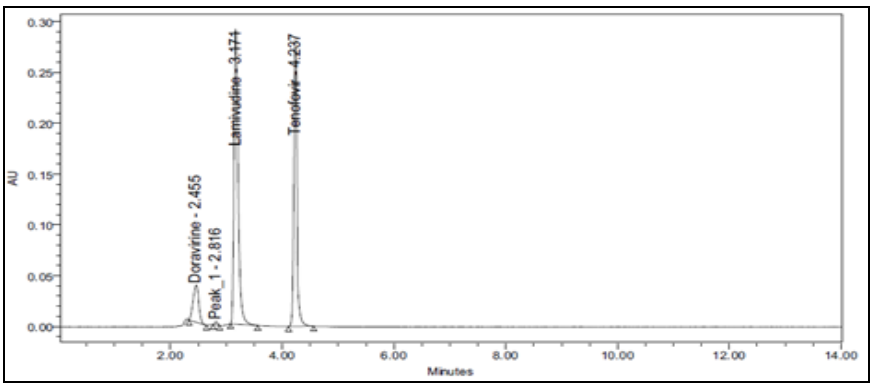


FIG. 11: ALKALI DEGRADATION STUDIES (DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE)

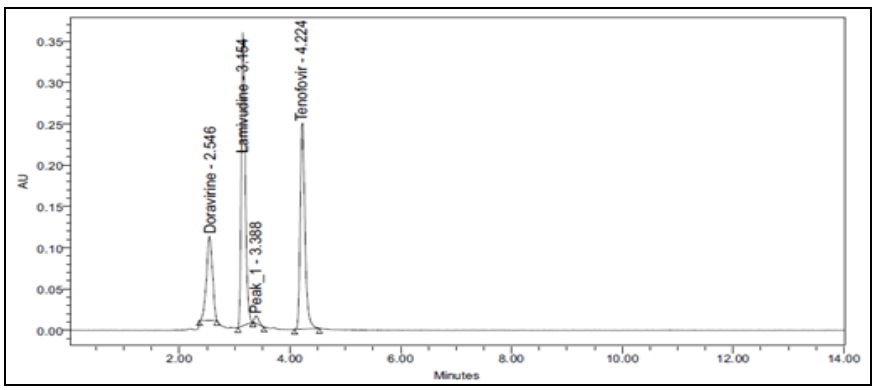


FIG. 12: PEROXIDE DEGRADATION STUDIES (DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE)

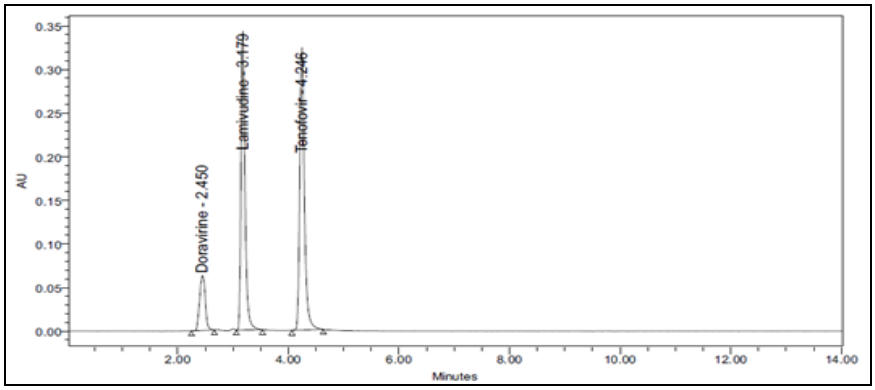


FIG. 13: THERMAL DEGRADATION STUDIES (DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE)

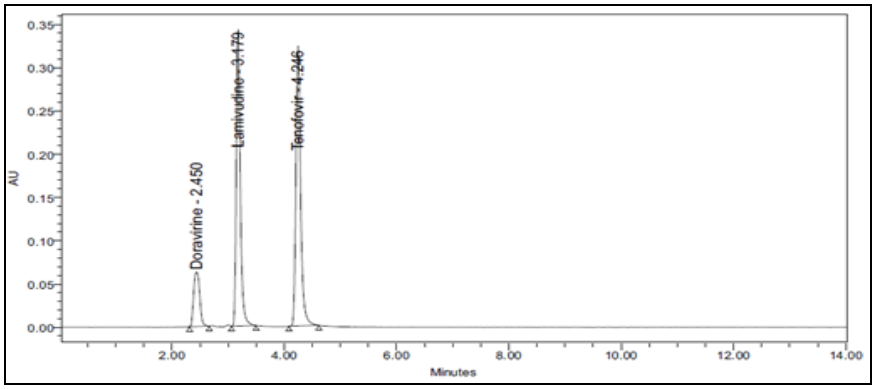
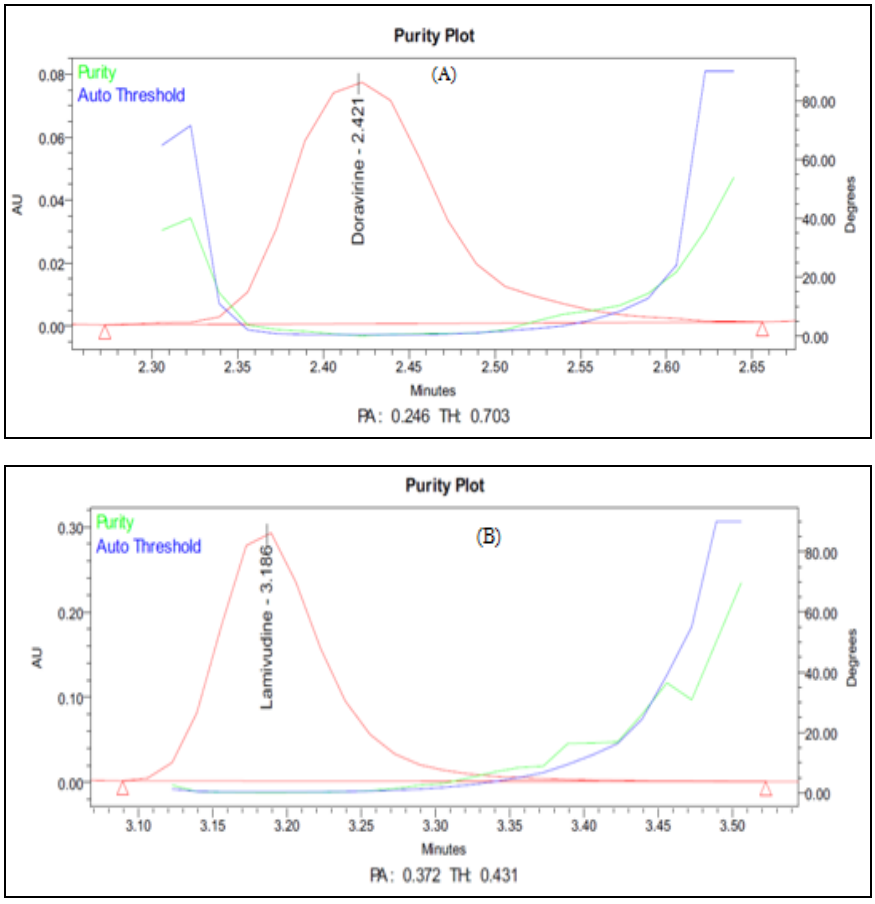


FIG. 14: PHOTOLYTIC DEGRADATION STUDIES (DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE)



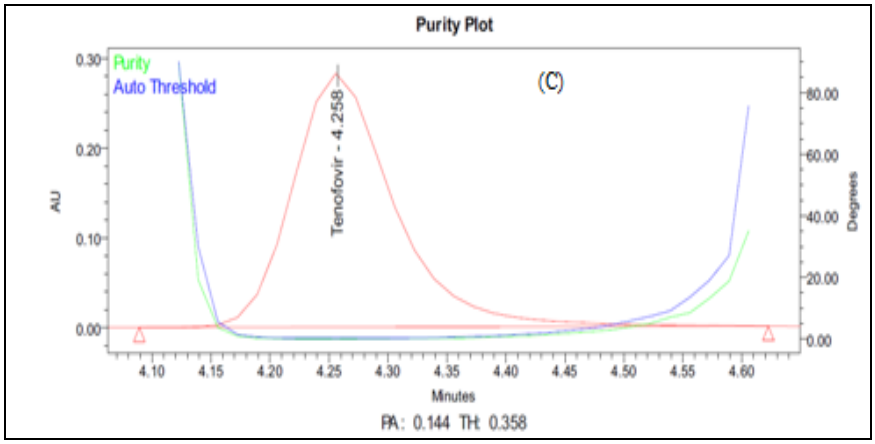
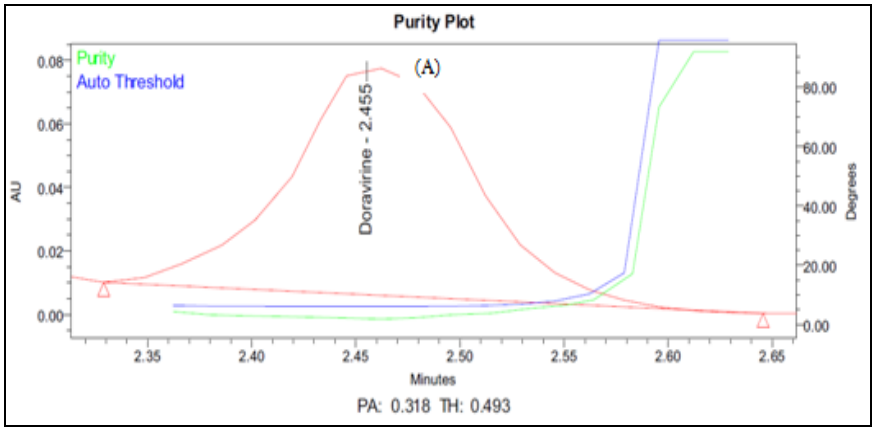
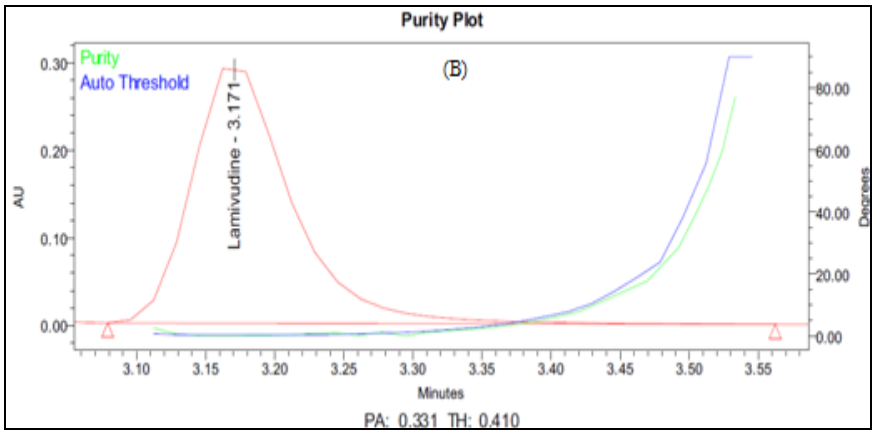


FIG. 15: (A), (B), (C) PURITY PLOTS FOR ACID DEGRADATION STUDIES OF DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE RESPECTIVELY





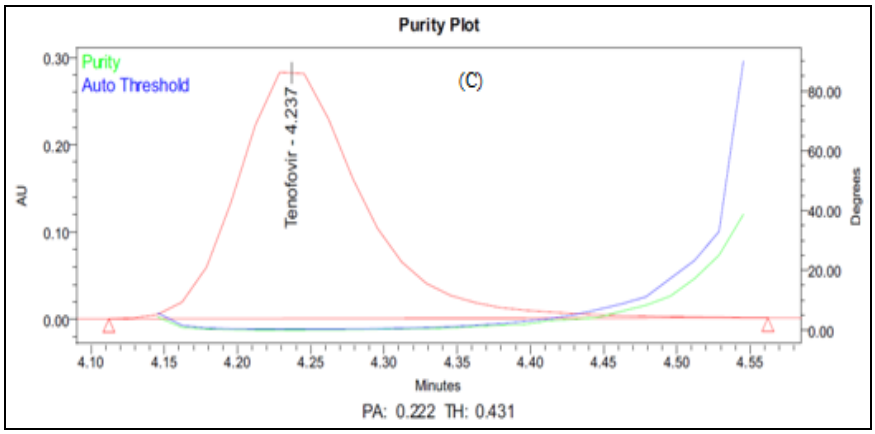


FIG. 16: (A), (B), (C) PURITY PLOTS FOR ALKALI DEGRADATION STUDIES OF DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE RESPECTIVELY

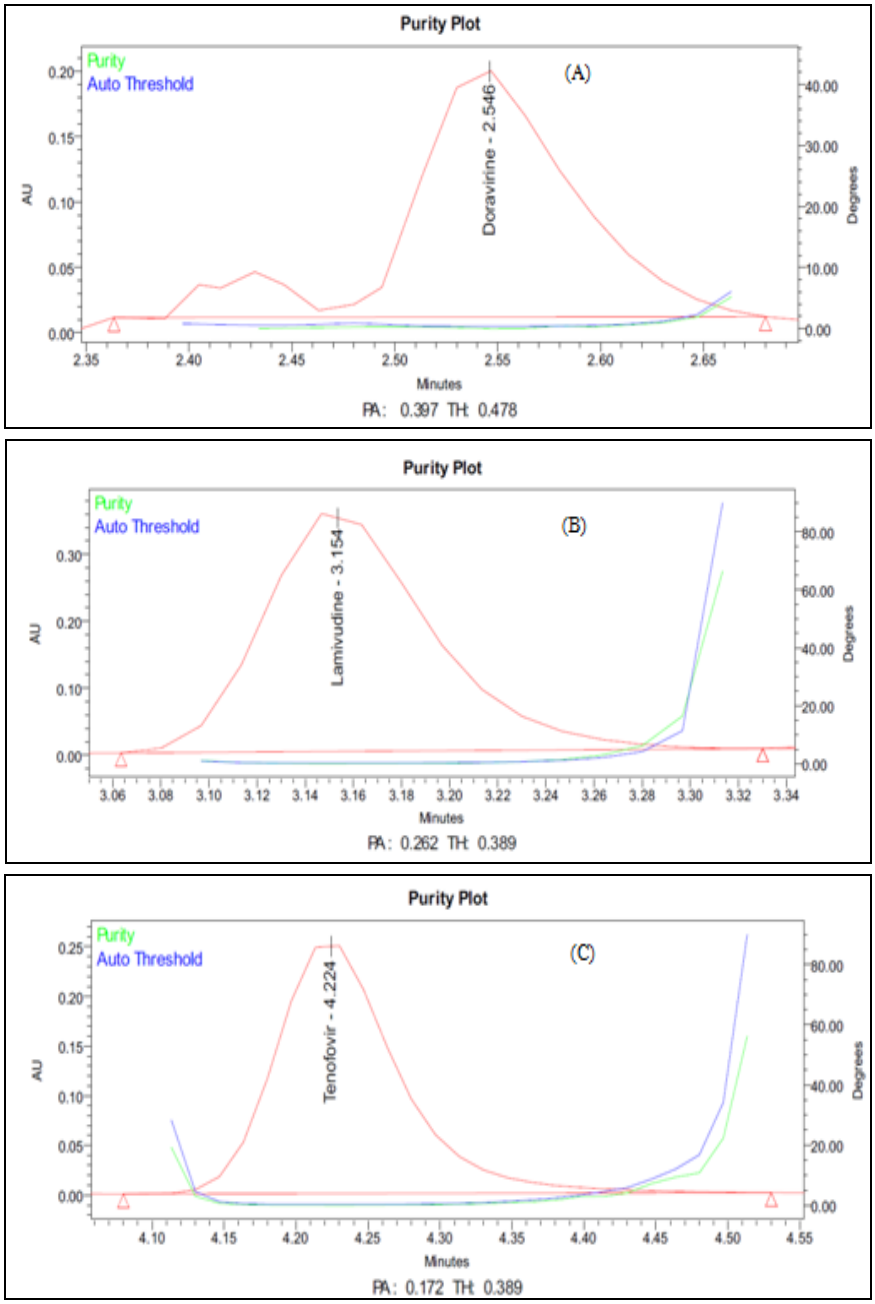


FIG. 17: (A), (B), (C) PURITY PLOTS FOR PEROXIDE DEGRADATION STUDIES OF DORAVIRINE, LAMIVUDINE, AND TENOFOVIR DISOPROXIL FUMARATE, RESPECTIVELY

CONCLUSION: A novel, simple and specific stability-indicating Reverse Phase High-Performance Liquid Chromatographic method was developed effectively to estimate Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate in the marketed tablet dosage form Delstrigo. The optimized LC method was validated as per the ICH guidelines for the parameters of linearity, method precision, intermediate precision, accuracy, the limit of detection, the limit of quantification, robustness, assay, and the reported results were found to be within the acceptance criterion as per the specified guidelines. The forced degradation studies were also performed to indicate the stability of the pharmaceutical dosage form as per the guidelines. The stress conditions for the stability testing of active pharmaceutical ingredients comprised of acid degradation studies, alkali degradation studies, peroxide degradation studies, photolytic degradation, thermal degradation and neutral degradation studies.

There were degradation peaks in the alkali and peroxide media, but no inter reference of the degradation peak with the API was found. Hence, the proposed method can be stated as specific with

homogeneity of the peaks of API. Finally, it can be concluded that the present method for determination of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate can be effectively adopted for the routine analysis of dosage forms.

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CONFLICTS OF INTEREST: The authors reported no potential Conflicts of Interest.

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