Research Article

Stability Indicating RP-HPLC Method for the Simultaneous Quantification and Validation of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate in Bulk and Tablet Dosage Form and its Applications in Dissolution Studies

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ABSTRACT

The study established and afterwards validated an "stability indicating RP–HPLC" stratagem for assessing lamivudine (LAD), doravirine (DOR), & tenofovir (TED). RP-HPLC separation & assessment of LAD, DOR & TED was accomplished with a mobile phase of KH2PO4 (0.1M concentration, 5.2 units pH) plus methanol solvent solutions at a 65:35 v/v percentage and 1 ml/min flow stream. The detector arranged at 237 nm. Elution times for LAD is 2.531 min, DOR is 5.691 min & TED is 3.530 min. The "stability indicating RP–HPLC" stratagem was proved quantity varied from 150 to 450 µg/ml for LAD, from 50 to 150 µg/ml for DOR & from 150 to 450 µg/ml for TED. Acceptable assessments were documented in precision study (RSD% - 0.230% for LAD; RSD% - 0.187% for TED; & %RSD – 0.264% for DOR) and accuracy study (recovery % - 98.98% for LAD; recovery% - 99.16% for TED; & recovery% – 98.53% for DOR). Robustness of "stability indicating RP–HPLC" stratagem also was discovered to be acceptable. Also, during degradation check, obstruction in the evaluation of the LAD, DOR & TED wasn't really discovered. The created "stability indicating RP–HPLC" stratagem also proved significant in a dissolution assessment of Delstrigo tablets comprising LAD, DOR, & TED as active components in combo.

Keywords: DOR, Degradation, Dissolution, LAD, RP-HPLC, Stability, TED.

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INTRODUCTION

HPLC

HPLC has unquestionably become an important analytical method for separating, detecting, or qualifying constituents of a mixture.

The split equilibrium among the polar stationary phase and indeed the non-polar mobile phase provides the basis for normal phase chromatography[9, 10]. The stationary phase has a higher polarity over the mobile phase. According to the retention process, the least polar solutes are retained first, followed by the more polar solutes. In generally, silica makes up the stationary phase, while chloroform, hexane, isopropanol, and other solvents make up the mobile phase.

The split equilibrium among the non-polar (hydrophobic) stationary phase and indeed the polar mobile phase, when the mobile phase has a greater polarity than that of the stationary phase, is the basis for reverse phase chromatography[9, 10]. Despite NPC, greater polar solutes were eluted first, followed by less polar solutes. Reverse phase chromatography separations are built on the reversible adsorption or desorption of solutes with variable amounts of hydrophobicity to something like a hydrophobic stationary phase, pursuant to the reverse phase chromatography concept.

HIV

A virus, HIV that attacks immunological structure cells which is really the instinctual safeguard of one's body against any and all illness. In its immune system, this virus destroys a kind of white blood cell known as a T- helper cell and aids in the replication of itself among such cells[1, 2]. T-helper cells can even be cited as CD4 cells.

HIV gradually weakens a body's immune system by destroying quite so many CD4 cells & causing it to duplicate themselves[3]. This means that those infected with HIV who don't even seek antiretroviral medication would have a harder time combating infections and illnesses.

If HIV is not treated, it can take up to 15 years for the immune system in becoming drastically weakened to the point where it can no more tolerate. The rate, at which HIV advances, on the other hand, is determined by the patient's age, overall fitness, and medical history.

AIDS

AIDS is a set of HIV-related symptoms / syndrome contrastingly to a virus. If an individual's immune system is extremely weak to fight infection and they acquire particular symptoms and illnesses, they are considered to have AIDS. If the infection has advanced far enough, this is the last degree of HIV which can lead to mortality if left uncontrolled[4].

The term AIDS refers to procured immune deficient

syndrome, which is also known as chronic or late- stage HIV. AIDS is a collection of symptoms and illnesses caused by chronic HIV infection which has debilitated the immune system[5, 6] AIDS is currently being contracted by fewer people; however, HIV treatment implies that more individuals are staying healthy.

Delstrigo Tablets

Delstrigo is a one-pill HIV treatment designed to treat HIV-1 infection in adults who've not taken HIV-1 medications or to replace existing HIV-1 medications for patients who meet specific requirements as determined by their doctor[7, 8.] The virus that triggers acquired immune deficiency syndrome is HIV-1 (AIDS).

Delstrigo is a 3-drug combination of doravirine (DOR), lamivudine (LAD), and tenofovir disoproxil fumarate (TED) that is proposed as part of a grownup HIV-1 therapy regimen[7, 8]:

- has never received antiretroviral therapy,
- Supplanting a stable antiretroviral regimen with no history of therapeutic failure and no identified substitutions imparting resistance to the individual Delstrigo components for individuals who are virologically stifled (HIV-1 RNA less than 50 copies per ml).

EXPERIMENTAL MATERIALS

Chemical Compounds

Hydrochloric acid, NaH_2PO_4, Methanol, Peroxide, K_2HPO_4, Sodium hydroxide.

Equipment Systems

Waters alliance's HPLC equipment, Waters alliance's Empower 2 software, Waters alliance's photodiode detector, Waters 150 mm length C18 column (4.6 mm, 5 µ dimension particle)

Drug Molecules

Lamivudine (LAD), Tenofovir (TED), Doravirine (DOR)

Tablets Employed

- Tabelt Product name: Delstrigo™
- Characterized claim: LAD – 300 mg, TED – 300 mg & DOR – 100 mg
- Mark Company: Merck & Co., NJ, USA.

HPLC Set Values For LAD, TED & DOR Assay:

- Column: Waters, C18, 150mm × 4.6mm, 5µm
- Column temp: 25°C
- Mobile phase: KH_2PO_4: Methanol (55:45)
- $P^{H:}$ 5.2
- Rate of flow: 1ml/min
- Size of injection sample: 10 µl
- Wavelength: 237 nm
- Run time: 8 min

➢ **Reagents**

Mobile Phase

The mobile phase is formulated by blending KH_2PO_4 (0.1M concentration, 5.2 units pH) plus methanol solvent solutions at a 65:35 v/v percentage.

Diluent Employed

The diluent is formulated by blending KH_2PO_4 (0.1M concentration, 5.2 units pH) plus methanol solvent solutions at a 65:35 v/v percentage.

Columns Tested

YMC C18, Intersil C18, Thermo C18, Supelco C18, Waters C18

Stock LAD, TED & DOR Solution

300 mg LAD, 300 mg TED, and 100 mg DOR were reliably metered and placed to a 100 ml flask, to which 75 ml diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage) was poured and blended to a volume mark of 100 ml diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). Concentration: 3000 µg/ml LAD, 3000 µg/ml TED & 1000 µg/ml DOR.

Working LAD, TED & DOR Solution

2.50 ml of LAD, TED & DOR stock solution (3000 µg/ml LAD, 3000 µg/ml TED & 1000 µg/ml DOR) diluted to volume mark of 25 ml by diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). Concentration: 300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR.

Linearity LAD, TED & DOR Solution

Five levels of linearity LAD, TED & DOR solutions (LS) were set ready with concentrations like below:

- Level 1 linearity: Concentration: 150 µg/ml LAD, 150 µg/ml TED & 50 µg/ml DOR.
- Level 2 linearity: Concentration: 225 µg/ml LAD, 225 µg/ml TED & 75 µg/ml DOR.
- Level 3 linearity: Concentration: 300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR.
- Level 4 linearity: Concentration: 375 µg/ml LAD, 375 µg/ml TED & 125 µg/ml DOR.
- Level 5 linearity: Concentration: 450 µg/ml LAD, 450 µg/ml TED & 150 µg/ml DOR.

DELSTRIGO™ Sample Solution

776 mg of DELSTRIGO™ powder made were reliably metered and placed to a 100 ml flask, to which 75 ml diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage) was poured, ultrasonicated (nearby 15 min) and blended to a volume mark of 100 ml diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). Concentration: 3000 µg/ml LAD, 3000 µg/ml TED & 1000 µg/ml DOR.

2.50 ml of LAD, TED & DOR stock DELSTRIGO™ solution (3000 µg/ml LAD, 3000 µg/ml TED & 1000 µg/ml DOR) diluted to volume mark of 25 ml by diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). This test DELSTRIGO™ solution was employed in evaluation Concentration: 300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR.

Evaluation of LAD, TED & DOR DELSTRIGO™ Sample Solution

The Waters C18 fitted HPLC device was feed with 10 µl measure volume of test DELSTRIGO™ solution and experimented under settings revealed in "HPLC Set values for LAD, TED & DOR Assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR content in DELSTRIGO™ sample.

Stability Tests On LAD, TED & DOR

The stability investigations been performed out employing 10 ml of DELSTRIGO™ stock solution (3000 g/ml LAD, 3000 g/ml TED, & 1000 g/ml DOR) utilizing ICH eligibility requirement conditions like [12].

- Degradation accelerated by acid
- Degradation accelerated by alkali
- Oxidation accelerated by peroxide
- Degradation accelerated by temperature
- Degradation accelerated by sun

Degradation Accelerated By Acid:

10 ml of DELSTRIGO™ stock solution (3000 g/ml LAD, 3000 g/ml TED, & 1000 g/ml DOR) was blended, in ultrasonicator (25°C) for nearby 30 min, with 10 ml measured portion of 0.1 N HCl. The mixture blended to a volume mark of 100 ml by diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). Theoretic concentration: 300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR. The Waters C18 fitted HPLC device was feed with 10 µl measure volume of stressed DELSTRIGO™ solution and experimented under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR content remained in DELSTRIGO™ sample.

Degradation Accelerated By Alkali

10 ml of DELSTRIGO™ stock solution (3000 g/ml LAD, 3000 g/ml TED, & 1000 g/ml DOR) was blended, in ultrasonicator (25°C) for nearby 30 min, with 10 ml measured portion of 0.1 N NaOH. The mixture blended to a volume mark of 100 ml by diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). Theoretic concentration: 300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml

DOR. The Waters C18 fitted HPLC device was feed with 10 µl measure volume of stressed DELSTRIGO™ solution and experimented under settings revealed in "HPLC set values for LAD, TED & DOR assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR content remained in DELSTRIGO™ sample.

Oxidation Accelerated By Peroxide

10 ml of DELSTRIGO™ stock solution (3000 g/ml LAD, 3000 g/ml TED, & 1000 g/ml DOR) was blended, in ultrasonicator (25°C) for nearby 30 min, with 10 ml measured portion of peroxide (30% strength). The mixture blended to a volume mark of 100 ml by diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). Theoretic concentration: 300 µg/ml LAD, 300 µg/ml TED &

100 µg/ml DOR. The Waters C18 fitted HPLC device was feed with 10 µl measure volume of stressed DELSTRIGO™ solution and experimented under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR content remained in DELSTRIGO™ sample.

Degradation Accelerated by Temperature

10 ml of DELSTRIGO™ stock solution (3000 g/ml LAD, 3000 g/ml TED, & 1000 g/ml DOR) was set down in oven (60 °C) for nearby 30 min. Afterwards the sample blended to a volume mark of 100 ml by diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). Theoretic concentration: 300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR. The Waters C18 fitted HPLC device was feed with 10 µl measure volume of stressed DELSTRIGO™ solution and experimented under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR content remained in DELSTRIGO™ sample.

Degradation Accelerated By Sun

10 ml of DELSTRIGO™ stock solution (3000 g/ml LAD, 3000 g/ml TED, & 1000 g/ml DOR) was set down in sun light for nearby six hr. Afterwards the sample blended to a volume mark of 100 ml by diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage). Theoretic

concentration: 300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR. The Waters C18 fitted HPLC device was feed with 10 µl measure volume of stressed DELSTRIGO™ solution and experimented under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR content remained in DELSTRIGO™ sample.

Dissolution Assessment of Delstrigo^{tm} Tablet Comprising LAD, DOR, &TED: media for LAD, DOR, & TED dissolution

KH_2SO_4 (13.609 gm) was blended with sodium hydroxide (0.9 gm) with 1000 ml measured portion of water in container. Apply HCl, if required, to tune pH. Degass the media for LAD, DOR, & TED dissolution at 41 °C for nearby 10 min once it had being readied to clear air bubbles. Then, place this media for LAD, DOR, & TED dissolution into six discrete bowls. Plump for appropriate program for DELSTRIGO^{TM} tablet. Afterwards wait till the bowl touches 37 °C. When the temperature touches 37 °C within all bowls, pause the equipment and instantly dump the DELSTRIGO^{TM} tablets and execute the tester.

Conditions: Dissolution Assessment of Delstrigo^{TM} Tablet ➢

- Dissolution media: 6.5 pH. phosphate buffer
- Dissolution medium quantity : 900 ml
- Dissolution Bath Temperature : 38 °C
- Dissolution bowl Temperature : 37 °C
- Dissolution Apparatus : USP type 2 (paddle)
- RPM : 50
- Sample collection time interval : 30 min
- Sample collection volume : 10 ml
- Rinse volume : 3 ml
- Replenish : No

Evaluation Of LAD, TED & DOR in Dissolution Delstrigo™ Sample

The Waters C18 fitted HPLC device was feed with 10 µl measure volume of test dissolution DELSTRIGO™ solution made under settings revealed in "CONDITIONS: DISSOLUTION ASSESSMENT OF DELSTRIGO^{TM} TABLET". Experimented dissolution DELSTRIGO™ solution under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR content in dissolution DELSTRIGO™ sample.

Selection of Wavelength:

1STPEAK-LAMIVUDINE is noted at 228.5 nm

2nd PEAK-TENOFOVIR is noted at 245.0 nm

3rd PEAK-DORAVIRINE is noted at 273.5nm

✓ The isobestic point is noted at **237 nm**

Optimized Trial

Optimized Chromatographic Conditions

Mobile Phase: KH_2PO_4: Methanol (55:45, v/v, pH 5.2)
Column : Waters, C18, 150 × 4.6mm, 5µm Flow Rate : 1.0ml/Min

Temperature : 25˚C Volume : 10µl

Run time : 8min

Detector : 237

Validation

The validation investigations been performed out employing ICH eligibility requirement[13].

Table 1: Optimized Chromatographic Parameters.

Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
LAD	2.531	2612389	33.54	538600	-	1.29	6542
TED	3.530	3491241	44.83	563581	6.82	1.20	7692
DOR	5.691	1684218	21.63	180692	10.42	1.17	8718

Note: LAD, TED & DOR is the drugs Lamivudine, Tenofovir & Doravirine.

System Suitability

Table 2: Results for system suitability.

Parameters	Tenofovir	Lamivudine	Doravirine	Acceptance criteria
RT	3.424	2.429	5.574	✓
Area	3505120.8	2626822.3	1695077.3	✓
Plate count	7718	6572	8645	(NLT-2500)
Resolution	6.81	--	10.41	✓ (NLT – 2)
Tailing	1.20	1.29	1.17	✓ (NMT-2)

Note: RT is Retention Time, NLT is Not Less Than, NMT is Not More Than.

Selectivity

The Waters C18 fitted HPLC device was feed with 10 µl measure volume of:

- Test DELSTRIGO™ solution (300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR)
- Working LAD, TED & DOR solution (300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR)
- Diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage)

Above specimen solutions are experimented under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Chromatograms for LAD, TED & DOR were marked off and afterwards used to compare. No LAD, TED & DOR peaks seen in diluent (blend of KH_2PO_4 0.1M concentration, 5.2 units pH plus methanol solvent solutions at a 65:35 v/v percentage) chromatogram. No surplus peaks viewed in test DELSTRIGO™ solution chromatogram other than LAD, TED & DOR peaks.

Precision

Experimented working LAD, TED & DOR solution (300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR) in six replicates under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Chromatograms for LAD, TED & DOR were marked off and afterwards used to get measure of mean, deviation and %RSD of LAD, TED & DOR peak area readings.

The **%RSD** for LAD, TED & DOR is found to be **0.230, 0.187** & **0.264** respectively.

Linearity

A plot of LAD, TED & DOR quantity (g/ml) versus consecutive LAD, TED & DOR peak area readings determined

LAD, TED & DOR assay system linearity. By assessing five various LAD, TED & DOR quantities of produced linearity samples, the linearity LAD, TED & DOR curves were generated. The quantity varied from 150 to 450µg/ml for LAD, from 50 to 150µg/ml for DOR & from 150 to 450 µg/ml for TED. Above linearity specimen solutions are experimented under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Chromatograms for LAD, TED & DOR were marked off and afterwards used to evaluate linearity by a least square lined regression course.

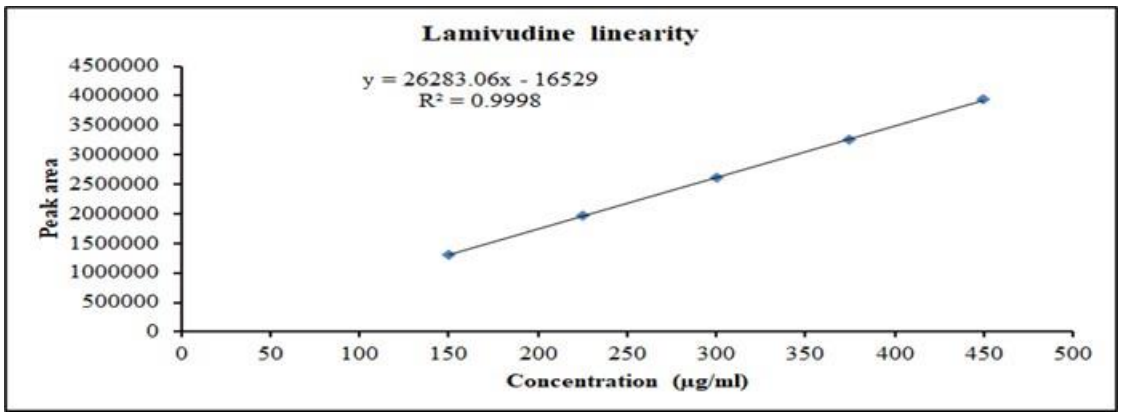

Figure 1: Linearity graph of Lamivudine

Note: Lamivudine - Lined Regression Equation is **y = 26283.06x – 16529** and Correlation Coefficient is **R^2 = 0.9998**

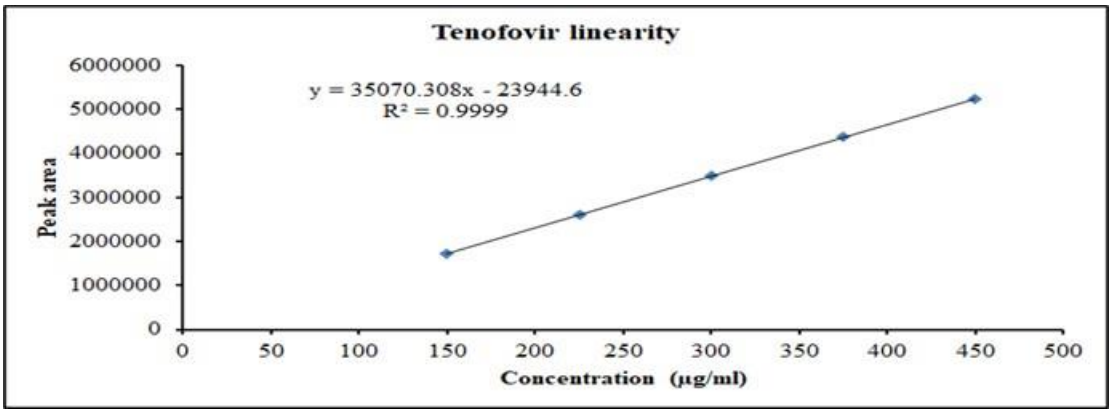

Figure 2: Linearity graph of Tenofovir

Note: Tenofovir- Lined Regression Equation is **y = 35070.308x - 23944.6** and Correlation Coefficient is **R^2 = 0.9999**

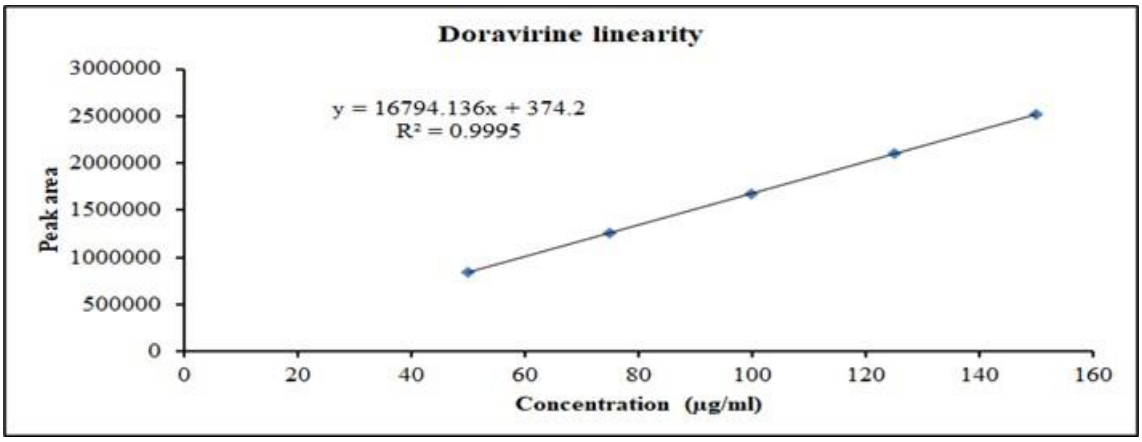

Figure 3: Linearity graph of Doravirine

Note: Doravirine- Lined Regression Equation is **y = 16794.136x + 374.2** and Correlation Coefficient is **R^2 = 0.9995**

Accuracy

Experimented working LAD, TED & DOR solution (300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR) in six replicates under settings revealed in "CONFIGURATED HPLC SET VALUES FOR LAD, TED & DOR ASSAY" section. Chromatograms for LAD, TED & DOR were marked off and afterwards used to get measure of assay mean percentile of LAD, TED & DOR.

The accuracy readings taking six samples of each drug LAD, TED & DOR is found to be **98.98, 99.16** & **98.53%** respectively.

Recovery

Renowned amounts of LAD, TED & DOR were appended to Test DELSTRIGO™ solution (300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR).

- 50% appended level: 148.50 µg/ml LAD, 148.50 µg/ml TED & 49.50 µg/ml DOR
- 100% appended level: 297.00µg/ml LAD, 297.0 µg/ml TED & 99.0 µg/ml DOR
- 150% appended level: 445.50 µg/ml LAD, 445.50 µg/ml TED & 148.5 µg/ml DOR Experimented above appended DELSTRIGO™ specimen solutions under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR recoveries in appended DELSTRIGO™ specimen solutions.

Table 3: Recovery readings.

Concentration added	Lamivudine mean % recovery	Doravirine mean % recovery	Tenofovir mean % recovery
	HPLC Method	HPLC method	HPLC Method
50%	99.66%	100.33%	99.81%
100%	100.01%	99.67%	100.08%
150%	100.34%	99.87%	100.18%

Note: The Results of Recovery is expressed in percentages at three levels 50%, 100% & 150% is within the limits.

LOD (Limit of Detection)

LOD for the HPLC: LAD, TED & DOR assay system was evaluated seeing the LAD, TED & DOR concentration via the S/N rating. The LOD is quantity of LAD, TED & DOR which might upshot in a S/N rating of 3. LOD = 0.775 µg/ml for LAD 0.740 µg/ml for TED 0.769 µg/ml for DOR S/N rating = 3.65 for LAD 3.60 for TED 3.30 for DOR

LOQ: (Limit of Quantification)

LOQ for the HPLC LAD, TED & DOR assay system was evaluated seeing the LAD, TED & DOR concentration via the S/N rating. The LOD is quantity of LAD, TED & DOR which might upshot in a S/N rating of 10. LOQ = 2.582 µg/ml for LAD 2.467 µg/ml for TED 2.565 µg/ml for DOR S/N rating = 10.97 for LAD 10.66 for TED 10.51 for DOR

Robustness

Experimented working LAD, TED & DOR solution (300 µg/ml LAD, 300 µg/ml TED & 100 µg/ml DOR) in robustness study. The HPLC LAD, TED & DOR assay procedure's robustness was make certain by inspecting alterations in methanol percentage, pH, temperature, flow rate, and wavelength for HPLC system device suitability facets for LAD, TED & DOR peaks.

All Parameters values are within the limits although changes are made in Flow rate, Methanol Percentage, Temperature, Wavelength & pH.

Table 4: Results of Robustness

Drugs	Parameter	Flow		Methanol Percentage %		Temperature		pH		Wavelength	
		0.9ml/min	1.1ml/min	40%	50%	23ºC	27ºC	5.0	5.4	235	239
Tenofovir	RT	2.744	4.901	2.744	4.108	3.075	4.108	3.416	3.414	3.432	3.430
	Area	2762890	5024081	2762890	4159321	3115661	4159321	3509831	3505099	3789430	3222177
	Plate count	6680	7894	6680	7751	7033	7751	7647	7720	7667	7649
	Resolution	6.64	7.05	6.64	7.04	6.77	7.04	6.78	6.78	6.81	6.78
	Tailing	1.19	1.19	1.19	1.19	1.20	1.19	1.21	1.21	1.21	1.21
Lamivudine	RT	1.904	3.465	1.904	2.884	2.148	2.884	2.426	2.425	2.433	2.433
	Area	2058961	3715428	2058961	3095482	2321092	3095482	2631410	2631937	2351516	2783830
	Plate count	5499	6701	5499	6419	5973	6419	6562	6565	6546	6533
	Tailing	1.29	1.28	1.29	1.28	1.29	1.28	1.30	1.30	1.30	1.30
Doravirine	RT	4.568	8.028	4.568	6.742	5.095	6.742	5.553	5.548	5.593	5.586
	Area	1323692	2387623	1323692	1979347	1492711	1979347	1699238	1694646	1544062	1808418
	Plate count	7576	8750	7576	8548	7860	8548	8664	8632	8680	8516
	Resolution	10.10	10.71	10.10	10.59	10.24	10.59	10.37	10.31	10.43	10.38
	Tailing	1.17	1.17	1.17	1.16	1.17	1.16	1.17	1.18	1.17	1.19

Note: RT is Retention Time

ASSAY

The percentage assay of all the three drugs (Lamivudine, Tenofovir and Doravirine) is found to be **99%.**

Stability Indicating Study/ Stability Tests On LAD, TED & DOR:

The stability investigations been performed out employing 10 ml of DELSTRIGO™ stock solution (3000 g/ml LAD, 3000 g/ml TED, & 1000 g/ml DOR) utilizing ICH eligibility requirement conditions like: Degradation accelerated by acid, Degradation accelerated by alkali, Oxidation accelerated by peroxide, Degradation accelerated by temperature, and Degradation accelerated by sun. The results for LAD, TED & DOR stability study are in below table.

Table 5: LAD, TED & DOR Stability indicating/ stability readings

Condition accelerated	LAD area readings	Percent LAD assay	Percent LAD loss
0.1 N HCl accelerated	2351630	89.26	10.74
Sun light accelerated	2425161	92.05	7.95
0.1N NaOH accelerated	2451035	93.03	6.97
60 ºC accelerated	2379349	90.31	9.69
Peroxide accelerated	2507492	95.17	4.83
Condition accelerated	**TED area readings**	**Percent TED assay**	**Percent TED loss**
0.1 N HCl accelerated	3196283	90.82	9.18
Sun light accelerated	3206331	91.11	8.89
0.1N NaOH accelerated	3251683	92.40	7.6
60 oC accelerated	3152991	89.59	10.41
Peroxide accelerated	3316659	94.24	5.76
Condition accelerated	**DOR area readings**	**Percent DOR assay**	**Percent DOR loss**
0.1 N HCl accelerated	1499065	88.08	11.92
Sun light accelerated	1594372	93.68	6.32
0.1N NaOH accelerated	1588961	93.36	6.64
60 oC accelerated	1535445	90.22	9.78
Peroxide accelerated	1600995	94.07	5.93

Note: Percentage loss is less than 12% with different stress conditions.

Directive of LAD stability

Peroxide > Base > Photo > Dry heat > Acid

Directive of TED stability:

Peroxide > Base > Photo > Acid > Dry heat

Directive of DOR stability:

Peroxide > Photo > Base > Dry heat > Acid

Evaluation of LAD, TED & DOR in Dissolution Delstrigo™ Sample

The test dissolution DELSTRIGO™ solution made under settings revealed in "CONDITIONS: DISSOLUTION ASSESSMENT OF DELSTRIGO™ TABLET" was experimented under settings revealed in "HPLC set values for LAD, TED & DOR Assay" section. Peak areas for LAD, TED & DOR were marked off and afterwards used to get measure of LAD, TED & DOR content in dissolution DELSTRIGO™ sample.

The percentage of drug release for three drugs LAD, TED, & DOR in six sample collection is found to be **99.99%.**

CONCLUSION

The study established and afterwards validated an "stability indicating RP–HPLC" stratagem for assessing LAD, DOR, & TED. Rendering to validation analysis, HPLC LAD, DOR, & TED evaluation methodology is relevant for measuring LAD, DOR, & TED content in pill formulations with no influence from excipients in tablets and also from degradants of accelerated stability tests. The created "stability indicating RP–HPLC" stratagem also proved significant in a dissolution assessment of Delstrigo tablets comprising LAD, DOR, & TED as active components in combo.

REFERENCES

1. Brew BJ, Garber JY. Neurologic sequelae of primary HIV infection. Handbook of Clinical Neurology, 2018; 152: 65-74.
2. Capriotti T. HIV/AIDS: An Update for Home Healthcare Clinicians. Home Healthcare Now, 2018; 36 (6): 348-355.
3. Justiz Vaillant AA, Gulick PG. HIV Disease. [Updated 2019 Oct 13]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534860/
4. Poorolajal J, Hooshmand E, Mahjub H, Esmailnasab N, Jenabi E. Survival rate of AIDS disease and mortality in HIV-infected patients: a meta-analysis. Public Health, 2016; 139: 3-12.
5. Becerra JC, Bildstein LS, Gach JS. Recent Insights into the HIV/AIDS Pandemic. Microbial Cell, 2016; 3 (9): 451-475.
6. Waymack JR, Sundareshan V. Acquired Immune Deficiency Syndrome (AIDS) [Updated 2019 Sep 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537293/
7. InformedHealth.org [Internet]. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG); 2006-. Doravirine / lamivudine / tenofovir disoproxil fumarate (Delstrigo) for the treatment of HIV: Overview. 2019 May 9. Available from: https://www.ncbi.nlm.nih.gov/books/NBK541968/
8. Delstrigo 100 mg /300 mg /245 mg film-coated tablets, Accessed on June 2020, Available at: https://www.medicines.org.uk/emc/product/9694/smpc
9. Robards K. Principles and practice of modern chromatographic methods. Haddad, P. R., Jackson, P. E. Amsterdam: Elsevier/Academic Press, 1994.
10. Ismail BP. Basic Principles of Chromatography. In: Nielsen S. (eds) Food Analysis. Food Science Text Series. Springer, Cham, 2017; pp 185-211.
11. Margaret ELC, William RLC. General instrumentation in HPLC. Liquid Chromatography (2nd Ed) Fundamentals and Instrumentation, 2017; p. 417-429.
12. International Conference on Harmonization (ICH) of technical requirements for the registration of pharmaceutical for human use stability testing of new drugs substance and products Q1A (R2), Geneva, Switzerland, 2003.
13. International Conference on Harmonization; Validation of Analytical Procedures: Text and Methodology, Q2 (R1). IFPMA, Geneva, Switzerland, 200.

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