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Original Article

RP-HPLC (STABILITY-INDICATING) BASED ASSAY METHOD FOR THE SIMULTANEOUS ESTIMATION OF DORAVIRINE, TENOFOVIR DISOPROXIL FUMARATE AND LAMIVUDINE

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ABSTRACT

Objective: In this study, a RP-HPLC (stability-indicating) based assay method for the estimation of doravirine (DRV), tenofovir disoproxil fumarate (TFF) and lamivudine (LMV) simultaneously in the tablets was described.

Methods: The simultaneous analysis of DRV, TFF and LMV was done with HPLC system (Agilent 1100 series) and Luna Phenomenex C18 (250 mm × 4.6 mm × 5µ) column with isocratic mobile phase (35% volume ratio of methanol and 65% volume ratio of 20 mmol ammonium formate, pH 5). Validation of assay method was done on sensitivity, linearity, accuracy, selectivity, precision, robustness and specificity.

Results: The calibration curves were linear through the range of 25-200 µg/ml for DRV and 75-600 µg/ml for TFF and LMV. The percent relative standard deviation for intraday variation/precision, interday variation/precision, intermediate precision/ruggedness and robustness were lower than 2%. The recovery of LMV (99.09-99.76%), TFF (99.10-99.41%) and DRV (98.65-99.28%) confirmed the good accuracy. The stability of LMV, TFF and DRV in 0.1N NaOH, 3% peroxide, 0.1 N HCl, UV light and dry heat of 60 °C was determined.

Conclusion: The results have allowed the method to be implemented in the tablets to quantify DRV, TFF, and LMV.

Keywords: Doravirine, Tenofovir disoproxil fumarate, Lamivudine, Stability, RP-HPLC

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INTRODUCTION

The human immunodeficiency virus is categorized in the Lentivirus genus, Retroviridae family and Orthoretrovirinae subfamily [1]. The human immunodeficiency virus is categorized into two groups, HIV type 1 and HIV type 2, based on genetic features and variations in the viral antigens [2]. Worldwide, the assessed number of people active with HIV infection as of 2019 is 38 million [3, 4]. Among 38 million, 1.8 million were children below 15 y age and 36.2 million were adults. As of 2019 end, there had been global access to antiretroviral treatment for 25.4 million individuals with HIV infection. This means that still 12.6 million people are waiting.

Delstrigo tablets, which was approved by FDA in 2018, were suggested as a full medication regimen for HIV type 1 infection in adults with no previous history of antiretroviral therapy [5, 6]. Delstrigo tablet contains fixed dose of doravirine (DRV-100 mg), tenofovir disoproxil fumarate (TFF-300 mg) and lamivudine (LMV-300 mg) [7]. DRV is a non-nucleoside analogue while TFF and LMV are nucleoside analogues. These three drugs (DRV, TFF and LMV) act as an inhibitor for reverse transcriptase enzyme in HIV, causes viral DNA chain termination that leads to the inhibition of viral replication [8-13].

To quantify LMV, spectrophotometry [14, 15] and HPLC [16-19] based methods were proposed. TFF was quantified using spectrophotometry [20], HPLC [21-24] and LC-MS [25, 26] based methods. To determine DRV, LC-MS [27] based method was proposed. There is still no method for estimating the combination of DRV, TFF and LMV in tablets. The combination of DRV, TFF and LMV is not endorsed in any Pharmacopeia. It is therefore prerequisite to develop a simple quantitative method for estimating the combination of DRV, TFF and LMV in tablets. Current work describes a specific RP-HPLC (stability-indicating) method for estimating the content of DRV, TFF and LMV simultaneously in tablets.

MATERIALS AND METHODS

Instrument

The simultaneous analysis of DRV, TFF and LMV in the tablets and bulk materials was done with the HPLC system (Agilent 1100 series, G1311 A Quaternary pump, G1316 A thermostat column, G1329 A autosampler and programmable G1314 A UV detector) and Luna Phenomenex C18 (250 mm × 4.6 mm × 5 µ).

Chemicals

DRV, TFF and LMV reference samples were collected from Merck Pharmaceutical Ltd (Mumbai), Cipla Ltd (Hyderabad) and Aurobindo Pharma Ltd (Hyderabad), respectively. Delstrigo tablets (Merck and Co., Inc., NJ, USA) claimed to have 100 mg of DRV, 300 mg of TFF and 300 mg of LMV were procured locally. Methanol (HPLC class, Merck, Mumbai), ammonium formate (AR grade, Qualigens Fine Chemicals Ltd. Maharashtra), formic acid (AR grade, Qualigens Fine Chemicals Ltd. Maharashtra), water (HPLC class, Milli-Q, Merck), hydrochloric acid (AR grade, Rankem Chemicals Ltd, Maharashtra), sodium hydroxide (AR grade, Rankem Chemicals Ltd, Maharashtra) and peroxide (AR grade, Rankem Chemicals Ltd, Maharashtra) were used.

HPLC conditions

Separation and assay of DRV, TFF, LMV and their forced degraded products were achieved on the Luna Phenomenex C18 (250 mm × 4.6 mm × 5 µ). The mixture of methanol (35% volume ratio) and 20 mmol ammonium formate (65% volume ratio, pH 5.0 set with 0.1% formic acid) was opted as mobile phase. The elution was operated in an isocratic system with 1 ml/min flow rate of opted mobile phase and 20 µl of sample injection volume. The temperature at the column was ambient. The detection and quantification of DRV, TFF, LMV were done with the UV detector tuned at 234 nm. Methanol (50% volume ratio) and water (45% volume ratio) was employed as the solvent system (diluent) for preparing sample and standard solutions.

DRV, TFF, LMV combination solutions

Standard stock combination solution (DRV-1000 µg/ml, TFF-3000 µg/ml, LMV-3000 µg/ml) was made ready by dissolving accurate quantities of DRV (100 mg), LMV (300 mg) and TFF (300 mg) in 30 ml of the diluent solvent system followed by dilution with the opted mobile phase to 100 ml.

10 ml of the above standard stock combination solution was further diluted to 100 ml with opted mobile phase to get working

combination solution with concentration 100 µg/ml of DRV, 300 µg/ml of TFF and 300 µg/ml of LMV.

Calibration curves of DRV, TFF and LMV

Standard stock combination solution (DRV-1000 µg/ml, TFF-3000 µg/ml, LMV-3000 µg/ml) was diluted serially with the opted mobile phase to obtain seven solutions. The concentrations of DRV, TFF and LMV in seven solutions were:

Solution 1: DRV-25 µg/ml, TFF-75 µg/ml, LMV-75 µg/ml

Solution 2: DRV-50 µg/ml, TFF-150 µg/ml, LMV-150 µg/ml

Solution 3: DRV-75 µg/ml, TFF-225 µg/ml, LMV-225 µg/ml

Solution 4: DRV-100 µg/ml, TFF-300 µg/ml, LMV-300 µg/ml

Solution 5: DRV-125 µg/ml, TFF-375 µg/ml, LMV-375 µg/ml

Solution 6: DRV-150 µg/ml, TFF-450 µg/ml, LMV-450 µg/ml

Solution 7: DRV-200 µg/ml, TFF-600 µg/ml, LMV-600 µg/ml

20 µl of each solution was applied to the HPLC system and analysed through employing the proposed methodology. The peak areas of DRV, TFF and LMV at every concentration were recorded. The calibration curves were charted in the range of 25-200 µg/ml for DRV and 75-600 µg/ml for TFF and LMV.

Estimation of DRV, TFF and LMV in tablets

Weight of ten tablets (each having 100 mg of DRV, 300 mg of TFF and 300 mg of LMV) was measured and crushed to powder. A powder weight equal to 100 mg of DRV, 300 mg of TFF and 300 mg of LMV was dissolved in diluent solvent system by sonication for 20 min. After sonication, the resulting solution was and diluted to a volume of 100 ml with opted mobile phase system. 10 ml of the above stock tablet solution was further diluted to 100 ml with opted mobile phase to get working tablet solution for assay. 20 µl samples of working tablet solution were applied to the HPLC system. Applying the proposed methodology, chromatograms and peak areas for DRV, TFF, and LMV were recorded. The quantities of DRV, TFF, and LMV present in tablets were obtained from DRV, TFF, and LMV calibration curves or their regression equations, respectively.

Degradation studies

Degradation study was conducted on the tablet solution using 0.1N NaOH, 3% peroxide, 0.1 N HCl and on tablet powder using UV light and Dry heat of 60 °C [28].

Hydrolytic degradation with 0.1N HCl

10 ml of stock tablet solution (DRV-1000 µg/ml, TFF-3000 µg/ml, LMV-3000 µg/ml) was left for 24 h with 50 ml of 0.1N HCl solution at room temperature. The solution was then neutralized using 0.1N NaOH solution, filtered and the volume was diluted to 100 ml with opted mobile phase system. 20 µl samples of 0.1N HCl degraded tablet solution were applied to the HPLC system and analysed by applying the proposed methodology. It was determined how much quantities of DRV, TFF, and LMV remained in tablets.

Hydrolytic degradation with 0.1N NaOH

10 ml of stock tablet solution (DRV-1000 µg/ml, TFF-3000 µg/ml, LMV-3000 µg/ml) remained at room temperature for 24 h with 50 ml of 0.1N NaOH solution. The solution was then neutralized using 0.1N HCl solution, filtered and the volume was diluted to 100 ml with opted mobile phase system. 20 µl sample of 0.1N NaOH degraded tablet solution was added to the HPLC device and analysed using the suggested methodology. The quantities of DRV, TFF, and LMV remained in tablets were determined.

Oxidative degradation with 3% peroxide

10 ml of stock tablet solution (DRV-1000 µg/ml, TFF-3000 µg/ml, LMV-3000 µg/ml) was mixed with 50 ml of 3% peroxide for 24 h, filtered, and completed to 100 ml volume with opted mobile phase system. 20 µl sample of peroxide degraded tablet solution was added to the HPLC device, analysed using the methodology suggested. The quantities of DRV, TFF, and LMV remained in tablets were determined.

Thermal degradation at 60 °C

The tablet powder weight equal to 100 mg of DRV, 300 mg of TFF and 300 mg of LMV was taken in petridish and placed in oven for 6 h at 60 °C. Upon 6 h the thermal degraded tablet sample was treated as described in the section "Estimation of DRV, TFF and LMV in tablets" to determine the quantities of DRV, TFF and LMV remained.

Photodegradation using UV light (254 nm)

The tablet powder weight equal to 100 mg of DRV, 300 mg of TFF and 300 mg of LMV was taken in petridish and placed in UV chamber for 6 h at 254 nm. Upon 6 h the photo degraded tablet sample was treated as described in the section "Estimation of DRV, TFF and LMV in tablets" to assess the amounts of DRV, TFF and LMV remained.

RESULTS

Validation

The quantitative approach developed for the combined assay of DRV, TFF and LMV in the tablets has been validated in accordance with the standards of the International Conference on Harmonization [29, 30].

System suitability

The values of tailing factor, peak areas, theoretical plates and resolution for DRV, TFF and LMV peaks obtained with the optimized assay conditions were displayed in table 1 and a typical chromatogram of DRV, TFF and LMV combination was furnished in fig. 1.

Table 1: System suitability values for DRV, TFF and LMV obtained with optimized assay conditions

Parameter	Results*	RSD**	Acceptable limit
RT	LMV-3.351 min	0.945	-
	TFF-4.952 min	0.668	
	DRV-8.156 min	0.343	
Resolution	LMV-----	-	Value should exceed 2.0
	TFF-5.33	0.432	
	DRV-11.95	0.200	
Area	LMV-490870	0.097	-
	TFF-595353	0.080	
	DRV-149204	0.192	
Theoretical Plates	LMV-3528	0.081	Value should exceed 2000
	TFF-5182	0.304	
	DRV-8070	0.024	
Tailing Factor	LMV-0.91	1.247	Value should be equal or less than 2.0
	TFF-1.05	0.795	
	DRV-0.95	0.744	

*mean for five values; **RSD for five values

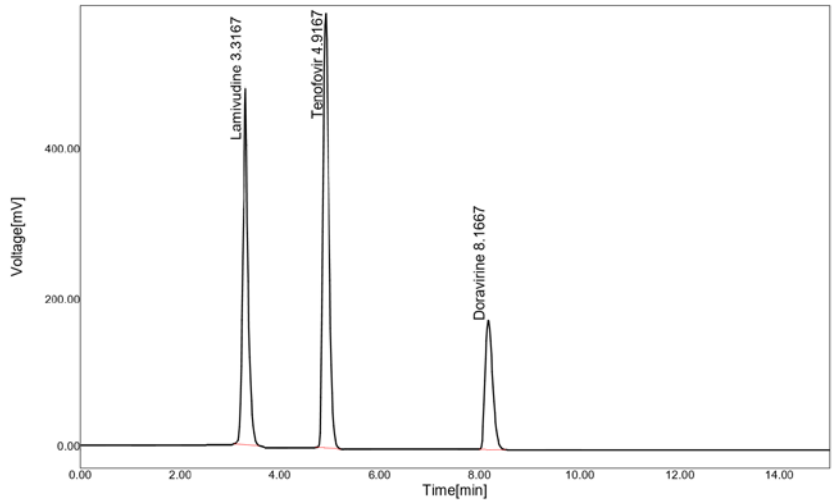

Fig. 1: DRV, TFF and LMV chromatogram obtained with optimized assay conditions

Selectivity

Specificity was demonstrated by assessing the chromatograms attained with the analysis of working combination solution (DRV-100 µg/ml, TFF-300 µg/ml, LMV-300 µg/ml), blank diluent solvent system and working tablet solution (DRV-100 µg/ml, TFF-300 µg/ml, LMV-300 µg/ml). Fig. 2 shows the representative chromatograms. No peaks were identified in the chromatogram (fig. 2a) of the blank diluent solvent system. The retention times of LMV, TFF and DRV in the chromatogram (fig. 2c) of working tablet solution was confirmed with retention times of LMV, TFF and DRV in the chromatogram (fig. 2b) of working combination solution. Additional peaks other than LMV, TFF and DRV peaks were not identified in the chromatogram (fig. 2c) of working tablet solution.

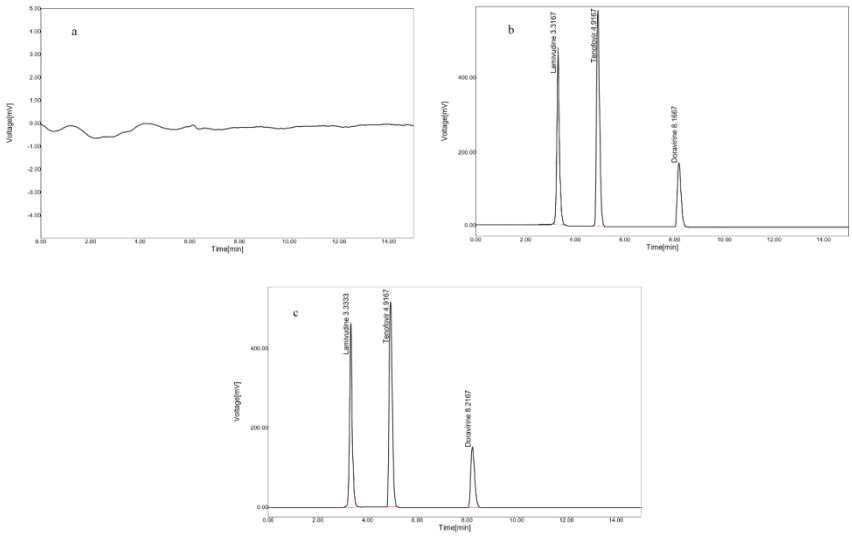

Fig. 2: Chromatograms of (a) Blank diluent solvent system (b) Working combination solution (c) Working tablet solution

Linearity

LMV, TFF, and DRV calibration curves were generated by a linear regression system. The range of linearity investigated was 25-200 µg/ml for DRV and 75-600 µg/ml for TFF and LMV with coefficients of regression 0.9991 (LMV), 0.9989 (TFF) and 0.9998 (DRV). The equations obtained with linear regression system were:

Peak response area = 1571.3 *x*+27624 for LMV

Peak response area = 1795.6 *x*+38515 for TFF

Peak response area = 1523.6 *x*–3814 for DRV

Quantification limit and detection limit

For this assay procedure, the quantification and detection limits were estimated on the basis of the standard deviation (STD) of area response and slope (SE) of calibration graph by using the formula given here [29, 30]:

$$\text{Detection limit} = 3.3 \times \frac{\text{STD}}{\text{SE}}$$

$$\text{Quantification limit} = 10 \times \frac{\text{STD}}{\text{SE}}$$

The detection limits for LMV, TFF and DRV were 1.623 µg/ml, 3.326 µg/ml and 0.705 µg/ml, respectively. The quantification limits were 4.918 µg/ml, 10.078 µg/ml and 2.136 µg/ml for LMV, TFF and DRV, respectively. The method presented was, therefore, sufficiently sensitive for the combined assay of LMV, TFF and DRV in tablets.

Precision

The precision of the assay method was determined as intraday variation/precision, interday variation/precision and intermediate

precision/ruggedness. Intraday variation/precision was demonstrated using six measurements of the working combination solution (DRV-100 µg/ml, TFF-300 µg/ml and LMV-300 µg/ml) on the same day. Intraday variation/precision was demonstrated by measurements of the working combination solution (DRV-100 µg/ml, TFF-300 µg/ml and LMV-300 µg/ml) on 3 different days. Intermediate precision/ruggedness was demonstrated by measurements of the working combination solution (DRV-100 µg/ml, TFF-300 µg/ml and LMV-300 µg/ml) by different analysts. All details are mentioned in table 2.

Table 2: Repots of precision investigation

Parameter	LMV	TFF	DRV
Intraday variation/precision			
Mean peak area* (n=6)	489481.37	593863.02	148831.17
RSD of peak areas** (n=6)	0.158	0.305	0.219
Interday variation/precision–Day 1			
Mean peak area$ (n=3)	491195.50	594005.73	148971.97
RSD of peak areas$$ (n=3)	0.108	0.441	0.422
Interday variation/precision–Day 2			
Mean peak area$ (n=3)	489190.10	596195.33	150010.37
RSD of peak areas$$ (n=3)	0.421	0.719	0.892
Interday variation/precision–Day 3			
Mean peak area$ (n=3)	489338.87	593807.03	149069.80
RSD of peak areas$$ (n=3)	0.153	0.197	0.108
Over all Interday variation/precision			
Mean peak area$ (Days 1,2,3)	489908.16	594669.36	149350.71
RSD of peak areas$$ (Days 1,2,3)	0.228	0.223	0.384
Intermediate precision/ruggedness–Analyst 1			
Mean peak area$ (n=3)	490770.80	595747.07	148936.20
RSD of peak areas$$ (n=3)	0.631	0.196	0.792
Intermediate precision/ruggedness–Analyst 2			
Mean peak area$ (n=3)	488962.70	595556.70	146049.03
RSD of peak areas$$ (n=3)	0.257	0.286	1.082
Over all Intermediate precision/ruggedness			
Mean peak area# (Analysts 1 and 2)	489866.75	595651.89	147492.62
RSD of peak areas## (Analysts 1 and 2)	0.261	0.023	1.384

*mean for six values; **RSD for six values; $mean for three values; $$RSD for three values; #mean for two values; ##RSD for two values

Robustness

The robustness of the assay method was checked by making marginal and deliberate modifications to experimental parameters, for example methanol ratio and pH in the mobile phase solvent system, and detection wavelength. The working combination solution (DRV-100 µg/ml, TFF-300 µg/ml and LMV-300 µg/ml) was analysed with changed conditions and optimized conditions. Analysed the working combination solution (DRV-100 µg/ml, TFF-300 µg/ml and LMV-300 µg/ml) with modified assay conditions and optimized assay conditions. All details are mentioned in table 3.

Table 3: Repots of robustness investigation

Parameter	LMV	TFF	DRV
Mobile phase			
Optimal assay condition (methanol 35% vol and 20 mmol ammonium formate 65% vol)	490020.3	595154.8	149673.8
Modified assay condition 1 (methanol 40% vol and 20 mmol ammonium formate 60% vol)	491395.1	591246.3	148239.1
Modified assay condition 2 (methanol 30% vol and 20 mmol ammonium formate 70% vol)	486267.9	590362.7	149582.6
Mean peak area$ (n=3)	489227.77	592254.60	149165.17
RSD of peak areas$$ (n=3)	0.542	0.431	0.539
pH			
Optimal assay condition (pH 5.0)	490020.3	595154.8	149673.8
Modified assay condition 1 (pH 4.9)	488987.2	596316.8	150021.4
Modified assay condition 2 (pH 5.1)	493636.2	589956.4	148991.1
Mean peak area$ (n=3)	490881.23	593809.33	149562.10
RSD of peak areas$$ (n=3)	0.497	0.570	0.350
Detection wavelength			
Optimal assay condition (234 nm)	490020.3	595154.8	149673.8
Modified assay condition 1 (229 nm)	494591.8	597974.5	147676.9
Modified assay condition 2 (239 nm)	493263.5	590467.1	148582.4
Mean peak area$$ (n=3)	492625.20	594532.13	148644.37
RSD of peak areas$$(n=3)	0.477	0.638	0.673

$mean for three values; $$RSD for three values

Accuracy

To demonstrate accuracy, LMV, TFF and DRV spiked tablet sample solutions were analysed. The recovery of spiked LMV, TFF and DRV concentrations at different levels are given in table 4. The obtained recovery is in the range of 98.65-99.76% for LMV, TFF and DRV.

Stability of LMV, TFF and DRV

The stability of LMV, TFF and DRV in the applied degradation conditions like 0.1N NaOH, 3% peroxide, 0.1 N HCl, UV light and dry heat of 60 °C was studied through degradation studies. The percentage quantity of LMV, TFF and DRV degraded was shown in table 5.

Table 4: Reports of accuracy investigation

50% spiked accuracy			100% spiked accuracy			150% spiked accuracy		
Concentration (µg/ml)		Recovery percent	Concentration (µg/ml)		Recovery percent	Concentration (µg/ml)		Recovery percent
Spiked	Quantified		Spiked	Quantified		Spiked	Quantified	
Lamivudine accuracy reports								
150	148.14	98.76	300	297.45	99.149	450	449.10	99.8
150	149.58	99.72	300	297.83	99.275	450	448.43	99.65
150	149.04	99.36	300	296.57	98.857	450	449.19	99.82
Mean$ recovery		99.28	Mean$ recovery		99.09	Mean$$ recovery		99.76
RSD$$ of recovery		0.488	RSD$$ of recovery		0.216	RSD$$ of recovery		0.093
Tenofovir disoproxil fumarate accuracy reports								
150	149.07	99.38	300	297.45	99.15	450	448.56	99.68
150	148.52	99.01	300	299.08	99.69	450	442.58	98.35
150	148.37	98.91	300	298.12	99.37	450	446.72	99.27
Mean recovery$		99.10	Mean$$ recovery		99.41	Mean$$ recovery		99.10
RSD$$ of recovery		0.250	RSD$$ of recovery		0.274	RSD$$ of recovery		0.687
Doravirine accuracy reports								
50	49.67	99.33	100	98.49	98.49	150	147.72	98.48
50	49.64	99.28	100	99.05	99.05	150	148.38	98.92
50	49.62	99.23	100	99.46	99.46	150	147.84	98.56
Mean$ recovery		99.28	Mean$ recovery		99.00	Mean$ recovery		98.65
RSD$$ of recovery		0.050	RSD$$ of recovery		0.492	RSD$$ of recovery		0.238

$mean for three values; $$RSD for three values

Table 5: Reports of stability investigation

Condition	LMV			TFF			DRV		
	Percent degraded	Percent stability	RSD*	Percent degraded	Percent stability	RSD*	Percent degraded	Percent stability	RSD*
0.1N HCl	5.86	94.14	0.431	6.88	93.12	0.585	6.86	93.14	0.333
Dry heat of 60 °C	2.10	97.90	0.884	2.83	97.17	1.555	2.27	97.73	1.075
0.1N NaOH	7.69	92.31	0.140	8.19	91.81	0.124	6.45	93.55	0.660
3% Peroxide	6.08	93.92	1.371	5.62	94.38	0.967	5.04	94.96	0.999
UV Light	8.92	91.08	0.162	9.31	90.69	0.104	8.14	91.86	0.628

*mean for three percent degraded values

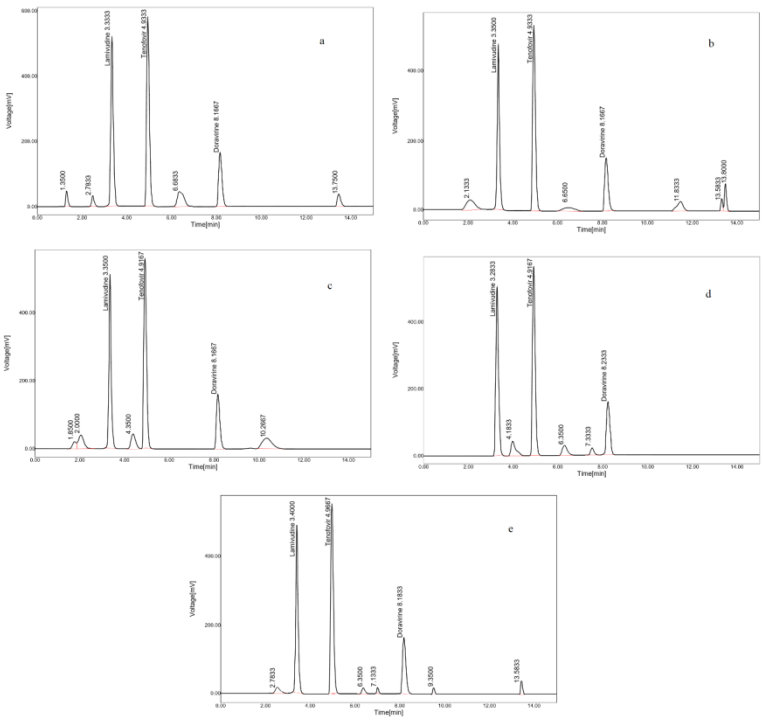

Fig. 3: Chromatograms of (a) 0.1N HCl (b) 0.1N NaOH (c) 3% peroxide (d) Dry heat (e) UV light degraded tablet solution

Table 6: Repots of tablet analysis

Parameter	LMV	TFF	DRV
Content (mg) in tablet	300	300	100
Mean[$] quantified (mg) (n=3)	298.08	296.04	99.14
Mean[$] assay percent (n=3)	99.36	98.68	99.14
RSD[$$] of assay percent (n=3)	0.118	0.062	0.433

[$]mean for three values; [$$]RSD for three values

Specificity

The resolution of LMV, TFF, DRV and its degradation components, as shown in chromatograms (fig. 3a-e) of degraded tablet sample solutions, showed stability, indicating characteristic feature and specificity of assay method for the LMV, TFF and DRV combined analysis.

Application of assay method to the tablets

The assay method established was employed to evaluate the LMV, TFF, and DRV content in the Delstrigo tablets (DRV-100 mg, TFF-300 mg and LMV-300 mg). The reports of LMV, TFF and DRV content evaluation in the Delstrigo tablets was displayed in table 6. The obtained recovery (98.68-99.36%) values and RSD of percent assay (0.062-0.433%) values of LMV, TFF and DRV confirming the good accuracy and precision of the assay method for the LMV, TFF and DRV combined analysis in tablets.

DISCUSSION

For quantification of LMV, TFF and DRV, methods using spectrophotometry [14, 15, 20], HPLC [16-19, 21-24] and LC-MS [25-27] were found. But these methods [14-27] were used to quantify LMV, TFF and DRV individually in samples. These methods [14-27] were not applied for the combined quantification of LMV, TFF and DRV. Therefore, a stability-indicating HPLC methodology was established in this study to analyse DRV, TFF and LMV in combination. During development, essential parameters like the solvent combination as mobile phase, pH and column as stationary phase were examined. The column as stationary phase examined include Lichrospher ODS RP-18 (4.5 mm × 150 mm × 5 µm), Waters RP-18 column having dimensions of 4.5 mm × 150 mm × 5 µm and 4.5 mm × 250 mm × 5 µm, and Phenomenex luna C18 having dimensions of 4.5 mm × 150 mm × 5 µm and 4.5 mm × 250 mm × 5 µm. The solvent combinations examined were methanol: buffer of ammonium acetate (0.1M, pH 5.2) in 70:30 vol/vol ratio, acetonitrile: buffer of phosphate (0.1M, pH 5.5) in 50:50 vol/vol ratio, methanol: buffer of phosphate (0.1M, pH 5.0) in 30:70 vol/vol ratio, methanol: buffer of acetate (0.1M, pH 5.7) in 40:60 vol/vol ratio, methanol: ammonium formate (20 mmol, pH 5.0, 5.8, 6.1) in ratios like 50:50 vol/vol, 70:30 vol/vol and 35:65 vol/vol. The flow rate remains constant all along the trails at 1.0 ml per min. During trails, the results were checked for values of tailing factor, peak areas, theoretical plates and resolution for the DRV, TFF and LMV peaks. Better, satisfactory and acceptable results were achieved with Luna phenomenex C18 (4.5 mm × 250 mm × 5 µm) stationary phase at ambient temperature with methanol: ammonium formate (20 mmol, pH 5.0) in ratio of 35:65 vol/vol as mobile phase. Better sensitivity (peak areas) for DRV, TFF and LMV was obtained with UV detector set at 234 nm.

The method presented was selective for the combined assay of LMV, TFF and DRV in tablets without interruption with diluent solvent system components and tablet excipients [14-27,29,30]. The coefficients of regression higher than 0.99 indicating a lined correlation between the LMV, TFF, and DRV concentration and their respective area responses [14-27,29,30]. The method was sufficiently sensitive for the combined assay of LMV, TFF and DRV in tablets because of low values of quantification and detection limits [14-27,29,30]. The precision results were below 2% RSD. The reports indicated that the assay method was precise for the LMV, TFF and DRV combined analysis [14-27,29,30]. The robustness results were below 2% RSD. It was verified that there were no significant changes in the reports showing that the presented assay method was robust for the LMV, TFF and DRV combined analyses. The obtained recovery (98.65-99.76%) values of LMV, TFF and DRV confirming the good accuracy of assay method for the LMV, TFF and DRV combined analysis [14-27,29,30]. During degradation, the order of stability was [28]:

LMV: Dry heat of 60 °C > 0.1N HCl > 3% Peroxide > 0.1N NaOH > UV light

TFF: Dry heat of 60 °C > 3% Peroxide > 0.1N HCl > 0.1N NaOH > UV light

DRV: Dry heat of 60 °C > 3% Peroxide > 0.1N NaOH > 0.1N HCl > UV light

CONCLUSION

Current work described a RP-HPLC (stability-indicating) method for estimating DRV, TFF and LMV simultaneously. This method can be preferred for the quantification of DRV, TFF and LMV simultaneously in the tablets because of its sensitivity, accuracy, preciseness, robustness, selectivity, suitability and specificity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

None exists in the current study.

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