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RESEARCH ARTICLE

Development and Validation of Stability Indicating Related Substances Method for Dolutegravir/Lamivudine/Tenofovir Disoproxil Fumarate (DLT) Tablets using High Performance Liquid Chromatography

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ABSTRACT:

A novel, accurate, specific, linear, precise and robust RP-HPLC method (stability indicating) has been developed and validated for the related substances (impurities) analysis of Dolutegravir, Lamivudine and Tenofovir Disoproxil Fumarate in tablet formulation. This research paper presents the developed method and the outcome of validation challenges. The RP-HPLC method was developed on a 250 x 4.6 mm, 5 µm, C18 column, with a gradient mode using combination of phosphate buffer and phosphoric acid in methanol and water as mobile phase, the detection was performed at 265nm and 235nm. The method was subjected to validation challenges of specificity, precision, linearity, accuracy, robustness and is demonstrated to be suitable for testing of stability samples.

KEYWORDS: Dolutegravir Sodium, Lamivudine, Tenofovir Disoproxil Fumarate, Liquid chromatography, Stability Indicating, Related Substances.

INTRODUCTION:

Incidence of HIV infections globally is very high and health agencies have been constantly in pursuit of providing HIV patients with improved therapeutic alternatives. WHO recommends fixed dose multidrug treatment regimens in an endeavor to enhance the patient compliance (one of the major challenges of multidrug treatment regimens) and thus help achieving key goals of treatment i.e., increase in longevity of patient's life along with improvement in Quality of life. The WHO 2019 guideline recommends oral triple combination of DLT as first line of treatment for HIV infection in adults.

The development of stability indicating related substances method is challenging for this triple combination product as the number of impurities that needs to be separated are high. In this research work a common RP-HPLC method for determination of related substances of all the three active components i.e., Dolutegravir, Lamivudine and Tenofovir disoproxil fumarate in oral solid dosage form was successfully developed and validated.

Dolutegravir:

Dolutegravir is an integrase strand transfer inhibitor[1] belonging to second generation of antiretrovirals. It is a preferred drug of choice due to no requirement of dose adjustments when combined with the NRTI class[2,3]. Dolutegravir is chemically (4R,12aS)-N-[(2,4-difluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido [1',2':4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide (WHO) has a chemical formula of $C_{20}H_{18}F_2N_3NaO_5$ with Mol. Wt of 419.3788.

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Lamivudine:
Lamivudine is L-enantiomeric analog of Cytosine having reverse transcriptase inhibitor activity against hepatitis B and Human immunodeficiency virus. It is first line antiretroviral drug in treatment guidelines[4]. Lamivudine is indicated for the treatment of Human Immunodeficiency Virus and chronic HBV infection[5]. The chemical name of Lamivudine is 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one[6] and molecular weight of 229.254 with a chemical formula of $C_8H_{11}N_3O_3S$.

Tenofovir Disoproxil Fumarate:
Tenofovir DF is a bis-alkoxyester pro drug and is acyclic nucleotide analog with reverse transcriptase inhibitor activity against Human Immunodeficiency Virus and HBV. Tenofovir is released into systemic circulation by cleavage of promoieties during adsorption[7]. It is chemically 9- [R- (2[[bis]] isopropoxycarbonyl) oxy] methoxy] phosphonyl] methoxy] propyl] adenine fumarate (1:1) and has molecular weight of 635.52 and a chemical formula of $C_{23}H_{34}N_5O_{14}P$.

Development of cost effective, precise, specific, linear, robust and accurate stability indicating quantitative method that simultaneous estimation of 13 known impurities of Dolutegravir, Lamivudine and Tenofovir DF is a challenging task and was accomplished successfully by applying the underlying theoretical concepts and expertise of developers.

MATERIALS AND METHODS:

Waters Quaternary pump HPLC equipped with PDA detector, Waters Sunfire C18, 250 x 4.6mm, 5µm analytical column, HPLC grade KH_2PO_4, o-phosphoric acid, HPLC grade H_2O, HPLC grade SD fine methanol and Millipore 0.45µm filter.

Several trials were undertaken and the below mentioned method was finalized and subjected to validation challenges as per ICH guidelines[8].

Method subjected to analytical method validation:
Chromatographic conditions: Mobile Phase A: 10mM Potassium dihydrogen phosphate pH 4.0, Mobile Phase B: 0.1% o-phosphoric acid in methanol and water (75:25) Column: Waters Sunfire C18, 250 x 4.6mm, 5µm, Flow Rate: 1.4 mL/min, Wavelength: 265 and 235 nm, column oven temperature: 30°C±2°C, Sample Cooler Temperature: 10°C±2°C, Injection Volume: 20µL, Injection delay 10 mins, Diluent: 10mM KH_2PO_4 pH 2.5 and methanol in the ratio of 80:20 respectively.

Flow Program:

Time (min)	% MP A	% MP B
0	95	5
9	95	5
27	88	12
45	57	43
55	45	55
82	42	58
100	23	77
108	2	98
116	95	5
120	95	5

Table:1 Chemical name of Drug substances and its impurities with its retention times

Name of drug substance/Impurity Name	Drug substance/Impurity Identification Name	Retention time (in mins)
Lamivudine	Lamivudine	21.20
Tenofovir Disoproxil Fumarate	Tenofovir Disoproxil Fumarate	72.21
Dolutegravir Sodium	Dolutegravir Sodium	94.52
Tenofovir Disoproxil Fumarate Impurities		
Adenine Impurity	Impurity A (Imp A)	7.83
Tenofovir Impurity or TDF-II Impurity	Impurity B (Imp B)	17.68
Mono ester impurity	Impurity C (Imp C)	45.89
Mono POC Dimer Impurity	Impurity D (Imp D)	69.71
Mixed Dimer Impurity	Impurity E (Imp E)	101.21
Dimer Impurity	Impurity F (Imp F)	110.43
Lamivudine Impurities		
Carboxylic acid Impurity	Impurity G (Imp G)	5.55
Diastereomer Impurity	Impurity H (Imp H)	20.51
Dolutegravir Sodium Impurities		
Hydroxy impurity	Impurity I (Imp I)	88.73
Methyl Dolutegravir Impurity	Impurity J (Imp J)	91.14
2-Fluoro impurity	Impurity K (Imp K)	91.73
Des Fluoro or 4-Fluoro impurity	Impurity L (Imp L)	89.62
Isomer-1 and 2	Impurity M (Imp M)	95.32

Validation Outcomes:

Linearity:
The Linearity of the test method was established from LOQ to 150% of limit concentration (0.2%) as tabulated below. All the three active pharmaceutical ingredients and its impurities exhibited linear behavior in the specified range. The linearity data is presented below in Table:2. Refer Fig:2 for corresponding Linearity plots.

Table:2 Linearity Data (Dolutegravir, Lamivudine, Tenofovir DF and its impurities).

Component	Concentration (µg/mL)	Regression equation	R^2
Impurity A	0.071 – 6.921	y = 66160x+234.94	0.9999
Impurity B	0.092 – 7.044	y = 36521x -491.96	0.9999
Impurity C	0.112 – 7.012	y = 66377x- 2269.7	0.9999
Impurity D	0.106 –7.033	y = 67982x- 1127.9	0.9997
Impurity E	0.104 – 6.982	y = 16441x+ 1146	0.9998
Impurity F	0.114 – 7.013	y = 61661x - 749.2	0.9996
Impurity G	0.091 – 7.084	y = 39493x + 192.5	0.9998
Impurity H	0.102 – 7.023	y = 23770x+706.91	0.9999
Impurity I	0.153 – 7.503	y = 32986x -564.03	0.9999

Impurity J	0.073 – 7.113	y = 66294x -490.95	0.9998
Impurity K	0.076 – 7.234	y = 65911x -907.64	0.9999
Impurity L	0.071 – 7.084	y = 65838x -699.53	0.9999
Impurity M	0.074 – 7.183	y = 65892x -1069.9	0.9998
Tenofovir Disoproxil Fumarate	0.082 – 7.044	y = 36504x + 6015	0.9997
Lamivudine	0.126 – 7.118	y = 73907x -4296.6	0.9994
Dolutegravir	0.108 – 7.243	y = 99770x+123.41	0.9997

Accuracy:

The accuracy of an analytical process expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found[8].

The study was performed at 50%, 100% and 150% (triplicate preparations at each level) of limit concentration (0.2%). The individual and mean accuracy at each level for all the impurities was found to be between 80.0 to 120.0%. The accuracy data is presented in Table:3

Table:3 Accuracy

Criteria	Accuracy Level	Impurities												
		A	B	C	D	E	F	G	H	I	J	K	L	M
Average % Recovery	50%	87.2	90.2	88.6	89.4	88.2	90.6	100.2	101.1	83.8	95.4	100.4	86.6	93.1
	100%	95.4	99.8	92.1	94.3	95.0	94.3	93.1	98.2	97.7	99.3	98.2	96.4	99.7
	150%	97.4	99.7	94.0	98.3	96.1	99.8	102.5	103.2	98.3	99.1	100.5	96.3	99.1
RSD (%Recovery)	50%	3.0	3.1	2.8	4.8	2.6	3.8	3.4	4.7	4.0	4.3	1.4	3.6	4.4
	100%	2.9	1.8	3.9	4.8	3.0	4.1	1.5	2.3	2.7	3.5	3.2	3.8	3.2
	150%	1.2	1.4	4.4	1.9	3.6	2.4	2.9	2.0	1.0	1.9	2.0	4.1	3.7

Specificity: It is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.[8]

Table:4 Forced Degradation Study

Control sample (No treatment)		**Dolutegravir Peak Purity**		**Lamivudine Peak Purity**		**Tenofovir Disoproxil Fumarate Peak Purity**	
		PA	PT	PA	PT	PA	PT
		0.144	0.362	0.221	0.312	0.156	0.329
Forced Degradation Study							
Samples	**Condition**	**% Degradation**	**Dolutegravir Peak Purity**		**Lamivudine Peak Purity**		**Tenofovir Disoproxil Fumarate Peak Purity**	
			PA	**PT**	**PA**	**PT**	**PA**	**PT**
Acid Degradation	1N HCl for 30 mins at room temperature	10.2	0.208	0.424	0.118	0.301	0.224	0.386
Alkali Degradation	1N NaOH for 30 mins at room temperature	8.3	0.147	0.318	0.281	0.424	0.108	0.264
Peroxide Degradation	30% H_2O_2 for 90 mins at room temperature	4.6	0.228	0.543	0.192	0.284	0.233	0.414
Thermal Degradation	105°C for 2 hours	9.8	0.486	0.814	0.226	0.374	0.118	0.304
Humidity Degradation	25°C/95%RH/72Hrs	0.6	0.108	0.364	0.174	0.304	0.224	0.364
Water Degradation	60°C for 3 hours	0.3	0.222	0.501	0.443	0.628	0.321	0.484
Photo stability	Exposed to UV light at 200-watt hrs/Sq.mt and white light for about 1.2 million lux hours	0.2	0.133	0.308	0.267	0.474	0.208	0.389

PA=Purity Angle, PT=Purity Threshold

Specificity of the method was established by spiking all the available known impurities of all the three active pharmaceutical ingredients in sample. All the peaks were observed to be specific. The peak purity was evaluated, purity angle for Dolutegravir, tenofovir and lamivudine peaks was observed to be less than auto purity threshold in the spiked sample. The method met the validation challenge of specificity.

Forced degradation studies were performed. Prominent degradation was observed during acid, alkali and thermal degradation. Since PA is less than the PT for Tenofovir Disoproxil fumarate, Lamivudine and Dolutegravir peaks in all degradation conditions, the method met the criteria of stability indicating for related substances of impurities in Dolutegravir/Lamivudine/Tenofovir Disoproxil Fumarate tablets. The results from forced degradation study are tabulated in Table: 4