Research Article

Stability Indicating Gradient RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Abacavir and Dolutegravir in Bulk and their Combined Dosage Form

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ABSTRACT

The objective of this research was to develop simple, rapid, precise, accurate and economical stability-indicating reversed phase (RP) HPLC assay method and validated for simultaneous estimation of Lamivudine, Abacavir and Dolutegravir in bulk laboratory synthetic mixture and their combined dosage form. The method has shown adequate separation of Lamivudine (LAM), abacavir (ABA) and Dolutegravir (DOL) from their degradation products. Separation was achieved on Luna Phenyl Hexyl, (250 mm x 4.6mm, 5µm) column, using mobile phase acetonitrile and 0.1 M Ortho phosphoric acid buffer using a gradient program with a flow rate of 1ml/min, throughout the gradient program with a detection wavelength of 258 nm for all the compounds with injection volume of 20µl. The retention times for Lamivudine, Abacavir and Dolutegravir were found to be 3.3, 4.5 and 6.3 min respectively. The linearity range of LAM, ABA and DOL were 3-45 µg/ml, 6-90 µg/ml and 0.5-7.5 µg/ml respectively with a correlation coefficient of 0.999. The method was validated as for ICH Q2 R1 guidelines. The method was validated for selectivity, linearity, accuracy, robustness, precision and specificity and the obtained results are within acceptance range. The results were indicating the method was selective in analysis of all Lamivudine, Abacavir and Dolutegravir in the presence of degradation products formed under various stress conditions.

Keywords: Lamivudine, Abacavir, Dolutegravir, Luna Phenyl Hexyl, Acetonitrile, ICH Guideline.

INTRODUCTION

Lamivudine (LAM)

chemically- (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H) pyrimidinone[1], is a synthetic nucleoside analogue with potent activity against human immune deficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reverse transcriptase activity. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight- 229.3 g/mol, soluble in water, sparingly soluble in methanol and practically insoluble in acetone.

Abacavir (ABA)

chemically-(1S, cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt) (2:1). The IUPAC name of the abacavir is [(1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl] cyclopent-2-en-1-yl] methanol[2]. The molecular formula is $(C_{14}H_{18}N_6O)$ $2\bullet H_2SO_4$, molecular weight 286.33 g/mol and is soluble in water, methanol and buffers, fine crystal form with white in color. The mechanism of action by inhibiting the activity of HIV-1 reverse transcriptase (RT).

Dolutegravir (DOL)

chemically-(RS)(4R,12aS)-N-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido[11,21,4,5]pyrazino[2,1-b][1,3]oxazine-9-caboxamide[3]. Molecular Formula $C_{20}H_{19}F_2N_3O_5$ and molecular weight. 419.37 g/mol. It is slightly soluble in water and methanol. Dolutegravir is an FDA approved drug for the treatment of HIV infection. Dolutegravir is an integrase inhibitor. DTG is an integrase strand transfer inhibitor (INSTI) that does not require ritonavir for cytochrome P450 3A4 inhibition, and preferentially blocks the strand transfer step of integration of the viral genome into the host cell's DNA, which is a two-step process mediated by the viral integrase enzyme. TRIUMEQ (LAM-300mg, ABA-600 mg and DOL-50 mg) is the marked formulation of all these three drugs.

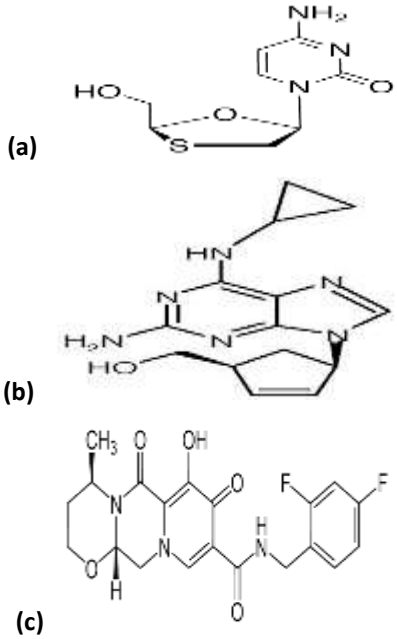


Figure 1a: Chemical structure of (a) Lamivudine (b) Abacavir (c) Dolutegravir

The literature survey reveals that there was spectrophotometric[4-6], HPLC-MS/MS Method[7], and chromatographic methods established for individual and simultaneous estimation of abacavir[8,9] and lamivudine[10-15], in bulk and combined dosage forms. There were no reported analytical methods for simultaneous estimation LAM, ABA & DOL in bulk and their combined dosage forms in presence of their degradation products. Hence an author made an attempt to develop stability indicating specific, sensitive, accurate and precise RP-HPLC method for simultaneous estimation of these drugs using gradient elution mode. The developed method was validated as per ICH Q2 R1 guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Lamivudine (99.5%), Abacavir (99.6%) and Dolutegravir (99.4%) are obtained as gift samples from Hetero laboratories, Hyderabad, India. HPLC grade acetonitrile, Ortho Phosphoric Acid were purchased from Merck (Mumbai, India), HPLC grade Water (Milli Q or equivalent) all chemicals (AR Grade) were used for entire study.

Instrumentation

All HPLC experiments were carried out on a Waters Alliance 2695 separation module, with waters 2996 photodiode array detector in gradient mode using Auto sampler. Data collection and processing was done using EMPOWER PDA 2 software. The analytical column used for the separation was Luna phenyl hexyl (250mm x 4.6mm, 5µm) Column, Other equipments used were ultra-sonicator (model 3210) Analytical balance (contech balance).

Preparation of solutions

Diluent: Mix Buffer and Acetonitrile in the ratio of 50:50, is used as a diluent.

Mobile phase: Acetonitrile: Buffer mixture used as a mobile phase injected into the system through a gradient flow indicated in Table 1a.

Table 1a: Grad+aunient Program

S. No	Time	Flow	Acetonitrile (ACN)	Buffer
1	0.01	1.00	10.0	90.0
2	3.00	1.00	80.0	20.0
3	5.00	1.00	10.0	90.0
4	15.00	1.00	10.0	90.0

Preparation of standard solution

Solution A: Lamivudine

Weigh accurately about 30 mg of Lamivudine working standard into a 100 ml volumetric flask. Add 70 ml of diluents (Mix Buffer and acetonitrile in the ratio of 50:50), sonicate to dissolve and dilute to volume with diluent.

Solution B: Abacavir

Weigh accurately about 60mg Abacavir working standard into a 100 ml volumetric flask. Add 70 ml of diluents (Mix Buffer and acetonitrile in the ratio of 50:50), sonicate to dissolve and dilute to volume with diluent.

Solution C: Dolutegravir

Weigh accurately about 5mg Dolutegravir working standard into a 100 ml volumetric flask. Add 70 ml of diluents (Mix Buffer and acetonitrile in the ratio of 50:50), sonicate to dissolve and dilute to volume with diluent.

Further dilute each 5ml of Solution-A, B and C to 50 ml with diluent to get the required concentration of 30 µg/ml Lamivudine, 60 µg/ml Abacavir and 5 µg/ml Dolutegravir.

Chromatographic conditions

The determination was carried out on Waters HPLC 2690 equipped with PDA 996 as detector using data handling system – waters empower 2.0 software. The column used in the development for the determination is Luna Phenyl Hexyl, (250mm x 4.6mm, 5µm). The detection wavelength at 258 nm with a flow rate of 1ml/min was used for the determination of Lamivudine, Abacavir and Dolutegravir. The 20µl sample was injected into HPLC system. The corresponding peak and retention times were recorded for each drug. From the chromatogram retention times for Lamivudine, Abacavir and Dolutegravir were found to be 3.35, 4.55 and 6.31 mins respectively. Typical chromatogram of LAM, ABA & DOL show in fig. 1b and optimized chromatographic conditions as shown in below.

Column: Luna Phenyl Hexyl (250 x 4.6mm, 5µm)

Flow rate: 1ml/min

Wavelength: 258nm

Column temperature: Ambient

Injection volume: 10µl

Run time: 15min

Diluents: Mix Buffer and acetonitrile in the ratio of 50:50, is used as a diluent.

Elution: Gradient

Mobile phase: ACN+ Buffer

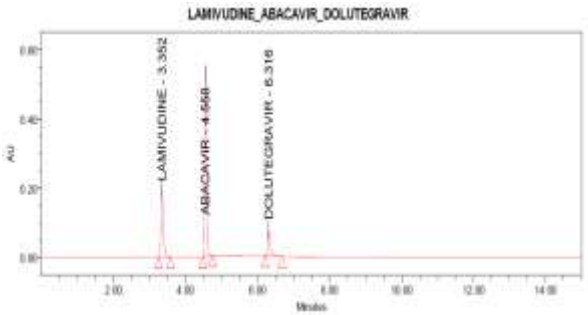


Figure 1b: Typical chromatogram of LAM, ABA & DOL

Method Development

To saturate the column, the mobile phase was pumped for about 30 minutes thereby to get the base line corrected. The separate standard calibration lines were constructed for each drug. A series of aliquots were prepared from the above stock solutions using HPLC grade water to get the concentrations 3-45µg/ml for Lamivudine, 6-90µg/ml for Abacavir and 0.5-7.5µg/ml Dolutegravir. Each concentration 6 times was injected in to chromatographic system. Each time peak area and retention time were recorded separately for all the drugs. Calibration curves were constructed as by taking average peak area on Y-axis and concentration on X-axis separately for all the drugs. From the calibration curves regression equations were calculated, these regression equations were used to calculate drug content in formulation.

Estimation of Lamivudine, Abacavir and Dolutegravir in laboratory synthetic mixture

For the estimation of Lamivudine, Abacavir and Dolutegravir, laboratory synthetic mixture was prepared with Lamivudine, Abacavir and Dolutegravir APIs and excipients (Mannitol) with the strength of 300 mg of Lamivudine, 600 mg of Abacavir, 50 mg of Dolutegravir and 300 mg of mannitol in glass motor and pestle, after proper mixing, weigh accurately about a quantity of powder which was equivalent to 60 mg of Abacavir was transferred to100 ml volumetric flask add 70 ml of diluent, sonicate to dissolve and dilute to volume with diluent. Further dilute 5 ml to 50 ml with the diluent. Filter through 0.45µ Nylon syringe filter. This solution was estimated by above developed method. The assay procedure was repeated 6 times (n=6) the drug content was estimated using above calculated regression equation; the results of laboratory mixture are shown in the table 2.

Estimation of LAM, ABA and DOL in tablet dosage forms

Weigh 20 tablets and crush to powdered then take powder equivalent 30 mg LAM (60 mg ABA & 5 mg DOL) into a 100 ml volumetric flask. Add 70 ml of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5 ml to 50 ml with the diluent. Filter through 0.45µ Nylon syringe filter. This solution was estimated by above developed method. The assay procedure was repeated 6 times (n=6) the drug content was estimated using above calculated regression equation; the results of laboratory mixture are shown in the table 3.

Table 2: Results of laboratory synthetic mixture

Compound name	Test concentration (µg/ml)	Amount found (µg/ml)	%Assay	%RSD
Lamivudine	60	60.85	101.41	0.517
Abacavir	30	31.02	103.4	1.056
Dolutegravir	5	5.02	100.4	0.613

Table 3: Results of tablet dosage form

Compound name	Brand name	Label claim(mg)	Test concentration (µg/ml)	Amount found (µg/ml)	%Assay	%RSD
Lamivudine		600	60	60.43	100.7	0.640
Abacavir	TRIUMEQ	300	30	30.22	100.7	0.0277
Dolutegravir		50	5	5.04	100.8	0.674

Table 4: Linearity studies of proposed method

Parameters	Lamivudine	Abacavir	Dolutegravir
Linearity range (µg/ml)	3-45	6-90	0.5-7.5
Regression equation	y = 74605x + 3256	y = 49626x + 3006	y =61114x – 536
Slope	74605	49626	61114
Intercept	3256	3006	536
Correlation coefficient (r)	0.999	0.999	0.999
Accuracy (%Recovery)	100.58	100.27	100.11
System Precision (RSD)	0.5173	1.0559	0.6126
Method precision(RSD)	0.1227	0.9794	1.7265
LOD (µg/ml)	0.036	0.065	0.021
LOQ (µg/ml)	0.112	0.198	0.071

Table 5: Robustness studies for LAM, ABA & DOL

Method Parameters	Conditions	Retention Time(R_t)			Area			%Recovery		
		LAM	ABA	DOL	LAM	ABA	DOL	LAM	ABA	DOL
Flow +	+20 %	2.807	3.868	5.583	781731	1857962	353024	100.3	100.6	100.5
Flow -	-20 %	4.203	5.624	7.723	1209647	2823142	475278	100.4	1005	100.4
Organic +	+2 %	3.065	4.475	6.189	963117	531075	391990	100.4	100.5	100.2
Organic -	-2 %	3.609	4.674	6.503	954998	2255410	394685	100.3	100.3	100.2
Wavelength +	+5 nm	3.382	4.569	6.319	1015544	2366158	396451	100.6	100.2	100.5
Wavelength -	-5 nm	3.389	4.576	6.329	1015544	2369497	396451	100.5	100.5	100.4

Table 6: System suitability parameters of Lamivudine, abacavir and Dolutegravir

Parameters	Values obtained (n=6)		
	Lamivudine	Abacavir	Dolutegravir
Plate count	13062	30724	39984
Tailing Factor	1.35	1.28	1.89
R_t (min)	3.393	4.576	6.326
Resolution	0	10.47	14.52

Table 7: Stability studies for Lamivudine, Abacavir and Doluegravir

Stress condition	Drugs	% Label Claim	% Degradation	Purity Angle	Purity Threshold
Control	Lamivudine	99.95	---	7.831	90
	Abacavir	99.96	---	10.332	31.878
	Doluegravir	99.98	---	2.029	17.581
Acid (5N Hcl)	Lamivudine	76.7	23.7	27.058	90
	Abacavir	74.8	26	7.576	27.37
	Doluegravir	76.9	23.3	2.747	26.874
Alkali (5N NaoH)	Lamivudine	77.1	23.3	28.94	90
	Abacavir	75.4	25.4	7.279	28.155
	Doluegravir	77.9	22.3	2.474	20.251
Peroxide (30% H_2O_2)	Lamivudine	79.9	20.5	29.92	90
	Abacavir	77.9	23.9	6.779	28.101
	Doluegravir	80.1	20.1	2.238	18.224
Reduction (10% Sodium Bisulphate)	Lamivudine	79.2	21.2	29.439	90
	Abacavir	76.3	24..5	6.652	28.176
	Doluegravir	79.7	20.5	2.38	19.01
Thermal (105°C / 72 Hrs)	Lamivudine	76.7	23.7	17.93	90
	Abacavir	79.4	21.4	7.654	27.154
	Doluegravir	70.8	29.4	2.138	20.586
Photolytic (1.2 Million Lux Hours)	Lamivudine	78.3	22.1	8.594	90
	Abacavir	75.1	25.7	6.556	26.02
	Doluegravir	74.6	25.6	1.906	16.518
Humidity (25°C / 60% Rh For 72 Hrs)	Lamivudine	80	20.4	8.025	90
	Abacavir	77.8	23	5.994	25.134
	Doluegravir	76.6	23.6	1.844	15.326
Hydrolysis	Lamivudine	77.8	22.6	8.004	90
	Abacavir	72.7	28.1	6.249	26.109
	Doluegravir	72.6	27.6	1.719	14.915

Method Validation

The analytical method was validated for various parameters as per ICH guidelines

Accuracy

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed method, recovery studies were carried out in different recovery levels (50%, 100% and 150%) by adding placebo to the pre-analyzed formulation. The solutions were suitably diluted in the range and then each of the dilution was observed 6 times. The % recovery of the drug was found to be 100.58, 100.27 & 100.11 % in Lamivudine, Abacavir and Dolutegravir respectively. The results were shown in the table 4.

Linearity

The linearity of the method was determined in concentration range of 3-45 µg/ml for LAM, 6-90 µg/ml for ABA and 0.5-7.5 µg/ml for DOL. Each solution was injected in triplicate. The average peak area versus concentration data of all drugs was treated by least squares linear regression analysis and the results obtained from as shown in table 4.

Specificity and Selectivity

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by examining blank matrix samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of any other placebos. Two different samples were injected and studied with respective placebos. The HPLC chromatograms recorded for the drug matrix (mixture of the drug and placebos) showed almost no interfering peaks with in retention time ranges. The obtained figures shows that the selected drugs were cleanly separated. Thus, the HPLC method proposed in this study was selective.

Precision

Precision is the degree of repeatability of an analytical method under normal operation conditions.

Precision is of 3 types

1. System precision

2. Method precision

3. Intermediate precision

a. Intraday precision

b. Inter day precision

Method precision was achieved by repeating the same procedure of preparation solution six times and injecting.

System precision is checked by using standard chemical substance to ensure that the analytical system is working properly. In this peak area and % of drug of six determinations is measured and % RSD should be calculated.

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD. And the results are shown in the table 4.

LOD and LOQ

LOD

It is lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is usually expressed as the concentration of analyte. The standard deviation and response of the slope.

LOD = 3.3 × standard deviation (б) /s

LOQ

The quantitation limit of an analytical procedure is the lowest amount of an analyte of a sample which can be quantitatively determined with suitable precision and accuracy. The standard deviation and response of the slope and the results obtained.

LOQ= 10× standard deviation (б) /s

The results of LOD & LOQ are shown in the table 4.

Robustness

To evaluate the robustness of the method, the chromatographic conditions were deliberately altered and degree of reproducibility was evaluated. During robustness testing each condition was varied separately, all other conditions being held constant at the optimized values. Robustness of the proposed method was assessed with respect to small alterations in the flow rate (1.0 ± 0.2 ml/min), organic composition and wavelength (258±2) and the results obtained from as shown the table 5.

System suitability parameters

For assessing system suitability, six replicates of working standards samples of LAM, ABA and DOL were injected and studied the parameters like plate number(N), tailing factor(K), resolution, relative retention time and peak asymmetry of samples. The results were tabulated in table 6.

Forced Degradation Studies

Preparation of Sample Stock Solution

For the forced degradation studies of Lamivudine, Abacavir and Dolutegravir, laboratory synthetic mixture was prepared with Lamivudine, Abacavir and Dolutegravir APIs (Mannitol) with the strength of 300 mg of Lamivudine, 600 mg of Abacavir, and 50 mg of Dolutegravir in glass motor and pestle, and crush into fine powder from this Weigh accurately 125mg (equivalent to 30 mg LAM 60 mg ABA and 5mg DOL) of powdered

sample into a 100ml volumetric flask dissolve and dilute to volume with diluents and filter the solution using 0.45µ Nylon syringe filter.

Acid Degradation (5N HCl)

Procedure

From the test stock solution 5ml was taken in 50ml volumetric flask, add 5ml of 5N HCl and heated at 60°C for 30 mins on a water bath.

The flask was removed from the water bath and allowed to cool at room temperature. Add 5ml of 5N NaOH to neutralize the solution cooled at room temperature and diluted to volume with diluent and mixed.

Base Degradation (5N NaOH)

Procedure

From the test stock solution 5ml was taken in 50ml volumetric flask, add 1ml of 5N NaOH and heated at 70°C for 1 hour on a water bath. The flask was removed from the water bath and allowed to cool at room temperature. Add 1ml of 5N HCl to neutralize the solution cooled at room temperature and diluted to volume with diluent and mixed.

Peroxide Degradation (30% H_2O_2)

Procedure

From the test stock solution 5ml was taken in 50ml volumetric flask, add 1ml of 30% H2O2 and heated at 70°C for 1 hour on a water bath. The flask was removed from the water bath and allowed to cool at room temperature and diluted to volume with diluent and mixed.

Reduction Degradation (10% Sodium Bisulphate)

Procedure

From the test stock solution 5ml was taken in 50ml volumetric flask, add 1ml of 10% Sodium Bisulphate and heated at 70°C for 1 hour on a water bath. The flask was removed from the water bath and allowed to cool at room temperature and diluted to volume with diluent and mixed.

Hydrolysis Degradation

Procedure

From the test stock solution 5ml was taken in 50ml volumetric flask, add 10ml of water and sonicated to disperse, dissolve and heated at 70°C for 3 hours on a water bath. The flask was removed from the water bath and allowed to cool at room temperature and diluted to volume with diluent and mixed.

Note: If the degradation is not achieved between 10 – 25%, further heat the solution on water bath at 70°C or change the strength of degradation medium to achieve the minimum level of degradation.

Thermal Degradation (105°C / 72 hrs)

Procedure

For the Thermal Degradation of Lamivudine, Abacavir and Dolutegravir, laboratory synthetic mixture was prepared with Lamivudine, Abacavir and Dolutegravir APIs and excipients (Mannitol) with the strength of 300 mg of Lamivudine, 600 mg of Abacavir, 50 mg of Dolutegravir and 300 mg of diluents (mannitol) in glass motor and pestle, and crush into fine powder transfer powder to 250mg (60 mg LAM 120 mg ABA and 10mg DOL) powder into petridish. Heat the sample in oven for about 72hrs at 1050C. From this Weigh accurately 125mg (equivalent to 30 mg LAM 60 mg ABA and 5mg DOL) of powdered sample into a 100ml volumetric flask dissolve and dilute to volume with diluent. Filter the solution using 0.45µ Nylon filter.

Transfer 5ml of above stock solution to 50ml volumetric flask and make up the volume with HPLC grade water to get the concentration of 30 µg/ml LAM 60 µg/ml ABA and 5 µg/ml DOL.

Humidity Degradation (25°C / 92% RH for 72 hrs)

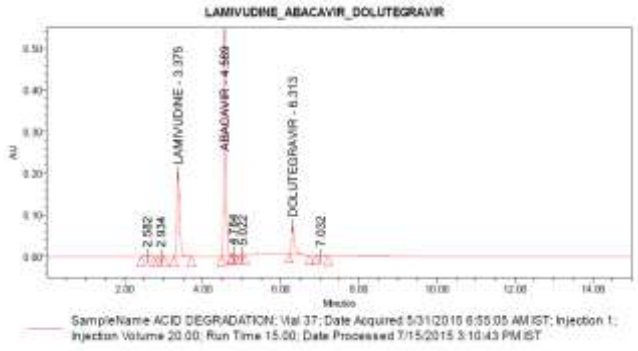
Procedure

Sample was exposed at 25°C / 92% RH for at least 72 hrs and the exposed sample was analysed as per Appendix A.

Photolytic Degradation (1.2 Million lux hours)

Procedure

Photolytic degradation study was carried out by exposing the accurately weighed 250mg (60 mg LAM 120 mg ABA and 10mg DOL) powder into petridish to UV light in a photolytic chamber at 1.2 Million lux hours for 24 hr., After 24hrs weigh accurately 120 mg of powdered sample into a 100ml volumetric flask dissolve and dilute to volume diluent. Transfer 5ml of above stock solution to 50 ml volumetric flask and filter the solution using 0.45µ Nylon syringe filter. Using the peak purity test, the purity of the drugs peaks were checked at every stage of above-mentioned studies. And the results are shown in the table 7.



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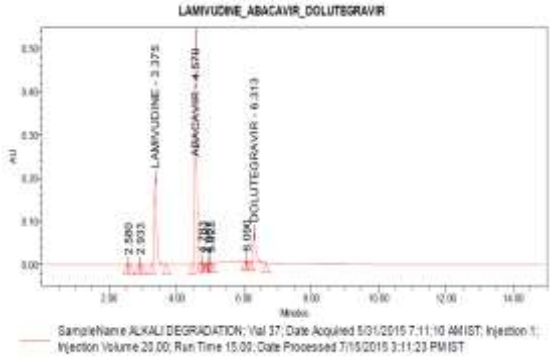

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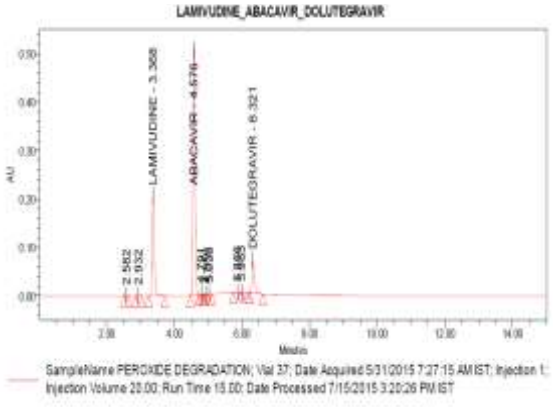

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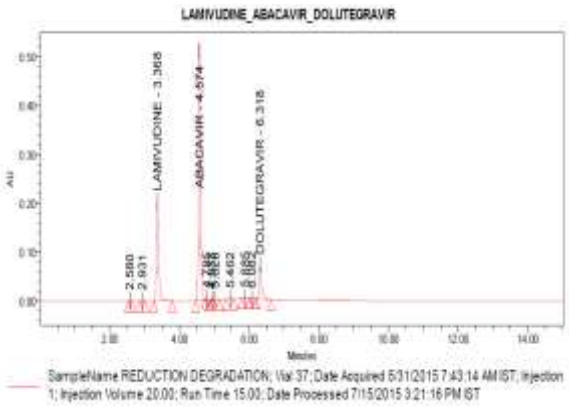

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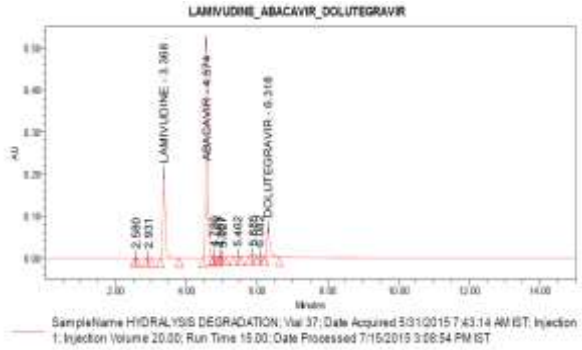

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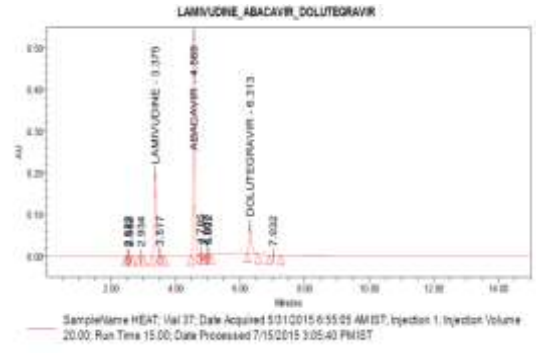

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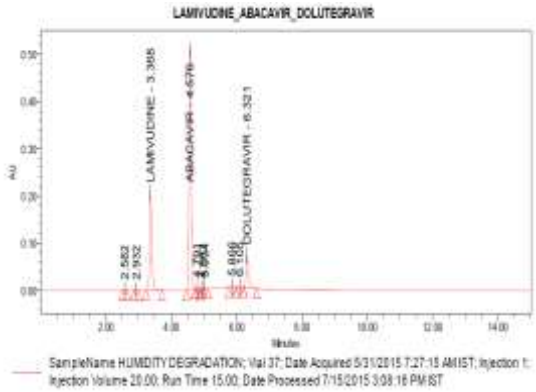

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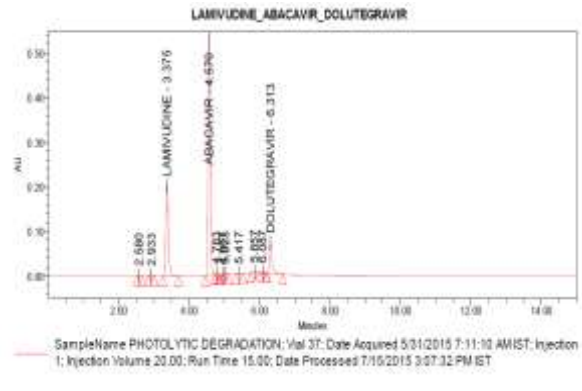

(h)

Figure 2: chromatograms of degradation-(a) acid degradation (b) Alkali degradation (c) of peroxide degradation (d) Reduction degradation (e) Hydrolysis degradation (f) thermal degradation (g) Humidity degradation (h) Photolytic degradation

RESULTS AND DISCUSSION

The conditions tested for method development indicates that all the system suitability parameters according to ICH guidelines was achieved by using Luna Phenyl Hexyl (250 x4.6mm, 5µm) Column using mobile phase acetonitrile and buffer by a gradient program with a flow rate of 1ml/min throughout the gradient program with a detection wavelength of 258 nm for all the compounds with injection volume of 20µl. To validate the RP-HPLC method, a series of tests were made using the most promising conditions. A calibration curve was made and concentration examined within the detection range of 3-45µg/ml, 6-90 µg/ml & 0.5-7.5 µg/ml for LAM, ABA & DOL and correlation coefficient was found to be 0.999, 0.999, and 0.999 for all the compounds respectively. The precision (expressed as the relative standard deviation (RSD) was determined for LAM, ABA & DOL for repeated analysis and the values are presented in Table 4. The assay values obtained by proposed method and recovery experiment values obtained were performed by adding different amounts placebo to pre-analysed concentration summarized in Table 4.

The stability of sample was checked by forced degradation in different conditions and % of degradation was calculated. The peak purity of the analyte was passed in all conditions (purity angle should be less than the threshold value). The results as shown in Table 8 indicate that any other impurity is not merging with the main peak (Figure-2).

The analyte sample solution was stable up to 24hrs at room temperature. The reliability of the method was determined by made small deliberate variations in method parameters and the %RSD values (Table 5) obtained, an indication of its reliability on normal usage. A method was developed for the determination of LAM, ABA & DOL in tablets which is rapid, stable & specific. The results indicate that the described method can be used for quantitative analysis of the compounds.

Accuracy and Precision

Accuracy as recovery was evaluated by spiking previously analyzed test solution with additional Placebo at three different concentration levels (table 4). Recovery of previously analyzed test solution drug concentration added was found to be 100.58 % for LAM, 100.27 % for ABA and 100.11% for DOL with the value of RSD less than 2% indicating that the proposed method is accurate for the simultaneous estimation of all drugs from their combination drug products in presence of their degradation products.

Linearity, LOD and LOQ

The calibration plot was linear over the concentration range investigated (3-45µg/ml; n= 3), (6-90µg/ml; n= 3) and (0.5-7.5µg/ml; n = 3) for LAM, ABA and DOL respectively. Average correlation coefficient r=0.999 for all the drugs with %RSD values ≤2.0 across the concentration ranges studied was obtained from regression analysis. The LOD that produced the requisite precision and accuracy was found to be 0.036µg/ml for LAM, 0.065µg/ml for ABA and 0.02µg/ml for DOL. The resultant %RSD values were ≤1.00% (Table 4). The LOQ for LAM, ABA and DOL were found to be 0.112, 0.198 and 0.071µg/ml respectively. The Regression results indicate that method was linear in the concentration range studied and can be used for detection and quantification of LAM, ABA and DOL in a very wide concentration range.

Specificity and Selectivity

Specificity is checked in each analysis by examining blank and placebo samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of any other excipients.

Robustness

Results of the robustness (table 5). The elution order and resolution for all components were not significantly affected. RSD of peak areas were found to be well within the limit of 2.0%.

System suitability

The system suitability parameters were found to be within acceptance criteria. Good peak with resolution between two drugs is >1.5, asymmetric factor <2 shows that the three drugs were better separated. Results are tabulated in table 6.

Degradation studies

Acid hydrolysis

Upon performance of acid degradation studies 23.7 % of LAM, 26 % ABA and 23.3% of DOL was degraded.

Base hydrolysis

Upon performance of base degradation studies 23.7 % of LAM, 25.4% ABA and 22.3% of DOL was degraded.

Peroxide hydrolysis

Upon performance of peroxide degradation studies 20.5 % of LAM, 23.9 % ABA and 20.9 % of DOL was degraded.

Reduction degradation

Upon performance of Reduction degradation studies 21.2 % of LAM, 24.5 % ABA and 20.5 % of DOL was degraded.

Photolytic degradation

Upon performance of Photolytic degradation studies 22.1 % of LAM, 25.7% ABA and 25.6% of DOL was degraded.

Humidity degradation

Upon performance of Humidity degradation studies 20.4 % of LAM, 23 % ABA and 23.6 % of DOL was degraded.

Hydrolysis degradation

Upon performance of Hydrolysis degradation studies 22.6 % of LAM, 28.1% ABA and 27.6 % of DOL was degraded.

Thermal degradation

Upon performance of Thermal degradation studies 23.7 % of LAM, 21.4% ABA and 29.4 % of DOL was degraded.

All the stability studies results were shown in table 7 and figure-2.

CONCLUSION

A simple, rapid, accurate and precise stability-indicating gradient RP-HPLC analytical method has been developed and validated for the quantitative analysis of Lamivudine, Abacavir and Doluegravir in bulk drugs and combined dosage forms. The newly developed gradient RP-HPLC method for separation of different degradation products along with the pure drugs were found to be capable of giving faster retention times while still maintaining good resolution than that achieved with conventional HPLC. This method exhibited an excellent performance in terms of sensitivity and speed. The results of stress testing undertaken according to the ICH guidelines reveal that the method is specific and stability-indicating. The proposed method has the ability to separate these drugs from their degradation products in tablet dosage forms and hence can be applied to the analysis of routine quality control samples and samples obtained from stability studies.

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