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Analytical Method Development and Validation of Stability Indicating Rp-Hplc Method for the Simultaneous Estimation of Abacavir and Dolutegravir

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ABSTRACT

Special, effective high pressure liquid chromatography method has been developed for the simultaneous quantification of Abacavir and Dolutegravir.By using Waters HPLC e-2695 quaternary pump with a PDA detector of 2998 instrument the chromatographic separation of Abacavir and Dolutegravir was achieved on the column of Inertsil ODS150X4.6mm, 3.5µ using an isocratic elution with a buffer containing 0.1percentformic acid and acetonitrile at a rate of 30:70 as a mobile phase with a flow rate of 1 ml/min at ambient temperature. A detector wavelength of 236 nm utilizing the PDA detector were given in the instrumental settings. The linearity was studied between the concentration range of 30-450 µg/ml of Abacavir and 5-75 µg/ml of Dolutegravir were injected.The plotted calibration curves were linear with a regression coefficient of R2> 0.999, indicates that the linearity was with in the limit. As a part of method validation the parameters like specificity, linearity, accuracy, ruggedness, robustness were determined and the results were found to be within the allowable limit. The method developed was found to be applicable to routine analysis and to be used for the measurement of both active pharmaceutical ingredients (i.e, Abacavir and Dolutegravir).Validation of the proposed method was carried out according to an International Conference on Harmonization (ICH) guidelines.

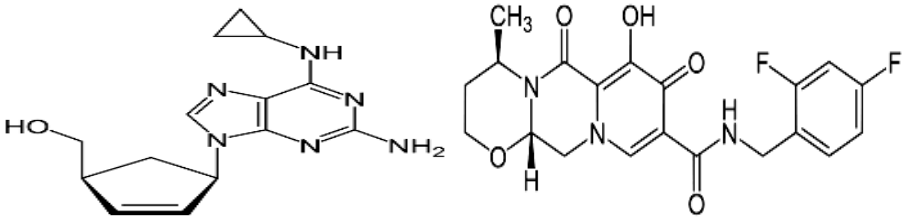
Keywords: *Abacavir, Dolutegravir, HPLC, Development, Validation*

1. INTRODUCTION

Abacavir, sold under the brand name Ziagen, is a medication used to prevent and treat HIV/AIDS[1,2]. Similar to other nucleoside analog reverse-transcriptase inhibitors (NRTIs) [3,4], abacavir is used together with other HIV medications, and is not recommended by itself. It is taken by mouth as a tablet or solution and may be used in children over the age of three months [5]. Abacavir is generally well tolerated. Common side effects include vomiting, trouble sleeping[6,7], fever [8], and feeling tired. More severe side effects include hypersensitivity[9], liver damage[10,11], and lactic acidosis[12,13], hypertriglyceridemia[14,15], and lipodystrophy[16,17]. Genetic testing can indicate whether a person is at higher risk of developing hypersensitivity. Symptoms of hypersensitivity include rash, vomiting, and shortness of breath [18,19]. Abacavir is in the NRTI class of medications, which work by blocking reverse transcriptase, an enzyme needed for HIV virus replication. Within the NRTI class, abacavir is a carbocyclic nucleoside[20].

Dolutegravir (DTG), sold under the brand name Tivicay, is an antiretroviral medication[21] used, together with other medication, to treat HIV/AIDS. It may also be used, as part of post exposure prophylaxis[22], to prevent HIV infection following potential exposure. It is taken by mouth. Common side effects include trouble sleeping, feeling tired, diarrhea[23], high blood sugar[24,25], and headache. Severe side effects may include allergic reactions[26] and liver problems. There are tentative concerns that use during pregnancy can result in harm to the baby. It is unclear if use during breastfeeding is safe. Dolutegravir is an HIV integrase strand transfer inhibitor which blocks the functioning of HIV integrase which is needed for viral replication.There are tentative concerns that use during pregnancy can result in harm to the baby. Effective birth control is thus recommended while on dolutegravir, with pregnancy testing before starting

treatment. Use during the first trimester should only occur if there is no alternative.



A B

FIG. 1: CHEMICAL STRUCTURE OF (A) ABACAVIR (B) DOLUTEGRAVIR

2. MATERIALS AND METHOD

Chemicals: Acetonitrile, HPLC-grade ortho phosphoric acid, water were purchased from Merck India Ltd, Mumbai, India. APIs of Abacavir and Dolutegravir standards were procured from Glenmark,Mumbai.

The Instrumentation: Waters alliance liquid chromatography (model e-2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study.

Preparation of buffer: 1 ml of formic acid is dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

Chromatographic conditions: The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% formic acid, and Inertsil ODS150X4.6mm, 3.5µ with a flow rate of 1 ml /min.

Diluent:0.1% formic acid and Acetonitrile in the ratio (30:70) is used as diluent.

Preparation of the standard stock solution: For standard stock solution preparation, add 70ml of diluents to 300mg of Abacavir and 50 mg of Dolutegravir taken in a 100 ml volumetric flask and sonicate for 10 minutes to fully dissolve the contents and then make up to the mark with diluent.

Preparation of Standard solution: 5 ml of solution is drawn from the above normal stock solution into a 50ml volumetric flask and diluted up to the level.

3. RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide a good performance the chromatographic conditions were optimized.

Method optimization: To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally 0.1% formic acidbuffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as C_8, C_{18} phenyl and amino,luna phenyl columns were tested. From these trials the peak shapes were relatively good with a Inertsil ODS150X4.6mm, 3.5µ with a PDA detector. The mobile phase flow rate has been done at 236nm in order to obtain enough sensitivity. By using above conditions we get retention times of Abacavir and Dolutegravir were about 2.936 and 4.535 min with a tailing factor of 1.41&1.27. The number of theoretical plates for Abacavir and Dolutegravir were in acceptable limit which indicate the column's successful output the % RSD for six replicate injections was around 0.32% and 0.28%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the established method was validated.

Method validation

The optimized RP-HPLC validated method according to ICH guidelines in terms of system suitability, linearity, accuracy, precision and robustness.

System suitability: Device suitability was performed by injecting standard solution containing 300 µg/ml of Abacavir and 50 µg/ml of Dolutegravir in six replicates. The results show that the machine fitness parameter is within the limit provided by ICH. The results were shown below table 1. Figure 2 represents the chromatogram of standard.

TABLE 1: RESULTS OF SYSTEM SUITABILITY

System suitability parameter	Acceptance criteria	Drug name	
		Abacavir	Dolutegravir
USP Plate count	NLT 2000	3322	7236
USP Tailing	NMT 2.0	1.41	1.27
USP Resolution	NLT 2.0	-	7.53
% RSD	NMT 2.0	0.32	0.28
Retention Time	NLT 2.0	2.936	4.535

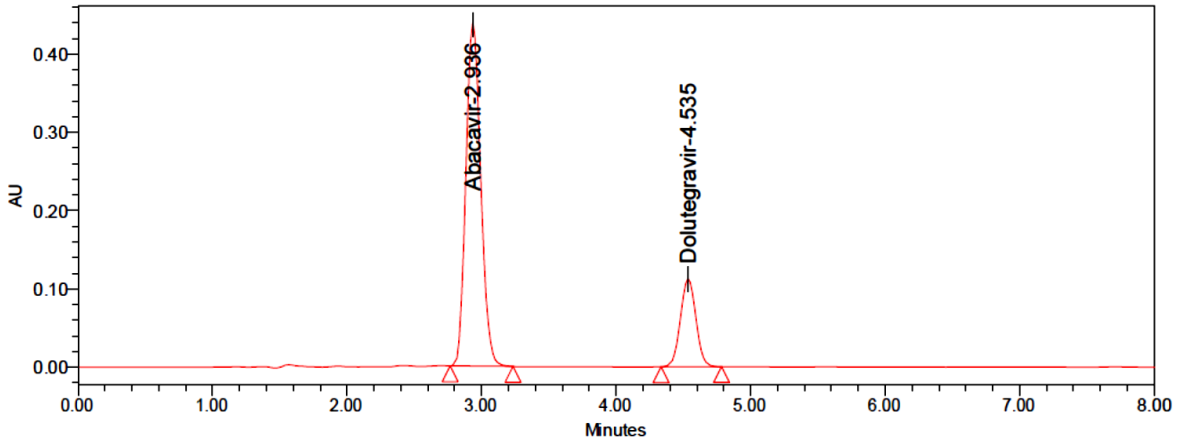


FIG. 2: CHROMATOGRAM OF STANDARD

Specificity: There was no interference from blank at the retention time of Abacavir and Dolutegravir. This proves the technique is specific. Figure 3 shows the chromatogram of blank.

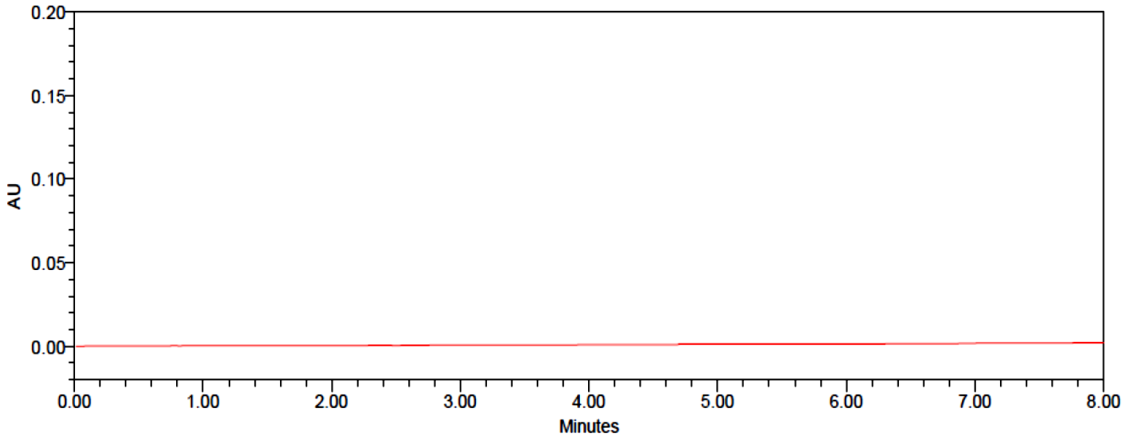
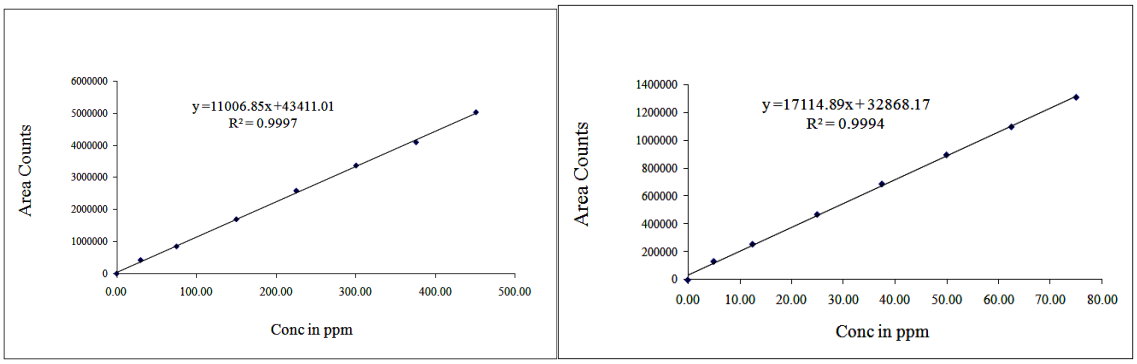


FIG. 3: CHROMATOGRAM OF BLANK

Linearity: Linearity was calculated by plotting a calibration curve of the peak area against its respective concentration, linearity was determined. From this calibration curve, it was noticed that the curve was linear between the range of 30-450µg/ml of Abacavir and 5-75µg/ml of Dolutegravir. The regression equations for calibration curve was y = 11006.85x +43411.01 (R^2=0.9996) for Abacavir and y =17114.89x + 32868.17 (R^2=0.9994) for Dolutegravir respectively and the results of linearity were shown in table 2. Calibration plots were shown in figure 4.

TABLE 2: LINEARITY RESULTS

Linearity	Abacavir		Dolutegravir	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
Linearity-1	30.00	417808	5.00	132581
Linearity-2	75.00	844753	12.50	255651
Linearity-3	150.00	1691560	25.00	468128
Linearity-4	225.00	2582175	37.50	687473
Linearity-5	300.00	3366503	50.00	895033
Linearity-6	375.00	4087600	62.50	1095570
Linearity-7	450.00	5022881	75.00	1306743
Slope	11006.85		17114.89	
Intercept	43411.01		32868.17	
CC	0.9996		0.9994	



A **B**

FIG. 4: CALIBRATION PLOTS OF (A) ABACAVIR (B) DOLUTEGRAVIR

Accuracy: The accuracy of the system was achieved by measuring the recovery experiments at three stages (50 percent, 100 percent and 150 percent). APIs with concentrations of150, 300 and 450µg/ml of Abacavir and 25, 50 and 75µg/ml of Dolutegravir were prepared. For each spike stage, the test solution was injected three times and the test was performed according to the test process. The recovery results were similar to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and relative standard deviations were determined. Recovery values shown within the desired range were correct. The results are summarized below. Accuracy findings have been shown in table 3.

TABLE 3: RESULTS OF ACCURACY

S. No.	% Level	Abacavir % Recovery	Dolutegravir % Recovery
1	50	99.5	99.7
2	100	100.1	100.2
3	150	99.0	100.2

Precision: The precision of the analytical technique is the degree of proximity of the sequence of measurements obtained from multiple homogeneous mixture samplings. The accuracy of the process of the drugs were calculated by injection of six

individual determinations of Abacavir (300 µg/ml) and Dolutegravir (50µg/ml). Method precision results were shown in table 4 and method precision chromatogram was shown in figure 5.

TABLE 4: RESULTS OF INTRADAY PRECISION

S. No.	Abacavir			Dolutegravir		
	Conc. (µg/ml)	Area	% Assay	Conc. (µg/ml)	Area	% Assay
1	300	3362891	100.4	50	894314	100.2
2		3313789	99		892141	99.9
3		3335810	99.6		885739	99.2
4		3351608	100.1		899352	100.7
5		3389396	101.2		891343	99.8
6		3375312	100.8		893561	100.1
% RSD	0.80			0.50		

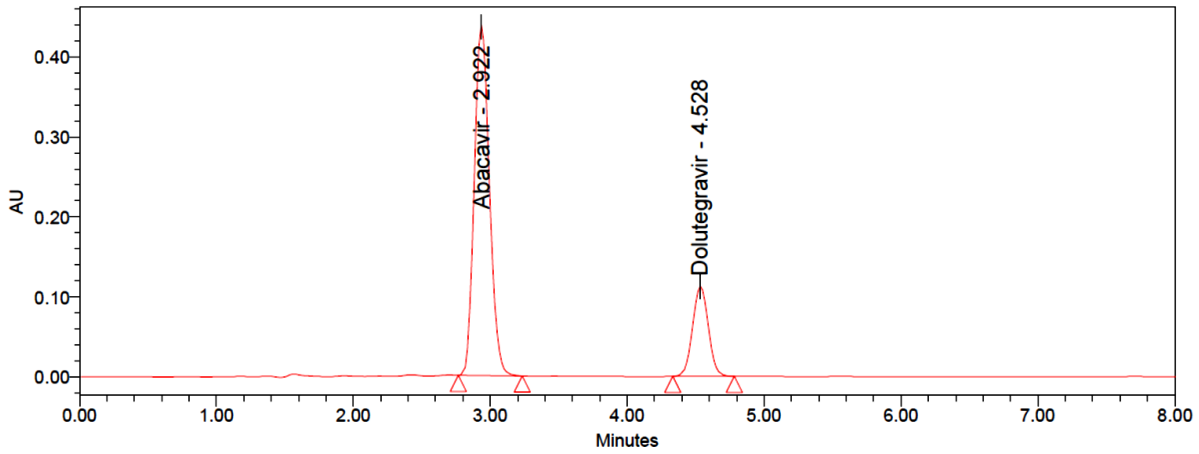


FIG. 5: CHROMATOGRAM OF METHOD PRECISION

Intermediate Precision: Six replicates of the standard solution were analyzed bydifferent researchers and different tools were checked on separate days. The peak regions used to assess the average percent of RSD values have been determined. The findings are shown in the table 5.

TABLE 5: INTER-DAY PRECISION RESULTS

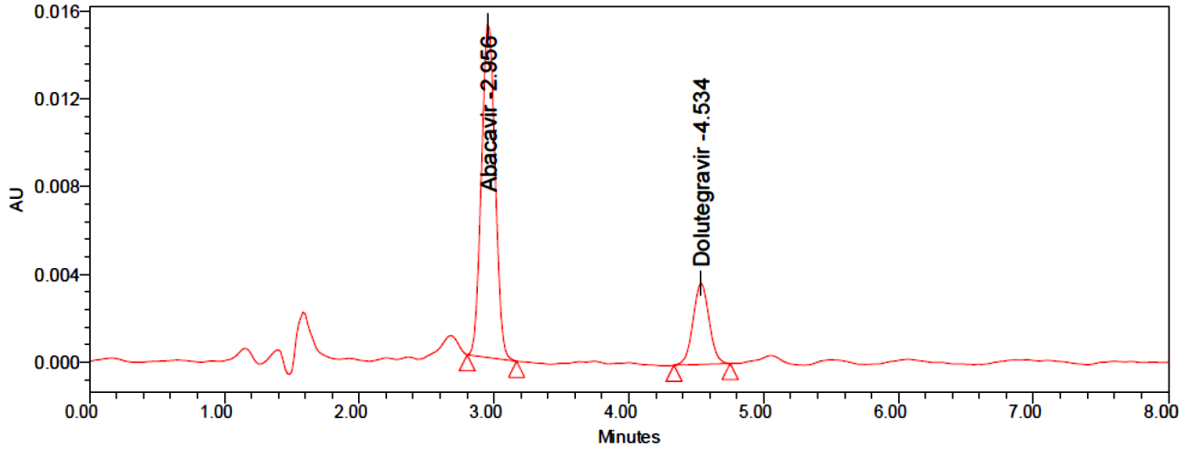
S. No.	Abacavir			Dolutegravir		
	Conc. (µg/ml)	Area	% Assay	Conc. (µg/ml)	Area	% Assay
1	300	3322889	99.2	50	890420	99.8
2		3353794	100.1		895346	100.3
3		3305815	98.7		893047	100.0
4		3374611	100.8		899359	100.8
5		3393597	101.3		888940	99.6
6		3341324	99.8		887936	99.5

%CV	0.97	0.49

LOD and LOQ: LOD and LOQ were determined separately using the calibration curve technique. The LOD and LOQ of the compound were measured using the developed RP-HPLC method by injecting lower and lower concentrations of the standard solution. The LOD and LOQ concentrations and their s/n values of Abacavir and Dolutegravir were represented in the following table 6 and the chromatograms of LOD and LOQ were shown in figure 6.

TABLE 6: LOD AND LOQ RESULTS

Abacavir				Dolutegravir			
LOD		LOQ		LOD		LOQ	
Conc. (µg/ml)	s/n	Conc. (µg/ml)	s/n	Conc. (µg/ml)	s/n	Conc. (µg/ml)	s/n
9	7	30	25	1.5	4	5	28



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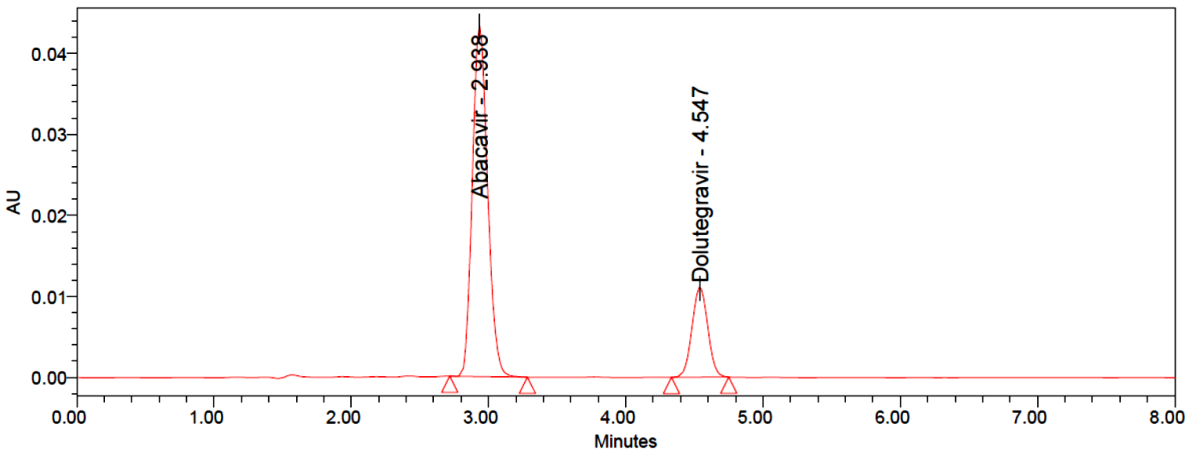

B

FIG. 6: CHROMATOGRAM OF (A) LOD (B) LOQ

Robustness: The conditions of the experiment was designed to measure the robustness of the intentionally changed conditions such as flow rate, organic percentage in mobile phase. Results of robustness were shown in table 7.

TABLE 7: ROBUSTNESS RESULTS

Parameter name	% RSD	
	Abacavir	Dolutegravir
Flow rate (0.8 ml/min)	0.60	0.46
Flow rate (1.2 ml/min)	0.35	0.36
Org Plus (77:23)	0.90	0.40
Org Minus (63:37)	0.68	0.47

Degradation studies: Abacavir and Dolutegravir standard was subjected to various conditions of forced degradation in order to induce partial degradation of the compound. Forced degradation experiments have been performed to establish that the process is acceptable for degradation materials. In addition the studies include information on the condition under which the drug is unstable, such that the steps are also taken during formulation to prevent possible instabilities. Forced degradation results were shown in table 8.

Acid degradation:5 ml of standard stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 1N HCl and left it for 15 min. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark.

Alkali degradation: 5 ml ofstandard stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 1N NaOH and left it for 15 min. After 15 min add 1 ml of 1N HCl and make up to the mark.

Peroxide degradation:5 ml of standard stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 30% hydrogen peroxide solution and make upto the mark with diluents.

Reduction degradation:5 ml of standard stock solution was moved to a volumetric flask of 50 ml and add 1 ml of 30% sodium bi sulphate solution and make upto the mark with diluents.

Thermal degradation: The standard solution was set in an oven at 110°C for 24 hrs. The resultant solution was injected into HPLC system.

Photolytic degradation: The standard solution was placed in sun light for 24 hrs. The resultant solution was injected into HPLC system.

TABLE8: FORCED DEGRADATION RESULTS

Degradation condition	Abacavir		Dolutegravir	
	% Assay	% deg	% Assay	% deg
Control	100	0	100	0
Acid deg	83.7	16.3	84.1	15.9
Alkali deg	85.5	14.5	83	17
Peroxide deg	85.1	14.9	83.3	16.7
Reduction deg	87.2	12.8	88.6	11.4
Thermal deg	89.8	10.2	86.8	13.2
Photolytic deg	99.1	0.9	98.2	1.8
Hydrolysis deg	98.7	1.3	98.5	1.5

4. CONCLUSION

This method described the quantification of Abacavir and Dolutegravir in bulk and pharmaceutical formulation as per ICH guidelines. The evolved technique was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of standard preparation and reproducibility data are satisfactory. The evolved chromatographic method can be effectively applied for regular investigation in drug research.

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CONFLICTS OF INTEREST

Author declares that there has been no conflicts of interest.

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