CONSECUTIVE DETERMINATION OF EMTRIVA AND TIVICAY IN BULK AND COMBINED DRUGS FORMS BY RP-HPLC METHOD

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ABSTRACT:

The New Analytical method was to develop and validate a rapid, sensitive and accurate method for estimation of Emtriva and Tivicay in drug product by liquid chromatography. The chromatographic separation was achieved on C18 or Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5um) at ambient temperature .The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoro acetic acid in water: Methanol (30:70). The flow rate was 0.8ml/ minute and ultra violet detector at 260nm. The average retention time for Emtriva and Tivicy found to be 3.595 min and 3.263 min. The proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 50.0 – 150.0µg/mL for Emtriva and 50.0-150.0 µg/mL of Tivicy.

Key words: Emtriva, Tivicay , Isocratic, HPLC, Eclipsc XDB-Phenyl, Trifluoro acetic acid, Acetonitrile, Methanol and validation.

1. INTRODUCTION

Emtriva:

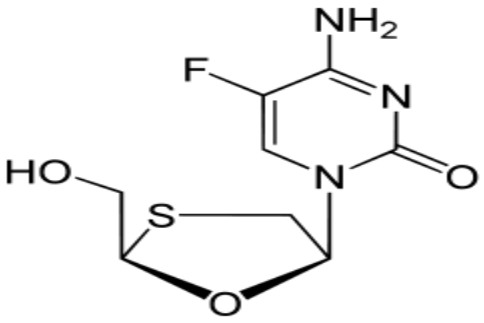
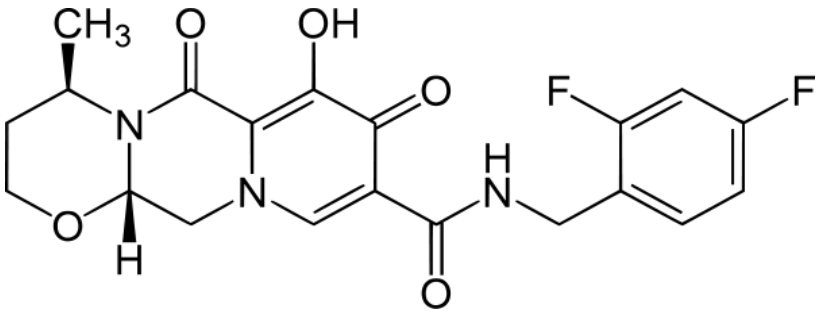


Fig.1. Chemical structure: Emtriva

Emtriva (**2'-deoxy-5-fluoro-3'thiacytidine**, **FTC**), (formerly **Coviracil**), is a nucleoside reverse transcriptase inhibitor (NRTI) for the prevention and treatment of HIV infection in adults and children.Emtriva is chemically designated as 4-amino-5-fluoro-1-[(2*R*, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. Its molecular formula is $C_8H_{10}FN_3O_3S$, and its molecular weight is 247.248 g/mol.

Tivicay:



Tivicay (DTG) [3-4] is a medication used for the treatment of HIV infection. Tivicay is an integrase inhibitor. Tivicay is approved for use in a broad population of HIV-infected patients. It can be used to treat HIV-infected adults who have never taken HIV therapy (treatment-naïve) and HIV-infected adults who have previously taken HIV therapy (treatment-experienced), including those who have been treated with other integrase strand transfer inhibitors. Tivicay is also approved for children ages 12 years and older weighing at least 40 kilograms (kg) who are treatment-naïve or treatment-experienced but have not previously taken other integrase strand transfer inhibitors.

Tivicay is chemically designated as (4R,12aS)-N-(2,4-difluorobenzyl)-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. Its molecular formula is C20H19F2N3O5, and its molecular weight is 419.38 g/mol.

2. MATERIALS AND METHODS

2.1 Equipments:

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5µm) as stationary phase ,Ultrasonic cleaner, Scaletech analytical balance and Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

2.2 Materials:

Pharmaceutically pure sample of Emtriva/Tivicay were obtained as gift samples from Fortune pharma training institute, Sri Sai nagar colony, KPHB, Hyderabad, India.
HPLC-grade Methanol and Acetonitrile were obtained from qualigens reagents pvt ltd. Trifluoro acetic acid (AR grade) was from sd fine chem.

2.3 Chromatographic conditions The sample separation was achieved on a (Eclipse XDB-Phenyl 250*4.6, 5µm) Phenyl column, aided by mobile phase mixture of 0.1%v/v Trifluoro acetic acid in water: Methanol (30:70). The flow rate was 0.8ml/ minute and ultra violet detector at 260nm that was filtered and degassed prior to use, Injection volume is 10µl and ambient temperatures.

Preparation of mobile phase:

Buffer Preparation: Taken accurately 1ml of Trifluoro acetic acid in 1000ml of water
Mobile phase: Then added 30 volumes of buffer and 70 volumes of Methanol mixed well and sonicated for 5 min.

Diluents: Water: Methanol: 50:50 v\v

2.4 Preparation of solutions

2.4.1 Standard solution: 40 mg of pure Emtriva and 5 mg of Tivicay were weighed and transferred to 10 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluents to give a primary stock solution. From the above solution 0.25ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 100.0µg/ml of Emtriva and 12.5µg/ml tivicay .

2.4.2 Preparation of sample solution: Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 40mg of emtriva and 5mg of tivicay sample and transferred to 10 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 0.25 ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 100.0µg/ml of emtriva and 12.5µg/ml tivicay .

2.5 Method validation

2.5.1. System suitability

The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system

2.5.2. Linearity

Linearity was studied by analyzing five standard solutions covering the range of 50.0 - 150.0µg/ml for emtriva and 6.3 -18.8µg/ml tivicay. From the primary stock solution 0.125ml,0.187ml,0.25ml,0.312ml,0.375 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 50.0 µg /mL , 75.0µg/mL , 100.0µg/mL ,125.0µg/mL and 150.0µg/mL of emtriva and 6.3g/mL, 9.4µg/mL, 12.5µg/mL , 15.6µg/mL and 18.8 µg/mL of tivicay. Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

2.5.3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

2.5.4. Method precision

The precision of the method was checked by repeated preparation(n=6) of 100.0µg/ml of emtriva and 12.5µg/ml tivicay without changing the parameter of the proposed chromatographic method, and measured the peak areas and retention times.

2.5.5. Accuracy

The accuracy of the method was determined by calculating the recoveries of emtriva and tivicay by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Emtriva and tivicay.

2.5.6. Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min.

3. RESULTS AND DISCUSSIONS:

Determination of Working Wavelength (λ max): 10 mg of the emtieva and tivicay standard drug is taken in a 10 ml volumetric flask and dissolved in diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml volumetric flask and made up to the mark with the Water to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using Water as blank. The λmax was found to be 260nm

After several initial trails with mixtures of methanol, water, Acetonitrile and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Trifluoro acetic

acid in water: Methanol (30:70). At flow rate was 0.8mL/ minute brought sharp peaks. The Chromatogram was shown in Fig 3.

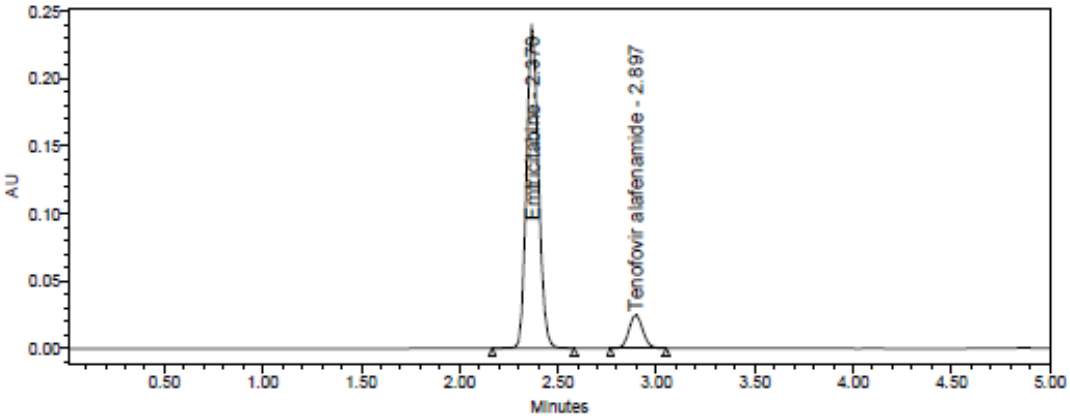


Fig 3 Chromatogram of emtriva and tivicay

System suitability

The system suitability of the method was checked by repeated preparations for tivicay and Emtriva. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system, System suitability data of **Emtriva** and tivicy are shown in Table 1.

Parameter	Emtriva	Tivicay	Acceptance criteria
Retention time	3.596	4.671	+-10
Theoretical plates	9708	9841	>3000
Tailing factor	1.18	1.13	<1.50
% RSD	0.21	0.19	<2.00

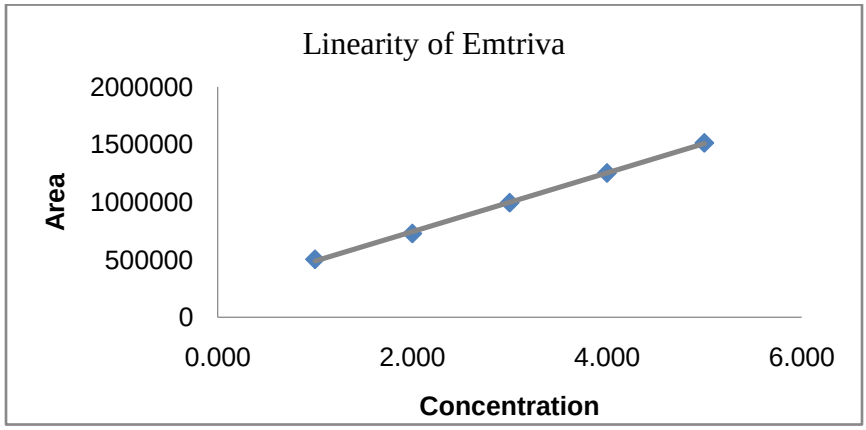
Table 1 System suitability data of emtriva and tivicay

Linearity:

Linearity was studied by analyzing five standard solutions covering the range of 50.0 -150.0µg/ml for Emtirva and 6.3 -18.8µg/ml tivicay. From the primary stock solution 0.125ml,0.187ml,0.25ml,0.312ml,0.375 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 50.0 µg /mL , 75.0µg/mL , 100.0µg/mL ,125.0µg/mL and 150.0µg/mL of emtriva and 6.3g/mL, 9.4µg/mL, 12.5µg/mL , 15.6µg/mL and 18.8 µg/mL of tivicay.

A linear relationship between peak areas versusconcentrations was observed for emtrivaand tivicayin the range of 50% to 150% of nominal concentration. Correlation coefficient was 1.000 and 0.9999 for emtriva and tivicay .

A



B

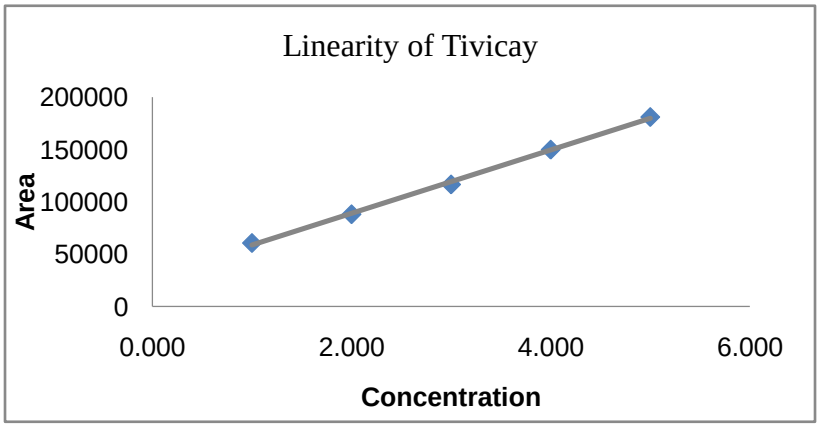


Fig. 4 Calibration curve: (A) Emtriva: (B) Tivicay

Method precision (repeatability)

The precision of the method was checked by repeated preparation(n=6) of 50.0µg/ml of Emtriva and 12.5µg/ml Tivicay without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times. The precision of the method (% RSD) was found to be <1% showing good repeatability. The values of percentage RSD for Emtriva and Tivicay are shown in Table 2 and Table 3

	Retention time	Peak area	% Assay
1	3.597	1536410	98.9
2	3.598	1538297	99.2
3	3.597	1528266	99.5
4	3.598	1526039	99.2

5	3.597	1538164	99.4
6	3.597	1542609	99.9
Mean	3.597	1534964	99.4
%RSD	**0.01**	**0.42**	**0.34**

Table 2: Summary of peak areas for method precision of Emtriva

Sample No	Retention time	Peak area	% Assay
1	4.673	182071	99.1
2	4.672	182342	99.6
3	4.677	181025	99.3
4	4.678	180623	98.7
5	4.678	182125	99.5
6	4.677	182444	100.3
Mean	4.676	181772	99.5
%RSD	**0.06**	**0.42**	**0.56**

Table 3: Summary of peak areas for method precision of Tivicay

Accuracy (recovery study):

The accuracy of the method was determined by calculating the recoveries of Emtriva and Tivicay by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Emtriva and Tivicay. The percentage recovery results obtained are listed in Table 4 &5

LEVEL	S.NO	%Recovery of Emtriva	Average
50	1	99.6	99.4%
	2	99.0	
	3	95.5	
100	1	98.5	99.2%
	2	99.2	
	3	99.5	
150	1	100.1	99.9%
	2	99.7	
	3	99.9	

Table 4: Recovery data of Emtriva

LEVEL	S.NO	%Recovery of tivicay	Average
50	1	99.6	99.1%
	2	99.2	
	3	98.5	
100	1	99.1	99.3%
	2	99.8	
	3	99.0	
150	1	99.7	99.6%
	2	99.6	
	3	99.3	

Table 5: Recovery data of tivicay

CONCLUSION

From the above experimental results it was concluded that, newly developed method for the simultaneous estimation of EMTRIVA and TIVICAY was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in pharmaceutical industries, approved testing laboratories.

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