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Method Development and Validation for the Simultaneous Estimation of Trifluridine and Tipiracil in Bulk and Tablet Dosage Form

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Trifluridine and Tipiracil bulk and pharmaceutical formulations. Separation of Trifluridine and Tipiracil was successfully achieve Dona:Supelco 250X4.6mm, 5µm, C18or equivalent in an isocratic mode utilizing Na_2SO_4: Acetonitrile (60:40)at a flow rate of 1.0 mL/min and eluatewas monitored at 265nm, with a retention time of 2.697 and 3.166 minutes for Trifluridine and Tipiracil respectively. The method was validated and the re sponsewas found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Trifluridine and 50µg/ml to150 µg/ml for Tipiracil. The values of the correlation coefficient were found to 1.000 for Trifluridine and 1.000for Tipiracil respectively. The LOD and LOQ for Trifluridine were found to be 0.031 and 0.105 respectivly. The LOD and LOQ for Tipiracil were found to be 0.019 and 0.064 respectively. This method was found to be good percentage recovery for Trifluridine and Tipiracil were found to be 100 and 100 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Accuacy, Precision, Specificity and Robustness.

Keywords: Trifluridine,Tipiracil, High performance liquid chromatography.

I. INTRODUCTION

The Trifluridine is drug is an antiviral agent has not been fully elucidated, but appears to involve the inhibition of viral replication.Trifluridine gets incorporated into viral DNA during replication, which leads to the formation of defective proteins and an increased mutation rate.

Trifluridine is used for the treatment of primay keratoconjunctivitis and recurrent epithelial keratitis due to herpes simplex virus, types 1 and 2 in ophthalmic solutions.Trifluridine, in combination with tipiracil, is indicated for the treatment of adult patients with metastatic colorectal cancer who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF biological therapy, and if RAS wild-type, an anti-EGFR therapy.

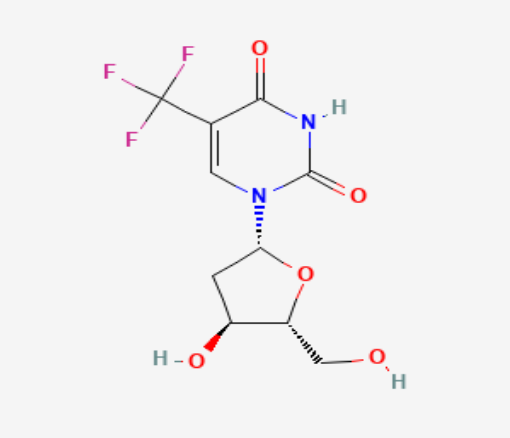

Figure 1: Structure of Trifluridine

Tipiracil, in combination with trifluridine, is indicated for the treatment of adult patients with metastatic colorectal cancer who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF biological therapy, and if RAS wild-type, an anti-EGFR therapy.Tipiracil is a thymidine phosphorylase inhibitor. Its function prevents the breakdownof the active component of trifluridine, thus increasing the bioavailability of trifluridine and boosting its systemic presence. In addition, it is reported that thymidine phosphorylase is an

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angiogenic factor usually overexpressed in solid tumors.

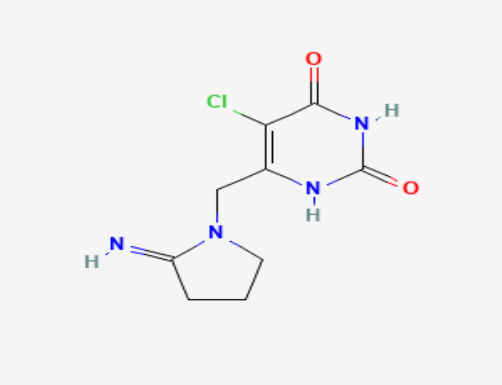

Figure 2: Chemical structure of Tipircil

The Existing literature reveals that Trifluridine and Tipiracil can be analyzed by UV detection, HPTLC, HPLC individually and combination with other drugs in bulk material and pharmaceutical form.A comprehensive, validated and simple analytical simultaneous method development and validation of Trifluridine and Tipiracil is, therefore, crucial. No economic, simple and precise HPLC method was there for simultaneous estimation of CR Trifluridine and Tipiracil in bulk and pharmaceutical dosage form.

II. EXPERIMENTAL

Chemicals and reagents: Trifluridine and Tipracil Pure Drugs [API],Comnination of both drugs from Tablet [LONSURF] Acetonitrile[Merck],Distled Water[LobaChemi]Potassium Dihydrogen phosphate,[Dr.Reddy"s] sodium sulphate[NA_2SO_4] From Finar

Equipments: Electronics Balance-Ascoset pH meter –ADWA, India Ultrasonicator-Enertech, WATERS HPLC E 2695 SYSTEM Photo diode array detector (PDA) 2998, with an automated sample injector. The output signal was monitored and integrated using Empower 2 software.Supelco C18 (250mm X 4.6mm,5µm,) column was used for separation, Heating Mantle-Bio Technics India,Thermal oven Narang , Filter paper 0.45 Microns.

Chromatographic condition: The mobile phase used was 0.01N Sodium sulphate and Methanol in the gradient mode employing flow rate at 1 ml/min. The analytical column SupelcoC18 (4.6 x 250 mm, 5 µm). The detection was carried out at a wavelength of 265 nm with a run time of 6 min. Water and Acetonitrile in the ratio of 60:40 v/v used as diluent

Preparation of solutions:
Preparation of mobile phase: Transfer 1000ml of HPLC water into 1000ml of beaker and Na_2SO_4 adjust pH 3.8
Transfer the above solution 600ml $Na_2SO_4$of, 400ml of Methanol is used as mobile phase. They are mixed and Sonicate for 20min.

The diluent
Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50 as diluent.

Preparation of standard solution: Accurately weigh and transfer 20mg Trifluridine and 8.19 mg Tipiracilinto 100ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with water.

Preparation of sample solution: Commercially available 20 tablets ware weighed and powdered the powdered equivalent to the 284mg of (20mg Trifluridine and 8.19mg Tipiracil) of active ingredients were transfer into a 100ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with water.
Transfers above solution 1ml into 10ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1 ml/min for 5 minutes to equilibrate the column at ambienttemperature. Chromatographic separation was achieved by injecting a volume of 10 µl of standard into Inspire C18 (4.6 x 250 mm, 5 µm) column, the mobile phase of composition 0.01N Na_2SO_4 buffer and Methanol in the gradient mode was allowed to flow through the column at a flow rate of 1 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in Table 1 and figure 3.

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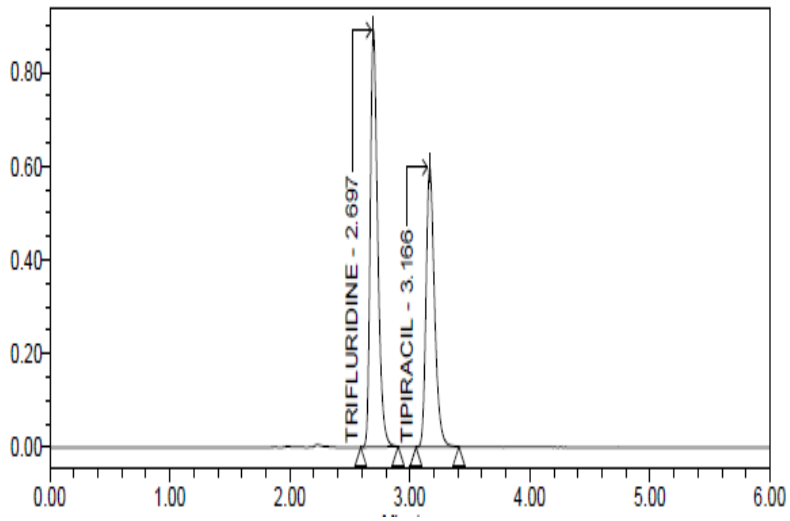

Figure 3: Standard chromatogram

Table 1: System suitability parameters

Parameter	Trifluridine	Tipiracil	Acceptance Criteria
Retention time	2.697	3.166	+-10
Theoretical plates	9302	9788	>2500
Tailing factor	1.36	1.30	<2.00
% RSD	0.1	0.1	<2.00

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Trifluridine andTipiracl simultaneously in their pharmaceutical dosage form. The result obtained for Trifluridine andTipiraclwas comparable with the corresponding labeled amounts and they were shown in Table-2 and figure-4.

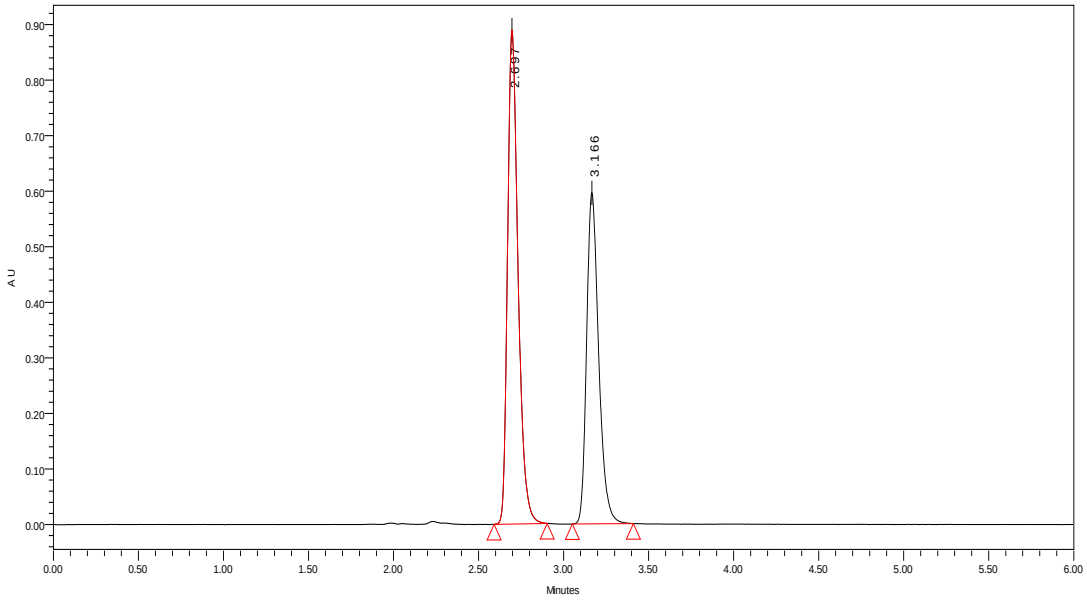

Figure 4: Sample chromatogram

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Table 2: Assay results for netarsudil and latanoprost

	Label Claim (mg)	% Assay
Trifluridine	20 mg	99.6%
Tipiracil	8.19mg	99.7%

Method validation

The method was validated in accordance with ICH guidelines.

Linearity: Linearity of the method was studied by injecting Five concentrations of the drugs in triplicate prepared in the range of 50-150µg/mlinto the HPLC system. Linear graphs were plotted by using the peak areas against concentration in µg/ml from which the correlation coefficients, slopes and Y-intercepts of the calibration curves were determined. The results were shown in Table 3& 4 and figure 5 & 6.

Table 3: Linearity results for Trifluridine

S.No	Conc (µg/ml)	RT	Area
1.	50	2.681	1890874
2.	75	2.687	2853835
3.	100	2.691	3810718
4.	125	2.696	4770755
5.	150	2.703	5736226
Correlation coefficient (r^2)			1.000

Table No.4:Linearity results for Tipiracil

S.No	Conc (µg/ml)	RT	Area
1.	50	3.146	1441861
2.	75	3.152	2173303
3.	100	3.155	2911300
4.	125	3.161	3643960
5.	150	3.169	4375104
Correlation coefficient (r^2)			1.000

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Figure 5: Linearity graph for Trifluridine

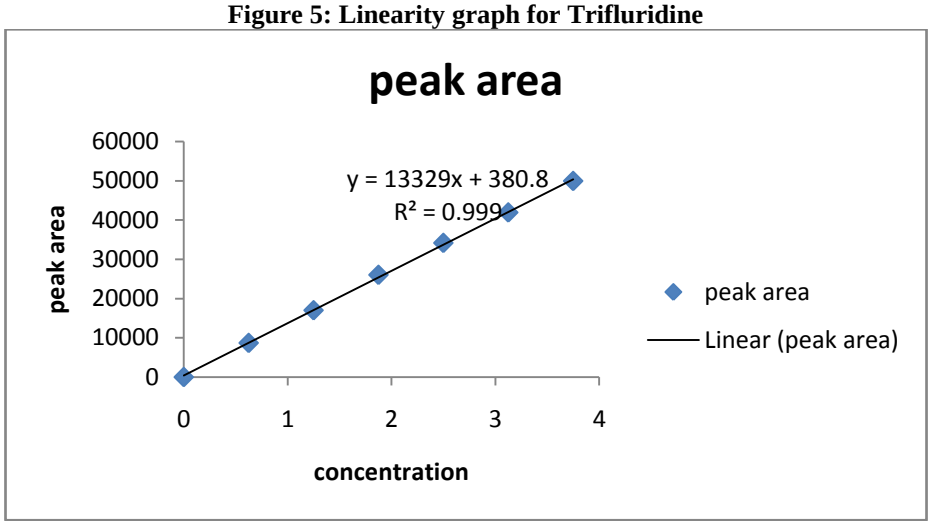
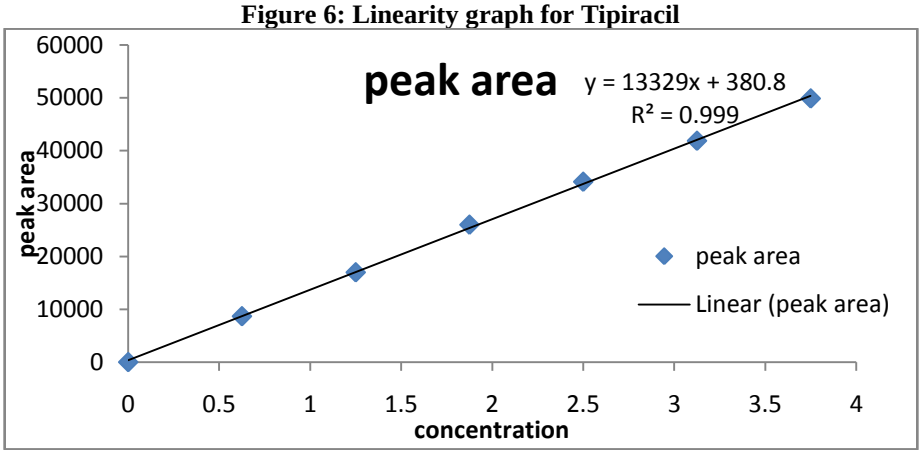


Figure 6: Linearity graph for Tipiracil



Accuracy: The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed sample. Percent recovery was calculated by comparing the area with pre analysed sample. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount.

% Recovery was 100% for Trifluridineand 100% forTipiracil. All the results indicate that the method is highly accurate**.**Table-5 and6.

Table 5: Accuracy%recovery results

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	142.00	9.900	9.85	99	100
		2	142.00	9.900	9.89	100	
		3	142.00	9.900	9.87	100	
2	100%	1	284.00	19.800	19.81	100	100
		2	284.00	19.800	19.83	100	

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		3	284.00	19.800	19.88	100	
3	150%	1	426.00	29.700	29.78	100	100

Table 6: Accuracy (%recovery) results ofTipiracil

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	142.00	4.054	4.03	99	100
		2	142.00	4.054	4.03	99	
		3	142.00	4.054	4.06	100	
2	100%	1	284.00	8.108	8.12	100	100
		2	284.00	8.108	8.15	100	
		3	284.00	8.108	8.12	100	
3	150%	1	426.00	12.162	12.18	100	100
		2	426.00	12.162	12.19	100	
		3	426.00	12.162	12.21	100	

Precision: For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. The sample was injected in six replicate for The peak area for injections was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed HPLC method was found to be precise. The Precision results were shown in Table-6 and 8.

Table 7: Precision results for Trifluridine

S.No	RT	Area	%Assay
injection1	2.695	3815896	99
injection2	2.697	3823703	99
injection3	2.692	3815712	99
injection4	2.692	3819778	99
injection5	2.694	3811724	99
injection6	2.692	3823743	99
Mean			99
Std. Dev.			0.13
% RSD			0.13

Table 8: Precision results for Tipiracil

S.no	RT	Area	%Assay
injection1	3.162	2928139	100
injection 2	3.164	2916515	99
injection 3	3.157	2915183	99
injection 4	3.159	2913661	99
injection 5	3.160	2917779	99
injection 6	3.158	2911788	99
Mean			99
Std. Dev.			0.20
%RSD			0.20

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Robustness: Robustness of the method was checked by making deliberate changes in chromatographic conditions like mobile phase ratio (±10%), and flow rate (1.2 ml/min). It wasobserved that there were no marked changes in system suitability parameters, which demonstrated that the developed HPLC method is robust.

Limit of detection and Limit of quantification:
The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method.

III. CONCLUSION:

In the present work a new, accurate, precise and robust HPLC method was developed and validated for simultaneous estimation of netarsudil and latanoprostin pharmaceutical dosage form in accordance with the ICH Guidelines. The method gives good resolution between both the compounds with a short analysis time (2.6 min). Linearity is observed in the concentration range of 50-150µg/ml for Trifluridineand 50-150µg/ml for Tipiracil. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the Trifluridine and Tpiracilin combined dosage form without any interference from excipients.

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