DEVELOPMENT AND VALIDATION IN SIMULTANEOUS ESTIMATION OF TRIFLURIDINE AND TIPIRACIL IN PURE FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

Objective: A simple, precise, accurate method was developed for the simultaneous estimation of Trifluridine and Tipiracil in bulk and marketed pharmaceutical dosage form by RP-HPLC technique.

Methods: Methanol, Acetonitrile and Water (50:35:15%v/v) in the ratio of 50:35:15%v/v/v) used as mobile phase run through X-Terra C18 ODS (4.6 ×150mm, 5µm particle size) column with a flow rate of 1.0 ml/min. The temperature of the column oven was maintained at 42 °C. Wavelength was selected 280 nm. Stock and working solutions were prepared by using the diluents. Runtime was fixed to 8 min.

Results: Trifluridine and Tipiracil were eluted at 1.688 and 3.266min with good resolution the plate count, tailing factor and all system suitability parameters are within ICH range. Trifluridine and Tipiracil were found to be linear low in concentration range of 20-100µg/ ml and 5-25µg/ ml respectively in the linearity study, regression equation and coefficient of correlation for Trifluridine and Tipiracil were found to be ($y = 22649x+35680$ $r^2=0.999$) and ($y=19445+3220.1$ $r^2 = 0.9991$) Percentage recovery for both Trifluridine and Tipiracil was found in range of 98%-102% indicating accuracy of the proposed work. Assay of the tablet was performed and found as 98.99%.

Conclusion: All the parameters were within the ICH guidelines and the method was economical and simple as retention times were less than in literature and decreased run time.

Key Words: Trifluridine and Tipiracil, RP-HPLC, ICH Guidelines, Accuracy, Precision.

INTRODUCTION

Trifluridine is a fluorinated pyrimidine nucleoside that is structurally related to idoxuridine 1. It is an active antiviral agent in ophthalmic solutions used mainly in the treatment of primary keratoconjunctivitis and recurrent epithelial keratitis due to herpes simplex virus. It displays effective antiviral activity against Herpes simplex virus type 1 and 2. Trifluridine[1] exhibits an antiviral effect against herpes simplex virus, types 1 and 2 and vacciniavirus both in vitro and in vivo. Some strains of adenovirus that contribute to the pathology of keratoconjunctivitis were shown to be susceptible to Trifluridine[2] in vitro 1. While there is evidence from a study that cross-resistance may develop between Trifluridine and idoxuridine or vidarabine, Trifluridine[3] was shown too effective in treating dendritic ulcers in patients with herpetic keratitis who are unresponsive to idoxuridine or vidarabine based on the results from masked comparative trials 1. The IUPAC Name of Trifluridine is 1-[(2R, 4S, 5R)-4-hydroxy-5-(hydroxy methyl) oxolan-2-yl]-5-(trifluoromethyl) pyrimidine-2, 4-dione. The Chemical Structure of Trifluridine is as follows

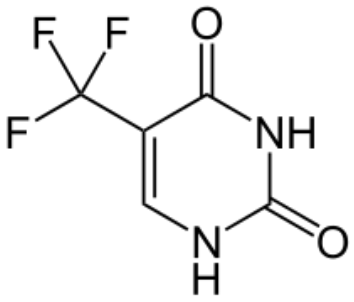

Fig-1: Chemical Structure of Trifluridine

Tipiracil is a thymidine phosphorylase inhibitor. It is used in combination with Trifluridine, in a ratio of 1:0.5, to form TAS-102. The main function of Tipiracil[4] in TAS-102 is to increase Trifluridine bioavailability by inhibiting its catabolism.2 TAS-102 is indicated for the treatment of metastatic colorectal cancer which has been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, or with an anti-VEGF or anti-EGFR therapy. Tipiracil[5] prevents Trifluridine conversion into 5-trifluoromethyl-2, 4 (1H, 3H)-pyrimidinedione, which is an inactive major metabolite, by inhibiting the enzyme thymidine phosphorylase. Thus, Tipiracil is able to increase Trifluridine bioavailability. On the other hand, thymidine phsophorylase is a known platelet-derived endothelial cell growth factor and its inhibition generates an indirect antiangiogenic benefit. Tipiracil[6] is a thymidine phosphorylase inhibitor. Its function prevents the breakdown of 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 angiogenic factor usually overexpressed in solid tumors. There is a direct association of thymidine phosphorylase with a poor prognosis; where the tumors with an elevated expression of this enzyme tend to present an increased angiogenesis and ergo, be more malignant. Therefore, it has been suggested that Tipiracil presents an additional function by down regulating tumoral angiogenesis. The IUPAC Name of Tipiracil is 5-chloro-6-[(2-iminopyrrolidin-1-yl) methyl]-1H-pyrimidine-2, 4-dione. The Chemical Structure of Tipiracil is as following

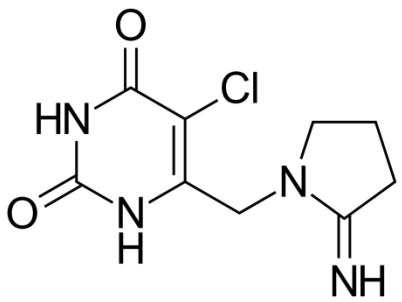

Fig-2: Chemical Structure of Tipiracil

EXPERIMENTAL

Table-1: Instruments used

S.No.	Instruments and Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA Detector.
2	pH meter	Labindia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Trifluridine (Pure)	Local Market
2	Tipiracil (Pure)	Local Market
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Trifluridine and Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1875ml of Trifluridine and 0.15ml of the Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[7-9].

Method Validation
Preparation of Mobile Phase:
Accurately measured 500 ml (50%) of Methanol and 350 ml of Acetonitrile (35%) and 150ml (15%) of Water were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

Validation Parameters
System Suitability
Accurately weigh and transfer 10 mg of Trifluridine and 10mg of Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1875ml of Trifluridine and 0.15ml of the Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:
Preparation of Standard Solution:
Accurately weigh and transfer 10 mg of Trifluridine and 10mg of Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1875ml of Trifluridine and 0.15ml of the Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:
Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Trifluridine and Tipiracil sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1875ml of Trifluridine and 0.15ml of the Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the three replicate injections of standard and sample solutions and calculate the assay[10] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:
Accurately weigh and transfer 10 mg of Trifluridine and 10mg of Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20ppm of Trifluridine & 5ppm of Tipiracil):
Pipette out 0.2ml of Trifluridine and 0.05ml of Tipiracil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (40ppm of Trifluridine & 10ppm of Tipiracil):
Pipette out 0.4ml of Trifluridine and 0.1ml of Tipiracil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (60ppm of Trifluridine & 15ppm of Tipiracil):
Pipette out 0.6ml of Trifluridine and 0.15ml of Tipiracil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (80ppm of Trifluridine & 20ppm of Tipiracil):
Pipette out 0.8ml of Trifluridine and 0.2ml of Tipiracil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (100ppm of Trifluridine & 25ppm of Tipiracil):
Pipette out 1.0ml of Trifluridine and 0.25ml of Tipiracil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[11].

Precision

Repeatability

Preparation of Trifluridine and Tipiracil Product Solution for Precision:

Accurately weigh and transfer 10 mg of Trifluridine and 10mg of Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1875ml of Trifluridine and 0.15ml of the Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Trifluridine and 10mg of Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.9375ml of Trifluridine and 0.75ml of the Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Trifluridine and 10mg of Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1875ml of Trifluridine and 0.15ml of the Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Trifluridine and 10mg of Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.2812ml of Trifluridine and 0.2262ml of the Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Trifluridine and Tipiracil and calculate the individual recovery and mean recovery values.

Robustness:
The analysis was performed in different conditions[12] to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Trifluridine and 10mg of Tipiracil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1875ml of Trifluridine and 0.15ml of Tipiracil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: Acetonitrile: Water was taken in the ratio and 55: 30:15, 45:35:20 instead 50:35:15, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Method Development

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Orthophosphoric acid and Phosphoric acid (pH 3): Acetonitrile and Methanol: ACN with varying proportions. Finally, the mobile phase[13] was optimized to Methanol: Acetonitrile: Water in the proportion of 50:35:15%v/v respectively.

Optimization of Column:

The method was performed with various columns like C_{18} column, ODS and Zodiac column. X-Terra (4.6 ×150mm, 5µm particle size) was found to be ideal as it gave good peak shape and resolution[14] at 1ml/min flow.

Optimized Chromatographic Condition:

Table-3: Shows Optimized Chromatographic Conditions

PARAMETER	OPTIMIZED CHROMATOGRAPHIC CONDITIONS
Mobile phase :	Methanol: Acetonitrile: Water (50:35:15%v/v)
Column :	X-Terra (4.6 ×150mm, 5µm particle size)
Flow rate :	1ml/min
Diluent	Methanol: Acetonitrile: Water (50:35:15%v/v)
Injection Volume	10 µl
Wavelength:	280 nm
Column temp:	42ºC
Run mode	Isocratic
Runtime	8minutes

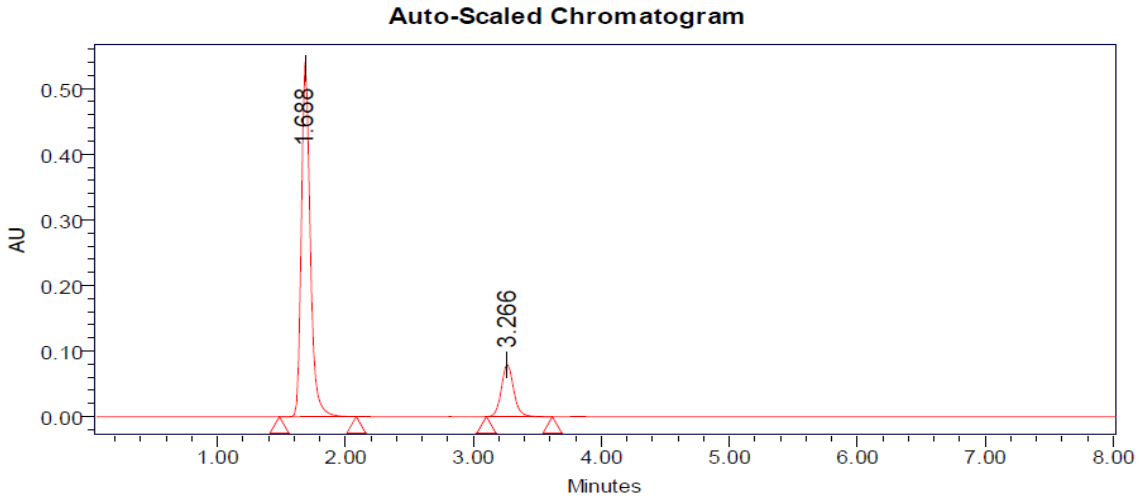

Fig-3: Optimized Chromatogram Condition

Validation of Method:

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines[15].

Specificity (Assay)

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Table-4: Assay Results

Label Claim	% Purity
Trifluridine and Tipiracil	98.99%

System Suitability Parameters:

In System suitability[16] injecting standard solution and reported USP tailing and plate count values are tabulated in table 5.

Table-5: Observation of system suitability parameters

S. NO	Parameter	Trifluridine	Tipiracil
	Retention Time (min)	1.694	3.234
	Theoretical Plates	6993	5735
	Tailing factor	1.23	1.12
	Area	1429524	300414
	Resolution	10.69	

The system suitability parameters were found to be within the specified limits for the proposed method.

Accuracy:

The accuracy[17] of the developed method was done by recovery studies by the addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100, and 150%. The percentage recovery and percentage mean recovery were determined for the drugs and shown in Table 6 and 7.

Table-6: Accuracy Observation of Trifluridine

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	713835.3	9.375	9.3	100	99.3%
100%	1406995	18.75	18.92	99.1	
150%	205468	28.12	27.86	99.05	

Table-7: Accuracy Observation of Tipiracil

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	150840	7.5	7.59	100	100.7%
100%	300215	15	15.27	101.82	
150%	443109	22.5	22.62	100.5	

The accuracy studies were shown as % recovery for Trifluridine and Tipiracil at 50%, 100% and 150% the limits of % recovery should be in range of 98-102%.

The results obtained for Trifluridine and Tipiracil were found to be within the limits. Hence the method was found to be accurate.

The accuracy studies showed % recovery of the Trifluridine 99.3%-and Tipiracil 100.7%.

The limits of % recovery of drugs were 98-102 % and from the above results it indicates that the commonly used excipients present in the pharmaceutical formulation do not interfere in the proposed method.

Precision:

Equivalent concentration solutions of Trifluridine and Tipiracil were injected five times, and % RSD was determined for both the drugs. The results are shown in table-8.

Table-8: Observation of System Precision

S. No.	Sample Area 1	Sample Area 2
1	1429456	300557
2	1422446	300364
3	1424679	300377
4	1425211	300817
5	1426102	300227
Mean	1425578.8	300468.4
Std.dev	2552.73	227.4
%RSD	0.1	0.07

Acceptance Criteria:

In the precision study[18] %RSD was fond to be less than 2%. For Trifluridine 0.1% and Tipiracil 0.07% which indicates that the system has good reproducibility.

For precision studies 5 replicated injections of Trifluridine and Tipiracil formulation was performed. %RSD was determined for peak areas of Trifluridine and Tipiracil.

The acceptance limits should be not more than 2% and the results were found to be within the acceptance limits.

Ruggedness:

The ruggedness[19] was determined by measuring the analyst to analyst variation assay of two different analysts.

Day 1:

Table-9: Observation of Robustness Day1

S. No.	Sample Area 1	Sample Area 2
1	1428595	300757
2	1426785	300826
3	1426465	300668
4	1426588	300119
5	1427757	300599
6	1426622	300181

Mean	1427135	300525
Std. Dev.	854.665	301.1717
% RSD	0.059887	0.100215

Day 2:

Table-10: Observation of robustness Day2

S. No.	Sample Area 1	Sample Area 2
1	1426363	300918
2	1420494	300848
3	1428474	300199
4	1428574	300188
5	1426563	300198
6	1426568	300194
Mean	1426173	300424.2
Std. Dev.	2954.815	356.1204
% RSD	0.207185	0.118539

Linearity

The area of the linearity peak versus different concentrations has been evaluated for Trifluridine and Tipiracil, as 20, 40, 60, 80, and 100 & 5, 10, 15, 20 and 25 µg/ml respectively. Linearity[20] was performed in the range of 20-100µg/ml & 5-25µg/ml of Trifluridine and Tipiracil respectively. The correlation coefficients achieved was greater than 0.999 & 0.9991 for Trifluridine and Tipiracil respectively.

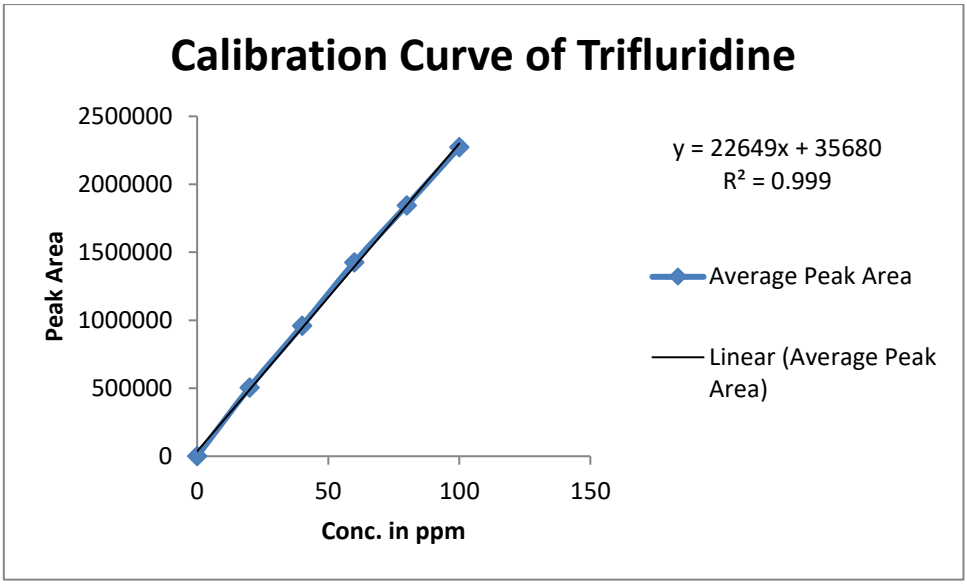

Fig-4: Calibration curve for Trifluridine

Table-11: Linearity Observation of Trifluridine

S. No.	Concentration Level (%)	Concentration µg/ml	Average Peak Area
	I	20	504954
	II	40	958753
	III	60	1426583
	IV	80	1845498
	V	100	2272948
Correlation coefficient			0.999

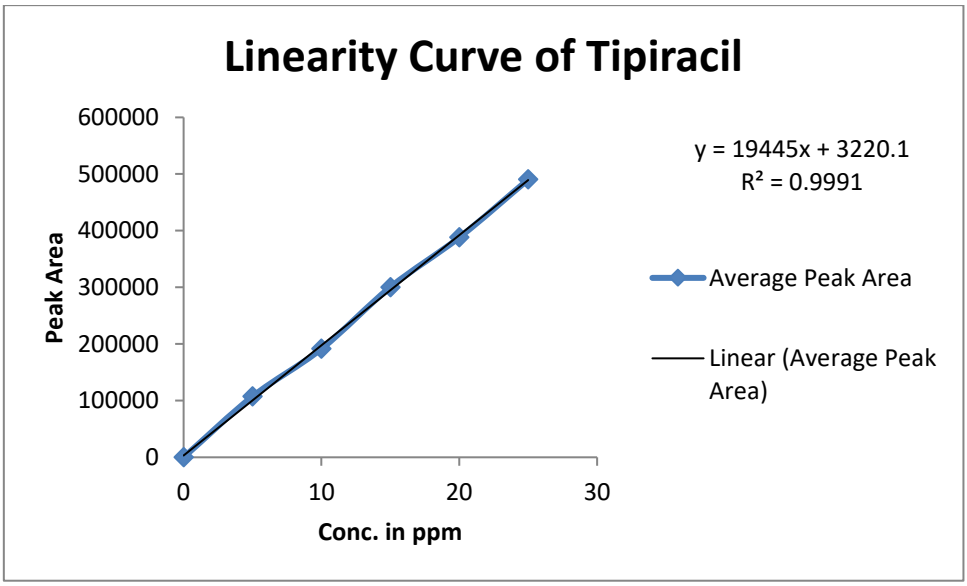

Fig-5: Calibration Curve for Tipiracil

Table-12: Linearity Observation of Tipiracil

S. No.	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	I	5	107359
2	II	10	191497
3	III	15	300389
4	IV	20	388105
5	V	25	490352
Correlation coefficient			0.999

The linearity range was found to be 50-150 µg/ml for both Trifluridine and Tipiracil respectively. Calibration curves were plotted and correlated Co-efficients for both the drugs found to be 0.999.

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × S.D / Slope

Table-13: LOD results of the method

Drug	Amount(µg/ml)
Trifluridine	2.58
Tipiracil	1.08

From the above, the LOD values of Trifluridine and Tipiracil were found to be 2.58 and 1.081 µg/ml respectively.

Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10× S.D / Slope

Table-14: LOQ results of the method

Drug	Amount(µg/ml)
Trifluridine	7.84
Tipiracil	3.27

From the above, the LOQ values of Trifluridine and Tipiracil were found to be 7.84 and 3.27 µg/ml respectively.

Robustness

The robustness[21] was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Tipiracil, Trifluridine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Tipiracil, Trifluridine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

System suitability Results for Trifluridine

Table-15: Flow rate Observation of Trifluridine

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6728	1.23	1.868
Actual Flow rate	1	6993	1.23	1.694
More Flow rate	1.2	5285	1.21	1.544

* Results for actual flow rate have been considered from assay standard.

System suitability Results for Tipiracil

Table-16: Flow rate Observation of Tipiracil

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	4402	1.11	3.621
Actual Flow rate	1	5735	1.12	3.234
More Flow rate	1.2	6509	1.12	2.998

*****On evaluation of the above results, it can be concluded that the variation in flow rate not affect the method significantly.

Table-17: System Suitability Results Trifluridine

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	45:35:20	6728	1.23	1.868
Actual organic phase	50:35:15	6993	1.23	1.694
More organic phase	55:40:5	6996	1.24	1.675

Table-18: System Suitability Results Tipiracil

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	45:35:20	4402	1.11	3.621
Actual organic phase	50:35:15	5735	1.12	3.234
More organic phase	55:40:5	4831	1.15	2.302

Stability Studies

Results of Forced Degradation Studies: The results of the forced degradation studies[22] indicated the specificity of the developed method that has been developed. Trifluridine and Tipiracil were stable only in alkaline and thermal stress conditions. The results of stability studies are given in the following Table-19.

Table-19: Results of Forced Degradation Studies of Trifluridine and Tipiracil API

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	83.105	16.895	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	80.148	19.852	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	82.411	17.589	100.00
UV (254nm)	24Hrs.	91.066	8.934	100.00
3% Hydrogen Peroxide	24Hrs.	83.108	16.892	100.00

SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The simultaneous estimation of Trifluridine and Tipiracil was done by RP-HPLC. The mobile phase was optimized with consists of Methanol, Acetonitrile and Water was taken in the ratio of 50:35:15%v/v. A X-Terra (4.6 ×150mm, 5µm particle size), Make: Waters) or equivalent chemically bonded to porous silica particles was used as stationary phase.. The solutions were chromatographed at a constant flow rate of 1.0ml/min. the linearity range of Trifluridine and Tipiracil were found to be from 20-100ppm and 5-25 µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery was found to be within the limits of Trifluridine and Tipiracil. LOD and LOQ were found to be within limits.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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