A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TRIFLURIDINE AND TIPIRACIL HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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Abstract:
Trifluridine and Tipiracil Hydrochloride are anti-cancer drugs. A new HPLC method has been proposed for the simultaneous determination of Trifluridine and Tipiracil Hydrochloride tablets on Isocratic mode with 1.0 mL/min flow rate and a diluent consisting of a mixture of Acetonitrile: Water: Methanol (60:20:20v/v) was used for the study. And it used column Hypersil C18 (4.6×150mm, 5.0 µm) A Waters Model 2695 separation module HPLC system equipped with 996 PDA detector was used. The detection was done at 230 nm. The retention time were found to be 2.842 min, 3.878 min for Trifluridine and Tipiracil Hydrochloride respectively. Trifluridine showed linearity in the concentration range of 10-50 µg/mL with linear regression (R2 = 0.9999). The % RSD for precession and accuracy studies were found to be < 2%.The LOD and LOQ were found to be 0.6 µg/ml,12.8 and 1.9 & 38.90 µg/ml, respectively.. The proposed method was observed to be simple, economical and was validated according to the ICH guidelines for linearity, precision, accuracy and stability. This proposed method is highly sensitive, precise and accurate which reduces cost of analysis; hence recommended for routine quality analysis in laboratories.

Keywords: Trifluridine and Tipiracil Hydrochloride, RP-HPLC, Robustness and ICH Guidelines.

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Introduction:

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. The current good manufacturing practice (CGMP) and food drug administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Development of a method of analysis is usually based on prior art (or) existing literature, using the same (or) quite similar instrumentation. It is rare today that an HPLC-based method is developed that does not in same way relate (or) compare to existing, literature based approaches. Today HPLC (high performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for routine assay of drugs. Hence for the assay of the selected drugs HPLC and UV spectroscopy has been chosen for these proposed methods. The developed chromatographic methods further validated as per ICH or USFDA guidelines for all the critical parameters. To access the precision and to evaluate the results of analysis the analyst must use statistical methods. These methods include confidence limit, regression analysis to establish calibration curves. In each analysis the critical response parameters must be optimized and recognized if possible. Trifluridine and Tipiracil Hydrochloride are anti cancer drugs. Trifluridine chemically 1- [(2R, 4S, 5R)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-(trifluoromethyl)-1,2, 3, 4-tetra hydropyrimidine-2,4-dione., Tipiracil Hydrochloride chemically 5-Chloro-6-[(2-imino-1- pyrrolidinyl)methyl]-2,4 (1H,3H)-pyrimidinedione. The literature survey reveals that there are few HPLC and spectroscopic methods available for the determination individual Trifluridine and Tipiracil in bulk and dosage forms. There were less reported analytical methods for simultaneous estimation Trifluridine and Tipiracil in bulk and pharmaceutical dosage forms. Hence an attempt to develop specific, sensitive, accurate and precise RP HPLC method for simultaneous estimation of these drugs. The developed method was validated as per ICH Q2 guidelines.

MATERIAL AND METHODS

Trifluridine from Sura Labs, Tipiracil hydrochloride from Sura labs, KH2PO4 from FINER chemical LTD, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck, Triethyl amine from Sura labs.

HPLC METHOD DEVELOPMENT:

Mobile Phase Optimization: Initially the mobile phase tried was Acetonitrile: Water and Acetonitrile: Sodium dihydrogen phosphate buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile with Sodium dihydrogen phosphate buffer (pH 6.8), in proportion 20:80 v/v respectively. Optimization of Column: The method was performed with various columns like C18 column, X- bridge column, Xterra, and C8 m, Make: Waters) was found to be ideal as it gaveμcolumn. Phenomenex Luna C18 (4.6mm x 250mm, 5 good peak shape and resolution at 1ml/min flow. Validation methods procedures followed as per ICH guidelines.

RESULTS AND DISCUSSION

(Optimized Chromatographic Condition)

Mobile phase: Acetonitrile: Water: Methanol (60:20:20v/v)
Column: Hypersil C18 (4.6×150mm, 5.0 µm)
Flow rate:1 ml/min
Wavelength: 230 nm
Column temp: Ambient
Sample Temp: Ambient
Injection Volume:10 µl
Run time: 7 minutes

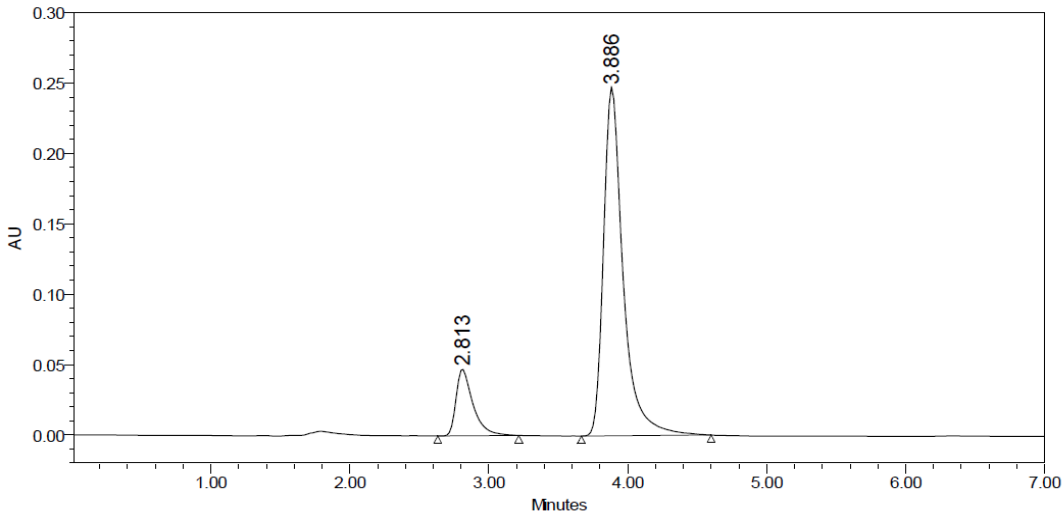

Fig.1. Typical Chromatogram for optimized Tipiracil and Trifluridine

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Tipiracil	2.813	399693	47206		1.6	2667.2
2	Trifluridine	3.886	2431064	247830	4.5	1.5	4142.6

Observation:

From the above chromatogram it was observed that the Tipiracil and Trifluridine peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Retention time of Tipiracil–2.813min

Retention time of Trifluridine – 3.886 min

SYSTEM SUITABILITY:

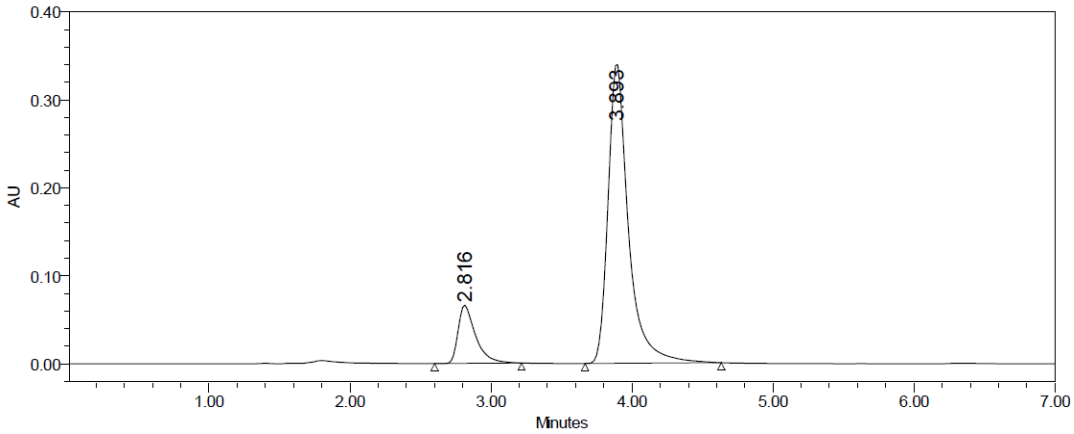

Fig.2. Chromatogram for system suitability

Table: Results of system suitability parameters for Tipiracil and Trifluridine

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Tipiracil	2.816	572745	66043		1.5	2642
2	Trifluridine	3.893	3423737	340922	4.5	1.5	4153

Acceptance criteria:

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

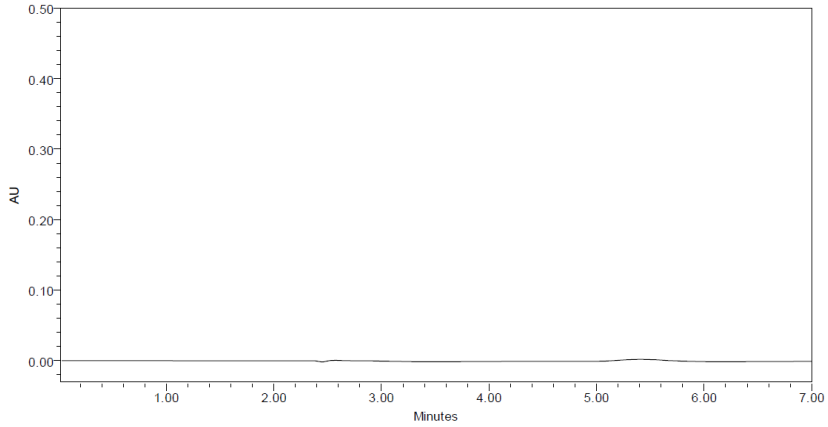

Fig.3 Chromatogram showing blank (mobile phase preparation)

VALIDATION PARAMETERS:

Assay (Standard):

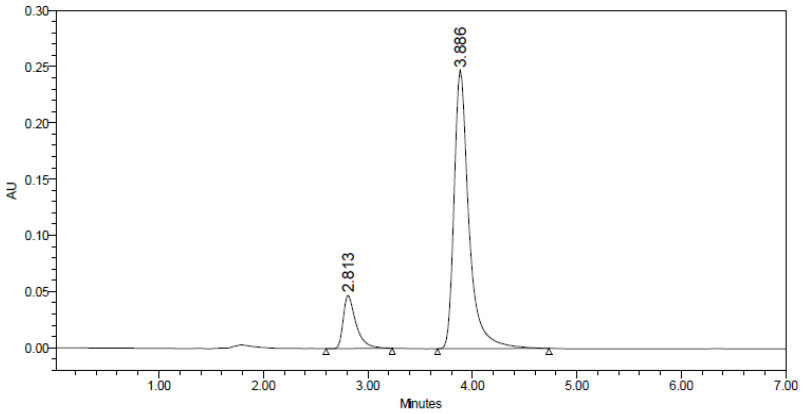

Fig:4. Chromatogram showing assay of standard injection-1

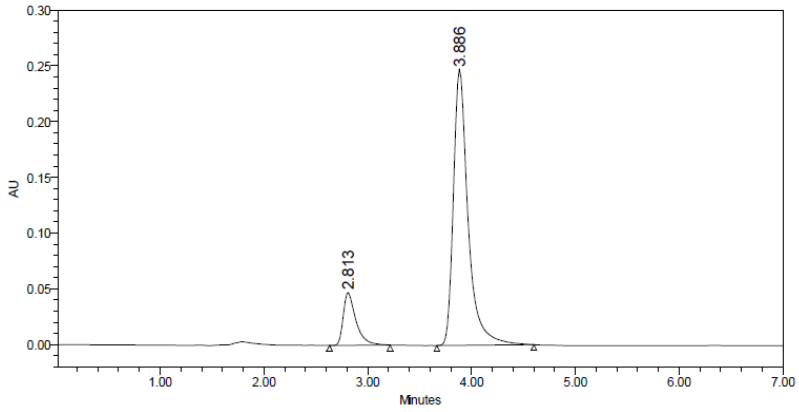

Fig.5. Chromatogram showing assay of standard injection-2

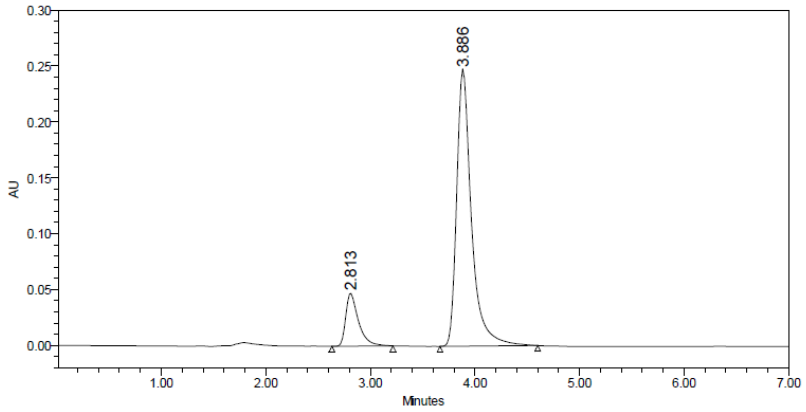

Fig.6. Chromatogram showing assay of standard injection-3

Table.1. Showing assay standard results

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Tipiracil	2.813	401018	47231		1.6	2663.4	1
2	Trifluridine	3.886	2444315	247928	4.53	1.5	4137.0	1
3	Tipiracil	2.813	399693	47206		1.6	2667.2	2
4	Trifluridine	3.886	2431064	247830	4.53	1.5	4142.6	2
5	Tipiracil	2.813	414561	43298		1.6	2632.9	3
6	Trifluridine	3.886	2321452	246128	4.53	1.5	4167.5	3

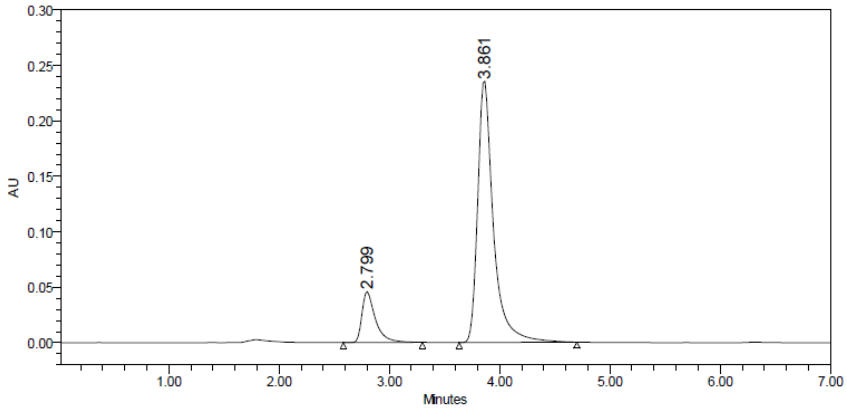

Fig.7. Chromatogram showing assay of sample injection -1

Table 2. Showing assay sample results

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Tipiracil	2.799	386989	45887		1.6	2423.3	1
2	Trifluridine	3.863	2342352	237080	4.5	1.5	4641.3	1
3	Tipiracil	2.799	387093	45772		1.6	2846.4	2
4	Trifluridine	3.861	2335575	236195	4.5	1.5	4631.8	2
5	Tipiracil	2.799	384362	45932		1.6	2691.1	3
6	Trifluridine	3.863	2395231	239412	4.5	1.5	4264.0	3

Table 3. Showing assay results

S.No	**Name of compound**	**Label claim**	**Amount taken(from combination tablet)**	**%purity**
1	Tipiracil	500mg	499.8	99.6 %
2	Trifluridine	600mg	599.9	99.8%

The retention time of Tipiracil and Trifluridine was found to be 2.860mins and 3.886mins respectively. The % purity of Tipiracil and Trifluridine in pharmaceutical dosage form was found to be 99.6% and 99.8% respectively.

6.3.2: precision:

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table 4. Results of method precession for Tipiracil:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tipiracil	2.808	368013	46097	4536	1.6
2	Tipiracil	2.808	372552	46244	4236	1.6
3	Tipiracil	2.808	367873	46092	4565	1.6
4	Tipiracil	2.808	375555	46312	4682	1.6
5	Tipiracil	2.808	374843	46275	4521	1.6
6	Tipiracil	2.808	368013	46097	4561	1.6
Mean			371767			
Std. Dev			3663.5			
% RSD			0.9			

Table 5. Results of method precession for Trifluridine:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Trifluridine	3.880	2321302	241739	4641.3	1.5	4.5
2	Trifluridine	3.880	2308016	241530	4632.2	1.5	4.5
3	Trifluridine	3.880	2326058	241796	4621.6	1.5	4.5
4	Trifluridine	3.880	2334897	241910	4695.3	1.5	4.5
5	Trifluridine	3.880	2326143	241799	4691.7	1.5	4.5
6	Trifluridine	3.880	2324512	241639	4685.1	1.5	4.5
Mean			2323283				
Std. Dev			9845.8				
% RSD			0.42				

Intermediate precision/Ruggedness:

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Table 6. Results of Intermediate precision for Tipiracil:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tipiracil	2.808	377409	45431	4536	1.6
2	Tipiracil	2.808	371977	45316	4521	1.6
3	Tipiracil	2.808	376191	45418	4596	1.6
4	Tipiracil	2.808	372169	45329	4587	1.6
5	Tipiracil	2.808	378930	45466	4536	1.6
6	Tipiracil	2.808	378624	45325	4562	1.6
Mean			375335			
Std. Dev			3132.9			
% RSD			0.83			

Table 7. Results of Intermediate precision for Trifluridine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Trifluridine	3.882	2268108	234269	4673.2	1.5	4.5
2	Trifluridine	3.882	2275775	234323	4628.3	1.5	4.5
3	Trifluridine	3.882	2254168	234089	4698.7	1.5	4.5
4	Trifluridine	3.882	2285916	234469	4612.9	1.5	4.5
5	Trifluridine	3.882	2296220	234557	4636.5	1.5	4.5
6	Trifluridine	3.882	22984261	234362	4678.2	1.5	4.5
Mean			2276037				
Std. Dev			16171.8				
% RSD			0.71				

6.3.4: ACCURACY:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 8. Results of Accuracy standard values:

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Tipiracil	2.860	753538	87418		1.63	2632.62	1
2	Trifluridine	3.949	4517559	450206	4.4	1.37	4050.28	1
3	Tipiracil	2.860	759356	87510		1.65	2624.44	2
4	Trifluridine	3.949	4586952	450917	4.4	1.41	4038.66	2
5	Tipiracil	2.860	752361	88142		1.6	2691.1	3
6	Trifluridine	3.949	4576321	452063	4.4	1.5	4264.0	3

Table 9.accuracy (recovery) data for Tipiracil

%Concentration (at specification Level)	**Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	569325	5	4.9	98%	99.8%
100%	753538	10	10.1	101%	
150%	955999	15	15.1	100.6%	

Table 9. Accuracy (recovery) data for Trifluridine

%Concentration (at specification Level)	**Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	3441832	5	4.9	98%	99.4%
100%	4517559	10	10.1	101%	
150%	5738638	15	14.9	99.3%	

Table 10. Results of Accuracy sample 50% values:

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Tipiracil	2.816	572745	66043		1.4	2423.3	1
2	Trifluridine	3.893			4.4	1.5	4641.3	1
3	Tipiracil	2.816	569325	65987		1.5	2846.4	2
4	Trifluridine	3.893			4.5	1.4	4631.8	2
5	Tipiracil	2.816	565848	65927		1.4	2691.1	3
6	Trifluridine	3.893			4.4	1.5	4264.0	3

Table 11. Results of Accuracy sample 100% values:

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Tipiracil	2.860	759356	87510		1.4	2465.3	1
2	Trifluridine	3.949	4586952	450917	4.5	1.4	4326.3	1
3	Tipiracil	2.860	753538	87418		1.5	2654.3	2
4	Trifluridine	3.949	4517559	450206	4.4	1.6	4329.1	2
5	Tipiracil	2.860	753318	87408		1.6	2146.9	3
6	Trifluridine	3.949	4551951	450625	4.5	1.4	4823.1	3

Table 12. Results of Accuracy sample 150% values:

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Tipiracil	2.824	952422	107607		1.5	2695.2	1
2	Trifluridine	3.914	5747649	553605	4.5	1.5	4268.3	1
3	Tipiracil	2.824	955999	107658		1.6	2687.1	2
4	Trifluridine	3.914	5738638	553502	4.4	1.4	4623.1	2
5	Tipiracil	2.824	953769	107627		1.5	2674.1	3
6	Trifluridine	3.914	5720937	553368	4.5	1.4	4861.3	3

Linearity:

The linearity range was found to lie from 10-50ppm of Tipiracil, 66.6µg/ml to 330µg/ml of Trifluridine and chromatograms are shown below.

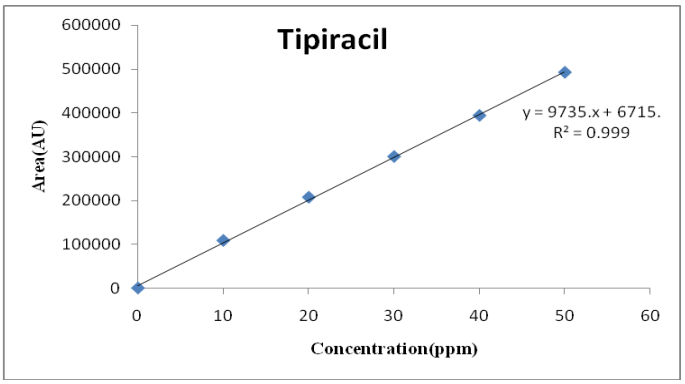

Fig.8. calibration graph for Tipiracil

Linearity Results: (for Tipiracil)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	10	108407
2	II	20	206978
3	III	30	299892
4	IV	40	393459
5	V	50	491862
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.999

Linearity Results: (for Trifluridine)

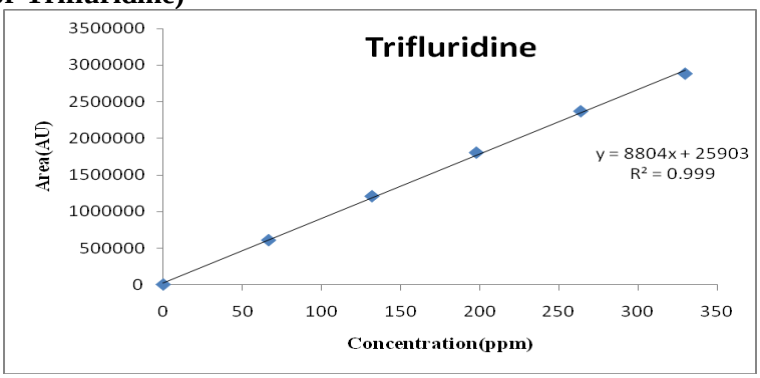

Fig. 9. Calibration graph for Trifluridine

S.No	Linearity Level	Concentration(ppm)	Area
1	I	66.6	606125
2	II	132	1208367
3	III	198	1804843
4	IV	264	2371642
5	V	330	2885708
Correlation Coefficient			0.999

Table-9 Analytical performance parameters of Tipiracil and Trifluridine

Parameters	Tipiracil	Trifluridine
Slope (m)	9735	8804
Intercept (c)	6715	25903
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria:
Correlation coefficient (R^2) should not be less than 0.999

LIMIT OF DETECTION
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 × σ / s
Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Tipiracil:
Result:
= 3.3×1921.9/9735
= 0.6µg/ml

Trifluridine:
Result:
=3.3×34259/8804
= 12.8µg/ml

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

LOQ=10×σ/S
Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Tipiracil:
Result:
=10×1921.9/9735
=1.9µg/ml

Trifluridine:
Result:
=10×34259/8804
=38.9µg/ml

ROBUSTNESS:
The standard and samples of Tipiracil and 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.

Variation in flow

Table 10. System suitability results for Tipiracil:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2741.14	1.71
2	1.0	2423.3	1.6
3	1.1	2543.21	1.58

***** Results for actual flow (1.0 ml/min) have been considered from Assay standard.

Table 11. System suitability results for Trifluridine:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4162.06	1.57
2	1.0	4641.3	1.5
3	1.1	3921.45	1.49

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

Table 12. System suitability results for Tipiracil

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2980.49	1.60
2	*Actual	2423.3	1.6
3	10% more	2423.52	1.64

Table 13. System suitability results for Trifluridine:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	9407	1.01
2	*Actual	4641.3	1.5
3	10% more	4457.17	1.44

REFERENCES:

1. Sharma B K, (1999). Instrumental method of chemical analysis Meerut. PP 175-203. 17.
2. Breaux J and Jones K: (2003). Understanding and implementing efficient analytical method development and validation. Journal of Pharmaceutical Technology, 5, PP 110-114. 18.
3. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A (1991). "Instrumental methods of analysis" 7th edition CBS publisher and distributors, New Delhi, PP 436-439. 19. ICH Q2A, (1999).
4. "Validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline. 20. Sura, R. S., CVS, S., & Rachamalla, S. S. (2022).
5. Bioanalytical RP-HPLC Method Development and Validation Of Clopidogrel Bisulfate In Wistar Rat Plasma And Its Application To Pharmacokinetic Study. International Journal of Applied Pharmaceutics, 14(1), 106–111.
6. Asha Eluru and K Surendra Babub, A New Selective Separation method development and Validation of Trifluridine and Tipiracil and its degradents were characterized by LC-MS/MS/QTOF, /J. Pharm. Sci. & Res. Vol. 12(1), 2020, 199-205
7. https://doi.org/10.22159/ijap.2022v14i1.43328 21.
8. https://pubchem.ncbi.nlm.nih.gov/compound/Tipiracil-hydrochloride 22.
9. https://pubchem.ncbi.nlm.nih.gov/compound/Trifluridine
10. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126. 5.
11. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.