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ORIGINAL ARTICLE **OPEN ACCESS**

Simultaneous Estimation of Trifluridine and Tipiracil Hydrochloride in Bulk and Tablet Dosage form by Using RP-HPLC Method

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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 Phosphate buffer (0.01M) pH 6.8: Acetonitrile (80:20% v/v) was used for the study. A Waters Model 2695 separation module HPLC system equipped with 996 PDA detector was used. The detection was done at 268 nm. The retention time were found to be 2.242 min, 3.678 min for Trifluridine and Tipiracil Hydrochloride respectively. Trifluridine showed linearity in the concentration range of 15-75 µg/mL with linear regression equation y = 83208x + 97468 (R^2 = 0.9999). The % RSD for precession and accuracy studies were found to be < 2%.The LOD and LOQ were found to be 0.8 µg/ml and 2.4 µg/ml, respectively. Tipiracil Hydrochloride showed linearity in the concentration range of 7-31 µg/mL with linear regression equation y = y = 11933x + 5454 (R^2 = 0.9999). The % RSD for precession and accuracy studies were found to be < 2%.The LOD and LOQ were found to be 0.3 µg/ml and 1.19 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. Trifluridinechemically 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 column. Phenomenex Luna C18 (4.6mm x 250mm, 5µm, Make: Waters) was found to be ideal as it gave 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: Phosphate buffer (0.01M) pH 6.8: Acetonitrile (80:20%v/v)
Column: Phenomenex Luna C18 (4.6mm x 250mm, 5µm Particle size Make: Waters) or equivalent
Flow rate: 1 ml/min
Wavelength: 268 nm
Column temp: Ambient
Sample Temp: Ambient
Injection Volume: 10 µl

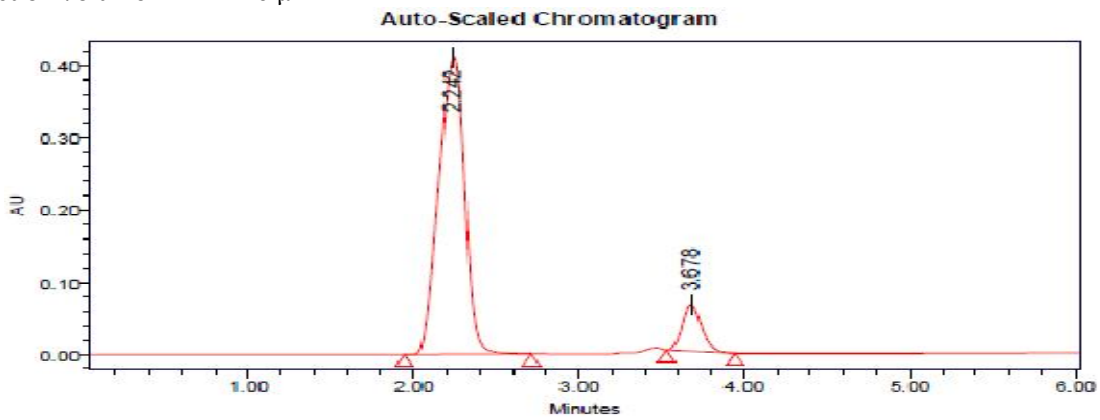

Fig-1: Optimized Chromatographic Condition (Trifluridine + Tipiracil hydrochloride)

Table1-: Results of Optimized Chromatographic Condition (Trifluridine + Tipiracil hydrochloride)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Trifluridine	2.242	4256351	565842		0.68	6584
2	Tipiracil hydrochloride	3.678	265284	285441	3.6	1.47	4857

From the above chromatogram it was observed that the Trifluridine and Tipiracil hydrochloride peaks are well separated

Retention time of Trifluridine – 2.242 min

Retention time of Tipiracil hydrochloride – 3.678 min

SYSTEM SUITABILITY:

Table 2-: Results of system suitability parameters for Trifluridine and Tipiracil hydrochloride

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Trifluridine	2.242	4263524	545145		0.85	7568
2	Tipiracil hydrochloride	3.679	267412	27582	3.9	1.26	4214

Assay Standard

Table 3-: Peak Results for assay standard of Trifluridine + Tipiracil hydrochloride

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Trifluridine	2.241	4256587	545145		0.86	6352
2	Tipiracil hydrochloride	3.680	268547	27547	3.6	1.48	4652
3	Trifluridine	2.245	4268541	545241		0.69	6541
4	Tipiracil hydrochloride	3.683	265412	26854	3.6	1.49	4684
5	Trifluridine	2.245	4258417	552415		0.75	7684
6	Tipiracil hydrochloride	3.683	269854	26859	3.6	1.47	4365

Assay Sample

Table 4-: Peak Results for assay Sample of Trifluridine + Tipiracil hydrochloride

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Trifluridine	2.241	4236521	545847		0.62	6522
2	Tipiracil hydrochloride	3.680	275874	27521	3.6	1.44	4412
3	Trifluridine	2.245	4298542	546541		0.68	6632
4	Tipiracil hydrochloride	3.683	278547	27452	3.6	1.45	4754
5	Trifluridine	2.245	4265844	556352		0.63	6851
6	Tipiracil hydrochloride	3.683	278798	26425	3.6	1.66	4632

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

The % purity of Trifluridine and Tipiracil hydrochloride in pharmaceutical dosage form was found to be 100.14% and 99.85%

ACCURACY:

Accuracy STD:

Table 5-: Accuracy (recovery) data for Trifluridine:

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3372980	22.5	21.893	99.732%	100.41%
100%	4285059	45	45.617	101.234%	
150%	5085059	67.5	67.563	100.271%	

Table 6-: Accuracy (recovery) data for Tipiracil hydrochloride

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	209948	9.5	9.412	99.03%	99.58%
100%	262097	19	18.538	99.076%	
150%	318874	28.5	28.583	100.638%	

LINEARITY:

Linearity Results: (forTrifluridine)

Table 7-: Results for Linearity for Trifluridine

S.No.	Linearity Level	Concentration	Area
1	I	15 ppm	1414547
2	II	30 ppm	2658542
3	III	45 ppm	3831546
4	IV	60 ppm	5127547
5	V	75 ppm	6274451
Correlation Coefficient			0.999

Linearity Results: (for Tipiracil hydrochloride)

Table 8-: Results for Linearity for Tipiracil hydrochloride

S.No.	Linearity Level	Concentration	Area
1	I	7 ppm	94547
2	II	13 ppm	162475
3	III	19 ppm	234284
4	IV	25 ppm	299866
5	V	31 ppm	375214
Correlation Coefficient			0.999

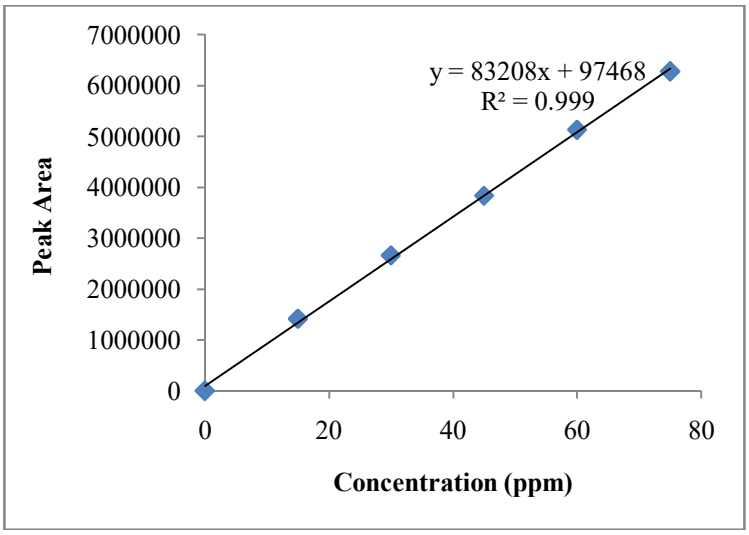

Fig 2-: Calibration graph for Trifluridine at 268 nm

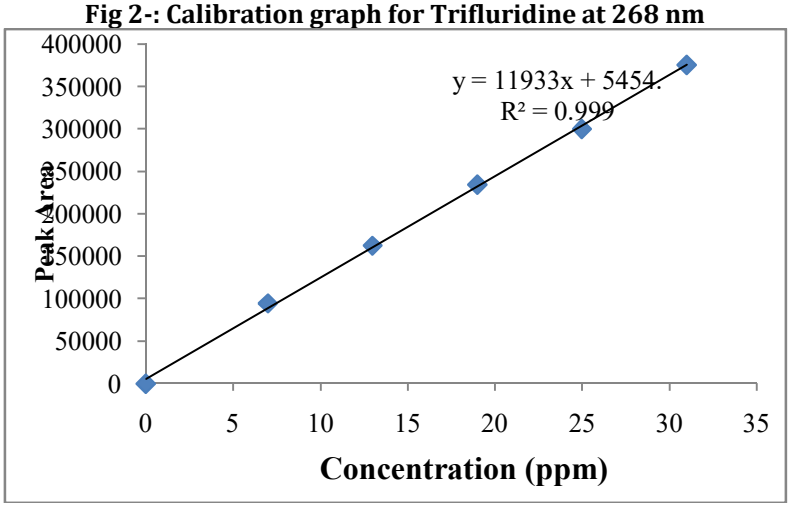

Fig 3-: Calibration graph for Tipiracil hydrochloride at 268 nm

LIMIT OF DETECTION FOR TRIFLURIDINE AND TIPIRACIL HYDROCHLORIDE

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

Result:

Trifluridine:

0.8µg/ml

Tipiracil hydrochloride:

0.3µg/ml

LIMIT OF QUANTITATION

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

Result:

Trifluridine:

2.4µg/ml

Tipiracil hydrochloride:

1. 19µg/ml

ROBUSTNESS:

System suitability results forTrifluridine:

Table 9-: System suitability results forTrifluridine:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	6686	0.69
2	1.0	6584	0.68
3	1.2	6785	0.67

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

System suitability results forTipiracil hydrochloride:

Table 10-: System suitability results forTipiracil hydrochloride:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	4986	1.49
2	1.0	4857	1.47
3	1.2	4998	1.53

System suitability results for Trifluridine:

Table 11-: 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	6087	0.59
2	*Actual	6584	0.68
3	10% more	6989	0.57

System suitability results for Tipiracil hydrochloride:

Table 12-: System suitability results for Tipiracil hydrochloride

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4169	1.39
2	*Actual	4857	1.47
3	10% more	4468	1.38

CONCLUSION

A new method was established for simultaneous estimation of Trifluridine and Tipiracil hydrochloride by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Trifluridine and Tipiracil hydrochloride by using Phenomenex Luna C18 (4.6mm x 250mm, 5µm, Make: Waters) or equivalent, flow rate was 1ml/min, mobile phase ratio was (20:80 v/v) Acetonitrile:

Phosphate bufferpH 6.8 (pH was adjusted with orthophosphoric acid), detection wave length was 268 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.242mins and 3.678 mins. The % purity of Trifluridine and Tipiracil hydrochloride was found to be 100.14% and 99.85% respectively. The system suitability parameters for Trifluridine and Tipiracil hydrochloride such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study on Trifluridine and Tipiracil hydrochloride was found in concentration range of 15µg-75µg and 7µg-31µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.41% and 99.83%, %RSD for repeatability was 0.207 and 0.534. The precision study was precise, robust, and repeatable. LOD value was 0.8 and 0.3, and LOQ value was 2.4 and 1.19 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Trifluridine and Tipiracil hydrochloride in API and Pharmaceutical dosage form.

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