Journal of Pharma Research
Available online through
www.jprinfo.com
Vol. 14 Issues 06, 2025 ISSN: 2319-5622

Original Article

RP- HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TIPIRACIL AND TRIFLURIDINE IN TABLET DOSAGE FORM

P. Sri Harsha[1]*, P. Vivek Sagar[1], S. HemaLatha[1], Jutta Swathi[1]
Department of Pharmaceutical Analysis, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Tarnaka, Secunderabad

Received on: 01-10-2025 **Accepted on: 03-11-2025**

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Tipiracil and Trifluridine in Tablet dosage form. Chromatogram was run through C18Hypertsil 5µ, 250mm×4.6mm column using 0.1% ortho phosphoric acid in water: acetonitrile (pH 4 using sodium hydroxide) as mobile phase. The retention time for Lamivudine and Tipiracil and Trifluridine were 2.1 min and 4.2 min respectively. The method is linear over a concentration range of 22.5 – 135 µg/ml for Tipiracil and 50 to 300 µg/ml for Trifluridine. The method was precise with % RSD within the acceptance limits. The method was validated for system suitability, accuracy, precision, linearity and ruggedness. The system suitability parameters were within limit, hence it was concluded that the method was suitable to perform the assay.

Keywords: *Tipiracil, Trifliridine, RP- HPLC, Simultaneous estimation, Method validation.*

INTRODUCTION

Colorectal cancer (CRC) remains a significant global health burden, demanding the development of effective therapeutic strategies. The combination of Tipiracilland Trifluridine(TFD) has emerged as a promising treatment option for patients with metastatic CRC. Tipiracil, acting as a thymidine phosphorylase inhibitor, potentiates the antitumor activity of TFD, a nucleoside analog that disrupts DNA synthesis in cancer cells. While both Tipiracil and TFD play crucial roles in this therapeutic approach, there is a paucity of robust and selective methods for their simultaneous quantification in commercially available tablet dosage forms. Existing literature primarily focuses on individual drug analysis or methods applicable to other drug combinations. This scarcity of readily available analytical methods for the Tipiracil-TFD combination presents a challenge for pharmaceutical quality control and potentially hinders optimal patient care.

This present study aims to bridge this critical analytical gap by developing and validating a Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Tipiracil and TFD in tablets. This optimized method offers several key advantages like enhanced efficiency by reducing analysis time and laboratory resource consumption compared to separate methods for each drug.

The method was meticulously designed to achieve chromatographic separation of Tipiracilland TFD from potential degradation products and common tablet excipients, ensuring accurate and reliable quantification.The method was optimized for short analysis times and user-friendliness, facilitating its routine application in quality control laboratories for the tipiracil-TFD combination product.

2. MATERIALS AND METHODS

• Tipiracil and Trifluridinepure drugs (API) received as gift sample from NATCO pharma ltd.Marketed tablets of Tipiracil and Trifluridine (Lonsurf) was purchased from Indiamart

Corresponding author:
Jutta Swathi
Department of Pharmaceutical Analysis
Sarojini Naidu Vanita Pharmacy MahaVidyalaya,
Tarnaka, Secunderabad, Telangana, India
Email: swathijutta9100@gmail.com
DOI: https://doi.org/10.5281/zenodo.17555981

Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogenortho phosphate buffer, Ortho-phosphoric acid. Alltheabovechemicals andsolvents are from Rankem

2.1. Solutions:

2.1.1. Preparation of Standard solutions:

Twenty milligrams (20 mg) of trifluridine and 9 milligrams (mg) of tipiracil were accurately weighed. The weighed substances were then transferred to a 10 mL volumetric flask. Seven and one-half milliliters (7.5 mL) of diluent were added to the flask. The solution was sonicated for 10 minutes using a laboratory or handheld sonicator. Following sonication, the volume of the solution in the flask was brought to 10 mL with diluent. This flask was labeled as the "standard stock solution".

Next, 1.0 mL of the standard stock solution was pipetted into a new 10 mL volumetric flask. This solution was then diluted to 10 mL with diluent and labeled as the "30 µg/mL solution". Likewise, 1.5 mL of the standard stock solution was pipetted into another new 10 mL volumetric flask. This solution was also diluted to 10 mL with diluent and labeled as the "13.5 µg/mL solution".

2.1.2. Samples Preparation

A sample solution containing trifluridine and tipiracil can be prepared from tablets following a defined procedure. Ten tablets are first weighed to determine the average weight of a single tablet. A portion of the crushed tablets, equivalent to the weight of one tablet, is then transferred to a 10 mL volumetric flask. After adding a few milliliters of diluent, the mixture is sonicated for 25 minutes to ensure proper extraction. The volume of the solution is then brought to 10 mL with the same diluent used previously. Finally, the solution is passed through HPLC filters to remove any unwanted particles. This process yields a sample solution with a final concentration of 2000 µg/mL trifluridine and 900 µg/mL tipiracil.

2.1.3. Cc standards;

A stock solution of Trifluridine and Tipiracil was prepared by accurately weighing 20 mg of Trifluridine and 9 mg of Tipiracil. These weighed substances were then transferred to a 10 mL volumetric flask. Subsequently, 7.5 mL of diluent was added to the flask, and the solution was sonicated for 10 minutes. Following sonication, the volume of the solution in the flask was brought to 10 mL with diluent.

Next, aliquots of appropriate volume were pipetted from the stock solution into separate 10 mL volumetric flasks. The aliquots were chosen to achieve concentrations ranging from 50 to 300 µg/mL for trifluridine and 22.5 to 135 µg/mL for tipiracil within these flasks. Finally, the volume of the solutions in each volumetric flask was made up to 10 mL with diluent. These resulting solutions served as the calibration curve standards.

2.2. Diluent:Mobile phase is used as diluent.

2.3. Chromatographic conditions:

The new HPLC method for estimation of Trifluridine and 9 mg Tipiracil was developed and validated using a Hypersil ODS C18 column (150 x 4.6mm 3.5mm). The mobile phase for this method was prepared by mixing 0.1% ortho phosphoric acid in water (adjusted to pH 4) and acetonitrile in a ratio of 65:35. Isocratic elution mode with a flow rate of 1.0 mL/min was employed for separation, and the eluent was monitored at a wavelength of 292 nm.

2.4. System suitability:

In order to assess system suitability, standard solutions of Trifluridine and Tipiracil were prepared and injected six times. From these injections, several parameters were evaluated including peak tailing, resolution, and USP plate count. The acceptance criteria for system suitability dictated that the relative standard deviation (RSD) for the peak area of both drugs across the six replicate injections should not exceed 2%. Additionally, the tailing factor for both Trifluridine and Tipiracil should be less than or equal to 2.

2.5. Method validation

The method validation was performed in accordance with ICH guidelines

2.5.1. Linearity

Each concentration level of the calibration standards was injected into the chromatographic system, and the corresponding peak area for each drug was measured. A calibration curve was then constructed by plotting peak area on the y-axis versus concentration on the x-axis. The correlation coefficient of this calibration curve was subsequently calculated

2.5.2. Accuracy

Each concentration level of the calibration standards was injected into the chromatographic system, and the corresponding peak area for each drug was measured. A calibration curve was then constructed by plotting peak area on the y-axis versus concentration on the x-axis. The correlation coefficient of this calibration curve was subsequently calculated

2.5.3. Precision

The precision of an analytical procedure is defined by the closeness of agreement between a series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed conditions. It reflects the degree of repeatability or reproducibility of the analytical method. Typically, precision is expressed through variance, standard deviation, or coefficient of variation calculated from a series of

measurements. To assess the overall precision of the method, both system precision and method precision are determined.

2.5.4. Robustness

The analytical method was subjected to controlled modifications to evaluate its impact on system suitability parameters. The flow rate was systematically varied by ±0.2 mL/min. Additionally, the mobile phase composition was adjusted to two different ratios: 65:35 and 55:45. For each modification, USP tailing factor and USP plate count were measured following six replicate injections of standard solutions. Subsequently, the system suitability parameters were assessed to determine if they remained within acceptable limits

2.5.5. Specificity:

The specificity of the method was evaluated by assessing potential interference from placebo on the analyte peak. A placebo sample was injected into the HPLC system following the established test procedure. The resulting chromatogram of the placebo injection was examined to ensure the absence of any peaks at the retention time corresponding to the analyte peak.

3. RESULTS AND DISCUSSION

3.1 Assay of formulation:

The assay of the formulation was carried out following the standard procedure in triplicate to ensure accuracy. The amount of each drug (tipiracil and trifluridine) present in the formulation was then calculated using a pre-constructed standard calibration curve. These calculations yielded assay percentages of 101.25% and 101.1% for tipiracil and trifluridine, respectively. Representative chromatograms for the standard solution, the test sample, and a blank were included in the figures. Additionally, the corresponding peak areas for each chromatogram were tabulated in Table 1.Representative chromatograms for standard, test and blank was given in figures 3,4&5. Peak areas were given in table no. 1.

3.2 System suitability

System suitability parameters were determined according to ICH guidelines. Plate count was more than 2000, tailing factor was less than 2 and resolution was more than 2. All the system suitable parameters were passed and were within the limits. The results showing system suiability parameters were given in table no. 2

3.3 Validation

3.3.1. Linearity

Linearity was assessed using six concentration levels for Trifluridine (ranging from 50 µg/mL to 300 µg/mL) and six concentration levels for Tipiracil (ranging from 22.5 µg/mL to 135 µg/mL). Peak areas were plotted against concentration for each drug, and a calibration curve was constructed. This calibration curve is presented in Figure 3. The correlation coefficient (r^2) for both Trifluridine and Tipiracil exceeded 0.99 within the tested concentration ranges. The linearity data is summarized in Table 3.

3.3.2. Accuracy

To assess the method's accuracy, the standard addition method was employed at three different concentration levels. Triplicate injections were performed at each level, and the percentage recoveries were subsequently calculated. The mean percent recoveries obtained were 100.01% and 99.55% for Tipiracil and Trifluridine, respectively. A summary of the accuracy data is presented in Table 4

3.3.3. Precision:

The method's precision was evaluated by examining both system precision and method precision.Six replicate injections of the same homogeneous standard solution were analyzedforSystem Precision. Peak areas for each injection were determined, and the average area, standard deviation, and % RSD were calculated for both Tipiracil and Trifluridine. The results, presented in Table 5, revealed %RSD values of 0.15% and 0.20% for the retention time of Tipiracil and Trifluridine, respectively. Additionally, the %RSD values for peak area were 0.20% and 0.05% for Tipiracil and Trifluridine, respectively.

Six replicate injections of the test solution were analyzedfor Method Precision. Similar to the system precision assessment, peak areas were determined for each injection, and the average area, standard deviation, and % RSD were calculated for both drugs. The results, presented in Table 6, indicated a %RSD value of 0.02% for both the retention time and peak area of both Tipiracil and Trifluridine.

3.3.4. Robustness:

The method's robustness was assessed by deliberately introducing variations in flow rate, column oven temperature, and mobile phase ratio. Following each modification, the system suitability parameters were evaluated by injecting standard solutions six times and recording the resulting chromatograms. These parameters were found to be relatively unaffected by the changes, with all values remaining within acceptable limits. Additionally, the percent RSD (% RSD) remained within the established threshold. A summary of the robustness testing results is provided in Table 7.

3.3.5. Specificity

A comparison of the standard and sample chromatograms revealed nearly identical profiles with matching retention times for the analytes. The absence of any peaks at the retention time of the analytes in the placebo and sample chromatograms further confirmed the method's specificity. This indicates that there is no

interference from either the placebo or the sample matrix at the point of detection for the analytes.

Table 1: Results of Assay

S. No	TIPIRACIL		TRIFLURIDINE	
	Standard Area	Sample Area	Standard Area	Sample Area
1	1093054	1090989	2818065	2816897
2	1092896	1091034	2817988	2817039
Mean	1092975	1091012	2818027	2816968
Regression equation	y = 12700x + 5639.9		y = 14883x - 35183	
% Assay	101.1 %		101.25	

Table 2: Systemsuitability parameters forTrifluridine and Tipiracil

SAMPLE	Rt	Peak Area	USP plate count	USP Tailing
Tipiracil	2.135	1159123	4574	1.21
Trifluridine	4.154	2944187	4162	1.16

Table 3: Linearity data for Tipiracil and Trifluridine

% Level	Tipiracil		Trifluridine	
	Concentration (µg/ ml)	Peak Area	Concentration (µg/ ml)	Peak Area
25%	22.5	284422	50	711503
50%	45	576734	100	1446573
75%	67.5	869635	150	2200347
100%	90	1158026	200	2943219
125%	112.5	1432105	250	3685676
150%	135	1713823	300	4428409

Table 4: Accuracy data of Trifluridine and Tipiracil

% Level	Trifluridine			Tipiracil		
	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery
50%	100	101.02	101.02	45	44.21	98.2444
	100	100.05	100.05	45	44.84	99.6444
	100	100.15	100.15	45	45.36	100.8
100 %	200	200.02	100.01	90	88.68	98.5333
	200	200.26	100.13	90	89.14	99.0444
	200	198.62	99.31	90	91.02	101.133
150 %	300	298.54	99.5133	135	133.58	98.9481
	300	299.47	99.8233	135	134.98	99.9852
	300	300.41	100.137	135	134.58	99.6889

Table 5: Method Precision data of Trifluridine and Tipiracil

Injection no.	Tipiracil	Trifluridine
Average Peak Area	1089920.667	2827528
% RSD	0.02	0.02

Table 6: System Precision data of Trifluridine and Tipiracil

Injection no.	Tipiracil	Trifluridine
Average Peak Area	1093497.833	2817036
% RSD	0.03	0.03

Table 7: Robustness data of Trifluridine and Tipiracil

Variation Of Flow rate		Tipiracil	Trifluuridine
		Rt.	Rt.
	0.9ML/MIN	2.161	4.422
	1.1ML/MIN	2.137	3.880
Variation In Buffer Concentration Ratio	60 : 40	2.161	4.422
	70 : 30	2.137	3.880

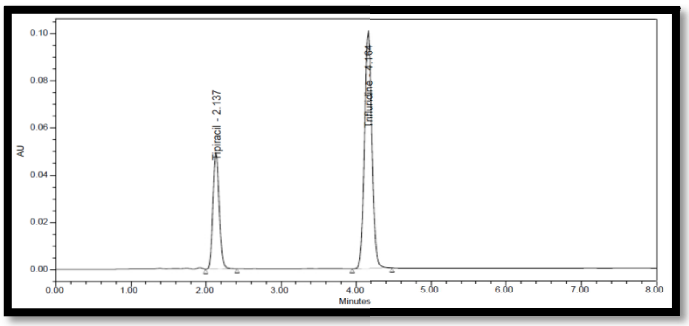

Fig 1: Representative Chromatogram of working standard solution

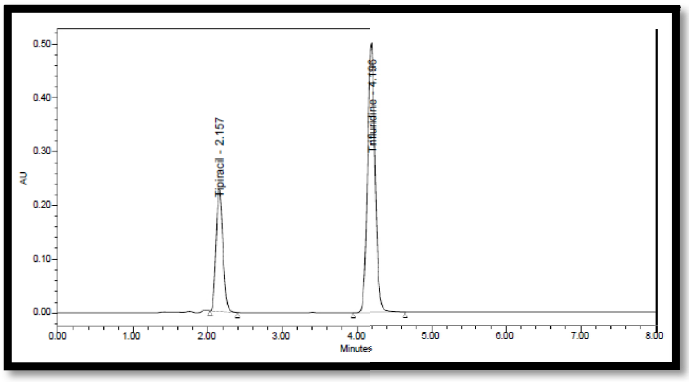

Fig 2: Representative Chromatogram of working sample solution

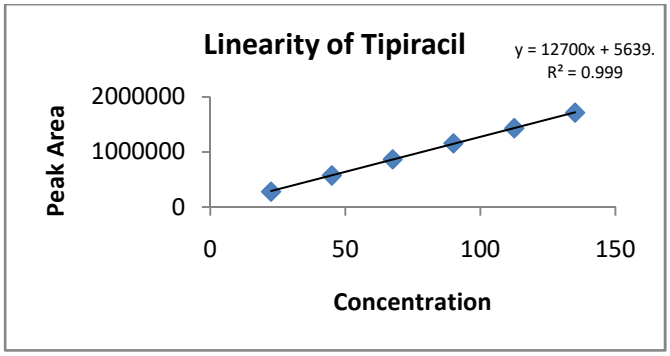

Fig 3: Calibration curve of Tipiracil

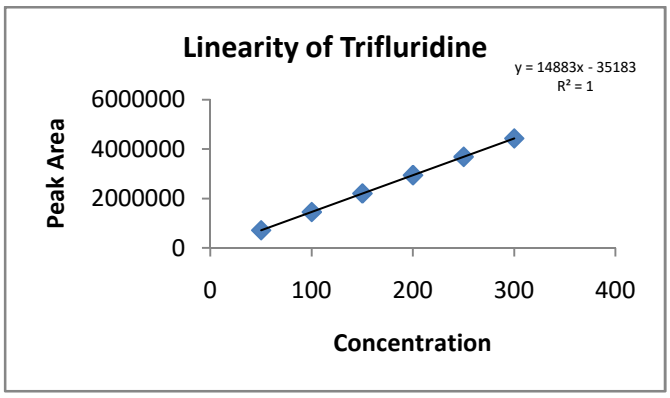

Fig 4: Calibration curve of Trifluridine

4. CONCLUSION

The present work describes the successful development and validation of a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of Tipiracil and Trifluridine in both bulk and tablet dosage forms. The method demonstrated satisfactory performance in terms of accuracy, precision, linearity, and reproducibility.The analysis is performed on a Hypersil C18 column (250mm x 4.6mm, 5µ) with a mobile phase composed of 0.1% ortho phosphoric acid in water adjusted to pH 4 with sodium hydroxide (65%) and acetonitrile (35%). This mobile phase effectively separated Tipiracil and Trifluridine, achieving retention times of 2.1 min and 4.2 min, respectively.

A comprehensive validation process was conducted, encompassing system suitability, accuracy, precision, linearity, and ruggedness. The system suitability parameters consistently met established criteria, confirming the method's suitability for the intended assay of Tipiracil and Trifluridine.

5. REFERENCES:

1. World Health Organization. Cancer. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer. Accessed July 3, 2024.

2. American Cancer Society. What Is Cancer? Available from: https://www.cancer.org/cancer/cancer-basics/what-is-cancer.html. Accessed July 3, 2024.

3. National Cancer Institute. Cancer Stat Facts: Common Cancer Sites. Available from: https://seer.cancer.gov/statfacts/html/common.html. Accessed July 3, 2024.

4. Mayo Clinic. Cancer. Available from: https://www.mayoclinic.org/diseases-conditions/cancer/symptoms-causes/syc-20370588. Accessed July 3, 2024.

5. Cancer Research UK. What Is Cancer? Available from: https://www.cancerresearchuk.org/about-cancer/what-is-cancer.

6. American Cancer Society. Colorectal Cancer: Detailed Guide. Available from: https://www.cancer.org/cancer/colon-rectal-cancer.html. Accessed July 3, 2024.

7. Mayo Clinic. Colon Cancer: Symptoms & Causes. Available from: https://www.mayoclinic.org/diseases-conditions/colon-cancer/symptoms-causes/syc-20353669. Accessed July 3, 2024.

8. National Cancer Institute. Colon Cancer Treatment (PDQ) – Health Professional Version. Available from: https://www.cancer.gov/types/colorectal/hp/colon-treatment-pdq. Accessed July 3, 2024.

9. Cancer Research UK. Colon Cancer Treatment. Available from: https://www.cancerresearchuk.org/about-cancer/colon-cancer/treatment. Accessed July 3, 2024.

10. American Society of Clinical Oncology (ASCO). Colon Cancer: Treatment Options. Available from: https://www.cancer.net/cancer-types/colorectal-cancer/treatment-options.

11. National Center for Biotechnology Information. PubChem Compound Summary for Trifluridine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/6253

12. Hazra BB, Vageesh NM, Kistayya C and Shahanaz B: Analytical method development and validation for simultaneous estimation of trifluridine and tipiracil in pure and pharmaceutical dosage form. Innovat International Journal of Medical and Pharmaceutical Sciences 2018; 3: 55-58.

13. Mastanamma SK, Nagaraju K, Reehana SK and Radhakrishnaveni V: Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of trifluridine and tipiracilin bulk and their combined dosage form. International Journal of ChemTech Research 2019; 12: 117-26.

14. Jogi K, Rao MB and Raju RR: An effective and sensitive stability indicating chromatographic approach based on RP-

HPLC for trifluridine and tipiracil in bulk and pharmaceutical dosage form. International journal of research in pharmacy and chemistry 2017; 7: 63-70.

15. Eluru A and Babub KS: A new selective separation method development and validation of trifluridine and tipiracil and its degradents were characterized by LCMS/MS/QTOF. J Pharm Sci and Res 2020; 12: 199-205.

16. Goday S, Abdulrahaman SK and Prameelarani A: Development and validation of stability indicating RPHPLC method for the simultaneous estimation of combination drugs trifluridine and tipiracil in bulk and pharmaceutical dosage forms. International Journal of Research in Applied, Natural and Social Sciences 2017; 5: 93-104.

17. Prathap B, Haribaskar V, Kumar B, Raghu PS and Reddy SBK: Method development and validation for the simultaneous estimation of trifluridine and tipiracil in tablet dosage form by RP-HPLC method. J Global Trends Pharm Sci 2017; 8: 4514-21

18. U.S. National Library of Medicine. Trifluridine and Tipiracil. Available from: https://medlineplus.gov/druginfo/meds/a615037.html.

19. National Center for Biotechnology Information. PubChem Compound Summary for Tipiracil. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/3058776.

20. U.S. National Library of Medicine. Trifluridine and Tipiracil. Available from: https://medlineplus.gov/druginfo/meds/a615037.html.

21. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva: ICH; 2005. Available from: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf. Accessed July 3, 2024.

22. United States Pharmacopeia and National Formulary (USP-NF). General Chapter <1225> Validation of Compendial Procedures. Rockville, MD: USP; 2020. Available from: https://www.uspnf.com/uspnf/pub/index?usp=43&nf=38&s=1&officialOn=August%201,%202020.

23. European Medicines Agency. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. London: European Medicines Agency; 1995. Available from:

24. U.S. Food and Drug Administration (FDA). Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics. Rockville, MD: FDA; 2015. Available from: https://www.fda.gov/media/71711/download.

25. European Pharmacopoeia Commission. European Pharmacopoeia 10th Edition. Strasbourg: Council of Europe; 2020. Available from: https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-10th-edition

How to cite this article:

Jutta Swathi *, RP- HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TIPIRACIL AND TRIFLURIDINE IN TABLET DOSAGE FORM J Pharma Res, 2025; 14(06): 27-32.
DOI: https://doi.org/10.5281/zenodo.17555981

Conflict of interest: The authors have declared that no conflict of interest exists.
Source of support: Nil

