



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Research Paper

Development and Validation of a Reversed-Phase HPLC Method for Simultaneous Quantification of Trifluridine and Tipiracil in a Combined Tablet Dosage Form

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ARTICLE INFO

Published: 09 Oct 2026

Keywords:

Trifluridine; Tipiracil; RP-HPLC; Method development; Analytical validation; Simultaneous estimation; Tablet assay

DOI:

10.5281/zenodo.23255967

ABSTRACT

Background: Trifluridine and tipiracil are combined in a fixed-dose oral tablet and require a selective analytical procedure for simultaneous quantitative determination. Objective: To develop and validate a rapid isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) procedure for simultaneous assay of trifluridine and tipiracil in bulk-related analytical solutions and the marketed combined tablet dosage form. Methods: Chromatographic development was performed by screening aqueous/organic mobile-phase systems, buffer pH, organic modifier proportion, flow rate, column temperature and injection volume. The optimized procedure used an octadecylsilane C18 column (5 μ m, 4.6 $\times$ 150 mm), 0.02 M potassium dihydrogen orthophosphate buffer adjusted to pH 3.75 and acetonitrile (65:35, v/v), isocratic elution at 1.0 mL/min, 20 μ L injection volume, 25 $\pm$ 2 $^{\circ}$ C column temperature and detection at 267 nm. The total run time was 6 min. Validation included system suitability, specificity, linearity, accuracy, precision, sensitivity and robustness. Results: Trifluridine and tipiracil eluted at approximately 2.90 and 4.21 min, respectively, with a resolution of 7.13. Calibration ranges were 10.00–30.00 μ g/mL for trifluridine and 4.095–12.290 μ g/mL for tipiracil, with r^2 values of 0.999943 and 0.999989, respectively. Mean recoveries were 99.83% and 99.49%. System precision (%RSD, n=6) was 0.054% and 0.062%, while independent tablet-preparation precision (%RSD, n=3) was 0.16% and 0.17%. Experimentally verified signal-to-noise LOD values were 0.70 and 0.32 μ g/mL and LOQ values were 0.233 and 0.175 μ g/mL for trifluridine and tipiracil, respectively. Assay of three independent tablet preparations gave 99.85% and 99.45% of label claim. Conclusion: The developed RP-HPLC procedure provides rapid chromatographic separation and reproducible simultaneous quantification of the two

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



analytes in the investigated tablet matrix. The data support its use for assay-oriented pharmaceutical analysis of the studied formulation

INTRODUCTION

Trifluridine is a nucleoside metabolic inhibitor and tipiracil is a thymidine phosphorylase inhibitor. The two active components are administered together as a fixed-dose oral combination, in which tipiracil limits the rapid degradation of trifluridine and thereby increases systemic exposure to trifluridine. The marketed 20 mg/8.19 mg strength is documented in current product information.^[1,2]

Analytical procedures for simultaneous determination of the two components have been reported using different reversed-phase chromatographic systems. Published methods demonstrate that the pair can be separated by isocratic RP-HPLC, but reported conditions differ in stationary phase, buffer chemistry, organic modifier, temperature and detection wavelength.^[3-5] These differences make method-specific development and validation important when a procedure is intended for a particular formulation and laboratory configuration.

The present work was focused exclusively on the chromatographic component of the submitted study. The objective was to establish a short, reproducible RP-HPLC procedure capable of physically separating trifluridine and tipiracil and quantifying both analytes at concentrations corresponding to the marketed tablet. The analytical procedure was developed using a C18 stationary phase and optimized through systematic screening of mobile-phase composition, pH, flow rate, column temperature and injection volume.

The final procedure was assessed for system suitability, specificity, linearity and range, detection and quantitation capability, accuracy, precision, robustness and assay of the marketed formulation. Current ICH terminology is used

where applicable. ICH Q2(R2) states that analytical procedure validation is intended to demonstrate fitness for the intended purpose, while ICH Q14 provides the framework for science- and risk-based analytical procedure development.^[6,7] The marketed 20 mg/8.19 mg strength used in the study is consistent with current product information for trifluridine/tipiracil tablets.^[1,2]

2. AIM AND OBJECTIVE

The aim of the chromatographic part of the study was to develop and validate a simple, rapid and selective isocratic RP-HPLC procedure for simultaneous quantification of trifluridine and tipiracil in the combined tablet dosage form.

Specific objectives were:

- (i) To select an appropriate reversed-phase stationary phase
- (ii) To optimize the aqueous buffer, pH and organic modifier
- (iii) To optimize flow rate, column temperature and injection volume
- (iv) To establish a common detection wavelength
- (v) To prepare calibration standards that reproduce the drug ratio present in the marketed tablet.
- (vi) To establish chromatographic system suitability
- (vii) To evaluate specificity, linearity and range, accuracy, precision, detection and quantitation capability and robustness.
- (viii) To determine the assay of trifluridine and tipiracil in the marketed formulation.

The study was designed around the nominal working concentrations reached after sample preparation: 20.00 µg/mL for trifluridine and 8.19 µg/mL for tipiracil. Calibration levels were therefore prepared at 50%, 75%, 100%, 125% and 150% of these working concentrations. This design avoided extrapolation of the routine assay result beyond the calibration range.



3. LITERATURE AND METHOD-DEVELOPMENT RATIONALE

Previously published RP-HPLC procedures for trifluridine and tipiracil demonstrate the feasibility of simultaneous chromatographic estimation. Valli Kumari and Vardhani reported an isocratic procedure using acetonitrile and dipotassium hydrogen phosphate, with a C18 column and UV detection. [3] Reddy and co-workers later reported another RP-HPLC method using a C18 column with methanol and triethylamine buffer. [4] These published procedures differ from the optimized procedure developed in the present study.

During the present development work, the aqueous phase pH was found to have a strong effect on the peak shape of tipiracil. Screening showed that acetonitrile:water (50:50, v/v) produced insufficient retention, whereas acetonitrile:water (30:70, v/v) increased retention but produced marked tipiracil tailing. Methanol:water (50:50, v/v) gave broad, incompletely resolved peaks. Phosphate buffer at pH 6.8 with acetonitrile produced a broad/fronting tipiracil peak, while pH 4.5 improved peak shape but caused trifluridine to elute close to the void volume.

At pH 3.75, a 70:30 buffer:acetonitrile mixture gave excellent separation but unnecessarily extended the run. Reducing the aqueous phase slightly to 65% and using 35% acetonitrile retained good resolution while shortening the analysis. The final condition was therefore selected as 0.02 M potassium dihydrogen orthophosphate buffer, pH 3.75, and acetonitrile at 65:35 (v/v).

The development observations in the source study also showed that lower pH reduced tipiracil tailing. The selected pH was retained for its observed chromatographic performance rather than because of a claim of universal applicability to all formulations.

4. MATERIALS AND INSTRUMENTATION

Trifluridine reference standard was reported as procured from Carbanio, Lab Chemicals, Hyderabad. Tipiracil hydrochloride API was reported as purchased from Sigma-Aldrich Pvt. Ltd. The investigated marketed formulation was TIPANAT 20, containing 20 mg trifluridine and 8.19 mg tipiracil per tablet as stated in the submitted study.

Acetonitrile was obtained from Loba Chemie Pvt. Ltd. Methanol, acetone and ethanol were listed from Rankem Pvt. Ltd. and Sisco Research Laboratories Pvt. Ltd., respectively. HPLC-grade water was listed from Thermo Fisher Scientific India Pvt. Ltd.; purified water was obtained using the laboratory water-purification system.

Chromatographic analysis was performed using a Waters HPLC system equipped with a Waters 515 HPLC pump and Waters 2489 UV/Visible detector, with Waters Empower 3 software. The column used for the final procedure was an octadecylsilane (C18) column, 5 μ m particle size, 4.6 $\times$ 150 mm. A Rheodyne injector with a 20 μ L injection volume was used.

Analytical glassware, a calibrated pH meter, microbalance, sonicator and membrane-filtration equipment were used for solution preparation. All solvents and reagents used for chromatographic analysis were treated as appropriate for their intended analytical use.

The source materials identify the final HPLC system as a Waters platform with Empower 3 software, a Waters 515 pump and Waters 2489 UV/Visible detector, using a 5 μ m C18, 4.6 $\times$ 150 mm column and a 20 μ L Rheodyne injector.

5. CHROMATOGRAPHIC METHOD DEVELOPMENT

Method development began with selection of the organic modifier and aqueous component. Methanol was initially considered because it



provided acceptable solution handling and chromatographic behavior. Acetonitrile was subsequently screened in combination with water and phosphate buffer. The screening results showed that the aqueous pH was critical for obtaining an acceptable tipiracil peak shape while retaining trifluridine sufficiently on the C18 stationary phase.

With acetonitrile:water (50:50, v/v), both analytes eluted at or near the void region and adequate separation was not obtained. Increasing the aqueous fraction to 70% improved retention but resulted in severe tipiracil tailing. Methanol:water (50:50, v/v) produced broad and unsymmetrical peaks and incomplete separation.

Phosphate buffer at pH 6.8 with acetonitrile (65:35, v/v) produced a broad and fronting tipiracil peak. At pH 4.5, both peaks became sharper and resolution increased, but trifluridine eluted too close to the void volume. At pH 3.75, a 70:30 buffer:acetonitrile mixture provided high resolution but increased the analysis time. The 65:35 composition at pH 3.75 gave the required

balance between retention, peak shape, resolution and total run time.

Flow rate was subsequently examined. A flow rate of 0.8 mL/min extended the run beyond 7 min without a useful improvement in resolution, whereas 1.2 mL/min reduced resolution and increased column back-pressure. A flow rate of 1.0 mL/min was therefore selected. The column was thermostatted at 25 °C, and 20 µL was selected as the injection volume.

Detection was performed at 267 nm, the common wavelength used for simultaneous monitoring in the submitted procedure. The optimized chromatogram showed two well-resolved peaks within a 6-min total run.

6. FINAL CHROMATOGRAPHIC CONDITIONS

Table 1 summarizes the final chromatographic conditions adopted from the experimental study. The procedure uses isocratic elution and mobile phase as the diluent.

Table 1. Final chromatographic conditions adopted for simultaneous RP-HPLC estimation.

Parameter	Final condition
Stationary phase	Octadecylsilane (C18), 5 µm, 4.6 × 150 mm
Mobile phase	0.02 M potassium dihydrogen orthophosphate buffer, pH 3.75 ± 0.05 : acetonitrile (65:35, v/v)
Elution mode	Isocratic
Flow rate	1.0 mL/min
Injection volume	20 µL
Column temperature	25 ± 2 °C
Detector	UV-visible / photodiode-array detector
Detection wavelength	267 nm
Run time	6 min
Diluent	Mobile phase
Retention time	Trifluridine ~2.90 min; tipiracil ~4.21 min

7. MOBILE-PHASE PREPARATION AND STANDARD SOLUTIONS

Potassium dihydrogen orthophosphate (2.72 g) was dissolved in 1000 mL HPLC-grade water to prepare 0.02 M buffer. The pH was adjusted to 3.75 using dilute orthophosphoric acid with a calibrated combination electrode. The buffer was filtered through a 0.45 μm nylon membrane. A 650 mL portion of buffer was mixed with 350 mL acetonitrile to obtain the final 65:35 (v/v) mobile phase. The mixture was degassed by sonication for 15 min and was used as both mobile phase and diluent. The source procedure states that the mobile phase was prepared fresh daily.

Trifluridine stock solution: 25 mg of trifluridine was accurately weighed into a 25 mL volumetric flask, dissolved in mobile phase and diluted to volume to obtain 1000 $\mu\text{g/mL}$. Tipiracil stock solution: 28.77 mg of tipiracil hydrochloride was weighed into a 25 mL volumetric flask and diluted with mobile phase to obtain a solution corresponding to 1000 $\mu\text{g/mL}$ of tipiracil on the free-base basis used for the analytical calculations. Combined intermediate standard: 10.0 mL of the trifluridine stock and 4.095 mL of the tipiracil stock were transferred to a 100 mL volumetric flask and diluted to volume with mobile phase. The resulting intermediate contained 100 $\mu\text{g/mL}$ trifluridine and 40.95 $\mu\text{g/mL}$ tipiracil, maintaining the 2.442:1 analytical mass ratio used for the tablet.

8. CALIBRATION AND SAMPLE PREPARATION

Five calibration standards were prepared by transferring 10.0, 15.0, 20.0, 25.0 and 30.0 mL of the combined intermediate standard into separate 100 mL volumetric flasks and making each to volume with mobile phase. The resulting concentrations were 10.0000, 15.0000, 20.0000, 25.0000 and 30.0000 $\mu\text{g/mL}$ for trifluridine and 4.0950, 6.1400, 8.1900, 10.2375 and 12.2900 $\mu\text{g/mL}$ for tipiracil. These corresponded to 50%, 75%, 100%, 125% and 150% of the working concentration. CS3 (20.00 $\mu\text{g/mL}$ trifluridine and 8.19 $\mu\text{g/mL}$ tipiracil) was used as the working standard for system suitability, repeatability and comparison with tablet sample preparations.

For formulation analysis, 20 tablets were weighed individually and the average tablet weight was calculated. The tablets were powdered in a glass mortar. Powder equivalent to 20 mg trifluridine, corresponding to 8.19 mg tipiracil on the analytical basis used in the study, was transferred to a 100 mL volumetric flask. Approximately 60 mL mobile phase was added and the flask was sonicated for 20 min with intermittent swirling. The solution was allowed to reach room temperature and diluted to volume with mobile phase. The solution was filtered through a 0.45 μm nylon membrane, discarding the first 5 mL of filtrate. A 5.0 mL aliquot was transferred to a 50 mL volumetric flask and diluted to volume with mobile phase, producing nominal concentrations of 20 $\mu\text{g/mL}$ trifluridine and 8.19 $\mu\text{g/mL}$ tipiracil. Three independent sample preparations were analyzed.

Table 2. Calibration standards for the RP-HPLC procedure.

Level	TFL ($\mu\text{g/mL}$)	TPR ($\mu\text{g/mL}$)	% Working
CS1	10.0000	4.0950	50
CS2	15.0000	6.1400	75
CS3	20.0000	8.1900	100
CS4	25.0000	10.2375	125
CS5	30.0000	12.2900	150



9. SYSTEM SUITABILITY AND SPECIFICITY

Five replicate injections of the working standard were used for system suitability before the analytical sequence. The final chromatographic system produced retention times of approximately 2.90 min for trifluridine and 4.21 min for tipiracil. The reported theoretical plate counts were approximately 5894 and 6697, tailing factors were 1.32 and 1.30, and the resolution was 7.13.

The source study used acceptance criteria of theoretical plates >2000 , tailing factor ≤ 2.0 and resolution ≥ 2.0 . The observed values met these criteria with substantial margin. Peak-area repeatability was 0.054% RSD for trifluridine and

0.062% RSD for tipiracil from six replicate injections.

Specificity was evaluated by injecting the diluent, placebo extract, working standard and tablet sample under identical chromatographic conditions. Chromatograms were examined for responses at the analyte retention times and for agreement between standard and sample retention times. The submitted study reports absence of relevant interference at the analyte peaks.

Because the reported specificity experiment involved blank, placebo, standard and sample solutions, but did not document forced-degradation experiments, the present manuscript does not describe the method as stability-indicating.

Table 3. Selected chromatographic performance characteristics.

Parameter	Trifluridine	Tipiracil
Retention time (min)	2.90	4.21
Theoretical plates	5894	6697
Tailing factor	1.32	1.30
Resolution	—	7.13

10. LINEARITY AND RANGE

Linearity was evaluated at five concentration levels covering 50% to 150% of the nominal working concentration. Each calibration level was injected in triplicate. The calibration series were prepared from a common intermediate standard so that the trifluridine:tipiracil ratio remained constant at every level.

Trifluridine showed a linear response from 10.00 to 30.00 $\mu\text{g/mL}$. Mean peak areas at the five levels were 1,040,797; 1,562,069; 2,091,201; 2,608,116; and 3,115,318, respectively. The slope was 103,901.78 area units per $\mu\text{g/mL}$ and the intercept was +5,464.60. The correlation coefficient was 0.999971 and r^2 was 0.999943.

Tipiracil showed a linear response from 4.095 to 12.290 $\mu\text{g/mL}$. Mean peak areas were 1,253,258;

1,883,077; 2,514,133; 3,145,682; and 3,767,369, respectively. The slope was 307,056.35 area units per $\mu\text{g/mL}$ and the intercept was -2,241.23. The correlation coefficient was 0.999995 and r^2 was 0.999989.

The working concentrations of 20.00 $\mu\text{g/mL}$ trifluridine and 8.19 $\mu\text{g/mL}$ tipiracil were centered within the respective calibration ranges. The source data therefore support direct interpolation of the routine assay concentrations.

Calibration-response data



Level	TFL concentration	TFL mean area	TPR concentration	TPR mean area
CS1	10.0000	1,040,797	4.0950	1,253,258
CS2	15.0000	1,562,069	6.1400	1,883,077
CS3	20.0000	2,091,201	8.1900	2,514,133
CS4	25.0000	2,608,116	10.2375	3,145,682
CS5	30.0000	3,115,318	12.2900	3,767,369

11. LIMIT OF DETECTION AND LIMIT OF QUANTITATION

Detection and quantitation limits were investigated experimentally using the signal-to-noise approach. Progressive dilutions of the working standard were injected until the analyte signal was approximately three times and ten times the surrounding baseline noise, respectively.

For trifluridine, the experimentally observed LOD was 0.70 µg/mL at a retention time of 2.887 min with a signal-to-noise ratio of 3.71. The LOQ was 0.233 µg/mL at 2.878 min with a signal-to-noise ratio of 10.54. For tipiracil, the LOD was 0.32 µg/mL at 4.201 min with a signal-to-noise ratio of 3.60, and the LOQ was 0.175 µg/mL at 4.201 min with a signal-to-noise ratio of 10.02.

The study also calculated alternative estimates using $3.3\sigma/S$ and $10\sigma/S$. These produced LOD/LOQ values of 0.31/0.93 µg/mL for trifluridine and 0.054/0.165 µg/mL for tipiracil. For the assay-oriented procedure, the experimentally demonstrated signal-to-noise values are reported as the primary LOD/LOQ values.

An internal consistency check in the source data shows that the signal-to-noise concentrations are 0.70 and 0.32 µg/mL, not 0.070 and 0.032 µg/mL. The latter values appearing in one consolidated table are inconsistent with the corresponding concentrations and S/N values and have therefore been corrected in this manuscript.

Table 4. Detection and quantitation limits. The experimentally demonstrated signal-to-noise values are used as the primary reported limits.

Analyte	LOD by S/N (µg/mL)	LOQ by S/N (µg/mL)	Alternative calculated LOD	Alternative calculated LOQ
Trifluridine	0.70	0.233	0.31	0.93
Tipiracil	0.32	0.175	0.054	0.165

12. ACCURACY AND PRECISION

Accuracy was assessed by standard addition at 50%, 100% and 150% of the working concentration, using three independent preparations at each level and maintaining the tablet drug ratio.

Trifluridine recoveries at 50%, 100% and 150% were 99.87%, 99.83% and 99.80%, respectively. The overall mean recovery was 99.83% with an

overall %RSD of 0.21%. Individual determinations ranged from 99.60% to 100.20%. Tipiracil recoveries at the corresponding levels were 99.41%, 99.43% and 99.62%, with an overall mean recovery of 99.49% and overall %RSD of 0.23%. Individual determinations ranged from 99.02% to 99.76%.

System precision was established from six replicate injections of CS3. The %RSD values were 0.054% for trifluridine and 0.062% for tipiracil. Method precision based on recovery



preparations was 0.21% and 0.23%, respectively. Independent tablet-preparation precision based on three sample preparations gave %RSD values of 0.16% for trifluridine and 0.17% for tipiracil.

The submitted experimental record explicitly notes that intermediate precision across different days, analysts and preferably a second instrument/column was not established. Accordingly, this manuscript reports repeatability and method precision as demonstrated, without claiming a full intermediate-precision study.

13. ROBUSTNESS AND ASSAY OF MARKETED TABLETS

Robustness was evaluated by deliberate variation of chromatographic conditions. The source study varied operational parameters including flow rate, organic modifier proportion, aqueous-phase pH, column temperature and detection wavelength. Across the reported robustness conditions, tailing factors remained between 1.22 and 1.33,

theoretical plate counts remained between 4951 and 7076, and resolution remained between 6.62 and 7.23. The predefined criteria were tailing ≤ 2.0 , plate count ≥ 2000 and resolution ≥ 2.0 .

Assay was performed on three independent preparations of the marketed TIPANAT 20 tablet. For trifluridine, the amounts found were 20.006, 19.958 and 19.944 mg/tablet, corresponding to 100.03%, 99.79% and 99.72% of label claim. The mean was 19.969 mg/tablet, equivalent to 99.85% label claim, with %RSD 0.16%.

For tipiracil, the amounts found were 8.131, 8.146 and 8.158 mg/tablet, corresponding to 99.28%, 99.46% and 99.61% of label claim. The mean was 8.145 mg/tablet, equivalent to 99.45% label claim, with %RSD 0.17%.

These results demonstrate reproducible assay performance for the investigated tablet preparation. The assay results should be interpreted as results for the specific formulation and laboratory procedure studied.

Table 5. Assay of the marketed TIPANAT 20 tablet.

Analyte	Prep 1 %LC	Prep 2 %LC	Prep 3 %LC	Mean %LC	%RSD
Trifluridine	100.03	99.79	99.72	99.85	0.16
Tipiracil	99.28	99.46	99.61	99.45	0.17

DISCUSSION

The optimized RP-HPLC procedure achieved physical separation of trifluridine and tipiracil within a short 6-min analytical run. The final retention times of approximately 2.90 and 4.21 min, respectively, were sufficiently separated to provide a reported resolution of 7.13. Theoretical plate counts and tailing factors were within the study acceptance criteria, supporting adequate column efficiency and peak symmetry for quantitative work.

The calibration design is a notable feature of the procedure. Rather than constructing independent calibration series with unrelated concentration ratios, a combined intermediate was prepared at 100 $\mu\text{g/mL}$ trifluridine and 40.95 $\mu\text{g/mL}$ tipiracil. Every calibration level therefore preserved the 2.442:1 analytical ratio of the marketed tablet. This placed the routine assay concentrations near the center of the calibration ranges.

The accuracy and precision results were consistent across validation experiments. Mean recoveries were close to 100%, while system, method and



independent sample-preparation %RSD values were substantially below the stated 2.0% precision criterion. Robustness testing likewise showed that the key chromatographic performance characteristics remained well separated from their predefined acceptance limits.

The method should, however, be described precisely according to the evidence generated. The submitted data establish assay-oriented specificity through blank/placebo/standard/sample comparison, but do not establish stability-

indicating capability through documented forced-degradation studies. Similarly, intermediate precision was not established in the presented dataset. Current ICH Q2(R2) emphasizes demonstrating fitness for the intended purpose and evaluating appropriate performance characteristics based on intended use. [6] Thus, the present data support the stated assay application while identifying the boundaries of the validation evidence.

Table 6. Consolidated assay-oriented validation results.

Validation parameter	Trifluridine	Tipiracil
System precision %RSD (n=6)	0.054	0.062
Method precision %RSD	0.21	0.23
Independent assay precision %RSD (n=3)	0.16	0.17
Mean recovery	99.83%	99.49%
Experimental LOD	0.70 µg/mL	0.32 µg/mL
Experimental LOQ	0.233 µg/mL	0.175 µg/mL
Assay	99.85%	99.45%

CONCLUSION AND REFERENCES

Conclusion: A rapid isocratic RP-HPLC procedure was developed for simultaneous quantification of trifluridine and tipiracil in the investigated combined tablet dosage form. The procedure uses a C18 column, 0.02 M potassium dihydrogen orthophosphate buffer at pH 3.75, acetonitrile (65:35, v/v), 1.0 mL/min flow rate, 20 µL injection volume, 25 ± 2 °C column temperature and detection at 267 nm. The analytes eluted at approximately 2.90 and 4.21 min with a reported resolution of 7.13. Linearity, accuracy, precision, sensitivity and robustness data support assay application to the studied formulation. The manuscript deliberately avoids calling the procedure stability-indicating because forced-degradation data were not included.

Conflict of Interest

The authors declare that there are no conflicts of interest related to the preparation, conduct, analysis, or publication of this study.

Funding

No specific external funding was received for this study.

Author Contributions

All authors contributed to the conception and design of the analytical study. Experimental work, data collection, analysis, interpretation of results, and manuscript preparation were carried out collaboratively. All authors reviewed and approved the final manuscript.



Data Availability Statement

The data supporting the findings of this study are contained within the manuscript and the associated experimental records. Additional information may be made available from the corresponding author upon reasonable request, subject to applicable institutional requirements.

Ethical Approval

Ethical approval was not required because the study involved analytical method development and validation of pharmaceutical drug substances and a marketed tablet formulation and did not involve human participants or experimental animals.

Acknowledgements

The authors acknowledge the Department of Pharmaceutical Analysis and the supporting laboratory personnel for providing the facilities and technical support required for completion of the analytical studies.

Consent for Publication

Not applicable.

REFERENCES

1. DailyMed. LONSURF (trifluridine and tipiracil) tablets, prescribing information. Taiho Pharmaceutical Co., Ltd.; revised 2023.
2. Electronic Medicines Compendium. Trifluridine/Tipiracil Servier 20 mg/8.19 mg film-coated tablets: Summary of Product Characteristics. Updated 2026.
3. Valli Kumari RV, Vardhani G. RP HPLC method development and validation for simultaneous determination of trifluridine and tipiracil in pure and tablet dosage form. *Int J Pharm Anal Res.* 2019;8(3):377-386. doi:10.61096/ijpar.v8.iss3.2019.377-386.

4. Reddy MP, Reddy RR, RamyaSri S. Reverse phase-high performance liquid chromatography method for the simultaneous estimation of trifluridine and tipiracil in pure form and tablet dosage form. *Int J Pharmacol Clin Res.* 2022;6(4).
5. Sahu R, et al. Development and validation of a RP-HPLC-PDA method for simultaneous determination of trifluridine and tipiracil in pure and pharmaceutical dosage form. *Int J Novel Trends Pharm Sci.* 2017;7(5):145-151.
6. International Council for Harmonisation. ICH Q2(R2): Validation of Analytical Procedures. Final version adopted 1 November 2023; current corrected version.
7. International Council for Harmonisation. ICH Q14: Analytical Procedure Development. Final version adopted 1 November 2023.
8. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken: Wiley; 2010.
9. Dong MW. Modern HPLC for Practicing Scientists. Hoboken: Wiley-Interscience; 2006.
10. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. Hoboken: Wiley-Interscience; 2007.

HOW TO CITE: Poovarasani. S, Kanmani. R, Development and Validation of a Reversed-Phase HPLC Method for Simultaneous Quantification of Trifluridine and Tipiracil in a Combined Tablet Dosage Form, *Int. J. of Pharm. Sci.*, 2026, Vol 4, Issue 10, 1338-1347, <https://doi.org/10.5281/zenodo.23255967>

