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Development And Validation Of A Stability-Indicating Uplc Method For The Simultaneous Estimation Of Tipiracil And Trifluridine In Bulk And Pharmaceutical Dosage Forms

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ABSTRACT

A rapid, sensitive and stability-indicating UPLC method was developed and validated for the simultaneous estimation of Trifluridine and Tipiracil in bulk drug substances and pharmaceutical dosage forms. Chromatographic separation was achieved using an ACQUITY BEH C18 column with a mobile phase comprising 0.1% orthophosphoric acid and acetonitrile (50:50, v/v). The method was validated according to ICH Q2(R2) guidelines and demonstrated excellent specificity, linearity, precision, accuracy, robustness and sensitivity. Forced degradation studies confirmed its stability-indicating capability. The developed method is suitable for routine quality control, assay determination and stability analysis of pharmaceutical formulations containing Trifluridine and Tipiracil.

INTRODUCTION

Gastric and colorectal cancers remain major causes of cancer-related morbidity and mortality worldwide, with advanced disease frequently associated with poor prognosis and limited therapeutic options after failure of standard treatment. The management of metastatic gastrointestinal malignancies has progressively evolved through the introduction of targeted agents and oral chemotherapeutic combinations; however, patients who progress after multiple treatment lines continue to represent a substantial therapeutic challenge. Consequently, the development of effective fluoropyrimidine-based strategies remains an important component of treatment for advanced gastrointestinal cancers (1,2).

Trifluridine/tipiracil (FTD/TPI; TAS-102)

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is an orally administered antineoplastic combination developed to overcome some limitations associated with conventional fluoropyrimidine therapy. Trifluridine is a thymidine-based nucleoside analogue that becomes incorporated into DNA following intracellular phosphorylation, resulting in DNA dysfunction and inhibition of tumour cell proliferation. Its clinical utility is enhanced by tipiracil, a potent inhibitor of thymidine phosphorylase that prevents the rapid degradation of trifluridine and thereby increases its systemic exposure. The complementary actions of the two components provide sustained antitumour activity and form the pharmacological basis of the TAS-102 formulation (6–9).

Clinical and regulatory evaluations have established trifluridine/tipiracil as an important therapeutic option for patients with previously treated advanced or metastatic gastrointestinal malignancies. The combination has demonstrated clinical benefit in heavily pretreated patients with metastatic colorectal cancer and has subsequently been incorporated into treatment strategies for advanced gastric and gastroesophageal cancers. Its established clinical application and oral administration have increased the importance of reliable pharmaceutical quality-control procedures capable of accurately determining both active components in finished dosage forms (1,3–7).

The simultaneous quantitative determination of trifluridine and tipiracil presents an analytical challenge because both compounds possess distinct physicochemical and chromatographic characteristics. A suitable analytical procedure must therefore provide adequate selectivity and resolution while maintaining accuracy and precision in the presence of formulation excipients and potential degradation products. Stability-indicating methods are particularly important because degradation may occur during manufacturing, storage, transportation, and exposure to environmental stress. Forced degradation studies provide information regarding degradation behaviour and help demonstrate that an analytical procedure can distinguish the intact drug substances from their degradation products (20–22).

Several analytical procedures have been reported for the simultaneous determination of trifluridine and tipiracil. RP-HPLC methods have been developed for assay determination and stability assessment in bulk drug substances and combined pharmaceutical formulations, with reported methods demonstrating satisfactory linearity, precision, accuracy, and chromatographic separation (12–16). A selective LC–MS/MS/QTOF approach has also been reported for the separation and characterisation of trifluridine, tipiracil, and their degradation products, providing valuable information regarding degradation behaviour (11). Despite these developments, conventional HPLC procedures may involve comparatively longer analysis times and higher mobile-phase consumption. Furthermore, continued advances in pharmaceutical quality control require rapid and efficient analytical procedures capable of supporting high-throughput analysis.

Ultra-performance liquid chromatography (UPLC) provides enhanced chromatographic efficiency through the use of small particle-size stationary phases, enabling improved resolution, shorter analysis times, and lower solvent consumption compared with conventional HPLC. These characteristics make UPLC particularly attractive for routine pharmaceutical analysis and stability studies. A validated UPLC procedure can therefore provide an efficient analytical platform for simultaneous determination of trifluridine and tipiracil while maintaining the selectivity required for stability-indicating applications.

Analytical method validation is essential to establish the reliability and suitability of a procedure for its intended purpose. ICH Q2 guidelines recommend evaluation of parameters including specificity, linearity, accuracy, precision, and robustness, whereas ICH Q1A(R2) provides guidance concerning stability testing and degradation assessment of pharmaceutical products (17,18,21).

Therefore, the present study was undertaken to develop and validate a rapid, accurate, precise, robust, and stability-indicating UPLC method for the simultaneous determination of trifluridine and tipiracil in bulk drug substances and pharmaceutical dosage forms. The developed method was further evaluated through forced degradation studies to establish its ability to resolve the drug substances from their degradation products and to demonstrate its suitability for routine quality control and stability assessment of the combined formulation.

2. MATERIALS & METHODS

Chemicals and Reagents

Certified reference standards of trifluridine and tipiracil were obtained from a recognised pharmaceutical source. A marketed fixed-dose tablet formulation containing trifluridine and tipiracil was procured from the commercial market. HPLC-grade acetonitrile, methanol, and purified water were used for chromatographic analysis. Orthophosphoric acid of analytical-reagent grade was used for preparation of the aqueous mobile-phase component. All reagents and solvents were of suitable analytical or chromatographic grade and were used without further purification.

Instrumentation

Chromatographic analysis was performed using a UPLC system equipped with a binary solvent delivery system, autosampler, column oven, and photodiode-array (PDA) detector. Chromatographic data acquisition and processing were performed using the corresponding chromatography data system. An analytical balance, ultrasonic bath, calibrated volumetric glassware, pH meter, and membrane filtration unit were used during sample preparation and method development. Mobile phases and sample solutions were filtered through 0.22 µm membrane filters before chromatographic analysis.

Chromatographic Conditions

Chromatographic separation of trifluridine and tipiracil was achieved using a **reversed-phase C18 UPLC column** with suitable dimensions and sub-2 µm particle size. The aqueous phase consisted of **0.1% orthophosphoric acid**, which was combined with acetonitrile as the organic modifier. The final mobile-phase composition, flow rate, column temperature, detection wavelength, injection volume, and total run time were optimised during method development to achieve satisfactory resolution, peak symmetry, and analysis time.

The developed chromatographic method was operated under isocratic elution conditions, with detection performed using a PDA detector at the selected analytical wavelength. Prior to sample analysis, the column was equilibrated with the mobile phase until stable baseline and reproducible system-suitability characteristics were obtained.

Preparation of Mobile Phase

The required volume of purified water was measured and acidified with orthophosphoric acid to prepare the aqueous mobile-phase component. The aqueous phase was mixed with acetonitrile in

the optimised proportion and filtered through a 0.22 µm membrane filter. The mobile phase was subsequently degassed by sonication before use. Freshly prepared mobile phase was used for chromatographic analysis.

Preparation of Standard Stock Solutions

Accurately weighed quantities of trifluridine and tipiracil reference standards were separately transferred into suitable volumetric flasks. Each standard was dissolved in the selected diluent with the aid of sonication and diluted to volume to obtain individual stock solutions. Appropriate aliquots of the respective stock solutions were subsequently combined and diluted with the diluent to prepare a mixed standard solution containing both analytes at the desired working concentrations.

Preparation of Sample Solution

Twenty tablets were accurately weighed individually, and the average tablet weight was calculated. The tablets were finely powdered and an accurately weighed portion of the powder equivalent to the required label claim of trifluridine and tipiracil was transferred into a volumetric flask. A suitable quantity of diluent was added, followed by sonication to facilitate complete extraction of the active pharmaceutical ingredients. The solution was allowed to cool, diluted to volume with the diluent, and mixed thoroughly. An aliquot of the resulting solution was filtered through a 0.22 µm membrane filter and further diluted to obtain the final sample concentration suitable for UPLC analysis.

Method Development and Optimisation

Method development was initiated by evaluating different reversed-phase stationary phases, aqueous-organic mobile-phase compositions, flow rates, detection wavelengths, and column temperatures. The chromatographic conditions were systematically optimised based on retention behaviour, peak shape, theoretical plate count, tailing factor, resolution, baseline stability, and total analysis time. The final conditions were selected to provide reproducible separation of trifluridine and tipiracil within a short chromatographic run while maintaining adequate resolution from potential degradation products.

Method Validation

The developed UPLC method was validated in accordance with **ICH Q2(R1)** recommendations (17,18). The analytical characteristics evaluated included system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantitation (LOQ), solution stability, and stability-indicating capability.

System Suitability

System suitability was assessed by injecting the mixed standard solution repeatedly under the optimised chromatographic conditions. Retention time, peak area, theoretical plate count, tailing factor, and chromatographic resolution were evaluated. The percentage relative standard deviation (%RSD) of replicate injections was calculated to verify the reproducibility of the chromatographic system.

Specificity

Specificity was evaluated by separately analysing the diluent, placebo, standard solution, sample solution, and stressed sample solutions. The chromatograms were examined for any interference at the retention times of trifluridine and tipiracil. PDA peak-purity analysis was additionally employed to establish the spectral homogeneity of the analyte peaks.

Linearity

Linearity was assessed by preparing a series of standard solutions covering the selected concentration ranges of trifluridine and tipiracil. Each concentration was analysed under the optimised chromatographic conditions. Calibration curves were constructed by plotting the corresponding peak areas against nominal concentrations, and linear regression analysis was performed to obtain the slope, intercept, and correlation coefficient.

Accuracy

Accuracy was evaluated using the standard-addition technique at three concentration levels corresponding to **50%, 100%, and 150%** of the nominal concentration. Each level was analysed in triplicate. Percentage recovery and %RSD were calculated to establish the closeness of the measured values to the theoretical concentrations.

Precision

Precision was investigated as repeatability and intermediate precision. Repeatability was determined by analysing multiple independently prepared sample solutions under identical conditions on the same day. Intermediate precision was evaluated on different days and, where applicable, by different analysts. The results were expressed as %RSD of the assay values.

Limit of Detection and Limit of Quantitation

LOD and LOQ were determined from the standard deviation of the response and the slope of the calibration curve using the following equations:

$$\mathbf{LOD = 3.3\sigma/S}$$

$$\mathbf{LOQ = 10\sigma/S}$$

where **σ** represents the standard deviation of the response and **S** represents the slope of the calibration curve.

Robustness

Robustness was assessed by introducing deliberate but minor variations in selected chromatographic parameters, including flow rate, mobile-phase composition, detection wavelength, and column temperature. The effect of these changes on retention time, peak shape, resolution, and assay results was evaluated to determine the resilience of the method under normal laboratory variations.

Solution Stability

The stability of standard and sample solutions was investigated by storing the prepared solutions under laboratory conditions for a predetermined period. The solutions were analysed at selected time intervals and the results were compared with those obtained from freshly prepared solutions. Solution stability was considered acceptable when no significant change in assay response or chromatographic performance was observed.

Forced Degradation Studies

Forced degradation studies were performed to establish the stability-indicating capability of the proposed UPLC method in accordance with the principles of ICH Q1A(R2) (21). The drug product was subjected to different stress conditions, including acidic hydrolysis, alkaline hydrolysis, oxidation, thermal degradation, photolytic degradation, and neutral hydrolysis. Appropriate stress conditions and exposure times were selected to produce measurable degradation without complete loss of the parent compounds.

Following stress treatment, acidic and alkaline samples were appropriately neutralised where required, diluted with the selected diluent, filtered, and analysed using the optimised UPLC method. The chromatographic profiles were compared with those of unstressed samples. The ability of the method to separate trifluridine and tipiracil from

their respective degradation products was assessed based on retention behaviour, resolution, peak purity, and overall chromatographic performance. The results were used to confirm that the proposed method was capable of selectively quantifying the intact drug substances in the presence of their degradation products.

3. RESULTS AND DISCUSSION

Method Development

Table 1: Preliminary Trials for UPLC Method Development

Trial No.	Column	Mobile Phase Composition	Flow Rate (mL/min)	Observation	Decision
Trial 1	ACQUITY BEH C18 (100 × 2.1 mm, 1.7 µm)	Water : Acetonitrile (60:40, v/v)	0.3	Trifluridine eluted with broad peak, while Tipiracil showed poor retention and inadequate separation.	Rejected
Trial 2	ACQUITY BEH C18 (100 × 2.1 mm, 1.7 µm)	0.1% Orthophosphoric Acid : Methanol (50:50, v/v)	0.3	Baseline instability with increased back pressure and distorted peak shape.	Rejected
Trial 3	ACQUITY BEH C18 (100 × 2.1 mm, 1.7 µm)	Phosphate Buffer (pH 3.0) : Methanol (45:55, v/v)	0.3	Peak fronting observed for Trifluridine and poor resolution between analytes.	Rejected
Trial 4	ACQUITY BEH C18 (100 × 2.1 mm, 1.7 µm)	0.1% Orthophosphoric Acid : Acetonitrile (40:60, v/v)	0.3	Elution was rapid; however, incomplete separation and slight peak overlap were observed.	Rejected
Trial 5	ACQUITY BEH C18 (100 × 2.1 mm, 1.7 µm)	0.1% Orthophosphoric Acid : Acetonitrile (55:45, v/v)	0.3	Resolution improved, but retention time of Tipiracil was comparatively longer with moderate peak tailing.	Further optimisation required
Trial 6 *(Optimised)*	**ACQUITY BEH C18 (100 × 2.1 mm, 1.7 µm)**	**0.1% Orthophosphoric Acid : Acetonitrile (50:50, v/v)**	**0.3**	**Excellent peak symmetry, complete resolution, acceptable retention times, high theoretical plates and stable baseline.**	**Selected for validation**

Table 2: Optimized Chromatographic Conditions for the Developed UPLC Method

Parameter	Optimised Condition
Instrument	Waters ACQUITY UPLC H-Class

Detector	Photodiode Array (PDA) Detector
Column	ACQUITY BEH C18 (100 mm × 2.1 mm, 1.7 µm)
Mobile Phase	0.01 M Potassium Dihydrogen Phosphate Buffer : Acetonitrile (55:45, v/v)
Buffer pH	3.0 (Adjusted with Orthophosphoric Acid)
Flow Rate	0.30 mL/min
Detection Wavelength	210 nm
Injection Volume	2 µL
Column Temperature	30°C
Diluent	Water : Acetonitrile (50:50, v/v)
Run Time	5.0 minutes
Elution Mode	Isocratic

Method Validation: **System Suitability**

Table 3: System Suitability Test Parameters of the Developed UPLC Method

Parameter	Acceptance Criteria	Trifluridine	Tipiracil
Retention Time (min)	—	**1.482**	**2.134**
Peak Area (Mean, n = 6)	—	**842615**	**354278**
% Relative Standard Deviation (%RSD)	NMT 2.0%	**0.28**	**0.34**
Theoretical Plates (N)	NLT 2000	**8124**	**9687**
Tailing Factor	NMT 2.0	**1.09**	**1.12**
Resolution	NLT 2.0	—	**6.84**

Linearity:

Table 4: Linearity Studies of the Proposed UPLC Method

Linearity Level (%)	Trifluridine Concentration (µg/mL)	Mean Peak Area	Tipiracil Concentration (µg/mL)	Mean Peak Area
25	12.5	214385	5	89362
50	25	424917	10	178246
75	37.5	636582	15	267458
100	50	846934	20	355714
125	62.5	1058721	25	445362
150	75	1267428	30	534218

Table 5: Linear Regression Data

Regression Parameter	Trifluridine	Tipiracil
Linearity Range (µg/mL)	12.5–75.0	5.0–30.0
Regression Equation	**Y = 16892x + 2896**	**Y = 17792x + 610**
Slope	16892	17792
Intercept	2896	610

Correlation Coefficient (R^2)	**0.9999**	**0.9998**

Precision:

Table 6: Results of Repeatability Studies (Intra-Day Precision)

Injection No.	Trifluridine Peak Area	Assay (%)	Tipiracil Peak Area	Assay (%)
1	847265	100.18	356142	100.26
2	845914	100.02	355278	100.01
3	846582	100.1	355864	100.18
4	844976	99.91	354916	99.91
5	846128	100.05	355492	100.08
6	845387	99.97	354781	99.88
Mean	**846042**	**100.04**	**355412**	**100.05**
Standard Deviation	**852.6**	**0.1**	**528.9**	**0.14**
%RSD	**0.1**	**0.1**	**0.15**	**0.14**

Table 7: Results of Intermediate Precision (Inter-Day Precision)

Injection No.	Trifluridine Peak Area	Assay (%)	Tipiracil Peak Area	Assay (%)
1	846794	100.12	355948	100.21
2	844832	99.89	354862	99.91
3	846235	100.06	355416	100.07
4	845216	99.94	354975	99.94
5	846598	100.1	355781	100.17
6	845048	99.92	355164	100
Mean	**845787**	**100.01**	**355358**	**100.05**
Standard Deviation	**859.8**	**0.11**	**460.7**	**0.12**
%RSD	**0.1**	**0.11**	**0.13**	**0.12**

Accuracy (% Recovery):

Table 8: Accuracy Studies at 50% Recovery Level

Replicate	Trifluridine			Tipiracil		
	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Amount Added (mg)	Amount Recovered (mg)	% Recovery
1	25	24.96	99.84	10	9.98	99.8
2	25	25.04	100.16	10	10.02	100.2
3	25	24.99	99.96	10	9.99	99.9
Mean			**99.99**			**99.97**
SD			**0.16**			**0.21**
%RSD			**0.16**			**0.21**

Table 9: Accuracy Studies at 100% Recovery Level

Replicate	Trifluridine			Tipiracil		
	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Amount Added (mg)	Amount Recovered (mg)	% Recovery
1	50	49.94	99.88	20	19.98	99.9
2	50	50.08	100.16	20	20.03	100.15
3	50	50.02	100.04	20	20	100
Mean			**100.03**			**100.02**
SD			**0.14**			**0.13**
%RSD			**0.14**			**0.13**

Table 10: Accuracy Studies at 150% Recovery Level

Replicate	Trifluridine			Tipiracil		
	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Amount Added (mg)	Amount Recovered (mg)	% Recovery
1	75	74.91	99.88	30	29.97	99.9
2	75	75.11	100.15	30	30.05	100.17
3	75	75.03	100.04	30	30.01	100.03
Mean			**100.02**			**100.03**
SD			**0.14**			**0.14**
%RSD			**0.14**			**0.14**

Table 11: Summary of Accuracy (% Recovery) Studies

Recovery Level	Trifluridine Mean Recovery (% ± SD)	%RSD	Tipiracil Mean Recovery (% ± SD)	%RSD
50%	99.99 ± 0.16	0.16	99.97 ± 0.21	0.21
100%	100.03 ± 0.14	0.14	100.02 ± 0.13	0.13
150%	100.02 ± 0.14	0.14	100.03 ± 0.14	0.14
Overall Mean Recovery	100.01	0.15	100.01	0.16

Robustness:

Table 12: Robustness Study by Variation in Flow Rate

Flow Rate (mL/min)	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor	Assay (%)	%RSD
0.28	Trifluridine	1.561	8048	1.11	99.82	0.32
	Tipiracil	2.241	9586	1.15	99.94	0.29
0.30 (Optimised)	Trifluridine	1.482	8124	1.09	100.04	0.28

	Tipiracil	2.134	9687	1.12	100.05	0.34
0.32	Trifluridine	1.408	7986	1.13	100.18	0.31
	Tipiracil	2.046	9512	1.16	100.11	0.3

Table 13: Robustness Study by Variation in Mobile Phase Composition

Mobile Phase (Buffer:ACN, v/v)	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor	Assay (%)	%RSD
52:48:00	Trifluridine	1.548	8064	1.1	99.89	0.3
	Tipiracil	2.226	9621	1.13	99.95	0.33
50:50 (Optimised)	Trifluridine	1.482	8124	1.09	100.04	0.28
	Tipiracil	2.134	9687	1.12	100.05	0.34
48:52:00	Trifluridine	1.426	7998	1.12	100.15	0.31
	Tipiracil	2.058	9548	1.15	100.08	0.29

Table 14: Robustness Study by Variation in Detection Wavelength

Detection Wavelength (nm)	Drug	Peak Area	Theoretical Plates	Tailing Factor	Assay (%)	%RSD
208	Trifluridine	849286	8096	1.1	99.91	0.31
	Tipiracil	358412	9634	1.14	99.98	0.3
210 (Optimised)	Trifluridine	846934	8124	1.09	100.04	0.28
	Tipiracil	355714	9687	1.12	100.05	0.34
212	Trifluridine	844512	8062	1.11	100.09	0.29
	Tipiracil	353468	9582	1.15	100.1	0.32

Table 15: Robustness Study by Variation in Column Temperature

Column Temperature (°C)	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor	Assay (%)	%RSD
25	Trifluridine	1.523	8012	1.12	99.9	0.33
	Tipiracil	2.198	9568	1.15	99.94	0.31
30 (Optimised)	Trifluridine	1.482	8124	1.09	100.04	0.28
	Tipiracil	2.134	9687	1.12	100.05	0.34
35	Trifluridine	1.446	8056	1.11	100.12	0.3
	Tipiracil	2.082	9602	1.14	100.09	0.29

Table 16: Summary of Robustness Studies

Parameter Varied	Condition	Trifluridine Assay (%)	Tipiracil Assay (%)	Overall Observation

Flow Rate	0.28 mL/min	99.82	99.94	No significant variation observed
	0.30 mL/min	100.04	100.05	Optimised condition
	0.32 mL/min	100.18	100.11	Method remained unaffected
Mobile Phase	52:48:00	99.89	99.95	Acceptable chromatographic performance
	50:50:00	100.04	100.05	Optimised condition
	48:52:00	100.15	100.08	No significant effect observed
Detection Wavelength	208 nm	99.91	99.98	Detector response acceptable
	210 nm	100.04	100.05	Optimised condition
	212 nm	100.09	100.1	Method remained robust
Column Temperature	25°C	99.9	99.94	Chromatographic performance acceptable
	30°C	100.04	100.05	Optimised condition
	35°C	100.12	100.09	No appreciable effect observed

Specificity:

Table 17: Specificity (Interference Studies) of the Proposed UPLC Method

Solution Injected	Observation	Trifluridine Retention Time (min)	Tipiracil Retention Time (min)	Interference at Analyte Retention Time	Peak Purity	Result
Blank	No chromatographic peaks observed	—	—	No	—	Pass
Diluent	No interfering peaks detected	—	—	No	—	Pass
Placebo	Excipients did not produce any interfering peaks	—	—	No	—	Pass
Standard Solution	Sharp and symmetrical peaks obtained	**1.482**	**2.134**	No	Passed	Pass
Sample Solution	Well-resolved analyte peaks without interference	**1.484**	**2.136**	No	Passed	Pass

Acceptance Criteria

Parameter	Acceptance Criteria	Observed Result
Blank Interference	No peak at analyte retention time	Complies

Placebo Interference	No interference	Complies
Peak Purity	Peak purity should pass	Passed
Peak Resolution	Complete separation of analytes	Achieved
Method Specificity	No co-eluting peaks	Confirmed

LOD & LOQ

Table 18: LOD & LOQ

Parameter	Trifluridine	Tipiracil
Slope of Calibration Curve	16892	17792
Standard Deviation (σ)	308.52	148.26
Limit of Detection (LOD) (µg/mL)	**0.06**	**0.028**
Limit of Quantification (LOQ) (µg/mL)	**0.182**	**0.083**

Assay of Pharmaceutical Formulation

Table 19: Assay of Pharmaceutical Formulation

Sample No.	Trifluridine Peak Area	Assay (% Label Claim)	Tipiracil Peak Area	Assay (% Label Claim)
1	846218	99.92	355684	100.08
2	847164	100.14	356042	100.19
3	845972	99.86	355128	99.93
4	846756	100.05	355847	100.13
5	846084	99.89	355416	100.01
6	847008	100.11	355962	100.17
Mean	**846534**	**99.99**	**355680**	**100.09**
Standard Deviation	**491.8**	**0.12**	**363.7**	**0.11**
%RSD	**0.06**	**0.12**	**0.1**	**0.11**

Acceptance Criteria

Parameter	Acceptance Criteria	Observed Result
Assay	98.0–102.0%	Complies
%RSD	NMT 2.0%	Complies
Peak Symmetry	Tailing factor ≤ 2.0	Complies
System Suitability	As per ICH Q2(R2)	Complies

Forced degradation studies:

Table 20: Forced Degradation Studies

Stress Condition	Stress Applied	Trifluridine Assay (%)	Trifluridine Degradation (%)	Tipiracil Assay (%)	Tipiracil Degradation (%)	Peak Purity	Observation
Control	Untreated Sample	100	0	100	0	Passed	No degradation observed
Acid Hydrolysis	1 N HCl, 60°C, 30 min	94.68	5.32	95.24	4.76	Passed	Moderate degradation with well-resolved degradants
Alkaline Hydrolysis	1 N NaOH, 60°C, 30 min	93.92	6.08	94.37	5.63	Passed	Highest degradation observed under alkaline conditions
Oxidative Degradation	3% H_2O_2, 30 min	95.81	4.19	96.18	3.82	Passed	Mild oxidative degradation observed
Thermal Degradation	105°C, 6 h	97.28	2.72	97.64	2.36	Passed	Slight degradation following heat exposure
Photolytic Degradation	UV light, 24 h	97.82	2.18	98.15	1.85	Passed	Minimal degradation under UV exposure
Neutral Hydrolysis	Water, 60°C, 6 h	98.46	1.54	98.71	1.29	Passed	Very slight degradation observed

CONCLUSION

A rapid, sensitive, precise, accurate, and stability-indicating RP-UPLC method was successfully developed for the simultaneous determination of trifluridine and tipiracil in bulk drug substances and pharmaceutical dosage forms. The optimised chromatographic conditions using an ACQUITY BEH C18 column provided efficient separation of both analytes within a 5 min run time, with retention times of 1.482 and 2.134 min for trifluridine and tipiracil, respectively, and satisfactory resolution and peak symmetry.

The method demonstrated excellent linearity, accuracy, repeatability, intermediate precision, robustness, and sensitivity, with all validation results complying with the predefined acceptance criteria. The low LOD and LOQ values further demonstrated the adequate sensitivity of the procedure for quantitative analysis. The assay results of the pharmaceutical formulation were within the specified limits, confirming the applicability of the method for routine product analysis.

Forced degradation studies produced controlled degradation of the drug substances, with degradation products adequately separated from the principal analyte peaks and acceptable peak purity observed. These findings establish the stability-indicating capability of the proposed method. Overall, the developed RP-UPLC procedure offers a reliable, rapid, and efficient

analytical approach for routine quality control, assay determination, stability assessment, and batch-release testing of pharmaceutical formulations containing trifluridine and tipiracil.

REFERENCES

1. Chan WL, Lam KO, So TH. Third-line systemic treatment in advanced/metastatic gastric cancer: A comprehensive review. Ther Adv Med Oncol. 2019;11:1–11.
2. Smyth EC, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2016;27(Suppl 5):v38–v49.
3. Taiho Oncology Inc. Lonsurf® (Trifluridine and Tipiracil) Tablets: US Prescribing Information. Princeton, NJ: Taiho Oncology Inc.; 2019.
4. European Medicines Agency. Lonsurf® (Trifluridine/Tipiracil): Summary of Product Characteristics. Amsterdam: EMA; 2017.
5. Pharmaceuticals and Medical Devices Agency. Lonsurf Combination Tablets T15/T20: Japanese Prescribing Information. Tokyo: PMDA; 2017.
6. Burness CB, Duggan ST. Trifluridine/tipiracil: A review in metastatic colorectal cancer. Drugs. 2016;76(14):1393–1402.
7. Temmink OH, Emura T, De Bruin M, Fukushima M, Peters GJ. Therapeutic potential of the dual-targeted TAS-102 formulation in the treatment of gastrointestinal malignancies. Cancer Sci. 2007;98(6):779–789.
8. Peters GJ, Bijnsdorp IV. TAS-102: More than an antimetabolite. Lancet Oncol. 2012;13(12):e518–e519.
9. Matsushita S, Nitanda T, Furukawa T, Sumizawa T, Tani A, Nishimoto K, et al. The effect of a thymidine phosphorylase inhibitor on angiogenesis and apoptosis in tumours. Cancer Res. 1999;59(8):1911–1916.
10. Hoff PM, Cassidy J, Schmoll HJ. The evolution of fluoropyrimidine therapy: From intravenous to oral. Oncologist. 2001;6(Suppl 4):3–11.
11. Eluru A, Babub KS. A new selective separation method development and validation of trifluridine and tipiracil and its degradants characterised by LC–MS/MS/QTOF. J Pharm Sci Res. 2020;12(2):199–205.
12. Mastanamma SK, Nagaraju K, Reehana SK, Radhakrishnaveni V. Development and validation of a stability-indicating RP-HPLC method for simultaneous estimation of trifluridine and tipiracil in bulk and combined dosage form. Int J ChemTech Res. 2019;12(3):117–126.
13. Jogi K, Rao MB, Raju RR. An effective and sensitive stability-indicating RP-HPLC method for simultaneous estimation of trifluridine and tipiracil in bulk and pharmaceutical dosage form. Int J Res Pharm Chem. 2017;7(1):63–70.
14. Goday S, Abdulrahaman SK, Prameelarani A. Development and validation of a stability-indicating RP-HPLC method for simultaneous estimation of trifluridine and tipiracil in bulk and pharmaceutical dosage forms. Int J Res Appl Nat Soc Sci. 2017;5(7):93–104.
15. Hazra BB, Vageesh NM, Kistayya C, Shahanaz B. Analytical method development and validation for simultaneous estimation of trifluridine and tipiracil in pure and pharmaceutical dosage form. Innovat Int J Med Pharm Sci. 2018;3(3):55–58.
16. Prathap B, Haribaskar V, Kumar B, Raghu PS, Reddy SBK. Method development and validation for simultaneous estimation of trifluridine and tipiracil in tablet dosage form by RP-HPLC. J Global Trends Pharm Sci. 2017;8(4):4514–4521.

17. International Council for Harmonisation (ICH). ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.
18. International Council for Harmonisation (ICH). ICH Q2B: Validation of Analytical Procedures: Methodology. Geneva: ICH; 1996.
19. Trivedi CD, Mardia RB, Suhagia BN, Chauhan SP. Development and validation of a spectrophotometric method for estimation of ritonavir in tablet dosage form. Int J Pharm Sci Res. 2013;4(12):4567–4572.
20. Reddy BA, Alam MDI, Khanam N, Krishnanand PR. An innovative RP-HPLC method development and forced degradation studies for simultaneous estimation of sofosbuvir and ledipasvir. Int J Pharm Pharm Sci. 2019;11(2):21–26.
21. International Council for Harmonisation (ICH). ICH Q1A(R2): Stability Testing of New Drug Substances and Products. Geneva: ICH; 2003.
22. Ngwa G. Forced degradation studies as an integral part of HPLC stability-indicating method development. Drug Deliv Technol. 2010;10(5):56–59.
23. Vanitha C, Reddy B, Satyanarayana SV. Quality-by-design approach to selective stability-indicating RP-HPLC method development and validation for estimation of sofosbuvir in bulk drug. Int J Res Pharm Sci. 2018;9(2):298–308.

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