IJPSR (2021), Volume 12, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 July 2020; received in revised form, 20 November 2020; accepted, 08 May 2021; published 01 July 2021

# DEVELOPMENT OF STABILITY-INDICATING UPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF TIPIRACIL ANDTRIFLURIDINE IN FORMULATION

S. Lakshmi Maneka [*1], R. T. Saravanakumar [1] and Ch. K. V. L. S. N. Anjana [2]

Department of Pharmacy [1], Annamalai University, Chidambaram - 608002, Tamil Nadu, India.
Department of Pharmaceutical Chemistry and Phytochemistry [2], Nirmala College of Pharmacy, Guntur - 522503, Andhra Pradesh, India.

**Keywords:**

Tipiracil, Trifluridine, Isocratic elution, UPLC, Validation, Stability

**Correspondence to Author:**
**S. Lakshmi Maneka**

Research Scholar,
Department of Pharmacy,
Annamalai University, Chidambaram - 608002, Tamil Nadu, India.

**E-mail:** slmanu91@gmail.com

**ABSTRACT:** Aim of the present research work was to develop a sensitive, precise, and robust stability-indicating UPLC method for the simultaneous estimation of tipiracil and trifluridine in formulations. The chromatographic separation of a mixture of tipiracil and trifluridine was attained in isocratic method utilizing a mobile phase of 0.1% orthophosphoric acid: acetonitrile in the proportion of 50:50% v/v utilizing a Hibera C18 column which has dimensions of 100 × 2.1 mm, 1.8µ particle size and the flow rate of 1.0 ml/min. The detection system was monitored at 230nm wavelength maximum with 1.5 µl injection volume. The retaining time for trifluridine and tipiracil was achieved at 0.549 min, and 1.251 min. respectively. Tipiracil and trifluridine and their combined drug formulation were exposed to thermal, acidic, oxidative, photolytic, and alkaline conditions. The present method was validated as per the guidelines given by the ICH for specificity, accuracy, sensitivity, linearity, and precision. The developed method was highly sensitive, rapid, precise, and accurate than the earlier reported methods. The total run time was decreased to 3.0 min; hence, the technique was more precise and economical. Stability studies were directed for the suitability of the technique for degradation studies of tipiracil and trifluridine. The projected method can be utilized for routine analysis in the quality control department in pharmaceutical trades.

**INTRODUCTION:** Gastric cancer is the fifth most frequently diagnosed cancer worldwide and the third leading cause of cancer-related mortality, resulting in 783,000 deaths globally during 2018. The aim of treating metastatic gastric cancer is to prolong survival and maximize the health-related quality of life (HR-QOL).

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(7).3701-08

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(7).3701-08

Unresectable metastatic gastric cancer has traditionally been treated with palliative therapies in combination with best supportive care (BSC). A fixed-dose combination tablet comprising trifuridine and tipiracil (hereafter referred to as trifuridine/ tipiracil) 1584 C. Kang *et al*., [Lonsurf®] is approved worldwide for use in metastatic colorectal cancer, including in the USA, the EU, and Japan, data for which have been reviewed previously and are beyond the scope of this review [1-6].

The drug consists of cytotoxin trifluridine and the thymidine phosphorylase inhibitor (TPI) tipiracil [7].

Trifluridine is incorporated into DNA during DNA synthesis and inhibits tumor cell growth. Trifluridine (TFT) is incorporated into DNA by phosphorylation by thymidylate kinase (TK) to TF-TMP; TF-TMP then covalently binds to tyrosine 146 of the active site of thymidylate synthase (TS), inhibiting the enzyme's activity. TS is vital to the synthesis of DNA because it is an enzyme involved in the synthesis of the deoxynucleotide, thymidine triphosphate (dTTP). Inhibition of TS depletes the cell of dTTP and causes accumulation of deoxyuridine monophosphate (dUMP), which increases the likelihood that uracil gets misincorporated into the DNA [8]. Also, subsequent phosphorylations of TF-TMP cause an increased level of TF-TTP within the cell, which results in it being incorporated into DNA. Even though the exact mechanism of how TFT causes DNA damage is not completely understood, it is hypothesized that the incur-poration TF-TTP in DNA leads to DNA strand break formation [8]. Tipiracil prevents the degradation of trifluridine *via* thymidine phosphorylase (TP) when taken orally and also has antiangiogenic properties [9, 10].

Tipiracilchemically designated as 5-Chloro-6-[(2-imino-1-pyrrolidinyl)methyl]-2, 4(1*H*, 3*H*)- pyrimidinedione with molecular weight of 242.67 g/ mole **Fig. 1**. Trifluridine chemically designated as1-[4-Hydroxy- 5 -(hydroxymethyl) oxolan- 2- yl]- 5-(trifluoromethyl)-(1*H*,3*H*)-pyrimidine-2,4-dione with molecular weight of 296.2 g/mole **Fig. 1**.

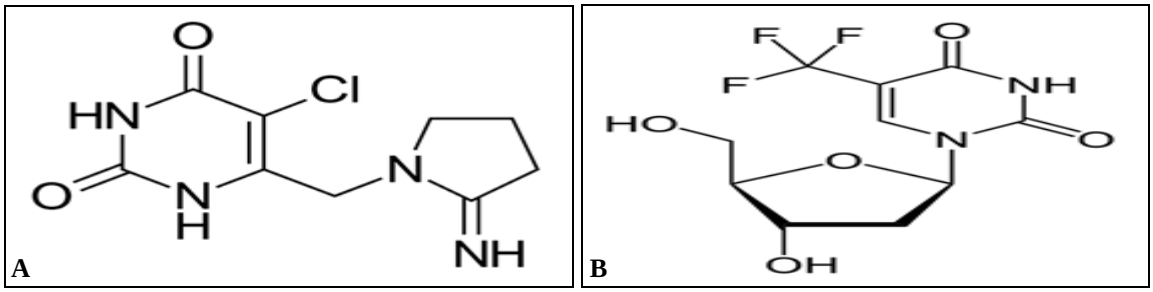


**FIG. 1: STRUCTURES OF A) TIPIRACIL AND B) TRIFLURIDINE**

The literature review discloses that very few LC-MS/MS [11] and high-performance liquid chromatographic [12-16] techniques have been reported for the estimation of tipiracil and trifluridine. Based on the reported HPLC methods, there is a need to develop a rapid, sensitive reversed-phase UPLC method for simultaneous estimation of tipiracil and trifluridine in bulk and formulations.

## MATERIALS AND METHODS:

**Chemicals and Reagents:** The standard components of tipiracil and trifluridine were provided as a gift sample from spectrum Pharma Research Solutions, Hyderabad. Lonsurf film-coated tablets labeled to contain tipiracil8.9 mg, and trifluridine 20 mg were procured from the local market. HPLC grade methanol was obtained from A.B enterprises, Mumbai, India. Orthophosphoric acid was bought from Ranchem, Mumbai, India. HPLC grade water was processed by utilizing Milli-Q Millipore water purification system used during the method development.

**Liquid Chromatography:** Chromatographic system of Waters UPLC system furnished with photodiode array detector, auto-sampler, and Hibera C18 column which have dimensions of 100 × 2.1 mm, 1.8µ particle size. The output signal was monitored and integrated utilizing water Empower-2.0 software. The isocratic mobile consisting of 0.1% ortho phosphoric acid: acetonitrile in the proportion of 50:50% v/v, pumped through the Hibera C18 (100 × 2.1mm, 1.8µ) column at a fixed flow of 1.0 ml/ min. The injection volume of1.5µL was utilized to measure the chromatograms at 230 nm as wavelength maximum in the detection system. Water and acetonitrile in the ratio of 50:50% v/v was utilized as diluent.

**Preparation of Buffer:** 1ml of orthophosphoric acid solution in a 1000ml of the volumetric flask was transferred, and about 100ml of milli-Q water was added. The final volume was made up to 1000 ml with milli-Q water.

**Preparation of Standard Stock Solution:** Accurately Weighed and transferred 20 mg of trifluridine and 8.19 mg of tipiracil working standards into 100 ml clean dry volumetric flasks, 3/4$^{th}$ volume of diluent added and sonicated for 10

minutes. The final volume was made up to 100 ml with diluent to get 200µg/ml of trifluridine and 89µg/ml of tipiracil.1ml from the above two stock solutions were taken into a 10ml volumetric flask and made up to 10ml with diluent.

**Preparation of Sample Solution:** 20 tablets were weighed and calculated the average weight of tablets, and then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask containing 50mL of diluent and sonicated for 25.0 min. Further, the volume made up with diluent and subjected for filtration by HPLC filters (200µg/ml of trifluridine, and 89µg/ml of tipiracil). From the filtrate, 1.0 ml solution was pipetted out into a 10.0 ml volumetric flask and made upto 10.0 ml with diluent to get 20µg/ml of trifluridine, and8.9µg/ml of tipiracil.

**Analytical Method Validation:** The developed method for trifluridine and tipiracil was subjected for validation for the parameters like limit of detection (LOD), the limit of quantification (LOQ), linearity, robustness, precision, system suitability, and accuracy as per the guidelines of ICH [17-20].

## RESULTS AND DISCUSSION:

**Optimized Chromatographic Conditions:** After systematic trials with different mobile phase compositions and other parameters involved in the technique, the following chromatographic conditions were employed:

| | | |
|---|---|---|
| Mobile phase | : | Buffer: acetonitrile (50:50% v/v) |
| Flow rate | : | 1.0 ml/min |
| Column | : | Hibera C18 100 × 2.1mm, 1.8 µ. |
| Detector wavelength | : | 230nm |
| Column temperature | : | 30°C |
| Injection volume | : | 1.5µl |
| Run time | : | 3min |
| Diluent | : | Water: acetonitrile (50:50) |

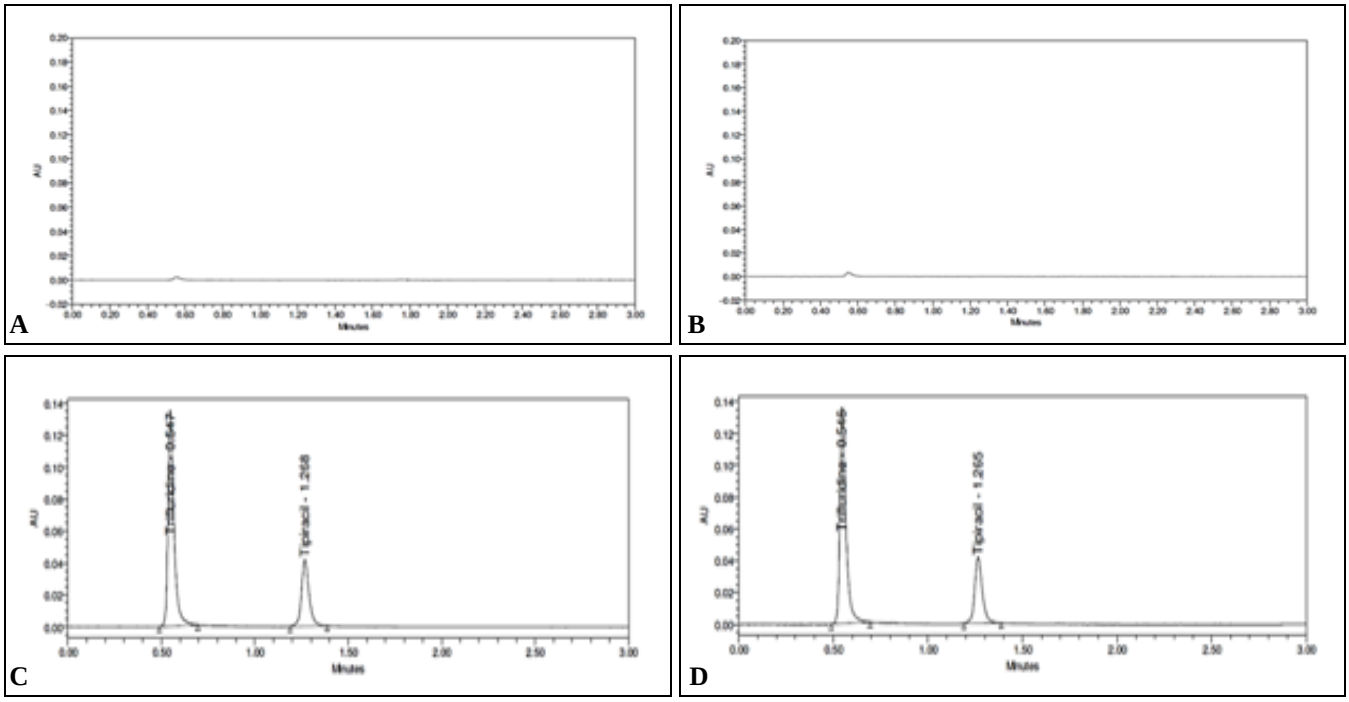


**FIG. 2: CHROMATOGRAMS OF A) BLANK, B) PLACEBO, C) STANDARD AND D) FORMULATION**

**Specificity:** It is the ability of a method to unequivocally evaluate the analyte components in the presence of other components like impurities, degradants and excipients, *etc*., expected to be present. This parameter was estimated by injecting and evaluating the blank, placebo, standard, and sample solutions and chromatograms, respectively [18, 19]. Chromatograms of blank, placebo, and sample solution shown no peaks at the retaining time of tipiracil and trifluridine peaks. The chromatograms of tipiracil and trifluridine of standard, blank, formulation, and placebo were represented in **Fig. 2**.

**Linearity:** Aliquots of 0.25, 0.50, 0.75, 1.0, 1.25, and 1.50 ml of standard stock solution were pipetted out from the standard stock solution of concentration 200µg/ml of trifluridine, and 89µg/ml of tipiracil and made up to 10.0 ml mark with diluent. The resulting solutions came into 5 to 30µg/ml of trifluridine and 2.225 to 13.35 µg/ml of tipiracil concentration range. The resulting linearity solutions were infused into a chromatographic system, and form the chromato-grams linearity graph was plotted by taking the peak area on Y-axis and concentration on X-axis [18, 19]. The calibration graphs were shown in **Fig. 3, 4,** and **Table 1**, and all findings were within limits.

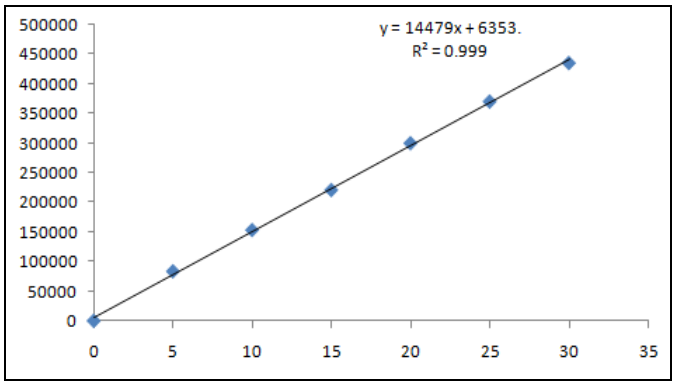


**FIG. 3: LINEARITY OF TRIFLURIDINE**

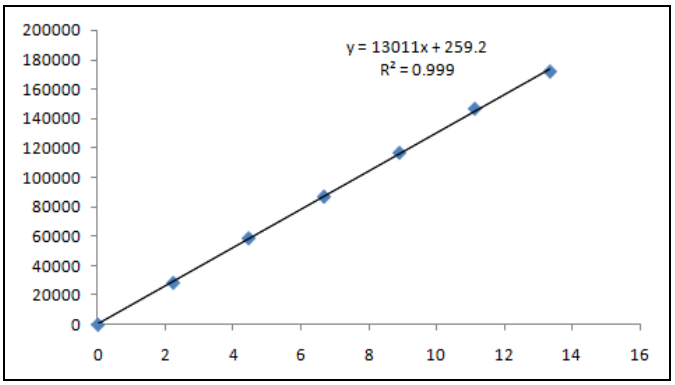


**FIG. 4: LINEARITY OF TIPIRACIL**

**TABLE 1: CALIBRATION CURVE DATA OF TRIFLURIDINE AND TIPIRACIL**

| Trifluridine | | Tipiracil | |
|---|---|---|---|
| **Concentration (µg/ml)** | **Peak area** | **Concentration (µg/ml)** | **Peak area** |
| 5 | 83808 | 2.225 | 28500 |
| 10 | 153476 | 4.45 | 58717 |
| 15 | 221136 | 6.675 | 86987 |
| 20 | 300174 | 8.9 | 116781 |
| 25 | 370527 | 11.125 | 146761 |
| 30 | 435636 | 13.35 | 171994 |
| **Regression equation** | | | |
| y = 14479x + 6353.2 | | y = 13011x + 259.2 | |
| **Correlation coefficient ($R^2$)** | | | |
| 0.9991 | | 0.9996 | |

**System Suitability:** Six replicates of the standard reference solution were processed and infused to perform the system suitability parameter, and the resulting chromatograms peak area, retention time, resolution, plate count, and tailing were measured. The findings of the system suitability parameter were shown in **Table 2,** and related chromatograms were given in **Fig. 2**.

**TABLE 2: TIPIRACIL AND TRIFLURIDINE SYSTEM SUITABILITY RESULTS**

| S. no. | Peak name | Peak area | Retention time | Plate count | Resolution | Tailing |
|---|---|---|---|---|---|---|
| 1 | Trifluridine | 316866 | 0.551 | 2231 | | 1.61 |
| 2 | Tipiracil | 115812 | 1.287 | 5803 | 10.9 | 1.13 |

**TABLE 3: LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION RESULTS**

| Parameter | Measured concentration (µg/ml) | |
|---|---|---|
| | **Tipiracil** | **Trifluridine** |
| LOD | 0.04 | 0.20 |
| LOQ | 0.13 | 0.61 |

**LOD and LOQ:** LOD and LOQ parameters for tipiracil and trifluridine were calculated to form the linear regression equation 18. Linearity values, graph and regression equation were got from the linearity study and the LOD and LOQ values were represented in the **Table 3**.

**Precision:** Analytical method precision is defined as closeness of agreement between the replicate measurements of the analyte. It is expressed as the percentage coefficient of correlation or relative standard deviation (RSD) of the replicate measurements.

**System Precision:** Working standard preparation of 1.5 µl solution was infused six times into the chromatographic system, and chromatograms were obtained. %RSD of the peak area was calculated. The findings of system precision were shown in **Table 4**.

**TABLE 4: SYSTEM PRECISION DATA**

| S. no. | Peak area response of analytes | |
|---|---|---|
| | Trifluridine | Tipiracil |
| 1 | 316866 | 900762 |
| 2 | 317985 | 908579 |
| 3. | 316370 | 908664 |
| 4 | 316076 | 903595 |
| 5 | 315612 | 903033 |
| 6 | 315727 | 903096 |
| Average | 316439 | 904622 |
| STDV | 883.2 | 3249.7 |
| % RSD | 0.3 | 0.4 |

STDV: Standard deviation; RSD: Relative Standard deviation

**Method Precision:** Working sample solutions of 1.5µl were infused 6 times into the chromatographic system, and chromatograms were obtained. The %RSD of the assay result of six preparations was determined. The findings achieved for assay were represented in **Table 5**.

**TABLE 5: METHOD PRECISION RESULTS**

| S. no. | Peak area response of drugs | |
|---|---|---|
| | Trifluridine | Tipiracil |
| 1 | 318868 | 114573 |
| 2 | 310675 | 115752 |
| 3. | 311032 | 116657 |
| 4 | 314925 | 115702 |
| 5 | 318121 | 114195 |
| 6 | 316105 | 115188 |
| Average | 314954 | 115345 |
| STDV | 3474.7 | 889.7 |
| % RSD | 1.1 | 0.8 |

STDV: Standard deviation; RSD: Relative Standard deviation

**Intermediate Precision:** Working standard preparation of 1.5 µl was infused six times test preparations into the chromatographic system, and chromatograms were obtained. The %RSD was evaluated for peak areas. The findings of the intermediate precision study were represented in **Table 6**.

**TABLE 6: INTERMEDIATE PRECISION RESULTS**

| S. no. | Peak area response of drugs | |
|---|---|---|
| | Trifluridine | Tipiracil |
| 1 | 308692 | 107501 |
| 2 | 309105 | 107405 |
| 3. | 305533 | 106828 |
| 4 | 303456 | 105906 |
| 5 | 302503 | 106985 |
| 6 | 301938 | 105679 |
| Average | 305205 | 106717 |
| STDV | 3114.8 | 762.5 |
| % RSD | 1.0 | 0.7 |

**Accuracy:** A known amount of tipiracil and trifluridine at each three concentration levels of 50%, 100%, and 150% was added to a pre-analyzed sample solution and injected in triplicate at each level into the chromatographic system [17, 18]. The mean percentage recovery of tipiracil and trifluridine at each level was estimated. The findings were represented in **Table 7**.

**Robustness:** Working standard solution prepared as per test method was infused into the chromatographic system at variable conditions such as flow rate at±0.1 ml/min, mobile organic phase composition by ±10%, and column temperature by ±5°C. The results of robustness study parameters like peak area, retention time, plate count, and tailing factor were within limits.

**TABLE 7: PERCENTAGE RECOVERY RESULTS**

| | Trifluridine | | | | Tipiracil | | | |
|---|---|---|---|---|---|---|---|---|
| Spiked level | spiked (µg/ml) | recovery (µg/ml) | % recovery | Mean % recovery | spiked (µg/ml) | recovery (µg/ml) | % recovery | Mean % recovery |
| 50% | 10 | 9.92 | 99.17 | 99.87 | 4.45 | 4.47167 | 100.49 | 99.98 |
| | 10 | 9.86 | 98.55 | | 4.45 | 4.435086 | 99.66 | |
| | 10 | 9.99 | 99.96 | | 4.45 | 4.425094 | 99.44 | |
| 100% | 20 | 19.80 | 99.02 | | 8.9 | 8.917985 | 100.20 | |
| | 20 | 19.74 | 98.72 | | 8.9 | 8.812612 | 99.02 | |
| | 20 | 19.88 | 99.38 | | 8.9 | 8.90953 | 100.11 | |
| 150% | 30 | 30.09 | 100.29 | | 13.35 | 13.37744 | 100.21 | |
| | 30 | 30.57 | 101.89 | | 13.35 | 13.34777 | 99.98 | |
| | 30 | 30.55 | 101.84 | | 13.35 | 13.31788 | 99.76 | |

**Forced Degradation Studies:**

**Acid Degradation Studies:** To 1 ml of stock s solution tipiracil and trifluridine, 1ml of 2N Hydrochloric acid was added and refluxed for 30 min. at 60 °C [19-21]. The resultant solution was diluted to obtain 20µg/ml of trifluridine, and

8.9µg/ml of tipiracil solution and 10 µl solution was injected into the chromatographic system, and the chromatograms were recorded to assess the stability of the sample **Fig. 5** and **Table 8**.

**TABLE 8: RESULTS OF STRESS DEGRADATION STUDY**

| S. no. | Degradation condition | Trifluridine | | Tipiracil | |
|---|---|---|---|---|---|
| | | % recovery | % Degraded | % recovery | % Degraded |
| 1 | Acid hydrolysis | 93.36 | 6.64 | 94.41 | 5.59 |
| 2 | Base hydrolysis | 94.43 | 5.57 | 93.14 | 6.86 |
| 3 | Peroxide | 95.45 | 4.55 | 94.04 | 5.96 |
| 4 | Dry heat | 96.05 | 3.95 | 96.42 | 3.58 |
| 5 | Photo stability | 97.69 | 2.31 | 97.49 | 2.51 |
| 6 | Water sample | 97.69 | 2.31 | 99.58 | 0.42 |

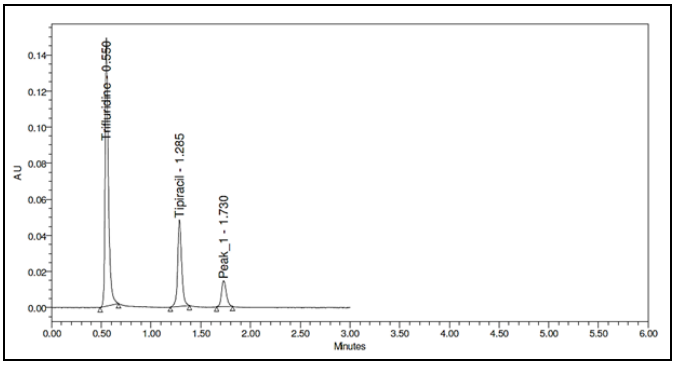


**FIG. 5: CHROMATOGRAM FOR ACID DEGRADATION STUDY**

**Oxidation**: To 1 ml of stock solution of tipiracil and trifluridine, 1 ml of 20% hydrogen peroxide ($H_2O_2$) was added separately. The solutions were kept for 30 min at 60 °C. For UPLC study, the resultant solution was diluted to obtain 20µg/ml of trifluridine, and 8.9µg/ml of tipiracil solution and 10 µl solution was injected into the chromatographic system, and the chromatograms were recorded to assess the stability of the sample **Fig. 6** and **Table 8**.

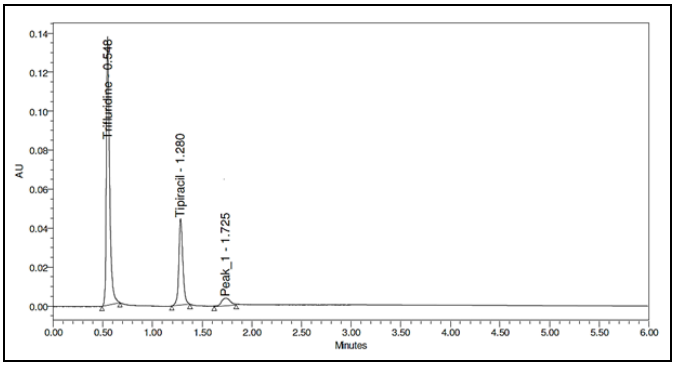


**FIG. 6: CHROMATOGRAM FOR OXIDATION DEGRADATION STUDY**

**Alkali Degradation Studies:** To 1 ml of stock solution OMTR, PRTR, and RTNR, 1 ml of 2N sodium hydroxide was added and refluxed for 30 min. at 60°C [20]. The resultant solution was diluted to obtain 20 µg/ml of trifluridine, and 8.9 µg/ml of tipiracil solution and 10 µl solution was injected into the chromatographic system, and the chromato-grams were recorded to assess the stability of the sample **Fig. 7** and **Table 8**.

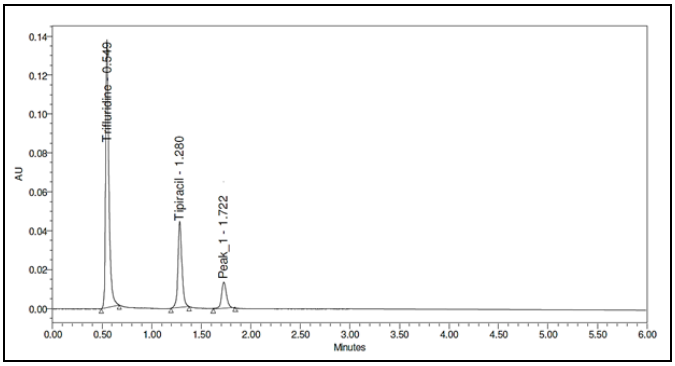


**FIG. 7: CHROMATOGRAM FOR ALKALI DEGRADATION STUDY**

**Dry Heat Degradation Studies:** The standard drug solution was placed in an oven at 105 °C for 6 h to study dry heat degradation. For the UPLC study, the resultant solution was diluted to get 20µg/ml of trifluridine, and 8.9µg/ml of tipiracil solution and 10 µl solution was injected into the chromatographic system, and the chromatograms were recorded to assess the stability of the sample **Fig. 8** and **Table 8**.

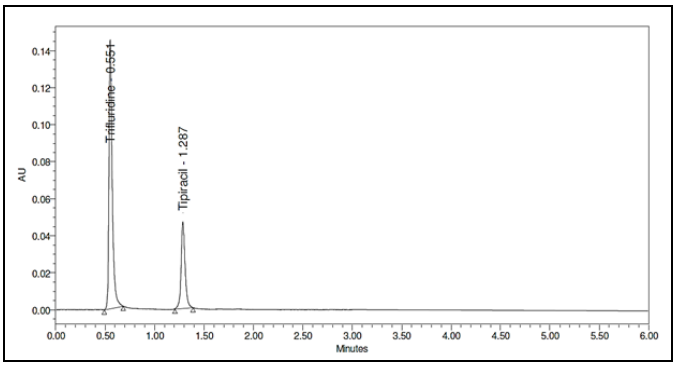


**FIG. 8: CHROMATOGRAM FOR DRY HEAT DEGRADATION STUDY**

**Photo Stability Studies:** The photochemical stability of the drug was also studied by exposing the (200µg/ml of trifluridine and 89µg/ml of tipiracil) solution to UV Light by keeping the beaker in UV Chamber for 3 days or 200 Watt h/$m^2$ in photostability chamber [21-23]. For UPLC study, the resultant solution was diluted to obtain 20µg/ml of trifluridine, and 8.9µg/ml of tipiracil solution and 10 µl solution was injected into the chromato-graphic system, and the chromatograms were recorded to assess the stability of the sample **Fig. 9** and **Table 8**.

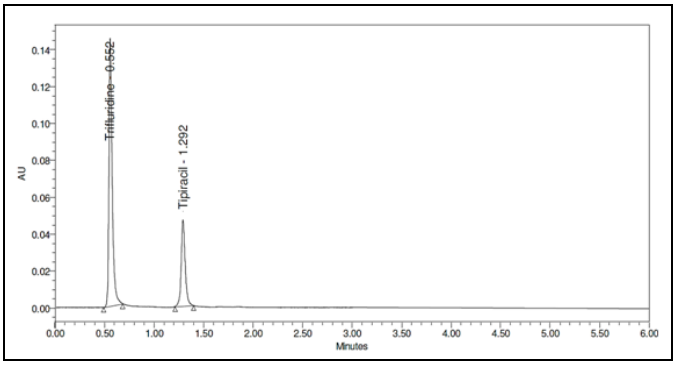


**FIG. 9: CHROMATOGRAM FOR PHOTO STABILITY STUDY**

**Neutral Degradation Studies:** Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60 °C. For UPLC study, the resultant solution was diluted to get 20µg/ml of trifluridine, and 8.9µg/ml of tipiracil solution and 10 µl solution was injected into the chromatographic system, and the chromato-grams were recorded to assess the stability of the sample **Fig. 10** and **Table 8**.

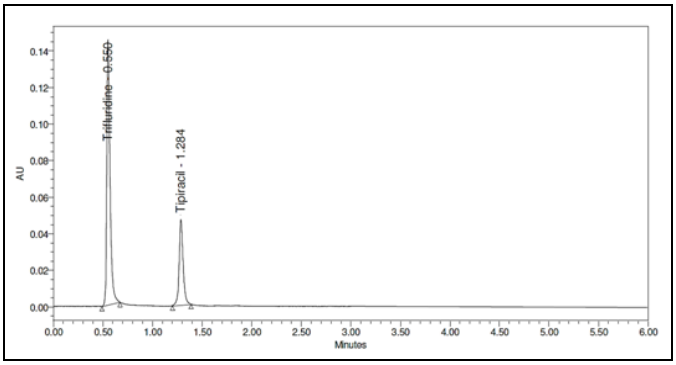


**FIG. 10: CHROMATOGRAM FOR NEUTRAL DEGRADATION STUDY**

**CONCLUSION:** A sensitive, rapid and accurate, stability-indicating RP-UPLC method for the simultaneous estimation of trifluridine and tipiracilin formulations was developed and validated as per the ICH guidelines. Retention times for trifluridine and tipiracil were achieved at 0.549 min. and 1.251 min. respectively. The mean percentage recovery of trifluridine and tipiracil were found to be 99.87% and 99.98%, respectively. LOD/LOQ values obtained from regression equations of tipiracil and trifluridine were found to be 0.04µg/ml/0.13µg/ml and 0.20µg/ml/0.61 µg/ml respectively. Regression equation of trifluridine and tipiracil were: y = 14479x + 6353.2 and y = 13011x + 259.2 respectively. Stability studies of these drugs proven that the percentage degradation of analytes was found in between 0.42% to 6.86%. Retention time and total run times of analytes were decreased. Hence, the developed method was rapid and economical that can be applied in routine analysis of these drugs in the quality control department of pharmaceutical trades.

**ACKNOWLEDGEMENT:** Nil

**CONFLICTS OF INTEREST:** The authors declare that there are no conflicts of interest regarding the paper's publication.

## REFERENCES:

1. Chan WL, Lam KO and So TH: Third-line systemic treatment in advanced/metastatic gastric cancer: a comprehensive review. TherAdv Med Oncol 2019; 11: 1-11.
2. Smyth EC, Verheij M, Allum W: Gastric Cancer: ESMO Clinical Practice Guidelines. Ann Oncol 2016; 27(Suppl 5): v38-49.
3. Taiho Oncology Inc. Lonsurf (trifuridine and tipiracil) tablets for oral use: US prescribing information. 2019. https://www.fda.gov. Accessed 29 Aug 2019.
4. European Medicines Agency. Lonsurf® (trifuridine/ tipiracil): summary of product characteristics. 2017. https://www.ema.europ a.eu. Accessed 29 Aug 2019.
5. Pharmaceuticals and Medical Devices Agency. Lonsurf Combination Tablets T15/Lonsurf Combination Tablets T20: Japanese prescribing information 2017. https://www.pmda.go.jp. Accessed 29 Aug 2019.
6. Burness CB and Duggan ST: Trifuridine/tipiracil: a review in metastatic colorectal cancer. Drugs 2016; 76: 1393-402.
7. Temmink OH, Emura T, De Bruin M, Fukushima M and Peters GJ: Therapeutic potential of the dual-targeted TAS-102 formulation in the treatment of gastrointestinal malignancies. Cancer Science 2007; 98: 779-89.
8. Peters GJ and Bijnsdorp IV: TAS-102: more than an antimetabolite. The Lancet. Oncology 2012; 13: e518-9.
9. Matsushita S, Nitanda T, Furukawa T, Sumizawa T, Tani A and Nishimoto K: The effect of a thymidine phosphorylase inhibitor on angiogenesis and apoptosis in tumors. Cancer Research 1999; 59: 1911-6.
10. Hoff PM, Cassidy J and Schmoll HJ: The evolution of fluoropyrimidine therapy: from intravenous to ora. The Oncologist 2001; 6: 3-11.

11. Eluru A and Babub KS: A new selective separation method development and validation of trifluridine and tipiracil and its degradents were characterized by LC-MS/MS/QTOF. J Pharm Sci and Res 2020; 12: 199-205.
12. 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.
13. 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.
14. Goday S, Abdulrahaman SK and Prameelarani A: Development and validation of stability indicating RP-HPLC 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.
15. 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.
16. 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.
17. ICH: Q2 (R1), Validation of analytical procedures: text and methodology; 2005.
18. ICH: Q2B. Harmonized Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva; 1996.
19. Trivedi CD, Mardia RB, Suhagia BN and Chauhan SP: Development and validation of spectrophotometric method for the estimation of ritonavir in tablet dosage form. International Journal of Pharmaceutical Sciences and Research 2013; 4: 4567-72.
20. Reddy BA, Alam MDI, Khanam N and Krishnanand PR: An innovative method development and forced degradation studies for simultaneous estimation of sofosbuvir and ledipasvir by RP HPLC .International Journal of Pharmacy and Pharmaceutical Sciences 2019; 11: 21-26.
21. ICH Guidelines Q1A (R2), Stability Testing of New Drug Substances and Products, International Conference on Harmonization; 2003.
22. Ngwa G: Forced degradation studies as an integral part of HPLC stability indicating method development. Drug Delivery Technol 2010; 10: 56-9.
23. Vanitha C, Reddy B and Satyanarayana SV: Quality-by-design approach to selective stability indicating RP-HPLC method development and validation of estimation of sofosbuvir in bulk drug. Int J Res Pharm Sci 2018; 9: 298-308.


**How to cite this article:**
Maneka SL, Saravanakumar RT and Anjana CKVLSN: Development of stability-indicating UPLC method for the simultaneous determination of tipiracil andtrifluridine in formulation. Int J Pharm Sci & Res 2021; 12(7): 3701-08. doi: 10.13040/IJPSR.0975-8232. 12(7).3701-08.