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Development and quantification of UPLC method for the simultaneous determination of tipiracil and trifluridine in formulation

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ABSTRACT

Aim of the present research work was to develop a sensitive, precise and robust UPLC method for the simultaneous estimation of tipiracil and trifluridine in formulations. The chromatographic separation of mixture of tipiracil and trifluridinewas attained in isocratic method utilizing a mobile phase of 0.1% ortho phosphoric acid: acetonitrile in the proportion of 50:50%v/v utilizing a Hibera C18 column which has dimensions of 100 x 2.1 mm, 1.8µparticle 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 tipiracilwas achieved at 0.549min, and 1.251 min respectively. 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. The projected method can be utilized for routine analysis in quality control department in Pharma industries.

Keywords: Tipiracil, Trifluridine, Isocratic elution, UPLC.

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 health-related quality of life (HR-QOL). 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 the 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 are 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 incorporation 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].

Tipiracil chemically 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). Trifluridinechemically designated as1-[4-Hydroxy-5-(hydroxymethyl) oxolan-2-yl]-5-(trifluoromethyl)-(1*H*,3*H*)-pyrimidine-2,4-dionewith molecular weight of 296.2 g/mole (Fig.2).

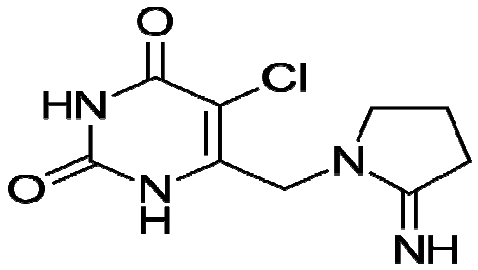

Fig.1: Structure of Tipiracil

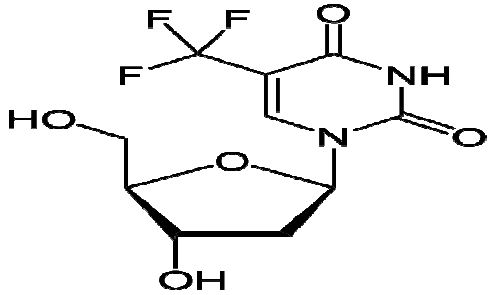

Fig.2: Structure of Trifluridine

The literature review discloses that a very few high performance liquid chromatographic[12-15] 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

Instrumentation (UPLC)

Chromatographic measurements were made on a Waters alliance (Waters Corporation, Milford, MA, USA) 2695 model which consisted of two (AD/LC/32) solvent delivery modules, a PDA (AD/LC/77) detector, quaternary gradient pumps, in built an auto injector valve with a 20 µL loop. The system was controlled through a system controller (SCL-10A) and a personal computer using Empower 2 software installed on it. The mobile phase was degassed using a Shimadzu sonicator (Model: Power sonic 405). Absorbance spectra were recorded using Shimadzu UV-Visible spectrophotometer (Model: UV-1800) employing quartz cell of 1.0 cm of path length and weighing was done with Shimadzu balance (Model: AUX220).

Chemicals and reagents

The standard components of tipiracil and trifluridinewere provided as a gift sample from Spectrum Pharma Research Solutions, Hyderabad. Lonsurf film coated tabletslabeled to contain tipiracil 8.19 mg and trifluridine 20 mg were procured from the local market. HPLC grade methanol was obtained from A.B enterprises, Mumbai, India. Ortho phosphoric 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.

Preparation of buffer

1ml of ortho phosphoric acid solution in a 1000ml of 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 a 100 ml clean dry volumetric flasks, 3/4th 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 81.9µg/ml of tipiracil. 1.0ml 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 81.9µg/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 and 8.19 µg/ml of tipiracil.

RESULTS AND DISCUSSION

Method development

The sample solution of TFD (10 µg/mL) and TPR (10 µg/mL) in methanol was prepared individually and sample was scanned over the range of 200 - 400 nm individually. During the method development various mobile phase compositions consisting of methanol, acetonitrile, water, phosphate buffers and different stationary phases were executed to get fine chromatographic conditions like theoretical plates, resolution, tailing and peak shape. The sample solution of TFD (10 µg/mL) and TPR (10 µg/mL) in methanol overlay graph showed that both drug absorb at 230 nm (Fig.3). So, the wavelength selected for the estimation of TFD and TPR were at 230 nm. With different mobile phase compositions and stationary phases five different trials were executed and sixth trail was optimized. In all the five trials there was only one peak was eluted in trial -1 and trial -2 (Fig.4, Fig.5), fronting observed in tipiracil peak in trail -3 (Fig.6), peak shape was poor in the trail -4 (Fig.7) and there was extra peaks in the trial -5 (Fig.8) and the major trails details represented in Table.1.

Table.1. The major trials details

S.No	Trials	Column	Mobile Phase	Observation
01	**Trial -1**	Zorbax C18 Column (100 x 3 mm, 2.1 µm)	Methanol:Water (1:1)	Peak was eluted
02	**Trial -2**	Inertsil C18 Column (100 x 3 mm, 2.1 µm)	ACN:Water (70:30)	Peak was eluted
03	**Trial -3**	Hypersil C18 (100 x 3 mm, 2.1 µm)	Methanol: ACN: water (1:1:1)	Fronting observed in tipiracil peak
04	**Trial -4**	Zorbax SB C18 (100 x 3 mm, 2.1 µm)	0.1% OPA: methanol (30:70 v/v)	Peak shape was poor
05	**Trial -5**	Acquity BEH C18 (100 x 3 mm, 1.7 µm)	0.1% OPA: ACN (30:70 v/v)	There was extra peaks

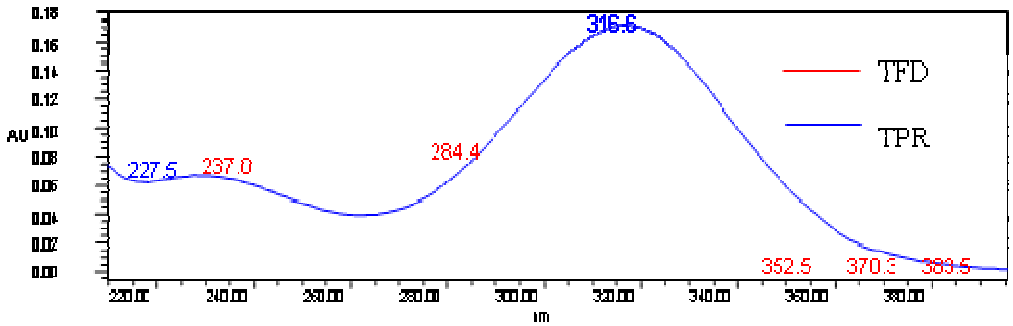

Fig.3: Overlay UV spectra for TFD and TPR

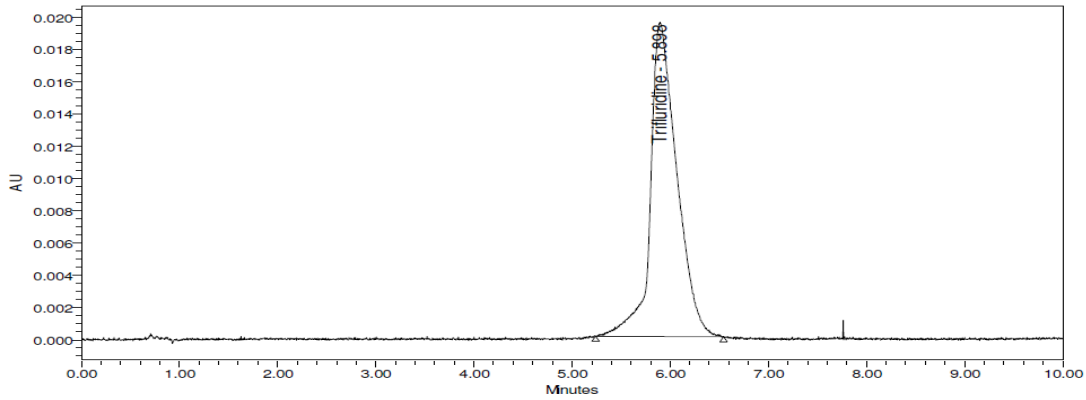

Fig.4: Representative chromatogram of trial-1.

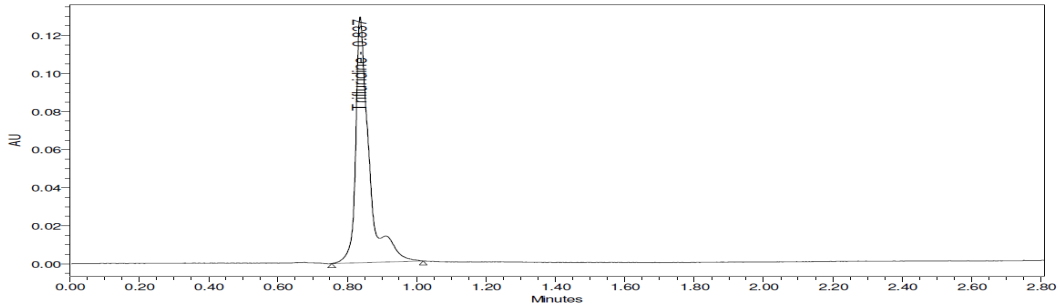

Fig.5: Representative chromatogram of trial-2.

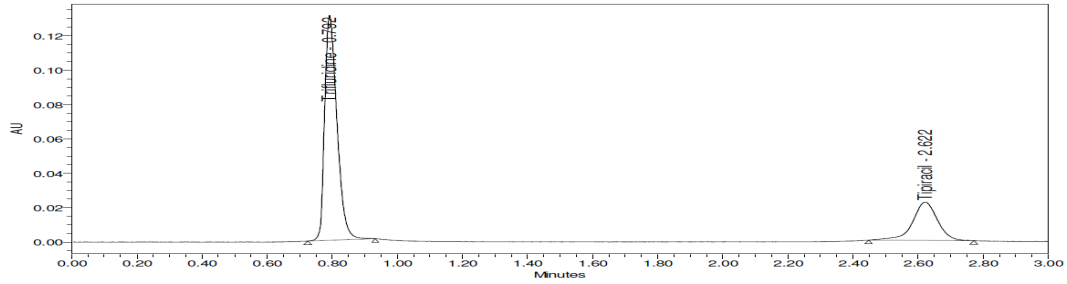

Fig.6: Representative chromatogram of trial-3.

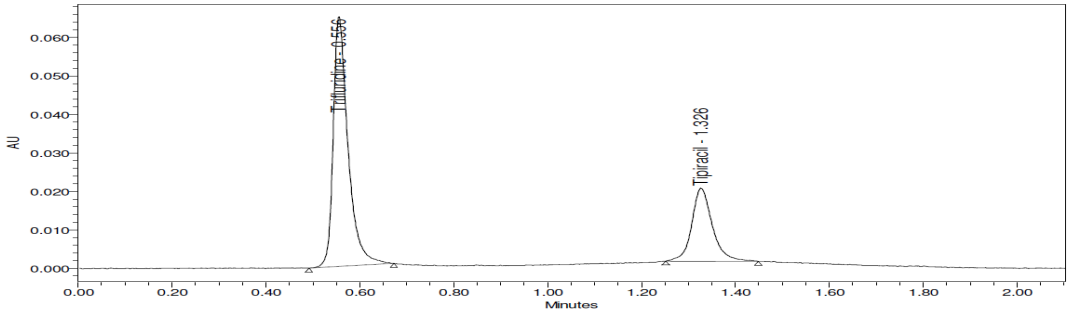

Fig.7: Representative chromatogram of trial-4.

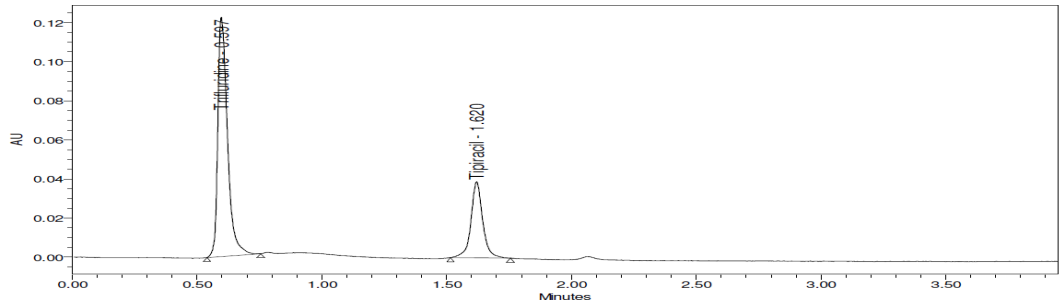

Fig.8: Representative chromatogram of trial-5.

It is the ability of a method to unequivocally evaluate the analyte components in 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 [16,17]. 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.9.

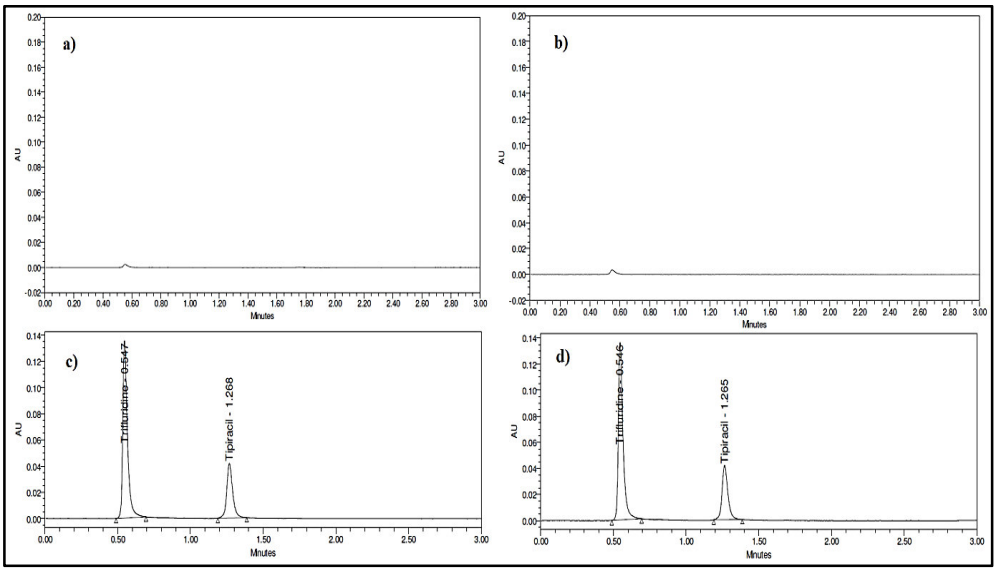

Fig.9: Chromatograms of a) blank, b) Placebo, c) Standard and d) Formulation.

Optimized Chromatographic Conditions

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

- Column : Hibera C18 100 x 2.1 mm, 1.8 µm
- Mobile Phase **:** Buffer: acetonitrile (50:50 %v/v) (Buffer: 1.0 mL of ortho phosphoric acid solution in a 1000 mL of milli-Q water to 0.1% OPA)
- Diluent **:** Water: acetonitrile (50:50 v/v)
- Flow Rate **:** 1.0 mL/min
- Column Temperature **:** 30 °C
- Injection Volume **:** 1.5 µL
- Wave Length **:** 230 nm
- Run Time **:** 3 mins

Assay of marketed formulation

Themarketed formulation of Lonsurf (film coated tablet) was evaluated by infusing 1.5 µl of reference and analyte solutions six times into the chromatographic system and the resulting chromatograms of analytes were documented. The quantity of anaytes existed in the marketed

formulation was estimated by equating the peak area of reference and analyte. The % assay of TFD and TPR were found to be 99.0–101.0%.

CONCLUSION

A sensitive, rapid and accurate RP-UPLC method for the simultaneous estimation of trifluridine and tipiracil in formulations was developed and validated as per the ICH guidelines. Retention times for trifluridine and tipiracilwas achieved at 0.549 min and 1.251 min respectively. Mean percentage assay of trifluridine and tipiracil were found to be 99.87%, and 99.98% respectively. Retention time and total run times of analytes were decreased. Hence, the developed method was rapid and economical that can be applicable in routine analysis of these drugs in quality control department of pharma industries.

REFERENCES

[1]. Chan WL, Lam KO, and So TH: Third-line systemic treatment in advanced/metastatic gastric cancer: a comprehensive review. TherAdv Med Oncol; 11, 2019, 1–11.

[2]. Smyth EC, Verheij M, Allum W: Gastric Cancer: ESMO Clinical Practice Guidelines. Ann Oncol 27(5), 2016, 38–49.

[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, Duggan ST:Trifuridine/tipiracil: a review in metastatic colorectal cancer. Drugs; 76, 2016, 1393–402.

[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 Science; 98, 2007, 779–89.

[8]. Peters GJ,Bijnsdorp IV: TAS-102: more than an antimetabolite. The Lancet. Oncology; 13, 2012, e518-9.

[9]. Matsushita S, Nitanda T, Furukawa T, Sumizawa T, Tani A, Nishimoto K: The effect of a thymidine phosphorylase inhibitor on angiogenesis and apoptosis in tumors. Cancer Research; 59, 1999, 1911–6.

[10]. Hoff PM, Cassidy J, Schmoll HJ: The evolution of fluoropyrimidine therapy: from intravenous to ora. The Oncologist; 6, 2001, 3–11.

[11]. Mastanamma Sk. Nagaraju K, ReehanaSk, 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; 12, 2019, 117-126.

[12]. KusumaJogi, MandavaBasaveswaraRao, Rundraraju Ramesh Raju. 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 ;7, 2017, 63-70.

[13]. SwapnaGoday, Abdul rahaman SK, 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; 5, 2017, 93-104.

[14]. 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 International Journal of Medical and Pharmaceutical Sciences; 3, 2018, 55-58.

[15]. Prathap B, HariBaskarV, Kumar B, Raghu PS, Bharath Kumar Reddy S: 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; 8, 2017, 4514 – 4521.

[16]. ICH: Q2B. Harmonized Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva; 1996.

[17]. 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; 4, 2013, 4567-4572.