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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR STIMULTANEOUS ESTIMATION OF TRIFLURIDINE AND TIPIRACIL BY RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

K. Swapna[*1], D. Naresh[1], G.VIjaya Kumar[1], M.A.Haneef[2]

[1]KGR Institute of technology and management,Sy. No. 419, Rampally, Keesara ,Secunderabad, Telangana 501301,India.
[2]Research Scholar, Sri SatyaSai University of Technology and Medical Sciences, Sehore.M.P.466001.

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Tipiracil and Trifluridine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6 x 250mm, 5µm) column using a mixture of Acetonitrile: Water:Methanol (60:20:20v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 230nm. The retention time of the Tipiracil and Trifluridine was 2.8, 3.8 min respectively. The method produce linear responses in the concentration range of 10-50µg/ml for Tipiracil, and 66.6-330µg/ml of Trifluridine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Tipiracil, Trifluridine, RP-HPLC, PDA Detection, Method validation.

Corresponding Author: - **K. Swapna** Email: jsnraj2015@gmail.com

INTRODUCTION

Lonsurf is a novel oral nucleoside antitumor agent that consists of trifluridine (FTD) Figure.1 and tipiracil (TPI) Figure 2. Lonsurf is specifically indicated for patients with metastatic colorectal cancer. Trifluridine is Nucleoside Analog Antiviral a fluorinated thymidine analog with potential antineoplastic activity. Trifluridine is incorporated into DNA and inhibits thymidilate synthase, resulting in inhibition of DNA synthesis, inhibition of protein synthesis, and apoptosis [1]. Tipiracil is a drug used in the treatment of cancer and is Tipiracil helps to maintain the blood concentration of trifluridine by inhbiting the enzyme thymidine phosphorylase which metabolizes trifluridine**.** Trifluridine (FTD) is an antineoplastic nucleoside analog discovered by Heidelberger and others at the University of Wisconsin as a drug that inhibits thymidylate synthetase (TS) similarly to existing fluoropyrimidines but exerts a growth inhibitory effect mainly by being incorporated into DNA of tumor cells. A assay is reported in Literature that is An effective and sensitive chromatographic approach based on RP-HPLC for trifluridine and tipiracil in bulk and pharmaceutical dosage forms [2-4].

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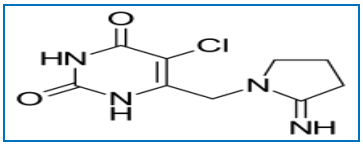
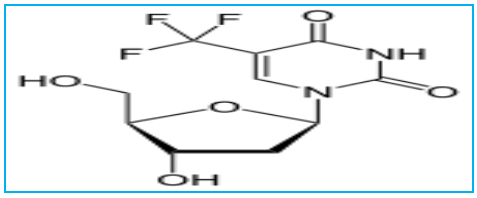
TIPIRACIL
Structure



Chemical name/ Nomenclature / IUPAC Name : 5-Chloro-6-[(2-imino-1-pyrrolidinyl)methyl]-2,4(1H,3H)-pyrimidinedione
Molecular Formula : C9H11ClN4O2
Molecular Weight : 242.67 g/mol [5]

TRIFLURIDINE
Drug category : Anti-Infective Agents.
Structure



Chemical name/ Nomenclature / IUPAC Name :1-[4-Hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-(trifluoromethyl)-(1H,3H)-pyrimidine-2,4-dione
Molecular Formula : C10H11F3N2O5
Molecular Weight : 296.2 g/mol [6].

EXPERIMENTAL

MATERIALS

Trifluridine (FTD) and tipiracil (TPI) standard supplied by Surapharma Lab, Dilshuknagar Hyderabad, India.Lonsurf containing 20 mg of FTD and 9mg of TPI were purchased from local market Hyderabad, India. Potassium dihydrogen phosphate, Hydrogen peroxide, Sodium hydroxide, HPLC methanol, Acetonitrile, orthophosphoric was purchased from Merk and S.D Fine chemical, India. MilliQ water was used throughout the experiment [7].

Equipments

Analysis was performed on a chromatographic system of Waters Alliance, with photodiode array detector. A chromatographic separation was achieved on kromasil (250 mm × 4.6 mm, 5 µm) analytical column. Data acquisition was made with Empower 2 software. The peak purity was checked with the Photo diode array detector [PDA] [8, 9].

Preparation of mobile phase

Accurately measured 700 ml (70%) of Acetonitrile and 300 ml of Water (30%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Optimized Chromatographic Condition

Mobile phase : Acetonitrile: Water:Methanol (60:20:20v/v)
Column : Hypersil C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 230 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume : 10 µl
Run time : 7 minutes

VALIDATION PARAMETERS

Precision

Preparation of Standard Solution

Accurately weighed and transferred 10 mg of Tipiracil and 10mg of Trifluridine working standard into a 10 ml of clean dry volumetric flasks individually and add about 7ml of Diluents to each volumetric flasks and sonicate to dissolve it completely and make up the volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml and 1.98ml of the above Tipiracil, Trifluridine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Accurately weigh 10 combination tablets crush in mortor and pestle and transfer equivalent to 10 mg ofTipiracil, Trifluridine (marketed formulation-dose of Tipiracil is 25 mg, Dose of Trifluridine is 50 mg in combination tablet) sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 1.98ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.The standard and sample solutions of 30µg/ml of Tipiracil, 198µg/ml of Trifluridine were injected for five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution

Accurately weighed 10 combination tablets crush in mortor and pestle and transfer equivalent to 10 mg ofTipiracil, Trifluridine (marketed formulation-dose of Tipiracil is 3mg, Dose of Trifluridine is 20mg in combination tablet) sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) .Further pipette 1.98ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.The standard and sample solutions of containing

concentrations were 30µg/ml of Tipiracil and 198µg/ml of Trifluridine.

Accuracy

Accurately weigh and transfer 10 mg of Tipiracil and 10mg of Trifluridine working standard into a 10 mL and 10 ml of clean dry volumetric flasks add about 7mL and 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml and 1.98ml of the above Tipiracil, Trifluridine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Linearity

The linearity range was found to lie from 10-50ppm of Tipiracil, 66.6µg/ml to 330µg/ml of Trifluridine and chromatograms are shown below.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Tipiracil LOD= 3.3×1921.9/9735 = 0.6µg/ml
Trifluridine LOD =3.3×34259/8804 **=** 12.8µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
Tipiracil: LOQ =10×1921.9/9735 =1.9µg/ml
Trifluridine: LOQ=10×34259/8804 =38.9µg/ml

ROBUSTNESS

The standard and samples of Tipiracil and Trifluridine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 1. Precession data of Tipiracil

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tipiracil	2.808	368013	46097	4536	1.6
2	Tipiracil	2.808	372552	46244	4236	1.6
3	Tipiracil	2.808	367873	46092	4565	1.6
4	Tipiracil	2.808	375555	46312	4682	1.6
5	Tipiracil	2.808	374843	46275	4521	1.6
6	Tipiracil	2.808	368013	46097	4561	1.6
Mean			371767			
Std. Dev			3663.5			
% RSD			0.9			

Table 2.precession data of Trifluridine

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Trifluridine	3.880	2321302	241739	4641.3	1.5	4.5
2	Trifluridine	3.880	2308016	241530	4632.2	1.5	4.5
3	Trifluridine	3.880	2326058	241796	4621.6	1.5	4.5
4	Trifluridine	3.880	2334897	241910	4695.3	1.5	4.5
5	Trifluridine	3.880	2326143	241799	4691.7	1.5	4.5
6	Trifluridine	3.880	2324512	241639	4685.1	1.5	4.5
Mean			2323283				
Std. Dev			9845.8				
% RSD			0.42				

Table 3. Accuracy data of Tipiracil

%Concentration (at specification Level)	Peak Area	Amount Added(mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	569325	5	4.9	98%	99.8%
100%	753538	10	10.1	101%	
150%	955999	15	15.1	100.6%	

Table 4. Accuracy data of Trifluridine

%Concentration (at specification Level)	Peak Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3441832	5	4.9	98%	99.4%
100%	4517559	10	10.1	101%	
150%	5738638	15	14.9	99.3%	

Table 5. Linearity data of Tipiracil

S.No	Linearity Level	Concentration(ppm)	Area
1	I	10	108407
2	II	20	206978
3	III	30	299892
4	IV	40	393459
5	V	50	491862
Correlation Coefficient			0.999

Table 6. Linearity data of Trifluridine

S.No	Linearity Level	Concentration(ppm)	Area
1	I	66.6	606125
2	II	132	1208367
3	III	198	1804843
4	IV	264	2371642
5	V	330	2885708
Correlation Coefficient			0.999

Table 7. Robustness data of Tipiracil

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2741.14	1.71
2	1.0	2423.3	1.6
3	1.1	2543.21	1.58

Table 8. Robustness data of Trifluridine

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4162.06	1.57
2	1.0	4641.3	1.5
3	1.1	3921.45	1.49

Fig 1. Typical Chromatogram of Mixed Working standard solution

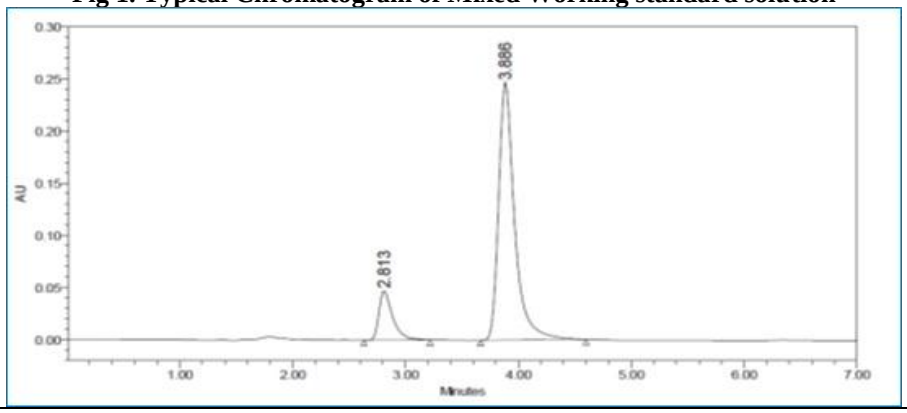


Fig 2. Calibration graph for Tipiracil

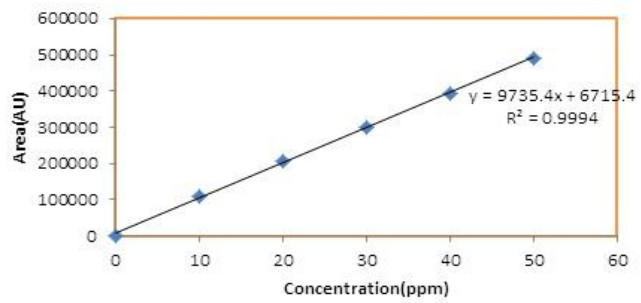
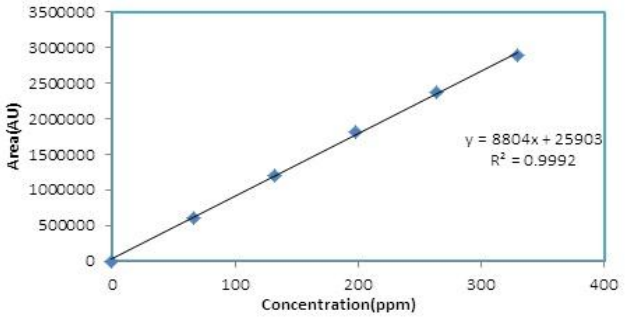


Fig 3. Calibration graph for Trifluridine



SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Tipiracil and Trifluridine was done by RP-HPLC. The mobile phase was optimized with consists of Acetonitrile: Water. Acetonitrile mixed in the ratio of 70:30 % v/ v. A Hypersil C_{18} column (4.6 x 150mm, 5µm, Make: Waters) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1ml/min. the linearity range of Tipiracil and Trifluridine were found to be from 10-50ppm, 66.6-330ppm respectively. Linear regression coefficient was not more than 0.999, 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery of Tipiracil is 99.6 and Trifluridine is 99.8%. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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CONFLICT OF INTEREST

No interest

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