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DEVELOPMENT OF SIMULTANEOUS SPECTROPHOTOMETRIC METHODS OF TRIFLURIDINE AND TIPIRACIL HCL BY VIERORDT'S METHOD IN PURE FORM AND COMBINED MICROSPHERE DOSAGE FORM

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ABSTRACT

Introduction: A simple specific spectrophotometric method that is accurate, and suitable for the simultaneous determination of Trifluridine and Tipiracil HCl in the combined dosage form. For the treatment of metastatic gastric cancer, a combination of TFD and TP HCl is already in use. **Materials and Method:** According to ICH guidelines, the simultaneous equation method was used to determine the concentrations of Trifluridine and Tipiracil HCl in the combined dosage form. 0.1 N HCl (pH 1.2) was used as a solvent for the spectrophotometric method development. **Results:** λmax of Trifluridine and Tipiracil HCl was found to be 262 and 276 nm respectively. Beer's law was followed over the 5- 30 µg/ mL concentration ranges for TFD and 2.5-15µg/ mL for TP HCl. The linearity of the proposed methods was tested between 2.5 and 15 µg/ml for TP HCl and 5 and 30 µg/ml for TFD. The % assay for the formulation was found to be 99.12±0.002 for TFD and 99.42±0.002 for TP HCl by the methods. The accuracy was observed to be 101%for TFD and 99.9%for TP HCl by simultaneous equation method. LOD and LOQ were 0.61 and 1.87µg/ml for TFD, 1.15µg/ml, and 3.5 µg/ml for TP HCl.**Conclusion:** The simultaneous determination of TP HCl, TFD, and pharmaceutical dosage form was successfully accomplished using Vierordt's method, and the resulting results were confirmed to be accurate, quick, and valid.

Keywords: Trifluridine; Tipiracil HCl; Vierordt's Method; Validation, Microsphere

INTRODUCTION

The growth of cells that begins in the stomach is called gastric cancer. Metastatic or localized advanced gastric cancer are both possible. A mutation in the structure of DNA in cells triggers gastric cancer, which can disrupt the cells' growth. Around 782,685 people worldwide die from gastric cancer each year, making it the fourth leading cause of cancer-related death worldwide. **[1, 2]**. The potent cytotoxic agent trifluridine was first produced in the 1960s. In the liver and gastrointestinal tract, Trifluridine is rapidly metabolized by thymidine phosphorylase (TPase) to inactive forms with decreased cytotoxicity, resulting in its poor bioavailability and toxicity when taken alone. Trifluridine's metabolic degradation is stymied by the thymidine phosphorylase (TPase) inhibitor Tipiracil HCl. As a result, the required cytotoxicity of Trifluridine is increased when it is combined with Tipiracil HCl. In order to inhibit Trifluridine's catabolism and increase Trifluridine's bioavailability, tipiracil is combined with Trifluridine in a 1:0.5 ratio. Trifluridine/tipiracil is the drug of choice for treating gastric cancer in the third or second line **[3, 4]**.

Literature survey: The literature review reveals that while HPTLC, RP-HPLC HPLC,and LC-MS methods are reported for the determination of Trifluridine and Tipiracil individually in various pharmaceutical dosage forms, no UV spectrophotometric method was reported for the simultaneous estimation of trifluridine and Tipiracil. However, there are a few methods reported for the combination of trifluridine and Tipiracil HCl with another drug in the pure and dosage form. This work proposes a modestly validated analytical method for simultaneously estimating Trifluridine and Tipiracil in their microsphere and pure dosage forms **[5-7]**.

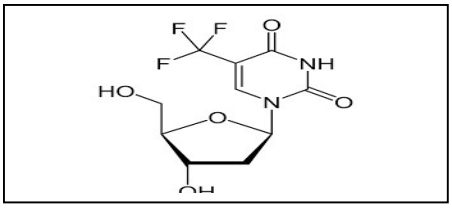

Figure 1: Structure of Trifluridine

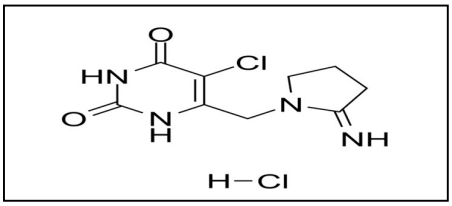

Figure 2: Structure of Tipiracil HCl

MATERIALS AND METHODS

Instrumentation

UV Spectrophotometry

A Shimadzu Model UV 1800 double beam UV-visible spectrophotometer with a pair of 1 cm-matched quartz cells and a spectral slit width of 2 nm.

Chemicals and Reagents

Trifluridine and Tipiracil HCl were kindly supplied as Emcure pharmaceuticals, Ahmedabad, Gujarat, and HCl (S.D fine

chemical Ltd., Mumbai, India) were used. All chemicals and reagents were of analytical reagent grade.

Method development by simultaneous equation method

Scanning of Trifluridine and Tipiracil HCl in 0.1 N HCl

Using a double beam UV/Vis spectrophotometer, the standard solutions of Trifluridine (20 µg/ml) and Tipiracil HCl (10 µg/ml) were scanned between 200 and 400 nm to record the spectra against 0.1N HCl as a blank. The wavelength maxima of Trifluridine and Tipiracil HCl were determined from the spectra recorded. The λ_{max} for Trifluridine and Tipiracil HCl were 262nm and 276nm, as shown in **Figure 3 [7, 8]**.

Preparation of standard solution for Tipiracil Hcl and Trifluridine (1000µg/ml)

Tipiracil HCl and Trifluridine weighed individually 100 mg and transferred in 100 ml of 0.1 N HCl in two separate volumetric flasks to get a concentration of 1000µg/ml. From these standard stock solutions, 10 ml solution was withdrawn and transferred to a 100 ml volumetric flask and the volume was made up of 0.1 N HCl up to 100 ml to get 100µg/ml. The series of further dilution for Tipiracil HCl was made in the range of 2.5 - 15 µg/ml whereas Trifluridine was from 5-30 µg/ml.

Vierordt's Simultaneous Equation Method Development.

The simultaneous equation approach was used to analyze both medications using their overlay spectra at wavelengths of 262 nm for TFD and 276 nm for TP, respectively, which correspond to the maximum wavelengths for each drug. In light of this, it might be able to identify both medications using the simultaneous equation method. In 0.1 N HCL, five standard solutions with concentrations of 5- 30 g/mL for TFD and 2.5-15 g/mL for TP HCl were made, and their corresponding absorbances were measured at 276 nm and 262 nm. The formula below was used to calculate the amount of drug x (TP) and y (TFD) present in sample solutions.

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

where Cx is the concentration of TP and Cy is the concentration of TFD, $ax1$ absorptivity of TP at 276 nm and $ax2$ absorptivity of TFD 262 nm, and $ay1$ and $ay2$ are absorptivity's of TFD at 276 nm and 262 nm, respectively $A1$ and $A2$ are the absorbance of sample solution at 276 nm and 262 nm, respectively **[9]**.

RESULTS

Validation of the developed method [10-17]

The following parameters are considered under the ICH guidelines for the validation of the method

Linearity: Fresh aliquots were prepared from the stock solution of TP (100 µg/ml) ranging from 2.5 – 15 µg/ml and TFD (100 µg/ml) ranging from 5 – 30 µg/ml and they were transferred into a 10 ml volumetric flask and diluted up to 10 ml using 0.1 N HCl as a solution. The absorbance of the solution was then measured at 276 and 262 nm. The calibration curve was constructed by plotting absorbance vs concentration and the regression coefficient equation was calculated. The results of the same are shown in **Table 3**.

Precision: Inter-day precision was performed by analyzing three different concentrations of drugs for three different days. Different concentrations of a standard solution of TP and TFD were measured for intraday and interday. It is expressed as relative standard deviation (%RSD).

Limit of detection and Limit of quantification: The limit of detection (LOD) and limit of quantitation (LOQ) of Tipiracil HCl and Trifluridine by proposed methods were determined using calibration standards. "LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding calibration curve using the equations". LOD and LOQ were calculated as

$LOD = 3.3 \times \sigma/s$, $LOQ = 10 \times \sigma/s$

Where s is the slope of the calibration curve and σ is the standard deviation of the y-intercept of the regression lines (six independent measurements of a sample with very low concentrations).

Accuracy: The recovery method determined the accuracy of the proposed method**.** A known quantity of Trifluridine and standard Tipiracil HCl at three distinct concentration levels—80%, 100%, and 120%. The percentage of recovery was calculated by comparing the absorbance before and after the addition of the standard drug.

Assay: The microspheres were accurately weighed before being crushed into powder. A 100 ml volumetric flask containing powder equivalent to 45/90 mg of TP HCl/TFD was weighed, the volume was filled to the mark with 0.1 N HCl after 24 hours, and the solution was filtered. To get the concentration of 10 µg/mL of TP HCl and 20 µg/mL of TFD, an aliquot of 1 mL of the sample stock solution was moved to a 10-ml standard volumetric flask, and the volume was changed in accordance with mark with 0.1 N HCl.

DISCUSSION

Table 1: Absorptivity value for Tipiracil HCl

conc(µg/ml)	Absorbance λ1- 276 nm	Absorptivity λ1- 276 nm	Absorbance λ2- 262 nm	Absorptivity λ2- 262 nm
2.5	0.070	280	0.0533	213.5
5	0.126	252	0.0899	179.8
7.5	0.188	250.66	0.134	178.66
10	0.254	254	0.182	182
12.5	0.308	246.4	0.220	176
15	0.373	248.66	0.267	178
Absorptivity for 255.28		Absorptivity 184.66		

Table 2: Absorptivity value for Trifluridine

conc(µg/ml)	sorbance λ1- 276 nm	osorptivity λ1- 276 nm	sorbance λ2- 262 nm	Absorptivity λ2- 262 nm
5	0.0942	188	0.175	350
10	0.192	192	0.362	362
15	0.277	184.66	0.530	353.33
20	0.372	186	0.708	354
25	0.450	180	0.866	346.4
30	0.541	180.33	1.04	347.66
Absorptivity for 185.16		Absorptivity for 352.23		

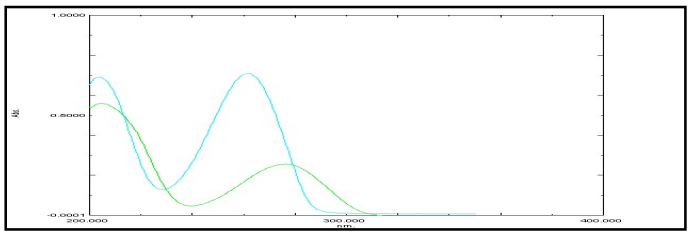

Figure 3: overlain simultaneous equation spectra of TP HCl (10µg/ml) and TFD for (20µg/ml)

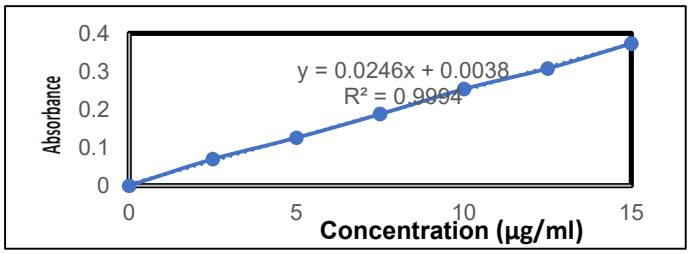

Figure 4: Calibration Curve of TP in 0.1N HCl at 276 nm

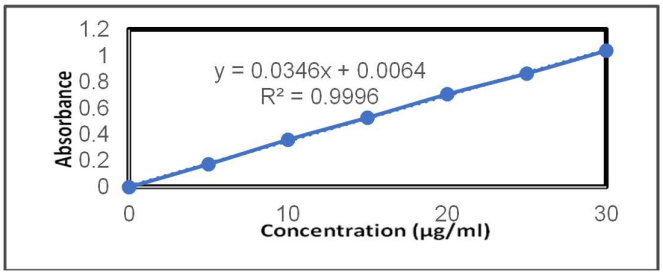

Figure 5: Calibration Curve of TFD in 0.1N HCl at 262 nm

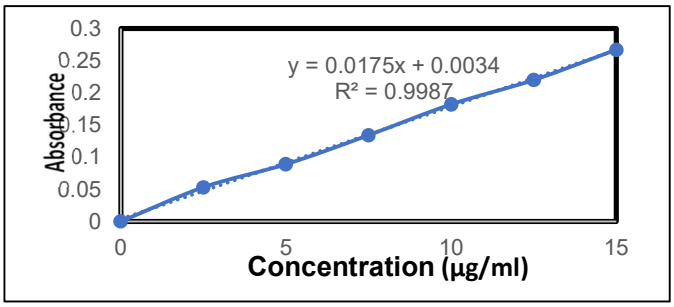

Figure 6: Calibration Curve of TP in 0.1N HCl at 262nm

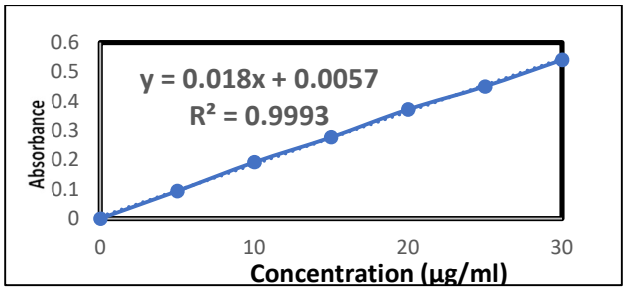

Figure 7: Calibration Curve of TFD in 0.1N HCl at 276 nm

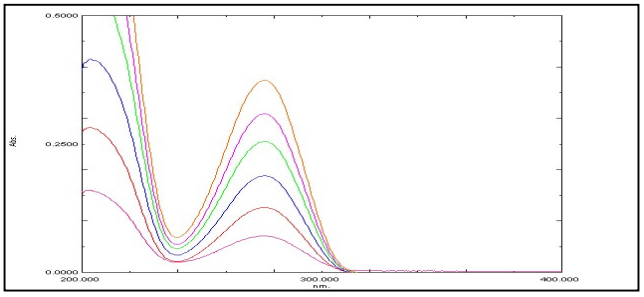

Figure 8: overlain spectra of Trifluridine

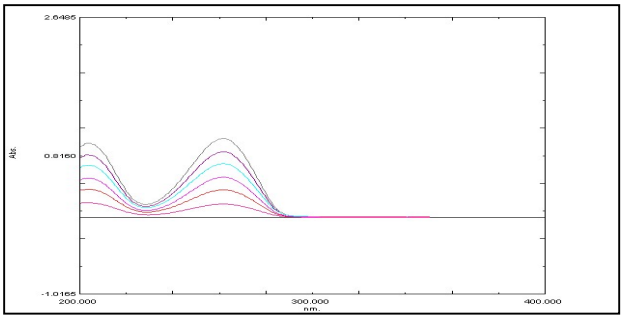

Figure 9: overlain spectra of Tipiracil HCl

Table 3: Linearity of TP

Conc(µg/ml)	Absorbance ±SD	%RSD
2.5	0.160 ± 0.001789	1.118034
5	0.329±0.004355	1.323731
7.5	0.479±0.003933	0.821037
10	0.640±0.007763	1.212994
12.5	0.814±0.011737	1.441942
15	0.974±0.010727	1.101326

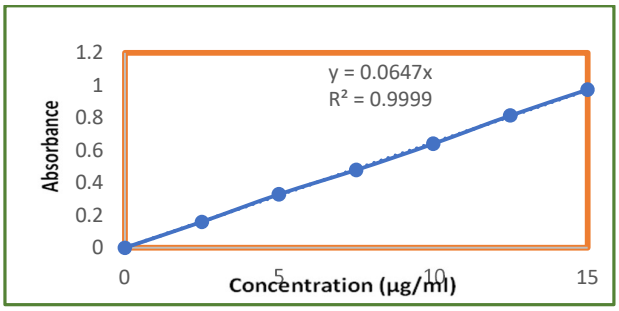

Figure 10: calibration curve of TP at 276nm

Table 4: Linearity of TFD

Conc(µg/ml)	Absorbance ±SD	%RSD
5	0.222 ± 0.003011	1.356347
10	0.453±0.004517	0.99705
15	0.671±0.006463	0.963147
20	0.883±0.013688	1.550192
25	1.11±0.02137	1.925204
30	1.3±0	0

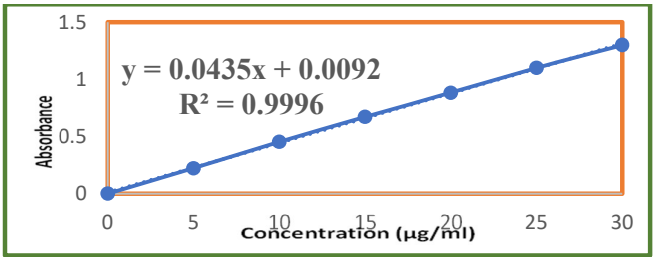

Figure 11: calibration curve of TP at 276nm

The correlation coefficients for TP and TFD were found to be 0.9998 and 0.9996, respectively. In the given range, the method was found to be linear as a result **(Table 6)**. By analyzing the three distinct drug concentrations three times on the same day, an intraday study was carried out **(Table 7)**. % RSD of TP was found to be 0.96 to 1.53 at 276 nm. For TFD was found to be 1.04 to 1.55 at 262 nm. Thus the result was found to be within the acceptable criteria (<2) **(Table 7)**.

Table 6: Interday precision

Conc.(µg/ml)		Mean absorbance ± SD (n=3)		% RSD	
TP	TFD	TP	TFD	TP	TFD
2.5	5	0.165±0.003055	0.223±0.004583	1.84	1.99
5	10	0.331±0.003512	0.447±0.001528	1.05	1.72
7.5	15	0.480±0.02658	0.680±0.03572	1.11	1.91

Table 7: Intraday precision

Conc. (µg/ml)		Mean absorbance ± SD (n=3)		% RSD	
TP	TFD	TP	TFD	TP	TFD
2.5	5	0.164±0.0025	0.226±0.003512	1.53	1.55
5	10	0.329±0.004619	0.449±0.006429	1.40	1.43
7.5	15	0.476±0.004583	0.665±0.006928	0.96	1.04

Accuracy:

Table 8: Results of accuracy

Drug	% Conc	Conc (µg/ml)	Amount recovery ± SD (n=3)	% Recovery	RSD (%)
TP HCL	80	10	9.92±0.106	99.2	1.06
	100		9.9±0.110	99	1.11
	120		10.2±0.116	102	1.13
TFD	80	20	19.96±0.20	99.8	1.00
	100		20.2±0.203	101	1.00
	120		20.5±0.104	102.5	0.507

According to ICH, the recovery of TP and TFD was found to be within acceptance criteria of 98 to 102% **(Table 8)**.

Limit of Detection and Limit of Quantitation: For TP and TFD, the LOD was determined to be 1.15 µg/ml at 276 nm and 0.61 µg/ml at 262 nm, respectively, and the LOQ was determined to be 3.5 µg/ml at 276 nm and 1.87 µg/ml at 262 nm.

Discussion

The % assay for the formulation was found to be 99.12±0.002 for TFD and 99.42±0.002 for TP HCl by the methods **(Table 9)**.

Table 9: Results of the assay

Drug	Label claim (mg)	Amount found(mg)	%Drug content	SD	% RSD
TFD	90	89.22	99.12	0.020	0.02
TP HCl	45	44.59	99.42	0.102	0.28

Table 10: Regression and optical characteristics of Tipiracil HCl and Trifluridine

Parameters	TP HCl at 276 nm		TFD at 262 nm	
λmax	276	262	262	276
Beer's Law range µg/ml	2.5-15	2.5-15	5-30	5-30
Slope	0.0246	0.0175	0.0346	0.018
Intercept	0.0038	0.0034	0.0064	0.0057
Regression coefficient	0.999	0.998	0.999	0.999
LOD	1.15	-	0.61	-
LOQ	3.5	-	1.87	-
Accuracy	9.92±0.106		19.96±0.20	
	9.9±0.110		20.2±0.203	
	10.2±0.116		20.5±0.104	

CONCLUSION

In accordance with ICH guidelines, simultaneous estimation of Trifluridine and Tipiracil HCl in their microsphere dosage form was developed and validated. For Trifluridine, linearity ranged from 5 to 30 µg/ml, while for Tipiracil HCl, linearity ranged from 2.5 to 15 µg/ml. Precision for Tipiracil HCl and Trifluridine is exemplified by a relative standard deviation of 0.96 to 1.53 at 276 nm. For TFD was found to be 1.04 to 1.55 at 262 nm. The percentage of Mean recovery for TP and TFD was found to be in the range of 98-102, during accuracy studies. By reviewing all the validation parameters (Linearity, Precision, Accuracy, LOD, and LOQ). For the purpose of determining Trifluridine and Tipiracil HCl, we discovered that the procedures were precise, and quick.

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