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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TRIFLURIDINE AND TIPIRACIL IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, specific, accurate and precise reverse phase HPLC method has been developed and validated for the simultaneous estimation of Trifluridine (FTD) and Tipiracil (TPI) in Tablet dosage form. Chromatographic separation of FTD and TPI were successfully achieved on an Agilent ODS C 18 (150 X 4.6mmX5µm) analytical column. A mixture of phosphate buffer and methanol in the ratio of 30:70 %v/v (pH 3.0) was used as the mobile phase at a flow rate of 1.0ml/min and detection wavelength at 240nm. Calibration curve showed correlation coefficient of 0.999 for both drugs. The percentage recovery was 100.15% and 100.51% respectively.FTD and TPI indicating accuracy and reliability of method. The method was found to be precise as indicating by the repeatability of the analytical procedure, showing %RSD < 2%. Both drugs were eluted within 6 minutes and gave sharp peaks with high theoretical plate count and low tailing factor. The HPLC method was fully validated as per International Conference on Harmonization (ICH). The developed method can be used for routine analysis.

INTRODUCTION

Drug analysis plays an important role in the development of drugs, their manufacture and therapeutic use. The number of drugs and drug formulations introduced into the market by pharmaceutical industries is increasing at an alarming time. These drugs and formulations may be either or partial modification of the existing one or they may be novel or multicomponent dosage forms. The complexity of these dosage forms possesses considerable challenge to the analytical chemist during the development of assay procedures. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and the final products obtained meet the required specification. Trifluridine is a nucleoside analog antiviral fluorinated thymidine analog with potential antineoplastic activity. Trifluridine is incorporated into DNA synthesis, inhibition of protein

synthesis and apoptosis. Tipiracil is a drug used to treatment of cancer and is Tipiracil help to maintain concentration of trifluridine by inhibiting 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. Lonsurf is a novel oral nucleoside antitumor agent that consists of trifluridine (FTD) Figure.1. and tipiracil (TPI) Figure.2. Lonsurf is specifically indicated indicated for patients with metastatic colorectal cancer. FTD is chemically 1-[(2R, 4S, 5R) – 4-hydroxy-5-(hydroxymethyl) oxolan-2-yl]-5-(trifluoromethyl)-1, 2, 3, 4-tetrahydropyimidine-2, 4-Dione. TPI is chemically 5-Chloro-6-[(2-iminopyrrolidin-1yl) methyl]-1, 2, 3, 4-tetrahydropyrimidine-2, 4-dione [1-10]. There are few methods reported in the literature of trifluridine and tipiracil alone or in combination with other drugs in the pure and pharmaceutical formulation by UV Spectrophotpmeter, HPLC, HPTLC and LC-MS [13-14]. In view of the need for suitable, cost-effective RP-HPLC method for routine analysis of simultaneous estimation of Trifluridine and Tipiracil in pure and tablet dosage form, attempts we made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Trifluridine and Tipiracil. The proposed method will be validated as per ICH guidelines.

EXPERIMENTAL

Materials

Trifluidine (FTD) and tipiracil (TPI) standard supplied by PharmaTrain Laboratories, Hyderabad, India. Lonsurf (tablet dosage form) containing 15mg of FTD and 6.14mg of TPI were purchased from local market. The purity of the drug was evaluated and no impurities were found. The drug was used without further purification. All the reagents used in the experimental work were of analytical grade was purchased from Merk and Rankam, India. HPLC grade water and Methanol was made from Milli-Q USA. Mobile phase was used as solvent.

Chromatographic conditions (instrumentation and analytical conditions)

An Alliance 2695 Waters, chromatographic system was used equipped with quaternary pump and water 2487 UV detector, Agilent C 18 250 X 4.6 mm, 5µ, auto sampler thermostat and degasser. Chromatographic software Empower was used for data collection and processing separations were performed using Agilent C18 analytical column, 250X4.6mm packed with 5µm particle size. Injection volume was 10µL. Separations and simultaneous determination of FTD and TPI were performed using the mixture of Methanol: buffer pH 3 (70:30%v/v) as a mobile phase. Mobile phase was filtered through a 0.45µm Millipore filter. The flow rate was 1.0mL/min and UV detection was performed at 240nm.

Method Development

Selection of Detection wavelength

The UV spectrum of diluted solutions of various concentrations of Trifluridine and Tipiracil in mobile phase was recorded using UV spectrophotometer. The wavelength of the maximum absorbance was observed at 240nm. This wavelength was used for detection of Trifluridine and Tipiraci.

Preparation of standard solutions

10mg of Trifluridine and 6mg of Tipiracil standard was accurately weighed and transferred into 10ml clean dry

volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. The solutions were filtered through a 0.45µm membrane filter before injection into the HPLC system.

Preparation of sample solution

10 tablets were carefully weighed and powdered to get a homogenous fine powder in a mortar. An appropriate weight of this powder equivalent to one tablet content was weighed, transferred into the calibrated volumetric flask and dissolved in sufficient mobile phase in an ultrasonic bath. Further dilute 0.1ml of the above solution to 10ml with diluent. Filter through 0.45µm Nylon syringe filter to obtain the certain concentration in the linearity range of FTD and TPI for HPLC.

VALIDATION PROCEDURE[11-12]

Chromatographic separation was optimized in the aim to obtain a resolution above 1.5 between all components with the respect of stationary and mobile phase compositions, flow rate, sample volume, detection wavelength and temperature. The method was validated for linearity, range, accuracy, precision (repeatability and intermediated precision), specificity, limit of quantization, limit of detection and robustness.

Linearity and Range

Standard calibration curve were prepared with five calibrations over a concentration range of 25 to 125µg/ml for Trifluridine and 15 to 75µg/ml for Tipiracil. The data of peak area versus drug concentration were treated by linear least square regression analysis. The standard curves were evaluated for linearity.

Accuracy (Recovery study)

The accuracy of the method was determined by calculating the recoveries of Trifluridine and Tipiracil by the standard addition method. Known amounts of standard solutions of Trifluridine and Tipiracil were added at (50%, 100% and 150%) concentration to pre-quantified sample solutions of Trifluridine and Tipiracil and the amount of drug recovered was estimated.

Precision

The precision of the assay was studied with respect to both repeatability and intermediated precision. Repeatability was calculated from five replicate injections of freshly prepared solution in the same equipment on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration on two additionally consecutive days to determine intermediate precision. Precision was expressed by the % of the relative standard deviation (R.S.D.) of the analyte peaks.

Specificity

In an assay demonstration of specificity requires to be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials. There should be no interference of the diluents and placebo at retention time of drug substance.

Limit of detection and Quantization

Limits of detection (LOD) and limits of quantization (LOQ) were provided and calculation was made with the following equations:

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

When σ was the standard deviation of the response (estimated from the standard deviation of y – intercepts or regression lines) and S was the slope of the standard curve.

Sensitivity

The sensitivity of an analytical method is defined by the minimum variation that requires to be applied to the magnitude measured in order to obtain a significant variation in the signal measured.

Robustness

Robustness of method was investigated by varying the chromatographic conditions such as change of flow rate, organic content in mobile phase. Robustness of the developed method was indicated by the overall %RSD between the data at each variable condition.

RESULTS AND DISCUSSION

Optimization of HPLC conditions

Finally HPLC conditions were optimized to obtain a desired peak with high purity and resolution. Therefore the various parameters affecting the peak shape, retention time and resolution of FTD and TPI were investigated in detail. The separation efficiency of Agilent C18 (150X4.6mm, 5µ) was compared to the Intersil ODS (150X4.6mm,5µ) for the determination of FTD and TPI under the same conditions and the proposed column was chosen for the further optimization of parameters. During our preliminary experiments the series of aqueous mobile phases containing buffer solutions with the different pH values in combination with different organic modifiers including the different ratios of acetonitrile, methanol and triethylamine were tested for obtaining the optimum separation conditions. Methanol and phosphate buffer solution were selected as the eluents. The chromatographic analysis time of FTD and TPI was shortened with high organic solvent content and also the buffer solutions in the mobile phase ensured stable chromatographic retention times preventing broad peaks. The effect of the mobile phase pH on the retention time and peak shape of the analyte was studied especially in the acidic region. The best retention time and peak shape of FTD and TPI were achieved with phosphate buffer pH 3. The best separation was achieved with the mobile phase consisting of phosphate buffer pH 3: methanol (30:70%v/v). The retention time of Trifluridine and Tipiracil was found to be 2.262 mins and 4.260 mins respectively.

METHOD VALIDATION

The method was validated for linearity, precision, accuracy, robustness and ruggedness of the FTD and TPI. Linearity was prepared in the range of 25 to 125µg/ml for Trifluridine and 15 to 75µg/ml for Tipiracil solutions are analyzed through the high pressure liquid chromatographic techniques. The peak area were plotted against concentration was subjected to linear plots shown in figures 4 and 5. The calibration data is presented in table 1 and 2. Good recoveries (99 -100%) of the drug were obtained at each added concentration indicating that the method was accurate. The percentage of recovery data had shown in table 3 and 4. Precision of this method was studied in inter day and intraday variation. The precision of intraday studies of five different concentration of the drug was repeated thrice in a day and in the inter day variation studies of five different concentration of the drug was repeated on three consecutive days. The developed method was found to be precise as the percentage of RSD values for inters day and intraday precision studies were found to be less than 2%. The results of precision studies were shown in table 5 and 6.

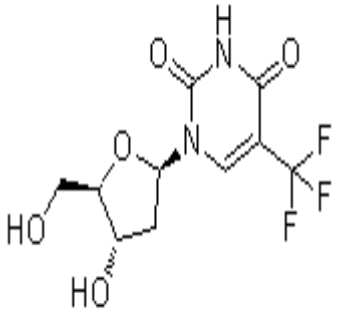

Figure 1: Structure of Trifluridine

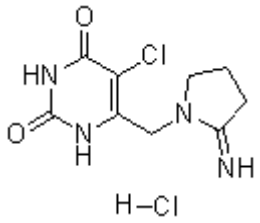

Figure 2: Structure of Tipiracil

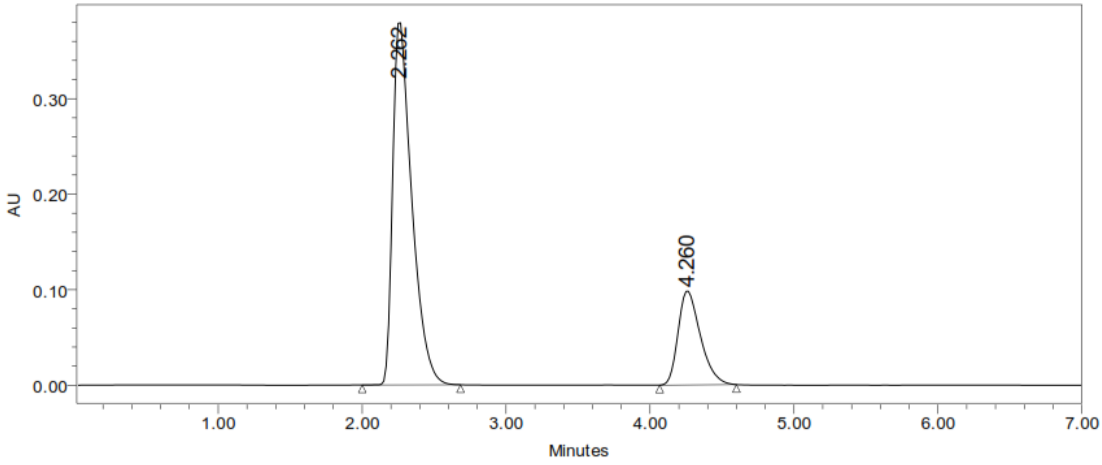

Figure 3: Chromatogram of Trifluridine and Tipiracil

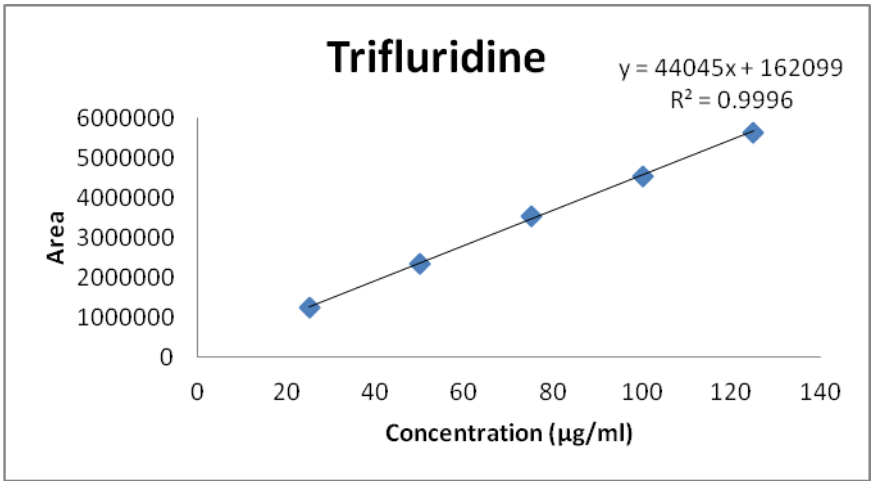

Figure 4: Calibration curve of Trifluridine

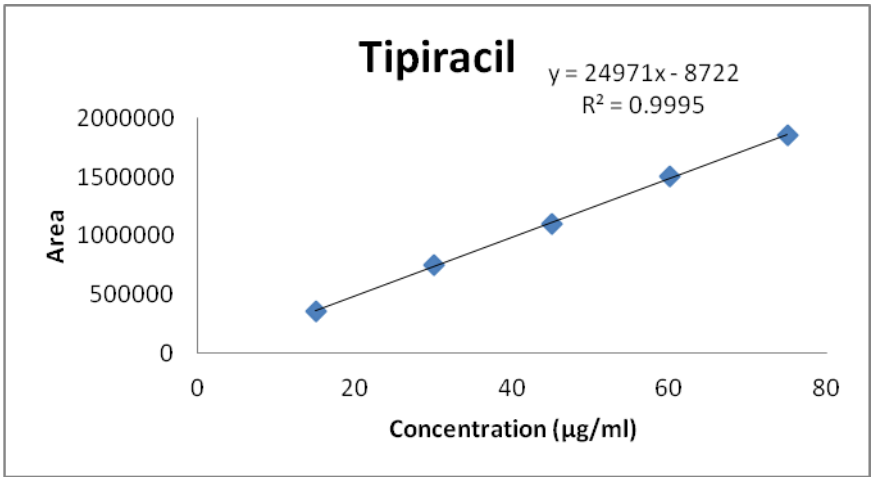

Figure 5: Calibration curve of Tipiracil

Concentration (µg/ml)	Area
25	1246587
50	2248079
75	3529801
100	4553376
125	5649583

Table 1: Calibration data for Trifluridine

Concentration (µg/ml)	Area
15	361744
30	751959
45	1068815
60	1503717
75	1858720

Table 2: Calibration data for Tipiracil

Recovery Level	Amount added (mg)	Amount found(mg)	Mean Area	Recovery	Mean recovery
50%	5.0	5.01	1829739	100.20%	100.15%
100%	10.0	9.96	3642243	99.60%	
150%	15.0	15.1	5535371	100.66%	

Table 3: Recovery Data for Trifluridine

Recovery Level	Amount added (mg)	Amount found(mg)	Mean Area	Recovery	Mean recovery
50%	5.0	4.98	564367	99.60%	100.51%
100%	10.0	10.2	1115445	102.00%	
150%	15.0	14.99	1682465	99.93%	

Table 4: Recovery Data for Tipiracil

Injection	Area	Area
	Trifluridine	Tripiracil
1	3367917	1025541
2	3324161	1023214
3	3390163	1023881
4	3323428	1020840
5	3329454	1026447
Mean	3347025	1023985
SD	30354.9	2178.2
%RSD	0.9	0.21

Table 5: Precision (Repeatability) data for Trifluridine and Tripiracil

Injection	Area	Area
	Trifluridine	Tripiracil
1	3663690	1059395
2	3609383	1056248
3	3642290	1054757
4	3653384	1058139
5	3635880	1057066
Mean	3540925	1057121
SD	98052.9	1772
%RSD	2.0	0.16

Table – 6: Intermediate Precision data for Trifluridine and Tripiracil

The limit of detection (LOD) for FTD and TPI were found to be 3.17 and 0.1372 µg/ml calculated from related equation (S/N = 3). The similar study claimed that a narrow working range (LOQ) such as 5.60 and 0.132µg/ml for FTD and TPI were obtained at the excitation wavelength of 240nm. Robustness of the method includes small changes in chromatographic conditions such as change in flow rate (± 20%) and organic content in mobile phase (± 5%). To determine the robustness of the method for the analysis of FTD and TPI the above mentioned changes has been undertaken and the USP plate count and tailing values were found to be reliable. The chromatographic data has been shown in figure 3.

CONCLUSIONS

A highly sensitive and effective validated reversed phase HPLC method was successfully developed for FTD and TPI assay. This method was validated for linearity, accuracy, precision and robustness of FTD and TPI drug. The RSD values for all parameters were found to be less 2 which indicates the validity of method and results obtained by this method are in fair agreement. Finally this method can be used as better analytical tool for pharmaceutical formulations for FTD and TPI.

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