Ind. J. Pharm. Edu. Res., 2024; 58(3s):s1041-s1052.
https://www.ijper.org

Original Article

A Rapid UPLC Technique for Quantification of Tegafur, Oteracil and Gimeracil in Bulk and Pharmaceuticals and its Validation

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ABSTRACT

Background: Tegafur (TEG) is a prodrug of 5-Fluorouracil (FU) that is mainly employed in the treatment of colorectal tumors combined with Gimeracil (GIM) and Oteracil (OTE) enhances its stability by enhancing the antineoplastic activity and reducing gastric irritation. **Aim:** The key focus of this research was to design a rapid Ultra Performance Liquid Chromatography (UPLC) and validate that it is uncomplicated, exact, responsive and reliable for quantifying the concentrations of tegafur, oteracil and gimeracil in both their pure state and pharmaceutical formulations. **Materials and Methods:** The UPLC method was developed using HSS C8 (100 mmx2.1 mm, 1.8 µm) column and the mobile phase was composed of phosphoric acid at a concentration of 0.1% v/v, with a pH of 2.0 and methanol in an 80:20 ratios. The process was carried out at a flow rate of 0.2 mL min^{-1}, with injection volume of 5 µL and absorbance maxima of 282 nm. The technique was validated following the ICH standards. UPLC is a superior technique regarding performance thus it was chosen. **Results and Discussion:** The elution time obtained was found to be below 2 min for all three drugs. The validated method's linearity was determined to be between 40 and 240 µg/mL for tegafur, 31.6-189.6 µg/mL for oteracil and 11.6-69.6 µg/mL for gimeracil. Tegafur, oteracil and gimeracil were found to have Detection Limit (LOD) and Quantitation Limit (LOQ) values of 0.3 µg/mL and 1 µg/mL, 0.2 µg/mL and 0.8 µg/mL, 0.1 µg/mL and 0.3 µg/mL, respectively. The developed method demonstrated high accuracy, as indicated by the RSD being less than 2%. **Conclusion:** Thus, it can be employed as a method for evaluating stability and conducting regular quality control inspections for tegafur, gimeracil and oteracil.

Keywords: Tegafur, Gimeracil, UPLC, Oteracil, Stability.

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Received: 04-02-2024;
Revised: 26-02-2024;
Accepted: 31-03-2024.

INTRODUCTION

Tegafur (TEG) is a prodrug of 5-Fluorouracil (FU) that is mainly employed in the treatment of colorectal tumors combined with other chemotherapeutic agents.[1,2] Chemically tegafur (Figure 1A) is 1-[2-tetrahydrofuranyl]-5-fluoro uracil.[2] The liver degrades a significant quantity of tegafur via dihydroxy pyrimidine dehydrogenase, producing the inactive metabolite dihydro fluorouracil. To impede this degradation, tegafur is combined with Gimeracil (GIM), inhibiting degradation and Oteracil potassium (OTE), which inhibits the enzyme pyrimidine phosphoribosyl transferase, which leads to a decrease in the phosphorylation of 5-fluorouracil in the gastrointestinal tract decreasing the toxicity and unpleasant reactions associated with oral administration in the gastrointestinal tract thus both substantially enhances its stability by enhancing the antineoplastic activity and reducing gastric irritation.[3,4,6] Chemically oteracil potassium (Figure 1B) is 1, 4, 5, 6-tetrahydro-4, 6-dioxo-1, 3, 5-triazine-2-carboxylic acid potassium and Gimeracil (Figure 1C) is 5-chloro-2,4-dihydroxypyridine and Oteracil.[5,6] The combination of three pharmacological compounds was authorized By European Medicines Agency and Central Drugs Standard Control Organization (CDSCO) as antineoplastic agent for colorectal cancers. Additional clinical trials are anticipated to be conducted to advance the use of these combination drugs as the primary treatment regimen for advanced gastric malignancies.[7]

Prior analyses of tegafur, oteracil potassium and gimeracil were conducted using a variety of analytical techniques, including Reverse Phase High-Performance Liquid Chromatography (RP HPLC), Liquid Chromatography-Tandem Mass Spectrophotometry (LC-MS/MS) for bioanalytical quantification and HPLCs for the development of relative substance

DOI: 10.5530/ijper.58.3s.104

methods.[8-10] Furthermore, the literature also reports using Liquid Chromatography-Tandem Mass Spectrophotometry for the bioanalytical measurement of tegafur and Gimeracil. The literature review also uncovers the analysis of impurities using High-Performance Liquid Chromatography-Quadrupole Time of Flight Mass Spectroscopy (HPLC QTOF MS), as well as the determination of Tegafur alongside other medicinal substances using various liquid chromatographic techniques.[11-14] There is one method reported for simultaneous estimation of gimeracil, oteracil and tegafur by Ultra Performance Liquid Chromatography (UPLC) in the reverse phase mode.[15]

In the current study, A relatively rapid UPLC method was utilized to develop and validate the assay method for TEG, OTE and GIM in formulation. This method offers numerous benefits in comparison to reported UPLC and other conventional analysis methods, including a substantial reduction in analysis time and better mean resolution and sensitivity. Stability studies were also required as part of the experimental method development, encompassing forced acidic, alkaline, thermal and oxidative degradation processes as per International Conference of Harmonization (ICH) Q1A(R2) and Q1B and validation performed as per ICH Q2 (R1) guidelines.[16-18]

MATERIALS AND METHODS

Experimental Materials

TEG, OTE and GIM reference standards were procured from Venkatasai Life Sciences, Hyderabad, India. (97.5-99.0% purity). Tegonat® 20 (capsule dosage form containing 20 mg Tegafur, 5.8 mg of gimeracil and 15.8 mg of oteracil potassium) was purchased from Varun medicals, Nagpur, India. HPLC grade Acetonitrile and Methanol obtained from Merck India, ltd. All other solvents and reagents were of analytical reagent grade obtained from SD fine Chem, Mumbai. Milli-Q water was used throughout the analysis obtained from Millipore Milli Q purification system, Bangalore.

Instruments used

Acquity UPLC™ from Waters Corporation equipped with a sample manager, binary solvent manager, injector, temperature-controlled column and Photo Diode Array (PDA) detector was used to develop a rapid method. To process signals, the Empower 2 software was utilized HSS C8 Columns with dimensions of 100x2.1 mmx1.8 µm were utilized during the process of method development. In addition to that, we made use of a weighing balance (PI-214), a pH meter Thermo Fischer), a vortex mixer (Remi CM-101) and a centrifuge (CM 01).

Chromatographic conditions

ACQUITY UPLC HSS C8 column (100 mm(length)x2.1 mm (internal diameter)x1.8 µm (particle size)) regulated to 30°C, with mobile phase proportion of 80:20 of 0.1% phosphoric acid, (pH 2.0) and methanol was used for separation. The analysis was performed for 2.0 min at 282 nm with an injection load of 5 µL and a flow velocity of 0.2 mL/min.

Preparation of mobile phase

0.1%v/v phosphoric acid was made with Milli Q water. The mobile phase is made by combining phosphoric acid and methanol at a proportion of 80:20 (v/v) cleaned up through 0.22 µm PVDF fine porosity membrane filters. The mobile phase is also utilized as a diluent.

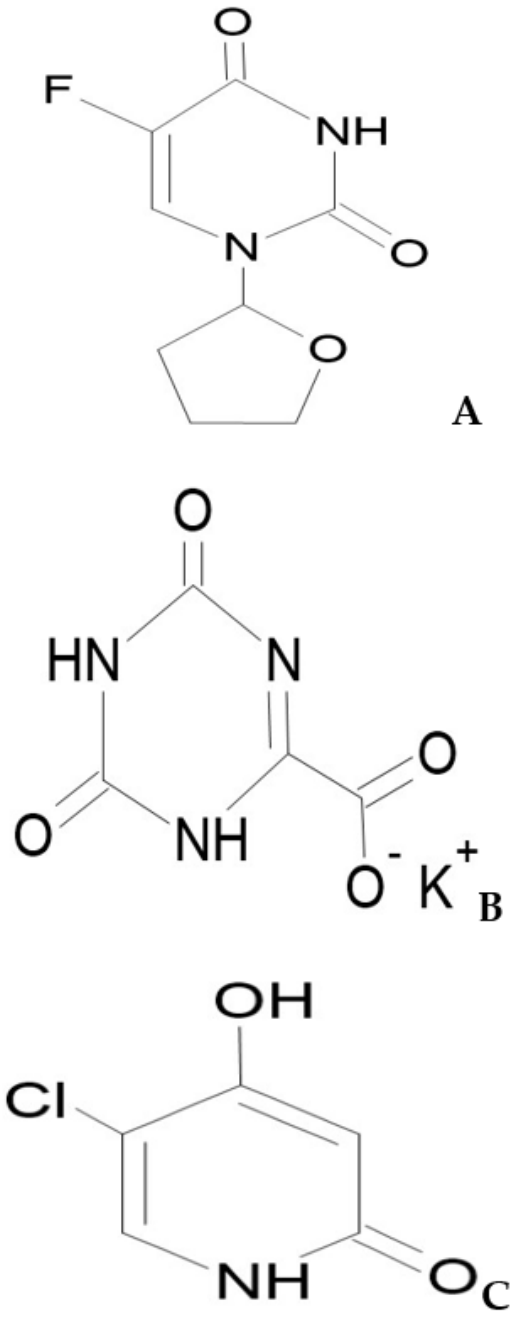


Figure 1: Molecular Structure of Tegafur (A), Oteracil Potassium (B) and Gimeracil (C).

Tegafur Standard Stock Solution

Weigh exactly 20.00 mg of tegafur standard in a 50 mL standard flask that has been cleaned and dried. Add 10 mL of the diluent and homogenize for 10 min. Then, increase to final volume using a diluent and mix thoroughly to achieve a concentration of 400 µg/mL.

Oteracil potassium Standard stock solution

Weigh exactly 15.8 mg of oteracil potassium standard in a 50 mL standard flask that has been cleaned and dried. Add 10 mL of the diluent and homogenize for 10 min. Then, increase to the final volume using a diluent and mix thoroughly to get a concentration of 316 µg/mL.

Gimeracil Standard stock solution

Weigh exactly 5.8 mg of gimeracil standard in a 50 mL standard flask that has been cleaned and dried. Add 10 mL of the diluent and homogenize for 10 min. Then, increase to the final volume using a diluent and mix thoroughly to achieve a concentration of 116 µg/mL.

Standard mixed test solution (100% level)

5 mL of the standard stock solution containing tegafur, gimeracil and oteracil potassium was taken into a dried and clean 10 mL volumetric flask. The solution was then diluted to a total volume of 10 mL using a diluent in a volumetric flask and thoroughly mixed. The concentrations of the combined standard solution were 200.0 µg/mL of TEG, 158.0 µg/mL of OTE and 58.0 µg/mL of GIM.

Sample stock solution

The mean weight of 10 capsules was calculated by measuring the mass of the formulation. We emptied one capsule (containing 20 mg of tegafur, 5.8 mg of gimeracil and 15.8 mg of oteracil potassium) in a 50 mL standard flask. Subsequently, we introduced 5 mL of diluent into the mixture and subjected it to sonication. A diluent was added to attain the final volume. The mixture was thoroughly blended and thereafter passed through a PVDF membrane filter with a finer porosity of 0.22 µm. The concentration of a stock solution of the sample obtained was 400 µg/mL of TEG 316 µg/mL of OTE and 116 µg/mL of GIM.

Sample test solution

5 mL of the initial stock solution was carefully placed into a standard flask with a capacity of 10 mL and subsequently diluting with mobile phase, ensuring thorough mixing. The resulting concentrations were 200.0 µg/mL of TEG, 158 µg/mL of OTE and 58 µg/mL of GIM.

Placebo solution

A placebo solution was made by precisely weighing 70.2 mg of lactose monohydrate and 0.187 mg of Magnesium stearate in a 100-standard flask. Approximately After adding 50 mL of diluent, the mixture was subjected to 15 min of sonication with periodic shaking. The solution was then cooled and further diluted to the desired level with diluent. Apply centrifugal force at a velocity of 3000 rotations per minute for 5 min.[19,20]

Selection of wavelength for Analysis

The standard working solution was diluted to obtain concentration, 20 µg/mL of TEG, 15.8 µg/mL of OTE and 5.8 µg/mL of GIM and examined in the wavelength region of 200-400 nm and spectral data acquired was used to ascertain the suitable operating wavelength.

Method Optimization

Different buffer pH, stationary phase and mobile phase chromatographic parameters were utilized in the trials. Utilizing the data of retention time, high plate count and symmetry in peak, resolution the approach was completely optimized.

Validation

The method optimized was validated regarding linearity, specificity, precision, accuracy, detection and quantification limit and system suitability parameters as per ICH standards.

System suitability

It ensures that the developed method is suitable for its intended purpose. The chromatograms obtained under optimal conditions were analyzed using the system suitability test to assess several parameters, such as column efficiency (with a minimum of 2000 plates), resolution (more than 1.5), capacity factor and peak tailing. System suitability factors were tested by introducing six replicates of a standard solution comprising, 200 µg/mL, 158 µg/mL of OTE and 58 µg/mL of GIM into the system.

Specificity

The specificity of the UPLC method was evaluated by analyzing a blank sample, a placebo, the sample test solution and a deteriorated sample acquired from the degradation investigation, as per ICH standards.

Linearity and range

The calibration curve exhibited linearity at the concentration ranges of 40-240 µg/mL, 31.6-189.6 µg/mL and 11.6-69.6 µg/mL for TEG, OTE and GIM, respectively by taking 1 mL, 2 mL, 3 mL, 4 mL, 5 mL and, 6 mL of standard solution and diluted to 10 mL with diluent to prepare 20%-120% level. The solutions were introduced into the UPLC apparatus and a correlation coefficient was calculated by plotting the concentration against the peak area.

The range was determined by conducting precision, accuracy and linearity tests at concentration levels ranging from a lower of 80% to a high of 120% relative to the sample concentration.

Precision

The analytical procedure precision refers to the reproducibility among multiple measurements taken from the same homogenous material under specific conditions. The repeatability of the test method was confirmed by generating 6 sample test solutions with 200 µg/mL of tegafur, 158 µg/mL of oteracil potassium and 58 µg/mL of gimeracil which was at a 100% concentration level. The test solution was introduced into a UPLC system following the procedure. The test findings were used to calculate %RSD. Intermediate precision was achieved by producing six sample test solutions, which were then injected into a UPLC machine according to the proposed method. This was done on a different day to ensure consistency and reliability. The test findings were compared by computing the Relative Standard Deviation (RSD) obtained over two days.

Accuracy

The method's accuracy defines the degree of correlation between a measurement's result and its actual value. By ICH standards, a recovery study was conducted to confirm the accuracy. Take 1 mL of placebo solution, add 4 mL, 5 mL and 6 mL of standard stock solution of TEG, OTE and GIM to get 80%, 100% and 120% of concentrations, after 10 min of sporadic shaking while using a sonicator, solutions were dilute to get the desired volume.

Robustness

The robustness study consisted of making slight modifications to the parameters of the developed method, such as the mobile phase ratio and flow rate These adjustments included mobile phase ratio by +/-10% (0.1% orthophosphoric acid(A) and methanol(B)) to 82:18 and 78:22, the flow rate by +/-10% to 0.55 mL/min and 0.45 mL/min. The sample preparations were injected five times using the aforementioned changes and the Relative Standard Deviation (RSD) values of the peak areas were calculated.

Detection limit and Quantitation limit

The LOD and LOQ were established through the process of repeated dilutions of TEG, OTE and GIM stock solutions. The objective was to achieve a Signal-to-Noise (S/N) ratio of 3:1 for LOD and 10:1 for LOQ.

Analysis of commercial formulation

The quantity and percentage purity of the marketed formulation of TEG, OTE and GIM were determined by applying the subsequent equation to the analysis.

$$\text{The concentration of the drug in the dosage form} = A/B \times C/D \times \text{Average weight}$$

Where A is sample area, B is standard area, C is standard dilution, D is sample dilution.

$$\text{Percentage purity} = \text{Amount of drug present/Label claim} \times 100$$

Forced Degradation Experiments

The ICH guidelines advocate for stress testing as a means to assess the inherent stability of pharmacological compounds. These treatments included acid hydrolysis (using 0.1M HCl, refluxed at 50°C for 1 day), alkali hydrolysis (using 0.1 M NaOH, refluxed at 50°C for 1 day), peroxide oxidation (using 3% hydrogen peroxide heated at 50°C for 1 day), heat exposure (samples were placed in a heat chamber at 60°C for 1 day), exposure to UV light (samples were placed in a Ultraviolet cabinet for 1 day) and decomposition in water (refluxing at 60°C for 1 day).[21] Forced degradation experiments are conducted on the blank, placebo and sample solutions. After subjecting the solutions to stress testing, they are diluted to avoid any additional degradation. To ensure that TEG, GIM and OTE do not contain blank, placebo, or degradation contaminant peaks during their retention time, peak purity was evaluated for each of the substances.

RESULTS AND DISCUSSION

Method Optimization

The UV instrument detected an isobestic point at 282 nm when scanning standard solutions of drugs (Figure 2). The wavelength of 282 nm was used for the analysis. The primary goal of the method's development was to enhance the separation efficiency, reduce the runtime and maximize the sensitivity. Upon analyzing their structures, it was concluded that all three drugs displayed polar properties. OTE is acidic compound and TEG and GIM are neutral compounds. Consequently, the reverse phase approach was chosen, necessitating the use of a non-polar HSS C8 column. Various dimensions of the column were used in the trials. 50x2.1 mmx1.8 µ columns shows poor retention, hence 100x2.1 mmx1.8 µ column was selected. When improving and verifying a UPLC method, it is customary to optimize the composition of the mobile phase after choosing a suitable column. This is done to achieve the intended outcomes. Methanol was employed as an organic solvent in various proportions, in conjunction with 0.1% phosphoric acid at a pH of 2 to improve retention. A mobile phase ratio of 50:50 gave poor resolution. Decreasing the ratio of the modifier (methanol) gave good resolution peaks.

Acquity HSS C8 column from Waters Corporation, USA of dimensions 100×2.1 mm, 1.8 µm. with a rate of flow of 0.2mL/min at the temperature of the column oven at 30°C and a 0.1%phosphoric acid (pH 2) and methanol in 80:20 (v/v) as mobile phase and absorbance maxima of 282 nm was used to obtain final optimized conditions and faster elution below 2 min. (Figure 3).

Validation

The optimized technique was verified by the ICH criteria, which included a system suitability test, specificity through mixed degradation tests, linearity, precision, accuracy, robustness, detection and quantification limit, to show the method's adequacy.

System suitability

The system suitability characteristics were assessed using six repetition injections of the standard test solution. The retention times for TEG, OTE and GIM were 0.418, 0.923 and 1.502 min, respectively. The Resolution (Rs) was determined to be 5.39 and 6.57. The number of plates was equivalent to 6553, 5649, 8828. The tailing factor was measured to be 0.85, 0.82 and 0.96. The RSD (Relative Standard Deviation) values for retention time, tailing factor and plate count were significantly below 2% (Table 1).

Specificity

At a wavelength of 282 nm, the specificity of the method demonstrated that there were no interventions caused by the presence of additives or eluent. This demonstrated that the optimized method was specific for TEG, OTE and GIM. At the retention times of the medications, there were no peaks formed because of the presence of excipients (placebo), eluent, or degradation products, if any were present (Figure 4).

Linearity and range

The method's linearity was established in the concentration ranges of 40-240 µg/mL, 31.6-189.6 µg/mL and 11.6-69.6 µg/mL for TEG, OTE and GIM, respectively. This suggests that the responses obtained were exactly proportionate to the drug solution's concentration within the specified range. Regression coefficients and linear regression equations were computed (Figure 5). The method performed well in terms of precision,

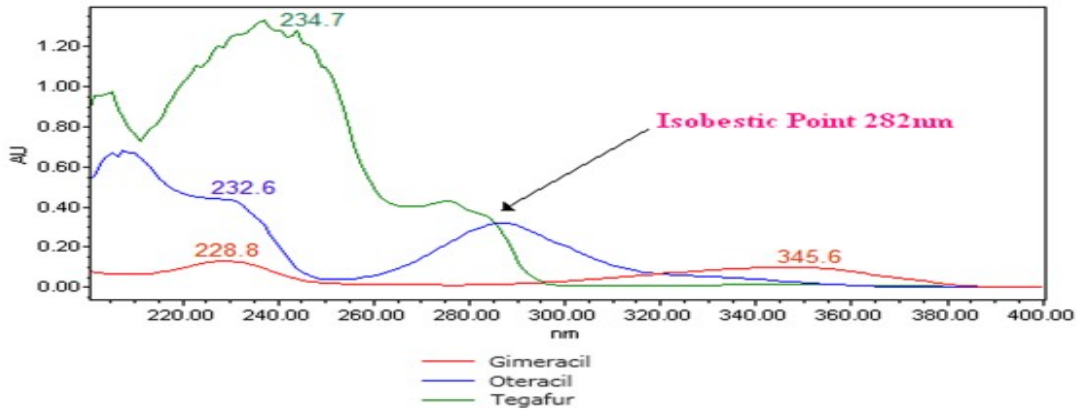


Figure 2: UV spectrum of Tegafur, oteracil potassium and gimeracil

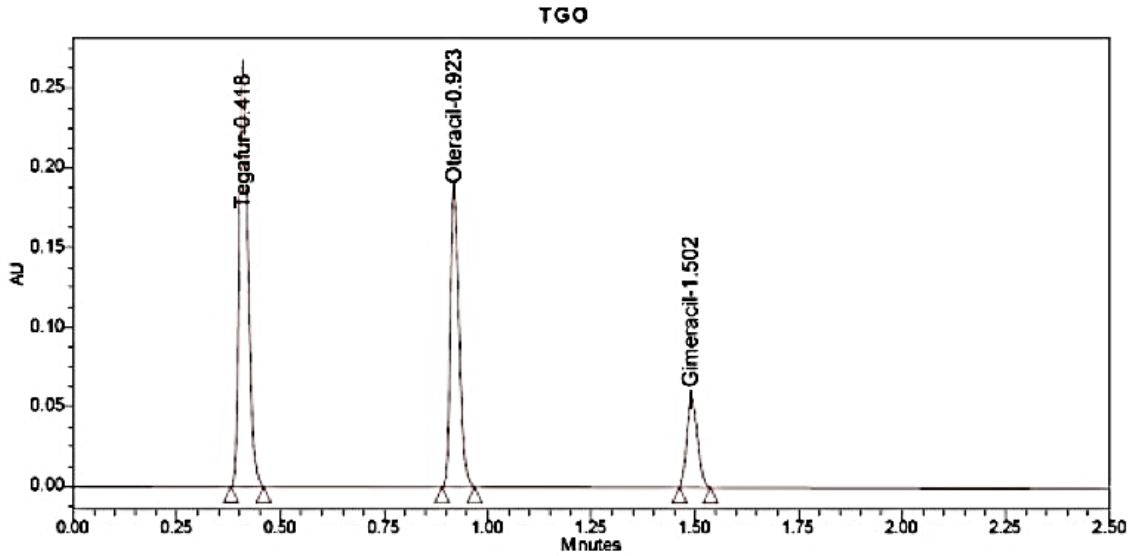


Figure 3: Optimized Chromatogram of TEG, OTE and GIM.

linearity and accuracy concerning drug concentration, showing a range from 80% to 120% at the lower and higher limits, respectively.

Precision

The precision of the RP UPLC assay methods was assessed as part of the evaluation of repeatability. The Relative Standard Deviation (RSD) values for TEG, OTE and GIM were calculated as 0.6, 0.5 and 0.2, respectively. The Relative Standard Deviation (RSD) values for TEG between two distinct days were determined to be 0.578 and 0.578. For OTE, the RSD values were 0.211 and 0.47 and for GIM, the RSD values were 0.47 and 0.24. The calculated %RSD was found to be below 2% (Tables 2a and 2b).

Accuracy

The methods accuracy of the TEG, OTE and GIM was evaluated utilizing the % recovery approach. The recovery rates for TEG, OTE and GIM were determined and summarized (Table 3). These values are within the range of 98.0% to 102.0%.

Robustness

To assess the method's robustness, variations in mobile phase ratios and flow rate were considered. Table 4 provides a summary of the results. The percentage RSD between the initial results and the robustness test sample results was less than 2.0%, indicating that the test was successful.

LOD and LOQ

For the LOD and LOQ, the signal-to-noise ratio was found to be 3:1 and 10:1, respectively. Here are the limits of detection and quantification for TEG: 0.3 µg/mL and 1 µg/mL; for OTE they are 0.2 µg/mL and 0.8µg/mL and for GIM they are 0.1 µg/mL and 0.3 µg/mL. The results show that the method is very sensitive to the methods that were already reported (Figure 6).

Table 1: System Suitability Parameter Results.

Sl. No.	TEG			OTE			Rs	GIM			Rs
Inj	Rt (min)	N	Tf	Rt (min)	N	Tf		Rt (min)	N	Tf	
1	0.418	65536	0.85	0.923	8828	0.85	5.39	1.502	5649	0.96	6.57
2	0.412	65525	0.84	0.925	8847	0.85	5.28	1.507	5639	0.98	6.43
3	0.411	65555	0.88	0.922	8813	0.85	5.34	1.508	5679	0.94	6.51
4	0.415	65548	0.84	0.929	8866	0.89	5.48	1.503	5632	0.98	6.73
5	0.416	65551	0.85	0.924	8839	0.87	5.39	1.505	5682	0.96	6.58
6	0.419	65518	0.84	0.925	8835	0.87	5.39	1.501	5669	0.94	6.57
Avg	0.42	65538	0.85	0.92	8838	0.86	5.39	1.50	5658	0.96	6.57
SD	0.0	15.0	0.0	0.0	17.9	0.0	0.1	0.0	21.2	0.0	0.1
%RSD	0.8	0.0	1.8	0.3	0.2	1.9	1.2	0.2	0.4	1.9	1.5

Inj: Injection: Retention time, N: Plate count, Tf: Tailing factor, Rs: Resolution, Avg: Average, SD: Standard Deviation, %RSD: Percentage Relative Standard Deviation.

Table 2a: Method Precision Results.

Sample	Sample Area			Percentage purity		
	TEG	OTE	GIM	TEG	OTE	GIM
1	2315784	1931547	713354	100.2	99.7	99.8
2	2333485	1949548	714063	100.9	100.6	99.7
3	2300918	1926017	715874	99.5	99.4	99.6
4	2325985	1931254	717148	100.6	99.6	99.8
5	2305948	1940587	712389	99.7	100.1	99.5
6	2302514	1944857	715140	99.6	100.3	100.1
AVG	2314106	1937302	714661	100.1	100	99.8
SD	13374.03	9109.15	1738.61	0.578	0.459	0.207
%RSD	0.578	0.47	0.243	0.6	0.5	0.2

AVG: Average, SD: Standard Deviation, %RSD: Relative Standard Deviation.

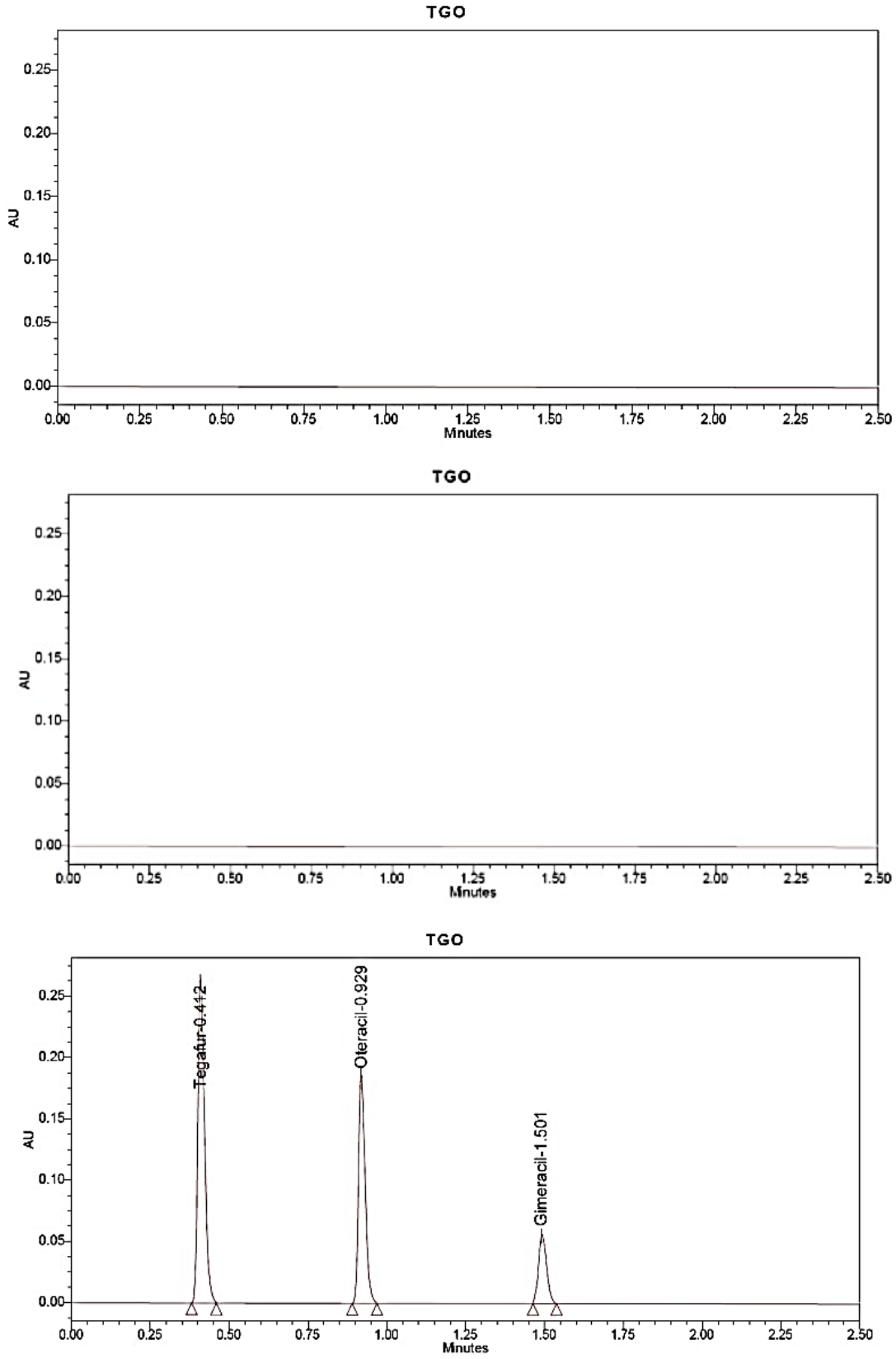
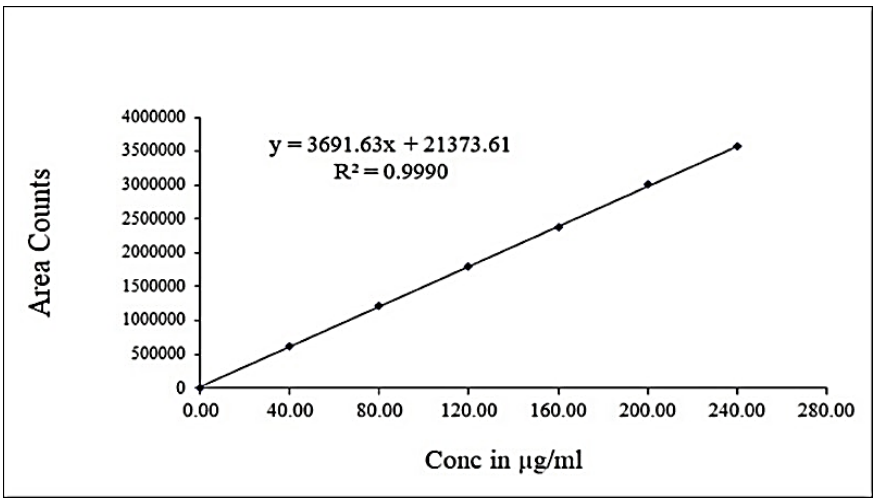
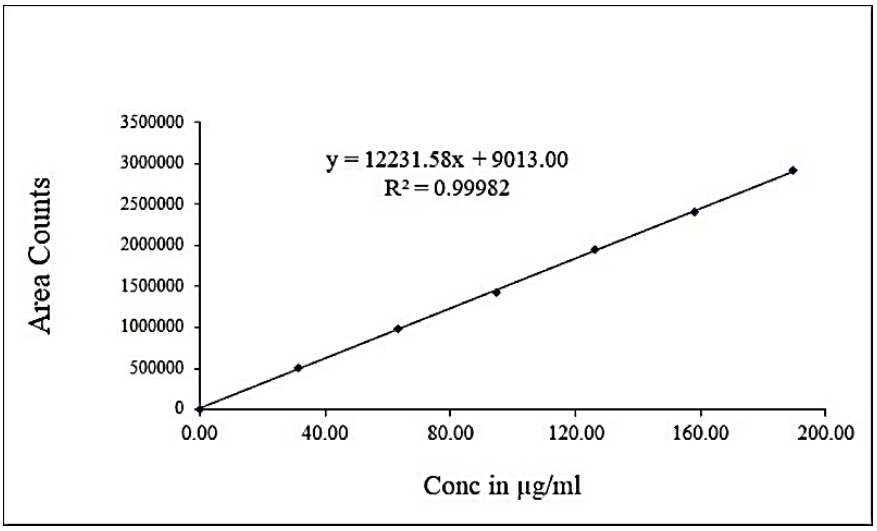


Figure 4: Chromatogram of Blank (A), Placebo (B), Sample solution (C).





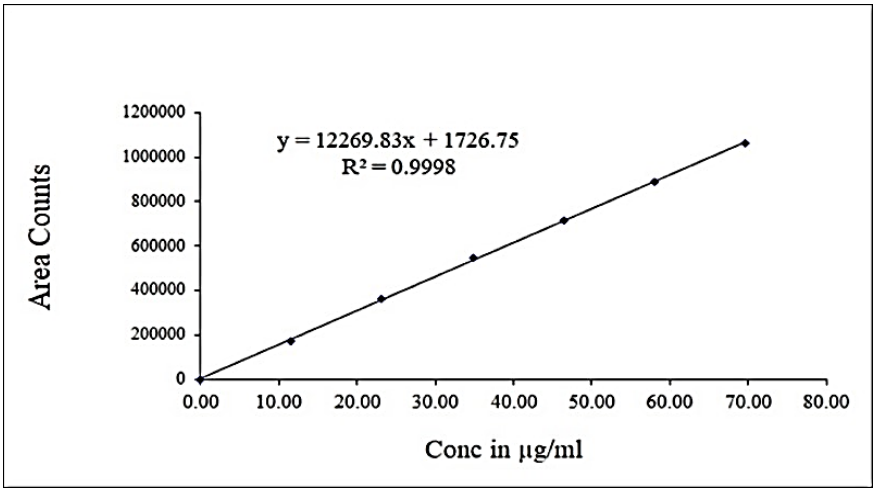


Figure 5: Linearity of Tegafur (A), Oteracil (B), Gimeracil (C).

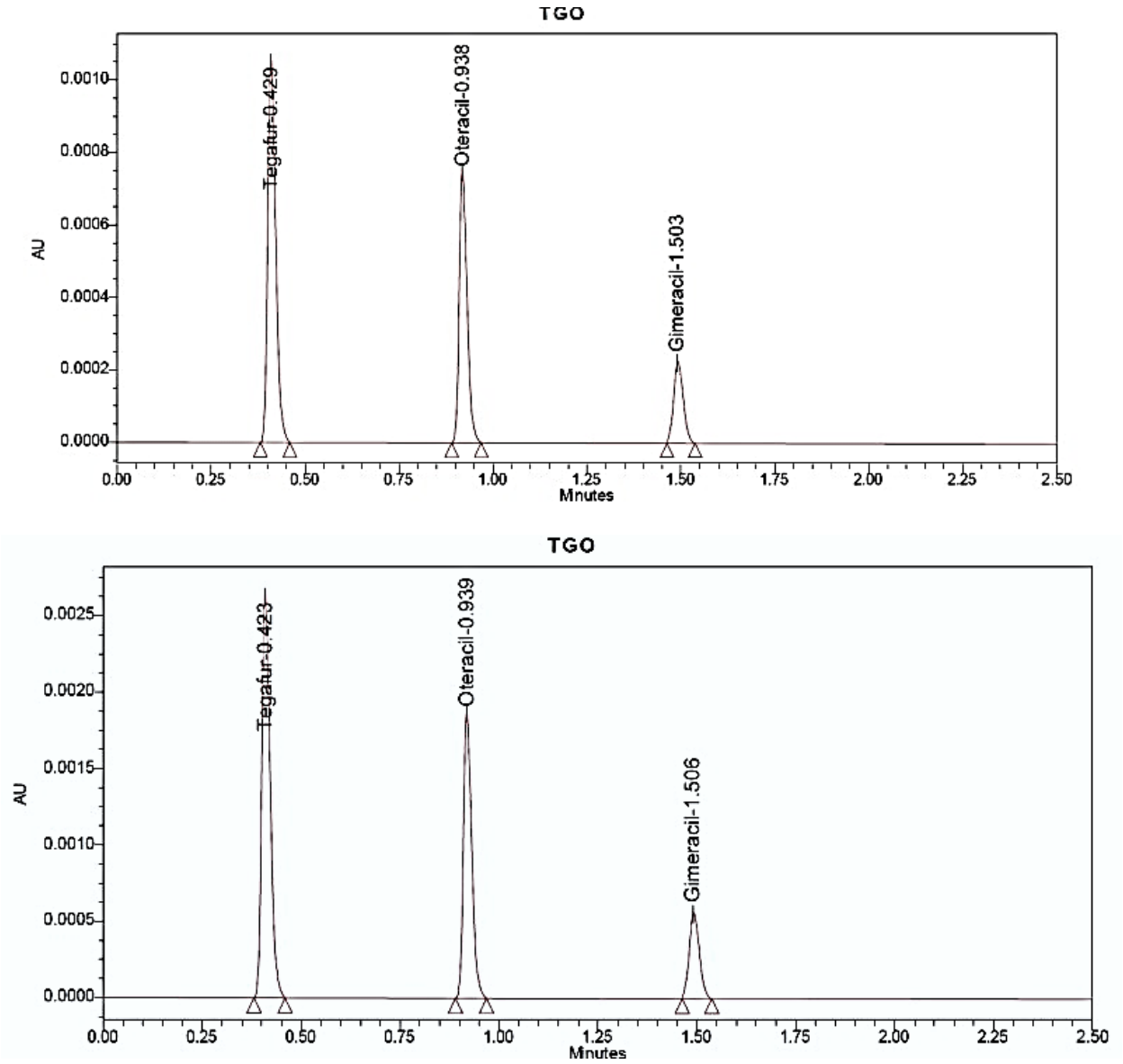


Figure 6: Chromatogram of Detection Limit (A) and Quantitation limit (B).

Table 2b: Intermediate Precision Results.

Sample	Day 1			Day 2		
	Sample Area			Sample Area		
	TEG	OTE	GIM	TEG	OTE	GIM
1	2315784	714254	1931547	2315784	1931547	713354
2	2333485	713361	1949548	2333485	1949548	714063
3	2300918	712842	1926017	2300918	1926017	715874
4	2325985	714454	1931254	2325985	1931254	717148
5	2305948	712154	1940587	2305948	1940587	712389
6	2302514	716441	1944857	2302514	1944857	715140
AVG	2314106	713918	1937302	2314106	1937302	714661
SD	13374.03	1506.35	9109.15	13374.03	9109.15	1738.61
%RSD	0.578	0.211	0.47	0.578	0.47	0.243

AVG: Average, SD: Standard Deviation, %RSD: Relative Standard Deviation.

Table 3: Percentage Recovery Accuracy Results.

Sample	% level	Quantity injected (µg/mL)	Quantity recovered (µg/mL)	%Recovery
TEG	80%	160.02	159.98	99.97
	100%	200.04	200.05	100.00
	120%	240.02	240.01	99.97
OTE	80%	126.42	126.39	99.97
	100%	158.01	158.03	100.00
	120%	189.64	189.58	99.96
GIM	80%	46.43	46.38	99.89
	100%	58.02	57.99	99.94
	120%	69.65	69.69	100.05

Table 4: Results of Robustness.

Parameter	Variation made	Avg Sample area			%RSD		
		TEG	OTE	GIM	TEG	OTE	GIM
Mobile phase	18B:82A	2012010	1583632	686479	0.40	1.00	0.70
	22B:78A	2747974	2213880	749788	0.56	0.99	0.46
Flow rate	0.55 mL/min	2181142	1725029	707479	0.87	1.31	0.40
	0.45 mL/min	2516494	2056263	734217	0.25	0.5	0.30

B: Methanol, A: Phosphoric acid, %RSD: Percentage Relative Standard Deviation.

Table 5: Assay results.

Drug	ASA	Std. wt.(mg)	LA (mg)	AF (µg/mL)	% Purity
Tegafur	2317688	20	20	20.05	100.2
Gimeracil	717066	5.8	5.8	5.81	100.2
Oteracil	1954288	15.8	15.8	15.93	100.9

ASA: Average sample area, **LA:** Label amount, **AF:** Amount found.

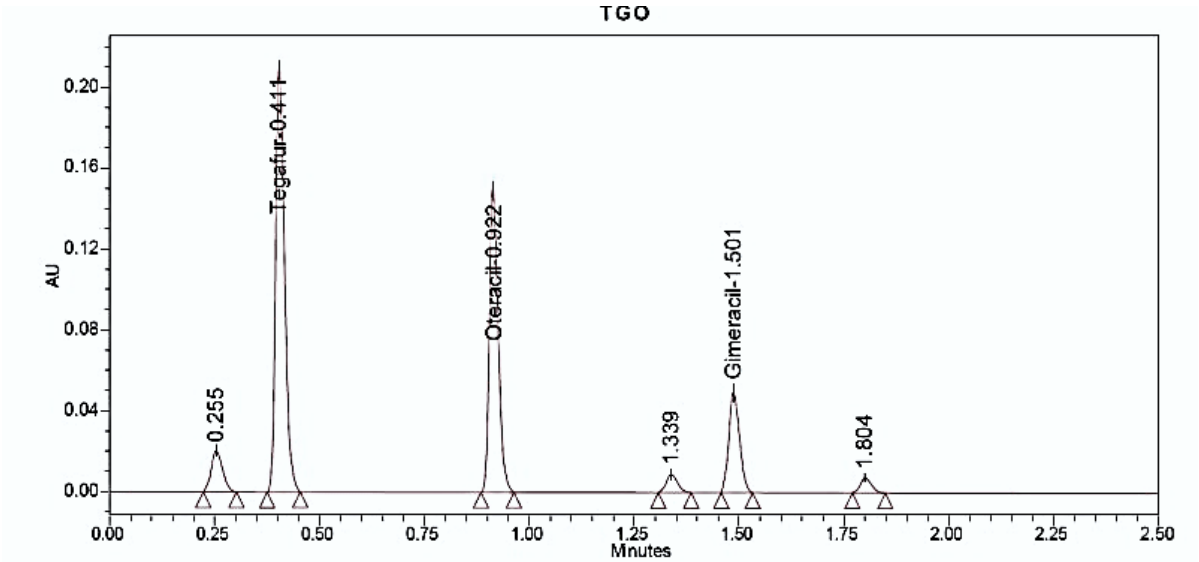


Figure 7: Representative chromatogram of Forced Degradation studies (Acid hydrolysis).

Table 6: Forced Degradation Studies Results.

Sample	Compound	Rt (min)	Peak angle	Purity threshold	Peak purity
Sample	TEG	0.413	10.328	26.118	Pass
	OTE	0.922	8.334	17.248	Pass
	GIM	1.504	14.183	33.247	Pass
Acid hydrolysis	TEG	0.411	10.358	26.746	Pass
	OTE	0.922	8.143	17.745	Pass
	GIM	1.502	14.532	33.604	Pass
Alkali hydrolysis	TEG	0.413	10.547	26.654	Pass
	OTE	0.925	8.228	17.154	Pass
	GIM	1.501	14.147	33.427	Pass
Peroxide oxidation	TEG	0.412	10.234	26.339	Pass
	OTE	0.923	8.546	17.293	Pass
	GIM	1.500	14.236	33.333	Pass
Heat	TEG	0.414	10.634	26.123	Pass
	OTE	0.926	8.842	17.258	Pass
	GIM	1.503	14.058	33.852	Pass
UV Chamber	TEG	0.413	10.229	26.427	Pass
	OTE	0.920	8.629	17.161	Pass
	GIM	1.502	14.427	33.199	Pass
Water Hydrolysis	TEG	0.413	10.328	26.118	Pass
	OTE	0.922	8.334	17.248	Pass
	GIM	1.504	14.183	33.247	Pass

Assay

A total of six replicates of the sample solution were prepared and assessed. The recorded outcomes of the test were 9100.2%, 100.9% and 100.2% for TEG, OTE and GIM, as depicted in Table 5.

Forced Degradation Results

The forced deterioration studies of TEG, OTE and GIM findings were presented in Table 6 and Figure 7. Under all stress conditions, the peak area of the sample solution changes significantly, but not its retention time. The good separation of the degraded product from the parent peak demonstrates that the method is stability-indicating.

CONCLUSION

Using Ultra-Performance Liquid Chromatography (UPLC) technique in reverse phase mode, a rapid technique has been generated for the simultaneous quantification of TEG, OTE and GIM in dosage form. This technique was rapid, accurate, precise and reproducible and it was validated by the ICH standards. The current approach achieves a shorter runtime of less than 2 min for quantifying three compounds, in comparison to previously documented methods that require 3.5 min.[15] The implementation of the UPLC technique led to a decrease in the total analysis time and the quantity of solvents used. The reduced retention time of less than 2 min led to cost savings in the analysis of TEG, OTE and GIM in both Active Pharmaceutical Ingredient (API) and dosage form, while simultaneously improving sensitivity and speed. A robust and rapid Reversed-Phase Ultra-Performance Liquid Chromatography (RP-UPLC) approach has been created and confirmed for the determination of TEG, OTE and GIM, with the capacity to detect any degradation or instability. After conducting peak purity analysis on stressed samples using the specified method, it can be finalized that the method is specific for measuring compounds in the presence of degradation products. This is supported by the lack of a co-eluting peak alongside the main peak of TEG, OTE and GIM. The suggested RP UPLC method is very rapid and has exceptional sensitivity, precision and reproducibility. This approach lowers the cost of analysis by using less solvent because of its two-minute run time, which also allows for faster analysis and higher job throughput. As a result, bulk and pharmaceutical dosage forms stability samples and quality control samples can be routinely assayed using the established approach.

ACKNOWLEDGEMENT

Professor Galla Rajitha conceptualized and designed the research work. Charumathi Salva collected the essential data and performed the appropriate analysis. All contributors collaborated in the authorship of the manuscript. All authors have given their permission to the final version of the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TEG: Tegafur; **OTE:** Oteracil; **GIM:** Gimeracil; **RT:** Retention Time; **Rs:** Resolution; **UV:** Ultraviolet; **RP UPLC:** Reverse Phase Ultra Performance Liquid Chromatography; **%RSD:** Relative Standard Deviation; **ICH:** International Conference of Harmonization.

SUMMARY

The key focus of this research was to design a rapid Ultra Performance Liquid Chromatography (UPLC) and validate that it is uncomplicated, exact, responsive, and reliable for quantifying the concentrations of tegafur, oteracil, and gimeracil in both their pure state and pharmaceutical formulations. The method utilized an HSS C8 column (100mm x 2.1mm, 1.8µm) and a mobile phase consisting of 0.1% phosphoric acid (pH 2.0) and methanol in an 80:20 ratio, This approach facilitated elution times of less than 2 minutes for all three substances. The flow rate was maintained at 0.2 ml/min, with an injection volume of 5 µl, and detection at 282 nm. Linearity was established for tegafur (40-240 µg/mL), oteracil (31.6-189.6 µg/mL), and gimeracil (11.6-69.6 µg/mL). The detection limits were determined to be 0.3 µg/mL for tegafur, 0.2 µg/mL for oteracil, and 0.1 µg/mL for gimeracil, with quantitation limits of 1 µg/mL, 0.8 µg/mL, and 0.3 µg/mL, respectively. The method demonstrated high accuracy and precision, with relative standard deviations (RSD) below 2%. This validated UPLC method is deemed suitable for stability assessment and routine quality control of tegafur, gimeracil, and oteracil.

REFERENCES

1. Mittal B, Tulsyan S, Kumar S, Mittal R D, Agarwal G. Cytochrome P450 in Cancer Susceptibility and Treatment. In: Gregory S. Makowski, Editors. Advances in Clinical Chemistry. Newington, CT: Elsevier, 2015;71:77-139. doi: doi.org/10.1016/bs.acc.2015.06.003.
2. Peer CJ. McManus TJ, Hurwitz HI, Petros WP. Development and utilization of a combined LC-UV and LC-MS/MS method for the simultaneous analysis of tegafur and 5-fluorouracil in human plasma to support a phase I clinical study of oral UFT®/leucovorin. Journal of Chromatography B, 2012;898:32-7. doi: 10.1016/j.jchromb.2012.04.010.
3. Tegafur. In: J.K. Aronson, editor. Meyler's Side Effects of Drugs, Sixteenth Edition. Elsevier; 2016;711-2. doi: 10.1016/B978-0-444-53717-1.00185-2.
4. Avendano C, Menéndez JC. Antimetabolites. In: Avendano C, Menéndez JC, editors. Medicinal Chemistry of Anticancer Drugs. Third Edition. Elsevier; 2023;37-100. doi: 10.1016/B978-0-12-818549-0.00001-7.
5. Li XL, Zhu H, Zhan L, Liu T, Cheng G, Liu N, *et al.* A Convenient synthesis of Gimeracil. Journal of Chemical Research. 2018;42:33-4. doi: 10.3184/174751918X15166150010920.
6. Honggang W, Anguo Zinventors; Lunan Pharmaceutical group Corp, assignee. Preparation method of oteracil potassium. China Patent CN110655429A. 2020.
7. Kobayakawa M, Kojima Y. Tegafur/gimeracil/oteracil (S-1) approved for the treatment of advanced gastric cancer in adults when given in combination with cisplatin: a review comparing it with other fluoropyrimidine-based therapies. Onco Targets Ther. 2011;4:193-201. doi: 10.2147/OTT.S19059.
8. Mailamuri HK, Khandapu BMK, Rudraraju rb liquid chromatography dependent stability indicating methodology: development and authentication for formulations of capsule type containing tegafur, gimeracil and oteracil. International Journal of Applied Pharmaceutics. 2023;15(4):71-81. doi: 10.22159/ijap.2023v15i4.47831
9. Liu K, Zhong D, Zou H, Chen, X Determination of tegafur, 5-fluorouracil, gimeracil and oxonic acid in human plasma using liquid chromatography-tandem mass spectrometry. Journal of pharmaceutical and biomedical analysis, 2010;52:550-6. doi: 10.1016/j.jpba.2010.01.026.
10. Haoyu X, Jianghua L, Sumin Qi, Liying Z, Miao S, Quinquin Q, *et al.* River Pharmaceutical group Co. Ltd. China Patent CN102253152B. 2013.
11. Yang WF, Xü MJ, Shi YF, Zhu PX. Study of main impurities in oteracil potassium by HPLC-QTOF-MS. Chin J Pharm Analysis, 2016;36:2041-5.
12. Jarugula VR, Boudinot FD. High-performance liquid chromatographic determination of 5-fluorouracil and its prodrugs, tegafur and 4-deoxy-5-fluorouracil, in rat plasma. J Chromatogr B Biomed Sci Appl, 1996;677:199-203. doi: 10.1016/0378-4347(95)00438-6.
13. Cao Y, Sun X, Zhang Q. Determination of the relevant substances in tegafur injection by HPLC. China Pharm, 2008;31:2451-2.
14. Remaud G, Boisdron-Celle M, Morel A, Gamelin A. Sensitive MS/MS-liquid chromatography assay for simultaneous determination of tegafur, 5-fluorouracil and 5- fluorodihydrouracil in plasma. J Chromatogr B Analyt Technol Biomed Life Sci, 2005;824:153-60. doi: 10.1016/j.jchromb.2005.07.023.
15. Kunala A, Gummadi S. Simultaneous Determination of Gimeracil, Oteracil and Tegafur in Pure Blend and their Combined Capsules by Stability-indicating RP-UPLC Method. Asian Journal of Pharmaceutics, 2023;17(3):470-7. doi: 10.22377/ajp.v17i03.4989.
16. ICH Harmonized Tripartite. Stability testing of new drug substance and products. Q1A(R2); International Conference on Harmonization, Geneva: 2023. Accessed 21 August 2023.
17. ICH Harmonized Tripartite. Stability testing: Photostability testing of new drug substances and products Q1B; International Conference on Harmonization, Geneva: 1996 Accessed 21 August 2023.
18. ICH Harmonized Tripartite Validation of analytical procedures: text and methodology Q2 (R1); International Conference on Harmonization, Geneva: 2005.Accessed 21 August 2023.
19. Li J, Wu Y. Lubricants in Pharmaceutical Solid Dosage Forms. Lubricanst. 2014;2(1):21-43. doi:10.3390/lubricants2010021.
20. European Medicines Agency, Medicines. Teysuno: EPAR-Product information [online] Accessed 22 August 2023.
21. Blessy M, Patel R D, Prajapati P N, Y.K. Agrawal. Development of forced degradation and stability indicating studies of drugs-A review. Journal of Pharmaceutical Analysis, 2014;4(3):159-65. doi: 10.1016/j.jpha.2013.09.003.

Cite this article: Salva C, Galla R. A Rapid UPLC Technique for Quantification of Tegafur, Oteracil and Gimeracil in Bulk and Pharmaceuticals and its Validation. Indian J of Pharmaceutical Education and Research. 2024;58(3s):s1041-s1052.