Original Article

Characterization of Forced Degradants of Tegafur, Gimeracil, and Oteracil Potassium by Liquid Chromatographic-Electrospray Ionization-Mass Spectrometry and Simultaneous Estimation of Triple Combination in Drug Substance and Finished Pharmaceutical Dosage Form

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ABSTRACT

Tegafur, gimeracil, and oteracil potassium are widely used pharmaceuticals to treat lung cancers of the gastrointestinal tract, such as those of the oral cavity, esophagus, colon and rectum, and pancreas, as well as non-small cell lung cancers. The literature review revealed that no study has yet offered a completely stability-demonstrating, validated liquid chromatography-mass spectrometric approach for the concurrent estimation of tegafur, gimeracil, and oteracil potassium, along with all known degradation products. The simultaneous detection of tegafur, gimeracil, and oteracil potassium and their forced degradation product characterization necessitated the invention of a simpler, faster, and less expensive method. Therefore, this study aimed to follow the ICH method validation standards to develop and validate a fast, easy, and rugged liquid chromatography-mass spectrometry (LC-MS) technique for the concurrent estimation of tegafur, gimeracil, and oteracil potassium in the drug substance and the finished dosage form. Tegafur, gimeracil, and oteracil potassium were examined on the Waters HPLC Alliance system, coupled to the SCIEX QTRAP 5500 mass spectrometer, and endowed with an interface capable of carrying electrospray ionization. The tegafur, gimeracil, and oteracil peaks eluted at retention times of 2.338 min, 3.756 min, and 5.338 min, respectively. The limit of detection values of tegafur, gimeracil, and oteracil were detected to be 0.6, 0.174, and 0.474 µg/mL, respectively. The results for the quantification limit were calculated at 2.0, 0.58, and 1.58 µg/mL concentrations, respectively. Tegafur, gimeracil, and oteracil had linear ranges of 50-300 µg/ml, 14.5-87 µg/ml, and 39.5-237 µg/ml, with regression coefficients of 0.99956, 0.99986, and 0.999479, respectively. The accuracy values of tegafur, gimeracil, and oteracil in the ranges of 50%, 100%, and 150% were determined at 99.9%, 99.9%, and 99.4%, respectively. The RSD for the six replicates was less than 2% for precision. According to the ICH Q2 guidelines, this approach was effectively evaluated with LC-MS to validate the chemical structures of the freshly created tegafur, gimeracil, and oteracil degradation products. An accurate and sensitive LC-MS technique was developed and validated for the concurrent quantification of tegafur, gimeracil, and oteracil potassium in the drug material and the medicinal dosage form.

Keywords: Degradation study, Gimeracil, Liquid chromatography mass-spectrometry technique, Oteracil, Tegafur

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1. Introduction

The cytochrome P-450 enzyme in the liver progressively transforms the prodrug tegafur into the antibiotic fluorouracil. Fluorouracil's catabolic enzyme, dihydropyrimidine dehydrogenase (DPYD), is competitively inhibited by uracil, increasing the serum levels of the drug (1). Gimeracil (5-chloro-2,4-dihydroxypyridine) is an inhibitor of DPYD that prevents the blood from degrading pyrimidines such as 5-fluorouracil. The oral fluoropyrimidine derivative S-1 is first combined with gimeracil to produce sustained 5-fluorouracil (5-FU) in the body, specifically in serum and tumor tissues (2). Oteracil potassium is a chemoprotective agent that modulates the action of 5-FU and inhibits the enzyme orotate phosphoribosyl-transferase (OPRT). The gastrointestinal (GI) tract is where oteracil potassium is primarily localized. By inhibiting OPRT, it slows down the conversion of 5-FU into its active metabolite, 5-fluorouridine-5'-monophosphate. This lessens the GI toxicity brought on by the activated 5-FU (3). For the chemical structure, refer to figure 1. Tegafur, gimeracil, and oteracil potassium are widely used pharmaceuticals to treat lung cancers of the GI tract, such as those of the oral cavity, esophagus, colon and rectum, and pancreas, as well as non-small cell lung cancers. They also received approval as a therapy for head and neck tumors (progressive or recurrent) in 2001 (4,5). The literature survey for tegafur showed that tegafur drug substance was analyzed by high-pressure liquid chromatography (HPLC) (6,7), gas-liquid chromatography (8), and liquid chromatography-mass spectroscopy (LC-MS) (9). Gimeracil was estimated by HPLC (10) and LC-MS (11-13) mostly in human plasma and blood samples withdrawn from subjects. Oteracil potassium was always used in combination with tegafur and gimeracil for treating cancer. Tegafur, gimeracil, and oteracil potassium combinations were estimated by LC-MS (14-19). Tegafur, gimeracil, and oteracil potassium were estimated simultaneously; nevertheless, the stability and forced degradation tests have not been satisfactorily carried out yet. None of the previous studies reported the characterization of the degradation products formed during the shelf life of the formulation. Moreover, no forced degradation studies were reported on the finished pharmaceutical dosage form. The information presented above can help understand the chemical stability of tegafur, gimeracil, and oteracil potassium, create a suitable formulation, and check for optimal storage conditions. The literature review revealed that no study has yet offered a completely stability-demonstrating, validated liquid chromatography-mass spectrometric approach for the concurrent estimation of tegafur, gimeracil, and oteracil potassium, along with all known degradation products. The simultaneous detection of tegafur, gimeracil, and oteracil potassium and their forced degradation product characterization necessitated the invention of a simpler, faster, and less expensive method. The goal of the study was to follow the ICH method validation standards to develop and validate a fast, easy, and rugged liquid LC-MS technique for the concurrent estimation of tegafur, gimeracil, and oteracil potassium in the drug substance and the finished dosage.

2. Materials and Methods

2.1. Chemicals and Drugs

Merck supplied all of the solvents, which were HPLC-grade. All of the solvents and solutions used were filtered using 0.45 µm PVDF filters. Shree Icon Labs sent tegafur, gimeracil, and oteracil potassium medicinal substance samples as a gift. Tegafur, gimeracil, and oteracil potassium drug products (Tegonat 20) were manufactured by Natco Pharma LTD (Hyderabad, Iran) and bought from the market.

2.2. Instruments

HPLC (Make: Waters, Model: Alliance HPLC e-2695) combined with the mass spectrometer of the SCIEX QTRAP 5500 was used and endowed with an interface capable of electrospray ionization. The SCIEX software was used for the interpretation of the data from the chromatogram.

2.3. Method

The separation of tegafur, gimeracil, and oteracil potassium was successfully achieved by an Inertsil ODS column with dimensions of 150 mm×4.6 mm, 3.5µm, a mobile phase composition of formic acid (0.1%) and acetonitrile in the proportion of 40:60 in isocratic mode, with a flow rate of the mobile phase of 1.0 ml/min, and an auto-injector injection volume of 10 µL. The autosampler and column maintained a temperature of 25°C (ambient or room temperature). In the forced degradation study, the liquid chromatography system was linked to a mass spectrometer. On the positive electrospray ionization mode, the usual operational basic settings for mass spectrometer scans of tegafur, gimeracil, and oteracil potassium were improved. The collision energy was set at 15 V, the ion spray voltage was set at 5500 V, and the de-clustering potential was set at 40 V. The collision gas was ultrapure nitrogen gas. The source temperature was 550°C, the drying gas temperature was 120-250°C, and the drying gas flow rate was 5 l/min. The entrance potential was 10 V while the exit potential was 7 V. The dwell time was set at 1 sec.

2.4. Diluent Preparation

In this stage, 0.1% formic acid and acetonitrile were mixed in a ratio of 40:60.

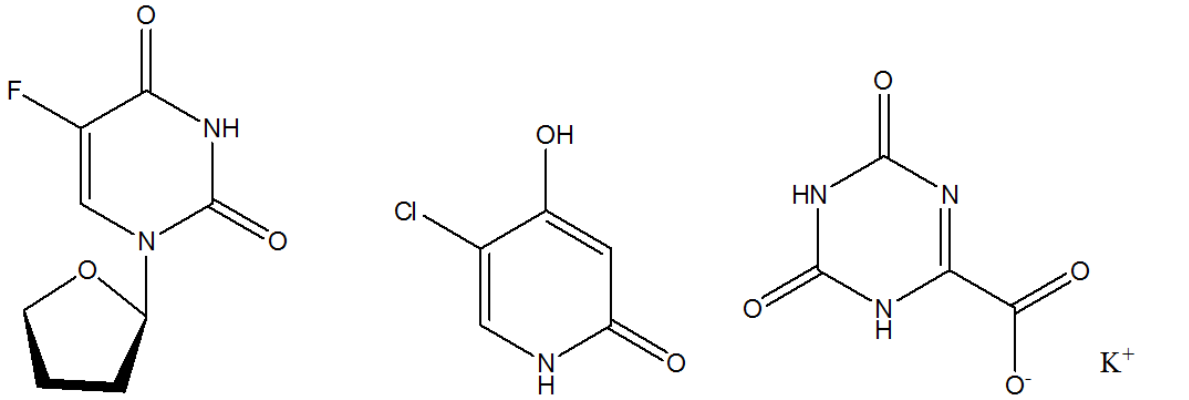


Fig.1. Structure of Tegafur, gimeracil, and oteracil potassium

2.5. Preparation of the Standard Solution

2.5.1. Preparation of the Standard Stock Solution

Tegafur 200 mg, gimeracil 58 mg, and oteracil 158 mg working standards were carefully weighed and put into a 100-cleaned dried volumetric flask. A total of 70 ml of diluent was included and sonicated for over 30 min. The remaining volume was then made up using a diluent and used as the default stock solution.

2.5.2. Standard Solution Preparation

A total of 5 ml of the above-prepared stock was transferred using a pipette into a 50 ml volumetric flask, which was then filled with the diluent to the final volume, which was considered the standard solution.

2.6. Sample Solution Preparation

A total of 327 mg of the sample was weighed and put in a 10 ml cleaned and dried volumetric flask. It was then filled with 7 ml of diluent, sonicated over 30 minutes, and diluted up to the final volume using the diluent. After that, 1 ml of the aforesaid stock solution was pipetted into a 10 ml volumetric flask, and the remaining volume was filled up with the diluent.

2.7. Method Validation Activity

2.7.1. System Suitability Parameter

Six replicate injections of tegafur, gimeracil, and oteracil standard solutions were introduced to the system to validate system suitability, and parameters such as plate count-USP (N), resolution, tailing factor, and analyte peak asymmetry were studied.

2.7.2. Linearity

Six distinct concentrations of tegafur, gimeracil, and oteracil were synthesized in various amounts of 50-300 µg/ml, 14.5-87 µg/ml, and 39.5-237 µg/ml, respectively. The peaks of each solution were recorded when they were injected into the instrument. After that, the regression coefficient was computed by drawing a line from the average peak area to the concentration.

2.7.3. Accuracy

Recovery experiments were carried out to confirm the procedure's accuracy at three levels of 50%, 100%, and 150%. Tegafur, gimeracil, and oteracil were discretely introduced into the pre-analyzed samples in the specified amounts. After each spike level was delivered into the liquid chromatographic system, the recovery percentage of each level was determined.

2.7.3. Method Precision

Tegafur, gimeracil, and oteracil samples were spiked at 100% of the concentration of the sample specification limit in six replicate preparations to demonstrate method precision. Six identical replicates were injected. The relative standard deviation was then calculated using the amounts of tegafur, gimeracil, and oteracil.

2.7.4. Intermediate Precision

In six preparations, the intermediate precision was obtained by sample spiking with tegafur, gimeracil, and oteracil at 100% of the prescribed limit in terms of sample concentration. The intermediate precision study was carried out over several days by numerous experts.

2.7.5. Sensitivity of the Method

The signal-to-noise ratios of 3:1 for the limit of detection (LOD) and 10:1 for the limit of quantification (LOQ) were used for evaluation.

2.7.6. Robustness

To verify the method's durability, small changes were made in chromatographic parameters, such as organic phase (+) (65B:35A) (-) (55B:45A) and mobile phase flow (+) (1.1 ml/min) (-) (0.9 ml/min).

2.7.7. Specificity

Two distinct samples were administered for comparison to placebo controls. Any interference peaks found in LC chromatograms for the therapeutic matrix (combination of medication and placebos) were investigated.

2.8. Forced Degradation Studies

2.8.1. Preparation of Buffer

In this stage, formic acid (1 ml) was dissolved in 1 liter of water.

2.8.2. Mobile Phase Preparation

The mobile phase was made by combining acetonitrile and buffer at a 60:40 ratio. The mobile phase was further filtered through 0.45 µm membrane filter paper.

2.8.3. Preparation of Standard Stock Solution for Forced Degradation

A sample of 327 mg was measured and then added to a 10 ml volumetric flask. Afterward, 7 ml of the diluent was added. Later, this solution was further sonicated for 15 min to dissolve the contents before they were diluted to a final volume of 10 ml with the diluent and combined.

2.8.4. Acid Degradation Procedure

In this stage, 1 ml of the sample stock solution was taken into a 10 ml volumetric flask, and 1 ml of 1 N HCl was added. It was placed at room temperature (RT) for 30 min. After that, 1 ml of 1 N NaOH was added slowly and diluted to volume with the diluent.

2.8.5. Base Degradation Procedure

A 10 ml volumetric flask was taken. The sample stock solution quantity of 1 ml was transferred, and 1 ml of 1 N NaOH was added. It was then placed at RT for 30 min. Further, 1 ml of 1 N HCl was added and diluted to volume with the diluent.

2.8.6. Peroxide Degradation Procedure

A 10 ml volumetric flask was taken. The sample stock solution quantity of 1 ml was transferred, and 1 ml of 10% H_2O_2 was added. It was then placed at RT for 30 min and diluted to volume with the diluent.

2.8.7. Reduction Degradation Procedure

A 10 ml volumetric flask was taken. The sample stock solution quantity of 1 ml was transferred, and 1 ml of 10% NaHSO4 was added. It was then placed at RT for 30 min and diluted to volume with the diluent.

2.8.8. Thermal Degradation (105°C/6 h) Procedure

A total of 500 mg of the sample was exposed for 6 h at 105°C, and the exposed standard was examined. Afterward, 327 mg of the exposed sample was placed in a 10 ml flask and diluted with the diluent according to volume. Finally, 1 ml of the above stock was diluted to 10 ml with the diluent.

2.8.9. Photodegradation Procedure

A total of 500 mg of the sample was exposed to sunlight for 6 h, and the exposed sample was analyzed. A total of 327 mg of the exposed sample was placed in a 10 ml flask and diluted with the diluent according to volume. After that, 1 ml of the above stock was diluted to 10 ml with the diluent.

2.8.10. Hydrolysis Degradation Procedure

A 10 ml volumetric flask was taken. A sample stock solution quantity of 1 ml was transferred, and 3 ml of HPLC grade water was added. It was then placed at room temperature (RT) for 3 min. Finally, 1 ml of the above stock was diluted to 10 ml with diluent. The forced degradation studies are summarized in table 1.

3. Results

3.1. Method Development and Optimization

The analytical method development activity was initiated by selecting a mobile phase with varied combinations of acid and organic phases. Trials were conducted on 0.1% orthophosphoric acid (OPA) with acetonitrile in varied concentration ratios. It was observed that in most of the trials with 0.1% orthophosphoric acid with acetonitrile, the baseline was not stable and had high noise. Finally, trials were conducted on 0.1% formic acid with acetonitrile in varied concentration ratios, and the peak response was found to be better than the OPA and acetonitrile combination. Various C_{18} columns were also scanned for better separation. The final optimized LC-MS method was developed on an Inertsil ODS column with dimensions of 150 mm×4.6 mm, 3.5 µm, and a mobile phase composition of formic acid (0.1%) and acetonitrile in the proportion of 40:60 injected in isocratic mode. The mobile phase flow rate was 1.0 ml/min, and the auto-injector volume was 10 µl. The column oven temperature was maintained at 25°C (ambient or room temperature). The wavelength maxima of tegafur, gimeracil, and oteracil were 224, 219, and 212 nm, respectively. The mobile phase was used as the diluent. The isosbestic point was found to be 220 nm (refer to figure 2), and this wavelength was selected for estimating the combined response of tegafur, gimeracil, and oteracil. In the optimized chromatographic conditions, tegafur, gimeracil, and oteracil peaks elute at 2.3, 3.7, and 5.3 min, respectively. Therefore, each injection was run for only 7 min. The resolution between tegafur and gimeracil and the resolution between gimeracil and oteracil were greater than 2.0. The USP plate count (N) and the resolution for tegafur, gimeracil, and oteracil were more than 5000 each, and the peak asymmetry of tegafur, gimeracil, and oteracil ranged from 0.9 to 1.1, which was optimum. The optimized chromatogram is shown in figure 3.

3.2. Method Validation

ICH Q2 standards were used to validate the optimized analytical technique. The estimated approach was validated well using ICH principles, and the results were within acceptable limits. Therefore, this validated analytical technique can be used for the estimation of tegafur, gimeracil, and oteracil.

Table1- Forced Degradation Studies conditions

S. No.	Name	Conditions
1	Acid Degradation	1 ml of 1 N HCl at room temperature (RT) for 30 min
2	Base Degradation	1 ml of 1 N NaOH at room temperature (RT) for 30 min
3	Peroxide Degradation	1 ml of 10% H_2O_2 at room temperature (RT) for 30 min
4	Reduction Degradation	1 ml of 10% NaHSO4 at room temperature (RT) for 30 min
5	Thermal Degradation	exposed for 6 h at 105°C
6	Photodegradation	exposed to sunlight for 6 h
7	Hydrolysis Degradation	3 ml of HPLC grade water at room temperature (RT) for 30min

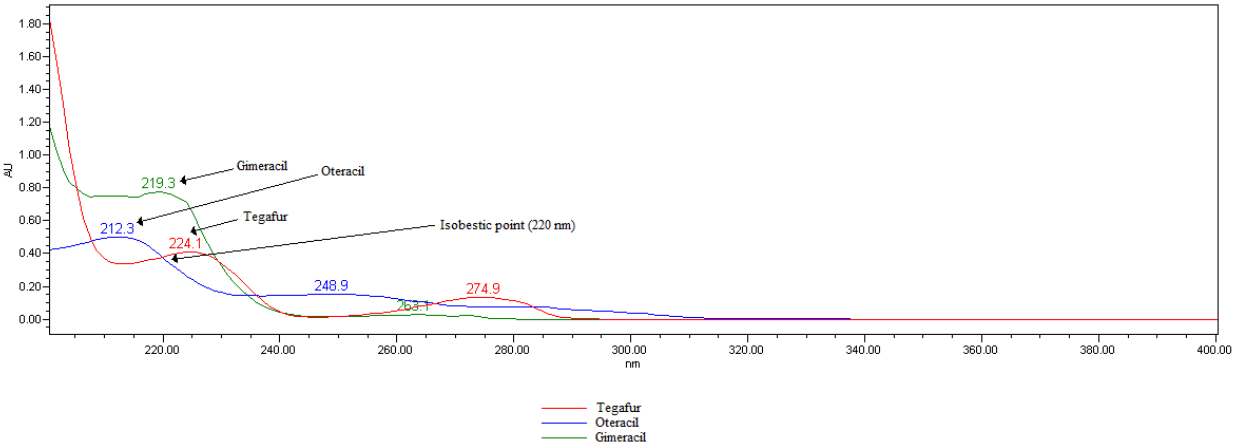


Fig. 2. Spectra showing Isosbestic point for Tegafur, Gimeracil and Oteracil

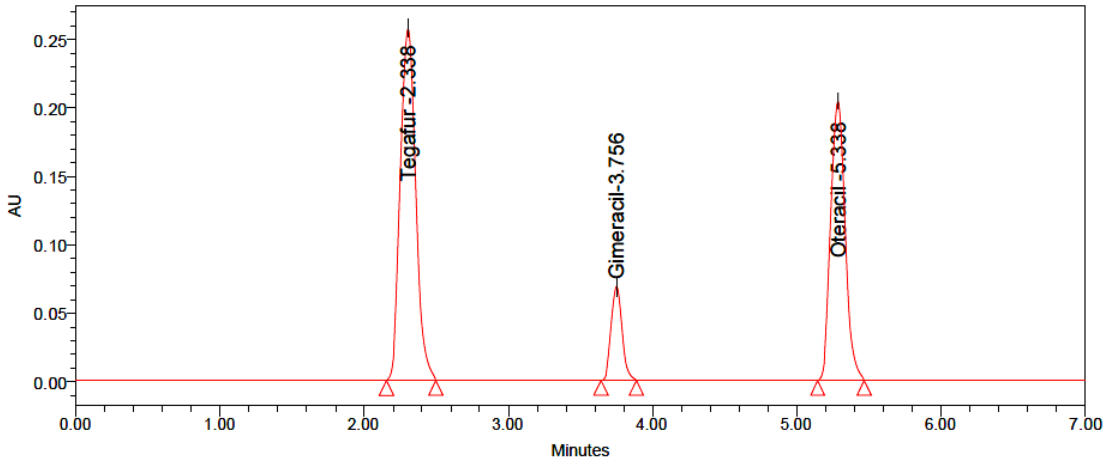


Fig.3. Final Optimized HPLC chromatogram for tegafur, gimeracil and oteracil potassium.

3.2.1. System Suitability Test

Analytical techniques include workability testing of the system as the primary step of any analysis. To find the appropriate settings, the system suitability indicators were examined and used. For this objective, the retention duration, the number of theoretical plates, and the tailing factor were investigated. The theoretical plate number count exceeded 2000, which was judged adequate. According to the standards, the tailing factor was within the specified limitations. These results demonstrate that the suggested approach may deliver findings of satisfactory quality. All of the system's proper parameters were agreed upon and found to be within the parameters. The findings are summarized in table 2.

3.2.2. Linearity

Linearity parameters displayed a direct proportionate connection between the concentration and test outcome. Tegafur, gimeracil, and oteracil linearity were examined in the ranges of 50-300 µg/ml, 14.5-87 µg/ml, and 39.5-237 µg/ml, respectively. The regression coefficient (R^2) values were calculated using the calibration curve. The calibration curve was made by calculating the acquired peak area and concentrations. Tegafur, gimeracil, and oteracil calibration curve R^2 values were 0.99956, 0.99986, and 0.99979, respectively, which were within the acceptable limits (NLT 0.99). As a result, the data demonstrated a high association between the peak area and analyte concentration. The outcomes are depicted in figure 4 and table 3.

3.2.3. Accuracy

The average recovery of tegafur, gimeracil, and oteracil from varied amounts of spiked sample solutions was 99.9%, 99.9%, and 99.4%, respectively. The percentage of recovery was estimated to be between 98 and 102%. This means that the suggested method was very accurate, and the findings were within the permissible limits of the ICH recommendations. The results are tabulated in tables 4 to 6.

3.2.4. Precision and Intermediate Precision

For tegafur, gimeracil, and oteracil method precision results, the percent relative standard deviation values were 0.52%, 0.26%, and 0.72%, respectively. Tegafur, gimeracil, and oteracil intermediate precision findings were 0.93%, 0.36%, and 1.01%, respectively. For tegafur, gimeracil, and oteracil method precision results, the total percent relative standard deviation values were 0.75%, 0.30%, and 0.84%, respectively. The results were substantially below the commonly accepted 2% limit. Consequently, the precision of the new procedure has been proven. Tables 7 and 8 display the results.

3.2.5. Limit of Detection and Limit of Quantitation

The LOD results of tegafur, gimeracil, and oteracil were estimated at 0.6, 0.174, and 0.474 µg/ml, respectively. Tegafur, gimeracil, and oteracil exhibited LOQs of 2.0, 0.58, and 1.58 mg/ml, respectively, suggesting that the procedure was sensitive. The LOD and LOQ values for tegafur, gimeracil, and oteracil are shown in table 9.

3.2.6. Robustness

To examine the robustness of the experimental process, the influence of minor adjustments in chromatographic parameters was considered. In all of the purposely modified chromatographic conditions, the RSD percentage of tegafur, gimeracil, and oteracil was less than 2.0. The system suitability requirements were not changed significantly and were all passed. The results are shown in table 10.

3.2.7. Specificity

The technique's specificity to one analyte is assessed in each analysis by looking for interference peaks in blank matrix samples. The approach's specificity was assessed with regard to interference resulting from the existence of extra placebo peaks. In LC-MS, the blank and placebo chromatograms exhibited essentially no intrusive peaks throughout the retention time ranges. As a consequence, the LC-MS technique presented in this article was particular and discriminating. The chromatograms of the placebo and blank solutions are shown in figures 5 and 6, respectively.

3.3. Forced Degradation Studies

According to the ICH guidelines, many forms of stressful circumstances have been investigated. During the investigation, a few degradation products (DP) were discovered. System suitability parameters, such as plate count, tailing factor, percentage relative standard deviation, and percentage deteriorated, were all within the parameters for forced degradation investigations. This shows that the method was accurate and stability-demonstrating. The findings are summarized in table 11, and chromatograms are shown in figures 7-13. No significant degradation was found in gimeracil.

3.3.1. Forced Degradation Studies of Tegafur

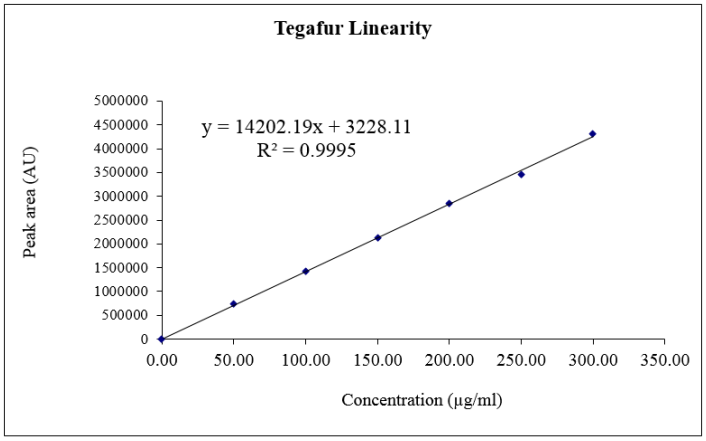
DP1: DP1 disintegration process is presented in figure 14a. For the strongest [M+H] ion, the m/z was -236.03. This peak was observed after degeneration by acid stress, as indicated by the ESI spectrum. Several degradative product (consequential) ions were indicated at m/z of -165.99 (loss of C_8H_8O) and -117.02 (loss of NH_3Cl) by the MS spectra of DP1.

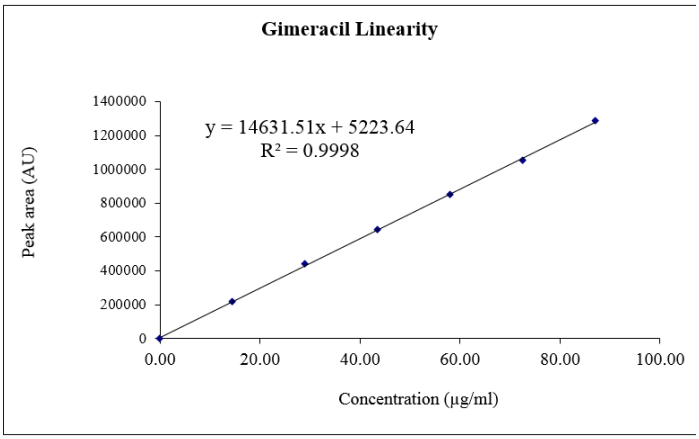
DP2: Under alkali degeneration conditions, the ESI fields confirmed the greatest [M+H] ion of m/z -186.08. The fragmentation process of DP2 is illustrated in figure 15a. Numerous product (consequential) ions were identified at m/z of -113.03 (expulsion of C_4H_8O) and -61.03 (expulsion of C_2H_5NO) in the DP2 MS spectra.

DP3: The process of disintegration of DP3 is depicted in figure 16a. The most powerful [M+H] ion of m/z -216.05

Table-2 System suitability results

S. No	Tegafur			Gimeracil			Oteracil			Resolution between Tegafur and Gimeracil	resolution between Gimeracil and Oteracil
	RT in (min)	Plate Count-USP	Tailing	RT in (min)	Plate Count-USP	Tailing	RT in (min)	Plate Count-USP	Tailing		
1	2.338	9865	1.08	3.756	5082	0.96	5.338	12247	0.94	5.58	6.45
2	2.334	9877	1.02	3.762	5068	0.95	5.353	12235	0.98	5.63	6.49
3	2.342	9886	1.04	3.758	5052	0.98	5.364	12246	0.91	5.64	6.52
4	2.364	9877	1.06	3.776	5063	0.96	5.383	12251	0.94	5.68	6.37
5	2.381	9886	1.04	3.745	5054	0.92	5.368	12239	0.98	5.74	6.29
6	2.374	9867	1.08	3.752	5058	0.96	5.385	12243	0.92	5.82	6.33





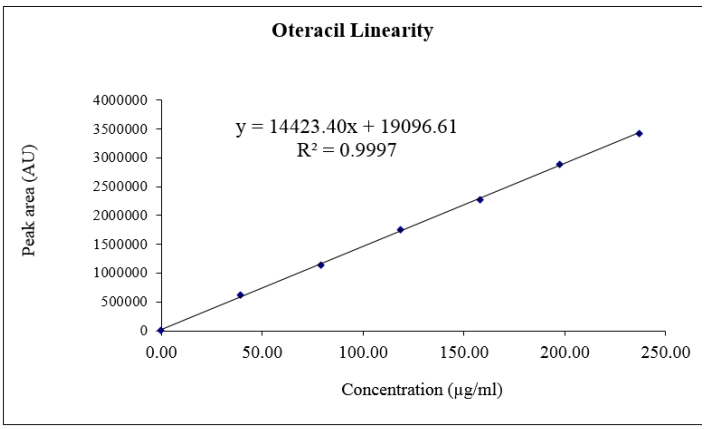


Fig. 4. Linearity graph for Tegafur, gimeracil and oteracil

Table-3 Linearity results of tegafur, gimeracil and oteracil

Tegafur			Gimeracil		Oteracil	
S. No	Concentration (µg/mL)	Peak area (AU)	Concentration (µg/mL)	Peak area (AU)	Concentration (µg/mL)	Peak area (AU)
1	0.00	0	0.00	0	0.00	0
2	50.00	728745	14.50	218543	39.50	618258
3	100.00	1426337	29.00	438957	79.00	1136981
4	150.00	2134596	43.50	641523	118.50	1754263
5	200.00	2851997	58.00	854715	158.00	2273451
6	250.00	3464774	72.50	1052647	197.50	2892475
7	300.00	4328451	87.00	1285476	237.00	3422459
Regression coefficient (R^2)		0.99956		0.99986		0.99979
Slope		14202.19		14631.51		14423.40
Intercept		3228.11		5223.64		19096.61

Table-4 Recovery results of tegafur

Recovery level	Amount Spiked (mg)	Amount recovered (mg)	% Recovery	Mean %Recovery	Overall Mean % recovery
50%	10	9.93	99.3	99.4	99.9
	10	10.02	100.2		
	10	9.86	98.6		
100%	20	20.09	100.5	100.4	
	20	20.08	100.4		
	20	20.05	100.3		
150%	30	29.87	99.6	99.9	
	30	30.06	100.2		
	30	29.98	99.9		

Table-5 Recovery results of gimeracil

Recovery level	Amount Spiked (mg)	Amount recovered (mg)	% Recovery	Mean %Recovery	Overall Mean % recovery
50%	2.9	2.888	99.6	99.9	99.9
	2.9	2.915	100.5		
	2.9	2.891	99.7		
100%	5.8	5.83	100.5	100.1	
	5.8	5.81	100.2		
	5.8	5.78	99.7		
150%	8.7	8.717	100.2	99.8	
	8.7	8.652	99.4		
	8.7	8.677	99.7		

Table-6 Recovery results of oteracil

Recovery level	Amount Spiked (mg)	Amount recovered (mg)	% Recovery	Mean %Recovery	Overall Mean % recovery
50%	7.9	7.798	98.7	99.5	99.4
	7.9	7.863	99.5		
	7.9	7.916	100.2		
100%	15.8	15.855	100.3	99.5	
	15.8	15.715	99.5		
	15.8	15.576	98.6		
150%	23.7	23.741	100.2	99.3	
	23.7	23.559	99.4		
	23.7	23.287	98.3		

Table-7 Results for Method Precision

S. No	Area of tegafur	% Recovery of tegafur	Area of gimeracil	% Recovery of gimeracil	Area of oteracil	% Recovery of oteracil
1	2826357	99.3	856623	100.2	2231454	99.4
2	2815434	98.9	853625	99.8	2259745	100.6
3	2822235	99.2	854718	99.9	2265963	100.9
4	2817459	99.0	855958	100.1	2264415	100.8
5	2854147	100.3	852956	99.7	2228971	99.3
6	2833542	99.6	851152	99.5	2241523	99.8
Mean		99.4		99.9		100.1
S.D		0.512		0.258		0.72
%RSD		0.52		0.26		0.72

Table-10 Results of robustness

S. No	Condition	%RSD of tegafur	%RSD of gimeracil	%RSD of oteracil
1	Mobile Phase Flow rate (+) 0.9ml/min	1.15	0.26	0.80
2	Mobile Phase Flow rate (-) 1.1ml/min	0.66	0.35	0.32
3	Mobile Phase Organic phase (-) 35	0.82	0.10	0.71
4	Mobile Phase Organic phase (+) 45	0.47	0.27	0.86

Table-8 Results for Intermediate precision

S. No	Area of tegafur	% Recovery of tegafur	Area of gimeracil	% Recovery of gimeracil	Area of oteracil	% Recovery of oteracil
1	2856321	100.2	850084	99.3	2231454	101.2
2	2815047	98.8	858254	100.3	2259745	100.9
3	2846303	99.9	855230	99.9	2265963	99.5
4	2835986	99.5	857485	100.2	2264415	100.9
5	2885614	101.3	853952	99.8	2228971	99.9
6	2817589	98.9	856634	100.1	2241523	98.6
Mean		99.8		99.9		100.2
S.D		0.929		0.361		1.011
%RSD		0.93		0.36		1.01
Overall Mean		99.6		99.9		100.15
Overall S.D		0.742		0.302		0.837
Overall %RSD		0.75		0.30		0.84

Table-9 Limit of detection and limit of quantification

Drug	LOD (µg/ml)	LOQ (µg/ml)
Tegafur	0.6	2
Gimeracil	0.174	0.58
Oteracil	0.474	1.58

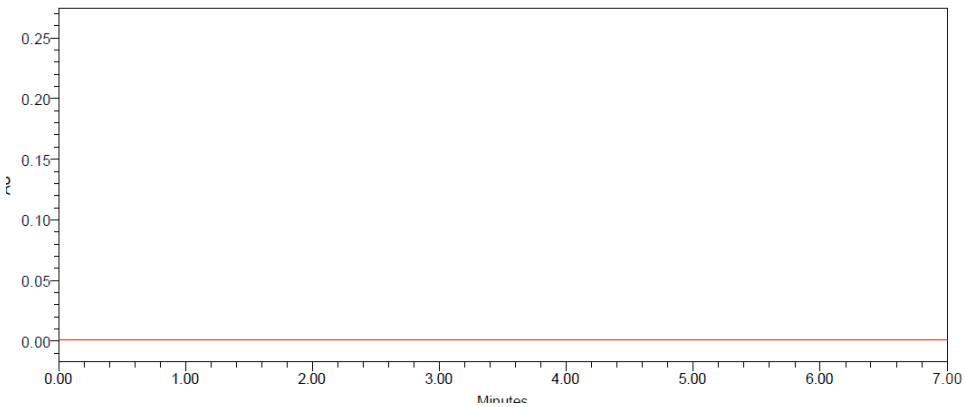


Fig.5. Chromatogram of Blank

was detected under peroxide deterioration conditions indicated by the ESI field. Many product ions were indicated at m/z of -146.01 (removal of C_4H_6O) and -61.01 (removal of C_3H_4FNO) in the DP3 MS spectra.

DP4: The ion with an m/z of -204.09 was the strongest [M+H] ion that was observed in the ESI spectrum under thermal degeneration conditions. Figure 17 illustrates the mechanism for DP4 fragmentation. The DP4 MS spectra at m/z of -134.04 (removal of C_4H_8O) and -61.03 (removal of $C_2H_7NO_2$) indicated many product ions.

3.3.2. Forced Degradation Studies of Oteracil

DP5: Figure 14.5 depicts the DP5 breakup mechanism. The strongest ion of [M+H] with m/z -230.94 was observed after acid degeneration, as indicated by the ESI spectrum. The MS spectra of DP5 indicated multiple product (consequential) ions at m/z -148.99 ($CHKO_2$ removal) and m/z-100.02 (NH_3Cl removal).

DP6: A prominent [M+H] ion was discovered when subjected to alkali degeneration studies. The ESI range was m/z -180.98 for the prominent alkali degradant, and the process of DP6 fragmentation is depicted in figure 15b. Several product ions were found in the DP6 aMS ranges at m/z -99.04 (elimination of $CHKO_2$) and m/z -44.03 (elimination of C_2H_5NO).

DP7: The most prominent [M+H] ion found in peroxide deterioration was at m/z -210.96 in the ESI spectra. Figure 16b depicts the DP7 fragmentation process. The existence of multiple product (consequential) ions at m/z -129.01 (elimination of $CHKO_2$) and m/z -72.03 (elimination of CH_3NO_2) was indicated by the MS spectra of DP7.

4. Discussion

An accurate and sensitive LC-MS technique was developed and validated for the concurrent quantification of tegafur, gimeracil, and oteracil potassium in the drug material and the medicinal dosage form. The proposed method was extremely simple, exact, and robust. The present method is superior to the existing method as it is a stability-indicating chromatographic method where characterization of the degradants was performed. During the stability investigations, just a handful of degradation products were found. The system suitability parameters, such as plate count, tailing factor, percentage relative standard deviation, and percentage deteriorated, were all within the parameters for forced degradation investigations. This illustrates how exact and reliable the technique was. Furthermore, no gimeracil degradation products were found. As a result, this technique can be utilized in the quality control departments to detect tegafur, gimeracil, and oteracil potassium.

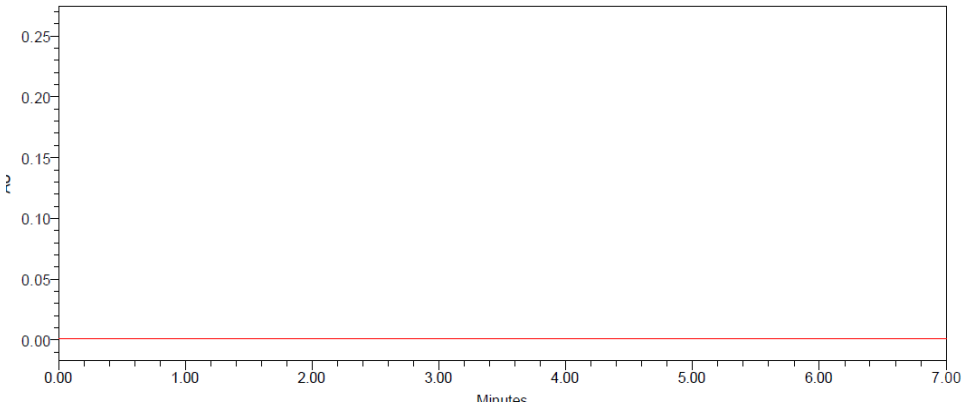


Fig.6. Chromatogram of Placebo

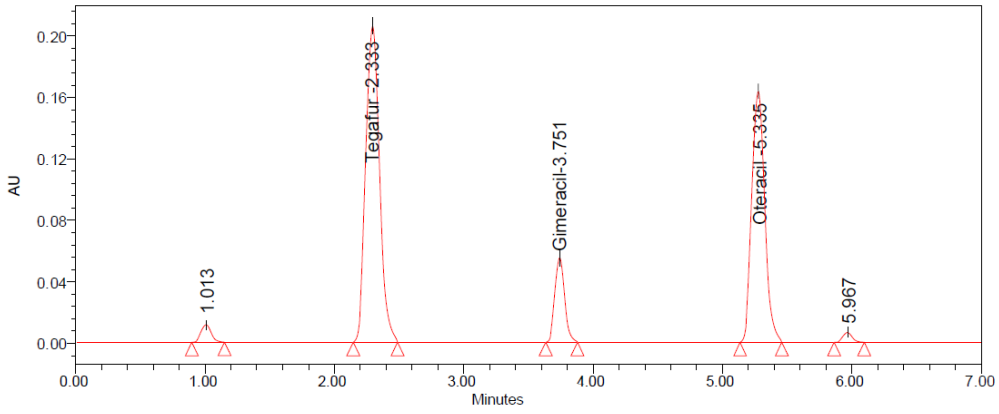
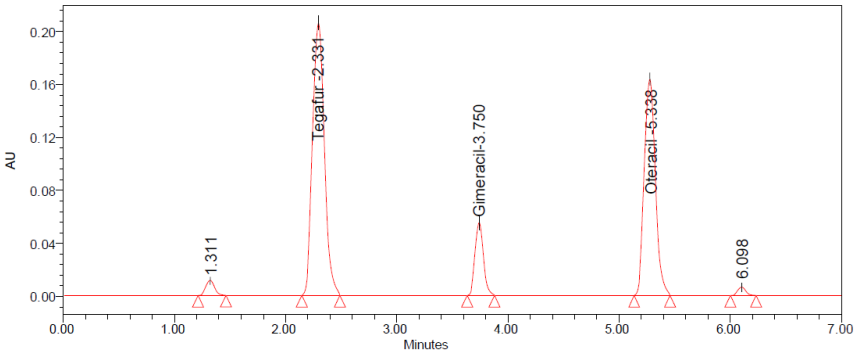


Fig.7. Chromatogram for Acid degradation

Table-11 Forced degradation study results

Name	Degradation Condition	% Recovered	% Degraded
Tegafur	Acid	86.5	13.5
	Alkali	87.2	12.8
	Peroxide	84.1	15.9
	Reduction	96.0	4.0
	Photo	95.3	4.7
	Thermal	90.7	9.3
	Hydrolysis	96.4	3.6
Gimeracil	Acid	98.0	2.0
	Alkali	96.7	3.3
	Peroxide	95.3	4.7
	Reduction	98.1	1.9
	Photo	97.0	3.0
	Thermal	97.1	2.9
	Hydrolysis	99.2	0.8
Oteracil	Acid	86.8	13.2
	Alkali	88.0	12.0
	Peroxide	86.0	14.0
	Reduction	96.1	3.9
	Photo	97.3	2.7
	Thermal	95.3	4.7
	Hydrolysis	98.7	1.3

Fig.8. Chromatogram for Base degradation



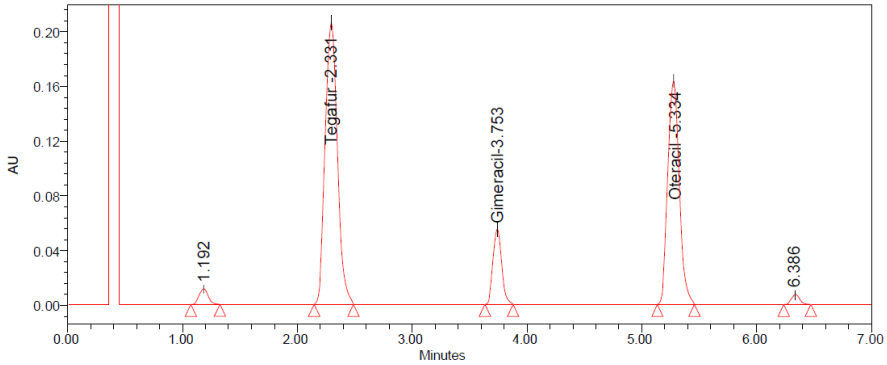


Fig.9. Chromatogram for Peroxide degradation

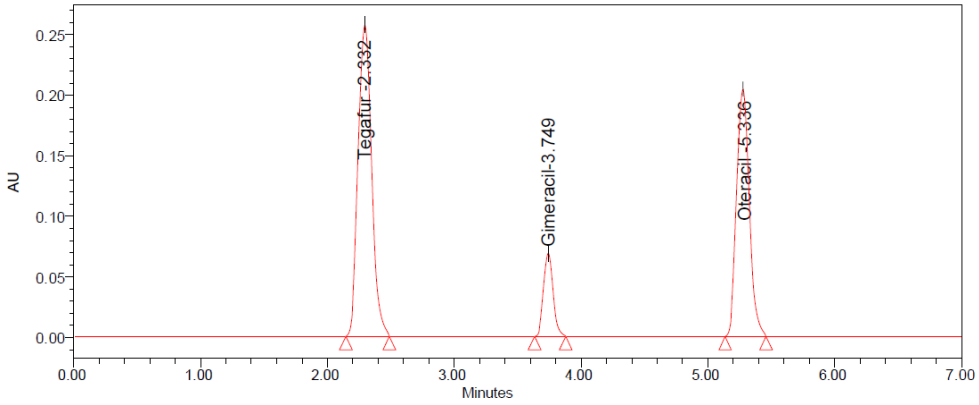


Fig.10. Chromatogram for Reduction degradation

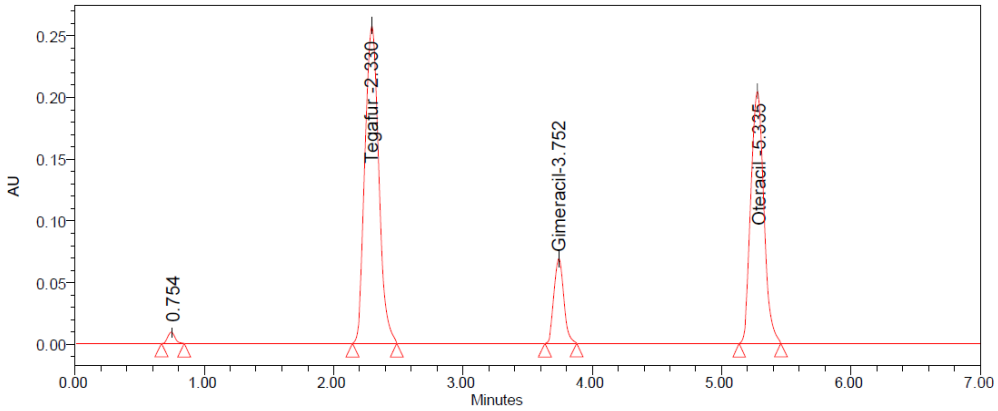


Fig.11. Chromatogram for Thermal degradation

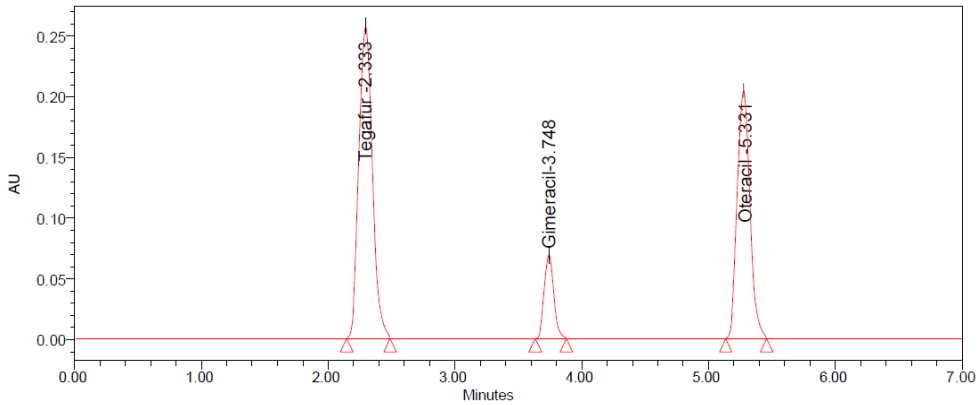


Fig.12. Chromatogram for Photo stability degradation

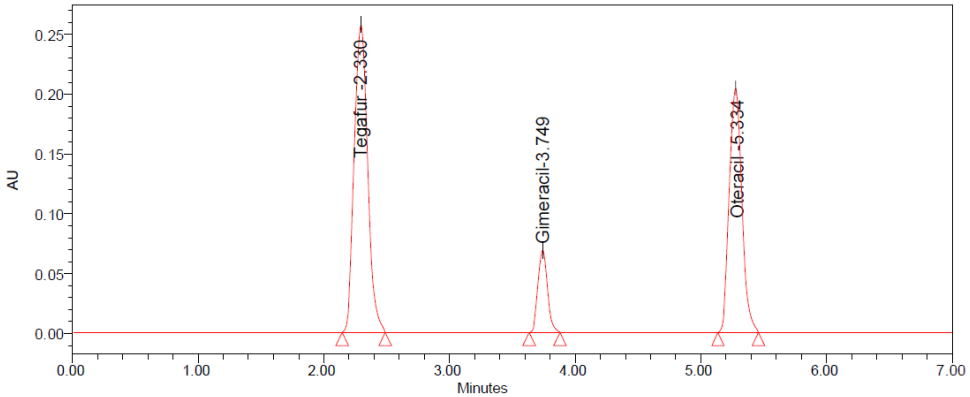
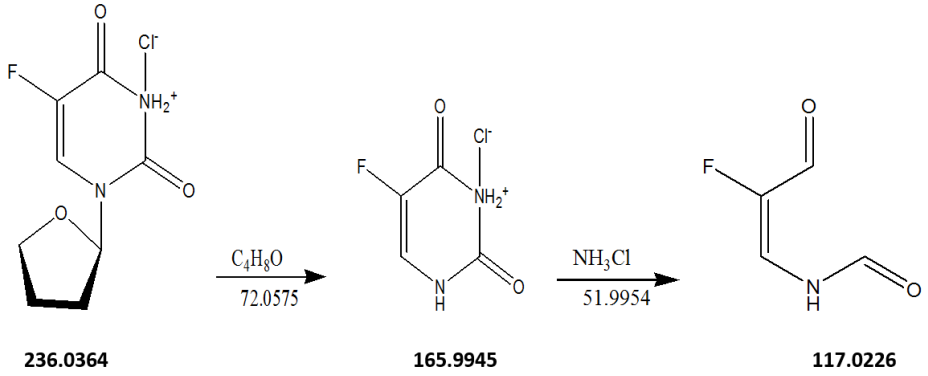


Fig.13. Chromatogram for Hydrolysis degradation

Fig.14. Degradation products of acid impurity for

a) tegafur impurity- DP1



b) oteracil acid impurity-DP5

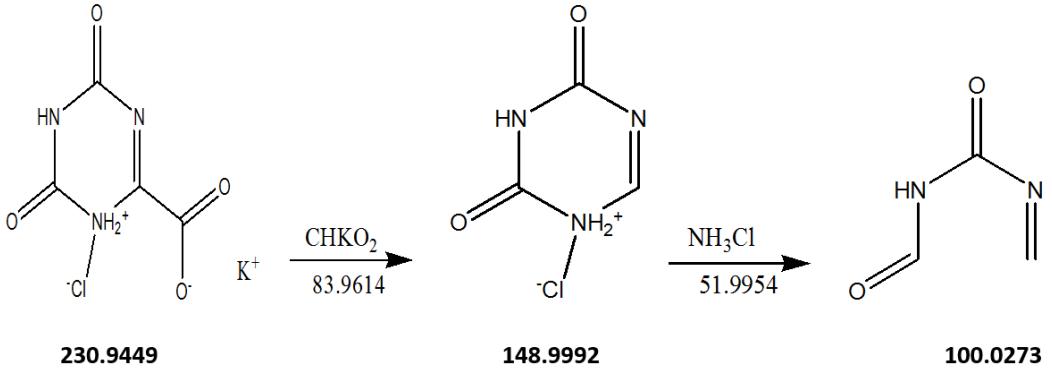
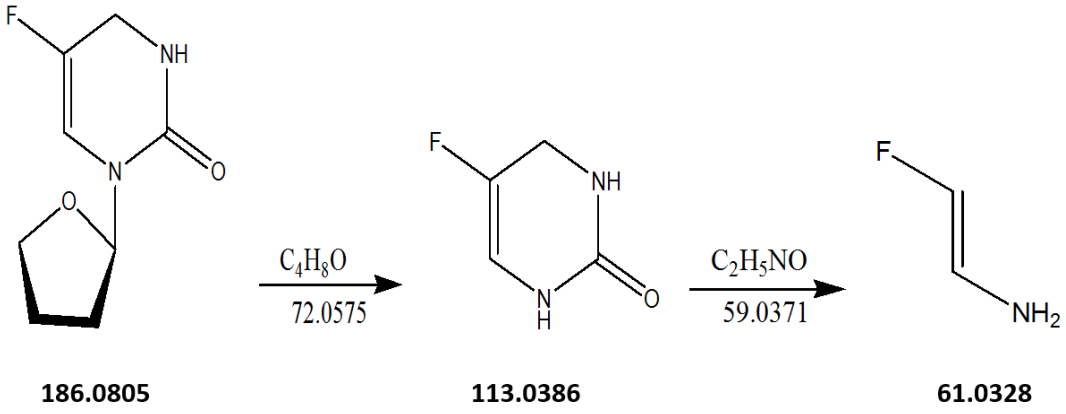


Fig.15. Degradation products of alkali impurity for

a) tegafur impurity-DP2



b) oteracil impurity-DP6

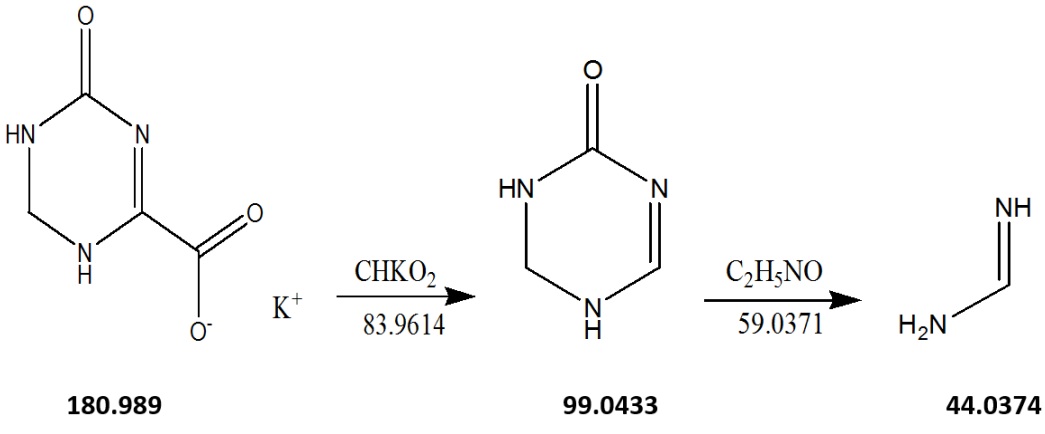
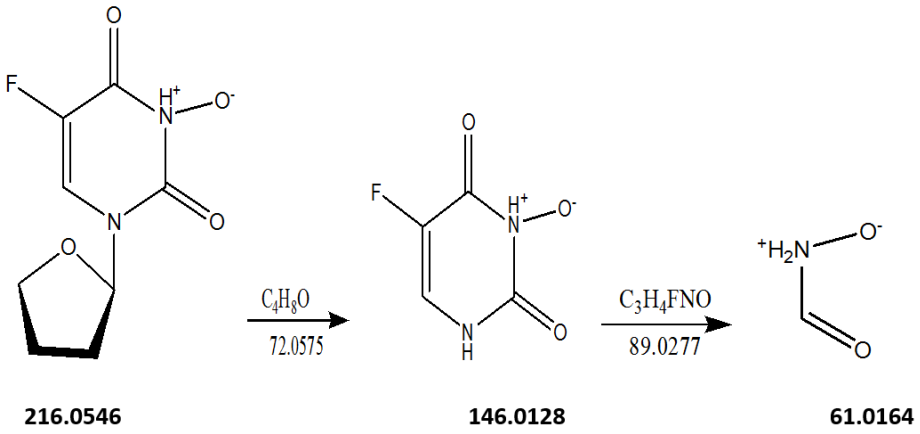
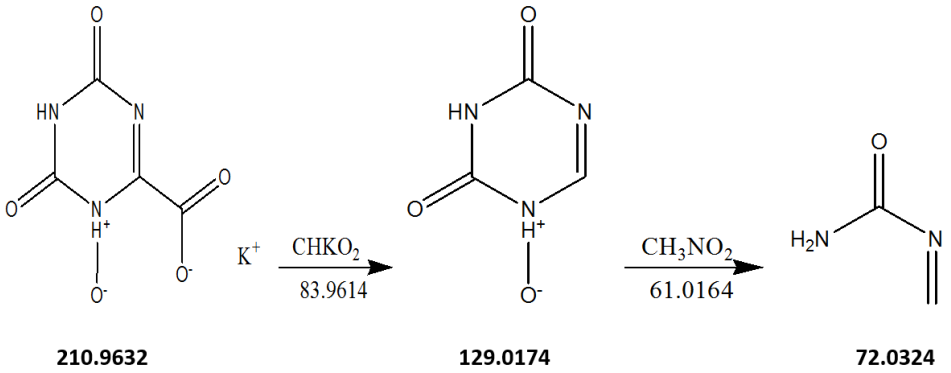


Fig.16. Degradation products of peroxide impurity for

a) tegafur impurity-DP3



b) oteracil impurity-DP7



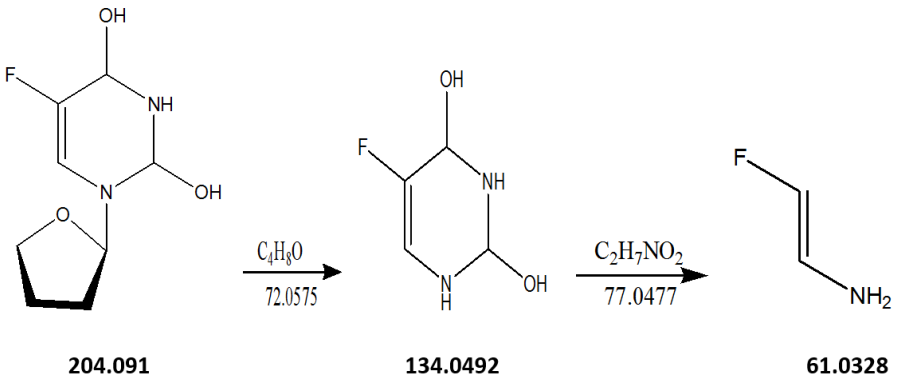


Fig.17. Degradation products of tegafur thermal impurity-DP4

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Authors' Contribution
Ashish Kumar Pal: Conceptualization, methodology, and original draft preparation
Raja Sundararajan: Supervision, reviewing, and editing.

Ethics
We declare that all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest
The authors state that they do not have any known conflicting financial interests or personal connections that may seem to have influenced the work disclosed in this study.

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