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Research Article

A SIMULTANEOUS ESTIMATION, VALIDATION AND FORCED DEGRADATION STUDIES OF 5-FLUOROURACIL AND TEGAFUR IN A PHARMACEUTICAL DOSAGE FORM USING REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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ABSTRACT

Objective: The present study describes the stability indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous estimation of 5-fluorouracil and tegafur in pharmaceutical dosage forms.

Method: 5-fluorouracil and tegafur the propose RP-HPLC method were developed by using Shimadzu Prominence-i LC-2030 HPLC system equipped with UV detector and chromatographic separation was carried on shim-pack gist c18 (250 × 4.6 mm, 5 µ) column at a flow rate of 1 ml/min and the run time was 10 min. The mobile phase consisted of methanol and water in the ratio of 50:50% v/v and elements were scanned using a UV detector at 271 nm.

Result: The retention time of 5-fluorouracil and tegafur was found to be 2.74 and 3.66 min, respectively. A linearity response was observed in the concentration range of 13.4 µg/ml–31.3 µg/ml for 5-fluorouracil and 6 µg/ml–14 µg/ml for tegafur, respectively. Limit of detection and limit of quantification of 5-fluorouracil were 10.97 µg/ml and 33.26 µg/ml and for tegafur are 4.89 µg/ml and 14.83 µg/ml, respectively.

Conclusion: The stability indicating that the method was developed by subjecting drugs to stress conditions such as acid and base hydrolysis, oxidation, photo and thermal degradation, and degraded products formed were resolved successfully from samples.

Keywords: 5-Fluorouracil, Tegafur, Reversed-phase high-performance liquid chromatography, Degradation and validation.

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INTRODUCTION

5-Fluorouracil (Fig. 1) (5-fluoro-pyrimidine-2,4-dione) has been widely used in the chemotherapy of a variety of human carcinomas, including the head, neck, and gastrointestinal tract and breast using various schedules [1]. However, administration of the compound often caused severe gastrointestinal toxicity and myelosuppression. To overcome the toxicity, many derivatives and related compound have been synthesized [4].

Tegafur (Fig. 2) [5-fluoro-1-(oxolan-2-yl) pyrimidine-2,4-dione] a 2-tetrahydrofuranyl derivative of 5-fluorouracil has been shown to have a broad spectrum of antitumor activity when administered intravenously or orally [1]. It acts as a prodrug of 5-fluorouracil and produces comparatively little myelosuppression [2,3].

Literature survey reveals HPLC for estimation 5-fluorouracil and tegafur is not available for pharmaceutical dosage form.

METHODS

5-fluorouracil and tegafur standard were provided by Yarrow Chem. Products, Dombivali, Mumbai, Maharashtra, India. Commercial tablet dosage form tegafur (5-fluorouracil 224 mg and Tegafur 100 mg) was purchased from local markets. The HPLC grade methanol and water were purchased from Thomas Fisher scientific Pvt. Ltd., Powai, Mumbai, Maharashtra, India. Hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from S.D. Fine chemicals.

HPLC instrument

The chromatographic separation was carried out by Shimadzu Prominence-i LC-2030 HPLC system equipped with UV detector and autosampler. The lab solution software was used for signal monitoring and processing, UV chamber has been used for photolytic degradation. Hot air oven was used for thermal degradation.

Chromatographic conditions

The chromatographic separation of analytes was carried out using Shimadzu reversed-phase high-performance liquid chromatography (RP-HPLC) system with Shim-pack GIST C18 (250 × 4.6 mm) column. The mobile phase consists of water and methanol in the ratio 50:50 %v/v, and column temperature was maintained at 30°C the analytes were detected at 271 nm using UV detector. The runtime was set at 10 min at a flow rate of 1 ml/min.

Preparation of standard stock solution

Standard stock solution of 5-fluorouracil and tegafur was prepared separately by dissolving 22.4 mg of 5-fluorouracil and 10mg of tegafur in 100 ml of volumetric flask with water:methanol (50:50) as diluents and sonicated for 10 min. From the above solution, 1 ml of 5-fluorouracil and 1 ml of tegafur were transferred separately to 10 ml volumetric flask, made up the volume to get 24 µg/ml and 10 µg/ml of stock solution of 5-fluorouracil and tegafur respectively.

Preparation of sample solution

Ten tablets (Tegafur capsule; 224 mg 5-fluorouracil and 10 mg tegafur) were weighed and the average weight of each tablet was calculated; then the weight equivalent to 10 tablets was transferred into 100 ml volumetric flask; 50 ml of diluents were added and sonicated for 30 min; further the volume made up with diluents and filtered. From the filtered solution, 1 ml was pipette out into 10 ml volumetric flask and sonicated for 10 min, and volume made up to 10 ml with diluents.

Forced degradation studies

Forced degradation studies were carried out in the presence of acid, alkali, H_2O_2, and heat. With the sample bearing concentration 22.4 µg/ml and 10 µg/ml of 5-fluorouracil and tegafur, respectively, these studies help to know the inherent stability characteristic of the active molecule in drug product and possible degradation products [6,7].

Table 1: Optimized chromatographic conditions

S. No	Parameter	Optimized conditions
1	Column	SHIM-PACK C-18 (250×4.6 mm, 5 µ) column
2	Mobile phase	Methanol and water 50:50% v: v
3	Flow rate	1 ml/min
4	Wavelength	UV detector 271 nm
5	Injection volume	20 µL
6	Temperature	30°C
7	Retention time	5-fluorouracil 2.74 min and tegafur 3.66 min

Table 2: System suitability parameters

Sr. No	Parameters	5-fluorouracil	Tegafur
1	Retention time	2.74	3.66
2	USP plate count	3765	4652
3	USP tailing	1.21	1.16

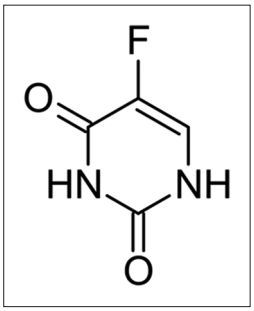

Fig. 1: 5-fluorouracil

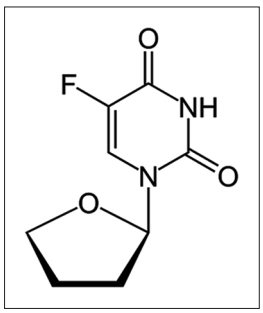

Fig. 2: Tegafur

Acid degradation

Acidic degradation was carried out by adding 5 ml of 1N HCl and after 60 min neutralizing the mixture by adding 5 ml of 1N NaOH.

Alkali degradation

Alkali degradation was carried out by adding 5 ml of 1N NaOH and after 60 min neutralizing the mixture by adding 5 ml of 1N HCl.

Oxidative degradation

Oxidative degradation was performed by exposing the drug to 5 ml of 10% (v/v) H2O2 for 60 min.

Photolytic degradation

Photolytic degradation was carried out by exposing the drug content to UV light inside a UV chamber for 1d.

Thermal degradation

Thermal degradation was performed by placing the drug in an oven at 105°C for 24 h to study dry heat degradation.

Statistical analysis

To evaluate the contribution of each factor with different levels of responses, two-way analysis of variance was performed using GraphPad Prism 7.04 Software.

RESULTS AND DISCUSSION

Method development

A number of trials were conducted with different columns, with various combination mobile phases to develop a suitable RP-HPLC method for estimation of 5-fluorouracil and tegafur in tablet dosage form. Then, finally a typical chromatogram was obtained with water and methanol in the ratio 50:50% v/v. The chromatographic separation was performed on SHIM-PACK C-18 (250 × 4.6 mm, 5 µ) column on injecting 20 µL, and the analysts were detected with UV detector at 271 nm. The retention time of 5-fluorouracil and tegafur was found to be 2.7 min and 3.6 min, respectively. The force degradation study was also carried out using the developed method. The optimized conditions were given in Table 1.

Method validation

The validation was performed with an above developed RP-HPLC method for simultaneous estimation of 5-fluorouracil and tegafur according to ICH guidelines. Various parameters were evaluated such as system suitability, precision, accuracy, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

System suitability

System suitability was performed to verify the acceptability of the resolution and repeatability of the system. System suitability was

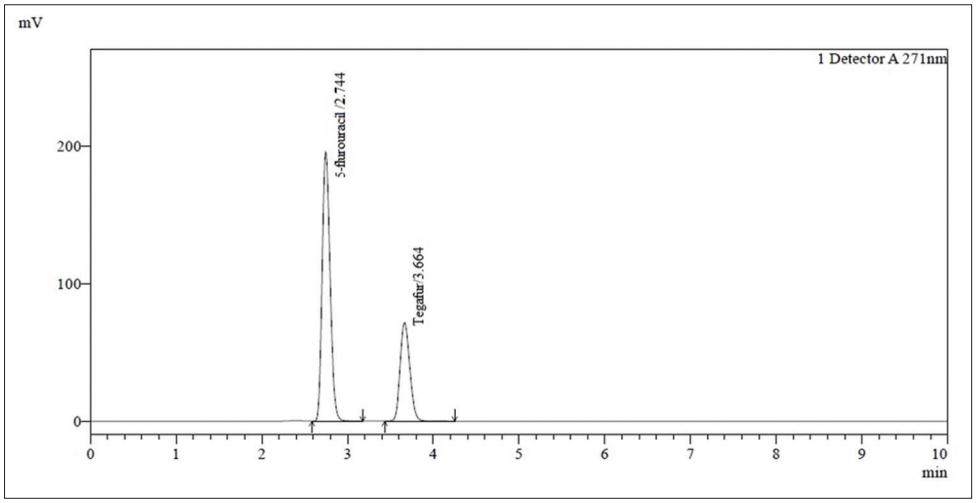

Fig. 3: Chromatogram of standard 5-fluorouracil and tegafur

performed by injecting six replicate injections of the standard solution (100%) and parameters such as peak area, USP tailing, theoretical plates, retention time, and peak asymmetry were evaluated. The percentage RSD was determined and reported within limits (Table 2 and Fig. 3).

Accuracy

The accuracy of the proposed method was evaluated by calculating the recovery studies of the test drug at three different concentration levels (80%, 100%, and 120%) by the standard addition method. A known amount of 5-fluorouracil and tegafur was added to the pre quantified sample solution, and three replicates of each concentration were injected into developing chromatographic conditions. The mean percentage recovery of 5-fluorouracil and tegafur was varied between 99.5 and 100.4% indicating that the developed method was found to be accurate (Table 3).

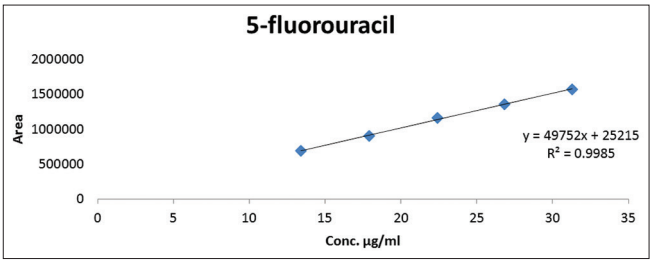

Fig. 4: Linearity graph of 5-fluorouracil

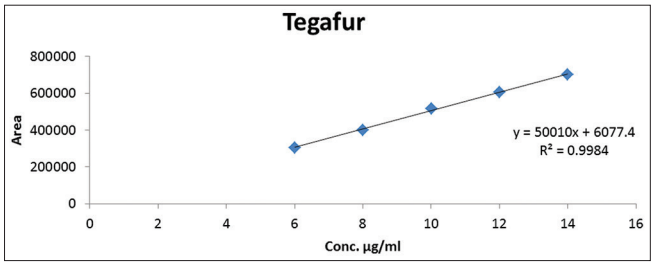

Fig. 5: Linearity graph of tegafur

Precision

The precision of an analytical procedure may be defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The method precision and system precision studies were carried out by injecting six replicates of both standard and test solutions with the same concentration. The percentage RSD was calculated from the chromatograms and the results obtained were within the limits of 2%, and the proposed method was found to be precise (Table 4).

Linearity

The linearity of the method was determined at different concentration levels ranging from 13.44 µg/ml to 31.36 µg/ml of 5-fluorouracil and from 6 µg/ml to 14 µg/ml of tegafur. All the concentrations were prepared and injected into the system. The linearity curve was constructed by plotting peak area versus concentration of the analyte. From the results obtained that the proposed method was found to be linear. The regression coefficient was found to be 0.998 and 0.998 for 5-fluorouracil and tegafur, respectively (Figs. 4 and 5).

LOD and LOQ

In the present study, the LOD and LOQ 5-fluorouracil and tegafur were evaluated based on the standard calibration curve method. LOD is performed to know the lowest concentration level of the analysts that give a measurable response. LOD and LOQ for 5-fluorouracil are 10.97 µg/ml and 33.26 µg/ml and for tegafur are 4.89 µg/ml and 14.83 µg/ml, respectively.

Robustness

Robustness of the proposed method has been evaluated by small deliberate changes in the system parameters such as flow rate,

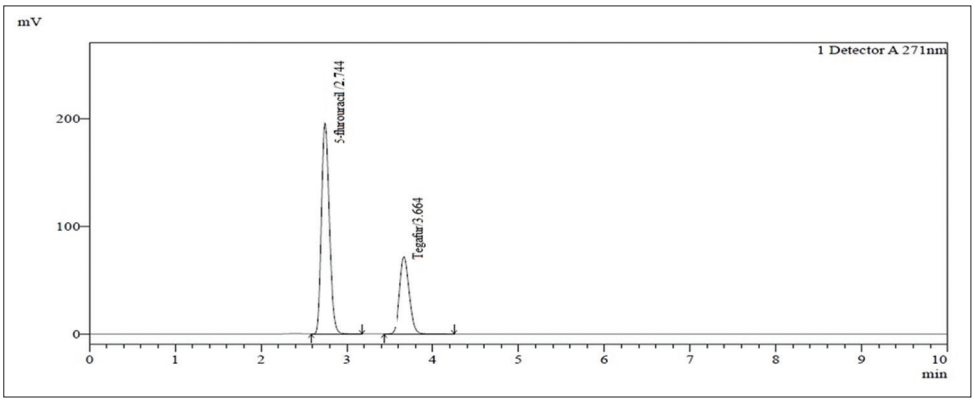

Fig. 6: Chromatograph of untreated capsule (sample)

Table 3: Percentage recovery results of 5-fluorouracil and tegafur

S. No	Spiked (%)	Percentage recovery		Mean percentage recovery	% RSD	
		5-fluorouracil	Tegafur		5-fluorouracil	Tegafur
1	80	99.56	99.67	99.66	0.05	0.05
		99.61	99.71			
		99.67	99.78			
2	100	99.86	99.84	99.90	0.06	0.04
		99.92	99.92			
		99.98	99.89			
3	120	99.98	100.26	100.35	0.49	0.24
		100.46	99.98			
		100.97	100.46			

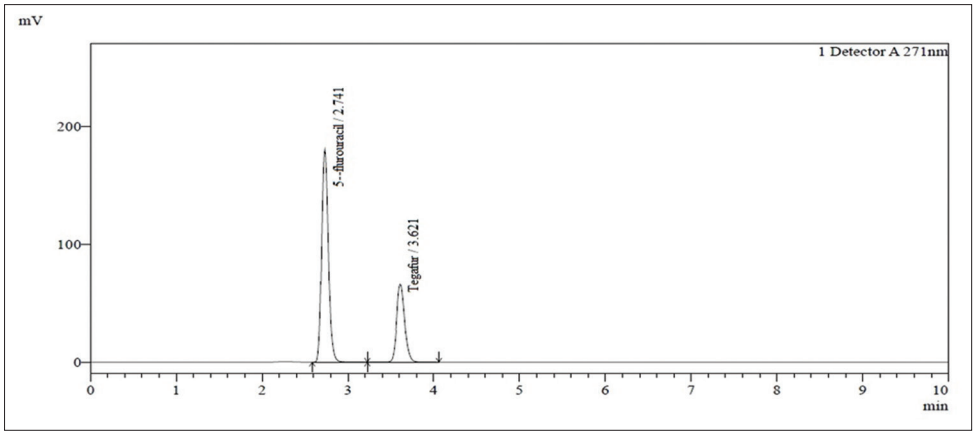

Fig. 7: Chromatograph of acid degradation

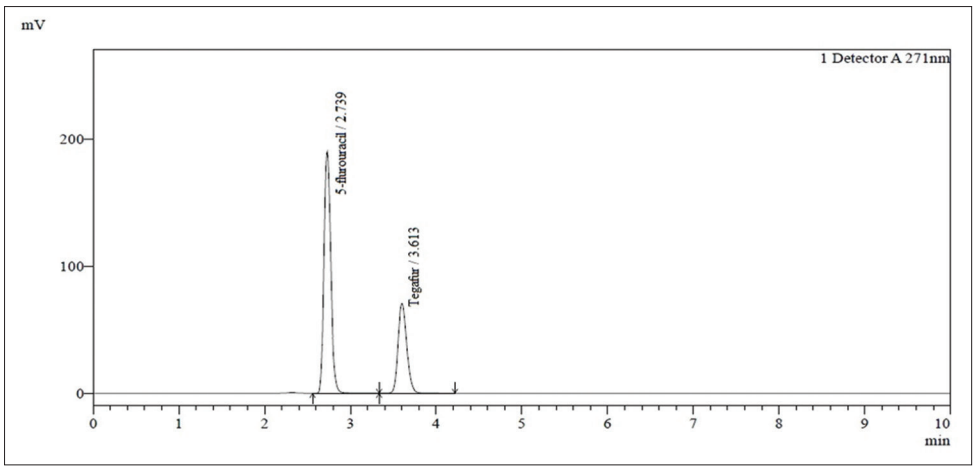

Fig. 8: Chromatograph of base degradation

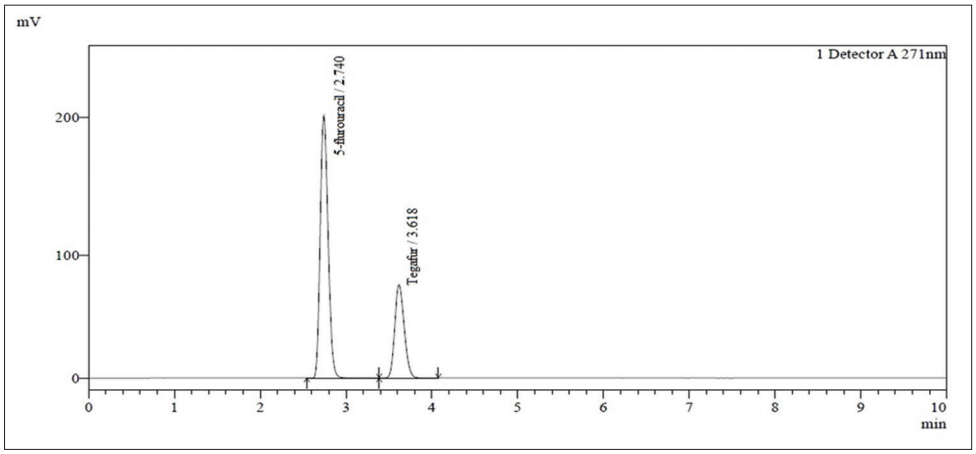

Fig. 9: Chromatograph of ultraviolet degradation

wavelength, and temperature. It was found that none of the above parameters caused an alteration in the peak area, retention time, and USP tailing by small changes such as ±0.2 ml change in flow rate, ±2 nm wavelength, and ±2°C change in temperature. The percentage RSD was found to be within limits, and the method was found to be robust (Table 5).

Assay of marketed formulation

Analysis of the marketed formulation (tegafur capsule: 5-fluorouracil is 224 mg and tegafur is 100 mg) was purchased from the local pharma market. 10 capsules weighed and average weight were calculated; weight equivalent to 1capsules was transferred into 100 ml volumetric flask, 50 ml of diluents was added and sonicated for 30 min, and further volume was made up with diluents and filtered. From filtered solution, 1 ml was a pipette out into 100 ml volumetric flask and made up to 100 ml with diluents. From the resulting solution, 20 µL was injected into HPLC system, and peak areas were recorded. The percentage assay of the marketed formulation was found to be 98.7 for 5-fluorouracil and 98.6 for tegafur (Table 6).

Table 4: Results of method precision for 5-fluorouracil and tegafur

S. No	Sample no	5-fluorouracil	Tegafur
		% Assay	% Assay
1	Injection 1	99.82	99.59
2	Injection 2	99.83	99.60
3	Injection 3	99.77	99.57
4	Injection 4	99.88	99.61
5	Injection 5	99.83	99.60
6	Injection 6	99.85	99.61
7	Average	99.83	99.60
8	SD	0.038	0.015
9	% RSD	0.038	0.015

Table 5: Results of robustness

S. No.	Parameters	5-fluorouracil			Tegafur		
		RT	NTP	TF	RT	NTP	TF
1	Flow rate 0.9 ml	3.03	4138	1.21	4.04	4953	1.17
	Flow rate a 1.1 ml	2.50	3755	1.20	3.33	4568	1.15
2	Temperature 28	2.75	3915	1.21	3.67	4755	1.16
	Temperature 32	2.74	3933	1.21	3.64	4739	1.16
3	Wavelength 268	2.74	3948	1.21	3.65	4757	1.16
	Wavelength 273	2.74	3929	1.21	3.65	4754	1.16

RT: Retention time, NTP: Number of theoretical plate, TF: Tailing factor

Forced degradation studies (Figs. 6-11)

ICH degradation was attempted to various stress conditions such as acid hydrolysis (using 1N HCl), base hydrolysis (using 1N NaOH), oxidative hydrolysis (using 5% H_2O_2), thermal degradation (heated at 100°C for 24 h), and photolytic degradation (using UV light inside a UV chamber for 48 h). The results of stress studies were shown in Table 7.

CONCLUSION

In the present study, a method has been developed using RP-HPLC and validated for simultaneous estimation with stability indication of 5-fluorouracil and tegafur in tablet dosage form. The validated method was successfully used for stress testing, analysis of 5-fluorouracil and tegafur. The proposed method was proved selective, accurate, precise, and rapid and it can be used for the routine analysis of 5-fluorouracil and tegafur in the formulation.

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AUTHORS' CONTRIBUTIONS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this

Table 6: Percent content of marketed formulation

S. No	Tablet	Drug	Amount taken (mg)	Amount found (mg)	% assay
1	Tegafi plus (5-fluorouracil 224 mg and tegafur 100 mg)	5-fluorouracil	224	223.61	99.83
		Tegafur	100	99.60	99.60

Table 7: Forced degradation studies of 5-fluorouracil and tegafur

S. No	Stress condition	5-fluorouracil		Tegafur	
		% Assay	% Difference W.R.T control	% Assay	% Difference W.R.T control
1	Control	98.6	NA	99.4	NA
2	Acid degradation	91.52	8.47	90.19	9.80
3	Base degradation	94.37	5.62	93.29	6.70
4	Oxidative degradation	96.48	3.51	96.06	3.93
5	Photolytic degradation	97.24	2.75	96.58	3.41
6	Thermal degradation	98.87	1.12	98.56	1.43

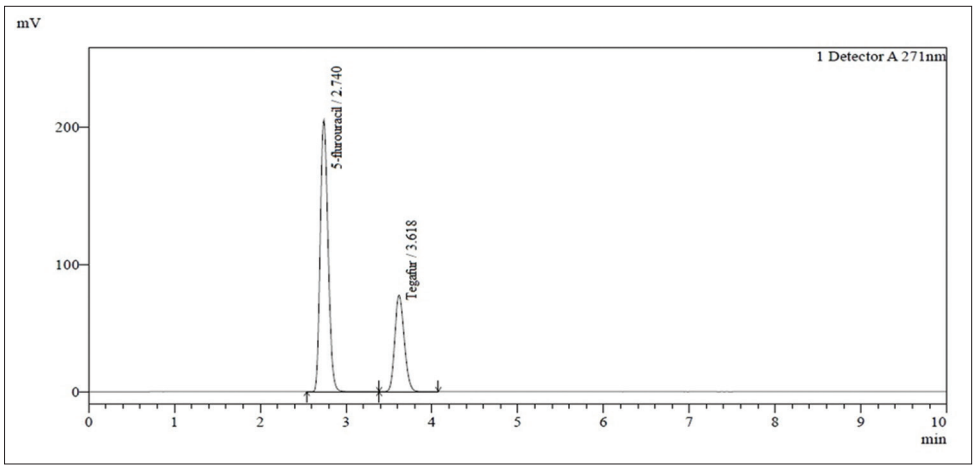

Fig. 10: Chromatograph of thermal degradation

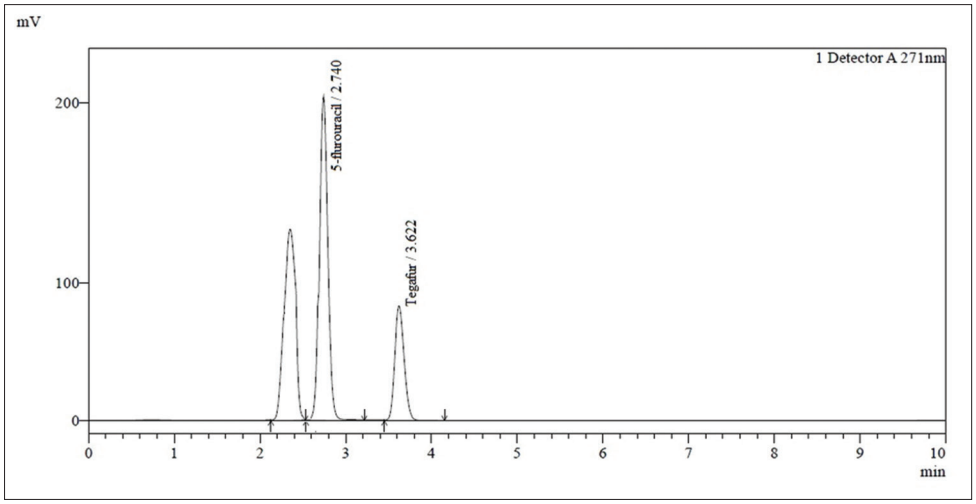

Fig. 11: Chromatograph of oxidative degradation

article will be borne by the authors. Mr.AkshayYelwe collected the data, analyzed the data, all the laboratory work performed, and wrote the introduction, discussion, the material and method part. Mrs.Vaishali Mistry proofreads the whole manuscript, and Mr. Amey Deshpande helps in designing and conducting the study.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interests regarding the publication of this paper.

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