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Original Article

LIQUID CHROMATOGRAPHY DEPENDENT STABILITY INDICATING METHODOLOGY: DEVELOPMENT AND AUTHENTICATION FOR FORMULATIONS OF CAPSULE TYPE CONTAINING TEGAFUR, GIMERACIL, AND OTERACIL

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ABSTRACT

Objective: This investigation entitles the development and authentication of a rapid, selective and explicit RP-HPLC technique to assay tegafur (TGR), gimeracil (GMR), and oteracil (OTR) simultaneously in bulk and formulations of capsule type.

Methods: The separation, detection and assessment of TGR, GMR and OTR were achieved using a C18 Agilent Zorbax (25 cm; 4.6 mm; 5 µm particle dimension) reverse phase column. The acetonitrile (40% by volume) and 0.1% triethylamine in distilled water (pH 2.5, 60% by volume) was utilized as mobile phase. The validation of the method and degradation study was performed as per the strategy given by ICH.

Results: The retention periods in Agilent Zorbax column for OTR, TGR, and GMR were 2.458 min, 7.236 min and 8.629 min, respectively. Linearity was seen in the concentration series of 5.0-30.0 µg/ml (TGR), 1.45-8.70 µg/ml (GMR), and 3.95-23.70 µg/ml (OTR). The regression coefficient was greater than 0.999. The LOQ values were 0.606 µg/ml (TGR), 0.175 µg/ml (GMR), and 0.478 µg/ml (OTR). The percent comparative standard deviation (exactness) values were bestowed to be 0.243%-0.676%, 0.293%-1.894% and 0.269%-0.615% for TGR, GMR and OTR, respectively. The percent recoveries (accuracy) were in the range of 100.044%-100.493 for TGR, 99.730%-100.335% for GMR and 100.064%-100.543% for OTR.

Conclusion: The research results of the degradation investigation proved the technique's specificity as well as stability indicating feature. The process could be used for routine evaluation of OTR, TGR, and GMR in formulations of capsule type.

Keywords: Tegafur, Tegonat capsule, Gimeracil, HPLC, Oteracil, Stability

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INTRODUCTION

Gastric cancer is the fourth largely prevalent cancer in the overall world and is the second leading cause of cancer-related mortality globally [1, 2]. Once the gastric cancer condition has advanced, symptoms start to appear. The incidence probability of gastric cancer varies greatly by region. More than half of all new cases are figured-out in underdeveloped nations. The high-jeopardy regions included East Asia, Central America, Eastern Europe, and South America. The low-jeopardy regions included North America, North Africa, New Zealand Southern Asia, East Africa, and Australia [3].

The clinical therapy of metastatic gastric cancer needed the use of innovative therapeutic medications with greater effectiveness. To manage severe gastric cancer, a set of three pharmaceutical substances-tegafur (TGR), gimeracil (GMR), and oteracil (OTR) was authorized [4, 5]. After being taken orally, TGR progressively transforms into the DNA synthesis-inhibiting compound 5-fluorouracil. Additionally, RNA function is disturbed by the amalgamation of 5-fluorouracil into RNA [6]. GMR, a dihydropyrimidine dehydrogenase blocker, stops 5-fluorouracil against being degraded. In order to lessen toxicity to the healthy gastrointestinal mucosa, OTR, an orotate phosphoribosyltransferase blocker, is designed to limit the action of 5-fluorouracil in the stomach [7, 8]. The chemical architectures of TGR, GMR, and OTR appear in fig. 1.

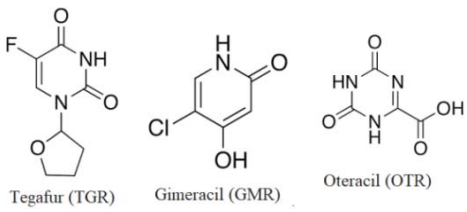

Fig. 1: Chemical architectures of TGR, GMR, and OTR

Pharmaceutical assessment in drug development largely focuses on ways to discover as well as quantify prospective novel drug candidates, assess purity, recognize bye-products and degradation components in compatibility, including stability studies, and to establish the destiny of the drug ingredient in humans [9, 10]. These kinds of difficult activities need advanced approaches, specialized tools, and procedures. Liquid chromatography, specifically high-performance liquid chromatography, has recently emerged as the paramount analytical method employed in both normal quality control agencies and drug development [11, 12]. The fundamental benefit of liquid chromatography is its extensive use in academics, education, and routine development over decades, leading to generally recognized and extensively implemented ways of method innovation, method improvement, and problem-solving. Given that liquid chromatography methodologies are the preferred analytical strategy, many researchers have worked to develop a variety of liquid chromatography techniques for the simultaneous estimate of various active elements in multi-constituent medications [13-21].

The quantification of TGR in combination with uracil and 5-fluorouracil by UPLC-MS/MS [22]; TGR in combination with uracil by LC-MS/MS andLC-UV [23]; TGR alone by competitive ELISA [24]; and TGR in combination with 5-fluorouracil, GMR and oxonic acid by LC–MS/MS [25] were reported. The aforementioned procedures were used only on human plasma [22-25]. The simultaneous estimate of TGR, GMR, and OTR in the pharmaceutical capsule preparation hasn't yet been recorded using any approach.

This study intends to set up an efficient, simple, accurate, and reproducible RP-HPLC approach for the quantitative investigation of TGR, GMR, and OTR. Another objective of the study is to evaluate the validity of the devised RP-HPLC technique and by what means this designed new RP-HPLC approach can identify the stabilities of these three molecules (TGR, GMR, and OTR) under different ICH-mentioned stress situations.

MATERIALS AND METHODS

Drug and capsule formulations

The active pharmaceutical ingredients TGR, GMR and OTR were supplied by "Natco Pharma Limited", India. Tegonat capsules from Natco Pharma Limited, India, were bought at a local market and were branded as having amounts of TGR, GMR, and OTR of 20 mg, 5.8 mg, and 15.8 mg, respectively.

Chemicals

Acetonitrile, triethyl amine, NaOH, HPLC grade water, orthophosphoric acid, HCl, and peroxide were supplied by "Merck Life Science Private Limited" India.

Instruments

The analytes (TGR, GMR and OTR) were investigated employing HPLC system ("Water-2695/Alliance") couple with PDA detector ("Water-2998/Alliance"). The photostability compartment ("Newtronic NLPS4SI") was employed to investigate TGR, GMR and OTR photodegradation. Hot air oven ("Sun life") was employed to investigate TGR, GMR and OTR thermal degradation. The pH of the analytes solutions and the mobile phase was checked with pH meter ("Eutech Instruments-ECPH70042GS"). "Unichrome sonicator" was used to sonicate TGR, GMR and OTR samples.

TGR, GMR and OTR solutions

The active pharmaceutical ingredients TGR (200 mg), GMR (58 mg) and OTR (158 mg) were dissolved in ultrapure acetonitrile to create stock solutions of TGR (2000 µg/ml), GMR (580 µg/ml), and OTR (1580 µg/ml), which were then completed in 100 ml calibrated measurement flasks. Acetonitrile was used to further dilute aliquots of the produced stock TGR, GMR and OTR solutions, resulting in a final volume of 100 ml. These diluted TGR, GMR and OTR solutions were used as working samples having 20 µg/ml, 5.8 µg/ml and 15.8 µg/ml quantities of TGR, GMR and OTR, respectively.

Conditions for TGR, GMR and OTR analysis

The analytes (TGR, GMR and OTR) were separated employing C18 Agilent Zorbax type column (dimensions: 250 mm × 4.6 mm, particle dimension: 5 µm) arranged with ambient temperature using isocratic kind elution, with the mobile phase being a mixture of acetonitrile (40% by volume) and 0.1% triethylamine in distilled water (pH 2.5, 60% by volume) with 1.0 ml per min flow scale. For the quantitative study, the areas of TGR, GMR, and OTR obtained at 282 nm utilising PDA sensor module were employed. For analysis, 10 µl of TGR, GMR, and OTR samples were infused.

Calibration curves of TGR, GMR and OTR

Standard solutions containing 5.0–30.0 µg/ml TGR, 1.45–8.70 µg/ml GMR and 3.95–23.70 µg/ml OTR, were made ready separately in ultra-pure acetonitrile. Measured the peak areas of 5.0–30.0 µg/ml TGR, 1.45–8.70 µg/ml GMR and 3.95–23.70 µg/ml OTR solutions at 282 nm employing the suggested HPLC approach. The peak areas of TGR, GMR, and OTR are exactly proportionate to the corresponding concentrations of each; therefore, calibration graphs for TGR, GMR, and OTR were built and regression equations for TGR, GMR, and OTR were calculated.

Analysis of TGR, GMR and OTR in capsule formulation

One Tegonat capsule's worth of material (20 mg TGR, 5.8 mg GMR, and 15.8 mg OTR) was precisely placed into a calibrated flask of volume 100 ml, and 25.0 ml of acetonitrile was added while the mixture was continuously stirred in a sonicator for almost 30 min. The flask is allowed to cool before being filled with acetonitrile to finish the volume and filtrated with 0.45 µ syringe nylon filter. Acetonitrile was used to further dilute an aliquot (1 ml) of the produced stock Tegonat capsule solutions, resulting in a final volume of 10 ml. These diluted Tegonat capsule solutions were used as working Tegonat capsule samples having 20 µg/ml, 5.8 µg/ml and 15.8 µg/ml quantities of TGR, GMR and OTR, respectively. The suggested approach was then applied to analyse a pharmaceutical Tegonat preparation, and the concentrations of TGR, GMR, and OTR in Tegonat capsule were determined using the associated regression equations.

Stress investigation TGR, GMR and OTR

The tegonat capsule material underwent stress examinations in conformity with ICH norms [26].

Acid degradation

One Tegonat capsule's worth of material (20 mg TGR, 5.8 mg GMR, and 15.8 mg OTR) was appended with 5 ml of acid (1N strength HCl) in a volumetric flask (100 ml capacity) and boiled in a water bath thermostated at 60 °C for 30.0 min. Then, once it had reached room temperature, 5 ml of alkali (1N strength NaOH) was added. Finally, 70 ml of acetonitrile was appended, and the mixture was ultrasonically processed for 20 min before being made up to the required level (100 ml) with acetonitrile.

Alkali degradation

One Tegonat capsule's worth of material (20 mg TGR, 5.8 mg GMR, and 15.8 mg OTR) was subjected to alkali (1 N strength NaOH, 5 ml) and a temperature of 60 °C in a thermostated water bath for 30.0 min. Once the contents had reached room temperature, 5 ml of acid (1N strength HCl) was added. Finally, 70 ml of acetonitrile was appended, and the mixture was ultrasonically processed for 20 min before being made up to the required level (100 ml) with acetonitrile.

Oxidative degradation

This was carried out on one Tegonat capsule's worth of material (20 mg TGR, 5.8 mg GMR, and 15.8 mg OTR) under an oxidant (30% peroxide, 5 ml) at 60 °C in a thermostated water bath for 30.0 min. After bringing the contents to ambient temperature, 70 ml of acetonitrile was appended, and the mixture was ultrasonically processed for 20 min before being made up to the required level (100 ml) with acetonitrile.

Hydrolytic degradation

This was made on one Tegonat capsule's worth of material (20 mg TGR, 5.8 mg GMR, and 15.8 mg OTR) with water (Milli Q, 5 ml) at 60 °C in a thermostated water bath for 30.0 min. After adding 70 ml of acetonitrile, the mixture was ultrasonically treated for 20 min before being topped off with acetonitrile to the necessary level (100 ml). The prepared sample was then filtered using a 0.45 syringe nylon filter.

Photodegradation

A Tegonat capsule comprising 20 mg TGR, 5.8 mg GMR, and 15.8 mg OTR was used during these experiments. The substance (Tegonat capsule powder) was treated in a photostability chamber over 72 lux h. The stressed Tegonat capsule sample was brought to ambient thermal conditions and precisely placed into a calibrated flask of volume 100 ml, and twenty-five ml of acetonitrile was added while the mixture was continuously stirred in a sonicator for almost 20 min. The flask is allowed to cool before being filled with acetonitrile to finish the volume and filtrated with 0.45 µ syringe nylon filter.

Thermal degradation

A Tegonat capsule comprising 20 mg TGR, 5.8 mg GMR, and 15.8 mg OTR was used during these experiments. The substance (Tegonat capsule powder) was treated at 105 °C in the thermostated oven for three hr. The sample of the stressed Tegonat capsule was cooled to ambient temperature and carefully placed in a calibrated flask with a capacity of 100 ml. An 25.0 ml of acetonitrile was further added, and the mixture was constantly agitated in ultrasonication for almost 20 min. After allowing the flask to cool, the remaining volume is replenished with acetonitrile. The prepared sample was then filtered using a 0.45 syringe nylon filter.

In each instance, an appropriate amount of the stressed Tegonat capsule (1 ml) was diluted to 10 ml with acetonitrile before being analysed using the indicated HPLC methodology.

RESULTS

To separate TGR, GMR and OTR well within a manageable run time, the

chromatographic conditions first needed to be optimised. The isocratic mode was adopted for the HPLC assessments of TGR, GMR, and OTR.

Inertsil ODS (dimensions: 250 mm × 4.6 mm, particle dimension: 5 µm), Waters symmetry C18 (dimensions: 250 mm × 4.6 mm, particle dimension: 5 µm), Aligent C18 zorbax (dimensions: 250 mm × 4.6 mm, particle dimension: 5 µm) were put on trails as stationary phase. With Aligent C18 zorbax (dimensions: 250 mm × 4.6 mm, particle dimension: 5 µm), smoother symmetrical peaks, excellent system efficiency are attained. Improved separation selectivity for TGR, GMR and OTR are also attained.

In the proposed investigation, the mobile phase was made up of an eluent that included varied amounts of 0.1% phosphoric acid buffer (pH 2.5) and 0.1% triethyl amine buffer (pH 2.5) with acetonitrile. Adequate retention, better system efficacy, symmetrical peak shapes, and selectivity in a 12 min separation time for TGR, GMR and OTR were obtained with a mixture of acetonitrile (40% by volume) and 0.1% triethylamine in distilled water (pH 2.5, 60% by volume). The same mixture was maintained with 1.0 ml per min flow scale. The elution times were 2.456 min for OTR, 7.236 min for TGR and 8.629 min for GMR (fig. 2).

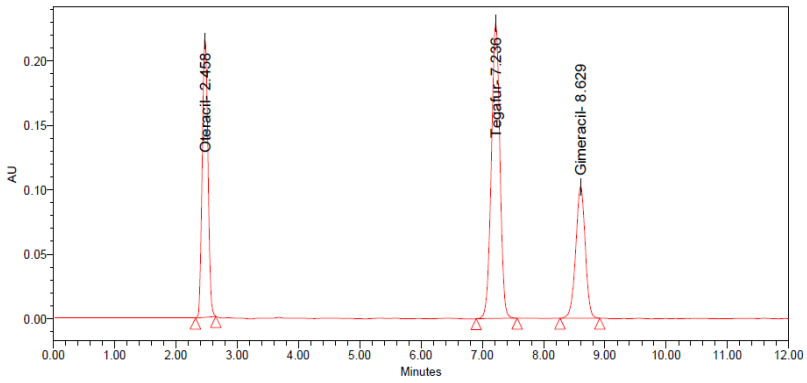

Fig. 2: TGR, GMR and OTR chromatogram

As depicted in fig. 3, the absorbance spectrum of the TGR, GMR, and OTR got scanned across the 200-400 nm range. The data information obtained was saved in the computer. The 282 nm was shown to represent the ideal wavelength for determining TGR, GMR, and OTR.

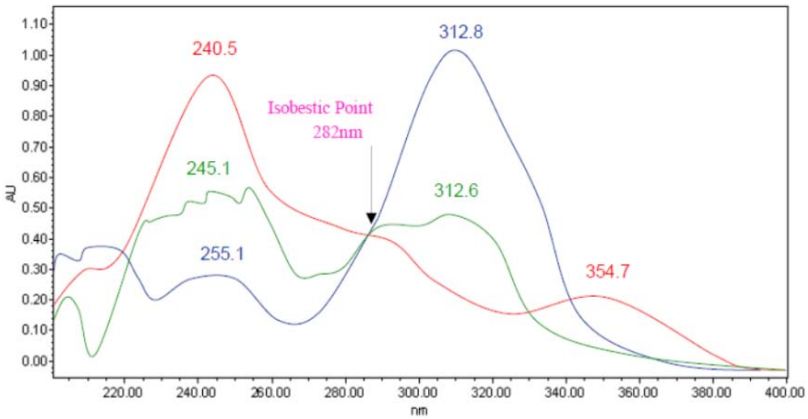

Fig. 3: Absorbance spectrum of the TGR, GMR, and OTR

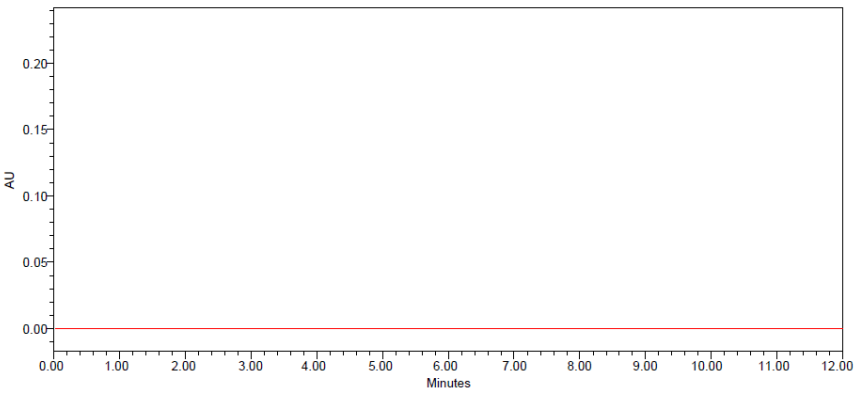

Fig. 4a: Mobile phase blank (without TGR, GMR, and OTR) chromatogram

Validation

When validating the established HPLC: TGR, GMR, and OTR analysis technique, the ICH recommendations were considered taking into account [27, 28].

Selectivity

The selectivity was evaluated in order to identify compounds that might interfere with TGR, GMR, and OTR elution in the chromatogram. Mobile phase blank, working TGR, GMR, and OTR solution (20 µg/ml TGR, 5.8 µg/ml GMR and 15.8 µg/ml OTR) and Tegonat capsule solution (20 µg/ml TGR, 5.8 µg/ml GMR and 15.8 µg/ml OTR) were evaluated to look after selectivity. Fig. 4a to 4c show the comparable chromatograms.

System suitability

Five injections of the working TGR (20 µg/ml), GMR (5.8 µg/ml), and OTR (15.8 µg/ml) sample solution were made in a volume of 10 µl. Peak areas, theoretical plate numbers, elution durations, tail factors for TGR, GMR and OTR were calculated. The standard deviation (SD) and relative standard deviation (RSD) of these variables for TGR, GMR and OTR were calculated were also calculated (table 1).

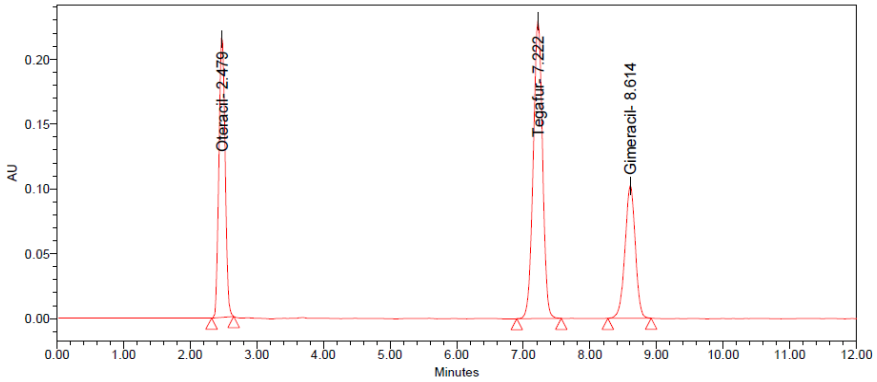

Fig. 4b: Working TGR, GMR, and OTR solution chromatogram

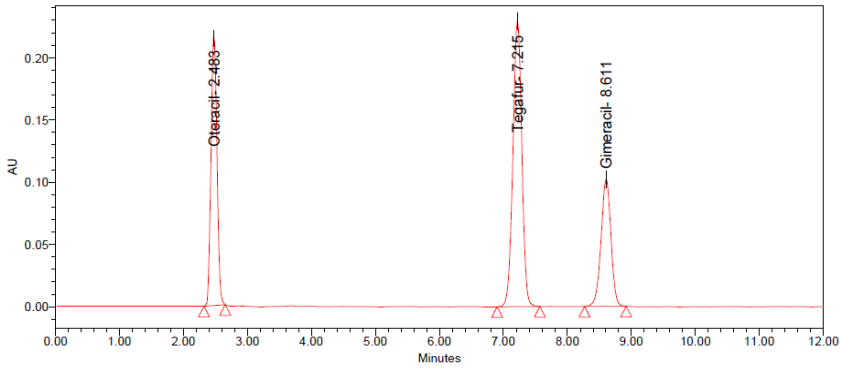

Fig. 4c: Tegonat capsule solution chromatogram

Table 1: System suitability of HPLC: TGR, GMR, and OTR analysis technique

Statistics ↓	Drug area	Drug tailing	Column plate number	Resolution among drugs	Drug's elution time
OTR (15.8 µg/ml)					
Mean†	2269534	1.126	5083.6	-	2.482
SD‡	15440.18	0.016733	5.176872	-	0.002236
%RSD	0.6803	1.4861	0.1018	-	0.0901
TGR (20.0 µg/ml)					
Mean†	2652826	0.974	12832.8	21.784	7.2244
SD‡	17495.66	0.011402	177.7321	0.126016	0.007987
%RSD	0.6595	1.1706	1.3850	0.5785	0.1106
GMR (5.8 µg/ml)					
Mean†	1263457	0.976	14619.6	5.14	8.596
SD‡	22650.61	0.013416	222.8493	0.057446	0.040305
%RSD	1.7927	1.3746	1.5243	1.1176	0.4689

SD–standard deviation; † three number of experiment average; ‡ Deviation for three number of experiment; RSD–relative standard deviation

Linearity

While the linearity test, six calibration solution standards from the ranges of 5.0-30.0 µg/ml TGR, 1.45-8.70 µg/ml GMR, and 3.95-23.70 µg/ml OTR were analysed for the calibration curves of TGR, GMR, and OTR. The peak area and drug quantity were plotted in order to create the drug calibration curve. The intercept, R^2 and slope for TGR, GMR, and OTR were obtained by the application of linear regression. Fig. 5, illustrates the data's linear fit. Linear equation obtained were: y = 14080*x*+12823 for OTR; y = 13236*x*+8507.6 for TGR; and y = 21422*x*+12540 for GMR. The R^2 for OTR was 0.9994; for TGR was 0.9996; and for GMR was 0.9995.

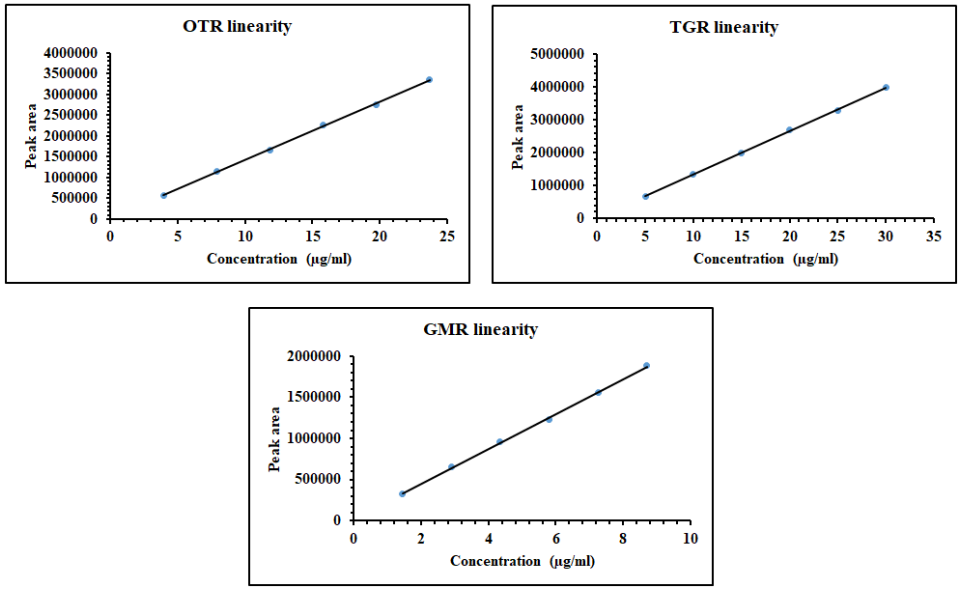

Fig. 5: Data's linear fit for TGR, GMR, and OTR

Sensitivity

In order to fig. out the LOD and LOQ values for TGR, GMR, and OTR, the "signal-to-noise" (STR) ratio was exercised. The LOD of TGR, GMR, and OTR were 0.2 µg/ml, 0.058 µg/ml, and 0.158 µg/ml, respectively. The LOQ of TGR, GMR, and OTR were 0.606 µg/ml, 0.175 µg/ml, and 0.478 µg/ml, respectively. Fig. 6 illustrates the chromatograms of TGR, GMR, and OTR at corresponding LOD and LOQ quantities.

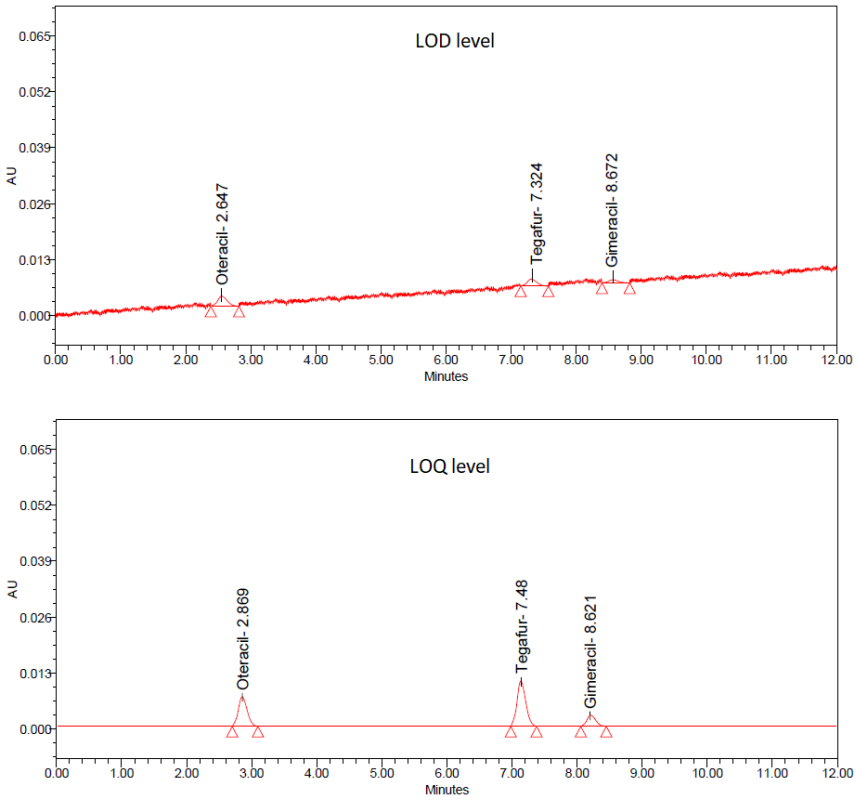

Fig. 6: TGR, GMR, and OTR chromatograms at corresponding LOD and LOQ concentrations

Precision

Mixed standard TGR, GMR, and OTR solutions (TGR-20 g/ml; GMR–5.8 g/ml; and OTR–15.8 g/ml) were evaluated six times within a single day for evaluating system precision and method precision. The peak area and assay of TGR, GMR, and OTR on a single day were used to compute the RSD percentile of TGR, GMR, and OTR. Regarding system precision, the RSD percentile of peak areas of TGR, GMR, and OTR were employed. But for method precision, the RSD percentile of TGR, GMR, and OTR assay was used (table 2).

Table 2: Precision of HPLC: TGR, GMR, and OTR analysis technique

Statistics	System precision		
↓	TGR	GMR	OTR
Mean†	2649280	1268729	2270375
SD‡	17898.44	24024.98	13962.59
%RSD	0.676	1.894	0.615
Statistics	Method precision		
↓	TGR	GMR	OTR
Mean††	99.227	100.860	99.871
SD‡	0.2416	0.2952	0.2684
%RSD	0.243	0.293	0.269

SD–standard deviation; † six number of experiment average for peak area; †† six number of experiment average for percentile assay; ‡ Deviation for six number of experiment; RSD–relative standard deviation

Ruggedness

The investigation of ruggedness used conscious and significant observable changes, such as analyst-analyst and day-day, while keeping the other experimental HPLC: TGR, GMR, and OTR analysis technique circumstances and parameters constant. Mixed standard TGR, GMR, and OTR solutions (TGR-20 g/ml; GMR–5.8 g/ml; and OTR–15.8 g/ml) were evaluated six times for evaluating ruggedness. In table 3, data for ruggedness were displayed.

Table 3: Ruggedness of HPLC: TGR, GMR, and OTR analysis technique

Experiment	GMR assay (%)		OTR assay (%)		TGR assay (%)	
	Analyst 1 Lab 1	Analyst 2 Lab 2	Analyst 1 Lab 1	Analyst 2 Lab 2	Analyst 1 Lab 1	Analyst 2 Lab 2
1	101.098	100.086	100.139	101.487	99.294	98.848
2	101.066	99.813	100.102	101.172	99.44	99.031
3	101.098	100.154	100.091	101	99.531	99.191
4	100.979	99.962	99.915	100.821	98.988	98.896
5	100.775	99.086	99.798	100.74	99.103	98.406
6	100.382	99.008	99.448	100.293	99.074	98.027
Mean†	100.292		100.417		98.986	
SD‡	0.7456		0.6175		0.4207	
%RSD	0.743		0.615		0.425	

SD–standard deviation; † twelve number of experiment average for percentile assay; ‡ Deviation for twelve number of experiment; RSD–relative standard deviation

Table 4: Recovery of TGR with HPLC: TGR, GMR, and OTR analysis technique

µg/ml TGR added	µg/ml TGR found	% TGR recovered	Recovered (%) mean†	SD‡	RSD%
50% study level					
10.19	10.250	100.589	100.493	0.1703	0.169
10.15	10.180	100.296			
10.11	10.170	100.593			
100% study level					
19.89	19.890	100.000	100.168	0.2102	0.210
19.85	19.870	100.101			
19.78	19.860	100.404			
150% study level					
30.05	30.160	100.366	100.044	0.2829	0.283
30.21	30.160	99.834			
30.02	30.000	99.933			

SD–standard deviation; † three number of experiment average for percentile assay; ‡ Deviation for three number of experiments; RSD–relative standard deviation

Table 5: Recovery of GMR with HPLC: TGR, GMR, and OTR analysis technique

µg/ml GMR added	µg/ml GMR found	% GMR recovered	Recovered (%) mean†	SD‡	RSD%
50% study level					
2.99	2.990	100.000	100.335	0.3355	0.334
2.98	3.000	100.671			
2.99	3.000	100.334			
100% study level					
5.89	5.920	100.509	100.226	0.3520	0.351
5.91	5.930	100.338			
5.94	5.930	99.832			
150% study level					
8.65	8.610	99.538	99.730	0.1760	0.176
8.62	8.610	99.884			
8.58	8.560	99.767			

SD–standard deviation; † three number of experiment average for percentile assay; ‡ Deviation for three number of experiments; RSD–relative standard deviation

Recovery study/accuracy

The recovery study of TGR, GMR, and OTR was determined by incorporating additional quantities of TGR, GMR, and OTR into the Tegonat capsule solution, which contains 20 µg/ml TGR, 5.8 µg/ml GMR, and 15.8 µg/ml OTR. TGR recovery (table 4) was seen to range from 100.044% to 100.493, with a minimal RSD (0.169%-0.283%). The recoveries of GMR (table 5) ranged from 99.730% to 100.335%, with an RSD percentile of 0.176% to 0.351%. OTR recovery (table 6) was seen to range from 100.064% to 100.543%, with a percentile minimal RSD of 0.131% to 0.260%.

Robustness

The flow rate as well as the acetonitrile percentage in the mobile phase, were tweaked to assess robustness. Mixed standard TGR, GMR, and OTR solutions (TGR-20 µg/ml; GMR–5.8 µg/ml; and OTR–15.8 µg/ml) were evaluated in each case three times for evaluating robustness. In each case, the assay percentiles of TGR, GMR, and OTR were determined (table 7). The percentage recoveries were 98.987% to 100.467% for GMR, 99.233% to 100.267% for OTR and 98.500% to 100.733% for TFR. The RSD percentiles were found as 0.058% to 0.304% for GMR, 0.058% to 1.202% for OTR and 0.115% to 0.703% for TFR.

Table 6: Recovery of OTR with HPLC: TGR, GMR, and OTR analysis technique

µg/ml OTR added	µg/ml OTR found	% OTR recovered	Recovered (%) mean†	SD‡	RSD%
50% study level					
7.99	8.040	100.626	100.543	0.2616	0.260
8.01	8.030	100.25			
7.97	8.030	100.753			
100% study level					
15.80	15.790	99.937	100.064	0.1684	0.168
15.76	15.760	100.000			
15.68	15.720	100.255			
150% study level					
23.34	23.400	100.257	100.286	0.1314	0.131
23.31	23.410	100.429			
23.34	23.380	100.171			

SD–standard deviation; †three number of experiment average for percentile assay; ‡Deviation for three number of experiments; RSD–relative standard deviation

Table 7: Robustness of HPLC: TGR, GMR, and OTR analysis technique

Robustness parameters →	Flow (+): 1.1 ml a min	Flow (-): 0.9 ml a min	Acetonitrile (+): 45% by volume	Acetonitrile (-): 35% by volume
GMR (5.8 µg/ml)				
Recovered (%) Mean†	98.967	100.467	100.033	100.367
SD‡	0.0577	0.3055	0.1155	0.2082
%RSD	0.058	0.304	0.115	0.207
OTR (15.8 µg/ml)				
Recovered (%) Mean†	99.933	99.767	100.267	99.233
SD‡	0.0577	0.0577	0.2082	1.1930
%RSD	0.058	0.058	0.208	1.202
TGR (20.0 µg/ml)				
Recovered (%) Mean†	100.033	100.733	100.367	98.500
SD‡	0.3215	0.5686	0.1155	0.6928
%RSD	0.321	0.564	0.115	0.703

SD–standard deviation; † three number of experiment average for percentile assay; ‡ Deviation for three number of experiments; RSD–relative standard deviation

Degradation studies

On treatment with 5 ml of acid (1N strength HCl) in a water bath thermostated at 60 °C for 30.0 min, 13.913% of GMR, 18.672% of OTR and 15.159% of TFR were found degraded. The acid-degraded Tegonat capsule chromatogram showed two additional peaks with elution times 3.874 min and 6.167 min (fig. 7). At a temperature of 60 °C in a thermostated water bath for 30.0 min, TGR, GMR, and OTR peaks showed nearly12.832%, 15.681% and 17.836% degradation underneath the alkaline (1 N strength NaOH, 5 ml) condition, respectively. Fig. 7 displays the elution patterns of the degradation products TGR, GMR, and OTR. Two additional peaks with elution times 3.774 min and 6.057 min were seen. Degradations of TGR (15.159%), GMR (18.548%), and OTR (19.600%) were also detected over oxidative condition (30% peroxide, 5 ml) at 60 °C in a thermostated water bath for thirty min. The oxidized Tegonat capsule chromatogram disclosed one additional peaks with an elution time of 4.941 min (fig. 7). An 13.814% degradation of GMR, 10.004% degradation of OTR and 9.651% degradation of TFR were found with exposure of TGR, GMR, and OTR to photostability chamber over 72 lux hrs. After degradation of TGR, GMR, and OTR in the photostability chamber over 72 lux hrs, two degradant peaks with 19.328 min retention time and 20.751 min retention time (fig. 7). GMR degradation was 2.097%, OTR degradation was 12.549% and TFR degradation was 13.049% at 105 °C in a thermostated oven for three hr. One degradant peak having 14.724 elution time (fig. 7) was found with TGR, GMR, and OTR degradation at 105 °C in a thermostated oven for three hrs. Hydrolytic degradation of TGR (0.070%), GMR (3.804%), and OTR (0.040%) at 60 °C in a thermostated water bath for 30.0 min did not yield significant degradation. The chromatogram of hydrolytic degradation of TGR, GMR, and OTRat 60 °C in thermostated water bath for 30.0 min did not display any additional peaks (fig. 7).

Selectivity

By assessing the peak purities of TGR, GMR, and OTR using LabSolution software, the method's selectivity was explored. The peak purities of TGR, GMR, and OTR were evaluated to make absolutely sure that neither comigration components affected the responsiveness of the TGR, GMR, and OTR peaks. The analytes (TGR, GMR, and OTR) were clearly segregated across all degradation compounds (fig. 7), and the peak purities, as well as peaks of TGR (fig. 8), GMR (fig. 9), and OTR (fig. 10) obtained across all stress conditions throughout forced degradation experiments, were pure and homogenous.

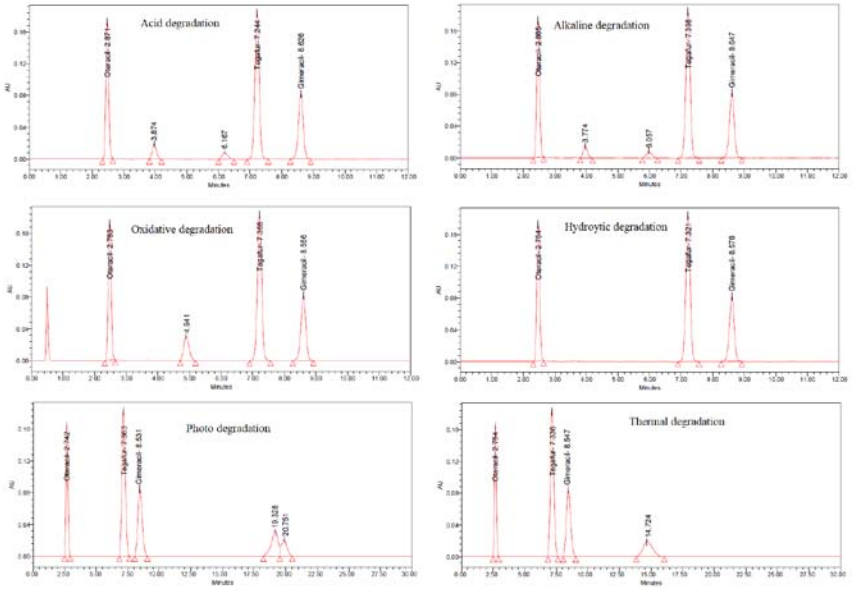

Fig. 7: TGR, GMR, and OTR chromatograms after accelerated degradation tests

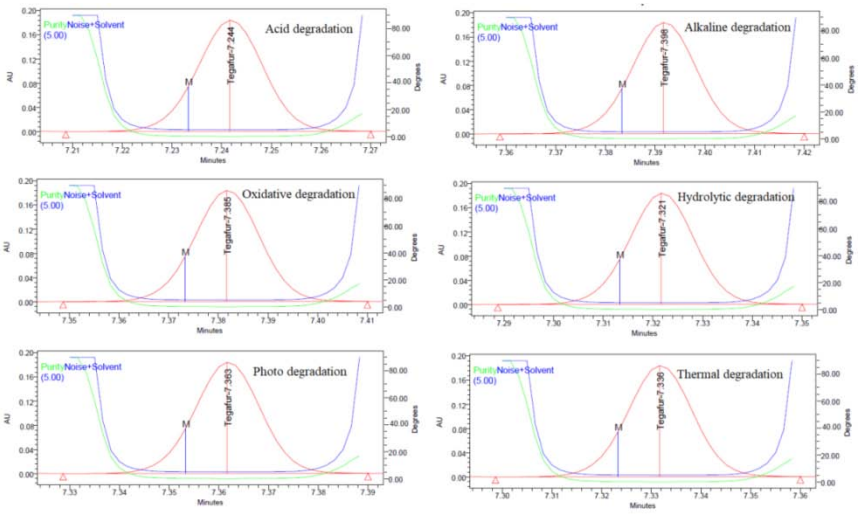

Fig. 8: Peak purity plots of TGR after accelerated degradation tests

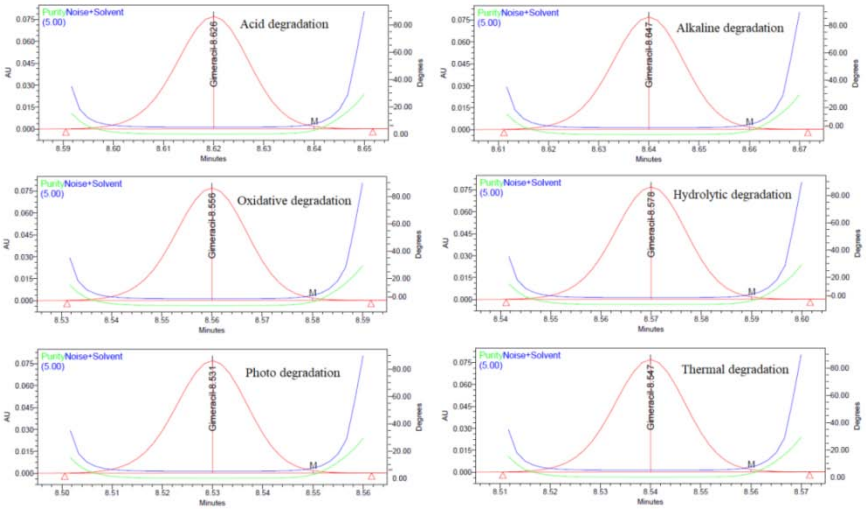

Fig. 9: Peak purity plots of GMR after accelerated degradation tests

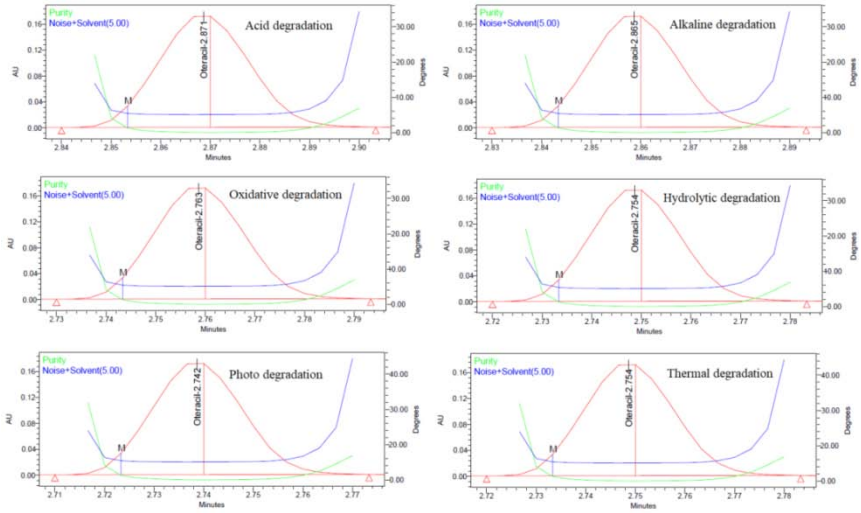

Fig. 10: Peak purity plots of OTR after accelerated degradation tests

Application

Commercially accessible capsule products containing TGR, GMR, and OTR were analysed in order to assess the usability of this newly devised HPLC approach. The TGR, GMR, and OTR recovery values were ascertained in an attempt to appraise the method's applicability. The contents of two determinations, expressed as percentages, were 100.185% and 100.147%, with an RSD percentile of 0.026 and mean content being 100.166% for OTR. The percentages were 99.491% and 99.637%, with RSD percentile of 0.104 and mean content being 99.564% for TGR. The percentages were 100.510% and 100.478%, with RSD percentile of 0.022 and mean content being 100.494% for GMR.

DISCUSSION

Shiraiwa *et al.* reported UPLC-MS/MS [22], Peer *et al.* and Ki *et al.* reported LC-MS/MS [23, 25], Marta *et al.* reported immunoassay [24] methodologies to quantity TGR in uracil/5-fluorouracil/GMR/oxonic acid on human plasma. The simultaneous measurement of TGR, GMR, and OTR was not addressed by any of the described approaches [22–25]. The HPLC method developed in our work is the first to concurrently measure TGR, GMR, and OTR in bulk and formulations of the capsule type.

The ICH recommendations were considered taking into account while validating the established HPLC: TGR, GMR, and OTR analysis technique [27]. Excipients in capsule formulation and mobile phase ingredients were not observed to interfere with TGR, GMR, and OTR elution. The selectivity was supported by the chromatograms (fig. 4a to 4c) of selectivity [29].

After analysing the results of five analytical replications, it was concluded that there were no discernible variations in the responses. The high degree of instrument precision was reflected by the relative standard deviation (RSD) during system appropriateness testing of the approach, which was ascertained to be<2 (0.0901% to 1.7927%) [30]. The data disclosed an acceptable fit to the regression line with R^2 values of 0.9994 for OTR, 0.9996 for TGR, and 0.9995 for GMR [31]. The LOD is the quantity where the signal intensity of the TGR/GMR/OTR is at minimum three times that of the baseline signal noise [32]. The LOQ is the quantity where the signal intensity of the TGR/GMR/OTR is at minimum ten times that of the baseline signal noise [32]. The low fig. of LOD and LOQ for TGR, GMR, and OTR disclosed ample sensitivity of HPLC: TGR, GMR, and OTR analysis technique

For TGR, GMR, and OTR, the RSD percentile of peak area variability varied from 0.615% to 1.894%, demonstrating admissible system precision [33, 34]. While the RSD percentile of assay variability for TGR, GMR, and OTR varied from 0.243% to 0.293%, exhibiting excellent technique precision. The RSD percentile of assay variability for TGR, GMR, and OTR was consistently determined to be under 2%, exhibiting excellent technique ruggedness [33, 34]. The TGR, GMR, and OTR mean recoveries, which are close to 100%, and the lower RSD (lower than 1%), show that the HPLC: TGR, GMR, and OTR analysis technique was accurate [33, 34]. The RSD (0.058%-1.202%) and percentile assays (98.987%-100.733%) for TGR, GMR, and OTR obtained under all robustness experimental circumstances fell within allowable ranges for all modifications to the analytic conditions. The procedure is hence robust [33, 34].

The accelerated degradation tests demonstrate the TGR, GMR, and OTR's vulnerability to deterioration in basic, heat, UV, acidic, as well as oxidative circumstances [35, 36]. Chromatographic separation of opted analytes (TGR, GMR, and OTR) from distinct degradation products was achieved. This shows that the new approach has stability indicating efficiency and specificity for determining TGR, GMR, and OTR in pharmaceutical capsule formulations and bulk raw samples.

CONCLUSION

The established HPLC: TGR, GMR, and OTR analysis methodology is quick and simple, and it has been successfully used to assure the TGR, GMR, and OTR in their formulation capsule form meet quality standards. The time-and cost-efficient, well-established HPLC: TGR, GMR, and OTR analytical approach stand out as a potent method for achieving effective and reliable separation of TGR, GMR, and OTR. These benefits, which have been scientifically verified, support the quality control laboratories' attempts to use this HPLC: TGR, GMR, and OTR analytical approach. Another benefit of this approach is that it accomplished excellent separation of all analytes (TGR, GMR, and OTR) and degradation products, as seen by the chromatograms of TGR, GMR, and OTR following degradation tests.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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